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Phytochemical composition and GC-MS profiling of the bark of *Syzygium tamilnadense*, (Rathakr. & Chitra)

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Abstract
Plants have long evolved to synthesize a diverse array of natural compounds with notable therapeutic potential, many of which continue to inform modern drug discovery. In this study, we investigate *Syzygium tamilnadense* (Rathakr. & Chitra), an underexplored species endemic to the Sholur and Nanjanad Reserve Forests of the Nilgiri Biosphere in the Western Ghats. This marks the first scientific exploration of its medicinal properties. Preliminary phytochemical screening of solvent extracts revealed that the ethanol extract was particularly rich in bioactive constituents, including alkaloids, flavonoids, catechins, glycosides, phenols, saponins, steroids, tannins, terpenoids, sugars, and xanthoproteins. Quantitative analyses indicated high levels of phenolics (152.09 ± 0.96 mg GAE/g), flavonoids (51.26 ± 0.26 mg QES/g), and tannins (41.20 ± 0.56 mg QAE/g), suggesting strong antioxidant potential. Furthermore, Gas Chromatography–Mass Spectrometry (GC–MS) analysis identified 25 distinct bioactive compounds, including caryophyllene oxide, neophytadiene, squalene, phytol, linoleic acid ethyl ester, copaene, hexadecane, p-camphorene, and henicosanal—many of which are known for their antidiabetic, antioxidant, anti-inflammatory, antimicrobial, and anticancer properties. These findings highlight *S. tamilnadense* bark as a promising source of pharmacologically active compounds, validating its traditional medicinal use and supporting its potential in future pharmaceutical development.

Keywords: *Syzygium tamilnadense*, phytochemicals, gc-ms, caryophyllene oxide squalene, neophytadiene, caryophyllene, copaene, henicosanal

Introduction

Medicinal plants constitute an essential source of therapeutic agents and have historically played a pivotal role in global healthcare due to their broad spectrum of pharmacological activities. Their contributions extend beyond disease management to encompass overall health promotion. The sustained global reliance on herbal medicine is attributed to its cultural acceptance, favorable safety profile, and biocompatibility compared to synthetic drugs (Newman and Cragg, 2007) [35]. Traditional medicinal systems such as Ayurveda, Siddha, and Unani have long utilized plant-derived formulations, drawing upon indigenous knowledge accumulated over centuries. Today, approximately 20,000 plant species are recognized for their medicinal value, and scientific interest in phytochemical research continues to grow due to its potential to inform modern pharmacotherapy (Hossain *et al*., 2021 [20]; Ozioma and Chinwe 2019) [36]. While traditional remedies are often administered as crude preparations—including decoctions, tinctures, essential oils, and powders—modern natural product research aims to isolate and characterize individual phytoconstituents for enhanced pharmacological efficacy and bioavailability. Plants produce a diverse array of secondary metabolites such as alkaloids, flavonoids, terpenes, glycosides, saponins, steroids, tannins, coumarins, catechins, xanthoproteins, quinones, and phenols. These compounds are responsible for various biological activities, and their scientific investigation has gained momentum in recent years. Among the analytical tools available, Gas Chromatography–Mass Spectrometry (GC–MS) has proven particularly effective for phytochemical profiling, offering high sensitivity and specificity for compound identification (Kumar *et al*., 2019). Various plant parts, including leaves, stems, roots, flowers, seeds, and bark, are traditionally employed for their medicinal value.

The family Myrtaceae, encompassing over 55,000 species across 142 genera, is well-represented in tropical and subtropical regions and is taxonomically divided into two subfamilies and 17 tribes (Rahman *et al*., 2018) [18]. Named after the shrub Myrtus, this family includes both shrubs and trees, and many of its members are recognized for their ecological, morphological and anatomical and pharmacological importance (Mitra *et al*., 2012 [33]; Khandaker et.al 2018) [25]. Species in this family typically thrive in waterlogged and humid rainforest environments and are often characterized by bisexual, actinomorphic flowers and fleshy or dry fruits. Ethnobotanical uses of Myrtaceae members are well-documented across indigenous systems worldwide. Within this family, the genus Syzygium represents the largest and most diverse group, comprising approximately 1,800 species predominantly distributed across South and Southeast Asia, Australia, and parts of Africa and the Pacific Islands (da Costa *et al*., 2020). Many *Syzygium* species are integral to traditional medicine Despite the therapeutic promise of this genus, several endemic species remain underexplored. *Syzygium tamilnadense* (Rathakr. & Chitra), an endemic species of the Western Ghats, is one such plant that has yet to be thoroughly examined for its phytochemical and pharmacological potential. In light of the increasing demand for plant-based bioactive compounds—particularly from endemic and lesser-known species—the present study aims to investigate the phytochemical composition of *S. tamilnadense* bark using GC–MS analysis. This research seeks to identify bioactive constituents that may contribute to its potential use in pharmaceutical and nutraceutical applications.

Study area

(a). Study area – Sholur, Nanjanad;

(b). Study species- *Syzygium tamilandense* – Habit

The study was conducted in the Sholur–Nanjanad region, located within Udhagamandalam Taluka of The Nilgiris District, Tamil Nadu, India. Positioned approximately 13 km from Udhagamandalam—the administrative headquarters for both the district and sub-district—the village of Nanjanad encompasses a total geographical area of 13,064.23 hectares. Geographically, the study site is situated at coordinates 125,478.30 N and 2,531,453 E. The region is renowned for its dense forest cover, particularly the Cairn Hill Reserve Forest, which comprises a mosaic of shola forests, subtropical, and temperate vegetation types. This forested landscape supports a rich diversity of flora, including endemic shola species, as well as cypress, pine, and other tree species typical of the southern Western Ghats.

