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Phytochemical Analysis and Antimicrobial Activity of Ethanolic Extract of Dried Fruit Rind of *Garcinia gummi-gutta*

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ABSTRACT

Garcinia gummi-gutta is an evergreen medium sized tree of the family *Clusiaceae* with remarkable traditional value which has been indicated for the treatment of bowel complaints, rheumatism, intestinal parasites and also as a culinary and preservative agent for fish preparations in Southern India and Srilanka. The extract of dried fruit rind of *Garcinia gummi-gutta* obtained through Soxhlet extraction using ethanol was subjected to preliminary phytochemical analysis along with *in vitro* evaluation of antimicrobial activity against common food borne pathogens. Preliminary phytochemical analysis of the rind extract of the plant showed the presence of phytochemical constituents such as alkaloids, phenolic compounds, steroids, tannins, flavonoids, glycosides, diterpenes and triterpenes. The plant extracts were evaluated for the antimicrobial activity by in vitro agar well-diffusion at a dose rate of 5mg/ml, 10 mg/ml and 15mg/ml against the microbial strains namely *Staphylococcus aureus*, *Vibrio parahemolyticus*, *Listeria monocytogenus* and *Salmonella tymphimurium*. The results of the antimicrobial activity showed effective inhibitory activity against all the tested organisms in a dose dependant manner.

Keywords: *Garcinia gummi-gutta*, Phytochemical analysis, Antimicrobial.

INTRODUCTION

Food-borne intoxication and infection acquired through contaminated food consumption are considered major health burdens leading to high morbidity and mortality, accounting for the most common causes of food-borne diseases worldwide. *Staphylococcus aureus, Vibrio parahemolyticus, Escherichia coli*, and *Salmonella* sp. are some of the most common food-borne microorganisms that cause infection and intoxication. Chemical preservatives with antimicrobial properties are used widely in the food industry for the prevention of the growth of a wide range of spoilage and pathogenic microorganisms in foods. However, food manufacturers were forced to explore potentially effective, healthier and safer alternative sources of antimicrobial compounds as food preservative due to the onset of increased demand for organic foods, human food safety implications, extended shelf life foods, and adverse effects of chemical preservatives [1]. In this regard, medicinal plants provide a new remedial source, as plants have been utilized by human beings for basic preventive and curative healthcare since time immemorial. According to World Health Organization (WHO), 80% of the population in developing countries of the world depends on traditional and complementary medicine for their primary health care with about 85% involves the use of medicines of plant origin. (WHO, 2002). Several researchers have demonstrated the antimicrobial activity of medicinal plants against microorganisms causing food poisoning [2, 3, 4]. The secondary metabolites derived from medicinal plants sources have led to the discovery of new medicinal drugs that are highly effective in treating various pathophysiological disorders and infectious diseases. In recent years the trend of using drugs and secondary metabolites derived from plants to treat infectious diseases increased owing to lower adverse effects, cost effectiveness and due to high incidence of antimicrobial resistance [5].

Garcinia gummi-gutta. Roxb Syn. *Garcinia cambogia*, popularly known as Malabar tamarind/kudampuli, belonging to the family Cluisaceae is a dicotyledonous medium sized tree distributed widely in the evergreen forest of Southwest India, predominantly in Western Ghats. The fruits of the tree are ovoid in shape with six to eight seeds surrounded by a succulent aril that is now popularly used in developed countries as a dietary supplementary ingredient for weight loss [6]. It flowers during the hot season and the fruits ripen during the rainy season. The fruits have been reported to be used in traditional Ayurvedic medicine for the treatment of aliments like delayed menstruation, diarrhoea, haemorrhoids, dysentery, ulcer, rheumatism and heart complaints [7, 8]. The sun-dried smoked rind ofthe fruit has beenextensively used for centuries throughout Kerala and Sri Lanka as a flavour condiment in fish curries and as

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preservative agent during curing of fish [9]. Experimental studies have shown that *G. gummi-gutta* possesses hypolipidemic [10], immunomodulatory [11], antioxidant [12], anti-cancer [13], anti-parasitic [14], antifungal [15], anti-inflammatory [16] and anthelminthic properties [17]. However, till date only a few studies have been focused on the therapeutic potential of medicinal plants as source of antimicrobial agent that could inhibit food borne pathogenic microorganisms and to develop a natural, non-toxic herbal food preservative.

