

In Vitro Comparative Antimicrobial Potential of Bioactive Crude and Fatty acids Extracted from Abundant Marine Macroalgae, Egypt

Hager Ahmed^{1,*}, Samia Heneidak¹, Gihan Ahmed El Shoubaky², Abdel-Hamied M. Rasmey¹

¹ Botany and Microbiology Department, Faculty of Science, Suez University, Suez, Egypt.

² Botany and Microbiology Department, Faculty of Science, Suez Canal University, Ismailia, Egypt.

ARTICLE INFO

Article history:

Received 24 July 2023

Received in revised form 9 August 2023

Accepted 10 August 2023

Available online 16 August 2023

Keywords

Marine macroalgae,
Fatty acids extracts,
Antimicrobial activity,
Resistant microbes,
crude extracts.

ABSTRACT

This research examined the antimicrobial activity of both crude extracts and fatty acid extract of nine different marine macroalgae species (*Caulerpa racemosa*, *Ulva fasciata*, *Halimeda tuna*, *Galaxura rugosa*, *Hypnea cornuta*, *Jania rubens*, *Turbinaria tubinata*, *Hormophysa cuneiformis* and *Polycladia myrica*). The antibacterial activity of these extracts was examined against *Klebsiella pneumoniae*, *Bacillus Subtilis*, *Escherichia coli* and *Staphylococcus aureus*, while the antifungal activity was achieved against *Aspergillus niger* and *Candida albicans*. The fatty acid macroalgal extracts recorded the highest antimicrobial activities against *Candida albicans*, *Staphylococcus aureus* and *Bacillus Subtilis* compared to the crude macroalgal extracts. Most of the brown macroalgae extracts exhibited significant antimicrobial activity against the tested fungi and bacteria. The crude and fatty acid extracts of the selected macroalgae species showed no inhibition zones against *Aspergillus niger*. *Caulerpa racemosa*, *Hypnea cornuta*, *Turbinaria tubinata* and *Hormophysa cuneiformis* showed antimicrobial activity against all the tested pathogenic microorganisms. The brown macroalga *Polycladia myrica* recorded the maximum inhibition zone. The fatty acids extract of the brown alga *Hormophysa cuneiformis* and the red alga *Jania rubens* recorded the highest inhibition zones against the fungus *Candida albicans*. This study established that the macroalgal fatty acid extracts exhibited significant efficacy against both pathogenic fungi and bacteria than the crude macroalgal extract as well as the standard commercial antibiotic. Marine macroalgae have the potential to be an essential resource for natural pharmaceutical products against infectious diseases.

Introduction

In the present time, the alarming rise in antimicrobial resistance poses a pressing public health threat, diminishing the effectiveness of conventional antibiotics in combating infectious diseases. Additionally, globalization and human migration have played a significant role in the dissemination of drug-resistant microorganisms. More than 35 000 people die from antimicrobial-resistant infections in the EU/EEA each year, according to estimates presented in a new report released today. The estimated number of deaths in the report examines the years 2016-2020 and shows an increase from previous estimates. The health impact of antimicrobial resistance (AMR) is comparable to that of influenza, tuberculosis and HIV/AIDS combined (European Centre for Disease Prevention and Control, 2022, November 17).

The estimated global economic losses because of antimicrobial resistance are estimated to be \$100 trillion USD (Schwartz *et al.*, 2018).

* Corresponding author at Suez University

E-mail addresses: hagerkamal82@gmail.com (Hager Ahmed)

So, the intervention to discover novel natural antimicrobial agents with different modes of action than the traditional antibiotics is necessary to overcome the global microbial resistance.

Although, antibiotics derived from terrestrial sources have been extensively used as therapeutic agents to treat various diseases, the vast biodiversity of the oceans presents significant potential for discovering novel compounds with commercial value. (Smit, 2004). Because of the diluting effect of seawater and the hard climate they survive, some chemicals produced by algae possess high biological activity. These compounds exhibit antibacterial potential, as they are specifically developed by algae to combat stresses. (Hughes and Fenical, 2010).

Marine macroalgae, being a rich source of structurally diverse and biologically active secondary metabolites, play a crucial role (Rajasulochana *et al.*, 2012). These secondary metabolites serve various functions, including defense against herbivores, fouling organisms, and pathogens (Watson and Cruz-Rivera, 2003). In recent decades, the bioactive compounds obtained from marine organisms have led to the development of a plethora of

extracts with applications in the pharmaceutical and industrial sectors. The various classes of macroalgae encompass a diverse range of secondary metabolites with antimicrobial activity, such as polysaccharides, polyunsaturated fatty acids, phlorotannins, phenolic compounds, and carotenoids (Abu-Ghannam & Rajauria, 2013 and Rizzo *et al.*, 2016). Seaweeds produce an extensive array of metabolites, many of which possess bacteriocidal properties. The antimicrobial compounds derived from seaweeds exhibit diverse functional groups, leading to bacteriostatic effects, such as brominates, phenols, oxygen heterocyclics, terpenols, sterols, fatty acids, polysaccharides, and polyacetylenes. (Blunt *et al.*, 2015 and Chojnacka & Kim, 2015). Algae produce certain chemicals to combat environmental stresses, and many of these compounds possess antibacterial potential. The remarkable biological activity of these compounds can be ascribed to the diluting effect of seawater and the demanding conditions in which algae flourish (Hughes and Fenical, 2010). Fatty acids are organic compounds characterized by their carboxylic acid functional group and long aliphatic chains, which may contain unsaturated fatty acids. (Desbois and Smith, 2010). The antimicrobial characteristics of fatty acids have been acknowledged for a considerable period, and it is noteworthy that plants and algae produce fatty acids as a defense mechanism against pathogens, including multidrug-resistant bacteria. (Venkata *et al.*, 2015 and Desbois, 2012). They act as precursors in the biosynthesis of eicosanoids, which function as bioregulators involved in numerous cellular processes (Khotimchenko, 2005). Furthermore, polyunsaturated fatty acids (PUFAs) play vital roles in cellular and tissue metabolism, including the regulation of membrane fluidity, facilitation of electron and oxygen transport, and enabling thermal adaptation (Funk, 2001). Marine macroalgae exhibit an intricately diverse lipid composition, with some species containing relatively high concentrations of PUFAs, making them of particular interest for pharmaceutical and food applications (Elenkov *et al.*, 1996).

