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Phytochemical Screening, Antibacterial Evaluation and Antioxidant Activity of Ethyl Acetate Extract of Leaves of *Avicennia marina*

Vasantha Kumar K.[1], Jayaseelan K.[1]*, Murugesan R.[2] and Dinesh Kumar G.[3]

[1]PG and Research Department of Zoology, A.V.V.M. Sri Pushpam College (Autonomous) (Affiliated to Bharathidasan University, Trichy), Thanjavur, Tamil Nadu, India
[2]Department of Zoology, Annai Vailankanni Arts and Science College (Affiliated to Bharathidasan University, Trichy), Bishop Sundaram Campus, Thanjavur 07, Tamil Nadu, India
[3]Department of Zoology, Sri Vidya Mandir Arts and Science College, Katteri, Uthangarai, Salem, Tamil Nadu, India

**Corresponding Author*

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Abstract: Extracting natural compounds from mangroves is common practise, with many of these compounds finding applications in traditional medicine and the pharmaceutical industry. The plant *Avicennia marina* (Forssk.) has been used for centuries in conventional and alternative medicine. *A. marina* is a member of the family Acanthaceae, which consists of evergreen trees. The Indo-West Pacific is home to more of this species of mangrove than any other tropical or subtropical region on Earth. Recent research supports the use of *A. marina* for its medicinal effects. The plant's pharmacological effects are attributed to the presence of a wide variety of phytochemical classes. An initial phytochemical screen of *A. marina's* ethyl acetate leaf extract revealed the presence of alkaloid, flavonoids, cardiac glycosides, steroids, terpenoids, tannins, and anthraquinones, but no protein or saponins. Treatment for 48 h at 800 ppm resulted in 93.567% antioxidant activity, and experimentation over 24 h at the same concentration yielded a rate of 93.234% antioxidant activity. The 48 h experiment with the lowest antioxidant treatment recorded a value of 14.267%. The *A. marina* tree may live for hundreds of years and thrive in a wide range of climates. It includes a collection of promising phytochemicals that, after further investigation, may be exploited in the development of new medicines. Scientists need to invest time and resources into researching these renewable and natural medication sources and developing therapeutic formulations in order to address today's difficult-to-treat health concerns and combat the drug-resistant era.

Keywords: *Avicennia marina*, Phytochemicals, Bacteria, Antioxidant, Mangroves

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Introduction

Many people are abandoning synthetics in favor of natural environments because they feel safer there. In order to alleviate the negative effects of synthetic medications, herbal remedies are the only option (Murugesan *et al.*, 2022). The green mangrove, *Avicennia marina* (Forsk.) is primarily found in tropical climate regions. It does, however, have distribution in several temperate regions of the world. *A. marina* as a small evergreen tree growing up to 10 m tall and commonly known as gray or white marsh plant (Karami *et al.,* 2012). *A. marina* is highly resistant to environmental challenges and can grow in unfavourable environments such as high salinity, strong winds, anaerobic soil and high temperature (Das *et al.,* 2016). *A. marina* is a salt marsh that is twice as salty as seawater (Moore *et al.,* 2015). *A. marina* is the only species with well-developed morphological, ecological, biological and physiological adaptations to changing environmental conditions. Due to the unique adaptation and history of such plants, learning about the possibilities of developing new drugs and standardized products to deal with contemporary health issues such as drug-resistant diseases, are made possible. These types of medicinal plants are alternate against chemical pesticides (Murugesan *et al.,* 2021).

Medicinal herbs have been used by people in this world for thousands of years. Similarly, *A. marina* has long played an important role in folk medicine. Ulcers, burns and rheumatism have all been treated with the leaves of the plant (Nabila *et al.,* 2019). It has long been used to relieve joint pain (Shafi *et al.,* 2013). It is used to treat snakebite and smallpox around the world. Fish stings, ringworms, skin lesions boil and scabies have been treated with leaves, bark and fruits (Libecite and Rao, 2006; Ju *et al.,* 2009).

