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Research Article

Larvicidal Activities of the Extract and fractions of *Paullinia pinnata* Linn leaf

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ABSTRACT: **Background and objectives:** The use of plants and their extracts in vector control for malaria has received recent attention. *Paullinia pinnata* leaf is used in folklore medicine for the treatment of fever and was investigated in this study for its larvicidal activity. **Materials and methods:** Effects of the methanol extract, pet. ether and chloroform fractions of *P. pinnata* leaf was been investigated against the third and fourth instar larvae of *Anopheles gambiae* by adopting the WHO method with little modification. **Result:** All the tested extract and fractions exhibited significant larvicidal effects against *A. gambiae* larvae. The pet. ether displayed the strongest response after 24 hr exposure, while the methanol extract exhibited a dose-dependent effect 48 hr after exposure. The Phytochemical analysis of *P. pinnata* revealed the presence of alkaloids, tannins, saponins. **Discussion and Conclusion:** This study indicates that *P. pinnata* leaf possesses larvicidal activity against *Anopheles gambiae* and may be a possible source for control of mosquito vectors.

KEYWORDS: *Paullinia pinnata,* larvicidal activity, *Anopheles gambiae*, malaria

INTRODUCTION

Mosquitoes are vectors responsible for the transmission of various diseases[1] such as malaria, filariasis, yellow fever, dengue fever and other infections.[2,3] More than 700 million people annually suffer from malaria.[4,5]

The effective control of the vectors, in both the aquatic and adult stages, is important in controlling malaria. The use of chemical insecticides is currently the method of control adopted for eliminating adult mosquitoes although this is considered to have adverse effect on the environment, toxicity, as well as enhancing resistance of the mosquito population to the insecticide[6,7] and causing undesirable effects on non-target organisms.[4,8]

There is an increased interest in the search for insecticides from natural sources as they are considered safer, more cost effective, biodegradable and are target specific against mosquitoes.[9,2] Several studies have identified and reported plants and plant extracts effective against mosquitoes at various stages of development.[10-18]

Paullinia pinnata Linn (Sapindaceae) commonly known as Sweet gum, Hannu biyar (Hausa)[19] and Itakun- okere (Yoruba) is native to Cuba, Central America, South America and Tropical Africa.[20] It is a climbing shrub characterised by serrated leaflets with prominent veins, winged rachis and petiole. The flowers are white and fruits red or dark pink when ripe.[20] In traditional medicine, various parts of *P. pinnata* are used for treating various diseases. ln South West Nigeria, the juice of the leaf of *P. pinnata* is used for the treatment of sore throat and black coated tongue, an infusion is used for fever while the roots are used for the treatment of leprosy, jaundice, snake bites,[21] nausea and vomiting.[22]

Previous studies have reported the wound healing,[23] the antivenom,[24] and inhibitory effects against several infectious organisms,[25] as well as the effect of the leaf ethanolic extract on malaria infections in rats infected with *Plasmodium berghei berghei*.[26]

This study aims to investigate the effect of the methanol extract, pet ether and chloroform fractions of *P. pinnata* leaf on the third and fourth instars larvae of *Anopheles gambiae*.

MATERIALS AND METHODS

Plant Collection and Authentication

The leaves of *P. pinnata* were collected from Sagamu, Ogun State and authenticated at the Forestry Research Institute of Nigeria, Ibadan where voucher specimen (voucher number FHI 108892) was deposited.

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Extraction

The air dried powder of P. *pinnata* leaves were macerated in methanol for three days, filtered and dried under reduced pressure in a rotary evaporator (Gallenkamp, UK). The dried extract was re-constituted in distilled water and successively partitioned with petroleum ether and chloroform to yield the pet. ether and chloroform fractions.

Phytochemical Screening

The Phytochemical screening was carried out using standard procedure[27]

Larvicidal activity

Larvae of A. *gambiae* were collected from stagnant water in Ojoo district of Ibadan, Oyo state, Nigeria. Collected larvae were washed in clean well water, stored in several plastic bowls and fed with commercial biscuits (Sumal Foods, Ibadan. Nigeria).

The effects of five doses (62.5 µg/ml, 125 µg/ml, 250 µg/ml, 500 µg/ml, and 1000 µg/ml) of the extract and fractions were tested for their larvicidal activities.

The larvicidal bioassay used in this study followed the WHO standard protocols[28] with little modification. Five doses of the crude methanol extract, pet ether and chloroform fractions were prepared in 1% ethanol and transferred into sterile glass petri dishes. Twenty third and fourth instars larvae of *Anopheles gambiae* were introduced into the petri dishes containing appropriate concentrations. Mortality rates were recorded after 24 hr, 48 hr exposure and the larvae were considered dead when they failed to move after probing with needle. The experiment was carried out in triplicates at 25 °C and 80% relative humidity. 1% ethanol was used as control.

Statistics

The results are presented as Percentage mortality (PM) ± S.D. The Percentage mortality (PM) was determined using the equation:

$$\%PM = \frac{\text{Death count of larvae}}{\text{Initial larvae population}} \times 100$$

RESULTS

Phytochemical screening of the crude extract of *Paullinia pinnata* leaf revealed the presence of saponin, tannin, alkaloids (Table 1). Larvicidal properties were exhibited by the methanol crude extract, petroleum ether and chloroform fractions of the leaf against the third and fourth instar larvae of *Anopheles gambiae* (Table 2).