Many of marine macroalgal species found in abundance biomass in Mediterranean Sea, Suez Canal and Red Sea, Egypt coasts. Therefore, the primary objective of this study was to assess the comparative biological potential of the abundant three green macroalgae (*Caulerpa racemosa*, *Ulva fasciata*, *Halimeda tuna*), three red macroalgae (*Galaxura rugosa*, *Hypnea cornuta*, *Jania rubens*) and three brown algae (*Turbinaria tubinata*, *Hormophysa cuneiformis*, *Polycladia myrica*) as a source of fatty acids which harvested from Port Said, Ismailia and Hurghada coastline, Furthermore, in addition to assessing the antimicrobial activity of crude and fatty acid extracts, the study also conducted evaluations against four pathogenic bacteria and two fungal strains.

Materials and Methods

Study area

Marine macroalgae were harvested manually at the intertidal zone during the low tide on November-December

2022 from three sites. The collection data provides information on the macroalgae collection used in this study (Table 1 and Fig. 1).

Macroalgae samples collection and preparation

The Nine marine macroalgae were harvested manually from various areas representing three classes (Phaeophyceae, Rhodophyceae and Chlorophyceae) to represent the diverse of macroalgae functional groups as shown in Table 2. Upon collection, the samples were carefully washed with seawater to remove epiphytes and other marine organisms. Subsequently, the macroalgal species were transported to the laboratory in sterile polythene bag for additional analysis and experimentation, the selected macroalgae had large biomass were identified according to Gribb (1983), Womersley (1984 & J987) and Aleem (1993). To remove salt, epiphytes, and sand particles, the collected samples underwent a two-step rinsing process: first with tap water and then with distilled water. Afterward, the samples were air-dried in a shaded area at room temperature. Once fully dried, the samples were cut into small pieces and powdered using a mixer grinder.

The extract preparation of Macroalgal crude

Crude extraction involved the utilization of 95% ethyl alcohol, while a chloroform-methanol mixture (50-50, v/v) was employed to extract the fatty acids. In this process, each 5g sample of powder was soaked in 40 ml of the respective solvent for a duration of three days. The resulting crude extracts underwent filtration and subsequent concentration in a rotatory evaporator at a temperature of 40 degrees Celsius. These crude extracts were then weighed and stored in a deep freeze at -20 degrees Celsius until they were ready for testing. (Wong *et al.*, 1994).

Target pathogenic microorganisms

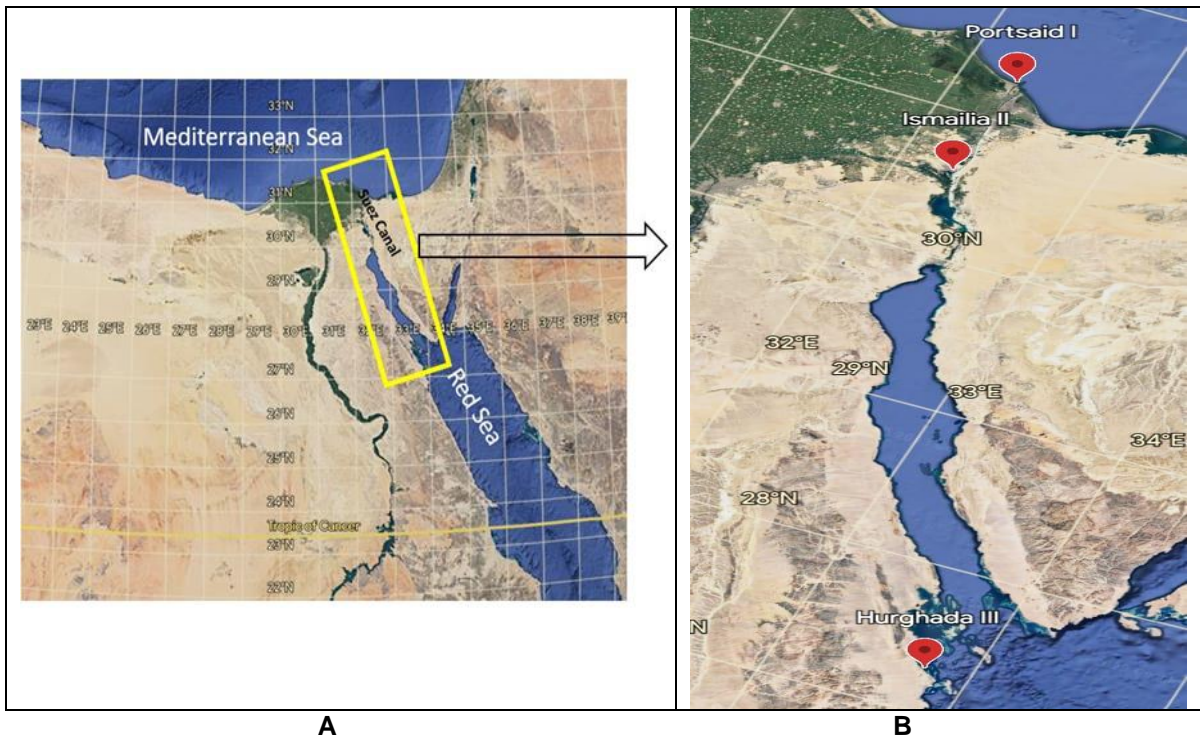
The antibacterial assay conducted by utilizing the following *Bacillus Subtilis* (ATCC 6633), *Klebsiella pneumoniae* ATCC13883, *Escherichia coli* ATCC8739 and *Staphylococcus aureus* ATCC6538. The antifungal test conducted by utilizing the following *Aspergillus niger* ATCC16404 and *Candida albicans* ATCC10231. Microbial strains were obtained from Ultra Biotechnology Research Lab. The bacterial stock cultures were preserved and managed using Muller Hinton Agar medium, and the fungal cultures were sustained on Saboured dextrose agar medium.

Antimicrobial assay

The agar well diffusion method applied to assess the antimicrobial activities of the chosen marine macroalgal extracts. (Magaldi *et al*, 2004). To initiate the agar plate experiment, we spread 100 µl of the microbial inoculum evenly across the entire agar surface. Subsequently, using a sterile cork borer, we aseptically punched a hole with a diameter of 6 to 8 mm. Then, we introduced a desired volume (ranging from 20 to 100 mL) of the antimicrobial agent or extract solution into the well.

