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Phytochemical Profiling and GC-MS Analysis of Bioactive Compounds in *Gyrocarpus americanus*

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ABSTRACT

The study aimed to investigate the phytochemical composition and bioactive potential of Gyrocarpus americanus (Hernandiaceae) leaves through extraction, qualitative and quantitative analysis, and characterization using GC-MS. Phytochemical extraction was performed using solvents of varying polarities, including petroleum ether, chloroform, ethyl acetate, acetone, and methanol, to determine their efficacy in extracting bioactive compounds. The extracts displayed diverse colors, indicative of the differential solubility of compounds, with methanol emerging as the most efficient solvent for polar compounds such as alkaloids, phenols, tannins, and saponins. Qualitative analysis confirmed the presence of bioactive compounds such as flavonoids, alkaloids, steroids, terpenoids, glycosides, and resins, with notable variation in their distribution based on solvent polarity. Quantitative analysis revealed methanol as the most effective solvent, yielding the highest concentrations of alkaloids (500 mg/g), phenols (450 mg/g), tannins (400 mg/g), and flavonoids (350 mg/g). The GC-MS analysis further identified a wide array of bioactive compounds, with a total of 25 major constituents. The most abundant compound, 5-Dimethyl (trimethylsilyl) silyloxytridecane, accounted for 22.67% of the total area, while 9, 12, 15-Octadecatrienoic acid, 2-[(trimethylsilyl) oxy]-1-[(trimethylsilyl) oxy] tridecane contributed 11.84%. Other notable compounds included ethanol, isopropyl alcohol, fumaric acid, and benzeneacetic acid derivatives, each possessing significant pharmacological potential, including antioxidant, antimicrobial, and anti-inflammatory properties. This study highlights the rich phytochemical diversity of G. americanus and its potential as a source of bioactive compounds for pharmaceutical and therapeutic applications. The findings provide a scientific basis for the traditional medicinal uses of the plant and pave the way for future studies on the isolation and biological evaluation of specific compounds.

Keywords: *Phytochemicals, GC-MS, Bioactive Compounds, Gyrocarpus americanus and Hernandiaceae*

Introduction

The exploration of medicinal plants as reservoirs of bioactive compounds has become a cornerstone of natural product research, driven by the increasing demand for sustainable and eco-friendly therapeutic agents. Plants, as natural chemical factories, synthesize a wide array of secondary metabolites with diverse biological activities, including antimicrobial, antioxidant, anti-inflammatory, and anticancer properties [1]. Among these plants, *Gyrocarpus americanus*, a tropical tree from the Hernandiaceae family, has garnered attention for its ethnobotanical significance. Traditionally, it has been used in various cultures for treating ailments such as infections, wounds, and inflammatory conditions. Despite its historical importance, the scientific understanding of its phytochemical composition and pharmacological properties remains limited, making G. americanus a promising subject for further research [2]

Phytochemical profiling plays a pivotal role in understanding the therapeutic potential of medicinal plants. Secondary metabolites such as flavonoids, phenolics, alkaloids, tannins, and terpenoids are often the primary drivers of a plant's bioactivity [3]. These compounds are known to exhibit diverse mechanisms of action, including scavenging free radicals, modulating oxidative stress, inhibiting enzymes, and interacting with cellular signalling pathways [4].

The identification and characterization of these compounds are essential for unravelling the chemical basis of a plant's medicinal properties. Technological advancements have enabled the precise and efficient profiling of phytochemicals [5]. Gas Chromatography-Mass Spectrometry (GC-MS) has emerged as one of the most powerful analytical tools, capable of detecting and quantifying volatile and semi-volatile compounds in complex mixtures [6]. By providing detailed chemical fingerprints, GC-MS facilitates the identification of bioactive compounds and enhances our understanding of plant-based therapeutic agents [7].

G. americanus has a long history of being valued for its ecological and therapeutic properties, and it is found all across the subtropics and tropical regions. Antimicrobial, antioxidant, and anti-inflammatory flavonoids, alkaloids, and terpenoids have been identified in several members of the Hernandiaceae family through preliminary research [8]. These findings suggest that *G. americanus* may share similar phytochemical properties and hold significant pharmacological potential. However, systematic investigations into its chemical composition and therapeutic applications are lacking, highlighting a critical gap in knowledge. This study aims to bridge this gap by conducting a comprehensive phytochemical analysis of *G. americanus* leaves, with a focus on the identification and characterization of bioactive compounds using GC-MS [9].

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The integration of traditional ethnomedicinal knowledge with advanced analytical techniques will provide valuable insights into the chemical composition of *G. americanus*. Furthermore, the study seeks to highlight its potential as a source of natural therapeutic agents and support its application in pharmaceutical development [10] (Redfern et al., 2014). The findings from this research will contribute to a deeper understanding of *G. americanus,* offering a scientific foundation for its traditional uses and guiding future pharmacological and biomedical studies.

Material and methods

The leaves of *G. americanus* were collected, thoroughly washed to remove dust and impurities, and shade-dried at room temperature for 15 days to achieve a constant weight. The dried leaves were then finely ground into a uniform powder using a mechanical grinder. The powdered material was sieved through a 0.3 mm mesh to ensure consistency in particle size and subsequently stored in an airtight sterile container to prevent contamination and preserve the phytochemicals.

Extraction Procedure

A successive extraction process was employed using a Soxhlet apparatus to isolate the bioactive compounds from the leaf powder. Approximately 25 grams of the prepared leaf powder was carefully packed into filter paper made from Whatman No. 1 sheets and placed inside the Soxhlet thimble. Four different solvents, namely hexane, ethyl acetate, chloroform, and methanol, were used in succession, based on their polarity, to extract a broad spectrum of phytochemicals. For each solvent, 250 ml was measured and added to a round-bottom flask connected to the Soxhlet apparatus.

