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Antibacterial Activity of Corallina officinalis Seaweed Extracts Against Some Pathogenic Bacteria

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Abstract: The adverse effects of antibiotics and the evolving resistance strategies of bacteria have made their use extremely challenging in recent times. Therefore, ongoing research is required to find novel antimicrobial chemicals so that safer and more effective medications can be created. This study evaluates the antibacterial activities of crude extracts from *Corallina officinalis* as alternative antibiotics. *Corallina officinalis* seaweed was collected from Alexandria, Egypt (31◦21′46″N, 29◦88′49.4″E) during March 2022. Different solvents (ethanol, methanol and acetone) were used to extract the antibacterial substances from this seaweed and were examined against three pathogenic bacteria, *Staphylococcus aureus*, *Citrobacter* sp. and *Klebsiella pneumoniae* by agar well diffusion method. The maximum activity was associated with the appearance of a clear zone (30.2 ±0.08mm) which was recorded when using 200mg/ml of acetone-extract against *Klebsiella pneumonia* and minimum activity (15.9 ±0.08mm) was recorded when using 200mg/ml of ethanol-extract against *Citrobacter* sp. Scanning electron microscope showed the cells were damaged when treated with seaweed extracts. The GC-MS chromatographic analysis of the *Corallina officinalis* revealed the presence of various bioactive compounds such as 1-iodotridecane (5.142%), cholesterol (19.365%), nonadecane (4.806%) %), pentadecane (6.237%), docosane (1.639%), tetramethyl-5'-thymidylic acid (7.042%). The results of FTIR analysis confirmed the presence of phenol, alcohols, alkanes, carboxylic group, ketone group, aromatics and aliphatic amines. Our results suggest the potential of *Corallina officinalis* extracts as natural and effective promissing source of new antibiotics.

Keywords: Antimicrobial activity, Seaweeds, Pathogenic bacteria, Scanning Electron Microscope, Gas Chromatography–Mass Spectrometry, FTIR analysis.

1. Introduction

Pathogenic bacteria are among the most annoying organisms that have the ability to cause numerous deadly diseases in both humans and animals, for example, *Klebsiella pneumonia*, *Staphylococcus aureus*, and *Escherichia coli* can cause illnesses such as upper respiratory problems, mastitis, and abortions [1]. According to (Kandasamy and Arunachalam) [2], *K. pneumonia* is a common bacterium that can cause life-threatening disease in burn victims. Antimicrobial medicine use is currently fraught with difficulty because of adverse effects and evolving disease resistance strategies. For this reason, it's imperative to keep looking for novel antibacterial agents in order to create medications that are both safer and more effective. As a result, the pharmaceutical industry is placing more value on substances that come from natural sources [3]. The majority of the resources found in terrestrial plants have been mostly depleted by both traditional and contemporary medicine. These days, it's thought that the marine environment is a great place to find novel medical drugs [4-6]. It has been shown that several of the new chemicals that have recently been identified from marine species exhibit intriguing biological activity [7-10]. According to (Dawczynski et al) [11], seaweeds are actually the most fascinating aquatic organisms that have historically been used as food for humans and animals. Furthermore, they are recognized for generating a variety of structurally varied and physiologically active secondary metabolites that serve as a defensive mechanism against herbivores and fouling organisms [12]. According to (Shanab, Kolanjinathan et al., and Lavanya et al) [13-15], the majority of bioactive chemicals derived from seaweeds are utilized in the pharmaceutical industry for their antibacterial, antiviral, antifungal, anticancer, and antioxidant properties.

Our study was undertaken to examine the antibacterial effect of crude extracts of *Corallina officinalis* seaweed, against pathogenic bacteria such as *Klebsiella pneumonia*, *Citrobacter* sp. *Staphylococcus aureus*. Also, the efficacy of three different solvents (acetone, methanol and ethanol) to obtain the most effective crude extract from seaweed was evaluated.

2. Materials and method

2.1 Sample Collection and Preparation

The red seaweed *Corallina officinalis* sample morphological shape as shown in (Fig1) was collected from Alexandria, Egypt (31◦21′46″N, 29◦88′49.4″E) during March 2022. The algal biomass was hand picked collected and washed several times with seawater to remove epiphytes, debris, and sand particles followed by using distilled water. After that, it was allowed to be air dried before being ground into powdered form using an electrical blender. The seaweed was identified in accordance with Guiry & Guiry, Kanaan & Belous and Aleem [16-18].

