International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.4, No.1, pp 334-342, Jan-Mar 2012

In vitro antioxidant activity of *Garcinia pedunculata*, an indigenous fruit of North Eastern (NE) region of India.

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Abstract: *Garcinia pedunculata* (GP, Family: Guttiferae) is an important predominant fruits of North Eastern (NE) region of India, having numbers of medicinal properties and used against various diseases in folk medicine. Present study was undertaken to investigate the antioxidant properties of the dried pulp of *Garcinia pedunculata* (DPGP) as few scientific studies regarding its antioxidant activity has been reported. Antioxidant activity of the methanolic extract of DPGP with reference to standard antioxidants have been investigated employing various well-established *in vitro* methods *i.e.* 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, H_2O_2 radical scavenging activity, reducing power and *in vitro* lipid peroxidation. Chemical composition analysis of DPGP revealed that it is one of the rich sources of ascorbic acid, phenolics and flavonoid compounds. FTIR analysis of DPGP revealed presence of some functional groups like carboxylic acids, amines, amides, lactone, phenols and carbohydrate which demonstrates that DPGP may be rich sources of alkaloids, polyphenolic compounds, quinines, amino acids etc. DPGP extract showed potential antioxidant activity against DPPH and H_2O_2 free radicals. Besides DPGP extract inhibited the lipid peroxidation induced by Fe^{2+}-ascorbate in rat liver homogenate in a dose dependent manner. Total reducing-power assay revealed the potential reducing power of DPGP. Phenolics and ascorbic acid might contribute to its free radical scavenging potential. Trace elements like iron, copper, potassium and zinc were detected and it was found that DPGP may be a good source of iron. So DPGP might be used as an economical source of natural antioxidants.
Keywords: Antioxidant, *in vitro*, Fruits, North East India.

Introduction

About 80 % of the world population relies on traditional plant medicines as also in India by various rural and tribal communities through Indian Systems of Medicine and other undocumented traditional medicine. The growing interest in the substitution of synthetic food antioxidants by natural antioxidants and in the health implications of antioxidants as nutraceuticals has fostered research on fruit and vegetable sources and the screening of raw materials for identifying antioxidants. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant.

Garcinia is a large genus of polygamous trees or shrubs distributed in tropical Asia, Africa, and Polynesia. *Garcinia pedunculata* (GP) is a large evergreen tree with fluted trunk with short spreading branches, leaves lanceolate, midrib stout. Fruit is globose, 8-12 cm in diameter with fleshy aril. The mature fruit is eaten cooked or raw[1]. It is mostly available in North Eastern (NE) region of India and locally known as Borthekera in Assam, a state of NE region of India. It has got many medicinal properties. GP is used as an antiscorbutic, astringent, cooling, cardiotonic, emollient. Cold water infusion of dry pericarp is taken by folk people of NE region of India as antidiarrhoeic, antidysentric, in dyspepsia and in flatulence[2]. Dry fruits (pericarp) contain the benzophenones, pedunculol, garcinol and cambogin and the heartwood gives benzophenone and xanthone[3]. Fruit rinds of GP are rich in (-)-hydroxycitric acid (HCA), which is a proven natural antiobese agent. The hexane and chloroform extracts showed antioxidant activity studied through β-carotene-linoleate model system and 1, 1-diphenyl-2-picrylhadrazyl (DPPH)[4]. As there is no study available regarding the antioxidant activity of the methanolic extract of DPGP and metal contents, therefore the present study was designed to determine the *in vitro* antioxidant properties and phytochemical analysis of DPGP using some standard methods and discussed in this article.

Materials and Methods

Chemicals

1, 1-Diphynyl-2-picrylhydrazyl (DPPH), Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid), thiobarbituric acid (TBA), cathechin, quercetin were obtained from Sigma Chemicals (St Louis MO, USA). All other chemicals used in the study were obtained from Merck, India.

Plant materials

Fresh fruits are sliced and dried in sunlight. Dried pulps were used for further experiment.

