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Original Article

# *In vitro* antifungal and antibacterial potentials of organic extracts of *Avicennia marina* collected from Rabigh Lagoon, Red Sea Coasts in Saudi Arabia

## Potenciais antifúngica e antibacteriana in vitro de extratos orgânicos de *Avicennia marina* coletados da Lagoa Rabigh, Red Sea Coasts na Arábia Saudita

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**Abstract**

Mangrove shrub *Avicennia marina* (Forsk.) Vierh was used to test the antifungal and antibacterial activities of aerial fractions *in vitro*. *Aspergillus sp*, *Candida sp* and Gram positive bacteria have all been found to be sensitive to mangrove extracts, whereas Gram negative bacteria have been found to be resistant to them. Agar disc diffusion and well-cut diffusion were employed to conduct antifungal and antibacterial activities. The MICs (minimum inhibitory concentrations) for each assay have been established. Several extracts from Mangrove reduced fungus growth (diameters fluctuated between 11 and 41 mm). The Ethyl acetate fraction showed particularly strong inhibition of *C. tropicalis*, *C. albicanis*, and *A. fumigatus*. They had 41, 40, and 25 mm-diameter inhibition zones, respectively. Nesoral, a synthetic antifungal medication, showed no significant changes in its MICs compared to different extracts. *Enterococcus faecalis* and *Bacillus subtilis* were inhibited by Petroleum Ether extracts at MICs of 0.78 and 0.35 mg/mL, respectively. It is possible that *A. marina* extracts may be exploited as a viable natural alternative that may be employed in the management of various infections, notably nosocomial bacterial infections, as anti-candidiasis and as anti-aspergillosis agents.

**Keywords:** antifungal, antibacterial, *Avicennia marina* (Forsk.) Vierh., *Aspergillus sp*, *Candida sp*, MIC.

**Resumo**

Arbusto de mangue Avicennia marina (Forsk.) Vierh foi usado para testar as atividades antifúngicas e antibacterianas de frações aéreas in vitro. As bactérias Aspergillus sp, Candida sp e Gram-positivas mostraram-se sensíveis aos extratos de mangue, enquanto as bactérias Gram-negativas mostraram-se resistentes a eles. Difusão em disco de ágar e difusão bem cortada foram empregadas para realizar atividades antifúngicas e antibacterianas. Para cada ensaio foram estabelecidas as CIMs (concentrações inibitórias mínimas). Vários extratos de mangue reduziram o crescimento do fungo (os diâmetros variaram entre 11 e 41 mm). A fração acetato de etila mostrou inibição particularmente forte de C. tropicalis, C. albicanis e A. fumigatus. Eles tinham zonas de inibição de 41, 40 e 25 mm de diâmetro, respectivamente. Nesoral, um medicamento antifúngico sintético, não apresentou alterações significativas em suas CIMs em comparação com diferentes extratos Enterococcus faecalis e Bacillus subtilis foram inibidos por extratos de éter de petróleo em MICs de 0,78 e 0,35 mg/mL, respectivamente. É possível que os extratos de A. marina possam ser explorados como uma alternativa natural viável que pode ser empregada no manejo de várias infecções, notadamente infecções bacterianas nosocomiais, como agentes anti-candidíase e anti-aspergilose.

**Palavras-chave:** antifúngico, antibacteriano, *Avicennia marina* (Forsk.) Vierh., *Aspergillus sp*, *Candida sp*, MIC.

## 1. Introduction

Medical practitioners in the present day are seeking to duplicate herbal treatments' success in treating and preventing infectious diseases. Plants include a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which all display antimicrobial action *in vitro*, as have been established in numerous

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studies on natural medicinal substances (Arshad et al., 2024;Compean and Ynalvez, 2014; Ananthavalli and Karpagam, 2017). A growing number of pharmaceutical corporations and researchers have turned their attention to therapeutic plants in the last few decades. Antibacterial and antifungal capabilities have been shown to be present in these plant-derived chemicals (Silva and Fernandes Júnior, 2010; Chassagne et al., 2021; Nigussie et al., 2021). Natural remedies, diet, health, and culturally distinct folk health practices are all intertwined in Saudi Arabian traditional herbal therapy (Ullah et al., 2020).

