Diss. ETH No. 12324

Medical Ethnobotany of the Isthmus-Sierra Zapotecs (Oaxaca, Mexico) and Biological-Phytochemical Investigation of Selected Medicinal Plants

A dissertation submitted to the

SWISS FEDERAL INSTITUTE OF TECHNOLOGY ZURICH

for the degree of

Doctor of Natural Sciences

Presented by

BARBARA FREI

Eidg. dipl. Apothekerin
born February 6, 1964
citizen of Au (SG)

Accepted on the recommendation of
Prof. O. Sticher, examiner
Dr. M. Heinrich, co-examiner

Zürich 1997

marmota
Barbara Frei
Runatsch 137
CH-7530 Zernez, Switzerland

Published by *marmota* 1997

ISBN 3-9521454-0-8

Printed in Switzerland by MIKRO + REPRO AG,
Haselstr. 16, CH-5400 Baden

¡Ay! Santo Domingu
bele guidaguiétina zih tu de líí,
gunaba lo Dios para ná
di gula'ala guihquiluna
dso'si da xqueala na'
di zuzedazi galan
ñah capa nuna zih tu de lii
di dziah la da guiqui na li.

Augustín Olivera Estudillo

A los Zapotecos de la Sierra del Istmo de Tehuantepec

I went to the woods

because I wished to live deliberately.

I wanted to live deep and suck out all the marrow of life.

To put to rout all that was not life.

And not, when I came to die, discover that I had not lived.

Henry D. Thoreau

Curriculum Vitae

1964	Born February 6, Zurich, Switzerland
1971-1976	Primary school, Oberlunkhofen, Switzerland
1976-1981	College, Bremgarten, Switzerland
1981-1985	Gymnasium, Wettingen, Switzerland; Matura Type D (modern languages)
Summer 1985	Au-pair for 6 months in Irvine, CA, USA
1985-1987	Swiss Federal Institute of Technology, ETH Zurich, Switzerland, studies in pharmaceutical sciences (Scientific basic course, pharmaceutical basic course)
1987-1988	Practical training at the Pharmacy of Gumligen, and accompanying courses at the University of Berne, Switzerland
1988-1990	Swiss Federal Institute of Technology, ETH Zurich, Switzerland, studies in pharmaceutical sciences (Pharmaceutical specialist courses)
December, 1990	Diploma: *Eidg. Dipl. Apothekerin*
1990-1992	Pharmacist at the Koenigstein pharmacy of Kuttigen, Switzerland; Spanish classes in Mexico and searching scolarships for field work of the future doctoral thesis
1992-1997	Ph. D. candidate at the Swiss Federal Institute of Technology, ETH Zurich, Switzerland, Department of Pharmacy, Section Pharmacognosy and Phytochemistry (Prof. O. Sticher) in collaboration with: -University of Freiburg, Germany; anthropological and pharmaceutical/phytochemical collaboration (Dr. M. Heinrich, Prof. H. Rimpler) -Universidad Nacionál Autónoma de México, Mexico-City, Mexico, anthropological and botanical collaboration during ethnobotanical fieldwork (Dr. P. Davila, Dr. C. Viesca, Dr. R Campos, Dr. R. Bye)
1992-1993, 1994	Ph.D. field studies: Medical, ethnobotanical fieldwork (during 15 and 2 months respectively) in Oaxaca, Mexico
1993-1997	Part-time employment as pharmacist, at the Koenigstein-pharmacy of Kuttigen, Switzerland
September 1997	Final exame to obtain the degree of Doctor of Natural Sciences, ETH Zurich, Switzerland

To **Doña Modesta** and **Don Zacarias** with their family I am thankful for the pleasant stays at their house while doing research in Santa María Guenagati. For the hospitality in Guevea de Humboldt I am thankful to **Doña Artemia** and **Sheila Cabrera O.**

Thanks are extended to **Nereyda Antonio B.**, **Dr. Ignacio Bernal T.**, and **Dr. Arturo Argueta V.** of the Instituto Nacional Indigenista (INI) for their help in botanical, medicinal and teaching matters as well as for the protection and authorization of my journeys to the Isthmus.

I am also indebted to the professional botanists who identified many of my collections and helped to solve other "botanical-related" problems. These are at the Universidad Nacional Autónoma de México (UNAM) in the Herbario Nacional: **Dra. Patricia Davila A.**, **Leticia Torres C.**, **Francisco Ramos M.**, **Oswaldo Tellez V.**, **Dr. Jose-Luis Villaseñor R.** (Asteraceae), **Dr. Mario Sousa S.** (Leguminosae), **Rafael Torres C.** (Flora de Oaxaca), **Dr. Rafael Lira S.** (Cucurbitaceae), and **Gilda Ortiz C.**; and at the Jardín Botanico: **Dr. Robert Bye Jr.** and **Teodolinda Baltazar S.** For the identifications of some tricky collections I would like to thank **Dr. Roger McVaugh** (Myrtaceae) and **Dr. Michael Heinrich** (Lamiaceae).

I express my sincere thanks to the Sociedad Para El Estudio De Recursos Bióticos De Oaxaca (SERBO, A.C.), but especially to **Silvia H. Salas M.** and **Leo Schibli** for the infectious motivation for ethnobotany, development and conservation matters, as well as for their hospitality and friendship. In addition, I will not forget to include my friend and Spanish teacher, **Guadalupe Jiménez O.** and **her parents** in the Isthmus who helped me to make the long travelling to the field a bit easier.

For anthropological discussions as well as their hospitality and friendship I thank **Dr. Carlos Viesca T.**, **Dr. Roberto Campos N.**, and his wife **Adriana** in Mexico (Instituto de Filosofia e Historia de la Medicina, UNAM) and **Liselotte Kuntner** in Küttigen, Switzerland (Institute of Ethnology, University of Zurich).

I wish to thank sincerely **Dr. Jimmy Orjala** for the motivating support, the fruitful discussions and the advice on chromatographic matters. Thanks are due to

Acknowledgements

This present study was carried out in the Sierra del Istmo de Tehuantepec in Oaxaca, Mexico and at the Department of Pharmacy, Division Pharmacognosy/ Phytochemistry, Swiss Federal Institute of Technology (ETH) Zurich, Switzerland.

I wish to specially acknowledge those persons who not only tolerated my insistent questions and plant collecting, but also revealed to me their professional secrets by teaching me their art of healing and for their friendship and hospitality: Healers, midwives, and the inhabitants of Santo Domingo Petapa (SDP), Santa María Petapa (SMP), Guevea de Humboldt (GdH), and Santa María Guenagati (SMG). But specially my principal informants: **Doña Eleuteria, Don Sixto, Don Estanislao, Doña Crisófora, Doña Rosa, Doña Francisca, Don Cipriano, Doña Adela, Don Constanzio,** and **Doña Eufrosina.**

My sincere gratitude goes to **Victoria Ramirez L.** and **Victor de la Cruz** who opened for me the most important doors for the field work.

I wish to express my gratitude to **Prof. Dr. Otto Sticher**, my supervisor, for giving me the possibility to carry out an ethnobotanical-phytochemical thesis at the Department of Pharmacy, for encouraging me at several difficult stages of the work and for providing excellent working facilities. I am most grateful to **Dr. Michael Heinrich**, my co-supervisor, for introducing me to anthropology and for all the valuable discussions during the course of this most challenging Mexican adventure.

I am indebted to the whole family of **Doña Eleuteria** and **Don Jorge** for their unforgettable hospitality, to **Doña Amelia**, **Don Efren,** and their families, for adopting me, for raising me a second time by teaching me to behave like a Zapotec woman.

My thanks go also to **Don Jorge** and **Maestra Maurilia Velazquez V.** for their patience in teaching me the Zapotec language.

I wish to thank my friends **Petra Cruz de Juarez** and **Ismael Juarez** for the pleasant time in the paradise, called Aurora, and for accompanying me on difficult journeys to the ranchos.

corporae sano", así como a **Michael Vasescha** por su apoyo técnico en varias ocasiones y por entrenarme a ser puntual.

Me gustaría agradecer especialmente a la **Dra. Beatrix Falch** quien revisó este manuscrito y me apoyó con sus conversaciones alentadoras durante todo el período de mi tesis. Igualmente le doy las gracias a **David McLaughlin** quien corrigió el inglés en este trabajo.

Mi agradecimiento se dirige también al **Prof. Horst Rimpler** por dejarme participar en el proyecto Friburgo-México. Además les estoy agradecida al jardinero **Harald Bunz**, y las **colegas** de la Universidad Albert Ludwigs en Friburgo/Alemania por su cooperación múltiple en este proyecto.

Gracias igualmente a **todos mis amigos** (¡a uno en especial!) quienes llenaron hasta arriba mi apartado postal en el Zócalo, hicieron trabajar el fax público de Oaxaca e incluso vinieron a visitarme.

Por fin quiero extender mi agradecimiento a **mis padres** y a todos los miembros de **las familias Haller y Frei** por su apoyo y su continuo estímulo a lo largo de este trabajo.

Y como último en orden, pero no en importancia, le quedo profundamente agradecida a **Ruedi Haller** por su apoyo personal y por darme ánimos cuando los necesitaba – espero que el producto final de estos estudios llegue a justificar de algún modo el sacrificio hecho.

UNAM) y **Liselotte Kuntner** en Küttigen, Suiza (Instituto de Etnología de la Universidad de Zurich).

Mi profundo agradecimiento lo dirijo también al **Dr. Jimmy Orjala** por su apoyo tan alentador, por las conversaciones provechosas y los consejos en asuntos cromatográficos. Asimismo le debo las gracias al **Dr. Oliver Zerbe** quien me introdujo a la resonancia magnética nuclear (RMN) moderna y me asistió con mucha paciencia cuando elaboré estudios RMN uni- y bi-dimensionales (1D y 2D).

Además estoy agradecida al **Dr. Joachim Schmitt** y **Dr. Dieter Herrmann** de Boehringer-Mannheim por llevar a cabo unos ensayos biológicos así como por las interesantes conversaciones sobre citotoxicidad y líneas celulares.

Al **Dr. Matthias Baltisberger** (quien me procuró material de mucha ayuda para secar plantas), al **Dr. Walter Amrein** y a su equipo (mediciones de espectrometría de masas) y al **Dr. Engelbert Zass** (búsqueda de literatura) quiero dar mis gracias por ayudarme a cumplir con muchas de las tareas que el trabajo de campo y las investigaciones fitoquímicas llevan consigo.

Unas palabras de agradecimiento muy especiales se las dedico a **Selma** y **Horst Riede**, a **Sandra Feldmeier** y a **Edgar Partida P.** Me ayudaron a soportar mejor el choque cultural cuando tuve que viajar a México D.F., sabiendo que iba a poder pasar unos momentos maravillosos con ellos en Cuernavaca o Puebla.

A la **Agencia Suiza para el Desarrollo y la Cooperación** (COSUDE) y a la fundación **Barth Fonds** del Instituto Tecnológico Federal de Suiza en Zúrich les debo mi agradecimiento por su generoso apoyo financiero de mis estudios etnobotánicos en la Sierra de Oaxaca.

Además quiero expresar mi gratitud a mis **colegas** y al **personal** en el Departamento de Farmacia del Instituto Tecnológico Federal de Suiza en Zúrich quienes contribuyeron a que el tiempo durante el que trabajé en el laboratorio fuera – ¡en su mayoría! – muy agradable. Mi agradecimiento especial se lo expreso en este sentido a **Deniz Tasdemir** por "mantener viva la llama de la fitoquímica" y por las muchas e interminables conversaciones, no sólo científicas. Gracias también a mis **colegas de footing** con quienes sudaba para un "anima sana in

Además quisiera darles las gracias a mis amigos **Petra Cruz de Juarez** e **Ismael Juarez** por los lindos momentos que pasamos en aquel lugar paradisíaco llamado Aurora y por acompañarme en algunos viajes difíciles a los ranchos.

Estoy igualmente agradecida a **Doña Modesta** y a **Don Zacarias** y sus familias por acogerme de una manera tan amable mientras llevaba a cabo investigaciones en Santa María Guenagati. La hospitalidad que recibí en Guevea de Humboldt se la debo a **Doña Artemia** y a **Sheila Cabrera O.**

Mi agradecimiento se dirige también a **Nereyda Antonio B.**, al **Dr. Ignacio Bernal T.** y al **Dr. Arturo Argueta V.** del Instituto Nacional Indigenista (INI) por su ayuda en asuntos botánicos, medicinales y pedagógicos así como por autorizar y proteger mis viajes al Istmo.

Igualmente les estoy agradecida a los botánicos profesionales quienes identificaron muchas de mis colecciones y me ayudaron a resolver otros problemas relacionados con la botánica. Ellos son, en la Universidad Nacional Autónoma de México (UNAM): la **Dra. Patricia Davila A.**, **Leticia Torres C.**, **Francisco Ramos M.**, **Oswaldo Tellez V.**, el **Dr. José-Luis Villaseñor R.** (Asteraceae), el **Dr. Mario Sousa S.** (Leguminosae), **Rafael Torres C.** (Flora de Oaxaca), el **Dr. Rafael Lira S.** (Cucurbitaceae) y **Gilda Ortiz C.** Y en el Jardín Botánico son: el **Dr. Robert Bye Jr.** y **Teodolinda Baltazar S.** Por la identificación de algunas colecciones difíciles quisiera darles las gracias al **Dr. Roger McVough** (Myrtaceae) y al **Dr. Michael Heinrich** (Lamiaceae).

Deseo expresar mi gratitud a la Sociedad Para El Estudio De Recursos Bióticos De Oaxaca (SERBO, A.C.), y en especial a **Silvia H. Salas M.** y a **Leo Schibli** por su manera contagiosa de motivarme por la etnobotánica y los temas del desarrollo y la conservación, así como por su hospitalidad y amistad. Además no olvidaré incluir en este agradecimiento a mi amiga y profesora de español, **Guadalupe Jiménez O.** y a sus padres, que viven en el Istmo y me ayudaron a hacer algo más fácil mis largos viajes al campo.

Agradezco asimismo las conversaciones sobre antropología y la hospitalidad y amistad que me ofrecieron el **Dr. Carlos Viesca T.**, el **Dr. Roberto Campos N.** y su esposa **Adriana** en México (Instituto de Filosofía e Historia de la Medicina,

Agradecimientos

Los estudios que aquí se presentan se llevaron a cabo en la Sierra del Istmo de Tehuantepec en Oaxaca, México, así como en el Departamento de Farmacia, Sección de Farmacognosia/Fitoquímica, del Instituto Tecnológico Federal de Suiza en Zúrich (ETH).

Me gustaría agradecer especialmente a todos aquellos quienes no sólo toleraron mis eternas preguntas y mi insistencia en coleccionar plantas, sino también me revelaron sus secretos profesionales, enseñándome su arte de curar, y me hicieron sentir su amistad y hospitalidad: los curanderos, las parteras y todo el pueblo de Santo Domingo Petapa, Santa María Petapa, Guevea de Humboldt y Santa María Guenagati, pero ante todo a mis principales informadores, **Doña Eleuteria, Don Sixto, Don Estanislao, Doña Crisófora, Doña Rosa, Doña Francisca, Don Cipriano, Doña Adela, Don Constancio** y **Doña Eufrosina**.

También deseo dar las gracias a **Victoria Ramirez L.** y a **Victor de la Cruz**, quienes abrieron para mí las puertas más importantes hacia el trabajo de campo.

Además quisiera expresar mi gratitud al **Prof. Dr. Otto Sticher**, mi supervisor, por haberme dado la posibilidad de elaborar esta tesis etnobotánica-fitoquímica en el Departamento de Farmacia, por infundirme ánimos en varias fases difíciles de los estudios y por proporcionarme unas excelentes facilidades de trabajo. También le estoy muy agradecida al **Dr. Michael Heinrich**, mi cosupervisor, por introducirme a la antropología así como por todas las valiosas conversaciones a lo largo de esta emocionante aventura mexicana.

Mi profundo agradecimiento se extiende a toda la familia de **Doña Eleuteria** y **Don Jorge** por su hospitalidad inolvidable, así como a **Doña Amelia**, a **Don Efren** y a sus familias por adoptarme y criarme una segunda vez, enseñándome a comportarme como una mujer zapoteca.

También a **Don Jorge** y a **Maestra Maurilia Velazquez V.** quiero expresar mi gratitud por haber tenido tanta paciencia enseñándome la lengua zapoteca.

Dr. Oliver Zerbe, for introducing me to modern NMR spectroscopy and the patient assistance while I was performing 1D- and 2D-NMR studies.

In addition, I am grateful to **Dr. Joachim Schmitt** and **Dr. Dieter Herrmann** from Boehringer-Mannheim for performing bioassays and for the interesting discussions about cytotoxicity and cell lines.

For covering many of the duties while doing field work and chemical prospecting I would like to thank **Dr. Matthias Baltisberger** (for providing me with very helpful material for plant drying), **Dr. Walter Amrein** and his staff (MS measurements), and **Dr. Engelbert Zass** (literature searches).

A special word of thanks is due to **Selma** and **Horst Riede, Sandra Feldmeier**, and **Edgar Partida P.** who made it easier for me to overcome the cultural shock when travelling to Mexico City, knowing that I would be able to enjoy a wonderful time with them in Cuernavaca or Puebla.

For generous financial support of my ethnobotanical research in the Oaxacan Sierra, I am indebted to the **Swiss Agency of Development Cooperation** (**SDC**) and the **Barth Fonds** of ETH Zurich.

I express my sincere thanks to those **colleagues** and **staff** at the Department of Pharmacy, ETH Zurich who made most (!) of the period I worked in the laboratory very enjoyable. Special thanks therefore go to **Deniz Tasdemir** for "keeping the flame flickering" for phytochemistry and for the endless, not always scientific, discussions! Many thanks go to my **running colleagues**, where we sweat for "anima sana in corporae sano". Thanks to **Michael Wasescha** for the technical assistance in several situations and for training me in being punctual.

Special thanks are due to **Dr. Beatrix Falch** for revising this manuscript and motivating discussions throughout the period of my thesis. Many thanks are due to **David McLaughlin** for checking the English usage in this present work.

I would like to thank **Prof. Dr. Horst Rimpler** for let me participate in the Freiburg-Mexican project. I am grateful to the gardener **Harald Bunz**, and to the **colleagues** from the Albert-Ludwigs-University of Freiburg/Germany for cooperating with this project in many ways.

Sincere thanks are also due to **all my friends** (one especially!) who jammed my P. O. Box at the Zocalo and fed the public fax of Oaxaca or even came to visit me.

Finally, my warmest thanks are due to **my parents** and the **Frei-** and **Haller-families** for their support and encouragement during this work.

Last but not least, I am deeply grateful to **Ruedi Haller** for private support and motivation and I can only hope that the final product of this study in some way justifies the sacrifices.

Contents

	page
Resumen	19
Summary	21
Zusammenfassung	23
Foreword	25
Background and Objectives	27
Section 1	33
1 Introduction	35
1.1 Ethno-science	35
1.2 Ethnobotany	36
1.3 Medicinal Ethnobotany in Mexico	37
1.4 Studies among the Isthmus-Sierra Zapotecs	39
2 Publications	45
2.1 Publication I	45
2.2 Publication II	69
2.3 Publication III	109
3 Additional Results and Discussion	138
3.1 Geographical and historical-ethnological background	138
3.2 Methodology	139
3.3 Evaluation of data	151
3.3.1 Why is a plant medicinal for the Zapotecs? Why is a plant a food plant?	151
3.3.2 Ranked lists	152
3.3.3 Category of indigenous uses - Part of plant used - Frequency of plant parts used	153
3.3.4 Parts of plant used	159
3.3.5 Frequency of plant parts used	163
3.3.6 Ethno-ecological considerations	165
3.3.7 Evaluations, what for?	165
4 Conclusion	166
4.1 To improve in future ethnobotanical research	166
4.2 Negative and positive impacts from the research	168
4.3 Feedback so far from the research	168
Section 2	171
5 Introduction	173

5.1 Strategies in plant screening 173
5.2 Requirements for bioassays 175
5.3 Strategies in the present work 177
6 Publication IV 179
7 Additional Results and Discussion 208
7.1 Phytochemical screening with spray reagents 208
7.2 Bioassays: Problems and advantages 210
7.2.1 Extraction and preparation of samples for assays 210
7.2.2 Alleged properties and their corroboration in bioassays 210
7.2.3 Additional factors affecting bioassay's results 211
7.2.4 Activity threshold in bioassays 214
8 Conclusion 215
8.1 Why *Begonia heracleifolia*? 215
8.2 New trends in plant screening for drug development 216
8.2 Is there an ethical and moral basis in plant screening? 218
Section 3 219
9 Introduction 221
9.1 Botanical and phylogenetic aspects of Begoniaceae 222
9.2 Botany and Medicinal Uses of *Begonia heracleifolia* 224
9.2.1 Botany (Irmscher, 1960; Thorne, 1983; Mabberley, 1996) 224
9.2.2 Zapotec medicinal uses 227
9.3 Botanical and chemical investigations of *Begonia* sp. 229
10 Publication V 233
11 Additional Results and Discussion 244
11.1 Isolation procedures 244
11.1.1 Extraction 244
11.1.2 Fractionation 245
Vacuum liquid chromatography (VLC) 246
Medium pressure liquid chromatography (MPLC) 246
Liquid-liquid partition (LLP) 247
High pressure liquid chromatography (HPLC) 247
Open column chromatography 247
Thin layer chromatography (TLC) 248
Gas Chromatography (GC) 248
11.2 Structure elucidation 251

11.2.1 Methods 251
Nuclear Magnetic Resonance spectroscopy (NMR) 251
Mass spectrometry (MS) 253
Optical rotation 254
Chemical derivation 255
11.3 Compounds isolated from *Begonia heracleifolia* 256
Sterols 256
Fatty acids 258
Cucurbitacins 259
Oligosaccharide 277
11.4 Biological activities and chemotaxonomic considerations of isolated compounds 277
12 Conclusion 279
Epilogue 281
References 283
Appendix 299
I Questionnaire from field work 300
II List of plants collected, medicinal uses, application and preparation 302
II.1 Location 302
II.2 Plant part used 303
II.3 Preparation 304
II.4 Application 306
II.5 Categories of indigenous uses 306
II. 6 Culture-bound syndromes and symptoms 307
II.7 Classification, qualities 310
II.8 Categories of "Importance" 311
III List of healers participating in the study 342
IV Medicinal plant garden in Santo Domingo Petapa 344
V NMR-Spectra of isolated compounds 345
VI Abbreviations 365
VII Note on orthography of the Zapotec language 368
VIII Glossary of Spanish and Zapotec expressions 369
IX List of Poster 372
X List of Oral Presentations 372
XI List of Publications 373

XII List of scholarships obtained 374
XIII List of Figures 374
XIV List of Tables 376
Map of the area of research 381
Curriculum Vitae 382

Indice (abreviación)

página
Resumen 19
Prólogo 25
Trasfondo y objetivos 27
Sección 1: Etnobotánica medicinal sobre los Zapotecos de la Sierra del Istmo 33
1 Introducción 35
1.1 Etno-ciencias 35
1.2 Etnobotánica 36
1.3 Etnobotanica medicinal en México 37
1.4 Estudios sobre los Zapotecos de la Sierra del Istmo de Tehuantepec 39
2 Publicaciones 45
2.1 Publicación I: Plantas como medicina y alimento: Criterios de selección entre los Zapotecos (Medicinal and food plants: Zapotec criteria for selection) 45
2.2 Publicación II: Etnobotánica medicinal sobre los Zapotecos de la Sierra del Istmo (Oaxaca, México): Documentación y evaluación (Medicinal ethnobotany of the Isthmus-Sierra Zapotecs (Oaxaca, Mexico): Documentation and evaluation) 69
2.3 Publicación III: Estrategias de manejo indigena de plantas medicinales en el Istmo de Tehuantepec (México): Diversidad botánica e importancia cultural (Indigenous medicinal plant management in the Isthmus of Tehuantepec (Mexico): Botanical diversity and cultural importance) 109
3 Resultados adicionales y discusión 138
4 Conclusión 166
Sección 2: Rastreo biológico y actividades en los bioensayos 171

5 Introducción 173
6 Publicación IV: Rastreo biológico multiple de plantas medicinales de Oaxaca, México: Etnobotánica y bioensayos como base de investigaciones fitoquímicas (Multiple Screening of Medicinal plants from Oaxaca, Mexico: Ethnobotany and Bioassays as a Basis for Phytochemical Investigation) 179
7 Resultados adicionales y discusión 208
8 Conclusión 215
Sección 3: Investigaciones fitoquímicas 219
9 Introducción 221
10 Publicación V: Investigaciones fitoquímicas y biológicas *de Begonia heracleifolia*: Estudios sobre mecanismos de estructura-actividad de cucurbitacinas (Phytochemical and Biological Investigation of Begonia heracleifolia: Structure-Activity Studies on cucurbitacins) 233
11 Resultados adicionales y discusión 244
12 Conclusión 279
Epílogo 281
Bibliografía 283
Apéndice 299
II Lista de plantas medicinales, usos medicinales, aplicaciones y preparaciones 302
II.1-8 Abreviaciones de la lista de plantas medicinales 302
III Lista de curanderos y parteras paticipantes en estos estudios 342
IV Jardín de plantas medicinales en Santo Domingo Petapa 344
Mapa del área de investigación 381

Resumen

Los zapotecos, habitantes de la Sierra del Istmo de Tehuantepec (Oaxaca, México), viven en una zona tropical con una gran diversidad botánica. Para este grupo indígena, las plantas y su uso tradicional juegan un rol importante tanto en la vida diaria como en los tratamientos médicos. Los curanderos, las parteras y los hierberos usan en sus tratamientos empíricos y sus rituales más de 445 especies de plantas diferentes procedentes de 110 familias botánicas.

En el marco de unos estudios de investigación etnobotánico-fitoquímica, a lo largo de 17 meses se documentaron, con trabajos etnológicos de campo, el uso y la aplicación de plantas medicinales y la manera de pensar medicinal de los curanderos, las parteras y los hierberos zapotecos. Se lograron describir 3611 posibles aplicaciones, integrando en el herbario 445 plantas medicinales. Como los conceptos de enfermedad más frecuentes entre los zapotecos pudieron averiguarse en primer lugar diversos conceptos dualísticos (p. ej. caliente-frío, dulce-agrio, húmedo-seco) así como la doctrina de signaturas y algunas clasificaciones organolépticas. Los zapotecos coleccionan plantas procedentes de seis zonas ecológicas que se caracterizan según criterios indígenas y se diferencian en cuanto a su forma de explotación y localización. Para estos estudios también se documentaron y se discutieron las posibilidades y las vías de suministro así como los criterios de selección para plantas medicinales. Estos datos son de gran importancia a la hora de establecer estrategias sostenibles para el uso de la vegetación tropical.

Tras un análisis cuantitativo de los datos coleccionados en el trabajo de campo se evaluaron las enfermedades epidemiológicas más importantes. En esa región, los problemas medicinales más frecuentes son las enfermedades dermatológicas y las afecciones gastrointestinales. En una segunda parte de los estudios, mediante unos bioensayos se analizaron los extractos crudos de 11 especies botánicas indicadas para las enfermedades dermatológicas y las afecciones gastrointestinales según su actividad antimicrobiana, antifúngica, citotóxico-antitumoral, antiinflamatoria, inmunomodulatoria y antisecretoria. En estos bioensayos se llegaron a encontrar diversas actividades que respaldan las aplicaciones tradicionales, suministrando al mismo tiempo información adicional sobre esas plantas. Además se llevó a cabo un rastreo químico con cromatografía en capa fina (CCF) y con reactivos de aspersión por las sustancias secundarias más importantes.

Begonia heracleifolia Schltdl. & Cham. (Begoniaceae) mostró en los análisis preliminares unas actividades antibacteriana y citotóxico-antitumoral, dando así evidencias farmacológicas de su uso tradicional en la medicina zapoteca. Con diversos métodos cromatográficos (VLC, MPLC, HPLC, CCF) se aislaron seis cucurbitacinas, tres esteroles, un glicósido de esterol, cinco ácidos grasos y un oligosacárido. El elucidación estructural de estas sustancias se llevó a cabo mediante métodos espectrométricos (DCI-MS, EI-MS, FAB-MS) y con técnicas RMN uni- y bi-dimensionales (DFQ-COSY, HBMC, HMQC, ROESY, TOCSY).

Tres de las cucurbitacinas aisladas mostraron efectos citotóxico-antitumorales de diferente intensidad, mientras que los tres restantes eran inactivos. Estas actividades selectivas pueden explicarse con mecanismos de estructura-actividad.

La investigación sobre plantas medicinales procedentes de regiones tropicales y que se usan en los sistemas medicinales indígenas evoca grandes esperanzas a la hora de buscar nuevas sustancias para el tratamiento de problemas patológicos locales y globales. En este tipo de estudios no puede olvidarse, sin embargo, que el fundamento para un eventual éxito de investigación se basa en una selección indígena empírica elaborada a lo largo de los siglos. En cuanto al problema de los derechos de propiedad intelectual ("intellectual property rights"), hay que encontrar una solución rápida y justa para las dos partes – la de los informadores, generalmente indígenas, y la de los investigadores –, para que la importante búsqueda de productos naturales con efectos biológicos siga contribuyendo a solucionar problemas medicinales.

Summary

The Zapotec inhabitants of the Isthmus' Sierra of Tehuantepec in Oaxaca (Mexico) live in a tropical area of great botanical diversity. In daily subsistence and in response to illness, plants still play a major role. Healer, midwives, and herbalists use more than 445 different plant species belonging to 110 different botanical families during empirical therapies and ritual healing sessions.

For the present ethnobotanical and phytochemical study the indigenous uses of medicinal plants and the healing specialists' way of reasoning in medicinal matters were documented during a 17-month stay in the field. 3,611 medicinal uses were documented and sets of 445 voucher specimens have been deposited in several herbaria. The Zapotec employ binary forms, usually opposing systems, of classifying illnesses (e.g. hot-cold, sweet-sour, wet-dry). This dichotomy is dominant, but also doctrines of signatures, and taste and smell properties were used to classify plants and illnesses. The Zapotecs gather plants for healing purposes from six indigenous ecological zones which are defined by their respective distance to the community and by the way they are managed. These ecological data are valuable concerning sustainable resource management of the tropical vegetation.

In a quantitative approach, the epidemiologically most important plants were evaluated. Dermatological illnesses and gastrointestinal complaints are the most frequent medical problems in this region. In a second part of the study, extracts of eleven plant species were prepared and subjected to biological assays for the evaluation of antimicrobial, antifungal, cytotoxic/antitumoral, anti-inflammatory, immunomodulatory, and antisecretory activity. Several activities have been evaluated corroborating the alleged properties as described in traditional medicine and additional information on the plants was obtained. Also, a chemical screening on thin-layer chromatography (TLC) and with spray reagents has been conducted.

Begonia heracleifolia Schltdl. & Cham. (Begoniaceae) showed antibacterial and cytotoxic/antitumor activity in the screening corroborating the medicinal use of this plant among the Zapotec healers. With different chromatographic methods, (VLC, MPLC, HPLC, TLC), six cucurbitacins, three sterols, a sterol glycoside, five fatty acids, and an oligosaccharide have been isolated. Structure elucidation was conducted mainly by spectrometric methods (DCI-MS, EI-MS, FAB-MS) and 1D- and 2D-NMR techniques (COSY, HMBC, HMQC, ROESY, TOCSY).

Three of the isolated cucurbitacins showed varying cytotoxic/antitumoral activity, while three were inactive. These selective activities may be due to specific structural features of the respective compounds.

Research on traditionally used medicinal plants from tropical countries shows great promise in the search for new drugs in the treatment of local and global medicinal problems. In case of successful results, it should never be forgotten that the primary selection has been based on empirical indigenous criteria applied over centuries. Solutions to the "intellectual property rights" problems must be found soon, since it is necessary to carry on the important search for natural products which are valuable for medicine.

Zusammenfassung

Die Zapoteken, Bewohner der Sierra im Isthmus von Tehuantepec (Oaxaca, Mexiko) leben in einer tropischen Zone mit hoher botanischen Vielfalt. Pflanzen und ihre traditionelle Verwendung spielen für diese indigene Gruppe eine wichtige Rolle im täglichen Leben und somit auch in medizinischen Behandlungen. Heiler, Hebammen und Arzneipflanzenspezialisten verwenden in empirischer Therapie und Ritualen über 445 verschiedene Pflanzenspezies aus 110 botanischen Familien.

In Rahmen einer ethnobotanischen - phytochemischen Forschungsarbeit wurde während 17 Monaten in ethnologischer Feldforschung der Gebrauch und die Anwendung von Medizinalpflanzen und die medizinische Denkweise der zapotekischen Heiler, Hebammen und Arzneipflanzenspezialisten dokumentiert. Es konnten 3611 mögliche Anwendungen beschrieben und 445 Medizinalpflanzen herbarisiert werden. Als wichtige Krankheitskonzepte konnten in erster Linie verschiedene dualistische Konzepte (z.B. heiss-kalt, süss-sauer, feucht-trocken) und im Weiteren die Signaturenlehre und einzelne organoleptische Klassifizierungen eruiert werden. Die Zapoteken verwenden Pflanzen aus sechs nach indigenen Kriterien eingeteilten ökologischen Zonen, die in Abhängigkeit von der jeweiligen Nutzung und Entfernung vom Ort unterschieden werden. Die Möglichkeiten und Wege der Beschaffung, sowie die Selektionskriterien für Medizinalpflanzen wurden dokumentiert und diskutiert. Diese Daten sind wichtig hinsichtlich nachhaltiger Nutzungsstrategien der tropischen Vegetation.

In einer quantitativen Auswertung der Felddaten wurden die epidemiologisch wichtigsten Erkrankungen evaluiert. Entzündliche Hauterkrankungen und gastrointestinale Beschwerden sind die wichtigsten medizinischen Probleme in dieser Region. In einem zweiten Teil der Forschung wurden Rohextrakte von 11 Pflanzenspezies aus den Indikationsgebieten Hauterkrankungen und gastrointestinale Beschwerden in biologischen Tests auf antimikrobielle, antifungale, zytotoxische/antitumorale, anti-inflammatorische, immunmodulierende und antisekretorische Aktivität untersucht. In diesen Bio-

Assays wurden verschiedene Aktivitäten gefunden, die die traditionellen Verwendungen untermauern, aber auch zusätzliche Informationen über diese Pflanzen liefern. Ein chemisches Screening mit Dünnschichtchromatographie (DC) und Sprühreagenzien auf hauptsächliche Sekundärpflanzenstoffe wurde zusätzlich durchgeführt.

Begonia heracleifolia Schltdl. & Cham. (Begoniaceae) zeigte in den Voruntersuchungen antibakterielle und zytotoxische-tumorhemmende Aktivitäten, die pharmakologische Belege für die traditionelle Anwendung in der zapotekischen Medizin liefern. Mit Hilfe verschiedener chromatographischer Methoden (VLC, MPLC, HPLC, DC) wurden sechs Cucurbitacine, drei Sterole, ein Sterolglykosid, fünf Fettsäuren, und ein Oligosacharid isoliert. Die Strukturaufklärung dieser Substanzen erfolgte mit Hilfe spektrometrischer Methoden (DCI-MS, EI-MS, FAB-MS) und eindimensionalen und zweidimensionalen NMR-Techniken (DFQ-COSY, HMBC, HMQC, ROESY, TOCSY).

Drei der isolierten Cucurbitacine zeigen unterschiedlich starke zytotoxisch/tumorhemmende Wirkungen, während drei weitere inaktiv waren. Diese selektive Aktivität kann durch Struktur-Wirkungs-Mechanismen erklärt werden.

Forschung über Medizinalpflanzen aus tropischen Gebieten, die in indigenen Medizinalsystemen verwendet werden, birgt grosse Hoffnungen in der Suche nach neuen Wirkstoffen für die Behandlung von lokalen und globalen Krankheitsproblemen. Dass das Fundament für einen eventuellen Erfolg in der Forschung auf einer über Jahrhunderten, empirischen indianischen Auswahl basiert, sollte bei solchen Studien nicht vergessen werden. Für die Problematik der „intellectual property rights“ muss für beide Seiten, die der (meist) indigenen Informanten und die der Forscher eine schnelle und faire Lösung gefunden werden, damit die wichtige Suche nach neuen Naturstoffen mit biologischer Wirkung weiterhin ihren Beitrag zur Lösung von medizinischen Problemen leisten kann.

Foreword

> I keep thinking that the best ethnobotanist would be a member of an cultural minority and, trained as a botanist and as an ethnologist, would study, from within and as part of it, the traditional knowledge, the cultural significance, and the traditional management and uses of the flora. And it would be even better if his studies could bring economic and cultural benefit to his own community.
>
> (Barrera, 1972).

From the beginning, I, as a non-native, had no chance to become the "best ethnobotanist". Nevertheless, the scope of the present work is to make the best possible contribution to ethnobotany and phytochemistry by an interdisciplinary approach. Many different academic disciplines are interrelated with this kind of research I have chosen: Botanical knowledge is necessary for the documentation of plants, anthropological/ ethnological understanding is the basis of the interviews and participating observation. My pharmaceutical and medical education helps to face medicinal problems, to some extent to understand traditional curing and healing systems and was the introduction to phytochemistry. Linguistic studies are important to be able to talk from the same thing. Through my pharmaceutical education I was trained in some of these disciplines and used to do interdisciplinary work. With the help and collaboration of the Zapotecs I had good prerequisites to realize this ethno-pharmaco-botanical study.

In the last decades, ethnobotanical documentation has become an important science because the scientific community realized that traditional knowledge and biodiversity is being rapidly lost by influences of environmental and social changes and technological development. This "list making of plants used in traditional societies" is valuable from so many ethical and scientific points of view: For the documentation of so far only orally transmitted knowledge, for the improvement of the medical situation of poor people, and for studies on efficacy and toxicity of traditionally used plants. It is further precious for understanding of, learning from, and interchanging of traditional healing practices and concepts, for conservation programmes and community development, search for chemically new compounds and lead structures for the development of new drugs, and chemotaxonomy. Some of these topics were chosen and will be discussed in the following with the aim to make an interdisciplinary contribution to "ethno"-science and pharmaceutical sciences, and to hopefully improve the living situation of indigenous people.

The present thesis is divided into three main sections. Every section is subdivided into a general introduction to the respective topic, followed by (a) publication(s) and followed by a discussion and forward-looking conclusions. **Section 1** presents anthropological/ethnological, botanical and ecological results. **Section 2** describes the biological plant screening and its activity-based evaluation for further investigations. In **Section 3** the results of the phytochemical investigations of a selected medicinal plant are discussed. The **Appendix** provides longer data compilations from the field and laboratory work.

In a multilingual region, such as the Isthmus of Tehuantepec, where indigenous people do not speak Spanish perfectly, and others (children, members of other indigenous groups, and ethnobotanists) do not speak Zapotec well, it is understandable that language use varies. Also this present work will vary in several languages: If not marked differently, *words in italics* are in *Zapotec* and <u>underlined words</u> are in <u>Spanish</u>. A **Glossary** is provided in the Appendix for translations.

Background and Objectives

Drugs are derived from a number of sources and in a variety of ways. Some of the earliest medicines were differently prepared extracts, obtained from plants. The use of plants as a source for medicine can be traced back to written documents of early civilizations, but there is no doubt that it must go back to the beginning of human existence. It is estimated that 370,000 to 750,000 species of higher plants (angiosperms, gymnosperms) and 200,000 lower plants (algae, fungi, lichens, bryophytes and pteridophytes) exist on earth (Williamson et al. 1996). Up to now, only a relative few number has been thoroughly studied for all aspects of their potential therapeutic value in medicine (Williamson et al. 1996). Hence, the true potential of plants as sources for new drugs is largely unknown.

Several approaches to choose plants for the discovery of plant-derived medicines are followed today. In a random approach plants are collected on a large scale, but arbitrarily/indiscriminately. In the chemotaxonomic approach the strategy is to target for collecting those plant families or genera known to be rich in certain biologically active secondary metabolites, such as alkaloids, di- or triterpens or flavonoids. The information-based approach relies on information available through databases containing natural products literature, such as NAPRALERT (NAtural PRoduct ALERT), BA (Biological Abstract), Medline, or CA (Chemical Abstracts) (Cordell, 1995). The goal of this approach is to match data on plants with known medicinal use and biological activity, but from which so far no active principle has been isolated. In the ethnobotanical/-pharmacological approach the selection of plants is based on their traditional, mostly therapeutical use by an indigenous or "cultural" group, often a minority. An alternative approach would be to scan ancient texts of the classicists (Greek and Latin) or of monasteries, often sites of medievel hospitals, for clues to new biological agents (Cordell, 1995). Poisonous plants known for their toxicity but excluded in medicinal uses yielded many important drugs (e.g. curare) and therefore, ethnobotanical information used for natural product research should not only be reduced to medicinal plants but drawn from ethnobotany in general (see Section 1).

There is an on-going discussion about the most successful approach for chemical prospecting. Farnsworth (1988) showed a 74% correlation between the current clinical use of compounds derived from plants and the traditional use of the plant from which they were isolated to confirm the value of the ethnobotanical approach. Whereas the fact that 26% of today's plant-based commercial drugs were found serendipitiously might suggest that the screening of all plants, medicinal or not, is also very likely to yield valuable information (Coombs, 1992). In drug discovery programmes the question about the more successful approach will always end with the economic calculations and/or be answered by the cost-effective solution. While the ethnobotanical approach has the potential for many more chances for significant contributions such as economic development and use of local plant resources, development of strategies for sustainable economic growth and biodiversity conservation and ultimately, cultural survival of traditional people themselves is of the greatest importance.

As a result of the "green revolution" and because of the lack of new efficient drugs to cure many current medical problems (infectious diseases, cancer, AIDS, cardio-vascular ailments, malaria), the value of medicinal plants in treatment in western health care is receiving increasing attention worldwide. In 1985, in Europe about 50% and in the USA more than 25% of the drugs in prescription medicine have their origin in plants (Farnsworth et. al., 1985), mainly in higher plants and to a lesser extent in fungi. In the 1991 ranking list of the World's 25 best selling pharmaceuticals, 12 natural plant derived products (such as cyclosporin, salbutamol, captopril, enalapril, diclofenac, oestrogens) can be found (O'Neill and Lewis, 1993). The total of phytopharmaceuticals sold in Germany in 1996 is estimated to 1,357 million US$, covering 10% of the German drug market (Gruenwald, 1997).

Plant extracts, pure compounds derived from plants, and synthetics (where secondary metabolites served as templates for design, found by computer modelling) are prepared as pharmaceuticals. They are well distributed in pharmacies, affordable for everyone to cure many minor or severe illnesses. These facts fit for the western world, the situation in developing countries is very different. While inhabitants of urban areas have access to doctor consultations, hospitals and pharmacies, in rural, often remote areas, access to western medicine is difficult and, if at all, not affordable due to the bad economic situation of the people. Additionally, indigenous people prefer traditional healing to western therapy in the case of culture-bound syndromes because modern medicine often does not respect and understand the traditional reasoning of the illness and, therefore, is not believed to be able to cure it. On the other hand, many rare illnesses striking few people still do not have an effective drug to cure, because the pharmaceutical industry is not interested in developing such noneconomical products (orphan drugs). Therefore, in most developing countries it is to the traditional practitioner or to the indigenous medicine that the majority of the population turns when sick. The treatment they receive is largely based on plants recipes and often accompanied by ritual healing.

According to the World Health Organization (WHO), as many as 80% of the world's people depend on traditional medicine for their primary health care needs (WHO, IUCN, WWF, 1993). Recognizing its potential value for the expansion of health services in the developing world, the World Health Assembly (WHA), in 1976, drew attention to the manpower reserve constituent by traditional practitioners (Resolution WHA29.72). The next year, WHA urged countries to utilize their traditional systems of medicine (Resolution WHA30.49). Then in the following year, 1978, it called for a comprehensive approach to the subject of medicinal plants (Resolution WHA31.33) by including inventories and therapeutic classifications of medicinal plants used in different countries, by assessing safety, efficacy and quality, and by disseminating information through coordinated training of health workers. At the Alma-Ata Conference (1978) WHO passed a resolution for future action in the field:

-to give higher priority to traditional medicine and its systems by strengthening the position of the healers, by integration of efficient traditional remedies into primary health care systems,

-to initiate comprehensive programmes for the identification, evaluation, preparation, cultivation and conservation of medicinal plants used in traditional medicine,

-to ensure quality control of drugs derived from traditional plant remedies,

-to support research on traditional ways of treating ailments and to promote family health, nutrition, and well-being,

-to extend cooperation and exchange of experts, skills and training in this and related fields (Akerele, 1990).

In 1988, IUCN (The International Union for Conservation of Nature and Natural Resources) and WWF (World Wide Fund for Nature) joined WHO for collaboration in "The Chiang Mai Declaration-Saving Lives by Saving Plants" which affirms the importance of medicinal plants and calls for action to be taken for their conservation (WHO, IUCN, WWF, 1993). With this declarations many important steps have been initiated to promote local resources to be used more efficiently since they are of low-cost, accessible for many people, part of the culture and, therefore, based on broad acceptance to improve the health situation of developing regions and the conservation of plants.

Over centuries healers, midwives and herbalists tested plants for curing, selected them when effective and passed this empirically acquired knowledge of preparation and use, orally to the next generation. Hence, in many regions of the world no written documentation on traditional medicinal plant use is available. This knowledge, valuable for locals as well as for westerners, is endangered by the ongoing destruction of natural ecosystems and cultures.

The objectives of this study were therefore:

-documentation of traditional medical knowledge – to make it available to coming generations;

-documentation of the health care situation (as outlined by traditional healers and midwives) of a developing rural area and evaluation of main medical problems – to become aware of local problems and make a contribution to improve the living situation of indigenous people;

-ethno-ecological and ethnobotanical documentation of plants of the endangered tropical/subtropical flora – to make a little contribution to the preservation of biodiversity;

-quantitative field data evaluation – to assess culturally important plants and to select those appropriate for further biological and phytochemical investigations and for a possibly regional cultivation;

-biological investigation of medicinal plants used to cure main local medical problems – to select the most promising plants for further investigations on primary health problems with local importance;

-preparation of plant extracts for investigations along traditional and phytochemical methods – in order to follow up the traditional mode of preparation and to investigate the plant as fully as possible;

-phytochemical characterisation of a selected medicinal plant by bioactivity-guided isolation – to search for new compounds or lead structures for the development of new drugs and to investigate the toxicity for further save use of the medicinal plant and additionally to provide information to chemotaxonomy;

-publication and dissemination of collected information and obtained results of the study – so that the host country has access to it;

-sensibilization of persons and institutions to recognize intellectual property rights and to become aware of the responsibility by using such information in research – to assure appropriate returns of benefit to indigenous peoples in case of successful findings based on the data of this ethnobotanical-phytochemical study.

Section 1

Medical Ethnobotany of the Ishmus-Sierra Zapotecs

La medicina indígena va a desaparecer

el día que termine la natuarleza;

la medicina indígena va creciendo con el tiempo

porque se sigue aprendiendo cada vez más

y ningún doctor puede terminar con ella.

Autor anónimo

1 Introduction

1.1 Ethno-science

"Ethno" is currently a very popular prefix used to express "of a particular cultural group". If it is used in combination with a discipline of the natural sciences or medicine (e.g. ecology, botany, taxonomy, zoology, medicine, psychology, pharmacology) or of the humanities (e.g. archaeology, geography), it implies that researchers are exploring the perception, reasoning, and knowledge of a specific cultural group in the respective scientific field. Ethno-ecology, for example, is the study how local people interact with all aspects of the natural environment, including plants, animals, land forms, forest types and soils as well as many others (Martin, 1995).

To explain the importance of studies in the inter- and trans-disciplinary fields of ethno-science, another popular word of today will be used: "Global" seems to be of total contradiction in the field of ethno-science. By applying knowledge globally (e.g. Western agricultural land management in tropical zones) instead of considering locally, empirically developed methods, mismanagement in developing programmes is predictable. But on the other hand, while studying the relationship between a specific cultural group and their environment in a specific geographical zone, new findings can be of global importance, for the benefit of local people and people in industrial countries in terms of development, conservation and sustainable progress.

"Ethno" is also connected with a sense of "urgency". Recording ethnobotanical information and collecting biological material are today a race against time, against the continuous destruction of natural ecosystems and cultures. Ethno-research can, therefore, play a useful role in rescuing disappearing knowledge and protecting it by returning it to local communities. While maintaining pride for local cultural knowledge and practices, links between communities and their environment will be reinforced, the most important prerequisite for conservation.

1.2 Ethnobotany

Although plants have always been important to people, and the study of the knowledge and use of plants goes back to the beginning of human existence, only in our century has ethnobotany evolved as a distinct branch of the natural sciences. In 1895, John W. Harshberger first coined the word "ethnobotany" for a discipline at least as old as Aristotle's research on plants (Schultes and von Reis, 1995). In the following, ethnobotany has undergone a real evolution, especially in the 20th century. There was a long way to get rid of the popular image of ethnobotany of the old colonialist and neo-colonialist times, of ethnobotanists as adventurers or missionaries exploring virgin forests to contact isolated groups of indigenous people and to make unending lists of medicinal and hallucinogenic plants, documenting the secrets of witch doctors (Martin, 1995). Romanticized myths had to make way for modern natural science. Today ethnobotany is concerned with the "totality of the place of plants in a culture" (Ford, 1978), including social, political, biological, medicinal, economic, and ecological factors and, is therefore, interrelated with many different disciplines of natural sciences and the humanities.

A related field but not a synonym for ethnobotany is "economic botany", the study of useful, sometimes novel, plant species appropriate to commercialisation and possible domestication as alternative sources of income for sustainable development.

"Medicinal ethnobotany", a subsection of ethnobotany, deals with plants used by a specific indigenous group to treat illnesses and for the general well-being. Consequently, in a lesser extent, also food plants must be included (e.g. Etkin, 1982, 1994; Johns 1990, 1995). Furthermore, perception, reasoning, and knowledge in traditional curing are studied. Botany, anthropology/ethnology, pharmaceutical science, especially phytochemistry, medicine, linguistics are the scientific disciplines involved in such a study. I prefer not to use the term "ethnopharmacology" as a synonym for this kind of studies. Along the definition of pharmaceutical science, "pharmacology" is the science of nature, structure, and mode of action on the organism, of endogenous and exogenous agents and their uses as drugs [= *pharmakon* (Greek)]. In this present study "ethnopharmacology" will not be applied when refering to the interdisciplinary field of "medicinal ethnobotany", since this connotation does not include all aspects of the term as it is used here.

1.3 Medicinal Ethnobotany in Mexico

Botanically and culturally, Mexico is a country favored with enourmous diversity. The two major floristic kingdoms - the Holartic and the Neotropic (Rzedowski, 1988) - of the New World intersect in Mexico and generate ten basic vegetation types and a flora of about 30,000 species, the second largest in the Western Hemisphere. Despite a drastic decline of the indigenous population, caused by the Spanish Conquest, Mexico ranks first in the total number of native speakers, divided within the 54 languages surviving from 120 (Bye et al. 1995). For Bye (1993), the synergism between plants and humans, resulting in a rich ethnobotanical diversity, is not only due to a simple overlay of richness spheres. Plants respond to humans, whereas humans also change when plants are domesticated. The utilization of over 5,000 vascular plants in Mexican ethnobotany (Caballero, 1987) is therefore considered as a result of plant-human interactions and adaptations, as well as of dramatic modifications, diversifications and empirical selections in the Mexican plant kingdom.

Ethnobotanical documentation of Mexican medicinal plants has a long tradition and can be traced back to the early colonial period. Martin de la Cruz from Tezcoco prepared a herbal with Nahuatl text and colored illustrations known as the "Libellus de Medicinalibus Indorum Herbis" in 1552. Or Fray Bernardino de Sahagún collected and translated indigenous information about medicinal plant use condensed with other information in the Códice Florentino in 1570 (Bye et al., 1995). In addition to these inventories made by the native healers und herbalists and church ethnographers, Farnciso Hernández (physician of King Phillip II from Spain) also made a systematic study of the medicinal flora of New Spain (Historia de las plantas de Nueva España between 1571 and 1576). A manuscript called Jardin Americana by Fray Juan Navarro is a source from the 18th century. From the early years of the 20th century Roys' study on lowland Mayan groups (1931) and Reis Altschul's informations found on voucher specimens (1973) have to be mentioned (Heinrich, 1996). More recent publications on Mexican ethnobotany are numerous (e.g. Alcorn, 1984, Aguilar et al., 1994, Argueta, 1994, Heinrich 1996; and see References Publication I).

By evaluating publications, Díaz (1976) and Bye (carried on until 1993) counted 3,352 Mexican plants to be used as medicinals. The potential for enlarging this list is obvious when taken into consideration that as much as 34 % of the plants were recorded in the evaluation by Bye (1993), in the time after the study of Díaz.

Many regions of Mexico and its inhabitants bear an enormous potential of information, but no ethnobotanical inventory is available yet.

1.4 Studies among the Isthmus-Sierra Zapotecs

The Zapotecs, the third most numerous indigenous group of Mexico, live in three major regions of the state of Oaxaca: In the northern sierra, la Sierra Norte, in the central valley, el Valle de Oaxaca, and in the southern isthmus, el Istmo (de Tehuantepec). Since this indigenous group occupies such a enormous territory it is not surprising that the Zapotecs are not a culturally homogenic group. Life in the highlands, in the Sierra Norte and the Valle de Oaxaca (Messer, 1978; Leslie, 1981; Nader, 1990; Stephen, 1991) differs in many aspects from life in the Isthmus Sierra and lowlands (Brueske, 1976; Covarrubias, 1986; Newbold Chiñas, 1992, 1993; Campbell et al. 1993). As a consequence, cultural variations, such as women dresses, agricultural practices or healing methods are noticeable. Reasons may be geographical factors (climate, elevation above sea level and vegetation, types), as well as adaptation to and communication with neighbouring indigenous groups. In parallel, also the Zapotec language is divided into several independent dialects or languages (Mellado et al., 1994).

Mexico's narrowest point and only coast to coast stretch of low-lying land is the 200 km-wide Isthmus of Tehuantepec. In the southern Isthmus from the Pacific Ocean to the Sierra de Oaxaca at least two different Zapotec languages are distinguishable. The majority of the Zapotec inhabitants lives in and around the big cities (Juchitán, Tehuantepec, and Ixtepec), in the alluvial isthmus land, speaking el Zapoteco del Istmo. A smaller number of little municipios is found in the mountainous regions, where an older dialect is spoken, related to el Zapoteco de Valle (Brueske, 1976; Nader, 1976). Although these mountain villages have busy trade and further social contacts with the lowland isthmus inhabitants, their dialect was not much influenced by them. Due to the geographical isolation from the central valley for the last 600 years and the restricted contact with the "upper-class" Zapotecs of the big cities, the Isthmus-Sierra-Zapotecs developed into a different cultural branch.

The Isthmus-Sierra-Zapotec communities integrated in this study are documented for the first time in the 16th century in el lienzo de Guevea (Seler, 1986), which is either a map (Seler, 1986) or a calendar (Reko, 1945). Two or three copies exist of this lienzo, illustrating the kinship between the inhabitants. It documents the warlike migration of the Zapotec king *Cosijoeza* and the Zapotec nobility (Figure 1-S1) from the Central Valley of Oaxaca to the Southern Isthmus of Tehuantepec by displacing Huave, Zoque, Mixe and Chontal Indians (Campbell et al., 1993). The lienzo shows the communities of Santiago Guevea (later called: Guevea de Humboldt) and Santo Domingo de Guzman de la Cruz (later called: Santo Domingo Petapa). Although the main communities are situated in two different valleys, separated by mountain ranges of elevations up to 1,560 m above sea level, many contacts have later been possible due to their nearby coffee plantations and its ranchos.

Nowhere is culture static, but it is an ongoing, dynamic, changing and responding process over time (Brett, 1992) and so it was and still is in the Isthmus of Tehuantepec. The wish of all anthropologists and ethnobotanists to stay with an isolated and still very "original" indigenous group had never been dreamt. The turbulent history of this region, at least after the Spanish conquest, is well known and the ethnic heterogeneity is quite remarkable. In Pre-Columbian times Zapotec, Huave, Zoque, Mixe and Chontal Indians settled in this region, while Zapotec rulers were permanently interested in enlarging their tributaries and their trade routes. After the Spanish Conquest in 1521 the Isthmus was incorporated in Hernán

Cortés' own property, the Marquesado del Valle (Gerhard, 1972). People of African descent were brought as slaves to work the ranchos of the Marquesado. French people reached the Isthmus during the French intervention in 1866. With the construction of the transisthmic railroad (opened in 1907) and of the transisthmus highway (in the 1950s) descendants of Chinese, European, North American and Mideastern immigrants arrived and settled mostly in the cities. Transportation had contributed to the dispersion of such different ethnic groups, and to the mestizization of the region. In Santo Domingo Petapa, family names such as the Chinese "Loo" or legends narrated in the village, as well as traces of Mulatto features give evidence of the history of the region.

Figure 1-S1. A detail of el lienzo de Guevea, showing the limits of the municipiality of Sto. Domingo Petapa and Guevea de Humboldt.

So, why would it be interesting to do medico-ethnobotanical field work in this area and how much "original culture" was left there? Mexicans can be very traditional when it comes to illness. When specific symptoms of an illness occur, even a Chilango (a modern resident of the 25-million City of Mexico D.F.) will consult a traditional healer. The traditional reasoning and beliefs about illnesses as well as medicinal plant use for curing are still an important part of the Mexican culture. For example, in the Herbolaria of the Sonora market in Mexico City fresh and dried plant material is sold from all over Mexico, some of them still not known in the literature as medicinals (see investigations of Linares and Bye, e.g. 1990). Although drugs are available in pharmacies throughout the country and Mexican health services (IMSS, INI, COPLAMAR, ISSSTE, SSA, DIF) have subsidiaries in the rural communities, traditional medicine is still first choice for many poor campesinos and their large families.

Furthermore, it is well known that "the Mexico of today" is built on a Pre-Columbian and Spanish (and more recently a North American) "basement". Many of these diverse features continue to exist simultaneously with dominance of one or the other in rural or urban, northern or southern regions. The result is a broad cultural diversity. Due to poverty and geographical reasons many indigenous groups still live rather isolated with deep relationship to their mixed Pre-Columbian-Spanish traditions and depend for subsistence on local resources. Although with electricity, "objects of progress", such as television sets, reached remote areas, the health care situation is often disastrous. Infant death rate is still well over the world's average. Cholera, tuberculosis, meningitis and less threatening infectious diseases are still main reasons for early death.

The motivation to chose the Isthmus-Sierra for investigation was the so far uninvestigated medical situation in this poor rural area and the hope to improve it in one way or another with this study. Futhermore, the fascinating cultural background of the region in combination with its botanical diversity looked as the ideal situation for field studies. The proximity to an already investigated zone (the Mixe of San Juan Guichicovi; Heinrich, 1989) seemed interesting for comparative studies.

Publication I discusses anthropological-botanical results of the field study, documenting cultural criteria applied by the Sierra Zapotecs for selecting plants as food and/or medicine. By studying these cultural aspects its aim was to understand as fully as possible the classification of plants, their use, and the traditional way of conceptualizing and reasoning in Zapotec cosmic vision concerning food and medicine.

In **Publication II** a quantitative evaluation of data on individual responses concerning medicinal and additionally non-medicinal uses for different plant species is presented. Studies confirming the attributed properties or a scientific explanation of therapeutic use, as well as toxicological data, are still lacking for many of these species. The uses were grouped into 10 categories of illnesses and the responses for each species were summed up in each of these 10 groups to yield ranked lists. Ethnobotanical, phytochemical and pharmacological data in the literature were used to evaluate the the taxa which rank highest and therefore are considered to be culturally important species.

Based on two independent ethnobotanical inventories among neighbouring Mixe Indians, **Publication III** analyzes ethno-ecological strategies of obtaining medicinal plants. Six ecologically important zones, along indigenous definition, are discussed. These data are compared with the vegetation types, along the definition of natural sciences, present in the area of study. Mixe and Zapotec perceive their environment by differentiating several zones, whereas these ecological habitats do not necessarily correspond to Western scientific systems of classification. This indigenous classification is based (a) on the distance from the house, the centre of the daily life and the family and (b) on the type of management applied to the respective area. Ethno-ecological data and medicinal ethnobotany in particular may offer important links between tradition and modernization. Sustainable management, conservation of biodiversity and local development ought to be based on such data.

2 Publications

2.1 Publication I

MEDICINAL AND FOOD PLANTS: ZAPOTEC CRITERIA FOR SELECTION [1]

Paper accepted for printing,

Ecology of Food and Nutrition, 1997

Barbara FREI [1)], Otto STICHER [1)], Carlos VIESCA T. [2)],

Michael HEINRICH [3) 4)]

[1)]Department of Pharmacy, Swiss Federal Institute of Technology (ETH) Zurich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland;

[2)]Institute of Medical History and Anthropology, UNAM, Calle Brasil 33, Mexico, D.F., Mexico

[3)]Institute of Pharmaceutical Biology, Albert-Ludwigs-University, Schaenzlestrasse 1, D-79104 Freiburg, Germany

[4)]Author to whom correspondence should be addressed

ABSTRACT

The Zapotec inhabitants of the Sierra de Juarez foothills in Oaxaca (Mexico) live in an area of great biological diversity. As farmers (campesinos), and occasional gatherers, hunters and fishermen, Zapotecs have a deep relationship with, and detailed knowledge of, their natural environment. Consequently in daily subsistence and in response to illness, plants play major roles. This paper examines cultural criteria applied by the Sierra Zapotecs for selecting plants as food and/or medicine. These criteria are based on binary forms of classification. While the "hot"/"cold" dichotomy is dominant, other opposing systems exist, such as amargo/simple (bitter/neutral). Whether a plant is regarded as frio ("cold"), caliente ("hot"), or (in some rare cases) templado ("temperate"), depends mainly on one of the following criteria: habitat and/or growing and collecting seasons, analogy in appearance to aspects of the illness being treated, or features associated with well-being (doctrine of signatures), and taste and smell properties.

Criteria for plant selection are not based on a single classificatory system, but are integrations of several. A comparison with ethnobotanical data from neighbouring Mixe clearly showed differences due to another cultural background.

INTRODUCTION

Inhabitants of the Sierra de Juarez foothills in Oaxaca (Mexico) live in an area of great botanic diversity. As farmers, occasional gatherers, hunters, and fishermen Zapotecs have an intimate relationship with, and detailed knowledge of, their natural environment. In daily subsistence and in response to illness, plants play major roles. Historically, and to the present time, selection guided by cultural criteria, empiricism and special needs has led to their native diet and their traditional pharmacopoeia. All plant use depends on social and cultural factors. While diet additionally depends on factors such as climate, biodiversity, and nutritional needs (for calories, vitamins, and trace elements), medicinal plants become culturally important due to demonstrated medical efficacy (as perceived in a specific culture). A number of studies in medical ethnobotany demonstrate that traditional herbal curing is often effective (Etkin, 1994; Etkin and Ross.; 1982; Foster, 1984a,1985; Heinrich, 1989; Johns, 1990; Messer, 1991; Ortiz de Montellano, 1986), and show that plant selection in native pharmacopoeias is a non-random process with emphasis on certain botanical families (Moerman, 1979, 1996).

The authors scientific interest in these medicinal plants is twofold and interrelated: First, we try to understand as fully as possible the classification of plants, their use, and the traditional way of conceptualizing and reasoning in Zapotec cosmic vision concerning food and medicine (see below), then we study the pharmacological effects of indigenous medicinal plants and isolate new bioactive compounds.

This paper focuses on the cultural criteria applied by the Sierra Zapotecs for selecting plants as food and/or medicine. Criteria are not based on a single classificatory system, but represent the integration of several systems. While the "hot"/"cold" dichotomy[2] is dominant, other opposing systems exist as well as selection based on physicochemical properties of the plant. Some important causes of illness as they are perceived by the Zapotecs and the indigenous methods for diagnosis are also discussed in this paper. A comparison with ethnobotanical data of a neighbouring Mixe group (Heinrich, 1989; 1997) clearly shows differences due to another cultural background.

ETHNOGRAPHIC BACKGROUND

The Zapotec are the most numerous group in the state of Oaxaca, Mexico. Historically, Zapotecs settled in the highland Valley of Oaxaca. Forced by Aztec and Mixtec invasions, some groups moved to the Istmo de Tehuantepec in the middle of the 14th century (Campell et al., 1993). This geographic dislocation was a major cause of different cultural and linguistic development. This paper deals with the inhabitants of the foothills of the Sierra Madre del Norte, precisely, with four isolated communities, of the so-called Istmo Zapotecs. Fieldwork in the communities of Sto. Domingo Petapa and Sta. Maria Petapa, as well as Sta. Maria Guienagati and Guevea de Humboldt was conducted between January 1992 and March 1993, and in October and November of 1994. The Petapa communities border on the Mixe-speaking community of San Juan Guichicovi (Heinrich, 1989).

1% to 5% of all inhabitants older than 5 years of age are Zapotec monolinguals, 50% to 70% are bilingual Indians and there are a considerable number of mestizos (ladinoized Zapotecs) in some locations (INEGI, 1993). Today many inhabitants have migrated or have seasonal jobs in other parts of Mexico, but agriculture especially maize, coffee, and citrus fruits, is still the basis of subsistence for most families.

According to linguistic classification, Zapotec belongs to the Otomanguean family (Josserand, Winter, and Hopkins, 1984). Six closely related languages or dialects are recognized (Nader, 1976). The dialect spoken by Zapotecs living in the foothills is most closely related to the "Valle" dialect of highland Oaxaca, but due to its geographical isolation for the last 600 years and due to their proximity to the Isthmus Zapotecs of the region of Juchitán and Tehuantepec, there are a large number of derived features in their speech. Vowels and consonants are generally pronounced as in Spanish. In the following discussion, Zapotec is transcribed as used by the bilingual teachers of Santo Domingo Petapa.

METHODS

The data presented in this paper were collected in 1992 and 1993 (15 months) as well as 1994 (2 months) mainly in the villages of Santo Domingo Petapa and Santa María Petapa and during several short stays in Santa María Guenagati and Guevea de Humboldt. The information is based on open and structured interviews with local specialists, such as traditional healers and midwives. Observation and participation in the healers daily work (plant collection, preparation, and healing sessions) yielded further information (Frei et al., submitted). Additionally, a large part of the population (farmers, housewives, children, and old people) was interviewed on popular use, treatment, and beliefs about medicinal plants.

To collect plant material, excursions were made with the informants to the different vegetation zones of the subdistrict (municipio). Detailed documentation about location, use, preparation, application, and healing concepts were obtained (Martin, 1995). Voucher specimens were collected and identified. Complete documented sets are deposited in the following herbaria: Mexico D.F., Mexico

(MEXU); Institute of Pharmaceutical Biology, Freiburg, Germany; and ETH Zurich, Switzerland (ZT).

RESULTS

Classification and Medicinal Plants

As campesinos (farmers), the Zapotecs have a deep relationship with, and detailed knowledge of, their natural environment. In daily subsistence and in response to illness, plants always had and will continue to play a major role. To cite one of the informants, Doña Crisofora:

"¡Todas las plantas que huelen, o que tienen goma y leche, todas sirven para curar. Las que tienen forma diferente o un color bonito sirven también; no más de algunos todavía no sabemos para que enfermedad, pero nos dice que podemos ocuparlas!" (All plants which smell or which have gum or milk sap, all of them are medicine. Those [plants] with a special form or color are also medicinal, but we still do not know all of them and what illness to cure with them, but they tell us that we could use them (authors translation)).

This statement from the curandera and partera (healer and midwife) expresses one major aspect of classifying and selecting plants in Sierra Zapotec society. The central aspects of Zapotec classification and selection clearly appear to be dichotomies and are based on perception and conceptualization. *Naj'lej'*, "hot", and *naj'galaj'*, "cold", play dominant roles. *Nala'aj'*, bitter, and *nababa*, neutral, are two other frequently mentioned aspects. Classification is further based on metaphoric terms concerning culturally defined expectations. Whether one of these systems is built upon the others or if they exist in parallel with similar importance is discussed below.

Healing and food preparation are largely based upon the principle of opposites, the humoral theory (Foster, 1988). While "cold" medicinal plants are chosen to cure "hot" illnesses and vice versa, food plants must have either a balanced composition or a compensating effect for "hot"/hot and "cold"/cold exposure in the course of daily life. The determination of a plant or a plant part as frio ("cold") or caliente ("hot"), in some rare cases templado ("temperate"), depends on the following three criteria:

First, **habitat** and / or **season and time** of growing and collecting;

second, **analogy in appearance** to some aspects of the illness being treated or features associated with well-being, namely **doctrines of signature;** and

third, on **taste** and **smell properties**. (While the first criterion is only used to classify a plant as "hot", "cold" or "temperate", the second and the third criteria are additionally used as separate and independent classifications.) The three criteria require detailed explanation:

Habitat refers to the characteristics of the places where a certain plant species typically grows. *Xanthosoma robustum* Schott (Araceae), in Zapotec called *biu'luj'*, has its ecological optimum in shady, humid places, close to rivers. Therefore Zapotecs believe this plant has "cold" properties. *Hua'a* (*Piper auritum* Kunth., Piperaceae), on the other hand, grows, as the Zapotec informants explain, en el sol, exposed to a lot of direct sunlight, and *guiere* (*Pinus oocarpa* Schiede, Pinaceae), which grows on the dry and sunny hills, are classified as "hot" plants.

If the plant is flowering in the humid but also hot rainy **season** it is a "cold" medicine because of the "cold" properties of water. The different stages of the moon indicate to the healer whether to collect a plant or not, because of the different degrees of "coldness" of the moon. It is also important to collect bark on the side where the tree is exposed to the sun in order to get the more powerful "hot" medicine. As these examples show, humoral and temperature aspects are not always strictly separated. Prepared medicine is often to be kept overnight in the sereno - right under the roof - to collect the power of the freshness of the night for a "cold" preparation.

Analogy concerns some aspects of the illness being treated or features associated with well-being, namely **doctrines of signature**, which may relate to either color, form or texture.

Guixa'a riene (*Lantana camara* L., Verbenaceae), literally "flower of blood," has red petals and is used to cure all kinds of haemorrhages (menstruation, nosebleeds, etc.). Blood is classified "hot", but the cause of a haemorrhage is usually "cold", so the red "hot" plant will cure the illness. *Tulipan duendi*, (*Malvaviscus arboreus* Cav., Malvaceae) with its red, fire-like, "hot" flower is used to prepare a syrup against "cold" cough. The inflorescence of *guixa'a cancer* (*Tournefortia densiflora* Mat. & Gal., Boraginaceae) shows the form of the ovarian tubes and indicates to a midwife its power in curing female illness like inflammation and infertility. Mango (a Spanish loan word, *Mangifera indica* L., Anacardiaceae) produces a reddish "hot" sap when its bark is injured. Zapotecs cure the "cold" type of diarrhoea with this sap.

Taste and smell can indicate whether a plant is "hot" or "cold". But often these are also used as direct descriptions and classifications.

Smell: Usually, strong smelling aromatic plants are classified as "hot" and considered valuable in cases of aire, a "cold" illness caused by supernatural winds which mainly cause pain and swelling.

