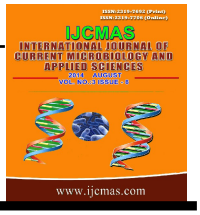
*Int.J.Curr.Microbiol.App.Sci* (2014) 3(9) 800-804

*International Journal of Current Microbiology and Applied Sciences*
***ISSN: 2319-7706*** **Volume 3 Number 9 (2014) pp. 800-804**
**http://www.ijcmas.com**




**Original Research Article**

# Anti Microbial, Antifungal and Anti Oxidant Activity of Ethanolic Extracts of *Garcinia indica* Fruit

**K.Nagendra, R.Kusum and H.D.Ramachandran***

Department of Biochemistry, Central College Campus, Bangalore University, Banglore-560001, India
**Corresponding author*

**A B S T R A C T**

The intention of the present research was to evaluate the *in vitro* anti microbial, anti fungal and antioxidant activity of the methanolic extract from fruit of *Garcinia Indica* collected. They were examine for the *in vitro* antibacterial activity against 2 Gram-positive *Bacillus subtilis* and *Staphylococcus aureus* and 2 Gram-negative bacteria *Pseudomonas aeruginosa* and *Escherichia coli*, using agar well diffusion method and antifungal activity for *Aspergillus niger* and *Candida albicans* was estimated, using agar well diffusion method. The antioxidant activity was determined by DPPH assay. The minimum inhibition concentration of the *Garcinia Indica* methanolic extracts was 0.5 mg/mL for *Escherichia coli*. The growth of *Bacillus subtilis* with the maximum zone of inhibition of 2.6mm the slightest zone of inhibition of 0.7mm on *Staphylococcus aureus* and in fungal 12.0 mm in *Candida albicans* and nil in *Aspergillus niger*. The methanolic fruit extract of Garcinia indica demonstrated antioxidant potential dose dependently with best activity at 100 μg/ml. The results obtained from this study specify that fruit of *Garcinia indica* extract has both antifungal and antibacterial properties and has a potential for use as a biopreservative in food applications and can be used as therapeutic agent and antioxidant thus could prevent many radical diseases and could be used as neutraceuticals.

**Keywords**

*Garcinia indica*, Anti oxidant activity, Anti microbial, Antifungal.

## Introduction

Spices and herbs have been used for thousands of centuries by many cultures to enhance the flavor and aroma of foods. Early cultures also recognized the value of using spices and herbs in preserving foods and for their medicinal value. Scientific experiments since the late 19th century have documented the antimicrobial properties of some spices, herbs, and their components [1] [2]. Many herbs and spices are known to exert antioxidant activity and are useful for preventing lipid oxidation in living organisms as well as in foods. It is estimated that there are 250,000 to 500,000 species of plants on [3]. Relatively small percentages (1 to 10%) of these are used as food by both humans and other animal species. It is possible that even more are used for medicinal purposes [4].

Species show evidence of antibacterial, antifungal and anti oxidant activity. Microbiologists have conducted laboratory experiments that involve numerous challenging food-borne bacteria, fungi, and yeasts with phytochemicals extracted from spice plants. Multiple techniques have been used to investigate antimicrobial activity, and the primary data vary considerably in quality and quantity among different spices. Nevertheless, it is now clear that many spices have potent antimicrobial properties [1] [5] - [8].The fruits of Garcinia indica have been suggested in the Indian system of medicine for a number of diseases. These include its usefulness as an infusion, in skin rashes caused by allergies, treatment of burns, to relieve sunstroke, remedy for dysentery and mucous diarrhea, an appetizer, liver tonic, to allay thirst and as a cardiotonic. Garcinol a polyisoprenylated benzophenones, has antioxidative, chelating, free radical scavenging, anti glycation, anticancer, anti inflammatory, and anti ulcer activities [9] – [12]. This study explores the antimicrobial activity of methanolic extracts from the fruit of *Garcinia indica* against Gram-positive and Gram negative bacteria and fungi. And to discover antioxidant properties of *Garcinia indica* methanolic fruit extract *invitro.*

## Materials and Methods

### Plant collection and extraction

The fruits were collected separated from matured fruits, shade dried, broken into small pieces and powered coarsely. The 500 g of dry fruits sample which was later coarsely powdered in a Willy Mill to 60-mesh size and used for solvent extraction in Soxhlet apparatus and successively extracted with methanol by for 24 hrs. The extract was concentrated using rotary vacuum evaporator. The extract was used for total phenol and flavonoids content and also for estimation of antioxidant through various chemical assays [13] [14].