Study Specimen

Class: Magnoliopsida
Order: Myrtales
Family: Myrtaceae
Genus: *Syzygium*
Species: *tamilnadense*, Rathakr. & Chitra

Habit: Trees up to 15 m tall.
Trunk &Bark: Bark grey, scaly; blaze cream.
Branches and branchlets: Branchlets stout, quadrangular and narrowly winged, glabrous.
Leaves: Leaves simple, opposite, decussate; petiole 0.4-1.2 cm long, canaliculate above, glabrous; lamina 6.5-12.5 x 3.3-7.5 cm, elliptic to broad elliptic or obovate, apex obtuse to rounded; base cuneate, margin entire, pellucid gland dotted, coriaceous, glabrous; midrib distinctly canaliculate above; intramarginal nerve present; secondary nerves ca. 12 pairs; tertiary nerves obscure.
Inflorescence / Flower: Flowers small in terminal corymbose cyme.
Fruit and Seed: Berry, globose, purple, 0.8 cm across, crowned with persistent calyx; seed one.

Materials and Methods

Collection of plant sample

Fresh leaves of *Syzygium tamilnadense* Rathakr & Chitra were gathered from Sholur, Nanjanad forest, Western Ghats, in the Nilgiris District, Tamil Nadu. The plant specimens were identified by comparing them to the local flora and authenticated by the Botanical Survey of India, Southern Circle, Coimbatore. The collected stems were chopped into small pieces and shade-dried to ensure uniformity and smoothness in the drying process. Once dried, the stems were ground into a fine powder using a blender and then sieved to obtain a uniform consistency. This powder was then used for the extraction of the plant's active constituents.

Preparation of extract for phytochemical analysis

A required amount of leaf powder was weighed and transferred to Stoppard flasks, where it was mixed separately with different solvents (petroleum ether, benzene, ethyl acetate, methanol, ethanol, and water) until the powder was fully submerged. The flasks were shaken every hour for the first six hours. Afterward, the extracts were filtered using Whatman No. 1 filter paper. All extracts underwent qualitative testing following standard procedures to identify various phytochemical constituents. The ethanol extract was then used to estimate the total phenolic, flavonoid, and tannin contents, as well as for GC-MS analysis.

Preparation of powder

The stem, bark and leaf of *S. tamilnadense* were cut into small fragments and shade dried until the fracture was uniform and smooth. The dried plant materials were powdered separately by using a blender and sieved to get uniform particles by using sieve No. 60. The final uniform powder of stem and leaf were used for various experimental studies.

Physicochemical characteristics

The percentage of total ash, acid insoluble ash, water soluble ash, sulphated ash and extractive values in various solvents were obtained by employing standard method of analysis described in Pharmacopoeia of India.

Determination of total ash

Three grams of the powdered drugs (stem, bark and leaf) was accurately weighed in a silica crucible, which was previously ignited and weighed. The powdered drug was spread as a fine layer on the bottom of the crucible. The crucible was incinerated at a temperature not exceeding 450°C until free from carbon. The crucible was cooled and weighed. The procedure was repeated to get the constant weight. The percentage of total ash was calculated with reference to the air-dried powder.

Determination of acid insoluble ash

The ash obtained as described in the determination of total ash was boiled with 25 ml of 2N Hydrochloric acid for 5 minutes. The insoluble ash was collected on an ashless filter paper and washed with hot water. The insoluble ash was transferred to a preweighed silica crucible. The procedure was repeated to get constant weight. The percentage of acid insoluble ash was calculated with reference to the air-dried drug.

Determination of water-soluble ash

The ash obtained in the determination of total ash was boiled for 5 minutes with 25 ml of water. The insoluble matter was collected on an ashless filter paper and washed with hot water. The insoluble ash was transferred into a pre-weighed silica crucible and ignited for 15 minutes at a temperature not exceeding 450°C. The procedure was repeated to get the constant weight. The weight of the insoluble matter was subtracted from the weight of the total ash. The difference in weight was considered as the water-soluble ash. The percentage of water-soluble ash was calculated with reference to the air-dried powder.

Determination of sulphated ash

A silica crucible was heated to redness for 10 minutes and allowed to cool in a desiccator. One gram of sample was weighed, transferred to the crucible and reweighed the crucible and the contents accurately. It was ignited gently at first, until the substance was thoroughly charred. It was cooled, moistened with 1 ml of sulphuric acid, heated gently until the white fumes were no longer evolved and ignited at 800°C ± 25°C until all black particles disappeared. The ignition was done in a place protected from air currents. The crucible was cooled, a few drops of sulphuric acid was added and heated. Again, it was ignited as before and allowed to cool and weighed. This was repeated until two successive weighing did not differ by more than 0.5 mg.

Extractive values

The extractive values of the stem, bark and leaf of *Syzygium tamilnadense* in various solvents (petroleum ether, benzene, chloroform, ethyl acetate, methanol, ethanol and water) were determined by employing the methods of analysis described in Pharmacopoeia of India. About 5 g of air-dried sample was taken in a stoppered flask. 100 ml of the respective solvent were added, shaken well and allowed to stand for 24 hrs with occasional shaking. Then the content was filtered. 50 ml of the filtrate were pipette out into a clean, previously weighed china dish and evaporated on a water bath. Finally, it was dried at 105°C, cooled and weighed. The percentage of solvent soluble extractive with reference to the air - dried sample was calculated.

Fluorescence analysis

The drug powders were treated with acids like 1N HCl, conc. HCl, 50% H2SO4, conc. H2SO4, 50% HNO3, conc. HNO3, acetic acid and conc. HNO3+NH3; alkaline solutions like aqueous sodium hydroxide, 40% NaOH + 10% lead acetate and alcoholic sodium hydroxide; solvents like acetone, benzene, chloroform, petroleum ether, methanol and ethanol; other chemical reagents like ferric chloride and ammonia. They were subjected to fluorescence analysis in daylight and in short UV light (254 nm) and long UV light (365 nm). The fluorescence analysis was carried out as per the standard procedures.

