

Original Research Article

Insecticidal Potentials and Chemical Composition of Oil Extracts from the Leaves of Two Nigerian Plants

Abstract

This research evaluated the n-hexane extracts of *Phyllanthus amarus* and *Stachytarpheta cayennensis* for their insecticidal efficacy on *Periplaneta americana*, *Schistocerca gregaria* and *Culiseta longiareolata*. In all cases, the insects were introduced to same concentration of the extracts (0.5g), placed in well perforated plastic containers and mortality observed at 4 hours intervals for 24 hours. Both extracts were able to cause 100% mortality in all test insects at different exposure rates. No mortality was recorded in the control groups during the 24 hours. Mortality was fastest in mosquito larval; 60% mortality at 8 hours exposure for *P. amarus* and 73% at 8 hours for *S. cayennensis*. Grasshoppers exhibited the slowest mortality rate to both extract as compared to the other insects. *S. cayennensis* proved to be quicker in activity than *P. amarus*. The result of the GC-MS analysis carried out on both plant extract revealed for *S. cayennensis* the presence of the following secondary bioactive metabolites in the following concentrations: Decanoic acid, ethylester (Ethyl decanoate) 6.02%, Dodecanoic acid, ethylester (Ethyl dodecanoate) 11.26%, Tetradecanoic acid, ethylester (Ethyl tetradecanoate) 9.22%, Hexadecanoic acid, ethylester (Ethyl hexadecanoate) 10.16%, Phytol 28.52%, 9, 12, 15-Octadecatrienoic acid, ethylester (Ethyl linolenate) 11.34%, Stigmasta-7,25-dien 3-ol 7.95%. For *P. amarus* we had; Decanoic acid, ethyl ester (Ethyl decanoate) 6.05%, Dodecanoic acid, ethyl ester (Ethyl dodecanoate) 10.89%, Tetradecanoic acid, ethyl ester (Ethyl tetradecanoate) 8.32%, Hexadecanoic acid ethyl ester (Ethyl palmitate) 5.01%, Phytol 8.27%, Linoleic acid, ethyl ester (Ethyl linolenate) 5.79%, 9,12,15-octadecatrienoic acid, ethyl ester (Ethyl linolenate) 5.79%, Vitamin E 8.29%, Stigmasterol 9.38% and beta-Sitosterol 16.67%. Phytol was implicated as one of the very important compounds likely responsible for insecticidal activity in both plant extracts with concentration of 8.27% and 28.52% in *P. amarus* and *S. cayennensis* respectively. The result from this study indicate that these two plants may be exploited as alternative insecticidal agents that are available, environmentally friendly, cheap, and effective in the control of insect pests as against the harsh synthetic chemicals currently in use.

Keywords: insecticidal, potentials, chemical, composition, extracts, plants

Introduction

Insecticides are essential tools for preventing or minimizing insect damage to, and significantly increasing the quality and quantity of crops, as well as for improving the quality of life for humans, domestic animals and livestock [7]. Insects are invertebrate animals that

are part of the larger group of animals called arthropods. They are known to be the most successful and diverse animals on earth. Insects are the principal vectors of the pathogens causing many human, animal and plant diseases. Insect-transmitted pathogens, such as those causing malaria, dengue fever, yellow fever and leishmaniasis kill millions of people annually throughout the world. Malaria alone affects over 300 million people per year, thriving disproportionately in areas of poverty and lower economic growth [29]. Synthetic insecticides developed for dealing with insect pests have been associated with attendant hazards like low affordability, resistance, environmental pollution and other biohazards [6]. However, botanical insecticides have proven to be comparatively less toxic (or in most cases nontoxic at all) than the synthetic insecticides conventionally in use. This paved the way for the search of more botanical insecticides in the management and control of pests [10]. Again, plant extract mixtures may act synergistically [8], showing greater overall bio-activity as compared to their individual constituents [9]. Insect resistance and desensitization is also less likely to develop with mixtures of plant extracts [15][11] as pest resistance is one of the problems associated with intensive use of synthetic insecticides.