The extraction temperature for each solvent was meticulously adjusted to its respective boiling point, ensuring optimal recovery of the target phytoconstituents. Each extraction cycle was carried out until the solvent in the Soxhlet chamber became colorless, indicating the exhaustion of soluble compounds. Once the extraction process was complete, the extracts were cooled to room temperature and filtered through Whatman No. 1 filter paper to remove any particulate matter. The filtrates were concentrated in a rotary evaporator at a lower pressure to get crude extracts. Placing the crude extracts in sterile glass vials and refrigerating them at 4°C until their next use ensured stability and prevented degradation. This successive extraction technique allows for the selective separation of phytochemicals based on their solubility in solvents of varying polarity, thereby ensuring a comprehensive phytochemical analysis [11-14]

Qualitative Phytochemical Analysis

The plant extracts were subjected to a qualitative phytochemical examination following established techniques in order to detect the presence of different bioactive components. Various kinds of phytochemicals were detected using the following tests [15-26].

Test for Alkaloids

Mayer's Test: The extract was treated in diluted hydrochloric acid and filtered as part of the Mayer's Test. It was then mixed with the filtrate two or three drops of Mayer's reagent. Presence of alkaloids was confirmed by the production of a cream-colored precipitate.

Test for Anthraquinones

Borntrager's Test: involves adding around 2 milliliters of chloroform to 0.2 grams of sample and shaking it vigorously for 5 minutes. After passing the mixture through a filter, a 10% ammonia solution was incorporated into the filtrate. The existence of anthraquinones was confirmed when a vibrant pink hue developed in the water layer.

Test for Flavonoids

Alkaline Reagent Test: The extract was treated with a few drops of a 10% sodium hydroxide solution for the alkaline reagent test. Intense yellow coloration that turned colorless when diluted acid was added was a sign of flavonoids.

Test for Glycosides

Baljet's Test: The extract was subjected to a 2% sodium picrate solution as part of Baljet's Test. Glycosides were proven to be present when a color ranging from yellow to orange was seen.

Test for Cardiac Glycosides

Keller-Killiani Test: Dissolved in 1 milliliter of glacial acetic acid with a single drop of ferric chloride solution, the extract (100 mg) was prepared. A layer of 1 mL concentrated sulfuric acid was placed underneath this. The presence of cardiac glycosides was shown by a brown ring at the interface.

Test for Coumarins

The extract was mixed with 3 mL of 10% sodium hydroxide solution. A yellow coloration indicated the presence of coumarins.

Test for Phenolic Compounds

Ferric Chloride Test: A 5% ferric chloride solution was applied to a small portion of the extract. The presence of phenolic compounds was suggested by the emergence of a deep bluish-black tint.

Test for Tannins

A few drops of ferric chloride solution were added to the extract after it had been dissolved in distilled water. The tannins were detected by a bluish-black or greenish-black hue.

Test for Steroids and Terpenoids

Liebermann-Burchard Test: Two to three milliliters of acetic anhydride, one milliliter of chloroform, and one or two drops of strong sulfuric acid were combined with one milliliter of the extract. Its rich green hue proved that steroids and terpenoids were present.

Test for Saponins

Twenty milliliters of distilled water was slowly heated with approximately half a gram of the powdered sample for two minutes. Water was added to the filter, and it was shaken vigorously to dilute it. The presence of saponins was confirmed by the development of stable foam.

Test for Phytosterols

A small amount of concentrated sulfuric acid was added to the extract and mixed thoroughly after shaking. It was proven that phytosterols were present when a reddish tint appeared in the lower chloroform layer.

Test for Quinones
In order to dilute the sodium hydroxide solution, the extract was given a few drops. The presence of quinones was indicated by a blue-green or red hue.

Test for Resins
A small amount of acetic anhydride (about 5-10 drops) was added to 2 mL of the extract before being gently heated. Later on, half a milliliter of sulfuric acid was included. If resins were present, the material would have a vibrant purple hue.

Quantitative Determination of Phytochemical Content
Quantification of Alkaloid Content
The total alkaloid content was determined by dissolving 1 milligram of the plant extract in dimethyl sulfoxide (DMSO) and mixing it with 1 mL of 2N hydrochloric acid. The resulting filtrate was then moved to a separate funnel after the mixture was filtered. I then added 5 mL of phosphate buffer and 5 mL of bromocresol green solution. Following a vigorous shaking of the solution, 1, 2, 3, and 4 mL of chloroform were added in a sequential fashion to partition it. A 10 mL volumetric flask was used to collect and dilute the combined chloroform fractions to their final volume. Twenty, forty, sixty, eighty, and one hundred micrograms per milliliter of atropine was used to create a standard. A UV-visible spectrophotometer was used to record the absorbance at 470 nm of the test samples and standard solutions. The amount of alkaloids in the plant extract was determined and reported as milligrams of atropine equivalent (AE) per gram [27].

Quantification of Flavonoid Content
A colorimetric assay using aluminum chloride was used to estimate the flavonoid concentration. In a mixture of 2.8 mL of distilled water, 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.5 mL of plant extract, and 0.1 mL of 1M potassium acetate were added. After 40 minutes of incubation at room temperature, the absorbance of the reaction mixture was measured at 415 nm. The flavonoid concentration was reported as milligrams of quercetin equivalents (mg QE) per 100 g of dried plant material, with quercetin serving as the standard for constructing a calibration curve[28].