Estimation of total phenolics

The total phenolic content was determined using a Folin-Ciocalteau reagent-based assay, with slight modifications. To 1 ml of each extract (100 µg/ml), 5 ml of diluted (ten-fold) Folin-Ciocalteau reagent and 4 ml of 75 g/L Na_2CO_3 were added. The mixture was allowed to stand at 20°C for 30 minutes, and the absorbance of the resulting color was measured at 765 nm using a UV-VIS spectrophotometer. To create a calibration curve, 1 ml aliquots of methanolic gallic acid solutions at concentrations of 20, 40, 60, 80, and 100 µg/ml were used as standards. The absorbance of the sample solutions was then compared with the gallic acid calibration curve. The total phenolic content was expressed as milligrams of gallic acid equivalent per gram (mg GAE g^{-1}).

Estimation of flavonoids

The total flavonoid content was determined following the method 0.5 ml aliquot of the sample was mixed with 0.1 ml of 10% aluminium chloride and 0.1 ml of 1M potassium acetate. To this mixture, 4.3 ml of 80% methanol was added to bring the total volume to 5 ml. The mixture was vortexed, and the absorbance was measured spectrophotometrically at 415 nm. The optical density value was used to calculate the total flavonoid content in the sample. The flavonoid content was expressed as milligrams of quercetin equivalent per gram (mg QES g^{-1}).

Estimation of tannin content

Tannins were quantified using the Folin-Ciocalteu method. To a 10 ml volumetric flask containing 7.5 ml of distilled water, approximately 0.1 ml of the plant extract was added. Then, 0.5 ml of Folin-Ciocalteu reagent and 1 ml of 35% Na_2CO_3 solution were introduced. The mixture was diluted to 10 ml with distilled water, thoroughly shaken, and allowed to stand at room temperature for 30 minutes. A set of reference standard solutions of gallic acid was prepared in the same manner as described earlier. The absorbance of both the test and standard solutions was measured against a reagent blank at 725 nm using a UV-visible spectrophotometer. The tannin content was expressed as milligrams of gallic acid equivalent per gram of sample (mg GAE g^{-1}).

Result and Discussion

The determination of ash content is a fundamental aspect of evaluating the purity and quality of plant-derived materials. The ash values obtained for the analyzed sample are presented in Table 1 and Figure 1, representing the total amount of inorganic residue remaining after complete incineration of the plant material. The results include

physicochemical parameters such as total ash, water-soluble ash, acid-insoluble ash, and sulphated ash, which provide essential information regarding the mineral composition and presence of extraneous matter in the sample.

Table 1: Ash values of powdered Bark

SI. No.	Types of ash	Ash value %[a]
1	Total ash	10.68±0.11
2	Water soluble ash	2.54±0.03
3	Acid insoluble ash	1.52±0.02
4	Sulphated ash	9.88±0.06

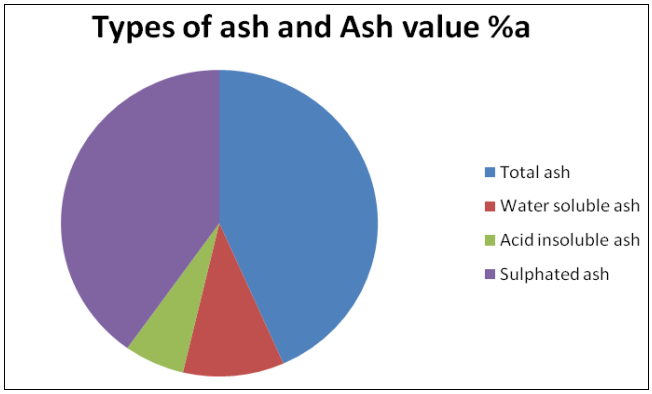


Fig 1: Types of Ash and Corresponding Ash Values in Powdered Bark

[a] All values are the mean of triplicate determinations expressed on dry weight basis.
±Standard error.

The total ash value indicates the total amount of inorganic residue remaining after incineration of the plant material. A value of 10.68% suggests a moderate mineral content, which includes both physiological ash (originating from the plant tissue) and non-physiological ash (derived from external sources such as soil or dust). The low standard deviation reflects the reliability and consistency of the measurements. Water soluble ash represents the portion of total ash that is soluble in water, typically composed of soluble minerals and inorganic salts. This value is an important parameter for detecting the presence of soluble adulterants or the loss of natural constituents during processing. The relatively low value indicates limited water-soluble inorganic matter. The acid insoluble ash provides an estimate of siliceous matter, primarily sand and other acid-insoluble residues, which are typically considered contaminants. The low value of 1.52% indicates minimal contamination with earthy materials and reflects good post-harvest handling and processing quality. Sulphated ash is determined by treating the sample with sulfuric acid prior to ignition, ensuring complete oxidation of organic matter. The value of 9.88% corresponds closely to the total ash, suggesting that most of the inorganic components are retained as stable sulphated residues. This further confirms the low presence of volatile inorganic impurities.

Extractive values are indicative of the presence of active phytochemicals soluble in specific solvents, thus providing insight into the nature and polarity of constituents present in the plant material. The extractive values obtained using various solvents are summarized in Table 2 and Fig. no. 2. The results reveal significant variability in extractive values, depending on the solvent used.

Table 2: Extractive values of powdered Bark

SI. No.	Extract	Extractive value %[a]
1	Petroleum ether	5.22±0.03
2	Benzene	5.42±0.04
3	Chloroform	5.98±0.06
4	Acetone	6.56±0.08
5	Methanol	8.28±0.09
6	Ethanol	9.26±0.14
7	Water	8.21±0.10

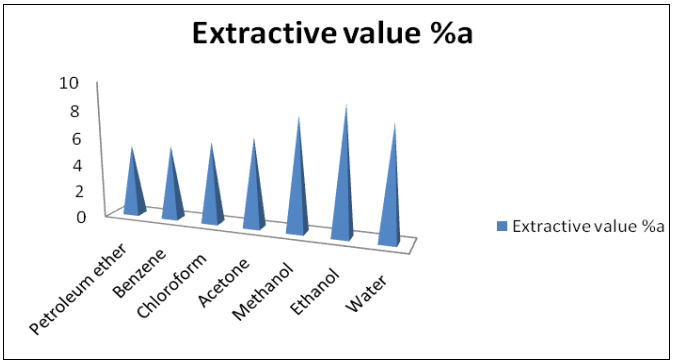


Fig 2: Comparative Analysis of Extract and Extractive Values of the Powdered Stem

[a] All values are the mean of triplicate determinations expressed on dry weight basis.
±Standard error.

