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An Investigation of the Lethality of *Picralima Nitida*, Family *Apocynaceae* in Malaria Vector Control

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Abstract

Insecticides resistance and the corresponding health and environmental challenges that arises as a result of the use of synthetic chemical based insecticides prompts the search for better alternative control measures which are more effective, specific in action and less toxic. The 4th instar larvae of *Anopheles spp*, the vector of the deadly *plasmodium* were evaluated in this research against aqueous and methanolic leaf extracts of the plant *Picralima nitida.* Results revealed that aqueous leaf extract of the test plant had a mean mortality of 11 at 24hrs exposure and concentration of 5.0mg/ml. 95% mortality was also recorded at 5.0mg/ml after 48hrs exposure. Methanolic leaf extract had a mean mortality of 7.7 at 48hrs exposure time and same concentration of 5.0mg/ml. however, at 72hrs exposure, (concentration 5.0mg/ml), the mean mortality increased to 19.3 (97% mortality). The Median Lethal Time evaluated using probit analysis at 95% Confidence Limit showed the average lethal time of the test organism Anopheles larvae to the methanolic extract to be 55hrs and 29hrs for the aqueous leaf extract. This result hence supports the fact that leaf extracts of *P. nitida* can be used as a source of eco-friendly alternatives in the control of mosquito vectors, if developed.

Keywords: Resistance, Insecticides, *Anopheles spp*, Eco-friendly, Larviciding

1. Introduction

Mosquitoes are vectors of disease causing agents found within almost all tropical and subtropical countries. They are responsible for the transmission of pathogens causing some of the most life-threatening and debilitating diseases of man, like malaria, yellow fever, dengue fever, filariasis, encephalitis, etc (Chandra *et al.*, 2008 and ICMR Bulletin, 2003).Which has put 55% of the world's population at risk in 124 countries (Beatty *et al.*, 2007). According to WHO/UNICEF, 2005 in their first comprehensive report of the Roll Back Malaria partnership, malaria is endemic in 117 countries with some 3.2 billion people living in risk areas all over the world. Anopheline mosquitoes are the vector responsible for the transmission of malaria (Wendy *et al.*, 2012). Around the world, the medical and economic burden caused by vector-borne diseases continues to grow as current control measures fail to cope (Radhika *et al.*, 2011). There is therefore an urgent need to identify new control strategies that will remain effective, even in the face of growing insecticide and drug resistance (Achs and Malaney, 2002). Repetitive use of man-made insecticides for mosquito control disrupts natural biological control systems and lead to reemergence of mosquito populations (Radhika *et al.*, 2011). It also resulted in the development of resistance, detrimental effects on non-target organisms and human health problems and subsequently this initiated a search for alternative control measures (Das *et al.*, 2007 Zhang *et al.*, 2011). The use of biological control agents such as predatory fish (Legner, 1995), bacteria (Becker & Ascher, 1998), protozoa (Chapman, 1974), fungus (Murugesan, 2009), nematodes (Kaya & Gaugler, 1993) and plant products (Mathur, 2003) had shown promising results in the control of mosquito populations. The development of new strategies, including naturally occurring larvicides in the control of mosquitoes, is important in order to counter the evolution of resistance in target populations and the possible effects on non-target organisms (Cetin and Yanikoglu, 2006). The work An Investigation of the Lethality of *Picralima nitida*, Family *Apocynaceae* In Malaria Vector Control was therefore carried out to explore the possibility of using this plant against these deadly insect vectors. The plant *Picralima nitida*, family Apocynaceae has for many years been used by rural dwellers in Oguta and other parts of southeastern Nigeria for the treatment of various diseases like malaria and other forms of fever. The use of this plant against pathogenic diseases was examined by (Nwakile *et al*., 2011 and Okokon *et al.*, 2007) examined its use against protozoan infections (Dibua *et al.*, 2013 and Ubulom *et al.*, 2012) examined the larvicidal effect of the leaf samples while (Ubulom *et al.*, 2012) also evaluated the antifungal effects.

2. Materials and Methods

2.1. Collection of Plant samples

Fruits and leaves of the Plant *Picralima nitida* were collected from Oguta Local Government Area of Imo State, Nigeria. The plant was identified by a professional plant traxonomist at the herbarium section of the International Center for Ethno–Medicine and Drug Development, Nsukka, Enugu State, Nigeria.

2.2. Photochemical Screening

Screening was conducted in accordance with (Evans and Treas, 2002). The phytochemical tested for includes; saponin, steroid, tannins, terpenes, alkaloids, glycosides and flavonoids.

2.3. Preparation of Samples

Leaf and seed samples were collected washed and air dried under shady condition at room temperature ($28\pm2^{O}C$). 1g of each dried samples was then pulverized and extracted using a soxhlet extractor. Extracts were then evapourized using a rotary evaporator 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.

2.4. Stock Preparation

2.5g of the extracts was solubilized in 5ml dimethylsulphuroxide (DMSO), the mixture was then made up with 495ml of water to get a stock solution of 5mg/ml. Graded concentrations of 4mg/ml, 3mg/ml, 2mg/ml, 1mg/ml and 0.5mg/ml.

