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Research Article

Antimicrobial Activity of Red Sea Weed *Ceramium diaphanum* Against Pathogenic Bacteria

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Abstract

In the present study, antimicrobial activity of the extracts against pathogenic bacteria and fungi was performed by agar diffusion method. It exhibited potent activity against all tested microorganisms. The ethanol and chloroform extracts demonstrated significant antibacterial activity against various pathogenic bacteria, including *E. coli, P. aeruginosa, S. aureus, V. cholerae*, and methicillin-resistant *S. aureus* (MRSA). These results may be helpful for use of this seaweed in the health care.

Keywords: Antimicrobial; *Ceramium diaphanum;* MRSA

Introduction

Macroalgae are a widely diverse group of photosynthetic organisms, with approximately 15,000 described species. Known as "seaweeds" and "sea vegetables", marine macroalgae have been exploited throughout the ages, with seaweed harvesting and usage being activities that are deeply rooted in the tradition and history of many cultures scattered around the world.

The bioactive compounds held by seaweed are responsible for the different bioactivities already researched by many authors. Numerous studies and reviews have referred to the antioxidant [1], antimicrobial, anti-fungal [2], anti-inflammatory [3], anti-cholesterol [4], anti-neurodegenerative [5], anti-tumor [6] and prebiotic [7] properties of these bioactive compounds extracted from seaweeds. On industrial and commercial levels, while seaweed bioactives remain relatively unexploited, efforts are being undertaken to promote the use of seaweeds in food ingredient applications.

Antimicrobial and antioxidant activities have been particularly interesting among the various biological properties attributed to red seaweed extracts. The antimicrobial potential of these extracts has been evaluated against a range of pathogenic microorganisms, with studies reporting significant inhibitory effects against both Gram-positive and Gram-negative bacteria [8].

In this study, we aim to investigate antimicrobial properties, of extracts obtained from red seaweed. By employing various extraction techniques and conducting comprehensive bioactivity assessments.

Materials and Methods

Red Sea weed extract preparation

The specimen of *Ceramium diaphanum*, a type of red seaweed, was obtained from the Kunkeshwar coastline in Maharashtra, India. The crushed plant material (10g) was placed into a thimble and subsequently positioned inside a Soxhlet extractor. The thimble was filled with 250 mL of ethanol/chloroform as the organic solvent for extraction. The side arm of the extractor was insulated with glass wool. The solvent was heated using a heating mantle, initiating evaporation and movement through the apparatus to the condenser. The condensate dripped into the reservoir containing the thimble. Once the solvent level reached the siphon, it returned to the flask, restarting the cycle. This extraction process was carried out for a total of 8 hours. After completing 7 extraction cycles, the extracted plant samples were air-dried and collected in the extraction collector for further use.

Antimicrobial activity

Antifungal activity

The inoculum of the microorganism (*A. flavus ATCC 9643*) was prepared from bacterial cultures. A volume of 25 ml of Sabouraud agar (HiMedia) medium was poured into clean, sterilized Petri plates and allowed to cool and solidify. A 100 µl aliquot of the fungal strain broth was pipetted out and evenly spread over the solidified medium using a sterile spreading rod until it dried completely. Wells with a diameter of 6 mm was bored using a sterile cork borer. Solutions of the test compounds (100 µl/ml) were prepared in DMSO. Subsequently, 100 µl of each test solution and the standard were added to the wells. The concentrations utilized were 1 mg ml^{-1} for positive control and samples. The Petri plates were then incubated at 37°C for 24 hours. Miconazole (1 mg/ml) was used as a positive control, and DMSO served as the negative control. Antibacterial activity was assessed by measuring the diameters of the zones of inhibition (ZI). All determinations were performed in triplicate.

