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Original Article

Spectral analysis and Antibacterial activity of the bioactive principles of *Sargassum tenerrimum J. Agardh* collected from the Red sea, Jazan, Kingdom of Saudi Arabia

Análise espectral e atividade antibacteriana dos princípios bioativos de *Sargassum tenerrimum J. Agardh* coletados no mar Vermelho, Jazan, Reino da Arábia Saudita

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Abstract

Seaweeds are a major marine resource that can be explored to develop novel pharmaceutical molecules. The present study showed the presence of unique bioactive components in the petroleum ether extract (PEE) and methanolic extract (ME) of *Sargassum tenerrimum*. The gas chromatography-mass spectrometry analysis suggested that the PEE of *S. tenerrimum* contained antibacterial biomolecules: hexadecanoic acid, methyl ester, 17-pentatriacontene, dasycarpidan-1-methanol, and acetate (ester). However, the ME of *S. tenerrimum* exhibited better antibacterial effect than the PEE due to the presence of the bioactive compounds 1,2-benzenedicarboxylic acid, diisooctyl ester, tetratetracontane, 1-docosene, 1,2-benzenediol, and benzoic acid. Thus, promising antibacterial molecules can be isolated from *S. tenerrimum* for better therapeutic use.

Keywords: brown alga, *Sargassum tenerrimum*, bioactive constituents, antibacterial effect.

Resumo

As algas marinhas são um importante recurso marinho que pode ser explorado para desenvolver novas moléculas farmacêuticas. O presente estudo mostrou a presença de componentes bioativos únicos no extrato etéreo de petróleo (PEE) e no extrato metanólico (ME) de *Sargassum tenerrimum*. A análise por cromatografia gasosa-espectrometria de massa sugeriu que o PEE de *S. tenerrimum* continha biomoléculas antibacterianas: ácido hexadecanoico, éster metílico, 17-pentatriaconteno, dasycarpidan-1-metanol e acetato (éster). Entretanto, o ME de *S. tenerrimum* exibiu melhor efeito antibacteriano do que o PEE devido à presença dos compostos bioativos ácido 1,2-benzenodicarboxílico, éster diisooctil, tetratetracontano, 1-docosene, 1,2-benzoenodiol e ácido benzoico. Assim, moléculas antibacterianas promissoras podem ser isoladas de *S. tenerrimum* para melhor uso terapêutico.

Palavras-chave: alga marrom, *Sargassum tenerrimum*, constituintes bioativos, efeito antibacteriano.

1. Introduction

The pharmaceutical importance of seaweeds has been gaining attention in recent years among various researchers globally (Salehi et al., 2019; Maria et al., 2016). Seaweeds constitute a significant resource from which secondary metabolites can be isolated; those that have specific pharmaceutical properties (Namvar et al., 2014; Bhutiya et al., 2018). *Sargassum tenerrimum* J. Agardh belonging to the Sargassaceae family, commonly found in the Red Sea, Jazan province, Jazan, Kingdom of Saudi Arabia (KSA), can be studied to develop novel pharmaceutical molecules. To the best of our knowledge, there are very few scientific publications on the pharmaceutical value of *S. tenerrimum*. Previous studies show that resistance to antibacterial agents is mainly exhibited by *Staphylococcus aureus*, *Streptococcus pyogenes*, *Mycobacterium tuberculosis*, *Escherichia coli*, and *Pseudomonas aeruginosa* (Pelgrift and Friedman, 2013; Riley et al., 2012). Recently, a study reported that the methanolic extract of *S. tenerrimum* contains steroids, saponins, alkaloids, tannins, flavonoids, terpenoids proteins, and phenolic compounds (Kumar et al., 2012). In continuation of our earlier works (Moni et al., 2019; Moni et al., 2018), the present study was designed to characterize the bioactive principles found in *S. tenerrimum* using gas chromatography-mass spectrometry (GC-MS)

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and Fourier-transform infrared (FT-IR) techniques. We also evaluated the antibacterial effect of the extracts *in vitro* against both gram-positive and gram-negative bacteria.

2. Material and Methods

2.1. Chemicals and reagents

All chemicals and solvents used in this study were purchased from Sigma Aldrich (USA), and supplied by Bayouni Trading Co (Jeddah, KSA). Bacteriological media were products of Scharlau supplied by Somatco (Jeddah, KSA).

