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PHYTOCHEMICAL PROFILING AND ANTIBACTERIAL ACTIVITY OF *Garcinia pedunculata* Rox. ex Buch. HAM LEAF CRUDE EXTRACTS USING GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS) ANALYSIS

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ABSTRACT

The current research was conducted to study the phytochemical contents and antimicrobial activity of the leaf extracts of *Garcinia pedunculata* using various solvent systems. Preliminary qualitative phytochemical screening revealed the presence of significant bioactive compounds such as alkaloids, flavonoids, phenolic compounds, saponins and phytosterols and methanol extract was found to have the most abundant phytochemical profile. GC–MS analysis further revealed the presence of a wide range of secondary metabolites including terpenoids, phytosterols, fatty acids, flavonoids, tocopherols and phenolic acids. Significant pharmacological potential was attributed to the major compounds identified, including campesterol, ellagic acid, apigenin, betulinic acid, oleanolic acid, β-sitosterol and α-humulene. The antimicrobial activity of the extracts was determined against pathogenic bacterial strains including *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Bacillus anthracis* by agar well diffusion method. The positive control showed a high antibacterial activity with zones of inhibition between 19.33 ± 0.57 mm and 27.33 ± 1.15 mm. Among the solvent extracts, ethanol extract showed moderate antibacterial activity against *E. coli*, *P. aeruginosa* and *B. anthracis*, but no inhibition was observed against *S. aureus*. The antimicrobial activity observed may be due to the presence of flavonoids, phenolics, terpenoids and phytosterols identified in the phytochemical analysis. In conclusion, the results suggest the existence of valuable bioactive components in *Garcinia pedunculata* leaves with promising antimicrobial activity, supporting their potential application in pharmaceutical and nutraceutical products. Further studies including purification, structural characterization and in vivo validation are recommended to explore their therapeutic efficacy.

Keywords **:** *Garcinia pedunculata,* GC–MS analysis, Phytochemical screening, Antimicrobial activity, Bioactive compounds.

Introduction

Antimicrobial resistance is prominent global crises that have necessitated the research for new therapeutic agents. According to WHO, in developing countries, people still use traditional plant-based medicines as primary healthcare (Anand *et al.,* 2019). The medicinal plants with their rich phytochemical diversity are a potential new area in the discovery of antimicrobial agents against multidrug-resistant pathogens (Balunas & Kinghorn, 2005). The reliance of the pharmaceutical industry on natural products still persists, with over half of modern medicines directly or indirectly producing some form of a plant-derived product, and the systematic phytochemical studies are regarded as important as ever (Anand *et al.,* 2019). *Garcinia* is a genus of the *Clusiaceae* family that has more than 300 species; it is found in tropical areas of Asia, Africa, and Polynesia, and in India, it has about 30 species (Hemshekhar *et al*., 2011). The genus has been of significant scientific interest because of its outstanding therapeutic potential and the large variety of bioactive compounds that it has, such as xanthones, benzophenones, flavonoids, and organic acids. The different species of *Garcinia* have been shown to have

remarkable pharmacological effects, which include antimicrobial, antioxidant, antiphlogmatory, antitumor and antidiabetic (Hemshekhar *et al*., 2011). The high phytochemical diversity of the genus *Garcinia* makes the genus a promising source of drug discovery, especially in the fight against an increasing menace of antimicrobial resistance. *Garcinia pedunculata Roxb*. ex Buch.-Ham., also called Bor Thekera in Assam, Hebung in Manipur, is a fruit-bearing underutilized tree that is mainly located in the northeastern parts of India and specifically in Assam, Meghalaya, and Manipur (Sarma and Devi, 2015). This is an important ethnomedicinal plant used by native people who have used different portions of the plant to treat different diseases. *G. pedunculata* fruit has widely been used in traditional medicine to treat gastrointestinal diseases, dysentery, infections, and inflammation (Sarma and Devi, 2015). The survey on ethnopharmacology of *G. pedunculata* was organized in six districts of Assam and demonstrated that native people have been using the plant since ancient times to treat stomach diseases, cough, cold, and to serve as an appetizer (Sarma and Devi, 2015). In addition to its medicinal use, the dried fruit slices have common commercial uses in the culinary preparation as a souring agent to the nutritional and cultural heritage of the area. Earlier phytochemical studies of *G. pedunculata* have shown that it contains a number of bioactive compounds. Specifically, fruit rinds are rich in hydroxycitric acid that is a combination of organic acids, phenolic compounds and xanthone derivatives. The extracts of the rinds in hexane and chloroform have strong antibacterial action, able to inhibit food-borne pathogens and food spoilers, including *Bacillus cereus, Bacillus subtilis, Staphylococcus aureus* and *Escherichia coli.* Minimum concentration inhibitory was between 300 and 1250 mg/ml (Negi *et al*., 2008). Notably, these extracts were stronger with Gram-positive bacteria than Gram-negative bacteria (Negi *et al*., 2008). In addition, the extracts produced great antioxidant properties, which can be explained by the presence of polyphenols (Sarma *et al*., 2016). The further application of the experimental animal models showed the antihyperglycemic and antidiabetic effects of the compounds where the hyperglycemia, lipid profiles and antioxidant enzyme activities were significantly improved (Ali *et al*., 2017). The Medicinal plant leaf is a significant but often ignored source of bioactive phytochemicals. Secondary metabolites are different chemicals produced and stored in the tissues of leaves in defense against the attacks of the herbivores and microbial diseases (Dahiya and Purkayastha, 2012). Screenings of leaf extracts of diverse medicinal plants have demonstrated a significant antimicrobial potential, which is usually equal to or even higher than that of fruits and other parts of plants (Dubale *et al*., 2023). The study of leaf extracts is especially useful to the sustainable use because leaf harvest does not harm the plant as much as does bark harvest, root harvest or harvesting the entire plant. Though, there is very little scientific knowledge available on systematic phytochemical study and antimicrobial screening of *G. pedunculata* leaves, which leads to the gap in knowledge that should be filled with systematic research. The current advent and subsequent increase in the prevalence of antimicrobial resistance threaten the public health in the world significantly, and it is estimated that the prevalence of antimicrobial-resistant infections may result in 10 million deaths every year by 2050 if no action is taken (Anand *et al*., 2019). Excessive consumption and abuse of traditional antibiotics have increased the rate of development of resistance mechanisms by pathogenic microorganisms and many of the currently used antimicrobial agents are ineffective. This is an alarming scenario that has rejuvenated the effort to find other sources of antimicrobials, especially medicinal plants that have been effective in the traditional medicine systems (Srivastava *et al*., 2014). The antimicrobial compounds found in plants usually have multiple mechanisms of action, which have complex chemical structures, thereby limiting the chances of developing resistance, which is likely to occur with single-target synthetic antibiotics. Moreover, it is possible that due to the synergistic action of various bioactive compounds found in plant extracts, antimicrobial activity can be improved and the range of action expanded (Kebede *et al*., 2021). Thus, given the traditional medicinal value of *Garcinia pedunculata*, its reported bioactivities and the necessity to find new antimicrobial agents, the present study was aimed at the purpose of carrying out comprehensive phytochemical evaluation of the leaf extracts of *G. pedunculata* using polar and non-polar solvents. This study will determine and describe the bioactive compounds in the extracts using GC-MS and determine their antimicrobial activity against pathogenic microorganisms that have clinical importance.

Materials and Methods

Chemicals and Reagents

Methanol (99.8% purity), ethanol (99.8% purity), petroleum ether (99% purity) and n-hexane (99% purity) analytical grade were procured from SRL (India) and utilised as extraction solvents. Dimethyl sulfoxide (DMSO, 99.9% purity) was procured from Sigma-Aldrich (St. Louis, MO, USA). Streptomycin

was procured from HiMedia Laboratories (Mumbai, India). HiMedia Laboratories was also the source of Mueller-Hinton Agar (MHA), Mueller-Hinton Broth (MHB). Sigma Aldrich supplied the BSTFA + 1% TMCS (silylation, N,O-bis (trimethylsilyl) trifluoroacetamide with 1% trimethylchlorosilane).

