Life Science Informatics Publications
Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences
Journal Home page http://www.rjlbpcs.com/
RJLBPCS
ISSN 2454-6348

Original Research Article DOI:10.26479/2019.0501.59

EFFECT OF ESSENTIAL OILS OF *AEGLE MARMELOS* (L.) CORREA AND *PSIDIUM GUAJAVA* L. ON LARVAE OF MALARIA VECTOR *ANOPHELES STEPHENSI* LISTON.

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ABSTRACT: Larvicides are main tool to inhibit the outbreak of mosquito-borne diseases that represents a threat for millions of people worldwide. Investigation of natural products for mosquito larvicidal activity against the major Malarial vector *Anopheles stephensi* resulted in identification of two potential plant essential oils (EO) viz., *Aegle marmelos* (L.) Correa and *Psidium guajava* L. variety Navalur. Experiments were conducted according to the World Health Organization (WHO) standard protocols with slight modifications against *Anopheles stephensi* larvae. During the test of larvicidal activity, five different concentrations of the EOs were prepared and tested against the late third to early fourth instar larvae. The experiment proved that the larval mortality was directly related to the exposure time and concentration of the EOs. Based on the Log - Probit analysis, the LC_{50} and LC_{90} values of *Aegle marmelos* (EO) at 24h was 54.95 ppm, 85.11 ppm and at 48h was 53.95 ppm, 74.30 ppm, respectively. And the same values for *Psidium guajava* were 40.27 ppm, 56.49 ppm and 38.02 ppm, 51.52ppm, respectively. It was observed that the larvae of *Anopheles stephensi* were highly susceptible to the essential oils of *Psidium guajava* than *Aegle marmelos* and could consequently be used to reduce the prevalence of malaria in the endemic areas.

KEYWORDS: *Aegle marmelos* (L.) Correa, *Psidium guajava* L., Essential oils, *Anopheles stephensi,* Larvicidal activity.

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1. INTRODUCTION

Mosquitoes are dominant vectors of tropical diseases and irritant pests. It can transmit numerous diseases than other group of arthropods and attack millions of people worldwide. Malaria is the most predominant among the mosquito-borne diseases of the developing world [1, 2, 3]. The poor communities of developing countries mainly affected by malarial infection which imposes the socio-economic burden on population [4, 5]. *Aegle marmelos* commonly called Indian Bael is a sacred tree, native to the Indian subcontinent and South Asia. This plant is only member of the genus *Aegle* and belongs to the family Rutaceae. However, the leaf extracts of the plant *Aegle marmelos* act against *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* larvae [6, 7]. The copper nanoparticles from *Aegle marmelos* aqueous leaf extract shows mosquito larvicidal efficacy [8] and it also possess anti-microfilarial, antifungal, immunomodulatory, antiproliferative, wound healing, antifungal, analgesic, anti-inflammatory, antipyretic, hypoglycaemic, antidyslipidemic, antifertility, antidiabetic and insecticidal activity [9, 10,35]. *Psidium guajava* is a useful medicinal plant and exhibit antioxidant, antimicrobial, antidiabetic, cardioprotective, neuroprotective, hepatoprotective and anticancer activities [11, 12]. The Essential oil of *Psidium guajava* shows the repellent activity against the malarial vector *Anopheles stephensi* [13]. Furthermore essential oil of *Psidium guajava* from Brazil possess larvicidal activity against *Aedes aegypti* (Diptera: Culicidae) [14].The leaf extract of the *Psidium guajava* act against *Aedes aegypti* mosquitoes [15]. Resistance to all kinds of insecticides resulting in the increasing reports of malarial vector control breakdown and also synthetic insecticides like DDT, DEET have been responsible for environmental pollution. Therefore, there is an urgent need for the invention of environment-friendly and biodegradable active compounds. The plant world contains innumerable source of biochemicals that could be used as insecticides. The present study was undertaken to check the larvicidal activity of essential oils from the plants, *Aegle marmelos* and *Psidium guajava* against medicinally important species of the malaria vector *Anopheles stephensi* and would be useful in encouraging research aiming at the invention of a new agent for mosquito control based on bioactive chemical compounds from indigenous plant sources as an alternative to synthetic chemical larvicides.

