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EVALUATION OF ANTIMICROBIAL ACTIVITY OF LEAVES OF GARCINIA GUMMI GUTTA - A PLANT OF ETHNOMEDICINAL IMPORTANCE

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ABSTRACT

Garcinia gummi gutta Linn. is a plant of immense importance in ethnobotany. The plant has been used by the tribes singly or in combination with other plant parts to cure various ailments. The present study investigated the antibacterial activity of aqueous, acetone and methanolic extracts of leaves of *G. gummi gutta* on eight bacterial strains *viz Staphylococcus aureus, Salmonella typhi*, *Salmonella typhimurium*, *Bacillus* sp., *Proteus* sp., *Klebsiella* sp., *Serratia* sp. and *Pseudomonas* sp. and two fungal stains viz. *Aspergillus* and *Penicillium*, respectively. All the three extracts potentially inhibited the growth of the test bacteria. However, the acetone extract was superior in antibacterial activity, followed by the methanol and aqueous extracts. Significant antibacterial activity was observed against *S. aureus*, *Bacillus* sp. and *Serratia* sp. In the antifungal assay the aqueous extract exhibited minimal antifungal activity against *Penicillium* sp., but did not inhibit the growth of *Aspergillus* sp. Acetone extract inhibited the growth of *Aspergillus* alone, without having any inhibitory activity on *Penicillium* sp. Methanol extract inhibited the growth of both the test fungi.

KEYWORDS: *Garcinia gummi gutta*, secondary metabolites, antibacterial activity, antifungal activity, ethnomedicine.

INTRODUCTION

Garcinia gummi gutta Linn., commonly known as 'Kodampuli' or 'Malabar tamarind', is a dicotyledonous tropical tree belonging to the family Clusiaceae (Guttiferae). *G. gummi gutta* is a semi-domesticated crop, with wide distribution in semi-evergreen to evergreen forests. The genus *Garcinia* comprises around 200 species throughout the world, out of which 36 species have been reported from India.[1] It is grown in India, Sri Lanka, Africa and Malaysia. In India, it is commonly found in the evergreen and Shola forests of Western Ghats, Karnataka and Kerala and also in the states of Maharashtra, Goa and Tamil Nadu. It is a cultivated crop in Kerala.[2]

Garcinia gummi gutta is a small or medium-sized evergreen tree growing to a height of 20 metres; also seen as shrubs. The bark is dark and smooth. The branches are thin and soft, drooping or horizontal. Leaves are opposite, petiolate, dark green, shining, elliptic to obovate and glabrous, ranging from 2½ to 3½ inch in length and 1 to 1½ inch broad. The tree flowers during December to February and it fruits from March to June.[1] Flowers are seen in clusters of 4-20, usually red, though some trees have yellow ones also. Fruit is a green, ovoid berry, having 1 to 1½ inch in diameter. The fruits are yellow or red in colour when ripe, having 6-8 grooves. Fruit pulp contains 5 to 8 big seeds which are surrounded by a succulent aril.

Clusiaceae family contain four major classes of compounds such as xanthones, coumarins, biflavonoids and benzophenones. *Garcinia* is a good source of medicine and has been investigated extensively by many researchers in reference to its medicinal properties and various other cyototoxic effects. It has been shown to contain a variety of secondary metabolites such as xanthones, flavonoids and benzophenones.[1, 3, 4]

The leaves of *Garcinia* contain hydroxyl citric acid.[5] The presence of phytochemicals such as tannin, phlobatannin, saponin, flavanoids, terpenoids and cardiac glycosides has been reported in the crude extract of *Garcinia gummi gutta* leaves.[6] Most of these compounds contribute to the pharmacognostic properties of this plant against gastrointestinal infections. Several authors have linked the antimicrobial properties of the crude extracts to the presence of these bioactive compounds.[7, 8, 9] Much research has been carried out on the anti-inflammatory, anti-bacterial and anti-cancer properties of *Garcinia*.[10, 11, 12, 13]

Madappa and Bopaiah have extensively studied the phytochemical profile of leaves of *G. gummi gutta*.[14] In their study they have identified the presence of alkaloids,

tannins, phenolic compounds, flavonoids, carbohydrates and proteins in higher amounts in leaves of *G. gummi gutta*. Maridass et al have reported the presence of alkaloid, terpenoid, steroid, oil, catachin and phenols in the leaf extracts of *G. gummi gutta*.[15] Dhanya and Benny have reported the antifungal activity of flavonoids present in the methanol extract of leaves against *Phytophthora* sp. *Curvularia* sp. and *Corynespora* sp.[13]

