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Research Article

# *IN VITRO* ASSESSMENT OF ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF FRUIT RIND EXTRACTS OF *GARCINIA CAMBOGIA. L.*

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**ABSTRACT**

Background: *Garcinia cambogia* is a native species of Asia and Indonesia and it is widely distributed in the subtropical region. The plant is well known for its weight reducing property especially in controlling obesity. In the present we have studied the antioxidant and antibacterial activity of *G. cambogia*.

Materials and method: Fruit rind was dried and extracted in four different solvents (hexane, ethyl acetate, ethanol and hydro-alcoholic extracts). Each extract was individually screened for its activity against different human bacterial pathogens by agar well diffusion method. The *in vitro* antioxidant assay of the four different extracts of *G. cambogia* was also evaluated and compared to check the extract showing highest activity.

Results: The ethyl acetate extract of *G. cambogia* exhibited better antibacterial activity where as hexane extract showed on activity against any of the test pathogens. Antioxidant assay conclude that the hydro-alcoholic extract of *G. cambogia* possesses the highest antioxidant activity followed by ethanol, and with hexane having no or least activity.

Conclusion: The results indicate that the fruit rind extract of the plant has an excellent antibacterial activity and antioxidant property.

**Keywords:** *Garcinia cambogia*, Antibacterial, Antioxidant, Anti- obesity.

## INTRODUCTION

*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 [1]. The colour can vary considerably. When the rinds are dried and cured in preparation for storage and extraction, they are dark brown or black in colour.

The fruit rind of the plant is commonly used in various food preparations in southern India especially, mainly in Kerala. Aside from the use of food preparation and preservation, the extracts of *G. cambogia* are used as purgatives in traditional system. Gambogia 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 [2].

The living system is damaged by free radicals like ROS, RNS, $O_2$, OH, RCOO (peroxyl radicals) which are formed during excessive metabolism leading to early aging of the cells [3]. ROS are highly reactive and have short life span, known to cause damage to cellular components including lipid, DNA, protein, carbohydrate, and other biological molecules, leading to many pathological processes such as aging, cancer, cardiovascular diseases, diabetes, inflammation and neurodegenerative diseases [4-9]. Biological system has its own defence mechanism against these free radicals by producing antioxidants. Antioxidants are of medicinal interest because they protect the organism against the damage caused by the free radicals [10]. Antioxidant inhibits generation of (ROS) reactive oxygen species and (RNS) reactive nitrogen species, or it directly scavenges free radicals, but the deficiency or excess oxidative stress in the body requires an external source.

Plants are vital source of antioxidants in nature; they contain chemical compounds like flavonoids, phenols, and other compounds which show high antioxidant activity. Researches are being carried out to find natural antioxidants from plants [11-12]. Plants are safe and effective natural antioxidants, especially spices and herbs [13]. Polyphenols from food is important to prevent the oxidative stress due to over production of ROS [14-15]. ROS causes membrane damage, lipid peroxidation, protein oxidation and fragmentation, carbohydrate damage, mutagenesis and carcinogenesis [15-16].

*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 [17-20]. *G. cambogia* which used traditionally is also a well established plant for reducing weight.

In the present study the *in vitro* anti oxidant and anti bacterial activity against human pathogens (*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus substilis,)* was evaluated.

## MATERIALS AND METHODS

### Reagents

Diphenyl-picrylhydrazyl (DPPH), Linoleic acid, Ascorbic acid (Vitamin C) and α- tocopherol. Culture media's used was purchased from HIMEDIA laboratories, Mumbai, India. All other chemicals and solvents used for the analysis were of Analytical grade.

### Plant Material

The plant *Garcinia cambogia was* collected from Trivandrum, Kerala; it was then identified by Dr. N.K. Dubey, Plant Taxonomist, Department of Botany, Banaras Hindu University, Varanasi, India. The fruits of *Garcinia cambogia were* collected and rind was removed, shade dried and then finely powdered using mechanical grinder.

