

Antioxidant and antibacterial activities of selected marine algae and Egyptian aromatic herbs regarding to their phytochemical characteristics and mass spectrometry evaluation

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ABSTRACT

In this study, three algae (*Corallina officillica*, *Ulva lactuca*, and *Pterocaldia capallicia*) and three aromatic herbs (*Ocimum basilicum*, *Mentha longifoila*, and *Salvia officinalis*) were investigated. Phytochemicals, *P. capallicia* had the highest total carbohydrate content (17.50%), while *M. longifoila* had the highest total proteins ratio (17.95%). *Mentha longifoila* possessed the maximum total lipids percent (32.14%), while *C. officillica* had the highest ash content (58.83%). As well as, *M. longifoila* had the highest phenolics and carotenoids values, while *U. lactuca* had the highest total flavonoids. To antioxidant activity estimated, the *S. officinalis* (M6) extract showed the highest scavenging activity (89.88%). Regarding antibacterial activities, the *C. officillica* extract exhibited the most powerful activity against *P. aeruginosa* ATCC 9027 (44 mm), followed by *S. officinalis* extract against the same bacterium (40 mm). The GC-MS assessment revealed the existence of several bioactive constituents, involving fatty acids, terpenoids, steroids, flavonoids, carotenoids, phenolic compounds, and other compounds.

INTRODUCTION

The botanical resources for both modern and traditional medicines have been extensively utilized. Moreover, the marine environment has the potential to serve as a valuable reservoir of unique bio-agents that can be utilized to combat cancer and infectious diseases, owing to its extensive biological and chemical diversity (Mayer *et al.*, 2007; Chew *et al.*, 2008). Over the past few decades, numerous new compounds were extracted from organisms in the marine, and a significant proportion of these demonstrate intriguing biological activities (Ely *et al.*, 2004; Dubber and Harder 2008). The marine organisms, therefore, present an abundant source of highly bioactive secondary metabolites that may serve as promising starting points in the development of novel pharmaceutical agents (Zbakh *et al.*, 2012). Marine macroalgae demonstrate a diverse

range of biological activities, including antimicrobial (Bouhlal *et al.*, 2011), antiviral, anti-allergic (Na *et al.*, 2005), antifungal (De Felício *et al.*, 2010), anticancer (Kim *et al.*, 2011), anticoagulant (Shi *et al.*, 2010), antioxidant activities (Devi *et al.*, 2011), and antifouling (Bhadury and Wright, 2004). These algae generate a multitude of chemically energetic metabolites in response to their environment, serving as a defense mechanism compared to other settling organisms (Bhadury and Wright, 2004). Marine algae have been found to contain a diverse range of chemically unique compounds that exhibit antimicrobial properties. These compounds include, but are not limited to, brominated sterols, phenols, polysaccharides, terpenoids, proteins, peptides, terpenes, acrylic acid, phenols, chlorophyllides, and heterocyclic carbons. This has been reported in studies conducted by Manivannan *et al.* (2011) and Priyadharshini *et al.* (2011). Dried seaweed samples have been found to possess more potent medicinal antioxidant and antimicrobial properties compared to their fresh counterparts. Marine macroalgae are a rich source of antimicrobial agents and biomedical antioxidant, which include chlorellin derivatives, acrylic acid, carotenoids, xanthophylls, terpenoids, and sulfur-containing heterocyclic components and halogenated aliphatic, in addition to phycocolloids as agar, carrageenan, and alginate. These compounds had been found to possess scavenging free radicals, bacteriostatic, and bactericidal characteristics, as reported by Rattaya *et al.* (2015) and El-Sheekh *et al.* (2020). According to Alagan *et al.* (2017), seaweed is also rich in several bioactive phytochemicals including riboflavin, vitamins, fatty acids of polyunsaturated, minerals, sterols, polysaccharides, tocopherols, pigments and proteins. Throughout history, botanical extracts and their biological active ingredients have served as a valuable supplier of natural products that have contributed significantly to disease prevention and treatment, thereby promoting human health (Karuppiah and Mustafa, 2013). This is due, in part, to the widely held belief that they are safe and have been traditionally utilized in conventional medicine since ancient times to cure various illnesses and diseases (Rasamiravaka *et al.*, 2015), thereby establishing a long-standing tradition of their use in medicinal practices. As a result, the current study was conducted to investigate the antibacterial and antioxidant effects of methanol and hexane crude extracts of three different species of marine algae (Chlorophyceae, Rhodophyceae, and Phaeophyceae) collected from the Egyptian Mediterranean coasts in addition to three different species of Egyptian aromatic herbs against bacterial pathogens of humans and fish. Furthermore, the quantitative phytochemical constituents of the tested species (total phenolics, total carotenoids, and total flavonoids) were evaluated in this study.

MATERIALS AND METHODS

1.1. Reference bacterial strains

The indicator strains of bacterial (*Pseudomonas aeruginosa* ATCC 9027, *Aeromonas hydrophila* ATCC 13037, *Staphylococcus aureus* ATCC 25923, *P. fluorescens* ATCC 13525, *Vibrio damsela* ATCC 33539, *Streptococcus agalactiae* ATCC 13813,

Escherichia coli ATCC 8739, *V. fluvialis* ATCC 33812, 29212 *Klebsiella pneumoniae* ATCC 13883, and *Enterococcus faecalis* ATCC) were offered by Marine Microbiology Dep., NIOF, Alexandria, Egypt.

1.2. Collection of samples

Three marine algae (*Coralina officillica*, *Ulva lactuca*, and *Pterocaldia capallicia*) were collected from the Egyptian marine environment, while three plants (*Ocimum basilicum*, *Mentha longifoila*, and *Salvia officinalis*) were purchased from aromatic herbs market (Fig.S1). The plant and algal specimens were collected and subsequently subjected to multiple rinses with tap water to eliminate any extraneous sand or debris. A final rinse with DIW (deionized water) was performed to eliminate any residual salts. The specimens were cut to approximately 2 mm dimensions to facilitate subsequent analytical procedures. The drying process of each specimen was conducted under dark ambient conditions and packaged in Kraft paper, as described by **De Pádua et al. (2004)**.



Fig.S1: General features of algae and aromatic plants under the current investigation collected from different Egyptian marine locations and herbs market, respectively.

1.3. Chemical and culture media.

The chemicals employed for biochemical analyzing and extracting of antimicrobial activity were procured from Sigma chemicals-USA with were of high purity. The media materials utilized in the study were of analytical grade and were obtained from established chemical suppliers, with a primary focus on Oxoid Co. The quantification of media composition was conducted in grams per liter, and pH adjustment was performed (pH=7.5) through autoclaving at 121°C for 15 minutes prior to sterilization. The

composition of the Nutrient agar medium consisted of 1 g of beef extract, 2 g of yeast extract, 5 g of peptone, 15 g of agar, and 5g of NaCl, as reported by **Atlas (2005)**.

1.4. Phyto-chemical analysis

1.4.1. Organic matter, ash, and moisture content

After drying at 100°C, the mass losses from a sample of 1 g were considered to determine humidity. The remaining portion of this sample was heated to 600°C to determine the ash percentage. The determined ash value was the difference between 100% and the sum of humidity.

1.4.2. Lipids, carbohydrates, and total proteins

In this study, three separate replicates of each sample, measuring 100 µL each, were subjected to testing. To ensure protein solubilization, each sample was 100-fold diluted utilizing PBS buffer with pH=7.4, then sonicated for duration 30 minutes. The resulting solution was filtered, and 150 µL of the filtrate was subjected to evaluation using the Micro BCA Protein Assay Kit (Thermo Scientific). To perform carbohydrate analysis, the percentage of carbohydrate content was determined by deducting the sum of protein, humidity, ash, fibers, and lipids values from 100. The quantification of lipid content was performed through the extraction of 5 grams of the sample with ethyl ether in a Soxhlet apparatus for a duration of 5 hours, after which the solvent was evaporated. The lipid content was determined by measuring the mass of the residue on the balloon, as reported by **El-Naggar *et al.* (2014)**.

1.4.3. Total flavonoids and total phenolics

The present study involves the utilization of initially prepared Gallic acid and rutin standards to determine the total phenolic and total flavonoid content, respectively. By dissolving 1 mg/mL of the gallic acid in methanol, a stock solution was prepared, followed by the preparation of seven serial dilutions of concentrations 1000, 800, 600, 400, 200, 100, and 50 µg/mL (**Attard, 2013**). Similarly, a stock solution of rutin was prepared by dissolving 1 mg/mL of the compound in methanol, followed by the preparation of seven serial dilutions of concentrations 1000, 600, 400, 200, 100, 50, and 10 µg/mL (**Kirnanmai *et al.*, 2011**). Subsequently, the roots and leaves of the samples were extracted by adding 10 g of plant material to 100 mL of methanol, resulting in an ending concentration of 100 mg/mL. A volume of 200 µL was extracted from each sample and subsequently transferred to a volumetric flask (10 mL). Methanol was then added to the flask until the volume was completed, reaching 2 mg as final concentration of plant material per mL of methanol. The data were then documented using a microplate reader, FluoStar Omega. Six replicates of each of 7 standards of Gallic acid and 2 samples were pipetted into the wells of the plate. The assessment was conducted at a wavelength of 630nm. Similarly, for each of 7 rutin standards and 2 samples, 6 replicates were pipetted into the plate wells, and the analysis was performed at 420 nm.

1.5. Extraction of bioactive agents from marine algae and herb terrestrial plants

For the extraction of bioactive agents, the material was soaked in methanol, a polar solvent, at a proportion of 1:10 (w/v) in ambient temperature containers on a rotator shaker that was continuously shaken and stirred for 96 h. After completion of the soaking period, the extract was collected and filtered using 420 filter papers and cotton. After undergoing filtration, the methanol was subjected to evaporation. The resulting crude extracts were then concentrated and evaporated utilizing a rotary evaporator under vacuum conditions within a temperature range of 45-65°C. The extract solutions of algae that has been concentrated and hydrated, were subjected to lyophilization using a lyophilizer. The resulting freeze-dried algal types were weighed and stored at a temperature of -20°C until they were assayed, as per the procedure described by **El-Sheekh *et al.* (2020)**.

