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SECONDARY PLANT METABOLITES OF LEAF EXTRACTS OF COMMON WIRE WEED (*Sida acuta*), CATNIP (*Nepeta cataria*) AND NEEM (*Azadirachta indica*) AND THEIR LARVICIDAL ACTIVITIES AGAINST MOSQUITO (*Aedes aegypti*)

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ABSTRACT

Dengue is a viral disease caused by dengue virus transmitted by Aedes mosquitoes. Extensive use of chemical insecticides for control of diseases vectors has created problems related to pesticide resistance to vectors, adverse environmental effects, high operational cost, pest resurgence and vertebrate toxicity. Hence the emphasis should be given on botanical insecticides which are more eco-friendly and effective. This study was conducted to evaluate the potential of some indigenous plant extracts to control the larva of mosquito in the laboratory of Sher-e-Bangla Agricultural University, Dhaka. The bioassay of the extracts revealed their high potential as larvacides which increase with the increase in concentration of 2, 5 and 10 mg/L respectively. The *Nepeta cataria* showed highest mortality rate at different concentration of the extract with LC_{50} of 0.98 mg/L. The phytochemical analysis shows the three extract to contain secondary plant metabolites like alkaloids, flavonoids, saponins and terpenoids at different concentrations. The study revealed that the extracts are very potent against the larva of the mosquito, therefore more effort should be made to harness the potential of these available raw material as botanical pesticide.

Keywords: Dengue, plant extract, larvacidial.

INTRODUCTION

Principal way of controlling dengue and chikungunya in the tropics and sub-tropics is by attacking the vector of the disease; mosquito. *Aedes aegypti*. The resistance of the vector to the most notable form of the pesticide has been a major concern hence, the promotion of botanical pesticides which are known to be safe and active. The toxicological effects of plant extracts or pure compounds on insects can be manifested in several manners including toxicity, mortality, anti-feedant growth inhibitor, suppression of reproductive behaviour and reduction of fecundity and fertility (Jbilou *et al.*, 2006). The misuse and excessive use of synthetic insecticides may cause some undesirable effects not only to the agricultural ecosystem but also to human health due to insecticide residue in food (Dadang *et al.*, 2009). Apart from this, there has been a major concern for the promotion of botanical pesticides as environmental friendly pesticides although there could still be a need to depend on chemical insecticides in case of epidemic outbreak (Abdelouaheb *et al.*, 2009). Prior to the discovery of the Organochlorine and Organophosphate insecticides in the late 1930s and early 1940s, botanical insecticides were important sources for pest management in industrialized countries (Isman 1997). Neem plants (Azadirachta indica) is one of these plants of interests, the plants has been used as botanical insecticides and are relatively safe towards non-target organism, less likely to induce resistance, due to their multiple modes of action on insects (Umar *et al.*, 2007). Apart from the extract, dried neem leaves and bark are commonly used in villages for protection against infestation of stored grain and other products by insects. *Sida acuta* and *Nepeta cataria* are plants that their insecticidal potency has been reported by various authors (Adeniyi *et al.*, 2010; Karou *et al.*, 2007). Interest in plants with insecticidal properties has been on the increase around the world today, either singly or in conjunction with synthetic pesticide and many of these extracts have been reported to be highly effective. This study aim to compare the effectiveness of the three local medicinal plants for their effectiveness to control mosquito larva.

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MATERIALS AND METHODS

Larvicidal impact of three plants was conducted at the Entomology Laboratory of Sher-e-Bangla Agricultural University, Dhaka from June-October, 2015.

Sample collection and preparartion

Fresh leaves of three native plants (*A. indica, S. acuta and N. cataria*) were collected from the botanical garden Mirpur, Dhaka where they grow as wild plants. The plants were identified by the Department of Crop Botany, Sher-e-Bangla Agricultural University, Dhaka. The leaves were air dried at room temperature (29°C) for 3 weeks, crushed and stored in air tight polythene bags

Extraction of plant materials

Methanol extraction was carried on the plants. 250g each of the ground samples was suspended in 4000ml methanol for a period of 72 h at room temperature. The mixture stirred thoroughly and filtered through whatman filter paper and the extracts were concentrated using a rotary evaporator. The concentrates were evaporated to dryness in a water bath (40°C) and stored in labelled specimen bottle for bioassay.