Taste: Amargo/simple, bitter/neutral or in Zapotec *naj'laj'/ nababa* (see Table I-P1), seems to be another dichotomical system additional to "hot"/"cold". It has a lot of similarity to the Mixe classification (Heinrich, 1994). Bitter plants are used in the treatment of gastrointestinal illnesses and for abortion and childbirth. Astringent plants are applied to treat diarrhoea and dysentery. Sour drugs are employed to cure respiratory ailments. Some of these applications are comparable to uses of plants which have similar properties and which are included in European pharmacopoeias (for example, Pharmacopoeia Helvetica 8). Several phytochemical components of these Zapotec plants have been isolated and various biological and pharmacological activities have been demonstrated in *in vitro* and *in vivo* tests.

There is some evidence that amargo (bitter) corresponds with "cold" and simple (neutral) with "hot", because the terms are often given at the same time in the same combination. If the "hot"/"cold" classification is not used, the second most frequently mentioned system of classification is amargo or simple. This usage may also indicate the existence of an independent system on its own. Additional data are required to substantiate this idea. The properties of medicinal plants are summarized in Table I-P1.

Table I-P1. Qualities of Zapotec medicinal and food plants

Zapotec	Spanish	English	Plant
naj'le'ej'	caliente	"hot"	*Malvaviscus arboreus* Cav. (Malvaceae)
nagaaj'la'aj'	frio	"cold"	*Xanthosoma robustum* Schott (Araceae)
nala'aj'	amargo	bitter	*Artemisia ludoviciana* ssp. *mexicana* Nutt. (Asteraceae)
digapahuaj dibeettiij / nababa	simple	tasteless/neutral	*Mirabilis jalapa* L. (Nyctaginaceae)
naj'shiej lindahuaj	huele bonito	smells nice, aromatic	*Cymbopogon citratus* (DC.) Stapf (Cyperaceae)
rala'a fieruhuaj	huele feo	smells bad	*Chromolaena collina* (DC.) R.M. King & H. Rob. (Asteraceae)
niij	agrio, acido	sour/acid	*Hibiscus sabdariffa* L. (Malvaceae)
nabara'a	"estitico"/astringente	astringent	*Mimosa tenuiflora* (Willd.) Poir. (Mimosaceae)
naruchu'u	resvaloso	slimy	*Zebrina pendula* Schnizl. (Commelinaceae)
naya'naj	picoso	hot/spicy (like chili)	*Capsicum baccatum* L. (Solanaceae)
na'aj''xi'	dulce	sweet	*Lippia nodiflora* (L.) Greene (Verbenaceae)
guguegue'	quemoso	burning	*Argemone mexicana* Sw. (Papaveraceae)
gugue nadii	baboso	sticking	*Plumeria rubra* L. (Apocynaceae)
gudedisejdxu'u	salado	salty	*Gonolobus* aff. *barbatus* Kunth (Asclepiadaceae)
rej'abadorana	seco	dry	*Jatropha curcas* L. (Euphorbiaceae)
bedxij'na'ahuaj	espumoso	foaming	*Enterolobium cyclocarpum* (Jacq.) Griseb. (Mimosaceae)
****alcanforado***	alcanforado	camphoric	*Tagetes lucida* Cav. (Asteraceae)

(*No Zapotec expression could be elucidated; loan word from Spanish.)

There also exist many intermediate stages such as agriodulce, sweet-and-sour or modifications of the basic terms such as muy picoso, very hot/spicy.

As stated by Heinrich (1997) with respect to the Mixe, the Zapotecs also want to see or feel the effect on the human body of the plant applied, so-called culturally defined expectations. A physiological effect is believed to be the first step toward recovery. This may be sweating, diarrhoea, forced flow of urine, vomiting, aching, or flushing. But also visible transformation of the applied plant material during treatment indicates effectiveness. According to indigenous interpretation a "hot" illness will burn the "cold" plant by changing the color from green to black or by changing their nice smell into a bad one.

All these examples probably give the impression that there must be a strict rule to classify all plants and diseases into either "hot" or "cold" or some other opposing systems, and the term once given will always be connected with this particular plant. Molony (1975) suggested in her work from a Spanish-Zapotec community in Oaxaca that peasants apply a stereotypic code for determining relevance of food plants. Following such a code for each patient and situation, an individual explanation will meet Zapotec expectations and cosmovision. In the case of the Isthmus Sierra Zapotecs the code would be the three criteria discussed above. The approach of Molony is surely more valid than the approach still encountered in ethnobotanical studies of making lists of plants with allegedly precisely defined uses and properties. The cure must change depending on the type of illness, its humoral classification in a certain situation and its causes.

Several illnesses can be "cold" or "hot" . The plants themselves can also be divided into various categories according to a variety of factors mentioned above. Not only is the plant as a whole classified, but also the plant parts can be divided into different sections with special properties. Plant parts and their humoral classification:

-**roots** are usually considered to be "hot" because they are not exposed to the "cold"/cold winds (mal aire) of the night and grow in the "hot"/hot ground.

-**leaves** are "cold", because they are exposed to winds.

-**twigs**: if they are green they are not as strong as red ones, which are considered "hot" (for example, *Chenopodium ambrosioides* L.; Chenopodiaceae)

-**flowers** can be "hot" or "cold", depending on their stage of maturity or their colour.

-**gum, milk sap** are usually "cold".

Plants recently introduced at regional healer-meetings or through travel are rapidly classified without apparent doubt. This flexibility also helps understanding of the process of selecting new plants and learning from other healers that is an ongoing process based on the three criteria described above. But not only verbal reasoning indicates which new plants to use in the traditional pharmacopoeia. Dreams also play an important role in plant selection for some healers.

Classification and Food Plants

Medicinal plants are taken to restore a highly imbalanced body and to alter conditions in one distinct direction. Food plants, on the other hand, should be consumed as balanced compositions, or should have compensating effects for the usually slighter "hot" or "cold" exposure in the course of daily life. Food can cause illness if it is not eaten at a proper time. "Cold"/cold water instead of "hot"/hot coffee in the early morning will affect the "hot" body which has just awakened. Cold soft drinks in the evening after "hot" work will weaken the body. Waiting past the usual hours of the meals is bad too, as every excess causes imbalance. Taste and smell properties which are also used for classifying food plants are shown in Table I-P1 and are analogous to those used for medicinal plants following the above mentioned three criteria.

Illnesses

Indigenous medicine always tries to explain the causes of illness as does western medicine. While allopathic medicine interprets many smaller or grave symptoms easily as an isolated illness, traditional Zapotec medicine looks at various symptoms conceived as one single problem and describes a small group of six main causes. These causes are frequently seen as the illness itself (Table II-P1).

All these illnesses can be caused by "cold"/cold or "hot"/hot influences and consequently **are** "cold" or "hot". There is no other classification of illness which corresponds to plant description as does the "hot"/"cold" system. Other descriptions such as color, texture and sensory perceptions will be transcribed into "hot" and "cold" as well.

Table II-P1. Main causes of illness in Zapotec medicine

Zapotec	Spanish	English
dzieebi	susto	sudden fright
stu	verguenza	shame
guelereza'ga'	cansancio	fatigue
gueleraaj'qui	daño de la comida / empacho	food
mbe'	aire	supernatural winds
*golpe	golpe	physical and / or supernatural blow

(*No Zapotec expression could be elucidated; loan word from Spanish.)

Illnesses can have various stages of "cold" and their intermediates such as "very cold", "cold," and not "very cold". The same variation is possible for "hot" and even "temperate". This is important to know because there are also different stages of gravity of an illness that have to be cured by the right plant and an accurate dose of the plant material. "Hot" cures are more dangerous and have to be applied with more caution. In "cold" cures one can employ a lot of plant material without danger.

Zapotec Reasoning of Causes and Symptoms ("Diagnosis")

"Diagnosis" is a term of western medicine and does not match exactly the Zapotec reasoning of causes and symptoms of an illness. This fact shows again that Zapotec concepts of illness are very complex and interrelated.

Usually curanderos (healers) do not ask many questions to the patient or his/her relatives. They look at the sick person, check his eyes, pulse, feel the temperature and tension of the muscles in different regions of the body, and soon start the healing rituals. These are similar for different illnesses; only - and this is essential for our ethnopharmacological studies - the plant species and amount is varied. The recommendation at the end concerning food, medicine to apply at home, and behaviour, is individual and often based on "hot"/"cold" principles. Water - a central aspect in the Zapotec cosmic vision as it is in several South and Meso American religions - is a "cold" principle and opposite to the more dangerous "hot" medicine. The way to deal with water influences the progress of a disease. The affected person is not allowed to take a bath or to eat "cold" food for three days after a treatment with very "hot" medicine.

DISCUSSION

Classification criteria for plants in Sierra Zapotec society are not based on a single system, but are the integration of several complementary systems. While the "hot"/"cold"/"temperate" aspect is dominant, there exist on a "lower" level other dichotomous systems as well as approaches based on sensory perception and observation. Where possible such second-place criteria will frequently be translated into "hot" and "cold" concepts. Classification rooted in perception or observation is never translated into "temperate". This indicates, as suggested by Foster (1984b), that the term "temperate" does not actually belong to the humoral system. Because in humoral terms, "temperate" illness would correspond to health and "temperate" medicinal plants have no effect on either "cold" or "hot" illnesses, it is rather an expression for not knowing the plant or its qualities.

In the last 60 years many papers have been published about whether humoral theory is either Prehispanic or fully adopted after the Spanish conquest. "Hot"/"cold" is a Spanish verbalization of part of the Zapotec cosmic vision. The Zapotecs had developed and worked out a widespread system of dichotomous reasoning in Prehispanic times (Lopez Austin, 1980). We do not know this system and therefore a discussion of the origin of the Zapotec "hot"/"cold" concepts remains speculative. While Spanish-Mexican humoral theory as described by Foster (1994) mainly focuses on medicine, Zapotec dichotomous reasoning is omnipresent in the course of everyday life:

- to express seasons of the year,
- to describe segments of life,
- to distinguish between genders,
- to express religious traditions,
- to give definitions of the environment.

These and the data presented above, corroborate the importance of duality as the basic classificatory system. The suggestion of Foster that the humoral theory is the only valid framework in traditional medicine in Meso America is not confirmed for the Istmo Sierra Zapotecs.

It is of interest to evaluate data about Zapotec-Mixe and Mixe-Zapotec influences. It is astonishing that two distinct ethnic groups in a similar vegetation zone with common community borders do not correspond more in their medicinal systems. Why was there so little interchange? And why does this continue up to the present day? The main reason seems to be the different cultural backgrounds:

Otomanguean versus Macro-Mayan linguistic roots, Highland Zapotec who migrated into the region from far away versus Lowland Mixe who had settled there earlier, and therefore dominant intruding Zapotecs versus displaced Mixe. Today there still exists a continuing dispute about language. Mixe and Zapotec do not have common vocabulary and for communication Spanish is spoken. During inter-societal meetings of healers, there appears to be a general reluctance to speak in Spanish, the only common language; hence, exchange of ideas is limited. Additionally, the two communities have been enemies since the Zapotec invasion into Mixe land over five hundred years ago. Today the struggle continues about community borders and agricultural dominance. As for medicinal plants and their uses, there is a lot of correlation (Heinrich and Frei, unpublished data), but the ways of reasoning about them are totally different. The main aspect in Mixe classification seems to be sensory perceptions (Heinrich, 1997). On the other hand, Zapotec clearly have a dichotomous reasoning which is based - besides other criteria - on sensory perceptions (see above: criteria for taste and smell).

Surprising correlations with the data of Messer (1991) about systematic and medicinal reasoning in Mitla Highland Zapotec botany show again the importance of the cultural background for the construction of belief systems.

Future work in this field will be the comparison of data from ethnobotanical fieldwork among different Mexican ethnic groups (Mixe, Nahua, Maya, and Zapotec; by Heinrich et al., submitted) in order to understand in greater detail the classification of plants and the medicinal system. We also try to trace the pharmacological effects of indigenous medicinal plants and their phytochemical components, in order to better understand the biological and pharmacological effects of plants from these medicinal systems using ethnological and pharmaceutical studies. The results should be used to upgrade the indigenous knowledge and present an easy and inexpensive possibility for providing appropriate and effective medicine to poor and remote areas.

ACKNOWLEDGEMENTS

We are very grateful to the healers, midwives and inhabitants of Sto. Domingo and Sta. María Petapa, Sta. María Guenagati and Guevea de Humboldt, Oaxaca for teaching their art of healing, for their friendship and hospitality. We would like to thank Dr. Patricia Davila, National Herbarium (MEXU), Dr. Robert Bye, Botanical Garden, and Dr. Carlos Viesca, Institute of Medical History and Anthropology, all of the Universidad Nationál Autónoma de México UNAM; Prof. Dr. H. Rimpler, Institute of Pharmaceutical Biology, Freiburg/Germany; and Dr. Matthias Baltisberger, Geobotany, ETH Zurich/Switzerland for their help at various stages of the project. Many thanks are due to John A. Brett, Department of Anthropology, University of Colorado at Denver for stimulating discussions. This research would not have been possible without the financial support of the Swiss Agency for Development and Cooperation (SDC, Bern, Switzerland) and a travel grant for the annual meeting of the American Anthropological Association from the Barth Fonds of ETH Zurich, Switzerland. And last but not least, many thanks are due to Dr. Ch. Pryce for checking the English usage in this paper.

FOOTNOTES

1 Expanded version of a paper presented at the annual meeting of the American Anthropological Association. Atlanta, USA, Dec. 1st, 1994.

2 In order to differentiate between hot and cold in the context of humoral medical concepts and other hot-cold concepts, the former ones are put in quotation marks.

REFERENCES

Campell, H., L. Binford, M. Bartolomé, and A. Barabas (1993). Zapotec Struggles. Smithsonian Institution Press, Washington, London, pp. 285.

Etkin, N. L. (1994). Eating on the Wild Side. University of Arizona Press, Tucson.

Etkin, N. L., and P. J. Ross (1982). Food as medicine and medicine as food. Soc. Sci. Med., 16, 1559-1573.

Foster, G. M. (1984a). How to stay well in Tzintzuntzan. Soc. Sci. Med., 19, No. 5, 523-533.

Foster, G. M. (1984b). The concept of "neutral" in humoral medical systems. Med. Anthropol., 8/3, 180-194.

Foster, G. M. (1985). How to get well in Tzintzuntzan. Soc. Sci. Med., 21, No. 7, 807-818.

Foster, G. M. (1988). The validating role of humoral theory in traditional Spanish-American therapeutics. Am. Ethnol., 15/1, 120-135.

Foster, G. M. (1994). Hippocrates' Latin American Legacy. Humoral Medicine in the New World. Gordon and Breach, Langhorne (PA, USA).

Frei, B., M. Baltisberger, O. Sticher, and M. Heinrich (submitted). Medicinal Ethnobotany of the Isthmus-Sierra Zapotecs (Oaxaca, Mexico): Documentation and Evaluation. J. Ethnopharmacol.

Heinrich, M. (1989). Ethnobotanik der Tieflandmixe (Oaxaca, Mexico) und phytochemische Untersuchung von Capraria biflora L. (Scrophulariaceae). Dissertationes Botanicae No. 144. J. Cramer in Gebr. Borntraeger Verlagsbuchhdlg., Berlin und Stuttgart.

Heinrich, M. (1994). Herbal and symbolic medicines of the Lowland Mixe (Oaxaca, Mexico); Disease concepts, healer's roles, and plant use. Anthropos 89, 73-83.

Heinrich, M., Ankli, A., Frei, B., Weimann, C. and O. Sticher (submitted). Medicinal Plants in Mexico: Healer's Consensus and Cultural Importance. Soc. Sci. Med.

Heinrich, M. (in press, 1997). Indigenous concepts of medicinal plants: The example of the Lowland Mixe (Oaxaca, Mexico). Ecol. Food Nutr.

Instituto Nacional de Estadística, Geografia Informática, INEGI (1993). Region Istmo, Oaxaca, perfil sociodemografico, XI censo general de población y vivienda, 1990. Instituto Nacional de Estadística, Geografia Informática, Aguascalientes.

Johns, T. (1990). With Bitter Herbs They Shall Eat It. The University of Arizona Press, Tucson.

Josserand, J. K., M. Winter, and N. Hopkins (1984). Essays in Otomanguean culture history. Vanderbilt University, Publications in Anthropology, No. 31, Nashville, Tennessee, pp. 30.

Lopez Austin, A. (1980). Cuerpo Humano e Ideologia. La Conception de los Antiquos Nahuas. UNAM, Mexico D.F..

Martin, G. (1995). Ethnobotany. Chapman & Hall, London.

Messer, E. (1991). Systematic and medicinal reasoning in Mitla folk botany. J. Ethnopharmacol., 33, 107-128.

Moerman, D. E. (1979). Symbols and selectivity: A statistical analysis of native American medicinal ethnobotany. J. Ethnopharmacol., 1, 111-119.

Moerman, D. E. (1996). An analysis of the food plants and drug plants of native North America. J. Ethnopharmacol., 52, 1-22.

Molony, C. H. (1975). Systematic valence coding of Mexican "hot"-"cold" food. Ecol. Food Nutr., 4, 67-74.

Nader, L. (1976). The Zapotecs of Oaxaca. In R. Wauchope (Ed.), Handbook of Middle American Indians. University of Texas Press, Austin, Ethnology, part 1, pp. 329-359.

Ortiz de Montellano, B. (1986). Aztec sources of some Mexican folk medicine. In R. P. Steiner (Ed.), Folk Medicine, The Art and The Science. Am. Chem. Soc., Washington DC., chapter 1, pp. 1-22.

2.2 Publication II

Medicinal Ethnobotany of the Isthmus-Sierra Zapotecs (Oaxaca, Mexico): Documentation and Evaluation

Paper submitted, *Journal of Ethnopharmacology*

Barbara FREI [a], Matthias BALTISBERGER [b], Otto STICHER [a],

Michael HEINRICH [c, d]

[a] Department of Pharmacy, Swiss Federal Institute of Technology (ETH) Zurich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland

[b] Institute of Geobotany, Swiss Federal Institute of Technology (ETH) Zurich, Zollikerstrasse 107, CH-8008 Zürich, Switzerland

[c] Institute of Pharmaceutical Biology, Albert-Ludwigs-University, Schaenzlestrasse 1, D-79104 Freiburg, Germany

[d] Author to whom correspondence should be addressed

ABSTRACT

The Zapotec inhabitants of the Sierra de Oaxaca foothills (Mexico) live in an area of great botanic diversity. In daily subsistence and in response to illness, plants play a major role. An inventory of the Zapotec medicinal ethnobotany was carried out during 17 months of fieldwork. 3,611 individual responses concerning medicinal and additionally non-medicinal uses for 445 different species were documented. The uses were grouped into 10 categories and the responses for each species were summed up in each of these 10 groups to yield ranked lists. Ethnobotanical, phytochemical and pharmacological data in the literature were used to evaluate the high ranking and therefore culturally important species. Studies confirming the attributed properties or a scientific explanation of therapeutic use, as well as toxicological data, are still lacking for many of these species. The quantitative approach described will be the basis for future studies on efficacy and safety of Zapotec traditional plant use. Finally these data may also be of importance for biodiversity conservation and community development.

INTRODUCTION

The documentation of popular and indigenous plant uses has a long tradition in botany. Voucher specimens of many early explorers - biologists, botanists, geographers, ethnologists, and anthropologists - frequently bear such information (e.g. Humbolt, 1849; cf. Schultes, 1983). In Mexico this tradition has partly been preserved in several colonial codices (Codice Florentino, 1950; Cruz, 1991; Ortiz de Montellano, 1975). Today pre-Columbian and Spanish colonial influences are still observable, but modern influences are constantly altering the traditional medical systems and medicinal plant use. There are several (often multidisciplinary) recent publications on Mexican ethnobotany (Alcorn, 1984; Browner, 1985; Linares and Bye, 1987; Heinrich, 1989; Aguilar et al., 1994; Argueta, 1994).

In the declaration of Alma-Ata (1978), the World Health Organization (WHO) calls for upgrading of traditional medicine and plant use, as well as the integration of modern and traditional medicine (Farnsworth et al., 1985; Tortoriello and Aguilar-Santamaría, 1996). In Mexico there are over 5,000 species of plants used medicinally (Bye, 1993). Therefore the selection of the most important taxa is a prerequisite for initiating ethnopharmacological, phytochemical, and toxicological studies. Such a selection will also facilitate the preservation of ancient information and empirical knowledge. This approach thus focuses on traditional uses of plants and their cultural importance. It is therefore different from the one used in many (ethno-) botanical projects, which exclusively focus on the search for new bioactive compounds from plant sources (Hamburger and Hostettmann, 1991).

In order to assess the cultural importance of a medicinal plant, we quantified the number of recorded uses of each species. Up to the mid-1980's ethnobotanical reports were based on compilation of data and no quantitative approach was used. In recent years some quantitative and semi-quantitative studies in medicinal ethnobotany (Alcorn, 1984; Friedman et al., 1986; Johns et al., 1990; Moerman, 1996) were published but only Johns et al. (1990) and Friedman et al. (1986) analyzed their data with respect to the cultural importance of the individual plant in a specific culture. However, other recent publications using quantitative methods focus on traditional knowledge of forest management (e.g. Philips et al., 1994).

Therefore, the goal of this paper is to quantify the data of medicinal plant use of the Isthmus-Sierra Zapotecs in the Southern Mexican state of Oaxaca. The quantification is based on the number of positive responses for each species obtained in the interviews with 13 healers. The positive responses were grouped into 10 major categories of indigenous medical uses. Ranked plant lists for each category are presented. Relevant bioactive compounds and pharmacological activities of culturally important native plants are discussed.

BACKGROUND AND METHODOLOGY

Geographic overview and vegetation types - - The research area is situated in the southern part of the state of Oaxaca (Isthmus of Tehuantepec), in the foothills of the Sierra Madre de Oaxaca. It includes a small plateau 200 m above sea level up to the mountainous Sierra with elevations of 2000 m above sea level. The accentuated relief with its changing altitudes determines the climate and the vegetation types. In a global view, based on modifications of Köppen (Heyer, 1988), the climate is classified as the As type: A tropical climate, with a year-round average temperature over 18 ° C and one rainy season from June to September. Local types of vegetation include humid forests (tropical ombrophilous, evergreen, and cloud), subhumid forests (conifer or oak), and relatively dry deciduous lowland and submontane forest, as well as extended areas of secondary vegetation.

Ethnographic background - - The Zapotecs are the most numerous indigenous group in the state of Oaxaca. Historically, Zapotecs settled in the highland Valley of Oaxaca. Forced by Aztec and Mixtec invasion, some groups moved to the Isthmus of Tehuantepec in the middle of the 14th century (Campell et al., 1993). This geographical dislocation was one of the major causes of different cultural and linguistic development within the ethnic group itself. This paper deals with four linguistically isolated communities (Santo Domingo Petapa, Santa María Petapa, Santa María Guenagati, Guevea de Humboldt) of the foothills of the Sierra Madre de Oaxaca (Istmo Sierra-Zapotecs). 1% to 5% of all inhabitants older than five years are Zapotec monolinguals, 50% to 70% are bilingual Indians and there are a considerable number of mestizos in some parts (INEGI, 1993). Today many inhabitants have migrated or have seasonal jobs in other parts of Mexico. But agriculture - part of it as shifting and seasonal cultivation - especially corn, coffee, and citrus fruits, is still the basis of subsistence for most families (Brueske, 1976).

According to linguistic classification, Zapotec belongs to the Otomanguean family (Josserand et al., 1984). Six closely related languages or dialects are recognized (Nader, 1976). The dialect spoken by the Zapotecs living at the foothills is most closely related to the "*Valle*" dialect of highland Oaxaca, but due to their geographical isolation for the last 600 years and due to their proximity to the Isthmus Zapotecs of the region of Juchitán and Tehuantepec, there are a large number of derived features. Vowels and consonants are generally pronounced as in Spanish. In the following, Zapotec is transcribed as used by the bilingual teachers of Santo Domingo Petapa.

In the Zapotec medical system various specialists to cure different illnesses are distinguished. The most important ones are healers (curanderos) who cure culture-bound syndromes in ritual ceremonies (limpias). Midwives (parteras) accompany women during pregnancy, birth and childbed. Specialists of medicinal plants (hierberos) nearly exclusively recommend the use of plants but do not perform healing sessions with such plants. Experts in illnesses of the skeleto-muscular system (hueseros) cure sprains, fractures, and bruises. Many healers have experience in several forms of treatment.

Ethnobotanical methods and evaluation - - The data presented in this paper were collected from January 1992 to March 1993 and in October and November 1994 mainly in the villages of Santo Domingo Petapa and Santa María Petapa and during several short stays in Santa María Guenagati and Guevea de Humboldt. The information is based both on open and structured interviews with 13 local specialists, such as traditional healers, herbalists, and midwives. Observation and participation in their daily work (plant collection, preparation, and healing sessions) completed the interviews and discussions. To collect plant material field trips were made with the practitioners to the different vegetation zones of the subdistrict (municipio). For each species detailed documentation (ethnomedical information) about location, use, preparation, application, and healing concepts were obtained. Voucher specimens were collected and identified. Complete sets (FREI 1-544) were deposited in the following herbaria: Mexico-City, Mexico (MEXU); Institute of Pharmaceutical Biology, Freiburg, Germany; and ETH Zurich, Switzerland (ZT).

In order to analyze the cultural importance of an individual species, 10 major categories of indigenous uses are distinguished. These categories mostly refer to the part of the human body that is affected by an illness (e. g. respiratory system, skin, gastrointestinal tract). Systemic illnesses (especially fever) and culture bound syndromes form other groups. The medicinal uses of the plant species were grouped quantitatively into these categories and the individual responses for each species were summed up in each of these 10 groups to yield ranked lists. A species may be listed in more than one category. Species with the largest numbers of positive responses are listed first. For the same number of positive responses species are arranged alphabetically first by family, second by genus. The resulting ranking lists are based on cited species with five (in two cases: four) or more positive responses by specialists (healers, herbalists or midwives). For species with the highest ranking, literature searches (in BIOSIS, Medline, NAPRALERT, Chemical Abstracts, and Excerpta Medica) were performed in order to obtain phytochemical and pharmacological information. Non-native species were not evaluated since many of these plants have been investigated in detail and the data are easily available (e. g. Steinegger and Hänsel, 1992; Hänsel et al. 1992, 1993, 1994).

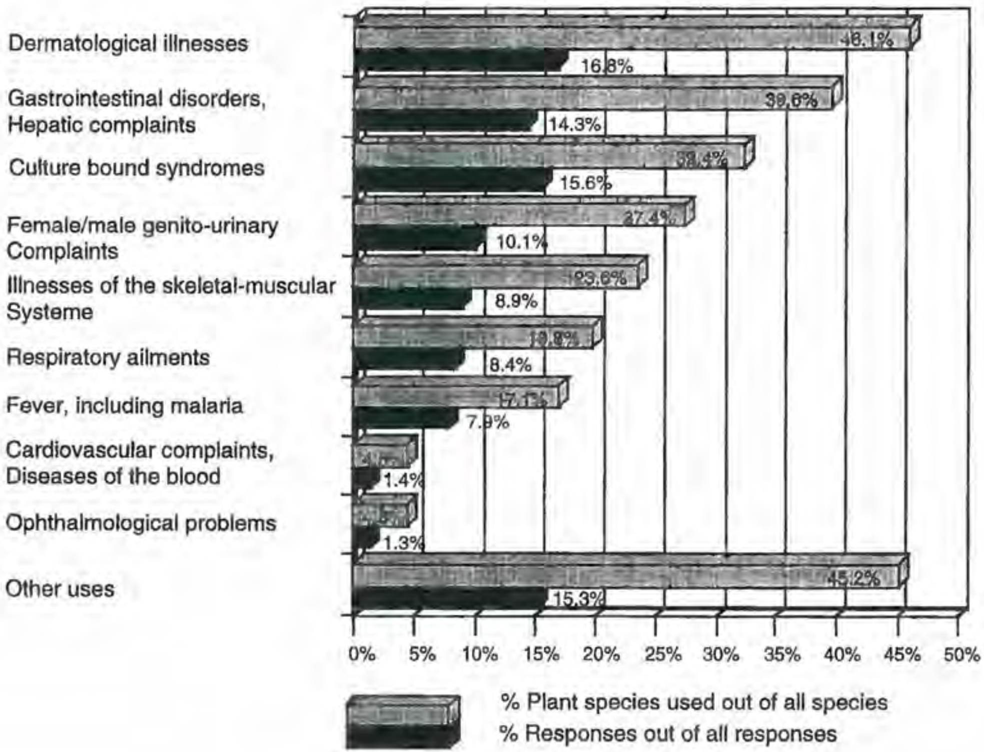
RESULTS AND DISCUSSION

445 different botanical species of medicinal plants with 3,611 positive responses/uses were collected from 13 specialists and grouped into ten major categories of indigenous uses (Figure 1-P2). The classification proposed here tries to reflect as far as possible traditional healing concepts, but some western medical influences could not be avoided. Zapotec understanding of illness considers the body as one unit and illnesses are regarded as being caused by imbalance. While these categories are based on our (Western) understanding of the Zapotec medical system, the categories correspond well to the symptoms recognized by the indigenous people.

Dermatological conditions (205 species (46.1%)) along with gastrointestinal disorders (176 species (39.6%)) are the most frequently mentioned medical problems for the Zapotecs (Figure 1-P2). These results correspond to the unpublished epidemiological records and statistics of the responsible health service (e.g. Instituto Nacional Indigenista, INI; pers. comm.). 144 species (32.4%) are mentioned for culture-bound syndromes (folk illnesses), 122 species (27.4%) for female and male genito-urinary complaints, 105 species (23.6%) for illnesses of the skeleto-muscular system, 88 species (19.8%) for respiratory ailments, 76 species (17.1%) for fever, including malaria, 20 species (4.5%) for cardiovascular complaints and diseases of the blood, and 20 species (4.5%) for ophthalmologic problems. Additional citation of 201 species (45.2%) for rare illnesses, for medico-religious uses, and non-medical uses were documented.

Tables 1-P2 to 9-P2 present the results in the 9 categories of indigenous uses. They are discussed below in detail for each group. Only species with more than five (in two cases four) use reports are considered of higher ethnobotanical importance (for details, see methodology).

Figure 1. 445 different botanical species (=100%) of medicinal plants with 3611 positive responses (=100%) were collected. Plants were grouped by their (frequently multiple) use into 10 categories of indigenous uses.



Dermatological illnesses - - This category of indigenous uses covers all diseases and injuries located on skin or mucous membranes (e.g. bacterial and viral infections, eczema, dermatitis, psoriasis, acne, and rash) and the underlying tissues (snakebites, bullet wounds), and their accompanying symptoms such as pain, itching, bleeding, swelling, and bruises. The medicinal preparations are usually applied externally as infusions used for shower baths, as washings, or as steam baths (in Zapotec: vaoh), as well as creams, shampoo, or medicinal soap. Often, fresh plant material is crushed or ground and applied as a compress or is toasted and inserted into the wound. Fresh material is also used in ritual healing sessions (limpias) by striking the body. 605 responses (16.8%) classify 205 species useful to treat dermatological conditions (Table 1-P2). The most important but introduced plant is *Aloe barbadensis* (in Zapotec and Spanish: zabila) used to treat fresh and bleeding or infected wounds, bums, eczema and dandruff.

Leaves of *Tournefortia densiflora* (in Zapotec: ***guixa'a cancer*** / Spanish: hoja de cancer) are applied externally as infusions to wash or bathe the respective part of the skin. Toasted plant material is applied to dry a wet wound and to accelerate the healing process. The Boraginaceae are known for containing many species with wound healing properties, probably due to the keratolytic - osmotic, granulating, and cell regenerating effect of allantoin. They are also noted for hepatotoxic effects of the pyrrolizidine alkaloids when applied internally (Steinegger and Hänsel, 1992). An internal use should therefore not be recommended.

T. densiflora is also used in combination with *Piper auritum* (***hua'a*** / hierba santa). Together with *P. tuberculatum* (***gui'iquuimberu'u*** / hoja de alacran) there are two culturally important plants of the family of the Piperaceae used to treat dermatological conditions. Piperamides (cepharadione A and B from *P. auritum* and presumably piperovatine in *P. tuberculatum*) with antifungal and anaesthetic properties are reported (Hegnauer, 1962-1990). The alkaloid piplartine-dimer A is known from *P. tuberculatum* (Braz et al., 1981). No data on the biological activity of this compound are available. Some compounds of the essential oil from the two species posses antibacterial properties (cyclohexane epoxides; Orjala, 1993). Not only in the Isthmus of Tehuantepec but in many other regions of Mexico and South America *Tithonia diversifolia* (***rula'a*** / arnica) is used to treat bruises, wounds and skin infections applied as powder from the toasted leaves or in creams. Sesquiterpene lactones (Baruah et al., 1979) such as the tagitinins and constituents of the essential oil (more than 20 identified compounds of the monoterpene type; Lamaty et al. 1991) may be responsible for the antiphlogistic effect of this medicinal plant (Lin et al., 1993). The sesquiterpene lactones tagitinin A-F are reported to have significant antitumor activity (Pal et al., 1976). The ethanolic extract of the aerial parts showed inhibitory activity on the transcription factor NF-κB (Bork et al., 1996).

Table1-P2. Ranking list of spontaneously mentioned plants which elicited 5 or more positive responses for the treatment of dermatological illnesses. Total plant species = 205; total positive responses = 605 (100%). The species listed present 37% of all reported uses in this category. For each number of positive responses the plants are listed alphabetically according to (1) family, (2) genus, and (3) species. Abbreviations: aep = aerial parts, bar = bark, ear = unripe ear of corn, flo = flower, frp = fruit pulp, fru = fruit, hus = husk, inf = whole inflorescence, jui = juice of fruits, lea = leaf, uor = underground organs, pet = petal, res = resin, sap = xylem and phloem sap, sed = seed, sho = shoot, tur = turpentine, whp = whole plant, wod = wood; indet. = not determined.

Plant species	Family	No. respo.	%	Plant part(s) used
Aloe barbadensis Mill.	Asphodelaceae	13	2.1	lea, sap
Tournefortia densiflora Mart. & Gal.	Boraginaceae	12	2.0	aep, lea
Piper auritum Kunth	Piperaceae	9	1.5	aep, lea
Piper tuberculatum Jacq.	Piperaceae	7	1.2	aep, lea
Tithonia diversifolia (Hemsl.) Gray	Asteraceae	7	1.2	lea, sho
Comocladia engleriana Loes.	Anacardiaceae	6	1.0	bar, sap
Nerium oleander L.	Apocynaceae	6	1.0	sap, lea
Thevetia thevetioides Schum.	Apocynaceae	6	1.0	sap
Zebrina pendula Schnitzl.	Commelinaceae	6	1.0	whp
Jatropha curcas L.	Euphorbiaceae	6	1.0	sap, lea, sed
Hyptis verticillata Jacq.	Lamiaceae	6	1.0	lea
Swietenia humilis Zucc.	Meliaceae	6	1.0	lea, sed
Pinus oocarpa Schiede	Pinaceae	6	1.0	res, tur, wod,
Hamelia patens Jacq.	Rubiaceae	6	1.0	flo, lea
Capraria biflora L.	Scrophulariaceae	6	1.0	aep
Solanum torvum Sw.	Solanaceae	6	1.0	lea
Allium cepa L.	Alliaceae	5	0.8	uor
Xanthosoma robustum Schott	Araceae	5	0.8	sap, uor
Chameodorea tepejilote Liebm.	Arecaceae	5	0.8	fru,lea,uor,sap
Epaltes mexicana Less.	Asteraceae	5	0.8	aep, lea, uor
Crescentia alata Kunth	Bignoniaceae	5	0.8	fru, jui
Crescentia cujete L.	Bignoniaceae	5	0.8	bar, frp
Tabebuia impetiginosa (Mart. ex DC.) Standl.	Bignoniaceae	5	0.8	bar
Sechium edule (Jacq.) Sw.	Cucurbitaceae	5	0.8	fru, lea, sho

Croton ciliataglanduliferus Ortega	Euphorbiaceae	5	0.8	sap, lea
Croton soliman Cham. & Schltdl.	Euphorbiaceae	5	0.8	aep
Hyptis suaveolens (L.) Poit.	Lamiaceae	5	0.8	aep, lea
Phoradendron cf. *amplifolium* Nutt.	Loranthaceae	5	0.8	aep, lea
Sida acuta Burm. f.	Malvaceae	5	0.8	aep, lea
Acacia farnesiana (L.) Willd.	Mimosaceae	5	0.8	bar, lea, uor
Adiantum trapezoides Fée	Pteridaceae	5	0.8	lea, uor
Coffea arabica L. / *C. liberica* W. Bull. / *C. canephora* Pierre	Rubiaceae	5	0.8	lea, sed
Ruta chalepensis L.	Rutaceae	5	0.8	aep, lea
Lygodium verustrum Sw.	Schizaceae	5	0.8	aep, lea
Brugmansia suaveolens Bercht. & Presl	Solanaceae	5	0.8	flo, lea
Datura stramonium L.	Solanaceae	5	0.8	aep, bar
Heliocarpus appendiculatus Turcz.	Tiliaceae	5	0.8	bar, sap
Albahacar del monte (in Spanish)	indet.	5	0.8	aep, lea

Gastrointestinal disorders and hepatic complaints - - Gastrointestinal disorders and hepatic complaints include diarrhoea, dysentery, colic, and less frequently cited spasms, gastritis, ulcers, nausea, vomiting, hepatic, and liver problems. Accompanying symptoms are pain, flatulence, loss of appetite, and fatigue. Polluted water and food and in the dry (and windy) season dust frequently cause bacterial, viral, protozoal, and helmintic infections. Some of these illnesses are highly dangerous to new-borns, infants, and children (cholera also to adults), if they are not treated rapidly. Teas are the predominant mode of application and provide the body with the necessary liquid often essential to overcome these illnesses.

Plants are also applied externally as compress (hot or cold), bath and massage or internally as syrup, alcoholic tinctures, or are eaten fresh.

518 responses (14.3%) of 176 plants were recorded for the treatment of gastrointestinal disorders and hepatic or liver complaints (Table 2-P2). The introduced species *Ruta chalepensis* is reported very frequently for treatment of various gastrointestinal disorders. Species with astringent properties (*Psidium* spp., *Juliania adstringens* [syn. *Amphipterygium adstringens*], *Guazuma ulmifolia*) and with high levels of tannins are dominant in this group. Tannin-containing drugs are widely used to treat diarrhoea and related disorders. Wagner (1988) suggests a tanning of the intestine's mucous membrane for decreasing a prior irritation, stopping inflammation and secretion, acting as a weak anaesthetic and bacteriostatic. No pharmacological studies confirm this mode of action. Recent investigations point to a specific effect of the tannins. Hör et al. (1995) demonstrate an inhibitory effect of polymeric proanthocyanidins on the (cholera) toxin induced chloride secretion causing diarrhoea. Antibacterial and anti-inflammatory effects have been demonstrated (Scholz, 1994).

Three taxa of the Myrtaceae [*Psidium guajava* (***guixa'a nguetuj'*** / hoja de guayaba), *P. salutare* and *P.* x *hypoglaucum* (***bihuishuba'aj*** / raiana)] are mentioned very frequently by the informants. Leaf, root, and fruit are used in teas and eaten fresh, respectively to treat diarrhoea and dysentery. All over Mexico the vitamin rich fruit of *P. guajava* L. is sold on markets and consumed daily in refreshing juices. The fruit may play an important role in preventive medicine (Pisha and Pezzuto, 1994). The Myrtaceae also frequently contain high contents of essential oils - mostly monoterpenes and sesquiterpenes (Steinegger and Hänsel, 1992) - which may explain the spasmolytic, antimicrobial, and antiphlogistic action. Morales et al. (1994) report spasmolytic effects due to a calcium-antagonist effect of quercetin glycosides. *P. guajava*'s use as an efficient antidiarrhoeal remedy has been demonstrated in a series of pharmacological studies (e.g. Ponce, 1994; Lutterodt, 1994). Also bitter tasting taxa (e.g. *Artemisia mexicana*) are frequently used especially to treat strong gastrointestinal pain. Bitter sesquiterpene lactones are known (Ruiz et al., 1993).

Well established are the anthelmintic properties of *Chenopodium ambrosioides* (**biajta'a** / epazote; syn.: *Teloxis ambrosoides*, Weber (1985)) Chenopodiaceae. Zapotecs add *Ch. ambrosioides* as seasoning to their food like *Phaseolus vulgare* L., Fabaceae (**bizandxa'a** / frijol) or to soup. *Chenopodium* spp. are known from many regions of Mexico, belonging to the quelites, a group of edible weed seedlings regularly collected and integrated in diet as vegetables, and seasonings or preventive and curing remedies. The species is rich in flavonoids, triterpene saponins, betacyanins, and essential oils. *Ch. ambrosioides* contains ascaridol which acts as a vermicide (Heinrich, 1989; Schultes and Raffauf, 1990).

Table 2-P2. Ranking list of spontaneously mentioned plants which elicited 5 or more positive responses for the treatment of gastrointestinal disorders and hepatic complaints. Total plants = 176; total positive responses = 518 (100%). The species listed present 36,3% of all reported uses in this category. For abbreviations see Table 1-P2.

Plant species	Family	No. respo.	%	Plant part(s) used
Psidium guajava L.	Myrtaceae	10	1.9	fru, uor, sho
Ruta chalepensis L.	Rutaceae	10	1.9	aep, lea
Chenopodium ambrosioides L.	Chenopodiaceae	9	1.7	aep
Psidium salutare (Kunth) Berg	Myrtaceae	8	1.5	fru, lea, uor
Artemisia mexicana Willd. ex Spreng.	Asteraceae	7	1.4	aep

Pluchea symphytifolia (Mill.) Gillis	Asteraceae	7	1.4	flo, lea
Citrus limon (L.) Burm. f.	Rutaceae	7	1.4	jui, lea, sho
Juliania adstringens (L.) Schltdl.	Anacardiaceae	6	1.2	bar, lea
Annona reticulata L.	Annonaceae	6	1.2	lea, sho
Anethum graveolens L.	Apiaceae	6	1.2	sed
Equisetum sp.	Equisetaceae	6	1.2	aep
Mentha x piperita L.	Lamiaceae	6	1.2	lea
Byrsonima crassifolia (L.) Kunth	Malpighiaceae	6	1.2	bar, lea
Psidium x hypoglaucum Standl.	Myrtaceae	6	1.2	uor
Cocos nucifera L.	Arecaceae	6	1.2	bar, sap
Zea mays L.	Poaceae	6	1.2	ear, hus
Guazuma ulmifolia Lam.	Sterculiaceae	6	1.2	fru, sed
Aloe barbadensis Mill.	Asphodelaceae	5	1.0	lea, sap
Tecoma stans L. & Griseb.	Bignoniaceae	5	1.0	lea
Brassica sp.	Brassicaceae	5	1.0	sed
Tamarindus indica L.	Caesalpiniaceae	5	1.0	fru, lea
Carica papaya L.	Caricaceae	5	1.0	fru, sed
Ricinus communis L.	Euphorbiaceae	5	1.0	bar, lea, sed
Rosmarinus officinalis L.	Lamiaceae	5	1.0	sed
Magnolia schiedeana Schltdl.	Magnoliaceae	5	1.0	flo
Talauma mexicana G. Don	Magnoliaceae	5	1.0	flo
Cecropia peltata L./*C. obtusifolia* Bert.	Moraceae	5	1.0	lea, sho
Dorstenia drakena L.	Moraceae	5	1.0	uor
Piper amalago L.	Piperaceae	5	1.0	aep, lea
Lippia nodiflora (L.) Greene	Verbenaceae	5	1.0	inf
Lucema (in Spanish)	indet.	5	1.0	sed

Culture-bound syndromes (folk illnesses) - - These are illnesses only found in a certain culture and describe often ancient indigenous concepts, which are difficult to translate into Western medical terms. According to western medical understanding various symptoms are observable during such illness episodes and they may vary from one patient to another. The dominant Zapotec folk illnesses are ***dzieebi*** (in Spanish: susto / sudden fright), ***stu*** (verguenza / shame), ***guelereza'ga'*** (cansancio / fatigue), ***gueleraaj'qui*** (empacho / harm from food) and golpe (physical and / or supernatural blow).

563 responses (15.6%) for 144 plants to treat folk illnesses were recorded (Table 3-P2). Fresh twigs of *Gliricidia sepium, Ocimum basilicum* or *Piper amalago* are used in ritual healing sessions by striking the body (Zapotec: ***radij'ni guixa'a*** / Spanish: limpias). Resin of *Hymenaea courbaril* or toasted leaves of *Piper auritum* are burned as incense. Predominant are strong smelling plants with high amounts of essential oils. Ritual healing is culture specific and involves sensory perceptions and the psychological effects. Therefore a pharmacological - biological evaluation of this group is not feasible.

Table 3-P2. Ranking list of spontaneously mentioned plants which elicited 5 or more positive responses for the treatment of culture bound syndromes. Total plants = 144; total positive responses = 563 (100%). The species listed present 55.7% of all reported uses in this category. For abbreviations see Table 1-P2.

Plant species	Family	No. respo.	%	Plant part(s) used
Tagetes erecta L.	Asteraceae	12	2.1	aep
Hymenaea courbaril L.	Caesalpiniaceae	12	2.1	fru, res, sed
Ocimum basilicum L.	Lamiaceae	12	2.1	aep, lea

Piper amalago L.	Piperaceae	12	2.1	aep, lea
Piper auritum Kunth	Piperaceae	12	2.1	aep, lea
Tithonia diversifolia (Hemsl.) Gray	Asteraceae	11	2.0	lea, sho
Ruta chalepensis L.	Rutaceae	11	2.0	aep, lea
Bursera cf. *penicillata* (Sessé. et. Moç. ex DC.) Engl.	Burseraceae	10	1.8	bar, lea, res
Gliricidia sepium (Jacq.) Steud.	Fabaceae	10	1.8	lea, sho
Allium sativum L.	Alliaceae	8	1.4	uor
Dysodia appendiculata Lag.	Asteraceae	8	1.4	aep, lea
Siparuna andina (Tul.) A. DC.	Monimiaceae	8	1.4	lea
Anethum graveolens L.	Apiaceae	7	1.2	sed
Crescentia cujete L.	Bignoniaceae	7	1.2	bar, frp
Sambucus mexicana Presl ex DC.	Caprifoliaceae	7	1.2	flo, lea
Datura stramonium L.	Solanaceae	7	1.2	aep, bar
Solanum lanceolatum Cav.	Solanaceae	7	1.2	lea
Solanum torvum Sw.	Solanaceae	7	1.2	lea
Porophyllum pringlei Rob.	Asteraceae	6	1.1	lea
Porophyllum ruderale (Jacq.) Cass. ssp. *macrocephalum* (DC.) R.R. Johnson	Asteraceae	6	1.1	lea
Crescentia alata Kunth	Bignoniaceae	6	1.1	fru, jui
Sechium edule (Jacq.) Sw.	Cucurbitaceae	6	1.1	fru, lea, sho
Cyperus articulatus L.	Cyperaceae	6	1.1	uor
Poiretia punctata (Willd.) Desv.	Fabaceae	6	1.1	aep
Petiveria alliacea L.	Phytolaccaceae	6	1.1	lea, uor
Capraria biflora L.	Scrophulariaceae	6	1.1	aep
Capsicum baccatum L.	Solanaceae	6	1.1	aep, fru, lea
Cissus sicyoides L.	Vitaceae	6	1.1	aep, fru
Zingiber officinale Roscoe	Zingiberaceae	6	1.1	uor

Spondias mombin L.	Anacardiaceae	5	0.9	fru, lea
Aristolochia sp.	Aristolochiaceae	5	0.9	aep, uor
Matricaria recutita L.	Asteraceae	5	0.9	aep, flo
Brassica sp.	Brassicaceae	5	0.9	sed
Senna atomaria (L.) Irwin & Barneby	Caesalpiniaceae	5	0.9	bar, fru, lea
Chenopodium ambrosioides L.	Chenopodiaceae	5	0.9	aep
Cucurbita argyrosperma Huber ssp. *sororia* (L. H. Bailey) Merr. & Bats.	Cucurbitaceae	5	0.9	frp, fru
Erythrina folkersii Krukoff & Moldenke	Fabaceae	5	0.9	flo, lea, sed
Erythrina sp.	Fabaceae	5	0.9	flo, lea, sed
Indigofera suffruticosa Mill.	Fabaceae	5	0.9	aep
Rosmarinus officinalis L.	Lamiaceae	5	0.9	sed
Malvaviscus arboreus Cav.	Malvaceae	5	0.9	flo, lea
Eucalyptus camaldulensis Dehn.	Myrtaceae	5	0.9	lea
Eucalyptus sp.	Myrtaceae	5	0.9	lea
Cocos nucifera L.	Arecaceae	5	0.9	bar, sap
Ternstroemia pringlei Standl.	Ternstroemiaceae	5	0.9	flo

Female and male genito-urinary complaints - - 364 responses (10.1%) of 122 species refer to the treatment of illnesses of the female and male genital organs and infections of the kidneys and bladder. Plants indicated for contraception (*Tagetes lucida*) and reproduction are included as well (Table 4-P2). The number of responses are in general lower than in other groups. Most of the reported uses are for gynaecological problems and are only dealt with by the 8 midwives (one of them a male) out of the 13 main informants. Plants (e.g. *Ocimum basilicum*) are used in massages during pregnancy to reduce pain and to "adjust the position of the foetus" close to the date of birth. The majority of the plants is cited for acceleration of labor (*Aristolochia ovalifolia, Pinus oocarpa*) and to expel the placenta (*Chrysanthemum parthenium*).

Vaginal, uterine, and ovarian inflammation are serious problems for Zapotec women. *Tournefortia densiflora* (***guixa'a cancer*** / hoja de cancer) is reportedly a potent medicine applied as vaginal douches, vapor bath (vaoh) and bath. As reported in the part of skin diseases many Boraginaceae have wound healing properties. The pharmacologically interesting group of the pyrrolizidine alkaloids is common in species of *Tournefortia*. Hegnauer (1962-1990) describes supinine and tourneforcine among others.

Table 4-P2. Ranking list of spontaneously mentioned plants which elicited 5 or more positive responses for the treatment of female and male genito-urinary complaints. Total plants = 122; total positive responses = 364 (100%). The species listed present 35.4% of all reported uses in this category. For abbreviations see Table 1-P2.

Plant species	Family	No. respo.	%	Plant part(s) used
Tournefortia densiflora Mart. & Gal.	Boraginaceae	8	2.2	aep, lea
Aristolochia sp.	Aristolochiaceae	7	1.9	aep, uor
Chrysanthemum parthenium (L.) Bernh.	Asteraceae	7	1.9	aep, flo
Chaemecrista fagonioides (Vogel) L. et B. var. *fagonioides*	Caesalpiniaceae	7	1.9	aep, lea
Ocimum basilicum L.	Lamiaceae	7	1.9	aep
Equisetum sp.	Equisetaceae	6	1.6	aep
Pinus oocarpa Schiede	Pinaceae	6	1.6	tur, res, wod
Piper auritum Kunth.	Piperaceae	6	1.6	aep, lea

Juliania adstringens (L.) Schltdl.	Anacardiaceae	5	1.4	bar, lea
Cocos nucifera L.	Arecaceae	5	1.4	bar, lea, sap
Artemisia absinthium L.	Asteraceae	5	1.4	aep
Gnaphalium roseum Kunth	Asteraceae	5	1.4	aep, lea
Tagetes lucida Cav.	Asteraceae	5	1.4	aep
Brassica sp.	Brassicaceae	5	1.4	sed
Quercus oleoides Schltdl. & Cham.	Fagaceae	5	1.4	bar
Rosmarinus officinalis L.	Lamiaceae	5	1.4	sed
Litsea glaucescens Kunth	Lauraceae	5	1.4	aep, lea
Mimosa pudica L.	Mimosaceae	5	1.4	aep, lea, uor
Piper dioica L.	Piperaceae	5	1.4	sed
Oryza sativa L.	Poaceae	5	1.4	sed
Zea mays L.	Poaceae	5	1.4	ear, hus
Punica granatum L.	Punicaceae	5	1.4	bar, fru
Lucema (in Spanish)	Indet.	5	1.4	sed

Illnesses of the skeletal-muscular system - - It includes any form of dysfunction and trauma associated with the joints, muscles, or skeleton (rheumatism, arthritis, fractures, bruises, and sprains). Due to hard physical work and the seasonal humid climate such illnesses are very common. The most common treatment includes massages with herbal creams (*Ocimum basilicum*), poultices (*Poiretia punctata*), and shower baths (*Porophyllum pringlei*) to alleviate pain and accelerate the healing process. Internally, teas (*Allium cepa*), tinctures, and syrup (*Crescentia cujete*) are applied. Many of the preparations are mixtures of several plants. 321 responses (8.9%) of 105 plants are reported (Table 5-P2).

The application of the fruit pulp (internally and externally) of *Crescentia cujete* is widely observed to accelerate the healing of bruises and sprains. Heltzel et al. (1993) isolated furanonaphthoquinones with anticancer activity. The Bignoniaceae have not been investigated profoundly. Hegnauer (1962-1990) lists quinones (lapachol), iridoid glycosides, and polyphenols.

Table 5-P2. Ranking list of spontaneously mentioned plants which elicited 5 or more positive responses for the treatment of illnesses of the skeletal-muscular system. Total plants = 105; total positive responses = 321 (100%). The species listed present 29.9% of all reported uses in this category. For abbreviations see Table 1-P2.

Plant species	Family	No. respo.	%	Plant part(s) used
Ocimum basilicum L.	Lamiaceae	8	2.5	aep
Aloe barbadensis Mill.	Asphodelaceae	7	2.2	lea, sap
Allium sativum L.	Alliaceae	6	1.9	uor
Crescentia cujete L.	Bignoniaceae	6	1.9	bar, frp
Cyperus articulatus L.	Cyperaceae	6	1.9	uor
Poiretia punctata (Willd.) Desv.	Fabaceae	6	1.9	aep
Petiveria alliacea L.	Phytolaccaceae	6	1.9	lea, uor
Zingiber officinale Roscoe	Zingiberaceae	6	1.9	uor
Allium cepa L.	Alliaceae	5	1.6	uor
Cymbopetalum cf.	Annonaceae	5	1.6	pet
Porophyllum pringlei Rob.	Asteraceae	5	1.6	lea
Porophyllum ruderale (Jacq.) Cass. ssp. *macrocephalum* (DC.) R. R. John.	Asteraceae	5	1.6	lea
Tithonia diversifolia (Hemsl.) Gray	Asteraceae	5	1.6	lea, sho
Anredera ramosa (Moq.) Eliasson	Basellaceae	5	1.6	uor

Boussingaultia leptostachys Moq.	Basellaceae	5	1.6	uor
Senna atomaria (L.) Irwin & Barneby	Caesalpiniaceae	5	1.6	bar, fru, lea
Heliocarpus appendiculatus Turcz.	Tiliaceae	5	1.6	bar, sap

Respiratory ailments - - Malnutrition, poverty (lack of footwear and clothes) and seasonal heavy rains favor respiratory ailments. Tuberculosis is still a serious problem. In the treatment plants are prepared as tea (*Allium sativum, Citrus limon*), syrup (*Bougainvillea glabra*), drops of the fresh plant sap (*Crescentia alata*), cream (*Eucalyptus camadulensis*) and as shower baths.

303 responses (8.4%) of 88 plants were recorded for this category (Table 6-P2). High amounts of resin make *Pinus oocarpa* (***guiere*** / ocote) a medically important plant. Uses of wood and oleoresin from *Pinus* sp. are known from many regions of the world and listed in many pharmacopoeias (of Switzerland, Austria, Germany, the Homeopathic Pharmacopoeia of the US). The essential oil from the needles and the turpentine is rich in mono- and sesquiterpenes. Diterpenes are found in the colophony and the oil of turpentine. Proanthocyanidins, catechine tannins and flavones are reported from the bark. The wood contains flavones, stilbenes, lignans, shikimic acid and sugars (Hänsel et al., 1992-1994). Hänsel et al. describe antimicrobial activity of related species (e. g. on *Streptococcus* sp., *Staphylococcus* sp.; on *Bacillus tuberculosis* only inhibiting), enhancement of bronchial secretion and expectoration, a bronchospasmolytic and a general counterirritant effect

.

Table 6-P2. Ranking list of spontaneously mentioned plants which elicited 5 or more positive responses for the treatment of respiratory ailments. Total plants = 88; total positive responses = 303 (100%). The species listed present 48.8% of all reported uses in this category. For abbreviations see Table 1-P2.

Plant species	Family	No. respo.	%	Plant part(s) used
Eucalyptus camaldulensis Dehn.	Myrtaceae	11	3.6	lea
Bougainvillea glabra Choisy	Nyctaginaceae	10	3.3	flo
Citrus limon (L.) Burm. f.	Rutaceae	10	3.3	lea, jui, sho
Allium sativum L.	Alliaceae	8	2.6	uor
Crescentia cujete L.	Bignoniaceae	8	2.6	bar, frp
Pinus oocarpa Schiede	Pinaceae	8	2.6	res, tur, wod
Punica granatum L.	Punicaceae	8	2.6	bar, fru
Gnaphalium roseum Kunth	Asteraceae	7	2.3	aep, lea
Crescentia alata Kunth	Bignoniaceae	7	2.3	fru, jui
Cinnamomum zeylanicum Nees	Lauraceae	7	2.3	bar
Mangifera indica L.	Anacardiaceae	6	2.0	fru,sap,lea,sed
Cassia grandis L.	Caesalpiniaceae	6	2.0	bar, sed
Piper amalago L.	Piperaceae	6	2.0	aep, lea
Citrus sp.	Rutaceae	6	2.0	aep,bar,jui,sed
Allium cepa L.	Alliaceae	5	1.7	aep
Nerium oleander L.	Apocynaceae	5	1.7	sap, lea
Plumeria rubra L.	Apocynaceae	5	1.7	bar, flo
Thevetia thevetioides Schum.	Apocynaceae	5	1.7	sap
Bursera cf. *penicillata* Engl.	Burseraceae	5	1.7	bar, lea, res
Hymenaea courbaril L.	Caesalpiniaceae	5	1.7	fru, res, sed
Hibiscus rosa-sinensis L.	Malvaceae	5	1.7	flo
Lippia nodiflora (L.) Greene	Verbenaceae	5	1.7	inf

Fever and malaria - - Here we describe a symptom (with the exception of malaria) which is due to various diseases, but often considered as a separate group of illnesses by the Zapotecs (Table 7-P2): 76 plants were mentioned 285 times (7.9%). *Ocimum basilicum* and *Gliricidia sepium* are widely used in ritual healing (limpias). A large number of fresh twigs soaked in alcohol are struck over the body. Their strong aromatic odor (essential oils) extracted with the alcohol as well as the alcohol itself are perceived as having a cooling and relaxing effect on the body. Other treatments are ritual baths with lukewarm decoctions of *Bursera grandifolia* (***yalaj'guettu'u*** / palo mulato) or *Juliania adstringens*, (syn. *Amphipterygium adstringens*; ***ya'guiaj'*** / huachinala): starting at the feet, then up to the knees and in two more steps until the whole body is washed. This as well as enemas (***badiaj'raj'li*** / lavados intestinal) are ancient Mesoamerican concepts to cleanse the body of the "heat" (Dr. Viesca T., C.; pers. comm.). In addition to the external application, teas are prepared.

Several triterpene acids (with reported anticancer activity), three alkyl anacardic acids and long chain phenols (active on serum cholesterol level) have been isolated from *Juliania adstringens* (Dominguez et al., 1983; Navarrete et al., 1989; Mata et al., 1991). Studies to evaluate the potential anti-inflammatory and antipyretic activity have not been performed yet. *Bursera* spp. have been investigated phytochemically in greater detail (Hegnauer, 1962-1990). Polyphenols like lignans, coumarins, flavanones, procyanidins, and tannins are known. Many members of this family are rich in balsam and resin-containing triterpenes. *Bursera grandifolia* has not yet been investigated for antipyretic activity.

From remote ranchos (little farms, about 200 to 400 m above sea-level) malaria is still reported and usually rapidly treated by medical doctors. Bathing and teas with *Acosmium panamense* (in Zapotec and Spanish: **guayuacan**) and

Turnera diffusa (guixa'a / hoja de fiebre) were mentioned to treat malaria in ancient times. No parasitological data are available to substantiate this claim.

Table 7-P2. Ranking list of spontaneously mentioned plants which elicited 5 or more positive responses for the treatment of fever (including malaria). Total plants = 76; total positive responses = 285 (100%). The species listed present 51.2% of all reported uses in this category. For abbreviations see Table 1-P2.

Plant species	Family	No. respo.	%	Plant part(s) used
Ocimum basilicum L.	Lamiaceae	10	3.5	aep
Bursera grandifolia (Schltdl.) Engl.	Burseraceae	9	3.2	bar
Gliricidia sepium (Jacq.) Steud.	Fabaceae	8	2.8	lea, sho
Juliania adstringens (L.) Schltdl.	Anacardiaceae	7	2.5	bar, lea
Bryophyllum pinnatum (Lam.) Kurz	Crassulaceae	7	2.5	lea
Loeselia mexicana (Lam.) Brand	Polemoniaceae	7	2.5	aep
Turnera diffusa Willd. ex Schult.	Turneraceae	7	2.5	aep
Tamarindus indica L.	Caesalpiniaceae	6	2.1	fru, lea
Siparuna andina (Tul.) A. DC.	Monimiaceae	6	2.1	lea
Cymbopogon citratus (DC.) Stapf	Poaceae	6	2.1	lea
Salix sp.	Salicaceae	6	2.1	bar, lea
Solanum diflorum Vell.	Solanaceae	6	2.1	lea
Guazuma tomentosa Kunth	Sterculiaceae	6	2.1	bar, fru, sed
Anethum graveolens L.	Apiaceae	5	1.8	sed
Epaltes mexicana Less.	Asteraceae	5	1.8	aep, lea, uor
Tagetes erecta L.	Asteraceae	5	1.8	aep
Cassia grandis L.	Caesalpiniaceae	5	1.8	bar, sed

Zebrina pendula Schnitzl.	Commelinaceae	5	1.8	aep
Acosmium panamense (Benth.) Yakov.	Fabaceae	5	1.8	bar
Phoradendron cf. *amplifolium* Nutt.	Loranthaceae	5	1.8	aep, lea
Sida acuta Burm. f.	Malvaceae	5	1.8	aep, lea
Swietenia humilis Zucc.	Meliaceae	5	1.8	lea, sed
Capsicum baccatum L.	Solanaceae	5	1.8	aep, fru, lea
Lippia nodiflora (L.) Greene	Verbenaceae	5	1.8	inf

Cardiovascular complaints and diseases of the blood, ophthalmologic problems - - The last two categories are rather small: 52 responses (1.4%) of 20 plants useful to treat cardiovascular complaints and diseases of the blood were recorded (Table 8-P2). 48 responses (1.3%) of 20 plants are listed to treat ophthalmologic problems (Table 9-P2). Only three, respectively two species with five or more positive responses are reported. Zapotecs use *Magnolia schiedeana* and *Talauma mexicana* (as teas) to cure cardiovascular complaints. Phytochemical publications list essential oils (monoterpenes, sesquiterpenes, and phenylpropanoids), alkaloids (phenylalanine type), polyphenols (coumarins, flavonoids, and lignans), cyclites, sesquiterpene lactones and saponins for the two genera. Sesquiterpene lactones with an α-methylene butanolide substituent and cardenolides are presumably responsible for the cardioactivity confirmed in pharmacological tests (Hegnauer, 1962-1990).

Table 8-P2. Ranking list of spontaneously mentioned plants which elicited 4 or more positive responses for the treatment of cardiovascular complaints and diseases of the blood. Total plants = 20; total positive responses = 52 (100%). The species listed present 44.2% of all reported uses in this category. For abbreviations see Table 1-P2.

Plant species	Family	No. respo.	%	Plant part(s) used
Magnolia schiedeana Schltdl.	Magnoliaceae	7	13.5	flo
Talauma mexicana G. Don	Magnoliaceae	7	13.5	flo
Ternstroemia pringlei Standl.	Ternstroemiaceae	5	9.6	flo, fru
Haematoxylum brasiletto Karst.	Caesalpiniaceae	4	7.7	bar, wod

Table 9-P2. Ranking list of spontaneously mentioned plants which elicited 4 or more positive responses for the treatment of ophthalmological problems. Total plants = 20; total positive responses = 48 (100%). The species listed present 39.6% of all reported uses in this category. For abbreviations see Table 1-P2.

Plant species	Family	No. respo.	%	Plant part(s) used
Rosa centifolia L.	Rosaceae	6	12.5	pet, flo
Capsicum baccatum L.	Solanaceae	5	10.4	aep, fru, lea
Sambucus mexicana Presl ex DC.	Caprifoliaceae	4	8.3	flo, lea
Cissus sicyoides L.	Vitaceae	4	8.3	aep, fru

The huge family of the Solanaceae provided many pharmacological active compounds to western medicine (atropine, hyoscyamine, nicotine, and scopolamine) some of which are widely used in ophthalmology. In *Capsicum* spp. (Solanaceae) the main compound is capsaicine, a vanillylamide with hyperemic, local irritating, and neurotransmitter activating properties. Consequently vasodilatation, enhanced permeability and anti-inflammatory activity are observed (Hänsel et al., 1992-1994). Whether these amide or Solanaceae-alkaloids are the pharmacologically active compounds of the leaves of *Capsicum baccatum* (***balagaguina'a*** / hoja de chile) has not yet been investigated. *Rosa centifolia* is ranked highly but introduced into the area and therefore not discussed.

Other uses - - 552 responses (45.2%) of 201 plants are cited for a few diseases which could not be sorted into the categories mentioned above. Included are also uses in rituals as well as non-medicinal uses as ornaments, construction material, or food. Since this group is too heterogeneous, no ranking list was drawn up. Also included is "diabetes" of which a growing number of older people is affected. No laboratory data to confirm these popular diagnoses are available. Type 2 diabetes mellitus (non-insulin dependant) is controllable with appropriate diet, reduction of body weight and medicinal plants (although infant malnutrition is a serious problem, Isthmus-Zapotec women are often overweight (cultural status symbol)). With the Zapotecs as well as in other parts of Mexico the use of *Tecoma stans, Opuntia* sp., *Cecropia peltata* and *C. obtusifolia* to control the disease is widespread.

Hallucinogens are of minor cultural importance for the Isthmus-Sierra Zapotecs. The knowledge about uses is mainly distributed among healers of other ethnic groups, settled in the area. Therefore only few data are available and are not discussed here.

CONCLUSION

The Isthmus-Sierra Zapotecs live in an area of great botanic diversity that provides potent phytotherapeutic remedies. People have access to pharmacies and health service in the region. But - because of lack of money and the remoteness of some areas - plants still play an important role. The Zapotec medical system is inexpensive and plant-based remedies are easily available. In this article we selected the ethnobotanically important species from a longer and interesting medico-botanical inventory using a semi-quantitative method. The grouping of the plant species into 10 categories of indigenous uses is a methodologically useful approach in order to meaningfully organize extensive field data. The analysis of the available phytochemical and pharmacological literature shows that experimental studies confirming the attributed properties do exist for many species. However, many of the plant species have not been studied for their phytochemical composition in detail. Data on the chemical characteristics of related genera or the botanical families may yield additional information for a scientific explanation of a medical use and may point to the need for further phytochemical and/or pharmacological studies. Toxicological results also have to be taken into consideration not only for pharmaceutical purposes but especially for applying the information on traditional plant use (e.g. as discussed for *Tournefortia densiflora*).

Interdisciplinary research on ethnobotany requires the collaboration between indigenous people, botanists, phytochemists, ethnologists, pharmacologists, ecologists and medical doctors and will be for the benefit of traditional and western medicine. Consequently, indigenous plant use and healing concepts will be better understood and could lead to an upgrading of traditional medicine. Its combination with modern western medicine may give new inputs to both of them. Phytochemists should choose ethnobotanically important and phytochemically less investigated plant species (e.g. *Xanthosoma robustum*, Kato et al., 1996) for the search for new compounds or lead structures for the development of new drugs.

The collected ethnobotanical and pharmaceutical data could be one basis for biodiversity conservation and community development. Small plantations with the production of medicinal plants for regional use may - after a profound evaluation - provide remote areas with inexpensive and potent therapeutics and small scale additional income.

ACKNOWLEDGEMENTS

We are very grateful to the healers, midwives and the inhabitants of Sto. Domingo and Sta. Maria Petapa, Sta. Maria Guienagati and Guevea de Humboldt, Oaxaca for teaching their art of healing, for their friendship and hospitality. We would like to thank Dr. P. Davila A., O. Tellez. V., L. Torres C., R. Torres C., F. Ramos M., Dr. R. Lira S., and Dr. J.L. Villaseñor R. at the National Herbarium (MEXU), Dr. R. A. Bye Jr., T. Balcazar S. at the Botanical Garden, Dr. C. Viesca T., Institute of Medical History and Anthropology, all Universidad Nacional Autónoma de México UNAM; Prof. Dr. H. Rimpler, Institute of Pharmaceutical Biology, Freiburg/Germany; for their help at various stages of the project. This work would not have been possible without the financial support by the Swiss Development Cooperation (SDC, Bern).

LITERATURE CITED

Aguilar C., A., Chino, J. R., Jaquez P., S. and Lopez, M. E. (1994) *Herbario medicinal del Instituto Mexicano del Seguro Social.* Instituto Mexicano del Seguro Social (IMSS). México D.F.

Alcorn, J. B. (1984) *Huastec Mayan ethnobotany.* University Texas Press, Austin, TX.

Argueta V., A. (coordinador) (1994) *Atlas de las plantas de la medicina tradicional Mexicana.* 3 vols.. Instituto Nacional Indigenista, México D.F..

Baruah, N.C., Sharma, R.P., Madhusudanan, K.P. and Thyagarajan, G. (1979) Sesquiterpene lactones of *Tithonia diversifolia.* Sterochemistry of the tagitinins and related compounds. *Journal of Organic Chemistry* 44, 1831-1835.

Bork, P. M., Schmitz, M.L., Weimann, C., Kist, M. and Heinrich, M. (1996) Nahua Indian Medicinal Plants (Mexico): Inhibitory Activiy on NF-kB as an Antiinflammatory Model and Antibacterial Effects. *Phytomedicine*, in press.

Braz F., R., de Souza, M. P. and Mattos, M. E. O. (1981) Piplartine - dimer A, a new alkaloid from *Piper tuberculatum*. *Phytochemistry* 20, 345-346.

Browner, C. H. (1985) Plants used for reproductive health in Oaxaca, Mexico. *Economic Botany* 39, 482-504.

Brueske, J. (1976) The Petapa Zapotecs of the Inland Isthmus of Tehuantepec. Thesis UCLA, Los Angeles.

Bye, R. Jr. (1993) The Role of Humans in the Diversification of Plants in Mexico. In: Ramamorthy, T.P., Bye, R. , Lot, A. and Fa, J. , *Biological Diversity of Mexico, Origins and Distribution*. Oxford University Press, p. 707.

Campell, H., Bindford, L., Bartolome, M. and Baraba, A. (1993) *Zapotec struggles*. Smithsonian Institution Press, Washington, London, p. 285.

Codice Florentino (1950-1969) Dibble, Ch. and Anderson, A. (eds.), 12 vols.. The School of American Research and The University of Utah. Santa Fe, N. Mexico.

