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Drug development strategy for antimicrobial applications from nine different aquatic bio-based plants against human and fish pathogens

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ABSTRACT: A study was carried out to explore the antibacterial properties of aquatic plants study often associated with breeding grounds for disease vectors like mosquitoes. These plants have recently been recognized for their potential as sources of antibacterial compounds. The purpose of the study was to assess the antibacterial effectiveness of local aquatic plant species against particular bacterial strains. Nine different aquatic plants were collected from ponds near Kandiyaperi Lake in Palaya Pettai, Tirunelveli district, Tamil Nadu, India. Crude extracts of these plants were prepared using water, ethyl acetate, and methanol. The antibacterial activity of these extracts was subsequently evaluated against eight bacterial strains, four of which were aquatic and four were humans using the agar well diffusion method. Additionally, antibiotic sensitivity testing was performed to compare the effectiveness of these extracts with standard antibiotic discs. Results indicated that among the three types of crude extracts, those prepared with ethyl acetate exhibited the highest antibacterial potential. Aqueous extracts had no antibacterial action against any of the studied bacterial strains, whereas methanolic extracts exhibited moderate activity. This study yielded promising findings, highlighting a few selected aquatic plant extracts as potent natural antimicrobials. The final results suggest that these plant extracts could be utilized in the development of drugs to treat bacterial infections. The discovery opens up new avenues for exploring natural resources in combating infectious diseases caused by bacteria. Overall, this research underscores the importance of aquatic plants not only as breeding grounds for disease vectors but also as valuable sources of antimicrobial compounds.

1. INTRODUCTION

For thousands of years, sophisticated traditional medical systems have been based on plants, leading to the isolation of different chemical compounds and used for various applications (Nasim et al., 2022). Plant-based medications are becoming more popular due to the fact that they are safe, clinically effective, tolerated well by patients, less expensive,

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and competitive worldwide (Pratt et al., 2019). Aquatic plants, such as bryophytes, pteridophytes, and angiosperms, grow entirely or in part on the water's surface. They negatively impact aquatic habitats, causing financial losses. Research on aquatic plants has revealed that they are abundant in bioactive compounds and have strong antibacterial effects (Bushmann and Ailstock, 2006). As a result, aquatic plant species may offer various sources for lead compounds that are potent antimicrobials.

Out of 0.4 million plant species worldwide, 47,513 species (11.4% of the total flora) are found in India, which makes up 2.4% of the world's landmass (Singh and Dash, 2014). India is home to most of the world's aquatic angiosperms, which are mainly distributed in the Nymphaeaceae, Podostemaceae, Najadaceae, Hydrocharitaceae, Lemnaceae, Alismataceae, Potamogetonaceae, and Ceratophyllaceae families, including 107 aquatic angiosperm species (Arisdason and Lakshminarasimhan, 2016).

Numerous authors have documented the antibacterial qualities of aquatic plants (Hossain et al., 2018), but to our knowledge, most of the investigations were to evaluate the antibacterial potential of wild plants (Bhaigybati et al., 2020; Verma et al., 2021). Nonetheless, research has indicated that aquatic plants from India possess antimicrobial qualities (Pushkareva et al., 2017). The reports on the ethnomedical survey provided some of the information on aquatic plants used for traditional medical purposes in rural India (Dabur et al., 2007). Understanding the chemical composition of aquatic plants is crucial as it varies considerably based on the species, season, and location (Lata and Dubey, 2010). This study set out to evaluate the antibacterial qualities of different solvent extracts for nine distinct aquatic plants (Figure 1).

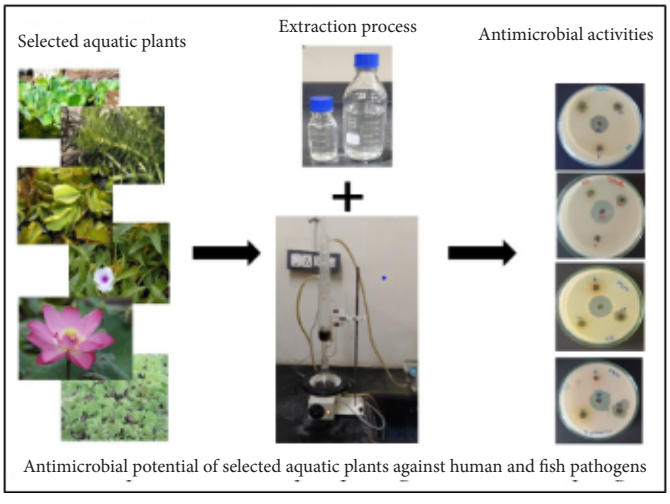

Figure 1 The schematic diagram represents the antimicrobial activity of aquatic plants

