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Solvent-Dependent Phytochemical Extraction, Antioxidant Potential & Bioactive Compound Identification in Asparagus Racemosus Tuberous Roots Using GC-MS Analysis

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Abstract: ***Asparagus racemosus Wild. is a well-known medicinal plant belonging to the family Asparagaceae. It is commonly known as Shatavari in Sanskrit and is widely used in traditional Indian systems of medicine such as Ayurveda, Siddha, and Unani. The present study was aimed to evaluate the phytochemical composition of different solvent extract of Asparagus racemosus root, determine their antioxidant potential, and identify bioactive compounds using GC-MS analysis. Overall, the results of phytochemical analysis indicate that A. racemosus tuberous roots are phytochemically rich, and ethanolic and methanolic extracts contain a broader spectrum of secondary metabolites. Among the various solvent extracts analyzed, the ethanolic extract of Asparagus racemosus (tuberous roots) exhibited the presence of the maximum number of phytochemicals, justifying its selection for quantitative estimation The GC–MS analysis revealed the presence of ten bioactive compounds in the extract, indicating a chemically diverse profile comprising aromatic hydrocarbons, alkanes, esters, sulfur-containing compounds, and pharmacologically active molecules. GC–MS profile indicates that the extract contains multiple bioactive constituents with potential antioxidant, antimicrobial, anti-inflammatory, and therapeutic applications. Overall, the findings provide a strong scientific basis for the development of standardized herbal formulations and nutraceutical applications of Asparagus racemosus, while also paving the way for future research aimed at isolation, characterization, and clinical validation of its bioactive constituents.***

Keywords: Asparagus racemosus, Phytochemicals, Alkaloids, Flavonoids, Ethanol, Methanol, GC-MS analysis and Antioxidant potential

1. Introduction

Asparagus racemosus Wild. is a well-known medicinal plant belonging to the family Asparagaceae. It is commonly known as Shatavari in Sanskrit and is widely used in traditional Indian systems of medicine such as Ayurveda, Siddha, and Unani. The name "Shatavari" literally means "the plant with a hundred roots," indicating its extensive root system and medicinal significance. The plant is highly valued for its rejuvenating, adaptogenic, and nutritive properties and is traditionally classified as a "Rasayana" drug in Ayurveda, which is used to promote longevity, vitality, and overall health.The word "Asparagus" is derived from the Greek word meaning "shoot" or "stalk." Asparagus species belonging to the subfamily, Asparagoideae, and family, Asparagaceae are perennial herbaceous, climbing shrubs. The genus Asparagus comprising about 300 species have been cultivated almost throughout the world, mostly in dry places. Among all the variants, Asparagus Racemosus (also called "Shatavari") is the commonly used traditional herbal medicine A. Racemosus wild. Is a tall, woody climber, reaching up to a Height of 1–3 m. It is an extensively scandent spinous, highly Branched undershrub.

Phytochemical investigations of Asparagus racemosus have revealed the presence of various secondary metabolites such as steroidal saponins (shatavarins), alkaloids, flavonoids, tannins, phenolic compounds, and glycosides. Among these, steroidal saponins are considered the major bioactive constituents responsible for many of its pharmacological effects. These phytochemicals exhibit a anti-inflammatory, immunomodulatory, antimicrobial, antidiabetic, antiulcer, and neuroprotective properties. The presence of such diverse bioactive compounds make Asparagus racemosus a promising candidate for the development of plant-based therapeutic agents. anti-inflammatory, immunomodulatory, antimicrobial, antidiabetic, antiulcer, and neuroprotective properties. The presence of such diverse bioactive compounds make Asparagus racemosus a promising candidate for the development of plant-based therapeutic agents.

Figure 1: Asparagus racemosus plant

Overall, literature evidence indicates that Asparagus racemosus possesses significant therapeutic potential supported by diverse phytochemical constituents.Systematic phytochemical screening along with GC-MS analysis forms a robust methodological framework for detailed investigation, standardization, and future drug development studies.Hence the present study was aimed to evaluate the phytochemical composition of different solvent extract of Asparagus racemosus root, determine their antioxidant potential, and identify bioactive compounds using GC-MS analysis.

2. Materials And Methods:

Collection And Preparation of Sample:

Asparagus racemosus (tuberous roots) were collected from the local area near Koyambedu, Chennai. Roots were dried, grounded to a fine powder and dried again. Extraction process, infusion, a type of maceration was utilized for the extract preparation. After grinding into fine powder, they were placed in a clean container. Solvents used were water, 50% ethanol, 50% methanol, 50% petroleum ether, and 50% chloroform. Then the extraction solventswere then separately poured on top of the sample material kept in 5 different containers, soaked, and kept for a short period of time (24h) to extract the bioactive constituents that are readily soluble. Then, the samples were filtered with grade 1 Whatman paper. 2% extracts were prepared using the solvent; stored and utilized for further analysis.