2.2. Study area, collection, and identification

Jazan, which is a coastal region along the Red Sea, is located in the southwest part of Saudi Arabia and is rich in varieties of seaweeds, especially brown algae. *S. tenerrimum* is abundantly available in the Red Sea. The seaweed was collected from Al Murjan beach in Jazan, Saudi Arabia, in November 2019. The Al Murjan beach is located 10 kilometers from the city, and the seaweed was harvested by going 10 to 20 meters within the sea from the beach's coastline. Figure 1 representing the study area of Al Murjan beach, Jazan. The collected alga was thoroughly washed twice in seawater at the seashore and exposed to open air to drain the water. The seaweeds were packed in polythene biohazard yellow bags, tied and then transported to the laboratory. The seaweeds were again thoroughly washed under normal tap water to remove adhered impurities. Thereafter, the washed seaweed was air-dried in a well-ventilated room for 15 days. The seaweed was authenticated by Dr. Remesh Moochikkal, herbarium curator, Jazan University herbarium (JAZUH), on depositing a voucher specimen; the identification reference number was JAZUH 1631. The air-dried *S. tenerrimum* was cut into small pieces and finely powdered using a grinder to obtain a coarse powder. The powdered samples were pooled and packed in an airtight container for further experimental use.

2.3. Organic solvent extraction

Hot continuous percolation by Soxhlet apparatus was used to extract active principles from *S. tenerrimum* using petroleum ether and methanol, separately, at 60 °C for 4 h. After cooling, the extracts were transferred to glass beakers and air-dried. The dried extracts were subjected to GC-MS and FT-IR spectral analysis to determine the various bioactive compounds present.

2.4. GC-MS analysis

GC-MS analysis of the organic solvent extracts of *S. tenerrimum* was performed using a Thermo Scientific GC-MS with the AS 3000 autosampler and ISQ detector. Capillary TR-5MS column (30 m × 0.25 mm ID × 0.25 µm) and helium carrier gas at a flow rate of 1.2 mL/min was used to achieve the separation of components. Petroleum ether and methanolic extracts were diluted ten times with

Figure 1. The map of study area of Al Murjan beach, Jazan.

their respective extraction solvents, soicated, and filtered through 0.45 µm filter and injected using splitless mode (2µL). The oven temperature was set initially at 70°C and hold for 5 min, subsequently ramped to 205°C, 280°C, 290°C and 300°C at a rate of 5°C/min with a holding time of 5 min at each ramp. The injection port, ion source and MS line transfer temperatures were set at 290°C, 320°C and 340°C respectively with helium gas flow rate of 1.2 mL/min and spectra were recorded using positive ion mode within the mass range of 60-900 amu with delay time of 5 minutes. Structural interpretation of the mass spectrum of the bioactive compounds was carried out using the built-in NIST, Mainlib, and Replib software libraries. The percentage areas of each peak were obtained using Xcalibur software and are uncorrected. The compounds were identified by using match and reverse match factor thresholds of nine hundred and above between measured and standard library spectrum. The identified compounds were further confirmed by their retention indices data obtained by running the mixture of n-alkanes (C_6-C_{40}) and comparing with the standard literature values. (Ardrey and Moffat, 1981)

2.5. Fourier transform infrared spectroscopy studies (FT-IR)

The functional groups of the organic solvent extracts of *S. tenerrimum* were elucidated using a Nicolet iS10 FT-IR spectrophotometer. Sample was prepared using the KBr pellet technique, and the spectra were obtained by scanning through the range 4000-400 cm^{-1}, with a resolution of 4 cm^{-1}.

2.6. Antibacterial analyses

Human pathogenic bacteria *Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae,* and *Pseudomonas aeruginosa* were used in this study. The stock was sub-cultured in nutrient broth and incubated at 37 °C for 24 h and designated as the 24 h working culture (WC). The WC was standardized by calculating the colony-forming unit per ml (CFU/ml) as described by Cappuccino and Sherman, 2014. Standardized cultures were utilized for antibacterial spectral studies. The agar-well diffusion technique was used to determine the activity of both, the methanolic and petroleum ether, extracts of *S. tenerrimum* on aseptically prepared Mueller-Hinton (MH) agar plates. The activity of both the methanolic and petroleum ether extracts of *S. tenerrimum* was determined by arranging individual sets. After uniformly spreading the standardized cultures (100 µl) on individual MH agar plates, the plates were placed under laminar airflow for 30 min to allow for diffusion. Wells of 10 mm diameter were made on the MH agar plates using a sterilized standard borer. The extracts were then placed in the corresponding wells, and the plates were ideally positioned for the initial diffusion and incubated at 37 °C for 24 h. The antibacterial activity was determined by measuring the zone of inhibition around the well; the values were expressed in millimeters (mm). The Kirby Bauer technique was used to determine the antibacterial potential of a standard streptomycin disc (10 µg/disc) (Cappuccino, Sherman, 2014; John et al., 2018). The antibacterial activity was assessed after the 24 h incubation by measuring the diameter of inhibitory zones around the wells. The level of activity and potency is directly proportional to the diameter of the zones of inhibition.

2.7. Statistical analysis

Statistical analyses were performed using the Graph Pad Instat software system, version 3.10. All the experiments were performed six times (n = 6), and the values were represented with a standard deviation. Values of $p < 0.001$, were highly significant. The test values were compared with the standard drug values using Dunnett's post hoc test.