Petroleum ether (5.22 ± 0.03%), a non-polar solvent, primarily extracts lipophilic compounds such as fats, waxes, and certain non-polar terpenoids. The relatively low extractive value suggests a limited presence of non-polar constituents in the sample. Benzene (5.42 ± 0.04%) and chloroform (5.98 ± 0.06%), both moderately polar solvents, yielded slightly higher extractive values than petroleum ether. These solvents typically extract alkaloids, certain flavonoids, and other medium-polarity compounds, indicating a moderate presence of such phytochemicals in the plant material. Acetone (6.56 ± 0.08%), a polar aprotic solvent, showed a relatively higher extractive value. This suggests a broader solubility range, likely encompassing phenolic compounds, flavonoids, and other polar to semi-polar secondary metabolites. Methanol (8.28 ± 0.09%) and ethanol (9.26 ± 0.14%), both highly polar solvents, exhibited significantly higher extractive values, with ethanol yielding the maximum. These results imply that the plant material is rich in polar constituents such as glycosides, phenolics, flavonoids, and tannins, which are efficiently extracted by alcoholic solvents. Water (8.21 ± 0.10%), the universal solvent, also produced a high extractive value, comparable to methanol. This reflects a substantial content of hydrophilic compounds, including carbohydrates, tannins, saponins, and water-soluble alkaloids. Among all tested solvents, ethanol proved to be the most effective in extracting phytoconstituents, followed closely by methanol and water. This trend highlights the predominance of polar bioactive compounds in the plant sample, which may contribute significantly to its therapeutic efficacy. The extractive values not only guide the selection of appropriate solvents for phytochemical analysis and herbal formulation

development but also serve as key indicators in identifying exhausted or adulterated plant materials, as emphasized by Jayendran *et al*. (2025) [21].

Fluorescence analysis

Fluorescence analysis serves as a rapid and dependable technique for the preliminary detection of phytochemical constituents, relying on their characteristic color responses under visible and ultraviolet (UV) light. The powdered plant material, both in its native form and following treatment with various chemical reagents, was examined under daylight, short-wave UV (254 nm), and long-wave UV (365 nm) conditions. The results are presented in Table 3.

Table 3: Fluorescence analysis of *S. tamilnadense* bark

SI. No.	Experimental	Visible / Day light	VV light	
			254 mm (Short wave length)	365 mm (Long wave length)
1	Powder as such	Brown	Brown	Dark brown
2	Powder + 1 N Aqueous NaOH	Brown	Yellowish green	Dark black
3	Powder + 1 N Alcoholic NaOH	Pale brown	Fluorescent green	Black
4	Powder + 1 N HCl	Pale brown	Pale brown	Black
5	Powder + Conc. H_2SO_4	Dark brown	Fluorescent green	Black
6	Powder + Conc. HNO_3	Dark brown	Fluorescent green	Black
7	Powder + Conc. HCl	Yellowish green	Fluorescent green	Dark black
8	Powder + 50% HNO_3	Greenish yellow	Fluorescent green	Dark black
9	Powder + 50% H_2SO_4	Greenish yellow	Fluorescent green	Dark brown
10	Powder + 40% NaOH + 10% Lead acetate	Yellowish green	Fluorescent green	Dark brown
11	Powder + HNO_3 + NH_3	Pale brown	Yellowish green	Dark brown
12	Powder + NH_3	Dark brown	Dark green	Dark black
13	Powder + Acetic acid	Dark brown	Dark green	Dark black
14	Powder + Ferric acid	Dark brown	Dark brown	Black
15	Powder + Benzene	Pale brown	Pale brown	Black
16	Powder + Petroleum ether	Pale brown	Pale brown	Black
17	Powder + Acetone	Brown	Fluorescent green	Dark brown
18	Powder + Chloroform	Dark brown	Greenish yellow	Black
19	Powder + Methanol	Dark brown	Greenish yellow	Black
20	Powder + Ethanol	Dark brown	Dark brown	Black

Under visible light, the untreated powder exhibited a brown coloration, with only slight variations observed upon treatment with different reagents. However, under UV illumination, distinct and diagnostically significant changes in fluorescence were recorded. Under short-wave UV (254 nm), pronounced color changes were observed particularly with both alkaline and acidic reagents. Samples treated with aqueous and alcoholic sodium hydroxide, concentrated sulfuric acid (H_2SO_4), nitric acid (HNO_3), hydrochloric acid (HCl), and organic solvents such as acetone and chloroform exhibited transitions from brown to fluorescent green or yellowish green. These changes are indicative of the presence of phenolics, flavonoids, and other conjugated aromatic compounds, which are known to exhibit characteristic fluorescence. Under long-wave UV (365 nm), the fluorescence effects were even more prominent. Treatments with alcoholic NaOH, concentrated acids, 50% acid dilutions, and mixtures containing lead acetate resulted in intense dark black or fluorescent green coloration. Such vivid fluorescence under 365 nm suggests the presence of highly conjugated and reactive phytoconstituents, which are chemically active under strong acidic or basic conditions.

Noteworthy fluo rescent reactions included

- Powder + alcoholic NaOH → Fluorescent green (254 nm), black (365 nm)
- Powder + conc. H_2SO_4 / HNO_3 / HCl → Fluorescent green (254 nm)
- Powder + acetone → Fluorescent green (254 nm)
- Powder + 40% NaOH + lead acetate → Fluorescent green (254 nm)

These findings suggest the presence of fluorophores or their precursors within the plant matrix, which may function as chemotaxonomic indicators or markers of bioactive compounds. The fluorescence response of the powdered sample to various chemical reagents demonstrates the potential of this method as a reliable diagnostic tool for the authentication of crude drug materials, particularly in their powdered or adulterated forms. Moreover, fluorescence analysis supports the standardization of herbal formulations, as highlighted by several researchers.

