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Anti-microbial activity of garcinia gummigutta fruit rind

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Abstract
The objective of the present study was to evaluate the in-vitro anti microbial activity of fresh and dry rinds of Garcinia gummigutta fruit in methanolic extract against food borne illnesses causing pathogens. The fruit rinds extracts were examined to evaluate the antimicrobial activity against 3 Gram-positive Bacteria i.e. *Bacillus subtilis* and *Staphylococcus aureus and Enterococcus fecalis*, 2 Gram-negative bacteria *Escherichia coli* and *Shigella boydii*, using Agar well diffusion antimicrobial assay. The zone of inhibition was nil at the concentration of 1g/100ml. However the minimum zone of inhibition was found at the concentration rate of 10g/100ml in *Enterococcus fecalis* (13.59mm), *Shigella* (12.89mm), *Bacillus subtilis* (12.69mm), *Escherichia coli* (11.68mm) *and Staphylococcus aureus* (9.75mm) respectively. Since the extracts showed a broad spectrum of antimicrobial activity on both gram positive and negative bacterial isolates, *Garcinia gummigutta* can be used as a bio preservative or neutraceuticals or therapeutic agent to prevent several diseases.

Keywords: Antimicrobial activity, Agar well diffusion, *Garcinia gummigutta*, Zone of inhibition

Introduction
Medicinal plants and herbs are of great importance to the health of individual and communities. The use of traditional medicines holds a great promise as an easily available source as effective medicinal agents to cure several diseases. The World Health Organization (WHO) estimated that 80% of the population of developing countries still relies on traditional medicines, mostly plant drugs, for their primary health care needs (GuribFakim, 2006) [3]. Globally researchers are using extracts of plants for their antibacterial, antifungal and antiviral activities (Bakht *et al.*, 2011) [1].

Despite of the existence of herbal medicines over several centuries, only few numbers of species has been studied for their application. However, in the recent past, increase in research evidence indicates the positive role of traditional medicinal plants in the prevention or control of some of the metabolic disorders like Obesity, Diabetes, Coronary diseases etc. The steadily increasing bacterial resistance to existing drugs is a serious problem in antimicrobial therapy and necessitates continuing research into new classes of antimicrobials. Biomolecules of plant origin appears to be one of the alternatives for the control of these antibiotic resistant human and plant pathogens and hence in the present investigation fresh and dry fruit rind extract of *Garcinia gummigutta* was tested for its efficacy against the inhibition of human harmful pathogens.

***G**arcinia gummi-gutta*, the Malabar tamarind, commonly known by its previous scientific name Malabar tamarind is a native to South eastern Asia. These trees are mainly distributed in tropical Asia, and Africa. Garcinia gummi-gutta is one of the most medicinally important member of the Clusiaceae or Guttiferae family. It is a small or medium size tree of up to 12 m tall with a rounded crown and drooping branches. Flowering occurs during the summer (March–May) while fruiting occurs during the rainy season(June–September).The ovoid fruits can be yellow, orange or red when ripe and has 6 to 8 seeds surrounded by a succulent aril. It has a limited native global distribution, being restricted to India, Nepal and Sri Lanka.

These trees are found mainly in the semi-evergreen to evergreen forests of Southwest India, predominantly in the Western Ghats (Maharashtra, Karnataka, Kerala and Tamil Nadu).

This tree can grow on both hill tops and plain lands but grows best in dry or occasionally waterlogged or flooded soils in riverbanks and valleys. It is tolerant to fluctuating water tables and drought. *It* looks more like a small yellowish, greenish, or sometimes reddish pumpkin. The color can vary considerably. When the rinds are dried and cured in preparation for storage and extraction, they are dark brown or black in color.

Along the west coast of South India, *G. gummi-gutta* is popularly termed "Malabar tamarind", and shares culinary uses with the tamarind. It is also called *goraka* or, simply *kattchapuli* (souring fruit) in Tamil and Kudampuli /Kodampuli in Malayalam. It is called uppage or murugalu in Kannada and fruits are collected and dried for selling to dealers in Chickamagalurand Sirsi region of Karnataka.