Soil Sample Collection

Fresh soil samples were collected form the Basistha, Silviculture Garden, Guwahati, Assam (Table-1). The collected soil samples were subjected to analysis for pH, EC, moisture, Organic content, Average Nitrogen, Phosphorous and Potassium.

Table 1 : Geographic Coordinates and Soil Sample Collection Details of the Lower Soil Layer (6"–12" Depth) at Site 02 (Basistha Silviculture Garden)

Soil sample collection	Latitude	Longitude
TS01S2L (Test sample 01, site number S2, lower layer soil)	26.10160705	91.79124358
TS02S2L	26.10180204	91.79120798
TS03S2L	26.10158718	91.79111876
TS04S2L	26.10153094	91.79131926
TS05S2L	26.10178258	91.79138679
TS06S2L	26.10177256	91.7912027
TS07S2L	26.1016007	91.79112178

Plant Material Collection

Fresh leaves of *Garcinia pedunculata* were collected in March 2025 from the Basistha, Silviculture Garden, Guwahati, Assam, located at coordinates latitude 26.10159828°N, and longitude 91.79119722°E (Figure-1).

Fig. 1: Plant specimen collection site.

Fig. 2: Fruit of *G. pedunculata*

The collection was conducted under the authorization of Memo No. WL/FG.31/RS/Misc. The leaves were thoroughly washed under running water to remove dust and debris, followed by a rinse with distilled water to eliminate residual contaminants. The plant material was identified as *Garcinia pedunculata* by Professor Kumananda Tayung, Department of Botany, Gauhati University, Assam. The identification was verified by comparison with standard herbaria

Preparation of Extracts

The shade dried leaves of *Garcinia pedunculata* were coarse powdered using an electric grinder. A standardized powder was obtained by sieving (40 mesh) the dried leaves coarse powder. Solvents extraction was then done on the powder to get polar and non-polar extracts. To extract it directly, 10g of leaf powder was first moistened in 100 mL of methanol, ethanol, petroleum ether and n-hexane, swirled together on an orbital shaker at room temperature over 24 hours followed by filtration through Whatman No. 1 filter paper and evaporation under reduced pressure at 40°C (non-polar) or 50°C (polar) using a rotary evaporator (Buchi, Switzerland) to produce dry extracts. The extracts were also stored at 4°C. The extract powders were dissolved in 10% DMSO to make extract solutions.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The GC-MS analysis of the extracts of the *Garcinia pedunculata* was done through the PerkinElmer (USA) GC-MS system (model: Clarus 680 GC and Clarus 600C MS, fitted with liquid auto-sampler). The TurboMass version 6.4.2 system software was used, and the NIST-2014 data analysis

library was used to identify peaks. The length of the capillary column was an Elite-5MS (capillary length: 60 m, internal diameter: 0.25 mm, film thickness: 0.25 um) and the stationary phase was 5% diphenyl-95% dimethyl polysiloxane. The carrier gas was the helium gas at the constant flow rate of 1 mL/min and splitless mode was used to inject the samples (1uL) with injector temperature of 280℃ and ion source temperature of 180℃. The program of the oven was as follows: the initial hold at 60℃ (1 min), the ramp (7℃/min) to 200℃ (3 min), the ramp (10℃/min) to 300℃ (5 min). The overall run time had been about 39 min, with an 8 min solvent delay to ensure that the solvents should not interfere. Mass spectrometry was performed in electron impact (EI+) ionization mode at 70eV and the temperature of the ion source kept at 180℃. Mass spectra were measured in a mass-to-charge (m/z) ratio range of 50-600 amu with an 8-min solvent delay that was calibrated with the conditions of the GC. The GC chromatograms were compared to the NIST-2014 library database to identify the peaks in the chromatograms using the mass spectra. The phytochemicals were tentatively identified through the best quality of spectral matches, which was considered necessary to ensure the high accuracy of the characterization of the chemical constituents of the extract (Enerijiofi *et al*., 2021).

Qualitative Screening of Phyto-compounds

The prepared extracts of *Garcinia pedunculata* leaves were screened qualitatively using standard methods to identify the occurrence of phytochemicals like alkaloids, flavonoids, phenolic compounds, saponins, phytosterols, terpenoids and tannins. All the tests were done in triplicates to be reproducible. The approaches used were as follows:

Alkaloid Detection

- ***Mayer's Test:*** 1 mL filtrate was added with 1-2 drops of Mayer's reagent along the sides of test tube; formation of a creamy white or yellow precipitate shows the presence of alkaloids[1].
- ***Wagner Test:*** 1ml of each extract added with 1-2 drops of Wagner's reagent (iodine in potassium iodide)along the sides of test tube; formation of a brown or reddish precipitate shows the presence of alkaloids. The alkaloids were identified by a reddish-brown precipitate.

Flavonoids Detection

- ***Alkaline Reagent Test:*** 1 mL of each extract was added to with few drops of 10% sodium hydroxide solution. Presence of flavonoids was indicated by the development of an intense yellow color which turned colourless when a few drops of dilute hydrochloric acid were added.

Phenolic Compounds Detection

Iodine Test: Few drops of 1% Iodine solution were added to 1 mL of each extract. The formation of a red color indicated the presence of phenolic compounds.

Saponins Detection

Foam Test: 1mL plant extract filtrate was added with 2mL water and shaken vigorously in vortex. Formation and persistence of foam for 5 min shows the presence of saponins.

Phytosterols and Terpenoids Detection

Salkowski Test: 1 mL of each extract was dissolved in chloroform followed by addition of 2 mL of concentrated sulfuric acid (H_2SO_4) carefully added along the sides of the test tube. A reddish-brown color at the interface indicated the presence of phytosterols, while a red color in the chloroform layer confirmed terpenoids.

Ferric Chloride Detection

Tannins Test: ***10% NaOH Test:*** To 1 mL of plant extract was mixed with 2 mL of 10% sodium hydroxide (NaOH) solution. The formation of a white precipitate that turned yellowish upon standing indicated the presence of tannins .

Assessment of Antibacterial Activity: The assessment of the antibacterial activity was done using gram-positive bacteria (*Staphylococcus aureus, Bacillus anthracis*) and gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*). The bacterial inoculum was made by the suspension of the isolated colonies in the sterile saline to provide the standard of 0.5 McFarland (1.5x108 CFU/mL). Bacterial inoculum of each strain (100ul) was placed on a separate plate of MHA agar that was prepared with autoclave sterilized (121℃ for 15 minutes) media. Each agar plate was punched with a sterile cork borer and vertical wells were punched into it. Complementary positive control, negative control and 100 ul of extract solutions were introduced in each plate. The plates were incubated for 24hrs 37℃. Measurement of the zones of the inhibition was done by a digital caliper after incubation.

Statistical Analysis: All experiments were performed in triplicates. The extraction yield and the antibacterial zone of inhibition were reported as mean ± SD. The antimicrobial activity (zone diameters) of extracts and controls were compared using one-way analysis of variance (ANOVA). Multiple comparisons were then

made with Tukey post hoc test based on one way ANOVA (GraphPad Prism Software version 9.0, GraphPad Software, San Diego, CA, USA). A p-value below 0.05 ($p < 0.05$) was taken as statistical significance.

