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RESEARCH ARTICLE

Nutritional and antioxidant properties of two species of edible scarab beetles (*Cetonia aurata* and *Oryctes rhinoceros*)

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Abstract

Edible scarab beetles are important repositories for nutritive and bioactive compounds, but this information remains largely unexplored. This study explored the nutritional and therapeutic properties of two edible scarab beetles from three different locations (Embu, Murang'a and Nairobi Counties) in Kenya. Morphological and molecular characterization of the wild collected larvae revealed two important edible beetle species (*Cetonia aurata* L. and *Oryctes rhinoceros* L.). The larvae of both species are excellent source of crude proteins (44% for *O. rhinoceros* and 63% for *C. aurata*). The larvae of *O. rhinoceros* were rich in minerals, particularly calcium (15.75-22.65 mg/g) and potassium (13.62-22.88 mg/g). The anti-radical activity of *C. aurata* larvae ranged between 91% and 92% across the various target sites. Lysine was the most important and abundant amino acid in both larvae (>5 mg/g). The larvae of *C. aurata* and *O. rhinoceros* are a good source of unsaturated fatty acids like Methyl-8*Z*, 11*Z*, 14Z-eicosatrienoate (32 mg/g) and Methyl-9*Z*-octadecenoate (9.47-120.84 mg/g). The entomochemicals of phenolic nature derived from the beetle larvae portray a unique opportunity to supply and improve high quality nutritious food with enhanced ability to get-rid of free radicals, thus promoting good health and well-being of the consumers. Future development of foods supplemented with scarab beetle larvae derived products must consider the preservation of potential benefits of not only nutrients, but also nutraceuticals. However, in-depth research is still needed to guarantee the bioactivity in processed foods and ensure quality control test before the release of these products to the market.

Keywords

antioxidant properties – edible insects – entomochemicals – functional foods – nutritional composition

1 Introduction

The world population is projected to reach 9.2 billion by the year 2050, with most of the unprecedented demographic dynamism reportedly occurring in low-income countries (Bongaarts, 2009). This presents a challenge to food supply chain disruption and nutrition security, as was intensified by the recent pandemic of COVID-19. It became evident that adequate nutrition plays a fundamental role in strengthening the immune sys-

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tem, thereby reducing mortality rates (Béné *et al.*, 2021). Ensuring food security by providing nutrient-rich foods is of utmost concern due to the increased prevalence of lifestyle disorders such as obesity, cognitive disorders, and cases of malnutrition.

Globally, approximately 827 million people are reportedly hunger-stricken with 24.8% prevalence of undernourishment being reported (McGuire, 2013). For instance, local inhabitants of Africa, Asia and Latin America continue to suffer from under-nutrition, particularity protein energy malnutrition (PEM) due to insufficient palatable sources of protein (Ajobiewe *et al.*, 2021). This situation is projected to worsen as the demand for animal-based protein increases, alongside growing food insecurity. Unfortunately, the efforts to tackle food insecurity have greatly focused on the conventional patterns of food production, which have proven unsustainable due to their ecological impact on natural ecosystems and biodiversity (Steenson and Buttriss, 2020). Therefore, there is need for people to start embracing alternative food systems (Roohani *et al.*, 2013; Van Huis *et al.*, 2013).

Entomophagy serves as a sustainable alternative to traditional sources of food and feed benefiting the environment, health, and livelihoods (Van Huis *et al.*, 2013). Insects have served as sources of food and medicine since ancient times (Laureati *et al.*, 2016). These insects are highly diverse, abundantly present in nature for sustainable harvesting and are endowed with several therapeutic and functional food properties (Mudalungu *et al.*, 2021; Rumpold and Schlüter, 2013). Apart from the ecological benefits, insects such as beetles are known to contain high protein content with excellent essential amino acids, micronutrients, antinutrients, fat-soluble tocopherols and bioactive molecules which are essential in combating metabolic disorders (Anaduaka *et al.*, 2021; Mudalungu *et al.*, 2021; Omotoso, 2018). For instance, adult *Holotrichia parallela* Motschulsky is known to be an excellent source of protein (70.27%), minerals (3.61%) especially potassium (2,851 mg/kg) and low crude fat (10.36%) (Hu *et al.*, 2010). Additionally, the larval stage of *Protaetia brevitarsis* Lewis, traditionally used as medicine in Asia, is documented as an excellent source of bioactive components with excellent antioxidant activities (Suh *et al.*, 2011). Insects also have better protein digestibility compared with conventional sources of protein such as soya beans and milk protein casein (Akinnawo and Ketiku, 2000). The coconut rhinoceros beetle (*Oryctes rhinoceros* L.) and the green rose chafer (*Cetonia aurata* L.) (Coleoptera: Scarabaeidae) are also considered a delicacy in many parts of the world (Félix, 2019; Van Huis, 2021). Although, some documented information exists on the nutritional composition of *O. rhinoceros*, no similar knowledge on *C. aurata* has been reported. This study sought to explore novel functional properties of two scarab beetles (*C. aurata* and *O. rhinoceros*) from three different Counties (Embu, Murang'a and Nairobi) in Kenya. We hypothesized that the bio-functional properties of the two beetle species' larvae are influenced by the sampling geographical location and the species type. The intention was to bridge the scarce knowledge gap on the medicinal properties and nutrient profiles of these beetles, highlighting their possible use as functional ingredients in food fortification.

2 Materials and methods

Sample collection

A quasi-experimental design was conducted on *C. aurata* and *O. rhinoceros* larvae collected from Embu, Murang'a and Nairobi Counties in Kenya during the off-rainy season in January and February 2023. These three Counties were chosen based on their agricultural activities and diversity of organic waste suitable (cattle and poultry manure) as food substrates for the beetle larvae. From each county, random site where larvae naturally occurred was chosen, and the next sampling site was set to be distant from the previous one. Three sites were sampled in each county: Embu County (Nthangaiya (S00°27′58.9″, E037°33′58.6″), University of Embu (S00°30′41.3″, E037°27′29.5″) and Gachururiri (S00°42′25.6″, E037°28′58.7″)); Murang'a County (Kiunyu (S00°57′21.8″, E037°1′9.2″), Njoguini (S00°43′17.7″, E037°7′38.2″) and Kairi (S00°36′48.3″, E037°0′44.3″)) and Nairobi County (Mwiki (S01°13′47.2″, E036°57′1.0″), Ruai (S01°17′27.7″, E037°0′29.6″) and Kangemi (S01°15′53.9″, E036°44′37.5″)) (Figure 1). Morphological identification of the beetle larvae was performed using taxonomic keys from Bedford (1974) and CABI (2023) (Figure 2). About 500-600 larvae were collected at each site (Murang'a and Nairobi) and 250-300 were found in Embu. Those from the same area were merged for further processing. The collected larvae samples were then transported in perforated plastic containers with wet substrate within 24 hours to the Animal Rearing and Containment Unit (ARCU) at the International Centre of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya for further processing and analysis.