Quantitative estimation of total phenolic, flavonoid and tannin

The quantification of key bioactive constituents—total phenolics, flavonoids, and tannins—was performed to assess the phytochemical richness of the plant material. The results are summarized in Table 4. and Fig.3.

Table 4: Total phenolic, flavonoid, tannin contents of *S. tamilnadense* Bark *

Sl. No.	Bioactive compounds	Quantification value mg/g
1	Total phenolic mgGAE/g	152.09±0.96
2	Flavonoid mgQES/g	51.26±0.26
3	Tannin mgQAE/g	41.20±0.56

* Values are mean ± SD (n = 3)

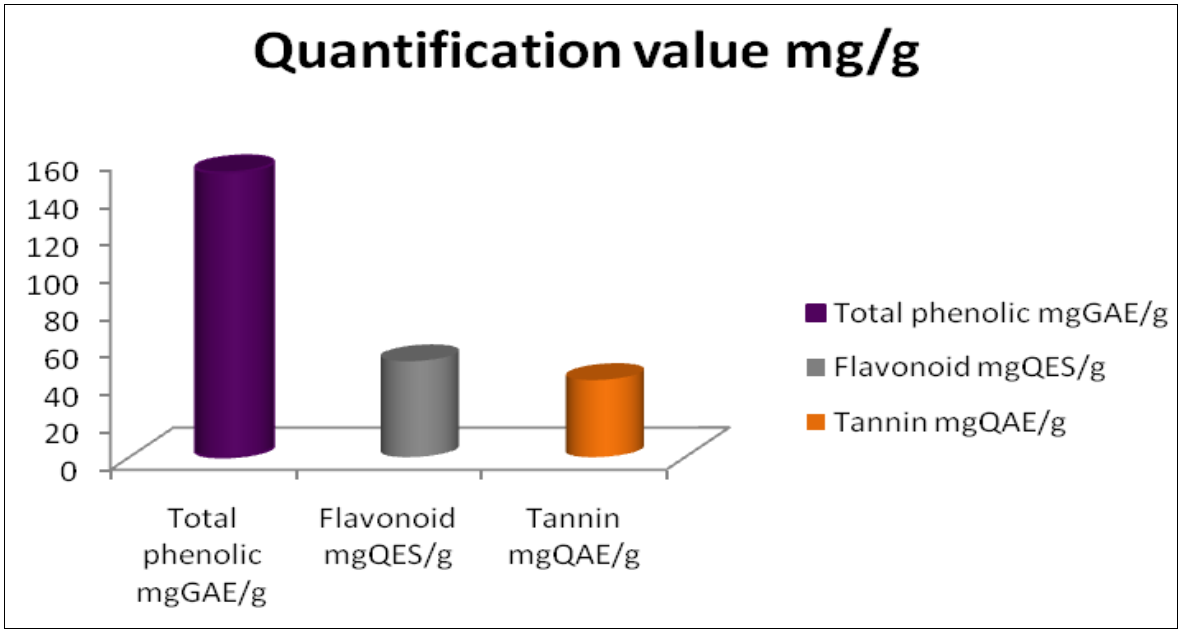


Fig 3: Total phenolic, flavonoid, tannin contents of *S. tamilnadense* stem

The total phenolic content of the ethanolic extract of *S. tamilnadense* bark was found to be 152.09 ± 0.96 mg GAE/g, indicating a high concentration of phenolic constituents. These secondary metabolites are well-documented for their strong antioxidant properties and are known to play an essential role in the plant's defense mechanisms and therapeutic potential (Sankhalkar & Vernekar, 2016; Jayendran *et al*., 2025) [21]. The flavonoid content was estimated at 51.26 ± 0.26 mg QE/g, reflecting a substantial presence of these polyphenolic compounds, which contribute to a wide spectrum of pharmacological effects including anti-inflammatory, antimicrobial, and cardioprotective activities (Jayendran *et al*., 2025) [21]. Additionally, the tannin content was determined to be 41.20 ± 0.56 mg TAE/g, suggesting a notable presence of astringent and antioxidant compounds commonly associated with wound healing, antimicrobial, and gastroprotective properties (Jayendran *et al*., 2025) [21]. Plant barks of both roots, and stems have different biomolecules such as alkaloids, glycosides, polyphenols, steroids, vitamins, terpenes, etc. that tend to serve as potential therapeutic agents to cure bone ailments (Singh *et al*., 2020) There are several plants barks described and used in different traditional therapy to heal the bone, which act as a safe, economic, and effective alternate treatment (Singh *et al*., 2016 [45], Singh 2017) [46]. The adoption of herbals as an alternative medicine to accelerate the bone mending process, reduce the healing period and treatment cost, and quickly regain good health is still a matter of detailed phytopharmacological investigation (Devi Datt Joshi, et.al 2023) [16]

The substantial concentration of these bioactive constituents enhances the therapeutic value of the ethanolic extract of *S. tamilnadense* bark. Collectively, the high levels of phenolics, flavonoids, and tannins indicate a rich phytochemical composition, highlighting the plant's potential as a natural source of antioxidants and pharmacologically active compounds. These findings not only validate its ethnopharmacological importance but also support its application in the development of herbal formulations and pharmaceutical products. The results strongly warrant further investigation into its pharmacodynamic mechanisms and clinical efficacy.