2.5. Larvae Identification

Larvae used for this research were identified by a professional parasitologist from the Department of Zoology, University of Nigeria, Nsukka. They lacked the air tube (Siphon) which is possessed by other genus of mosquito. They lay parallel to the water surface unlike other genus that stays at an angle to the water surface.

2.6. Procedures for Insect Rearing:

About 100 unparasitalized 4th instar larvae of the insect were collected from flooded grass field. The larvae were identified by a parasitologist as Anopheles by a parasitologist at the department of Zoology University of Nigeria, Nsukka. Larvae collected were reared in a bowl protected with net to trap the adults when they emerge. The larvae were fed with quaker oat. Emerged adult mosquitoes were transferred to an oviposition cage with potted plants. Adult mosquitoes were fed with 10% glucose solution, laboratory reared albino rats were also provided as source of blood meal for the adults. Ovitraps were placed within the cage for laying of eggs. Eggs laid were collected on daily basis and were recycled to the 4^{th} instar before they were used for the bioassay.

2.7. Bioassay

Larvicidal bioassay of individual plant extracts was tested against 4th instar larvae of *Anopheles spp*. The tests were conducted, in accordance with (WHO, 2005) protocol with slight modification. Three replicates and a control were run simultaneously during each trial. Twenty healthy larvae were introduced into each glass beaker and mortality was observed at 24, 48 and 72 hrs. Larvicidal activity of each extract was determined, by counting the number of dead larvae on daily basis (24hrs interval). The dead larvae in the three replicates were combined and expressed as percentage mortality for each concentration. Larvae were recorded dead when they failed to move after probing with a needle.

When the control mortality ranged from five to twenty per cent, the observed percentage mortality was corrected by Abbott's (Abbott, 1925) formula.

$$P = \frac{\%\ Po - \%\ Pc}{100 - \%\ Pc} \quad X\ 100$$

Where P is the corrected mortality, Po is the observed mortality and Pc is the control Mortality, all expressed in percentages.

2.8. Statistical Analysis

Results obtained from research were analysed using probit analysis as developed by [24]. Statistical package foe social sciences (SPSS) version 16 was used for the analysis.

3. Results

Phytochemical screening of the plant revealed the presence of bioactive ingredients in the leaf extract of the test plant. Test showed that P. nitida is rich in alkaloids, flavonoids, saponins, tannins, terpenes and glycosides.

3.1. Bioassay

The bioassay results as shown in table 1 and 2 shows the mean mortality values of the two extracts. Their percentage mortality at the various time of exposure is also shown in the tables. Result shows that the aqueous leaf extract showed a mean mortality of 11 after 24 hours exposure at 5.0mg/ml, but at 48 hours exposure the mean mortality increased to 19 which indicates 95% of the test larvae. Mean mortality for the methanolic leaf extract after 24 hours exposure at 5.0mg/ml was 3, but increased to 19.3 after 72 hours exposure at the same concentration. This amounts to a percentage mortality of 97%.

3.2. Lethal Median Time

The lethal median time of extracts at the various concentrations, expressed as log of time is shown in Figure 1. The average lethal time for which approximately 50 percent of larvae exposed would die was 55 hours for methanolic extract were as that of the aqueous leaf extract is approximately 29 hours.

4. Discussion

Larviciding as a strategy in the fight against insect pest is gaining grounds as most research focuses on the elimination of pest at their immature stage rather than their adult forms. The use of plants and plant products has also been a focus research area in the search for possible alternative to chemical based insecticides. Shaalan (2005) , reviewed the application of larvicide from botanical origin as an essential part of IMM, and various mosquito control agents such as ocimenone, rotenone, capllin, quassin, thymol, eugenol, neolignans, arborine and goniothalamin. The larvicidal effect of the plant P. nitida has been previously studied by (Ubulom *et al*., 2012 Dibua *et al*., 2013). The use of plants belonging to the family *"Apocynaceae"* was also reported to exhibit significant cidal effect on the larvae of various insect pest by (Al-Doghairi *et al.*, 2004). Phytochemical screening of the plant revealed the presence of phytochemicals corresponding to those reported by previous works. The mortality was more with the aqueous leaf extract, showing a mean percentage mortality of 95% at 48hrs exposure at a concentration of 5.0mg/ml and a mean percentage mortality of 100% after 72hrs exposure at 4.0mg/ml (Table 1), while the methanolic extracts showed 38% mortality at 48hrs exposure and concentration of 5.0mg/ml, and 95% mortality at 72hrs exposure with concentration of 3.0mg/ml (Table 2). This confirms the findings of (Dibua *et al.*, 2013) who gave LC_{50} and LC_{95} values of 3.141mg/ml and 42.154mg/ml, 0.352mg/ml and 4.730mg/ml and 0.164mg/ml and 2.201mg/ml for aqueous leaf extracts at 24, 48 and 72h. LC_{50} values for methanolic leaf extract were 48.383mg/ml, 15.817mg/ml and 0.333mg/ml at 24h, 48h and 72 h. This hence suggests that *P. nitida* has a higher proportion of methanol soluble bio- active components than water soluble components. The phytochemical compounds detected in the leaf of *P. nitida* have been reported to have biological activity (Lee, 2000 and Wiesman, 2006). Evaluation of the Median lethal time of the test larvae to the various extracts revealed the average LT_{50} values to be 55 hours for methanolic extract and 29 hours for aqueous extract (Figure 1). The results of this work therefore suggest that *P. nitida* is rich in bioactive components which are active against the insect vector *Anopheles spp*. It further calls for more work on the specific effect of the individual phytochemicals present in this plant. Again the effect of environmental factors like turbidity of water, temperature and pH should also be investigated as these factors has been reported by (Paaijmans, 2008) to have an effect on the survival rate of larva.

