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ABSTRACT

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Keywords: GC-MS, *Hippocratea africana,* Histology, Phytochemistry**,** *Sitophilus zeamais*.

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ABSTRACT

Phytochemicals in methanolic leaf extract of *Hippocratea africana* using qualitative and Gas Chromatography-Mass Spectrum (GC-MS) analysis to determine the phytochemicals present and its effect on the histology of midgut of *Sitophilus zeamais*. Insects were administered with 10mg/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 minutes. They were collected into foil processing paper and fixed in Bouins fluid for 24 hours, repacked after 24 hours 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 *H. africana* 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 showed eight major compounds as shown on Table 4. They were: 5-amino-1- tetrazolylacetic acid [RT-83.55017, Peak Percentage- 1.173%], 2-amino-4-(2-methylpropenyl) -pyrimidin-5 carboxylic acid [RT- 83. 978, Peak Percentage 1.713%], Cedrandiol [RT-87.201, Peak Percentage-2.445%, Malic acid [RT 88. 740, Peak Percentage 1.431%], 1, 2 benzenedimethanethiol [RT-91.634, PeakPercentage - 2.045%], ethyl 5- (furan-2-yl)-1, 2-oxazole-3-carboxylate [RT-89.693, Peak ercentage-1.446%], Mephenesin [RT-92.587, Peak Percentage-1.911%]. The findings indicated that methanol extract of *H. africana* 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: GC-MS, *Hippocratea africana,* Histology, Phytochemistry, *Sitophilus zeamais.*

I. INTRODUCTION

Despite encouraging efforts over the past 2-3 decades to isolate botanicals with enhanced insecticidal potential for insect management as an alternative to synthetic insecticides, there is still inadequate information available in terms of their synergistic potential, toxicology, optimum use and species specificity (1, 2). Many botanicals are used by small scale farmers as insecticides in both homes and subsistence farming. Certain of the compounds derived from plants are screened and marketed as insecticides. Since plant materials are rich in phytochemicals, their extracts and secondary metabolite have been used to control insect pests of various orders (3, 4, 5).

Hippocratea africana (Willd.) Loes.ex Engl. (Celastraceae) syn. *Loeseneriella africana* (Willd.) N.Hallé is a perennial, hairless (glabrous) green forest climber, reproducing from seeds (Figure 1), (6). It is commonly referred to as 'African paddle-pod.' The Nigerian tribe 'Efik' and 'Ibibio' call it "Eba enang enang" while Oro people

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calls it 'Mkpak oyo'ananang'. Tropical Africa is home to this plant. The Efik and Ibibios Niger Delta region in Nigeria have long utilized the root of this plant to treat different maladies such as fever, convulsions, malaria, bodily discomfort, diabetes, and diarrhea (7). Decoction of the plant's root is also employed as an antidote or antipoison for the treatment of liver and inflammatory illnesses such as jaundice and hepatitis, according to an ethnobotanical survey (8, 9). The root was found to have anti-plasmodial activity (6), anti-inflammatory and analgesic (10), anti-diarrheal, antiulcerative (11), anti-diabetic and hypolipidemic (12, 13). Other biological activities that have been express by this plant include: beta-cell cytotoxicity, anti-oxidant burst and anti-leishmanial, hepatoprotective activity (14), anticonvulsant and antibacterial activities (15). Plants have evolved a variety of defense mechanisms to reduce insect attack, both constitutive and inducible, however, there are few investigation on the application of plant as a natural insecticide. Hence, this study reports the phytochemical constituents of *Hippocratea africana* using Gas Chromatography- Mass spectroscopy and determine the effect of the extracts on the histology of insect midgut.

Figure 1: Hippocratea africana (Dalziel, 1956)

II. MATERIAL AND METHODS

2.1 Collections and Identification of Plant Materials

Fresh leaves of *Hippocratea africana* (Figure 1) were obtained from Faculty of Pharmacy Medicinal Farm of University of Uyo, Akwa Ibom State and authenticated by a taxonomist in the Department of Botany and Ecological Studies, University of Uyo. Voucher specimens with number: UUH/3689 was deposited in their herbarium for further referencing.