Phytochemical analysis

The qualitative phytochemical screening of various solvent extracts revealed the differential presence of bioactive compounds across the tested solvents—petroleum ether, benzene, ethyl acetate, methanol, ethanol, and aqueous extracts (Table 5). The results indicate that solvent polarity significantly influences the extraction efficiency of specific phytoconstituents. Alkaloids were detected in ethyl acetate, methanol, and ethanol extracts, while being absent in petroleum ether, benzene, and aqueous extracts. Anthraquinones were not detected in any of the solvent systems tested. Catechins were present in all organic solvent extracts but absent in the aqueous extract, suggesting their higher solubility in organic solvents. Similarly, coumarins were found in benzene, ethyl acetate, and methanol extracts, but not in petroleum ether, ethanol, or aqueous extracts. Flavonoids, glycosides, and phenols were universally present across all organic extracts, and notably, flavonoids and glycosides were also detected in the aqueous extract, indicating their wide solubility range. In contrast, quinones were detected only in the ethyl acetate extract, signifying their selective solubility. Saponins showed a broader solubility profile, being detected in all extracts except petroleum ether, indicating their amphiphilic nature. Steroids were present in petroleum ether, ethyl acetate, methanol, and ethanol extracts but were absent in benzene and aqueous extracts. Tannins and terpenoids were consistently present in all organic solvents except the aqueous extract, reflecting their higher affinity for non-polar to moderately polar solvents. Sugars were detected in all extracts, underscoring their broad solubility. Lastly, xanthoproteins were found in all extracts except benzene, implying a moderate polarity-based solubility. These results demonstrate that methanol and ethyl acetate were the most effective solvents for extracting a wide range of phytochemicals, indicating their suitability for further bioactivity-guided fractionation.

Table 5: Phytochemical screening of *S. tamilnadense* bark

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	+	-	+	+	+	+

+ Present – Absent

Plants function as biosynthetic reservoirs, producing a diverse array of secondary metabolites, including alkaloids, glycosides, saponins, steroids, quinones, tannins, flavonoids, terpenoids, sugars, catechins, and coumarins, many of which are known to exert significant physiological and therapeutic effects. These secondary metabolites, characterized by specific chemical structures, are largely responsible for the medicinal properties attributed to various plant species.

Syzygium is the largest woody genus of flowering plants, with many of its species recognized for their medicinal applications (Uddin *et al*., 2022) [50]. The genus possesses a strong phytochemical profile, as reported by several studies (Duyen *et al*., 2019 [17]; Jhansi *et al*., 2021 [22]; Pham et.al 2020) [37]. Numerous investigations have highlighted the diverse pharmacological activities of *Syzygium* species in the treatment and prevention of various diseases (Abdulrahman, 2022 [1]; Stalin & Swamy, 2018) [47]. These species are rich in flavonoids, tannins, phenols, steroids, and alkaloids, which significantly contribute to their therapeutic properties (Aung *et al*., 2021 [5]; Zulcafli *et al*., 2020) [53].

Traditionally, *Syzygium* species have been widely used in Ayurvedic medicine for treating ailments such as cough, colds, diarrhea, dysentery, fever, inflammation, pneumonia, wounds, ulcers, and various infections (Cock & Cheesman, 2018) [13]. In particular, they have shown promise in the management of diabetes, gastrointestinal disorders, and other health conditions (Hanif *et al*., 2020). Numerous researchers have employed a range of analytical techniques to identify these phytoconstituents in different plant parts. Preliminary phytochemical investigations across various species of the same genus have consistently reported the occurrence of diverse bioactive compounds (Margaret et.al. 2015 [30]; Maregesi et.al 2016 [31]; Jayendran *et al*., 2025;) [21]. Alkaloids are particularly well-studied due to their broad pharmacological spectrum, including analgesic, antiasthmatic, anticancer, antihypertensive, antipyretic, antihyperglycemic, anti-inflammatory, antimicrobial, antioxidant, acetylcholinesterase inhibitory, antimalarial, antidiabetic activities and Alzheimers disease (Chrisanta, et.al 2022; Devi Datt Joshi, et.al 2023) [16].

Plant-derived steroids exhibit a broad spectrum of biological activities, including antitumor, anticancer, immunosuppressive, hepatoprotective, antibacterial, plant hormone-regulating, cytotoxic, cardiotonic, and antihelminthic effects (Dean et.al 2017) [14]. Catechins, primarily found in tea leaves, are potent antioxidants and play significant roles in preventing or minimizing skin damage, among other physiological functions (Lima *et al*., 2016) [27]. Coumarins, members of the benzopyrone class, are widely distributed in plants, typically in the form of glycosides or esters. They have been extensively studied for their antimicrobial (Alshibl *et al*., 2020) [3], anti-inflammatory, antidiabetic, antioxidant, and enzyme-inhibitory (Wu *et al*., 2009) [51] properties. Quinones are another important class of phytochemicals known for their cytotoxic and antimicrobial activities, with anthraquinone derivatives demonstrating significant potential as chemotherapeutic agents in cancer treatment.

Xanthoproteins and sugars have also been associated with various pharmacological benefits, including hypoglycemic, vasorelaxant, hepatoprotective, antidiabetic, anti-inflammatory, antioxidant, weight management, and antibacterial effects (Maheshwaran *et al*., 2024) [29]. According to (Kusriani *et al*.2019) [26], flavonoids from *Syzygium* species represent a highly valued group of phytochemicals due to their diverse therapeutic potential, including antioxidative, anti-inflammatory, antimutagenic, anticancer, and anticarcinogenic properties. These compounds are widely utilized in the nutraceutical, pharmaceutical, medicinal, and cosmetic industries (Fernandez *et al*., 2021). Additionally, phenolic and triterpenoid compounds play crucial roles in human health by offering antioxidant, antibacterial, cardioprotective, anticancer, immune-enhancing, and UV-protective effects (Sun & Shahrajabian, 2023 [49]; Jayendran *et al*., 2025) [21]. Tannins have recently gained attention for their multiple health benefits, including gastrointestinal protection, antimicrobial and antiviral activities, antioxidant capacity, and chemopreventive potential against various cancers (Hossian *et al*., 2021). Saponins are recognized for their anticancer, hepatoprotective, and antioxidant properties, as well as their involvement in managing osteoporosis, obesity, and diabetes, although their mechanisms of action are not yet fully elucidated (Sharma *et al*., 2023) [42]. Glycosides, too, exhibit a broad spectrum of pharmacological effects, including antibacterial, anticancer, anti-inflammatory, cardiovascular, and neuroprotective properties (Riaz *et al*., 2023) [40]. The present study confirms that *S. tamilnadense*

bark is rich in diverse secondary metabolites. The abundance of these bioactive compounds underscores the plant's considerable potential for pharmacological applications and supports its candidacy as a promising source for the development of novel therapeutic agents.

