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An evaluation of larvicidal activity of acetone extracts of *Aegle marmelos* (L.) Correa (Rutaceae) and *Andrographis paniculata* (Burm.f.) Wall ex Nees (Acanthaceae) leaves against larvae of *Culex quinquefasciatus*

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Abstract
Mosquitoes are the most serious problem in public health, which are responsible for spreading a various number of serious diseases, viz., malaria, filariasis, dengue and Japanese encephalitis. Aim of this present study is evaluation of mosquito larvicidal activity of both plants of *Andrographis paniculata* (Burm.f.) Wall ex Nees (Acanthaceae) and *Aegle marmelos* (L.) Correa (Rutaceae) studied for 24h. The both leaf powder materials of *A. paniculata* (Burm.f.) Wall ex Nees (Acanthaceae) and *A. marmelos* (L.) were extracted with acetone for 4h in soxhlet apparatus and excess of solvent was removed rotary evaporator under reduced pressure. The crude extracts were dissolved in 1ml acetone and different concentrations of extract prepared and mosquito larvicidal activity was studied for 24 h in laboratory conditions. The results of acetone extract of leaves of *A. paniculata* (Burm.f.) Wall ex Nees (Acanthaceae) and *A. marmelos* (L.) Correa (Rutaceae) were showed in the LC_{50} values of 25.156, and 28.355 respectively active against the mosquito larvae of *Culex quinquefasciatus*. The conclusion of the present study reported that acetone extracts of both plants of *Andrographis paniculata* (Burm.f.) Wall ex Nees (Acanthaceae) and *Aegle marmelos* (L.) Correa (Rutaceae) have a good larvicidal activity.

Keywords: Phytochemicals, malaria, medicinal plants, extracts, larvicidal activity, *Culex quinquefasciatus*

1. INTRODUCTION

Insect-Borne Diseases (IBD) are serious health problems in human and animal health in worldwide[1]. Mosquitoes are responsible for spreading serious diseases for malaria, dengue fever, chikungunya, zika virus, yellow fever and Rift Valley fever were seriously affected in the human[2-4]. Laboratory production of synthetic insecticides have been used for past decades, the controlling pests and vectors of various human diseases. Indiscriminate use of pesticide resulted in the several problems in worldwide viz. environmental hazards, elimination of natural enemies, toxic residues in food, and also produced insecticidal resistance in major vector species [5]. To overcome from these problems there is an persistent need for search and development of new insecticides[6]. Plant based active principles are most promising alternatives to synthetic insecticides in controlling insect pests. Most of the active constitutes are used for various medicinal purposes and which of these phytochemicals are environmentally safe, biodegradable, low cost and vector control [7-12]. About 2000 species of plants have been reported for their insecticidal activities[13-15]. Several herbal products were obtained for plant parts, such as nicotine obtained from tobacco leaves; anabasine and lupinine, the alkaloids extracted from Russian weed, *Anabasis aphylla* [12], rotenone from *Derris eliptica* [16] and pyrethrums from *Chrysanthemum cinererifolium* flowers [17] have been used as natural insecticides. The literature review of both study plants of *Andrographis paniculata* (Burm. f.) Wall. ex Nees and *Aegle marmelos* is an important medicinal plant and it is widely used for around the world. *Andrographis paniculata* (Burm. f.) Wall. ex Nees is belong to the family Acanthaceae. Previous studies, highest amount of andrographolide isolated from the *A. paniculata* [18]. Dua et al. [2004] isolation of four xanthones compounds (1,2-dihydroxy-6,8-dimethoxyxanthone; 1,8-dihydroxy-3,7-dimethoxyxanthone; 3,7,8-trimethoxy-1- hydroxyxanthone; 4,8-dihydroxy-2,7-dimethoxyxanthone) from roots of AP[19]. Xu et al. isolated for 5 rare types of noriridoids with a known iridoid curvifloruside F from the ethanol extracts of roots [20]. Arifullah et al. [2013] demonstrated moderately potent

