

RESEARCH ARTICLE

PRELIMINARY PHYTOCHEMICAL QUALITATIVE AND QUANTITATIVE ANALYSIS OF *BRASSICA OLERACEA* LEAF EXTRACTS ON FOURTH INSTAR LARVAE OF *AEDES AEGYPTI* (CULICIDEA: DIPTERA)Loganathan, R.^{1*}, S. R. Vasugi¹, D. Rajmohan², S. Senthilkumar³ and A. Karthikeyan⁴¹Department of Zoology, Periyar EVR College, Tiruchirapalli, Tamil Nadu, India.²Department of Zoology, Kongunadu Arts and Science College, Coimbatore, Tamil Nadu, India.³Department of Zoology, Kandasamy Kandar's College, Parmathivelur, Tamil Nadu, India⁴Department of Zoology, Govt Arts College, Karur, Tamil Nadu

ABSTRACT

Aedes aegypti, dengue fever mosquito, is primarily associated with the transmission of dengue in tropical and subtropical regions of the world. The present investigations was carried out to assess the larvicidal efficacy of aqueous and ethanol leaf extract of *Brassica oleracea* var. *botrytis* L against 4th instar larvae of *Ae.aegypti*. The concentration of extracts prepared from the leaf of plant *Brassica oleracea* var. *botrytis* L. were screened for their larvicidal activity against early fourth instars of dengue vector. The mortality rate of *Ae.aegypti* against aqueous and ethanol extracts of *B.oleracea* as follows 72% and 96%. Ethanol extract exposed to IVth larvae of *Ae.aegypti* is more efficiency than the aqueous extract. The present investigation suggest the possible use *B. oleracea* L. as an agent for the control of dengue vector, *A. aegypti*.

Keywords: Phytochemical analysis, *Brassica oleracea*, *Aedes aegypti*.

1. INTRODUCTION

Mosquitoes can distributing a number of diseases than any other group of arthropods and affect more than 700 million people worldwide annually, including arboviruses responsible for yellow fever, dengue hemorrhagic fever, epidemic polyarthritis, several forms of encephalitis bancroftian filariasis (1) and pathogens which continue to have devastating effect on human beings (2). Personal protection from mosquito bites is currently the most important way to prevent transmission of this disease (3). To prevent these mosquito borne diseases and to improve quality of environment and public health, mosquito control is essential. Larvicide is successful way of reducing mosquito densities in their breeding places before they emerge into adults. Pesticides are indeed very effective in its use. However, the use of chemical insecticides are often toxic to both human and non-target animals. The intensive use of chemical insecticides led to the development of resistant insect populations, resulting in reduced control, environmental pollution resulting in bio-amplification in food chain and contamination (4).

Plants have the major advantage of still being the most effective and cheaper alternative green measure for the control of arthropods of public health importance (5,6). Natural products of plant origin are safe to use than the synthetic insecticides (7). *B. oleracea*, the sole species in the genus *Brassica* of the plant family *Brassicaceae* is

widely cultivated. *Brassica* is a small herb-like plant, with a single stem growing from 3 to 5 feet short with spiral leaves. It is used as remedy against a variety of diseases (8,9). The leaf *Brassica oleracea* extracts have larvicidal and antioxidant activities (10). The main objective of the study was to test the larvicidal ability of aqueous and ethanolic leaf extracts of *Brassica oleracea* var. *botrytis* (Cauliflower) against *Aedes aegypti* mosquito larvae.

2. MATERIALS AND METHODS

2.1. Location of the study

This research was conducted at the Research Laboratory of P.G and Research Department of Zoology, Periyar E.V.R College, Tiruchirappalli.

2.2. Plant collection and Processing

The fresh leaves of plant *Brassica oleracea* var. *botrytis* were collected from weekly market Muthur, Tiruppur, district, Tamil Nadu, India. The selected plant parts were separated washed, dried, powdered and the extracts were filtered using whatman filter paper. Chemical test were carried out on the crude aqueous and ethanolic extracts using standard procedures to identify the phytochemical constituents like alkaloids, carbohydrates, proteins, coumarins, phenols, saponins, tannins, flavonoids, as described by Sofowora (11); Trease and Evans (12); Horborne (13).

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2.3. Mosquito species

Mosquito larvae of *Aedes aegypti* were collected from ICMR (Indian Council for Medical Research) Madurai, Tamil Nadu, India. *Ae. aegypti* was obtained an egg rafts on the filter paper and were reared in trays containing tap water and maintained at $28 \pm 2^\circ\text{C}$. When the eggs were hatched out into first instar larvae, they were fed with a mixture of yeast powder and dog biscuits in the ratio of (1:3). On the third day after hatching the first instar larvae moulted into second instar larvae on the fifth day, third instar larvae observed, which moulted into fourth instar larvae on the seventh day (14). The 4th instar of *Aedes aegypti* was experimented for the present study.

2.4. Larvicidal Assay

The larvicidal assay was conducted according to (15). In the Larvicidal assay on 4th instar larvae of *Aedes aegypti* were exposed to test concentration of 1% extract of crude aqueous and ethanol of *B. oleracea* L. separately. 1ml of solution was taken in separate bowl made up to 100 ml with distilled water and respective solvent separately for crude aqueous and ethanol extracts. Five concentrations of Aqueous and Ethanol leaf extracts were taken for experiments (2, 4, 6, 8 and 10%) and 20 numbers of 4th instars larvae were transferred gently to the test medium separately, simultaneously a control was maintained without extracts. The larval mortality in both treated and control were recorded after 6, 12, 24, 48, 72, and 96 hrs. Dead larvae were collected by tip of thin brush. This experiment was repeated five times. The percentage mortality was calculated by $\text{No of larvae Dead/Total No. of larvae} \times 100$.