Hence, the goal of the study was to evaluate the *in vitro* antimicrobial property of ethanolic dried fruit rind extract of *Garcinia gummi-gutta* against common pathogens implicated in food borne poisoning. The plant extract was also profiled for the presence of various phytochemical constituents with the ultimate focus on further research for the isolation of active constituents that can lead to synthesis of a novel antimicrobial herbal food preservative.

MATERIALS AND METHODS

Plant material

The fresh fruits of *G. gummi-gutta* (Malabar tamarind) were procured locally from Thrissur district of Kerala and authentication was done at NISCAIR, New Delhi.

Preparation of fruit rind extract

The fruit rind was separated, cut into small pieces; shade dried and were pulverized. The dried powder obtained was extracted with 95% ethanol using Soxhlet apparatus, followed by concentration in a vacuum rotary evaporator and kept under refrigeration for further use. The yield of the extract was 44% on dry matter basis.

Phytochemical analysis

Qualitative phytochemical screening of the ethanolic extract of *G. gummi-gutta* fruit rind was carried out to determine the flavonoids, phenolic compounds, alkaloids, glycosides, tannins and saponins according to the standard protocols as described by Harborne [18].

Tests for detection of steroids

Salkowski test

3ml of chloroform was mixed with 5 mg of the extract followed by shaking with 3 ml concentrated sulphuric acid. The presence of steroids was confirmed by the development of red colour.

Lieberman Burchardt test

The extract (5mg) was mixed with 3 ml of chloroform in a test tube, followed by addition of 1 ml of concentrated sulphuric acid and five drops of acetic anhydride through the sides of the test tube. The presence of steroids was confirmed by the development of a reddish ring at the junction of two layers.

Tests for Detection of Alkaloids

The extract (0.5 g) was mixed with 5 ml of ammonia, 5ml of chloroform and 5 ml dilute hydrochloric acid. The acid layer obtained was used for the following chemical tests for alkaloids.

Mayer's test

A few drops of Mayer's reagent were added to 1ml of acid layer. The presence of alkaloids was confirmed by the development of a creamy white precipitate. Mayer's reagents was made by mixing 1.358 g of mercuric chloride dissolved in 60 ml of water and 5 g of potassium iodide disolved in 10 ml of water and make up the final volume to 100 ml with distilled water)

Wagner's test

1 ml of the acid extract was mixed with a few drops of Wagner's reagent. The presence of alkaloids was indicated by the development of reddish brown precipitate. (Wagner's reagent was prepared by mixing 2 g of iodine and 6 g of potassium iodide followed by dissolving in 100 ml of water)

Hager's test

1ml of the acid extract was mixed with a few drops of Hager's reagent were mixed. Development of yellow precipitate indicated the presence of alkaloids. (Hager's reagent was made by mixing 1 g of picric acid in 100 ml of water)

Dragendroff's Test

1ml of acid extract was mixed with a few drops of Dragendroff's reagent. Development of a reddish brown precipitate indicated the presence of alkaloids. (Dragendroff's reagent was prepared by mixing Stock solution (1) and Stock solution (2) which was then mixed with 7ml of concentrated hydrochloric acid and 15 ml of water. The final volume was made up to 400 ml with distilled water. Stock solution (1) was made by dissolving 0.6 g of bismuth sub nitrate in 2 ml of concentrated hydrochloric acid and 10 ml of water. Stock solution (2) was made by dissolving 6 g of potassium iodide in 10 ml of water).

Test for detection of phenolic compounds

Five drops of 10 per cent ferric chloride was added to the plant extract (5gm), dissolved in 1ml of water. The presence of phenolic compounds was confirmed by the **d**evelopment of dark blue colour.

Test for detection of tannins

Gelatin test

The extract (0.5 g) was mixed with a few drops of 1% solution of gelatin dissolved in 10% sodium chloride. The presence of tannins was confirmed by the development of a white precipitate.

Ferric chloride test

3ml of 1% ferric chloride solution was mixed with 2mg of the plant extract. The presence of tannins was confirmed by the development of a blue, green or brown colour indicated

Tests for detection of flavonoids

Lead acetate test

The alcoholic solution (2ml) of the extract was mixed with a few drops

of neutral 10 % lead acetate. Development of yellow precipitate confirmed the presence of flavonoids. The alcoholic solution of the extract was prepared by dissolving 0.5 g extract in 10 ml methanol.

