International Journal of Drug Development and Research

Philip Jacob et al., Int J Drug Dev & Res 2015, 7:3

Research Article

Evaluation of Antibacterial and Antioxidant Activity of *Garcinia gummi-gutta*

Kizhakedathil Moni Philip Jacob*, Mahuwala Arif Ali, Hemant Vishnu, Gunasekar Shylaja, Sathiavelu Mythili and Arunachalam Sathiavelu

School of Biosciences and Technology, VIT University, Vellore-632 014, Tamil Nadu, India

***Corresponding author:** Kizhakedathil Moni Philip Jacob, School of Biosciences and Technology, VIT University, Vellore-632 014, Tamil Nadu, India, Tel: 918122036830; Email: kmoni.philipjacob2013@vit.ac.in; asathiavelu@vit.ac.in

Received September 03, 2015; **Accepted** September 23, 2015; **Published** September 28, 2015

Abstract

Antibiotic resistance in microbes is a great problem in the modern world. Everyday new drugs are being used to overcome this problem. The aim of this study was to examine Antibacterial and Antioxidant properties of the *Garcinia gummi-gutta*. The dried fruits of *G. gummi-gutta* are used in Kerala, a southern state of India for preparation of curries. The extract of dried fruit of *G. gummi-gutta*, was prepared by using three different solvents such as acetone, ethanol and distilled water. These prepared extracts were used for evaluation of the antibacterial property against two gram negative bacterial strains *Escherichia coli, Pseudomonas aeruginosa* and two gram positive bacterial strains *Bacillus subtilis, Staphylococcus aureus.* The Antibacterial property was evaluated by Disc diffusion assay and the results of the test were compared with Chloramphenicol (30 mcg) as positive control. *P. aeruginosa* was sensitive to all the extracts of *G. gummi-gutta* tested. Whereas the other organisms showed good to moderate activity. In this study, the Minimum Inhibitory Concentration (MIC) for the *G. gummi-gutta* extract was also determined. The Antioxidant property of *G. gummi-gutta* was evaluated by Ferric chloride reducing power assay and compared with Ascorbic acid (Vitamin C) solution. The results indicated that *G. gummi-gutta* extract had more antioxidant property than Ascorbic acid solution.

Keywords: Antibacterial activity; *Garcinia gummi-gutta*; Disc diffusion method; Antioxidant property; Minimum inhibitory concentration; *Pseudomonas aeruginosa*

Introduction

The Genus Garcinia contains around 200 species, where 35 species are found in the two ecosystems of India, the Western Ghats and The Himalayan foot hills [1]. *Garcinia gummi-gutta* is a most common species found in Western Ghats [1,2]. *G. gummi-gutta* belongs to the family Guttiferae. It is a hardwood underutilized medicinal fruit crop, where its fruits are used in food preparations, especially in curries (Figure 1a and 1b) [3,4]. Previous studies had shown that, the leaf extract of the plant had shown strong antifungal activity on three fungal species namely *Phytopthora sp., Curvularia sp.,* and *Corynesporia sp.*, which could be used as natural fungicide [5]. *G. gummi-gutta* had also shown to increase the levels of erythrocytes, leucocytes, thrombocytes, hemoglobin and was found to decrease the level of glucose, total cholesterol and LDL level in catfish. This was due to the presence of a Hypolipidaemic compound, Hydroxy citric acid. It also increased immunity by increasing cellular immunological indicators [6-11].

Figure 1: (a) Garcinia tree. **(b)** Dried fruits of Garcinia.

The phytochemical analysis of *G. gummi-gutta* revealed the presence of high content of alkaloids, tannins, phenolic flavonoids, flavonoids, carbohydrates and proteins and low content of steroids, terpenoids, phlobatannin and Cardiac glycosides [12]. The bioactive molecules like hydroxyl citric acid (HCA), flavonoids, terpenes, polysaccharides, procyanidines and polyisoprenylated benzophenone derivatives like garcinol, xanthochymol and guttiferone were isolated from the genus Garcinia. It was found that the polyisoprenylated benzophenone and xanthone derivatives have antioxidant, apoptotic, anti-cancer, anti-inflammatory, antibacterial, anti-viral, anti-fungal, anti-ulcer and anti-protozoal properties [13]. The long term medication of antibiotics such as tetracycline, erythromycin etc., and over usage of synthetic antioxidants like Propyl gallate etc., has a possibility for causing health hazards and side effects [7-9]. The main aim of this study was to find a natural therapeutic compound which has antibacterial property and antioxidant property so that it can be an alternative in treating drug resistant bacteria.