2.1.2 Seaweed extracts preparation:

The following modified versions of previously published

methods [19-20] used for preparing three different extracts of *Corallina officinalis* seaweed, 10g of seaweed powder was extracted with 150 ml of the solvent (methanol, ethanol or acetone), using a Soxhlet extraction apparatus at 50ºC for 24h. The extracted components were further heated to 40ºC in an oven to cause the solvent to evaporate. The leftovers, or crude extract was gathered and kept in sealed vials at -20°C until use.

Fig1: Morphological shape of Corallina officinalis seaweed

2.2. Bacterial Strains

Bacterial strains used for the present study were obtained from microbiology laboratory, Faculty of medicine, Sohag University Hospital. They are *Klebsiella pneumoniae*, *Citrobacter* sp and two isolates of *Staphylococcus aureus* (SA1, SA2). The morphology and biochemical test were caried out continuously to ensure purity [21]

2.3. Screening for antibacterial activity

Using the agar well diffusion method, the antibacterial activity of the red seaweed *Corallina officinalis* extracts was measured [22]. On sterile Petri dishes with 15 ml of nutrient agar media, the bacterial suspension (1 ml) was dispersed. Agar wells were created using a stainless-steel cork borer that had been previously sanitized. 100µl of seaweed extract was added to each well. The plates were incubated at 37°C for a whole day. The evaluated seaweed extract antibacterial activity is indicated by the diameter of the inhibition zones. Gentamicin was used as a positive control (50µg/100µL).

2.4. Scanning electron microscopy

The Electron Microscopy Unit at Assiut University captured this scanning electron microscopy (SEM) image demonstrating the impact of the seaweed *Corallina officinalis* on the bacteria *Klebsiella pneumonia* and *Staphylococcus aureus* (SA1). SEM is a popular microscopy method for characterizing forms and surface morphology in nanoscale materials as well as high-resolution surface imaging. On nutrient agar (NA) (peptone 5 g/L, yeast extract 3 g/L, sodium chloride 3 g/L, agar 15 g/L, final pH 6.5 ± 0.2), bacterial isolates were re-inoculated for two days at 35 ± 2 °C. The sterile cork bore was used to create 0.3mm diameter holes with 100 µL of extracts. Every plate underwent 37°C incubation. Plates were examined 24 hours later to see if a clear zone had formed. Cuts of small agar were made from the inhibitory zone, and after being fixed for one hour at room temperature in 3% (v/v) glutaraldehyde buffered with 0.1 M sodium phosphate buffer (pH 7.2), the pieces were rinsed four times in the sodium phosphate buffer. After post-fixing the pieces for an hour in 1% (w/v) osmium tetroxide (OsO4), they were rinsed four times in the buffer. They underwent a series of graded alcohol dehydration. Propylene oxide was used for the last stages of dehydration (CH3CH.CH2.O). Following drying and mounting of the specimens on stubs with double-sided carbon tape, a Polaron SC 502 sputter applied a thin layer of gold to the specimens.

2.5. Gas chromatography–mass spectrometry (GC–MS)

The analytical technique known as gas chromatography-mass spectrometry (GC-MS) combines the capabilities of mass spectrometry and gas chromatography to identify various compounds present in a test sample [23]. The following acquisition parameters were used for the chromatographic separation in the Assiut University GC-Ms (7890-5975), column Hp-5ms (30 m × 0.25mm × 0.25 µm). The GC oven temperature was programmed to go from 40C to 280C at a rate of 10ºC/min to 150ºC for six minutes, with helium serving as the carrier gas (1 ml/min). The chromatogram and GC analysis report that are obtained for every sample.

2.6. Fourier transform infrared spectroscopy (FTIR)

FT-IR spectrum of *Corallina officinalis* seaweed was scanned at a resolution of 2 cm^{-1}, in the spectral region of 4000–400 cm^{-1} with a JASCO FT-IR spectrophotometer (FT-IR-6100; JASCO, Tokyo, Japan). The employed technique offers advantages over conventional transmission mode FTIR, including faster sampling without preparation, excellent reproducibility, and ease of use.

