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CHEMICAL COMPOSITION AND GC-MS PROFILING OF THE STEM OF *SYZYGium TAMILNADENSE* (RATHAKR. & CHITRA), AN ENDEMIC SPECIES OF THE WESTERN GHATS, NILGIRIS, TAMIL NADU, INDIA

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ABSTRACT: Plants have evolved to produce a vast array of natural compounds with significant health-promoting properties. Medicinal plants remain crucial resources for contemporary drug discovery and development. This study presents the first scientific investigation into the medicinal potential of *Syzygium tamilnadense* (Rathakr & Chitra), a rare and endemic species of the Myrtaceae family native to the Sholur-Nanjnad Reserve Forests in the Nilgiri Biosphere, Western Ghats, Tamil Nadu, India. Various solvent extracts were prepared, with ethanol yielding the highest concentration of phytochemicals. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, catechins, coumarins, glycosides, phenols, saponins, steroids, tannins, terpenoids, sugars, and xanthoproteins compounds known for their diverse pharmacological activities. Quantitative analysis showed high contents of total phenolics (128.14 ± 0.76 mg GAE/g), flavonoids (44.20 ± 0.28 mg QE/g), and tannins (38.26 ± 0.48 mg QAE/g), indicating strong antioxidant capacity. Gas Chromatography– Mass Spectrometry (GC–MS) analysis identified 25 bioactive compounds, including caryophyllene oxide, neophytadiene, squalene, phytol, and linoleic acid ethyl ester, which are associated with antioxidant, anti-inflammatory, antimicrobial, and anticancer properties. These findings highlight *S. tamilnadense* as novel and valuable source of pharmacologically active phytoconstituents, validating its traditional use and underscoring its potential for pharmaceutical and nutraceutical applications.

INTRODUCTION: Plants have long been a vital source of medicinal compounds and continue to play a crucial role in global healthcare due to their therapeutic efficacy. They are integral to health systems around the world, contributing significantly to the treatment and prevention of various diseases^{7, 37, 44, 97}. Beyond their role in disease management, medicinal plants are valued for promoting overall health and well-being. Their widespread use is attributed to cultural acceptance, biocompatibility, affordability, and a generally

lower incidence of side effects compared to synthetic drugs. As a result, many countries, particularly in the developing world, rely heavily on herbal medicine as a primary healthcare resource^{16, 23, 47}. For centuries, plant-based remedies have formed the backbone of traditional medicine systems such as Ayurveda, Siddha, and Unani. These systems, grounded in indigenous knowledge, have made extensive use of natural bioactive compounds derived from various plant parts to treat a wide range of ailments.

The medicinal use of plants is a practice observed across cultures and civilizations. Today, approximately 20,000 plant species are classified as medicinal, and ongoing research continues to uncover new applications for plant-derived compounds in modern medicine^{45, 46, 112, 118}. Phytochemicals, the biologically active constituents

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of plants, are well recognized for their pharmacological properties. According to the World Health Organization, nearly 75–80% of the global population and over 90% in developing countries depend on herbal medicine for primary healthcare needs¹¹⁴. This reliance stems not only from the accessibility of these resources but also from the wealth of traditional knowledge handed down through generations. Many plant species are traditionally believed to possess therapeutic efficacy against chronic and infectious diseases, including cancer, diabetes, and microbial infections^{68, 83}. In addition to primary metabolites, medicinal plants produce a wide variety of secondary metabolites such as alkaloids, terpenes, glycosides, tannins, saponins, steroids, and phenols compounds that are largely responsible for their pharmacological activities. Interest in the identification and analysis of these bioactive constituents has grown considerably in recent years^{69, 71}. Among the analytical techniques used, Gas Chromatography–Mass Spectrometry (GC–MS) is particularly effective for phytochemical profiling. It enables precise separation, detection, and identification of plant-derived metabolites by combining chromatographic and mass spectral data, making it a valuable tool in natural product research⁵⁷. Various parts of plants including leaves, stems, roots, flowers, and seeds have been traditionally utilized for their health benefits and therapeutic applications (Shakri *et al.*, 2020). The *Syzygium* genus, a member of the Myrtaceae family, comprises approximately 1,100–1,200 species and is widely distributed across tropical and subtropical regions^{5, 23, 54}. It is the largest genus of woody flowering plants, with numerous species known for their medicinal value⁸⁰. Extensive studies have documented the diverse biological activities of *Syzygium* species, including antimicrobial, anti-inflammatory, antioxidant,

antidiabetic, and anticancer properties^{1, 42}. These therapeutic effects are largely attributed to the rich presence of flavonoids, tannins, phenols, steroids, and alkaloids^{14, 124}. In traditional Ayurvedic medicine, *Syzygium* species have been used to treat a variety of ailments, such as coughs, colds, diarrhea, dysentery, fever, inflammation, pneumonia, wounds, ulcers, and infectious diseases²⁶. They have also been employed in the management of chronic conditions, including diabetes and gastrointestinal disorders⁴³. Given the therapeutic potential of this genus, the present study aims to conduct a comprehensive phytochemical screening and identify bioactive compounds from the stem of *Syzygium tamilnadense* (Rathakr. & Chitra), an endemic and underexplored species, using GC–MS analysis. This investigation marks the first scientific exploration of its medicinal properties and may support its potential application in pharmaceutical and nutraceutical development.

Study Area: The study was conducted in the Sholur–Nanjanad region, located within Udthagamandalam Taluka of The Nilgiris District, Tamil Nadu, India. This area lies approximately 13 kilometers from Udthagamandalam (Ooty), the administrative center of both the district and sub-district. The village of Nanjanad spans a total geographical area of 13,064.23 hectares. Geographically, the study site is positioned at coordinates 125,478.30 N and 2,531,453 E. The region is ecologically significant due to its dense forest cover, particularly within the Sholur hidden valley, which comprises a mosaic of shola forests, subtropical, and temperate vegetation. This diverse landscape harbors a rich assemblage of flora, including endemic shola species, as well as cypress, pine, and other representative tree species of the southern Western Ghats.

