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DEFENSIVE POTENTIAL OF *AVICENNIA MARINA* AGAINST ECONOMICALLY SIGNIFICANT FUNGAL AND BACTERIAL PATHOGENS

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ABSTRACT

Although *Avicennia marina* (Forssk.) Vierh. (*A. marina*) has long been known to have therapeutic benefits, little is known about its bioactive compounds and anti-pathogenic properties. The purpose of this study is to examine the antioxidant components and antimicrobial potential of *A. marina* in order to evaluate its suitability for managing common human and fruit crop pathogens. In Sandspit, Karachi, Pakistan, fresh plant samples were gathered from the mangrove forests. Soxhlet extraction techniques were used in the lab to create ethanolic and methanolic extracts. Important secondary metabolites with antioxidant (DPPH) and nutritional qualities, such as flavonoids, saponins, tannins, terpenoids, carbohydrates, and proteins, were found through phytochemical screening. The disc diffusion method was used to assess the extracts' antimicrobial activity against specific bacterial pathogens (*Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*) and fungal pathogens (*Aspergillus niger*, *Penicillium italicum*, and *Rhizopus stolonifer*). Efficacy was assessed by measuring the zones of inhibition. The findings showed that the tested pathogens were significantly inhibited by both ethanolic and methanolic extracts.

These results imply that, despite being halophytic, *A. marina* has promising antimicrobial qualities that could be used to create medicinal agents for human health and as plant pest bio-control agents, potentially increasing crop yields. In order to better understand the mechanisms underlying the bioactive compounds responsible for the observed activities, future research should concentrate on quantitative phytochemical analyses, such as high-performance liquid chromatography (HPLC).

Keywords: antimicrobial, pathogens, *Avicennia marina,* phytochemicals, antioxidant

1. Introduction

Mangroves are a distinct collection of plants and shrubs that are primarily tropical and thrive in the marine intertidal zone. They have been referred to as tidal forests, mangrove forests, and coastal wetlands (Aziz and Khan, 2000). Mangrove conservation efforts are expanding globally; about 42% of the remaining mangroves are located inside protected areas. However, they may suffer losses due to natural disasters and inadequate encirclement management (Spalding and Leal, 2024).

A. marina (Forssk.) The mangrove tree Vierh. is primarily found in tropical and subtropical regions of the Indo-West-Pacific. It is said to be a well-known example of mangroves whose possible health advantages have been extensively studied (Bandaranayake, 1998). In the Mediterranean regions of Africa and Europe, the Arabian Peninsula, southwestern Asian countries like Pakistan, India, and Afghanistan, and East Asian countries like China and Thailand, halophyte species have long been used as traditional medicines to treat or alleviate the symptoms of both infectious and non-infectious diseases (Ferreira *et al.,* 2022).

A. marina is a great source of several active compounds with important pharmacological effects (Okla *et al*., 2021). Halophyte extracts are rich in phenolic compounds, which have a range of biological characteristics, including anti-inflammatory, anti-cancer, antioxidant, antifungal, and antibacterial qualities (Petropoulos *et al.*, 2018).

Cerri *et al*. (2022) state that because of *A. marina's* exceptional resistance, scientists are currently investigating the plant's potential for pharmacological uses and the development of new drugs (Cerri *et al*., 2022). Additionally, using bioactive compounds found in plants is the only effective strategy to counteract the detrimental consequences of antibiotic resistance in pathogenic microorganisms (Priyatharsini *et al.,* 2015). Mangroves are the second most fertile maritime environment, after coral reefs, according to Dahibhate *et al.* (2019). He added that mangroves contain bioactive substances with good bioactivities, including pesticides, such as steroids, alkaloids, terpenoids, saponins, and tannins.

Flavonoids, alkaloids, terpenoids, tannins, sterols, and saponins from the Thatta region of Pakistan were found in A. marina, according to Mangrio *et al*. (2016) qualitative investigation of phytochemical constituents.

In particular, *A. marina*, the most common species in the Red Sea region, is regarded as one of the richest sources of bioactive compounds (Al-Mur, 2021). Some scientific studies have demonstrated the medicinal properties of *A. marina*. It is believed that the species' pharmacological actions are caused by a variety of bioactive phytochemicals (El Dohaji *et al.*, 2020).

