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ORIGINAL PAPER

Elemental, fatty acid, volatile and amino acid profiles coupled with antidiabetic, antioxidant, and enzyme inhibitory activities of selected edible insects from Uganda

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Abstract

Edible insects are traditionally consumed in Uganda and are increasingly recognized as nutritionally valuable and environmentally sustainable food resources. In this study, the nutritional composition, chemical profiles, biological activities, and safety of three commonly consumed edible insects—*Ruspolia differens* (green and brown morphs) and *Macrotermes nigeriensis*—were comprehensively evaluated. Extraction yields differed markedly among species, with *R. differens* morphs showing substantially higher methanolic yields (46.74–48.93%) than *M. nigeriensis* (22.57%). Fatty acid analysis revealed oleic acid as the predominant component (42.2–44.1%), followed by palmitic and linoleic acids, indicating a lipid profile rich in mono- and polyunsaturated fatty acids. Volatile profiling demonstrated species-specific aroma signatures, with *M. nigeriensis* characterized by acetic acid and pyrazines, while *R. differens* morphs exhibited high levels of isovaleric acid and diverse aromatic compounds. Amino acid analysis showed that methanolic extracts generally contained higher concentrations and broader profiles than aqueous extracts, with taurine, glutamic acid, alanine, and ethanolamine as the dominant components. Elemental analysis by ICP–MS indicated appreciable levels of essential minerals (K, Ca, Fe, Zn, and Se), while potentially toxic elements (As, Cd, Hg, and Pb) were detected at low and variable concentrations depending on species and morph. In enzyme inhibition assays, *R. differens* methanolic extracts exhibited strong α-glucosidase inhibition (up to 88.16%), comparable to acarbose, whereas α-amylase and cholinesterase inhibition remained limited. Antioxidant activity was moderate, with *M. nigeriensis* aqueous extract showing the highest DPPH• and $ABTS^{\bullet+}$ scavenging capacities among samples. Antimicrobial activity was generally weak to moderate, with methanolic extracts showing greater efficacy, particularly against *Candida albicans*. Cytotoxicity and genotoxicity assessments using human dermal fibroblasts and lymphocytes revealed no significant adverse effects at the tested concentrations, with cell viability remaining above 80% and chromosomal aberration indices comparable to negative controls. Overall, the results demonstrate that Ugandan

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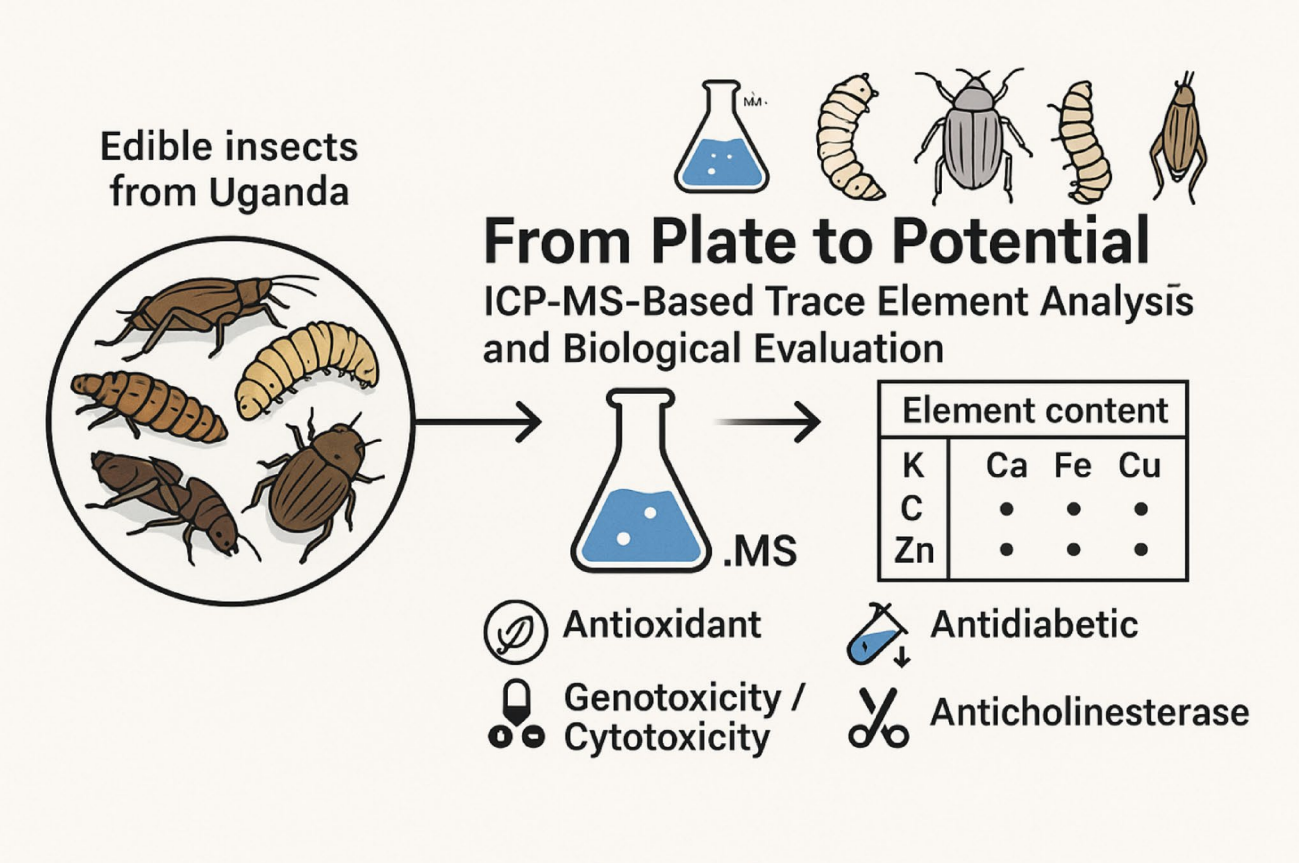
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edible insects possess favorable nutritional profiles, selective bioactivities, and an acceptable preliminary safety profile, supporting their potential as alternative food resources. However, controlled rearing, standardized processing, and further studies on bioavailability and long-term consumption are warranted.

Graphical abstract



Keywords Amino acid · anticholinesterase · antidiabetic · cytotoxicity · element content · Uganda edible insects

Introduction

Entomophagy, the consumption of insects, is a long-established dietary practice in many traditional cultures worldwide and has recently gained increased scientific and commercial interest. Edible insects are nutritionally rich, providing high-quality protein, essential amino acids, lipids, vitamins, minerals, and energy, often comparable to conventional livestock on a dry weight basis. Moreover, their high feed conversion efficiency, rapid growth, and low environmental footprint make insects a promising sustainable alternative protein source. To date, more than 2 000 edible insect species have been reported globally, with consumption being most prevalent in tropical and subtropical regions, while interest in Western countries has risen in response to projected increases in global protein demand driven by population growth, urbanization, and changing dietary patterns [1].

Edible insects have attracted increasing scientific interest due to their high nutritional value, environmental sustainability, and potential role in future food systems. Previous studies have demonstrated that commonly consumed insect species are rich sources of high-quality protein, essential amino acids, unsaturated fatty acids, and bioavailable micronutrients such as iron and zinc. For example, winged termites traditionally consumed in East Africa were reported to possess favorable nutrient profiles comparable to conventional animal-based foods [2]. Beyond nutritional aspects, edible insects have been discussed as promising alternatives to conventional livestock in the context of global food security, although challenges related to safety, processing, and consumer acceptance remain [3]. Recent advances in processing technologies—including drying, extraction, and

fermentation—have further expanded the potential applications of edible insects in functional foods and nutraceuticals, while also improving their safety and shelf-life [4]. Within this framework, comprehensive chemical and safety evaluations of traditionally consumed insect species are essential to support their wider utilization.

Edible insects offer notable health benefits due to their rich nutrient composition, being high in polyunsaturated fatty acids such as linoleic (ω-6) and α-linolenic acid (ω-3), essential amino acids, vitamin B12, iron, zinc, fiber, and antioxidants, and although they lack long-chain omega-3 fatty acids (EPA and DHA) typical of fish, their high PUFA-to-saturated fat ratio supports a heart-friendly, low-cholesterol nutritional profile [5].

Diabetes mellitus (DM) and related metabolic disorders are increasingly recognized as major global health challenges, affecting both adults and children across diverse populations [6]. DM is marked by persistent high blood sugar due to insufficient insulin or impaired signaling. With the global population expected to exceed 9 billion by 2050, the search for sustainable food sources is critical. Edible insects offer not only high-quality protein but also bioactive properties, including antidiabetic effects. Species like *Musca domestica*, *Alphitobius diaperinus*, and *Gryllodes sigillatus* inhibit DPP-IV, an enzyme that degrades glucose-regulating incretin hormones, suggesting potential benefits in managing type 2 diabetes [7].

Insect-derived fats are rich in polyunsaturated fatty acids, particularly linoleic and α-linolenic acids, which have been associated with improved insulin sensitivity, enhanced glycemic control, reduced blood pressure, and a lower risk of metabolic disorders, and traditional practices further suggest antidiabetic potential, as insects such as *Thasus gigas* in Mexico and Ulomoides dermestoides in parts of Africa and Argentina are consumed for managing DM, although scientific evidence supporting these uses remains limited [8].

Alzheimer's disease (AD) is a prevalent age-related neurodegenerative disorder and a growing public health issue. Its progression is closely associated with β-amyloid (Aβ) accumulation and resulting neurotoxicity. Current treatments are limited to a few FDA-approved drugs, including acetylcholinesterase inhibitors and NMDA receptor antagonists, which offer only short-term symptom relief in mild to moderate stages and are often accompanied by side effects [9].

Emerging evidence indicates that diabetes mellitus significantly increases the risk of Alzheimer's disease (AD), with affected individuals showing approximately a 1.57-fold higher likelihood of developing AD, and although the precise mechanistic link remains under investigation, both disorders share overlapping pathological features including impaired glucose metabolism, insulin resistance, blood–brain barrier dysfunction, chronic neuroinflammation, progressive cognitive decline, and frequently associated neuropsychiatric symptoms such as depression [10].

All extant termites are Isoptera within the order Blattodea. A typical termite colony comprises three main social castes: worker, soldier, reproductive/queen castes, and the most frequently eaten form is the adult winged alates. Termites are found all over the world and are considered valuable food sources in many countries especially in Africa. A total of 53 termite species are documented as edible [11]. Edible termites are an important part of East African food culture. In underdeveloped countries, where malnutrition is widespread, these insects are particularly important because of their nutritional properties [12]. Uganda is one of the 20 countries in the world with the highest prevalence of undernourishment [13]. So much so that in some areas termite mounds are privately owned. They are part of the inheritance when people die [11]. Termites appear in abundance during the first rains. In general, during the rainy season in April and October, the termites are collected. Typically, light traps or mercury lamps were used to collect termites. Traditionally, individuals collect them for food by setting bowls of water beneath light sources to use as traps during the night [14]. The *M. nigeriensis* also known as the "African winged termites" can be consumed in powdered form and is usually served with maize meal porridge. Raw termites were described as one of the cheapest luxuries in Uganda [15]. In order to meet the high market demand during dry seasons, individuals employ various processing methods such as salting, sun-drying, frying, blanching, roasting, toasting, or grinding into powder [16]. Termites are eaten raw directly or are prepared by blanching in boiling water, then sun-dried and finally fried in their own fat.

Ruspolia differens also known as the "cone-head" bush cricket is well adapted to the grassland habitat. In Uganda, *R. differens* goes by the name "nsenene" in the Luganda and Ganda language, also known in some places as "senesene". This is the word for the month of November, when they are abundant. It is native to sub-Saharan Africa. It is a highly prized, the most widely consumed and commercialised edible insect especially in Central Uganda [17, 18]. It's a delicacy, often fried and enjoyed as a crunchy snack during certain seasons. Also they can be eaten raw after removing the wings. It is primarily gathered from natural sources, both for domestic consumption and trade, although efforts are being made to mass produce it on an industrial scale. The roads can be completely blocked by people coming from the countryside to collect nsenene in Kampala, Uganda [19]. *R. differens* is harvested in the morning, before the sun rises, between 06.00 and 07.00 am, when they are inactive and unable to fly. They are collected by hand-picked from their

host plants or by light trapping soon after dark. Also, they are attracted to light, even though streetlights in the evening, making them easy to collect. For mass collection, local light traps are used between 10:00–2:00 pm [18]. It is sold in their raw state or processed. Before washing and cooking, the appendages, wings, legs and ovipositor are removed. Dry frying or pan frying, air drying, boiling and sundrying methods are used to process *R. differens*. For long-term storage, it is salted, then cooked and sun-dried. As the insects are only seasonally available, sun drying and roasting insects are often the methods of preservation. Sautéing and deep frying are often preferred by commercial processors and are often seen in outdoor markets. It's more expensive than beef [18, 20]. As well as taste and nutritional value, customs, ethnic preferences or prohibitions can also determine whether insects can be eaten or not [21]. For this reason, religion and tradition have had an impact on people's consumption patterns of nsenene. For example, women of the Baganda tribe in Uganda are prohibited to consume nsenene. In addition, in Uganda, the customs and traditions of the Baganda tribe prohibit the consumption of raw nsenene [22]. The *R. differens* exhibits colour polymorphism, up to eight colours forms have been reported, with green and brown being the most common [21, 23]. In this study green and brown coloured nsenene were studied.

According to researchers the most commonly consumed insects in Uganda are termites and grasshoppers. So much so that in Uganda, grasshoppers are sold at 40% more than beef [11, 20, 24]. According to a study, common insect orders used as food in Lango sub-region, northern Uganda included: Isoptera, Orthoptera, and Hymenoptera [25]. Whereas in this study, the authors systematically searched Web of Science and Google Scholar for studies on edible insect species in Uganda, listing 52 edible insect species from nine orders (Fig. 1).

Of the edible insects, Orthoptera represents the major group (17 species), followed by Lepidoptera (13 species), Isoptera (10 species), Coleoptera (3 species), Diptera (3 species), Ephemeroptera (2 species), Hymenoptera (2 species), Hemiptera (1 specie), Mantodea (1 specie).

The consumption of edible insects by various peoples in Africa is noted in numerous publications, old as well as recent. According to the anthropoentomophagic literature in Africa, a taxonomic and geographic analysis of edible insects that are consumed by people has not yet been completed. In this regard, approximately 464 to 619 edible insect species have so far been recorded in Africa as a part of traditional diets [17, 26, 27]. Reports indicate that up to 20 insect species have been consumed in Uganda [18]. In addition, in Uganda, there are knowledge gaps and very little published information on edible insects [17, 20, 25]. Therefore, in this study, the list of edible insects of Uganda is revised based on international peer-reviewed literature (Table 1) [26–40]. Each data indicates the scientific name, order, family, English and local name, edible life stage and consumed countries according to the world peer-reviewed literature. The table shows that even in the neighboring countries of Uganda, there are differences in the species of edible insects [17, 26, 27]. An annotated systematic list is given below.