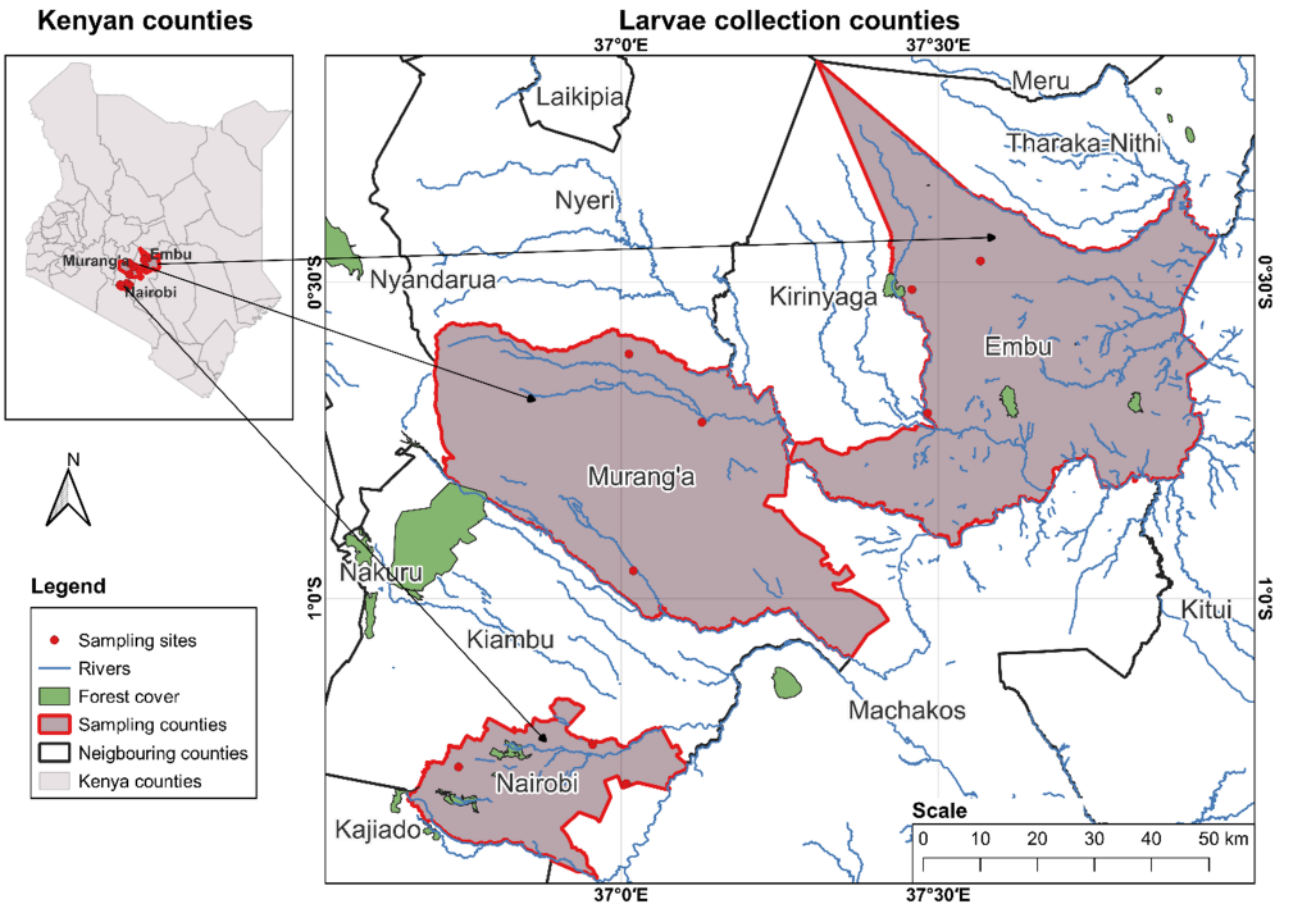

FIGURE 1 Map of Kenya showing the three countries in which the larvae collection was done. The red dots in each county represent the exact location in which the larvae were obtained.

Molecular identification of the beetle larvae from the various target sites

Molecular tools employed included DNA extraction from individual legs of the beetle larvae collected from the various sites using Isolate II Genomic Kit (Bio line), following the manufacturer's instructions. The obtained DNA was then amplified by polymerase chain reaction (PCR) using two primers (one for each insect) due to lack of universality of a single primer to target the COI mitochondrial gene in the *O. rhinoceros* beetle and 28S ribosomal RNA in the *C. aurata*. The COI gene section was targeted using LCO 1490 (forward, 5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO 2198 (reverse, 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) primers (Wilson, 2012). The mixture for PCR reaction contained 4 µL of *O. rhinoceros* DNA, 6 µL 5× HOT FIREPOL® Master Mix, 20.8 µL RNA free water, 0.6 µL forward primer and 0.6 µL of reverse primer (10 mM). The PCR program was set at 95 °C for 15 mins followed by 40 cycles of 95 °C for 45 secs, 53 °C for 1 min, 72 °C for 1 min and final extension at 72 °C for 5 mins. On the other hand, 28s rRNA primers were Lep D 2 forward (5′-AGTCGTGTTGCTTGATAGTGCAG-3′) and Lep-D2 reverse (5′-TTGGTCCGTGTTTCAAGACGGG-3′). For this reaction, the PCR mixture contained 2 µL of *C. aurata* DNA, 6 µL 5× HOT FIREPOL® Master Mix, 11 µL PCR water, 0.5 µL forward (10 mM) and 0.5 µL reverse primer (10 mM). The PCR program was set at 95 °C for 15 mins, followed by 40 cycles of 95 °C for 30 secs, 58.8 °C for 30 secs, 72 °C for 1 min and final extension of 72 °C for 10 mins. The resulting PCR amplicons were confirmed using gel electrophoresis (2% agarose), which was viewed under UV light using Kodak Gel Logic 200 Imaging System (SPW Industrial, Laguna Hills, CA, USA). The remaining PCR amplicons were cleaned using ExoSAP-IT (Affymetrix, Santa Clara, CA, USA) then sequenced by Macrogen Inc. (Amsterdam, the Netherlands). A BLAST tool (Sonnhammer and Durbin, 1995) was used to reveal the identity by querying the obtained sequences against the known sequences in Gene bank nr (https://blast.ncbi.nlm.nih.gov/Blast.cgi; accessed on 19 May 2023). Alignment was done using the MAFFT plugin in Geneious Prime software version 2020.2.2 (Kearse *et al.*, 2012), where the obtained sequences were aligned with available beetle related sequences sourced from Gene bank nr Database.

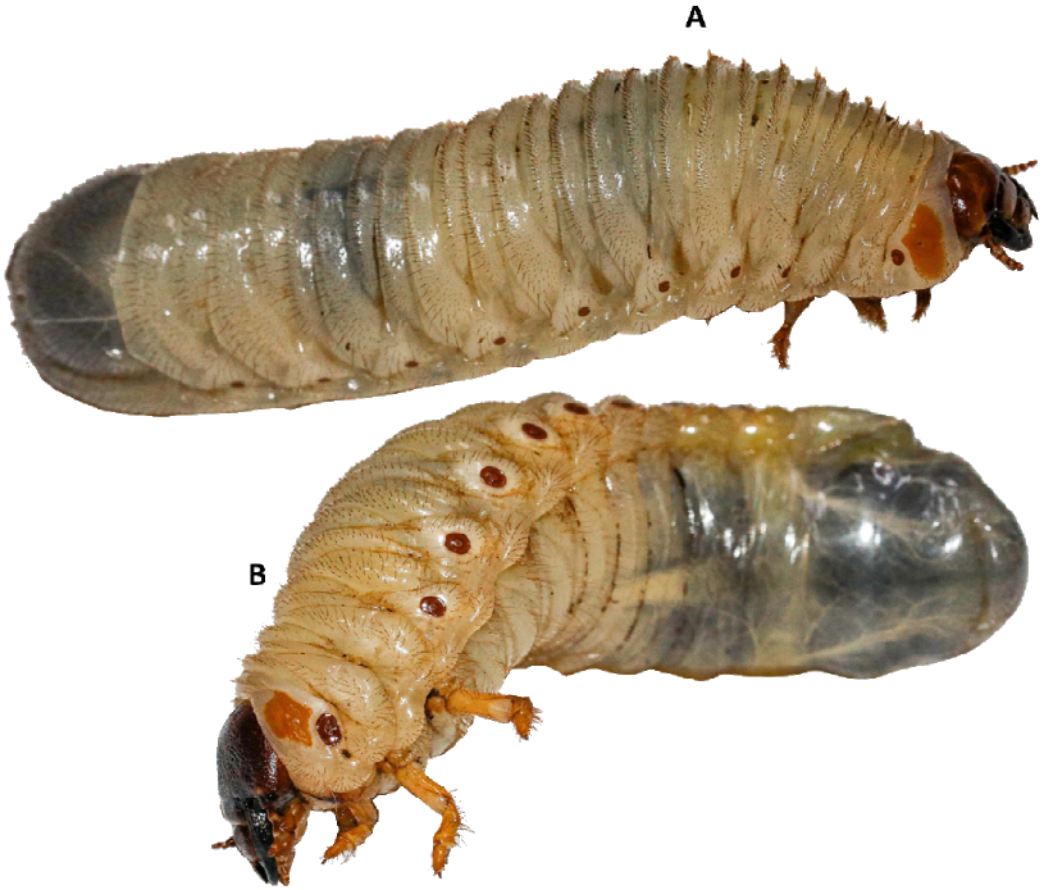

FIGURE 2 Morphologically distinct Scarab larvae; *C. aurata* (A, under 1.9× magnification) and *O. rhinoceros* (B). At the third instar, the larvae of *C. aurata* has a length of 55 mm, a diameter of 35 mm and three pairs of limbs, while *O. rhinoceros* has a length of 85 mm, a diameter of 45 mm and also three pairs of limbs.

PhyML 3.0 was used to create maximum-likelihood phylogenies, with automated model selection based on the Akaike information criteria (Guindon *et al.*, 2010).

Beetle larvae sample preparation for proximate analysis

The beetle larvae were thoroughly rinsed with tap water before being asphyxiated in –20 °C freezer for 3 hours to immobilize them. Thereafter, approximately 250-600 larvae per sampling site were degutted and then oven dried (WTB binder, Tuttlingen, Germany) for 24 hours at 60 °C. The dried samples were then ground using a GRT-750A(K) grinder (Zhejiang, China). The proximate parameters such as crude protein, moisture content, dry matter, fiber and ash were estimated following the protocols by Association of Official Analytical Chemists (Helrich, 1990). The moisture and dry matter content were determined by oven drying at 135 °C for 2 hours. Ash content was determined by igniting 1 g of the samples at 550 °C in muffle furnace (Heraeus-Kundendienst, Düsseldorf, Germany). Kjeldhal method was applied for the estimation of crude protein content. A conversion factor (K_p) of 4.76 was considered for nitrogen to protein conversion in the whole larvae to avoid overestimation due to presence of nonprotein nitrogen content as reported in the darkling beetle; *Alphitobius diaperinus* and the yellow mealworm; *Tenebrio molitor* by Janssen *et al.* (2017). Crude fiber was determined by acid and base digestion in a fiber analyzer SA30520200 (FIWE, Velp Scientifica, Usmate, Italy).

