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Insecticidal Potentials and Chemical Composition of Ethanol Extracts from the Leaves of *Acanthus Montanus* on Selected Insect Pests

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ABSTRACT

Many plant species produce substances that protect them by killing or repelling the insects that feed on them hence effective natural insecticides could be extracted from such plants. Insects are the principal vectors of the pathogens causing many human, animal and plant diseases. The synthetic pesticides formulated for the control of these insect pests have been associated with some forms diseases in humans. There is need therefore to develop safe alternatives. Acanthus montanus is a plant who from observation seem to possess chemical compounds with insecticidal properties. This research evaluated the insecticidal potentials of ethanol extracts from the leaves of Acanthus montanus. Selected insects were exposed to ethanol extracts from the leaves and observed for 24 hours. Percentage mortality was calculated and compared with that of "SWAN" a synthetic insecticide. A GC-MS analysis of the plant extract was also carried out to determine the active constituents present in the extract. The result showed that the ethanol extract was substantially effective as an insecticidal agent as revealed by the mortality rates observed for insects treated with the plant extract as against those treated with the positive and negative controls. The GC-MS result of the extract showed a total of 22 peaks. Predominant among them were; 9, 12, 15 Octadecatrienoic acid (30.91%), followed by squalene (12.39%), and n hexadecanoic acid (11.68%). Other constituents were; Phytol (8.68%), Octadecanoic acid (7.06%) and 1-Octadecyne (5.62%). The plant has proven to be a potential source of organic pesticide which can be exploited in combating agricultural and domestic pests.

Keywords: Insecticidal; Potential; Chemical; Composition; Extract; Insect

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INTRODUCTION

Insecticides are pesticides that are formulated to kill, harm, repel or mitigate one or more species of insects. Insecticides work in different ways, some insecticides disrupts the nervous system whereas others may damage their exoskeletons, repel them or control them by some other means [1]. Agricultural crops are under constant assault by insect pests, making insecticides essential to reduce losses. Synthetic insecticides such as organophosphates are effective tools in modern crop management; however they pose serious threats to the environment and to people. There is therefore need to encourage the production and effective use of natural insecticides. While some natural insecticides may be toxic, most are actually much safer and more eco-friendly than conventional pesticides. They are equally very effective when used correctly. It is common knowledge that insects have affected human life and agricultural produce negatively for decades now; the most common problems encountered being malaria in the case of mosquitoes, food poisoning and allergies in the case of cockroaches and crop destruction in the case of grasshoppers. Spraying the inside of houses with insecticides is the main method used by the World Health Organization (WHO) to reduce the number of our household insects while the manipulation of environment such as provision of shades by trees and removal of course vegetation from fallow land and field margins as well as the use of pesticides will defer the activities of grasshoppers [2].

Acanthus montanus, also known as *Bear's Breech* or *Mountain Thistle,* is a thinly branched perennial plant which possesses basal clusters of oblong shaped glossy, dark green leaves of about 12 inches long. It belongs to the family; *acanthacease*. It is called Leopard's tongue in English. Other common names include; white ginger and bear's breech. In Yoruba Nigeria it is called *Ahon-ekun* or *ikunmu-arugbo* [3]. The leaves are silver marked with wavy margins. It's up to 6 feet tall and about 61 cm wide. It prefers shady situations and occasional deep watering, but tolerates sunny, dry situations too. Its aggressive roots make it perfect for slopes [4]. Among bio-pesticides, botanical ones are experiencing a revival due to the eco-toxicological properties of synthetic ones. Plants play pivotal roles in ecological systems because they constitute a rich source of bioactive chemicals [5]. Risk and problems associated with the use of chemicals lead to increasingly stringent environmental regulation of pesticides. There is therefore an urgent need to develop safer, more environmentally friendly and efficient alternatives that have the potential to replace synthetic pesticides and are convenient to use. Many secondary plant metabolites are known for their insecticidal properties, and in many cases plants have a history of use as home remedies to kill or repel insects [6]. More so, plants being of natural origin are likely to contain many complex chemicals which could work in synergy to alleviate the pest resistant properties of some insect pests.

