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Medicinal Relevance Of Bioactive Compounds in *Rhynchophorus Ferrugineus* (Red Palm Weevil) Larva

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Abstract
The aim of this research was to enhance human and insect relationships from ordinary nutritional delicacy to medicinal and pharmaceutical use by identifying medicinally relevant bioactive compounds in the insects. Matured and healthy *R. ferrugineus* larvae harvested from infested palm trees in Bayelsa State, Nigeria, were washed thrice using distilled water, asphyxiated by freezing for 24 hours, and blended using a Binatone BLG 450 electric blender. Ten grams of the ground sample, staple-sealed in Whatman no.4 filter paper, was extracted in a Soxhlet extractor using dichloromethane as solvent. The extract obtained was concentrated, and the bioactive compounds were determined using an Agilent Technology manufactured Gas Chromatograph model HP 6890 and Mass Spectrometer model 5973. The highest peaks in the chromatogram of bioactive compounds were observed at retention times of 15.301 and 15.469 mins. n-Hexadecanoic acid and Octadecanoic acid have the highest percentage concentrations with values of 39.900 and 22.230 amongst the saturated fatty acids, while Octadec-9-enoic acid and (Z)-octadec-9-enoic acid were the highest unsaturated fatty acids with percentage concentrations of 13.452 and 4.207. Bis(2-ethylhexyl) phthalate was the highest amongst the ethers, with a percentage concentration of 1.116. while Tetracosane, 3-ethyl and Naphthalene, 1,2,3,4-tetrahydro-1-nonyl- were the only aliphatic and polycyclic aromatic hydrocarbons with percentage concentration of 1.422 and 0.314, respectively. The presence and antimicrobial activities of these bioactive compounds present this insect larva as a major source of natural antimicrobial compounds, which may be of relevance in the pharmaceutical development of broad-spectrum antimicrobial medicine.

Keywords: Insects, Bioactive compounds, Dichloromethane, Extract, Antimicrobial Properties.

Introduction

Insects and humans have enjoyed both friendly and unfriendly relationships. Though most insects are often regarded as pests, some insects are important in adding aesthetic values, while others are consumed by man as food. For decades, edible insects have been consumed as a highly nutritious traditional food, especially in terms of fat, protein, and mineral elements, thus serving as alternative food, feed formulation sources, and potential substitutes for fish and animal meal formulae. Though one major problem associated with insect consumption is its acceptability, *Rhynchophorus ferrugineus* (Red palm weevil) is one insect whose edibility has gained wide acceptance due to its amino acids, fatty acids, and mineral element content (Ayensu et al., 2020). Aside from their edibility, they are also used in traditional medicine for skin ailments, cosmetic formulation, and in the production of antimicrobial peptides (Niode et al., 2024). It is a major pest of palm plantations due to its ability to bore through palm tree stems, feed on their sap and cellulose, and eventually cause the death of palm trees. Scientific reports have shown significant level of nutrient composition, protein solubility, minerals, functional and anti-nutritional factors in both its larval and the pupal stages (Abdel-Moniem et al., 2017), however, larval stage is highly desirable for its meat and in powdered food

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production than the pupal stage (Abdel-Moniem et al., 2017) *R. ferrugineus* larvae has been successfully integrated into the human delicacy list especially within the Southern region of Nigeria, where they are served as fried or toasted pepper garnished delicacy. Trevor (2017) reported it as a rich Vietnamese delicacy, often eaten alive with fish sauce or by toasting, frying, and steaming, while Kohler et al. (2020) also reported its consumption in other countries such as East Malaysia and New Guinea. Indigenous people in eastern Nigeria also use R. ferrugineus larvae in biological water treatment or as a possible immune booster by dropping a healthy, viable organism into a glass of water and subsequently administering the water to infants after 24 hours. This cultural process may be attributed to the ability of *R. ferrugineus* larvae to release antimicrobial peptides (AMPs), potential antimicrobial properties for both the treatment of microbial infections and the prevention of antibiotic-resistant microorganisms (Niode et al., 2024). In this present study, the percentage concentrations of the bioactive components in healthy *R. ferrugineus* larvae will be determined using Gas Chromatography- Mass Spectroscopy (GC-MS).

