Special Conference Edition, November, 2017 http://dx.doi.org/10.4314/bajopas.v10i1.91S

Bayero Journal of Pure and Applied Sciences, 10(1): 477 - 480
ISSN 2006 – 6996

COMPARATIVE LARVICIDAL EFFICACIES OF *Nymphae lotus* FRACTIONS AGAINST *Anopheles* MOSQUITO LARVAE

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ABSTRACT
This study aimed at comparing the larvicidal efficacy of five extract of the leaves of Nymphaea lotus against Anopheles (Culicidae). Ethanolic extract of the dried leaves of Nymphaea lotus was partitioned with chloroform, methanol, n-hexane and water to yield fractions E001, E002, E003, E004 and E005 respectively. The larvicidal efficacies of each of the five fractions were tested in triplicates by exposing the larvae to 1000, 500, 250, 125, 62.5 and 31.25µg/ml respectively, the result obtained was subjected to One Way Analysis of Variance and Probit analysis using SPSS 16.0. Phytochemical screening of the five fractions reveals the presence of alkaloids, steroid, flavonoids, terpenoids, tannins and saponins. GCMS analysis of the five fractions shows the presence of over forty five (45) pre-cursor compounds of interest. The result obtained shows that the n-hexane fraction (E004) has the highest lethal effect against both the Anopheles culicidae mosquito larvae and Brine Shrimp with LC_{50} of 81.30 and159µg/ml, respectively.In light of the above findings, the n-hexane fraction of Nymphaea lotus could be of immense potentiality in the search for a bio-friendly larvicide for the fight against malaria as it has been found to be harmless to other aquatic organisms at lower concentration.
KEYWORDS: Larvicidal, Nymphaea lotus, Anopheles, leaves extracts, fractions, Culicidae

INTRODUCTION

Mosquito species are well known vectors of diseases affecting human beings particularly malaria and filariasis (Durga and Muruga, 2013). These vectors occurs mainly in tropical countries where more than two billion people live in endemic regions with about one million deaths been claimed yearly from malaria and filariasis (WHO Malaria Report, 2005; Southgate, 1984). An estimated 50 million people are infected with dengue each year caused by *An. aegypti*. Malaria, caused by *An. gambiae*, has a crippling effect on Africa's economic growth and perpetuates vicious cycles of poverty (World Malaria Report, 2013).
Malaria is a serious threat to the lives of over three point two (3.2) billion people worldwide, about half of the world population (WHO, 2015). However, the vector of this disease is ever becoming more resistant to synthetic insecticides which are also a threat to environmental safety. Recent researches show that, plant-based insecticides are proving to be more viable and environmentally friendly alternative for the control of mosquito. Therefore, the present research is geared toward investigating larvicidal potential of *Nymphaea lotus* against *Anopheles* (Culicidae).
Natural products of plant origin are safer to use than the synthetic insecticides, and hence they formed a broad research area for the control of vectors and microbes of medical importance (Mousumi *et al.,* 2013). A special feature of higher plants is their capacity to produce a large number of organic chemicals of high structural diversity called secondary metabolites (Castello *et al.,* 2002). And about 80% of plants selected for analysis on the basis of ethno-medicinal information have demonstrated significant pharmacological activity (Fatope, 2001). Screening of compounds obtained from plants origin for their pharmacological assay has indeed been the vast source of innumerable therapeutic agents representing molecular diversity engineered by nature (Mothana and Lindequist, 2005). One of the ways to prevent antibiotic resistance of pathogenic species is to use new compounds that are not based on existing synthetic antimicrobial agents (Shah, 2005).

MATERIALS AND METHODS

Collection and Identification of Plant Material

Fresh leaves of *Nymphaea lotus* were collected in September, 2014 in Kano city; around Dan Bare and Hauran Shanu pondsin Kano metropolis. The plant was identified using their morphological features such as aquatic habit, floating leaves, flamboyant flowers, several petals, many stamens and carpels and endospermous seeds with small embryo (Hutchinson and Dalziel, 1954), and confirmation was made at the Herbarium of the Department of Plant Biology, Bayero University, Kano as *Nymphaea lotus* leaves with Kano Herbarium Accession number BUKHAN0356.