Previous research has found that distinct extracts of multiple medicinal plants obtained from various locations around the Kingdom of Saudi Arabia and belonging to different Botanic families exhibit interesting antibacterial and antifungal properties. Among them species from Boraginaceae, Asteraceae, Polygonaceae, Rhamnaceae, Resedaceae, Euphorbiaceae Combretaceae and Acanthaceae (Moni et al., 2023; Aly and Bafeel, 2010; Alamri and Moustafa, 2012; Shahat et al., 2017; Okla et al., 2021).

The Saudi Pharmacopeia makes use of 96 species from 47 families, as reported by (Ullah et al., 2020). Traditional Saudi Arabian medicine usually referred to plants in the *Acanthaceae* family. In the Kingdom of Saudi Arabia, *Avicennia marina* (Forsk.) Vierh., often known as grey mangrove, is a member of the *Acanthaceae* family (Chaudhary and Al-Waṭanīyah, 1999). In tropical and subtropical intertidal zones and coastal areas, it is a tropical plant and a woody shrub that thrives (Asaf et al., 2021).

The enormous range of traditional *Avicennia* products made and used by coastal populations in the Pacific and Asia is widely documented. *A. marina*'s soft leaves, seeds, and seedlings are often consumed as vegetables. As a soap alternative, the ash of *Avicennia* species, which are high in "sodium compounds," is employed in East Asia. It is also used in traditional medicine against rheumatism, small pox and ulcers. In Persian traditional medicine, this herb has also been used to cure a variety of infectious disorders (Field, 1995; Bandaranayake, 1998; Namazi et al., 2013). Although there were no verified traditional medical applications of *A. marina* in Saudi Arabia, it was utilized as a diarrhoea and dysentery treatment in Andhra Pradesh, India (Dahdouh-Guebas et al., 2006). Moreover, *A. marina* fruits have traditionally been used to treat digestive issues such as constipation. The paste made from the leaves and roots was used to cure wounds (Khasim et al., 2020). For instance, previous investigations demonstrated that *A. marina* possesses well-known bioactive activities such as antiviral, anticancer, and antimicrobial properties (Namazi et al., 2013; Gupta and Roy, 2012; Albinhassan et al., 2021). Because of the presence of a wide range of bioactive chemicals, there is a growing interest in mangrove plants for their medicinal properties, particularly the antimicrobial effects (Yompakdee et al., 2012; Bakshi and Chaudhuri, 2014; Patra and Mohanta, 2014; Ibrahim et al., 2022; Saad et al., 2012; Abeysinghe, 2010). Surprisingly antimicrobial properties of *A. marina* from red seacoasts of Saudi Arabia have not been enough investigated (Okla et al., 2021; Afzal et al., 2011; Behbahani et al., 2013).

This research focuses on the antifungal and antibacterial properties of aerial sections of *A. marina* as a means of preserving and valuing Saudi Arabia's natural resources. Antibacterial and antifungal capabilities of new plant extracts are the focus of this study, which aims to identify new medications to prevent the spread of antibiotic resistance.

## 2. Material and Methods

### *2.1. Plant material collection*

*Avicennia marina* (Forsk.) Vierh (family: *Acanthaceae)* was collected during flowering stage in Rabigh (East of Saudi Arabia), near red seacoasts region (22° 47' 55" North, 39° 1' 56" East). Aerial parts were cleaned and dried in the shade with tap water at room temperature. A sterile and airtight container was used to store the fine powder after they had dried.

### *2.2. Plant extract preparation*

100 grams of dry powder were soaked for three days in a series of increasing polarity organic solvents, yielding the following extracts: petroleum ether, ethyl acetate, butanol, and methanol. to filter the resultant solution, membrane filters with a diameter of 1 mm were used. For further processing, the dry extracts were always kept at 4°C in the dark after vacuum evaporation in a rotary evaporator (BUCHI, Germany).