Antibacterial activity

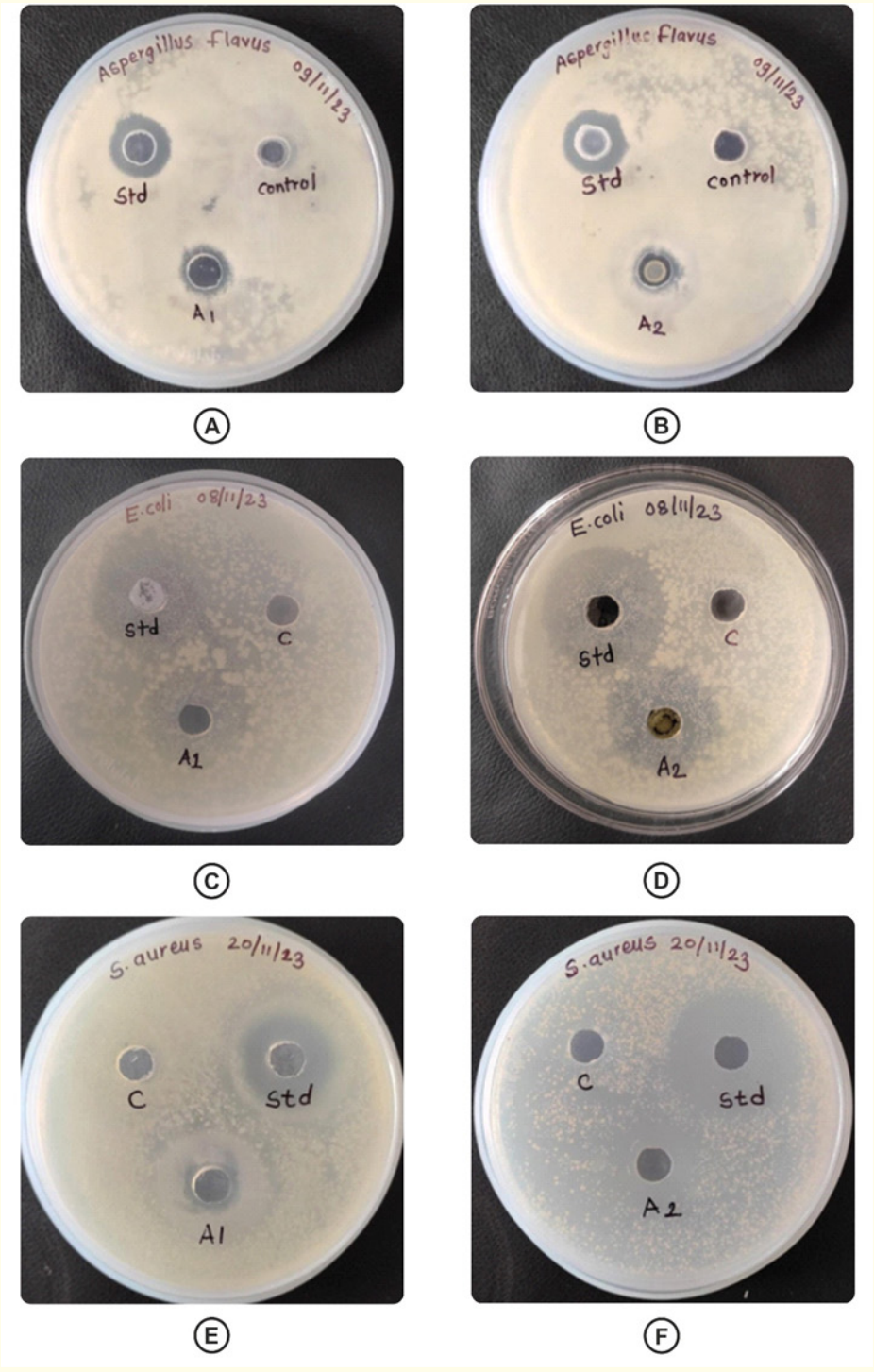
The inoculum of the microorganisms (*E. coli ATCC 25922*, *P. aeruginosa ATCC15442*, *S. aureus ATCC 23235*, *V. cholerae ATCC 9459*, *S. aureus ATCC 23235, S. aureus* Methicillin-resistant (MRSA) were prepared from the bacterial cultures. A volume of 25 ml of nutrient agar medium (HiMedia) was poured into clean, sterilized Petri dishes and allowed to cool and solidify. A 100 µl aliquot of the bacterial strain broth was pipetted out and evenly spread over the solidified medium using a sterile spreading rod until it dried completely. Wells of 6 mm in diameter were created using a sterile cork borer. Solutions of the compounds (100 µg/ml) were prepared in DMSO, and 100 µl of these test solutions and standards were added to the wells. The Petri dishes were incubated at 37°C for 24 hours. Streptomycin antibiotic used as a positive control because it is broad spectrum antibiotic, while DMSO was used as a negative control. The concentrations utilized were 1 mg ml^{-1} for positive control and samples. Antibacterial activity was assessed by measuring the diameters of the zones of inhibition (ZI). All determinations were performed in triplicate.