The leaves were dried under shade at room temperature of 25 ± 3°C and grounded into powder using electric grinder until the powder passed through a 0.4 mm mesh sieve and then stored in a refrigerator at – 4°C.

Extraction and Fractionation of Plant Materials

The extraction scheme (Appendix I) was performed according to the standard method adopted by Okoye and Osadede (2009).

Five hundred grams (500g) of powdered plant material was subjected to solvent extraction for a period of two weeks by cold maceration in 95% ethanol with periodic shaking at least twice a day. The crude ethanol extract was then drained, filtered and concentrated almost to dryness under reduced pressure at 40°C using rotary evaporator RE300 (ROTAFLO, England).

Fractionation

The ethanol fraction labeled E001 was sequentially partitioned using methanol, chloroform, n-hexane, and water (Appendix I and Figures 1and 2). Ten grams (10g) of E001 was partitioned between 150ml each of chloroform and water respectively. The chloroform soluble fraction (E002) was drained and evaporated to dryness using rotary evaporator to yield 6.55g while the water soluble fraction (E005) was freeze dried to yield 3.15g using Freeze-drying machine (Lyophilizer); manufactured by York Scientific Industries Pvt. Ltd. (India). Fifteen grams (15g) of E001 was partitioned between n-hexane and 10% methanol (300ml each). Both the n-hexane soluble fraction (E004) and the methanol soluble fraction (E003) were evaporated to dryness using rotary evaporator to yield 1.27g and 2.67g respectively. The extract and fractions were stored in a refrigerator at - 4°C.

RESULTS

Physical Properties

The physical properties of the five fractions were presented in Table 1. The ethanolic extract (E001) obtained was dark green, tasteless, odorless, sticky and weigh 51.58g.

The chloroform fraction (E002) is dark green, bitter, smooth, with a minty odor and weighs 6.55g. The methanol fraction (E003) is dark green, bitter, smooth and odorless and weighs 2.67g. The n-hexane fraction (E004) is dark green in color, oily, tasteless, and odorless and weighs 1,27g. While the water fraction is light green, tasteless odorless and frothy and weigh 3.15g.

Table 1: Physical Properties and Appearances N*ymphaea lotus* of Leaves Fractions

Sample ID	Color	Taste	Odor	Texture	Volume
E001	Dark Green	Tasteless	Odorless	Sticky	50ml
E002	Dark Green	Bitter	Minty	Smooth	17ml
E003	Dark Green	Bitter	Odorless	Smooth	5ml
E004	Dark Green	Tasteless	Odorless	Oily	5ml
E005	Light Green	Tasteless	Odorless	Frothy	100ml

E001:- Ethanol Extract, E002:- Chloroform Extract, E003:- Methanol Extract E004:- n-Hexane Extract, E005:- Water Extract.

Table 2 shows the result of preliminary phytochemical screening of *N. lotus* which shows that fraction E001 contains low amounts of alkaloids, steroids, and saponins, moderate amounts of tannins and terpenes while flavonoids are absent. Fraction E002 contains low amounts of alkaloids, steroids, tannins and terpenes, moderate tannins and terpenes, while alkaloids, flavonoids, steroids, and saponins are absent. Fraction E003 contains low amounts of tannins and terpenes, while alkaloids, flavonoids, saponins and steroids are absent. Fraction E004 contains low amounts of steroids, saponins and terpenes, moderate amounts of alkaloids and tannins and high amounts of flavonoid. Fraction E005 contains low amounts of tannins, moderate amount of saponins, while alkaloids, flavonoid, steroids and terpenes are absent.

