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Screening and Larvicidal Activity of the Ethanol Root and Leaf Exract of *Combretum molle* Against *Anopheles gambiae*

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ABSTRACT

Mosquitoes are of public health importance as they constitute serious biting nuisance and transmit most deadly and life-threatening diseases. Plants have become the focus of intense study in terms of conservation and their pharmacological use. This study aimed at evaluating the larvicidal activity of stem extract of *Combretum molle* on *Anopheles gambiae* mosquito. The phytochemical analysis of the ethanol extract of the stem revealed the presence of biological active compounds including alkaloid, saponin, tannin, steroid and flavonoid. The effect of the ethanol extracts of the stem of *Combretum molle* (Bush willow) against the larvae of *anopheles gambiae* mosquito was further investigated in accordance to the World Health Organization's guidelines for laboratory and field testing of mosquito larvicides (2005). Early second and third instars larvae of *anopheles gambiae* mosquitoes were exposed for up to 48 Hours to a concentration of 1, 25, 50 and 75ppm extracts of the roots and leaf. All tested extracts showed larval mortality, however, larval mortality was greatest with the ethanol leaf extract at an increasing concentration. This is supported by the abundance of flavonoid and tannin compound on the stem tissue. The study concluded that these parts of the *Combretum molle* contain larvicidal properties and could be implore as a natural larvicidal agent.

Keywords: Medicinal Plants, *Combretum molle, Anopheles gambiae,* Larvicidal.

INTRODUCTION

Mosquitoes are of public health importance as they constitute serious biting nuisance and transmit most deadly and life-threatening diseases. The brunt of these diseases is mostly felt in Africa due to the poor socio-economic conditions and large expanse of the aquatic habitats which provide conducive breeding sites for the mosquitoes (Monsuru *et al.,* 2013). The control of mosquito by chemical substance is not safe at present because of insecticide resistance by the vectors and environmental imbalance. Application of chemical or synthetic insecticides leads to deleterious effects in the long term, hence it does not provide absolute results. Mosquitoes in the larval stage are attractive targets for pesticides because they breed in water sources. However, introduces many risks to people and the environment and due to the continuous increase in their resistance to familiar synthetic insecticide, better alternative means are sought (Hag *et al.,*1999). Natural pesticides, especially those derived from plants are more promising in this aspect (Ameer and Mehlhorn, 2006). A considerable plant derivative has shown to be effective against mosquito larva with a safer manner.

Pesticides are compounds that are used to kill pests. They include compounds labeled as insecticides e.g., Organophosphates (OP), Organochlorides, Carbamates, Rodenticides (e.g., anticoagulants), Herbicides (e.g., paraquat, diquat, 2,4-dichlorophenoxyacetic acid (2,4-D), Fungicides (e.g., dithiocarbamates, captan), and Fumigants

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(e.g., ethylene dibromide, methyl bromide, phosphine). These toxic chemicals have become an integral part of the ecosystem, although many of them are extremely toxic to mammals and other non-target creatures. Phytochemicals (from Greek *phyto*, meaning "plant") are chemicals produced by plants through primary or secondary metabolism. They generally have biological activity in the plant host and play a role in plant growth or defense against competitors, pathogens, or predators. (Bresline and Andrew, 2017). Plants are being used as main source of therapeutics for the human beings since centuries till to date. Medicinal plants play a key role in the human health care. About 80% of the world population relies on the use of traditional medicine, which is predominantly based on plant material. Scientific studies available on a good number of medicinal plants indicate that

Phytochemicals are the non-nutritive secondary metabolites that have defensive or disease preventive properties (Tan *et al.,* 2010). They synthesize these chemicals to protect themselves, for example some plants are reported to produce phytoalexins in response to attack by bacteria and fungi (Hammerschmidt, 2011). Mosquitoes are of public health importance as they constitute serious biting nuisance and transmit most deadly and life-threatening diseases. The brunt of these diseases is mostly felt in Africa due to the poor socio-economic conditions and large expanse of the aquatic habitats which provide conducive breeding sites for the mosquitoes (Monsuru *et al.,* 2013).

Pesticides are substances that are used to control pests. They include; herbicides, insecticides, nematicides, fungicid es, and many others. Pesticides control, repel or kill their designated targets. These targets are usually are problem either in every day today life or in farms. Commonly used pesticides are herbicides to control or destroy weeds and other unwanted organisms (FAO, 1989). Control programs generally depend on the use of chemicals, Sprays, Impregnated Nets (ITNs) or Indoor Residual Spraying (IRS). Over the years, many insecticides have been introduced, but recent control methods are largely dependent on synthetic pyrethroids, which are the only WHO-recommended insecticides for ITNs (WHO, 2006).