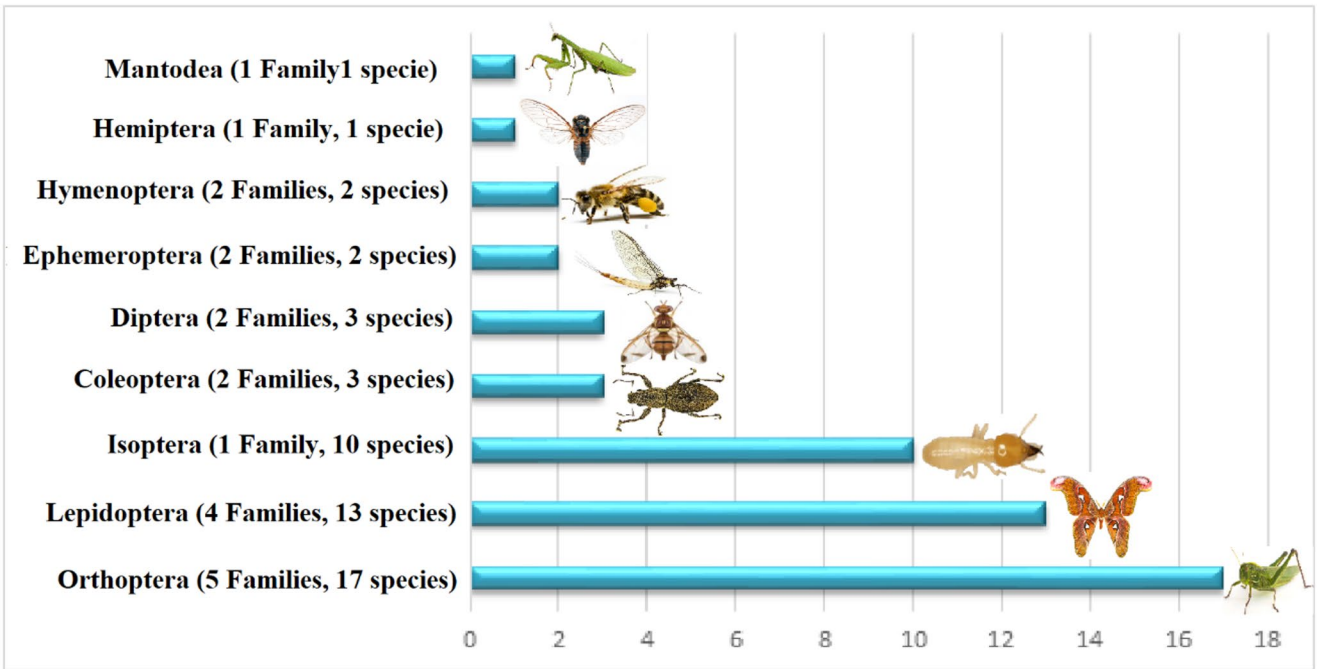


Fig. 1 Commonly consumed insects by 9 order, 20 families and 52 species in Uganda

Table 1 Common edible insects consumed by various indigenous people in Uganda

Order	Family	Scientific name	English and Vernacular Name	Edible Life Stage	Countries Eaten	References
Coleoptera	Curculionidae	*Rhynchophorus phoenicis* (Fabricius, 1801)	Palm weevil, Munimuni Eruru, Tata, Erungwo, Kwaron dabino, Ụkpana, Fara, Ipe Ibo Ebe	Larva Pupa Adult	Uganda, Benin, D.R. Congo, Liberia, Niger, Sao Tome, Togo, Guinea Bissau	[26, 28, 29]
	Scarabaeidae	*Oryctes* sp. Hellwig, 1798		Larva	Uganda, Burkina Faso	[28]
		Pachnoda ephippiata Gerstaecker, 1867	Sun beetle or African sun chafer	Adult	Uganda	[28]
Hymenoptera	Apidae	*Apis mellifera* Linnaeus, 1758		Egg Larva Pupae	Northern Uganda, Malawi Angola, Kenya,	[15, 30]
	Formicidae	*Carebara vidua* Smith, 1858	black ant		Kenya, Uganda, Zambia, Congo, Zimbabwe, South Africa, Botswana, Malawi, Sudan, Namibia, Mozambique	[29]
Hemiptera	Cicadidae	*Ugada limbalis* (Karsch, 1890)			Uganda, Angola Malawi, South Africa	[18, 26]
Orthoptera	Acrididae	*Acanthacris ruficornis* (Fabricius, 1787)	Golden locust	Adult	Uganda, Benin Zambia, South Africa, Malawi, Zimbabwe, Togo, Mali, Cameroon, D.R. Congo, Burkina Faso, Nigeria,	[18, 26]
		Cyrtacanthacris aeruginosa (Stoll, 1813)		Adult	Uganda	[26]
		Cytacanthacris naeruginosus Brunner von Wattenwyl 1898	Short horned grasshopper	Adult	Uganda, Nigeria Cameroon, DRC Congo, Cote D'ivioire	[26]
		Schistocerca gregaria Forsskål, 1775	desert locust	Adult Nymph	Uganda, Congo, Botswana, Cameroun, Tanzania, South Africa, Sudan, Sierra Leone Lesotho, Kenya, Ethiopia, Nepal Guinea, Somalia, Morocco, Mauritania, Zambia, Eritrea, Guinea Bissau,	[28, 29, 31, 32]
		Nomadacris septemfasciata Audinet-Serville, 1883	Red locust	Adult	Uganda, Congo, South Africa, Nepal, Nigeria Eastern Africa, Botswana, Cameroun, Tanzania, Zambia, Malawi Zimbabwe, Mozambique	[11, 17]
		Locusta migratoria (Linnaeus, 1758)	migratory locust	Adult	Congo, Tanzania, (Brazzaville), Uganda, Zambia	[15]
	Pyrgomorphidae	*Zonocerus variegatus* (Linnaeus, 1758)	Tata, Fara, Yoruba-Tata, Ibo-Abuzu, Ukpana	Adult	Nigeria, Ghana Uganda, Cameroon, D.R. Congo, Cote D'ivioire, Guinea, Liberia, Sao Tome, Guinea Bissau	[26]
	Tettigoniidae	*Ruspolia differens* Serville, 1838 Syn. *Homorocoryphus nitidulus vicinus*	longhorn grasshopper, katydid grasshopper, "nsenene" in Luganda or "senene" in Kiswahil-Swahili	Adult	Uganda, The whole of East and Southern Africa, D.R. Congo, Malawi Cameroon, Tanzania, Mauritius, Angola, Ghana, Ivory Coast, Madagascar, Zambia, Burkina Faso, Zimbabwe Kenya, Rwanda, Guinea, Benin	[18, 33–35]

Table 1 (continued)

Order	Family	Scientific name	English and Vernacular Name	Edible Life Stage	Countries Eaten	References
		Ruspolia nitidula (Scopoli, 1786) syn. *Homorocoryphus nitidulus*	long-horned grasshopper	Larva Adult	Uganda, Botswana Zambia, South Africa, D.R. Congo	[26]
		Acanthoplus sp. Stål, 1873			Uganda	[28]
	Gryllidae	*Acheta* sp. Fabricius, 1775		Adult	Uganda	[28]
		Acheta domesticus (Linnaeus, 1758)		Adult	Uganda, Cote D'ivioire, Nigeria, Ghana	[26, 32]
		Brachytrupes spp. Serville, 1838	Odir	Adult	Uganda, Cameroon	[26, 30]
		Brachytrupes membranaceus (Drury, 1770)	Giant African cricket, Odir, Yoruba- Ire	Adult Nymph	Uganda, Malawi Nigeria, Angola, Zimbabwe, Cameroon, D.R. Congo, Burkina Faso, Tanzania Togo, Benin	[18, 25]
		Gryllus bimaculatus De Geer, 1773		Adult	Sub-Saharan Africa, Kenya, Uganda	[32]
		Scapsipedus icipe Hugel and Tanga 2018		Adult	Sub-Saharan Africa, Kenya, Uganda	[32]
	Gryllotalpidae	*Gryllotalpa africana* (Palisot de Beauvois, 1805)	Mole cricket	Adult	Uganda, Nigeria, Zimbabwe, Malawi, Kenya, Tanzania, Dem. Rep. of Congo	[26, 36]
Isoptera	Termitidae	*Syntermes* sp. Holmgren, 1909		Adult	Uganda	[25, 26]
		Acanthotermes acanthothorax (Sjöstedt, 1898)			Uganda	[36, 37]
		Macrotermes spp. Holmgren, 1910	Ngwen, Ibo-Aku, Yoruba-Esunsun	Adult	Uganda, Ghana, Burundi, Malawi, Zambia, CoteD'Ivoire, D.R. Congo, Togo, Guinea, Nigeria, Senegal, Tanzania, Zimbabwe	[17, 25, 29]
		Macrotermes bellicosus (Smeathman, 1781)	white ants Sisi, Aku, Mbe, Enswa	Adult	Uganda, Kenya, Nigeria, Togo, D.R. Congo, Cameroon, São Tomé, Senegal, Cote D'ivioire, Príncipe Guinea, Ghana Bissau, Burundi, Liberia, Tanzania, Zimbabwe	[29, 30, 38]
		Macrotermes subhyalinus (Rambur, 1842)	Agoro	Adult	Uganda, Cote D'ivioire, Burkina Faso, Tanzania, Senegal, Kenya Rwanda, Togo, Burundi, Zimbabwe, Zambia, Angola	[26, 29, 37]
		Macrotermes niger Macrotermes nigeriensis (Sjöstedt, 1898)	Esusun, Aku, Shinge Mbube	Adult	Nigeria, Kenya, Uganda, D.R. Congo	[26]
		Macrotermes falciger (Gerstäcker, 1891)		Adult	Uganda, Benin, Zimbabwe, Burundi, Zambia, Burkina Faso	[19, 26]
		Odontotermes kibarensis (Fuller, 1923)		Adult	Uganda	[22, 29, 37]
		Pseudacanthotermes militaris (Hagen, 1858)		Adult	Uganda, Kenya, Angola, Tanzania	[22, 29, 37]

Table 1 (continued)

Order	Family	Scientific name	English and Vernacular Name	Edible Life Stage	Countries Eaten	References
		Pseudacanthotermes spiniger (Sjoestedt, 1900)		Adult	Uganda, Congo, Zambia, Tanzania, Kenya, Burundi	[22, 29, 37]
Lepidoptera	Hesperiidae	*Coeliades libeon* (Druce, 1875)	Mitoko, Batôh		Uganda, Kenya, Ghana, Liberia, Zambia, Benin, Sierra Leone, Togo, Guinea-Bissau, Congo, Ivory Coast, Zimbabwe, Nigeria, Angola, Cameroon, Central African Rep., Gabon, Equatorial Guinea, Mozambique D.R. Congo, Rwanda, Burundi, Malawi, Namibia, Botswana, Lesotho, South Africa, Swaziland, Tanzania	[39]
	Notodontidae	*Anaphe panda* (Boisduval, 1847)	Silkmoth	Larva	Uganda, D.R. Congo, Nigeria, Tanzania	[26, 40]
	Saturniidae	*Bunaea alcinoe* (Stoll, 1780)	cabbage tree emperor moth, Egu	Larva Pupa Adult	Uganda, Congo, Zambia, Sierra Leone, Liberia, Ghana, Ivory Coast, Togo, Benin, Nigeria, Cameroon, Central African Republic, Equatorial Guinea, Gabon, Tanzania, D.R. Congo, Rwanda, Burundi, Angola Guinea-Bissau, Malawi	[39]
		Gonimbrasia belina Westwood, 1849	mopane worm	Larva	Uganda	[28]
		Gonimbrasia anthinoides (Rougeot, 1978)	Binkélé, Inkèlè, Mpôsô		Uganda, Kenya, Gabon, D.R. Congo, Central African Rep., Rwanda, Zambia, Angola Burundi, Cameroon, Equatorial Guinea, Congo,	[39]
		Gonimbrasia alopia (Westwood, 1849)		Larva	Uganda, Kenya, Congo, D.R. Congo, Central African Rep. Cameroon, Gabon, Rwanda, Equatorial Guinea, Zambia, Togo, Angola, Benin, Nigeria, Liberia, Ivory Coast, Ghana, Sierra Leone, Guinea Bissau	[39]
		Gonimbrasia dione (Fabricius, 1793)	Nzangala, Motikaliké, Baetsuka	Larva	Uganda, Kenya, Congo, Malawi, Benin, Zambia, Angola, Togo, Rwanda, Sierra Leone, Burundi, D.R. Congo, Central African Republic, Ghana, Guinea Bissau, Liberia Tanzania, Ivory Coast, Gabon, Cameroon, Equatorial Guinea, Nigeria	[39]
		Imbrasia epimethea (Drury, 1773)	Kuluka, Nzamba, Epak, Ipak, Guk, Embii, Mbinzu	Larva	Uganda, Ghana, Rwanda, Gabon, D.R. Congo, Central African Rep., Zambia, Angola, Congo, Equatorial Guinea, Benin, Cameroon, Nigeria, Togo, Liberia, Ivory Coast, Sierra Leone, Guinea-Bissau	[39]
		Imbrasia forda (Westwood, 1849) Syn. *Cirina forda*	Ngbanda, Ngwanda, Elèr, Ilir, Milun, Mpuâmpuala, Ajôh Gbangbana, Yoruba-kanni, Munimuni		Uganda, Kenya, Chad, Tanzania, Cameroon, Zambia, Congo, Zimbabwe, Swaziland, Central African Rep., South Africa, Gabon, Equatorial Guinea, Malawi, Mozambique, D.R. Congo, Rwanda, Sudan, Burundi, Angola Ethiopia, Lesotho, Namibia, Botswana	[39]

Table 1 (continued)

Order	Family	Scientific name	English and Vernacular Name	Edible Life Stage	Countries Eaten	References
		Cirina butyrospermi Vuillet, 1911	Chitoumou	Adult Larva	Burkina Faso, Uganda, Sudan, Guinea	[33]
		Lobobunaea phaedusa (Drury, 1782)	Kungunu, Mbaah	Larva	Uganda, Gabon, Rwanda, Kenya, D.R. Congo, Central African Rep. Zambia, Angola, Congo, Tanzania, Equatorial Guinea, Guinea-Bissau, Ghana, Cameroon, Nigeria, Benin, Togo, Liberia, Ivory Coast, Sierra Leone	[39]
		Pseudantheraea discrepans (Butler, 1878)	Kanga, Ekeso, Ikwô, Mikwo, Embânyeh Mbônah Ibaniah	Larva	Uganda, Gabon, Congo, TanzaniaD.R. Congo, Central African Repub., Togo, Angola, Sierra Leone, Ivory Coast, Ghana, Cameroon, Equatorial Guinea, Nigeria, Benin, Liberia, Guinea-Bissau	[39]
	Noctuidae	*Spodoptera littoralis* (Boisduval, 1833)	Mabilu, Ahihi, Ayihi, Babili		Uganda, Kenya, Ghana, Liberia, Zambia, Benin, Sierra Leone, Togo, Guinea-Bissau, Congo, Ivory Coast, Zimbabwe, Chad, Guinea, Gabon, Nigeria, Somalia, Rwanda, Cameroon, Central African Rep., Equatorial Mozambique D.R. Congo, Mali, Burundi, Malawi, Angola Namibia, Algeria, Burkina Faso, Ethiopia, Lesotho, Eritrea Botswana, South Africa, Madagascar, Swaziland, Tanzania, Tunisia, Egypt, Sudan, Niger, Senegal, Libya, Gambia Morocco Mauritania	[39]
Diptera	Chaoboridae	*Chaoborus edulis* Edwards, 1930	Nkhungu	Adult	East African lakes, Tanzania, Uganda, Malawi Kenya	[15, 36]
		Chaoborus pallidipes (Theobald, 1911)			East African lakes, Uganda	[31, 36]
	Chironomidae	*Chironomus* sp. Meigen, 1803	non biting midges	Adult	Uganda, Kenya, Tanzania	[19]
Ephemeroptera	Polymitarcyidae	*Povilla adusta* Navás, 1912	mayfly	Adult	Uganda, Kenya, Tanzania	[31]
	Caenidae	*Caenis kungu* Eaton, 1879	kungus	Adult	Uganda, Kenya, Tanzania	[19]
Mantodea	Mantidae	*Mantis religiosa* (Linnaeus, 1758)	African mantis	Adult	Uganda, Nigeria South Africa, Angola, Congo, Zimbabwe, Cameroon	[26]

It should be noted that in the edible insect literature, there is taxonomic confusion in the names of some edible insect species. There are gaps in the knowledge of the names of edible insects. Some names are not available in the literature or some taxonomic names are misspelled. For example, termites are eusocial polymorphic insects, thus they have three main social castes: worker, soldier, reproductive castes. *Syntermes soldiers* is among the edible insects eaten in Uganda. However, there is a lack of taxonomic information regarding it. Cast name (soldiers) may have been used as the name of a species. This species is therefore not included in Table 1. The species name *Anacanthotermes acanthothorax* has been corrected to *Acanthotermes acanthothorax*. It is therefore necessary to make a clear taxonomic identification and a proper determination.