Table 2: Preliminary Phytochemical Screening of *Nymphaea lotus* Fractions

Sample ID	Alkaloid	Flavonoid	Saponin	Steroid	Terpenes	Tannin
E001	+	--	+	+	++	++
E002	+	++	--	+	+	+
E003	--	--	--	--	+	+
E004	++	+++	+	+	+	++
E005	--	--	++	--	--	+

(+):- Present at low Concentration; (++):- Present at moderate Concentration; (+++):- Present at high Concentration; (--):- Not Present.
E001:- Ethanol Extract, E002:- Chloroform Extract, E003:- Methanol Extract
E004:- n-Hexane Extract, E005:- Water Extract.

Table 3: Lists of Identified Organic Compounds Obtained from *N. lotus* Fractions Based on their Mass/Charge Ratio with Reference to Library

S/N	Compounds	Sample Fraction				
		E001	E002	E003	E004	E005
1	3, 5-dihydro-6-methyl-2, 3-dihydro-4H-Pyran-4-one	+	-	+	-	+
2	Dihydro-2-methyl-3(2H) furanone	+	-	+	-	-
3	Phthalic acid, di-(-1-hexen-5-yl) ester	+	+	+	-	+
4	N (5-Methyl-3-isoxazoyl) phthalimide	+	-	-	-	-
5	Alpha, 3-dihydroxy Benzene acetic acid	+	-	-	-	-
6	4-methyl-3-(1H-tetrazol-5yl)-1oxa-Spiro [4.4] non-3-en-2-one	+	-	-	-	-
7	1-(4, 5-dimethy) l-2-nitrophenyl)-1H-tetrazole	-	-	-	+	-
8	2- Phenyl propylbutarate	-	-	-	+	-
9	trans-3- caren-2-ol	-	-	-	+	-
10	2-nitrocumene	-	-	-	+	-
11	Palmitic acid, beta-monoglyceride	-	-	-	+	-
12	Squalene	-	-	-	+	-
13	Famesol isomer a	-	-	-	+	-

DISCUSSION

The physical properties of the five fractions show that E001, E002, E003 and E004 are all dark green in color, while E005 is light green. E001, E004 and E005 are tasteless, while E002 and E003 are bitter to the taste. All the fractions are odorless except E002 which has a minty smell. E001 has a sticky texture, E002 and E003 are smooth to the touch, and E004 is oily while E005 is frothy.

It has been shown that the mosquito larvae have respiratory siphon which they breathe through on spiracles located on the 8th abdominal segment and therefore must come to the surface frequently to breathe, hence, the oil could block the spiracles, resulting in asphyxiation and death of the larvae. Fraction E004 (n-hexane fraction) in this study is also oily (Table 1) and happens to be the most toxic of the five fractions.

Phytochemical analysis of *Nymphaea lotus* indicates the presence of tannin (+++), saponin (+++), resin (++), flavonoid (+) and phlobatannin (+). (Imam and Tajuddeen, 2013). These compounds may be responsible for the observed mortality in the test organisms. Saponins and alkaloids had been reported to be responsible for toxicity of seed coat of *Cassia sophera* on all instar larvae of *Cx. quinquefasciatus* (Mousumi *et al.,* 2013).

According to a research, tannins and alkaloids in *Pistia stratiotes*; tannins, alkaloids and steroid glycosides in *Typha latifolia*; tannins, saponins and steroid glycosides in *Leucas martinicensi*; alkaloids, saponins and tannins in *Cynodon dactylon* and saponins and tannins in *Nymphaea lotus* have been reported to be responsible for larval toxicity of *Anopheles* mosquitoes (Imam and Tajuddeen 2013).

CONCLUSION

In conclusion, the result of the present study revealed that n-hexane (E004) fraction of *Nymphae lotus* has the highest toxicity against the test organisms out of the five fractions analyzed, with LC_{50} values of 81.30µg/ml (Table 3) and 159µg/ml (Table 4) for Mosquito larvae and Brine shrimp respectively. Hence, n-hexane fraction of *Nymphaea lotus* leave extract could be useful in managing field populations of *Anopheles* mosquitoes. Application of the fraction to mosquito breeding habitats may yield a favorable result in malaria and mosquito management programs.

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