2. MATERIALS AND METHODS

Collection and Identification of plant materials: Fresh and green leaves of *Aegle marmelos* (L.) Correa collected from Kasaragod, Kerala and *Psidium guajava* L. variety Navalur were collected from in and around of Karnatak University, Dharwad, Karnataka state, India. The taxonomical identification of *Psidium guajava* L. variety navalur was identified by Dr. G. Mulugund with taxonomic keys. Herbarium specimens were prepared and lodged in the Ward Herbarium (RKC/KUD-BOT/04) at Department of Botany, Karnatak University, Dharwad, Kranataka India. The herbarium specimen of *Aegle marmelos* are stored in the herbarium of Department of Applied Botany, Mangalore University.

Extraction of plant Essential oils (EO): The dried leaves (100g) were powdered mechanically using commercial electrical stainless steel blender and essential oils were extracted with the help of hydrodistillation method by Clevenger apparatus. 1% stock solution is prepared by adding 1 ml of oil from each plant in 99 ml of acetone [16, 17].

Mosquitoes Rearing Technique: The egg of *Anopheles stephensi* were obtained from National Institute of Malaria Research Centre (NIMR) Field Unit, Bangalore, Karnataka state, India. They were placed in a white tray containing Reverse osmosis (RO) water. Larvae were fed with powdered yeast and dog biscuits in the ratio 3:1 and maintained at (27 ± 2) °C, (75%-85%) RH, under 14 L: 10D photoperiod cycles [18, 19]. It takes about 6- 8 days for larval development and pupae were separated using the droppers. Obtained pupae were kept inside the mosquito cage for emergence. The cotton balls or pads were prepared and soaked in 10 % glucose solution and placed inside the cage for the nourishment of mosquitoes after three days of emergence. The Wistar rat blood meal was given to adult female mosquitoes. The ovi-traps containing water is placed in the cage. The eggs were laid in the ovi-trap after three days of blood meal [20, 21].

Larvicidal bioassay: The extracted oils from two different plants *Aegle marmelos* and *Psidium guajava* were used in 5-6 different concentrations (50,60,70,80,90 ppm and 30,35,40,45,50,55 ppm respectively) and their activity was evaluated by standard WHO method with slight modifications [22, 23, 24]. The activity was conducted at (27 ± 2) °C, (75%-85%) RH, under 14 L: 10 D photoperiod cycles. The 20 late third to early fourth instar larvae are taken in each trial of the bioassay. Five replicates of each concentration were set up with a control containing acetone. The control mortality was corrected by using Abbot's Formula [25] and the LC_{50} and LC_{90} values were calculated by using log- probit analysis [22, 23, 26]. The whole experiment of larvicidal bioassay was carried out at ICMR-NIMR, Bangalore, Karnataka, India.

Statistical analysis: The mortality observed (ppm) was corrected using Abbott's formula during the observation of the larvicidal effect of plant essential oils. Statistical analysis of the experimental data was performed using the computer software Microsoft excel 2010 to find the LC_{50}, LC_{90} values by log- probit analysis. Differences between means were considered significant at $P < 0.05$[27, 28].

3. RESULTS AND DISCUSSION

The successive extraction of essential oils from the leaf samples of *Aegle marmelos* and *Psidium guajava* yielded 2ml/50gm of the powdered sample and 1.7ml/50gm of powdered sample respectively. Larvicidal bioassay conducted with both the essential oils indicated that the essential oil of *Psidium guajava* has maximum larvicidal activity than the essential oil of *Aegle marmelos* (figure: I & II).

