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Evaluation of Antioxidant Properties and Antimicrobial Activity of *Garcinia cambogia* Hort. Ex Boerl (Clusiaceae) fruit extracts.

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Abstract

Garcinia cambogia (Clusiaceae) is a traditionally used medicinal plant claimed to possess antioxidant properties and antimicrobial activity. The present study was aimed to evaluate the *in vitro* antioxidant property and antimicrobial activity. The antioxidant property of the methanolic fruit extracts were evaluated using two methods. DPPH Radical Scavenging Assay, Total antioxidant *phosphomolybdenum method* and Total Reducing Capacity. The results revealed that free radical scavenging activity of methanolic fruits extracts of *G. cambogia* has significant radical scavenging ability on DPPH with IC_{50} value of 39. 45 µg/ml respectively. The positive control ascorbic acid showed the IC_{50} values of 32.42 µg/ml. The phytochemical screening showed the presence of Terpenoids, Tannins, Saponins, Phenols, Flavonoids and Steroids compounds. Methanol, Ethyl acetate and chloroform extracts of the plant was tested for antimicrobial activity against Gram positive bacteria such as *Bacillus subtilis, Staphylococcus aureus, Streptococcus faecalis,* Gram negative bacteria such as *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and a fungus *Candida albicans* using agar well plate method. The results obtained in the present study indicated that the fruit of *G. cambogia* showed the best antimicrobials activity against the *C. albicans*, *S. aureus* ($G^{+)}$ and *K. pneumoniae* (G^{-}). Gentamicin is used as a positive control. In conclusion, the results indicate antimicrobial activity of the extract which could be further explored for purification of antioxidant compounds.

Keywords: *Garcinia cambogia*, Clusiaceae, Antioxidant, DPPH, Antimicrobial activity.

Note: All the figures and tables are listed in supplementary article

Introduction

Medicinal plants based traditional systems of medicines are playing important role in providing health care to large section of population, especially in developing countries. Interest in them and utilization of herbal products produced based on them is increasing in developed countries also (Ravishankar *et al*., 2007). India has the unique distinction of having six recognized systems of medicine in this category. They are- Ayurveda, Siddha, Unani and Yoga, Naturopathy and Homoeopathy. Though Homoeopathy came to India in 18th Century, it completely assimilated in to the Indian culture and got enriched like any other traditional system hence it is considered as part of Indian Systems of Medicine (Prasad, 2002). All the above systems of medicine in India are largely based on herbal drugs. Despite of their efficient effects, much of the ancient knowledge and many valuable medicinal plants of India are being lost at an alarming rate. With the rapid depletion of forests, impairing the availability of raw drugs, Ayurveda, like other systems of herbal medicines has reached a very critical phase. In this context, scientific validation of Indian medicinal plants is the need of hour. The medicinal value of plants can be attributed to some chemical substances which produce a definite physiological action on the human body. The most important of such bioactive compounds are alkaloids, flavonoids, tannins and phenolic compounds (Ramesh *et al*., 2012).

Oxidation is a normal physiological and metabolic process in the cell. During the process, approximately 5% oxygen gets reduced to the oxygen based free radicals, includes superoxide, hydrogen peroxide, hydroxyl and nitric oxide radicals (Halliwell and Gutteridge, 1989). These free radicals are collectively known as Reactive Oxygen Species (ROS). Free radicals formed during metabolism are electrically charged and react with nucleic acids, mitochondria, proteins and enzymes and resulting in their damage (Halliwell, 1996; Morrissey and O'Brien, 1998). The antioxidant defense system protects the cells against the free radicals. The antioxidant defense mechanism is affected by age, diet and health condition of individual (Yu, 1994). When formation of free radicals overtakes the antioxidant defense system, the free radicals start attacking the cell and resulting in several physiological disorders like Alzheimer's disease, cancer, atherosclerosis, diabetes, liver cirrhosis and rheumatism (Frankel *et al*., 1993; Goodwin and Brodwick, 1995).

G. cambogia is a native species of Asia and Indonesia and belongs to the family Culsiaceae. It is widely distributed in the sub- tropical regions and it is commonly known as Brindleberry, Malabar tamarind, Kodumpuli and Goraka. With thin skin and deep vertical lobes, the size of the fruit of *G. cambogia* is that of an orange; it looks more like a small yellowish, greenish or sometimes reddish pumpkin (Uphof, 1968).

G. cambogia are used as purgatives in traditional system. *G. cambogia* extracts are an ingredient in some herbal appetite suppressant and energy products, though there is no formal evidence to support its effectiveness. It is used in weight-loss supplements (Lobb, 2009). *G. cambogia* has been used traditionally for the treatment of edema, delayed menstruation, constipation, ulcers, hemorrhoids, diarrhoea, dysentery, fever, open sores, intestinal parasites, anti-microbial agent, anti-fungal, and as an anti-cancerous (Mahendran *et al*., 2001; Mackeen *et al*., 2002; Ho *et al*., 2002 and Pan *et al*., 2001). Malabar tamarind has been shown to contain a variety of secondary metabolites such as xanthones, flavonoids and benzophenones (Koshy *et al*., 2001 and Masullo *et al*., 2008).