Total flavonoids content (TFC)

Aluminum chloride colorimetric assay was used to measure the TFC following the protocol described by Mokaya *et al.* (2022) with minor modifications. To 50 mg of each sample, 1 mL 50% methanol was added, vortexed using Vortex-Genie 2 then centrifuged at 4,200 rpm for 10 mins. The supernatant (500 µL) was then mixed with 3.2 mL of 50% methanol followed by introduction of 0.15 mL of 5% $NaNO_3$. After 5 mins of incubation at room temperature, 0.15 mL of 10% $AlCl_3$ was added and left for 1 min before subsequently adding 1 mL of 1M NaOH. The absorbance was measured at

510 nm against a blank made of all the components of the assay minus sample, using Evolution™ Pro UV-Vis Spectrophotometer (Thermo-Fisher Scientific, Maddison, WI, USA). Authentic standard quercetin (QE) (20-250 μg/mL) was also analyzed by the UV-Vis Spectrophotometer to generate a calibration curve for external quantification of the TFC, expressed in mg of QE equivalent per 100 g.

Total phenols content (TPC)

The TPC was measured in consonance with methods previously described by Mokaya *et al.* (2022). For each sample, 50 mg of the larvae powder were weighed and dissolved in 50% methanol, vortexed briefly and centrifuged for 10 mins at 4,200 rpm. Upon centrifugation, 500 μL of the supernatant was picked and mixed with 2.5 mL of 0.2 N Folin-Ciocalteu then left to stand for 5 mins at room temperature. After incubation, 2 mL of a 75 g/L Na_2CO_3 solution was added and left to stand for 2 hours. The absorbance was read at 760 nm using Evolution™ Pro UV-Vis Spectrophotometer (Thermo-Scientific, Maddison, WI, USA) against a blank made with all other reagents without the sample. Authentic standard gallic acid (GA) (20-250 μg/mL) was also analyzed by the UV-Vis Spectrophotometer to generate a calibration curve for external quantification of the TPC, expressed in mg of QA equivalent per 100 g.

Radical scavenging activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was adopted for the assessment of radical scavenging activity according to methods reported by Mokaya *et al.* (2022). Each sample (2.5 mg) was dissolved in 1 mL of 50,% methanol, vortexed for 1 min followed by centrifugation at 4,200 rpm for 10 mins. The supernatant (0.75 mL) was mixed with 1.5 mL of 5 mg/100 mL methanol DPPH and left to stand for 5 mins at room temperature. The blank comprised 0.75 mL of extract solution mixed with 1.5 mL of methanol whereas the control comprised 0.75 mL methanol mixed with 1.5 mL DPPH solution. The absorbance was read at 517 nm using the Evolution™ Pro UV-Vis Spectrophotometer (Thermo-Scientific, Maddison, WI, USA). The same procedure was repeated using extract dissolved in 50% hexane with a resultant concentration of 30 mg/mL. Methanol (50%) and hexane (50%) were chosen based on their effectiveness in extracting entomochemicals and lipids, respectively. The inhibition percentage was obtained using the following formula:

$$\% \text{ Inhibition} = (\text{Control absorbance} - \text{Sample absorbance})/(\text{Control absorbance} * 100) * 100.$$

Amino acids determination

The amino acid profile of the beetle larvae was determined according to previous methods reported by Murugu *et al.* (2021). Ground larvae (100 mg each) were hydrolyzed with 2 mL of 6 N HCl at 110 °C for 24 hours under nitrogen gas. Afterwards, the hydrolysates were concentrated *in vacuo* and the residues reconstituted in 1 mL of 0.01% formic acid/acetonitrile 95%. These mixtures were then vortexed for 30 secs, sonicated for 30 mins and finally centrifuged at 14,000 rpm for 15 mins. The supernatant was analyzed on an Agilent single quadrupole LC-MS 1200 series (Agilent Technologies, Inc., Santa Clara, CA, USA). The chromatographic separation was achieved using a Zorbax RX-C18, 4.6 × 250 mm, 5 μm column, operated at 40 °C. The mobile phase comprised of water (A) and acetonitrile (B) all supplemented with +0.01% formic acid. The gradient elution adopted was as follows: 0-6 min, 10% B; 6-7.5 min, 10-80% B; 7.5-10.5 min, 80% B; 10.5-13 min, 80-100% B; 13-18 min, 100% B; 18-20 min, 100-10% B; 20-25 min, 10% B. The flow rate was programmed as follows: 0-13 min; 0.25 mL/min, 13-25; 0.5 mL/min and the injection volume was 5 μL. The mass spectrometer was operated on API-positive mode at a mass range of *m/z* 50-600 at 70 eV cone voltage. An authentic standard of amino acids (Sigma-Aldrich, St. Louis, MO, USA) was also analysed by LC-MS and used to externally quantify the amino acids. All the analyses were performed in triplicates.

Fatty acids determination

The extraction of total lipids and methylation to fatty acids methyl esters (FAMEs) was conducted using a protocol similarly described by Ochieng *et al.* (2022). Folch-based extraction method, involving extraction of 1 g of each sample with 10 mL of dichloromethane/methanol (2:1 *v*/*v*) containing 0.05 mg/mL butylated hydroxytoluene (BHT), was applied. Upon centrifugation for 15 mins at 4,200 rpm, the supernatants were vacuum evaporated to remove the solvents and recover fats of ~250 mg. Subsequently, 100 mg of fat extracts were methylated by adding 1 mL of 100 mg/mL sodium methoxide, vortexing for 1 min, sonicating for 10 min

and incubation for 1 hour in a 70 °C water bath. The reaction was quenched by adding 100 µL of deionized water and vortexed for another 1 min. The FAMEs were extracted with 1 mL of GC-grade hexane, centrifuged at 14,000 rpm for 15 min and the supernatant dried over anhydrous sodium sulphate. The dry supernatant (1.0 µL) was analyzed using GC-MS on a 7890A GC (Agilent Technologies Inc., Santa Clara, CA, USA) coupled with a 5975C mass selective detector (Agilent Technologies Inc., Santa Clara, CA, USA). The column fitted on the GC was (5%-phenyl)-methylpolysiloxane (HP5 MS) low bleed capillary column (30 m × 0.25 mm i.d., 0.25 µm; J&W, Folsom, CA, USA). The carrier gas used was Helium flowing at the rate of 1.25 mL/min. The initial temperature was programmed to begin at 35 °C and end at 285 °C with a rising rate of 10 °C per min. The holding time for initial and final temperatures was 5 and 20.4 mins, respectively. The temperatures for the ion source and quadrupole mass selective detector were held at 230 °C and 180 °C, respectively. Spectral masses were acquired using electron impact (EI) at an acceleration energy of 70 eV and using full scan mode, fragment ions in the 40-550 *m/z* mass range were examined. A 3.3-min filament delay duration was chosen. In order to create a linear calibration curve (peak area vs concentration) with the following equation:

$$Y = 5\text{E} + 0.7X + 2\text{E} + 07,$$

serial dilutions of pure methyl octadecenoate standard (0.2-125 ng/L) were made from octadecanoic acid (95% purity) (Sigma-Aldrich, St. Louis, MO, USA) and analyzed by GC-MS in full scan mode. The resulting calibration curve was used to quantify the various fatty acids in the processed samples and had its coefficient of determination value as 0.9997. The data acquisition was done using ChemStation B.02.02 software where the integration parameters were set with initial threshold of 3, initial peak width of 0.01, initial area reject of 1 and shoulder detection was turned on. The identification of the fatty acids was done by comparing their retention times and mass spectral data with that of standard as well as from the published MS-libraries (National Institute of Standards and Technology (NIST) 05, 08, and 11).

Minerals determination

The mineral composition was determined according to the protocol previously described by Tanga *et al.* (2023). Briefly, the samples were processed by dry ashing in muffle furnace (Heraeus-Kundendienst, Düsseldorf, Germany) at 550 °C for 3 hours. Upon cooling, 5 mL of 6 N HNO_3 acid was added to 1 g of each sample and subjected to microwave-assisted digestion for 20 mins. The hydrolysates were tested for calcium, magnesium, iron, sodium, manganese, zinc and potassium using inductively coupled plasma emission mass spectrometer (Agilent 7900 ICP-MS, Inc., Santa Clara, CA, USA).

Statistical analysis

All statistical analyses were achieved using R Core Team (2017). The Shapiro-Wilk test was employed to establish the suitability of data sets for subsequent analyses. Data sets from any of the assays that violated the Shapiro Wilks test hypothesis of normal distribution were subjected to Kruskal-Wallis's test and means compared using Dunns' test using Agricole package (De Mendiburu and Simon, 2015). Conversely, data-sets that were normally distributed were subjected to one-way ANOVA and mean separated using Student-Newman-Keuls (SNK) test with the aid of Fisheries Stock Assessment (FSA) (Ogle and Ogle, 2017) and Agricole packages (De Mendiburu and Simon, 2015). All the assays were performed in triplicates.

3 Results

Morphological identification, molecular identification and phylogenetic analysis of the collected larvae

The larvae of both edible dung beetle species were creamy-white in colour, with a soft grub-like body. Their soft skin was covered in many setae (hair-like structures that perform sensory functions). They possessed three bodily parts: the head, the thorax and an enlarged dark abdomen housing the hindgut. They also featured three pairs of short functioning legs on the thoracic segment and large mandibles designed to devour decaying organic materials. *Cetonia aurata* larvae measured 55-60 mm long, 35-40 mm in diameter and had a brown head capsule that was 5.2-6.0 mm wide. On the other hand, *O. rhinoceros* larvae measured 85-96 mm in length, 45-50 mm in diameter, and had a brown head capsule that was 10.7-11.1 mm broad.