This study explores the toxic metal(oid) content and bioactive potential of several commonly consumed edible insects in Uganda. To date, available studies addressing edible insect diversity and safety in this region are limited and fragmented, and an integrated evaluation remains lacking. Therefore, this research includes an updated inventory of edible insect species, an evaluation of their biodiversity,

and detailed taxonomic identification. The biological activities of insect extracts were assessed. Specifically, their cholinesterase inhibitory potential was evaluated against both acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), alongside their α-amylase and α-glucosidase inhibitory activities, which are relevant to antidiabetic effects. Furthermore, the antimicrobial, antioxidant, and genotoxic properties of aqueous and methanolic extracts from five insect species were investigated. In addition, the samples underwent comprehensive amino acid profiling (43 amino acids) via LC-MS/MS, and their volatile and volatile compositions were analyzed using GC-MS.

Materials and methods

Insect materials

In collaboration with international undergraduate and graduate students from Atatürk University (Türkiye), insect samples were procured [1. Nsenene (*R. differens*-green), 2. Nsenene (*R. differens*-brown), 3. Nswa (*M. nigeriensis)*], which were directly consumed by the students. For each species/form, approximately 10–15 individual insects were collected by local community members in Uganda. A total of three insect species/varieties commonly consumed, with a focus on Uganda were sourced. To prepare the extracts, the collected insect samples underwent a meticulous process of drying at temperatures below 40 °C. Subsequently, they were finely ground into powder for further analysis. The insect specimens were identified by Prof. Dr. Ümit İncekara and Assoc. Prof. Dr. Zeynep Aydoğan. Voucher samples have been preserved in alcohol and are maintained within the laboratory collection of Atatürk University for future reference.

The fieldwork was performed from March to September 2023 in Uganda. It is a country in East Africa (Fig. 2). The climate is tropical throughout the country. However, the north-eastern part of the country has a semi-arid climate. Agriculture, livestock, forestry, and fishing are largely based in the south of the country, where rainfall is higher and the soil more fertile. Lakes and rivers cover almost 20% of Uganda, and fishing holds considerable potential for the country. Nowadays, insect farming is an emerging industry [20, 41].

Collection and taxonomic identification of edible insect samples

Fieldwork was conducted in Uganda. Adult edible insects were freshly collected by Denis Waiswa, a Ugandan national, during the rainy season from their natural habitats. In the present study, the edible species from two orders were collected in early morning hours during the raining season from their natural habitat, namely *Macrotermes nigeriensis* (Blattodea: Termitidae) and *Ruspolia differens* (Orthoptera: Tettigoniidae) (brown and green locally known as Nsenene). Collection of insects was usually in the early hours of the day. *R. differens* were collected using handpicking, early in the morning between 6.00 am and 7.00 am, when



Fig. 2 The locations of broader and zonal geographic sampling sites in Uganda

they were inactive and therefore unable to fly, and placed in a sterile zip lock bag. *M. nigeriensis* are very attracted to light, collected from termiterium (termite nest) by cutting some section. The adult insects (*M. nigeriensis* and *R. differens* (green, brown)) were collected randomly from the wild in Uganda, and were labelled before preparation. Then, harvested insects were transported to Atatürk University in Türkiye. Taxonomic identification and further analysis were carried out at Atatürk University.

Preparation of methanol and water extracts from insects

To obtain bioactive compounds from the insect samples, methanolic and aqueous extracts were prepared using standardized extraction procedures. Prior to extraction, dried insect samples were finely milled to obtain a homogeneous powder.

For methanolic extraction, 10 g of dried insect powder was accurately weighed and mixed with 100 mL of methanol (solid-to-solvent ratio 1:10, w/v). The mixture was subjected to cold maceration at room temperature (25 ± 2 °C) for 72 h with occasional stirring. Following extraction, the mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator at 40 °C until complete solvent removal. The resulting methanolic extract was transferred into airtight containers and stored at −20 °C until further analysis.

For aqueous extraction, 10 g of dried insect powder was mixed with 100 mL of distilled water (solid-to-solvent ratio 1:10, w/v). The extraction was performed at room temperature (25 ± 2 °C) for 24 h with gentle stirring, followed by filtration to separate the liquid phase. The aqueous extract was subsequently concentrated by freeze-drying (lyophilization). The dried extracts were stored in sealed containers at −20 °C under dark conditions until use for biological and chemical analyses.

GC-MS analysis of volatile compounds

Volatile constituents were analyzed using gas chromatography–mass spectrometry (GC-MS) on a Hewlett Packard GCD system. The separation was performed using an HP-Innowax FSC capillary column (60 m × 0.25 mm i.d., 0.25 µm film thickness), with helium as the carrier gas at a constant flow rate of 1 mL/min. The GC oven temperature program began with an initial hold at 60 °C for 10 min. The temperature was then increased to 220 °C at a rate of 4 °C/min and held for another 10 min. Following this, the temperature was further raised to 240 °C at a slower rate of 1 °C/min. All injections were performed in splitless mode, and the injector temperature was set at 250 °C.

Mass spectral data were acquired in electron ionization (EI) mode at 70 eV, scanning a mass range of 35–425 *m/z*. Volatile compounds were identified based on their mass spectra and retention indices, using comparison with library databases and published literature.

Identification of components

Component identification was carried out using a combination of approaches. Initially, the retention times of the detected compounds were compared with those of authentic reference standards. In addition, relative retention indices (RRIs) were calculated using a homologous series of *n*-alkanes and compared with published data. To enhance identification accuracy, mass spectral matching was performed using commercial spectral libraries, including the Wiley GC/MS Library and MassFinder Software 4.0). Furthermore, compound identities were cross-verified using the in-house Başer Library of Essential Oil Constituents, developed from authenticated reference substances and well-characterized essential oils. This library follows the identification principles described by Hochmuth (2008) and McLafferty & Stauffer (1989) [42, 43].

Chemical compositions of fatty acids

Fatty oils were isolated from dried insect samples (0.5 g) using Soxhlet extraction with *n*-hexane as the solvent. The extracted lipids were then subjected to methylation prior to analysis, following the protocol described by Azcan et al. (2004) [44].

Amino acid measurement analysis by LC-MS/MS

A volume of 50 µL from both experimental and control samples was transferred into Eppendorf tubes. Subsequently, 50 µL of an internal standard solution was added to each tube, followed by vortexing for 10 s to ensure homogeneity. Then, 700 µL of an amino acid extraction solvent-prepared by mixing mobile phase A and mobile phase B in a 1:4 volumetric ratio-was added. The mixture was vortexed again for 1 min to facilitate extraction.

Following this, samples were centrifuged at 4 °C for 8 min. The resulting supernatants were carefully collected and passed through 0.45 µm syringe filters to remove particulates. Amino acid profiling was then performed using an LC-MS/MS system (Agilent 6460 Triple Quadrupole, USA) in accordance with the method described by Atila et al. (2021) [45].

ICP-MS

Elemental analysis was performed to evaluate both essential nutrients and potentially toxic elements in insect species traditionally consumed as food. The elements were categorized into two main groups:

- **Nutritional elements**: Sodium (Na), Magnesium (Mg), Potassium (K), Calcium (Ca), Manganese (Mn), Iron (Fe), Zinc (Zn), Cobalt (Co), Copper (Cu), Chromium (Cr), Strontium (Sr) and Selenium (Se).
- **Toxicologically relevant elements**: Aluminum (Al), Scandium (Sc), Cadmium (Cd), Arsenic (As), Lead (Pb), Mercury (Hg), Cesium (Cs), Barium (Ba), Lanthanum (La), Rubidium (Rb), Cerium (Ce), Samarium (Sm), and Uranium (U).

To prepare the samples, all insect materials were oven-dried at 40 °C for 15 h. The dried insects were then finely ground, and 1.0 gram of each powdered sample (on a dry weight basis) was precisely weighed for analysis. Elemental quantification was carried out using inductively coupled plasma mass spectrometry (ICP-MS) at the Eastern Anatolian High Technology Application and Research Center (DAYTAM), Atatürk University.

The results were reported on a dry weight basis and evaluated against maximum permissible intake levels recommended by international health and safety authorities, such as the Environmental Protection Agency (EPA) and the World Health Organization (WHO). This comparison provided insights into both the nutritional contributions and the safety profile of the insect species analyzed, supporting their potential as sustainable food resources.

Biological activity studies

α-glucosidase enzyme inhibition assay

The α-glucosidase inhibitory activity of the insect extracts was evaluated with slight modifications to the method described by Bachhawat et al. (2011) [46]. All assays were carried out in the Pharmacognosy Research Laboratory, Atatürk University, Erzurum, Turkey, under controlled laboratory conditions (25 ± 2 °C, relative humidity 50–60%, and ambient laboratory lighting; no specific light–dark cycling was applied during the assay). The assay was performed in 96-well microplates. Briefly, 50 µL of phosphate buffer (50 mM, pH 6.9) was added to each well, followed by 10 µL of α-glucosidase enzyme solution (1 U/mL) and 20 µL of insect extract at various concentrations (1–5000 µg/mL). The reaction mixtures were pre-incubated at 37 °C for 5 min. Subsequently, 20 µL of 3 mM *p*-nitrophenyl-α-D-glucopyranoside (pNPG) was added as the substrate, and the plates were incubated at 37 °C for 30 min. The reaction was terminated by adding 50 µL of 0.1 M sodium carbonate.

All reagents were prepared in the same phosphate buffer system. The release of *p*-nitrophenol was quantified by measuring absorbance at 405 nm using a microplate spectrophotometer. Acarbose was used as the positive control. All experiments were performed in triplicate, and percentage inhibition was calculated using the equation: % Inhibition = $[(A_{control} - A_{sample}) / A_{control}] \times 100$.

α-amylase enzyme inhibition assay

The *α*-amylase inhibitory activity of the insect extracts was assessed based on the method described by Nampoothiri et al. (2011), with minor modifications [47]. All assays were carried out in the Pharmacognosy Research Laboratory, Atatürk University, Erzurum, Turkey, under controlled laboratory conditions (25 ± 2 °C, relative humidity 50–60%, and ambient laboratory lighting; no specific light–dark cycling was applied during the assay). The assay was conducted using a 96-well microplate format. Equal volumes (100 µL each) of the test extract, prepared at concentrations ranging from 1 to 5000 µg/mL, and a 1% starch solution prepared in 20 mM sodium phosphate buffer (pH 6.9 containing 6 mM NaCl) were added to each well and thoroughly mixed. The plate was then incubated at 25 °C for 10 min to allow interaction between the extract and the starch substrate. Following the initial incubation, 100 µL of porcine pancreatic α-amylase (0.5 mg/mL) was added to each well. The reaction mixture was incubated again at 25 °C for another 10 min to facilitate enzymatic hydrolysis. To terminate the reaction, 200 µL of dinitrosalicylic acid (DNS) color reagent was added to each well. The microplate was then incubated in a boiling water bath (100 °C) for 5 min to develop color. After cooling the samples to room temperature, 50 µL from each reaction tube was transferred to a fresh 96-well microplate. Each well was subsequently diluted with 200 µL of distilled water. The absorbance of the resulting solution was measured at 540 nm using a microplate spectrophotometer. A blank (containing all reagents except the test sample) served as the control, and acarbose, a standard *α*-amylase inhibitor, was used as the positive control. All experiments were performed in triplicate to ensure the accuracy and reproducibility of the results. The percentage inhibition of *α*-amylase activity was calculated using the following formula: % Inhibition = $[(A_{control} - A_{sample}) / A_{control}] \times 100$.

Acetylcholinesterase and butyrylcholinesterase enzyme inhibition assays

The inhibitory activities of insect extracts against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes were evaluated using a modified version of the method developed by Ingkaninan et al. [48]. All assays were carried out in the Pharmacognosy Research Laboratory, Atatürk University, Erzurum, Turkey, under controlled laboratory conditions (25 ± 2 °C, relative humidity 50–60%, and ambient laboratory lighting; no specific light–dark cycling was applied during the assay). The assay was performed in a 96-well microplate format.

Each well was loaded with a reaction mixture containing 125 µL of Ellman's reagent [5,5'-dithiobis-(2-nitrobenzoic acid), DTNB, 3 mM], 25 µL of substrate [acetylthiocholine iodide for AChE or butyrylthiocholine iodide for BChE, both at 15 mM], 50 µL of Tris-HCl buffer (50 mM, pH 8.0), and 25 µL of the test extract. The mixture was gently mixed, after which 25 µL of the appropriate enzyme (AChE or BChE) was added to initiate the reaction.

The AChE reaction mixtures were incubated for 10 min, while the BChE assays were incubated for 15 min at room temperature. Following incubation, the absorbance was measured at 405 nm using a microplate spectrophotometer to determine enzyme activity based on the formation of the yellow-colored 5-thio-2-nitrobenzoate anion. Donepezil, a well-established AChE inhibitor, was used as a positive control. All experiments were conducted in triplicate to ensure reliability and statistical validity. The percentage of enzyme inhibition was calculated using the following equation: % Inhibition = $[(A_{control} - A_{sample}) / A_{control}] \times 100$.

Antioxidant activity assay

ABTS radical scavenging capacity assay

The ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) cation radical scavenging activity of the insect extracts was assessed using a modified version of the method described by Re et al. [49]. All assays were carried out in the Pharmacognosy Research Laboratory, Atatürk University, Erzurum, Turkey, under controlled laboratory conditions (25 ± 2 °C, relative humidity 50–60%, and ambient laboratory lighting; no specific light–dark cycling was applied during the assay). This assay was performed in a 96-well microplate format to evaluate the antioxidant potential of the samples. To generate the $ABTS^{\bullet+}$ radical cation, equal volumes of 2 mM ABTS solution and 2.45 mM potassium persulfate solution were mixed and allowed to react in the dark at room temperature for 12–16 h. Test extracts were prepared at varying concentrations (10–150 µg/mL) through serial dilution. The extract solution and $ABTS^{\bullet+}$ working solution were mixed according to procedure in each well of the microplate. The mixture was incubated at room temperature to allow the reaction to proceed. The absorbance was recorded at 734 nm using a microplate spectrophotometer, with the buffer solution serving as the blank. *α*-Tocopherol and trolox were used as standard antioxidants for comparative purposes. All measurements were carried out in triplicate to ensure reproducibility. The percentage of ABTS radical scavenging activity was calculated using the following formula: $ABTS^{\bullet+}$ scavenging capacity (%) = $[(A_{control} - A_{sample}) / A_{control}] \times 100$.

DPPH radical scavenging capacity assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of the insect extracts was evaluated using a modified version of the method originally developed by Blois (1958) [50]. All assays were carried out in the Pharmacognosy Research Laboratory, Atatürk University, Erzurum, Turkey, under controlled laboratory conditions (25 ± 2 °C, relative humidity 50–60%, and ambient laboratory lighting; no specific light–dark cycling was applied during the assay). The assay was performed in a microplate format to assess the antioxidant capacity of the samples. A 1 mM solution of $DPPH^{\bullet}$ was prepared in ethanol and used as the radical source. Insect extracts were serially diluted to prepare stock solutions at various concentrations ranging from 10 to 150 µg/mL. In each well of the 96-well microplate, the extract solution and $DPPH^{\bullet}$ solution were added according to the procedure and mixed well. The reaction mixtures were incubated in the dark at room temperature for a predetermined time to allow for radical scavenging interaction. After incubation, the absorbance was measured at 517 nm using a microplate spectrophotometer, with ethanol serving as the blank. *α*-Tocopherol and trolox were used as reference antioxidants for comparison. All tests were performed in triplicate to ensure accuracy and reproducibility. The percentage of DPPH radical scavenging activity was calculated using the following formula: DPPH radical scavenging capacity (%) = $[(A_{control} - A_{sample}) / A_{control}] \times 100$.

