JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

PHYTOCHEMICAL ANALYSIS AND GC-MS PROFILING OF *SYZYGIUM TAMILNADENSIS*, (RATHKR & CHITRA) -LEAVES

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Abstract

Medicinal plants play a crucial role in pharmacological research, and the pharmaceutical industry relies on them for raw materials. *Syzygium tamilnadensis*, a member of the Myrtaceae family, was the focus of the present study, which aimed to evaluate the phytochemical profile and potential bioactive compounds in the ethanol extract of its leaves using GC-MS analysis. The phytochemical screening of both methanol and ethanol extracts from *S. tamilnadensis* leaves revealed the presence of alkaloids, catechins, flavonoids, glycosides, phenols, saponins, steroids, tannins, terpenoids, sugars, and xanthoproteins. The study also conducted quantitative analysis of total phenolic content, flavonoids, and tannins. GC-MS analysis identified 26 bioactive compounds, including dodecanoic acid, 1,2,3-propanetriyl ester, neophytadiene, caryophyllene oxide, 9,12,15-octadecatrienoic acid ethyl ester (z,z,z), hexadecanoic acid ethyl ester, 14-hydroxycaryophyllene, squalene, aromandendrene, and caryophyllene, as the major compounds. The phytochemical and GC-MS profiling of the ethanol extract of *S. tamilnadensis* leaves indicates the presence of bioactive compounds that likely contribute to the plant's medicinal properties. Therefore, these phytochemicals could be responsible for the therapeutic effects observed.

Keywords: *Syzygium tamilnadensis*, phytochemicals, GC-MS, neophytadiene, caryophyllene.

Introduction

Around 80% of the global population, and over 90% of people in developing countries, rely on herbal medicine for primary healthcare. Medicinal plants have been utilized for centuries to treat a wide range of ailments, with their use dating back to the dawn of human civilization, well before written records. Due to the widespread availability of these plants, their use is also deeply rooted in various cultures. The continued use of these traditional medicines is often based on indigenous knowledge passed down through generations or shared by traditional health practitioners. These practitioners are typically the primary sources of knowledge about which plants can be used to treat or manage specific health conditions. As a result, many medicinal plants are traditionally believed to treat a variety of conditions, including cancer, diabetes, stress, and a host of other infectious and non-infectious diseases (Ozioma and Chinwe, 2019; Mathe *et al*., 2024).

In addition to primary metabolites, medicinal plants also produce secondary metabolites such as alkaloids, terpenes, glycosides, tannins, saponins, steroids, and phenols, which are believed to be responsible for the plants' medicinal properties. There is a growing interest in the scientific community to identify these secondary metabolites and assess their potential in

treating various diseases (Marquardt *et al*., 2020; Mapfumari *et al*., 2022). Gas Chromatography-Mass Spectrometry (GC-MS) has become an essential technique for analyzing the various phytochemicals present in plant extracts and determining their structures. This method offers exceptional separation capabilities, providing highly accurate and precise chemical fingerprints. Additionally, GC-MS provides quantitative data and a mass spectral database, which is invaluable for linking bioactive compounds to their pharmacological applications (Kumar *et al*., 2019).

The genus *Syzygium*, part of the Myrtaceae family, includes 1,200 to 1,800 species distributed across the globe. Species within this genus have been used for a wide range of medicinal purposes by traditional healers from diverse ethnic and cultural backgrounds. Among the most well-documented uses are those in traditional Indian medicine, particularly in Ayurveda. Ayurvedic practitioners utilize various *Syzygium* species for their therapeutic properties, treating conditions such as coughs and colds, diarrhea, dysentery, fever, toothaches, inflammation, pneumonia, sexually transmitted diseases, wounds, hemorrhages, ulcers, and as general tonics (Cock and Cheesman, 2018). In light of this, the present study aims to conduct phytochemical analyses and identify the bioactive compounds in *Syzygium tamilnadensis* Rathkr & Chitra leaves using GC-MS analysis.