### Preparation of Extracts

The fruit rind of *G. cambogia* was extracted in different solvents namely hexane, ethyl acetate, ethanol and hydro- alcohol (60:40) of (water: ethanol) by cold extraction, in which powdered plant material was macerated in various solvents for 48 hrs. This was then filtered and the residue was dried and stored. All extracts were stored in sterilized containers at room temperature until used for laboratory testing. At the time of testing, the extracts were reconstituted to a concentration of 25mg/ml in Dimethyl Sulphoxide (DMSO).

### Test organisms

All the bacterial strains were obtained from Madras type cell culture (MTCC) *P. aeruginosa* (2599), *E. coli*(443)*, K. pneumonia*(3384), *B. subtilis* (441), *S. aureus*(7443).

**DPPH assay**

The DPPH assay is used to measure the free radical scavenging capacity of the plant extracts. DPPH is a convenient and accurate method for titrating the oxidisable groups of natural or synthetic antioxidants. 200 µL of 0.004% DPPH methanolic solution was pipetted into each well of a 96-well plate followed by 20 µL of sample, or solvent for the blank. The mixture was incubated at 30 °C for 1 h, and the absorbance at 515 nm was measured with a microplate reader [21-22]. The inhibition percentage of the radical scavenging activity was calculated using the equation.

$$\text{Inhibition (\%)} = 100 - 100\ (A_s \div A_o)$$

Where (A0) is absorbance of the blank and (AS) is absorbance of the sample at 515 nm.

The assay was conducted in triplicate.

**Hydroxyl radical scavenging activity**

2 ml of 6mM ferrous sulphate was added to different concentration of 2 ml of plant sample, and then 2 ml of 6mM hydrogen peroxide was added to it and kept for 10 minutes incubation, to this mixture 2 ml of 6mM sodium salicylate was added. This was kept for incubation at 37 degree for 30 minutes [23]. After the incubation period the samples were checked for absorbance at 510 nm and the % of inhibition was calculated using following equation.

$$\text{Inhibition \%} = 1 - (A_s - A_w / A_o) * 100.$$

Where, ($A_s$) is absorbance of the sample with sodium salicylate. ($A_w$) is Absorbance of the sample without sodium salicylate and ($A_o$) is Absorbance of the reagent.

**Ferric Thiocyanate (FTC) Assay**

The standard method as described [24]was used. A mixture of 4.0 mg of plant extract in 4 ml of absolute ethanol, 4.1 ml of 2.52% linolenic acid in absolute ethanol, 8.0 ml of 0.05 M phosphate buffer (pH 7.0), and 3.9 ml of water was placed in a vial with a screw cap and then placed in a dark at 40 C. To 0.1 ml of this solution were added 9.7 ml of 75% ethanol and 0.1 ml of 30% ammonium thiocyanate. Precisely 3 min after the addition of 0.1 ml of 0.02 M ferrous chloride in 3.5% HCl to the reaction mixture, the absorbance of red colour was measured at 500nm every 24 h until one day after the absorbance of control reached its maximum. α- tocopherol was used as positive controls, while a mixture without a plant extract was used as the negative control.

**Antibacterial activity**

The antibacterial activity was tested by agar-well diffusion method. Bacterial strains were grown and diluted using Mueller-Hinton broth. Bacterial strains were grown to exponential phase in Mueller-Hinton at 37$^0$C for 18hrs and adjusted to a final density of $10^8$ CFU/ml by diluting fresh cultures and comparison with Mc Farland density.

The antibacterial activity was tested by inoculating 500µl of Mueller- Hinton broth into 25 ml of nutrient agar and allowed to cool under strict aseptic conditions. On solidification of the medium wells were made in petriplates with the help of a sterile metal borer (7mm). 100µl of each extracts were filled in each after that the plates were incubated at 37$^0$ C for 24hrs. After proper incubation, antibacterial activity was determined by measuring the diameter of the zone of the inhibition around the well by using metric scale. Three replicates were carried out for each extract against each of the test organism.

**RESULTS**

**DPPH Assay**

Free radical scavenging potential of the four extracts and ascorbic acid at different concentrations was tested by DPPH method.