Result and Discussion

Soil Physiochemical Properties

The soil physicochemical analysis revealed that the soil possessed moderately favorable characteristics for plant growth and microbial activity. The soil pH was recorded as 6.04, indicating a slightly acidic nature, which is considered suitable for nutrient availability and healthy plant development. Electrical conductivity (EC) was found to be 0.04 dS/m, suggesting very low salinity and absence of harmful salt accumulation in the soil. The moisture content of the soil was 9.3%, indicating moderate water retention capacity that can support microbial metabolism and nutrient transport. Organic content was relatively low (0.6%), reflecting limited organic matter and suggesting the need for organic amendments to improve soil fertility and structure. The macronutrient analysis showed that nitrogen content was comparatively moderate at 199.14 ppm, which may support vegetative growth and chlorophyll synthesis. Phosphorus content was low (7.75 ppm), indicating possible phosphorus deficiency that may limit root development and plant productivity. Potassium content was measured at 38.75 ppm, representing an adequate level for enzyme activation, osmotic regulation, and stress tolerance in plants. Textural analysis revealed that the soil contained 52.64% sand, 16% silt, and 31.36% clay, classifying it as sandy loam soil. This texture favors good aeration, drainage, and root penetration, although nutrient retention may be comparatively lower than clay-rich soils. Overall, the soil characteristics suggest moderate fertility with a requirement for improved organic matter and nutrient management strategies to enhance agricultural productivity (Table-2). The measured pH 6.04, is in the optimum range required by most plant species and thus making nutrient solubility and microbial activity easy (Sharma *et al.,* 2018). A pH ranging from 6.0 to 7.0 usually improves the availability of phosphorus and reduces the hazards of aluminum and manganese toxicity (Zhang and Wienhold, 2002). Electrical conductivity (EC) was 0.04 (dS/m) indicating little salinity and low salt accumulation. Such low EC levels favor the growth of plants by the avoidance of osmotic stress, and are typical for non-saline soils with sufficient drainage and limited dissolution of ion. The soil moisture content was found to be 9.3%; which implies that the soil has a moderate water-retention capacity upon sampling. The moisture that is available has a key effect on the microbial activity, transport of nutrients, and the general dynamic of the soils in terms of their fertility (Zhang & wyshienhold, 2002). The organic matter was 0.60% which is relatively low, when compared with fertile agricultural soils. Organic matter serves as a pool for critical nutrients, enhances soil structure, coastal the water retention and microbial communities that are essential for nutrient roundup (Abah & Petja, 2015; Sharma *et al*., 2018).

Macronutrient concentrations were as follows: Average Nitrogen was at 199.14 ppm, a moderate concentration to promote vegetative growth. Nitrogen, as the limiting macronutrient in many ecosystems, is a key component in the synthesis of proteins and chlorophyll formation (Gudla *et al*., 2023). Average Phosphorus concentration was 7.75 ppm, that is low at availability. Phosphorus plays a key role for energy bearing, photosynthesis, root development. Phosphorus deficit is important as it limits plant productivity (Abah & Petja, 2015; Sharma *et al*., 2018). Average Potassium was 38.75 ppm which is in the adequate range for plant nutrition. Potassium in plants controls stomatal functioning, enzyme activation, and osmoregulation's, which improves drought resistance and disease resistance (Gudla *et al*., 2023).

Texture analysis classified the soil as sandy loam with 52.64%, 31.36% and 16.00% sand, silt and clay respectively as per the USDA textural triangle. This texture is associated with good drainage, moderate water holding capacity and ease of cultivation, although it may be limited with respect to nutrient holding capacity of the soil compared to soils rich in clay (Beretta *et al*., 2014; Kettler *et al*., 2001). Sandy loam soils are well-aerated, and encourage root penetration and microbial activity, yet may need the addition of supplemental organic materials for enhanced fertility and moisture retention (Huluka and Miller 2014; Mwendwa, 2022). Collectively, as the slightly acidic pH, low organic matter and moderate nutrient availability, it appears the soil would benefit much from organic fertilization and targeted nutrient management strategies related to optimizing plant growth and productivity.

Table 2: Soil Physiological properties.

pH	EC (dS/m)	Moisture (%)	Organic Content (%)	Average Nitrogen (ppm)	Average Phosphorus (ppm)	Average Potassium (ppm)	Sand %	Silt %	Clay %
6.04	**0.04**	**9.3**	**0.60**	**199.14**	**7.75**	**38.75**	**52.64**	**16.00**	**31.36**

Qualitative Phytochemical Profiling

The phytochemical screening of the *Garcinia pedunculata* leaf extracts was qualitatively done using different solvent extracts, which showed different profiles (Table 3). The methanol extract was positive in flavonoids, phenolic compounds, saponins, and phytosterols, the ethanol extract was positive in flavonoids and phytosterols, the hexane extract was positive in flavonoids, and the petroleum ether extract was negative for most compounds except alkaloids. It is noteworthy that the Wagner test showed presence of alkaloids in all the extracts, but the Mayer test was negative in all solvents. The methanol extract was the only extract that produced saponins using the foam test. The results of the Salkowski test for terpenoids and the 10% NaOH test for tannins were negative indicating that both terpenoids and tannins were not detected in any of the extracts. These results indicate that the polarity of the solvent plays a major role in the kind of phytochemicals extracted with methanol more effective in extracting polar compounds such as saponins.

Table 3: Qualitative Phytochemical Profiles of *Garcinia pedunculata* leaf extracts.

Compound to be detected	Test Name	Methanol extract	Ethanol extract	Hexane extract	Petroleum ether extract
Alkaloids	Mayers test	negative	negative	negative	negative
	Wagners test	positive	positive	positive	positive
Flavonoids	Alkaline reagent test	positive	positive	positive	negative
Phenolic compounds	Iodine test	positive	negative	negative	negative
Saponins	Foam Test	positive	negative	negative	negative
Phytosterols	Salkowski test	positive	positive	negative	negative
Terpenoids	Salkowski test	negative	negative	negative	negative
Tannins	10% NaOH test	negative	negative	negative	negative

Alkaloids were detected in all four extracts through Wagner's test, while Mayer's test showed negative results. The selectivity of bioactive compounds by the solvents with different polarity exemplifies the role of solvent polarity in determining phytochemicals recovery. The identification of alkaloids in the screened leaves of the plant indicated that the alkaloid content is widespread, and a positive test of the alkaline reagent in the methanol, ethanol, and hexane extracts showed a wide distribution of the alkaloids (Adaramoye *et al*., 2005). Flavonoids were largely found in methanolic and ethanol extracts in garcinia kola and this is due to its high solubility in polar solvents (Adaramoye *et al*., 2005). The abundance of flavonoids refers to their significance as the secondary metabolites with antioxidant, anti-inflammatory and anti-microbial, and hence, complementing the pharmacological profile of the plant (Saikia *et al*., 2024). Components of the iodine test appeared as blue-black coloration in the methanolic extract and saponin foam was positive only in the methanolic but not in the ethanol, hexane and petroleum ether extracts. Parekh & Chanda (2007) came up with similar results. Therefore, methanol was found to be the most effective solvent in the removal of polar compounds like saponins, flavonoids and phenolics. The fact that terpenoids and tannins are not present in any of the extracts is an indication that these classes are either present at insignificant concentrations, or are insoluble under the given set of conditions. The fact that the Wagner test was positive to alkaloids in all extracts and the Mayer test negative shows that certain types of alkaloids are present, perhaps quaternary or tertiary alkaloids that are selectively revealed by Wagner reagent. Its polar nature is in agreement with the selective extraction of saponins by methanol since saponins are glycosides containing hydrophilic sugar moiety (Sparg *et al*., 2004). These results are in agreement with other studies conducted on *Garcinia* species, i.e., *G. kola*, which also reported the presence of flavonoids, phenolic compounds, and phytosterols as important components (Adaramoye *et al*., 2005).

Phytochemical Quantitative Analysis

GC-MS Chromatogram

The Total Ion Chromatogram (TIC) of *Garcinia pedunculata* extract displayed distinct peaks at

retention time between 8.09 to 38.87, corresponding to different phytochemical constituents separated by gas chromatography. Among these, the peaks at 9.90, 10.08, 12.19, 14.26, 15.30, 16.72, 18.33, 19.38, 26.68, 28.62, 33.23, 33.73 min and, 34.53 min showed the highest intensity, indicating that these compounds are present in greater abundance compared to others. The extended chromatogram highlighted late-eluting compounds, typically less volatile, higher molecular weight, or thermally stable phytochemicals such as phytosterols, diterpenoids, and triterpenoids. Early-eluting peaks (before 15 min, not shown) are generally attributed to volatile terpenes. Mass spectrometry and library matching (NIST/Wiley) was applied to each chromatographic peak to obtain the accurate identification of the compound.