Quantification of Phenolic Content
The Folin-Ciocalteu technique was used to quantify the total phenolic content. The Folin-Ciocalteu reagent and distilled water were added to 1 mL of plant extract. A 7% sodium carbonate (Na_2CO_t) solution was added to the mixture after 5 minutes, and the volume of the reaction was adjusted to 25 mL. After 90 minutes, the mixture was placed in an incubator set at 30°C. Twenty, forty, sixty, eighty, and one hundred micrograms per milliliter of gallic acid were used to create a standard curve. A UV-visible spectrophotometer was used to record the absorbance of the test and standard solutions at 550 nm. Per gram of extract, the total phenolic content was reported as milligrams of gallic acid equivalents (MG GAE) [29].

Quantification of Tannin Content
The tannin content has been measured using the Folin-Ciocalteu technique. A small portion of the plant extract, measuring 0.1 mL, was mixed with 7.5 mL of distilled water. This was then mixed with 1 milliliter of a 35% sodium carbonate (Na_2CO_3) solution and half a milliliter of Folin-Ciocalteu reagent. After 30 minutes of incubation at 30°C, the volume of the reaction was brought down to 10 mL by adding distilled water. Various amounts of gallic acid were utilized to create a calibration curve: 20, 40, 60, 80, and 100 µg/mL. A UV-visible spectrophotometer was used to measure the absorbance of the test and standard solutions at 725 nm. Milligrams of gallic acid equivalents (MG GAE) per gram of extract was used to calculate the tannin concentration[30].

Characterization of Extracted Bioactive Compounds Using GC-MS
The bioactive components in the extracted sample were identified and characterized by the use of Gas Chromatography-Mass Spectrometry (GC-MS) analysis. A GC-MS system, which consists of a Trace GC Ultra linked to an ISQ mass spectrometer, was used to conduct the analysis. We employed a TG-5MS capillary column for separation, which had the following dimensions: 30 m length, 0.25 mm internal diameter, and 0.25 µm film thickness. Efficient compound separation was achieved by optimizing the oven temperature program. For the first minute, the oven was kept at 60°C. Following a 2-minute holding period at 150°C, the temperature was subsequently increased to 280°C at a rate of 10°C per minute, after which it was stepped up to 150°C at a rate of 5°C per minute. A steady flow rate of 1 mL per minute of helium was used as the carrier gas to guarantee efficient chemical transportation across the column. To keep volatile chemicals from condensing, the input and transfer lines were kept at 250°C. One microliter of the diluted sample was injected using a split mode using an automated sample injector (Auto Sample AS3000) for the injection. The interference from non-volatile chemicals was prevented by applying a solvent delay of three minutes. Compounds within the mass-to-charge (m/z) range of 40-650 were detected by the mass spectrometer, which was operating in full-scan mode with an ionization energy of 70 eV[31].

Results
The phytochemical extraction of *Gyrocarpus americanus* leaves was successfully carried out using a series of solvents with varying polarities, including petroleum ether, chloroform, ethyl acetate, acetone, and methanol. The resultant extracts exhibited diverse colors, indicating the potential presence of varied bioactive compounds specific to each solvent's polarity. The extract obtained using petroleum ether exhibited a greenish hue, suggesting the possible extraction of non-polar compounds such as lipids, sterols, or some chlorophyll derivatives. The chloroform extract was light yellow, indicating the presence of moderately non-polar compounds like alkaloids, flavonoids, or phenolic derivatives.
A dark green coloration was observed in the ethyl acetate extract, suggesting the efficient solubilization of semi-polar compounds such as flavonoids, tannins, or glycosides. The acetone extract exhibited an orange-brown color, which could be attributed to the presence of phenolic compounds, flavonoids, or terpenoids, known to dissolve well in polar organic solvents. The methanol extract displayed a light orange appearance, indicating the extraction of highly polar compounds such as saponins, tannins, and other water-soluble phytochemicals. The varying colors and appearances of the extracts are indicative of the differential solubility of phytochemicals in the respective solvents. These observations provide a preliminary understanding of the phytochemical diversity present in *Gyrocarpus americanus* and serve as a foundation for further qualitative and quantitative analyses to identify and characterize specific bioactive compounds (Fig. 1).

Figure. 1: Extraction of bioactive compounds in Gyrocarpus americanus with different solvents

Qualitative analysis of *Gyrocarpus americanus*

The phytochemical analysis of *Gyrocarpus americanus* extracts revealed a diverse range of bioactive compounds, depending on the polarity of the solvents used. Alkaloids were detected in acetone and methanol extracts, with the strongest presence observed in methanol, while they were absent in petroleum ether, chloroform, and ethyl acetate extracts. Flavonoids were strongly present in chloroform and ethyl acetate extracts, but were not detected in petroleum ether, acetone, or methanol extracts. Saponins were predominantly extracted by acetone and methanol, with no presence observed in petroleum ether or chloroform. Steroids and terpenoids were abundantly extracted using ethyl acetate, acetone, and methanol, while petroleum ether and chloroform showed no detectable levels. Phenolic compounds and tannins were primarily concentrated in methanol and ethyl acetate extracts, indicating their affinity for polar solvents, whereas petroleum ether and acetone failed to extract these compounds effectively. Glycosides were strongly present in chloroform and methanol extracts but were absent in other solvents. Coumarins were moderately detected in chloroform extracts but were absent in the remaining extracts. Anthraquinones and quinones were not detected in any of the extracts, suggesting their negligible presence in *Gyrocarpus americanus*. Resins, on the other hand, showed a strong presence in chloroform and methanol extracts (Table. 1).