3. Results and Discussion

The Red Sea of Jazan, KSA, is rich in many taxonomically diverse seaweeds, especially brown algae. Here, we studied *S. tenerrimum,* a brown alga, for the presence of bioactive constituents through organic solvent extraction.

3.1. GC-MS analysis

Figure 2 shows the bioactive compounds of the PEE of *S. tenerrimum* determined by GC-MS analysis. Their corresponding names, molecular formulae, retention times, retention index, and molecular weights are listed in Table 1. Our results suggested the presence of the following compounds, benzoic acid, 3,5-dicyclohexyl-4-hydroxyester, and a unique methyl ester, called methyl 3,5-dicyclohexyl-4-hydroxybenzoate, having a retention time of 63.37 min. A similar compound has been reported in the methanolic extract of *Buchholzia Coriacea* Engler with a retention time of 48.65 min (Chukwunonye et al., 2015). This compound has not yet been reported in seaweeds and its biological properties have not been published. Isomethadone was identified at 44.11 min with lesser probability. Cholesterol was another unique molecule observed in the PEE of *S. tenerrimum*. The presence of cholesterol has been reported in the ethyl acetate extract of the marine alga *S. oligocystum* collected from the Persian Gulf (Permeh et al., 2012). Squalene, a triterpene which is an intermediate produced during cholesterol biosynthesis was observed at 50.26 min. Megha et al. (2016) reported that the methanolic extract of *U. lactuca* and *L. obtusa* contained squalene. An earlier review suggested the cosmetic use of squalene (Huang et al., 2009) and another use as a vaccine adjuvant, especially in the influenza vaccine adjuvant known as MF 59 (Giuseppe et al., 2006). It also possesses anti-cancer properties and reduces blood LDL and cholesterol levels (Lozano-Grande et al., 2018).

Octatriacontyl pentafluoropropionate was detected in the PEE of *S. tenerrimum* at a retention time of 52.42 min with the highest molecular weight of 696. Recently, this compound was reported in the GC-MS analysis of the methanolic extract of *Dendrobium moniliforme* with a retention time of 13.036 min (Paudel et al., 2018). Seaweeds are rich sources of polyunsaturated fatty acids (Moni et al., 2019). The hexadecanoic acid derivative, 9-hexadecenoic acid, eicosyl ester, (Z)-, otherwise