Sample preparation

DPGP was extracted with methanol. The extracts were filtered and the solvent was removed by a rotavapor apparatus (Bûchi, Switzerland) to recover soluble components of the pulp.

DPPH radical scavenging activity

The free radical scavenging activity of DPGP fruit was determined by DPPH method[5] at different concentrations *i.e.* 5:25, 5:50, 5:100 and 5:200 (pulp and methanol ratio). The solution of DPPH in methanol (6×10^{-5}M) was prepared just before UV measurements. Samples were added to DPPH solution in 1:1 ratio followed by vortexing. The reaction was allowed to take place in the dark at room temperature under nitrogen atmosphere. The absorbance at 515 nm was measured at different time intervals. A decreasing intensity of the purple colour was taken as increasing scavenging activity. Trolox served as a standard and results of extracts were also expressed relative to trolox interms of trolox equivalent (TE). The inhibition % of radical scavenging activity was calculated using the following equation.

Inhibition (%) = $[(A_0\text{-}A)/A_0] \times 100$

where A_0 is the absorbance of DPPH in the absence of the sample and A is the absorbance of DPPH in the presence of sample.

Hydrogen Peroxide radical scavenging activity

The radical scavenging activity of DPGP against hydrogen peroxide was determined using the method of Ruch *et al.*[6]. A 43mM solution of

hydrogen peroxide (H_2O_2) was prepared in 0.1 M phosphate buffer solution (pH 7.4). Samples (1mL) were mixed with 43 mM hydrogen peroxide solution (0.6 mL). After 10 min, the reaction mixture absorbance was determined at 230 nm. The phosphate buffer without hydrogen peroxide was used as a blank. Ascorbic acid was used as a reference compound. The percentage inhibition activity was calculated as:

Percentage scavenged (H_2O_2)
= $[(A_0 - A_1) / A_0] \times 100$
Where A_0 is absorbance of the control and A_1 is absorbance of juice or standards.

Reducing power
Reducing power of samples was determined according to the method of Oyaizu[7] (1988); 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide were added to 1 mL sample solution and mixed gently. The mixtures were incubated at 50°C in a water bath for 20 min. Reaction was stopped by adding 2.5 mL of 10% trichloroacetic acid (TCA) and the mixtures were centrifuged at 4000 rpm for 10 min. From the top layer, 2.5 mL was transferred into tubes containing 2.5 mL distilled water and 0.5 mL of 0.1% ferric chloride ($FeCl_3.6H_2O$). The resulting solutions were mixed well and, after 5 min, the absorbance was measured at 700 nm against blanks.

***In vito* lipid peroxidation**
Lipid peroxidation induced by Fe^{2+}-ascorbate system in rat liver homogenate by the method of Bishayee and Balasubramaniyam (1971)[8] was estimated as thiobarbituric acid reacting substances (TBARS) by the method of Okhawa *et al.* (1979)[9]. The reaction mixture contained rat liver homogenate 0.25 mL (10% w/v in 0.05 M phosphate buffer, pH-7.4), 0.1 mL Tris-HCl buffer (150 mM, pH 7.2), 0.05 mL ascorbic acid (0.1 mM), 0.05 mL $FeSO4.7H_2O$ (4 mM) and 0.05 mL of GP extract. The mixture was incubated at 37°C for 1 h and then 1.5 mL 2-thiobarbituric acid (TBA, 0.8 % w/v), 1.5 mL acetic acid (20 %) and 0.2 mL sodium dodecyl sulfate (SDS, 8.1 % w/v) were added to the reaction mixture. The mixture was made up to 4.0 mL with distilled water and heated at 95°C for 60 min. After cooling with tap water, 1.0 mL distilled water and 5.0 mL of a mixture of n-butanol and pyridine (15:1, v/v) were added. The mixture was shaken vigorously and centrifuged at 5000 g for 10 min. After centrifugation, the optical density of the butanol layer was measured at 532 nm in a spectrophotometer.

