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Full Length Research Paper

Qualitative phytochemical screening and larvicidal potencies of ethanolic extracts of five selected macrophyte species against *Anopheles* mosquitoes (diptera: culicidae)

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Abstract

The study aimed at qualitative phytochemical screening and larvicidal potencies of ethanolic extract of five selected macrophyte species. The ethanolic extracts were obtained through percolation process and were used in carrying out phytochemical test for alkaloids, saponins, resins, flavonoids, steroid, reducing sugar, tannins and phlobatannins. The result showed that all the aforementioned phytochemicals were present in all the extracts with the exception of resins. The toxicity test was carried out using mosquito larvae and the result obtained showed that extracts of *Pistia stratiotes*, *Typha latifolia*, *Leucas martinicensis, Cynodon dactylon*, and *Nymphaea lotus* has LC_{50} of 63.3, 68.1, 68.4, 68.1 and 62.8 mg/L respectively. This shows that *N. lotus* has the highest lethal effect while *L. marticinensis* has the least. Considering the side effect of chemical insecticides to human health, it is suggested that the use of organic insecticides should be encouraged so as to ameliorate health problems, since it is eco-friendly in nature.

Keywords: Macrophytes, phytochemical, ethanolic, toxicity.

INTRODUCTION

Aquatic plants are often an integral component of aquatic ecosystems and can be of ecological importance since they represent the major structural component of littoral habitats, acting as shelter, nesting, and feeding grounds for a wide variety of macro invertebrates, fish and waterfowl (Hudon *et al*., 2000). Various ways have been used in repellant of insect pest like mosquitoes, termites, millipede, earwigs, slugs, ants, cockroaches etc. These include the use of organic pesticides, inorganic pesticides, general methods, bio-control etc (Chevillon *et al*., 1999). Organic pest control does seem to be less harmful to humans they still work wonders in eliminating pests. Some organic garden pest control products contain citrus oils which are good at killing flying pests while others use things such as silica aerogel which absorbs all the moisture from insect and insect tends to die of dehydration (Ware, 1994). In modern times, the active ingredients and curative actions of medicinal plants were first investigated through the use of European Scientific methods (Herborn, 1998). The most important ingredients present in plant communities turn out to be alkaloids, terpenoids, steriods, phenols glycosides and tannins (Abayomi, 1993).

Phytochemicals are botanicals which are naturally occurring insecticides obtained from floral resources. Applications of phytochemicals in mosquito control were in use since the 1920s (Shahi *et al*., 2010) but the discovery of synthetic insecticides such as DDT in 1939 side tracked the application of phytochemicals in mosquito control programme. Several groups of phytochemicals such as alkaloids, steroids, terpenoids, essential oils and phenolics from different plants have been reported previously for their insecticidal activities (shaalan *et al*., 2007). At present phytochemicals make up to 1 percent of world's pesticide market (Isman, 1997). . Kishore *et al.,* (2011) reviewed the efficacy of phytochemicals against mosquito larvae according to their chemical nature and described the mosquito larvicidal potentiality of several plant derived secondary materials, such as, alkanes, alkenes, alkynes and

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Table 1. Plant evaluated for Insecticidal Activity

Scientific name	Common name	Family	Parts used
P. stratiotes	Water lettuce	Araceae	Leaves
T. latifolia	Cattail	Typhaceae	Leaves
L. martinicensis	Whitewort	Lamiaceae	Leaves
C. dactylon	Bermuda grass	Poaceae	Leaves
N. lotus	Waterlily	Nymphaeaceae	Leaves

Table 2. Preparation of stock and standard solutions according to dilution ratios

Dilution(v/v)	Concentration (mg/l)	Required amount (ml)
1:1	500	25
1:2	250	12.5
1:3	125	6.25
1:4	62.5	3.13
1:5	31.3	1.56

generally the active toxic ingredients of plant extracts are secondary metabolites that are evolved to protect them from herbivores. Sukumar *et al.,* (2001) have described the existence of variations in the level of effectiveness of phytochemical compounds on target mosquito species *vis-à-vis* plant parts from which these were extracted, responses in species and their developmental stages against the specified extract, solvent of extraction, geographical origin of the plant, photosensitivity of some of the compounds in the extract, effect on growth and reproduction.

This study is aimed qualitative phytochemical screening and larvicidal potencies of five selected macrophytes against *Anopheles* mosquitoes (Diptera: Culicidae).

MATERIALS AND METHOD

Collection and Identification of Macrophytes

The five selected macrophyte species were collected at Hauren shanu, Hauren wanki and Ruwan madara burrowpits along BUK road in urban Kano. Plant species were handpicked with the aid of hand gloves and identified based on Walker, *et al*., (2001); and Quattrochi, (2000) protocols. The species identified were *Pistia stratiotes* (A), *Typha latifolia* (B) *Leucas martinensis* (C), *Cynodon dactylon* (D) and *Nymphaea lotus* (E) (Table 1).