The fruit of Malabar tamarind has been traditionally used in food preparation and cooking, having a distinctive taste. It is mainly used to coat pork and fish as a fat cutter.Garcinia has gained lot of attention of late as a popular natural weight loss aid. The reason is that the rind of this fruit is rich in a substance called hydroxycitric acid / HCA. Beside from its use in food preparation and preservation, extracts of G. cambogia are sometimes used in traditional medicine as purgatives.

Kudampuli is known for its medicinal values in Ayurveda. The fruit rind is commonly used as a food preservative, flavouring agent or food-bulking agent, and as a traditional remedy to treat constipation, piles, rheumatism, oedema, irregular menstruation and intestinal parasites in many Asian countries. Numerous scientific studies have indicated biological activity such as anti-obesity, hypercholestremea, antioxidant and anticancer activity. Commercial products containing G.cambogiain the market received considerable positive media attention.

Hence in the present study we tried to evaluate the in-vitro anti microbial activity of fresh and dry rinds of Garcinia gummigutta fruit in methanolic extract against food borne illnesses causing pathogens. The fruit rinds extracts were examined to evaluate the antimicrobial activity against 3 Gram-positive Bacteria i.e. *Bacillus subtilis* and *Staphylococcus aureus* and Enterococcus *fecalis*, 2 Gram-negative bacteria *Escherichia coli* and *Shigella*, using Agar well diffusion antimicrobial assay.

Materials and method

Disease free samples were collected from ZAHRS, Mudigere. The aril (i.e. the white part of the fruit) was removed. Collected material was washed thoroughly in running tap water, rinsed in distilled water and the rind was crushed using mortar and pestle, then stored in airtight closed bottles for two days before used for analysis. Food borne cultures of 3 Gram-positive Bacteria i.e. *Bacillus subtilis* and *Staphylococcus aureus and Enterococcus fecalis*, 2 Gram-negative bacteria *Escherichia coli* and *Shigella* (Clinical isolates) were obtained from Auriga research pvt. Ltd, Bangalore. The strains were maintained on agar slant at 4°C and activated at 37 °C for 24 h on Nutrient agar before any susceptibility test.

Extraction of plant material aqueous extraction

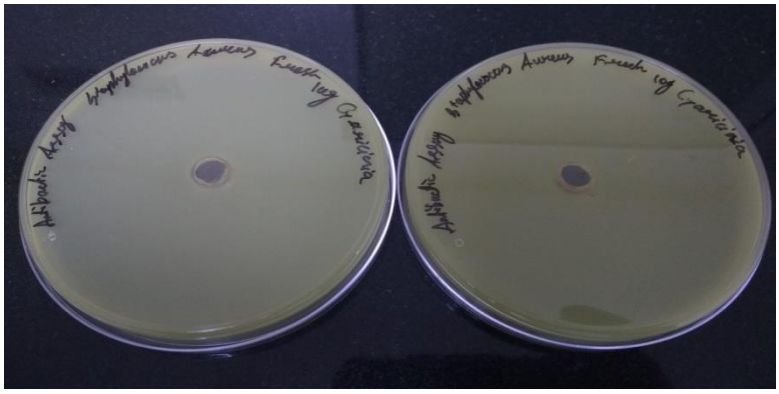
Ten gram of dry powder sample was dissolved in ram of dry powder of samples was dissolved in 30 ml of water. The residue was then removed by filtering through muslin cloth; the filtrate was then centrifuged at 8,100 x g, for 5 min.

Media preparation

Twenty three grams of nutrient agar was dissolved in 1000 ml of distilled water and bring to boil. Agar was then autoclaved for 15 min at 121° C and left to cool at room temperature. Once the media was cooled (about 45°C), it was poured into Petri dishes. Each Petri dish was left on the flat surface for 30–40 min until completely set.