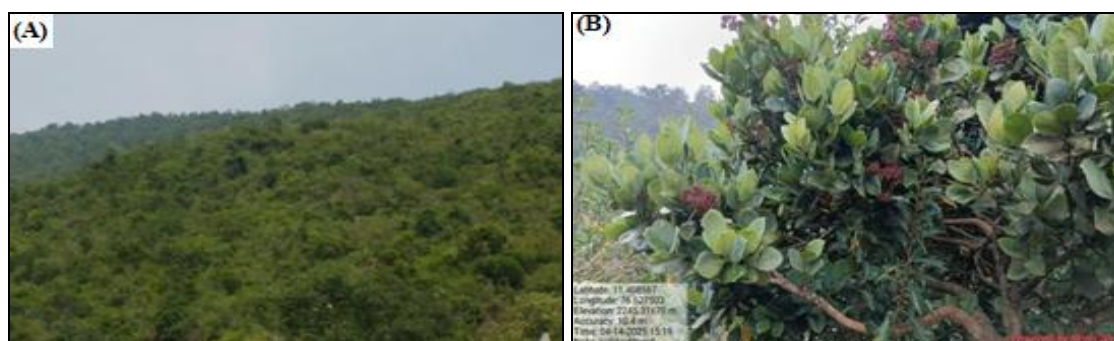


FIG. 1: (A). STUDYAREA-SHOLUR, NANJANAD (B). STUDYSPECIES-SYZYGIUM TAMILANDENSE-HABI

Study Specimen:**Class:** Magnoliopsida**Order:** Myrtales**Family:** Myrtaceae**Genus:** *Syzygium***Species:** *Syzygium tamilnadense* Rathakr & Chitra**Morphological Description:****Habit:** A medium-sized tree, reaching up to 15 meters in height.**Trunk & Bark:** Bark is grey and scaly; the blaze is cream-colored.**Branches and Branchlets:** Branchlets are stout, quadrangular, narrowly winged, and glabrous.**Leaves:** Simple, opposite, and decussate in arrangement. Petiole measures 0.4–1.2 cm, canaliculate above, and glabrous. Lamina is 6.5–12.5 × 3.3–7.5 cm, elliptic to broadly elliptic or obovate in shape. Apex is obtuse to rounded; base is cuneate. Margin entire, with pellucid gland dots; leaves are coriaceous and glabrous. The midrib is distinctly canaliculate on the upper surface. Intramarginal nerve present, with approximately 12 pairs of secondary nerves; tertiary nerves are obscure.**Inflorescence/Flower:** Flowers are small, arranged in terminal corymbose cymes.**Fruit and Seed:** The fruit is a globose berry, purple in colour, approximately 0.8 cm in diameter, and crowned with a persistent calyx. Each fruit contains a single seed. Your Materials and Methods section is well-detailed and scientifically robust. To enhance clarity, readability, and consistency in formatting (particularly important for journals and academic submissions), here's a refined and structured version:**MATERIALS AND METHODS:****Collection of Plant Material:** Fresh leaves of *Syzygium tamilnadense* Rathakr. & Chitra were collected from the Sholur–Nanjanad forest region in the Nilgiris District, Western Ghats, Tamil Nadu, India.

Botanical identification was carried out by comparing morphological characteristics with the local flora and was further authenticated by the Botanical Survey of India (BSI), Southern Circle, Coimbatore. Voucher specimens were preserved for future reference.

Preparation of Plant Material: The collected stems, bark, and leaves were cleaned, chopped into small pieces, and shade-dried at room temperature until a uniform, smooth fracture was achieved. The dried materials were ground separately using a mechanical blender and sieved through sieve No. 60 to obtain fine, uniform powders. These powdered forms of stem and leaf were stored in airtight containers and used for various experimental analyses.**Extraction Procedure for Phytochemical Screening:** A weighed quantity of leaf powder was placed in separate Stoppard flasks and soaked in different solvents (petroleum ether, benzene, ethyl acetate, methanol, ethanol, and water) until fully submerged. The mixtures were agitated hourly for the first six hours to facilitate maximum extraction. After 24 hours, each mixture was filtered through Whatman No. 1 filter paper. The obtained filtrates were used for preliminary qualitative phytochemical screening, following standard procedures^{18, 59, 95}. Among the solvents tested, ethanol yielded the highest concentration of phytochemicals and was selected for further quantitative and GC–MS analysis.**Physicochemical Analysis:** Physicochemical parameters, including total ash, acid-insoluble ash, water-soluble ash, sulphated ash, and extractive values, were determined using standard methods outlined in the Pharmacopoeia of India¹².**Determination of Total Ash:** Three grams of powdered sample (stem, bark, or leaf) were accurately weighed into a pre-ignited silica crucible. The sample was incinerated at a temperature not exceeding 450°C until carbon-free. The crucible was cooled in a desiccator and weighed. This process was repeated until a constant weight was obtained. The percentage of total ash was calculated with reference to the air-dried sample.

Determination of Acid-Insoluble Ash: The total ash obtained above was boiled with 25 mL of 2N hydrochloric acid for five minutes. The mixture was filtered through an ashless filter paper. The residue was washed with hot water, transferred to a pre-weighed silica crucible, and incinerated. The process was repeated to ensure constant weight. The percentage of acid-insoluble ash was calculated relative to the air-dried sample.

