Available online at http://www.journalcra.com

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

International Journal of Current Research
Vol.2, Issue, 1, pp. 027-031, January, 2011

ISSN: 0975-833X

RESEARCH ARTICLE

ANTIMICROBIAL, ANTIOXIDANT AND ANTITUMOUR ACTIVITY OF THE *Garcinea mangostana*

[1]Maleeka Begum, S.F* and [2]Kavitha

Department of Biotechnology, Sri Krishna Arts and Science College, Kuniamuthur, Coimbatore - 641 008, India.

ARTICLE INFO

Article History:
Received 15th September, 2010
Received in revised form
28th September, 2010
Accepted 11th December, 2010
Published online 6th January, 2011

Key words:
Garcinea mangostana,
Methanol,
Ethanol,
Butanol,
Antimicrobial,
Antiproliferative,
Cytotoxic activity.

ABSTRACT

The exocarp of *Garcinea mangostana* was dissolved in various organic solvents namely Methanol, Ethanol and Butanol was tested for antimicrobial, antiproliferative and/or cytotoxic activity. Significant antimicrobial activity was found with *Garcinea mangostana* against *Pseudomonas aeroginosa* and *Staphylococcus aureus*. The extracts were also found to possess antifungal activity against *Candida albicans.* Out of different extracts tested, methanolic extract was found to be the most active against *Pseudomonas aeruginosa* followed by *S. aureus* and *P.mirablis.* Total Polyphenolic content ranged from 102.4to 138.6mg GAE/g of extract. Among the three solvent extracts, methanol extract showed maximum Polyphenol content at 6 hr extraction time followed by ethanol and butanol respectively. The extractive yield of sample was higher in methanol, compared to ethanol and butanol. The yield obtained decreased with decrease in polarity. The LD_{50} values of the plant extract against HEp2 cells was determined by MTT assay. The LD_{50} value of *Garcinea mangostana* was $\geq$ 120µg,92 µg and 85 µg for, methanol, ethanol, and butanol extracts respectively and the study revealed that the exocarp possessed free radical scavenging activity. Methanolic extract, Ethanolic extract, Butanolic extract were found to reduce the GSH level than the control. TBARS of stress induced HEp2 cells was reduced in the presence of Ethanolic extract and Butanolic extract. All extracts were found to reduce GPx activity.

INTRODUCTION

Traditional medicine has a long history of serving peoples all over the world. India is without doubt a herbal hub, hold large reservoir of plant genetic diversity, which can provide novel biomolecules.

***Corresponding author:** *javeed_94@yahoo.co.in*

Recently, considerable attention has been focused on identifying naturally occurring chemo preventive substances capable of inhibiting, retarding, or reversing the multi stage carcinogenesis. The majority of these naturally occurring phenolics retain antioxidative and

anti-inflammatory properties which appear to contribute to their chemo-preventive or chemo-protective activity. Natural products either as standardized plant extracts provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity (Parekh and Chand, 2006). The cost of production of synthetic drugs is also high and they produce adverse effect, when compared to plant derived drugs. Hence much attention has been paid recently, to the biologically active compounds derived from plants used in herbal medicine. The objective of our work is to evaluate antimicrobial, antiproliferative and/or cytotoxic activity of the exocarp of the fruit *Garcinea mangostana* , using MTT assay

MATERIAL AND METHODS

Plant Used: *Garcinea mangostana,* the selection of the plant species for the present study was mainly based on the traditional uses of these species for the treatment of various diseases including skin infections, ulcer, tumor, cancer etc. *Garcinia* is a plant genus of the family Clusiaceae. *Garcinia* species are evergreen trees and shrubs, dioecious and in several cases apomictic.Many species of *Garcinia* have fruit with edible arils. The best-known species is the Purple Mangosteen (*G. mangostana*), which is now cultivated throughout Southeast Asia and other tropical countries.

Extracts of the exocarp of certain species – typically Gambooge, but also Purple Mangosteen – are often contained in appetite suppressants such as Hydroxycut, Leptoprin or XanGo. But their effectiveness at normal consumption levels is unproven, while at least one case of severe acidosis caused by long-term consumption of such products has been documented.

Preparation of exocarp of fruit*:* The exocarp of fruit was shade dried. The dried exocarp of fruit were immersed in 100 ml of organic solvent namely Methanol, ethanol and Butanol. It was incubated at room temperature at different time intervals of 6, 12 and 24 hr. After incubation time, the suspension was filtered and the solvent was evaporated. The extract was concentrated to dryness and dissolved in 0.25% Dimethyl Sulphoxide (DMSO, Merck) to the concentration of 100 mg / ml.

