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Investigating of antibacterial properties of polar and non-polar extracts of brown algae (*Iyengaria stellata*) from Qeshm Island, Persian Gulf

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ABSTRACT

Algae can be used as a potential source of natural compounds for the development of medicine production and treatment of many diseases due to the production of secondary metabolites. The present study was performed to determine the antibacterial activity of brown algae (*Iyengaria stellata*) extract from the Qeshm island coasts. The collected algae were transferred to the Persian Gulf and Oman Sea Ecological Research Institute and the samples were identified according to their physical characteristics. Four solvents of N-hexane, diethyl ether, acetone and methanol were used for extraction, and then their antibacterial properties were investigated at concentrations of 50 µg/ml, 100 µg/ml, 200 µg/ml, 500 µg/ml, 1000 µg/ml, 2000 µg/ml, 3000 µg/ml, 4000 µg/ml and 5000 µg/ml on 4 bacterial species *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Listeria monocytogenes*. The results of this study showed a significant antibacterial effect of N-hexane and Estonian extracts, they were followed by diethyl ether extract, but methanolic extract had no antibacterial affect. According to the results, *Iyengaria stellata* has significant antimicrobial effects against gram-positive bacteria *Listeria monocytogenes* and *Staphylococcus aureus* and can possibly be used as a natural source in the treatment of infections. Therefore, it is suggested that while purifying the compounds, identifying and determining the specific role of each of them, be used to make medicinal compounds and replace them with industrial antibiotics.

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Introduction

More than 70% of the earth's surface is covered by saline water sources (seas and oceans), which has mostly remained unexplored (Hasani-Azhdari *et al.*, 2022). While the biodiversity in lands is great, the largest biodiversity exists in the saline water sources characterized by 226,000 eukaryotic marine species. Though, it has been likely only a small part of the whole organisms that have not yet been explored (Appeltans *et al.*, 2012). Solving the problem of continuous emergence of new strains of antibiotic-resistant pathogenic bacteria is one of the difficult and great challenges of modern medicine (Azhdari *et al.*, 2023). Presently, about 700,000 people die each year due to antibiotic-resistant infections, which is estimated to increase to 10 million a year (O'neill, 2014; WHO, 2020; Azhdari *et al.*, 2022).

Food poisoning is a concern for both consumers and the food industry, despite the use of various methods of food protection. The prevalence of diseases caused by some pathogenic microorganisms and spoilage in food is growing. Nowadays, consumers want fresh foods that are at least processed. In addition, consumers are concerned about the safety of foods containing artificial preservatives. These cases have put pressure on the food industry and led to increased research into the discovery of alternative natural antimicrobials (Nazir *et al.*, 2017). Food spoilage due to the presence of bacteria causes economic damage on a global scale. Since many seaweeds have developed good defense mechanisms against bacteria, there is a tendency to use seaweed as a natural antimicrobial source. To meet the needs, in addition requirement for develop new antimicrobial chemical compounds, extensive research is necessary to discover natural antimicrobial sources and compounds in order to reduce the dependence of modern medicine on traditional antimicrobial compounds. Brown algae are natural source of biologically active substances. The unique property of these compounds is a wide range of biological activities such as: antiviral, antibacterial, anticoagulant, immuno stimulating, anti-inflammatory, and antitumor. (Cumashi *et al.*, 2007; Dashtiannasab *et al.*, 2012; Morya *et al.*, 2012; Mohammadi *et al.*, 2020). These bioactive compounds in macroalgae are produced in the difficult and complex conditions of their habitat so that they are not found in other living organisms (Lordan *et al.*, 2011).

Iyengaria stellata is a species of brown algae belonging to the class Phaeophyceae, order Scytosiphonales and family Scytosiphonaceae. This alga grows in the area between the tides, especially in the lower to shallow parts below the tides and on the surface of rocky beds. *I. stellata* usually grows in groups with the brown alga *Csinuosa sinuosa* (Mohammadi *et al.*, 2016). Due to the disadvantages and harms caused by the use of chemical antibacterial drugs and the emergence of antibiotic-resistant strains, the need to use natural compounds with antimicrobial ability has become more than before. On the other hand, one of the important and accessible sources for the extraction these natural compounds is seaweeds. Therefore, due to the high potential of the Persian Gulf to access a variety of marine algae with high bioactive compounds, this study investigates the antibacterial properties of *I. stellata* extract.