Results and Discussion

Antimicrobial activity of red sea algae

Samples	Zone in diameter (mm)
Antifungal activity (A1: Ethanol extract; A2: Chloroform extract)	
A. flavus ATCC 9643	
Control	0
Standard (Miconazole)	15 ± 0
A1	9.33 ± 0.57
A2	8.66 ± 0.57
Antibacterial activity (A1: Ethanol extract; A2: Chloroform extract)	
E. coli ATCC 25922	
Control	0
Standard (Streptomycin)	24 ± 0
A1	12.66 ± 0.57
A2	13.33 ± 1.15
P. aeruginosa ATCC15442	
Control	0
Standard (Streptomycin)	24 ± 0
A1	9 ± 0
A2	11.66 ± 0.15
S. aureus ATCC 23235	
Control	0
Standard (Streptomycin)	24 ± 0
A1	10.66 ± 0.57
A2	20 ± 0
V. cholerae ATCC 9459	
Control	0
Standard (Streptomycin)	24 ± 0
A1	17.66 ± 0.57
A2	19 ± 0
Methicillin-resistant *S. aureus* (MRSA)	
Control	0
Standard (Streptomycin)	24 ± 0
A1	15.66 ± 0.57
A2	14 ± 0

Table 1: Antimicrobial activity assessment of red algae chloroform extract.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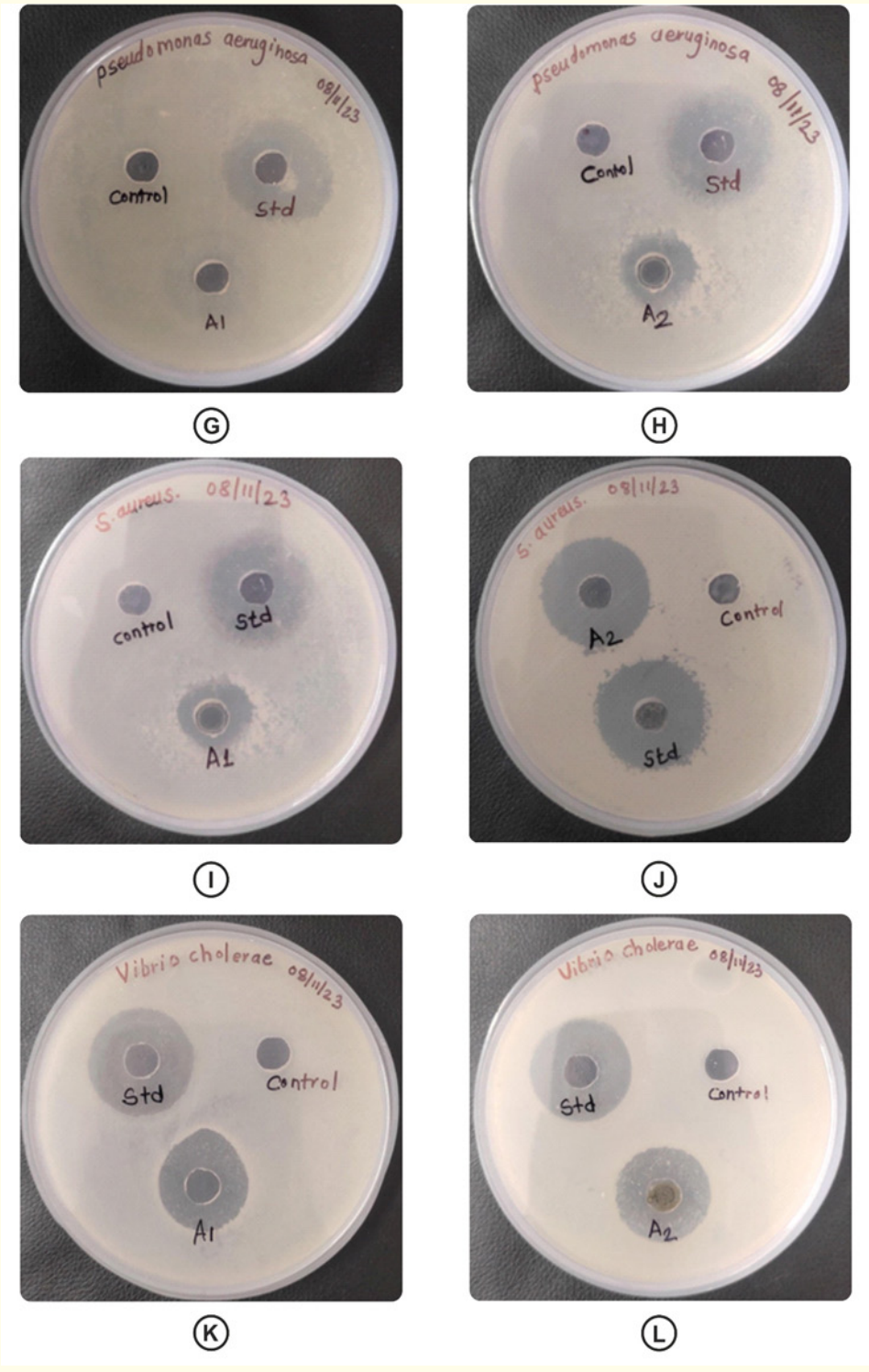