### Test strains and culture media

Strains of bacteria and fungi were obtained from MTCC (Microbial Type Culture Collections, Chandigarh, India). Antimicrobial activity of methanolic extracts against *Bacillus subtilis* (MTCC 7419), *Staphylococcus aureus* (MTCC 3381), *Escherichia coli* (MTCC 1652), *Pseudomonas aeruginosa* (MTCC 2453), *Candida albicans* (MTCC 3071), and *Aspergillus niger* (MTCC *3323)* was studied. The species of bacteria were grown in Nutrient agar at 37°C and the fungi were grown on Potato Dextrose Agar at room temperature

### Antibacterial assay

The customized agar well diffusion method [15] was used; nutrient agar media were used for bacteria. Once the agar was solidified, 50µl of the different bacterial cultures were spread onto the plates using a sterile spreader. The plates were punch with six millimeter diameter wells and filled with 25µl of the plant extract and blank distilled water which served as the negative control. Simultaneously, streptomycin (100µg/ml) was used as positive controls. The tests were carried out in duplicates. The bacterial plates were incubated at 37°C for 24 hrs. The diameter of the zone of inhibition was measured in millimeters at 24 hrs.

### Antifungal assay

The customized agar well diffusion method [15] was used; potato dextrose agar media were used for fungi. Once the agar was solidified, 50µl of the different fungal cultures were spread onto the plates using a

sterile spreader. The plates were punch with six millimeter diameter wells and filled with 25µl of the plant extract and blank distilled water which served as the negative control. Simultaneously, nystatin (100µg/ml) was used as positive controls. The tests were carried out in duplicates. The fungal plates were incubated at room temperature. The diameter of the zone of inhibition was measured in millimeters at 120 hrs.

**Antioxidant activity**

Antioxidant activity (DPPH free radical scavenging activity) of the extract *Garcinia indica* fruits was assessed on the basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH)-free radical activity by modified method[16]. 1, 1-Diphenyl-2-picryl-hydrazyl (DPPH) was obtained from Sigma Aldrich. The diluted working solutions of the test extracts were prepared in methanol. Ascorbic acid was used as standard in 1-100 µg/ml solution. 0.002% of DPPH was prepared in methanol and 1 ml of this solution was mixed with 1 ml of sample solution and standard solution separately. These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm using UV Spectrophotometer. Methanol (1 ml) with DPPH solution (0.002%, 1 ml) was used as blank. The optical density was recorded and % inhibition was calculated using the formula given [17].

Percent (%) inhibition of DPPH activity = (A-B/A) 100
Where A = optical density of the blank and B = optical density of the sample.

## Results and Discussion

The antibacterial activity of the extract from the test samples in condition of minimum inhibitory concentrations and inhibition zones are reported (Table 1). The crude extract of *Garcinia indica* on four different bacteria showed that the extract was inhibiting the growth of *Bacillus subtilis* with the highest zone of inhibition of 2.6mm the least zone of inhibition of 0.7mm on *Staphylococcus aureus.* Moderate inhibition was shown in other bacteria 1.20mm and 1.5mm in *Pseudomonas aeruginosa and Escherichia coli* respectively. Minimum inhibition concentration results revealed that 0.5mg/ml of *Garcinia indica* concentration was enough for inhibiting *Escherichia coli* whereas the MIC of *Garcinia indica* was 5mg/ml for both *Bacillus subtilis* and *Pseudomonas aeruginosa* and 50mg/ml concentration was required to inhibit *Staphylococcus aureus.*

Among the fungi, *Garcinia indica* showed highest inhibition zone of 12.0 mm in *Candida albicans* and it could not inhibit the growth of *Aspergillus niger*. The MIC of the extract for *C. albicans* was 0.5mg/ml (Table 2). A freshly prepared DPPH solution exhibited a purple color with a maximum absorption at 517 nm. This purple color disappears when an antioxidant is present in the medium. Thus, antioxidants molecules can quench DPPH free radicals and convert them to a colorless product, resulting in a decrease in absorbance at 517 nm. The RSA values of *Garcinia indica* are presented in (Table 3) results are expressed as IC50 values. Methanolic fruit extract of *Garcinia indica* dose dependently demonstrated antioxidant potentials by scavenging DPPH radical scavenging activity. The DPPH scavenging potential of extract might be due to its reducing actions, which might donate hydrogen to a free radical, reducing it to nonreactive species [18]. Higher DPPH scavenging potential of Garcinia indica might be due to the higher reducing potential.

**Table.1** Minimum inhibitory concentrations and inhibition zones for Bacterial species

| Organisms | *Minimum Inhibitory Concentration* | | Inhibition zone (mm) |
|---|---|---|---|
| | ***Garcinia indica*** | **Streptomycin** | |
| *Bacillus subtilis* | 5mg/ml | 100µg/ml | 2.6 |
| *Staphylococcus aureus* | 50mg/ml | 100µg/ml | 0.7 |
| *Pseudomonas aeruginosa* | 5mg/ml | 100µg/ml | 1.2 |
| *Escherichia coli* | 0.5mg/ml | 100µg/ml | 1.5 |

