

Research Article

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Phytochemical screening and GC-MS analysis of *Rosmarinus officinalis* L. leaves

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Abstract

Rosmarinus officinalis L. (rosemary) is an aromatic medicinal plant widely used in traditional medicine and herbal formulations. The present study focuses on the phytochemical screening and gas chromatography-mass spectrometry (GC-MS) analysis of *R. officinalis* leaves to identify major bioactive constituents. Shade-dried leaves were extracted using methanol, ethyl acetate, and aqueous solvents. Preliminary phytochemical screening revealed the presence of tannins, saponins, flavonoids, alkaloids, terpenoids, phenols, and coumarins, with the methanolic extract showing the richest phytochemical profile. GC-MS analysis of the methanolic leaf extract identified several biologically active compounds, including terpenoids, fatty acids, and phenolic derivatives known for antimicrobial, antioxidant, anti-inflammatory, and therapeutic properties. The findings provide scientific evidence supporting the medicinal value of *R. officinalis* leaves and highlight their potential as a natural source of bioactive compounds for pharmaceutical and nutraceutical applications.

Keywords

Rosmarinus officinalis,
Phytochemical screening,
GC-MS analysis,
Medicinal plant

Introduction

Medicinal plants have been used as therapeutic agents since ancient times and remain an important component of traditional and complementary healthcare systems worldwide. The World Health Organization reports that a large proportion of the global population

continues to depend on plant-based medicines for primary healthcare due to their accessibility, affordability, and perceived safety. In recent years, growing concerns regarding the side effects and resistance associated with synthetic drugs have renewed scientific interest in medicinal plants and their bioactive constituents (WHO, 2000). Plants produce a wide array of secondary

metabolites, commonly referred to as phytochemicals, which play a crucial role in plant defense mechanisms against pathogens, herbivores, and environmental stress. These compounds include alkaloids, flavonoids, phenols, terpenoids, tannins, saponins, quinones, and coumarins, many of which are associated with significant biological activities such as antimicrobial, antioxidant, anti-inflammatory, and anticancer effects (Harborne, 1998; Patwardhan et al., 2004). Phytochemical screening is therefore a vital step in evaluating the medicinal potential of plant species and identifying compounds of pharmacological relevance.

The family Lamiaceae is well known for its aromatic and medicinal plants, which are rich in essential oils and phenolic compounds. Members of this family are widely used in traditional medicine, food preservation, and the pharmaceutical industry. *Rosmarinus officinalis* L., commonly known as rosemary, is an evergreen perennial shrub native to the Mediterranean region and cultivated in many parts of the world. The plant is characterized by narrow, aromatic leaves and has long been valued for both culinary and medicinal purposes. Traditionally, rosemary has been used to treat digestive disorders, headaches, muscle pain, rheumatism, inflammatory conditions, and nervous disorders. It has also been associated with memory enhancement and improved circulation. These therapeutic properties have been attributed to the presence of diverse phytochemicals, particularly phenolic diterpenes, monoterpenes, and other volatile compounds present in the leaves (Bakkali et al., 2008). Previous studies have demonstrated that rosemary extracts exhibit antimicrobial, antioxidant, and anti-inflammatory properties, supporting its extensive use in herbal medicine.

Gas chromatography–mass spectrometry (GC–MS) is a powerful and widely used analytical technique for the identification of volatile and semi-volatile compounds in plant extracts. By combining the separation efficiency of gas chromatography with the detection capability of mass spectrometry, GC–MS enables accurate

characterization of complex phytochemical mixtures. This technique is extensively employed in phytochemical research to profile bioactive constituents and to support the quality control and standardization of medicinal plants (Sasidharan et al., 2011). Preliminary phytochemical screening, when used in conjunction with GC–MS analysis, provides comprehensive information on the chemical composition of plant extracts. Such integrated approaches help validate traditional medicinal claims and contribute to the identification of compounds with potential pharmaceutical applications. In this context, the present study aims to investigate the phytochemical constituents of *Rosmarinus officinalis* L. leaves through qualitative phytochemical screening and GC–MS analysis, with an emphasis on identifying major bioactive compounds present in the methanolic extract. The findings are expected to provide scientific evidence supporting the medicinal relevance of rosemary and its potential utilization in herbal and pharmaceutical formulations.