2. MATERIALS AND METHODS

2.1. Plant materials

Nine different aquatic plants were used in this study and collected from ponds close to the Department of Animal Science, Manonmaniam Sundaranar University, and the Kandiyaperi Lake, Palaya Pettai in Tirunelveli district (Table 1). The collected plants were cleaned, rinsed in distilled water and shade-dried.

2.2. Preparation and preservation of plant extract

The water extraction process was carried out by Rahman et al. (2009). Using the percolation method, 100 g of fresh leaves from each plant were weighed, ground, and immersed in 400 mL of distilled water for 7 days, shaking occasionally. After filtering, the supernatant was lyophilized at low pressure. The dried extracts were diluted in dimethyl sulfoxide (DMSO) to reach a concentration of 50 mg/mL. The dried extract was kept at 4°C for the antibacterial activity test.

Methanol and ethyl acetate (1:10%) together with 100 g of air-dried plant powder were combined. The combination

Table 1
Antibiotic sensitivity profile of aquatic pathogens.

Antibiotics _Aquatic bacterial pathogens/Zone of inhibition (mm)	ET	AH	AC	VC
Streptomycin	13.00	18.00	32.00	20.00
Ampicillin	NZ*	NZ	32.00	NZ
Tetracycline	22.00	22.00	21.00	18.00
Erythromycin	NZ	NZ	12.00	NZ
Penicillin-G	NZ	NZ	37.00	NZ
Doxycycline hydrochloride	18.00	20.00	26.00	20.00
Gentamicin	22.00	24.00	26.00	22.00
Neomycin	20v	20.00	22.00	20.00
Cloxacillin	17.00	16.00	20.00	16.00
Chloramphenicol	28.00	30.00	18.00	30.00
Oxacillin	NZ	NZ	24.00	NZ
Azithromycin	20.00	20.00	16.00	18.00
Methicillin	NZ	NZ	NZ	NZ
Vancomycin	08.00	10.00	18.00	14.00
Amikacin	18.00	20.00	26.00	20.00
Polymyxin	10.00	12.00	12.00	12.00
Rifampicin	14.00	16.00	18.00	17.00
Ciprofloxacin	26.00	30.00	26.00	30.00
Kanamycin	17.00	18.00	16.00	20.00
Bacitracin	08.00	08.00	08.00	10.00

*NZ: no zone

(residue) was macerated overnight in a percolator. After filtering, the extract was boiled in a water bath at 40°C. The concentration of each extract (50 mg/mL) was determined after the dry extract was weighed. For an antibacterial activity test, the resulting extracts were stored at 20°C. (The extraction procedures for two solvents are the same.)

2.3. Test microorganisms

Eight bacterial strains were used in this study. It includes *Aeromonas hydrophila, A. caviae, Vibrio cholerae*, and *Edwardsiella tarda* (aquatic pathogen); *Pseudomonas aeruginosa, Acinetobacter baumannii, Staphylococcus aureus*, and *Klebsiella pneumonia* (human pathogen). All the examined strains were taken from the Department of Microbiology, Manonmaniam Sundaranar University, Tirunelveli. The bacterial cultures were maintained at 4°C on nutrient agar slants and 37°C in nutrient broth, respectively.

2.3.1. Inoculum preparation

Each bacterial strain was subcultured in Mueller Hilton agar (MHA) slants overnight at 37°C. A spectrophotometer was used to harvest the bacterial growth using 5 mL of sterile saline water, correct for absorbance at 580 nm, and diluted to obtain a viable cell count of 1 × 106 Colony Forming Units (CFU)/mL.

2.3.2. Determination of antibacterial activity

To evaluate the antibacterial activity of the aforementioned extracts, the agar well diffusion method was employed with a few modifications by Patra et al. (2009). Five 8.0 mm diameter wells were aseptically created on the assay plates using the wide end of sterile 1 mL tips and the target microbe was then seeded into each well. The crude extracts were reconstituted in DMSO to a final concentration of 50 mg/mL to avoid the test strains negatively impacting the high toxicity of the two solvents. Following that, 100 mL of each extract was loaded into each well. The negative control was DMSO. After 2 hours to allow for full diffusion, the plates were incubated for the entire night at 37°C. The diameter of the negative control was compared to the diameter of the inhibitory zones, which included the well's (6 mm) diameter. Each test was performed in triplicate and the average of the three repetitions for each extract was determined.