Material and Methods

The algae (*I. stellate)* were collected from the Shib Deraz area on Qeshm Island and transferred to the Persian Gulf and Oman Sea Ecology Research Institute. FAO identification keys was performed. *Escherichia coli* and *Staphylococcus aureus* strains were obtained from the Food

Science Department of the University of Tehran Campus of Agriculture and Natural Resources, and *Pseudomonas aeruginosa* and *Listeria monocytogenes* strains were obtained from the Science and Technology Park of the University of Tehran Campus of Agriculture and Natural Resources. After drying in the freezer (24 hours, 70°C), the algae were extracted using 4 solvents: N-hexane, diethyl ether, acetone and methanol (Nazemi and Wheater, 2014).

The strains were cultured to obtain their colonies. The colonies were inoculated into broth medium in test tubes. This was repeated until the opacity of the broth medium was equal to that of the half McFarland tube (0.5 McFarland). The antibacterial properties of the extracts with concentrations of 50 µg/ml, 100 µg/ml, 200 µg/ml, 500 µg/ml, 1000 µg/ml, 2000 µg/ml, 3000 µg/ml, 4000 µg/ml and 5000 µg/ml were investigated by agar diffusion method (well) and tubular dilution (Bacterial Broth Dilution Methods) and also by well dilution method by ELISA reader.

Minimum Inhibitory Concentration (MIC) and also Minimum Bactericidal Concentration (MBC) were obtained (Rosenblatt, 1991). Also in this study, Tetracycline and Ampicillin antibiotics were used to consider a positive control and no biologically active substance was added to one of the tubes as a negative control. In a tube, only the culture medium without active ingredient and bacteria was placed to determine the test error in case of environmental contamination. SPSS 25 software was used for data analysis.

Results

Based on the results, the concentration of N-hexane extract required for growth inhibitory effect in *P. aeruginosa* 5000 µg/ml, on *L. monocytogenes* 3000 µg/ml, in *Escherichia coli* 4000 µg/ml and in *S. aureus* 2000 µg/ml. According to these results, N-hexane extract has a greater inhibitory effect on the growth of *L. monocytoge* and *S. Aureus*. Diethyl ether extract at a concentration of 5000 µg/ml had an inhibitory effect on *P. aeruginosa*, while this extract had no effect on any of the other bacteria and did not inhibit their growth. Estonia extract at a concentration of 1000 µg/ml has the ability to inhibit the growth of *S. aureus*. It also has growth inhibitory effect for *Escherichia coli* at a 4000 µg/ml. But in the case of the other bacterias, the Estonian extract has no inhibitory effect. In fact, the Estonian extract has the highest growth inhibitory power on *S. aureus*, followed by *E. coli*. Methanolic extract had no inhibitory power on bacterial growth and turbidity was observed in all test tubes (Table 1).

Table 1. MIC results of antibiotic testing of N-hexane, diethyl ether, acetone and methanolic extracts of I.stellata:

		Staphylococcus aureus								
	concentration	5000 µg/ml	4000 µg/ml	3000 µg/ml	2000 µg/ml	1000 µg/ml	500 µg/ml	200 µg/ml	100 µg/ml	50 µg/ml
N-hexane	Turbidity	-	-	-	-	+	+	+	+	+
Diethyl ether	Turbidity	+	+	+	+	+	+	+	+	+
Acetone	Turbidity	-	-	-	-	-	+	+	+	+
Methanol	Turbidity	+	+	+	+	+	+	+	+	+
		Escherichia coli								
	concentration	5000 µg/ml	4000 µg/ml	3000 µg/ml	2000 µg/ml	1000 µg/ml	500 µg/ml	200 µg/ml	100 µg/ml	50 µg/ml
N-hexane	Turbidity	-	-	+	+	+	+	+	+	+
Diethyl ether	Turbidity	+	+	+	+	+	+	+	+	+
Acetone	Turbidity	-	-	+	+	+	+	+	+	+