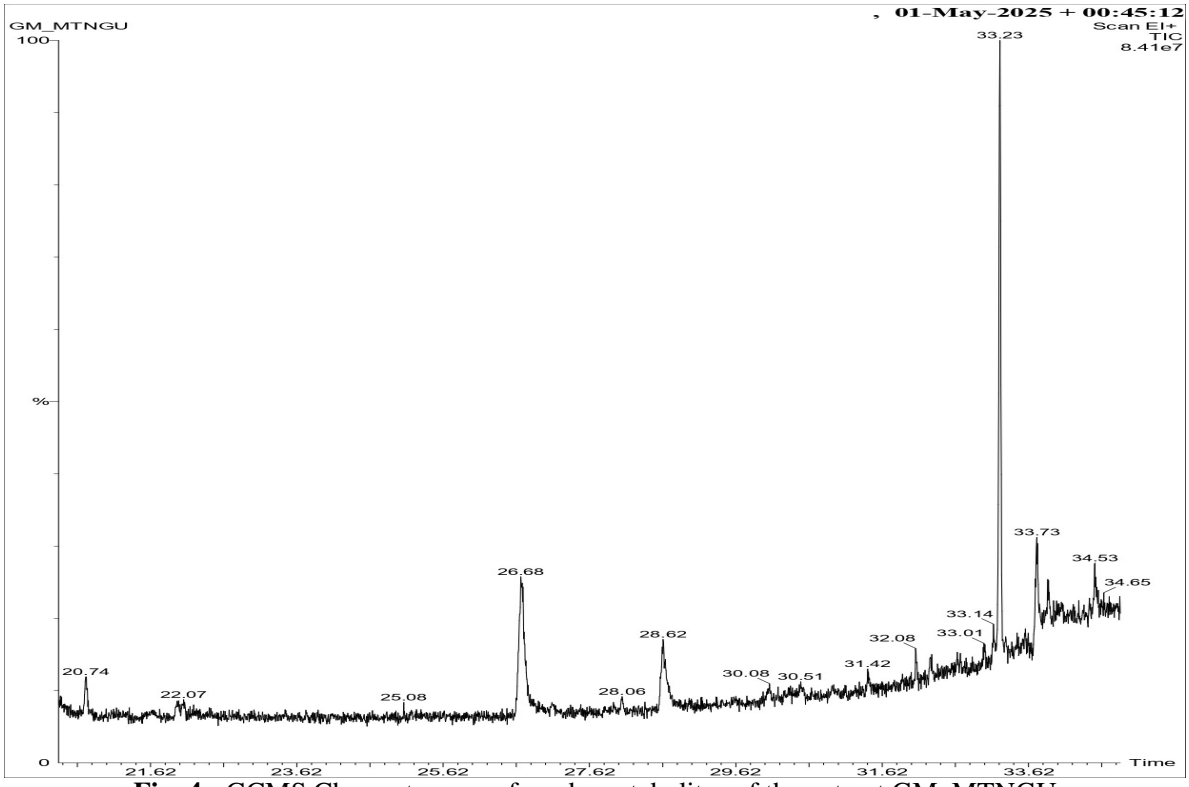


Fig. 4 : GCMS Chromatogram of crude metabolites of the extract GM_MTNGU

Phytochemicals Identification

Tentative identification of 100 phytochemicals in the extract using GC-MS analysis of the extract of the plant was achieved based on retention time, percentage peak area, percentage normalised and normalisation against the NIST -2014 spectral library (Table-3). The identified compounds had a wide range of chemical classes, such as terpenoids, phytosterols, fatty acids, phenolic acids, flavonoids, tocopherols and long-chain hydrocarbons, which results in complex secondary-metabolite profile typical of *Garcinia* species (Brahma *et al*., 2024; Chouni *et al*., 2021).

The profile was predominantly terpenoid and included monoterpenes as well as sesquiterpenes. Linalool (RT 8.018 min, 9.19%), camphor (RT 8.754 min, 5.94%), borneol (RT 8.894 min, 2.27 %), and terpineol (RT 8.989 min, 1.78 %) were the main monoterpenes. Other monoterpenes in low concentration were; α pinene (RT 8.314 min, 1.50%), 8-pinene (RT 8.404 min, 3.84%), limonene (RT 9.084 min, 1.06%), and citronellal (RT 9.359 min, 1.06%). Monoterpenes are commonly recognized as antimicrobial, anti-inflammatory and antioxidant agents (Pokajewicz, 2023). The major components of sesquiterpenes were 2-caryophyllene (RT 10.079 min, 6.15 %), 2 -humulene (RT 12.190 min, 7.03 %), nerolidol (RT 14.256 min, 2.41 %) and farnesol (RT 15.301 min, 1.61 %). Sesquiterpenes, which are generally heavier and less volatile than monoterpenes, are associated with strong antimicrobial and anti-inflammatory abilities (Al-Mterin *et al*., 2021). The identified compounds included a large proportion of phytosterols and triterpenoids. Campesterol (RT 20.006 100 % Norm) was the most common component, then, followed by β-sitosterol (RT 19.806 min, 4.77%), stigmasterol (RT 20.146 min, 1.60%), and lupeol (RT 20.236 min, 1.31%). Structurally

related to cholesterol, phytosterols have received much attention in cholesterol-lowering, anti-inflammatory, and anticancer (Gachumi & El-Aneed, 2017; Tan *et al*., 2019). Pentacyclic triterpenoids detected were oleanolic acid (RT 26.679 min, 8.54 %), betulinic acid (RT 35.517 min, 9.27 %), ursolic acid (RT 26.829 min, 0.78 %) and 8 -amyrin (RT 20.736 min, 1.12 %). Trierpenoids are also famous due to a wide range of pharmacological effects, such as antimicrobial effects, hepatoprotective effects, and antitumour effects (Brahma *et al*., 2024). Another important chemical group was fatty-acid derivatives, including both unsaturated and saturated compounds. Linoleic acid (RT 19.376 min 1.50%), oleic acid (RT 19.476 min 1.30%), palmitic acid (RT 19.506 min 1.08%), stearic acid (RT 19.606 min 1.20%), etc. were highly detected. Among the long-chain fatty acids, there were arachidic acid (RT 36.057 min 2.06 %), behenic acid (RT 36.102 min 1.29 %) and lignoceric acid (RT 36.217 min 1.36 %). Fatty acids are known to be a part of the membrane integrity, energy storage, and signalling pathways, with unsaturated fatty acids, including linoleic acid, specifically reputed to have antioxidant and anti-inflammatory properties (Zhang & Zuo, 2004). The important bioactive constituents were detected to be phenolic acids and flavonoids. The phenolic compound that was most abundant was ellagic acid (RT 35.467 min, 43.25 %), then apigenin (RT 33.226 min, 12.31 %), and hesperetin (RT 33.7 31 min, 2.94 %). Others of interest were quercetin (RT 32.686 min, 0.43 %), kaempferol (RT 33.011 min, 0.52 %), rutin (RT 33.141 min, 0.80 %) and naringenin (RT 33.576 min, 0.44 %). It was also found to contain phenolic acid including ferulic acid (RT 30.505 min, 0.57%), caffeic acid (RT 31.42 min, 0.67%), chlorogenic acid (RT 34.527 min, 1.31%) and gallic acid (RT 34.066 min, 0.52%). The potent antioxidant, antimicrobial and anti-inflammatory properties of phenolic compounds have been highly appreciated; in this regard, ellagic acid and apigenin have been shown to possess powerful free-radical scavenging properties (Proestos *et al*., 2006; Acquadro *et al*., 2020). Tocopherol isoforms were detected as well, α-tocopherol (RT 28.624 min, 3.93%) and γ-tocopherol (RT 30.08 min, 0.85%). These vitamin E analogues have well-defined antioxidant effects. A phenylpropanoid, eugenol (RT 9.904 min, 3.04%), which is known to have both antimicrobial and analgesic effects was also identified. A triterpene hydrocarbon, known as squalene (RT 28.064 min, 0.47%) which is the precursor of steroid biosynthesis. Diterpene alcohol phytol (RT 19.301 min, 0.59%) that is the precursor to vitamins E and K was also identified (Khallouki *et al*., 2020).