2.3.3. Antimicrobial susceptibility test

The antimicrobial susceptibility assay was performed in MHA plates using the disc diffusion method (Kirby-Bauer disc diffusion susceptibility test protocol). Eight (four human and four aquatic) pathogens were evaluated individually against 16 antibiotics. The National Committee For Clinical Laboratory Standards' (NCCLS') disc diffusion method (NCCLS, 2000) was used to determine the results. Following are the tested antibiotics and their respective concentration ranges: ampicillin (10 µg), streptomycin (10 µg), erythromycin (10 µg), penicillin (10 µg), tetracycline (10 µg), doxycycline hydrochloride (10 µg), gentamycin (10 µg), neomycin (10 µg), cloxacillin (10 µg), chloramphenicol (10 µg), oxacillin (10 µg), azithromycin (10 µg), methicillin (10 µg), vancomycin (10 µg), amikacin (10 µg), polymyxin (10 µg), rifampicin (10 µg), ciprofloxacin (10 µg), kanamycin (10 µg), and bacitracin (10 µg). The NCCLS had established the same resistance breakpoints (NCCLS, 2000).

2.3.4. Statistical analysis

The results are shown as three replicates' mean and standard deviation (SD). SAS (v 6. 12) was used to conduct the statistical analysis. A one-way analysis of variance (ANOVA) at probability level $p \leq 0.05$ was used to statistically examine the collected data to determine the level of significance.

3. RESULTS

3.1. Antibacterial activity against tested bacterial pathogens

3.1.1. Azolla pinata

The water extract showed no efficacy against any of the pathogens. The methanol extract exhibited no activity against *V. cholera, A. baumannii*, or *K. pneumonia*, but it was most active against *S. aureus* (20 mm) (Figure 2A), followed by *A. caviae* (14 mm). The ethyl acetate extract exhibited negligible efficacy against *K. pneumonia* but had the greatest effectiveness against *V. cholera* and *E. tarda* (15 mm). The antibiotic tetracycline showed the greatest antibacterial activity against *E. tarda* (27 mm) and minimum activity against *K. pneumoniae* and *A. baumannii* (16 mm).

3.1.2. Salvinia molesta

The aqueous extraction showed no antibacterial activity against any of the pathogens. The methanol extract was most effective against *A. caviae* (11 mm) and *E. tarda* (18 mm). *A. hydrophila* (8 mm) exhibited the least amount of activity against ethyl acetate extract, while *E. tarda* (21 mm) exhibited the most. Tetracycline was the antibiotic with the highest action against *V. cholera* (29 mm) and the lowest against *K. pneumoniae* (15 mm) (Figure 2B).

3.1.3. Pistia stratiotes

None of the pathogens in this study showed any action in the water extract. *V. cholera* (8 mm) and *A. caviae* (10

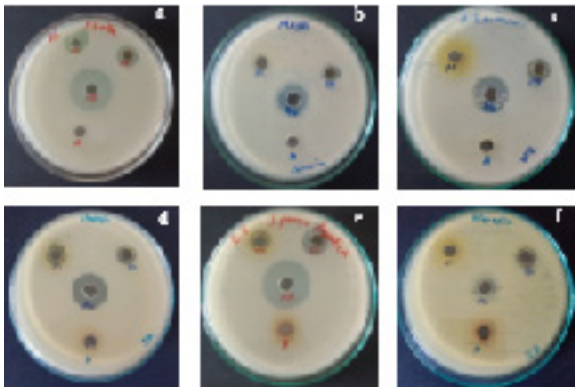

Figure 2. Antibacterial activity of selected aquatic plants agauut microbial pathogens

