ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF *GARCINIA CAMBOGIA* LEAF EXTRACTS

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Abstract: The study has been undertaken to evaluate the antibacterial and antioxidant activity of various solvent extracts (petroleum ether, chloroform, ethyl acetate and methanol) of *Garcinia cambogia* leaves. Antibacterial activity was performed by agar well diffusion method. Chloramphenicol was used as the positive control. All extracts showed antibacterial activity for the entire organism tested. Methanol extract showed highest activity than the other extracts. Among the four extract the highest activity obtained against *Streptococcus pneumonia* (19mm) at 50%concentration. The lowest activity obtained in the chloroform extract. Antioxidant activity was estimated by DPPH free radical scavenging activity. Among the four extracts, Methanol leaf extract of *G. cambogia* showed highest antioxidant activity. This study provided scientific evidence on the traditional use of *G. cambogia* leaf extract in treating bacterial diseases. Further, the leaf extract can possibly be used to produce alternative forms of antibacterials.

Keywords: *Garcinia cambogia,* antibacterial activity, antioxidant activity, methanol extract, agar well diffusion method.

I. INTRODUCTION

India is one of the main centers of the ancient human Civilization in the world where wild plants have been utilized for various purposes including herbal medicines [1]. Medicinal plants which from the backbone of traditional medicine have in the last few decades been the subject of very intense pharmacological studies. Medicinal plants are considered as rich resources of ingredients which can be used in drug development pharmacopoeial, non- pharmacopoeial or synthetic drugs. Apart from that, these plants play a critical role in the development of human cultures around the whole world. Over the past two decades, there has been a tremendous increase in the use of herbal medicine; however, there is still a significant lack of research data in this field [2]. A large number of plants are used in traditional system of medicine, which is wild in nature. Approximately, 3000 plants species are known to have medicinal properties in India [3]. Medicinal plants are used locally in the treatment of infections caused by fungi, bacteria, viruses and parasites. 60% of people in rural areas depend on the traditional medicine for the treatment of their ailments. Different plants have been used as a source of inspiration in the development of novel drugs [4]. World Health Organization (WHO) has defined medicinal plants as plants that contain properties or compounds that can be used for therapeutic purposes or those that synthesize metabolites to produce useful drugs. Several screening studies have been carried out in different parts of the world. There are several reports on the antimicrobial activity of different herbal extracts in different regions of the world [5].

Medicinal plants have been tested for biological, antimicrobial and hypoglycemic activity. They have also tested for antiulcerogenic, antihelminthic, hepatoprotective, analgesic, antipyretic, antileishmania and insecticidal activities [6]. Scientific investigations of medicinal plants have been initiated in many countries because of their contributions to health care. The primary benefits of using plant-derived medicines are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment [7].

Garcinia cambogia, also known as Malabar tamarind, and known as Garcinia, is a plant native to Southeast Asia. The leaves and fruits are sour, astringent, thermogenic, constipating and digestive. The leaves and fruits are sour, astringent, thermogenic, constipating and digestive. The herbal preparations made from *Garcinia* leaves and rinds are used in the treatment of inflammatory ailments, for rheumatic pains and bowel complaints [8].

Plant extracts have great potential antimicrobial compound against microorganisms [9]. The medicinal value of plants lies in the bioactive compounds such as alkaloids, flavonoids, tannins, and phenolic compounds that produce a definite physiological action on the human body. The increasing use of plant extracts in the food, cosmetic, and pharmacological industries suggests that in order to extract active compounds, a systematic study of medicinal plants is very important [10].The use of crude extracts of plants

parts and phytochemicals, of known antimicrobial properties, can be of great significance in the therapeutic treatments. In recent years, a number of studies have been conducted in various countries to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are due to the secondary metabolites synthesized by the plants. These products are known by their active substances like, phenolic compounds which are part of the essential oils, as well as in tanning. The screening of plant products for antimicrobial activity has shown that the higher plants represent a potential source of novel antibiotic prototypes [11].