Determination of Water-Soluble Ash: The total ash was boiled for five minutes with 25 mL of distilled water. The resulting mixture was filtered through ashless filter paper, and the insoluble matter was washed with hot water, then transferred to a pre-weighed crucible and incinerated at temperatures not exceeding 450°C. The weight of the water-insoluble ash was subtracted from the total ash to calculate the water-soluble ash percentage.

Determination of Sulphated Ash: A clean silica crucible was ignited at red heat for 10 minutes, cooled in a desiccator, and weighed. One gram of the air-dried powdered sample was transferred to the crucible and weighed accurately. The sample was gently ignited until thoroughly charred. After cooling, 1 mL of concentrated sulfuric acid was added. The crucible was then heated gently until white fumes ceased and further ignited at 800°C $\pm$ 25°C until all black particles disappeared.

The crucible was protected from air currents during ignition. After cooling, a few more drops of sulfuric acid were added, and the sample was reheated and ignited until a constant weight was achieved. The sulphated ash content was expressed as a percentage relative to the air-dried sample.

Determination of Extractive Values: Extractive values were determined in various solvents (petroleum ether, benzene, chloroform, ethyl acetate, methanol, ethanol, and water) following standard procedures described in the Pharmacopoeia of India (Anonymous, 2002).

Five grams of the air-dried plant material was placed in a stoppered flask, and 100 mL of solvent was added. The mixture was shaken intermittently and allowed to stand for 24 hours. After filtration, 50 mL of the filtrate was evaporated to dryness in a pre-weighed porcelain dish on a water bath, dried

at 105°C, cooled in a desiccator, and weighed. The extractive value was calculated as a percentage of the air-dried sample.

Fluorescence Analysis: Fluorescence characteristics of the powdered drug were observed under daylight and UV light (254 nm and 365 nm) after treatment with various chemical reagents, including:

- Acids: 1N HCl, conc. HCl, 50% H₂SO₄, conc. H₂SO₄, 50% HNO₃, conc. HNO₃, acetic acid, conc. HNO₃ + NH₃
- Alkaline reagents: aqueous NaOH, 40% NaOH + 10% lead acetate, alcoholic NaOH
- Solvents: acetone, benzene, chloroform, petroleum ether, methanol, ethanol
- Other reagents: ferric chloride, ammonia

The analysis was conducted following the procedure of Chase and Pratt (1949).

Quantitative Phytochemical Analysis:

Estimation of Total Phenolics: Total phenolic content was determined using the Folin–Ciocalteu method ⁷⁴. One milliliter of ethanol extract (100 µg/mL) was mixed with 5 mL of diluted Folin–Ciocalteu reagent and 4 mL of 75 g/L sodium carbonate solution. After 30 minutes at room temperature, absorbance was measured at 765 nm using a UV-Vis spectrophotometer. A gallic acid calibration curve was used, and results were expressed as mg gallic acid equivalents per gram of extract (mg GAE g⁻¹).

Estimation of Flavonoids: Flavonoid content was estimated as per Eom et al. (2007). A 0.5 mL sample was mixed with 0.1 mL of 10% aluminium chloride, 0.1 mL of 1M potassium acetate, and 4.3 mL of 80% methanol. The mixture was vortexed, and absorbance was measured at 415 nm. Results were expressed as mg quercetin equivalents per gram of extract (mg QES g⁻¹).

Estimation of Tannins: Tannin content was measured using a modified Folin–Ciocalteu method (Marinova et al., 2005; Miean & Mohamed, 2001). To a 10 mL volumetric flask, 0.1 mL of extract was added to 7.5 mL distilled water, followed by 0.5 mL of Folin–Ciocalteu reagent and 1 mL of 35%

sodium carbonate solution. The volume was made up to 10 mL with distilled water, mixed thoroughly, and allowed to stand for 30 minutes. Absorbance was read at 725 nm. Tannin content was calculated as mg gallic acid equivalents per gram (mg GAE g⁻¹).

RESULTS AND DISCUSSION: The physicochemical parameters, including total ash, water-soluble ash, acid-insoluble ash, and sulphated ash, are summarized in **Table 1** and **Fig. 1**. These values indicate the quantity of inorganic residue present in the plant materials after complete incineration, which is essential for assessing the purity and quality of the crude drug. Variations among the stem, bark, and leaf samples reflect differences in mineral composition and potential contamination with inorganic substances.

TABLE 1: ASH VALUES OF POWDERED STEM

S. no.	Types of ash	Ash value % ^a
1	Total ash	10.12±0.06
2	Water soluble ash	2.48±0.05
3	Acid insoluble ash	1.72±0.02
4	Sulphated ash	9.78±0.10

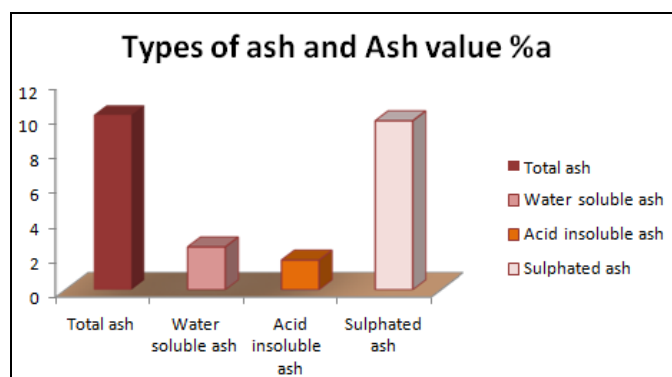


FIG. 2: TYPES OF ASH AND CORRESPONDING ASH VALUES IN POWDERED STEM

Physicochemical Analysis:

Ash Values: The total ash content of *Syzygium tamilnadense* was found to be 10.12%, indicating a moderate level of inorganic constituents. Total ash represents the sum of physiological ash (originating from plant tissue) and non-physiological ash (from external contaminants such as soil, sand, or other inorganic matter). This value should be evaluated against pharmacopeial standards to determine the overall quality and purity of the crude drug. The water-soluble ash content was recorded at 2.48%, which reflects the fraction of the total ash that is soluble in water. This parameter provides insight into the presence of water-soluble inorganic salts.

