ISSN 1996-3351

Asian Journal of Biological Sciences

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OPEN ACCESS Asian Journal of Biological Sciences

ISSN 1996-3351
DOI: 10.3923/ajbs.2020.223.227

Research Article
Entomotoxicological Potencies of Extracts of the Weaver Ant (*Oecophylla smaragdina*) Against *Anopheles* spp.

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Abstract

Background and Objective: The endemicity of mosquito-borne diseases in more than 100 countries recently, as a result of mosquito complex behaviour dynamics coupled with the challenges of costs, environmental toxicity and most especially insecticide resistance associated with indiscriminate chemical vector control Therefore this study aimed to elucidate the mosquito larvicidal efficacies of crude and fractionated extract of the weaver ant against *Anopheles* mosquito spp. **Materials and Methods:** The entomochemical composition of the crude methanolic extract was analyzed following standard procedures. The larvicidal activities of both the crude and fractionated extract of the weaver ant against 4th instar larvae of *Anopheles* mosquito spp. were carried out using slightly modified WHO standard protocols and larvae mortality were recorded after 24 h exposure period. Graded concentrations, ranging from 0.1-1.2 mg L^{-1} of both the crude and fractionated extracts were tested against 20 batches of healthy 4th instar larvae of *Anopheles* mosquito spp. **Results:** The entomochemical screening revealed the presence of saponins and cardiac glycosides in the crude methanol extract. Percentage larvae mortality was directly dependent on extract concentration in both the crude and fractionated forms. All the tested extract concentrations showed moderate to high larvicidal activities. However, the lowest lethal concentration was recorded with the n-hexane fraction, followed by the ethyl acetate fraction. The LC_{50} and LC_{90} of n-hexane and ethyl acetate fraction were recorded as 0.03, 0.34 and 0.463, 0.718 mg L^{-1}, respectively. There was strong correlation between larvicidal activities of the crude methanolic extract and n-hexane fraction is an indication of similarity in their mode of larvicidal action. **Conclusion:** The findings of this study concluded that bioactive metabolites from weaver ants is a promising source alternative highly potent and perhaps, eco-friendly insecticidal agents for sustainable malaria vector control and, hence should be thoroughly investigated for that purpose.

Key words: Larvicidal activities, weaver ant, malaria vector, mosquito-borne diseases, *Anopheles* spp.

Citation: Adeniyi Kamoru Abdulazeez, Olayemi Israel Kayode, Shittu Kudirat Oluwatosin, Ukubuiwe Azubuike Christian, Oyibo-Usman Khadijat Ahuoiza and Garba Yusuf, 2020. Entomotoxicological potencies of extracts of the weaver ant (*Oecophylla smaragdina*) against *Anopheles* spp. Asian J. Biol. Sci., 13: 223-227.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Despite the giant stride recorded against mosquitoes in the last few decades, the vector has remained a major public health threat, worldwide[1,2] and has been appreciated as the greatest notoriety among arthropods[3]. According to Hemalatha *et al.*[2], more than 100 species of mosquitoes have potentials to vector various diseases in human and other vertebrates. Their menace contributes significantly to diseases burden, death, poverty and social debility all over the world, especially in Africa, south of the Sahara. For instance, as at 2015, malaria infection transmitted by infected female *Anopheles* mosquitoes, remains the most virulent vector borne disease, affecting between 300-500 million people and causing 1.4-2.6 million deaths annually world-wide. In 2016, it was estimated globally that 3.3 billion people were at the risk of malaria infection, with populations living in sub-Saharan Africa having the highest risk of acquiring the disease[4]. In Nigeria, malaria is the foremost public health threat, accounting for up to 50% of all attendants at healthcare facilities, across the six geopolitical zones of the country and resulting in over 300, 000 deaths annually[5-7].

