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EVALUATION OF LARVICIDAL ACTIVITIES OF STEM BARK AND SEED EXTRACTS OF *PICRILIMA NITIDA* (STAPF.) T. DURAND AND H. DURAND (APOCYNACEAE) ON *AEDES AEGYPTI* LARVAE

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ABSTRACT

This work evaluates the larvicidal activity of Picralima nitida stem bark and seed extracts on Aedes aegypti larva, a mosquito species which is a vector for yellow fever, dengue fever, zika, and, chikungunya viruses. The acetone and methanol extracts were obtained by maceration method after 48hours respectively, A stock solution of 10mg/ml was prepared for each extract from which six different concentrations (0.5, 1.0, 2.0, 3.0, 4.0, &5mg/ml) were made and used for the test in accordance with World Health Organization (WHO) guideline, with slight modification. A control was also prepared for each extract by adding 1ml of the solvent to 100ml of water. A total of 20 larvae were used for each of the concentration prepared in triplicate. Mortality was recorded at 24, 48 and 72 hours. After 72 hours, the LC_{50} of methanol and acetone, seed extracts were 1.5238 and 2.5524mg/ml respectively, while the LC_{50} of methanol and acetone stem bark extracts were 102.6714 and 71.9657mg/ml respectively. The result shows that methanol and acetone extracts of the seed of Picralima nitida are more active than stem bark extracts, and could be useful source of Larvicide.

KEY WORDS

piralima nitida, Aedes aegypti, larvicide, larvae.

INTRODUCTION

Mosquito has been described by WHO as one of the most dangerous animal, [1] this is because of its ability to carry and transmit dangerous parasites that causes disease and sickness in man. Thus, mosquito borne diseases constitute a major health challenges all over the world more especially in third world tropical countries of Africa. Their ability to carry and spread diseases to humans causes millions of deaths every year. Annually, millions of people and man-hours with attendant economic implications are lost to this pandemic. Different genera of mosquito are known to transmit different diseases. The genus of interest in this study is the *Aedes* and the species is *Aedes aegypti*. The diseases transmitted by *Aedes aegypti* include yellow fever, dengue fever, zika virus fever and chikungunya. Many types of methods have been used to deal with the vectors transmitting these diseases. These have suffered certain limitations. This limitation justified the need to search for environmentally safe, degradable, affordable and target-specific compounds against these insect-vectors. The search for such compounds has been directed to the plant kingdom.[2]

Before the discovery of synthetic insecticides, natural insecticides such as pyrethrum, rotenone, nicotine, sabadilla, ryania, among others, have been extensively used for insect control.[3] Searching for new control agents from natural products such as plant secondary metabolites have gained popularity among researchers in countries with a strong herbal tradition, and large numbers of plants have been reported to possess insecticidal activity.[4]

Larvicides are insecticides that are specifically targeted against the larval life stage of an insect. Their most common use is against mosquitoes. Larviciding is a preferred option in mosquito control because the larvae occur in specific areas and can thus be more easily controlled.

Picralima nitida (Apocynaceae) with common name Akuamma is a medicinal plant which is use in herbal medicine for the treatment of various ailments. Many herbalists have claimed to use the leaves, roots, seeds or stem bark for the treatment fevers, hypertension, jaundice, gastrointestinal disorders and malaria.[5] The larvacidal properties of the leaf, pulp and seed extracts of the plant on *Anopheles gambiae* species of mosquito have been investigated by Ubulom et al, [6] and Nwabor et al [7] respectively with significant result.

The aim of this work is to investigate the larvicidal activities of the stem bark and seed extracts of *Picralima nitida* on another genus of mosquito, the *Aedes* and particularly on the species *Aedes aegpyti*

MATERIALS AND METHODS

Materials: some of the materials use includes; rotary evaporator (Labsicience England), Water bath (Techmel and Techmel USA) Heating mantle (Labsience, England) pestle and mortar. Chemicals: methanol (JHD China), acetone (Loba Chemie, India), maceration bottle containers, etc.