The relatively low value suggests minimal contamination with such salts and is within an acceptable range for herbal raw materials. The acid-insoluble ash value was 1.72%, representing the portion of the ash that remains insoluble in dilute hydrochloric acid. This is primarily indicative of the presence of silica, siliceous earth, or other acid-insoluble impurities, such as sand. The low percentage points to a limited presence of such contaminants; however, further investigation may be warranted to ensure full compliance with pharmacopoeial purity standards.

The sulphated ash content was determined to be 9.78%. This method involves treating the sample with concentrated sulfuric acid before incineration, promoting the oxidation of organic matter and conversion of inorganic components into more stable sulphate forms. The slightly lower value compared to total ash suggests the possible volatilization of some inorganic components during sulphation. Sulphated ash is a more reliable indicator for detecting metallic impurities and provides a clearer estimate of total inorganic content. Ash values are critical indicators of crude drug quality. They provide quantitative data on the presence of inorganic radicals such as phosphates, carbonates, and silicates of sodium, potassium, calcium, and magnesium elements that naturally occur in specific ratios within unadulterated plant materials. Any significant deviation from these expected values may indicate contamination or adulteration^{21, 93}.

Extractive Value Analysis: The extractive value analysis, as shown in **Table 2** and **Fig. 2**, serves as a vital quality control parameter in pharmaceutical and phytochemical research. This analysis quantifies the number of bioactive constituents that can be extracted from plant materials using different solvents, thereby providing important insights into the polarity and solubility characteristics of the phytochemicals present.

TABLE 2: EXTRACTIVE VALUES OF POWDERED STEM

S. no.	Extract	Extractive value % a
1	Petroleum ether	5.18±0.04
2	Benzene	5.76±0.05
3	Chloroform	6.18±0.03
4	Acetone	6.48±0.04
5	Methanol	8.08±0.09
6	Ethanol	8.92±0.11
7	Water	8.18±0.06

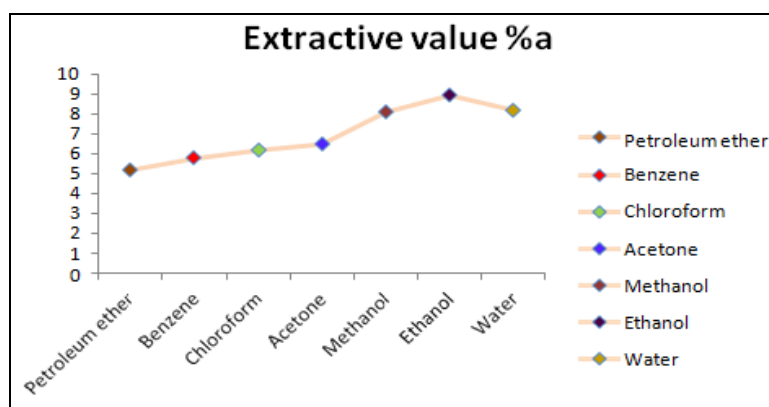


FIG. 3: COMPARATIVE ANALYSIS OF EXTRACT AND EXTRACTIVE VALUES OF THE POWDERED STEM.^a
All values are the mean of triplicate determinations expressed on dry weight basis. ± Standard error.

The selection of solvents with varying polarities (e.g., petroleum ether, benzene, chloroform, ethyl acetate, methanol, ethanol, and water) allows for the targeted extraction of different classes of compounds, such as non-polar lipids, moderately polar flavonoids, and highly polar glycosides and tannins. Higher extractive values are generally indicative of a greater concentration of soluble active constituents, which may correlate with the plant's therapeutic efficacy. These values are particularly important in the standardization of herbal drugs, as they assist in identifying suitable extraction solvents for formulation development and ensuring batch-to-batch consistency. The extractive value can also be used as a diagnostic tool to detect adulteration or substandard samples lacking sufficient active phytochemicals. Your write-up is detailed, well-researched, and scientifically appropriate. Here's a refined and publication-ready version of your extractive value analysis and fluorescence analysis sections. These revisions aim to enhance clarity, scientific tone, and logical flow without altering the core content:

Extractive Value Analysis: The extractive values of *Syzygium tamilnadense* using various solvents are presented in **Table 2** and **Fig. 2**. These values reflect the efficiency of each solvent in extracting phytoconstituents based on their polarity. The extract obtained with petroleum ether ($5.18 \pm 0.04\%$) highlights its effectiveness as a non-polar solvent, primarily extracting lipophilic substances such as fixed oils, fats, sterols, and certain terpenoids. The relatively low value indicates a moderate presence of such non-polar compounds in the plant material. Benzene extraction yielded a slightly higher value ($5.76 \pm 0.05\%$) than

petroleum ether, suggesting the presence of additional lipophilic flavonoids, alkaloids, or essential oils soluble in this mildly polar solvent.

Chloroform, being moderately polar, resulted in a greater extractive value ($6.18 \pm 0.03\%$). This solvent is known to dissolve alkaloids, some glycosides, and other semi-polar constituents, indicating a notable presence of these bioactives in the plant sample. Acetone, with slightly higher polarity, gave an extractive value of $6.48 \pm 0.04\%$, suggesting its capability to extract a broader range of moderately polar compounds, including flavonoids, phenolics, tannins, and alkaloids. Methanol, a highly polar solvent, produced a significantly higher yield ($8.08 \pm 0.09\%$). This is consistent with its known efficacy in extracting a wide range of phytochemicals, such as alkaloids, glycosides, tannins, flavonoids, and polyphenols. The ethanol extract exhibited the highest extractive value ($8.92 \pm 0.11\%$) among all tested solvents. Ethanol's intermediate polarity makes it suitable for extracting both polar and moderately non-polar phytoconstituents, underlining its effectiveness in phytochemical investigations and supporting its common use in herbal formulation.