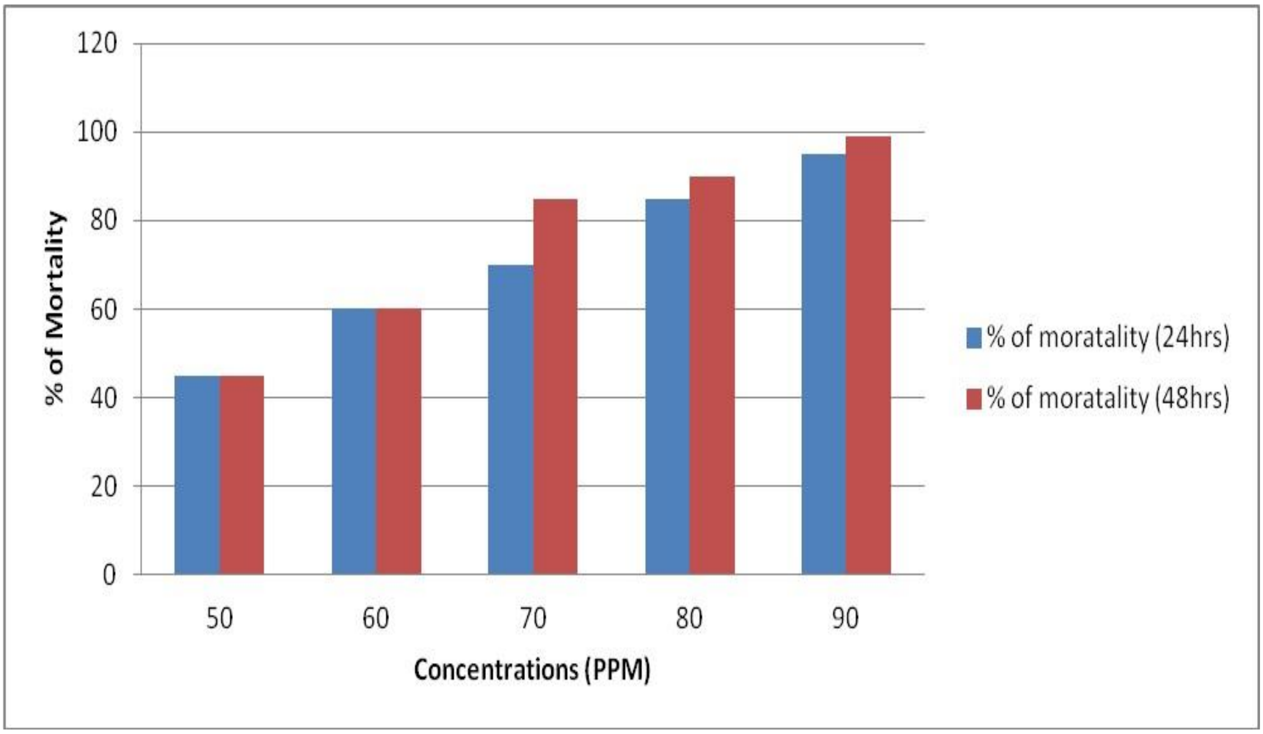

Figure I: Larvicidal activity of the essential oil of *Aegle marmelos* against *Anopheles stephensi*

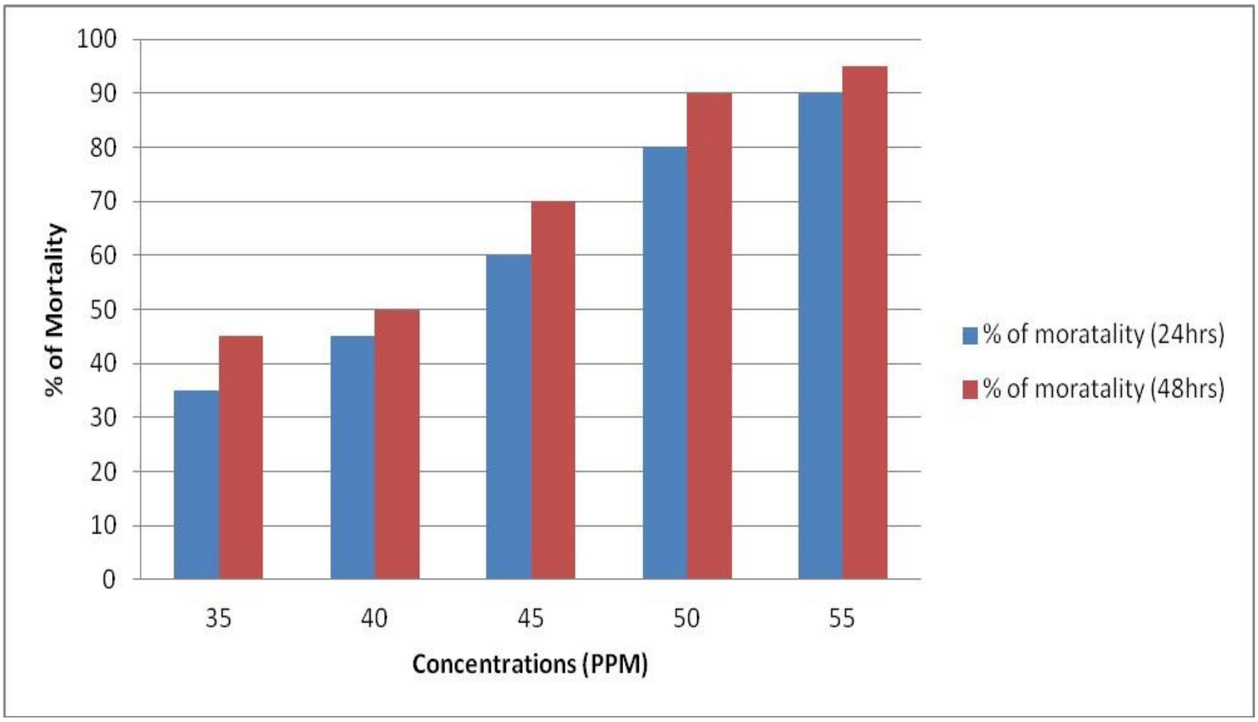

Figure II: Larvicidal activity of the essential oil of *Psidium guajava* against *Anopheles stephensi.*