Collection of Plant Materials

Plant samples (stem bark and seeds) of *Picralima nitida* was collected from Awomamma community in Oru East local government of Imo state, Nigeria. The plant was identified by Dr. Oladele of Department of Forestry, University of Port Harcourt. It was deposited at the Department of Pharmacognosy herbarium, University of Port Harcourt with the voucher number UPHA0293

Sample Preparation The stem bark and the seed collected was spread on a clean surface and allowed enough time to air-dry under shade at the normal environmental temperature. The dried plant sample was then pulverized using clean mortar and pestle. The pulverized sample was then used for extraction.

Extraction

The extraction was done by maceration method. The solvents used for both the stem bark and seed are methanol and acetone. 180mg of stem bark and 150mg of seed samples were used for each of the solvents. The maceration was allowed for 48hour with intermittent shaking. After 48hour they were filtered and concentrated with rotary evaporator and finally dried in the water bath at temperature of about 50°C. The extracts obtained were transferred to air tight containers and stored in desiccators until the time of use. The percentage yields of the extracts were determined.

Phytochemical Screening

Phytochemical tests were carried out on the pulverized sample using standard phytochemical screening method according to Harborne, J.B,[8] Sofowora[9] and Trease and Evans.[10] The phytochemuicals that were screened for includes; saponins, Tannins, phlobatannins, flavonoids, anthraquinones, cardiac glycosides, terpenes, carbohydrates alkaloids and steroids

Test organism

Third instar larvae of *Aedes aegypti* used in this investigation were provided by National Arbovirus and Vectors Research Centre (NAVRC), Enugu, Nigeria.

Larvicidal Bioassay

The larvicidal tests were carried out against 3rd instar larvae of *Aede* saegypti in accordance with WHO guidelines for larvicidal testing, [11] with slight modification. Stock solution of 10mg/ml was prepared. From the stock solution, different concentrations (0.5, 1.0, 2.0, 3.0, 4.0, & 5.0mg/ml in 100ml volume) were made for each extract. A control was also prepared for each extract by adding 1ml of the solvent used for extraction to 100ml of water. The tests were conducted in plastic containers of 100ml. Three replicates and a control were run simultaneously for each concentration, and a total of 20 healthy larvae were used for each container. The tests were carried out at room temperature (28± 20c). Mortality was observed at 24, 48 and 72 hours. Larvicidal activity of each extract was determined by counting the number of dead larvae on daily basis (24 hours interval). Dead larvae were recorded when they failed to move after probing with a needle.

Statistical Analysis

The LC50 and LC90 of each of the extracts were calculated using standard method of probits by finney, [12] and Ldp line software by Ehab, [13]

RESULT AND DISCUSSION

Result

The yield value of the methanol and acetone seed extracts of *Picralima nitida* are 17.1% and 5.13% respectively and that of stem bark of *Picralima nitida* are 9.88% and 3.5% respectively.

The phytochemical detected from seed of the plant extract are; alkaloids, tannins, phlobatannins, cardiac glycosides, carbohydrates, terpenes and steroids, while that of the stem bark are alkaloids, flavonoids, saponin, tannins, terpenes, steroids and cardiac glycosides.

From table 1 below, the result of the activity of the methanol seed extract showed that after 24 hours the LC_{50} of the extract is 1.8621mg/ml while the LC_{90} is 15.7789mg/ml. after 48 hours the LC_{50} and LC_{90} became 1.713mg/ml and 16.0563mg/ml respectively with little difference from that of the first 24hours, but after 72 hours the LC_{50} and LC_{90} reduced to 1.5238mg/ml and 13.7326mg/ml respectively.

Table 1: Larvicidal activity of methanol seed extract of *Picralima nitida* on *Aedes aegypti.* Average of 20 larvae per concentration.

