EVALUATING THE SYNERGISTIC AND ANTIBACTERIAL EFFECTS OF FRESHWATER PLANTS AND SEAGRASS EXTRACTS ON *ACINETOBACTER BAUMANNII*

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Abstract. *Acinetobacter baumannii* has become one of the most dangerous global health pathogens in nosocomial infections, exhibiting increasing resistance to a wide range of antimicrobial agents. This resistance presents challenges in treatment and highlights the pressing need for innovative approaches to address these infections. Therefore, our investigation focuses on the ability of extracts produced from specific freshwater plants and seagrasses to inhibit the growth of *A. baumannii*. Moreover, we explore the potential synergistic effects of these natural extracts when used together with certain antibiotics. Our study evaluates the synergistic antibacterial effects between these extracts and antibiotics via the agar diffusion assay. The results showed that extracts from some species of freshwater plants were more effective in inhibiting the growth of *A. baumannii* than extracts from seagrasses. The variation in effectiveness is due to the solvents used for extraction and their differing capabilities. It is well known that *A. baumannii* has an inherent resistance to various classes of antibiotics, but this study also showed that certain plant extracts had substantial synergistic effects against the tested pathogenic microbe. This work demonstrates the potential of natural plant extracts as a complementary adjunct options to standard antibiotic therapy.
Keywords: *natural extract, pathogenic bacteria, synergism, resistance, protection*

Introduction

Bacterial infections remain a major global health concern due to its harmful impacts resulting in significant morbidity and mortality rates (Eissa et al., 2025; Mathew et al., 2025). Antibiotics are among the most important treatments in modern medicine, used to combat infections in various scenarios, including cancer treatments, organ transplantation, neonatal care, and major surgeries (Laxminarayan et al., 2013; Eissa et al., 2023; Dighiesh et al., 2024; Abd El-Aziz et al., 2024). Recent studies suggest that bioactive plant extracts may help to combat antibiotic resistance by enhancing antibiotic activity against drug-resistant bacteria (Ahmed et al., 2025). Thus, these extracts potentially providing a novel approach to improving treatment efficacy (Eissa et al., 2024). By combining natural plant extracts with antibiotics, it is possible not only to increase the effectiveness of infection treatment but also to reduce negative effects by lowering the required doses of synthetic drugs. Gram-negative bacteria, including *A. baumannii* responsible for almost 2% of all hospital-acquired infections in the US and Europe. It also has higher rates reported from Asia and the Middle East. Its multidrug resistance, biofilm formation, and hosts of virulence factors classifies it as a particularly insidious pathogen, especially in hospitals (Harding, 2018; Ayoub and Hammoudi, 2020; Wei et al., 2024). In recent years, several studies have investigated the antimicrobial activity of different plant extracts showing significant anti-bacterial action against pathogenic bacteria (Han et al., 2023), while others focus on the antibacterial potential of

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specific plants such as *Nigella sativa* (Abdullah et al., 2021) and *Clitoria ternatea* (Zulkamal et al., 2023). These studies highlight the need for further research on natural compounds as possible additives or replacements for traditional antibiotics, especially against multidrug-resistant organisms such as *A. baumannii*. Furthermore, microbial biofilms may invade plants in nutrient-rich habitats and plants in habitats with many bacteria unless plants possess a means to control them (Vattem et al., 2007; Tariq et al., 2019). Thus, researchers have focused their attention on aquatic plants that have been found to have a very good antibacterial compounds (Ertürk et al., 2019).

Freshwater plants have caught the attention of researchers as they have potential as antibacterial agents (Bushmann and Ailstock, 2006; Morales et al., 2006). Plant species have been used for food, aquaculture, and cattle feed, or gathered as wild stock in flooded fields. All plant parts, including stems, roots, rhizomes, tubers, and seeds, have been utilized for construction materials, mulch, and medicine (Edwards, 1980; Bablee et al., 2024).

In all seas, with the exception of the polar regions, seagrasses are numerous submerged marine angiosperms that grow in the tidal and subtidal zones. Human food is made from seagrass biomass, particularly by coastal people (Hossain et al., 2021). According to de la Torre-Castro and Rönnbäck (2004), seagrasses have been utilized for a range of therapeutic purposes, including the treatment of fever, skin conditions, muscle pains, wounds, stomach issues, medicine against ray stings, and sedatives for infants.