It is used for contraception by inducing an endocrine function (Duke, 1983; Bandaranaike, 1998). The juice of the seeds is also used to treat sore throats. Furthermore, leaf tincture is widely used to treat food poisoning (Bandaranaike, 1998). The fruits of *A. marina* are traditionally used to treat digestive problems such as constipation. A paste made from the leaves and roots is used to heal wounds (Qasim *et al.,* 2020). Many rural populations have traditionally relied on *A. marina* for wood and non-wood goods such as charcoal, fish traps, timber, fuelwood, house poles and boats (Rasolofo, 1997). The leaves and seeds of *A. marina* are used as animal feed for goats, camels and sheep along the Red Sea coast and in the Gulf of Aden.

Many active compounds in the plant have been proven to have major pharmacological actions (Okla *et al.,* 2019). According to Osman and Abkar (2016), *A. marina* contains a wide range of phytochemicals, including carbohydrates, carotenoids, hydrocarbons, alkaloids, aliphatic alcohols, esters, free fatty acids, phenolics, steroids, glycosides, triterpenes, tannins, acids and amino acids (Bandaranayake, 2002; Sun *et al.,* 2008; Li *et al.,* 2010).

The presence of flavonoids, phenols and tannins in *A. marina* has been linked to its anti-diabetic benefits. Through an anti-hyperglycemic effect, these chemicals cause the pancreas to increase insulin output (Babu, 2014). Antiglycation action was also seen in Stigmasterol-3-O-D galactopyranoside (Mahera *et al.,* 2011). Antimicrobial activity was observed against *Staphylococcus aureus*, *Mycobacteria* species and *Candida albicans* using stenocarproquinone B, avicennones A, C, E and F isolated from the twigs of *A. marina* (Han *et al.,* 2007).

A. marina leaf extracts has shown antibacterial activity against *Staphylococcus aureus, Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Devi *et al.,* 2012). Antibacterial activity of *A. marina* leaf extracts is made evident through studies conducted in India, Pakistan, Indonesia and Sri Lanka against *A. tumefaciens*, *P. aeruginosa, Bacillus cereus, B. subtilis, S. mutans* (Sharief *et al.,* 2014).

A. marina was found to have DNA polymerase and antiviral activity. Hepatitis B virus surface

antigen and reverse transcriptase were all inhibited by ethanol leaf extracts. The presence of phenolics, flavonoids and tannins in *A. marina* leaf extract was found to be responsible for its inhibitory effect (Beula *et al.,* 2012). The methanol extract of *A. marina* has strong anthelmintic action, causing worms to be paralysed and die (Moghal *et al.,* 2016).

Khafagi *et al.* (2003) found that aqueous extracts of the plant seedlings' shoots and roots haveantiplasmodial and antimalarial properties. According to studies, the chloroform and hexane extract of *A. marina* can boost male rats' sexual activity in a similar way to the common drug sildenafil (Al-Rehaily *et al.,* 2015). Pyrrolidine has been discovered in the methanol extract of *A. marina* and has been shown to have anti-cholinergic and anti-convulsant properties (Prabhu and Guruvayoorappan, 2012).

The biotechnological industry is interested in medicinal plants, and most pharmaceutical companies rely on plant parts for future manufacturing of pharmaceutical substances. In light of this, the goal of this study was to discover the phytoconstituents found in *Avicennia marina*.

Materials and Methods

Collection and Extraction of Avicennia marina:

Avicennia marina leaves were procured from their natural habitat of Muthupettai mangrove in Thiruvarur district, Tamil Nadu, India and verified by professionals from the Department of Botany, St. Joseph's College, Tiruchirappalli, Tamil Nadu, India. The plant's herbarium number is KVK003. The leaves were washed with distilled water and shade dried, powdered and extracted in ethyl acetate. Plant powder (20 g) was steeped in 100 ml of solvents for 24 h and then shaken. The solvent phase was separated and evaporated after centrifugation at 5000 rpm. The crude was kept at 40º C and used for further research study.

Preliminary Phytochemical Analysis:

The presence of phytochemical screening in ethyl acetate leaf extracts was determined using conventional procedures (Debiyi *et al.,* 1978: Sofowora, 1993: Roopashree *et al.,* 2008).

Antibacterial activity:

The disc diffusion method was used for the antibacterial activity of the ethyl acetate leaf extract of *A. marina*. One loop of both bacterial stock culture medium was sub-cultured on Mueller-Hinton agar and then the Whatman filter paper 6 mm dipped in different types of extract concentrations, were laid on the surface of the bacterial medium. Using sterile distilled water, extract concentrations of 5, 10, 15, 20, 25, 30, 35 and 40 mg/ml were generated. The diameter of the growth inhibition zone was carefully measured using a ruler after all of the culture media had been cultured for 24 h at 37 °C. All of the experiments were carried out three times.