Methanol	Turbidity	+	+	+	+	+	+	+	+	+
		Listeria monocytogene								
	concentration	5000 µg/ml	4000 µg/ml	3000 µg/ml	2000 µg/ml	1000 µg/ml	500 µg/ml	200 µg/ml	100 µg/ml	50 µg/ml
N-hexane	Turbidity	-	-	-	+	+	+	+	+	+
Diethyl ether	Turbidity	+	+	+	+	+	+	+	+	+
Acetone	Turbidity	+	+	+	+	+	+	+	+	+
Methanol	Turbidity	+	+	+	+	+	+	+	+	+
		Pseudomonas aeruginosa								
	concentration	5000 µg/ml	4000 µg/ml	3000 µg/ml	2000 µg/ml	1000 µg/ml	500 µg/ml	200 µg/ml	100 µg/ml	50 µg/ml
N-hexane	Turbidity	-	+	+	+	+	+	+	+	+
Diethyl ether	Turbidity	-	+	+	+	+	+	+	+	+
Acetone	Turbidity	+	+	+	+	+	+	+	+	+
Methanol	Turbidity	+	+	+	+	+	+	+	+	+

+ : Turbidity observed (no inhibition of bacterial growth)
- : Turbidity not observed (inhibition of bacterial growth)

The effects of the different solvent extracts of *I. stellata* on bacterial growth and proliferation over a 24-h period are presented in Figures 1-3 and summarized in Table 2. The antibacterial activity of n-hexane, diethyl ether, acetone, and methanol extracts at concentrations of 2000 to 5000 µg/mL after 24 hours of incubation exhibited a concentration-dependent inhibitory effect against the studied strains (P<0.05). The highest concentration of diethyl ether and methanol extracts significantly reduced the growth of *S. aureus*. Among the gram-negative strains, *E. coli* showed the highest sensitivity to n-hexane and acetone extracts and was relatively resistant to other solvents. However, the most striking finding was the inhibition of *P. aeruginosa* by the diethyl ether extract; at a concentration of 5000 µg/mL, this extract exhibited a much stronger and more effective performance than the antibiotic.

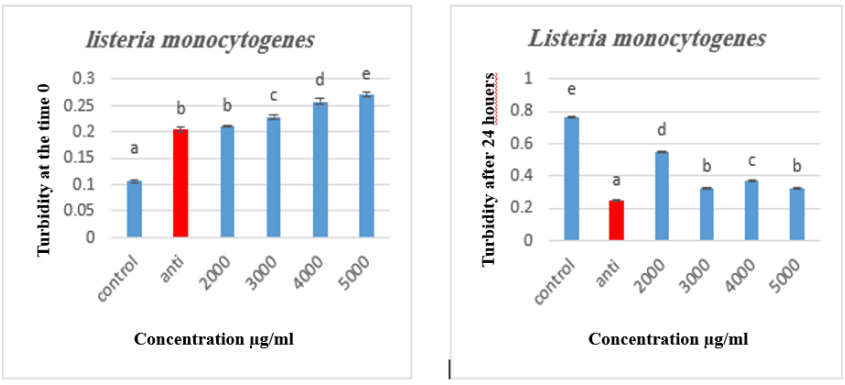

Figure 1. The effect of N-hexane extract on *Listeria monocytogenes after 24 hours using microplate reader, the second column shows the tetracycline antibiotic at the MBC concen*tration of each bacterium. The presence of dissimilar letters in each row indicates the existence of a significant difference.

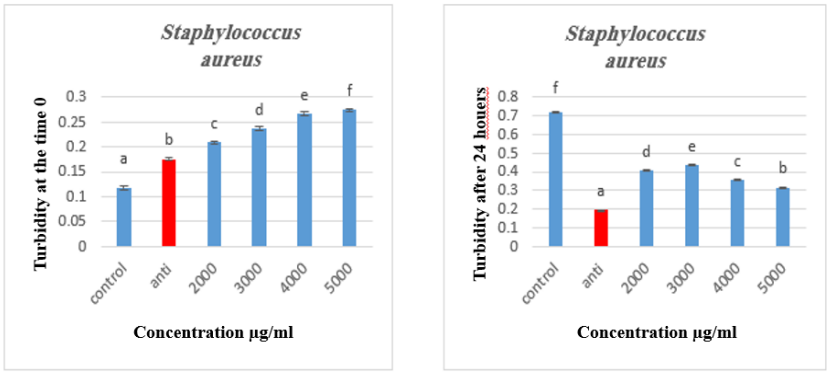

Figure 2. The effect of N-hexane extract on *Staphylococcus aureus* after 24 hours using microplate reader, the second column shows the tetracycline antibiotic in the MBC concentration of the bacterium. The presence of dissimilar letters in each row indicates the existence of a significant difference.