Figure 1: Antimicrobial activity of red seaweed chloroform extract on different microorganisms (A1: Ethanol extract; A2: Chloroform extract). In the image, the activity is against pathogenic bacteria where A and B: *A. flavus* ATCC 9643; C and D: *E. coli* ATCC 25922; E and F: *S. aureus* (Methicillin-resistant); G and H: *P. aeruginosa* ATCC 15442; I and J: *S. aureus* ATCC 23235; K and L: *V. cholerae* ATCC 9459.

Antibacterial activity

Red algae, scientifically known as Rhodophyta, are a significant natural reservoir of bioactive compounds with potential antimicrobial properties. These marine organisms, comprising around 6000 species, are rich in secondary metabolites that exhibit promising alternatives to traditional antibacterial agents [9]. Red algae are recognized as a vital source of biologically active metabolites, surpassing other algal classes in terms of the diversity and abundance of these compounds [10]. The genus Laurencia, a type of red algae, is particularly noteworthy for producing a wide array of secondary metabolites with diverse biological activities, including antimicrobial properties against various organisms [11]. Studies have highlighted that red algae, with their high diversity of families and genera, are among the oldest eukaryotic algae and represent a rich source of bioactive secondary metabolites [12].

Red algae have been studied for their antimicrobial properties, with extracts showing potent activity against various pathogens [13]. The chemical defences of red algae, such as halogenated furanone, have been shown to inhibit bacterial colonization, highlighting their role in protecting against microbial threats. Moreover, red algae have been found to contain rare acetogenins with anti-inflammatory effects, further expanding the potential applications of these bioactive compounds [14]. Many organic solvents, including ethanol, methanol, and chloroform, are frequently used to assess the antibacterial activity of marine algae extracts [15]. The differences in antibacterial activity between the ethanol and chloroform extracts of red algae that have been found may be attributed to the choice of solvent, which can influence the extraction of bioactive substances with antibacterial activities. Additionally, the solvent used for extraction can influence the antibacterial activity of plant extracts, with chloroform extracts often exhibiting notable inhibitory effects against bacteria [16].

E. coli ATCC 25922

The control showed no zone of inhibition. The ethanol extract produced a zone of inhibition of 12.66 ± 0.57 mm, indicating some antibacterial activity. The chloroform extract produced a slightly higher zone of inhibition at 13.33 ± 1.15 mm, suggesting it was marginally more effective than the ethanol extract. The slight difference in activity indicated that the chloroform extract may contain bioactive compounds with better efficacy against *E. coli ATCC 25922*, possibly due to its ability to extract more hydrophobic antibacterial agents.

P. aeruginosa ATCC 15442

There was no zone of inhibition was observed in the case of control. The ethanol extract had a zone of inhibition of 9 ± 0 mm, indicating minimal antibacterial activity. The chloroform extract showed a zone of inhibition of 11.66 ± 0.15 mm, which was slightly better than the ethanol extract but still indicated limited effectiveness. This outcome suggested that while both extracts have some antibacterial properties, the chloroform extract is slightly more effective against *P. aeruginosa ATCC 15442*, potentially due to its extraction of compounds that target this particular bacterium more effectively.