Materials and Methods

Collection of plant sample

Fresh leaves of *Syzygium tamilnadensis* Rathkr & Chitra were gathered from Nanjanad village 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 leaves were chopped into small pieces and shade-dried to ensure uniformity and smoothness in the drying process. Once dried, the leaves 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 (Brinda *et al*., 1981; Lala, 1993). The ethanol extract was then used to estimate the total phenolic, flavonoid, and tannin contents, as well as for GC-MS analysis.

Estimation of total phenolics

The total phenolic content was determined using a Folin-Ciocalteau reagent-based assay, as described by McDonald *et al*. (2001), 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 described by Eom *et al*. (2007). A 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}) (Marinava *et al*., 2005; Miean & Mohamed, 2001).

Gas chromatography – mass spectrometry

The GC-MS analysis of the ethanolic extract of *Syzygium tamilnadensis* leaves was conducted using a Perkin-Elmer GC Clarus 500 system, interfaced with a mass spectrometer (GC-MS). The system was equipped with an Elite-I fused silica capillary column
(30 × 0.25 mm ID × 1 μM df), made of 100% dimethyl polysiloxane. For GC-MS detection, an electron ionization system with an ionizing energy of 70 eV was used. Helium (99.999%) served as the carrier gas, maintaining a constant flow rate of 1 mL/min, and a 2 μL injection volume (split ratio of 10:1). The injector temperature was set at 250°C, and the ion-source temperature was 280°C. The oven temperature program began at 110°C (isothermal for 2 minutes), then increased by 10°C per minute until reaching 200°C, followed by a further increase of 5°C per minute to 280°C, and concluded with a 9-minute isothermal period at 280°C. Mass spectra were recorded at 70 eV, with a scan interval of 0.5 seconds and a mass range from 45 to 450 Da. The total GC run time was 36 minutes. The relative percentage of each component was determined by comparing its peak area to the total area. The software TurboMass was used to process the mass spectra and chromatograms.

Result and Discussion

Phytochemical analysis

The distribution of various phytoconstituents in the petroleum ether, benzene, ethyl acetate, methanol, ethanol, and aqueous extracts of *Syzygium tamilnadensis* leaves was evaluated qualitatively, and the results are summarized in Table 1. The ethyl acetate, methanol, and ethanol extracts of *S. tamilnadensis* leaves showed the presence of alkaloids, catechins, flavonoids, glycosides, phenols, saponins, steroids, tannins, terpenoids, sugars, and xanthoproteins. Similarly, the aqueous extract of *S. tamilnadensis* leaves exhibited the presence of catechins, flavonoids, glycosides, phenols, saponins, tannins, sugars, and xanthoproteins. The total phenolic, flavonoid, and tannin contents in the ethanol extract of *S. tamilnadensis* leaves were found to be 268.64 ± 1.21 mg GAE/g, 86.81 mg GAE/g, and 46.14 mg GAE/g, respectively.

Table 1. Phytochemical screening of *S. tamilnadensis* leaf

Bioactive components	Petroleum ether	Benezene	Ethyl acetate	Methanol	Ethanol	Aqueous
Alkaloids	-	-	+	+	+	-
Anthraquinones	-	-	-	-	-	-
Catechins	+	+	+	+	+	+
Coumarins	-	+	-	-	-	-
Flavonoids	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+
Phenols	+	+	+	+	+	+
Quinones	-	-	-	+	+	-
Saponins	-	+	+	+	+	+
Tannins	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	-
Sugars	+	+	+	+	+	+
Xanthoproteins	+	+	-	+	+	+