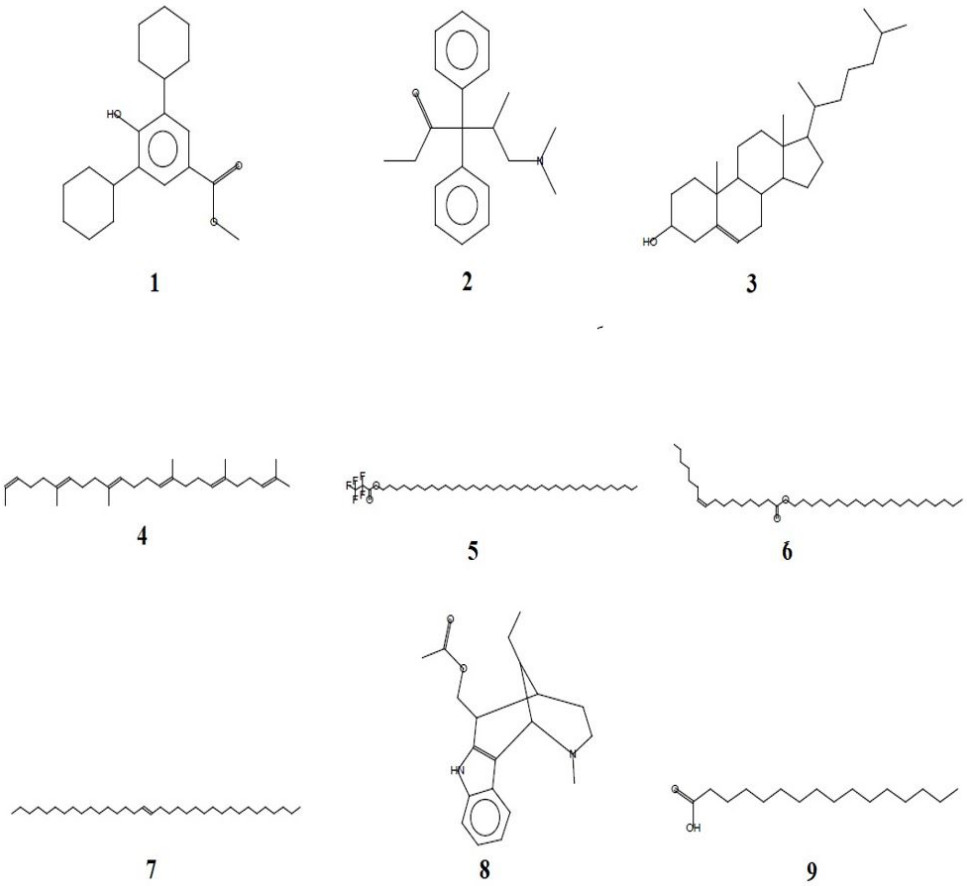

Figure 2. Bioactive compounds of petroleum ether extracts of *Sargassum tenerrimum was* identified through by GC-MS analysis. **(1)** Benzoic acid, 3,5- dicyclohexyl-4-hydroxy-methyl ester **(2)** Isomethadone **(3)** Cholesterol **(4)** Squalene **(5)** Octatriacontyl pentafluoropropionate **(6)** 9-Hexadecenoic acid, eicosyl ester, (Z)-, **(7)** 17-Pentatriacontene **(8)** Dasycarpidan-1-methanol, acetate (ester) **(9)** Hexadecanoic acid, methyl ester.

Table 1. Bioactive compounds of petroleum ether extracts of *Sargassum tenerrimum* identified through by GC-MS analysis.

S.no	Bioactive compound	Molecular formula	Retention time (Minutes)	Molecular weight	Retention Index
1	Benzoic acid, 3,5-dicyclohexyl-4-hydroxy-, methyl ester	$C_{20}H_{28}O_3$	63.37	316	2613
2	Isomethadone	$C_{21}H_{27}NO$	44.11	309	2110
3	Cholesterol	$C_{27}H_{46}O$	49.63	386	2999
4	Squalene	$C_{30}H_{50}$	50.26	410	2806
5	Octatriacontyl pentafluoropropionate	$C_{41}H_{77}F_5O_2$	52.42	696	Unconfirmed
6	9-Hexadecenoic acid, eicosyl ester, (Z)-	$C_{36}H_{70}O_2$	56.74	534	3763
7	17-Pentatriacontene	$C_{35}H_{70}$	70.55	490	3501
8	Dasycarpidan-1-methanol, acetate (ester)	$C_{20}H_{26}N_2O_2$	70.92	326	2446
9	Hexadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	31.81	256	1925

called methyl palmitoleate was detected in the PEE of *S. tenerrimum*. An earlier report showed that this compound has anti-inflammatory properties (Astudillo et al., 2018). A previous study showed that hexadecanoic acid, the major fatty acid in *Sargassum granuliferum,* prevents the growth of biofilm-forming bacteria (Bakar et al., 2017). The present study demonstrated that the PEE of *S. tenerrimum* contains 17-pentatriacontene, which has been reported to have anti-inflammatory, anticancer, antibacterial, and antiarthritic properties (Dinesh et al., 2018). Dasycarpidan-1-methanol acetate (ester) is an alkaloidal compound identified in the PEE of *S. tenerrimum* in this study. This bioactive compound has been reported to have anti-inflammatory, anti-bacterial, anti-fungal, and anti-cancer properties (Al Rubaye et al., 2017).

Table 2 shows the results of the GC-MS analysis of the methanolic extracts of *S. tenerrimum*, and the structures of the compounds identified are presented in Figure 3. A steroidal compound cholest-5-en-3-ol, 24- propylidene-, (3á)- was detected in the ME of *S. tenerrimum* with the highest retention time of 59.50 minutes. Also, a similar compound has been reported in the ethanolic extract of *Ipomoea staphylina* Roem & Schult (Padmashree et al., 2018). The ME of *S. tenerrimum* showed the presence of 1,2-benzenedicarboxylic acid, diisooctyl ester, which is also called diisooctyl phthalate, and phthalic acid. An earlier study suggested that 1,2-benzenedicarboxylic acid and diisooctyl ester, isolated and extracted in methanol from the unripe fruits of *Nauclea latifolia*, exhibited antibacterial properties (Padmashree et al., 2018); they were also reported to be present in *Jania rubens,* a red alga (Soad et al., 2016) . Another study reported its antioxidant and anti-cholinesterase activities in the dichloromethane extract of *Sargassum wightii* (Syad et al., 2013).