The insects used in this study were selected because they represent some of the fiercest attackers of both human and agricultural products. While cockroaches do not cause any disease by themselves, they carry millions of bacteria and infectious agents that can lead to an array of diseases from diarrhea to food poisoning. Grasshoppers are plant eaters, sometimes becoming serious pests of cereals, vegetables and pastures, especially when they swarm in their millions as locusts and destroy crops over wide areas. They eat large quantities of foliage both as adults and during their development. They protect themselves from predators by camouflage; when detected, many species attempt to startle the predator with a brilliantly-coloured wing-flash while jumping and launching them into the air, usually flying for only a short distance [7]. Diseases like filariasis, dengue, yellow fever, malaria, Japanese encephalitis and chikungunya are some of the deadly diseases spread by mosquitoes [8]. This research therefore aims to access the insecticidal potentials of ethanol extract from the leaf of *Acanthus montanus* on some selected insect pests and vectors and compare same to a commonly used synthetic insecticide "SWAN" in order to assess its efficacy as an insecticidal agent and advice on its use. The research will also find out the chemical components of the ethanol extract which may be responsible for any insecticidal action observed in this study.

EXPERIMENTAL SECTION

Equipments and Reagents

Electric blender- (AKAI TOKYO JAPAN); Model BD0011DA-1033M made in PCR, Weighing balance- (symmetry Colle-parmer Instrument Co, USA), separating funnel, Memmert WNB 7 water bath, Gas chromatography-Mass spectrometer analyser (GCMS-QP2010 PLUS SHIMADZU, JAPAN). Ethanol-BDH chemicals limited Poole England.

Materials and Methodology

The leaves of *Acanthus montanus* was harvested from a farm in Uyo city, Akwa Ibom state and identified at the Department of Botany University of Calabar. The leaves were washed with clean water and air dried for about two (2) weeks and then milled to powder using an electric blender. About 150 g of the powdered leaf was soaked in 900 mls of anhydrous ethanol for 48 hours with continuous shaking at intervals. The solution was thereafter filtered using whatman filter paper. The filtrate was subsequently concentrated by evaporation at 80°C using a water bath. About 15.0 g of oily extract was recovered amounting to 10% yield. The oil extract obtained was used for the insecticidal studies. American cockroach (*Periplaneta americana*), great green hoppers (*Tettigonia virridissima*) and African malaria mosquito (*Anopheles gambiae*) were used for this research. *Periplaneta americana* were caught from septic tanks in Satellite town Ete-agbor, Calabar, while *Tettigonia virridissima* were caught at the football field of University of Calabar, Calabar Cross Rivers State. 10 insects each (*Periplaneta americana* and *Tettigonia virridissima*) were placed in a cylindrical plastic container perforated at one end to allow for proper ventilation. The mosquito larvae were trapped in a plastic bowl kept for about two weeks in a swampy grass field in the University of Calabar, they were viewed under a light microscope and each basin contained over ten mosquito larvae. The insects were provided with food from the time they were captured until the end of the experiment. The grasshoppers were provided with fresh green grass and vegetables, cockroaches were given milk products with cassava flakes (garri) and mosquitoes were fed with Tetramin baby fish food, ground into fine powder and dispersed in the water surface, made in Germany. All insects were active and feeding properly at the time of commencement of the experiment. The insects were divided into three different groups containing ten (10) insects each making a total of about thirty (30) insects for each group. Group A was treated with *A. montanus* extract while groups B and C were treated with

SWAN and distilled water respectively. About 500 mg of ethanol extract from *A. montanus* was soaked in cotton wool and placed inside the perforated cylindrical containers (10 cm height, 5 cm diameter) containing the insects, for the mosquito 500 mg of the oil extract was measured and put into the water containing the larvae. The same procedure was repeated with SWAN and distilled water (Tables 1-4). Experiments were carried out three times and the average mortality rates were recorded. The GC-MS analysis of extracted oil was carried out described by Sparkman et al. at the National Research Institute for Chemical Technology (NARICT) Zaria, Kaduna State [9].