Antioxidants scavenge free radicals and reactive oxygen species and can be extremely important in inhibiting oxidative mechanisms that lead to degenerative diseases [12]. Many herbs contain antioxidant compounds which protect the cells against the damaging effects of reactive oxygen species [13]. 2, 2-diphenyl-1-picrylhydrazyl is a stable (in powder form) free radical with purple colour which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. Antioxidants react with DPPH and reduce it to DPPH-H and as consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability. Free radicals have been implicated as playing a role in the etiology of cardiovascular disease, cancer, Alzheimer's disease and Parkinson's disease. The antioxidant capacity of most plant food sources is usually associated with their phenolic contents [14]. The objective of the present study is to evaluate the potential antibacterial activities and DPPH activity of *Garcinia cambogia* leaf extract.

II. MATERIALS AND METHODS

Collection and preparation of plant extracts

The leaves used in the present study (*Garcinia cambogia*) were collected from in and around Kerala. The *leaves* were brought to the laboratory immediately after collection and washed with tap water thoroughly followed by a final rinse with dechlorinated water, following which, they were shade dried at room temperature (21± 2˚C) for 48-72 hours, depending on the plant. The dried plants were ground to coarse powder (< 2mm) using an electric blender. For extraction 250 g of the plant powder was extracted with 2.5 litres methanol using a Soxhlet apparatus for 48 hours. Prior to extraction with methanol, the plant material was defatted with Petroleum ether, Chloroform, Ethyl acetate.

2.1 ANTIBACTERIAL ASSAY

2.1.1 *Test microorganisms*

The bacterial isolates used were the clinical isolates obtained from P.S.G. Institute of Medical Science and Research, Coimbatore. The bacterial isolates used were Gram positive bacteria: *Staphylococcus aureus, Streptococcus pneumoniae* and *Bacillus cereus* whereas, Gram negative bacteria: *Escherichia coli, Klebsiella pneumoniae* and *Salmonella typhi.*

2.1.2 *Agar well diffusion method*

The activity of various solvent extracts of leaves of *Garcinia cambogia* on selected bacterial species were assayed by agar well diffusion method. 20 ml of sterilized Muller Hinton Agar was poured into sterile petriplate, after solidification, 100 µl of fresh culture of human pathogens were swabbed on the respective plates. The wells were punched over the agar plates using sterile gel puncher at various concentration (10 µl, 20 µl, 30 µl, 40 µl and 50 µl) of each plant extract were added into the wells. Chloramphenicol was used as the positive control. The plates were incubated for 24 hours at 37˚C. After incubation the diameter of inhibitory zones formed around each well were measured in mm and recorded.

2.2 ANTIOXIDANT ACTIVITY

2.2.1 *DPPH (2, 2-diphenyl-1-picrylhydrazyl) scavenging assay*

To 0.01ml to 0.05ml of the fraction, 0.5ml of an ethanolic solution of DPPH and 0.49ml of ethanol were added. The mixture was allowed to react at room temperature for 30 minutes in the dark. Ethanol served as a blank and DPPH in ethanol, without plant extract fraction, served as the positive control. After 5 minutes of incubation, the decolourization of the purple to yellow colour was measured at 517 nm. The radical scavenging activity was calculated as,

$$\% \text{ RSA} = \{(\text{Abs control} - \text{Abs sample})\} / (\text{Abs control}) \times 100$$

Where, RSA is the Radical Scavenging Activity; Abs control is the absorbance of DPPH radical + methanol; Abs sample is the absorbance of DPPH radical + plant extract.

III. RESULTS AND DISCUSSION

3.1 *Antibacterial activity*

The leaves of *Garcinia cambogia* are used for the treatment of several infectious diseases. The choice of this plant for the present study was based on its medicinal properties and use in traditional medicinal system. The juice of the leaf is used to treat diarrhea, dysentery and glandular tumour. The potential beneficial

effects of *G. cambogia* include its anti-oxidant property, anti-helminthic, antimicrobial and anti-obesity and weight reducing agent.