Cruz, M. de la. (1991) Libellus de medicinalibus indorum herbis. Fondo de Cultura Económica/Instituto Mexicano de Seguro Social, México. [orig.: Códice De la Cruz-Badiano, 1552].

Dominguez, X. A., Franco, R., Garcia, S., Porras, MA. E., Vasquez, G. and Amezcua, B. (1983) Plantas medicinale Mexicanas. XLVIII: Estructura del ácido instipolinácico separado de la corteza del cuachalalate (*Amphyterygium adstringens*). *Revista Lationamericana de Quimica* 14, 99-100.

Farnsworth, N. R., Akerele, O., Bingel, A. S., Soejarto, D. D. and Guo, Z. (1985) Medicinal plants in therapy. *Bulletin of the World Health Organization* 63, 965-981.

Friedman, J., Yaniv, Z., Dafni, A. and Palewitch, D. (1986) A preliminary classification of the healing potential of medicinal plants, based on a rational analysis of an ethnopharmacological field survey among bedouins in the Negev Desert, Israel. *Journal of Ethnopharmacology* 16, 275-287.

Hamburger, M. and Hostettmann, K. (1991) 7. Bioactivity in plants: The link between phytochemistry and medicine. *Phytochemistry* 30, 3864-3874.

Hänsel, R., Keller, K., Rimpler, H. and Schneider, G. (Hrsg.) (1992-1994) *Hagers Handbuch der pharmazeutischen Praxis*, Drogen A-D, Drogen E-O, Drogen P-Z, 5. Auflage, Bde. 4-6. Springer-Verlag, Berlin.

Hegnauer, R. (1962-1990) *Chemotaxonomie der Pflanzen*, Bd. 1 - 9. Birkhäuser-Verlag, Basel, Boston, Berlin.

Heinrich, M. (1989) *Ethnobotanik der Tieflandmixe (Oaxaca, Mexico) und phytochemische Untersuchung von Capraria biflora L. (Scrophulariaceae).* Dissertationes Botanicae No. 144. J. Cramer, Berlin und Stuttgart.

Heltzel, C. E., Guantilaka, A. A. L., Glass, T. E. and Kingston, D. G. I. (1993) Bioactive fuaranonaphthoquinones from *Crescentia cujete. Journal of Natural Products* 56, 1500-1505.

Heyer, E. (1988) *Witterung und Klima.* 8. Auflage, BSB B.G. Teubner Verlagsgesellschaft, Leipzig.

Hör, M., Rimpler, H. and Heinrich, M. (1995) Inhibition of intestinal chloride secretion by proanthocynidins from *Guazuma ulmifolia* bark. *Planta Medica* 61, 208-212.

Humbolt, A. von (1849) *Ansichten der Natur.* 3. Auflage, Bd. 2, 119. Stuttgart und Tübingen.

INEGI (1993) Region Istmo, Oaxaca, Perfil sociodemografico, XI Censo general de población y vivienda, 1990. Instituto Nacional de Estadística, Geografia Informática, Aguascalientes.

Johns, T., Kokwaro, J. O. and Kimanani, E. K. (1990) Herbal remedies of Luo of Siaya District, Kenya: Establishing quantitative criteria for consensus. *Economic Botany* 44, 369-381.

Josserand, J. K., Winter, M. and Hopkins,N. (1984) Essays in Otomanguean culture history. *Publications in Anthropology* 31, 30. Vanderbilt University, Nashville, Tennessee.

Kato, T., Frei, B., Heinrich, M. and Sticher, O. (1996) Antibacterial hydroperoxysterols from *Xanthosoma robustum. Phytochemistry* 41, 1191-1195.

Lamaty, G., Menut, CH., Zollo, P.-H., A., Kuiate, J.R., Bessiere, J.-M. and Koudou, J. (1991) Aromatic plants of tropical Central Africa. III. Constituents of the essential oil of the leaves of *Tithonia diversifolia* (Hemsl.) A. Gray from Cameroon. *Journal of Essential Oil Research* 3, 399-402.

Lin, C. C., Lin, M. L. and Lin, J. M. (1993) The Antiinflammatory and liver protective effect of *Tithonia diversifolia* (Hemsl.) Gray and *Dicliptera chinensis* Juss. extracts in rats. *Phytotherapy Research* 7, 305-309.

Linares, E. and Bye, R. A. Jr. (1987) A study of four medicinal plant complexes of Mexico and adjacent United States. *Journal of Ethnopharmacology* 19, 153-183.

Lutterodt, G. D. (1994) Effect on electrolyte and water transport by *Psidium guajava* extract in a rat secretory diarrhoea model. *Asia Pacific Journal of Pharmacology* 9, 189-193.

Mata, R., Calzada, F., Navarrete, A., Del Rio, F. and Delgado, G. (1991) Long-chain phenols from the bark of *Amphypterygium adstringens. Journal of Ethnopharmacology* 34, 147-154.

Moerman, D. E. (1996) An analysis of the food plants and drug plants of native North America. *Journal of Ethnopharmacolgy* 52, 1-22.

Morales, M. A., Tortoriello, J., Mekes, M., Paz, D. and Lozoya , X. (1994) Calciumantagonist effect of quercetin and its relation with the spasmolytic properties of *Psidium guajava* L. *Archives of Medicinal Research* 25, 17-21.

Nader, L. (1976) The Zapotecs of Oaxaca. In: R. Wauchope (Ed.), *Handbook of Middle American Indians*, part Ethnology, 1329-359. University of Texas Press, Austin, TX.

Navarrete, A., Mata, R. and Delgado, G. (1989) Alkylanacardic acids from *Amphypterygium adstringens*. *Planta Medica* 55, 579.

Orjala, J. (1993) Phytochemical and biological investigation of *Piper aduncum*, a traditional remedy from Papua New Guinea. Thesis ETH Zurich, no. 10116.

Ortiz de Montellano, B. (1975) Empirical Aztec medicine, *Science* 188, 215-220.

Pal, R., Kulshreshta, D.K. and Rastogi, R.P. (1976) Antileukemic and other constituents of *Tithonia tagitiflora* Desf., *Journal of Pharmaceutical Sciences* 65, 918-919.

Philips, O., Gentry, A. H., Reynel, C., Wilkin, P. and Galvez-Durand, B. C. (1994) Quantitative ethnobotany and Amazonian conservation. *Conservation Biology* 8, 225-248.

Pisha, E. and Pezzuto, J.M. (1994) Fruits and vegetables containing compounds that demonstrate pharmacological activity in humans. In: H. Wagner and N. R. Farnsworth [eds.], *Economic and medicinal plant research*. Vol. 6, chapt. 5, 189-233.

Ponce M., M., Navarro A., I., Martinez G., M. N. and Alvarez C., R. (1994) Efecto antigiardiasico *in vitro* de 14 extractos de plantas. *Revista de Investigacion Clinica* 46, 343-347.

Ruiz-Cancino, A., Cano, A.E. and Delgado, G. (1993) Sesquiterpene lactones and flavonoids from *Artemisia ludoviciana* ssp. *mexicana*. *Phytochemistry* 33, 1113-1115.

Scholz, E. (1994) Pflanzliche Gerbstoffe. *Deutsche Apotheker Zeitung* 34, 17-29.

Schultes, R. E. (1983) Richard Spruce: An early ethnobotanist and explorer of the Northwest Amazon and Northern Andes. *Journal of Ethnobiology* 3, 139-147.

Schultes, R. E. and Raffauf, R. F. (1990) *The healing forest*. Dioscorides Press, Portland, Oregon.

Steinegger, E. and Hänsel, R. (1992) *Pharmakognosie*, 5. Auflage. Springer Verlag, Berlin.

Tortoriello, J. and Aguilar-Santamaría (1996) Evaluation of the calcium-antagonist, antidiarrhoeic and central nervous system activities of *Baccharis serraefolia*. *Journal of Ethnopharmacology* 53, 157-163.

Wagner, H. (1993) Pharmazeutische Biologie, Bd. 2, *Drogen und ihre Inhaltsstoffe*. 5. Auflage. Gustav Fischer Verlag, Stuttgart.

Weber, W. A. (1985) The genus *Teloxys* (Chenopodiaceae). *Phytologia* 58, 477-478.

2.3 Publication III

Barbara FREI (Eidg. dipl. Apothekerin.) [1], Rudolf M. HALLER (dipl. geogr.) [2]

Otto STICHER (Prof. Dr. sc. nat.) [1], Michael HEINRICH (Dr. rer. nat.) [3]

[1] Department of Pharmacy, Swiss Federal Institute of Technology (ETH) Zurich,
Winterthurerstrasse 190, CH-8057 Zürich, Switzerland;

[2] GIS Swiss National Park, Chasa dal Parc, CH-7530 Zernez, Switzerland;

[3] Institute of Pharmaceutical Biology, Albert-Ludwigs-University,
Schaenzlestrasse 1, D-79104 Freiburg, Germany

Paper submitted, *AMBIO*

Indigenous Medicinal Plant Management in the Isthmus of Tehuantepec (Mexico):

Botanical Diversity and Cultural Importance

Author to whom correspondence should be addressed:
Michael HEINRICH
Institute of Pharmaceutical Biology, Albert-Ludwigs-University,
Schaenzlestrasse 1, D-79104 Freiburg, Germany
phone: +49-761-203-2806

fax: +49-761-203-2803

ABSTRACT

The tropics represent a zone rich in medicinal plant species and ethnomedical knowledge. It is well known that these biotic and cognitive resources are endangered by deforestation and cultural changes. The documentation of indigenous knowledge and concepts of the environment may be one approach for developing sustainable forms of resource use in these areas. Based on two independent ethnobotanical inventories among neighboring Mixe and Zapotec Indians in Mexico, ethno-ecological strategies for obtaining medicinal plants are analyzed. Six ecologically important zones (along indigenous definitions) are discussed. These ethnobotanical data on the anthropogenetic vegetation are compared with the main vegetation types (along western scientific definition), present in the area of study. Most ethnomedically important species are cultivated in the "house yard" or gathered in the community or its immediate surroundings. The results of the study show that the strategies depend on the cultural differences between the two groups as well as on environmental aspects.

INTRODUCTION

The tropical ecosystems and the biology of many of its plant species are still not well understood. Technological changes and expansion of agriculture as well as other economic interests daily cause monumental loss of knowledge and understanding about the world's richest ecosystems. For centuries indigenous people have managed tropical forests for subsistence as well as for medico-pharmaceutical purposes ([1, 2, 3, 4]). Understanding traditional strategies of use and native people's perceptions of the natural environment may provide many new insights.

Ethno-ecology is the study of native people's perceptions of "natural" divisions in the biological world and soil-plant-animal-human relationships within each division ([5]). Ethnobotany focuses on the importance of plants in a culture and

---

1 Alcorn, J. B. 1984. Development policy, forests, and peasant farms: Reflections on Huastec-managed forests' contributions to commercial production and resource conservation. *Econ. Bot. 38*. 389-406.

2 Posey, D. A. 1985. Native and indigenous guidlines for new Amazonian development strategies: Understanding biological diversity through ethnoecology. In: *Changes in the Amazon Basin. Vol. I. Man's Impact on forests and rivers.* Hemming, J. (ed.). Manchester University Press, Manchester, UK. pp. 156-181.

3 Irvine, D. 1989. Succession management and resource distribution in an Amazonian rain forest. *Adv. Econ. Bot. 7*, 223-237.

4 Balée, W., and Gély, A. 1989. Managed forests succession in Amazonia: The Ka'apor case. *Adv. Econ. Bot.7*, 129-158.

5 Frechione, J., Posey, D. A., and Francelino da Silva, L. 1989. The perception of ecological zones and natural resources in the Brazilian Amazon: An ethnoecology of Lake Coari. *Adv.*

medical ethnobotany deals specifically with plants used for curing purposes and for well-being. Documentation of endangered knowledge was the main goal in the early ethnobotanical studies, while today many projects collect data for biodiversity conservation and community development focusing on the ecological feasibility of the indigenous management strategies ([6, 7, 8, 9, 10, 11]). Systematic investigation of indigenous ecological knowledge systems will not only provide useful scientific data about the heterogeneity of tropical ecosystems, but can also justify new

---

Econ. Bot.7, 260-282.

6 Akerele, O., Heywood, V., and Synge, H. 1991. *Conservation of Medicinal Plants.* Cambridge University Press, Cambridge, UK.

7 Martin, G. J., Hoare, A. L., and Posey, D. A. (eds.) 1996. *Protecting Rights.* People and Plants Handbook Issue 2, WWF, UNESCO, RGB, Kew, Paris, France.

8 Cunningham, A. B. 1993. *African Medicinal Plants.* WWF, People and Plants, United Nations Educational, Scientific and Cultural Organization, UNESCO Press, Paris, France. Working paper 1.

9 Cunningham, A. B., and Mbenkum, F. T. 1993. *Sustainability of Harvesting Prunus africana Bark in Cameroon.* WWF, People and Plants, United Nations Educational, Scientific and Cultural Organization, UNESCO, Paris, France. Working paper 2.

10 Aumeeruddy, Y. 1994. *Local Representations and Management of Agroforests on the Periphery of Kerinci Seblat National Park Sumatra, Indonesia.* WWF, People and Plants, United Nations Educational, Scientific and Cultural Organization, UNESCO, Paris, France. Working paper 3.

11 Gómez-Pompa, A., Whitmore, T. C., and Hadley, M. (eds.) 1991. *Rain Forest Regeneration amd Management.* MAB Series, Vol. 6. The Parthenon Pub. Group Washington, D. C., USA.

directions for natural resource utilization. Furthermore, it can be valuable in rural development based on a sustainable silvicultural and agricultural management.

This study consequently looks at the vegetation types in one geographical area and contrasts these data with the management strategies for one specific resource: medicinal plants. Based on two independent botanical inventories, strategies of Mixe and Zapotec Indians plant use are analyzed. The ecologically important areas of gathering and cultivating medicinal plants are discussed using six ecological zones, which are described based on the indigenous concepts of the environment. These anthropogenetic zones are (1) the *house yard* (solar [12]), (2) *inside the borderlines of the village* (en el pueblo), *outside of yards*, along streets and water streams, (3) *secondary vegetation* along *roadsides* leading out of the village (camino), (4) *fields* and *forests with nomadic agriculture, coffee plantations* (milpa, cafetál), *pasture land* (corral, potreros), (5) *primary or less managed forest* (bosque) and for completeness, (6) *markets* and *peddlers* (mercado, and comerciantes) which are not actually an ecological zone. By comparing data from two ethnic groups concerning the criteria for plant selection and management strategies of indigenous ecological zones, the results provide interesting information on environmental and cultural influences. Additionally, differences and parallels in the indigenous groups' approach to plant resources are outlined. When no specific ethnic group is mentioned, the data refer to both groups, the Zapotec and Mixe.

[12] Underlined words are in Spanish if not otherwise marked.

BACKGROUND AND METHODS

Ethnographic background

The Zapotecs are the most numerous group in the state of Oaxaca (ca. 350 000). Historically, the Zapotecs settled in the highland Valley of Oaxaca where the archaeological sites of Monte Alban and Mitla give evidence of the advanced Zapotec civilization. In the middle of the 14th century some groups moved to the Isthmus of Tehuantepec, forced by Aztec and Mixtec invasion ([13]), settling until today in communities and affiliated ranchos (seasonal occupied settlements). This geographical dislocation was one of the major causes of different cultural and linguistic development among Istmo Sierra Zapotec of the foothills of the Sierra Madre de Oaxaca as compared to the highland groups. 1% to 5% of all inhabitants older than 5 years are Zapotec monolinguals, 50% to 70% are bilingual Indians and there are a considerable number of mestizos (ladinoized Zapotecs) in some parts ([14]).

The neighboring Mixe are the fourth numerous group (ca. 70 000) among the 15 indigenous groups in the state of Oaxaca. They spread over the central region of the state populating a well-defined area, the so-called distrito Mixe in the humid and cold mountains of the Sierra de Juarez. Only one municipio (subdistrict) is situated in the subtropical/tropical lowland of the Isthmus de Tehuantepec. Under

---

[13] Campell, H., Bindford, L., Bartolome, M., and Barabas, A. 1993. *Zapotec Struggles.* Smithsonian Institution Press, Washington, London, UK. pp. 285.

[14] INEGI 1993. *Region Istmo, Oaxaca, Perfil Sociodemografico, XI Censo General de Población y Vivienda, 1990.* Instituto Nacional de Estadística, Geografia Informática, Aguascalientes, Mexico. (In Spanish).

Spanish reign lowland Mixe were resettled into one central community called San Juan Guichicovi (Guichicovi = new village [in Zapotec]). A large proportion of the inhabitants live permanently in the cabecera (main village). Today more than 20% of all inhabitants older than 5 years are Mixe monolinguals, 75% are bilingual Indians and only less than 5% are mestizos ([15]).

Mixe and Zapotec subsistence is based on shifting and seasonal cultivation (corn), cash cropping (coffee, citrus fruits), gathering and wage labor. Today many members of both groups have migrated or have seasonal jobs in other parts of Mexico or as undocumented workers across the US border. Furthermore, the production of the Isthmus Zapotec-style women's blouses, the huipils (which are today part of the Mixe dress too) by the Mixe and the wide-spread cultivation of achiote (*Bixa orellana* L., Bixaceae) by the Zapotecs, provide additional income for both of these indigenous groups.

Ethnobotanical methods and evaluation

The data presented on San Juan Guichicovi, the Mixe community, were collected from November 1985 to March 1986 and during several short stays since then ([16, 17, 18]). The data from the Zapotec communities - Santo Domingo Petapa and

---

15 Unpublished data of Mexican Government's agencies.

16 Heinrich, M. 1989. *Ethnobotanik der Tieflandmixe (Oaxaca, Mexico) und phytochemische Untersuchung von Capraria biflora L. (Scrophulariaceae)*. Dissertationes Botanicae No. 144. J. Cramer, Berlin und Stuttgart, Germany. (In German).

17 Heinrich, M. and Antonio B., N. 1993. Medicinal plants in a lowland Mixe Indian community

Santa María Petapa - were collected from January 1992 to March 1993 and in October and November 1994 ([19]). Both are based on open and structured interviews with local specialists such as traditional healers, herbalists and midwives. Additionally, observation of and participation in their daily work (plant collection, preparation and healing sessions) were made, in order to understand as fully as possible the classification of plants, their use, and the traditional way of conceptualizing and reasoning in indigenous cosmic vision ([20, 21]). To collect plant material, excursions were made with the informants to the different vegetation zones of the subdistrict. For each plant, detailed documentation on the area of collection, uses, preparation, application and healing concepts were obtained. Voucher specimens were collected, identified and complete sets have been deposited in the following herbaria: Mexico D. F., UNAM Mexico D. F. (MEXU), Institute of Pharmaceutical Biology, Freiburg, Germany (collections *Heinrich and Antonio 1-320* and *Frei 1-554*) and ETH, Zurich, Switzerland (ZT; only *Frei 1-554*).

(Oaxaca, Mexico): Management of important resources. *Angew. Bot. 67*,141-144.

18 Heinrich, M., Rimpler, H. and Antonio Barrera N. 1992. Indigenous phytotherapy of gastrointestinal disorders in a lowland Mixe community (Oaxaca, Mexico): Etnopharmacological evaluation. *J. of Ethnopharmacol. 36*, 63-80.

19 Frei, B. 1997. Medical Ethnobotany of the Isthmus-Sierra Zapotecs (Oaxaca, Mexico) and Biological-Phytochemical Investigation of Selected Medicinal Plants. Thesis. ETH. in preparation.

20 Heinrich, M. 1997. Indigenous concepts of medicinal plants: The example of the Lowland Mixe (Oaxaca, Mexico). *Ecol. Food Nutr.* in press.

21 Frei, B., Sticher, O., Viesca T., C., and Heinrich M. 1997. Medicinal and food plants: Zapotec criteria for selection. *Ecol. Food Nutr.* in press.

RESULTS

Vegetation Types

Both research areas are situated in the southern part of the state of Oaxaca, in the Isthmus of Tehuantepec and its foothills and lowlands of the Sierra Madre de Oaxaca (Figure 1-P3). The area under investigation includes a small plateau 200 m to 260 m above sea level in the area of Matías Romero, Santo Domingo Petapa and Santa María Petapa as well as the mountainous Sierra with elevations up to 1600 m above sea level. The accentuated relief with its changing altitudes determines the climate and the vegetation types. In a global view, based on classifications of Köppen ([22]), the climate is defined as the As type. This means a tropical climate, all months of the year show an average temperature above 18° C with one rainy season from June to September.

---

[22] Heyer, E. 1988. *Witterung und Klima.* 8. Auflage, BSB B.G. Teubner Verlagsgesellschaft, Leipzig, Germany. pp.58-67 (In German).

Figure 1-P3. General map of Mexico with the State of Oaxaca and the research area.

Several attempts to classify the vegetation of Mexico, including Oaxaca, have been made by Leopold in 1950 ([23]), by Miranda and Hernández in 1963 ([24]),

23 Leopold, A. 1950. Vegetation zones of Mexico. *Ecology 31*, 507-518.

24 Miranda, F., and Hernández, X. 1963. Los tipos de vegetación de México y su clasificación. *Bol. Soc. Bot. México 28*, 29-179. (In Spanish).

by Flores and co-workers in 1972 ([25]), by Rzedowski in 1978 ([26]), and the COTECOCA (Comisión Tecnico Consultiva para la Determinación Regional de los Coeficientes de Agostadero) in 1980 ([27]). Each of these has used physiognomic and floristic criteria to define communities or formations and, in addition, characteristics of climate, topography and soil have been included as a part of the overall vegetation description ([28]). All of these publications contain either inaccuracies due to extrapolation from topographical and ecological data or show too much generalization for Oaxaca's complex topography resulting in the mentioned microclimates and microhabitats. In Figure 2-P3 (see map in Appendix: Zapotec Area of the Isthmus Sierra: Research area) the vegetation types in the area of study (as of approximately 1990) are shown along the classification of Lorence and Mendoza ([28, 29]). They are based primarily on COTECOCA, Miranda

[25] Flores, M. G., Jiménez L., J., Madrigal S., X., Moncayo R., F., and Takaki T., F. 1972. *Mapa y descripciones de los tipos de vegetación de la República Mexicana.* Secretaría de Agricultura y Recursos Hidráulicos. México D.F., Mexico. (In Spanish).

[26] Rzedowski, J. 1978. *Vegetación de México.* Editorial Limusa, México D.F., Mexico. pp. 423. (In Spanish).

[27] COTECOCA 1980. *Oaxaca.* Impreso por las memorias de COTECOCA-SARH.V.1,2, with map of vegetation, scale of 1:500,000. pp. 295. Unpublished. (In Spanish).

[28] Lorence, D.H., and Mendoza, A. G. 1989. Oaxaca, Mexico. In: *Floristic Inventory of Tropical Countries.* Campbell D. G., and Hammond, H. D. (eds.). New York Botanical Garden, Bronx, New York, USA. pp. 253-269.

[29] This map includes the area of a medical-ethnobotanical study carried out among *four* Zapotec municipalities. The relevant data for this present evaluation refer to the Mixe municipality of San Juan Guichicovi and the Zapotec municipalities Santo Domingo Petapa and Santa María Petapa.

and Hernández, and Rzedowski, while modified to fit the UNESCO system ([30]). Therefore 12 original vegetation types are recognized to cope with Oaxaca's highly accentuated relief and the fact that the two major floristic kingdoms, the Holartic and the Neotropic, of the New World intersect in the area of study.

The original vegetation in the area is tropical ombrophilous forest in the humid lowlands to the east and north (violet color in Figure 2-P3; with *Vochysia hondurensis* Sprague, Vochysiaceae; *Swietenia macrophylla* King, Meliaceae; *Terminalia amazonia* L., Combretaceae), and drought deciduous lowland (and submontane) forest in the south (dark-green; with *Plumeria rubra* L., Apocynaceae; *Pithecellobium* sp., Mimosaceae; *Bursera* sp., Burseraceae). However most of the study area was originally covered by evergreen conifer and oak forest (light-green: with *Pinus oocarpa* Schiede and other *Pinus* spp., Pinaceae; *Quercus* spp., Fagaceae) as well as (sub-)-tropical evergreen, partly submontane (broad-leaved) seasonal forest types (light-pink; with *Manilkara zapota* (L.) van Royen, Sapotaceae; *Coccoloba barbadensis* Jacq., Polygonaceae; *Enterolobium cyclocarpum* (Jacq.) Griseb., Mimosaceae). In higher elevations tropical ombrophilous forests (broad-leaved cloud forests and montane forests) have replaced the above mentioned vegetation types (blue-green; with *Liquidambar* sp., Hamamelidaceae; *Podocarpus* sp., Podocarpaceae; *Hymenaea courbaril* L., Caesalpiniaceae) ([28]).

This primary vegetation has been modified by indigenous manipulation for at least 600 years. Since no archaeological studies have been conducted in the area, the occupation prior to the historical record is uncertain. Nevertheless,

[30] UNESCO 1974. Tentative physiognomic-ecological classification of plant formations of the earth (Revised from Ellenberg and Mueller-Dombois). In: *Aims and Methods of Vegetation Ecology*. Mueller-Dombois, D., and Ellenberg, H. (eds.). John Wiley & Sons, New York, USA.

several studies suggest that indigenous populations in the New World tropics were considerably larger than previously assumed and their agricultural and ecological management systems have been shown to be more sophisticated and productive than expected ([31]). Therefore the human impact in this region may have been much more intensive and long-lasting than previously assumed. Also the current vegetation is heavily influenced by the agricultural activities of the Mixe and Zapotec. The milpa (cornfield) provides the culturally most important crop, maize (*Zea mays* L., Poaceae), which is mostly produced with slash and burn agriculture, but along the rivers also by permanent forms of agriculture. Coffee (*Coffea* sp., Rubiaceae) was introduced into the area in the 1930s. It largely replaced the Zapotec cash crops vainilla (*Vanilla planifolia* Andr., Orchidaceae), añil (*Indigofera suffruticosa* Mill., Fabaceae), zarsaparilla (*Smilax medica* Schl., *Smilax* sp., Smilacaceae) ([32]) and cacao (*Theobroma cacao* L., Sterculiaceae) as well as the Mixe root products (presumably *Colocasia esculenta* (L.) Schott, Araceae and others, indet.), añil and a large number of minor products (mostly fruits like *Tamarindus indica* L., Mimosaceae).

The current vegetation types are accordingly heavily influenced by human impact and in the context of our research the question from which vegetation zone a medicinal plant comes from is of interest. For such a study the ecological concepts of the Zapotec and Mixe must be considered and are discussed below.

---

31 Posey, D. A. 1993. The importance of semi-domesticated species in post-contact Amazonia: Effects of the Kayapó Indians on the dispersal of flora and fauna. In: *Tropical Forests, People and Food*, Hladik, C. M., Hladik, A., Linares, O F., Pagezy, H., Semple, A., and Hadley, M. (eds.). Man and the Biosphere Series Vol. 13, UNESCO, Paris. pp. 63-71.

32 Brasseur, Ch. 1992. *Viaje por el Istmo de Tehuantepec 1859-1860*. Secretaria de Educación Publica, Fondo de Cultura Economica, Mexico D.F., Mexico. (In Spanish).

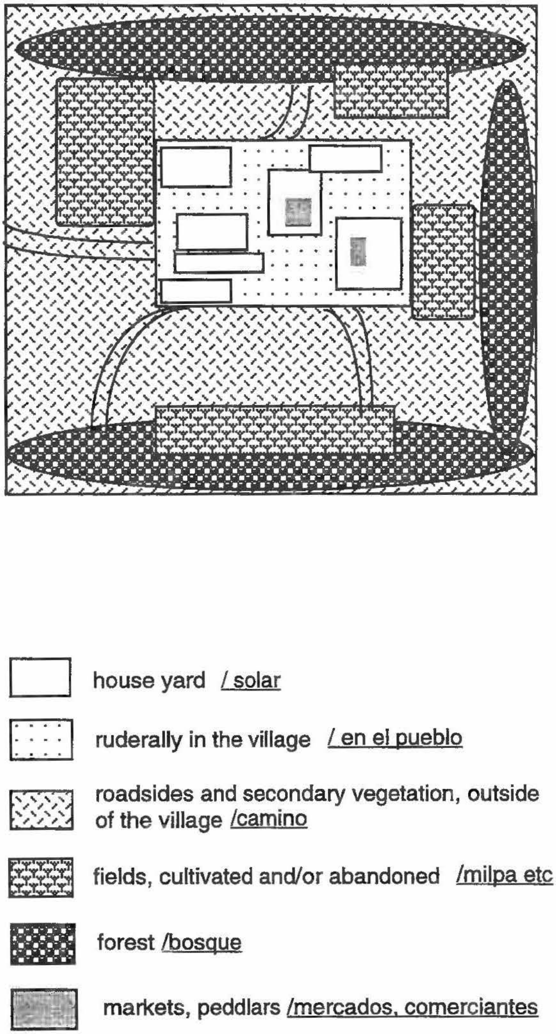


Figure 3-P3. Organization of the Mixe and Zapotec concept of the environment.

Table 1-P3: Medicinal plants in the indigenous ecological zones.

	Mixe			Zapotecs		
Indigenous ecological zones	number of species	%	cum. %**	number of species	%	cum. %**
House yard / solar	67	31.8	31.8	96	26.2	26.2
In the village / en el pueblo	28	13.3	45.0	48	13.1	39.3
Outskirts / camino, monte	56	26.5	71.6	73	19.9	59.2
Fields /milpa, cafetál, corral, potreros	18	8.5	80.1	57	15.5	74.7
Wood / bosque	33	15.6	95.7	72	19.6	94.3
Market, peddlers / mercado *	9	4.3	100.0	21	5.7	100.0
Total	211	100.0		367	100.0	

* not actually an ecological zone in the sense of an area, but necessary for the complete description of Mixe and Zapotec medicinal plant use. ** cumulative %.

Ecological zones according to indigenous criteria: indigenous ecological zones

Mixe and Zapotec perceive their environment by differentiating several zones, although these ecological habitats do not necessarily correspond to western scientific systems of classification (Figure 4-P3). This indigenous classification is based on two major criteria:

- a) on the distance from the house, the centre of the daily life and the family and

- b) on the type of management applied to the respective area.

Six different zones are distinguished (Figure 3-P3).

Solar

The central and most important area according to the indigenous concept of the environment is the house yard (solar). This is the meeting place of daily life, of the family and the major sphere of activity of the healers. Housework like processing corn, drying and roasting coffee, as well as breeding animals and commercial activities take place here. It is the most important and most intensively managed zone. Usually a few (two to five) trees, either planted or spared and protected when spontaneously grown, are observed with multiple function. While giving shade and demarcating the yard, they also are sources of fresh food and medicine at special times of the year. In Mixe as well as in Zapotec yards the following species are found: *Annona* spp., Annonaceae; *Citrus* spp., Rutaceae; *Crescentia alata* Kunth, Bignoniaceae; *Terminalia catappa* L., Combretaceae; *Tamarindus indica* L., Caesalpiniaceae, and *Ficus incipida* Willd., Moraceae. Shrubs and herbs are also planted. These serve for example as ornamentals or as medicinal sources. These plants often are transplanted from zones too far away for convenient usage (e.g. plants from the tropical omphrophilous forests) or from other regions of Mexico. They receive special attention and care. The most frequent plants in both Mixe and Zapotec yards are: *Aloe barbadensis* Miller, Aloeaceae; *Ocimum basilicum* L., Lamiaceae; *Piper* spp., Piperaceae; *Chenopodium ambrosioides* L., Chenopodiaceae; *Jatropha curcas* L., Euphorbiaceae; *Kalanchoe pinnata* (Lam.) Persoon, Crassulaceae. 31.8% or 67 species in Mixe yards and 26.2% or 96 species in Zapotec yards belong to this first zone (Table 1-P3). Mixe cultivate a larger percentage (+5.6%) of medicinal plants in their private gardens than the Zapotecs.

<u>En el pueblo</u>

Plants growing outside of yards, along streets and streams inside the borderlines of the village belong to the second zone. Some of these 'good' or 'bad' non-crops ([33]) growing on poor soils in the community <u>(en el pueblo)</u> are esteemed for medicinal purposes but also as fodder for animals. Little attention is paid to these plants since they grow abundantly without special care and are only removed twice a year on the official days of the collective cleaning of the villages. In one case, *Tournefortia densiflora* Mart. & Gal., Boraginaceae, seeded by a Zapotec healer in his community, intentional introduction of plants which grow too far away for convenient usage, was observed. 28 medicinal species (13.3%) in

[33] Chacón, J. C., and Gliessman, S. R. 1982. Use of the "non-weed" concept in traditional tropical agroecosystems of South-Eastern Mexico. *Agro-Ecosys. 8*, 1-11.

Mixe and 48 (13.1%) in Zapotec communities grow in these open spaces (Table 1-P3). Examples are mostly herbs and little shrubs such as *Heliotropium indicum* L., Boraginaceae; *Hyptis verticillata* Jacq., Lamiaceae; *Sida* spp., Malvaceae; *Melochia* spp., Sterculiaceae; *Rauwolfia tetraphylla* L., Apocynaceae; *Capraria biflora* L., Scrophulariaceae, and *Plumeria rubra* L., Apocynaceae. Since the plants in this group, and plants in the solar are prone to be eaten and contaminated by animals, some healers prefer to have them in special medicinal gardens inside of their yards (Figure 3-P3).

Camino

The second most important zone with 56 species (26.5%) in Mixe and 73 species (19.9%) in Zapotec areas (Table 1-P3) are the immediate surroundings of the communities. Plants are found along the paths or roads (camino) which are leading out of the village to the fields, the rivers or to neighbouring communities, or in places where no cultivation is possible like gorges and areas where firewood is collected. Trees, shrubs, especially climbers and to a lesser degree herbs, are collected in this zone (e. g. *Tithonia diversifolia* (Hemsl.) Gray, Asteraceae; *Guazuma ulmifolia* Lam., Sterculiaceae; *Thevetia* spp., Apocynaceae; *Malvaviscus arboreus* Cav., Malvaceae; *Gonolobus* spp., Asclepiadaceae; *Croton* spp., Euphorbiaceae, and *Xanthosoma robustum* Schott, Araceae). This area, with the exception of the sides of the main roads, is considered to be better for collecting clean plants. Healers believe that these "wilder" plants have more healing power than cultivated plant material. Since this area is common property, everybody is allowed to gather plants. Nevertheless, places of rare species are well known among the healers and are spared when the area is cleaned. Non-healers consider this zone of lesser importance whereas medical specialists manipulate the vegetation intentionally and influence the structure of this zone.

Milpa, corral, potrero, cafetál

Fields and forests with nomadic or shifting cultivation, pasture land and the coffee plantations (milpa, corral, potrero, and cafetál,) form a fourth, rather heterogeneous zone. These areas are also important for collecting timber and foods. This group is conceptionally further differentiated by our informants because on one hand, it includes the sacred ground of the milpa, where above all corn (the most important crop for the Zapotecs and Mixe since ancient times) grows. On the other hand, it includes zones for animals. Since the differentiation was especially made for cultivates and yielded only 18 and 57 medicinal plants (8.5% and 15.5%), respectively for the Mixe and Zapotec, these data were combined into one group. Only the owner of a plot of land or members of the ejido ([34]), who currently plant crops there are allowed to collect plants. These areas often are hours away from the village and therefore many of these plants are additionally brought into the house yards. *Poiretia punctata* (Willd.) Dev., Fabaceae; *Zebrina pendula* Schnizl., Commelinaceae; *Annona* spp., Annonaceae; *Quercus* spp., Fagaceae but also crop and medicinal plants like *Theobroma cacao* L., Sterculiaceae; *Coffea* sp.,

---

34 The ejido system is a product of post-revolutionary land reforms. Land under the law of ejido tenure, was until recently a non-negotiable resource. Ejidatarios were granted a plot which was neither sold, transferred nor mortgaged and subject to confiscation if left for more than two years.

Rubiaceae; *Musa* spp., Musaceae, and *Cucurbita pepo* L., Cucurbitaceae, are found there.

Bosque, montañas

Directly, sometimes difficult to distinguish from the previous area is the last zone, the managed and unmanaged forest (bosque) or the mountains (montañas). It includes "primary" and secondary vegetation of forest with trees up to 30 m and more in height, in gorges or on steep slopes. For the Zapotecs this zone is more important (72 species; 19.6%) than for Mixe healers (33 species; 15.6%). Plants in this area which is not managed intensively include *Dioscorea* spp., Dioscoreaceae; *Piper* spp., Piperaceae; *Psidium* spp., Myrtaceae; *Pinus oocarpa* Schiede, Pinaceae; *Critonia quadrangularis* (DC.) R. M. King & H. Rob. (syn. *Eupatorium quadrangulare* DC.), Asteraceae; *Begonia heracleifolia* Schltdl. & Cham., Begoniaceae, and *Siparuna andina* (Tul.) A.DC., Monimiaceae.

Mercado, comerciantes

The markets (mercados) and peddlers (comerciantes) are not actually an ecological zone in the sense of an area, but are necessary for the complete description of Mixe and Zapotec medicinal plant use. Only a few apecies with medicinal purposes like *Matricaria recutita* L., Asteraceae; *Capsicum* spp., Solanaceae, or *Cinnamomum ceylanicum* Sw., Lauraceae, are sold in the small shops in the villages. A larger supply is available in the nearby cities of Matías

Romero, Juchitán, or Tehuantepec (one to three hours away by bus) or farther away in the state capital Oaxaca (a one day journey). Almost every week peddlers pass by the villages selling all kind of things including medicinal plants. The plants are grown all over Mexico, purchased on the Sonora market of Mexico City and redistributed in the country ([35]). Nine species (4.3%) and 21 species (5.7%) were recorded for the Mixe and Zapotec, respectively.

35 Heinrich, M., Antonio Barrera N., and Kuhnt, M. 1992. Arzneipflanzen in Mexiko: Der Markt von Matías Romero. *Dtsch. Apoth. Ztg. 132/8*, 351-358. (In German).

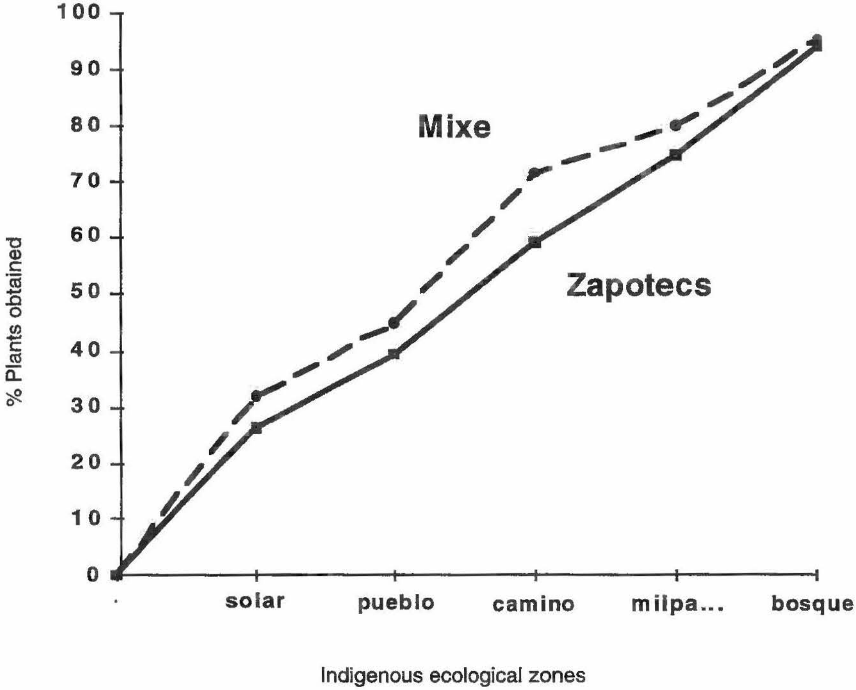


Figure 4-P3. Cumulative % of obtained plants from the indigenous ecological zones.

A Statistical Analysis of the Species Numbers in the Ecological Zones

A statistical comparison of the results presented in Table 1-P3 and Figure 4-P3, shows that the Mixe collect significantly more species in the first three zones closer to the house [solar, pueblo, and camino, Mixe = 151 plants and Zapotecs = 217 plants; P-value = 0.0051, χ^2=7.839 ([36])] as compared to the Zapotec. On the other hand, the Zapotecs prefer plants from the zones which are farther away (milpa, cafetál, etc. and bosque, Zapotecs = 129 plants and Mixe = 51 plants). By comparing the five individual zones for general independence ([37]), the data shows a significantly different pattern of preference for collection by the two indigenous groups (P-value = 0.036, χ^2= 10.3). A comparison of the number of species used as medicinals with the total number of species growing in each indigenously defined zone is not possible (although desirable), since no data on the total numbers are available. The sixth "zone" (mercado, comerciantes) was not included in the statistical analysis.

DISCUSSION

The Mixe and Zapotec communities under investigation are located in an area of great botanical diversity. While the indigenous inhabitants categorize their environment into at least six ecological zones (for comparative purposes some groups, such as milpa, cafetál etc., were reduced to a single group), based on aspects of distance and the type of management applied, western natural science classifies it into 12 different, non-corresponding, vegetation zones referring to the plant species found at a specific location. Westerners categorize plants as wild,

---

[36] Pearson's χ^2 test for 2x2-tables with Yates' continuity correction, whereas the *first three* and the *last two* indigenous ecological zones (see Table 1) were combined into two groups (first group: „close to the house"; second group: „faraway from the house").

[37] Pearson's χ^2 test without Yates' continuity correction with the data of *five* (not combined) indigenous ecological zones.

domesticated, or weed, while indigenous people view vegetation primarily as resources ([38]). Through management, selection or adaptation the flora has been manipulated to create habitable zones for their needs. From different zones a variety of plants for daily subsistence and also in response to illness is obtained. It is noteworthy that both the Zapotec and the Mixe obtain most (59.2% and 71.6%, respectively) of their medico-botanical resources from their immediate environment. These phytotherapeutic preparations are used to treat the common illnesses of the region ([18, 19]). The comparatively lower number of medicinally important species collected from the secondary and primary forest vegetation contradicts the common popular assumption that these vegetation types are the principal sources of indigenously used medicinal plants. Recently, Voeks ([39]) reported on the relative importance of primary as compared to secondary forest vegetation in Bahia, Brazil. While primary vegetation is essential for obtaining timber, the secondary forests yielded a much larger number of medicinal plants. Since this author did not look at species from non-forest vegetation zones, a direct comparison is not possible. Some of the species grown near the house originate from these forest habitats, but because they have been regarded as a useful remedy, they have been brought to the solar of the healer or its corral and grown there. Having easy access to the resources is therefore a factor with much influence on the diversity of the zones closer to the house. Comerford ([40]) showed in the Petén region in Guatemala that regrown forests and intensively managed zones are more important for medicinal plant gathering and therefore traditional

---

38 Alcorn, J. B. 1981. Huastec noncrop resource management: Implications for the prehistoric rain forest management. *Human Ecol. 9*, 395-417.

39 Voeks, R. A. 1996. Tropical forest healers and habitat preference. *Econ. Bot. 50*, 381-400.

40 Comerford, S. C. 1996. Medicnal plants of two Mayan Healers from San Andrés, Petén, Guatemala. *Econ. Bot. 50*. 327-336.

medicine would not be seriously threatened by loss of primary forests. This conclusion is only of relevance with respect to the plant's immediate importance, because plant selection for medicinal use is an on-going process. Next to old knowledge, new findings by traditional healers are endangered as well. Future higher population densities will disturb and manipulate as well as explore the primary forests of today. Therefore these forests are invaluable not only for medicine, but also for traditional societies such as the Mixe and Zapotecs, whose subsistence activities still rely on renewable local resources.

Historical developments and the cultural background determine the pattern of settlement and are therefore factors to be taken into consideration. While Mixe live concentrated in the cabecera [principal community San Juan Guichicovi, and other populated places: El Ocotal, El Chocolate, and Río Pachiñe; (Figure 2-P3; Appendix)], the Zapotec settlements are widely dispersed over their territory (Figure 2-P3, Appendix: populated places marked in green and blue). Zapotecs have more and faster access to different vegetation zones and the plant diversity is exploited in a greater variety. Mixe have a much larger proportion of plants in cultivation in their private yards in order to have the plants at hand when needed. This seems to be one of the major reasons for the different patterns of collecting plants in the various indigenous ecological zones (Table 1-P3). Whether there are other socio-cultural reasons for these different preferences remains to be elucidated in subsequent studies.

This article also raises several methodological questions. While ethnobotanists and ecologists usually look at one scientifically defined vegetation zone (especially forests), this article shows the utility of an approach based on the indigenous concept of their environment and what resources are collected in which part of the environment. The approach thus relies more heavily on a botanico-anthropological method as compared for example to the mere enumeration of useful species in one zone. Consequently, the natives' perspective of the environment is more central to our approach as to other ones. The scientific classification (Figure 2-P3; Appendix) is based on the structure of the vegetation, while the indigenous one is based on the uses the area has. These botanico-anthropological data in combination with the scientific classification are an useful empirical basis for the further development of the area.

Simultaneously, we are interested in the empirical basis of the Zapotec plant use and have conducted several studies on the efficacy and safety of Zapotec and Mixe herbal remedies ([41, 42,43, 44]). The approach may accordingly provide the basis for small plantations producing medicinal plants for regional use. After a systematic evaluation it may provide remote areas with inexpensive therapeutics and small scale additional income. Ethnoecology and medicinal ethnobotany in particular are therefore important links between tradition and modernization on the one hand and sustainable management, conservation and local development on the other.

---

[41] Kato, T., Frei, B., Heinrich, M. and Sticher, O. 1996. Antibacterial hydroperoxysterols from *Xanthosoma robustum*. *Phytochemistry 41*, 1191-1195.

[42] Frei, B., Heinrich, M., Bork P. M., Hermann, D., Jaki, B., Kato. T., Kuhnt, M., Schmitt, J. , Schühly, W., Volken, C., and Sticher, O. 1997. Multiple screening of medicinal plants from Oaxaca, Mexico: Ethnobotany and bioassays as a basis for phytochemical investigation. *Phytomedicine.* submitted.

[43] Bork, P., Schmitz, M. L., Weimann, C., Kist, M., and Heinrich, M. 1996. Nahua Indian medicinal plants (Mexico): Inhibitory activiy on NF-kB as an anti-inflammatory model and antibacterial effects. *Phytomed. 3*, 263-269.

[44] Bork, M. P., Schmitz, M., Kunth, M., Escher, C., and Heinrich, M. 1997. Sesquiterpene lactone containing Mexican Indian medicinal plants and pure sesquiterpene lactones as potent inhibitors of transcription factor NF-kB. *FEBS Letters 402*, 85-90.

The authors are very grateful to the healers, midwives and the inhabitants of the villages of the research area. We would like to thank Dr. P. Davila A.; O. Tellez. V.; L. Torres C.; R. Torres C.; F. Ramos M.; Dr. R. Lira S. and Dr. J.-L. Villaseñor R. at the National Herbarium (MEXU); Dr. R. A. Bye Jr. and T. Balcazar S. at the Botanical Garden; Dr. C. Viesca T. at the Institute of Medical History and Anthropology; all at the Universidad Nacional Autónoma de México UNAM; Prof. Dr. H. Rimpler, Institute of Pharmaceutical Biology, Freiburg/Germany and Dr. M. Baltisberger, Geobotany, ETH Zurich for their help at various stages of the project. We like to thank Prof. Dr. E. Spiess and his staff, Department of Cartography, ETH Zurich for the support during the elaboration of the map of the research area. Thanks are due to C. J. Luchsinger, Institute of Mathematics, University of Zurich, Switzerland for statistical support. This work would not have been possible without the financial support by the Swiss Agency for Development Cooperation (SDC, Bern). And last but not least, many thanks are due to David McLaughlin for checking the English usage in this paper.

3 Additional Results and Discussion

In the course of Section 1, field data on medicinal ethnobotany were evaluated. Including all the anthropological/ethnological information on Zapotec life would have gone beyond the scope of this first part of the thesis. Nevertheless, while discussing the Publications I to III and aspects presented in a poster at a congress (Title see Appendix), some additional results will enter these following paragraphs.

3.1 Geographical and historical-ethnological background

The original intention to spend an equal amount of time for research in all four historically related communities, north and south of the dividing mountain ranges, soon had to be abandoned. It was not possible to conduct "complete" research in both regions in the available time. The majority of time on research was spent in the communities of SDP and SMP and their ranchos. The Zapotec language used in the publications (I to III) is the one spoken in these two villages, although some divergence is found already here. For the same reason when comparing data with results from the neighbouring Mixe community of San Juan Guichicovi (Publication III), the language of the same two locations is cited.

When discussing results from GdH and SMG, they refer only to plants in and of the surroundings (up to 1h walking time) of the cabecera. Unfortunately, the political situation was too unstable at that time to visit their ranchos. I decided it too risky to visit the ranchos, after being for a short time in these two communities. Repeated presence of the army was due to the unstable political situation caused by coming regional and national elections (for detailed description of the political situation in the Isthmus of Tehuantepec, see Campbell, 1993 and 1994) and due to frequent assaults on buses and trucks. In addition, military expeditions into the surrounding area of the ranchos took place to destroy hidden marihuana and opium fields.

An initial goal of the present study was to compare the plant uses among the southern (GdH, SMG) and the northern communities (SDP, SMP). Its aim was to evaluate possible correlations in medicinal plant use in order to get information whether people with the same cultural background, but living in slightly differing vegetation zones, have selected the same plants for curing purposes over time. The comparison of plant uses and of Zapotec plant names between the historically related communities showed high correlation and small linguistic variations. Since the community borders of SMG and GdH also include a large area of the dry vegetation zone of bosque tropical caducifolio (Appendix), 78 additional plants (17.5 % of a total of the 445 taxa used in all four communities) are only used in their native "pharmacopoeia". The principal 367 plants (82.5 %) show many similar uses and modes of application throughout the four communities. As for the linguistic studies, while people from SDP say *guixa* for leaf, a Guveano uses the shorter word *guix*, trees are called *yaga* in SDP and *yag* in GdH. These illustrating examples are part of a larger (probably uncompleted; reasons see above) compilation of ethnobotanical-linguistic data, which are not further discussed in this thesis. There seems to be still a lot of potential for future research and evaluation.

3.2 Methodology

The anthropological/ethnological and botanical data presented in Section 1 were collected during a total of 17 months of field work. In order to study the botanical and cultural aspects in the course of a whole year, the first period of field work was a 15-months stay. In addition, 1 1/2 years later, another 2 months were spent in the field in order to obtain additional field data and for collecting plant material for biological-phytochemical investigation (see Section 2 and 3). The initial method of gathering information was to talk with local people, to observe, and participate in the everyday activities. Several anthropological field techniques where applied in the study.

-"Participant observation" refers to living with people by sharing, learning and observing many parts of their daily life, from subsistence activities (farming, gathering, cooking, hunting) to ritual occasions (healing sessions, religious celebrations, marriages, funerals).

-In "open-ended" or "semi-structured" surveys some general questions, prepared in advance, about plants, myths or cosmology, lead into an open conversation with spontaneously arising questions (informal or qualitative methods).

-"Structured interviews" refers to asking a selected group of people to respond to the same set of questions (formal or quantitative method). The last approach yields the most complete set for statistical evaluation (Cotton, 1996).

In a first approach, in participant observation and with open-ended and structured interviews, local specialists in traditional medicine (Table 1-S1) took place in the healer's house or garden, "in the field" or while visiting patients.

Table 1-S1. Specialization of Zapotec traditional healers.

Specialists (in Spanish)	**Specialization**	**Gender**
partera/o	midwifery, illnesses of children and women	female healer curing women's "illnesses", in rare cases also men assist at birth
curandero/a	susto, verguenza, cansancio, daño de la comida/empacho, golpe, aire (culture-bound syndromes) (see Publication I)	female and male practitioners
culebrero	snake bites	male healers, because they are more confronted with it while working in the fields and woods
rezador	leading prayers during ritual occasions	men and to a lesser extent women
huesero	curing fractures and strains	male healers
hierbera/o	specialist in plant remedies, selling plants, performing only few ritual ceremonies	women and men as healers
sobador/a	performing massages	women and men as practitioners
masajista	performing massage treatment, sometimes accompanied by ritual ceremonies	male healers

While accompanying the healers, they were questioned about the following ethnomedical, ethnobotanical, and demographic issues:

-medicinal plant use, collection, preparation, and application (Publication I to III, Poster I)

-classification of plants, illnesses and the environment (Publication I and III, Table 2-S1-S1)

-healing practices (Table 2-S1-S1)

-illnesses most frequently treated; important folk illnesses (culture-bound syndromes), (Table 2-S1-S1)

-"objects" used while curing and/or handed to the patient for further curing at home (Table 2-S1-S1)

-beliefs in supernatural powers (Table 2-S1-S1)

-places to perform ritual ceremonies (Table 2-S1-S1)

-symbols (sacred places, persons and objects, (Table 2-S1-S1))

-mode of "payment" for a cure

-relationship between food and medicinal plants (Publication I and III)

-story of becoming a traditional healer

-existing networks between healers

-socio-demographic profile: sex, age, place of birth, languages spoken, education, other professions, number of persons in the household

Table 2-S1. Zapotec ethnomedical and ethnobotanical characteristics.

Ethnomedical and ethnobotanical aspects	**Explanations from Zapotec healers**
Classification of medicinal plants	-hot/cold and in general dichotomial systems -analogies -taste and smell criteria (of inferior importance)
Important folk illnesses (culture bound syndromes)	-susto -verguenza -cansancio -encono -daño de la comida/empacho -golpe: all of these illnesses are weakening / unbalancing the body and -aire can then enter it
Beliefs in supernatural powers	-naguales (each person is living as one or several animal(s)/soul(s) in the surroundings of the village, in the forest, or closer mountains) -Catholic saints (also corresponding to Pre-Columbian gods)
Places for ritual ceremonies	-family altars in the private house -sacred caves -sacred mountains -springs -crossings of rivers and ways -little templos (churches) for special Saints in the village, affiliated with a private house -main church (important on Easter and All Saints Day, church patrons celebration and for madrina de vela-rituals)

Table 2-S1: continued.

Ethnomedical and ethnobotanical aspects	**Explanations from Zapotec healers**
Symbols (sacred places, persons and objects)	-water -3 blue-green crosses ("water crossing the earth", in chapel close to the springs, "ojo de agua") -Catholic saints -selected plants (see Publication I) -holy days of the calendar (performed by a healer with Zoque background, Mixe/Zoque origin?)
Objects used during healing ceremonies	-plants (fresh, dried or as drugs in creams, alcohol, soaps, syrups, drops, teas, bath, shampoos; ashes) -animals and animal related objects (snake's skin, eggs and shell, armadillo's shell, chicken, frog, scorpions in alcohol, goat's milk, cattle's seburn, bee's wax, deer horns, grasshoppers, crickets) -candles, -alcohol, -minerals
Objects used in amulets	-plants, especially seeds -animal-derived objects (bone, powder, bees wax) -other objects borrowed from other cultures (figure of Buddha, hand of Fatima, horseshoe, etc.)
Practices during ritual healing	-"diagnosis": asking a few questions, checking eyes, pulse and tension of muscles, feeling skin temperature -limpia -egg used in the limpia is dropped in a glass of water and reasons for the illness are "explained" -prayers, murmured or sung, -massages
Corn oracle	-not known among Zapotecs (performed by one healer with Zoque origin)
Rituals with hallucinogenic plants and mushrooms	-rarely performed,selling of ololiuqui seeds (*Rivea corymbosa* (L.) Hall. f. witnessed to Mixe neighbours

For every plant mentioned during the observations, conversations, and interviews a record with the plant's ethnobotanical profile was created and completed during further interviews (Table 3-S1). It was possible to complete 554 records, whereas 445 different species were identified by their scientific botanical names. In a second approach, five key informants were interviewed about the indigenous classification of 300 medicinal plants collected so far (Table 4-S1). These data were used as a basis for the Publications I and II.

Table 3-S1. Ethnobotanical plant-profile-record. *Names in italics* = English translation.

Nombre(s) popular(es): *Popular name(s)*	Nombre científico: *Scientific name*	Numero: *Number* FREI ..... Herbarium: *Collection*
Informador(es), profesión: *Informant(s), profession(s)*	Familia: *Botanical family*	Semilla, fruta: *seed, fruit* Hojas: *Leaf*
Usos, enfermedades: *Uses, illnesses*	Recolección: *Recollection* Identificación: *Identification*	Raíz: *Root* Foto: *Picture*
Partes utilisadas: *Used plant parts*	Utilización diferente que en el lugar del entrevista: *Uses in other locations*	
Preparación: *Preparation*	Descripción: *Description of the plant (by the ethnobotanist)*	
Dosis, forma, duración de la aplicación: *Doses, form and duration of application*	Habitat (tipo de vegetación, geografia etc.): *Conditions of growth (vegetation type, geography etc.)*	
Efecto: *Effects*	Observaciónes: *Observations*	
Efecto secundario, contra-indicación: *Side effect, contra-indication*	Importancia: *Cultural importance of the plant* I .... uso observado *uses observed*	
Classificación: *Classification*	II .... se usa *is used* III .... es posible usarla *it could be used*	
Descripción de la planta según el informador: *Description of the plant by the informant*	IV .... ningún uso medicinal *no medicinal uses*	
Importancia por el informador: *Importance of the plant for the informant*		

Table 4-S1. Questionnaire for the evaluation of indigenous plant classification and cultural importance of a plant. *Names in italics* = English translation.

<table>
<tr><td colspan="8">Importancia y classificación de las plantas en la medicina Zapoteca
Cultural importance and classification of medicinal plants in the Zapotec medicinal system</td></tr>
<tr><td colspan="4">Nombre(s) popular(es): Popular names</td><td colspan="4">Numero: number
FREI</td></tr>
<tr><td>Importancia
Importance</td><td>Informador
Informant</td><td>Curandero
Healer</td><td>Fecha
Date</td><td>Uso
Uses</td><td>Partes utilisadas
used plant parts</td><td>frio caliente
hot-cold</td><td>otros nombres
other names</td></tr>
<tr><td>I
uso observado
uses observed</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>II
se usa
is used</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>III
es posible usarla
it could be used</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>IV
ningún uso medicinal
no medicinal uses</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
</table>

In a later period of the field work a large part of the population in the villages of SDP and SMP was interviewed. 140 people (farmers, housewives, children, old people) in 98 households were confronted with a structured interview (see Appendix: Questionnaire). This questionnaire provided among other information:

-home remedies used in the treatment of ten important illnesses of the region

-popular uses of 16 common medicinal plants (specimens were shown to the informants)

-socio-demographic information about the interview partners.

Results on popular use, treatment and beliefs about the medicinal plants are partly described in Publication I to III. Based on the data collected through both questionnaires, a medicinal plant garden with 25 important plant species was established on the grounds of the escuela bilingue (bilingual primary school) at El Barrio de Santa Cruz of Santo Domingo Petapa (List of plants, see Appendix). The same information will also be integrated in future evaluations for a local plant booklet (folleto).

The excursions to collect plant material were usually made with the key informants. In some other cases, relatives of the informants were accompanied on trips to the mountains and forests or women were accompanied while collecting firewood. If no key informant was leading the excursion, the collected material was later sorted and identified by them. Five examples of every plant species was then pressed on the same day collected, dried in a secadora (field dryer; Figure 2-S1) over two light bulbs at a maximum temperature of 40° C. Voucher specimens were labelled with detailed documentation in Spanish and German (Figure 3-S1). One set of voucher specimens was prepared with pupils of the escuela secundaria

(College) of SDP while introducing them to ethnobotany during several classes in botany. The plants were donated to the school for teaching matters. More sets are deposited at SERBO's (Sociedad para el Estudio de los Recursos Bióticos de Oaxaca, A.C.) in Oaxaca, at the INI (Instituto Nacional Indigenista) in Oaxaca, at the Herbario Nacional MEXU de la UNAM (National Herbarium of Mexico D.F., National Autonomous University of Mexico D.F.), at the Institute of Pharmaceutical Biology of the Albert-Ludwigs-University of Freiburg/Germany, and at the Herbarium of ETH Zurich/Switzerland. Some fragments (fruits, seeds, tubers only, or examples purchased from peddlers or at markets and from a healer's home pharmacy) are deposited in the fragment collection of the Jardín Botanico de la UNAM (Botanical Garden, National Autonomous University of Mexico D.F.).

Figure 2-S1. A wooden frame, constructed by a local carpenter, covered with a lona (tarpaulin) at the bottom with two light bulbs, was used as a secadora (field dryer) for drying the plants in the plant presses or in cotton bags.

PLANTAS MEDICINALES DE LA ZONA ZAPOTECA DEL ISTMO DE TEHUANTEPEC, MEXICO
HEILPFLANZEN AUS DER ZAPOTEKISCHEN REGION DES ISTHMUS VON TEHUANTEPEC, MEXICO

Familia/Familie: ....Numero/Nummer: FREI ....
Nombre latino/Latein. Name: ....
Nombre(s) popular(es)/Populärname(n):.........................(español)................(zapoteco)
Uso/Verwendung:...
Partes utilisadas/Verwendete Pflanzenteile: ....
Habitat/Wuchsform: ....
Lugar de recoleccción/Fundort: ....
Habitat/Standort: ....
Observaciones/Bemerkungen: ....
Herbario/Herbar: la comunidad, MEXU, FB, TZ, JB

Recolector y fecha/Einlegung und Datum: ....
Identificador y fecha/Wiss. Identifikation u. Datum: ....
Barbara FREI y/und Dr. M. Heinrich, Departement Pharmazie ETH ZH, 8057 Zürich, Suiza y/und Inst. f. pharm. Biol., Universität, 79104 Freiburg, R.F.A.

Figure 3-S1. Label for voucher specimens in Spanish and German

3.3 Evaluation of data

3.3.1 "Why is a plant medicinal for the Zapotecs? Why is a plant a food plant?"

All publications presented a focus on different aspects of medicinal ethnobotany. While Publication II evaluates medico-botanical-phytochemical, Publication III discusses ethno-ecological-conservational aspects. In Publication I anthropology/ ethnology is the central topic when discussing Zapotec perception, selection criteria and cosmovision. By exploring indigenous reasoning, its aim is to understand and describe the emic categorization, avoiding, as far as possible, the researcher's (the etic) interpretation. A central aspect in Publication I is therefore the question "Why is a plant medicinal for the Zapotecs? Why is a plant a food plant?" As culture is an ongoing, dynamic, changing and responding process, also traditional curing is permanently subject to development and improvement within a certain culture. Until a medicinal plant becomes part of the pharmacopoeia of a healer or of a whole culture it has to meet expectations: the plant must alleviate or influence the course of the illness. Plants which do not fulfill these expectations will be rejected. This process of accepting or rejecting is not just a "trial and error" situation, but requires an underlying cultural logic as stated by Brett (1992). Zapotec logic is expressed in the selection criteria and reasoning concerning medicinal and food plants. It makes no sense to list only plants and their uses, when the underlying cultural logic is not understood and passed on as well. Knowledge, expectations, beliefs about illness, what happens to one when afflicted with illness, etiology, and how one needs to be cared for when ill (Brett, 1992) are all aspects forming the Zapotec (or in general, a traditional) healing concept.

In Publication I (Table II) the six main causes of illnesses in Zapotec society are described. Whether these concepts picture the entire Zapotec belief system of illnesses and how much Spanish influence is found within these concepts can not be answered. So far, it was not possible to interpret comparative Zapotec data from Pre-Columbian documents. The results discussed here represent "the state of art" at time of the field work in 1992/3. Any further interpretation would be a speculative or a hypothesis to be verified in future field work.

3.3.2 Ranked lists

In Publication II the resulting ranked lists are based on the plant list as shown in the Appendix, which was created based on the information from the larger data set of the plant-profile-records (FREI 1-554). The systematic classification of the plant list was carried out in accordance with Frohne and Jensen (1992). Information on the mode of application, the location in which it is used, and a classification into ten categories of indigenous uses is listed. Classification on plant perceptions (see Publication I) and the relative importance of the plants (see Table 4-S1) was included as well as their Zapotec and Spanish names. The ranked lists are presented in an emic point of view of classification (first order = scientific plant names). Nevertheless the etic (Zapotec) point of view is the basis of the ten categories of indigenous uses (see discussion Publication II). The classification tries to respect as far as possible traditional healing concepts but some western medical influences could not be avoided due to my pharmaceutical background. As long as ethnobotanists of foreign cultures with training in western natural science conduct research in other societies, their influence on the interpretations of data will remain a fact for criticism on their work. As stated in the preface, the "individual best" or "truest" information may rather be recorded by a native ethnobotanist due to the deeper relationship to the language and the culture. The presented work is a contribution with no claim of completeness.

3.3.3 Category of indigenous uses - Part of plant used - Frequency of plant parts used

In a poster presentation for a botanical congress (Title see Appendix, Poster I) a further data evaluation was presented and will be discussed in the following, based on larger data sets (Table 5-S1, Table 6-S1, and Table 7-S1).

-Plant species were grouped by the botanical family and their category of indigenous uses (Table 5-S1).

-Plant species were grouped by their botanical family and part of the plant used (Table 6-S1) and

-a general ranking list of the frequency of plant parts used was carried out (Table 7-S1).

All of these evaluations receive more importance when compared with data from other ethnic groups. Moerman (1991, 1996) analyzed with a method of regression residuals, the distribution of medicinal species utilized by North American Indians in subclasses and families as well as in groups defined in terms of growth habit and life pattern. He verified an all-over, highly non-random distribution. Saying, that there is a correlation of cognitive selected plant species by indigenous people and the distribution of these plants within the plant families. A direct comparison to the evaluation in this work is not possible, due to the diffrent methodological approaches and the varying North American and Oaxacan flora. In order to get information about the proportional distribution of medicinal uses among the 110 different botanical families collected with Zapotec healers and to set up priorities for a special category of indigenous uses, the most relevant outcomes of this comparison will be discussed.

18 families, namely the Anacardiaceae, Apocynaceae, Asteraceae, Bignoniaceae, Boraginaceae, Caesalpiniaceae, Cucurbitaceae, Euphorbiaceae, Fabaceae, Lamiaceae, Malvaceae, Mimosaceae, Moraceae, Myrtaceae, Piperaceae, Poaceae Rutaceae, and Solanaceae showed 20 or more uses in different Zapotec categories of indigenous uses, and are therefore considered of special importance (Table 5-S1). Remarkable parallels can be found in the treatment of gastrointestinal complaints when compared to Mixe (Heinrich, 1989), Mayan (Ankli et al., 1996), and North American Indian uses (Moerman, 1991). Plant utilization within the botanical families of the Asteraceae and Lamiaceae show high priority in all of these studies. On the other hand, while the Zapotecs, Mixe, and Maya Indians mentioned Euphorbiaceae and Mimosaceae (two botanical families with broad distribution in the tropics), as important plants to cure dermatological problems, Moerman reports less uses among these families due to the different composition of the vegetation of North America. These comparisons give evidence of a high potential for interesting bioactivity to be found among these families. And Bye (1995) believes that the continued use of plants over time, as well as the cross-cultural acceptance of those effective plants by people with different ethnomedical concepts is a measure of the important value of vegetal remedies, showing that traditional medicinal plant use is not random. Such data evaluation can therefore be an additional hint "in the jungle of 445 different plant species" on an ethnobotanical approach for further phytochemical investigations.

Table 5-S1. Plant species grouped by the botanical family and their category of indigenous uses. sp. = species; other abbreviations see Appendix.

Botanical division and family	categories of indigenous uses										Σ uses	Σ sp.
	GH	RT	ST	SD	F/M	fmG	Oph	CO	FI	OU		
Mycophyta												
AURICULARIACEAE				1						1	2	1
Pteridophyta												
ADIANTACEAE				2					2		4	2
CYATHEACEAE	1										1	1
EQUISETACEAE	1				2	1		1	1		6	1
PTERIDACEAE				1		1					2	1
SCHIZAEACEAE				1							1	2
THELYPTERIDACEAE						1					1	1
Spermatophyta												
PINACEAE		1	1	1		1			1	1	6	1
Dicotyledonae												
ACANTHACEAE	1	1		1					2	1	6	1
AMARANTHACEAE	1									3	4	3
ANACARDIACEAE	2	3	1	4	3	3		1	1	3	21	5
ANNONACEAE	3	1	4	1	1	1			4	4	19	5
APIACEAE	2	2	1	1	1				2	1	10	3
APOCYNACEAE	2	5	1	8	1	3				3	23	9
ARISTOLOCHIACEAE	2		2	2	2	2		2	2		14	2
ASCLEPIADACEAE										2	2	2
ASTERACEAE	26	8	9	15	8	13	1		10	8	98	42
BASELLACEAE			3	2					3		8	2
BEGONIACEAE			1	1							2	1
BIGNONIACEAE	3	3	4	5		1			3	3	22	8
BIXACEAE	1		1	1	1	1				1	6	2
BOMBACACEAE	1			1		1			2	2	7	2
BORAGINACEAE	5	1	2	4	1	5	1	1	1	3	24	8

(Table 5-S1.:continued)

Botanical division and family	categories of indigenous uses										Σ uses	Σ sp.
	GH	RT	ST	SD	F/M	fmG	Oph	CO	FI	OU		
BRASSICACEAE	2			1		2			1	1	7	3
BURSERACEAE	2	1	1	3	2	2			2	1	14	4
CACTACEAE	2	1	1			1				3	8	1
CAESALPINIACEAE	8	5	7	5	6	1		1	5	5	43	12
CANNABACEAE			1						1	1	3	1
CAPRIFOLIACEAE			1	1	2	1	1		1		7	1
CARICACEAE	1					1			1	1	4	1
CELASTRACEAE	1			1		1					3	1
CHENOPODIACEAE	1	1		1			1		1	1	6	1
COMBRETACEAE	1									1	2	1
CONVOLVULACEAE	3		1	4					2	1	11	6
CRASSULACEAE			2	2	2	1				1	8	4
CUCURBITACEAE	4	1	1	4		2			4	7	23	7
DILLENIACEAE										1	1	1
EBENACEAE				1						2	3	2
ERICACEAE	1							1			2	1
EUPHORBIACEAE	6	1	1	12	1	4			2	5	32	18
FABACEAE	2	1	4	5	6	4		1	7	11	41	16
FAGACEAE	1					2				1	4	2
FLACOUTIACEAE	1	1		1					1	1	5	1
HAMAMELIDACEAE	1									1	2	1
KRAMERIACEAE	1					2					3	2
LAMIACEAE	8	2	3	3	1	5		1	5	4	32	12
LAURACEAE	3	1	1	1	1	3	2	1	2	3	18	3
LOGANIACEAE		1		1		1				1	4	1
LORANTHACEAE			1	2	2						5	2
LYTHRACEAE			1		1						2	2
MAGNOLIACEAE	3					1		2	1		7	3
MALPIGHIACEAE	1	1		1		1				1	5	1
MALVACEAE	3	3	3	4	7	5		1	2	5	33	7
MELASTOMATACEAE	1									1	2	2

(Table 5-S1: continued)

Botanical division and family	categories of indigenous uses										Σ uses	Σ sp.
	GH	RT	ST	SD	F/M	fmG	Oph	CO	FI	OU		
MELIACEAE	1	1	1	2	2	1			1	1	10	2
MIMOSACEAE	7	4		10	1	6	2		5	9	44	15
MONIMIACEAE	1		1	1	2	1			1		7	2
MORACEAE	3	2	4	3	1	1		1	4	3	22	6
MORINGACEAE			1		1				1		3	1
MYRTACEAE	6	4	3	1		4			3	3	24	8
NYCTAGINACEAE		3	1	3	1				1	3	12	4
ONAGRACEAE		1		1		1					3	1
PAPAVERACEAE				1			1				2	1
PASSIFLORACEAE	1			1	2	1			3	2	10	4
PEDALIACEAE						1				1	2	1
PHYTOLACCACEAE	1	1	2	1					2		7	2
PIPERACEAE	6	1	6	3	3	4		1	6	1	31	7
PLANTAGINACEAE							1				1	1
PLUMBAGINACEAE				1							1	1
POLEMONIACEAE	1			3	6	2			1	4	17	4
POLYGONACEAE	2	4	1	4		4				3	18	5
PORTULACACEAE										1	1	1
PUNICACEAE	1	1	1	1		1					5	1
RHAMNACEAE						3					3	3
ROSACEAE	1				1	1	1		1		5	1
RUBIACEAE	1	1		3	1	3			1	3	13	4
RUTACEAE	4	4		5	4	2	2	1	3	5	30	7
SALICACEAE	1		1	1	2						5	2
SAPINDACEAE	1			2						2	5	3
SAPOTACEAE	1		1	1					1	2	6	2
SCROPHULARIACEAE	1			2					1		4	2
SIMAROUBACEAE	1		1	1							3	2
SOLANACEAE	2	2	5	11	7	4	2	2	10	9	54	15
STERCULIACEAE	2	1		3	3	3				1	13	7
THEACEAE	2							2	2		6	1
TILIACEAE	1		1	4						1	7	4

(Table 5-S1: continued)

Botanical division and family	categories of indigenous uses										Σ uses	Σ sp.
	GH	RT	ST	SD	F/M	fmG	Oph	CO	FI	OU		
TURNERACEAE	1			1	2	1					5	2
ULMACEAE				1							1	1
URTICACEAE		1	1								2	1
VERBENACEAE	6	2		1	3	1			1		14	7
VITACEAE				2			1		2	2	7	2
ZYGOPHYLLACEAE	1		1	1						1	4	2
Monocotyledoneae												
AGAVACEAE	1	1		1					1	2	6	1
ALLIACEAE	2	2	2	2					4	3	15	4
ARACEAE			1	1					1	4	7	4
ARECACEAE	3		1	2		1			1	3	11	4
ASPHODELACEAE	1	1	1	1	1			1		1	7	1
BROMELIACEAE	1									1	2	1
COMMELINACEAE	2		2	7	1	1			2		15	5
CYPERACEAE			1	1	3	3			1	1	10	4
DIOSCOREACEAE			1			1			1	1	4	1
SMILACACEAE				2							2	2
LILIACEAE				1							1	1
MARANTHACEAE				2	2	2				2	8	2
MUSACEAE	1	1		1		1			1	2	7	2
ORCHIDACEAE										1	1	1
POACEAE	3	3		1	1	3			2	7	20	6
PONTEDERIACEAE	1										1	1
SMILACACEAE				2							2	2
ZINGIBERACEAE	1	1	1	1		1			2	1	8	2
indetermined plant sp.	7	3	4	11	3	4	3	1	12	9	54	32
Total: 110 families	187	90	105	197	96	136	19	22	150	191	1193	445

3.3.4 Parts of plant used

Further evaluations were carried out by grouping plants by their botanical family and plant part used (Table 6-S1) and in a general ranking list of the frequency of plant parts used (Table 7-S1).