Plant extracts, either directly or through the creation of nanoparticles, have been used in numerous investigations that have found antidiabetic, anti-inflammatory, antioxidant, cytotoxic, and anticancer properties. However, antioxidants have a critical role in preventing human illnesses (EL Haouari *et al.*, 2021).

The DPPH method is one of the most widely used methods for assessing a sample's antioxidant capacity and a compound's potential to function as a hydrogen donor or free radical scavenger (Sulmartiwi *et al.*, 2018).

The pathogenic organisms damage the livelihood and economics of the people living in Pakistan's Karachi district, one of the country's most well-known cities, by causing a variety of diseases in humans as well as in fruit crops. Thus, a bioactive phytochemical found in marina can effectively be used to manage and treat various infectious diseases in the region. Therefore, the purpose of the current study was to assess *A. marina's* antioxidant, qualitative phytochemical screening, and antimicrobial activity against fruit pathogens *P. italicum*, *A. niger*, and *R. stolonifera* as well as economically significant pathogens *S. aureus*, *K. pneumoniae*, and *S. epidermidis*. These mangroves are located on Sindh's coast at Sandspit. The study postulated that because *A. marina* contains beneficial antioxidants to strengthen the human immune system, it may be efficient against certain diseases. By examining these common human and agricultural illnesses, the study seeks to better understand A. marina's potential to enhance human health and agricultural disease prevention. This study emphasises the potential of coastal plants, particularly halophytes, to treat common ailments by highlighting their broader usage in medicine.

MATERIALS AND METHOD

1.1. Collection Site:

In February 2022, samples were taken from the Sandspit backwater, which is roughly eighteen kilometres southwest of Karachi (24° 85' N, 66° 90' E). More than 400 hectares of mangrove swamps, intertidal mud flats, and shallow tidal lagoons make up the Sandspit backwaters. Saltwater reaches the backwaters from the south through Karachi Harbour, while freshwater from the Lyari River enters from the east. The location is amid a mangrove forest that is thought to be reasonably pure and has a higher influx of seawater than the Wetland Centre (Rizwan *et al.*, 2017). The University of Karachi's herbarium number 99742 was assigned to the taxonomically identified *A. marina* specimen utilized in this investigation.

1.2. Plant extract preparation:

A. marina dried leaves were powdered into a fine powder. 25 mL of either 99% ethanol or methanol were combined with 1 g of the powdered material to prepare the extract. For two and a half hours, the mixtures were shaken at 100 rpm at room temperature. After that, the extracts were centrifuged for 15 minutes at 6000–8000 rpm to extract the supernatant. For later examinations, the obtained clear extracts were kept at 4°C. Preliminary experiments showing successful extraction without solvent saturation led to the selection of the concentrations employed (1 g in 25 mL).

Microbes collection:

The University of Karachi's Department of Microbiology provided pathogenic strains such as *Aspergillus niger*, *Penicillium italicum*, *Rhizopus stolonifer*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*. Before testing, these strains were cultivated and kept up to date using normal microbiological procedures.

1.3. Phytochemical Screening

Terpenoids, flavonoids, tannins, saponins, carbohydrates, and proteins were detected in the *A. marina* leaf extracts by qualitative phytochemical screening. Tests were carried out using suitable positive and negative controls in accordance with the approach outlined by Pant *et al*. (2017). In particular:

Ferric chloride test for tannins
Mayer's and Dragendorff's reagent tests for alkaloids
Molisch's test for carbohydrates
Froth test for saponins

Shinoda, alkaline reagent, and zinc hydrochloride tests for flavonoids
Salkowski's test for terpenoids
Biuret assay for proteins