Fig. 1: A representative picture of healthy *R. ferrugineus* larvae used for this study

Materials and Methods

Sample Collection and Preparation

Matured and healthy *R. ferrugineus* larvae were harvested from infested palm trees in Bayelsa State, Nigeria, and twenty of the most viable larvae were placed in an open glass container, and a moist palm tree stem fiber from their host tree was also introduced into the glass container (to create their pseudo-home) and transported to the Department of Biochemistry Laboratory, University of Port Harcourt. These life and viable larvae were removed from the glass and washed in thrice, using distilled water, and asphyxiated by freezing for 24 hours and blended using a Binatone BLG 450 electric blender. Ten grams (10 g) of the ground sample placed in Whatman no.4 filter paper was staple-sealed and placed in a Soxhlet extractor mounted on a dried distillation flask. Fifty milliliters (50 ml) of analytical Dichloromethane were introduced into the distillation flask, and the flask was set up using a retort stand. A continuous jet of cold water was allowed into the condenser as the hot dichloromethane (at 50 ºC) refluxed for 6 hours. This process was repeated twice to obtain a clear solvent, and the extracts obtained were separated from the solvent, concentrated by evaporation at 23 ºC under a Labotronics LB-12DFH Ducted Fume hood, and placed in a labeled dry sterile universal sample bottle.

GC-MS Determination of Bioactive Compounds

The bioactive compounds present in the *R. ferrugineus* larvae extracts were determined using the method described by Ohiri & Bassey (2016). An Agilent Technology manufactured Gas Chromatograph model HP 6890, and Mass Spectrometer model 5973 were fitted with a capillary column of HP-5 MS of 30.0 m x 250 µm x 0.25 µm packed with

5 % phenylmethylsiloxane, using helium as the carrier gas. An initial column temperature of 120 ºC was kept for 5 minutes and subsequently increased to 320 ºC at 5 ºC per minute and held for 5 minutes. Electron impact ionization for mass spectroscopy was done at an ionization energy of 70 eV, and a volume of 0.5 ml of the pure extract was diluted using 98 % of dichloromethane, and 2.0 µl of the diluted extract was auto-injected into the Agilent Tech model 5973 Mass Spectrometer. The bioactive compounds present in the extract were identified against their pure standards, using Chem-Office software attached to the MS library, while the National Institute of Standards and Technology database was used to establish the molecular formulas, weights, and names of the bioactive compounds.

Results

The result of this study showed the presence of ten (10) bioactive compounds. The chromatogram of bioactive compounds has its highest peaks at retention times of 15.301 mins, 15.469 mins, and 17.057 mins, which translates to percentage concentrations of 16.627, 15.513, and 12.470 for Octadecanoic acid, n-Hexadecanoic acid, and 9-Octadecenoic acid (E)- respectively (fig. 2).