Figure 2: Asparagus racemosus sample

Preliminary Phytochemical Screening

The following protocol was followed for the preliminary phytochemical screening of aqueous, ethanol, methanol, petroleum ether and chloroform extracts.

S. No	Experiments	Observations
1	**TEST FOR ALKALOIDS** **WAGNER"S TEST** A few drops of Wagner"s reagent are added to few ml of plant extract along the sides of test tube.	A reddish- Brown precipitate
2	**TEST FOR AMINO ACID** **NINHYDRIN TEST** Two drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone) are added to 2 ml of aqueous filtrate.	Appearance of purple colour
3	**TEST FOR CARBOHYDRATE** **MOLISCH' S TEST** To 2 ml of plant sample extract, two drops Molish' s reagent are added. The mixture is shaken well and few drops of concentrated sulphuric acid is added slowly along the sides of test tube **BENEDICT' S TEST** To 0.5 ml of filtrate, 0.5 ml of Benedict"s reagent is added. The mixture is heated on a boiling water bath for 2 minutes **FEHLING'S TEST** 1mL each of Fehling's solution A &B + 1mL filtrate+ boiled in water bath	A violet ring A characteristic red/yellow/green colored precipitate A red precipitate
4	**TEST FOR PHENOLIC COMPOUNDS** **FERRIC CHLORIDE TEST** The extract (50 mg) is dissolved in 5 ml of distilled water. To this few drops of neutral 5% ferric chloride solution are added	A dark green colour
5	**TEST FOR FLAVONOIDS** **ALKALINE REAGENT TEST** An aqueous solution of the extract is treated with 10% ammonium hydroxide solution. **ACID TEST** Plant extract + Conc. HCl	Yellow fluorescence Red color
6	**TEST FOR STEROLS** **SALKOWSKI'S TEST** Filtrate+ few drops of conc. H_2SO_4 (Shaken well and allowed to stand) (Ayoolaet al., 2008).	Red colour (in lower layer)
7	**TEST FOR PROTEINS** The extract (100 mg) is dissolved in 10 ml of distilled water and filtered through Whatmann No. 1 filter paper and the filtrate is subjected to test for proteins **BIURET TEST**	Pink colourethanolic layer

	2 ml of filtrate is treated with 1 drop of 2% copper sulphate solution. To this 1 ml of ethanol (95%) is added, followed by excess of potassium hydroxide pellets	
8	**TEST FOR GUM AND MUCILAGES** The extract (100 mg) is dissolved in 10 ml of distilled water and to this 2 ml of absolute alcohol is added with constant stirring.	White or cloudy precipitate
9	**TEST FOR DITERPENES** Plant extract is dissolved in distilled water + 3-4 drops of copper acetate solution	Emerald green colour
10	**TEST FOR TANNINS** **10% NaOH TEST** 0.4mL plant extract + 4mL 10% NaOH + shaken well	Formation of emulsion
12	**TEST FOR QUINONES** **CONC. HCL TEST** Plant extract + conc. HCl	A green colour
13	**TEST FOR GLYCOSIDES** For 50 mg of extract is hydrolysed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate is subjected to the following tests. **BORNTRAGER'S TEST** To 2 ml of filtered hydrolysate, 3 ml of choloroform is added and shaken, choloroform layer is separated and 10% ammonia solution is added to it.	Pink colour

Phytochemicals Quantification:

QUANTIFICATION OF ALKALOIDS: The extract was dissolved in 2 N of Hydrochloric acid (HCl) and then filtered. 1 ml of test extract solutions was transferred to separating funnel and added 5 ml of phosphate buffer pH 4.7 and 5 ml of bromocresol green (BCG) solution. The mixture was shaken and complex formed was extracted with 5 ml of chloroform. Chloroform layer was collected in 10 ml of volumetric flask and volume was made up to mark with chloroform. Absorbance was taken at 470 nm against blank. The alkaloid content was expressed in terms of mg of atrophine equivalents/ g of extract.

QUANTIFICATION OF TANNINS: The tannins were determined by Folin-Ciocalteu method. About 0.1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent, 1 ml of 35% sodium carbonate solution and diluted to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of gallic acid (20, 40, 60, 80, 100 µg/ ml) were prepared as described earlier. Absorbance for test and standard solutions were measured against the blank at 720 nm with an UV/ Visible spectrophotometer. The estimation of the tannin content was carried out in triplicate. The tannin content was expressed in terms of mg of gallic acid equivalents/ g of extract.