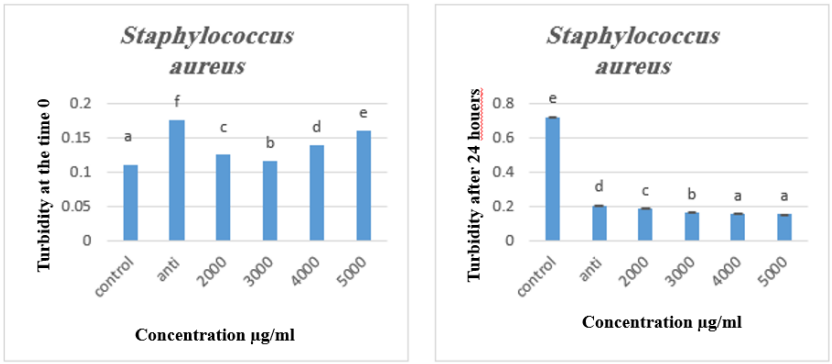

Figure 3. Effect of Estonian extract on *Staphylococcus aureus* 24 After 24 hours using microplate reader, the second column shows the antibiotic tetracycline MBC of the bacterium. The presence of dissimilar letters in each row indicates the existence of a significant difference.

Table 2. Effect of N-hexane, diethyl ether, acetone and methanolic extracts of *Iyengaria stellata* on *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogene* and *Pseudomonas aeruginosa*, after 24 hours using microplate reader, Anti: Tetracycline antibiotics at MBC concentrations related to each bacterium

Staphylococcus aureus							
N-hexane	Concentration	0	Anti	2000	3000	4000	5000
	Time 0	–	–	–	–	–	–
	Time 24 hours	–	–	–	–	–	–
Diethyl ether	Time 0	0.11233±0.003^{a} b	0.23400±0.003 c	0.10900±0.002 a	0.11700±0.004 b	0.11967±0.005 b	0.11900±0.005 b
	Time 24 hours	0.65967±0.006^{d}	0.24767 ± 0.002^{a}	0.64267 ± 0.010^{c}	0.64133 ± 0.010^{c}	0.63933 ± 0.003^{c}	0.61867 ± 0.015^{b}
Acetone	Time 0	–	–	–	–	–	–
	Time 24 hours	–	–	–	–	–	–
Methanol	Time 0	0.12200 ± 0.003^{c}	0.25567 ± 0.003^{d}	0.11033 ± 0.001^{a}	0.12167 ± 0.001^{c}	0.11533 ± 0.001^{b}	0.11667 ± 0.002^{b}
	Time 24 hours	0.67733 ± 0.002^{e}	0.24833 ± 0.002^{a}	0.65333 ± 0.002^{d}	0.63500 ± 0.005^{c}	0.61633 ± 0.005^{bc}	0.61467 ± 0.004^{b}
Escherichia coli							
N-hexane	Concentration	0	Anti	2000	3000	4000	5000
	Time 0	0.10567 ± 0.004^{a}	0.19567 ± 0.003^{b}	0.21033 ± 0/001^{c}	0.22933 ± 0.003^{d}	0.25433 ± 0.007^{e}	0.27500 ± 0.003^{f}
	Time 24 hours	0.75367 ± 0.003^{f}	0.29433 ± 0.002^{a}	0.61900 ± 0.016^{e}	0.50833 ± 0.010^{d}	0.33933 ± 0.010^{b}	0.34167 ± 0.008^{c}
Diethyl ether	Time 0	0.10933 ± 0.002^{a}	0.24133 ± 0.002^{c}	0.11200 ± 0.000^{a}	0.11567 ± 0.008^{ab}	0.11600 ± 0.005^{ab}	0.12133 ± 0.002^{b}

