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Milky Mangrove *Excoecaria agallocha* L. Plant as a source for potential mosquito larvicides

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ABSTRACT

Mosquitoes are one of the most dangerous vectors among the group of arthropods. Infections due to mosquitoes are a major worldwide health problem, with high endemicity in developing countries. In the present study, we determined the larvicidal activity of Methanol, Acetone, Hexane, Chloroform and Aqueous extracts of a mangrove plant *Excoecaria agallocha* against (4th instar larvae) *Aedes aegypti and Culex quinquefasciatus*. Mosquito larvicidal assays were conducted and their mortality rate was identified after 24hours to evaluate the larvicidal activity of the crude extract of *Excoecaria agallocha*.

INTRODUCTION

Mosquitoes are medically important arthropod vectors transmitting many diseases like Malaria, Encephalitis, Dengue fever, Yellow fever, filariasis, schistosomiasis, Chikungunya and Japanese encephalitis (Das and Ansari, 2003; Kamaraj *et al.,* 2011; Tennyson *et al.,* 2012). Larviciding is a successful way of reducing mosquito densities in their breeding places before they emerge into adults (Tiwary *et al.,* 2007). Synthetic chemical insecticides such as organochlorine, organophosphorus, carbamates, pyrethrins and pyrethroids are currently used for larviciding purposes (Tiwary *et al.,* 2007; Ali *et al.,* 2013). However, their indiscriminate use resulted in several problems such as environmental pollution, insecticide resistance and toxic, hazardous which affect human health and disrupt the ecosystem (Devine, 2007; Bansal *et al.,* 2011). Plants may be alternative sources of mosquito control agents (Hedlin *et al.,* 1997). The investigation of insecticidal properties of plant-derived extracts concluded that they are environmentally safe, degradable, and target specific (Senthil Nathan and Kalaivani,2005). *Excoecaria agallocha* (Figure 1) (Family: Euphorbiaceae) is a medicinal mangrove plant found in many tropical countries including India, Myanmar, Malaysia, Indonesia, Thailand, Philippines and Sri Lanka (Jayaweera,1980). It is commonly known as Thillai in Tamil and milky mangrove in English. The milky sap of this tree can cause temporary blindness if it enters the eyes (Vadlapudi *et al.,* 2009). This plant has traditionally been used as purgative and in treatment of epilepsy, dermatitis, leprosy, toothache (Ramanathan, 2000). The potential medicinal value of this plant has been reported for antinociceptive, gastroprotective, antimicrobial, antioxidant, antihyperglycemic and anticancer properties (Simlai and Roy, 2013). The extract of various plant parts of the mangrove *Excoecaria agallocha* has been proved as a potential source of a mosquito larvicidal agent (Thangam and Kathiresan,1996, Thirunavukkarasu *et al.,* 2011, Satyan *et al.,* 2012). With this background, the present study assessed the role of mosquitoe larvicidal activities of hexane, chloroform, ethyl acetate, acetone, and methanol leaf extracts of *Excoecaria agallocha* leaves against the fourth instar larvae of *Aedes aegypti and Culex quinquefasciatus.*

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Fig. 1: Mangrove Plant - *Excoecaria agallocha Linn.*

MATERIALS AND METHODS

Collection of plant materials

The matured leaves of *Excoecaria agallocha* were collected from Parangipettai, Tamil Nadu in the month of January 2014. The collected plants were taxonomically identified and the leaves were carefully examined. Old, insect damaged and fungus infected leaves were removed. Healthy leaves were washed with dechlorinated water and spread out.

Preparation of plant extracts:

The collected leaves were shade dried for 7-10 days under room temperature (27-37 ^{o}C) (Figure 2). The dried leaves were powdered mechanically using commercial electrical stainless steel blender (Figure 3). One kilogram dried powder was extracted by using five different solvents namely aqueous, Methanol, Acetone, Chloroform, Hexane individually based on increasing polarity by cold percolation (Figure 4). The extracts were filtered through a Buchner funnel with Whatman number 1 filter paper. The extract was then subjected to evaporation until solvents were completely evaporated to get the solidified crude extracts. The crude extracts thus obtained was stored and maintained at 4 ^{o}C in a refrigerator.