Taken together, the GC-MS profiling highlights a diverse and abundant phytochemical makeup among the GC-MS profiling of *Garcinia pedunculata* which is mainly composed of terpenoids, phytosterols, phenolics and fatty acids. The identification of bioactive compounds including campesterol, ellagic acid, apigenin, β-sitosterol and oleanolic acid highlights the potential of this species in both pharmaceutical and nutraceutical use as medicine. The provisional identification, which was made on the basis of mass-spectral similarity with the NIST-2014 library, gives a background on which other validation is proceeded through the use of authentic standards and other superior techniques of analysis like LC-MS/MS and NMR spectroscopy (Proestos *et al*., 2006; Gachumi & El-Aneed, 2017; Rahman *et al*., 2024).

Table 4: Tentative Identification of Phytochemicals from GC-MS

Sr. No.	Retention Time (min)	Area %	Norm %	Tentative Phytochemical
1	8.018	2.245	9.19	Linalool
2	8.314	0.367	1.5	α-Pinene
3	8.404	0.939	3.84	β-Pinene
4	8.509	0.77	3.15	Camphene
5	8.574	0.169	0.69	Sabinene
6	8.754	1.451	5.94	Camphor
7	8.894	0.556	2.27	Borneol
8	8.989	0.434	1.78	Terpineol
9	9.024	0.166	0.68	α- Terpinene
10	9.084	0.259	1.06	Limonene
11	9.109	0.12	0.49	γ- Terpinene
12	9.164	0.427	1.75	ρ-Cymene
13	9.219	0.178	0.73	α- Phellandrene
14	9.269	0.137	0.56	Myrcene
15	9.314	0.145	0.59	α- Terpinolene
16	9.359	0.259	1.06	Citronellal
17	9.444	0.287	1.17	Citral
18	9.519	0.106	0.44	Geranial
19	9.589	0.174	0.71	Linalyl acetate
20	9.639	0.153	0.63	Geranyl acetate
21	9.904	0.744	3.04	Eugenol
22	10.079	1.504	6.15	β-Caryophyllene
23	12.19	1.719	7.03	α-Humulene
24	12.335	0.171	0.7	α-Copaene
25	12.39	0.154	0.63	β- Bour bonene
26	12.845	0.303	1.24	δ-Cadinene
27	13.481	0.106	0.43	Germacrene D
28	13.921	0.149	0.61	β-Selinene
29	14.256	0.588	2.41	Nerolidol
30	15.301	0.393	1.61	Farnesol
31	15.711	0.123	0.5	o-Bisabolol
32	15.802	0.176	0.72	β- Farnesene
33	16.722	0.401	1.64	Caryophyllene oxide
34	17.302	0.148	0.61	Spathulenol
35	17.522	0.16	0.66	Viridiflorol

36	17.98	0.118	0.48	β- Eudesmol
37	18.325	1.408	5.76	α- Terpineol
38	19.301	0.143	0.59	Phytol
39	19.346	0.167	0.68	Geranylgeraniol
40	19.376	0.366	1.5	Linoleic acid
41	19.476	0.318	1.3	Oleic acid
42	19.506	0.263	1.08	Palmitic acid
43	19.606	0.294	1.2	Stearic acid
44	19.806	1.165	4.77	β-Sitosterol
45	20.006	24.431	100	Campesterol
46	20.146	0.391	1.6	Stigmasterol
47	20.236	0.319	1.31	Lupeol
48	20.736	0.273	1.12	β-Amyrin
49	22.072	0.122	0.5	o-Amyrin
50	26.679	2.087	8.54	Oleanolic acid
51	26.829	0.191	0.78	Ursolic acid
52	26.974	0.126	0.52	Betulin
53	27.104	0.135	0.55	Friedelin
54	28.064	0.115	0.47	Squalene
55	28.624	0.96	3.93	α- Tocopherol
56	30.08	0.208	0.85	γ- Tocopherol
57	30.45	0.102	0.42	Phytosterol acetate
58	30.505	0.138	0.57	Ferulic acid
59	31.42	0.164	0.67	Caffeic acid
60	32.076	0.222	0.91	ρ-Coumaric acid
61	32.296	0.238	0.97	Vanillin
62	32.646	0.143	0.59	Syringaldehyde
63	32.686	0.105	0.43	Quercetin
64	33.011	0.126	0.52	Kaempferol
65	33.141	0.195	0.8	Rutin
66	33.226	3.008	12.31	Apigenin
67	33.306	0.137	0.56	Luteolin
68	33.576	0.107	0.44	Naringenin
69	33.731	0.719	2.94	Hesperetin
70	33.881	0.281	1.15	Catechin
71	34.001	0.104	0.43	Epicatechin
72	34.066	0.127	0.52	Gallic acid
73	34.527	0.32	1.31	Chlorogenic acid
74	34.822	0.102	0.42	Rosmarinic acid
75	35.172	0.112	0.46	Cinnamic acid
76	35.467	10.567	43.25	Ellagic acid
77	35.517	2.265	9.27	Betulinic acid
78	35.597	0.335	1.37	Maslinic acid
79	35.667	0.747	3.06	Corosolic acid
80	35.697	0.759	3.11	Asiaticoside
81	35.792	0.139	0.57	Madecassic acid
82	35.847	0.113	0.46	α- Linolenic acid
83	36.057	0.502	2.06	Arachidic acid
84	36.102	0.316	1.29	Behenic acid
85	36.217	0.331	1.36	Lignoceric acid
86	36.292	0.128	0.53	Cerotic acid
87	36.317	0.11	0.45	Montanic acid
88	36.342	0.102	0.42	Melissic acid
89	36.682	0.13	0.53	Triacontanol
90	36.783	0.146	0.6	Hentriacontane
91	36.833	0.105	0.43	Dotriacontane
92	37.218	0.104	0.43	Trityacontane
93	37.848	0.106	0.43	Tetratriacontane
94	38.023	0.153	0.63	Pentatriacontane
95	38.153	0.124	0.51	Hexatriacontane
96	38.728	0.137	0.56	Heptatriacontane
97	38.758	0.116	0.48	Octatriacontane
98	38.778	0.127	0.52	Nonatriacontane
99	38.838	0.121	0.5	Triacontanol derivative
100	38.878	0.123	0.5	Long-chain alkane

