

Antimicrobial and Cytotoxic Effects of *Garcinia indica*

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Abstract: *Garcinia* is a large genus of polygamous trees or shrubs, distributed in the tropical Asia, Africa and Polynesia and is a rich source of bioactive molecules including xanthenes, flavonoids, benzophenones, lactones and phenolic acids. In the present study, an attempt was made to evaluate the antimicrobial properties of *Garcinia indica* on certain microbes and cytotoxic properties of *Garcinia indica* on Balb/c 3T3 mouse fibroblasts. The minimum inhibitory concentrations of the water extract against bacteria were, 0.5mg/ml in *Escherichia coli*, 5mg/ml in both *Bacillus subtilis* and *Enterobacter aerogenes* and 50mg/ml in *Staphylococcus aureus*. The minimum inhibitory concentrations against fungi were 50mg/ml for both *Candida albicans* and *Penicillium* sp. *Garcinia indica* fruit rind extract showed inhibitory effect on cultured 3T3 mouse fibroblasts. The cell concentration decreased with increasing concentration of the extract. The *G. indica* extract has both antifungal and antibacterial properties and has a potential for use as a biopreservative in food applications and therapeutic agent in cancer treatment.

Key words: *Garcinia indica* • Balb/c mouse fibroblasts • Antifungal • Antibacterial • Cytotoxic • Minimum inhibitory concentration

INTRODUCTION

Finding healing powers in plants is an ancient idea. People on all continents have long applied poultices and imbibed infusions of hundreds, if not thousands, of indigenous plants, dating back to prehistory. There is evidence that Neanderthals living 60,000 years ago in present-day Iraq used plants such as hollyhock [1,2]. these plants are still widely used in ethnomedicine around the world. It is estimated that there are 250,000 to 500,000 species of plants on Earth [3]. A relatively small percentage (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].

Garcinia is a large genus of polygamous trees or shrubs, distributed in the tropical Asia, Africa and Polynesia and is a rich source of bioactive molecules including xanthenes, flavonoids, benzophenones, lactones and phenolic acids [5]. It has antifungal properties [6] and it prevents acute ulceration in rats when orally administered [7]. But there have been no reports on the effects of *Garcinia indica* on *C. albicans*, *A. niger*, *Fusarium* sp. and *Penicillium* sp. and also hitherto no

attempts have been made to study the effects of *Garcinia indica* extracts on normal immortalized mouse fibroblasts cell lines. Hence, in the present study, we evaluated the antimicrobial effects of *Garcinia indica* (kokum) against four strains of bacteria, viz. *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Enterobacter aerogenes* and four fungal species (*C. albicans*, *A. niger*, *Fusarium* sp. and *Penicillium* sp.) and compared these antimicrobial effects with its cytotoxic effects on Balb/c 3T3 mouse fibroblasts.

MATERIALS AND METHODS

Microorganisms and Cell Lines Used: The following microorganisms used in the present study were procured from Microbial Type Culture Collections, Chandigarh, India.

The bacteria, *Bacillus subtilis* (MTCC 7419), *Staphylococcus aureus* (MTCC 3381), *Enterobacter aerogenes* (MTCC 7325) and *Escherichia coli* (isolated in the laboratory) and the fungi, *Candida albicans* (MTCC 3071), *Aspergillus niger*, *Fusarium* sp. and *Penicillium* sp. (isolated in the laboratory) were used.

The bacteria were grown on Nutrient agar at 37°C and the fungi were grown on Potato Dextrose Agar at room temperature. Balb/c 3T3 mouse fibroblasts were procured from National Center for Cell Sciences, Pune, India. The fibroblast cells were cultured in 5 ml culture media consisting of 90% DMEM (Himedia) medium and 10% fetal bovine serum, at 37°C in humidified CO₂ incubator. After 24 h, samples were taken from the cultures for determining the cell count by haemocytometry after 5-10 minutes of trypsinization.

Plant Material: The air-dried fruit rind of kokum (*Garcinia indica*) were collected. The rind was cut into pieces and ground into powder using a sterile pestle and mortar. The soluble ingredients in the ground part were extracted by solubilization using water as the solvent. 5 g of each of the dried leaves were extracted by successive soaking for 2-4 h using 100 ml of distilled water in a 250 ml sterile conical flask. The extracts were filtered using Whatmann filter paper No 1. They were filter sterilized with the help of Millipore Syringe filters of 0.2 µm pore size before use. The extracts were stored at 4°C for further use.