Mosquito culture:

The *Aedes aegypti* and *Culex quinquefasciatus* mosquito larvae (Figure 5) were collected from stagnant water areas of Parangipettai coastal environment, Tamil Nadu. The larvae were maintained at 25-27°C, 75-85% relative humidity under 14:10 light and dark photo period cycle. They fed with a mixture of dog biscuits and yeast powder (3:1) and the water in the tray was changed daily (Kamaraj *et al.*, 2009).

Larvicidal bioassay

Mosquito larvicidal activity of various solvent extracts of *Excoecaria agallocha* leaves was assessed at varying grades of concentration such as 20, 40, 60, 80, 100 mg/ml of Tween 80 and the samples were diluted with 99ml of distilled water. Standard World Health Organization protocol (World Health Organization, 1996) was employed with modification for the bioassay procedure. Ten larvae of same stage (i.e. 4th instar) were put into beakers containing the test solution of each above mentioned concentration. Distilled water (99 ml) containing Tween 80 (1 ml) was used as control. For each concentration and control, three replicates were used and each test was repeated three times. Observation on larval mortality was recorded after 24hrs. Larvae were considered dead, when they failed to move after probing with a needle/brush. The number of dead larvae in each vial was counted and the percent mortality of larvae was calculated using the Abbott's formula (Abbott, 1925).

$$\% \text{ corrected percentage} = 1 - \frac{\text{n in test after treatment X 100}}{\text{n in control after treatment}}$$

RESULTS AND DISCUSSION

The marine environment is incomparable reservoirs of bioactive natural products, many of which exhibit structural features that have not been found in terrestrial natural products (Ali *et al.,* 2013). Mangroves are biochemically unique vegetation that produce wide array of natural products with immense medicinal potential (Patra *and* Mohanta, 2014). They have natural metabolites with diverse biological activities such as antibacterial, antiviral activity, antidiarrhoeal, antifeedant activity, insecticidal activity and cytotoxic activity (Manilal *et al.,* 2009). In this context, mangroves have received much attention as potentially useful natural larvicides (Thangam and Kathiresan,1988; Thangam and Kathiresan,1988(a); Thangam and Kathiresan,1989; Renugadevi *et al.,* 2012). Crude hexane extract from the dried roots of *E. agallocha* inhibited 50% of the growth of third instar larvae of *Culex quinquefasciatus* within 24 h (LC_{50}: 315 ppm) (Satyan *et al.,* 2012).

The highest mortality was found in Methanol and aqueous extract of mangrove plant extracts of *Excoecaria* agallocha bark against the larvae of *Anopheles stephensi* (LC_{50} =12.69, 11.35, and LC_{90} =38.33, 36.42), *C. quinquefasciatus*(LC_{50} =10.45, 09.98 and LC_{90} = 37.85, 35.67), *Aedes aegypti* (LC_{50} 14.87, 12.67 and LC_{90} 40.67, 41.25) (Thirunavukkarasu *et al.,* 2011).The studies on mosquito larvicidal activities of leaf extracts of *Excoecaria agallocha* are too restricted. Hence, the present study was investigated the larvicidal action of various solvent extracts of the mangrove plant *Excoecaria agallocha.*

The larvicidal studies of *Excoecaria agallocha* leaf extracts have been tested among two species of mosquito larvae (i.e) *Aedes aegypti* and *Culex quinquefasciatus*. The percentage of mortality was calculated by using Abbott's formula. Controls did not reveal the effect on larvae. No mortality was observed in the control group of the experiment. The figures 6&7 will step out the mortality rate of both the species arrived at the end of 24hrs and they are tabulated based on their increasing polarity. A dose dependent lethality was observed in all the extracts. Methanol and hexane extracts showed the best mortality rate (100%) against the larvae of *Aedes aegypti* and *Culex quinquefasciatus*.