Fresh Rind

Bacillus Subtilis

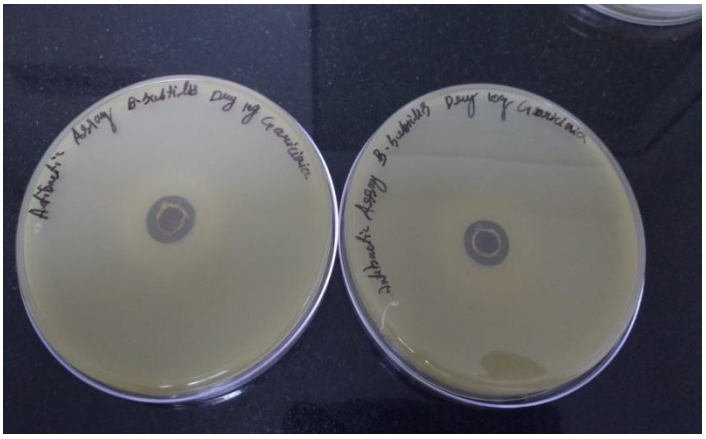


Staphylococcus Aureus

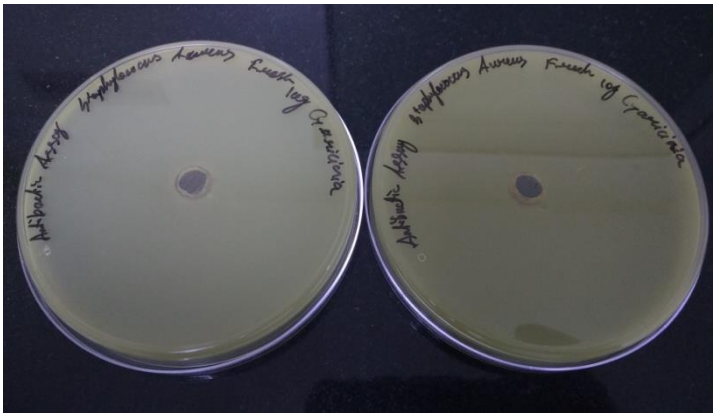
Enterococcus Fecalis

Fig 1: Zone of inhibition of fresh rind of Garcinia Gummigutta on gram positive human pathogens