2.2 Rearing of Test Organisms

Sitophilus zeamais cultures were established to provide equivalent age weevils for the experiment. A total of ten (10 kg) bean seeds were obtained and cleaned to remove any seeds that had evident damage. To prevent field infestation, the clean seeds were kept in a sealed container in the refrigerator at 4°C for a month. Seeds were placed in plastic bags and stored at room temperature for two weeks. *S. zeamais* were taken from contaminated bean grains and their sexes were established by inspecting their snouts. Females have a longer and thinner snout, while males have a shorter and fatter snout. In addition, females have smooth textured bodies, whilst males have rough textured bodies (16). The insects were cultivated in jars holding 100 weevils per 400 g of seeds that had been cleaned and sterilized. To allow aeration and prevent weevil escape, the jar was covered with muslin cloth and held in place with a rubber band at room temperature. All parent weevils in each jar were removed seven days after oviposition (17). To distinguish the sexes, the dimorphic rostral features were used (18, 19, 20, 21). The jars were kept in an insect rearing cage at the University of Uyo's Entomology Laboratory, Department of Animal and Environmental Biology. The experiment used two day-old newly emerging insects.

2.3 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 an electric blender (Braum Multiquick Immersion, B White Mixer MR 5550CA, Germany), the dried leaves were grinded and fine powder was then kept in an airtight container for further analysis. The bioactive components in the leaves were extracted using methanol according to the reported standard procedures (21, 22. 23). Briefly, 50 g of the powder was soaked for 48 – 72 hours at room temperature in 95 % methanol. The crude extract

were then filtered using rotary evaporator, then stored in the refrigerator for use.

2.4 Phytochemical Analysis of the Plants

The preliminary phytochemical screening of the plant was carried out in Pharmacognosy Laboratory of University of Uyo, Akwa Ibom State using the standard procedures as described by (24, 25, 26, 27).

2.5 Gas Chromatography- Mass Spectroscopy Analysis

A GC Clarus 500 Perkin Elmer system and gas chromatograph were interfaced with a mass detector (Turbo mass gold Perkin Elmer) (GC-MS) according to (28, 29). Column: Elite-5MS (5 percent diphenyl/95 percent dimethyl poly siloxane), 30 x 0.25 mm x 0 25m 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 70eV, 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 minutes. The GC- MS was conducted in Multi- User Science Research Laboratory, Department of Chemistry, Ahmadu Bello University (ABU) Zaria Kaduna Nigeria.

2.6 Histopathological Assay of Insects

Using the method of Humason (32), insects were administered with 10g the plant extract and observed for 5 minutes 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 hours, repacked after 24 hours and folded in fresh foil immersed in buffered formalin for histopathological studies. After 48 hours 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 hours. 1 hour 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 hours, 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 um 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 minutes, 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 minutes, then section were washed briefly and dehydrated, cleared in xylene, mounted with DPX, cover-slipped and observed under digital microscope for pathological changes.

III. RESULTS

To investigate the importance of any medicinal plant, the initial or first step is to screen for its phytochemicals, as it gives a broad knowledge with respect to the nature of the compounds present in it. In the present study, the methanol leaf extracts of *H. africana* 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.

Table 1: Qualitative phytochemical analysis of the different extracts

	H. africana	Test
Anthraquinones	-	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

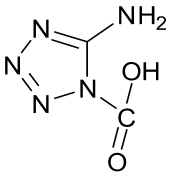
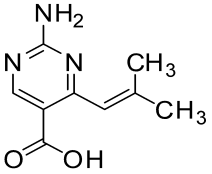
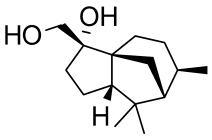
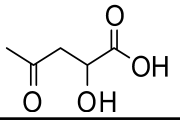
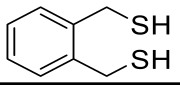
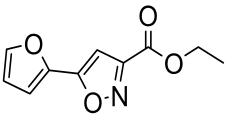
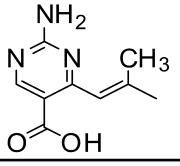
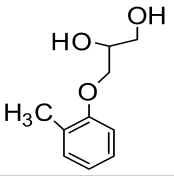
+++= Strongly present; ++ = moderately present; += trace; - = absent + = present

The results of the qualitative phytochemicals revealed the presence of different metabolites and their intensity was determined based on colours as shown in Table 1a. Flavonoids, Steroids and terpenes were stongly present in *H. africana*. Anthraquinones were absent in *H. africana*. Cardiac glycosides, Tannins and Phenols, alkaloids, phlobatannins and saponin were moderately present.