Materials and methods

Plant materials

Plants were collected from natural population growing in the Courtallum forest area. Tirunelveli District, Tamil Nadu, India, during October 2015. The plant sample was carried to the Botany Research Laboratory; Voucher specimen of the plant was deposited in the Botany research laboratory V.H.N.S.N. College (Autonomous) for further references.

Preparation of fruit extracts

The fruits were cleaned and cut into small pieces before being dried in a hot air-blowing oven at 50ºC. All samples, after drying, water contents below 10%. They were ground to a fine powder in a mechanical blender and kept at room temperature prior to extraction. The dried fruits extracts were prepared by sequential extraction method using three organic solvents in the basis of polarity of solvents (Chloroform, Ethyl acetate and Methanol). 30g of the fruits sample was taken in a conical flask and 200 ml of Chloroform was added. The conical flask was kept on mechanical shaker for 24 hours, after that the extract was filtered through what man filter paper 1 and the pellet was allowed for drying and this pellet was used for the next solvent extraction (Ethyl acetate and Methanol). The dried extract was recovered and stored in Refrigerator for further analysis.

Phytochemical Screening

The collected plant fruits extracts were subjected to Antioxidant activity and Antimicrobial analysis for identification of various classes of active chemical constituents were carried out using standard methods.

Test for Alkaloids (Mayers Test)

To 1 ml of fruits extract, 6 drops of Mayer's reagent was added. The formation of yellowish creamish precipitate indicated the presence of alkaloids (Edeoga *et al*., 2005; Harbone, 1973).

Test for Tannins (Braymers Test)

1ml of the fruits extract was added mixed with 2ml of water. To these 2 drops of 5% ferric chloride solution was added. Appearance of dirty green precipitate indicated the presence of tannins (Edeoga *et al*., 2005; Harbone, 1973).

Test for steroids (Salkowski Test)

To 2ml of the extract, 2ml of chloroform was added and followed by concentrated sulphuric acid. Formation of reddish brown ring at the junction showed the presence of steroids (Yadav *et al*., 2014).

Test for terpenoids

2ml of the extract was added with 2ml acetic acid. Then concentrated sulphuric acid was added. Deep red color development showed the presence of terpenoids (Yadav *et al*., 2014).

Test for Coumarins

Take 2ml of the extract and added 3ml of 10% sodium hydroxide. Formation of yellow coloration indicates the presence of coumarins ((Yadav *et al*., 2014).

Test for Catachins

2ml of alcoholic extract solution was treated with few drops of Echrilich reagent and few drops of concentrated HCL. The pink color formation indicates the presence of catachin (Yadav *et al*., 2014).

Test for phenols

1ml of the extract was treated with 3% ferric chloride. The appearance of deep blue color, then it shows the presence of phenol (Kokate, 2000; Harborne, 1999).

Test for flavonoids

1mi of the extract was added with 1ml of sulphuric acid. Orange color formation confirmed the presence of flavonoids (Kokate, 2000; Harborne, 1999).

Test for Quinones

1ml of the extract was treated with 5ml of HCL. Formation of yellow color precipitate indicated the presence of quinine (Kokate, 2000; Harborne, 1999).

Quantitative phytochemical analysis

Estimation of total phenol content

The amount of total phenol was determined using the Folin-Ciocalteu reagent method of Lister and Wilson, 2001. A standard curve was prepared by using gallic acid. Different concentrations of gallic acid were prepared in 80% methanol, and their

absorbance was recorded at 760 nm. 100ml of sample was dissolved in 500ml (1/10 dilution) of Folin-Ciocalteu reagent and 1ml of distilled water. The contents were mixed and incubated at room tempreture for 1 min. After 1 min, 1.5 ml of 20% sodium carbonate solution was added. The final mixture was shaken and incubated for 2h in the dark at room temperature. The absorbance of all samples was measured at 760 nm using a UV-Vis spectrophotometer (Model. U.2800, Hitachi). The results were expressed in mg gallic acid equivalents (GAE) per milligram of dry weight of the plant. The amount of phenol in plant extracts were calculated by the following formula;

$$X= (A.m_o)/(A_o.m)$$

Where X is the phenol content, mg/mg plant extract in GAE, A is the absorbance of plant extract, A_o is the Absorption of standard gallic acid solution, is the m is the weight of plant extract, and m_o gallic acid in the solution.