The GC-MS results revealed the presence of different types of long-chain fatty acids, such as tetratetracontane and hexatriacontane in the ME of *S. tenerrimum*. A previous study suggested the presence of tetratetracontane and hexatriacontane in the diethyl ether and ethanolic extracts, respectively, of *Sargassum fusiforme* (El Shafay et al., 2016). Tetratetracontane was identified as one of the bioactive constituents in the hexane extract of the aerial parts of *Quisqualis indica*, and the extract was also demonstrated to have antibacterial activity (Agarwal et al., 2017). Hentriacontane, also called untriacontane, is a long-chain alkane hydrocarbon that was previously reported in the hexane extract of *T. ornate* and exhibited larvicidal effects (Sowmiya et al., 2017). Kim et al. (2011) reported that hentriacontane from *Oldenlandia diffusa* exhibited anti-inflammatory, antioxidant, and anti-tumor activities. 1-Docosene and 1-nonadecene are alkenes that were identified in the ME of *S. tenerrimum* in this study. Recent reports show that the dichloromethane extract of the red seaweed *Corallina officinalis* contains 1-docosene and exhibits antibacterial properties (Sahar and Aida, 2018). Studies have suggested that the chloroform extract of *C. racemose*, a green alga, contains 1-nonadecene that exhibits antioxidant, anti-inflammatory, and anti-cancer activities (Sivakumar and Balaraman, 2018). Interestingly, the compound 1-nonadecene was identified in the wastewater of a leather industry (Natarajan et al., 2013). 1-Hexadecanol, also called cetyl alcohol or palmityl alcohol, is a fatty acid that has a moderate retention time and is widely used in the cosmetic industry as a non-gelling thickening agent (Ribeiro et al., 2004). Recently, hexadecane was identified in seaweeds by Mohy El-Din and Alagawany (2019). In this study, 1,2-Benzenediol and were detected with the lowest retention times at 13.73 and 13.25 minutes, respectively, in the ME of *S. tenerrimum*. 1,2-Benzenediol, also known as pyrocatechol, and benzoic acid found in the methanolic extract and crude extract, respectively, of the red alga *Rhodomela confervoides* exhibited antibacterial properties (Nianjun et al., 2003). Benzoic acid was one of the bioactive constituents in *Sargassum wightii* (Balachandran et al., 2016); it has found use as a food preservative that inhibits macroautophagy (Park et al., 2001), and its derivatives showed antimicrobial effects against screened microorganisms (Hazan et al., 2004).

3.2. FT-IR spectroscopy analysis

The preliminary phytochemical investigation of the ME and PEE of *Sargassum tenerrimum* showed the presence

Table 2. Bioactive compounds of methanolic extracts of *Sargassum tenerrimum* identified through by GC-MS analysis.

S.no	Bioactive compound	Molecular formula	Retention time (Minutes)	Molecular weight	Retention Index
1	Cholest-5-en-3-ol, 24- propylidene-, (3á)-	$C_{30}H_{50}O$	59.50	426	2861
2	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	45.78	390	2698
3	Tetratetracontane	$C_{44}H_{90}$	45.03	618	Unconfirmed
4	Hentriacontane	$C_{31}H_{64}$	42.98	436	3116
5	1-Docosene	$C_{22}H_{44}$	32.35	308	2174
6	1-Nonadecene	$C_{19}H_{38}$	28.33	266	1878
7	1-Hexadecanol	$C_{16}H_{34}O$	18.93	242	1854
8	1,2-Benzenediol	$C_6H_6O_2$	13.73	110	1189
9	Benzoic acid	$C_7H_6O_2$	13.25	122	1156