Table 6-S1. A selection of interesting plant species grouped by their botanical family and used part of the plant.

Botanical family	plant part	frequency
CAESALPINIACEAE	**leaf**	**32 %**
	involucre	16 %
	bark	12 %
	inflorescence	8 %
	fruit	8 %
	herb, twig	8 %
	seed	8 %
	resin	4 %
	wood	4 %
	Total	100%(=25)
FABACEAE	**leaf**	**32 %**
	seed	18 %
	inflorescence	14 %
	herb, twig	13 %
	bark	9 %
	involucre	9 %
	fruit	5 %
	Total	100%(=22)

(Table 6-S1: continued)

Botanical family	plant part	frequency
MIMOSACEAE	**leaf**	**31 %**
	bark	27 %
	root, rhizome	15 %
	involucre	11 %
	herb, twig	8 %
	seed	8 %
	Total	100%(=26)
MYRTACEAE	**leaf**	**36 %**
	root, rhizome	**36 %**
	fruit	14 %
	bark	7 %
	inflorescence	7 %
	Total	100%(=14)
ASTERACEAE	**leaf**	**48 %**
	herb, twig	34 %
	whole plant	5 %
	inflorescence	7 %
	creeper, climber	3 %
	root, rhizome	3 %
	Total	100%(=59)

(Table 6-S1: continued)

Botanical family	plant part	frequency
CUCURBITACEAE	**fruit**	**36 %**
	leaf	22 %
	seed	14 %
	bark	7 %
	fruit pulp	7 %
	herb	7 %
	latex, sap	7 %
	Total	100%(=14)
EUPHORBIACEAE	**leaf**	**37%**
	bark	21 %
	herb, twig	13 %
	latex, sap	13 %
	seed	12 %
	root, rhizome	4 %
	Total	100%(=24)
APOCYNACEAE	**latex, sap**	**53 %**
	leaf	20 %
	inflorescence	13 %
	bark	7 %
	whole plant	7 %
	Total	100%(=15)

(Table 6-S1: continued)

Botanical family	plant part	frequency
SOLANACEAE	**leaf**	**55 %**
	fruit	17 %
	bark	10 %
	herb, twig	10 %
	inflorescence	4 %
	seed	4 %
	Total	100%(=29)
BIGNONIACEAE	**bark**	**29 %**
	leaf	22 %
	fruit	14 %
	fruit pulp	14 %
	creeper, climber	7 %
	inflorescence	7 %
	juice	7 %
	Total	100%(=14)

Ethnobiological systems of classification are usually not based on the taxonomic categories of families, subclasses, genus, etc. like in Western natural science. Indigenous people, the Zapotecs included, categorize the plants based on life forms and "habits" (Berlin et al. 1973), like *guixa* (hierba, hoja = herb, leave), *yaga* (arbol = tree), *hijtu'* (zacate = grass), *lube'e* (bejuco = creeper), but also *ruj'dxu* (goma = latex). Habit is a character which, for some species, might vary with climate or habitat, notably trees and shrubs. The Zapotec classification seems descriptive of the predominant habit of the plant or of a characteristic feature.

Preference of utilization of specific plant parts used for medicinal purposes depends on one hand on the vegetation zone and the resulting frequency of the respective plant forms. On the other hand, distinguished family characteristics can determine the frequency of the utilized plant part. It is not surprising that latex/sap (53.3%), a family characteristic of the Apocynaceae, is the plant "part" mostly used for curing purposes (Table 6-S1; and see Heinrich, 1989). Or, the characteristic fruits (35.7%) of the Cucurbitaceae are the most frequently used part within this family. The family of the Solanaceae yielded important drugs still used in allopathic medicine, such as the alkaloids, scopolamine or atropine. Therefore, many European Pharmacopoeias list the mother plants of these natural products. The plant part described mostly is "folium", is the same drug part ranking high in Zapotec utilization within this family. Western phytochemical analytical methods and Zapotec empirical selection conclude to use the plant part with the appropriate concentration of the bioactive compound in curation. In course of the selection approach for further investigation this may be a hint to chose the evaluated plant parts.

3.3.5 Frequency of plant parts used

In a general ranking list of the frequency of plant part used, herbal aerial plant parts are the most preferred habit for curing. This may be due to its ease of preparation, abundant occurrence within the surroundings, and simple storage (Table 7-S1). Herbs or twigs are ranked second, probably due to their application in the important ritual healing session of the limpia (the body is stricken with twigs or the whole herb to "wash off" the "illness"). Since bathing the body with a lukewarm preparation of cooked bark, e.g. for feverish illnesses, an ancient and frequent recommendation (see Publication II), this plant part is ranked in third position. Consequently, determing indicators for the order of the presented list are traditional circumstances and the advantages in the mode of handling the plant parts.

Table 7-S1. Frequency of plant parts used for medicinal purpose of 445 species. (*special ritual prepartation, therefore separated from "wood").

leaf, shoot	**206**	**32.8 %**
herb, twig	94	14.9 %
bark	69	11.0 %
fruit	49	7.8 %
root, rhizome	48	7.6 %
seed	41	6.5 %
inflorescence, flower	33	5.3 %
latex (milky)	21	3.3 %
creeper, climber	11	1.8 %
whole plant	11	1.8 %
involucre	10	1.6 %
ear	6	1.0 %
juice (watery)	6	1.0 %
wood	6	1.0 %
trunk*	5	0.8 %
resin	4	0.6 %
fruit pulp	3	0.5 %
part of fungus	3	0.5 %
petal	2	0.3 %
"cotton"	1	0.2 %
Total	**629**	**100 %**

3.3.6 Ethno-ecological considerations

It is estimated that during the first fifty years after the Spanish Conquest, the population in the Isthmus has declined by 90 % in comparison to the pre-conquest era (Campbell et al., 1993). Pre-Columbian societies, with higher population densities, probably had an even greater effect on forest composition and structure than do modern indigenous groups today. The impact of today's indigenous peoples is nevertheless overwhelmed by other forces like logging, Western agricultural methods, creation of pastures for meat production, interests of oil companies, "questinable" projects for electricity production, and other forms of resource exploitation (e.g. in the area of research: coppermining, cement production and over-collection of *Dioscorea* sp.). Disturbances originating in modern industrial societies can be clearly distinguished from those originating in egalitarian societies (like the Zapotecs) or the pre-Columbian chiefdoms (Balée, 1993). Traditional management relies on renewable and local resources, whereas modern management is expanding, exploiting and destructive. In Publication III ecological aspects of Zapotec ethnobotany are evaluated. The data should be a contribution to ethno-ecology, conservation, and management of the Isthmus-Sierra habitat with its challenging compilation of differing indigenous ecological zones and vegetation types within such a limited area.

3.3.7 Evaluations, what for?

The goals are manifold by applying such evaluations and discussions of characteristics as done in the previous paragraphs. Since the approach for phytochemical investigation here is ethnobotanical, the field data evaluation is thought to be an instrument to reduce the immense data set. Out of the 445 plants used in the Zapotec pharmacopoeia, the evaluations should systematically tackle a group of the most important plants.

The results are additionally valuable when compared with chemotaxonomic information in the literature. Such studies may give hints for relationships between plant families, genus' and species.

Indigenous plant classification and the frequency of the plant parts used are important for the understanding of the indigenous perception of the environment and therefore essential for conservation and management matters (see also Publication III). All in all, these ethnobotanical evaluations are interesting links between indigenous empiricism and Western biochemical findings.

4 Conclusion

4.1 To improve in future ethnobotanical research

Section 1 presented and discussed anthropological/ethnological, botanical and ecological aspects of this study. Problems occurring in the beginning while assessing these data were often communication based. In several situations, when specific questions about medicinal plants or illnesses were asked to several key informants, different information was received. The longer the field work took place the lesser the alliteration in Zapotec language was to blame. Then by suddenly realizing that Zapotec plant knowledge has not been developed in a literate context and the impossibility to scrutinize it systematically by the informants themselves (in an analytical, natural science trained manner), I had to make changes in the interview methods.

This example underlines the importance of the appropriate methods applied in the field as well as the importance of "talking the same language" and speaking "in" the same language while discussing a topic. Today, Zapotecs are mostly bilingual and only in one case I had to do interviews with the help of an interpreter. Although Spanish is spoken, the way of thinking was in a Zapotec manner. I worked many hours with a bilingual teacher to learn Zapotec names of plants and illnesses as well as easy conversation terms. Additionally, an older man helped me to make long vocabulary lists of Zapotec terms of many aspects concerning plants. He also assisted me in the identification of synonymous information (e.g. several indigenous terms for the same plant species). Nevertheless, I still think I was not literate enough in Zapotec. Therefore in future investigation I would put even more emphasis on learning the local language as fast as possible.

I still do not understand exactly why in an area and climate with ideal requirements for planting and growing almost every plant (e.g. vitamin and mineral rich vegetables), people are still suffering of malnutrition. The small range of the diet (above all: corn, beans, tomato, eggs and condiments) is astonishing. Since this diet is a tradition, it will not be easy to change this attitude. It is frustrating to realize that recently the range of food was "enriched" by some modern junk-foods such as soft drinks and sweets. When planning medical-ethnobotanical studies it is recommended to put more emphasis on the relationship between medicinal and food plants. Also for the Zapotecs, illness and nutrition is interrelated (see Publication I). Many recommendations and taboos exist (e.g. for mothers after giving birth), concerning nutrition in relation to health. I, therefore, think an integrated study of food and medicinal plants could help to change this awkward situation by outlining the local variety and possibilities of plants.

4.2 Negative and positive impacts from the research

While doing field work, I was often asked why " for heaven's sake" I came to learn plant remedies from poor people instead of being interested in all the good medicines the western health system is providing. To the end of the field work people said to me: "When people from outside spend a lot of time for something, it is usually important. Our plants must be very valuable then, to spend so much time on them." I was very happy with this reaction. I probably had awaken their pride for local cultural knowledge, an important link to conserve it for future generations.

In the first pages of this thesis (Background and Objectives) I proposed to upgrade traditional medicine e.g. through ethnobotanical studies for the improvement of the primary health care situation. Besides all the benefits, there are also some bad impacts to be taken into consideration. If plant species are collected not only for regional uses they may be endangered by over-collection. As described in Publication III, people of traditional societies have management strategies of their environment and so do healers when collecting medicinal plants, (e.g. not collecting bark from the same tree every time, not collecting all plants of a group of plants). Recommendations from outside for the collection and repeated utilization of plants can therefore disturb such management strategies. Considerations for the production of medicinal plants in plantations should be discussed when returning such data. This strategy could also have socio-ecological influences on a region by creating new employment or/and additional income.

4.3 Feedback so far from the research

In collaboration with healers and mothers and fathers of pupils (comité de padres de familia) I established a garden for medicinal plants on the grounds of a school, considered to be an exhibition garden for classes. While healers, teachers and pupils are caring for the plants, the plant material may be used by the healers. Additional room inside the fence was planed to be used by the teachers and pupils for a tree nursery.

I organized work shops with the healers to revive an older traditon of preparing medicine based on ancient recepies and others introduced from the INI. The material donated to the healers in work shops on production of creams, drops, syrups, soaps, and shampoos was considered to be a basis for further production.

Furthermore, I have planned to compile a booklet about the most important plants and their preparations to be distributed among the inhabitants or healers, depending on the range of information put in the booklet. An young artist from SDP has already drawn some pictures of medicinal plants to illustrate this folleto.

These are the feed backs so far from the research. Throughout the spent on reserach I was very much concerned about questions of "intellectual property rights" or "payback of benefits" in case of successful results. I have not found an answer yet to these questions. Especially tricky is the mode of the payback. Whom should get them (government, communities, healer organizations, individual people?) and should it be in the form of money or resources? Resolving, or at least clarifying these problems by making clear agreements before planning ethnobotanical studies should be done in the future.

Section 2

Plant Screening and Biological Activities

Screening is about what you find,

not about what you miss.....!

Anonymous phytochemist

5 Introduction

In the present section (2), plant screening and biological activities are discussed. This section is the link between Section 1 and Section 3, between ethnobotany and phytochemistry, between empirical knowledge and natural sciences. In an ethnobotanical approach to natural product isolation, the following step after field data evaluation is the systematic biological and chemical screening of the medicinal plants. The goal of this strategy is to select, out of the large ethnobotanical data, the most promising plant species for further phytochemical investigations.

5.1 Strategies in plant screening

The scientific strategies for the study of natural products from plants have changed substantially in the past few years for a number of reasons. The combined impacts of technological advances (e.g. chromatography, biotechnology) and the impact of new theoretical approaches (e.g. ethno-sciences, bioactivity-directed isolation) as well as the increased awareness of the biological diversity and its importance to human life have modified the strategies. In earlier years the phytochemist's approach was directed by taxonomic aspects. A phytochemical screening with spray reagents (e.g. Dragendorff reagent for alkaloids; "Nasschemische Methoden") was used as a selection criterium for further procedures. A working group of a laboratory was specialized on the isolation of a specific chemical class of compounds (e.g. iridoids, flavonoids). Since testing with specific spray reagents or the chemotaxonomic approach do not necessarily lead to bioactive compounds, the strategy has been discontinued recently in many laboratories.

Today, bioassay-guided natural product isolation is widely used, allowing one to trace and follow a specific activity found in the plant extract. Both of the strategies have their advantages and disadvantages. While the older approach concentrated in a laboratory team a lot of know-how concerning the isolation of a specific class of compounds, the newer one confronts the single phytochemists with a broader number of chemically different substances. When expertise is lacking, the process leading from the plant to a pure compound can be even longer and more tedious than it is anyway. Fortunately, advances in methods for structure elucidation (especially NMR techniques) help to solve many problems.

Bioassays allow one to screen for a specific target (e.g. anti-cancer, AIDS) as extensively conducted in the random and non-random screening programmes of the National Cancer Institute (NCI). In an ethnobotanical approach to natural product isolation, the bioassays are especially helpful for selecting specific methods to follow the activity suggested by the ethnobotanical study. Unfortunately, the laboratory facilities required for performing certain specific bioassays are becoming more sophisticated and more expensive. They are faraway from being applied in the field or being established in poorer countries. On the other hand, many newly developed bioassays are preferable also from an ethical point of view, because newer *in vitro* test systems can often replace older *in vivo* methods, at least, in early stages of the screening.

5.2 Requirements for bioassays

Assays should be simple, "bench-top", rapid, reproducible, and inexpensive. Since active principles occur generally at low concentration in the crude extract, the test system must be sensitive enough to detect them reliably (Hamburger and Hostettmann, 1991). On the other hand, the assays should be ideally insensitive to possible interferences from ubiquitous plant compounds such as polyphenols, fatty acids, chlorophyll, and other pigments causing many false positive results. The targets for bioassays are usually lower organisms such as microorganisms, insects, crustaceans, or molluscs. Examples of simple "bench-top" and inexpensive assays, used in this study, are the brine shrimp toxicity assay, with a crustacean as target for the evaluation of general toxicity (Meyer et al. 1982), and the bio-autographic TLC assays for the search of antimicrobial activity (Hamburger and Cordell, 1987).

Cell cultures for assay purposes became important first in the search for anti-cancer agents, where continuous cell lines (e.g. KB, P-388) were used for primary screening of extracts and further fractionation (Hamburger and Hostettmann, 1991). In this study, the KB and Caco-2 cell lines are applied in the pre-screening for the evaluation of cytotoxicity/anti-tumor activity. The KB cell line, derived from a human nasopharyngal carcinoma, has been useful for many years as a preliminary screen, being more sensitive to most anti-tumor agents than *in vivo* assays (Perdue, 1982). The Caco-2 cell line, obtained from a human colon adenocarcinoma, was chosen in order to get a broader information on cytotoxicity/ anti-tumor activity. Whether there is a specific anti-tumor activity or a general toxicity cannot be evaluated with only two cell lines as test models. The NCI developed a method to detect unlike patterns of differential cytotoxicity by performing a screening with 60 different cell lines (Fuller et al., 1994).

Consequently, in combination with the immunomodulating tests, based on several cell lines, a more detailed interpretation is then also possible in our study (see Publication IV).

Although cell cultures often can replace pharmacological assays on isolated organs, many models have not yet found an adequate substitute. Consequently, in this study two extracts have been tested in the "Ussing chamber", on isolated rabbit distal colon (Hör et. al., 1995) in order to get information on inhibition of intestinal secretion.

With a deeper understanding of cell biology and molecular pharmacology, mechanism-based bioassays have become increasingly important also in drug targeting. Due to their selectivity, sensitivity, good reproducibility, high sample throughput, (and high costs !), this type of assays are widely applied in the industry or in programmes as such as the NCI's. Targets in such assays are isolated subcellular systems, like enzymes, receptors, organelles, or cultured cells of human or animal origin (Hamburger and Hostettmann, 1991). Extracts containing compounds with unknown modes of action and non-specific interactions (e.g. enzyme inhibition by tannins) may lead to false positive results. Examples of mechanism-based bioassays applied in this study are the band-shift experiments to evaluate potential inhibitory effect on the activation of transcription factor NF (nuclear factor)-κB for the evaluation of anti-inflammatory potential (Bork et. al., 1996) or the immunomodulating assays (**Publication IV**).

5.3 Strategies in the present work

In the present work, we were particularly interested in selecting plants of major ethnomedical importance within the Isthmus-Sierra Zapotec's region (see Section 1). Therefore, specific assays were chosen to get broad information concerning dermatological and gastrointestinal disorders, the major medical problems occurring in the Zapotec villages and ranchos. The bioassay systems applied in this project were:

- bioautographic and agar-diffusion techniques for testing for antimicrobial activity
- permanent cell lines and brine shrimp for the evaluation of the cytotoxic and/or anti-tumor potential
- NF-κB and HET-CAM tests to assess the anti-inflammatory activity
- the Ussing chamber to measure intestinal secretion.

For reasons of current research interest (e.g. organ transplantation, AIDS) additional immunomodulation assays were performed in collaboration with the pharmaceutical industry:

- various *in vitro* assays using murine and human lymphoid cells in order to evaluate the proliferation and stimulation.

The bioassays were applied to a large pre-screening of eleven plant species (**Publication IV**). Additionally, a phytochemical screening was performed with several spray reagents (see Additional results and Discussion).

6 Publication IV

Multiple Screening of Medicinal Plants from Oaxaca, Mexico: Ethnobotany and Bioassays as a Basis for Phytochemical Investigation

Paper submitted, *Phytomedicine*

B. FREI [1], M. HEINRICH [2], P. M. BORK [2], D. HERRMANN [3], B. JAKI [1], T. KATO [1,5*], M. KUHNT [2, 4], J. SCHMITT [3], W. SCHÜHLY [1], C. VOLKEN [1], and O. STICHER [1]

[1] Department of Pharmacy, Swiss Federal Institute of Technology (ETH) Zurich, Winterthurerstr. 190, 8057 Zürich, Switzerland

[2] Institute of Pharmaceutical Biology, Albert-Ludwigs-University, Schänzlestr. 1, 79104 Freiburg, Germany

[3] Boehringer Mannheim GmbH, Dept. of Molecular Pharmacology/New Indications, Sandhofer Str. 116, 68305 Mannheim

4 Current Address: Agro GES mbH, Brunnerstr. 59, 1235 Vienna, Austria

[5] Faculty of Pharmaceutical Sciences, Josai University, 1-1 Keyakidai, Sakado, Saitama, 350-02 Japan; deceased August 1995

* This paper is dedicated to the memory of Takeshi Kato, deceased August 1995

Summary

Based on ethnobotanical data collected among Zapotec Indians in Mexico, nine species traditionally applied to treat skin diseases and two species used to treat gastrointestinal disorders were subjected to several bioassays as further selection criteria for phytochemical investigation. Ten were active against at least one of the pathogenic and/or non-pathogenic bacteria and one against a non-pathogenic fungus in bioautographic TLC and agar diffusion tests. Cytotoxic/antitumor potential was found for one plant species with cell lines (KB, Caco-2) and for six with the brine shrimp assay. In the NF-κB- and the HET-CAM-test used to test for anti-inflammatory potential, two respectively one plant extract showed noteworthy activity. Furthermore, a potentially immunomodulating activity was investigated by evaluating the influence of extracts in various *in vitro* assays using murine and human lymphoid cells. In addition to the reported biological activities of the eleven plant species, comparisons of the ethnobotanical data and strategies for the selection for further phytochemical investigations are discussed.

Keywords: Zapotec Indians (Mexico), ethnobotany, medicinal plants, traditional medicine, antimicrobial, KB, Caco-2, brine shrimp, NF-κB, HET-CAM, immunomodulation, dermatological problems, gastrointestinal illnesses.

Introduction

Medicinal plants are used by the Zapotecs to treat a large number of illnesses including a variety of infections (Frei et al., submitted/a). Observations in the field indicated that the major medical problems for the Zapotecs are dermatological conditions and gastrointestinal disorders. As a part of our study on the ethnobotany of the Zapotec Indians (Frei et al., in press; Frei et al., submitted/a) in the Isthmus-Sierra of Tehuantepec (Oaxaca) 28 medicinal plant species were collected for biological screening and phytochemical investigation. Based on the documented uses and preparations as well as on a literature search, nine species traditionally applied to treat skin diseases and two species used to treat gastrointestinal illnesses were selected.

There exists a large number of potential pharmacological and microbiological targets with possible relevance for the interpretation and possibly the justification of the indigenous uses. Therefore, a multiple screening of the ethanolic extracts was conducted in order to select taxa for phytochemical studies:

- antimicrobial activity against several facultatively pathogenic (Biosafety Level 2) and non-pathogenic (Biosafety Level 1) bacteria, fungi, and a yeast
- anti-inflammatory activity [activation of transcription factor NF (nuclear factor)-κB and HET-CAM-test (Hen Eggs Test-ChorioAllantoic Membrane)]
- cytotoxic activities (KB cells, Caco-2 cells) in combination with the brine shrimp lethality bioassay.
- proliferation and stimulation of human and murine cells to investigate immunomodulation.

The two plant species traditionally used to treat gastrointestinal complaints were further subjected to the Ussing-chamber-experiment to determine the effect on intestinal secretion.

This paper discusses biological activities of eleven plant species and compares the data to the corresponding ethnobotanical uses. Both ethnobotanical importance and activity in bioassays are applied as selection criteria for further phytochemical investigation. So far, *Begonia heracleifolia*, *Epaltes mexicana*, and *Xanthosoma robustum* have been investigated phytochemically. Other plant species are currently under investigation. Structure elucidation employing mainly spectroscopic methods lead to several new and known compounds. Further biological testing of the pure compounds yielded additional data which corroborate some of the indigenous uses.

Materials and Methods

Plant Material

Plant material was collected near Santo Domingo Petapa (Mexico). Authenticated voucher specimens (*FREI 23, 28, 36, 66, 68, 137, 151, 169, 190, 279, 293*) are deposited at the following herbaria: MEXU (UNAM, México D.F.), ZT (ETH Zurich, Switzerland), and at the Institute of Pharmaceutical Biology, University of Freiburg, Germany.

Extract Preparation

Shade dried, powdered plant material (10 g) was successively extracted (Ultra Turrax) with ethanol 96% (V/V) for 3 min. in an ice bath. The same plant material was then further extracted, once with ethanol 96% (V/V) and twice with ethanol 70% (V/V), under reflux for 15 min. Crude extracts were obtained by removing the solvents in vacuo (30°C) followed by lyophilisation (Table 2-P4).

Assays for bioactivity

1. Antibacterial and antifungal activity: Antibacterial and antifungal activity against non-pathogenic test-organisms (Biosafety Level 1) was evaluated with bioautographic-TLC-assays (Baumgartner et al., 1990) using a modified procedure. Test organisms used were the bacteria *Bacillus subtilis* (ATCC 6633, gram +; Abbreviation: Bs), *Micrococcus luteus* (ATCC 9341 (gram +; Ml), *Escherichia coli* (ATCC 25922, gram -; Ec), and the fungus *Pencillium oxalicum* (CBS 219.30; Po). Crude extracts (≤ 200 µg) were applied several times on analytical TLC plates (Merck Si 60 F_{254}, aluminium) and developed using appropriate solvent systems. One chromatogram of the TLC plate was then dipped into a Desaga staining tube, containing a suspension of an overnight culture of test-organism in BBL nutrient broth (Becton & Dickinson Co. 11479) or a conidial suspension in Czapex-Dox broth (Difco, USA), respectively. The bacterial inhibition zones were detected with 0.5% *p*-iodonitrotetrazolium chloride in H_2O (Fluka, Switzerland) after incubation, whereas conodial inhibition zones were obvious without treatment. Concurrently, the other chromatograms on the TLC plate were visualized with different spray reagents and under UV light (254 and 366 nm). Chloramphenicol (Siegfried AG, Switzerland) and miconazole nitrate salt (Sigma, USA) were used as antibacterial and as antifungal standard, respectively. Antimicrobial activity is expressed as minimum growth inhibitory amount in µg extract on TLC-plates (Table 2-P4).

Further antibacterial and antifungal activity against facultatively pathogenic test-organisms (Biosafety Level 2) was evaluated in two agar-diffusion-assays

(Grimm, 1974; Rahalison, 1994). Test organisms used were the bacteria *Bacillus cereus* (ATCC 10720, gram +; Bc), *Mycobacterium fortuitum* (* , gram +; Mf), *Staphylococcus aureus* (ATCC 25923, gram +; Sta), *Staphylococcus epidermidis* (ATCC 12228, gram +; Ste), *Streptococcus Group A hemolis* (**, gram +; Sth), *Pseudomonas aeruginosa* (ATCC 27853, gram -; Pae), *Salmonella enteritidis* (**, gram -, Se), *Shigella flexneri* (**, gram -; Sf), and the yeast *Candida albicans* (H29 ATCC 26790; Cal). Some of the test organisms have been isolated from material of investigation at the Institute of Microbiology, Zurich* or Freiburg**.

Crude extract (≤ 200 µg or ≤ 1 mg for *Salmonella* and *Shigella,* respectively) dissolved in an appropriate solvent was applied on paper discs (Ø 0.6 cm; BLANK DISCS, Oxoid, UK) and left to dry. Alternatively, crude extract was applied into a hole (Ø 0.6 cm) of an agar plate and diluted with sterile water up to 100 µl. In both cases nutrient agar plates were plated with 25 to 100 µl of microbial culture in the exponential growing phase (Mueller-Hinton Agar, Oxoid, UK; Malt Extract Agar, Oxoid, UK, for *C. albicans,* respectively). As antibacterial standards chloramphenicol (Siegfried, Switzerland), tetracycline hydrochloride (Fluka, Switzerland) or ampicillin (SIGMA, USA), and as an antifungal miconazole nitrate salt (Sigma, USA) were used. Antimicrobial activity is expressed as minimum growth inhibitory amount in µg extract on agar (Table 2-P4).

2. <u>Cytotoxicity/antitumor-activity assays</u>: Cytotoxic or antitumor potential was assessed using two cultured cell lines [Swanson et al. (1990) and Orjala et al. (1994)]:

- KB cells (ATCC CCL 17; human nasopharyngal carcinoma), modified for cultivation in 24-well plates.

- Caco-2 cells (ATCC HTB-37; human colon adenocarcinoma) modified for cultivation in 24-well plates and with Dulbecco's Modified Eagle's Medium (31966; Gibco, Life Technologies, Switzerland).

Cells growth was quantified using the protein determination by Lowry. The results are presented as IC_{50} values (50% inhibition of cell growth in comparison to the control) in [µg/ml]. Extracts are considered active (Perdue, 1982) with IC_{50} values ≤20 µg/ml (Table 2-P4).

Further testing for toxicity was made by applying extract (100 µg/ml) to the brine shrimp lethality-assay (*Artemia salina* Leach; Brs) as performed by Meyer et al. (1982). Extracts were considered to show a general toxicity (Table 2-P4) if the death value reached 50%.

3. <u>Anti-inflammatory potential</u>: In a physiological model for anti-inflammatory activity - the HET-CAM-assay, utilizing incubated hen's eggs (10 days), described by Luepke and Kemper (1986) and Kuhnt et al. (1995) - the delay in the onset of capillary reactions of the allantois membrane was tested. The test sample's ability to stabilize the capillary membrane is expressed as the time difference until haemorrhage is recorded, in percent compared to untreated controls. Samples with a more than 20% change are considered anti-inflammatory (Table 2-P4; III = inactive or nonsignificant increase < 10% of time of onset of haemorrhage; II = 10 - 20% increase of time of onset of haemorrhage; I = > 20% increase).

The potential inhibitory effect on the activation of transcription factor NF-κB as an indicator for anti-inflammatory activity, was tested as performed by Bork et al. (1997). Activity is expressed as negative interference with the activation of factor

NF-κB revealed in band-shift experiments (Table 2-P4; + = crude extract which act as inhibitors of NF-κB).

4. Assays for potential immunomodulation by evaluating proliferation and stimulation of human and murine cells:

General procedure: Stock solutions of extracts were made in DMSO (10 mg/ml) and concentrations of 100, 10, 1, 0.1, 0.01, 0.001 µg/ml were tested. Negative controls with the respective concentration of DMSO were tested in parallel. All experiments were performed in 96-well plates and conducted at least twice. Cell proliferation was measured by incubation with a radio-labelled precursor of DNA ([^{3}H]-thymidine). The amount of radioactivity incorporated into the cells was determined by liquid scintillation counting (Nowel, 1960; Farrant et al., 1980; Maurer, 1981). Results are presented as IC_{50} values (50% inhibition of cell proliferation in comparison to the control). The number of experiments varied between n = 1 and n = 3; SD <10%. Results are shown in Table 3-P4.

4.1 Influence on primary murine and human lymphocytes

4.1.1 Polyclonal stimulation on murine spleen cells

Murine spleen cells (3.6x10^5/well) were incubated with various concentrations of the extracts and stimulated with 2.5 µg/ml of mitogen concanavalin A (ConA) in microtiter plates (Schlossmann et al., 1971). Cultures were incubated for 48 h and [^{3}H]-thymidine added for another 5 h. Incorporated radioactivity was measured by scintillation counting at the end of the incubation period.

4.1.2 Allogenic stimulation of human peripheral blood cells

Allogenic stimulation of human PBL was studied in a two-way mixed lymphocyte reaction. Lymphocytes from male and female donors were isolated by Ficoll gradient centrifugation using peripheral blood. Female and male cells ($1x10^5$ each/well) and various concentrations of the extracts were added to microtiter plates. After four days of incubation [^{3}H]-thymidine was added for another 5 h and incorporation of radioactivity was measured (Wagner et al., 1988, Koehn et al., 1994).

4.1.3 Stimulation of activated human T-lymphocytes with Interleukin-2

Human lymphocytes were obtained from peripheral blood by Ficoll gradient centrifugation and incubated for three days with the mitogen ConA (2.5 µg/ml). Cells were then washed three times, adjusted to a density of $8x10^4$/well and incubated with Interleukin-2 (20 U/ml) and the various concentrations of the extracts. Cell proliferation was measured by the amount of radioactivity incorporated after 48h (Martin et al., 1992).

4.2 Influences on growth of permanent cell lines:

4.2.1 Spontaneous cell proliferation of tumor cell lines

MethA cells (murine MethylcholAntrene-induced fibrosarcoma) and THP-1 cells (human acute monocytic leukemia) were passaged as permanent cell lines. MethA or THP-1 cells ($9x10^3$/well) were incubated with different concentrations of the extracts for 48 h. Cell proliferation was measured by the addition of [^{3}H]-thymidine and incorporated radioactivity was determined after another 5 h of incubation (Ignotz, 1992, Mashiba et al., 1993).

4.2.2 Spontaneous proliferation of murine fibroblast cell lines

SC-1 and 3T3 cells (murine embryonic fibroblasts) were passaged as adherent cultures. SC-1 or 3T3 cells ($9x10^3$/well) were incubated with various concentrations of the extracts. To quantify cell proliferation the amount of radioactivity incorporated after 48 h of incubation was determined by the addition of [^{3}H]-thymidine (Bonin et al., 1993; Park et al., 1994).

4.2.3 Cytokine-dependent proliferation of NFS-60 cells

The murine myeloid leukemic cell line NFS-60 was passaged permanently in the presence of 1 ng/ml recombinant human G-CSF. NFS-60-cells ($1.3x10^4$/well) were incubated for 24 h with various concentrations of the extracts in the presence of G-CSF (1 ng/ml). [^{3}H]-thymidine was added for another 16 h and cell proliferation was then determined by the amount of incorporated radioactivity (Nakoinz et al., 1990).

Potential immunomodulatory activity is investigated on lymphocytes in the tests 4.1.1 to 4.1.3, while the four tests on fibroblasts (4.2.1 to 4.2.3) are performed to get further information on unspecific reactions against other cell types. "The pattern" of the combined results (varying values or similar values) may give additional information on specific and unspecific cytotoxicity.

5. Antisecretory activity: The extracts of *Dorstenia drakena* and *Krameria pauciflora* were also examined in rabbit distal colon mounted in an Ussing-chamber (Greger et al., 1991; Hör et al., 1994) to evaluate the influence on intestinal secretion. Chloride secretion was stimulated by cholera toxin on the luminal side and by prostaglandin E_2 on the serosal side and expressed by the calculation of the short-circuit current I_{SC} (equivalent to the electronic transepithelial ion transport). Samples (200 µg/ml; 400 µg/ml) were tested for their inhibition of induced I_{SC}.

Results and Discussion

Ethnobotany as a Basis for Phytochemical Investigation

In our research on medicinal plant use among Zapotec Indians and on pharmacological-biological activities of extracts as well as isolated compounds, we are particularly interested in selecting plants of major ethnomedical importance within the research area. A number of different healing specialists such as *curanderos* (healers), *parteras* (midwives), and *hierberos* (herbalists) use more than 440 different plant species for healing purposes. Preparations of plant material, fresh, dried, toasted, soaked in alcohol, or extracted with alcohol are applied in ritual healing sessions or recommended as home remedies. Hence, a large evaluation of the field data by a quantitative ethnobotanical approach (Frei et al., submitted/a) was performed. Table 1-P4 shows the ethnobotanical information on medicinal use, preparation, and plant parts used of the eleven plant species. The listed "ethnobotanical importance" is based on ranking lists in which the frequency of usage is employed as a quantitative criterion. More frequently cited plants are regarded to be of greater ethnobotanical importance than the ones cited only by a few healing specialists. Thus, the higher the value cited, the more important is a plant for Zapotec medicinal use.

In a further step, specific assays were chosen to get pharmacological and microbiological information regarding the ethnobotanical information on plants used to treat dermatological and gastrointestinal disorders. In addition, assays for immunomodulation were conducted. The main selection criteria for further phytochemical investigation of a plant are: High ethnomedical importance along with promising activities resulting from the biological screening.

Table 1-P4. Ethnobotanical information on 11 plant species used in Zapotec traditional medicine in Oaxaca, Mexico.

* Ethnobotanical importance as evaluated in Frei et al. (n.d./a): the higher the value, the higher the importance of the plant.

Plant species, family [Voucher number]	Zapotec medicinal use	Plant part used	Preparation	ethno-importance*
Begonia heracleifolia Cham. & Schltdl. BEGONIACEAE [FREI 66]	wounds, local infections (e.g. acne, comedo, pustule, insect bite, sting), rheumatism, pain, tumor	rhizome, petioles	fresh plant material ground and applied topically; fresh plant material in alcohol, applied topically	2
Dysodia appendiculata Lag. ASTERACEAE [FREI 36]	Candida mycosis, pain (head, stomach, gums), culture bound syndromes	leaf	fresh plant material ground and applied topically; fresh plant material in alcohol, applied topically; fresh plant material mixed with vaseline	8
Epaltes mexicana Less. ASTERACEAE [FREI 151]	mycosis, "espinilla" (purulent or dry pustules on chest and neck), swelling, oedema, dermatobiasis (furunculosis due to *Dermatobia hominis* (Cuterebridae)), fever, ulcus	herb, root	local bath with water of cooked plant material; toasted and ground, applied topically	5
Phoradendron amplifolium Nutt. LORANTHACEAE [FREI 293]	erysipelas (deep red inflammation, usually *Streptococcus* sp., with high fever), local infections, mumps, tumor, rheumatism	herb	fresh plant material ground and applied topically with or without alcohol; bath	4
Psittacanthus calyculatus (DC.) G. Don LORANTHACEAE [FREI 23]	erysipelas	leaf or herb with flower	fresh plant material ground and applied topically	4

Table 1-P4. (Continues)

Table 1-P4. (Continued)

Plant species, family [Voucher number]	Zapotec medicinal use	Plant part used	Preparation	ethno-importance*
Solanum diflorum Vell. SOLANACEAE [FREI 137]	erysipelas, swelling, oedema, fever	leaf	general bath; tea; fresh plant material ground and applied topically	6
Solanum lanceolatum Cav. SOLANACEAE [FREI 169]	local infections, "espinilla", wounds	leaf	local bath with water of cooked plant material; local massage with fresh leaf; ground plant material applied topically	7
Xanthosoma robustum Schott. ARACEAE [FREI 28]	bleeding wounds, local infections, swelling, oedema, wart, snake bite, culture bound syndromes	tuber or leaf with latex	fresh tuber cut in half, applied on bleeding wounds; fresh plant material ground and applied topically	5
Zebrina pendula Schnizl COMMELINACEAE [FREI 68]	erysipelas, local infections, pain, swelling, oedema, tumor	herb	local bath with water of cooked fresh plant material; fresh plant material ground and applied topically	6
Dorstenia drakena L. MORACEAE [FREI 279]	diarrhoea, dysentery, pain, stomach ache	root	tea, in alcohol, applied topically	5
Krameria pauciflora (Moç. & Sessé) ex DC. KRAMERIACEAE [FREI 190]	vaginitis, general infections, pain (birth), diarrhoea	herb	bath; tea	2

Tabel 2-P4. Results of the biological screening from nine plant species used to treat skin diseases and two species against gastrointestinal disorders.

Plant Species (% plant extract out of total crude drug)	Antibacterial / antifungal activity assays									Cytotoxicity assays			Anti-inflammatory potential	
	Bs [µg]	Ml [µg]	Ec [µg]	Po [µg]	Bc [µg]	Mf [µg]	Sta [µg]	Ste [µg]	Pae [µg]	KB IC_{50} [µg/ml]	Caco-2 IC_{50} [µg/ml]	BrS [%]	HET-CAM [%]*	Transcription factor NF-κB**
B. heracleifolia (11.2 %)	20	20	20	--	--	--	150	200	--	3.8	7.5	100	II	tox.
D. appendiculata (24.5 %)	--	100	--	--	200	--	--	200	200	--	--	75	III	--
E. mexicana (24.3 %)	--	25	--	--	--	--	--	200	--	--	--	< 50	II	--
P. amplifolium (26.3 %)	--	--	--	25	200	--	--	200	--	--	--	87	nt	--
P. calyculatus (25.8 %)	--	100	--	--	200	--	--	--	--	27.8	--	80	II	--
S. diflorum (23.5 %)	--	100	--	--	200	--	--	--	200	28.2	--	50	III	+
S. lanceolatum (25.6 %)	--	200	--	--	--	--	--	--	--	--	--	< 50	III	(+)
X. robustum (25.5 %)	25	25	--	--	200	--	--	--	--	--	--	94	I	--
Z. pendula (13.6 %)	200	--	--	--	--	--	--	200	--	--	--	100	II	(+)
D. drakena (6.2 %)	--	--	--	--	200	--	--	200	--	--	--	< 50	nt	--
K. pauciflora (33.1 %)	--	25	--	--	200	200	--	200	200	--	--	< 50	nt	--
Control	0.04 Cc 0.5 Tc	0.04 Cc 0.5 Tc	0.04 Cc 0.5 Tc	5 Mc	0.5 Tc	0.5 Tc	0.1 Ac	0.5 Tc	0.5 Tc	0.05 Pt	0.05 Pt	100 Pt	16.0 sec 0.9 % NaCl, 5 % DMSO	100 µM PDTC

nt = not tested; -- = no activity; Ac = Ampicilline; Cc = Chloramphenicol; Mc = Miconazol; Pt = Podophyllotoxin; Tc = Tetracycline, PDTC = Ammonium pyrrolidinedithiocarbamate. * III = inactive or nonsignificant increase < 10% of time of onset of haemorrhage; II = 10 - 20% increase of time of onset of haemorrhage; I > 20% increase. **+ = strong, (+) = weak, -- = no inhibitory effect on the transcription factor NF-κB at 100 µg/ml.

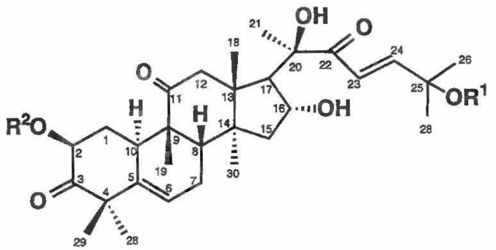
Biological activities and their relevance to the ethnobotanical data

Eleven ethanolic plant extracts from taxa of eight different families were investigated. The results of the biological screening are summarized in Table 2-P4. None of the extracts showed activity in the antimicrobial assays against *Candida albicans*, *Salmonella enteritidis*, *Shigella flexneri*, or *Streptococcus Group A hemolis*. Consequently, these data are not listed. The evaluation of an immunomodulating activity is discussed for four plant species (Table 3-P4) which showed particularly prominent activity. The data of each plant species will be discussed separately.

Field data on *Begonia heracleifolia* are partially supported by the promising results of the antibacterial assays (*B. subtilis, E. coli, M. luteus, S. aureus*; Table 2-P4). The rather strong cytotoxicity/antitumor activity (KB, Caco-2, brine shrimp assay) indicated possible pure compounds with general toxicity or potent specific cell toxicity. The results of the tests for proliferation and stimulation of human and murine cells (Table 3-P4) supported the latter assumption showing a varying pattern in this cell line assays. Especially noteworthy is the simultaneous strong inhibition of the only two cytokine-dependant cell lines as shown in the tests 4.1.3 and 4.2.3 after stimulating the T-lymphocytes and the NFS 60 cells with Interleukin-2 or G-CSF, respectively. So far, bioactivity-guided isolation with the KB cell lines yielded six cucurbitacins. Three compounds (**1-3**; Figure 1-P4) showed strong cytotoxic activity against the KB cell line (Frei et. al., submitted/b) and strong activity in the series of the immunomodulating tests.

Table 3-P4. Selected results from the assays of four plant extracts for potential immunomodulation by evaluating proliferation and stimulation of human and murine cells. Experiment numbers (4.1.1 - 4.2.3) see "Material and Methods".

Plant species	IC_{50} (µg/ml)							
	Immunomodulatory activity			Influences on growth of permanent cell lines				
	4.1.1 (ConA)	4.1.2 (PBL)	4.1.3 (IL-2)	4.2.1 (MethA)	4.2.1 (THP-1)	4.2.2 (SC-1)	4.2.2 (3T3)	4.2.3 (NFS-60)
B. heracleifolia	28.6	27.3	3.2	33.0	5.3	38.7	>100	2.1
P. calyculatus	50.3	30.7	21.7	34.8	51.3	64.0	8.8	36.2
S. diflorum	65.9	25.6	20.4	36.0	>100	>100	>100	43.2
Z. pendula	>100	96.1	69.7	66.2	>100	60.0	11.4	>100
Cyclosporin A	0.025	0.00021	>1	>1	0.29	>1	0.29	0.91
No. of experiments	n =2	n =2	n =2	n =2	n =1	n =3	n =1	n =2



1 R^1 = Ac, R^2 = H	Cucurbitacin B
2 R^1 = H, R^2 = H	Cucurbitacin D
3 R^1 = H, R^2 = H, 23,24-dihydro	23,24-Dihydrocucurbitacin D

Figure 1-P4. Bioactivity-guided isolation yielded three cucurbitacins from *Begonia heracleifolia* showing strong cytotoxic activity in the KB cell line assay.

With the crude extract of *Dysodia appendiculata*, the Zapotec medicinal usage to treat Candida mycosis could not be corroborated *in vitro*. Whereas interesting activity (Table 2-P4) against *M. lutes, B. cereus, S. epidermidis,* and the often antibiotica-resistant bacterium *P. aeruginosa* was detected [references of inhibition zones of tetracycline in disc assays against *P. aeruginosa*: 0 mm (1 µg), 2 mm (10 µg) in comparison to 3.5 mm (1 µg) and 10 mm (10 µg) for *M. luteus,* respectively]. The ethnobotanical evaluation by a quantitative approach showed that *D. appendiculata* is on the other hand important in curing culture- bound syndromes (folk illnesses). According to western medical understanding, various symptoms are observable during such illness episodes. Ritual healing is predominant involving sensory perceptions and psychological effects. The latter may be explained by the high content of volatile substances such as monoterpenes and thiophenols in this plant genus (Bohlman and Zdero, 1979).

Phoradendron amplifolium and *Psittacanthus calyculatus*, both belonging to the Loranthaceae family, are used to treat erysipelas (infection with *Streptococcus*) and for general wound healing. *P. amplifolium* shows high activity in the antifungal assay and weak activity in the antibacterial assays (Table 2-P4). In this case, ethnobotanical data are weakly corroborated. While chemotaxonomic considerations (the family of the Loranthaceae is known for its lectines and viscotoxines) and the resulting pattern of *P. calyculatus* in the immunomodulating cell-tests (Table 3-P4) as well as in the KB cell test are stimulating for further investigations of these plants.

Solanum diflorum is traditionally used to treat fever and infected wounds. Anti-inflammatory effects were documented both in the correlating data from the HET-CAM assay and the NF-κB-test (Table 2-P4). Thus, phytochemical investigation by bioactivity-guided fractionation following the NF-κB-test might in this case lead to potent anti-inflammatory compounds. Whereas the plant *S. lanceolatum* of the same genus seems to be of minor relevance according to the results of the selected assays.

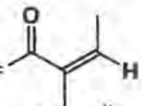
The results of the two cytotoxicity assays of the extract of *Zebrina pendula* (Table 2-P4) do not show correlation. The pattern of the results of the cell-based immunomodulation tests (Table 3-P4) shows varying IC_{50} values. This may indicate mechanism-based cell toxicity to be studied in bio-activity-guided fractionation with the easy-to-handle assay evaluating brine shrimp lethality.

Ethnobotanical data documenting the uses of *Dorstenia drakena*-roots and of the herb of *Krameria pauciflora* as gastrointestinal remedies are not supported by the experiment in the Ussing-chamber. Nevertheless, broad antibacterial activity corroborates traditional treatment for skin diseases.

Bioactivity-guided isolation yielded eight sesquiterpenes (Figure 2-P4; compounds **4-11**) from *Epaltes mexicana* confirming antibacterial activities as shown in the screening (Kato et al., 1996 a).

4 R^1=epoxyang, R^2=H, R^3=OH
5 R^1=epoxyang, R^2=Ac, R^3=OH
6 R^1=ang, R^2=H, R^3=OH
7 R^1=ang, R^2=H, R^3=H
8 R^1=epoxyang, R^2=H, R^3=H
9 R^1=epoxyang, R^2=Ac, R^3=H

10 R^1=epoxyang, R^2=H
11 R^1=epoxyang, R^2=Ac

ang = 

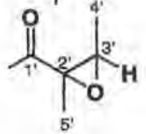
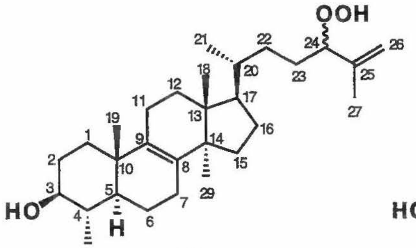
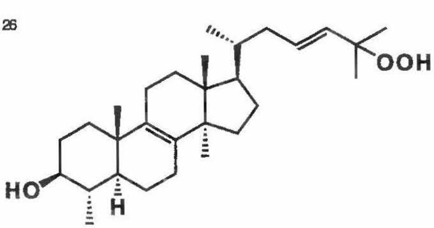
epoxyang = 

Figure 2-P4. Eight sesquiterpenes with antibacterial activity from *Epaltes mexicana*.

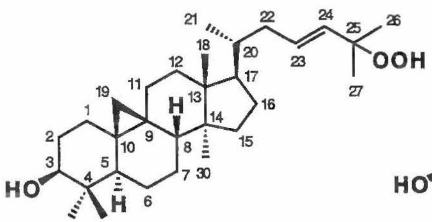
From *Xanthosoma robustum* eight sterols, four (Figure 3-P4; compounds **12-15**) with noteworthy antibacterial activity have been isolated (Kato et al., 1996 b). Both, roots of *Xanthosoma robustum* and leaves of *Epaltes mexicana,* are used to treat external wounds with bacterial and/or fungal infections. The biological results presented here of the crude extracts and the activity of the isolated compounds support the indigenous uses.



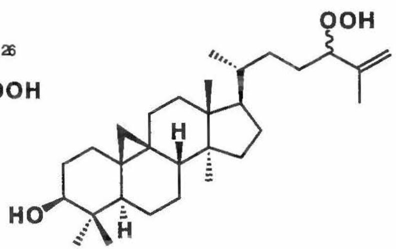
12



13



14



15

Figure 3-P4. Bioactivity-guided isolation yielded four hydroperoxysterols from *Xanthosoma robustum.*

Conclusion

The Isthmus-Sierra Zapotecs live in an area of vast botanical diversity that provides them with a broad range of medicinal plants. However, many of the medicinal plants used have not been studied for their phytochemical composition in detail. The data presented in this work show that a rational approach in the search for bioactive compounds includes a combination of ethnobotanical, biological, and phytochemical research. Furthermore, indigenous plant use and healing concepts will be better understood and could lead to an upgrading of traditional medicine. Its combination with modern western medicine may give new inputs to both of them.

References

Baumgartner, B., Erdelmeier, C. A. J., Wright, A. D., Rali, T., Sticher O.: An antimicrobial alkaloid from *Ficus septica*. *Phytochemistry 29*: 3327-3330, 1990.

Bohlmann, F., Zdero, C.: Ueber dimere Terpenketone aus *Tagetes gracilis*. *Phytochemistry 18*: 341-343, 1979.

Bonin, P. D., Singh, J. P., Gammill, R. B., Erickson, L. A.: Inhibition of fibroblast and smooth muscle cell proliferation and migration in vitro by a novel aminochromone U-67154. *J. Vasc. Res. 30*: 108-115, 1993.

Bork, P., Schmitz, M. L., Weimann, C., Kist, M., Heinrich, M.: Nahua Indian Medicinal Plants (Mexico): Inhibitory activiy on NF-κB as an anti-inflammatory model and antibacterial effects. *Phytomedicine 3*: 263-269, 1996.

Bork, P. M., Schmitz, M. L., Kuhnt, M., Escher, C., Heinrich, M.: Sesquiterpene lactone containing Mexican Indian medicinal plants and pure sesquiterpene lactones as potent inhibitors of transcription factor NF-κB. *FEBS Letters 402*: 85-90, 1997.

Farrant, J., Clark, J. C., Lee, H., Knight, S. C., O'Brien, J.: Conditions for measuring DNA synthesis in PHA stimulated human lymphocytes in 20 µl hanging drops with various cell concentrations and periods of culture. *J. immunol. Meth. 33*: 301-312, 1980.

Frei, B., Sticher, O., Viesca T., C., Heinrich, M.: Medicinal and food plants: Zapotec criteria for selection. *Ecol. Food Nutr.*:In press, 1997.

Frei, B., Baltisberger, M., Sticher, O., Heinrich, M.: Medicinal ethnobotany of the Isthmus-Sierra Zapotecs (Oaxaca, Mexico): Documentation and evaluation. Submitted/a.

Frei, B., Heinrich, M., Sticher, O.: Phytochemical and Biological Investigation of *Begonia heracleifolia*: Structure-Activity Studies on Cucurbitacins. Submitted/b.

Grimm, H.: Untersuchungen mit Sulfadiazin, Nitrofurantoin und der Kombination beider Wirkstoffe im Reihenverdünnungs- und Agardiffusionstest. *Int. J. Clin. Pharmacol. 9*: 232-242 , 1974.

Hamburger, M.O., Cordell, G.A.: A direct bioautographic TLC assay for compounds possessing antibacterial activity. *J. Nat. Prod. 50*: 19-22, 1987.

Hör, M., Rimpler, H., Heinrich, M.: Inhibition of intestinal chloride secretion by proanthocyanidines from *Guazuma ulmifolia*. *Planta med. 61*: 208-212, 1995.

Ignotz, R. A.: Transforming growth factor-beta 1 induces expression of statin during differentiation of human promonocytic leukemia cells. *J. Cell. Biochem. 50*: 285-292, 1992.

Kato, T., Frei, B., Heinrich, M., Sticher O.: Sesquiterpenes with antibacterial activity from *Epaltes mexicana*. *Planta med. 62*: 66-67, 1996 a.

Kato, T., Frei, B., Heinrich, M., Sticher O.: Antibacterial hydroperoxysterols from *Xanthosoma robustum*. *Phytochemistry 41*: 1191-1195, 1996 b.

Koehn, F. E., McConnell, O. J., Longley, R. E., Sennett, S. H., Reed, J.K.: Analogues of the marine immunosuppressant microcolin A: preparation and biological activity. *J. Med. Chem. 37*: 3181-3186, 1994.

Kuhnt, M., Pröbstle, A., Rimpler, H., Bauer, R., Heinrich, M.: Biological and pharmacological activities and further constituents of *Hyptis verticillata*. *Planta med.* *61*: 227- 232, 1995.

Luepke, N. P., Kemper, F. H.: The HET-CAM test: An alternative to the Draize Eye Test. *Fd. Chem. Toxic.* *24*: 495, 1986.

Martin, R., Schwulera, U., Menke, G., Rudolph, W., Buch, K., Fasold, H., Lissner, R., Thrun, A., Krauseneck, P., Bogdan, U.; Interleukin-2 and blood-brain barrier: Pharmacokinetics and tolerance following intrathecal and intravenous administration. *Eur. Cytokine Netw.* *3*: 399-406, 1992.

Mashiba, H., Matsunaga, K.: Augmented inhibition of MethA tumor cell proliferation in combined use of diethyldithiocarbamate with catalase or by a non-dialysable fraction from co-incubation. *Toxicol. Lett.* *66*: 97-104, 1993.

Maurer, H. R.:Potential pitfalls of [^{3}H] thymidine techniques to measure cell proliferation.*Cell Tissue Kinet.* *14*: 111-120, 1981.

Meyer, B. N., Ferringni, N. R., Putman, J. E., Jacobsen, L. B., Nichols, D. E., McLaughlin, J.L.: Brine shrimp: A convenient general bioassay for active plant constituents. *Planta med.* *45*: 31-34, 1982.

Nakoinz, I., Lee, M. T., Weaver, J. F., Ralph, P.: Differentiation of the IL-3-dependent NFS-60 cell line and adaption to growth in macrophage colony-stimulating factor. *J. Immunol.* *145*: 860-864, 1990.

Nowel, P. L.: Phytohemagglutinin: An initiator of mitosis in cultures of normal human leukocytes. *Cancer Res.* *20*: 462-466, 1960.

Orjala, J., Wright, A. D., Behrends, H., Folkers, G., Sticher, O.: Cytotoxic and antibacterial dihydrochalcones from *Piper aduncum*. *J. Nat. Prod. 57*: 16-26, 1994.

Park, B. H., Matuschke, B., Lavi, E., Gaulton, G. N.: A point mutation in the *env* gene of a murine leukemia virus induces syncytium formation and neurologic disease. *J. Virol. 68*:7516-7524, 1994.

Perdue, R. E.: KB cell culture. I. Role in discovery of antitumor agents from higher plants. *J. Nat. Prod. 45*: 418- 426, 1982.

Rahalison, L.: Mise au point et applications d'une méthode de dépistage d'activité antifongique (*Candida albicans*) dans des extraits végétaux. Diss. Université de Lausanne, Institute de Pharmacognosie et Phytochimie, Lausanne. 1994.

Schlossman, S. F., Levin, H. A., Rocklin, R. E., David, J. R.: The compartmentalization of antigen reactive lymphocytes in desensitized guinea pigs. *J. Exp. Med. 134*: 741-749, 1971.

Swanson, S. M., Pezzuto, J. M.: Bioscreening technique for cytotoxic potential and ability to inhibit macromolecule biosynthesis, in: Drug Bioscreening: Drug Evaluation Techniques in Pharmacology. Thompson, E. B. (ed.), VCH, New York, Weinheim, Basel, Cambridge. p. 273, 1990.

Wagner, H., Kreher, B., Jurcic, K.: In Vitro stimulation of human granulocytes and lymphocytes by pico- and femtogram quantities of cytostatic agents. *Arzneimittelforsch.. 38*: 273-275, 1988.

Acknowledgement

We are very grateful to the healers, midwives and the inhabitants of Sto. Domingo and Sta. Maria Petapa, Sta. Maria Guenagati, and Guevea de Humboldt, for their friendship and hospitality. We would like to thank Dr. P. Davila A., O. Tellez. V., L. Torres C., R. Torres C., F. Ramos M., Dr. R. Lira S., and Dr. J.L. Villaseñor R. at the National Herbarium (MEXU), Dr. R. A. Bye Jr., and T. Balcazar S. at the Botanical Garden, Dr. C. Viesca T., Institute of Medical History and Anthropology, all at the Universidad Nacional Autónoma de México UNAM; Prof. Dr. H. Rimpler, Institute of Pharmaceutical Biology, Freiburg/Germany; for their help at various stages of the project. Special thanks are due to M. Wasescha for technical assistance and D. McLaughlin for checking the English usage in this paper. This work was financially supported by Swiss Development Cooperation (SDC, Bern) and Barth Fonds of ETH Zurich.

7 Additional Results and Discussion

7.1 Phytochemical screening with spray reagents

In addition to the biological screening also a phytochemical screening was performed. On analytical TLC plates (Merck Si 60 F_{254}, aluminium) the extracts were developed with appropriate solvent systems and visualized with several spray reagents and under UV light (254 and 366 nm). In order to get information on the plant's content of main phytochemical classes of compounds, methods were followed as described in Wagner and Bladt (1996) (Table 8-S2 and 9-S2).

Table 8-S2. Spray reagents used in the phytochemical screening.

Classes of compounds	Spray reagent
Amino acids, biogenic amines	Ninhydrin reagent
N-containing compounds	Iodplatinate reagent
Alkaloids, heterocyclic N compounds	Dragendorff
Essential oils (terpenoids, phenylpropanoids)	Vanillin/H_2SO_4 and NH_2
Flavonoids	Natural products/PEG reagent
Phenol glycosides	Millons reagent
Triterpenes/steroids (saponins, bitter principles)	Liebermann-Burchard reagent

Table 9-S2. Results of the phytochemical screening with seven spray reagents.

Plant species	Amino acids, biog. amines	N-containing compounds	Alkaloids, heterocyc. N compounds	Essential oils	Flavonoids	Phenol glycosides	Triterpenes/ steroids
B. heracleifolia	+	++	-	++	(+)	-	++
D. appendiculata	+	+	++	+++	+++	+	++
E. mexicana	++	++	++	++	+++	+	++
P. amplifolium	++	+	++	+	+++	-	++
P. calyculatus	++	++	++	++	++	-	++
S. diflorum	++	++	+	++	++	+	++
S. lanceolatum	++	+	+	+++	++	-	++
X. robustum	(+)	++	++	+	+	-	(+)
Z. pendula	(+)	+	++	+	+	-	(+)
D. drakena	++	(+)	-	++	+++	-	(+)
K. pauciflora	+	(+)	-	++	++	-	-

7.2 Bioassays: Problems and advantages

7.2.1 Extraction and preparation of samples for assays

There is an ongoing discussion about the preparation of assay samples. In an ethnobotanical approach, the traditional procedure of preparation has to be taken into consideration, otherwise this strategy is useless. In Zapotec traditional medicine, drugs are usually prepared with water as infusions, decoctions, or cold mazerations. Furthermore, fresh plant material is applied as such, ground, or toasted. Mazeration in alcohol (ethanol, mezcal, pulque) is less frequently employed. Extracts for phytochemical studies are prepared with solvents of increasing polarity in order to extract apolar and polar compounds. For this study from each plant species one crude extract was prepared combining the traditional and the phytochemical aspects of extraction. Ethanol 90% and 70% were used as solvents. Forced successive extraction (Ultra Turrax) at room temperature was combined with repeated exhaustive extraction under reflux. To remove the solvent completely, the extracts were concentrated under vacuo and then dried by lyophilisation.

7.2.2 Alleged properties and their corroboration in bioassays

Publication IV reports a broad selection of bioassays applied to crude extracts of eleven plant species selected along ethnobotanical information. Although assays were chosen specifically to the alleged properties of the plants, only a few expected results were corroborated in the test systems. This is not surprising when considering that a plant is composed of hundreds of different substances in varying concentrations. In a bioassay, plant compounds can induce, mask, or prevent a result and can also give no reaction at all. Synergistic and antagonistic mechanisms as well as aspects of concentration must be taken into consideration. It is estimated that a bioassay sample may comprise 30 to 40 assayable compounds among others, present at too low a level for "detection" (Cordell, 1995). Therefore, the screening starts already when selecting the assay and its target. Plant extracts screened in very specific tests systems (e.g. mechanism-based assays) may be considered as "uninvestigated" with respect to any other pharmacological activity.

7.2.3 Additional factors affecting bioassay's results

There are additional factors which may affect a bioassay's result dramatically:

- pH
- solvent residue
- poor solubility of an extract
- interference from ubiquitous plant compounds.

Microoroganisms are very sensitive to pH changes. A pH range from 6 to 8 is required for optimal growth in the bio-autographic assays and may be arranged with a buffer when necessary.

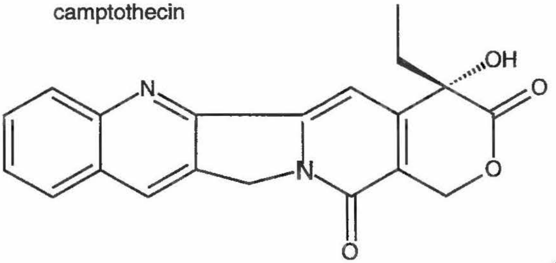
Solvent residue, especially toluene, on TLC plates disturbs microorganism growth as well. It is necessary to remove it as well as possible from the TLC plates, e.g. with a blow dryer or a heating plate before performing the assay.

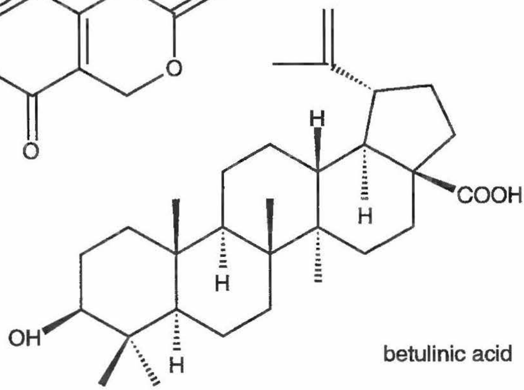
Dimethylsulfoxide (DMSO) is widely used as a solubilizing agent in aqueous sample preparations for bioassays (e.g. KB cell line, Brine shrimp). Yet many apolar substances (e.g. diterpenes) are insoluble in water. Poor solubility of an extract may produce a false negative result due to concentration factors. Hence, such extracts may be dissolved in 100% DMSO and the concentration will be adjusted while adding it to the test system. With pure compounds solubility problems have been improved with mainly two methods. Very apolar substances have been subjected to assays as co-precipitate with polyvinylpyrrolidine (Pisha et al., 1995). Likewise, clathrates with cyclodextrine are induced (Lehner, 1993) to increase solubility and bio-availability.

While testing crude extracts in sensitive assays, it is recommended to insert a first purification step before testing to remove ubiquitous plant compounds in order to reduce the possibility of "false" results. A sample can be pre-separated, over a cartridge, filled with an adsorbent or purified by precipitation of the disturbing compounds. For example, tannins may be eliminated by caffeine precipitation or polyamide, Sephadex LH20, or silica gel chromatography, but also with collagen or polyvinylpyrollidone (Wall et al., 1993). Anionic polysaccharides may be removed by precipitation from an aqueous solution with an equal volume of ethanol (Cordell, 1995). There has been no definitive study as to which method is the most efficient. Nor has there been a study on the selectivity of the procedures for the various classes of polyphenolic compounds (flavonoids, xanthones, lignans, etc.) which might also be removed (Cardellina et al., 1993).

There are many possibilities to induce false positive or false negative results and to weaken reproducibility while performing bioassays. Nevertheless, many important newer findings have been isolated on the bioassay-guided approach to drug development. Paclitaxel, the former taxol, from *Taxus brevifolia* Nutt. (Taxaceae) and camptothecin from *Camptotheca acuminata* Decne (Nyssaceae) were isolated as a result of KB cell activity-directed fractionation (Cordell, 1995; Kinghorn, 1996). From *Ziziphus mauritiana* Lam. (Rhamnaceae) betulinic acid was isolated using cultured human melanoma cells (Pisha et al., 1995). Also cyclosporin A, isolated from two fungi imperfecti, was traced by bioassays.

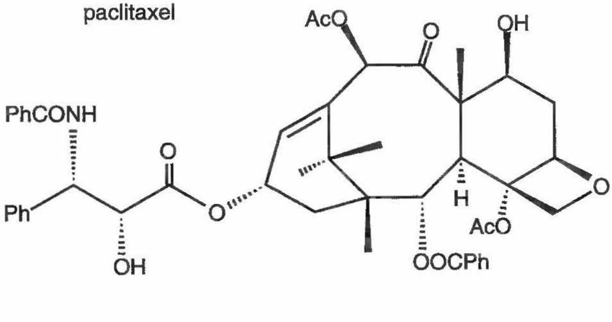
camptothecin





betulinic acid

paclitaxel



7.2.4 Activity threshold in bioassays

The detailed procedure for preparing the test samples may vary from laboratory to laboratory. Problem-oriented solutions must be found from case to case. Many research groups use a different set of microorganisms in bio-autographic assays as a result of national regulations on the handling of microorganisms. In some countries test kits are available, whereas their use may be restricted in others, due to federal laws (e.g. Bundesgesundheitsgesetz, Richtlinien der Schweizerischen Kommission für biologische Sicherheit, Tierschutzgesetz). Moreover, when reviewing scientific journals, activity is assigned to extracts with a minimum growth inhibitory amount between 2000 µg and >100 µg for bio-autographic assays! The criteria for activity seems to be a high "hit rate" instead of a selective screening! A comparison of the published data is therefore rarely possible. A literature study was performed in order to verify useful concentrations for a possible comparison with published data. As a consequence, in this work doses of ≤ 200 µg where chosen as an activity threshold in bio-autographic tests.

Does it make any sense calling for a "standardization" of bioassay procedures? Due to the numerous reasons mentioned above, a standardization of the procedures seems almost impossible. In order to strengthen reproducibility of bioassays it may be useful to establish GBP's (good bioassay practices). Furthermore, it could be interesting to compare bioassay data from the same taxonomic families. Similar chemical composition may lead to comparable reactions and may detect a reproducible pattern among several bioassays. Standardized information on a taxonomic group could help to define activity thresholds within this group or in general, for a specific assay. It will then be possible to compare future published data.

As described earlier in this chapter, the NCI has broad experience in cytotoxicity *in vitro* - screens with cell lines. Due to this fact, they were able to established a -since then often referred to- activity threshold for the KB cell assay [IC_{50} values ≤ 20 µg/ml for extracts, ≤ 4 µg/ml for pure compounds (Perdue, 1982)]. These criteria had been approached empirically and with the intention to yield about 10% active extracts. They had been confirmed since then by the isolation of several promising compounds. The difference between the value for extracts and for pure compounds seems small, when keeping in mind that a plant is composed of hundreds of substances and the active compounds may occur in too low a concentration for a reaction. On the other hand due to possible synergistic effects as often occur in extracts, the threshold must be defined at a lower value than one for pure compounds.