Phytochemical analysis

Determination of Vit C content
Vit C content was determined by the iodine titration method[10].

Determination of total polyphenol content
Total phenolic content of methanolic extract of GP (5:100 mL concentration) was determined by Folin and Ciocalteu method with slight modifications[11]. Methanolic extract (0.5 mL) was mixed with Folin-Ciocalteu reagent (2.5 mL, diluted 10 times) and incubated for 2 min at room temperature followed by addition of sodium carbonate solution (2 mL, 7.5% w/v). The mixture was then allowed to stand for 30 min at room temperature and absorbance was measured at 765 nm. The amount of total polyphenol was calculated as a cathechin equivalent from the calibration curve of cathechin standard solutions and expressed as mg cathechin/100 gm dried pulp of the sample.

Determination of total flavonoid content
The total flavonoids were estimated according to the Dowd method as adapted by Arvouet-Grand *et al*. (1994)[12]. A diluted 2 mL of DPGP extract was mixed with 2 mL of aluminium trichloride ($AlCl_3$) in methanol (2 %). The absorbance was read at 415 nm after 10 min. Quercetin was used as reference compound and the results were expressed as mg of quercetin equivalents (QE)/100 gm dried pulp of GP.

FTIR analysis of DPGP
The IR spectral analysis was carried out using FT-IR spectrophotomer (Perkin Elmer).

Data analysis
Results were expressed as mean ± SE. All measurements were done in triplicate.

Table: 1 DPPH radical scavenging activity of methanolic extract of dried pulp of *Garcinia pedunculata.*

Quantity of fruit pulp(g/ml solvent)	Quantity of extract (gm)	% Activity	TE (Trolox Equivalent mM)
5 gm/25 ml	1.5670	91.81	0.0483
5 gm/50 ml	2.0860	92.30	0.0485
5gm/100 ml	2.6625	91.89	0.0483
5 gm/200 ml	2.6712	91.97	0.0483

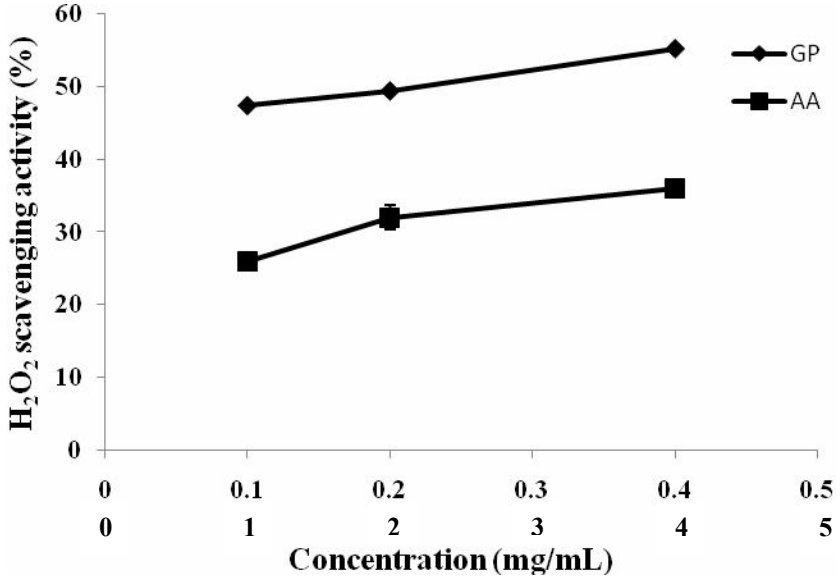

Fig: 1- H_2O_2 scavenging activity of methanolic extract of dried pulp of *Garcinia pedunculata* and ascorbic acid.