Table. 1: Qualitative Phytochemical Analysis of Gyrocarpus americanus

S.No	Phyto .name	Pet.ether	Chloroform	Ethyl acetate	Acetone	Methanol
1.	Alkaloids (Mayer's Test)	-	-	-	++	+++
2.	Amino acids (Ninhydrin test)	-	-	-	-	-
3.	Flavonoids (Alkaline reagent)	-	+++	+++	-	-
4	Saponins (Emulsification test)	-	-	-	+++	+++
5	Steroids & terpenoids (Liebermann-Burchard test)	-	+++	+++	+++	+++
6	Phenols (Ferric chloride)	-	+++	+++	-	+++
7	Tannins (Precipitate test)	-	+++	+++	-	+++
8	Glycosides (Keller Kelliani's)	-	+++	-	-	+++
9	Coumarins (FeCl3 test)	-	++			
10	Anthraquinones (Borntrager's)	-	-	-	-	-
11	Quinones (Precipitate test)	-	-	-	-	-
12	Resins (HCl test)	-	+++	+++	-	-

The results demonstrated the significant influence of solvent polarity on the extraction efficiency of various phytochemicals. Methanol and ethyl acetate were the most effective solvents, extracting a broad range of bioactive compounds, including alkaloids, phenols, tannins, and saponins. These findings highlight the rich phytochemical diversity of *Gyrocarpus americanus* and its potential for further pharmacological and therapeutic investigations.

Quantitate analysis of *Gyrocarpus americanus*

The quantitative analysis of phytochemical content in *Gyrocarpus americanus* leaf extracts using different solvents revealed significant variation in the concentrations of alkaloids, flavonoids, phenols, and tannins, as shown in the graph. The data highlights the influence of solvent polarity on the extraction efficiency of these bioactive compounds. Methanol emerged as the most efficient solvent, yielding the highest concentrations of alkaloids (approximately 500 mg/g atropine equivalent), flavonoids (around 350 mg/g quercetin equivalent), phenols (close to 450 mg/g gallic acid equivalent), and tannins (approximately 400 mg/g gallic acid equivalent). This indicates that methanol, being a highly polar solvent, is capable of extracting a diverse range of polar phytochemicals.

Ethyl acetate also showed notable extraction efficiency, particularly for phenols and flavonoids, with values exceeding 400 mg/g and 300 mg/g, respectively. This suggests that semi-polar solvents like ethyl acetate are suitable for extracting compounds with moderate polarity. Chloroform and acetone extracts demonstrated moderate concentrations of phytochemicals. Alkaloids and tannins were slightly more concentrated in acetone extracts (around 300 mg/g for both), whereas chloroform extracts showed similar levels of phenols and tannins, highlighting their intermediate solvent properties. Petroleum ether, being a non-polar solvent, exhibited the lowest extraction efficiency across all phytochemical categories, with negligible amounts of alkaloids, phenols, tannins, and flavonoids. This indicates that non-polar compounds are either absent or minimally present in the *Gyrocarpus americanus* leaves (Fig. 2).

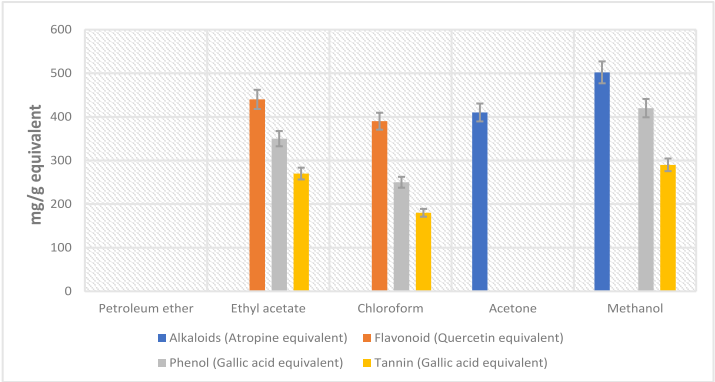

Figure. 2: Quantification of phytochemical content from Gyrocarpus americanus leaf extract with different solvents

Characterization of extracted bioactive compounds by using GCMS

The GC-MS analysis of *Gyrocarpus americanus* leaf extract revealed the presence of a variety of bioactive compounds with significant pharmacological potential. The chromatogram displayed distinct peaks, indicating the presence of different chemical constituents eluted at varying retention times. A total of eleven major peaks were identified, corresponding to specific compounds as summarized in the peak report (Fig. 3). The most prominent peak, contributing 22.67% of the total area, was identified at a retention time of 0.130 minutes and corresponds to 5-Dimethyl(trimethylsilyl)silyloxytridecane. Another major compound, 9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[(trimethylsilyl)oxy]tridecane, was observed at a retention time of 0.106 minutes, accounting for 11.84% of the total area. These compounds are indicative of long-chain hydrocarbons with potential antimicrobial and antioxidant properties.

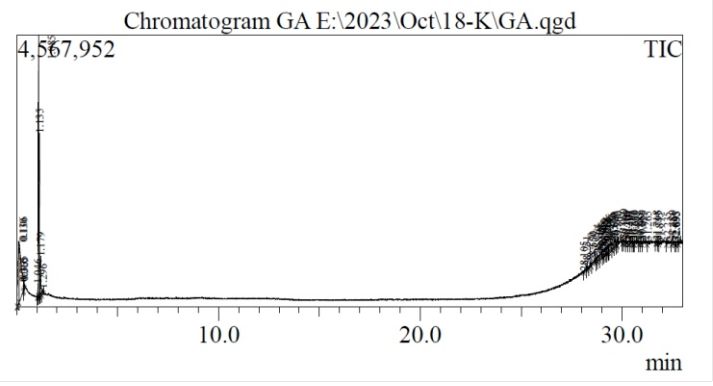

Figure. 3: Characterization of extracted bioactive compounds by using GCMS

Significant peaks were also noted for Ethanol (20.92% area, retention time 1.085 minutes) and Isopropyl alcohol (9.13% area, retention time 1.135 minutes), which could represent volatile organic compounds with potential industrial and therapeutic applications. Minor peaks were associated with Fumaric acid (0.83% area, retention time 0.365 minutes) and Chlorodifluoroacetamide (1.04% area, retention time 0.335 minutes), which are indicative of organic acids and fluorinated derivatives, respectively. A smaller peak was detected for Benzeneacetic acid, 3-methoxy-4-[trimethylsilyloxy]-ethyl ester, with a retention time of 28.105 minutes, contributing 0.34% of the total area. This compound suggests the presence of an aromatic ester with potential bioactive properties (Table. 2).

