Journal of Pharmaceutical and Scientific Innovation
www.jpsionline.com
Research Article

IN VITRO ANTIOXIDANT PROPERTIES OF *GARCINIA INDICA* LINN ALCOHOLIC FRUITS EXTRACTS

Rajesh Kumar Rawri[1], K. Bharathi[2], K.N. Jayaveera[3], SMB Asdaq[4]*
[1]Department of Pharmaceutical Chemistry, Krupanidhi College of Pharmacy, Bangalore, India
[2]Institute of Pharmaceutical Technology, Sri Padmavati Mahila Visvavidyalayam, Tirupati, India
[3]Department of Chemistry, Jawaharlal Nehru Technical University, Ananthpur, India
[4]Department of Pharmacology, Al-Maarefa College of Science and Technology, Riyadh, KSA
*Corresponding Author Email: basheer_1@rediffmail.com/sasdaq@gmail.com
DOI: 10.7897/2277-4572.02328
Published by Moksha Publishing House. Website www.mokshaph.com

Received on: 15/04/13 Revised on: 20/05/13 Accepted on: 01/06/13

ABSTRACT
The current research was an attempt to reiterate the antioxidant potential of alcoholic fruit extract of *Garcinia indica* invitro. The fruits of *Garcinia indica* were collected and shade dried at room temperature. The powdered mass was defatted with petroleum ether then filtered and residue was extracted with ethanol (95%) into soxhlet apparatus. The suspension of extract was prepared freshly in normal saline with the help of 0.5% w/v carboxymethylcellulose (CMC). Varying concentration of extract (10, 25, 50 and 100 µg/ml) was tested for invitro antioxidant properties. Antioxidant activity was determined by DPPH assay, reducing power ability, hydrogen peroxide scavenging assay and hydroxyl radical (OH^-) scavenging activity. The ethanolic fruit extract of *Garcinia indica* demonstrated antioxidant potential dose dependently with best activity at 100 µg/ml. In conclusion, traditionally claimed medicinal benefits of *Garcinia indica* might be due to its potent anti-oxidant nature. However, further studies to be carried out on animal models using their biological tissues before exploiting for its clinical benefits.
Key words: *Garcinia indica*, antioxidant, In-vitro, DPPH

INTRODUCTION

Free radical induced damages are combated by the help of antioxidants. Oxidative free radicals are chemical species, which contains one or more unpaired electrons due to which they are highly unstable and cause damage to macromolecules by extracting electrons from them in order to attain stability. Reactive oxygen species (ROS) formed in vivo, such as superoxide anion, hydroxyl radical and hydrogen peroxide, are highly reactive and potentially damaging transient chemical species. These are continuously produced in the human body, as they are essential for energy supply, detoxification, chemical signaling and immune function. ROS are regulated by endogenous superoxide dismutase, glutathione peroxidase and catalase but due to over-production of reactive species, induced by exposure to external oxidant substances or a failure in the defense mechanisms, damage to cell structures, DNA, lipids and proteins occur[1] which increases risk for several disease processes[2].

The alleviation of chronic diseases can be achieved by either enhancing the body's natural antioxidant defenses or by supplementing with proven dietary antioxidants[3]. This is one of the underlying reasons for increased discoveries and synthesis of novel antioxidants. However, synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) commonly used in processed foods have side effects and are carcinogenic[4,5]. Hence use of natural antioxidants present in food and other biological materials has attracted considerable interest due to their presumed safety, nutritional and therapeutic value[6].

Natural antioxidants mainly come from plants in the form of phenolic compounds (flavonoids, phenolic acids and alcohols, stilbenes, tocopherols, tocotrienols) ascorbic acid and carotenoids. The quest for natural antioxidants for dietary, cosmetic and pharmaceutical uses has become a major industrial and scientific research challenge over the few decades. Efforts to gain extensive knowledge regarding the power of antioxidants from plants and to tap their potential are therefore on the increase.

Garcinia indica Linn belonging to family Clusiaceae commonly recognized as 'Kokum' is found in Maharashtra and particular in Konkan, Goa and the western region of India. The fruits of *Garcinia indica* Linn 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, antiulcer, liver tonic, to allay thirst and as a cardiotonic. Garcinol a polyisoprenylated benzophenones, has antioxidative, chelating, free radical scavenging, antiglycation, anticancer, antiinflammatory, and antiulcer activities[7-10]. One of the ingredients of kokum, hydroxycitric acid (HCA), has been patented for use as a hypocholesterolaemic agent [11-13]. The antioxidant potential of *Garcinia indica* could be responsible for its potential benefits in ameliorating number of diseases. Hence present study was designed to explore antioxidant properties of *Garcinia indica* alcoholic fruit extract invitro.