S. aureus ATCC 23235

The zone of inhibition was completely absent in the control well. The ethanol extract showed a zone of inhibition of 10.66 ± 0.57 mm, indicating moderate antibacterial activity. The chloroform extract exhibited a zone of inhibition of 20 ± 0 mm, suggesting a higher effectiveness compared to the ethanol extract. This significant difference highlights that the chloroform extract contains more potent antibacterial compounds against *S. aureus ATCC 23235*, which could be due to the extraction of more effective hydrophobic bioactive agents.

V. cholerae ATCC 9459

The control well demonstrated no zone of inhibition. The ethanol extract had a zone of inhibition of 17.66 ± 0.57 mm, indicating substantial antibacterial activity. The chloroform extract showed a zone of inhibition of 19 ± 0 mm, which was higher than the ethanol extract and suggested a significant potential as an antibacterial agent. The close efficacy of both extracts suggested that both solvents are effective at extracting compounds that inhibit *V. cholerae ATCC 9459*, though the chloroform extract shows a slightly better performance.

Methicillin-resistant *S. aureus (MRSA)*

No inhibitory zone was observed in the control. The ethanol extract showed a zone of inhibition of 15.66 ± 0.57 mm, indicating considerable antibacterial activity against MRSA. The chloroform extract produced a zone of inhibition of 14 ± 0 mm, which

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No inhibitory zone was observed in the control. The ethanol extract showed a zone of inhibition of 15.66 ± 0.57 mm, indicating considerable antibacterial activity against MRSA. The chloroform extract produced a zone of inhibition of 14 ± 0 mm, which

was slightly less effective than the ethanol extract but still showed notable activity against MRSA. The findings revealed that, despite the usual pattern of chloroform extracts exhibiting stronger antibacterial activity, both extracts are efficient; nevertheless, the ethanol extract may include some chemicals that are particularly effective against MRSA. The study demonstrated that both ethanol and chloroform extracts of red algae exhibited varying antibacterial activity against different bacterial strains. Based on the results, chloroform extracts showed higher inhibition zones than ethanol extracts, suggesting they might be more effective. The differences in antibacterial activity between the two extracts can be attributed to the distinct chemical compositions extracted by each solvent. Chloroform, a non-polar solvent, likely extracted more hydrophobic compounds [17], which can have stronger antibacterial properties [18]. In contrast, ethanol, a polar solvent, extracts polar compounds that may not be as effective against certain bacteria. Although none of the extracts matched the efficacy of the standard antibiotics, the findings suggest that the chloroform extracts have a higher potential for antibacterial activity compared to the ethanol extracts.

The variation in antibacterial activity between chloroform and methanolic extracts of red algae can be attributed to their distinct chemical compositions and properties. Karabay-Yavasoglu., *et al*. found that chloroform extracts of red macroalgae exhibit higher antibacterial activity compared to methanolic extracts. This observation is consistent with earlier report, where the chloroform extract of red algae demonstrated significant antimicrobial activity [19]. The presence of complex mixtures, volatile oils, and antiinfective agents in the chloroform extract may have contributed to its enhanced antibacterial properties [20]. Extracts with various antibacterial properties are typically produced by different solvents; against some bacteria, often chloroform extracts are more effective than methanolic extracts. Moreover, Yap., *et al*. highlighted that chloroform extracts' DPPH radical scavenging activity can be higher than that of methanolic extracts in specific algae species [21]. This difference in antioxidant potential may influence the observed variation in antibacterial activity between the two types of extracts. The presence of phytochemicals with antioxidant properties in the chloroform extract, as shown in a report, could contribute to its superior antibacterial effects [22]. Additionally, Karimzadeh and Zahmatkesh indicated that chloroform extracts of algae exhibit significant antioxidant abilities, which may indirectly enhance their antibacterial activity [23]. The choice of extraction method and solvents used significantly impacts the composition and efficacy of the extracts. Furthermore, Gonfa., *et al*. provided evidence supporting the superior antibacterial activity of chloroform extracts, as they demonstrated the highest inhibition zones against *Escherichia coli* compared to other extracts [24]. This finding aligns with the notion that chloroform extracts possess potent antibacterial properties, potentially explaining their increased efficacy compared to ethanolic and methanolic extracts.