+ Present - Absent

Alkaloids have a wide range of pharmacological applications, including analgesic, antiasthmatic, anticancer, antihypertensive, antipyretic, and antihyperglycemic effects (Ng *et al*., 2015), as well as anti-inflammatory, antimicrobial, antioxidant, acetylcholinesterase inhibitory, antimalarial, and antidiabetic activities (Aryal *et al*., 2022). Flavonoids are linked to a broad spectrum of health benefits and are essential in various nutraceutical, pharmaceutical, medicinal, and cosmetic products. Their antioxidative, anti-inflammatory, antimutagenic, and anticarcinogenic properties, along with their ability to modulate key cellular enzyme functions, make them highly valuable (Panche *et al*., 2016). Plant phenolics have significant effects on human health, including antioxidant effects, antibacterial activity, cardioprotective benefits, anticancer properties, immune system support, anti-inflammatory effects, and protection against UV radiation (Sun and Shahrajabian, 2023). Recently, tannin compounds have been shown to have several beneficial effects on the gastrointestinal tract, such as antioxidant activity, free radical scavenging, antimicrobial, antiviral, antimutagenic, anticarcinogenic, anthelmintic, hepatoprotective effects, inhibition of harmful pro-oxidative enzymes, and antinutrient properties. Additionally, various tannins have demonstrated chemopreventive effects against cancers of the breast, oral cavity, prostate, stomach, and skin (Hossian *et al*., 2021).

Saponins exhibit a range of biological activities, including anticancer, hepatoprotective, and antioxidant effects, and are involved in the treatment of conditions such as osteoporosis, obesity, and diabetes. However, their precise mode of action remains unclear, and much is still unknown about them (de Hierro *et al*., 2018; Sharma *et al*., 2023). Glycosides have demonstrated impressive health benefits for various ailments, including antibacterial and anticancer properties, as well as anti-inflammatory, cardiovascular, and neurological effects (Riaz *et al*., 2023). The present study indicates that *S. tamilnadensis* leaves are rich in chemodiversity. The presence of these valuable secondary metabolites suggests that the plant holds significant potential for treating a range of human diseases and could serve as a promising source for the development of new therapeutic drugs.

GC-MS analysis

The phytocompounds in *S. tamilnadensis* leaves were analyzed using GC-MS. The chromatogram for the ethanol extract of *S. tamilnadensis* leaves is shown in Figure 1. The identified phytocompounds, along with their retention time (R.T.), molecular formula, molecular weight (MW), and peak area percentage, are listed in Table 2, along with their corresponding chemical structures. The GC-MS analysis revealed the presence of twenty-six phytocompounds in the ethanol extract of *S. tamilnadensis* leaves. The predominant compounds identified were dodecanoic acid, 1,2,3-propanetriyl ester (23.37%), neophytadiene (12.13%), caryophyllene oxide (11.21%), 9,12,15-octadecatrienoic acid, ethyl ester (z,z,z) (6.25%), tetradecanoic acid, ethyl ester (5.13%), 14-hydroxycaryophyllene (4.68%), squalene (4.00%), aromandendrene (3.75%), caryophyllene (3.72%), linoleic acid ethyl ester (3.06%), phytol (2.61%), and beta-ocimene (2.41%).

