INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
21–0820/2021/26–6–695–701
DOI: 10.17957/IJAB/15.1884
http://www.fspublishers.org

Full Length Article

Gas Chromatography-Mass Spectroscopy and Histopathological Effects of Methanol Leaf Extract of *Uvaria chamae* on the Midgut of *Sitophilus zeamais*

Diligent Oboho[1*], Samson Oyebadejo[2], Innocent Edagha[5], Peace Ubulom[4], Basil Ita[3], Akwaowo Nelson[4], Akaninyene Akpan[4] and Joseph Eyo[1]

[1]*Department of Zoology and Environmental Biology, University of Nigeria, Nsukka Enugu State, Nigeria*
[2]*Department of Biotechnology, Molecular Pathology Laboratory, Shri Jagdish Prasad Jhabarmal Tibrewala University, Jhunjhunu, Rajasthan 333001, India*
[3]*Department of Chemistry, University of Uyo, Nigeria*
[4]*Department of Animal and Environmental Biology, University of Uyo, Nigeria*
[5]*Department of Human Anatomy, University of Uyo, Nigeria*
[*]For Correspondence: sweetdili4life@yahoo.com
Received 05 August 2021; Accepted 25 September 2021; Published 15 December 2021

Abstract

The present study was carried out to assess the effect of methanol leaf extract of *Uvaria chamae* using Gas Chromatography-Mass Spectrum (GC-MS) to determine the phytochemicals present and its effect on the histology of midgut of maize weevil, *Sitophilus zeamais*. Insects were administered with 10 mg/kg of the plant extract using diffusion method where insects were put in a petri dish containing various concentrations and observed to see the stage they begin to die due to toxicity and observed for 5 min. They were collected into foil processing paper and fixed in Bouins fluid for 24 h, repacked after 24 h and folded in fresh foil immersed in buffered formalin for histopathological studies. Result revealed that a severe degeneration de-arrangement of the respiratory tract epithelial lining, secretory lining cells and gastrointestinal layers with the destruction of the muscular layer when compared with the control. The methanol leaf extracts of *U. chamae* were preliminary screened for the phytochemicals. The extract shows the presence of cardiac glycosides, saponin, steroids/terpenes, flavonoids, alkaloids and phenols. GC-MS analysis of the extract showed the presence of 2-nitrobenzaldehyde (4.00), malic acid (2.04), L-aspartic acid (2.00), 1, 1, dimethylhydrazine (1.86), Cedrandiol (1.75), 2-amino-4-(2-methylpropenyl)-pyrimidin-5-carboxylic acid (1.56), thiirane (1.54), mercaptoethanol (1.11) and some minor compounds. The findings indicated that methanol extract of *U. chamae* is rich in phyto-compounds having biological activities on the midguts' histology of *S. zeamais.* Therefore, it is recommended as an alternative for the synthetic insecticide used by farmers for the preservation of stored grains.

Keywords: Phytochemistry; GC-MS analysis; Histopathology; *Sitophilus zeamais*; *Uvaria chamae*

Introduction

Man grieves significantly both in agriculture and health due to the attack of the insect population. In agriculture, insects disturb growing crop parts and cause severe damage to stored products, leading to a loss in revenue. Insects are the invertebrate group's most essential living organisms, beneficial to humans and other species as well as harmful, causing diseases. The adjustable tropical biotopes provide a perfect environment for many arthropods, mainly insects. Botanical insecticides as undoubtedly used in crop protection are possibly likened to have started when agriculture began (Thacker 2002).

Extracts from local plants, used alone or in mixtures, have conventionally been used in Africa as crop protection agents. The combination of efficiency, speediness of action, easiness of use, and low cost of manmade insecticides has led many botanicals in most developed countries to near oblivion. However, Twenty years after manmade insecticides were decisively enshrined in 'modern' agricultural invention, the reported and so-called problems of general environmental degradation, harmfulness to non-target organisms, most significantly, adverse effects on human health led to a reappearance of interest in 'natural' pest control measures, including extended searches for new sources of plant insecticides. Consequently, several reports on the use of phytochemicals to control the hazard from insects exist (Regnault-Roger 2005; Isman 2006).

