EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 12
Issue: 9
Year: 2025

ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF MARINE MACROALGAE COLLECTED FROM THE COASTLINE OF RAS AL KHAIMAH: TOWARDS GREEN PHARMACEUTICAL SOLUTIONS

***Vibha Bhardwaj**

Director Environment Laboratories Ras Al Khaimah Municipality, United Arab Emirates.

***Corresponding Author: Vibha Bhardwaj**
Director Environment Laboratories Ras Al Khaimah Municipality, United Arab Emirates.

Article Received on 21/07/2025 **Article Revised on 12/08/2025** **Article Accepted on 01/09/2025**

ABSTRACT
Numerous natural antimicrobial compounds have been identified in marine environments than in terrestrial. Marine organisms, like marine algae, are the source of structurally distinct natural products with biological, pharmacological, and nutraceutical properties. The current study examined the antibacterial and antioxidant properties of methanolic extracts from five different seaweed species: *Ulva intestinalis* (green), *Padina gymnospora* (brown), *Sargassum vulgare* (brown), *Kappaphycus alvarezii* (greenish brown), and *Chondrus crispus* (red). The DPPH radical scavenging assay was used to measure antioxidant activity, and *Salmonella enterica*, *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis* were used to measure antibacterial activity. At 1000 µg/ml, the maximum inhibition ranged from 65.19% to 68.20%, indicating dose-dependent radical scavenging activity in all seaweed extracts. *Sargassum vulgare* and *Kappaphycus alvarezii* exhibited the strongest DPPH scavenging properties among them. Species-specific inhibitory patterns were shown by antibacterial assays: *Padina gymnospora* was most effective against *S. aureus* (32 mm) and *S. enterica* (26 mm), while *Ulva intestinalis* showed the strongest activity against *S. aureus* (58 mm zone of inhibition). Interestingly, *Sargassum vulgare* exhibited broad-spectrum inhibition, especially against *B. subtilis* (19 mm) and *E. coli* (25 mm). The results support the potential use of these macroalgae in pharmaceutical and functional food applications by highlighting their therapeutic potential as natural sources of antioxidants and antibacterial agents.

KEYWORDS: Marine macroalgae, Antioxidant activity, Antibacterial activity, *Chondrus crispus*, *Kappaphycus alvarezii*, *Padina gymnospora*, *Sargassum vulgare*, *Ulva intestinalis*, DPPH assay, Natural bioactive compounds.

1. INTRODUCTION
The marine environment is a vast reservoir of naturally occurring compounds that are both structurally unique and biologically active, often producing more diversity than terrestrial ecosystems. (Ireland et al., 1988). As possible sources of pharmacologically significant compounds with antimicrobial, antioxidant, and other therapeutic properties, macroalgae, or seaweeds, have garnered a lot of attention among marine organisms (Schwartsmann et al., 2001; Manilal et al., 2010). Numerous secondary metabolites that seaweeds produce have been shown to have bioactivities of biomedical significance and to play ecological roles in defense and competition (Chiheb et al., 2009). Antibacterial, antioxidant, antifungal, and anti-inflammatory properties have been reported for crude extracts and purified metabolites from a variety of algal species (Lustigman & Brown, 1991; Tuney et al., 2006; Patra et al., 2008). Furthermore, several studies have highlighted their bacteriostatic and bactericidal effects, as well as their ability to inhibit the growth of pathogenic bacteria (Hornsey & Hide, 1985; Gorban et al., 2003; Kolanjinathan et al., 2009).

Antibiotics derived from terrestrial microorganisms have long been the mainstay of treatment for infectious diseases. However, the emergence of resistant bacterial strains has been accelerated by the overuse and careless application of these agents (Bacon et al., 2000; Levy, 2002). A major global health concern is the emergence of multidrug-resistant (MDR) pathogens, also known as "superbugs," since they are becoming more difficult, if not impossible, to treat (Sande-Bruinsma et al., 2008). Finding novel antimicrobial agents from other sources is crucial given the sluggish pace of new antibiotic discovery and the quick evolution of resistance mechanisms (Smit, 2004; Peters et al., 2008).

