

RESEARCH ARTICLE

GC-MS profile of phytoconstituents from leaves of *Avicennia marina* (Forssk.) Vierh.

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Article No.: SAJBR173; Received: 16.07.2025; Peer-reviewed: 2.9.2025; Accepted: 15.09.2025; Published: 30.09.2025

Doi: <https://doi.org/10.5281/zenodo.18662471>

Abstract

Avicennia marina leaf extracts were used in the current work to evaluate their phytoconstituents. The leaves were gathered from their natural habitats, washed, let to air dry, and then grounded. Using acetone, benzene, chloroform, distilled water, hexane, and methanol, the solvent extracts of leaves were made using Soxhlet apparatus. According to established protocols, both qualitative and quantitative phytochemical analyses were performed on the extracts. Utilizing GC-MS technology, an analysis using gas chromatography and mass spectrometry was carried out. In accordance with the findings, all six extracts included alkaloids, flavonoids, phenols, saponins, and steroids but lacked tannins and reducing sugars. Methanol and hexane extracts of *A. marina* leaf yielded the highest number of phytochemicals (eight), whereas chloroform and distilled water extracts yielded the lowest number (six). Quantitative analysis results showed that methanol extracts had the highest concentration of terpenoids (3.763%), alkaloids (3.841%), flavonoids (1.963 mg QE/g), phenols (1.764 mg GAE/g), tannins (0.098 mg TAE/g), and flavonoids (1.963 mg QE/g). Six peaks representing the identification of six chemicals were found after the GC-MS analysis of the methanolic leaf extract of *A. marina*. The findings of this study support the need for more clinical research to ascertain a specific phytochemical's potential in vivo efficacy.

Keywords: *Avicennia marina*; GC-MS analysis; Leaf extracts; Phytochemical screening.

1. Introduction

The natural bioactive substances present in plants are known as phytoconstituents. These phytoconstituents function as an integral part of the body's defensive mechanism against a variety of diseases and stress-related ailments when combined with nutrients and fibres (Saravanan et al., 2015). As this methodology has proven to be an effective way to analyse phytoconstituents, GC-MS investigations have been used more frequently in recent years for the research of medicinal plants (Praveen Kumar et al., 2010).

Avicennia marina is a species of mangrove tree that belonging to the Acanthaceae plant family (formerly the Verbenaceae or Avicenniaceae) (APG IV, 2016). It grows in the intertidal regions of estuary habitats. It was categorized as Least Concern species as per IUCN 3.1 (Duke et al., 2010). The paste made from the leaves of *A. marina* is prescribed for the treatment of leprosy and skin eruption, which is most commonly followed medicinal treatment in many rural areas of Thoothukudi coastal region in Tamil Nadu, India. Hence, the present research work was conducted to screen the phytochemicals found in *A. marina* leaves by GC-MS.

2. Material and method

2.1. Plant sample collection

The healthy leaves of *A. marina* were brought to the lab after being harvested from their wild habitats in the coastal region close to Ratchanyapuram (77.3097° E longitude and 8.2880° N latitude) in Tamil Nadu, India. To achieve a constant weight, the leaves were properly washed with tap water and shade dried at room temperature. In preparation, the air dried samples were pulverized in an electric blender and kept in plastic bags for further examination. According to APG IV classification, the plant was botanically verified and authenticated (APG IV, 2016).

2.2. Preparation of plant extract

Six different solvents viz. acetone, benzene, chloroform, distilled water, hexane, and methanol were used in succession to extract the dry powder material. Using a Soxhlet apparatus, 15 g of the dried and powdered plant material was extracted individually for 6 to 8 hours at a temperature below the boiling point of the solvents in 150 ml of acetone, benzene, chloroform, distilled water, hexane, and methanol. The acquired crude extracts were purified using Whatman No. 1 filter paper before being concentrated using a rotary evaporator at 40° C while under vacuum, and they were then kept in storage at 4° C for subsequent use.

2.3. Qualitative phytochemical analysis

In order to ascertain the presence of various phytochemicals like alkaloids, flavonoids, glycosides, phenols, quinones, reducing sugars, saponins, steroids, tannins, terpenoids, and triterpenoids, preliminary phytochemical analyses of the leaves of *A. marina* were conducted as per standard procedures (Trease and Evans, 1996; Brain and Turner, 1975; Harborne, 1998; Mukherjee, 2010).

