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Full Length Research Paper

Pulp extracts of *Picralima nitida*: A larvicidal agent in malaria vector control

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The methanolic and aqueous pulp extracts of *Picralima nitida* as an eco-friendly alternative in malaria vector control were investigated. The sample yielded a 3.93% extract with methanol and 0.89% with aqueous. The phytochemicals constitutes of crude methanolic and aqueous extracts of *P. nitida* pulp includes the alkaloids, cardiac glycosides, saponins, tannins, flavonoids, terpenes and steroids. Laboratory reared larvae of *Anopheles gambiae* 4th instar were exposed to varying concentrations of the *P. nitida* pulp extracts ranging from 0.5 to 5.0mg/ml, according to WHO Bioassay Methods for susceptibility. Probit analysis using statistical package for social sciences version 16.0, at (P<0.05) gave time dependent LC_{50} and LC_{95} values of 4.979 mg/ml and 18.541mg/ml, 4.299 mg/ml and 16.008 mg/ml and 2.792 mg/ml and 10.397 mg/ml for aqueous pulp extracts at 24, 48 and 72 hours while methanolic extract values were 12.285 and 96.993 mg/ml, 9.700 and 76.584 mg/ml and 6.490 and 51.236 mg/ml at 24h, 48h and 72 h respectively. The Relative Potency estimate revealed that methanolic extract has 1.525, 1.554 and 1.880 times the potency of aqueous extract at 24 h, 48 h and 72 h respectively.

Keywords: *Picralima nitida,* eco-friendly, insecticides, resistance, larvicidal.

INTRODUCTION

Picralima nitida belongs to the family *Apocynaceae* (Meyer *et al.,* 2006). Extracts of the plant have been used in the treatment of pathogenic diseases (Ubulom *et al.,* 2011), protozoan infections (Okokon *et al.,* 2007) and non pathogenic diseases (Kouitcheu *et al.,* 2006). Diabetes mellitus is a major endocrine disease that is treated with the extracts of the plant (Inya-Agha *et al.,* 2006). The Larvicidal and Antifungi properties of leaf samples of *P. nitida* has been investigated by Ubuloma *et al*., 2012, and was reported to show a significant larvicidal effect on *Anopheles gambiae*. Larvicidal is a general term for killing immature insects by applying agents, collectively called larvicides, to control larvae and/or pupae stages of these insects (Flourida Mosquito Control, 2009).

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Larvicidal approach is a more proactive, proenvironment, target specific and safer approach than controlling adult mosquitoes. Application of larvicide from botanical origin was extensively studied as an essential part of IMM, and various mosquito control agents such as ocimenone, rotenone, capllin, quassin, thymol, eugenol, neolignans, arborine and goniothalamin were developed (Shaalan *et al.,* 2005). Larval control is the foundation of most mosquito control programs. Whereas adult mosquitoes are widespread in the environment, larvae must have water to develop; control efforts therefore can be focused on such breeding sites (Dibua *et al.*, 2013). Minimizing the number of adults that emerge is crucial to reducing the incidence and risk of disease.

Development of resistance to readily available insecticide and simultaneous environmental problems posed by synthetic inorganic insecticides necessitated the 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 (Mathur, 2003). Phyto-products, on account of their minimal hazardous effect on the environment and wide range of availability offer promise in future mosquito control programs. Phytochemicals with insecticidal and larvicidal activities are now recognized as potential alternatives to replace synthetic insecticides in mosquito control programs due to their excellent cidal properties (IRAC, 2005).

METHODOLOGY

Collection of Plant Materials

Plant sample (fruit) of *Picralima nitida* was collected from Umuagwu Akabor in Oguta Local Government Area of Imo State State, Nigeria. The plant was identified by A.O. Ozioko, a professional plant taxonomist at the Herbarium section of the International Center for Ethno-medicine and Drug Development, Nsukka.

Sample Preparation and Extraction

The pulps were extracted from the fruit; then pulverized using an electrical blender.