According to their pigmentation and biochemical makeup, seaweeds—primitive non-flowering plants devoid of true roots, stems, and leaves—are divided into three main groups: Rhodophyta (red algae), Chlorophyta (green algae), and Phaeophyta (brown algae). Many people believe that they are promising sources of bioactive substances that have antiviral, antibacterial, antifungal, and antitumor properties (Gonzalez et al., 2001; Chakraborthy et al., 2010a). Algal species (Valchos et al., 1997), extraction techniques (Tuney et al., 2006), solvent polarity (Cox et al., 2010), and sample preparation are some of the variables that affect seaweed's antimicrobial potency. According to studies, dried seaweed extracts frequently have higher antimicrobial activity than fresh samples (Padmini Sreenivasa Rao et al., 1986; Campos-Takaki et al., 1988; Manivannan et al., 2011), and methods like lyophilization can improve compound recovery and bioactivity even more (Salvador et al., 2007).

In addition to their antimicrobial properties, seaweeds are abundant in flavonoids, phenolic compounds, and other antioxidants that have been linked to improved longevity, a lower risk of chronic illnesses, and protection against disorders brought on by oxidative stress (Hodgson & Croft, 2006; Halliwell, 2007; Moraes-de-Souza et al., 2008; Yan & Asmah, 2010). Seaweeds are therefore a low-toxicity, sustainable source of medicinal substances with antibacterial, and antioxidant uses.

This study assesses the in vitro antioxidant and antimicrobial properties of five marine algae species that were collected from the coastal waters of Ras Al Khaimah, United Arab Emirates: *Ulva intestinalis* (green), *Padina gymnospora* (brown), *Sargassum vulgare* (brown), *Kappaphycus alvarezii* (greenish-brown), and *Chondrus crispus* (red). The DPPH radical scavenging assay was used to evaluate the antioxidant potential of methanolic extracts of dried algal samples and test them against specific Gram-positive and Gram-negative human pathogens.

2. MATERIAL AND METHODS

2.1 Algal Collection and processing

Between February and June 2025, five distinct seaweed species representing green, brown, and red algae were collected from Ras Al Khaimah's coastline in the United Arab Emirates. After being carefully chosen, the algal samples were thoroughly cleaned with seawater to get rid of contaminants, sand fragments, and epiphytic organisms. To preserve their freshness, the samples were sent right away in sterile iceboxes to the Environment Laboratory's Microbiology section in Ras Al Khaimah Municipality (Figure 1).

(a) AS1-*Chondrus crispus (R)* | **(b) AS2-*Kappaphycus alvarezii (R)*** | **(c) AS3- *Padina gymnospora* (P)**

(d) AS4-*Sargassum vulgare* (P) **(e) AS5-*Sargassum vulgare* (P)**

Figure 1: (a) *Chondrus crispus* (b) *Kappaphycus alvarezii* (c) *Padina gymnospora* (d) *Sargassum vulgare* (e). *Sargassum vulgare* (Source: Wikipedia)

To make sure that no contaminants or debris remained, the seaweeds were rinsed in the lab with tap water first, then with sterile distilled water. Following cleaning, the samples were allowed to air dry for a week at room temperature. A laboratory blender was used to grind the dried algal material into a fine powder after it had been cut into small pieces. Following the procedure outlined by Bhardwaj (2021a,b), the powdered samples were then kept in sterile containers for additional examination and screening of their therapeutic qualities.

2.2 Preparation of the extracts

The extraction solvent used was methanol. Five grams of powdered material were soaked in fifty milliliters of methanol for three days at room temperature, with periodic shaking, for every type of algae. Following extraction, the mixtures were filtered, and a rotary evaporator set to 25 °C was used to concentrate the filtrates. A vacuum pump was used to remove any remaining moisture. After weighing, the resultant crude extracts were dissolved in DMSO to reach a final concentration of 50 mg/mL and kept at 4 °C until they were needed again (Mohanta et al., 2007; Patra et al., 2008).

2.3 Test organisms

The antibacterial activity of the algal extracts was evaluated against four reference bacterial strains: *Bacillus subtilis*, *Escherichia coli*, *Salmonella enterica* and *Staphylococcus aureus*. All strains were obtained from the American Type Culture Collection (ATCC, USA) through LTA srl, Italy.

The bacterial isolates were maintained as pure cultures on nutrient agar (NA) slants at 4 °C and sub-cultured periodically to ensure viability and purity before experimental use. Working cultures for antimicrobial assays were freshly prepared by inoculating single colonies into nutrient broth (NB) and incubating at 37 °C for 18–24 h to achieve the required turbidity for standard inoculum preparation.