GC-MS analysis

The GC-MS chromatographic analysis of the ethanolic bark extract of the endemic plant species *S. tamilnadense* revealed a chemically diverse profile, characterized by multiple prominent peaks within the retention time range of 4.0 to 29.0 minutes (Fig. 4 and Table 6). The most intense peak, observed at 17.409 minutes, corresponds to a major phytochemical constituent—likely a bioactive compound of therapeutic significance. The presence of numerous well-resolved peaks highlights the complex chemical matrix of the extract, which is rich in secondary metabolites such as terpenoids, flavonoids, phenolics, sterols, and fatty acid derivatives. These phytochemical classes are widely recognized for their antioxidant, anti-inflammatory, antimicrobial, and anticancer activities. Notably, peaks at retention times of 20.295, 21.746, and 29.035 minutes correspond to compounds of pharmacological interest, which may act synergistically to enhance the overall therapeutic efficacy of the extract. Preliminary compound identification suggests the presence of phytoconstituents commonly associated with the Syzygium genus, many of which have demonstrated antidiabetic, hepatoprotective, and cardioprotective effects in previous studies.

Quantitative analysis indicated that caryophyllene oxide (14.19%) was the predominant constituent. This sesquiterpene oxide is well-documented for its anti-inflammatory, antifungal, and anticancer activities, highlighting its potential as a lead therapeutic agent. Neophytadiene (11.17%) was also found in significant concentration and is associated with antimicrobial and anti-inflammatory properties. Other notable constituents included 9,12,15-octadecatrienoic acid ethyl ester (6.99%) and hexadecanoic acid ethyl ester (6.79%), both of which exhibit antioxidant and hypocholesterolemic effects. Additionally, squalene (6.44%), a triterpene hydrocarbon with known antioxidant and skin-protective properties, was detected. Sesquiterpenes such as caryophyllene (4.60%) and aromadendrene (4.46%) contribute to the anti-inflammatory and antibacterial potential of the extract. Phytol (2.63%) and linoleic acid ethyl ester (3.59%) were also identified, further enriching the pharmacological profile due to their anticancer and antimicrobial activities.

The following compounds were uniquely found in the bark of *S. tamilnadense;* copaene (1.28%) – antioxidant and anti-inflammatory hexadecane (0.74%) – antimicrobial and insecticidal (Chandrashekar *et al*.,2020) [10] 1H-cycloprop[e]azulen-4-ol, decahydro-1,1,4,7-tetramethyl-, [1aR-(1aα,...)] (2.25%) – antioxidant and anti-inflammatory (Yadav *et al*., 2021 [52],; p-camphorene (0.06%) – antibacterial and analgesic (Karthikeyan *et al*., 2018) [23]; and henicosanal (1.33%) – antibacterial and antioxidant. Monoterpenes such as β-myrcene (3.00%) and β-ocimene (2.88%)—both known for their analgesic, antioxidant, and antimicrobial activities—were also present. Several minor compounds, including γ-muurolene, α-guaiene, 14-hydroxycaryophyllene, dodecane, hexadecane, henicosanal, and various naphthalene derivatives, were identified, contributing to the chemical complexity and potentially synergistic bioactivity of the extract.

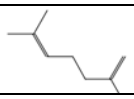
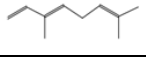
Caryophyllene oxide, the most abundant compound, has been reported to possess antioxidant (Gyrdymova & Rubtsova, 2021) [19], anticancer, analgesic, anti-inflammatory, and antimicrobial properties. Caryophyllene itself is known for its antioxidant, antimicrobial, antitumor, antirheumatic, hypocholesterolemic, hepatoprotective, and cosmetic applications. It has also shown efficacy against fungal infections such as candidiasis and even dengue (Alighiri, 2022) [2].

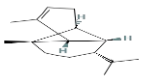
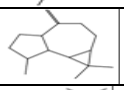
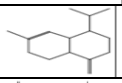
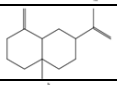
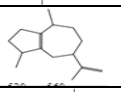
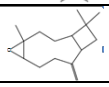
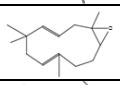
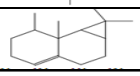
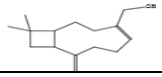
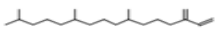
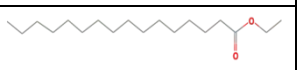
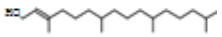
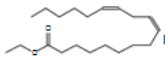
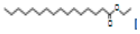
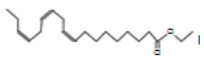
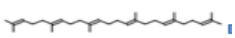
Squalene and phytol are bioactive compounds with a broad spectrum of biological activities, including antioxidant, anti-inflammatory, anticarcinogenic, antidiabetic, hypolipidemic, detoxifying, skin-hydrating, and emollient effects. Other monoterpenes such as β-ocimene, humulene, and β-myrcene have demonstrated antimicrobial, anticancer, and immunostimulant effects. Aromadendrene has been reported to exhibit antimicrobial, anticancer, and antiproliferative properties (de Medeiros *et al*., 2024) [15], while γ-muurolene has shown antioxidant, antiviral, and antiproliferative activity (Sena *et al*., 2024) [41].