The BLAST analyses of the sequences of the two beetle larvae revealed 97% and 96.30% match with *C. aurata* and *O. rhinoceros*, respectively (Figure 3). These sequences obtained from *C. aurata* and *O. rhinoceros* have been deposited in the GenBank nr database under the accession numbers OQ925397.1 and OR115609.1, respectively.

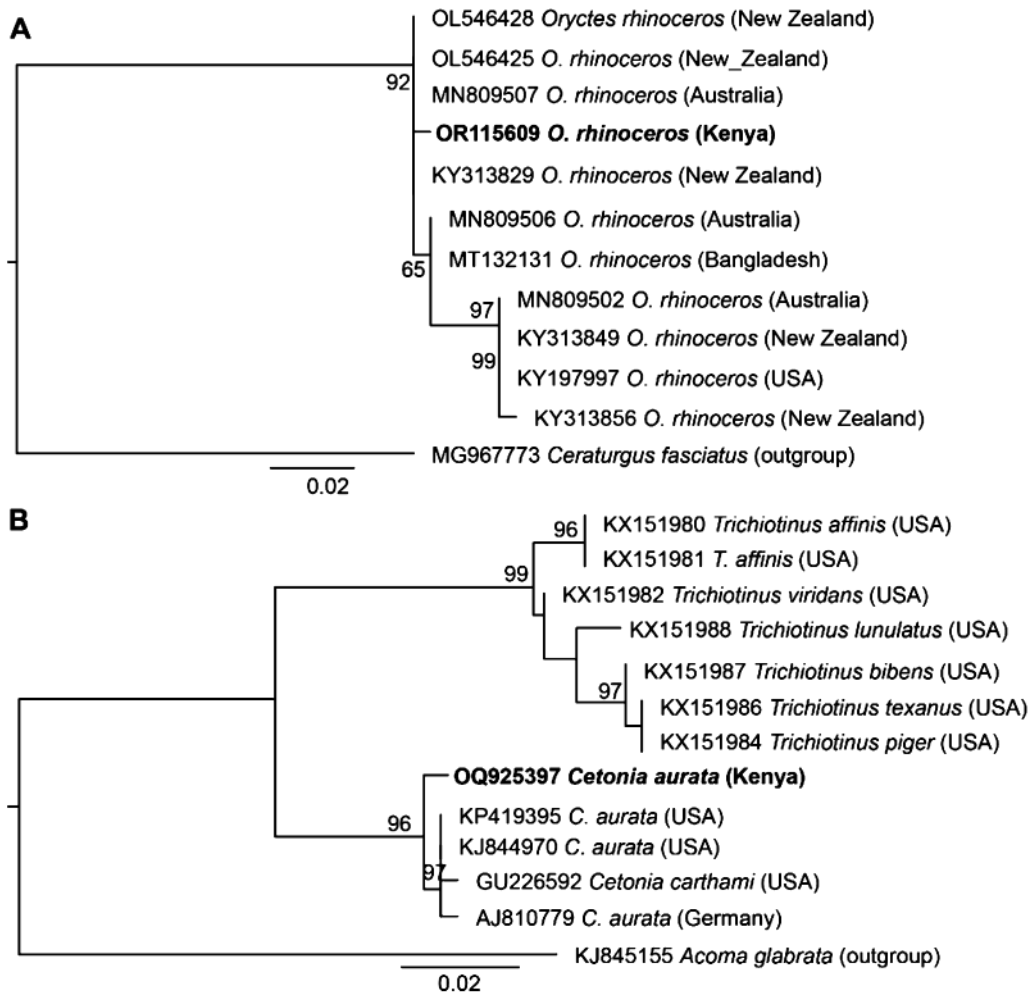

FIGURE 3 (A) Maximum likelihood phylogenetic trees of cytochrome oxidase subunit 1 (COI) gene sequences from *O. rhinoceros* larvae, and (B) 28s rRNA sequences from *C. aurata* beetle larvae. Sequences of *O. rhinoceros* and *C. aurata* in this study is indicated in bold. Bootstrap values are of percentage agreement among 1000 bootstrap replicates. Substitutions per site are indicated by branch length. The outgroup sequence at the bottom of each tree was used for rooting.

Radical scavenging activities

The radical scavenging activities (RSA) of methanolic extracts of *C. aurata* larvae ranged between 91.1-92.7%, while that of *O. rhinoceros* ranged between 55-60% (Figure 4A). The RSA of *O. rhinoceros* was significantly ($P < 0.05$) lower than that of *C. aurata* larvae. The RSA of the two beetle larvae did not vary significantly across the various locations but differed significantly ($F = 16.02$, $df = 5$, $P < 0.05$) between the species (Table 1). The RSA for hexane extracts of *C. aurata* and *O. rhinoceros* ranged between 33.83-88.60% and 71.60-86.70%, respectively (Table 1). Unlike the methanolic extracts, the RSA from the hexane extracts of *C. aurata*, varied significantly ($F = 10.60$, $df = 5$, $P < 0.05$) across the sampling locations with no discernible species effects (Figure 4B and Table 1).

Total flavonoids content and total phenols content

The total flavonoids content (TFC) values for *C. aurata* ranged between 17.78-34.90 mg QE/100 g, while that for *O. rhinoceros* ranged between 28.64-35.02 mg QE/100 g (Figure 4C). The total phenols content (TPC) of *O. rhinoceros* and *C. aurata* ranged between 44.10-50.82 and 50.19-54.60 mg GAE/100 g, respectively (Figure 4D). There was no influence of TPC and TFC across the species and target location (Table 1).

Proximate composition

The proximate components of the two beetle larvae species from the three collection sites are presented in Table 2. The ash, crude fiber and crude protein content of the larvae varied significantly across the sampled locations and species type. *Cetonia aurata* from Murang'a and Nairobi recorded significantly higher protein levels than *O. rhinoceros* in Murang'a and Embu. The

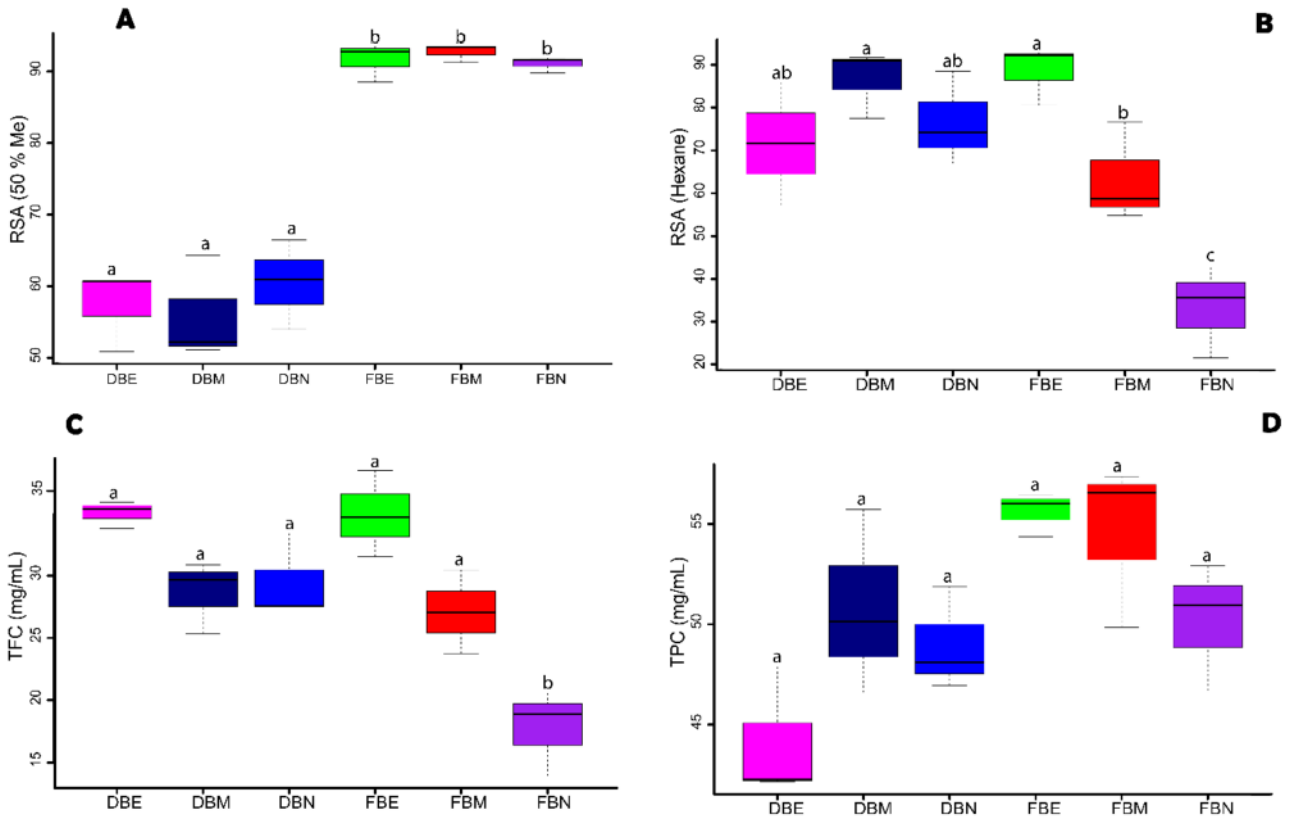

FIGURE 4 Box plots showing variations in radical scavenging activity (RSA); (A) in 50% methanol, (B) hexane and bio functional compounds; (C) total flavonoids and D-total phenols content; FB represents *C. aurata* larvae while DB is *O. rhinoceros* larvae. The last letter at end of FB or DB represents the site of collection. *C. aurata* from Nairobi (FBN), Murang'a (FBM), Embu (FBE), *O. rhinoceros* from Nairobi (DBN), Murang'a (DBM) and Embu (DBE). Boxplots with different letters on top are significantly different at $P < 0.05$.