Materials and Methods

Plant Material Collection

Fresh, healthy, and disease-free leaves of *Rosmarinus officinalis* L. were collected during October 2023 from Kanchipuram District, Tamil Nadu, India. The plant material was authenticated based on morphological characteristics. Collected leaves were thoroughly washed with distilled water to remove dust and debris, shade-dried at room temperature, and ground into a fine powder using a sterile pestle and mortar.

Preparation of Leaf Extracts

Approximately 100 g of powdered leaf material was separately extracted with methanol, ethyl acetate, and distilled water using maceration. Each solvent (200 mL) was added to the powdered material in a conical flask, sealed, and kept at room temperature for 24 h with occasional shaking. The extracts were filtered using Whatman No. 1 filter paper, and the filtrates were

concentrated using an air condenser apparatus. The concentrated crude extracts were stored at 4 °C until further analysis.

Preliminary Phytochemical Screening

Qualitative phytochemical screening of the methanolic, ethyl acetate, and aqueous extracts was carried out using standard procedures described by Vogel et al. (1958). The extracts were tested for the presence of carbohydrates, tannins, saponins, flavonoids, alkaloids, quinones, glycosides, cardiac glycosides, terpenoids, phenols, coumarins, steroids, phlobatannins, and anthraquinones using specific reagents and color reactions.

GC-MS Analysis

GC-MS analysis of the methanolic extract was performed using a Shimadzu GC-2010 Plus gas chromatograph coupled with a quadrupole mass spectrometer operating in electron ionization mode. Helium was used as the carrier gas at a constant flow rate of 2 mL/min. A capillary column (EC-5, 15 m × 0.25 mm, 0.25 µm film thickness) was employed for separation.

The oven temperature was programmed to start at 35 °C and held for 2 min, followed by an increase at 20 °C/min to 280 °C and held for 5 min. The injector temperature was maintained at 250 °C. Mass spectra were recorded in the range of m/z 25–1000 with a scan interval of 0.3 s.

Table 1. Qualitative phytochemical constituents of *R. officinalis* leaf extracts

| Phytochemical | Methanol | Ethyl acetate | Aqueous |
|----------------|----------|---------------|---------|
| Carbohydrates | – | – | – |
| Tannins | + | + | + |
| Saponins | + | – | + |
| Flavonoids | + | – | – |
| Alkaloids | + | + | + |
| Quinones | + | + | – |
| Terpenoids | + | + | + |
| Phenols | + | + | + |
| Coumarins | + | + | + |
| Steroids | – | – | + |
| Anthraquinones | – | – | – |

Identification of compounds was carried out by comparing the obtained mass spectra with those available in the National Institute of Standards and Technology (NIST) mass spectral library.

Results

Preliminary Phytochemical Screening

Qualitative phytochemical screening of *Rosmarinus officinalis* L. leaf extracts revealed the presence of several important secondary metabolites. Among the three solvents tested, the methanolic extract exhibited the richest phytochemical profile, followed by ethyl acetate and aqueous extracts. The methanolic extract tested positive for tannins, saponins, flavonoids, alkaloids, quinones, terpenoids, phenols, and coumarins. In contrast, the ethyl acetate extract showed the presence of tannins, alkaloids, quinones, terpenoids, phenols, and coumarins but lacked flavonoids and saponins. The aqueous extract contained tannins, saponins, alkaloids, terpenoids, phenols, coumarins, and steroids, while flavonoids and quinones were absent. Carbohydrates, glycosides, phlobatannins, and anthraquinones were not detected in any of the extracts. The consistent detection of phenols and terpenoids across all extracts indicates that these compounds are major constituents of *R. officinalis* leaves. The qualitative phytochemical profile is summarized in Table 1.