The plant *G. gummi gutta* is a lower risk near threatened plant. The tribal people belonging to Kurichia, Kuruma, Kattunaika, Adiya and Paniya groups of Wayanad use *Garcinia gummi gutta* as ethnomedicine. *G. gummi* gutta, either singly, or in combination with other plant parts, has been by used these tribals to cure various ailments and also as a source of food and other value added products.[16, 17] Its leaves have been used by the Kurichia tribes of Wayanad district to prepare medicine for dysentery, diarrhoea, tonsillitis, ulcer and bleeding piles.[18] The dried fruit is an essential ingredient in fish and prawn preparations in Kerala. Besides imparting flavour, it also improves the keeping quality of the products. A decoction made from leaves of *Garcinia* is administered for rheumatism and bowel complaints. In cattle, it is used as a wash for mouth diseases. Hydroxy citric acid extracted from the mature fruit rind is used against obesity.[18]

In view of the antimicrobial properties of *Garcinia gummi gutta*, the present study was indented to focus on assaying the antimicrobial activity of the phyto-constituents present in the leaf extracts of *Garcinia gummi gutta*.

MATERIALS AND METHODS

Collection of Sample

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

Bacterial Stains Used

Bacterial cultures used in this study were obtained from the culture collections of Doctors Diagnostic Research Laboratory, Kottayam, Kerala. The bacterial strains *viz. Staphylococcus aureus, Salmonella typhi, Salmonella typhimurium, Bacillus* sp., *Proteus* sp., *Klebsiella sp., Serratia sp.* and *Pseudomonas sp.* were included in this study. The bacterial strains were maintained on Nutrient Agar (Hi Media) plates or slants and were stored at 4°C before use. The fungal strains, *Aspergillus* and *Penicillium*, were isolated by air exposure of Potato Dextrose Agar (PDA) and were isolated and purified. The fungal strains were maintained on PDA till use at 4°C.

Surface Cleaning and Sterilization of the Samples

The leaves of *Garcinia gummi gutta* were surface sterilized following the modified procedure of Aneja.[19] The leaves were washed in 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 were 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 Extracts

Water, methanol and acetone were used for preparing the extracts. Fresh, sterile samples of leaves of *G. 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, tied in a clean cheese cotton cloth, was used for extraction using Soxhlet Apparatus. An initial volume of 250 ml of each solvent (water/methanol/acetone) was taken in a 500 ml round bottom flask for the extraction. The aqueous extract was prepared at an extraction temperature of 100°C whereas the acetone and methanol extracts were prepared using an extraction temperature of 40°C. The extraction was carried out continuously for 8 hrs after which each extract was concentrated by evaporation and made up to a final volume of 20 ml. The extracts were stored at room temperature, in sterile screw capped containers, till use.

Determination of Antimicrobial Activity

Sensitivity Discs

Sterile sensitivity discs of 5 mm diameter were prepared from Whatman No. 1 filter paper. The discs were sterilized by autoclaving at 121°C for 15 minutes and stored at room temperature till use. The discs were soaked in the extracts for 10 minutes and allowed to dry. The dried discs carrying the bioactive compounds were used for disc diffusion assay.

Preparation of Bacterial Suspension

Pure isolated colonies of bacterial colonies were inoculated into peptone water and incubated at 37°C for 48 hrs and were used as inoculum for lawn culture on Mueller Hinton Agar (Hi Media) for assaying the antibacterial activity of the extracts.

Preparation of Fungal Spore Suspension

Fungal strains were cultured on PDA till fine confluent growth and high sporulation was evident. The spores were rubbed off from the surface of agar into sterile water using a fine sterile brush. These spore suspensions were used as inoculum for lawn culture on PDA for assaying the antifungal activity of the extracts.

Disc Diffusion Assay

The antimicrobial activity was tested comparatively using aqueous, methanol and acetone leaf extracts of *Garcinia gummi gutta*. Mueller Hinton Agar (MHA) was used as the base medium for assaying the antibacterial activity and 0.1% peptone water for preparation of inoculums. About 15 to 20 ml of MHA medium was poured in sterile Petri dishes and allowed to solidify.

Using sterile cotton swab, 0.2 ml of 24 hr old culture was inoculated evenly on to the surface of MHA to make a lawn culture. For analysing the antibacterial activity of *Garcinia gummi gutta* extracts, the discs carrying the respective extract were impregnated on the seeded agar plate (2 discs per plate). Discs carrying the respective solvents alone were used as controls. The experiment was performed in duplicates. The plates were incubated at 37°C for 24 hrs and observed for zone of inhibition of growth around the discs.