Two kilogram of each pulverized plant sample (pulp of *P. nitida),* was subjected to extraction using soxhlet extractor (Electrothermal heating mantle, Model Ms-9506 with Pyrex soxhlet column, condenser and round bottom flask). The samples were packed into the soxhlet column to 2/3 its volume. The column was then inserted into the flask and filled with the solvents. The column was filled until the solvents began to siphon. The soxhlet was then placed on the heating mantle, and heat adjusted to 40^{o}c. The solvents were allowed to reflux repeatedly, until refluxing solvent was clear and free from extracts. 2.5L of 95% methanol and 3.0L distilled water were used respectively. The extracted content was then subjected to rotary evaporator (Bibby Sterlin Ltd, England, RE. 200) until solvents were completely evaporated to get the solidified crude extracts. The crude extracts thus obtained was stored in sterilized amber coloured bottles and maintained at 4^{o}c in a refrigerator.

1000mg each of the methanolic and aqueous extracts were solubilised 5ml of dimethylsulphoxide (DMSO). The extracts were then diluted in 200ml of distilled water to obtain a stock solution of 5.00mg/ml. From the stock, graded concentrations of 4.00mg/ml, 3.00mg/ml, 2.00mg/ml, 1.00mg/ml and 0.50mg/ml were then obtained.

Larvae Rearing and Identification

The 4th instar Larvae of *A. gambiae* used in this investigation were raised through the assistance of technicians at the Arbo-Viral Research Laboratory, Enugu. Following emergence, the mosquitoes were identified by a professional Parasitologist, Dr Goddy Ngwu of the Department of Zoology, University of Nigeria, Nsukka. Cyclic generations of *A. gambiae* were maintained in a 29 cm x 21.5 cm x 56.5 cm cages with potted plants. Mean room temperature of (27 ± 2^{0}C) and a relative humidity of 70-80 percent were maintained in the insectary. The adult mosquitoes were fed on ten per cent glucose solution. For continuous maintenance of mosquito colony, the adult female mosquitoes were blood fed with laboratory reared albino mice. Ovitraps were placed inside the cages for egg laying. The eggs laid were then transferred to enamel larval trays maintained in the larval rearing chamber. The larvae were fed with larval food (Quaker oat and yeast in the ratio 3:1). 4th instar larvae were then picked for larvicidal bioassay.

Larvicidal Bioassay

Larvicidal bioassay of extracts was tested against 4th instar larvae of *A. gambiae.* The tests were conducted in glass beakers, in accordance with (WHO, 2005) protocol with slight modification. Three replicates and a control were run simultaneously during each trial. For control, 5ml of DMSO in 195ml of distilled water was used. Twenty healthy larvae were released in each glass beaker and mortality was observed at 24, 48 and 72 hrs after treatment with extract concentrations of 5.00, 4.00, 3.00, 2.00, 1.00, and 0.5mg/ml. The treatments were maintained at room temperature. Larvicidal activity of each extract was determined, by counting the number of dead larvae on daily basis (24hrs interval). The moribund and dead larvae in the three replicates were combined and expressed as percentage mortality for each concentration. Dead larvae were recorded when they failed to move after probing with a needle. The percentage mortality was calculated and analysis of data was carried out by employing probit analysis.

Phytochemical Screening

The crude methanolic and aqueous extracts of the pulp of *P. nitida* were screened for their phytochemical components using the methods described by Harborne (1984) and Evans (2002).

Statistical Analysis

The Median Lethal Concentration values (LC_{50} and LC_{95}), Median Lethal Time (LT_{50}) and Relative Potency were estimated using probit analysis as described by Finney, (1971). SPSS version 16.0

RESULT

Percentage Yield of Sample

The sample showed a 3.93% extract with methanol and 0.89% with aqueous.

