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RESEARCH ARTICLE

IN-VITRO ANTIOXIDANT ACTIVITY OF *GARCINA CAMBOGIA* FRUITS

Shukla Ajay[1], Shukla Rajesh*[2], Vikas Pandey[2], Dilip Golhani[2] and Jain C. P.[1]

[1]Dept. of pharmaceutical Science, Mohan Lal Sukhadiya University, India

[2]Dept. of pharmaceutical Science, Suresh Gyan Vihar University, India

Correspondence
Mr. Rajesh Shukla
Department of p'ceutical science, Mohan lal sukhadiya university, Udaipur,RJ, India.
Email Id:
Rajeshshukla2628@gmail.com

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ABSTRACT

The object of this research work to isolate the total phenol and flavonoid content and to determine in-vitro antioxidant activity of water fruit extract of *garcina cambogia*. The raw, dry fruit powder was extract with distilled water by microwaves method of green technology. Test shows that extract contains higher level of total phenol and flavonoid. The total phenolic acid equivalent (r^2 = 0.985) and total flavonoid content was found to be 137.27 µg/g of extract calculated as Gallic quarcetin equivalent (r^2 = 0.997). The water extract was evaluated for potential antioxidant activities using by hydroxyl radical-scavenging activity method, reducing power activity, and hydrogen peroxide-scavenging activity. The in-vitro antioxidant assay showed *garcina cabbogia* possess potent antioxidant activity when compared with standard compound ascorbic acid. *garcina cabbogia* are useful in various preparation of neutraceuticals, and as potent source of antioxidant to treat various human diseases .

INTRODUCTION

Various natural sources of antioxidants presents in the plants scavenge harmful free radicals from our body. Recently, natural plants have received much attention as sources of biological active substances including antioxidants (1). The free radicals are free highly active species capable of independent existence that contains one r more unpaired electrons which reacts with other molecule by taking or giving electrons and involved in many pathological conditions (2). It is possible to reduce the risk of chronic diseases and prevent diseases progression by either enhancing the body's natural antioxidant defenses or by supplementing with proven dietary antioxidants (3). Synthetic antioxidants like ascorbic acid, butylatedanisole commonly used in the foods have side effects and are carcinogenic (4). Plant poly-phenol compounds, such as flavonoids are described as scavengers of reactive oxygen species. Recently the ability of phenolic substances including flavonods and phenolic acids to act as antioxidants has been extensively investigated (5,6-7). Most sources of natural antioxidants originate from plants materials (8-9). The plant *garcinia cambogia* belongs to family Clusiaceae popularly known as Vrikshamala or vilayati emali in hindi, Kkum in English. It's native to India, from the Western Ghats region of India, along the western coast. It is found in forest lands, riversides, and wastelands, and also gets cultivated on a small scale. The plants have been used in hindu medicines from very early times. In ayurveda the plants is considered beneficial for the flatulence, odema, chronic alcoholism, digestive power, quenching thirst, mouth diseases, substitute for daadima punica granatum, carminative, astringents, healing ulceration, dysentery. Thus present study was undertaken to evaluate the in-vitro antioxidant effects of water extract of *garcinia cmbogia* 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 has lactones, citric acid and oxalic acid. The fruit of *garcinia cambogia* contains other compounds including malic acid, polyphenols, carbohydrates, anthrocynin, pigments and ascorbic acid (10). Garcinol shows strong antioxidants activity since it contains booth phenolic hydroxyl groups as well as β-dike tone moiety.

MATERIAL AND METHOD

Plant material

Dried fruits were collected from the Amir-chem. company pithampur Indore (M.P.)

Extraction procedure

The dry fruits were separated from mature fruits. Broken in to small pieces and powdered coarsely. About 5oo gm of air dried powdered material was extraction carried out at 250w for 20 minutes, 350 minutes for 15 minutes, and 450 for 10 minutes heating with distilled water of in a microwave extraction unit. The extract was concentrated to dryness under reduced pressure and evaporator. The water extract yielded a brown sticky mass found to be 7.5%w/w. the extract was used directly for total phenol and capacity through various chemical assays.

Phyto-chemical evaluation

The water extract of *garcinia combgia* fruits was subjected to the following chemical tests for the identification of various active constituents.

Estimation of total phenol content

The total phenol content of *garcinia cambogia* was estimated according to the method of Makkar et.al (1997) (11).The water 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 (1:1with water).then 2.5 ml of sodium carbonate solution (20%) were added the sequentially to the test tube. then after over mixture, the test tube were placed in the dark room for 40 minutes. 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 phenol content was calculated and expressed as gallic acid equivalent in mg/g of extract.

Estimation of total flavonoid content

Flavones and flavonols in the water extract of *Garcinia cambogia* fruit were determined as Quercetin equivalent. quercetin was used to make the calibration curve (10,20,30,40,50,60,70,80,90,100 in 99.9 % water v/v).the standard solutions or extracts (0.1 ml 10% aluminum chloride 42ml (w/v),0.1 ml of 1ml/1 sodium acetate and 2.8 ml water. The volume of 10% aluminum chloride was substituted by the same volume of distilled water in dark. After incubation at room temperature for 30 minutes, the absorbance of the reaction mixture was measured at 415 nm.