The application of plant products as alternative substitutes to the conventional chemical agents has been reported by many researchers like [4]. Over 120 plants and plant products have been shown to have insecticidal or deterrent activity against common insect pests of humans, higher animals and agricultural products [13]. Many of the plants used to preserve crops, control or deter insect pests in household management of insect pests have been found to be safe and non-toxic to human upon consumption or inhalation [18]. However, only few of these plants have been scientifically evaluated and characterized successfully [23]. The traditional belief and practice that *Stachytarpheta cayennensis* and *Phyllanthus amarus* leaves when placed on a local lantern repel mosquitoes and other insects such as cockroaches led to

the investigation of these two plants. The plants were investigated for their insecticidal potential on selected insect pests.

Phyllanthus amarus is a widely distributed small erect tropical annual herb whose stem has green capsule, and grows up to 10-50 cm high and bears flowers with 5 white sepals and apical acute anther. It is locally called iyin-olobe by the Yorubas of South-West Nigeria [2]. *Starchytaroheta cyaennensis* is an erect, perennial, branching somewhat angular fibrous sub-shrub that is very resistant to traction. It usually has opposite, ovate leaves with a distinct petiole and serrated and indented edges, an acute or sub-acute apex, a slightly wrinkled appearance [2].

The aim of the study is to observe the insecticidal efficacy of oil extracts of *Phyllanthus amarus* and *Starchytarpheta cayennensis* on mosquito larvae, cockroaches and grasshoppers. To quantitatively determine the chemical compounds that may be responsible for their insecticidal action if any.

Materials and methods

The equipments used for the research include; soxhlet assemblies set comprising of; electric heating mantle, Gallenham, conical flasks, soxhlet extractor and a liebig condenser, electric blender, weighing balance, water bath, oven, Hotplate, Gas Chromatography-Mass Spectrometry analyzer.

Identification, Collection and Preparation of Plant Extracts

Fresh leaves of *Phyllanthus amarus* and *Starchytarpheta cayennensis* were obtained from University of Calabar botanical garden and were taken to the Herbarium room in Botany department, University of Calabar where they were identified by a botanist. After identification, the leaves were washed with clean tap water and air-dried for a period of 3

weeks. The dried plant materials were blended into powder using an electric blender. Two hundred (200) grams of each powdered sample was weighed for extraction. The oils were extracted by continuous extraction in soxhlet apparatus for 12 hours using 500ml of n-hexane (50°C) as solvent according to the method described by the association of official analytical chemists [5] After evaporation using a water bath at 60°C, 11.3g and 8.5g of oils were recovered for *Phyllanthus amarus* and *Stachytarpheta cayennensis* respectively corresponding to a percentage oil yield of 5.65% and 4.25% for *Phyllanthus amarus* and *Stachytarpheta cayennensis* respectively. The extracted oils were stored in a dark amber container and kept in a dark, cool environment for further analysis.

Collection of test insects

A total of forty (40) live cockroaches and grasshoppers were obtained from an old building structure at Uwanse, and football field of the government secondary school Uwanse respectively, both in Calabar South LGA. All insects were very active and showed no signs of disease or disability as observed from their quick response to stimuli. The insects were kept in four (4) separate well perforated clean transparent plastic containers of 6 inches diameter and 12inches height each for a period of 24 hours at room temperature. Fresh food and green grasses were provided for them inside the containers throughout the experiment.

Collection of Mosquito Larvae

Four (4) separate medium sized plastic basins filled with natural rain water were kept at a lawn in front of the chemistry laboratory, University of Calabar, for 3 weeks. At the end of 3 weeks a good number of mosquito larvae were seen swimming in the water.