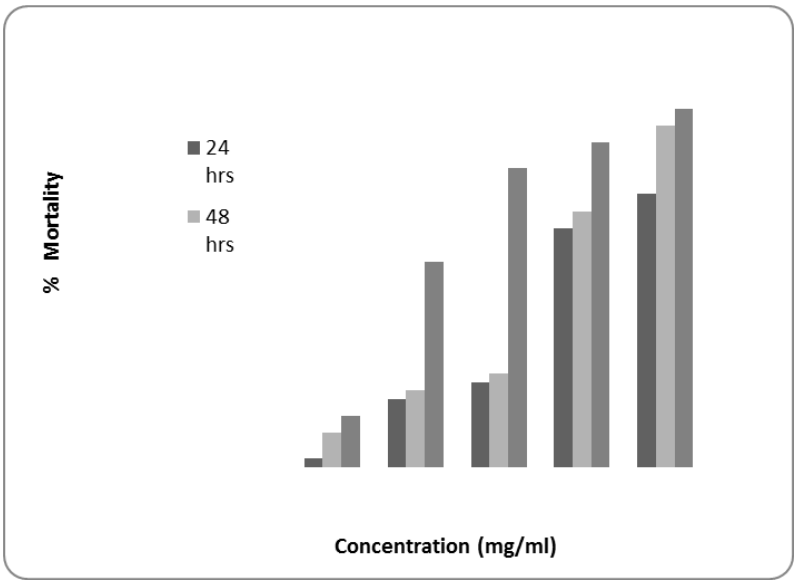

Figure 1. Mortality of *A. gambiae* to aqueous pulp extract of *P. nitida*

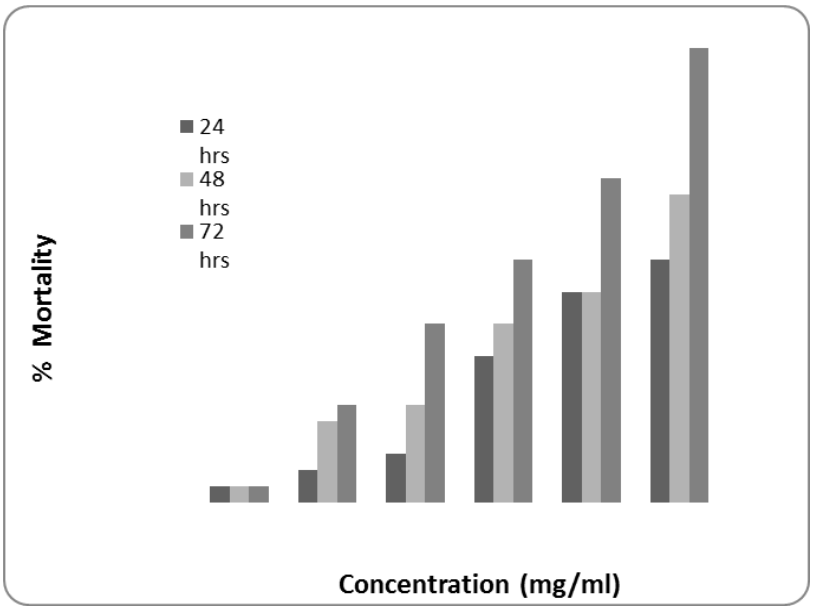

Figure 2. Mortality of *A. gambiae* to methanolic pulp extract of *P. nitida*

Phytochemistry

The phytochemicals constitutes of crude methanolic and aqueous extracts of *P. nitida* pulp presented. The presence of alkaloids, cardiac glycosides, saponins, tannins, flavonoids, terpenes and steroids was observed.

Lethality Assay

Figure 1 shows the lethality pattern of the aqueous pulp extract of *P. nitida* on *Anopheline* Larva. The aqueous pulp extract shows a moderate larvicidal activity against the *Anopheline* larva. At concentrations of 5.00, 4.00 and 3.00mg/ml a percentage mortality of 53, 50 and 58 respectively were observed over an exposure time of 24 hours, 48 hours and 72 hours respectively.

Figure 2 shows the lethality pattern of methanolic pulp extract of *P. nitida* on *Anopheline* Larva. The figure shows a fairly moderate effect of the extract on the test organism. However at concentrations used in this research, the extract failed to exhibits a mortality of at least 50% notwithstanding the time of exposure. The

Table 1. Time Dependent Median Lethal Concentrations of the Various Extracts.

	24hrs Lethal Conc. (mg/ml)		48hrs Lethal Conc. (mg/ml)		72hrs Lethal Conc. (mg/ml)	
SAMPLES	**LC_{50}**	**LC_{95}**	**LC_{50}**	**LC_{95}**	**LC_{50}**	**LC_{95}**
Aqueous Pulp	4.979	18.541	4.299	16.008	2.792	10.397
Methanolic Pulp	12.285	96.993	9.700	76.584	6.490	51.236

Table 2. Relative Median Potency Estimates at Various Time Intervals.