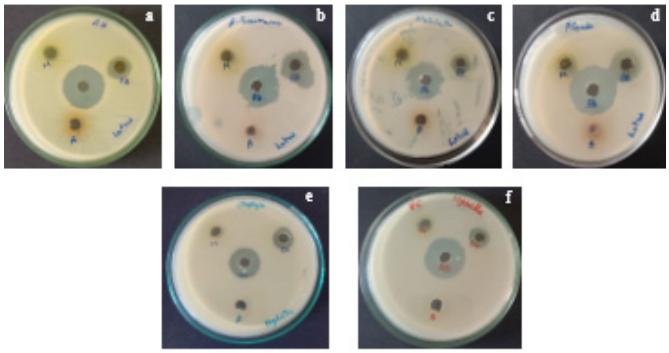

Figure 3. Antibacterial activity of selected aquanc plaits against microbial padiozer.

mm) were the pathogens against which the methanol extract had the greatest action, whereas other pathogens exhibited no activity. Ethyl acetate extract displayed maximum antibacterial efficacy against *P. aeruginosa* and *A. baumannii* (12 mm) (Figure 2C) and none against *A. hydrophila* and *S. aureus*. Tetracycline was the most effective antibiotic against *V. cholera* (27 mm) but did not affect *A. caviae*.

3.1.4. Ipomea aquatica

All the pathogens exhibited no activity in the aqueous extract. Methanol extract had little efficacy against *P. aeruginosa* and *K. pneumonia*; however, it was highly active against *E. tarda* (13 mm) and *S. aureus* (12 mm). The ethyl acetate extract exhibited the least amount of activity against *K. pneumonia* and *S. aureus* (12 mm) and the most against *A. baumannii* (17 mm). Tetracycline, an antibiotic, exhibited the least amount of efficacy against *K. pneumonia* (12 mm) and the most against *V. cholera, E. tarda*, and *A. hydrophila* (26 mm) (Figure 2D–F).

3.1.5. Nelumbo nucifera

The aqueous extract had no effect against any of the pathogens in *Nelumbo nucifera*. The methanol extracts showed little effect against *A. baumannii* and *E. tarda*, but they were most active against *P. aeruginosa* (13 mm) and *S. aureus* (12 mm). Maximum activity was demonstrated by the ethyl acetate extract against *P. aeruginosa* (16 mm), followed by *A. baumannii* (15 mm); the least activity was demonstrated against *E. tarda* (11 mm). Tetracycline had the highest activity against *P. aeruginosa* (27 mm) and the lowest against *A. hydrophila* and *A. caviae* (22 mm) (Table 2 and Figure 3A–D).

3.1.6. Hydrilla verticillate

The aqueous extraction in this study had no efficacy against any of the pathogens. The methanol extract had no activity against *A. hydrophila, A. baumannii*, or *S. aureus*; however, it did exhibit maximum activity against *A. caviae* and *E. tarda* (11 mm). Ethyl acetate extract had the least activity against *A. hydrophila* (9 cm) and the most against *P. aeruginosa* (15 mm). Tetracycline showed the least activity against *K. pneumonia* (15 mm) and *A. baumannii* (16 mm) and the most against *A. caviae, V. cholera*, and *E. tarda* (26 mm) (Table 2 and Figure 3E–F).

3.1.7. Eichhornia crassipes

Aqueous extracts showed no efficacy against any of the pathogens in this study. Maximum activity against *A. hydrophila* (10 mm) and *P. aeruginosa* (10 mm) was demonstrated by the methanol extract. A high level of activity against *P. aeruginosa* (12 mm) was demonstrated using ethyl acetate extracts, but no activity against *S. aureus* was detected. Tetracycline exhibited the least activity against *A. hydrophila* (23 mm) and the most against *A. baumannii* (26 mm) (Table 2).

3.1.8. Nymphaea alba

The aqueous extracts of *N. alba* displayed no activity against all pathogens. The petroleum ether extract had the maximum antibacterial activity against *E. tarda* (12 mm), whereas the minimum against *V. cholera* (9 mm) and none was found in *A. baumannii* and *K. pneumonia*. The highest activity was shown against *V. cholera, E. tarda*, and *P. aeruginosa* (13 mm) with ethyl acetate extract; no activity was detected against *A. baumannii* and *S. aureus.* Tetracycline revealed the maximum activity against *P. aeruginosa* (27 mm), followed by *S. aureus*

Table 2
Antibacterial activity of aquatic plants extracts against aquatic pathogens.