Grouping of insects and administration of treatments

Insects were grouped into four major groups each, with group A (control) receiving food only, B receiving 0.5g of *P.amarus* oil extract, C 0.5g of *S.cayennensis* oil extract and D 0.5g of Synthetic insecticide (Raid). Mortality was observed for a period of 24hours and recorded at 4 hours interval.

Results

Table 1: Percentage mortality of cockroach (*Periplanata amaricana*) exposed to 0.5g of each treatment.

Groups	Time (Hours)								
	0	4	8	12	16	20	24	TM	M%
A (control)	-	-	-	-	-	-		0/10	0%
B (0.5g <i>P.amarus</i>)	-	-	-	6	3	1		10/10	100
C (0.5g <i>S.cayennenses</i>)	-	-	7	2	1			10/10	100
D (0.5g Raid)	-	10						10/10	100

Table 2: Percentage mortality of grasshopper (*Schistocerca americana*) exposed to 0.5g of each treatment.

Groups	Time (Hours)								
	0	4	8	12	16	20	24	TM	M%
A (control)	-	-	-	-	-	-	-	0	0
B (0.5g <i>P.amarus</i>)	-	-	-		4	4	2	10/10	100
C (0.5g <i>S.cayennenses</i>)	-	-	-	6	3	1		10/10	100
D (0.5g Raid)	-	10						10/10	100

Table 3: Percentage mortality of mosquito (*Culiseta longiarcolata*) larval exposed to 0.5g of each treatment.

Groups	Time (Hours)								
	0	4	8	12	16	20	24	TM	M%
A (control)	-	-	-	-	-	-	-	0	0

B (0.5g <i>P.amarus</i>)	-	-	20	10				30	100
C (0.5g <i>S.cayennenses</i>)	-	-	22	8				30	100
D (0.5g Raid)	-	26	4					30	100

Key: TM= mortality, M%= percentage mortality.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of extracted compounds.

GC-MS analysis of both plant extracts was performed using a Perkin-Elmer Gas Chromatography GC Clarus 500 system (Perkin-Elmer Scientific Co. Norwalk, CT06859, and USA), interfaced to a Mass Spectrometer MS equipped with an Elite- 1,5 fused silica capillary column composed of 100% Dimethyl Polysiloxane and an electron ionization system with ionizing energy of 70eV. The carrier gas used was Helium (99.999%) at a constant flow rate of 0.5ml/min. One microlitre (1 μ) sample injection volume (Split ratio of 10:1); the inlet/injection temperature was maintained at 250⁰C, Ion source temperature 300⁰C for 2min, then an increase to 120⁰C, then programmed to increase to 280⁰C at a rate of 20⁰C for 5mins. Total run time was 77min. The MS transfer line was maintained at a temperature of 300⁰C for 5min. The Mass Spectra were taken at 70Ev and a scan interval of 0.5 seconds with fragments from 45 to 415.00KD.

Identification of Components: This was based on Interpretations using the database of National Institute Standard and Technology [24] mass spectral library having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the component stored in the NIST library version, software, turbomas 5.2 [14].