Uvaria chamae Beauv is a part of the Annonaceae family. It is commonly known as 'bush banana' or 'finger

To cite this paper: Oboho D, S Oyebadejo, I Edagha, P Ubulom, B Ita, A Nelson, A Akpan, J Eyo (2021). Gas Chromatography-Mass Spectroscopy and histopathological effects of methanol leaf extract of *Uvaria chamae* on the midgut of *Sitophilus zeamais*. *Intl J Agric Biol* 26:695–701

root.' Additionally, the leaves are arranged with simple leaf structures, lanceolate in the shape of a whole lamina and net veined. Leaves are stipulated, leaf apex cuminate and the vestiture of the leaf is glabrous (Bongers *et al.* 2005). It is a climbing plant found primarily in West Africa's tropical rainforest (Okwu 2004). It's common in Nigeria's savannas and rain forests, as well as other African countries. Among the Igbos, Hausas and Yorubas respectively, it is called "*Mmimiohia*," "*Kaskaifi*," and "*Akisan*" (Adetunji 1999; Ogueke *et al.* 2007). *U. chamae* is known for its medicinal and nutritional value. However, Okwu and Iroabuchi (2009), reported that extracts of *U. chamae* are exhibits mutagenic effect. However, there are less research outcomes on its use as a natural insecticide. The aim of this work was to carry out the phytochemical constituents of the plant using Gas Chromatography-Mass spectroscopy and to evaluate the effect of the extracts on histology of the insect's midgut.

Materials and Methods

Collections and identification of plant materials

The fresh leaves of *Uvaria chamae* were obtained from Faculty of Pharmacy Medicinal Farm of University of Uyo, Akwa Ibom State and validated by a taxonomist in the Department of Botany and Ecological Studies, University of Uyo. Voucher specimens with number: UUH/3687 was deposited in their herbarium for further referencing.

Rearing of test organisms

To provide comparable age weevils for the experiment, *S. zeamais* cultures were established. A total of ten (10 kg) maize seeds were purchased and cleaned to remove any seeds with visible damage. To prevent potential field infestation, the clean seeds were kept in a sealed container in the fridge at 4°C for a month. Seeds were placed in soft bags and stored at room temperature for two weeks. Sexes of unruffled *S. zeamais* were determined by probing the snout of pest-infested corn grains. Females have a longer and thinner snout, while males have a shorter and fatter snout. Furthermore, females have smooth bumpy bodies, whilst males have rough bodies (Kranz *et al.* 1977). The insects were cultured on clean seeds, with 100 weevils per 400 g of seeds in each jar. To allow airing and prevent weevil escape, the jar was covered with muslin cloth and secured with a rubber band and kept at room temperature. All parent weevils were removed from each jar seven days after oviposition (Walgenbach *et al.* 1983). The dimorphic rostral characteristics were used to separate the sexes (Halstead 1963; Odeyemi and Daramola 2000; Adedire 2001). The jars were placed in an insect rearing cage kept in the Entomology Laboratory, Department of Animal and Environmental Biology, University of Uyo, Uyo. Newly emerged, two day-old insects was used for the experiment.

Preparation of plant powder and extract

After collection, the plant leaves were washed and chopped into pieces and room dried to a constant weight. Using a power-driven blender (Braum Multiquick Immersion Hand Blender, B White Mixer MR 5550CA, Germany), the dry plants were melded into fine powder and then kept in an airtight container pending use. The crude leaf extracts were then prepared using standard procedures as outlined by (Fatope *et al.* 1999; Mukhtar and Huda 2005; Santana *et al.* 2013). This involved soaking 50 g of the powder for 48 – 72 h at room temperature in 95 percent methanol. This was followed by filtrate evaporation using a rotatory evaporator to obtain the crude extract.

Phytochemical analysis of the plants

The initial phytochemical screening of the different plants was carried out in Pharmacognosy Laboratory of University of Uyo, Akwa Ibom State using the standard procedures as described by (Harborne 1984; Evans 2002; Kokate *et al.* 2008; Prashant *et al.* 2011).