Table (1): The collection sites, areas and their coordinates

Site	Area	Latitude	Longitude
The Hunting Club I	Port Said Mediterranean Sea	31.26941" N	32.31513" E
Aldunfah Beach Club II	Ismailia Suez Canal	30.58973"N	32.30508"E
El Ahyaa District III	Hurghada Red Sea	27.1703"N	33.4618"E

**Fig. (1):** A- Map of Egypt showing the studying area and b- The study area showing the selected sites location in Port Said, Suez Canal, and Hurghada.**Table (2):** Samples of macroalgae species in the selected areas

Sample No.	Macroalgal species	area
	<u>Chlorophyta</u>	
H1	<i>Caulerpa racemosa</i> (Forsskål) J. Agardh	Hurghada, Red Sea
H2	<i>Ulva fasciata</i> Delile	Port Said, Mediterranean Sea
H3	<i>Halimeda tuna</i> (J.Ellis & Solander) J.V.Lamouroux	Hurghada, Red Sea
	<u>Rhodophyta</u>	
H4	<i>Galaxura rugosa</i> (J. Ellis & Solander) J.V.Lamouroux	Hurghada, Red Sea
H5	<i>Hypnea cornuta</i> (Kützting) J. Agardh C	Ismailia, Suez Canal
H6	<i>Jania rubens</i> (Linnaeus) J.V. Lamouroux	Hurghada, Red Sea
	<u>Phaeophyta</u>	
H7	<i>Turbinaria turbinata</i> (Linnaeus) Kuntze	Hurghada, Red Sea
H8	<i>Hormophysa cuneiformis</i> (J.F. Gmelin) P.C.Silva	Hurghada, Red Sea
H9	<i>Polycladia myrica</i> (S.G. Gmelin) Draima, Ballesteros, F.Rousseau & T. Thibaut	Hurghada, Red Sea

Following this, the agar plates were stored for 24 hours at 37°C for bacteria and for 72 hours at 28°C for fungi. The measured values for the diameter of the inhibitory zones around the wells were recorded in millimeters. (Espinel-Ingroff et al., 2011).

Statistical assessments

All experiments conducted three times independently, and the data were provided as the mean \pm standard deviation.

Results

Antimicrobial Activities of crude macroalgae extracts

In our study, we conducted tests to determine antimicrobial activity of nine macroalgal species against pathogenic microorganisms. These tests included a comparison with a standard commercial antibiotic (control) as illustrated in Table 3. Aside from the differences in antimicrobial effects throughout ethanolic macroalgae crude extracts (H1-H9), each species presented various degree of inhibition against different pathogenic microorganisms (Fig. 2). The in vitro antibacterial activity of the extracts evaluated by monitoring the appearance of inhibition zones surrounding the filter paper discs. (Photo 1). The selected macroalgae species (H1-H9) not recorded antimicrobial effects (no inhibition zones) with the pathogenic fungus *Aspergillus niger*.

The green macroalga *Caulerpa racemosa* (H1), the red alga *Hypnea cornuta* (H5) and the brown algae (*Turbinaria tubinata*, H7 & *Hormophysa cuneiformis*, H8) showed antimicrobial activity against all the tested pathogenic microorganisms except *Aspergillus niger*. *Caulerpa racemosa* showed the largest inhibition zone (27 \pm 0.1 mm) against *Bacillus subtilis* and *Candida albicans*. The brown alga *Polycladia myrica* (H9) recorded

the highest inhibition zone (25 \pm 0.6) against *Staphylococcus aureus* while the red alga *Galaxura rugosa* (H4) showed the highest inhibition zone diameter (25 \pm 0.4) against *Escherichia coli*. The green macroalga *Ulva fasciata* registered the highest antibacterial activity (25 \pm 0.9) against *Klebsiella pneumonia*.

Antimicrobial Activities of extracted fatty acids

The organic solvents of chloroform-methanol showed antimicrobial activities of fatty acids efficiency with the most selected species extracts than the standard commercial antibiotic (control) (Table, 4; Fig., 3 and Photo, 2). The brown macroalgae (H7, H8 & H9) corroborated antibacterial activities against all the tested bacteria species, beside *Candida albicans* as a tested fungus species. *Polycladia myrica* (H9) recorded the maximum inhibition zone 36 \pm 0.3. *Escherichia coli* registered inhibition zones with the fatty acids of the green alga *Ulva fasciata* H2, red alga *Hypnea cornuta* H5 and the brown alga *Polycladia myrica* H9 to record 34 \pm 0.8, 33 \pm 0.3 and 36 \pm 0.3 respectively. As the same of the crude extract, the selected macroalgae fatty acids extracts (H1-H9) not recorded any inhibition zone with the pathogenic fungi *Aspergillus niger*. The calcareous algae *Halimeda tuna* H3 and *Jania rubens* H6 displayed no activity against the tested bacteria species *Bacillus subtilis* and *Staphylococcus aureus*. The red alga *Galaxura rugosa* not recorded any inhibition zones against the tested bacteria *Escherichia coli* and *Klebsiella pneumoniae*. *Hormophysa cuneiformis* H8 and *Jania rubens* H6 recorded the highest inhibition zones against the fungus *Candida albicans* (26 \pm 0.5 and 25 \pm 0.5 respectively), while the green algae *Ulva fasciata* H2 and *Halimeda tuna* H3 not registered any activity.

Table (3): Standard deviation of sample macroalgae crude extracts (H1-H9) and standard commercial antibiotic (control) against pathogenic microorganisms

Pathogenic microorganism	Green algae			Red algae			Brown algae			Control
	H 1	H 2	H 3	H4	H5	H6	H7	H8	H9	
Pathogenic Bacteria species										
<i>Bacillus subtilis</i> (ATCC 6633)	27 \pm 0.1	NA	24 \pm 0.6	25 \pm 0.1	24 \pm 0.1	22 \pm 0.1	22 \pm 0.6	18 \pm 0.1	25 \pm 0.5	23 \pm 0.3
<i>Staphylococcus aureus</i> (ATCC 6538)	20 \pm 0.3	22 \pm 0.8	20 \pm 0.6	NA	19 \pm 0.1	17 \pm 0.6	23 \pm 0.2	22 \pm 0.8	25 \pm 0.6	30 \pm 0.1
<i>Escherichia coli</i> (ATCC 8739)	21 \pm 0.1	21 \pm 0.2	NA	25 \pm 0.4	23 \pm 0.5	NA	21 \pm 0.4	22 \pm 0.9	17 \pm 0.3	16 \pm 0.5
<i>Klebsiella pneumoniae</i> (ATCC 13883)	21 \pm 0.4	25 \pm 0.9	22 \pm 0.1	NA	22 \pm 0.8	18 \pm 0.9	19 \pm 0.1	20 \pm 0.2	NA	15 \pm 0.6
Pathogenic Fungi species										
<i>Candida albicans</i> (ATCC 10231)	24 \pm 0.2	16 \pm 0.1	18 \pm 0.6	NA	20 \pm 0.3	18 \pm 0.5	17 \pm 0.2	18 \pm 0.3	NA	18 \pm 0.1
<i>Aspergillus niger</i> (ATCC 16404)	NA	NA	NA	NA	NA	NA	NA	NA	NA	17 \pm 0.9