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antimicrobial and antioxidant activity of andrographolide and echiodinin extracted from acetone and methanol extracts of *in vitro* leaf callus of AP[21]. Zhang et al. reported 3 new ent-labdane diterpenoids, namely, 19-norandrographolides A, B, and C from the ethanol extracts of the aerial parts of AP[22]. *Aegle marmelos* is belonging to the family Rutaceae. It is an important medicinal plant in the traditional Indian system of medicine, the Ayurveda. Active compounds of tannins, skimmianin, essential oils like caryophyllene, cineole, citral, cuminaldehyde, citronella, p-cymene, d-limonene, and eugenol, sterols and/or triterpenoids, including lupeol, β- and γ-sitosterol, α- and β-amyrin, flavonoids like rutin and coumarins, including aegeline, marmesin, umbelliferone marmelosine, marmelin, o-methyl halfordinol, alloimperatorin methyl ether, o-isopentenyl halfordinol, phlobatannins, flavon-3-ols, leucoanthocyanins, and anthocyanins [23-24]. Aim of the present investigation has been potential of mosquito larvicidal activity of leaf extracts of *Andrographis paniculata* (Burm.f.) Wall ex Nees (Acanthaceae) and *Aegle marmelos* (L.) Correa (Rutaceae) active against *Culex quinquefasciatus.*

2 MATERIALS AND METHODS

2.1 Plant Material

Fresh leaf materials of *Andrographis paniculata* (Burm.f.) Wall ex Nees (Acanthaceae) and *Aegle marmelos* (L.) Correa (Rutaceae) were collected from their natural habitat of Tirunelveli District,Tamilnadu in December, 2018. Both plant materials were wrapped with plastic sheets during transport. Herbarium prepared and voucher specimen were deposited in the Department of Conservation Ecology (Rice- FISSD'S). The plant specimen voucher number are Rice-234, and Rice-234261.

2.2 Preparation of plant extracts

The dried plant materials of *A. paniculata* and *A. marmelos* were grounded into powder using Wiley Laboratory Mill apparatus. 100 gm of both plant samples were extracted with 250 ml of acetone using the Soxhlet apparatus for 4 h. Each extract was dried by rotary evaporator under reduced pressure and stored in a dry state until use.

2.3 Mosquito larvicidal activity

The mosquito larvicidal activity of selected species of *Culex quinquefasciatus* larvae were collected from the field and colonized in the laboratory. The larvae of *C. quinquefasciatus* were maintained at room temperature (25 ± 2 °C) and kept in dechlorinated tap water in an enamel bowl. Larvae of *C. quinquefasciatus* were fed on the dog biscuit and yeast powder in prepared for 3:1 ratio. The acetone extracts of *A. paniculata* and *A. marmelos* leaves were dissolved in 1 mL of acetone and prepared into different concentrations (25, 50, 75, 100 125, and 150 ppm). The experimental animals of ten fourth-instar stage of *C. quinquefasciatus* larvae were used for the larvicidal assay and five replicates were maintained for each concentrations. During this experiment no food was provided for the larvae of *C. quinquefasciatus*. The larval mortality of *C. quinquefasciatus* was calculated after the 24h exposure periods.

2.4 Statistical analysis

The statistical analysis of average larval mortality data were subjected to probit analysis for calculating LC_{50} values and other statistics at 95% fiducial limits of lower and upper confidence limit and Chi square values were calculated by SPSS 19.0 for windows, the significance level was set at $P < 0.05$.

3. RESULTS AND DISCUSSION

The larvicidal activity of both plants of *A. paniculata* and *A. marmelos* was tested for fourth larvae of *Culex quinquefasciatus* for 24h and results of the mortality of larvae are seen in the table-1 and 2.