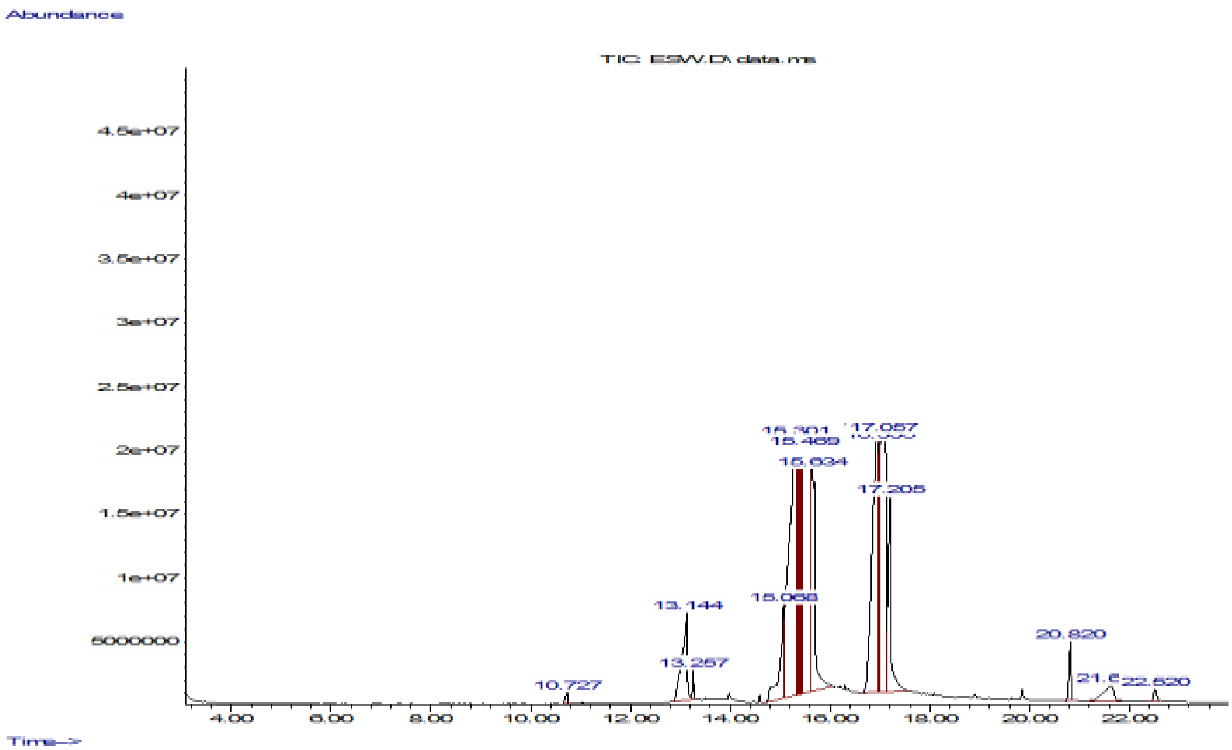

Figure 1: GC-MS Chromatogram of Bioactive Compounds in *R. ferrugineus* larvae extracts

Amongst the four (4**) saturated fatty acids observed in this study,** n-Hexadecanoic acid and Octadecanoic acid have the highest percentage concentrations with values of 39.900 and 22.230, respectively, while Tetradecanoic acid and Dodecanoic acid had the least percentage concentrations with values of 4.363 and 0.243, respectively (Table 1).

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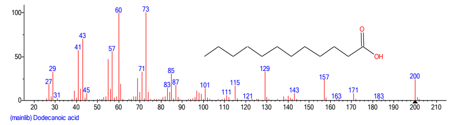
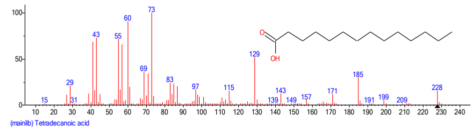
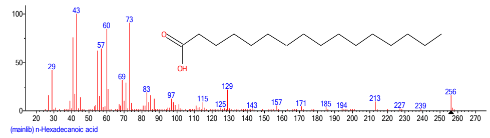
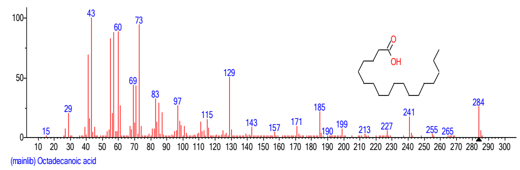
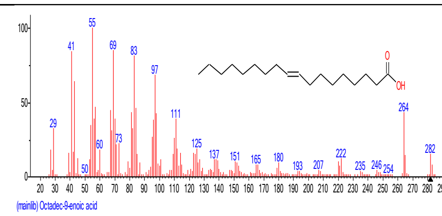
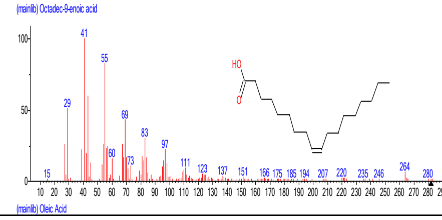
Table 1: Saturated Fatty acid Content of *R. ferrugineous*

S/N	Compound	Retention Time (min)	Percentage Concentration	Molecular formula	Molecular weight	Structure
1	Dodecanoic acid	10.727	0.243	$C_{12}H_{24}O_2$	200.3178	
2	Tetradecanoic acid	13.144	4.363	$C_{14}H_{28}O_2$	228.3709	
3	n-Hexadecanoic acid	15.388	39.900	$C_{16}H_{32}O_2$	256.4241	
4	Octadecanoic acid	16.253	22.230	$C_{18}H_{36}O_2$	284.4772	

Octadec-9-enoic acid and (Z)-octadec-9-enoic acid were the only unsaturated fatty acids observed in this study with retention times of 16.943 and 4.207, and percentage concentration of 13.452 and 4.207 (table 2).

