EVALUATION OF EFFICACY OF SELECTED ZIMBABWEAN REPELLENT PLANT SPECIES AGAINST LABORATORY REARED FEMALE *Aedes aegypti* (WEID) MOSQUITOES (DIPTERA; CULICIDAE)

BY

GATAMA GICHINI

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M. Sc. THESIS

SUPERVISORS

Dr. T. MDULUZA

UNIVERSITY OF ZIMBABWE

FACULTY OF SCIENCE

BIOCHEMISTRY DEPARTMENT

Dr. A.M. MABVENI

UNIVERSITY OF ZIMBABWE

FACULTY OF SCIENCE

BIOLOGICAL SCIENCE DEPARTMENT

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DEDICATION

To my dear family members and my beloved wife Lydiah Wanjiku for bearing such a long separation during the study.

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ABSTRACT

Rural people in Zimbabwe residing in malaria endemic areas claims that the following plants have mosquito repellent properties and use them for personal protection against mosquitoes; *Adansonia digitata* L. (Bombacaceae), *Colophospermum mopane* (J Leopard) (Fabaceae), *Dicoma anomala* Sond. (Compositae), *Epaltes alata* (Sond.) Steetz (Compositae) *Eucalyptus spp* (Myrtaceae), *Friesodielsia obovata* (Benth.) Verdc (Annonaceae) *Lippia javanica* (SPRENG) (Verbenaceae) and *Ocimum urtifolium* Roth (Labiatae). Following this report a field survey was conducted in Gokwe with the aim of collecting these plants. A botanist from the Botanic Gardens in Harare. Zimbabwe confirmed their identity. Use of intact branches as way of repelling insects in the houses and cattle shed was evident in most of the homesteads visited.

Partitioning of the solvent system in the fractionation scheme resulted in non-selective extraction, hence availability of all plants compounds that were in the plant. Despite all the efforts of homogenizing the extraction procedures, extraction of plants through fractionation scheme demonstrated different bioavailability indexes. Besides affecting the types of plants compounds recorded by each extract, it was found that, bioavailability was linked to the repelling properties of each extract. Fractions from *Eucalyptus spp* were more readily extracted through the aqueous and the organic pathways compared to fractions of the other repellent plants. Silica gel, which coated the TLC plate, was found compatible with the wide range solvent systems and spraying reagents used. Fractions of the eight repellent plants extracted were found to contain alkaloids, bitter tasting substances, essential oils and saponins.

Mosquitoes, which were already into the biting behavior (7-10 days), were found to be better biters than the freshly emerged ones. Fractions that offered maximum protection immediately they were applied recorded the lowest proportions of mosquitoes that landed and sucked. Crude extract of *L. javanica* was the most effective because it protected beyond the 6.5 hrs recorded by the synthetic repellent DEET. Fractions with desirable mosquito repellent properties were found to contain essential oils or bitter tasting substances.

In conclusion local people are already into the practice of using repellent plants meaning that promotion of their use will yield significant contribution to the vector control. Scientists in the field of plant-based mosquito repellent have a role to play in their improvement, focusing on the issue of improving plants which have been reported to offer better protection against the vector than the synthetic repellents (DEET). Let us also not forget the aspect of repelling vector mosquitoes to those who cannot afford improved repellents.

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Special thanks go to staff of Blair research institute who not only supplied mosquitoes for the bioassay experiments but repellent plants extracts were tested on their hands. I am indebted to Mr. Lukwa Nzira for the vital information he availed on plant based mosquito repellents.

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CHAPTER ONE-INTRODUCTION

Tremendous progress has been achieved over the last century in the understanding the fight against malaria, but it's worth noting that despite all these efforts malaria is still the biggest medical problem to mankind. In sub-Saharan Africa, malaria causes about 4 000 death everyday among children who are under 5 years old, which far exceeds mortality from AIDS (Louis, 1999). Björkman (1999) describes malaria as `a poor man's disease' whilst Touré (1999) stated that the malaria problem mainly concerns rural areas, which accounts to 75 % of African countries. In areas where there is low literacy, lack of basic health-care facilities and safe water supplies, malaria threats human life meaning that it is highly linked to poverty.

Touré (1999) cited factors that have contributed to less success in the control of malaria in sub-Saharan Africa. These include the spreading of drug resistant strains of malaria parasite, the development of insecticide resistance (behavioral avoidance) by the malaria vectors, inadequate health infrastructure and lack of sufficiently well trained health personnel and scientists in malaria control. It is evident that a lot has been done in research than in control implementation, thus calling for the immediate need of addressing this imbalance.

Reduction of human and vector contact ensures that malaria transmission is interrupted and in so doing mortality and morbidity from malaria is prevented.

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Use of insect repellents has been shown to protect against malaria and has resulted in incidence of malaria variation between adjacent communities living in high malaria endemic areas (Greenwood, 1999). Besides that in many instances personal protection is much more economical than area control measures (Schreck, 1977). Before the advent of synthetic chemicals, people used plant-derived substances to repel or kill mosquitoes (Curtis *et al.*, 1991). Plant based mosquito repellents are highly used in the rural areas because they are relatively cheap and locally available.

Lukwa *et al.* (1999) observed that the use of commercially available options for mosquito control in Zimbabwe was related to employment, with those working being able to afford expensive control methods. Govere *et al.* (2000) working with mosquito repellent plants in Mpumalanga Province, South Africa reported that N, N-diethyl -M- toluamide (DEET) like most other synthetic repellents is not affordable for widespread use by the majority of people resident in tropical African countries where malaria occurs. In Zimbabwe, although traditionally repellent plants have been documented to work in the rural areas, very little has been done to scientifically prove the activities of these repellent plants.

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1.1 Rationale of the project

Recent reports on activity of DEET as a solvent of paints, varnishes, plastic and synthetic fibers (Sukumar *et al.*, 1991; Curtis *et al.* 1991) have raised great concern over its long-term future use as a mosquito repellent. This fact calls for focus on research that will yield inexpensive and environmentally sound repellents appropriate for the community. In the search for effective affordable cheap alternatives to synthetic repellents, there has been much interest in natural plant extracts (Curtis *et al.*, 1991). Lukwa *et al.* (1996) working with *Lippia javanica* and *Ocimum canum,* indigenous plants in Zimbabwe, demonstrated that the ethanolic extracts of these plants have a repellent activity of less than 5 hours to *Aedes aegypti* mosquitoes.

There is a certain urgency associated with the study of these plant based mosquito repellents because plant habitats are rapidly being changed or destroyed hence calling for an immediate reason of studying much of the disappearing plants materials before the opportunities are lost (Hostettmann *et al.,* 1996). Besides that, most of these repellent plants used as protection against the vector insects are associated with elderly people in the rural areas. Therefore the elders who die before their recipe knowledge for a plant based vector repellents have been taped for documentation is equivalent to the disappearance of vital libraries (Hostettmann *et al.,* 1996). Researchers in the field of plant based mosquito repellents must not allow this to happen because it will lead to

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retrogressing so many steps backward in the field of ethno-pharmaceutical research, which will lead to identification of the alternatives for the synthetic vector repellents.

According to Curtis *et al.* (1991), the base of most popular commercial repellent DEET is as a result use of oils found in cassia, camphor, citronella, lemongrass, clove, thyme, geranium, bergamot, pine, wintergreen, pennyroyal and eucalyptus. Oils obtained from these plants were mixed and tested until many different varieties were produced resulting in DEET, which is the most common commercial mosquito repellent to date.

Phytochemical evaluation of plants used as repellents of mosquitoes will provide information that will enhance the planning of conservation programmes of plants with medicinal value. An investigation on the side effects of the plants is also required. Some of the plants used as repellents by the rural community have been reported to have some bad side effects. Lukwa *et al.* (1996) reported that *Ocimum canum* has got an oily substance in its extract, which causes severe burning on the skin once applied. This effect has not been reported in the rural areas because the traditional way of preparing extracts does not involve organic solvents. In other parts of the world some people have marketed the Citrosa plant (*Pelargonium citrosum* 'van Leenii') as a mosquito repellent until Matsuda *et al.* (1996), scientifically evaluated the plant he revealed that plant doesn't have any mosquito repellent properties.

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Literature has revealed that very little has been done in advancing the understanding of the relationship between chemical properties of plant based repellent extracts and the repellence properties they have against blood-sucking arthropods (Davis, 1985). In this project, the efficacy of some ethno-botanically selected mosquito repellent plants used in the rural Zimbabwe was scientifically evaluated.

1.2 General objective

a) To assess the effect of repellent plant extracts on laboratory reared female *Aedes aegypti* mosquitoes.

1.3 Specific Objectives

a) Collection and correct identification of plant material claimed to have mosquito repellency properties.
b) Preparation of fractions from crude extracts through fractionation scheme (Tunon *et al.,* 1994) and identification of essential oils, saponins, alkaloids and bitter tasting substances through Thin Layer Chromatography (Hidebert and Sabine, 1996).

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c) To determine the duration taken by each of the repellent plant extracts to retain its repellency properties to the female *Aedes aegypti* mosquitoes.

d) To investigate the anti-feedant activity (deterrent effect) of plant extracts to female *Aedes aegypti* mosquitoes.

e) To determine the behavior response of female *Aedes aegypti* mosquitoes to the repellent plant extracts.

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CHAPTER TWO- LITERATURE REVIEW

2.1 Repellency and its history

Dethier (1956) defined repellency in terms of the specific behavior patterns that they invoke in the insects targeted " Repellents are chemicals that cause an insect to make an oriented movement away from its source" that's any stimulus which elicits an avoiding reaction (Dethier, 1956). The net result of the oriented movement away from the treated surface is the prevention from biting.

Use of repellency as a way of making humans less attractive to vector insects dates back to the Second World War period. Bed nets and indoor house spraying with insecticides was not an effective means of protection to the military personnel because they spent most of their time outdoor. This factor led to the development of focus on scientific research that was to make humans less attractive to mosquitoes and other biting arthropods. Dethier (1956) reported that during this period, oil of Citronella was used widely. Some synthetic repellents such as dimethyl phthalate, indalone and rutgers 612 were used at a low scale against biting arthropods.

Use of citronella oil against vectors dates back to 1901. Its role was overshadowed by the synthetic repellents just like any other natural product from plants, used against insect pests.

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Failure of synthetic repellents against biting arthropods dates back to when endeavors were made to mix dimethyl phthalate, indalone and Rutgers 612 in order to get a perfect synthetic repellent (Dethier, 1956), explaining the reason why efforts of sourcing an alternative mosquito repellent are long overdue.

2.2 Mode of action of repellents

One may assume that mosquitoes are repelled by the bad smell or taste of the repellent. Research on the concepts of the mode of action of repellents in relation to potential sensory mechanisms has revealed that the repelling action is by the subtle process that interferes with the sensory functions of the mosquitoes and also the reduction of the emission of the attractants from the host (Davis, 1985). Davis (1985) reports that very little is known about how repellents affect the behavioral response of insects and how they prevent the vector insect from finding and biting the host.

Study of the behavior of the insects in the presence of the repellent is most likely to yield great contribution to the behavior responses, which then can be linked to the mechanisms involved in the mode of action of the insects' repellent. Daykin *et al.* (1965) reported that repellents cause blocking of the pores of the mosquito antennal sensilla hence making it lack olfactory sense. Davis (1985) disputes these findings on the practicability basis, logically neither the repellent nor the

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host odour would have been perceived by the female mosquito in order to make the apparent response, if at all the sensilla pores are blocked.

Concluding on the mode of action of repellents Davis (1985) and Barnhard (1999) pointed out that there is no clear picture of how insect repellents work. Chemical repellents do not behave as a single class of compounds having a common mode of action. The situation becomes more complicated when repellents known to repel at certain concentrations alters the responses of the insect from repulsion to attraction at lower concentrations. Davis (1985) reported that DEET at low concentrations attracts *Aedes aegypti* and repellency was observed only when the amount of DEET in the vapor phase was increased. Lactic acid, which normally attracts mosquitoes at higher concentration levels, repelled female *Aedes aegypti* at lower concentration levels (Davis, 1985).

Another school of thought proposes the mechanism of the repellents affecting the sensory hairs on the mosquito's mouthparts and the antennae in the head. Willes and Roth (1958) showed that one of the small neurons supplying the club shaped pegs of the maxillary palps is sensitive to small and abrupt changes of carbon dioxide concentration. Most likely these hairs detect changes in carbon dioxide content, humidity and temperature of the air surrounding the host. If this sensor detects change of this vital stimulus, the female mosquito turns aside to look for where there are no repellents. It is this turning to seek for repellent free surfaces that is interpreted as the working of the repellents.

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2.3 Properties of a good repellent

Davis (1985) reports that several authors have sought to gain some insight into what really constitutes a good repellent. To understand the constituents of a good repellent, the aspects of the chemical, structural and physical properties comes into the scene. Skinner and Johnson (1980) reported that vapor pressure has been exhaustively correlated with repellent properties, because it is an obvious property. Most likely the entire chemical that are going to repel insects acts in the vapor phase. The repellent should not evaporate very fast otherwise the ability to protect is lost.

Davis (1985) reported that the range of boiling point temperatures into which most repellents work have been defined by Dethier (1956) who pointed out that effective repellents should have a boiling point of approximately 280°C. Davis (1985) further noted that partition coefficients, molecular weights (apart from those linked to the boiling point), infrared absorption, viscosity, surface tension, molecular polarizability and hammett substituent constants have no correlation with the repellency. Garson and Winnike (1968) stated that 4308 compounds have been examined for mosquito repellent properties and amides, imides, alcohols and phenols were found to be most effective, while Davis (1985) further pointed out that an oxygen function seems to be a necessity for the repellency properties.

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2.4 Synthetic insect repellents

Synthetic organic insecticides have long been known to be highly efficacious against all the target insect species such as mosquitoes, but can be detrimental to animals including man (Sukumar, 1991). Besides the adverse environmental impacts these synthetic insecticides have, most malaria vector species have become physiologically resistant to many of these compounds (Brown, 1986). Though synthetic insect repellents such as DEET are widely used and generally believed to be safe and effective, reports of adverse effects dates back to 1961.

Gryboski *et al.* (1961) pointed out the case of a three-year-old girl who was always crying and shaking any time her nightclothes were sprayed with synthetic insect repellents. According to Gryboski *et al.* (1961) the young girl ended up being confused and finally had slurred speech. Elke *et al.* (1985) reported a case of toxic encephalopathy in a young girl aged eight years when she was exposed to DEET. According to the parents of the young girl, the family had no history of seizure disorders or other neurological diseases and all the other family members were well. The child developed a raised erythematous pruritic rash on the face and extremities where the lotion had been applied and the parents of the young girl noticed the altered behavior with unusual restlessness. In the late seventies another five year old girl died after her severe toxic encephalopathy deteriorated despite all the medical intervention (Zadikoff, 1979).

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The severe encephalopathy developed after the young girl was sprayed nightly for three months with synthetic insect repellents (Zadikoff, 1979).

According to the above-cited cases it may be assumed that DEET could be having adverse effects on young girls only. Miller (1982) highlighted the case of 42-year-old woman who produced an anaphylactic shock when her skin came into contact with insect repellent, DEET. All the above-cited cases happened in developed countries where the living standards are quite high. Considering that these synthetic repellents are extensively used in the tropical countries where malaria is a killer disease, more cases of side effects should be known, however there is massive under reporting or the cases resolve before being documented. Those which are reported are attributed to other factors due to lack of proper capacity of carrying out the screening and confirmatory tests.

2.5 Role of plant phytochemicals in mosquito control

Plants, insects and other organisms have co-existed for more than three hundred million years. During this time, plants have been under continuous selection pressure from predators and numerous environmental factors (Tsao *et al.,* 2002). Plants are known to be immobile and this factor makes them rely on both physical and chemical defense mechanisms. This mainly involves production of toxic metabolites in order to deter some of the predatory attacks from other higher and lower organisms such as insects, bacteria and fungi. Sutherst *et al.* (1982) states

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that 5-10% of the higher plants phytochemically evaluated, more than 30,000 secondary metabolites have been reported in them. These defense chemicals or secondary metabolites of plants serve as insecticidal and antimicrobial against bacteria, fungi and virus (Tsao and Coats, 1995). In the search for human and environmentally safe repellents against mosquitoes, a lot of focus has been extensively directed towards the plant kingdom (Curtis *et al.,* 1991). Phytochemicals derived from plants have provided numerous beneficial uses ranging from repellency, botanical insecticides and pharmaceuticals (Sukumar *et al.,* 1991).

Thorsell *et al.* (1970) reported that extracts from *Ledum palustre, Lycopersicon lycopersicon* and *Myrica gale* showed repellency against *Aedes aegypti* adults. Discovery and development of pyrethrin insecticides is credited to the lady of Ragusa Dalmatia who discovered dead insects on the bouquet of pyrethrin flowers, (Hartzell and Wilcoxon, 1941 cited in Sukumar, 1991). Essential oils from certain plants such as *Ocimum suave* have been reported to exhibit repellent properties to mosquitoes (Chogo and Crank, 1981). Campbell, *et al.* (1933) reported the earliest use of plant extracts against mosquito larvae. In his study he found out that alkaloids such as nicotine, anabasine methyl anabasine and lupinine extracted from *Anabasis aphylla* (Russian weed) killed *Culex pipiens* Linn. larvae. Minijas and Sarda, (1986) showed that crude extracts from fruits pods of *Swartzia madagascariensis* containing saponins produced high mortality of *Anopheles gambiae* Giles larvae.

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Plant chemicals have been reported to interfere with growth and reproduction of mosquitoes. Exposing mosquitos' larvae to precocene from *Ageratum spp* prevented pupae formation and adult emergence (Cupp *et al*, 1977). Kelly and Fuchs (1978) demonstrated the effect of prococene, when they exposed adult female mosquitoes after a blood meal. It resulted in inhibition of trypsin synthesis and retarded ovarian maturation, hence abnormal oviposition. Saxena *et al.* (1979) demonstrated the sterility effect of aristolochic acid from *Aristolochia bracteata* to the mosquitoes.

According to Sukumar *et al.* (1991) numerous plants have shown tendencies to interfere with growth and reproduction of mosquitoes. *Azadirachta indica* occupies the central role because of its strong action in inducing toxicity through inhibition of growth and reproduction (Sukumar *et al.*, 1991). Literature has revealed that the exact mode of action of *Azadirachta indica* components present in the seed kernels is not clearly documented. Sukumar *et al.* (1991) suggests the likelihood of hormonal interference in the mosquito endocrine system. This followed the earlier suggestion from Zebitz (1984a, 1984b) that cited azadirachtin as an anti-ecdysteroid that affects the neuroendocrine control of the ecdysteroids.

In conclusion, effects of plant phytochemicals on mosquitoes provide a basis for the emergence of promising mosquito control programs that are specific and ecologically sound.

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2.6 Mosquito behavior in the presence of the host

2.6.1 Mosquito feeding drive

Laarman (1958) came up with the term "feeding drive", to describe the condition of the mosquito when it responds to host stimuli by orienting to and attacking the host. Mosquitoes are known to have a rhythmical nature of host seeking activity, which Clements (1963) believes is controlled by endogenous mechanisms within the insect. According to Haddow (1961) biting activity is induced by favorable microclimate thus further supporting the idea that it is controlled endogenously. Clements (1963) demonstrated that mosquitoes would respond to host stimuli over a fairly wide range of climatic conditions. Any condition, that depresses or inhibits mosquito activity, in general, will have the same effect on the biting activity of the mosquito.

Hamon *et al.* (cited by Clements, 1963) proved that females of *Anopheles gambiae* and *Anopheles funestus* caught biting in nature had oviposited the same night thus implicating the gonotrophic cycle in the biting activity of the female mosquitoes. Lavoipierre, (cited by Clements, 1963) reported that when a virgin female *Aedes aegypti* containing mature oocytes was mated, biting drive was temporarily inhibited but it was gradually developed in the next two hours. Clements (1963) demonstrated a decline in the biting activity of inseminated female *Aedes aegypti* late in the gonotrophic cycle, which rose immediately after

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oviposition. He further pointed out that biting fell sharply after the oocytes had reached stage three. Clements (1963) reported that biting activity was less inhibited by the presence of mature eggs in the ovary than it was by insemination of the female.

2.6.2 The role of carbon dioxide in mosquito orientation

Gilles (1980) reported that the normal levels of atmospheric carbon dioxide ranges from 0.03-0.04% but at night due to tropical forests it rises up to 0.06-0.1%. Tregear (1966 cited in Gilles 1980) stated that excretion of carbon dioxide through the skin of the human beings is very low, ranging from 0.3-1.5% of the lungs. Besides this low volume of carbon dioxide emitted, the total surface area of the body over which excretion takes place is very large. Brown 1952 cited in Gilles (1980) argued that there is no evidence of implicating these small quantities of carbon dioxide as far as orientation of the mosquito is concerned. Gilles (1980) disputes these findings, and reported that carbon dioxide is the most important olfactory stimulus involved in host finding by mosquitoes.

The role played by carbon dioxide as far as host finding is concerned is poorly understood as some scientists have claimed that it repels, attracts and sometimes it inactivates. Khan *et al.* (1972) a researcher in the field of chemical control of insect behavior showed that carbon dioxide acts as mosquito locomotor's stimulant. Snow (1970) demonstrated that there was a reduction in the absolute

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numbers of mosquitoes approaching a subject with a reduced carbon dioxide output, hence implicating carbon dioxide as a long-range attractant to mosquitoes. Khan *et al.* (1972) concluded that carbon dioxide acts as an activator whereas other authors such as Brown *et al.* (1951) maintained that carbon dioxide acts as an attractant. Carbon dioxide has been used in the past for trapping mosquitoes hence supporting its role as an attractant, leading to mosquito orientation. Snow (1970) basing his argument on the published data concluded that carbon dioxide stimulates flight and contributes to the orientation of the mosquitoes to a host.

Having established that carbon dioxide is a vital stimulus of the vector mosquito' s orientation to the host, the next question is the threshold concentration of carbon dioxide at which mosquitoes respond. Kellogg (1970) established that for mosquito orientation to occur, the crucial factor is the change of the concentration of carbon dioxide and not the volume level. The findings of Mayer and James (1969) further support Kellogg's findings, that additional concentration of as little as 0.05% carbon dioxide to the room air, elicited responses from *Aedes aegypti* in a wind tunnel. It can therefore be concluded that mosquitoes respond to alterations of carbon dioxide concentration and expect that very small changes of carbon dioxide influence their behavior.

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2.6.3 Heat effects

Besides carbon dioxide and human odour, heat and moisture are other factors well known to orientate mosquitoes to the host. As early as (1910) Howlet had established that mosquitoes are highly attracted to objects which are heated above the air temperature, both under laboratory and field conditions, hence implicating the body temperature as an important factor in host seeking. It was further observed that several species of the vector mosquito responded by orientation and probing when they were held in a cage over a warm object. While analyzing the behavior of mosquitoes towards its host, Hocking (cited by Snow 1970) demonstrated that carbon dioxide and human odour are relatively long range wind borne attractants, with warmth and moisture contributing to orientation to the host at very short range. This finding is supported by work of Kingscote and Francis, (Cited by Clements, 1963) who investigated the effect of cooling rats below room temperature before exposing them to *Aedes aegypti.* The rats, which were cooled, were less attractive compared to those, which were not cooled. Laarman (1958) established that heat stimulates alighting when he observed that *Anopheles labrachiae atroparvus* alighted in response to dry air current 2.5 °C above the room temperature, while the same insect hovered around when the temperature of a warm moist air bearing body odour was reduced from 34 °C to 26 °C.

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Some people are more attractive to mosquitoes than others, Smart and Brown (cited by Clements, 1963) observed that when two hands of individuals only differing in skin temperatures were exposed to *Aedes aegypti* in a cage, more females landed on the warmer hand. When the warmer hand was cooled and the cold hand warmed more females landed on the warmer hand than on the cold hand.

2.6.4 Response to moisture

Clements (1963) reported that air with less moisture (dry air) acts as a repellent to mosquitoes; these findings are in line with Laarman (1958) who demonstrated in his experiments that moistened air lead to large numbers of mosquitoes alighting in the cage. Water vapour has been shown to play a crucial role as far as alighting of the mosquitoes is concerned. Laarman (1958) demonstrated that when unheated air containing human odours was dried high numbers of *Anopheles labranchiae atroparvus* hovering was observed. But when water vapour was restored it led to a reduced proportion hovering, and an increase in the proportion alighting. Water vapour in the air is not always attractive to the mosquitoes. Brown (1958) showed that *Aedes aegypti* showed a highly significant preference for landing on hand, which transpired less water. Mosquitoes orientate towards moist air and show a strong tendency to alight at the source provided that the relative humidity is not raised to near saturation.

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2.6.5 Mosquito probing activity over the human arm

Clements (1963) highlights several factors that have been implicated in the location and recognition of the host during the probing activity over the human arm. These are heat, moisture, carbon dioxide, odour and various visual factors. Under the influence of these factors, mosquito behavior in the presence of the host arm can be categorized into the following steps; activation (changes which make mosquito respond more strongly to the orienting stimuli), orientation to the host and alighting.

According to Gilles (1980) activation in mosquitoes is purposely meant for flight induction activity, hence divided into two phases; take-off and sustained flight. Daykin *et al.* (1965) showed that in the absence of the host stimuli, the rate of take off by resting mosquitoes is simply a random process. Unlike heat and moisture, which are very difficult to investigate in complete isolation, various scientists have made a lot of endeavors to link the mosquito behavioral pattern resulting from variation of carbon dioxide levels. Gilles (1980) reported that when mosquitoes were exposed to an air stream, which contained 0.2% carbon dioxide, the rate of take-off was greatly increased for two minutes before it fell again to low levels. He described carbon dioxide as an activator, which he further pointed out that if the stimulus is provided in a still air, mosquitoes cannot orientate to the source because of lack of the orientation cues for steering the insect up. This fact explains the reason of using cages, which are open on two of its sides to ensure

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that carbon dioxide, which is a natural stimulus, is supplied in a more natural way hence facilitating the orientation of the mosquitoes.

2.7 Mosquito repellents testing in the laboratory

Biological assays are used to test mosquito repellents and questions about plant based mosquito repellents are answered. Bar-Zeev and Smith (1959) and Hill *et al.* (1979) categorized biological assays into in *vitro* and in *vivo* methods. *In vitro* methods involves use of cloth, filter paper, animal membrane and olfactometry while *in vivo* methods involves use of human beings or animals subjects in evaluating plant based mosquito repellents. Though *in vitro* methods are safe more so when testing toxic substances, results obtained are problematic because they have no relevance to the end-user of the repellent, human beings. In search for repellents whose ultimate beneficiary is the human beings the *in vivo* method is the recommended one. *In vivo* methods are accurate and results obtained are relevant to the end user of the repellent. Schreck (1977) reported that use of human beings as test person when evaluating mosquito repellent is widely accepted around the world. The reason for this is because all those other methods proposed over the years have not been able to replace the human as the primary bait in repellent evaluation (Schreck, 1977). After bioassays are completed the candidate plant is keyed as a repellent or not and the quantity of the plant needed to repel is also known and the duration of the repellence.

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It is conventionally accepted to use *Aedes aegypti* mosquitoes for mosquito repellents tests because it is easily reared in the laboratory, it is an avid blood feeder, a good feeder in the laboratory and generally test persons show milder reactions than *Anopheles* bites (Schreck, 1977). This factor has contributed to fewer publications on the other species as far as mosquito repellence is concerned. Schreck (1977) observed that exclusive use of *A. aegypti* in repellency studies has made it possible for data produced by different laboratories to be compared on common grounds. Relying on only one species for testing repellents has led to many compounds being overlooked because they were not effective against the only used species (Schreck, 1977).

Mosquitoes used in the laboratory for testing should be reared under standardized conditions so that grounds for comparison are set. Besides that the density of the laboratory population of test mosquitoes is very important. Schreck (1977) reported that when large numbers of mosquitoes are used (1500-3000) the biting rate was found to be more predictable than when 6-500 was used. Though some scientists have recommended the use of large numbers of mosquitoes, Khan *et al.* (1972) and Shimmin *et al.* (1975) recommended use of small numbers of female mosquitoes. This is because most plant-based mosquito repellent scientists use very small test areas. WHO recommends the test area to be 25 cm^2; this ensures that the treatment rate is always the same from one test person to the next.

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Despite all these efforts of homogeneity when conducting the bioassay experiments, Schreck (1977) observed that variation are brought by the skin characteristics of test person i.e. texture, color absorption, evaporation, moisture, dryness and hairiness. Considering that these factors vary with each test person in a nearly infinite combination, Schreck (1977) recommend use of a large number of test persons and more test replicates in order to gain greater validity in the test results. In conclusion the use of a well-trained team of test subjects when evaluating the repellents will add reliability to the data collected during the study.

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CHAPTER THREE- MATERIALS AND METHOD

3.1 Description of the collection site

Repellent plants were collected from Gokwe region, which lies in the Midlands Province in the western side of Zimbabwe (Appendix A). The region is divided into Gokwe North and Gokwe South for administrative reasons (Figure 3.1). The actual collection site of the repellent plants was Gokwe North and it lies within the agro-ecological regions III, IV and V (Appendix B). Repellent plants were collected in Region 5, which is about 10 % of the entire area (Appendix B). The rainy season normally starts as from late October and continues until mid-March and rainfall ranges from 450 mm to 800 mm. The main economic activities are livestock and cash crop farming, the main crop being cotton. These two major economic activities promote the existence of mosquitoes with anthrophagic and zoophilic behavior in the region, hence explaining the reason why various sympatric species have been documented to exist in previous studies. Mahon *et al.* (1976) reported the existence of the four siblings species *Anopheles arabiensis*, *An. gambiae*, *An. merus* and *An. quadriannulatus* in this region.

The vegetation comprises extensive areas of *Colophospermum mopane* woodlands with other plant species. The woodland products provide essential goods and services for the communal people living in this area, i.e. wild fruits, bee farming, medicinal plants, home for wild animals, besides being used for timber.

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Most of the houses are made of mud and wattle walls roofed with grass or plant leaves. Due to the expansion of the population, change of farming patterns and the current land allocation exercises, vegetation in the area is being cleared at a very high rate. The current economic hardships have forced farmers in the region to extensively pay more attention to cotton in comparisons to their indigenous subsistence farming hence accelerating the process of deforestation. Adverse effects of the saline water to the vegetation were also noted where the repellent plants collection sites were near minor or major water bodies (Figure 3.2 and Appendix C). Presence of saline water in the region enhances the breeding of mosquito species such as *An. merus*, which only breeds in salty water.

The type of soil found in Gokwe is derived from the parent rock type called Madumabisa lower Karoo (Appendix D FAO soil classification). The residual soils derived from this mudstone are moderately to well drained, moderately shallow, sandy clay loams to clays over greyish brown sometimes calcareous sandy clays or clays (Appendix D FAO soil classification). Presence of clay type of soil in the region enhances the breeding activities of the mosquitoes in the region, because it traps water in depressions hence acting as the breeding sites. Soils with vertic and sodic properties on the moderate and lower slopes respectively are particularly known to be prone to erosion. High rates of erosion during the rainy season create depressions, which trap water hence resulting in mosquito-breeding sites. Type of soils found in Gokwe is generally rich with mineral plant nutrients such as calcium, magnesium and potassium though the organic nitrogen

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content is low (Appendix D FAO soil). This factor promotes the farming activities in the region.

Generally, Gokwe is a diverse region in terms of altitude, vegetation and resources. Evidence of great potentiality in terms of agriculture, rural industry and wildlife was noted during our repellent plants collection exercise.

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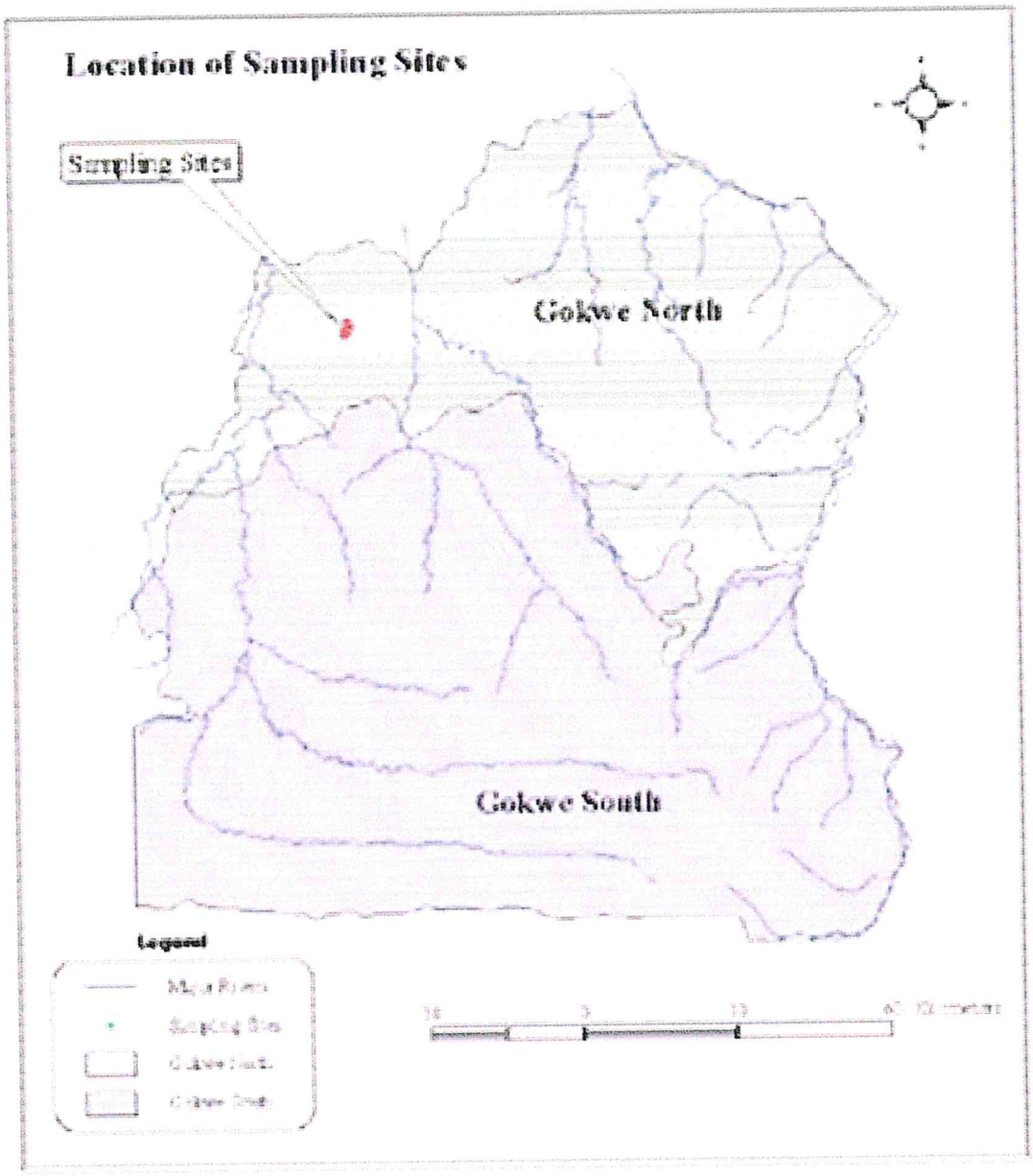

Figure 3.1 Gokwe North and Gokwe South

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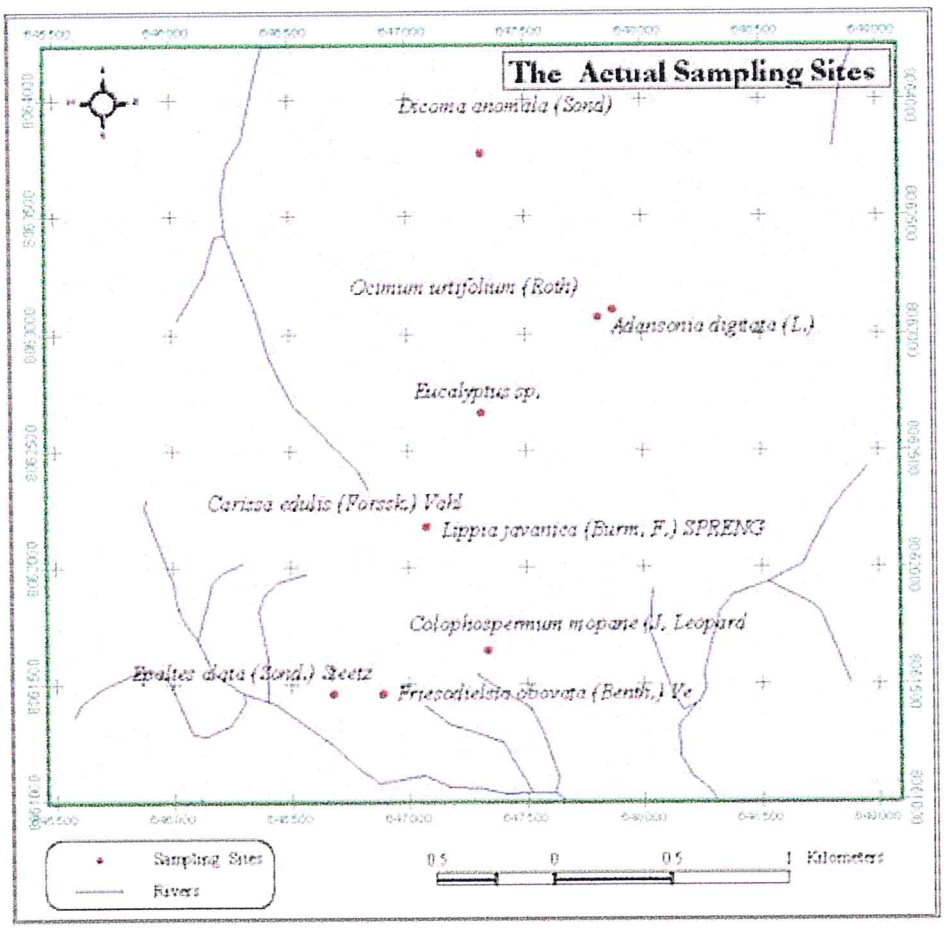

Figure 3.2 Point Map of the repellent plants actual collection sites (scientific names used)

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3.2 Plant collection and identification

Use of the following plants as mosquito repellent in rural areas of Gokwe and Honde valley was reported by Lukwa *et al.* (1999); *Adansonia digitata* (L.) (Muuyu) Bombacaceae, *Bersama abyssinica* Fresen (Munyahava) Melianthaceae, and *Colophospermum mopane* J. Leopard (Mupani) Fabaceae, *Dicoma anomala* (Sond) (Chifumoro) Acanthaceae, *Dodanae viscosa* (Jacq) (Mukonachando) Sapindaceae, *Epaltes alata* (Sond.) Steetz (Mutsangidze) Asteraceae, *Eucalyptus sp.* (Mupuranga) Myrtaceae, *Hexalobus monopetalus* (A.Rich) (Munyani) Annonaceae, *Leucas marticensis* (R. Br) Bobbin weed, (Bisikavana) Lamiaceae and *Ocimum urtifolium* (Roth) (Mutandamasenya) Lamiaceae.