Neophytadiene exhibited a broad range of biological activities, including anti-inflammatory, analgesic, antimicrobial, antipyretic, and antioxidant properties (Bhardwaj *et al*., 2020) [6]. Hexadecanoic acid ethyl ester has been linked to anti-inflammatory, anti-arthritic, cancer-preventive, hepatoprotective, and antihistaminic effects (Chukwunweike *et al*., 2024) [12], while linoleic acid ethyl ester is associated with hypocholesterolemic, insect-repellent, anti-acne, 5-alpha-reductase inhibitory, and anti-eczema effects. Copaene, p-camphorene, and henicosanal exhibit a wide range of biological activities such as wound healing, anti-inflammatory, antinociceptive, antiparasitic, trypanocidal, antileishmanial, anticancer, and gastroprotective effects.

GC-MS and HPTLC analysis has also been conducted on species within the same genus (Amitha *et al*., 2018; Mabel Parimala & Antilin Salomi, 2021 [28]; Neshar Uddin *et al*., 2022) [34, 50], as well as on the leaf parts of *S. tamilnadense* (Jayendran *et al*., 2025) [21]. Collectively, the diverse bioactivities reported for these phytocompounds underscore the therapeutic potential of *S. tamilnadense* and provide scientific validation for its traditional medicinal use.

Table 6: Bioactive compounds found in the ethanol extract of *S. tamilnadense* bark

S. NO	R. Time	Name of the Compound	Molecular Formula	Molecular Weight g/mol	Peak Area %	Structure
1	7.266	. beta. -Myrcene	$C_{10}H_{16}$	136	3.00	
2	8.529	. beta. -Ocimene	$C_{10}H_{16}$	136	2.88	

3	11.578	Dodecane	$C_{12}H_{26}$	170	0.44	
4	14.522	Copaene	$C_{15}H_{24}$	204.35	1.28	
5	14.769	Hexadecane	$C_{16}H_{34}$	226.44	0.74	
6	15.200	Caryophyllene	$C_{15}H_{24}$	204	4.60	
7	15.471	Aromandendrene	$C_{15}H_{24}$	204	4.46	
8	15.945	. gamma. -Muurolene	$C_{15}H_{24}$	204	1.61	
9	16.188	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4	$C_{15}H_{24}$	204	1.42	
10	16.275	. alpha. -Guaiene	$C_{15}H_{24}$	204	1.26	
11	16.531	1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene	$C_{15}H_{24}$	204	2.42	
12	17.495	Caryophylleneoxide	$C_{15}H_{24}$	220	14.19	
13	17.848	(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo [9.1.0] dodeca-3,7-diene	$C_{15}H_{24}O$	220	2.11	
14	18.212	1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-	$C_{15}H_{24}$	204	2.12	
15	18.618	14-Hydroxycaryophyllene	$C_{15}H_{24}O$	220	2.93	
16	20.295	Neophytadiene	$C_{20}H_{38}$	278	11.17	
17	20.403	1H-Cycloprop[e]azulen-4-ol, decahydro-1,1,4,7-tetramethyl-,[1aR-(1a.alpha.,	$C_{15}H_{26}O$	222.37	2.52	
18	21.566	p-Camphorene	$C_{20}H_{32}$	272.46	0.06	
19	21.988	Hexadecanoicacid, ethylester	$C_{18}H_{36}O_2$	284	6.79	
20	23.223	Phytol	$C_{20}H_{40}O$	296	2.63	
21	23.680	Linoleicacidethylester	$C_{20}H_{36}O_2$	308	3.59	
22	23.746	9,12,15-Octadecatrienoicacid, ethylester, (Z, Z, Z)-	$C_{20}H_{34}O_2$	306	6.99	
23	23.973	Octadecanoicacid, ethylester	$C_{20}H_{40}O_2$	312	1.04	
24	29.235	Squalene	$C_{30}H_{50}$	410	6.44	
25	29.435	Henicosanal	$C_{21}H_{44}$	296.6	1.33	

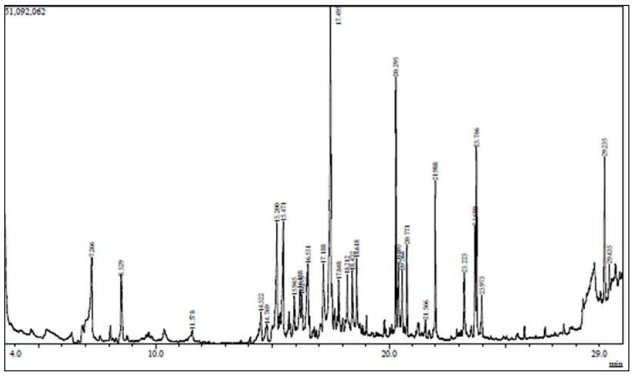


Fig 4: GC-MS Chromatogram of ethanol extract of *Syzygium tamilnadensis* bark

Conclusion

The present study provides comprehensive pharmacognostic, phytochemical, and GC-MS-based profiling of the ethanolic bark extract of Syzygium tamilnadensis, highlighting its rich composition of therapeutically relevant secondary metabolites. The ash values and fluorescence characteristics confirm the sample's purity and the presence of a diverse array of phytoconstituents, reinforcing its quality and standardization potential for herbal drug development.

The extract demonstrated a notable abundance of phenolics, flavonoids, and tannins, aligning with its strong antidiabetic, antioxidant and therapeutic properties. GC-MS analysis revealed the presence of several pharmacologically active compounds, including caryophyllene oxide, neophytadiene, hexadecanoic acid ethyl ester, copaene, hexadecane, p-camphorene, henicosanal, and linoleic acid ethyl ester. These constituents have been previously reported to exhibit a wide range of bioactivities, such as anti-inflammatory, antimicrobial, antioxidant, and anticancer effects.

The phytochemical complexity observed in the chromatographic profile underscores the potential of *S. tamilnadensis* as a promising source of novel bioactive agents. These findings provide a scientific rationale for its traditional medicinal use and support its application in the development of herbal therapeutics and nutraceutical products. Future work should focus on bioassay-guided fractionation, in *vivo* pharmacological evaluations, and clinical validations to fully elucidate the mechanisms of action and therapeutic efficacy of its key constituents.

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Competing interests

The authors declare no competing interests.

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