1.6. Antioxidant activity

The 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical scavenging activity was estimated in this study. The samples were prepared by subjecting 10 grams of the plant material to extraction with 100 milliliters of methanol at 100 mg/mL concentration. A 200 µL aliquot was extracted from each sample and transferred to a 10 mL volumetric flask. Methanol was added to achieve a final 2 mg concentration of plant material per mL of methanol for the leaves and roots. Furthermore, as per previous approaches, 1 mL of the solution was transferred to a volumetric flask with a capacity of 10 mL, then methanol was incorporated to achieve the desired volume, reaching to 0.2 mg final concentration of plant material per mL of methanol, as reported by **Duan (2006)**. In the second step, the IC₅₀ value was estimated for every sample. Extracts that demonstrated more than 50% inhibition in any of the initial screening step concentrations were serially diluted to generate 5 concentrations. The third step included conducting the DPPH free radical assay as per the methodology presented by **Boly *et al.* (2016)**. In this experiment, 100µL of freshly prepared DPPH solution (0.1ml in 100ml methanol) were added to 100µL of the sample or substances (which was dissolved in methanol) on a 96-well plate (n = 6). The mixture was then incubated for thirty minutes in the absence of light at RT. The reduction in DPPH chromatic intensity was assessed at a wavelength of 540 nm after the incubation period, and the results were computed as the mean value with standard deviation. The outcomes were documented utilizing a FluoStar Omega microplate reader. The data underwent analysis through the IC₅₀ value and Microsoft Excel were determined by altering the concentration values to their log value and choosing the non-linear regression model that involves the logarithmic transformation of the inhibitor variable and the normalization of the response-variable slope equation using GraphPad Prism 5, as outlined by **Chen *et al.* (2013)**.

1.7. Antibacterial activity

In this study, the crude extracts were dissolved in dimethyl sulfoxide (DMSO), and their

antibacterial effects were tested against 10 different types of bacteria. A quantity of 100 milliliters of sterilized nutrient agar was subjected to cooling in a water bath until it reached a temperature of 50°C, followed by the addition of 1 mL of inoculum containing 108 colony-forming units (CFU). The contents of each test tube were subjected to vortex mixing for a duration of 15-30 seconds, following which they were dispensed onto a sterile Petri dish with a diameter of 100 mm for the purpose of solidification, as described by **Amer and Ibrahim (2019)**. The well diffusion technique was employed to assess the activity, wherein a sterile cork-borer measuring 0.5 cm was utilized to perforate wells in nutrient agar plates that harbored the microorganisms under investigation. All algal and plant extracts, as well as profitable antibiotics, were filtered within a 0.22- μ m membrane filter prior to use. Around 100 μ L of each of the crude extract was additional to each well, and the plates were incubated at 4°C for 2 h, followed by incubation at 37°C for 24 h. The diameter of the zone of inhibition, measured in millimeters, surrounding each well was recorded as a positive result (**Ibrahim *et al.*, 2020**).

1.8. Antibiotic sensitivity against reference strains

The present investigation involved the selection of five commercially available antibiotics to assess their efficacy in inhibiting bacterial strains. The antibiotics utilized in the study were rifampicin (RF, 30 μ g), metronidazole (MTZ, 20 μ g), cephalexin (CL, 30 μ g), piperacillin (TZP, 10 μ g), and amikacin (AMK, 30 μ g). The sterilized prepared medium was inoculated with microbial strains, and the application of small discs of twelve antibiotics was utilized rather than crude extracts of aquatic invertebrates for each microbial strain. The plates were incubated at a temperature of 4°C for a duration of 2 hours, after which they were incubated at a temperature of 37°C for a period of 24 hours, as reported by **Khan *et al.* (2019)** and **Shaaban *et al.* (2020)**. The outcomes were assessed through the measurement of the diameter of the zone of inhibition, which was conducted thrice for everyone well and subsequently expressed in millimeters.

1.9. GC-MS analysis of methanolic crude extracts

Along with this study, the filtered extract of crude methanolic of all investigated plants were analyzed using GC-MS analysis, following the method outlined by **Thakur and Pandey (2016)**. The GC-MS tools were equipped with a detector of TR-5 MS column and thermos mass spectrometer, which had dimensions of 30 meters in length and 0.32 millimeters in internal diameter, and a film thickness of 0.25 micrometers. The carrier gas utilized in the experiment was helium, at a 1.0 mL/min flow rate and a about 1:10 splitting ratio. The temperature program employed for the experiment is as follows: The sample was subjected to a thermal treatment protocol consisting of an initial heating step at 60°C for a duration of 1 minute, followed by a gradual increase in temperature at a rate of 4°C per minute until reaching a final temperature of 240°C. The sample was then maintained at this temperature for a further 1 minute. The temperature of both the injector and detector was adjusted to 210°C. Samples that were diluted using hexane were subjected to injection using a volume of 1 μ L. The identification of the chemical

components was accomplished through deconvolution utilizing the AMDIS software. The components were characterized by their retention indices in relation to C8-C22 (n-alkanes), related mass spectrum, and reference to the NSIT library and Wiley spectral library collection database.

1.10. Statistics

The statistical analysis was conducted using the software SPSS 17. A One-way analysis of variance (ANOVA) test was conducted to determine the distinctions among different groups. A significance level of $P < 0.05$ was utilized.

RESULTS AND DISCUSSION

3.1. Bioactive compounds from marine algae and aromatic plants

For many years, natural products have been used to treat various diseases. Such products are known to demonstrate efficacy, cost-efficiency, and readily available. On consideration of the increasing demand for naturally products in the manufacture of therapeutic drugs, there is an increasing attention to aquatic organisms, particularly algal communities (Suleiman *et al.*, 2019). According to Rashad and El-Chaghaby (2000) and Alagan *et al.* (2017), marine macroalgae are regarded as a rich resource of bio-active secondary metabolites with powerful biological activities, as anticoagulant, antioxidant, antibacterial, antifungal, antiviral, anticancer, and antimutagenic effects. According to Poore *et al.* (2013), seaweed synthesizes potent secondary metabolites like a mechanism of defense in extreme and challenging habitats. There are plentiful reports of chemical compounds derivative from macroalgae that have a wide-ranging of biological activities, several of these have been utilized in the pharmaceutical industry.

It is predictable that there are around 2,500,000 various higher plant species worldwide, and the common of them not thoroughly studied regarding their pharmacological properties. However, for several decades, there has been increasing interest in the use of plants and the identification of their ingredients with antibacterial properties. The main reason for this is that infections consider one of the principal causes of mortality and illness worldwide, mostly infections caused by *Staphylococcus* and *Enterococcus* species, that are responsible for many hospital-acquired infections (Soberón *et al.*, 2007).

Consequently, the present study attempted to investigate and evaluate the antibacterial and activities of antioxidant of selected plants that are rich in bioactive substances (marine algae and aromatic herbs) based on their phytochemical compositions. The phytochemical analyses of six plants collected from the Egyptian coastline and herbs market were conducted using recommended methods.

3.2. Phytochemical analysis of algae and plants

Results presented in Table 1 displayed that the diverse ratios of numerous parameters had a clear flocculation and recorded higher values for moisture content, total lipids, total proteins, total carbohydrates, and ash content either in algal samples (C.

officillica, *U. lactuca* and *P. capillicia*) or herbal samples (*O. basilicum*, *M. longifoila* and *S. officinalis*), respectively.

Collectively, *P. capillicia* was the most alga recorded total carbohydrates content (17.50%) due to its abundance of polysaccharide known as agar. On the contrary, values of other algae and herbs had very low carbohydrates content. The highest total proteins ratio was of *M. longifoila* (17.95%) then *S. officinalis* (14.58%) followed by *O. basilicum* (13.66%), while the algal samples had very low ratios of proteins. Moreover, the highest total lipids percentage was recorded in *M. longifoila* (32.14%), then *P. capillicia* (9.22%), followed by *O. basilicum* (8.30%). Furthermore, the highest ash content was recorded in *C. officillica* (58.83%) followed by *S. officinalis* (31.56%) then *P. capillicia* (10.32%). This referees to its high minerals content.

3.3. Total phenolic compounds, flavonoids, and carotenoids

The data presented in Table 2 demonstrated that different concentrations of main bioactive groups had an obvious variation and recorded higher values for total phenolics and total carotenoids in herbal samples than those of algal sample. However, the highest phenolics concentration was detected in *M. longifoila* (4.14 mg/g) followed by *S. officinalis* (3.35 mg/g) then *O. basilicum* (2.64 mg/g). As well as, the maximum concentration level of total flavonoids was recorded in *U. lactuca* (22.03 mg/g), then *M. longifoila* (12.50 mg/g) followed by *O. basilicum* (11.43 mg/g). Briefly, this result means that *M. longifoila* possessed the highest phenolics and carotenoids values. As well as *U. lactuca* had the highest total flavonoids. Previous study has indicated that algae possess high levels of naturally phytochemical antioxidants, tannins, glycosides, including phenolics, alkaloids, and flavonoids, which have been linked to the management of diverse chronic ailments (El-Sheekh *et al.*, 2020). Farasata *et al.* (2014) determined the levels of total flavonoids and phenolics in 4 *Ulva* species cultivated in various regions of the Persian Gulf's northern littoral in southern Iran. The data presented by the authors indicates that the species *U. clathrate* exhibited the greatest flavonoid content (33.094 mg RE g⁻¹) and phenolic content (5.080 mg GAE g⁻¹) among the analyzed samples.

Table 1: Phytochemical analysis of marine algae and aromatic herbs under investigation.

Algal / Herbal species	Moisture content (%)	Total carbohydrates (%)	Total proteins (%)	Total lipids (%)	Ash content (%)
<i>C. officillica</i>	0.80 ± 0.25	0.036 ± 0.10	3.55 ± 0.20	0.01 ± 0.07	58.83 ± 0.03
<i>U. lactuca</i>	8.89 ± 0.05	0.166 ± 0.25	0.80 ± 0.15	0.09 ± 0.18	8.00 ± 0.05
<i>P. capillicia</i>	3.54 ± 0.60	17.50 ± 0.05	1.66 ± 0.75	9.22 ± 0.10	10.32 ± 0.22
<i>O. basilicum</i>	5.40 ± 0.35	0.259 ± 0.35	13.66 ± 0.05	8.30 ± 0.25	9.06 ± 0.11
<i>M. longifoila</i>	4.93 ± 0.07	0.238 ± 0.85	17.95 ± 0.40	32.14 ± 0.09	7.98 ± 0.17
<i>S. officinalis</i>	6.11 ± 0.28	0.054 ± 0.15	14.58 ± 0.06	7.85 ± 0.45	31.56 ± 0.25

Abou Gabal et al. (2020) compared the biochemical structure of 4 seaweeds (*U. fasciata*, *U. compressa*, *C. officinalis*, and *C. elongate*), gathered from Eastern Harbor Mediterranean coast, Egypt. The findings of the study indicate that *U. compressa* exhibited the greatest concentration of phenols (12.7 mgGAg^{-1} dry wt.) and flavonoids (9.42 mgCAG^{-1} dry wt.). The highest levels of carotenoids were observed in *C. officinalis* (1.04 mgg^{-1} FW), with *C. elongate* following closely behind (0.86 mgg^{-1} FW). The species *U. fasciata* exhibited the lowest ratio, measuring 0.45 mgg^{-1} FW. The results recorded by **Khairy and El-Sheikh (2015)** showed the highest rise of β -carotene in *P. capillacea* in summer. Practical analysis of flavonoids has shown a large changeability depending on the organic solvent and the species utilized for the extraction. There are differences in total phenolic content observed between data obtained by various researchers. Therefore, the recovery of phenolic compounds' contents in different samples is affected by the polarity of the extracting solvents and the solubility of these compounds in the solvent utilized for the extraction process (**Hsaine et al., 2019**).