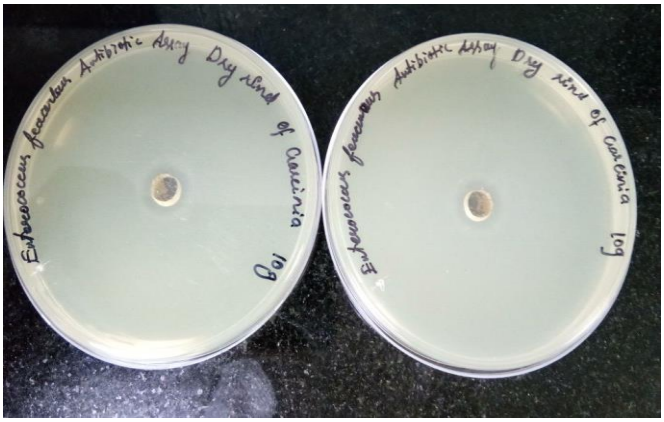
Dry Rind



Bacillus Subtilis



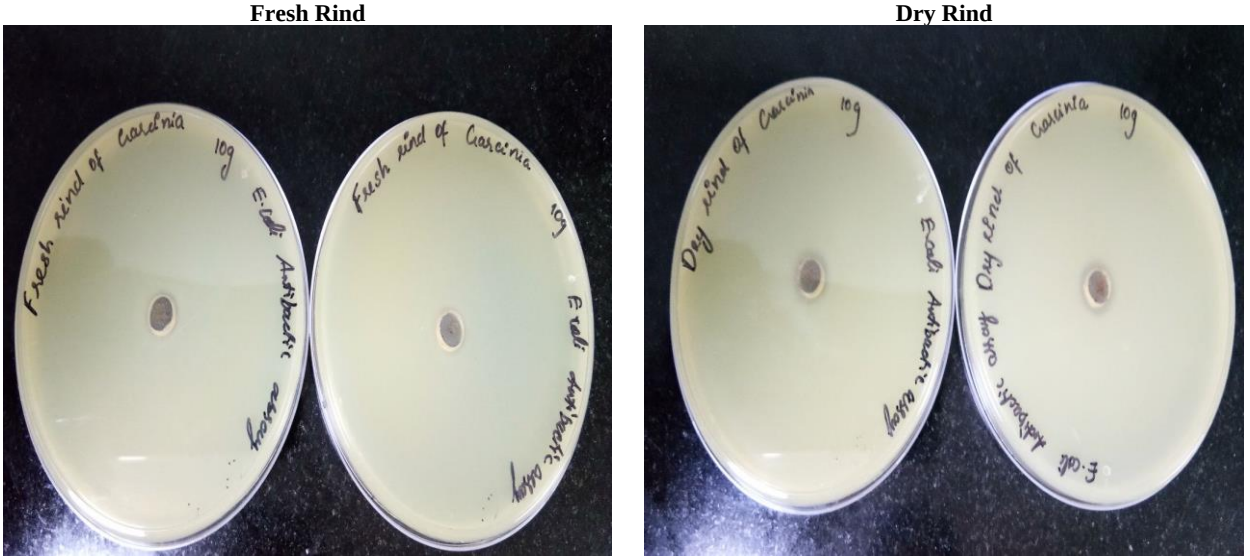
Staphylococcus Aureus



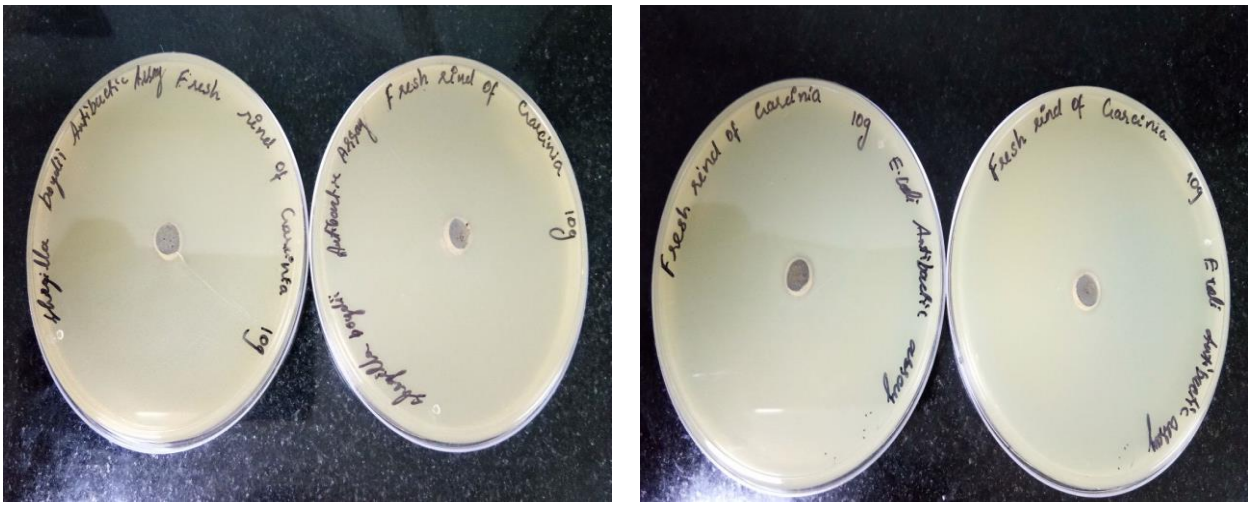
Enterococcus Fecalis

Fig 2: Zone of inhibition dry rind of Garcinia Gummigutta on gram positive human pathogens

Fresh Rind **Dry Rind**



Escherichia Coli ***Escherichia Coli***



Shigella Boydii ***Shigella Boydii***

Fig 3: Zone of inhibition of Fresh and Dry rind of Garcinia gummigutta on Gram Negative Human pathogens

Table 1: Anti microbial activity of Fresh rind of *Garcinia gummigutta* on Human pathogens

S. No	Human pathogens	Zone of inhibition (mm)	
		Rind Concentration at 1%	Rind concentration at 10%
Gram Positive Bacteria			
1.	*Bacillus subtilis*	Nil	9.33
2.	*Staphylococcus aureus*	Nil	8.71
3.	*Enterococcus fecalis*	Nil	12.99

Gram Negative Bacteria			
1.	*Escherichia coli*	Nil	9.49
2.	*Shigella boydii*	Nil	Nil

Table 2: Anti microbial activity of Dry rind of *Garcinia gummigutta* on Human pathogens