GC-MS: The compound name, molecular formulae, molecular weight, peak area and retention time of the bioactive compounds were ascertained. The relative percentage amount of each component was calculated by comparing its average peak area to the total mass. The result 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.

Histologic section of the *S. zeamais* treated with concentrated *Hippocratea africana* and 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.

Table 2: Chemical Composition of Methanol Extract of *H. africana*

S/N	Compound	RT	Area (%)	Chemical Formula	Molecular Weight	Structure
1	5-amino-1-tetrazolylacetic acid	83.55017	1.173	$C_2H_3N_5O_2$	129.08	
2	2-amino-4-(2-methylpropenyl)-pyrimidin-5-carboxylic acid	83.978	1.713	$C_9H_{11}N_3O_2$	193.20	
3	Cedrandiol	87.201	2.445	$C_{15}H_{26}O_2$	238.37	
4	Malic acid	88.740	1.431	$C_5H_8O_4$	132.11	
5	1,2-benzenedimethanethiol	91.634	2.045	$C_8H_{10}S_2$	170.29	
6	Ethyl 5-(furan-2-yl)-1,2-oxazole-3-carboxylate	89.693	1.446	$C_{10}H_9NO_4$	207.18	
7	2-amino-4-(2-methylpropenyl)-pyrimidin-5-carboxylic acid	89.949	1.868	$C_9H_{11}N_3O_2$	193.20	
8	Mephenesin	92.587	1.911	$C_{10}H_{14}O_3$	182.22	