Ahmed et al. (2019) observed total phenolic acid, flavonoids, and tannin content was observed in ethanol extracts of *O. basilicum*. The observed total phenolic contents demonstrated significant DPPH radical scavenging ability, as evidenced by the IC₅₀ value of 1.29 mg/mL . **Gulluce et al. (2007)** detected that the whole phenolic component of the *M. longifolia* extract was 4.5 g/100 g , that equivalent to gallic acid. According to **Stanisavljevic et al. (2012)**, the naturally dried extract of *M. longifolia* exhibited the highest phenolic content ($113.8 \pm 2.0 \text{ mg}$ of gallic acid of dehydrated extract) and flavonoid content ($106.7 \pm 0.3 \text{ mg}$ of rutin/g of dry extract) among all the extracts tested. The study conducted by Iqbal and colleagues in 2013 revealed that the methanol extract of *M. longifolia* displayed significant rise in the total phenolics and total flavonoids levels compared to the dichloromethane and hexane extracts. The study accompanied by **Hassan et al. (2020)** revealed that the *M. longifolia* ethyl acetate fraction exhibited the highest concentration of total phenolic content, measuring at 228.36 mg GAE/g . In contrast, the pet. ether fraction demonstrated the lowest concentration of total phenolic content, measuring at 154.56 mg GAE/g . Conversely, the pet ether and butanol fractions exhibited a range of $293.86\text{--}153.39 \text{ mg QE/g dw}$ in terms of total flavonoid content. As reported by **Jasicka-Misiak et al. (2018)**, the total phenolic content varied between 63.9 to 134.4 mg GAE/g across the *Salvia* species that were investigated. The tissues of Polish sage varieties exhibit a higher concentration of flavonoids. Phenolic compounds, specifically flavonoids, have been documented to exhibit antimicrobial features against both Gram-negative and Gram-positive bacterial strains. Flavonoids are synthesized by plants regards to microbial infection, and it is not remarkable that they have been found to be active antimicrobial substances in vitro contrary to a wide range of microorganisms. According to **Basile et al. (2000)**, the process of glycosylation serves to enhance the polarity of flavonoids, thereby facilitating their storage in the vacuoles of plant cells and augmenting their solubility in water.

Table 2: Main bioactive groups in marine algae and aromatic herbs under investigation.

Algal / Herbal crude extract & Code	Total phenolics (%)	Total carotenoids (mg/g)	Total flavonoids (mg/g)
<i>C. officillica</i> (M1)	1.89 ± 0.42	0.25 ± 0.24	6.70 ± 0.32
<i>U. lactuca</i> (M2)	1.66 ± 0.14	0.94 ± 0.18	22.03 ± 0.40
<i>P. capallicia</i> (M3)	0.93 ± 0.23	0.82 ± 0.14	7.14 ± 0.31
<i>O. basilicum</i> (M4)	2.64 ± 0.15	1.85 ± 0.25	11.43 ± 0.14
<i>M. longifoila</i> (M5)	4.14 ± 0.50	2.07 ± 0.07	12.50 ± 0.21
<i>S. officinalis</i> (M6)	3.35 ± 0.81	1.20 ± 0.27	3.63 ± 0.35

3.4. Antioxidant properties of different crude extracts

The activity of antioxidant of various crude extracts was estimated via DPPH technique. Slightly, low differences in the activity among different algal and herbal species were observed. Amazingly, the data presented in Table 3 recorded activity values ranged between 72.33% and 89.88%. As shown, *S. officinalis* crude extract (M6) exhibited the highest scavenging activity, followed by 88.12% conducted by *M. pulegium* crude extract (M5). The IC₅₀ is calculated by extrapolating the results of a linear regression study; a lower IC₅₀ indicates a compound's better antioxidant activity. All algal and herbal extracts were less effective in scavenging free radicals than the reference substance Torolox (a common antioxidant medication) (IC₅₀ = 59.1 gmL⁻¹). In comparing the antioxidant activity of various samples to Torolox, all samples under our investigation showed very promising antioxidant properties. This indicated particularly that *U. lactuca*, *M. pulegium*, and *S. officinalis* have strong antioxidant activity, so they are effective agents.

Table 3: Antioxidant activity (%) recorded by bioactive substances in various crude extracts via DPPH method.

Crude extract sample	IC ₅₀ (µg/mL)	DPPH activity (%)
<i>C. officillica</i> / M1	99.7 ± 0.29	74.30 ± 0.07
<i>U. lactuca</i> / M2	94.3 ± 0.31	87.35 ± 0.52
<i>P. capallicia</i> / M3	105.1 ± 0.26	72.33 ± 0.35
<i>O. basilicum</i> / M4	100 ± 0.83	74.11 ± 0.13
<i>M. pulegium</i> / M5	95.5 ± 0.08	88.12 ± 0.22
<i>S. officinalis</i> / M6	92.2 ± 0.05	89.88 ± 0.56
Torolox	59.1 ± 0.25	98.00 ± 0.19

Macroalgal bio-active metabolites possess powerful antioxidant efficacy that may support and protect animal and human health (Varijakzhan *et al.*, 2021). According to Ali *et al.* (2021), the metabolites of a certain number of seaweeds from the Red Sea act as

free radical metal chelators and eliminators, with potent nitric oxide and hydrogen peroxide scavengers, making them naturally occurring antioxidants that reduce lipid peroxidation. Additionally, **Takó et al. (2020)** have reported the antioxidant activity of seaweed-derived metabolites.

Benattouche et al. (2017) determined the antioxidant activity of *C. officinalis* sulfated polysaccharide, showing a moderate effect in inhibiting the formation of radicals. **Abou Gabal et al. (2020)** found that *C. elongate* had the highest radical scavenging of DPPH capacity and whole antioxidant capacity evaluate, despite containing the smallest phenols quantities (5.9 mgGAg^{-1} dry wt.) and flavonoids (8.29 mgCAg^{-1} dry wt.). In a study conducted by **Shivaprasad, et al. (2021)**, it was found that both *C. officinalis* and *C. elongate* demonstrated a noteworthy increase in their capacity to scavenge radicals in DPPH (with IC₅₀ values of 5.41 and 5.51 mg/mL, correspondingly). The crude extract derived from *S. aquifolium* and *C. tomentosum* demonstrated notable antimicrobial activity despite exhibiting the lowest hemolytic activities.

Farasata et al. (2014) observed that the methanolic extracts of *U. clathrata* confirmed greater antioxidant capability with a reduced IC₅₀ (0.881 mg/mL). **Khairy and El-Sheikh (2015)** evaluated the antioxidant activity of *U. lactuca*, *P. capillacea*, and *J. rubens* collected from Boughaz El-Maadya Abu-Qir Bay of Alexandria, Egypt seasonally from spring to autumn 2010. *Ulva lactuca* exhibited the highest antioxidant scavenging activity, particularly during summer. **Ismail (2017)** examined the biochemical composition of 3 seaweeds: *Sargassum linifolium*, *U. fasciata*, and *C. officinalis*. The highest total carotenoid content was found in *C. officinalis* with value 3.8 mgg^{-1} dry wt., and it had the maximum β -Carotene at 3940.12 IU/100 g.

The maximum level of carbohydrates was found in *C. officinalis* (27.98% of dry wt.), besides proteins were highest in *S. linifolium* (14.89%). Additionally, *U. fasciata* encompassed the maximum quantities of phenols (11.95 mgGAg^{-1} dry wt.), lipids (2.96%), and flavonoids (7.04 mgCA/g dry wt.). Moreover, the *U. fasciata* exhibited the highest antioxidant activities at 81.3%, while *S. linifolium* and *C. officinalis* followed at 79.8% and 72.6%, respectively.

Prasedya et al. (2019) estimated the antioxidant activity of *U. lactuca* ULA, *U. lactuca* ULB, and *U. lactuca* ULS with an activity of IC₅₀ $522.23 \pm 43 \text{ }\mu\text{g/mL}$, $534.76 \pm 14 \text{ }\mu\text{g/mL}$, and IC₅₀ $682.23 \pm 23 \text{ }\mu\text{g/mL}$, respectively. **Ismail et al. (2020)** screened the crude extracts of *U. lactuca*, *P. capillacea*, and other algae for their antioxidant properties. The findings of the study indicated that the seaweeds that were analyzed demonstrated antioxidant properties as determined by the DPPH antioxidant assays.

Ahmed et al. (2019) detected a high correlation between antioxidant activity and total phenolic contents of the *O. basilicum* extracts. Additionally, **Patil et al. (2011)** reported that the ethanol extract of *O. basilicum* exhibited higher antioxidant activity than the standard antioxidant. **Gülçin et al. (2007)** demonstrated that the total antioxidant

activity of *O. basilicum* was 70.4% inhibition, using α -tocopherol as a reference antioxidant.

The present study investigates the antioxidant potential of the methanolic extract obtained from *M. longifolia* were assessed by Gulluce *et al.* (2007). The extract exhibited favorable antioxidant activity in DPPH assays, as evidenced by an IC₅₀ value of 57.4 μ g/mL. **Stanisavljevic *et al.* (2012)** found that the maximum antioxidant activity estimated by the DPPH assay for *M. longifolia* was estimated in the extracts derived from naturally dry herbs (EC₅₀ = 0.022 \pm 0.001 mgmL⁻¹). The authors **Iqbal *et al.* (2013)** informed that the methanol extracts of *M. longifolia* demonstrated noteworthy antioxidant activity, as assessed through the DPPH scavenging free radical assay. According to **Elansary *et al.* (2020)**, the leaf extracts of *M. longifolia* exhibited significant antioxidant properties attributed to the presence of major polyphenols such as rosmarinic, cynaroside and cryptochlorogenic acids.