The present study of antibacterial activity was conducted with various leaf extracts against six human pathogenic bacteria. The various solvent leaf extracts of *G. cambogia* showed significant antibacterial activity against *Bacillus cereus, Streptococcus pneumoniae, Staphylococcus aureus, Salmonella typhi, Klebsiella pneumoniae* and *Escherichia coli*. When compared to standard drug (positive control) the results observed were, methanol extract of the leaves of *G. cambogia* showed highest activity at 50% against *Bacillus cereus* (18.5mm), *Streptococcus pneumonia* (19mm), *Staphylococcus aureus* (15.75mm), *Salmonella typhi* (13.5mm), *Klebsiella* pneumoniae (13mm) and *Escherichia coli* (18.75mm).The chloroform extract showed the lowest activity against *Bacillus cereus* (7.75mm), *Streptococcus pneumonia* (8.5mm), *Staphylococcus aureus* (8.25mm), *Salmonella typhi* (8.25mm), *Klebsiella pneumoniae* (8.5mm) and *Escherichia coli* (8.5mm).

Maridass *et al*., [15] have also reported similar levels of antibacterial activity of *G. gummigutta* against *Aeromonas hydrophila* (28 mm), *Bacillus subtilis* (26 mm), *Staphylococcus aureus* (19 mm), *Salmonella typhi* (17 mm), *Pseudomonas auruginosa* (23mm) and *Klebsiella pneumoniae* (16 mm). Diethyl ether and methanol extract of the leaves of *G. gummigutta* do not inhibit the growth of *E. coli* whereas aqueous extract recorded highly significant inhibitory activity against *E. coli* reported by Devi Prasad *et al*., [16]. Iinuma *et al*., [17] have reported the antibacterial activity of *Garcinia* against methicillin resistant *S. aureus*. The significant levels of antibacterial activity of ethanol and methanol extracts of fruit rind of *Garcinia* have been reported against *Micrococcus aureus, B. megaterium* and *P. aeruginosa* studied by Sutar *et al*., [18].

Lakshmi *et al*., [19] have reported the antibacterial activity of stem bark extract of the *G. indica* against *Staphylococcus aureus, Escherichia coli* and *Salmonella typhi*. Sankar Kumar Dey [20] reported that, alcoholic extract of leaves showed antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* and aqueous extract showed activity against *Staphylococcus aureus*. The plant extracts in organic solvent (methanol) provided more consistent antimicrobial activity compared to those extracted in water. Mahato and Chaudhary [21] reported that, the ethanol and chloroform extracts of *Adhathoda vasica* significantly inhibited the growth of *E. coli, Salmonella typhi, S.aureus* and *Bacillus subtilis*. Methanolic extract of *Adathoda vasica* exhibited positive antimicrobial activity against *Pseudomonas aeruginosa, S. aureus* and *B. subtilis*. These results supports the studies carried out by Brantner and Chakraborty [22], methanolic extract of *Adhatoda vasica* showed antibacterial activity against the microorganisms greater than the aqueous extract of *Adhatoda vasica*.

3.2 *DPPH free radical scavenging activity*

Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties [23]. A rapid,simple and inexpensive method to measure antioxidant capacity of food involves the use of the free radical, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) which is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity [24]. In the current study, the methanol extract of *G.a cambogia* showed the maximum DPPH free radical scavenging activity. The methanol extract of *G. cambogia* demonstrated significant DPPH radical scavenging activity. The DPPH radical scavenging activity of the extract was found to be increasing with increase in the dose. Antioxidant activity of methanolic extracts of *G. gummigutta* leaf showed both anti-oxidant and anti-hepatotoxicity activity was reported by Thamizh Selvam *et al*.,[25]. Likewise Kaur *et al*., [26], evaluated the antioxidant and antimicrobial activities of aqueous and methanolic extracts of *Adhatoda vasica*.