	Time 24 hours	0.75000 ± 0.003^{b}	0.25900 ± 0.002^{a}	0.75600 ± 0.006^{b}	0.75067 ± 0.011^{b}	0.73300 ± 0.030^{c}	0.75833 ± 0.001^{b}
Acetone	Time 0	0.11533 ± 0.004^{a}	0.25567 ± 0.003^{b}	0.21033 ± 0/001^{c}	0.25967 ± 0.003^{d}	0.27033 ± 0.007^{e}	0.29567 ± 0.003^{f}
	Time 24 hours	0.72567 ± 0.003^{f}	0.29433 ± 0.002^{a}	0.62333 ± 0.016^{e}	0.54867 ± 0.010^{d}	0.38933 ± 0.010^{c}	0.41167 ± 0.008^{b}
Methanol	Time 0	0.12200 ± 0.002^{b}	0.27567 ± 0.002^{c}	0.10533 ± 0.005^{a}	0.11100 ± 0.005^{a}	0.11000 ± 0.000^{a}	0.12100 ± 0.001^{b}
	Time 24 hours	0.83400 ± 0.003^{d}	0.28000 ± 0.001^{a}	0.83500 ± 0.011^{c}	0.82933 ± 0.011^{bc}	0.82633 ± 0.001^{b}	0.82567 ± 0.007^{b}
Listeria monocytogene							
N-hexane	Concentration	0	Anti	2000	3000	4000	5000
	Time 0	–	–	–	–	–	–
	Time 24 hours	–	–	–	–	–	–
Diethyl ether	Time 0	0.11267 ± 0.004^{ab}	0.22400 ± 0.006^{d}	0.10667 ± 0.003^{a}	0.11567 ± 0.001^{b}	0.11833 ± 0.005^{b}	0.12567 ± 0.004^{c}
	Time 24 hours	0.76100 ± 0.004^{c}	0.23800 ± 0.005^{a}	0.75933 ± 0.005^{c}	0.75100 ± 0.014^{b}	0.74967 ± 0.017^{b}	0.74833 ± 0.007^{b}
Acetone	Time 0	0.11667 ± 0.003^{a}	0.20100 ± 0.002^{f}	0.12133 ± 0.000^{b}	0.13300 ± 0.001^{c}	0.15933 ± 0.003^{d}	0.16333 ± 0.002^{e}
	Time 24 hours	0.77533 ± 0.002^{f}	0.25100 ± 0.002^{a}	0.67633 ± 0.001^{e}	0.64733 ± 0.002^{d}	0.63133 ± 0.003^{c}	0.62367 ± 0.001^{b}
Methanol	Time 0	0.11800 ± 0.003b^{c}	0.23000 ± 0.003^{d}	0.10867 ± 0.005^{a}	0.11467 ± 0.002^{ab}	0.11933 ± 0.002^{bc}	0.12333 ± 0.004^{c}
	Time 24 hours	0.78600 ± 0.003^{f}	0.23833 ± 0.002^{a}	0.65067 ± 0.001^{e}	0.64200 ± 0.007^{d}	0.63667 ± 0.011^{c}	0.62533 ± 0.012^{b}
Pseudomonas aeruginosa							
N-hexane	Concentration	0	Anti	2000	3000	4000	5000
	Time 0	0.09933 ± 0.004^{a}	0.19267 ± 0.003^{b}	0.21000 ± 0.007^{c}	0.23033 ± 0.001^{d}	0.26433 ± 0.006^{e}	0.28133 ± 0.002^{f}
	Time 24 hours	0.85667 ± 0.004^{f}	0.21700 ± 0.002^{a}	0.77200 ± 0.012^{e}	0.63400 ± 0.006^{d}	0.56833 ± 0.020^{c}	0.36800 ± 0.009^{b}
Diethyl ether	Time 0	0.11300 ± 0.004^{ab}	0.24733 ± 0.002^{c}	0.11067 ± 0.006a	0.11633 ± 0.001^{ab}	0.11767 ± 0.003^{ab}	0.11967 ± 0.005^{b}
	Time 24 hours	0.72500 ± 0.004^{f}	0.24033 ± 0.002^{b}	0.67067 ± 0.004^{c}	0.62333 ± 0.007^{e}	0.53533 ± 0.007^{d}	0.2200 ± 0.005^{a}
Acetone	Time 0	0.10100 ± 0.002^{a}	0.17633 ± 0.002^{e}	0.10633 ± 0.003^{b}	0.13067 ± 0.001^{c}	0.16500 ± 0.005^{d}	0.18033 ± 0.001^{e}
	Time 24 hours	0.69700 ± 0.004^{e}	0.22733 ± 0.001^{a}	0.67233 ± 0.006^{d}	0.64933 ± 0.010^{c}	0.63867 ± 0.001^{c}	0.61633 ± 0.000^{b}
Methanol	Time 0	0.11233 ± 0.002^{a}	0.23500 ± 0.002^{c}	0.10800 ± 0.008^{a}	0.11733 ± 0.002^{ab}	0.11733 ± 0.005^{ab}	0.12633 ± 0.005^{b}
	Time 24 hours	0.78733 ± 0.002^{b}	0.23600 ± 0.002^{a}	0.78633 ± 0.003^{b}	0.78700 ± 0.003^{b}	0.78600 ± 0.000^{b}	0.78433 ± 0.003^{b}

The presence of dissimilar letters in each row indicates the existence of a significant difference (P<0.05). Anti: Tetracycline antibiotic in MBC concentration for each bacterium.