Antimicrobial activity assay

The antimicrobial activity of insect-derived extracts from Uganda was evaluated against three standard microbial strains: *Escherichia coli* ATCC 8739 (Gram-negative), *Staphylococcus aureus* ATCC 6538 (Gram-positive), and *Candida albicans* ATCC 10,231. For comparison, standard antimicrobial agents moxifloxacin and fluconazole were tested at concentrations ranging from 0.125 to 128 µg/mL. All assays were carried out in the Pharmacognosy Research

Laboratory, Afyokarahisar Health Sciences University University, Afyokarahisar, Turkey, under controlled laboratory conditions (25 ± 2 °C, relative humidity 50–60%, and ambient laboratory lighting; no specific light–dark cycling was applied during the assay). The evaluation followed a modified microdilution technique based on the Clinical and Laboratory Standards Institute (CLSI) protocols: M100-S16 for aerobic bacteria and M27-A2 for fungi. Before testing, the strains were revived on Mueller Hinton Agar (MHA) for bacteria and Potato Dextrose Agar (PDA) or Sabouraud Dextrose Agar (SDA) for *Candida albicans*, followed by incubation at 37 °C for 24 h. Single colonies were transferred into Mueller Hinton Broth (MHB) for bacteria or RPMI 1640 medium for *Candida* and incubated for another 24 h at 37 °C. The microbial suspension was adjusted to a turbidity equivalent to McFarland standard No. 0.5 (approximately 1.5×10^8 CFU/mL). Extract dilutions and microbial suspensions were dispensed into 96-well U-bottom microplates. After incubation at 35–37 °C for 16–20 h, 15 µL of 0.5 mg/mL resazurin solution was added to each well as a colorimetric indicator of viability. The plates were further incubated at 37 °C for 1–2 h. The Minimum Inhibitory Concentration (MIC) was determined as the lowest concentration of the extract that prevented a color change from blue to pink, indicating inhibition of microbial growth [51].

Human fibroblast cell culture

The human dermal fibroblast cell line (HDFa, ATCC® PCS-201-012™) was used to evaluate the cytotoxic and genotoxic effects of the insect-derived extracts. All cell culture and toxicity experiments were carried out in the Cell Culture Laboratory of Erzurum Technical University (Erzurum, Turkey) under controlled laboratory conditions (25 ± 2 °C, relative humidity 50–60%, ambient laboratory lighting with no specific light–dark regime).

HDFa cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin, and maintained in a humidified incubator at 37 °C with 5% CO_2 until reaching confluency. For cytotoxicity and genotoxicity assessments, cells were seeded into 48-well plates and exposed to insect extracts at concentrations ranging from 1.5 to 200 µg/mL, a dose range commonly applied in in vitro toxicity studies. Untreated cells were used as the negative control.

All experiments were performed in triplicate to ensure reproducibility and statistical reliability. The HDFa cell line was selected due to its non-transformed nature and well-characterized physiological properties, providing a robust model for assessing potential cytotoxic and genotoxic effects of insect-derived extracts on healthy human cells.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

Cell viability was assessed in vitro using the MTT assay [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], a colorimetric method based on mitochondrial activity in living cells. All MTT assay was carried out in the Cell Culture Laboratory of Erzurum Technical University (Erzurum, Turkey) under controlled laboratory conditions (25 ± 2 °C, relative humidity 50–60%, ambient laboratory lighting with no specific light–dark regime).

Human fibroblast cells were seeded into 48-well plates at a density of 10^5 cells per well in 100 µL of complete culture medium. Experimental groups included cells treated with various concentrations of insect extracts, while untreated cells served as negative controls. Following seeding, the plates were incubated at 37 °C in a humidified 5% CO_2 atmosphere for 24 h to allow for attachment and treatment response. After the incubation period, 10 µL of MTT solution was added to each well. The plates were briefly shaken on a circular orbital shaker for 1 min to ensure proper mixing, and then returned to the incubator for an additional 3–4 h to facilitate formazan crystal formation. As a result of mitochondrial reduction of MTT, insoluble purple formazan crystals formed and accumulated at the bottom of the wells. The culture medium was carefully aspirated from each well to avoid disturbing the cell monolayer. For any non-adherent cells, centrifugation at 400×g for 10 min was performed prior to medium removal. To dissolve the formazan crystals, 100 µL of dimethyl sulfoxide (DMSO) was added to each well. The absorbance of the resulting solution was measured at 570 nm using a microplate reader. The absorbance values directly reflected the number of metabolically active (viable) cells, with higher absorbance indicating greater cell viability.

Nuclear structure analysis with Hoechst 33,258 fluorescent staining

To evaluate potential nuclear abnormalities and detect mutated nuclei, Hoechst 33,258 staining was utilized. The tests were carried out in the Cell Culture Laboratory of Erzurum Technical University (Erzurum, Turkey) under controlled laboratory conditions (25 ± 2 °C, relative humidity 50–60%, ambient laboratory lighting with no specific light–dark regime). Human fibroblast cultures were treated with varying concentrations of insect-derived extracts, while untreated cells served as the negative control group. Following treatment, the cells were incubated at 37 °C with 5% CO_2 for 24 h to allow for the development of possible nuclear changes. After incubation, the cells were fixed with 4% paraformaldehyde prepared in phosphate-buffered

saline (PBS) and kept at 4 °C for 30 min. Post-fixation, the cells were gently washed with PBS to remove any residual fixative. The nuclear DNA was then stained by incubating the cells with Hoechst 33,258 at a final concentration of 1 µM for 5 min at room temperature, protected from light. Fluorescent nuclear staining was visualized using a fluorescence microscope (e.g., Leica® DM IL LED) at 20× magnification. Images were captured for subsequent analysis. This method enabled clear visualization of nuclear morphology, allowing the identification of chromatin condensation, fragmentation, or other nuclear abnormalities indicative of apoptotic or genotoxic effects.

Chromosomal aberration test

The chromosomal aberration assay was carried out with slight modifications from the protocol described by Türkez et al. (2010) to assess the potential genotoxic effects of edible insect extracts [52]. The tests were carried out in the Cell Culture Laboratory of Erzurum Technical University (Erzurum, Turkey) under controlled laboratory conditions (25 ± 2 °C, relative humidity 50–60%, ambient laboratory lighting with no specific light–dark regime). To initiate the assay, 6 mL of pre-warmed Karyotyping Medium B was added to sterile cell culture tubes, followed by the addition of 0.5 mL of freshly collected peripheral blood. The insect extracts were then introduced into the cultures at concentrations corresponding to their previously determined IC_{50} values. The tubes were gently inverted to ensure homogenous mixing and incubated at 37 °C in a CO_2 incubator. After 70 h and 15 min of incubation, 0.1 mL of colchicine solution (10 µM/mL) was added to each culture to arrest cells at the metaphase stage of mitosis. At the 72-hour mark, the cultures were vortexed and centrifuged at 900 rpm for 10 min. The supernatant was discarded, and the cell pellets were gently resuspended with 8 mL of hypotonic solution, added dropwise to induce cellular swelling. After 15–20 min of incubation at room temperature, the samples were centrifuged again under the same conditions. The supernatant was removed, and 5 mL of freshly prepared fixative (typically methanol: acetic acid, 3:1) was added dropwise to the pellets. This fixation process was repeated three times to ensure complete stabilization of chromosomal material. After the final centrifugation step, the cell pellets were thoroughly resuspended using vortexing. Prepared cell suspensions were carefully dropped onto pre-cleaned and pre-labeled microscope slides, which had been previously washed and stored at 4 °C. The slides were allowed to air dry at room temperature for 72 h. Subsequently, the chromosomal preparations were stained with Giemsa for 15 min, followed by another 2–3 days of drying prior to microscopic evaluation. For each experimental group, 30 metaphase spreads were examined under the microscope. Chromosomal structures were analyzed for evidence of aberrations, including chromatid and chromosome gaps, breaks, and other structural anomalies. The mean frequency of chromosomal aberrations was calculated for each group to determine the extent of genotoxic damage induced by the insect-derived extracts.

Apoptosis-necrosis test

For the evaluation of apoptosis and necrosis, Annexin V-FITC/Propidium Iodide (PI) dual staining was employed in fibroblast cultures following experimental treatments. The tests were carried out in the Cell Culture Laboratory of Erzurum Technical University (Erzurum, Turkey) under controlled laboratory conditions (25 ± 2 °C, relative humidity 50–60%, ambient laboratory lighting with no specific light–dark regime). Cells were first detached from the culture plate using trypsin at room temperature for 5 min. After detachment, the cell suspension was collected and centrifuged. A total of 5×10^4 cells were resuspended in 500 µL of 1X binding buffer to prepare for staining. Subsequently, 5 µL of Annexin V-FITC and 5 µL of PI (at a concentration of 50 µg/mL) were added to each cell suspension. The samples were incubated for 5 min in the dark at room temperature to allow for specific binding of Annexin V to phosphatidylserine on the outer leaflet of apoptotic cell membranes and for PI to penetrate cells with compromised membranes. Following incubation, the stained samples were analyzed by flow cytometry equipped with appropriate filter sets for FITC (green fluorescence) and PI (red fluorescence) detection. This allowed for the quantitative discrimination of viable, early apoptotic, late apoptotic, and necrotic cells, based on fluorescence emission profiles. The resulting data provided detailed insights into cellular viability and the extent of apoptosis or necrosis induced by the tested insect extracts.

Statistical analysis

Statistical analyses of group data were performed using one-way analysis of variance (ANOVA). To compare the cytotoxic effects of insect isolates with those of the control group, Dunnett's post hoc test and Tukey's multiple comparison test were applied, as appropriate. A significance threshold of $p < 0.05$ was established for all statistical evaluations. All analyses were conducted using GraphPad Prism software (version 7.0). To ensure the robustness and reproducibility of findings, each experimental assay was carried out in triplicate. In addition to parametric tests, the Kruskal–Wallis non-parametric test was employed to confirm statistical significance

Table 2 Amount of powdered material and extract yields of edible insect samples

Samples	Nsenene (*Ruspolia differens*, green)	Nsenene (*Ruspolia differens*, brown)	Nswa (Macrotermes nigeriensis)
Powdered material (g)	7.44	7.21	10.90
Methanolic extract (g)	3.64	3.37	2.46
Aqueous extract (mg)	724.45	814.00	936.62

Table 3 Extraction yields (%) of methanolic and aqueous extracts

Extract type	Nsenene (*R.* differens, green)	Nsenene (*R.* differens, brown)	Nswa (M. nigeriensis)
Methanol (%)	48.93	46.74	22.57
Aqueous (%)	9.74	11.29	8.60

where appropriate. Further statistical assessments were performed using IBM SPSS Statistics software (version 20.0; IBM Corp., Armonk, NY, USA), with the significance level set at $p = 0.05$. In the results section, data are presented as mean ± standard deviation (SD), including percent inhibition values and calculated IC_{50} values for each tested sample.

Results

Extraction

Tables 2 and 3 presents the powdered biomass used for extraction, the resulting extract amounts in both methanol and aqueous solvents, and the corresponding extraction yields for three edible insect samples: *Ruspolia differens–green*, *Ruspolia differens–brown*, and *Macrotermes nigeriensis*. Among the samples, *M. nigeriensis* had the highest initial powdered biomass at 10.90 g, compared to 7.44 g and 7.21 g for the green and brown variants of *R. differens*, respectively. Despite its larger mass, *M. nigeriensis* yielded the lowest methanol extract amount at 2.46 g, corresponding to a methanolic yield of 22.57%, which was significantly lower than the yields of both *R. differens–green* (48.93%) and *R. differens–brown* (46.74%). In terms of aqueous extraction, *M. nigeriensis* again produced the highest mass of extract (936.62 mg), followed by *R. differens–brown* (814.00 mg) and *R. differens–green* (724.45 mg). However, when calculated as a percentage of the initial powdered material, the aqueous yield of *M. nigeriensis* was the lowest at 8.60%, compared to 11.29% for *R. differens–brown* and 9.74% for *R. differens–green*.

Table 4 Fatty acid compositions of the insect samples from Uganda

Fatty acids	Nswa (*Macrotermes nigeriensis*) (%)	Nsenene (*Ruspolia differens*)-brown (%)	Nsenene (*Ruspolia differens*-green) (%)
Myristic (14:0)	0.1	0.3	0.1
Palmitic (16:0)	33.5	31.3	30.6
Palmitoleic (16:1)	1.7	1.6	1.5
Margaric (17:0)	0.1	0.1	-
Stearic (18:0)	4.9	8.9	8.8
Oleic (18:1)	42.2	42.5	44.1
Elaidic (18:1)	0.3	0.3	0.4
Linoleic (18:2)	12.9	13.1	13.0
Linolenoic (18:3)	1.6	1.5	1.4
Arachidic (20:0)	0.5	0.3	-
Total	97.8	99.9	99.9

% calculated from FID d

Fatty oil extraction and analysis

Table 4 presents the detailed fatty acid compositions (% of total fatty acids) of three insect samples: *M. nigeriensis*, *R. differens* (brown variant), and *R. differens* (green variant), all consumed in Uganda. Across all samples, oleic acid (C18:1) was the most abundant fatty acid, ranging from 42.2% in *M. nigeriensis* to 44.1% in *R. differens–green*. This monounsaturated fatty acid is known for its cardiovascular benefits and stability, indicating the insects may serve as a source of heart-healthy fats.

Palmitic acid (C16:0), a saturated fatty acid, was the second most abundant component, with the highest proportion in *M. nigeriensis* (33.5%) and slightly lower levels in *R. differens–brown* (31.3%) and *green* (30.6%). While common in animal fats, high palmitic acid intake is associated with increased LDL cholesterol, so its levels warrant consideration in nutritional evaluations. Stearic acid (C18:0) content was higher in *R. differens* variants (8.9%–8.8%) than in *M. nigeriensis* (4.9%). Notably, stearic acid is considered more neutral in its impact on blood cholesterol compared to other saturated fats. Linoleic acid (C18:2), an essential omega-6 polyunsaturated fatty acid (PUFA), ranged between 12.9% and 13.1% across all three insect species, supporting their nutritional relevance for essential fatty acid intake. The presence of α-linolenic acid (C18:3), an omega-3 PUFA, was also consistent (~1.4–1.6%), though in lower amounts, indicating a modest contribution to anti-inflammatory lipid intake. Palmitoleic acid (C16:1), a less common monounsaturated fatty acid with potential metabolic benefits, was detected in all samples (~1.5–1.7%). Additionally, trace levels of myristic, margaric, elaidic, and arachidic acids were observed, contributing minimally to the overall profile. The total identified fatty acids accounted for

97.8% in *M. nigeriensis* and 99.9% in both *R. differens* variants, indicating excellent chromatographic resolution and comprehensive profiling.