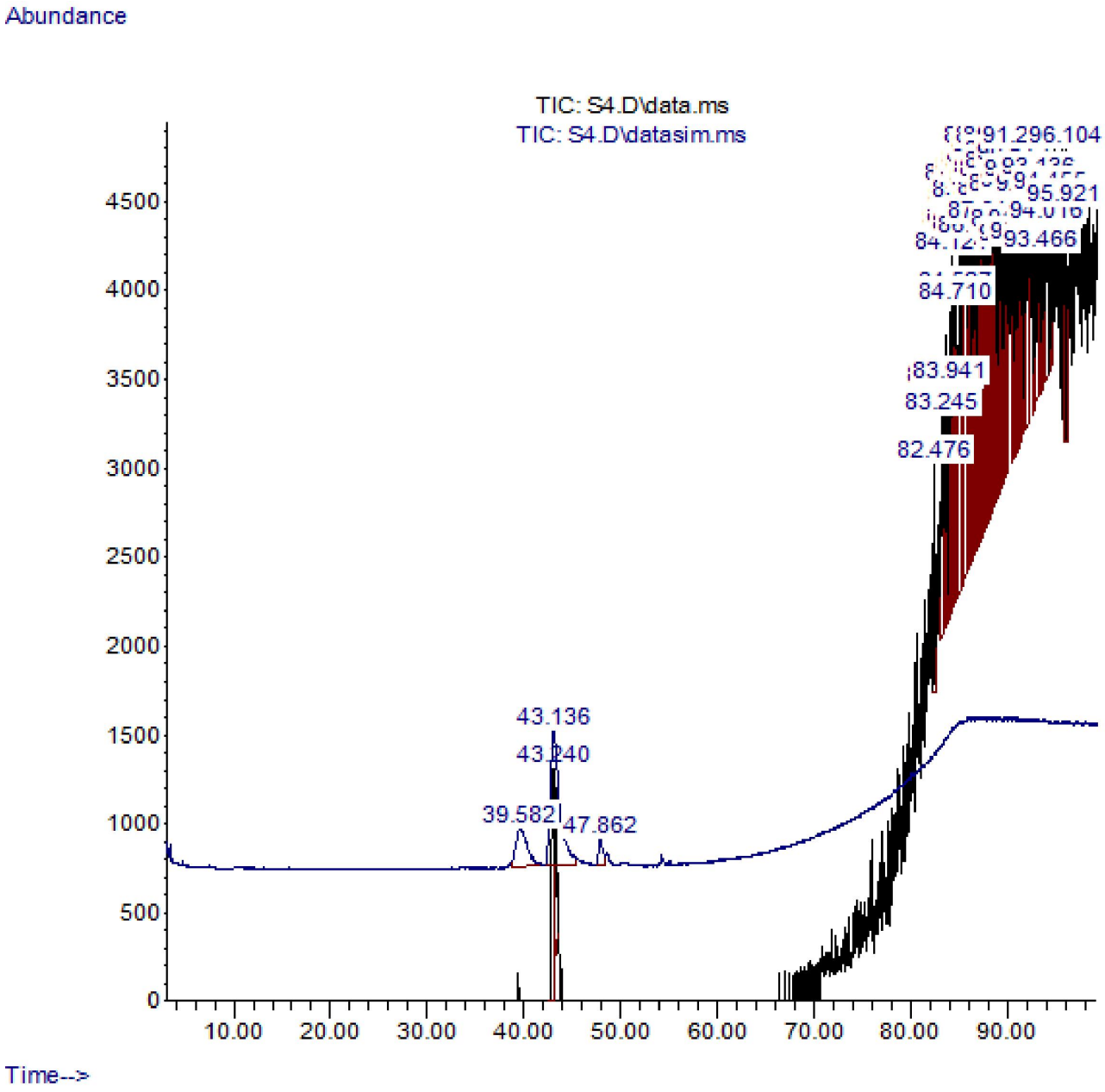