The insects have defied several control measures deployed to control or regulate them, but the increase in insects that are resistant to pesticides has called for the need to search for more viable but environmentally friendly methods of controlling vectors of diseases (Monsuru *et al*., 2013). Continual use of conventional pesticides such as organophosphates, carbamate insecticides, insect growth regulators and bacterial larvicides has often resulted in the widespread development of resistance and has undesirable effects on non-target organisms (WHO, 2006; Rozendal, 1997).

MATERIALS AND METHODS

Sample Collection

Fresh stem sample of *Combretum molle* was collected from Mararaban Pindiga, Akko local government, Gombe state. The samples were then identified and authenticated at the Herbarium, Botany Department, Gombe state university.

Sample Preparation

The samples were collected in a wrapped in big brown envelopes and labeled. Only the fresh stem samples in good conditions were collected in order to produce good quality dried product (Audu and Lawal, 2005). The stem bark was air dried (25°C) and pounded by hand in a mortar. A quantity of 50g of the powder was subjected to maceration procedures using 250ml of ethanol. The plant

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and solvent mixture were placed on an orbital shaker (at 160 rpm) for 72 hours at room temperature. The extract was filtered and concentrated in a rotary evaporator at a temperature of 40^{o}C. The filtrate was concentrated to dryness on a water bath at 100^{0}C so as to obtain the dry extract after which was then finally stored in a freezer for further studies.

Mosquito Larva collection

The larval stage of mosquito was collected at Gadan Doma Brigde, Gombe state. Mosquito larval were picked using soup ladle dipper in the stagnant water. The larvae collected were taken to the laboratory in a container and were allowed to acclimatize, placing baking powder as their feed in the water bowl. All the samples collected were then identified according to Gillies and Coetzee (1987) before subjecting them to bioassay. The larvicidal bioassay test was carried out following procedures for insecticides resistance monitoring in mosquitos' vectors (WHO 2013) with slides modifications. The assay was performed by detecting the mortality and susceptibility of the larvae by direct observation of the larvae movement.

Stock Preparation and Experimental Design

1gram of each the extract was dissolved in 100ml of ethanol solvent to prepare the stock solution, the mixture was kept in a screw-cap vial, with vigorous shaking to enhance dissolution of the material in the solvent. A serial doubling dilution was used to obtain concentrations of 75, 50, 25 and 1ppm were prepared using the stock solution with a sterile distilled water.

Three replicates of twenty-five larva each, of the second and third instar were placed in transparent disposable plastic containers containing different concentrations of the treatment solution. After adding the larvae, the plastic containers containing the larva were kept in the laboratory and maintained at a room temperature. 20 samples of the larvae for each replicate were made using distilled water as control. The effects of the extracts were monitored by counting the number of dead larvae for up to 48 hours. No adult emergence was observed.

Qualitative Phytochemical Analysis

The qualitative phytochemical analysis of the plant extracts was performed using the standard methods described by Sofowora A. (2006) to determine the presence of Alkaloid, Saponin, Tannin, Flavonoid and Steroid.

RESULTS AND DISCUSSION

Table 1. Phytochemical screening of the root and leaf extract of *Combretum molle*

Phytochemical compound	Root	Leaf
Alkaloid	+	+
Saponin	-	+
Tannin	++	+++
Flavonoid	++	++
Steroid	-	-

Keys: Trace = + Moderate = ++ Excess = +++ Absent = -

Table 2: Mortality of *Anopheles gambiae* larva exposed to four concentrations of ethanol extract of the leaves of *Combretum Molle*

Microorganism	Mortality (Mean±SE)/ Time of exposure				
	Concentration	5hr	1hr	24hrs	48hrs
Anopheles gambiae	1ppm	-	-	9±1.0	13±1.0
Anopheles gambiae	25ppm		1±1.0	9±1.0	18±1.0
Anopheles gambiae	50ppm	2±1.0	7±1.0	18±1.0	25±1.0
Anopheles gambiae	75ppm	7±1.0	13±1.0	20±1.0	25±1.0
Control	Saline water	-	-	-	-

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Key = - Means no activity, Negative Control is distilled water.