2.4 Estimating Antioxidant activity

2.4.1. DPPH free radical scavenging assay

The plant methanolic extracts were prepared in different concentrations 125, 250, 500, 1000 µg/mL to analyse the antioxidant property by using DPPH radical scavenging assay method which was described in our previous research paper (Bhardwaj. V 2021c). The measurement of the DPPH radical scavenging activity was performed according to methodology described by Brand-Williams et al. 1995. The scavenging activity percentage (AA%) was determined according to Mensor et al. 2001:

Scavenging activity (%) = ([A_{517} of control − A_{517} of test sample] / A_{517} of control) × 100.

Where A_{517} control is the absorbance of DPPH radical+ methanol; A_{517} test sample is the absorbance of DPPH radical+ sample extract.

2.5 Methodology for detection of antibacterial activity

2.5.1 Inoculums preparation

The bacterial pure culture isolates were first grown in 5 ml of nutrient broth into sterile test tubes for 18 h before use.

2.5.2 Agar well diffusion assay

The agar well diffusion method was used to evaluate the methanolic algal extracts' antibacterial activity. Using a sterile spreader, 100 µL of fresh bacterial inocula of each test strain was evenly distributed on Mueller-Hinton Agar (MHA) plates, and the plates were left to settle at room temperature. A sterile cork borer was used to punch wells into the agar that were 6 mm in diameter.

The five algal species' methanolic extracts were made at the appropriate 10% concentrations, and 30 µL of each extract was cautiously added to each well. The negative control was methanol, and the positive control was ciprofloxacin (30 µg/disc). To allow the extracts to diffuse, the inoculated plates were first maintained at 2–8 °C for 1 hour. They were then incubated at 37 °C for 24h.

After incubation, zones of inhibition (mm) was measured to the closest millimeter. Significant antibacterial activity was thought to be indicated by a clear inhibition zone of ≥7 mm (Sohel, 2010; Uddin et al., 2007; Bhardwaj, 2022). Three duplicates of each assay were run, and the mean values were noted. To prevent contamination, the experiments were conducted under strict aseptic conditions.

2.6 Statistical analysis

The tests were performed in triplicates. Data are expressed as mean. Pair wise comparisons were made. Experimental error was determined for triplicate and expressed as standard deviation (SD).

3. RESULTS AND DISCUSSION

3.1 Antioxidant Activity (DPPH Assay)

The DPPH radical scavenging assay was used to measure the antioxidant activity of the methanolic extracts of the five algal samples (AS1–AS5) at concentrations ranging from 125 to 1000 µg/mL (Table 1; Fig. 2). Every extract demonstrated a dose-dependent rise in radical scavenging, demonstrating their capacity to neutralize free radicals by donating electrons or hydrogen atoms. At 1000 µg/mL, the scavenging activities ranged from 65 to 68 percent. AS4 (68.20 %) had the greatest effect, followed by AS2 (67.16 %) and AS5 (66.20%). These results confirm earlier findings that seaweeds are rich in phenolic compounds, flavonoids, and other metabolites with strong antioxidant potential (Hodgson & Croft, 2006; Halliwell, 2007).

Table 1: DPPH Antioxidant scavenging activity of methanolic extract of Algae samples at different concentration.

S No	Concentration (µg/ml)	*DPPH scavenging activity of different Algae Samples (AS) (%)				
		AS 1	AS 2	AS 3	AS 4	AS 5
1	125	22.3 ± 3.05	33.51 ± 0.03	21.76 ± 0.05	20.19 ± 0.05	24.60 ± 0.06
2	250	32.18 ± 0.02	43.31 ± 1.02	25.32 ± 0.042	41.43 ± 2.02	47.43 ± 1.02
3	500	45.46 ± 2.04	54.47 ± 0.015	46.32 ± 0.04	54.34 ± 0.01	50.34 ± 0.04
4	1000	65.199 ± 0.02	67.16 ± 0.05	67.12 ± 1.03	68.20 ± 1.01	66.20 ± 1.02

***All values are expressed as mean ± SEM for triplicates**

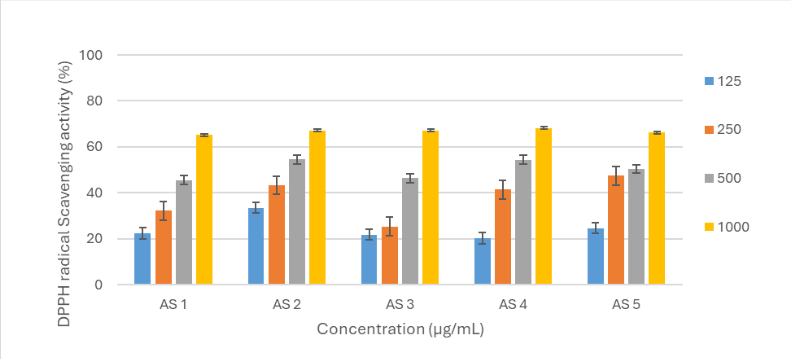


Figure 2. DPPH scavenging activity (%) of methanolic extract of Algal samples at different concentration.