Materials and Methods

Sample collection

The dried fruits of *Garcinia gummi-gutta* which was readily available in the shops (Vernacular Name: Kodampuli) was bought from a local merchant in Kerala and the sample was authenticated by a local botanist. The dried fruits were stored in room temperature for future use.

Extract preparation

For the preparation of extract, three different solvents, viz Water, Acetone and Ethanol were used. The dried fruits of *G. gummi-gutta* were cut into very small pieces using a sterile knife. Six grams of the dried fruits were transferred into separate flasks containing 30 ml of the solvents. The mixture was kept in a shaker at room temperature (30°C) at 170 rpm for 24 hours for continuous shaking. The extracts were collected in sterile falcon tubes in a laminar air flow chamber and were centrifuged at 4900 rpm for 10 minutes to remove the debris. The supernatant containing the extracts were collected in a sterile falcon tube and stored at 4°C for further use.

Antibacterial test assay

Antimicrobial susceptibility test was carried out by the Disc diffusion method [10]. The Petri-dish containing Muller Hinton Agar was plated with 0.1 ml culture of different bacterial strains (*E. coli, B. subtilis, S. aureus, P. aeruginosa*). The plates inoculated with bacteria, were made in triplicate. The discs containing the *G. gummi-gutta* extracts were placed on the agar using sterile forceps. Chloramphenicol (30 mcg) was used as positive control. The plates were incubated at 37°C for about 18-24 hours. The diameter of resultant Zone of inhibition was measured in millimeters.

Evaluation of antioxidant property by reducing power assay

Reducing power assay was done by the modified method of Nikhat et al. Initially 1% (w/v) Ascorbic acid and 1% (w/v) *G. gummi-gutta* extract were prepared in sterile distilled water and then further diluted to a working concentration ranging from 200 to 1000 µg/ml by with phosphate buffer (pH- 6.6). Then 2.5 ml of 1% (w/v) Potassium ferricyanide solution was added. The mixture was incubated at 50°C for 20 minutes in a water bath. 2.5 ml of 10% (w/v) Trichloroacetic acid were added into the mixture followed by centrifugation at 4900 rpm for 12 minutes. The supernatant of the solution was transferred into sterile test tubes and equal volume of distilled water and 1 ml of freshly prepared 1% (w/v) Ferric chloride solution was added. The tests were done in triplicate. The absorbance of the reaction mixture was measured at 700 nm using a UV-Visible Spectrophotometer. The averages of the values were plotted on a graph with O.D in the Y- axis and Concentration in the X-axis. The Absorbance of standard Ascorbic acid solution was compared with *G. gummi-gutta* extract solution [11].

Minimum inhibitory concentration

Different concentration of *G. gummi-gutta* extracts (w/v) i.e., 1%, 2%, 5%, 10%, 20%, 30%, 40% and 50% were prepared in sterile distilled water. 1 ml of each of the different concentration of the extract was added to a sterile test tube containing 5 ml of nutrient broth that contained 10^{-5} cells of *E. coli*, which was obtained after serial dilution. The Control test tube did not contain *G. gummi-gutta* extract. The tubes were then incubated at 37°C for about 18-24 h. Based on Spectrophotometric analysis, the lowest concentration of the extract which inhibited the growth of the organism was considered as Minimum Inhibitory Concentration which was compared with Blank and Control. Blank contained only the broth without the inoculum whereas, the Control contained inoculum.

Statistical analysis

The results of the Disc diffusion test were expressed as Mean ± S.D. The statistical significance was measured using one way ANOVA. The 'P value' was found to be <0.05, which is considered to be significant.

Results and Discussion

Antibacterial test assay

The different extracts of *G. gummi-gutta* showed good to moderate Antibacterial effect on all the strains that was tested. Various extract showed different effects on the bacteria, which were summarized in Table 1. For this study two gram positive bacteria (*B. subtilis, S. aureus*) and two gram negative bacteria (*E. coli, P. aeruginosa*) were used. Chloramphenicol (30 mcg) was used as a positive control. The main reason to use Chloramphenicol was because of its broad-spectrum antibiotic activity and it was seen that number of multi drug resistant bacteria were still sensitive to Chloramphenicol [14]. From the 'Standard Zone Size Interpretative Chart for Chloramphenicol', the results were interpreted. For Chloramphenicol (30 mcg), Resistant: 12 mm and less, Intermediate: 13-17 mm and Sensitive: 18 mm and more.