### *2.3. Antifungal activity*

#### *2.3.1. Fungal strains*

*Aspergillia flavus*, *Aspergeria fumigata*, *Aspergillus niger*, *Candida albicans*, and *Candida tropicalis* were obtained from the King Fahd Hospital in Jeddah to conduct this experiment. The fungus was grown on SDA-prepared petri plates, Inoculums were made by injecting a little portion of each fungus into sterile water (10 ml). As much as one ml ($10^6$ cells/ml) of the suspension was used to seed a flask with 50 ml of warm, sterile medium (45°C). After a good shake, the flask was emptied into Petri dishes where the mixture could solidify.

#### *2.3.2. Antifungal assay*

The well-cut diffusion approach was utilized, according to (Balouiri et al., 2016). Wells were drilled into the plate using a cork borer. The plates were incubated at 4°C for two hours with 50 µl of plant extract in each well. It was then incubated for 48 hours at 27°C. The diameter of the growth inhibitory holes was measured in millimetres. The three replicate experiments were used to calculate the means and standard deviations for each experiment.

#### *2.3.3. Determination of Minimal Inhibitory Concentrations (MIC) by using serial broth dilution method*

A minimum inhibitory concentration (MIC) was defined as the lowest concentration of antimicrobials that inhibit any bacterial growth (MIC). The steps in this technique were completed in accordance with (Balouiri et al., 2016). Freshly generated standard cell concentrations of fungus isolates (1.5 x $10^8$ spores/ml) were placed on sterilized plates before being loaded with the extract and serially diluted.

*2.4. Antibacterial activity*

*2.4.1. Bacteria strains*

Gram-negative strains of bacteria including *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 35218 and five Gram-positive strains of bacteria, including *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis CIP 106510, Enterococcus faecalis* ATCC 29212, *Bacillus anthracis* CIP 66.17, and *Bacillus subtilis* CIP 52.62 were used in this study. Glycerol stock was used to preserve strains at -80°C (20%). Before any antimicrobial testing was done, all strains were resuspended three times.

*2.4.2. Antibacterial assay*

*2.4.2.1. Method of disc diffusion*

The antibacterial activity of several *A. marina* extracts was studied using the traditional disc diffusion method (Dhayanithi et al., 2012; Abamecha et al., 2015). Using a DENSIMAT, the bacterial inoculum was calibrated to meet turbidity criteria of 0.5 McFarland (Biomerieux). Using Petri dishes with Muller Hinton agar media, we placed sterile Whatman No. 3 paper discs (6 mm) imbibated with 50 mg/ml extracts. Controls included an antibiotic disc GENTAMICIN® (20 g/disc) and a solvent control, a disc impregnated with DMSO (10%). All the Petri dishes had the plant extract fully diffused after two hours of incubation at 4°C. The plates were incubated at 37°C for 24 hours. The inhibitory zone's millimeter diameter was used to calculate the antibacterial activity (mm).

*2.4.2.2. Minimum inhibitory and bactericidal concentrations (MIC and MBC)*

*Avicennia marina* organic extracts were tested using the serial dilution method with some modifications to find the minimum inhibitory concentration (MIC) (Marroki and Bousmaha-Marroki, 2022). The Minimum Bactericidal concentration (MBC is the lowest possible concentration at which 99.9% of the bacteria are eliminated (Dellavalle et al., 2011).

*2.5. Statistical analysis*

The one-way analysis of variance was performed on three sets of data in SPSS Ver. 20. (ANOVA). The standard deviation is calculated using the mean of the replicates (SD).

## 3. Results

*3.1. Antifungal activity*

The well-cut diffusion technique revealed variable antifungal activity against *A. fumigatus, A. flavus, A. niger, C. tropicalis*, and *C. albicanis* when using Ethyl Acetate, Petroleum Ether, Methanol, and Butanol extracts from *A. marina* (Figure 1).

Different extracts inhibited the development of test fungus (fluctuated between 11 and 41 mm). The Ethyl acetate fraction exhibited the strongest inhibitory effects on *C. tropicalis, C. albicanis*, and *A. fumigatus*, with the highest inhibition zones reaching 41, 40 and 25 mm, respectively. Followed by Petroleum ether extract, against *C. tropicalis* and *C. albicanis,* with inhibition zones of 30 and 29 mm respectively (Figure 1 and Figure 2).