3.2 Antibacterial Activity

Salmonella enterica, *Staphylococcus aureus*, *Escherichia coli, and Bacillus subtilis* were the four human pathogens used to test the algal extracts' antibacterial efficacy (Table 2). Although the range and strength of antibacterial activity varied significantly between species, all extracts showed antibacterial activity. According to Ibtissam et al. (2009), the methanol extracts of *S. vulgare* do not exhibit antibacterial activity against the growth of *S. aureus* and *E. coli*, which is consistent with our investigation. Silva et al. (2013) discovered that only the ethanolic extract of the brown seaweed *Padina gymnospora* influenced *E. coli* and *P. aeruginosa*. Methanolic extracts of the brown seaweed species *Sargassum latifolium B* and *Sargassum platycarpum A* were found to be more effective against gram positive bacteria than gram negative bacteria, according to a different study by Nadine et al. (2017).

Table 2: **Antibacterial Activity of methanolic extracts of Algal Samples.**

SNo.	Microorganisms	AS 1	AS 2	AS 3	AS 4	AS 5
1	*Bacillus subtilis* (ATCC 6633)	18 ± 0.0	15 ± 0.5	16± 0.2	19± 0.3	14± 0.5
2	*E.coli* (ATCC 8739)	24 ± 0.5	18± 0.4	17± 0.4	25± 0.5	No zone
3	*Salmonella enterica* (ATCC 14028)	25 ± 0.2	19± 0.1	26± 0.5	22± 0.1	No Zone
4	*Staphylococcus aureus* (ATCC 6538)	22 ± 0.1	19 ± 0.1	32± 0.1	30± 0.2	58± 0.2

AS1 (*Chondrus crispus* – Red Alga)

The antibacterial properties of AS1 were moderate to strong. The extract exhibited inhibition zones of 22 mm against *S. aureus*, 25 mm against *S. enterica*, 24 mm against *E. coli*, and 18 mm against *B. subtilis*. Additionally, its antioxidant activity rose gradually with concentration, peaking at 65.19% at 1000 µg/mL. These results imply that AS1 is a good candidate for broad-spectrum applications since it has both antioxidant and antimicrobial properties (Table 2, figure 3).

AS2 (*Kappaphycus alvarezii* – Greenish-Brown Alga)

With one of the highest scavenging activities (67.16% at 1000 µg/mL), AS2 was one of the most successful extracts in antioxidant tests. With inhibition zones that ranged from 15 mm (*B. subtilis*) to 19 mm (*S. aureus*), its antibacterial activity was, nevertheless, relatively moderate. Given its dual characteristics, AS2 might be more promising for nutraceutical development as an antioxidant-rich source than as a potent antimicrobial agent (table 2, Figure 3).