GCMS Conditions

The analysis was performed using a QP2010 PLUS GC-MS SHIMADZU JAPAN equipped with GC-2010 capillary column with Viscosity Comp Time: 0.2 sec, Pumping time: 5 sec, Injection. Port Dwell Time: 0.3 sec, Washing volume: 8 uL, Column oven temperature: 80.0°C, Injection Temp: 250.00°C, Flow control Mode: Linear velocity, Pressure: 108.0 KPa, Total flow: 6.2 mL/min, Column flow; 1.58 mL/min, Linear velocity: 46.3 cm/sec, Purge Flow: 3.0 mL/min, Split Ratio: 1.0. The oven temperature was set between 80.0°C to 280.0°C, Hold time between 1.00-5.00 min at a rate of 10.00. The equilibrium time was 3.0 min, Ion source temperature: 230.00 °C, Interface temp: 250.00°C, Solvent Cut Time: 250 mins, Threshold: 1000, Start time: 3.00 min, End time: 28.00 min, Event Time: 0.50 sec, Scan speed: 1250, Sample Inlet Unit: GC. The chemical compounds in the oils were identified based on GC retention time on GC-2010 capillary column matched with EI MS library of the NIST/EPA/NIH Mass Spectral Library 2005 (NIST, 2005) as the reference library.

RESULTS

Table 1: Percentage mortality of *Tettigonia virridissima* (grasshoppers) exposed to insecticidal agents after 24hours

Group A				Group B			Group C		
No of Hrs	*A. montanus* extract (500 mg)			SWAN (500 mg)			Water (500 mg)		
	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3
4	-	-	+	+	+	+	-	-	-
8	+	+	+	+	_	++	-	-	-
12	+	+	-	+	++	+	-	-	-
16	-	-	-	++	-	-	-	-	-
20	-	-	-	-	-	-	-	-	-
24	-	-	-	-	-	-	+	-	-
Total no of deaths	4/10	3/10	2/10	7/10	4/10	6/10	1/10	0/10	0/10
Mean mortality (%)	30%			57%			3%		

Table 2: Result of observation on *Periplaneta american* (American cockroach) exposed to insecticidal agents after 24hours

Group A				Group B			Group C		
No of Hrs	*A. montanus* extract 500 mg			SWAN 500 mg			Water 500 mg		
	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3
4	-	-	+	+	+	+	-	-	-
8	++	+	+	+	+	++	-	_	-
12	+	+	+	+	++	++	-	+	-
16	-	-	-	++	+	+	-	-	-
20	-	-	-	-	-	-	-	-	-
24	-	-	-	-	-	-	-	-	-
Total number of deaths	3/10	3/10	5/10	8/10	6/10	7/10	0/10	1/10	0/10
Mean mortality (%)	37%			70%			3%		

Table 3: Result of observation on *Anopheles gambaie* (mosquitoes) exposed to insecticidal agents after 24hours

Group A		Group B				Group C			
No of Hrs	*A montanus*			SWAN		Control			
	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3
4	+	-	+	-	+	+	-	-	-
8	++	++	+	++	++	++	-	-	-
12	+	++	+	++	++	+	-	-	-
16	-	-	-	++	+	++	-	+	-
20	-	-	-	-	-	+	-	-	+
24	-	-	-	-	-	-	-	-	-
Total number of deaths	5/10	6/10	5/10	7/10	7/10	8/10	0/10	1/10	1/10
Mean mortality (%)	53%			73%			6%		

Table 4: Showing result of GC-MS characterization of ethanol leaf extract of *A.montanus*

