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Phytochemical Composition and Insecticidal Effects of Extract of Some Selected Medicinal Plants against Malaria Mosquito, *Anopheles Gambiae.* Giles.

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ABSTRACTS

The use of synthetic insecticides against mosquitoes have led to the development of resistance in insects and they are potential health hazards in humans and the environment. Consequently, there is a need to search for other alternative botanical insecticides that could prevent insecticide resistance in insect management program. This study aimed at assessing the larvicidal, pupacidal and adulticidal effects of ethanol leaf extracts of *Chromolaena odorata, Vernonia amygdalina, Datura metel and and Acalypha wilkesiana* on *Anopheles gambiae*. Ethanol was the solvent used for the extraction of the leaves using Soxhlet extractor at 60^{o}C to determine the secondary metabolites. The larvae, pupa and adult mosquitoes were exposed to 1.0, 2.0, 3.0, 4.0 and 5.0 mL of the extract at 28 ± 2^{o}C, 75 ± 5 % RH. Phytochemical screening was also conducted on the selected plant species to determine the secondary metabolites. Results showed that the extracts of the test plants caused high mortality of larvae, pupae and adults of *An. gambiae*. The most effective extract was *V. amygdalina* which caused 100 % mortality of larvae and pupae on exposure to 5.0 mL dosage within 48 h, while 100 % mortality of adult *An. gambiae* was observed on exposure to 5.0 mL dosage within 4 h. The results of the qualitative phytochemical screening of the test plants revealed the presence of most phytochemicals in *C. odorata* (saponins, tannins, flavonoid, glycosides and phenol), *V. amygdalina* (saponins, flavonoid, glycosides, phenol and alkaloids), *D. metel* (saponins, tannins flavonoid, glycosides, phenol and alkaloids), *A. wilkesiana* (tannins, flavonoid, glycosides and alkaloid). The results suggest that the leaf extracts of the test plants have the potential of being used for the control of *An. gambiae* larvae, pupa and adult instead of synthetic insecticides.

Keywords: *Anopheles. Gambiae,* larvicidal, pupacidal and adulticidal, Phytochemicals, Soxhlex extractor.

INTRODUCTION

Mosquitoes (Diptera: Culicidae) are major arthropod groups that existed over 180–220 million years ago (Gabriel *et al*., 2014; Bird and Mc Elroy 2016; Benelli and Durggan 2018; Hillary and Ceasar 2021). Mosquitoes belong to two subfamilies (Gabriel *et al*., 2014): Anophelinae (*Anopheles*) and Culicinae (*Aedes*, *Culex*, oils used and *Mansonia*) which pose severe threat to humans and animals because of their widespread occurrence. These two subfamilies are vectors that transmit diseases like dengue, chikungunya, zika, yellow fever, malaria, Japanese encephalitis, and filariasis ago (Gabriel *et al*., 2014; Bird and Mc Elroy 2016; Benelli and Durggan 2018; Hillary and Ceasar 2021; Obembe *et al*., 2024). They endanger people's lives in tropical and subtropical areas of the world. It has been confirmed that half of the world's population is at higher risk due to these disease-borne mosquitoes (WHO, 2015).

Generally, mosquitoes are among the most important groups of arthropods belonging to the class Insecta which are of medical significance. They transmit several important parasitic and arboviral diseases, such as malaria, filariasis, dengue, yellow fever, and Rift Valley fever (Juliano and Philip 2005). Malaria fever which is caused by protozoans (*Plasmodium* spp.) can lead to high mortality and morbidity (WHO, 2020). In 2019 there were 229 million reports of malaria cases (WHO, 2020). Malaria cases that occurred in 2020 were estimated to be 241 million, with 627,000 deaths reported from 85 countries of the world. Around 95% of the malaria cases and 96% of malaria deaths were found in sub-Saharan Africa, with 80% of all malaria deaths in Africa estimated to be among children under the age of five (WHO, 2021).

