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Research Article

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Partition and Evaluation of Crude Methanol Seed Extract of *Picralima nitida* (Stapf.) T. Durand and H. Durand (Apocynaceae) for Larvicidal Activity on Aedesaegypti Larvae

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Abstract In this study the active crude methanol seed extract of *pricralimanitida* was partition and evaluated for larvicidal activity against *Aedes aegypti*. About 50g of the active crude methanol extract was dissolve in sufficient quantity of water. This was extracted with sufficient quantity of n-hexane exhaustively; after this it was also exhaustively extracted with sufficient quantity of ethyl acetate. The n-hexane, ethyl acetate and the aqueous portions were dried and the extracts evaluated for larvicidal activities. The evaluation of the larvicidal activities on *Aedes aegypti* larva was carried in accordance to the method of WHO, 2005 guideline. The result showed that after the partition extraction, the yield was 8.5, 17.2 and 74.3% for n-hexane, ethyl acetate and the aqueous portions respectively. The result of the evaluation the larvicidal activities showed that the n-hexane portion gave the highest activity with LC_{50} of 0.03, 0.013 and 0.0048mg/ml after 24, 48 and 72 hours respectively. Ethyl acetate portion has LC_{50} of 0.98, 0.53 and 0.26mg/ml after 24, 48 and 72 hours respectively. While the aqueous part has LC_{50} of, 10.05, 4.35 and 3.42mg/ml after 24, 48 and 72 hours respectively. From the result the n-hexane and the ethyl acetate portion are recommended for further evaluation and isolation of the active constituent(s).

Keywords seed extract, larvicidal, n-hexane, ethyl acetate, partition

Introduction

Mosquitoes are the most important single group of insects in terms of public health as they serve as vectors for various subtropical and tropical diseases which have adverse effects on humans. According to WHO, 2015, [1] mosquitoes are one of the deadliest animals in the world. They do not only transmit pathogens and parasites but also serve as sources of allergic reactions that include local skin and systemic sensitivity. The most common diseases associated with mosquitoes are malaria, yellow fever, dengue fever, chikungunya, filariasis, etc. *Aedes aegypti* (Linnaeus) is one of these mosquito species and a principal tropical vector that transmit the arboviruses responsible for dengue haemorrhagic fever (DHF), chikungunya, yellow fever, zika fever and other diseases. Due to the pathogenic diseases and serious harms caused by mosquitoes, the need to control them is imperative. The technique used in controlling mosquitoes depends on the growth stages (egg, larvae, pupae and adult) on target. Mosquito control includes targeting the adult mosquito through spraying chemical insecticides or by killing the mosquito

larvae before they emerge into adults via using synthetic larvicides or botanical extracts as an alternative larvicide. [2]

Picralima nitida (Stapf) T. Durand and H. Durand is the only known species of the genus *Picralima.* It is a fruit-bearing tree plant in the family Apocynaceae. Its common names include Akuamma, Picralima or Pile plant and it is native totropical Africa (Benin, Ghana, Ivory Coast, Nigeria, Gabon, Cameroon, Central African Republic, Republic of Congo, Zaire, Uganda).

P. nitida has been used in systems of traditional medicine for various purposes. Preparations from different parts of the plant are employed as crude drug or crude herbal extract as remedy for various kinds of human diseases. For instance, the seeds are widely used in West Africa especially in Nigeria, Ghana and Cote d'ivoire as antipyretic, aphrodisiac, for the treatment of malaria, pneumonia and other chest conditions [3-9]. Apart from its medicinal uses, Ubulom, et al., (2012) [10] has shown that the leaf extracts have larvicidal activity against Anopheles gambiae. Also Nwabor *et al* 2014 [11] showed that the pulp extract has larvicidal activites. The aim of this study is to carry out partition separation on aqueous methanol crude extract of *Picralima nitida* seed using ethyl acetate and n-hexane, and to run larvicidal assay on the three extracts of the partition fractions (aqueous, ethyl acetate and n-hexane fractions) on *Aedes aegypti* mosquito larvae to ascertain the level of their activities.

Materials and Method

Reagents and Solvents used

Methanol (JHD, China), Ethyl acetate (JHD, China), N-hexane (JHD, China), Dimethyl sulphoxide (DMSO), Industrial distilled to analytical Methanol, Industrial distilled to analytical N-hexane, Industrial distilled to analytical Ethyl acetate.