Aquatic Plants	Solvents	Zone of inhibition (mm)			
		AH	AC	VC	ET
Nelumbo nucifera (lotus)	Aqueous	NA*	NA	NA	NA
	Methanol	9.00	11.00	9.00	NA
	Ethyl Acetate	14.00	13.00	14.00	11.00
Positive control (streptomycin)		26.00	22.00	25.00	25.00
Nymphaea alba (water lily)	Aqueous	NA	NA	NA	NA
	Methanol	11.00	10.00	9.00	12.00
	Ethyl Acetate	11.00	11.00	13.00	11.00
Positive control (streptomycin)		24.00	23.00	23.00	23.00
Eichhornia crassipes (water hyacinth)	Aqueous	NA	NA	NA	NA
	Methanol	10.00	NA	NA	9.00
	Ethyl Acetate	10.00	10.00	9.00	9.00
Positive control (streptomycin)		23.00	24.00	25.00	25.00
Hydrilla verticillate	Aqueous	NA	NA	NA	NA
	Methanol	NA	11.00	9.00	11.00
	Ethyl Acetate	9.00	13.00	13.00	11.00
Positive control (streptomycin)		24.00	26.00	26.00	26.00
Ceratophyllum demersum (Coontail)	Aqueous	NA	NA	NA	NA
	Methanol	NA	12.00	NA	8.00
	Ethyl Acetate	10.00	12.00	11.00	10.00
Positive control (streptomycin)		25.00	25.00	26.00	26.00
Ipomea aquatica	Aqueous	NA	NA	NA	NA
	Methanol	10.00	11.00	9.00	13.00
	Ethyl Acetate	13.00	15.00	13.00	15.00
Positive control (streptomycin)		26.00	25.00	26.00	26.00
Azolla pinnata	Aqueous	NA	NA	NA	NA
	Methanol	9.00	14.00	NA	10.00
	Ethyl Acetate	12.00	13.00	15.00	15.00
Positive control (streptomycin)		26.00	25.00	26.00	27.00
Salvinia molesta	Aqueous	NA	NA	NA	NA
	Methanol	NA	11.00	9.00	18.00
	Ethyl Acetate	8.00	11.00	11.00	21.00
Positive control (streptomycin)		22.00	25.00	26.00	24.00
Pistia stratiotes	Aqueous	NA	NA	NA	NA
	Methanol	NA	10.00	8.00	10.00
	Ethyl Acetate	NA	11.00	12.00	10.00
Positive control (streptomycin)		26.00	26.00	29.00	27.00

*NA: no activity

(25 mm), and the minimum antibacterial activity against *K. pneumonia* (20 mm) (Table 2).

3.1.9. *Ceratophyllum demersum*

C. demersum water extract has shown little efficacy against all pathogens. *A. caviae* (12 mm) and *E. tarda* (8 mm) were the two pathogens against which the methanol extracts exhibited the highest activity, whereas the rest of the pathogens exhibited no activity at all. *A. caviae* (12 mm) and *V. cholera* (11 mm) were the two organisms against which ethyl acetate extract had the highest antibacterial activity. Tetracycline displayed the maximum activity against *V. cholera* and *E. tarda* (26 mm) and the minimum against *K. pneumonia* (15 mm) (Table 2).

3.2. Antibiotics sensitivity test

The results of standard antibiotic sensitivity tests on aquatic and human pathogens are given in Table 3. All aquatic pathogens from this study showed resistance against erythromycin and methicillin, whereas gentamicin, chloramphenicol, amikacin, ciprofloxacin, and neomycin were found to be sensitive. Among the four aquatic pathogens, three such as *A. caviae, V. cholera*, and *E. tarda* were found to be intermediate in kanamycin. Likewise, all human pathogens exhibited resistance against ampicillin, penicillin-G, oxacillin, and methicillin. In this study, gentamicin and amikacin showed sensitivity against all human pathogens whereas polymyxin exhibited intermediate results against all human pathogens.

Table 3
Antibiotic sensitivity profile of human pathogens.

