British Journal of Pharmaceutical Research
2(4): 259-268, 2012

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Potential Larvicidal Properties of *Blighia sapida* Leaf Extracts against Larvae of *An. gambiae*, *Cu. quinquefasciatus* and *Ae. aegypti*

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Authors' contributions

This work was carried out in collaboration between all authors. Author PMEU designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author GNI managed the analyses of the study and supervised the work. Authors EOE and CEU managed the literature searches, presentations and improved the manuscript. All authors read and approved the final manuscript.

Research Article

Received 23rd July 2012
Accepted 14th October 2012
Published 10th December 2012

ABSTRACT

Blighia sapida is a medicinal plant used in Southern Nigeria for the treatment of some eye ailments and headache. The Centre for Scientific Research into Plant Medicine (CSRPM), Ghana, has used this plant for the treatment of diarrhea for over 20 years.
Objective: This study was designed to investigate the lethal effect of aqueous, ethanol, and ethyl acetate extracts of the leaf of *B. sapida* on fourth instar larvae of *An. gambiae, Cu. quinquefasciatus* and *Ae. aegypti*.
Methods: The lethal effect of aqueous, ethanol and ethyl acetate extracts of the leaves of *B. sapida* at concentrations of 0.15, 0.30, 0.45, 0.60 and 0.75% w/v each were investigated in static bioassays on 4th 15 instar larvae of *An. gambiae*, *Cu. quinquefasciatus* and *Ae. aegypti*.
Results: The 72hLC_{50} values of the aqueous extract were 0.393, 0.488 and 0.423%w/v

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for larvae of *An. gambiae*, *Cu. quinquefasciatus* and *Ae. aegypti* respectively, while the values for the ethanol extract were 0.319, 0.407 and 0.384%w/v for *An. gambiae*, *Cu. quinefasciatus* and *Ae. aegypti* larvae respectively. For the ethyl acetate extract tested against larvae of *An. gambiae*, *Cu. quinquefasciatus* and *Ae. agypti*, the 72hLC_{50} values were 0.135, 0.177 and 0.133% w/v respectively. As judged by the 72hLC_{50} values ethyl acetate extract was the most potent of the three extracts.
Conclusions: Results obtained demonstrate that the leaves of B. sapida have marked larvicidal potential against mosquito larvae used in this study.

Keywords: Blighia sapida; leave extract; mosquito larvae.

1. INTRODUCTION

Many devastating diseases are the result of infection with parasites and some of these parasites are vectored by mosquitoes. Mosquitoes occur throughout the tropical and temperate regions and constitute a major public health menace. Females of the dapple – winged mosquitoes of the genus *Anopheles,* transmit malaria, a life – threatening disease caused by *Plasmodium* parasites. Vector control is the main way to reduce malaria transmission at the community level and it is the only intervention that can reduce malaria transmission from very high levels to close to zero [1]. Lymphatic filariasis, commonly known as elephantiasis is caused by infection with nematodes of the family filariodidea and these nematodes are transmitted by some mosquito species including *Anopheles, Culex* and *Aedes*. More than 1.3 billion people in 81 countries world-wide are threatened by lymphatic filariasis [2]. Yellow fever, a major public health challenge occurring in epidemic patterns is vectored by *Aedes* mosquitoes, notably *Aedes aegypti* [3]. Dengue is an infection that causes a severe flu-like illness and sometimes a potentially lethal complication called dengue haemorrhagic fever (DHF). According to WHO [4], dengue has become a major international public health concern in recent decades. Dengue and dengue hemorrhagic fever are caused by four distinct, but closely related viruses, which are transmitted to humans through the bites of infected female *Aedes mosquitoes* and *Ae. aegypti* is the primary dengue vector [4]. There is no specific treatment for dengue and there is no vaccine to protect against dengue. The only way to prevent dengue virus transmission is to combat the vector mosquitoes.

The giant stride in scientific advancement and efforts of the health services in most countries notwithstanding, effective control of certain diseases transmitted by vectors has remained a mirage. This is due to the fact that the strategies which have been devised to reduce the prevalence of these diseases have limitations such as environmental pollution by synthetic insecticides and the development of physiological resistance to these insecticides by the vectors. These limitations have resulted in the continued search for environmentally safe, effective and target-specific insecticides. Extracts from plant sources have demonstrated promising potential as insecticidal/larvicidal agents [5, 6, 7]. On this premise, the lethal effect of extracts of the leaf of *Blighia sapida* on fourth instar larvae of *Anopheles gambiae, Culex quinquefasciatus* and *Aedes aegypti* was investigated.