Previous studies have identified mosquitoes of *Anopheles gambiae* (principally, *A. gambiae* s.s. and *A. arabiensis*) and *Anopheles funestus* complexes as the principal vectors of malaria and Lymphatic filariasis[8,9]. The complexities of the bio-ecology of the adult mosquito, as well as, biting behaviour have greatly hampered their effective management and control over the years. Experts have, therefore, identified the larval stage as an attractive target for insecticide development because they breed in water[10,11].

Although, the use of synthetic chemicals with insecticidal potency such as organochlorines, organophosphates, carbamates and pyrethroid have recorded novel successes in controlling mosquitoes and other insect pests worldwide, their extensive and indiscriminate use have nurtured a number of environmental and health concerns such as high cost, introduction of toxicant to the food chain, unwanted lethal effect on non-target organisms and most especially development of resistance by mosquitoes[12]. Those limitations have led to aggressive search for ecofriendly, cost-effective, biodegradable, less liable to resistances and target specific insecticides against mosquito vectors[2,10,13]. Besides, most of the natural product derived insecticides have been majorly from plants and marine organisms; sources which are vulnerable to resistance. There is, therefore, need to search from other natural products with promising bioactive compounds. From time immemorial, insects and bioactive compounds derived from them have been used directly and/or indirectly in the medical system of different human cultures throughout the world. Weaver ants, for instance are commonly found on mango trees where they construct their nest by webbing the leaves. The antibacterial potency of the ants and their secretions have been earlier reported by Singh and Padmalatha[14]. Weaver ant is also documented to be effective in the treatment of cold and cough[15], so the present study was designed to explore the potential bioactive metabolites of weaver ant and efficacies of the crude and fractionations against the malarial vector, *Anopheles* spp.

MATERIALS AND METHODS

Duration of study: The study was carried out at the Applied Entomology Unit of the Department of Animal Biology, Federal University of Technology, Minna, Nigeria. The duration of the study was from February 2017-April, 2018.

Sources of insect specimen and preparation of crude extract: Weaver ant colonies were collected from mango trees in Gidan Mangoro area of Minna, Nigeria. The insect samples were collected in a covered container with little access to air and to prevent their escape. The samples were transferred to the Laboratory of the Department of Animal Biology, Federal University of Technology, Minna, where the insects were freeze-killed and oven-dried at 25°C for complete removal of the ice droplets. The samples were allowed to dry at room temperature for 2 weeks. The dried insect samples were pulverized using an electric grinder (Model: WPB80BC) and the powdered sample stored at room temperature until extraction. The extraction of the powdered insect material was carried out as described by O'Neill *et al.*[16] and Najafi *et al.*[17]. Briefly, 200 g of the weaver ant powdered sample was percolated in 1600 mL of methanol and kept in the shade for 48 h. The filtrate was harvested in a beaker and concentrated using a rotary vacuum evaporator[11]. The crude extract was preserved at -4°C until fractionation and bio-assay.

Preparation of solvent fractions: Solvent-solvent extraction has been described as one of the most popular methods for partial purification (i.e., group separation according to polarity) of crude extract[18]. Therefore, the weaver ant crude methanolic extract was dissolved in 100 mL of methanol in a beaker; after which, the mixture was poured into 1 L of separating funnel and successfully portioned using n-hexane and ethyl acetate. This gave 3 fractions namely, n-hexane, ethyl acetate and methanol.

Collection and maintenance of mosquitoes: The *Anopheles gambiae* mosquito larvae used for the study were obtained from the wild in Bosso area of Minna, Nigeria, at their first instar larval stage and maintained in the Insectary of Applied Entomology and Parasitology Unit, Department of Animal Biology, Federal University of Technology, Minna. Laboratory handling and maintenance of the mosquitoes followed standard procedure at 27.00+2.50°C, 75.00+9.00% relative humidity and 12:12 light: darkness and photo-period[19].

Entomochemical screening of crude extract: The entomochemical constituents in the crude methanolic extract of the weaver ant were determined using the methods described by Evans[20] and Sofowora[21]. The entomochemical tests determined the presence or absence of alkaloids, anthraquinones, flavonoids, tannins, phlobatannins, terpenes and saponins.