From antioxidant study the DPPH activity observed were maximum in methanolic extract when compared to other extracts. Kumar *et al*., [27] reported that the antioxidant activity of *Adhatoda vasica*, due to the presence of tannins and saponins contributes to the high antioxidant activity. Singh *et al*., [28] investigated and reported that the methanolic extracts have high antioxidant activity than the standard ascorbic acid in the DPPH and Hydrogen peroxide radical scavenging environments.

Study on DPPH scavenging ability of *A. paniculata* aerial parts by Arash *et al*. [29] had shown 86.87% scavenging of DPPH radicals at a concentration of 10 mg/ml of ethanol extract. Study carried out by Hasan *et al*. [30] has shown the DPPH radical scavenging activity of *T. cordifolia* aerial parts with an IC50 value of 0.02 mg/ml. The difference in the IC50 value can be attributed to the distribution of secondary metabolites that may fluctuate between different plant organs by Lisiewska *et al*., [31].

The presence of phytoconstituents such as flavonoids and phenolic compounds may be responsible for the above radical scavenging activities by Kawsar *et al.*, [32]. Kim and Lee, [33] reported that the antioxidant activity of *M. uniflorum* was due phenolic acid content which can facilitate electron delocalization between the aromatic ring and propenoic acid. Ravishankar and Vishnu Priya, [34] and

Marathe *et al*., [35] reported that ethnolic extracts of *M. uniflorum* was responsible for radical scavenging activity by DPPH methods.

IV. CONCLUSION

Garcinia cambogia are medicinal plants that contain the phytochemical constituents such as alkaloids, flavonoids, terpanoids, phlobatannins and reducing sugars. Medicinal plants have vital role in preventing various diseases. *G. cambogia* are important plant with several medicinal uses in folk and traditional medicinal system. In the present study it was revealed that the antibacterial, antioxidant properties of various extract of these selected plant leaves have a great potential to be developed as drugs in pharmaceutical industries. From the results it could be concluded that *G. cambogia* contain various bioactive compounds and further study of these compounds by using advance analytical techniques may prove the medicinal importance in future.

Table 1- Antibacterial activity of *Garcinia cambogia* in petroleum ether leaf extract

SL. NO	BACTERIA	ZONE OF INHIBITION(mm)					
		CONCENTRATION					
		10%	20%	30%	40%	50%	CONTROL
1	*Bacillus cereus*	7.00±0.0	8.75±0.43	10.00±0.0	11.25±0.43	12.50±0.50	39.00±0.0
2	*Streptococcus pneumoniae*	13.00±0.50	13.00±1.00	13.75±2.68	14.50±1.11	17.50±1.11	43.00±0.1
3	*Staphylococcus aureus*	8.00±0.00	8.50±0.50	8.50±0.50	8.50±0.50	9.00±0.0	41.00±0.0
4	*Salmonella typhi*	8.00±0.0	8.25±0.43	8.25±0.43	8.50±0.50	9.00±0.0	42.50±2.1
5	*Klebsiella pneumoniae*	8.00±0.0	8.00±0.0	8.25±0.43	8.25±0.43	8.50±0.50	40.00±0.0
6	*Escherichia coli*	7.25±0.43	7.50±0.50	8.25±0.43	8.50±0.50	8.75±0.43	45.00±0.0

Table 2- Antibacterial activity of *Garcinia cambogia* in chloroform leaf extract