Hassan *et al.* (2020) found that the ethyl acetate fraction of *M. longifolia* had the maximum antioxidant activity, with a lower IC₅₀ of 107.01 μ g/mL. The study conducted by **Abd El Fattah *et al.* (2011)** determined that the traditional utilization of *S. officinalis* as an antioxidant is innocuous and may provide advantageous characteristics. Moreover, it was observed that *S. officinalis* could potentially demonstrate regulatory effects on oxidative damage caused by γ -rays in rats. **Walch *et al.* (2011)** showed that the antioxidant potential of *S. officinalis* L. was highly reliant on rosmarinic acid and its derivatives. **Kolac *et al.* (2017)** confirmed that *S. officinalis*, which has a high flavonoid and phenolic acid content, is a powerful antioxidant herb. **Vieira *et al.* (2020)** suggested that *S. officinalis* is a natural supply that can allow the development of efficient treatments for oxidative strain.

Conclusively, polyphenolics are considered bioactive agents that contribute to the antioxidant and antimicrobial properties of plants and seaweeds. Chidambararajan *et al.* (2019) measured the scavenging activity for selected seaweeds and found it to be 78.5% in the best case. This may be attributed to the high polyphenolic compounds and flavonoid contents of the extract, which showed maximum growth inhibition of 74.4%.

3.5. Antibacterial properties of different crude extracts

Complementarily, the antibacterial activity of bio-active substances included in crude extracts of both marine algae and aromatic herbs was detected by utilizing well cut-diffusion procedure. On focus, data in Fig.2S & Table 4 revealed that few methanolic crude extracts of tested algae and fewer crude extracts of the aromatic herbs had potent activities. However, crude extracts showed considerable value against both *P. aeruginosa* ATCC 9027, which was the biggest inhibited bacterium and *S. aureus* ATCC 25923, which was the most affected bacterium. The antibacterial activity was recorded against *S. aureus* ATCC 25923 as 22, 18 and 16 mm by *C. officillica*, *U. lactuca* and *P. capillicia* extract, respectively. Whiles, it was recorded as 44 mm against *P. aeruginosa* ATCC 9027 by *C. officillica* extract. In addition, the algal extracts exhibited low antibacterial

activity against *E. faecalis* ATCC 29212 as 10, 14 and 12 mm, respectively. Moreover, the *C. officillica* extract showed activity against *V. fluvialis* ATCC 33812, *B. subtilis* ATCC 6633 and *A. hydrophila* ATCC 13037 as 24, 18 and 14 mm, respectively.

On the other side, crude extracts of *O. basilicum*, *M. pulegium*, and *S. officinalis* recorded high antibacterial activities against *S. aureus* ATCC 25923 and their values were 34, 36, and 38 mm, respectively. Additionally, the same extracts assessed noticeably high activities against *P. aeruginosa* ATCC 9027 with values of 38, 34 and 40 mm, respectively. Moreover, *E. coli* ATCC 8739 was affected by *M. pulegium* extract with inhibition zone recorded as 20 mm. As for the rest of the cases, it had no effect at all. Furthermore, this finding can summarize that the very weak value was detected against *E. faecalis* ATCC 29212 (10 mm) by *C. officillica* extract, while it was also the most powerful crude extract against *P. aeruginosa* ATCC 9027 (44 mm) then *S. officinalis* extract against the same bacterium (40 mm).

Basically, methanol was chosen as the extraction solvent due to it is considered one of the highly effective solvents for the extraction of antimicrobial compounds from plants. Methanol's polarity ensures the extraction of polar and slightly polar active substances from plants regarding microorganisms, as tannins, terpenoids, polyphenols, and flavones (Sánchez *et al.*, 2016). The well dispersion assay is a qualitative procedure besides, is mainly utilized for choosing extracts with antimicrobial activity, particularly when the diameter zones of inhibition are exhibiting a minimum diameter of 10mm or greater (Usman *et al.*, 2009). It is crucial to realize that the variation in the size of inhibition zones among different extracts may be attributed to the polarity of the compounds obtained. This is because a diffusible but less efficient extract may yield a larger diameter of inhibition compared to a non-diffusible but more potent extract (Savaroglu *et al.*, 2011).

Table 4: Screening of antibacterial activity of 6 methanol crude extracts from selected marine algae and aromatic herbs against human and fish bacterial pathogens via well-cut diffusion techniques.

Microorganisms	Algal / Plant methanol-crude extract concentration (50 µg) / well						CIP (100 µg/well)
	M1	M2	M3	M4	M5	M6	
<i>S. aureus</i> ATCC 25923	22 ± 0.6	18 ± 0.10	16 ± 0.05	34 ± 0.05	36 ± 0.13	38 ± 0.03	38 ± 0.10
<i>E. coli</i> ATCC 8739	ND	ND	ND	ND	20 ± 0.09	ND	34 ± 0.15
<i>P. aeruginosa</i> ATCC 9027	44 ± 0.07	ND	ND	38 ± 0.09	34 ± 0.14	40 ± 0.01	36 ± 0.11
<i>E. faecalis</i> ATCC 29212	10 ± 0.10	14 ± 0.07	12 ± 0.15	ND	ND	ND	26 ± 0.13
<i>K. pneumoniae</i> ATCC 13883	ND	ND	ND	ND	ND	ND	28 ± 0.06
<i>V. damsela</i> ATCC 33539	ND	ND	ND	ND	ND	ND	30 ± 0.05
<i>V. fluvialis</i> ATCC 33812	24 ± 0.06	ND	ND	ND	ND	ND	16 ± 0.17
<i>P. fluorescens</i> ATCC 13525	ND	ND	ND	ND	ND	ND	30 ± 0.31
<i>S. agalactiae</i> ATCC 13813	ND	ND	ND	ND	ND	ND	20 ± 0.25
<i>A. hydrophila</i> ATCC 13037	14 ± 0.11	ND	ND	ND	ND	ND	22 ± 0.11

ND = Not Detected, 1 = *C. officillica*, 2 = *U. lactuca*, 3 = *P. capallicia*, 4 = *O. basilicum*, 5 = *M. pulegium*, and 6 = *S. officinalis*.

CIP = Ciprofloxacin antibiotic.

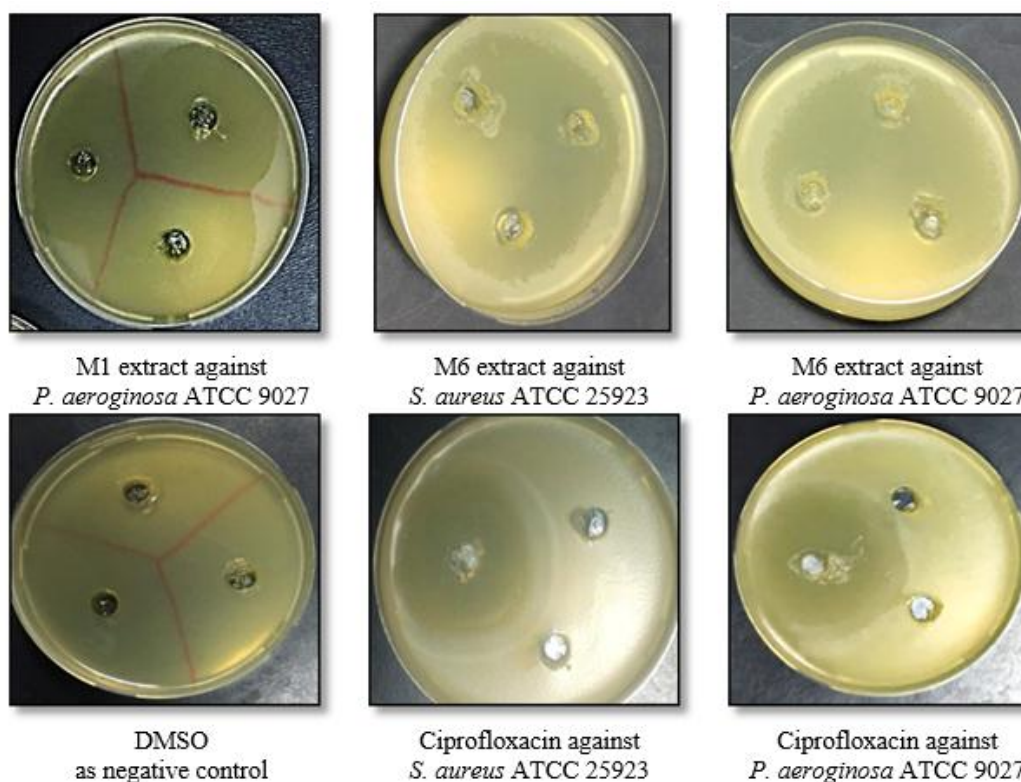


Fig. S2: Antibacterial activity of methanol crude extracts against tested bacterial pathogens. Notice: M1 = *C. officillica*, M2 = *U. lactuca*, M3 = *P. capillicia*, M4 = *O. basilicum*, M5 = *M. pulegium*, and M6 = *S. officinalis*.

Preliminarily, **Zbakh *et al.* (2012)** found that methanolic extracts from 20 Moroccan species of macroalgae exhibited antibacterial activity against *E. coli*, *E. faecalis*, and *S. aureus*. The red algae extracts are significantly subdued the growing of the 3 tested bacterial strains, with inhibition zones ranging from 20 to 24 mm.

Several studies have reported findings that support our own. For example, **Osman *et al.* (2013)** demonstrated the antimicrobial activity of different 3 macroalgal species (*Sargassum vulgare*, *U. fasciata* Delile, and *Jania rubens* Lamouroux) from Abu-Qir Bay, Alexandria, Egypt. The 70% acetone extract of *U. fasciata* exhibited the highest level of antimicrobial activity against all microorganisms tested. Results from **Sánchez *et al.* (2016)** showed quite variable antimicrobial activity among each algal extract, fluctuating from 0 to 2.8 cm. The extract of *P. laevigata* was the most active against all the clinical isolates, and the highest inhibition diameter was 2.8 ± 0.5 cm against the *S. aureus* strain. Additionally, both *K. pneumoniae* and *E. faecalis* were only subdued by *O. ficus-indica* and *P. laevigata*, with inhibition regions ranging between 1.3 ± 0.2 and 0.7 ± 0.08 , respectively. **Mohy El-Din and El-Ahwany (2016)** evaluated the antibacterial activity of methanolic extracts of *P. capillacea*, *J. rubens*, and *C. mediterranea* versus *P. capillacea* and *V. fluvialis* was found to be the most effective at controlling its growth.

