



## Antifungal Effect of Methanolic Extracts of Leaves of *Garcinia Gummi-Gutta.L.*

Dhanya.P., Benny P.J\*.

Department of Zoology, St.Thomas College Palai under Mahatma Gandhi University, Kottayam, Kerala, India.

\*Corresponding author's E-mail: [sudhidhanya09@gmail.com](mailto:sudhidhanya09@gmail.com)

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### ABSTRACT

The antifungal effect of methanolic extract of leaves of *Garcinia gummi-gutta* were tested *in vitro* against three plant pathogenic fungi. For the antifungal evaluation we adopted well-diffusion method. The results revealed that the plants extracts had a strong antifungal activity with significant inhibition on the growth of the 3 tested fungi *Phytophthora sp.*, *Curvularia sp.* and *Corynespora sp.* Of these, the *Phytophthora sp.*, exhibited maximum zone of inhibition, whereas *Corynespora sp.* exhibited the least zone of inhibition. The inhibitory effect of plant extracts may be due to the antifungal principles / compounds present in them. Findings from this study revealed that plant extracts can be used as natural fungicides to control pathogenic fungi which causes diseases in plants, thus reducing the dependence on the synthetic fungicides.

**Keywords:** Antifungal, *Garcinia gummi-gutta*, methanolic extract, fungicides.

### INTRODUCTION

Fungi plays an important role in our day to day life both directly or indirectly. Some of them are beneficial to us, whereas some others may be harmful. Harmful effects include causing diseases in plants, animals and human beings, considerable loss of food, vegetables and fruit crops, destruction of valuable timbers, spoilage of food and many other useful articles etc. Fungi can destroy foodstuffs and grains during storage by producing mycotoxins which is toxic for human consumption. It can also deteriorate the nutritive value of food as well. A significant portion of the agricultural produce in the country and the world over become unfit for human consumption due to mycotoxins contamination of grains, especially those produced by species of *Aspergillus*<sup>1-3</sup>. During the past several years, there has been an increasing incidence of fungal infections in plants thus paving the way for great agricultural losses. Many of the parasitic fungi can cause diseases in plants like smuts, rusts, mildews, blight, rots and wilts. Several species of fungus can cause post harvest diseases in fruits and vegetables, thus shortening their storage life. A wide array of phytopathogenic fungi have been isolated, of which includes *Phytophthora sp.*, *Curvularia sp.* and *Corynespora* species. *Phytophthora* is a genus of plant-damaging Oomycetes, whose member species are capable of causing enormous economic losses on crops worldwide, as well as environmental damage in natural ecosystems. *Curvularia* and *Corynespora* are hyphomycete fungus which is a facultative pathogen of many plant species and of the soil<sup>4</sup>.

An enormous varieties of chemicals have been developed to control many plant diseases. The continuous and injudicious application of synthetic products paved the way for consequences like decreased resistance to pathogens, accumulation of the residual chemicals in the environment causing environmental pollution and food

contamination. Even though effective and efficient control of seed borne fungi of seeds can be achieved by the use of synthetic chemical fungicides, the same cannot be applied to grains for reasons of pesticide toxicity<sup>5,6,7</sup>. Excessive usage of pesticides in agriculture to over come the pre-harvest and post-harvest problems also resulted in many toxic epidemics. Thus, there aroused the need to search for alternative approaches for prevention of biodeterioration of grains during storage without any toxicity to the consumer and also for the development of fungicides that are safe, ecofriendly and cost effective. Plant metabolites and plant based pesticides appear to be one of the best alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to the synthetic pesticides<sup>8</sup>.

