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LARVICIDAL ACTIVITY OF EXTRACTS FROM *MYXOPYRUM SERRATULUM* A.W.HILL AGAINST *Aedes Aegypti* L

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ABSTRACT

The larvicidal potential of extracts from *Myxopyrum Serratulum* was evaluated against larvae of *Aedes aegypti*. The results from the present study showed that the petroleum ether and methanolic extract from *M. Serratulum* exhibited significant toxic effects against *Ae. aegypti* larvae with LC₅₀ value of 134.2 µg/ml and 172.47 µg/ml, respectively. Two compounds myxopyroside (1) and kaemferol (2) were isolated from methanolic extract. However, the synergetic effect of Kaemferol with Phenolic compound of methanolic extract shows higher larvicidal activity than petroleum ether extract.

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INTRODUCTION

Dengue is an infectious disease caused by an arbovirus transmitted to humans by the bite of female mosquitoes of the genus *Aedes*. Among many vectors, *Aedes aegypti* L. (Diptera: Culicidae) is the one of utmost epidemiological importance [1,2]. Over the past 60 years the incidence, distribution, and clinical severity of dengue have increased dramatically; resulting in the most important viral disease transmitted by arthropod vectors in terms of mortality and morbidity [3,4].

In the absence of effective and safe vaccines for dengue prevention, the epidemics control is accomplished by monitoring mosquitoes breeding sites and by the use of synthetic chemical insecticides to control the spreading of adult or larval forms of *Ae. aegypti* [5,6]. Although the chemical products are effective, the continuous use in large scale has contributed to the emergence of many negative consequences, such as, the appearance of insecticide resistance in dengue vectors leading to decreased efficacy and adverse effects on non-target species, including humans (Zahran and Abdelgaleil, 2011). These problems have persuaded numerous researchers in order to develop alternative strategies using natural products to control *Ae. aegypti* proliferation. In this direction, the plant extracts and their major constituents have received much attention as potential bioactive agents against mosquito vector [7-12].

Myxopyrum serratum (Oleaceae) commonly known as “Chaturamulla” is a large woody climbing shrub. The leaves are astringent, acid, sweet, thermogenic, anodyne, febrifuge and tonic. They are useful in vitiated conditions of kapha and vata, cough, asthma, rheumatism, cephalalgia, nostalgia, consumption, fever, otopathy, neuropathy and cuts and wounds [13]. Earlier the plant has been studied for its antimicrobial [14], wound healing [15] and anti-inflammatory activity [16].

Pharmacognostical evaluation has been made for the plant and reported for the presence of terpenoids, flavones, anthraquinones, sugars, alkaloids, phenols, tannins, and saponins. Antimicrobial study has been carried out in leaves. [17] Previous studies have shown the presence of triterpenoidursolic acid in leaves [18] and the iridoid glycoside myxopyroside. [19] Various secondary metabolites present in the plant are responsible for its medicinal value. Few species from Oleaceae has reported to have larvicidal activity against the Dengue and Chikungunya Vector *Aedes aegypti* L. [20]

Therefore, the present study aims to evaluate the larvicidal activity of Petroleum ether and methanol extract of *M. Serratum* against third instar larvae of *Ae. aegypti*. Additionally, isolate the phytoconstituents from active extract of *M.Serratum*.

MATERIALS AND METHODS

Plant material:

M. Serratum belongs to *Oleaceae* family was collected from kasaragod district, kerala in the month of November 2015. The plant was authenticated by Dr. R. Gopalan, Department of Botany and herbarium was preserved in the Department of botany, Karpagam University.

Extraction procedure:

Fresh leaves of *M. Serratum* (3 kg) was washed, shade dried and ground in to coarse powder and extracted with petroleum ether and methanol by cold percolation method for three times (72hrs each) to yield the extract. The two extracts were concentrated by distillation and monitored by thin layer chromatography using petroleum ether and ethyl acetate (8:2) solvent.