Larvicidal bioassay: Bioassay of the crude and fractionated extracts were performed against early 4th instar larvae of *Anopheles gambiae* complex, according to the standard method for testing the efficacy of bio-insecticides[22], with slight modification. Stock solution was prepared by adding 1g of the extract into10 mL solvent of the extraction. From the stock solution, 2 mL was dissolved in 18 mL distilled water; thereafter, graded concentrations of both the crude extract and fractions were prepared to obtain 0.4, 0.6, 1.0 and 1.2 mg L^{-1} for crude and 0.1, 0.2, 0.3, 0.5 and 0.5 mg L^{-1} for the fractions, respectively, in the final volume of 100 mL distilled water. Batches of 20 healthy 4th instar larvae of the *Anopheles* mosquitoes were separately exposed to each extract grated concentration assay medium. Five replicates comprising of 20 larvae each were used in the experiment. A control experiment containing only 1 mL of the solvent for each assay in 100 mL of distilled water was set up. The experiment was repeated twice and conducted under laboratory conditions of 25- 30°C and 80-90% relative humidity. Mortality of the larvae was noted and recorded after 24 h exposure period. The larvae were considered death when they did not show any response to prodding with a Pasteur pipette[23].

Statistical analysis: Medial lethal concentration (LC_{50}) and upper lethal concentration (LC_{90}), i.e., 50 and 90% mortality, respectively, in the mosquito larvae were determined by the use of Probit linear regression analysis. Significant differences in mortalities recorded among the tested concentration were done using one-way Analysis of Variance (ANOVA), coupled with Duncan Multiple Range test. The percentage mortality for each concentration was computed and correction for mortalities when necessary was done using Abbot's formula. Pearson correlation was employed to determine the relationship between the larvicidal activity of crude and fractionated extracts. A p-value of <5% was assumed significant. Analysis was done using Microsoft Excel, 2010 and Statistical Packages for Social Sciences, 20th version.

RESULTS

Qualitative analysis: The entomochemical that were detected in the methanolic crude extract of the weaver ant were saponins, phlobatannins, cardiac glycoside, terpenoids and oxalate, while flavonoids, tannins, alkaloids, steroids and phenol were not detected (Table 1).

Larvicidal activities: The larvicidal effects of the crude methanolic extract and the solvent fractions of the weaver ant are detailed in Table 2. Exposure of the 4th instar larvae of the *Anopheles* mosquitoes to both the crude and fractionated extracts of weaver ant, after 24h-exposure period showed a dose-dependent mortality effect (i.e., the higher the concentration the more the mortality caused to the larvae). Further, larvae mortality increased with increase in extract concentration and lower concentrations were more effective in the fractions than the crude extract. For the crude methanol extract, at 0.4 mg L^{-1}, only 12% mortality was recorded in the larvae, 24h-post exposure. Larval mortality, thereafter, increase significantly ($p<0.05$) with increase in concentration of the extract. The highest mortality (84.70%) was recorded at 1.2 mg L^{-1} crude extract concentration.

Similarly, the toxicity of the fractionated extract varied significantly ($p<0.05$) among the different solvents of fractionation. For n-hexane, >90% (i.e., 92.00%) larvae mortality was recorded at 0.3 mg L^{-1}.

Table 1: Qualitative entomochemical constituents of the crude methanolic extract of weaver ant (*Oecophylla smaragdina*)

Entomochemical constituents	Results
Flavonoids	-
Tannin	-
Saponins	+
Alkaloid	-
Steroid	-
Phlobatannins	+
Cardiac glycosides	+
Anthraquinones	-
Total phenol	-
Terpenoids	+
Oxalate	+
Phytate	-

-: Absent, +: Present

Table 2: Larvicidal activities of crude and fractionated extracts of *Oecophylla smaragdina* (weaver ant) extract against 4th instar larvae of *Anopheles* spp. after 24 h exposure periods