Following this report a field survey was conducted in Shamva and Gokwe with the aim of collecting the same plants used in these rural villages. Plants collected were used to conduct laboratory experiments to scientifically validate the repellent effects they are claimed to have against the vector mosquitoes. Through the help of the traditional healers well versed with most of the medicinal plants used locally, the following plants were collected: *Adansonia digitata* (L.) (Muuyu) Bombacaceae, *Carissa edulis* (Forssk.) Vahl (Mudyabveni) Apocynaceae, *Colophospermum mopane* (J. Leopard) (Mupani) Fabaceae, *Dicoma anomala* (Sond) (Chifumuro) Acanthaceae, *Epaltes alata* (Sond.) Steetz (Mutsangidze) Asteraceae, *Eucalyptus sp.* (Mupuranga) Myrtaceae, *Ficus capensis* Thunb

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(Mukuyu) Moraceae, *Friesodielsia obovata* (Benth.) Verdc (Munyani) Annonaceae, *Lippia javanica* (Burm. F.) SPRENG (Zumbani) Verbenaceae and *Ocimum urtifolium* (Roth) (Mutandamasenya) Lamiaceae. Voucher specimens were prepared soon after collection and sent to Botany lecturers at the University of Zimbabwe, Biological Sciences Department for identification, before being confirmed at the National Botanical Gardens Harare-Zimbabwe by Mr. Anthony Mapaura.

During the plant collection exercise, coordinates (longitudes and latitudes) of the repellent plants collection sites were recorded using the GPS 300 satellite navigator (Megellan), in order to prepare a point map of where they were collected. The main reason of preparing the point map is to aid future plant based mosquito repellent researchers who would be interested in collecting these repellent plants to narrow down their collections to these areas, assuming that the repellent plants will be still intact. Using Arcview GIS Version 3.1, repellent plants collection sites were linked to small and big water bodies, which are important as mosquito breeding sites. The type of soil was also linked to the repellent plants collection sites. Clay type of soil is known to have low drainage capacity, which leads to trapping of water in the small drenches made by either vehicle tyres or by other means. Water trapped in those drenches acts as temporarily breeding sites of the vector mosquitoes. The collection site was linked to the farming region it fell in; this was an attempt to show evidence of human activities where the repellent plants were collected.

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Other uses, description and the ecology of the repellent plant were as well recorded during the repellent plant collection exercise.

3.3 Plants preparation, extraction and fractionation

3.3.1 Plants preparation

Leaves of these plants were separated from the rest of the plant parts and then dried under room temperature and relative humidity. Dried leaves were then ground into fine powder using a pulverizer machine (Siebtechnik) from the Institute of Mining Research, University of Zimbabwe, through consultation with Mr. Spencer, the analytical chemist from the Institute. The finely ground powder was then stored in airtight paper bags in readiness for the extraction procedure.

3.3.2 Extraction

Using a digital weighing balance (Melter AE 260 Delta range) four grams of each finely ground powder of the repellent plant species was weighed and digested with 80% ethanol for 18 hrs in a 40°C water bath (P selecta). Whiteman filter papers with filtering circles of 110 mm diameter were used for filtering the extract obtained after the digestion. The weight of each of the filter papers used was noted prior to filtering.

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The residue obtained was dried under the room temperature and it was used to determine the amount of the fine powder that was dissolved by the absolute ethanol into fraction A of the fractionation scheme.

3.3.3 Fractionation Scheme

The filtrate obtained was used to perform the fractionation on the basis of acidic, basic and neutral properties with reference to work done by Lalonde *et al.* (1980). Adjusting of the pH was done by NAOH 1M, $NAHCO_3$ 1M and concentrated HCL; PH meter GLP 21 was used to determine the required pH in each fraction. Fractionation was performed with two parts of petroleum ether/ ether, (B.P 40 ° C to 60 ° C) and one part of distilled water. In order to determine the percentage availability in each fraction the organic phase was evaporated by Vacuo Rotavapor-RE BUCHI at 80°C-100°C and then weighed using the digital balance.

The hydrophilic C1 fraction was subjected to acetone precipitation to eliminate carbohydrates and peptides then the supernatant passed through cation exchanger for further separation in reference to work done by Tunon *et al.* (1994). The different fractions obtained were stored in airtight culture bottles at -10°C awaiting thin layer chromatography analyses and the bioassays with the laboratory bred vector mosquitoes. Based on the amount of plant material available in each fraction the percentage availability in each fraction was

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calculated and indicated in the extraction flow chart of each repellent plant species and availability of different fractions compared in bar graphs.

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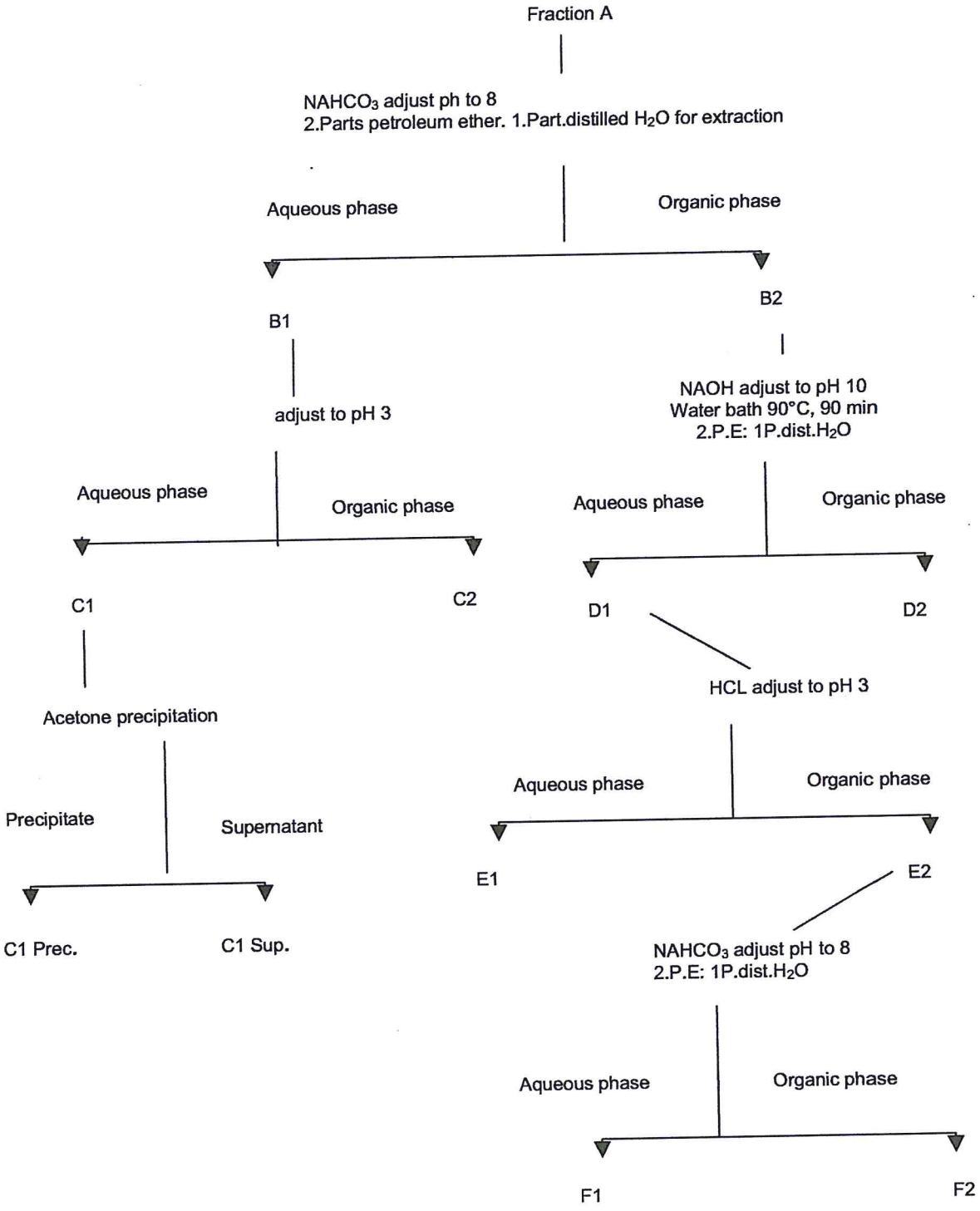
Figure 3.3 Fractionation flow chart. Adopted from Tunon *et al.* (1994)



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3.4 Thin Layer Chromatography

Hoerhammer *et al.* (1964) cited in Harbone (1984) stated that using Thin layer Chromatography TLC as the only separation technique, it is possible to analyze most of the plant compounds. TLC is very useful in study of plant-based mosquito repellent because we are targeting the less volatile plant compounds. Silica gel, which coats the TLC plate, is the most widely used absorbent as it is compatible with a wide range of solvents (Harbone, 1984). Unlike Gas Liquid Chromatography, which yields in one operation both qualitative and quantitative analyses, TLC only shows the presence or absence, hence making it very useful in the initial screening of the repellent plants. After confirmation, other advanced studies i.e. isolation of the specific plant compounds linked to repellency can be done.

Fractions obtained from the repellent plants were all subjected to alkaloids, bitter tasting substances, coumarins, essential oils and saponins screening. Twenty microlitres of each fraction were loaded on TLC Silica gel 60 F_{254} (Merck). The samples were applied at the start, as a line and the distance separating each spot of the sample were 0.5-1.5 cm. In order to get sharp bands an air blower was used to concentrate the spots while loading. The loaded plates were then placed in the development chamber (20 X 9 X 20) cm for the vertical development.

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3.4.1 Alkaloids

Alcoholic solution of 1 % Atropine was used as the references; 10μl was loaded in the same plate with the fractions. Solvent system was prepared by mixing Toluene, Ethyl acetate and Diethylamine in the following proportions (70 Toluene: 20 ethyl acetate: 10 Diethylamine). It was poured into the chromatography tank, and allowed to remain in the closed tank for 15 minutes for the saturation to be achieved. The loaded TLC plates were then placed in position, and chromatography was allowed to proceed, developing vertically.

Visual detection of the alkaloids was done using Dragendorff reagent, which was prepared by making stock solution A and B. The stock solution A was prepared by dissolving 1.7g of basic bismuth nitrate in 20 ml glacial acetic acid and 80 ml of distilled water under a water bath for 2 hours and then filtered. Stock solution B was prepared by dissolving 16g potassium iodide in 60 ml water. The two stock solutions were mixed in the ratio of 1:1. Mixing 10 ml of the two-mixed stock solutions to 20 ml of glacial acetic acid, and then topping it up to 100 ml with distilled water prepared the final spraying reagent.

The spraying reagent was used to spray the TLC plates after their development. A spraying gun was connected to the spraying pump compressor and the spraying was conducted under the fume hood. The sprayed plates were dried under the fume hood as the bands developed fully. The plate was scanned or photographed

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using a digital camera immediately in order to capture the actual colors of bands before fading. The color of the different bands obtained and the calculated retention factor value (R_f value) was used to determine the type of alkaloids present in each fraction. Atropine was used to determine the correction factor that was used in adjusting the calculated R_f values of the fractions to the book values obtained by Hidebert and Sabine, (1996).

3.4.2 Bitter tasting substances

Methanolic solution of 0.1 % glucose was used as the reference; 10μl was loaded in the same plate with the fractions. Solvent system was prepared by mixing chloroform, methanol and water in the following proportions (60 chloroform: 40 methanol: 4 water). It was poured into the chromatography tank, and allowed to remain in the closed tank for 15 minutes for the saturation to be achieved. The loaded TLC plates were then placed in position, and chromatography was allowed to proceed developing vertically.

Visual detection of the bitter tasting substances was done by using Anisaldehyde sulphuric acid reagent, which was prepared by mixing 0.5 ml of anisaldehyde with 10 ml glacial acetic acid, 85 ml of methanol and 5ml of concentrated sulphuric acid in that order. About 10 ml of the spraying reagent was used to spray the TLC plates after their development. A spraying gun was connected to the spraying pump compressor and the spraying was conducted under the fume hood. The

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sprayed plates were dried under the fume hood before being heated at 100 ° C for 5-10 minutes then evaluated for presence or absence of bitter tasting substances. The plates were scanned or photographed using a digital camera immediately to avoid fading before bands were captured. The color of the different bands obtained and the calculated R_f values were used to determine the type of bitter tasting substance present in each fraction. Glucose (reference) was used to determine the correction factor. The correction factor was used in adjusting the calculated R_f values of the fractions to the book values obtained by Hidebert and Sabine (1996).

3.4.3 Essential oils

Thymol and cineole were used as the references and they were loaded in the same plate with the fractions. They were prepared in toluene in the ratio of 1:30. A solvent system was prepared by mixing toluene and ethyl acetate in the following proportions (93 Toluene: 7 ethyl acetate). The solvent was placed in the chromatography tank, and allowed to stand in the closed tank for 15 minutes for the saturation to be achieved. The loaded TLC plates were then placed in position, and chromatography was allowed to proceed developing vertically. Visual detection of the essential oils was done by using anisaldehyde spraying reagent; it was prepared by first preparing the acid alcohol which was prepared by mixing 90 % methanol to 5 % concentrated sulphuric acid and then 5 % acetic acid, then the 100 ml of the prepared acid alcohol was mixed with 0.5 ml of p-

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anisaldehyde to make the anisaldehyde-spraying reagent. The spraying reagent was used to spray the TLC plates after their development. A spraying gun was connected to the spraying pump compressor and the spraying was conducted under the fume hood. The sprayed plates were dried under the fume hood before being heated at 100 ° C in a dry heat oven for 7-10 minutes until full development of the bands occurred. The plates were scanned or photographed using a digital camera, in order to capture the bands developed before fading. The color of the different bands obtained and the calculated R_f value was used to determine the type of essential oils present in each fraction. Thymol and Cineole (references) were used to determine the correction factor that was used in adjusting the calculated R_f values of the fractions to the book values obtained by Hidebert and Sabine (1996).

3.4.4 Saponins

Methanolic solution of 0.1 % quillajae was used as the reference; 10μl was loaded in the same plate with the fractions. Solvent system was prepared by mixing chloroform, glacial acetic acid, methanol and water in the following proportions: 64 chloroform: 32 glacial acetic acid: 12 methanol: 8 water. The solvent was poured into the chromatography tank, and allowed to stand in the closed tank for 15 minutes for the saturation to be achieved. The loaded TLC plates were then placed in position, and chromatography was allowed to proceed developing vertically.

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Visual detection of the bitter saponins was done by using Anisaldehyde sulphuric acid reagent, which was prepared by mixing 0.5 ml of anisaldehyde with 10 ml glacial acetic acid, 85 ml of methanol and 5ml of concentrated sulphuric acid in that order. About 10 ml of the spraying reagent was used to spray the TLC plates after their development. A spraying gun was connected to the spraying pump compressor and the spraying was conducted under the fume hood. The sprayed plates were dried under the fume hood before being heated at 100 ° C for 5-10 minutes then evaluated for presence or absence of saponins. The plates were immediately scanned or photographed using a digital camera to capture the bands before fading. The color of the different bands obtained and the calculated R_f values were used to determine the type of saponins present in each fraction. Quillajae (reference) was used to determine the correction factor that was used in adjusting the calculated R_f values of the fractions to the book values obtained by Hidebert and Sabine (1996).

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3.5 Bioassay experiments

3.5.1 Mosquitoes used for the bioassay

Mosquitoes (*Aedes aegypti*) were reared and maintained at 27± 2 °C temperature and 80±10 % relative humidity under a 12 hrs photoperiod. Larvae were reared on a diet of dog biscuits enriched with yeast health tablets. Female mosquitoes aged 10-14 days were used for the bioassay and they were maintained on 10 % sugar solutions during the experimentation period.

3.5.2 Preparations

The hands of the human used as test subject were cleaned with 70% ethanol to remove sweat, perfumes and soap residues. Floral fragrance from perfumes, soaps, lotions, and hair-care have been reported to act as mosquitoes' attractants (Foster and Hancock,1994). After the hand was cleaned it was air-dried and put into a latex surgical glove with a top aperture measuring 25 cm ^{2} as per the World Health Organizations Guidelines (Figure 3.4).

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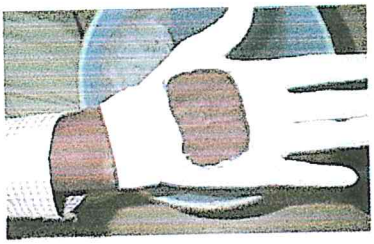

Figure 3.4 Human hand inside the latex surgical glove with a top aperture (25 cm^2) (World Health Organizations Guidelines)

The treatments (repellent plant fractions) were then assigned randomly to the test persons. The fractions were then spread as evenly as possible over the open aperture of the human hand, after which the hand was air-dried before being introduced into a mosquito cage containing the starved mosquitoes as shown in Figure 3.5.

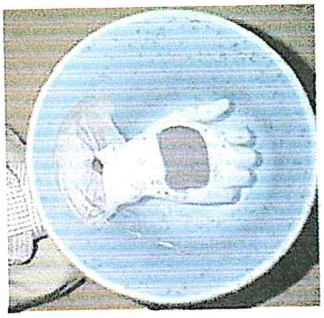

Figure 3.5 Human hand introduced to starved female mosquitoes enclosed into a plastic cage

Following the findings of our preliminary experiments 50 female mosquitoes were isolated from the colony and starved for 24-48 hrs prior to all the experiments. Plastic cages cylindrically shaped measuring (22.5 X 25 X 18.5) cm and having a net opening top were used (Figure 3.6a and 3.6b). On the side of the cage there was an opening of 15 cm diameter covered with a net (Figure 3.6a).

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Human hands were inserted into this opening during the dose finding, residual effect and the antifeedant experiments as shown in Figure 3.5.

Figure 3.6a side view

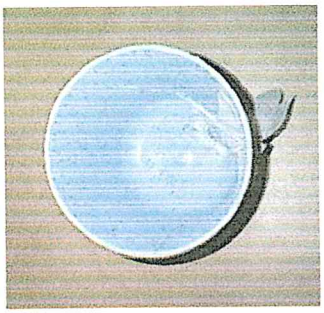

Figure 3.6b top view

Behavioral (orientation) experiments were conducted using three joined cages each measuring (20 X 20 X 20) cm (Figure 3.7). The cages were made up of iron frame which was covered by a net, to ensure even distribution of the light. An opening 12 cm diameter connected each of the three cages joined together. The middle chamber had an aperture opening of 15 cm diameter covered with a net arm through which mosquitoes were introduced. The middle apertures were always closed with a piece of paper once the mosquitoes were introduced prior to the exposure of the treated and the untreated arms.

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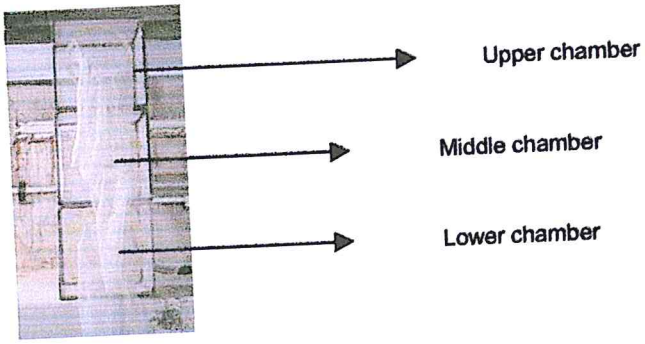

Figure 3.7 Experimental cage for the orientation experiments

3.5.3 Preliminary experiments

Preliminary experiments were conducted using untreated hands in order to establish the best group of mosquitoes to use during the experiments. Mosquitoes, which were already into the biting behavior, were found to be better biters than the freshly emerged ones. Therefore a decision was made to use 7-10 days old mosquitoes, in the subsequent experiments. In order to establish the duration of exposing the treated hand to the starved mosquitoes, preliminary experiments were conducted using untreated hands. It was established that 30 seconds was more than enough for the mosquitoes, to have oriented and settled for feeding on the untreated hand. We therefore decided to expose the treated hand for 60 seconds after which the number of mosquitoes on it was noted.

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3.5.4 Control experiments

Control experiments were done using hands untreated and treated with water, ethanol, the commercial available repellence N, N-diethyl -M- Toluamide (DEET). The test person held his arm in a cage as shown in Figure 3.5 and described in the preparations section. At the end of every minute the number of mosquitoes on the hand was noted and shaken off. To check for any decline in the feeding avidity of the populations, untreated hands were exposed in between exposures of treated hands.

Control experiments for the behavior experiment were done using the cage shown in Figure 3.7. Test persons subjected their two untreated hands to the upper and lower chambers of the cage as shown (Figure 3.8). The objective of this experiment was to establish if there was any significant difference between mosquitoes that oriented to the upper or lower chamber of the experimental cage.

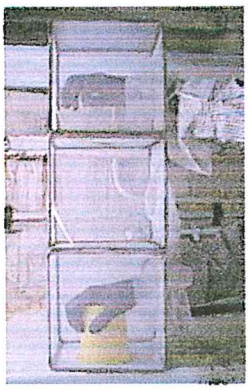

Figure 3.8 Human hands inserted on the lower and the upper chamber of the experimental cage

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The Cage used is described in the preparations sections. The number of female mosquitoes orientating to the lower and upper cages in the presence of untreated hands was noted. Data obtained was subjected to statistical analysis (t-test) to find out if there is any significant difference between female mosquitoes orienting to the upper and lower chambers in presence of untreated hands.

3.5.5 Residual effect experiments

The dose that was found to offer maximum protection was applied on the human hand and the duration of protection was investigated. The treated human hand was exposed to a plastic cage containing starved female mosquitoes as shown in (Figure 3.5). The number of mosquitoes landing was noted at the end of every minute as described in the preliminary experiments section. After every one-minute exposure mosquitoes that were found settled on the human hand were shaken off and the exposure replicated three times. After every 30 minutes the treated human hand was subjected to the starved mosquitoes and the number landing on the human hand was noted. The exercise was repeated until the number of mosquitoes landing on the treated arm exceeded those landing on the control. Data obtained on the control and treated arm was used to calculate the percentage repellence as described under the data analysis section.

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3.5.6 Anti-feedant experiments

The treated human hand was exposed to a plastic cage containing starved female mosquitoes as described in the preparations section (Figure 3.5). The number of female mosquitoes landing but not sucking and landing and sucking was noted at the end of every minute. After every one-minute of treated hand exposure, mosquitoes that were found settled on the human hand were shaken off and the exposure replicated three times. After every 30 minutes the treated human hand was subjected to the starved mosquitoes and the number of female mosquitoes landing but not sucking and landing and sucking on the human hand was noted. The exercise was repeated until the end of two hours. Data obtained in this experiment was used to investigate presence of antifeedant properties in the crude and lower fractions of the repellent plants.

3.5.7 Behavioral experiments

The treated human hand was exposed to the three-chambered cage as described in the preparation section (Figure 3.7). The cage contained 50 starved female mosquitoes as described in the preliminary experiment section. Control experiment was conducted as described in the control experiment section. The aim of the experiment was to establish the trend of orientation to the lower and upper chambers. Starved female mosquitoes were given the choice of deciding where to go in this experiment.

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Both treated and untreated hands were introduced simultaneously into the cage and held at about 10 cm from the aperture, which connected the middle, to lower and upper chamber. Starved female mosquitoes were introduced and confined into the middle chamber prior to the simultaneously introduction of the treated and untreated hands (Figure 3.8). Soon after the two hands were introduced, starved female mosquitoes confined in the middle chamber were released through the middle openings. After every one-minute of treated and untreated hand exposure, upper and lower chambers were closed hence trapping the female mosquitoes into any of the three chambers. Using an aspirator (sucking tube) mosquitoes were sucked and counted from the lower and upper chambers and reintroduced back to the middle chamber. The exercise was replicated three times and the data obtained was used to investigate the effect of different crude extracts and fraction to the orientation of mosquitoes.

3.6 Statistical analysis

Data obtained in the bioassay experiments was in count and percentage form. Using SAS Version 8.1 the distribution of the data was checked using the Proc Univariate Procedure. Normality test revealed that the data was either skewed to the right or left. Considering that the mosquito response was affected by plant, fraction and time (3 independent variables), there is no known non-parametric test that can handle data with such a high number of independent variables.

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Limitation to only using the parametric tests meant that an attempt was to be made to normalize the data.

The percentage repellence was determined as 100 X (mean control - mean treatment) / Control (Mehr *et al.*, 1985 and Govere *et al*, 2000). This data was transformed by getting the arcsine of the percentage repellence (Y = arcsine (X). {Where Y = Transformed repellence and X = percentage repellence}. The count data i.e. number of mosquitoes orienting to the treated or untreated hands was transformed by getting the natural logarithm of the values + 1. Y = Log (X + 1) Where Y = transformed observation, X = number of mosquitoes i.e. orienting to the treated or untreated hands.

All the three replications done in the bioassay experiments were meant to account for the experimental variability that could have contributed to experimental error. During the analyses replication was considered as a blocking factor because of the response variability it could have created. Different models were run using the PROC GLM procedure of SAS Version 8.1, in order to address the objectives of the various experiments conducted. For the residual effect and antifeedant experiment the model [$Y = \mu + r\ p + t + f + (t \times r\ p) + (r\ p \times f) + (t \times f) + (r\ p \times t \times f) + e$] was used to establish the effect of each repellent plant, time post application, fractions and all the possible interaction effect of the independent variable. For the behavior experiment the model [$Y = \mu + r\ p + f + (r\ p \times f) + e$] was used to establish the effect of each repellent plant, fractions and all the

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possible interaction effect of the independent variable. {Where μ = population mean, t = time post application, r p = repellent plant, t x p = interaction effect of time and plants, f = fractions and e = the error term}.

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CHAPTER FOUR-RESULTS

4.1 Plant collection and identification

4.1.1 *Adansonia digitata*

Identification of *Adansonia digitata* (Figure 4.1) by traditional healers was confirmed at the National Botanic Gardens. The plant belongs to the family Bombacaceae and it is a large deciduous tree attaining height of up to 15 meters. It was found growing in a very close proximity to *Eucalyptus spp* and *O. urtifolium*, (Figure 3.2). The environment was hot and dry surrounded with poorly drained clay-sandy soils. The plant has got a wide array of uses ranging from medicinal to food. People in the northern part of Gokwe claimed that aqueous extract of the bark of *A. digitata* cures stomach pains. Powdered bark of the plant mixed with porridge cures malaria. The fruit of the plant contains kernel, which has got the high valued edible oil. Fruits of this plant are commonly seen at market places of rural Zimbabwe and high-density suburbs of Harare.

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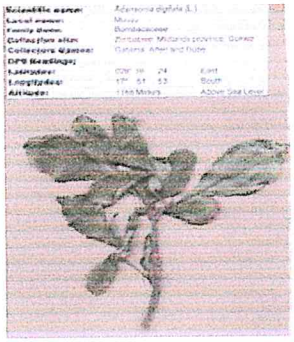

Figure 4.1 *Adansonia digitata* L. (Muuyu)

4.1.2 *Colophospermum mopane* (Mupani)

Identification of *Colophospermum mopane* (Mupani) (Figure 4.2) by traditional healers was confirmed at the National Botanical Garden. The plant belongs to the Leguminosae family and it is a deciduous tree growing up to 8 meters. Leaves of the plant provide livestock feeds besides being used for roofing. The vegetation cover of Gokwe is composed of this plant. Not much information was provided in terms of the importance of the plant as a medicinal plant. There was evidence of destruction of the plant by the mopane worms, which is a popular delicacy to most of the people we talked to. The plant was collected in a close proximity to minor water bodies (breeding sites for mosquitoes) (Figure 3.2)

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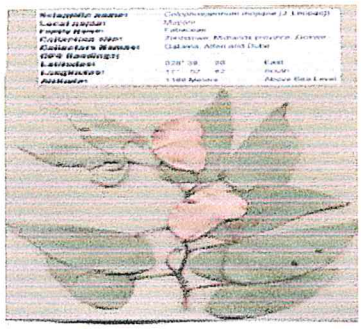

Figure 4.2 *Colophospermum mopane* (Benth.) J. Leonard (Mupani)

4.1.3 *Carissa edulis* (Forssk.)

Misidentification of *Carissa edulis* (Forssk.) (Figure 4.3) Vahl (Mudyabveni) (Apocynaceae) as *Bersama abyssinica* Fresen (Munyahava) (Melianthaceae) by the traditional healers adds to the list of known mosquito repellents in Zimbabwe. This plant was identified and confirmed to be *Carissa edulis* at the National Botanical Gardens. *Carissa edulis* is a shrub with green branches with sharp spines towards the end and it produces edible fruits. The plant contains a lot of oils both in its leave and the fruits. Rural people of Gokwe confirmed burning the plant in order to scare away mosquitoes at night. Fruits of the plants as well contain pungent extracts and people claimed that when smeared on the body of human bodies mosquitoes and other biting insects are scared off. Farmers argued that they hang the branches of the plants near cattle sheds as a way of scaring off sucking insects.

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Evidence of the plant containing plenty of oils was demonstrated in the laboratory after grinding the leaves of the plant. Extraction and analysis of the extract was not done in this project because of resource and time factor limitations.

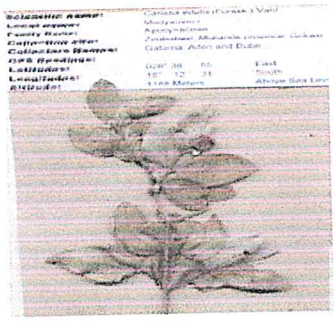

Figure 4.3 *Carissa edulis* (Forssk.) (Mudyabveni)

Though the previous study had reported presence *of Bersama abyssinica* in this region, when we conducted this field survey, the plant was not available. May be this could be the reason why the local community is using and referring to *C. edulis* as *Bersama abyssinica.* Activities such as burning and intensive farming could have resulted in the extinction of the plant from these regions. Figure 3.2 shows that the plant was collected in the same spot with *L. javanica.*

4.1.4 *Dicoma anomala* Sond. (Chifumuro)

Identification of *Dicoma anomala* Sond. (Chifumuro) (Figure 4.4) by traditional healers was confirmed at the National Botanical Garden. The plant belongs to the family Compositae and it grows on wooded grasslands with sandy soils.

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It is a perennial herb highly valued for its medicinal role it plays in the community. The tuber of the plant is known to cure tumors and also reduces stomach pain during the pregnancy period. The plant was only spotted in a single place about 200 km from Gokwe centre, where the owners of the land expected us to buy it. Figure 3.2 shows that the plant was collected in an isolated place compared to the rest. Because of its value to the community uprooting of the plant was through the aid of an elder who was also a traditional healer from the community.

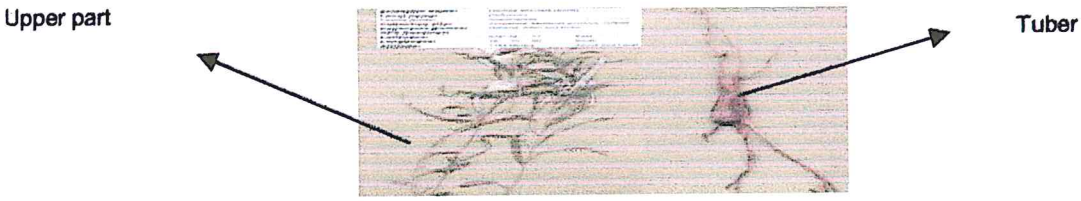

Figure 4.4 *Dicoma anomala* Sond. (Chifumuro)

4.1.5 *Epaltes alata* (Sond.) Steetz

Epaltes alata (Sond.) Steetz (Mutsangidze) (Asteraceae) (Figure 4.5) was identified as *Leucas marticensis* (R. Br) (Bobbin weed) (Bisikavana) Lamiaceae by the traditional healers. At the National Botanical Garden the plant was identified and confirmed to be *Epaltes alata.* It is a perennial plant ranging from 0.2 to 0.5 m in height. The plant appears bushy and has got a very strong aromatic scent, which is reduced when it has flowered its purplish flowers. The plant was collected from disturbed land where burning was done in the previous

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seasons. The surrounding vegetation was of mopane and *F. obovata* (Figure 3.2). The plant was claimed to have other uses besides repelling mosquitoes. Burning of the leaves produces ash, which is used as salt. Grinding of its leaves with water produces an aqueous extract, which is used to bath the young babies to avoid bad odors from their bodies.

Figure 4.5 *Epaltes alata* (Sond.) (Mutsangidze)

Leucas marticensis (R. Br) (Bobbin weed) (Bisikavana) (Lamiaceae) is a common annual weed, which grows in an erect and un-branched form. The flowers are white in color. The traditional healers might have misidentified *E. alata* as *L. marticensis*, because the two plants have similar medicinal values. When this survey was conducted *L. marticensis* was not available in the field.

4.1.6 *Eucalyptus spp*

Identification of *Eucalyptus spp* (Figure 4.6) by traditional healers was in agreement to that of National Botanic Gardens.

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However it was not possible to identity this plant up to the species level. The plant belongs to the family Myrtaceae and it attains great heights with straight poles. The leaves were found to have aromatic smells when crushed for the extraction. Leaves are known to have medicinal properties. Other uses of the plant includes fire wood, making of furniture, production of poles used for telephone and electricity transmission and for ornamental purpose.

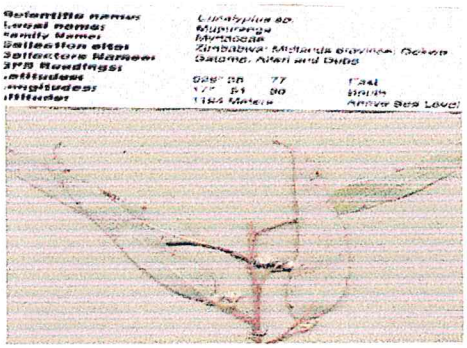

Figure 4.6 *Eucalyptus spp* (Mupuranga)

4.1.7 *Ficus capensis* Thunb (Mukuyu)

Ficus capensis Thunb (Mukuyu) (Moraceae) (Figure 4.7) was as well identified as *Dodanae viscosa* (Jacq) (Mukonachando) (Sapindaceae) by the traditional healer. The plant was confirmed to be *Ficus capensis* at the National Botanic Gardens. It was identified as a mosquito repellent plant by the traditional healers; the local community also claimed that direct burning of the plant repels vector mosquitoes.

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The bark of the plant is used to make ropes while the stem is used to make mortars. The plant was found growing near a swampy area with evidence of mosquito breeding. Removing of the bark of the plant to make ropes was found to be the main cause of damage to the plant. *Dodanae viscosa,* which we had targeted to collect during this exercise, was not spotted in all the areas visited.

