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ORIGINAL RESEARCH ARTICLE

Larvicidal efficacy of crude extracts of *Emblica officinalis* and *Eucalyptus citriodora* against *Aedes aegypti* L.

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ABSTRACT

Background & objectives: Natural products of plant origin possess larvicidal potential against mosquitoes with safety. Presently research studies have been undertaken to test the larvicidal activity of plants. The aim of the present study was to test the larvicidal efficacy of fruits of *Emblica officinalis* and leaf of *Eucalyptus citriodora* against third instar larvae of *Aedes aegypti*.

Methods: The plant powder was sequentially extracted using various solvent from non polar to polar solvents such as hexane, dichloromethane (DCM), ethanol, methanol and water. Each crude extract was dissolved in dimethyl sulfoxide (DMSO) and acetone (1:1v/v) and test concentration was prepared. Larvicidal bioassay was carried out and mortality data were analysed by log probit regression analysis to calculate 50% lethal dose (LD_{50}) at 95% fiducial limit.

Results: The bioassays with crude extracts of both plants revealed that the dichloromethane extract of *E. officinalis* and *E. citriodora* exhibited higher larvicidal effect than other solvent extracts of both plant after 24 hours of exposure period and LD_{50} values were 166.64 ppm and 176.29 ppm respectively. Hexane, ethyl acetate, methanol and aqueous extracts of *E. officinalis* had higher larvicidal activity against third instar larvae of *Ae. aegypti* than the same extracts of *E. citriodora*.

Interpretation & conclusion: The findings of present study suggest that DCM extract of *E. officinalis* and *E. citriodora* exhibited higher larvicidal potential against *Ae. aegypti*. Further study is warranted to isolate and purify the novel bioactive molecule from the dichloromethane extract of *E. officinalis* and *E. citriodora.*

Key words: *Aedes aegypti, Emblica officinalis, Eucalyptus citriodora,* larvicidal, sequential extraction, vector control.

INTRODUCTION

Mosquito-borne diseases such as malaria, dengue, and chikungunya are of public health importance in Sri Lanka [1, 2]. *Aedes aegypti* is the principal vector of dengue and dengue hemorrhagic fever in many South Asian countries. Over the years the mosquito control programmes have been carried out by using synthetic insecticides. Repeated application of synthetic insecticides for mosquito control has disrupted natural biological control systems, killed non-target organisms and caused harmful effects on human [3]. It has also resulted in the development of resistance in some medically important mosquito vectors against insecticide [4]. In recent times many approaches have been made to search alternative method to control mosquito vectors through the use of bioactive, eco-friendly and biodegradable plant materials [3]. Large numbers of plant products have been reported to have mosquito larvicidal activity [5]. Plant essential oils have been recognized as important natural resources of insecticides[6]. Some important phytochemical products have been used as insecticides such as pyrethrum, derris, quassia, nicotine, hellebore, anabasine, azadirachtin, dlimonene, camphor, and terpenes [7].

Emblica officinalis (Indian gooseberry) belonging to family Euphorbiaceae is a deciduous tree and fruit of this plant are diuretic and laxative [8]. This

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plant is said to be native to tropical South-Eastern Asia, mainly southern and central India and it is also found through out Sri Lanka, Bangladesh, China and Malayan peninsula [8, 9]. Fruits are used in traditional medicine for the treatment of dysentery, diarrhoea, cough and anemia [10] and its fruits and seeds are used to treat asthma, bronchitis, biliousness [11]. Fruits of *E. officinalis* had antibacterial activity against some bacteria [10, 12]. *Eucalyptus citriodora* (Lemon scented gum) belongs to family Myrtaceae. *Eucalyptus* is native to Australia and genus of the *Eucalyptus* contains about 600 species. In 18th century *Eucalyptus* were introduced to Sri Lanka from Australia and these were planted in Some important Sri Lankan gardens such as Uva and Dimbulla and Hakgala Botanical Gardens[13]. *Eucalyptus* leaves can be applied to infection of upper respiratory tract, skin diseases, burns and rheumatism [10]. It has been reported that *Eucalyptus* oil possessed antibacterial activity against bacteria causing respiratory disorders [14]. The *Eucalyptus* extracts were found to be effective against pathogens causing food poisoning, acne and athlete's foot, and wide range of commercial application [15]. Inhalation of *Eucalyptus* derivatives has been used to treat pharyngitis and bronchitis [16].