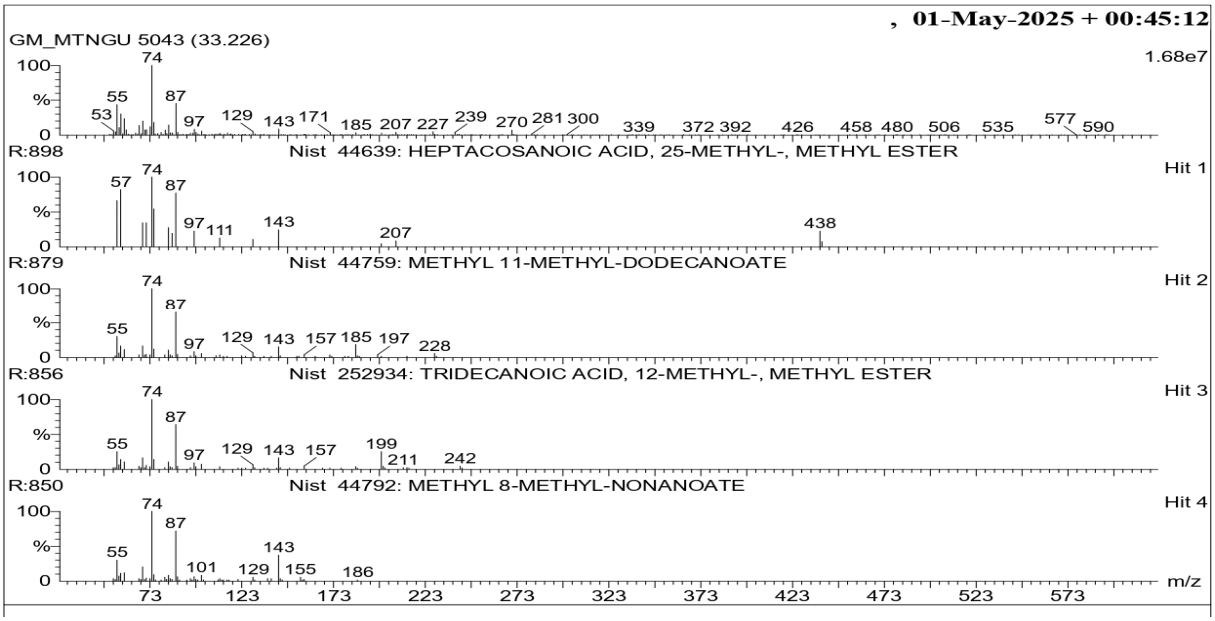


Fig. 5 : Spectrum of major identified componds in the crude metabolites of the extract GM_MTNGU at Retention time 33.226

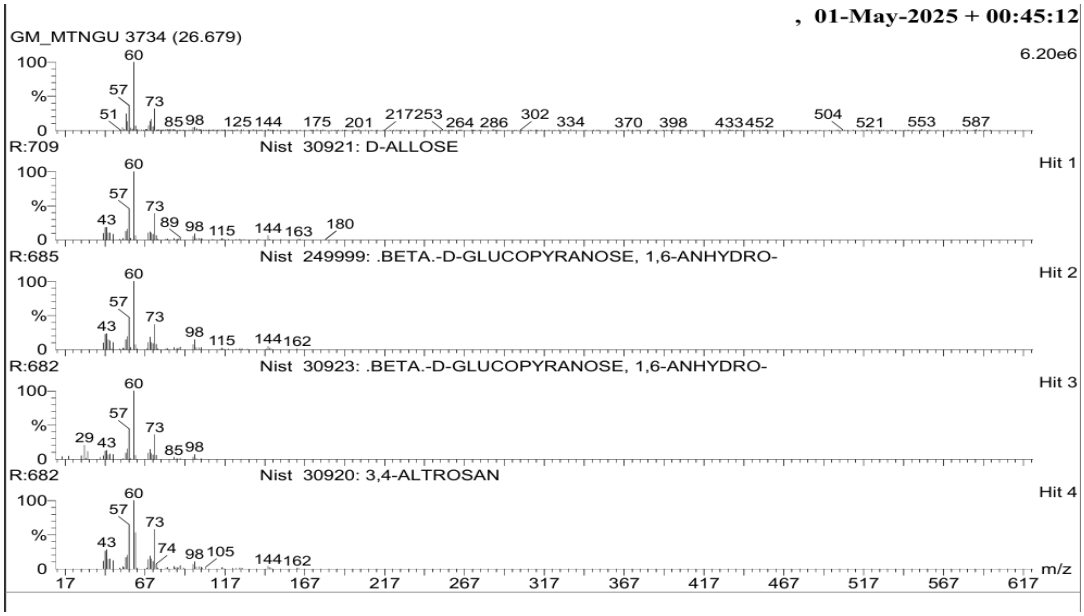


Fig. 6 : Spectrum of major identified componds in the crude metabolites of the extract GM_MTNGU at Retention time 26.679

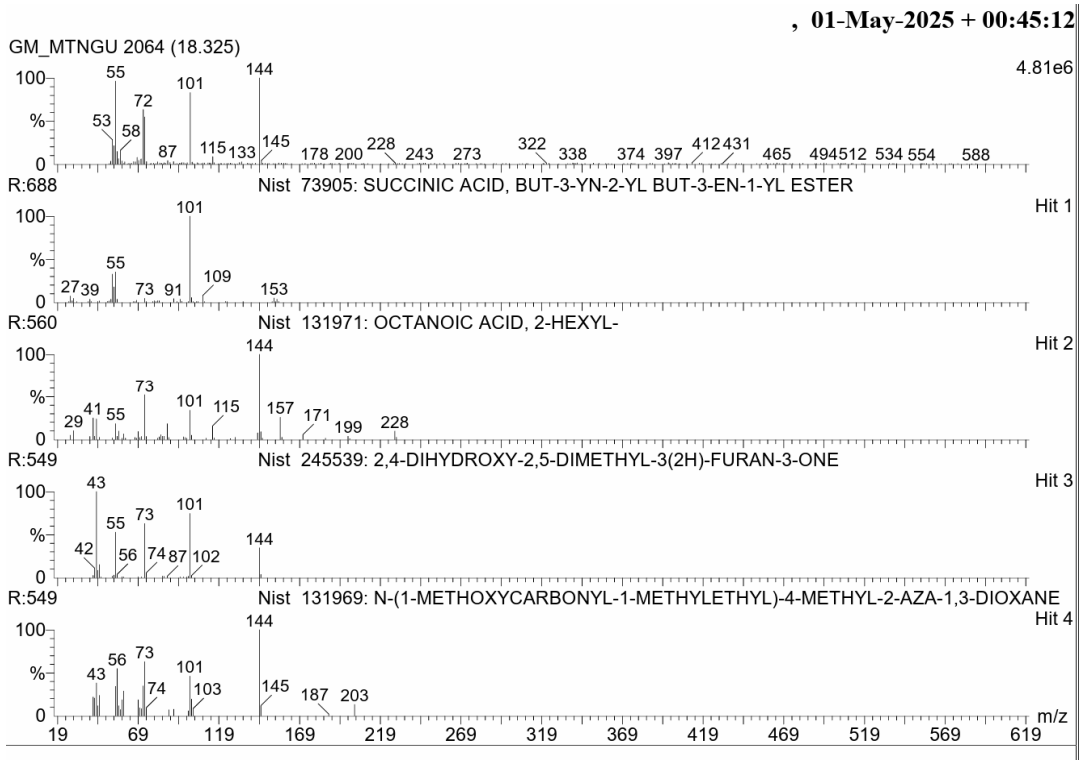


Fig. 7 : Spectrum of major identified componds in the crude metabolites of the extract GM_MTNGU at Retention time 18.325