Anopheles gambiae is one of the notorious species which have been incriminated for transmitting malaria parasites (Roll Back Malaria 2011; WHO 2015). The female *Anopheles gambiae* is responsible for the transmission of the malaria parasite called Plasmodim in most countries of the world. There are other malaria vectors such as *Anopheles arabiensis, Anopheles pharoensis, Anopheles funetus, Anopheles nili, and Anopheles stephensi* which also transmit malaria parasite in some countries of the world (Zein, 2021; Balkew *et al*., 2021). Various species of mosquitoes are abundant in more than 100 countries infecting over 700 million people every year globally (Akinkulorere *et al*., 2011; Rahuman, 2011). Presently, there are over three hundlred species of the Anopheles in the world, grouped into 39 genera and 135 subgenera (Remia *et al*., 2017).

Presently, to curb the incidence of malaria fever, the major agent of control of mosquito has been through the use of conventional chemical insecticides. Malaria control programs in Nigeria and beyond face a growing challenge, such as increasing insecticide resistance in major mosquito vectors (Shililu *et al*., 1998). Synthetic insecticides, though effective, posed environmental and health risks to both human and other animals, and their overuse have contributed to resistance development (Charlwood *et al*., 1995; Omar *et al*., 2022). As a result of the aforementioned shortcomings associated with the conventional chemical insecticides, the need for alternative insecticides has become a pressing issue (Olayemi *et al*., 2009). Moreover, the absence of available alternative insecticides for vector control has exacerbated the issue (Onyido *et al*., 2009).

Many researches are presently on-going regarding the use of botanicals as a control measures of mosquitoes. Plant-based insecticides offer a promising solution, as they comprise complex blends of chemical compounds that act on both behavioral and physiological processes of the insects (Lehane, 2005). These plant materials are ecologically friendly, cheap, readily available among others Obembe *et al*., (2024).

The naturally occurring medicinal plants contain various active ingredients (phytochemicals, extracts, and oils), potentially interfering in mosquitoes' life stages (egg, larva, pupa, and adult). In addition, researchers prefer plant-based products that work against vector mosquitoes as alternatives to harmful chemical insecticides. Several articles have been published on the insecticidal properties of botanicals against vector mosquitoes (Remia *et al*., 2017; Hikal *et al*., 2017; Bekele, 2018; Gharsan, 2019; Noronha *et al*., 2020; Ganesan *et al*., 2023) .

This present research was carried out to investigate the phytochemicals present in the leaves of *Chromolaena odorata, Vernonia amygdalina, Datura metel and and Acalypha wilkesiana* and to determine the bio-efficacy of their extracts on the developmental stages of *An. gambiae*

Plate 1: *Chromolaena odorata* plant

Plate 2: *Vernonia amygdalina* Plant

Plate 3: *Datura metel* plant

Plate 4: *Acalypha wilkeiana* plant

MATERIALS AND METHODS

Collection of plant materials

The fresh leaves of Devil Weed (*Chromolaena odorata*), Bitter leaf (*Vernonia amygdalina*), Angel's Trumpet (*Datura metel*) *and* copperleaf (*Acalypha wilkesiana*) were procured from the campus of Ekiti State University, Ado Ekiti, Nigeria. The leaves were authenticated by the herbarium curator in the Department of Plant Science and Biotechnology, Ekiti State University, Ado Ekiti, Nigeria.

Preparation of extract from the plant materials

The fresh leaves of *C. odorata, V. amygdalina, D. metel and and A. wilkesiana* used for this study were washed with tap water. They were cut into small pieces with a sharp knife and air-dried in a tray for fifteen days under the laboratory condition. The dry leaves were pulverized into fine powders using a Binatone, electric grinder (Model BL-400). The powders were kept in labeled black polythene bags and stored at the ambient temperature of 28 ± 2ºC until required for use. One hundred grams (100g) of each ground leaf powder was weighed into a thimble and the extract was extracted using absolute

ethanol at the temperature of 60ºC, using a Soxhlet extractor. The resulting extract was concentrated using rotary evaporator and then air-dried to remove any trace of ethanol. The extracts were poured into specimen bottles, labeled and stored in the refrigerator at -20ºC until needed for the experiment.