The antibacterial potential of different extracts of *I. stellata* algae was evaluated by determining the MIC and MBC values compared to standard antibiotics (tetracycline and ampicillin) (Tables 3, 4 and 5). According to the results in Table 3, the antibiotics exhibited similar, potent inhibitory activity against all four bacterial strains (MICs ranging from 750 to 1500 µg/mL). Among the solvents used for extraction, the N-hexane extract exhibited the broadest antibacterial spectrum, inhibiting the growth of all Gram-positive and Gram-negative strains. The lowest inhibitory concentration of this extract was reported to be 3000 for Gram-positive bacteria (*S. aureus* and *L. monocytogenes*) and 4000 and 5000 µg/mL for Gram-negative bacteria (*E. coli* and *P. aeruginosa*), respectively. On the other hand, the acetone extract, although it had a more limited spectrum of action, exhibited the strongest inhibitory potential against S. aureus, with a MIC of 1000 µg/mL, which is highly competitive with standard antibiotics (MIC=750). The diethyl ether extract inhibited the resistant strain of *P. aeruginosa*

only at 5000 µg/mL, and the methanol extract showed no inhibitory activity against the evaluated pathogens at the tested concentrations (MIC > 5000).

Examination of the bactericidal properties of the extracts (Tables 4 and 5) revealed that the algal extracts eliminated certain bacterial strains. The reference antibiotics at concentrations of 1500 to 3000 µg/mL caused complete bacterial death. Regarding the extracts, the acetone extract was bactericidal against *S. aureus* at 3000 µg/mL, reducing the colony counts to zero. Also, the n-hexane extract at the highest concentration evaluated (5000 µg/mL) showed absolute bactericidal effects (zero colonies) against both Gram-positive bacteria (*S. aureus* and *L. monocytogenes*). The lack of MBC values for other extracts against Gram-negative bacteria indicates that these extracts at the aforementioned concentrations are primarily bacteriostatic (growth-inhibitory) and that concentrations higher than 5000 are required for bactericidal effects.

Table 3. Comparison of MIC results of antibiotic test of two Tetracycline and Ampicillin antibiotics and algal extracts tested in micrograms per milliliter.

	S. ureus	*E. coli*	*L. monocytogenes*	*P. aeruginosa*
Tetracycline	750	750	1000	1500
Ampicillin	750	750	1000	1500
Acetone	1000	4000	-	-
N-hexane	3000	4000	3000	5000
Diethyl ether	-	-	-	5000
Methanol	-	-	-	-

Table 4. MBC results from I.stellata antibiotic test.

Concentration of algal extract	Bacteria	Colonies number
N-hexane 5000 µg/ml	*S. aureus*	0
N-hexane µg/ml 5000	*L. monocytogenes*	0
Acetone µg/ml 3000	*S. aureus*	0

Table 5. Comparison of MBC results from antibiotic test of two tetracycline and ampicillin antibiotics and algal extracts tested in micrograms per milliliter.

	S. aureus	*E. coli*	*L. monocytogenes*	*P. aeruginosa*
Tetracycline	1500	1500	2000	1500
Ampicillin	1500	1500	2000	3000
Acetone	3000	-	-	-
N-hexane	5000	-	5000	-

Discussion

Due to the vastness of saline water sources and the diversity of aquatic species and their bioactive compounds, these species need to be carefully studied. Research into the antibacterial properties of marine organisms began around 1950 (Ryan and Ray, 2004). Between 1960 and 1990, about 300 marine organisms with the ability to produce bioactive compounds were identified and 6,500 bioactive compounds were obtained from them (Hickman *et al.*, 2006). In recent years, research into the chemistry of algae (or marine organisms in general) has increased dramatically due to the need for compounds that have biological activity, potential medicinal applications, or other potential economic properties.