2.4. Quantitative phytochemical analysis

The content of alkaloids, flavonoids, phenols, tannins and terpenoids were determined as per the methodology of Harborne (1998) and the results were expressed as percentage (%) for alkaloids, mg Quercetin Equivalent (QE)/g for flavonoids, mg Gallic Acid Equivalent (GAC)/g for phenols, mg Tannic Acid Equivalent (TAC)/g for tannins and percentage (%) for terpenoids.

2.5. Gas Chromatography–Mass Spectrometry (GC-MS) analysis

Shimadzu GCMS-QP 2010 ultra-gas chromatographic equipment was used to perform GC-MS about analysis. The capillary column (BPX5: 5% phenyl, 95% methyl polysilphenylene, 30 m 0.25 mm 0.25 mm) was used to analyze the methanolic leaf extract of *A. marina*. The column temperature program was set at 50°C (0 min) with an

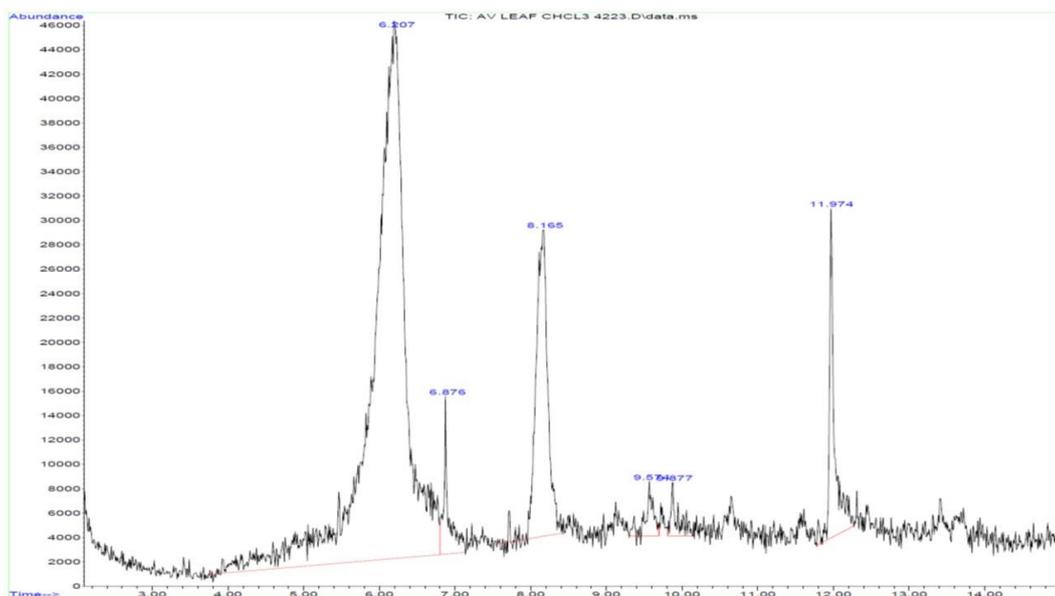


Figure 1. Mass spectrum of methanolic leaf extract of *A. marina* by GC-MS.

Table 1. Qualitative phytochemical screening of *A. marina* leaf.

Phytoconstituents	Solvent extracts					
	Acetone	Benzene	Chloroform	Distilled water	Hexane	Methanol
Alkaloids	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Glycosides	-	+	-	-	-	+
Phenols	+	+	+	+	+	+
Quinones	-	+	-	+	+	-
Reducing sugars	-	-	-	-	-	-
Saponins	+	+	+	+	+	+
Steroids	+	+	+	+	+	+
Tannins	-	-	-	-	-	-
Terpenoids	+	-	+	-	+	+
Triterpenoids	+	-	-	-	+	+

Table 2. Quantitative phytochemical screening of *A. marina* leaf.

Phytoconstituents	Solvent extracts			
	Acetone	Benzene	Hexane	Methanol
Alkaloids (%)	3.336	3.703	3.560	3.841
Flavonoids (mg qe/g)	0.786	1.178	1.963	0.928
Phenols (mg gae/g)	1.176	1.138	1.029	1.764
Tannins (mg tae/g)	0.079	0.086	0.067	0.098
Terpenoids (%)	3.643	3.456	3.676	3.763

Table 3. GC-MS profile of methanolic leaf extract of *A. marina*.