QUANTIFICATION OF TOTAL PHENOLS: The total phenolic contents in the extracts were measured using Folin-Ciocalteu reagent method (Makkar et al., 1993). Extract of 0.02 ml, 0.05 ml and 0.1 ml were transferred into test tubes and the volume was made up to 0.5 ml with distilled water. To this solution, 0.25 ml of Folin- Ciocalteu reagent (1N) and then 1.25 ml of 20% sodium carbonate was added, and the tubes were vortexed thoroughly. Absorbance was recorded at wavelength of 550 nm using UV- visible spectrophotometer after 40 minutes. The concentration of total phenolic compounds in the extract was calculated as Gallic acid equivalent (GAE) from standard curve. The total phenolic content was expressed as GAE/g of extract.

QUANTIFICATION OF TOTAL FLAVONOIDS: Total flavonoid content of the extracts was determined according to method of Nabavi et al. (2008). The ethanolic plant extract (0.5 ml) was mixed with distilled water (2 ml) and subsequently with 5% $NaNO_2$ solution (0.15 ml). After 6 mins of incubation, 10% AlCl3 solution (0.15 ml) was added and then allowed to stand for 6 min, followed by addition of 1M NaOH solution (2 ml) to the mixture. Then, distilled water was added to the sample to make up the volume to 5 ml and the mixture was thoroughly mixed and allowed to stand for another 15 min. The mixture s absorbance was determined at 510 nm using UV-Visible spectrophotometer. The test was performed in triplicate and the flavonoid content was expressed as mg of quercetin equivalent per g of extract (mg QE/g).

Phosphomolybdate Assay

(TOTALANTIOXIDANT CAPACITY): The total antioxidant capacity of the fractions was determined by phosphomolybdate method using ascorbic acid as a standard (Umamaheswari and Chatterjee, 2008). An aliquot of 0.1 ml of sample solution was mixed with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 765 nm against a blank. A typical blank contained 1 ml of the reagent solution and the appropriate volume of the solvent and incubated under the same conditions. Ascorbic acid was used as standard. The antioxidant capacity was estimated using following formula:

Antioxidant effect % = control absorbance – sample absorbance / control absorbance × 100

GAS Chromatography-Mass Spectrometer Analysis (GC-MS):

GC-MS (Gas Chromatography-mass spectrometer) analysis was carried out to identify the bioactive compounds present in the jackfruit rag extract. It was performed using the GCMS-TQ8040 NX Triple Quadrupole Gas Chromatograph Mass Spectrometer from Shimadzu Scientific Instruments. The injector was kept at 270 °C, split ratio at 2 and high-pressure injection at 200 kPa for 0.50 min.

After the high-pressure injection period, the fibre was retracted and removed from the injector. The chemical profile of the resulting extracts was performed by injecting 1 µL of

the sample volume on the aforementioned GC–MS, equipped with a splitless injector and cross-bonded fused column (30 m × 0.25 mm, 0.25 µm film thickness) to low polarity phases. The oven temperature was programmed as follows: 60 °C for 15 min, then gradually increased to 280 °C at 3 min. Bioactive compounds were identified based on the database available in the Library NIST20M2.lib and the results obtained have been tabulated.

3. Statistical Analysis

In this study, all the experiments were carried out in three independent biological replicates, and data were reported as mean ± standard deviation for each set of conditions. Statistical significance of the data was tested through one - way analysis of variance (ANOVA) using least significant difference with $p<0.05$, $p<0.01$, $p<0.001$.

4. Results

Table 1: Preliminary Phytochemical Screening of Aqueous, Ethanolic, Methanolic, Petroleum Ether and Chloroform Extracts of Asparagus Racemosus (Tuberous Roots).

S. No	Experiments	Aqueous	Ethanol	Methanol	Chloroform	Petroleum Ether
1	Alkaloids	Absent	Present	Absent	Present	Present
2	Amino acid	Absent	Present	Present	Present	Present
3	Carbohydrates - Molisch's Test	Present	Present	Present	Present	Present
4	Carbohydrates - Benedict's Test	Present	Present	Present	Present	Present
5	Phenols	Absent	Present	Absent	Absent	Absent
6	Flavonoids	Present	Present	Absent	Present	Absent
7	Sterols	Present	Present	Present	Present	Present
8	Proteins	Absent	Absent	Absent	Absent	Present
9	Diterpenoids	Absent	Present	Absent	Present	Absent
10	Gum and Mucilages	Absent	Absent	Present	Absent	Present
11	Tannins	Absent	Present	Present	Absent	Present
12	Quinones	Absent	Present	Present	Absent	Present
13	Glycosides	Absent	Present	Present	Absent	Absent

Table 1 demonstrates that Asparagus racemosus (tuberous roots) contains a wide range of phytochemicals distributed across different solvent extracts, indicating that solvent polarity significantly influences the extraction of bioactive compounds.Alkaloids were detected in ethanolic, chloroform, and petroleum ether extracts, suggesting their better solubility in semi-polar and non-polar solvents. Amino acids were present in all extracts except the aqueous extract, while carbohydrates and sterols were consistently observed in all solvent extracts, indicating their widespread distribution in the plant material.