Our data showed the antimicrobial influence of plant extract was appeared to be very different in terms of effectiveness since some fungal species were highly resistant and some other were more sensitive. So, Candida species showed higher sensitivity by Ethyl acetate and Petroleum ether than *Aspergillus sp.* that diameters of inhibitions fluctuated between 11.0 and 15.5 mm, except *A. fumigatus* that recorded 25 mm.

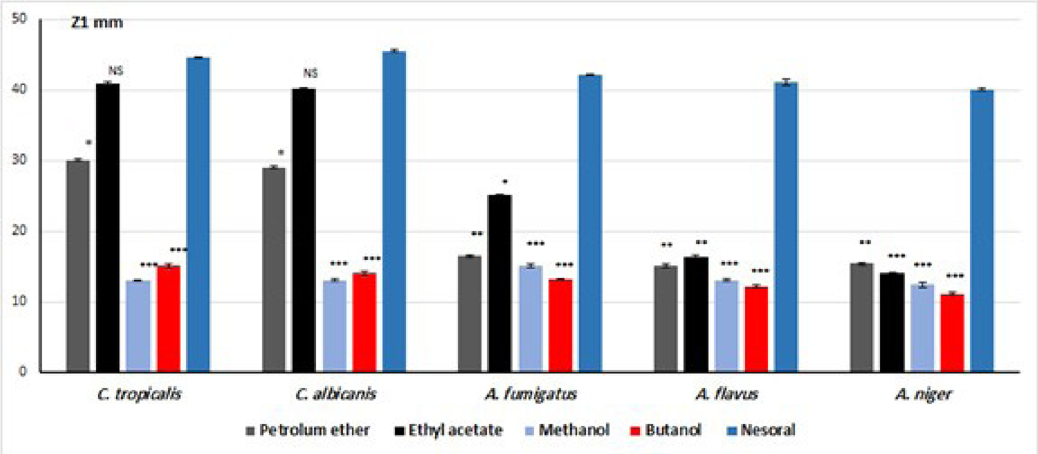


**Figure 1.** The antifungal effect of plant extracts from *Avicennia marina* against some pathogenic fungi expressed by zones of inhibition (ZI in mm). All tests were performed in triplicates (n = 3) and the error bars represent the SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ with respect to the control. NS: not significant.

The antifungal effects of Nizoral, a synthetic antifungal drug, were found to be greater against the yeasts *C. albicans* and *C. tropicalis* (45.5 and 44.7 mm) and *A. niger*, *A. flavus*, and A. fumigatus (40.0, 41.0, and 42.0 mm), respectively than those found in other plant extracts (Figure 1).

MIC values of *Avicennia marina* extracts against *A. Fumigates*, *C. albicans* and *C. tropicalis* are shown in Figure 3. The unique chemical's antifungal efficacy was further confirmed in this work, as was a new method of assessing the sensitivity of organisms to the extract. The MIC, or minimum inhibitory concentration, is a crucial element in determining the appropriate dose of extract for a certain bacterium. In our investigation, the MIC values ranged from 0.25 to 3.00 mg/ml.

According to Figure 3, Ethyl acetate extract of *A. marina* was the most potent and recorded the lowest MIC values that inhibited both *C. tropicalis* and *C. albicans* by 0.50 mg/ml and *A. fumigatus* by 1.50 mg/ml. Whereas, Petroleum ether extract recorded a MIC of 3.00 mg /ml against *A. fumigatus* and 1.00 mg /ml for both *C. tropicalis, and C. albicans*. Our extracts exhibited significant activities compared with the synthetic antifungal agent Nesoral (MIC 0.25 to 0.50 mg/ml).

### *3.2. Antibacterial activity*

Diverse extracts of *A. marina* have been proven to have different antibacterial potentials against the majority of examined microorganisms, as illustrated in Figures 4-5. In disc diffusion method, Ethyl acetate and Petroleum ether extracts exhibited significantly stronger antibacterial potentials than Butanol and Methanol extracts, as it was revealed in the antifungal tests. *Staphylococcus epidermidis* and *Enterococcus faecalis* have inhibition zones of 7 to 13.5 mm around the discs, respectively (Figure 4).