8 Conclusion

8.1 Why *Begonia heracleifolia*?

The method of selecting plants for further phytochemical investigation has been described in Publication IV. As a conclusion, *Begonia heracleifolia* Schltdl. & Cham (Begoniaceae) was chosen for detailed investigation. Although the ethnobotanical importance shows "only" a value of 2 for the Zapotecs, it was selected because of its regional and even international cultural importance. Additional information on the plant's medicinal use is, for example available from the neighboring Mixe communities (Heinrich, 1989), from the Huastecs of Veracruz (Alcorn, 1984), from the Popoluca of Veracruz (Aguilar et al, 1994), from various places of Central Mexico (Martinez, 1989), and from southern Mexico to Peru (Morton, 1981).

Furthermore, *B. heracleifolia* is a plant of "local", Middle American origin and it is well distributed from Mexico to Belize to Guatemala (Smith and Schubert, 1973; see also Section 3).

The pattern of the results distributed among the several antimicrobial assays indicates an interesting, not-overall activity (Publication IV, Table 2-P4). In addition, the low IC_{50} values of the cytotoxicity assays and the correlating results of the brine shrimp assay (high percentage of lethality) lead to this selection from a biological point of view. As a result, the KB cell assay was the first choice for bioactivity-guided fractionation and isolation. Simultaneously, the fractions were tested for antimicrobial activity.

8.2 New trends in plant screening for drug development

As stated in earlier paragraphs of Section 1 and Section 2 there are several approaches for plant collection and there are two fundamental strategies of *in vitro* screening: "shot gun" screening and "target-directed" screening. In the former of these strategies, the mechanism underlying an assay is not well known and the results are hints for general activities (e.g. cytotoxicity, brine shrimp lethality, molluscicidal activity). The latter method is clearly directed to one target, or in other words, the activity searched for is molecularly target-directed (Wagner, 1988). For example, high-priority target systems in the pharmaceutical industry are such as enzyme activity (e.g. protein kinases, acyl-CoA synthase), receptor binding (estrogen), receptor function (PAF, insulin), ion channel modulation (Ca^{++}), transcription events (NF-κB), or non-protein targets (blood clots) (Trueb, 1995). The advantages of target-directed screenings are: (a) such tests are specific and sometimes even organ-specific, and (b) they lend themselves very readily to automatization (Wagner, 1988). Today the pharmaceutical industry is working with automated screening robots, with through-puts up to 15,000 samples (novel and known pure compounds, plant extracts) in one week for one test system (Trueb, 1995). To process these loads of data, efficient computer programs are necessary. The strategy of the industry is to "push serendipity" by systematically exploiting all chemical, technical, and biological possibilities and their combinations. It is obvious that such methods are very expensive and out of reach of the possibilities in research programmes at the universities. While the industry has the capacity to test "everything against everything", the trend at the university could be a more new-compound-directed one, following strategies of combined ethnological, analytical, and biological methods. An other area of interest for university research could be the search for new compounds for the development of orphan drugs.

While a pure bioassay-guided approach will miss interesting lead compounds not exhibiting the tested activity, a pure chemical-analytical approach instead misses active compounds. In order to avoid isolation of known compounds techniques such as LC/MS, LC/MS/MS, LC/UV, or LC/NMR could be used at the earliest stages of separation. This on-line produced spectroscopical data may provide molecular weight and structural information on secondary metabolites of interest within the crude plant extract. By comparing such data with compound-libraries, re-isolation could be avoided. Fractions will then be tested in general or specific bioassays and those with both interesting results will be worked up and analysed (Wolfender and Hostettmann, 1996; Hostettmann, 1997a and 1997b).

Moreover, there is still the question if the isolation of pure compounds should be the only goal in drug development. Well-defined extracts also seem to be of high relevance in modern medicine (e.g. *Crataegus monogyna, Ginkgo biloba, Hypericum perforatum, Valeriana officinalis*) as well as in medicinal ethnobotany. Rational phytotherapy should not be of minor priority in future phytochemical investigation.

8.2 Is there an ethical and moral basis in plant screening?

Developing countries usually do not, and in the future will not have the money to keep up with these technological advances described above. Yet the majority of uninvestigated plant material is from poor tropical regions. Likewise as in this study, many natural product research programmes located in the northern hemisphere have extracts from the south under investigation. As described in Publication IV, an ethnobotanical approach selects specific methods to follow the activity of a plant presumed by the ethnobotanical information. However, in this study also the advantage was taken to screen the extracts on other targets such as the immunomodulating activity and others. Going the ethnobotanical approach does not mean leaving aside all other screening targets. Since there is usually enough crude extract available for multiple testing, as many chances as possible should be taken to provide to new drug development. But exactly this procedure bears some dangers concerning the so far unprotected intellectual property. Will a company pay back benefits to indigenous people when their information on the plant's use and application is irrelevant to the subsequent application of the developed drug? Usually, when it comes in research to ethics and morals, things are "getting very complex" and are "too expensive" an aspect anyway! Plant screening for drug development is too important for the future to lose its ethical and moral basis.

Section 3

Phytochemical Investigations

What is a weed?

A plant whose virtues have not yet been discovered.

Ralph W. Emerson

9 Introduction

One goal of phytochemical investigation is the isolation of secondary metabolites from plants. The driving force of such studies is to find compounds with a unknown chemical structure and/or with promising biological activity(ies) for a possible use as new drugs or as chemical leads, facilitating the development of new therapeutic agents. Ethnobotanical information is one possible approach to select plant drugs and pharmacological-biological targets for a bioassay-guided phytochemical investigation. In addition to "the revelation of a plant's virtues" by isolating pure, biologically active compounds, it is of special interest to simultaneously corroborate ethnomedicinal uses of the respective taxa. Moreover, phytochemical investigations of a plant species may yield interesting additional chemotaxonomic information on the plant's affiliation to the respective botanical family and genus.

In this present section the detailed phytochemical investigation of *Begonia heracleifolia* Schltdl. & Cham., Begoniaceae, is described. The plant was chosen based on the results described in the two previous sections. The bioactivity-guided fractionation of the crude extract and the subsequent isolation of biological active pure compounds was mainly directed by the activity detected in the cytotoxicity/anti-tumor activity assays. After forced flow extraction with solvents of increasing polarity, various chromatographic methods were employed for the fractionation of the solvent fractions as well as for the subsequent isolation of the pure compounds (see Publication V and Additional Results and Discussion). The structure elucidation employing mainly spectroscopic methods lead to several classes of compounds such as triterpenes, sterols, an oligosaccharide, fatty acids, flavonoids, and procyanidines. Four compounds were isolated for the first time in *Begonia* sp. The biological testing of the pure compounds yielded interesting data which corroborate some of the medicinal uses and applications as performed by the Zapotec healers. Furthermore, additional information on biological activity of *B. heracleifolia* against other targets not directly selected on the basis of the primary ethnobotanical information was obtained. The predominate compounds, isolated from *B. heracleifolia* confirmed chemotaxonomic information as it is known from the literature.

9.1 Botanical and phylogenetic aspects of Begoniaceae

The family of the **Begoniaceae** is divided into five genera. *Begonia*, with at least 900 species, is widespread in the tropics and subtropics. *Begoniella* is found with three species in Colombia and *Semibegoniella* with three species in Ecuador. The monotypic *Hillebrandia* with *H. sandwicensis* on Hawaii and *Symbegonia* with 10 to 12 (Smith, 1986) species in New Guinea represent the two Pacific genera (Irmscher, 1960; Takhtajan, 1996). The independent listening of *Begoniella*, *Semibegoniella* and *Symbegonia* is questionable. In more recent publications (Smith et al., 1986; Mabberly, 1993) they are all united with the genus of *Begonia*, whereas Takhtajan (1996) describes *Begonia*, *Hillebrandia*, *Semibegoniella*, and *Symbegonia* as seperate genera. Nevertheless, the South American *Semibegoniella* is very close to the almost pantropic *Begonia*. The information about the total number of species of the Begoniaceae varies. Barkley and Golding (1974) mention over 1200 different species with the addition of several "cultivars", plants that are derived from hybridization, mutation, or selection.

The **phylogeny of the Begoniaceae** is rather unclear. Usually they are botanically considered allied to the family of the Datiscaceae but plurilocular ovaries are rather uncommon in the order of the Violales and centripetal stamens are "aberrant" in the subclass of the Dilleniidae (Mabberley, 1993). Takhtajan

(1996) classifies the Begoniaceae as rather closely related to the Datiscaceae but being more advanced. Hegnauer (1964) suggested to take into consideration as well, the broad occurrence of the procyanidines in the family of the Begoniaceae. Furthermore, Hegnauer (1989) places the Begoniaceae close to the Cucurbitaceae, because of cucurbitacin, tricyclic triterpenes, found in both families. On the other hand Gershenzon and Mabry (1984) believe that these compounds, although chemically complex, are of systematic little value at higher levels of classification, while they show greater promise as taxanomic characters for intrafamilial work.

The genus ***Begonia*** is subdivided into 60 sections (Irmscher, 1960). More than 900 species are known. With nearly pantropical distribution, especially in northern parts of South America and in tropical Asia, but absent in Australia and Polynesia, begonias are considered to be of South American origin (Smith and Schubert, 1973). Plant species and members of one section normally grow more or less regionally, only three species are known to be distributed on more than one continent (Irmscher, 1960). Due to their large colorful leaves and attractive flowers they have been cultivated for ornamental purposes world-wide for many centuries.

Begonia sp. had already been mentioned in the 16th century in Aztec-Spanish sources, such as Martin de la Cruz' "Libellus de Medicinalibus Indorum Herbis" (1552) or in Fray Bernardino de Sahagún's Códice Florentino (1570). "Quauhtla-xoxocoyollin" means wild (= quauhtla), very sour (= xoxo), tuber (= coyollin), and was identified as *Begonia* sp. Sahagún listed three as edible, one as edible but extremely sour, and several others as powerful purgatives (Delfeld, 1996).

9.2 Botany and Medicinal Uses of *Begonia heracleifolia*

9.2.1 Botany (Irmscher, 1960; Thorne, 1983; Mabberley, 1996)

Division: Spermatophyta

Subdivision: Angiospermae

Class: Dicotyledonae (=Magnoliatae)

Subclass: Dilleniidae

Superorder: Violanae

Order: Violales

Suborder: Begoniineae

Family: Begoniaceae

Genus: Begonia

Section: Magnusia

Species: ***Begonia heracleifolia* Schlechtendal & Chamisso**

The name of this plant species may be translated and explained as:

- begoniinus = H.C.C. 6.19 (Horticultural Color Chart), a color near coral pink
- heracleifolia = leaves similar to *heracleum* (the bear's paw).

Macroscopic and microscopic botanical description (Figure 1-S3):

Herbaceous, very variable in indument, coloration of leaves, size of inflorescence, and form of capsule-wings; tuberous repent rhizome (7-15 cm long), 2 cm thick, simple, internodes very short; leaves developing with the flowers, stipules triangular, setose-acuminate, entire, 12-20 cm long, succulent petioles, erect, stout, 3-40 cm long, from densely hirsute to nearly glabrous, red-punctuate; trichomes especially large and dense at the apex of the petiole, leaf-blade palmately 7-lobed 1/2 to 2/3 toward base, subcordate, 8-26 cm wide, irregularly sinuate-dentate with

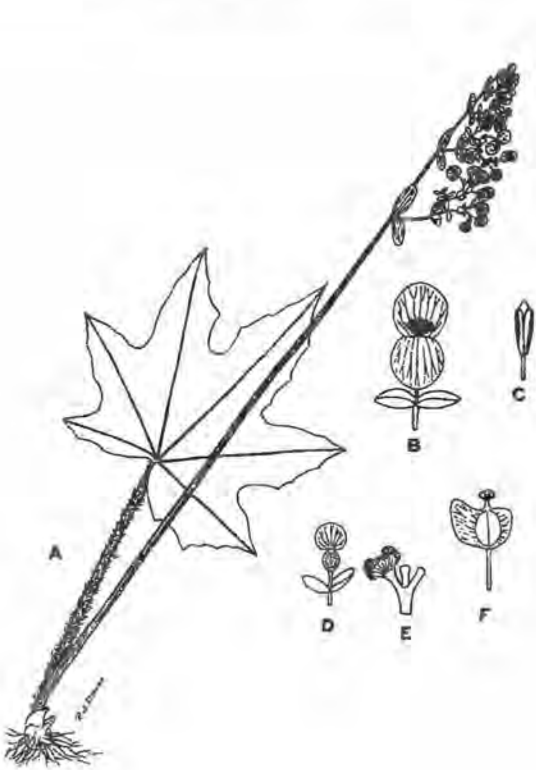


Figure 1-S3. *Begonia heracleifolia*. A: Habit (x1/4). B: Staminate flower and bracts (x1). C: Stamen (x5). D: Pistillate flower and bracts (x1). E: Style (x5). F: fruit (x1). (from Smith and Schubert, 1973)

acuminate lobes, nearly or quite glabrous above, more or less hirsute beneath on the nerves and margin, thin, color green and red or with broad, nearly black margins; peduncles mostly exceeding the leaves, 2-7 cm long, more or less hirsute; inflorescence tending to develop on one side much more than on the other, bisexual, up to 25 cm long, many-flowered, sometimes red-punctuate, bracts persistent, broadly ovate or elliptic, entire or serrate, green or red, the lowest 2 cm long; pedicles slender, 8-18 mm long; staminate tepals 2, suborbicular, 10-14 mm, white or rose; stamens numerous, subfree, filaments short, anthers oblong; pistillate flowers ebracteolate; pistillate tepals 2; ovary 3-celled, placenta bifid, ovuliferous on all sides, styles connate at base, stigmatic tissue lunate at their apices; capsule erect or somewhat nutant, suborbicular, 8-12 cm long, wings unequal, seeds ellipsoid, blunt (Smith and Schubert, 1973). Habitat (Figure 2-S3): saxicolous in forests, 50-1500 meters over see level, Guatemala, Belize and Southern Mexico (Smith and Schubert, 1973).

Figure 2-S3. *B. heracleifolia* growing saxicolously in the shady and humid tropical ombrophilous forest near Sto. Domingo Petapa. Blooming with many-flowered light pinkish inflorescence (Voucher specimen FREI 66).

9.2.2 Zapotec medicinal uses

Information from Don Estanislao Márquez Jiménez from Sto. Domingo Petapa

Uses: **rheumatism, pain, wounds, tumor, and local infections (e.g. acne, comedo, pustule, insect bite, sting)**

Plant part used: **rhizomes and succulent petioles**

Preparation and application:

(a) fresh plant material ground and applied topical as a cataplasm,

(b) fresh plant material macerated in alcohol, extract applied topical.

Other uses of *B. heracleifolia* in Mexico: infusion against constipation, flower stalk eaten as greens (Martinez, 1984), mouth sores (Alcorn, 1984), gonorrhea (Heinrich, 1989), and syphilis (Martinez, 1989).

Other medicinal and non-medicinal uses of *Begonia* sp. in different places of the world:

Uses	Preparation and Application	Location
• coughs, colds, tuberculosis, fever	• syrup, tea	• Middle/South America[1]
• inflammation, bilious fever	• tea	• Haiti[1]
• tumor, furuncles	• poultice	• Haiti[1]
• eye ailments	• fresh juice	• Venezuela, Mexico, Colombia[1]
• cuts, contusions, ulcer, snakebites, stomach	• fresh, powdered, poulticed, decoction,	• Southern Mexico to Peru[1], Guatemala[1]
• vegetable	• fresh	• Phillipines[2]
• intestinal worms	• tea	• Amazonian region[3]
• urinary disorders	• fresh, chewed, juice	• India[4]
• cancer	• poulticed	• China[5]
• antidote (*Dioscorea*)	• tea	• Phillipines[6]
• purgant, vomit	• fresh, ground	• Mexico[7]
• ear, eye, nose, throat, moth sores,	• fresh, bath	• Mexico[8]
• contraceptive	• --	• Central America[9]
• repellent	• fresh	• [5]
• ornamental	• fresh	• Middle/South America[1], Sri Lanka[1]

[1] Morton, 1981; [2] von Reis and Lipp, 1982; [3] Duke and Vasquez, 1994; [4] Jamir and Rao 1990; [5] EthnobotDataBase (http://www.ars-grin.gov/~ngrlsb/ethnobot.htlm, 1996); [6] von Reis Altschul, 1973; [7] Martinez, 1989; [8] Alcorn, 1984; [9] de Laszlo and Henshaw, 1954.

9.3 Botanical and chemical investigations of *Begonia* sp.

Biological and chemical investigations of *Begonia* sp. started in 1944 by Rodriguez with a general chemical analysis, but she only investigated the classes of compounds present. **Biotechnological publications** on growth regulation, habit, and hybridization undertaken on *Begonia* sp. are on-going and numerous (e.g. Myster et al. 1997), probably also due to the plant's importance as an ornamental. *Begonia* species have also been investigated in *in vitro* **systems for enhanced production of phytochemicals** (George et al., 1994). From 1957 until now, studies on **anthocyanin** pigments of the plants have been conducted in many laboratories (identification by comparison on paper chromatography and TLC, isolation and structure elucidation: Bopp, 1957; Harborne, 1963; Langhammer and Grandet, 1974; Ensemeyer, 1980; Ensemeyer et al., 1980; Ensemeyer and Langhammer, 1984; George et al., 1994; Chirol and Maurice, 1995). **Branched trisaccharides** in the anthocyanins and in relation to the occurring **flavonoid glycosides** have been investigated by Harborne (1963, 1964) and Hansmann (1990) with paper chromatography, by isolation and were as well characterized after synthesis. Phytochemical studies on isolation and structure elucidation of pure compounds are published on the species *B. tuberhybrida* (horticulturally) (Nordal and Resser, 1966), *B. tuberhybrida* Voss var. *alba* (Doskotch et al., 1969; Doskotch and Hufford, 1970), *B. malabarica* Lamk. (Desai et al., 1975), *B. glabra* Aubl. (Ensemeyer, 1980; Ensemeyer et al., 1980), *B. fagifolia* Fischer (Ensemeyer and Langhammer, 1984), *B. erythrophylla* Neum. (Vereskovskii et al., 1988a, 1988b). The compounds have been isolated from either leaves, petioles or from all aerial parts and include:

- anthocyanin glycosides of cyanidin and pelargonidin with one to three sugar moieties, e.g. such as the branched 2-*O*-(β-D-glucopyranosyl)-6-*O*-(α-L-rhamnopyranosyl)-α-D-glucopyranoside
- monomeric and dimeric procyanidins: catechin, epichatechin, procyanidin-B1, procyanidin-B2
- flavone C-glycosides: vitexin, isovitexin, orientin, iso-orientin
- flavonol O-glycosides: rutin, quercitrin, cynaroside
- flavonoids: 3-O-methyl quercetin, 3-O-methyl kaempferol, quercetin, luteolin
- lipophilic flavonols: quercetin-3,3',7-trimethylether; 8-methoxy-quercetin-3,3',7-trimethylether (ternatin)
- sterol: stigmasterol
- triterpenes: cucurbitacin B, D, dihydrocucurbitacin B, hexanor-cucurbitacin D, A
- organic acids: oxalic acid (sap: pH 1.6-1.3), fumaric acid, citric acid, succinic acid, malic acid, phorbic acid
- saccharides: glucose, fructose, saccharose

Many of the compounds listed, occur rather ubiquitously in higher plants and therefore, do not contribute much to chemotaxonomy. Exceptions may be the triterpenes and the branched trisaccharides (see Phylogeny of the Begoniaceae). Investigation on biological activity is only available for the cucurbitacins, where strong cytotoxicity was described (Doskotch et al., 1969). Nevertheless, general pharmacological information on natural products [e.g. flavonoids --> anti-inflammatory; procyanidins --> anti-inflammatory, weak anesthetic and bacteriostatic (see Publication II), antitumor activity (Ensemeyer, 1980)] corroborate many of the listed ethnomedicinal uses.

With only five plant species "investigated", seven studies performed until the early eighties and most of them with special emphazes on anthocyane glycosides, as well as minimal studies on bioactivity, the plant species *Begonia* was considered as phytochemically less explored. Based on the promising results of the biological screening and due to ethnobotanical importance *B. heracleifolia* was chosen for further phytochemical investigation. **Publication V** describes briefly the isolation of the most important compounds and their biological activities. Additional results and more details on procedures can be found in the discussion part of this section.

10 Publication V

Phytochemical and Biological Investigation of *Begonia heracleifolia*: Structure-Activity Studies on Cucurbitacins

Paper submitted, *Planta Medica*

Barbara Frei [1], Michael Heinrich [2], Dieter Herrmann [3], Jimmy E. Orjala [1, 4], Joachim Schmitt [3], and Otto Sticher [1,5]

[1] Department of Pharmacy, Swiss Federal Institute of Technology ETH Zurich, CH-8057 Zürich, Switzerland

[2] Institute of Pharmaceutical Biology, Albert-Ludwigs-University, D-79104 Freiburg, Germany

[3] Boehringer Mannheim GmbH, Dept. of Molecular Pharmacology/New Indications, D-68305 Mannheim, Germany

[4] Present address: AgraQuest Inc., Davis, CA 95616, USA

[5] Address for correspondence

Abstract: From the rhizomes of *Begonia heracleifolia* six known cucurbitacins (**1**-**6**) were isolated. Based on spectral data (1D and 2D ^{1}H-, ^{13}C-NMR, ESI- and CI-MS) the structures were established as cucurbitacin B (**1**), cucurbitacin D (**2**), 23,24-dihydrocucurbitacin D (**3**), 23,24-dihydrocucurbitacin F (**4**), 2-O-β-glucopyranosyl-cucurbitacin B (**5**), and 2-O-β-glucopyranosyl-cucurbitacin D (**6**). Four of them (**3-6**) were so far not reported as constituents of *Begonia* spp. Further isolation and structure elucidation yielded known compounds including three sterols, a sterol glycoside, five fatty acids, and an unidentified tetrasaccharide. Strong antiproliferative activity for tumor cells and immune cells was observed for three compounds (**1-3**), which may be due to common structural features. All of the isolates (**1-6**) were further tested in antifungal and antibacterial assays and found to be inactive.

In continuation of an ethnobotanical study on plants used in traditional medicine in Mexico, we have selected *Begonia heracleifolia* Schltdl. & Cham. (Begoniaceae) for further phytochemical investigation due to the evaluated bioactivity of the crude extract in a multiple screening ([45]). *Begonia heracleifolia* is a native species of humid Southern Mexico ([46]). Fresh rhizomes and petioles are used by the Zapotec Indians of Oaxaca to treat wounds, local infections (such as acne, comedo, pustules, insect bites, and stings), rheumatism, pain, and "tumors" ([47]). In the course of the biological screening of *B. heracleifolia* several

---

References

[45] Frei, B., Heinrich, M., Bork, P. M., Herrmann, D., Jaki, B., Kato, K., Kuhnt, M., Schmitt, J., Schühly, W., Volken, C., Sticher, O. (1997) Phytomedicine. submitted.

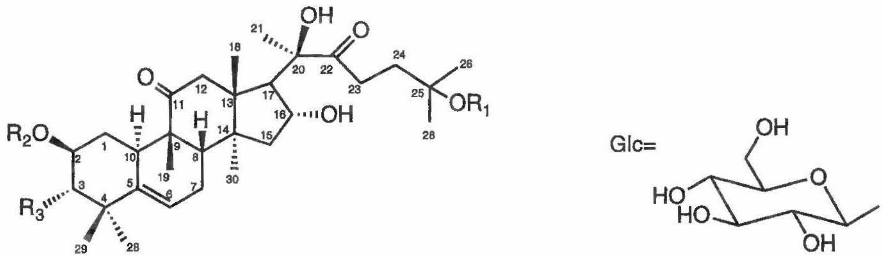
[46] Smith, L.B., Schubert, B.G. (1973) Fieldiana Bot. 24, 157-185.

[47] Frei, B. (1997) Medical Ethnobotany of the Isthmus-Sierra Zapotecs (Oaxaca, Mexico) and Biological-Phytochemical Investigation of Selected Medicinal Plants. Thesis ETH. in preparation.

fractions showed antibacterial and cytotoxic/antitumor activity with noteworthy specific inhibition of cytokine-dependant cell lines ([45]). Bioactivity-guided fractionation yielded six cucurbitacins (**1**-**6**). We report the isolation and biological activity of these compounds; **3-6** were isolated for the first time from the genus of *Begonia*. Further isolation and structure elucidation yielded the compounds stigmasterol, the C-24 epimers spinasterol (24α) and chondrillasterol (24β), 3-O-β-D-glucopyranosyl stigmasterol, esters of five fatty acids (arachidic, behenic, linoleic, palmitic, and stearic acid), and a so far unidentified tetrasaccharide composed of α- and β- glucose and α- and β-fructose.

All compounds showed no activity (> 20 μg) against several bacterial, fungal and yeast targets in an agar overlay method ([48]) [*Bacillus subtilis* (ATCC 6633), *Micrococcus luteus* (ATCC 9341), *Escherichia coli* (ATCC 25922), *Bacillus cereus* (ATCC 10720), *Mycobacterium fortuitum* (Inst. of Microbiology, University of Zurich), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Pseudomonas aeruginosa* (ATCC 27853), *Candida albicans* (H29 ATCC 26790)]. The antibacterial activity of the crude extract ([45]) must therefore be due to other compounds.

[48] Rahalison, L. (1994) Mise au point et applications d'une methode de depistage d'activité antifongique (*Candida albicans*) dans des extraits vegetaux. Thesis Université de Lausanne, Institute de Pharmacognosie et Phytochemie, Lausanne.



Compound	R_1	R_2	R_3	other	name
1	$COCH_3$	H	=O	$\Delta^{23,24}$	cucurbitacin B
2	H	H	=O	$\Delta^{23,24}$	cucurbitacin D
3	H	H	=O	-	23,24-dihydro-cucurbitacin D
4	H	H	OH	-	23,24-dihydro-cucurbitacin F
5	$COCH_3$	Glc	=O	$\Delta^{23,24}$	2-glc-cucurbitacin B
6	H	Glc	=O	$\Delta^{23,24}$	2-glc-cucurbitacin D

The potential of the compounds **1-6** to influence various further biological targets was assessed with several in vitro test systems by liquid scintillation counting after thymidine incorporation (exception: KB cells by protein determination along Lowry method). For the determination of cytotoxic/antitumor activity cultured KB (human nasopharyngal carcinoma), 3T3 (murin embryonic fibroblasts), PC3 (human prostate carcinoma), and MethA (murine MethylcholAntrene-induced fibrosarcoma) cells were tested with the test substances and processed as described previously ([45]). In addition, potential immunomodulation was investigated by evaluating the influence on proliferation of mitogen ConA activated, Interleukin-2 dependent murine lymphoblasts and mitogen ConA activated murine spleen cells ([45]). Compounds **1-3** showed cytotoxic/antitumor activity against KB, 3T3, PC3, and MethA cell lines (Table 1-P5). The immunomodulatory activity observed *in vitro* is not of interest due to the activity pattern and its combination with the results of the compounds' influence on growth of permanent cell lines. Compounds **4-7** showed no activity (IC_{50} > 20 µg/ml) in the test systems mentioned.

Due to their cytotoxicity against various tumor cell lines ([49]) with unusual potency, cucurbitacins have been investigated extensively. Recently, pharmacological studies on their possible mode of action (e.g. actin disruption, antiproliferative, antimitotic) have been reported ([50, 51, 52]) showing evidence of

[49] Cassady, J. M., Suffness, M. (1980) Terpenoid antitumor agents. In: Cassady, J. M., Douros, J.D. (eds.), Anticancer Agents Based on Natural Products Models, Academic Press, New York, 247-254.

[50] Ryu, S. Y., Choi, S. U., Lee, S. H., Lee, Ch., O., No, Z., Ahn, J. W. (1995) Arch. Pharm. Res. 18, 60-61.

[51] Duncan, K. L. K., Duncan, M. D. Alley, M. C., Sausville, E. A. (1996) Biochem. Pharmacol. 52, 1553-1560.

[52] Bar-Nun, N., Mayer, A. M. (1989) Phytochemistry 28, 1369-1371.

structure-activity relationship. The structural sites specifically associated with cytotoxicity are: α,β-unsaturated ketone in position C-22, a 25-acetate group, free 16 α-OH, free 20 β-OH, ring A with either diosphenol or 3-ketol structure ([49, 50, 51]).

Table 1-P5 clearly shows the decrease in activity parallel to each loss of a structural requirement for cytotoxicity. Compound **1** with all of the prerequisites for cytotoxicity shows strongest inhibition in all test systems. The elimination of the acetyl group at C-25 of compound **2** results in lower toxicity. Furthermore, compound **3** without an α,β-unsaturated ketone moiety in the C-17 side chain and hydroxylation at C-25 demonstrates further notable reduction in its toxicity. The biological activity is totally lost when ring A shows a 2,3-diol structure as in compound **4**. Moreover, the isolated 2-O-β-glycosides (**5** and **6**) of the highly toxic compounds **1** and **2** show no inhibition on tumor growth *in vitro*.

The biological results presented here strongly support previous assumptions based on pharmacological and structure-activity studies about cucurbitacins: The importance of the side chain at C-17, mediating specific affinity interactions by its α,β-unsaturated ketone in position C-22 and/or the 25-acetate group (**1**, **2**). Yet the results additionally outline the strong influence of the substitution pattern in position C-2 and C-3 of ring A (**3**-**6**). Not only the presence of a 3-ketol but in combination with a 2-OH seems essential due to complete loss of activity when hydroxylated in position C-3 (**4**) or glycosidated in position C-2 (**5**, **6**). This is further supported by the fact, that cucurbitacins with a glycosidic bond in position C-16 still show strong activity ([53]).

[53] Kupchan, S. M., Sigel, C.W., Guttman, L.J., Bryan, R.F. (1972) J. Am. Chem. Soc. 94, 1353-1354.

Table 1-P5. Effect of compounds **1-6** and reference drugs on proliferation and stimulation of human and murine cells.

	IC_{50} (µg/ml)					
	Immunomodulatory activity		Influences on growth of permanent cell lines			
Compounds	IL-2 depend. lympho-blasts	ConA activated spleen cells	KB	3T3	PC3	MethA
1	0.017	0.03	0.01	1.8	0.003	0.15
2	0.25	0.09	0.032	2.75	0.04	0.65
3	1.0	0.46	0.021	> 10	0.4	3.81
4	> 20	> 20	> 20	> 20	> 20	> 20
5	> 20	> 20	> 20	> 20	> 20	> 20
6	> 20	> 20	> 20	> 20	> 20	> 20
cyclosporin A	>1	0.0001	--	>1	>1	>1
cis-platin	0.44	0.84	--	0.21	0.77	0.12
podophyllotoxin	--	--	0.01	--	--	--

Three of the isolated compounds were shown to be biological active and presumably are responsible for at least part of the cytotoxicity of the crude extract.

Pharmacological and biological effects of cucurbitacins including cytotoxicity have been reviewed recently ([54]) and are numerous. Restricting the external use of *Begonia heracleifolia* seems to be unfounded, but reduced oral application should be recommended.

Materials and Methods

Rhizomes of *B. heracleifolia* were collected in October 1994 in a rocky area of the tropical forest of Sto. Domingo Petapa (Oaxaca, Mexico). Voucher specimens (FREI 66) have been deposited at the following herbaria: MEXU (UNAM, México D.F.), ZT, (ETH Zurich, Switzerland), and the Inst. Pharm. Biol., University of Freiburg, Germany.

Compounds **1-6** were obtained from the methanol extract (50.7 g) of air-dried, powdered rhizomes (505 g). A combination of partition between BuOH - H_2O and $CHCl_3$ - MeOH/H_2O followed by MPLC (column: 26 x 800; RP-18; ACN - H_2O, gradient 3 : 7 to 1 : 1; 60 ml/fraction) yielded compound **1** (12.9 mg). Purification with HPLC (Spherisorb ODS II S5; column: 16 x 250; ACN-H_2O, 3.5 : 6.5) of fr. 38 from MPLC led to compound **5** (36.2 mg) and a mixture of the compounds **2** (8.4 mg) and **3** (7.4 mg). The latter were purified by open column separation (hexane-EtOAc-iso-PrOH, 10 : 2 : 1 to 80 : 2 : 1). Purification of fr. 13 from MPLC with HPLC (Spherisorb S5 ODS II; column: 16 x 250; ACN:H_2O, 2.5 : 7.5) yielded compound **4** (4.6 mg) and **6** (5.7 mg). Identifications of **1-6** were performed by 1D-, 2D-NMR (^1H, ^{13}C, DFQ-COSY, HMBC, HMQC, ROESY) and MS-data (ESI, CI) and by the

[54] Miró, M. (1995) Phytotherapy Res. 9, 159-168.

comparison of spectroscopic data with those reported ([55, 56, 57, 58, 59, 60, 61, 62, 63]). Copies of the original spectra are obtainable from the author of correspondence.

---

55 Jacobs, H., Singh, T. (1990) J. Nat. Prod. 52, 1600-1605.

56 Che, C. T., Fang, X., Phoebe, C. H., Kinghorn, A. D.; Farnsworth, N. R. (1985) J. Nat. Prod. 48, 429-434.

57 Halaweish, F. T. (1993) J. Chem. Ecol. 19, 29-37.

58 Vande Velde, V., Lavie, D. (1983) Tetrahedron 39, 317-321.

59 Fang, X., Phoebe, C.H., Pezzuto, J. M., Fong, H.S., Farnsworth, N.R. (1984) J. Nat. Prod. 47, 988-993.

60 Kasai, R., Matsumoto, K. Nie, R., Morita, T., Awazu, A., Zhou, J., Tanaka, O. (1987) Phytochemistry 26, 1371-1376.

61 Bauer, R. Berganza, L.H., Seligmann, O., Wagner, H. (1985) Phytochemistry 24, 1587-1591.

62 Yamada, Y., Hagiwara, K., Iguchi, K., Suzuki, S., Hsu, H.Y. (1978) Chem. Pharm. Bull. 26, 3107-3112.

63 Liu, J., Davidson, R.S. (1994) J. prakt. Chem. 336, 16-18.

11 Additional Results and Discussion

The most important phytochemical results from the investigation of *B. heracleifolia* have been outlined in Publication V. Additional results as well as more detailed information on isolation, structure elucidation, and biological activity will be given in these following paragraphs.

11.1 Isolation procedures

11.1.1 Extraction

505 g (~ 15 kg fresh plant material) of rhizomes, dried in the secadora, were powdered, moistened with n-hexane, and mixed with 50 g of quartz sand. A Büchi® MPLC column (26x800 cm) was filled with quartz sand approximately 1 cm deep. The plant material was poured into the column and covered with a final layer of quartz sand. Forced flow successive extraction was performed by pumping solvents of increasing polarity (n-hexane, dichloromethane, ethyl acetate, methanol, 50% aqueous methanol) through the column with a Laboprep-MPLC pump (Labomatic®). The pump was stopped overnight to allow maceration. After four days the solvent was changed. The extracts were dried under evaporation and stored in the freezer at -20°C (Figure 3-S3).

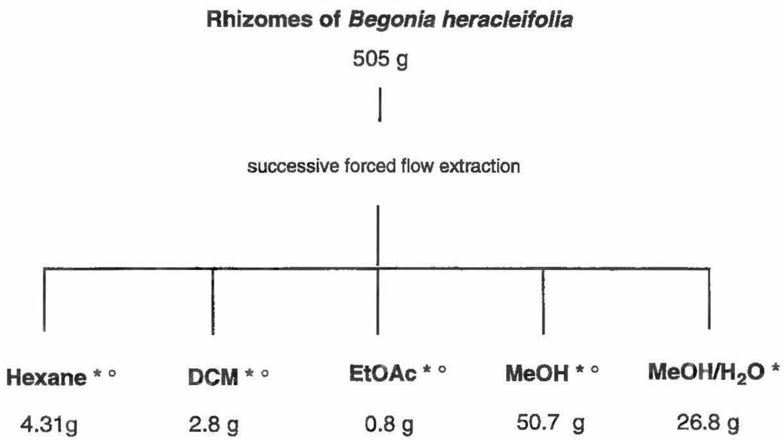


Figure 3-S3. Extraction scheme. * with antibacterial activity, ° with KB cell activity

11.1.2 Fractionation

Fractionation of extracts and isolation of pure compounds were conducted by various chromatographic methods with appropriate solvent systems as in general described below. Detailed conditions are given in the individual steps of the separation table (Figure 4 and 5-S3) and in Publication V. After each step, the fractions obtained were tested in the KB cell assay and in the bioautographic TLC assay against the bacteria *M. luteus*, *B. subtilis*, and *E. coli* as well as being monitored with ^{1}H-NMR spectroscopy in order to locate the activity and to provide direction for further separation procedures.

Chromatographic methods:

Vacuum liquid chromatography (VLC)

VLC was used as initial separation procedure of the crude extracts and as well for pre-purification prior to separation on RP-HPLC. Materials employed as stationary phases were normal (NP: silica gel) and reversed (RP: RP-18) phases with TLC grade. In one case, aminopropyl bonded (NH_2; Bond Elut®) phases in prepacked cartridges were used. Extremely important for a successful separation was the dry packing of the stationary phase under applied vacuum in order to obtain a hard cake [column size in this study: l = 220 mm; ø 40 mm (hexane-extract); l = 220 mm; ø 70 mm (DCM-extract)]. Extracts were applied either dissolved in the starting mobile phase or adsorbed on quartz sand or on kieselguhr. The separation was forced by a water suction pump producing a vacuum (Coll and Bowden, 1986).

Medium pressure liquid chromatography (MPLC)

MPLC was applied as a subsequent purification step after VLC for large and complex fractions. In this study, the materials used as stationary phases were reversed phases (RP-18) of a particle sizes 40 - 63 µm. The column (l = 800 cm; ø 26 cm) was slurry packed (Hostettmann, Hostettmann, and Marston, 1986) and the sample dissolved in the mobile phase was injected via a 4-way valve. A Büchi® piston pump was connected producing flow rates of the mobile phase from 3 - 160 ml/min.

Liquid-liquid partition (LLP)

The LLP method makes use of the different partition coefficients of compounds in two non-miscible phases. This mild fractionation was employed to separate crude extract as well as less complex fractions and guaranteed minimal loss of substances. Non-miscible systems, as used in this study, were butanol-water, and methanol/water-chloroform.

High pressure liquid chromatography (HPLC)

HPLC was usually applied as final separation step. The guard columns employed and the main separation columns were both filled either with normal (NP: silica gel) or reversed (RP: RP-18) phase material. The method employed is called semipreparative HPLC due to the used column sizes (l = 250 mm; ø 8 - 16 mm) and the particle size (5-10 µm) of the stationary phase. These allow to load samples from 1 to 100 mg in each injection (Unger and Weber, 1995).

Open column chromatography

Conventional open column chromatography was useful for the separation of small fractions when the desired compound was present in high amounts [column size: l = 42 cm, ø 3.5 cm (sterol); l = 29 cm ø 2.5 cm (oligosaccharide)]. It was also successfully used in the purification of a very small, HPLC-unseparable, mixture of two triterpenes (column size: l = 30 cm, ø 1.65 cm). Since in open column chromatography the system is "open", no additional pressure is applied and separation works by gravity. The materials used as stationary phase were normal phases (NP: silica gel).

Thin layer chromatography (TLC)

Normal phase and reversed phase TLC was used as a method to optimize the mobile phases for open column chromatography, MPLC, and HPLC. In combination with the "PRISMA" model (Nyiredy et al., 1985), which is based on Snyder's solvent characterization (Snyder, 1978), it was possible to establish a suitable mobile phase in three steps. TLC was further employed for small scale preparative separation, in bioautographic TLC assays, for monitoring chemical derivation (acetylation, acid hydrolysis), and chromatographic separations. Cucurbitacins were visualized with the specific spray reagent vanillin/H_3PO_4 (Bauer and Wagner, 1983).

Gas Chromatography (GC)

Gas liquid chromatography in combination with mass spectrometry (GC-MS) was applied in order to study a VLC fraction of the hexane extract which showed signals of eicosanoids in preliminary NMR measurements. Due to the instability of the eicosanoids the identification was not possible but yielded information on the fatty acid composition of the fraction. A nonpolar capillary column, Ultra 1 (Hewlett Packard), of 100% dimethylsiloxane was used. The sample was analyzed after methylation.

Isolation tables:

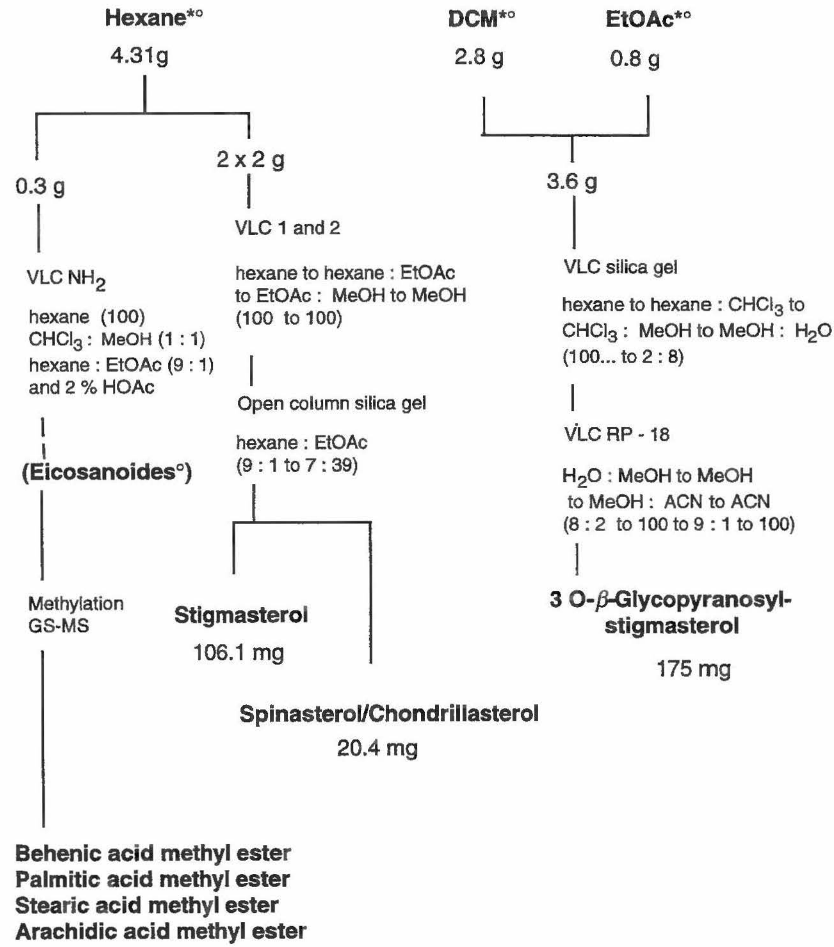


Figure 4-S3. Isolation table of the hexane and the combined dichloromethane and ethyl acetate extracts. * with antibacterial activity, ° with KB cell activity

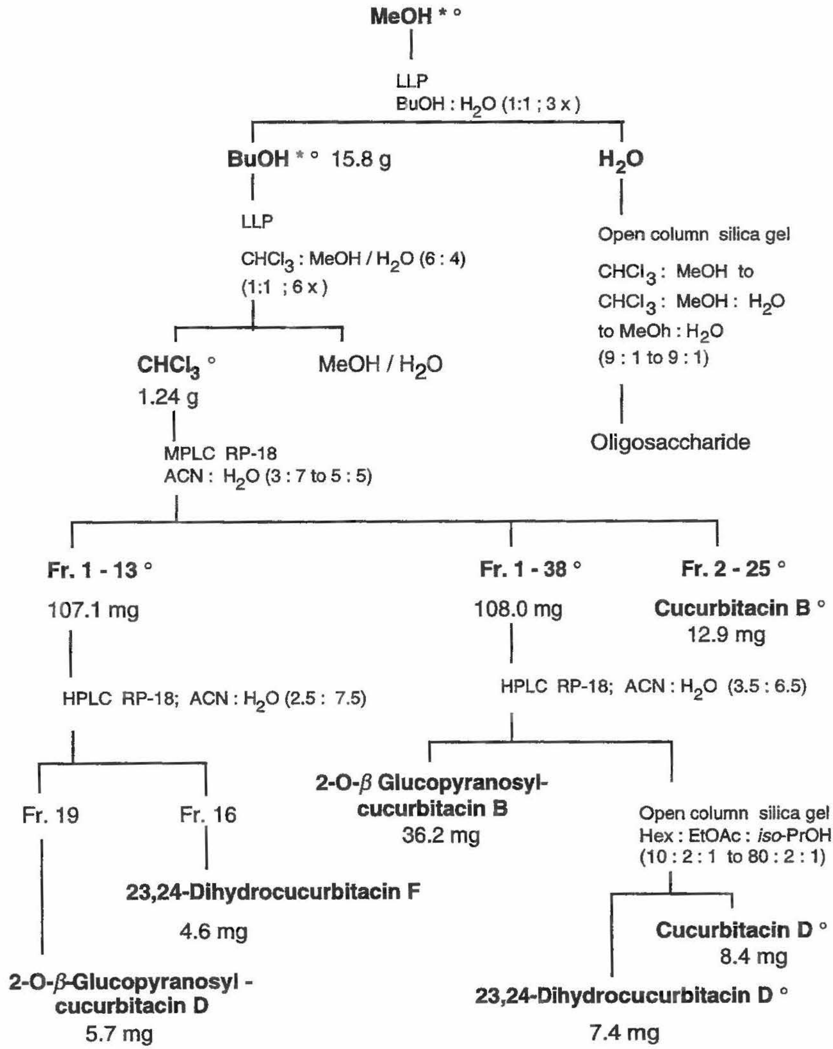


Figure 5-S3. Isolation table of the methanol extract. * with antibacterial activity, ° with KB cell activity.

11.2 Structure elucidation

Various spectroscopic and chemical methods were employed in this study for the structure elucidation of the pure compounds. Due to the fact that all isolated compounds were previously characterized from other plant species, the usual strategy for structure elucidation of unknown compounds was restricted. The used strategy and methods are described as follows.

11.2.1 Methods

Nuclear Magnetic Resonance spectroscopy (NMR)

NMR spectroscopy has become the most important method for the determination of molecular structures by studying nuclear magnetic moments. When placed in a strong magnetic field, nuclei that have a nonzero spin quantum number (in this study: **^{1}H, ^{13}C** = 1/2) are able to absorb energy from the radio frequency range of the electromagnetic spectrum. The magnetic field strengths applied corresponded to the ^{1}H resonance frequency of 300 MHz and the ^{13}C frequency of 75.5 MHz (Bruker AMX-300 spectrometer, Spectrospin AG, Fällanden, Switzerland).

In the pulsed Fourier transformation NMR spectroscopy, all nuclei of a particular isotope are activated simultaneously by a short-duration radio frequency pulse. The absorbed energy is subsequently lost to the surrounding or to other nuclei over a period of time (relaxation). The resulting magnetization is monitored and the respective signal (free induction decay; FID) is digitized. This process is repeated until sufficient signals have been added into a computer memory. They are then converted mathematically (Fourier transformation) into interpretable spectra. A first estimation of the number of protons and carbons is possible.

The frequency at which a nucleus is able to absorb energy is characteristic of the environment of the particular nucleus (δ; chemical shift in ppm). Functional groups or structural fragments having characteristic ^{1}H and ^{13}C chemical shift values may be identified with data from the literature. The pattern of signals (singulet or split into multiplets) provides further information of the interconnection of coupled nuclei. The frequency difference between such multiple lines is known as the coupling constant J (Hz) and depends on the number and nature of bonds and the angular relationship of the coupled nuclei (Byrne, 1993).

The **DEPT** (distortionless enhancement by polarization transfer) method, a third one-dimensional (1D) experiment, transfers the magnetization from protons to their directly attached carbons and allows the determination of carbon multiplicities. The resonances can be distinguished by either positive (CH, CH_3) or negative (CH_2) signals, while fully substitute carbons do not appear.

Two dimensional (2D) NMR experiments provide further information on the interaction between nuclei such as connectivities over one or more bonds and through space. The scalar coupling techniques (see 1, 2, 3) provide the direct connectivities between the atoms, while dipolar coupling techniques (see 4) give the internuclear distances. In the present study, the experiments described below (Zerbe, 1996) have been employed and information for structure elucidation was gained following this strategy (1-4) of interpretation:

1) Heteronuclear multiple quantum correlation (HMQC): providing correlation of directly bonded protons and carbons,

2) Homonuclear, double-quantum filtered correlation spectroscopy (DQF-COSY): showing vicinal/geminal proton correlation,

3) Heteronuclear multiple bond correlation (HMBC): detecting proton, carbon correlation via long-range (^2J, ^3J) couplings,

4) Homonuclear, rotating frame nuclear Overhauser effect spectroscopy (ROESY): providing correlation via dipolar interaction of nuclei close in space.

Mass spectrometry (MS)

To corroborate the structural information obtained from NMR measurements, the mass of the respective molecule was investigated by mass spectrometrical methods. Mass spectrometry involves the generation of gas-phase positive or negative ions from molecules. Subsequent separation sorts the ionic fragments by their masses. The mass spectra provides the molecular ion (M^+), structural information regarding functional groups and the abundance of fragments (Bloor and Porter, 1993). Techniques employed in this study are (Bloor and Porter, 1993; Baldwin, 1995):

- (D)CI^+-MS, direct chemical ionization: generating positive ions of involatile or labile compounds (dissolved in methanol) with the reagent gas ammonia (NH^+_4) resulting in proton transfer or electrophilic addition

- ESI-MS, electronspray ionization: involatile sample injected in a liquid stream of methanol-water (acidified), N_2 encourages the evaporation and ions are formed by proton attachment

- FAB^+-MS, fast atom bombardment mass spectrometry: suspension of involatile sample in a liquid matrix (3-NOBA), bombarded by fast argon or xenon atoms displacing ions (observed ions: + Na^+, +K^+, +H^+)

Optical rotation

Molecular chirality results in the phenomenon of optical activity, the ability to rotate the plane of polarized light. The angle (α) through which the plane of polarization has been rotated was measured under defined conditions (c [g/100 ml]; t [°C]; l [cm]) using light corresponding to the D line of sodium (λ 589 nm). The specific rotation $[\alpha]_D$ is as characteristic property of any chiral compound and is of (–) configuration for a left turned or of (+) configuration for a right turned plane.

Chemical derivation

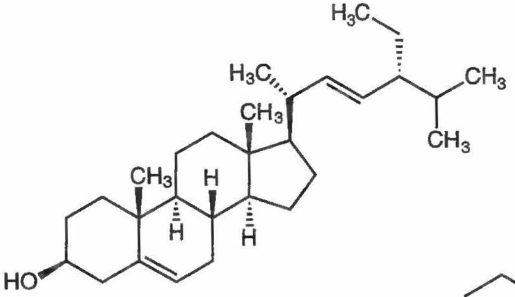
-Acetylation: In order to change solubility properties (stigmasterol glycoside) or to determine glycosidation patterns (oligosaccharide), compounds were acetylated for facilitating the structure elucidation. A quantity (10 mg) of compound was dissolved in pyridine (0.5 ml) and Ac_2O (0.5 ml) and kept at room temperature overnight. The reaction was then quenched with ice water (2 ml) and extracted four times with 2 ml of $CHCl_3$. The chloroform was evaporated and the reaction was monitored with TLC (benzol-acetone; 5:1) and spay reagent (vanillin/H_3PO_4).

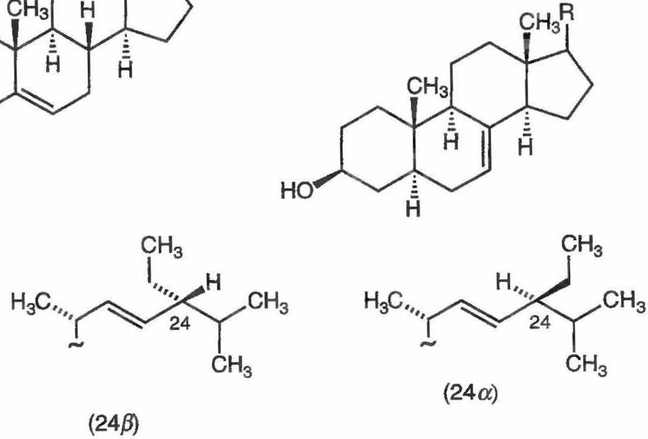
-Acid hydrolysis: In order to characterize the configuration of the single sugar moieties of the oligosaccharide, an acidic hydrolysis was performed. For this purpose, a 15-mg quantity of sample was refluxed with 6 ml of 2M HCl in MeOH (6 ml) for 4 h. The reaction solution was evaporated under reduced pressure, neutralized with alkali solution and again concentrated at reduced pressure. Subsequent analysis by TLC ($CHCl_3$-MeOH-H_2O; 32:18:4 and iso-PrOH-EtOAc-H_2O; 70:20:10) and by specific spray reagents (thymol/H_2SO_4 and anisic aldehyde/H_2SO_4) in comparison with standard sugars, provided additional information to the NMR data.

11.3 Compounds isolated from *Begonia heracleifolia*

Sterols

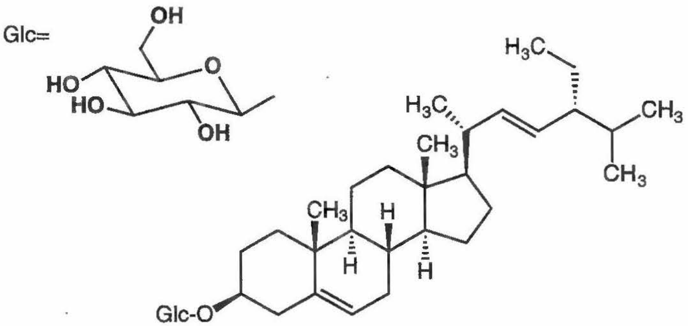
1 Stigmasterol





2 Chondrillasterol (24β) **3** Spinasterol (24α)

Identification of compound **1** was performed with two data base searches (Specinfo® Similarity Search, Beilstein Crossfire). ^{1}H, ^{13}C NMR data, and specific rotation $[\alpha]_D$ were compared with those reported in the literature (Nicotra et al., 1985). The structure of **1** was established as stigmasta-5,22-dien-3*β*-ol. A second fraction of the chromatographic separation (see Figure 4-S3) yielded a mixture of a C 24-epimer. Compound **2** and **3** were identified as 24*α*- and 24*β*-ethyl-5*α*-cholesta-7, *trans*-22-dien-3*β*-ol (Sucrow et al., 1976; Iida et al., 1980; Itoh et al., 1981). Co-occurrence of chondrillasterol **2** and spinasterol **3** is also often found in seeds of Cucurbitaceae (Sucrow et al., 1976; Itoh et al., 1981; El-Fattah et al., 1989).



4 3-O-*β*-D-glucopyranosyl-stigmasterol

Comparison with TLC showed a very similar composition of the primary fractions extracted with dichloromethane and ethyl acetate. Additionally, due to their small amounts, they were combined for further investigation (see Figure 4-S3). From an unsuccessful VLC with silica gel the major fraction was separated on VLC with reversed phase material (RP-18) and yielded compound **4**, only soluble in pyridine and purely crystallizing. Acetylation enabled structure elucidation based on ^{1}H and ^{13}C NMR data and showed **4** to be the acetate of 3-O-*β*-D-glucopyranosyl-stigmasterol.

Fatty acids

GC-MS was performed at the College of Pharmacy, Oregon State University in Corvallis (USA) in order to analyze a VLC fraction of the hexane extract. Promising signals in preliminary NMR measurements were expected to be of eicosanoids. Due to the instability of the eicosanoids (daylight, O_2) the identification was not possible, even with repeated isolation and following specific work-up procedures. Nevertheless, the measurements yielded information on the fatty acid composition (**5-7**, **9**) of the fraction. Due to preliminary methylation, the fatty acids were determined as methyl esters. Linoleic acid **8** was found to be of general occurrence in the hexane fraction.

5 Arachidic acid

6 Behenic acid

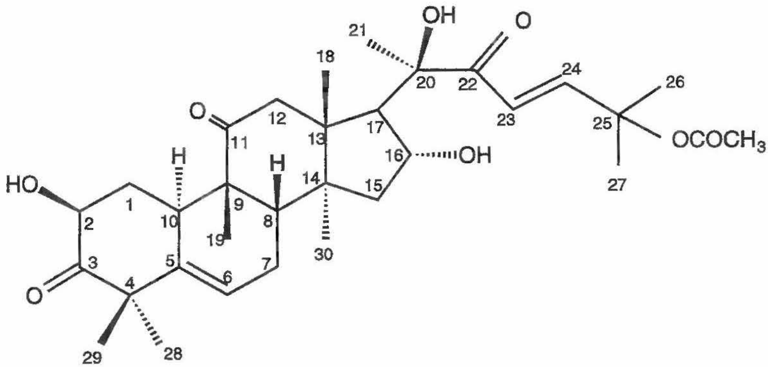
7 Palmitic acid

8 Linoleic acid

9 Stearic acid

Cucurbitacins

The isolation procedures of the compounds **10-15** has been described in the isolation table and in Publication V. Additional data is given as follows. Many cucurbitacins show UV activity in the range of 200 and 300 nm. A molecule with an α,β-unsaturated side chain absorbs at 230 nm, whereas several different structure features show a weak maximum at the value of 210 nm (Bauer and Wagner, 1983). MPLC and HPLC were therefore monitored by UV detectors at a wave length of 210 nm, a value also possible for chromatography with acetonitrile.



1 0

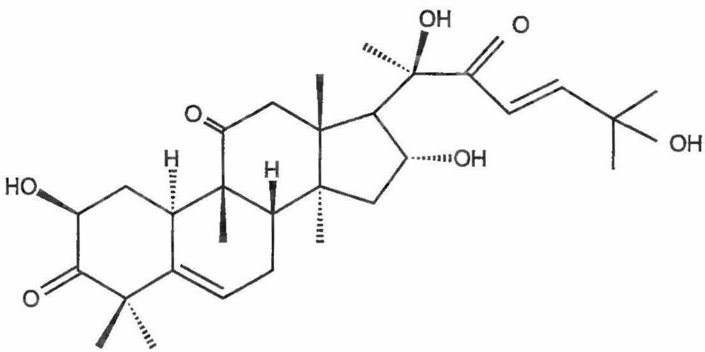
Cucurbitacin B $C_{32}H_{46}O_8$ **(M_r 558)**

White amorphous powder: $[\alpha]_D^{20}$ +79.5° (c=1.5, 95% EtOH); 1D-NMR: ^{1}H-NMR (300 MHz, 298K, $CDCl_3$) and ^{13}C-NMR (75.5 MHz, 298K, $CDCl_3$); data see Table 1-S3 and spectra in Appendix.

Table 1-S3. ^{1}H- and ^{13}C-NMR data of cucurbitacin B. [a] signal pattern unclear due to overlapping; * pairs of methyl groups not differentiated by connectivity experiments.

Position of C	^{1}H-NMR ppm (*J* Hz)	^{13}C-NMR ppm
1	1.30-2.31 [a]	35.99 t
2	4.42 dd	71.65 d
3		213.05 s
4		50.23 s
5		140.40 s
6	5.79 br. d	120.44 d
7	1.99-2.41 [a]	23.87 t
8	1.96 d	42.39 d
9		42.39 s
10	2.71 d	33.75 d
11		212.12 s
12	2.69 d_{AB} (14.6) 3.24 d_{AB} (14.2)	48.65 t
13		50.67 s
14		48.11 s
15	1.46-1.87 [a] m	45.33 t
16	4.37 dd	71.28 d
17	2.50 d	58.22 d
18	0.98 s	19.83 q
19	1.08 s	20.04 q
20		78.26 s
21	1.44 s	23.93 q
22		202.49 s
23	6.48 d_{AB} (15.6)	120.33 d
24	7.07 d_{AB} (15.6)	151.94 d
25		79.31 s
26	1.55 s*	26.41 q*
27	1.57 s*	25.96 q*
28	1.28 s*	29.36 q*
29	1.34 s*	21.25 q*
30	1.35 s	18.88 q
O<u>C</u>OMe		170.23 s
$O_2C\underline{C}H_3$	2.01 s	21.91 q

OH	^{1}H-NMR ppm (*J* Hz)	^{13}C-NMR ppm
OH	4.25 s	
OH	3.60 br. s	
OH	2.75 s	



11

Cucurbitacin D $C_{30}H_{44}O_7$ **(M_r 516)**

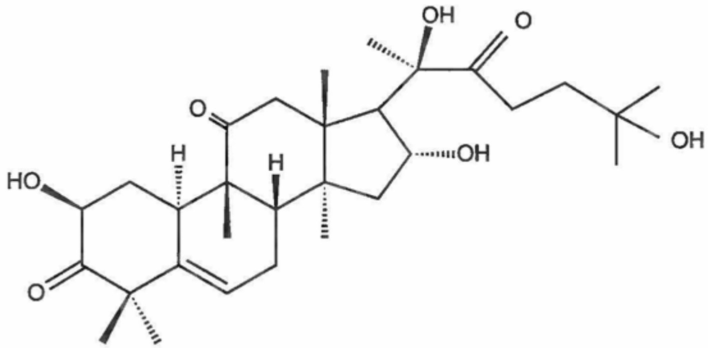
synonym: elatericin A

White amorphous powder: $[\alpha]_D^{20}$ +52° (c=1.5, 95% EtOH); 1D-NMR: ^{1}H-NMR (300 MHz, 298K, $CDCl_3$) and ^{13}C-NMR (75.5 MHz, 298K, $CDCl_3$). 2D-NMR: DQF-COSY, HMBC, ROESY; data see Table 2-S3 and spectra in Appendix. NH_4-DCI, in CH_3OH, m/z (rel. int.): 534 (8) $[M^+ + NH_4^+]$, 517 (19) $[M^+ + H^+ - NH_3]$, 516 (11) $[M^+]$, 501 (32), 500 (35) $[M^+ - H_2O + 2H^+]$, 499 (100) $[M^+ - H_2O + H^+]$, 498 (13) $[M^+ - H_2O]$, 485 (12), 484 (14), 483 (48), 482 (26), 481 (54) $[M^+ - 2H_2O + H^+]$, 467 (15), 465 (17), 455 (11), 387 (10), 385 (13), 369 (20), 325 (11), 113 (19), 112 (26), 111 (29), 96 (42).

Table 2-S3. NMR data of cucurbitacin D. [a] signal pattern unclear due to overlapping. */+ pairs of methyl groups not possible to differentiate. °° hydrogenic bonds possible, long side chain freely rotating.

Position of C	^{1}H-NMR ppm (*J* Hz)	^{13}C-NMR ppm	HMBC correl.	DQF-COSY	ROESY
1	2.33 m [a] 1.21 [a]	36.02 t	2	1,2, 10	6, 10, 7
2	4.46 [a]	71.65 d	1, 2(OH), 28	1, 2(OH)	6, 29
3		213.02 s	28, 29		
4		50.26 s	6, 8, 28, 26/7°°		
5		140.52 s	7, 29, 28		
6	5.79 br d	120.30 d	7, 8	7	2, 7/8, 30
7	2.44 m [a] 1.96 [a]	23.90 t	6, 8	6,7, 8	7/8-6, 1, 12, 30, 7, Me
8	1.96 [a]	42.37 d	7, 6, 19, 30	7	7/8-6, 1, 12, 30, 18
9		48.39 s	7/8, 30		
10	2.78 br	33.80 d	6, 8	1	
11		212.06 s			
12	3.29 d_{AB} (14.4) 2.71 d_{AB} (14.6)	48.64 t	12, 17, 18, 15		6, 16, 25 (OH)°°, 12, 17, 7, 19, 18, 30
13		50.84 s+	12,17, 18, 20, 7/8, 19, 30		
14		48.27 s+	12, 17, 18, 20, 8/7, 19, 30		

Position of C	^{1}H-NMR ppm (J Hz)	^{13}C-NMR ppm	HMBC correl.	DQF-COSY	ROESY
15	1.84 [a] 1.35 [a]	45.45 t	8, 19	16, 15	-Me, 18
16	4.42 [a]	71.45 d	17	17, 15	23, 16, 25
17	5.11 d	57.49 d	20(OH), 21, 18, 21	16	24, 23, 20(OH), 12, 18
18	0.99 s	19.92 q	12		20(OH), 12, 17, 15, 7/8
19	1.36 s	19.14 q	8, 15, 2		6, 12,
20		78.10 s	21, 20(OH)		
21	1.42 s	23.90 q	20(OH)		16
22		202.76 s	24, 23, 16, 21		
23	6.66 d_{AB} (15.2)	119.00 d		24	24,16, 25(OH), 17, 21
24	7.13 d_{AB} (15.2)	155.65 d	26/7	23	23, 21
25		71.27 s			
26	1.38 s	29.20 q*			26*-24, 23, 16, 17
27	1.26 s	29.54 q*			27*-24, 23, 16, 17
28	1.35 s	21.24 q	29		28-2
29	1.30 s	29.33 q	28		6, 2
30	1.09 s	20.08 q	7, 11, 12, 17, 8		6, 7/1, 7/8
OH (20)	4.37 s				15, 12, 17, 18, 21
OH (25)	3.61 s				23, 26/7, 29
OH (16)	1.63 s				16-23



12

23,24-Dihydrocucurbitacin D $C_{30}H_{46}O_7$ **(M_r 518)**

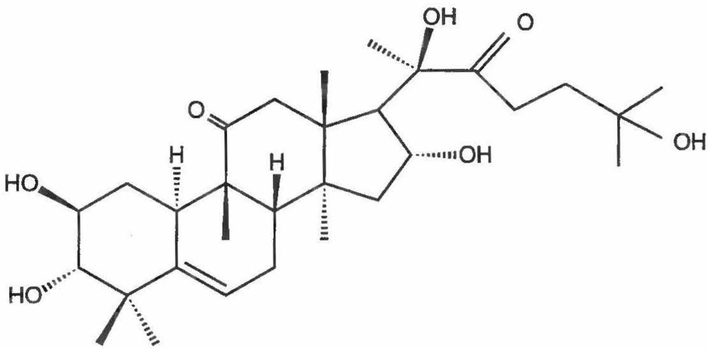
synonym: Cucurbitacin R

White amorphous powder: $[\alpha]_D^{20}$ +97° (c=1.5, $CHCl_3$); 1D-NMR: ^{1}H-NMR (300 MHz, 298K, $CDCl_3$) and ^{13}C-NMR (75.5 MHz, 298K, $CDCl_3$). 2D-NMR: HMBC, DQF-COSY, ROESY; data see Table 3-S3 and spectra in Appendix. NH_4-DCI, in CH_3OH m/z (rel. int.): 518 (2) [M^+], 502 (33) [M^+-H_2O+2H^+], 501 (100) [M^+-H_2O+H^+], 500 (5) [M^+-H_2O], 485 (14) , 484 (16), 483 (50) [M^+-2H_2O+H^+], 482 (14) [M^+-2H_2O], 142 (12), 113 (41).

Table 3-S3. NMR data of 23, 24-dihydrocucurbitacin D. * / "/+ pairs of methyl groups not possible to differentiate. [a] signal pattern unclear due to overlapping. °° hydrogenic bonds possible, long side chain freely rotating

Position of C	^{1}H-NMR ppm (J Hz)	^{13}C-NMR ppm	HMBC correl.	DQF-COSY	ROESY
1	2.33 m [a] 1.31 [a]	36.00 t	2	2, 1, 10	2, 10, 12, 19
2	4.42 q [a]	71.64 d		1	6, 10, 1, 2(OH)-28/9
3		213.04 s			
4		50.25 s	6, 28/9, 21		
5		140.44 s	7, 1		
6	5.79 br t	120.38 d	7	7	2, 12, 1, 7, 30, 28/9, 19, 8
7	2.42 [a] 1.98 [a]	23.88 t	7	7, 8	6, 7, 10, 12, 19, 18, 8
8	1.99 [a]	42.35 d	6, 19, 30	7	6, 10, 7, 18, 19
9		48.40 s	7, 30, 19		
10	2.74 [a]	33.78 d	6, 7, 26/7, 19	1	2, 1, 28/9, 19, 8
11		212.17 s	12, 19		
12	3.25 d_{AB} (14.4) 2.69 d_{AB} (14.7)	48.70 t	14, 19	12	6, 12, 10, 17, 12, 7, 30, 1, 26/7, 17, 23, 18, 19, 1, 7
13		50.81 s	12, 17, 18, 30, 21		

Position of C	^{1}H-NMR ppm (J Hz)	^{13}C-NMR ppm	HMBC correl.	DQF-COSY	ROESY
14		48.31 s	16, 17, 30, 18, 7		
15	1.86 [a]; 1.43 [a]	45.39 t	30	16	16,23,18, 26, 21
16	4.32 t [a]	71.04 d	21, 30	17, 15	17, 7, 18, 21
17	2.62 d	57.80 d	21, 18	16	0H(20/16),12,15
18	0.98 s	19.77 q	12, 17		16, 8
19	1.38 s "	20.03 q"	7		7/1, 8
20		79.22 s	21		
21	1.44 s	24.50 q			16, 23, 18
22		215.51 s	20(OH), 23, 21		
23	2.96 m; 2.69 [a]	30.85 t		23, 24	15, 21, 30, 24
24	1.83 [a]	36.90 t	23, 26/7	23	26/7, 17, 23
25		70.32 s	26/7		
26	1.22 s*	29.87 q*			23, 1*
27	1.25 s*	28.77 q*			23, 1*
28	1.28 s+	29.32 q+	29, 28/9-19!		6, 2, 12+, 10
29	1.34 s+	21.23 q+	28, 28/9-19°°		6, 2, 12, 10
30	1.08 s "	18.88 q"	30-7, 30-15, 7		
OH (20/16)	4.38 s				
OH (25)	3.62 s				
OH	2.05 s				
OH	1.70 s				



1 3

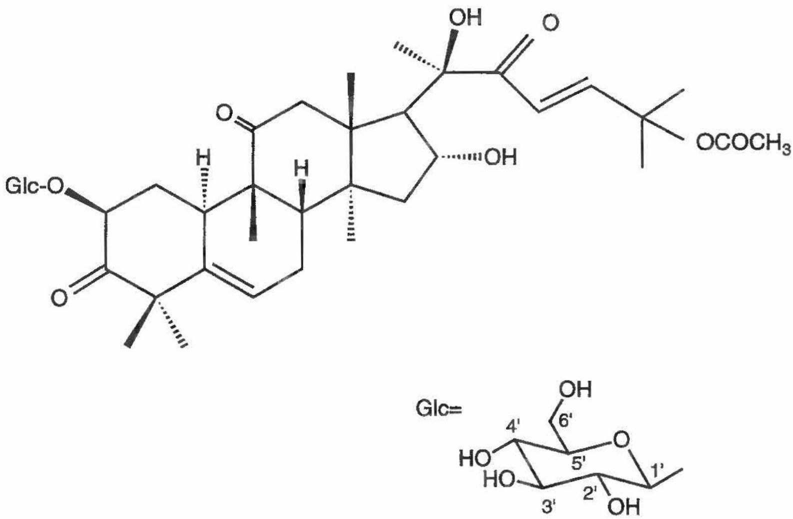
23,24-Dihydrocucurbitacin F $C_{30}H_{48}O_7$ **(M_r 558)**

White amorphous powder: $[\alpha]_D^{20}$ + 41.2° (c=0.1, EtOH); 1D-NMR: ^{1}H-NMR (300 MHz, 298K, $CDCl_3$) and ^{13}C-NMR (75.5 MHz, 298K, $CDCl_3$). 2D-NMR: HMBC, DQF-COSY, ROESY; data see Table 4-S3 and spectra in Appendix. NH_4-DCI, in CH_3OH, m/z (rel. int.): 520 (1.3) [M^+], 503 (66) [M^+-H_2O+H^+], 487 (14), 486 (33) [M^+-2H_2O+ 2H^+], 485 (100) [M^+-2H_2O+H^+], 469 (11), 467 (23), 441 (15), 113 (74).