The aqueous extract also demonstrated a high extractive value ($8.18 \pm 0.06\%$), indicating efficient solubilization of highly polar compounds such as glycosides, saponins, tannins, and carbohydrates. This finding supports the traditional use of water-based preparations in ethnomedicine. Overall, extractive value analysis serves as a reliable indicator of phytochemical richness, helping to assess drug quality, detect adulteration, and guide solvent selection for formulation development^{49, 93}.

TABLE 3: FLUORESCENCE ANALYSIS OF *S. TAMILNADENSE* STEM

S. no.	Experimental	Visible / Day light	VV light	
			254 mm (Short wave length)	365 mm (Long wave length)
1	Powder as such	Brown	Brown	Black
2	Powder + 1 N Aqueous NaOH	Yellowish green	Fluorescent green	Dark black
3	Powder + 1 N Alcoholic NaOH	Pale brown	Yellowish green	Dark black
4	Powder + 1 N HCl	Pale brown	Greenish yellow	Black
5	Powder + Conc. H ₂ SO ₄	Brown	Fluorescent green	Dark black
6	Powder + Conc. HNO ₃	Greenish yellow	Fluorescent green	Black
7	Powder + Conc. HCl	Greenish yellow	Fluorescent green	Dark black
8	Powder + 50% HNO ₃	Greenish yellow	Fluorescent green	Dark black
9	Powder + 50% H ₂ SO ₄	Dark brown	Fluorescent green	Dark green
10	Powder + 40% NaOH + 10% Lead acetate	Dark brown	Florescent green	Dark green
11	Powder + HNO ₃ + NH ₃	Greenish yellow	Yellowish green	Dark green
12	Powder + NH ₃	Greenish yellow	Greenish yellow	Dark brown
13	Powder + Acetic acid	Dark brown	Dark green	Dark brown
14	Powder + Ferric acid	Dark brown	Fluorescent green	Dark brown
15	Powder + Benzene	Greenish yellow	Yellowish green	Black
16	Powder + Petroleum ether	Greenish yellow	Yellowish green	Black
17	Powder + Acetone	Pale brown	Fluorescent green	Dark brown
18	Powder + Chloroform	Pale brown	Pale brown	Black
19	Powder + Methanol	Dark brown	Dark brown	Black
20	Powder + Ethanol	Dark brown	Dark brown	Dark green

When treated with various chemical reagents such as acids, alkalis, organic solvents, and specific detecting agents the samples exhibited distinct colour changes and fluorescence responses under UV light. These reactions are indicative of the presence of specific classes of phytochemicals, including flavonoids, coumarins, alkaloids, and phenolic compounds, many of which are known for their natural fluorescence properties. This method, widely used in pharmacognosy, aids in detecting crude drug adulteration, evaluating consistency across batches, and establishing a diagnostic fingerprint for quality control. The combination of visible and UV-light observations enhances the specificity and reliability of the technique²⁰.

Fluorescence Analysis and Its Pharmacognostic Significance: In the present study, the fluorescence behaviour of *Syzygium tamilnadense* stem powder was evaluated under visible light and ultraviolet (UV) radiation at wavelengths of 254 nm and 365 nm. The powder exhibited distinct colour changes upon treatment with various chemical reagents, indicating the presence of diverse phytochemical groups such as flavonoids, alkaloids, tannins, and phenolics.

Notably, fluorescent green and dark black hues observed under UV light suggest the occurrence of pharmacologically active constituents, particularly

phenolic compounds and alkaloids, which are known to exhibit natural fluorescence. Strong fluorescence responses upon treatment with reagents such as sulfuric acid, nitric acid, sodium hydroxide, and ferric chloride further confirm the interaction of these reagents with phenolic hydroxyl groups and nitrogen-containing alkaloidal structures.

These findings contribute significantly to the Pharmacognostic profiling of *S. tamilnadense*, supporting its authentication, standardization, and quality control for use in traditional and modern herbal formulations. Fluorescence analysis remains a non-destructive, rapid, and cost-effective technique, widely employed for the preliminary identification and purity assessment of crude drugs^{11, 93, 95}.

Despite the growing demand for herbal therapeutics, a key challenge in the herbal drug industry is the absence of standardized quality control profiles for raw botanical materials and their derivative products^{113, 120}. Thus, standardization spanning from cultivation to clinical application is vital for ensuring consistency, efficacy, and safety of herbal drugs^{40, 92}.