Larvae rearing

Aedes aegypti mosquitoes were collected from Sher-e-Bangla Agricultural University, Dhaka. Adult of both sexes were fed with 5g/250ml of sucrose solution in a caged plastic covered with net. Eggs of these mosquitoes were subsequently cultured in the Entomology Laboratory of Sher-e-Bangla Agricultural University, Dhaka. Larvae were reared in plastic jar containing tap water and maintained at 25 – 27°C, cool environment and dark photo period cycle. They were fed with fresh food containing mixure of Cabin Biscuit and Regal – dried yeast (75 – 25 by weight) until reached the 4^{th} instars larvae.

Phytochemical screening

Chemical tests were carried out on the powdered samples using standard procedures to identify the constituents as described by Sofowora (2003), Trease and Evans (1989) and Harborne (1973). This was done qualitatively and quantitatively in the Microbiology and Biochemistry Laboratory of North South University, Dhaka.

Procedure of qualitative and quantitative analysis

Test for Alkaloids

Add 3 ml of each plant extract and stir with 3 ml of 1% HCl on steam bath. Mayer and Wagner's reagent was then added to the extract. Turbidity of the resulting precipitate was taken as an evidence for the presence of alkaloids.

Test for Flavonoids

To 3ml of each plant extract, 1 ml of 10% lead acetate solution was added. The formation of a yellow precipitate was taken as a positive result for flavonoids.

Test for Saponins

3 ml of each plant extract was shaken vigorously with an equal volume of distilled water in a test tube and the mixture was warmed. The formation of stable foam was taken as an indication of presence of Saponins.

Test for Steroids

1g of each plant extract sample was shaken vigorously with 2 ml of Lieberman–Burchard reagents in a test tube. The formation of a green or green-blue colour after a few minutes was the positive test of steroids.

Test for Tannins

3 ml of each plant extract was stirred with 3 ml of distilled water and few drops of FeCl3 solution were added. The formation of green color precipitate indicates the presence of tannins.

Test for Phlobatannins
3 ml of each plant extract was added to 2 ml of 1%HCl and the extract was boiled. Deposition of a red precipitate was taken as an evidence for the presence of phlobatannins.

Test for Terpenoids
3 ml of each plant extract was dissolved in 2 ml of chloroform and evaporated to dryness. 2 ml of concentrated sulphuric acid was then added and heated for about 2 min. Development of greyish color indicates the presence of terpenoids.

Test for Cardiac Glycosides
1.0 g of each plant extract was dissolved in 1 ml of glacial acetic acid containing one drop of ferric chloride solution. This was then underlayered with 1 ml of concentrated sulphuric acid ($H_2 SO_4$).

Reducing Sugar
To 0.5 ml of each plant extract solution, 1 ml of water and heated after adding 5–8 drops of Fehling's solution. Brick red precipitation indicated the presence of reducing sugar.

Bioassay and larva mortality
Bioassays were performed with 4th instar larvae stages using concentrations from 2, 5 and 10mg/L for each of the extracts and untreated water for control. A minimum of 15 larvae per concentration were used for all the experiments to maintain uniformity of batches of larvae. Larvae mortality was assessed after every 24 h of exposure and moribund larvae were counted dead (Azmi *et al.*, 1998). The experiments were repeated four times for each concentration of the extracts and percentage average mortality was calculated by using.

$$\% \text{ mortality} = \frac{\text{Number of dead larvae}}{\text{Total number of larvae introduced}} \times 100$$

Mortality data were corrected using the Abbot`s (1925) formula.

$$\% \text{ Corrected mortality} = \left(1 - \frac{\text{Population in treatment after treatment}}{\text{Population in control after treatment}}\right) \times 100$$

RESULTS AND DISCUSSION

Table 1. shows that the alkaloids, saponins, tannins, terpenoids and flavonoids were present in all the extracts of *A.indica, S. acuta and N. cataria*. Steroids and cardiac glycosides were present in the extracts of *A. indica* and *S. acuta* but absent in *Nepeta cataria* while phlobatannins was present in the extracts of *N. cataria* only.