Figure 5a-5b illustrates the *A. marina* extracts' MIC values (in mg/ml) against seven different bacterial strains. Microdilution is expected to be more effective than other antibacterial techniques due to miscibility issues. Ethyl acetate and petroleum ether extracts were found to inhibit all the bacteria examined. Petroleum Ether extracts had MICs of 0.78 mg/mL for *Enterococcus faecalis* and 0.35 mg/mL for *Bacillus subtilis*. When

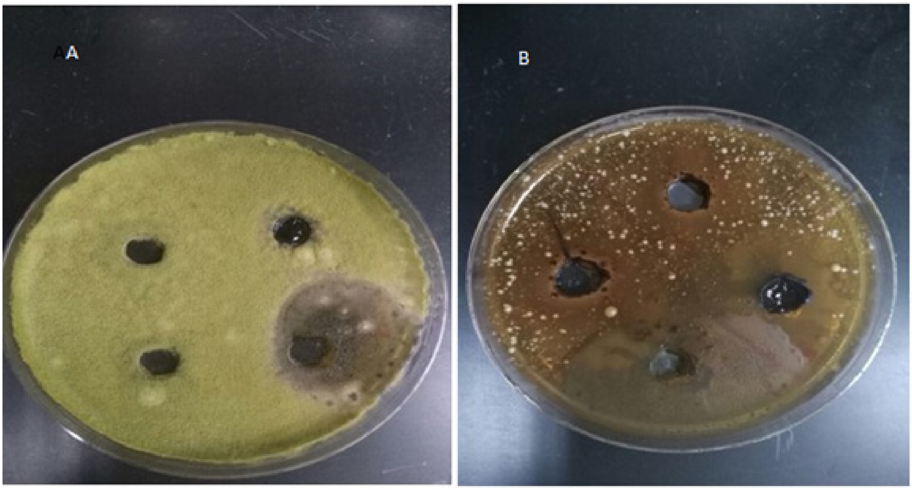


**Figure 2.** Antagonistic effect expressed by the inhibition zone of Ethyl acetate extract on the growth of: (A): *A. fumigatus*; (B): *C. albicanis*.

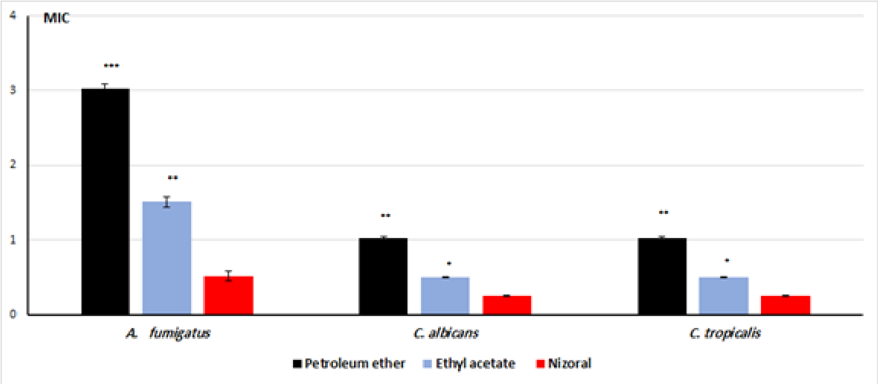


**Figure 3.** Comparison of MIC values (mg/mL) of *A. marina* different extracts. All tests were performed in triplicates (n = 3) and the error bars represent the SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ with respect to the control.

compared to the positive control gentamicin, the differences in activity across different bacterial strains are stark (p less than 0.001, Figure 5a-5b). Except for *Enterococcus* and *Bacillus subtilis* (MIC 0.78 and 0.35mg /mL respectively; very high significant at $p < 0.001$), the Ethyl Acetate appears to be more active than the Petroleum Ethyl Extract. Comparable to MIC values, the MBCs of organic extracts of *A. Marina* were determined to be (Figure 5a-5b). *Escherichia coli* was also substantially resistant to extracts of mangrove with MBC over 25 mg/ mL when compared to positive Gram bacteria tested to the extracts of mangrove extract.