The volatile composition analysis

The volatile profiles of three edible insect species from Uganda-*M. nigeriensis*, *R. differens* (brown), and *R. differens* (green)-reveal significant diversity in both compound presence and aromatic character (Table 5). Among the three, *Macrotermes nigeriensis* exhibited the highest total volatile contribution, with 99.4%, followed closely by *R. differens* brown (97.8%) and green (88.8%), indicating that all species are chemically rich in aroma-active components. Each species demonstrated a unique balance of volatile compounds that shaped their odor signatures. For example, *M. nigeriensis* was particularly rich in acetic acid (25.6%), contributing a pungent vinegar-like odor, and tetramethyl pyrazine (8.7%), which provides roasted and fermented cereal-like notes. These compounds suggest a dominant umami or savory sensory impression. In contrast, *R. differens* (green morph) had an exceptionally high level (38.0%) of isovaleric acid, a compound known for its intense cheese-like aroma. This dominant presence greatly influences its overall sensory profile. The brown morph of *R. differens* contained a broader range of aromatic molecules, including phenol, indole, and methyl isoeugenol, which added musky, spicy, and floral notes to its aroma.

Across all species, several pyrazines (e.g., 2,5-dimethylpyrazine, trimethylpyrazine, ethylpyrazine) were detected, providing chocolate-like, nutty, or roasted attributes, which are desirable in food flavor development. These compounds, commonly associated with thermally processed foods, suggest that the insects may offer familiar and appealing aromatic qualities when used in culinary contexts.

Other volatiles such as limonene, styrene, and terpinene introduced fruity, citrusy, and herbal characteristics, particularly in *R. differens* samples. Meanwhile, compounds like 2-methylpropanoic acid and methyl decanoate contributed notes of rancid butter and fermented fruit, reflecting the lipid metabolism and microbial interactions in insect tissues.

Quantitative analysis of amino acids

The amino acid compositions of three edible insect species from Uganda—*Macrotermes nigeriensis*, *Ruspolia differens* (brown morph), and *Ruspolia differens* (green morph)—were quantitatively analyzed in both methanolic and aqueous extracts. The results were given in Table 6. Among these, the methanolic extract of *M. nigeriensis* demonstrated remarkably high concentrations of taurine (2502.58 mmol/mL), glutamic acid (634.97 mmol/mL), ethanolamine (429.90 mmol/mL), alanine (320.13 mmol/mL), and proline (326.12 mmol/mL), indicating a particularly rich amino acid profile. Although the aqueous extract contained lower overall values, it still showed notable levels of taurine (286.81 mmol/mL).

In *R. differens* (brown morph), the methanolic extract exhibited high levels of arginine (571.02 mmol/mL), glutamic acid (593.71 mmol/mL), and ethanolamine (593.22 mmol/mL). The aqueous extract of this morph was especially rich in alanine (3011.72 mmol/mL) and taurine (594.69 mmol/mL), highlighting the efficiency of aqueous extraction for specific hydrophilic amino acids.

By contrast, the green morph of *R. differens* displayed very low amino acid concentrations in the aqueous extract, with many compounds undetectable or at trace levels. Nonetheless, the methanolic extract retained substantial levels of glutamic acid (592.70 mmol/mL), ethanolamine (583.22 mmol/mL), and alanine (3011.17 mmol/mL), showing a profile similar to that of the brown morph.

In summary, methanolic extracts generally yielded higher concentrations and a broader spectrum of amino acids compared to aqueous extracts, particularly in *M. nigeriensis* and *R. differens* (brown). Taurine, glutamic acid, alanine, and ethanolamine were the most abundant amino acids across the samples, supporting the nutritional relevance of these Ugandan insect species as promising sources of dietary amino acids.

ICP-MS analysis

This study investigated the elemental composition (Na, Mg, Al, K, Fe, Sr, Ca, Rb, Cr, Mn, Zn, Co, Ba, La, Ce, Se, As, Sc, Cu, Cd, Cs, Hg, Pb, Sm, U) of three edible insect species widely consumed in Uganda: *M. nigeriensis*, *R. differens* (brown), and *R. differens* (green). Elements were classified into major, trace, and ultratrace categories and compared with international recommended dietary intake (RDI) values established by WHO, IOM, and USEPA [53–62]. The results were given in Tables 7 and 8.

Elemental composition showed clear variation among the investigated insect species and between the color morphs of *Ruspolia differens*. The brown form of *R. differens* contained higher levels of potassium (K), iron (Fe), and zinc (Zn) compared to the green form. Arsenic (As) concentrations ranged from 0.023 to 0.102 mg/kg, with the highest value observed in *R. differens* (green). Cadmium (Cd) levels varied between 0.007 and 0.044 mg/kg, reaching the maximum concentration in *Macrotermes nigeriensis*. Mercury (Hg) concentrations ranged from 0.001 to 0.091 mg/kg, with the highest level again

Table 5 The volatile composition of the edible insects from Uganda

Component	RRI	Nswa (*Macrotermes nigeriensis*)	Nsenene (*Ruspolia differens*)-brown	Nsenene (*Ruspolia differens*-green)	Odor description
Methyl hexanoate	1197	-	4.2	-	Fruity
Limonene	1203	47.9	17.9	16.1	Citrus type odor
Ethyl hexanoate	1239	-	1.1	-	Fruity
γ-Terpinene	1255	1.8	-	-	Herbaceous-citrusy
Styrene	1272	1.4	-	-	Pungent, gassy; slightly fresh-aromatic
p-Cymene	1280	4.1	-	-	Woody and terpy-like
2-Methyl butyl isovalerate	1299	-	0.6	1.3	Fruity type odo
2,5-Dimethyl pyrazine	1332	-	-	-	Chocolate type odor
2,6-Dimethyl pyrazine	1337	1.0	0.6	1.2	Chocolate type odor
2,3-Dimethyl pyrazine	1358	1.3	-	-	Chocolate type odor
Methyl octanoate	1399	-	2.1	0.9	Waxy type odor
Trimethyl pyrazine	1409	8.7	4.8	7.2	Nutty type odor
Ethyl octanoate	1444	-	0.3	-	Strong odor of fruit and flowers
2-Ethyl-3,5-dimethyl pyrazine	1452	-	0.4	0.7	Burnt type odor
3-Ethyl-2,5-dimethyl pyrazine	1468	-	0.6	0.9	Burnt type odor
Acetic acid	1475	-	0.3	0.5	Strong vinegar-like odor
Tetramethyl pyrazine	1482	25.6	3.8	6.0	Musty, fermented, coffee odor
2,3,5-Trimethyl-6-ethyl-pyrazine	1492	0.9	0.5	1.0	Roasted nut, baked potato odor
Methyl nonanoate	1500	-	0.3	-	Fruity
3,5-Octadien-2-one	1520	-	-	-	Fruity fatty mushroom
Methyl 3-methylthio propionate	1541	-	1.0	0.2	Sulfurous type odor
Benzaldehyde	1541	0.6	-	-	Bitter almond odor
Propanoic acid	1559	-	0.3	0.6	Pungent, disagreeable, rancid odor
2-Methylproponoic acid (=*Isobutyric acid*)	1591	0.6	6.6	6.9	Rancid butter
Methyl decanoate	1601	-	1.3	0.3	Fermented type odor
2-Undecanone	1604	-	-	0.3	Waxy, fruity, creamy, fatty, orris, floral
Butanoic acid (=*Butyric acid*)	1631	-	1.4	-	Unpleasant, similar to vomit or body odor
Methyl dec-4-enoate	1634	-	1.3	-	Fruity
γ-Butyrolactone	1651	0.2	-	-	Sweet, pleasant, and creamy
Isovaleric acid	1685	1.9	33.8	38.0	Cheesy type odor
α-Terpinyl acetate	1709	-	1.2	-	Sweet floral and herbal aroma with bergamot, pine and lavender nuances
γ-Hexalactone	1726	0.3	-	-	Tonka
Acetamide*	1769	3.1	-	0.3	Odorless mouse-like with impurities
Methyl dodecanoate	1815	-	1.1	0.2	Fatty, floral, winey odor
Methyl hydrocinnamate	1856	-	1.9	0.5	Honey type odor
Ethyl-3-phenyl propionate	1904	-	0.3	-	Ethereal, rum, fruity, floral
Methyl tetradecanoate	2022	-	0.8	-	Waxy type odor
Methyl eugenol	2030	-	0.7	-	Spicy earthy odor
Phenol	2033	-	3.1	4.1	Sickeningly sweet and tarry
2-Piperidinone (=*Valerolactam*)*	2162	-	0.2	0.3	Ammoniacal, pepper-like
Methyl hexadecanoate	2226	-	1.2	0.2	Oily waxy fatty orris
Methyl palmitate	2226	-	0.8	-	Waxy type odor
Methyl dihydrojasmonate (=*Hedione*)	2308	-	-	0.3	Buttery-floral scent
Methyl oleate	2456	-	2.2	-	Pleasant fatty ester odor
Indole	2471	-	0.5	0.8	Animalic, floral-heady, faecal, naphthelene / moth-balls

Table 5 (continued)

Component	RRI	Nswa (*Macrotermes nigeriensis*)	Nsenene (*Ruspolia differens*)-brown	Nsenene (*Ruspolia differens*-green)	Odor description
Methyl linoleate	2509	-	0.6	-	Low oily, fatty, woody
Total		99.4	97.8	88.8	

RRI Relative retention indices calculated against *n*-alkanes

% calculated from total ion chromatogram (TIC)

*From Wiley; tentative identification

Table 6 Amino acid composition of the insect samples from Uganda (nmol/mL)

No	Compound	Nswa (*Macrotermes nigeriensis*) MeOH	Nswa (*Macrotermes nigeriensis*) Aqueous	Nsenene (*Ruspolia differens*)-brown MeOH	Nsenene (*Ruspolia differens*)-brown Aqueous	Nsenene (*Ruspolia differens*-green) MeOH	Nsenene (*Ruspolia differens*-green) Aqueous
A. Proteinogenic amino acids							
2	Tryptophan	27.6977	27.7000	25.0452	37.6367	58.3006	0.0000
3	Phenylalanine	77.4605	23.1875	21.8146	122.2625	49.5110	0.0000
4	Tyrosine	91.4359	251.8766	60.5230	237.3034	177.3125	44.0525
5	Leucine	321.3312	266.6134	184.9772	418.9155	320.7667	0.0000
6	Isoleucine	236.3328	192.1152	144.4419	205.0211	201.8307	0.0000
7	Methionine	0.0000	29.0959	0.0000	101.6416	0.0000	0.0000
12	Valine	357.9950	339.1446	160.7466	188.5382	383.7683	0.0000
13	Glutamic acid	634.9768	220.2789	362.1942	440.1623	593.2005	98.9519
16	Aspartic acid	84.6242	94.4426	77.9165	88.6595	32.6403	0.0000
18	Serine	115.4455	0.0000	36.4951	183.4001	158.2212	0.0000
19	Threonine	219.5301	0.5202	96.7032	173.7117	138.3094	0.0000
20	Alanine	1302.1234	1596.0636	730.3127	1060.4002	3011.172	137.9407
21	Glycine	379.8144	94.9212	138.8096	228.2375	247.3226	72.9418
22	Trans-4-Hydroxyproline	24.7007	19.1969	26.0764	24.4464	20.5692	24.2535
23	Asparagine	230.7113	0.0000	220.8030	85.0032	215.1981	0.0000
24	Proline	326.1185	0.0000	133.3276	29.3417	223.1752	0.0000
35	Arginine	117.107	45.7851	81.924	571.0163	173.464	11.8771
37	Histidine	0.0000	0.0000	0.0000	0.0000	40.1098	0.0000
B. Non-proteinogenic amino acids and derivatives							
1	Taurine	2502.5838	286.8079	1419.6109	594.6859	0.0000	0.0000
8	3-Aminoisobutyric acid	236.1846	4.6354	86.1665	13.5224	56.7964	0.0000
9	GABA	148.4745	7.3438	64.5613	13.2824	39.6216	0.0000
10	2-Aminoadipic acid	9.2259	3.7586	0.0000	6.6734	0.0000	0.0000
15	Beta-alanine	79.3732	13.9398	25.3307	9.1942	16.7102	0.0000
27	Sarcosine	43.5065	31.9322	40.7950	32.0213	83.7530	39.4895
29	Citrulline	275.4372	13.9910	92.6966	108.0108	2.8377	0.0000
38	Ornithine	40.9134	48.1956	35.5353	131.9980	28.3922	12.2948
C. Dipeptides and small peptides							
40	Carnosine	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
43	Anserine	2.8615	11.5467	7.3733	4.2980	11.2988	10.2955

detected in *M. nigeriensis*. Lead (Pb) values ranged from 0.105 to 0.394 mg/kg, with *R. differens* (brown) exhibiting the highest concentration. Selenium (Se) was detected at levels between 0.13 and 0.54 mg/kg, with *M. nigeriensis* showing the highest Se content. In addition, several ultra-trace elements, including Sc, Cs, La, Ce, Sm, and U, were detected, with a relatively higher accumulation of Sm observed in *R. differens* (green).

Enzyme inhibition assays

The results of enzyme inhibition assays are given in Table 9. At a concentration of 5000 µg/mL, the methanolic extract of *R. differens*-green exhibited the highest *α*-glucosidase inhibitory activity (88.16%), followed by the methanolic extract of *R. differens*-brown (82.85%) and *M. nigeriensis* (46.16%). These values were comparable

Table 7 Element composition of the edible insect species commonly eaten in Uganda

Element group	Elements	Nswa (*Macrotermes nigeriensis*)	Nsenene (*Ruspolia differens*)-brown	Nsenene (*Ruspolia differens*-green)	Recommended Daily Intake	Source
Major elements (mg/kg dry weight)	Na	13905.418	32168.058	95111.035	≤ 2300 mg	[53]
	Mg	13650.061	12276.587	17920.126	310–420 mg	[54]
	Al	5896.533	3344.224	3778.563	1–2 mg (tolerable limit)	[55]
	K	5830.02	6629.74	5640.00	4700[b]	[56]
	Fe	305.10	343.34	319.13	8–18[c]	[54]
	Ca	105.25	72.44	99.43	1000–1300[c]	[57]
Trace elements (mg/kg dry weight)	Sr	272.647	238.113	239.223	No data available	-
	Rb	35.037	23.000	24.906	No data available	-
	Cr	106.976	104.763	335.832	0.025–0.035 mg	[54]
	Mn	39.345	66.479	136.650	1.8–2.3 mg	[54]
	Zn	73.47	80.12	72.72	8–11[a]	[54]
	Co	20.219	42.998	53.758	5–8 µg	[58]
	Ba	79.412	15.320	14.505	1–2 mg	[59]
	La	26.949	8.140	38.911	No data available	-
	Ce	22.033	13.488	43.410	No data available	-
Ultra-trace elements (µg/kg dry weight)	Se	0.54	0.13	< 0.000	0.055[c]	[60]
	As	0.023	0.087	0.102	0.01–0.1[b]	[59]
	Sc	< 0.000	< 0.000	< 0.000	No data available	-
	Cu	6.09	14.50	58.35	0.9[c]	-
	Cd	0.044	0.007	0.007	0.007[b]	[59]
	Cs	< 0.000	< 0.000	< 0.000	No data available	-
	Hg	0.091	0.002	0.001	0.004[b]	[61]
	Pb	0.105	0.394	0.326	0.2[a]–2[b]	[59]
	Sm	2.973	< 0.000	7.592	No data available	-
	U	1.542	0.897	< 0.000	0.6 µg/kg/day	[59]

PTWI refers to the Provisional Tolerable Weekly Intake, while IOM stands for the Institute of Medicine, a branch of the U.S. National Academies. USEPA is the United States Environmental Protection Agency, and WHO represents the World Health Organization. Certain rare earth elements such as samarium (Sm), cerium (Ce), lanthanum (La), and scandium (Sc), along with rubidium and cesium, currently lack established Recommended Daily Intake (RDI) values due to their non-essential status or the absence of sufficient data regarding their biological functions in humans