Antimicrobial activity: Antimicrobial activity of the exocarp of fruit extracts was determined by Agar disc diffusion method (Bauer et al., 1966). Clinical bacterial isolates like *E.coli, Pseudomonas aeroginosa, Proteus mirabilis and Staphylococcus aureus* were used for the study. For antifungal activity, *Candida albicans* isolated from the clinical sample were used.

Determination of total polyphenol contents**:** Total polyphenol contents in the extracted powder were determined by the Folin-Ciocalteu colorimetric method (Ough and Amerine, 1988 and Kumazawa et al., 2002). Various solvent extracts of the sample (1 mg/ml) were mixed with 1 ml of the Folin-Ciocalteu reagent and 1 ml of 10% Na2CO3, and the absorbance was measured at 760 nm after 1 hr incubation at room temperature. The standard curve was calibrated covering a range of 50 to 250 mg/ml of gallic acid. Total polyphenol contents were expressed as mg GAE/ g of extract.

Antitumor assay: The antitumor assay was performed on human laryngeal epithelioma (HEp2) cells. The cells were grown in 24 well plate in Eagle's Minimum Essential Medium (Hi Media) supplemented with 10% fetal bovine serum(Hi Media) and 1% antibiotics (streptomycin, penicillin-G). The cell suspension (100 cells / ml) was seeded in every well and incubated at 37oC for 48 hr in 5% CO2 for the formation of monolayer. The monolayer of cells in 24 well plates was exposed to various dilutions of the Methanol, Ethanol and Butanol extracts. The cell viability was measured using MTT assay as described by Mosmann (1983) using MTT (5 mg / ml) and DMSO. Cell control was maintained throughout the experiment and the assay was performed in replicates.

MTT assay: Cytotoxicity was evaluated against HEp2 by MTT assay (Mosmann, 1983) and Lethal Dose 50 (LD_{50}) of all the extracts were determined by % cytotoxicity

$$\% \text{ Cytotoxicity} = \frac{\text{Mean OD of test (alcoholic extract)}}{\text{Mean OD of Control}} \times 100$$

Free radical scavenging activity: The free radical scavenging activity of the exocarp of fruit extracts was evaluated by DPPH method (Brant-Williams, 1995).

RESULTS AND DISCUSSION

The present investigation establishes the vital role of plants and plants products as still reservoir of many pharmaceuticals industry in treating infectious diseases. Antimicrobial activity of methanol, Ethanol and Butanol extracts are presented in Table 1. Significant antimicrobial activity was exhibited by Garcinea *mangostana* against Pseudomonas aeroginosa and Staphylococcus aureus. It may be due to the presence of secondary metabolites. Higher concentration of flavonoids, are found to exhibit antibacterial activity (Mohan et al., 2008). The extracts were also found to possess antifungal activity against *Candida albicans.* Out of different extracts tested, methanolic extract was found to be the most active against *Pseudomonas aeruginosa* followed by *S. aureus* and *P.mirablis*.

The yield of extracts from the exocarp of fruit of *Garcinea mangostana* using ethanol, methanol, and butanol as solvents were 12.4, 18.6 and 6.3 % respectively, on dry weight basis. Total Polyphenolic content ranged from 102.4to 138.6mg GAE/g of extract (Table 2). Among the three solvent extracts, methanol extract showed maximum Polyphenol content at 6 hr extraction time followed by ethanol and butanol respectively. Phenolic acids constitute a large group of naturally occuring organic compounds with a broad spectrum of pharmaceutical activities. It was found that they possess not only antioxidant but also antiviral and antibacterial properties. The antioxidant activity of phenolics is generally combined with hydroxyl groups on their molecules (Perchellet, 1989 and Dragsted, 1998). These natural antioxidants can exert considerable protection, in humans, against aging and cancer caused by free radicals, and can replace synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), which are suspected to have toxic and carcinogenic effect on humans (Beier and Oertli, 1983, Afek *et al.,* 1986, Zaat *et al.,* 1987 and Laks and Pruner, 1989).

The extractive yield of sample was higher in methanol, compared to ethanol and butanol. The yield obtained decreased with decreasing polarity. Since methanol has high polarity, it could dissolve both the polar and non polar compounds in it. The methanol extracts of sample inhibited nearly 100 % of HEp2 cells up to 1:4 dilution of the crude extract and started decreasing with increase in dilution. Arpornsuwan and Punjanon (2006) reported that the methanolic extract of *M. citrifolia* fruit was much more effective on breast cancer cells and neuroblastoma cells. Mayalen Zubia (2009) also reported that crude extracts of *B. bifurcata, C. tamariscifolia, Desmarestia ligulata, Dictyotadichotoma and H. siliquosa* exhibited strong cytotoxic activities against three different tumor cells lines (Daudi, Jurkat and K562).