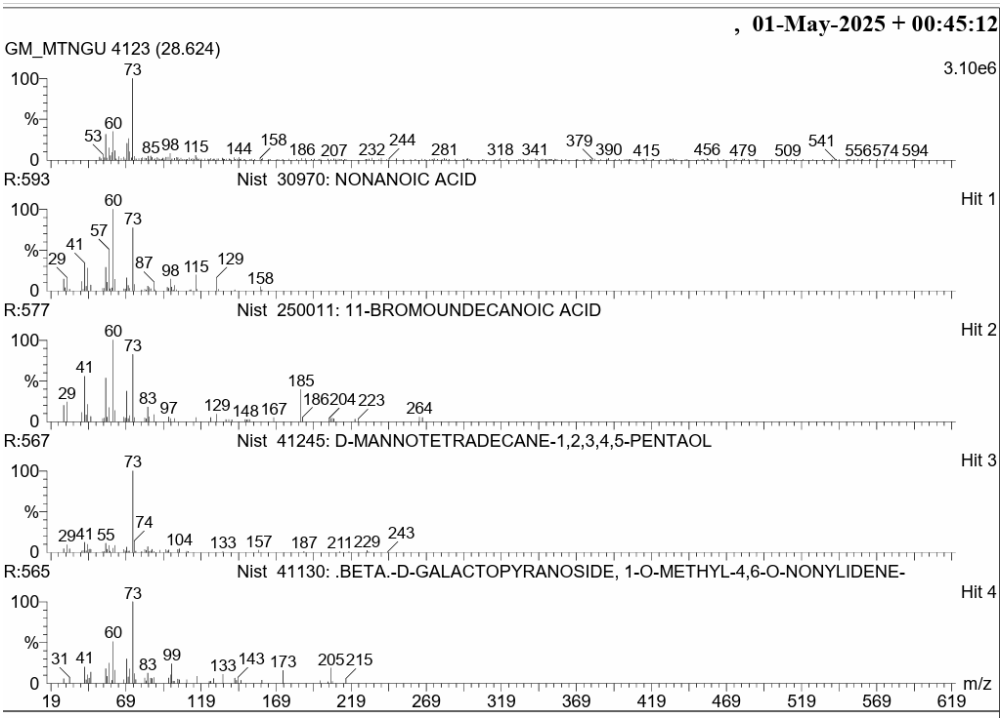
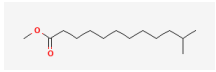
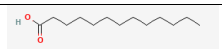
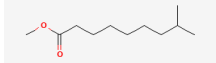
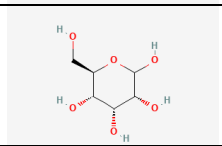
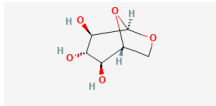
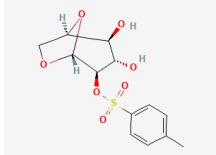
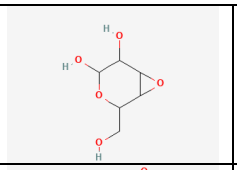
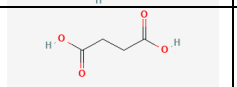
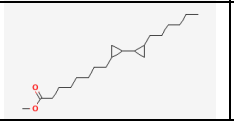
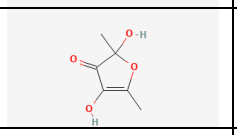
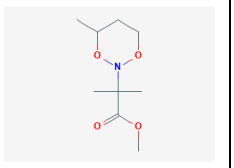
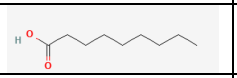
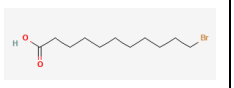
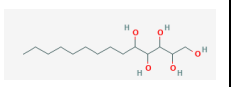
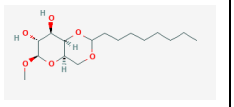


Fig. 8 : Spectrum of major identified compounds in the crude metabolites of the extract GM_MTNGU at Retention time 28.624

Table 5: Major Compounds identified in the crude metabolites of the extract GM_MTNGU at Retention time 33.226 with their chemical structure and bioactivity.

Retention time	Compound name with chemical formula	Chemical Structure	Bioactivity	Reference
33.226	Heptacosanoate, 25-methyl-methyl ester ($C_{29}H_{58}O_2$)		Antimicrobial, Cytotoxicity	Youssef *et al*., 2016; El Sawi *et al*., 2014
	Methyl 11-methyldodecanoate ($C_{14}H_{28}O_2$)		Diuretic potential, Laxative, Antioxidant	Islam *et al*., 2022; Asamara *et al*., 2025
	Tridecanoic acid ($C_{13}H_{26}O_2$)		Antimicrobial	Chowdhury *et al*., 2021
	Methyl 8-methylnonanoate ($C_{11}H_{22}O_2$)		Antimicrobial	Deka & Jha, 2020
26.679	D-ALLOSE $C_6H_{12}O_6$		Antioxidant, Anicancer	Shintani & Sato, 2020
	BETA.-D-GLUCOPYRANOSE, 1,6-ANHYDRO- $C_6H_{10}O_5$		Antibacterial	Igwe & Okwu, 2013
	BETA.-D-GLUCOPYRANOSE, 1,6-ANHYDRO- $C_{13}H_{16}O_7S$		Antibacterial	Igwe & Okwu, 2013

	3,4-ALTROSAN $C_6H_{10}O_5$		Fungicide	Suriati *et al*., 2025
18.325	SUCCINIC ACID $C_4H_6O_4$		Antibacterial, Anti-inflamatory	Kuchana *et al*., 2020; Yang *et al*., 2007
	OCTANOIC ACID, 2-HEXYL $C_{21}H_{38}O_2$		Antimicrobial, Antioxidant	Aziz *et al*., 2026; Shawer *et al*, 2022
	2,4-DIHYDROXY-2,5-DIMETHYL-3(2H)-FURAN-3-ONE $C_6H_8O_4$		Antioxidant	Dhami & Pant, 2024
	N-(1-METHOXYCARBONYL-1-METHYLETHYL)-4-METHYL-2-AZA-1,3-DIOXANE $C_9H_{17}NO_4$ $C_9H_{17}NO_4$		Antifungal	Debi, 2020
28.624	NONANOIC ACID $C_9H_{18}O_2$		Antifungal, Antimicrobial	Sahin *et al*., 2006; Zanki *et al*., 2026
	11-BROMOUNDECANOIC ACID $C_{11}H_{21}BrO_2$		Antimicrobial, Antioxidant	Yasa *et al*., 2017, Gandhi *et al*., 2021
	D-MANNOTETRADECANE-1,2,3,4,5-PENTAOL $C_{14}H_{30}O_5$		Antioxidant	Bora *et al*., 2025
	BETA.-D-GALACTOPYRANOSIDE, 1-O-METHYL-4,6-O-NONYLIDENE- $C_{16}H_{30}O_6$		Antioxidant	Augustini *et al*., 2025

Antimicrobial Activity

The leaf extracts of *Garcinia pedunculata* were examined for antibacterial activity against four bacterial strains: *Escherichia coli* (ATCC2412), *Staphylococcus aureus* (ATCC 2408), *Pseudomonas aeruginosa* (ATCC2080), and *Bacillus anthracis* (ATCC4453). The results, expressed as inhibition zones (Mean ± SD in mm), are presented in Table 6.

Table 6: The inhibition zones of the extracts of *Garcinia pedunculata* leaves against the tested microorganisms.

Extract / Control	***Escherichia coli* (ATCC 2412) Mean ± SD (mm)**	***Staphylococcus aureus* (ATCC 2408) Mean ± SD (mm)**	***Pseudomonas aeruginosa* (ATCC 2080) Mean ± SD (mm)**	***Bacillus anthracis* (ATCC 4453) Mean ± SD (mm)**
Positive control	19.33 ± 0.57	27.33 ± 1.15	23.66 ± 0.57	19.33 ± 2.08
Negative control	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Ethanol	17 ± 1	0 ± 0	9 ± 1	6.66 ± 0.57
Methanol	19.33 ± 1.52	17.66 ± 0.57	16.33 ± 1.15	14 ± 1
Petroleum ether	13 ± 1	13.5 ± 1	14 ± 1	1.66 ± 0.57
Hexane	14.33 ± 1.15	0 ± 0	0 ± 0	8.33 ± 1.15

Positive control: Streptomycin; Negative control: 10% DMSO.

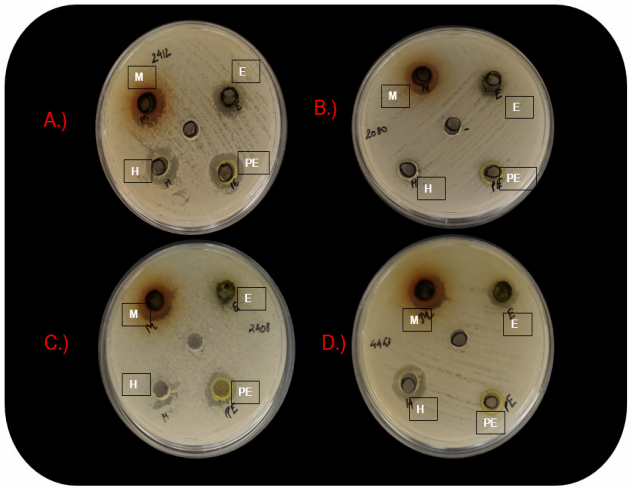


Fig. 9: Photo plates showing antimicrobial activity of crude extract against four different test pathogens.