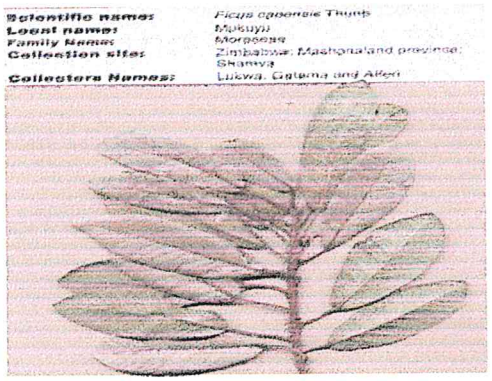

Figure 4.7 *Ficus capensis* Thunb (Mukuyu)

4.1.8 *Friesodielsia obovata* (Benth.)

Friesodielsia obovata (Benth.) Verdc (Munyani) Annonaceae (Figure 4.8) misidentified by the traditional healers as *Hexalobus monopetalus* (A.Rich) (Munyani) (Annonaceae) was identified and confirmed to be *Friesodielsia obovata* at the National Botanical Gardens. The two plants have got the same local names besides being in the same family Annonaceae. The two are shrubs attaining a maximum height of up to 5 meters. The only difference between the two plants is that *F. obovata* is a semi climbing plant, unlike *H. monopetalus,* which is not a

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climber. Traditional healers and local communities implicating use of *F. obovata* as a repellent plant against the vector mosquitoes adds to the list of plants claimed to have mosquito repellent properties in Zimbabwe. The repellent properties of *F. obovata* were confirmed after the bioassay experiments were conducted and the results obtained will be presented latter.

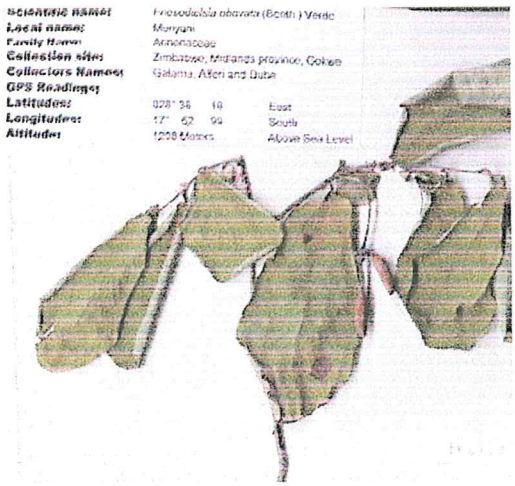

Figure 4.8 *Friesodielsia obovata* (Benth.) Verdc (Munyani)

4.1.9 *Lippia javanica* (Burm.f.) Spreng (Zumbani)

Traditional healers identified *Lippia javanica* (Burm.f.) Spreng (Zumbani) (Figure 4.9), and was confirmed at the National Botanic Gardens. *L. javanica* belongs to the Verbenaceae family and it grows as a shrub attaining a maximum height of 1.7meters. The leaves of the plant produce an aromatic smell, which is believed to repel most insects.

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Besides smearing the fresh juice from the leaves of this plant on the skin, the branches are hanged against the wall as a way of repelling mosquitoes. The plant was found growing in wooded grasslands where there was evidence of disturbance. Figure 3.2 shows that the plant was collected in the same spot with *C. edulis*. Local people in this area claimed to use intact branches of the two plants as a way of repelling insects in the cattle sheds.

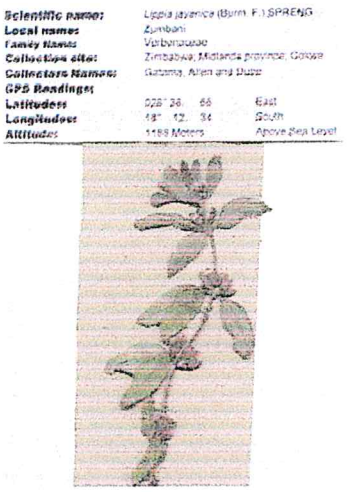

Figure 4.9 *Lippia javanica* (Burm.f.) Spreng (Zumbani)

4.1.10 *Ocimum urtifolium* Roth (Chinyamupfukidzi)

Identification of *O. urtifolium* (Figure 4.10) by the local community and traditional healers was confirmed at the National Botanic Gardens. The plant is a herb and belongs to the family Labiatae. It was found growing in very close proximity to *A. digitata* (Figure 3.2). The environment was hot and dry, surrounded with poorly drained clay-sandy soils.

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The plant has a wide array of uses ranging from medicinal to ornamental. The plant was found on the edges of live fences in most of the households we visited. The leaves and the tender stem of the plant are strongly aromatic, hence explaining why the plant is used as a mosquito repellent.

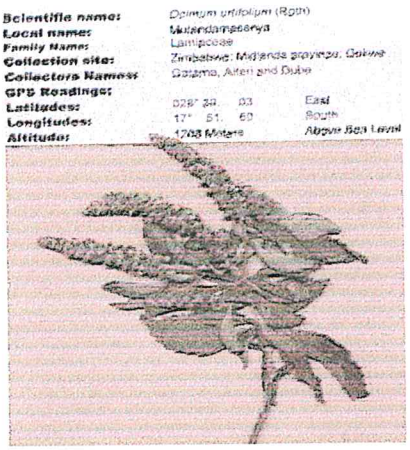

Figure 4.10 *Ocimum urtifolium* (Roth) (Mutandamasenya)

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4.2 Fractionation

Figure 4.11 shows availability in milligrams of fraction A of the different repellent plants extracted. Bioavailability of fraction A is of importance because all the lower fractions were derivatives from it. Three main classes that varied significantly existed amongst the fraction A (Table 4.1). Availability of fraction A of *Eucalyptus spp* varied significantly ($P < 0.05$) to all fractions A of the other repellent plants (Figure 4.11 and Table 4.1). No significant difference ($P > 0.05$) existed between availability of fraction A in *L. javanica* and *O. urtifolium* (Figure 4.11 and Table 4.1). Availability of fraction A of *F. obovata*, *C. mopane*, *D. anomala*, *A. digitata*, *E. alata* and *L. javanica* was not significantly different ($P > 0.05$) (Figure 4.11 and Table 4.1).

Comparison across different B1 fractions extracted reveals that four classes existed among the eight repellent plants extracted (Table 4.1). Fraction B1 of *Eucalyptus spp* varied significantly from the rest, indicating that most of its compounds were dissolving in the aqueous phase easily (Table 4.1). Looking at the variety of plant compounds recorded by this fraction, it did contain a number of bitter tasting substances and essential oils (Table 4.6, Plate 16 and 17). Fraction B1 of *Eucalyptus spp*, offered the longest residual effect compared to the rest of the other B1 fractions (Appendix E). Fraction B1 of *C. mopane* was in its own class of having the least yield (Appendix E). No plant compound was recorded from this fraction besides offering insignificant protection.

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Fraction B1 of *L. javanica* was not significant different ($P > 0.05$) to fraction B1 of *Eucalyptus spp* and *F. obovata* (Figure 4.11). Besides recording a variety of plant compounds all these B1 fractions offered desirable mosquito repellent properties (Appendix E).

Fraction B2 of *Eucalyptus spp* varied significantly to the rest of the B2 fractions, hence making it record the highest availability index compared to the rest of B2 fraction (Table 4.1). The rest of the B2 fractions were not significantly different in regard to the yield recorded (Table 4.1).

Most of the yield of B1 from *E. alata* went to fraction C1 pathway explaining the reason why it differed significantly ($P < 0.05$) from the rest of the fractions (Table 4.1). Lower fractions of *E. alata* (F1 and F2) were not significantly different in regard to availability (Table 4.1) and protection against the vector mosquito (Appendix M and N). Most lower fractions of the repellent plants protected for less than 0.5 hrs after they were applied most likely.

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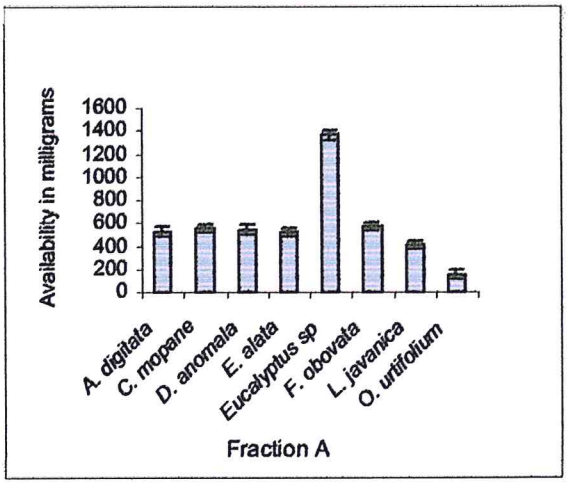

Figure 4.11 Bioavailability of fraction A of the different repellent plants
Standard error ±37.5 milligrams

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Table 4.1 Bioavailability of fractions (milligrams) of the repellent plants extracted through the fractionation scheme

Fractions / R. Plants	A	B1	B2	C1	C2	D1	D2	E1	E2	F1	F2
A. digitata	530.27^{b}	374.67^{abc}	155.30^{b}	317.00^{abc}	42.07^{a}	267.17^{b}	397.83^{a}	193.33^{b}	19.83^{a}	18.33^{a}	22.50^{af}
C. mopane	558.37^{b}	59.70^{d}	259.75^{b}	26.60^{c}	33.10^{a}	284.00^{b}	213.00^{ab}	250.30^{ab}	33.70^{a}	32.80^{a}	1.97^{a}
D. anomala	546.77^{b}	348.77^{abcd}	198.00^{b}	201.20^{abc}	164.23^{a}	47.33^{b}	150.67^{ab}	28.67^{b}	18.67^{a}	13.23^{a}	8.11^{a}
E. alata	521.90^{b}	463.23^{a}	49.34^{b}	423.90^{a}	38.33^{a}	32.67^{b}	26.00^{b}	18.23^{b}	14.43^{a}	9.67^{a}	11.10^{a}
Eucalyptus	1363.73^{a}	528.67^{a}	837.00^{a}	371.50^{ab}	92.90^{a}	694.90^{a}	177.10^{ab}	530.77^{a}	164.13^{a}	63.47^{af}	100.67^{af}
F. obvata	570.70^{b}	514.07^{a}	63.57^{b}	452.53^{a}	61.53^{a}	200.40^{b}	18.60^{b}	168.69^{b}	31.71^{a}	11.00^{a}	20.72^{a}
L. javanica	405.10^{bc}	376.10^{ab}	29.00^{b}	331.43^{ab}	44.67^{a}	19.57^{b}	9.43^{b}	14.47^{b}	9.00^{a}	3.50^{a}	3.17^{a}
O. urtifolium	153.90^{c}	133.00^{bcd}	20.90^{b}	128.00^{bc}	5.00^{a}	9.80^{b}	11.10^{b}	8.80^{b}	1.00^{a}	1.00^{a}	1.00^{a}

$^{a\ b\ c\ d}$ LSMEANS in milligrams within a column with different superscript letters were significantly different ($p < 0.05$).

Standard error ± 0.32

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4.3 Alkaloids, Bitter Tasting Substances, Essential oils and Saponins

4.3.1 *Adansonia digitata*

Fractions A, B1 and B2 of *A. digitata* contained alkaloids (Table 4.2 and Plate 4.1). The two types of alkaloids recorded by these fractions were brucine and chelidonine. Brucine and chelidonine are characterized by retention factors of 0.53, 0.88 and grey-violet colour bands respectively (Plate 4.1). Fraction A (crude extract) and fraction B1 extracted through the aqueous phase pathway recorded the highest number of alkaloids.

Out of the 13 fractions of *A. digitata* subjected for bitter tasting substances analyses fractions A, B1, C1 and C1S tested positive (Table 4.2 and Plate 4.2). Melittoside characterized by retention factor of 0.2-0.4 and grey colour band was recorded by fraction A, B and C1S, making it the most common fraction. Aucubin characterized by retention factor of 0.20 and grey colour band was recorded in fraction C1 only (Table 4.2 and Plate 4.2). Cynaropicrin and gentiopicroside characterized by retention factor of 0.31, 0.41 and colour band of violet respectively was recorded in fraction C1S (Table 4.1 and Plate 4.2). Fractions extracted through the aqueous phase pathway recorded higher number of bitter tasting substances compared to those of the organic phase (Table 4.1 and Plate 4.2).

Fractions A, B1, B2, D1, D2 and E2 of *A. digitata* recorded various types of essential oils out of the 13 fractions submitted for the analysis (Table 4.2 and

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Plate 4.3). The most common type of essential oil recorded was the THC compound, while alpha santonin, nerolidol and citronellal were the least common (Table 4.2 and Plate 4.3). Fractions A, B1, D1, D2 and E2 were found to contain THC compound which was characterized by forming a violet blue band at the solvent front (Table 4.2 and Plate 4.3). Alpha santonin, nerolidol and citronellal were characterized by a retention factor of 0.30, 0.38, 0.65 and colour band of blue and dark blue were recorded in fractions B1, A and B2 respectively.

Citral, characterized by a retention factor of 0.41 and band color of blue violet was recorded in fractions B1, B2 and D2 (Table 4.2 and Plate 4.3). Isomenthone with its retention factor of 0.55 and blue green colour band was recorded in fractions B2 and D2 (Table 4.2 and Plate 4.3). Fractions B2 and D2 recorded three different types of essential oils compared to fraction D1 that recorded only one type (Table 4.2 and Plate 4.3). Fractions from the organic phase recorded 7 different types of essential oils compared to 3 to 4 types recorded by fractions from the aqueous phase (Table 4.2 and Plate 4.3).

Out of the 13 fractions of *A. digitata* subjected to saponins analysis only fractions A and C1S tested positive (Table 4.2 and Plate 4.4). Quillaic acid characterized by a retention factor of 0.05-0.15 and a color band of Dark brown was recorded in fractions A and C1S (Table 4.2 and Plate 4.4), while Parillin characterized by a retention factor of 0.86 and a colour band of Yellow brown was recorded in fraction C1S (Table 4.2 and Plate 4.3). Compared to the other fractions of *A.*

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digitata fraction C1S recorded the highest number of different types of saponins (Table 4.2 and Plate 4.4). The two types of saponins recorded in the two fractions of *A. digitata* each had two bands (Table 4.2 and Plate 4.4).

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Table 4.2 *A. digitata* alkaloids, bitter tasting substances, essential oils and saponins

Fraction / Standard	Alkaloids	R_f value	Band colour
A, B1	Brucine	0.53	Grey violet
A, B1, B2	Chelidonine	0.88	Grey violet
Standard	Atropine (At)	0.33	Orange brown
Fraction / standard	Bitter tasting substances	R_f value	Band colour
A, B1, C1S	Melittoside	0.2-0.4	Grey
C1	Aucubin	0.20	Grey
C1S	Cynaropicrin	0.31	Violet
C1S	Gentiopicroside	0.41	Violet
Standard	Glucose (G)	0.32	Grey
Fraction / Standard	Essential oils	R_f value	Band colour
A	Nerolidol	0.38	Dark blue
A, B1, D1, D2, E2	THC compounds	Solvent front	Violet blue
B1, B2, D2	Citral	0.41	Blue violet
B1	Alpha santonin	0.30	Blue
B2, D2	Isomenthone	0.55	Blue green
B2	Citronellal	0.65	Dark blue
Standard	Thymol (T)	0.76	Red violet
Fraction / Standard	Saponins	R_f value	Band colour
A, C1S	Quillaic acid	0.05-0.15	Dark brown
C1S	parillin	0.86	Yellow brown
Standard	Quillajae (Qu)	0.14-0.31	Brown

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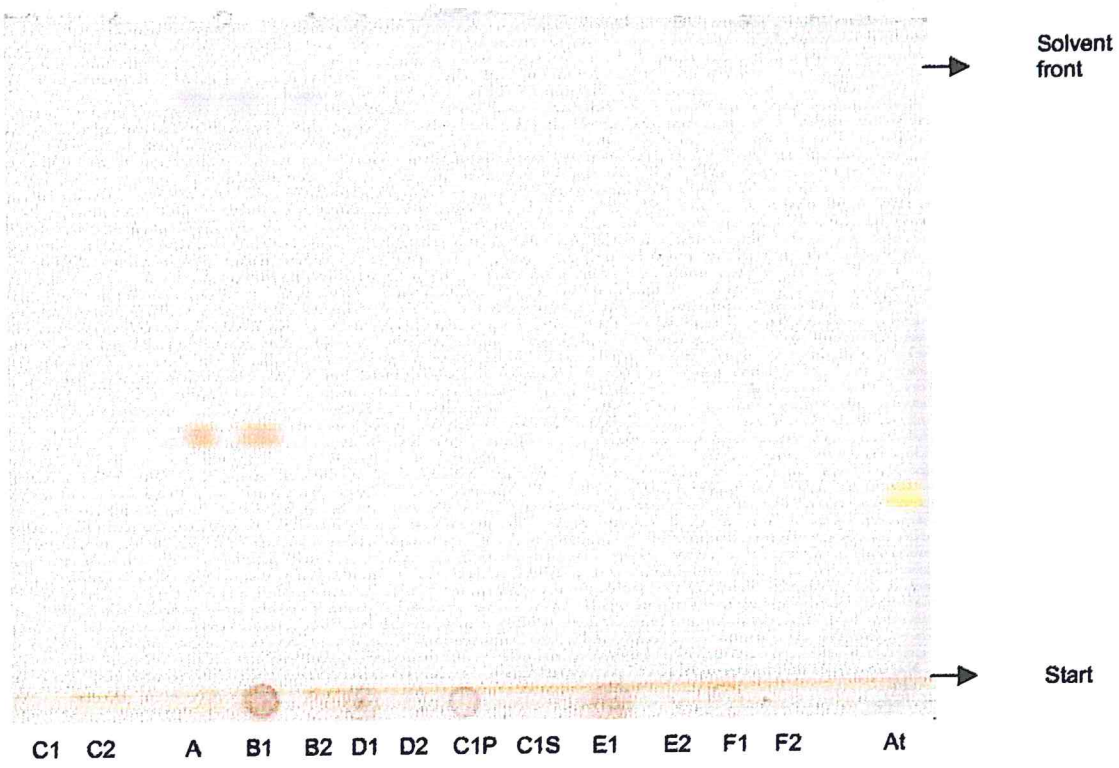

Plate 4.1 *Adansonia digitata* alkaloids

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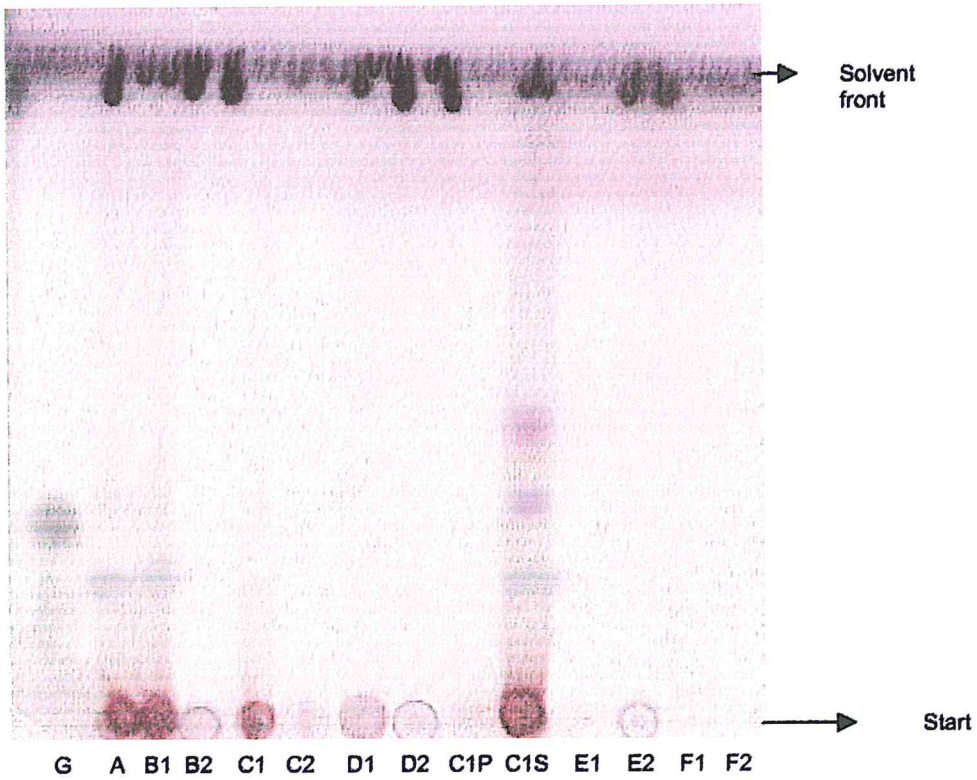

Plate 4.2 *A. digitata* bitter tasting substances

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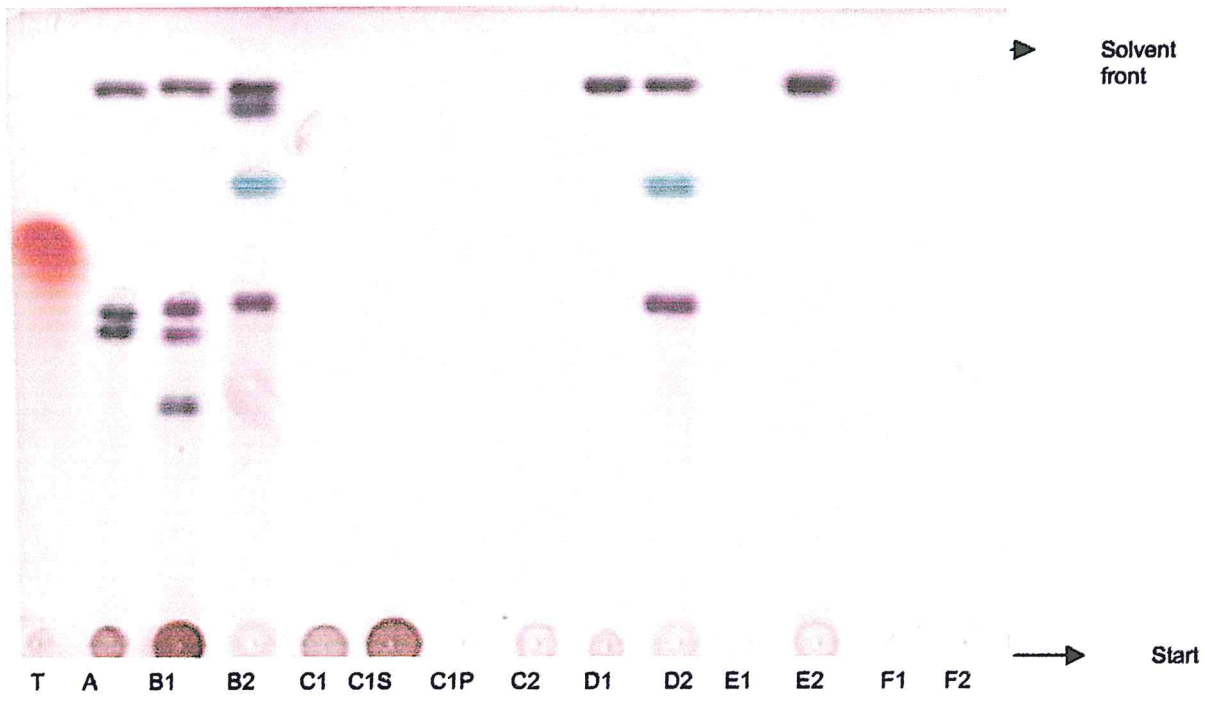

Plate 4.3 *A. digitata* essential oils

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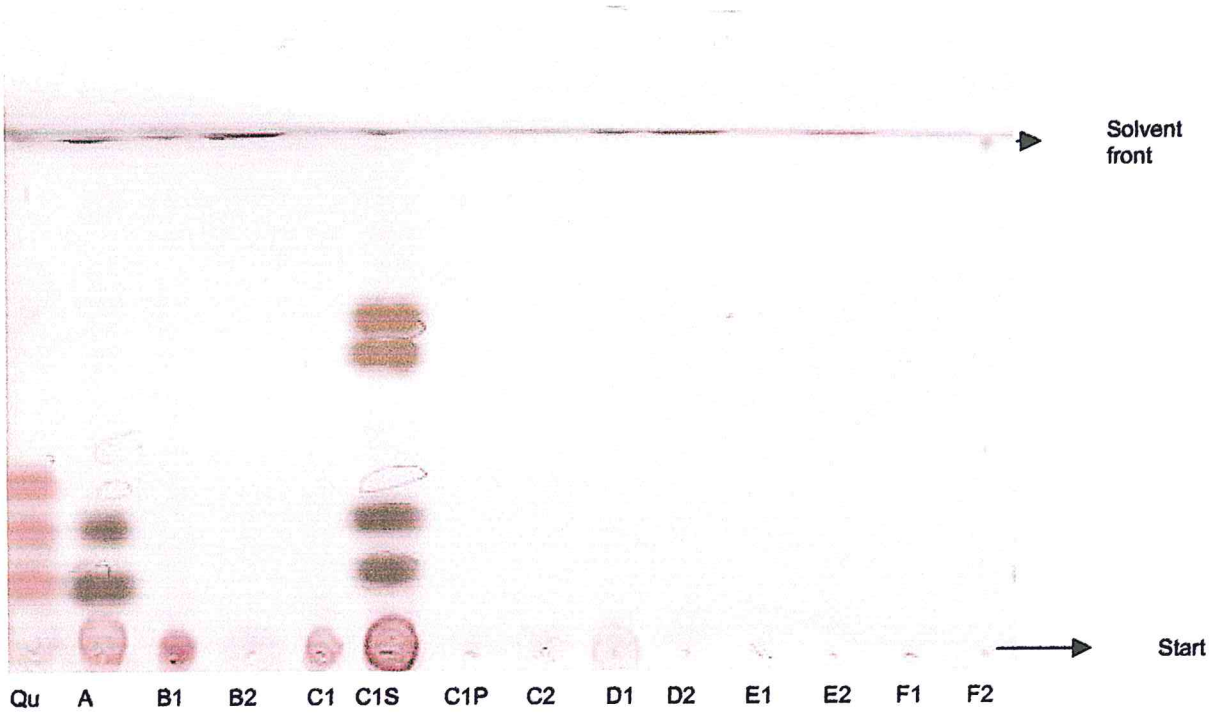

Plate 4.4 *A. digitata* saponins

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4.3.2 *Colophospermum mopane*

Out of the 13 fractions of *C. mopane* analyzed for the different types of alkaloids, fractions A, C2 and D2 tested positive (Table 4.3 and Plate 4.5). The three fractions recorded only one type of alkaloid. Colchine, the alkaloid recorded is characterized by a retention factor of 0.60 and blue colour band (Table 4.3 and Plate 4.5).

No fraction of *C. mopane* recorded bitter tasting substance. Fractions A, B1 and C1 appeared to contain traces of bitter tasting substances, but the bands were not clearly visible for identification (Plate 4.6).

Out of the 13 fractions analyzed for essential oils only fractions C2, D2 and E2 tested positive (Table 4.3). Crude extract (fraction A) did not record any essential oil (Table 4.3). Spathulenol characterized by a retention factor of 0.36 and violet colour band was recorded in fractions C2, D2 and E2 (Table 4.3). Peperitone with a retention factor of 0.52 and orange red colour band was recorded by fractions D2 and E2 (Table 4.3). *C. mopane* recorded the least number of essential oils compared to other extracts from the rest of the plants. Only fractions extracted through the organic phase pathway recorded essential oils (Table 4.3). Comparison of higher (i.e. crude extract) and lower fractions (i.e. E2) of *C. mopane* reveals that the latter recorded more essential oils (Table 4.3)

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Fractions A, C2, C1P and E1 tested positive for the saponins (Table 4.3, Plate 4.7). In total four different types of saponins were recorded, (Table 4.3 and plate 4.7). Parillin, characterized by a retention factor of 0.75 and colour band of brown yellow was recorded by fractions A and E1 (Table 4-2, Plate 4-7). Quercetin and sarsaparillae characterized by retention factors of 0.36 and 0.10 and yellow-brown colour band were recorded by fractions A and C1P respectively (Table 4.3, Plate 4.7). Fraction C2 recorded quillaic acid, which was characterized by a retention factor of 0.10 and dark-brown colour band. Compared to the other fractions, the crude extract recorded more saponins than the rest. Fractions extracted through the aqueous phase pathway recorded more saponins than fractions of the organic phase (Table 4.3, Plate 4.7).

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Table 4.3 *C. mopane* alkaloids, essential oils and saponins

Fraction / Standard	Alkaloids	R_f value	Band colour
A, C2, D2	Colchine	0.60	Blue zone
Standard	Atropine (At)	0.33	Orange brown
Fraction / Standard	Essential oils	R_f value	Band colour
C2,D2, E2	Spathulenol	0.36	Violet
D2, E2	Peperitone	0.52	Orange red
Standard	Thymol (T)	0.67	Red-violet
Standard	Cineole (Cin)	0.59	Blue
Fraction / Standard	Saponins	R_f value	Band colour
A	Quercetin	0.36	Yellow-brown
A, E1	Parillin	0.75	Brown-yellow
C1P	Sarsaparillae	0.10	Sarsaparillae
C2	Quillaic acid	0.05-0.15	Dark-brown
Standard	Quillaja (Qu)	0.14-0.31	Brown

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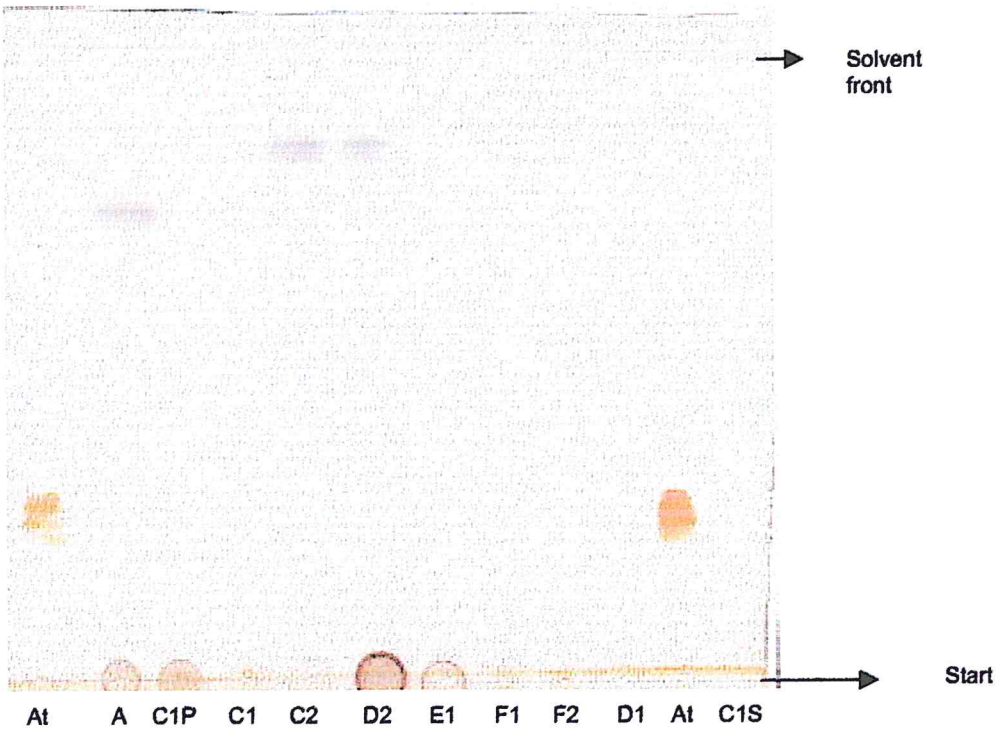

Plate 4.5 *C. mopane* alkaloids

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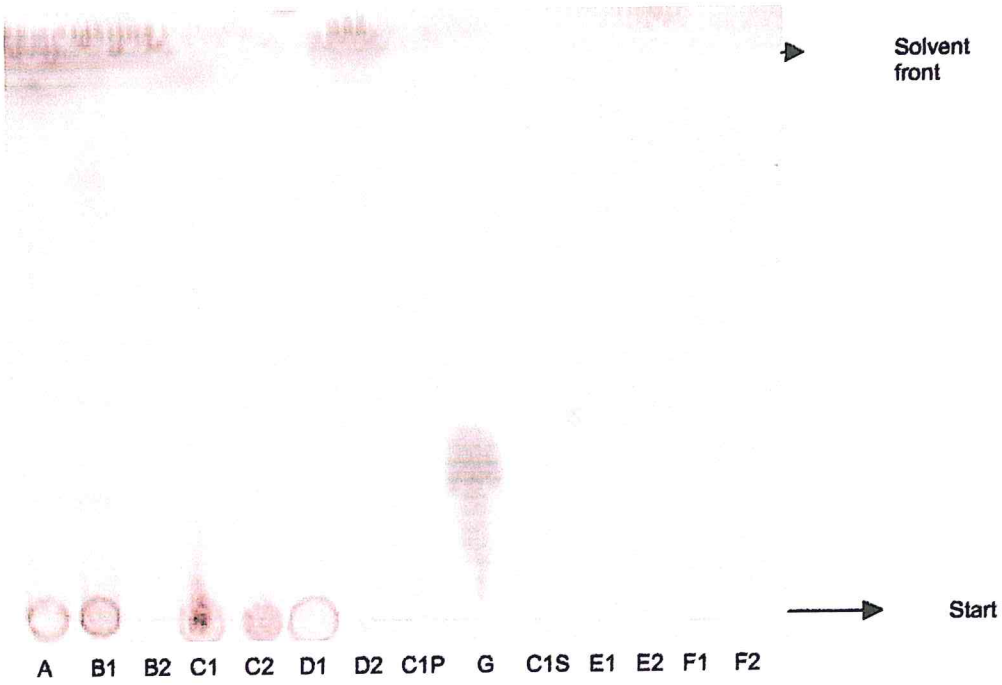

Plate 4.6 *C. mopane* bitter tasting substances

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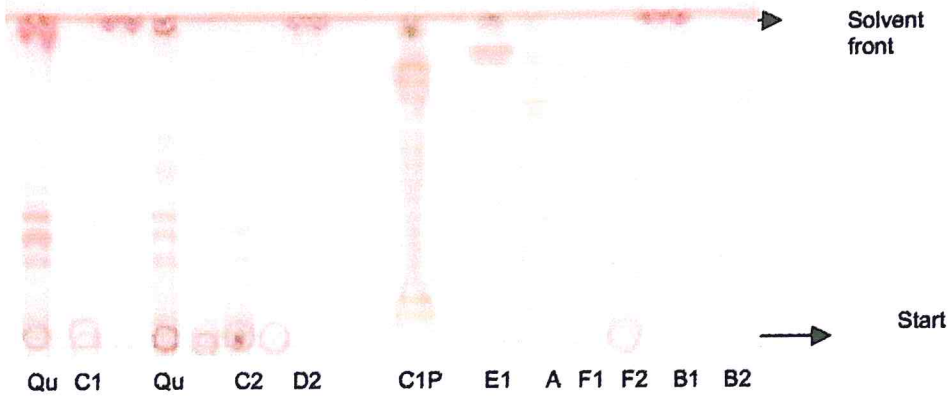

Plate 4.7 *C. mopane* saponins

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4.3.3 *Dicoma anomala*

Fractions A, C2 and E2 tested positive for the alkaloids (Table 4.4 and Plate 4.8), out of the 13 fractions submitted for the analyses. Fraction A recorded monovincine and anabasine characterized by retention factor of 0.94, 0.35 and colour band of brown and orange-brown respectively (Table 4.4 and Plate 4.8). Other fractions that contained monovincine were C2 and E2 (Table 4.4 and Plate 4.8). Availability of monovincine to the lower fractions suggests that it was either in large quantity or it was not negatively affected by the extraction procedures involved for the lower fractions extraction. Alkaloids recorded by fractions of *D. anomala* are of medium and high migration power (Table 4.4 and Plate 4.8). Lower fractions that recorded alkaloids are from the organic phase (Table 4.4 and Plate 4.8).

Out of the 11 fraction of *D. anomala* that were subjected to bitter tasting substances analyses only fraction B1 tested positive (Table 4.4 and Plate 4.9). Gentionoside and cutapol characterized by retention factor of 0.10, 0.30 and colour band of green brown and violet, respectively, was recorded by fraction B1 (Table 4.4 and Plate 4.9). Presence of only two types of bitter tasting substances suggests that either they were affected during the extraction process or they are in low concentration.