The present study was carried out to examine the larvicidal activity of different solvent extracts of *Emblica officinalis* and *Eucalyptus citriodora,* which were sequentially extracted from non polar to polar solvent such as hexane, ethyl acetate, ethanol, methanol and water extracts of both plants against third instar larvae of *Aedes aegypti*,

MATERIAL AND METHODS

The fruit of *Emblica offcinalis* and leaf of *Eucalyptus citriodora* were used in this study. These plant parts were collected and identified with the help of the preserved voucher specimen at the Department of Botany, University of Jaffna, Sri Lanka. The plants materials were washed thoroughly with distilled water and they were allowed to dry under sun light for 1-2 weeks. Dried materials were ground to make powder using blender (kanchan, TYCOON) and powder were stored in airtight bottles until use. One hundred grams of each powder was soaked into 200 ml of hexane in a stopppered bottle separately for 3 days at room temperature on electric shaker. Then these were filtered using Whatman No.1 filter paper and filtrate was collected into conical flask. Again 200 ml of hexane was added and the above procedure was repeated thrice. Once hexane extraction was over resultant residue of each plant powder was dried at room temperature. These powders were used for the sequential extraction using other solvent such as dichloromethane (DCM) ethyl acetate, ethanol, methanol, and water [17].

The filtrates were dried under reduced pressure using rotary evaporator (BUCHI, Rotavapor, R-114). The dried crude extracts were stored at 4ºC in airtight bottles. The concentrated each extract of each plant was dissolved in mixture of dimethyl sulfoxide (DMSO) and acetone (1:1v/v) and it was used as stock solution. From the stock solution test samples with the concentration of 50, 75, 100, 125, 150, 175, 200, 225, and 250 ppm were prepared by diluting the stock solution with distilled water.

The laboratory reared (29±2ºC and 75±5 % RH) third instar larvae of *Ae. aegypti* were used for experiments. The different solvent extracts of the selected medicinal plants were bioassayed against third instar larvae of *Ae. aegypti*. Bioassays were conducted, according to method of Singh *et al* [18]. Briefly, 20 third instar larvae were transferred to into 300 ml capacity plastic cup that contain 200 ml of test samples with appropriate concentration. The mixture of acetone and DMSO (1:1 v/v) were maintained as control test. For each concentration three replicates were run at the same time. The each experimental set up was maintained at ambient temperature (29±2ºC). The larvae were considered dead if they were immobile and unable to reach the water surface. The number of larvae surviving at the end of 24 h was recorded and mortality values were calculated. Mortality data were subjected to log probit regression analysis to determine 50% and 90% of lethal concentration using MINITAB version 14 computer statistical package. LD_{50} and LD_{90} value were expressed with fiducial limits.

RESULTS

The results of larvicidal bioassay (**Table 1**) revealed that among the different solvent extracts of *E. officinalis* and *E.citriodora*, DCM extracts of both plants exhibited higher toxic effect than other solvent extracts against third instar larvae of *Ae. aegypti* at 24 hours exposure period. The determined LD_{50} values of DCM extracts of *E.*

officinalis and *E. citriodora* were 166.64 ppm and 176.29ppm respectively. Hexane, ethyl acetate, methanol and aqueous extracts of *E. officinalis* had higher larvicidal activity against third instar larvae of *Ae. aegypti* than corresponding solvent extracts of *E. citriodora* with the LD_{50} values of 202.92, 197.19, 208.77 and 226 ppm respectively. However ethanol extract of *E. citriodora* exhibited higher larvicidal activity than ethanol extract of *E. officinalis*. Control experiment indicated that the mixture of acetone and DMSO (v/v1:1) did not show any effect on the mortality of larvae.

Table 1: Larvicidal effect of different solvent extracts of *Emblica officinalis* and *Eucalyptus citriodora* against third instar larvae of *Aedes aegypti* after 24 hours of exposure period.

Plant	Part used	Extracted Solvent	LD_{50} (ppm) (fiducial limit)	LD_{90} (ppm) (fiducial limit)	Chi-Square	DF
Emblica officinalis	Fruit	Hexane	202.92 (192.33-215.33)	305.89 (283.60-337.03)	5.692	7
		DCM	166.64 (159.45-173.96)	236.21 224.99-250.38)	10.611	7
		Ethyl acetate	197.19 (188.30-207.25)	283.65 (266.47-306.90)	13.455	7
		Ethanol	239.35 (225.38-258.55)	343.19 (313.85-387.22)	4.2482	7
		Methanol	208.77 (192.61-230.55)	369.74 (327.82-436.40)	9.5011	7
		Aqueous	226.35 (213.62-243.01)	332.660 (305.23-372.97)	17.950	7
Eucalyptus citriodora	Leaf	Hexane	231.69 (215.75-253.80)	363.89 (327.17-420.61)	17.5771	7
		DCM	176.29 (168.37-184.62)	256.05 (242.35-273.80)	14.0882	7
		Ethyl acetate	255.22 (235.84-284.02)	387.09 (344.85-454.82)	3.6197	7
		Ethanol	188.99 (180.94-197.80)	267.15 (252.57-286.30)	3.5017	7
		Methanol	221.35 (208.81-237.53)	330.77 (303.46-370.64)	15.4384	7
		Aqueous	254.34 (235.42-282.36)	383.90 (341.98-448.81)	6.5555	7