Well Diffusion Assay

The antifungal activity of the extracts was assayed using the well diffusion assay. PDA was used as the base medium for assaying the antifungal activity. Wells of 8 mm were dug out using a sterile cork borer in solidified PDA medium. The spore suspensions of *Aspergillus* and *Penicillium* were inoculated evenly on to the surface of the PDA plate to make a lawn culture. A volume of 100 µl each of the various extracts was added to the respective wells. The plates were incubated at room temperature for 5 days and observed for zone of inhibition of growth around the wells.

Zone Analysis

After incubation the antibacterial and antifungal activity of the extracts against each bacterial or fungal strain was assayed by measuring the diameter of zone of inhibition to the nearest mm. The results were recorded and compared.

RESULTS AND DISCUSSION

Garcinia gummi gutta Linn. is a rich source of secondary metabolites and hence the plant is a potential source of herbal medicine. Earlier studies have revealed the anti-inflammatory, antibacterial and anticancer properties of the medicinally active phytochemicals present in *G. gummi gutta*. It has been reported earlier that the leaves of *G. gummi gutta* contain high content of alkaloids, tannins, phenolic flavonoids, carbohydrates and proteins. Iinuma et al have reported the antibacterial activity of garcinol against methicillin resistant *S. aureus*.[20] The fruit and fruit rind of *G. gummi gutta* are reported to possess antimicrobial activity against *E. coli*, *Bacillus subtilis*, *Enterobacter aerogenes*, *S. aureus*, *Bacillus megaterium* and *Pseudomonas aeruginosa*.[21,22] The present study also aimed at elucidating the antibacterial and antifungal activity of leaf extracts of *G. gummi gutta* against eight bacterial strains *viz S. aureus, S. typhi, S. typhimurium, Bacillus* sp., *Proteus* sp., *Klebsiella* sp., *Serratia* sp. and *Pseudomonas* sp. and two molds *viz. Aspergillus* and *Penicillium*. The extracts inhibited the growth of all the test bacteria with wider zones of growth inhibition, except for *Bacillus* sp. which exhibited less sensitivity to the aqueous extract (Table 1). Aqueous extract maximally inhibited the growth of *Klebsiella* sp. with a diameter of 12 mm of zone of growth inhibition. The extract equally inhibited the growth of *Pseudomonas* sp. and *S. aureus*, with zone of clearance of average diameter 11.5 mm each. Least activity of antibiosis was observed for *Bacillus* sp. The growth of all the other test bacteria of this study was considerably affected by the aqueous extract (Table 1). On the contrary, *Proteus* sp. exhibited resistance to the bioactive compounds in the aqueous extract. Concordant results have been reported where the medicinal use of leaves of this plant as potential anti-salmonella agents - against *Salmonella typhi*, *Salmonella paratyphi* A and *Salmonella typhyrimurium* - have been identified.[23]

In this study the acetone extract of leaves of *G. gummi gutta* exhibited maximal antibiosis against all the eight strains of test bacteria. The acetone leaf extract inhibited the growth of *S. aureus* - a gram positive bacterium - more effectively, with an average diameter of zone of inhibition of 22.5 mm (Table 1). Comparable antibiosis was observed for *Bacillus* sp. (21.5 mm), yet another gram positive bacterium. The extract also inhibited the growth of *Serratia* and *Proteus* sp. effectively (19.5 mm each). The antibacterial activity of acetone extract was observed least against *Klebsiella* sp. (12 mm). Devi Prasad et al have reported the antibacterial activity of diethyl ether extract of this plant against *S. aureus*.[18]

Table 1. *In vitro* Antibacterial Activity of Leaf Extracts of *Garcinia gummi gutta* Assayed by Disc Diffusion Method

Bacterial strains used	Average Diameter of Zone of Inhibition of Growth in mm		
	Aqueous	Acetone	Methanol
Klebsiella sp.	12	12	13
Salmonella typhimurium	8	15.5	11.5
Proteus sp.	0	19.5	11.5
Bacillus sp.	7	21.5	13.5
Staphylococcus aureus	11.5	22.5	13.5
Serratia sp.	8.5	19.5	14
Pseudomonas sp.	11.5	18	12
Salmonella typhi	9	14	14.5

Significant levels of antibacterial activity of ethanol and methanol extracts of fruit rind of *Garcinia* have been reported against *Micrococcus aureus, B. megaterium and P. aeruginosa*.[22] Lakshmi et al have reported the antibacterial activity of stem bark extract of the plant against *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi*.[24] In the present study methanol extract exhibited maximal antibacterial activity against *S.*

typhi (14.5 mm). The extract significantly inhibited the growth of *Bacillus* sp. and *S. aureus* with zones of diameters of 13.5 mm each. In this study the growth of *Serratia* (14 mm) and *Klebsiella* (13 mm) were also found to be considerably affected by the methanolic extract. The growth inhibitory activity of the methanol extract was minimal for *S. typhimurium* and *Proteus* sp. (Table 1). Maridass et al. (2010) have also reported similar levels of antibacterial activity of *Garcinia gummi gutta* against *Aeromonas hydrophila* (28 mm), *Bacillus subtilis* (26 mm), *Staphylococcus aureus* (19 mm), *Salmonella typhi* (17 mm), *Pseudomonas auruginosa* (23mm) and *Klebsiella pneumoniae* (16 mm).

