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In -Vitro antioxidative activity of phenolic and flavonoid compounds extracted from fruit of *Garcinia indica*

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Abstract

The aim of this work was to estimate the total phenolic and flavonoid content, and to evaluate *in-vitro* antioxidant activity of Methanolic fruit extract of *Garcinia Indica*. The raw, dry fruit powder was extracted with 99.9% of methanol. Phytochemical test shows that extract contains higher level of total phenol and flavonoids. Total phenolic compound in methanolic fruit extract of *Garcinia Indica* was found to be 0.348 mg/g of extract calculated as gallic acid equivalent (R^2 = 0.985) and total flavonoids compound was found to be 137.27 µg/g of extract calculated as quercetin equivalent (r2=0.997). The extract was screened for its potential antioxidant activities using tests such as hydroxyl radical-scavenging activity, reducing power activity, and hydrogen peroxide-scavenging activity. The *in-vitro* antioxidant assay showed *Garcinia Indica* posses potent antioxidant activity when compared with reference compound ascorbic acid. *Garcinia Indica* could be useful for preparation of neutraceuticals as potent antioxidant to treat various human diseases and its complications.

Key-Words: *In vitro* antioxidant activity, Phenol, Flavonoid, Reducing power activity, Hydrogen peroxide-scavenging activity, *Garcinia Indica*.

Introduction

Natural antioxidants present in the plants scavenge harmful free radicals from our body. Free radical is any species capable of independent existence that contains one or more unpaired electrons which reacts with other molecule by taking or giving electrons and involved in many pathological conditions[1]. It is possible to reduce the risk of chronic diseases and prevent disease progression by either enhancing the body's natural antioxidant defenses or by supplementing with proven dietary antioxidants[2]. Synthetic antioxidants like ascorbic acid, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) commonly used in foods have side effect and are carcinogenic[3]. Plant polyphenolic compounds, such as flavonoids are described as scavengers of reactive oxygen species[4]. Recently, the ability of phenolic substances including flavonoids and phenolic acids to act as antioxidants has been extensively investigated[5]. Most sources of natural antioxidants originate from plant materials[6].

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The plant *Garcinia indica* belongs to family Clusiaceae popularly known as Vrikshamala in Hindi, Kokum in English. It is native to India, from the Western Ghats region of India, along the western coast. It is found in forest lands, riversides, and wasteland, and also gets cultivated on a small scale. The plant has been used in Hindu medicines from very early times. In ayurveda the plant is considered beneficial for the Flatulence, oedema, chronic alcoholism, cordil, digestive power, quenching thirst, mouth disease, substitute for daadima (Punica granatum), Carminative, astringent, Anticorbutic, healing ulceration, dysentry. Thus, present study was undertaken to evaluate the *in- vitro* antioxidant effect of methanolic extract of *Garcinia indica* fruit. The main constituent present in the fruit are especially from its rind, are rich in polyisoprenylated benzophenone derivatives such as Garcinol and its colorless isomer, Isogarcinol. The rind also contains hydroxycitric acid (HCA), hydroxycitric acid lactones, citric acid and oxalic acid. The fruit also contains other compounds including malic acid, polyphenols, carbohydrates, anthocyanin, pigments and ascorbic acid. Garcinol shows strong antioxidant activity since it contains both phenolic hydroxyl groups

as well as a β-diketone moiety, and in this respect it resembles with the well known antioxidant of plant origin, viz. Curcumin[7].

Material and Methods

Plant material

Fruit were collected from the Pinnacle Biomedical Research Institute Bharat Scaud Guide Campus Shyamala Hills Bhopal.

Extraction procedure

The fresh fruit were separated from matured fruits, shade dried, broken into small pieces and powered coarsely. About 500 gm of air dried powdered material was extracted with 99.9% of methanol in a soxhlet extractor for 7 days. The extract was concentrated to dryness under reduced pressure and controlled temperature (40-50° C) using rotary evaporator. The methanolic extract yielded a brown sticky mass weighing 9.2g (10.3% w/w) and extractive value was found to be 3.326% w/w. the extract was used directly for total phenol and flavonoid content and also for assessment of antioxidant capacity through various chemical assays[8-9].

Phytochemical evaluation

The methanolic extract of *Garcinia indica* fruit was subjected to the following chemical tests for the identification of various active constituents.

Estimation of total phenolic content

The total phenolic content of *Garcinia indica* was estimated according to the method of Makkar et.al (1997)[10]. The aliquots of the extract was taken in a test tube and made up to the volume of 1 ml with distilled water. Then 0.5 ml of Folin-Ciocalteu reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) were added sequentially to the test tube. Soon after over texing the reaction mixture, the tubes were placed in the dark for 40 min. and the absorbance was recorded at 725 nm against the reagent blank. Using gallic acid monohydrate, a standard curve was prepared. The linearity obtained was in the range of 1-10 µg/ml. using the standard curve, the total phenolic content was calculated and expressed as gallic acid equivalent in mg/g of extract.

Estimation of total flavonoid content

Flavones and flavonols in the methanolic extracts of *Garcinia indica* fruit were estimated as Quercetin equivalent. Quercetin was used to make the calibration curve [10, 20, 30, 40, 50, 60, 70, 80, 90, 100 in 99.9% methanol (v/v)]. The standard solutions or extracts (0.5 ml) were mixed with 1.5 ml 95% methanol (v/v), 0.1 ml 10% aluminum chloride 42(w/v), 0.1 ml of 1 mol/l sodium acetate and 2.8 ml water. The volume of 10% aluminum chloride was substitued by the same volume of distilled water in blank. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm.