Gas chromatography-mass spectrometry analysis

A GC Clarus 500 Perkin Elmer system and gas chromatograph were interfaced with a mass detector (Turbo mass gold Perkin Elmer) according to Mishra *et al.* (2015) and Hema *et al.* (2010) (GC-MS). Column: Elite-5MS (5 percent diphenyl/95 percent dimethyl poly siloxane), 30 x 0.25 mm × 0.25 mm df, Carrier gas: Helium (99.999 percent) with constant flow rate of 1 mL per min, (Split ratio: 10:1), Sample Injection volume 2 L, Software: Turbo mass 5.2, Oven operating in electron impact mode at 70 eV, oven temperature was fixed from 110°C (isothermal The injector was set to 250°C, the ion source to 280°C, and the total GC run time was 36 min. The GC-MS was conducted in Multi-User Science Research Laboratory, Department of Chemistry, Ahmadu Bello University (ABU) Zaria, Kaduna, Nigeria.

Determination of LC_{50}

The acute toxicity (LC_{50}) of the extract of the extract types used in this study were established using the method of Ousman *et al.* (2007) and Abbott (1925) where LC_{50} of the extract types were obtained during preliminary studies until a concentration that will have effects 50% of the tests after 24 h was obtained.

Histopathological assay of insects

Using the method of Humason (1979), insects were administered with 10 kg the plant extract and observed for 5 min using diffusion method where insects were put in a petri

dish containing various concentrations and observed to see the stage they begin to die due to toxicity. They were collected into foil processing paper and fixed in Bouins fluid for 24 h, repacked after 24 h and folded in fresh foil immersed in buffered formalin for histopathological studies. After 48 h of fixations, samples were labeled according to the groups and process to paraffin wax by passing the basket of insects through 10% formal saline for 2 h. 1 h in 3 changes of alcohol for dehydration ranging from 70 to 100%, 2 changes of xylene for clearing, 2 changes of melted paraffin wax at 56°C for impregnation for 2 h, samples were embedded in melted paraffin wax to create support for the tissues in the embedding cassettes. Then microtomy was carried out using Rotary Microtome by sectioning the embedded tissues at 5 μm and mounted the cut sections in ribbons from water bath on the labeled glass slide, drained of excess water, allowed to dry using hot plate and stained with hematoxylin and Eosin technique by dewaxing with xylene, taking the section to water, by passing through descending grade of alcohol, stained for nuclear content in hematoxylin for 10 min, washed in water, differentiate in 1% acid alcohol and blue in saturated solution of lithium carbonate solution, washed in water and counter stained briefly in eosin, for 3 min, then section were washed briefly and dehydrated, cleared in xylene, mounted with DPX, cover-slipped and observed under digital microscope for pathological changes.

Results

To examine the significance of some therapeutic plant, the first step is to screen for its phytochemicals, as it gives a wide knowledge with respect to the type of the compounds present in it. In the current study, the methanol leaf extracts of *U. chamae* were preliminary screened for the phytochemicals. The extract shows the presence of cardiac glycosides, saponin, steroids/terpenes, flavonoids, alkaloids and phenols as shown in Table 1.

GC-MS: Compound's name, molecular formulae, molecular weight, peak area and retention time of the bioactive compounds were established. The relative proportion amount of each constituent was calculated by relating its average peak area to the total mass. The results of Gas Chromatography-Mass Spectroscopy of the extracts of *U. chamae* are as shown on Table 2. The extracts of *U. chamae* showed eight (8) major compounds: Thiirane [RT-40.712, Peak Percentage 1.539%], 1,1, dimethylhydrazine [RT-41.115, Peak Percentage-1.861%], malic acid [RT-91.304, Peak Percentage- 2.040%], 2-amino-4-(2-methylpropenyl)-pyrimidin-5-carboxylic acid [RT-84.846, Peak Percentage-1.554%], L-aspartic acid [RT-85.846, Peak Percentage- 2.001%], 2- nitro benzaldehyde [RT-86.505, Peak Percentage- 3.903%], Cedrandiol [RT-87.055, Peak Percentage- 1.751%] and Mercaptoethanol [RT- 88.300, Peak Percentage- 1.115%]. The phytochemicals from the extract are known to control insects by eroding the cuticle layer and causing dehydration. These phytochemicals are known to block the spiracles of insect and causing death by asphyxiation hence, the insecticidal efficacy of the plant.