Three marine extracts from *Avicennia marina*, *Sargassum* sp., and *C. mediterranea* have been shown to have antibacterial activity against *Arthrobacter sulfureus* YACS14, *S. aureus*, *E. coli*, and *V. anguillarum* MVM425 by **Hamdona et al. (2018)**. **Negm et al. (2018)** detected the antibacterial activity of four algal species (*C. mediterranea*, *Grateloupia* sp., *U. fasciata*, and *P. capillacea*) against bacterial pathogens using a well-cut diffusion procedure. However, the antibacterial activity exhibited a range of positive records, with values ranging from 2.4 to 23.6 AU.

Ghareeb et al. (2019) reported potential antibacterial activities of *U. lactuca* extracts and concluded that the phenolic content, detected from HPLC, plays a role in the antioxidant activity. The results obtained by **Cadar et al. (2022)** emphasized the antioxidant and antibacterial activity of the studied *U. lactuca* L extracts. On the other side, **Early and Grein (1994)** conducted a study on the antibacterial activity of plant extracts from 28 families. Their results revealed that approximately 60 percent of the tested plant extracts displayed some level of antibacterial action. **Oliveira et al. (2007)** screened 26 Brazilian plant extracts to identify antibacterial properties against *A. hydrophila*, *B. subtilis*, *P. aeruginosa*, and *S. aureus*. It was observed that 13 of the tested extracts showed antibacterial activity. Additionally, **Weckesser et al. (2007)** demonstrated the effectiveness of *S. officinalis* extracts against a panel of bacteria. Due to variations in the methodology used, it is difficult to experimentally compare the findings for antibacterial plant components or extracts from various research (**Burt, 2004**). However, Natural products possess an unparalleled range of chemical diversity, rendering them a promising source for distinctive and permissible additives and pharmacological interventions, as indicated by **O'Bryan et al. (2008)**.

Kaya et al. (2008) found that methanol extracts of *O. basilicum* showed antimicrobial activity against strains of *P. aeruginosa*, *Shigella* sp., *Listeria monocytogenes*, *S. aureus*, and *E. coli*. **Patil et al. (2011)** investigated methanol, hexane, and ethanol extracts from *O. basilicum* Labiatae for their *in vitro* antimicrobial properties. Equally the methanol and hexane extracts, but not the ethanol extracts, inhibited three isolates that were studied. **Salleh et al. (2021)** found that methanol extracts of the *O. basilicum* L. species were most active against Gram-positive bacteria and least effective against *E. coli*. The extract showed the highest diameter inhibition zone of 16.33 ± 1.53 mm against *B. cereus*. The study conducted by **Hassan et al. (2020)** established the antimicrobial efficacy of the methanolic extract of *M. longifolia* against a fungal isolate, one yeast isolate, two Gram-positive bacterial isolates, and two Gram-negative bacterial isolates.

Elansary et al. (2020) found that leaf extracts of both *M. longifolia* and *M. piperita* had antibacterial impacts against most bacterial species (with *P. aeruginosa* being the most sensitive), that were related to the polyphenols present in the extracts. **Doudi et al. (2014)** reported that *S. officinalis* ethanol extracts showed a significant inhibitory impact on the progress of multi-drug resistant bacteria (*S. aureus*, *E. coli*, *P. aeruginosa*, and *K. pneumoniae*) in a well diffusion method. **Beheshti-Rouy et al. (2015)** found that a Sage

mouthwash containing *S. officinalis* extracts significantly reduced *S. mutans* causing dental plaque in school-aged children. **De Oliveira *et al.* (2019)** revealed that the *S. officinalis* extract provided antimicrobial activity against entirely isolates of *S. aureus*, *S. mutans*, and *Candida* spp. **Mendes (2020)** assessed the antimicrobial activity of the *S. officinalis* dichloromethane crude extract against *Porphyromonas gingivalis* and found that it is a promising antibacterial agent.

Despite of these findings, the crude extracts bioactivities of tested samples may be qualitatively not quantitatively. This means that the most effective antibacterial and/or antioxidant activities might be due to the presence of highly effective bioactive ingredient in the extract. Surprisingly, flavonoids in plants may be responsible for their antimicrobial activity (**Wu *et al.*, 2009**), although further enrichment and characterization are needed to confirm the bioactive compound(s). The present finding is significant as previous research on the influence of crude extracts has demonstrated a higher level of efficacy contrary to Gram-positive bacteria in comparison to Gram-negative bacteria (**Palombo and Semple, 2001**). According to **Parekh *et al.* (2006)**, polar compounds with low water solubility are responsible for the antimicrobial activity of bioactive substances, and therefore, organic solvent extracts exhibit higher potency. The study conducted by **Yompakdee *et al.* (2012)** has revealed the existence of antimicrobial activity in methanol extracts, which suggests a polar configuration. This finding is in line with the observed greater antibacterial efficacy against Gram-negative bacteria compared to Gram-positive bacteria.

3.6. Sensitivity and susceptibility of reference strains towards antibiotics

Considering their characteristics, mode of action, and frequent prescription for combating microbial agents, the activity (mm) of 12 commercial antibiotics [Chloramphenicol (Chl), Cloxicillin 10 (Clx), Cefeprem (Cef), Colistine (Col), Nolidiric acid (Nol), Gentamicin (Gen), Clonithmycin (Cln), Ampcilin (Amp), Amoxicillin 30 (Amo), Meropenem 10 (Mer), Cefterixaxone 30 (Ceft), and Ceftazidene (Cefz)] were investigated and then compared to the data of potent crude extracts (mm) (Table 5).

Fundamentally, few Gram-positive and Gram-negative bacteria exhibited clear susceptibility to most of the experienced antibiotics. The former was influenced by 15 commercial antibiotics, while the latter was inhibited by 20 commercial antibiotics. Specifically, the most affected bacterial pathogen by commercial antibiotics was *E. faecalis* ATCC 29212 (7 antibiotics) followed by *S. aureus* ATCC 25923 (6 antibiotics) then *P. aeruginosa* ATCC 9027 (5 antibiotics) with high records and *P. fluorescens* ATCC 13525 (5 antibiotics) with low records.

In some details, *E. faecalis* ATCC 29212 was very sensitive towards Chloramphenicol, Colistine, Gentamicin, Clonithmycin, Amoxicillin, Meropenem, and Cefterixaxone 30, while it was resistant towards Cloxicillin 10, Cefeprem, Nolidiric acid, Ampcilin, and Ceftazidene. Also, *S. aureus* ATCC 25923 was sensitive towards Chloramphenicol, Gentamicin, Clonithmycin, Meropenem 10, and Cefterixaxone 30, while

it was resistant towards Cloxicillin 10, Cefeprem, Colistine, Nolidiric acid, Amoxicillin 30, and Ceftazidene. As well as *P. aeruginosa* ATCC 9027 was sensitive toward Chloramphenicol, Cloxicillin 10, Gentamicin, Clonithmycin, and Meropenem 10, while it was resistant towards Cefeprem, Colistine, Ampicilin, Amoxicillin 30, Ceftaxone 30, and Ceftazidene. In addition, *P. fluorescens* ATCC 13525 and *V. fluvialis* ATCC 33812 exhibited moderate susceptibility towards different 4 antibiotics only, while *S. agalactiae* ATCC 13813, *A. hydrophila* ATCC 13037, and *E. coli* was susceptible against only one antibiotic and resistant to the others. On the contrary, *K. pneumoniae* ATCC 13883 displayed clear resistance to all tested antibiotics.

Table 5: Sensitivity test of several antibiotic discs against reference bacterial strains.

Bacterial pathogen	Positive activity (+)											
	Chl	Clx	Cef	Col	Nol	Gen	Cln	Amp	Amo	Mer	Ceft	Cefz
<i>S. aureus</i> ATCC 25923 (+ve)	+++	-	-	-	-	+++	+++	+++	-	+++	+++	-
<i>E. faecalis</i> ATCC 29212 (+ve)	+++	-	-	+++	-	+++	+++	-	+++	+++	+++	-
<i>K. pneumonia</i> ATCC 13883 (+ve)	-	-	-	-	-	-	-	-	-	-	-	-
<i>S. agalactiae</i> ATCC 13813 (+ve)	-	-	-	-	+	-	-	-	-	-	-	-
<i>E. coli</i> ATCC 8739 (-ve)	-	-	-	+		+		-	-	-	-	-
<i>P. aeruginosa</i> ATCC 9027 (-ve)	+++	+	-	-	-	+++	+++	-	-	+++	-	-
<i>V. damsela</i> ATCC 33539 (-ve)	++	-	-	+++	-	+++	+++	-	-	-	-	-
<i>V. fluvialis</i> ATCC 33812 (-ve)	++	-	-	++	-	++	++	-	-	-	-	-
<i>P. fluorescens</i> ATCC 13525 (-ve)	++	+	-	++	-	++		-	-	++	-	-
<i>A. hydrophila</i> ATCC 13037 (-ve)	-	-	+	-	+	-	-	-	-	-	-	-

Chl = Chloramphenicol, Clx= Cloxicillin, Cef = Cefeprem, Col = Colistine, Nol = Nolidiric acid, Gen = Gentamicin, Cln = Clonithmycin, Amp = Ampicilin, Amo = Amoxicillin, Mer = Meropenem 10, Ceft = Ceftaxone, Cefz = Ceftazidene.

Reasonably, the commercial antibiotics used in the studies represented a wide range of effectiveness. For example, Ampicillin is a highly effective antibiotic that is commonly utilized in clinical settings to treat a diverse array of bacterial infections. Cefotaxime, a third-generation cephalosporin, is employed in the treatment of nosocomial infections caused by Gram-negative bacteria. Imipenem is a member of the carbapenem group of β -lactam antibiotics, which is primarily employed in the treatment of multi-organism and Gram-negative infections (Klastersky, 2003). According to Jubeh *et al.* (2020), Oxacillin is a type of β -lactam antibiotic that is commonly employed to combat Gram-positive bacterial strains, including *S. aureus*. Additionally, vancomycin has been found to be a reliable and secure antibiotic for treating a range of severe Gram-positive infections.