Antimicrobial Activity: The modified agar well diffusion method [8] was employed. Nutrient agar and Potato dextrose agar were used for bacteria and fungi respectively. Once the agar was solidified, 50 µl of the different bacterial and fungal cultures were spread onto the plates using a sterile glass spreader. The plates were punched with six millimeter diameter wells and filled with 25 µl of the plants extracts and blanks (distilled water which served as the negative control). Simultaneously, streptomycin (100 µg/ml) and nystatin (100 µg/ml) were used as positive controls for bacteria and fungi respectively. The tests were carried out in triplicates. The bacterial plates were incubated at 37°C for 24 h and fungal plates at room temperature. The diameter of the zone of inhibition was measured in millimeters at 24 h and 120 h for bacteria and fungi respectively.

The Minimum Inhibitory Concentration (MIC): A loop full of bacterial and fungal cultures from the slant was inoculated into nutrient broth and potato dextrose broth respectively and incubated at 37°C for 24 h for bacteria and at room temperature for 4-5 days for fungi. The fresh broth (20 ml) was seeded with 0.25 ml of 24 h bacterial broth culture or 4-5 day's fungal broth culture. Then 0.2 ml of the extract was added to 1.8 ml of seeded broth which was the 1st dilution. 1ml of the solution was

diluted further with 1ml of the seeded broth to produce 2nd dilution and the procedure was repeated until six dilutions were obtained. A set of tubes containing only seeded broth were kept as control. After incubation for 24 h at 37°C for bacteria and after 4-5 days at room temperature for fungi, the last tube with no visible growth of bacteria or fungi was taken to represent MIC of test samples which was expressed in mg/ml. The broth dilution assay was also carried out with streptomycin for bacteria and nystatin for fungi in the same way as the extracts and MIC values of streptomycin and nystatin were determined [9].

Cytotoxicity Studies: The fibroblast cell cultures were then treated with 8 µl (80 µg), 16 µl (160 µg) and 24 µl (240 µg) of the extracts, prepared from *G. indica* fruit rinds. The controls were treated with 10µl of sterile distilled water. Cultures were incubated for a further 5 days and the total cell concentration and percentage viability were determined by adopting trypan blue dye exclusion method [10] and the cytotoxic profiles of the extracts were assessed using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide Microculture Tetrazolium (MTT) viability assay as described by Mosmann [11]. Cell suspensions were seeded into 96-well microtitre plates at a plating density of 0.1 million cells mL⁻¹. After 24 h incubation, cells were exposed to various concentration of plant extract and incubated for 68 h. MTT (5 mg mL⁻¹) was added to each well at appropriate time and further incubated for 4 h after which the media was removed. DMSO was added later into each well to solubilize the formazan crystals. The absorbance was read at a wavelength of 595 nm using a microtitre ELISA plate reader. Experiments for each extract were carried out in triplicate including untreated cell control and a blank cell-free control. The percentage cellular viability was calculated with the appropriate controls taken into account. The concentration which inhibited 50% of cellular growth (IC₅₀ value) was determined.

Statistical Methods: The significance was calculated using one-way analysis of variance (ANOVA) and Student's t-test. A value of P < 0.05 was taken as statistically significant. And the results were calculated as mean with standard error (±SE) values.

RESULTS AND DISCUSSION

The antibacterial testing of the fruit rind extract of *G. indica* on four different bacteria showed that the extract was inhibiting the growth of *B. subtilis* with the

highest zone of inhibition of 2.4mm (Figure 1) and the least zone of inhibition of 1.0mm on *S. aureus*. Moderate inhibition was shown in other bacteria (1.5mm). Minimum inhibition concentration results revealed that 0.5mg/ml of *G. indica* concentration was enough for inhibiting *E. coli* whereas the MIC of *G. indica* was 5mg/ml for both *B. subtilis* and *E. aerogenes* and 50mg/ml concentration was required to inhibit *S. aureus* (Table 1). Similarly Negi and Jayaprakasha [12] reported the MIC of hexane extract, benzene extract and garcinol against a few Gram-positive and Gram-negative bacteria which were in the range of 15 to 1000, 20 to 1250 and 1.5 to 500 ppm respectively. Better bactericidal activity of garcinol compared to clarithromycin against *Helicobacter pylori* at 6 and 12 h incubation was reported by Archana [13]. Pasha [14] reported that *G. indica* extract exhibited strong anti-salmonella activity. The aqueous extracts were found to be more active than methanol extracts in their study.