Histological section of the *S. zeamais* administered with 10 mg/kg extract concentration of *Uvaria chamae* treatment at magnification X400 revealed severe de-arrangement of the respiratory, secretory and gastrol intestinal layer with destruction of the muscular layer when compared to the control group (Fig. 1a, b). The effect observed were the parting of the epithelial cells from the basement membrane with mutilation of the peritrophic membrane.

Discussion

The preliminary phytochemical screening of the plant extract of *Uvaria chamae*, revealed the presence of alkaloids, saponins, tannins, flavonoids, phenols and cardiac glycosides. This result was in agreement with the report of Udoh *et al.* (2019) who reported similar result from the crude extracts of *U. chamae* against wound isolated strains of *Pseudomonas aeruginosa* and *Proteus mirabilis.* Okokon *et al.* (2006), Okon *et al.* (2013), Kone *et al.* (2015), also reported similar result from the ethanolic root extract of *U. chamae* for its antibacterial, haematological and *in vivo* antimalarial activities respectively. Folawewo *et al.* (2017) and Bassey *et al.* (2014) who carried out a phytochemical screening of some methanolic plants extracts also found tannins, flavonoids, alkaloids and saponins to be the most abundant phytochemical present. Anthraquinone was not present in this study, while Phlobotannins was moderately present. The report of this study was in agreement with the results of Ekanem *et al.* (2016), Ebana *et al.* (2016) who carried out the phytochemical screening of extract of *L. africana* and *H. africana* and reported no trace of anthraquinone and moderately presence of Phlobatannins.

The study of organic chemicals found in plants, as well as their actions, has grown in popularity. GC-MS is an ideal technique for qualitative study of volatile and semi-volatile bioactive chemicals because it combines the best separation technique (GC) with the best identification technique (MS) (Grover and Patni 2013). The identified compounds possess some important biological potentials for future insecticide development especially those of botanical origin with low residual effects in the environment and low mammalian toxicity. Insecticides disrupt the natural functions of certain cells, making it harder for insects to survive. Many researchers have investigated the impact of different insecticides on the gut of insects from various orders, such as Orthoptera (Singh 1990).

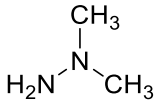
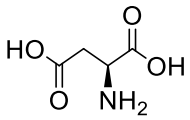
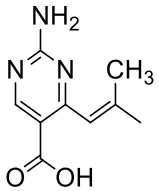
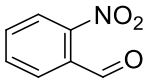
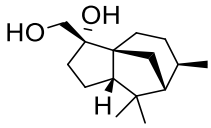
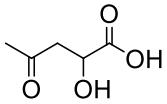
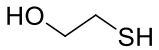
Histopathologically, evidence of disintegration of columnar epithelial cells and severe detachment of cells from their basement membrane, in addition to nuclear degeneration, cytoplasmic material granulation, and vacuolization was observed in this study. This is similar to the work by Rawi *et al.* (2011) who stated that the larval mid-gut of *S. littoralis* had undergone histological changes when treated with *Azadirachta indica* and *Citrulus colocynthesis*

Table 1: Qualitative phytochemical analysis of the different extracts

	U. chamae	Test
Anthraqunones	-	Borntrager
Steroids/terpenes	+	Liebermann- Burchard
Cardiac glycoside	+	Keller-kiliani, Salkowsiki
Saponin	+	Frothing, Fehling solution, Na_2CO_3
Tannins and Phenols	+	Ferric Chloride, Pb acetate
Flavonoids	+	NaOH, Mayer, Wagner
Alkaloids	+	NaOH, Shinda
Phlobatannins	+	Dragendoff, Mayer, Wagner

+ = Present
Absent

Table 2: Chemical composition of methanol extract of *U. chamae*

S/N	Compound	RT	Area (%)	Chemical Formula	Molecular Weight	Structure
1	Thiirane	40.712	1.539	C_2H_4S	60.12	
2	1,1 dimethyl hydrazine	41.115	1.861	$C_2H_8N_2$	60.10	
3	L - aspartic acid	85.846	2.001	$C_4H_7NO_4$	133.10	
4	2-amino-4-(2-methylpropenyl)-pyrimidin-5-carboxylic acid	84.747	1.554	$C_9H_{11}N_3O_2$	193.20	
5	2- nitro benzaldehyde	86.505	3.903			
6	Cedrandiol	87.055	1.751	$C_{15}H_{26}O_2$	238.37	
7	Malic acid	91.304	2.040	$C_5H_8O_4$	132.11	
8	Mercaptoethanol	88.300	1.115	C_2H_6OS	78.13	