3. Results and discussion

3.1. Antibacterial activity by the agar well diffusion method

The antibacterial activity of each extract differs from one another depending on the targeted bacterial strain where each bacterial strain showed different response to each extract and activity strength increased as the concentration of extract increased as the following: in case of *Citrobacter* sp., all the used extracts showed high activity against the bacterial strain, methanolic extract showed the highest activity where inhibion zone reached to 23.1±0.12 mm at the highest concentration we have used in the experiment (200mg/ml), followed by acetone extract (22 mm), where each of the two extracts showed inhibition activity much more than that of the positive control (20.1±0.08 mm) and also ethanolic extract showed inhibition zone reached to 15.9±0.08 mm, it was very close to that of the positive control as shown in (Table1). In case of *Klebsiella pneumonia*, no one of the used concentrations (50 to 200mg/ml) from methanolic and ethanolic extracts showed any inhibition activity against this bacterial strain, while acetone extract showed high activity where inhibition zone reached to 30.2±0.08 mm at 200mg/ml. In case of *Staphylococcus aureus* (SA1), it was affected by two types of extracts; methanolic extract which showed high activity where inhibition zone was 20.4±0.08 mm at 200mg/ml, it was much more than that of the positive control (19.9±0.08 mm) and also acetone extract showed inhibition zone reached to 19.9±0.08 mm, while all concentrations (50 to 200mg/ml) from ethanolic extracts did not show any inhibition. *Staphylococcus aureus* (SA2), was affected by two types of extracts, methanolic extract which showed the highest activity where inhibion zone reached to 20.13±0.12 mm at 200mg/ml followed by acetone extract (20 mm), each of the two extracts showed inhibition activity much more than that of the positive

control (12.4±0.08 mm) as shown in (Fig 2) and (Table1). while all of the used concentrations from (50 to 200mg/ml) from ethanolic extracts did not show any inhibition.

A similar observation was recorded by Salem et al. [24], who found that Gram negative bacteria (*E. coli*, *P. aeruginosa*, *Enterococcus feacalis and Salmonella* sp.) were the most resistant bacteria to most tested seaweed extracts while Gram positive bacteria *(S. aureus and Bacillus cereus*) were the most sensitive to all seaweed extracts, also similar observation was recorded by Bhuyar et al**. [**25], who found Compared to gram-positive bacteria, gram-negative bacteria seem to be less vulnerable to antibacterial agents.because of its structural form, negative bacteria and their arrangement **[26]**. This is because outer membrane of the gram-negative bacteria consists of lipopolysaccharide which serves to impede and prevent the entry of agents that are both antibacterial and antimicrobial.

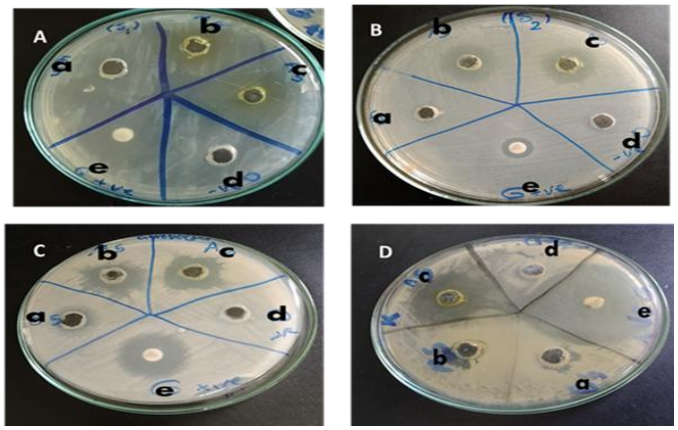

Fig 2. Antibacterial effectiveness of *Corallina officinalis* extracts against (A) *S. aureus* (SA1), (B) *S. aureus* (SA2), (C) *Citrobacter* sp. and (D) *Klebsiella pneumonia* (a = 200 mg/ml ethanolic extract, b = 200 mg/ml methanolic extract, c = 200 mg/ml acetone extract, d = DMSO (negative control) and e = 10µg/ml Gentamicin (positive control).