Larvicidal bioassay

Larvicidal bioassay of individual plant extracts was tested against third instar larvae of *Ae. aegypti*. The tests were conducted in glass beakers. Mosquito immature particularly early third instar larvae were obtained from NCDC mettupalayam. The concentration ranges were determined by a preliminary curve concentration–response with 20 larvae. Stock solution (1000 µg/ml) was prepared by mixing 1g petroleum ether extract along with traces of acetone, which was further dissolved in 1000 ml of distilled water. From the stock solution, a several dilutions were prepared ranging are 40, 80, 120, 160 and 200µg/ml. Twenty larvae were collected and placed on a 250 ml of different concentrations of test solutions separately in a glass beaker. Three replicates were used for each concentration. The mortality was recorded after 24 h exposure to different concentration of testing solutions. Larvae were considered dead when they did not respond to stimulus. The same method has been carried out for methanol extract also. Without sample is used as a negative control. However, when the control mortality ranged from five to twenty per cent, the observed percentage mortality was corrected by Abbott’s formula.

$$\text{Percent mortality} = \% \text{ of test mortality} - \% \text{ control mortality} / 100 - \% \text{ control mortality} \times 100$$

Isolation of compounds from methanolic extract of *M. Serratum*:

The crude methanolic extract of *M. Serratum* was mixed with about 3.0g of silica gel and subjected to column chromatography, eluting with petroleum ether and polarity gradually reduced by addition of ethyl acetate followed by rechromatography (solvent system 7:3 petroleum ether and ethyl acetate) of appropriate partially resolved fractions. It was further purified by preparative TLC using petroleum ether and ethyl acetate 7:3.

RESULTS AND DISCUSSION

Larvicidal activity

Table 1: Larvicidal activity of different concentrations of methanol and petroleum ether extract of *M. Serratulum* against *Ae. aegypti*.

<i>M.Serratulum</i> extracts	Concentration($\mu\text{g/mL}$)				
	40	80	120	160	200
	% of mortality\pm standard deviation				
Methanol extract	20.08 \pm 1.17	37.5 \pm 2.04	45.83 \pm 1.17	57.5 \pm 2.04	71.66 \pm 1.17
*Pet. ether extract	14.16 \pm 1.178	21.66 \pm 1.178	31.66 \pm 1.178	45.83 \pm 3.11	60 \pm 2.04

*Petroleum ether extract.

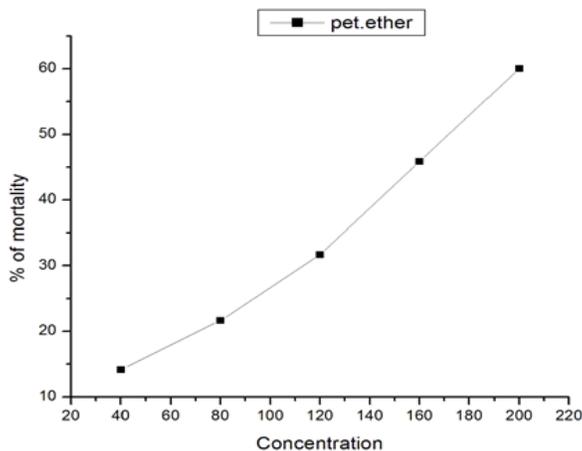


Fig. 1: Petroleum ether extract of *M. Serratulum* shows Larvicidal activity with LC_{50} value 172.47 $\mu\text{g/mL}$.