Instruments and Glassware used

Measuring cylinder, (Jinotech, Minghe) (5ml, 10ml, 50ml, 100ml, 1000ml)

Beakers (50ml, 100ml, 250ml) (G.G 17, England), Separating funnel (Jinotech), Capillary tubes (75mm) (Maxicom), Spatula, evaporating dishes, dropping pipette (3ml), Rotary evaporator (RE-52A) (Labscience, England) Digital thermostatic water bath, (Jinotech), Weighing balance (HCK 500 X 0,1g) (Dipse.com), Glass rods (20cm x 2cm), (G.G 17, England).

Experimental organisms

The experimental/test organisms were the larvae of *Aedes aegypti* at their third instar. The eggs laid by the mosquito specie were obtained from the National Arbovirus and Vectors Research Center, Enugu state, Nigeria and hatched to the required larval stage in the Department of Pharmacognosy and Phytotherapy laboratory, Faculty of Pharmaceutical sciences, University of Port Harcourt.

Partitioning of Methanol crude seed extract of *P. nitida*

The dried methanol crude extract (50g) was dissolved in 500ml of water and partitioned with 200ml N-hexane by vigorously shaking the aqueous methanol extract with n-hexane. The n-hexane layer was collected and the aqueous portion was further extracted with more n-hexane until it was exhaustive. The process was repeated with ethyl acetate. The n-hexane, ethyl acetate and aqueous portions so obtained were dried, weighed; the percentage yields were calculated and noted. These were stored in a desiccator till time of use.

Preparation of stock solution

The stock solution was prepared by dissolving 6g of the n-hexane and ethyl acetate portions each in about 5ml of Dimethyl sulphoxide (DMSO) and making the volume up to 600ml with water to achieve a stock concentration of 10mg/ml.

Larvicidal bio-assay

The bio-assay followed standard WHO protocols for testing the susceptibility of mosquito larvae to insecticides [12]. From the stock solution, different concentrations (0.5, 1.0, 2.0, 3.0, 4.0, & 5.0mg/ml) were made for each extract. A control was also prepared for each concentration containing only 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 third instar larvae of *Aedes aegypti* were released into each container of the test extract and the control. The larvae were then monitored for mortality at 24-hour intervals for 3 days (72 hours).

Statistical Analysis

The statistical tools that were used in this study is the Ldp line software by Ehab, [13] based on the standard method of probits by Finney [14] to calculate LC_{50} values of each extract after 24, 48 and 72 hours of treatment.

Results

Table 1: Percentage yield of extracts obtained from partition of crude methanol seed extracts of *Picralima nitida*

Weight of crude methanol extract = 50g

Partition fractions	Yield (g)	Percentage yield (%)
n-hexane	4.25	8.5
Ethyl acetate	8.6	17.2
Aqueous	37.15	74.3

The table 1 above showed the percentage yield of n-hexane, ethyl acetate and water. Aqueous portion gave the highest yield than the other two with percentage of 74.3%, ethyl acetate gave 17.2% while n-hexane gave the lowest yield of 8.5%. This was expected since methanol is polar solvent and the more the polarity of the solvent the more the will extract from the methanol. Water which is more polar than the ethyl acetate and n-hexane extracted most of the extract and ethyl acetate which is more polar than n-hexane extracted more than n-hexane.

Table 2: Result for larvicidal assay of the n-hexane partition portion of crude methanol extract of *Picralima nitida* seeds on *Aedes aegypti* larvae

		After 24 hours			After 48 hours			After 72 hours		
Conc. mg/ml	**Number of larvae**	**% mortality**	**LC_{50} mg/ml**	**Control death**	**% mortality**	**LC_{50} mg/ml**	**Control death**	**% mortality**	**LC_{50} mg/ml**	**Control death**
0.5	20	96.7±2.4	0.03	0	98.4±2.4	0.013	0	98.4±2.4	0.0048	0
1	20	80±8.2		0	88.4±2.4		0	90±0		0
2	20	71.7±6.2		0	100±0		0	100±0		0
3	20	100±0		0	100±0		0	100±0		0
4	20	100±0		0	100±0		0	100±0		0
5	20	100±0		0	100±0		0	100±0		0

From the table 2 the n-hexane partition extract from the crude methanol seed extract of *P. nitida* gave good larvicidal activity against the *Aedes aegypti* mosquito larvae with 100% mortality from the concentration of 3mg/ml within 24 hours with LC_{50} of 0.03 mg/ml. By 48 hours it gave 100% mortality from concentration of 2mg/ml and LC_{50} of 0.0048 mg/ml. thus indicating high activity.