Antibiotics	Human bacterial pathogens/Zone of inhibition (mm)			
	KP	AB	PA	SA
Streptomycin	12.00	16.00	16.00	22.00
Ampicillin	10.00	10.00	NIZ*	NIZ
Tetracycline	13.00	18.00	16.00	19.00
Erythromycin	NIZ	22.00	10.00	14.00
Penicillin	11.00	NIZ	9.00	12.00
Doxycycline hydrochloride	13.00	16.00	14.00	19.00
Gentamicin	19.00	22.00	18.00	24.00
Neomycin	14.00	18.00	18.00	22.00
Cloxacillin	NIZ	NIZ	NIZ	11.00
Chloramphenicol	18.00	18.00	16.00	19.00
Oxacillin	NIZ	9.00	NIZ	NIZ
Azithromycin	10.00	23.00	14.00	14.00
Methicillin	NIZ	NIZ	NIZ	NIZ
Vancomycin	NIZ	15.00	14.00	18.00
Amikacin	18.00	21.00	25.00	25.00
Polymyxin	10.00	10.00	10.00	10.00
Rifampicin	NIZ	10.00	9.00	13.00
Ciprofloxacin	20.00	18.00	19.00	28.00
Kanamycin	19.00	17.00	14.00	18.00
Bacitracin	NIZ	NIZ	NIZ	9.00

*NIZ: no inhibition zone.

4. DISCUSSION

Research on novel bioactive compounds for antibacterial properties has increased due to pathogen resistance to antibiotics. Numerous manufacturers of unidentified natural substances in the aquatic ecosystems are thought to have the ability to reduce or control bacterial infections (Aboukhalaf et al., 2020). Some water plants have antibacterial properties that are reported for future applications (Fareed et al., 2008). One advantage of exploring aquatic plants is their quick growth that facilitates the production of large extracts to isolate active chemicals (Wolff et al., 2008). A majority of aquatic plants possess an abundance of bioactive substances that are used in biological processes (Abu-Lafi et al., 2019). The aquatic plants selected for this study are found throughout India. When compared to terrestrial plants, aquatic plants have fewer reports. Therefore, it is important to investigate their applications and conduct comprehensive, broad research to understand their therapeutic properties.

Table 4
Antibacterial activity of aquatic plant extracts against human pathogens.

Aquatic Plants	Solvents	Human pathogens*/Zone of inhibition (mm)			
		PA	AB	SA	KP
Nelumbo nucifera (lotus)	Aqueous	NA	NA	NA	NA
	Methanol	13.00	NA	12.00	10.00
	Ethyl Acetate	16.00	15.00	14.00	12.00
Positive control (streptomycin)		26.00	26.00	25.00	25.00
Nymphaea alba (water lily)	Aqueous	NA	NA	NA	NA
	Methanol	10.00	NA	10.00	NA
	Ethyl Acetate	13.00	NA	NA	11.00
Positive control (streptomycin)		27.00	20.00	25.00	20.00
Eichhornia crassipes (water hyacinth)	Aqueous	NA	NA	NA	NA
	Methanol	10.00	NA	NA	NA
	Ethyl Acetate	12.00	9.00	NA	10.00
Positive control (streptomycin)		25.00	26.00	21.00	25.00
Hydrilla verticillate	Aqueous	NA	NA	NA	NA
	Methanol	10.00	NA	NA	9.00
	Ethyl Acetate	15.00	12.00	13.00	11.00
Positive control (streptomycin)		23.00	16.00	20.00	15.00
Ceratophyllum demersum (Coontail)	Aqueous	NA	NA	NA	NA
	Methanol	NA	NA	NA	NA
	Ethyl Acetate	10.00	NA	NA	NA
Positive control (streptomycin)		18.00	20.00	20.00	15.00
Ipomea aquatica	Aqueous	NA	NA	NA	NA
	Methanol	NA	10.00	12.00	NA
	Ethyl Acetate	15.00	17.00	12.00	12.00
Positive control (streptomycin)		25.00	20.00	20.00	17.00
Azolla pinnata	Aqueous	NA	NA	NA	NA
	Methanol	10.00	NA	20.00	NA
	Ethyl Acetate	10.00	11.00	13.00	NA
Positive control (streptomycin)		18.00	16.00	20.00	16.00
Salvinia molesta	Aqueous	NA	NA	NA	NA
	Methanol	NA	10.00	NA	NA
	Ethyl Acetate	NA	10.00	NA	NA
Positive control (streptomycin)		18.00	20.00	20.00	15.00
Pistia stratiotes	Aqueous	NA	NA	NA	NA
	Methanol	NA	NA	NA	NA
	Ethyl Acetate	12.00	12.00	-	10.00
Positive control (streptomycin)		19.00	20.00	20.00	16.00