3.7. GC-MS evaluation of various crude extracts

The GC-MS data of different methanolic crude extracts presented in both Fig.1& Table 6. Initially, the *C. officinalis* extract (Coded as M1) confirmed presence of numerous active compositions, with a total 25 major compounds. However, the chemical profiles of them are mainly: Dodecanoic Acid, Methyl Ester; 8-Heptadecene; Tetradecanoic Acid,

Methyl Ester; Tetradecanoic Acid; 2,6,10-Trimethyl,14-Ethyle Ne-14-Pentadecne; 2-Pentadecanone, 6,10,14-Trimethyl-; 2-Pentadecanone, 6,10,14-Trimethyl-; 3,7,11,15-Tetramethyl-2-Hexadecen -1-Ol; 9-Hexadecenoic Acid, Methyl Ester, (Z)-; Hexadecanoic Acid, Methyl Ester; Hexadecanoic Acid, Ethyl Ester; Hexadecanoic Acid; 9,12-Octadecadienoic Acid, Methyl Ester, (E,E)-; 9-Octadecenoic Acid (Z)-, Methyl Ester; Octadecanoic Acid, Methyl Ester; Cis-5,8,11,14,17-Eicosapentaenoic Acid; Oleic Acid; Hexadecanoic Acid, 1-(Hydroxymethyl)-1,2-Ethanediy Ester; Tributyl Acetylcitrate; Cis-13-Eicosenoic Acid; Eicosanoic Acid, Methyl Ester; Docosanoic Acid, Methyl Ester; Diisooctyl Phthalate; Cholesta-4,6-Dien-3-Ol, (3á)-; Ethyl Iso-Allocholelate; and Cholest-5-En-3-Ol (3á)-. Also, the methanolic extract from *U. lactuca* (Coded as M2) showed the existence of numerous bioactive constituents, with totally 14 major compounds, mainly are: à-Tocopherol nicotinate; 1-Butoxy-1-isobutoxy-butane; n-Butyric acid 2-ethylhexyl ester; Sulfurous acid, dodecyl; 6-Tridecanol, 3,9-diethyl-; Methyl tetradecanoate; Pentadecanoic acid, methyl ester; L-Sorbose; Cyclopropaneoctanoic acid,; Heptadecanoic acid, methyl ester; 9-Octadecenoic acid (Z)-, methyl ester; Methyl stearate; 8,11-Octadecadienoic acid, methyl ester; Cyclopropaneoctanoic acid, 2-octyl-, methyl ester.

As well as, the chemical profiles of methanolic extract from *P. capallicia* (Coded as M3) revealed the existence of numerous bioactive constituents, with totally 10 major, mainly are: à-Tocopherol nicotinate; Heptadecane; 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester; Hexadecanoic acid, methyl ester; Octadecanal; Methyl stearate; Bis(2-ethylhexyl) phthalate; 3-Hydroxypropyl palmitate, TMS derivative; Cholesta-4,6-dien-3-ol, (3á)-; and Cholesterol.

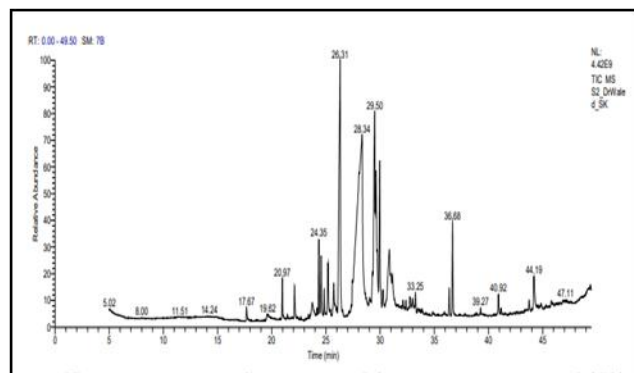
In addition, GC-MS data of methanolic that extract from *O. basilicum* (Coded as M4) recorded the existence of several effective compositions, with a total of 8 major compounds. However, the chemical profiles of them are mainly: 4-Bromophenethyl alcohol; Linalool; 2-Propenoic acid, 3-phenyl-, methyl ester; 2-Propenoic acid, 3-phenyl-, methyl ester, (E)-; Hexadecanoic acid, methyl ester; Methyl stearate; Dimethyl *u-truxinate; and 2-Propenoic acid, methyl ester, 3-phenyl-. Moreover, the results of GC-MS of *M. pulegium* extract (Coded as M5) confirmed the existence of several active configurations, with a total of 16 major compounds. However, their chemical profiles are mainly: 2-(2-Aminoethyl)pyridine; Dimethyl sulfone; à-Terpineol; Cyclohexanone, 2-isopropyl-2,5-dimethyl; Cyclohexanone, 5-methyl-2-(1-methylethylidene)-; Cyclohexene, 1-(1,1-dimethylethoxy)-6-methyl; 1-Oxaspiro[2.5]octan-4-one, 2,2,6-trimethyl-, trans; Cyclohexanone, 2-(1-mercapto-1-methylethyl)-5 -methyl; 2-Oxabicyclo[3.3.0]oct-7-en-3- one, 7-(1-hydroxypentyl)-; 2-Cyclohexen-1-one, 3-methyl-6-(1-methylethyliden e)-; 2-Propenoic acid, 3-phenyl-, methyl ester; 1H-Indene, 1-ethylideneoctahydro-7a-meth yl-, (1E,3aà,7aá)-; 1,2-Cyclohexanediol, 1-methyl-4-(1-methylethenyl)-; (3E,10Z)-Oxacyclotrideca-3,1 0-diene-2,7-dione; Hexadecanoic acid, methyl ester; and Methyl stearate. Furthermore, the data exhibited that the *S. officinalis*

extract (Coded as M5) has the existence of numerous active compositions, by totally 20 major chemical profiles, mainly as: 2-(2-Aminoethyl)pyridine; (+)-2-Bornanone; α -Terpineol; Bicyclo[3.1.1]hept-2-ene-2-methanol, 6,6-dimethyl; 2-Oxabicyclo[2.2.2]octan-6-ol, 1,3,3-trimethyl; exo-2-Hydroxycineole; Cyclohexanone, 5-methyl-2-(1-methylethylidene)-; Cyclohexene, 5-methyl-3-(1-methylethenyl)-, trans(-)-; 2-Propenoic acid, 3-phenyl-, methyl ester, (E)-; Aromadendrene; Caryophyllene oxide; Tricyclo[5.2.2.0(1,6)]undecan-3-ol; Hexadecanoic acid, methyl ester; Alloaromadendrene oxide-(1); Methyl stearate; 12-O-Methylcarnosol; Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl]; 6-Hydroxy-3'-methoxyflavone, trimethylsilyl ether; 1,25-Dihydroxyvitamin D3, TMS derivative; Octadecanoic acid, 2-hydroxy-1,3-propanediyl di-ester.

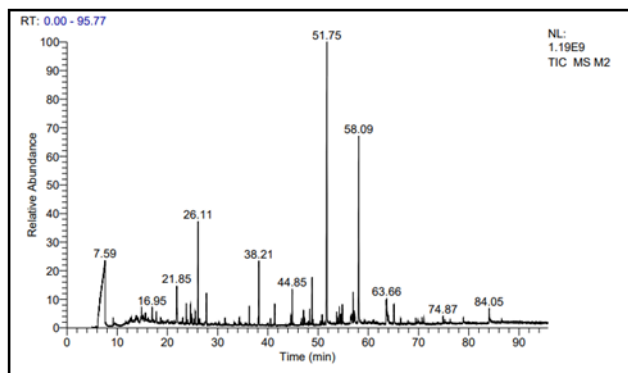
The current examination reveals that various crude extracts contain a significant number of major compounds that exhibit biological activities, particularly those with antioxidant and antibacterial properties. Nevertheless, these findings were verified by means of the GC-MS analysis of the extracts. Focus mainly on; fatty acids, derivatives of fatty acids, terpenoids, steroids, flavonoids, carotenoids, phenolic compounds, sugars, and other compounds such as; quinone's, alkanes, cholesterol, and alcohols. **Mohy El-Din and El-Ahwany (2016)** characterized the chemical constituents of seaweeds (*J. rubens*, *C. mediterranea*, and *P. capillacea*) using GC-MS data, which displayed that they comprise organic compounds like 1,2-benzenedicarboxylic acid. Ismail et al. (2020) also used GC-MS to analyze algal extracts (*U. fasciata*, *C. officinalis*, and *Sargassum linifolium*), and found the presence of different bioactive compounds in the tested seaweeds, mostly cyclotrisiloxane and hexamethyl, that could be accountable for their antidiabetic and antioxidant activities. **Elansary et al. (2020)** investigated the polyphenol profiles and biological activity of methanolic extracts derived from the leaves of various populations of *M. longifolia*. Polyphenols were detected in *M. longifolia* through chromatographic analyses. These polyphenols include rosmarinic acid (781.6 mg/100 g DW), cryptochlorogenic acid (191.1 mg/100 g DW), p-coumaric acid (113.0 mg/100 g DW), chlorogenic acid (63.8 mg/100 g DW), and m-coumaric acid (112.2 mg/100 g DW). Many of these structures have been reviewed as bioactive agents with various biological potencies, including antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory, and anticancer activities.

The structures of the most usual compounds are shown in Fig.2. Among the prevailing compounds are fatty acids (such as hexadecanoic acid, tetradecanoic acid, oleic acid, pentadecanoic acid, eicosanoic acid, cyclopropaneoctanoic acid, cis-5,8,11,14,17-eicosapentaenoic acid, etc.), derivatives of fatty acids (such as hexadecanoic acid ethyl ester, hexadecanoic acid methyl ester, docosanoic acid methyl ester, dodecanoic acid methyl ester, 2-propenoic acid, 3-phenyl-, methyl ester, octadecanoic acid, 2-hydroxy-1,3-propanediyl di-ester), terpenoids (such as monoterpenoids like α -terpineol, diterpenes

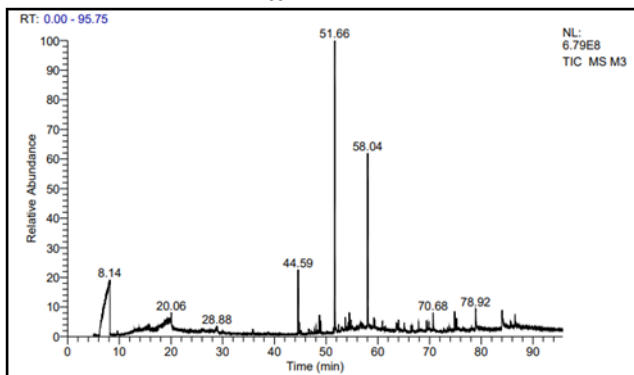
like 12-O-methylcarnosol, terpene alcohols like linalool, (+)-2-bornanone, etc.), steroids (such as sesquiterpenoids like aromandendrene, ethyl iso-allocholate), flavonoids, carotenoids, phenolic compounds, sugars like L-sorbose, and other compounds such as quinones, alkanes, cholesterol, alcohols (such as 4-bromophenethyl alcohol).