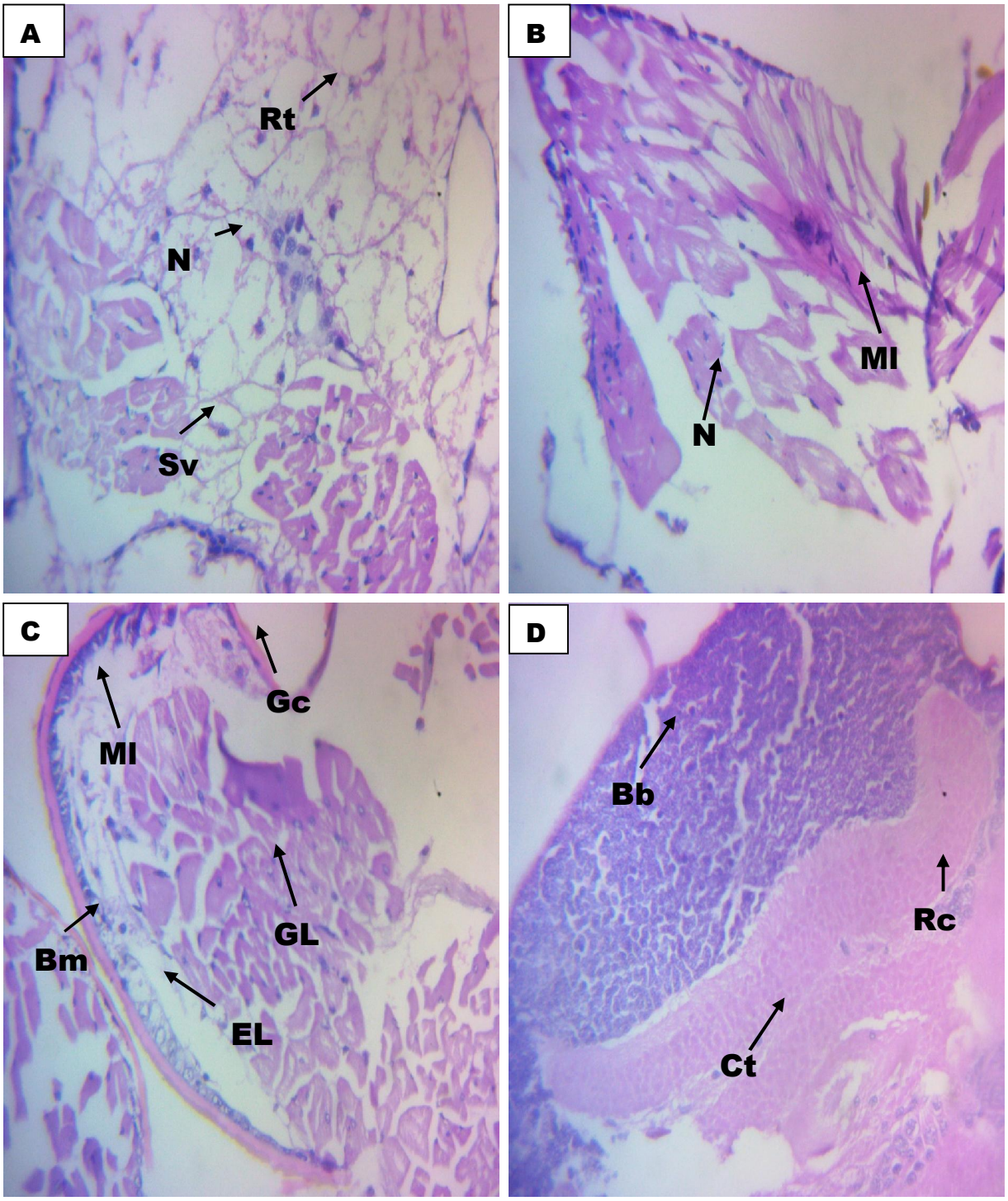