Ferric chloride test

2ml of alcoholic solution of the extract was mixed with a few drops of neutral ferric chloride solution. The presence of flavonoids was confirmed by the development of green colour.

Tests for detection of glycosides

Sodium hydroxide test

5-6 drops of sodium hydroxide solution (10 %) was added to 5mg of the plant extract dissolved in 1 ml water. Development of yellow colour indicated the presence of glycosides.

Benedict's test

5ml of Benedict's reagent was added to the extract (0.5 g) was dissolved in 1ml of water. The presence of glycosides was confirmed by the development of brown or red colour on boiling for two minutes.

Tests for detection of diterpenes

The extract (5mg) was mixed with 3 ml of 5 per cent copper acetate solution. the presence of diterpenes was indicated by the development of green colour.

Tests for detection of triterpenes

Salkowski test

The extract (3 mg) was added to 3 ml of chloroform followed by shaking with 3ml conc. sulphuric acid. The presence of triterpenes was indicated by the development of yellow colour in lower layer on standing.

Lieberman Burchardt test

The extract (3 mg) was mixed with 3 ml of chloroform in a test tube. 1 ml of concentrated sulphuric acid and 5 drops of acetic acid were added slowly along the sides of the test tube. The presence of triterpenes was indicated by the development of a deep red ring at the junction of two layers.

Tests for detection of saponins

Foam test

The extract (5 mg) was shaken with equal volume of water. The presence of saponins was confirmed by development of the foam that persisted for 10 minutes.

Screening for Antimicrobial Activity

Test microorganisms

The organisms used in this study consisted of four bacterial strains. These included *Staphylococcus aureus*(MTCC 1144), *Vibrio parahemolyticus* (MTCC 451), *Listeria monocytogenes*(MTCC 1143) and *Salmonella enterica* (MTCC 98). The organisms were obtained as freeze dried pure cultures from the Microbial Type Culture Collection, Institute of Microbial Technology (IMTECH), Chandigarh, India and were maintained at 4°C on nutrient agar slants.

Antimicrobial assay

Antimicrobial activity was determined by the modified agar well diffusion method [19] by using Muller Hinton Agar. The microorganisms were inoculated in nutrient broth and kept for incubation at 37^0 C for 24 h and microbial broth cultures with 0.5 McFarland standard turbidity was used as the seed culture for the assay. For screening, the molten and cooled media (Muller Hinton Agar) was poured in sterilized petri dishes (20 ml/dish) and were incubated at 37°C for 24 hrs for checking sterility. Sterile petri plates containing 20ml Muller Hinton medium (MHA) were seeded with bacterial inoculum using sterile cotton swab by streaking while turning the plate 60^0 angle between each streaking.

20 µl of the ethanolic extracts diluted in dimethyl sulphoxide (DMSO) at a concentration of 5 mg/ml, 10 mg/ml and 15mg/ml were added into wells that were cut using a sterile 6mm well bore. The incubation of the plates was done at 37°C for 24 hours. After incubation, the antibacterial activity of the extract was evaluated by measuring the inhibition zone (diameter) formed around each well using a transparent ruler in millimetre. The assays were performed in triplicate. Chloramphenicol disc was used as a positive control.

RESULTS AND DISCUSSION

The phytochemical screening and antimicrobial evaluation of ethanolic extract of *G. gummi-gutta* is an effort to unravel the pharmacological benefits (if any) derivable against food pathogens. The microbes used in this assay were chosen on the basis of their insinuation as agents of food–borne infectious diseases and food spoilage. Our finding showed that the presence of different secondary metabolites such as alkaloids, phenolic, flavonoids, saponins, and glycosides (Table 1). The present findings are in accordance with the previous results that reported the presence of bioactive secondary metabolites like flavonoids, phenols and tannins in the fruit rind of *G. cambogia* [20]. The secondary metabolites present in plants are often regarded as an adaptive and defensive mechanism against predatory attacks, pathogenic invasion by microorganisms, and environmental stress. These plant components are found to possess diverse pharmacotherapeutic potential with experimental evidences of antimicrobial activity against food borne pathogenic organisms. Hence, preliminary screening for the presence of biologically active phytochemical constituents is considered to be an effective initial step in predicting the therapeutic potential of plant extracts [21].

