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Original Article

GC-MS/HPTLC Analysis, Antioxidant, Anti-inflammatory and Antimicrobial Efficacy of *Garcinia cambogia*

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ABSTRACT

Background: Natural substances that were initially obtained from plants were once regarded as a beneficial source of possible medicinal agents and were recognized as crucial in the development of human diseases. The current study sought to determine whether hydroalcoholic extracts of *Garcinia cambogia* plant fruits included any bioactive substances. **Materials and Methods:** The plant fruits were collected, shade-dried, ground into a fine powder, and then extracted using Soxhlet extractors and organic solvents. After that, the extracts were examined for the presence of phytochemicals using GC-MS/HPTLC, antioxidant, anti-inflammatory, and antibacterial techniques. According to the GC-MS studies, the hydroalcoholic extracts of *G. cambogia* contained 25 bioactive components. **Results:** The secondary metabolites identified in the plant, discovered by GCMS/HPTLC, are what give the plant extracts their powerful medicinal qualities. The results of the antimicrobial activity demonstrated outstanding antioxidant and anti-inflammatory properties as well as effective dose-dependent inhibitory activity against all of the tested species. **Conclusion:** The current study provided early data on the bioactive components present in *G. cambogia* fruit extracts, which have outstanding antimicrobial, antioxidant, and anti-inflammatory action, which could provide a significant platform for pharmaceutical companies to create different *Garcinia* species-based medications.

Keywords: *Garcinia cambogia*, GCMS, HPTLC, Antibacterial, Antioxidant, Anti-inflammatory.

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INTRODUCTION

The primary metabolism, which is critical to a plant's existence, is the generation of metabolites required for growth and function. Secondary metabolism, which is produced in reaction to physical, chemical, and biological elements formed in the plant's contact with its environment, is not required for survival. Secondary metabolism protects plants from biotic and abiotic stress by producing secondary metabolites and activating the plant's defence mechanism.[1,2]

More than 200,000 secondary metabolites are thought to be produced by plants; the most diverse ones include flavonoids, alkaloids, and polyphenols.[3,4] A well-known class of substances with antioxidant capabilities called polyphenols is found in plants as secondary metabolites. The walls and vacuoles of epidermal and sub-epidermal cells have higher concentrations of polyphenols. The fundamental building block of polyphenols is a kind of phenyl ring with a hydroxyl group. Due to their fundamental design, polyphenols can be divided into several subfamilies, such as tannins and flavonoids.[5-7]

The 15-carbon phenyl benzopyran skeleton of flavonoids is present in plants.[8] They are produced in all plant sections and are in charge of giving flowers their colour. The plant is shielded by polyphenols from some insects and UV light. Tannins are high molecular weight (500–20,000 Daltons) compounds that are formed in seeds, roots, bark, wood, and leaves. They have an astringent flavor that makes them resistant to predators like birds, herbivores, and some insects.[9]

Alkaloids are secondary metabolites that were initially classified as pharmacologically active molecules largely made of nitrogen. They are created from lysine, tyrosine, and tryptophan, three of the few commonly occurring amino acids. Plants have been shown to contain more than 12,000 alkaloids, representing more than 150 families, and about 20% of the "species of flowering plants" include alkaloids. Alkaloids are typically found in plants as salts of organic acids, including acetic, malic, lactic, citric, oxalic, tartaric, and tannic acids. Some weak basic alkaloids, like nicotine, are present in nature without restriction. Some alkaloids can also be found as glycosides of sugars like glucose, rhamnose, and galactose, such as the solanum group alkaloid solanine, as amides (piperine), and as esters of organic acids (atropine,

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cocaine). Reactive Oxygen Species (ROS), Reactive Nitrogen Species (RNS), and Reactive Sulphur Species (RS) are just a few examples of free radicals that scavenge these metabolites. These radical species influence cells by a variety of processes, including the disturbance of homeostasis, interference with signaling pathways, modification of DNA integrity, and production of organismal imbalance. Cardiovascular and chronic degenerative illnesses, as well as various types of cancer, can all be caused by these pathways.[10]

Due to bacterial resistance to several medications, many patients in clinics have difficulties brought on by microorganisms, which causes issues and delays in patients' ability to recover from their illnesses.[11] We are at a phase that could be critical since antibiotics are losing their efficacy and previously treatable common bacterial diseases are becoming more challenging. These issues result from bacterial strains becoming more and more resistant to antibiotics. As this phenomenon worsens and there is a lack of adequate and efficient treatment, many people may risk dying. Plants are one of the key sources of novel active compounds with antibacterial action, which is why this is necessary.

Plants are a great source of chemical compounds that are renewable and have a variety of advantages for people. It is challenging to pinpoint individual chemicals linked to a given trait since plants create a vast range of molecules. One of the chromatographic techniques is designed to isolate and identify the compounds of complex substances using Gas Chromatography coupled to Mass Spectrometry (GC-MS) using various plant parts of the plant and with various kinds of solvents.[12-16]

To identify the volatile chemicals in the extracts, several studies used GC/MS to assess the chemical constituents of the aerial parts of the plants. For instance, 77 different chemicals were discovered to be present in the mint extract, with menthol and P-menthan-3-one exhibiting the greatest levels of antibacterial action.[17] According to certain studies,[18-21] antioxidant activity and plant volatile profile are related. On the other hand, there aren't many GC/MS characterisation investigations of *Garcinia gummi-gutta.*

The Malabar tamarind, also known as *Garcinia gummi-gutta* (L.) Roxb. or *Garcinia cambogia* (Gaertn.) Desr. (Clusiaceae), is a native of Southeast Asia. In many Asian nations, the peel of the fruit has traditionally been used to cure oedema, irregular menstruation, rheumatism, piles, constipation, and intestinal parasites. It is also commonly employed as a food preservation, flavour, or bulking agent.[22] Numerous scientific research has indicated biological activity, like anti-obesity,[23,24] hypolipidemic,[25] and anti-cancer.[26] More additional phytochemical information on the plant directed to the isolation of various organic acids,[27] benzophenones,[28] and xanthones,[29] as major constituents.

Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacillus subtilis, and *Staphylococcus aureus* are only a few examples of the varied biological properties that are not unique to the species *G. cambogia*. The *G. cambogia* extract did not affect any of the pathogens tested. This work aims to undertake the phytochemical, antioxidant, anti-inflammatory, and GC-MS/TLC profile of the hydroalcoholic extracts of *G. cambogia* fruit to assess the changes in antioxidant compound concentrations among them. This was done to see which concentration has the most anti-inflammatory, antioxidant, and antibacterial action.

MATERIALS AND METHODS

Reagents

All of the compounds were of analytical quality and could be used right away without additional purification. All chemicals were prepared using deionized water.

Preparation of Extracts

Harvesting and Identification of G. cambogia

The plant was gathered from several conservatories in Tamil Nadu, India, between February and June 2022.

Cleaning and Drying

Plants were split into fruit portions, and after the common dirt was removed, drinkable water was utilized to rinse the plants. To minimize humidity buildup, the plant material was dried at ambient temperature in the lack of light using printing paper that was replaced every 24 hr.

Extracts Preparation

Dry *G. cambogia* fruit can be used as a test sample, crushed and filled in a porous cellulose thimble. PHF and 70% ethanol are added to a Soxhlet extractor and condenser, which evaporate for 4 hr. The extract is kept in the refrigerator for future use.[30]

Total Ash

In the incineration dish, weigh 1 g of the test sample after it has been heated for at least 30 min at 550°C in the muffle furnace, cooled in the desiccator, and weighed to the nearest 0.001 g.[31]

The crude ash, expressed as a percentage test sample, is equal to by mass of the 100,

$$(m2 – mo) \times ml —MO$$

Phytochemical screening

A preliminary qualitative analysis of the plant extracts was used to check for the presence of bioactive compounds in the *G. cambogia* fruit. Recognizing Resins, Carboxylic acid, tannins, steroids, flavonoids, glycosides, proteins (Bradford Method), phenol (ferric chloride test), saponin and saponification tests,

gum tests, flavanoglycoside detection, Mayer's test for alkaloids, biuret test, and carbohydrate detection are all kinds of substances that can be detected by the methods.[32-36]

Quantitative phytochemical screening

Quantification of Alkaloids

G. cambogia fruit extract was mixed with 10% acetic acid in ethanol for 4 hr, then heated to reduce its original volume, filtered, and added 15 drops of ammonium hydroxide. After 3 hr, the precipitate was collected and the supernatant was discarded. 20mL of 0.1M was used to wash the precipitates, and the residue was collected using ammonium hydroxide and filtered, which quantitatively indicates the amount of alkaloid present in the sample.[37]

Quantification of Saponin

G. cambogia fruit extract was added to 10mL of 20% aqueous ethanol and heated to 55°C for 4 hr. After filtering, the residue was extracted using 10mL of 20% aqueous ethanol and evaporated to 40mL at 90°C. Diethyl ether was added and mixed vigorously in the separating funnel. The NaCl layer was removed after extracting 6mL of n-butanol twice with 1mL of 5% NaCl. After heating for 30 min, the residual solution was dried in a crucible.[38]

Quantification of Phenol

The total content of phenol in *G. cambogia* fruit extract was estimated using the Folin Ciocalteu technique. 1 mL of aliquots and standard gallic acid, 5 mL of distilled water and 0.5 mL of Folin Ciocalteu's reagent, 10 mL of distilled water and 1.5 mL of 20% sodium carbonate were placed in test tubes and set at room temperature for 2 hr. A deep blue hue was created and a UV-visible spectrophotometer was used to detect the absorbance at 750 nm. Triplicate tests on the *G. cambogia* fruit extract were conducted. Reagent blank with solvent was used to complete the blank. According to data from Bhalodia *et al.*[39] the phenolic content of the plant was represented as mg of Gallic Acid Equivalent weight (GAE)/100 g of dry mass.

Quantification of Flavonoid

The aluminium chloride colorimetric assay was employed to quantify the total flavonoid concentration in *G. cambogia* fruit extract. Test tubes were filled with 4 mL of distilled water, 0.3 mL of 5% sodium nitrite solution, 1 mL of aliquots, and 1 mL of standard quercetin solution. 5 min later, 0.3 cc of 10% aluminium chloride was added and 2 mL of 1 M sodium hydroxide was added. The absorbance at 510 nm was measured with UV-visible equipment. Triplicate tests were conducted to visualize the calibration curve and express the total flavonoids in *G. cambogia* fruit extract according to the method from Patela *et al.*[40]

Quantification of Terpenoids

G. cambogia fruit extract weighing 1g was steeped in ethanol for 24 hr. After filtering the solution, petroleum ether was used to extract the filtrate. The ether extract was gathered and calibrated. The number quantifies how many terpenoids are present in the samples.[41]

Total Terpenoids = Initial weight – Final weight/ Initial weight

Gas Chromatography-Mass Spectrometry Analysis

The volatile and semi-volatile components of *G. cambogia* fruit extracts were analyzed using an Agilent 7890B Gas Chromatograph (GC) and an Agilent 5977A Mass Selective Detector (MSD), using helium as the carrier gas and a flow rate of 1 mL/min.