Antioxidant Activity Test:

DPPH (2, 2-diphenyl-1-picrylhydrazyl) technique was used to assess antioxidant activity. When compared to other antioxidant activity assay methods, DPPH methods were easy, simple and required few samples. Mangrove ethyl acetate extracts were made at different concentrations of 40, 100, 200, 400, 600 and 800 ppm. 160 µl of ethyl acetate leaf extract was poured into the wells, along with 40 µl of DPPH solution. 40 µl of ethyl acetate was added to 160 µl of each leaf extract examined as a control. Ethyl acetate was used as a negative control, whereas 20 µl ethyl acetate was used to make the blank. Ascorbic acid was employed as a positive control. After 30 min of incubation at room temperature, the absorbance was measured with a UV Spectrophotometer at 517 nm.

Statistical Analysis:

All data were calculated using Graphed Prism 8.0.1.

Results and Discussion

Phytochemical constituents such as alkaloid, flavonoids, cardiac glycosides, steroids, terpenoids, tannins, anthraquinones, protein and

Table 1: Qualitative phytochemical analysis of ethyl acetate leaf extract of *Avicennia marina*

S. No.	Test	Results
1.	Alkaloids	+
2.	Flavonoids	+
3.	Cardiac glycosides	+
4.	Steroids	+
5.	Terpenoids	+
6.	Tannins	+
7.	Anthraquinones	+
8.	Protein	-
9.	Saponins	-

+ Presents; - Absents

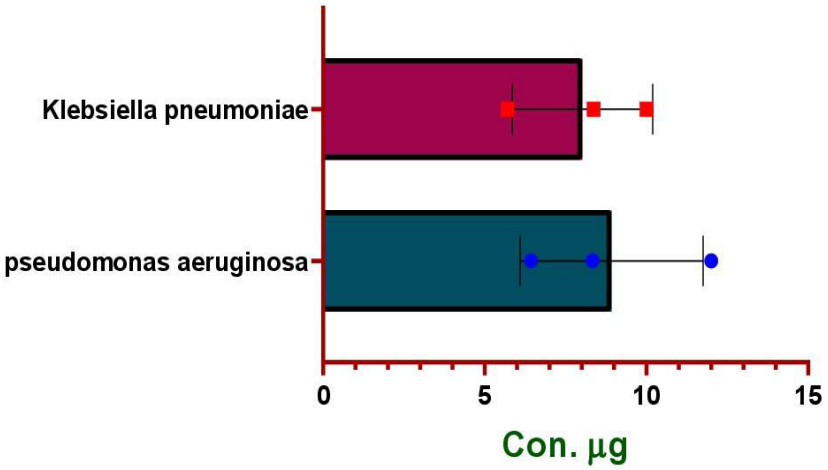

Fig. 1: Antibacterial activity of ethyl acetate leaf extract of *Avicennia marina.*

saponins were tested in *A. marina*. In the preliminary phytochemical analysis alkaloid, flavonoids, cardiac glycosides, steroids, terpenoids, tannins and anthraquinones were present and protein and saponins were absent in ethyl acetate leaf extract of *A. marina* (Table 1).

Antibacterial activity revealed that when the concentration increased, the inhibition zones grew significantly. The antibacterial activity of the ethyl acetate leaves extract of *A. marina* was shown to be considerable against the pathogens tested, with *Pseudomonas aeruginosa* (11.68 mm) showing the greatest growth suppression, followed by *Klebsiella pneumoniae* (9.87 mm). Tambekar reported similar findings, stating that *Dashmula churna* has antibacterial power against *S. epidermidis*, *P. vulgaris*, *S. aureus*, *K. pneumoniae, E. aerogenes*, *S. typhi*, *B. subtilis*, *E. coli,* and *P.*