Gono et al. (2022) has showed that various bioactive compounds derived from seagrasses, have promising pharmacological activities. These molecules have been recognized for their potential in the development of potent drugs for a variety of diseases, including fevers, mental disorders, wounds, skin diseases, muscle pain, and gastrointestinal ailments. Besides, the report demonstrates the historical uses of seagrasses for applications in traditional medicine and lays the groundwork for future drug discovery and development from these underutilized marine resources.

Besides, Prajoko et al. (2024) has done a study to test the bioactive molecules profiles from *E. acoroides* and their direct biological anticancer effects. The group has examined the chemical composition of the various extracts using HPLC-ESI-HRMS/MS to characterize the powerful active metabolites that may impair the HER2/EGFR/HIF-1α pathway, which is a central mechanism in the breast cancer progression. The research group has applied through the MTT assay to detect the anti-proliferative activity of extracts on different cancer cell lines, in parallel to their potential antioxidant properties. This research not only corroborates the uses of seagrasses in traditional medicine but also paves the way for future drug discovery and development from these underexplored marine resources, specifically in the realm of cancer therapeutics (Prajoko et al., 2024).

Recent studies have also highlighted the antimicrobial potential of actinobacteria associated with seagrasses, suggesting a new avenue for the discovery of antibiotic compounds. Since pathogens frequently develop drug resistance as a result of the careless use of antibiotics, bioactive chemicals must be used with great care to eradicate or control the bacteria. As a result, compared to seaweed, seagrasses have received less attention in bioassay. Therefore, it is important to focus on secondary metabolite investigations of seagrasses with antibacterial and antifungal capabilities (Yuvaraj et al., 2012; Siro and Pipite, 2024). However, studies have yet to look into their capacity to manufacture antibacterial chemicals. In this work, the antibacterial potential of extracts from these plants against harmful Gram-negative bacteria (*A. baumannii*) was evaluated for the first time. The objective of this paper to evaluate the antibacterial effects of *Juncus rigidus*,

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Nasturtium officinale, *Veronica anagallis-aquatica* as well as Seagrass extracts on *Acinetobacter baumannii*. Every extract was tested alone or in combination with antibiotics to examine the synergistic possibility.

Materials and methods

Plant material and extraction process

Freshwater plants collection

Three freshwater plants, namely *Juncus rigidus, Nasturtium officinale,* and *Veronica anagallis-aquatica*, were collected from Albahah city, and *Mentha longifolia* was sourced from Al Taif city in Saudi Arabia.

Seagrass collection

During autumn, four types of seagrass leaves—*Cymodocea rotundata*, *Halophila uninervis, Halophila stipulacea,* and *Thalassia hemprichii*—were harvested from various locations along the Red Sea coast of Saudi Arabia.

The identification of these seagrasses was conducted according to El Shaffai (2016). The methodology for processing the collected plant materials followed the established procedures by Harborne (1998), which involved cleaning, drying in the shade, and pulverization. The powdered plant material underwent solvent extraction using ethanol, chloroform, ethyl acetate, and petroleum ether. The solvents were then removed through vacuum evaporation to yield the crude extract of each fraction. These extracts were freshly diluted in dimethyl sulfoxide (DMSO, Merck, Germany) and stored at 4°C until further use.

Bacterial isolates

The study focused on *Acinetobacter baumannii*, a Gram-negative bacterium, clinically isolated from King Fahad Hospital in Al-Madinah al-Munawwarah City.

Antibacterial activity assessment

Agar diffusion assay

The antimicrobial activity was evaluated using the agar diffusion assay. A 100 µL suspension containing 10^6CFU/mL was spread onto Petri dishes containing Müeller-Hinton agar (Himedia®). Holes of approximately 5 mm in diameter and 3 mm in height were created in the agar and filled with 30 µL of the plant extracts at a concentration of 50 mg/mL. DMSO and ampicillin (5 mg/mL, Sigma®, A9518) served as negative and positive controls, respectively. The inhibition zones were measured in millimetres after incubating the plates for 24 hours at 37°C. Zones larger than 7 mm were considered indicative of positive inhibition (Han et al., 2023).

Evaluation of synergistic effects

To evaluate antibiotic and natural antimicrobials interaction and to quantify the synergistic inhibition issued. Müeller-Hinton-deficient agar plates were inoculated with each bacterium. Discs (5 mm in diameter) were added onto each inoculated plate, adding 20 µL of the extract on top (200 mg/mL). This design was used to observe the possible

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synergistic effects between the plant extract and antibiotics. The filter paper disc was then air-dried for an hour before submerging the plastic plates in an incubator at 37°C for twenty-four hours, after which the clearing zones were measured (Breijyeh and Karaman, 2024).