For *E. coli*, the water extract showed an inhibition zone of about 18 mm (approx), which indicates that the water extract had shown a good Antibacterial activity. The ethanol and acetone extracts which had a zone of about 17 mm and 16.5 mm (approx) respectively, indicates a moderate activity on the microorganisms. For *B. subtilis* and *S. aureus*, both the water and ethanol extracts showed a zone of inhibition of about 15.6 mm and 15.3 mm (approx) respectively, indicating a moderate activity on both the organisms, whereas, the acetone extract with an inhibition zone of about 18.3 mm (approx) for both the organisms showed a good Antibacterial activity. For *P. aeruginosa* - the water, ethanol and acetone extracts showed inhibitory zones of about 41 mm, 44 mm and 38 mm respectively. This indicates that *P. aeruginosa* is highly sensitive to all the extracts.

In a nutshell, *E. coli* was sensitive to the water extract. Both *B. subtilis* and *S. aureus*, were sensitive to acetone extract, whereas, *P. aeruginosa* was highly sensitive to all the extracts. As stated by Naveen et al. *G. gummi-gutta* contains many Antibacterial compounds which could have contributed for the good activity against microbes. Further research is essential to isolate and identify the compound for therapeutic purpose.

Reducing power assay

For the measuring the Reducing power of any compound, the

Organism	Control	Water	Ethanol	Acetone
Escherichia coli	0.00	18.0 ± 1.00	17.0 ± 1.73	16.6 ± 1.52
Bacillus subtilis	0.00	15.6 ± 0.57	15.6 ± 0.57	18.3 ± 0.57
Pseudomonas aeruginosa	0.00	41.0 ± 1.00	44.0 ± 3.46	38.0 ± 6.35
Staphylococcus aureus	0.00	15.3 ± 2.08	15.3 ± 1.52	18.3 ± 0.57

*All the measurements are in millimeters.

Table 1: *In vitro* Antimicrobial activity of *G. gummi-gutta* extracts on Test organisms (determined by diameter of inhibition zones).

Serial No	Concentration of ascorbic acid (Vitamin C) (µg/ml)	Concentration of Garcinia extract (µg/ml)	Absorbance at 700 nm	
			Vitamin C	Garcinia
1	200	200	0.252	1.372
2	400	400	0.375	1.696
3	600	600	0.493	1.935
4	800	800	0.596	3.102
5	1000	1000	0.812	3.217

Table 2: Reducing power activity of Vitamin C and Garcinia extract.

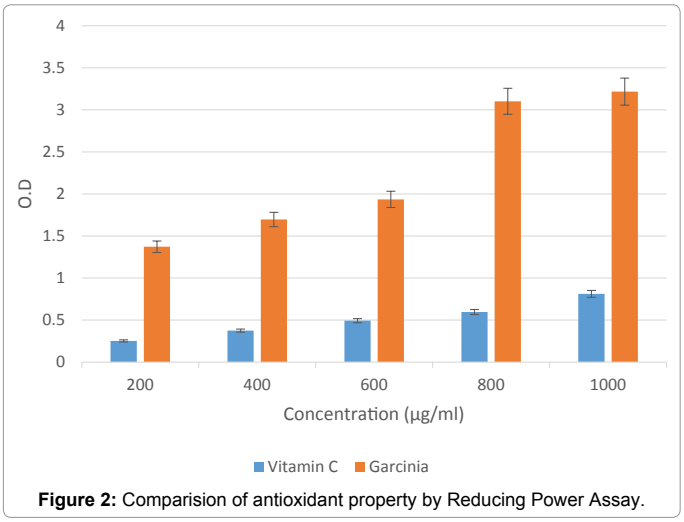

Figure 2: Comparision of antioxidant property by Reducing Power Assay.

capability of the compound to convert the Ferric ion (Fe^{+3}) to Ferrous ion (Fe^{+2}) was measured. In this assay, Yellow colour of the solution changes to Blue colour as the Ferric ion (red) to Ferrous ion (blue). Absorbance was measured at 700 nm. Higher absorbance indicates that the compound has more Antioxidant property.

In this study, *G. gummi-gutta* extract was compared with Ascorbic acid (Vitamin C) for determining its Reducing power. From the results obtained, it was found that the *G. gummi-gutta* extract had a higher Antioxidant property than Ascorbic acid solution. Ascorbic acid was used to test because of its natural Antioxidant potential. The Antioxidant property of the *G. gummi-gutta* extract and Vitamin C has been summarized in Table 2 and graphically represented in Figure 2.