characterized with ep ithelial lining de-arrangement, necrosis and vacuolisation resulted from cell elongation and molecular decomposition of the nuclear and cytoplasmic constituents. Also, after 48 and 72 h, the brush border and some epithelial cells were apically degenerated, and the majority of the cells completely disintegrated and vacuolated, according to Assar and El-Sokby (2003) who observed that the water extract of *Eichlornia crassipes* had a severe effect on larval midgut as the brush border and some epithelial cells were apically degenerated. The digestive system of insects is well known as one of the primary physiochemical barriers to numerous poisons and pathogenic agents. The gut is the key organ responsible for food digestion, assimilation, and absorption; any abnormalities in the gut region could impair the insect pests' growth and development, as well as their survival. The result of this study showed absorptive epithelial linings, shrunken nuclei and slanted epithelium when treated with extracts likened to control. Similar results were obtained by Prasad and Roy (2011), when completely shrunken mid-gut tissues with shrunken columnar epithelial cells and withered nuclei was reported in *H. armigera* fed with diet containing ethanol leaf extracts of *Lantana camara.* It also confirmed the work by Adel *et al.* (2010) who observed histological effects of *Artemisia monosperma* extract on

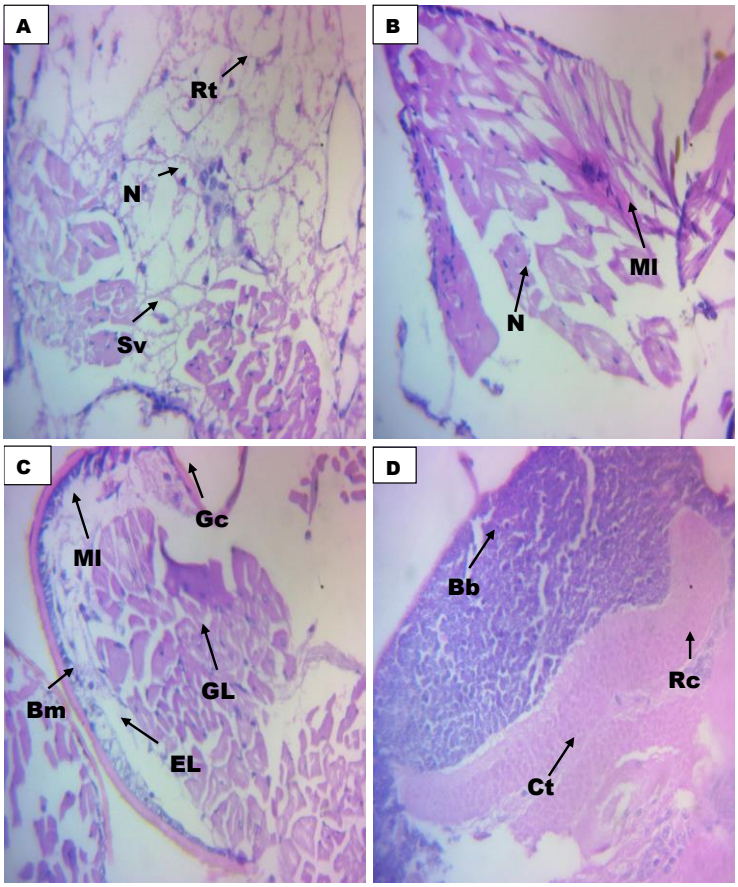


Fig. 1a: (Control) *Sitophilus zeamais*
Photomicrographs of Weevils without treatment at magnification x400 stained with H&E method
Keys: Epithelium Lining (**EL**), Basement membrane (**BM**), Regenerative Cells (**Rc**), Gut Lumen (**GL**), Muscular Layer (**ML**), Secretory Vesicles (**SV**), Goblet cells (**Gc**), Connective Tissue **(Ct)** Respiratory tract (**Rt)** and Nucleus (**N**)
A= Respiratory Tract, **B**= Muscle, **C**=Gastro-intestinal Tract and **D**= Excretory system

S. littoralis thereby resulting in the damage of the mid-gut epithelium. Also, the histological disturbances in the mid-gut cells of *S. littoralis* with vacuolization and destruction of nuclear contents were recorded. Degenerated columnar epithelial cells and detachment from the basement membrane was observed by Adel *et al.* (2010) when he treated it with crude extracts of *Azadirachta indica* and *Citrullus colocynthis.* The result was also similar to that of Mishra *et al.* (2015), who observed slight but distinctive disappearance and alteration of absorptive epithelial cells joined with reformed shape and structure when extract of *Thevetia neriifolia* was tested on *Helicoverpa armigera* early fourth instars larvae.