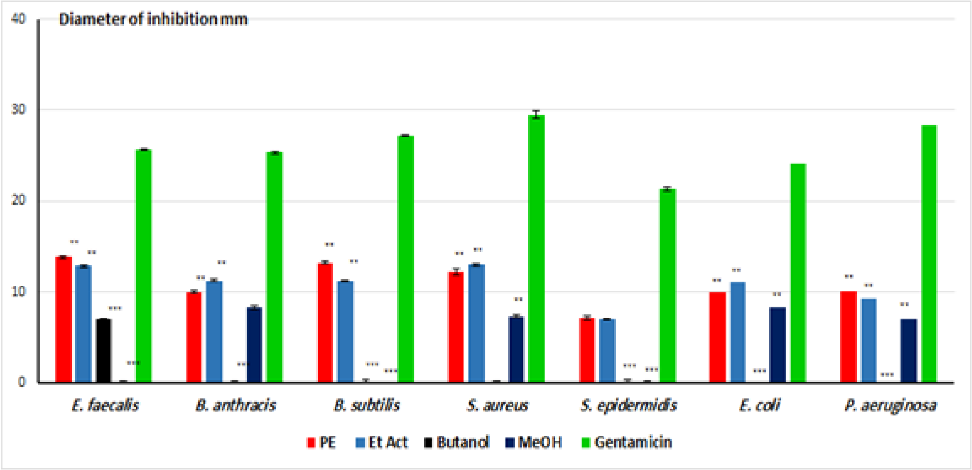


**Figure 4.** Comparison of the Diameters of inhibition in mm of *A. marina* extracts. All tests were performed in triplicates (n = 3) and the error bars represent the SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ with respect to the control.

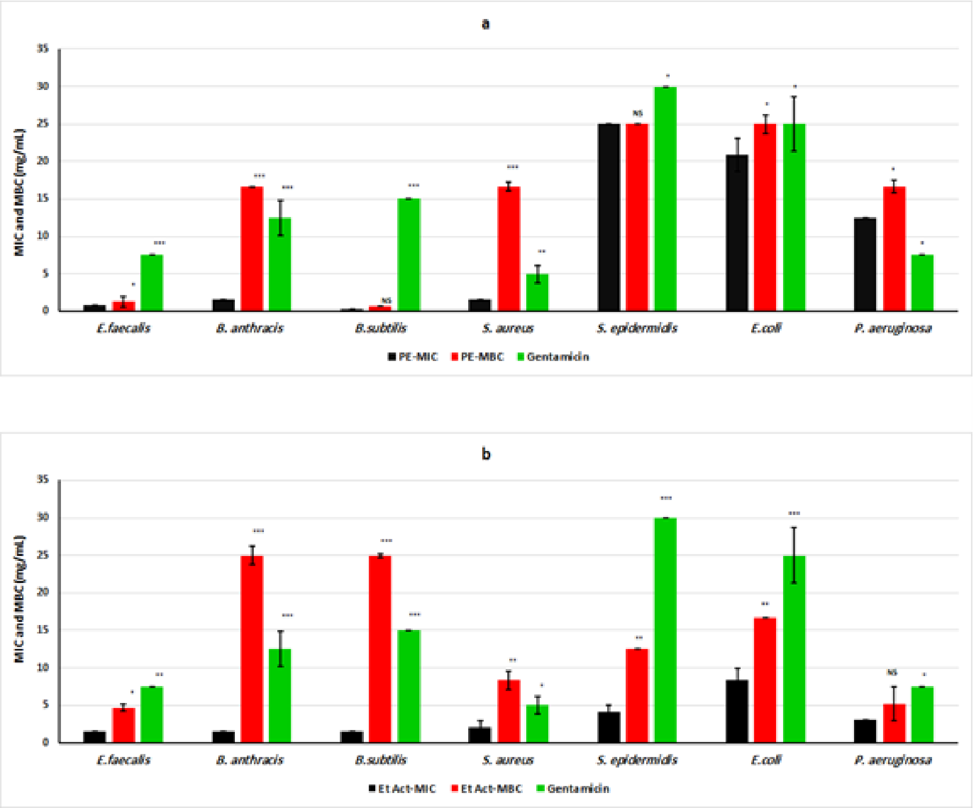


**Figure 5.** Comparison of MIC with MBC values (mg/mL) of *A. marina* in different solvents: a: Petroleum ether; b: Ethyl acetate. All tests were performed in triplicates (n = 3) and the error bars represent the SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ with respect to the control. NS: not significant.