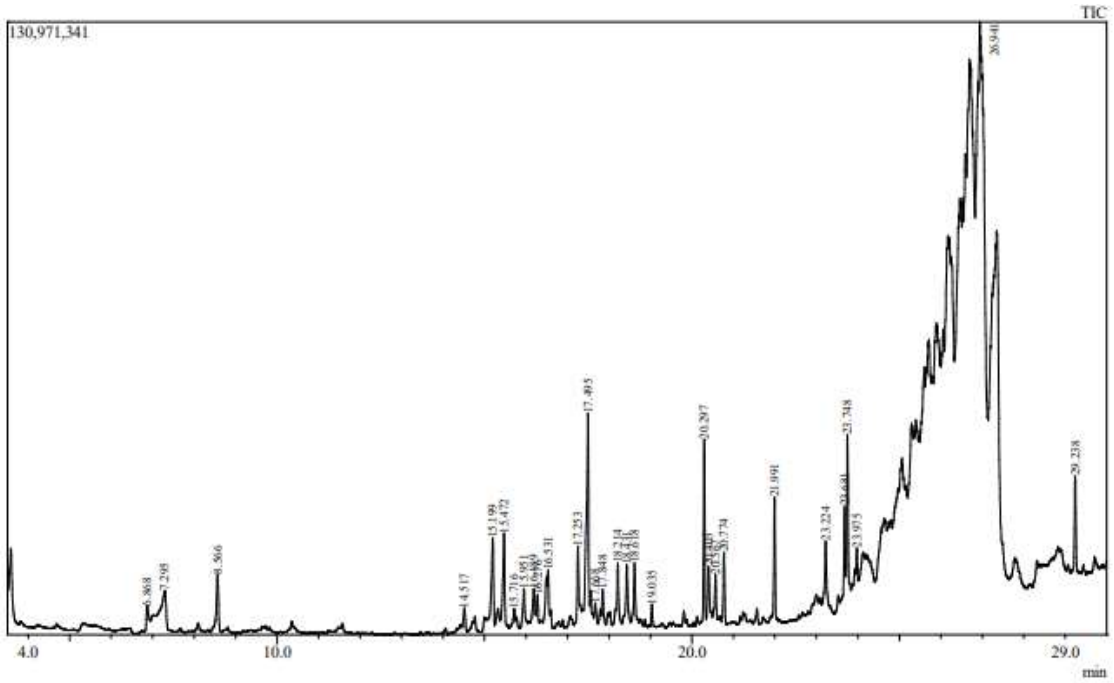
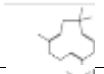
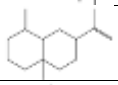
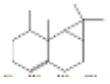
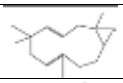
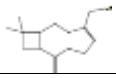
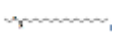
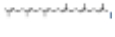


Figure 1: GC-MS Chromatogram of ethanol extract of *Syzygium tamilnadensis* leaf

Among the identified phytocompounds, dodecanoic acid 1,2,3-propanetriyl ester is known for its antioxidant, antibacterial, antiviral, antifungal (candidiasis), hypocholesterolemic, antiarthritic, hepatoprotective, mosquito repellent, and cosmetic properties (Sujatha *et al*., 2020). Neophytadiene displays a range of activities, including anti-inflammatory, antimicrobial, antioxidant, analgesic, and antipyretic effects (Bhardwaj *et al*., 2020; Mustapa *et al*., 2015). Hexadecanoic acid, ethyl ester has been reported to have nematicidal, antihistaminic, anti-eczema, anti-arthritic, anti-inflammatory, cancer-preventive, hepatoprotective, and anti-coronary properties (Karthika and Paulsamy, 2014). Linoleic acid, ethyl ester is associated with hypocholesterolemic, nematicidal, anti-arthritic, antihistaminic, anti-coronary, insect-repellent, hepatoprotective, anti-androgenic, 5-alpha reductase inhibiting, anti-eczema, and anti-acne activities (Sudha *et al*., 2013).

Table 2: Bioactive compounds found in the ethanol extract of *S. tamilnadensis* leaf