Where, * NA : No activity, Inhibition Zone (mm)

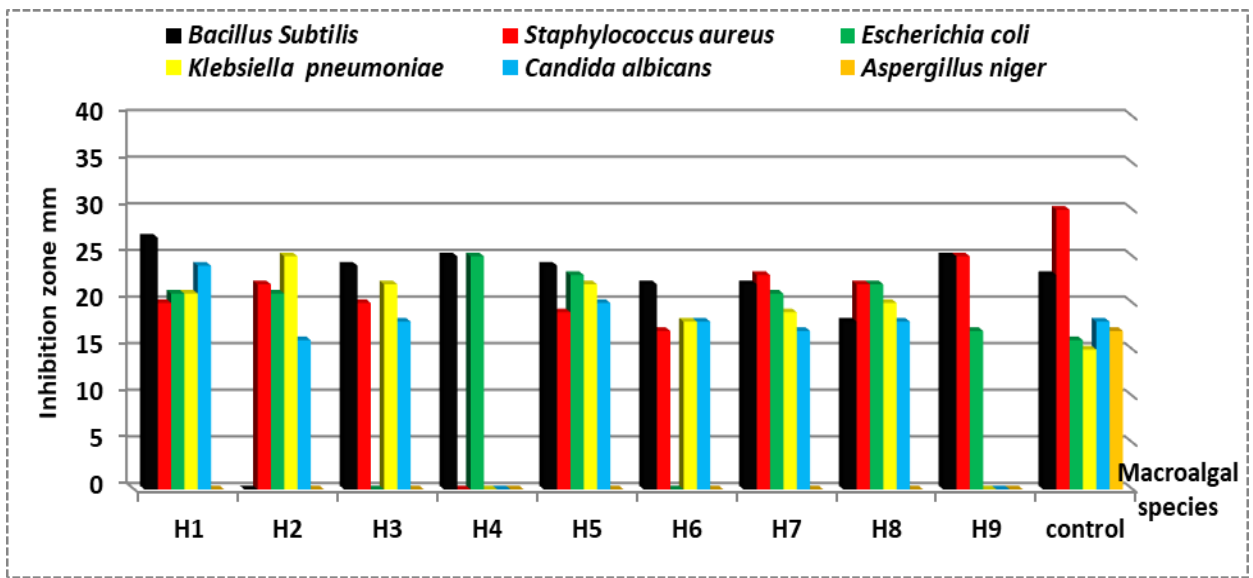


Fig. (2): Antimicrobial activity of crude macroalgal extracts (H1-H9) against six pathogens in comparison with the standard commercial antibiotic (control)

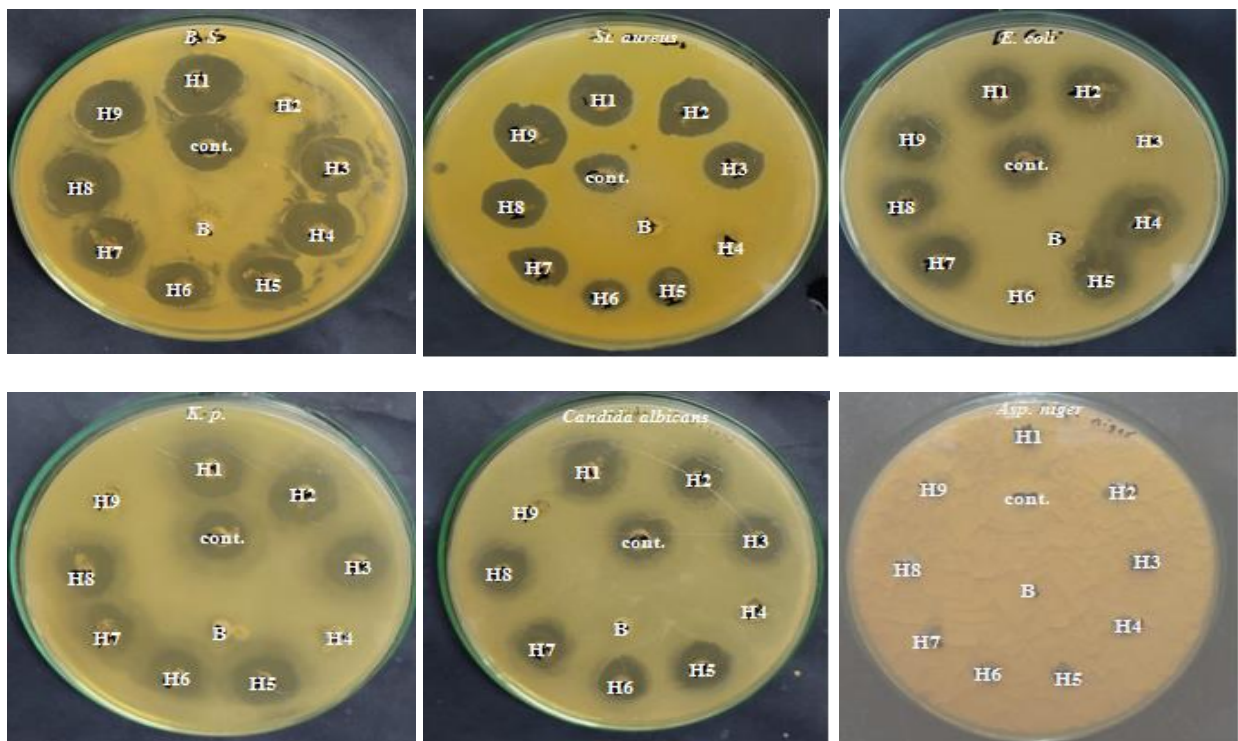


Photo (1): Inhibition zone (mm) of nine macroalgae crude extracts against six pathogens in the presence of cont. (control): Standard commercial antibiotic and B (blank): solvent

Comparative Assessment of Antimicrobial Potential: Crude & Fatty Acids Extracts

Antimicrobial activities percentage of both crude and fatty acids extracts was estimated as shown in Fig. 4. In general, the fatty acid extracts recorded the highest antimicrobial activities against most of the tested pathogenic microbes as *Staphylococcus aureus*, *Candida albicans* and *Bacillus subtilis* than the crude macroalgal extracts. *Escherichia coli* showed high effective with the

crude macroalgal extracts. *Klebsiella pneumoniae* evaluated frequently moderate activity between both crude and fatty acids macroalgal extracts. The crude and fatty acid extracts of the selected macroalgae species (H1-H9) not recorded any inhibition zone with the pathogenic fungi *Aspergillus niger*.