Peak No.	Retention Time (min.)	Area %	Compound Name	Molecular Formula	Molecular weight (g/mol)
1	6.210	72.17	6-Octadecenoic acid	C ₁₈ H ₃₄ O	282.46
2	6.881	2.94	Oxirane, [[4-(1,1-dimethylethyl)phenoxy]methyl]-	C ₁₃ H ₁₈ O	206.28
3	8.167	14.96	Octadecanoic acid	C ₁₈ H ₃₆ O	284.48
4	9.576	1.64	1-Docosene	C ₂₂ H ₄₄	308.59
5	9.878	1.38	9-Eicosene, (E)-	C ₂₀ H ₄₀	280.53
6	11.977	6.91	Tridecanoic acid	C ₁₃ H ₂₆ O ₂	214.34

increase rate of 3°C/min to 300°C (10 min), and the injector temperature was set at 250°C. With a flow rate of 0.8 mL/min, pure helium gas (99.999%) was used as the carrier gas with the split ratio of about 1:10. Electron ionization (EI) and 70eV were utilized as the ion source and ionization voltage, respectively. The detecting voltage was set at 0.87kV and the ion source temperature at 200°C. The solvent cut-off duration was 2.0 minutes, and the interface temperature was 250°C. The start and end times were set at 2.5 and 93 minutes respectively. For the interpretation of the mass spectrum of GC-MS, the National Institute of Standards and Technology (NIST) database, which contains more than 62,000 patterns, was employed. A comparison between the mass spectra of the unknown and known components recorded in the NIST library was made (NIST, 2011).

3. Result

3.1. Qualitative phytochemical screening of *A. marina* leaves

According to the results of the qualitative phytochemical screening of *A. marina* leaf, acetone extract contained alkaloids, flavonoids, phenols, saponins, steroids, terpenoids, and triterpenoids, while benzene extract contained alkaloids, flavonoids, glycosides, phenols, quinones, saponins, and terpenoids, while chloroform extract contained alkaloids, flavonoids, phenols, saponins and steroids. The hexane extract simultaneously contained alkaloids, flavonoids, phenols, quinones, saponins, steroids, terpenoids, and triterpenoids, whereas the methanol extract also contained alkaloids, flavonoids, glycosides, phenols, saponins, steroids, terpenoids, and triterpenoids. Alkaloids, flavonoids, phenols, saponins, and steroids were present in all six extracts, while reducing sugars and tannins were absent (Table 1). In methanol and hexane extracts of *A. marina* leaf, the greatest number of phytochemicals (eight) were found, and the fewest (six) in chloroform and distilled water extracts (Table 1).

3.2. Quantitative phytochemical screening of *A. marina* leaves

Four different solvents (acetone, benzene, hexane, and methanol) were chosen for quantitative analysis because they showed the greatest concentration of phytoconstituents in *A. marina* leaf. The findings of quantitative phytochemical studies showed that the methanol leaf extract of *A. marina* had the highest alkaloid content (3.841%), followed by the benzene extract (3.703%), and the acetone extract (2.633%). In terms of flavonoids, hexane leaf extract had the maximum concentration (1.963 mg QE/g), whereas acetone extract had the lowest (0.786 mg QE/g). The results showed that the phenol concentration of the extracts was highest in methanol (1.764 mg GAE/g) and lowest in hexane (1.029 mg GAE/g). The results of this investigation also showed that the maximal tannin content of methanolic extract was found to be around 0.098 mg TAE/g. Next to this, 0.086 mg TAE/g and 0.067 mg TAE/g of TAE, respectively, were found in benzene and hexane extracts, respectively. Terpenoid content was discovered to be present in methanol extract at a level of 3.763%, which was higher than that observed in other solvent extracts (Table 2).