High-Performance Thin Layer Chromatography

The tissue lyophilizes was reconstituted in distilled water at a concentration of 10 mg/mL. Then, using a Camag syringe of 100 mL and an autosampler called a Linomat 5, 15 mL of the solutions were placed in bands measuring 8 mm in length on aluminium backing plates covered with silica gel $60F_{254}$ (20 10 cm).

The plates were placed in a glass container that had been pre-saturated for 30 min with the mobile phase, which is made up of water, methanol, and ethyl acetate in the ratios of 10:14:76 v:v. Then the dried plates were then examined in a UV chamber at two different wavelengths of 254 and 366 nm. TLC plates were also made with precise solutions for each kind of compound: trichloroacetic acid solution (glycosides), ferric chloride solution (tannin), Dragendorff's reaction (alkaloids), and aluminium chloride solution (flavonoids).

The retention factor was calculated using the following equation:

$$R_f = \text{Distance the band / Distance of the solvent}$$

Antioxidant Activity

Dilutions of the freeze-dried extracts of *G. cambogia* were generated from 13 to 1000 g/mL in order to assess the antioxidant activity of the hydroalcoholic extract of *G. cambogia* fruit extract using the DPPH, ABTS, H_2O_2, and Nitric Oxide Radical Scavenging Assay procedures. These samples served as antioxidant tests. Using the statistical tool Graph Pad version 9, a straightforward ANOVA analysis was used to determine the half-maximal inhibitory concentration (IC_{50}).[42-44]

Anti-inflammatory Activity

Inflammation is primarily brought on by denaturing of proteins. The method of Mizushima, Kobayashi, and Sakate *et al.*,[45] was slightly modified to assess protein denaturation inhibition. The test sample of *G. cambogia* fruit extract (500, 250, 100, 50, and 10 g/mL) was mixed with 500 L of 1% bovine serum albumin.

Antimicrobial Activity

The antimicrobial experiments included bacterial (*Staphylococcus aureus*-902) and fungal (*Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus,* and *Cryptococcus neoformans*) microbial strains (*Pseudomonas aeruginosa*-1688, *E. coli*-443, *Propionibacterium acnes*-1951). By Agarwell-diffusion methods according to the standard protocols.[46-49]

RESULTS

Phytochemical Evaluation

The majority of the compounds that plants make are secondary metabolites that serve as defence against predators, give them their distinctive smells, and give them their distinctive pigmentation. The majority of phytochemicals are widely employed as pharmaceutical ingredients to treat a variety of diseases around the world.

Table 1: Qualitative phytochemical screening of *G. cambogia*.

Sl. No.	Phytochemical compound	Results
1	Resins	+
2	Carboxylic acid	+
3	Tannins –	–
4	Steroids	–
5	Flavanoids	+
6	Carbohydrates	+
7	Glycosides	–
8	Saponification	+
9	Proteins	+
10	Phenol	+
11	Biuret	–
12	Saponin	+
13	Gum	–
14	Flavanoglycoside	–
15	Alkaloids	+

+ Positive; – Negative

The quantitative analysis of the phytochemical components in *G. cambogia* leaves ethanol extract showed the presence of resins, carboxylic acid, flavonoids, carbohydrates, proteins, saponification, phenol, saponin, and alkaloids, while tannins, steroids, glycosides, biuret, gum, and flavanoglycoside are absent, as shown in (Table 1).

Quantification of Phenols, Terpenoids, Alkaloids, Saponins and Flavonoids

Spectrophotometrically using the Folin-Ciocalteu technique. For each gram of *G. cambogia*, the outcomes were represented as mg of Gallic Acid (G.A.) equivalents. The *G. cambogia* fruit had the greatest phenol concentrations, with 2.5 mg/mL and 5 mg/mL, respectively (Table 2 and Figure 1) display the values that have been graphed.

The aluminium trichloride technique was used to determine the flavonoid concentration. The results were given in mg Quercetin Equivalents (Q.E.) per gram of *G. cambogia*. With 0.49 g/mL, the *G. cambogia* fruit had the highest concentration of flavonoids. Table 3 and Figure 2 displays the values that have been graphed.

The vanillin-sulfuric acid colour reaction was used to determine the saponin concentration. Results showed that *G. cambogia* saponin was % Saponin = Weight of saponin/Weight of sample 100. With 0.17 g/mL, the *G. cambogia* fruit had the highest concentration of saponin. % Saponin = 0.17 / 0.5* 100 = 39%.

Dragondroff's method was used to determine the alkaloid content. Results were given in terms of mg% Alkaloid, which is calculated as follows: weight of alkaloid/weight of sample 100 of *G. cambogia*. With 0.87 g/mL, the *G. cambogia* fruit had the highest concentration of alkaloids. % Alkaloid = 0.87/ 1.25* 100 = 69.6%.

The Salkowski methods were used to determine the terpenoid content. The findings were presented as mg Total Terpenoids=

Table 2: The total phenol content was present in the extract *G. cambogia*.

Sl. No.	OD value at 750 nm	Total Phenol content	Mean value of total phenol content
1	0.073	0.24	0.32
2	0.090	0.32	
3	0.083	0.42	

Table 3: The total flavonoids content was present in the extract *G. cambogia*.

Sl. No.	OD value at 510 nm	Total Flavonoid content	Mean value of Total Flavonoid content
1	0.184	0.403	0.49
2	0.190	0.498	
3	0.196	0.593	

Initial weight - Final weight/ Initial weight 100 of *G. cambogia*. With 0.87 g/mL, the *G. cambogia* fruit had the highest concentration of Terpenoids.

Determination of total ash

The outcomes of plant ash's physicochemical investigation, The *G. cambogia* ash was displayed as a fine powder. *G. cambogia* 0.39 (grams) had the highest percentage of loss during drying.