DISCUSSION

Recently many approaches have been conducted to assess the larvicidal potential of many plants against different stages of larvae of *Ae. aegypti* [19]. Testing the plant crude extracts against mosquito can lead to identify potential bioactive compounds that can be used as larvicides to control mosquito[20]. Mosquito control programmes can be easily carried out targeting larval stages as they are confined to water bodies which are mainly man made and can be located[21].

The findings of present investigation indicate that DCM extract of *E. officinalis* and *E. citriodora* show higher larvicidal activity against third instar larvae of *Ae. aegypti*. The higher activity of DCM extract of *E. officinalis* and *E. citriodora* may be due to the presence of bioactive components against third instar larvae of *Ae. aegypti*. Further semi-polar solvents had ability to dissolve polar and non polar compounds [22]. Here DCM is semi-polar solvent and polar and non polar compounds can be dissolved in the DCM crude extracts of both tested plants. Therefore the reason for the higher larvicidal activity of DCM crude extract may also be due to presence of both non polar and polar active compounds in this extract.

Few mosquito larvicidal activity studies have been conducted using the sequentially extracted

different solvent extracts of medicinal plants[20]. In this study sequentially extracted crude extracts of two plants were tested against mosquito larvae. Sequential extraction technique is directed to minimize the number of compounds in each solvent extract. As the number of compounds increased in the extracts, the action of one compound can be interfered another compounds present in the extract. This interfere effect is known as antagonistic effect[23]. In present study each extract may possess less number of compounds, therefore the antagonistic effect can be reduced and also the isolation of and purification can easily be carried out to find out active novel compound against mosquito.

The methanol extract of leaves of *E. officinalis* was tested against forth instar larvae of *Culex quinquefasciatus* and this extract did not cause mortality against this larvae. However other plants which were member of family Euphorbiaceae showed larvicidal activity [24]. The genera of Family Euphorbiaceae such as *Phyllanthus amarus* was tested by Rahuman *et al* [25] against the early fourth instar larvae of *Culex quinquefasciatus* (Say) and *Aedes aegypti* L. and by Vinayagam *et al* [26] against fourth instar larvae of *Anopheles stephensi*. The present study shows that the fruits of *E. officinalis* possess larvicidal activity against third instar larvae of *Ae. aegypti*.

Pervious study indicated the larvicidal effect of hexane extract of *Eucalyptus* against mosquito larvae, particularly *Anopheles* species [27]. It has been reported that leaf extracts of *E. citriodora* and *E. camaldulensis* had larvicidal activity against larvae of *Cx. quinquefasciatus* [28]. In the present study compared to other solvent extracts of *E. citriodora* DCM and ethanol extracts showed more larvicidal activity against third instar larvae of *Ae. aegypti*.

It has been reported that the natural phytochemicals such as saponin, isoflavanoids, alkaloids, and tannin are found to possess larvicidal activity against mosquito larvae [29]. Further some phytochemicals from medicinal plants, saponin from *Clorophytum briririlianum* [30] and alkaloids from *Piper longum* [31] had ability to cause mortality against third instar larvae of mosquitoes. Previous phytochemical analysis pointed out that *E. officinalis* possessed flavanoids, glycoside, phenol, tannin and saponin and *Eucalyptus* sp. possessed alkaliod, phenol and tannin [10]. Therefore presence of these phytochemicals in *E. officinalis* and *E. citriodora* may be responsible for the larvicidal activity of both plants.

CONCLUSION

Dichloromethane extract of *E. officinalis* and *E. citriodora* possess higher larvicidal activity than other solvent extracts of both plants against third instar larvae of *Ae. aegypti*. Further studies on isolation and purification of bioactive molecule from the dichloromethane extract of *E. officinalis* and *E. citriodora* are warranted. Further studies on the larvicidal efficacy of these plant extracts against different vector mosquitoes of human diseases are needed.

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