Discussion

Marine macroalgae are abundant in natural bioactive substances, making them valuable sources for potential

biological activities. Their research has made a significant contribution to the study of natural therapeutic compounds that are utilized in biomedical and pharmaceutical fields. (Fayzi *et al.*, 2020). In our study, we evaluated the antimicrobial activities of both crude and fatty acids of nine marine macroalgal species belonging to three different classes (Rhodophyceae, Chlorophyceae and Phaeophyceae) were tested against four pathogenic bacteria and two fungi species in comparison with the standard commercial antibiotic (control). The differences in antimicrobial effects across ethanolic macroalgae crude extracts exhibited diverse levels of inhibition zone (mm) against different pathogenic microorganisms. Among the screened algal crude samples for antimicrobial activity, the green macroalga *Caulerpa racemosa*, the red alga *Hypnea cornuta* and the brown algae (*Turbinaria tubinata* & *Hormophysa cuneiformis*) showed antimicrobial activity against all the tested pathogenic microorganisms except *Aspergillus niger*. *Caulerpa racemosa* displayed the greatest zone of inhibition against *Bacillus subtilis* and *Candida albicans*. This is coincided with Arunkumar *et al.* (2010) and Oumaskour *et al.* (2012).

The brown alga *Polycladia myrica* recorded the highest inhibition zone against *Staphylococcus aureus* while the red alga *Galaxura rugosa* showed the highest inhibition zone diameter against *Escherichia coli*. Earlier research has indicated that the significant antibacterial activity observed in brown seaweeds may be attributed to the presence of a variety of bioactive compounds, including sulphated polysaccharides, peptides, amino acids, lipids, and polyphenols (Vallinayagam *et al.*, 2009; Gupta and Abu-Ghannam, 2011). In our study, the green macroalga *Ulva fasciata* registered high antibacterial and antifungal activities. This is agreeing with Fayzi *et al.*, (2020). The

green marine macroalga *Ulva fasciata* displayed noteworthy antibacterial activity, although higher concentrations were needed to efficiently control bacterial growth. These results are consistent with earlier research emphasizing the presence of abundant bioactive compounds in *Ulva fasciata* that act as antibacterial agents (Christabell *et al.*, 2011 and Chandrasekaran *et al.*, 2014). The selected macroalgae species not recorded antimicrobial effects (no inhibition zones) with the pathogenic fungus *Aspergillus niger*.

The multifaceted antimicrobial properties discovered in seaweeds can be attributed to their diverse inhibitory mechanisms. Numerous studies have suggested that these beneficial bioactivities arise from the synergistic effects of various active compounds found in the same macroalgal extract. (Kotnala *et al.*, 2009).

The ethanolic extract of calcareous red alga *Galaxura rugosa* showed the highest inhibition zone diameter against *Escherichia coli* and *Bacillus subtilis*. On the contrary Fayzi *et al.* (2020) observed the two red algae, particularly the calcareous *Corallina elongata*, exhibited the lowest antimicrobial activity in our study. This result is in line with earlier studies that reported the seaweed species' inability to inhibit the growth of the mentioned microorganisms (Salvador *et al.*, 2007 and Farid *et al.*, 2012). However, it is noteworthy that Osman *et al.* (2012) found a significant antibacterial effect of the methanolic extract of *Corallina elongata* collected from the Egyptian Mediterranean coast. These variations in activity could potentially be attributed to geographical differences, developmental stages of the seaweed, or differences in extraction methods.

Table (4): Standard deviation of samples macroalgae fatty acids extracts (H1-H9) and standard commercial antibiotic (control) against pathogenic microorganisms

Pathogenic microorganism	Sample	Green algae			Red algae			Brown algae			Control
		H 1	H 2	H 3	H4	H5	H6	H7	H8	H9	
<u>Pathogenic Bacteria species</u>											
<i>Bacillus subtilis</i> (ATCC 6633)		25±0.2	26±0.6	NA	28±0.1	29±0.4	NA	25±0.5	24±0.6	27±0.1	23±0.3
<i>Staphylococcus aureus</i> (ATCC 6538)		28±0.5	29±0.4	NA	32±0.2	32±0.6	NA	30±0.6	30±0.2	29±0.4	30±0.1
<i>Escherichia coli</i> (ATCC 8739)		32±0.6	34±0.8	30±0.4	NA	33±0.3	30±0.4	28±0.3	28±0.4	36±0.3	16±0.5
<i>Klebsiella pneumoniae</i> (ATCC13883)		29±0.1	24±0.2	25±0.1	NA	28±0.8	NA	27±0.7	28±0.2	30±0.5	15±0.6
<u>Pathogenic Fungi species</u>											
<i>Candida albicans</i> (ATCC 10221)		21±0.3	NA	NA	22±0.5	22±0.3	25±0.5	18±0.2	26±0.5	19±0.2	18±0.1
<i>Aspergillus niger</i> (ATCC 16404)		NA	NA	NA	NA	NA	NA	NA	NA	NA	17±0.9