GC-MS Analysis of *G. cambogia*

We chose to use Gas Chromatography combined with Mass Spectrometry (GC-MS) to screen for volatile and semi-volatile chemicals to determine the chemical makeup of the *G. cambogia* extracts. hydroalcoholic extracts were subjected to GC-MS analysis, which revealed a total of 25 chemicals (Table 4). Since the root is the plant's covert portion, whereas the stem, leaf, and flower are its aerial portion, this distinction in chemical constituents may be explained by the qualities of the samples.

High-Performance Thin Layer Chromatography

The *G. cambogia* was separated using Thin-Layer Chromatography (TLC) with a mobile stage of water, methanol, and ethyl acetate with a ratio of 10:14:76. The plates were made employing the unique chemicals of each group of chemicals and then subjected to ultraviolet light at two different wavelengths including 254 and 366 nm. To conceive the tannins, 1% ferric chloride solution; the plates indicated the existence of bands in all of the FDE. The existence of two specific bands with R_f = 0.015 and R_f = 0.036, suggests that the combinations discovered in these retention factors are present in all tissues of the plant. Flavonoids were created in a similar manner using 1% aluminium chloride. All of the extracts had responsive bands to this solution, with FDE-Flower having the most elaborate banding pattern (18 bands). The R_f for each experiment's bands. Only two chromatoplate extracts revealed any bands when visualized at 254 nm, but all extracts from banding patterns visualized at 366 nm revealed complicated banding patterns (Figure 3). Compounds containing the alkaloid developer were not discovered.

Antioxidant Activity of *G. cambogia*

Using the four methods outlined above for measuring antioxidant capacity, we examined the fractions that were extracted with the solvents. The fractions of *G. cambogia* fruit extract that had the highest antioxidant activity were those that were employed in all four of the techniques.

In the DPPH, ABTS, Hydrogen peroxide, and Nitric oxide radical assays, a hydroalcoholic extract made from the fruit exhibited *in vitro* antioxidant properties. The extract demonstrated ABTS, DPPH radical, hydrogen peroxide, and nitric oxide radical scavenging activities with IC_{50} values of 134.0 g/mL, 82.95 g/mL, 107.3 g/mL, and 138.7 g/mL, respectively. Fractions of the plant had the most suitable profiles of antioxidant capacity, phenol content, and flavonoids. The graphs of the reported antioxidant activity are shown in Figures 4A-D.

Evaluation of anti-inflammatory activity

Protein stabilization concerning the control is measured by the protein denaturation percentage inhibition. Figure 5 displays the test's outcomes. The findings demonstrated that hydroalcoholic extract has increased anti-inflammatory action.

Table 4: The report of GC-MS analysis showing 25 bioactive compounds present in the hydroalcoholic extract of *G. cambogia* fruits.

Peak	Peak	Peak	Peak	Area %	Height	Height %	A/H	Name
1	10.004	TIC	27214	5.7	15910	6.68	1.71	Benzoic acid, 2,5-bis(trimethylsiloxy)-, trimethylsilyl ester
2	13.931	TIC	75659	15.85	42031	17.64	1.8	Cyclohexasiloxane, dodecamethyl-
3	17.545	TIC	38636	8.09	22568	9.47	1.71	3-Butoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane
4	20.786	TIC	29286	6.13	18368	7.71	1.59	Benzoic acid, 2,4-bis(trimethylsiloxy)-, trimethylsilyl ester
5	23.584	TIC	16043	3.36	10668	4.48	1.5	Phosphonous dibromide, [2,2,2-trifluoro-1-(trifluoromethyl)-1-[(trimethylsilyl)oxy]ethyl]-
6	28.347	TIC	8972	1.88	4487	1.88	2	1-(3,4-ditrimethylsiloxyphenyl)-2-isopr opylaminoethanol
7	30.411	TIC	10789	2.26	5950	2.5	1.81	Phosphonous dibromide, [2,2,2-trifluoro-1-(trifluoromethyl)-1-[(trimethylsilyl)oxy]ethyl]-

Peak	Peak	Peak	Peak	Area %	Height	Height %	A/H	Name
8	32.318	TIC	11164	2.34	7405	3.11	1.51	Naphtho[2,1-b]furan-6-carboxylic acid, 3a-(1,3-dioxolan-2-yl)dodecahydro-2-hydroxy-6,9a-dimethyl-, methyl ester, [2r-(2.alpha.,
9	34.117	TIC	23690	4.96	11649	4.89	2.03	2,2,4,4,6,6,8,8,10,10,12,12,14,14,16,16,18,18,20,20- Icosamethylcyclodecasiloxane#
10	34.868	TIC	54612	11.44	22024	9.24	2.48	1,2-benzenedicarboxylic acid, diisooctyl ester
11	34.935	TIC	1690	0.35	2296	0.96	0.74	Cyclohexadecane-1,2,9,10-tetraone
12	35.808	TIC	25543	5.35	12195	5.12	2.09	Cyclononasiloxane, octadecamethyl-
13	37.393	TIC	34416	7.21	14432	6.06	2.38	Cyclononasiloxane, octadecamethyl-
14	37.515	TIC	10853	2.27	2596	1.09	4.18	5,10-ethanobenzocycloocten-11-ol, 5,6,7,8,9,10-hexahydro-, (5.alpha.,10.alpha.,11s*)-
15	37.905	TIC	1679	0.35	1932	0.81	0.87	Chlorine dioxide
16	38.216	TIC	1648	0.35	2876	1.21	0.57	3,4-pentadien-2-one, 1,1,1-trifluoro-
17	38.345	TIC	6728	1.41	4575	1.92	1.47	Dimethyl-flubendazole
18	38.45	TIC	8207	1.72	2646	1.11	3.1	Dimethyl-flubendazole
19	38.621	TIC	7670	1.61	3507	1.47	2.19	1,3-propanediol, 2-[1-(dimethoxyamino)-1-methylethyl]-
20	38.67	TIC	9709	2.03	4105	1.72	2.37	2'-deoxy-guanosine-triacetyl derivative
21	38.76	TIC	8772	1.84	3332	1.4	2.63	2'-deoxy-guanosine-triacetyl derivative
22	38.903	TIC	40724	8.53	13168	5.53	3.09	Cyclononasiloxane, octadecamethyl-
23	39.065	TIC	6560	1.37	3259	1.37	2.01	N-methyl-3-(ethylcarbonyloxy)-4,5-epoxy-6-methoxy morphinan-1-ene
24	39.12	TIC	3473	0.73	2697	1.13	1.29	.Alpha.-(p-methoxyphenyl)-.alpha.,.alpha.'-dimethoxypropanone-2,4-dinitrophenylhydrazone
25	39.16	TIC	13697	2.87	3555	1.49	3.85	Cis-fused 1-benzyl-1,4,4a,5,6,7,8,8a-octahydro-3,2,1-benzoxathiazine 2-oxide