S. No	Human pathogens	Zone of inhibition (mm)	
		Rind Concentration at 1%	Rind concentration at 10%
Gram Positive Bacteria			
1.	*Bacillus subtilis*	Nil	12.69
2.	*Staphylococcus aureus*	Nil	9.75
3.	*Enterococcus fecalis*	Nil	13.59
Gram Negative Bacteria			
1.	*Escherichia coli*	Nil	11.68
2.	*Shigella boydii*	Nil	12.89

Antibacterial activity

The agar well diffusion method was employed to know the anti microbial activity. Nutrient agar was used for both Gram positive and Gram negative bacteria. The cooled molten medium was seeded with respective organism. On solidification of the medium, 1 gram and 10 gram Malabar Tamarind rind samples were added into 100ml of sterile water. 0.1ml of that sample was pipette out into the wells of pre poured incubated media (Antibiotic assay medium of *Bacillus subtilis, Staphylococcus aureus, Enterococcus fecalis, Escherichia coli and Shigella boydii)* respectively. The petri plates were then incubated in an upright position at 31°C for 24 to 48 hours. After the incubation the diameter the inhibition zone (mm) was measured in an transparent scale and was recorded. Each extract was analyzed in triplicate, the mean values are presented.

Results and discussion

The antibacterial activity of methonolic extract of *G. gummigutta* was assayed *in vitro* by agar disc diffusion method against 5 bacterial species. Table 1 and 2 summarizes the microbial growth inhibition. The significant antibacterial activity of the active plant extract was comparable to Kanamycin disc (30 µg/disc).

Anti microbial activity of all the bacterial strains were pound to be nil from both fresh and dry rind extract at the concentration level 1 percent. Whereas, at the concentration level of G. gummigutta fresh fruit rind extract at 10 per cent showed maximum zone of inhibition Enterococcus fecalis (12.99) fallowed by E.coli (9.49mm), Bacillus subtilis (9.33mm) and S. aureus (8.71mm) respectively. S. boydii did not show any zone of inhibition even at the concentration level of 10 per cent.

From the dry fruit rind E. fecalis (13.59mm) showed more zone of inhibition fallowed by S. boydii(12.89mm), B. subtilis (12.69mm), E. coli (11.65mm) and S. aureus (9.75mm) respectively.

G. gummigutta Kokum showed antibacterial activity against gram positive and gram negative organisms. Antimicrobial activity of methonolic extract against gram positive and gram negative organisms increases as the volume of extracts increases. Antimicrobial activity is primarily due to presence of furfural in G. Gummigutta extract. In many cases the main cause of indigestion has been attributed to imbalance in the normal microbial flora of the alimentary tract, like increased population of one type or intrusion of a harmful microbe. Similar results were found acco to (Miller *et al*., 2009) [5]. Restabilization of the flora resets the digestion and thus removes the problem associated with indigestion. If the problem is not a severe one then kokum juice can help to bring about the restabilization of the flora and thus rectify the problem of indigestion. These finding are concurrent with the similar results obtained earlier: ethanologenic E. coli, Saccharomyces cerevisiae (Taherzadeh *et al*., 1997 [8]; Palmqvist and Hahn-Hagerdal, 2000) [7]. However, the quantity of the juice to be consumed should be just sufficient (depending on the individual). If consumed in excess, it can be harmful like furfural can inhibit some of the liver function like inhibiting alcohol dehydrogenase, aldehyde dehydrogenase and pyruvate dehydrogenase in the liver (Tobiaset *et al*., 2002) [9].

The Anthocyanins present in G. gummigutta may possess the antimicrobial property. The anthocyanins present (mass spectrographic results not shown) are also significant as antimicrobial agents. The prominent anthocyanin present is cyanidin -3- glucose. This is actually known for its anti inflammatory actions, but is significantly antibacterial in its characteristics against gram positive bacteria. That is why the extracts of kokum are often used to cure dermatitis or other mild skin infections, when applied topically. Similar anthocyanin has been reported from berry fruits (Cavanagh *et al*., 2003) [2], Punica granatum (Naz *et al*., 2007) [6].

Conclusion

Garcinia gummigutta possess significant antibacterial activity. Further research is needed for the isolation and identification of active principles present in the extracts which could possibly be exploited for pharmaceutical use.

Acknowledgement

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