From the table 1, it is seen that all the four extracts of the plant *G. cambogia* are able to reduce the free radicals. The percentage of DPPH inhibition was found more ethanol extract of the plant which had 87±4.2% of inhibition at the highest concentration which was comparable with Ascorbic acid which had 94±2.8% of inhibition at the some concentration. Inhibition percentage of other extracts was hexane 59±2.3%, ethyl acetate 64±3.1%, and hydro alcohol had 79±2.1% each at their highest concentration.

**OH radical scavenging**

The plant extracts were tested for hydroxyl radical scavenging activity and the results are given in figure-1. It is seen that the percentage of OH radical scavenging activity was higher in hydro alcoholic extract of 82% followed by ethanol and ethyl acetate extract. Hexane showed minimum activity of 12%. The OH radical activity was comparable to standard used Ascorbic acid.

**FTC Assay**

The FTC method measures the amount of peroxide produced during the initial stages of lipid oxidation. The ferric ion combines with ammonium thiocyanate and produce ferric thiocyanate. The substance is red in colour. The four plant extracts were tested and showed strong antioxidant activity or differential capacity to inhibit LPO by FTC method which is indicated by their low absorbance values as seen in figure- 2.

**Anti microbial activity**

The antibacterial activity of crude extracts (hexane, ethyl acetate, ethanol and hydro alcoholic cold extracts at a concentration of 25mg/ml). It was seen that the hexane extract of *G. cambogia* showed no activity against all the organism. The positive results were seen for all the other extracts. The results of diameters of the zones of inhibition of extracts and antibiotic are presented in Table-2, and were interpreted as sensitive (18 mm), intermediate (14-17 mm) and resistant (<14 mm).