Table 1. Qualitative analysis of the phytochemicals contents of plant extracts

Phytochemicals	*Azadiracta indica*	*Sida acuta*	*Nepeta cataria*
Alkaloids	+	+	+
Flavonoids	+	+	+
Saponins	+	+	+
Steroids	+	+	-
Tanins	+	+	+
Phlobatannins	-	-	+
Terpenoids	+	+	+
Cardiac Glycosides	+	+	-
Reducing sugar	-	-	-

+ = Presence of constituent
- =Absence of constituent

Table 2. shows the quantitative phytochemicals determination of the three plant extracts of *A. indica, S. acuta* and *N. cataria*. Alkaloids, saponin, and flavonoids were present at different level in all the extract. Tables 3, 4 and 5 show the percentage larvae mortality when treated with the extract of the *A. indica, S. acuta* and *N. cataria,* respectively at concentration of 2, 5 and 10mg/L and control. The probit analysis at LC_{50} of the extract shows 6, 5 and 0.98mg/L respectively.
Identification of various plant extract that have larvicidal potential activities against aedes mosquito can be of advantage in reducing the problem of resistance and concern for the environmental safety.

Table 2. Quantitative analysis of the phytochemicals contents of three plant extracts.

Phytochemicals	***Azadiracta indica (%)***	***Sida acuta (%)***	***Nepeta cataria (%)***
Alkaloids	3.5660	1.2210	4.0220
Flavonoids	1.7605	1.8400	1.1720
Saponins	4.0720	2.0070	3.4900

Table 3. Larvae mortality at different concentrations *Azadiracta indica* from 24h to 192h.

Conc.(mg/L)	**% Mortality**	**Corrected mortality**	**LC_{50}**
2	43.33±1.85	41.4±0.37	
5	50±2.412	48.3±0.48	6mg/L
10	61.6±1.16	60.7±0.23	
Control	3.3±%		

Each value (X±S.E) represents mean of four values for the period of 192h

Table 4. Larvae mortality at different concentrations *Sida acuta* from 24h to 192h.

Conc.(mg/L)	**% Mortality**	**Corrected mortality**	**LC_{50}**
2	33.3±1.62	3.33±0.29	
5	50±2.44	50±0.48	5mg/L
10	71.6±1.83	71.6±0.36	
Control	0.0		

Each value (X±S.E) represents mean of four values

Control of virus bearing vectors is a common way of disease control. Larva control of mosquito can reduce the population of the insect which could reduce the burden of the disease. Aedes mosquito breeds in water where it hatches into larva until adult. The use of conventional pesticide in water posses many risks and health hazards to the people and the environment. *A. indica, S. acuta* and *N.cataria* extracts that were focused in this study all proved to hold good insecticidal promises against dengue vector. The potential of these extract either having larvicidal or insecticidal activities has earlier been explored by various authors. Also, many authors have widely reported the chemotherapeutic ability of some of these extract as dengue herbs or other medicinal uses (Abdelouabeb *et al.*, 2009; Umar *et al.*, 2007). The phytochemical screening results indicated that the leaves extracts of these plants were rich in alkaloids, flavonoids and tannins and saponins which are responsible for the insecticidal properties observed in these plants. These phytochemical have earlier been reported to have larvicidal and insecticidal abilities by other authors (Sofowora 1993).

Table 5. Larvae mortality at different concentrations *Nepeta cataria* from 24h to 192h.

Conc.(mg/L)	**% Mortality**	**Corrected mortality**	**LC_{50}**
2	71.6±0.75	71±0.15	
5	75.0±0.53	74.5±0.13	0.98mg/L
10	91±0.76	90.8±0.76	
Control	**1.7±0.25**		

Each value ±S.E) represents mean of four values

Neem crude extract or oil has specifically been reported to inhibit metamorphosis thereby disallowing pupation or adult emergent of the mosquito (Kabaru and Gichia, 2001).The result of this study agreed with the finding of Okumu *et al.* (2007) where it was reported that neem is highly toxic to aedes mosquito and delay pupation. Exposure of *Aedes aegypti* larvae to sub-lethal doses of neem and catnip leaves extract in the laboratory prolonged larvae development and pupation (Su and Mulls 1999).This view is in consistent with this present study. The probit analysis of percentage mortality of the three extract at LC_{50} shows moderate to average level of concentration. This is also in agreement with the finding of Shaalan *et al.* (2005) and Zhu *et al.*, (2008). In conclusion, the three plants have shown great potential as botanical pesticides. The plants are abundant in Bangladesh therefore cheap raw material that could be harnessed for the control of dengue and chikungunya in Bangladesh.

CONCLUSION

In conclusion, the three plants have shown great potential as botanical pesticides showed low resistance for vector of dengue and chikungunya. The plants are abundant in Bangladesh therefore cheap raw material that could be harnessed for the control of dengue and chikungunya in Bangladesh by saving and maintaining contamination free environment.

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