Antimicrobial activity of the ethanolic extract from the dried fruit rind of *G. gummi-gutta* against the tested bacteria strains was assessed by the presence and absence of zone of using inhibition by agar well diffusion method. *In vitro* antimicrobial assay of the ethanolic extract of *G. gummi-gutta* revealed that the plant exhibited a concentration dependant antimicrobial activity against all the tested microorganisms. The zone of inhibition produced by the extract obtained against all the four micro-organisms tested was recorded in millimeters (mm) and depicted in (Table 2 and Figure 1). The zone of inhibition produced by

various concentration of ethanolic extract on different bacterial strains was between 10 mm to 17mm. The highest antibacterial activity was noted with 15 mg/ml concentration with a zone of inhibition (mm) of 15.66, 17, 15.33 and 16.66 against *Staphylococcus aureus, Vibrio parahemolyticus, Listeria monocytogenes* and *Salmonella enterica* respectively. However, the inhibition zone produced by standard disc was larger than was larger than those produced by plant extract. (Table 2). Previous studies have indicated that leaf extract of the plant possess similar antimicrobial properties [22]. The presence of various secondary phytochemicals in *G. gummi-gutta* fruit rind extract can be the reason for the potent antibacterial property.

Phytochemical constituents like flavonoids, simple phenolics, alkaloids, saponins, tannins and terpenes are effectual antimicrobial agents against a wide range of pathogenic microorganisms [23]. Previous research studies have shown that several species of Garcinia potent antimicrobial and antioxidant activity, due to the presence of different kinds of pharmacologically bioactive secondary metabolites [24, 25, 26, 27, 28]. These metabolites present in the plant extracts have the ability to destroy the bacterial cell wall and inhibit the synthesis of nuclear materials and protein by obstructing the enzymes functions in specific pathways. Flavonoids are highly effective scavenging compounds with polyphenolic structure that oxidizes various free radical molecules implicated in the cellular pathophysiology of several diseases [29, 30, 31]. Alkaloids are nitrogen containing organic compounds with diverse array of pharmacological activity including antimicrobial property [32]. Hence, the study ascertains the therapeutic value of the plant extract which could be of substantial interest in the development of a new novel antimicrobial herbal food preservative agent.

Table 1: Phytochemical screening of fruit rind of *Garciniagummi-gutta*

Active principle	Result
Steroids	Present
Alkaloids	Present
Phenolic compounds	Present
Tannins	Present
Flavonoids	Present
Glycosides	Present
Diterpenes	Present
Triterpenes	Present
Saponins	Absent

Table 2: Antimicrobial activity of ethanolic extract of *Garcinia gummi-gutta* by Agar well disc diffusion method

Microorganism	Zone of inhibition (mm)		
	5mg/ml	10mg/ml	15mg/ml
Staphylococcus aureus	11.66	14	15.66
Vibrio parahemolyticus	10.33	12.33	17
Listeria monocytogenes	12	14.33	15.33
Salmonella enterica	13	15.66	16.66

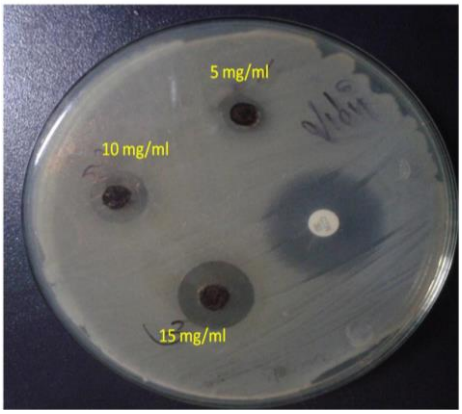

Figure 1: The inhibition zone (mm) of ethanolic extract of *Garcinia gummi-gutta* at concentrations of 5 mg/ml, 10 mg/ml and 15 mg/ml against *Vibrio parahemolyticus*

CONCLUSION

The demonstration of antimicrobial activity and presence of secondary bioactive principles in the extract of fruit rind of *G. gummi-gutta* provides a scientific need for future research focusing on the pharmacological action of plant extract against a wider range of bacterial and fungal strains, toxicological evaluation and purification of the extract for the isolation of active constituents that can lead to synthesis of a novel antimicrobial herbal food preservative agent.

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Conflict of Interest

None declared.

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