Crude extract concentration (mg L^{-1})	Larval mortality (%)	Fractionated extract concentration (mg L^{-1})	Larval mortality (%)		
			n-hexane	Ethyl acetate	Methanol
0.4	2.41±0.35^{b*} (12.00)**	0.1	11.60±1.21^{b*} (58.00)**	0.60±0.25^{a*} (3.00)	1.00±0.45^{a} (5.00)
0.6	8.60±0.75^{c} (43.00)	0.2	15.60±0.87^{c} (78.00)	1.60±0.25^{b} (8.00)	3.60±0.60^{b} (18.00)
0.8	14.80±0.73^{d} (74.00)	0.3	18.40±0.51^{d} (92.00)	2.20±0.37^{c} (11.00)	4.20±0.74^{b} (21.00)
1.0	15.23±0.00^{d} (76.15)	0.4	20.00±0.00^{e} (100)	8.79±0.37^{d} (28.95)	5.80±0.92^{c} (29.00)
1.2	16.94±0.00^{d} (84.70)	0.5	20.00±0.00^{e} (100)	14.12±0.45^{e} (35.60)	11.80±0.37^{d} (59.00)
Positive control	0.91±0.00^{b} (4.50)	Positive control	0.41±0.88^{a} (2.05)	0.30±0.10^{a} (1.50)	0.57±0.01^{a} (0.00)
Negative control	0.00±0.00^{a} (0.00)	Negative control	0.20±0.10^{a} (2.00)	0.95±0.09^{b} (4.75)	0.43±0.22^{a} (2.15)

*Values followed by the same superscript alphabets in a column are not significantly different at $p \geq 0.05$, **Values in parenthesis represent the percentage mortality after 24 h exposure period, (n = 20/replicate)

Table 3: Lethal concentrations of crude and fractionated extracts of weaver ants against 4th instar larvae of *Anopheles* spp.

Type of extract	LC_{50} (mg L^{-1})	LC_{90} (mg L^{-1})	R^2	Regression equation
Crude extract	0.711	1.159	0.876	y = 89.27x-13.45
n-hexane fraction	0.03	0.342	0.8811	y = 106x+53.8
Ethyl acetate fraction	0.463	0.718	0.794	y = 156.9x-22.69
Methanol fraction	0.498	0.834	0.8703	y = 119x-9.3

Table 4: Correlation coefficients of the larvicidal activities of crude and fractionated extracts of *Oecophylla smaragdina* (weaver ants) against 4th instar larvae of *Anopheles* spp.

Parameters	Crude methanol	n- hexane fraction	Ethyl acetate fraction	Methanol fraction
Crude methanol	1			
n-hexane fraction	0.990**	1		
Ethyl acetate fraction	0.818	0.847	1	
Methanol fraction	0.798	0.782	0.915*	1

* Correlation is significant at the 0.05 level (2-tailed), ** Correlation is significant at the 0.01 level (2-tailed)

This was significantly higher than the mortality recorded for 0.2 mg L^{-1} crude extract concentration. However, 100% larvae mortality after 24 h exposure was observed at 0.4 mg L^{-1} of the n-hexane fraction. Although, increase in the concentration of ethyl acetate and methanol fraction yielded an increase in larvae mortality, the mortality was lower than that of the crude extract and n-hexane fraction (Table 2).

Lethal concentrations: The lethal concentrations (LC) of the crude and fractionated extracts are presented in Table 3. The study revealed an LC_{50} of 0.711, 0.03, 0.463 and 0.498 mg L^{-1}, respectively for the crude methanol extract, n-hexane, ethyl acetate and methanol fractions. While the equivalent LC_{90} were 1.159, 0.342, 0.718 and 0.834 mg L^{-1}, respectively.

