

In vitro larvicidal activity of aqueous leaf extract of *Garcinia gummi gutta* Linn. against mosquito larvae

*Lincy Sara Varghese, Shehma Shukkoor, Jaina P James, Beena Habel, Aiswarya Tom, Shalia Joseph

Department of Botany, Bishop Kurialacherry College for Women, Amalagiri, Kottayam, Kerala, India

Abstract

The present study focussed on the larvicidal activity of bioactive compounds in the crude aqueous extract of leaves of *Garcinia gummi gutta* against the late III or early IV instar mosquito larvae. The aqueous extract was used at varying concentrations of 20%, 40%, 60%, 80% and 100% of crude extract, containing 45, 90, 133, 180 and 225 mg/ml of the extract, respectively. Of the five test concentrations used, 100% mortality rate was observed for the extracts containing 180 and 225 mg/ml, at 25 and 15 minutes, respectively. For the test samples at 90, 133, 180 and 225 mg/ml concentrations LC50 values were obtained at 30, 20, 15 and 10 minutes, respectively.

Keywords: *Garcinia gummi gutta*, leaf extract, larvicidal activity, mosquito larvae, mortality rate

1. Introduction

Mosquitoes are the major vectors for the transmission of serious human diseases such as malaria, dengue fever, yellow fever, filariasis, schistosomiasis and Japanese encephalitis, and results in millions of deaths every year [1, 2]. Hence new ways of reducing the incidence of such diseases has been undertaken by health institutions and medical agencies in a large scale; at the primary health centres and even at individual households. This is mainly aimed at blocking disease transmission by either killing, preventing mosquitoes to bite human beings (by using repellents) or by causing larval mortality in a large scale at the breeding centres of the vectors. Many approaches have been developed to control mosquito, the primary approach being based on the use of synthetic insecticides. Nevertheless they are effective; they have created many problems like insecticide resistance, pollution and toxic side effects on human beings [3, 4]. Besides, they have also caused the problem of pesticide resistance in some mosquitoes [5]. This has led to the use of plants as alternative sources of mosquito control agents. The phytochemicals or other secondary metabolites present in plants have recently been given much emphasis as pesticides and insecticides since many of them are eco-friendly. Many plant extracts or allelochemicals show a broad spectrum of activity against pests that such products have long been used as attractive alternatives to synthetic chemical pesticides for pest management.

Garcinia gummi gutta Linn. is a semi-domesticated crop, with wide distribution in semi-evergreen to evergreen forests. It is a tropical tree belonging to the family Guttiferae (Clusiaceae); commonly known as 'Kodampuli' or 'Malabar tamarind'; grown in India, Sri Lanka, Africa and Malaysia. *Garcinia* is found in the evergreen forests of Western Ghats; commonly seen in the states of Maharashtra, Goa, Karnataka, Kerala and Tamil Nadu. It is a cultivated crop in the Kerala state of India⁶. Malabar tamarind has been shown to contain a variety of secondary metabolites such as xanthenes, flavonoids and benzophenones [7, 8, 9].

The leaves of *Garcinia* contain hydroxyl citric acid [10]. The presence of tannin, phlobatannin, saponin, flavanoids, terpenoids and cardiac glycosides has been reported in the crude extract of *Garcinia gummi gutta* leaves [11]. Several authors have linked the antimicrobial properties of the crude extracts to the presence of these bioactive compounds [12, 13, 14]. The presence of saponins, cardiac glycosides and flavonoids has been attributed for the anti-tumour activity and also for their action against gastrointestinal disorders [11]. Much research has been done on the anti-inflammatory, anti-bacterial, and anti-cancer properties of *Garcinia*. Few studies have addressed the antifungal aspects of *Garcinia*. Hitherto there have been no reports on the larvicidal activity of aqueous extract of *G. gummi gutta*. The present study aimed at elucidating the larvicidal activity of aqueous leaf extract of *G. gummi gutta* against mosquito larvae.

2. Materials and Methods

Collection of Sample

Fresh leaves of *Garcinia gummi gutta* were collected from Kottayam District, Kerala, India.

Surface Cleaning and Sterilization of the Leaf Samples

The leaves of *Garcinia gummi gutta* were surface sterilized following the modified method of Aneja [15]. The leaves were washed under running tap water for 10 minutes followed by detergent wash in 10% Extran (Merck) for 10 minutes. The leaves were rinsed with distilled water, rinsed in 70% ethanol for 30 seconds and washed again in distilled water till the ethanol smell completely diminished. The leaves were finally spread out in clean trays for oven drying.