The EO of *Psidium guajava* exhibited 95% mortality at 55 ppm whereas the *Aegle marmelos* showed at 90 ppm after 48hrs. The EO of *Psidium guajava* exhibited the 50% of mortality between 40-45 ppm at 24hrs and 35-40 ppm at 48 hrs in larvae. But in the case of *Aegle marmelos* showed the 50 % of mortality between 50-60 ppm at 24 and 48 hrs. The LC_{50} (LC_{90}) values of *Psidium guajava* and

Aegle marmelos by log- probit analysis are 40.27(56.49) ppm, 54.95 (85,11) ppm at 24 hrs and 38.02 (51.52) ppm, 53.95(74.3) ppm at 48 hrs respectively (Table: I).

Table I: Larvicidal activity of essential oils from *Aegle marmelos* (L.) Correa and *Psidium guajava* L. against *Anopheles stephensi*

Plant essential oil	Time (hrs)	LC_{50} and (LC_{90}) (ppm)	Regression equation	Chi-square value (X^2)
Aegle marmelos (L.) Correa,	24	54.95 (85,11)	Y=6.69x-6.61	3.0276
	48	53.95 (74.3)	Y=9.21x-10.95	2.999824
Psidium guajava L.	24	40.272 (56.49)	Y=8.70x-8.962	2.930944
	48	38.02 (51.52)	Y=9.715x-10.35	2.4964

The essential oils are complex blend of biochemical compounds and there could be more than one compound behind the mosquito - larvicidal activity. Larvicides of plant origin have received attention as agents for vector control. Chalannavar *et al*., (2013) reported the insecticidal and repellent activity of *Psidium guajava* L. against *Anopheles arabiensis* [29]. Rajkumar and Jebanesan (2007) reported repellent activity of *Psidium guajava* against *Anopheles stephensi* using the human-bait technique [13]. Elango *et al*., (2011) reported that methanol extract of the *Aegle marmelos* shows ovicidal deterrence activity against *Anopheles subpictus* at 500 ppm was -0.91 [30]. Rathy *et al*., 2015 studied aqueous extract of *Aegle marmelos* against *Aedes albopictus* larvae and the 55% larvicidal property found 8ml of the extract at 96 hours [31]. Sarma *et al*., (2017) studied the essential oil from the leaves of *Aegle marmelos* and investigated for their larvicidal, ovicidal, adulticidal and repellent properties against *Aedes aegypti* and *Culex quinquefasciatus* [32]. Plants are a source of ecologically sensitive pesticides, generally considered safe to humans and other mammals [33]. Vector control is an essential part of reducing malaria transmission and became less effective in recent years [4, 34]. The plant essential oils of both the species exhibited larvicidal effects against *Anopheles stephensi* and may be helpful for minimizing the dependency on synthetic chemicals.

4. CONCLUSION

In the present study, the essential oils extracted from *Aegle marmelos* and *Psidium guajava* exhibited excellent larvicidal activity. The bunch of chemicals of present in essential oils active against larval stage is helpful to control malaria-transmitting *Anopheles stephensi* population in their breeding site.

These plant essential oils are easy to prepare, inexpensive and safe for mosquito control which possesses enough potential in larvicide could be used directly as larvicidal agents in small volume at aquatic habitats or in/ around human dwellings after confirming the toxicity level. Results from this study have initiated further isolation and purification of major compounds from the essential oils of leaves of *Aegle marmelos* and *Psidium guajava*.

ACKNOWLEDGEMENT

The authors are grateful to the Dr. S K Ghosh, Director of the National Institute of Malaria Research, Field Unit, Bengaluru for providing laboratory facility to conduct the Larvicidal activity. We are also thankful to Visiting Scientist, Dr. Ravindra B. Malabadi, Miller Blvd, NW, Edmonton, AB, Canada for the valuable guidance and support.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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