Conclusion

Following the chemical composition as illustrated by the GS-MS Chorography and the histopathological destruction caused by the investigated plant insecticide, it suggests that this extract are capable of causing death of an insect when it enters into tissues in sufficient amounts. In conclusion, the extract exhibited good insecticidal efficacy for the control of *Sitophilus zeamais.* Since this plant preparation is non-toxic to the non-target organisms, ecologically safe and freely obtainable, it can be incorporated into integrated pest management programmes.

Acknowledgements

The authors like to thank the laboratory technicians (Celestina Nwankwo and Wesley Okorie) of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka; Mr. Kokoette Raymond of Chemistry Department, University of Uyo; Dr. Bashir Musa, Multi- User Science Research Laboratory, Ahmadu Bello University, Zaria for their dedications to this work.

Author Contributions

DO and JE designed and supervised the study. DO, SO and AN carried out the laboratory studies. DO, IB and AA wrote

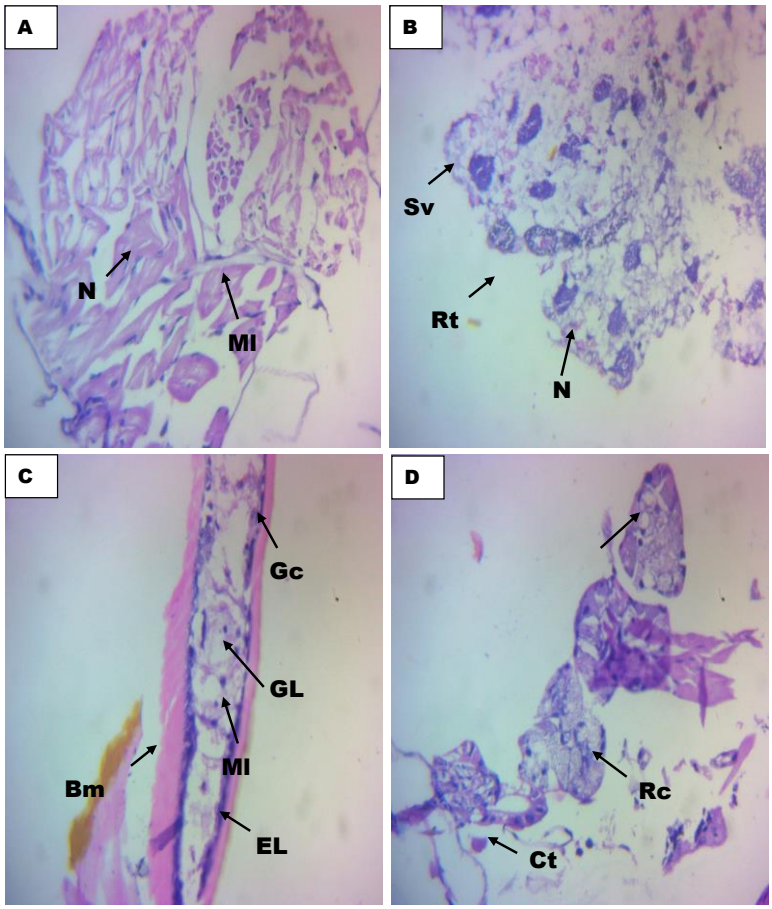


Fig. 1b: -2 (UC-CS)
Photomicrographs of maize weevils treated with 10 mg/kg of *U, chamae* at magnification x400 stained with H&E method
Keys: Epithelium Lining (**EL**), Basement membrane (**BM**), Regenerative Cells (**Rc**), Gut Lumen (**GL**), Muscular Layer (**ML**), Secretory Vesicles (**SV**), Goblet cells (**Gc**), Connective Tissue **(Ct),** Respiratory tract (**Rt**) and Nucleus (**N**)
A= Respiratory Tract, **B**= Muscle, **C**=Gastro-intestinal Tract and **D** = Excretory system

the first manuscripts; JE, DO, IB, IE and PU critically reviewed the manuscripts. All authors read and approved the final manuscripts.