3.2. Scanning electron microscopy

SEM was used to identify the morphological changes in *Klebsiella pneumonia* and *Staphylococcus aureus* (SA1) when treated with the effective concentration (200mg/ml) of *Corallina officinali*s extracts. The photomicrographs given in (Fig 3 and 4) were representative of the samples observed on the microscopic sample's holder. The rod-shaped morphology of untreated control *Klebsiella pneumonia* and cocci- shape morphology of untreated control *Staphylococcus aureus* (SA1) appears to be normal, intact and with a smooth continuous outer membrane (Fig 3(A) and Fig 4 (A)). After treatment with 200 mg/ml of *Corallina officinalis* extracts, the general structure of the bacterial envelope seems to be damaged (Fig 3(B) and Fig 4(B)). Some *Klebsiella pneumonia* and *Staphylococcus aureus* (SA1) cells were completely deformed (shown by arrow) and parts of bacterial envelope and intracellular content were lost. our results are correlated with the result obtained by jelan et al. [27] who's reported degraded *S. aureus* and *E. coli* cells which appeared shrunk, ragged, wrinkled when treated 100µg/ml of *Corallina officinalis* extracts for 12 h and similar reported by Ilhan et al. [28] when treated *Staphylococcus aureus* and *Pseudomonas aeruginosa* with methanol extracts of *Ocimum basilicum* to observed when the bacterial cells treated with plant extracts (*O. basilicum*), the cells appeared to be shrinking and there was a degradation of the cell walls. It was formerly believed that the mechanisms of action were coagulation, protein binding, cytoplasmic membrane protein damage, cell wall degradation, and leakage of cell contents. of cytoplasm and depletion of the proton motive force [29].

All these findings indicate that *Corallina officinalis* extracts possess antibacterial activity against *Klebsiella pneumonia* and *Staphylococcus aureus* (SA1) and they cause lysis and eradicate bacteria by degrading bacterial cell walls. and action mechanism of its active chemicals will build upon the findings of this study.

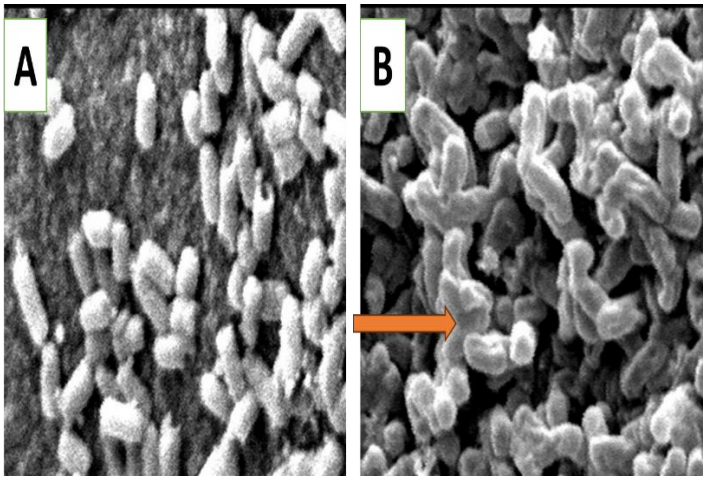

Fig 3. Scanning electron microscope of *Klebsiella pneumonia* where (A): untreated control cells, (B): damaged cells after treatment with acetone extract of *Corallina officinalis*.

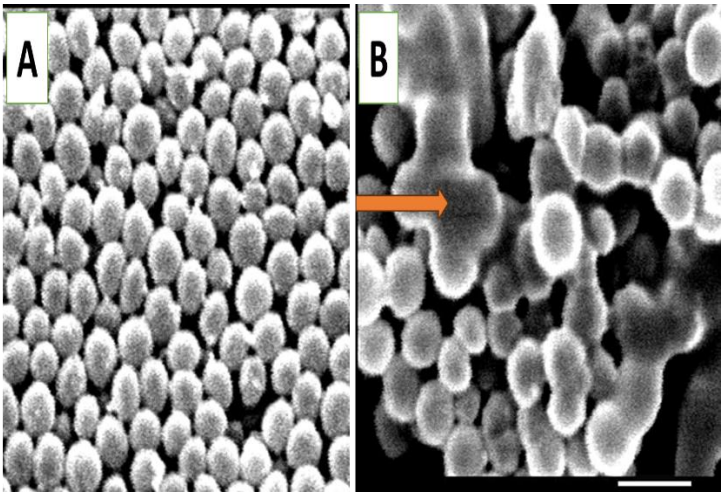

Fig4. Scanning electron microscope of *Staphylococcus aureus* (SA1) where (A): untreated control cells, (B): damaged cells after treatment with acetone extract of *Corallina officinalis*.