Statistical analysis

All tests were conducted twice in triplicate to ensure the reliability of the results. The inhibition zones obtained from the extracts were compared with those from the positive control using SPPS software using ANOVA followed by a post hoc analysis test where($p<0.05$) refer to significant difference.

Results

Antibacterial activity of freshwater plant leaves extracts

A systematic evaluation regarding the antibacterial activity of freshwater plant leaves extracts against *A. baumannii* is thoroughly summarized in (*Table 1*). Four fresh water plants namely *M. longifolia, J. rigidus, V. anagallis-aquatica* and *N. officinale* were used in this study to prepare a variety of solvent extracts. Of all the solvent extracts, extracts from petroleum ether showed the best activity in showing antibacterial activity. In particular, the *M. longifolia* leaves petroleum ether extract exhibited the maximum antibacterial effect, resulting the largest diameter zone of inhibition (21.33 ± 0.58 mm). This was followed by *J. rigidus* extracts with 19.67 ± 0.58 mm, *V. anagallis-aquatica* 19.00 ± 0.58 mm, and *N. officinale* 18± 0.10 mm.

Table 1. *Antibacterial activity of freshwater leaves extracts against A. baumannii bacteria, tested using well diffusion assay (Data are represented as means ±SD; Different superscript letters refer significant results where P≤0.05)*

Type of aquatic plant	Mean diameter of the inhibition zone (mm) ± SD				
	Ethanol	Chloroform	Ethyl acetate	Petroleum Ether	Distilled water
J. rigidus	19.00 ± 1.73^{a}	19.33 ± 0.58^{a}	18.00 ± 1.00^{a}	19.67 ± 0.58^{a}	0.00 ± 0.00^{b}
V. anagallis-aquatica	18.67 ± 1.15^{a}	18.00 ± 0.00^{a}	17.00 ± 0.00^{a}	19.00 ± 0.58^{a}	0.00 ± 0.00^{b}
N. officinale	16.33 ± 1.55^{a}	17.33 ± 1.55^{a}	16.33 ± 1.55^{a}	18.00 ± 0.10^{a}	0.00 ± 0.00^{b}
M. longifolia	18.00 ± 1.53^{a}	18.67 ± 0.58^{a}	18.00 ± 0.00^{a}	21.33 ± 0.58^{a}	$0.00 \pm 0,00^{b}$

M. longifolia possessed the highest antibacterial activity among the extracts. This indicates that *M. longifolia* has rich bioactive composition. In addition, it was found that the effectiveness of extracts was directly correlated with the polarity of the solvent used, being the non-polar solvents like petroleum ether more effective than polar solvents. However, aqueous extracts of these freshwater plants did not exhibit antibacterial activity against *A. baumannii*. The aqueous extracts may be lacking or have only a low concentration of water-soluble bioactive compounds of the plant with antibacterial properties, resulting in the absence of antibacterial activity.

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Antibacterial activity of seagrasses leaves extracts

Table 2 summarizes the analysis of seagrass leaf extracts' antibacterial properties, specifically with respect to *T. hemprichii, C. rotundata, H. uninervis*, and *H. stipulacea*. *T. hemprichii* leaves extract displayed an inhibition zone of 20 ± 0.20 mm, confirming a robust presence of chloroform soluble anti-bacterial compound. *H. uninervis* and *C. rotundata* exhibited inhibition zones of 19.67 ± 0.58 mm and 18.67 ± 0.58 mm, respectively, and *H. stipulacea* had a zone of inhibition of 18.33 ± 1.53 mm. Unlike the freshwater plant extracts, some antibacterial activity was observed in the aqueous extracts of seagrasses, but less. The water soluble antibacterial compounds exhibited by seagrasses can vary; the aqueous extract from *H. uninervis* leaves for example did not inhibit *A. baumannii*. This suggests that some of the water-soluble antibacterial compounds are less effective than the antibacterial compounds that can be extracted from non-polar solvents.