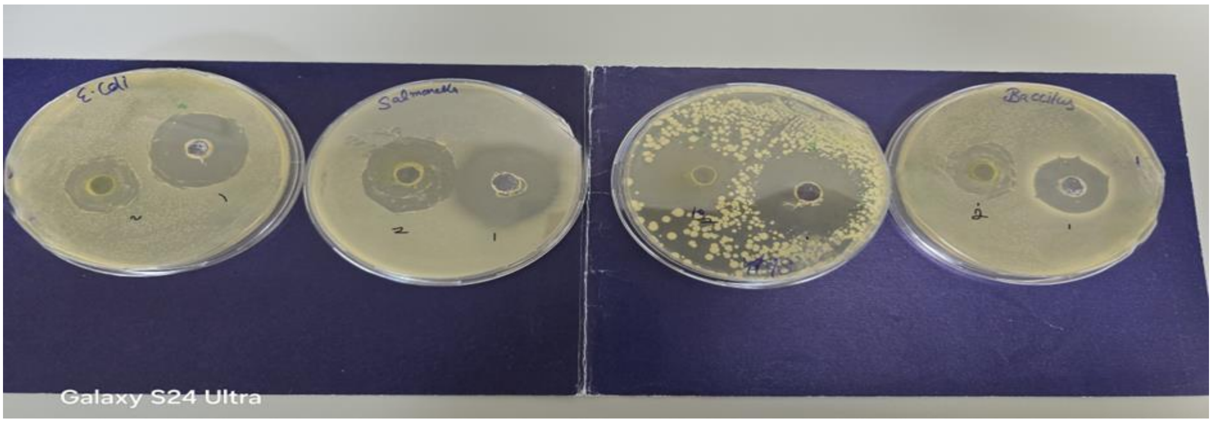


Figure 3: Extracts of AS1 and AS2 showed antibacterial activity as indicated by the zone of inhibition against different microorganism's strain.

AS3 (*Padina gymnospora* – Brown Alga)
AS3 showed the strongest inhibition of *S. aureus* (32 mm) and the highest antibacterial activity against *S. enterica* (26 mm). With 67.12% scavenging at 1000 µg/mL, its antioxidant activity was marginally lower than that of AS2 and AS4. These results suggest that AS3 has potential as a natural antimicrobial agent, especially against foodborne pathogens like *S. enterica* and Gram-positive bacteria (Table 2, Figure 4).

AS4 (*Sargassum vulgare* – Brown Alga)
In both tests, AS4 showed steady activity. It demonstrated potent effects against *E. coli* (25 mm) and *S. aureus* (30 mm), and it generated the largest inhibition zone against *B. subtilis* (19 mm). It had the highest scavenging activity (68.20% at 1000 µg/mL) in the antioxidant assay. According to these findings, AS4 has strong antioxidant and antimicrobial properties, which makes it extremely relevant for use in pharmaceutical applications (Table 2, Figure 4).

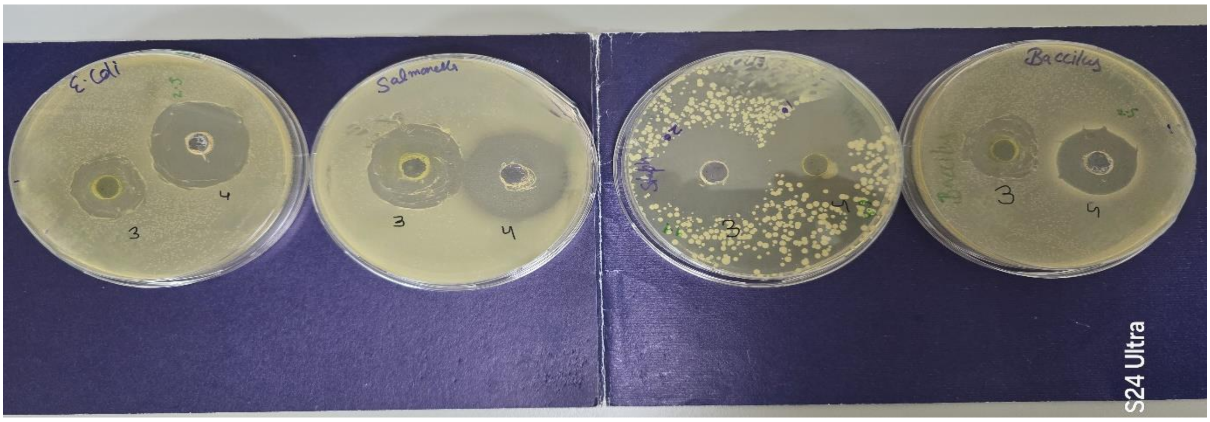
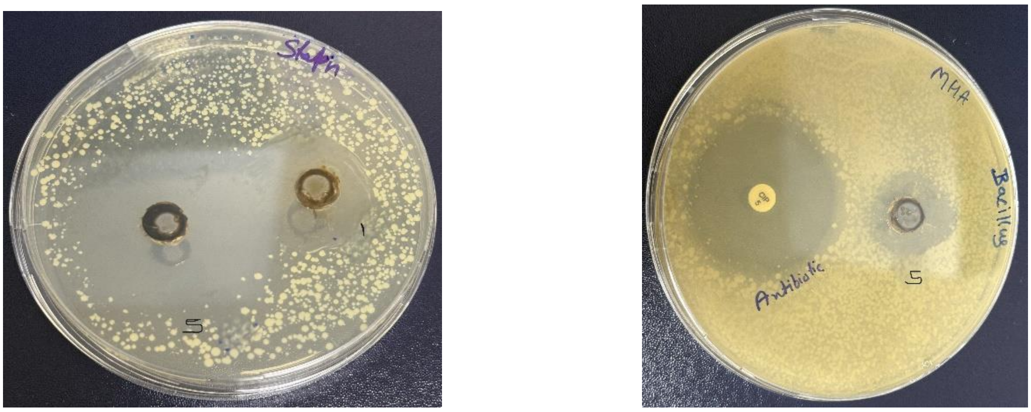