Estimation of total flavonoid content

The flavonoid content in the extract was determined spectrometrically by the method of Quettier-Deleu *et al*., 2000. This method was based on the formation of a complex, flavonoid-aluminium, with the absorbance maximum at 430 nm. Rutin was used as standard to make the calibration curve. 1ml of diluted sample was separately mixed with 1ml of diluted sample was separately mixed with 1ml of 2% aluminium chloride methnolic solution. After incubation at room temperature for 15 min, the absorbance of the reaction mixture was measured at 430 nm in a UV-Vis spectrophotometer. The flavonoid content was expressed in mg per mg of rutin equivalent (RE). The amount of flavonoid in the plant extracts in RE was calculated by the following formula;

$$X= (A.m_o)/(A_o.m)$$

Where X is the flavonoid content, mg/mg plant extract in RE, A is the absorbance of plant extract, A_o is the Absorption of standard Rutin acid solution, is the m is the weight of plant extract and m_o is the weight of Rutin acid in the solution.

Free Radical Scavanging Ability (DPPH)

The scavenging ability of methanol extract on 1,1-diphenyl-2-picrylhydrazyl free radicals was estimated according to the method of Shimada *et al*., 1992. This method depends on the reduction of purple DPPH to yellow colored diphenyl picryl hydrazine. 2ml of the various concentrations (10-100 µg/ml) of test sample was mixed with 0.5 ml of 0.001M DPPH in methanol. An equal amount of methanol and DPPH served as control. The mixture was shaken vigorously and then steadily kept for 30 min at room temperature in dark. The absorbance of the resulting solution was measured at 517nm against the blank using UV-Vis

spectrophotometer. The experiment was performed in triplicates. The DPPH radical scavenging activity was calculated by the following equation;

% DPPH radical scavenging activity= $(A_o-A_1)/A_o x100\%$

Where A_o is the absorbance of the control reaction and A_1 is the absorbance of the sample of the tested extracts. Percentage of free radical activity was plotted against the corresponding antioxidant substance concentration to obtain the IC_{50} value, which is defined as the amount of antioxidant substance required to scavenge the 50% of free radicals present in the assay system. IC_{50} values are inversely proportional to the antioxidant potential.

Total antioxidant activity by phosphomolybdenum method

Total antioxidant capacity was expressed as ascorbic acid equivalent and was calculated by the phosphomolybdenum method (Prieto and Pineda, 1999). Antioxidant present in the sample reduce the Mo(VI) to Mo(V) which react with phosphate group of sodium phosphate to form green coloured Mo(V)- Phosphate complex (phosphomolybdenum complex in an acetic medium. This complex then spectrophotometrically measured at 695 nm. The tubes containing 0.2 ml of extract was mixed with 1.8ml of distilled water and 2ml of phosphomolybdenum reagent solution. Incubate at 95ºC for 90 minutes. The absorbance of the resulting solution was measured at 695nm. The total antioxidant capacity was expressed as equivalents of Ascorbic acid using the Ascorbic acid standard graph.

Reducing power ability

The reducing power ability of methanolic extract was determined by method of Oyaizu, 1986. Reaction mixture was prepared by a addition of 2.5ml of phosphate buffer (0.2M, PH 6.6) with 2.5ml potassium ferricyanide (0.1%) followed by the concentrations of extracts (250µg/ml). Then the reaction mixture was incubated at 50ºC in for 30min and allowed to cool at room temperature. Then 2.5 ml of 10% Trichloro acetic acid was added to each reaction mixture and centrifuged at 2000 rpm for 10min. The supernatant (2.5 ml) was taken in a test tube and a added with 2.5ml of distilled water and 0.5 ml of Fecl3 (1.0%). After 10min incubation at room temperature, the absorbance was measured at 700 nm. Ascorbic acid solution was used as standard. All the tests were performed in triplicate and the graph was plotted with the average of the three determinations.

Statistical Analysis

All analysis was carried out in triplicates. The results of scavenger activity and total phenol and total flavonoid contents were performed from the averages of all samples reading Mean+- SD used Excel 2003.

Results and Discussion

Percentage yield of crude extracts

The course powder of plant material was extracted by mechanical method using methanol, Ethyl acetate and chloroform successively. The extract values were found to be the difference between four solvent extract separately. The yield of extract depends upon the solvent system showed in Table 1.

Preliminary phytochemical screening

The results of qualitative test revealed the presence of Alkaloids, Saponins, Tannins, Flavonoids, Terpenoids, Coumarins, Phenols and Steroids (Table 2). The phytoconstituents catechin and quinone were not reported in any of these three solvent extracts. The chloroform extract showed the presence of Terpenoids and Steroids. The ethyl acetate extract tested positively for phenols, Steroids and Saponins. The methanol extract showed positive colour reaction for the phytoconstituents like Alkaloids, Saponins, Tannins, Flavonoids, Terpenoids, Coumarins, Phenols and Steroids.