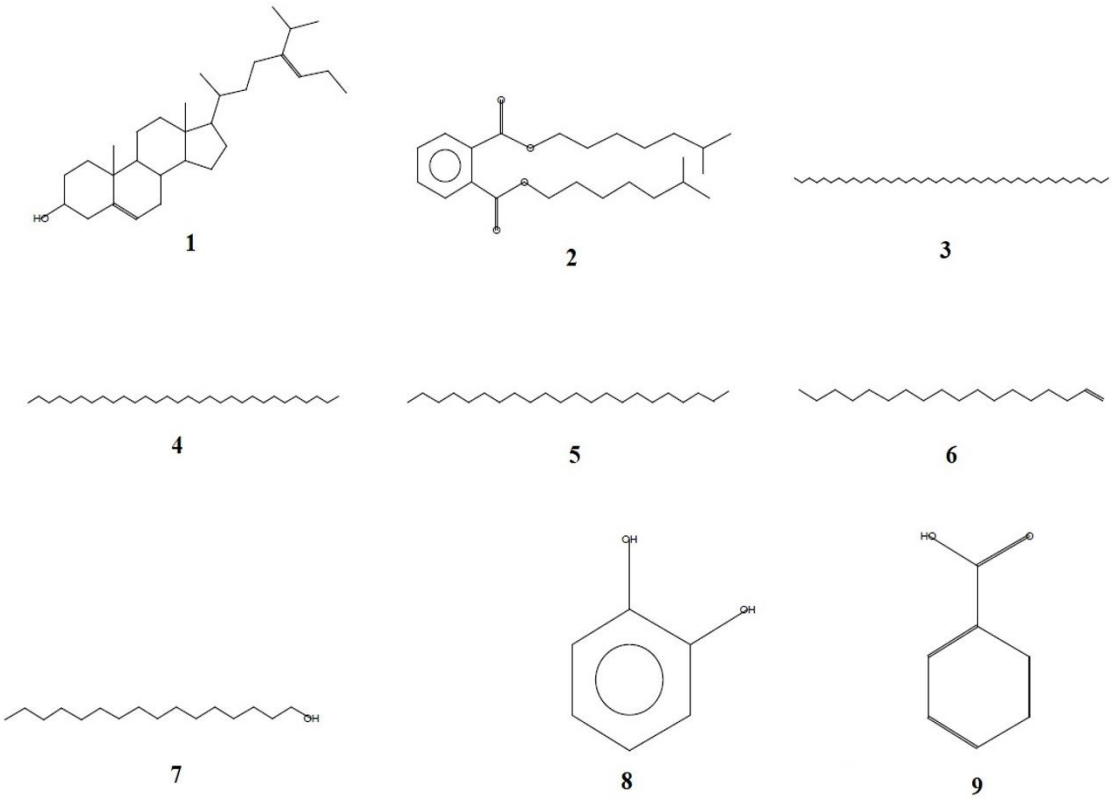

Figure 3. Bioactive compounds of methanolic extract of *Sargassum tenerrimum* identified through by GC-MS analysis **(1)** Cholest-5-en-3-ol, 24- propylidene-, (3á)-, **(2)** 1,2-Benzenedicarboxylic acid, diisooctyl ester **(3)** Tetratetracontane **(4)** Hentriacontane **(5)** 1-Docosene **(6)** 1-Nonadecene **(7)** 1-Hexadecanol **(8)** 1,2-Benzenediol **(9)** Benzoic acid.

of polysaccharides, flavonoids, terpenoids, amino acids, proteins, phenolic compounds, and sterol alkaloids, as shown in Table 3. FT-IR predicted the prominent functional groups present in the extract. Characteristic peaks were observed at 3355, 2959, 2945, 2927, 2873, 2832, 2359, 2051, 1652, 1451, 1030, 881 and 739 cm^{-1} (Figures 4, 5). The broad peak conforming to 3355 cm^{-1} indicates the presence of polymeric hydroxyl groups in unsaturated fatty acids, steroids, saponins, flavonoids etc. The peaks corresponding to 2959, 2945, 2927, 2873, and 2832 cm^{-1} indicate symmetrical and asymmetrical C-H stretching in aliphatic compounds, polysaccharides, steroids, squalene, and tannins. The small peak at 2359 cm^{-1} corresponds to S-H stretching vibration that indicates different amino acids and proteins. The characteristic peaks present at 2051 cm^{-1} are attributed to alkynes (C ≡ C) and alkenes (C=C) in ketones (Dovbeshko et al., 2000). The carbonyl stretching vibrations around 1652 cm^{-1} in the extract indicate the presence of aldehydes, ketones, acids, and esters (Zhou et al., 2010). A medium-intensity peak at 1451 cm^{-1} reveals the existence of asymmetric CH_3 bending vibrations of the methyl groups of proteins (Nyquist, 2001).

The peak corresponding to 1030 cm^{-1} is characteristic of the C-O stretching of glycogens. The peaks at 881 cm^{-1} and 739 cm^{-1} indicate the presence of C-H out of plane bending and C-Cl stretching of polysaccharides and halogenated compounds, respectively. The analysis of the algal extract through FT-IR showed the presence of prominent functional groups of the biologically active alcohols, acids, phenols, esters, ethers, and alkanes found. Antimicrobial activity has generally been attributed to long-chain unsaturated fatty acids (C16-C20), such as oleic, linoleic, and, palmitic acid, and saturated fatty acids, such as stearic and palmitic acid (Zheng et al., 2005).

3.3. Antibacterial studies

The results presented in Table 4 summarize the antibacterial effects of the PEE and ME of *S. tenerrimum*. The PEE of *S. tenerrimum* exhibited antibacterial activity due to the presence of hexadecenoic acid, methyl ester, 17-pentatriacontene, dasycarpidan-1-methanol, and acetate (ester). The ME of *S. tenerrimum* showed activity against the selected human pathogenic bacteria. The antibacterial activity of the ME of *S. tenerrimum* was due to the presence of 1,2-benzenedicarboxylic acid, diisooctyl ester, tetratetracontane, 1-docosene, 1,2-benzenediol and, benzoic acid. The ME showed better activity than the PEE against both, gram-positive and gram-negative bacteria. Although the extracts showed good activity against the screened organisms, their efficacy was significantly lower than that of the standard streptomycin disc (10 µg/disc).

Table 3. Chemical composition of *Sargassum tenerrimum* as obtained from FTIR Spectroscopy of both the PEE and ME extracts.