Preparation of Extract

Fresh sterile samples of leaves of *Garcinia gummi gutta* were oven dried at 60°C, continuously, for 7 days. The dried samples were powdered using a clean grinder. The powder was stored in air tight container at room temperature before extraction. A fixed weight of 30 gm of the powdered material

was weighed out in aseptic condition and was tied in a clean cheese cotton cloth. Extracts were prepared using Soxhlet Apparatus at an extraction temperature of 100°C. An initial volume of 250 ml of distilled water was taken in a 500 ml round bottom flask for the extraction. The extraction was carried out continuously, for 8 hrs after which it was concentrated by evaporation and made up to a final volume of 20 ml. The extract was stored at room temperature, in sterile screw capped containers, till use.

Larvicidal Bioassay

The larvicidal bioassay was carried out using the modified procedure of Dhanya and Benny [16]. For the assay the crude aqueous extract was dissolved in sterile water, so as to solubilise the compounds as well as to prepare graded series of concentrations. From the crude aqueous extract, different concentrations ranging from 20%, 40%, 60%, 80% and 100 %, containing 45, 90, 133, 180 and 225 mg/ml of the extract were prepared. Control was maintained by adding 5 ml of distilled water. The assay was carried out in duplicates.

The larvicidal bioassay was carried out in Petri plates. For the treatment the late III or early IV instar mosquito larvae were used. The larvae were exposed to 5 ml each of 20%, 40%, 60%, 80% and 100% of crude aqueous extracts of leaves of

G. gummi gutta for a time period of 30 minutes. The larvae were considered dead if, at the end of 30 minutes, they showed no signs of swimming movements even after touching with a glass rod. The data were analysed and the mortality rates were recorded.

3. Results and Discussion

Garcinia gummi gutta Linn., a member of the family Guttiferae, is a rich source of secondary metabolites and a potential source of herbal medicine. Earlier studies have revealed the anti-inflammatory, antibacterial and anticancer properties *G. gummi gutta*. The presence of high levels of alkaloids, tannins, phenolic flavonoids, carbohydrates and proteins has been reported in the leaves of *G. gummi gutta gutta* [11, 17]. Madappa and Boppaiah [17] have reported the presence of tannins, in high quantities in methanol and ethanol extracts of *Garcinia*, which have been found toxic to fungi, bacteria and viruses in plants. Dhanya and Benny [18] have reported the antifungal activity of methanolic extracts of leaf of *G. gummi gutta* against *Phytophthora* sp., *Curvularia* sp. and *Corynespora* sp. Leaves of *G. gummi gutta* contain bioactive phytochemicals and medicinally active compounds with activity against bacteria, fungi and larvae.

Table 1: Larvicidal Effect of Aqueous Leaf Extract of *Garcinia gummi gutta*

% composition of the crude aqueous extracts	Concentration of the extracts (mg/ml)	No. of dead larvae											
		5 minutes		10 minutes		15 minutes		20 minutes		25 minutes		30 minutes	
		T	C	T	C	T	C	T	C	T	C	T	C
20	45	0	0	0	0	0	0	0	0	0	0	1	0
40	90	0	0	0	0	1	0	1	0	1	0	2	0
60	133	0	0	1	0	1	0	2	0	2	0	2	0
80	180	0	0	1	0	3	0	3	0	4	0	4	0
100	225	1	0	3	0	4	0	4	0	4	0	4	0

T - Test sample, C- Control

Shaded portion shows the maximum mortality rate with respect to time at each test concentration of the extract

This is a preliminary study aimed at elucidating the larvicidal activity of bioactive compounds in the leaves *G. gummi gutta*. In this study the aqueous extract of leaves of *G. gummi gutta*, at varying concentrations 20%, 40%, 60%, 80% and 100% of the crude extract (containing 45, 90, 133, 180 and 225 mg/ml respectively), were tested against late III or early IV instar mosquito larvae. The mortality rate was calculated, after verifying the number of dead larvae, starting from the moment of exposure of the larvae to the extract, to the final stage of the experiment, i.e, at the end of 30 minutes. The mortality rates were calculated for the varying concentrations of the extracts as test samples (T) and were compared with the larvicidal activity of water as control (C). The crude aqueous extract (225 mg/ml) exhibited 25% mortality within 2 minutes of exposure. A 50% larvicidal activity (LC50) was observed within 5-10 minutes of exposure to the crude extract (Table 1). Total killing or 100% mortality of the larvae was observed within 10-15 minutes after exposure to the crude aqueous extract. A 50% mortality rate was observed for all the higher concentrations. The test samples containing 90, 133, 180 and

225 mg/ml exhibited LC50 at times 30, 20, 15 and 10 minutes, respectively, following the addition of the extracts (Table 1 and Figure 1).