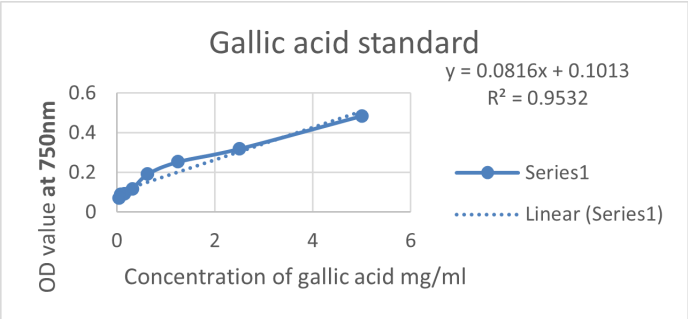


Figure 1: The total phenol content was present in the extract *G. cambogia*.

IC_{50} 53.88 g/mL is the value of the *G. cambogia* fruit extract. It is common knowledge that tissue protein denaturation results in inflammatory situations.

Antimicrobial Activity of *G. cambogia*

The hydroalcoholic extract of *G. cambogia* dried fruit rind was tested for antimicrobial activity against the tested bacteria strains by detecting the presence or absence of a zone of inhibition utilizing the agar well diffusion technique. The hydroalcoholic extract of *G. gummi-gutta* underwent *in vitro* antimicrobial testing, and the results showed that the plant demonstrated concentration-dependent antibacterial activity against all of the tested pathogens. The extract's zone of inhibition against each of the four microorganisms tested was measured in millimeters (mm) and shown in (Figures 6 A-H).

The hydroalcoholic extract from *G. cambogia* zone of inhibition on various bacterial strains ranged in size from 10 mm to 17 mm. The concentrations with the highest antibacterial activity against

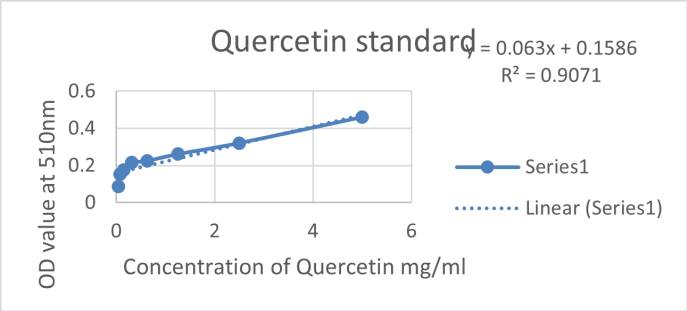


Figure 2: The total flavonoids content was present in the extract *G. cambogia*.

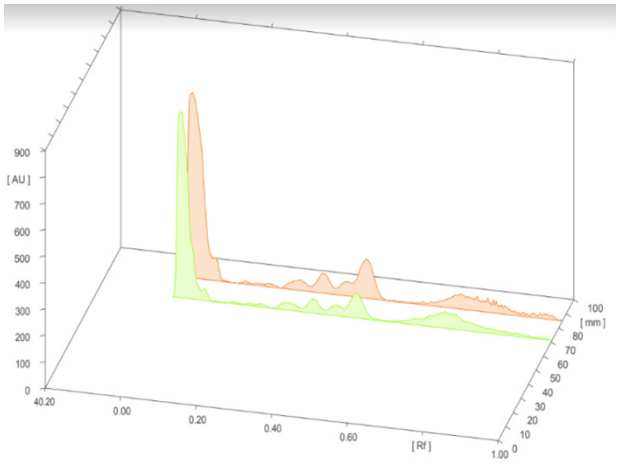
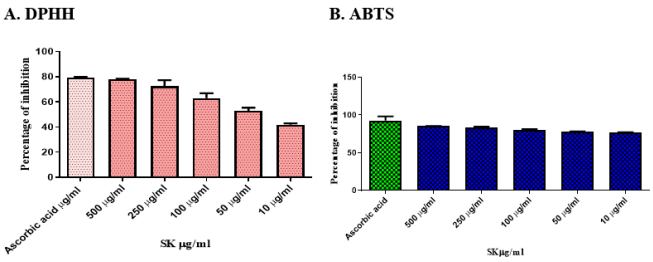
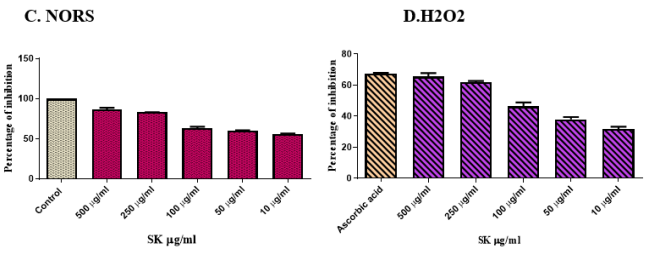


Figure 3: HPTLC.