According to the IUPAC Compendium of Chemical Terminology (2nd edition), a trace element is defined as one with an average concentration below approximately 100 parts per million (ppm), or 100 micrograms per gram (µg/g). However, advances in analytical instrumentation have greatly enhanced detection capabilities, rendering this traditional threshold outdated. To better describe and measure extremely low concentrations, the concept of *ultra-trace analysis* has emerged. Although no universally fixed definition exists, ultra-trace typically refers to elements found in quantities less than 10^{-6} to 10^{-8} grams per gram, or below 1 ppm to 10 parts per billion (ppb) [62]

Table 8 Element composition of *M. nigeriensis* and *R. differens* according to literature (mg/kg dry weight)

Elements	Ca	Fe	Zn	Cu	K	Se
Nswa *(Macrotermes nigeriensis)*	331-530[a]	707- 1118[a]	44-162[a]	3-14[a]	92-126[a]	0.04[b]
	1.00[b]	9.56[b]	0.97[b]	0.72[b]	3360[b]	
	14.2[c]	563.3[c]	72[c]	8.6[c]		
Nsenene (*Ruspolia differens*)-brown	245[d]	130[d]	124[d]	5[d]	2597[d]	0.5[f]
	357-1280[e]	332-690[e]	81.1-137[e]	16.8-20.8[e]	4540-6100[e]	
	11,240[f]	2587[f]	142.4[f]	16.7[f]	8344[f]	
Nsenene (*Ruspolia differens*-green)	274[d]	166[d]	173[d]	6[d]	3706[d]	ND[f]
	349-1060[e]	324-536[e]	85.1-124[e]	16-22.3[e]	4460-6730[e]	
	9676[f]	1744[f]	150[f]	16[f]	7240[f]	

ND Not determined

[a][12]; [b][79]; [c][16]; [d][21]; [e][80]; [f][81]

to the positive control acarbose (77.61%). For α-amylase inhibition (5000 µg/mL), the most pronounced effect was again observed with *R. differens*-green MeOH (22.68%), whereas both *R. differens*-brown extracts showed modest inhibition (9.07% for MeOH and 9.24% for aqueous). Acarbose displayed significantly higher α-amylase inhibition (62.74%) as expected (Table 9). No acetylcholinesterase inhibition was detected in any sample at

Table 9 The results of enzyme inhibition assays

Samples	α-Glucosidase Inhibition (%) (5000 µg/mL) (mean ± SD)	α-Amylase Inhibition (%) (5000 µg/mL) (mean ± SD)
Nsenene (*Ruspolia differens*-green) MeOH	88.16 ± 3.85	22.68 ± 8.43
Nsenene (*Ruspolia differens*-green) Aqueous	ND	ND
Nsenene (*Ruspolia differens*)-brown MeOH	82.85 ± 2.61	9.07 ± 1.02
Nsenene (*Ruspolia differens*)-brown Aqueous	ND	9.24 ± 4.00
Nswa *(Macrotermes nigeriensis)* MeOH	46.16 ± 2.77	ND
Nswa *(Macrotermes nigeriensis)* Aqueous	ND	ND
Acarbose	77.61 ± 4.70	62.74 ± 1.85
Donepezil	-	-

ND Not determined, *SD* Standard Deviation

Table 10 The results of $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ scavenging activity of samples

Samples	$ABTS^{\bullet+}$ Scavenging Activity (%) (100 µg/mL) (mean ± SD)	$DPPH^{\bullet}$ Scavenging Activity (%) (100 µg/mL) (mean ± SD)
Nsenene (*Ruspolia differens*-green) MeOH	10.837 ± 0.007	8.768 ± 0.003
Nsenene (*Ruspolia differens*-green) Aqueous	9.429 ± 0.002	1.644 ± 0.001
Nsenene (*Ruspolia differens*)-brown MeOH	10.380 ± 0.006	2.575 ± 0.004
Nsenene (*Ruspolia differens*)-brown Aqueous	11.013 ± 0.014	3.133 ± 0.004
Nswa *(Macrotermes nigeriensis)* MeOH	5.413 ± 0.003	1.523 ± 0.001
Nswa *(Macrotermes nigeriensis)* Aqueous	19.412 ± 0.002	44.528 ± 0.018
α- Tocopherol	89.707 ± 0.001	88.264 ± 0.001
Trolox	99.258 ± 0.001	93.018 ± 0.0003

SD Standard Deviation

5 µg/mL, while the positive control donepezil achieved 93.03% inhibition, validating the assay. Similarly, butyrylcholinesterase inhibition was negligible, with only *M. nigeriensis* MeOH showing weak activity (6.46% at 500 µg/mL), compared to complete inhibition by donepezil (100.00%).

Table 11 Minimum inhibitory concentrations of the Insect Samples from Uganda (MIC), µg/mL

Water extracts	Escherichia coli ATCC 8739	S. aureus ATCC 6538	C. albicans ATCC 10,231
Nsenene (*Ruspolia differens*-green)	> 2500	> 2500	> 5000
Nsenene (*Ruspolia differens*)-brown	> 2500	> 2500	> 5000
Nswa *(Macrotermes nigeriensis)*	> 2500	> 2500	> 5000
Moxifloxacin	0.125>	0.125>	-
Fluconazole	-	-	0.125>

$ABTS^{\bullet+}$ and $DPPH^{\bullet}$ scavenging activity

Antioxidant activity of extracts is presented in Table 10. In DPPH radical scavenging tests, when the % inhibition values of the standards (α-tocopherol (TC), troloks (TR)) and extracts were compared at a concentration of 100 µg/mL, it was determined that especially water extract of *M. nigeriensis* (MN) had a higher % inhibition value on $DPPH^{\bullet}$ [(TR)93.0> (TC)88.2> (MN)44.5> (RDB)3.1> (RDG)1.6%; 100 µg/mL % inhibition for water extracts of insects species], [(TR)93.0> (TC)88.2> (RDG)8.7> (RDB)2.5> (MN)1.5%; 100 µg/mL % inhibition for methanol extracts of insects species].

When the % inhibition values of standards and extracts in ABTS cation radical scavenging tests were compared at 100 µg/mL concentration, it was seen that the *M. nigeriensis* water extract came to the fore in terms of antioxidant effect. In this context, the results were found to be compatible with DPPH radical scavenging capacity tests [(TR)99.2> (TC)89.7> (MN)19.4> (RDB)11.0> (RDG)9.4%; 100 µg/mL % inhibition for water extracts of insects species], [(TR)99.2> (TC)89.7> (RDG)10.8> (RDB)10.3> (MN)5.4%; 100 µg/mL % inhibition for methanol extracts of insects species]. The antioxidant capacities of the extracts were found to be average compared to the standards.

Antimicrobial activity

Antimicrobial activity of water and methanol extracts of insect species/forms Nsenene (*R. differens*-green), Nsenene (*R. differens*-brown), and *M. nigeriensis*, in Uganda were tested against *E. coli*, *S. aureus and C. albicans.*

The minimum inhibitory concentrations (MICs) of insects for water extracts were found generally > 2500 µg/mL against tested microorganisms. Results are given in Tables 11 and 12.

The MICs value were obtained between 312.5 and 2500 µg/mL for methanol extracts of insects. All extracts of insects were more active against *Candida albicans* with MIC = 625 µg/mL. The Nsenene (*R. differens*-green)

Table 12 Minimum inhibitory concentrations of the Insect Samples from Uganda (MIC), µg/mL

Methanol extracts	Escherichia coli ATCC 8739	S. aureus ATCC 6538	C. albicans ATCC 10,231
Nsenene (*Ruspolia differens*-green)	2500	312.5	625
Nsenene (*Ruspolia differens*)-brown	2500	625	625
Nswa *(Macrotermes nigeriensis)*	2500	1250	625
Moxifloxacin	0.125>	0.125>	-
Fluconazole	-	-	0.125>

methanol extract was found effectible aginst *S. aureus* with MIC = 312.5 µg/mL. The Nsenene (*R. differens*)-brown methanol extract was also effective against *S. aureus* with MIC = 625 µg/mL. Methanol extract of *M. nigeriensis* was found at MIC = 1250 µg/mL against *S. aureus.* All methanol insect exracts showed MIC = 2500 µg/mL against *E. coli.*

Cytotoxicity and genotoxicity analyses

Cytotoxicity analyses on the human dermal fibroblasts (HDFa) after 24 h of exposure to aqueous and methanolic extracts of *P. helleri*, *V. nigricornis*, *G. bimaculatus*, *H. puera* (caterpillar), and *H. puera* (cocoon) were investiagted by using MTT cell viability analysis. Most extracts caused no significant reduction in viability at the tested concentrations, with cell viability remaining above 80% in most cases. Some exceptions were observed for certain methanolic extracts (e.g., *G. bimaculatus*) that induced a statistically significant decrease compared to the negative control (Fig. 3). MTT analysis on lymphocyte viability after 24 h treatment with insect extracts was generally well preserved, with only isolated reductions reaching statistical significance. Both aqueous and methanolic extracts of most species maintained viability above 80%, indicating low acute cytotoxicity (Fig. 4).

Nuclear abnormalities (micro-nuclei, notched, and lobed nuclei) were analyzed by using Hoechst 33,258 staining on HDFa cells. Across all insect extracts, both aqueous and methanolic forms induced very low nuclear abnormality indices (NAi $\leq 0.007 \pm 0.003$), close to the negative control (0.004 ± 0.002) and far below the positive control (0.017 ± 0.006). No extract caused a dose-dependent increase in abnormalities, and values remained within background variation (Fig. 5; Table 13). Furthermore, the frequency of structural chromosomal aberrations after 72 h exposure to insect extracts at a concentration of 1000 µg/mL did not show a significant increase compared to the control group. All insect extracts exhibited mean chromosomal aberration indices (CAi) ranging from 0.4 ± 0.02 (*G. bimaculatus* methanol) to 1.2 ± 0.06 (*P. helleri* aqueous), values comparable to the negative control (0.8 ± 0.04) and much lower than the positive control (3.62 ± 0.10). Aberrations observed were sporadic and included chromosomal gaps, breaks, and occasional complex abnormalities (ring, fused chromosomes). No consistent pattern of extract- or solvent-specific genotoxicity was detected (Table 13). Representative metaphase chromosome spreads used for the evaluation of chromosomal aberrations were shown in Fig. 6. Generally, genotoxicity evaluation using Hoechst 33,258 staining revealed minimal nuclear abnormalities in fibroblasts, with NAi values nearly identical to untreated controls, indicating no detectable induction of micronuclei or chromatin morphology changes. Similarly, chromosomal aberration analysis in lymphocytes confirmed the absence of significant clastogenic or aneugenic effects, as all extracts produced CAi values within normal background ranges. Notably, *G. bimaculatus* methanolic extract yielded the lowest aberration index (0.4 ± 0.02).

Discussion

Extraction

Water and methanol are two of the most commonly used solvents in natural product extraction, each with distinct properties influencing the phytochemical profile and biological activity of the resulting extracts.

Water is a polar, non-toxic, and eco-friendly solvent that closely mimics traditional preparation methods such as decoction and infusion. It is particularly effective in extracting hydrophilic compounds such as polysaccharides, glycosides, certain flavonoids, and phenolic acids [63]. However, its extraction efficiency is generally lower compared to organic solvents, and it is less effective for isolating non-polar bioactive compounds such as lipids, terpenoids, and some alkaloids [64]. Additionally, aqueous extracts may be more susceptible to microbial contamination and degradation if not processed promptly.

Methanol, a polar organic solvent, offers broader extraction capabilities, efficiently solubilizing both polar and moderately non-polar compounds. It is widely used for phenolic and flavonoid extraction due to its ability to disrupt plant cell walls and solubilize secondary metabolites [65]. Methanol extracts often yield a higher concentration of bioactive constituents and demonstrate stronger antioxidant and enzyme inhibitory activities. However, methanol is toxic and requires complete removal before any potential application in food or pharmaceutical formulations.

Overall, these results suggest that *R. differens*, particularly the green variant, is more efficient in terms of both methanol and aqueous extraction yields, despite starting

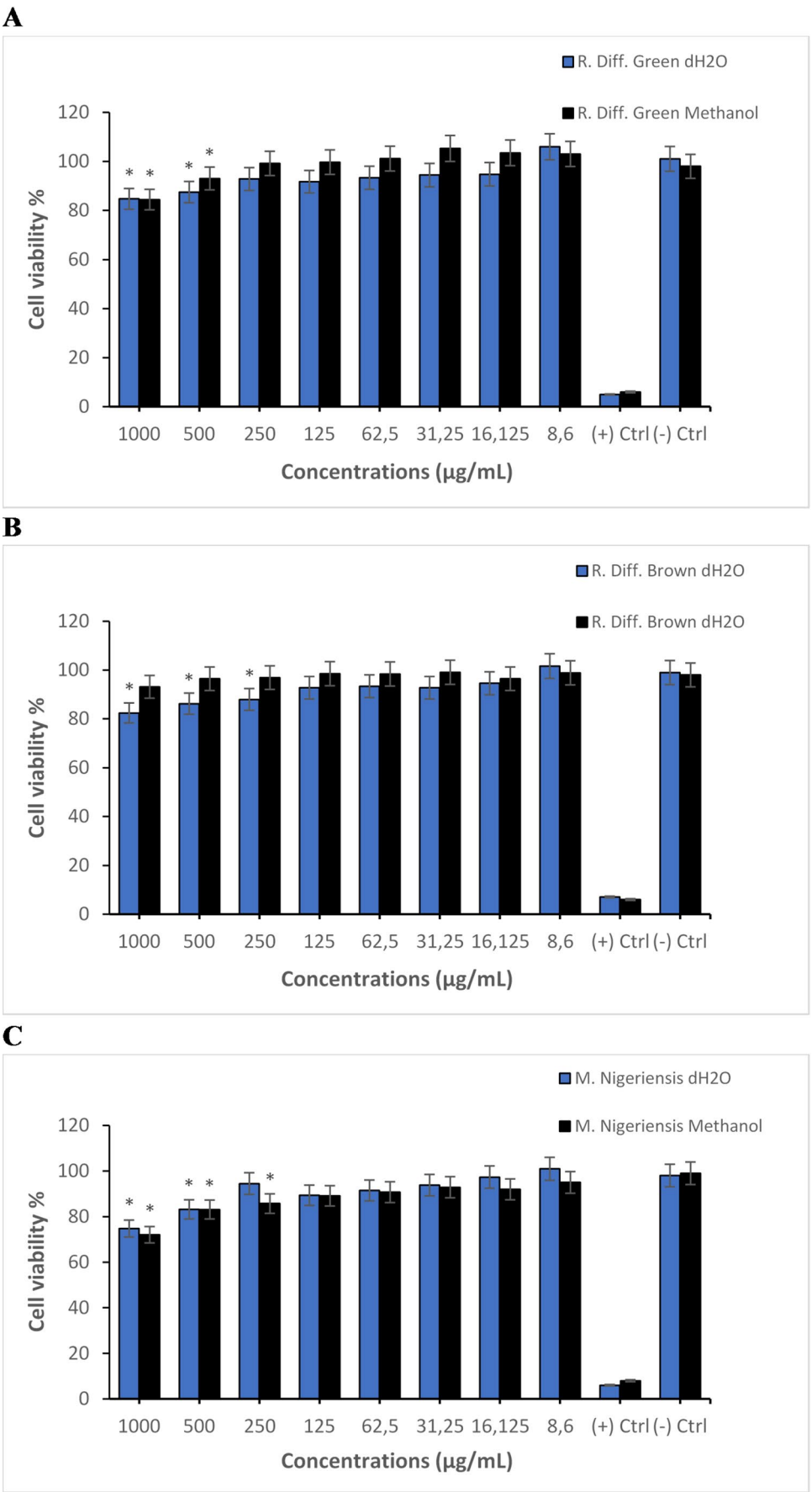


Fig. 3 Cytotoxic effects of insect extracts (water and methanol) on the human fibroblast (HDFa) cell culture for 24 h of applications. **A-** Nsenene (*R. differens*-green), **B-** Nsenene (*R. differens* and **C-***M. nigeriensis*. Cell viability is expressed as percentage relative to the negative control ((–) Ctrl). Data are presented as mean ± SD ($n = 3$). Statistical analysis was performed using one-way ANOVA followed by appropriate post hoc testing. Asterisks (*) indicate statistically significant differences compared with the negative control at $p < 0.05$