Similar results have been obtained during the investigations of Dhanya and Benny [16] on the larvicidal activity of flavonoids isolated from the methanolic extracts of leaves of *G. gummi gutta* against *Aedes aegypti*. In their study they observed that at higher concentrations (3 mg/ml) the extract showed 96% mortality of the larvae while at lower concentrations the extract had a stimulatory effect rather than the inhibitory effect. Likewise, in this study, the extract at the lower concentration of 45 mg/ml exhibited the lowest mortality rate of 25% even at the end of 30 minutes. Total killing or 100% mortality was observed at higher concentrations of 180 (80%) and 225 mg/ml (100%) of the extract after exposure for 25 and 15 minutes, respectively (Figure 1).The concentration of the crude extracts used in the present study are much higher than that was used in the studies of Dhanya and Benny [16] which could possibly be the major reason for the high mortality rate observed within the short test time of 30 minutes.

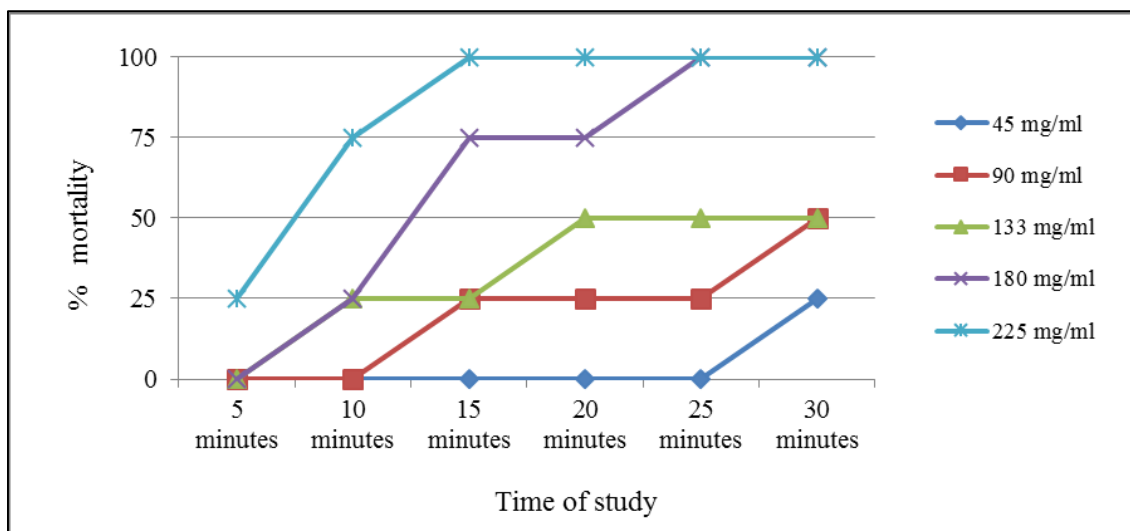


Fig 1: Percentage Mortality of Mosquito Larvae at Varying Concentrations of Aqueous Leaf Extract of *Garcinia gummi gutta*

4. Conclusions

The plant *Garcinia gummi gutta* has gained much importance as a promising source of ethnomedicine. The people belonging to Kurichia, Kuruma, Kattunaika, Adiya and Paniya tribes of Wayanad use the plant in the treatment of many diseases. The leaves of this plant have been used by the Kurichia tribes of Wayanad district to prepare medicine for dysentery, diarrhoea, tonsillitis, ulcer and bleeding piles^[19]. The phyto-chemical investigations of Madappa and Boppaiah^[17] have resulted in the identification of many compounds that have specific activity against wide range of microbial population. The present study investigated the larvicidal activity of aqueous leaf extract of *G. gummi gutta* against late III or early IV instar mosquito larvae. A 50% larvicidal activity was observed for all the test concentrations, except at the lowest concentration of 45 mg/ml. A 100% mortality rate was observed at concentrations of 180 and 225 mg/ml of the extract. The larvicidal effect of aqueous extract of leaves of *G. gummi gutta* has hitherto not been reported. The results of this study reveal the efficacy of *G. gummi gutta* as a potent larvicidal agent. The phytochemicals or the bioactive principles in this plant could be of immense application in combating many of the mosquito-borne diseases in the future.