1.4. Antioxidant Assay by DPPH:

The DPPH assay, as reported by Yen and Chen (1995), was used to evaluate the extracts' capacity to scavenge free radicals. In short, 25 mL of ethanol or methanol were mixed with 1 g of plant powder, and the mixture was shaken for 2.5 hours at room temperature. After being made, the DPPH solution (1 M) was refrigerated. A UV-visible spectrophotometer was used to assess absorbance at 517 nm after the extract was centrifuged for 15 minutes at 6000–8000 rpm. The supernatant was then combined with DPPH solution. The percentage of inhibition was computed as follows:
Percent of inhibition = (Control OD − Sample OD/Control OD) × 100)

1.5. Antimicrobial Assay:

The disc diffusion method was used to assess the antibacterial activity of *A. marina* leaf extracts. The plant extracts were diluted in ethanol or methanol at a concentration of 50 mg/mL to impregnate sterile Whatman filter paper discs (5 mm diameter). Standard antibiotic discs were utilized as positive controls, and sterile discs were used as negative controls. Microbial suspensions that were adjusted to the 0.5 McFarland standard were used to create inoculated agar plates. Plates were incubated at 37°C for a full day after discs were placed on the agar surface. The diameter was multiplied by two to get the zone sizes, and the zones of inhibition were measured in millimetres. The results are shown as mean ± standard deviation, and each experiment was run in triplicate.

2. RESULTS

The DPPH radical scavenging test was the main method used to assess the antioxidant ability of *A. marina* extracts. Bioactive substances such as proteins, carbohydrates, terpenoids, saponins, tannins, and flavonoids were identified using phytochemical screening.

With percent inhibition rising from 20.33% at 2 mg/mL to 50.8% at 5 mg/mL, the ethanolic extract demonstrated dose-dependent scavenging activity (Fig. 1, 2). On the other hand, the methanolic extract demonstrated better overall antioxidant activity, with inhibition values ranging from 39.48% at 3 mg/mL to 52.71% at 4 mg/mL. Notably, when it came to scavenging free radicals, the methanolic extract outperformed the ethanolic extract.

Zones of inhibition were measured to evaluate the extracts' antibacterial activity against different bacterial and fungus species. In comparison to the ethanolic extract, which produced a zone of inhibition measuring 15 ± 1.2 mm against *Aspergillus niger*, the methanolic extract produced a zone of inhibition measuring 27 ± 1.1 mm (Table1; Plate 1). The zones for *P. italicum* were 13 ± 1.1 mm for the ethanolic extract and 29 ± 1.06 mm for the methanolic extract. With zones of 6 ± 2.1 mm and 5 ± 1.3 mm for methanol and ethanol extracts, respectively, the efficacy against *Rhizopus stolonifer* was negligible.

Table 1. Zone of inhibition (mm) by applying solvent extracts of ethanol and methanol extract of *A. marina*.

S.NO.	Pathogens	Control E	Ethanol Extract	Control M	Methanol Extract
1.	*K. pneumoniae*	05 ± 1.05	10 ± 1.1	07 ± 1.06	09 ± 1.08
2.	*S. aureaus*	09 ± 1.05	12 ± 1.1	04 ± 1.2	15 ± 1.3
3.	*S. epidermidis*	08 ± 1.07	11 ± 1.2	08 ± 1.06	14 ± 1.9
4.	*A. niger*	09 ± 1.2	15 ± 1.2	17 ± 1.06	27 ± 1.1
5.	*P. italicum*	09 ± 1.05	13 ± 1.1	12 ± 1.2	29 ± 1.06
6.	*R. stolonifer*	02 ± 1.2	05 ± 1.3	01 ± 1.06	06 ± 2.1

Control E= Control Ethanol; Control M=Control Methanol

With zones of inhibition of 15 ± 1.1 mm and 14 ± 1.2 mm, respectively, the methanolic extract demonstrated significant efficacy against *Staphylococcus aureus* and *Staphylococcus epidermidis* in bacterial tests (Plate 2). With zones of inhibition of 15 ± 1.1 mm and 14 ± 1.2 mm, respectively, the methanolic extract demonstrated significant efficacy against *Staphylococcus aureus* and *Staphylococcus epidermidis* in bacterial tests (Table 1; Plate 2). With a zone of 10 ± 1.1 mm, the ethanolic extract showed modest efficacy against *Klebsiella pneumoniae*.