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Evaluation of the presence or absence of essential oils in each of the fraction obtained from *Dicoma anomala* was done visually (Plate 4.10). The colour of the different bands obtained and the calculated R_f value were used to determine the type of different essential oils present in each fraction (Plate 4.10). Fractions A, B1, B2 C1 D2 and F1 were found to contain at least one type of essential oils whereas with C2, D1 E1, E2 and F2 no essential oil was recorded (Plate 4.10). Borneol characterized by a blue grey zone and retention factor of 0.28 was the most common type of essential oil recorded in *D. anomala* extracts (Plate 4.10). Cinamic acid characterized by dark blue bands and retention factor of 0.13 and THC compounds forming at the solvent front with dark blue zones followed the list (Plate 4.10). Cinnamoyl cinnate characterized by a dark blue band and retention factor of 0.28 was only recorded in fraction A (Plate 4.10).

The most common type of essential oil borneol was recorded by fractions A, B1, B2, C1 and D2 whereas B1, C1 and F1 were found to contain cinamic acid (Plate 4.10). Fractions B1 B2 and D2 were the only ones found to contain the THC compounds (Plate 4.10). Fraction B1 contained the highest number of essential oils since all the three different types of essential oils were present, whereas fraction F1 recorded only one type of essential oil (Plate 4.10). The results indicate that essential oils were recorded both on the organic and aqueous phase fractions (Table 4.4 and Plate 4.10). None of the essential oils reported from the 11 fractions evaluated was found to have the same characteristics as that of the standard thymol (Plate 4.10). *D. anomala* can be said to contain essential oils with

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higher and lower migration power (Plate 4.10). Fraction F1 is the only lower fraction, which was found to contain essential oils (Plate 4.10) meaning that most of the essential oils went to the organic phase during fractionation process (Table 4.4). Fractions B1, C1 and F1 from the aqueous phase were found to contain essential oils (Table 4.4) compared to B2 and D2 from the organic phase (Table 4.4). Fraction B1 from the aqueous phase recorded the highest number of different essential oils (Table 4.4).

Out of the 11 fractions of *D. anomala* subjected for saponins analysis, B1, C1 and C2 tested positive (Table 4.4 and Plate 4.11). Hederin characterized by a retention factor of 0.7-0.8 and dark-grey-blue was recorded by fraction B1 (Table 4.4 and Plate 4.11). Isochlorogenic acid characterized by a retention factor of 0.75 and blue colour was recorded by fractions B1, C1 and C2 (Table 4.4 and Plate 4.11). Fraction C2 is the only one from the organic phase that recorded saponins compared to two fractions from the aqueous phase (Table 4.4 and Plate 4.11). Having recorded two types of saponins fraction B1 emerged as the best fraction in terms of recording variety of saponins. Fractions from *D. anomala* are the only one that recorded Isochlorogenic acid, suggesting that this type of saponins is specific to this plant.

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Table 4.4 *D. anomala* alkaloids, bitter tasting substances, essential oils and saponins

Fraction / Standard	Alkaloids	R_f value	Band colour
A, C2, E2	Monovincine	0.94	Brown zone
A	Anabasine	0.35	Orange-brown
Standard	Atropine	0.39	Orange brown
Fraction / standard	Bitter tasting substances	R_f value	Band colour
B1	Gentionoside	0.10	Green brown
B1	Cutapol	0.30	Violet
Standard	Glucose (G)	0.41	Grey
Fraction / Standard	Essential oils	R_f value	Band colour
A, B1, B2, C1 and D2	Borneol	0.28	Blue gray
B1, C1 and F1	Cinamic acid	0.13	Dark blue
B1 B2 and D2	THC Compounds	Solvent front	Dark blue
A	Cinnamoyl cinnamate	0.28	Dark blue
Standard	Thymol	0.79	Red-violet
Fraction / standard	Saponins	R_f value	Band colour
B1	Hederin	0.7-0.8	Dark-grey-blue
B1, C1 and C2	Isochlorogenic acid	0.75	Blue
Standard	Quillaja (Qu)	0.14-0.31	Brown

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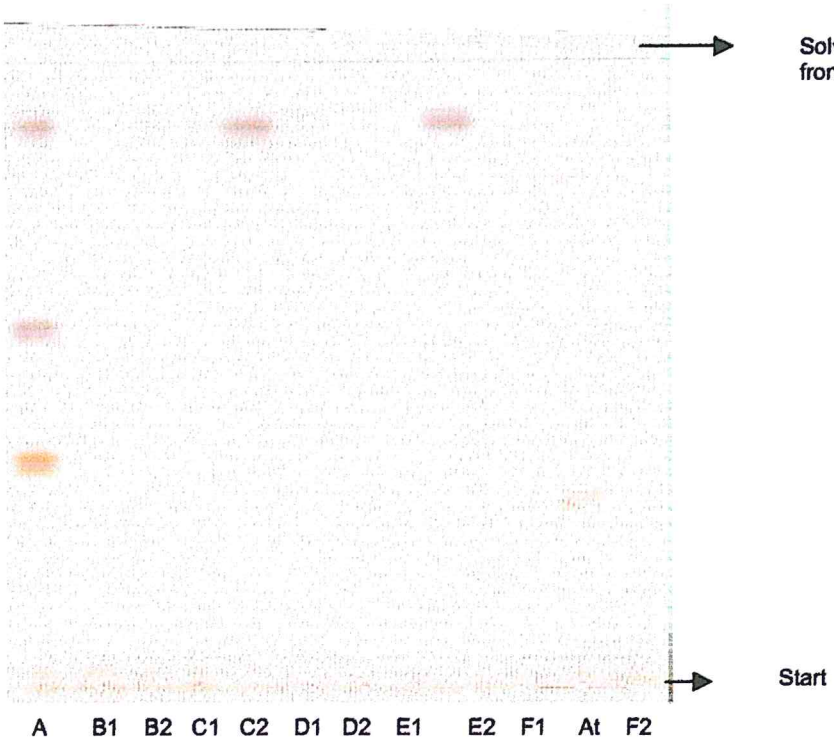

Plate 4.8 *D. anomala* alkaloids

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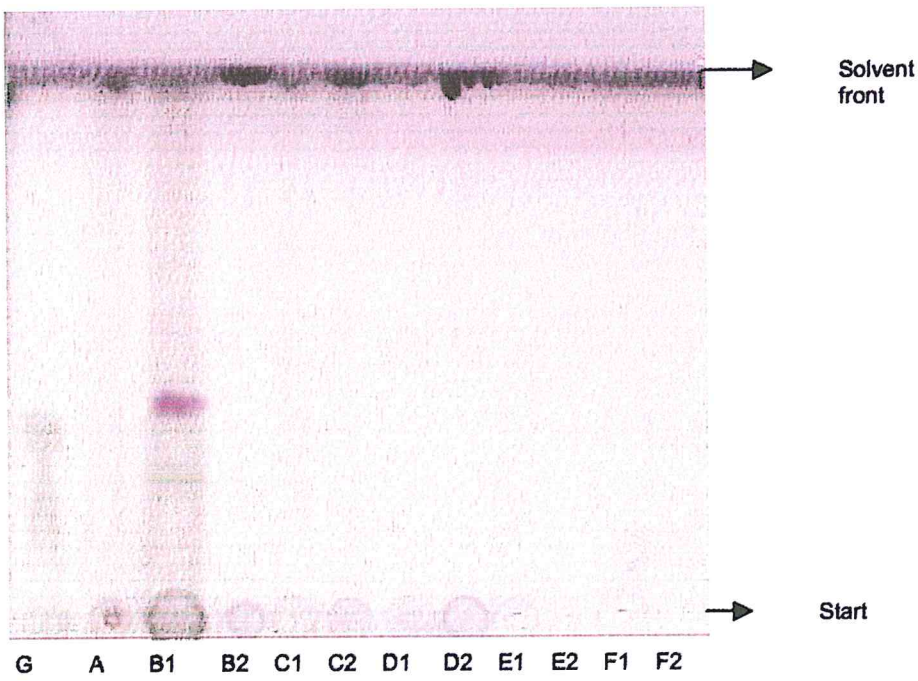

Plate 4.9 *D. anomala* bitter tasting substances

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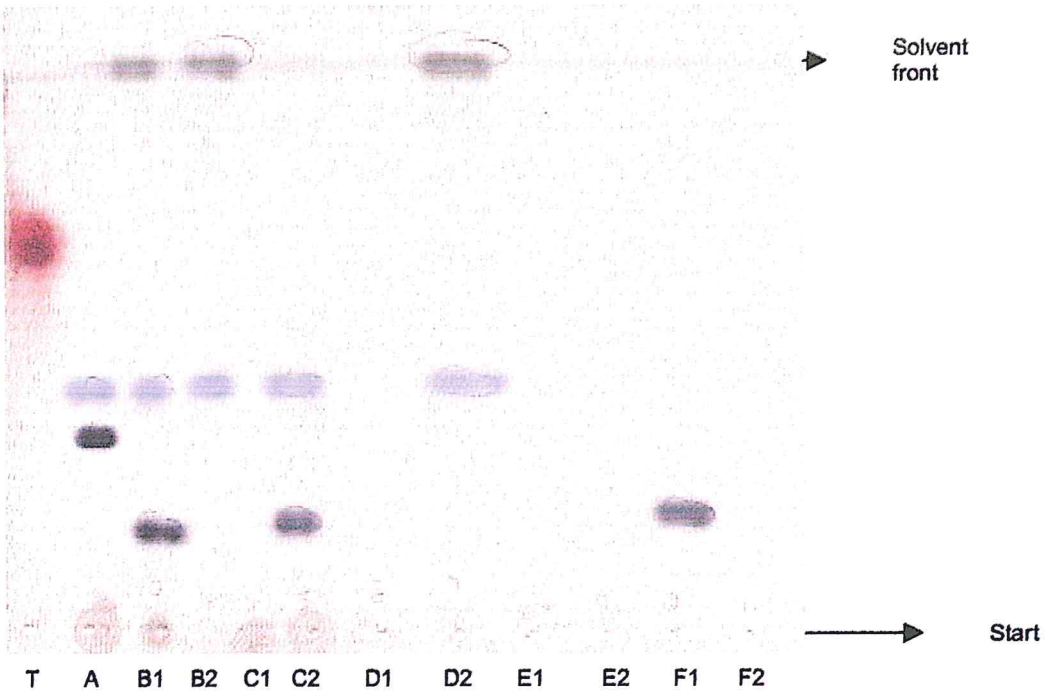

Plate 4.10 *D. anomala* essential oils

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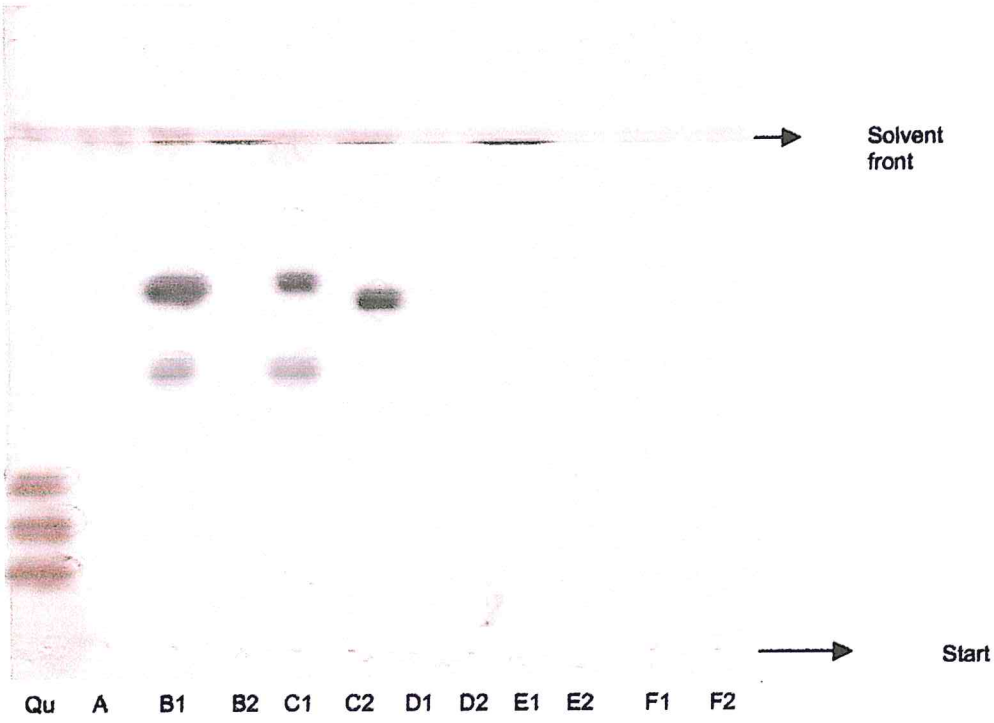

Plate 4.11 *D. anomala* saponins

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4.3.4 *Epaltes alata*

Crude extracts and lower fractions of *E. alata* subjected to alkaloids analysis tested positive for only two types of alkaloids (Table 4.5 and Plate 4.12). Crude extract (fraction A) and fraction B1 recorded two types of alkaloids compared to fractions C2, D2 and C1P that recorded only one type (Table 4.5 and Plate 4.12). Minovincine characterized by a retention factor of 0.79 and brown colour band was the most common type of alkaloid and it was recorded by fractions A, B1, C2, D2 and C1P (Table 4.5 and Plate 4.12).

Fractions A, B1, C1 and C1S of *E. alata* tested positive for the bitter tasting substances (Table 4.5 and Plate 4.13). Cynaropicrin and Curcurbitacin aglycones characterized by retention factor of 0.31, 0.85 and violet, yellow brown colour bands, respectively, were recorded by fractions A, B1, C1 and C1S (Table 4.5 and Plate 4.13). Only fraction C1S recorded gentiopicroside that was characterized by retention factor of 0.41 and violet colour band (Table 4.5 and Plate 4.13). Foliamenthrin characterized by retention factor of 0.64 and blue colour band was recorded by fractions A, B1 and C1 (Table 4.5 and Plate 4.13). Cynaropicrin and Curcurbitacin aglycones were the most common types of bitter tasting substances recorded by *E. alata* fractions (Table 4.5 and Plate 4.13). Fraction extracted through the aqueous phase pathway recorded the highest number of bitter tasting substances ((Table 4.5and Plate 4.13).

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Only fraction C2, D1 and D2 out of the 13 fractions obtained from *E. alata* contained essential oils (Table 4.5 and Plate 4.14). Linalool and borneol characterized by retention factor of 0.7, 0.23 and colour band of blue zone, violet blue respectively was the most common (Table 4.5 and Plate 4.14). Menthol cineole and linalyl acetate characterized by retention factor of 0.30, 0.39, 0.59 and colour band of red violet and blue, respectively, were recorded only once. Fraction C2 recorded the highest number of essential oils compared to fraction D1 that recorded only one type of essential oil (Table 4.5 and Plate 4.14). Fractions C2 and D2 from the aqueous phase recorded a total of five different types of essential oils compared to fraction D1 from aqueous phase that recorded only one type of essential oil (Table 4.5 and Plate 4.14). Higher fractions of *Epaltes alata* did not record any type of essential oil, this does not mean that they did not contain any but the chances are that they escaped due to their high volatilities before they could separate in the TLC plate. Lower fraction (C2) recorded essential oils with low to medium migration power (Table 4.5 and Plate 4.14).

No fraction of *E. alata* tested positive for the saponins.

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Table 4.5 *E. alata* alkaloids, bitter tasting substances and essential oils

Fraction / Standard	Alkaloids	R_f value	Band colour
A, B1	Corytaberine	0.34	Orange brown
A, B1, C2, D2, C1P	minovincine	0.79	Brown bands
Standard	Atropine (At)	0.31	Orange brown
Fraction / Standard	Bitter tasting substances	R_f value	Band colour
A, B1, C1, C1S	Cynaropicrin	0.31	Violet
C1S	Gentiopicroside	0.41	Violet
A, B1, C1,	Foliamenthrin	0.64	Blue
A, B1, C1, C1S	Curcurbitacin aglycones	0.85	Yellow brown
Standard	Glucose (G)	0.32	Grey
Fraction / Standard	Essential oils	R_f value	Band colour
C2 D1	Linalool	0.17	Blue zone
C2, D2	Borneol	0. 23	Violet blue
C2	Menthol	0.30	Red violet
C2	Cineole	0.39	Blue
D2	Linalyl acetate	0.59	Blue zone
Standard	Thymol (T)	0.77	Red-violet

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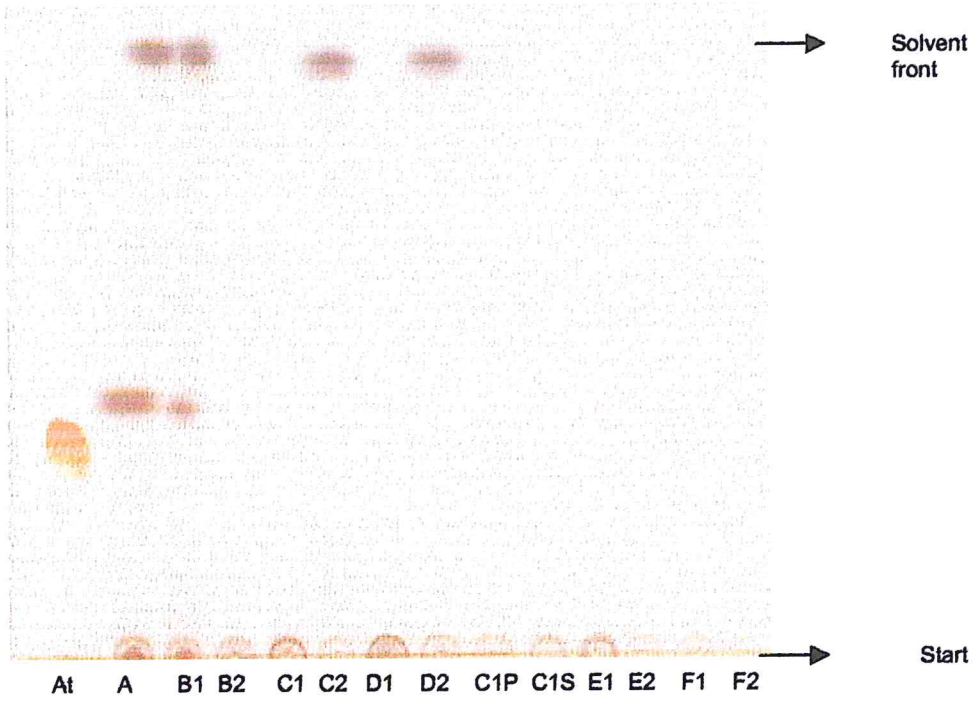

Plate 4.12 *E. alata* alkaloids

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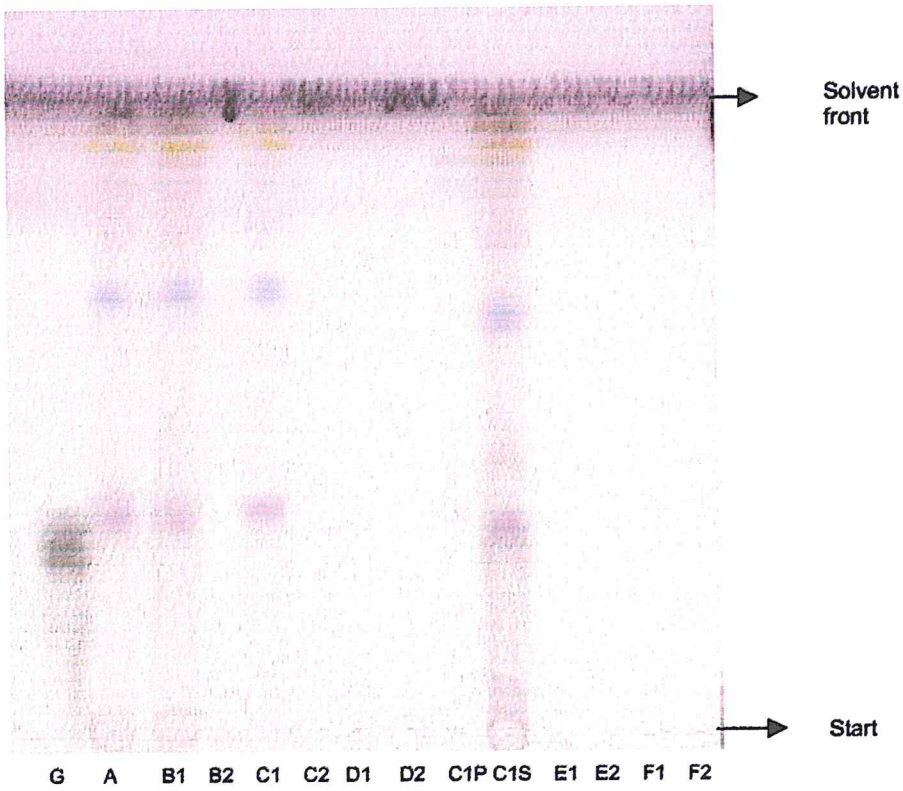

Plate 4.13 *E. alata* bitter tasting substances

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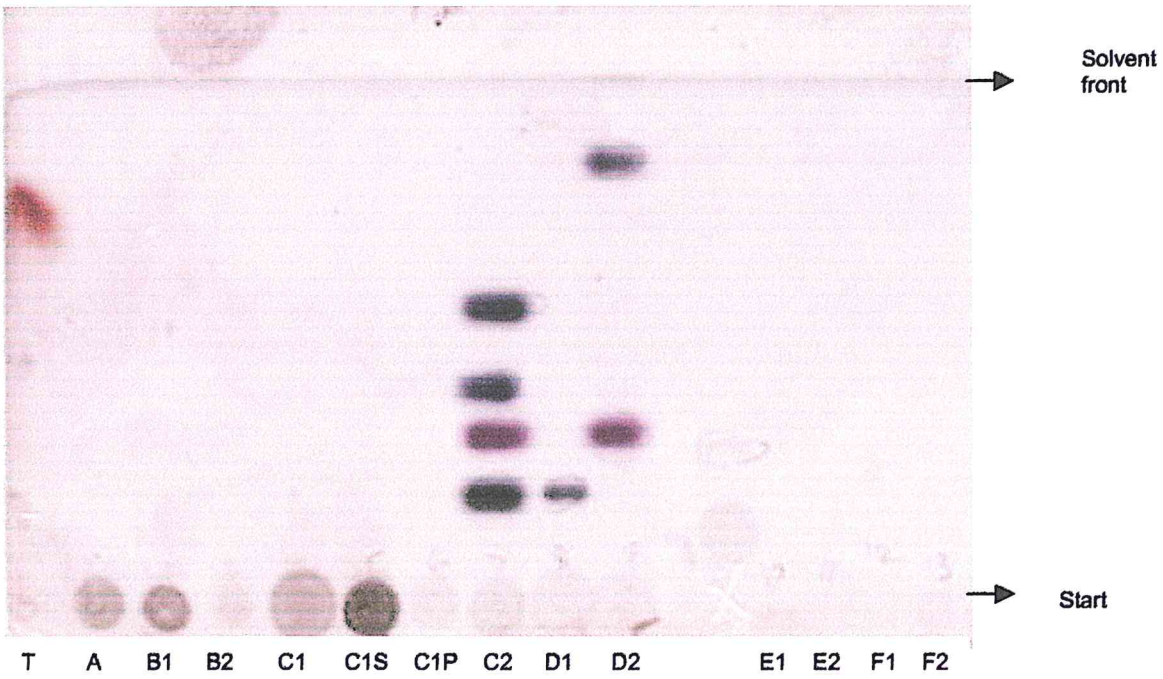

Plate 4.14 *E. alata* essential oils

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4.3.5 *Eucalyptus spp*

Out of the 13 fractions of *Eucalyptus sp*, fractions A, B2 and C2 tested positive for alkaloids (Table 4.6 and Plate 4.15). Fraction B2 and C2 recorded two types of alkaloids compared to the crude extract, which recorded one type (Table 4.6 and Plate 4.15). Berberine characterized by retention factor of 0.39 and yellow colour band was recorded in fractions A, B2 and C2 (Table 4.6 and Plate 4.15). Harmaline, characterized by retention factor of 0.57 and brown colour band was recorded in fractions B2 and C2 (Table 4.6 and Plate 4.15). Besides recording harmaline, C2 contained monovincine that was characterized by a retention factor of 1 and brown colour band (Table 4.6 and Plate 4.15). All the fractions of *Eucalyptus spp* that contained alkaloids were those extracted through the aqueous phase pathway (Table 4.6 and Plate 4.15). Fraction B2 recorded two types of alkaloids, hence making it the best fraction in terms of recording a variety of alkaloids (Table 4.6 and Plate 4.15).

Fractions A, B1, C1 and C1P of *Eucalyptus spp* tested positive for the bitter tasting substances out of the 13 fractions (Table 4.6 and Plate 4.16). Condurangins, quassin and dihydrofoliamenthin characterized by retention factor of 0.35, 0.64, 0.69 and violet colour band and minor blue, respectively, were recorded by fractions A, B1, C1 and C1P (Table 4.6 and Plate 4.16). Fraction A, B1 and C1P recorded cutapol characterized by retention factor of 0.20 and violet colour band (Table 4.6 and Plate 4.16). Condurangins, quassin and

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dihydrofoliamenthin were the most common types of bitter tasting substances recorded by fractions of *Eucalyptus spp* (Table 4.6 and Plate 4.16). Fractions A, B1 and C1P recorded 4 types of bitter tasting substances compared to fraction C1 which recorded 3 (Table 4.6 and Plate 4.16). Fractions extracted through the aqueous phase pathway recorded the highest number of bitter tasting substances compared to those of the organic phase (Table 4.6 and Plate 4.16).

Fractions A, B1, B2, C2, D1, D2 and C1P of *Eucalyptus spp* were found to contain essential oils out of the 13 fractions submitted for analysis (Table 4.6 and Plate 4.17). Alpha santonin, Bisabolol oxide, Piperitone and Spathulenol were the most common types of essential oils recorded in *Eucalyptus sp* fractions followed in THC compounds while Xanthoruhizol was the least common recorded only in fraction C1P (Table 4.6 and Plate 4.17). Alpha santonin characterized by its retention factor of 0.14 and blue colour band was recorded in fractions A, B1, B2, D1, D2 (Table 4.6 and Plate 4.17). Bisabolol oxide characterized by retention factor of 0.26 and green colour band was also recorded in the same fractions (Table 4.6 and Plate 4.17).

Piperitone characterized by a retention factor of 0.53 and orange red colour band was recorded by fractions B1, B2, C2, D1 and D2 (Table 4.6 and Plate 4.17). Fractions A, B2, C2, and D2 recorded the THC compounds forming at the solvent front with dark blue colour band (Table 4.6 and Plate 4.17).

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Fraction C1P is the only one, which contained Xanthoruhizol essential oil characterized by a retention factor of 0.83 and blue Violet colour bands (Table 4.6 and Plate 4.17). Fractions B2 and D2 recorded the highest number of different essential oils, while fraction C1P recorded the lowest number (Table 4.6 and Plate 4.17). Fractions A, B1, and D1 recorded the same number of essential oils (Table 4.6and Plate 4.17). Fractions extracted through the organic phase pathway recorded the highest number of essential oils compared to those of the aqueous phase (Table 4.6 and Plate 4.17).

No fraction out of the 13 fractions of *Eucalyptus spp* subjected to saponins screening tested positive (Plate 4.18).

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Table 4.6 *Eucalyptus spp* alkaloids; bitter tasting substances essential oils and standards

Fraction / Standard	Alkaloids	R_f value	Band colour
A, B2, C2	Berberine	0.39	Yellow
B2, C2	Harmaline	0.57	Brown
C2	Monovincine	1	Brown
Standard	Atropine (At)	0.33	Orange brown
Fraction / Standard	Bitter tasting substances	R_f value	Band colour
A, B1, C1P	Cutapol	0.20	Violet
A, B1, C1, C1P	Condurangins	0.35	Violet
A, B1, C1 C1P	Quassin	0.64	Violet
A, B1, C1, C1P	Dihydrofoliamenthin	0.69	Minor blue
Standard	Glucpse (G)	0.37	Grey
Fraction / Standard	Essential oils	R_f value	Band colour
A, B1, B2, D1, D2	Alpha santonin	0.14	Blue
A, B1, B2, D1, D2	Bisabolol oxide	0.26	Green
B1, B2, C2, D1, D2	Piperitone	0.53	Orange Red
A, B1, B2, D1, D2	Spathulenol	0.35	Violet
C1P	Xanthoruhizol	0.83	Blue Violet
A, B2, C2, D2	THC compounds	Solvent front	Dark blue colour
Standard	Thymol (T)	0.64	Red-violet
Standard	Cineole (Cin)	0.59	Blue

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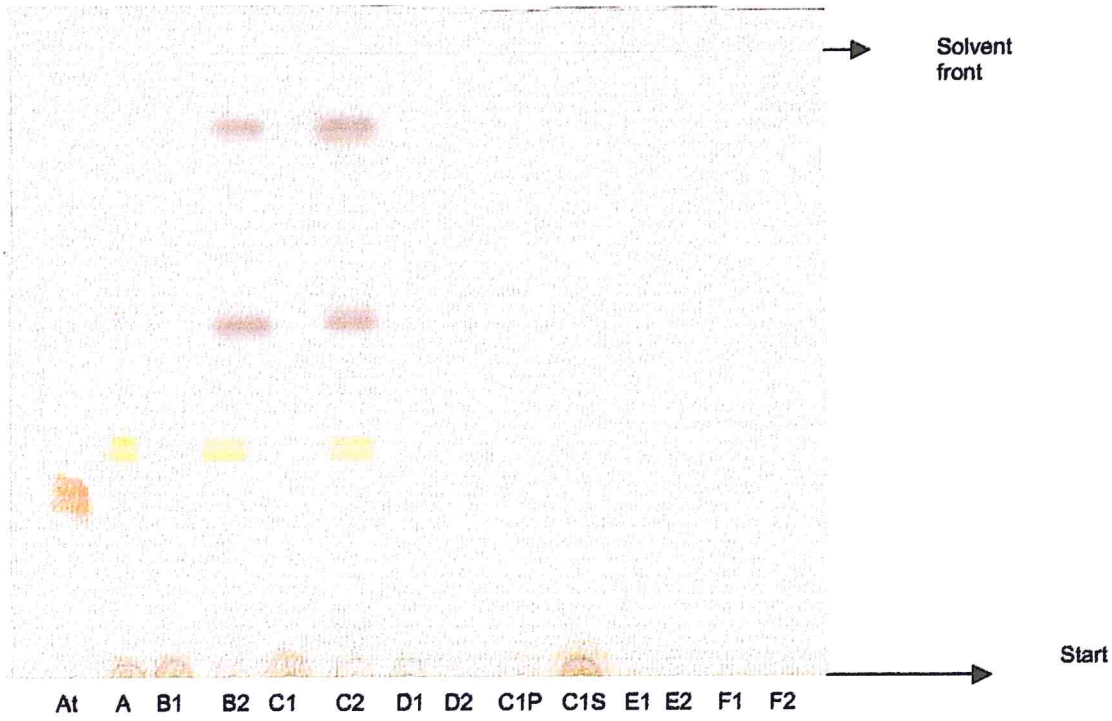

Plate 4.15 *Eucalyptus spp* alkaloids

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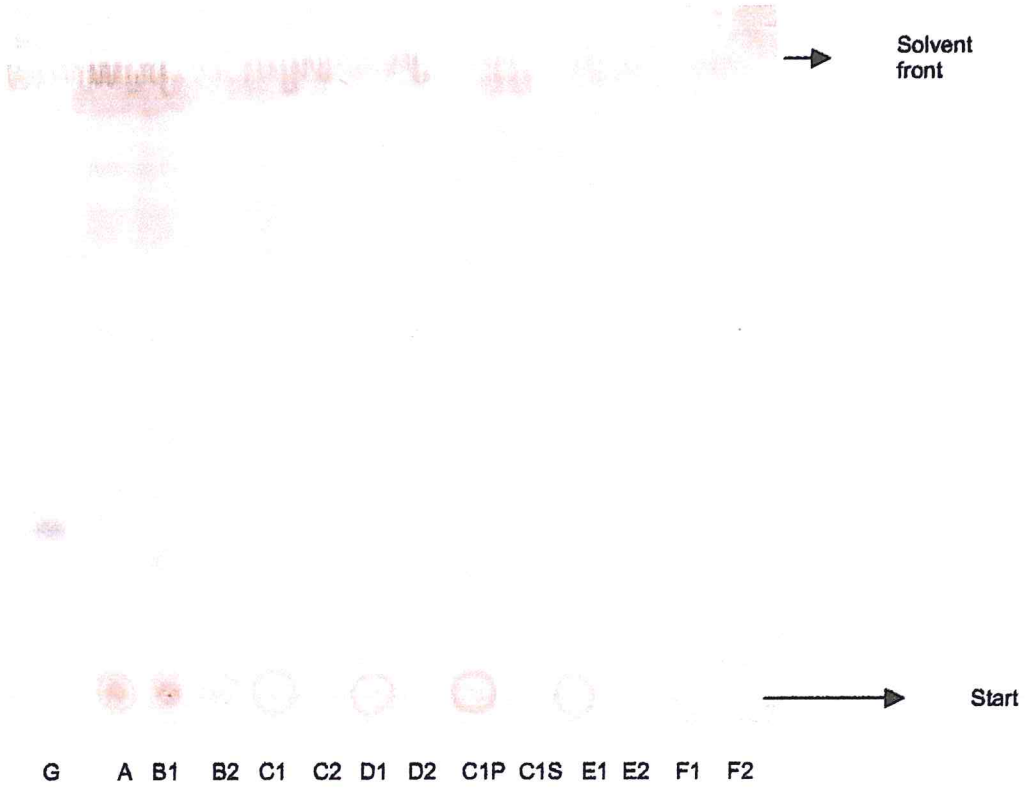

Plate 4.16 *Eucalyptus spp* bitter tasting substances

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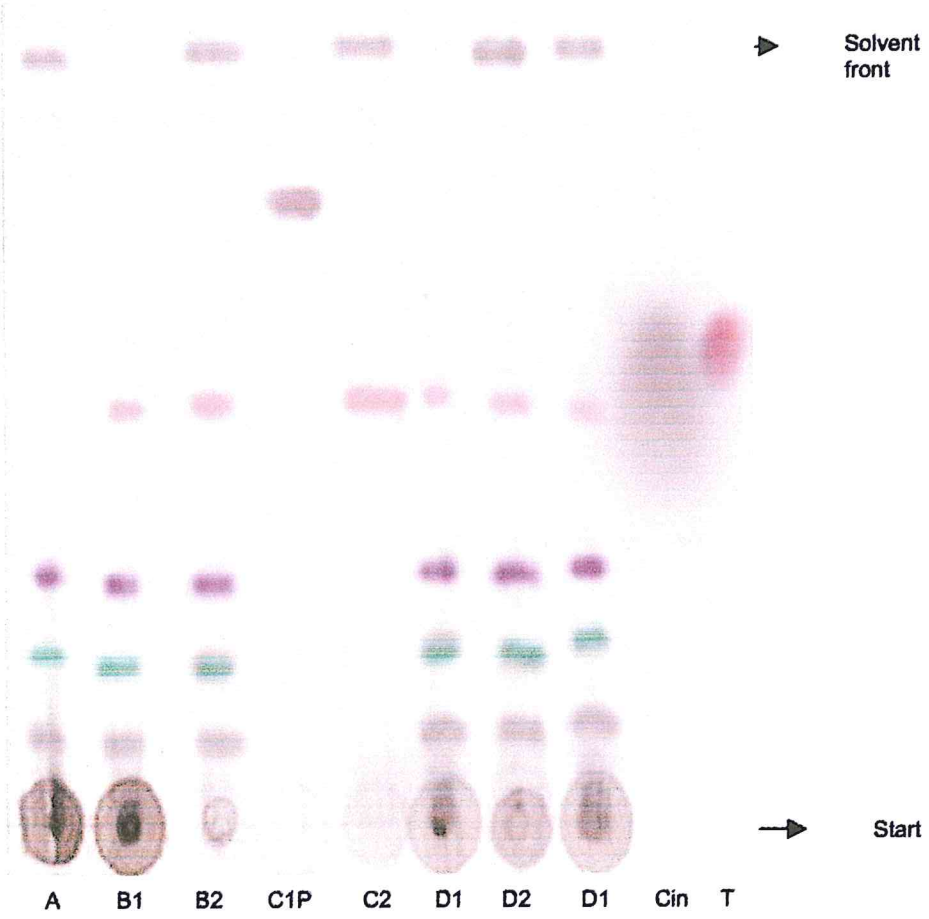

Plate 4.17 *Eucalyptus spp* essential oils

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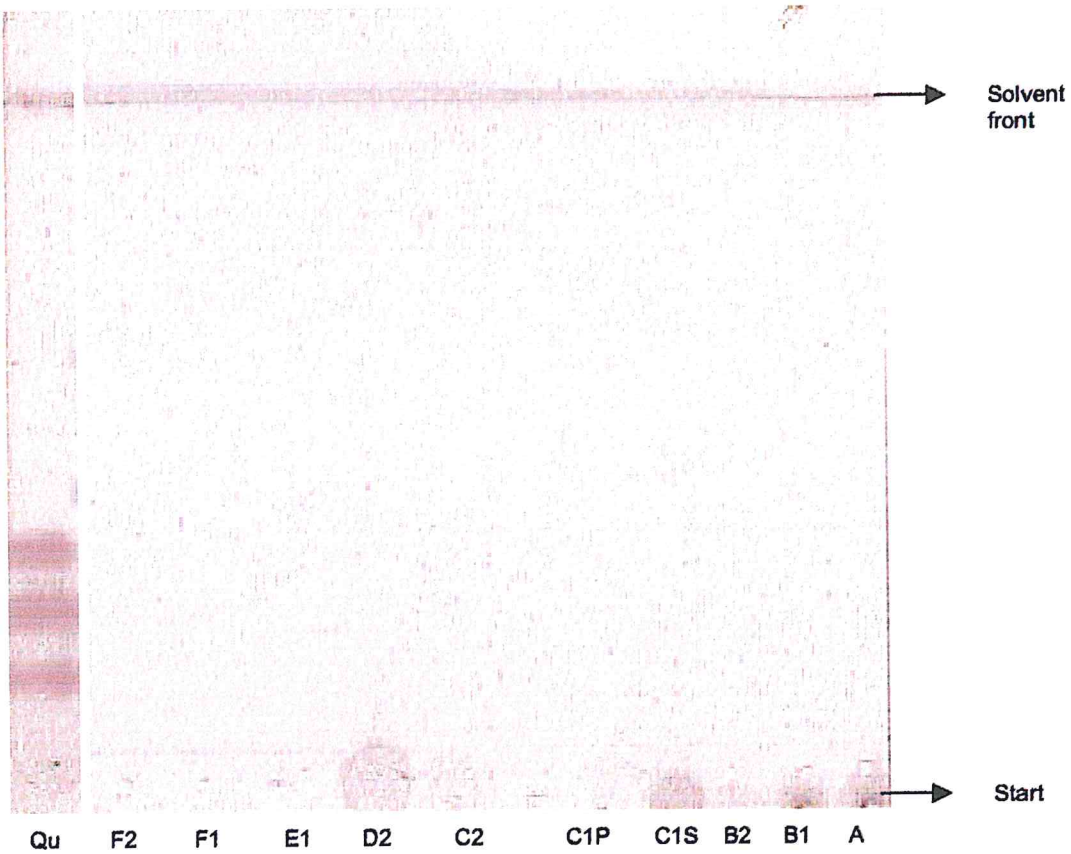

Plate 4.18 *Eucalyptus spp* saponins

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4.3.6 *Friesodielsia obovata*

Crude extract and the derivative fractions of *Friesodielsia obovata* recorded the highest number of different alkaloids compared to the other repellent plants. Out of the 13 fractions submitted for the alkaloids analysis fractions A, B1, B2, C1, C2, C1P, C1S and F2 recorded different types of alkaloids (Table 4.7and Plate 4.19). Crude extract recorded all the types of alkaloids recorded by the derivative fractions of this plant (Table 4.7and Plate 4.19). The most common type of alkaloid was Chelidonine characterized by a retention factor of 0.78 and blue violet colour band; it was recorded by fractions A, B1, B2, C1P, C2 and F2 (Table 4.7and Plate 4.19). While papaverine characterized by retention factor of 0.38 retention factor and weak brown zones was the least common (Table 4.7 and Plate 4.19), and it was recorded by fractions A and C1P (Table 4.7 and Plate 4.19).