## 4. Discussion

Medical research into the therapeutic benefits of a wide range of plant species is constantly evolving and improving around the world. Saudi Arabia is one of the world's most botanically diverse countries, with a deep history of alternative medicine. The screening and valorisation of antimicrobial chemicals identified in Saudi Arabia Flora is among the present study's objectives. Antifungal and antibacterial capacities were investigated in four *A. marina* extracts.

A variety of fungi have been tested for various medicinal plants for anti-fungal qualities (Abirami et al., 2021, Cruz et al., 2022). Depending on the plant extract and fungus type, antifungal activity differed significantly (Ibrahim and Al-Ebady, 2014). According to our finding, *A. marina* exhibited significant antifungal properties. In contrast, (Sohaib et al., 2022) studied the effect of several parts of the *A. marina* plant on 3 fungi. They concluded that the aerial part's extract had no effect on *C. albicans* and *A. niger*. *Rhizopus* sp. exhibited just a modest inhibition, on the other hand. Ethyl acetate extracts have been found to be more effective than Methanol and Butanol at preventing the growth of dangerous fungus, according to previous studies (Ademe et al., 2013). When Pandey tested the antifungal properties of methanol extracts of spices like cloves and dalchini, he discovered that they were more potent than less-polar solvents such as acetone, which contradicted this assertion (Pandey et al., 2013). For example, Menhem et al. (2021), Afata et al. (2022) argued that each material solvent system has a specific behaviour due to its unique chemical properties, its extraction process, and the diverse structural and compositional features of the natural products. According to polarity differences across solvents, the solubility of active plant features may be to fault. Solubility discrepancies among bioactive metabolites in various solvents could account for these variations (Osman et al., 2010). In the same context, the lowest MICs of 0.56 g/ml were found in upper half of Pneumatophores from *A. marina*, for *C. albicans* and *A. niger*. The investigation used absolute ethanol as a solvent (Sohaib et al., 2022). Antimicrobial activity varies depending on several conditions. The variability of antifungal activity as a function of the organ of the plant has been widely demonstrated. Indeed, Okla et al. (2021), deduced that only the ethanolic extract of *A. marina* fruits inhibited *C. albicans*. While that of roots and seeds showed no activity.

According to this study, A. marina also possesses effective anti-aspergillosis and anti-candidal properties. *C. albicans* is a commensal colonizer of the vagina and can cause oral candidiasis (Dias et al., 2018), one of the top four leading causes of hematogenous infections, as well as being present in the gastro-intestinal tract in healthy individuals (Alonso-Monge et al., 2021).

*A. fumigatus* is the principal pathogen responsible for many human illnesses and diseases. Aspergillosis, a hypersensitivity to fungal components, can occur in those with compromised lung function, such as asthmatics and cystic fibrosis sufferers, because of the Fungus (Dagenais and Keller, 2009).

Plant-derived natural chemicals have shown potential benefits in combating the establishment of resistance to antibiotics in pathogenic bacteria. It has recently been discovered through in vitro testing that several extracts and isolated components from medicinal plants are highly effective against a variety of pathogenic bacteria, including those found in the Flora of Saudi Arabia (Shahat et al., 2017; Al-Tamimi et al., 2021; Oueslati et al., 2021; AlMotwaa and Al-Otaibi, 2022). Extracts from several mangrove plants have been shown to be effective towards human and plant infections (Abeysinghe, 2010; Ibrahim et al., 2022; Chandrasekaran et al., 2009; Amirkaveei and Behbahani, 2011; Gurudeeban et al., 2013; Thatoi et al., 2016). Antibiotic activity against the investigated bacteria varies significantly depending on the extraction solvent used, as shown by our findings. Previous research, for example, can be compared to our findings, but there may be some differences. Ethanol extract of *A. marina* bark showed excellent antibacterial activity against *Proteus* and *S. aureus*, however neither petroleum ether nor aqueous, mature leaf extract of *A. marina* could suppress the development of *Proteus* sp. in prior experiments (Abeysinghe, 2010). *S. aureus, S. typhi, E. coli*, and *K. pneumoniae* have been shown to be resistant to the antibacterial effects of *A. marina* ethanolic extract (Ananthavalli and Karpagam, 2017). Mangrove leaves from Egypt's Red Sea have recently been collected. Testing on various human and fish pathogens and other organic extracts revealed that the ethyl acetate extract had the strongest antibacterial efficacy (Ibrahim et al., 2022). Dhayanithi, in contrast to our findings (Dhayanithi et al., 2012), reported that the greatest antibacterial activity was found in the methanolic extract of *A. marina* leaves. It was shown that *A. marina* leaf extract in ethyl acetate displayed antibacterial action against both *Staphylococcus aureus* and *Escherichia coli*, but no extracts in ethanol, petroleum ether, chloroform or water did (Okla et al., 2021).