B. sapida (Sapindaceae) is referred to as akee apple in English, or ackee or vegetable brain [8]. Its oily aril (an accessory covering of the seed) has a nutty taste and is usually eaten raw or cooked. The leaf juice is used as eye drops to treat some eye problems like conjunctivitis, iritis and trachoma [9]. *B. sapida* has been used by the Centre for Scientific Research into

Plant Medicine (CSRPM), Ghana, for the treatment of diarrhea for over twenty years [8]. The leaves and seeds of this plant have been reported by the people of Itak Ikot Akap Community, in Ikono Local Government Area of Akwa Ibom State, Nigeria to posses piscicidal properties (pers. comm.) but a scientific document on its mosquito larvicidal potential has not been encountered.

The aim of the study was to investigate the lethal effect of aqueous, ethanol, and ethyl acetate extracts of the leaf of *B. sapida* on fourth instar larvae of *An. gambiae, Cu. quinquefasciatus* and *Ae. aegypti.*

2. MATERIALS AND METHODS

2.1 Preparation of Plant Material

The plant, *B. sapida* was obtained from Itak Ikot Akap Community in Ikono Local Government Area of Akwa Ibom State, Nigeria. Identification was done by Prof. Rufus Ubom of the Department of Botany and Ecological Studies, University of Uyo, Nigeria. A voucher specimen (No. UUH 69a) has been deposited in the herbarium, of the Department of Pharmacognosy and Natural Medicine, University of Uyo, Nigeria.

Leaves of *B. sapida* were thoroughly washed and air-dried on laboratory tables for 21 days. They were pulverized using the crusher machine, Gondard 77260, type TN 20. The leaf powder obtained was divided into three parts and separately subjected to extraction by maceration; using water, ethanol and ethyl acetate. Maceration lasted for 72h with periodic stirring. At the end of maceration, filtration was done repeatedly using muslin cloth, non-absorbent cotton wool and Whatman No. 1 filter paper. After filtration, the liquid extracts were concentrated. The aqueous extract was concentrated using a lyophilizer (Amsco/Finn-Aqua Lyovac, Germany). Other extracts were first passed through a rotary evaporator (Buchi laboratoriums Technik AG, CH 92300) at a temperature of 40ºC. This was to evaporate the organic solvents, before using the lyophilizer for freeze-drying.

2.2 Phytochemical Screening

The leaf extracts of *B. sapida* were screened to ascertain the presence or absence of plant metabolites such as alkaloids, anthraquinones (free and combined), cardiac glycosides, flavonoids, tannins, phlobatannins, terpenes and saponins. This was done using the methods described by Harborne, Evans and Sofowora [10, 11, 12]. Chemicals and reagents used were all of analytical grade.

2.3 Bioassay for Larvicidal Activitiy

Larvae of *An. gambiae, Cu. quinquefasciatus* and *Ae. aegypti*, in their fourth in stars were used for experiments reported in this study. They were provided by National Arbovirus and Vectors Research Centre (NAVRC), Enugu, Nigeria. Assays were carried out in the Entomology unit of NAVRC, Enugu. The method used to assess the effect of extracts of the leaf of *B. sapida* on the three mosquito species was adopted from the methods described by Ojewole et al., [1] and WHO (14). A stock solution of 5% w/v of each extract was prepared and heated in a water bath at 40^0c for 2 minutes after which it was left to cool for about 30 minutes. From the stock solution graded concentrations of each extract was prepared to obtain 0.15, 0.30, 0.45, 0.60 and 0.75%w/v concentrations of each extract. Twenty five

larvae of each mosquito species were separately exposed to the bioassay medium in a final volume of 100ml formulation. Plastic assay cups containing larval nutrients (fine Quaker Oats in solution) were used for the experiments. Five replicates were set up for each extract concentration. The controls which were also replicated had twenty five larvae of the different mosquito species separately immersed in 100ml distilled water to which larval nutrient had been introduced. Both the test and control experiments were maintained at room temperature (28 ± 2ºC).

Observations were made at 24, 48 and 72h and the effect of the extracts on the larvae was noted. Larvicidal effect of each extract was determined by counting the number of dead larvae each day, until the end of the experiment. Larvae which neither moved nor responded to stimulus with a Pasteur pipette were considered dead.

2.4 Data Analysis

Microsoft excel was employed in analysis of data obtained from this study. Probit analysis was done using SPSS version 17.

3. RESULTS

Alkaloids, combined anthraquinones, cardiac glycosides, flavonoids, saponins, tannins, phlobatannins and terpenes were detected in the leaf of *B. sapida.* Free anthraquinones were not detected.