Fractions C1S and C1 did not record chelidonine but by both; while aglycone with its retention factor 0.91 and yellow colour band forming at the solvent front was recorded by fraction C1 only (Table 4.7and Plate 4.19). Fraction B1 was the second best from the crude extract in terms of recording different types of alkaloid while fraction F2 recorded only one type of alkaloid which was the most common (Table 4.7and Plate 4.19). The second most common type of alkaloid recorded was strychine and aglycone and they were recorded by fractions A, B1, C1, C1S and C1P (Table 4.7 and Plate 4.19). Fractions derived from the aqueous phase

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recorded the highest number of different types of alkaloids compared to fractions from the organic phase (Table 4.7 and Table 4.19). Fraction C1P derived from the aqueous phase and being a lower fraction

Fractions A, B1 C1 and C1S of *F. obovata* tested positive for the bitter tasting, out of the 13 fractions subjected analysis (Table 4.7 and Plate 4.20). Melittoside characterized by retention factor of 0.26 and colour band of grey was the only one recorded by the fractions, which tested positive (Table 4.7 and Plate 4.20). Apart from the crude extract (fraction A) all the other fractions that recorded bitter tasting substances were those extracted through the aqueous phase pathway (Table 4.7 and Plate 4.20).

Out of the 13 fractions of *F. obovata* submitted for essential oils analyses only fractions A, B1, B2, C1, C1P, F1 and F2 were found to contain essential oils (Table 4.7 and Plate 4.21). Citral characterized by a retention factor of 0.42 and violet colour band was the most common type of essential oil (Table 4.7 and Plate 4.21). Citral was recorded by fractions A, B1, B2, C1, F1 and C1P (Table 4.7 and Plate 4.21). Fractions A, B1, B2 and C1P recorded the THC compounds forming at the solvent front with a dark blue colour band (Table 4.7 and Plate 4.21).

Elemicin characterized by a retention factor of 0.39 and a red violet colour band was contained in fractions A, B1 and C1P (Table 4.7 and Plate 4.21).

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Nerol and linalool characterized by retention factor of 0.22, 0.32 respectively and blue colour band were recorded by fractions A, B1a and C1P (Table 4.7 and Plate 4.21). Alpha santonin characterized by retention factor of 0.13 and a blue colour band was recorded only in fraction C1P (Table 4.7 and Plate 4.21). Fraction A and C1P recorded the highest number of essential oils compared to the other fractions obtained from *F. obovata*.

Fraction A recorded all the types of essential oils with an exception of alpha santonin, which was contained in fraction C1P and F2 (Table 4.7 and Plate 4.21), while Fraction C1P recorded all types of essential oils except nerol that was contained in fraction A and B (Table 4.7 and Plate 4.21). Fractions C1, F1 and F2 recorded only one type of essential oil citral and alpha santonin, respectively (Table 4.7 and Plate 4.21). It is worthy noting that lowest fractions of *F. obvata* (F1 and F2) recorded essential oils (Table 4.7 and Plate 4.21). Fractions derived from the organic phase pathway recorded a total of 8 different types of essential oils compared to 6 recorded by the aqueous phase fractions (Table 4.7 and Plate 4.21).

Out of the 13 fractions of *F. obovata* submitted for the saponins screening only fractions A, B1, B2 and C1S tested positive (Table 4.7 and Plate 4.22*)*. *Friesodielsia obvata* recorded a total of three different types of saponins (Table 4.7 and Plate 4.22*)*. Gingenosides characterized by a retention factor of 0.05-0.15 and a colour band of red was recorded by fractions A, B1, B2, C1S (Table 4.7 and

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Plate 4.22). Aescinols recorded in fractions A, B1, B2 and C1S was characterized by a retention factor of 0.4-0.6 and colour band of brown (table 6 and TLC plate 6), while hederin with its retention factor of 0.7-0.8 and a colour band of dark-grey-blue was recorded in fractions A, B1, B2 and C1S (Table 4.7 and Plate 4.22). Fractions A, B1, B2 and C1S recorded the same type of type of saponin (Table 4.7 and Plate 4.22). None of the fractions that tested positive had a type of saponin close to the quillaja the standard saponin (Table 4.7 and Plate 4.22).

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Table 4.7 *F. obovata* alkaloids; bitter tasting substances, essential oils and saponins

Fraction / Standard	Alkaloids	R_f value	Band colour
A, B1, C1, C1S, C1P	Strychine	0.26	Brown zones
A, B1, B2	Cinchonidine	0.5	Grey violet
A, B1, B2, C1P, C2, F2	Chelidonine	0.78	Blue violet
A, B1, B2, C1, C1P	Aglycone	0.91	Yellow-solvent front
A, B1, C1S	Isorhychnophylline	0.74	Orange brown
A, C1P	Papaverine	0.38	Weak brown zones
Standard	Atropine (At)	0.33	Orange brown
Fraction / Standard	Bitter tasting substances	R_f value	Band colour
A, B1 C1 C1S	Melittoside	0.26	Grey
Standard	Glucose (G)	0.41	Grey
Fraction / Standard	Essential oils	R_f value	Band colour
A, B1,	Nerol	0.22	Blue zone
A, C1P	Linalool	0.32	Blue zone
A, B1, C1P	Elemicin	0.39	Red violet
A, B1, B2, C1, F1, C1P	Citral	0.42	Violet
A, B1, B2, C1P	THC compounds	Solvent front	Dark blue colour
C1P, F2	Alpha santonin	0.13	Blue
Standard	Thymol (T)	0.79	Red-violet
Fraction / Standard	Saponins	R_f value	Band colour
A, B1, B2, C1S	Gingenosides	0.05-0.15	Red
A, B1, B2, C1S	Aescinols	0.4-0.6	Brown
A, B1, B2, C1S	Hederin	0.7-0.8	Dark-grey-blue
Standard	Quillaja (Qu)	0.14-0.31	Brown

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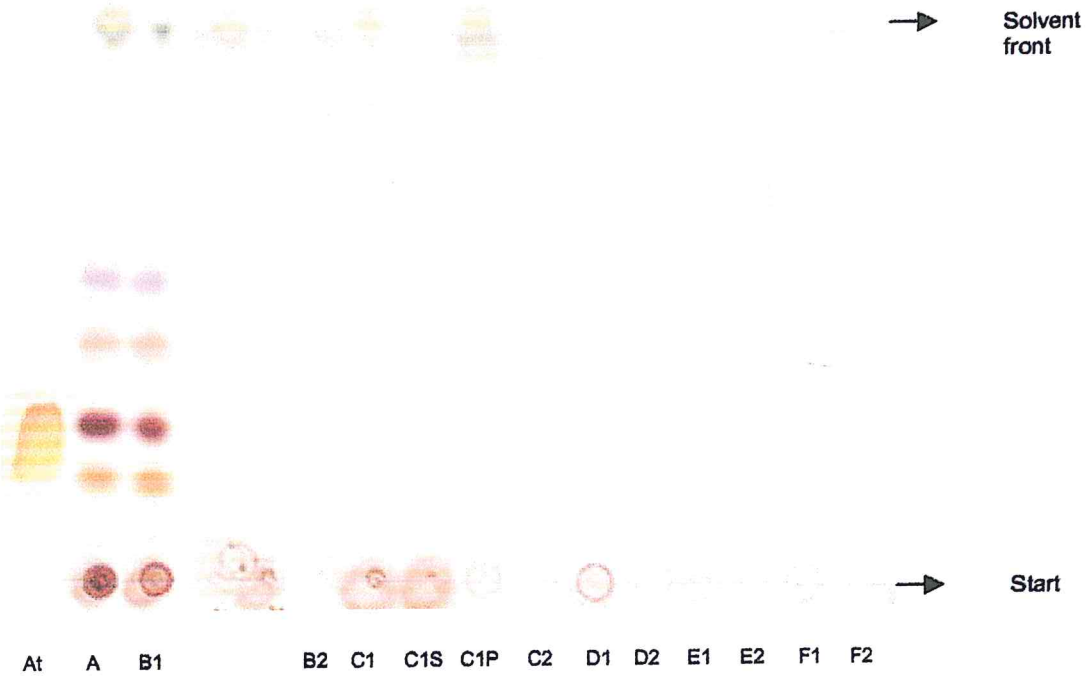

Plate 4.19 *F. obovata* alkaloids

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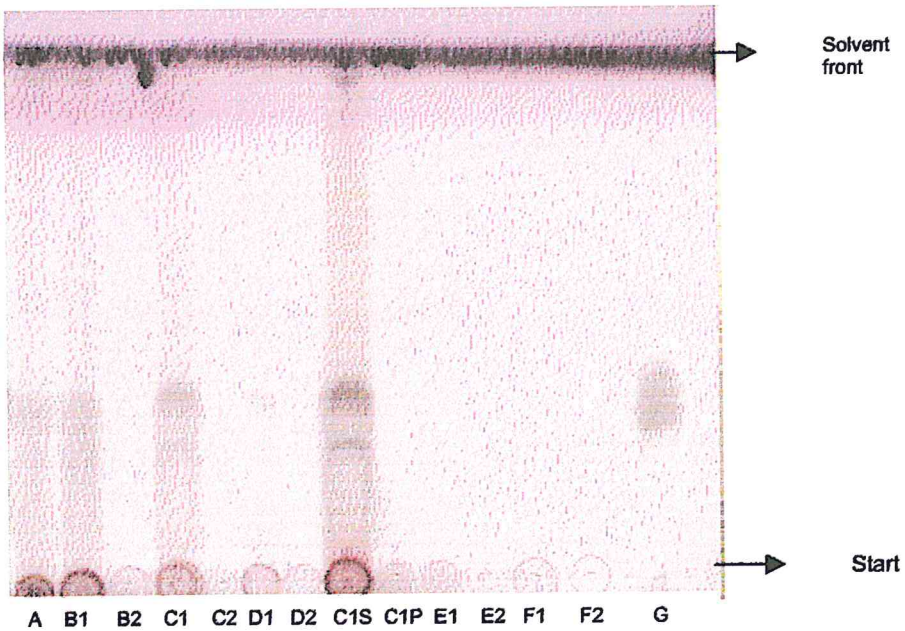

Plate 4.20 *F. obovata* bitter tasting substances

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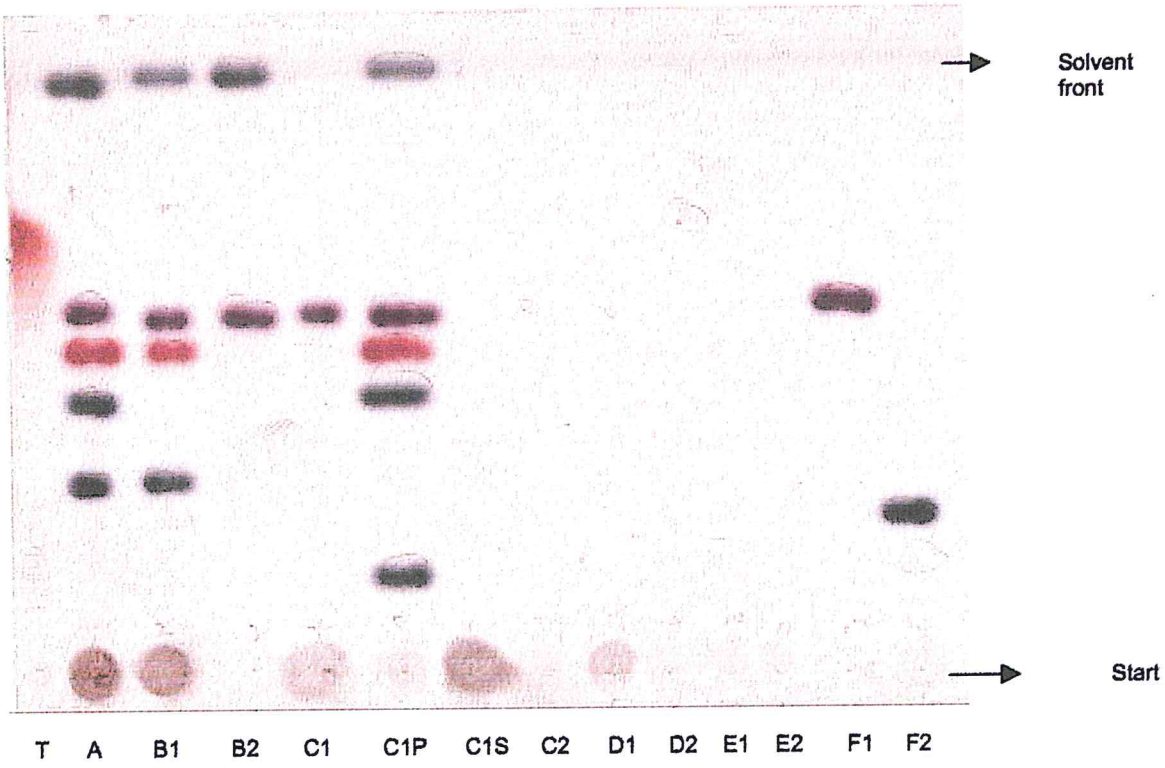

Plate 4.21 *F. obovata* essential oils

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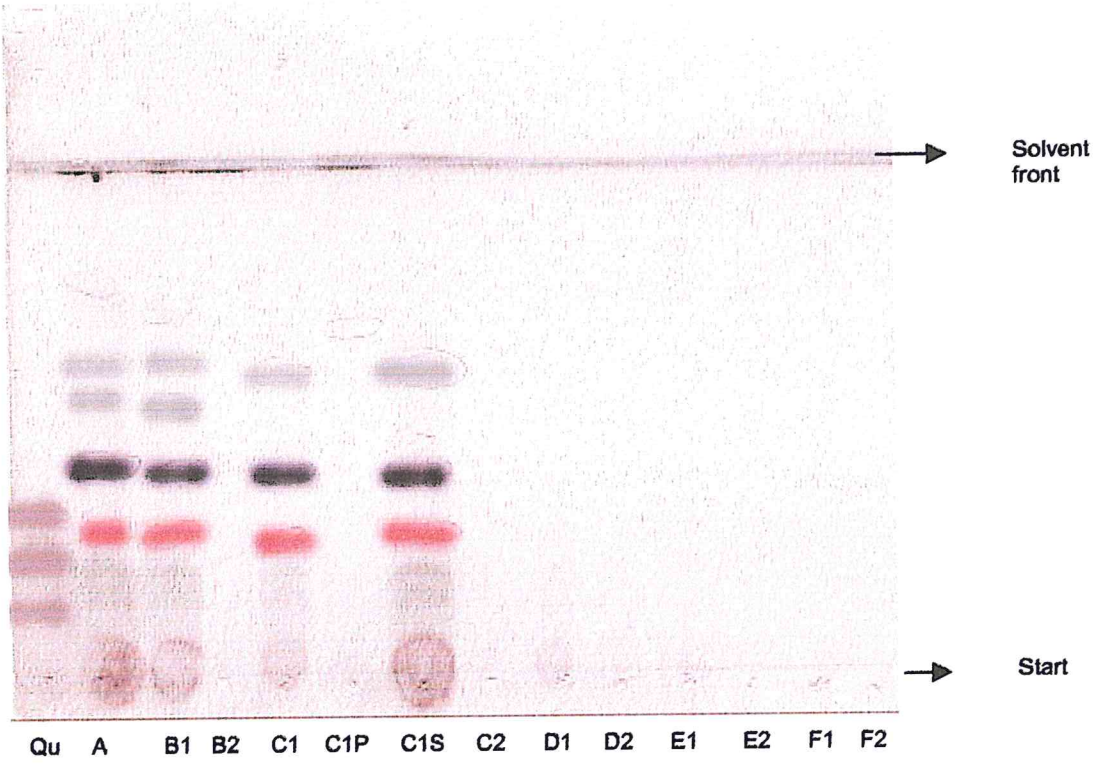

Plate 4.22 *F. obovata* saponins

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4.3.7 *Lippia javanica*

Crude extract (fraction A), B1, B2 and D1 are the fractions of *L. javanica* that tested positive for the alkaloids (Table 4.8and Plate 4.23). Crude extract and fraction B2 contained all the types of alkaloids recorded by *L. javanica* (Table 4.8 and Plate 4.23). Brucine and aconitine characterized by retention factor of 0.53, 0.6 and orange, brown colour band respectively was recorded by fractions A and B2 (Table 4.8 and Plate 4.23). Fractions A, B1 and B2 contained nornicotine, characterized by retention factor of 0.40 and orange brown colour band (Table 4.8 and Plate 4.23). Chelidonine characterized by a retention factor of 0.78 and blue violet colour band was recorded by fractions 0.78 (Table 4.8 and Plate 4.23). Fractions from the organic phase recorded higher proportion of alkaloids compared to those extracted through the aqueous phase pathway (Table 4.8 and Plate 4.23).

Out of the 11 fractions of *L. javanica* subjected to bitter tasting substances analysis, fractions A, B1 and C1 tested positive (Table 4.8 and Plate 4.24). Fractions that tested positive recorded the four types of bitter tasting substances (Table 4.8 and Plate 4.24). With an exception of fraction A (crude extract), fractions extracted through the aqueous phase pathway are the only ones, which recorded bitter tasting substances (Table 4.8 and Plate 4.24). Retention factor of Verbascosapine, Cutapol, Menthiafolin and Foliamenthin was 0.20, 0.27, 0.61 and 0.69 while the colour bands were blue grey, violet, minor blue and dark blue

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respectively (Table 4.8 and Plate 4.24). Comparison with the other plants *L. javanica* positive fractions recorded uniform bitter taste substances (Table 4.8 and Plate 4.24).

Out of the 11 fractions from *L. javanica* subjected for essential oils analysis only fractions A, B1 B2, C2 D1 and D2 were found to contain different types of essential oils (Table 4.8and Plate 4.25). The most common type of essentials recorded by fractions of L. *javanica* was Alpha santonin and Piperitone. While Bisabolol oxide and Borneol were the least common types of essential oils recorded (Table 4.8and Plate 4.25). Alpha santonin characterized by a retention factor of 0.14 and blue colour band was recorded by fractions A, B1 and C2 (Table 4.8 and Plate 4.25).

Fractions A, B1 and B2 recorded Piperitone which was characterized by retention factor of 0.53 and orange red band (Table 4.8 and Plate 4.25). Spathulenol, Neryl acetate and Geranyl acetate were the second common types of essential oils recorded by *L. javanica* fractions (Table 4.8 and Plate 4.25). Spathulenol characterized by its retention factor of 0.35 and violet colour band was present in fractions B1 and D2 (Table 4.8 and Plate 4.25). Neryl acetate with its retention factor of 0.85 and a violet colour band was recorded by fractions A and D2 (Table 4.8 and Plate 4.25). Geranyl acetate with its retention factor of 0.81 and a Blue band was recorded by fractions A, B2 and D1 (Table 4.8 and Plate 4.25).

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Fraction A is the only fraction that recorded Bisabolol oxide which was characterized by a retention factor of 0.26 and green colour band (Table 4.8 and Plate 4.25). Fraction B2 recorded Borneol which was characterized by a retention factor of 0.31 and violet blue colour band (Table 4.8and Plate 4.25). Most of the essential oils were recorded by fraction derived from the organic phase pathway (Table 4.8 and Plate 4.25). Six different types of essential oils were recorded by organic phase fractions, compared to only four on the aqueous phase fractions (Table 4.8 and Plate 4.25). The highest number of different types of essential oils was recorded by fraction A (five types), seconded by fraction B1 having three types (Table 4.8 and Plate 4.25). Fraction D1 recorded only one type of essential oil (Table 4.8 and Plate 4.25).

The only fraction of *L. javanica* that recorded saponins is fraction C1 (Table 4.8 and Plate 4.26). The three types of saponins recorded by fraction C1 are chlorogenic, quillaic, hederin and parillin (Table 4.8 and Plate 4.26). Quillaic acid and hederin were characterized by retention factor of 0.69, 0.80 and dark blue colour band respectively (Table 4.8and Plate 4.26). Chlorogenic was characterized by a retention factor of 0.45 and blue colour band (Table 4.8 and Plate 4.26), while Parillin was characterized by retention factor of 0.80 and yellow-brown colour band (Table 4.8 and Plate 4.26). Recording of saponins by lower fraction C1 suggests that they were not affected by the extraction procedures involved.

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Table 4.8 *L. javanica* alkaloids, bitter tasting substances, essential oils and saponins

Fraction / Standard	Alkaloids	R_f value	Band colour
A, B1, B2	Nornicotine	0.35-0.40	Orange-brown
A, B2	Brucine	0.53	Orange
A, B2	Aconitine	0.6-0.75	Brown
A, B2, D1	Chelidonine	0.78	Blue-violet
Fraction / Standard	Bitter tasting substances	R_f value	Band colour
A, B1, C1	Verbascosapine	0.20	Blue grey
A, B1, C1	Cutapol	0.27	Violet
A, B1, C1	Menthiafolin	0.61	Minor Blue
A, B1, C1	Foliamenthin	0.69	Dark blue
Standard	Glucose (G)	0.37	Grey
Fraction / Standard	Essential oils	R_f value	Band colour
A, B1 and C2	Alpha santonin	0.14	Blue
A	Bisabolol oxide	0.26	Yellow green
B2	Borneol	0.31	Violet blue
A, B1 and B2	Piperitone	0.53	Orange Red
B1 and D2	Spathulenol	0.35	Violet
A and D2	Neryl acetate	0.85	Violet
A, B2 and D1	Geranyl acetate	0.81	Blue
Standard	Thymol (T)	0.63	Red-violet
Standard	Cineole (Cin)	0.54	Blue
Fraction / Standard	Saponins	R_f value	Band colour
C1	Chlorogenic	0.45	Blue
C1	Quillaic acid	0.10	Dark-blue
C1	Hederin	0.69	Dark grey
C1	Parillin	0.80	Yellow-brown
Standard	Quillaja (Qu	0.14-0.31	Brown

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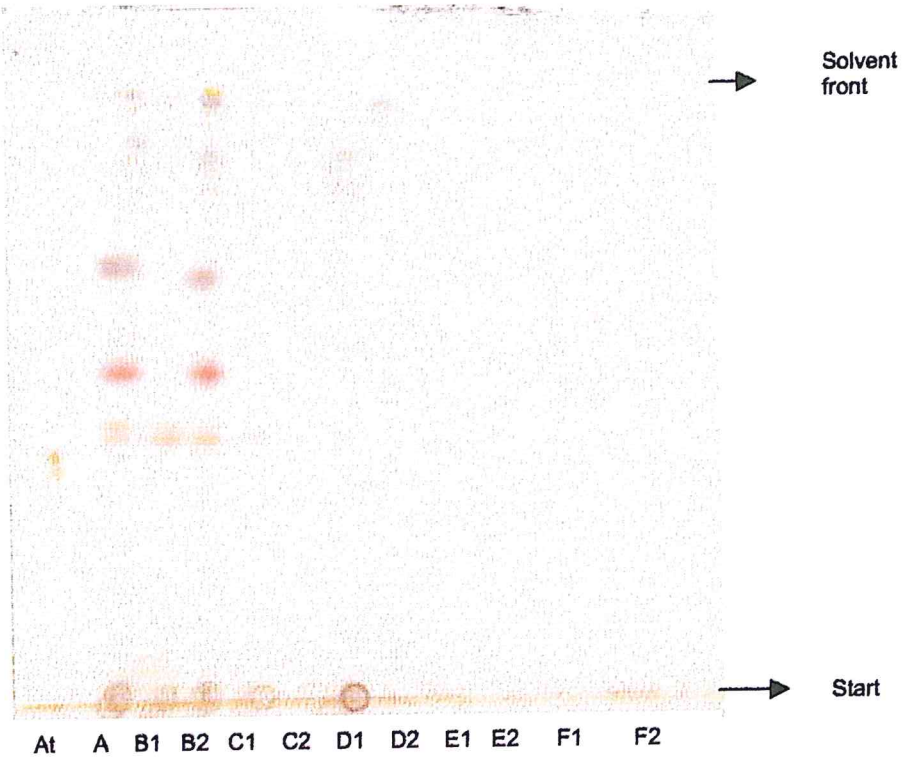

Plate 4.23 *L. javanica* alkaloids

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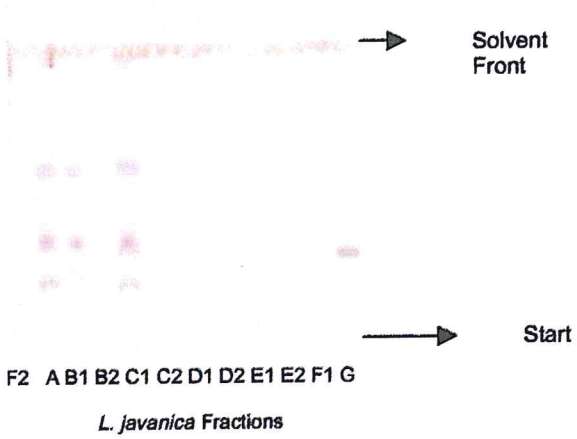

Plate 4.24 *L. javanica* bitter tasting substances

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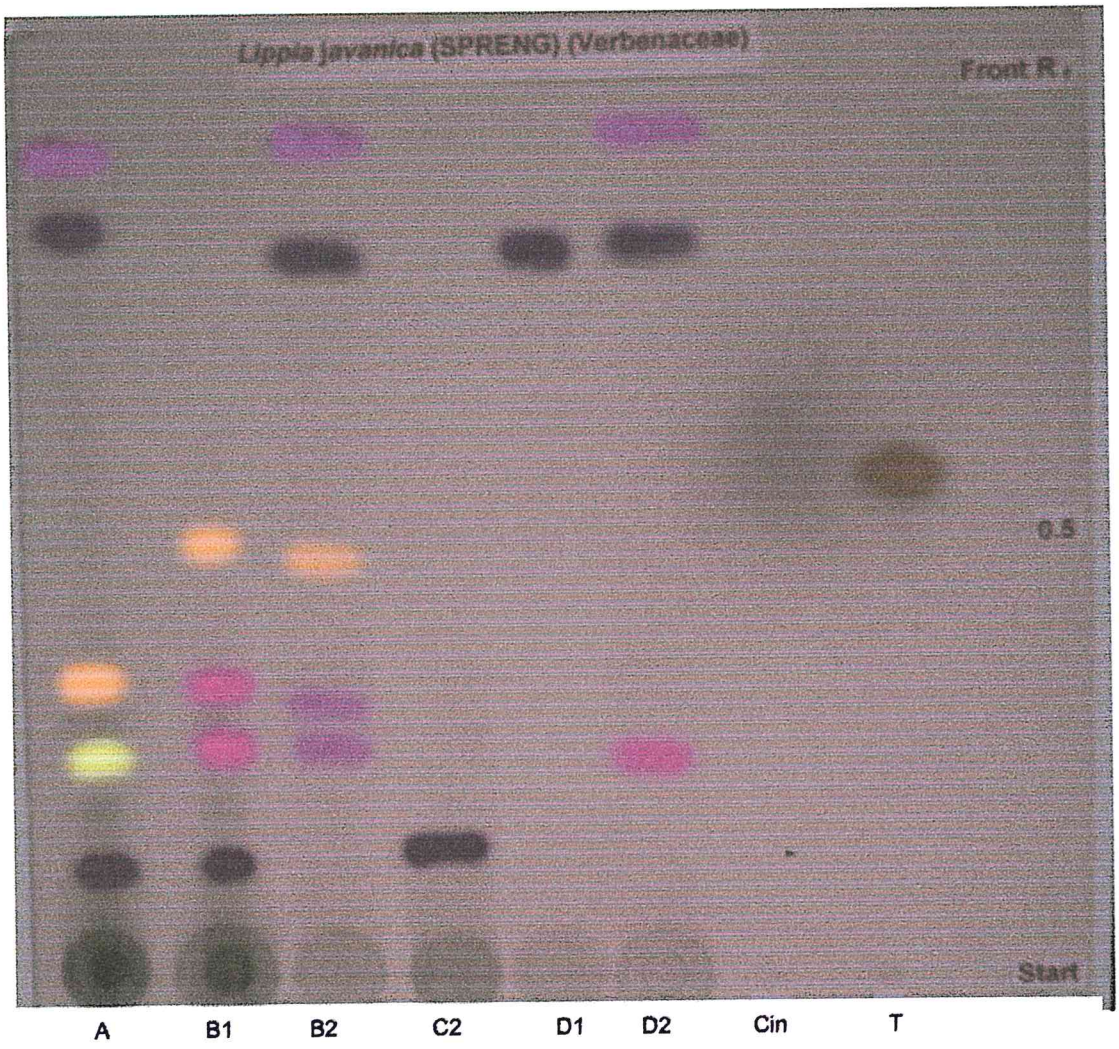

Plate 4.25 *L. javanica* essential oils

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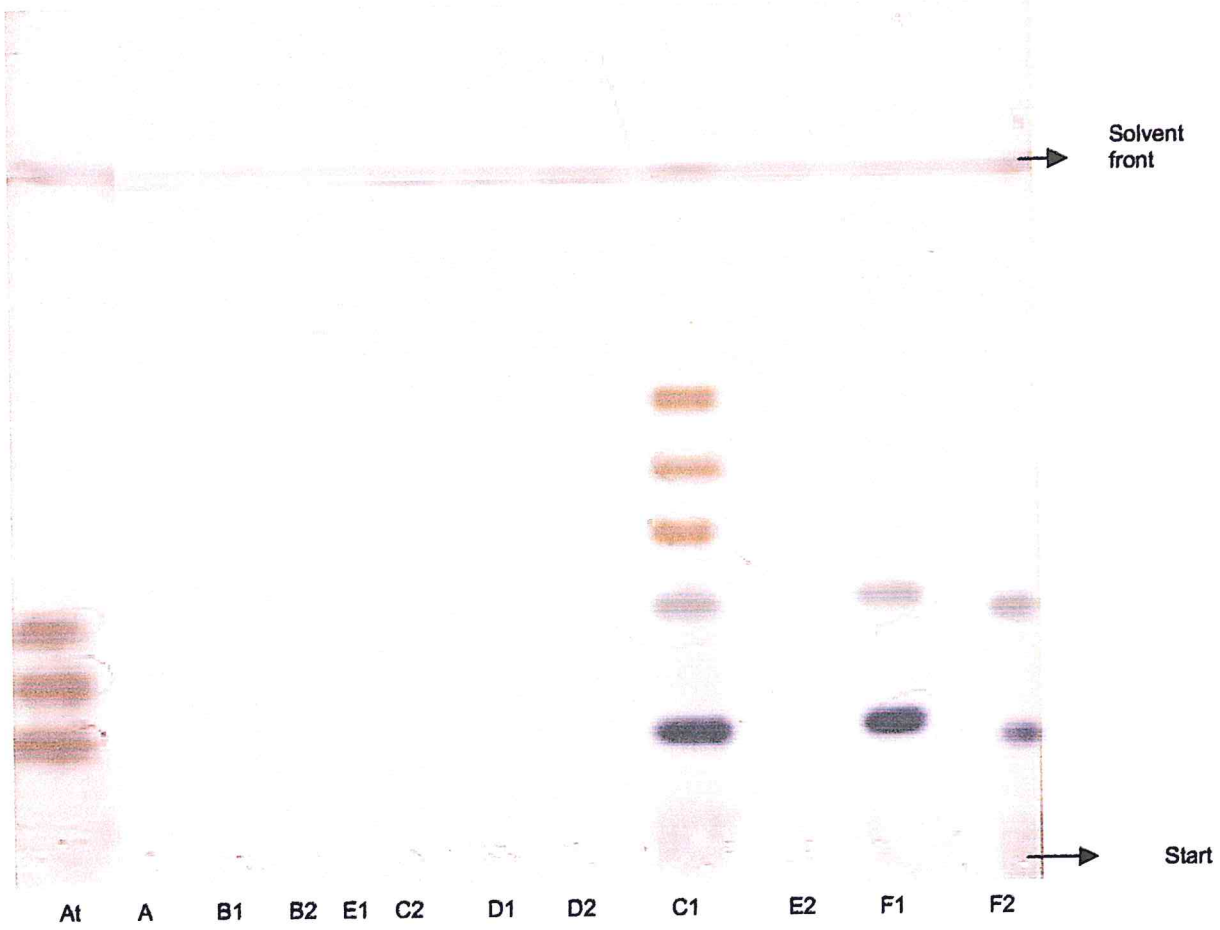

Plate 4.26 *L. javanica* saponins

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4.3.8 *Ocimum urtifolium*

Fractions B2 and C2 are the only fractions of *O. urtifolium* that tested positive for the alkaloids (Table 4.9 and Plate 4.27). Minovincine characterized by retention factor of 0.79 and brown colour band was recorded by fraction B2 and C2 (Table 4.9 and Plate 4.27). Besides recording minovincine fraction B2 also recorded corytaberine (Table 4.9 and Plate 4.27), which was characterized by retention factor of 0.34 and orange-brown colour band (Table 4.9 and Plate 4.27). All the fractions found to contain alkaloids, were extracted through the organic phase pathway (Table 4.9 and Plate 4.27). This suggests that alkaloids from this plant can best be extracted through the organic phase pathway.

Out of the 11 fractions of *O. urtifolium* subjected for bitter tasting substances analysis only fractions A, B1 and C1 tested positive (Table 4.9 and Plate 4.28). Melittoside characterized by retention factor of 0.20 and colour band of grey was recorded by fractions A, B1 and C1 (Table 4.9 and Plate 4.28). Previously mentioned fractions recorded Aucubin, which was characterized by a retention factor of 0.30 and a colour band of grey (Table 4.9 and Plate 4.28). With an exception of fraction A (crude extract) Only fractions extracted through the aqueous phase tested positive (Table 4.9 and Plate 4.28).