The phospholipid outer membrane of Gram-ve bacteria is impermeable to lipophilic substances. Hydrophobic chemicals, which may normally permeate across phospholipid membranes, are one of the key advantages of Gram-ve bacteria's lipopolysaccharides (LPS) layer, allowing them to evade antimicrobials and establishing the basis for their rising antibiotic resistance (Tommasi et al., 2015; Paracini et al., 2022). Such results may explain why *Escherichia coli* is more resistant to extracts of mangrove than the Gram+ve bacteria evaluated in the current study. To put it another way, we found that the plant extracts tested were particularly effective against Gram+ve *B. Subtilis*. This could be a significant finding. which has since been shown in subsequent research (Sohaib et al., 2022).

Tests on mangrove plants' alleged bioactive chemical have so far yielded positive results (Ananthavalli and Karpagam, 2017; Patra and Mohanta, 2014). Terpenoids, steroids, glycosides, and other phytochemicals are found in *A. marina*, which is commonly classified as flavonoids, phenols, and alkaloids (Abeysinghe, 2010; Khattab et al., 2012; Poompozhil and Kumarasamy, 2014). Phytochemicals with antibacterial activity include a wide variety of compounds (Khameneh et al., 2019; Mahizan et al., 2019). Furthermore, phytochemical investigations indicated that extracts with the greatest percentages of flavonoids and total phenolics, exerted the most potent antibacterial action. Its contents in *A. marina* collected from Red Sea- Egypt were determined to be 23 and 109 mg/g, respectively (Ibrahim et al., 2022). While in another study, the total flavonoids and the total phenolics of leaves from the same plant harvested from Jeddah, Saudi Red Sea, represent 21.7 and 190.8 mg/L, respectively (Al-Mur, 2021).The flavonoids found in *A. marina*, according to the authors, may be responsible of the antibacterial action (Ibrahim et al., 2022). Wu and Xiao isolated two antimicrobial Phytoconstituents, from *A. marina*: naphthoquinones and avicequinone (Wu et al., 2008). Such naturally produced substances have been discovered to be accumulated in the plant's leaves, stem and aerial roots. The antibacterial activity of leaves extract might be attributed to phytol (phytanic acid). Another chemical found in *A. marina* is stigma-sterol, which has been linked to lactamase inhibition, resulting in resistance to antibiotics in antibiotic resistant bacteria (Yenn et al., 2017).

A range of infectious diseases and wounds have been traditionally treated with the herb (Field, 1995; Bandaranayake 1998; Namazi et al., 2013; Khasim et al., 2020). However, before recommending the use of any plant as an antibacterial, in vitro cytotoxic testing should be carried out. Safety investigations conducted by (Ali and Bashir, 1998). found that in vivo delivery of *A. marina* was not associated with behavioural changes or increased mortality or morbidity. Certain aspects that may have a substantial impact on an extract's activity include, but are not limited to, plant parts, species, geographic location, extraction method, and the solvent employed in the extraction process, among others.

## 5. Conclusion

*A. marina* from Rabigh lagoon, Saudi Arabia's red seacoast has shown its ability to function as a novel source of antimicrobial product and may be considered as a feasible natural alternative for the management of various infections in the pharmaceutical and cosmetic industries. According to recent research, a thorough phytochemical investigation is needed to isolate chemical components from solvent extracts and determine their biological activity. Additionally, more research is required to determine whether or not they have any cytotoxic properties.

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