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Larvicidal Action of Garcinia Gummi-Gutta.Robs. Var. Gummi-Gutta on Dengue Victim Aedes Aegypti

KEYWORDS: Aedes aegypti, flavonoids, Garcinia gummi-gutta, larvicidal, leaf extract,percentage mortality

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ABSTRACT *Mosquitos are the vectors of many vector-borne diseases that outbursts at rainy seasons. Of these dengue fever is a common one caused by Aedes aegyptii. As a solution, many experiments were conducted in this study to evaluate the larvicidal potential of Garcinia gummi-gutta . Crude methanolic leaf extracts of Garcinia gummi-gutta were tested on Aeedes aegypti under in vitro condition. The results indicated that the leaf extract of Gracinia gummi-gutta showed more cytotoxic effect (inhibitory) on the third or fourth instar larva at higher concentrations as compared to control, while the lower concentrations of leaf extract showed negative larvicidal (stimulaitory) effect on growth parameters of mosquito larva. Presence of flavonoids have been observed and , LC/MSanalysis was carried out on methanol leaf extract to find out the active component.*

Introduction
Mosquitos are insects which are capable of transmitting diseases like malaria, filariasis,dengue, Japanese encephalitis, etc.[Becker et al.,2003]. These diseases are disastrous to human life. Among these diseases, dengue remains the most serious vector-borne disease affecting millions of people throughout the world and it is reported to infect more than 100 million people every year in more than 110 countries in the tropics [Halstead,2000]. *A. aegypti* is the principal vector of dengue fever and dengue haemorrhagic fever. At present no any promising medicines are avaiabe for dengue. New way of reducing the incidence is to block 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, among which the current approach is based on synthetic insecticides. Even though they are effective, they created many problems like insecticide resistance [Lixin et al.,2006], pollution and toxic side effects on human beings [Liu et al.,2005]. In addition, they have also caused the problem of pesticide resistance in some mosquitoes [Su and Mulla,1995].

Thus the use of natural products like phytochemicals and biopesticides, has recently been given much emphasis against the use of chemical fungicides and pesticides since they are eco-friendly. Many plant extracts or allelochemicals show a broad spectrum of activity against pests and such products have long been used as attractive alternatives to synthetic chemical pesticides for pest management because they pose little threat to the environment or to human health. The effects of botanical derivatives against mosquito have been reviewed [Sukumar et al.,1991].

Garcinia gummi-gutta var. *gummi-gutta* is a plant genus of the family Clusiaceae native to Asia, Australia, tropical and South Africa, and Polynesia.They are commonly known as Kodampuli or Malabar Tamarind. The Clusiaceae family is a rich source of secondary metabolites. Four major classes of compounds are found in the Cluisaceae family: xanthones, coumarins, biflavonoids, and benzophenones [Tharachand et al.,2013]. *Garcinia gummi-gutta* has been investigated extensively by many researchers in reference to its medicinal properties and various other cytotoxic effects [Nimbalet al.,1996b] Although many researchers have confirmed its antifungal [Duke et al.,1997], anticancer [Acuna et al.,2009], antimicrobial [Shara et al.,2004] and antiviral effects [Danziel JM,1937], making the plant a good source of medicine , only few researches have been done on its allelopathic aspect. So the purpose of the present study was to elucidate the alleopathic potential of of *Garcinia gummi-gutta* leaf extracts on some common agricultural crops.

Materials and Methods
The leaves of *Garcinia gummi-gutta* were collected,chopped into small pieces, oven-dried and were coarsely powdered using in a table model grinder. The ground plant leaves(50 g) were dipped in methanol solvents and were extracted with 250ml of methanol using a Soxhlet extractor for 72 h at a temperature not exceeding the boiling point of the solvent. The extracts were filtered using filter paper (Whatman No.1) and then concentrated in vacuo at 40°C using a Rotary Evaporator. The extract was considered as stock solution. The extracts obtained were stored in a freezer at -80°C until further tests [Fuji et al., 2003].

About 25 ml of the crude extract containig 2.430 g residue was subjected to purification by means of Normal Phase Coloumn chromatography using silica gel(60-120 mesh size) in successive elution with $CHCl_3$-MeOH in the ratio 2:1(30 ml each). The process of purification was repeated 2-3 times to increase the concentration. The fractions found to contain flavonoids (through phytochemical analysis) were pooled together (fraction 14-19), evacuated to dryness , thus obtained a concentration of 162.1.9 mg. Redissolved in 50 ml methanol. From this, a 5ml containing13.05mg was taken for larvicidal test.

Treatment of larvae with leaf extract **:** For the assay, dry extracts of the fraction (13.05mg in 5ml methanol) isolated from methanolic extract of the leaves were dissolved in 10ml dimethylsulphoxide (DMSO) to prepare graded series of concentrations. so as to solubilize the compound in water. From the stock solution, different concentrations ranging from 0,0 .5, 1.0, 2, 3,4 and 5ml /lit were prepared. Control medium was also maintained to 100 ml by adding 95 ml tap water and 5ml DMSO. Four replicates were maintained for each concentration.