GC-MS Analysis of Methanolic Leaf Extract

GC-MS analysis of the methanolic extract of *Rosmarinus officinalis* leaves resulted in the identification of multiple volatile and semi-volatile compounds with diverse chemical structures. A total of eleven major compounds were detected based on their retention times and mass spectral data, which were matched with the NIST mass spectral library. The identified compounds included terpenoids, fatty acids, alcohols, halogenated hydrocarbons, ketones, and heterocyclic compounds. The earliest eluting compound was dimethyl sulfoxide, detected at a retention time of 4.505 minutes. This was followed by the detection of 2-oxabicyclo[2.2.2]octane, 1,3,3-trimethyl- at 7.945 minutes. Terpineol, a monoterpane alcohol, was identified at a retention time of 10.676 minutes, indicating the presence of volatile aromatic constituents in the extract.

At a retention time of 15.684 minutes, cubenol, a sesquiterpenoid alcohol, was detected. Hexadecanoic acid, a saturated fatty acid, appeared at 20.403 minutes, representing one of the major lipid-derived components of the extract. Phytol, a diterpene alcohol, was identified at 22.034 minutes, followed by the detection of 1,3-dioxane at 22.470 minutes. Ferruginol, a phenolic diterpene, was detected at a retention time of 24.262 minutes. Diazoprogesterone appeared at 25.176 minutes, while 3-methyl-3-(2,5-dimethylphenyl)butanone was identified at 26.300 minutes. The final compound detected was 2-butene, 1,4-dibromo-, eluting at a retention time of 26.900 minutes. The presence of multiple terpenoid derivatives such as terpineol, cubenol, phytol, and ferruginol indicates that terpenoids constitute a major class of compounds in the methanolic leaf extract of *R. officinalis*. The detailed list of compounds identified, along with their retention times, molecular formulae, and reported biological activities, is presented in Table 2.

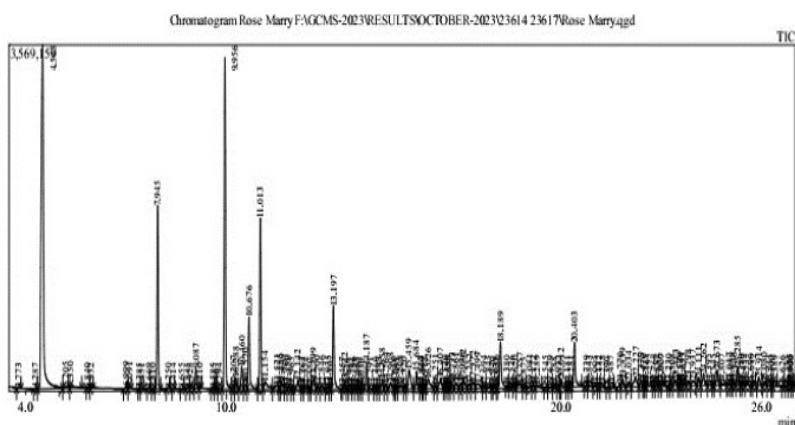


Table 2. Major compounds identified by GC-MS analysis of methanolic extract of *R. officinalis* leaves

| Retention Time (min) | Compound name | Molecular formula |
|----------------------|---------------------------------------------|---------------------------------------------------------------|
| 4.505 | Dimethyl sulfoxide | C ₂ H ₆ OS |
| 7.945 | 2-Oxabicyclo[2.2.2]octane, 1,3,3-trimethyl- | C ₁₀ H ₁₈ O |
| 10.676 | Terpineol | C ₁₀ H ₁₈ O |
| 15.684 | Cubenol | C ₁₅ H ₂₆ O |
| 20.403 | Hexadecanoic acid | C ₁₆ H ₃₂ O ₂ |
| 22.034 | Phytol | C ₂₀ H ₄₀ O |
| 22.470 | 1,3-Dioxane | C ₃ H ₆ O ₂ |
| 24.262 | Ferruginol | C ₂₀ H ₃₀ O |
| 25.176 | Diazoprogesterone | C ₂₁ H ₂₈ N ₂ O ₂ |
| 26.300 | 3-Methyl-3-(2,5-dimethylphenyl)butanone | C ₁₃ H ₁₈ O |
| 26.900 | 2-Butene, 1,4-dibromo- | C ₄ H ₆ Br ₂ |

The presence of these compounds suggests that *R. officinalis* leaves are a rich source of bioactive phytochemicals with diverse therapeutic properties.