Abundance

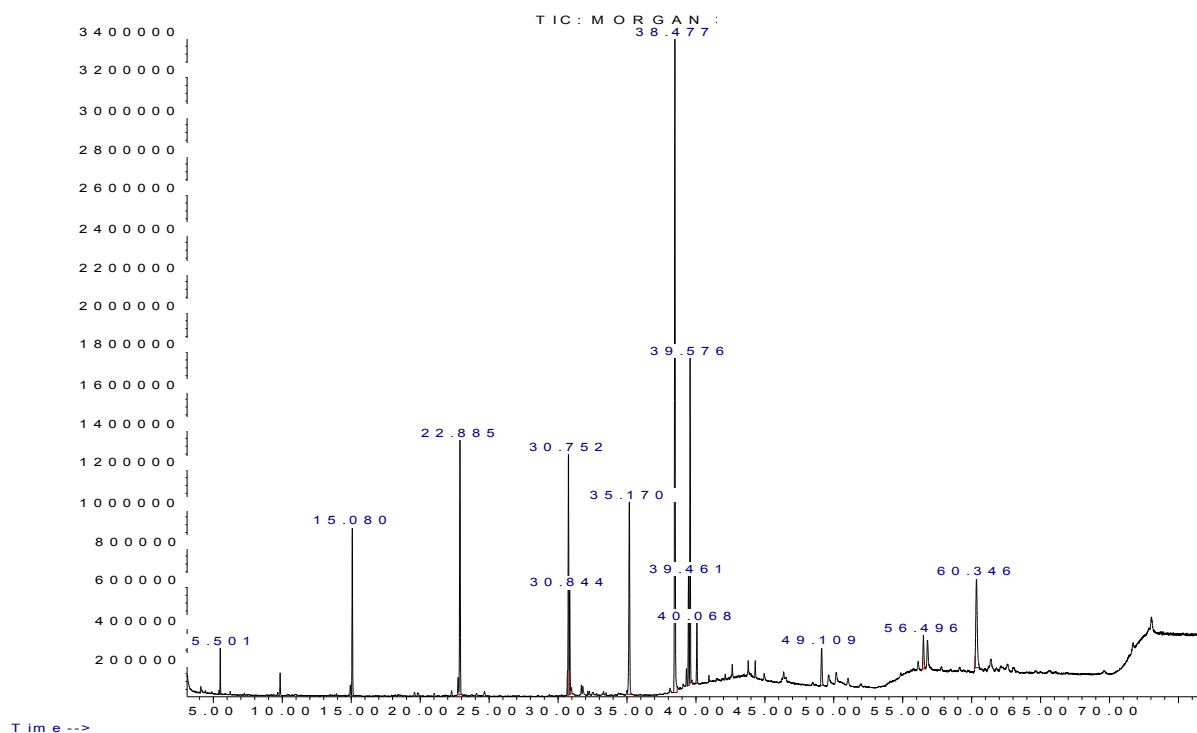


Figure 1: Chromatogram for *S.cayennensis*

The following secondary bioactive metabolites were discovered to be predominantly present in *S.cayanensis* in the following concentrations: Decanoic acid, ethylester (Ethyl decanoate) 6.02%, Dodecanoic acid, ethylester (Ethyl dodecanoate) 11.26%, Tetradecanoic acid, ethyl ester (Ethyl tetradecanoate) 9.22%, Hexadecanoic acid, ethylester (Ethyl hexadecanoate) 10.16%, Phytol 28.52%, 9, 12, 15-Octadecatrienoic acid, ethylester (Ethyl linolenate) 11.34%, Stigmasta-7,25-dien- β -ol 7.95%