Larvicidal bioassay was carried in 500 ml glass beakers containing 250 ml of water and 20 numbers of late III or early IV instar mosquito larvae for various concentrations. For the treatment of larvae with the leaf extract of *Gracinia gummi-gutta*,100 ml of tap water was kept in a series of glass beak-

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ers (of 200 ml capacity). Twenty numbers of 3rd or 4th instar larvae of *A. aegypti* were separately introduced into control and different concentrations of leaf extract. The mortality rate was evaluated 24 hours after the beginning of the experiment, verifying the number of dead larvae. After treatment, the larvae were considered dead if, at the end of 24 hrs, they showed no sign of swimming movements even after gentle touching with a glass rod, as described in the World Health Organization's technical report series [WHO,1981]. The number of larvae surviving at the end of 24 h was recorded and the per cent mortality values were calculated.

Results and Discussions

In the present investigation, the larvicidal potential of flavonoids isolated from methanolic extracts of leaves of the plant *Gracinia gummi-gutta* at varying concentrations against the larval stage of dengue vector *Aedes egypti* has been tested. The datas were recorded and mortality values were calculated. Results shows that the plant is possessing the activity at higher concentrations (inhibitory), whereas a negative larvicidal effect (stimulatory) was observed at lower concentrations. Of the seven concentrations used against *Aedes egypti* larvae of 95.6%, has been observed with a concentration of 3mg/ml. A 50% larvicidal effect (LC50)was exhibited by a concentration of 2.5mg/ml. Lower concentrations did not exhibit any larvicidal effect. The activity increased with an increase in the concentration of the compound (Table.1). So we can suggest that flavonoid compound isolated from *G.gummi-gutta* is possessing the ability to kill mosquito larvae at higher concentrations.

Table.1.Larvicidal Effect of the Flavonoids isolated from Garcinia gummi-gutta

Concentration of methanolic extract of leaf of *G.gummi-gutta(mg/ml)*	No.of larva dead after 48 hr (out of 20 larva)		Percentage mortality (%)	
	Control	Test	Control	Test
0.25	0.5±0.58	0.5±0.58	2.5	2.5
0.5	0.5±0.58	0.5±0.58	2.5	2.5
1	0.5±0.58	0.5±0.58	2.5	2.5
1.5	0.5±0.58	0.5±0.58	2.5	2.5
2	0.5±0.58	4.75±0.96	2.5	23.75
2.5	0.5±0.58	10.75±0.96	2.5	53.75
3	0.5±0.58	19.12±0.96	2.5	95.6

Four replicates were done.Values are expressed as mean±SD.

Flavonoids which are the pharmacologically active constituents in many herbal plant medicines are known to possess cytotoxic effects. Larvicidal effect of the plant *G.gummi-gutta* has not been tested before. The studies conducted on larvicidal effect of *Azadirachta indica* (Meliaceae) against *Aedes egypti* supports the idea [Dua et al., 1995]. Similar dose dependant effect of *Lantana camera* extracts on third instar larvae of *C. quinquefasciatus* showed larvicidal activity and cause maximum mortality rate at 3.0mg/ml concentration [Sathish Kumar and Maneemegalai, 2008]. Larvicidal activity of ethyl acetate, butanol, and petroleum ether extracts of five species of *Euphorbiaceae plants, Jatropha curcas, Pedilanthus tithymaloides, Phyllanthus amarus*, *Euphorbia hirta*, and *Euphorbia tirucalli*, were tested against the early fourth instar larvae of *Aedes aegypti L.* and *Culex quinquefasciatus* [Rahuman et al.,2008].

Plant extracts have offered many beneficial uses in applications ranging from pharmaceuticals to insecticides The combined effect of phenolic acids such a caffeic acid, vanillic acid, ansic acid, p-ansic acid, chlorogenic acid and parahydroxy benzoic acid isolated from the leaf extract of *P. hysterophous* revealed the larvicidal and pupicidal property on *A. aegypti* and *C. Quinquefasciatus* [Narasimhan and Keshava murthy,1984]. Parthenium isolated from the lant *Parthenium hysterophus* is also reported as promising remedy against hepatic amoebiasis [Sharma and Bhutani,1988;Dominguez and Sierra,1970]. The botanical insecticides are generally safer, readily biodegradable non-toxic. But they are found active against the insect pest, lack toxicity to higher animals and they do not leave any phytotoxic residues in the environment. Thus the observation made in the present study have come as yet another evidence for the significant influence of the plant derived botanical pesticide like *G.gummi-gutta* in the control of mosquito.

From the above results, it has been observed that that the flavonoids isolated from the leaf extracts of the plant *G.gummi-gutta* are promising as larvicidal agent against *Aedes egypti*. Further studies are needed to determine its mode of action, toxicity, stability , its impact on human health and non target organisms in mosquito feeding habitats when exploited as a larvicide etc.

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