Quantitative Estimation of Total Phenolics, Flavonoids, and Tannins: The results of the quantitative analysis for key bioactive constituents total phenolics, flavonoids, and tannins in the *Syzygium tamilnadense* stem extract are summarized as follows:

TABLE 4: TOTAL PHENOLIC, FLAVONOID, TANNIN CONTENTS OF *S. TAMILNADENSIS* STEM *

S. no.	Bioactive compounds	Quantification value mg/g
1	Total phenolic mg GAE/g	128.14±0.76
2	Flavonoid mg QES/g	44.20±0.28
3	Tannin mg QAE/g	38.26±0.48

* Values are mean ± SD (n = 3)

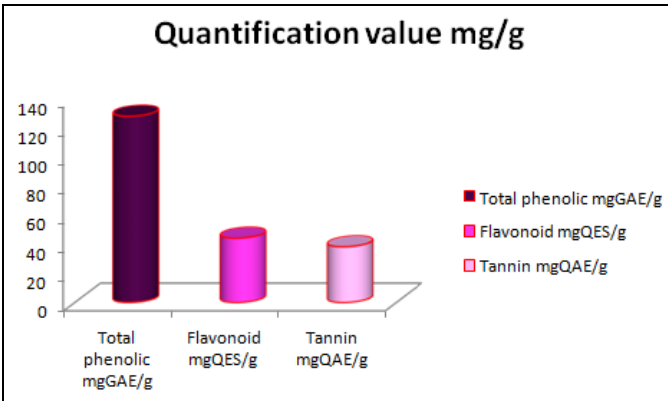


FIG. 4: TOTAL PHENOLIC, FLAVONOID, TANNIN CONTENTS OF *S. TAMILNADENSIS* STEM

Total Phenolic Content: The extract demonstrated a notably high concentration of phenolic compounds, quantified at 128.14 ± 0.76 mg GAE/g. Phenolic compounds are well recognized for their potent antioxidant activities, which are crucial in scavenging free radicals, mitigating oxidative stress, and offering a broad spectrum of pharmacological effects, including anti-inflammatory, antimicrobial, and anticancer properties^{11, 53, 77, 80, 93, 94, 104, 113, 49}.

Total Flavonoid Content: The flavonoid content was measured at 44.20 ± 0.28 mg QES/g, emphasizing the presence of this significant group of secondary metabolites. Flavonoids are celebrated for their diverse biological activities, particularly antioxidant, anti-inflammatory, antiviral, and cardioprotective effects^{11, 49, 53, 77, 80, 93, 94, 113, 116}.

Tannin Content: The tannin content was quantified at 38.26 ± 0.48 mg QAE/g, highlighting its role in the plant’s bioactive profile. Tannins are renowned for their astringent properties and are associated with various therapeutic applications, such as antimicrobial, antidiarrheal, and wound-healing activities^{11, 49, 53, 94}. The significant levels of phenolics, flavonoids, and tannins underscore the rich phytochemical composition of *S.*

tamilnadense. These findings not only enhance the ethnopharmacological value of the plant but also support its potential use in the development of herbal formulations and pharmaceutical products. The high concentration of antioxidant compounds positions the plant as a promising natural source for further pharmacological exploration.

Phytochemical Analysis: The phytochemical analysis of various extracts of *S. tamilnadense*, summarized in Table 5, revealed notable solubility patterns of bioactive constituents across solvents with varying polarity. The findings are as follows:

Flavonoids, Glycosides, Phenols, Tannins, and Sugars: These compounds were consistently detected across all tested solvents, with the exception of the aqueous extract. This suggests that these bioactive compounds are relatively non-polar and can be extracted effectively with a range of solvents.

Alkaloids: These were detected exclusively in ethyl acetate, methanol, and ethanol extracts, indicating a preference for solvents of intermediate to high polarity. This suggests that alkaloids in *S. tamilnadense* may possess nitrogenous structures that favour solubility in such solvents.

Saponins and Steroids: These compounds were predominantly extracted using polar and semi-polar solvents. While saponins were detected in the aqueous extract, steroids

± + Present – Absent were absent in water-based extractions, indicating their higher affinity for alcohol-based solvents.

Coumarins and Quinones: Both compounds showed selective solubility, with coumarins detected in ethyl acetate and benzene, and quinones in ethyl acetate alone. This suggests that these

constituents are present in lower quantities and are restricted to specific solvent environments.

presence at concentrations below the detection threshold.

Anthraquinones: These compounds were absent in all tested extracts, suggesting either their complete absence in the plant material or their

Xanthoproteins: These were identified in most solvent systems, except for benzene, underscoring their affinity for polar solvents.

TABLE 5: PHYTOCHEMICAL SCREENING OF *S. TAMILNADENSIS* STEM

Bioactive components	Nature of extract					
	Petroleum ether	Benzene	Ethyl acetate	Methanol	Ethanol	Aqueous
Alkaloids	-	-	+	+	+	-
Anthraquinones	-	-	-	-	-	-
Catechins	+	+	+	+	+	-
Coumarins	-	-	+	-	+	-
Flavonoids	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+
Phenols	+	+	+	+	+	+
Quinones	-	-	+	-	-	-
Saponins	-	+	+	+	+	+
Steroids	+	-	+	+	+	-
Tannins	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	-
Sugar	+	+	+	+	+	+
Xanthoproteins	+	-	+	+	+	+

The differential solubility of bioactive compounds across solvents highlights the importance of selecting appropriate solvents for efficient extraction, and points to the polar and semi-polar solvents (e.g., methanol and ethanol) as being particularly effective for extracting a broader spectrum of phytoconstituents.

Your section on Phytochemical Constituents and GC-MS Analysis provides a comprehensive overview of the bioactive compounds identified in *Syzygium tamilnadense* and their potential pharmacological applications. Here's a refined version that enhances clarity, flow, and readability while maintaining an academic tone:

Phytochemical Constituents and GC-MS Analysis of *Syzygium tamilnadense* Stem: Various researchers have employed different analytical methods to identify and characterize phytoconstituents in various parts of plants. Preliminary phytochemical investigations on diverse plant species consistently report the presence of various bioactive compounds ^{49, 92, 93}. These compounds exhibit a broad spectrum of pharmacological activities, including analgesic, anti-inflammatory, antimicrobial, anticancer, and antidiabetic effects, underscoring their potential therapeutic applications.