The highest growth inhibitory effect was related to N-hexane and acetone extracts from the studied algae on *S. aureus*. The methanolic extract had no antibacterial effect. But in a on the antibacterial activity of brown algae on the Vedalai coast, seven methods were used to extract, methanol, astonia, petroleum ether, ethanol, ethyl acetate, chloroform, and diethyl ether. As a result, methanolic extract was more effective than other extracts (Manivannak *et al.*, 2011). This result is not consistent with the present study, the discrepancy may be due to differences in the composition of the algae as well as factors such as the season of their collection, the environment and the different stages of algal growth, the extraction methods and the species of algae (Azhdari *et al.*, 2022). In another study, the biological effects of ethyl acetate, methanol and aqueous methanol extracts of the brown algae *Colpomenia sinousa* and *I. stellata* from the northern shores of the Persian Gulf were performed, All three extracts of ethyl acetate, methanol and aqueous methanol of two brown algae showed antibacterial effects, The ethyl acetate extract of *I. stellata* had a more inhibitory effect on *S. aureus*, *E. coli* and *P. aeruginosa* than *Colpomenia sinousa* (Farahani *et al.*, 2014), The result of the study is inconsistent with the results of the present study, the reason for this discrepancy may be due to differences in the extraction method. In the present study, the highest antibacterial effect was on *S. aureus*, but both Estonian and N-hexane extracts had a stronger inhibitory effect and methanolic extract of this alga showed no antibacterial effect.

According to studies, gram-positive bacteria are more sensitive to gram-negative bacteria than antibacterial extracts of algae because of the different cell wall structures between the two groups of bacteria. Gram-negative bacteria have an extra outer membrane that acts as a shield against substances such as antibiotics. This layer is composed of glycerol phospholipids and glycolipid lipopolysaccharides and has the ability to repair if damaged. In addition, gram-negative bacteria have purine channels in their walls that can be used to prevent the entry of toxic chemicals and antibiotics into the cell. All of this makes it much harder to kill gram-negative bacteria than gram-positive bacteria (Shaima et al., 2022). In the present study, the susceptibility of gram-positive bacteria (*S. aureus* and *L. monocytogenes*) was also demonstrated. Also, the results of this study showed the resistance of gram-negative bacteria to algal extracts. Studies have shown that *E. coli* gram-negative bacteria are resistant to most algal extracts, which overlaps with the results of a recent study (Satchasataporn et al, 2025; Silva et al., 2020). Several studies have shown that solvents with lower polarity have better results than solvents with higher polarity, probably due to its greater polarity, it also extracts most of the algal compounds and other components of the algae along with the active substance in the algae (including the abundance of chlorophyll). On the other hand, studies have shown that gram-positive bacteria are more sensitive to crude algal extracts (Kandhasamy and Arunachalam, 2008). In the present study, due to the fact that methanol has more polarity than N-hexane but showed weaker antibacterial results than N-hexane, in this regard, according to the results of other studies, ethyl acetate and N-hexane were the most effective solvents for the extraction of bioactive compounds compared to methanol (Soleimani *et al.,* 2018; Akbari *et al.*, 2022). In methanolic extract, the ratio of bioactive compounds with antibacterial properties to the total extracted solution decreases compared to n-hexane extract. The reason for this difference can be attributed to factors such as their collection season, environment and different stages of algae growth, extraction methods and algae species (Manivannak *et al.*, 2011).

Phenolic compounds in seaweed extract may have antimicrobial properties. Other researchers have reported that phenolic compounds from different plant sources can inhibit various food-borne pathogens (Smid and Gorris, 1999; Prashanth et al., 2001; Kim et al., 2005; Kuda et al., 2005; Plaza et al., 2010), the presence of phenolic compounds in *I. stellata* has been observed in studies such as Mohammadi et al. (2015, 2020), so the antibacterial properties of *I. stellata* can be attributed to the phenolic compounds in it.

Finally, the results indicate that the N-hexane and Estonian extracts used in this study have the highest antimicrobial effect compared to the other two extracts. In this study, it was shown that the antimicrobial effect of Estonian and N-hexane extracts of *Iyengaria stellata* on gram-positive bacteria is much higher than gram-negative bacteria, and in some cases, the extract has no antimicrobial effect on gram-negative bacteria. The results of this study also expressed that solvents with lower polarity have better results than solvents with higher polarity.

Based on the results, extract of *Iyengaria stellata* can be used as a source of natural antimicrobial to be replaced with synthetic antibacterial with commercial application in both pharmaceutical and food industries.

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