3.3 GC- mass analysis of *Corallina officinalis*

Thirty-five compounds were identified *in Corallina officinalis* the identification of the phytochemical compounds was confirmed based on the peak area, retention time and molecular formula (Figure 5 and Table 2). According to (Brown and Wang) [30], the biological activities of some of the identified

compounds are presented in (Table 3). The GC-MS chromatographic analysis of the *Corallina officinalis* revealed the presence of various bioactive compounds such as 1-iodotridecane (5.142%) are known for its antibacterial activity [31], cholesterol (19.365%) is known for its anticancer activity, anticardia activity, anti-inflammatory activity, antimicrobial activity, anti-psychotic activity, antioxidant activity [32], nonadecane (4.806%) %) is known for its antimicrobial and cytotoxic [33-34], eicosane (6.994%) is known for its antitumor activity [35], pentadecane (6.237%) is known for its antibacterial [36], docosane (1.639%) is known for its antibacterial activity enhances host egg parasitization [37-38], tetramethyl-5'-thymidylic Acid (7.042%) is known for its antibacterial, antifungal, insecticidal, herbicidal, antiviral, anti-inflammatory and antitumor [39-45], as shown in the (Table 3) . The majority of the chemical ingredients appear to be biologically active chemicals based on the GC-MS research.

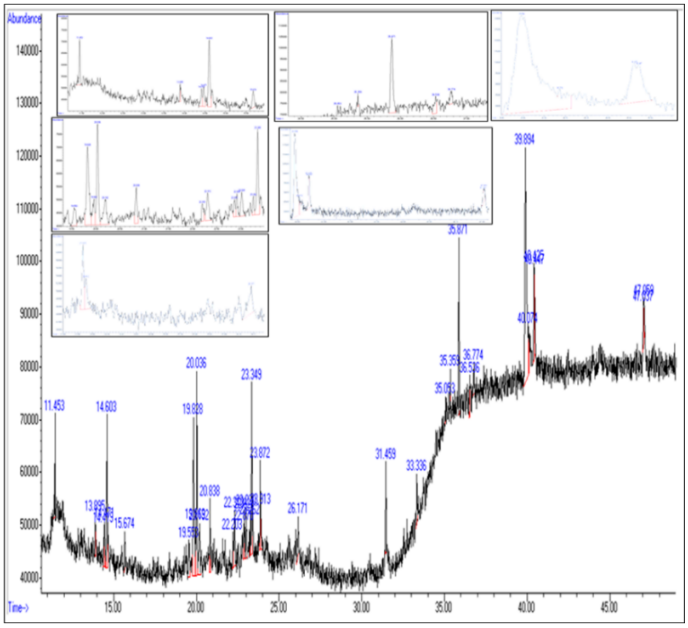

Fig 5: GC MS analysis *Corallina officinalis* extract.