Flavonoids were identified in aqueous, ethanolic, and chloroform extracts, reflecting their moderate polarity. Proteins were uniquely present in the petroleum ether extract, whereas diterpenoids were detected in ethanol and chloroform extracts, highlighting the effectiveness of these solvents in extracting terpenoid compounds. Gums and mucilages were found in methanol and petroleum ether extracts, and tannins and quinones were present in ethanol, methanol, and petroleum ether extracts. Glycosides were observed specifically in ethanol and methanol extracts. Overall, the results indicate that A. racemosus tuberous roots are phytochemically rich, and ethanolic and methanolic extracts contain a broader spectrum of secondary metabolites. This diversity of bioactive constituents supports the plant's potential therapeutic value and justifies further studies for isolation and characterization of active compounds.

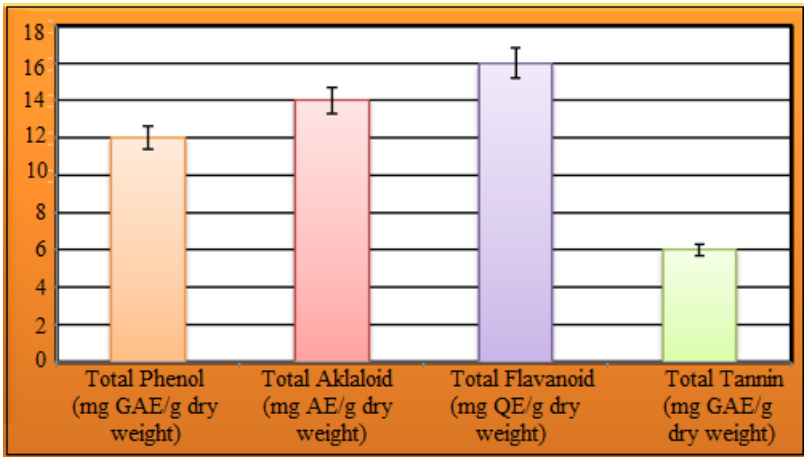

Figure 3: Quantification of Phytochemicals in Ethanolic Extract of Asparagus Racemosus (Tuberous Roots).

Since the ethanolic extract of Asparagus racemosus (tuberous root) exhibited the presence of the maximum number of phytochemicals in the preliminary screening, it was selected for quantitative analysis. Figure 3 shows the comparative concentration of major phytochemicals—phenols, alkaloids, flavonoids, and tannins—in the ethanolic extract.

The results indicate that flavonoids are present in the highest concentration, followed by alkaloids, phenols, and tannins, in the order: Flavonoids > Alkaloids > Phenols > TanninsThe predominance of flavonoids suggests strong antioxidant potential, as flavonoids are known for their effective free radical scavenging activity.

The substantial presence of alkaloids further supports possible pharmacological activities such as antimicrobial and adaptogenic effects. Although phenols and tannins are present in comparatively lower amounts, they still contribute significantly to the overall antioxidant and therapeutic properties of the extract. The quantitative results confirm that the ethanolic extract is rich in bioactive compounds, particularly flavonoids and alkaloids, which may be primarily responsible for the observed biological activities of A.racemosus.

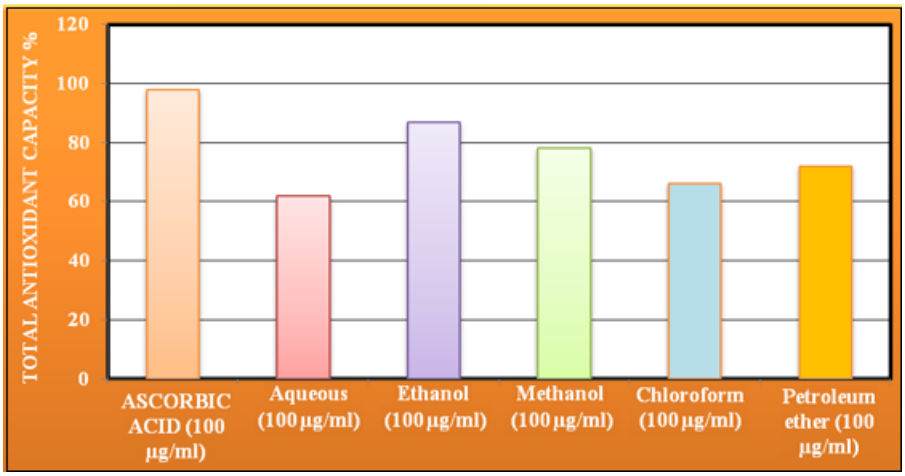

Figure 4: Total Antioxidant Capacity (TAC) of Aqueous, Ethanol, Methanol, Petroleum Ether and Chloroform Extracts of Asparagus Racemosus (Tuberous Roots). (N=5)

Figure 4 depicts the total antioxidant capacity of aqueous, ethanol, methanol, petroleum ether and chloroform extracts of Asparagus racemosus (tuberous roots).