Evaluation of in vitro antioxidant activity

Hydroxyl radical-scavenging activity

Hydroxyl radical scavenging activity of extract was measured according to the method of Halliwell et al. (1987)[12]. One milliliter of the final reaction solution consisted of aliquots (500 µl) of various concentrations of the extract, 1 mM $FeCl_3$, 1 mM EDTA, 20 mM H_2O_2, 1 mM L-ascorbic acid, and 30 mM deoxyribose in potassium phosphate buffer (pH 7.4). The reaction mixture was incubated for 1 h at 37 °C, and further heated in a boiling water bath for 15 min after addition of 1 ml of 2.8% (w/v) trichloroacetic acid and 1 ml of 1% (w/w) 2-thiobarbituric acid. The color development was measured of 532 nm against a blank containing phosphate buffer.

Reducing power activity

The reducing power of extract was determined by the method of Yen and Duh (1993)[13]. Different concentrations of extracts were mixed with 2.5 ml of phosphate buffer (200 mM, pH 6.6) and 2.5 ml of 1%potassium ferricyanide. The mixtures were incubated for 20 min at 50°C. After incubation, 2.5 ml of 10% trichloroacetic acid were added to the mixtures, followed bycentrifugation at 650×*g* for 10 min. The upper layer (5 ml) was mixed with 5 ml of distilled water and 1 ml of 0.1% ferric chloride and the absorbance of the resultant solution were measured at 700 nm.

Hydrogen peroxide-scavenging activity

The Hydrogen peroxide-scavenging activity of extract was determined by the method of Ruch et al., (1989)[14]. The extract was dissolved in 3.4 ml of 0.1M phosphate buffer (pH 7.4) and mixed with 600 µl of 43 mM solution of hydrogen peroxide. The absorbance value (at 230 nm) of the reaction mixture was recorded at 10 min intervals between zero and 40 min. for each concentration, a separate blank sample was used for background substraction.

Results and Conclusion

Phytochemical evaluations: The phytochemical methanolic extract shows the presence of following constituents.

S. No.	Phytochemical Methanolic extract of constituents *Garcinia indica fruit*
1.	Carbohydrates +ve
2.	Alkaloids +ve
3.	Steroids and sterols +ve
4.	Glycosides +ve
5.	Saponins -ve
6.	Flavanoids +ve

7.	Tannins and phenolic compound +ve
8.	Proteins and Amino acids +ve
9.	Fixed oil +ve
10.	Anthraquinone -ve

Total phenol content

Total phenolic compound in Methanolic fruit extract of *Garcinia indica* was found to be 0.348 of extract calculated as gallic acid equivalent. (r2=0.985).

Total flavonoid content

Total flavonoids compound in methanolic fruit extract of *Garcinia indica* was found to be 137.27 µg/g of extract calculated as quercetine equivalent. (r2=0.997).

Hydroxyl radical scavenging activity

DMSO and *Garcinia indica* showed hydroxyl radical scavenging activity with about 68.23-95.47% and 23.19-89.02% at concentration of 10µg/ml, 500µg/ml, (Table 2). A concentration dependent inhibition against hydroxyl radical induced deoxyribose degradation was observed in the deoxyribose assay. Because the *Garcinia indica* was high in its phenol and flavonoid content, its antioxidant compounds may well act as antioxidant and savenge hydroxyl radical generated from the Fenton reagent.

Table 2: Shows hydroxyl radical-scavenging activity of methanolic fruit extract of *Garcinia Indica*

Conc.	DMSO Inhibition %	*Garcinia Indica* Inhibition %
10 µg/ml	68.23	23.19
500 µg/ml	95.47	89.02

All the values are means of three independent determinations, n=3, analyzed in triplicate.

Hydrogen peroxide-scavenging activity

Scavenging activity of hydrogen peroxide in Ascorbic Acid (10µg and 500µg) and *Garcinia indica* (10µg and 500µg) as reference compound was shown to be 61.11-93.76% and 34.82-74.96% (Table 3). The composition of hydrogen peroxide into water may occur according to the antioxidant compounds as the antioxidant components present in the extract are good electron donors, they may accelerate the conversion of H_2O_2 to H_2O.

Table 3: Shows hydrogen peroxide scavenging activity of *Garcinia indica* (10 µg/mL)

Conc.	Ascorbic Acid Inhibition %	*Garcinia indica* Inhibition %
10 µg/ml	61.11	34.82
500 µg/ml	93.76	74.96

All the values are means of three independent determinations, n=3, analyzed in triplicate.

Reducing power activity

At concentration 10µg/ml and 500µg/ml Ascorbic Acid (Reference) and *Garcinia indica* showed absorbance with about 0.127-0.398 and 0.019-0.247 respectively (Table 4). Thus *Garcinia indica* exhibited reducing activity. The reducing power might be due to hydrogen donating ability.

Table 4: Shows reducing power activity of methanolic fruit extract of *Garcinia indica*

Conc.	Ascorbic Acid (Absorbance)	*Garcinia Indica* (Absorbance)
10 µg/ml	0.127	0.019
500 µg/ml	0.398	0.247

All the values are means of three independent determinations, n=3, analyzed in triplicate.

Based on the results obtained *Garcinia indica* showed antioxidant and free radical scavenging activity not remarkably different than reference compound Ascorbic Acid and major antioxidative component seems to be phenolic and flavonoids. Therefore, it can be concluded that the methanolic extract of *Garcinia indica* fruit could be considered for prevention and treatment of human diseases and its complications as potent antioxidant.

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