TABLE 1 The antioxidant activities and entomochemical contents of beetle larvae in three different counties

Sampling location	Larvae	Total flavonoids (mg QE/100 g)	Total phenols (mg GAE/100 g)	RSA in He (%)	RSA of 50% Me (%)
Murang'a	*C. aurata*	27.08 ± 1.94^{a}	54.60 ± 2.38^{a}	63.40 ± 6.70^{b}	92.73 ± 0.74^{b}
	O. rhinoceros	28.64 ± 1.68^{a}	50.82 ± 2.65^{a}	86.70 ± 4.61^{a}	55.89 ± 4.22^{a}
Nairobi	*C. aurata*	17.78 ± 1.99^{b}	50.19 ± 1.83^{a}	33.83 ± 6.20^{c}	91.10 ± 0.64^{b}
	O. rhinoceros	29.48 ± 1.96^{a}	48.98 ± 1.49^{a}	76.60 ± 6.35^{ab}	60.48 ± 3.60^{a}
Embu	*C. aurata*	34.90 ± 1.99^{a}	55.62 ± 0.64^{a}	88.60 ± 4.00^{a}	91.62 ± 1.57^{b}
	O. rhinoceros	35.02 ± 0.64^{a}	44.10 ± 1.89^{a}	71.60 ± 8.26^{ab}	57.45 ± 3.27^{a}
	P-value	0.00018	0.298	0.000408	0.0184
	F-value	12.84	1.384	16.02	10.60
	Df	5	5	5	5

Me = Methanol, QE = quercetin equivalent, He = Hexane, RSA = radical scavenging activity, GAE = gallic acid equivalent, df = degrees of freedom. Means are expressed as mean ± standard error. In each column means with same letters are not significantly different at $P < 0.05$.

fiber content of *O. rhinoceros* in Murang'a and Embu was significantly higher, while the ash content of *C. aurata* (8.35%) was higher in Embu only.

Amino acids profile

Eight essential amino acids (EAA) and four non-essential amino acids (NEAA) were detected from *C. aurata* and *O. rhinoceros* (Table 3). Methionine and lysine varied significantly between the species except across loca-

TABLE 2 Proximate composition (expressed in % of DM) of the two larvae collected from Murang'a, Embu and Nairobi counties

Sampling location	Larvae	Dry matter	Moisture	Ash	Crude fiber	Protein
Murang'a	*C. aurata*	94.00 ± 1^{a}	6.00 ± 1.00^{a}	7.57 ± 0.53^{ab}	4.74 ± 0.03^{d}	63.37 ± 0.73^{a}
	O. rhinoceros	93.67 ± 0.33^{a}	6.33 ± 0.33^{a}	7.22 ± 0.36^{ab}	7.71 ± 0.01^{a}	44.43 ± 0.24^{b}
Embu	*C. aurata*	93.67 ± 0.67^{a}	6.33 ± 0.67^{a}	8.35 ± 0.26^{a}	–	–
	O. rhinoceros	90.33 ± 3^{a}	10.00 ± 3.00^{a}	6.13 ± 0.41^{bc}	7.57 ± 0.02^{b}	44.40 ± 0.427^{b}
Nairobi	*C. aurata*	92.00 ± 0.57^{a}	8.00 ± 0.58^{a}	7.88 ± 0.41^{a}	4.13 ± 0.01^{e}	63.01 ± 0.73^{a}
	O. rhinoceros	93.33 ± 0.33^{a}	6.67 ± 0.33^{a}	4.98 ± 0.40^{c}	6.06 ± 0.01^{c}	44.60 ± 0.83^{b}
	Df	5	5	5	4	4
	χ^2	7.3667	7.7651	13.093	13.548	12.182
	P-value	0.1948	0.2558	0.02253	0.008885	0.01605

Values are presented as mean ± standard error. In each column means with different superscript letters are significantly different at $P < 0.05$.

tions. Glutamic acid, isoleucine, leucine, tyrosine and phenylalanine were the most abundant amino acids in the two beetle larvae species (Figure 5). The green colour in Figure 5 that the concentrations of the amino acids were comparable in the two larvae species. The concentration of histidine was lowest in the samples from the target locations (Table 3).

Fatty acids profile

A total of 19 fatty acids were identified with 6 monounsaturated fatty acids (MUFA), 5 polyunsaturated fatty acids (PUFA) and 8 saturated fatty acids (SFA) (Table 4). Stearic acid, margaric and palmitic acids of the SFAs, oleic acid and palmitoleic acid of the MUFAs and arachidonic and linoleic acids of the PUFAs were the most abundant fatty acids detected. Linoleic and arachidonic acids (PUFAs) were not detected from larvae sampled from Murang'a. The ω-6/ω-3 ratio was 3.44 and 6.64 for *C. aurata* and *O. rhinoceros* from Embu, respectively. Comparative total fatty acid values of *C. aurata* and *O. rhinoceros* to that of other animal-based sources and veal are shown in Figure 6.

Mineral composition

A total of eight minerals were detected from the two beetle larvae collected from the three sites (Table 5). Apart from zinc, the concentrations of all minerals varied significantly ($P < 0.05$) between the species and target location. Calcium (15.75-22.65 mg/g) and potassium (13.62-22.88 mg/g) were the most abundant mineral elements recorded in the larvae of the two beetle species. The larvae of *O. rhinoceros* from Nairobi and Murang'a exhibited significantly ($P > 0.05$) higher values of calcium (20.42-22.65 mg/g) and zinc minerals (0.28-0.3 mg/g).

4 Discussion

The morphological characteristics of the larvae were comparable to those described in literature (Bedford, 1974; CABI, 2023). The two major species identified included *C. aurata* and *O. rhinoceros*, which is consistent to that reported by Mckenna *et al.* (2015) and Marshall *et al.* (2017) in USA and Australia, respectively. This study unravels that the entomochemical compounds (flavonoids and phenols) of these beetle species, are known to be responsible for the prominent radical scavenging activities (Lange and Nakamura, 2021). The total phenols contents recorded in two beetle species superseded 38.3 mg GAE/100 g and 3.65 mg/100 g reported in other beetle species (*Rhynchophorus phoenicis* and *Oryctes owariensis*, respectively). However, the phenol levels are lower than the values (541 mg GAE/100 g) reported in silk moth (*Samia cynthia ricini*) and in flower beetle; *Protaetia brevitarsis* Lewis 73.53 mg GA g–1 extract (Botella-Martínez *et al.*, 2021; Mokaya *et al.*, 2022; Suh and Kang, 2012; Ukoroije and Bobmanuel, 2019b). The TFC levels in *C. aurata* and *O. rhinoceros* were comparably lower than 93-231 mg/g range previously reported in the wild silk moths: *Anaphe panda*, *Gonometa postica* and *Argema mimosae* (Mokaya *et al.*, 2022). The differences in the chemical composition described among the insect species can be attributed to the bioactive component levels present in the dietary sources, which explains why insects foraging exclusively on vegetarian diets have higher bioaccumulants than

TABLE 3 Amino acid profile (mg/g) of *C. aurata* and *O. rhinoceros* sampled from three counties; Embu, Murang'a and Nairobi