Overall, the findings show that *A. marina* extracts have strong antibacterial and antioxidant properties, particularly those made with methanol. In every test, the methanolic extracts outperformed the ethanolic extracts in terms of efficacy.

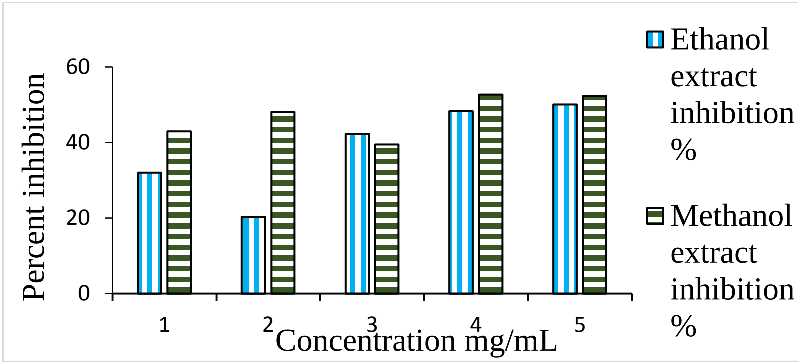

Fig. 1. Percentage inhibition of ethanolic and methanolic extracts of *Avicennia marina* leaves at various concentrations, illustrating their antioxidant activity.