Globally, numerous researchers have assessed the antibacterial properties of diverse aquatic plants (Baral and Vaidya., 2011; Vadlapuli, 2010). *E. crassipes* water extracts demonstrated strong antibacterial activity against every tested bacterium; these findings were consistent with those from a previous study by Chand et al. (2014). The antibacterial efficacy of water hyacinth methanolic and aqueous extracts against several bacterial strains was revealed by Rufchaei et al. (2021), which exhibited the strongest efficacy among them. These findings contradict the present results because there was no activity in aqueous extracts. The methanolic extracts of *E. crassipes* showed a significant zone of inhibition (12–17 mm) against the gram-positive bacteria *S. aureus* (Kiristos et al., 2018). This differed from the finding of our study because there was no antibacterial potential in methanolic extracts of *E. crassipes* and no recorded activity in methanolic extract against *S. aureus*. This result is similar to Gutiérrez -Morales et al. (2017) who reported the same results in methanolic extract against *S. aureus*. Haggag et al. (2017) studied the methanol extract of *E. crassipes* against *P. aeruginosa* in which the inhibition zone was less. These results were similar to our findings. Joshi and Kaur (2013) reported the antibacterial activities of aqueous extract in *E. crassipes*. The results revealed that aqueous extract has weak antibacterial potential but our study indicated no activity in aqueous extracts.

Al-Maliki et al. (2017) reported that *N. alba* ethanolic extract had high effectiveness against *S. aureus.* According to our study, *N. alba*'s methanolic extract was less effective against *S. aureus*. Daboor and Haroon (2012) recorded the alcoholic extract of *N. alba* against *S. aureus* showing that the inhibition zone was very high. The alcoholic extract of *N. alba* exhibited reduced effectiveness against *S. aureus* in our study. The effectiveness of *N. alba*'s aqueous extract against *S. aureus, K. pneumonia*, and *P. aeruginosa* was assessed by Yildirim et al. (2013). The findings showed that the aqueous extract exhibited a modest level of antibacterial activity against *S. aureus*, whereas no activity was found in *P. aeruginosa* and *K. pneumonia* compared to our study.

Saraswathi et al. (2019) revealed that water extracts of *N. nucifera* have moderate activity in *S. aureus*, but our study found that there was no activity in the water extracts of *N. nucifera*. Paudel and Panth (2015) reported aqueous and ethyl acetate extract of *N. nucifera* against *S. aureus*. The results revealed that aqueous extract has no activity and only moderate activity was found in ethyl acetate. This result was similar to our findings, with more or less the same activity against *S. aureus*. Kumar and Gitika (2014) studied and compared methanolic and aqueous extracts of *N. nucifera*, and the results indicated that the methanolic extract was the most effective since it demonstrated the largest inhibitory zone. However, according to our research, ethyl acetate has more potent antibacterial properties than methanol, while the aqueous extract showed no action. Arjun et al.'s (2012) documented methanol extracts showed moderate activity in *S. aureus*, and these findings agree with our study of methanol extracts against *S. aureus*. Our investigation found that ethyl acetate had considerable antimicrobial activity, while a similar study by Li and Xu (2008) showed that the methanol extract recorded the maximum antibacterial activity.

According to Pandi Prabha and Rajkumar (2015), *H. verticillata* ethanolic extract had a positive inhibitory activity for bacteria. In our investigation, methanol and ethyl acetate have shown moderate and strong antibacterial activity, while aqueous extracts showed none. Likewise, Kumari and Kumari (2013) documented the methanolic extracts of *H. verticilata* against *S. aureus* and *K. pneumonia*. The result revealed that the inhibition zone of two pathogens was moderate. But in our study, no activity was found in *S. aureus* and only moderate activity was present in *K. pneumonia*. The antibacterial properties of *C. demersum* stem and leaf methanolic extracts were reported by Omar et al. (2017). The results showed potent antimicrobial activity in pathogens, but our study revealed only moderate antimicrobial activity, and that the methanol extract of *C. demersum* against *S. aureus* had no inhibition zone. This result was similar to the study reported by Malathy and Stanley (2015) of methanol extract of *C. demersum* against *S. aureus*. Fareed et al. (2008) reported aqueous and methanolic extracts of *C. demersum* against *P. aeruginosa, K. pneumonia*, and *S. aureus*. The results revealed that aqueous extracts had good activity and that methanolic extracts had moderate activity.