2.4.1. Estimation of total Phenols

The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic components 15 mts. 5ml of the extract was pipette into a 50 ml flask, then 10 ml of distilled water was added. 2ml of ammonium hydroxide solution and 5ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 mts for colour development. This was measured at 503nm.

2.4.2. Estimation of total Flavonoids

Ten grams of sample was repeatedly extracted with 100ml of 80% aqueous and methanol at room temperature. The mixture was then filtered through a filter paper into a pre-weighed 250ml beaker. The percentage flavonoid was calculated by difference (16).

2.5. Statistical analysis

The concentration at which mortality observed (mg/ml) was corrected using Abbott's (17) formula. Statistical analysis of the experimental data was performed with MS Excel 2007 to find the Mean and Standard deviation values are tabulated.

3. RESULTS AND DISCUSSION

The present study has been carried out to assess the mortality of the aqueous and ethanolic extracts of plant *B. oleracea* on mosquito larvae. Details of plant extracts used for the present study of the larval mortality and the phytochemical constituents noticed are in the Table 1, 2 and 3. The effects of plant extracts on mosquito larvae exposed to 96 hrs, for confirming mortality as per WHO standards are given in Table 3. Mortality larvae differences were observed in the toxicity of the aqueous and ethanolic leaf extracts of plants consisting of leaf against the fourth instar larvae of to be inducing 72 to 96% larvicidal property at 10ml of the extract at 96 hrs respectively on mortality against mosquito larvae at varying concentrations.

As per the preliminary phytochemical investigation, the constituents like flavanoids, tannins, alkaloids, saponins, phenolic compounds, coumarins, terpinoids, quinones and carbohydrates are equally present in leaf, of aqueous and ethanolic extracts as mentioned in Table 1. According to Bowers *et al.* (18) the biological activity of the plant extract is due to various compounds like Phenols and Flavonoids etc. These findings were in agreement of similar nature of study conducted by Okoye (19). Moreover, flavanoids are very important constituent of natural product and have got apart antioxidant activity (20).

Table 1. Preliminary Qualitative analysis of *B. oleracea* var. *botrytis*.

Compound	Aqueous	Ethanol
Carbohydrates	+	+
Tannins	+	+
Flavonoids	++	++
Alkaloids	-	++
Phenols	++	++
Terpinoids	+	+
Quinones	-	-
Coumarins	+	+
Saponins	+	+
Steroids	-	-

Table 2. Preliminary Quantitative analysis of *B. oleracea* var. *botrytis* L.

Photochemical	Aqueous	Ethanol
TPC(CE/g)a	38.86 \pm 1.52	68.23 \pm 2.62
TFC(QE/g)b	26.76 \pm 0.95	42.33 \pm 1.92

a(mgCE/100g dry mass), C: Catechol, b(mg QE/100g of dry mass), Q: Quercetin, TPC: Total phenolic content, TFC: Total Flavonoid content.

Table 3. Mortality of larvae of mosquito, *Aedes aegypti* exposed to aqueous and ethanolic leaf extract of *Brassica oleracea* var. *Botrytis* L.

Concentration	2%		4%		6%		8%		10%	
Hrs	ALE	ELE	ALE	ELE	ALE	ELE	ALE	ELE	ALE	ELE
12	3	10	4	12	5	13	6	17	10	18
24	4	12	5	12	7	15	10	17	12	19
48	4	14	6	14	10	16	12	18	15	19
72	6	14	9	16	10	18	13	18	17	20
96	7	16	10	18	12	19	15	20	17	20
Tot	24	66	34	72	44	81	56	90	72	96
Mortality%	24	66	34	72	44	81	56	90	72	96
M	4.8	13.2	6.8	14.4	8.8	16.2	11.2	18	14.4	19.2
SD	1.6431	2.2803	2.5884	2.6076	2.7748	2.3874	3.4205	1.2247	3.3615	0.8366

Hrs= Hours., Tot= Total., % = Mortality percentage., M=Mean., SD=Standard Deviation.

ALE- Aqueous Leaf Extract., ELE- Ethanol Leaf Extract

Table 2 reveal the presence of total phenolic and flavanoid compounds in both aqueous and ethanol extracts of *B. oleracea*. High percentage of phenolic and flavanoids contents was reported in ethanol extract than aqueous extract. *B. oleracea* has been reported to possess potent mosquito larvicidal activity. The phytochemicals compounds present in the *B. oleracea* leaf extract show highest mortality of *Ae. aegypti* larvae (Table 3). The aqueous and ethanolic leaf extracts show 72% and 96% mortality of 4th instar of *A. aegypti* when compared to the control. High insecticidal activity exhibited by ethanolic leaf extract may be due to presence of phenolic and flavanoids (Table 2).

A survey of literature on control of different phytochemicals obtained from various plants has been carried out by number of researchers in the field of vector control (21). There are many studies of toxicity carried out with other plants that reflect a similar behaviour against *Aedes aegypti*. Plant could be an alternate source of bioactive chemicals and generally free from harmful effects. Use of these botanical derivatives in mosquito control instead of synthetic insecticides could reduce the cost and environmental pollution. Many of the defensive components of plants are biodegradable with non-residual effects on the biological environment. Hence an attempt has been made in the present investigation to identify the larvicidal potential of the locally available plant *B. oleracea*.

We can conclude from this study that in totality, the data collected show that *B. oleracea* indeed has larvicidal potential when treated to larvae in low concentrations, and can be used as substitute for commercial insecticides. Though the

presence of phytochemicals in *B. oleracea* could be studied further in detail and its beneficial effect to control mosquitoes.

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