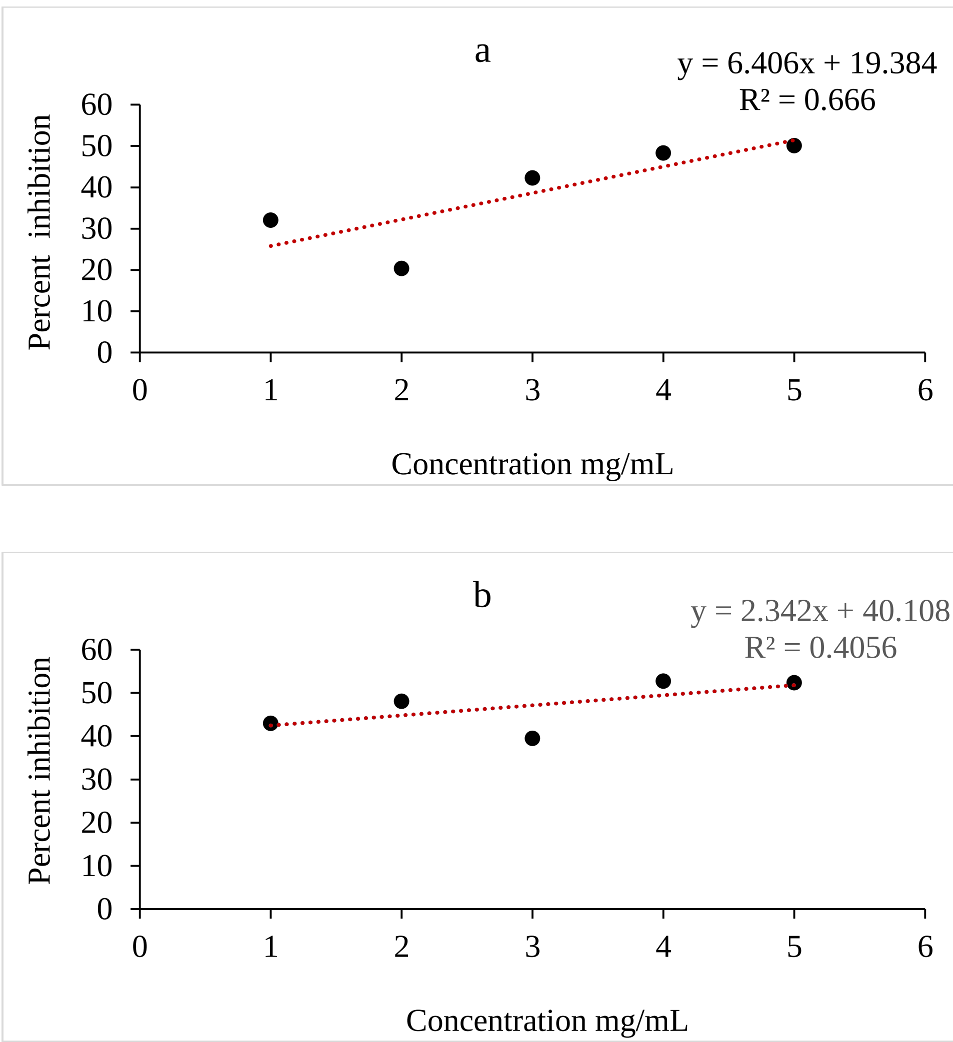

Fig. 2. The percentage inhibition of (a) ethanolic and (b) methanolic extracts of *Avicennia marina* leaves at various concentrations.

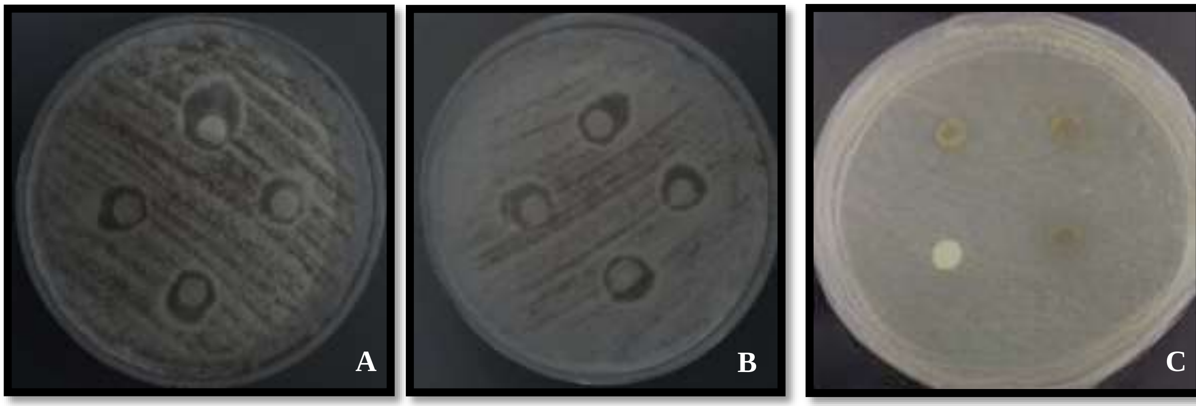

Plate 1. Antifungal activity of methanolic extracts of *Avicennia marina* and other plant extracts against various fungal pathogens: (A) *A. marina* extract against *P. italicum*, (B) against *A. niger*, and (C) *A. marina* extract against *R. stolonifera*. CM= Control Methanol, ME= Methanol Extract of Plant, EE= Ethanol Extract of Plant, CE= Control Ethanol.