Collection and **rearing of mosquito larvae and pupae**

Rain water was used in this research for the rearing of mosquito larvae and pupae. This was done by filling two (2) black (opaque) plastic water baths (200 liters) with large surface area with rain water. The importance of the rain water was to simulate the natural breeding environment of mosquitoes and to attract adult mosquitoes for the purpose of feeding and breeding. Fifty grams (50 g) of yeast was sprinkled on the surface of the water and allowed to dissolve. The important of the yeast was to feed the mosquito larvae.

The containers filled with rain water with dissolved yeast also served as bait for the adult mosquitoes. This attracts adult mosquitoes to visit the bait to lay their eggs which later metamorphosized into larvae and pupae. Afterward, the containers were moved to the laboratory. The insect larvae (wringler) were distinguished from the pupae through the absent of respiratory siphon and the presence of spiracle on the 8th abdominal segment. The spiracle is the breathing apparatus in mosquitoes larvae in contrast to the presence of siphon in the pupa stage which represent the breathing apparatus. Also, the larva is elongated while the pupa has a swollen anterior part. One of the plastic water-bath was kept in a large netted cage to allow complete metamorphosis to take place and after this there was emergence of numerous adult mosquitoes which were used for the fumigant effects of the extracts of the test plants on adult mosquitoes.

Toxic effects of leaves extract of the test plants on the larvae and pupae stages of *An. gambiae*

Desired concentration levels (1.0. 2.0, 3.0, 4.0 and 5.0 mL) of the leaves extract of the test plant were prepared. Some quantity of yeast powder was added to the extract to serve as food for the larvae. Afterward, 20 larvae or pupae of *A. gambiae* were introduced into beakers containing each of the extract concentration (1.0. 2.0, 3.0, 4.0 and 5.0 mL). Untreated control was also set-up as above. Larvae and pupae mortality were observed at 48 hours after treatment, by introducing the larvae and pupa into a beaker filled with distilled water to notice recovery. Larvae and pupa were considered dead when they were not coming to the surface for gaseous exchange.

Fumigant effects of leaves extract of the test plants on adult *An. gambiae*

Ten (10) adult mosquitoes were introduced into a test tube which was later plugged with cotton wool. Stripes of Whatman No. 1 filter paper (90 mm diameter) soaked in different extract dosages (1.0. 2.0, 3.0, 4.0 and 5.0 mL) were suspended in each test tube with the aid of a thread. Each treatment and the untreated control were replicated 4 times and arranged in Complete Randomization Design (CRD). Adult mosquitoes mortality was observed, counted and recorded after 4 hours of application.

QUALITATIVE PHYTOCHEMICAL SCREENING OF LEAF EXTRACTS OF THE TEST PLANTS

The phytochemical analyses were carried out using the method of Sofowora [1993]

Test for Tannins

Bromine Water test

Ten milliliters (10 mL) of bromine water was added to 0.5 g of extract. Discoloration of bromine water showed the presence of tannins.

Test for Saponins

Frothing test

Five milliliter (5.0 mL) of sterile distilled water was mixed with 0.5 g of plant extract in a test tube and it was mixed vigorously. The frothing was mixed with few drops of olive oil and mixed vigorously and the foam appearance showed the presence of saponins.

Tests for Flavonoids

Alkaline Reagent Test

Two milliliter (2 mL) of 2% NaOH mixture was mixed with 0.5g of plant extract; concentrated yellow colour was produced, which became colourless on addition of 2 drops of diluted acid to mixture. This result showed the presence of flavonoids.

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Test for Phenols

Lead acetate test

Three drops (3 drops) of lead acetate solution were added to the solution of the extract. A yellow-colour precipitation indicates phenols.