Where, NA: No activity

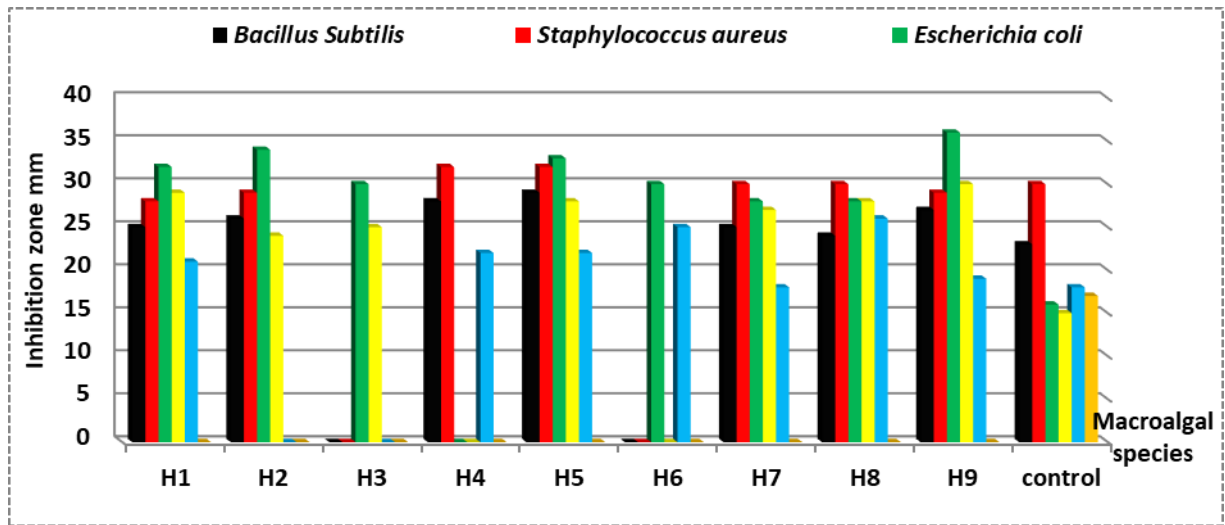


Fig. (3): Antimicrobial activity of fatty acid macroalgal extracts (H1-H9) against six pathogens in comparison with the standard commercial antibiotic (control)

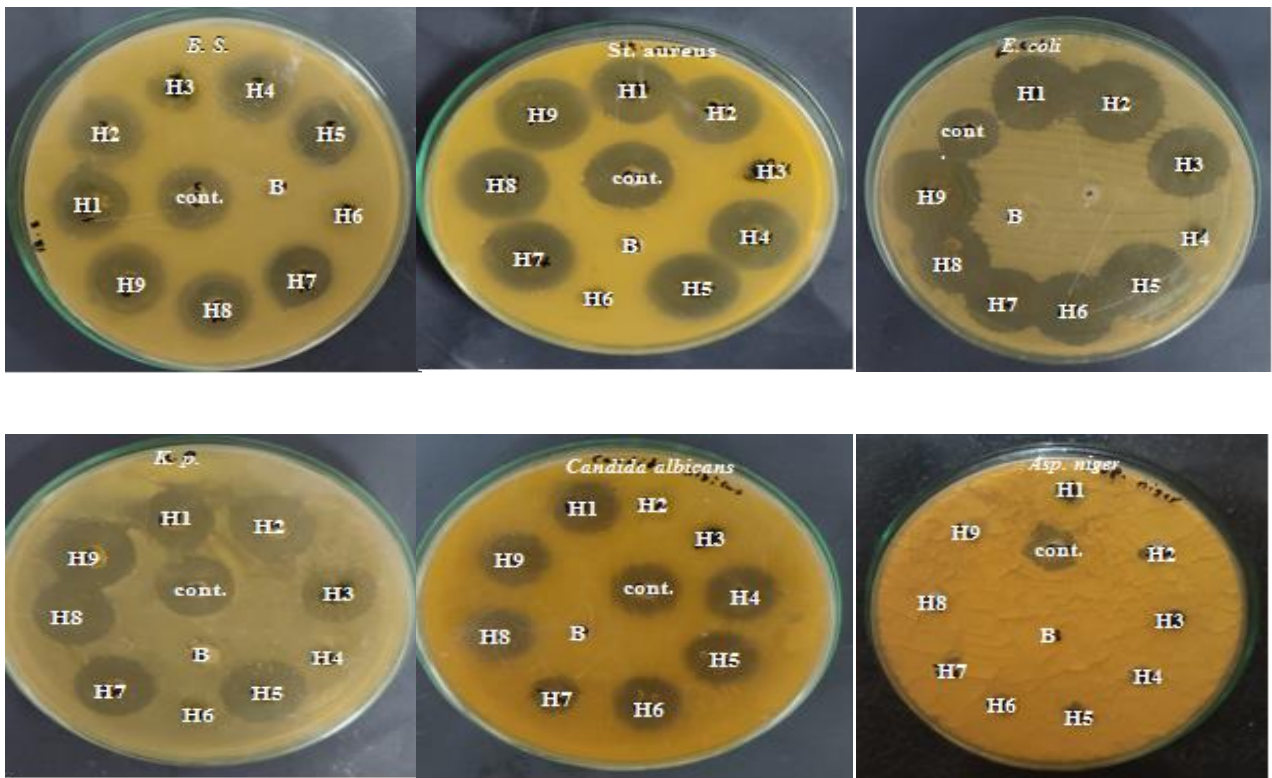


Photo (2): Inhibition zone (mm) of nine macroalgae fatty acid extracts against six pathogens in the presence of cont. (control): Standard commercial antibiotic and B (blank): solvent