Wave number (cm-1)	Intensity Estimation	Functional group	Type of vibration	Possible Compounds
3355	Weak	O-H	Stretching	Phenolic compounds, stigmasterol, 15-hydroxy progesterone, fucosterol, polysaccharides, myristic acid, arachidonic acid, flavanoids, saponins
2959	Strong	C-H	Asymmetrical Stretching	Aliphatic compounds, squalene, steroids, tannins
2945	Strong	C-H	Symmetrical Stretching	Aliphatic compounds, squalene, steroids
2927	Strong	CHO	Stretching	Aliphatic compounds
2873	Strong	CH_2 str. (Symmetrical)	Stretching	Carbohydrate, proteins
2832	Strong	OCH_3 str. (Symmetrical)	Stretching	Aliphatic compounds
2359	Weak	S-H	Stretching	Amino acids
2051	Weak	C≡C, C=C in ketones	Stretching	Steroids, hydroxy progesterone
1652	Strong	C=O, N-H	C=O Stretching NH bending	Myristic acid, esters like methyl palmitate, methyl oleate, aldehydes, aromatic ketones, myristic acid, arachidonic acid
1451	Strong	C-H	Asymmetrical bending	Proteins
1030	Medium	C-O	Stretching	Glycogens
881	Medium	C-H	Out of plane bending	Polysaccharides
739	Strong	C-Cl	Stretching	Halogenated compounds

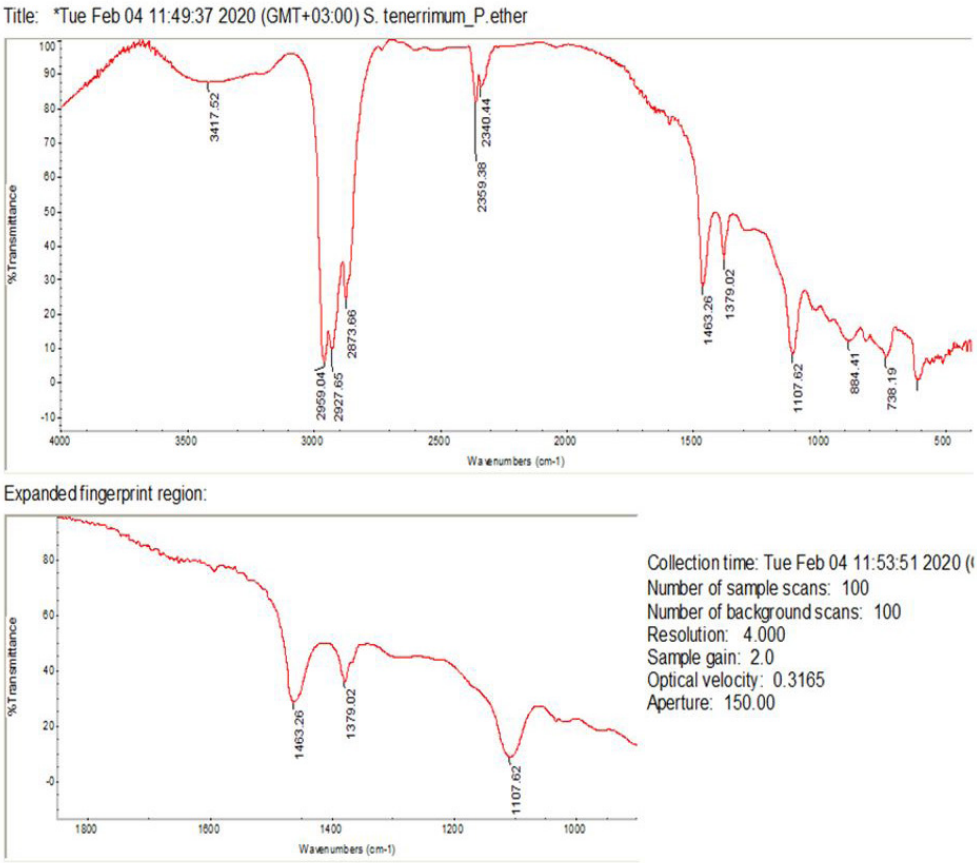

Figure 4. FT-IR spectral pattern of petroleum ether extract of *Sargassum tenerrimum*.