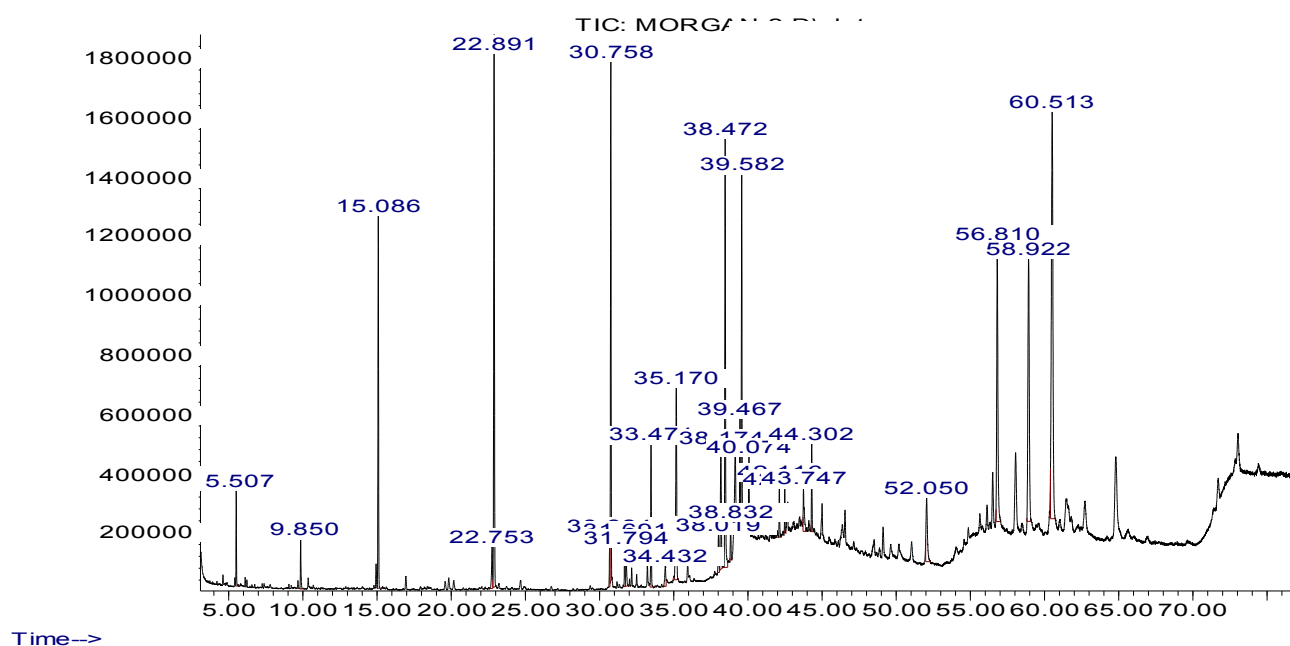


Fig 2: Chromatogram for *P. amarus* extract

The following compounds in the stated concentrations were found predominantly present in leaf extracts of *P. amarus* ; Decanoic acid, ethyl ester (Ethyl decanoate) 6.05%, Dodecanoic acid, ethyl ester (Ethyl dodecanoate) 10.89%, Tetradecanoic acid, ethyl ester (Ethyl tetradecanoate) 8.32%, Hexadecanoic acid ethyl ester (Ethyl palmitate) 5.01%, Phytol 8.27%, Linoleic acid, ethyl ester (Ethyl linolenate) 5.79%, 9,12,15-octadecatrienoic acid, ethyl ester (Ethyl linolenate) 5.79%, Vitamin E 8.29%, Stigmasterol 9.38%, beta-Sitosterol 16.67%.

Discussion and conclusion

In all cases, considerable differences in insect mortality were observed with different plant extracts and at different time intervals when the insects were exposed to the same amount (0.5g) of each treatment. The percentage mortality caused by plant extracts on cockroach, grasshopper and mosquito larva are shown in table 1, 2 and 3 respectively. Percentage mortality varied from plant to plant as well as from one insect to another at different time intervals. Even though all the plant extracts caused 100% mortality at different times, the mosquitoes were the most susceptible as they were all dead in the first 12 hours of treatment. All cockroaches were dead by the 20th hour while it took up till the 24th hour for some of the grasshoppers to die. The positive control however killed almost all the insects within the first 4 hours of exposure. Thus the insecticidal activity of these extracts was highest in mosquito larva and least in grasshoppers. Furthermore, in all cases *S.cayennensis* seemed to exhibit more insecticidal activity than *P.amarus*.

The high mortality rate recorded by the extracts could be as a result of the following predominant bioactive secondary metabolites present in the plants. For *P.amarus* we had; Decanoic acid, ethyl ester (Ethyl decanoate), Dodecanoic acid, ethyl ester (Ethyl dodecanoate), Tetradecanoic acid, ethyl ester (Ethyl tetradecanoate) , Hexadecanoic acid ethyl ester (Ethyl palmitate), Phytol, Linoleic acid, ethyl ester (Ethyl linolenate), 9,12,15-octadecatrienoic acid ethyl ester (Ethyl linolenate), Vitamin E, Stigmasterol and beta-Sitosterol. For *S.cayanensis* the following compounds were present in good concentrations; Decanoic acid, ethylester (Ethyl decanoate), Dodecanoic acid, ethylester (Ethyl dodecanoate), Tetradecanoic acid, ethylester (Ethyl tetradecanoate), Hexadecanoic acid, ethylester (Ethyl hexadecanoate), Phytol, 9, 12, 15-Octadecatrienoic acid, ethylester (Ethyl linolenate), Stigmasta-7,25-dien- β -ol.