Table-1: Larvicidal activity of plant extracts of *Aegle marmelos* (L.) Correa and *Andrographis paniculate* (Burm.f.) Wall ex Nee active against *Culex quinquefasciatus*

Sl. No.	Concentration of Extract mg/l (ppm)	Mortality (24h) *Aegle marmelos* (L.) Correa		*Andrographis paniculate* (Burm.f.) Wall ex Nee	
		Mortality counted (Nos)	% of Mortality	Mortality counted (Nos)	% of Mortality
1	0.0	0	00	1	10
2	10	2	20	3	30
3	20	4	40	4	40
4	40	5	50	5	50
5	60	7	70	7	70
6	80	8	80	9	90
6	100	10	100	10	100

Concentration of dose prepared 10 mg dissolved in1ml water

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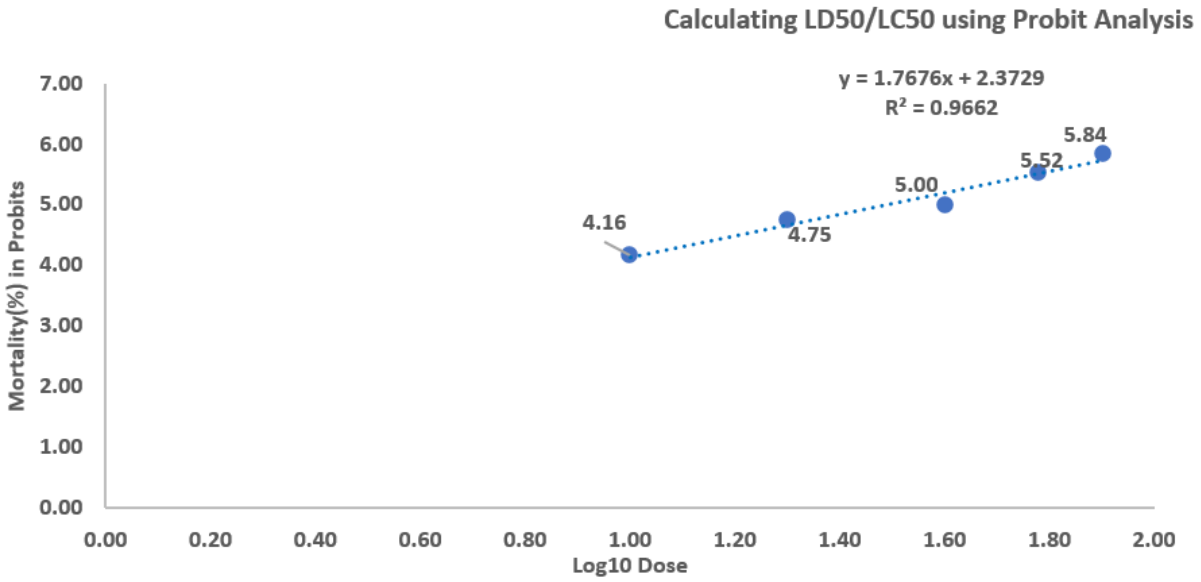

Fig.1: Larvicidal activity of plant extracts of *Aegle marmelos* (L.) Correa

Table-2: Larvicidal activity of plant extracts of *Aegle marmelos* (L.) Correa and *Andrographis paniculate* (Burm.f.) Wall ex Nee active against *Culex quinquefasciatus*

Sl.No.	Plant Name	LC_{50} Values (%)	LC_{50} Values (ppm)	95% fiducial Limit		Intercept	Slope	R^2	Chi-Test (x^2)
				Lower	Upper				
1	*Aegle marmelos* (L.) Correa	LC_{50}	28.355	18.170	44.251	2.373	1.786	0.966	0.539
2	*Andrographis paniculate* (Burm.f.) Wall ex Nee	LC_{50}	25.156	16.017	39.509	2.504	1.760	0.840	0.671