Amino acids	Murang'a		Embu		Nairobi		*P*-value	Df	χ^2
	C. aurata	*O. rhinoceros*	*O. rhinoceros*	*C. aurata*	*O. rhinoceros*	*C. aurata*			
Essential amino acids									
Valine (V)	14.37 ± 0.53[a]	9.22 ± 4.37[a]	13.73 ± 0.30[a]	13.15 ± 0.23[a]	14.31 ± 0.41[a]	14.20 ± 0.89[a]	0.2901	5	6.1696
Threonine (T)	5.56 ± 0.38[a]	4.67 ± 2.34[a]	7.08 ± 0.17[a]	6.00 ± 0.06[a]	7.32 ± 0.12[a]	6.35 ± 0.42[a]	0.06232	5	10.497
Phenylalanine (F)	18.17 ± 0.99[a]	10.66 ± 5.27[a]	16.94 ± 0.91[a]	15.57 ± 0.27[a]	16.60 ± 0.23[a]	17.21 ± 1.98[a]	0.2989	5	6.076
Methionine (M)	5.36 ± 0.23[a]	1.64 ± 1.10[b]	3.99 ± 0.11[a]	5.01 ± 0.11[a]	2.78 ± 1.14[a]	5.62 ± 0.25[a]	0.0135	5	14.357
Lysine (K)	10.96 ± 0.34[a]	5.40 ± 2.47[b]	8.23 ± 0.35[a]	9.64 ± 0.27[a]	8.01 ± 0.27[a]	9.95 ± 0.26[a]	0.01103	5	14.848
Isoleucine (I)	19.53 ± 1.31[a]	12.22 ± 5.92[a]	18.35 ± 0.34[a]	16.85 ± 0.27[a]	19.21 ± 0.60[a]	13.39 ± 5.86[a]	0.3056	5	6.0058
Leucine (L)	12.27 ± 0.59[a]	8.70 ± 4.14[a]	14.87 ± 1.06[a]	12.02 ± 1.06[a]	13.28 ± 1.47[a]	13.44 ± 0.31[a]	0.25881	5	6.521
Histidine (H)	3.69 ± 0.23[a]	2.29 ± 0.78[a]	2.96 ± 0.16[a]	3.70 ± 0.55[a]	2.86 ± 0.06[a]	3.54 ± 0.27[a]	0.1001	5	9.2339
Non-essential amino acids									
Glutamic acid (E)	17.50 ± 0.96[a]	12.63 ± 5.33[a]	18.12 ± 0.46[a]	17.26 ± 0.57[a]	18.37 ± 0.76[a]	19.07 ± 0.40[a]	0.2549	5	6.5673
roline (P)	15.09 ± 0.39[a]	12.60 ± 4.48[a]	16.49 ± 1.05[a]	15.18 ± 0.41[a]	18.52 ± 0.22[a]	15.39 ± 0.67[a]	0.1325	5	8.462
Serine (S)	9.15 ± 0.07[a]	6.71 ± 3.00[a]	9.47 ± 0.31[a]	9.84 ± 0.26[a]	9.96 ± 0.29[a]	9.58 ± 0.10[a]	0.4074	5	5.0702
Tyrosine (Y)	16.88 ± 0.43[a]	11.01 ± 5.18[a]	17.04 ± 0.05[a]	15.47 ± 0.61[a]	16.57 ± 0.11[a]	16.43 ± 1.13[a]	0.09022	5	9.5146

In each column means with different superscript letters are significantly different at $P < 0.05$.

the organic waste feeders (Di Mattia *et al.*, 2019). The polyphenolic compounds identified in the two scarab beetles have been previously been reported to counteract age-related illnesses such as Parkinson's disease, Alzheimer's disease and others affecting the locomotor system (Del Carmen Villegas-Aguilar *et al.*, 2023) through their radical scavenging potentials.

The larvae of *C. aurata* larvae exhibited high RSA activities, which could be due to the presence of high bioactive peptides, particularly with lysine (L) in their sequences which has been found to have high antioxidant activity as identified from GWLK peptide obtained from *Zizyphus jujuba* derived protein hydrolysates (Memarpoor-Yazdi *et al.*, 2013; Lange and Nakamura, 2021). Other reports have also shown that proteins might contribute significantly to the RSA (Mokaya *et al.*, 2022; Suh *et al.*, 2011). The RSA from *C. aurata* larvae are comparable to that reported for *Protaetia brevitarsis* (92%) and *Allomyrina dichotoma* (81.5%), respectively), but slightly lower than that reported by Suh *et al.* (2010, 2011). The similarity of RSA in the hexane extracts from *C. aurata* and *O. rhinoceros* signifies equal solubility of the compounds present (Di Mattia *et al.*, 2019). Nevertheless, the RSA of hexane from *C. aurata* and *O. rhinoceros* was higher than the 7.8% activity previously reported in *Allomyrina dichotoma* (Suh *et al.*, 2010), but similar to values reported by Mokaya *et al.* (2022). The outstanding bioactive components and RSA identified in

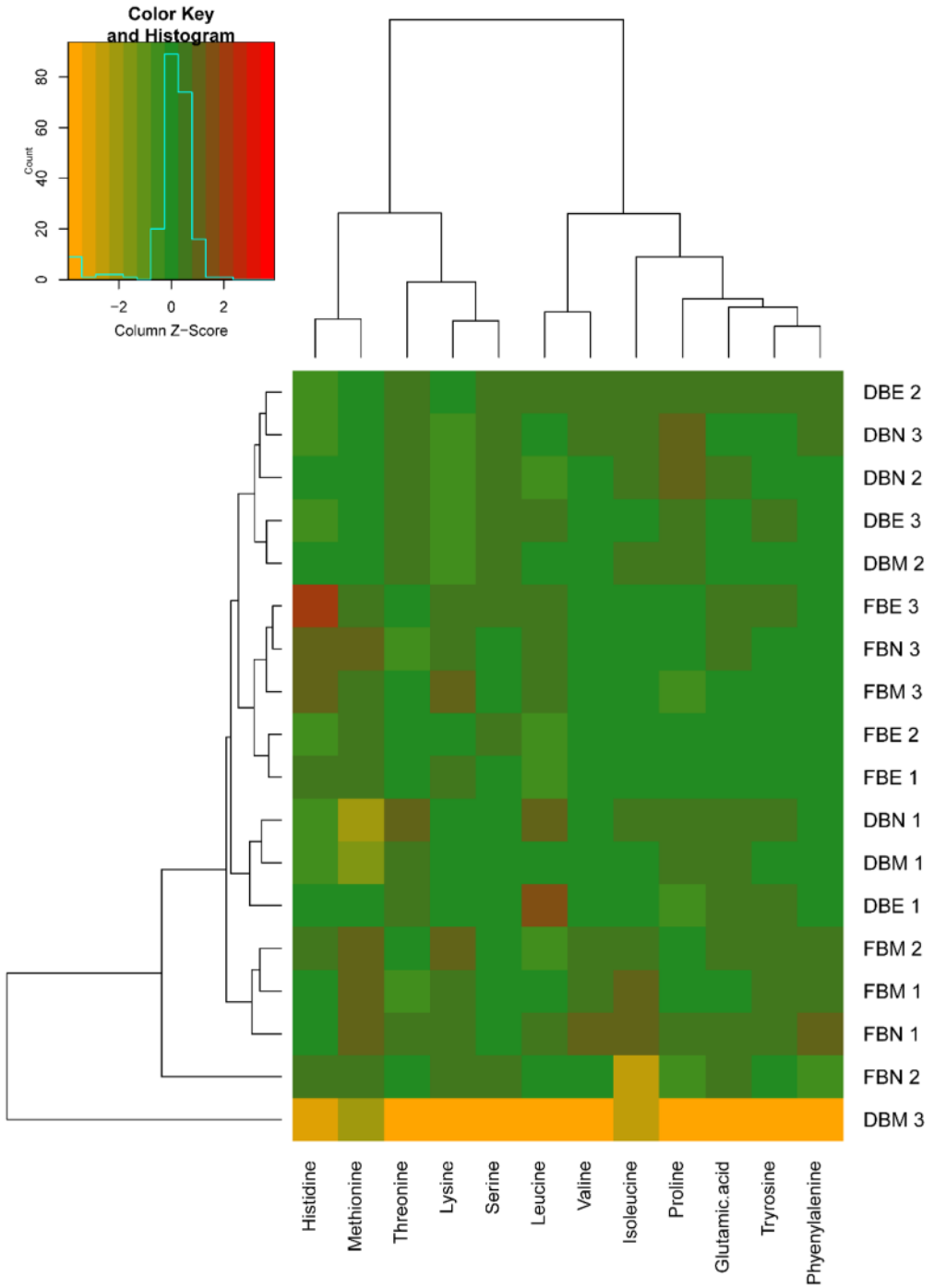

FIGURE 5 Heatmap showing concentrations of amino acids in *C. aurata* and *O. rhinoceros*. Dark-brown colour shows the highest concentration while orange and red colour represents the lowest concentration. The samples had comparable amounts since green colour was the most prevalent colour. The acronym FB represents *C. aurata* larvae while DB is *O. rhinoceros* larvae. The last letter at end of FB or DB represents the site of collection. The larvae of *C. aurata* from Nairobi (FBN), Murang'a (FBM), Embu (FBE), *O. rhinoceros* from Nairobi (DBN), Murang'a (DBM) and Embu (DBE).

this study have significant health implications. Therefore, the consumption of *C. aurata* and *O. rhinoceros* should be widely promoted as alternative food sources with reduced ecological impact (Fiebelkorn *et al.*, 2020).