Table 2. *Antibacterial activity of seagrasses leaves extracts against A. baumannii bacteria, tested using well diffusion assay (Data are represented as means ±SD; Different superscript letters refer to significant results where P≤0.05)*

Type of seagrasses	Mean diameter of the inhibition zone (mm) ± SD				
	Ethanol	Chloroform	Ethyl acetate	Petroleum Ether	Distilled water
H. uninervis	17.00 ± 0.00 ^{a}	19.67 ± 0.58 ^{a}	18.00 ± 0.00 ^{a}	17.33 ± 0.58 ^{a}	15 ± 0.00 ^{a}
C. rotundata	18.00 ± 0.00 ^{a}	19.00 ± 0.00 ^{a}	18.67 ± 0.58 ^{a}	17.67 ± 1.15 ^{a}	0.00 ± 0.00 ^{b}
T. hemperchii	18.33 ± 1.53 ^{a}	20.00 ± 0.20 ^{a}	19.00 ± 0.00 ^{a}	18.00 ± 1.00 ^{a}	0.00 ± 0.00 ^{b}
H. stipulacea	17.67 ± 0.58 ^{a}	18.33 ± 0.58 ^{a}	17.67 ± 0.58 ^{a}	17.00 ± 1.00 ^{a}	0.00 ^{b}

When comparing the antibacterial activity of freshwater plant and seagrass leaf extracts, we found that freshwater plant extracts yielded larger zones of inhibition. Implication of these observations is that freshwater plant species might have more quantity or potency of antibacterial compounds than seagrasses. The variation in the antibacterial potency between the groups can be explained by the dissimilar chemical make-up, driven by two different environments and solvents used for extraction. This indicates that the solvent choice is critical for this process as it determines both the extraction and the effectiveness of the bioactive compounds responsible for antibacterial activity.

Evaluation of synergistic effects

The investigation into the synergistic antibacterial effects of plant extracts and antibiotics against *A. baumannii* revealed notable findings, which are detailed below.

Synergistic activity of ethyl acetate extracts of freshwater plants with antibiotics

The effects of ethyl acetate extracts from four freshwater plants on antibiotics along with synergistic effects for the potential enhancement of antibacterial effect are shown in *Table 3*. In particular, *J. rigidus* leaves ethyl acetate extract exhibited a high synergistic effect with the antibiotic Imipenem, yielding a mean inhibition zone of 20.00 mm. The enhanced zone of inhibition (20.00 mm) shown by the combination of *J. rigidus* extract along with Imipenem, clearly indicates the previous inhibition zone versus the increase and thus, showing potentiation of Imipenem action against *A. baumannii*.

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*T**able 3.** Synergism Between Antibiotics and Leaves Extracts of Freshwater Herbs against A. baumannii (Different superscript letter refer significant results where P≤0.05)*

Antibiotics Names	Effects of Antibiotics	*J. rigidus*		*M. longifolia*		*N. officinale*		*V. anagallis-aquatica*		A+ 4F	4 F*
		PE*	PE +A	PE	PE+A	PE	PE +A*	PE	PE+A		
Tetracycline (TC)	0		0		0		0		0	0	
Cefepime (CPM)	0		0		0		0		0	0	
Gentamicin (GM)	15 [a]		0		0		0		0	0	
Amikacin (AK)	0		0		0		0		0	0	
Imipenem (IMI)	15 [a]		**25 [b]**		13		15 [a]		15 [a]	15 [a]	
Piperacillin (PRL)	0	19.67 [a] ± 0.58	0	20.33 [a]± 0.58	13	18± 0.6 [a]	0	19± 0.8 [a]	0	0	0
Ampicillin (AP)	0		0		0		0		0	0	
Augmentin (AUG)	16 [a]		0		0		0		0	0	
Cefoxltin (FOX)	15 [a]		0		0		0		0	0	
Cephalothin (KF)	10 [a]		0		0		0		0	0	
Cotrimoxazole (TS)	0		0		0		0		0	0	

*Pe = petroleum ether extract, A= antibiotics, 4F = leaves extracts of four freshwater herbs

Whereas, ethyl acetate extracts of *M. longifolia*, *V. anagallis-aquatica* and *N. officinale* did not show any effect upon co-administration of any of the antibiotics tested. This means that these extracts will not have an interaction with the antibiotics, and the extracts could be potentially employable in high-throughput combination therapies. Furthermore, it was recognized that *A. baumannii* had shown resistance to multiple antibiotics, such as Tetracycline, Cefepime, Amikacin, Piperacillin, Ampicillin, and Cotrimoxazole, which highlights the increasing issue of antibiotic-resistant pathogens. Nevertheless, the bacterium showed no resistance to the ethyl acetate extracts of the four freshwater plants, which suggests that the extracts could contain bioactive compounds with effective antibacterial activity.