Conflicts of Interest

The authors declared that there are no competing interests.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author

Ethics Approval

Not applicable in this paper

References

Abbott WS (1925). A method of computing the effectiveness of an insecticide. *J Econ Entomol* 18:65–66

Adedire CO (2001). Biology, ecology and control of insect pests of stored grains. *In: Pest of Stored Cereals and Pulses in Nigeria*, pp:59–94. Ofuya TI, NES Lale (Eds). Dave Collins Publications, Akure, Nigeria

Adel MM, FM El-Hawary, NF Abdel-Aziz, EA Sammour (2010). Some physiological, biochemical and histopathological effects of *Artemisia monosperma* against the cotton leafworm, *Spodoptera littoralis*. *Arch Phytopathol Plant Prot* 43:1098–1110

Adetunji LK (1999). *Great secret of Nature*, p:256. Natural Links Centre, Lagos

Assar AA, MM El-Sokby (2003). Biological and histopathological studies of some plant extracts on larvae of *Culex pipens* (Diptera: Culicidae). *J Egypt Soc Parasitol* 33:189–200

Bassey ME, EI Etuk, JU Ekpo (2014). Morphological diversity in the macrophyte genus *Lasianthera* (Icacinaceae) and the taxonomic implications. *Liv Sys Sustain Dev* 2:1–5

Bongers F, PME Parren, D Traore (2005). *Forest Climbing Plants of West Africa: Diversity, Ecology and Management*. CAB International, Publishing, Oxford, UK

Ebana RU, NU Asamudo, CA Etok, UO Edet, CS Onyebuisi (2016). Phytochemical screening, nutrient analysis and antimicrobial activity of the leaves of *Lasianthera africana* and *Dennettia tripetala* on clinical isolates. *J Adv Biol Biotechnol* 8:1–9

Ekanem NG, HOC Mbagwu, GI Harry (2016). Phytochemical screening and hypoglycaemic activity of *Lasianthera africana* Beauv. (Aquifoliales: Stemonuraceae) leaf extract on diabetic rats. *Braz J Biol Sci* 3:293–298

Evans WC (2002). *Trease and Evans' Pharmacognosy*, 15th edn. Elsevier, A Division of Reed Elsevier India Pvt. Limited, India

Fatope MO, H Ibrahim, Y Takeda (1999). Screening of higher plants reputed as pesticides using the brine shrimp lethality assay. *Intl J Pharmacogn* 31:250–254

Folawewo AD, AN Madu, FV Agbaje-Daniels, AO Faboyede, AR Coker (2017). Phytochemical screening and antibacterial activities of the root bark extracts of *Hippocratea africana* (Willd.) Loes. ex Engl. *Eur J Med Plants* 19:1–8

Grover N, V Patni (2013). Phytochemical characterization using various solvent extracts and GC-MS analysis of methanolic extract of *Woodfordia fruiticosa* (L.) Kurz. Leaves. *Intl J Pharm Sci* 5:291–295

Halstead DGH (1963). External sex differences in stored-products Coleoptera. *Bull Entomol Res 54*:119–134

Harborne JB (1984). *Phytochemical Methods*, 2nd edn. Chapman and Hall, New York, USA

Hema R, S Kumaravel, C Sivasubramanian (2010). GC-MS study on the potentials of *Syzygium aromaticum*. *Researcher* 2:1–4

Humason GL (1979). *Animal Tissue Techniques*, 4th edn. W.H. Freeman, San Francisco, USA

Isman MB (2006). Botanical insecticides, deterrents and repellents in modern agricultural and an increasingly regulated world. *Annu Rev Entomol* 51:45–56

Kokate CK, AP Purohit, SB Gokhale (2008). *Pharmacognosy*, 40th edn. Nirali Prakashan, India

Kone M, A Toure, K Outtara, A Coulibaly (2015). Phytochemical composition, Antioxidant and Antibacterial Activities of Root of *Uvaria chamae* P. Beauv (Annonaceae) used in Treatment of Dysentery in North of Cote d' Voire. *Intl J Pharmacogn Phytol Res* 7:1047–1053