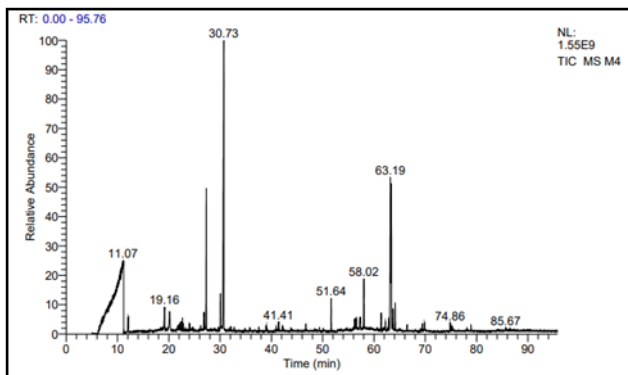
C. officinalis (M1)



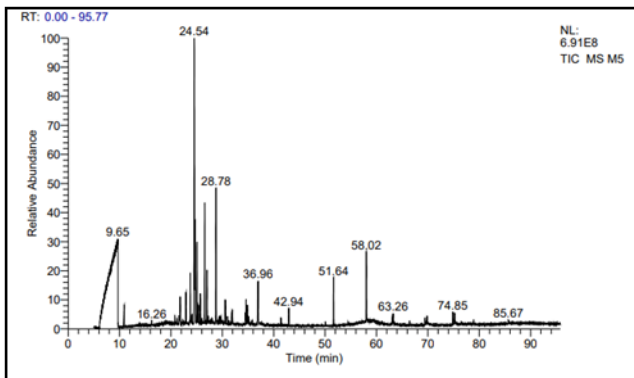
U. lactuca (M2)



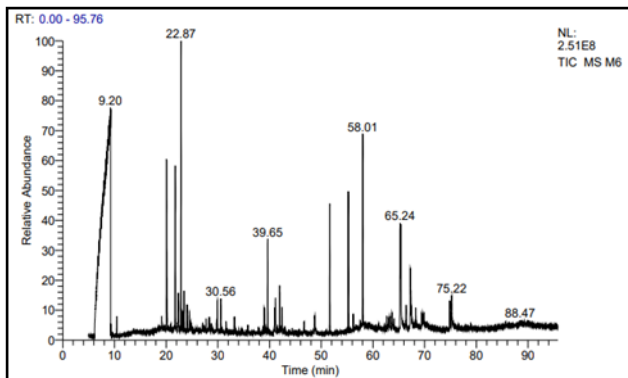
P. capallicia (M3)



O. basilicum (M4)



M. pulegium (M5)



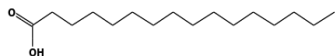
S. officinalis (M6)

Fig.1: Structures of the most common compounds in the methanol crude extracts from selected marine algae and aromatic herbs.

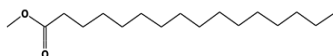
Table 6: Only major bioactive constituents detected in different methanol-crude extracts by GC-MS chromatographic tool.

Rt (Min)	Compound Name	Area %	Molecular Formula	Molecular Weight (M/Z)
<i>C. officinalis</i> extract (Coded as M1)				
17.67	Dodecanoic Acid, Methyl Ester	0.70	C ₁₃ H ₂₆ O ₂	214
20.97	8-Heptadecene	1.75	C ₁₇ H ₃₄	238
22.10	Tetradecanoic Acid, Methyl Ester	1.61	C ₁₅ H ₃₀ O ₂	242
23.74	Tetradecanoic Acid	0.82	C ₁₄ H ₂₈ O ₂	228
24.35	2,6,10-Trimethyl,14-Ethyle Ne-14-Pentadecne	3.47	C ₂₀ H ₃₈	278
24.55	2-Pentadecanone, 6,10,14-Trimethyl-	2.72	C ₁₈ H ₃₆ O	268
24.83	3,7,11,15-Tetramethyl-2-Hexadecen -1-Ol	1.16	C ₂₀ H ₄₀ O	296
25.71	9-Hexadecenoic Acid, Methyl Ester, (Z)-	1.72	C ₁₇ H ₃₂ O ₂	268
26.31	Hexadecanoic Acid, Methyl Ester	21.75	C ₁₇ H ₃₄ O ₂	270
27.44	Hexadecanoic Acid, Ethyl Ester	0.71	C ₁₈ H ₃₆ O ₂	284
28.04	Hexadecanoic Acid	0.52	C ₁₆ H ₃₂ O ₂	256
29.28	9,12-Octadecadienoic Acid, Methyl Ester, (E,E)-	0.53	C ₁₉ H ₃₄ O ₂	294
29.50	9-Octadecenoic Acid (Z)-, Methyl Ester	12.75	C ₁₉ H ₃₆ O ₂	296
29.96	Octadecanoic Acid, Methyl Ester	7.66	C ₁₉ H ₃₈ O ₂	298
30.25	Cis-5,8,11,14,17-Eicosapentaenoic Acid	0.98	C ₂₀ H ₃₀ O ₂	302
30.84	Oleic Acid	3.80	C ₁₈ H ₃₄ O ₂	282
32.10	Hexadecanoic Acid, 1-(Hydroxymethyl)-1,2-Ethanediyl Ester	0.60	C ₃₅ H ₆₈ O ₅	568
32.37	Tributyl Acetyl citrate	0.45	C ₂₀ H ₃₄ O ₈	402
32.75	Cis-13-Eicosenoic Acid	0.68	C ₂₀ H ₃₈ O ₂	310
33.25	Eicosanoic Acid, Methyl Ester	0.96	C ₂₁ H ₄₂ O ₂	326
36.37	Docosanoic Acid, Methyl Ester	1.39	C ₂₃ H ₄₆ O ₂	354
36.68	Diisooctyl Phthalate	4.53	C ₂₄ H ₃₈ O ₄	390
40.92	Cholesta-4,6-Dien-3-Ol, (3á)-	1.10	C ₂₇ H ₄₄ O	384
43.75	Ethyl Iso-Allocholate	0.57	C ₂₆ H ₄₄ O ₅	436
44.19	Cholest-5-En-3-Ol (3á)-	2.01	C ₂₇ H ₄₆ O	386
<i>U. lactuca</i> extract (Coded as M2)				
7.57	à-Tocopherol nicotinate	8.57	C ₃₅ H ₅₃ NO ₃	535
21.85	1-Butoxy-1-isobutoxy-butane	4.36	C ₁₂ H ₂₆ O ₂	202
26.10	n-Butyric acid 2-ethylhexyl ester	8.96	C ₁₂ H ₂₄ O ₂	200
36.28	Sulfurous acid, dodecyl	1.35	C ₁₅ H ₃₂ O ₃ S	292
41.36	6-Tridecanol, 3,9-diethyl-	1.69	C ₁₇ H ₃₆ O	256
44.85	Methyl tetradecanoate	2.41	C ₁₅ H ₃₀ O ₂	242
48.33	Pentadecanoic acid, methyl ester	1.12	C ₁₆ H ₃₂ O ₂	256
48.81	L-Sorbose	3.70	C ₆ H ₁₂ O ₆	180
54.24	Cyclopropaneoctanoic acid,	1.18	C ₁₁ H ₂₀ O ₂	184
54.82	Heptadecanoic acid, methyl ester	1.35	C ₁₇ H ₃₄ O ₂	270
56.97	9-Octadecenoic acid (Z)-, methyl ester	2.39	C ₁₉ H ₃₆ O ₂	296
58.09	Methyl stearate	16.39	C ₁₉ H ₃₈ O ₂	298
63.58	8,11-Octadecadienoic acid, methyl ester	1.61	C ₁₉ H ₃₄ O ₂	294
65.09	Cyclopropaneoctanoic acid, 2-octyl-, methyl ester	1.90	C ₂₀ H ₃₈ O ₂	310
<i>P. capallicia</i> extract (Coded as M3)				
8.16	à-Tocopherol nicotinate	11.03	C ₃₅ H ₅₃ NO ₃	535
44.59	Heptadecane	8.12	C ₁₇ H ₃₆	240
48.71	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	2.31	C ₁₆ H ₂₂ O	278
51.66	Hexadecanoic acid, methyl ester	38.59	C ₁₇ H ₃₄ O ₂	270
54.50	Octadecanal	2.33	C ₁₈ H ₃₆ O	268
58.04	Methyl stearate	25.46	C ₁₉ H ₃₈ O ₂	298