Table 2: Unsaturated Fatty acid content of *R. ferrugineous.*

S/N	Compound	Retention Time (min)	Percentage Concentration	Molecular formula	Molecular weight	Structure
1	Octadec-9-enoic acid	16.943	13.452	$C_{18}H_{34}O_2$	282.4614	
2	(Z)-octadec-9-enoic acid	16.995	4.207	$C_{18}H_{34}O_2$	282.4614	

While Tetradecanoic acid, ethyl ester observed at a retention time of 13.257 mins and Bis(2-ethylhexyl) phthalate observed at retention time of 20.820 were the only esters observed in this study, Bis(2-ethylhexyl) phthalate was of higher percentage concentration with a value of 1.116, Tetradecanoic acid, ethyl ester had a percentage concentration of 0.284 (table 3).

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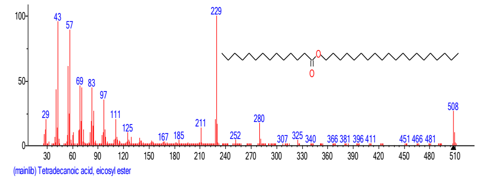
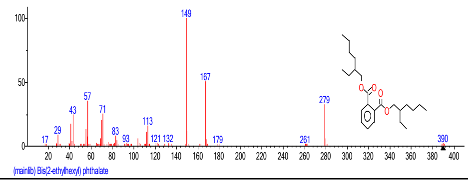
Table 3: Ester content of *R. ferrugineous.*

S/N	Compound	Retention Time (min)	Percentage Concentration	Molecular formula	Molecular weight	Structure
1	Tetradecanoic acid, ethyl ester	13.257	0.284	$C_{16}H_{32}O_2$	256.4241	
2	Bis(2-ethylhexyl) phthalate	20.820	1.116	$C_{24}H_{38}O_4$	390.5561	

While Tetracosane, 3-ethyl was the only aliphatic hydrocarbon observed in this study, with a retention time of 21.629 mins and a percentage concentration of 1.422, Naphthalene, 1,2,3,4-tetrahydro-1-nonyl- was the only polycyclic aromatic hydrocarbon observed in this insect, with a retention time of 22.520 mins and percentage concentration of 0.314 (tables 4 and 5).

Table 4: Aliphatic hydrocarbon content of *R. ferrugineous.*

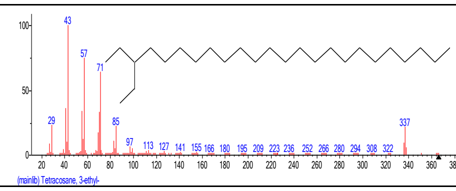
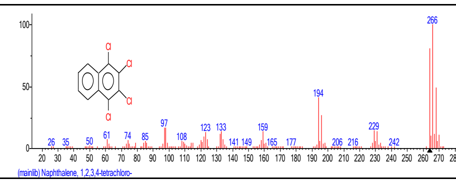
S/N	Compound	Retention Time (min)	Percentage Concentration	Molecular formula	Molecular weight	Structure
1	Tetracosane, 3-ethyl-	21.629	1.422	$C_{26}H_{54}$	366.7070	

Table 5: Polycyclic aromatic hydrocarbon Content of *R. ferrugineous.*

S/N	Compound	Retention Time (min)	Percentage Concentration	Molecular formula	Molecular weight	Structure
18	Naphthalene, 1,2,3,4-tetrahydro-1-nonyl-	22.520	0.314	$C_{19}H_{30}$	258.4415	

Discussion

The use of this larva in traditional medicinal practice may be attributed to the presence of these bioactive compounds. The high concentration of n-Hexadecanoic acid as observed in this study may be responsible for its antimicrobial properties and for the prevention of antibiotic-resistant microorganisms. Ganesan et al. (2024) found that n-hexadecanoic acid exhibits both antibacterial and antifungal properties and also acts as a natural inhibitor against both Gram-positive bacteria, such as S. aureus, B. subtilis and Gram-negative bacteria such as E. coli. Krishnan et al. (2019) reported that n-hexadecanoic acid isolated from Canthium parviflorum leaves not only inhibits Gram-negative and Gram-positive bacteria growth, but also inhibits fungal growth. These findings present n-hexadecanoic acid as a broad-spectrum antimicrobial compound, and its high concentration in this larva validates the use of this larva in traditional medicine for the treatment of different microbial infections. In addition to its antimicrobial effects, Ramamoorthy et