IC_{50} **Value: 82.95µg/ml**

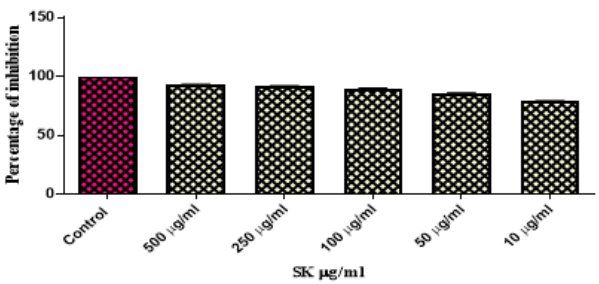
IC_{50} **Value: 134.0µg/ml**



IC_{50} **Value: 138.7µg/ml**

IC_{50} **Value: 107.3 µg/ml**

Figures 4 A – D: *In vitro* antioxidant activities.



IC_{50} Value of tested sample: 53.88 µg/mL.

Figure 5: Protein denaturation.

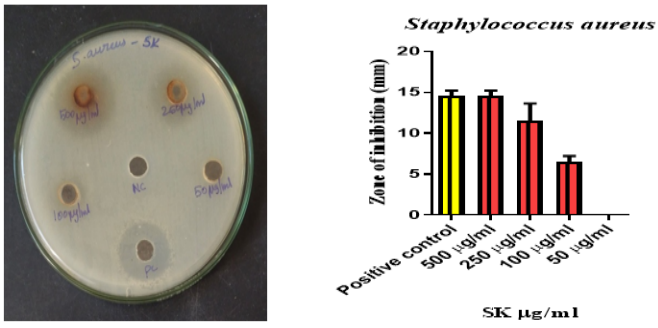


Figure 6 A: Effect of *G. cambogia* against *Staphylococcus aureus*.

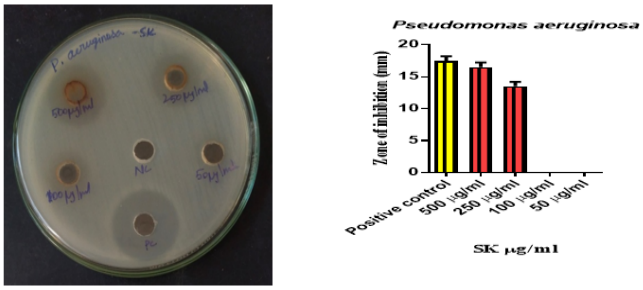


Figure 6 B: Effect of *G. cambogia* against *Pseudomonas aeruginosa*.

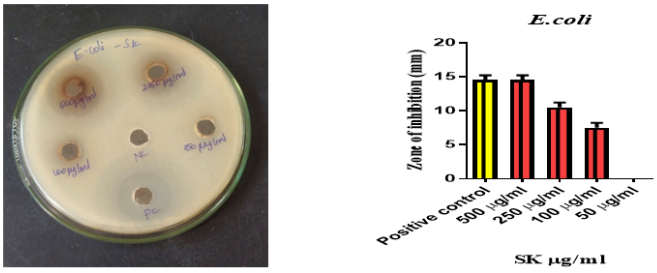


Figure 6 C: Effect of *G. cambogia* against *E. coli*.

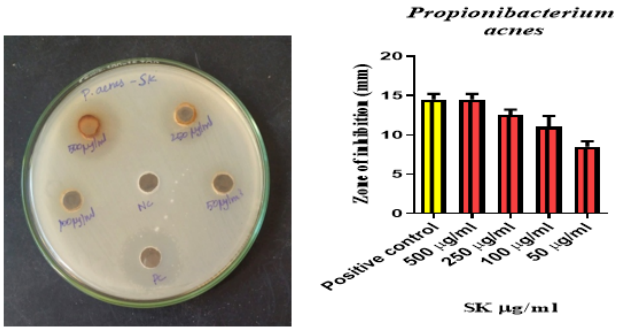


Figure 6 D: Effect *G. cambogia* against Propionibacterium acnes.

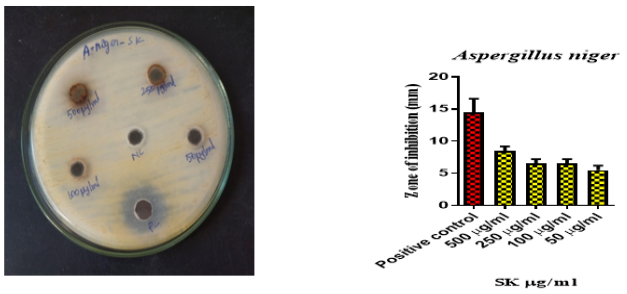


Figure 6 E: Effect of *G. cambogia* against Aspergillus niger.

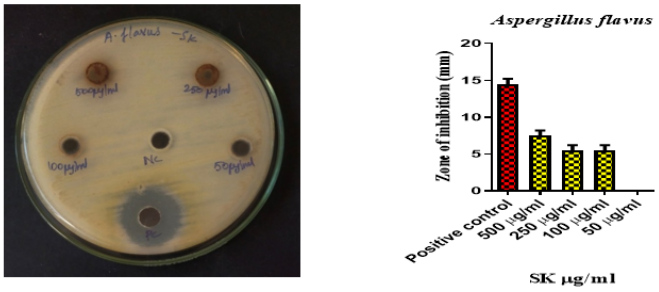


Figure 6 F: Effect of *G. cambogia* against *Aspergillus flavus*.