*Garcinia gummi-gutta.L.* 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 are a rich source of secondary metabolites. Four major classes of compounds are found in the Cluisaceae family: xanthenes, coumarins, biflavonoids, and benzophenones. Although many researches have been done on the anti-inflammatory, anti-bacterial, and even anti-cancer properties of *Garcinia*, making the plant a good source of medicine, only few researches have been done on its antifungal aspect. Qualitative analysis of phytochemicals in the leaf extracts of *G.cambogia* revealed that the leaf of the plant does contain medicinally active phytochemicals. The studies on the preliminary qualitative analysis of the plant showed that leaves have high content of alkaloids, tannins, phenolic flavonoids, flavonoids, carbohydrates and proteins<sup>9</sup>. Present study aims to elucidate the antifungal activity of the flavonoids isolated and purified from the methanolic extracts of leaves of *Garcinia gummi-gutta* extracts on some common phytopathogenic fungi.



## MATERIALS AND METHODS

### Preparation of the extract

The leaves of *Gracinia 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 250 ml 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 and were stored in a freezer at -80°C until further tests<sup>10</sup>.

About 25 ml of the crude extract containing 2.430 g residue were subjected to purification by means of Normal Phase Column chromatography using silica gel (60-120 mesh size) as the stationary phase. The process was done in successive elution with CHCl<sub>3</sub>-MeOH in the ratio 2:1(30 ml each) and repeated 2-3 times to increase the concentration.

The bioactive fractions, which were found to possess allelopathic effect found to contain flavonoids (through phytochemical analysis). They 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 containing 10 mg was taken for fungicidal test.

### Tested fungi

The following fungi were used for experiments:

1. *Phytophthora* sp.
2. *Curvularia* sp.
3. *Corynespora* sp.

### Antifungal susceptibility test

Antifungal susceptibility test was done by Agar Well Diffusion method<sup>11,12</sup>. The methanolic extracts which were evaporated to dryness were dissolved in 10ml of 2% DMSO. After adding the fungal spore's inoculum into the sterile SDA medium aseptically, it was poured to plates and allowed to set.

A well of 6mm diameter was made at the centre of each plate by a sterile cork borer. Depth of the well must be sufficient to accommodate 1 ml extract. 1 ml of extract was added separately into each plate. In control experiment, 1 ml of DMSO alone (without leaf extract) was added. Both test and control plates were incubated at 25°C - 29°C for 2-7 days. After the incubation period, antifungal activity was evaluated by measuring the diameter of zone of inhibition in centimeters. Test was repeated six times, mean value was calculated and recorded as the result.

Figure. i.a: *Phytophthora* sp.

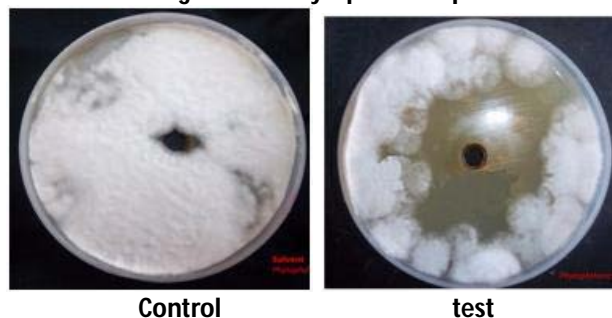


Figure. i.b: *Curvularia* sp.

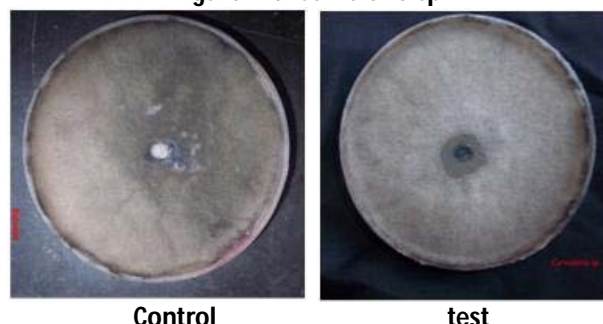
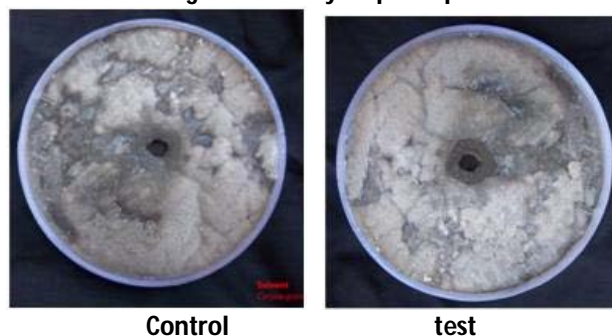


Figure. i.c: *Corynespora* sp.