The graph compares the total antioxidant capacity (%) of different solvent extracts of A. racemosusat 100 µg/ml with ascorbic acid (standard). Ascorbic acid shows the highest antioxidant capacity (~98–100%), confirming its strong free radical neutralizing ability (Shah et al., 2022).

Among the plant extracts, the ethanolic extract exhibits the highest antioxidant activity (~85–88%), followed by methanolic extract (~75–80%). Moderate activity is observed in the petroleum ether (~70–72%) and chloroform (~65–67%) extracts, while the aqueous extract shows the lowest activity (~60–62%).

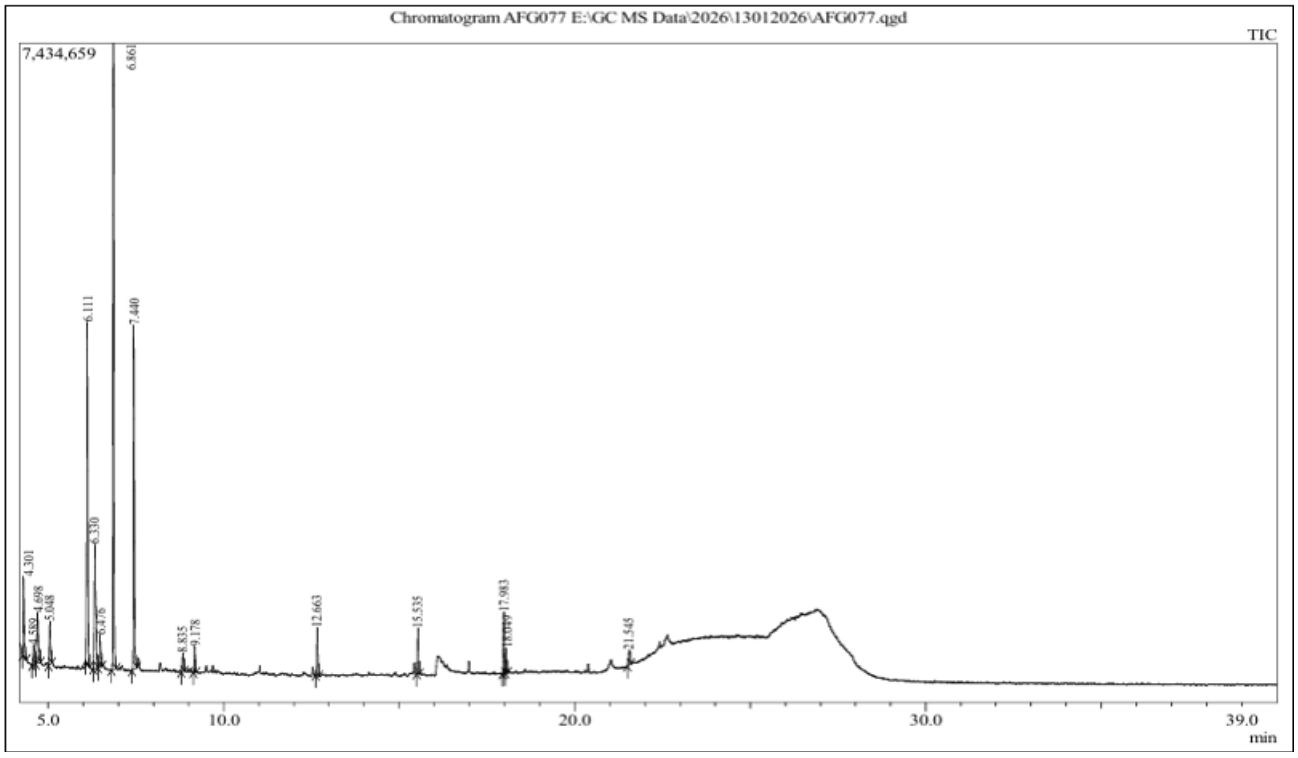

Figure 5: GCMS Spectrum of Ethanolic Extract of Asparagus Racemosus (Tuberous Roots).

GCMS spectrum depicted in Figure 5 shows the presence of 16 bioactive components with varied molecular weight and retention time.

Table 2: List of Bioactive Components in Ethanolic Extract of ASPARAGUS racemosus (TUBEROUS ROOTS).