Test for Alkaloids

Mayer's test

Few drops of Mayer's, reagent was added to the solution of the extract. The presence of cream precipitate indicates the presence of alkaloids (Trease and Evans 1989).

Tests for Glycosides

Keller-Kiliani Test

Four milliliter (4.0 mL) of glacial acetic acid solution and 1 drop of 2.0 % $FeCl_3$ mixture was mixed with the 10 mL plant crude extract and exactly 1 mL of concentrated H_2SO_4. A brown ring formed in between the layers which showed the presence of cardiac steroidal glycosides (Trease and Evans 1989)

DATA ANALYSIS

Data obtained were subjected to Analysis of Variance (Anova), while Tukey's test was used in separating the means.

RESULTS

Effect of leaves extracts of the test plants on mortality of larvae of *An. gambiae* at 48 hours post-treatment.

Mortality of *An. gambiae* larvae treated with the leaf extract of *Chromolaena odorata, Vernonia amygdalina, Datura metel and and Acalypha wilkesiana* is presented in table 1. Mortality of the mosquito larvae varied with the plant species used as well as the dosage levels of the extracts. Extracts of *V. amygdalina* and *D. metel* achieved 100 % mortality within 48 hours of treatment with 5.0 mL leaf extracts and it is significantly ($P < 0.05$) higher than the mortality recorded with other plants extracts. Leaf extracts of *A. wilkesiana* and *C. odorata* achieved 82.25 % and 75.78 % larvae mortality respectively on exposure to 5.0 mL extract dosage level within 48 hours. All levels of dosages recorded showed significantly higher larvae mortality than the control experiment.

Table 1: Percentage mortality of larva of *An. gambiae* within 48 h of exposure to different dosages of leaves extracts of the test plants

	Dosage (mL) / percentage Mortality				
Plant material	1.0	2.0	3.0	4.0	5.0
C. odorata	24.64 ± 2.13^{c}	32.25 ± 2.26^{c}	44.15 ± 3.04^{d}	66.50 ± 4.18^{c}	75.78 ± 3.73^{c}
V. amygdalina	28.35 ± 2.14^{a}	37.52 ± 2.52^{a}	58.25 ± 2.33^{a}	87.27 ± 3.13^{a}	100.00 ± 0.00^{a}
D. metel	27.25 ± 1.12^{b}	34.25 ± 2.41^{b}	54.20 ± 3.81^{b}	83.15 ± 4.53^{b}	100.00 ± 0.00^{a}
A. wilkesiana	23.34 ± 2.08^{c}	29.50 ± 2.13^{d}	46.25 ± 2.74^{c}	67.27 ± 3.12^{c}	82.25 ± 3.43^{b}
Untreated	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}

Means in the same column followed by the same alphabet(s) are not significantly different at $p < 0.05$ using Tukey's test**.**

Effect of leaves extract of the test plants on the mortality of pupae of *An. gambiae* at 48 hours post-treatment.

Mortality of *An. gambiae* pupae treated with the leaves extracts of *Chromolaena odorata, Vernonia amygdalina, Datura metel and and Acalypha wilkesiana* is presented in table 2. Pupae mortality varied with the species of plant used as well as the dosage levels of the extracts. Leaf extract of *V. amygdalina* was able to achieve 100 % mortality within 48 hours of exposure to 5.0 mL extract dosage and it is significantly ($P < 0.05$) higher than the mortality recorded in other dosages of plant extracts. *D. metel* achieve 88.26 % pupa mortality when treated with 5.0 mL dosage level of extract during the 48 hours of exposure followed by A. wilkesiana (85.25 %) and the least was *C. odorata* (74.10 %). All levels of dosages recorded showed significantly higher pupae mortality than the control experiments.