The MTT assay, which evaluates the mitochondrial enzyme succinate dehygrogenase activity, helps in determining the cytotoxicity. The LD_{50} values of the extracts against HEp2 cells were determined by MTT assay. The LD_{50} value of *Garcinea mangostana* was found to be $\geq$ 120µg, 92 µg and 85 µg for, methanol, ethanol, and butanol extracts respectively and the study revealed that the exocarp possessed free radical scavenging activity.(DPPH method). The activity was found be higher in the methanol extract when compared against ethanol and Butanol extract

The improper balance between reactive oxygen intermediates and antioxidants defense results in 'oxidative stress' and it may result in damage to the cells. Cellular antioxidant enzymes such as catalase, SOD and GPx normally challenge oxidative stress. Antioxidant activity of all plant extracts were represented in Table 4. Methanolic extract, Ethanolic extract, Butanolic extract were found to reduce the GSH level than the control. TBARS of stress induced HEp2 cells was reduced

in the presence of Ethanolic extract and Butanolic extract. All extracts were found to reduce GPx activity. Among all Butanolic found to have greater activity. None of the extracts were found to reduce catalase activity on HEp2 cells. Butanolic alone were found to reduce the SOD activity. All plant extracts were found to contain polyphenols, Ethanolic extract, Butanolic extract might be containing polyphenols in higher concentrations, where as polyphenols are found to possess antioxidant activity (Yoshida *et al.,* 1999). They have their redox property which plays an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen and decomposing peroxides (Mohan *et al.,* 2008).

Table 1. Antimicrobial Activity of *Garcinea mangostana*

Extract	*E.coli*	*Pseudomonas aeroginosa*	*Proteus mirabilis*	*Staphylococcus aureus*	Candida albicans
Methanol	-	+	+	+	+
Ethanol	-	+	+	+	+
Butanol	-	+	+	+	+

Table 2. Total Polyphenol content and extractive yield of crude extracts of *Garcinea mangostana exocarp*

Extraction Solvent	% Yield (%)	Extraction time (hr)	Total Polyphenol (mg GAE /g extract)
Ethanol	12.4	6	102.4
		12	105.3
		24	124.6
Methanol	18.6	6	124.3
		12	131.3
		24	138.6
Butanol	6.3	6	112.6
		12	120.1
		24	132.4

Table 3.. Free radical scavenging activity of the extracts from *Garcinea mangostana*

Extract	DPPH (%)
Ethanol	16.78 ± 0.92
Methanol	43.5 ± 0.64
Butanol	13.42 ±0.09

Table 4. Antioxidant activity of extracts from ***Garcinea mangostana***

Extract	*TBARS Mmol/ml*	*GSH*	*Catalase*	*SOD*	GPx
Ethanol	0.15±0.06	00.26±0.01	0.31 ± 0.06	0.14±0.01	0.31 ±0.06
Methanol	0.03±0.01	0.02 ±0.04	0.46 ±.01	0.21±0.04	0.27±0.01
Butanol	0.04±0.08	0.05 ±0.08	0.35 ±0.01	0.05 ±0.03	0.26±0.01

The antimicrobial and antitumor activity of different fractioned extracts of *Garcinea angostana* may be due to presence of saponins, which are the glycosides (Varshney and Sharma, 1996) present in plant. These plant glycosides are the polar compounds, therefore the methanolic extracts is showing the maximum activity. Oxidation is essential in many living organisms, for the production of energy to fuel biological processes. However, the uncontrolled production of oxygen derived from free radicals which are involved in the onset of many diseases such as atherosclerosis, rheumatoid arthritis and cancer as well as in degenerative processes associated with aging (Halliwell *et al.,* 1988). This free radical damage is well protected by enzymes such as superoxide

dismutase and catalase or compounds such as ascorbic acid, tocopherols and glutathione (Mau *et al.,* 2002). When the mechanism of antioxidant protection becomes unbalanced, deterioration of physiological functions may occur resulting in diseases. However, the antioxidants present in human diet are of great interest as possible protective agents to help the human bodies to reduce oxidative damage (Mohan *et al.,* 2008).