All extracts used in this investigation had notable toxicity signs on larvae of the three mosquito species. Prior to assays larvae were very agile and actively wriggling, but the introduction of the extracts resulted in reduction in activity (immobility) of the larvae. Examination of some of the dead larvae using a hand lens revealed disintegration of their integument (Plate 1).

Plate 1. Some dead larvae showing disintegration of integument

A gradient of increasing mortality with increase in extract concentration and increase in exposure time was observed in all treatments. No mortality was observed in the controls, rather larvae in the control experiments were agile and actively wriggling, throughout the duration of the experiment. Table 1 depicts the lethal effect of aqueous extracts of *B.sapida* leaves on larvae of *An. gambiae, Cu. quinquefasciatus* and *Ae. aegypti.* At the end of bioassays (72h) 0.60%w/v of the aqueous leaf extract of *B. sapida* resulted in 87.20, 60.80 and 64.80% mortality of larvae of *An.gambiae, Cu. quinquefasciatus* and *Ae. aegypti* respectively; whereas the highest concentration (0.75%w/v) resulted in 100% mortality of larvae of *An. gambiae* and *Cu. quinquefasciatus* at the end of experiment. The same concentration of extract produced 92.80% mortality in larvae of *Ae. aegypti.*

The ethanol leaf extract resulted in 100% mortality of An. gambiae at both 0.60 and 0.75%w/v concentrations of the extract, but the 0.75%w/v concentration resulted in 100% mortality of the larvae at 48h. For both larvae of *Cu. quinquefasciatus* and *Ae. aegypti*, 100% mortality of larvae was observed only at 72h (Table 2). 0.60% w/v of the ethanol leaf extract produced 100% mortality in larvae of An. gambiae at the end of experiment, whereas mortality values were 73.60 and 70.40% for *Cu. quinquefasciatus* and Ae. aegypti respectively.

Results obtained when larvae were exposed to the different concentrations of ethyl acetate extract of the leaves of *B. sapida* are shown in Table 3. Ethyl acetate extract produced 100% mortality in larvae of *An. gambiae* and *Ae. aegypti* at 0.60% w/v concentration, after 48h. At the same concentration and exposure time mortality of 80% was observed for larvae of *Cu. quinquefasicatus*. The highest concentration (0.75%w/v) resulted in 100% mortality of larvae of *An. gambiae* and *Ae. aegypti* at 24h, but 100% mortality was observed for larvae of *Cu. quinquefasciatus* at 48h. Results obtained when other concentrations of the extracts were tested against the larvae are also shown in Tables 1-3.

Table 4 depicts the 72hLC_{50} values of the extracts assayed against fourth instar larvae of *An. gambiae, Cu. quinquefasciatus* and *Ae. aegypti.* 72hLC_{50} values for the aqueous extract were 0.393, 0.488 and 0.423%w/v for larvae of *An. gambiae, Cu. quinquefasciatus* and *Ae. aegypti* respectively, whereas values for the ethanol extract were 0.319, 0.407 and 0.384%w/v for larvae of *An. gambiae, Cu. quinquefasciatus* and *Ae. aegypti* respectively. For the ethyl acetate extract 72hLC_{50} values obtained were 0.135, 0.133 and 0.177% w/v for larvae of *An.gambiae*, *Ae. aegypti* and *Cu. quinquefasciatus* respectively. These values were the lowest when compared to values obtained with aqueous and ethanol extracts. As judged by these 72hLC_{50} values the ethyl acetate extract was the most potent. Thus, potency of the extracts was in the order: Ethyl acetate > Ethanol > Aqueous.

Also based on the 72hLC_{50} values obtained, susceptibility was in the order:

Aqueous extract: *An. gambiae* > *Ae. aegypti* > *Cu. quinquefasciatus*
(0.393% w/v) (0.423%w/v) (0.488%w/v)

Ethanol Extract: An. gambiae > *Ae. aegypti* > *Cu. quinquefasciatus*
(0.319% w/v) (0.384%w/v) (0.407%w/v)

Ethyl acetate extract: *Ae. aegypti* > *An. gambiae* > *Cu. quinquefasciatus*
(0.133% w/v) (0.135%w/v) (0.177%w/v)

Table 1. Lethal effect of aqueous extract of the leaves of *B. sapida* on larvae of *An. Gambiae*, *Cu. quinquefasciatus* and *Ae. aegypti*