3.4. Fourier Transform-Infrared (FTIR) spectroscopic analysis

FT-IR is a valuable tool for measuring many chemical constituents in plants and seaweeds and it is used to reveal some qualitative aspects regarding the organic compounds **[46]**. The FTIR spectrum of *Corallina officinalis* results appeared strong broad bands at 3370.20 cm^{-1} assigned to N–H and O–H stretching vibrations corresponding to the amino acids and polysaccharides **[47-51]**, the band 2929.30 cm^{-1} can be attributed to the –CH3 and –CH2 stretching aliphatic vibrations of the chlorophyll compounds or C–H stretching symmetric aliphatic vibration was supposedly pointed to the secondary amines **[47-53]**, the band 2524.36 cm^{-1} may be due to C–O stretching band **[54]**, the band 1799.38 cm^{-1} may be due to C≡O Stretching and N≡O asymmetric stretching of esters and pectin complexes**[47, 51,55-59]**, the band 1434.35 cm^{-1} due to C–H stretch vibration of alkanes (methyl) **[52,60]**, the bands 1039.94, 1081.93 and 1153.74 cm^{-1} may be due to O–C–H, C–C–H, C–O–H, bending and rocking vibrations of carbohydrates **[47, 50, 55, 58, 61]**, the bands 873.70 cm^{-1} may be due to C–H bending mode of glucose & galactose **[46-47, 52]**, the band 717.13 cm^{-1} due to N–H vibration of fatty acid **[52]**, the bands 577.06 and 616.89 cm^{-1} may be due to Halogen compound **[62]**. *Corallina officinalis* may include biocompounds, a theory that was further supported by the primary functional groups found by FTIR. By recognizing amide, amino, hydroxyl, and ester groups in the infrared spectrum, it is shown that *Corallina officinalis* contains essential biological components (such as proteins, amino acids, polysaccharides, and lipids) **[63]**.

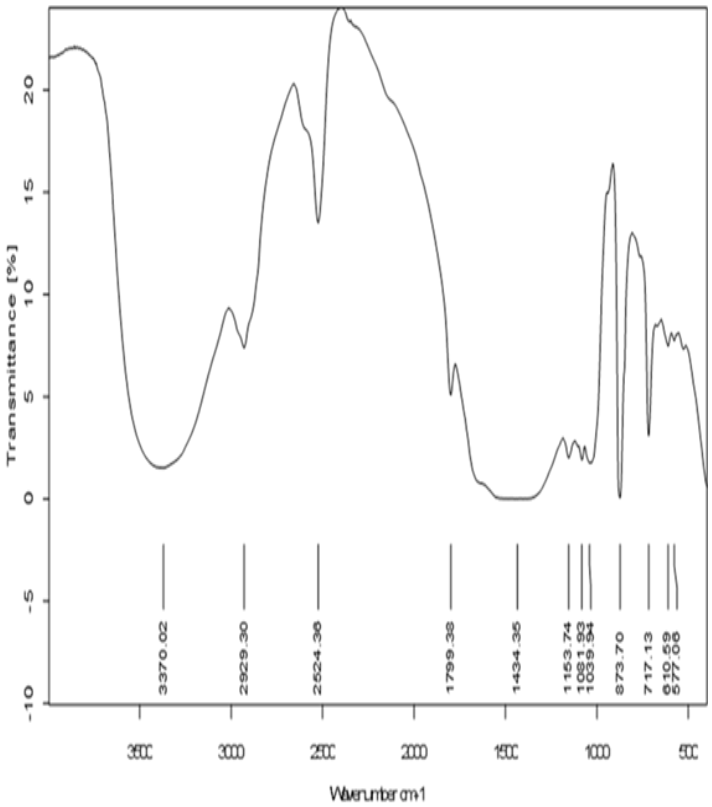

Fig 6. Fourier transform infrared spectrum of *Corallina officinalis.*

4. Conclusion

Conclusively, the current study showed appreciable antibiotic activity by *Corallina officinalis* seaweed against certain human pathogens. The present study revealed that *Corallina officinalis* is a promising alga, as there may be a potential to utilize its extract in food products to act as antimicrobial agent, which could potentially increase the shelf life and safety of a wide range of food products, or in pharmacology as new agent for therapeutic medical and veterinary applications. Therefore, it would be beneficial if it were to be used in the future to cure illnesses in humans or as a novel antibacterial agent to take the place of synthetic antimicrobial agents. By the findings and purification of the active agent that is present in the extract of *Corallina officinalis*, it will be possible to discover new natural drugs serving as chemotherapeutic drugs could be used to treat nosocomial diseases and manage germs that are resistant to antibiotics. Our future research will be on the sophisticated purification.

Table1. Antibacterial activity of *Corallina officinalis* seaweed extracts against pathogenic bacterial isolates. Data represented the average diameter of inhibition zone (mm)± S.D. N/A: No activity.