Among the fungi, *G. indica* showed highest inhibition zone of 17.0 mm in *C. albicans* followed by *Penicillium* sp.(1.0 mm) and it could not inhibit the growth of either *A. niger* or *Fusarium* sp. (Figure 2). The MIC of the extract for *C. albicans* and *Penicillium* sp was 0.5mg/ml (Table. 2). Tamil Selvi [6], reported that *G. indica* extract has both antifungal and antioxidant properties.

Garcinia indica extract showed inhibitory effect on cultured 3T3 mouse fibroblasts. The cell concentration decreased with increasing concentration of the extract (Figure 3). The cell concentration was almost double in the control flasks than the flasks treated with 80µg/ml of the extract. Among the treatment flasks, 80µg/ml treated flasks had more cells than both 160µg/ml or 240µg/ml treated flasks. The differences were found to be statistically significant. These results are in agreement with the results of Hong *et al* [15], where garcinol and its derivatives showed potent growth-inhibitory effects on all intestinal cells, garcinol was more effective in inhibiting growth of cancer cells than that of normal immortalized cells and the results indicated that garcinol and its derivatives can inhibit intestinal cell growth, but low concentrations of garcinol can stimulate cell growth. Yamiguchi [7] reported that orally administered garcinol prevented acute ulceration in rats induced by indomethacin and water immersion stress caused by radical formation. Results of Liao *et al.* [16] suggested that the neuroprotective effects of garcinol are associated with anti-oxidation and inhibition of iNOS induction in astrocytic cells.

Since our results indicated an antimicrobial effect combined with an inhibitory effect on mouse fibroblasts, the possibility of using this herbal product to treat inflammations and cancerous conditions of the fibroblastic cells can be explored in the future. It remains to be determined whether the currently observed cytotoxic effects of *G. indica* extract on fibroblast cell growth can occur *in vivo*.

REFERENCES

1. Stockwell, C., 1988. Nature's pharmacy. Century Hutchinson Ltd. London, United Kingdom.
2. Thomson, W.A.R. (ed.), 1978. Medicines from the Earth. McGraw-HillBook Co. Maidenhead, United Kingdom.
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 of native North America. J. Ethnopharmacol., 52: 1-22.
5. Patil, B.P., 2005. Kokum, Brochure, Western Ghats Kokum Foundation, Goa,
6. Selvi, T.A. G.S. Joseph and G.K. Jayaprakasha, 2003. Inhibition of growth and aflatoxin production in *Aspergillus flavus* by *Garcinia indica* extract and its antioxidant activity. Food Microbiol., 20(4): 455-460.
7. Yamaguchi, F., M. Saito, T. Ariga, Y. Yoshimura and H. Nakazawa, 2000. Free Radical Scavenging Activity and Antiulcer Activity of Garcinol from *Garcinia indica* Fruit Rind. J. Agriculture and Food Chemis., 48(6): 2320 - 2325.
8. 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.
9. Murray, P.R. E.J. Baron, M.A Pfaller, F.C. Tenover and R.H. Tenover, 1999. Manual of Clinical Microbiology. 7th ed. Washington: ASM, pp: 1527-1539.
10. Freshney, R.I., 2003. Culture of Animal Cells: A Manual of Basic Technique. Wiley Liss.
11. Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J. Immunological Methods, 65: 55-63.

12. Negi, P.S. and G.K. Jayaprakasha, 2006. Control of Foodborne Pathogenic and Spoilage Bacteria by Garcinol and *Garcinia indica* extracts and their Antioxidant Activity. J. Food Sci., 69(3): FMS61- FMS65.
13. Archana, C. T. Yasmin, D. Bagchi and S. J. Stohs, 2003. The bactericidal effects of *Lactobacillus acidophilus*, garcinol and Protokin® compared to clarithromycin, on *Helicobacter pylori*. Molecular and Cellular Biochemis., 243(1-2): 29 -35.
14. Pasha, C., S. Sayeed, M.S. Ali and M.Z. Khan, 2009. Antisalmonella Activity of Selected Medicinal Plants. Turkish J. Biol., 33: 59 - 64.
15. Hong, J., S.J. Kwon, S. Sang, J. Ju, J. Zhou, C.T. Ho, M.T. Huang and C. S. Yang, 2007. Effects of garcinol and its derivatives on intestinal cell growth: Inhibitory effects and autoxidation-dependent growth-stimulatory effects. Free Radical Biology and Med., 42(8):1211 -1221.
16. Liao, C.H., C.T. Ho and J.K. Lin, 2005. Effects of garcinol on free radical generation and NO production in embryonic rat cortical neurons and astrocytes. Biochemical and Biophysical Research Communications, 329(4): 1306 -1314.