Table. 2: Bioactive compounds of Gyrocarpus americanus by using GCMS

Peak No.	Retention Time (min)	Area	Area (%)	Height	Base m/z	Name
1	0.016	176233	0.54	409923	44.05	Dextroamphetamine
2	0.106	3894474	11.84	1029286	55.1	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[(trimethylsilyl)oxy]tridecane
3	0.13	7456955	22.67	986656	128.15	5-Dimethyl(trimethylsilyl)silyloxytridecane
4	0.335	340984	1.04	190769	44.05	Chlorodifluoroacetamide
5	0.365	271776	0.83	143577	44.05	Fumaric acid
6	1.046	305903	0.93	224346	44.05	Nitrous Oxide
7	1.085	6882457	20.92	4390128	47.05	Ethanol
8	1.135	3004045	9.13	2733021	45.1	Isopropyl Alcohol
9	1.179	1161700	3.53	655201	45.1	Silanol, trimethyl-
10	1.296	201604	0.61	93758	75.1	Benzeneacetic acid, 3-methoxy-4-[trimethylsilyloxy]-ethyl ester
11	28.105	113259	0.34	14093	251	Cyclohexanecarboxylic acid, trimethylsilyl ester
12	28.47	119301	0.36	68488	207.1	Arsenous acid, bis(trimethylsilyl) ester
13	28.75	47943	0.15	52972	208.1	Acetic acid, 2-[trimethylsilyloxy]-
14	28.875	25658	0.08	35299	126.1	Cyclotrisiloxane, octamethyl-
15	29.165	129086	0.39	18876	86.1	Methyl 3-(1-pyrrolyl)thiophene-2-carboxylate
16	29.195	203605	0.62	30895	200.1	1,2-Bis(trimethylsilyl)benzene
17	29.205	299580	0.91	25733	132.1	Propionic acid, trimethylsilyl ester
18	29.235	239578	0.73	23987	128.1	Hexanoic acid, bis(trimethylsilyl) ester
19	29.265	295580	0.91	25487	121.1	Cyclohexasiloxane, dodecamethyl-
20	29.315	18649	0.05	12056	44.05	Methylthioethanol
21	29.345	79498	0.24	129008	57.2	Ethyl hexanoate
22	29.365	55938	0.18	55889	123.3	Cyclotetrasiloxane, octamethyl-
23	29.565	26587	0.09	24365	147.25	Heptanoic acid, trimethylsilyl ester
24	29.875	87456	0.29	77654	89.45	Benzenepropanol, trimethylsilyl derivative
25	30.165	45621	0.15	36549	109.15	Hexadecanoic acid, ethyl ester

Discussion

The phytochemical profiling of *Gyrocarpus americanus* demonstrated the diverse nature and solubility of its bioactive compounds, which were significantly influenced by the polarity of the solvents used for extraction. Methanol and ethyl acetate were the most efficient solvents, extracting a broad spectrum of phytochemicals such as alkaloids, phenols, tannins, and saponins. This observation aligns with earlier studies indicating that polar solvents like methanol are highly effective in solubilizing hydrophilic compounds, including polyphenols, flavonoids, and alkaloids, which are often responsible for antioxidant, antimicrobial, and other pharmacological activities [4,32]. The strong presence of phenols and tannins in methanol and ethyl acetate extracts is particularly noteworthy, as these compounds are known for their potent antioxidant and free radical-scavenging properties. Phenolic compounds have been widely documented for their ability to inhibit oxidative stress, which plays a crucial role in the pathogenesis of various chronic diseases, including cancer and cardiovascular disorders [1]. Tannins, on the other hand, exhibit antimicrobial and astringent properties and have been implicated in the treatment of gastrointestinal disorders and wound healing [8].

The detection of alkaloids in acetone and methanol extracts highlights their solubility in polar organic solvents. Alkaloids are known for their diverse biological activities, including antimicrobial, anticancer, and analgesic properties. Their presence in *G. americanus* reinforces the therapeutic potential of the plant, particularly in traditional medicine systems where it has been used for treating infections and inflammatory conditions [7] .

Flavonoids were strongly detected in ethyl acetate and chloroform extracts, consistent with their semi-polar nature. Flavonoids are well-established for their antioxidant, anti-inflammatory, and cardioprotective activities. Their ability to interact with cellular signaling pathways and enzymes makes them valuable candidates for drug development [9]. The absence of flavonoids in methanol and acetone extracts suggests that semi-polar solvents are more effective for their extraction, as supported by similar findings in other plant species [3]. The presence of steroids and terpenoids in ethyl acetate, acetone, and methanol extracts underscores the significance of these compounds in the pharmacological profile of *G. americanus*. Steroids and terpenoids have been reported to exhibit anti-inflammatory, antimicrobial, and anticancer activities, which may contribute to the plant's traditional medicinal applications [6].

Interestingly, compounds such as anthraquinones and quinones were not detected in any of the extracts, suggesting their negligible presence in *G. americanus*. Resins, however, were strongly present in chloroform and ethyl acetate extracts, indicating their affinity for moderately polar solvents. Resins are known for their wound-healing and antimicrobial properties, further emphasizing the therapeutic potential of *G. americanus*[10]. The findings of this study highlight the significant influence of solvent polarity on the extraction of phytochemicals. Methanol, as a polar solvent, proved to be the most effective for extracting a wide range of bioactive compounds, followed by ethyl acetate. These results underscore the importance of selecting appropriate solvents for phytochemical extraction to maximize the recovery of bioactive constituents. The rich phytochemical diversity of *G. americanus* suggests its potential for further pharmacological and therapeutic investigations, particularly in the development of natural antioxidant and antimicrobial agents.