MATERIALS AND METHODS

Extraction procedures

The *Garcinia indica* Linn fruits were collected from the Devrukh region in Ratnagiri district, Tamil Nadu, India. The collected plant materials were recognized and authenticated by Dr. Shalini Kapoor Mehta, Department of Pharmacognosy, Krupanidhi College of Pharmacy, Bangalore. The fruits rinds were cut into pieces and shade dried at room temperature. The dried fruits were subjected to size reduction to a coarse powder by using mixer grinder. This powder was defatted with petroleum ether then filtered. The residue was allowed to dry at room temperature. This residue was extracted with ethanol (95%) into soxhlet

apparatus. The extract was dried at room temperature till semisolid mass was obtained. The sweet scented, chocolate colored semisolid residue formed after the complete dryness was kept in an airtight and waterproof container, which is stored in the refrigerator[16]. The suspension of ethanolic extract of *Garcinia indica* Linn fruit rind was prepared in 0.5% w/v carboxymethylcellulose (CMC) in normal saline.

Antioxidant activity (DPPH free radical scavenging activity) of extract

The antioxidant activity of the *Garcinia indica* Linn 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[15]. 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) was obtained from Sigma Aldrich Co., St. Louis, USA. 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 below[16]:

Percent (%) inhibition of DPPH activity = (A-B/A)100

Where A = optical density of the blank and B = optical density of the sample.

Reducing power ability

The extract of *Garcinia indica* Linn fruits was determined for their reducing power modifying the method of Oyaizu (1986)[17]. Reaction mixtures were prepared by adding 2.5 ml of phosphate buffer (0.2 M, pH 6.6), 2.5 ml Potassium Ferricyanide (1%) and varying concentrations of extracts (40-200 mg). After, the reaction mixtures were incubated at 50°C in water bath for 30 min, allowed to cool at room temperature (28°C), and 2.5 ml of 10% TCA (Trichloroacetic acid) were added to each reaction mixture, and then centrifuged at 2000 rpm for 10 min. The supernatant (2.5 ml) was separated in the test tube and added with 2.5 ml of distilled water and 0.5 ml FeCl3 (1.0%), and allowed to react for 10 min at room temperature and the absorbance was measured at 700 nm. Ascorbic acid solution (ASA) was used as standard.

Hydrogen peroxide scavenging assay

Hydrogen peroxide scavenging strengths of extracts of *Garcinia indica* Linn fruits was determined by the method described by Ruch *et al.* (1989) [18]. A solution of H_2O_2 (10 mM) was prepared in phosphate buffer (pH 7.4). Reaction mixtures contained 10mM of H_2O_2 and different concentrations of test samples, and absorbance values were measured at 0 min and after 60 min using wavelength of 240 nm. Ascorbic acid was used as the standard.

Hydroxyl radical (OH^-) scavenging activity[19]

Hydroxyl radical were generated by phenyl hydrazine in solution which was measured by appearance of pink colour (TBA) – MDA chromogen (due to OH- mediated decomposition of 2-Deoxyribose) (Halliwell et al., 1987). The reaction was performed in incubation mixture containing 50 mm phosphate buffer (PH 7.4), 1 mm deoxyribose, 0.2 mm phenylhydrazine hydrochloride and *Garcinia indica* Linn fruits extract (10, 25, 50 and 100 µg/ml) or (10 and 20) mm mannitol. The final reaction value was made up to 2ml. incubation was terminated after 1hr or 4 hrs with 2.8% trichloro acetic acid (1ml). Thiobarbituric acid (1%w/v) was then added to the reaction mixture followed by fitting for 10 min on a boiling water bath. The tubes were than cooled briefly and absorbance was taken at 532 nm. A decreased in absorbance indicated hydroxyl radical scavenging activity.

Table 1: In vitro antioxidant activity of *Garcinia indica*

Drug	Dose µg/ml	IC50 (µg/ml) DPPH (Mean ± SD)	Reducing power * (absorbance at 700 nm) (Mean ± SD)	H_2O_2 scavenging assay
Vitamin C	100	5.94 ± 0.11	1.98 ± 0.23	24.32 ± 1.46
	50	9.90 ± 0.12	1.04 ± 0.17	14.54 ± 1.33
Garcinia indica	100	4.09 ± 0.18	0.97 ± 0.38	22.36 ± 1.99
	50	6.65 ± 0.14	0.81 ± 0.43	19.33 ± 4.23
	25	9.99 ± 0.23	0.69 ± 0.34	16.39 ± 3.23
	10	12.5 ± 0.22	0.51 ± 0.12	12.37 ± 2.18

*Higher absorbance indicates higher reducing power

Table 2: Effect of *Garcinia indica* extract on Hydroxyl radical scavenging activity