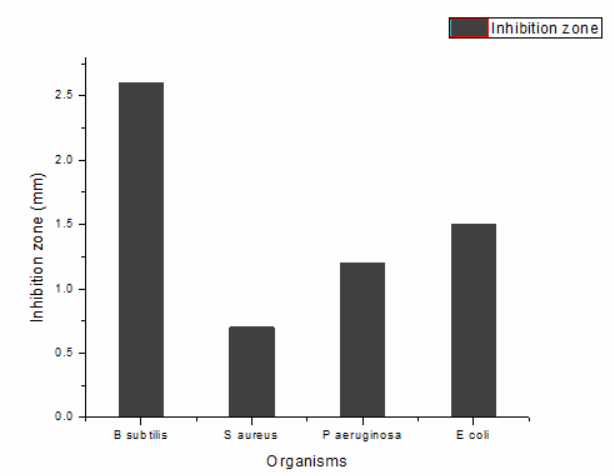



**Table.2** Minimum inhibitory concentrations and inhibition zones for fungal species

| Organisms | *Minimum Inhibitory Concentration* | | Inhibition zone (mm) |
|---|---|---|---|
| | ***Garcinia indica*** | ***Nystatin*** | |
| *Candida albicans* | 0.5mg/ml | 100µg/ml | 12 |
| *Aspergillus niger* | - | 100µg/ml | - |

**Table.3** In vitro antioxidant activity of *Garcinia indica*

| Garcinia indica Dose µg/ml | IC50 (µg/ml) DPPH(Mean±SD) |
|---|---|
| 100 | 3.87 ± 0.2 |
| 50 | 5.90 ± 0.2 |
| 25 | 8.34 ± 0.2 |
| 10 | 10.26 ± 0.2 |

## Acknowledgements

Nagendra K acknowledges the JRF under BRNS project no: DEV: D2: BRNS-RP: DR.HDR: BICH: 2009-10.

## References

1. Shelef, l.a. 1983. Antimicrobial effects of spices. J. Food Safety 6: 29–44
2. Zaika, l.l. 1988. Spices and herbs: their antimicrobial activity and its determination. J. Food safety 9: 97–118.
3. Borris, R.P., 1996. Natural products research: perspectives from a major pharmaceutical company. J. Ethnopharmacol., 51: 29-38.
4. Moerman, D.E., 1996. An analysis of the food plants and drug plants ofnative North America. J. Ethnopharmacol., 52: 1-22.
5. Hargreaves, L.L., Jarvis, B., Rawlinson, A.P. & Wood, J.M. 1975. The antimicrobial effects of spices, herbs and extracts from these and other food plants. Scientific and Technical Surveys, British Food Manufacturing Industries Research Association 88: 1–56.
6. Deans, S.G. & Ritchie, G. 1987. Antibacterial properties of plant essential oils. Int. J. Food Microbiol. 5: 165–180
7. Nakatani, N. 1994. Antioxidative and antimicrobial constituents of herbs and spices, pp. 251–272. In: Charalambous,G. (ed.) Spices, Herbs, and Edible Fungi. Elsevier, Amsterdam.
8. Hirasa, K. & Takemase, M. 1993. Spice Science and Technology. Marcel Dekker, New York.
9. Lin JK, Liao CH, Ho CT. Effects of garcinol on free radical generation

and NO production in embryonic rat cortical neurons and astrocytes. Biochem Bio Res Commun 2005; 329:1306-1314

10. Ho CT, Sang S, Liao CH, Pan MH, Rosen RT, Shiau SYL, et al Chemical studies on antioxidant mechanism of garcinol: analysis of radical reaction products of garcinol with peroxyl radicals and their antitumor activities. Tetrahedron 2002; 58:10095-10102
11. Yamaguchi F, Saito M, Ariga T, Yoshimura Y, Nakazawa H. Antioxidative and anti-Glycation activity of Garcinol from Garcinia indica fruit rind. J Agri Food Chem 2000;48:180-185.
12. Yamaguchi F, Saito M, Ariga T, Yoshimura Y, Nakazawa H. Free radical scavenging activity and antiulcer activity of Garcinol from Garcinia indica fruit rind. J Agri Food Chem 2000;48:2320-2325.
13. Mehta R.M. (1999). Textbook of Pharmaceutics, (II): 146-14.
14. Makkar H.P.S., Becker K., Abel H. and Pawelzik E. (1997). Nutrient contents, rumen protein degradability and antinutritional factors in some colour and white flowering cultivars of vicia faba beans. Journal of the Sciences of Food and Agriculture, 75: 511-520.
15. Perez, C., M. Pauli and P. Bazevque, 1990. An antibiotic assay by the agar well diffusion method. Acta Biologiae et Medicine Experimentalis, 15: 113-115.
16. Braca A, Sortino C, Politi M. Anti-oxidant activity of flavonoids from Licania licaniaeflora. J Ethnopharmacol 2002;79:379-381.
17. Bors W, Saran M, Elstner EF. Screening for plant antioxidants. In: Linskens HF, Jackson JF. editors. Modern Methods of Plant Analysis Plant Toxin Analysis New Series, Vol 13. Springer, Berlin; 1992: p. 277-295
18. Wang H, Gao XD, Zhou GC, Cai L, Yao WB. In vitro and in vivo antioxidant activity of aqueous extract from Choerospondias axillaries fruit. Food Chem 2008;106: 888-895