5. References

1. Das MK, Ansari MA. Evaluation of Repellent Action of *Cymbopogon martinii martinii* Stapf var Sofia Oil against *Anopheles sundiacus* in Tribal Villages of Car Nicobar Island, Andaman & Nicobar Islands, India. *J Vect Borne Dis.* 2003; 40:101-104.
2. Becker N, Petriae D, Zgomba M, Boase C, Dahl C, Lane J *et al.* Mosquitoes and their Control. New York, Kluwer Academic Plenum Publisher, 2003; 1-16.
3. Lixin S, Huiquin D, Chongxia G, Jin Q, Jing S, Lei M *et al.* Larvicidal Activity of Extracts of *Ginko biloba* Exocarp for Three Different Strains of *Culex pipiens pallens*. *J Med Entomol.* 2006; 43(2):258-261.
4. Liu H, Xu Q, Zhang L, Liu N. Chlorpyrifos Resistance Mosquito *Culex quinquefasciatus*. *J Med Entomol.* 2005; 42:815-820.
5. Su T, Mulla MS. Ovicidal Activity of Neem Products (Azadirachtin) against *Culex tarsalis* and *Culex quinquefasciatus* (Diptera: Culicidae). *J Am Mosq Control Assoc.* 1998; 14:204-209.
6. Abraham Z, Malik SK, Rao GE, Narayanan SL, Biju SL. Collection and Characterization of Malabar Tamarind [*Garcinia cambogia* (Gaertn.) Desr.]. *Genet Resour Crop Ev,* 2006; 53:401-406
7. Selvaraj TI, Avadhani M. Medicinal Properties of Malabar Tamarind [*Garcinia cambogia* (Gaertn.) DESR.]. *Int J Pharm Sci Rev Res.* 2013; 20:101-107.
8. Koshy AS, Anila L, Vijayalakshmi NR. Flavonoids from *Garcinia cambogia* Lower Lipid Levels in Hypercholesteremic Rats. *Food Chem,* 2001; 72(3):289-294.
9. Masullo M, Bassarello C, Suzuki H, Pizza C, Piacente S. Polyisoprenylated Benzophenones and an Unusual Polyisoprenylated Tetracyclic Xanthone from the Fruits of *Garcinia cambogia*. *J Agric Food Chem.* 2008; 56:5205-5210.
10. Hemshekhar M, Sunitha K, Santhosh MS, Devaraja S, Kemparaju K, Vishwanath BS *et al.* An overview on Genus *Garcinia*: Phytochemical and Therapeutical Aspects. *Phytochem Rev.* 2011; 10(3):325-351.
11. Devi Prasad AG, Raghavendra MG, Shyma TB. Antimicrobial Activity of Tribal Medicines Collected from Wayanad District, Kerala. *World J Pharm Res.* 2014; 3(2):2476-2492.
12. Adesokan AA, Akanji MA, Yakubu MT. Antibacterial Potentials of Aqueous Extract of *Enantia chlorantha* Stem Bark. *Afr J Biotechnol.* 2007; 6:2502-2505.
13. Oyeleke SB, Dauda BEN, Boye OA. Antibacterial activity of *Ficus capensis*. *Afr J Biotechnol.* 2008; 17:1414-1417.
14. Sahm DF, Washington JA. Antibacterial Susceptibility Tests: Dilution methods. In: Lennette, EH (eds). *Manual of Clinical Microbiology*, Edn 5, Washington DC; American Society of Microbiology, 1990, 1105-1118.
15. Aneja KR. *Experiments in Microbiology, Plant Pathology, Tissue Culture and Mushroom Production Technology.* Edn 3, Published by New Age International (P) Limited, New Delhi, India, 2002.
16. Dhanya P, Benny PJ. Larvicidal Action of *Garcinia gummi gutta* Robs. var. *gummi gutta* on Dengue Victim *Aedes aegypti*. *Biochemistry* 2014; 4(1):21-22.

17. Madappa MB, Bopaiah AK. Preliminary Phytochemical Analysis of Leaf of *Garcinia gummi gutta* from Western Ghats. J Pharm Biol Sci. 2012; 4:17-27.
18. Dhanya P, Benny PJ. Antifungal Effect of Methanolic Extracts of Leaves of *Garcinia gummi gutta* L. Int J Pharm Sci Rev Res. 2013; 59:330-333.
19. Devi Prasad AG, Shyma TB, Raghavendra MP. Informant Consensus Factor and Antimicrobial Activity of Ethnomedicines used by the Tribes of Wayanad District, Kerala. Afr J Microbiol Res. 2013; 7(50):5657-5663.