Interestingly, with the exception of stigmasta-7,25-dien- β -ol, all other compounds found in good concentrations in *S.cayanesis* were equally present in *P.amarus* in good concentration. *P amarus*

also contained; linoleic acid ethyl ester, vitamin E, stigmasterol and beta sitosterol in addition to the other compounds it shares with *S.cayannensis*.

Phytol; one of the compounds found in high concentration from the GC-MS result is an acyclic diterpene alcohol. Phytol and its metabolites (e.g phytanic acid) have been used as chemical deterrent agents against sumac flea beetles [31]. Recent reports have also revealed that phytol is the key bioactive compound in most botanical insecticides [21]. This could have contributed to the comparatively higher mortality exhibited by *S.cayennensis* in all cases compared to *P.amarus* as *S.cayennensis* contain higher concentration of phytol (28.52%) than *P.amarus* (8.27%).

Our results agree with that of [20], who reported the insecticidal activity of ethanolic extract of aerial and root parts of *Phyllanthus amarus* against stored grain pest; *Tribolium castaneum*. Aqueous and ethanol leaf extracts of *P. amarus*, has also been reported to show insecticidal activity against *M. bellicosus*. [27] . This confirms that *P. amarus* may actually possess some insecticidal properties as observed from our study. While there are not much reports on the insecticidal properties of *Stachytarpheta cayannensis*, reports have it that the plant is an inhibitor of acetyl cholinesterase [25] making it a potential neuromuscular pesticide . The plant is also used locally in the control of insect pests.

Some of the compounds found in reasonable concentrations and suspected to be responsible for the insecticidal activities observed in these plants include; Phytol which has been implicated as an insecticidal agent by some researchers in earlier studies [3][26] Also hexadecanoic acid ethyl ester and tetradecanoic acid ethyl ester have been reported to possess some insecticidal activities [22][17]. Stigmast-7-en 3 β -ol; one of the predominant compound found in *S.cayennensis* has also been reported to possess some insecticidal activity [1] [28]. These agree with our findings that these compounds may be largely responsible for the insecticidal actions observed in this study.

The compound with the highest concentration in *P.amarus* extract; sitosterol has been reported to possess some insecticidal properties [12]. The insecticidal properties of the crude extracts of the leaves and flowers of *Anemone pavonina* on *Pheidole pallidula* ants showed that the extract contained as major components; the sitosterol glycopyranoside lipids. Sitosterol is undoubtedly one of the greatest contributors to the insecticidal activity of this *P.amarus* [30]. Another major compound observed in *P.amarus* but not in *S.cayennensis* is Stigmasterol. It is an unsaturated plant sterol present in various medicinal plants. It is utilized in a number of chemical processes which are designed to yield numerous synthetic and semi-synthetic compounds for pharmaceutical industry [19]. Stigmasterol has been reported to inhibit acetyl cholinesterase activity and thus responsible for the larvicidal and repellent properties of *Chromolaena odorata* [16]. It is therefore not surprising that it is one of the active ingredients observed to contribute to the insecticidal ability of *P.amarus*.

Conclusion

If the major bioactive secondary metabolites observed in these plants are isolated, characterized and formulated into an industrially available form like we have in most synthetic pesticides, we may have in our hands another potent natural pesticide that is affordable, available and environmentally friendly. Field trials are also encouraged to further confirm the findings from this research.

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