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Out of the 11 fractions submitted for essential oils analysis only fractions A, B1 B2, C1, C2, F1 and F2 were found to contain essential oils (Table 4.9 and Plate 4.29a, b). Alpha santonin characterized by retention factor of 0.09 and blue zone band was recorded by 5 fractions compared to elemicin, THC compounds, borneol and mentho each recorded by only one fraction (Table 4.9 and Plate 4.29a, b). Elemicin characterized by a retention factor 0.39 and red violet colour band was recorded by fraction A (Table 4.9 and Plate 4.29a). Fraction A as well recorded the THC compounds forming at the solvent front with a violet blue colour band (Table 4.9 and Plate 4.29a).

Borneol and menthol characterized by a retention factor of 0.23, 0.30 and violet blue and blue band respectively were recorded by fraction C2 (Table 4.9 and Plate 4.29a). Fraction A recorded three different types of essential oils compared to fractions B1, C1, C2 and E2 which recorded only one type of essential oil ((Table 4.9 and Plate 4.29a). fraction F1 and F2 are the exceptional lower fractions that recorded essential oils (Table 4.9 and Plate 4.29b). Fractions extracted through the aqueous phase pathway recorded 4 different types of essential oils, those of the organic phase recorded only 1(Table 4.9 and Plate 4.29a).

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Out of the 11 fractions from *Ocimum urtifolium* subjected to saponins analysis only fractions A, B1 and C1 tested positive (Table 4.9 and Plate 4.30). These three fractions recorded quillaic acid characterized by a retention factor of 0.05-0.15 and a colour band of Dark-brown. Hiderin with its retention factor of 0.7-0.8 and dark-grey-blue colour band was recorded by fractions A, B1 and C1 (Table 4.9 and Plate 4.30). Same fractions recorded parillin which was characterized by a retention factor of 0.75 and yellow-brown colour band of (Table 4.9 and Plate 4.30). Results obtained show that only fractions extracted through the aqueous phase pathway were saponins (Table 4.9 and Plate 4.30).

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Table 4.9 *O. urtifolium* alkaloids, bitter tasting substances, essential oils and saponins

Fraction / Standard	Alkaloids	R_f value	Band colour
B2, C2	minovincine	0.79	Brown
B2	Corytaberine	0.34	Orange-brown
Fraction / Standard	Bitter tasting substances	R_f value	Band colour
A, B1, C1	Melittoside	0.20	Grey
A, B1, C1	Aucubin	0.30	Grey
Standard	Glucose (G)	0.32	Grey
Fraction / Standard	Essential oils	R_f value	Band colour
A, B1, C1, C2, E2	Alpha santonin	0.09	Blue zone
A	Elemicin	0.39	Red violet
A	THC compounds	Solvent front	Violet blue
C2	Borneol	0.23	Violet blue
C2	Menthol	0.30	Blue
Standard	Thymol (T)	0.79	Red-violet
Fraction / Standard	Saponins	R_f value	Band colour
A, B1, C1	Quillaic acid	0.05-0.15	Dark-brown
A, B1, C1	Hiderin	0.7-0.8	Dark-grey-blue
A, B1, C1	Parillin	0.75	Yellow-brown
Standard	Quillaja (Qu)	0.14-0.31	Brown

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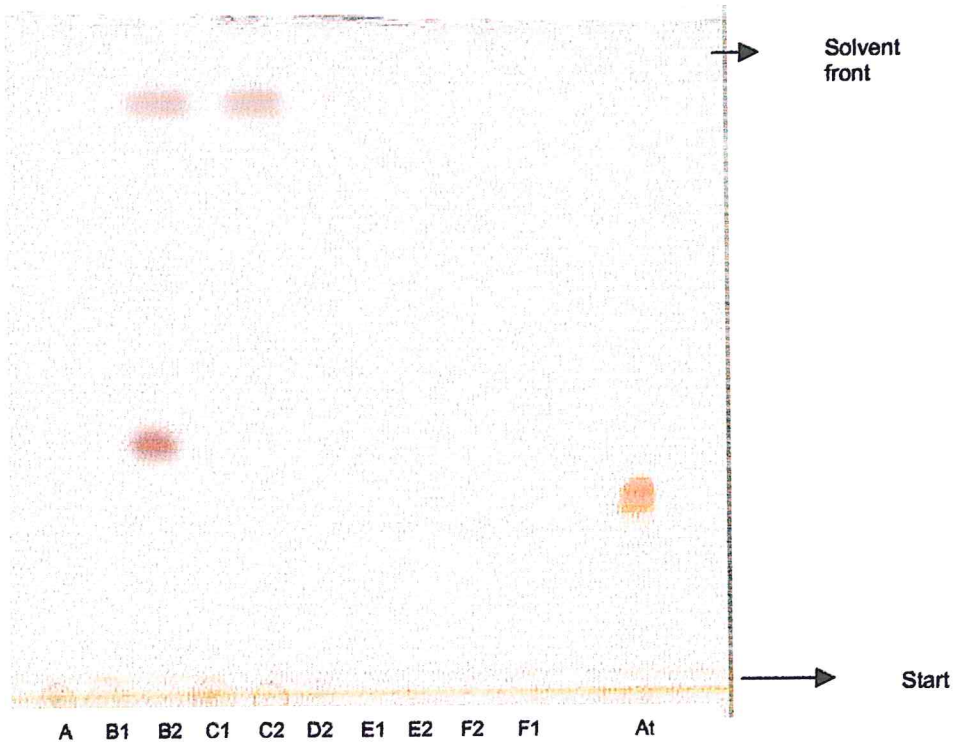

Plate 4.27 *O. urtifolium* alkaloids

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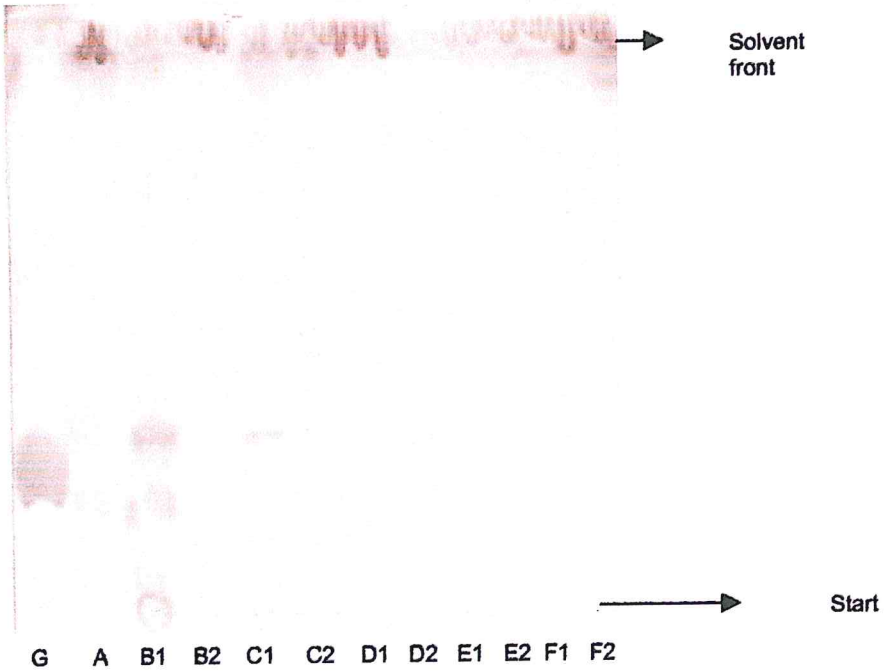

Plate 4.28 *O. urtifolium* bitter tasting substances

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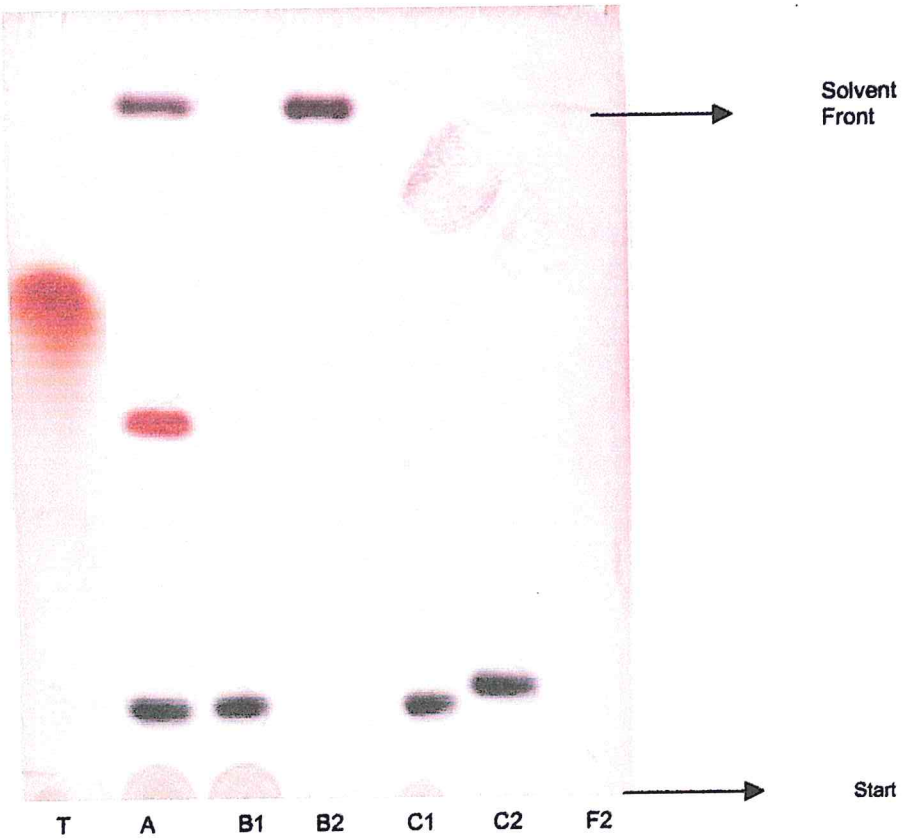

Plate 4.29a *O. urtifolium* essential oils

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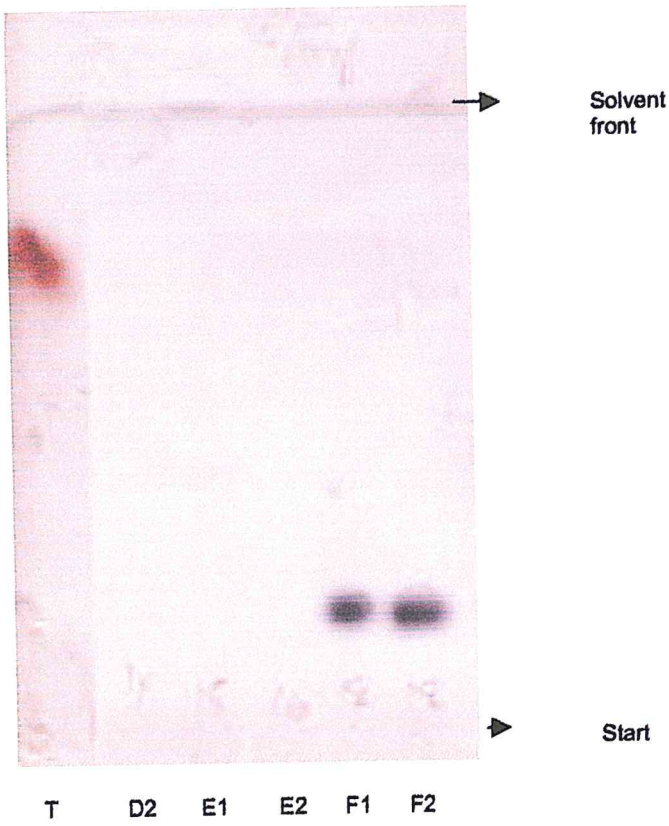

Plate 4.29b *O. urtifolium* essential oils

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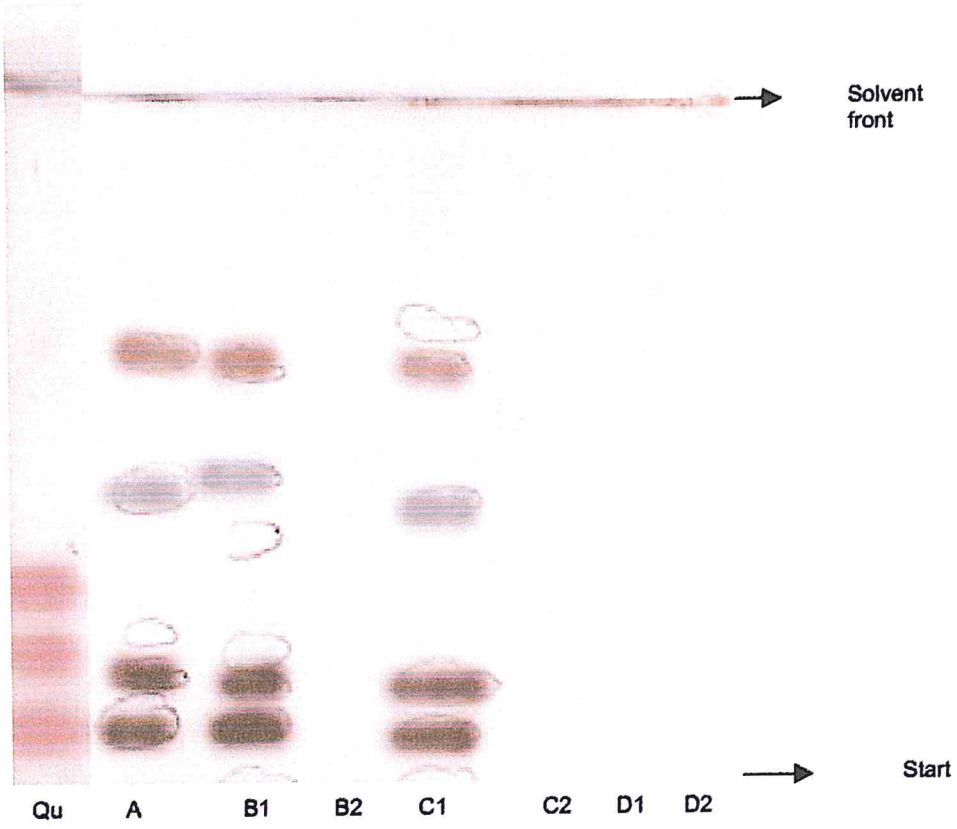

Plate 4.30 *O. urtifolium* saponins

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4.4 Residual effect experiment

Figure 4.12 shows the repellence of DEET and crude extracts of the plants in LSMEANS arcsine observed over the period of 0 to 6.5 hrs post application. The crude extract of *L. javanica* recorded the highest repellence compared to DEET and the rest of the crude extracts evaluated in this experiment (Figure 4.12 and Table 4.10). Repellence expressed by *L. javanica* varied significantly to that of DEET and other crude extracts over the period $P < 0.05$ (Figure 4.12 and Table 4.10). It was the best crude extract in terms of protection over that period (Figure 4.12).

The second best plants giving desirable protection were *F. obovata* and *Eucalyptus spp.* Repellence recorded by these plants was not significantly different to the control DEET ($P > 0.05$) (Figure 4.12 and Table 4.10). Third position was scored by *C. mopane* and *A. digitata,* which did not vary significantly from *Eucalyptus spp* ($P > 0.05$) (Figure 4.12 and Table 4.10). No significant difference existed between *Epaltes alata* and *D. anomala* ($P > 0.05$) (Figure 4.12 and Table 4.10). Crude extract of *O. urtifolium* was the least performer as a mosquito repellent since it was significantly different from the rest of the crude extracts recording the lowest effectiveness compared to the rest of the plants ($P < 0.05$) (Figure 4.12 and Table 4.10).

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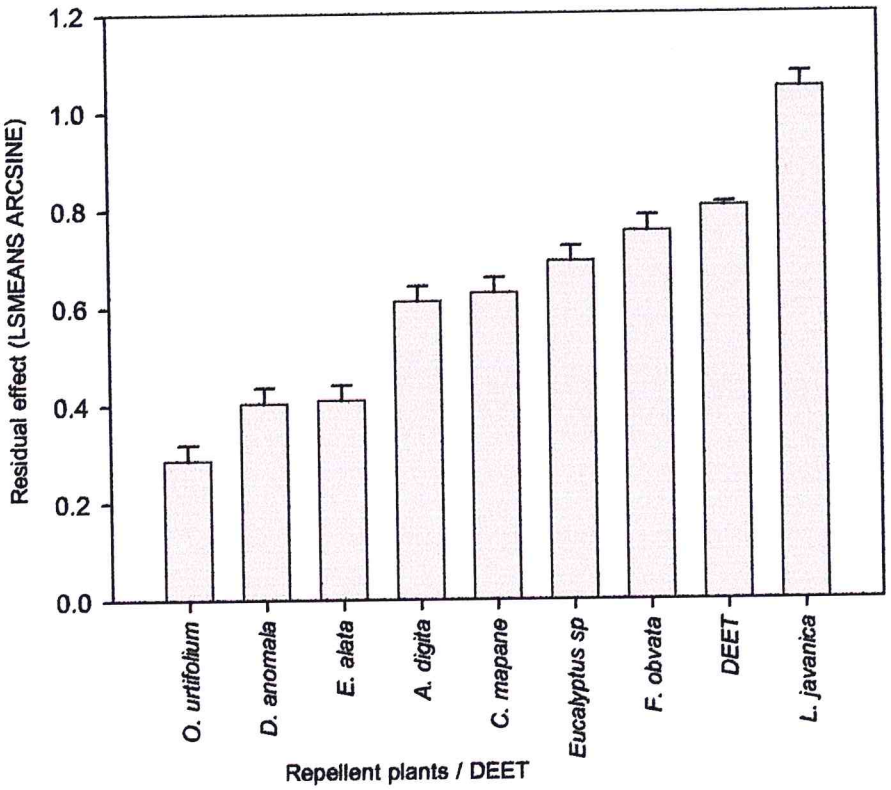

Figure 4.12 Residual effects recorded for DEET (control) and crude extracts of the repellent plants from 0 to 6.5 hrs post application

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4.4.1 Residual effect of DEET, within and across plants

Comparisons of protection post application of the crude extracts of *L. javanica* and DEET revealed that the well known synthetic repellent could only protect for less than 6.5 hours compared to the latter which protected for more than 6.5 hours (Figure 4.13). Repellence effect of DEET decayed at a faster rate compared to that of crude extract of *L. javanica,* indicating that DEET might be considered as a brief protector against the vector mosquitoes (Figure 4.13). Ability of the crude extract of *L. javanica* to protect for more than 6.5 hours recorded by DEET proves its great potential as a mosquito repellent (Figure 4.13). Though the effect of *L. javanica* declined gradually after 1.5 hours to 3 hours its effect remained stable until after 6.5 hours when protection was lost (Figure 4.13).

Protection time of *L. javanica* from 1 to 1.5 hours was not significantly different when compared to DEET ($P > 0.05$) Figure 4.13). Further comparison reveals that *L. javanica* showed two levels of protection which were significantly different ($P < 0.05$) within the post application period (0-2.5 hrs and 2.5-6.5 hrs), compared to DEET, which within the same time span expressed eight significant different phases ($P < 0.05$) (Figure 4.13).

O. urtifolium lost its protection time within the first 1.5 hours (Figure 4.13). Comparison of crude extract of *O. urtifolium* and *L. javanica* revealed that the two were significantly different (Figure 4.13). Crude extract of *O. urtifolium* gave four

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distinct phases (0-0.5, 0.5-1.5, 1.5-2.5 and 2.5-6.0 hours), which were significantly different besides protecting for only one hour (Figure 4.13). From 2.5 hours to 6.5 hours, *O. urtifolium* recorded no repellence hence making it the worst performer compared to the rest of the crude extracts (Figure 4.13). The possible reason for these differences is that *O. urtifolium* essential oils formed at the solvent front showing their high volatility nature (Plate 4.8). Besides that other types of essential oils recorded by this plant showed low migration power suggesting that when applied on the human skin there was no release of the volatiles that repels mosquitoes. Essential oils recorded by *L. javanica* showed medium retention factors, hence making them the best candidates in terms of repelling mosquitoes (Table 4.7).

With an exception of fraction B1 (protecting for 2.5 hrs) all the lower fractions of *O. urtifolium* protective properties decayed within the first hour post application (Appendix E). Comparison across the other B1 fractions suggests no significant difference to *L. javanica* 1.5 hours post application (Appendix E). Further comparison of B1 *D. anomala* (protected for 4 hrs) shows that no significant difference existed within the first 2.5 hrs post application (Appendix E). Fraction B1 of *O. urtifolium* contained alpha santonin, common essential oil that was recorded by most of the extracts, which showed desirable mosquito repellent properties (i.e. *L. javanica* crude extract) (Table 4.9).

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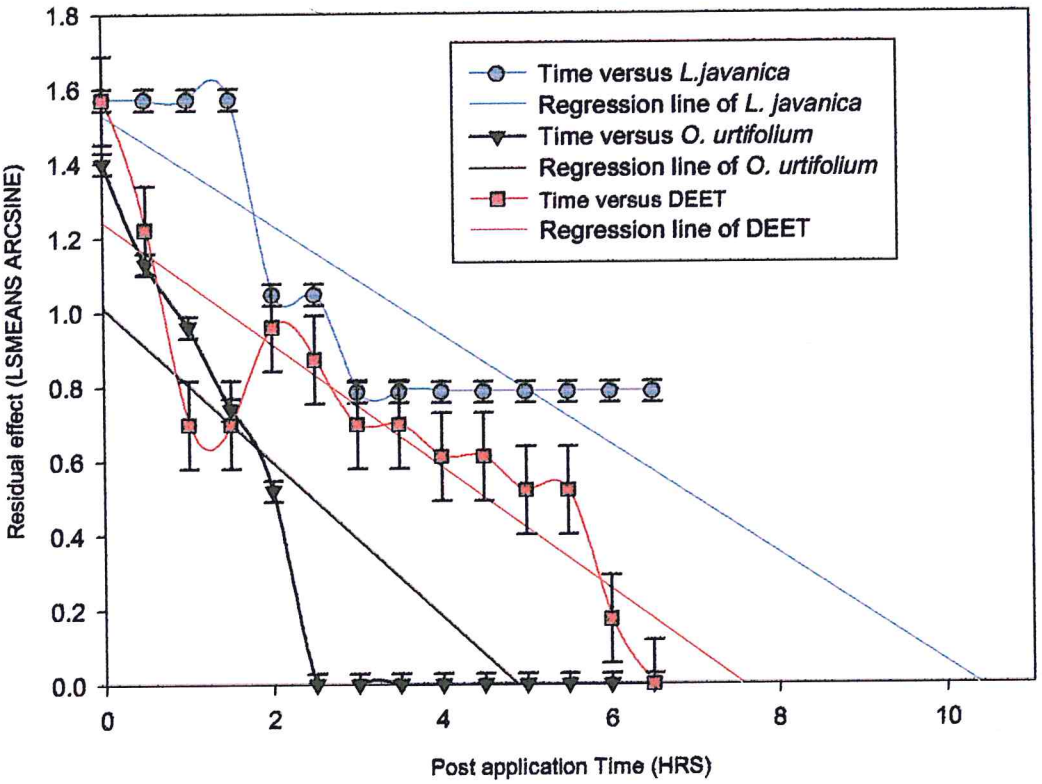

Figure 4.13 Residual effect of *L. javanica*, DEET and *O. urtifolium*

Notes

LSMEANS ARCSINE of 1.57, 1.18, 0.79 and 0.39 is equivalent to 100 %, 75 %, 50 % and 25 % residual effect, respectively.

Residual effect of *L. javanica* could have gone up to 10.5 hrs according to the regression line plotted

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Besides protecting for up to 4 hours, crude extract of *E. alata* was characterized by frequent fluctuations (Figure 4.14). Comparison of crude extract of *E. alata* to the other crude extract reveals that it failed to offer maximum protection immediately after application (Figure 4.14). In between its protection time it failed to protect, indicating that it is not a reliable protector. This was expected because the crude extract failed to record a single essential oil (Table 4.5). Protection recorded by this plant can be attributed to other plant compounds close to the group of the essential oils i.e. 3 types of bitter tasting substances recorded. Bitter tasting substances and essential oils are terpenoids of high volatility acting as mosquito repellents.

Except fraction B2 and B1 that protected up to 1.5 hrs, lower fractions of *E. alata* protected for only 30 minutes post application or failed to protect at all (Appendix E up to Appendix N). Most of the fractions that failed to protect did not record any essential oils and bitter tasting substances, besides recording very low availability index during extraction (Table 4.1). Neither fraction B1 nor B2 recorded essential oils but B1 recorded three types of bitter tasting substances.

Adansonia digitata repellence compared to that of *L. javanica* and DEET was low (Figure 4.14). Protection of *A. digitata* was better compared to that of *O. urtifolium* and *D. anomala*. Repellence expressed by *A. digitata* varied greatly from 0 to 6.5 hours (Figure 4.14). From 0 to 0.5 hours repellence appeared to decline and it varied significantly with *L. javanica* ($P < 0.05$) but not with DEET ($P > 0.05$)

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(Figure 4.14). Repellence recorded after 2 hrs was not significantly different from that of *L. javanica* ($P > 0.05$) but significantly different to that of DEET ($P < 0.05$) (Figure 4.14). The gradual decline of protection appeared to cease at 3.5 hours but it further picked up at the end of 3.5 hours (Figure 4.14). In conclusion we can say that *A. digitata* protected for up to 4 hours and it expressed different levels of protection (Figure 4.14). Evidence of lag phase was observed in this plant because it took some time before offering maximum protection (Figure 4.14).

Presence of THC compounds (high volatile compound) in the crude extract (fraction A) and all the other lower fractions which tested positive for the essential oils suggests that this compound was in large quantity in *A. digitata.* This could be the main reason it never offered maximum protection immediately it was applied (Figure 4.14). Presence of nerolidol essential oil, which was not present in the crude extract of *O. urtifolium* and *D. anomala,* could be the main reason, *A. digitata* protected for long hours soon it was out of the lag phase (Figure 4.14).

Except B1 and B2 all other lower fractions of *A. digitata* protected for less than one-hour post application (Appendix E to Appendix N). Comparison across the other B1 fractions revealed that B1 of *A. digitata* did not offer the best protection (Appendix E). Significant difference ($P < 0.05$) existed between B1 of *D. anomala* (protected for 4 hours) and B1 of A. *digitata* (Appendix E). Fractions B2 from the other plants i.e. (*L. javanica*, *F. obovata* and *Eucalyptus spp)* offered better

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protection than that of *A. digitata* (Appendix F). Significant difference existed between B2 of *D. anomala* and B2 of *A. digitata* (Appendix F).

Besides containing THC compounds (forming at the solvent front) fraction B1 *A. digitata* also recorded citral type of essential oils, which was not present in most of the extracts that were found to express desirable mosquito repellent properties. Fraction B2 of *A. digitata* contained three types of essential oils none of which was recorded by the crude extract (Table 4.2 to 4.9). Unlike crude extract of *A. digitata* which appeared to have a lag phase, fraction B1, started by offering maximum protection, possibly because of alpha santonin and citral types of essential oils which released volatiles readily.

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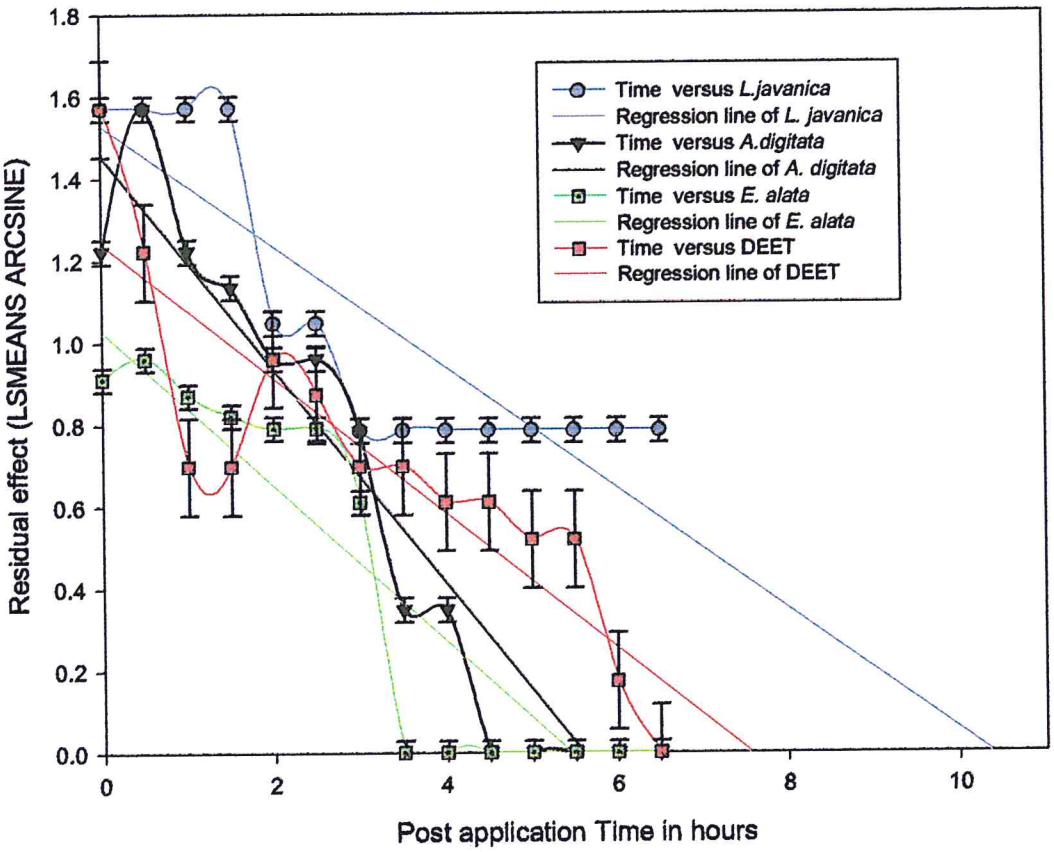

Figure 4.14 Residual effect of *A. digitata*, *E. alata* compared to that of *L. javanica* and DEET

Notes

LSMEANS ARCSINE of 1.57, 1.18, 0.79 and 0.39 is equivalent to 100 %, 75 %, 50 % and 25 % residual effect, respectively.

Residual effect of *L. javanica* predicted to go up to 10.5 hrs according to the regression line plotted

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Crude extract (fraction A) of *D. anomala* was significantly ($p<0.05$) to that of *L. javanica* and DEET (Figure 4.15). *D. anomala* performance was poor compared *to L. javanica* and DEET though it was better than *O. urtifolium* because the protection time went up to 3.5 hours (Figure 4.15). *D. anomala* protection time declined gradually from 0 to 2 hours before it shoot up at 2. 5 hours when it was expected to decline further (Figure 4.15). The protection time of *D. anomala* at 1 and 3.5 hours post application was not significantly different from that of DEET ($P > 0.005$) (Figure 4.15). From 2 to 4 hours, protection continued to decline in a similar way to the first declining phase of 1 to 2 hours up to 6.5 hours (Figure 4.15). *D. anomala* recorded repellence that was not significantly different from that *of L. javanica* ($P>0.05$) between 2 to 3 hours (Figure 4.15).

Most of the lower fractions of *D. anomala* with an except B1 protected for less than one hour post application (Appendix E up to (Appendix N). Protection of 4 hours post application offered by fraction B1 *D. anomala,* suggests that it was effective than some of the crude extracts i.e. *O. urtifolium* (crude extract) Figure 4.15 and 4.13). In addition to containing borneol, fraction B1 of *D. anomala*, THC compounds and cinamic acid were also present (Table 4.4). Considering that THC compounds forming at the solvent front were characterized by high migration power (highly volatile), there could be a possibility of synergistic effect i.e. THC compounds boosted the volatility of borneol in B1 than in the crude extract, hence explaining the observed differences between the two fractions containing borneol.

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Fraction B1 was extracted through the aqueous phase pathway, thus making most of the plant compounds present stick for sometime before escaping.

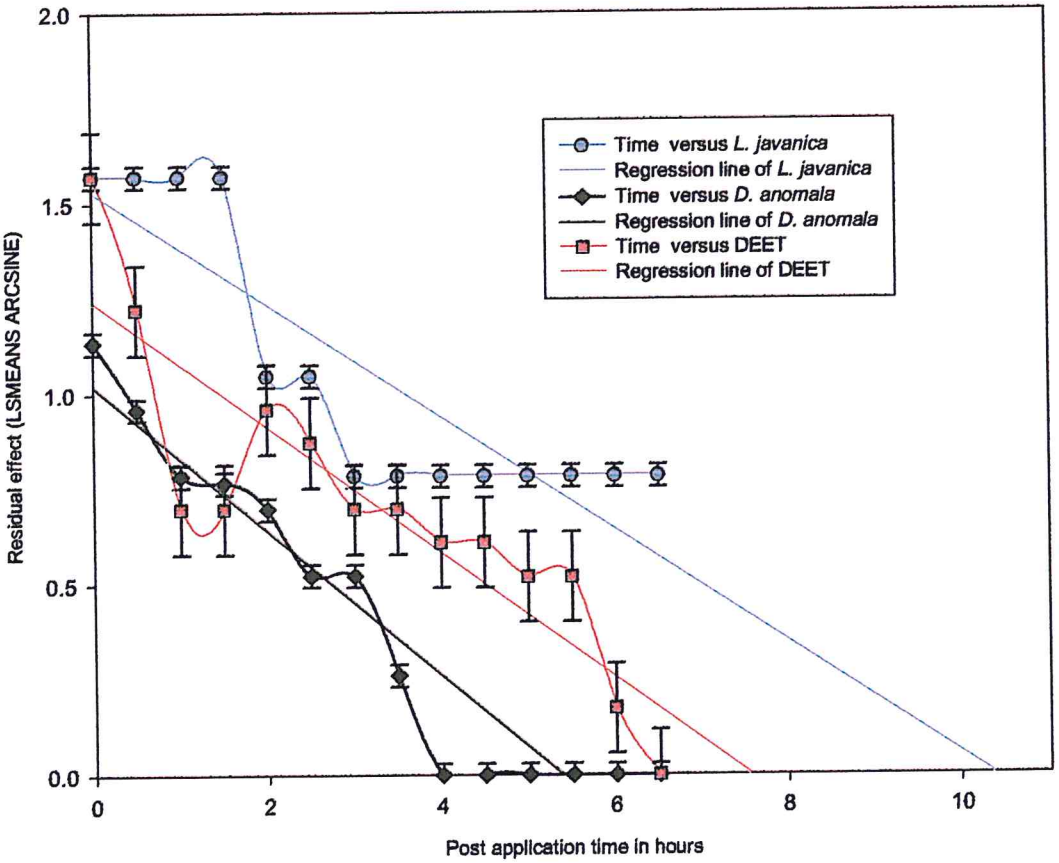

Figure 4.15 Residual effect of *D. anomala* compared to that of *L. javanica* and DEET

Notes

LSMEANS ARCSINE of 1.57, 1.18, 0.79 and 0.39 is equivalent to 100 %, 75 %, 50 % and 25 % residual effect, respectively.

Residual effect of *L. javanica* predicted to go up to 10.5 hrs according to the regression line plotted

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Eucalyptus spp and *C. mopane* varied significantly ($p < 0.05$) to *L. javanica* and DEET in terms of protection time (Figure 4.16). Crude extract (fraction A) of the two plants was not better than that of *L. javanica* and DEET, but they protected in a better way than *O. urtifolium, E. alata, D. anomala* and *A. digitata* (Figure 4.16, 4.15 and 4.14). At 0.5 and 1.5 hrs post application, the repellence expressed by *Eucalyptus spp* was not significantly different to that expressed by *L. javanica,* but was significantly different to that of *C. mopane* ($P < 0.05$) (Figure 4.16). The repellence expressed by *Eucalyptus* from 0 to 3.5 hrs appeared to fluctuate up and down and it stabilized at 3 hrs. Comparison of *Eucalyptus* and *L. javanica* showed that the latter maintained a repelling stability of 3 hrs, before the start of the rapid decline (Figure 4.16). Declining phase of *Eucalyptus sp* and that of DEET was significantly different ($P < 0.05$) (Figure 4.16). Repellency recorded by *C. mopane* from 0 to 3 hrs was significantly different to that recorded by *L. javanica* ($P < 0.05$) (Figure 4.16). Releasing of volatiles by *Eucalyptus spp* could have contributed to protective fluctuations.

Except for fraction C1, all the lower fractions of *C. mopane* protected for less than 1 hour post application (Appendix F up to Appendix N). Fraction C1 of *C. mopane* protected for 2.5 hours and was the best compared to C1 fractions of the other repellent plants (Appendix G). Fraction C1 was extracted through the aqueous phase pathway and protected long because of the low volatility nature of water. Repellent compounds present in this extract stuck for quite some time before escaping hence recording of high residual effect (Appendix G).