Table 2: Percentage mortality of pupae of *An. gambiae* within 48 hours of exposure to different dosages of leaves extracts of the test plants

	Dosage (mL) / percentage Mortality				
Plant material	1.0	2.0	3.0	4.0	5.0
C. odorata	25.20 ± 2.21^{c}	37.25 ± 2.35^{d}	47.42 ± 2.52^{d}	63.25 ± 3.66^{d}	74.10 ± 3.67^{d}
V. amygdalina	35.18 ± 1.72^{a}	48.35 ± 2.13^{a}	67.25 ± 3.33^{a}	83.65 ± 4.18^{a}	100.00 ± 0.00^{a}
D. metel	28.25 ± 1.78^{b}	44.84 ± 2.43^{b}	58.29 ± 3.14^{b}	75.25 ± 4.17^{b}	88.26 ± 4.15^{b}
A. wilkesiana	26.35 ± 1.31^{c}	39.50 ± 3.427^{c}	53.30 ± 3.14^{c}	72.45 ± 4.12^{c}	85.25 ± 3.37^{c}
Untreated	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}

Means in the same column followed by the same alphabet (s) are not significantly different at $p < 0.05$ using Tukey's test.

Fumigant effect of extracts of the test plants on mortality of adult *An. gambiae*

The fumigant effect of extracts of *Chromolaena odorata, Vernonia amygdalina, Datura metel and and Acalypha wilkesiana* on mortality of adult *An. gambiae* within 4 hours of exposure is presented in Table 3. Insect mortality increased with increased dosage levels of the extracts and varied with the species of plants used. *An. gambiae* adults exposed to extract of all the test plants extracts showed significantly higher ($P < 0.05$) mortality values than the control experiments. Extract of *V. amygdalina* at dosage level of 5.0 mL was able to achieve 100 % adult mortality within 4 hours of exposure. *D. metel* leaf extract achieved 92.25 %, followed by *A. wilkesiana* (87.68 %) and *C. odorata* (82.20 %) adult mortality on exposure to 5.0 mL dosage level during the 4 hours post-treatment. All levels of dosages recorded showed significantly higher adult mortality than the control experiments.

Table 3: Fumigant effect of test plants leaves extracts on the mortality of Adult *An. gambiae* within 4 hours post-treatment.

	Dosage (mL) / percentage Mortality				
Plant material	1.0	2.0	3.0	4.0	5.0
C. odorata	35.50 ± 2.16^{c}	43.20 ± 2.41^{d}	58.47 ± 3.15^{d}	72.25 ± 3.41^{c}	82.20 ± 4.22^{d}
V. amygdalina	40.25 ± 2.33^{a}	66.54 ± 3.31^{a}	76.15 ± 4.41^{a}	88.25 ± 4.12^{a}	100.00 ± 0.00^{a}
D. metel	37.67 ± 1.73^{b}	58.15 ± 3.18^{b}	72.11 ± 3.29^{b}	78.63 ± 3.84^{b}	92.25 ± 4.15^{b}
A. wilkesiana	40.30 ± 2.07^{a}	52.50 ± 2.12^{c}	63.18 ± 2.74^{c}	71.25 ± 3.32^{c}	87.68 ± 3.15^{c}
Untreated	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}

Means in the same column followed by the same alphabet(s) are not significantly different at $p < 0.05$ using Tukey's test.

Phytochemical screening of ethanol extracts of leaves of test plants

The results of the qualitative phytochemical screening of the test plants revealed the presence of most phytochemicals tested for: *C. odorata* (saponins, tannins, flavonoid, glycosides and phenol), *V. amygdalina* (saponins, flavonoid, glycosides, phenol and alkaloids), *D. metel* (saponins, tannins flavonoid, glycosides, phenol and alkaloids), *A. wilkesiana* (tannins, flavonoid, glycosides and alkaloid) (Table 4).