Conclusion

The extractive value, total polyphenolic content and antitumor activity and antimicrobial activity was at its peak in methanolic extract indicating that most of the active components are extractable with methanol. Cytotoxic changes observed were cell aggregation, cell rounding and cell death. The overall results indicate the promising baseline information for the potential uses of the methanol extracts of exocarp as an antitumor agent. The methanolic extract of *Garcinea mangostana* was found to have antimicrobial activity. The extracts showed cytotoxic activity as evident by the tumor cell suppression potential of various alcoholic extract of the sample on HEp2 cells and antioxidant effect against HEp2 cells. In future, this plants could represent antioxidant agents, which provide prophylaxis against various diseases related to oxidative stress. To conclude the obtained result are highly encouraging and could form a good basis for further investigation in the potential discovery of new valuable bioactive compounds.

REFERENCES

Afek, U., Sztejnberg, A. and Carmely, S. 1986. 6,7–Dimethoxycoumarin, a citrus phytoalexin conferring resistance against Phytophthora gummosis. *Phytochemistry*, 25:1855–1856.

Arpornsuwan, and Punjanon, T. 2006. Tumor cell selective antiproliferative effect of the extract from *Morinda citrifolia* fruits. *Phototherapy Res.,*20:515-517.

Bauer, Aw, Kirby, WMM, Sherris, JC. and Truck, M. 1966. Antibiotic susceptibility testing by standardized single disc method. *Am J Clin Pathol.,* 45: 493-496.

Beier, R.C. and E.H. Oertli, 1983. Psoralen and other linear furocoumarins as phytoalexins in celery. *Phytochemistry*, 22:2595–2597.

Brant-Williams, W. Cuvelier, M. E. Berset, C. 1995 Use of a Free Radical Method to Evaluate Antioxidant Activity. *Lebensm. Wiss. Technol.,* 28: 25-30.

Dragsted, L.O. 1998. Natural antioxidants in chemoprevention. *Arch. Toxicol.,* 20 ,209–226

Halliwell, B., Gutteridge, JM. and Grootveld, M. 1988. Methods for the measurements of hydroxyl radicals in biomedical systems; deoxyribose degradation and aromatic hydroxylation. *Methods of Biochem. Anal.,* 33: 59-90.

Kumazawa, S., Taniguchi, M., Suzuki, Y., Shimura, M., Kwon, M.S. Kumazawa, M. Taniguchi, Y. Suzuki, M. Shimura, M.S Kwon, T. Nakayama, 2002. Antioxidant activity of polyphenols in carob pods. *J. Agric. Food Chem.,* 50: 373– 377.

Laks, P.E. and Pruner, M.S. 1989. Flavonoid biocides: structure/activity relations of flavonoid phytoalexin analogues. *Phytochemistry*, 28:87–91.

Mau, JL, Lin, HC. and Song, S.F. 2002 Antioxidant properties of several specialty mushrooms. *Food Research International*., 35(6): 519-526.

Mayalen, Z., Marie, S.F. and Veronique, K, 2009. Antioxidant and antitumoural activities Bauer Aw, Kirby WMM, Sherris JC, Truck M. Antibiotic susceptibility testing by standardized single disc method. *Am J Clin Pathol.,* 1966; 45: 493-496.

Mohan, S., Abdul, AB., Wahab, SIA., Zubalri, ASL., Ethassan, MM. and Yousif, M. 2008. Antibacterial and antioxidant activity of Typhonium flagelliforiume (Lodd) Blumer Tuber. *American J Biochem and Biotechnol.,* 4(4): 402-407

Mosmann, T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods.,* 65: 55-63.

Ough, C.S. and Amerine, M.A. 1988. Methods for analysis of musts and wines. Wiley & Sons, New York, USA, 196-221.

Parekh, J. and Chand, SV. 2006. Invitro antimicrobial activity and phytochemical analysis of some Indian Medicinal Plants. *Turk J Biol.*, 2006; 31: 53-58.

Perchellet, J.P. and Perchellet, E.M., 1989. Antioxidants and multistage carcinogenesis in mouse skin. *Free Radic. Biol. Med.,* 7:377–408.

Yoshida H, Ishikawa T, Hosoai H, Suzukawa M, Ayaori M, Hisada T, Sawada S, Yonemura A,Higashi K, Ito T, Nakajima K, Yamashita T, et al., 1999. Inhibitory effect of tea flavonoids on the ability of cells to oxidize low density lipoprotein. *Biochem. Pharmacol.,* 58:1695–1703

Zaat, S.A.J., A.A.N. van Brussel, T. Tak, et al., 1987. Flavonoids induce Rhizobium leguminosarum to produce nod DABC gene-related factors that cause thick, short roots and root hair responses on common vetch. *Journal of Bacteriology,* 169:3388–3391.