Notably, when mixing four different ethyl acetate extracts before the application. They show no synergistic effects versus *A. baumannii*. This implies that the mixtures of extracts do not always have higher antibacterial activity, and that the synergistic effect was observed only in the presence of antibiotics and *J. rigidus*, highlighting the emerging nature of the active mixture from the plant fraction as one with a distinctive and unique chemical structure.

Following this leads us to discuss more specifically the phytochemical components on the extract of *J. rigidus* that exhibited this synergistic activity alongside Imipenem. Detection of these compounds may help the researchers understand the mechanism of action and develop novel antibacterial agents. Furthermore, knowledge regarding the impact in utilizing multiple plant extracts may contribute to enhanced strategies of incorporating phytochemicals in combination with antibiotics. Further research is also needed on the potential of other plant extracts to augment the action of antibiotics; this could be vital in combating multi-drug resistant bacterial strains. This study suggests that plant extracts can be used as adjuvants to antibiotics, and that examining their incorporation into current antibacterial approaches is a promising area of research.

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Seagrass extracts synergistically interact with antibiotics – chloroform extracts of seagrasses and antibiotics

In *Table 4*, the interactions of chloroform extracts from seagrass leaves with several antibiotics, aimed at detecting synergistic effects that increase antibacterial action are reported that could be used in therapeutic combination. Importantly, the chloroform extract from *C. rotundata* leaves exhibited synergistic impact against all administered antibiotics but Augmentin had no synergism. The chloroform extract of *T. hemprichii* showed a very strong synergistic effect with Imipenem, where the inhibition zone reached 23.00 mm versus 15.00 mm with only Imipenem. This shows a significant increase ($P \leq 0.05$) in the antibacterial effectiveness versus *A. baumannii* of Imipenem when combined with *T. hemprichii*.

Table 4. *Synergism Between Antibiotics and Leaves Extracts of Seagrasses Against A. baumannii (Different superscript letter refer significant results where P≤0.05)*

Antibiotics Names	Effects of Antibiotics	*C. rotundata*		*H. uninervis*		*H. stipulacea*		*T. hemprichii*		A+ 4 S	4 S
		CH*	CH +A	CH	CH +A*	CH	CH +A	CH	CH + A		
Tetracycline (TC)	0		20 ^{b}		0		0		0	0	
Cefepime (CPM)	0		20^b		0		0		0	0	
Gentamicin (GM)	15^a		20 ^{b}		0		0		0	0	
Amikacin (AK)	0		20 ^{b}		0		0		0	0	
Imipenem (IMI)	15^a		20 ^{b}		15 ^{a}		20^b		23^b	10^c	
Piperacillin (PRL)	0	19^a	20 ^{b}	18.6 ^{a}	0	18.33 ^{a}	0	20 ^{b}	0	0	0
Ampicillin (AP)	0		20 ^{b}		0		0		0	0	
Augmentin (AUG)	16 ^{a}		0		0		0		0	0	
Cefoxltin (FOX)	15 ^{a}		20 ^{b}		0		0		0	0	
Cephalothin (KF)	10 ^{a}		20 ^{b}		0		0		0	0	
Cotrimoxazole (TS)	0		20 ^{b}		0		0		0	0	

*Ch = chloroform extract, A= antibiotics, 4S = leaves extracts of four seagrasses

Furthermore, the study examined the co-treatment of Imipenem with chloroform extracts from three species of seagrasses (*T. hemprichii*, *C. rotundata*, and *H. stipulacea*), which exhibited interactions with inhibition zones of 23.00 mm, 20.00 mm, and 20.00 mm, respectively. Such synergistic interaction is not observed with *T. hemprichii* indicating the variability exhibited with different seagrass extracts in boosting the activity of antibiotics. As noted for freshwater plant extracts, susceptibility of *A. baumannii* to the chloroform extracts of the seagrasses was evident although it demonstrated resistance to similar antibiotics. When four different chloroform extracts were combined, no synergistic effects against *A. baumannii* were observed.

Supplementation with plant extracts may prove to be a useful alternative to improve the activity of antibiotics against *A. baumannii* as shown by the potent activity of plant extracts prepared from ethyl acetate and chloroform. Such results pave the way for future

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studies investigating the potential activity of plant derived compounds as adjuvants in antibiotic treatment. Following on from this, it is important to study which phytochemical compounds are responsible for each synergistic effect, as well as how these compounds work synergistically to make antibiotics more effective. Discovery of these compounds may contribute to the establishment of new therapeutic strategies utilizing the disparate natural antibacterial activities of plant extracts against antibiotic-resistant bacteria. Also, it would be useful to investigate the variability in synergistic potential between different plant species and extract types to determine the most efficient combinations of antibiotics to synergize. This research path is promising for combatting the growing threat of antibiotic resistance, and could lead to guidelines for more efficient antibacterial therapies.