Table 4: Qualitative phytochemical composition of the test plants

Phytochemicals	*C. odorata*	*V. amygdalina*	*Datura metel*	*A. wilkesiana*
Saponins	+	+	+	-
Tannins	+	-	+	+
Flavonoids	+	+	+	+
Glycoside	+	+	+	+
Phenols	+	+	+	-
Alkaloids	-	+	+	+

Key: + = Detected;

- = Not detected

Discussion

Conventional chemical insecticides play vital roles in insect vector control programs. However, due to environmental hazards, availability and the development of resistance to synthetic insecticides, there is a growing effort to explore plants to obtain bioactive compounds that are safe for non-target animals and which do not pose residue problems, but are still able to suppress insect vector populations. The present study evaluated the larvicidal, pupacidal and adulticidal activities of crude leaf ethanol extracts of *C. odorata, V. amygdalina, D. metel and and A. wilkesiana* against *An. gambiae*, an invasive malaria vector ravaging the world. The presence of some phytochemicals in the leaves of *C. odorata, V. amygdalina, D. metel and and A. wilkesiana* could have been responsible for the observed bioactivities against *An. gambiae* (Chore *et al*., 2014).

The results obtained from this work revealed that the extract of the leaves of the test plants possess the potential to control the developmental and adult stage of *An. gambiae.* The ability of these extracts to control the insects depends on the dosage level (Ghosh *et al*., 2012). Both the larva pupa and the adult stages were susceptible to the extract of the test plants. Moreover, the extracts of *V. amygdalina* was more potent on the larval and pupal stages of *An. gambiae* because it caused 100% mortality of these developmental stages when treated with 5.0 mL dosage level during 48 h of exposure, while in adult *A. gambiae,*100% mortality was recorded on exposure to 5.0 mL dosage level of the extract within 4 h. Ghosh *et al.*, (2012) reported that insecticidal

effects of plant extracts can vary due to the differences in plant species, mosquito species, geographical variation, extraction methodology, and polarity of solvents used during extraction which agrees with the findings of the current study.

The presence of secondary metabolites such as phenol, tannins, flavonoids, and glycosides claimed to have insecticidal activities have been identified in the extracts of the leaves in previous studies (Endalkachew and Halilu 2014; Tamiru *et al*., 2014). All these phytochemical have been reported to cause reduction in the growth and the larvae and pupa survival as well as disrupting the life cycle of the insects (Ileke *et al*., 2014). These phytochemicals could have contributed to the high effectiveness of the extracts of the test plants *against A. gambiae*. These plants have also been used as insecticides against stored beetles (Ileke *et al*., 2014; Obembe *et al*., 2017).

The high larvae and pupae mortality caused by the extracts of *V. amygdalina* and *D. metel* is similar to the result obtain by (Okumu, 2017) who obtained 95% larvae mortality on exposure to extract of *Azadirachta indica*. The 100% mortality recorded when the adult *An. gambiae* was exposed to 5.0 mL extracts of *V. amygdalina* is similar to the result obtain by (Okumu, 2017) who obtained 90 % adult mortality of *A. arabiensis* on exposure to ethanol leaf extract of *Ocimum lamiifolium.* The high mortality of the pupae and larvae may be due to the blockage of the respiratory apparatus by the extract thereby, preventing gaseous exchange and the consequent suffocation and death of the developmental stages (Akinkurolere *et al*., 2011; Obembe *et al*., 2024).

Also, Fafioye *et al*., (2004) reported that the ethanol extracts of *Parkia biglobosa* was more potent against the juveniles of *Clarias gariepinus* than the aqueous forms. This is due to the polarity, volatility and the ethanol's power to dissolve more of the active ingredients.

Although the statistical analysis revealed that the ethanol extraction is better in performance which does not mean that we cannot also use the aqueous form for such control. There is need to still investigate on the use of other volatile solvents in order to really discover the unknown properties of these plants. Invariably, botanical insecticides may serve as suitable alternatives to synthetic insecticides in future as they are relatively safe, degradable and are readily available in many areas of the world (Green *et al*., 1991).

Conclusion

In the present research, the extracts of *C. odorata*, *V. amygdalina*, *D. metel and and A. wilkesiana* have shown a great insecticidal potential against the larva, pupa and adult *An. gambiae*. Hence, they could be integrated into malaria vector strategies to replace the expensive and poisonous conventional chemical insecticides.

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