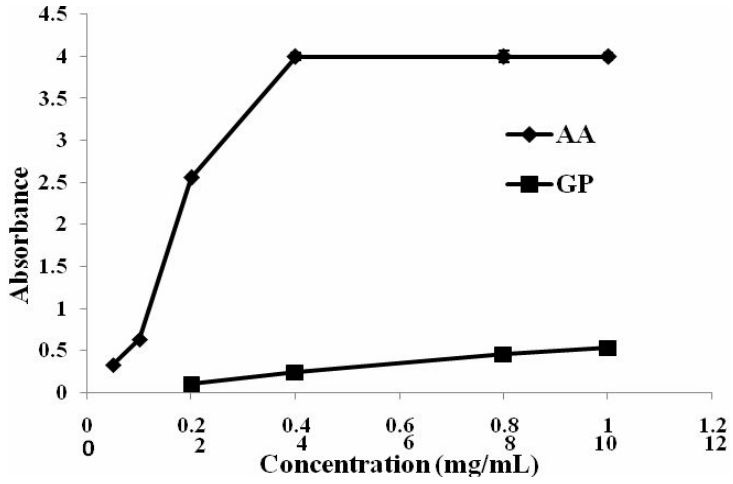

Fig:2-Reducing power of methanolic extract of dried pulp *Garcinia pedunculata* and ascorbic acid.

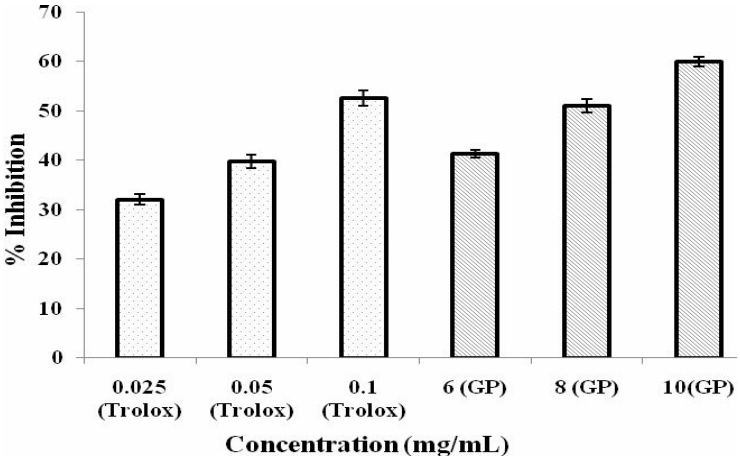

Fig: 3 -*In vitro* lipid peroxidation of methanolic extract of dried pulp of *Garcinia pedunculata* and trolox.

Table: 2 Total phenolic, flavonoid and ascorbic acid content of dried pulp of *Garcinia pedunculata.*

Parameters assayed	GP(mg/100 gm dried pulp)
Total polyphenol content	700.1 ± 10.74
Total flavonoid content	71.4 ± 0.84
Ascorbic acid content	80 ± 14.2
Potassium	127.72±0.4
Sodium	3.7±0.1
Iron	7 ±0.02
Copper	0.6±0.014
Zinc	13.2±0.3

Results

Free radical scavenging activity

Five-gram DPGP (as used traditionally) was dissolved in different amount of methanol *i.e.* 25, 50, 100, 200 mL. The maximum activity was found 92% when 5gm dried pulp was extracted with 50 mL of methanol and the TE was 0.0485 mM (Table: 1). The IC_{50} value (the concentration required to inhibit a radical formation by 50%) of methanolic extract of DPGP in DPPH assay is 5 mg/mL.

Hydrogen Peroxide radical scavenging activity

The hydrogen peroxide scavenging activity of methanolic extract of DPGP was given in Fig: 1. DPGP demonstrated hydrongen peroxide scavenging activity in a dose dependent manner with IC_{50} of 2 mg /mL while ascorbic acid (standard) showed 700 ug/mL.

Reducing power

Reducing power of DPGP and ascorbic acid were summarized in Fig: 2. The reducing power of DPGP increased with increasing concentration. Reducing power of ascorbic acid is much higher than DPGP.