S. No	Bioactive components	Retention time	Pharmacological property
1	1,3-Dimethylbenzene	6.330	1,3-Dimethylbenzene (m-xylene) is a naturally occurring aromatic hydrocarbon
2	Butyl lactate	4.589	Eco-friendly, bio-based solvent, biodegradable, non-toxic liquid commonly used in coatings, inks, and fragrances
3	3-Mercaptohexyl butanoate	4.698	Natural, sulfur-containing aroma compound, acts as a synthetic aroma compound in food industry applications
4	Anisole	7.440	Anisole (methoxybenzene) is a naturally occurring organic compound used to synthesize active pharmaceutical ingredients (APIs) for analgesics and anti-inflammatory drugs
5	Undecane	9.178	Natural alkanes compound, significant anti-allergic and anti-inflammatory properties
6	Dodecane	12.663	A hydrocarbon with limited direct medicinal use, used in cosmetics, its derivatives used as nicotinic acetylcholine receptor antagonists for treating nicotine-related neurological conditions
7	Tetradecane	15.535	Straight-chain alkane hydrocarbon, antibacterial, antifungal, and potential anti-inflammatory properties
8	Diethyl Phthalate	17.983	commonly used as a vehicle for fragrances and cosmetic ingredients
9	Heptadecane	18.049	Heptadecane is an organic compound, an alkane hydrocarbon, demonstrating notable antioxidant, anti-inflammatory, and potential anti-tuberculous properties
10	Lidocaine	21.545	widely used local anesthetic and antiarrhythmic medication

Table 2 depicts the bioactive components and their pharmacological value. The GC–MS analysis revealed the presence of ten bioactive compounds in the extract, indicating a chemically diverse profile comprising aromatic hydrocarbons, alkanes, esters, sulfur-containing compounds, and pharmacologically active molecules. The retention times ranged from 4.589 to 21.545 minutes, reflecting compounds of varying volatility and molecular weight.Among the identified compounds, 1,3-dimethylbenzene (m-xylene) and anisole represent aromatic hydrocarbons, which are often involved in the synthesis of pharmaceutical intermediates. Butyl lactate and diethyl phthalate are esters commonly used in cosmetic and fragrance formulations, suggesting potential industrial and cosmetic relevance of the extract.

The detection of 3-mercaptohexyl butanoate, a sulfur-containing compound, indicates the presence of aroma-active constituents, which may contribute to bioactivity through antioxidant or antimicrobial mechanisms. Straight-chain alkanes such as undecane, dodecane, tetradecane, and heptadecane were also identified. These hydrocarbons have been reported to exhibit antibacterial, antifungal, anti-inflammatory, and antioxidant activities, supporting the therapeutic potential of the extract. Notably, the presence of heptadecane suggests possible antioxidant and anti-inflammatory effects, while lidocaine, identified at the highest retention time (21.545 min), is a well-known local anesthetic and antiarrhythmic agent. The detection of such pharmacologically significant compounds highlights the potential medicinal relevance of the plant extract.GC–MS profile indicates that the extract contains multiple bioactive constituents with potential antioxidant, antimicrobial, anti-inflammatory, and therapeutic applications.

DISCUSSION: Preliminary phytochemical screening of different solvent extracts (aqueous, ethanolic, methanolic, petroleum ether, and chloroform) of Asparagus racemosus (tuberous roots) revealed a diverse range of bioactive constituents, demonstrating the influence of solvent polarity on phytochemical extraction efficiency. The qualitative analysis presented in Table 1 showed that carbohydrates and sterols were present in all extracts, indicating that these primary and secondary metabolites are widely distributed within the tuberous roots. Sterols are known for their anti-inflammatory and immunomodulatory properties, while carbohydrates contribute to nutritive and therapeutic functions (Pandey & Rizvi, 2009).

Alkaloids were detected in ethanolic, chloroform, and petroleum ether extracts, suggesting that semi-polar and non-polar solvents efficiently extract these nitrogen-containing compounds. Alkaloids are pharmacologically significant due to their antimicrobial, analgesic, and antioxidant activities (Harborne, 1998). The presence of flavonoids in aqueous, ethanolic, and chloroform extracts highlights the plant's antioxidant potential, as flavonoids are well documented for their free radical scavenging and anti-inflammatory properties (Pietta, 2000). Interestingly, proteins were observed only in the petroleum ether extract, while amino acids were present in all extracts except the aqueous extract. This variation may be attributed to differences in solvent affinity and extraction conditions. Diterpenoids were detected in ethanol and chloroform extracts, supporting the role of moderately polar solvents in isolating terpenoid compounds. Terpenoids are known for their antimicrobial, anti-inflammatory, and cytoprotective activities (Mahato& Sen, 1997).