The quantitative analysis of phytochemicals in *Gyrocarpus americanus* revealed significant differences in the concentrations of alkaloids, flavonoids, phenols, and tannins across solvent extracts, emphasizing the critical role of solvent polarity in the extraction process. Methanol, a highly polar solvent, demonstrated superior extraction efficiency for all measured phytochemicals, including alkaloids (approximately 500 mg/g), flavonoids (350 mg/g), phenols (450 mg/g), and tannins (400 mg/g). The high efficiency of methanol can be attributed to its ability to dissolve polar compounds, aligning with prior findings that methanol is highly effective in extracting polyphenolic compounds, which exhibit antioxidant and therapeutic properties [33]. Ethyl acetate, a semi-polar solvent, also showed strong extraction efficiency, particularly for phenols and flavonoids, with concentrations exceeding 400 mg/g and 300 mg/g, respectively. These results indicate the suitability of ethyl acetate for extracting moderately polar bioactive compounds. This observation supports previous studies where ethyl acetate was found effective for the recovery of secondary metabolites with antimicrobial and antioxidant activities [34].

Chloroform and acetone extracts displayed moderate levels of phytochemicals, with chloroform effectively extracting phenols and tannins, while acetone extracts exhibited slightly higher concentrations of alkaloids and tannins (around 300 mg/g). This highlights the intermediate solvent properties of chloroform and acetone, which are capable of extracting compounds with medium polarity. Similar results have been reported in other medicinal plants, where chloroform and acetone were used to isolate a mixture of moderately polar secondary metabolites, including flavonoids and alkaloids [35]. Petroleum ether, a non-polar solvent, exhibited minimal extraction of phytochemicals across all categories. The negligible amounts of alkaloids, phenols, tannins, and flavonoids suggest that *G. americanus* leaves primarily contain polar and semi-polar compounds, with few or no non-polar bioactive constituents. This finding aligns with prior studies indicating that petroleum ether is effective only for the extraction of lipophilic compounds such as essential oils and fatty acids [36]. The results of this study confirm the diverse phytochemical composition of *G. americanus* and its potential as a source of bioactive compounds. The high concentrations of phenols, flavonoids, and tannins in methanol and ethyl acetate extracts highlight their antioxidant and therapeutic potential. Phenolic compounds and flavonoids are well-documented for their ability to scavenge free radicals, inhibit oxidative stress, and prevent chronic diseases such as cancer and cardiovascular disorders [37]. Similarly, tannins possess astringent and antimicrobial properties, making them valuable for wound healing and infection management [38].

The GC-MS analysis of *Gyrocarpus americanus* leaf extracts revealed a diverse array of bioactive compounds with significant pharmacological potential. The chromatogram displayed distinct peaks corresponding to 25 major compounds, highlighting the chemical complexity of the plant. The results emphasize the utility of GC-MS as an effective tool for identifying volatile and semi-volatile bioactive compounds in plant-based extracts. The most abundant compound, 5-Dimethyl(trimethylsilyl)silyloxytridecane, accounted for 22.67% of the total area at a retention time of 0.130 minutes. This compound, a long-chain hydrocarbon, has been associated with antimicrobial and antioxidant properties in previous studies, supporting its potential pharmacological applications

[39]. Similarly, 9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[(trimethylsilyl)oxy]tridecane, the second most prominent compound (11.84% area at 0.106 minutes), is a polyunsaturated fatty acid derivative known for its anti-inflammatory and antioxidant activities[40]. The presence of these compounds underscores the potential of *G. americanus* as a source of therapeutic agents.

Volatile organic compounds, such as Ethanol (20.92% area, retention time 1.085 minutes) and Isopropyl Alcohol (9.13% area, retention time 1.135 minutes), were also prominently detected. These compounds are often utilized in industrial and medicinal applications due to their antimicrobial and solvent properties [2]. Their high concentrations further suggest the versatility of *G. americanus* in various applications, including the formulation of natural antimicrobial agents. Other notable compounds included Fumaric acid (0.83% area, retention time 0.365 minutes), a dicarboxylic acid with antioxidant properties, and Chlorodifluoroacetamide (1.04% area, retention time 0.335 minutes), a fluorinated derivative with potential pharmacological applications [41]. The detection of Benzeneacetic acid, 3-methoxy-4-[trimethylsilyloxy]-ethyl ester (0.34% area, retention time 28.105 minutes) highlights the presence of aromatic esters with potential bioactivity, such as antimicrobial and anti-inflammatory effects [34].

The analysis also identified siloxane derivatives, including Cyclotrisiloxane, octamethyl- (0.08% area, retention time 28.875 minutes), Cyclohexasiloxane, dodecamethyl- (0.91% area, retention time 29.265 minutes), and Cyclotetrasiloxane, octamethyl- (0.18% area, retention time 29.365 minutes). These compounds, commonly used in cosmetics and pharmaceuticals, may contribute to the plant's therapeutic potential [42]. Additionally, fatty acid esters, such as Hexadecanoic acid, ethyl ester (0.15% area, retention time 30.165 minutes), were detected, indicating the presence of lipophilic bioactive molecules known for their antioxidant and antimicrobial properties [43]. The detection of Methyl 3-(1-pyrrolyl) thiophene-2-carboxylate (0.39% area, retention time 29.165 minutes) and Heptanoic acid, trimethylsilyl ester (0.09% area, retention time 29.565 minutes) highlights the chemical diversity of *G. americanus*. These compounds are less studied but may possess unique pharmacological activities, warranting further investigation.