***In vitro* lipid peroxidation**

DPGP inhibited the *in vitro* lipid peroxidation with an IC_{50} of 7.84 mg/mL. Trolox showed the IC_{50} value 89 ug/mL. (Fig: 3)

Phytochemical analysis

Total phenolic content was found to be 700.1 ± 10.74 mg cathechin /100 gm DPGP and flavonoid content was 71.4 ± 0.84 mg quercetin /100 gm DPGP. Vitamin C (ascorbic acid) content was found quite high *i.e.* 80 ± 14.2 mg/100 gm DPGP (Table: 2). From table: 2 it was quite noticeable that Fe, Zn and K content were high in DPGP. FTIR of DPGP was shown in Fig: 4. The broad band around 3401 cm^{-1} that is characteristic for hydroxyl group indicates the presence of phenolic compounds. The observed absorption band at1633 cm^{-1} indicates the presence of amines. Absorption at 2930 cm^{-1} is characteristics of C-H stretching in aldehydes. Strong band in the region (1350-1000 cm^{-1}) confirms the presence of esters, aldehydes, ketones, lactones, carboxylic acids, amides, alcohols. Absorption at 1105 cm^{-1} is the most characteristics of ethers *i.e.* C-O stretching in C-O-C group. Strong band at 1195 cm^{-1} and another at 1401 cm^{-1} gives the presence of phenols. Appearance of strong band at 1672 cm^{-1} is due to C=O stretching which indicates the presence of quinines and amides. Absorption at 745 cm^{-1} may be due to carbohydrate.

Discussion

Phenolics are an important class of secondary plant metabolites possessing an impressive array of pharmacological activity with excellent radical scavenging ability. The antioxidant activities of phenolics are due to their redox properties. The phenol moiety (hydroxyl group on aromatic ring) helps them to work as reducing agents, hydrogen donors and singlet oxygen quenchers[13]. For total polyphenol estimation several methods including folin–Ciocalteu, permanganate titration, colorimetry with iron salts and ultraviolet absorbance have been used. But folin–Ciocalteu method has been mostly used by many researchers. In this method, phenols form a bluecolored phosphomolybdic–phosphotungstic–phenol complex in alkaline solution[14].