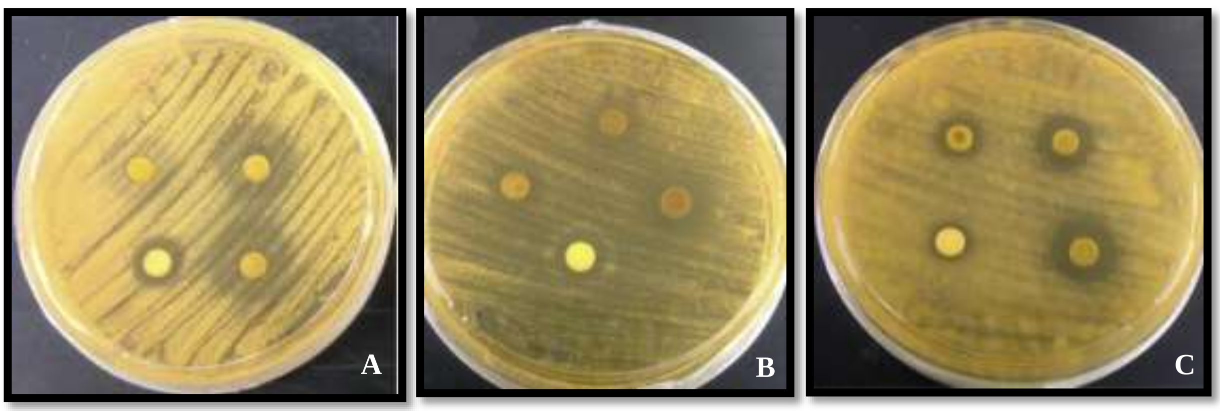

Plate 2. Antibacterial activity of plant extracts against pathogenic bacteria: (A) ethanol extract of *A. marina* against *K. pneumoniae*, (B) against *S. epidermidis*, and (C) methanol extract of *A. marina* against *S. aureus*. CM= Control Methanol, ME= Methanol Extract of Plant, EE= Ethanol Extract of Plant, CE= Control Ethanol.

3. DISCUSSION

Our study's results highlight *A. marina* potential as a useful source of bioactive substances with strong antibacterial and antioxidant qualities. The existence of important bioactive components, such as proteins, carbohydrates, terpenoids, saponins, tannins, and flavonoids, was verified by our qualitative phytochemical screening. The biological activities of these substances are well-documented, especially their functions in preventing microbial infections and oxidative stress (Harborne, 1998).

The DPPH radical scavenging experiment, which measures antioxidant activity, showed that the methanolic extract was more effective than the ethanolic extract. In particular, the ethanolic extract showed less activity, rising from 20.33% at 2 mg/mL to 50.8% at 5 mg/mL, whereas the methanolic extract showed inhibitory percentages ranging from 39.48% at 3 mg/mL to 52.71% at 4 mg/mL. These findings are consistent with earlier research showing that methanol extracts typically contain a wider range of phytochemicals with antioxidant activity (Takarina *et al.*, 2018), suggesting that methanol is a more efficient solvent for extracting antioxidant components from *A. marina*.

Our antimicrobial tests provided additional evidence for bioactivity of *A. marina*. Measuring 27 ± 1.1 mm against *Aspergillus niger* and 29 ± 1.06 mm against *Penicillium italicum*, the methanolic extract showed significant zones of inhibition. The ethanolic extract, on the other hand, created smaller zones, measuring 15 ± 1.2 mm and 13 ± 1.1 mm, respectively. These results support the idea that a greater variety of active phytochemicals are often present in methanol extracts, leading to increased antibacterial activity (Ncube *et al.*, 2008).

Furthermore, the broad-spectrum potential of *A. marina* was confirmed by the antibacterial activity against bacterial strains. The methanolic extract demonstrated noteworthy action against *Klebsiella pneumoniae* with a zone of 10 ± 1.1 mm, and significant inhibitory zones of 15 ± 1.1 mm and 14 ± 1.2 mm against *Staphylococcus aureus* and *Staphylococcus epidermidis*, respectively. These findings indicate that *A. marina* may be a viable natural substitute or supplement in antimicrobial therapy, which is especially pertinent in light of the growing worry over antibiotic resistance (Chan *et al.*, 2021).

All of our findings point to the ecological and therapeutic value of *A. marina*. Mangrove ecosystems are threatened by environmental degradation, therefore protecting these habitats is essential for both ecological balance and preserving their potential as sources of new medicinal agents. With a focus on sustainable collection and use in phytomedicine, more study should examine the particular phytochemicals causing these bioactivities and assess their mechanisms of action.

4. Conclusion

The findings show that *A. marina* leaf extracts have strong antibacterial efficacy against important pathogenic species, underscoring its potential for use in the treatment of infectious illnesses. With the exception of *K. pneumoniae*, where the plant extract exhibited significant resistance, methanolic extracts showed the strongest antibacterial action overall. On the other hand, *S. aureus* was significantly inhibited by the ethanolic extract. Furthermore, the greatest zone of inhibition against *P. italicum* was generated by the methanolic extract. These results imply that *A. marina* may be a useful natural source of antibacterial compounds, especially its methanolic extracts. To identify and describe the active phytochemicals causing these effects and assess their potential for use in therapeutic settings, more investigation is necessary.

Conflicts of Interest

This paper has no known conflicts of interest, and there hasn't been any major funding for the work that could have affected the results.

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