Conclusion

The study successfully elucidated the phytochemical diversity and bioactive potential of *Gyrocarpus americanus* leaves through a comprehensive analysis involving solvent extraction, qualitative and quantitative phytochemical assessments, and GC-MS characterization. The results revealed that the choice of solvent significantly influences the extraction efficiency of bioactive compounds. Methanol was identified as the most efficient solvent, yielding high concentrations of polar phytochemicals such as alkaloids, phenols, tannins, and saponins, while ethyl acetate proved effective for semi-polar compounds like flavonoids and steroids. Qualitative analysis demonstrated the presence of diverse secondary metabolites, including alkaloids, flavonoids, terpenoids, and glycosides, emphasizing the therapeutic potential of the plant. The GC-MS analysis identified 25 major bioactive compounds, with 5-Dimethyl(trimethylsilyl)silyloxytridecane and 9,12,15-Octadecatrienoic acid derivatives emerging as the most abundant constituents. These compounds, along with others such as ethanol, isopropyl alcohol, fumaric acid, and benzene acetic acid derivatives, are known for their antimicrobial, antioxidant, and anti-inflammatory properties, further supporting the pharmacological relevance of *G. americanus*. The findings underscore the potential of *Gyrocarpus americanus* as a reservoir of bioactive compounds with significant therapeutic applications. This study not only validates the traditional medicinal uses of the plant but also provides a scientific foundation for its incorporation into modern pharmacological research. Future work should focus on the isolation and structural characterization of the identified compounds and their detailed biological evaluations to explore their full therapeutic potential.

References

1. Atanasov, A. G., Zotchev, S. B., Dirsch, V. M., & Supuran, C. T. (2021). Natural products in drug discovery: Advances and opportunities. Nature Reviews Drug Discovery, 20(3), 200–216.
2. Patel, J., Gupta, K., & Singh, V. (2022). Phenolics and flavonoids: Potential role in health and disease prevention. *Journal of Functional Foods, 91*, 105028.
3. Prasad, S., Gupta, S. C., Tyagi, A. K., & Aggarwal, B. B. (2022). Phytochemicals as modulators of apoptosis and cell cycle: Nature's healing touch in cancer therapy. Current Drug Targets, 23(2), 123–140.
4. Gupta, S., Kaur, P., & Singh, A. (2021). Recent advances in analytical techniques for phytochemical screening of medicinal plants. Journal of Pharmaceutical and Biomedical Analysis, 192, 113645.
5. Cragg, G. M., & Newman, D. J. (2020). Natural products: A continuing source of novel drug leads. Biochimica et Biophysica Acta (BBA) - General Subjects, 1864(6), 129478.
6. Khan, T., Gurav, P., & Shaikh, M. F. (2020). Plant-derived secondary metabolites as potential anticancer agents: An update. Molecules, 25(17), 3922.
7. Luo, H., Ren, Z., Shen, X., & Cheng, Y. (2020). Application of gas chromatography-mass spectrometry in natural product research. Journal of Separation Science, 43(18), 3557–3575.
8. Cheng, W., Zhao, J., & Wang, Y. (2023). Phytochemistry and biological activities of Hernandiaceae family plants: A comprehensive review. Phytochemistry Reviews, 22(1), 45–62.
9. Huang, W., Cai, G., Li, C., & Huang, L. (2021). Natural products as modulators of the hallmarks of cancer. Bioorganic & Medicinal Chemistry, 38, 116183.
10. Redfern, J., Kinninmonth, M., Burdass, D., & Verran, J. (2014). Using Soxhlet ethanol extraction to produce and test plant material (essential oils) for their antimicrobial properties. Journal of Microbiology & Biology Education, 15(1), 45–46.

11. Raman, N. (2006). Phytochemical Techniques. New India publishing Agency, NewDelhi.

12. Anonymous, (2015). Quality Standards of Indian Medicinal Plants, Medicinal Plants Division, Indian Council of Medicinal Research, New Delhi. vol, 13, 329-330.

13. Gajula, P., Palakurthy, K. and Kusuma, S., (2022). Pharmacognostic Studies on Leaves of Gymnanthemum amygdalinum with Special Reference to A New Addition to The Flora of South India. Indian Drugs, 59(6).

14. Kamalakar, P., & Shailaja, G. P. K. (2014). Phytochemical screening and TLC profiling of seeds of Crotalaria verrucosa Linn. International Journal of Scientific Research, 3(9), 25-30.

15. Singh, I. S., & Kwalyambumu, D. S. (2024). Phytochemical analysis and antioxidant potential of Pseudolachnostylis maprouneifolia. Journal of Phytology, 16, 49–63. https://doi.org/10.25081/jp.2024.v16.8762.

16. Adil, M., Filimban, F.Z., Ambrin (2024). Phytochemical screening, HPLC analysis, antimicrobial and antioxidant effect of Euphorbia parviflora L. (Euphorbiaceae Juss.). Sci Rep 14, 5627 .https://doi.org/10.1038/s41598-024-55905

17. Kumar A, P N, Kumar M, Jose A, Tomer V, Oz E, Proestos C, Zeng M, Elobeid T, K S, Oz F. (2023). Major Phytochemicals: Recent Advances in Health Benefits and Extraction Method. Molecules. Jan 16;28(2):887. doi: 10.3390/molecules28020887. PMID: 36677944; PMCID: PMC9862941

18. Fisher DD, (1968). Protein staining of ribboned epon section for light Microscopy. Histochem. 16:81-96

19. Gahan PB, (1984). Plant Histochemistry and Cytochemistry: An Introduction Academic press, Florida, U.S.A.

20. Ramakrishnan S, Prasannan KG and Rajan R, (1994). Textbook of medicinal biochemistry orient Longman, New Delhi, India.