Gums and mucilages were identified in methanol and petroleum ether extracts, indicating the presence of polysaccharide-based components that may contribute to the plant's demulcent and soothing properties. Furthermore, tannins and quinones were present in ethanol, methanol, and petroleum ether extracts. Tannins possess strong antioxidant and antimicrobial properties, while quinones are associated with antimicrobial and cytotoxic activities (Cowan, 1999). Glycosides were observed in ethanol and methanol extracts, which is significant because glycosidic compounds are often responsible for cardioprotective, anti-inflammatory, and adaptogenic effects. Analysis of phytochemical profile of Asparagus racemosus tuberous roots demonstrates the presence of multiple classes of secondary metabolites with

established pharmacological relevance. The variation in phytoconstituents among different solvent extracts emphasizes the importance of solvent selection in phytochemical investigations. These findings support previous reports that attribute the therapeutic efficacy of A. racemosus to its rich phytochemical composition and provide a scientific basis for further isolation, characterization, and pharmacological evaluation of its bioactive compounds.

Among the various solvent extracts analyzed, the ethanolic extract of Asparagus racemosus (tuberous roots) exhibited the presence of the maximum number of phytochemicals, justifying its selection for quantitative estimation. Ethanol is widely recognized as an efficient solvent for extracting both polar and moderately non-polar phytoconstituents, including phenolics, flavonoids, alkaloids, and tannins (Harborne, 1998). The quantitative analysis (Figure 1) revealed that the phytochemical concentration followed the order: Flavonoids > Alkaloids > Phenols > Tannins.

The predominance of flavonoids in the ethanolic extract suggests a strong antioxidant potential, as flavonoids are known for their ability to scavenge free radicals, chelate metal ions, and inhibit lipid peroxidation (Pietta, 2000). Their high concentration may significantly contribute to the pharmacological properties of A. racemosus, including anti-inflammatory, immunomodulatory, and cytoprotective activities. Flavonoids also play a role in reducing oxidative stress, which is implicated in various chronic disorders.Alkaloids, which were found in the second highest concentration, are important nitrogen-containing compounds with diverse biological activities such as antimicrobial, analgesic, and adaptogenic effects (Harborne, 1998). The significant alkaloid content in the ethanolic extract further supports the traditional medicinal use of A. racemosus in managing various health conditions.

Phenolic compounds, though present in comparatively lower amounts than flavonoids and alkaloids, are well known for their antioxidant and anti-inflammatory properties. They act primarily through hydrogen donation and stabilization of reactive oxygen species (Pandey & Rizvi, 2009). Tannins, which were found in the least concentration among the quantified compounds, still contribute to therapeutic potential due to their astringent, antimicrobial, and antioxidant properties (Cowan, 1999).The quantitative predominance of flavonoids and alkaloids indicates that these compounds may play a major role in the biological activities associated with A. racemosus tuberous roots. The results align with previous phytochemical investigations that highlighted ethanol as an effective solvent for extracting bioactive constituents. The high concentration of secondary metabolites supports further studies aimed at isolating and characterizing specific active compounds responsible for the plant's pharmacological effects which may provide a scientific basis for developing standardized herbal formulations and nutraceutical applications.

In continuation, the total antioxidant capacities (%) of different solvent extracts of A. racemosus at 100 µg/ml with ascorbic acid (standard) were evaluated. Ascorbic acid shows the highest antioxidant capacity (~98–100%), confirming its strong free radical neutralizing ability (Shah et al., 2022). Among the plant extracts, the ethanolic extract exhibits the highest antioxidant activity (~85–88%), followed by methanolic extract (~75–80%). Moderate activity is observed in the petroleum ether (~70–72%) and chloroform (~65–67%) extracts, while the aqueous extract shows the lowest activity (~60–62%).

This pattern suggests that polar organic solvents like ethanol and methanol are more effective at extracting antioxidant phytochemicals compared with non-polar or water solvents. Phenolic compounds, flavonoids, and other polyphenols, which are known to confer antioxidant activity, are more soluble in ethanolic and methanolic media (Nabavi et al., 2015; Saini et al., 2023). The higher antioxidant capacity of these extracts therefore likely reflects their greater concentrations of these bioactive compounds. Ethanolic extracts of medicinal plants have frequently been reported to contain high levels of total phenols and flavonoids, which contribute to strong antioxidant effects (Zhang et al., 2021). The present results align with these findings and support the potential use of A. racemosus extracts as natural antioxidants in food, pharmaceutical, and cosmetic applications for managing oxidative stress and related disorders.

The GC–MS analysis of the extract revealed the presence of diverse volatile and semi-volatile bioactive compounds, including aromatic hydrocarbons, esters, sulfur-containing compounds, alkanes, phthalate derivatives, and a pharmacologically active compound (lidocaine). The detection of these compounds suggests a chemically complex profile that may contribute to the observed biological activities of the extract.