	After 24 hours			After 48 hours			After 72 hours		
Conc mg/ml	% mortality	LC_{50} mg/ml	LC_{90} mg/ml	% mortality	LC_{50} mg/ml	LC_{90} mg/ml	% mortality	LC_{50} mg/ml	LC_{90} mg/ml
0.5	20	1.86	15.78	20			20		
1	40	Lower	Lower	45	Lower	Lower	50	Lower	Lower
2	45	Limit	Limit	50	Limit	Limit	55	Limit	Limit
3	65	1.15	7.40	65	0.99	7.30	65	0.85	6.58
4	70			70			70		
5	70	Upper Limit 2.85	Upper Limit 134.37	70	Upper Limit 2.64	Upper Limit 166.14	75	Upper Limit 2.30	Upper Limit 113.42

Table 2 showed the activity of acetone seed extract. The LC_{50} and the LC_{90} are respectively 12.916mg/ml and 259.6926mg/ml after 24 hours, while after 48 hours the LC_{50} and LC_{90} became 10.1541mg/ml and 337.1818mg/ml. however, after 72 hours, the LC_{50} and LC_{90} reduced to 2.5524mg/ml and 23.2076mg/ml respectively with highest activity, less than that of methanol seed extract after 72hours.

Table 2: Larvicidal activity of acetone seed extract of *Picralima nitida* on *Aedes aegypti.* Average of 20 larvae per concentration

	After 24 hours			After 48 hours			After 72 hours		
Conc mg/ml	% mortality	LC_{50} mg/ml	LC_{90} mg/ml	% mortality	LC_{50} mg/ml	LC_{90} mg/ml	% mortality	LC_{50} mg/ml	LC_{90} mg/ml
0.5	5	12.916	259.6926	15	10.15	337.18	15	2.55	23.21
1	20			20			35	Lower	Lower
2	20			25			45	Limit	Limit
3	25			30			45	1.6742	9.539
4	30			35			60		
5	35			45			70	Upper Limit 4.48	Upper Limit 357.6

The activity of the stem bark methanol extract is shown in table 3 with low Larvicidal action. The LC_{50} and LC_{90} after 24 hours and 48 hours were not defined because of almost zero mortality, but after 72 hours, the LC_{50} and LC_{90} were 102.6714mg/ml and 5441.5668mg/ml which signified low activity.

Table 3: Larvicidal activity of methanol stem bark extract of *Picralima nitida* on *Aedes aegypti.* Average of 20 larvae per concentration

	After 24 hours			After 48 hours			After 72 hours		
Conc mg/ml	% mortality	LC_{50} mg/ml	LC_{90} mg/ml	% mortality	LC_{50} mg/ml	LC_{90} mg/ml	% Mortality	LC_{50} mg/ml	LC_{90} mg/ml
0.5	0			0			5	102.67	5441.57
1	0			0			5		
2	0			0			10		
3	0			0			15		
4	0			5			15		
5	0			10			15		

Table 4 showed the result of the activity of stem bark acetone extract. The result showed that acetone bark extract has more activity than that of methanol, even though on its own it has low activity as a lavicidal agent. The LC_{50} and LC_{90} after 24 hours are 75.0132mg/ml and 233.3835mg/ml respectively while after 48 hours they became 34.389mg/ml and 523.0789mg/ml respectively. However, after 72 hours the LC_{50} and LC_{90} became 71.9657mg/ml and 7517.1591mg/ml respectively. This is unexpected, but because of the same percentage mortality value for the last four concentrations it affected the LC_{50} and LC_{90} values in unexpected Way.