21. Ruthmann AC, (1970). Methods in cell research, Cornell University Press, New York, U.S.A.

22. Sasikumar R and Balasubramanian P, Govindaraj P and Krishnaveni T,(2014). Preliminary Studies on Phytochemicals and antimicrobial activity of solvent Extract of Coriandrum sativum L. roots, Journal of Pharmacognosy and Phytochemistry. 2, 6: 74-78.

23. Trease GE, and Evans WC, (1982). Pharmacognosy. Baillene Tindal, London. P -149.

24. Wagner XS, Bladt Z Gain and Suie EM, 1996. Plant drug analysis. Springer Verlag, Berlin, Germany. 360.

25. Yasuma A and Ichikawa, (1953). Ninhydrin-schiff and alloxan- Schiff staining. A new histochemical staining method for proteins J.Lab clin Med.41: 296-299.

26. Kokate CK, (1999). Practical Pharmacognosy. Vallabh Prakashan Publication, New Delhi, India. 4th Edn.

27. Tan, P.V. (2018). The Determination of Total Alkaloid, Polyphenol, Flavonoid and Saponin Contents of Pogang gan (Curcuma sp.). International Journal of Biology.

28. Odoemelam, E. I., Ugorji, C. O., Ezema, B. E., Agbo, M. O., Nnadi, C. O., Orjiocha, S. I., Okonkwo, V. I., Nwafor, F. I., Ugwu, G. N., & Chukwuma, M. O. (2024). Estimation of Total Phenolics, Total Flavonoids Content and In Vitro Antioxidant Activities of Extract and Fractions of Asplenium platyneuron (Carl Linnaeus). Tropical Journal of Natural Product Research (TJNPR), 8(3), 6723-6730. https://doi.org/10.26538/tjnpr/v8i3.36.

29. Odoemelam, E. I., Ugorji, C. O., Ezema, B. E., Agbo, M. O., Nnadi, C. O., Orjiocha, S. I., Okonkwo, V. I., Nwafor, F. I., Ugwu, G. N., & Chukwuma, M. O. (2024). Estimation of Total Phenolics, Total Flavonoids Content and In Vitro Antioxidant Activities of Extract and Fractions of Asplenium platyneuron (Carl Linnaeus). *Tropical Journal of Natural Product Research (TJNPR)*, *8*(3), 6723-6730. https://doi.org/10.26538/tjnpr/v8i3.36

30. Kalita Pallab, Barman Tapan K., Pal Tapas K., Kalita Ramen.(2013). Estimation of total flavonoids content (tfc) and anti-oxidant activities of methanolic whole plant extract of *Biophytum sensitivum* linn. Journal of Drug Delivery & Therapeutics; 2013, 3(4), 33-37.

31. Chache M, Das SS, Choudhury D, Sahariah BJ, Ashraf GJ, Sahu R, Dua TK, Majumder M, Dutta KN. GC-MS and HPTLC Fingerprinting Analysis and Evaluation of Antimicrobial Activity of Naga Chilli: An In Vitro and In Silico Approach. Biomed Chromatogr. 2025 Jan;39(1):e6058. doi: 10.1002/bmc.6058. PMID: 39709962.

32. Jain, D., Meena, M., Janmeda, P., Seth, C. S., & Arora, J. (2024). Analysis of Quantitative Phytochemical Content and Antioxidant Activity of Leaf, Stem, and Bark of Gymnosporia senegalensis (Lam.) Loes. *Plants*, *13*(11), 1425.

33. Yadav, N., Yadav, R., & Sharma, R. (2022). Role of methanol in the extraction of bioactive compounds from medicinal plants. *Asian Journal of Pharmaceutical Sciences, 17*(1), 125–134.

34. Ali, M., Khan, M., & Raza, S. (2021). Solvent-dependent extraction of phytochemicals and their antioxidant potential in medicinal plants. *Journal of Herbal Medicine, 27*, 100421.

35. Raza, S., Ahmad, R., & Malik, S. (2021). Comparative analysis of solvent polarity for phytochemical extraction in medicinal plants. *Journal of Plant Research, 134*(3), 245–257.

36. Sharma, A., Gupta, V., & Singh, P. (2020). Non-polar solvent extractions and their application in phytochemical studies. Plant Biochemistry Reviews, 15(2), 150–160.

37. Das, A., Biswas, S., & Chatterjee, S. (2022). Polyphenols as therapeutic agents: Current perspectives and challenges. *Food Chemistry, 367*, 130669.

38. Singh, G., Kumar, P., & Kumar, N. (2021). Tannins: Chemistry and therapeutic applications in modern medicine. Phytomedicine Reviews, 18(1), 45–60.

39. Das, A., Biswas, S., & Chatterjee, S. (2022). Polyphenols as therapeutic agents: Current perspectives and challenges. Food Chemistry, 367, 130669.

40. Singh, P., Gupta, A., & Kaur, G. (2023). Advances in fatty acid research for their therapeutic potential. Frontiers in Pharmacology, 14, 1183223.

41. Gupta, V., Sharma, P., & Singh, R. (2020). Role of volatile organic compounds in plant-based antimicrobial agents. *Current Research in Microbial Sciences, 12*, 56–66.

42. Kumar, S., Verma, D., & Patel, A. (2022). Siloxanes: Emerging natural compounds in pharmaceutical and cosmetic industries. *Pharmaceutical Biotechnology Reviews, 18*(3), 145–153.

43. Raza, S., Ahmad, R., & Malik, S. (2022). Comparative analysis of fatty acid esters in medicinal plants and their biological activities. *Molecules, 27*(8), 2545.