Table 3: Result for larvicidal activity of ethyl acetate partition portion of crude methanol extract of *Picralima nitida* seed. on *Aedes aegypti* larvae

		After 24 hours			After 48 hours			After 72 hours		
Conc. mg/ml	**Number of larvae**	**% mortality**	**LC_{50} mg/ml**	**control death**	**% Mortality**	**LC_{50} mg/ml**	**control death**	**% mortality**	**LC_{50} mg/ml**	**control Death**
0.5	20	15±12.3	0.98	0	50±4.1	0.53	0	71.7±2.4	0.26	0
1	20	70±4.1		0	75±4.1		0	100±0		0
2	20	63.3±6.2		0	93.3±2.4		0	98.3±2.4		0
3	20	95±7.1		0	100±0		0	100±0		0
4	20	100±0		0	100±0		0	100±0		0
5	20	88.3±10		0	100±0		0	100±0		0

From table 3 above it is very clear that the ethyl acetate extract of the partition crude methanol seed extract of *P. nitida* gave high activity against the mosquito larvae. The extract gave 100% mortality at concentration of 3mg/ml

after 48 hours with LC_{50} of 0.53 mg/ml. It also gave 100% mortality at concentration of 1mg/ml after 72 hours with LC_{50} of 0.26 mg/ml

Table 4: Result for larvicidal activity of the aqueous partition portion of crude methanol extract of *Picralima nitida* seed on *Aedes aegypti* larvae

		After 24 hours			After 48 hours			After 72 hours		
Conc. mg/ml	**Number of larvae**	**% mortality**	**LC_{50} mg/ml**	**Control death**	**% mortality**	**LC_{50} mg/ml**	**control death**	**% mortality**	**LC_{50} mg/ml**	**control death**
0.5	20	0±0	10.05	0	0±0	4.35	0	0±0	3.42	0
1	20	3.4±4.7		0	5±4.1		0	5±4.1		0
2	20	5±0		0	6.7±2.4		0	11.7±2.4		0
3	20	6.7±6.2		0	10±10.8		0	16.7±6.2		0
4	20	26.7±2.4		0	51.7±13		0	78.4±4.7		0
5	20	26.7±2.4		0	68.3±8.1		0	80±0		0

From table 4 above, the larvicidal activity of the aqueous extract from the partition of the crude methanol seed extract of *P. nitida* was low when compare to the those of n-hexane and ethyl acetate. After 24 hours we have 26.7% mortality and the LC_{50} was 10.05 mg/ml. after 72 hours the mortality stood at 80% at the concentration of 4mg/ml with LC_{50} of 3.42 mg/ml.

Discussion

Due the problem of mosquito resistance to insecticides that are synthesized and also couple with the fact that these synthesized chemicals may be poisonous to living things including human necessitated the search to alternative sources of insecticides to fight mosquitoes. The plant kingdom serves as an alternative source. Roark (1947) [15] showed that approximately 1,200 plant species have potential insecticidal value. Sukumar, *et al* (1991) [16] listed and discussed 344 plant species with toxic effect on mosquitoes.

Work on the larvicidal activity of the crude methanol seed extract of *P. nitida* on *Aedes aegypti* larvae showed that the crude extract had larvicidal activity [17]. In continuation, the activities of its partition extracts on *Aedes aegypti* larvae were carried out in this work.

From the results obtained, the percentage yield from the crude methanol seed extract of *Picralimanitida*seeds were 8.5%, 17.2%, 74.3% with respect to n-hexane, ethyl acetate and water (aqueous) portions respectively. These values show that most of the phytochemical constituents of the plant are polar as the yield obtained decreased with decrease in polarity of the solvents used. Hence, n-hexane had the least percentage yield of 8.5%.

The three portions obtained after partition of the crude methanol seed extract of *Picralimanitida* had larvicidal activity against the larvae of *Aedes aegypti*, which was concentration and time dependent. This was not un-expected as Okwubie and John, 2017[17] and Nwabor et al, 2014[11] have shown the seed extract had larvicidal activities which was time and concentration dependent. Ubulom et al, 2012[10] also showed that the leaf extract of *P. nitida* gave larvicidal activities which is also time and concentration dependent.

The n-hexane portion had the most significant larvicidal activity with percentage mortality of 100% at concentrations 3–5mg/ml and 96.65% at 0.5mg/ml just after 24 hours of exposure. After 72 hours, the same 0.5mg/ml concentration had an increased percentage mortality of 98.35%. An LC_{50} value of 0.0048 mg/ml was obtained after 72 hours. We also noticed some divergent on the percentage mortality at concentrations of 0.5mg/ml, 1mg/ml and 2mg/ml in table 2 in which the higher concentrations have lesser percentage mortalities, this may be due to reliance of some of the larvae to the toxic effects of the extracts. This also occurs in some other concentrations as shown in the tables but generally the activities are still concentration and time dependent.