Table 4-S3. NMR data of 23,24-dihydrocucurbitacin F. * pairs of methyl groups not possible to differentiate. [a] signal pattern unclear due to overlapping. °° hydrogenic bonds possible, long side chain freely rotating.

Position of C	^{1}H-NMR ppm (*J* Hz)	^{13}C-NMR ppm	HMBC correl.	DQF-COSY	ROESY
1	1.47 m- 1.62 m [a]	29.26 t	3	1, 2	2, 7, 19
2	3.92 m	68.38 d	1, 3	3	10, 1, 26
3	3.47 s	78.52 d	1, 27, 26 °°, 28/9, 26		27, 26
4		41.59 s	6, 26 °°, 28/9		
5		137.73 s	3, 7,1,28/9,26 °°		
6	5.73 br d	120.89 d	8, 7	7	27, 28/9, 7
7	1.93 d_{AB} (18.7) 2.43 d_{AB} (18.3)	23.89 t	6, 8	8, 7	19, 18, 15, 8, 7
8	1.95 d	42.68 d	6, 19, 30		6, 19, 18
9		48.42 s	8, 19		
10	2.35 br [a]	33.70 d	6, 8, 19	1	2, 12, 1, 28/9, 19, 26
11		212.89 s	19, 12, 19		
12	2.62 d_{AB} (14.1) 3.18 d_{AB} (14.4)	48.73 t	14, 12, 18	12	10, 17, 28/9°°
13		50.81 s*	12, 17, 18, 21, 30		
14		48.30 s*	16, 12, 8, 19, 30, 18		

Position of C	^{1}H-NMR ppm (*J* Hz)	^{13}C-NMR ppm	HMBC correl.	DQF-COSY	ROESY
15	1.82 d_{AB} (14.5) 1.40 d_{AB} (14.6)	45.46 t	8, 30	15	27, 21, 18
16	4.30 m	71.13 d	15, 17	17, 15	17, 18, 15
17	2.59 d	57.74 d	16, 15, 18, 20 (OH), 21		12, 24, 15, 21, 18
18	0.96 s	19.76 q	12, 17		7, 16, 17/24, 21, 19
19	1.14 s	20.17 q	18		7, 18, 1
20		79.23 s	21, 20(OH)		
21	1.42 s	24.50 q	20 (OH)		20 (OH), 17
22		215.50 s	23, 21, 20(OH), 24		
23	1.82 s 1.82 s	36.91 t	24, 28/9 °°		21, 15
24	2.62 m [a] 2.95 m [a]	30.86 t	23	24, 23	17, 27, 23
25		70.33 s	23, 24, 28/9°°		
26	1.03 s *	26.51 q *	unclear 23, 27		unclear 2, 3, 10
27	1.22 s *	28.83 q *	unclear23, 28/9 °°		unclear 3, 15, 18, 26
28	1.23 s *	25.33 q *	unclear 28/9-27°°	28/9-6	6, 15, 23°°
29	1.25 s *	29.83 q *	unclear	28/9-6	6, 15, 23°°
30	1.28 s	18.94 q	7, 8, 15		12, 7
OH (20)	4.35 s				21



14

2-O-β-D-glucopyranosyl cucurbitacin B $C_{38}H_{56}O_{13}$ **(M_r 720)**

synonym: Arvenin I

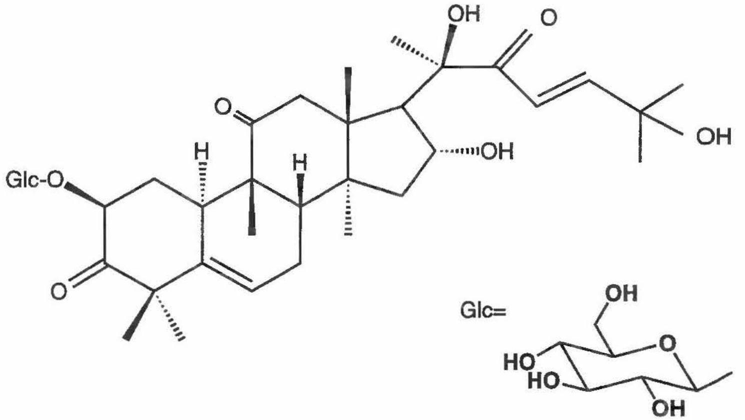
White amorphous powder: $[\alpha]_D^{20}$ + 40.6° (c=1.6, EtOH); 1D-NMR: ^{1}H-NMR (300 MHz, 298K, $CDCl_3$) and ^{13}C-NMR (75.5 MHz, 298K, $CDCl_3$). 2D-NMR: HMBC, DQF-COSY, ROESY; data see Table 5-S3 and spectra in Appendix. ESI, in CH_3OH/2% HOAc (1:1), m/z (rel. int.): 744 (6), 743 (13), 740, (10), 739 (21), 738 (46), (720, missing [M^+]), 499 (7) [M^+-CH_3OAc-Glucose+H^+].

Table 5-S3. NMR data of 2-O-β-D-glucopyranosyl cucurbitacin B. * carbonyl groups, and ** pairs of methyl groups not possible to differentiate. [a] signal pattern unclear due to overlapping. °° hydrogenic bonds possible, long side chain freely rotating.

Position of C	^{1}H-NMR ppm (J Hz)	^{13}C-NMR ppm	HMBC correl.	DQF-COSY	ROESY
1	1.45 [a] 2.41 [a]	34.58 t		1, 2, 10	
2	4.57 dd	79.35 d		1	28/9**
3		212.96 s*	29, 28		
4		48.06 s	28		
5		139.67 s	29, 28		
6	5.78 br d	120.50 d		7	30, 7/8 [a], 28/9**, -OCOCH$_3$
7	2.41 [a] 1.92 [a]	23.85 t	8	6, 7, 8	7 [a] -6, 21, 7/8 [a], 28/9 [a], 30
8	1.97 s [a]	42.33 d	30, 19	7	8 [a] -6, 26/7, 30
9		48.43 s	19		
10	2.77 d [a]	34.16 d	19, 8	1	28/9**
11		211.19 s*	12, 18		
12	3.28 d_{AB} (14.7) 2.71 d_{AB} (15.0)	48.89 t	18	12	12, 17, 30, 21, 18
13		51.30 s**	18, 30, 14, 15, 17		
14		50.60 s**	12, 7, 30, 18		

Position of C	^{1}H-NMR ppm (*J* Hz)	^{13}C-NMR ppm	HMBC correl.	DQF-COSY	ROESY
15	1.44 [a]	45.33 t	30	15, 16,	16,21/15, 18
16	4.35 [a]	71.25 d	30, 17	15, 17	20(OH), 18, 15, 17
17	2.51 d	58.29 d	21, 18	16	16, 12, 21, 30
18	0.98 s	19.92 q	17		16, 20(OH), 7/8 [a], 12
19	1.08 s	20.03 q			7/8 [a]
20		78.22 s	21, 1', 20(OH)		
21	1.44 s	24.06 q	20(OH)		24, 23, 20(OH), 19, 17, 15
22		202.52 s	24, 20(OH), 17, 21		
23	6.48 d_{AB} (15.6)	120.35 d	-OCOCH$_3$	24	24, 17, 21, 26/7**
24	7.05 d_{AB} (15.6)	151.94 d	26	23	23, 27/6**
25		79.46 s	26, 27, 23, 24		
26	1.55 s	26.44 q	24, 27		24, 23, 26-28/9**, 12
27	1.58 s	25.90 q	26		
28	1.29 s **	28.93 q	29		28**-6, 10
29	1.29 s **	21.26 q	28		29**-6, 10
30	1.35 s	18.83q	8		6, 12, 10, 17
O<u>C</u>OMe		170.27 s	-OCOCH$_3$		
$O_2C\underline{C}H_3$	2.02 s	21.95 q			

Position of C	^{1}H-NMR ppm (*J* Hz)	^{13}C-NMR ppm	HMBC correl.	DQF-COSY	ROESY
1'	4.31 a	103.63 d		2'	
2'	3.43 a	73.79 d		1' 3'	
3'	3.54 a	77.21 d		2'	
4'	3.52 a	70.14 d		5'	
5'	3.41 a	76.19 d		4', 6'	
6'	3.88 dd	62.47 t		6', 5'	
OH (20)	4.27 s				16, 21



15

2-O-β-D-glucopyranosyl cucurbitacin D $C_{36}H_{54}O_{12}$ **(M_r 678)**

synonym: Arvenin III

White amorphous powder: $[\alpha]_D^{20}$ + 16.7° (c=0.6, EtOH); 1D-NMR: ^{1}H-NMR (300 MHz, 298K, CD_3OD) and ^{13}C-NMR (75.5 MHz, 298K, MeOD and CD_3COCD_3;; data see Table 6-S3 and spectra in Appendix. ESI, in CH3OH, NH_4OAc, m/z (rel. int.): 734 (24), 733 (60), 717 (16), 704, (12), 702 (44) [M^++ Na^++H^+], 701 (100) [M^++Na^+], (678, missing [M^+]).

Table 6-S3. NMR data of 2-O-*β*-D-glucopyranosyl cucurbitacin D. [a] signal pattern unclear due to overlapping. */** pairs of methyl groups not possible to differentiate.

Position of C	^{1}H-NMR ppm (*J* Hz) (CD_3OD)	^{13}C-NMR ppm (CD_3OD)	^{13}C-NMR ppm (CD_3COCD_3)
1	2.35 m [a] 1.20 [a]	35.95 t	35.33 t
2	4.50 [a]	79.65 d	79.04 d
3		213.35 s	212.76 s
4		a (MeOH)	48.95 s
5		141.71 s	141.31 s
6	5.81 br d	121.28 d	120.61 d
7	2.40 m [a]; 1.90 [a]	24.85 t	24.58 t
8	1.95 [a]	44.14 d	43.50 d
9		a (MeOH)	49.11 s
10	2.60 br	35.03 d	34.26 d
11		215.70 s	213.97 s
12	~3.2 [a] (MeOH) 2.60 [a]	a (MeOH)	49.63 t
13		52.45 s	49.73 s
14		51.88 s	51.95 s
15	1.80 [a] 1.3 [a] (CH_3)	46.63 t	46.54 t
16	4.31 d	71.70 d	71.24 d
17	a	59.61 d	58.82 d
18	0.90 s*	21.81 q*	21.74 q
19	1.30 s*	19.14 q*	20.50 q
20		79.94 s	79.18 s
21	1.40 s*	20.13 q*	20.12 q
22		205.05 s	[a] (acetone)
23	6.81 d_{AB} (15)	121.40 d	120.97 d
24	6.98 d_{AB} (15)	155.36 d	155.31 d
25		71.51 s	71.13 s
26	1.30 s*	29.23 q**	[a] (acetone)
27	1.40 s*	29.23 q**	[a] (acetone)
28	1.30 s*	25.74 q	25.30 q
29	1.25 s*	29.36 q	[a] (acetone)
30	1.01 s*	19.47 q	19.14 q

Position of C	^{1}H-NMR ppm (*J* Hz)	^{13}C-NMR ppm (MeOD)	^{13}C-NMR ppm (CD_3COCD_3)
1'	4.30 [a]	104.343 d	104.23 d
2'	3.40 [a]	75.47 d	75.35 d
3'	3.60 [a]	78.26 d	78.10 d
4'	3.50 [a]	71.51 d	71.25 d
5'	3.40 [a]	77.96 d	77.72 d
6'	3.88 dd	62.93 t	62.80 t

Oligosaccharide

The aqueous fraction of the butanol-water partition yielded a fraction with one dominant spot in the TLC analysis. With open column chromatography (see Figure 5-S3) an oligosaccharide was isolated. Mesurement of NMR and mass spectrometry before and after acetylation as well as acidic hydrolization gave rise to the hypothesis that either a disaccharide and its two isomers or a tetrasaccharide has been isolated. Unclear signal patterns especially between 4.7 and 4.9 ppm in the ^{1}H-NMR spectrum made a final structure elucidation impossible. So far fructose and glucose in their α- and β-form have been corroborated.

11.4 Biological activities and chemotaxonomic considerations of isolated compounds

Cucurbitacins are of special interest in *B. heracleifolia*. Three of the isolated compounds (**10-12**) were shown to be cytotoxic and presumably are responsible for at least part of the cytotoxicity of the crude extract. The varying cytotoxicity of all isolated cucurbitacins (**10-15**) may be explained with structure-activity relationship (see Publication V). All cucurbitacins (**10-15**) showed no activity (> 200 µg) in the antimicrobial tests (methods and microbes see Section 2). The sterols (**1-4**) and the oliogosacchharide showed no activity in all assays described.

Pharmacological and biological effects of cucurbitacins have been reviewed recently (Miró, 1995) and include: gastrointestinal effects, cytotoxic and antitumor action, hepatoprotective and hepatocurative activities, anti-inflammatory activity, anovulatory activity, cardiovascular effects, effects on the central nervous system, antimicrobial activity, anti-helmintic activity, antigibberellin activity, and effects on insects. Therefore, cucurbitacins and the plant species that contain them will be of further interest. The widespread opinion of cucurbitacins being of general toxicity has to be revised. Especially, when taking into consideration that cucurbitacin E showed diverse results (not general toxic) in the anti-cancer research program of the NCI on 60 different cell lines (Fuller et al. 1994). Meanwhile, cucurbitacin E has even become a candidate for pre-clinical development for anti-cancer drugs (Cragg et al., 1997). Despite there toxicity, cucurbitacins are known as bitter principles of several plants. Recently, some sweet 3β-glycosides have been isolated. Structure-taste relationship show that 11α-hydroxy glycosides are of sweet taste, whereas 11-oxo glycosides are bitter or tasteless (Kasai et al. 1987). An additional ring from joining of C_5 and C_{19} by an oxigen cycle suspends the extreme bitterness as well (Miró, 1995). The isolated glycosides **14** and **15** showed bitter taste as expected due to their chemical composition.

So far, phytochemical investigations on 16 plant families yielded cucurbitacins (Agavaceae: *Phormium* ; Begoniaceae: *Begonia*; Cucurbitaceae: *Bryonia, Cayaponia, Citrullus, Cucumis, Cucurbita, Ecballium, Lagenaria, Luffa, Momordica, Peponium, Trochomeria,* a.o.; Brassicaceae: *Iberis, Lepidium*; Datiscaceae: *Datisca*; Desfontainiaceae: *Desfontainia*; Elaeocarpaceae: *Crinodendron, Elaeocarpus*; Euphorbiaceae: *Antidesma, Cleistanthus, Drypetes,* *Discoglypremma, Maprounea, Mareya, Trewia, Sapium, Spondianthus*; Polemoniaceae: *Ipomopsis*; Primulaceae: *Anagallis*; Rosaceae: *Purshia*; Rubiaceae: *Cigarrilla, Hintonia*; Scrophulariaceae: *Gratiola, Picria, Picrorhiza*; Sterculiaceae: *Helicteres*; Thymelaceae: *Gyrinops*; Tropaeolaceae: *Tropaeolum*). Therefore, it is questionable if these triterpenes are really of chemotaxonomic interest for the Begoniaceae. The co-occurrence of glycosides and aglyca classifies *B. heracleifolia* as a plant not containing elaterase (β-glucosidase). Elaterase containing plants make it impossible to isolate glycosides due to enzymatic hydrolysis. Up to now this enzyme has not been found in all cucurbitacin containing plants (Miró, 1995) and may be of interest in intrafamilial studies.

12 Conclusion

The phytochemical investigation of *B. heracleifolia* yielded biologically active compounds of the group of cucurbitacins. Thus some of the Zapotec ethnomedical uses could have been corroborated by these findings. The ethnobotanical approach in chemical prospecting seems, therefore, a reasonable one. There are still many studies lacking in numerous regions of the world on its inhabitants' individual plant uses and even more plant species are waiting to be investigated phytochemically. So far, from this study, eleven plant species out of 445 have been studied in the laboratory, while three of them have been investigated phytochemically. Not all of the remaining plant species are of high interest and others have been studied earlier, however, there are still some with promising pre-screening results. Besides the screened group of dermatological complaints, there are still more groups of indigenous uses to be analyzed. Nevertheless, I hope, based on this work here, either ethnobotanical or biological, phytochemical, or even medicinal data will help one day to improve the living situation of those Mexican people - and many more - who contributed to this thesis.

Epilogue

This thesis will be completed with these last words, but I am hesitating to declare that all the different anticipations put to this work had been fulfilled. Not talking from my expectations, but from the ones I awakened in the Zapotecs. I am sure they expected much less paper and more effective results! To finally close this rather long "monologue" of mine and not abandoning one tradition I have held throughout this thesis, I let someone else find the adequate words.

> The time has come when scientific truth must cease to be the property of the few, when it must be woven into the common life of the world; for we have reached the point when the results of science touch the very problem of existence.
>
> Louis Agassiz

References

2o. ENCUENTRO REGIONAL
de Médicos Indígenas
Tradicionales del Istmo
de Oaxaca.
24 y 25 DE NOVIEMBRE DE 1992
Sede: Sta. Ma. Guienagati Teh., Oax.
Organizan: Organización Indígenas Zapotecos, Mixes del Istmo
y Zoques de Chimalapa. I.N.I.

El médico verdadero: un sabio, da vida.

Conocedor experimentalmente las hierbas,

las piedras, los árboles, las raíces.

Tiene ensayados sus remedios, examina, experimenta, alivia enfermedades.

Da masaje, concierta los huesos.

Purga a la gente, la hace sentirse bien...

Fray Bernardino de Sahagún.

Textos nahuas del Códice Matritense

de la Real Academia de la Historia

References of Section I to Section III

(Refernces of Publication I to V not included, see at the end of each publication or in footnotes)

Aguilar, A., Argueta, A., Cano, L. (coords.) (1994) Flora Medicinal Indígena de México. Instituto Nacional Indigenísta (INI), México, D.F. Tomo I, II, III.

Akerele, O. (1990) Medicinal plants in traditional medicine. In: Economic and Medicinal Plant Research, Plants and Traditional Medicine. Wagner, H., Farnsworth, N.R. (eds.). Academic Press, London. 5-16.

Alcorn, J. B. (1984) Huastec Mayan Ethnobotany. University of Texas Press, Austin.

Ankli, A., Heneka, B., Orjala, J., Sticher, O., Heinrich, M. (1996) Plants in the treatment of gastrointesinal disorders in two Yucatec Maya communities (Mexico). Poster presented at Joint Meeting of the Society for Economic Botany and International Society for Ethnopharmacology: Plants for Food and Medicine, London, UK, June 30-July 5, '96.

Argueta V., A. (coord.) (1994) Atlas de Las Plantas de La Medicina Tradicional Mexicana. Instituto Nacional Indigenista, México D.F. Tomo 1-3.

Aumeeruddy, Y. (1994) Local representations and management of agroforests on the periphery of Kerinci Seblat National Park Sumatra, Indonesia. WWF, People an Plants, United Nations Educational, Scientific and Cultural Organization, UNESCO Press. Paper 3.

Baldwin, M. A. (1995) Modem mass spectrometry in bioorganic analysis. Nat. Prod. Report., 11: 33-44.

Balée, W. (1993) Footprints of the Forest. Ka'apor ethnobotany - The Historical Ecology of Plant Utilization by an Amazonian People Biology and Resource Management in the Tropics Series. Balick, M. J., Anderson, A.B., Redford, K. H. (eds.). Columbia University Press, New York.

Balick, M.J. (1990) Ethnobotany and the identification of therapeutic agents from the rainforest. In: Bioactive Compounds from Plants. Chadwick, D.J., Marsh, J. (eds.). Ciba Foundation Symposium No. 154, J. Wiley and Sons, Chichester. 154: 22-39.

Barkley, F. A., Golding, J. (1974) The Species of Begoniaceae. Northeastern University, Boston. 2ed. edition.

Barrera, A. (1972) La etnobotánica. In: La Etnobotánica: Tres Puntos de Vista y Una Perspectiva. Barrera, A. (ed.). INIREB, Mexico. 19-26.

Bauer, R., Wagner, H. (1983) Cucurbitacinhaltige Drogen. DAZ,123: 1313-1321.

Berlin, B., Breedlove, D.E., Raven, P.H. (1973) General principles of classification and nomenclature in folk biology. American Anthropologist, 75: 214-242.

Bloor, S.J., Porter, L.J. (1993) Mass spectrometry. In: Bioactive Natural Products: Detection, Isolation and Structure Determination. Colegate, S.M., Molyneux, R.J. (eds.). CRC Press, Boca Raton. Chapt. 5: 105-123.

Bopp, M. (1957) Untersuchungen über die Verteilung und Vererbung von Anthocyan in den Blättern von Begonien. Planta, 48: 631-682.

Bork, P., Schmitz, M.L., Weimann, C., Kist, M. Heinrich, M. (1996) Nahua Indian medicinal plants (Mexico): Inhibitory activiy on NF-κB as an antiinflammatory modell and antibacterial effects. Phytomedicine,3: 263-269.

Brett, J. A. (1992) Why is a plant medicinal: Medicinal plant selection criteria. Paper delivered at the III International Congress of Ethnobiology, Mexico City, 1992.

Brueske, J. M. (1976) The Petapa Zapotecs of the Inland Isthmus of Tehuantepec, Mexico: An Ethnographic Description and an Exploration into the Status of Women. University Microfilms International, Ann Arbor, Michigan.

Bye, R. (1993) The role of humans in the diversification of plants in Mexico. In: Biological Diversity of Mexico, Origins and Distribution, Ramamoorthy, T.P., Bye, R., Lot, A., Fa, J. Oxford University Press, Oxford. 707.

Bye, R., Linares, E., Estrada, E. (1995) Biological diversity of medicinal plants in Mexico. In: Recent Advances in Phytochemistry, Phytochemistry of Medicinal Plants, Arnason, J. T., Mata, R., Romeo, J.T. Plenum Press, New York, London. 29, Chap.4: 65-82.

Byrne, L. T. (1993) Nuclear magnetic resonance spectroscopy strategies for structural determination. In: Bioactive Natural Products: Detection, Isolation And Structure Determination. Colegate, S.M., Molyneux, R.J. (eds.). CRC Press, Boca Raton. Chapt. 4: 75-104.

Caballero, J. (1987) Etnobotánica y desarrollo: la busquedad de nuevos recursos vegetales. In: Memorias, IV. Congreso Latinoamericano de Botánica: Simposio de Etnobotánica, Instituto Colombiano para el Fomento de la Educación Superior. 79-96.

Campbell, H. (1994) Zapotec Renaissance. Ethics Politics and Cultural Revivialism in Southern Mexico University of New Mexico, Albuquerque.

Campbell, H., Binford, L., Bartolomé, M., Barabas, A. (eds.) (1993) Zapotec Struggles. Smithsonian Institution Press, Washington, London.

Cardellina II, J.H., Munro, M.H.G., Fuller, R.W., Manfredi, K.P., McKee, T.C., Tischler, M., Bokesch, H., Gustafson, K.R., Beutler, J.A., Boyd, M.R. (1993) A chemical screening strategy for the dereplication and prioritization of HIV-inhibitory aqueous natural products extracts. J. Nat. Prod., 56: 1123-1129.

Chadwick, D.J., Marsh, J. (eds.) (1990) Bioactive Compounds From Plants. Ciba Foundation Symposium, J. Wiley and Sons, Chichester. 154: 22-39.

Chirol, N., Maurice, J. (1995) Acylated anthocyanins from flowers of *Begonia*. Phytochemistry, 40: 275-277.

Coll, J.C., Bowden, B.F. (1986) The application of vacuum liquid chromatography to the separation of terpene mixtures. J. Nat. Prod., 49: 934-936.

Cordell, G., A. (1995) Chanching strategies in natural products chemistry. Review Article Number 109. Phytochemistry, 40: 1585-1612.

Cotton, C.M. (1996) Ethnobotany: Principles and Application. John Wiley & Sons, Chichester.

Covarrubias, M. (1986) Mexico South: The Isthmus of Tehuantepec. St. Edmundsbury Press, Bury-St.-Edmunds.

Cragg, G., M., Newman, D. J., Snader, K. M. (1997) Natural products in drug discovery and development. J. Nat. Prod., 60: 52-60.

Cunningham, A.B. (1993) African Medicinal Plants. WWF, People and Plants, United Nations Educational, Scientific and Cultural Organization, UNESCO Press. Paper 1.

Cunningham, A.B., Mbenkum, F.T. (1993) Sustainability of *harvesting Prunus africana* bark in Cameroon. WWF, People and Plants, United Nations Educational, Scientific and Cultural Organization, UNESCO Press. Paper 2.

de Laszlo, H, Henshaw, P.S. (1954) Plant materials used by primitive peoples to affect fertility. Science,119: 626-631.

Delfeld, M. A. (1996) Aztec sour herbs: *Begonia* and *Oxalis*. Paper Session at the Joint Meeting of the Society for Economic Botany and the International Society for Ethnopharmacology, Imperial College, London, UK, July 1- July 5. Paper 30.

Desai, H.K., Gawad, D.H., Govindachari, T.R., Joshi, B.S., Kamat, V.N., Parthasarathy, P.C., Ramachandran, K.S., Shanbag, M.N., Sidhaye, A.R., Viswanathan, N. (1975) Chemical investigation of some Indian plants: Part VIII. Indian J. Chem., 13: 97-98.

Diaz, J.L. (ed.) (1976) Usos de las Plants Medicinales de México. Instituto Mexicano para el Estudio de las Plantas medicinales, México. 329.

Doskotch, R. W., Hufford, C.D. (1970) Hexanor-cucurbitacin D, A degraded cucurbitacin from *Begonia tuberhybrida* var. *alba*. Can. J. Chem., 48: 1787-1788.

Doskotch, R. W., Malik, Y.M., Beal, J.L. (1969) Cucurbitacin B, the cytotoxic principle of *Begonia tuberhybrida* var. *alba*. Lloydia, 32: 115-122.

Duke, J. A., Vasquez, R. (1994) Amazonian Ethnobotanical Dictionary. CRC Press, Boca Raton.

El-Fattah, A.H., Zaghloul, A. M., Halim, A. F., Waight, E.S. (1989) Curbitacins and steroids from *Cucumis callosus* (Rottl) Cong. Acta Pharm. Jugosl., 39: 137-141.

Ensemeyer, M. (1980) Pharmakognostisch-Phytochemische Untersuchung von *Begonia glabra* Aublet. Inaugural -Dissertation, Fachbereich Pharmazie der Freien Universität Berlin, Berlin.

Ensemeyer, M., Langhammer, L. (1984) Phytochemische Untersuchung von *Begonia fagifolia* Fischer (Inhaltsstoffe von Begoniaceae, 5. Mitt.). Arch. Pharm., 317: 692-695.

Ensemeyer, M., Langhammer,L., Rauwald, H.-W. (1980) Isolierung und Konstitutionsaufklärung eines dimeren Proanthocyanidins in *Begonia glabra* Aubl. Arch. Pharm., 313: 61-71.

Etkin, N. L. (1994) Eating on the Wild Side. The pharmacologic, Ecologic and Social Implications of Using Noncultigens. The University of Arizona Press, Tucson.

Etkin, N.L., Ross, P.J. (1982) Food as medicine and medicine as food. Soc. Sci. Med., 16: 1559-1573.

Farnsworth, N.R. (1988) Screening plants for new medicines. In: Biodiversity. Wilson, E.O., Frances, M.P. (eds.). Washington D.C., National Academy Press, Chapter 9: 83-97.

Farnsworth, N.R., Akerele, O., Bingel, A.S., Soejarto, D.D., Guo, Z. (1985) Medicinal plants in therapy. Bull. WHO, 63: 965-981.

Fellows, L.E. (1992) Pharmaceuticals from taditional medicinal plants and others. Future prospects. In: New Drugs from Natural Sources. Coombes, J.D (ed.). IBC Technical Services Ltd., London. 93-100.

Ford R. I. (ed.) (1978) The Nature and Status of Ethnobotany. Anthropological Papers, Museeum of Anthropology, University of Michigan No. 67, Ann Arbor, Michigan.

Frohne, D., Jensen, U. (1992) Systematik des Pflanzenreichs, unter besonderer Berücksichtigung chemischer Merkmale und pflanzlicher Drogen. Gustav Fischer Verlag, Stuttgart, 4. Auflage.

Fuller, R.W., Cardellina, J.H. II., Cragg, G.M., Boyd,M.R. (1994) Cucurbitacins: Differential cytotoxicity, dereplication and firts isolation from *Gonystylus keithii*. J. Nat. Prod., 57, 1442-1445.

George, N.J., Madhavi, D.L., Smith, M.A.L. (1994) In vitro systems for enhanced production of valuable phytochemicals in *Begonia* sp. Hortscience, 29: 68.

Gerhard, P. (1972) A Guide to the Historical Geography of New Spain. Cambridge Latin American Studies, Cambridge University Press, London. 14.

Gershenzon, J., Mabry, T.J. (1984) Secondary metabolites and the higher classification of angiosperms. Nord. J. Bot., 3: 5-54.

Gruenwald, J. (1997) German medicine market segments with a high share of phytotherapeutics. Herbalgram, 39: 67-68.

Hamburger, M., Hostettmann, K. (1991) 7. Bioactivity in plants: The link between phytochemistry and medicine. Phytochemistry, 30: 3864-3874.

Hamburger, M.O., Cordell, G.A. (1987) A direct bioautographic TLC assay for compounds possessing antibacterial activity. J. Nat. Prod., 50: 19-22.

Hansmann, C. F. (1990) Synthesis and characterisation of methyl 2-O-(β-D-glucopyranosyl)-6-O(α-L-rhamnopyranosyl)-α-D-glucopyranoside. Carbohydr. Res., 204: 221-226.

Harborne, J.B., Hall, E. (1964) Plant polyphenols - XIII. The systematic distribution and origin of anthocyanins containig branched trisaccharides. Phytochemistry, 3: 453-463.

Harborne, J.B., Hall, E. (1963) Branched trisaccharides in the anthocyanins of *Begonia* and *Rubus*. Biochem. J., 88: 41-42.

Hegnauer, R. (1964) Begoniaceae. In: Chemotaxonomie der Planzen, Birkhäuser Verlag, Basel, Stuttgart. Band III, 237-239.

Hegnauer, R. (1989) Bombaceae. In: Chemotaxonomie der Planzen, Birkhäuser Verlag, Basel, Boston, Berlin. Band VIII, 142.

Heinrich, M. (1996) 38. Ethnobotany of Mexican Compositae: An analysis of historical and modern sources. In: Compositae: Biology & Utilization. Caligari, P.D.S., Hind, D.J.N. (eds.). Proceedings of the International Compositae Conference, Kew, 1994. 475-503.

Heinrich, M. (1989) Ethnobotanik der Tieflandmixe (Oaxaca, Mexico) und phytochemische Untersuchung von *Capraria biflora* L. (Scrophulariaceae). Disserationes Botanicae, J. Cramer, Gebrüder Borntraeger Verlagsbuchhandlung, Berlin, Stuttgart. No 144.

Hör, M., Rimpler, H., Heinrich, M. (1995) Inhibition of intestinal chloride secretion by proanthocynidins from *Guazuma ulmifolia*. Plant. Med., 61: 208-212.

Hostettmann, K. (1997a) Strategie in der Suche nach neuen Wirkstoffen aus Pflanzen. Oral presentation at the Institute of Organic Chemistry, University of Zurich, Switzerland, May, 6.

Hostettmann, K., Hostettmann, M., Marston, A. (1986) Preparative Chromatography Techniques, Applications in Natural Product Isolation. Springer-Verlag, Berlin, Heidelberg, New York.

Hostettmann, K., Wolfender, J.-L., Rodriguez, S. (1997b) Rapid detection and subsequent isolation of bioactive constituents of crude plant extracts. *Planta Med.*, 63: 2-10.

Iida,T., Ishikawa, T., Tamura, T., Matsumoto, T. (1980) Carbon-13 nuclear magnetic resonance spectroscopic evidence of chondrillasterol isolated from gourd seed oil. Yakagaku, 29: 345-346.

Irmscher, E. (1960) Begoniaceae. In: Die natuerlichen Pflanzenfamilien. Engler, A., Prantl, K. (ed.). Duncker & Humbolt, Berlin, Leipzig, 2. Auflage. Bd. 21: 548-588.

Itoh, T., Kikuchi, Y., Tamura, T., Matsumoto, T. (1981) Co-occurence of chondrillasterol and spinasterol in two Cucurbitaceae seeds as shown by ^{13}C NMR. Pytochemistry, 20: 761-764.

Jamir, N.S., Rao, R.R. (1990) Fifty mew or interesting medicinal plants used by the Zeliangs of Nagaland (India). Ethnobotany, 2: 11-18.

Johns, T. (1990) With Bitter Herbs They Shall Eat It. Chemical Ecological and the Origins of Human Diet and Medicne. University of Arizona Press, Tucson.

Johns, T., Chapman, L. (1995) Phytochemicals indigested in traditional diets and medicines as modulators of energy metabolism. In: Recent Advances in Phytochemistry, Phytochemistry of Medicinal Plants. Arnason, J. T., Mata, R., Romeo, J.T. Plenum Press, New York, London. 29, Chap.8: 161-188.

Kasai, R., Matsumoto, K., Nie, R., Morita, T., Awazu, A., Zhou, J., Tanaka, O. (1987) Sweet and bitter cucurbitane glycosides from *Hemsleya carnosiflora*. Phytochemistry, 26: 1371-1376.

Kinghorn, A. D., Seo, E.-K. (1996) Cultivating the pharmacopoeia. CHEMTECH, July: 46-54.

Langhammer, L., Grandet, M. (1974) Ueber die Verbreitung eines seltenen Anthocyans in der Familie der Begoniaceae. Plant. Med., 26: 260-268.

Lehner, S. (1993) Antimikrobielle Eigenschaften von Hydroxypropyl-β-cyclodextrin und seine Wechselwirkungen mit Konservierungsmitteln. Diss., Christian-Albrechts-Universität, Kiel.

Leslie, Ch. M. (1981) Now We Are Civilized: A Study of the World View of the Zapotec Indians of Mitla, Oaxaca. Greenwood Press Westport, Conn.

Linares, E., Bye, R., Flores, B. (1990) Tes Curativos de México. Instituto de Biologia, Universidad Nacional Autónoma de México (UNAM), Talleres Graficos de Cultura, Mexico D. F. Cuadernos 7.

Mabberley, D.J. (1993, and 1996) The Plant-Book. Cambridge University Press.

Martin, G. J. (1995) Ethnobotany. WWF International, UNESCO, Royal Botanic Gardens Kew, Chapman & Hall, London.

Martinez A., M.A. (1984) Medicinal plants used in a totonac community of the Sierra Norte de Puebla: Tuzamapan de Galeana, Puebla, Mexico. J. Ethnopharmacol., 11: 203-221.

Martinez, M. (1989) Las Plantas Medicinales de México. Ediciones Botas, México D.F.

Mellado C., V., Sanchez R., A., Feneia, P., Navarro M., A., Erosa S., E., Bonilla C., D.M., Domingez H., M. del S. (1994) La Medicina Tradicional de Los Pueblos Indigenas de México. INI (Instituto Nacional Indigenista), Mexico D. F. Tomo III.

Messer, E. (1978) Zapotec plant knowledge: classification, uses, and communication about plants in Mitla, Oaxaca, Mexico. In: Prehistoric and Human Ecology of the Valley of Oaxaca. Flannery, K.V., Blanton, R.E. (ed.). Memories of the Museum of Anthropology, University of Michigan No. 10, Ann Arbor. Vol. 5, part 2.

Meyer, B.N., Ferringni, N.R., Putman, J.E., Jacobsen, L.B., Nichols, D.E. McLaughlin, J.L. (1982) Brine shrimp: A convenient general bioassay for active plant constituents. Plant. Med., 45: 31-34.

Miró, M. (1995) Cucurbitacins and their pharmacological effects. Phytot. Res., 9: 159-168.

Moerman, D.E. (1991) The medicinal flora of native North America: An analysis. J. Ethnopharmacol., 31: 1-42.

Moerman, D. E. (1996) An analysis of the food plants and drug plants of native North America. J. Ethnopharmacol., 52: 1-22.

Morton, J.F. (1981) Atlas of Medicinal Plants of Middle America: Bahamas to Yucatan. Charles C. Thomas Publisher, Springfield.

Myster, J., Junttila, O., Lindgard, B., Moe, R. (1997) Temperature alternations and the influence of gibberllins and indoleacetic acid on elongation growth and flowering of *Begonia* x *hiemalis* Fotsch. Plant Growth Regulation, 21: 135-144.

Nader, L. (1976) The Zapotecs of Oaxaca. Handbook of Middleamerican Indians 7, Ethnology part 1: 329-359.

Nader, L. (1990) Harmony Ideology: Justice and Control in a Zapotec Mountain Village. Standford University Press, Standford CA.

Newbold Chiñas, B. (1992) The Isthmus Zapotecs. A matrifocal culture of Mexico. In: Case Studies in Cultural Anthropology. Spindler, G. and L. Spindler (eds.). Stanford University, Harcourt Brace Jovanovich College Publishers, Forth Worth. 2ed. ed..

Newbold Chiñas, B. (1993). La Zandunga. Of fieldwork and friendship in southern Mexico. Waveland Press, Prospect Heights, Illinois.

Nicotra, F., Ronchetti, F., Russo, G., Toma, L. (1985) Application of biosynthetic 13C enrichment using [1,2-^{13}C2] acetate as precursor for ^{13}C NMR spectral assignment of the isoporpyl methyl of (24β) etylsterols. Magn. Res. Chem., 23: 134.

Nordal, A., Resser, D. (1966) The non-volatile acids of succulent plants exhibiting a marked diurnal oscillation in their acid content- III. The acids of *Kleinia repens* (L.) Haw., *Begonia tuberhybrida* (Hort) and *Mesembryanthemum criniflorum* L. fil. Acta Chem. Scand., 20: 2004-2007.

Nyiredy, Sz., Meier, B., Erdelmeier, C.A.J., Sticher, O. (1985) "Prisma": Ein Modell zur Optimierung der mobilen Phase für die Dünnschichtchromatographie, vorgestellt anhand verschiedener Naturstoffrennungen. Plant. Med., 51: 242.

O'Neill, M.J., Lewis, J.A. (1993) The renaissance of plant research in the pharmaceutical industry. In: Human Medicinal Agents from Plants. Kinghorn, D.A., Balandrin, M.F. (eds.). ACS Symposium Series 534, American Chemical Society, Washington, DC.

Perdue, R. E. Jr. (1982) KB cell culture. I. Role in discovery of antitumor agents from higher plants. J. Nat. Prod., 45: 418-426.

Pisha, E., Chai, H., Lee, I.-S., Chagwedera, T., Farnsworth, N. R., Cordell, G. A., Beecher, Ch. W. W., Fong, H. H., Kinghorn, A. D., Brown, D. M., Wani, M. C., Wall, M. E., Hieken, T. J., Gupta, T. K., Pezzuto, J. M. (1995) Discovery of betulinic acid as a selective inhibitor of human melanoma that functions by induction of apoptosis. Nature Medicine, 1: 1046-1051.

Reko, B.P. (1945) Mitobotanica Zapoteca. M. Academia Nacional de Ciencias "A. Alzate", M. Soc. Botanica de Mexico, General León 9, Tacubaya, Mexico D.F.

Roys, R.L. (1931) The Ethno-Botany of the Maya. Middle American Research Series Publication No.2, The Departement of Middle American Research, The Tulane University of Lousiana, New Orleans.

Rzedowski, J. (1988) Vegetación de México. Editorial Limusa, Noriega Editores, Mexico D.F.

Schultes, R. E., von Reis, S. (eds.) (1995) Ethnobotany. Evolution of a Discipline. Chapman & Hall, London.

Seler, E. (1986) Plano Jeroglifico de Santiago Guevea. Ediciones *Guchachi'Reza*, A. C., Imprenta Madero, Mexico D.F.

Smith, L.B., Schubert, B.G. (1973) Begoniaceae. In: Fieldiana Botany (Flora of Guatemala, Standley, Williams), 24: 157-185.

Smith, L. B., Wasshausen, D. C., Golding, J., Karegeannes, C. (1986) Smithsonian Contribution to Botany. Begoniaceae. Part I: Illustrated Key. Part II: Annotated species List. Smithonia Institution Press, Washington D.C. Nr. 60.

Snyder, L.R. (1978) Classification of the solvent properties of common liquids. J. Chrom. Sci., 16: 223.

Stephen, L. (1991) Zapotec Women. University of Texas Press, Austin TX.

Sucrow, W., Slopianka, M., Kircher, H.W. (1976) The occurence of C-29 sterols with different configurations at C-24 in *Cucurbita pepo* as shown by 270 MHz NMR. Phytochemistry, 15: 1533-1535.

Takhtajan, A. (1996) Diversity and Classification of Flowering Plants. Columbia University Press, New York. 214-217.

Thorne, R. F. (1983) Proposed new realignments in the angiosperms. Nord. J. Bot., 3: 85-117.

Trueb, L. (1995) Systematische Suche nach neuen Wirkstoffen in der Arzneimittelforschung. Neue Zürcher Zeitung, Forschung und Technik, Mittwoch, 3. Nov., 256: 65-66.

Unger, K.K, Weber, E. (1995) Handbuch der HPLC. GIT Verlag, Darmstadt. Band I, II.

Vereskovskii, V.V., Gorlenko, S. V, Kuznetsova, Z.P., Dovnar, T.V. (1988a) Flavonoids of the leaves of *Begonia erythrophylla* I. Khim. Prir. Soedin., 4: 505.

Vereskovskii, V.V., Kuznetsova, Z.P., Dovnar T.V. (1988b) Flavone C-glycosides of *Begonia erythrophylla* II. Khim. Prir. Soedin., 6: 763-764.

von Reis Altschul, S. (1973) Drugs and foods from little-known plants. Notes on Harvard University Herbaria. Harvard University Press, Cambridge.

von Reis, S., Lipp, F. J. (jr.) (1982) New Plant Sources for Drugs and Foods from The New York Botanical Garden Herbarium. Harvard University Press, Cambridge, MS.

Wagner, H. (1989) Search for new plant constituents with potenial antiphlogistic and antiallergic activity. Plant. Med., 55: 235-241.

Wagner, H., Bladt, S. (1996) Plant Drug Analysis. Spinger Berlin. 2nd. ed.

WHO, IUCN, WWF (1993) Guidelines on The Conservation of Medicinal Plants. Media Natura, WHO, IUCN, WWF, Gland, Switzerland.

Williamson, E. M., Okpako, D. T., Evans, F. J. (1996) Selection, Preparation and Pharmacological Evaluation of Plant Material. Pharmacological Methods in Phytotherapy Research, John Wiley & Sons, Chichester.Vol. 1: 5.

Wolfender, J.-L., Hostettmann, K. (1996) Importance of LC/MS in plant analysis. Spectroscopy Europe, 8: 7-12.

Yamada, Y., Hagiwara, K., Iguchi, K., Suzuki, S., Hsu, *H.Y. (1978) Isolation an structures of arvenins from Anagallis* arvensis (Primulaceae). New cucurbitacin glucosides. Chem. Pharm. Bull., 26: 3107-3112.

Zerbe, O. (1996) Praktische Aspekte der NMR Spektroskopie. Vorlesungsunterlagen, Departement Pharmazie, ETH Zurich, Zürich.

Appendix


BIENVENIDOS
II ENCUENTRO DE MEDICOS INDIGENAS TRADICIONALES
STA. MA. GUIENAGATI, TEH. OAX. NOV. 24.25 DE 1992

I Questionnaire from field work

Knowledge and use of medicinal plants among the inhabitants

****

Cuestionario: Conocimiento de plantas medicinales y su uso

1) Explicar la finaldad de este cuestionario

2) ¿Usa Ud. plantas medicinales para curar? **si mucho poco no**

3) ¿De donde son las plantas?

jardin particular monte/selva mercado otro....................

4) ¿Cuales son las plantas y remedios caseros que utilizan? (nombre zapoteco)

a)	f)	k)	p)
b)	g)	l)	q)
c)	h)	m)	r)
d)	i)	n)	s)
e)	j)	o)	t)

5) Tratamiento de enfermedades

enfermedad té /baño /de asiento/ limpia/ purgar/ lavado vag./rect./ sobar /comida /ritual /frot.

a) diarrea/ desintería/ vomito..
b) calentura/ fiebre..
c) piel/ grano..
d) dolor de estomago/ de barriga..
e) dolor de cuerpo..
f) dolor de cabeza..
g) mal aire..
h) tos..
i) purgar..
j) bilis..

6) ¿Conoce Ud. las siguentes plantas ? Paraque se utilizan ? (muestras)

nombre si/no otros nomb. I/II/III/med. uso parte usada frío/caliente

Gordolobo...
Ocote...
Tulipán...
Empurga...
Frijolillo..
Barba de maíz...
Malva..
Cola de alacrán..
Guanábana..
Eücalipto...
Chomisu'...
Almendra...
Tamarindo..
Suelda con sueldo..
Zacate rojo....(*Euphorbia micromera* Boiss.)...
Gueyana.....(*Gomphrena nitida* L.)..

7) ¿A quien consulta Ud.?

curandero/a	**espiritualista**	**otro especialista**
....	....	....

médicos(INI/IMSS)	**parientes**	**vecinos**	**monjas**	**otros**
....	....	....	....	....

8) ¿Sabe Ud. que se quiere abrir una farmacia natural? si no

¿Va Ud. a consultar a una farmacia natural? si no

¿Porque?...

9) ¿Que vale para Ud. más, farmacia natural o quimica?..

....

10) Datos socio-demograficos:

sexo: M F **edad:**........ **ocupación:** .... **religión:**........................

casa: sin con luz **agua:** manguera río otro.......... **piso:** natural hormigón

muro: natural hormigón **techo:** ondulada hormigón teja otro........................

cocina: en casa aparte/ horno fuego

cafetal: si no **campo:** si no **rancho:** si no **trabajo tempor. afuera:** si no

Lugar:.......................................Fecha:.......................................Numero:..........................

****

II List of plants collected, medicinal uses, application and preparation

Explication of abbreviations in **PLANT LIST** (p. 312)

II.1 Location

Abbreviation of Location	Comunidad (Spanish)	Community (English)
todos	todos	all 4 communities
SDP	Santo Domingo Petapa	name of the community
SMP	Santa María Petapa	name of the community
GdH	Guevea de Humboldt	name of the community
SMG	Santa María Guenagati	name of the community
SDMP/G	SDP+SMP+SMG	in 3 communties

II.2 Plant part used

Abbreviation of plant part used	**Parte usada de la planta (Español/*Zapoteco*)**	**Plant part used (English)**
ag	agua	juice (watery)
al	algodón	cotton
ba	barba, espiga	ear
be	bejuco	creeper, climber
ca	cáscara	bark
cj	cogollo	shoot
co	coco	(coco)nut
co	corazón de la madera	medulla
fl	flor	flowers
fr	fruta	fruits
goma	goma	latex (milky)
hb	hierba	aerial parts
ho	hoja	leaves
inflo	inflorescencia	inflorescence
ju	jugo	juice (watery)
le	leche	latex (milky)
ma	madera	wood
mas	masa	fruit pulp
mag	magazo	rasped wood

Abbreviation of plant part used	Parte usada de la planta (Español/*Zapoteco*)	Plant part used (English)
pa	palo	trunk
pet	pétalo	petals
pl en	planta entera	whole plant
po	polvo	part of fungus
ra	rama	twig
re	resina	resin
ret	retoño, hoja tierna	shoot
rz	raíz, rizome, bulbo	root, rhizome, bulb
se	semilla	seeds
su	sumerio	resin
to	*totomoztle*	bracts of corn
tr	trementina	turpentine
va	vaina	legume

II.3 Preparation

Abbreviation of Preparation	Forma de preparación (Español/*Zapoteco*)	Mode of preparation (English)
1	té, cocimiento (p.e para lavar heridas)	tea, infusion (e.g. cleansing wounds)
2	baño de asiento, baño de todo el cuerpo	bath
3	lavado rectal o vaginal	rectal or vaginal douche

Abbreviation of Preparation	**Forma de preparación (Español/*Zapoteco*)**	**Mode of preparation (English)**
4	jarabe	syrup
5	macerada en alcohol, frotar	macerated in alcohol
6	horchata, atole	beverage (corn or other ground ingredients)
7	*vaoh*	steam bath
8	con sebo, pomada, jabón	cream, soap
9	tostada, polvo o freir	toasted, powder or fried
10	molida, machacada, parche, cataplasma (p.e. con sal)	ground, plaster, cataplasm (e.g. with salt)
11	ninguna forma de preparación, poner crudo o comer	crude plant applied
12	sahumar, bracadera	censer, incense
13	limpia	ritual cleansing with soaked plant
14	masaje	massage with crude plant
15	exprimir	make juice
16	tintura	tincture
17	fumar	to smoke

II.4 Application

Abbreviation for mode of application	Modo de aplicación (Español)	Mode of application (English)
gn	general, en todo el cuerpo	general, all over the body
lc	local	local
ns	nasal	nasal
or	oral	oral
re	rectal	rectal
vg	vaginal	vaginal

II.5 Categories of indigenous uses

Abbreviation	Categorías de usos medicinales	Categories of indigenous uses
DI or SD	Enfermedades dermatológicas	Dermatological illnesses, skin diseases
GH	Afecciones gastrointestinales Enfermedades hepáticas	Gastrointestinal disorders, hepatic complaints
CbS or FI	Enfermedades y sindromes folk	Culture-bound syndromes, folk illnesses
fm/G	Problemas uro-genitales femininas / masculinas	Female / male genito-urinary complaints
SMS or ST	Enfermedades del sistema músculo-esquelético	Illnesses of the skeletal-muscular system
RA or RT	Problemas respiratorias	Respiratory ailments

Abbreviation	Categorías de usos medicinales	Categories of indigenous uses
F/M	Fiebre, incluido paludismo	Fever, including malaria
CO	Problemas cardiovasculares, Enfermedades sanguíneas	Cardiovascular complaints, diseases of the blood
OPH	Enfermedades oftalmológicos	Ophtalmological problems
OU	Usos diferentes (p.e. diabetes, usos adicionales médico-religiosos	Other Uses (e.g. diabetes, and additional medico-religious uses)
s	con / de sangre	bloody
d	con dolor	with pain

II. 6 Culture-bound syndromes and symptoms

Abbreviation of culture-bound syndromes and some of its symptoms	Enfermedades y sindrome folk (Español/*Zapoteco*)	Culture-bound syndromes and symptoms (English)
aire	aire	supernatural winds
alf	alferecía	attacks of cramps, hemiplegia
ant	antojo	desire, appetite
asc	asco	disgust, nausea
ataq	ataque	attacks of cramps, fever
ca	cansancio	fatigue
cal	calor de sangre	sexual desire

Abbreviation of culture-bound syndromes and some of its symptoms	**Enfermedades y sindrome folk (Español/*Zapoteco*)**	**Culture-bound syndromes and symptoms (English)**
cam	no poder caminar	not being able to walk
can	cáncer	cancer, ulcer, sore
chib	*chibiguchi*	sexual desire
chin	*chineque*	spirit of water and/or cave
deb	débil	exhaustion, weakness
dorm	dormir	extensive sleeping
emp	empacho	food causing gastrointestinal problems
enc	*encono*	illnesses transmitted by a corpes
7 enf	7 enfermedades	7 illnesses
espa	espanto	sudden fright
espi	espinilla	pustules on the torso, dehydration
flac	flaco	meagerness, malnutrition
fri	frialdad (de parto)	infertility
fue	fuerza	strength, energy
gol	golpe	physical and / or supernatural blow
igua	iguana	illnesses of the eyes (glaucoma?)
jaq	jaqueca	pain or paralysis on one side of the head (migraines and stroke?)

Abbreviation of culture-bound syndromes and some of its symptoms	**Enfermedades y sindrome folk (Español/*Zapoteco*)**	**Culture-bound syndromes and symptoms (English)**
nag	naguales	alter ego, soul(s) of people as animals
nerv	nervios	nervousness
ojo	mal ojo	evil eye
pal	pálido	paleness
pens	pensativo	depression
prec	presiones	depression affecting the heart
rel	relajo	impotence
sal	salpuido	pustules
sus	susto	sudden fright
tris	tristeza	depression
verg	vergüenza	shame

Further abbreviations	**Español**	**English**
bora	borracho	drunken
chicalpestle	chical	painted bowl
curtir	curtir	to tan
div	divinanza	divination
escoba	escoba	broom, brush
mar	mareo	vertigo
pap	paperas	mumps
pescar	pescar	to fish
vet	uso veterinario	veterinary uses

II.7 Classification, qualities

Abbreviation of classification of plants	Clasificación, cualidad (Español)	Classification, quality (English)
f	frío	cold
c	caliente	hot
t	templado	temperate
ac	ácido	sour, acid
al	alcanforado	camphoric
am	amargo	bitter
an	anestésico	anaesthetizing
ba	baboso	sticking
ci	cítrico	citric
du	dulce	sweet
el	eléctrico	electrifying
es	"estítico"	astringent
esp	espumoso	foaming
fr	fresco	cold, fresh
ha	alucinógeno	hallucinatory
hu	huele bonito	smells nice, aromatic
pe	pesta	smells bad
pg	pega	sticking
pi	pica	hot, spicy (like chili)
qu	quema	burning

Abbreviation of classification of plants	Clasificación, cualidad (Español)	Classification, quality (English)
re	"resbaloso"	slimy
sa	salado	salty
sec	seco	dry
si	simple	tasteless, neutral
ve	venenoso	poisonous

II.8 Categories of "Importance"

Import.	Importancia	Importance
-	ningún uso	not used
I	uso observado	uses observed
II	se usa	is used
III	es posible usarla	it could be used
IV	ningún uso medicinal	no medicinal uses
( )	numero de respuestas	number of answers

PLANT LIST

Scientific name	Nr. FREI	Locat.	*Zapotec name* Spanish name	Part used	Prep.	Application	Groups of indigenous uses	Classification	Import.
EUKARYOTA									
Mycophyta									
AURICULARIACEAE									
Auricularia polytricha (Montagne) Sacc.	130	SDP	*yagambishia'a* oreja de la bruja	po	11	lc	Dlds	f c	II (2), III (3)
Pteridophyta									
equisetopsida									
EQUISETACEAE									
Equisetum sp.	144	todos	*shcool cavaij* cola de cavallo	hb	1 2 3	lc gn re vg	GH F/M fmG CO nerv	f	I (6)
filicopsida									
ADIANTACEAE									
Hemionitis palmata L.	362	GdH	hierba del gato	hb	1	lc	Dl espi	f c	I (2)
Hemionitis palmata L.	362 II	SDP	*guish misht'daj'* hierba del gato	hb	1	lc	Dl espi	f c	-
CYATHEACEAE									
Sphaeropteris myosuroides (Liebm.) R. M. Tryon	267	GdH	*shgola miigu'u* colandrillo, cola de chango	ho pa	1	or	GH	f	I (3)
PTERIDACEAE									
Adiantum trapezoides Fée	67	SDP	colandrillo	ho rz	1 11	lc gn or re vg	Dl fmG	f c	I,II (3)
THELYPTERIDACEAE									
Thelypteris sp.	421	SDP	*colandrillu*	ho rz	1	or vg	fmG	f c	I, III (1)
SCHIZAEACEAE									
Lygodium verustrum Sw.	131	SDP	*guixa'a mbala'a* hoja de la vibora	ho	1	lc gn or	Dlds	f	I (1),II (3), IV (2)

Aristolochia grandifolia Swarz	86	todos	huaco corriente	rz be	1 2	lc gn or	GH SMSd DIds F/M fmG CO aire ataq	c am	I (5), II (6)
papaverales: PAPAVERACEAE									
Argemone mexicana Sw.	34	SDP	*guedxe buloj* chacalote, chicalote	go	2 11 15	lc gn	DI OPH	c qu	I (4)
caryophyllales: AMARANTHACEAE									
Amaranthus hybridus L.	159	todos	*nagucho'o* /quintonil	ho	1 11	or	OU	f	I,IV (2)
Amaranthus spinosus L.	198	todos	*guedxe'e* /espina	hb ho	1	or	OU	f	IV (4)
Gomphrena nitida Rothr.	222	SDP	*gueyana*	hb	4	or	GHs OU	f c	II, IV (2)
BASELLACEAE									
Anredera ramosa (Moq.) Eliasson	18	SDP	*gu'* suelda con suelda	rz	10	lc	SMSd DUds aire	f c hu	I (3)
Boussingaultia leptostachys Moq.	321	SDP	suelda con suelda	rz	10	lc	SMSd DUds aire	f c hu	I (3)
	182	SDP	*gu'u lcu'uj* /camote qué huele feo	rz	10	lc	SMSd aire	f c	todos (1)
CACTACEAE									
Opuntia sp.	78	todos	*bia'aj* nopal	ho fr	6 9 10	or	GH RA SMSd fmG OU	f si re	I (7)
	338	SMG	organo	fr ho	pescar	or	OU	f c	II (1)
	317	SDP	tuna, nopal del cerro	fr pa	11	or	GH OU	f	II (2)
CHENOPODIACEAE									
Chenopodium ambrosioides L.	38	todos	*biajta'* epazote	hb	1 10 16	lc or	GH RA DI OPH ojo OU	f si pi	I (11)
NYCTAGINACEAE									
Boerhaavia coccinea Mill.	353	SMG	vuergüenzosa, pega	ra	1	lc or	RA DI	f c	II (1)
Bougainvillaea glabra Choisy	157	todos	bugambilia morada	fr	4	or	RA OU	c	I (5)
Mirabilis jalapa L.	83	todos	hoja de linda tarde, flor de china	ho	1 2	lc gn	RAd SMSd DI F/M nag OU	f c si hu	I (4)
Mirabilis sp.	344	SDMG	el compadre cimarron	ho fl	11	lc or	DI OU	f c ve	I (3)

Scientific name	Nr. FREI	Locat.	*Zapotec name* Spanish name	Part used	Prep.	Application	Groups of indigenous uses	Classification	Import.
PHYTOLACCACEAE									
Petiveria alliaceae L.	10	todos	*bete'a* hoja de zorillo	ho rz	1 5 11	lc or	GH RAd SMS DI aire	c pe	I (4)
Stegnosperma cubenae A. Rich.	332	SDMG	"niño qué no se puede levantar"	ra	11	lc or	SNS cam	f c	III (1)
PORTULACACEAE									
Portulaca oleraceae L.	240	SDP GdH	verdolaga	hb	1 11	or	OU	f	IV (5)
polygonales:									
POLYGONACEAE									
Antigonon flavescens S. Watson	298	SMG	*gulachiia*	ra fl	1	lc re vg	GHs		I,III,IV (1)
Coccoloba barbadensis Jacq.	311	SDP	*bidxuyej' shuga'a* totopotzle, carnero negro	fr	11	or	RAs DIs fmGs OU	f c	II (1)
Coccoloba barbadensis Jacq.	327	SMG	*shuubguijooj'* cinco negrito, carnero coyote	ca	1	or	RAs DIs fmGs OU	f c	I (4)
Coccoloba liebmannii Lindau	337	SMG	*shuug*	fr ca	1 10	lc or	DAd DIds fmG OU	f c	I, II, III (1)
Coccoloba sp.	395	SDP	*shuug nguio'o* carnero de coyote	ca	1	or	GHs RAs SMSs DIs fmGs	f c	I (2)
plumbaginales:									
PLUMBAGINACEAE									
Plumbago scandens L.	401	GdH	*guish chivat* hoja de chivato	ho	10	lc	DI	f c	I (2)
hamamelidales:									
HAMAMELIDACEAE									
Liquidambar sp.	315	SDP	*bijtu'u* , ocosote	ho ca	1	or	GH OU	f c	II (2)
fagales:									
FAGACEAE									
Quercus glaucescens Humb. & Bonpl.	418, A	SDP	*beshxii nagatzii* encino amarillo	ca	1 3 11	lc vg	fmG	f c	I (2)
Quercus oleoides Schltdl. & Cham.	132, A	SDMP	*beshxii qutzii* encino blanco, nanche	ca	1 3 11	lc gn or re vg	GH fmG OU	f am	I (4)

rosales:

CRASSULACEAE

Bryophyllum pinnatum (Lam.) Kurz	67	todos	*guish marauii*	ho	8 15	lc or	SMSd DI F/M	f si	I (5)
			siempreviva,		10				
			maravillosa				fmG OU		
	272	SDP	*lu'ujtzanguana'aj*	ho	10 11	lc	SMSd DI	f	I,II,III (1),
			lengua de mujer						IV (2)
ROSACEAE									
Rosa centifolia L.	51	todos	rosa blanca,	pet fl	3 4 15	lc or	GH F/M fmG	f si	I (6)
			rosa de castilla				OPH ojo		
fabales:									
CAESALPINIACEAE									
Caesalpinia pulcherrima (L.) Sw.	184	SMP	hoja de maravillia rojo	ho	10	lc	GHd SMSd	f	I,II (2)
Caesalpinia pulcherrima (L.) Sw. f. flava	301	SMP	hoja maravillia amarilla	fl	1	or	OU (epilep)	f c	II (1)
Cassia grandis L.	48	SDMP	cañafiste, cañafistula	se ca	1 2 4	gn or re	RA F/M OU	f c si am du hu	I (7)
Chamaecrista fagonioides	81	SDMP	*guixa'a sen*	ra ho	2 3 7	lc gn re vg	GH DI F/M fmG	f secc	I,II (7)
(Vogel)L. et B. var. fagonioides			hoja sen						
Haematoxylum brasiletto Karsten	379	SMG	palo brazil	ca co	1 4 5	or	GH COs fue pal OU	f c	I, II (2)
Hymenaea courbaril L.	15	SDMP	*biguu*	re fr se	6 10 13	lc or	GH RA SMSd	c hu	II (4)
			huapinol, guapinol				aire OU		
Senna atomaria (L.)	158	SDMP	frijolillo (arbol)	ho va ca	10 11	lc	GH SMSd DIs aire	f c	I (4)
Senna cobanensis	270	SDP	*bizandxa'a gueexii*	ho va	10 11	lc	GH RA SMSd aire	f c	III (1), IV(2)
(Britton & Rose) Irwin & Barneby			frijolillo del monte						
Senna fruticosa (Mill.) Irwin & Barneby	278	SDP	*bizandxa'a*	ho va	1 2 3	lc gn	DIds	f c	I (1), IV (2)
			cerilla						
Senna occidentalis (L.) Link	160	SDMP	frijolillo (planta)	va ho	1 10 11	lc gn or	GH RA SMSd	c hu	I,II (2)
Senna reticulata (Willd.) Irwin & Barneby	62	SDP	*guixa'a mbisundxi*	fl hb ho	1 2 11	lc gn or re vg	SMSd DI F/M	f du hu	I (3)
			flor de abejón						
Tamarindus indica L.	39	todos	*gubshnii'*	fr ho	6 11	lc or	GHd RA SMSd DI	f am du hu	I (6)
			tamarindo				F/M verg OU		
FABACEAE									
Acosmium panamense	247	SDP	*guassi* /guayuacan	ca mag	1	or	SMSd F/M fmG	f c am	I,II (3)
(Benth.) Yakovlev									

Scientific name	Nr. FREI	Locat.	*Zapotec name* Spanish name	Part used	Prep.	Application	Groups of Indigenous uses	Classification	Import.
Andira inermis (Sw.) Kunth	326	SMG GdH	*ngaash* lombricero	ca fr	1	or	GH OU	f c	I (3)
Calopogonium coeruleum (Benth.) Sauv.	285	SDMP	*batuujtza* zempantle	ho ret	1 2 10	gn or	ataq nerv dorm OU	(i)	II; IV (2)
Crotalaria longirostrata Hook & Arn.	264	SDP	*chepil*	ho	1 11	lc or	DI OU	f c	I (3)
Diphysa americana Mill.	236	SDP	ruda cimarron	ra	2	lc gn or	SMSd aire	f c	I,II,IV (1)
Dalbergia granadillo Pittier	432	SDP	guayacan	ca	1	or	SMSd F/M fmg	f c am	
Dalea carthagenensis (Jacq.) Macbr.	345	SMG	*guish gobüej* escoba	ra	escoba		OU		IV (1)
Erythrina folkersii Krukoff & Moldenke	194 B	todos	*guixa'a mindu'u* colorin, zempantle	ho fl se	1 9	or	nerv dorm OU	f c	II,(1),III(2), IV(4)
Erythrina sp.	194	todos	colorin	ho fl se	1 9	or	nerv dorm OU	f c	"
Gliricidia sepium (Jacq.) Stend.	35, II	todos	*guianixa'a* madre cacao	ho ret	2 11 13	lc gn or	DI F/M fmG espi aire ver	f am si	I (11)
Indigofera suffruticosa Mill.	239	SDP SMG	añil cimarron, hoja de tinta	ra	(indigo)	lc gn vg	RA DI fmG ojo ataq OU	f	I (4)
Phaseolus vulgaris L. (subsp.)	271	todos	*bizza'a* frijol	va	1 11	lc gn or	DIds COs OU	f c	I,II(1),IV(5)
Phaseolus vulgaris L. (supsp.)	356	GdH	frijol del palo	va se	1	or	OU		IV (2)
Poiretia punctata (Willd.) Desv.	14	SDP	*guixa'a* / hierba de malina	hb	1 2 16	lc gn or	GH SMSd DI F/M ojo aire	c si hu pe	I (4)
Stizolobium pruriens (L.) Medik. var. utilis (Wall. ex Wight)	415	SDP	nescafé, pica-pica	se	1	or	OU	hu	IV (3)
Vigna speciosa (HBK.) Verdc.	381	SMG	flor de guajalote	fl	1	or	OU	f c	IV (1)
MIMOSACEAE									
Acacia farnesiana (L.) Willd.	358	GdH	*guish guiatzj* espina blanca	ho	10 11 13	lc	DI ojo	f c	II (2)
Acacia farnesiana (L.) Willd.	106 II	SDP	*huisachi* , *sachi*	ca rz	5 9 10	lc ns	RAs DIds fmG	c si pi qu	I (4)
Acacia sp.	106	SDP	*huisachi* , *sachi*	ca rz	5 9 10	lc ns	RAs DIds fmG	c si pi qu	I (4)
Acacia cornigera (L.) Willd.	136	SDMP	*guedxebej* cacho del toro	ra rz	1 2	lc or vg	RAd fmGs	f c	I (2), III (2)

Enterolobium cyclocarpum (Jacq.) Griseb.	6	SDMP	*biguisha* oanacastle	ho ca	8 11	lc gn	DI OU	f esp	IV (5)
Inga sp.	201	SDP	*cahijnaquil* carnequil	va	1 11	or	OU	du	IV (6)
Leucaena lanceolata S. Watson	310	SDP	*laj'* / huaje de la peña, de agua	va	1 11	or	GH OU	(f)	II (1)
Leucaena leucocephala (Lam.) de Wit ssp. glabrata Rzed.	96	SDP	*laj' tza gutzii* huaje blanco	se	1 11	or	GH OU	c hu	I (1)
Mimosa albida H. et B. ex Willd.	294	SDP	huaje rosadito, la vergüenzosa	ho se	1	lc or	verg OU	f c	II (1), IV (2)
Mimosa pudica L.	138	todos	*guedzegumaj'alaj* dormilona	ho rz ra	1 2 3	lc or re vg	GHs DI fmGs OU	f	I (3)
Mimosa tenuiflora (Willd.) Poir.	13	SDMP/G	*gueedxe boog* tepesquehuite	ca ho	2 8 9	lc gn re vg	GH DI fmG	c am es	I (6)0
Mimosa c.f. rhodocarpa Britton & Rosé	296	SDP	tepehuaje cimarron	ho	9 10	lc re vg	DI fmG	(f)	II (1), IV (2)
Pithecellobium dulce (Roxb.) Benth.	330	SMG	*guamuchi*	ca va	1	lc or	GHs emp OU (cortir)	f	I (3)
Prosopis cf.	348	SMG	mesquite	ca ho ret	1	lc or	GHds DI OPH emp ojo OU	f c	-
Prosopis juliflora (Sw.) DC.	348 II	SMG	mesquite	ca ho ret	1	lc or	GHds DI OPH emp ojo OU	f c	I (3)
	351	SMG	toronjil	ra	1	lc or ns	RAs DIs F/M	f c hu	I (1)
myrtales:									
COMBRETACEAE									
Terminalia catappa L.	162	SDMP/G	hoja de almendra	ho se	1	or	GH OU	f c am	I (4)
LYTHRACEAE									
Heimia salicifolia (HBK.) Link & Otto	234	SDMP	sobadora	hb	5	lc gn	SMS	f c	I,III,IV (1)
Lawsonia inermis L.	295	SDP	rosedad	ra	2	gn	F/M	f hu	III (3)
MELASTOMATACEAE									
Arthrostemma ciliatum Ruiz & Pavon	265	SDP	*nida-ii* caña agria	ra	1	or	GH		II, IV (2)
Conostegia xalapensis D. Don.	419	SDP	moradito del cerro	ra ho	11	-	OU	-	IV (1)

Scientific name	Nr. FREI	Locat.	*Zapotec name* Spanish name	Part used	Prep.	Application	Groups of indigenous uses	Classification	Import.
MYRTACEAE									
Eucalyptus camaldulensis Dehnh.	37	todos	*gusha'a alcanfor* eúcalipto	ho	2 4 7	lc gn re vg	GH RA SMS fmG aire	c ac si	
Eucalyptus sp.	250	SDP	*ventulatu*	ho	2 4 7	lc gn re vg	"	c	I,III,IV (1)
Eugenia aromatica Berg	254	todos	*cuonagueleraajeu'u* clavo	inflo	1 5	lc gn or	RAd SMSd aire OU	c	I (4)
Eugenia capuli Schltdl.	171	SDMP	*yanaj'* cinco negrito	ca	1 2 3 4	lc gn or re vg	GHs RA Di fmG OU	f	I (4)
Psidium x hyploglaucans Standl.	168, A	SDMP	*behuishxuba'a* guaiabillo (no fuerte)	rz	1	or	GHs	f	I (3)
Psidium guajava L.	20	todos	*guisha nguetuj* hoja de guayaba	ho rz ret	1 2	gn or re vg	GHds fmGs OU	f c am es	I (7)
Psidium salutare (Kunth) Berg	63	SDP	*raiana* *bihuishuba'aj*	rz ho fr	1	or	GH d	f c am es	I (8)
ONAGRACEAE									
Epilobium sp.	365	GdH	*guish claab* clavo del monte	ra	16	or	RAs DIs fmGs	f c	I (2)
PUNICACEAE									
Punica granatum L.	79	todos	granada	fr ca	1 4 10	lc or vg	GH RA SMSds DIds fmG	f c am es	I (8)
rutales:									
ANACARDIACEAE									
Juliania adstringens (L.) Schltdl.	152	GdH SMG	*ya'guiaj* casc. de guachanalate	ca ho	1 2 16	lc gn or re vg	GHd SMSd F/M fmG CO	f secc	I (9)
Comocladia engleriana Loes.	343	todos	*laatz* / inchador, inchahuevo	ca ho	10 11	lc	DIds	f c ve	I,II (3)
Mangifera indica L.	22	todos	mango manolo, oro	ho go se	1 4	lc or	GH RA DIs F/M OU	c	I, II(3),IV(4)
Spondias mombin L.	105	todos	*biadxi* hoja de ciruelo	ho fr	1 2 5	lc or vg	RAs DIs fmGs	f am	I,II (3)
Spondias purpurea L.	104	todos	*biadxidu'u* *obo*	ho fr	11 15	lc or vg	RAs DIs fmGs OU espi salp OU	f am	II (3)