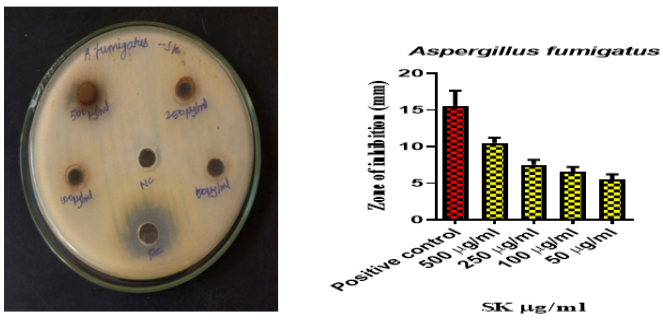


Figure 6 G: Effect of sample *G. cambogia* against *Aspergillus fumigatus*.

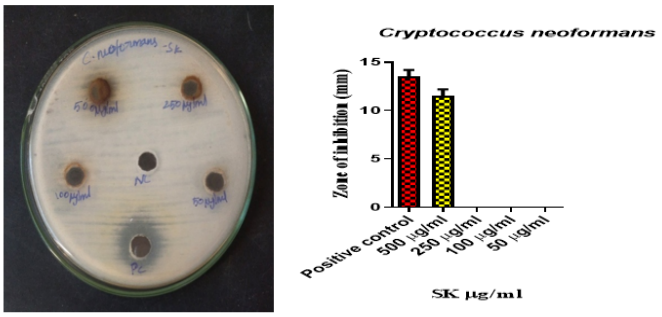


Figure 6 H: Effect of sample *G. cambogia* against *Cryptococcus neoformans*.

Staphylococcus aureus, P. aeruginosa, E. coli, P. acnes, A. niger, A. flavus, A. fumigates, and *C. neoformans* were 16.5 0.70, 14.5 0.70, and 14.5 0.70, respectively. However, compared to those created by plant extract, the inhibition zone produced by conventional disc was greater.

DISCUSSION

In both *in vitro* and *in vivo* settings, it has been demonstrated that a variety of extracts and pure chemicals, mostly derived from the *G. cambogia* fruit, display biological activity. The existing clinical trial data are mostly concerned with the HCA/*G. cambogia* supplement's ability to fight obesity.[50] Despite the fact that *G. cambogia* and supplements containing *G. cambogia* are most well-known for its ability to help people lose weight, numerous studies have shown that *G. cambogia* also has anti-inflammatory, anti-diabetic, antioxidant, antimicrobial, anti-ulcer, and hepatoprotective properties.

The current results are consistent with earlier research that found flavonoids, phenols, and tannins among other beneficial secondary metabolites in *G. cambogia's* fruit rind.[51] Plants produce secondary metabolites, which are frequently thought of as an adaptive and protective strategy against predatory attacks, pathogenic microbial invasion, and environmental stress. Experimental evidence of antibacterial action against pathogenic organisms indicates that these plant components have a wide range of pharmacotherapeutic potential. In order to determine the therapeutic potential of plant extracts, preliminary screening for the presence of physiologically active phytochemical ingredients is thought to be an effective first step.[52]

The study of phytochemical components for biological activity derived from natural sources is receiving more attention these days. A useful method for separating and identifying volatile and semi-volatile chemicals is the combination of gas chromatography and mass spectrophotometry.[53] We can infer from this study that GC-MS is quite dependable because it can extract chemicals in their purest form. This tool's capacity to produce more goods works in tandem with the solvent. In this investigation, GC-MS

analysis of *G. cambogia* leaves revealed the presence of several bioactive chemicals and hydroalcoholic extracts, which are shown in (Table 2). Any chemical compounds with less than 70% similarity are typically not reported due to low validity and less precision, according to the prior reports. Therefore, less than 70% of the compounds identified by GC-MS in the current study are reported as unknown. However, many plants also included chemicals with higher than 70%.

Recently, 120 compounds from *O. sinensis* and insect host-based products were reported by Qiu *et al*.[54] Their findings indicated that 2,6,11-trimethyldodecane was one of the chemicals present in the current investigation. Alfarhan *et al.*[55] study from 2019 identified 59 compounds from *Ziziphus nummularia*, of which the authors' findings for heneicosane, phytol acetate, and tetratetracontane were similar to those for the phytochemicals in the current study. Tetratetracontane, which is frequent in our extracts, was also observed in the ethanolic extract of *Brassica oleracea* reported by Jayalakshmi *et al.*[56]

Arora *et al.*[57] performed GC-MS profiling on *Ceropegia bulbosa's* tuber, stem, and leaf and found 43, 49, and 44 chemicals, respectively. Out of these, the present study found similarities between the 7,9-Di-tert-butyl-1-oxaspiro (4, 5) deca-6,9-diene-2,8-dione, eicosane, squalene, and phytol reported by authors.

From the leaf extract of *Pergularia daemia*, Rukshana *et al.*[58] screened phytochemicals and reported GC-MS data of 15 compounds, of which 7,9-Di-tert-butyl-1- oxaspiro (4,5) deca-6,9-diene-2,8-dione is present in the current study as well. In another investigation, 45 compounds were found in the leaves of *Syzyium jambos*, and only one of those compounds, 3,7,11,15-Tet ramethyl-2-hexadecen-1-ol, was found in the current study.