	An. gambiae **Percentage mortality**			***Cu. quinquefasciatus*** **Percentage mortality**			***Ae. Aegypti*** **Percentage mortality**		
Extract Conc. (%w/v)	**24**	**48**	**72**	**24**	**48**	**72**	**24**	**48**	**72**
0.15	0 (0.00%)	4(3.20%)	10(8.00%)	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)	2(1.60%)	12(9.60%)
0.30	3(2.40%)	10(8.00%)	27(21.60%)	0(0.00%)	7(5.60%)	18(14.40%)	4(3.20%)	13(10.40%)	30(24.00%)
0.45	9(7.20%)	25(20.00%)	59(42.40%)	7(5.60%)	20(16.00%)	40(32.00%)	12(9.60%)	27(21.60%)	59(47.20%)
0.60	28(22.40%)	65(52.00%)	109(87.20%)	22(17.60%)	48(38.40%)	76(60.80%)	18(14.40%)	43(34.40%)	81(64.80%)
0.75	51(40.80%)	107(85.60%)	125(100.00%)	31(24.80%)	71(56.00%)	125(100.00%)	25(20.00%)	65(52.00%)	116(92.80%)
Control	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)

24, 48 and 72 = Exposure period in hours
Numbers in brackets show percentage mortality

Table 2. Lethal effect of Ethanol extract of the leaves of *B. sapida* on larvae of *An. gambiae*, *Cu. quinquefasciatus* and *Ae. aegypti*

	An. gambiae **Percentage mortality**			***Cu. quinquefasciatus*** **Percentage mortality**			***Ae. Aegypti*** **Percentage mortality**		
Extract Conc. (%w/v)	**24**	**48**	**72**	**24**	**48**	**72**	**24**	**48**	**72**
0.15	0(0.00%)	7(5.60%)	19(15.20%)	3(2.40%)	9(7.20%)	17(13.60%)	0(0.00%)	3(2.40%)	16(12.80%)
0.30	7(5.60%)	18(14.40%)	36(28.80%)	4(3.20%)	12(9.60%)	26(20.80%)	6(4.80%)	17(13.60%)	35(28.00%)
0.45	18(14.40%)	40(32.00%)	79(63.20%)	10(8.00%)	24(19.20%)	47(37.60%)	15(12.00%)	33(26.40%)	66(52.80%)
0.60	36(28.80%)	79(63.20%)	125(100.00%)	25(20.00%)	53(42.40%)	92(73.60%)	21(16.80%)	47(37.60%)	88(70.40%)
0.75	53(42.40%)	125(100.00%)	-	39(31.20%)	90(72.00%)	125(100.00%)	30(24.00%)	84(67.20%)	125(100.00%)
Control	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)

24, 48 and 72 = Exposure period in hours

Table 3. Lethal effect of ethyl acetate extract of the leaves of *B. sapida* on larvae of *An. gambiae*, *Cu. quinquefasciatus* and *Ae. aegypti*

	An. gambiae Percentage mortality			*Cu. quinquefasciatus* Percentage mortality			*Ae. Aegypti* Percentage mortality		
Extract Conc. (%w/v)	**24**	**48**	**72**	**24**	**48**	**72**	**24**	**48**	**72**
0.15	13(10.57%)	36(28.80%)	77(61.60%)	12(9.60%)	31(24.80%)	59(47.20%)	18(14.40%)	45(36.00%)	77(61.60%)
0.30	30(24.00%)	62(49.60%)	99(79.20%)	19(15.20%)	45(36.00%)	82(65.60%)	29(23.20%)	65(52.00%)	106(84.80%)
0.45	58(46.40%)	111(88.80%)	125(100.00%)	36(28.80%)	78(62.40%)	119(95.20%)	54(43.20%)	107(85.60%)	125(100.00%)
0.60	88(70.40%)	125(100.00%)	-	48(38.40%)	100(80.00%)	125(100.00%)	98(78.40%)	125(100.00%)	-
0.75	125(100.00%)	-	-	85(68.00%)	125(100.00%)	-	125(100.00%)	-	-
Control	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)

24, 48 and 72 = Exposure period in hours

Table 4. The 72hLC_{50} values of aqueous, ethanol and ethyl acetate extracts of *B. sapida* leaves Assayed against larvae of *An. gambiae*, *Cu. quinquefasciatus* and *Ae. aegypti*