Table 3. Mortality of *Anopheles gambiae* larva exposed to four concentrations of ethanol extract of the roots of *Combretum Molle*

Microorganism	Mortality (Mean±SE)/ Time of exposure				
	Concentration	5hr	1hr	24hrs	48hrs
Anopheles gambiae	1ppm	-	-	-	-
Anopheles gambiae	25ppm	-	-	4±1.0	11±1.0
Anopheles gambiae	50ppm	3±1.0	6±1.0	6±1.0	13±1.0
Anopheles gambiae	75ppm	4±1.0	11±1.0	14±1.0	17±1.0
Control	Saline water	-	-	-	-

Key = - Means no activity, Negative Control is distilled water.

Table 4: $LC_{50,}$ LC_{90} and P-values of ethanol extract of the leaves of *Combretum Molle*

Time	LC_{50} (%)	LC_{90} (%)	P-values
15 Minutes	22.86 ± 0.95	37.97 ± 2.02	0.00
30 Minutes	9.11 ± 0.67	19.71 ± 1.11	0.00
1 Hour	2.60 ± 1.11	10.57 ± 0.91	0.01
24 Hours	1.80 ± 3.38	2.76 ± 1.47	1.00

Source: Minitab

Table 5: $LC_{50,}$ LC_{90} and P-values of ethanol extract of the roots of *Combretum Molle*

Time	LC_{50} (%)	LC_{90} (%)	P-values
15 Minutes	55.53 ± 9.77	88.64 ± 18.52	0.00
30 Minutes	21.54 ± 2.01	56.99 ± 7.77	0.00
1 Hour	12.43 ± 7.60	31.42 ± 5.11	0.01
24 Hours	2.80 ± 1.34	3.76 ± 1.47	1.00

Source: Minitab

In this study, the stem extract of *C. molle* were found to be effective against the *anopheles gambiae* larva at various concentration rates. The root and leaf were found to be active; this is attributed to the higher concentration of flavonoid and tannin present in the extracts as seen in table 1. This is in line with the finding of Wizman *et al.,* 2006 who reported that flavonoid and tannin is known by its toxicity to harmful insects. The interaction of flavonoid and tannin molecules with the cuticle membrane of the larvae, ultimately disarranging this membrane by the association of the flavonoid and tannin (table 1) molecule with these membranes, could be the most probable reason for the larvae death.

A total of 250 larva of *Anopheles gambiae* mosquito were used for the study. The mortality increased as the concentration and period of exposure to the extracts increased. During the first hour of observation, the highest mortality was seen in the leaf extract, with of 13 larva (at concentration of 75ppm), followed by the root extract with mortality of 11 larva. After twenty-four hours of observation, the highest mortality was seen in again in the ethanol leaf extract, with mortality of 20 larva, followed by the root extract, with mortality of 14 larva. After forty-eight hours the all the larva administered leaf extract at 75ppm died while only seventeen died in that of the root extract as shown in table 2 and 3.

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Table 4 provides the result of lethal concentration that is needed to kill at least 50 percent and 90 percent of mosquito larva exposed to ethanol extract of the leaves of *Combretum Molle* and P values according to time of exposure, at 15 minutes, 30 minutes, 1 hour and 24 hours, The minimum concentration in percentage that is needed to kill at least 50 percent of the larva is 22.86 ± 0.95, 9.11 ± 0.67, 2.60 ± 1.11 and 1.80 ± 3.38 respectively, LC_{50} decreases with increase in time, the same trend exist in the minimum concentration needed to kill at least 90 percent of the larva, at 15 minutes, 30 minutes, 1 hour and 24 hours the lethal concentrations are 37.97 ± 2.02, 19.71 ± 1.11, 10.57 ± 0.91 and 2.76 ± 1.47, from the table LC_{90} values across the time is greater than the LC_{50} values. The result for 15 minutes, 30 minutes and 1 hour shows that there is a significant difference ($p< 0.05$) between the mortality and concentration across the time, at 24 hours that there is no significance difference ($p> 0.05$) between mortality of the larva and concentration.