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al. (2025) reported anti-inflammatory efficacy of n-hexadecanoic, while its antioxidant capabilities were reported by Ghfil et al. (2025). Aside from n-hexadecanoic acid, octadecanoic acid was also observed in high concentration. Though octadecanoic acid may not directly inhibit microbial growth, its unsaturated derivatives, such as 9-octadecenoic acid, have been reported to exhibit antimicrobial properties against both bacteria and fungi (Pringgenies et al., 2023).

Scientific reports also indicate that tetradecanoic acid, which was also present in this larva, exhibits significant antimicrobial properties against bacterial and fungal infections (Kim et al., 2016). In 2021, Juárez-Rodríguez and his coworkers reported tetradecanoic acid amongst the carbon 12 to carbon 14 saturated fatty acids that are involved in the regulation of P. aeruginosa virulence. Dodecanoic acid, also observed in this larva, has also been reported to exhibit antimicrobial properties even at very low concentrations. In a study by Matsue et al. (2019), only a minute concentration of 0.062 micro mol/mL of dodecanoic acid was required to inhibit Pneumococcus infections, as against 1.45 and 0.218 micro mol/mL of capric acid and myristic acid needed to inhibit the same microbes. They also reported the ability of dodecanoic acid to modulate intestinal health by showing severe antimicrobial activity against gastrointestinal tract pathogenic organisms such as Bacteroides and Clostridium while exhibiting low activity on commensal lactic acid bacteria (Matsue et al., 2019).

Aside from the medicinal relevance of the saturated fatty acids observed in this larva, its unsaturated fatty acids have also shown varied degrees of antimicrobial activities. While octadec-9-enoic acid has been reported to exhibit broad spectrum antimicrobial activities, against different bacterial and fungal species such as S. aureus, E. coli, and C. albicans (Zahara et al., 2022), reports on (Z)-octadec-9-enoic acid shows its ability to inhibit microbial growth by destroying microbial cell membranes, thus causing depolarization and influx of calcium into microbial cells (Zhong-hui et al., 2010). Though low percentage concentrations of Tetracosane, 3-ethyl- (an aliphatic hydrocarbon) and Naphthalene, 1,2,3,4-tetrahydro-1-nonyl- (a polycyclic aromatic hydrocarbon) observed in this larva may be attributed to accumulation of these hydrocarbon in the host palm trees through hydrocarbon pollution via oil exploration activities in Bayelsa State and possible ingestion or topical absorption during the growth period of this larva, the presence of Bis(2-ethylhexyl) phthalate in this larva can also be attributed to environmental pollution (Rowdhwal 2014), while tetradecanoic acid, ethyl ester in this larva also adds to its antimicrobial potential. Mayser (2015) reported that tetradecanoic acid, ethyl ester, mostly of plant extracts, exhibits antimicrobial properties and has contributed to the antimicrobial effectiveness of natural products. The highest peaks in the chromatogram of bioactive compounds were observed at retention times of 15.301 and 15.469 mins. n-Hexadecanoic acid and Octadecanoic acid have the highest percentage concentrations with values of 39.900 and 22.230 amongst the saturated fatty acids, while Octadec-9-enoic acid and (Z)-octadec-9-enoic acid were the highest unsaturated fatty acids with percentage concentrations of 13.452 and 4.207. Bis(2-ethylhexyl) phthalate was the highest amongst the ethers, with a percentage concentration of 1.116. while Tetracosane, 3-ethyl and Naphthalene, 1,2,3,4-tetrahydro-1-nonyl- were the only aliphatic and polycyclic aromatic hydrocarbons with percentage concentration of 1.422 and 0.314, respectively.

Conclusion

The presence and antimicrobial activities of these bioactive compounds present this insect larva as a major source of natural antimicrobial compounds, which may be of relevance in the pharmaceutical development of broad-spectrum antimicrobial medicine.

Recommendations: Isolation, purification, and integration of these compounds in pharmaceutical products should be encouraged as a natural bioactive source, thereby reducing dependency on synthetic bioactive compounds.

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