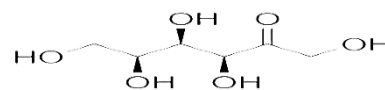
70. 68	Bis(2-ethylhexyl) phthalate	2.82	C ₂₄ H ₃₈ O ₄	390
74. 86	3-Hydroxypropyl palmitate, TMS derivative	2.61	C ₁₉ H ₃₈ O ₃	314
78. 92	Cholesta-4,6-dien-3-ol, (3â)-	2.81	C ₂₇ H ₄₄ O	384
84. 03	Cholesterol	2.92	C ₂₇ H ₄₆ O	386
<i>O. basilicum</i> extract (Coded as M4)				
11. 15	4-Bromophenethyl alcohol	15.05	C ₈ H ₉ BrO	201
19. 16	Linalool	2.40	C ₁₀ H ₁₈ O	154
26. 87	2-Propenoic acid, 3-phenyl-, methyl ester	1.21	C ₁₀ H ₁₀ O ₂	162
27.32	2-Propenoic acid, 3-phenyl-, methyl ester, (E)-	34.48	C ₁₀ H ₁₀ O ₂	162
51. 64	Hexadecanoic acid, methyl ester	2.32	C ₁₇ H ₃₄ O ₂	270
58. 02	Methyl stearate	4.45	C ₁₉ H ₃₈ O ₂	298
63. 38	Dimethyl *u-truxinate	11.02	C ₆ H ₁₀ O ₄	146
64. 13	2-Propenoic acid, 3-phenyl-, methyl ester	2.41	C ₁₀ H ₁₀ O ₂	162
<i>M. pulegium</i> extract (Coded as M5)				
9.68	2-(2-Aminoethyl)pyridine	10.04	C ₇ H ₁₀ N ₂	122
10. 91	Dimethyl sulfone	2.00	C ₂ H ₆ O ₂ S	94
21. 79	à-Terpineol	1.53	C ₁₀ H ₁₈ O	154
23. 77	Cyclohexanone, 2-isopropyl-2,5-dimethyl	3.30	C ₁₁ H ₂₀ O	168
24. 54	Cyclohexanone, 5-methyl-2-(1-methylethylidene)-	27.71	C ₁₀ H ₁₆ O	152
25. 06	Cyclohexene, 1-(1,1-dimethylethoxy)-6-methyl	5.40	C ₁₀ H ₁₈	138
25. 34	1-Oxaspiro[2.5]octan-4-one, 2,2,6-trimethyl-, trans	1.17	C ₁₀ H ₁₆ O ₂	168
25. 71	Cyclohexanone, 2-(1-mercapto-1-methylethyl)-5-methyl	2.24	C ₁₀ H ₁₈ OS	186
26. 57	2-Oxabicyclo[3.3.0]oct-7-en-3-one, 7-(1-hydroxypentyl)-	8.06	C ₅ H ₆ O ₂	210
28. 77	2-Cyclohexen-1-one, 3-methyl-6-(1-methylethylidene)-	9.57	C ₁₀ H ₁₄ O	150
30. 58	2-Propenoic acid, 3-phenyl-, methyl ester	1.90	C ₁₀ H ₁₀ O ₂	162
34. 61	1H-Indene, 1-ethylideneoctahydro-7a-methyl-, (1E,3aâ,7aâ)-	1.71	C ₁₂ H ₂₀	164
34. 87	1,2-Cyclohexanediol, 1-methyl-4-(1-methylethenyl)-	1.53	C ₁₀ H ₁₈ O ₂	170
42. 94	(3E,10Z)-Oxacyclotrideca-3,10-diene-2,7-dione	1.26	C ₁₂ H ₁₆ O ₃	208
51. 64	Hexadecanoic acid, methyl ester	3.23	C ₁₇ H ₃₄ O ₂	270
58. 02	Methyl stearate	5.56	C ₁₉ H ₃₈ O ₂	298
<i>S. officinalis</i> extract (Coded as M6)				
9.24	2-(2-Aminoethyl)pyridine	14.57	C ₇ H ₁₀ N ₂	122
20. 07	(+)-2-Bornanone	7.94	C ₁₃ H ₂₂ N ₂ O ₂	238
22. 87	à-Terpineol	10.67	C ₁₀ H ₁₈ O	154
23. 14	Bicyclo[3.1.1]hept-2-ene-2-methanol, 6,6-dimethyl	0.78	C ₁₀ H ₁₆ O	152
23. 46	2-Oxabicyclo[2.2.2]octan-6-ol, 1,3,3-trimethyl	1.54	C ₁₂ H ₂₀ O ₃	212
24. 04	exo-2-Hydroxycineole	0.98	C ₁₀ H ₁₈ O ₂	170
24. 52	Cyclohexanone, 5-methyl-2-(1-methylethylidene)-	1.07	C ₁₀ H ₁₆ O	152
29. 91	Cyclohexene, 5-methyl-3-(1-methylethenyl)-, trans(-)-	1.37	C ₁₀ H ₁₆	136
30. 56	2-Propenoic acid, 3-phenyl-, methyl ester, (E)-	1.29	C ₁₀ H ₁₀ O ₂	162
39. 64	Aromandendrene.	3.83	C ₁₅ H ₂₄	204
42. 42	Caryophyllene oxide	1.11	C ₁₅ H ₂₄ O	220
48. 72	Tricyclo[5.2.2.0(1,6)]undecan-3-ol,	0.74	C ₁₁ H ₁₈	352
51. 64	Hexadecanoic acid, methyl ester	4.92	C ₁₇ H ₃₄ O ₂	270
55. 24	Alloaromadendrene oxide-(1)	5.83	C ₁₅ H ₂₄ O	220
58. 02	Methyl stearate	8.63	C ₁₉ H ₃₈ O ₂	298
65. 25	12-O-Methylcarnosol	5.22	C ₂₁ H ₃₀ O ₄	344
66. 43	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl	1.09	C ₂₃ H ₃₂ O ₂	340
67. 24	6-Hydroxy-3'-methoxyflavone, trimethylsilyl ether	3.05	C ₂₁ H ₂₂ O ₉	418
74. 85	1,25-Dihydroxyvitamin D3, TMS derivative	1.23	C ₃₀ H ₅₂ O ₃ Si	488
75. 21	Octadecanoic acid, 2-hydroxy-1,3-propanediyl di-ester	1.50	C ₃₉ H ₇₆ O ₅	625



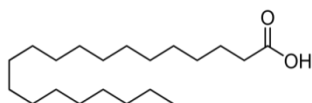
n-Hexadecanoic acid (Palmitic acid)



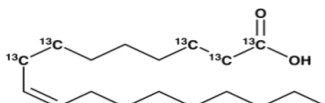
Hexadecanoic acid, methyl ester



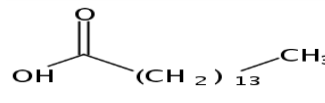
L-Sorbose



Eicosanoic acid



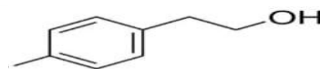
Oleic acid



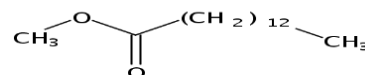
Pentadecanoic acid



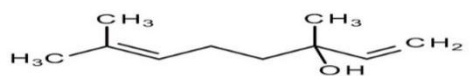
Tetradecanoic acid



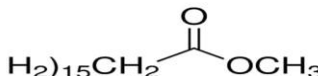
4-Bromophenethyl alcohol



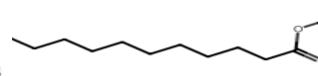
Methyl tetradecanoate



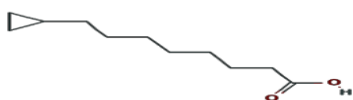
Linalool



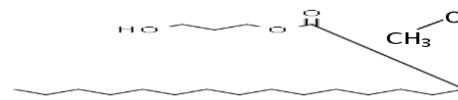
Methyl stearate



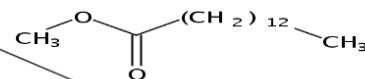
Dodecanoic acid methyl ester



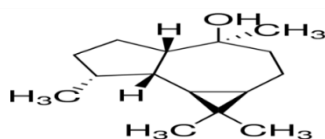
Cyclopropaneoctanoic acid



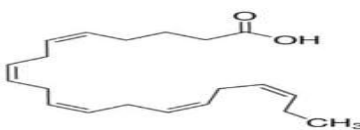
3-Hydroxypropyl palmitate



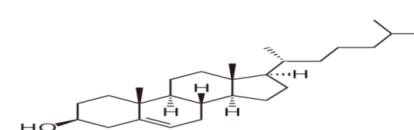
Methyl tetradecanoate



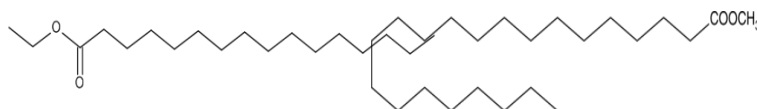
Aromandendrene



Cis-5,8,11,14,17-Eicosapentaenoic acid



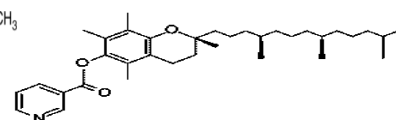
Cholesterol



Hexadecanoic acid ethyl ester



Docosanoic acid methyl ester



α-Tocopherol nicotinate

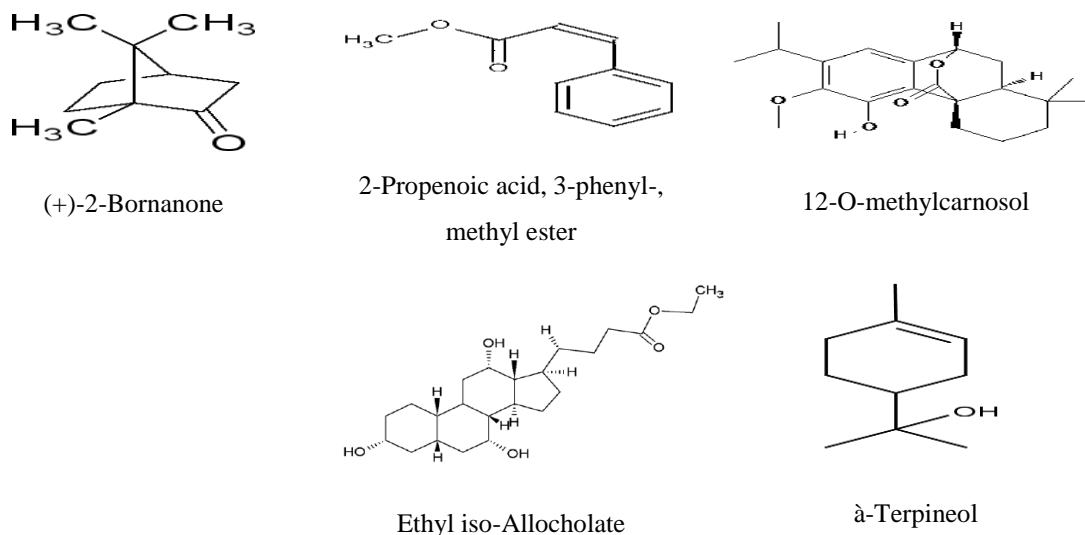


Fig. 2: structures of the most common compounds in the potent crude extracts of marine algae and aromatic plants.

CONCLUSION

The coasts of the Mediterranean harbor a vast array of biological diversity that remains largely unexplored. This untapped potential offers significant opportunities for the development of therapeutic and nutritional interventions that can benefit human populations. Marine algae have demonstrated considerable efficacy in this regard, holding promising implications for the health and wellbeing of current and future generations worldwide. Further, public aromatic plants available in the Egyptian market have been recognized as a treasured source of diverse bio-active compounds, providing an important avenue for their commercial and medicinal utilization. The present study's data, which investigated the bioactive substances in marine algae (*C. officillica*, *U. lactuca*, and *P. capallicia*) and Egyptian aromatic plants (*O. basilicum*, *M. pulegium*, and *S. officinalis*), revealed their richness in such substances, rendering them useful in the production of certain pharmaceuticals. The methanolic crude extracts of these plants exhibited potent bioactivities, including a good spectrum of antioxidant activity and antimicrobial activity against the examined microbes. Their efficacy against human bacterial pathogens specifically supports their potential applications that show promise in the clinical domain. Most of the chemical constituents in the crude extracts were identified as fatty acids and their corresponding esters, along with terpenoids, phenolic compounds, carotenoids, flavonoids, and other compounds as quinones, alkanes, cholesterol, and alcohols. The species investigated in this study have been found to be plentiful in carotenoids, phenolic compounds, antibacterial, and DPPH free radicals' agents. Collectively, these findings emphasize the potential of these species for medicinal applications, particularly in terms of their antioxidant and antimicrobial activities. The

results suggest that the marine algae species *C. officillica* and the aromatic plant species *S. officinalis* may be particularly promising candidates for the development of new therapeutic interventions.

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