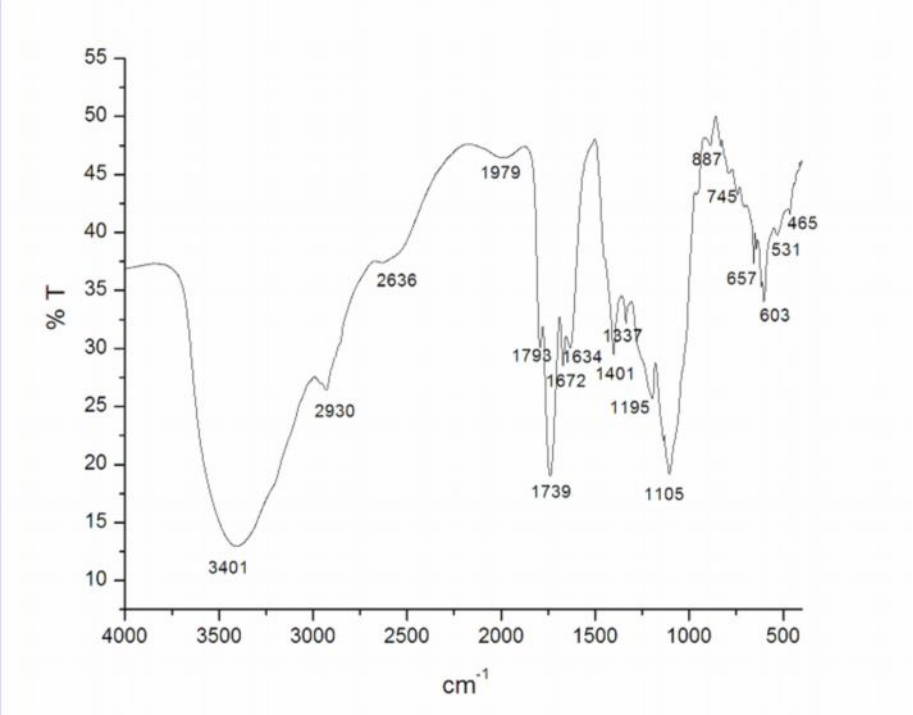

Fig: 4- FTIR analysis of dried pulp of *Garcinia pedunculata*

Our study showed high content of total polyphenols and flavonoid in DPGP. The physiological effects of flavonoids include possible antioxidant activity, therefore, suggesting their role in prevention of coronary heart diseases including atherosclerosis[15]. High content of ascorbic acid in DPGP may be responsible for chain breaking reaction. Ascorbic acid acts as a synergist with vitamin E, since vitamin C can donate a hydrogen atom to the vitamin E-derived phenolate radical, thus regenerating its activity[16].

In the present study we investigated the DPPH radical scavenging activity of methanolic extracts of DPGP, expressed in trolox equivalent (TE, mM). It was observed that the scavenging activity was increased with the increasing concentration of the sample. To find out the antioxidant potential of plant extract, DPPH radical scavenging activity is a very useful method as it is highly sensitive and rapid assay[17]. This assay is independent on substrate polarity where DPPH can accept an electron or hydrogen radical to become a stable diamagnetic molecule[18] . When an antioxidant scavenges the free radical by hydrogen donation, the purple color of DPPH in assay solution turns to yellow, which can be monitored spectrophotometrically at 517 nm[19]. Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite. An imbalance between antioxidants and reactive oxygen species results in oxidative stress leading to cellular damage. The results of the present study clearly indicate that DPGP extract has powerful antioxidant activity against DPPH free radical in *in vitro* condition.

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity[20]. The presence of reductants such as antioxidant substances causes the reduction of Fe^{3+}/ferricyanide complex to Fe^{2+}/ferrous form. Therefore, the reducing power of the sample could be monitored by measuring the formation of Perl's Prussian blue at 700 nm[21]. Samples with higher reducing power have better abilities to donate electrons. Free radicals form stable substances by accepting the donated electrons, resulting in the termination of radical chain reactions. It has been widely accepted that the higher the absorbance at 700 nm, the greater the reducing power. Our study also shows reducing power in a dose dependent manner though it was very less in comparision to that of a standard drug ascorbic acid. Addition of H_2O_2 to cells in cultures can lead to transition metal ion dependent $OH^{\surd}$ mediated oxidative DNA damage. In our study DPGP extract showed potential hydrogen peroxide scavenging activity.

Lipid peroxidation is one of the major causes of quality deterioration in lipid-containing foods. Plant phenolics can delay the onset of lipid peroxidation and decomposition of hydroperoxides in food products as well as in living tissues[22]. It is believed that lipid peroxidation is one of the causes of cardiovascular disease and cancer[23]. In our study DPGP extract showed inhibition of lipid peroxidation in *in vitro* condition though it was not that much as showed by synthetic antioxidant drug trolox.

Moreover, DPGP was found to be a good source of transition metals viz iron, copper and zinc. The presence of these transition metals is one of the important nutritional qualities of DPGP, because deficiency of transition metal ions continues to be the most prevalent nutritional deficiency disorder in the world, affecting two billion people of the globe[24].

Conclusion

On the basis of results presented in this study, it can be concluded that DPGP is a good source of natural antioxidant as it exhibited significantly higher antioxidant activity. It can be attributed to its high phenolic and flavonoid, and ascorbic acid content. Among the four tested methods, the highest activity was observed for H_2O_2 radical scavenging by DPGP.

Acknowledgement

The authors acknowledge the financial support from the Department of Science and Technology (DST), Government of India, New Delhi, India, (No SR/SO/PS-78/05dt 15.01.07) for this work.

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