S/N	R.Time (min)	Name of Comp	Molecular Formula	Molecular weight (g/mol)	Peak Area %
1	5.543	1,6-Octadien-3-ol,3,7-dimethyl	C10H18O	154	0.27
2	8.937	6-Octen-1-ol,3,7-dimethyl-, acetate	C12H22O2	198	0.3
3	9.085	2,6-Octadien-1-ol,3,7-dimethyl-,acetate	C12H20O2	196	0.32
4	9.333	2,6-Octadien-1-ol,3,7-dimethyl,acetate	C12H20O2		0.27
5	9.648	Cyclohexane,1-ethenyl -1-methyl-2,4-bis(1-methylethenyl)-	C15H24	204	2.14
6	10.084	Aromadendrene	C15H24	204	0.69
7	11.81	Undecanoic acid	C11H22O2	186	1.74
8	14.181	n-HEXADECANOIC ACID	C16H32O2	256	2.2
9	15.184	1-Octadecyne	C18H34	250	5.62
10	15.593	1- Octadecyne	C18H34	250	1.35
11	15.935	1-Nonyne	C9H16	124	2.14
12	17.823	n-Hexadecanoic acid	C16H32O2	256	11.68
13	18.108	Octadecanoic acid,ethyl ester	C20H40O2	312	2.32
14	20.132	pHHYTOL	C20H40O	296	8.68
15	20.714	9,12,15-Octadecatrienoic acid	C18H30O2	278	25.71
16	20.825	9,12,15-Octadecatrienoic acid	C18H30O2	278	5.2
17	20.934	Octadecanoic acid	C18H36O2	284	7.06
18	24.601	Hexadecanoic acid, 2,3-dihydroxypropyl ester	C19H38O4	330	3.9
19	25.267	Heptadecane,9-octyl	C25H25	352	0.63
20	26.319	(E)-13-Docosenoic acid	C22H42O2	338	3.69
21	27.321	Heptadecane,9-octyl	C22H42O2	352	1.7
22	27.729	Squalene	C30H50	410	12.39

DISCUSSION

From our research, it is obvious that ethanol oil extract of *A. montanus* possess reasonable insecticidal activity which could be harnessed and channeled appropriately into the management of insect pests and vectors in the nearest future. This can be seen from the mortality rates observed in insects treated with the plant extract within a period of 24 hours, when compared to the negative control which received no similar treatment. Even though its level of insecticidal activity was considerably lower than that of the positive control "SWAN", it is possible that when the active ingredients responsible for its insecticidal activity are isolated, purified and formulated into an industrially active form, it could perform even better that the already formulated synthetic pesticide "SWAN". *A. montanus* caused a 30% mortality rate in *T. virridissima*, 37% mortality rate in *P. americana* and 53% mortality in *A. gambiae*, this shows that the extract possesses reasonable insecticidal potential especially when compared with the control which did not show any significant level of mortality. The positive control; SWAN recorded a higher mortality rate of 57% for *T. virridissima*, 70% for *P. americana* and 73% for *A. gambaie*, this is understandable given that the insecticide already has its active ingredients isolated, purified and formulated into an industrially active form, it is therefore important that the active principles in *A. montanus* are isolated, purified and formulated into an industrially active form just like SWAN this could enhance its activity and make it perform even better than SWAN.

In all the treatments, grasshoppers had the least mortality as compared to other insects. This confirms a previous report by Okonkwo and Ohaeri [10] that grasshopper seem to possess some level of resistant towards insecticides whether natural or synthetic. Also, mortality was observed only after about 2 hours of treatment, this shows that the effect of the treatments on insects is not instantaneous but allowed some time before toxicity and death were manifested. The GC-MS analysis performed on the ethanol extract of *A. montanus* revealed the presence of 21 compounds, predominant among them were; 9,12,15-octadecatrienoic acid ($C_{18}H_{30}O_2$) with molecular weight 278 g/mol and concentration 30.91%, Squalene ($C_{30}H_{50}$) with molecular weight 410 g/mol and concentration 12.39% and n-Hexadecanoic acid ($C_{16}H_{32}O_2$) with molecular weight 256 g/mol and concentration 11.68%. Others include; phytol ($C_{20}H_{40}O$) mol weight 296/mol, concentration 8.68%, Octadecanoic acid ($C_{18}H_{36}O_2$) mol weight 284 g/mol, concentration;7.06%, 1-octadecyne ($C_{18}H_{34)}$ mol weight 250 g/mol, concentration; 5.62%, Hexadecanioc acid 2,3-dihydroxypropyl ester and (E)-13- Docosenoic acid, with concentrations; 3.90% and 3.69% respectively.