## RESULTS AND DISCUSSIONS

The present work evaluated the antifungal effects of flavonoids isolated from the methanolic extracts of *G.gummi-gutta*. The growth inhibition caused by flavonoids were found highly effective in suppressing the growth of all the three phytopathogenic fungi used (Table-1). Results suggest that the plant is having fungicidal effects (Figure1.A-C). Even though antifungal property of *G.mangostana*, a member of Cluciaseaea family has been studied earlier<sup>13</sup>, the fungicidal effect of *G.gummi-gutta* has not been tested before.

The acetonic fruit extract of *Flacourtia inermis* as an effective alternative agent against the dangerous pathogen, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus flavus*, *Mucor ramosissimus*, and *Chrysosporium* sp. supports the idea<sup>14</sup>. The present work is also in agreement with the work which reported the high potency of plant extracts for the control of pathogenic fungi of other crops<sup>15-17</sup>.

The growth of all the three species of fungi viz. *Phytophthora* sp., *Curvularia* sp. and *Corynespora* sp. were found inhibited. Of these, the *Phytophthora* sp.,

exhibited maximum zone of inhibition, whereas *Corynespora sp.* exhibited the least zone of inhibition. The order of growth inhibition is as *Phytophthora sp.* > *Curvularia sp.* > *Corynespora sp.*. Thus we can infer that the *Corynespora sp.* were more resistant to the plant extract than the other two fungal species tested.

Flavonoids are polyphenolic compounds, widely distributed in plants fulfilling many functions including protection from attack by microbes and insects. Flavonoids is known to possess cytotoxic effects of which includes fungicidal activity. They possess anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activity<sup>18</sup>. They are important for the physiology of plants to contribute to resistance to microorganisms, insects and herbivorous animals that can affect plants<sup>19</sup>, to help preserve the integrity of the plant with continuous exposure to environmental stresses, including ultraviolet radiation, relatively high temperatures and dehydration<sup>20</sup>. The fungicidal action may be due to a single flavonoid or due to the combined action of a group of flavonoids. Hence there occurs the need for purification and characterization of the active flavonoid present in this plant. Lemongrass essential oil efficacy against these species isolated from wagashi is thought to depend on specific toxicity of its single main active constituents or by its synergic effect<sup>21</sup>.

Biologically active plant derived pesticides are expected to play an increasingly significant role in crop protection strategies. They offer a source of new alternative compounds with broad-spectrum activity that may pose little threat to the environment or to human health. Exploitation of naturally available chemicals from plants, which retards the reproduction of undesirable microorganisms, would be a more realistic and ecologically sound method for plant protection and will have a prominent role in the development of future commercial pesticides for crop protection strategies, with special reference to the management of plant diseases<sup>8,22</sup>.

**Table 1:** Antifungal Effect of the methanolic leaf extract of *G.gummi-gutta*

Experimental material	Nature of leaf extract	Zone of Inhibition
<i>Phytophthora sp</i>	Control	1.3
	Test	5.2
<i>Curvularia sp.</i>	Control	0
	Test	2.9
<i>Corynespora sp.</i>	Control	0.5
	Test	2.6

Control is DMSO alone. Test is dried extract dissolved in DMSO. Values are the mean of six replicates.

The present study concludes that the flavonoids present in the plant *G.gummi-gutta* possess antifungal property for the control of *plant pathogens*. Further studies are needed to determine the chemical identity of the

bioactive flavonoid responsible for the observed antifungal activity, effect of concentration of the particular compound etc. The findings in this study also confirm that plant extracts can be used as natural fungicides to control pathogenic fungi and thus reduce the dependence on the synthetic fungicides.

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