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Fraction B1 of *Eucalyptus spp* offered the best protection up to 3.5 hrs (Appendix E). Besides being the best lower fraction of *Eucalyptus sp,* it was the second best protector among all the other B1 fractions (Appendix E). Fraction B1 of *Eucalyptus spp* contained four types of essential oils (Table 4.6), among them, three were similar to those of fraction A of *L. javanica* which protected beyond 6.5 hours as recorded by the synthetic repellent DEET (Table 4.6 and 4.8).

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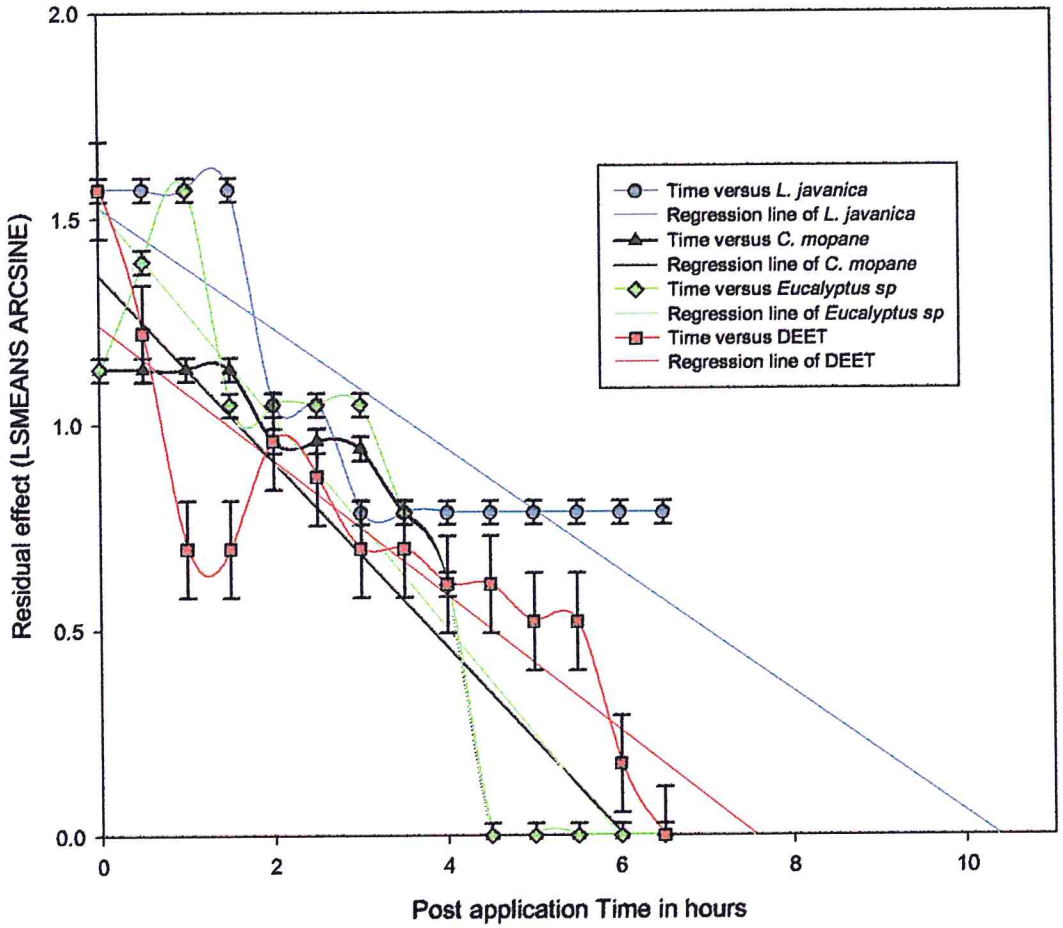

Figure 4.16 Residual effect of *C. mopane, Eucalyptus sp* compared to that of *L. javanica* and DEET

Notes

LSMEANS ARCSINE of 1.57, 1.18, 0.79 and 0.39 is equivalent to 100 %, 75 %, 50 % and 25 % residual effect respectively.

Residual effect of *L. javanica* predicted to go up to 10.5 hrs according to the regression line plotted.

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Comparison of *F. obovata* with the rest of the crude extracts evaluated revealed that it was a better candidate because it protected for 4.5 hours Figure 4.17). Performance of *F. obovata* as a mosquito repellent compared to that of *L. javanica* and DEET differed significantly ($p < 0.05$) (Figure 4.17). Unlike *Eucalyptus spp,* which had repellence on a lag phase, *F. obovata* offered immediate maximum protection (Figure 4.17). When *F. obovata* offered maximum protection, the repellence was significantly different to that recorded by *Eucalyptus spp* (Figure 4.17). Comparison of *Eucalyptus spp* and *F. obovata* revealed that the latter expressed well-defined protection trend because of limited fluctuations, besides not decaying rapidly (Figure 4.17).

Stationery phase of (1-2 hours) of *F. obovata* and *L. javanica* was not significantly different ($P > 0.05$) (Figure 4.17). *F. obovata* protected for 4.5 hours which made it the second best repellent plants protector against starved female mosquitoes (Figure 4.17). Though *F. obovata* contained 5 types of essential oils they were different from those recorded by *L. javanica* and *Eucalyptus spp* (Table 4.6, 4.7 and 4.8). This could be the main reason of the differences observed in terms of protection times.

Lower fractions of *F. obovata* protected for less than 1-hour post application, with an exception of fraction B2 that protected for 2.5 hours (Appendix E up to Appendix N). The lower fraction of *F. obovata* was the second best fraction

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among the other B2 fractions of the other repellent plants (Appendix F). Fraction B2, *F. obovata* contained only two types of essential oil (Table 4.7).

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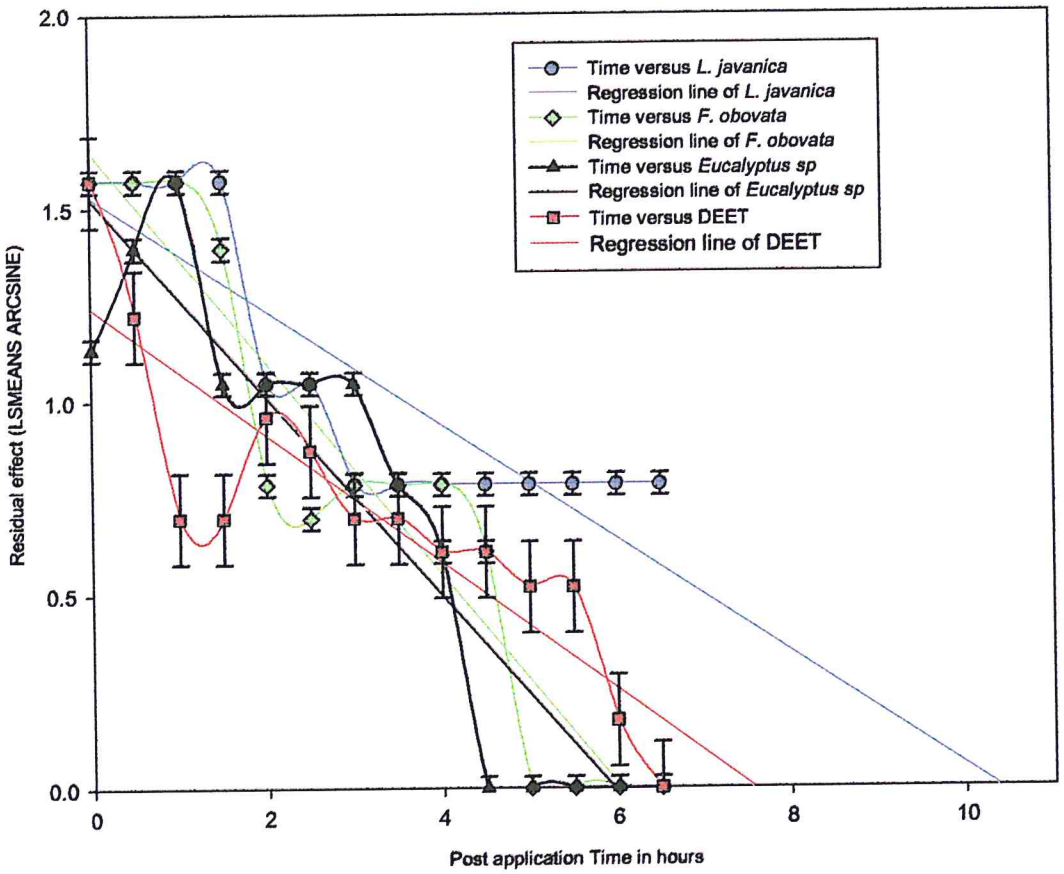

Figure 4.17 Residual effect of *F. obovata, Eucalyptus sp* compared to that of *L. javanica* and DEET

Notes

LSMEANS ARCSINE of 1.57, 1.18, 0.79 and 0.39 is equivalent to 100 %, 75 %, 50 % and 25 % residual effect, respectively.

Residual effect of *L. javanica* predicted to go up to 10.5 hrs according to the regression line plotted.

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4.5 Anti-feedant Experiments

Fraction B2 of *A. digiata* recorded the lowest proportion of mosquitoes that landed and sucked (Table 4.10), meaning that it was significantly different ($P < 0.05$) to the rest of the fractions (Table 4.10). The untreated hand, which acted as the control, was not significantly different ($p > 0.05$) to fractions A, B1, C1 and C2 (Table 4.10). Failure of some fractions to offer maximum protection soon after application contributed to sucking of mosquitoes that landed on the human hand.

No significant difference existed between fractions A (crude extract), B1, C1, C2 of *C. mopane* and the untreated hand ($P > 0.05$) (Table 4.10). Fraction B2 of *C. mopane* recorded the lowest proportions of starved mosquitoes that landed and sucked (Table 4.10). Fraction B2 of *C. mopane* recorded the highest availability index (Table 4.1), meaning that application of this extract coated the human skin hence preventing the mosquitoes that landed from biting. Neither essential oils nor bitter tasting substances were demonstrated in this fraction besides failing to record all the other plant compounds evaluated.

Treating the human hand with fraction A, B1, B2, C1 and C2 of *D. anomala* was not significantly different to the untreated hand ($P > 0.05$) (Table 4.10). Most of the fractions of *D. anomala* recorded very low availability index and did not offer maximum protection immediately after application. Fraction B2 and C1 of *E. alata* are the only two fractions that recorded low proportion of mosquitoes that landed

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and sucked (Table 4.10). No significant difference (P > 0.05) could be demonstrated amongst all the fractions of *E. alata* (Table 4.10). Besides recording a variety of plant compounds fractions B2 and C1 of *E. alata* recorded desirable availability indexes (Table 4.1).

Fraction A, B2, C1 and C2 of *F. obovata* were not significantly (P> 0.05) different in terms of the proportion of mosquitoes that landed and sucked (Table 4.10). Besides containing essential oils and bitter tasting substances the four fractions of *F. obovata* recorded alkaloids and saponins (Table 4.7). This could be the main reason why their effect against the orientation of the vector mosquito was of no significant difference (P> 0.05) (Table 4.10). Treatment of human hand with fraction B1 of *F. obovata* was not significantly different (p > 0.05) to the untreated (Table 4.10). Crude extract (fraction A) of *L. javanica* recorded the lowest proportion of mosquitoes that landed and sucked. Significant difference existed between fraction A and all the other fractions of *L. javanica* (P < 0.05) (Table 4.10).

No significant difference (P> 0.05) was demonstrated between human hands treated with fraction C1 and C2 of *L. javanica* to the untreated (Table 4.10). Fraction B1 and B2 of *L. javanica* did not vary significantly (Table 4.10). Variety of plant compounds demonstrated by fraction A of *L. javanica* in addition to its desirable availability index contributed its best performance. Fraction A of *O. urtifolium* had the lowest proportion of mosquitoes that landed and sucked (Table

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4.10). Fraction A and C2 of *O. urtifolium* varied significantly ($p < 0.05$) from the control, while the rest of fractions of the plant demonstrated no significant difference ($p > 0.05$) to the control (Table 4.10).

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Table 4.10 Comparison within and across repellent plant of proportions of mosquitoes that sucked after landing when human hands were treated with crude extract (fraction A), lower fractions of repellent plants versus the untreated hand (control)

Plants \ Fractions	A	B1	B2	C1	C2	Untreated Human hand (Control)
A. digitata	0.97^{ab3}	0.84^{ab123}	0.79^{a123}	0.86^{ab1}	0.85^{ab12}	1.03^{b1}
C. mopane	0.96^{b3}	1.02^{b34}	0.63^{a1}	0.85^{ab1}	0.95^{b123}	1.05^{b1}
D. anomala	0.75^{a23}	0.80^{a12}	0.89^{a23}	0.81^{a1}	0.90^{a123}	0.93^{a1}
E. alata	0.90^{ab3}	0.82^{a123}	0.75^{a123}	0.82^{a1}	1.10^{b3}	0.92^{ab1}
Eucalyptus sp	0.56^{a12}	0.94^{c234}	0.74^{ab12}	0.97^{c1}	1.04^{c23}	0.88^{bc1}
F. obvata	0.80^{a3}	0.92^{ba234}	0.81^{a123}	0.81^{a1}	0.80^{a1}	1.07^{b1}
L. javanica	0.49^{a1}	0.69^{ab1}	0.88^{bc23}	0.97^{c1}	1.04^{c23}	1.06^{c1}
O. urtifolium	0.76^{a23}	1.13^{c4}	0.95^{abc3}	0.97^{abc1}	0.85^{ab12}	1.08^{cb1}

^{abcd} LSMEANS of arcsine proportions of mosquitoes that landed and sucked within a row with different superscript letters were significantly different ($p < 0.05$).

^{123} LSMEANS of arcsine proportions of mosquitoes that landed and sucked within a column with different superscript numbers were significantly different ($p < 0.05$).

Standard error for crude extract, lower fractions and untreated hand are ± 0.081 and ± 0. 081 respectively.

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Comparison of fractions across repellents plants evaluated reveals that fraction A of *L. javanica* demonstrated the least number of mosquitoes that landed and sucked (Table 4.10). Except for fraction A of *L. javanica* the rest of the fractions did not vary significantly ($P > 0.05$) in terms of the mosquitoes that landed and sucked (Table 4.10). Comparison of fraction B1 across the repellent plants revealed that B1 of *L. javanica* recorded the least proportion of mosquitoes that landed and sucked (Table 4.10). Fraction B1 of *O. urtifolium* was in its own class of the poorest performers because the proportion of mosquitoes that landed and sucked exceeded that of the control (Table 4.10). Fraction B2 of *O. urtifolium* was significantly different ($P< 0.05$) to B2 fractions from the other repellent plants because it demonstrated the highest proportions of vector mosquitoes that landed and sucked. All fractions C1 of the repellent plants did not vary significantly ($P> 0.05$) in regard to the number of mosquitoes that landed and sucked (Table 4.10). Fraction C2 of *F. obovata* varied significantly ($P< 0.05$) to the rest of the fractions besides recording the least proportion of mosquitoes that landed and sucked (Table 4.10).

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4.6 Behavioral experiments

4.6.1 Control experiment

A t-test was performed using Statistix for windows software to determine if there was any significant difference between starved female mosquitoes, orienting to the lower or upper chamber of the experimental cage when only untreated hands were placed. Results obtained showed that there was no significant difference between mosquitoes that oriented to the lower or the upper chamber of the cage used to conduct the behavioral experiments (Table 4.11 and Appendix P). This was expected because no mosquito repellent was applied to mask the human cues, known to attract the host seeking female mosquitoes. Besides that transparent net was used to cover the lower, middle and upper chambers of experimental cage in order to ensure even distribution of light for the visual cues (Figure 3.7). The female mosquitoes responded to the untreated human hands placed on the lower and the upper chamber of the experimental cage and equal number of mosquitoes oriented towards the two sources of the attractant (Table 4.11 and Appendix P). Orienting towards the untreated hands of the human host lead to arrestment of almost all the starved female mosquitoes marking the end of host seeking behavior and the beginning of the probing behavior. This experiment proofed the hypothesis that all the starved female mosquitoes should orientate to untreated hands of the same test person evenly (Table 4.11 and Appendix P).

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Table 4.11 Mean of Log (number of mosquitoes + 1) that oriented to the upper and the lower chambers of the experimental cage

	Log_{10} (number of mosquitoes orienting to upper chamber +1)	Log_{10} (number of mosquitoes orienting to lower chamber +1
Sample size (N)	42	42
Mean	1.03	1.03
Standard error	0.024	0.025
P value	0.8892	0.8892

4.6.2 The choice experiment

Human hands treated with different fractions of the repellent plants showed significant differences ($P< 0.05$) in terms of the number of mosquitoes that oriented to them (Table 4.12 and 4.13). Comparison within the fractions of *L. javanica* reveals that fraction C1 and D2 were as effective as fraction A ($P > 0.05$) (Table 4.12) because no mosquitoes oriented to the human hand treated with these fractions.

The number of mosquitoes that oriented to human hands treated with fraction B1, B2 and C2 of *L. javanica* were not significantly different ($P > 0.05$) (Table 4.12). Fraction B1 of *L. javanica* recorded a lot of bitter tasting substances, which were of low, medium and high volatility (Table 4.8). Fraction B2 of *L. javanica* contained borneol type of essential oils, which was always positive in those fractions found to have desirable mosquito repellent properties.

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Fraction C2 of *L. javanica* contained alpha santonin, recorded by fraction A as well (Table 4.8). Considering that bitter tasting substances and essential oils are volatile compounds, this could have contributed to the similarities observed amongst the three fractions.

Fraction D1 was very much less effective because the number of mosquitoes that oriented to human hands treated with it was not significantly different from that of the control ($P > 0.05$) (Table 2). Fraction D1 of *L. javanica* recorded only one type of essential oil and did not record bitter tasting substances and saponins (Table 4.8).

No mosquitoes oriented to human hands treated with fraction A extract of *Eucalyptus spp* (Table 4.12). Although some mosquitoes oriented to hands treated with fraction B2 and C1 of this species no significant difference ($p > 0.05$) could be demonstrated between these two treatments and the crude extract treatment fraction A (Table 4.12). The number of mosquitoes that oriented to human hands treated with fractions C2, D1 and D2 were of not significantly ($P > 0.05$) different (Table 4.12). Human hands treated with fraction B1 and C2 did not differ significantly ($P > 0.05$) with regard to the number of mosquitoes that oriented towards them (Table 4.12). Both fractions B2 and C1 contained essential oils and bitter tasting substances, explaining the reason why they affected mosquito orientation in a similar way to fraction A which contained both.

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Human hands treated with crude extract (fraction A), B1, B2 C2 and D1 of *F. obovata* recorded no significant difference in terms of mosquitoes that oriented towards the hand (Table 4.12). In the previously mentioned fractions, only human hands treated with fraction D1 recorded no mosquitoes, hence making it the best fraction compared to the rest (Table 4.12). Fraction D1 of *F. obovata* offered the best residual, compared to all the other D1 fractions (Appendix I). Besides recording a variety of plant compounds, essential oils and bitter tasting substances included fraction D1 of *F. obovata* recorded the second best availability index (Table 4.1) hence explaining why mosquitoes failed to orient to hands treated with it. Mosquitoes that oriented to human hands treated with fractions C1 and D2 were not significantly different $P > 0.05$ (Table 4.12), but hands treated with fraction D2 resulted to least number of mosquitoes orienting (Table 4.12). There was a significant difference ($P < 0.05$) between the control (untreated hand) and all the hands treated with fractions from *F. obovata* (Table 4.12).

Treatment of human hands with fractions A, B2, D1 and D2 of *E. alata* was not significantly different ($p > 0.05$) with regard to the number of the mosquitoes that oriented (Table 4.12). Among the four previously mentioned fractions, fraction D2 was the best because no mosquitoes oriented to the hands treated with it (Table 4.12). Besides containing alkaloids of high migration power (R_f 0.79) Table 4.6 fraction D2 of *E. alata* contained borneol a type of essential oils that was always linked to extracts with desirable mosquito repellent properties. There was a

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significant difference ($P < 0.05$) between all the fractions from this plant and the control (untreated hand) (Table 4.12).

All the fractions from *A. digitata, C. mopane, D. anomala,* and *O. urtifolium* used to treat the human hands failed to stop orientation of the mosquitoes (Table 4.13). Combination of two factors could have contributed: recording of low availability index contributed to fewer amounts of plant compounds further resulting to minimal mosquito repellent properties. Human hands treated with fraction B1 of *D. anomala* were not significantly different ($P > 0.05$) to the untreated hands (Table 4.13).

Most fractions of *C. mopane* were of not significantly different to the control ($p > 0.05$) i.e. out of the seven fractions evaluated five showed the same orientation trend with the control (Table 4.13). Most of the fractions of *C. mopane* recorded desirable availability index but contained less plants compounds, meaning that what was available were in fibre form. Considering that the crude extract (fraction A) of *A. digitata* was found to have a lag phase in the residual effect experiments, none of its fraction was capable of stopping the orientation of the mosquitoes to the treated hands (Table 4.13). This explains the reason why there was no significant difference between the crude extract and the untreated in terms of mosquito orientation (Table 4.12).

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Table 4.12 Comparison within fractions of the repellent plants of the mosquitoes that oriented to the chamber containing the treated hand

Plants \ Fractions	A	B1	B2	C1	C2	D1	D2	Untreated Hand Control
A. digitata	1.06^{bcd}	0.96^{cb}	0.60^{a}	0.83^{cb}	0.73^{bc}	1.66^{cd}	0.60^{ab}	1.97^{d}
C. mopane	1.68^{b}	0.83^{a}	2.07^{cb}	0.96^{a}	1.73^{bc}	1.73^{bc}	1.69^{b}	2.21^{bc}
D. anomala	1.19^{bc}	1.79^{cd}	1.19^{bc}	0.37^{a}	0.83^{bc}	0.83^{bc}	0.54^{a}	2.03^{d}
E. alata	0.60^{abc}	0.92^{bc}	0.23^{ab}	0.70^{bc}	0.83^{bc}	0.23^{ab}	0.00^{a}	0.94^{d}
Eucalyptus sp	0.00^{a}	1.65^{cd}	0.23^{a}	0.23^{a}	1.06^{bc}	0.46^{ab}	0.60^{ab}	1.93^{d}
F. obvata	0.23^{ab}	0.23^{ab}	0.46^{ab}	1.23^{c}	0.23^{ab}	0.00^{a}	0.73^{bc}	1.93^{d}
L. javanica	0.00^{a}	0.96^{b}	1.06^{b}	0.00^{a}	1.13^{b}	1.98^{c}	0.00^{a}	2.23^{c}
O. urtifolium	1.84^{cd}	0.83^{ab}	0.46^{a}	1.36^{bc}	0.60^{a}	1.94^{cd}	0.23^{a}	2.08^{d}

^{abcd} LSMEANS of (Log mosquitoes + 1) within a row with different superscript letters were significantly different

0 LSMEANS of (Log mosquitoes + 1) means that starved vector mosquitoes were absolutely hindered from orienting

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Comparison across the plants reveals that there was a significant difference between the fractions of the repellent plants (Table 4.13). The best crude extracts that completely stopped orientation of the mosquitoes to treated hands was that of *L. javanica* and *Eucalyptus spp* (Table 4.13). This was expected because the two crude extracts performed so well during the residual effect experiment. The number of mosquitoes that oriented to human hands treated with crude extract of *F. obovata* and *L. javanica* were not significantly different ($P > 0.05$), despite the fact that each demonstrated different types of essential oil and availability indexes. Fraction A of the rest of the repellent plants failed to completely stop orientation of the mosquitoes hence making them less suitable candidates for mosquito control (Table 4.13).

The entire B1 fraction of the repellent plants failed to ensure zero orientation of the mosquitoes (Table 4.13). Fraction B1 of *F. obovata* was the best compared to the rest because a less number of mosquitoes oriented to the treated hands (Table 4.13). Both fractions B1 from *D. anomala* and *Eucalyptus sp* performed so poorly compared to the rest since they recorded the highest number of mosquitoes orienting to the treated hands (Table 4.13). There was no significant difference ($P > 0.05$) in terms of mosquito orientating to the treated hands with fraction B1 of *A. digitata*, *C. mopane*, *E. alata*, *L. javanica* and *O. urtifolium*, while those of *Eucalyptus spp* and *D. anomala* differed significantly ($P < 0.05$) to the rest of the fractions (Table 3). There was no significant difference ($P > 0.05$) in terms of mosquitoes that oriented to treated hands with fraction B2 of *O.*

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urtifolium, F. obovata, E. alata and *Eucalyptus spp* (Table 4.13). The least number of mosquitoes oriented to hands treated with fractions from *E. alata* and *Eucalyptus spp* (Table 4.13). Considering that most of these mosquitoes that oriented to the hands treated with fraction B2 might have started the probing stage, none of B2 fraction offered desirable orientation hindrance. Though there was no significant difference ($P > 0.05$) among hands treated with fraction C1 of *Eucalyptus spp, D. anomala* and *L. javanica*, the latter was the best because no mosquitoes oriented to the treated hands (Table 4.13). Following our conclusions that most of the mosquitoes that oriented to the treated hands might have gone up to the probing stage, C2 of all the repellent can be categorized in that category of fractions which never delivered in terms of protection against the vector mosquitoes. Treatment of human hands with fraction D2 of *L. javanica* and *E. alata* ensured no orientation of the mosquitoes (Table 4.13). Fraction D1 of *F. obovata* is the only one among all the D1 fractions that recorded zero orientation to hands treated with it (Table 4.13).

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Table 4.13 Comparison across the fractions of the repellent plants of the mosquitoes that oriented to the chamber containing the treated hand

Plants \ Fractions	A	B1	B2	C1	C2	D1	D2
A. digitata	1.06^{23}	0.96^{2}	0.60^{123}	0.83^{2345}	0.73^{1}	1.66^{3}	0.60^{12}
C. mopane	1.68^{34}	0.83^{12}	2.07^{4}	0.96^{345}	1.73^{2}	1.73^{3}	1.69^{3}
D. anomala	1.19^{234}	1.79^{3}	1.19^{3}	0.37^{123}	0.83^{1}	0.83^{2}	0.54^{12}
E. alata	0.60^{12}	0.92^{2}	0.23^{1}	0.70^{234}	0.83^{1}	0.23^{12}	0.00^{1}
Eucalyptus sp	0.00^{1}	1.65^{3}	0.23^{1}	0.23^{12}	1.06^{1}	0.46^{12}	0.60^{12}
F. obovata	0.23^{1}	0.23^{1}	0.46^{12}	1.23^{45}	0.23^{1}	0.00^{1}	0.73^{2}
L. javanica	0.00^{1}	0.96^{2}	1.06^{23}	0.00^{1}	1.13^{12}	1.98^{3}	0.00^{1}
O. urtifolium	1.84^{4}	0.83^{12}	0.46^{12}	1.36^{5}	0.60^{1}	1.94^{3}	0.23^{12}

^{123} LSMEANS of (Log mosquitoes + 1) within a row with different superscript letters were significantly different

0 LSMEANS of (Log mosquitoes + 1) means that starved vector mosquitoes were absolutely hindered from orienting to the treated hand.

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CHAPTER FIVE- DISCUSSION

5.1 Plants collection and identification

Repellent plants collected are easily available, besides being widely used and accepted by rural people, meaning that they act as a major supplement to other malaria control strategies in the country. The current economic hardships in the rural areas have forced people to move into forested areas aiming to achieve cash crop farming at the expense of indigenous subsistence farming. This practice has greatly increased the human and vector contacts besides limiting their chances of accessing skilled personnel for medication when they get infected with the parasite. Rural communities living under such conditions are left with no option other than to use plant based mosquito repellent as a measure of personal protection against the vector mosquitoes. Current farming practices and extensive use of plants as mosquito repellents is accelerating the process of deforestation, meaning that most of the repellent plants growing in natural environment are under the threat of extinction.

Integration of repellent plants and other medicinal plants to ways of live in rural areas has resulted in most of these plants being part of the major cultural context of the people in the areas. This explains the reason why almost all plants rural people claimed that they have medicinal properties were found to have a direct

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link to the superstitious rites that particular community that expressed value of the plant. A lot of history and religious beliefs is attached to these plants. Beside use of the plants as mosquito repellent, they also used them to treat other diseases common in the community. The dose to be applied, method of preparation and administration are within the fingertips of the rural people and traditional healers. One has to be keen and alert when gathering information from rural people and traditional healers because misinterpretation of explanations normally coded behind the traditional descriptions is the order of the day.

What was found out during the plant collection exercise is in agreement with the work conducted by Lukwa *et al.*, (1999). Laboratory validation of the efficacy of crude extract from these plants used as mosquito repellent in the rural households demonstrates that they act as repellent against the vector mosquitoes. Besides crushing the leaves and applying the extracts over their skin Lukwa *et al* (1999) in this study use of intact plants was found to be a common practice in malaria endemic areas of Zimbabwe (Gokwe). That is people cutting the branches and placing them inside their houses particularly around the beds. Some plants, which were acceptable as food by cattle, were fed and then dry cow dung from these animals was burnt at night in the fireplace as a way of repelling mosquitoes. Other plants i.e. *Carissa edulis* were burnt directly before feeding them to cattle to produce smoke that repels vector mosquitoes.

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In conclusion it would be very interesting to investigate the role of potted repellent plants and thermal expulsion as described by Seyoum *et al.* (2002). This will not only conserve our indigenous plants, which are currently under the threat of extinction (Hostettmann *et al.,* 1996) but also will provide a sustainable and affordable means of protecting and reducing human and vector contact, which leads to malaria transmission.

Take home message is local people are already into the practice of using repellent plants meaning that promotion of their use will yield significant contribution to the vector control. Scientists in the field of plant-based mosquito repellent have a role to play in their improvement, focusing on the issue of repelling mosquitoes to those who can not afford improved repellents.

5.2 Extraction of plants compounds and analysis

Bioassay guided fractionation scheme provides fluid extracts for chemical analysis and bioassay experiments. When solvent is partitioned during extraction, it leads to non-selective extraction i.e. extracts all plant compounds that are available within the plant. Unlike other methods such as steam distillation, which specifically extracts specific plant compounds such as essential oils. Use of fresh plants and proper storage after collection made the plants remain in good conditions for analysis. To emphasis the importance of proper storage, Harbone (1984) reported use of herbarium materials for essential oils analysis.

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The plant specimen was collected by Linneaus before 1800 and used in 1967 by Harley and Bell.

The interpretation we have from the fractionation results is that, despite all the efforts of homogenizing our extraction procedures bioavailability varied. Availability in milligrams of each of the fractions of the repellent plants to some extent determined type of plants compounds recorded by each fraction and mosquito repellent properties. This means that availability was linked to the repelling properties of fractions. The availability recorded by *Eucalyptus spp* suggests that a mixture of organic and aqueous solvents readily extracts it. Besides not having undesirable chemical residues, plants compounds that went to the aqueous phase pathway confirms their readily availability when crushed with water to protect against the mosquito bites. Availability results obtained for *L. javanica* are in agreement to those of Muya and Oguge, (2000), who found out that *L. javanica* showed the highest availability index when compared to the other plants they evaluated.

Crude extract of *L. javanica* tested positive for five different types of essential oils, making it the best repellent plant in terms of recording a variety of essential oils (Table 4.8). This could have been the main reason why the crude extract protected for longer time compared to the rest of the crude extracts. Besides recording the highest number of essential oils, crude extract of *L. javanica* recorded three different types of essential oils (piperitone, neryl acetate and

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geranyl acetate), which were not recorded by any of the crude extracts of the other repellent plants. Presence of these additional types of essential oils could have contributed to the outstanding good performance of *L. javanica*.

Except for *C. mopane* and *E. alata* all the crude extracts of the repellent plants contained essential oils (Table 4.2 to 4.9). Protection offered by the crude extract could have been contributed by the other plant compounds i.e. saponins. Besides not containing essential oils, extracts derived from *C. mopane* recorded no bitter tasting substances. This is strong evidence of supporting that *C. mopane* does not contain even the close associates of essential oils.

Crude extract of *Eucalyptus spp* contained a variety of essential oils (five types) (Table 4.6). Essential oils contained by crude extract (fraction A) of *Eucalyptus spp* ranged from the low volatile (low migration power) to high volatiles (high migration power) (Table 4.6). Protection offered by *Eucalyptus spp* appeared to fluctuate up and down, possibly because of the presence of these high volatile essential oils. Research on the methods of reducing escaping of volatile essential oils during extraction process will yield much because protection time offered will be increased besides raising the stability of the repellent extracts.

Crude extract of *D. anomala* recorded two types of essential oils compared to *L. javanica* that recorded five types (Table 4.4 and 4.8). Borneol, is a type of essential oil recorded by crude extract of *D. anomala* and was popular among the

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extracts found to have desirable mosquito properties. Following results obtained from the other extracts, it is possible to show that borneol contributed much to the protection of 3.5 i.e. fraction B1 of *D. anomala* is the only lower fraction that protected for up to 4 hrs and it contained borneol (Table 4.4). Going through most of the lower fractions that contained borneol i.e. C2, D2 of *E. alata* and C2 of *O. urtifolium* just to mention a few revealed, desirable protection effect compared to the rest of the fraction under the same pathway of extraction (Table 4.2 to 4.9).

The wide variety of plant compounds identified in the extracts of the repellent plants confirm the finding of Sutherst *et al.* (1982) who observed that plants have got a wide array of secondary metabolites. Phytochemicals extracted through the organic and aqueous phase pathway were found to have repellent properties confirming the findings of Sukumar *et al.*, (1991) who made similar observations. Plant extracts have been shown to be hostile to spider mites, besides wide range of herbivores (Agrawal, 2000). In this study plant extracts hostility towards the starved female mosquitoes was observed when their orientation to the treated human hands was stopped. Insects such as diabroticite beetles feed on bitter plant compounds such as sequestered cucurbitacins, in order to deter natural enemies from attacking them (Ferguson and Metcalf 1985). Plants extracts that acted and stopped feeding amongst the mosquitoes that landed could have acted in a similar manner. Presence of terpenoid compounds particularly those, which are bitter tasting in plants produced volatiles that repel both predatory and herbivorous insects i.e. predatory mites and spider mites (Anurag *et al.*, 2002).

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Plant extracts found to have either of the terpenoid i.e. essential oils and the bitter tasting substances, repelled mosquitoes.

Anurag *et al.,* (2002) found that predatory mites predating on eggs of spider mites reared on plants with plenty of bitter tasting substances laid less number of eggs over the same period of time compared to those which were not. It could be interesting to investigate the gonotrophic cycle of those female mosquitoes that landed and failed to initiate sucking. Harbone, (1984) reported that a typical monoterpene lactone isolated from the plant *Nepeta catari* (Labiatae) had a peculiar attraction for the domestic cat because of its odor. Mitchell, (1970) cited in Harbone, (1984) reported that sesquiterpenes C_{15} have the ability to act as insect and other living organisms allergens. Presence of curcurbitacin the most known bitter compounds (Anurag *et al.,* 2002) in some of the repellent plant extracts could have been the main reason of their antifeedant properties.

5.3 Bioassay experiments

Lower fractions of *L. javanica* offered different levels of protection, which lasted from 0 to 2.5 hours (Appendix E to Appendix N). From fraction B1 to fraction F2 there was a rapid decline of the protection, which could have been caused by different levels of yield obtained in each fraction (Table 4.1). Most of the volatile compounds known to repel mosquitoes could have been lost in the fractionation process. The best lower fraction of *L. javanica* to offer maximum protection was

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fraction B2 that protected up to 2.5 hours (Appendix F). Comparison across the other B2 fractions from the other repellent plants reveals that it offered the best protection from 0 to 1.5 hrs (Appendix F). Fraction B2 is the only fraction, which recorded geranyl acetate type of essential oil, which was also recorded by fraction A and D1 (Table 4.8). This could have been the reason why fractions demonstrated it expressed desirable protection properties.

Comparison of fraction B2 from *L. javanica* and *Eucalyptus spp* revealed that the latter recorded more types of essential oils but the duration under which they protected was the same (Table 4.6 to Table 4.8 and Appendix F). Both fractions B2 of *L. javanica* and B2 of *D. anomala* protected for 4.0 hours (Appendix F) and each contained borneol in addition to geranyl acetate types of essential oils.

Both fractions C1 and D2 of *L. javanica* contained similar plant compounds i.e. fraction D2 contained similar essential oils to fraction A while fraction C1 contained similar bitter tasting substances to fraction A (Table 4.8). This could be the reason why there was no significant difference, in terms of mosquitoes that oriented to hands treated with the three fractions. Besides containing bitter tasting substances fraction C1 of *L. javanica* is the only fraction within that plant that contained saponins (Table 4.8). Comparison across the other fraction D1 from the other repellent plants revealed that fraction D1 of *L. javanica* recorded the lowest availability index. Recording few plant compounds and the low availability index is

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the probable reason of mosquitoes orienting to hands treated with this fraction in a similar pattern to the control.