BURSERACEAE									
Bursera cf. penicillata (Sessé et. Moç. ex DC.) Engl.	17	todos	*ii'guiaj* copal	re ca ho	2 3 10 12	lc gn re vg	GH RA F/M fmG espa OU	f c am es	II (4)
Bursera grandifolia (Schltdl.) Engl.	12	todos	*yalajguettu'u* palo mulato	ca	1 2 3	lc gn or re vg	GH DI F/M fmG	f am si	I (6)
Bursera sp.	288	SMP	*yalanguiaj*	ho go	10 12	lc	SMSd DI aire	c	I (2), II (3)
Bursera sp.	376	todos	*guish yaguiyaj'* hoja de sumerio	fr	1	or	DI	f c	I (2)
MELIACEAE									
Cedrela odorata L.	129	todos	*yagadoo* cedro	ca ho	1 2	lc or	RAd SMSd DId aire OU	f	II (4)
Swietenia humilis Zucc.	16	todos	*gueleyexi'i* caoba	se ho	2 3 6 8 11	lc gn vg	GH DI F/M fmG OU	f am	II (4)
RUTACEAE									
Citrus sp.	153	todos	flor de, hoja de lima	ra ca	10	lc or	GH DI	c am du	II (2)
Citrus limon (L.) Burm. f.	11	todos	*cuanani'ij* limon	ju ho ret ret	4 15	lc or	GHsd RA DI F/M OPH verg OU	f ac si hu	I (6)
Citrus sp.	237	todos	limon dulce	ju	15	or	GH OU	f c	III(1), IV (2)
Citrus sp.	246	todos	*naraxa guayu'u* naranja agria, naranja de cavallo	ju su	10 15	lc or	RA DIds F/M OU	f am	I,II (2)
Citrus sp.	46	todos	*naraaxa'a* cascara de naranja	ca ho	1 11 15	lc or	RA DI F/M fmG ataq OU	c si	I (8)
Ruta chalepensis L.	41	todos	*shrud* ruda	ho ra	1	lc or re vg	GH RA DI F/M fmG OPH CO emp ataq OU	c si es hu	I (16)
sapindales:									
SAPINDACEAE									
Sapindus saponaria L.	2	SDP	*bijpiij*	ca (se)	1 8	lc gn	DI OU	f c am	IV (5)
Sapindus sp.	99	SDP	jaboncillo	se	8	lc gn	DI OU	c	I (1), IV(3)
Serjania racemosa Schum.	404	GdH	*riagshinguish* gulandrina	ho	1	or	GH	f c	I (2)
SIMAROUBACEAE									
Quassia c.f. amara L.	291	SDP SMG	cuassia	ca	1	or	GHd	c	I (2)
Simaba cedrón Planch.	164	SMP GdH	el cedron	se (ca)	1	or	SMSd DIds	c am	I,II (2)

Scientific name	Nr. FREI	Locat.	*Zapotec name* Spanish name	Part used	Prep.	Application	Groups of indigenous uses	Classification	Import.
polygalales:									
KRAMERIACEAE									
Krameria pauciflora (Moç. & Sessé) ex DC	384	SMG	*luu guiatzimbeer* hoja de disinteria	ra	1 3	or re vg	GHs fmGs	f c	I (3)
Krameria pauciflora (Moç. & Sessé) ex DC	190	SDP	romerito	hb	1 2	gn or re vg	fmGd	c hu	I (1), IV (2)
MALPIGHIACEAE									
Byrsonima crassifolia (L.) Kunth	117	todos	*mbatsi* nanche	ca ho	1 10	lc or vg	GHs RAd DI fmGs OU	f	I (8)
geraniales:									
ZYGOPHYLLACEAE									
Guaiacum sanctum L.	64	SDMP	balsamo	ca	5 8	lc gn	DI OU	f c hu	IV (5)
Larrea tridentata (DC.) Cav.	403	GdH	gobernadora	pl en	11	lc or	GHd	f c	I (2)
celastrales:									
CELASTRACEAE									
Hippocratea excelsa Kunth	275	SDMP	cancerina	ca	1	lc or re vg	GHd DIds fmG	f	I (2)
rhamnales:									
RHAMNACEAE									
Ziziphus amole (Sessé & Moç.) M. C. Johnston	346 I	SMG	pendeno	ca	1	lc vg	fmGs	c	I (4)
Ziziphus sp.	346 II	GdH	pendeno	ca	1	lc vg	fmGs	c	
VITACEAE									
Cissus sicyoides L.	306	GdH	*baladxi'i guexii* uva cimarron	ra	10 15	lc	DI nag	f c	II (2)
Cissus sicyoides L.	357	SDP	*elbüe sangu' laash* san julas	be	10 11	lc or	DI OPH nag	f	II (4)
LORANTHACEAE									
Phoradendron cf. amplifolium Nutt.	293	SDP	*shaguii nagitzi'i,* mata palo	ho ra	10	lc	SMSd DI F/M	f	I (3), II (4)
Psittacanthus calyculatus (DC.) G. Don	23	SDP	*guixa'a shaguii* / hoja de urraca, mata palo	ra fl	10	lc	DI F/M	f am re	I (4)

APIACEAE									
Anethum graveolens L.	60	todos	*neeld* eneldo	se	1 3 6 8 11	lc gn or re vg	GH DI F/M emp ataq aire	f si	I (4), II (3)
Cuminum cyminum L.	221	SDP	comino	se	1 6		GHv RAd	f c	I (1), IV (2)
Eryngium foetidum L.	134	SDP	cilandro cimaron	hb	1 9	lc or	RAd SMSd aire OU	c hu	todos (1)
asterales:									
ASTERACEAE									
Artemisia absinthium L.	74	todos	hierba maestra	hb	1	or	GH fmG	f am	I (2), III (4)
Artemisia mexicana Willd. ex Spreng.	26	todos	*fiat* / estafiate	ra	1	or	GH d	c am hu	I (9)
Artemisia stelleriana Buss.	324	SMG	*guichuch roob* estafiate blanco	ho	1	or	GHds	am hu	I (2)
Baccharis salicifolia (Ruiz & Pav.) Pers.	172, A	SDMP	*chomisù,badzu'umij* *guajgu'u*	hb ra	8 11	lc gn or re vg	DI fmGd OU	f	I (3), II (2)
Calea urticifolia (Mill.) DC.	100	SDP	prolijiosa	ho	1	or	GHd	c am	I(2)
Calea sp.	361	GdH	prodigiosa blanco	hb	1 16	or	GHs	f c am	I (2)
Calea ternifolia Kunth	361 B	GdH	prodigiosa blanco	hb	1 16				
Calea urticifolia (Mill.) DC.	100 II	SMG	prolijiosa	ho		or	GHd	c am	I (2), IV (3)
Calea urticifolia (Mill.) DC.	359 II	GdH	prodigiosa amarilla	ho pl en	1	or	GHd	f c am	I (3)
Chaptalia nutans (L.) Polák	398	GdH	*guish lay león* diente de león	ho	1	or	GHd	f c	I (2)
Chromolaena collina (DC.) R. M. King & H. Rob.	409	GdH	*guish petrol* flor de gas, hoja petrolio	ho fl	1 3	lc	DI	f c hu sec	I (2)
Chromolaena odorata (L.) R. M. King & H. Rob.	390	SDP	prodigiosa	ho	1	or	GHd	c am	-
Chromolaena odorata (L.) R. M. King & H. Rob.	367	GdH	*guish crush* hoja de cruz	ho	1	or	GHs	f c	I (2)
Chrysanthemum parthenium Bernh.	235	todos	*guixnash* flor de Sta. Maria	fl hb	1 7 12	lc or	GH fmG	c	I (7)
Critonia quadrangularis (DC.) R. M. King & H. Rob.	349, A	SDMP/G	*guish lujtz yuss* lengua de cierva	ho	10	lc	GHd SMSd aire	f c hu	I,II (1)
Dysodia appendiculata Lag.	36	todos	*guibiguadajnii* *zamposuche guesxi* flor de calandria	ho ra	5 8 11 13	lc or	GHd RAd DI OPH aire verg	c si pi pe	I (6)

Scientific name	Nr. FREI	Locat.	*Zapotec name* Spanish name	Part used	Prep.	Application	Groups of indigenous uses	Classification	Import.
Dyssodia decipiens (Bartel.) M. C. Johnst. ex Johnst. & Turner	372	GdH	*guibgui parloshaan* flor de angelina	pl en	1 2	lc gn or	GH F/M	f c	I (2)
Epaltes mexicana Less.	151	SDP GdH	*badxuumiij* sabañon, tabaquillo	ra rz ho	2 3 9	lc gn re vg	DI F/M fmg espi	f c	I (4)
Espejoa mexicana DC.	411	SMG	margarita del monte	ho	10	lc	DIds	f c	II (1)
Eupatorium sp.	359	GdH	prodigiosa amarilla	ho	1	or	GHd	f c am	-
Gnaphalium roseum Kunth	97	todos	*tafiatu gueexii* gordolobo	hb ho	1 2 3	lc or re vg	GHs RA DI F/M fmG	f c si	I (5)
Gnaphalium sp.	399	GdH	gordolobo	hb	1	lc or re vg	GHs RA DI F/M fmG	f c si	I (2)
Matricaria recutita L.	75	todos	manzanilla	ra fl	1 2	lc gn or re vg	GHd DI fmG emp	f c si	I (6)
Melanthera nivea (L.) Small	256	SDP	*guixa'a ruti yussu'u* mata ganado	pl en	veneno	-	-	ve	IV (3)
Montanoa grandiflora Hemsl.	266	SDP SMG	flor de paprea, flor de teresita	ho	10	lc	DI pap OU	f c	I(1), IV (2)
Parthenium hysterophorus L.	252	SDP	marijuana cimarron	ho	1 2	lc gn	SMSd DI OU	f	II,III,IV (1)
Pluchea sp. o Vernonia sp. ??	325	SMG	canela del rio	ho	1	or	SMSd	(f)	II (1)
Pluchea symphytifolia (Mill.) W. T. Gillis	156	SMP	*balagasana* flor de Santa Maria	fl ho	1 10	lc or	GHd SMSd fmG	c	II (2)
Pluchea symphytifolia (Mill.) W. T. Gillis	325 II	SMG	canela del rio	ho	1				
Pluchea symphytifolia (Mill.) W. T. Gillis	209 II	SDMP/G	*gui'xaan* hoja de canela	ho	1 5 10	lc gn or	GHd SMSd fmG	f c hu	I (5)
Porophyllum pringlei Rob.	187	SDP	*gixa'a mbeetzii* hoja de bruja,de piojo	ho	1 8 10 11	lc or	GHd SMSd ojo aire	f c hu	I (4)
Porophyllum punctatum (Mill.) S.F. Blake	319	SDP	hierba de piojo, de bruja chico	ho	1 8 10	lc gn	SMSd aire	(f) hu	I (1)
Porophyllum ruderale var. macrocephalum (DC.) R.P.John	187 B	SDP	hoja de bruja, de piojo	ho	1 8 10				
Salmea sp.	107, B	SDP	*yagaguina'a* palo de chile	be ca	10 11	lc or	RA DI OU	c pi an hu	I (2)
Salpianthus macrodonthus Standl.	333	SMG	*guish pileej* pie de paloma	ho	11	or	OU	hu	II (1)

Tagetes erecta L.	1	todos	*guibigua* zampozuche	hb	1 2 5	lc gn or re vg	GH RA DI F/M fmG ojo OU	f pi hu	I (4)
Tagetes filifolia Lag.	91	SDMP/G	anis del monte	hb ra	1 4	or	GHd RA	c si du	I (3)
Tagetes lucida Cav.	90	SDMP	*guiahuajsa'ac* pericón	hb rz	2 3 4	lc gn or vg	GH RA DI F/M fmG OU	c du pi al	I (6)
Tithonia diversifolia (Hemsl.) A. Gray	29, A	todos	*ru'ulá* arnica	ret hb	1 2 8 10	lc gn or re vg	GH RA SMS DI F/M fmG aire nag OU	f am	I (14)
Trixis inula Crantz	420	SMG		-	1	gn	GH		-
Vernonia deppeana Less.	209	SMP	*gui'xaan* hoja de canela	ho	1 5 10	lc gn or	GHd SMSd fmG		-
	123	SDP	*rula'a nidila'a* arnica de castilla	ho	2 9 10	lc gn	DI	c	I,II,III (1)
dilleniales:									
DILLENIACEAE									
Curatella americana L.	318	SDP	*balaga lujtza yussu'* hoja de lengua de vaca	ho	lija	lc	OU		IV (1)
violales:									
BEGONIACEAE									
Begonia heracleifolia Schltdl. & Cham. 66 A, 501 A, 507 A, 520 A, 540 A, 542 A	66	SDP	san nicola	rz	5 10	lc	SMSd DI can	f si	I (1), IV (3)
FLACOURTIACEAE									
Muntingia calabura L.	181	SDP	*mbe'e ze'e* capulin	ho fr ca	1 10	lc or	GH RA DI emp OU	c	I (3), II (2)
BIXACEAE									
Bixa orellana L.	82	SDP SMG	*mbeye'e* achiote	ho se	1 8	lc or	GH SMSd DI F/M fmG OU	f c si	I (5)
Cochlospermum vitifolium (Willd.) Spreng.	341	SDP SMG	*vapombu*	pa	1 6	or	GH F/M fmG OU	f	I (4)
CARICACEAE									
Carica papaya L.	80	todos	papaya	se fr go pa	11	or	GH fmG calor OU	f am du	II (3), IV (7)

Scientific name	Nr. FREI	Locat.	*Zapotec name* Spanish name	Part used	Prep.	Application	Groups of indigenous uses	Classification	Import.
PASSIFLORACEAE									
Passiflora sp.	176	SDP	granadita	fr	11	or	OU	f du	IV (2)
Passiflora sp.	213	GdH	passiflora	ho	1 11	gn or	F/M nerv aire cal	f	I,II,IV (1)
Passiflora sp.	350, II	SMP	pepe	be	1 3	lc or vg	fmG dorm	f c	I,II (1)
Passiflora sp.	225	SDP	*wapapa* granadita del monte	ho	1	lc or	GH DI aire OU	f	II (4)
TURNERACEAE									
Turnera ulmifolia L.	186	SDP SMG	*lexuuba'a quitzii* malva blanca	hb	1 2 3 11	lc or re vg	GHs DI fmG	f c	I,II (4)
Turnera diffusa Willd. ex Schult.	335, A	SMG	*guish fiebre* hoja de fiebre	ho ra	1 2 3	lc gn or re vg	F/M	f	I (4), II (3)
cucurbitales: CUCURBITACEAE									
Cayaponia racemosa (Mill.) Cogn.	417	SDP	*shuana'a soldadu* hierba de soldado	fr	1	or	GHd OU	f c	I (2), IV (1)
Cucurbita argyrosperma Huber ssp. sororia (L. H. Bailey) L. M. Derrick & Bates	224	SDMP	*guedu laac* calabaza amarga	fr ma	10	lc	DI fmG rel espi	f am	I (5)
Cucurbita cf. pepo L.	124	todos	*gueatu'u* calabaza	se go	2 6 11	lc gn or	GH DI espi asc OU	f c	II (3), IV (4)
Lagenaria siceraria (Molina) Standl.	303	SDP	*lobej' quiaj'ga chical pestle*	fr	*chical*		OU		IV (2)
Lagenaria siceraria (Molina) Standl.	202	todos	*beejru'u, guiajga* pumpo, lipo	ca ho	10	lc	GH RAd aire OU	f	II,IV (2)
Luffa aegyptiaca Mill.	206	SDP	estropajo	fr	3	lc	DI	fre am	I(2), IV(2)
Momordica charantia L.	31	SDP	manzanita, flor del chino	hb se	2 11	lc or	DI OU	f am	todos (2)
Sechium edule (Jacq.) Swarz	72	todos	hoja de chayote *balagayaappa*	ho fr ret	1 2 14	lc gn or	GH SMSd espi nerv OU	f si hu	I (5)
capparales: BRASSICACEAE									
Brassica sp.	219	todos	mustassa	se	1 7	lc or re vg	GH fmG aire	c	I (7)
Lepidium virginicum L.	364	GdH	*guish inguiedi* hierba de pollo	hb	1	lc or	DI fmGs	f c	I (2)

Nasturtium officinale R. Br.	243	SDP	*berro*	hb ho	1 9	or	GH OU	f	I (1), IV (4)
capparidales:									
MORINGACEAE									
Moringa oleifera Lam.	25	SDP	*jasintu'u* jasinto	fl ho	8	lc gn	SMSs F/M	f c si	III (3)
salicales:									
SALICACEAE									
Salix bonplandiana Kunth	413	SMG	hoja de saus	ho ret	1 5	lc or	GHd SMSd F/M	(f)	I (1)
Salix sp.	3	SDP	hoja de sauce	ho ca	1 3 13	gn vg	DI F/M	f si	I (4)
malvales:									
BOMBACACEAE									
Ceiba acuminata (S.Wats.) Rosé	214	SDP	*yaga shiene'e* ceiba	ho rz se	3 11	lc vg	fmG cal OU	f	III (3)
Ceiba pentandra Gaertn.	299	SDP	*biuug* pochote	ho rz se	1 2 6	lc gn or re vg	GHs DIs asc deb OU	f c	I (3)
MALVACEAE									
Gossypium hirsutum L.	304	SDP	*shilla'a* algodón	ho al	10	lc re vg	GHs SMSd DIds fmG OU	f c	I (3)
Hibiscus rosa-sinensis L.	212	todos	tulipan	fl	4	or	RA F/M aire	f	I (5)
Hibiscus sabdariffa L.	70	SDP	flor de jamaica	fl	1	or	GH COs OU	f am ac si hu	I (4)
Hibiscus uncinellus (Moq. & Sessé) ex DC.	424	SDP	tulipan del cerro	ho	4	or	RA	f c	III , IV (1)
Malvaviscus arboreus Cav.	253	SDMP	*tulipan duendi*	fl ho	1 4	lc or	RAd DI fmG sus	f	I (2), II (3)
Sida acuta Burm. f.	292	SDP	*lexuba'a nagatzii* malva amarilla	ho	1 2 3 8	lc gn re vg	SMSds DIds F/M fmG OU	f si	I (3), II (5)
Sida acuta Burm. f.	94	SDMP	*lexuba'a gutzii* malva blanca	ho ra	1 2 3 8	lc gn re vg	SMSds DIds F/M fmG OU	f	I (4)
STERCULIACEAE									
Guazuma tomentosa Kunth, (G. ulmifolia Lam.)	57	SDMP	*yana'a* caulote	se fr ca	1 3	or re	GH mar F/M	f si du es	I (11)
Melochia nodiflora Sw.	228	SDMP/G	*lexuba'a moradu'u* malva morada	ra ho	1 3 10 11	lc gn re vg	DIds F/M fmG	f	I (4)
Melochia tomentosa L.	352	SMG	malvarisco morado	ra	1	lc re vg	DI fmG	(f)	II (2)

Scientific name	Nr. FREI	Locat.	*Zapotec name* Spanish name	Part used	Prep.	Application	Groups of indigenous uses	Classification	Import.
Theobroma cacao L.	423	todos	cacao	se fr	9 10 11	or	OU	f c	IV (10)
Waltheria aff. conzattii Standl.	328	SMG	*alshoob nagatzi'* malvarisco amarillo	ra	10	lc	DI	f c	III (3)
Waltheria indica L.	329	GdH	*alshoob nagatzi'* malvarisco amarillo	rz	1	or	GHd fmGd	f c	I (2)
TILIACEAE									
Heliocarpus appendiculatus Turcz.	53	SDP GdH	*yaga lajsa'a* palo de majagua	go ca mag	10	lc	SMSs DIs	f t si pg	I (5)
Heliocarpus donnell-smithii Rose ex D. Don.	416	SDP GdH	*lajtz* majagua rojo	le mag	10	lc	DIds	f c	I (2)
Heliocarpus terebinthinaceus (DC.) Hochr.	305	SDP GdH	*lajsa'a boogui* majagua cabalin	ca mag	1 10	lc or	GHs DIds OU	f c	II (2)
Triumfetta speciosa Seem.	386	SDP GdH	*yag lass* majagua blanca	ca mag	10	lc	DIds	f c pg	I (1)
urticales:									
CANNABACEAE									
Cannabis sativa L.	290	todos	marijuana	ra	5 17	lc	SMSd aire OU	f ha	II (2)
MORACEAE									
Brosimum alicastrum Swarz	414	SDP	*viguiiru'u*	se	1	or	OU		IV (1)
Cecropia peltata L./ obtusifolia Bertol.	73	SDP GdH	*guarumbo, yagaba'aj* chancarro	ho ret	1 11	lc gn or	GHs RA SMSd DIs F/M fmG flac	f c si hu	I (5)
Dorstenia drakena L.	279, A	SDP	*yanayu'u*	rz	1 5	lc gn	GHs SMSd aire	f am	I (5)
Ficus goldmanii Standl.	307	todos	*dxuúmii* / higo	ho	10	lc	RAd SMSd DIds	f c	II (3)
Ficus insipida Willd.	98	todos	*dxuúmii* / higo	ho go le ca	8 10 11	lc	SMSds DIds nag OU	f du	I (3)
Ficus ovalis (Liebm.) Miq.	347	SMG	*amate* / higo	ca fr	1	or	GH CO nerv prec OU	f c si	I (2)
ULMACEAE									
Trema micrantha (L.) Blume	308	SDP GdH	*lajsa'a baagui'* majagua mixe, jonote	ca	10 11	lc	DIds	f c	II (2)

URTICACEAE									
Discocnide mexicana (Liebm.) Chew.	69	SDP	*balagadena* , *lagui*	le se hb	2 10 11	lc	RAd SMSd	c qu	I,II(1), III,IV(2)
euphorbiales:									
EUPHORBIACEAE									
Croton alamosanum Rose	392	GdH	*guaanashnash*	ho	11	lc	Dl	c am qu ve	II (1)
Croton arboreus Millsp.	161	SMP	*copalching*	ca	1 9	lc vg	Dlds fmG	f am	I,II,IV (1)
Croton ciliataglanduliferus Ortega	101	SDP	*guixaxunaashii* mata pescado, hoja de mesquino	le ho go	1 1	lc	Dl	c pi qu ve	II (3)
Croton draco Schltdl.	425	SDP	*yague riene*	ca	2 3	lc re	GH RA	f c	III (3)
Croton flavescens Greenm.	336	SMG	*guaanshnash*	ho	10	lc	Dl	c am qu ve	II (1)
Croton niveus Jacq.	161 B	SDMP	*copalching*	ca	1 9	lc re vg	fmG	(f)	-
Croton niveus Jacq.	300	SMG	*copa ching*		3	lc gn or re vg	GHs Dl F/M	f c	I (2)
Croton niveus Jacq.	385	SDP	*copa-chin*	ca	1	lc vg	Dlds fmG	f am	I,II,IV (1)
Croton soliman Cham. & Schltdl.	204	SDP	mata pescado, hoja de mesquino	hb	11	lc	Dl	c pi qu ve	-
Euphorbia pulcherrima Willd. ex Klotzsch	363	GdH	*guie chien* flor de noche buena	ho	10	lc	Dl OU	f	I (2)
Euphorbia hirta L.	370	GdH	*guish mbsia'a* golandrina	ra hb	1 3	lc or re vg	GHs fmG	f c	I,II (2)
Euphorbia heterophylla L.	388	SDP	-	le	1	lc	Dl		I (1)
Euphorbia micromera Boiss.	251	SDP	zacate rojo	ho	1 2 10	lc or re	GHs Dl	f c	I (3), IV (2)
Hura polyandra Baill.	155	SDMP	empurga	se	1 11	or	GH vet	c	I (3)
Jatropha curcas L.	121	SDMP	piñon	ho go se	1 2 11	lc	Dl OU	f qu secc	I (4)
Manihot sp.	276	SDMP/G	*guyaaajca'a* yucca, maniok	rz	1	or	OU	f	IV (4)
Pedilanthus pringlei Rob.	320	SDP	*nia'badu'u*	pe	2 11	lc	SMS espi cam OU	f c	II (4)
Ricinus communis L.	32	todos	*yaga huegu'u* hoja de higuerilla	ho se ca	1 8 11	lc or	GHd Dld	f ac si	I (8)
theales:									
THEACEAE									
Ternstroemia pringlei Standl.	21	todos	té de tila	fl	1	or	GH COs nerv	f am si	I (4)
Ternstroemia pringlei Standl.	92	todos	té de tila	fr	1	or	GH COs nerv	f am si	I (4)

Scientific name	Nr. FREI	Locat.	*Zapotec name* Spanish name	Part used	Prep.	Application	Groups of indigenous uses	Classification	Import.
ebenales:									
EBENACEAE									
Diospyros digyna Jacq.	108	SDP	*bila'huaj* zapote negro	ca fr ho	2 10 11	lc gn or	DI OU	f c am	II (3)
Diospyros verae-crucis (Standl.) Standl.	203	SDP	*ndxuuli'i* tachona	fr	11	or	OU	f c ci	IV (4)
SAPOTACEAE									
Manilkara zapota (L.) van Royen	340	SDP SMG	*guil ziji* chico zapote	ca(fr) go se	2 6	or	GH OU	c	IV (3)
Pouteria sapota (Jacq.) H.E. Moore & Stearn	165	todos	semilla de mamey *la'adxiguelexuunu'uj*	se	9 10	lc gn or	SMSs DI espi OU	f am	I (4)
dipsacales:									
CAPRIFOLIACEAE									
Sambucus mexicana Presl ex DC.	122	SDMP/G	flor de sauco	ho fl	1 2 13	lc gn re vg	SMSd DIs F/M fmG OPH	f	I (6)
ericales:									
ERICACEAE									
Arctostaphylos pungens Kunth	410	GdH	*pingüita*	fr ca	1	or	GH COs	f c	I (1)
gentianales:									
APOCYNACEAE									
Catharanthus roseus G. Don.	135	SDP	*parahuita*	ho	11	lc re	GH DI OU	f c	II (1), III (2)
Malouetia guatemalensis (Muell. Arg.) Standl.	231	SDMP	*mbiigu' moradu*	le	11	lc	DI	f	I (3)
Nerium oleander L.	215	SDMP	clabel	go ho	5 11	lc vg	RAs DI fmGs	c am qu	I (4)
Plumeria rubra L.	154	SDMP	*guiatzatzii quitzii* palo loco	fl ca	1 10 11	lc re	SMSds DIds fmGs OU	f am ba	II (2)
Pulmeria rubra L.	71	SDMP	*guiatzatzii nagatzii*	fl ca go	2 4 11	gn or	RAs OU	f am hu pg	I (5)
Rauwolfia tetraphylla L.	77, 95	SDP	*guanabajcu* veneno del perro	ho go fr	3 11	lc re vg	DI fmG	c am qu ve	I,IV (3)
Stemmadenia obovata (Hook. & Arn.) Schum.	178	SDMP	*mbiigu'* del monte	go	11	lc	RAd DI	f	II (4)

Thevetia plumeriaefoliae Benth.	170	SDMP	*mbiigu ' guexii /* del monte	go	11	lc	RAd DI	f c pi el	I (3)
Thevetia thevetioides Schum.	19	SDMP	*mbiigu'*	pl en	1 2 8 11	lc gn re vg	GH RAd DI F/M	f c si pi	I (3), II (3)
ASCLEPIADACEAE									
Gonolobus aff. barbatus Kunth	426	SDP	*cantuva*	fr	9 11	or	OU	f c hu sa	IV (2)
Gonolobus cf. fraternus Schltdl.	242	SDP	*batuguexe'e* candua, cantuva	fr	1 9	or	OU	f c hu	IV (8)
LOGANIACEAE									
Buddleja americana L.	407	SMG GdH	*gush blaad* lavatraste	ho	2 7	lc gn re vg	RA DI fmG OU	f c	I (3)
RUBIACEAE									
Alibertia edulis A. Rich. ex DC.	387	SDP	*shbola bishulu'* marimbola	fr	11	or	OU		IV (3)
Coffea arabica L. / C. liberica Bull./C. canephora Pierre	258	todos	*guish güe* / café	ho se	1 9	lc re	GH DIds fmGs OU	c am	I (5)
Hamelia patens Jacq.	233	SDP SMG	canserina del monte	fl ho	2 3 10	lc re vg	DI fmG	f ve	I (6)
Rondeletia leucophylla Kunth	9	SDP	huele de noche	fl ho	2 3 11	gn or	F/M pen tris OU	f si	II (2), IV (4)
boraginales:									
BORAGINACEAE									
Borago officinalis L.	226	todos	*borraj* / borraja	hb	1	or	RA fmG	c	I(3), II (2)
Cordia curassavica (Jacq.) Roem. & Schult.	118	SDP	*shubaruuba'a* /esco-billo,mais grande	ho	1 2	gn or	GHds F/M OU	f c	III (2)
Cordia dentata Poiret	179	SDP SMG	*gulaveri* flor de gulaveri	fr	10	lc	SNS OU	f c pg	I,II (1), IV (4)
Ehretia tinifolia L.	280	SDP	lambimbo	se ho fr	1 6	or	GH DI COs OU	(f)	II , IV (2)
Heliotropium angiospermum Murray	205	SDP	*guixamberu'u quit-zii* /de guajalote cabeza blanco	ra ho	1 3	lc re vg	GHs DIds fmG	f	I (5)
Heliotropium indicum L.	114	todos	*guixamberu'u* cola de alacran, hoja de guajalote	ho	1 3 9 10 11	lc or re vg	GHs DIds fmG aire	f	I,II (3)

Scientific name	Nr. FREI	Locat.	*Zapotec name* Spanish name	Part used	Prep.	Application	Groups of Indigenous uses	Classification	Import.
Tournefortia densiflora Mart. & Gall.	8 ,A	todos	*blajtu mshtajala* hoja de cancer	hb ho	2 3	lc gn or re vg	GH SMS DIs fmGs OPHs	f c si	I (6)
Tournefortia densiflora Mart. & Gall.	382	SMG	hoja de cancer	ho	1 2 3 7	lc gn or vg	SMS DIds fmGs	f c	I (4)
solanales:									
CONVOLVULACEAE									I,II,II(1),
Ipomoea batatas (L.) Lam.	166	SDP	camote del monte	rz	10	lc or	SMSd OU	(f) c	IV (2)
Ipomoea (sect. batatas) tiliacea Willd.	113 B	SDP	*guamol*	be	1 8	lc gn or	GH DI	f	I (1), IV (3)
Ipomoea alba L.	113	SDP	*guamol* rojo	be	1 8	lc gn or	GH DI	f	I (1), IV (3)
Ipomoea alba L.	147	SDP	*guamol* blanco	be	1 8	lc gn or	GH DI	f	I (1), IV (3)
Jacquemontia nodiflora (Desv.) G. Don	102	SDP	*badooj buishli* flor de virgen chico	se	6	or	divin	f	III (2)
Rivea corymbosa (L.) Hallier f.	89	SDP	*badooj* flor de virgen	se be fl ho	6	lc or	DI divin	f ha	II(2), III (4)
POLEMONIACEAE									
Loeselia mexicana (Lam.) Brand	139	todos	espinonzillo	hb ra	1 2 3 6	gn or re vg	GH DI F/M 7 enf	f	I (4)
Loeselia ciliata L.	282 B	SDMP	jaboncillo , espinonzillo de cerro	ho rz	1 2 3 8	lc gn vg	DI F/M fmGs	f	I (3)
Loeselia mexicana (Lam.) Brand	383	GdH	espinonzillo	ra	1	lc gn or	F/M	f	I (3)
Loeselia sp.	282	SDP	jaboncillo (palito)	ho rz	1	lc gn vg	DI F/M fmGs	f	
SOLANACEAE									
Brugmansia suaveolens Bercht & Presl	230	SDP GdH	hoja de campana, rosa	ho fl	1 10	lc or	RA SMSd DIds COs aire	f ve	I (6)
Capsicum annuum L. var. annuum	380	SMG	chile pasillo	fr	11	or	OU	(c) pi	IV (2)
Capsicum baccatum L.	112	todos	*balagaguina'a* hoja de chile	ho ra fr	2 11 13	gn or re vg	F/M OPH ojo verg igua OU	f c pi	I (4), II (3)
Datura candida (Pers.) Saff.	408	SDP GdH	floripondio, flor de campana blanco	ho	1 10	lc or	DIds CO	f c ve	I (3)
Datura metel L.	289	todos	*buuruj'hui moradu* toluache morado	se fr ho ca	7 9 11	lc	GHs DMSd DI vet	f ha ve	I (3)

Datura stramonium L.	85	todos	*mbuuruj'hui* toluache blanco	hb ca	2 10 11 16	gn or re vg	GHds SMSd DIds fmG verg dorm aire OU	f c am ve	I (7)
Nicotiana plumbaginifolia Viv.	115	SDP	*guiass yeen* tabaquillo	ho	1 2	gn or	SMSd F/M ataq bora OU	f (c)	I (3)
Nicotiana tabacum L.	116	SDP	*giasa'a* tabaco cimaron	ho	1 10 17	lc gn or ns	RA SMSd DI F/M sus nerv	c	I,II (2)
Solanum americanum Miller	103	todos	*ledxuxii* hierba mora	hb ho	1 2 10	lc or	DIds OU	f	I (3)
Solanum diflorum Vell.	137, A	SDP	*chacuaquillo,* cordilliera	ho	2 3 10	lc gn or re vg	DI F/M	f (c)	I (5)
Solanum hirtum Vahl	229	SDP	*guedxe baladu'u* lavaplato blanco	ho	9 14	lc gn re vg	DIds F/M fmGs espi OU	f	I (3)
Solanum lanceolatum Cav.	169, A	SDP	*guedxe baladu'u* lavaplato morado	ho	9 14	lc gn	DIds espi OU	f t secc	I (5)
Solanum lanceolatum Cav.	406	GdH	espina	ho	1 2 3 5 8	lc gn re vg	DI espi	f c	I (1)
Solanum seaforthianum Andr.	368	GdH	*guequelito* bejuco de iguana	fr ho	1	or	deb asc	f c	I (2)
Solanum torvum Sw.	5	SDP	*quitzii* /hoja de lavaplato blanco	ho	2 3 14	lc gn re vg	DI F/M fmGs espi OU	f am	I (5) , II (5)
	185	SMP	*chuchita*	ho ca fr	1 10	lc	DI OPH	f c am	II (4)
lamiales: LAMIACEAE									I,III (1),
Hyptis albida Kunth	120	SDP	*gujcu'u* pie de tortola	ho ca	1	or	GHs	c	IV (3)
Hyptis albida Kunth	334, II	SMG GdH	*guichu'*	ho fr	1	or	GHs	f c	I,II (2)
Hyptis mutabilis (Rich.) Briq.	50	SDP	hierba de toro	rz	1 2 3	lc gn or vg	fmGs	c hu	I (1)
Hyptis suaveolens (L.) Poir.	93	SDP	hierba de toro	ho	1 2 3	lc or vg	GHS RA SMSds DIds OU	c am si hu	I (8)
Hyptis tomentosa Poit.	389	SDP	*guichu'*	ho	1		GHs		II (5)
Hyptis verticillata Jacq.	7	SDMP	*guixa'a/* hierba de martina	ho	1 8 13	lc gn or vg	GH SMSd DI fmG aire	f si	
Mentha x piperitae L.	140	todos	*bedxestila'a* hierba buena	ho	1	or	GHd COd OU	f hu	I (7)

Scientific name	Nr. FREI	Locat.	*Zapotec name* Spanish name	Part used	Prep.	Application	Groups of indigenous uses	Classification	Import.
Ocimum basilicum L.	4	todos	*guiasharu'uj* albahaca	ra	1 8 13	lc gn or vg	GHd SMSd F/M fmGd ojo verg esp	fr secc si hu	I (10)
Pogostemon cablin (Blanco) Benth.	149	SDP	oregano grande	ho	1 15	lc or vg	GHd RAd fmGds OU	c hu	I (3)
Rosmarinus officinalis L.	218	todos	romero	se	1 7	lc or re vg	GH fmG emp rel	c am	I (7)
Salvia cf. tiliifolia Vahl	65	SDP	limpia	ra	13	lc gn	DI	f hu	I (1), IV (4)
Origanum sp.	260	todos	oregano	ra	1 11	or	OU	c	IV (2)
VERBENACEAE									
Lantana camara L.	193	SDP	*guxa'a riene'e* flor de sangre	ho	4 8	lc or	GHs DI	f c	II (4)
Lippia alba (Mill.) N.E. Br.	189	SDMP/G	salvia real, malva real	ra ho	1	or	GH fmG fri	f hu	I (5)
Lippia pringlei Briq.	109	SDP	*yagangucha'a* palo de gusano	ca ho	1	or	GH s	c	I (2), IV (3)
Lippia nodiflora (L.) Greene	197	SDMP	*guixa'a na'axii* hierba dulce	inflo	11	or	GHs RA F/M	c hu	II (3)
Stachytarpheta jamaicensis (L.) Vahl	309	SDP	*verchena*	hb	1	or	GHd F/M	(f)	III (1)
Verbena recta Kunth	360	SDP GdH	verbena	hb	1	or	GH	f c	I (2)
Vitex mollis Kunth	119, II	SDMP	*boyajsa'ac* ceresa negra	fr ho ca	1 4	or	RA	c	I (4)
scrophulariales:									
ACANTHACEAE									
Jacobinia spicigera L. H. Bailey	141	SMP SMG	*guxa'a tinta* hoja de tinta, de añil	hb	1 2	lc gn re vg	GH RA DI ataq alf OU	f	I (3)
BIGNONIACEAE									
Crescentia alata Kunth	174	todos	*buru boj* morrito	fr ju	1 4 10	lc or	RA SMS DI aire nag OU	f	I (6)
Crescentia cujete L.	173, A	todos	*buru shiiga'a* palo de morro	ca ma	4 10	lc or	RA SMS DI nag OU	f	I (5)
Parmentiera aculeata (HBK.) L. O. Williams	284, A	SMP	*biduaj guedxii* platano espina, uñ de tigre	fr fl	1 4 15	lc or	RAd	f c	I (4)
Pithecoctherum crucigerum (L.) A. H. Gentry	391	SDP	tres esquinas	be	8		OU (pescar)		IV (1)

Tabebuia impetiginosa (Mart. ex DC.) Standl.	55	SDP	palo roble	ca	1	lc (vet) or	SMS DIvet	c si	I (1), III (5)
Tecoma stans L. & Griseb.	394	GdH	hoja de san pedro	ho	1 5	lc or	GH DI	f c	I (2)
Tecoma stans L. & Griseb.	148	SDMP	*dze'ing*	ho	1 2	gn or	GH SMSd fmG fri	f	I,II,IV (1)
	402		trobadora	ho	1 5				
PEDALIACEAE									
Sesamum orientale L.	277	todos	ajonjoli	se	6 11	or	fmG OU	c	I (4)
PLANTAGINACEAE									
Plantago major L.	412	SMG	lantén	infl ho	1 15	lc	OPH	f c	I (3)
SCROPHULARIACEAE									
Capraria biflora L.	188	SDMP/G	*badxumij/* apazote de zorillo, sabañon	ra	1 2 10 11	lc gn or re vg	GH DIds espi	f pi	I (5)
Russelia sarmentosa Jacq.	405	SDP	*guish crii* hoja de cerilla	ra	1	lc re vg	DI	f c	I (1)
2. Klasse: Liliatae = Monocotyledoneae									
dioscoreales:									
DIOSCOREACEAE									
Dioscorea floribunda Mart. et Gall.	183	SDP	*ganabigujchii* barbasco	rz	1 5 10	lc gn vg	SMSd fmG aire OU	f c	II (3)
asparagales:									
AGAVACEAE									
Agave sp.	274	SDP SMG	*dobala'a* magey del cerro	go pe	1 10	lc or	RA DI chin OU	c am	I,IV(1),II(2)
	339	SMG	*bacüal* , gallo de peña	pe	11	or	GHd OU	f c	II (1)
ALLIACEAE									
Allium cepa L.	167	todos	*ceboy, cebul* cebolla blanca	rz	1 4 11	lc or	RA SMSd DIds gol ataq OU	c pi hu	I (4)
Allium cepa L.	175	todos	*ceboy moradu'u* cebolla morada	rz	1 2 4 5	lc gn or re	GHs RA SMS DIds cue ataq nag vet	c pi hu	I (5)

Scientific name	Nr. FREI	Locat.	*Zapotec name* Spanish name	Part used	Prep.	Application	Groups of indigenous uses	Classification	Import.
Allium sativum L.	54	todos	*aaxoj* / ajo	rz	1 4 10 16	lc or	GH RA SMSd DI ataq OU	c pi hu	I (5)
Allium sp.	354	GdH	*shitdx* cebollin	pl en	10	lc	OPH igua	f c	I (1)
ASPHODELACEAE									
Aloe barbadensis Mill.	44	todos	*zabila*	pe go	1 10 11	lc gn or	GH RA SMSd DI F/M COd OU	f c am hu	I(12)
SMILACACEAE									
Smilax sp.	183 II	SDP	barbasco	rz	1 5 10	lc or	DI	c am si es	II (5)
Smilax medica Schltdl.	49		zarzaparrilla	rz	1 16				
liliales:									
LILIACEAE									
	297 513	SMG SDP	elirio del rio	fl	5 3	lc	DI	f c	III (1),IV(2)
PONTEDERIACEAE									
Eichhornia crassipes (Mart.) Solms-Laub	378	GdH	rinoncillo	pl en	1	or	GH OU	(c)	I (2)
orchidales:									
ORCHIDACEAE									
Vanilla planifolia Andrews	429	SDP	vainilla	va	1 11	or	OU		IV
zingiberales:									
MARANTHACEAE									
Calathea lutea G. F. W. Meyer	56 II	todos	hoja blanca	ho po rz	1 3 10 11	lc or vg	DI F/M fmG OU	f si	todos (2)
Calathea ovandensis Matuda	56	todos	*balagaquitzii* hoja blanca	ho po rz	1 3 10 11	lc or vg	DI F/M fmG OU	f es	todos (2)
MUSACEAE									
Heliconia latispatha Benth.	283	SDP	*hua hua* platanillo (naranja)	ho fl	1 9	or	OU	f c	I (5)
Musa x paradisiaca L.	61	todos	*biduáj* platano	fl le fr ca rz	4 10 11	lc or	GH RA DI fmGs cal OU	f c t es	I (4)

ZINGIBERACEAE									
Costus pulverulentus Presl	133	SDP	*nida-ii* caña agria	pl en	1 15	or	GH fmG 7 enf	-	II (2), IV (2)
Zingiber officinale Roscoe	24	todos	jinhible, jengibre	rz	1 2 5	lc gn or	RAd SMSd DI aire OU	c pi	II (4)
bromeliales:									
BROMELIACEAE									
Ananas comosus (L.) Merr.	263	SDMP	piña	ju	1 11	lc or	GHs OU	f am	I,III,IV (2)
commelinales:									
COMMELINACEAE									
Commelina erecta L.	314	SDP	*madali*	pl en	1 10	lc	DI	f re	II (2)
Gibasis pellucida (Mart. & Gall.) D. R. Hun	312	SDP	*madali*	pl en	1 10	lc	DI	f re	II (2)
Rhoeo discolor (L'Hér.) Hance	142	SMP	zabila morado	ho	1 2 3	lc gn or re vg	GH SMSd DIds fmGs aire	f	I (4)
Tradescantia pallida (Rosé) D. R. Hunt	259	SMP	moradita	ho	1 15	or	GHs DId nag	f c	II (3)
Tripogandra serrulata (Vahl) Hand. - Mazz	313	SDP	madali blanco	pl en	1 10	lc	DI	f re	II (2)
	373	GdH	*guishambala* hoja de venado, de pescado	ho	5 10	lc	DI	f c	I (2)
Zebrina pendula Schnizl	68	SDMP/G	*madali*	hb	1 10	lc	SMSd DI F/M	f am si re	I (5)
cyperales:									
CYPERACEAE									
Cyperus articulatus L.	143	todos	*chantulli, shapandú*	rz	2 10	lc gn	SMSd DI aire jaq OU	f hu	I (8)
Cyperus diffusus Vahl	195	SDP	zacate	hb rz	1 3	or	F/M fmGd	f	II (3)
Cyperus lentiginosus Millsp .& Chase	322	SDP	zacate	hb rz	1 3	or	F/M fmGd	f	II (3)
Cyperus mutisii (H.B.K.) Griseb.	323	SDP	zacate	hb rz	1 3	or	F/M fmGd	f	II (3)
poales:									
POACEAE									
Cortaderia selloana (Schult.) Asch. & Graebn.	211	todos	*nida'a quitzii* caña blanca	ra	1 11	or	RA OU	c	II,IV (3)

Scientific name	Nr. FREI	Locat.	*Zapotec name* Spanish name	Part used	Prep.	Application	Groups of indigenous uses	Classification	Import.
Cortaderia sp.	210	todos	*nida'a xinil* caña roja	pe	1 11	or	RA OU	c	II,IV (3)
Cymbopogon citratus (DC.) Stapf	33	todos	*te guisa* / zacate, hoja de té, té de limon	ho	1	or	GH F/M nerv OU	f hu	I (7)
Oryza sativa L.	257	todos	arroz	se	6	or	GHs fmG OU	f	I (5)
Zea mays L.	150	todos	*bacuejlu'u moradu'u totomotzle moradu'u*	*to*	2 14	lc gn	DI espi OU	f	I (5)
Zea mays L.	40	todos	*xhuba'a* maíz, barba de, flor feminina	ba	1 2 6	gn or	GH fmG OU	f	I (7)
	273	SDP	*nida'a ngupil* caña dura, delgada, del armadillo	pe	1 4	or vg	RA fmGs OU	f c	II (1), IV (2)
arecales:									
ARECACEAE									
Acrocomia mexicana Karw. ex Mart.	245	SDMP/G	*biga'aj* coyól	rel	10	or	GH OU	f c du	II,IV (2)
Cocos nucifera L.	45	todos	coco	ag ca ho	4 6 11	or	GH fmG emp OU	f du	I (4), II (6)
Sabal mexicanum Mart.	87	SDP	palma real/*xi'inaaj*	rz	10	lc or	SMSds Dids	f c	II (1), IV(4)
Chamaedorea tepejilote Liebm.	199	todos	*gue'etzu'u* tepejilote	rz fr ho go	1 10 11	or	GH Dids OU	f c am	I(3), IV(4)
arales:									
ARACEAE									
Monstera deliciosa Liebm.	316	SDP	*chibaba* tripa de pollo	fr	11	or	OU	f c	IV (2)
Syngonium aff. angustatum Schott.	422	SDP	*buduaj gueexii* platano del monte	fr	11	or	OU	-	IV (2)
Xanthosoma robustum Schott.	28	todos	*blu'ulú* tequesquite	rz le	10 11 (1)12	lc or	SMS DI enc OU	f c pi pg	II (4)
Colocasia suculenta Schott.	430	todos	*malanga*	rz	1	or	OU	f	-

Undetermined plants

Spanish name	Nr. FREI	Locat.	*Zapotec name*	Part used	Prep.	Application	Groups of indigenous uses	Classification	Import.
albahacar del monte	366	GdH	*guish del gueex*	ho ra	8 10	lc	DI OPH ojo	f c	I (5)
balsamo	286	SDP		ho	1 10	lc	SMSd aire OU	f c	II(1), IV (2)
bejuco de chorizo	261	SDP	*longaniza'*	rz	10	lc	SMSd	f c	III, IV (1)
belladonna	177	SMP		ho	11 14	lc	GHs SMSd aire	c	II(1), IV (2)
cascara sagrada	536	GdH		ca	1	or	GH	-	-
	428	SDP	*chibaaba'*	ho	9 10	lc	DI	f c	II (2)
chinchong	244	SDP	*beroguguiachi*	ret co	11	or	OU	-	I (3)
chingolingo	342	SDP	*dxingolingu'*	ho	11	or	OU	f c	I (1)
cocolmeca	535	GdH		ho	1	or	GH	-	-
cola de borrego (Crassulaceae)	400	GdH	*guish pan burregu'*	ho	15	lc	OPH	f c	I (3)
cola de gato	393	GdH		pl en	1 11	lc or	DIds vet	f c	II (1)
colandrillo canoal	269	SDP		ho	1 2 11	lc gn or	F/M	-	II(1), IV(2)
colandrillo coralina	268	SDP		ho	1 11	lc or	DIds	f c	II(1), IV(2)
cuero viejo (hongo)	255	SDP	*guidigushu*	po	11	lc	DIds	f c	I(2),III,IV(1)
el asqueroso	127	SDP		ho	11	or	asc	-	II(1), IV(2)
espina de cavallo	532	GdH		fl	1 2	lc or	GH DI	-	-
flor de, cola de chango	377	GdH	*guish migu'u/*	pl en	1	lc gn	DI chib	f c	I (2)
flor de chivato	375	GdH	*guish chivat*	ho	8	lc	DI	f c	II (2)
flor de misericordia	374	GdH	*guish blaguiesh*	ho	1	or	GHd DOd OU	f c	I (2)
gangrina	216	SDP	*balagangrina*	ho	10	lc	DIds	f c	II(1), IV(2)
hierba amarga	512	SDP		fl	1	or	GHd	-	-
hierba buena del cerro, hierba dulce	511	SDP		hb	1	or	RA	-	-
hierba de cochino	128	SDP	*guixa'a mbehua'a*	rz	1 10	or	ant	-	II(1), IV(2)
hoja de algodon	530	GdH	*plactu mex*	ho	1	or	DI	-	-
hoja de antojo	503	SDP		ho	2 6	lc or gn	ant	-	-
hoja de malavar	180	SDP		ho	1	lc or	GHd emp	f c	I(1), IV(2)
hoja de manila	192	SDP		ho	11 13	lc gn	F/M aire nerv	-	II,IV (2)
hoja de pescado	125	SDP	*laxadù*	ho	1 2	lc gn or	ant	f c	II(1), IV(2)
hoja del camote del monte	249	SDP	*guixa'a guloatzii*	ho	11	or	OU	f	IV (2)
hoja del caldo	241	SDP	*guixa'a laguii, chaya*	ho	11	or	OU	f	I (4)
hoja de los angeles	369	GdH	*bindx daa'ij*	rz	1	or	RA	f c	I (2)

lacrima de juda	163	SMP		se	1	lc	OPH	f	II(1), IV(3)
lechuga	200	SDP	*lechu'u*	ho	2 10	lc gn	DIds F/M fmG	f c	III, IV (3)
lucema	217	todos	*cuanambidoxu'u*	se	1 7	lc or re vg	GH fmG emp relaj	f	I (7)
mariposa, naranja y amarillo	331	SMP		ho	1 3	lc or	DI	f c	I,II (1)
nuez de la montaña	262	SDP	*nuez lajtzii*	fr	11	or	GH	f c	II,IV (2)
palo de cepa	126	SDP		ca	1 2	or	CbS	-	I (1), IV (2)
panancillo	111	SDP		fl	11	lc	DI OPH ojo	f	I(1), IV(3)
papaloquelite	223	SDP		ho	2 11	lc gn or	aire	f	II(1), IV(2)
(hongo)	146	SDP	*paraguita*	pl en	11	or	divin	-	-
platano espina	287	SDP	*guamuchi*	fr ca	curtir	lc	OU	-	IV (3)
sabatía	396	GdH SMP	*zabadíla*	hb	2 7 10	lc gn re vg	fmG	f c	I (2)
sangre de drago	397	GdH	*shguid ladi angroagu'*	ca	16	lc re	GH RA	f c	I (2)
sarampion, hoja de	371	GdH	*guish lee*	ho	1	lc gn	DI	f c	I (2)
zacate chico	227	SDP		rz	1	or	GHs SMSd fmGs	c	III,IV (2)
zapotillo	355	SDP		fr	11	lc or	RA DI OU	f c	II (2)
zipress	533	GdH		se	1	lc	fmG	-	-

III List of healers participating in the study

Name	ethnic group	Languages	Location	Specialisation
Eleuteria Ramírez Antonio	Zapotec	bilingual	SDP	partera, curandera,
Estanislao Márquez Jiménez	Zapotec	bilingual	SDP	curandero, hierbero
Sixto Hernández Ramírez	Chontal (orig.)-Zapotec (born grown up)	bilingual (Chontal, Spanish)	SDP	curandero, hierbero
Constanzio Solano	Zoque (orig.) Zapotec (grown up, marriage)	bi- (tri-?) ilingual (Zapotec, Spanish, Zoque?)	SDP	curandero, com oracle, calendario
Antonia Rojas	Zapotec	bilingual	SDP	partera
Alba Gaspar Aguilar	Zapotec	bilingual	SDP	curandera
Pedro Jacób	Zapotec	mono-lingual (Zapotec)	SDP	curandero
Lencha, Lorenza Rueda	Zapotec	bilingual	SDP	partera,curandera
Sixta de Cena Estudillo	Zapotec	bilingual	SDP	partera, curandera
Silvina Ramírez	Zapotec	bilingual	SDP	hierbera
Efren Ramírez Antonio	Zapotec	bilimgual	SDP	hierbero
Alejandrina Aguilar Cabrera	Zapoteco	bilingual	SDP	partera curandera
Inés Juarez	Zapotec	bilingual	SDP	curandera, partera
Crisófora Vásquez Pérez	Zapotec	bilingual	SMP	partera, curandera
Erasto Lopez	Zapotec	bilingual	SMP	curandero
Jorge Navarro Gutiérrez	Zapotec	bilingual	SMP	curandero

Name	ethnic group	Languages	Location	Specialisation
Eufrosina Ruíz Vasquez	Zapotec	bilingual	SMP	hierbera
Francisca Gasca de Mendoza	Zapotec	bilingual	SMP	partera
Florencia Vasquez	Zapotec	bilingual	SMP	partera
Rosa Caranza Vasquez	north of Oaxaca	Spanish	SMG	partera, curandera
Dionísios Sibaja Rios	north of Oaxaca	Spanish	SMG	massajista
Erasmo Zaragoza Flores	Zapotec	bilingual	SMG	curnadero
Juan Jiménez Toledo	Zapotec	bilingual	SMG	curandero, huesero
Julia Granada Pérez	Zapotec	bilingual	SMG	hierbera
Bruno Jiménez Iglesias	Zapotec	bilingual	SMG	hierbero
Francisca Flores Hernández	Zapotec	bilingual	GdH	partera, curandera
Adela Guzmán Barrera	Zapotec	bilingual	GdH	partera, hierbera
Cipriano Alvarez Santiago	Zapotec	bilingual	GdH	curandero, partero, huesero, hierbero
Tomasa Toledo	Zapotec	bilingual	GdH	partera
Anselmo Lopez Flores	Zapotec	bilingual	GdH	hierbero

IV Medicinal plant garden in Santo Domingo Petapa

Plant species planted in the medicinal plant garden of the escuela bilingue (Primary school, with classes in Zapotec and Spanish) at El Barrio de St. Cruz of Santo Domingo Petapa:

Albahácar	*Ocimum basilicum* L.
Arnica	*Tithonia diversifolia* (Hemsl.) A. Gray
Bugambilia	*Bougainvillaea glabra* Choisy
Canela	*Cinnamomum zeylanicum* Nees
Chantulli	*Cyperus articulatus* L.
Cordonzillo	*Piper amalago* L.
Estafiate	*Artemisia mexicana* Willd. ex Spreng.
Flor de calandria/ Zamposuche del monte	*Dysodia appendiculata* Lag.
Frijolillo	*Senna occidentalis* (L.) Link
Gordolobo	*Gnaphalium roseum* Kunth
Hierba santa	*Piper auritum* Kunth
Hoja de alacrán	*Heliotropium indicum* L.
Hoja de cancer	*Tournefortia densiflora* Mart. & Gal.
Hoja de canela	*Pluchea symphytifolia* (Mill.)W.T.Gillis
Hoja de tinta	*Jacobinia spicigera* L.H. Bailey
Hoja de zorillo	*Petiveria alliaceae L.*
Laurel	*Litsea glaucescens* Kunth
Pericón	*Tagetes lucida* Cav.
Piñon	*Jatropha curcas* L.
Ruda	*Ruta chalepensis* L.
San Nicolás	*Begonia heracleifolia* Schltdl.&Cham.
Sauco	*Sambucus mexicana* Presl ex DC.
Siempreviva/Maraviliosa	*Bryophyllum pinnatum* (Lam.) Kurz
Yanayu'u	*Dorstenia drakena* L.
Zabila	*Aloe barbadensis* Mill.

V NMR-Spectra of isolated compounds

^{1}H of cucurbitacin B, 298 K, $CDCl_3$
^{13}C /dept 135 of cucurbitacin B, 298 K, $CDCl_3$

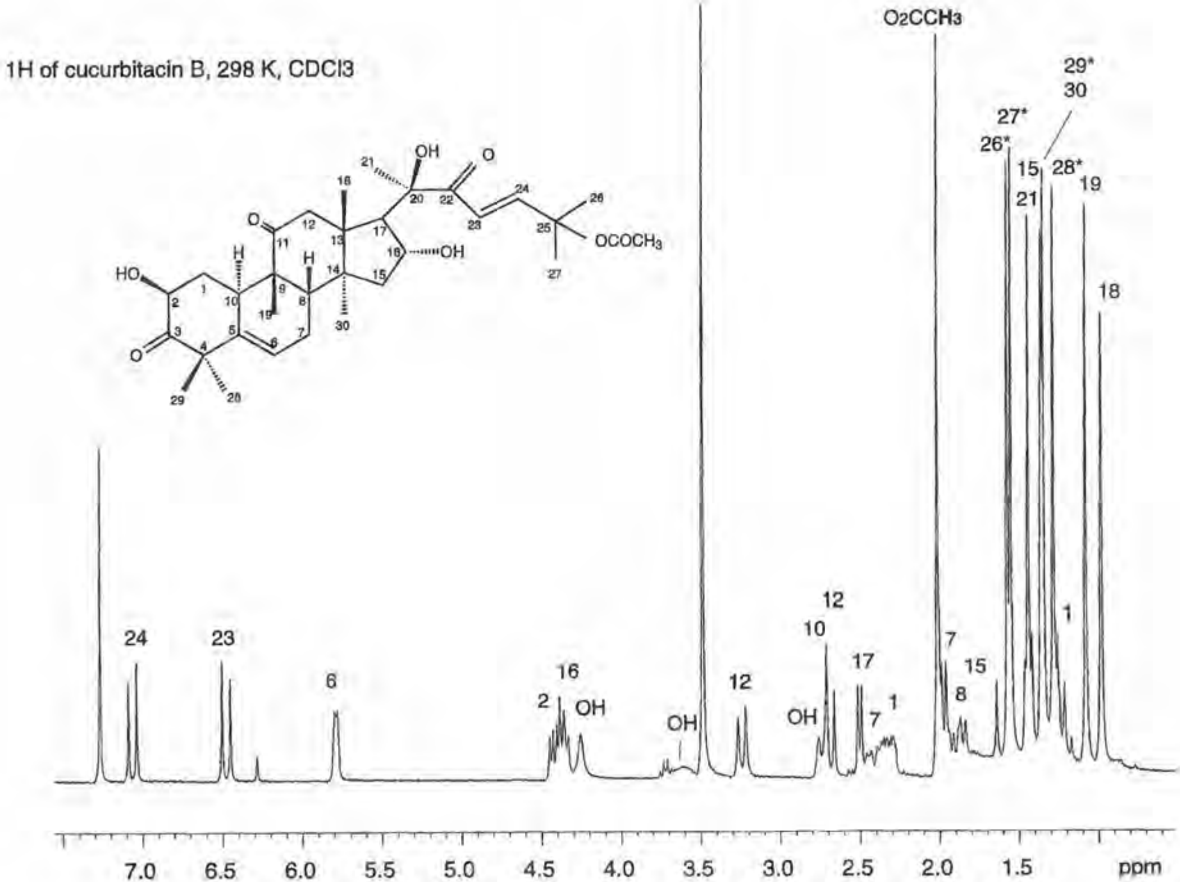
^{1}H of cucurbitacin D, 298 K, $CDCl_3$
^{13}C /dept 135 of cucurbitacin D, 298 K, $CDCl_3$
gs-HMQC of cucurbitacin D, 298 K, $CDCl_3$
gs-HMBC of cucurbitacin D, 298 K, $CDCl_3$
COSYDFQ of cucurbitacin D, 298 K, $CDCl_3$
80ms TOCSY of cucurbitacin D, 298 K, $CDCl_3$
500ms ROESY of cucurbitacin D, 298 K, CDCl3

^{1}H of 23,24-dihydrocucurbitacin D, 298 K, $CDCl_3$
^{13}C /dept 135 of 23,24-dihydrocucurbitacin D, 298 K, $CDCl_3$

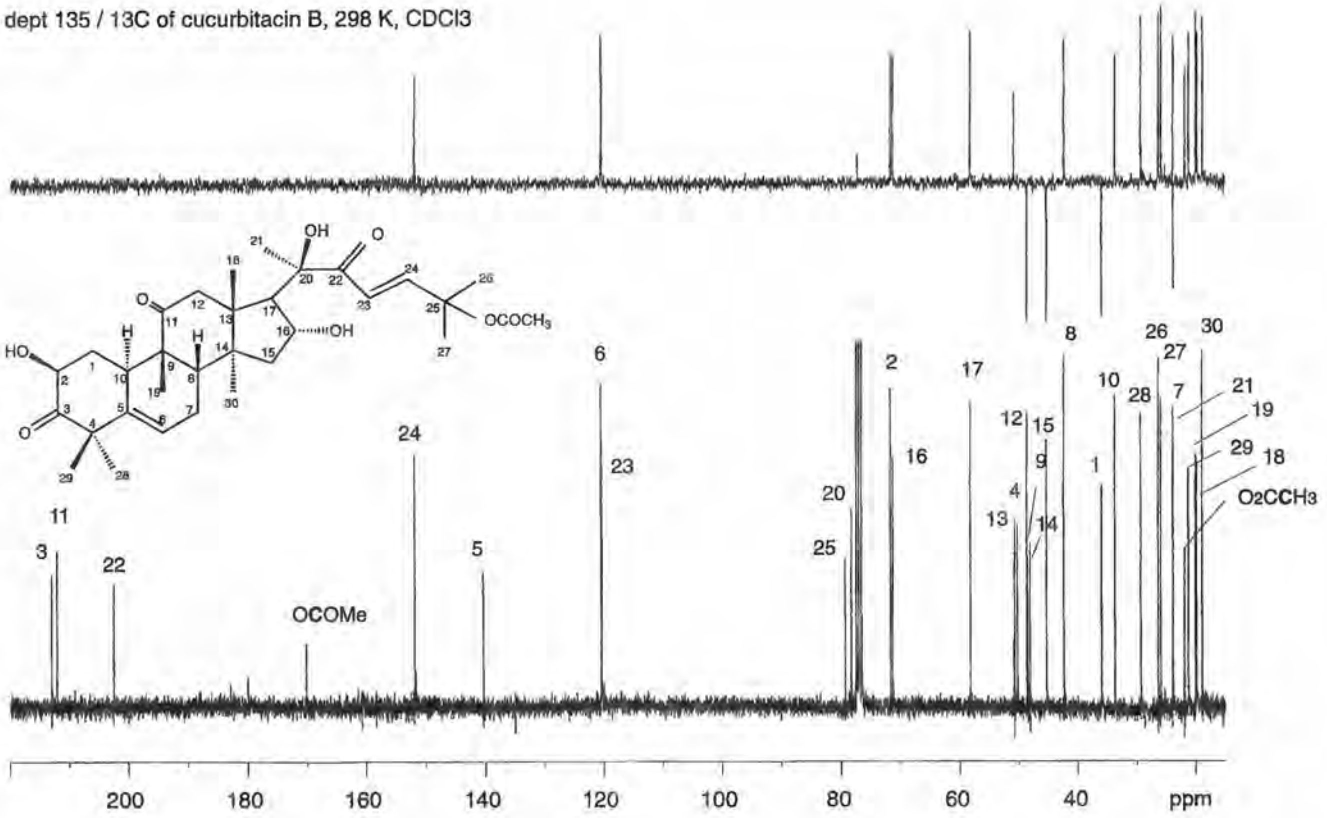
^{1}H of 23,24-dihydrocucurbitacin F, 298 K, $CDCl_3$
^{13}C /dept 135 of 23,24-dihydrocucurbitacin F, 298 K, $CDCl_3$

^{1}H of 2-O-β-D-glucopyranosyl cucurbitacin B, 298 K, $CDCl_3$
^{13}C /dept 135 of 2-O-β-D-glucopyranosyl cucurbitacin B, 298 K, $CDCl_3$

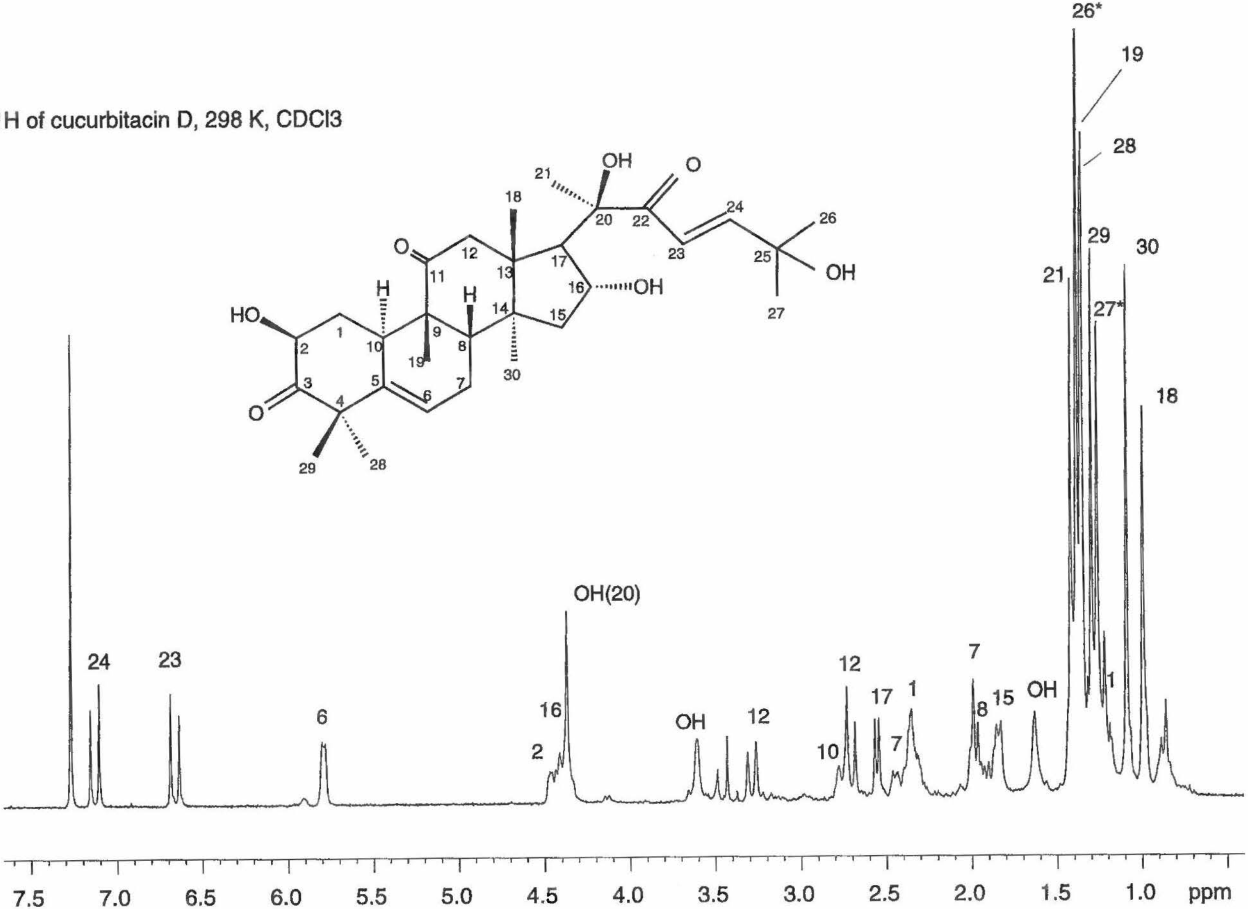
^{1}H of 2-O-β-D-glucopyranosyl cucurbitacin D, 298 K, CD_3OD
^{1}H of 2-O-β-D-glucopyranosyl cucurbitacin D, 298 K, CD_3COCD_3
^{13}C /dept 135 of 2-O-β-D-glucopyranosyl cucurbitacin D, 298 K, CD_3OD
^{13}C /dept 135 of 2-O-β-D-glucopyranosyl cucurbitacin D, 298 K, CD_3COCD_3


1H of cucurbitacin B, 298 K, CDCl3
O2CCH3
29*
30
27*
26*
15
28*
19
21
18
OH
O
21
18
20
22
24
26
23
25
OCOCH3
12
17
13
11
16
OH
H
H
HO
1
10
9
8
14
15
27
2
19
30
3
4
5
6
7
O
29
28
24
23
6
2
16
OH
OH
12
10
12
OH
17
7
1
7
15
8
1
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm

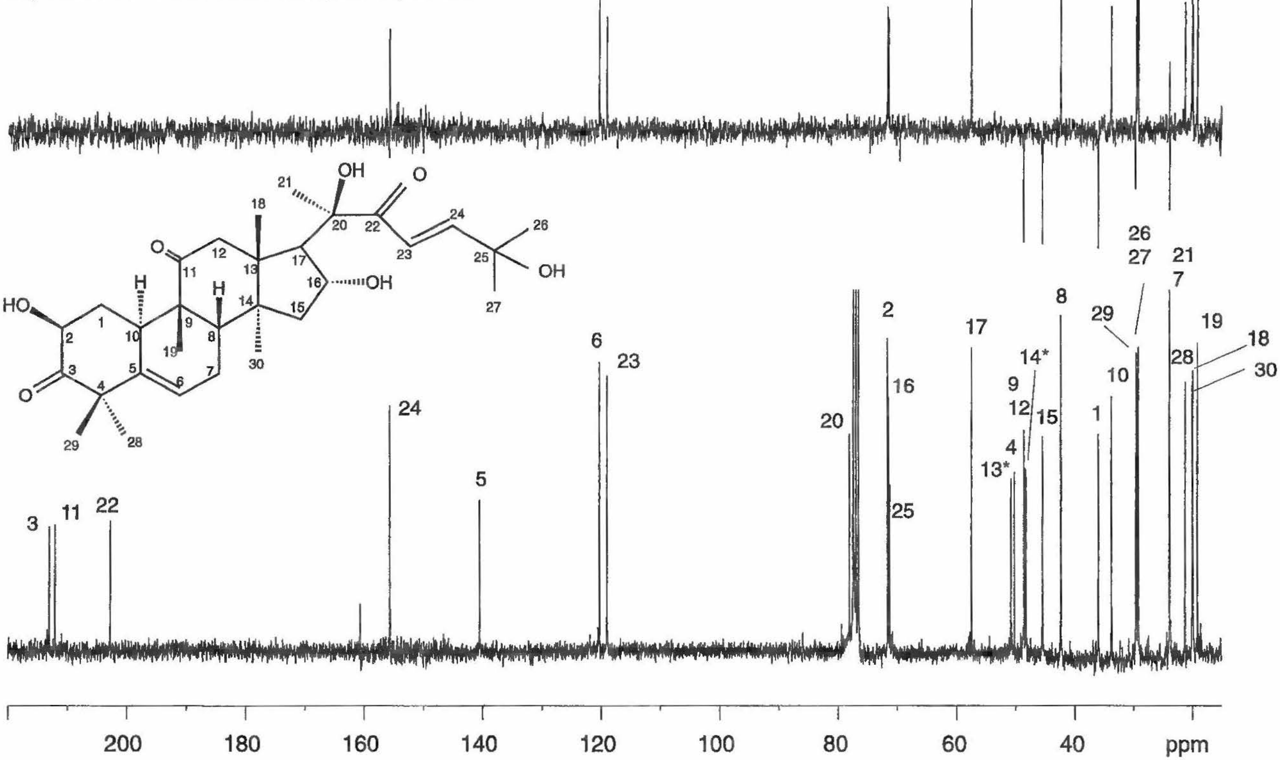
dept 135 / 13C of cucurbitacin B, 298 K, CDCl3