Figure 4: Extracts of AS 3 and AS4 showed antibacterial activity as indicated by the zone of inhibition against different microorganism's strain.

AS5 (*Ulva intestinalis* – Green Alga)
AS5 showed a distinct profile. It demonstrated remarkable antibacterial activity against *S. aureus* (58 mm), greatly outperforming all other extracts, but it was ineffective against *E. coli* and *S. enterica* (no inhibition zones). Additionally, it had a high level of antioxidant activity (66.20% at 1000 µg/mL). AS5 may contain highly specific bioactive compounds that could be further studied as leads against Gram-positive pathogens, especially multidrug-resistant *S. aureus*, as suggested by the selective but potent anti-*Staphylococcus* effect (Table 2, figure 5 a,b).



(a) **(b)**

Figure 5: (a) Extracts of AS5 and AS1showed antibacterial activity as indicated by the zone of inhibition against *Staphylococcus. aureus* (b) AS5 zone of inhibition and control with antibiotic.

3.3 Comparative Insights
When combined, the findings show that, albeit to differing degrees, all five algal species have strong antioxidant and antibacterial properties. The most well-balanced extract was AS4, which demonstrated potent antibacterial and antioxidant qualities against a variety of pathogens. AS5 showed remarkable potency against *S. aureus* and was very selective. Conversely, AS1 and AS3 demonstrated moderate to strong dual activities, whereas AS2 was notable mainly as a potent antioxidant source.

These discrepancies could be explained by species-specific differences in the type of active compounds, extraction efficiency, and secondary metabolites. Similar species-dependent variability in seaweed bioactivity has been documented in earlier research (Chiheb et al., 2009; Tuney et al., 2006). The results of this study support the potential of marine algae as substitute sources of naturally occurring antioxidants and antimicrobials, which may find use in the production of nutraceuticals, medications, and food preservation.

4. CONCLUSION

This study demonstrated that methanolic extracts of five marine algae species—*Chondrus crispus*, *Kappaphycus alvarezii*, *Padina gymnospora*, *Sargassum vulgare*, and *Ulva intestinalis*—possess significant antioxidant and antibacterial activities. All extracts showed concentration-dependent DPPH radical scavenging, with *Sargassum vulgare* and *Kappaphycus alvarezii* exhibiting the highest antioxidant potential. Antibacterial assays revealed species-specific effects, where *Ulva intestinalis* displayed exceptional inhibition against *Staphylococcus aureus*, while *Padina gymnospora* and *Chondrus crispus* were most effective against *Salmonella enterica* and *Escherichia coli*, respectively.

These results demonstrate the potential of marine algae as sources of bioactive compounds for use in natural antimicrobial formulations, pharmaceuticals, and nutraceuticals. Future studies should concentrate on identifying and characterizing active metabolites, clarifying antimicrobial mechanisms, and validating safety and efficacy in vivo in order to fully realize their therapeutic potential. Furthermore, to convert algal bioactive into workable solutions for battling oxidative stress and multidrug-resistant infections, developments in biotechnology, sustainable large-scale cultivation, and industrial formulation techniques will be crucial.

5. Abbreviations

SD, standard deviation; **MDR**, Multidrug resistant; **ATCC**, American Type Culture Collection; **E**, Extract; **h**, hours; **C**, ciprofloxacin; **AS**, Algae Samples

6. Ethics approval and consent to participate

Not applicable.

7. Consent for publication

Not applicable.

8. Availability of data and materials

The relevant data and materials are available in the present study.

9. Competing interests

The authors declare that they have no competing interests. All procedures followed were in accordance with the ethical standards (institutional and national).

10. Funding

Not applicable.

11. Acknowledgements

Authors would like to thank all individuals who provided their efforts for this research especially Aaesha Ahmed Alzaabi for her assistance during research work.

12. Authors' contributions

VB performed all the experiments. VB analysed the data and wrote the manuscript.

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