Bacterial isolates	Mean of diameter of inhibition zone (mm) ± Standard deviation										
	Concentrations of crude extracts mg/ml) with different solvents									Gentamicin(10µg) (positive control)	DMSO (negative control)
	Methanol			Ethanol			Acetone				
	50	100	200	50	100	200	50	100	200		
Citrobacter sp.	5.3± 0.16	14.8± 0.09	23.1± 0.12	N/A	5.36 0.12	15.9± 0.08	4.5± 0.08	13.63± 0.04	22± 0	20.1± 0.08	N/A
Klebsiella Pneumonia	N/A	N/A	N/A	N/A	N/A	N/A	10.73± 0.12	20.06± 0.16	30.2± 0.08	40.3± 0.16	N/A
Staphylococcus aureus (SA1)	3.8± 0.04	11.9± 0.09	20.4± 0.08	N/A	N/A	N/A	N/A	9.9± 0.08	19.9± 0.08	19.9± 0.08	N/A
Staphylococcus aureus (SA2)	4.1± 0.08	10.96± 0.04	20.13± 0.12	N/A	N/A	N/A	2.33± 0.12	12.23± 0.16	20± 0	12.4± 0.08	N/A

Table 2. List of compounds identified at various retention times from *Corallina officinalis* extract by GC MS analysis:

Peak	Name	R. Time	Area%	Molecular formula
1	(1. Alpha.,2. beta.,4a. alpha.,4b. beta.,10.be ta.)- Gibb-3-ene-1,10-dicarboxylic acid, 2,4a,7-trihydroxy-1-methyl-8-methylene-, 1,4a-lactone	35.359	2.501%	C19H22O6
2	(S)-L-Alanine, N-[N-[N-(2-Hydroxy-3-Methyl-1-Oxobutyl)-5-(4-Methoxyphenyl)-L-Norvaly L]-Dl-Alanyl]-,	15.675	1.416%	C22H29N3O6
3	1,2,5-Oxadiazol-3-carboxamide, 4,4'-azobis-, 2,2'-dioxide	22.312	2.345%	C6H4N8O6
4	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	23.916	1.197%	C16H22O4
5	11,20-Di-N-Decyltriacontane	22.868	0.917%	C50H102
6	11-N-BUTYLDOCOSANE	11.451	2.112%	C26H54
7	1-Bromotriacontane	19.556	1.593%	C30H61Br
8	1-Iodotridecane	14.601	5.142%	C13H27I
9	2-((E)[((E)2([(E)(2Hydroxyphenyl) Methylidene] Amino) Propyl) Amino] Methyl) Phenol	40.45	2.312%	C20H16N2O2
10	2-(4'-NITRO-2'-THIENYL) PYRIMIDINE	47.061	1.331%	C8H5N3O2S
11	2,6,10,15-tetramethyl Heptadecane	23.87	1.850%	C21 H44
12	2,6-Dichloro-3-phenyl-pyridine	23.262	0.854%	C11H7Cl2N
13	2-Amino-3-(2-Amino-2-Carboxy-Ethyldisulfanyl)-Propionic Acid	14.420	1.227%	C12H24N4O8S3
14	2-Cyanoacetamide	13.896	2.083%	C3H4N2O
15	2h-Pyrrol-2-One,4-Ethyl-5-[[2-[5-[(3-Ethyl-1,5-Dihydro-4-Methyl-5-Oxo-2h-Pyrrol-2-Ylidene) Methyl]-3,4-Dimethyl-2h-Pyrrol-2-Ylidene]-3,4-Dimethyl-2h-Pyrrol-5-Yl] Methylene]-1,5-Dihydro-3-Methyl-, (E, Z, Z)-	36.536	1.162%	C22H24N2O
16	3-(3-Oxo-3h-Benzo[F]Chromen-2-Yl)-2,4(1h,3h)-Quinolinedione	33.334	1.716%	C14H8O4
17	3,5-Dimethyl-2,6-bis(trimethylsiloxy)pyridine	40.424	2.487%	C13H25NO2Si2
18	3-Chloro-7-D-(2-phenylglycinamido)-3-cephem-4-carboxylic acid	14.472	1.323%	C7H7ClN2O3S
19	4,5. Alpha. -epoxy-3-methoxy-17-methyl-7. alpha. -(4-phenyl-1,3-butadienyl)-6. beta.,7. beta. (oxymethylene)	47.035	0.959%	C26H27NO8
20	5-(2-Oxohexahydro-1h-Thieno[3,4D] Imidazol-4-Yl) Pentanamide	22.92	1.278%	C10H17N3O2S
21	6 Methyl-2 Phenylindole	36.762	1.925%	C15H13N
22	Cholesterol	39.893	19.365%	C27H46O
23	Docosane	23.023	1.639%	C22H46
24	Glycyl-D-asparagine	20.837	2.036%	C6H11N3O4
25	Hexamethylcyclotrisiloxane	31.458	2.631%	C22H42O4
26	Hexanedioic acid, bis(2-ethylhexyl) ester	22.202	1.003%	C14H20ClN3S
27	Hydroxymethapyrilene	19.828	6.994%	C20H42