Table 5 summarizes the key findings and implications from the evaluation of synergistic effects between plant extracts and antibiotics, as detailed in the comparative analysis of the research study.

Table 5. *Comparative Analysis of Synergistic Antibacterial Effects Between Freshwater Plant and Seagrass Extracts with Antibiotics Against A. Baumannii*

Aspect	Freshwater Plant Extracts with Antibiotics	Seagrass Extracts with Antibiotics
Extracts and Antibiotics Evaluated	Ethyl acetate extracts from *J. rigidus*, *M. longifolia, V. anagallis-aquatica,* and *N. officinale*	Chloroform extracts from *T. hemprichii, C. rotundata*, and *H. stipulacea*
Synergistic Effects Noted	Significant synergistic effect with *J. rigidus* extract and Imipenem (IMI)	Significant synergistic effect with *T. hemprichii* extract and IMI; *C. rotundata* extract showed synergistic effects with all tested antibiotics except Augmentin
Antagonistic Effects Observed	No antagonistic effects with other extracts; no synergistic effects when extracts were combined	Antagonistic effects when IMI was combined with extracts from four seagrasses
Antibiotic Resistance	*A. baumannii exhibited* resistance to a broad spectrum of antibiotics but was susceptible to the ethyl acetate extracts	Similar resistance pattern to antibiotics; susceptibility to chloroform extracts from seagrasses
Implications for Antibiotic Therapy	*J. rigidus* extract could potentiate the efficacy of IMI against *A. baumannii;* potential for combination therapies	Variability in the potential of seagrass extracts to enhance antibiotic activity; importance of matching specific extracts with appropriate antibiotics
Future Research Directions	Investigate the phytochemical constituents of *Juncus rigidus* extract; explore other plant extracts for synergistic effects with antibiotics	Identify phytochemical constituents responsible for synergistic effects; understand mechanisms enhancing antibiotic efficacy

Table 5 provides a structured comparison of the interactions between plant extracts and antibiotics, highlighting their potential to enhance antibacterial efficacy. Freshwater plant ethyl acetate extracts and seagrass chloroform extracts alone and in combination with selected antibiotics were known to exhibit either synergistic or antagonistic effects as demonstrated through the bacteria enumeration analysis. The results showed that the combination of *J. rigidus* extract and imipenem had the most potentiating effect on *A. baumannii*. Likewise, *T. hemprichii* extract of seagrasses significantly enhanced the antibacterial activity of Imipenem. *A. baumannii* was resistant to a wide variety of

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antibiotic classes, but both of the groups suggested compatibility with commercial antibiotics under *in vitro* conditions, suggesting that they could be used as a combination therapy. One of our key observations is the lack of synergistic effects when combining multiple extracts, a finding reinforcing the specific nature of the interactions between specific plant extracts and specific antibiotics. This specificity comes at a time when there is a pressing need to explore how certain combinations of antibiotics might be effective in bypassing resistance mechanisms. The table highlights the importance of further investigating the phytochemical components that would be responsible for these activities and the differences in their synergy potential between various plant species or extract types. This comparative analysis emphasizes the potential of using plant extracts as additives in combing against the bacteria and that further studies should be performed for the development of superior antibacterial agent.

Discussion

The prevalence and increasing incidence of *A. baumannii* antibiotic resistance in the healthcare environment are currently a major public health issue. The ability of this pathogen to withstand a wide spectrum of conventional antibiotics makes the search for new antimicrobial strategies urgent. The preliminary exploration of the antibacterial strength of extracts derived from both freshwater plants and seagrasses reported in the present study is a prospective approach towards addressing this critical need and is in line with efforts to explore the information from nature, as potential sources to develop newer therapies to combat multidrug resistance.

The promising results of this study indicating the superior antibacterial activity of petroleum ether extracts from freshwater plants, particularly *M. longifolia*, against *A. baumannii*. The findings follow a growing body of literature that has shown essential oils and extracts of a wide array of plants to be effective against a variety of pathogens, including *Escherichia coli* and *Candida tropicalis* (Bukhari, 2023). The antibacterial action ethanolic extract of *Mentha arvensis* could be through of its impact to damage bacterial cell membrane and leakage of proteins, oxidative induction (Tafrihi et al., 2021).