Drug	Dose µg/ml	Hydroxyl radical scavenging activity	% of Inhibition
Control	-	0.390 + 0.0035	
Quercetin	10	0.114 + 0.029*	70.77
	25	0.131 + 0.026*	67.10
Vitamin C	50	0.078 + 0.026*	79.8
	100	0.068 + 0.058*	81.6
Garcinia indica	10	0.191 + 0.022*	51.03
	25	0.183 + 0.005*	53.08
	50	0.151 + 0.022*	61.29
	100	0.141 + 0.011*	63.85

RESULTS AND DISCUSSION

This study was carried out to determine the invitro antioxidant potential of alcoholic fruit extract of *Garcinia indica* Linn fruits. Antioxidant compounds in food and diet play an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and

phytoestrogens have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. Some compounds, such as gallates, have strong antioxidant activity, while others, such as the mono-phenols are weak antioxidants. The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases.

Garcinia indica (dried rind known as 'kokam') is an Indian spice used as traditional home remedy in case of flatulence, heat strokes and infections[20]. One of the ingredients of kokam, hydroxycitric acid (HCA), has been patented for use as a hypocholesterolaemic agent. HCA is a potential anti-obesity agent[21]. It suppresses fatty acid synthesis, lipogenesis, food intake and induces weight loss[22]. *Garcinia indica*, has traditionally been used in tropical regions and appreciated for centuries; however its biological properties are only beginning to be elucidated. Antioxidant potential of the fruit extract is attributed for its several therapeutic benefits.

A freshly prepared DPPH solution exhibited a deep 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 1; results are expressed as IC50 values (concentration of sample required to scavenge 50% of free radicals) of *Garcinia indica* and ascorbic acid are indicated Table 1. Alcoholic 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[23]. Higher DPPH scavenging potential of *Garcinia indica* might be due to the higher reducing potential.

The reducing power of ethanolic extract of *Garcinia indica* was found to be correlated with increasing absorbance (at 700 nm) as compared with ASA, a known antioxidant (Table 1). Similar observations were also reported earlier (Duh, 1998)[24]. The presence of reductones are responsible for reducing capacity, which involved in prevention of chain initiation, binding of metal ions, decomposition of peroxides and radical scavenging[25].

The scavenging ability of species of ethanolic extract of *Garcinia indica* with H_2O_2 is compared with the ascorbic acid and is depicted in Table 1. Though H_2O_2 itself not very reactive, it generates highly reactive molecule such as OH^- by reacting with metals (Fe^{2+} or Cu^{2+}), and superoxide anions in the Haber-Weiss reaction. Therefore, removing of H_2O_2 is very essential from the cell or food systems. A significant dose dependent H_2O_2 scavenging potential of ethanolic extract of *Garcinia indica* was observed during the present study. Electronic donors might accelerate the conversion of H_2O_2 to H_2O [26], which could possible to scavenge H_2O_2 in the ethanolic extract of *Garcinia indica*.

The hydroxyl radical (generated secondarily by the reaction of superoxide and hydrogen peroxide) actually brings about depolymerisation of hyaluromic acid as a OH^- scavenger can return the viscosity of hyaluromic acid solution. Hydroxyl radical (OH-) is closely associated with inflammatory disorder like arthritis where a progressive loss of hyaluromic acid in joint important feature of disease. In our study *Garcinia indica* extracts in concentration 10, 25, 50 & 100 µg/ml produces a dose dependent scavenging of OH- radical. The activity of extract was most effective in the first hour of the study.

In conclusion, ethanolic extract of *Garcinia indica* showed dose dependent antioxidant properties in different invitro evaluations. These revelations are significantly noticeable as *Garcinia indica* induced amelioration of numerous metabolic disorders and functional defects might be attributed to its antioxidant potential. However, scientific confirmation of traditional claims is necessary for exploiting the therapeutic benefits of this wonder grain.