Alkaloids, for instance, are known for their wide range of pharmacological applications, including analgesic, antiasthmatic, anticancer, antihypertensive, antipyretic, and antihyperglycemic effects (Ng *et al.*, 2015). They also exhibit anti-inflammatory, antimicrobial, antioxidant, acetylcholinesterase inhibitory, antimalarial, and antidiabetic activities ¹³. Catechins, primarily found in tea leaves, act as potent antioxidants that help prevent or minimize skin damage and provide other physiological benefits ^{15, 29}.

Coumarins, which belong to the benzopyrone class, are commonly found in plants as glycosides or esters. They have been studied for their antimicrobial ⁹, anti-inflammatory ⁶², antidiabetic ⁶¹, antioxidant ¹¹¹, and enzyme inhibitory properties ^{106, 107, 123}.

Flavonoids are associated with a wide array of health benefits, including antioxidant, anti-inflammatory, antimutagenic, and anticarcinogenic properties. These compounds also modulate critical cellular enzyme functions and are highly valuable in nutraceutical, pharmaceutical, medicinal, and cosmetic formulations ^{75, 84}. Glycosides exhibit significant therapeutic potential, contributing to the treatment of various ailments through antibacterial,

anticancer, anti-inflammatory, cardiovascular, and neuroprotective activities¹⁰². Plant phenolics show numerous health-promoting effects, including antioxidant, antibacterial, cardioprotective, anticancer, and anti-inflammatory properties, in addition to immune system support and protection against ultraviolet radiation^{49, 74, 117}.

Saponins display a broad range of biological activities, including anticancer, hepatoprotective, and antioxidant effects. They have also been implicated in the treatment of osteoporosis, obesity, and diabetes, although their mechanisms of action are still under investigation^{31, 110}. Plant steroids from various species in the *Syzygium* genus have demonstrated multiple bioactivities, including cytotoxic⁹¹, antibacterial^{73, 85}, antimicrobial⁹⁹, antioxidant⁷², and antidiabetic effects⁸⁶. These compounds are valued for their diverse medicinal, pharmaceutical, and agrochemical potential, which includes antitumor, anticancer, immunosuppressive, hepatoprotective, and sex hormone-related activities^{103, 111}.

Recent studies have highlighted the beneficial effects of tannins on the gastrointestinal tract, including antioxidant activity, free radical scavenging, antimicrobial, antiviral, antimutagenic, anticarcinogenic, anthelmintic, and hepatoprotective properties. Tannins have also been shown to inhibit pro-oxidative enzymes and possess antinutrient and chemo preventive properties against cancers of the breast, oral cavity, prostate, stomach, and skin¹¹⁹. Terpenoids, a class of organic compounds, demonstrate hepatoprotective, anti-inflammatory, antimicrobial, analgesic, and immunomodulatory activities^{7, 25, 44}. Additionally, xanthoproteins and sugars are linked to a variety of pharmacological benefits, including hypoglycemic, vasorelaxant, hepatoprotective¹⁰⁵, antidiabetic, anti-inflammatory, antioxidant, antibacterial, and weight management effects^{22, 38, 65, 66, 108, 122}. The findings of this study indicate that the stem of *Syzygium tamilnadense* is rich in chemo diversity, with the presence of these valuable

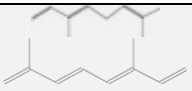
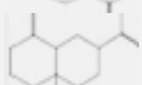
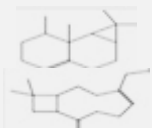
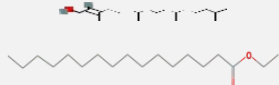
secondary metabolites suggesting significant potential for treating various human diseases. This plant could serve as a promising source for the development of novel therapeutic agents.

GC-MS Analysis: The phytochemical constituents of *S. tamilnadense* stem were analysed using Gas Chromatography-Mass Spectrometry (GC-MS). The Total Ion Chromatogram (TIC) of the ethanol extract is presented in **Fig. 1**, and the details of the identified compounds are listed in **Table 6**. These include retention time (R.T.), molecular formula, molecular weight (MW), peak area percentage, and corresponding chemical structures.

GC-MS analysis identified 25 chemical constituents, with retention times ranging from 7.273 to 29.237 minutes. These compounds belong to various chemical classes, including monoterpenes, sesquiterpenes, diterpenes, hydrocarbons, fatty acid esters, and alcohols. The molecular weights of these phytocompounds ranged from 134 to 410 g/mol, indicating the presence of both volatile and semi-volatile components in the ethanol extract. The most abundant compound identified was Caryophyllene oxide (12.01%), an oxygenated sesquiterpene known for its antimicrobial and anti-inflammatory properties. Other prominent constituents included Neophytadiene (10.39%), a diterpene hydrocarbon with antioxidant and insecticidal activities, and 9,12,15-Octadecatrienoic acid, ethyl ester (Z,Z,Z) (6.69%), which also demonstrated biological activity. Hexadecanoic acid, ethyl ester (6.52%), and Squalene (6.04%), a triterpene recognized for its antioxidant and emollient properties, were also identified. Monoterpenes such as β -Myrcene (3.17%) and β -Ocimene (4.10%), along with sesquiterpenes like Caryophyllene (5.35%), Aromandendrene (5.04%), γ -Muurolene (1.67%), and α -Guaiene (1.19%), contributed significantly to the chemical diversity of the extract. These compounds are known for their antimicrobial, anticancer, and immunomodulatory effects^{60, 67, 82, 89}.