Kranz J, H Schmutterer, W Koch (1977). *Diseases, Pests, and Weeds in Tropical Crops.* John Wiley and Sons, Incorporated, UK

Mishra M, KK Gupta, S Kumar (2015). Impact of the stem extract of *Thevetia neriifolia* on the feeding potential and histological architecture of the midgut epithelial tissue of early fourth instars of *Helicoverpa armigera* Hübner. *Intl J Ins Sci* 7:53–60

Mukhtar MD, M Huda (2005). Prevalence of *Tinea capitis* in primary school and sensitivity of etiological agents of *Pisti astratiotes* extracts. *Nig J Microbiol* 19:412–419

Odeyemi OO, AM Daramola (2000). *Storage Practices in the Tropics: Food Storage and Pest Problems*. Dave Collins Publications, Akure, Nigeria

Ogueke CC, JN Ogbulie, BN Anyanwu (2007). The effect of ethanolic and boiling water extracts of root-barks and leaves of *Uvaria chamae* on some hospital isolates. *J Amer Sci* 3:68–73

Okokon JE, BN Ita, AE Udokpoh (2006). The *in vivo* antimalarial activities of *Uvaria chamae* and *Hippocratea africana*. *Ann Trop Med Parasitol* 100:585–590

Okon JE, IR Udosen, EO Mbong (2013). Phytochemical screening and effect of ethanolic root extract of *Uvaria chamae* on haematological parameters on albino rats in Akwa Ibom State, Nigeria. *Merit Res J Environ Sci Toxicol* 1:16–20

Okwu DE (2004). Phytochemicals and vitamin content of indigenous species of southeastern Nigeria. *J Sustain Agric Environ* 6:30–37

Okwu DE, F Iroabuchi (2009). Phytochemical composition and biological activities of *Uvaria chamae* and *Clerodendoron splendus. J Chem* 6:553–560

Ousman A, MB Ngassoum, JJ Essia-Ngang, LST Ngamo, R Ndjouvenkwu (2007). Insecticidal activity of spicy plant oils against *Sitophilus zeamais* in stored maize in Cameroun. *Agric J* 2:192–196

Prasad A, SB Roy (2011). Histoarchitectural alterations in the mid-gut tissues of fourth instar larvae of grain pod borer, *Helicoverpa armigera* (Hub) fed with leaf extract of plant, *Lantana camara* (L.). *Intl J Pharm Biosci* 2:613–620

Prashant T, K Bimlesh, K Mandeep, K Gurpreet, K Harleen (2011). Phytochemical screening and extraction: A review. *Intl Pharm Sci* 1:98–106

Rawi SM, FA Bakry, MA Al-Hazmi (2011). Biochemical and histopathological effect of crude extracts on *Spodoptera littoralis* larvae. *J Evol Biol Res* 3:67–78

Regnault-Roger O (2005). Effects of plant secondary chemicals on insects. *J Stored Prod* 45:55–69

Santana PM, M Miranda, JA Payrol, M Silva, V Hernandez, E Peralta (2013). Gas Chromatography- Mass Spectrometry Study from the Leaves Fractions Obtained from *Vernonanthura patens* (Kunth) H. Rob. *Intl J Org Chem* 3:105–109

Singh MA (1990). Effect of some insecticide on the alimentary canal of surface grasshopper, *Chrotogonus trachypterus* Blanchard. (Pyrgomorhide: Orthoptera). *Ind J Entomol* 52:84–99

Thacker D (2002). *Global Neem Update 2: 1*. University Press, London

Udoh DI, US Ekong, BB Etang, NE Udo (2019). Qualitative assessment of *Uvaria chamae* crude extract against wound isolated strains of *Pseudomonas aeruginosa* and *Proteus mirabilis* alongside resistance and plasmid profiles determination of the isolates. *Afr J Microbiol Res* 13:520–530

Walgenbach CA, JK Phillips, DL Faustini, WE Burkholder (1983). Male-produced aggregation pheromone of the maize weevil, *Sitophilus zeamais*, and interspecific attraction between three *Sitophilus* species. *J Chem Ecol* 9:831–841