Ethanolic Extraction

The plant materials were washed, air-dried in a shade and powdered by a mechanical grinder. Ten (10) grams of powder of each sample was dispensed in 100 ml of ethanol kept for two weeks with shaking at regular intervals after which the content was filtered using Whatmann filter paper (N0 1), the extracts were kept in sterile bottles (Herborn, 1998).

Collection and Identification of Mosquito Larvae

The mosquito larvae were collected in a container from stagnant water at BUK old campus and identified by direct visualization based on their position on water surface and the size of their head. The species collected are those of Anophelene larva.

Preliminary Phytochemical Screening

The extracts were analyzed for the presence of alkaloids, reducing sugars, saponins, tannins, resins, flavonoids, phlobatannins and steriods according to the method described by (Trease and Evans, 1989; Sofowora, 1982) for testing phytochemical compounds

Evaluation of Toxicity using Mosquito Larvae

Preparation of Stock and Standard Solution

Ten (10) grams each of the extract was added to 100ml of Dimethylsulphoxide (DMSO) to make the stock solution. It is expressed in weight per volume (w/v). The standard solution was made by serial dilutions. The concentrations are shown in Table 2.

Three (3) replicates of each dose are made per ethanolic extract of macrophytes at different dilutions. To make the first dose which was in ratio of 1:1, 25ml of extract was added to 25ml of tap water in a 50ml container and 15 mosquito larvae were introduced. The second dose (ratio of 1:2), 12.5ml of extract was added to 37.5ml of tap water in a 50ml container and 15 mosquito larvae were added. The third dose (ratio of 1:3), 6.25ml of extract was added to 43.75ml of tap water and 15 mosquito larvae were added. The fourth dose (ratio 1:4), 3.13ml of extract was added to 46.87ml of tap water and 15 mosquito larvae were added. The fifth dose (ratio 1:5), 1.56ml of extract was added to 48.44ml of tap water and 15 mosquito larvae species were introduced, all are covered with a perforated cover to allow constant circulation of air and observed after intervals of 3hrs each for 24hrs to observe their mortality. The procedure protocol of Ibeh (2004) for test for toxicity was adapted.

Statistical analysis

Estimation of LC_{50} was done using probit analysis by employing SPSS software (version 16.0) statistical software so as to obtain 50% lethal concentration per each macrophytes extracts at $P \leq 0.05$.

RESULT AND DISCUSSION

The physical properties of the ethanolic extract of 5 selected macrophyte species are presented in Table 3. *P. stratiotes* are light green in color and odorless, *T. latifolia* has pale grayish green color and odorless, *L. martinicensis* are dark green and minty odor, *C. dactylon* are smooth in texture and has minty odor, *N. lotus* are dark green and odorless as compared and accepted with the works of Invasive Species Specialist Group (ISSG, 2005) of the IUCN species survival commission.

Table 4 shows the preliminary phytochemical screening which shows that all the plants contain tannins in high amount and alkaloids, saponin, flavonoids, steroid glycosides, phlobatannin, reducing sugar in moderate amount but resins is totally absent in all the samples. Several groups of phytochemicals such as alkaloids, steroids, terpenoids from different plants have been reported previously for their insecticidal activities (Shaalan *et al*., 2005). Saponins have been reported to be useful in reducing inflammation of the upper respiratory tract (Frantisek, 1991). Alkaloids compounds extracted from the skin of poison frog (dendrobatids) from the Smithsonian Institution, Virginia, were found to repel adult mosquitoes and that very little amount was required to have toxic effect. Alkaloids are known for their toxicity but not all alkaloids are toxic. They inhibit certain mammalian enzymes activites and are also known to affect glucagon and thyroid stimulating hormones (Okaka *et al*., 1991). Steroids are of importance and interest in pharmacy due to their relationship with such compounds as sex hormones (Okwu, 2001). Other compounds like saponins, flavonoids and tannins have larvicidal effect on mosquitoes (El Hag *et al*., 1999). It is also possible that repellency of the extract from the plant might arise through odor. Probably, certain compounds not exploited in the present investigation because of time and material constraints might play a role in repelling the mosquitoes. *Leucas martinicensis* has a repellent property against adult mosquitoes. It is indicated that adult mosquitoes are repelled by the plant leaf extract. The component such as flavonoid, alkanoid and volatile oil, might be responsible for repellency of the adult culex mosquitoes. The flavonoid and alkanoid compounds from *L. martinicensis* can be a potential candidate for use in the development of commercial mosquitocidal products that may be an alternative to conventional synthetic chemicals, particularly in integrated vector control application (Muhammad *et al*., 2012).