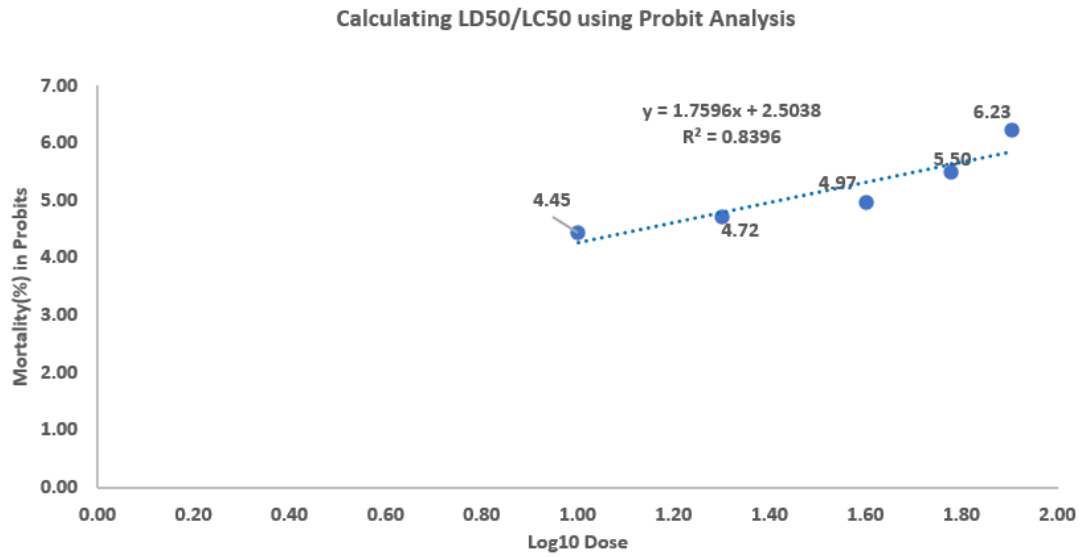

Fig.2: Larvicidal activity of plant extracts of *Andrographis paniculate* (Burm.f.) Wall ex Nee

The larvicidal activity of acetone leaves extracts of both plants of *A. paniculata* (Burm.f.) Wall ex Nees (Acanthaceae) and *A. marmelos* (L.) Correa (Rutaceae) were observed by the estimated LC_{50} values (95% confidence intervals), slope, Intercept, chi square and r^2 values are represented in the table-

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2 and fig.1. The observed and expected mortalities of larvae based on probit regression analysis for different concentrations of *A. paniculata* and *A. marmelos* (Linn.) are shown in Table -2. The LC_{50} values of *A. paniculata* less than *A. marmelos.* Earlier studies, Muthukrishnan et al. (1994) reported that ethyl acetate fractions of two plants *Solanum trilobatum* and *Letiota aspera* were observed by the LC_{50} values of 23.5 and 138.6 ppm against 2nd and 3rd larvae of *Culex quinquefasciatus* [25]. Methanolic fraction of leaves of *Menta piperita, Phyllanthus niruri and Letiota aspera* exhibited the LC_{50} values of 43.65, 1819.70 and 2818.38 respectively against the larvae of *Culex quinquefasciatus* [26]. According to Anuratha reported that petroleum ether extract of three plants viz. *Acacia nolotica* and *Citrullus colocynthis* showed 100 % mortality in *Culex quinquefasciatus* [27]. In the present study observed that acetone extract of *A. paniculata* and *A. marmelos* extract showed LC_{50} values of 25.156ppm and 28.355 respectively against the larvae of *C. quinquefasciatus* (Table-2 and Fig.1 and 2). Several plants have been reported for insecticidal properties are reported [28]. The plant based product are suitable for use in integrated pest management programs [29]. The conclusion of the finding report is acetone extracts of both plants of *A. paniculata* and *A. marmelos* good effect for control of mosquito *Culex quinquefasciatus.* Further studies will be going on identification of active compounds of both plants of leaves in *A. paniculata* and *A. marmelos.*

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