Correlation coefficients of the larvicidal activities: The correlation coefficients between the crude methanol and the fractionated extracts of weaver ant are represented in Table 4. There was a strong significant correlation between the toxic effects of crude methanol extract and those of the fractions, at 0.01 and 0.05 levels of significance. Similarly, methanol and ethyl acetate fractions showed strong significant correlation at 0.05 significant levels. Moderate correlation (r = 0.798, 0.782) was recorded between the methanol fraction and crude methanolic extract, as well as, methanol fraction and n-hexane fraction.

DISCUSSION

The crude methanolic extract of weaver ant tested in this study contained saponins, terpenoids, oxalate, phlobatannins and cardiac glycosides. The presence of these important entomochemical metabolites in the insect's extract may be attributed to foraging, which is basically plant species; a rich source of these phytochemicals. Also, these secondary metabolites, synthesized from the host plant

(i.e., the mango plant) can act as defense mechanism against predators[24]. Further, these bioactive metabolites harvested by insects from host plant during foraging may serve as precursor molecules for the bio-synthesis of other metabolites[25].

The present entomochemical result is novel as it marks the first experimental exploration of biochemical secondary metabolite in weaver ant. Previous investigations have reported such metabolites as phytochemicals in plants[1,26].

The mortality of the *Anopheles* mosquito larvae observed in the present study could be attributed to the potency of the weaver ant extract tested and possibly, the extracts entomo-therapeutic secondary metabolite constituents[1]. Their presence might have caused a significant deleterious lethal effect to the tested mosquito larvae. This could be corroborated by the fact that <5% mortality was observed in the control and the larvae remained agile throughout the experiment. The present larvae mortality could also be due to the toxicity caused by or the combine lethal effects of the bioactive metabolites detected in the extract. It was reported that saponins caused 100% mortality in larvae of *Aedes aegypti*[27]. Other bioactive phytochemicals such as phenol and steroid have been reported to exert significant toxicity on mosquito larvae[28-30]. By implication, the dose dependent lethal effect of the weaver ant extracts on the mosquito larvae could be attributed to increase in the concentration of anti-mosquito bioactive metabolites[31].

The lowest lethal concentrations were recorded for n-hexane fraction followed by the crude methanolic extract of the weaver ants. This indicated that n-hexane extract has the best larvicidal activities followed by crude methanolic extract. The LC_{50} and LC_{90} recorded in the present study are far lower better than those recorded for some plant species. Olayemi *et al.*[11] recorded higher LC_{50} of 2.65 and 2.33 mg L^{-1} for the extract of *Jatropha curcas* against *Culex pipiens* mosquito. The correlation analysis which shows significantly strong relationship between the n-hexane fraction and crude methanol extract of the insect indicates that there are similar accumulations of secondary metabolites in the two extracts. Also, this supports the earlier assertion that mortality in the tested anopheline larvae was due to toxicity of the extracts[23]. Additionally, the weaver ant extracts looks oily which may imply that the extract caused oxygen deficiency in the water medium[13]. It is, therefore, evident from the present results that the various graded concentrations of the weaver ant extracts were the main cause of mortality in the tested *A. gambiae* spp.

CONCLUSION AND RECOMMENDATION

The present investigation revealed, for first time, significant larvicidal efficacies of the extract of weaver ant against malaria vector, *Anopheline* mosquito spp. and it was concluded that the lowest lethal concentration was traced with the n-hexane fraction and there was strong correlation between larvicidal activities of the crude methanolic extract and n-hexane fraction. The LC_{50} and LC_{90} of n-hexane and ethyl acetate fraction were 0.03, 0.34 and 0.463, 0.718 mg L^{-1}, respectively. This implies that the insect possesses a number of bio-active metabolites that could be exploited for larvicidal purposes. The weaver ant extract could, therefore, be added to the library of biodegradable eco-friendly natural products with larvicidal potency. Further studies on the active lead-agents involved and their mode of action, as well as, field trials are needed to recommend weaver-ant extract as stand-alone anti-mosquito products in control programs.

ACKNOWLEDGMENT

The authors are grateful to the immeasurable supports from the technicians from the Laboratory of the Department of Animal Biology, Federal University of Technology, Minna, Nigeria.

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