9, 12, 15-octadecatrienoic acid also known as; α-Linolenic acid (ALA) is an n−3 fatty acid. It is one of the essential fatty acids so called because they are necessary for health and cannot be produced within the human body. They must be acquired through diet. ALA is an omega-3 fatty acid found in seeds, nuts, and many common vegetable oils. In terms of its structure, it is named all-cis-9, 12, 15-octadecatrienoic acid [11]. It has been implicated as an insecticidal agent in a study by Ramos -López et al. [12] which analysed the hexane extract of *R. communis* leaves. In this study, linolenic acid was identified as being insectistatic and insecticidal against *S. frugiperda*. Thus confirming linolenic acid as an insecticidal agent. Squalene is a hydrocarbon and a triterpene, and is a natural and vital part of the synthesis of all plant and animal sterols, including cholesterol, steroid hormones, and vitamin D in the human body [13]. It is a natural 30-carbon organic compound which has its origin from shark liver oil although plant sources are now used as well, including amaranth seed, rice bran, wheat germ, and olives. Also, yeast cells have been genetically engineered to produce commercially useful quantities of "synthetic" squalene [14].

There are reports that squalene can be used as pesticide and is particular good in controlling the fire ants and mosquitoes [15]. Little wonder it was one of the major components discovered in this plant extract. It is also beneficial and harmless to human and there is no residual damage reported yet. It has also been reported to be a strong acaricidal agent against mites [16]. N-hexadecanoic acid, another major component of *A. montanus* ethanol extract, has been reported to possess hemolytic pesticide properties and as an inhibitor of 5-alpha-reductase [17]. Its ester; hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester has also been reported as a haemolytic, pesticidal, nematicidal, and an inhibitor of 5-alpha reductase [18]. The presence of n-hexadecanoic acid in a significant amount in *A. montanus* plant oil extract shows that it may have contributed largely to the insecticidal effect exhibited by the oil. Also, hexadecanoic acid and its methyl ester was instrumental to the insecticidal activity exhibited by *Casimiroa edulis La Llave and Lex (Rutaceae)* leaf extract and its fractions against *Spodoptera litteralis* larvae [19]. Oladipupo et al. [20] also observed that the presence of hexadecanoic acid and n-hexadecanoic acid methyl ester in significant concentrations in the leaf extracts of *Chromolaena odorata* was accountable for the insecticidal action of the leaf towards adults of *S. zeamays*. It is not surprising therefore that this compound constitutes a major component of *A. montanus* oil extract. In another study, the GC-MS analysis of *J. curcas* leaf extract revealed the presence of trans-phytol (60.81%), squalene (28.58%), phytol (2.52%) and nonadecanone (1.06%) as major components that could be attributed for the insecticidal activity of J. curcas extracts [21]. Phytol is another compound that was found in significant quantity in *A. montanus* oil, It is an acyclic diterpene alcohol that can be used as a precursor for the manufacture of synthetic forms of vitamin E [22] and vitamin K [23]. In ruminants, the gut fermentation of ingested plant materials liberates phytol, a constituent of chlorophyll, which is then converted to phytanic acid and stored in fats [24]. Also phytol was one of the major components discovered in the oil of *Premna angolensis* and *Premna quadrifolia* leaves, which had insecticidal and repellent effects on *S. cerealella* [25].

CONCLUSION

It is obvious from this study that *A. montanus* oil possesses some level of insecticidal efficacy which could be utilized in the nearest future for the management of insect pests and vectors. Its usefulness as an insecticide should be maximized by carefully isolating the active principles identified in this study, purifying them and formulating them into an industrially active and acceptable form. The public should also be encouraged to use more of the natural products since most of them have little or no effect on human lives and the environment as a whole.

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