The pet. ether fraction was the most effective and exhibited the highest mortality rate of 100% at 125 µg/ml; 500 µg/ml and 1000 µg/ml; 87.5% at 62.5 µg/ml while the chloroform fraction exhibited mortality rate of 22.5, 7.5% 1000, 500 after 24 h exposure after 24 h exposure.

After 48 h exposure,(Table 3) the methanol crude extract exhibited a dose-dependent larvicidal activity of 92.5%, 67%, 65% and 25% mortality rate at a dose of 1000 µg/ml, 500 µg/ml, 250 µg/ml and 125 µg/ml respectively.

No mortality was induced in the control (1% ethanol) on the larvae.

Table 1: Phytochemical analysis of *Paullinia pinnata* leaf extract

Alkaloids	Saponin	Cardiac glycosides	Tannins	Anthracene derivative	
				Free	combined
++	++	–	++	– –	– –

++: Present in large amount +: Present –: Absent

Table 2: Effect of the methanol crude extract, Pet. ether fraction and Chloroform fractions of *Paullinia pinnata* on the third and fourth instar larvae *Anopheles gambiae* after 24 h exposure

Dose (µg/ml)	% Mortality ± S.D		
	Methanol Crude extract	Pet. ether fraction	Chloroform fraction
1000	17.5 ± 0.71	100 ± 0.00	22.5 ± 0.71
500	0 ± 0.00	100 ± 0.00	7.5 ± 0.71
250	0 ± 0.00	100 ± 0.00	7.5 ± .1
125	0 ± 0.00	100 ± 0.00	0 ± 0.00
62.5	0 ± 0.00	87.5 ± 0.71	0 ± 0.00
Control	0 ± 0.00	0 ± 0.00	0 ± 0.00

Results are the mean of three determinations ± S.D. 1% Ethanol was used as negative control.

Table 3: Effect of the methanol crude extract, Pet. ether fraction and Chloroform fractions of *Paullinia pinnata* on the third and fourth instar larvae *Anopheles gambiae* after 48 h exposure

Dose (µg/ml)	% Mortality ± S.D		
	Methanol Crude extract	Pet. ether fraction	Chloroform fraction
1000	92.5 ± 0.71	–	77.5 ± 3.54
500	67.5 ± 0.71	–	75.0 ± 0.00
250	65.0 ± 1.41	–	5.0 ± 0.00
125	25.0 ± 2.83	–	0 ± 0.00
62.5	7.5 ± 0.71	–	0 ± 0.00
Control	0 ± 0.00	0 ± 0.00	0 ± 0.00

Results are the mean of three determinations ± S.D. 1% Ethanol was used as negative control.

DISCUSSION

The adoption of larvae and adult control of the disease causing vectors of malaria could be a more effective and necessary in the management of malaria. This is because during the immature stages, mosquitoes are relatively immobile, remaining in more concentrated area than they are in adults.[29]

There is continued interest in plants and plant extracts which are effective as control against mosquitoes at various stages of development and these have led to the isolation of various active compounds including azadiractins, plumbagin, β-sitosterol and others which can exert toxic activity against mosquito species.[2,12-17, 30-35]

In this study, the methanol extract of *P. pinnata* leaf exhibited a dose-dependent activity which is similar to other studies which have also reported dose-dependency of plant against mosquito larvae.[36-38] The pet. ether fraction of the leaf was the most effective and exhibited the highest mortality rate of 100% of all the tested extract and fractions. The least activity was exhibited by the chloroform fraction with a mortality rate of 22.5, 7.5% at 1000 µg/ml, 500 µg/ml after 24 hr exposure; 77.5%, 75% at the same dose after 48 hr exposure.. This result revealed that the non-polar and polar compound(s) present in the pet. ether and chloroform fractions of *P. pinnata* leaf possess larvicidal activities and it could therefore be suggested that the non-polar compounds are mainly responsible for this bioactivity exhibited by the extract and fractions of *P. pinnata* leaf.

There was however no difference in the mortality rate exhibited by both the methanol crude extract and chloroform fraction 48 hr after exposure.

In this study, the active compound(s) in the methanol extract, chloroform and pet ether fractions of *P. pinnata* leaf might not possess the same or similar compounds. Whilst the anti-larvicidal mechanism of the *P. pinnata* leaf extract was not examined in this study, a previous study has reported that plant extracts and pure compounds may manifest their effects on insects in several ways: suppressing reproductive behaviour, toxicity, mortality, antifeedancy, growth inhibition, reduction of fecundicity and reduced fertility.[39]

The mechanism of action exhibited by *P. pinnata* leaf may therefore possibly be due to its toxic effects on the larvae. This is supported by a previous study which reported that an ethanol extract of *P. pinnata* exhibited toxic effects in the Swiss albino mice.[26] Other studies have also reported that plants that contain larvicidal agents may act in combination or independency.[40-42]

This study reports that the methanol extract, pet ether and chloroform fractions of *P. pinnata* have larvicidal activities.

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