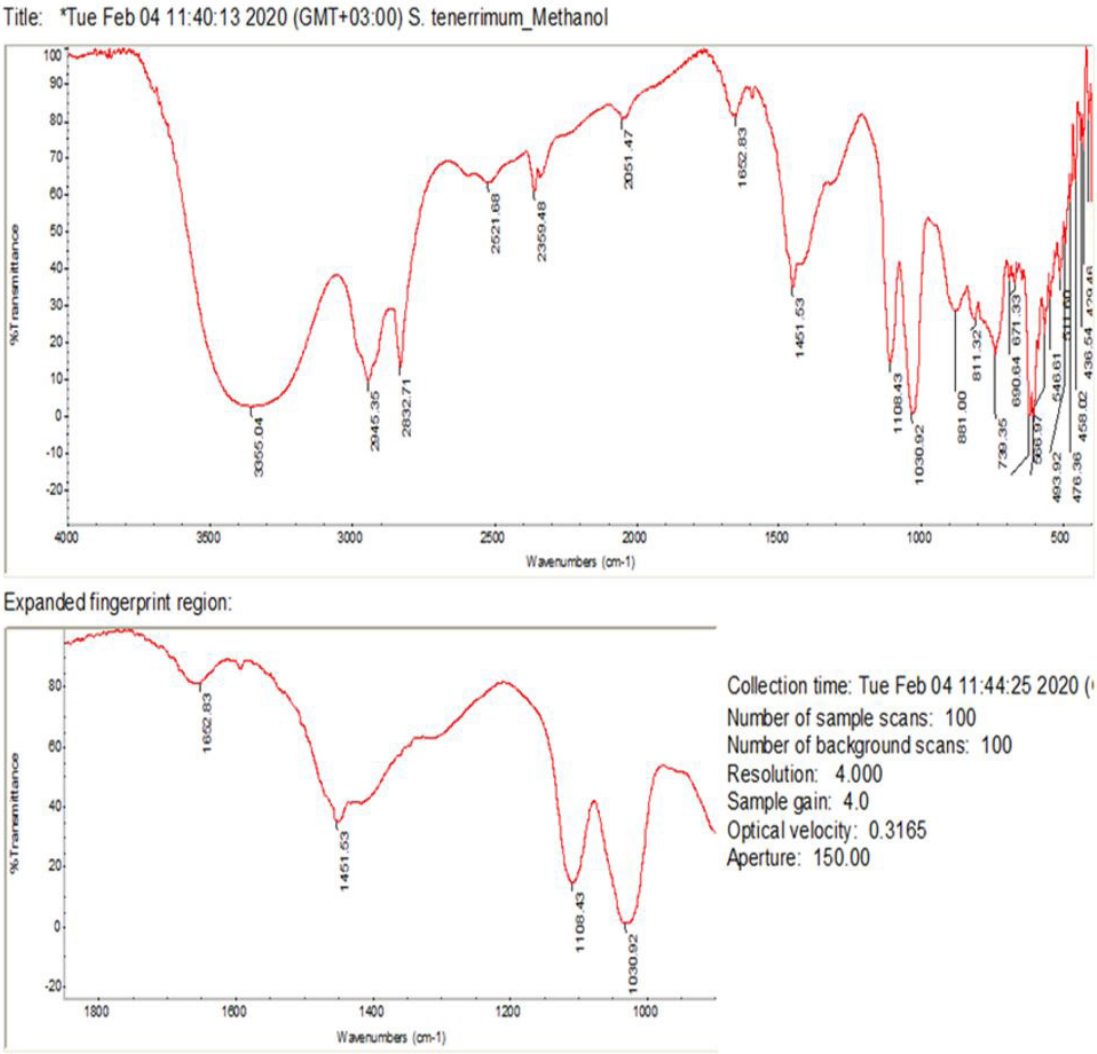

Figure 5. FT-IR spectral pattern of methanol extract of *Sargassum tenerrimum*.

Table 4. Antibacterial studies of the extracts of *Sargassum tenerrimum*.

Bacterial Organisms	Concentration of 24 h culture CFU /mL	Methanolic extract	Petroleum ether extract	Streptomycin 10 mcg/disc
Bacillus subtilis	2×10^{-4}	19.3 ± 1.2**	16.6 ± 2.5**	26.24 ± 1.6
Staphylococcus aureus	3×10^{-5}	20.3 ± 1.5*	17.8 ± 0.98**	23.26 ± 2.1
Streptococcus pyogenes	3×10^{-4}	11.8 ± 1.3**	8.3 ± 0.6**	22.6 ± 1.3
Escherichia coli	2×10^{-6}	20.6 ± 1.6**	12.5 ± 1.2**	28.2 ± 1.6
Pseudomonas aeruginosa	2×10^{-3}	17.3 ± 2.3**	14.3 ± 1.6**	25.3 ± 1.3
Klebsiella pneumonia	2×10^{-3}	22.8 ± 2.4*	19.5 ± 1.04**	26.6 ± 1.2

Each value is the mean of 6 batches with standard deviation by performing Dunnett multiple comparison test (Sample vs Standard), (post hoc test); **P < 0.01 (99% confidence interval) is very extremely significant and *P <0.05 (95% confidence interval) is significantly lesser when compared to standard streptomycin disc. CFU: Colony forming unit

4. Conclusion

The present study has documented the scientific evidence of the antibacterial properties of the petroleum ether (PEE) and methanolic (ME) extracts of *S. tenerrimum,* a brown alga found in the Red Sea, Jazan Province, Kingdom of Saudi Arabia. Bioactive molecules varied widely in their molecular structures in both ME and PEE. The ME of *S. tenerrimum* exhibited better antibacterial activity than the PEE. The results obtained in this study indicate that anti-bacterial active principles can be isolated from *S. tenerrimum;* they would be beneficial in the development of new antibacterial agents in the future.

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