Total Phenolic content

Total phenol content present in the plant material was estimated by the method of Lister and Wilson (2001). The values were represented in mg Gallic acid equivalent per gram dry weight of samples. The regression analysis of standard Gallic acid was obtained in $y = 0.330x$, $R^2 = 0.99$ (Figure 1). The maximum amount of total phenol was exhibited in methanol extracts of *G. cambogia* fruit (0.428± 0.002 mg/mg) followed by Ethyl acetate extract of (0.106±0.003mg/mg) and Chloroform extract (0.106±0.003mg/mg). Phenolic compounds are the key phytochemicals with high free radical scavenging activity. It has generated a great interest among the scientists for the development of natural antioxidant compounds from plants. Phenol compounds also possess anti-mutagenic and anti-tumor activities (Othman *et al*., 2007).

Total flavonoids contents

Total flavonoids content in various solvent extracts of plant were determined from regression equation of standard calibration curve ($y = 0.335x - 0.108$ $R^2 = 0.990$) and expressed in Rutin equivalents (RE) (Figure 2). The results revealed that the highest total flavonoid contents was found in Ethyl acetate extracts of *G. cambogia* (0.166±0.005 mg/mg) followed by methanol extract of (0.153±0.002mg/mg) and Chloroform extract (0.142±0.002mg/mg).

Effect of DPPH radical scavenging activity

DPPH is stable free radical with deep purple color, after receiving proton from a

proton donor such as phenolic compounds, it loses its chromophore and became yellow (Sanchez-Moreno *et al*., 1999). DPPH radical scavenging activity of *G. cambogia* fruit of methanolic extract compared with ascorbic acid. Fig 3 was showed that the *G. cambogia* had DPPH inhibition free radical activity was 88.42±1.15 at 100 µg/mL and IC_{50} was 39. 45 µg/ml of the methanolic extract and standard IC_{50} values of 32.42µg/ml. Shivakumar *et al*., 2013 reported Dpph radical scavenging activity of fruit rind extract showed high potent antioxidant activity where as Rawri *et al* ., 2013 reported the DPPH radical scavenging activity of fruit extract of *Garcinia indica*.

Total phospomolybdenum method

The phosphomolybdate method is quantitative, since the total antioxidant capacity (TAC) is expressed as ascorbic acid equivalents. The results showed antioxidant activity in dose dependent manner at concentration 25 to 250 µg/ml. *G. cambogia* (126 mg/g) had a significantly higher percentage of TAC activity. Strong antioxidant activity of methanol statistically similar to ascorbic acid indicates strong antioxidants in this fraction and these could be attributable to the presence of phenolic compounds.

Total Frap method

The frap method results showed antioxidant activity in dose dependent manner at concentration 50 to 250 µg/ml. *G. cambogia* (240 mg/g) had a significantly higher percentage of TAC activity. The frap method of total antioxidant capacity of *G. cambogia* has significant amount of TAC activity.

Antimicrobial activity

The results of antimicrobial activity of the fruits extracts of *G. cambogia* was investigated against *Streptococcus feacalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacillus subtilis, Staphyllococcus aureus*, and *Candida albicans* Table 3. All the extracts were inhibited growth of almost all the selected bacteria in the range between (8 -15.5mm) and selected fungi in the range between (12-15mm). Among them ethyl acetate extract showed great activity against *Candida albicans* (16.27±0.38mm) and moderated activity were reported against *Staphylococcus aureus* (14.33±0.29mm), *Klebsiella pneumoniae (*14.27±0.21mm), *Bacillus subtilis* (14.17±0.24mm), and *Streptococcus faecalis* (9.10±0.14mm) followed *Escherichia coli* (7.23±0.21mm). Antifungal activity of *G. cambogia* ethyl acetate extract showed great activity against *Candida albicans* (15.27±0.38 mm). The chloroform and ethyl acetate extracts have did not any activity similar organism like *Pseudomonas aeruginosa* and *Bacillus subtilis*. Another studies on antimicrobial screening of different parts of *G. cambogia revealed* its good antimicrobial activity in

the fruit rind different solvent extract. (Shivakumar *et al*., 2013).

Conclusion

The preliminary phytochemical screening of *G. cambogia* has revealed the presence of phenolics, flavonoids, alkaloid, steroids and saponins in high amounts. It is concluded that various extracts of *G. cambogia* exhibited a wide range of antioxidant capacities, thus making them a valuable source of natural antioxidants. Further isolation and purification of antioxidant components should be carried out for valuable utilization of these precious plant compounds. Still, further analyses on the mechanism of other beneficial compounds from various plants are needed for a better understanding to implement them into functional foods.

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Note: All the figures and tables are listed in supplementary article