Discussion

The present study provides qualitative evidence of the phytochemical richness of *Rosmarinus officinalis* L. leaves through preliminary phytochemical screening and GC-MS analysis. The detection of multiple secondary metabolites, particularly in the methanolic extract, supports the traditional medicinal value of rosemary and aligns with earlier reports on its chemical composition. Methanol proved to be an efficient solvent for extracting diverse phytoconstituents, which may be attributed to its polarity and ability to solubilize phenolic and terpenoid compounds.

Preliminary phytochemical screening revealed the presence of tannins, flavonoids, alkaloids, terpenoids, phenols, saponins, quinones, and coumarins. These classes of compounds are widely recognized for their pharmacological significance. Phenolic compounds and flavonoids are known to play a major role in plant defense mechanisms and are associated with antioxidant and antimicrobial properties (Rice-Evans et al., 1997). Tannins contribute to antimicrobial and anti-inflammatory activities, while alkaloids are well documented for their diverse therapeutic applications, including analgesic and antimicrobial effects (Harborne, 1998). The presence of terpenoids and coumarins further enhances the medicinal relevance of *R. officinalis*, as these compounds are frequently associated with antimicrobial and anti-inflammatory activities (Bakkali et al., 2008).

GC-MS analysis of the methanolic leaf extract enabled the identification of several volatile and semi-volatile bioactive compounds. Among the major constituents identified were terpineol, phytol, hexadecanoic acid, cubenol, and ferruginol. Terpineol is a monoterpenoid alcohol reported to exhibit antimicrobial and antioxidant properties and is commonly found in aromatic

medicinal plants (Santos et al., 2012). Phytol, a diterpene alcohol, has been reported to possess anti-inflammatory, antimicrobial, and anticancer activities, further supporting the therapeutic potential of rosemary leaves (Pejin et al., 2014). Hexadecanoic acid (palmitic acid), identified as one of the major compounds, has been reported to exhibit antioxidant, antimicrobial, and hypcholesterolemic activities (Yff et al., 2002). Ferruginol, a phenolic diterpene commonly reported in *R. officinalis*, has gained attention due to its antibacterial, antifungal, and anticancer properties (Ulubelen et al., 2000). The presence of cubenol, a sesquiterpenoid alcohol, further supports the antimicrobial potential of the extract. The combined presence of these phytochemicals suggests a synergistic effect that may enhance the overall biological activity of *R. officinalis* leaf extracts. The findings of this study corroborate previous reports indicating that rosemary is a valuable source of bioactive secondary metabolites. Thus, phytochemical screening coupled with GC-MS analysis serves as an effective approach for the characterization of medicinal plants and provides a scientific basis for the traditional use of *R. officinalis* in herbal medicine.

Conclusion

The present investigation highlights the phytochemical richness of *Rosmarinus officinalis* L. leaves through qualitative phytochemical screening and GC-MS analysis. Preliminary phytochemical evaluation revealed the presence of important secondary metabolites such as tannins, flavonoids, alkaloids, terpenoids, phenols, and coumarins, particularly in the methanolic extract. GC-MS profiling further confirmed the presence of several bioactive compounds, including terpineol, phytol, hexadecanoic acid, cubenol, and ferruginol, which are reported to possess diverse biological activities. The combined presence of these phytoconstituents supports the traditional medicinal use of rosemary and emphasizes its potential as a valuable source of natural bioactive compounds. Overall, this study provides a

scientific basis for the utilization of *R. officinalis* leaves in herbal and pharmaceutical applications and encourages further studies for isolation and functional validation of individual compounds.

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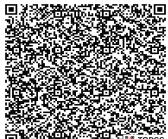
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