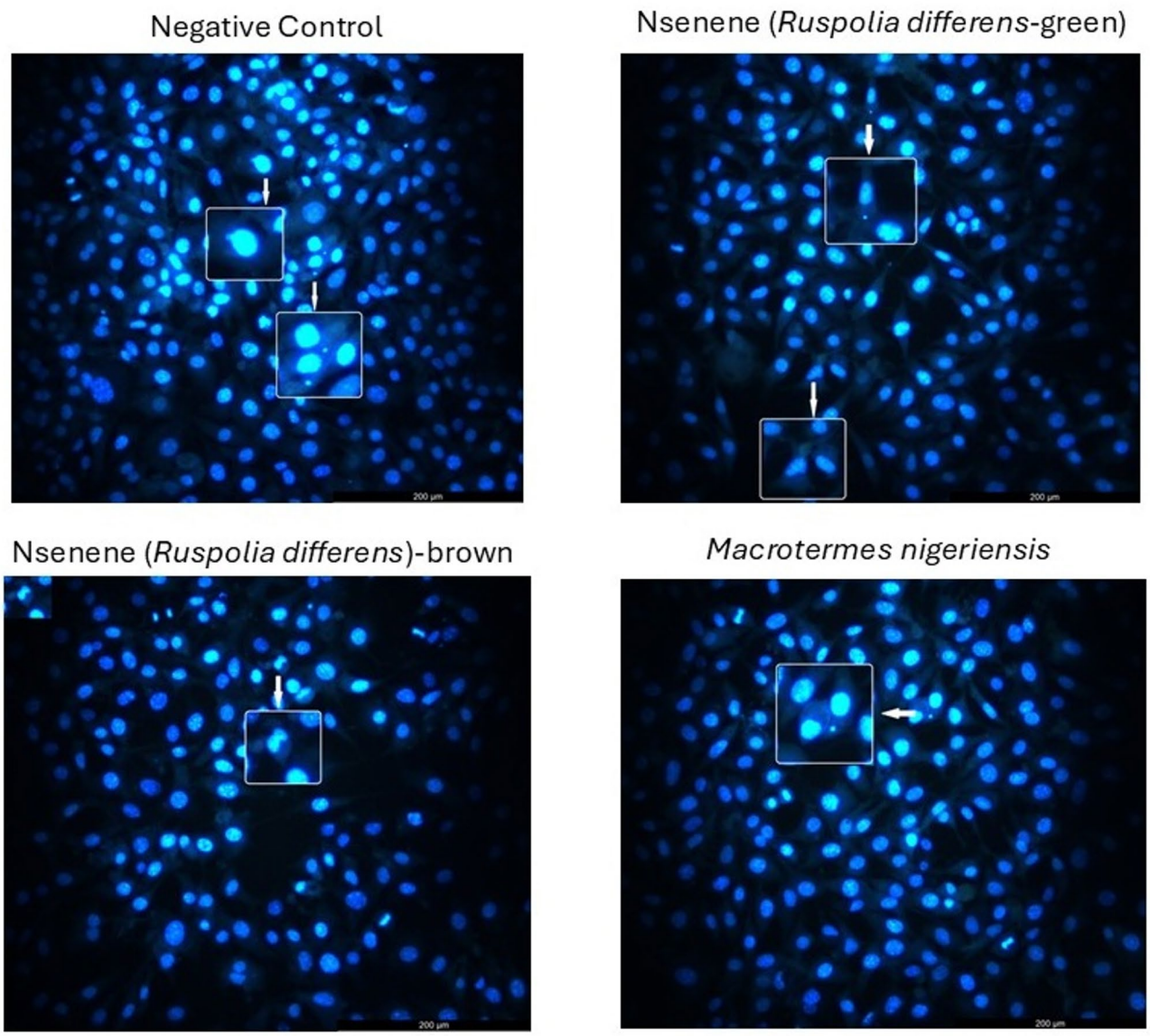


Fig. 4 Hoechst 33,258 staining analyses for the nuclear abnormality investigations. Sample images for Negative control group (non-treated HDFa cell culture), Nsenene (*R. differens*-green), Nsenene (*R. differens*)-brown, and *M. nigeriensis*. White arrows indicate nuclear abnormalities. Cell viability is expressed as percentage relative to the negative control ((–) Ctrl). Data are presented as mean ± SD ($n = 3$). Statistical analysis was performed using one-way ANOVA followed by appropriate post hoc testing. Asterisks (*) indicate statistically significant differences compared with the negative control at $p < 0.05$

with a smaller amount of biomass. This implies a potentially higher concentration of extractable phytochemical or biochemical components in *R. differens* compared to *M. nigeriensis* under the tested conditions. In summary, while water extracts are safer and more representative of traditional use, methanol extracts typically provide greater phytochemical richness and bioactivity. The choice of solvent should thus align with the target compounds, intended application, and safety considerations.

Fatty oil extraction and analysis

The fatty acid profiles of the studied insect species reveal a predominance of unsaturated fatty acids, particularly oleic acid (C18:1) and linoleic acid (C18:2). These findings align with previous studies highlighting the lipid richness of edible insects, especially in monounsaturated and polyunsaturated fatty acids (PUFAs), which are associated with favorable health outcomes [5, 66]. Oleic acid, in particular, is widely recognized for its beneficial role in reducing low-density lipoprotein (LDL) cholesterol while maintaining

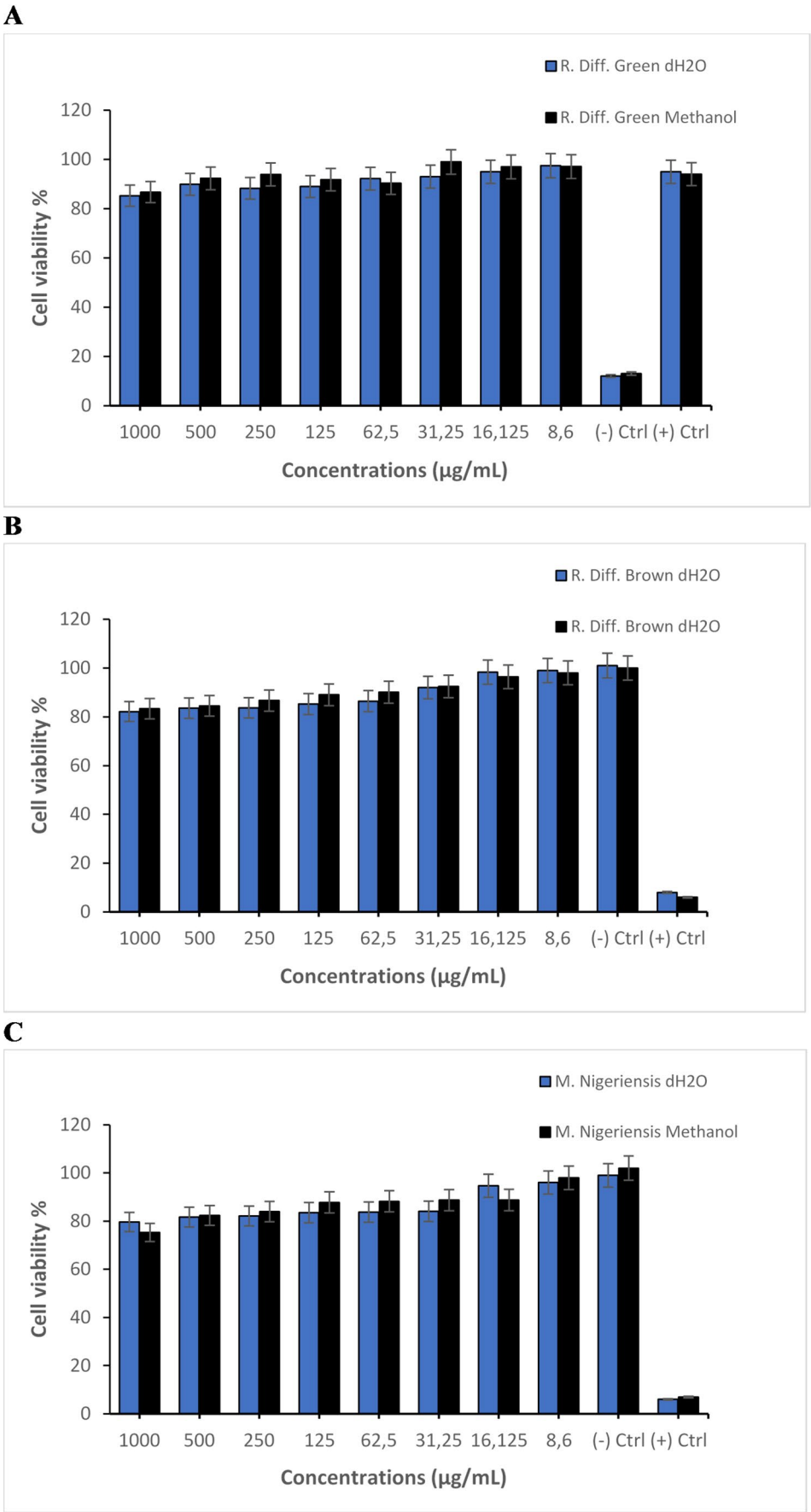


Fig. 5 Cytotoxic effects of insect extracts (water and methanol) on the lymphocyte cell culture for 24 h of applications. **A-** Nsenene (*R. differens*-green), **B-** Nsenene (*R. differens* and **C**-*M. nigeriensis*. Symbol (*) represents statistically significant difference compared to the negative control ((-) Ctrl)

Table 13 Frequency of chromosomal aberrations observed in human lymphocyte cell treated with 1000 µg/mL of all insect extraxts for 72 h

Groups	Methapahase Plaque	ChG	ChB	Ch'G	Ch'B	Others	Mean CAi + SD
Nsenene (*Ruspolia differens*-green) dH_2O	30	18	0	0	0	6	0.87 ± 0.04
Nsenene (*Ruspolia differens*-green) Methanol	30	0	0	0	12	24	1.1 ± 0.05
Nsenene (*Ruspolia differens*)-brown dH_2O	30	6	6	0	0	18	0.99 ± 0.04
Nsenene (*Ruspolia differens*)-brown Methanol	30	12	0	0	0	30	1.4 ± 0.07*
Nswa *(Macrotermes nigeriensis)* dH_2O	30	18	0	0	0	6	0.8 ± 0.04
Nswa *(Macrotermes nigeriensis)* Methanol	30	12	0	0	6	12	0.99 ± 0.04
Positive Control	30	18	0	6	6	18	3.62 ± 0.10
Negative Control	30	6	0	0	6	12	0.8 ± 0.04

ChG Chromosomal gaps, *ChB* Chromosomal breaks, *Ch'G* Chromatin gaps, *Ch'B* Chromatin breaks, *Others* including tetrad chromosome, ring chromosome, fused chromosome. *SD* Standard deviation

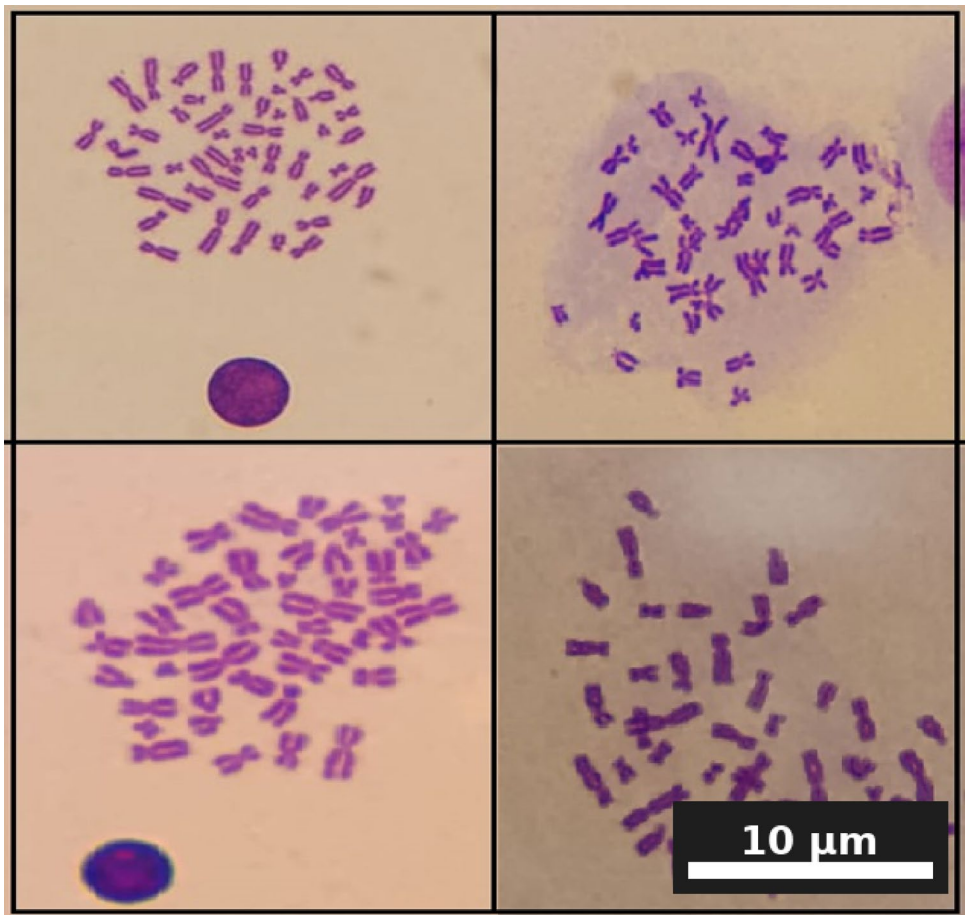


Fig. 6 Representative microscopic images of metaphase chromosome spreads used for the evaluation of chromosomal aberrations in the chromosomal aberration assay. Well-spread metaphase plates with clearly distinguishable chromosomes are shown. Scale bar = 10 µm

or even increasing high-density lipoprotein (HDL) cholesterol levels. Numerous clinical studies have demonstrated that diets rich in oleic acid—such as those based on olive oil—can reduce the risk of cardiovascular diseases [67, 68]. Linoleic acid, an essential omega-6 PUFA, was present in considerable amounts (~ 13%), suggesting that these insects can contribute to essential fatty acid intake. Additionally, *α*-linolenic acid (ALA, C18:3), an omega-3 fatty acid, was also identified (1.4–1.6%), though at lower concentrations. The presence of both linoleic and linolenic acids indicates a balanced lipid profile, which could support anti-inflammatory and neuroprotective effects when incorporated into human diets [69].

Palmitic acid (C16:0), a saturated fatty acid, was also relatively abundant, particularly in *M. nigeriensis* (33.5%). While saturated fats are often viewed as less desirable due to their potential to raise cholesterol levels, palmitic acid is a common component of many animal-derived and

plant-based foods, and its health effects depend on the overall dietary context [70].

The lower levels of myristic, margaric, stearic, and arachidic acids suggest that the overall saturated fat burden is moderate, especially in *R. differens–green*, which exhibited the highest oleic acid and lowest saturated fatty acid levels among the samples. This supports its potential as a functional food ingredient, particularly in the context of heart-healthy diets.