	An. Gambiae			*Cu. quinquefasciatus*			*Ae. Aegypti*		
Extract Used	**72hLC_{50} (%w/v)**	**95% confidence interval (%w/v)**	**Regression Equation**	**72hLC_{50} (%w/v)**	**95% confidence interval (%w/v)**	**Regression Equation**	**72hLC_{50} (%w/v)**	**95% confidence interval (%w/v)**	**Regression Equation**
Aqueous	0.393	0.055 – 0.906	Y =-12.682 + 4.888 x	0.488	0.310 – 0.721	Y = -17.823 + 6.630x	0.423	0.279 – 0.632	Y = -9.545 +3.634x
Ethanol	0.319	0.032-0.639	Y =-11.743 + 4.690 x	0.407	-	Y = -9.963 + 3.818x	0.384	0.198 – 0.617	Y=-10.223 + 3.955x
Ethyl acetate	0.135	0.004 - 0.209	Y=-7.651 + 3.589x	0.177	0.034-0.265	Y=-8.268 + 3.678x	0.133	0.044 – 0.184	Y =-8.138 + 3.834x

24, 48 and 72 = Exposure period in hours

4. DISCUSSION

Some plant metabolites were detected in the leaves of *B. sapida.* The mosquito larvicidal potential of plant metabolites have been documented by Francois et al. [15], Lee [16], Joseph et al. [17], Pelah et al. [18] and Wiesman and Chapagain [1]. Plant metabolites detected in the leaves of *B. sapida* may have exerted single, additive or synergistic effect on the larvae. Further investigation is needed to substantiate this.

The reduction in activity of larvae of the three mosquito species observed in this study corroborates the findings of Kaushik and Saini [20]. Disintegration of the integument of some dead larvae was observed in this study. Wiesman and Chapagain [19] also reported the same effect of their extract on their test mosquito larvae and they attributed it to the presence of saponins and tannins in the extract. Saponins and tannins were also detected in the leaves of *B. sapida.*

The biological activity of extracts is known to be dependent on some factors, one of which is the solvent used for extraction. Based on the 72hLC_{50} values obtained, the ethyl acetate extract was more potent than the other extracts used in this study, suggesting that the organic solvent (ethyl acetate) enhanced the extraction/release of the active principle(s) in the leaves.

The observed difference in susceptibility of mosquito larvae to the extracts corroborates the reports of other researchers. For instance, Mohan and Ramaswamy [21], reported that larvae of *Cu. quinquefasciatus* were more susceptible to the leaf extract of *Ageratina adenophora* (Asteraceae) than larvae of *Ae. aegypti,* with 24hLC_{50} values of 227.20ppm and 356.70ppm respectively. Similar trend of differential susceptibility of mosquito species to plant extracts was also reported by Kaushik and Saini (20), who evaluated the mosquito larvicidal activity of the leaf extract of *Millingtonia hortensis* (Bignoniaceae). From their studies they reported that the susceptibility order was observed to be *Cu. quinquefasciatus* > *Ae. aegypti* > *An. stephensi.* Pathak et al. [22] also reported on the differential susceptibility of mosquito larval species to their test plant extracts. This variation in susceptibility could be attributed to the difference in physiological response among species [23]. As aforementioned larvae of *An. gambiae* were more susceptible to the treatments with aqueous and ethanol extracts than larvae of the other species. This may have been due to a combination of factors such as the lethal effect of the extracts and the fact that larvae of this species often prefer clean water bodies as their habitat [24]. Their immersion in the test solutions thus amounted to an unfavourable environment for them. It represented an environment that was very different from their natural/preferred habitat. This certainly also posed a threat to their continued survival.

Finding out that some plants like B. *sapida* which are used freely in our communities contain compounds which can be used to control certain stages of mosquitoes in their development is of tremendous interest. This is considering the fact that mosquitoe has remained one of our greatest problems in Africa , killing and maiming both the young and old and eating deep into the meager finances of various governments as they invest so much in the procurement of drugs for the treatment of the resultant malaria.

5. CONCLUSION

Results obtained in this preliminary investigation demonstrate that the leaf extracts of *B.sapida* have marked larvicidal potential against the mosquito species, *An.gambiae, Cu. quinquefasciatus* and *Ae. aegypti.* This should be exploited in the formulation of agents for mosquito vector control. We are further interested in isolating and characterizing the active principle(s) responsible for the observed larvicidal action.

ACKNOWLEDGEMENT

Authors are grateful to Dr. Ibrahim Iliya and Mr. John Apev of the Department of Medicinal Plant Research and Traditional Medicine (MPRTM), National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria, and DR OKECHUKWU CHUKWUEKEZIE-Director national arbovirus and vectors research centre Enugu for their technical assistance. We also appreciate the assistance of Messrs Emmanuel O. Nwosu and E. Uwakwe of National Arbovirus and Vectors Research Centre (NAVRC) Enugu, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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