(A) *Escherichia coli* (ATCC2412)
(B) *Pseudomonas aeruginosa* (ATCC 2080)
(C) *Staphylococcus aureus* (ATCC 2408)
(D) *Bacillus anthracis* (ATCC 4453)
E= Ethanol, M= Methanol, PE= Petroleum Ether, H= Hexane, -ve =Negative control, +ve = Positive Control.

The antibacterial properties in the four leaf extracts of *G. pedunculata* were analyzed against four bacterial strains, and a spectrum of the extent and intensity of inhibitions were found varying significantly among the extracts. The methanol extract had the most stable antibacterial activities and had a broad-spectrum of activities with inhibition zone diameters of 19.33 ± 1.52 mm against *E. coli*, 17.66 ± 0.57 mm against *S. aureus*, 16.33 ± 1.15 mm against *P. aeruginosa*, and 14 ± 1 mm against *B. anthracis*. Particularly, the activity against the *E. coli* was practically virtually equivalent to the positive control, suggesting potent inhibitory compounds extractable by polar solvents. This is in line with previous studies of fruit extracts of *G. pedunculata*, which demonstrated that the methanol fraction was more potent against antibacterial activity than ethanol, and minimum inhibitory concentration ranged between 12.5 mg/mL against *S. aureus* and *P. aeruginosa* (Zoliansanga and Lalfakzuala, 2021). The extensive broad-spectrum activity of polar extracts is explained by their increased ability to dissolve and concentrate hydrophilic bioactives including phenolics, flavonoids, tannins, and alkaloids that have been reported in *G. pedunculata* (Sarma *et al*., 2016; Hemshekhar *et al*., 2011).

The ethanol extract also demonstrated reasonably good antibacterial activity, especially against the *E. coli* (17 ± 1 mm), but with a more selective profile: it was unable to generate a detectable inhibitory zone against the *S. aureus*, whereas it produced moderate effect against *P. aeruginosa* (9 ± 1 mm) and the *B. anthracis* (6.66 ± 0.57 mm). The dissimilar efficacy of the two polar solvents is probably the indication of qualitative and quantitative variability of the secondary metabolite contents extracted by them. Due to a higher polarization of methanol compared to ethanol, it has the capability to dissolve a wider range of polar molecules such as saponins and some glycosylated phenolics which may explain its more consistent activity with both Gram-positive and Gram-negative species (Jubair *et al*., 2021). A noteworthy finding is that even the petroleum ether and hexane extracts, which are generally considered to be non-polar fractions with high concentrations of fatty acids, sterols and terpenoids, exhibited some measurable antibacterial activity against some of the strains, unlike the non-polar fractions of many other medicinal plants which are mostly inactive. The petroleum ether extract demonstrated 13 ± 1 mm of activity against *E. coli*, 13.5 +1 mm against *S.aureus*, and 14 ± 1mm against *P.aeruginosa,* while negligible activity against *B.anthracis* (1.66 ± 0.57 mm). The hexane gave a zone of 14.33 ± 1.15 mm against *E. coli*, and 8.33 ± 1.15 mm against *B. anthracis*, but not detectable activity against *S. aureus* or *P. aureginosa*. The antibacterial activity observed in the non-polar fractions is due to the presence of xanthones or terpenoid derivatives which are known to be well-known constituents of the *Garcinia* genus and have been proven to exhibit antimicrobial activity even in the non-polar solvents (Hemshekhar *et al*., 2011; Nguyen *et al*., 2021). These results are contrary to previous studies with relatively high MIC values of hexane and chloroform extracts of the fruit rinds of the species, hexane and chloroform of extracts of the leaf of the species; Negi *et al*. (2008) reported that the extracts of the leaf part are potentially a better source of non-polar bioactive compared to the extracts of the fruit rind. The existing findings indicate that there is intricate, strain-specific pattern of susceptibility in the four extracts. All four extracts inhibited *E. coli* although it was relatively less susceptible to the methanol extract compared to the susceptibility of the methanol extract to *S. aureus*. The apparent predisposition of the Gram-positive *S. aureus* to the methanol extract (17.66 ± 0.57 mm) can be linked to the overall phenomenon that Gram-positive bacteria with no outer lipopolysaccharide (LPS) membrane are susceptible to plant-derived phenolic and flavonoid substances (Maher and Hassan, 2023). Gram-negative bacteria have an outer membrane which forms a major permeability barrier, consisting of an asymmetrical lipid bi-layer, where an outer LPS leaflet restricts passive diffusion of hydrophobic compounds

and active expels much of phytochemicals through the system of efflux pumps (Maher and Hassan, 2023). However, the methanol extract has shown remarkable activity in both Gram-negative test organisms (*E. coli* and *P. aureginosa*) implying that some hydrophilic bioactives found in *G. pedunculata* leaves/flavonoids and phenolic acids/may be diffusing through the porin channels or triggering their antimicrobial action by interfering with membrane integrity (Gorniak *et al*., 2019; Borges *et al*., 2013). The observed antibacterial activity highlights the phytochemical diversity of *G. pedunculata*. This plant is reported to produce a wide range of secondary metabolites, such as xanthones, benzophenones, flavonoids, phenolic acids and lactones (Hemshekhar *et al*., 2011; Sarma *et al*., 2016). The of flavonoids give antibacterial action through various mechanisms, e.g., the disruption of membrane, the inhibition of enzymes of cell-wall synthesis and the alteration of fatty-acid biosynthesis pathways (Gorniak *et al*., 2019), though the primary mode of phenolic acids is the disruption of bacterial lipid bilayer by partitioning (Borges *et al*., 2013). The broad spectrum antibacterial activity of *G. pedunculata* leaf extracts in this study is consistent with antimicrobial outcomes reported in closely related *Garcinia* species (i.e., *G. lancifolia*, where fruit juice and methanol extracts showed significant antimicrobial activity in the Gram-positive and Gram-negative bacteria) (Policegoudra *et al*., 2012) as well as many other *Garcinia* species (Nguyen *et al*., 2021). Taken together, these results prove that the leaves of the genus *G. pedunculata* is a prosperous source of bioactive compounds with a broad-spectrum antibacterial profile, and thus, it needs to be investigated to elucidate its bioactivity via the determination of the minimum inhibitory concentration, bioactivity-directed fractionation, and mechanisms of action

Conclusion

The present study supported that *Garcinia pedunculata* leaves are a good source of biologically active phytochemicals with considerable antimicrobial potential. The phytochemical qualitative analysis revealed the presence of alkaloids, flavonoids, phenolic compounds, saponins and phytosterols, especially in the methanol extract, indicating the efficiency of polar solvents to extract bioactive constituents. GC–MS analysis revealed a complex phytochemical profile such as terpenoids, flavonoids, phenolic acids, phytosterols, fatty acids, tocopherols and triterpenoids. Several important compounds such as ellagic acid, campesterol, apigenin, betulinic acid, β-sitosterol, and oleanolic acid have been shown to have antioxidant, anti-inflammatory, and antimicrobial activities. The antimicrobial assay showed that the ethanol extract inhibited the growth of *Escherichia coli, Pseudomonas aeruginosa* and *Bacillus anthracis*, but *Staphylococcus aureus* was resistant to the extract. The antibacterial activity observed may be attributed to the synergistic effects of phenolics, flavonoids and terpenoid compounds identified in the extract. *Garcinia pedunculata* has considerable phytopharmaceutical potential and may be a natural source of antimicrobial agents. The study provides scientific basis for the traditional medicinal use of the plant and its possible applications in drug development and nutraceutical industries. Additional studies are required to confirm its therapeutic value, including isolation of pure compounds, toxicity assessment, mechanism of action and clinical validation.

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Disclosure of Interest (Conflict of Interest): The authors declare that there are no competing interests.

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