Methanolic extracts of *I. aquatica* have shown modest efficacy against *S. aureus*, according to our investigation. These findings concurred with those of Manvar and Desai (2013), who found that *S. aureus* was less active. Methanolic and aqueous extracts of *I. aquatica* were shown to be effective against *S. aureus* and *P. aeruginosa* (Dhanasekaran et al., 2010). The findings demonstrated that the aqueous and methanolic extracts both exhibited good efficacy. However, neither the methanolic nor aqueous extracts of *I. aquatica* showed any efficacy against *P. aeruginosa* or *S. aureus* in our investigation. *I. aquatic*a aqueous extract exhibited moderate efficacy against *S. aureus, P. aeruginosa*, and *K. pneumonia* (Bhaigybati et al., 2020; Velmurugan and Suresh, 2020); however, no activity was detected in our investigation.

A. pinnata aqueous and methanolic extracts were shown to be effective against both *P. aeruginosa* and *S. aureus* by Farook et al. (2019). The findings showed that both aqueous and methanolic extracts had moderate activity. This result differed from our study because methanolic extract had good activity and aqueous extract showed none. The effectiveness of *A. pinnata* against *P. aeruginosa* and *S. aureus* was previously assessed by Kumar et al. (2017) using its methanol and ethyl acetate extracts. The activity was modest in the methanolic extract, whereas the inhibition zone was significant in the *S. aureus* ethyl acetate extract and moderate in *P. aeruginosa*. This finding runs counter to our investigation because the ethyl acetate extract has modest action against two pathogens, whereas the methanolic extract exhibits good activity against *P. aeruginosa*.

Mandal and Mondal (2011) reported aqueous extracts of *S. molesta* against *V. chlorae* and *K. pneumonia* in which no activity was present. Because of its moderate activity, this result differs from that of the methanolic extract and was comparable to our investigation in aqueous extract. *P. stratiotes* methanolic extract was shown to be effective against *P. aeruginosa, K. pneumonia*, and *S. aureus* by Abraham et al. (2014). The result showed less activity in the methanolic extract of *P. stratiotes.* This result was different from our study because no activity was found. Likewise, Mukhtar and Tukur, (2019) studied aqueous extract of *P. stratiotes* against *P. aeruginosa*, *K. pneumonia*, and *S. aureus*. According to the results, the aqueous extract had good efficacy against *S. aureus* while others showed no activity. However, according to our investigation, *P. stratiotes* aqueous extract is inactive against any infections. While there was no activity in our investigation, the aqueous extract of *P. stratiotes* showed modest effectiveness against *P. aeruginosa* and *S. aureus* (Rajapaksha et al., 2020). Rahman et al. (2011) reported a methanolic extract of *P. stratiotes* against *S. aureus* in which the inhibition zone was less. This result contradicts our study because no activity was found in the aqueous extract. Our study showed that ethyl acetate extract exhibited significant antimicrobial potential. More research is needed to discover the exact components responsible for the antimicrobial action of these extracts. Aqueous extracts exhibited no antimicrobial activity in our study. Future research could examine how these extracts perform against other infections.

4. CONCLUSIONS

The study conducted on the antibacterial properties of aquatic plants in the Tirunelveli district presents significant findings in the field of natural antimicrobials. Extracts made with ethyl acetate and methanol showed significant antibacterial action against a variety of bacterial species, including aquatic and human diseases, according to the study, which concentrated on nine distinct aquatic plants. The most striking outcome of this research is the superior antibacterial potential of the ethyl acetate extracts, followed by the moderate activity exhibited by the methanolic extracts. In contrast, the aqueous extracts showed no antibacterial activity. These findings are particularly relevant in the context of increasing antibiotic resistance, as they suggest that certain aquatic plants could be valuable sources of novel antibacterial compounds. The use of these plant extracts, especially those derived from ethyl acetate, could potentially lead to the development of new drugs for treating infectious diseases caused by bacterial pathogens. Furthermore, this research underscores the importance of exploring local biodiversity—in this case, aquatic plants from specific regions of southern India—for potential medical applications.

ETHICAL APPROVAL

The data of the manuscript will comply with the ethical standards.

DATA AVAILABILITY

This published article contains all the data created or analyzed during this investigation.

AUTHORS CONTRIBUTION

K.K.: Performed the experimental work and developed the overall study design. K.S.: Supervised the research, contributed to experimental planning, and fine-tuned methodology.

R.T.: Assisted in laboratory experiment. A.M.B.: Supported the drafting and organization of the manuscript content. V.A.: Provided critical corrections and refinement of the final article.

CONFLICTS OF INTEREST

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