Chloroform extracts of seagrasses demonstrated significant activity against *A. baumannii*, especially *T. hemprichii* chloroform extract, which showed the highest activity. This finding is in line with other studies showing antimicrobial activities of seagrass extracts, which have been pointed out as potential sources for new antibacterial agents. The bioactive components present in these extracts may have distinct action modes against bacterial pathogens (Lustigman and Brown, 1991; Umamaheshwari et al., 2009; Gumguje et al., 2018), such as hindering essential enzymes in bacteria, inhibiting nutritional uptake, or disrupting cell wall production.

Additionally, the study comments on the *A. baumannii's* resistance to commonly used antibiotics; Tetracycline, Cefepime, Amikacin, Piperacillin, Ampicillin, and Cotrimoxazole. The pattern of resistance, along with antimicrobial susceptibility testing results, mirrors that of the global situation surrounding antibiotic resistance and highlights an urgent need for alternative therapeutic strategies. Such synergistic effects of different plant extracts along with traditional antibiotics especially ethyl acetate extract of *J. rigidus* leaves along with Imipenem, can be promising candidates in overcoming resistance of several pathogenic bacteria and could be an alternative therapy in drug resistance scenario. This approach is based on the studies that have demonstrated

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synergism between plant extracts and antibiotics to develop new anti-infective drugs (Duarte et al., 2012; Knezevic et al., 2016; Boonyanugomol et al., 2017).

This study has important implications that can guide the development of novel antimicrobials. Researchers could then find what compounds in freshwater plants, or pits of seagrasses that can be inhibited, which could then lead to either direct inhibition of *A. baumannii* or potentiation of current antibiotics. The use of these extracts should be followed by isolating and characterizing the active ingredients in these extracts, determining their exact mode of action along with assessing their safety and efficacy in this context.

Of note, the growing resistance of *A. baumannii* to antibiotics constitutes an important risk factor for public health, which warrants the testing of alternative antimicrobial compounds. As such, this study illustrates the potential for freshwater-dwelling plants and seagrasses to serve as sources for new and or supplementary anti-biofilm and or bactericidal compounds. These synergistic effects that were observed between some of the plant extracts in conjunction with the antibiotics can prove beneficial in developing effective combination therapies against multidrug-resistant *A. baumannii*. Additional research, including studies-in-progress that extend into and beyond 2024, will be needed to validate these findings and to advance the search for potential treatments to combat this pathogen.

The current study demonstrated the antibacterial activity of petroleum ether (PET) extracts of freshwater plants, especially *M. longifolia*, against *A. baumannii* and adding to other studies correlating to the antibacterial potency of crude plant extracts which are comparable to essential oils derived from different plant sources (Saeedi et al., 2020). In addition, our observations of the significant activity of *T. hemprichii* chloroform extract provide new insights into the antimicrobial potential of seagrasses beyond the work of Gumgumjee et al. (2018); and Umamaheshwari et al. (2009) by assessing the antibacterial activities of these extracts against resistant pathogens. Furthermore, our study differs from previous works by showing a novel synergistic interaction between ethyl acetate extract of *J. rigidus* and Imipenem, highlighting the potential of combinations between active plant extracts and antibiotics to improve therapeutic efficacy, a concept that has been proposed but not widely explored as shown in the bulk of literature provided by Boonyanugomol et al. (2017); and AlSheikh et al. (2020). This overview highlights the emerging therapeutic potential of plant-derived antimicrobials and confirms expected antimicrobial activities of plant extracts, whilst revealing new therapeutic opportunities, suggesting further exploration of these plant-derived antimicrobials as novel antimicrobial strategies.

Our study provides insights supporting the notion that extracts obtained from these plants affect multiple targets, such as the bacterial cell membrane and oxidative pathways leading to bacterial lysis and cell death. These mechanisms are similar to those of traditional antibiotics, which target bacterial cell wall synthesis or DNA replication. This means that plant extracts have potentially greater amount of bioactive compounds that target multiple targets of bacteria cell. The ability of plant extracts to disrupt cell membranes, as demonstrated by the ethanolic extract of *M. arvensis* isolate, may increase permeability and ultimately cell lysis, a mechanism that has also been well documented for antibiotics such as polymyxins. Plant extracts also induce the production of reactive oxygen species (ROS), an oxidative stress mechanism causing damage to multiple cellular targets, which has been reported for other antibiotics like metronidazole (Boonyanugomol et al., 2017). Future investigation of these extracts could address

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whether they are broadly acting toward multiple bacterial species and pathways, or specifically target selective bacterial targets. This specificity could help reduce harm to helpful microbiota and the emergence of resistance. Detailing these mechanisms will not only elucidate the concept of using new antimicrobial therapies, but also provide a comparative basis to assess the efficacy and safety of plant-based extracts against traditional antibiotics.