Among the identified constituents, 1,3-dimethylbenzene (m-xylene) and anisole (methoxybenzene) are aromatic hydrocarbons commonly found in plant-derived volatile fractions. Anisole derivatives are frequently reported as intermediates in the synthesis of pharmaceutical agents with analgesic and anti-inflammatory properties (Zhang et al., 2021). Although aromatic hydrocarbons like m-xylene are primarily industrial chemicals, their occurrence in plant extracts may reflect natural volatile constituents or secondary metabolite derivatives.

The presence of butyl lactate, an eco-friendly and biodegradable ester, indicates potential applications in cosmetic and pharmaceutical formulations due to its low toxicity and solvent properties. Similarly, 3-mercaptohexyl butanoate, a sulfur-containing compound, is known for its aromatic and biological relevance in food and fragrance industries. Sulfur-containing phytochemicals have been associated with antimicrobial and antioxidant activities (Saini et al., 2023).

The identification of long-chain alkanes such as undecane, dodecane, tetradecane, and heptadecane is noteworthy. Several studies report that long-chain hydrocarbons exhibit antimicrobial, antioxidant, and anti-inflammatory activities, possibly through membrane interaction and modulation of oxidative stress pathways (Kumar et al., 2022). In particular, tetradecane and heptadecane have been linked to antibacterial and antifungal properties in plant extracts analyzed by GC–MS. Diethyl phthalate, commonly used as a vehicle in

cosmetic and fragrance industries, is often detected in GC–MS studies of plant extracts. While it may contribute to formulation stability, its presence should be carefully interpreted, as phthalates can sometimes arise from environmental contamination or laboratory materials (Net et al., 2015).

Interestingly, lidocaine, a well-known local anesthetic and antiarrhythmic drug, was also detected. The presence of such a compound may suggest either a naturally occurring analog or possible contamination; therefore, further confirmatory analysis (e.g., LC–MS/MS or NMR) is recommended to validate its origin. GC–MS profile indicates that the extract contains compounds with potential antioxidant, anti-inflammatory, antimicrobial, and therapeutic properties. The diversity of hydrocarbons, esters, sulfur compounds, and aromatic derivatives supports the biological activities observed in antioxidant and enzyme inhibition assays. However, further studies are required to isolate, purify, and confirm the bioactive constituents responsible for the pharmacological effects. Advanced spectroscopic characterization and in vivo validation would strengthen the understanding of the extract's therapeutic potential.

The present study demonstrates that Asparagus racemosus tuberous roots possess a rich and diverse phytochemical profile, significantly influenced by solvent polarity. Among the various extracts, the ethanolic extract exhibited the highest number and concentration of bioactive constituents, particularly flavonoids and alkaloids, which are likely responsible for its strong antioxidant activity. The total antioxidant capacity results further confirmed that polar solvents, especially ethanol and methanol, are more efficient in extracting antioxidant-rich phytochemicals. GC–MS analysis revealed the presence of multiple volatile and semi-volatile compounds with reported antioxidant, anti-inflammatory, antimicrobial, and therapeutic properties, supporting the biological activities observed in vitro. The combined phytochemical screening, quantitative estimation, antioxidant evaluation, and GC–MS profiling provide strong scientific evidence for the pharmacological potential of A. racemosus. Overall, these findings validate the traditional medicinal importance of A. racemosus and highlight its potential application in pharmaceutical, nutraceutical, and cosmetic industries. However, further studies involving isolation, structural characterization, mechanistic investigations, and in vivo validation are necessary to establish standardized formulations and confirm its therapeutic efficacy.

CONCLUSION: The present study comprehensively evaluated the phytochemical composition, antioxidant potential, and GC–MS profile of Asparagus racemosus tuberous root extracts using different solvents. Preliminary phytochemical screening revealed the presence of diverse secondary metabolites, including alkaloids, flavonoids, phenols, tannins, sterols, glycosides, diterpenoids, quinones, carbohydrates, amino acids, proteins, gums, and mucilages.

In conclusion, Asparagus racemosus tuberous roots are rich in bioactive phytochemicals with significant pharmacological relevance. The ethanolic extract, in particular, demonstrated superior phytochemical diversity and antioxidant capacity, emphasizing ethanol as an effective solvent for extracting therapeutically important constituents. The predominance of flavonoids and alkaloids suggests that these compounds may play a central role in the plant's biological activities.

The GC–MS profile further supports the potential therapeutic value of the extract, indicating the presence of compounds associated with antioxidant, antimicrobial, and anti-inflammatory properties. Nevertheless, advanced analytical techniques such as LC–MS/MS and NMR, along with in vivo studies, are required to confirm the identity of active compounds and validate their pharmacological efficacy. Overall, the findings provide a strong scientific basis for the development of standardized herbal formulations and nutraceutical applications of Asparagus racemosus, while also paving the way for future research aimed at isolation, characterization, and clinical validation of its bioactive constituents.

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