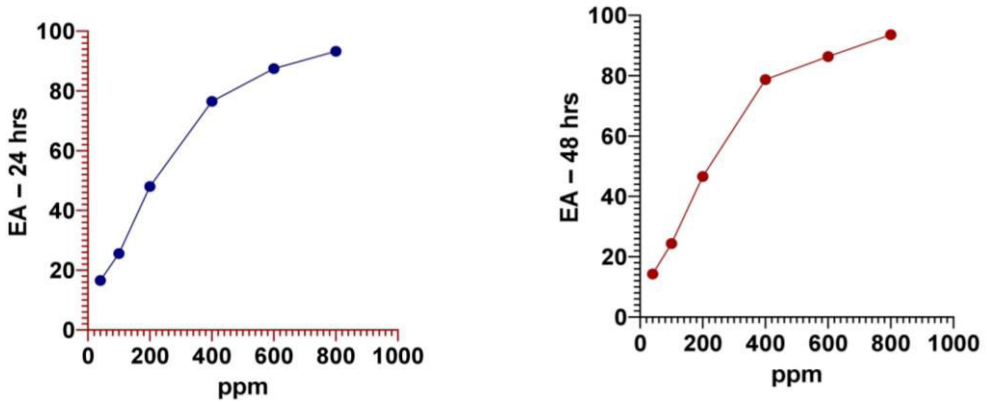

Fig. 2: Repeated analysis of ethyl acetate leaf extract of *Avicennia marina.*

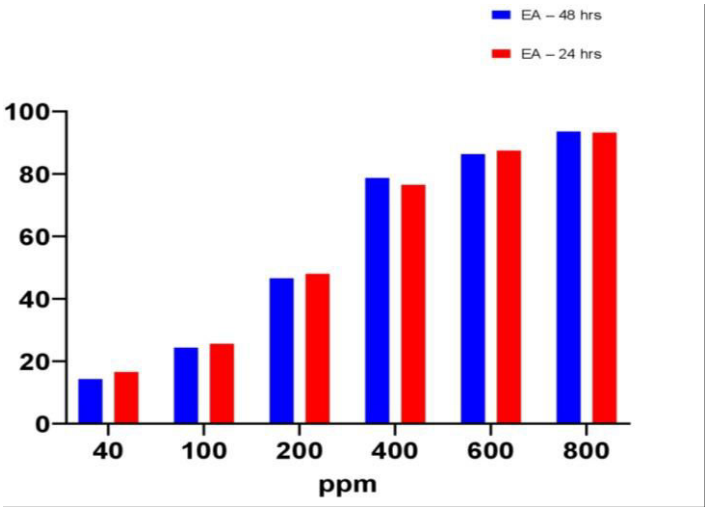

Fig. 3: Antioxidant activity of ethyl acetate leaf extract of *Avicennia marina.*

Table 2: Antioxidant activity of ethyl acetate leaf extract of *Avicennia marina*

Hours	40 ppm	100 ppm	200 ppm	400 ppm	600 ppm	800 ppm
EA – 24 h	16.535	25.5578	47.982	76.485	87.462	93.234
EA – 48 h	14.267	24.347	46.579	78.695	86.325	93.567

aeruginosa, as well as its utility in the treatment of bacterial illnesses (Tambekar *et al.,* 2011).

DPPH (1, 1-Diphenyl-2-picrylhydrazyl) techniques were used to test for antioxidants. DPPH may have filtered antioxidant compounds and interacted with free radicals that had been neutralised. DPPH has the ability to break the free radical chain reaction, allowing it to produce a nonradical molecule (Fig. 2). In terms of scavenging capacity, the findings of the free radical scavenging test revealed that none of the extracts were significantly similar to the positive control. Among the extracts tested, ethyl acetate extracts of mangrove had the greatest scavenging action (Table 2). Compounds derived from plants are an effective and environmentally friendly method for people (Murugesan *et al.*, 2021).

The current investigation found that the extraction solvent used in sample preparation has a significant impact on antioxidant activity. It was

also shown that the antioxidant activities of ethyl acetate extraction solvents differ significantly. The antioxidant activity of a mangrove ethyl acetate extract was higher in a DPPH scavenging assay.

Conclusion

The presence of several bioactive components in *A. marina* justifies traditional practitioners' use of the whole plant for various diseases. Isolation of particular phytochemical compounds and analysis of their biological activity, on the other hand, will almost certainly generate fruitful results. The findings of this work provide a foundation for adopting *A. marina* as herbal alternative for antibacterial agent production. According to the findings, *A. marina* contains a variety of bioactive chemicals. As a result, it is suitable for use as a phyto-pharmaceutical plant.

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