Furthermore, the ethyl acetate portion had more larvicidal activity against the test organism than the aqueous portion which manifested in the percentage mortalities of 15% and 88.33% at 0.5 and 5mg/ml concentrations respectively after 24 hours of exposure while the aqueous portion had percentage mortalities of 0% and 26.67% at the same concentrations and time of exposure.

The LC_{50} values decreased with increase in time as 0.98 and 0.26 mg/ml were obtained for the ethyl acetate portion while that of the aqueous portion was 10.05 and 3.42mg/ml after 24 hours and 72 hours respectively. The fact that n-hexane and ethyl acetate extracts exhibits far higher activities than that of aqueous extract showed that the active agent(s) are more of non-polar

Conclusion

In conclusion, the partition extracts of crude methanol seed extract of *P. nitida* have larvicidal activities as shown above, however, the n-hexane and ethyl acetate extracts of the partition gave much higher activities and can be further be worked on to isolate and identify the active agent(s)

References

[1]. WHO (2015). Mosquito borne diseases www.who.int/neglected_diseases/vector_ecology/mosquito-borne-diseases

[2]. Mohini Tiwary, S.N Naik, Dhananjay Kumar Tewary, P.K. Mittal, S. Yadav. (2007). Chemical composition and larvicidal activities of the essential oil of Zanthoxylum armatum DC (Rutaceae against three mosquito vectors. *Journal of Vector Borne Diseases* 44(3) 198-204

[3]. Kapadia GJ, Angerhofer CK, Ansa-Asamoah R. (1993). Akuammine:an antimalarial indolemonoterpene alkaloid of Picralimanitidaseeds. *Planta Medica* 59(6): 565-566.

[4]. Kouitcheu LB, Kouam J, Atangana P, Etoa FX. (2008). Phytochemical screening and toxicological profile of methanolic extract of *Picralima nitida* fruit-rind (Apocynaceae). *Toxicol Environ Chem*.90: 815-828.

[5]. Ainslie JR. (1937). A list of plants used in native medicine in Nigeria. Imperial Forestry Institute, Oxford Institute Paper: p. 7.

[6]. Oliver B. (1960). *Encyclopedia of medicinal plants*. Ibadan: College of Arts, Science and Tech.

[7]. Saxton J.E. (1965). Alkaloids of *Picralima nitida*. In: Manske RHF, editor. *The alkaloids: chemistry and physiology*. Academic Press, New York. 8: 119-158.

[8]. Ayensu ES. (1978). Medicinal plants in West Africa. Reference Publications Inc. Algonac, Michigan. 330.

[9]. Nkere CK, Iroegbu CU. (2005). Antibacterial screening of the root, seed and stem bark extract of Picralimanitida. *Afri J Biotechnol*, 4(6): 522-526.

[10]. Ubulom PME, Imandeh NG, Udobi CE, IIya I. (2012). Larvicidal and antifungal properties of *Picralima nitida* (Apocynaceae) leaf extracts. *Eur J Med Plants*, 2(2): 132-139.

[11]. Nwabor O.F, Dibua U.M, Nnamonu E. I, Odiachi Osita, Dickson I. Dickson, Okoro O.J. (2014). Pulp Extracts of *Picralima nitida*: ALarvicidal Agent in Malaria Vector Control. *Journal of Biology, Agriculture and Healthcare*, 4(8): 69-73.

[12]. WHO, (2005). *Guidelines for Laboratory and Field Testing of Mosquito Larvicides*. WHO communicable disease control, prevention and eradication. WHO pesticide evaluation scheme *WHO/CDS/WHOPES/GCDPP/2005.13.*

[13]. Ehab Mostafa Bakr; Ldp line available at http/www.ehabsoft.com, 2013

[14]. Finney, D.J. (1971). *Probit analysis*. Cambridge University Press, Cambridge.

[15]. Roark, R.C. (1947). Some promising insecticidal plants. *EconBot*, 1: 437-445.

[16]. Sukumar k, Perich M.J, Boobar L R. (1991). Botanical derivatives in mosquito control: a review. *Journal of the American Mosquito Control Association*, 7(2):2010-237.

[17]. Okwubie Lambert and Dan John. (2017).Evaluation of Larvicidal Activities of Stem Bark and SeedExtracts of *Picrilima nitida* (Stapf.) T. Durand and H. Durand (Apocynaceae) on Aedes Aegypti Larvae. *International Journal of Pharmacy and Biological Sciences*, 7(3): 65-70