REFERENCES

1. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radical metals and antioxidants in oxidative stress-induced cancer. Chem Bio Inter 2006;160:1–40. http://dx.doi.org/10.1016/j.cbi.2005.12.009 PMid:16430879
2. Aruoma OI. Free radicals, oxidative stress and antioxidants in human health and disease. J Am Oil Chem Soc 2008;75:199–212. http://dx.doi.org/10.1007/s11746-998-0032-9
3. Stanner SA, Hughes J, Kelly CN, Buttriss JA. Review of the epidemiological evidence for the 'antioxidant hypothesis'. Pub H Nut 2004;7:407–422.
4. Branen AL. Toxicology and biochemistry of butylated hydroxyl anisole and butylated hydroxytoluene. J Am Oil Chem Soc 1975;52:59–63. http://dx.doi.org/10.1007/BF02901825 PMid:805808
5. Ito N, Fukushima S, Hassegawa A, Shibata M, Ogiso T. Carcinogenicity of butylated hydroxyanisole in F344 rats. J Nat Can Ins 1983;70:343–347.
6. Ajila CM, Naidu KA, Bhat UJS, Rao P. Bioactive compounds and antioxidant potential of mango peel extract. Food Chem 2007;105:982–988. http://dx.doi.org/10.1016/j.foodchem.2007.04.052
7. 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. http://dx.doi.org/10.1016/j.bbrc.2005.02.110 PMid:15766569
8. 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. http://dx.doi.org/10.1016/S0040-4020(02)01411-4
9. 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.a
10. 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. B http://dx.doi.org/10.1021/jf990845y PMid:10888544
11. Heymsfield SB, Allison DB, Vasselli JR, Pietrobelli A. Garcinia cambogia (Hydroxycitric acid) as a potential antiobesity agent. J Am Med Asso 1998;280: 1596-1608. http://dx.doi.org/10.1001/jama.280.18.1596
12. Mattes RD, Bormann L. Effects of (-)-hydroxycitric acid on appetitive variables. Physiol Behav 2000;71:87-94 http://dx.doi.org/10.1016/S0031-9384(00)00321-8
13. Sakariah KK, Jena BS, Jayaprakasha GK, Singh RP. Chemistry and biochemistry of (-)-Hydroxycitric acid from Garcinia. J Agri Food Chem 2002;50: 10-22. http://dx.doi.org/10.1021/jf010753k PMid:11754536
14. Deore AB, Sapakal VD, Dashputre NL, Naikwade NS. Antiulcer activity of Garcinia indica linn fruit rinds. J App Pharm Sci 2011; 01 (05): 151-154.
15. Braca A, Sortino C, Politi M. Anti-oxidant activity of flavonoids from Licania licaniaeflora. J Ethnopharmacol 2002;79:379-381. http://dx.doi.org/10.1016/S0378-8741(01)00413-5
16. Bors W, Saran M, Elstner EF. Screening for plant anti-oxidants. In: Linskens HF, Jackson JF. editors. Modern Methods of Plant Analysis-

Plant Toxin Analysis-New Series, Vol 13. Springer, Berlin; 1992: p. 277-295. http://dx.doi.org/10.1007/978-3-662-02783-7_11
17. Oyaizu M. Studies on product of browning reaction prepared from glucose amine. Jap J Nut 1986;44:307-315. http://dx.doi.org/10.5264/eiyogakuzashi.44.307
18. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis 1989;10:1003-1008. http:/dx.doi.org/10.1093/carcin/10.6.1003 PMid:2470525
19. Halliwell B, Gutteridge J, Aruoma O. The deoxyribose method: a simple'test tube'assay for determination of rate constants for reaction of hydroxyl radicals. Anal Biochem 1987;165:215-219. http://dx.doi.org/10.1016/0003-2697(87)90222-3
20. Kirtikar KR, Basu BD. Indian Medicinal Plants, Vol. I, (eds Blatter, E. et al.), Allahabad, India, 1984.
21. Drury H. The Useful Plants of India, Allied Book Centre, Dehradun, 1991.
22. Jena BS, Jayaprakasha GK, Singh RP, Sakariah KK. Chemistry and biochemistry of (–)-hydroxycitric acid from Garcinia. J Agric Food Chem2002;50:10–22.http://dx.doi.org/10.1021/jf010753k PMid:11754536
23. Wang H, Gao XD, Zhou GC, Cai L, Yao WB. In vitro and in vivo antioxidant activity of aqueous extract from Choerospondias axillaris fruit. Food Chem 2008;106: 888-895. http://dx.doi.org/10.1016/j.foodchem.2007.05.068
24. Duh PD. Antioxidant activity of burdock (Arctium lappa Linne): its scavenging effect on free radical and active oxygen. J Am Oil Chem Soc 1998;75:455-461. http://dx.doi.org/10.1007/s11746-998-0248-8
25. Yildirim A, Mavi A. Determination of antioxidant and antimicrobial activities Rumaxscrispus L. extracts. J. Arric. Food Chem 2001;4: 4083-4089. http://dx.doi.org/10.1021/jf0103572
26. Ruch RJ, Chung SU, Klaunig JE. Spin trapping of superoxide and hydroxyl radicals. Meth Enzy 1984;105:198-209. http://dx.doi.org/10.1016/S0076-6879(84)05026-6

Source of support: Nil, Conflict of interest: None Declared

QUICK RESPONSE CODE	ISSN (Online) : 2277 –4572
	Website http://www.jpsionline.com

How to cite this article:
Rajesh Kumar Rawri, K. Bharathi, K.N. Jayaveera, SMB Asdaq. In vitro antioxidant properties of *Garcinia indica* Linn alcoholic fruits extracts. J Pharm Sci Innov. 2013; 2(3):9-12.