Table 5 shows the toxicity studies of mosquito larvae after been exposed to the extract for 9hrs. The table revealed that *N. lotus* has the highest mortality rate and *C. dactylon* has the least. All the larvae die at high concentrations within minimum time. This shows that the higher the concentration of extract, the more the mortality. *Nymphaea* spp has great degree of repelling mosquitoes as compared and accepted with the works of Melanie (2007).

Preliminary test with 15 mosquito larvae per dose level was conducted to establish the range of toxicity so that the proper dose level could be established for LC_{50} determinations. With the toxicity test, it was possible to establish the highest dose of the extract that killed all of the larvae (62.8mg/L) and the lowest dose that killed all the larvae (68.4mg/L). The dose levels used in the acute toxicity study ranged between these two doses extremes.

Highest LC_{50} (62.8mg/L) was observed from the *N. lotus* extract, while least LC_{50} (62.8mg/L) was observed from *L. martinicensis* extract. This result inferred that 50% mortality can best be achieved if *N. lotus* extract are used, whereas least lethality at 50% can be obtained when *L. martinicensis* extract are used. Although *L. martinicensis* showed a significant mosquito repellent potential, relatively it could not be best larvicidal agent. It is worthy of note that the LC_{50} of both the macrophytes extracts are comparatively less lethal when compared with methanolic extract of *Carica papaya* against larvae of *Culex pipiens pipiens* with LC_{50} of 25.49mg/L (Olayemi *et al*., 2013).

CONCLUSION

In conclusion, *Nymphaea lotus* has the highest lethal potential larvicide against mosquitoes and *L. martinicensis* has the least least among the five macrophytes spp. Today, environmental safety is considered to be of paramount importance. An insecticide

Table 3. Physical Properties of Ethanolic Extract of Five Selected Macrophyte Species

SAMPLE ID	AMOUNT (in ml)	COLOR	TEXTURE	ODOR
A	19.8	Light green	Smooth	Odorless
B	29.9	Pale grayish- green	Oily	Odorless
C	34.4	Dark green	Smooth	Minty
D	6.7	Dark green	Smooth	Odorless
E	8.7	Dark green	Smooth	Odorless

KEY:
A- *Pistia stratiotes*
B- *Typha latifolia*
C- *Leucas martinicensis*
D- *Cynodon dactylon*
E- *Nymphaea lotus*

Table 4. Preliminary Phytochemical Screening of Ethanolic Extract of Five Selected Macrophytes Species

Sample ID	Alk	Fla	Res	Rs	Sap	Sg	Tan	Phl
A	++	_	_	++	_	_	++	+
B	++	+	_	+	_	+	++	+
C	_	+	_	++	++	+	+++	+
D	+	_	_	_	+	_	++	+
E	_	+	_	++	+++	_	+++	+

KEY:
- : not detected; + : trace; ++ :moderate constituent; +++ : large constituent; Alk: alkaloid; Fla: flavonoid; Res: resin; Rs: reducing sugar; Sap: saponin; Sg : steroid glycosides; Tan: tannin; Phl: phlobatannin

Table 5. Larvicidal Potencies of Ethanolic Extract of Five Macrophytes against Mosquito Larvae

Sample ID	Dilution	Concentration (mg/L)	Number of Larvae Before (0HRS)	After (9HRS)	Mortality Rate (%)	LC_{50} (mg/L)
A	1:1	500.0	15	0	100	
	1:2	250.0	15	0	100	
	1:3	125.0	15	0	100	
	1:4	62.5	15	9	40	
	1:5	31.3	15	12	20	63.3
B	1:1	500.0	15	0	100	
	1:2	250.0	15	0	100	
	1:3	125.0	15	0	100	
	1:4	62.5	15	10	33.3	
	1:5	31.3	15	13	13.3	68.1
C	1:1	500.0	15	0	100	
	1:2	250.0	15	0	100	
	1:3	125.0	15	0	100	
	1:4	62.5	15	9	40	68.4
	1:5	31.3	15	11	26.7	
D	1:1	500.0	15	0	100	
	1:2	250.0	15	0	100	
	1:3	125.0	15	0	100	
	1:4	62.5	15	11	26.7	68.1
	1:5	31.3	15	13	13.3	
E	1:1	500.0	15	0	100	
	1:2	250.0	15	0	100	
	1:3	125.0	15	0	100	
	1:4	62.5	15	8	46.7	
	1:5	31.3	15	11	26.7	62.8
Control	0	0	15	15	0	

does not need to cause high mortality on target organisms in order to be acceptable but should rather be eco-friendly in nature.

RECOMMENDATION

- Further work should be carried out on other aquatic macrophytes to test for their larvicidal ability.
- Further phytochemical screening should be conducted to isolate active compounds in the extract responsible for mortality of the larvae.

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