Fig. 2: Shade dried leaves.

Fig. 3: Powdered sample.

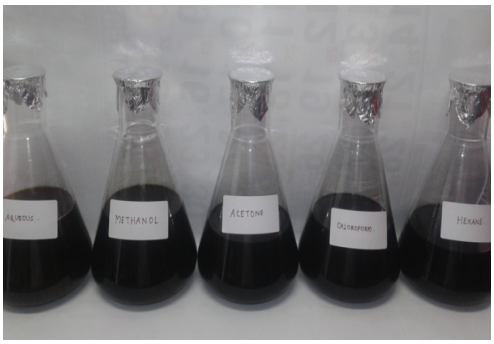

Fig. 4: Extraction with five different solvents.

Fig. 5: Collected larvae.

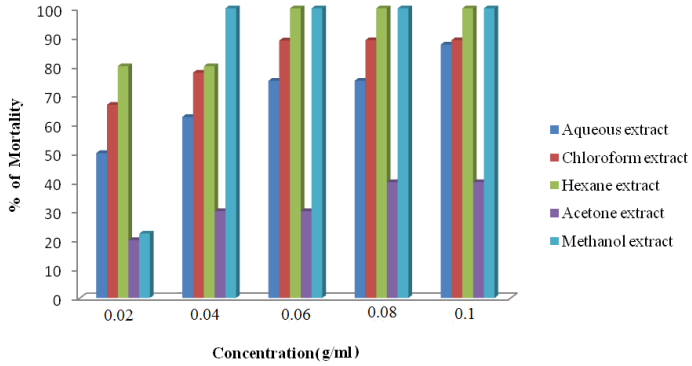

Fig. 6: Percentage mortality of *Aedes aegypti* treated with the various solvent extracts of *Excoecaria agallocha* leaves.

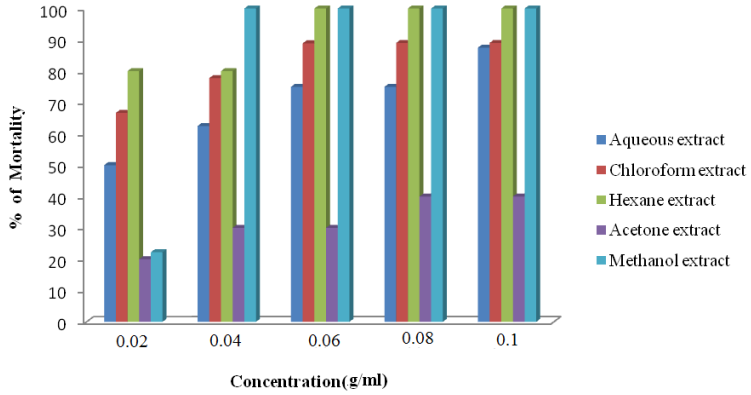

Fig. 7: Percentage mortality of *Culex quinquefasciatus* treated with the various solvent extracts of *Excoecaria agallocha* leaves.

Based on the mortality values, the potency of crude mangrove leaves extracts could be categorized as: Methanol> Hexane>chloroform > Aqueous > Acetone in the decreasing order.

In the present study, the higher percentage of mortality caused by the extracts is assumed to be caused by the active ingredients present in them. Further investigations are needed to elucidate the active ingredients of the extract responsible for larvicidal activity should be identified for field application of mosquito control.

CONCLUSION

It is concluded from the present study that methanol and hexane extracts of Excoecaria agallocha Linn. exhibited high mosquitoe larvicidal activities. Based on the earlier reports and the result of the present investigation, separation of active principles from *Excoecaria agallocha Linn* and studying their mode of action warrants further research for developing potent larvicidal compounds.

These results could be useful in the search for novel, ecofriendly larvicide for the control of *Aedes aegypti* and *Culex quinquefasciatus*. It may help in reducing the environmental side effects by the synthetic insecticides.

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