Minimum inhibitory concentration

The Minimum inhibitory concentration is the minimum concentration of the compound required to inhibit the growth of an organism, which was determined by comparing the growth of the bacteria in the control test tube, with the other test tubes by checking their turbidity in UV- Visible spectrophotometer at 600 nm. The organism tested was *E. coli*. From the results obtained, the *G. gummi-gutta* extract of concentration 1% and 2% showed growth, which indicated that there was no inhibition in the growth. Slight growth was observed at 5% extract concentration. The extract concentrations above 10% showed no growth of *E. coli.* From the results obtained, it was evident that the MIC of the extract for *E. coli* was about 10%.

Conclusion

From the above studies we can conclude that *G. gummi-gutta* showed a good Antibacterial activity on all the organisms tested. It also showed good Antioxidant activity. So *G. gummi-gutta* can be used as Antibacterial agent and also can be used as Antioxidant in Food and Pharmaceutical industries. Further investigations must be performed to examine the Antibacterial properties in other stains and other pathogenic bacteria. Cytotoxicity will be very minimal or null since *G. gummi-gutta* is already used in cooking. Since not much research has been done in this area, the other active compounds present, should be examined for the benefit of humans.

Acknowledgements

The authors thank the VIT University for providing the opportunity to carry out the research. They also thank Mrs. Roopa Rachel and Mr. Roshan P Shajan for providing the Garcinia fruits from Kerala for carrying out the research. The authors also thank Ms. Shoba Sundaramoorthy for her constant support throughout the research.

References

1. Parthsarathy U, Nanda Kishore OP (2014) A study on nutrient and medicinal compositions of selected Indian Garcinia species. Current Bioactive Compounds 10: 55-61.
2. Parthasarathy N, Karthikeyan R (1997) Biodiversity and population density of woody species in a tropical evergreen forest in Courtallum reserve forest, Wester Ghats. India Trop Ecol 38: 297-306.
3. Nair KKN, Mohanan C, Mathew G (2005) Preliminary observations on seed and plantation technology for Garcinia gummigutta (Guttiferae): An indigenous and economic tree crop of the Indian Peninsula. Forests Trees and Livelihoods 15: 75-87.
4. Drury Heber (1873) Garcinia gambogia (Desrous) Clusiaceae. The Useful Plants of India. 2nd edn. London: William H Allen & Co: 220.
5. Dhanya P, Benny PJ (2013) Antifungal effect of methanolic extracts of leaves of Garcinia gummigutta. Int J Pharm Sci Rev Res 21: 330-333.
6. Prasad G, Priyanka GL (2011) Effect of fruit rind extract of Garcinia gummigutta on haematology and plasma biochemistry of catfish Pangasianodon hypothalmus. Asian Journal of Biochemistry 6: 240-251.
7. Al-Shwyeh Hussah Abdullah, Mohamed Elwathig Saeed Mirghani, Parveen Jamal (2011) Antibacterial activity of Malaysian mango kernel. Afr J Biotechnol 10: 18739-18748.
8. Fang Tian, Bo Li, Baoping Ji, Jinhua Yang, Guizhi Zhang, Yang Chen, Yangchao Luo (2009) Antioxidant and antimicrobial activities of consecutive extracts from Gallachinensis: The polarity affects the bioactivities. Food Chem 113: 173-179.
9. Aziz Turkoglu, Mehmet Emin Duru, Nazime Mercan, Ibrahim Kivrak, Kudret Gezer (2007) Antioxidant and antimicrobial activities of Laetiporus sulphureus Murrill. Food Chem 101: 267-273.
10. Olila D, Olwa-Odyek, Opuda-Asibo J (2001) Antibacterial and antifungal activities of extracts of Zanthoxylum chalybeum and Warburgia ugandensis, Ugandan medicinal plants. Afr Health Sci 1: 66-72.
11. Nikhat F, Satynarayana D, Subhramanyam EVS (2009) Isolation, Characterization and Screening of Antioxidant Activity of the Roots of Syzygium cuminii (L) Skeel. Asian J Research Chem 2: 218-221.
12. Madappa MB, Bopaiah AK (2012) Preliminary phytochemical analysis of leaf of Garcinia gummi-gutta from Western Ghats. Int J Pharm Biol Sci 4: 17-27.
13. Naveen GPAN, Krishna Kumar G (2013) Traditional and Medicinal Uses of Garcinia gummi-gutta Fruit- A Review. Species 4: 4-5.
14. Fernández M, Conde S, de la Torre J, Molina-Santiago C, Ramos JL, et al. (2012) Mechanisms of resistance to chloramphenicol in Pseudomonas putida KT2440. Antimicrob Agents Chemother 56: 1001-1009.