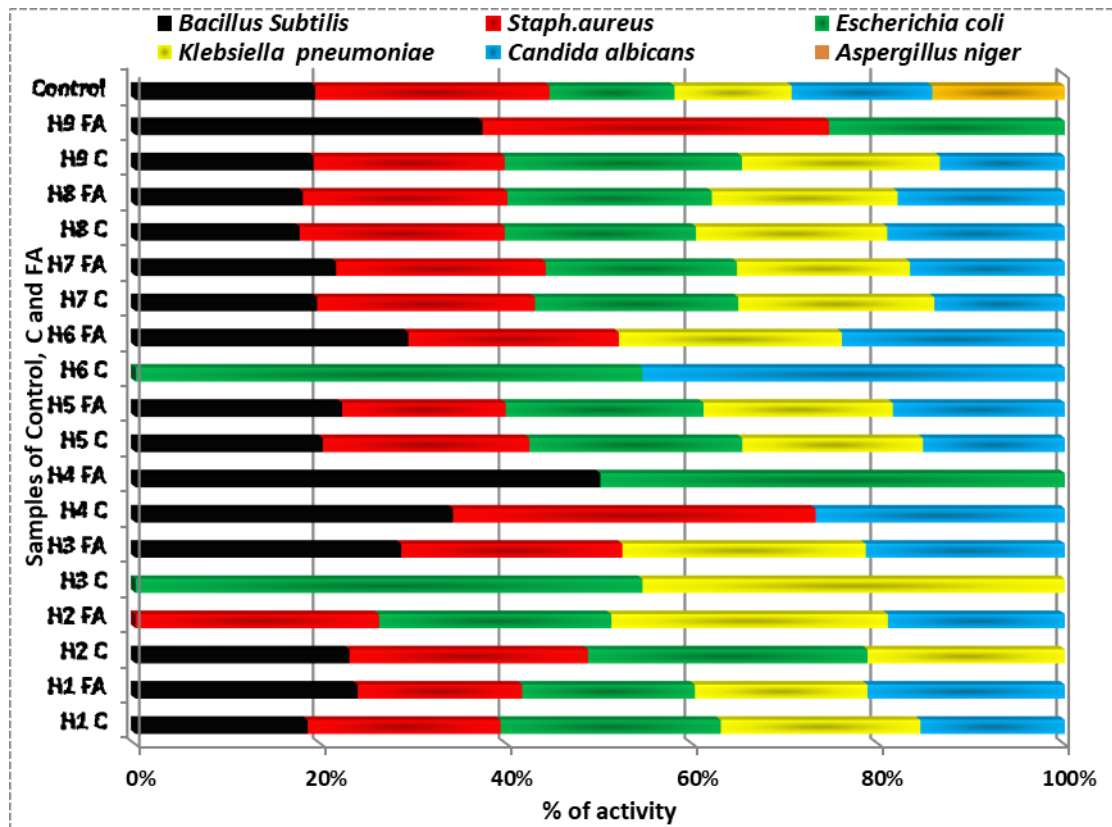


Fig. (4): The antimicrobial activity percentage of Crude (C), Fatty acids (FA) Extracts of the nine selected macroalgal samples (H1-H9) and Control (Standard commercial antibiotic) against six pathogens.

The fatty acids activities have roles in host defenses against potential pathogenic or opportunistic microorganisms. Our data showed that chloroform-methanol extracts of all the nine algae examined have antimicrobial activities of fatty acids efficiency with most selected species extracts than the crude extract and the control. The brown macroalgae corroborated antibacterial activities against all the tested bacteria species, beside *Candida albicans* as a tested fungus species. *Polycladia myrica* recorded the maximum inhibition zone. *Escherichia coli* registered inhibition zones with the fatty acids of the green alga *Ulva fasciata*, the red alga *Hypnea cornuta* and the brown alga *Polycladia myrica*. El Shoubaky and Salem (2014) reported that the brown algae *Padina pavonica* and *Hormophysa triquetra* possess a high number of efficient fatty acids and exhibit robust antibacterial activity, particularly *Hormophysa triquetra*. On the other hand, the calcareous algae *Halimeda tuna* and *Jania rubens* demonstrated no activity against the tested bacterial species *Bacillus subtilis* and *Staphylococcus aureus*. The red alga *Galaxura rugosa* not recorded any inhibition zones against the tested bacteria *Escherichia coli* and *Klebsiella pneumoniae*. *Hormophysa cuneiformis* and *Jania rubens* recorded the highest inhibition zones against the fungus *Candida albicans*, while the green algae *Ulva fasciata* and *Halimeda tuna* did not register any activity. Casillas-Vargas et al. (2021) mentioned that fatty acids have shown tremendous potential as the next generation of antibacterial agents for the treatment of bacterial infections in humans.

Significant strides have been achieved in the understanding of the relative potency and broad spectrum of antibacterial fatty acids, with a focus on identifying promising candidates for drug development. Additionally, recent years have seen biological research that complements these findings by documenting various possible mechanisms of action for traditional antibacterial fatty acids. These mechanisms include the inhibition of DNA/RNA replication, protein synthesis inhibition, cell wall inhibition, inhibition of metabolic pathways, and disruption of the cytoplasmic membrane.

In our study, we conducted a comparative assessment of the antimicrobial potential between crude extracts and fatty acids extracts. In general, the fatty acid macroalgal extracts recorded the highest antimicrobial activities against most of the tested pathogenic microbes as *Candida albicans*, *Staphylococcus aureus* and *Bacillus subtilis* than the crude macroalgal extracts. El Shoubaky and Salem (2014) reported that both the crude and fatty acid extracts of *Hormophysa triquetra* exhibited successful inhibition of six resistant pathogens. Furthermore, they found that the fatty acid and crude extracts of *Hormophysa triquetra* displayed significantly stronger antibacterial activity compared to *Padina pavonica*. Our study showed that *Escherichia coli* showed high effective with the crude macroalgal extracts. This is approving with El Shoubaky and Salem (2014). They mentioned that fatty acids extract of *Padina pavonica* did not demonstrate any inhibitory effect against *E. coli* and *S. aureus*. However, this lack of

inhibition could be related to the presence of other bioactive compounds within the extract that hindered the growth of these bacteria.

In the evaluation of *Klebsiella pneumoniae*, similar activity was observed between the crude and fatty acids macroalgal extracts. However, it was noted that certain extracts did not exhibit inhibitory effects against certain microorganisms, such as the pathogenic fungus *Aspergillus niger*. This observation might be plausibly clarified by the insolubility of particular bioactive compounds in certain solvents utilized for the extraction process.

Conclusion

The findings of this study indicate that marine macroalgal fatty acids exhibited a higher efficacy against pathogenic bacteria and fungi compared to the crude macroalgal extract and even the standard commercial antibiotic. Thus, marine macroalgae, along with their fatty acid extracts, could potentially serve as promising antimicrobial agents, making them potential candidates for pharmaceutical products targeting infectious diseases.