Food susceptibility to microbial spoilage is determined by its moisture content with lower levels known to correspond to longer shelf-life (Banjo *et al.*, 2006). The two larvae of *C. aurata* and *O. rhinoceros* investigated had similar moisture content, which were inconsistent to that reported on longhorn beetle (*Analeptes trifasciata*) and *Oryctes boas* (rhinoceros beetle) air-dried at 50 °C for 2 days (Yang *et al.*, 2014). The ash content mirrors the micronutrients levels in the insect biomass and the value reported in this study are similar

TABLE 4 Fatty acid composition (in mg/g of DM) of beetle larvae obtained from Embu, Murang'a and Nairobi counties

FAME	Common name	Murang'a		Nairobi	Embu		*P*-value	Df	χ^2
		C. aurata	*O. rhinocerous*	*C. aurata*	*C. aurata*	*O. rhinocerous*			
Methyl dodecanoate	Lauric acid	9.42 ± 0.26^{c}	–	14.24 ± 1.55^{bc}	29.68 ± 3.80^{a}	22.64 ± 1.75^{ab}	0.018785	3	9.9744
Methyl tetradecanoate	Myristic acid	–	1.39 ± 0.13^{c}	16.83 ± 3.82^{bc}	39.97 ± 1.33^{a}	38.34 ± 1.09^{a}	0.02162	3	9.6667
Methyl 8-heptadecenoate	Margaric acid	–	–	–	113.66 ± 4.04	–	–	–	–
Methyl undecanoate	Undecanoic acid	–	–	–	36.66 ± 3.22	–	–	–	–
11-Methyl octadecanoate	Stearic acid	91.53 ± 4.00^{b}	21.8 ± 1.18^{d}	87.21 ± 1.47^{bc}	37.20 ± 3.25^{cd}	102.64 ± 4.37^{a}	0.012	4	12.856
Methyl 10*Z*-nonadecenoate	Nonadecanoic acid	–	–	14.88 ± 1.19^{a}	–	28.52 ± 2.25^{b}	0.04953	1	3.8571
Methyl hexadecanoate	Palmitic acid	1.29 ± 0.05^{a}	28.37 ± 1.41^{b}	107.11 ± 1.70^{c}	35.64 ± 2.96^{b}	52.43 ± 3.32^{d}	0.009074	4	13.5
Methyl eicosanoate	Arachidic acid	–	–	–	–	46.53 ± 4.67	–	–	–
	ΣSFA	102.24	51.56	240.27	292.81	291.1	–	–	–
Methyl-5*Z*,8,11*Z*,14*Z*-eicosatetraenoate	Arachidonic acid	–	–	22.21 ± 1.76^{b}	96.40 ± 5.75^{a}	69.91 ± 4.67^{c}	0.02732	2	7.2
Methyl 9*Z*,12*Z*-octadecadienoate	Linoleic acid	–	–	24.52 ± 1.86^{b}	34.90 ± 3.53^{a}	12.70 ± 0.64^{c}	0.02732	2	7.2
Methyl 9*Z*,12*E*-octadecadienoate	Conjugated linoleic acid	10.62 ± 0.29	–	–	–	–	–	–	–
Methyl-8*Z*,11*Z*,14*Z*-eicosatrienoate	Dihomo-gamma linolenic acid (DGLA)	–	–	–	32.30 ± 12.99	–	–	–	–
5,14,23-Octadecatrien-14,15-diol	14,15-Dihydroxy-linolenic acid	–	–	–	47.62 ± 14.49^{a}	12.44 ± 0.42^{b}	0.01672	1	10.001
	ΣPUFA	10.62	–	46.73	211.22	95.05	–	–	–
Methyl-9*Z*-hexadecenoate	Palmitoleic acid	85.39 ± 2.84^{a}	2.07 ± 0.75^{d}	38.43 ± 1.38^{c}	9.11 ± 0.53^{b}	94.04 ± 2.89^{e}	0.009074	4	13.5

TABLE 4 (Continued)

FAME	Common name	Murang'a		Nairobi	Embu		*P*-value	Df	χ^2
		C. aurata	*O. rhinocerous*	*C. aurata*	*C. aurata*	*O. rhinocerous*			
13-Methyltetradec-9-enoate	*Myristoleic acid*	–	–	–	4.04 ± 0.18	–	–	–	–
Methyl-9E-octadecenoate	Ricinoleic acid	–	–	–	–	12.75 ± 0.53	–	–	–
Methyl-9*Z*-octadecenoate	Oleic Acid	120.84 ± 2.89[a]	9.74 ± 0.27[c]	54.92 ± 2.31[b]	–	20.00 ± 2.48[d]	0.01556	3	10.385
Methyl-11*Z*-eicosenoate	Gondoic acid	–	–	–	–	26.20 ± 5.03	–	–	–
Methyl 6-octadecenoate	Petroselinic acid	–	12.86 ± 0.53	–	–	–	–	–	
	ΣMUFA	206.23	24.67	93.35	13.15	152.99	–	–	–
	ΣFAs	319.09	–	380.35	517.18	539.14			
	% SFA	32.04	–	63.17	56.62	53.99			
	% PUFA	3.32	–	12.29	40.84	17.62			
	% MUFA	64.63	–	24.54	2.54	28.38			
	ω-6/ω-3 ratio	–	–	–	3.44	6.64			

In each row means with the same letter are not significantly different, – means not detected.

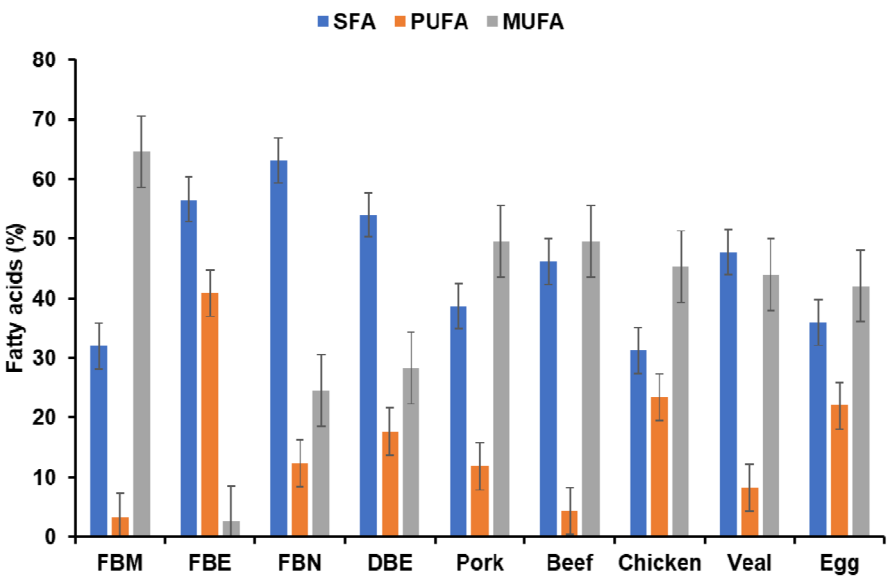

FIGURE 6 Bar chart showing comparative values of fatty acids found in beetle larvae (*C. aurata* and *O. rhinoceros*) to that of other animal and plant based sources (on a dry matter basis, or DM). Source: FAO/GoK (Ghosh *et al.*, 2017). FB represents *C. aurata* larvae while DB is *O. rhinoceros* larvae. The last letter at end of FB or DB represents the site collected. The larvae of *C. aurata* from Murang'a (FBM), Embu (FBE), Nairobi (FBN) and *O. rhinoceros* from Embu (DBE). SFA = saturated fatty acids, PUFA = polyunsaturated fatty acids, MUFA = Monounsaturated fatty acids.

TABLE 5 Mineral composition (in mg/g of DM) of *C. aurata* and *O. rhinoceros*

Sampling location	Larvae	Na	Mg	K	Ca	Mn	Fe	Cu	Zn
Nairobi	*C. aurata*	2.45 ± 0.01^{c}	3.15 ± 0.04^{d}	16.25 ± 0.23^{c}	17.95 ± 0.26^{bcd}	0.05 ± 0.01^{c}	0.55 ± 0.01^{c}	0.12 ± 0.01^{a}	0.27 ± 0.01^{a}
	O. rhinoceros	6.11 ± 0.03^{a}	5.04 ± 0.09^{a}	17.97 ± 0.06^{b}	22.65 ± 1.75^{a}	0.09 ± 0.01^{a}	0.74 ± 0.01^{a}	0.05 ± 0.01^{b}	0.30 ± 0.01^{a}
Murang'a	*C. aurata*	2.33 ± 0.01^{c}	3.33 ± 0.01^{d}	17.27 ± 0.13^{bc}	18.83 ± 0.19^{bc}	0.05 ± 0.01^{c}	0.59 ± 0.01^{b}	0.11 ± 0.01^{a}	0.28 ± 0.01^{a}
	O. rhinoceros	6.05 ± 0.30^{a}	4.59 ± 0.18^{b}	17.38 ± 0.89^{bc}	20.42 ± 0.83^{b}	0.07 ± 0.01^{b}	0.74 ± 0.02^{a}	0.04 ± 0.01^{b}	0.28 ± 0.02^{a}
Embu	*C. aurata*	2.50 ± 0.12^{c}	3.29 ± 0.04^{d}	22.88 ± 0.26^{a}	17.43 ± 0.36^{cd}	0.05 ± 0.01^{c}	0.53 ± 0.01^{c}	0.11 ± 0.01^{a}	0.28 ± 0.01^{a}
	O. rhinoceros	4.90 ± 0.04^{b}	3.57 ± 0.02^{c}	13.62 ± 0.02^{d}	15.75 ± 0.19^{d}	0.06 ± 0.01^{c}	0.54 ± 0.01^{c}	0.02 ± 0.01^{d}	0.22 ± 0.01^{a}
	P value	2.07e^{-07}	2.64e^{-06}	6.63e^{-06}	0.00161	0.000244	0.000244	3.7e^{-07}	0.1219845
	F value	378.4	160.8	117.9	17.51	34.3	34.3	311.2	8.69
	Df	5	5	5	5	5	5	5	5

Means are expressed as mean ± SE. In each column means with same letters are not significantly different at $P < 0.05$.