Table 4: Larvicidal activity of acetone stem bark extract of *Picralima nitida* on *Aedes aegypti.* Average of 20 larvae per concentration

	After 24 hours			After 48 hours			After 72 hours		
Conc mg/ml	% mortality	LC_{50} mg/ml	LC_{90} mg/ml	% mortality	LC_{50} mg/ml	LC_{90} mg/ml	% mortality	LC_{50} mg/ml	LC_{90} mg/ml
0.5	**0**	**75.01**	**2333.38**	**0**	**34.39**	**523.08**	**5**	**71.97**	**7517.16**
1	**5**			**5**			**15**		
2	**10**			**10**			**20**		
3	**10**			**10**			**20**		
4	**15**			**15**			**20**		
5	**15**			**20**			**20**		

Discussion

It has been shown that the plant kingdom is a reliable reservoir of strong phytochemicals which can be source of suitable, efficient, readily available and eco-friendly alternative in the fight against insect pest, like mosquito which is a vector responsible for the transmission of many disease-causing parasites. For instance, Roark describe about 1,200 plant species having potential insecticidal value.[14]

Based on this search for agents with excellent activity against insect pest, this work evaluates the larvicidal activities of crude acetone and methanol seed and stem bark extracts of *Picralima nitida* on the yellow fever, Zika virus fever, dengue fever and chikungunya diseases vector, Aedes aegypti.

From the result, the percentage yield of the extracts is; for seed 17.1% and 5.13% for methanol and acetone solvents respectively and for stem bark 9.88% and 3.5% for methanol and acetone solvents respectively. Methanol had the highest percentage yield. This could be as a result of the polarity index of methanol which confers on it the ability to extract both the polar and non-polar constituents of the sample. Acetone, been very less polar solvent extracted less Percentage of the extracts. This is because acetone extracts mostly the non-polar constituents of the sample

The phytochemical constituents of the seed of the P. nitida include alkaloids, tannins, phlobatannins, cardiac glycosides, carbohydrates, terpenes and steroids, while that of the stem bark are alkaloids, flavonoids, saponin, tannins, terpenes, steroids and cardiac glycosides. The larvicidal activities of seed extract may be as a result of

the action of one or more of these phytochemicals, which can be ascertain after proper isolation and characterization of the constituents of the extracts.

The result of this investigation showed that the methanol seed extracts exhibits significant activity from the concentration of 0.5mg/ml, which gave 20% mortality after 72 hours, on increasing the concentration to 1mg/ml, the percentage mortality increase to 55 after 72 hours. The highest percentage mortality of 75 occurs at the concentration of 5mg/ml after 72 hours. Examination of the results also showed that the percentage mortality increases with increase in concentration, this collaborate with the work by Dibua et al[15] and Nwabor et al on the larvicidal activity of the seed extract on *Anopheles gambea* species of mosquito. On the other hand, the activity gave little time dependent.

The acetone seed extract also gave larvicidal activity, with the highest concentration of 5mg/ml giving the highest percentage mortality of 70 after 72 hours. In this case the activity is both concentration and time dependent. The LC50 and LC90 also gave the same trends which are concentration and time dependent. This also collaborates with the activity of the larvicidal plant as reported by Ubulom et al, [6].

Examination of the activity of the stem bark extract showed low activity of both the methanol and acetone extracts when compare with that of seed extracts on the *A. aegypti* species of the mosquito larvae. However, the acetone extract gave better activity than that of the methanol. The acetone extract was able to give up to 20% mortality after 72 hours by 5mg/ml concentration. The LC50 and LC90 also gave high value indicating low larvicidal activity. On the other hand, the highest activity of 15% mortality of the methanol stem bark extract occur after 72 hour at the concentration of 5mg/ml. the values of the LC50 and the LC90 are also on the high side indicating low larvicidal action.

Though works have been done on parts of this plants like the larvicidal potentials of the leaf Ubulom et al [6] and pulp Nwbor et al [7] etc, not much has been done on the stem bark. However, evaluation of this show low activity of the stem bark on *Aedes aegypti* species while the seed extract showed good larvicidal activity.

CONCLUSION

The result obtained from this study showed that methanol and acetone seed extracts are more active than the stem bark extracts of *Picralima nitida* and could be develop and used as insecticide to combat *Aedes aegypti* which is the vector of the deadly yellow fever, Zika and dengue fever viruses as well as the chikungunya desease. It could also serve as an alternative to the synthetic insecticides.

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