Antifungal activity

Red algae have been recognized for their potential antifungal properties. Studies have shown that red algae extract possesses antifungal efficacy against various fungi. For instance, El-Bilawy., *et al*. investigated the antifungal potential of ethanolic extracts from red algae *Gracillaria chilensis* against *Phytophthora cinnamomi* [25]. Furthermore, red algae have been found to contain bioactive compounds such as sulfated polysaccharides, agar, and carrageenans, which contribute to their antifungal properties [26].

The antifungal activity of red algae extracts has been compared to conventional antifungal drugs like Nystatin and Clotrimazole, showing promising results [27]. Moreover, the presence of phytochemicals like tannins and phenols in algal extracts has been linked to their antifungal activity [28]. Studies have also highlighted the importance of organic solvents in extracting phenolic and lipid compounds from macroalgae to enhance their antifungal potential [29]. Overall, red algae extracts have demonstrated significant antifungal potential, making them a valuable source of natural compounds for combating fungal infections. The diverse bioactive compounds present in red algae contribute to their antifungal properties, showcasing their potential for pharmaceutical and medical applications.

Aspergillus flavus ATCC 9643

In the control, there was no evidence of a zone of inhibition. The standard (Miconazole) exhibited a zone of inhibition of 15 ± 0 mm, indicating its effectiveness as an antifungal agent. The ethanol extract demonstrated a zone of inhibition of 9.33 ± 0.57 mm, indicating moderate antifungal activity. The chloroform extract showed a zone of inhibition of 8.66 ± 0.57 mm, slightly lower than the ethanol extract. The results indicated that both ethanol and chloroform extracts of red algae possess antifungal activity against *Aspergillus flavus ATCC 9643*. The ethanol extract exhibited a slightly higher inhibition zone compared to the chloroform extract, suggesting it

may contain more effective antifungal compounds. The observed differences in antifungal activity between the ethanol and chloroform extracts can be attributed to their solvent properties and the chemical composition of the extracts. Ethanol, being a polar solvent, is likely more effective at extracting polar compounds such as phenolics and flavonoids, which are known for their antifungal properties [30,31].

Phenolic and flavonoid compounds have been shown to disrupt the cell membrane of fungi through various mechanisms. Flavonoids, such as sophoraflavone G, (-)-epigallocatechin gallate, and licochalcones A and C, have been reported to inhibit cytoplasmic membrane function and energy metabolism [32]. They interfere with fungal cell membrane integrity, disrupt ergosterol biosynthesis, and modulate critical signal transduction pathways, ultimately hindering fungal growth and pathogenicity [33]. Additionally, flavonoids have been found to inhibit the growth and proliferation of Candida species by disrupting fungal cell membrane integrity, interfering with fungal cell wall synthesis, and affecting fungal cell signalling pathways [34]. Moreover, flavonoids have been reported to interact with cell membranes, affecting their structure and function [35]. They have been shown to cause damage to the cell membrane, leading to the inhibition of macromolecular synthesis [36]. Flavonoids have also been highlighted for their antibacterial and antifungal activities, with evidence suggesting that they act on cell membranes, disrupting membrane integrity [37]; [38].

Conclusion

Both ethanol and chloroform extracts demonstrated significant antibacterial activity against various pathogenic bacteria, including *E. coli*, *P. aeruginosa*, *S. aureus*, *V. cholerae*, and methicillin-resistant *S. aureus* (MRSA). The chloroform extract generally showed higher inhibition zones than the ethanol extract, indicating its superior antimicrobial potential. This finding highlights the potential of red sea algae extracts as natural antimicrobial agents. The extracts exhibited moderate antifungal activity against *Aspergillus flavus ATCC 9643*, with the ethanol extract showing slightly higher efficacy. This suggests that red sea algae could be a source of natural antifungal compounds, although further research is needed to enhance their potency. The study revealed substantial bioactive compounds in the red sea algae extracts. The high content of these compounds correlates with the observed biological activities and further supports the potential of red sea algae as a source of valuable natural products. This research provides strong evidence for the potential of red sea algae as a rich source of bioactive compounds with significant antimicrobial, antifungal properties. The findings open new avenues for developing natural products in the pharmaceutical, nutraceutical, and cosmeceutical industries. However, further studies are needed to isolate and characterize specific bioactive compounds, evaluate their mechanisms of action, and assess their safety and efficacy in *in vivo* models. Additionally, research into optimizing extraction methods and exploring potential synergistic effects between different compounds could enhance the practical applications of these promising marine resources.

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