Thus, the inclusion of plant extracts as antibacterial agents into clinical use represents a promising strategy to address the growing threat of antibiotic resistance, a pressing problem that necessitates thorough safety and efficacy evaluation in the clinical context. Such assessment should include comprehensive toxicity studies, pharmacokinetics and pharmacodynamics, and establishment of standard operating procedures in the context of safe and effective use in patients. When considering plant materials for use as antimicrobials, their sustainability shouldn't be overlooked; sustainable harvesting practices and the cultivation of medicinal plants also need to be included to reduce the ecological consequences.; these practices can reduce the effects on wild populations, and biodiversity as a whole should be preserved. Equally important to address include ethical considerations such as the fair and equitable sharing of benefits with local communities, and respect for traditional knowledge when sourcing plant materials.

It'd be interesting to see more *in vivo* studies in animal models of disease to further explore the potential therapeutic benefits of these extracts as well as formulation strategies that would increase bioavailability and stability of the active compounds. Translation: Ultimately, human clinical trials to assess the effectiveness and safety of these herbal treatments will help determine whether they are adopted into routine medical practice and standard therapies. Hence, the global health impact of plant-based antimicrobials could be significant, especially for low- and middle-income countries where access is limited to standard antibiotics. Synthetic antibiotics, used for over 80 years, are reaching a critical point of offering a cost-effective and accessible alternative accessible to all and an improvement in global health security.

Interdisciplinary synergy is required to harness the potent antimicrobials in plants. The complexity of such plant extracts, owing to their plethora of bioactive components, necessitate veterinarians skilled in microbiology to define their antimicrobial mechanisms, phytochemists to chemical structure the active principles, pharmacologists to clinical properties, clinicians to use in therapy. This kind of cooperation is essential for enabling better insights into plant-based antimicrobials and the creation of new strategies for tackling the global risk caused by antibiotic-resistant pathogens.

Our research paper makes a significant contribution to the field of antibacterial research by investigating the potential of some freshwater plant and seagrass extracts as alternative treatments against the multidrug-resistant *A. baumannii,* a major cause of hospital-acquired infections. It addresses the global health challenge posed by antibiotic resistance by exploring the synergistic effects of natural compounds with existing antibiotics, potentially enhancing their efficacy and reducing adverse effects. This study fills a critical research gap by being among the first to assess the antibacterial capabilities of these aquatic plants against a formidable Gram-negative bacterium, thereby advancing our understanding of natural antibacterial agents. Additionally, it supports sustainable antibacterial approaches by seeking bioactive compounds from underutilized natural resources, which is consistent with measures to promote eco-friendly and prudent control of bacterial resistance.

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Conclusion

In this study, we have demonstrated that extracts from various freshwater plants and seagrasses exhibit potent antibacterial activity against *A. baumannii*, a Gram-negative bacterium responsible for numerous human infections. Notably, the ethyl acetate extract of *M. longifolia* and the chloroform extract of *T. hemprichii* emerged as the most effective antibacterial agents among the tested extracts. Given the widespread resistance of *A. baumannii* to a broad spectrum of antibiotics, the discovery that Imipenem exhibits a high degree of synergism with these potent extracts is particularly significant.

The global public health challenge posed by *A. baumannii* infections cannot be overstated. These infections are difficult to treat due to the bacterium's resistance to conventional antibiotics. Our findings underscore the urgent need to identify and characterize the specific bioactive compounds within these plant extracts that possess genuine antimicrobial properties. Such knowledge could pave the way for the development of new therapeutic agents or adjunctive treatments that can enhance the efficacy of existing antibiotics.

However, the battle against antibiotic resistance is complex, given the remarkable adaptability of bacteria to adverse conditions, including the presence of antimicrobial agents. Therefore, a thorough understanding of the resistance mechanisms employed by *A. baumannii* is crucial. By unraveling these mechanisms, we can develop targeted strategies to counteract the bacterium's resistance and mitigate the spread of antibiotic resistance. This study contributes to the growing body of evidence supporting the potential of natural plant extracts in the fight against multidrug-resistant pathogens and highlights the importance of continued research in this area.

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