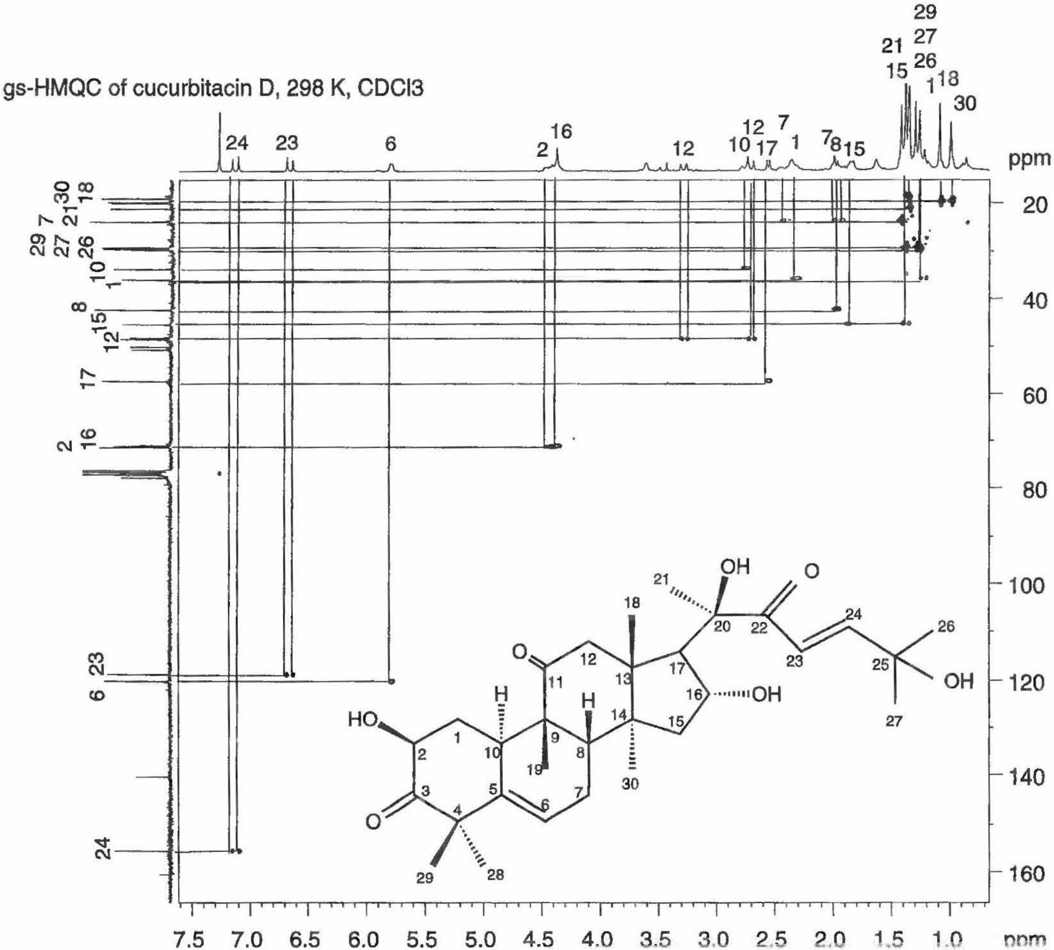


1H of cucurbitacin D, 298 K, CDCl3

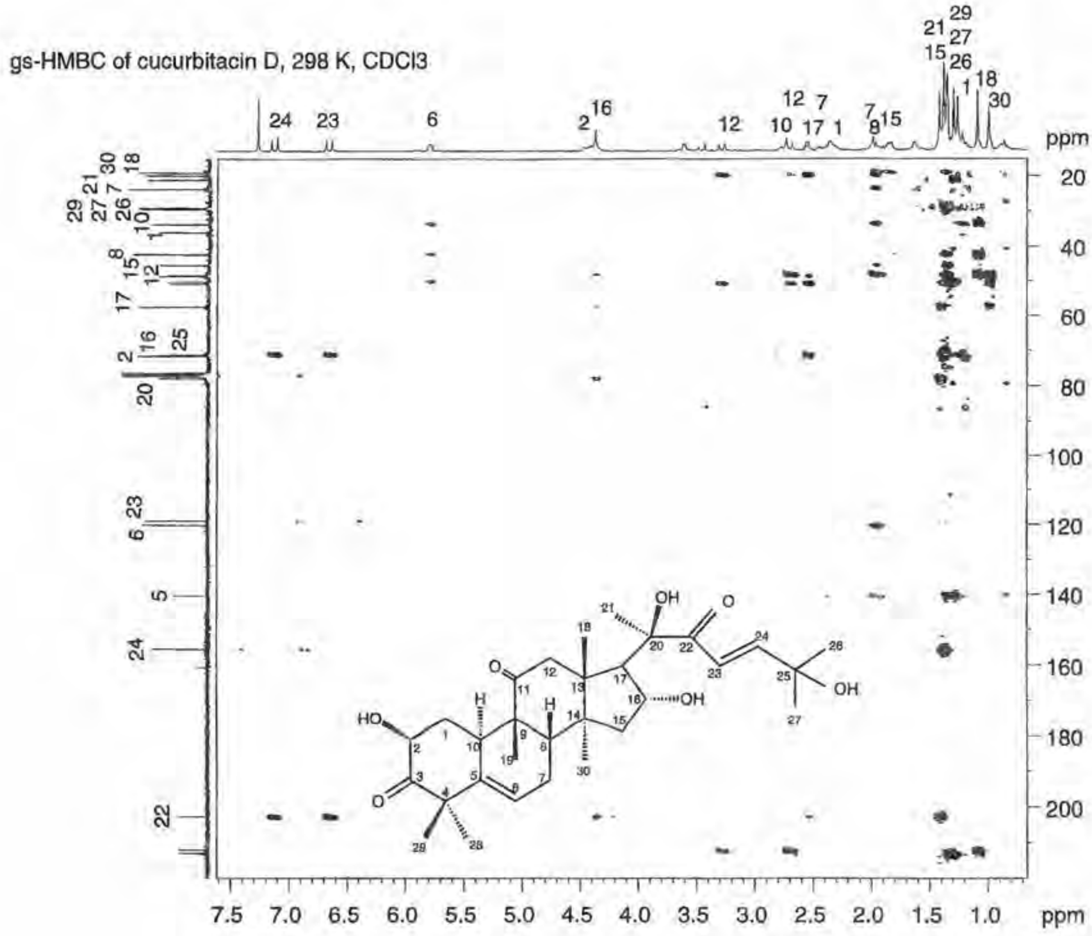


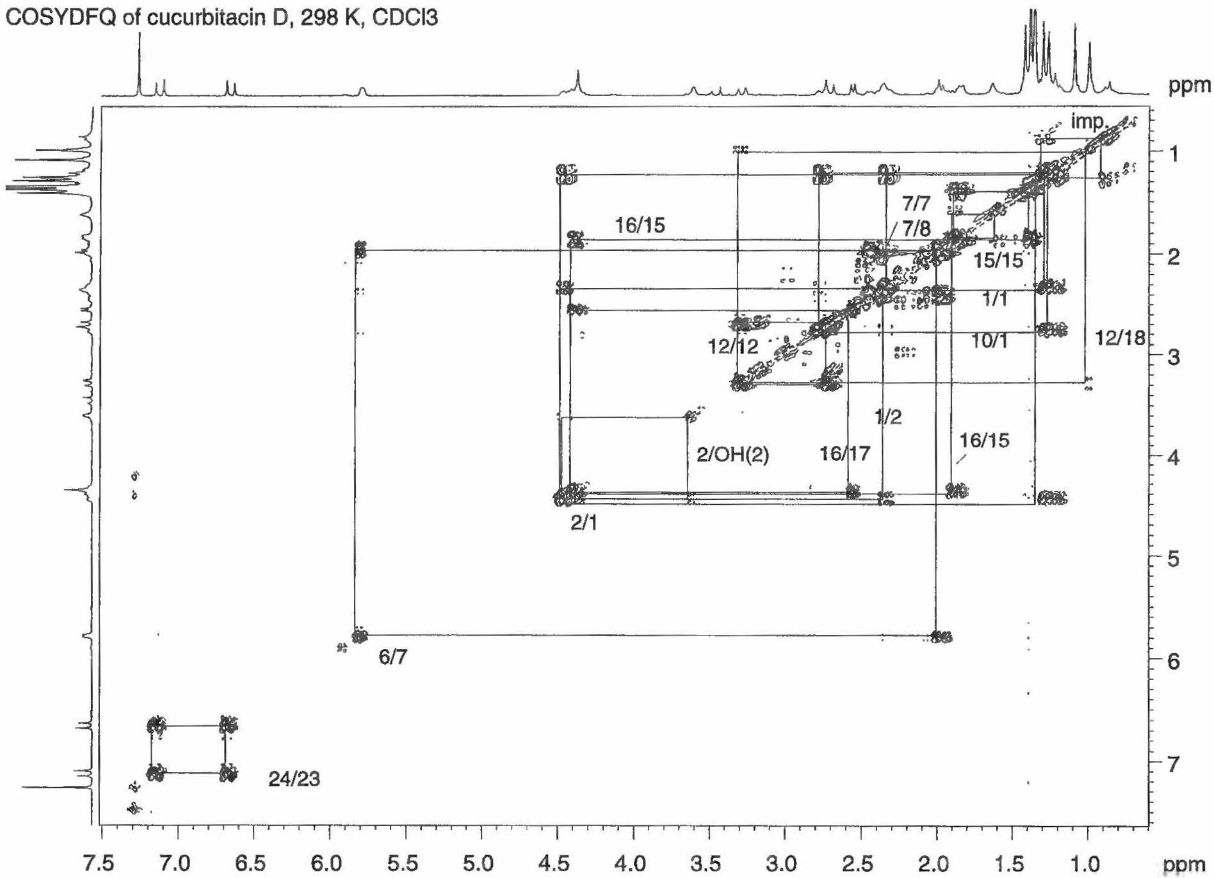
dept 135 / 13C of cucurbitacin D, 298 K, CDCl3



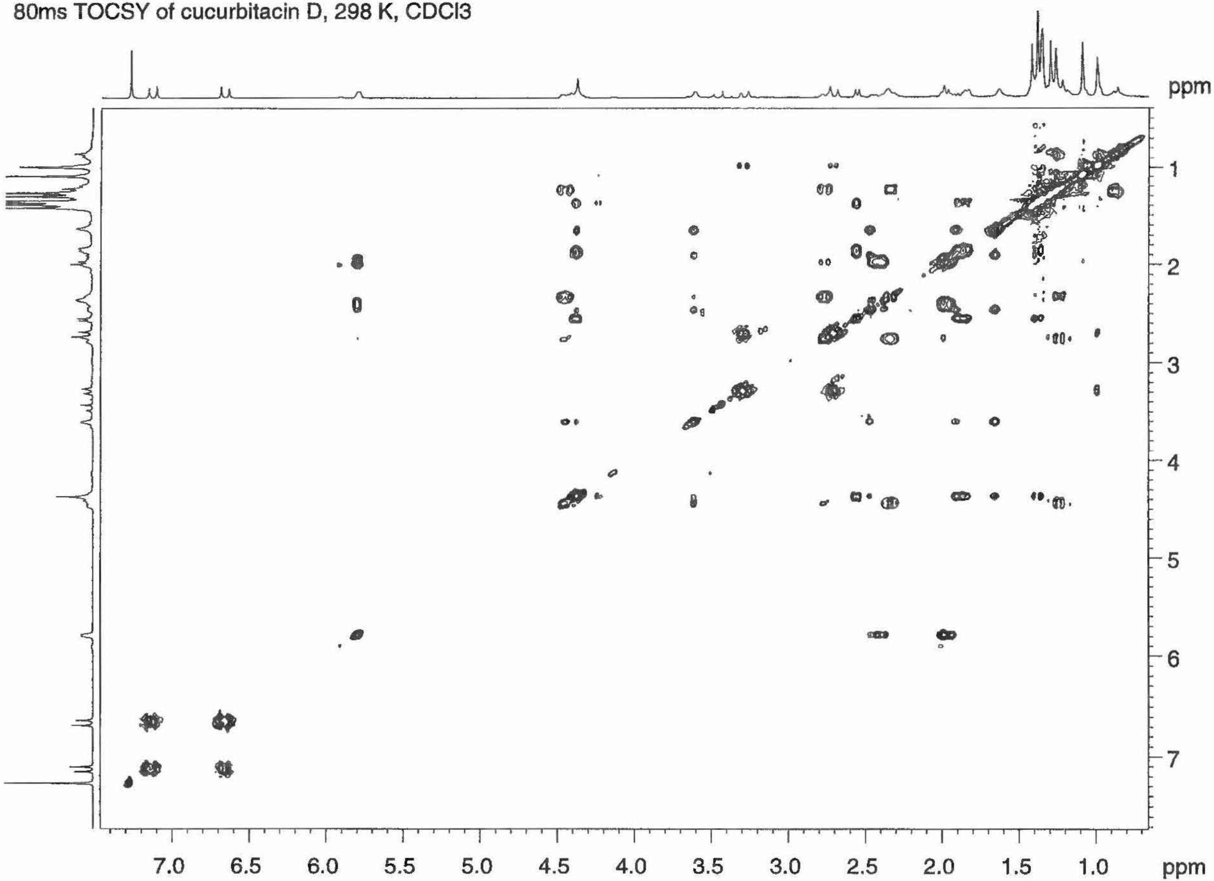


gs-HMQC of cucurbitacin D, 298 K, CDCl3
ppm
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
20 40 60 80 100 120 140 160
HO
OH
O
H

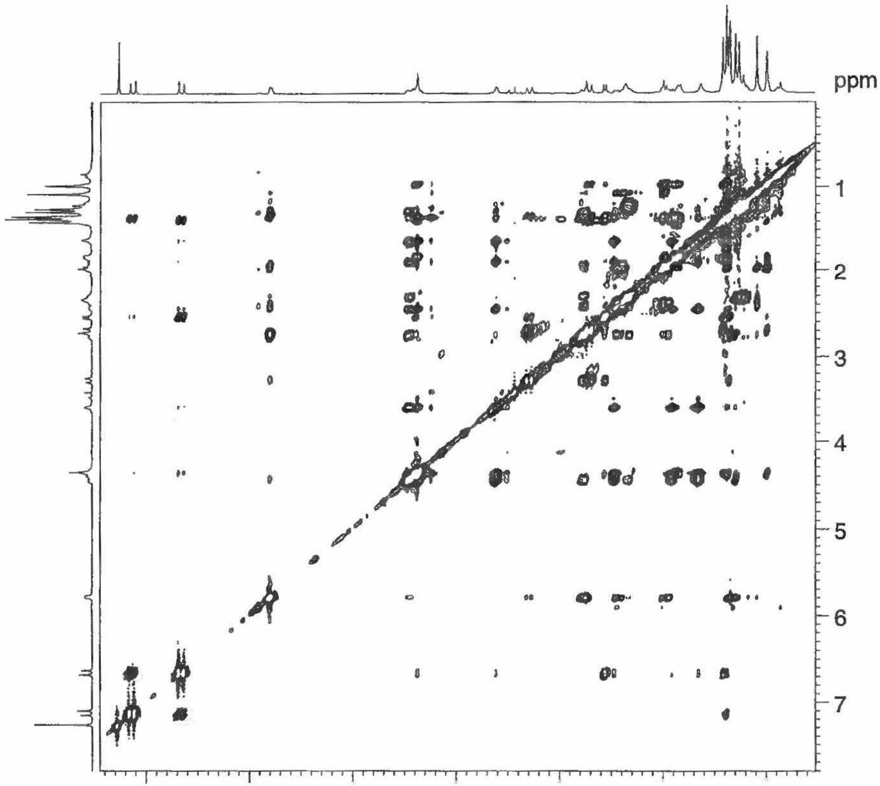

gs-HMBC of cucurbitacin D, 298 K, CDCl3
ppm
ppm


COSYDFQ of cucurbitacin D, 298 K, CDCl3
ppm
imp.
7/7
7/8
16/15
15/15
1/1
10/1
12/12
12/18
1/2
16/15
2/OH(2)
16/17
2/1
6/7
24/23
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
1
2
3
4
5
6
7

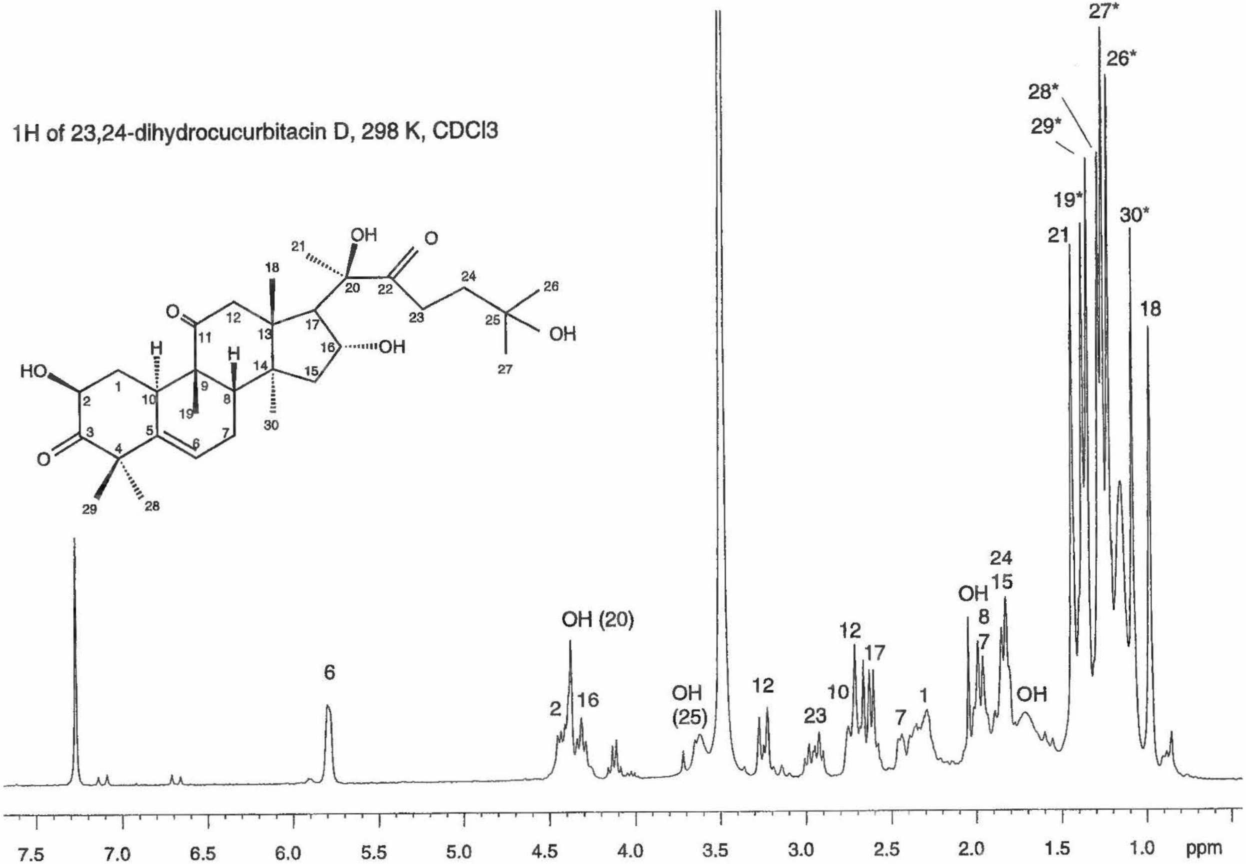
80ms TOCSY of cucurbitacin D, 298 K, CDCl3



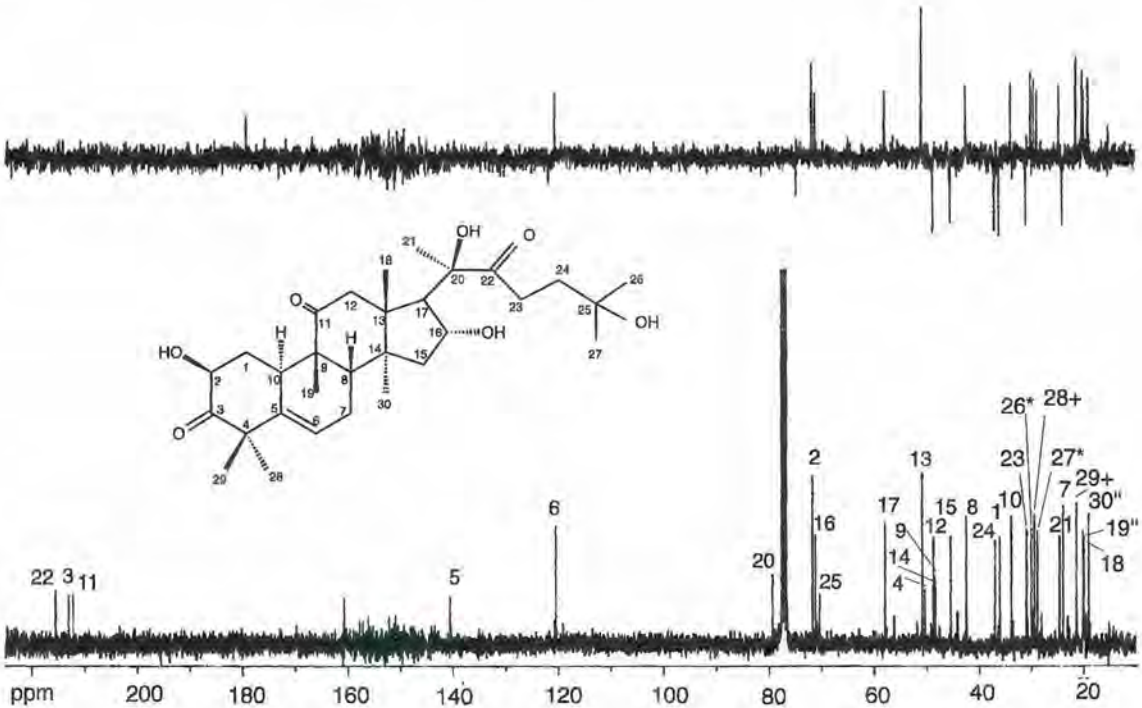
500ms ROESY of cucurbitacin D, 298 K, CDCl3

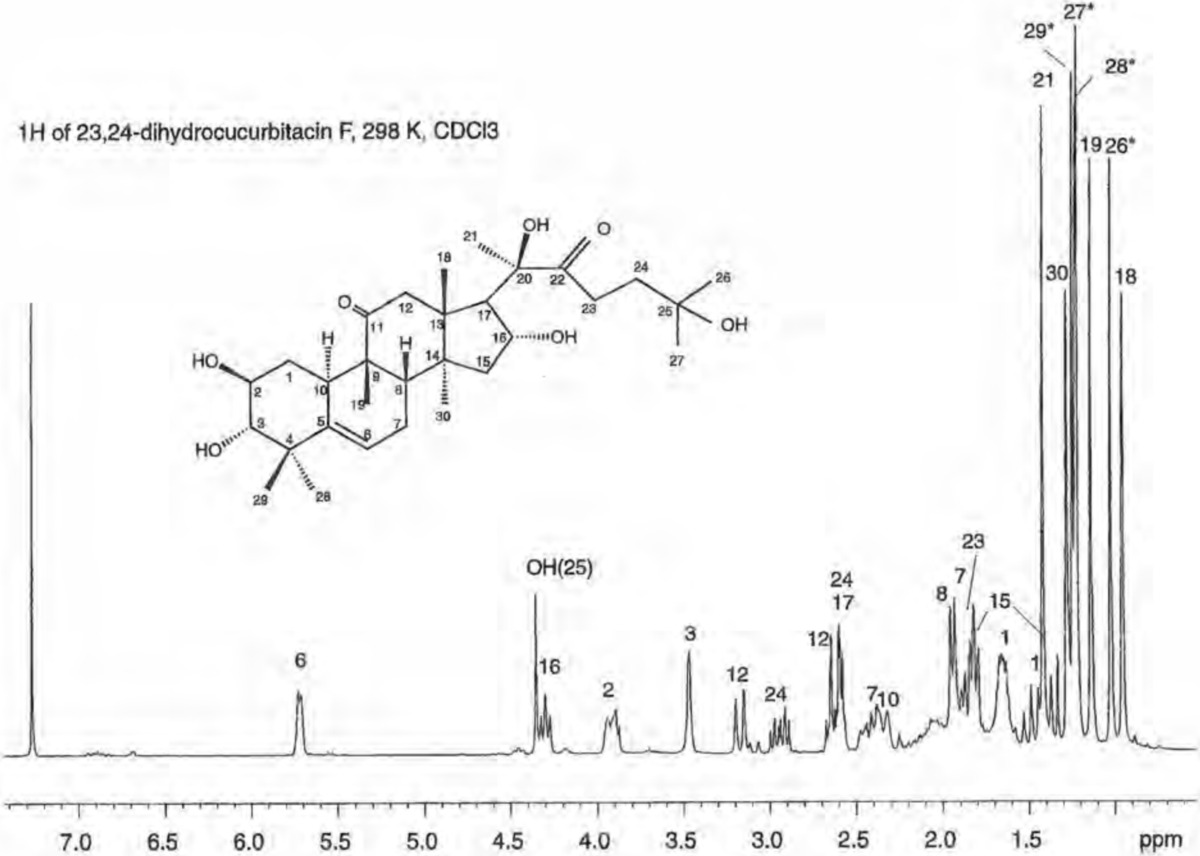


1H of 23,24-dihydrocucurbitacin D, 298 K, CDCl3



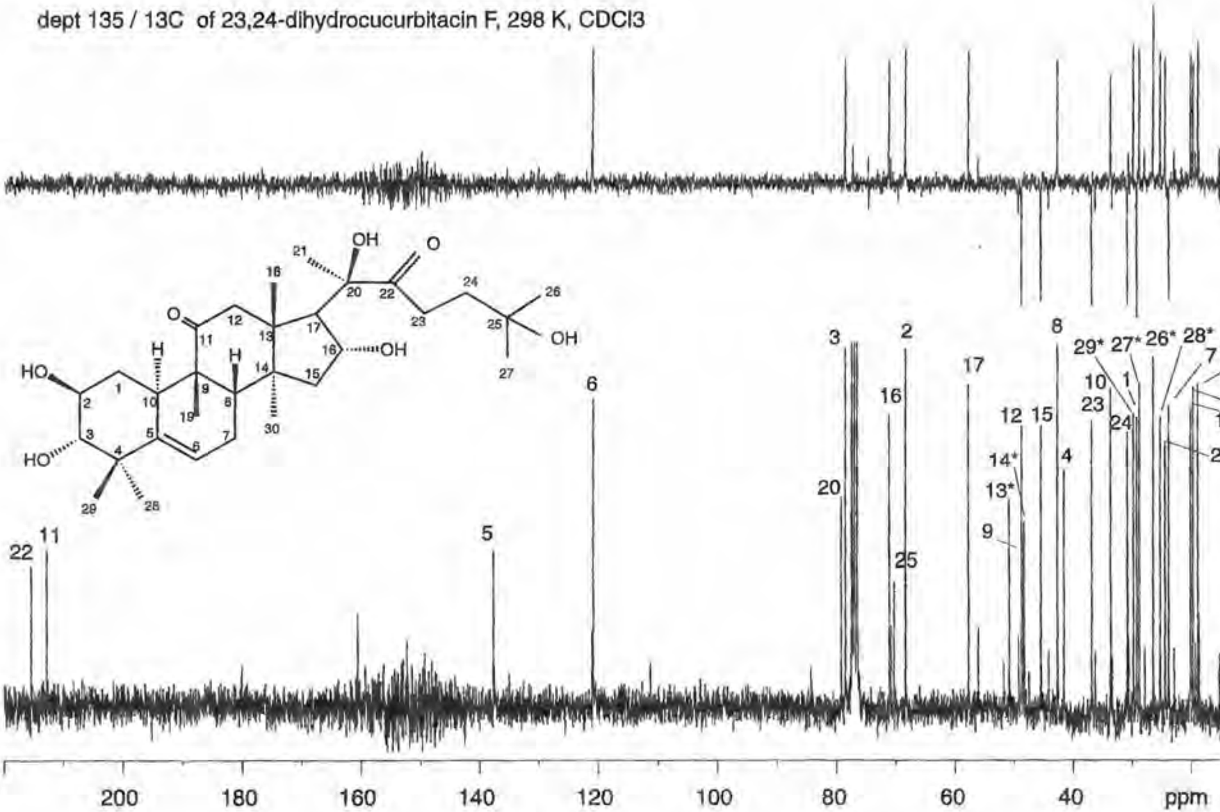
dept 135 / 13C of 23,24-dihydrocucurbitacin D, 298 K, CDCl3

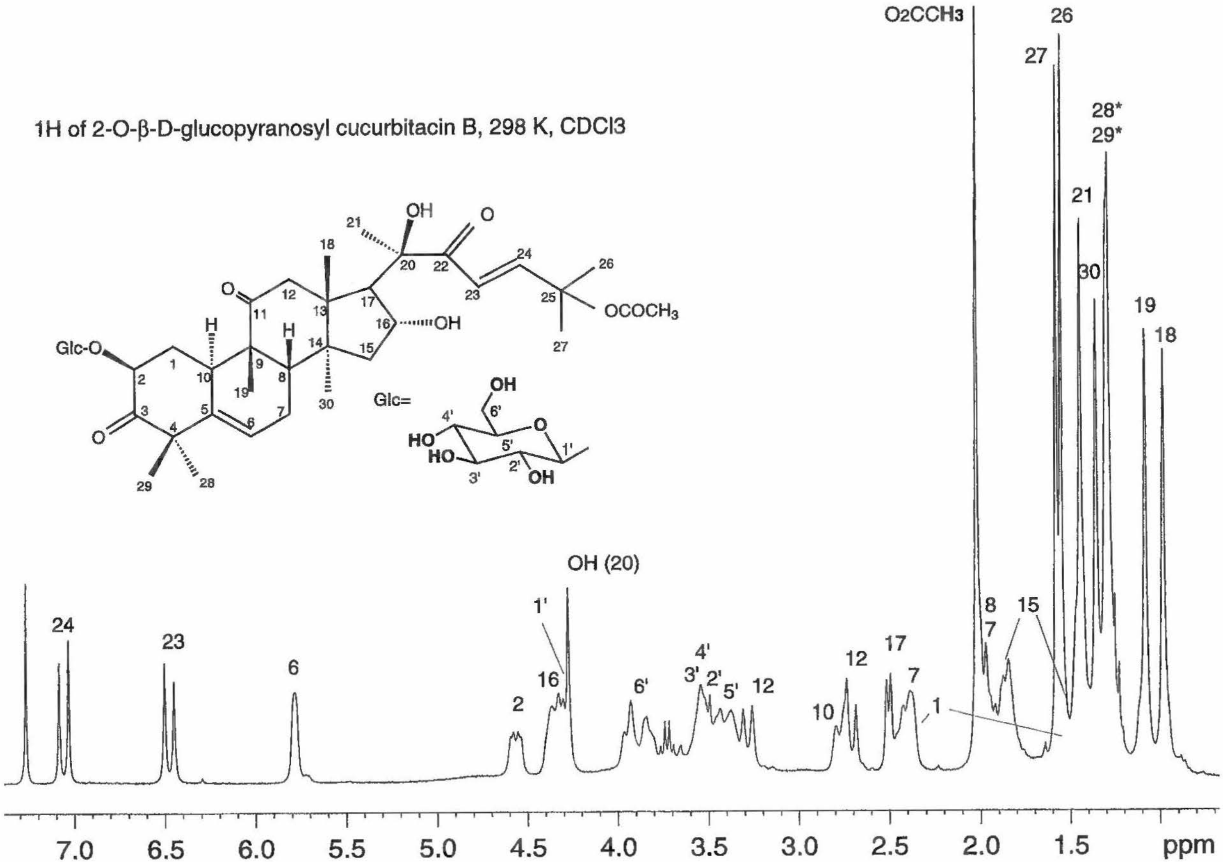




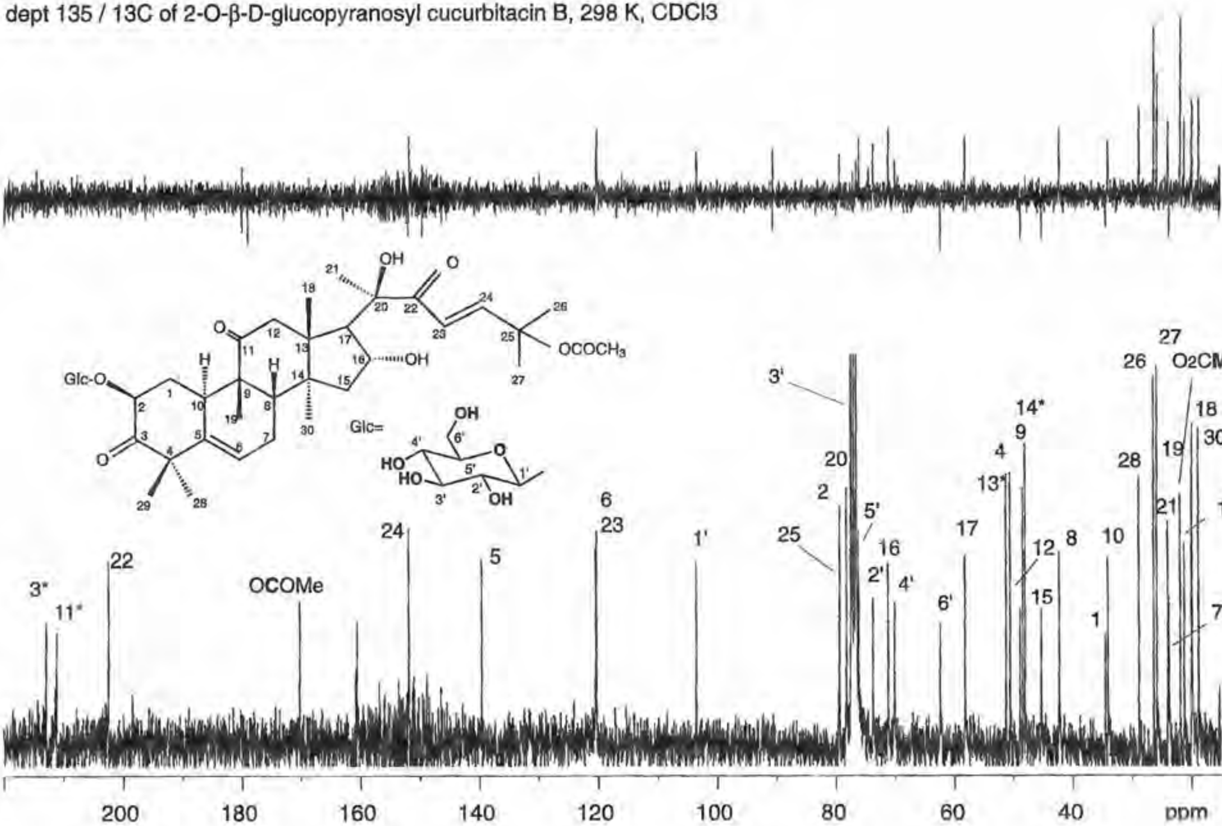
1H of 23,24-dihydrocucurbitacin F, 298 K, CDCl3
OH
O
21
18
20
22
24
26
12
11
13
17
23
25
OH
O
H
H
16
OH
HO
14
15
27
1
9
2
10
8
19
30
3
5
7
4
6
HO
29
28
27*
29*
28*
21
19
26*
30
18
23
OH(25)
24
17
8
7
15
3
12
1
6
16
1
2
12
24
7
10
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm

dept 135 / 13C of 23,24-dihydrocucurbitacin F, 298 K, CDCl3

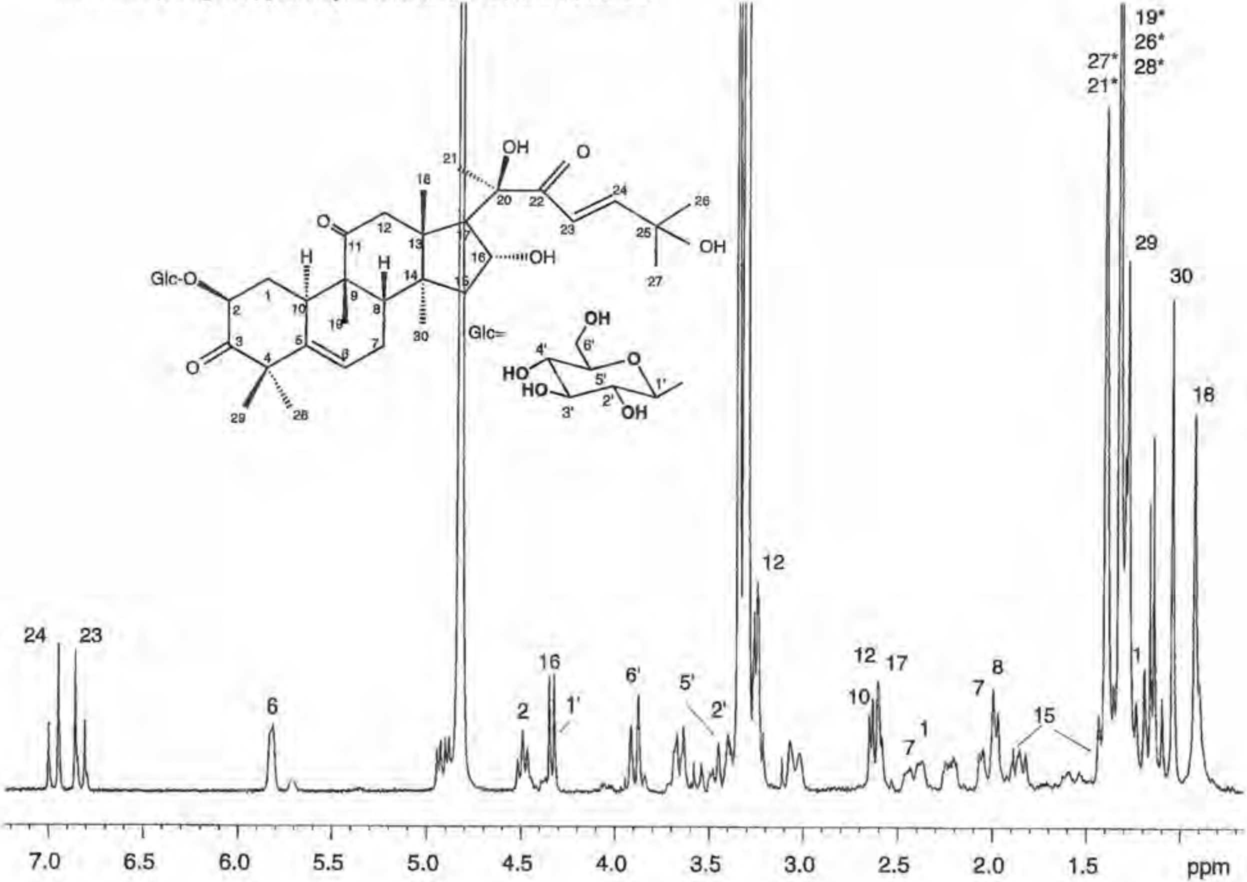



1H of 2-O-β-D-glucopyranosyl cucurbitacin B, 298 K, CDCl3
O2CCH3
26
27
28*
29*
21
30
19
18
OH (20)
1'
24
23
6
16
2
6'
4'
3'
2'
5'
12
10
12
17
7
1
8
7
15
OH
O
21
18
20
22
24
26
25
OCOCH3
27
23
17
13
12
11
O
16
OH
H
H
Glc-O
1
10
9
8
14
15
2
19
3
5
7
30
Glc=
4
6
O
29
28
OH
6'
4'
O
HO
5'
1'
HO
2'
3'
OH
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm

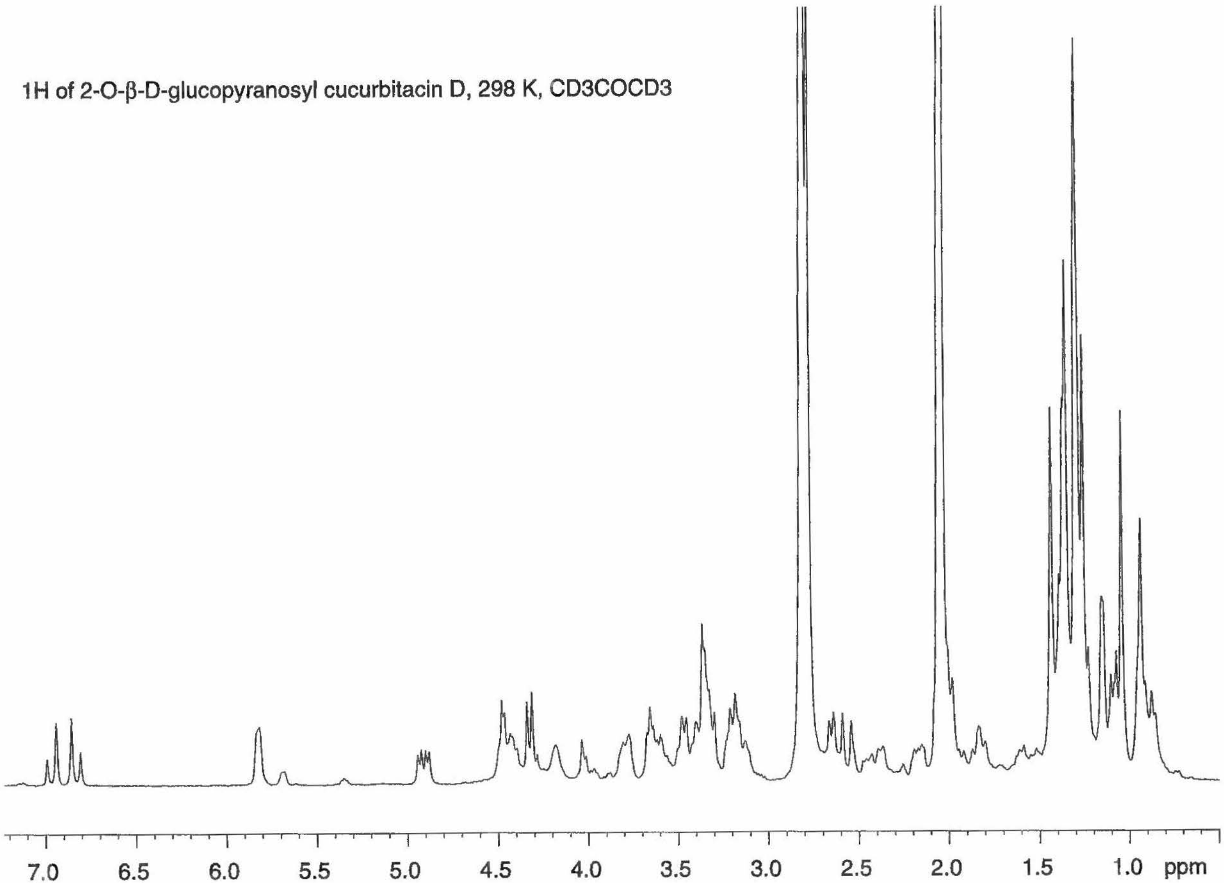
dept 135 / 13C of 2-O-β-D-glucopyranosyl cucurbitacin B, 298 K, CDCl3



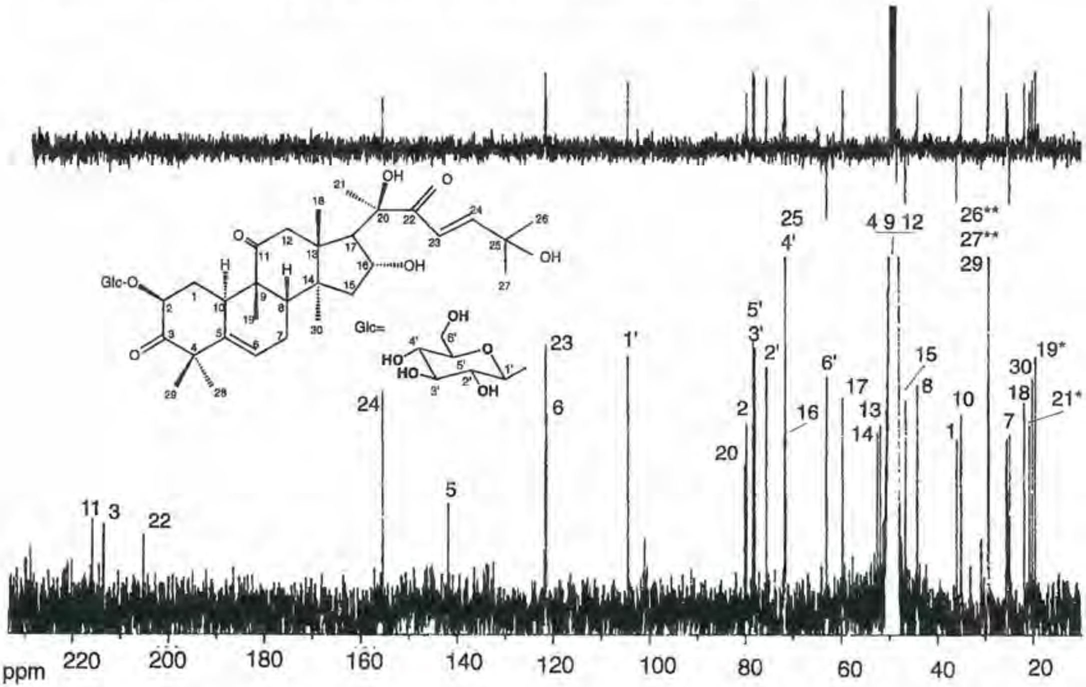
1H of 2-O-β-D-glucopyranosyl cucurbitac in D, 298 K, MeOD



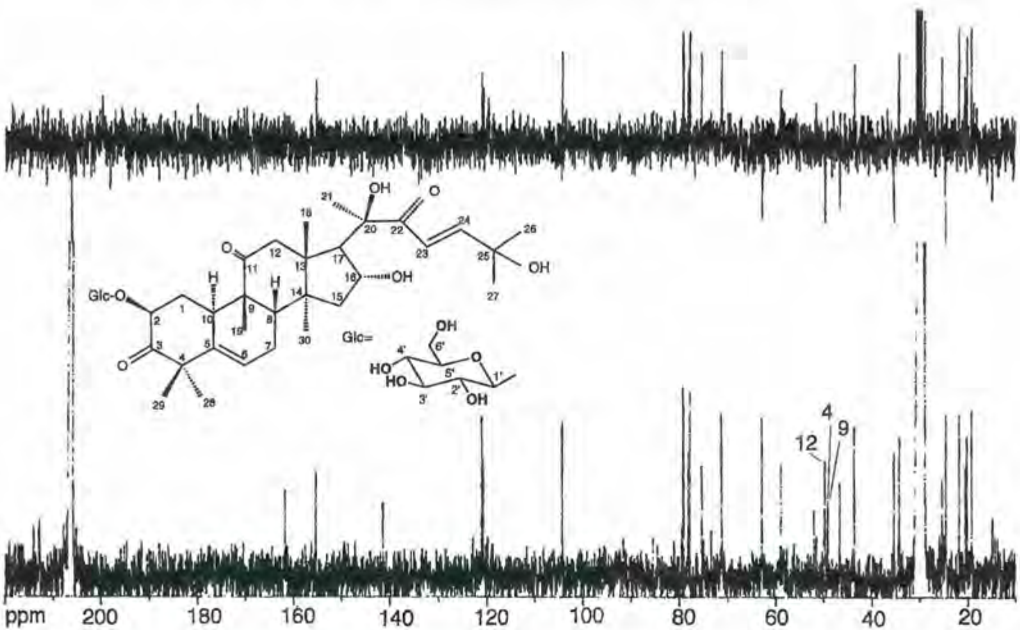
1H of 2-O-β-D-glucopyranosyl cucurbitacin D, 298 K, CD3COCD3



dept 135 / 13C of 2-O-D-glucopyranosyl cucurbitacin D, 298 K, MeOD



dept 135 / 13C of 2-O-D-glucopyranosyl cucurbitacin D, 298 K, CD3COCD3



VI Abbreviations

ACN	acetonitrile
Ac_2O	acetic anhydride
AIDS	acquired immunity deficiency syndrome
ATCC	American type cultures collection
br	broad
n-BuOH	n- or 1-butanol
Caco-2	human colon adenocarcinoma
CbS	culture bound syndromes
$CDCl_3$	deuterated chloroform
CD_3COCD_3	deuterated acetone
CD_3OD	deuterated methanol
$CHCl_3$	chloroform
CI-MS	chemical ionization mass spectroscopy
CO	cardiovascular complaints, diseases of the blood
COSY	correlated spectroscopy
d	doublet
1D	one dimensional
2D	two dimensional
DCM	dichlormethane
dept	distortionless enhancement by polarization transfer
DI	dermatological illnesses
DMSO	dimethylsulphoxide
EI-MS	electron impact - mass spectroscopy
ESI-MS	electrospray ionization - mass spectroscopy
EtOAc	ethyl acetate
FAB-MS	fast atom bombardment - mass spectroscopy

Abbreviations

F/M	fever, including malaria
fmG	female/male genito-urinary complaints
HMBC	heteronuclear multiple bond correlation
HMQC	heteronuclear multiple quantum correlation
GC	gas chromatography
GC-MS	gas chromatography couplet with mass spectrometry
GH	gastrointestinal disorders and hepatic complaints
GdH	Guevea de Humboldt
Hex	n-hexane
H_2O	water
HOAc	acetic acid
HPLC	high performance liquid chromatography
Hz	Hertz
IC_{50}	50 % inhibition concentration
INI	Instituto Nacional Indigenísta, governmental Indian organization
KB	human nasopharyngal carcinoma of patient K.B.
MeOH	methanol
MeOH-D_4	deuterated methanol
MEXU	Herbario Nacional en Mexico D.F./National Herbarium in Mexico-City
MHz	Megahertz
MPLC	medium pressure liquid chromatography
MS	mass spectrometry
NCI	National Cancer Institute, in Frederick, MA, USA

Abbreviations

NMR	nuclear magnetic resonance
NP	normal phase (e.g. silica gel)
OPH	ophthalmological problems
OU	other uses
ppm	part per million
iso-PrOH	iso-propyl alcohol
q	quartet
RP	reversed phase (e.g. n-octadecylsilyl=ODS)
RA	respiratory ailments
s	singulet
SDP	Santo Domingo Petapa
SERBO	Sociedad para el Estudio de los Recursos Bióticos de Oaxaca, A.C.
SMG	Santa María Guenagati
SMP	Santa María Petapa
SMS	illnesses of the skeletal-muscular systeme
sp.	species
spp.	sub-species
t	triplet
TLC	thin layer chromatography
UV	ultra violet
VLC	vacuum liquid chromatography
WHO	World Health Organization
ZT	Herbarium of ETH Zurich at the Botanical Garden of Zurich

VII Note on orthography of the Zapotec language

Vowels and consonants are generally pronounced as in Spanish. In this thesis, Zapotec is transcribed as used by the bilingual teachers of Santo Domingo Petapa.

Zapotec	**German**	**English**
j	ch (im Wort)	German ch as in "Chuchichäschtli"
j (end of word), *j'* (in word)	h oder ch'h	h as in how
tz	tz	ts
y	j	y as in you
e	ä	e as in ten
gue	ge	ge as in get
gui	gi	gi as in give
sh, x	sch	sh as in sherry
ii	i (lang)	ee as in see
i'i	i, i (endend: h gehaucht)	e'e with glottal stop (')
' (end of word)	h	h as in how
xhr	schr	shr as in shrimp
dx(u)	dsch(u)	ju as in June
qui	gih	gi as in Gibraltar ending with a h as in how

Lygodium verustrum Sw.	281	SDP	*guixa'a mbala'a* hoja de la vibora	pl ent	1 2 10	lc gn re vg	DIds	f	II (2)
Spermatophyta									
1. Gymnospermae pinatae PINACEAE									
Pinus oocarpa Schiede	88	todos	*guiere'ej* ocote	ma re tr	1 3 7 8	lc or re vg	RA SmSd DI fmG aire OU	c	I (6)
3. Angiospermae **Magnoliatae=Dicotyledonae** magnoliales ANNONACEAE									
Annona muricata L.	84	SDP	*balagahuanabana'a* hoja de guanabana	ho fr	1	lc or	GHd SMSd DI aire OU	f c si du	I (7)
Annona purpurea Moç. Sessé, ex Dunal	191	SDP	*guele bajna'a* condon, piñon-anona	fr ho	10 11	lc or	GHd SMSd OU	f	I(1), IV (3)
Annona reticulata L.	47	SDP	*guelebidxu'u* hoja de anona	ho cg	1 3 4 10 11	lc or re vg	GH SMSd F/M fmGs verg emp OU	f am du	I (8)
Annona squamosa L.	208	SDP	papause	ca fr	1	or	verg OU	f c du	II (2), IV (3)
Cymbopetalum sp.	232, II	SDMP	*yagamishu'u* oreja del gato	pet	5 10 15	lc	RAs SMSd jaq	f	I (6)
MAGNOLIACEAE									
Illicium verum Hook. f.	220	todos	anis estrella	inflo	1 7	lc or re vg	GH fmG emp	f	I (5)
Magnolia schiedeana Schltdl.	58 II	todos	flor de corazon, magnolia	fl	1	or	GH COd	c am si	II (6)
Talauma mexicana G. Don	58	todos	*yagabedxii* flor de corazon, magnolia	fl	1	or	GH COd	c am si	II (6)

Scientific name	Nr. FREI	Locat.	*Zapotec name* Spanish name	Part used	Prep.	Application	Groups of indigenous uses	Classification	Import.
laurales:									
LAURACEAE									
Cinnamomum zeylanicum Nees	207	todos	canela	ca	1 4	or	GHd RA fmG OU	c du hu	I (7)
Litsea glaucescens Kunth	42	todos	*guib diitz* laurel	ho ra	1 2 7 12	lc gn or re vg	GH fmG OPH aire espa OU	f c am si	I (7)
Persea americana Mill.	52	todos	*yeexu'u* hoja, semilla de aguacate	se ho	1 2 10 13	lc or	GH SMS DI F/M fmG OPH COs igua OU	c si hu	I (6)
MONIMIACEAE									
Peumus boldus Molina	302	SDP	boldo	ho	1	or	GHs	f c	II (1)
Siparuna andina (Tul.) A. DC.	59	todos	*balagamixii* hoja de zopilote, hoja mixe, negra	ho	2 11	lc gn	SMSd DI F/M fmGd nag	f c	I (3), II (3)
piperales:									
PIPERACEAE									
Piper amalago L.	27	todos	*guiadajna'a* cordonzillo	ho ra	1 11 13	lc or	GH RA SMSd CO enc aire	ft si hu	I (11)
Piper arboreum subsp. tuberculatum (Jacq.) M. C. Tebbs	238	todos	cordonzillo macho	ho	1 2 13	lc gn re vg	SMSd F/M fmG	f	I,(4)
Piper auritum Kunth	43	todos	*hua'a* hierba santa	hb ho	1 2 3 13	lc gn or re vg	GH SMS DIs F/M fmGs enc can gol OU	f c am si du pi hu	I (12)
Piper dioica L.	145	todos	*pimient rooj* pimienta grande	se	1	lc or re vg	GHd SMSd DId fmGds aire	c	I(8)
Piper minarum Standl. & Steyerm.	110	SDP	hierba santilla	ho	2 13	lc gn re vg	GH SMSd fmG	c	I,IV (2)
Piper tuberculatum Jacq.	30	SDP	*gui'iquimberu'u* cabeza de guajilote	ho rz	2 3	lc gn re vg	GH d DI aire	f	todos (2)
Piper yzabalanum C. DC. ex F.O. Smith	196	SDP GdH	cordonzillo grande *guiadajna'a rooj*	ho	13	lc gn	GH SMSd verg sus aire	f	II (2)
aristolochiales:									
ARISTOLOCHIACEAE									
Aristolochia ovalifolia Duch.	248	todos	guaco, huaco fino	ra be	1	lc gn or	GH SMSd DIds F/M fmG CO aire ataq	c am	I (4)

VIII Glossary of Spanish and Zapotec expressions

All terms from indigenous language appear in *italics* in the text, while those in Spanish are underlined.

achiote	*Bixa orellana* ; paste of the seeds for coloringtraditional meals and as a drug (gastrointestinal illnesses)
aire	supernatural winds (hot or cold) causing illness
cabecera	main village of a community
campesino	farmer
cansancio	fatigue
Códice Florentino	Florentine Codex
comité de padres de familia	mothers and fathers of pupils
culebrero	medicinal specialist for snake bites
curandero	healer
daño de la comida/empacho	food causing illness (gastrointestinal illnesses)
folleto	brochure
golpe	physical and supernatural blow
herbolaria	market for medicinal plants in Mexico-City
hierbero	herbalist

huipil	women traditional blouse
Historia de las plantas de Nueva España	history of the medicinal plants of New Spain
Istmo	isthmus, narrow land
Jardin Americana	American garden
lienzo	painted document of territory limits, on linen
limpia	ritual cleaning of the body or the house
madrina de vela	a often sick child choses (or its parents chose) a godmother (=madrina), to become responsible for the child after a ceremony in the church with a candle (=vela) and to protect it against further illness
mestizo	person of mixed blood (Indian and Spanish)
mezcal	alcohol destilated from leaves of maguey (Agave sp., Amarillidaceae)
municipio	municipiality
nahuatl	Aztec language
partera	midwife
pulque	alcohol distillated from juice of maguey (Agave sp., Amarillidaceae
rancho	little farms, seasonal occupied (e.g. coffee plantation)
rezador	leading prayers in ritual ceremonies
secadora	„box“ for plant drying with two light bulbs

sierra	mountain range
susto	sudden fright
templo, iglesia	church, while iglesia is the catholic church and templo is the house for ceremonies in general
valle	valley
verguenza	shame

IX List of Poster

A-1: Frei, B., Sticher, O., Heinrich, M. (1994) Medicinal Plants of the Lowland Zapotecs (Oaxaca, Mexico): Evaluation of an Indigenous Pharmacopoeia. Poster presented at Botanikertagung ´94, Bayreuth, Germany, 11.09.94-19.09.94.

A-2: Frei, B., Heinrich, M., Kato, T., and O. Sticher (1996) Biological Screening of Zapotec Medicinal Plants from Oaxaca (Mexico) and Correlating Phytochemical Results. Poster presented at Joint Meeting of the Society for Economic Botany and International Society for Ethnopharmacology: Plants for Food and Medicine, London, UK, July 1-July 5, '96.

X List of Oral Presentations

L-1: January, February 1994, two lectures of ethnobotany at the Institute for Ethnology of the University of Zurich. Title: 1) Einblick in die ethnobotanische Forschung und über den Umgang mit Heilpflanzen. 2) Über die Bedeutung und Anwendung traditioneller Heilpflanzen für die Gesundheit von Mutter und Kind am Beispiel der Zapoteken in Oaxaca, Mexiko und der Mafa in Guzda, Afrika.

L-2: B Frei, O. Sticher, M. Heinrich; Medicinal and Food Plants: Some Cultural Criteria for Selection; Paper presented at the annual meeting of the American Anthropological Association. Atlanta, USA. Nov 30 to Dec. 4, 1994

L-3: January 1996, lecture at the meeting of "Interdisziplinäre Kommission für Medizinethnologie (IKME)", Völkerkundemuseeum, Zurich. Title: Kulturelle und umweltbedingte Unterschiede der Arzneipflanzennutzung der Mixe und der Zapoteken im Isthmus von Tehuantepec (Mexiko).

L-4: April 1996: lecture at the 3rd Congres of Pharmacists, Pharma '96, Interlaken. Title: Ethnopharmazie in Oaxaca, Mexiko.

L-5: June 1996: lecture on ethnobotany and phytochemistry of Mexican medicinal plants at the Department of Pharmacy, in the weekly lecture of Pharmacognosy/Phytochemistry by Prof. O. Sticher (Abt. V), ETH Zurich.

L-6: November 1996: lecture about ethnobotany at the Institute for Ethnology of the University of Zurich. Title: Ethnobotanik der Mixe und Zapoteken in Oaxaca (Mexiko).

XI List of Publications

P-1: Frei, B., Sticher, O., Viesca T., C., Heinrich, M., Medicinal and food plants: Zapotec criteria for selection. Ecology of Food and Nutrition, *accepted for publication.*

P-2: Frei, B., Baltisberger, M., Sticher, O., Heinrich, M. Medicinal ethnobotany of the Isthmus-Sierra Zapotecs (Oaxaca, Mexico): Documentation and evaluation. Journal of Ethnopharmacology, *submitted.*

P-3: Frei, B., Haller, R.M., Sticher, O. Heinrich, M., Indigenous medicinal plant management in the Isthmus of Tehuantepec (Mexico): Botanical diversity and cultural importance. AMBIO, *submitted.*

P-4: Frei, B., Heinrich, M., Bork, P. M., Hermann, D., Jaki, B., Kato, T., Kuhnt, M., Schmitt, J., Schühly, W., Volken, C., Sticher, O. Multiple screening of medicinal plants from Oaxaca, Mexico: Ethnobotany and bioassays as a basis for phytochemical investigation. Phytomedicine, *submitted.*

P-5: Frei, B., Heinrich, M., Orjala, J. E., Sticher, O. Phytochemical and biological investigation of *Begonia heracleifolia* Schltdl. & Cham. and biological activities of its cucurbitacin constituents. Planta Medica, *submitted.*

P-6: Kato, T., Frei, B., Heinrich, M., Sticher, O. (1996) Sesquiterpenes with antibacterial activity from *Epaltes mexicana*. Planta Medica, 62, 66-67.

P-7: Kato, T., Frei, B., Heinrich, M., Sticher, O. (1996) Antibacterial hydroperoxysterols from *Xanthosoma robustum*. Phytochemistry 41, 1191-1195.

P-8: Heinrich, M., Ankli, A., Frei, B., Weimann, C., Sticher, O. Medicinal plants in Mexico: Healer's consensus and cultural importance. Soc. Sci. Med., *submitted.*

XII List of scholarships obtained

1992-1993	Swiss Agency for Development and Cooperation, (SDC), Swiss Federal Department of Foreign Affairs (15 months fieldtrip to Oaxaca, Mexico)
1994	Swiss Agency for Development and Cooperation (SDC), Swiss Federal Department of Foreign Affairs (2 months fieldtrip to Oaxaca,Mexico)
1994	Barth Fonds, ETH Zurich, Switzerland (travel grant)

XIII List of Figures

Figure 1-S1. A detail of el lienzo de Guevea, showing the limits of the municipiality of Sto. Domingo Petapa and Guevea de Humboldt.

Figure 1-P2. 445 different botanical species (=100%) of medicinal plants 3,611 positive responses (=100%) were collected. Plants were grouped by their (frequent multiple) use into 10 categories of indigenous uses.

Figure 1-P3. General map of Mexico with the State of Oaxaca and the research area.

Figure 2-P3 (see map in Appendix: Zapotec Area of the Isthmus Sierra: Research area)

Figure 3-P3. Organization of the Mixe and Zapotec concept of the environment.

Figure 4-P3. Cumulative % of obtained plants from the indigenous ecological zones.

Figure 2-S1. A wooden frame, constructed by a local carpenter, covered with a lona (tarpaulin) at the bottom with two light bulbs, was used as a secadora (field dryer) for drying the plants in the plant presses (between tied paper bundles).

Figure 3-S1. Label for voucher specimens in Spanish and German.

Figure 1-P4. Bioactivity-guided isolation yielded three cucurbitacins from *Begonia heracleifolia* showing strong cytotoxic activity in the KB cell line assay.

Figure 2-P4. Eight sesquiterpenes with antibacterial activity from *Epaltes mexicana.*

Figure 3-P4. Bioactivity-guided isolation yielded four hydroperoxysterols from *Xanthosoma robustum.*

Figure 1-S3. *Begonia heracleifolia.* A: Habit (x1/4). B: Staminate flower and bracts (x1). C: Stamen (x5). D: Pistillate flower and bracts (x1). E: Style (x5). F: fruit (x1). (from: Smith and Schubert, 1973)

Figure 2-S3. *B. heracleifolia* growing saxicolously in the shady and humid tropical ombrophilous forest near Sto. Domingo Petapa. Blooming with many-flowered light pinkish inflorescence (Voucher specimen FREI 66).

Figure 3-S3. Extraction scheme. * with antibacterial activity, ° with KB cell activity

Figure 4-S3. Isolation table of the hexane and the combined dichloromethane and ethyl acetate extracts. * with antibacterial activity, ° with KB cell activity

Figure 5-S3. Isolation table of the methanol extract. * with antibacterial activity, ° with KB cell activity.

XIV List of Tables

Table I-P1. Qualities of Zapotec medicinal and food plants

Table II-P1. Main causes of illness in Zapotec medicine

Table1-P2. Ranking list of spontaneously mentioned plants which elicited 5 or more positive responses for the treatment of dermatological illnesses. Total plant species = 205; total positive responses = 605 (100%). The species listed present 37% of all reported uses in this category. For each number of positive responses the plants are listed alphabetically according to (1) family, (2) genus, and (3) species. Abbreviations: aep = aerial parts, bar = bark, ear = unripe ear of corn, flo = flower, frp = fruit pulp, fru = fruit, hus = husk, inf = whole inflorescence, jui = juice of fruits, lea = leaf, uor = underground organs, pet = petal, res = resin, sap = xylem and phloem sap, sed = seed, sho = shoot, tur = turpentine, whp = whole plant, wod = wood; indet. = not determined.

Table 2-P2. Ranking list of spontaneously mentioned plants which elicited 5 or more positive responses for the treatment of gastrointestinal disorders and hepatic

complaints. Total plants = 176; total positive responses = 518 (100%). The species listed present 36,3% of all reported uses in this category. For abbreviations see Table 1-P2.

Table 3-P2. Ranking list of spontaneously mentioned plants which elicited 5 or more positive responses for the treatment of culture bound syndromes. Total plants = 144; total positive responses = 563 (100%). The species listed present 55.7% of all reported uses in this category. For abbreviations see Table 1-P2.

Table 4-P2. Ranking list of spontaneously mentioned plants which elicited 5 or more positive responses for the treatment of female and male genito-urinary complaints. Total plants = 122; total positive responses = 364 (100%). The species listed present 35.4% of all reported uses in this category. For abbreviations see Table 1-P2.

Table 5-P2. Ranking list of spontaneously mentioned plants which elicited 5 or more positive responses for the treatment of illnesses of the skeletal-muscular system. Total plants = 105; total positive responses = 321 (100%). The species listed present 29.9% of all reported uses in this category. For abbreviations see Table 1-P2.

Table 6-P2. Ranking list of spontaneously mentioned plants which elicited 5 or more positive responses for the treatment of respiratory ailments. Total plants = 88; total positive responses = 303 (100%). The species listed present 48.8% of all reported uses in this category. For abbreviations see Table 1-P2.

Table 7-P2. Ranking list of spontaneously mentioned plants which elicited 5 or more positive responses for the treatment of fever (including malaria). Total plants = 76; total positive responses = 285 (100%). The species listed present 51.2% of all reported uses in this category. For abbreviations see Table 1-P2.

Table 8-P2. Ranking list of spontaneously mentioned plants which elicited 4 or more positive responses for the treatment of cardiovascular complaints and diseases of the blood. Total plants = 20; total positive responses = 52 (100%). The species listed present 44.2% of all reported uses in this category. For abbreviations see Table 1-P2.

Table 9-P2. Ranking list of spontaneously mentioned plants which elicited 4 or more positive responses for the treatment of ophthalmological problems. Total plants = 20; total positive responses = 48 (100%). The species listed present 39.6% of all reported uses in this category. For abbreviations see Table 1-P2.

Table 1-P3: Medicinal plants in the indigenous ecological zones.

Table 1-S1. Specialization of Zapotec traditional healers.

Table 2-S1. Zapotec ethnomedical and ethnobotanical characteristics.

Table 3-S1. Ethnobotanical plant-profile-record. *Names in italics* = English translation.

Table 4-S1. Questionnaire for the evaluation of indigenous plant classification and cultural importance of a plant. *Names in italics* = English translation.

Table 5-S1. Plant species grouped by the botanical family and their category of indigenous uses. sp. = species; other abbreviations see Appendix.

Table 6-S1. A selection of interesting plant species grouped by their botanical family and used part of the plant.

Table 7-S1. Frequency of plant parts used for medicinal purpose of 445 documented species. (* special ritual prepartation, therefore separated from "wood").

Table 1-P4. Ethnobotanical information on 11 plant species used in Zapotec traditional medicine in Oaxaca, Mexico. * Ethnobotanical importance as evaluated in Frei et al. (n.d./a): the higher the value, the higher the importance of the plant.

Tabel 2-P4. Results of the biological screening from nine plant species used to treat skin diseases and two species against gastrointestinal disorders.

Table 3-P4. Selected results from the assays of four plant extracts for potential immunomodulation by evaluating proliferation and stimulation of human and murine cells. Experiment numbers (4.1.1 - 4.2.3) see "Material and Methods".

Table 8-S2. Spray reagents used in the phytochemical screening.

Table 9-S2. Results of the phytochemical screening with seven spray reagents.

Table 1-P5. Effect of compounds **1-6** and reference drugs on proliferation and stimulation of human and murine cells.

Table 1-S3. ^{1}H- and ^{13}C-NMR data of cucurbitacin B. ^{a} signal pattern unclear due to overlapping; * pairs of methyl groups not differentiated by connectivity experiments.

Table 2-S3. NMR data of cucurbitacin D. ^{a} signal pattern unclear due to overlapping. */+ pairs of methyl groups not possible to differentiate. °° hydrogenic bonds possible, long side chain freely rotating.

Table 4-S3. NMR data of 23,24-dihydrocucurbitacin F. * pairs of methyl groups not possible to differentiate. ^{a} signal pattern unclear due to overlapping. °° hydrogenic bonds possible, long side chain freely rotating.

Table 5-S3. NMR data of 2-O-β-D-glucopyranosyl cucurbitacin B. * carbonyl groups, and ** pairs of methyl groups not possible to differentiate. ^{a} signal pattern

unclear due to overlapping. °° hydrogenic bonds possible, long side chain freely rotating.

Table 6-S3. NMR data of 2-O-β-D-glucopyranosyl cucurbitacin D. [a] signal pattern unclear due to overlapping. */** pairs of methyl groups not possible to differentiate.