Label		95% Confidence Limits			95% Confidence Limits with LOG Transform		
(1)	**(2)**	**Estimate**	**Lower Bound**	**Upper Bound**	**Estimate**	**Upper Bound**	**Lower Bound**
24 Hours PROBIT							
AP	**MP**	.656	.428	.904	-.183	-.369	-.044
MP	**AP**	1.525	1.107	2.339	.183	.044	.369
48 Hours PROBIT							
AP	**MP**	.643	.445	.866	-.192	-.352	-.063
MP	**AP**	1.554	1.155	2.248	.192	.063	.352
72 Hours PROBIT							
AP	**MP**	.532	.398	.676	-.274	-.400	-.170
MP	**AP**	1.880	1.480	2.511	.274	.170	.400

highest concentration (5.00mg/ml), at the highest exposure time (72hours) only showed 47% mortality of the test organism.

Lethal Concentration Assay

The LC_{50} and LC_{95} assay of the two extracts at different time intervals indicated that the aqueous pulp extract has a lower median lethal concentration of 4.979mg/ml, 4.299mg/ml and 2.792mg/ml at 24, 48 and 72 h respectively. On the other hand, the methanolic pulp extract was observed to exhibit a higher median lethal concentration (12.285mg/ml, 9.700mg/ml and 6.490mg/ml) at the various time intervals respectively. A comparison of the two extracts therefore shows that the aqueous extract displayed more activity at equi- concentrations of the two extract.

Relative Potency

The Relative Median Potency of the extracts at time intervals of 24, 48 and 72 hours was estimated, result obtained is presented in Tables 2. The aqueous pulp extract showed a higher level of potency than the methanolic pulp extract at the varying exposure time intervals.

DISCUSSION AND CONCLUSION

Resistance of mosquitoes to chemical based insecticides and simultaneous toxicity of these insecticides to both human and other non- target organisms has become a major problem for researchers as they search for alternative measures that can probably replace already existing insecticides. The plant kingdom has proved to be a reliable source of larvicidal agents. Approximately 1,200 plant species having potential insecticidal value was described by Roark, (1947), while Sukumar *et al*., (1991) listed and discussed 344 plant species with cidal effect on mosquitoes. The larvicidal activity of pulp extracts of the plant *P. nitida* has not been investigated by any research, though Ubulom *et al.,* (2012) investigated the larvicidal potentials of crude ethanolic and aqueous extracts of the leaf and Dibua *et al.,* (2013) investigated the larvicidal effect of the seed and leaf methanolic and aqueous extracts. The phytochemical analysis showed the presence of bioactive components which includes alkaloids, cardiac glycosides, saponins, tannins, flavonoids, terpenes and steroids in the test plant extracts, as was also observed in Nwakile and Okore, (2011); Dibua *et al.,* (2013). The extracts showed a relatively high LC_{50} and LC_{95} values against the test organism in comparison to the activity of the seed and leaf extracts as reported by Ubulom *et al.,* (2012) and Dibua *et al.,* (2013) (Table 2). This however suggests higher efficacious activity of the leaf and seed which may be as a result of more bioactive ingredients or higher concentrations of specific components. However, LC_{50} and LC_{95} values of samples used decreased with increase in the time of exposure, showing a better activity

at 48 and 72hours trial (Table 1). Other plants belonging to the family Apocynaceae have been reported to exhibit significant cidal effect on the larvae of various insect pest. Al-Doghairi *et al.,* (2004) reported the LC_{50} values of *Solenostemna argel* against *Cx. Pipens* to be 0.037 and 0.031 ppm and the LC_{95} values were found to be 0.394 and 0.293 after 24 and 48hrs exposure respectively. These suggest the family Apocynaceae can be further explored as potential alternatives in the control of mosquitoes. Relative potency estimate demonstrated by the extracts was observed to be both concentration and time dependent. At 24, 48 and 72 hours, the potency of Methanolic pulp was 1.525, 1.554 and 1.880 times that of the aqueous pulp extract respectively. This finding gives credence to the use of chemicals of plant origin in the control of mosquito, and further justifies their use as an eco-friendly, readily available alternative to synthetic insecticides in the elimination of mosquitoes and other insect pests plaguing man and his environment.

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