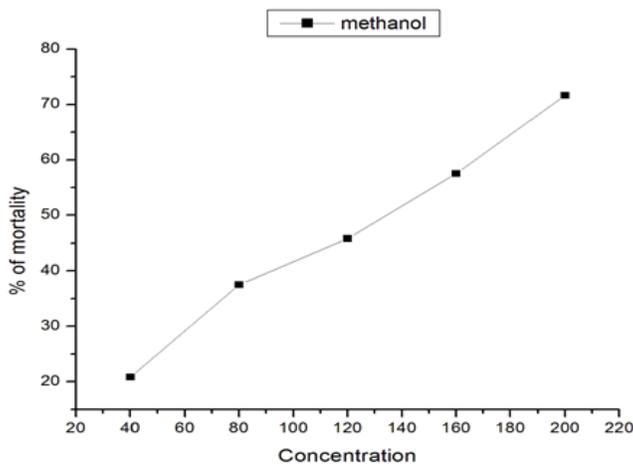


Fig. 2: Methanolic extract of *M. Serratulum* shows Larvicidal activity with LC_{50} value 134.2 $\mu\text{g/mL}$.

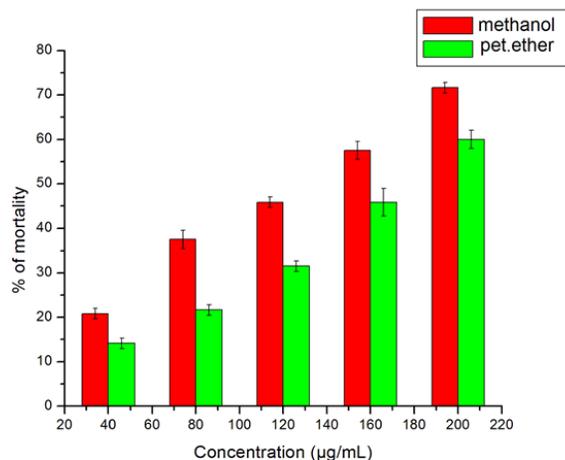
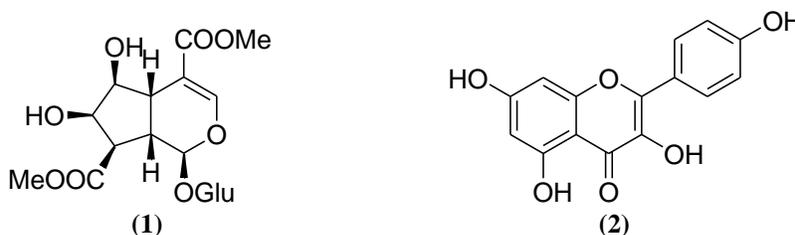


Fig. 3: Larvicidal activity of *M. Serratulum* aerial parts extracts against the third instar larvae of *Aedes aegypti*. Values are mean \pm SD of 3 replicates.

The result of the larvicidal activity of petroleum ether extract of *M. Serratulum* against *Ae. aegypti* larvae was evaluated by the LC_{50} values, expressed as ppm. The results of larvicidal assay revealed that the petroleum ether extract exhibited larvicidal activity against *Ae. aegypti* larvae and the rates of mortality were directly proportional to concentration. The petroleum ether and methanol extracts exhibited larvicidal activity with the LC_{50} value of 172.47 μ g/ml and LC_{50} value 134.2 μ g/ml respectively.

Phytoconstituents

The crude methanolic extract (3.0g) of *M. Serratulum* was mixed with about 4g of silica gel and subjected to column chromatography, eluting with petroleum ether and polarity gradually increased by addition of ethyl acetate. It is resulted in the isolation of myxopyroside (1) and kaemferol (2). The compound (1) and (2) were confirmed by comparison of their spectral data with literature data value [21]. To best of our literature survey (2) has been reported first time from this plant material.



Flavonoid extracts are reported to have larvicidal activity and flavonoids with other phenolic compound shows Synergetic larvicidal effect was [22]. The methanolic extract of *M. Serratulum* found to be more active against *Ae. aegypti* due to the presence of keamferol. However, further study is needed to substantiate this view.

CONCLUSION

The methanolic extract of *M. Serratulum* found to be more active against *Aedes aegypti* L. due to the presence of keamferol. However, further study is needed to substantiate this view. Hence methanol extract could be utilized for developing, ecofriendly insecticide as an alternative to synthetic insecticides.

Conflict of interest

The authors declare that there is no conflict of interest.

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