References

1. Abu-Ghannam, N., Rajauria, G. 2013. Antimicrobial activity of compounds isolated from algae. In: Dominguez H., editor. Functional Ingredients from Algae for Foods and Nutraceuticals. Woodhead Publishing Ltd.; Sawston, UK: pp. 287–306.
2. Aleem, A. 1984. The Suez Canal as a habitat and pathway for marine algae and seagrasses. Deep Sea Research A, 31(6), 901-918.
3. Arunkumar, K., Sivakumar, S., Rengasamy, R. 2010. Review on bioactive potential in seaweeds: a special emphasis on bioactivity against plant pathogens. Asian J. Plant Sci., 9(5): 227-240.
4. Blunt, J.W., Munro, M.H.G., Copp, B.R., Keyzers, R.A., Prinsep, M.R. 2015. Marine natural products. Nat. Prod. Rep. 32: 116–211.
5. Casillas-Vargas, G., Ocasio-Malavé, M., Medina, S., Morales-Guzmán, C., Del Valle, G., Carballeira, N.M., Sanabria-Ríos, D.J. 2021. Antibacterial fatty acids: mechanisms of action and implications in next-generation antibacterial agents. Progress in Lipid Research, 82, 101093.
6. Chandrasekaran, M., Venkatesalu, V., Raj, G.A., Krishnamoorthy, S. 2014. Antibacterial activity of *Ulva fasciata* against multidrug resistant bacterial strains. Int. Lett. Nat. Sci., 14: 40-51.
7. Chojnacka, K., Kim, S.-K. 2015. Marine Algae Extracts. Wiley-VCH; Weinheim, Germany. Introduction of Marine Algae Extracts; pp. 1–14.
8. Christabell, J., Lipton, A., Aishwarya, M., Sarika, A., Udayakumar, A. 2011. Antibacterial activity of aqueous extract from selected macroalgae of the southwest coast of India. Seaweed Res. Utilin., 33(1 & 2): 67-75.
9. Desbois, A., Smith, V. 2010. Antibacterial free fatty acids: activities, mechanisms of action, and biotechnological potential. Appl Microbiol Biotechnol, 85(6): 1629–42.
10. Desbois, A. 2012. Potential applications of antimicrobial fatty acids in medicine, agriculture, and other industries. Recent Pat Antiinfect Drug Discov, 7(2): 111–22.
11. El Shoubaky, G. A., Salem, E. AbdE. 2014. Active ingredients fatty acids as antibacterial agent from the brown algae *Padina pavonica* and *Hormophysa triquetra*. J. Coastal Life Med.; 2(6): 431-438.
12. Elenkov, I., Stefanov, K., Konaklieva, S.D., Popov, S. 1996. Effect of salinity on lipid composition of *Cladophora vagabunda*. Phytochem, 42: 39–44.
13. Espinel-Ingroff, A., Canton, E., Fothergill, A., *et al.* 2011. Quality control guidelines for amphotericin B, itraconazole, posaconazole, and voriconazole disk diffusion susceptibility tests with non-supplemented Mueller-Hinton agar (M51-A document) for nondermatophyte filamentous fungi. J Clin Microbiol, 49: 2568–71.
14. Farid, Y., Chennaoui, M., Assobhei, O., Etahiri, S. 2012. Screening of antimicrobial and anti-inflammatory activities in algae from Oualidia. Rev. Microbiol. Ind. San. Environn., 6(2): 192-209.
15. Fayzi, L., Askarne, L., Cherifi, O., Boufous, H., Cherifi, K. 2020. Comparative Antibacterial Activity of Selected Seaweed Extracts from Agadir Coastal Regions. Int.J.Curr.Microbiol.App.Sci, 9(6): 390-399.
16. Funk, C.D. 2001. Prostaglandins and leukotrienes: advances in eicosanoid biology. Science, 294: 1871–1875.
17. Gribb, A. 1983. Marine algae of the southern Great Barrier Reef. Part I. Rhodophyta. Australia Coral Reef Society.
18. Gupta, S., Abu-Ghannam, N. 2011. Bioactive potential and health effects of edible brown seaweeds. Trends Food Sci. Tech., 22(6): 315-326.
19. European Centre for Disease Prevention and Control. (2022, November 17). An Agency of the European Union: 35,000 annual deaths from antimicrobial resistance in the EU/EEA. <https://www.ecdc.europa.eu/en/news-events/agency-information>.
20. Hughes, C.C., Fenical, W. 2010. Antibacterials from the Sea. Chemistry, 16: 12512–12525.
21. Khotimchenko, S.V. 2005. Lipids from the marine alga *Gracilaria verrucosa*. Chem Nat Comp, 41: 285–288.
22. Kotnala, S., Garg, A., Chatterji, A. 2009. Screening for antimicrobial activity in Indian seaweeds. Pertanika J. Trop. Agric. Sci., 32(1): 69-75.
23. Magaldia, S., Mata-Essayaga, S., Hartung de Capriles, C., Perez, C., Colella, M.T., Olaizola, C., Ontiveros, Y. 2004. Well diffusion for antifungal susceptibility testing. Int. J. Infect. Dis., 8: 39–45.
24. Osman, M.E., Abu-Shady, A.M., Elshobary, M.E. 2012. Seasonal fluctuation of antimicrobial activity in macroalgae from Alexandria Coast, Egypt. In: B. Annous and J. Gurtler (Ed), Salmonella, Distribution, Adaptation, Control Measures and Molecular Technologies, Croatia, Tech. Publisher, pp.173-186.
25. Oumaskour, K., Boujaber, N., Etahiri, S., Assobhei, O. 2012. Screening of antimicrobial and antifungal activities in algae from Sidi Bouzid coast. African Journal of Biotechnology, 11(104): 16831-16837.
26. Rajasulochana, P., Krishnamoorthy, P., Dhamotharan, R. 2012. Isolation, identification of bromophenol compound and antibacterial activity of *Kappaphycus* sp. Int. J. Pharm. Bio. Sci., 3, 173-186.
27. Rizzo, L., Frascchetti, S., Alifano, P., Tredici, M.S., Stabili, L. 2015. Association of *Vibrio* community with the Atlantic

- Mediterranean invasive alga *Caulerpa cylindracea*. *J. Exp. Mar. Biol. Ecol.*, 475: 129–136.
28. Salvador, N., Gómez Garreta, M., Lavelli, L., Ribera Siguán, M.A. 2007. Antimicrobial activity of Iberian macroalgae. *Sci. Mar.*, 71(1): 101-113.
 29. Schwartz, K. L., & Morris, S. K. 2018. Travel and the spread of drug-resistant bacteria. *Current infectious disease reports*, 20, 1-10.
 30. Smit, A.S. 2004. Medicinal and Pharmaceutical uses of seaweed natural products: A review. *J. App Phycology*, 16: 245-262.
 31. Vallinayagam, K., Arumugam, R., Kannan, R.R.R., Thirumaran, G., Anantharaman, P. 2009. Antibacterial activity of some selected seaweeds from Pudumadam coastal regions. *Global J. Pharm.*, 3(1): 50-52.
 32. Venkata Mohan, S., Rohit, M., Chiranjeevi, P., Chandra, R., Navaneeth, B. 2015. Heterotrophic microalgae cultivation for biodiesel and waste remediation. *Bioresour Technol*, 184: 169–78.
 33. Watson, S.B., Cruz-Rivera, E. 2003. Algal chemical ecology: introduction to the special issue. *Phycologia*, 42: 319–323.
 34. Womersley, H. 1984. Marine benthic flora of Southern Australia. Part 1. University of Adelaide, South Australia 329pp.
 35. Womersley, H. 1987. Marine benthic flora of southern Australia. Part II. South Australian Government Printing Division Publ.
 36. Wong, W. H., Goh, S.H., Phang, S.M. 1994. Antibacterial properties of Malaysian seaweeds. *Algae Biotechnology in the Asia-Pacific region*. Phang *et al.*, (ed.) University of Malaya, pp. 75-80.