S. NO	R. Time	Name of the Compound	Molecular Formula	Molecular Weight	Peak Area %	Structure
1	6.868	1-Triazene,3,3-dimethyl-1-phenyl-	$C_8H_{11}N_3$	**149**	0.16	
2	7.295	.beta.-Myrcene	$C_{10}H_{16}$	**136**	0.55	
3	8.566	.beta.-Ocimene	$C_{10}H_{16}$	**136**	0.43	
4	14.517	Copaene	$C_{15}H_{24}$	**204**	0.13	
5	15.199	Caryophyllene	$C_{15}H_{24}$	**204**	0.74	
6	15.472	Aromandendrene	$C_{15}H_{24}$	**204**	0.56	
7	15.716	Humulene	$C_{15}H_{24}$	**204**	0.12	
8	15.951	.gamma.-Muurolene	$C_{15}H_{24}$	**204**	0.21	
9	16.189	Naphthalene,decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-,[4aR-(4	$C_{15}H_{24}$	**204**	0.12	
10	16.276	alpha.-Guaiene	$C_{15}H_{24}$	**204**	0.13	
11	16.531	1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene	$C_{15}H_{24}$	**204**	0.47	
12	17.253	Caryophyllene oxide	$C_{15}H_{24}O$	**220**	0.30	
13	17.668	1H-Cyclopropa[a]naphthalene,1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramet	$C_{15}H_{24}$	**204**	0.09	
14	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**	0.17	
15	18.214	1H-Benzocycloheptene,2,4a,5,6,7,8,9,9a-octahydro-3,5,5-trimethyl-9-methyle	$C_{15}H_{24}$	**204**	0.27	
16	18.431	14-Hydroxycaryophyllene	$C_{15}H_{24}O$	**220**	0.31	
17	19.035	1-Dodecanol,3,7,11-trimethyl-	$C_{15}H_{32}O$	**228**	0.09	
18	20.297	Neophytadiene	$C_{20}H_{38}$	**278**	0.80	
19	20.403	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{22}H_{42}O_2$	**338**	0.31	
20	21.991	Hexadecanoic acid,ethyl ester	$C_{18}H_{36}O_2$	**284**	0.55	
21	23.224	Phytol	$C_{20}H_{40}O$	**296**	0.35	
22	23.681	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	**308**	0.29	
23	23.748	9,12,15-Octadecatrienoic acid,ethyl ester,(Z,Z,Z)-	$C_{20}H_{36}O_2$	**308**	0.62	
24	23.975	Octadecanoic acid,ethyl ester	$C_{21}H_{38}O_2$	**322**	0.09	
25	26.941	Dodecanoic acid,1,2,3-propanetriyl ester	$C_{39}H_{74}O_6$	**638**	89.23	
26	29.238	Squalene	$C_{30}H_{50}$	**410**	0.48	

Caryophyllene and caryophyllene oxide are known to exhibit analgesic, anti-inflammatory, antioxidant, anticancer, cardioprotective, hepatoprotective, nephroprotective, immunomodulatory, gastroprotective, and antimicrobial activities (Gyrdymova and Rubtsova, 2021). Squalene is a bioactive compound with a variety of biological activities, including antioxidant, anti-inflammatory, anticarcinogenic, antidiabetic, hypolipidemic, detoxifying, skin-hydrating, and emollient properties (Kim and Karadeniz, 2012). 9,12,15-octadecatrienoic acid, ethyl ester (z,z,z), possesses anti-inflammatory, insect-repellent, hypocholesterolemic, cancer-preventive, nematicidal, hepatoprotective, antihistaminic, anti-eczema, anti-acne, 5-alpha reductase inhibiting, anti-androgenic, anti-arthritic, and anti-coronary activities (Sermakkani and Thangapandian, 2012; Guerrero *et al*., 2017). Phytol is recognized for its anticancer, antioxidant, anti-inflammatory, diuretic, antitumor, chemopreventive, and antimicrobial properties (Karthika and Paulsamy, 2014). The medicinal properties of *S. tamilnadensis* leaves may be attributed to the presence of these various bioactive compounds.

In the current study, *Syzygium tamilnadensis* leaves were found to contain a variety of secondary metabolites with numerous pharmacological properties. The GC-MS analysis identified 26 phytocompounds, which contribute to activities such as antimicrobial, antioxidant, anti-inflammatory, anticancer, hypocholesterolemic, anti-arthritic, antiviral, and others. Therefore, the presence of these phytocompounds is likely responsible for the plant's therapeutic effects. Further research is necessary to explore the potential development of novel drugs based on some of the bioactive compounds found in *S. tamilnadensis*.

Acknowledgement

The authors acknowledge the Manonmanium sundaranar university, Tirunelveli for providing GC-MS analysis facility.

Competing interests

The authors declare no competing interests.

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