3.3. GC-MS analysis of *A. marina* leaves

As maximum number and quantity of compounds were reported in methanol extract, the same was subjected for GC-MS analysis for present study. Six peaks were found in the GC-MS analysis of the methanolic leaf extract of *A. marina* (Figure 1) with the identification of 6 compounds. Table 3 lists the active principles' names along with their retention time, area percentage, molecular formula, and molecular weight (g/mol). Two substances, 6-Octadecenoic acid and Octadecanoic acid, were discovered to be the primary components in this fraction, with peak areas of 72.17% and 14.96%, respectively. Four other minor components were also found, including Tridecanoic acid (6.91%), Oxirane, [[4-(1,1-dimethylethyl)phenoxy]methyl]- (2.94%), 1-Docosene (1.64%) and 9-Eicosene, (E)- (1.38). These 6 compounds were detected at the retention time of 6.210, 8.167, 11.977, 6.881, 9.576 and 9.878 min. respectively (Table 3).

4. Discussion

From the findings of qualitative phytochemical analyses, it was clearly known that most of phytoconstituents were detected in

methanol extract than that of other solvent extracts subjected for present study. This can be attributable to the higher solubility of the phytochemicals of plant material in methanol than other solvents. Also, the recovery of phytochemical from plant sample could be influenced by dielectric constant, chemical structure of solvents used, and as well as chemical properties of phytochemicals (Felhi et al., 2017).

A. marina leaf extracts were also subjected to a quantitative phytochemical screening, which revealed that the extracts included their own unique phytoconstituents and that these phytochemicals had a variety of significant biological functions. According to studies by Cushnie et al. (2014), Raymond et al. (2010), and Qiu et al. (2014), alkaloids contain pharmacological properties such as antibacterial, antiarrhythmic, analgesic, and antihyperglycemic properties. Alpha-glucosidase activity, antioxidant activity, and anti-inflammatory activity of flavonoids were recognized to exist (Geng et al., 2007; Gil et al., 1999; Panthong et al., 1994). The anti-inflammatory, antibacterial, and antioxidant properties of phenolic compounds have also been well shown (Naczek and Shahidi, 2006; Giftson et al., 2010; Deng et al., 2015; Popa, 2015; Tanase et al., 2018). Tannins have a variety of physiological actions that have been noted, including anti-irritant and antiparasitic properties (Naveen Prasad et al., 2008). According to Wagner and Elmadfa (2003) and Rabbit and Bishayee (2009), terpenoids have been shown to have antimicrobial, anti-parasitic, antiviral, anti-allergenic, antispasmodic, anti-hyperglycemic, anti-inflammatory, and immunomodulatory properties that can be used in the treatment and prevention of a variety of diseases, including cancer. As *A. marina* leaves included the aforementioned biologically significant phytochemicals identified by the current investigation, all these research findings support the therapeutic uses of *A. marina* leaves.

According to earlier reports, each of the six substances isolated from *A. marina* leaves by GC-MS has significant biological activity. According to Rahman et al. (2014), 6-octadecenoic acid, methyl ester, for instance, possesses antibacterial and antioxidant effects. These include anti-microbial (Marco-Contelles et al., 2004), anti-cancer, and anti-tumor activity (Misra et al., 2008) properties of oxiranes. Octadecanoic acid possesses antibacterial properties (Gehan et al., 2009). According to Sahar and Aida (2018), 1-docosene has antimicrobial qualities. 9-Eicosene, (E)- has cytotoxic and antimicrobial efficacies (Sunita et al., 2017). Tridecanoic acid (TDA) exhibits a wide range of biological effects, including those against helminths, inflammation, bacteria, and cancer (Chowdhury et al., 2021). From the above research findings, it was clearly realized that the phytochemical compounds identified in *Avicennia marina* leaves by the present study have their own medicinal merit and biological significance.

5. Conclusion

The results of current research work highlighted that the *Avicennia marina* leaves make great attention on their use as therapeutic agent for treating various ailments and on other pharmacognostic activities. The examination of other biologically active potentials, such as the antibacterial, anti-diabetic, and anti-inflammatory actions of *Avicennia marina* leaf in vitro, also requires more study. In order to isolate and identify the specific chemical responsible for the desired pharmacological activity, large-scale isolation and spectral approaches are also needed in this case. Animal models should be used in the in vivo investigations to describe the mechanism of action of these chemicals in living systems.

Contribution of Authors

The first (AS) and third author (SV) have performed in data collection, material preparation and data collection. The second author (SS) designed the concept of the study, supervised the research work and finalized the manuscript. The fourth author (SS) has analyzed the data and support the drafting the manuscript.

Conflict of Interests

The authors declare that there is no conflict of interest.