In line with studies by Womeni et al. (2009) and Van Huis (2013), the favorable lipid composition observed in these insects reinforces the notion that entomophagy can provide not only sustainable protein but also a rich source of bioavailable, nutritionally valuable lipids [71, 72]. In summary, all three insect species demonstrated a high content of unsaturated fatty acids, particularly oleic and linoleic acids, suggesting favorable lipid profiles with potential functional food applications. *R. differens*—especially the green variant—showed slightly superior nutritional lipid quality due to its higher oleic acid content and lower saturated fat ratio.

The volatile composition analysis

The present study revealed considerable variation in the volatile compound composition among three edible insect species-*M. nigeriensis*, *R. differens* (brown), and *R. differens* (green)-collected from Uganda. These species exhibited distinct aroma profiles, characterized by species-specific combinations and concentrations of volatile compounds. Such differences are consistent with previous reports emphasizing the influence of species, developmental stage, and environmental factors on insect metabolite expression [66, 73]. The differences in compound distribution not only highlight the role of species-specific biochemistry but also underscore the influence of diet, environment, and physiology on volatile development. These findings demonstrate that edible insects possess complex and varied flavor profiles, with potential applications in food innovation as flavor enhancers or alternative protein sources enriched with distinct sensory properties.

Quantitative analysis of amino acids

Among all samples, *M. nigeriensis* exhibited the most diverse and abundant free amino acid profile, with remarkably high levels of taurine, glutamic acid, ethanolamine, and alanine in the methanolic extract. Taurine, in particular, is known for its antioxidant and osmoregulatory roles, and its abundance in this species enhances its functional food potential [74]. Similar to our findings, Finke (2013) reported high taurine and glutamic acid contents in various edible termites, underscoring their relevance in meeting essential amino acid requirements [75].

The brown morph of *R. differens* also demonstrated high free amino acid content in both methanolic and aqueous extracts, particularly alanine, arginine, and glutamic acid. Arginine, an amino acid involved in nitric oxide synthesis and immune function, was especially abundant in the methanolic extract, suggesting the potential immunonutritional benefit of this insect [76]. Moreover, alanine concentrations in the aqueous extract of *R. differens* (brown morph) exceeded 3000 mmol/mL, which is notable and consistent with previous studies identifying alanine as one of the dominant free amino acids in Orthopteran species [77].

In contrast, the green morph of *R. differens* showed poor aqueous extractability of free amino acids, which may be attributed to inherent compositional differences or variations in protein solubility. Nevertheless, this morph retained substantial amino acid concentrations in the methanolic extract, including glutamic acid and ethanolamine, mirroring the profile observed in the brown morph. These findings highlight the critical role of solvent selection in optimizing amino acid recovery from insect biomass, as also emphasized by Rumpold and Schlüter (2013) [66].

Overall, the dominance of glutamic acid, alanine, and ethanolamine across all methanolic extracts is noteworthy, as these amino acids contribute not only to human nutrition but also to umami taste perception and potential health-promoting properties of edible insects. Accordingly, these results further support the growing interest in insects as sustainable alternative protein sources for food and feed applications [72, 78].

In addition to absolute concentrations, the nutritional quality of insect proteins was evaluated based on amino acid scores relative to FAO/WHO reference patterns. The amino acid profiles of *M. nigeriensis* and *R. differens* (brown morph) indicated a generally balanced composition; however, sulfur-containing amino acids (methionine and cysteine), and in some cases lysine, appeared comparatively lower, suggesting their role as potential limiting amino acids. Similar trends have been widely reported for edible insects, particularly among Orthopteran and Isopteran species, where methionine is frequently identified as the first limiting amino acid. Despite this limitation, the overall amino acid scores of the studied insects remain nutritionally favorable and could be effectively improved through dietary complementation or formulation strategies combining insect proteins with cereal- or legume-based foods.

It should be emphasized that this section focuses exclusively on free amino acids contributing to nutritional quality, whereas bioactive oligopeptides and protein-derived fractions are discussed separately in the subsequent section.

ICP-MS analysis

The observed interspecific and intraspecific differences in elemental composition are consistent with earlier reports [12, 16, 21, 79–81] and may be explained by seasonal variability, differences between swarming and non-swarming phases, as well as environmental and biological factors such as geographic origin, life stage, diet, habitat, and agro-ecological conditions [80, 82]. Although arsenic concentrations were close to the WHO dietary guideline, some countries apply stricter limits to protect vulnerable populations [83]. The presence of cadmium and mercury, particularly in *M. nigeriensis*, is noteworthy given their toxicological significance and potential for long-term accumulation, possibly reflecting environmental contamination or harvesting practices [84, 85]. Lead concentrations approaching international safety thresholds further highlight the importance of monitoring heavy metals in edible insects. Conversely, selenium levels remained within safe intake ranges and may contribute positively to nutritional quality due to its protective role against heavy metal toxicity. Overall, despite variability among species and color morphs, the elemental profiles generally support the potential of these insects as safe and nutritionally valuable food sources, provided that environmental exposure and consumption patterns are carefully considered.

Enzyme inhibition assays

A recent study demonstrated that crude extracts of *Bombyx mori* and *Omphisa fuscidentalis*, rich in n-hexadecanoic and oleic acids as well as flavonoids such as quercetin and kaempferol, exhibited significant α-glucosidase, α-amylase, acetylcholinesterase, and tyrosinase inhibitory activities through mixed or non-competitive mechanisms, with in vitro findings supported by molecular docking analyses [86].

Another study showed that peptide fractions obtained from simulated gastrointestinal digestion of heat-treated edible insects, particularly *Schistocerca gregaria*, exhibited strong inhibitory activities against ACE, pancreatic lipase, and α-glucosidase, with several identified peptides displaying low IC_{50} values and heat treatment enhancing overall bioactivity [87].

A study investigating crude extracts of several edible cricket species reported that methanolic extracts rich in phenolics and flavonoids, as well as dichloromethane extracts, exhibited strong inhibitory activities against enzymes linked to DM, AD, and melanogenesis, with fatty acids identified by GC–MS as major contributors to the observed bioactivity [88].

A study on black cricket (*Gryllus assimilis*) protein hydrolysates showed that enzymatic hydrolysis markedly enhanced α-amylase, α-glucosidase, and ACE inhibitory activities, with low-molecular-weight peptides— including AGDDAPR and YPLDL—identified as the main contributors to the observed antidiabetic and antihypertensive effects [89].

Taken together, the present findings clearly demonstrate that edible insects represent a promising natural source of bioactive compounds with significant inhibitory potential against key metabolic and neurodegenerative disease-related enzymes. *R. differens* (green and brown morphs) and *M. nigeriensis* extracts exhibited potent *α*-glucosidase inhibition, with *R. differens*-green showing superior activity comparable to acarbose, while *α*-amylase and cholinesterase inhibition remained moderate to negligible. These results represented the first report of *α*-glucosidase, *α*-amylase, AChE, and BChE inhibitory activities in the genera *Ruspolia* and *Macrotermes* and within the families Conocephalidae and Termitidae, respectively, thereby filling a significant knowledge gap in entomopharmacology. When considered alongside previous studies on *B. mori*, *O. fuscidentalis*, *S. gregaria*, and various edible cricket species, a consistent pattern emerges indicating that insect-derived extracts, peptides, and fatty acids can act as mixed-type or non-competitive inhibitors of *α*-amylase, *α*-glucosidase, and AChE. Furthermore, enzymatic hydrolysis and fractionation processes were shown to enhance the release of low-molecular-weight peptides with improved bioactivity, underlining the importance of processing techniques in maximizing functional potential. Collectively, these results highlight edible insects as valuable candidates for the development of functional foods and nutraceuticals aimed at managing diabetes, hypertension, neurodegenerative disorders, and pigmentation-related conditions.

The enzyme inhibition profile observed in the present study clearly indicates that the bioactivity of *R. differens* (both green and brown morphs) and *M. nigeriensis* is primarily driven by strong α-glucosidase inhibition, while α-amylase and cholinesterase inhibition remained limited. This selective inhibitory pattern is particularly relevant from a metabolic perspective, as α-glucosidase inhibition is considered a key mechanism in the postprandial regulation of blood glucose levels and is often associated with reduced gastrointestinal side effects compared to strong α-amylase inhibition. Similar selectivity toward α-glucosidase over α-amylase has been reported in other edible insect studies, where phenolic compounds, fatty acids, or low-molecular-weight peptides were identified as the main contributors to enzyme inhibition [87–89]. However, unlike many previous reports that focused on isolated species or processed fractions, the present study provides the first comparative

evidence of this enzyme inhibition pattern within the genera *Ruspolia* and *Macrotermes*, thereby expanding the current understanding of insect-derived bioactives at the genus and family levels.

To the best of our knowledge, this is the first study to investigate the enzyme inhibitory activities—specifically *α*-glucosidase, *α*-amylase, acetylcholinesterase, and butyrylcholinesterase inhibition—of *R. differens* (green and brown morphs), of the genus *Ruspolia*, and, more broadly, within the family Conocephalidae. Similarly, no previous studies have reported these enzyme inhibitory activities for *M. nigeriensis*, for the genus *Macrotermes*, or for the family Termitidae. The available literature for both taxa has largely focused on nutritional composition and entomophagy-related aspects, with virtually no data on bioactivity profiles. Our work therefore represented the first systematic evaluation of these enzyme inhibitory activities in both groups, providing novel insights into the potential functional and pharmacological relevance of these edible insects as natural sources of enzyme inhibitors.

$ABTS^{\cdot+}$ and $DPPH^{\cdot}$ scavenging activity

Oxidative stress–induced cellular damage underlies aging and many chronic diseases, and although antioxidants from plant and synthetic sources are well documented, insects—despite being the largest animal group—remain an underexplored yet increasingly evidenced natural source of antioxidant compounds warranting further biological and pharmacological evaluation [90].

The DPPH radical scavenging test is a frequently used test to determine the antioxidant activity of food ingredients. The DPPH reagent is used to assess the neutralizing capacity of antioxidants, particularly through the electron transfer mechanism. DPPH is a stable free radical compound with maximum absorption at 517 nm in ethanol. In the presence of antioxidants, the characteristic purple color of DPPH changes to yellow upon reduction. In this process, the DPPH radical loses its radical properties by neutralizing it when it accepts an electron or hydrogen atom from an antioxidant molecule [91]. While the ABTS method provides a more suitable assessment environment for hydrophilic compounds, hydrophobic compounds can interact more effectively with peroxyl radicals such as DPPH [92]. Therefore, two different in vitro test methods were used in our study to comprehensively assess antioxidant capacity.

In our study, we evaluated the effects of the type of solvent used in the extraction process on the chemical composition of the resulting extract and its impact on antioxidant capacity. The findings indicate that different solvents have significant effects on the composition profile and antioxidant activity, with significant differences between groups clearly demonstrating this interaction.

Antimicrobial activity

Our literature search found only one dissertation on this subject matter. In this dissertation, *R. differens* was extracted at room temperature using a series of solvents *n*-hexane, ethyl acetate, methanol, and water, and evaluated for antibacterial activity against *E. coli* DH10B, *S. aureus* ATCC 65,385 and fungal strain *Candida albicans* SC5314 using the microdilution broth method. The *S. aureus* growth was successfully suppressed by *n*- hexane extract at a concentration of 0.01% (v/v) [93]. In terms of chemical content, the contents of water extract and methanol extracts differed due to their polarity. Especially the methanol extract seems to be effective against *S. aureus* and *C. albicans* among the strains tested in our study. The MIC value was found to be higher against *E. coli.* The reason for this fungi, Gram-positive bacteria, and Gram-negative bacteria have different cell wall compositions. As a result, the microbes were harmed differently by the antimicrobial drugs.

In this study, the amino acid content of methanol and water extracts of insect samples from Uganda was found to be high, especially alanine and glutamic acid. These amino acids may be responsible for the antimicrobial effect.

In earlier studies, using the bacteria *E. coli*, *Staphylococcus aureus*, and *Micrococcus luteus* as well as the yeast *Candida albicans*, alanine substitution peptides were synthesized, characterized, and their antibacterial efficacy assessed. The antimicrobial properties of analogues of alanine were in line with their structural characteristics [94].

Studies on glutamic acid show that it is antimicrobial. It was determined that the silver-glutamic acid complex mostly demonstrates antibacterial action against with minimum bactericidal concentrations (MBC) for *Salmonella glostrup*, *E. coli* 0151, *St. aureus* 209P, and *Candida albicans* being 10 g/mL [95].

In another study, L-glutamic acid's antibacterial properties were evaluated against Gram-negative bacteria (*Proteus*, *Shigella*, *Pseudomonas sps*, *and Vibrio cholerae*) and pathogenic strains such as *Bacillus cereus*, *Streptococcus sps*, *and Staphylococcus aureus*. The usefulness of L-glutamic acid polymorphs of *α* and *β* crystals as a novel alternative antibacterial treatment agent is highlighted in these investigations [96].

Cytotoxicity and genotoxicity analyses

As a result, the cytotoxicity assays on HDFa fibroblasts and primary human lymphocytes demonstrate that both aqueous and methanolic extracts of the tested edible insects have

generally low toxicity at concentrations up to 1000 µg/mL. While certain methanolic extracts (e.g., *G. bimaculatus*) caused a modest but statistically significant reduction in fibroblast viability, the majority of treatments preserved > 80% cell survival, aligning with previous findings that edible insect-derived extracts are well tolerated by mammalian cells at moderate doses [97, 98].

These findings suggest a potential absence, or even suppression, of chromosomal damage; however, this observation requires further confirmation through more detailed mechanistic studies. The lack of pronounced cytotoxic or genotoxic effects is encouraging from a food safety perspective, particularly in light of the growing interest in edible insects as sustainable alternative protein sources. The generally low toxicity profile observed in the present study is consistent with previous reports on other insect species commonly consumed in entomophagy [81, 99]. Nevertheless, the modest reductions in cell viability detected with certain methanolic extracts underscore the need for comprehensive toxicological evaluations, especially when insect-derived materials are considered in concentrated or processed forms for nutraceutical or pharmaceutical applications [99, 100].

Conclusion

Entomophagy constitutes an integral component of Ugandan food culture and provides clear nutritional and socioeconomic benefits, particularly for rural communities. In line with the specific objectives of this study, the present findings indicate that the investigated edible insects (*R. differens* green and brown morphs and *M. nigeriensis*) are valuable sources of essential minerals, including potassium, calcium, iron, zinc, and selenium, supporting their nutritional relevance. At the same time, non-essential and potentially toxic elements such as arsenic, cadmium, mercury, and lead were detected at generally low but variable levels, with concentrations influenced by species, color morph, and environmental conditions, highlighting the importance of continuous monitoring of metal(loid) exposure.

Beyond elemental composition, this study provides the first systematic evaluation of α-glucosidase, α-amylase, acetylcholinesterase, and butyrylcholinesterase inhibitory activities within the genera *Ruspolia* and *Macrotermes*. The pronounced α-glucosidase inhibition observed, particularly in *R. differens* extracts, suggests potential functional relevance in the modulation of postprandial glucose metabolism, while the limited α-amylase and cholinesterase inhibition reflects a selective enzyme inhibition profile. Furthermore, cytotoxicity and genotoxicity assessments using human dermal fibroblasts revealed no marked adverse effects at the tested concentrations, supporting a preliminary indication of safety for food-related applications.

Taken together, the results suggest that edible insects consumed in Uganda combine nutritional richness with functional bioactivity and a generally acceptable safety profile under the conditions tested. However, the observed variability in toxic metal content underscores the need for controlled rearing, standardized processing, and appropriate regulatory oversight. The key take-home message is that edible insects hold considerable potential as nutritious and functional food ingredients; however, this potential can only be responsibly realized through sustainable insect farming under controlled conditions, coupled with strict quality control, conservation practices, and legislation to minimize predictable health risks and ensure consumer safety.

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Data availability Data will be made available on request.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval There is no ethics approval.

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