Evaluation of in vitro antioxidant activity And Hydroxyl radical-scavenging activity

Hydroxyl radical scavenging activity of extract was measured to the method of Halliwell et al.(1987) (12). One milli-liter of the final reaction solution consisted of aliquots (500µl)of various concentrations of the extract,1 Mm FeCl3,1 mM EDTA, 20Mm H_2O_2,1Mm L-ascorbic acid, and 30Mm 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 minutes after addition of 1 ml of 2.8% (w/v) 2-thiobarbituric acid. The color development was measured of 532 nm againsed a blank containing phosphate buffer.

Reducing power activity

The reducing power of extract was determined by the method of Yen and Duh (1993).Different concentrations of extract were mixed with 2.5 ml of phosphate buffer (200mM, pH 6.6) and 2.5 ml of 1% potassium ferricynide. The mixtures were incubated for 20 minutes at 50 C .after incubation, 2.5 ml of 10% Trichloroacetic acid were added to the mixtures followed by centrifugation at 650×g for 10 minutes. The upper layer (5ml) was mixed with 5ml of distilled water and 1ml 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) (13).The extract was dissolved in 3.4 ml of 0.1 M phosphate buffer (pH 7.4) and mixed with 600 µml of 43 mM solution of hydrogen peroxide. The absorbance value (at 230 nm) of the reaction mixture was recorded at 10 minutes intervals between zero and 40 minutes. For each concentration, a separate blank sample was used for background subtraction.

RESULT AND CONCLUSION

Phytochemical evaluations:

The phytochemical water extract shows the presence of following constitutes in Table 1.

Total phenol content

Total phenol compounds in water fruit extract of *Garcinia cambogia* was found to be 0.348 of extract calculated as gallic acid equivalent.(r^2=0.985).

Total flavonoid content

Total flavonoids compound in water fruits extract of *Garcinia cambogia* was found to be 137.27 µg/g of extract calculated as quercetine equivalent (r^2=0.997)

Hydroxyl radical scavenging activity

DMSO and *Garcinia cambogia* showed hydroxyl radical scavenging activity with about 70.32-97.74% and 25.91-91.04% at concentration of 10µg/ml, 500µg/ml in Table 2. A concentration dependent inhibition against hydroxyl radical induced deoxyribose degradation was observed in the deoxyribose assay. Because the *Garcinia cambogia* was high in its phenol and flavonoids content, its antioxidant and scavenge hydroxyl radical generated from the Fenton reagent.

Hydrogen peroxide- scavenging activity

Scavenging activity of hydrogen peroxide in Ascorbic acid (10µg and 500µg) and *Garcinia cambogia* (10µg and 500µg) as reference compounds was shown to be 62.12-94.67% and 36.06-78.69% in Table 3.The composition of hydrogen peroxide into water may occur according to the antioxidant compounds as the antioxidant components presents in the extract are good electron donors, they may accelerate the conversion of H_2O_2 to H_2O.

Reducing power activity

At concentration 10µg/ml and 500µg/ml Ascorbic Acid (Reference) and *Garcinia cambogia* showed absorbance with about 0.128-0.399and 0.020-0.274respectively shown in Table 4. Thus *Garcinia cambogia* exhibited reducing activity. The reducing power might be due to hydrogen donating ability.

Based on the results obtained *Garcinia cambogia* showed antioxidant and free radical scavenging activity not remarkably different than reference compound Ascorbic Acid and major anti-oxidative component seems to be phenolic and flavonoids. Therefore, it can be concluded that the water extract of *Garcinia cambogia* fruits could be considered for prevention and treatment of human diseases and it is source of potent antioxidant.

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EXPERIMENTAL RESULT

Table 1. Phytochemical Test for *Garcinia cambogia*

S.N.	Phytochemical water extract of constituents *Garcinia cambogia* fruit
1	Carbohydrates +ve
2	Alkaloids +ve
3	Steroids and sterols +ve
4	Glycosides +ve
5	Saponins -ve
6	Flavanoids +ve
7	Tannins and phenol compounds +ve
8	Proteins and Amino acids +ve
9	Fixed oils +ve
10	Antraquinone -ve

Tables 2: Shows hydroxyl radical-scavenging activity of water extract of *Garcinia cambogia*

Concentration	DMSO Inhibition %	Garcinia cambogia Inhibition %
10µg/ml	70.32	25.91
500µg/ml	97.74	91.04

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

Table 3: Shows hydrogen peroxide scavenging activity of *Garcia cambogia* (10µg/ml)

Concentration	Ascorbic Acid Inhibition %	*Garcinia cambogia* Inhibition %
10µg	62.12	36.06
500µg	94.67	78.69

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

Table 4: Shows reducing power activity of water fruit extract of *Garcinia cambogia*

Concentration	Ascorbic Acid Absorbance	*Garcinia cambogia* Absorbance
10µg	0.128	0.020
500µg	0.399	0.274

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

Table 4: Shows reducing power activity of water fruit extract of *Garcinia cambogia*

Concentration	Ascorbic Acid Absorbance	*Garcinia cambogia* Absorbance
10µg	0.128	0.020
500µg	0.399	0.274

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