Figure 2: GC-MS of methanol leaf extract of Hippocratea africana

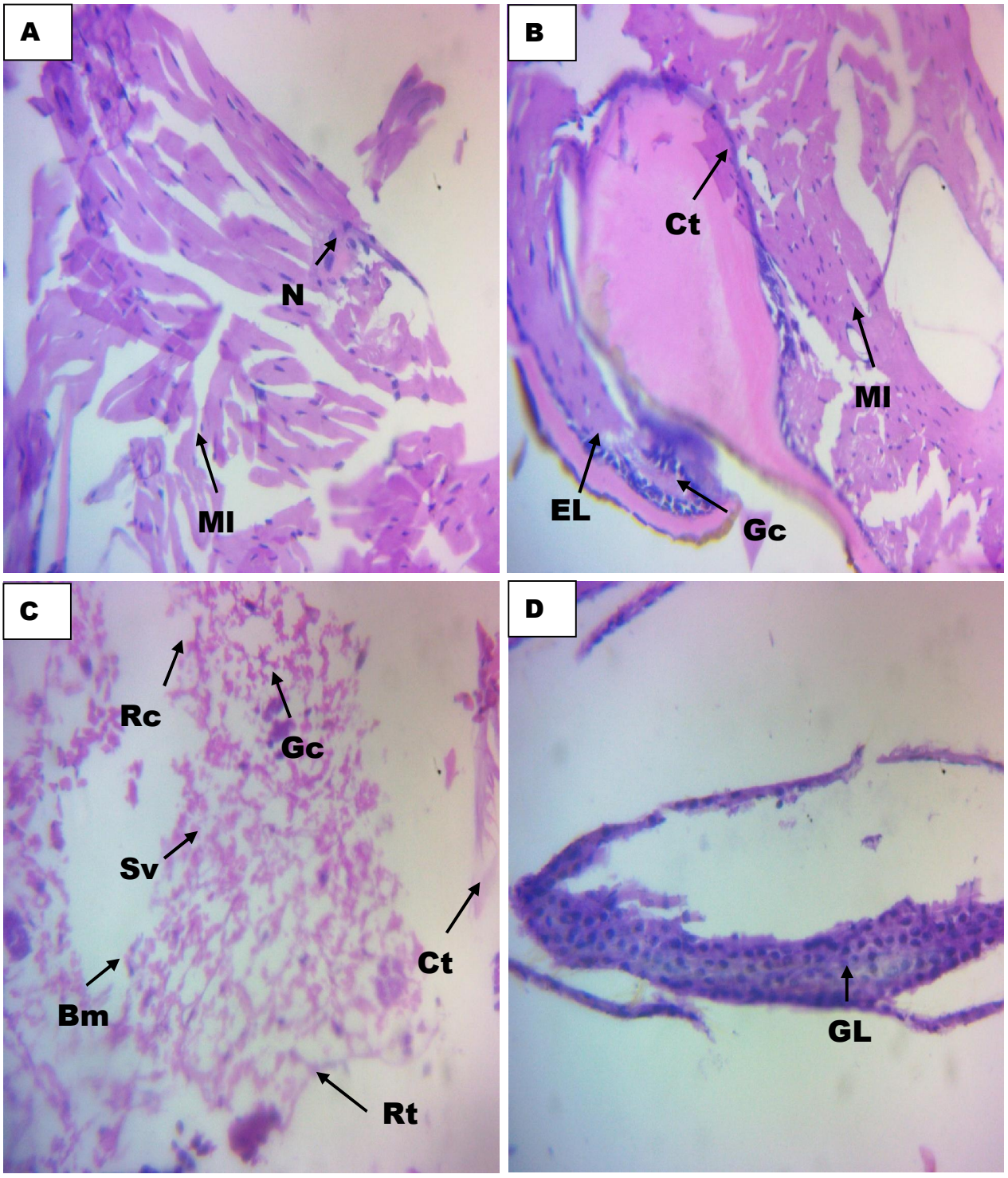
GROUP- 1 (CONTROL) *Sitophilus zeamais*



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

GROUP-3 (HA-CS)



Photomicrographs of maize weevils treated with 10mg/kg of *H. africana* at magnification x400 stained with H&E method

Keys: Epithelium Lining (**EL**), Basement membrane (**BM**),Brush Boader**(Bb)**, 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

IV. DISCUSSION

Anthraquinone was not present in this study while Phlobotannins was moderately present. This work is consistent with the findings of (33,34,35) 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 heavy presence of cardiac glycosides found in this study is consistent with the results of (33,34) who observed heavy presence of the same metabolites when carrying out the phytochemical screening of *L. africana and H. africana* but disagrees with the findings of (36) who did not detect any trace of cardiac glycosides when screening the extract of *H. africana*. It agrees with Mikali *et al.* (37) who observed similar phytoconstituents from methanolic leaf extract of *Ficus exasperate*.

There was great destruction of the mid-gut cells. The results was also in agreement with the findings of (38) who investigated the histological changes in the midgut of the larvae of *Agrotis ipsilon* treated with methoprene. The effect of the different treatment on the midgut epithelium was the exfoliation from the basemen membrane and partial destruction of cell lining. Meanwhile epithelial lining was strongly vacuolated and considerably elongated, lines in between cells disappear and the peritrophic membrane was moderately destroyed. The findings were consistent with those of (39) who found histopathological abnormalities, nuclei dissolving, and epithelial cell degeneration in two-day-old *Rynchophorus ferrugineus* larvae poisoned by two biopesticides, Boxus chinensis oil and precocene II. After two days of treatment with seven Essential oils (EO) concentrations, (40) discovered anomalies in the mid-gut and developing oocytes of female *Trogoderma granarium* treated as 4th instar larvae. The findings are likewise consistent with those of (41) who found significant effects on the alimentary canal and fat bodies of *H. littoralis* 1st nymphal instars after treatment with sub-lethal quantities of three oils from garlic, mint, and Eucalyptus. Epithelial cells were destroyed, microvilli were curled and ruptured, and the peritrophic membrane was curled and ruptured, compared to the control group. In the present investigation, the peritrophic membrane, striated border, secretory cells, and regeneration cells in the treated mid-gut sections of weevils with extract were significantly disrupted as compared to the control. This was in agreement with (42) who observed expansion of epithelial cells, development of vacuoles at the apical region of the cell, and breakdown of the peritrophic membrane in *Culex pipiens* larvae after exposure to chamomile oil extract. It also backed up the findings of (43) who found the same effect when *Datura alba* leaf extract was tested on the midgut of *Periplaneta americana*. Ranjini and Nambiar (44) also observed an elongated columnar cells, vacuolization of cytoplasm, increased goblet cells and thinning of the muscle layers when tested the effect of leaf extracts of *Clerodendrum infortunatum* and *Eupatorium odoratum* on the mid-gut tissue of sixth instar larvae of *Orthaga exvinacea*. The results also agreed with (45) who observed hypertrophy and lysis of epithelium intestinal cells when extract of *Ricinus communis* leaves were tested on the larvae of mosquito, *Culex pipiens*. The study therefore, identified *S. zeamais* economic role as a serious threat to maize production in Nigeria. Because the plant used to control the weevil is safe for animals at the dosages reported, more effort should be put into cultivating, packing, and using it as a botanical pesticide on a broad scale.

V. CONCLUSION

The disproportionate use of synthetic pesticides results in the secondary outbreak and rapid proliferation of the pests usually under natural control. The environmental concerns have become an inevitable part of human livelihood over the last few decades. As a result, an important quest of the day has become the hunt for safer and environmentally friendly implements for both agricultural and medical uses.

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