to that reported for most insects (Verkerk *et al.*, 2007). Fiber content in edible insects is chiefly comprised of the chitinous exoskeleton (Ozimek *et al.*, 1985; Hu *et al.*, 2010; Zhang *et al.*, 2000) and may vary depending on the species and the physiological needs. The fiber content of *O. rhinoceros* and *C. aurata* varied across the species and collection sites. The fiber content recorded for *C. aurata* and *O. rhinoceros* differed from that of *H. parallela* (Yang *et al.*, 2014) and *Rhynchophorus phoenicis f.* (Omotoso and Adedire, 2007). The results for crude protein reported in this study falls within the protein range (21-70 g/100 g of DM) estimated for many coleopteran beetles (Bukkens, 1997; Hu *et al.*, 2010; Verkerk *et al.*, 2007). Crude protein analysis was based on K_p of 4.76 to avoid overestimation from non-protein sources while using K_p of 6.25 (Janssen *et al.*, 2017). Considerable variation of crude protein in insects across different locations have previously been reported by several authors (Hu *et al.*, 2010) particularly for *H. parallela*. These large variations in protein content between the two larvae might be due to differences in growth conditions, and the differences in diet (organic waste) conversion efficiency to appreciable proportion of protein content (da Silva Lucas *et al.*, 2020; Thomas, 2018). Interestingly, the protein content of *C. aurata* and *O. rhinoceros* larvae compared favourably with 40-75 g/100 g of DM to conventional protein sources such as beef, fish, soya bean and pork thereby valorising the larvae of these beetle species as nutritious diets with potential to significantly contribute to over 85% of protein RDA. The larvae pose as a sustainable protein source with health benefits to young babies, elderly, pregnant and lactating women with malnourished conditions.

The characterization of amino acids profiles of novel food sources such as insects is paramount for making nutritionally informed decisions akin to combating malnutrition. The unravelling of isoleucine, phenylalanine, valine and lysine as the predominating essential amino acids in the two larvae corroborates findings by earlier studies on *O. rhinoceros* larvae (Okaraonye and Ikewuchi, 2008) and *H. parallela* (Hu *et al.*, 2010). These amino acids (isoleucine, phenylalanine and valine) comprise a list of hydrophobic amino acids which offer bioactive remedial cushions against some health risks (Atowa *et al.*, 2021). Lysine is a precious amino acid with deficient levels widely documented in cereal and plant-based products (Hu *et al.*, 2010). Its detection in appreciable levels indicates that *C. aurata* and *O. rhinoceros* larvae are nutritional resources that can be utilized to enrich commonly consumed plant-based food products with imbalanced nutrient profiles. Lysine is precursor in the synthesis of carnitine, which is instrumental in fat metabolism and also plays an important role in the production of hormones, antibodies and enzymes. Sulphur-containing amino acids thio-ether linkages such as methionine was the only detected sulphur containing

amino acid from *C. aurata* and *O. rhinoceros* larvae. Methionine are actively involved in the detoxification mechanisms and production of other metabolically important compounds such as choline (Ademola *et al.*, 2017; Fogang Mba *et al.*, 2017). The detection of glutamic acid as the most abundant non-essential amino acids concords to findings reported in other Coleopteran beetles (Oriolowo *et al.*, 2020; Hu *et al.*, 2010). Glutamic acid is an important savory amino acid, whose deficiency in food leads to glutamate formiminotransferase disorder. Based on the amino acids' profiles of the two beetle species larvae, it is admissible that such novel insects can be part of dietary diversification aimed at supplementing conventional low quality plant diets to alleviate malnutrition.

The ratio of total fatty acids, SFA: MUFA: PUFA mirrors the patterns previously reported in *Protaetia brevitarsis* larvae (Yeo *et al.*, 2013) and *O. rhinoceros* larvae (Okaraonye and Ikewuchi, 2008). Further, the detection of stearic and palmitic acids of the SFA, palmitoleic and oleic acids of the MUFAs as the abundant fatty acids ratifies similar findings from previous reports on edible beetles; *Apomecyna parumpunctata*, *O. monoceros*, *O. boas* (Thomas and Kiin-Kabari, 2022). Oleic acid is a common MUFA in living systems and is known to counteract risks of cancer, heart attack, atherosclerosis and dementia (Yeo *et al.*, 2013). Stearic and palmitic acids are important ingredients in the food industry for texture and tenderness modification of products. Higher palmitic acid levels, especially in *C. aurata* from Nairobi, portends high atherogenicity due to their propensity to increase low-density lipoprotein (LDL) cholesterol (Womeni *et al.*, 2009). Despite the negative health perceptions associated with saturated fatty acids, their coexistence with unsaturated fatty acids is paramount for a synchronous complementary functionality (Akpossan *et al.*, 2015). The threshold ratio of omega-6/omega-3 fatty acids (ω-6/ω-3 ratio) were recorded for *C. aurata* and *O. rhinoceros*, which implies that the larvae could be considered as targets of optimal health benefits (Fogang Mba *et al.*, 2017). However, these ratios were dependent on the detectable fatty acids from each larval species sampled from the three sites. Similar results have been reported from edible adult dung beetles like *Onthophagus seniculus*, *Copris nevinsoni*, and *Liatongus rhadamitus* (Bophimai and Siri, 2010). Variations in the fat content of edible insects are dependent on many factors including dietary sources, species, location, temperature and intra-tissue differences within an organism (Jantzen da Silva Lucas *et al.*, 2020; Manditsera *et al.*, 2019; Thomas and Kiin-Kabari, 2022).

Edible insects are reportedly credible sources of micro-nutrients (FAO and WHO, 2001; Van Huis *et al.*, 2021). The minerals found in this study had been previously detected from *Oryctes Owariensis*, *H. parallela* and *Rhynchophorus phoenicis f.* (Omotoso and Adedire, 2007; Ukoroije and Bobmanuel, 2019a; Yang *et al.*, 2014). The variabilities in the concentration of individual minerals detected from the larvae species may be as result of dietary sources, age and ecotype (Atowa *et al.*, 2021; Paiko *et al.*, 2014). Many studies have reported low calcium content of edible insects while alluding to their lack of mineralized skeleton (Kim *et al.*, 2019), however, in this study, calcium was an abundant mineral. The high calcium content maybe attributed to their detritus nature of devouring decaying organic matter and in the process, may sequestrate calcium from the soil (Banjo *et al.*, 2006). Abundance of calcium over other minerals has also been reported from larvae of long-horned beetle, *Apomecyna parumpunctata* Chev. (Thomas, 2018). In fact, the levels of calcium, iron and zinc found in this study supersedes the levels of 4.5, 1.8 and 4.6 mg/100 g, respectively, reported by Williams (2007) in beef. Owing to their cheap and sustainable accessibility, beetle larvae can be better sources of micro-nutrients than conventional sources of protein. The integration of larvae meal or powder or flour in food formulations would likely contributes to 68.88%, 50.5% and 18% of the RDA for iron, zinc and calcium, respectively. Hence, these larvae can be rich candidates for combating micronutrient deficiency common majority of the modern populace, especially children. Iron and zinc deficiencies are the leading causes of anemia and cognitive disorders in under five years old children. These cases are rampant in areas associated with over-reliance on cereals as staple food and minimal intake of animal products (Roohani *et al.*, 2013). Integrating sustainable protein sources such as the beetle larvae reported herein into cereal based staple products may be a great step towards alleviation of micro-nutrient related disorders among the undernourished population.

5 Conclusion

Here, it is experimentally concluded that the larvae of the two beetle species have the potential to serve as nutritious food and therapeutic source. The properties are owed to their high protein content, considerable levels of essential micro-nutrient (zinc, iron and calcium) as well as their entomochemical profiles. The larvae of *C. aurata* and *O. rhinoceros* could therefore substantially

contribute to the daily protein requirements for adults and children, aiding in the maintenance and growth of bones, respectively. Further, the presence of phenolic compounds would boosts the capacity to prevent oxidation of body molecules like lipids thereby fostering good health. Unlike the geographical location of the beetle species, the species type had a huge influence on the bio-functional composition of the larvae except for fatty acid value which might have been largely affected by diet and habitat. Further studies are required on protein digestibility, vitamin profile, potential allergenicity and safety aspects of the beetle species. Given the seasonality of occurrence of wild beetle species and limited supply and availability exploration of large-scale commercial rearing of the larvae, processing techniques and shelf-life research would be crucial to extend the nutritional advantages they confer to other communities across low and middle-income countries (LMIC). Furthermore, the detritivorous nature of the beetle would impact positively to the environment by reducing greenhouse gas emissions and waste management.

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Conceptualization, methodology, resources, project administration: S.S.M, C.M.M., N.M.K. and C.M.T.; software, investigation, supervision, funding acquisition: C.M.M. and C.M.T.; validation: S.S.M., C.M.M., H.O.M, J.M. and C.M.T.; formal analysis, data curation, visualization: S.S.M. H.O.M., B.O.O. and C.M.M.; writing – original draft preparation: S.S.M., writing – review and editing: S.S.M., C.M.M., H.O.M., B.O.O., N.M.K., J.M., and C.M.T. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors have no conflict of interest to declare.

Data availability

The 28s and COI sequences obtained for the larvae are available in the NCBI Sequence Read Archive (SRA) under accession numbers OQ925397.1 and ORI15609.1.

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Institutional review board statement

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