28	Eicosane	26.173	1.776%	C37H74ClNO
29	N, N-Dioctadecyl Carbamoyl Acetic Acid	20.19	2.976%	C29H60
30	Nonacosane	23.346	4.806%	C19H40
31	Nonadecane	20.035	6.237%	C15H32
32	Pentadecane	40.074	1.624%	C14H9NO
33	Pyrrolo[3,2-A] Dibenzofuran	35.876	7.042%	C10H15N2O8P
34	Tetramethyl-5'-Thymidylic Acid	19.963	2.029%	C(CH2OH)4
35	Trisoctoxy Monopentoxy Pentaerythritol	31.458	2.631%	C22H42O4

Table 3. Biological activities of phytochemical compounds identified in *Corallina officinalis* extract.

S/N	Compound name	Biological activity	Reference
1	Cholesterol	Anticancer activity, anticardia activity, anti-inflammatory activity, antimicrobial activity, anti-psychotic activity, antioxidant activity	Kong et al., 2021 [32]
2	Nonadecane	Antimicrobial and cytotoxic. A cuticular hydrocarbon of insects (chemical communication)	Hsouna et al., 2011; Colazza et al., 2007 [33-34]
3	Pentadecane	Antibacterial	Yogeshwari et al.,2012
4	Eicosane	Antitumor activity	Sivasubramanian and Brindha, 2013 [35]
5	Tetramethyl-5'-Thymidylic Acid	Antibacterial, antifungal, insecticidal, herbicidal, antiviral, anti-inflammatory and	Bhat et al., 2021; Wu et al., 2021; Liu et al., 2016; Yang et al., 2022; Kimura et al., 2006; Kumar et al., 2019; Thirumurugan et al., 2018 [39-45]
6	1-Iodotridecane	Antibacterial and bioactive	Nandhini et al., 2015 [31]
7	Docosane	Antibacterial activity	Gumgumjee and Hajar, 2015; Paul et al., 2002 [37-38]

Table 4. FT-IR absorption frequencies (cm^{-1}), intensity estimation and functional group of seaweed *Corallina officinalis*

.IR frequency (cm^{-1}) [Reference Article]	Bond	Functional groups	*C.officinalis* IRfrequency(cm^{-1})
3500-3200	N–H and O–H stretching	amino acids and polysaccharides	3370.02
3000-2850	Symmeteric stretching of -CH(CH2) vibration	Lipids, protein	2929.30
3500-2400	C–O Stretching	phosphine	2524.36
1800-1600	C≡O and N≡O asymmetric stretching	esters and pectin complexes	1799.38
1430-1350	C-H stretching vibration	Alkane	1434.35
1150-1020	O–C–H, C–C–H, C–O–H,	carbohydrates	1153.74- 1081.93- 1039.94
950-780	C–H bending	Glucose, galactose	873.70
720-715	N–H vibration	Secondary amine	717.13
690-550	Halogen compounds (bromo compound)	Aliphatic bromo compounds	610.59

CRediT authorship contribution statement

Conceptualization, A.E, M.A and H.A. designed the study; D.H. performed the experiments and H.A and A.E contributed to the writing. All authors have read and agreed to the published version of the manuscript."

Data availability statement

The data used to support the findings of this study are available from the corresponding author upon request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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