Though the effect of fraction B1 of *Eucalyptus spp* was not significantly different to that of fraction C2, it was also found not to be significantly different to the control ($p > 0.05$) (Table 4.12). From these observations we can conclusively regard fraction A, B2 and C1 of *Eucalyptus spp* as the best performers followed by C2, D1 D2 and B1 in that order (Table 4.12). Presence of a number of plant compounds in fraction B1 made it possible for it to express a certain degree of repellency, but it was not as effective as the rest of the fractions because they were of heavier molecular weight hence making their volatility very low.

Following points can be drawn from results obtained in the behavior experiment; host-seeking behavior of female mosquitoes was affected differently by various fractions of the repellent plants evaluated. When orientation of the female mosquitoes was not stopped after treatment almost all the mosquitoes that went to the human hands, entered the sucking stage. This explains the importance of understanding host-seeking behavior of female mosquitoes in our endeavors of controlling it. One would have expected the higher fractions i.e. crude extract (fraction A), B1 and B2 of all the repellent plants evaluated to be more effective than the lower fractions i.e. C1, D1 and D2.

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Evidence of higher fraction not significantly different ($P > 0.05$) to the untreated hand was reported, while on the other hand lower fraction i.e. D2 demonstrated significantly different ($P < 0.05$) from the control besides affecting the orientation of mosquitoes to the advantage of the test person than the crude fraction (fraction A). Fractions that recorded LSMEANS of zero absolutely stopped the orientation of the mosquitoes. Significantly different ($P < 0.05$) readings among the control suggests that fractions evaluated had different repelling powers i.e. besides female mosquitoes being attracted by the untreated human hands, female mosquitoes was as well repelled by the fractions applied on the human hands.

Fractions that recorded high availability index and a variety of plant compounds were found to absolutely hinder the orientation of the starved mosquitoes. When plants extracts were applied on the human hand, they masked the skin making it not to release carbon dioxide the well-known mosquito locomotor's stimulant (Khan *et al.,* 1972). Resulting to no orientation on the treated hand.

Protection against vector mosquitoes obtained from the extracts of the repellent plants evaluated is in agreement to other workers (Govere *at al,* 2000, Lukwa *et al.*, 1999 and Thorsell *et al.* 1970), who reported the use of plant extracts against the vector mosquitoes. Results obtained in this study revealed that DEET, crude extract (fraction A) and lower fractions of the repellent plants evaluated showed various levels of protection. The most effective crude extract was that of *L. javanica*, having protection beyond 6.5 hrs recorded by the synthetic repellent,

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DEET. This could be the reason why *L. javanica* is popular in malaria endemic areas of Zimbabwe and it was found to be in use by all age groups (Lukwa *et al.*, 1999).

Moore *et al.* 2002-reported better protection from *Eucalyptus spp* based mosquito repellent natural product compared to DEET. These findings are in agreement with the findings reported in this work because crude extract of *L. javanica* protected for longer hours than DEET. Our results are not in agreement to those of Fradin and Day, (2002), who reported that DEET based mosquito repellents provided complete protection for the longest duration and non-DEET repellents cannot be relied upon to provide prolonged protection against the vector mosquito.

There was a direct relationship between the level of protection offered by each extract and presence of essential oils. This is in agreement with findings of (Barnard, 1999). Thyme and clove essential oils were found to be most effective repellents against *Aedes aegypti* mosquito (Barnard, 1999). Synergistic effect was observed when clove was combined with geranium or thyme (Barnard, 1999). Investigations of the effects of each of the essential oils recorded in all the extracts found to offer desired protection will yield more information, in addition to picking out synergistic effects.

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Though crude extract of *O. urtifolium* in this experiment offered insignificant protection in terms of repellence, live intact plants of the same genus have been reported to repel vector mosquitoes (Seyoum *et al.,* 2002). Mechanically damaged plants have been reported to emit volatiles, which repels or attracts insects (Bolter *et al.*, 1997). Investigations on the repellence of the live potted plants, which have been implicated, as mosquito repellents in the rural Zimbabwe will address the issue of volatiles having been lost during the extraction process. Laboratory validation of *F. obovata* as a mosquito repellent, confirms that there are more repellent plants in Zimbabwe than they are documented.

In conclusion, results obtained in this study support the work of Govere *et al.,* (2000) who recommended use of *L. javanica* in place of DEET. Besides protecting for long hours against the vector mosquitoes, compared to DEET, L. *javanica* was more stable and predictable than DEET. If high-pressure liquid carbon dioxide extraction technique was the extraction method used pure repellent plants compounds would have been obtained, in addition to increasing stability of the extracts because the method takes care of most thermally unstable compounds.

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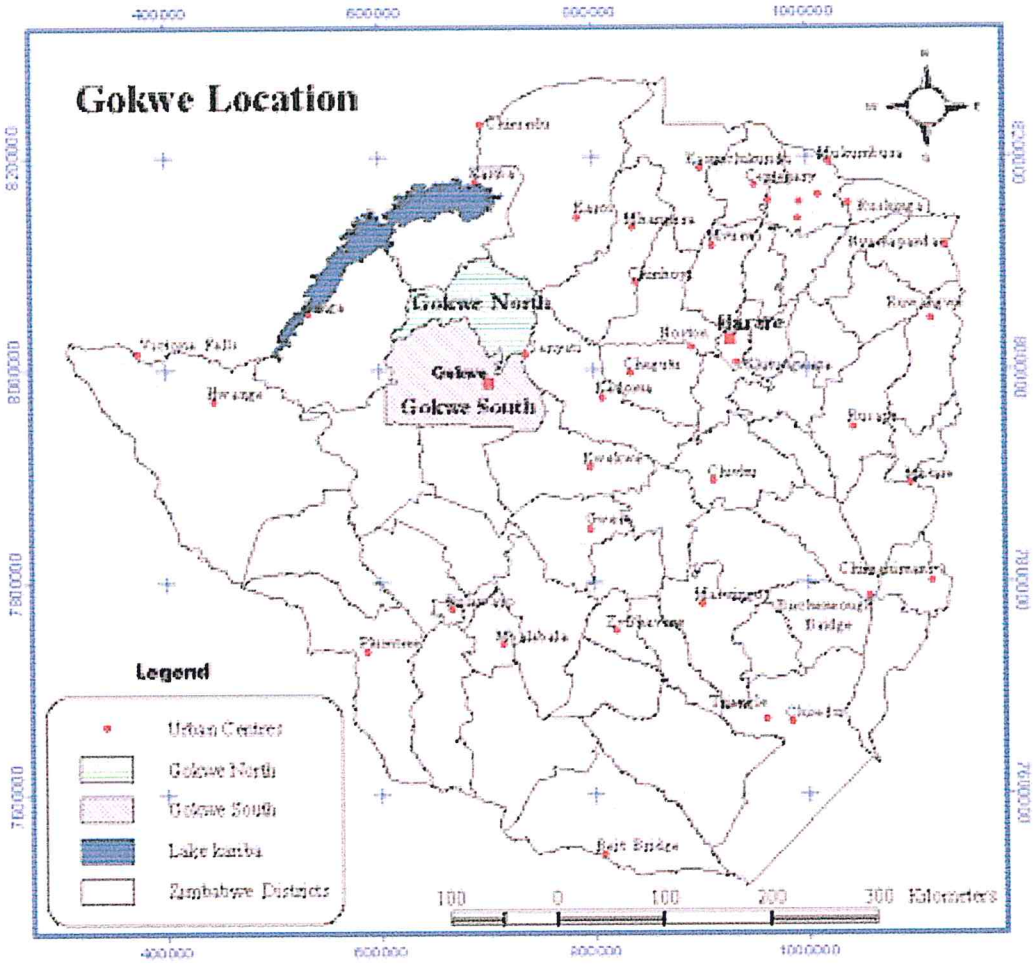
APPENDICES

Appendix A District Map of Zimbabwe



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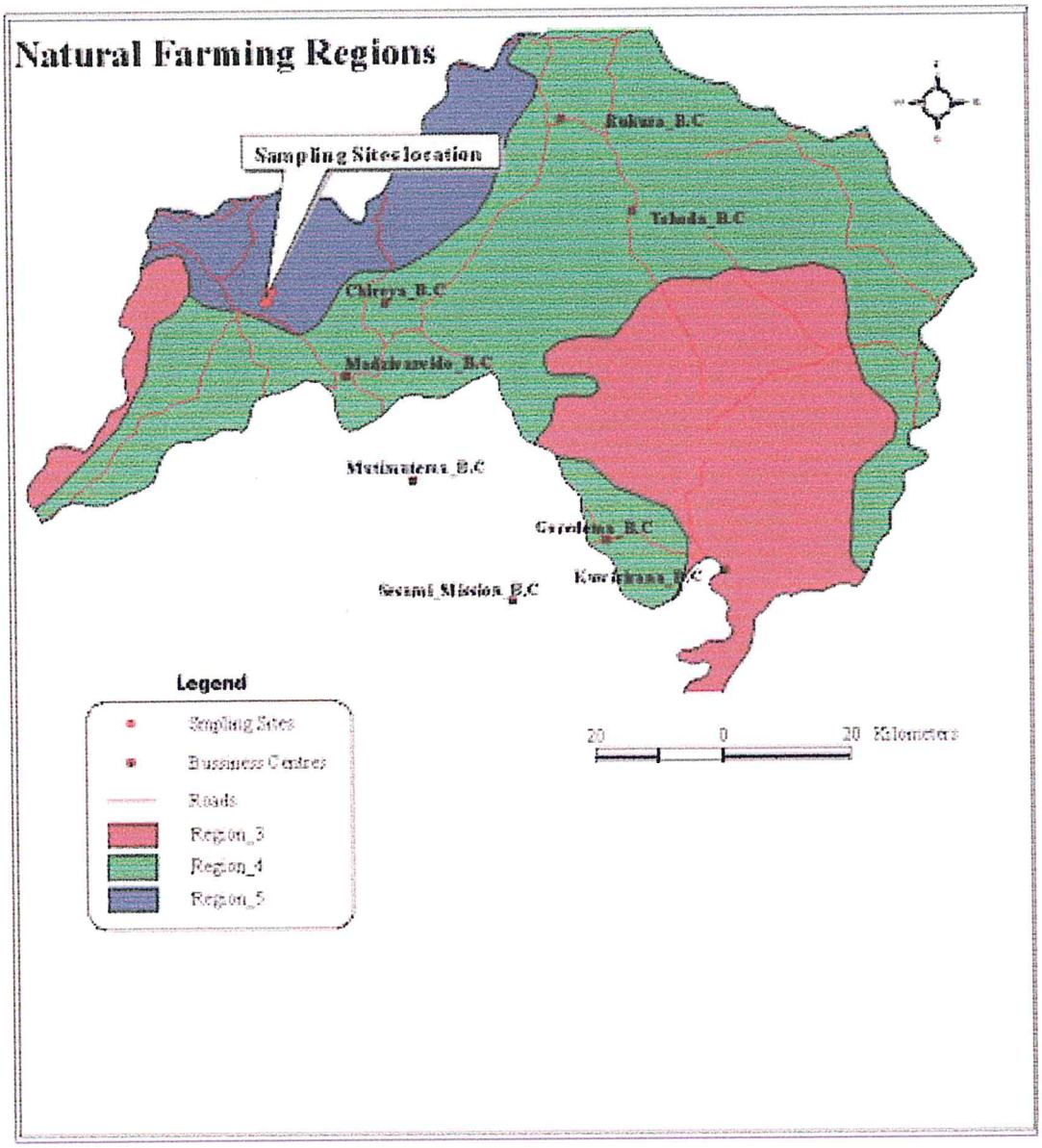
Appendix B Agro-ecological zones (Gokwe North and collection site)



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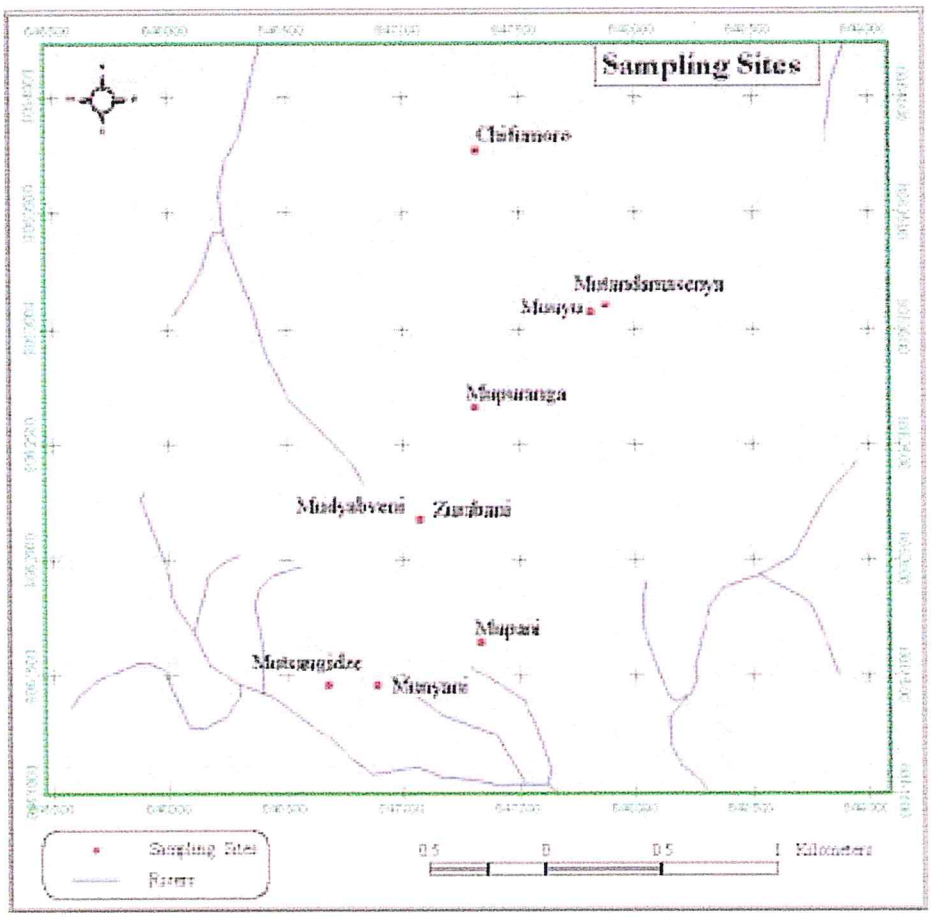
Appendix C Point Map of the repellent plants actual collection sites (local names used)



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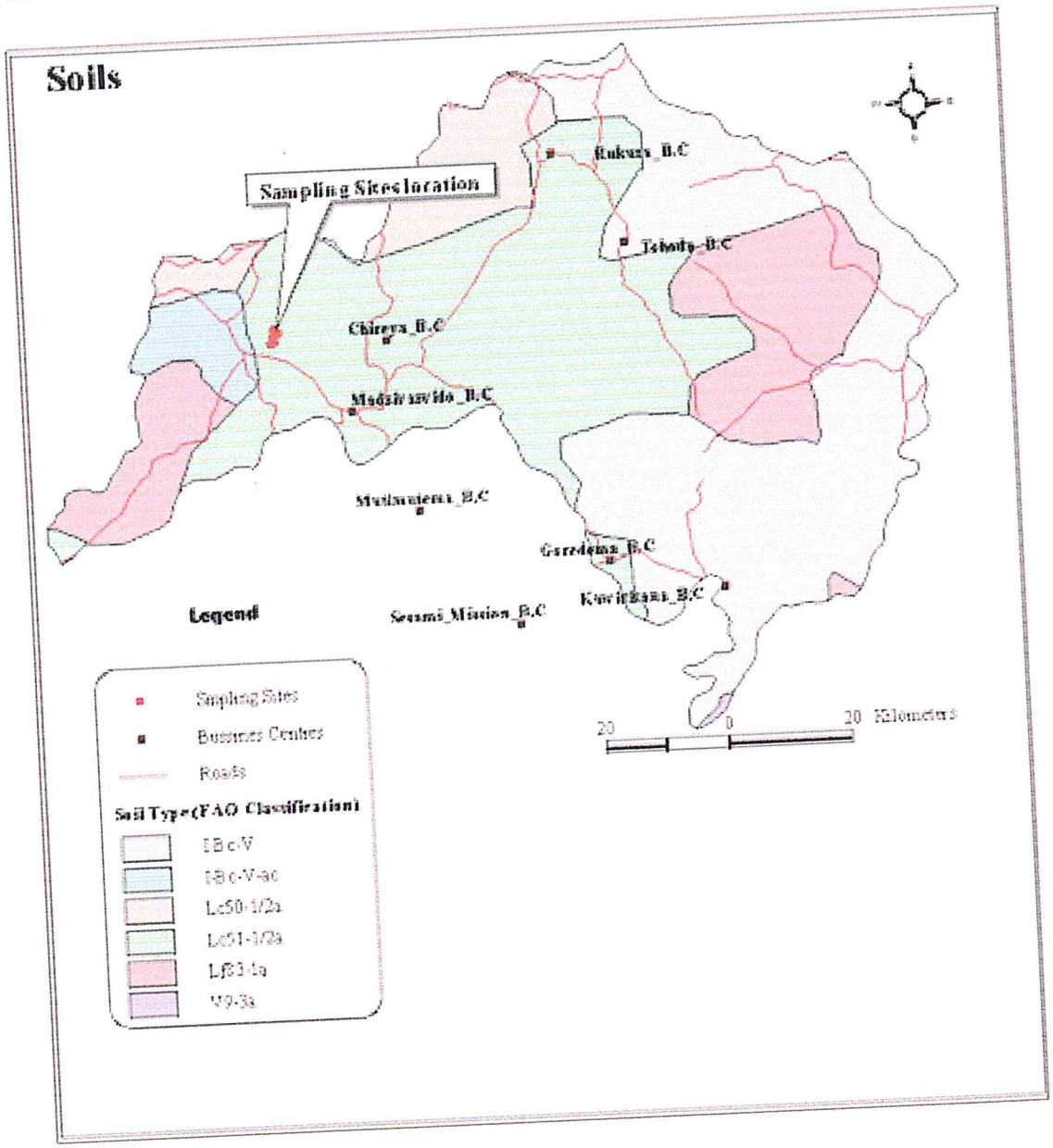
Appendix D FAO soil classification



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Appendix E Residual effect of fraction B1 of the different repellent plants evaluated for repelling mosquitoes versus that of DEET (control)

Time (hrs) / R. Plants	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6	6.5
A. Digitata	1.31^{a1}	1.05^{ab234}	1.05^{ab12}	0.79^{b23}	0.00^{c2}	0.00^{c3}	0.00^{c2}	0.00^{c3}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c1}
C. mopane	1.13^{a1}	0.79^{b4}	0.53^{b3}	0.00^{c4}	0.00^{c2}	0.00^{c3}	0.00^{c2}	0.00^{c3}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c1}
D. anomala	1.40^{a1}	1.40^{a1}	0.96^{b12}	1.05^{b12}	1.05^{b1}	0.61^{c2}	0.77^{bc1}	0.69^{c1}	0.52^{c1}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c1}
E. alata	1.40^{a1}	1.40^{a1}	0.79^{b23}	0.52^{b3}	0.00^{c2}	0.00^{c3}	0.00^{c2}	0.00^{c3}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c1}
Eucalyptus	1.57^{a1}	1.13^{b123}	1.13^{b1}	0.96^{bc12}	0.87^{bc1}	0.79^{dc12}	0.79^{dc1}	0.52^{d2}	0.00^{e2}	0.00^{e2}	0.00^{e2}	0.00^{e2}	0.00^{e2}	0.00^{e1}
F. obvata	1.40^{a1}	1.13^{ab123}	0.96^{bc12}	0.70^{c3}	0.00^{d2}	0.00^{d3}	0.00^{d2}	0.00^{d3}	0.00^{d2}	0.00^{d2}	0.00^{d2}	0.00^{d2}	0.00^{d2}	0.00^{d1}
L. Javanica	1.40^{a1}	0.79^{b4}	0.96^{b12}	0.70^{b3}	0.00^{c2}	0.00^{c3}	0.00^{c2}	0.00^{c3}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c1}
O. Urtifolium	1.13^{a1}	0.96^{ab34}	0.96^{ab12}	1.22^{a1}	0.79^{b1}	0.70^{b12}	0.00^{c2}	0.00^{c3}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c1}
DEET	1.57^{a1}	1.22^{b12}	0.7^{e3}	0.70^{e3}	0.96^{c1}	0.87^{d1}	0.61^{f1}	0.7^{ef1}	0.61^{f1}	1.22^{b1}	1.22^{b1}	0.7^{e1}	0.17^{g1}	0^{h1}

1,2,3 4 LSMEANS in arcsine within a column with different superscript numbers were significantly different ($p < 0.05$).
a b c d e f g h LSMEANS in arcsine within a row with different superscript letters were significantly different ($p < 0.05$).
Standard errors for residual effect of fraction B1 and that of DEET are ± 0.115 and ± 0.029 respectively.

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Appendix F Residual effect of fraction B2 of the different repellent plants evaluated for repelling mosquitoes versus that of DEET (control)

Time (hrs) / R. Plants	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6	6.5
A. Digitata	1.40^{a12}	1.13^{a234}	0.79^{b234}	0.35^{c34}	0.00^{d3}	0.00^{d6}	0.00^{d2}	0.00^{d2}	0.00^{d2}	0.00^{d2}	0.00^{d2}	0.00^{d2}	0.00^{d2}	0.00^{d1}
C. mopane	1.57^{a1}	0.79^{b5}	0.00^{c5}	0.00^{c5}	0.00^{c3}	0.00^{c3}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c1}
D. anomala	1.40^{a12}	1.22^{a23}	1.22^{a1}	1.05^{a1}	0.79^{b12}	0.79^{b12}	0.70^{b1}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c1}
E. alata	1.40^{a12}	1.40^{a12}	0.69^{b34}	0.17^{c45}	0.00^{c3}	0.00^{c3}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c1}
Eucalyptus	1.13^{a2}	0.96^{a345}	0.87^{ab23}	0.61^{bc23}	0.52^{c2}	0.17^{d3}	0.00^{d2}	0.00^{d2}	0.00^{d2}	0.00^{d2}	0.00^{d2}	0.00^{d2}	0.00^{d2}	0.00^{d1}
F. obvata	1.40^{a12}	0.87^{b45}	0.79^{b234}	0.79^{b2}	0.61^{b2}	0.61^{b2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c1}
L. Javanica	1.57^{a1}	1.57^{a1}	1.05^{b12}	0.79^{bc2}	0.70^{c2}	0.10^{d3}	0.00^{d2}	0.00^{d2}	0.00^{d2}	0.00^{d2}	0.00^{d2}	0.00^{d2}	0.00^{d2}	0.00^{d1}
O. Urtifolium	1.13^{a2}	0.87^{a45}	0.52^{b4}	0.00^{c5}	0.00^{c3}	0.00^{c3}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c1}
DEET	1.57^{a1}	1.22^{b23}	0.7^{e34}	0.70^{e2}	0.96^{c1}	0.87^{d1}	0.61^{f1}	0.7^{ef1}	0.61^{f1}	1.22^{b1}	1.22^{b1}	0.7^{e1}	0.17^{g1}	0^{h1}

1,2,3 4 LSMEANS in arcsine within a column with different superscript numbers were significantly different ($p < 0.05$).
a b c d e f g h LSMEANS arcsine within a row with different superscript letters were significantly different ($p < 0.05$).

Standard errors for residual effect of fraction B2 and that of DEET are ± 0.115 ± and 0.029 respectively.

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Appendix G Residual effect of fraction C1 of the different repellent plants evaluated for repelling mosquitoes versus that of DEET (control)

Time (hrs) / R. Plants	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6	6.5
A. digitata	1.13^{a2}	0.52^{b2}	0.00^{c3}	0.00^{c3}	0.00^{c3}	0.00^{c3}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c1}
C. mopane	1.13^{a2}	1.31^{a1}	0.96^{b1}	0.96^{b1}	0.79^{b1}	0.45^{c2}	0.00^{d2}	0.00^{d2}	0.00^{d2}	0.00^{d2}	0.00^{d2}	0.00^{d2}	0.00^{d2}	0.00^{d1}
D. anomala	0.44^{a32}	0.00^{b4}	0.00^{b3}	0.00^{b3}	0.00^{b3}	0.00^{b3}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b1}
E. alata	1.13^{a2}	0.17^{b34}	0.00^{c3}	0.00^{c3}	0.00^{c3}	0.00^{c3}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c1}
Eucalyptus	1.13^{a2}	0.61^{b2}	0.00^{c3}	0.00^{c3}	0.00^{c3}	0.00^{c3}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c1}
F. obvata	1.13^{a2}	1.05^{a1}	0.61^{b2}	0.61^{b2}	0.35^{b2}	0.00^{c3}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c1}
L. javanica	1.13^{a2}	0.35^{b23}	0.00^{c3}	0.00^{c3}	0.00^{c3}	0.00^{c3}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c1}
O. urtifolium	1.05^{a2}	0.17^{b3}	0.00^{b3}	0.00^{b3}	0.00^{b3}	0.00^{b3}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b1}
DEET	1.57^{a1}	1.22^{b1}	0.70^{e2}	0.70^{e2}	0.96^{c1}	0.87^{d1}	0.61^{f1}	0.70^{ef1}	0.61^{f1}	1.22^{b1}	1.22^{b1}	0.70^{e1}	0.17^{g1}	0.00^{h1}

1,2,3 4 LSMEANS in arcsine within a column with different superscript numbers were significantly different ($p < 0.05$).

a b c d e f g h LSMEANS in arcsine within a row with different superscript letters were significantly different ($p < 0.05$).

Standard errors for residual effect of fraction C1 and that of DEET are ± 0.120 and ± 0.030 respectively.

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Appendix H Residual effect of fraction C2 of the different repellent plants evaluated for repelling mosquitoes versus that of DEET (control)

Time (hrs) / R. Plants	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6	6.5
A. Digitata	0.00^{b4}	0.61^{a234}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b1}
C. *mopane*	0.87^{a2}	0.87^{a1}	0.61^{ab1}	0.52^{b1}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c1}
D. anomala	0.52^{a3}	0.00^{b4}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b1}
E. alata	0.61^{a23}	0.35^{a34}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c1}
Eucalyptus	0.52^{a3}	0.00^{b4}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b1}
F. obvata	0.52^{a3}	0.00^{b4}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b1}
L. Javanica	0.70^{a23}	0.79^{a2}	0.61^{a1}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b1}
O. *Urtifolium*	0.87^{a2}	0.61^{a2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b1}
DEET	1.57^{a1}	1.22^{b2}	0.70^{e1}	0.70^{e1}	0.96^{c1}	0.87^{d1}	0.61^{f1}	0.70^{ef1}	0.61^{f1}	1.22^{b1}	1.22^{b1}	0.70^{e1}	0.17^{g1}	0.00^{h1}

1,2,3 4 LSMEANS in arcsine within a column with different superscript numbers were significantly different ($p < 0.05$).

a b c d e f g h LSMEANS in arcsine within a row with different superscript letters were significantly different ($p < 0.05$).

Standard errors for residual effect of fraction C2 and that of DEET repellence are ± 0.119 and ± 0.030 respectively.

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Appendix I Residual effect of fraction D1 of the different repellent plants evaluated for repelling mosquitoes versus that of DEET (control)

Time (hrs) / R. Plants	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6	6.5
A. digitata	0.79^{a3}	0.35^{b34}	0.00^{c}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c1}
C. mopane	0.79^{a3}	0.70^{a2}	0.61^{a12}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c22}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c1}
D. anomala	0.87^{a23}	0.61^{ab23}	0.35^{b2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c1}
E. alata	0.79^{a3}	0.26^{b4}	0.00^{b3}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{c22}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c1}
Eucalyptus	1.13^{a2}	0.87^{a1}	0.00^{b3}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b1}
F. obvata	1.13^{a2}	0.87^{ab1}	0.70^{b1}	0.61^{b1}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c1}
L. Javanica	0.79^{a3}	0.52^{a234}	0.00^{b3}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b1}
O. urtifolium	0.79^{a3}	0.26^{b4}	0.00^{c3}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c1}
DEET	1.57^{a1}	1.22^{b1}	0.70^{e1}	0.70^{e1}	0.96^{c1}	0.87^{d1}	0.61^{f1}	0.70^{ef1}	0.61^{f1}	1.22^{b1}	1.22^{b1}	0.70^{e1}	0.17^{g1}	0.00^{h1}

Standard errors for residual effect of fraction D1 and that of DEET are ± 0.114 and ± 0.029 respectively.

1,2,3 4 LSMEANS in arcsine within a column with different superscript numbers were significantly different ($p < 0.05$).

a b c d e f g h LSMEANS in arcsine within a row with different superscript letters were significantly different ($p < 0.05$).

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Appendix J Residual effect of fraction D2 of the different repellent plants evaluated for repelling mosquitoes versus that of DEET (control)

Time (hrs) / R. Plants	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6	6.5
A. digitata	0.00^{a4}	0.00^{a3}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a1}
C. mopane	0.00^{a4}	0.00^{a3}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a1}
D. anomala	0.61^{a3}	0.17^{b23}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b1}
E. alata	0.79^{a23}	0.00^{b3}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b1}
Eucalyptus	1.05^{a2}	0.35^{b2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c1}
F. obvata	0.79^{a}	0.17^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b1}
L. Javanica	0.79^{a2}	0.17^{b2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c1}
O. urtifolium	0.17^{a4}	0.00^{a3}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a1}
DEET	1.57^{a1}	1.22^{b1}	0.70^{e1}	0.70^{e1}	0.96^{c1}	0.87^{d1}	0.61^{f1}	0.70^{ef1}	0.61^{f1}	1.22^{b1}	1.22^{b1}	0.70^{e1}	0.17^{g1}	0.00^{h1}

1,2,3 4 LSMEANS in arcsine within a column with different superscript numbers were significantly different ($p < 0.05$).

a b c d e f g h LSMEANS in arcsine within a row with different superscript letters were significantly different ($p < 0.05$).

Standard errors for the residual effect of fraction D2 and that of DEET are ± 0.111 and ± 0.028 respectively

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Appendix K Residual effect of fraction E1 of the different repellent plants evaluated for repelling mosquitoes versus that of DEET (control)

Time (hrs) / R. Plants	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6	6.5
A. digitata	0.79^{a3}	0.00^{b3}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b1}
C. mopane	0.17^{a56}	0.00^{b3}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b1}
D. anomala	0.61^{a34}	0.17^{b3}	0.00^{b22}	0.00^{b2}	0.00^{b22}	0.00^{b2}	0.00^{b22}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b1}
E. alata	0.44^{a45}	0.00^{b3}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b1}
Eucalyptus	1.13^{a2}	0.52^{b2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c2}	0.00^{c1}
F. obvata	0.00^{a6}	0.00^{a3}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a1}
L. Javanica	0.44^{a4}	0.00^{b3}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b1}
O. Urtifolium	0.34^{a45}	0.00^{b3}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b1}
DEET	1.57^{a1}	1.22^{b1}	0.70^{e1}	0.70^{e1}	0.96^{c1}	0.87^{d1}	0.61^{f1}	0.70^{ef1}	0.61^{f1}	1.22^{b1}	1.22^{b1}	0.70^{e1}	0.17^{g1}	0.00^{h1}

1,2,3 4 LSMEANS in arcsine within a column with different superscript numbers were significantly different ($p < 0.05$).

a b c d e f g h LSMEANS in arcsine within a row with different superscript letters were significantly different ($p < 0.05$).

Standard error s for residual effect of fraction E1 and that of DEET are ± 0.111 and ± 0.028 respectively.

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Appendix L Residual effect of fraction E2 of the different repellent plants evaluated for repelling mosquitoes versus that of DEET (control)

Time (hrs) / R. Plants	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6	6.5
A. Digitata	0.79^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a1}
C. *mopane*	0.61^{a23}	0.17^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b1}
D. anomala	0.79^{a2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b1}
E. alata	0.00^{a4}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a1}
Eucalyptus	0.70^{a23}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b1}
F. obvata	0.00^{a4}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a1}
L. Javanica	0.44^{a3}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b1}
O. Urtifolium	0.17^{a23}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b1}
DEET	1.57^{a1}	1.22^{b1}	0.70^{e1}	0.70^{e1}	0.96^{c1}	0.87^{d1}	0.61^{f1}	0.70^{e1f}	0.61^{f1}	1.22^{b1}	1.22^{b1}	0.70^{e1}	0.17^{g1}	0.00^{h1}

1,2,3 4 LSMEANS in arcsine within a column with different superscript numbers were significantly different ($p < 0.05$).

a b c d e f g h LSMEANS in arcsine within a row with different superscript letters were significantly different ($p < 0.05$).

Standard errors for residual effect of fraction E2 and that of DEET are ± 0.110 and ± 0.028 respectively

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Appendix M Residual effect of fraction F1 of the different repellent plants evaluated for repelling mosquitoes versus that of DEET (control)

Time (hrs) / R. Plants	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6	6.5
A. digitata	0.30^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a1}
C. mopane	0.00^{a3}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a1}
D. anomala	0.00^{a3}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a1}
E. alata	0.00^{a3}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a1}
Eucalyptus	0.35^{a2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b1}
F. obvata	0.35^{a2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b1}
L. javanica	0.52^{a2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b1}
O. urtifolium	0.00^{a3}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a1}
DEET	1.57^{a1}	1.22^{b1}	0.70^{e1}	0.70^{e1}	0.96^{c1}	0.87^{d1}	0.61^{f1}	0.70^{e1f}	0.61^{f1}	1.22^{b1}	1.22^{b1}	0.70^{e1}	0.17^{g1}	0.00^{h1}

1,2,3 4 LSMEANS in arcsine within a column with different superscript numbers were significantly different ($p < 0.05$).

a b c d e f g h LSMEANS in arcsine within a row with different superscript letters were significantly different ($p < 0.05$).

Standard error for residual effect of fraction F1 and that of DEET are ± 0.110 and ± 0.027 respectively.

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Appendix N Residual effect of fraction F2 of the different repellent plants evaluated for repelling mosquitoes versus that of DEET (control)

Time (hrs) / R. Plants	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6	6.5
A. digitata	0.00^{a3}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a1}
C. *mopane*	0.00^{a3}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a1}
D. *anomala*	0.00^{a3}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{11}
E. *alata*	0.00^{a3}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a1}
Eucalyptus	0.52^{a2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b1}
F. *obvata*	0.00^{a3}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a1}
L. *javanica*	0.52^{a2}	0.17^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b2}	0.00^{b1}
O. *urtifolium*	0.00^{a3}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a2}	0.00^{a1}
DEET	1.57^{a1}	1.22^{b1}	0.70^{e1}	0.70^{e1}	0.96^{c1}	0.87^{d1}	0.61^{f1}	0.70^{ef1}	0.61^{f1}	1.22^{b1}	1.22^{b1}	0.70^{e1}	0.17^{g1}	0.00^{h1}

1,2,3 4 LSMEANS in arcsine within a column with different superscript numbers were significantly different ($p < 0.05$).

a b c d e f g h LSMEANS in arcsine within a row with different superscript letters were significantly different ($p < 0.05$).

Standard error for residual effect of fraction F2 and that of DEET are ± 0.108 and ± 0.027 respectively.

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Appendix O Residual effect of the crude extract versus that of DEET (control) over the period 0 - 6.5 hours post application

Repellent plant / DEET	Residual effect	Standard error
O. urtifolium	0.29 [f]	± 0.032
D. anomala	0.40 [e]	± 0.032
E. alata	0.41 [e]	± 0.032
A. digitata	0.61 [d]	± 0.032
C. mopane	0.63 [d]	± 0.032
Eucalyptus sp	0.69 [dc]	± 0.032
F. obvata	0.75 [bc]	± 0.032
L. javanica	1.05 [a]	± 0.008
DEET (Control)	0.80 [b]	± 0.032

[abcdef] Residual effect in LSMEANS arcsine within a column with different superscript letters were significantly different ($p < 0.05$).

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Appendix P Summary of the raw data of the number of mosquitoes that oriented in the upper and lower chamber of the experimental cage

Replicates	Number of mosquitoes that oriented to upper chamber	Number of mosquitoes that oriented to lower chamber
1	15	16
2	14	15
3	15	16
1	10	11
2	11	12
3	2	2
1	6	6
2	7	7
3	8	8
1	9	8
2	10	10
3	7	7
1	6	6
2	22	23
3	27	27
1	11	12
2	13	13
3	12	12
1	7	6
2	8	5
3	7	6
1	10	11
2	10	11
3	9	11
1	8	8
2	7	8
3	8	8
1	11	11
2	11	10
3	11	11
1	8	9
2	9	10
3	11	11
1	13	13
2	13	12
3	12	12
1	7	8
2	8	9
3	7	8
1	11	11
2	11	10
3	11	11

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