In 2016, Gautam *et al.*[59] examined bioactive chemicals from Rhododendron arboretum flower extracts in various solvents and identified 70 compounds, three of which were present in our extracts: eicosane, tetratetracontane, and neophytadiene. The chemical components from the leaves of *Phoradendron mucronatum* and *Phoradendron microphyllum* were also assessed by Bastos *et al*. (2016).[60] They listed 51 compounds, some of which are found in the current study, such as methyl (9E,12E)-9,12-octadecadienoate, tetramethyl-2-hexadecen-1-ol, and 3,7,11,15-tetramethyl-2-hexadecen-1-ol. *A. papuana* root extract contained 49 different compounds, among which ethyl palmitate, eicosane, tetratetracontane, ethyl linoleate, and ethyl nonadecanoate were comparable in the current investigation.

Triethyl citrate was one of 19 chemicals found in the methanolic extract of *Melia azedarach* leaves that Al-Marzoqi *et al.*[61] reported included triethyl citrate. According to Hussein *et al.*[62] there was one additional investigation that demonstrated the triethyl citrate compound's presence in *R. communis* leaves. Tetratetracontane was one of the 27 compounds found in *Premna coriacea* leaves that Sadashiva *et al.*[63] reported, and it is also a prevalent phytochemical in the study under consideration. Two substances (3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol and squalene) from a different study that extracted 17 compounds from a methanolic leaf extract of *Cassia italica* were found to be comparable to our study's findings.

The overall antioxidant content of a plant sample could not be properly assessed using either one antioxidant assay method due to the varied characteristics of phytochemicals. The four distinct antioxidant tests used in the current research were chosen as a result. Utilizing straightforward and affordable antioxidant assays, antioxidant activity was measured in the current research effort. Using antioxidant molecules present in the sample to scavenge synthetic free radicals such as DPPH, ABTS, Hydrogen peroxide scavenging test, and Nitric Oxide Radical Scavenging provides a clear conclusion about the antioxidant's potential and prospective therapeutic applications.

In vitro antioxidant activity was demonstrated in the DPPH, hydroxyl radical scavenging, ferric thiocyanate, total peroxy radical trapping, and lipid peroxidation assays by an aqueous extract made from the fruit rind. With IC_{50} values of 36, 50, 44, and 62 g/mL, respectively, the extract demonstrated efficacy against the DPPH radical, hydroxyl radical, peroxy radical, and lipid peroxidation. Positive controls included ascorbic acid (10 and 24 g/mL IC_{50} against DPPH radicals and lipid peroxidation, respectively), quercetin (36 g/mL IC_{50} against hydroxyl radical), and TROLOX (18 g/mL IC_{50} against peroxy radical). According to the study, the action could be caused by phenolic components found in the extract. Using the DPPH, hydroxyl radical scavenging, and ferric thiocyanate assays, a later investigation by Shivakumar *et al.*[64] demonstrated that hydro-alcoholic and ethanolic extracts from the fruit rind displayed *in vitro* antioxidant activity. The extracts reduced DPPH radicals by 79% (hydro-alcoholic) and 87% (ethanol) at a concentration of 300 g/mL, whereas ascorbic acid had 94% activity at the same concentration. This study was relevant to our own.

Flavonoids, simple phenolics, alkaloids, saponins, tannins, and terpenes are just a few phytochemical components that are effective antibacterial agents against a variety of pathogenic microbes.[65] Due to the existence of many pharmacologically bioactive secondary metabolites, previous studies have demonstrated that numerous species of *Garcinia* possess strong antibacterial and antioxidant activity. These metabolites, which are found in plant extracts, have the power to break down bacterial cell walls and prevent the creation of protein and nuclear components by impeding the actions of particular enzymes. With their polyphenolic structure, flavonoids are powerful free radical scavengers that oxidize a variety of substances implicated in the cellular pathophysiology of numerous disorders. Alkaloids are chemical molecules with nitrogen that have a wide range of pharmacological properties,[66-68] including antibacterial action. As a result, the study determines the plant extract's medicinal

efficacy, which may be of great relevance in the creation of a new antibacterial herbal food preservative agent.

Overall, the findings of this study point to *G. cambogia* as a top candidate for additional research aimed at identifying the chemical constituents responsible for its considerable antioxidant capacity, anti-inflammatory properties, and potential antibacterial activity.

CONCLUSION

The nutraceutical benefits of *Garcinia* species' leaf extracts are well-known, but there are no publications on the plant's fruit's comprehensive phytochemical analysis. Hydroalcoholic extracts of the *G. cambogia* fruit were used in the current work to demonstrate the existence of volatile components. Plant extracts have medicinal qualities because secondary metabolites found through GCMS/HPTLC analysis are present. Pharmacological businesses can create a wide range of pharmaceuticals from plants that can be a good source of these drugs thanks to the extraction of chemicals utilizing GCMS/HPTLC. The majority of the chemicals found had significant biological properties like antibacterial, anti-inflammatory, and antioxidant activity. This demonstrates the therapeutic value of *G. cambogia* and its fruit in treating a variety of diseases. However, additional studies into their variety, therapeutic value, and intricate phytochemistry may bring fresh knowledge to the data in conventional medical systems.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

GC-MS/HPTLC: Gas chromatography-mass spectrometry/ High-performance thin- layer chromatography; **UV:** Ultraviolet; **ROS:** Reactive Oxygen Species; **RNS:** Reactive Nitrogen Species; **RS:** Reactive Sulphur Species; **DNA:** Deoxyribonucleic acid; **PHF:** Fluorobenzene; **M:** Molarity; **NaCl:** Sodium chloride; **MSD:** Mass Selective Detector; **TLC:** Thin Layer Chromatography; **R_f:** Retention factor; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **ABTS:** 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid; **H_2O_2:** Hydrogen Peroxide; **ANOVA:** Analysis of Variance; **IC:** Inhibitory concentration; **G.A:** Gallic Acid; **Q.E:** Quercetin Equivalents; **HCA:** Hydroxycitric acid.

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