TABLE 6: BIOACTIVE COMPOUNDS FOUND IN THE ETHANOL EXTRACT OF S. TAMILNADENSE STEM

S. no.	R. time	Name of the Compound	Molecular Formula	Molecular Weight	Peak Area %	Structure
1	7.273	.beta-Myrcene	C10H16	136	3.17	

2	8.540	.beta.-Ocimene	C10H16	136	4.10	
3	10.365	2,6-Dimethyl 1,3,5,7-octatetranene E,E-	C10H14	134	0.64	
4	11.581	Dodecane	C12H26	170	0.44	
5	15.200	Caryophyllene	C15H24	204	5.35	
6	15.471	Aromandendrene	C15H24	204	5.04	
7	15.946	.gamma.-Muurolene	C15H24	204	1.67	
8	16.188	Naphthalene,decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-,[4aR-(4	C15H24	204	1.45	
9	16.274	alpha.-Guaiene	C15H24	204	1.19	
10	16.531	1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene	C15H24	204	3.10	
11	17.495	Caryophyllene oxide	C15H24	220	12.01	
12	17.848	(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7- diene	C15H24O	220	2.00	
13	18.212	1H-Cycloprop[e]azulene,decahydro-1,1,7-trimethyl-4-methylene-14-Hydroxycaryophyllene	C15H24	204	2.28	
14	18.618		C15H24O	220	2.90	
15	20.298	Neophytadiene	C20H38	278	10.39	
16	20.400	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C20H40O	296	2.65	
17	21.990	Hexadecanoicacid, ethyl ester	C18H36O2	284	6.52	
18	23.223	Phytol	C20H40O	296	2.71	
19	23.681	Linoleic acid ethyl ester	C20H36O2	308	3.80	
20	23.748	9,12,15-Octadecatrienoic acid,ethyl ester,(Z,Z,Z)-	C20H34O2	306	6.69	
21	23.976	Octadecanoic acid,ethyl ester	C20H40O2	312	1.65	
22	25.801	Ethyl 14-methyl-hexadecanoate	C19H38O2	298	0.49	
23	26.695	Eicosane	C20H42	282	0.44	
24	27.490	1-Hexacosene	C26H52	364	0.44	
25	29.237	Squalene	C30H50	410	6.04	

Additional diterpenes such as Phytol (2.71%) and Neophytadiene (10.39%) were also detected, further highlighting the plant's bioactive potential. A substantial proportion of the extract was composed of long-chain fatty acid ethyl esters, including Hexadecanoic acid, ethyl ester (6.52%), Linoleic acid ethyl ester (3.80%), and 9,12,15-Octadecatrienoic acid, ethyl ester (Z,Z,Z) (6.69%).

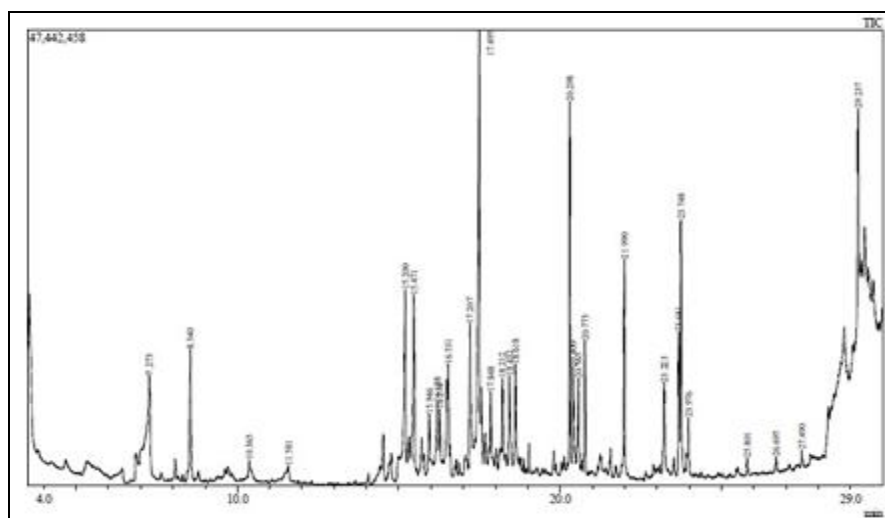


FIG. 5: GC-MS CHROMATOGRAM OF ETHANOL EXTRACT OF *SYZYGIUM TAMILNADENSE* STEM

Minor constituents such as Dodecane (0.44%), Eicosane (0.44%), and 1-Hexacosene (0.44%) were detected in trace amounts, contributing to the overall chemical profile.

Therapeutic Potential: The diverse bioactivities reported for these phytochemicals highlight the therapeutic potential of *S. tamilnadense*, validating its traditional medicinal use. Notably, Caryophyllene oxide, Squalene, and Phytol exhibit a range of antioxidant, anti-inflammatory, anticancer, and antidiabetic activities^{17, 27, 50, 55, 56, 90}.

Other identified compounds, such as Neophytadiene and Hexadecanoic acid, have broad anti-inflammatory, antimicrobial, and hepatoprotective effects^{17, 51}. These findings contribute to the growing body of evidence supporting the medicinal applications of *S. tamilnadense* and its promising future in the development of novel pharmaceutical agents.

CONCLUSION: The pharmacognostic and phytochemical evaluation of the endemic plant species *Syzygium tamilnadense* stem ethanolic extract highlights its substantial therapeutic potential and pharmaceutical relevance. The ash value and extractive studies suggest the presence of bioactive inorganic and organic constituents, with ethanol proving to be the optimal solvent for extraction. Fluorescence and GC-MS analyses revealed a diverse array of phytochemicals, further confirming the extract's antioxidant, antimicrobial, anti-inflammatory, and hepatoprotective activities. Key bioactive compounds identified, including

caryophyllene oxide, neophytadiene, hexadecanoic acid ethyl ester, and linoleic acid ethyl ester, emphasize the pharmacological significance of the species.

These findings lay a robust scientific foundation for the therapeutic use of *S. tamilnadense*, suggesting its potential application in drug development and nutraceutical formulations. Further studies and clinical trials are warranted to explore and confirm the full spectrum of its medicinal properties.

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