On comparing the antibacterial profile of the various extracts against the test bacteria it could be concluded that the acetone extract is superior in antibiosis since it inhibited the growth of the test bacteria with wider zones of growth inhibition, when compared to the methanol and aqueous extracts. Aqueous extract exhibited minimal growth inhibition against the test bacteria. Hence it could be concluded that water is not a good solvent for extracting out the phyto-constituents present in the leaves of *G. gummi gutta*. On the contrary, Devi Prasad et al have reported that diethyl ether and methanolic extract of the leaves of this plant do not inhibit the growth of *E. coli* whereas aqueous extract recorded highly significant inhibitory activity against *E. coli*.[6] They noted the inhibitory activity of diethyl ether against *Staphylococcus aureus* and have reported the presence of more than one active principle in the plant which is likely to act on Gram positive and Gram negative bacteria separately. These observations are concordant with the traditional knowledge of tribes in curing infectious diseases.

Many researchers have worked on the antibacterial activity; however a few have focused on the antifungal activity of the extracts. The present study elucidated the antifungal activity of aqueous, methanol and acetone extract of leaves of *G. gummi gutta* against *Penicillium* sp. and *Aspergillus* sp. In the antifungal assay the aqueous extract exhibited minimal antifungal activity against *Penicillium* sp., with zone of diameter of 5.5 mm (Table 2). On the contrary, *Aspergillus* sp. was proved resistant to the bioactive compounds in aqueous extract. Acetone extract inhibited the growth of *Aspergillus* sp. with a diameter of 17 mm of zone of growth inhibition. *Penicillium* sp. was resistant to the growth inhibitory activity of antifungal metabolites present in the acetone extract (Table 2). Methanol extract, on the contrary, inhibited the growth of both the test fungi considerably with zones of diameters 12 mm and 9 mm respectively, for *Aspergillus* and *Penicillium* sp. Dhanya and Benny reported the antifungal activity of methanolic extracts of leafs of *G. gummi gutta* against *Phytophthora* sp., *Curvularia* sp. and *Corynespora* sp.[13]

Table 2. *In vitro* Antifungal Activity of Leaf Extracts of *Garcinia gummi gutta* Assayed by Well Diffusion Method

Fungal strains used	Average Diameter of Zone of Inhibition of Growth in mm		
	Aqueous extract	Acetone extract	Methanol extract
Aspergillus sp.	0	17	12
Penicillium sp.	5.5	0	9

CONCLUSIONS

Garcinia gummi gutta Linn. has immense potential as a source of herbal and ethnommedicine The leaves of *G. gummi gutta* has been used by the Kurichia tribes of Wayanad district to prepare medicine for dysentery, diarrhoea, tonsillitis, ulcer and bleeding piles.[18] The present study assayed the antibacterial and antifungal activity of leaf extracts of *G. gummi gutta*. The extracts inhibited the growth of all the test bacteria with wider zones of growth inhibition, except for *Proteus* sp. which exhibited resistance to the aqueous extract. The acetone extract was superior in antibiosis, inhibiting the growth of test bacteria with wider zones of growth inhibition. Aqueous extract exhibited minimal growth inhibition against the test bacteria. In the antifungal assay the aqueous extract exhibited minimal antifungal activity against *Penicillium* sp., but did not inhibit the growth of *Aspergillus* sp. Acetone extract inhibited the growth of *Aspergillus* alone, without having any inhibitory activity on *Penicillium* sp. Methanol extract inhibited the growth of both the test fungi.

It is evident from the results of this study that the plant *Garcinia gummi gutta* Linn. has immense potential as a medicinal plant, rich in bioactive compounds, and having significant levels of antibacterial and antifungal activity. The plant has gained much importance as a promising source of ethnomedicine. The study should be extended to more number of pathogenic bacteria and fungi. Attempts should be made to selectively identify which species of bacteria or fungi is inhibited more effectively by the bioactive compounds in the extracts. Further studies have to be organized to determine the rate of toxicity, their mode of action and dose dependent activity against various strains of bacteria and fungi.

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