SL. NO	BACTERIA	ZONE OF INHIBITION(mm)					
		CONCENTRATION					
		10%	20%	30%	40%	50%	CONTROL
1	*Bacillus cereus*	7.00±0.0	7.00±0.0	7.25±0.43	7.50±0.50	7.75±0.43	39.00±0.0
2	*Streptococcus pneumoniae*	7.25±0.43	7.50±0.50	7.50±0.50	8.25±0.43	8.50±0.50	43.00±0.1
3	*Staphylococcus aureus*	7.00±0.0	7.50±0.50	7.75±0.43	8.00±0.0	8.25±0.43	41.00±0.0
4	*Salmonella typhi*	7.00±0.0	7.50±0.50	7.75±0.43	7.75±0.43	8.25±0.43	42.50±2.1
5	*Klebsiella pneumoniae*	7.25±0.43	7.25±0.43	7.50±0.50	7.50±0.50	8.50±0.50	40.00±0.0
6	*Escherichia coli*	7.25±0.43	7.75±0.43	8.00±0.0	8.25±0.43	8.50±0.50	45.00±0.0

Table 3- Antibacterial activity of *Garcinia cambogia* in ethyl acetate leaf extract

SL. NO	BACTERIA	ZONE OF INHIBITION(mm)					
		CONCENTRATION					
		10%	20%	30%	40%	50%	CONTROL
1	*Bacillus cereus*	7.50±0.0	8.25±0.43	10.00±0.0	10.50±0.50	11.00±0.0	39.00±0.0
2	*Streptococcus pneumoniae*	10.00±0.0	10.00±0.0	12.00±0.0	13.00±0.0	15.00±0.0	43.00±0.1
3	*Staphylococcus aureus*	9.00±0.0	10.50±0.0	10.50±0.50	12.00±0.0	13.00±0.0	41.00±0.0
4	*Salmonella typhi*	8.00±0.0	10.00±0.0	11.00±0.0	11.50±0.50	13.00±0.0	42.50±2.1
5	*Klebsiella pneumoniae*	10.00±0.0	10.00±0.0	11.00±1.0	11.25±0.43	11.50±0.50	40.00±0.0
6	*Escherichia coli*	9.75±0.43	10.50±0.50	11.00±1.0	11.25±0.43	12.00±1.00	45.00±0.0

Table 4 - Antibacterial activity of *Garcinia cambogia* in methanol leaf extract

SL. NO	BACTERIA	ZONE OF INHIBITION(mm) CONCENTRATION					
		10%	20%	30%	40%	50%	CONTROL
1	*Bacillus cereus*	8.00±0.0	10.00±0.0	11.00±1.0	14.50±0.50	18.50±0.50	39.00±0.0
2	*Streptococcus pneumoniae*	10.00±0.1	12.50±2.50	15.00±0.0	17.50±2.50	19.00±0.86	43.00±0.1
3	*Staphylococcus aureus*	9.00±0.0	11.00±0.0	11.00±0.0	15.00±0.0	15.75±0.82	41.00±0.0
4	*Salmonella typhi*	7.50±0.0	9.00±0.0	11.00±0.0	12.00±0.0	13.50±1.50	42.50±2.1
5	*Klebsiella pneumoniae*	8.00±0.0	10.00±0.0	10.50±0.50	12.00±0.0	13.00±0.0	40.00±0.0
6	*Escherichia coli*	10.00±0.0	11.25±2.16	12.50±0.50	14.25±0.43	18.75±4.14	45.00±0.0

Table 5 - DPPH free radical scavenging activity of *Garcinia cambogia*

Treatment Group	Plant extract (µl)	DPPH in Ethanol (µl)	Ethanol (µl)		OD at 517 nm			
Negative Blank	-	-	1000µl	Incubation at room temperature for 5 minutes	0.000			
Positive Control	-	500 µl	500 µl		0.224			
					PE	**CH**	**EA**	**MT**
Plant extracts	10 µl	500 µl	490 µl		95.53%	88.39%	95.08%	94.19%
	20 µl	500 µl	480 µl		94.19%	83.92%	91.96%	89.28%
	30 µl	500 µl	470 µl		92.85%	82.58%	84.37%	85.71%
	40 µl	500 µl	460 µl		89.28%	79.91%	71.42%	75.89%
	50 µl	500 µl	450 µl		76.78%	75.0%	57.14%	54.91%

PE-petroleum ether, CH- chloroform, EA-ethyl acetate, MT-methanol.

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