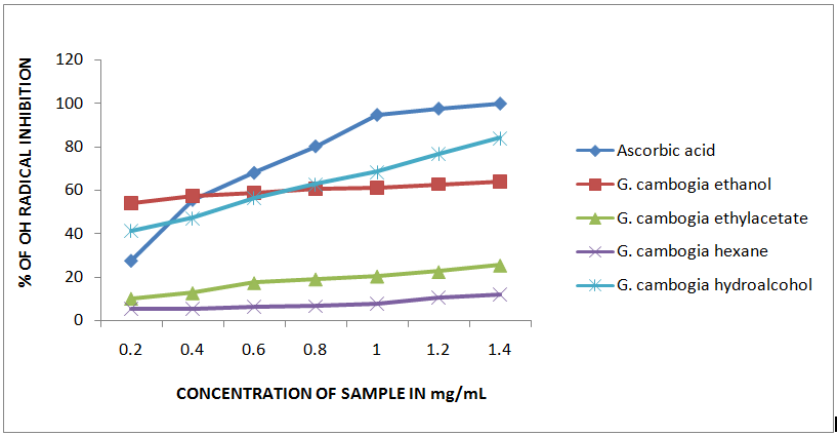


**Fig. 1: % Hydroxyl radical inhibition**

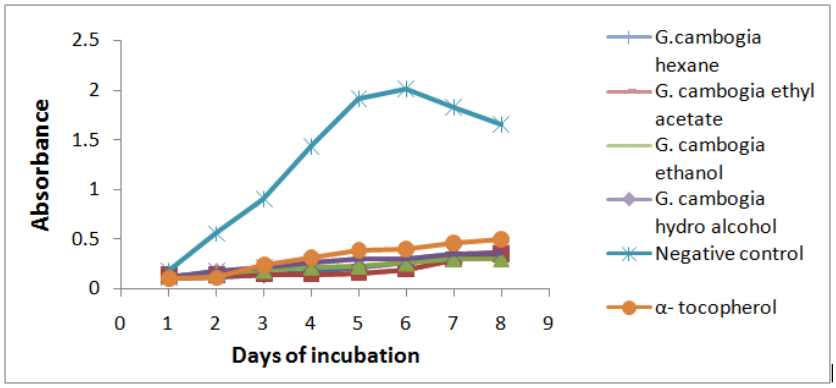


**Fig. 2: LPO inhibition by FTC method.**

**Table 1: % inhibition of DPPH radical in µg/ml**

| Extracts/standard | 10ug/ml | 50ug/ml | 100ug/ml | 200ug/ml | 300ug/ml |
|---|---|---|---|---|---|
| Hexane | 35.04 ±2.5 | 38.91±1.4 | 46.12 ±1.6 | 53.68 ±2.3 | 59.86 ±2.5 |
| Ethyl acetate | 37 ±1.2 | 43.49±1.9 | 52.01 ±2.4 | 58.41 ±1.7 | 64.08 ±3.1 |
| Ethanol | 29.92 ±3.1 | 66.05±2.3 | 72.55 ±1.2 | 82.63 ±2.5 | 87.98 ±4.2 |
| Hydro alcohol | 40.62 ±2.0 | 46.82±1.7 | 54.73 ±1.5 | 71.78 ±1. 8 | 79.81 ±2.1 |
| Ascorbic Acid | 26.33±1.7 | 43.5±2.6 | 60.27±3.1 | 76.81±1.9 | 94.53±2.8 |

**Table 2: Anti- bacterial Zone of inhibition of plant extracts in mm**

| Extracts | Zone of inhibition (mm) | | | | |
|---|---|---|---|---|---|
| | *E. coli* | *K .pneumonia* | *P .aeruginosa* | *B.subtilis* | *S. aureus* |
| Hexane | NA | NA | NA | NA | NA |
| Ethyl acetate | 34±2.3 | 23±4.2 | 32±3.4 | 27±1.2 | 23±1.4 |
| Ethanol | 27±1.9 | 21±2.1 | 24±1.8 | 22±2.4 | 19±4.8 |
| Hydroalcoholic | 21±3.5 | 19±1.6 | 18±2.7 | 16±1.3 | 15±3.9 |

NA- No activity. Values are the mean of three replicates.

## DISCUSSION

Reactive oxygen species (ROS), from both endogenous and exogenous sources, may be involved in diseases as arteriosclerosis, ischemic injury, cancer, and neurodegenerative diseases, as well as in processes like inflammation and ageing [25-27]. Antioxidants may be useful in preventing the deleterious consequences of oxidative stress and there is increasing interest in the protective biochemical functions of natural antioxidants contained in spices, herbs, and medicinal plants [28-29].

Free radicals like superoxide radicals, hydroxyl radicals, peroxyl radicals, and single oxygen are responsible for disease and ageing. Herbal drugs rich in antioxidants are gaining importance in treating such diseases. Many plants extract exhibit efficient antioxidant properties due to their phytoconstituents [30].

DPPH is a relatively stable radical [31], and one of the best methods to investigate the free radical scavenging activities of plants and its compounds. It is scavenged by antioxidants through the donation of proton forming the reduced DPPH. The colour changes from purple to yellow after reduction. Radical scavenging activity increases with increasing percentage of the free radical inhibition. Hydroxyl radical is a highly reactive oxidising agent that reacts with most biomolecules at diffusion controlled rates. They have a very short half life but are capable of causing damage within a small radius of its site of production. It damages the cell membrane extensively and causes cell death.

In the present study the plant fruit extract has shown the efficacy to inhibit the both DPPH and hydroxyl radical formation. It is also seen that the extract are able to prevent the lipid peroxidation through its low absorbance value in FTC assay.

Large evidence are available to demonstrate the potential of medicinal plants used in various traditional, complementary and alternate systems of treatment of human diseases [32]. It is known that the life span of antibiotics is limited, hence new sources are required especially from plant source which is used traditionally and there are several plants which are now used in common practice [33]. In our study preliminary screening of antibacterial activity of the fruit extract against human bacterial pathogenic strains demonstrated the ability of the extracts to inhibit the growth of the micro-organism hence can be further studied for isolation of individual component with antibacterial property.

## CONCLUSION

Conferring the great potentials of this plant used in traditional medicine, for various diseases like obesity, purgatives. And from the present study the plant had showed good antioxidant activity. The antimicrobial activity suggests us it is necessary to further investigation on isolation and purification of bioactive compounds responsible for the antibacterial activity.

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