Table 5 presents the result of lethal concentration that is needed to kill at least 50 percent and 90 percent of larva exposed to ethanol extract of the roots of *Combretum Molle* and P values in relation to time of exposure, at 15 minutes, 30 minutes, 1 hour and 24 hours, The minimum concentration in percentage needed to kill at least 50 percent of the larva is 55.53 ± 9.77, 21.54 ± 2.01, 12.43 ± 7.60 and 2.80 ± 1.34, the LC_{50} values increases as the time increases, the same trend exist in LC_{90} values, at 15 minutes, 30 minutes, 1 hour and 24 hours, The minimum concentration in ppm needed to kill at least 90 percent of the larva is 88.64 ± 18.52, 56.99 ± 7.77, 31.42 ± 5.11 and 3.76 ± 1.47, from the table, LC_{90} values across the time is greater than the LC_{50} values. The result for 15 minutes, 30 minutes and 1 hour shows that there is a significant difference ($p< 0.05$) between the mortality and concentration across the time, and at 24 hours there is no significance difference ($p> 0.05$) between mortality of the larva and concentration.

The third instar larvae of *anopheles gambie* complex were exposed to different concentrations of the leaf and root extracts of *Combretum molle* and their efficacies were evaluated in the laboratory. In this study, the ethanol leaf extract was the most effective larvicide, followed by the ethanol root extract. Similar work is that of Siti *et al*. (2018) that investigated the Susceptibility of third instar larvae of *Culex quinquefasciatus* against *Murraya koenigii* leaves extracts. They also observed high activity of the leaf extract at concentrations of 250 ppm). Analogous results have been obtained previously by El-Kamali (2001) where the 'LC_{50} value of was reported at higher concentrations of 490 ppm. However, the LC_{50} of the leaf extract observed in our study was greater than that reported by El-Kamali (2001). This variation can probably be attributed to different plants and concentration used. Further, the sensitivity of the larva could also play an important role in the difference observed. The WHO Global report on insecticide resistance in malaria vectors: 2010–2016 showed that resistance to the 4 commonly used insecticide classes – pyrethroids, organochlorines, carbamates and organophosphates – is widespread in all major malaria vectors across the WHO regions of Africa, the Americas, South-East Asia, the Eastern Mediterranean and the Western Pacific.

The lethal concentration needed to kill at least 50 percent of the larva exposed to ethanol leaf extract exposure at 24 hours, was 36%. A similar report is that of Fahd *et al.,* (2018) tested plant extracts from *Solenostemma argel* against larvae of Culex pipiens in the laboratory against 4th instar larvae of *Culex*

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quinquefasciatus (Diptera: Culicidae) under laboratory condition. A chloroform leaf extract of *S. argel* exhibited relatively high activity with a lethal concentration causing 50% mortality (LC_{50}) of 15.89 ppm, while chloroform and ethyl acetate extracts of *S. argel* fruits were 19.70 and 19.52 ppm, respectively.

El Ouali *et al*. (2016) evaluated the larvicidal properties of *Salvia officinalis* has remarkable larvicidal properties. The LC50 and LC90 lethal concentration measured for the extracts of *Salvia officinalis* appears to be effective with respective values of about 287 ppm and 487 ppm.

As shown by table5, lethal concentration decreases across time from higher at 15 minutes to lower at 24 hours, this indicates that mortality of the larva depends on the concentration of the chemical and time of exposure. The higher the time of exposure, the lower the concentration of the chemical. Similarly, the P values increase as the time increase from 0.00 to 1.00, this indicates that there is a statistically significance difference in the level of mortality at the different concentration, mortality increases with increase in concentration at 15 minutes, 30 minutes and 1 hour, whereas at 24 hours there is no statistically significance difference between the mortality and across the concentrations after 24 hours.

CONCLUSION

The results of the testing of the ethanol root and leaf extract of *Combretum molle* showed that they were all very effective against the larva of *anopheles gambiae* complex. The result also showed that the leaf extract proved to be the most effective extract, followed by the root extract respectively. For the result of the percentage mortality, ethanol leaf extract had the best performance with a mortality of 7 at concentration 75 ppm after 30 minutes of administration. There was 100% mortality observed for the leaf extract tested at 50 and 75 ppm concentrations after 48 hours.

The lethal concentrations that are needed to kill at least 50 percent of larva exposed to the leaf extract according to time of exposure, at 30 minutes, 1 hour and 24 hours is 22.8, 9.1 and 1.8 respectively, and that of 90 percent is 37.1, 19.2 and 2.7 respectively. lethal concentration that is needed to kill at least 50 percent of larva exposed to the root extract according to time of exposure, at 30 minutes, 1 hour and 24 hours is 55, 21 and 12, and that of 90 percent is 88, 56, and 31 respectively.

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