5. Conclusion

P. nitida is rich in bioactive components which are active against the insect vector *Anopheles spp.*This result hence supports the fact that leaf extracts of *P. nitida* can be used as a source of eco-friendly alternatives in the control of mosquito vectors, if developed.

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Table 1: Larvicidal Effect of Aqueous Leaf Extracts

Conc. (mg/ml)	Mean ± SD	24hrs Mean % Mortality	Mean ± SD	48hrs Mean % Mortality	Mean ± SD	72hrs Mean % Mortality
0.0	0.0 ± 0.0	0	0.0 ± 0.0	0	0.0 ± 0.0	0
0.5	2.3 ± 0.6	12	10.0 ± 1.7	50	12.0 ± 1.0	60
1.0	7.7 ± 1.2	38	17.0 ± 1.0	85	19.3 ± 1.2	97
2.0	8.0 ± 2.6	40	17.0 ± 1.7	85	19.7 ± 0.6	98
3.0	9.3 ± 1.5	47	18.3 ± 0.6	92	19.7 ± 0.6	98
4.0	9.7 ± 3.2	48	18.7 ± 1.5	93	20.0 ± 0.0	100
5.0	11.0 ± 1.0	55	19.0 ± 1.0	95	20.0 ± 0.0	100

Table 2: Larvicidal Effect of Methanolic Leaf Extracts

Conc. (mg/ml)	Mean ± SD	24hrs Mean % Mortality	Mean ± SD	48hrs Mean % Mortality	Mean ± SD	72hrs Mean % Mortality
0.0	0.0 ± 0.0	0	0.0 ± 0.0	0	0.0 ± 0.0	0
0.5	0.0 ± 0.0	0	2.7 ± 0.6	13	8.3 ± 1.5	42
1.0	0.0 ± 0.0	0	4.3 ± 1.2	22	16.7 ± 1.5	83
2.0	0.0 ± 0.0	0	5.0 ± 1.0	25	17.7 ± 1.5	88
3.0	0.3 ± 0.6	2	5.7 ± 1.5	28	19.0 ± 1.0	95
4.0	1.0 ± 1.0	5	7.0 ± 2.0	35	19.3 ± 0.6	97
5.0	3.0 ± 1.0	15	7.7 ± 0.6	38	19.3 ± 0.6	97

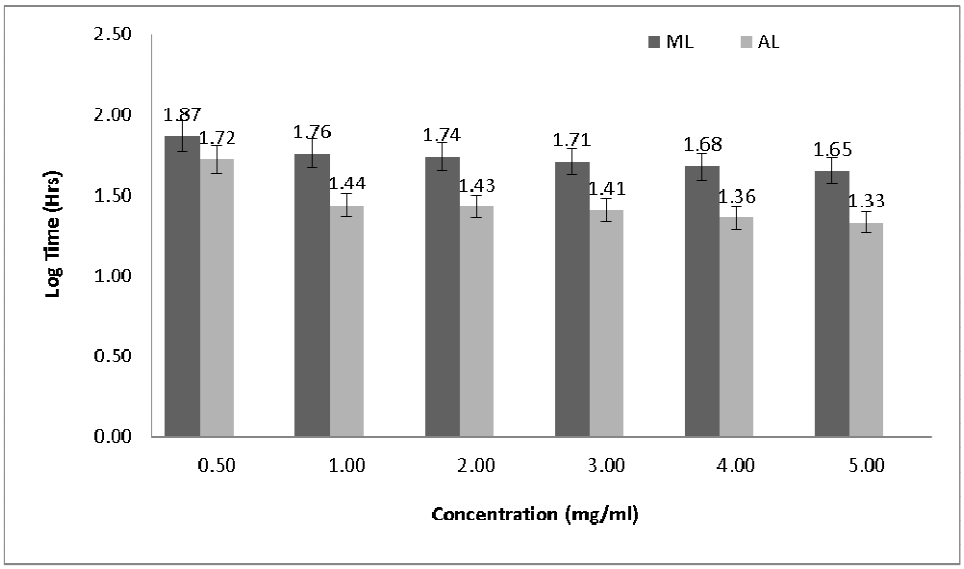

Figure 1: 50 Percent Lethal Concentration for Extracts

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