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Comparative Antimicrobial Activities of Some Mediterranean Sea Seaweeds-Derived Extracts

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Abstract

In the present study, a quantitative and qualitative comparison of major flavonoids and phenolic components of 6 species of Mediterranean Sea seaweeds collected from Stanley to Abu Qir Bay, Alexandria, Egypt (*Enteromorpha intestinalis, Ulva lactuca, Punctaria plantaginea, Colpomenia sinuosea*, *Dictyota dichotoma* and *Corallina officinalis*) were carried out using HPLC instrument. Also, the chemical profile of the non-polar components extracted by the petroleum ether solvent of these species was identified using GC-MS technique. *n*-hexadecanoic acid (8) was identified in all petrolum ether extracts (p1-p6) recording 18.0%, 17.2%, 17.2%, 20.2%, 19.2% and 16.8% for *E. intestinalis, U. lactuca, P. plantaginea, C. sinuosea*, *D. dichotoma* and *C.officinalis* (p1-p6), respectively. The antimicrobial activity of the obtained extracts was evaluated and *E. intestinalis* crude extract (M1) was the effective antimicrobial agent. Total phenolic and flavonoid contents were also evaluated for the selected six Mediterranean Sea seaweeds species. *D. dichotoma* and *C. officinalis* recorded the highest phenolic and flavonoid contents among all selected species, 31.42 mg/g, 23.42 mg/g for phenolic and 173.9 mg/g, 165.4 mg/g for flavonoid, respectively. These findings are compatible with HPLC analyses as *D. dichotoma* contains eight flavonoids and eleven phenolic constituents, in addition to *C. officinalis* also contains eight flavonoids and ten phenolic compounds.

Keywords: *Punctaria plantaginea*, *Colpomenia Sinuosea, Dictyota dichotoma*, Phaeophyceae.

1. Introduction

The tremendous growth of the world's population has strained available resources for medicines. Hence, pharmaceutical manufacturers are always looking for new resources to develop effective and safe medicines to meet the growing demand of the world population [1]. One of the most global concerns regarding health is antimicrobial resistance (AMR), which is a fatal problem facing us during the current century resulting from the lack of new antimicrobial medications and will result in about 10 million deaths by the year 2050, so we require exploring new antimicrobial agents [2]. From 1981 to 2008 around 68% of medication used to cure bacterial, viral, parasitic and fungal infections was derived from natural sources. Also, about 63% of anti-cancer drugs were derived from natural sources [3]. The marine environment is an exceptional source of novel bioactive secondary metabolites characterized by their different structural and chemical properties than those isolated from the terrestrial environment [1]. Seaweeds are considered a source of bioactive compounds as they are can produce a great variety of secondary metabolites like polysaccharides, polyphenols, carotenoids, proteins, peptides, sterols, terpenes and fatty acids [4]. Seaweeds are classified according to their photosynthetic pigment into green algae (chlorophyta), containing chlorophyll a and b with several carotenoids [5], brown algae (Ochrophyta); containing chlorophyll a and c and fucoxanthin carotenoid [6] and red algae (Rhodophyta) containing phycoerythrin and phycocyanin in addition to chlorophyll a, d, β-carotene, and xanthophylls [7, 8]. The Egyptian Mediterranean coast extends approximately 970 km from Rafah in the eastern north to

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Salloum in the western north [9]. In this extended area, about 187 species were recorded (red, green and brown algae; 107, 45 and 35 species; respectively), accounting for 16% of the Mediterranean seaweeds [10]. In this article, a quantitative and qualitative comparison of major bioactive components, total flavonoid and phenolic contents of six species of Mediterranean Sea seaweeds; Enteromorpha intestinalis, Ulva lactuca (green seaweeds), Punctaria plantaginea, Colpomenia sinuosea, Dictyota dichotoma (brown seaweeds) and Corallina officinalis (red seaweed) were carried out also, major flavonoids and phenolic compounds in crude extract were also evaluated using HPLC instrument and non-polar constituents of petroleum ether extracts were also identified using GC-MS analysis. The antimicrobial activities of both crude and non-polar extracts were also evaluated.

2. Experimental (Materials and Methods)

2 .1. Marine algae

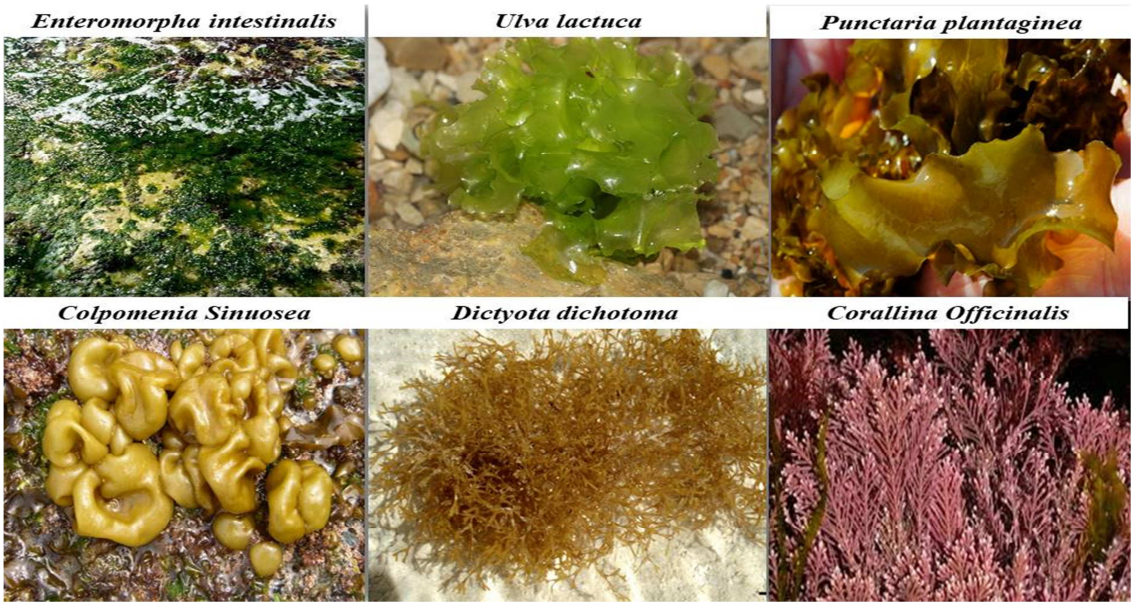

Figure 1: Mediterranean Sea seaweeds; *Enteromorpha intestinalis, Ulva lactuca* (green seaweeds), *Punctaria plantaginea, Colpomenia sinuosea*, *Dictyota dichotoma* (brown seaweeds) and *Corallina officinalis* (red seaweed).

Six species of green, red and brown seaweeds (**Fig. 1)** were collected from April to October from coastal areas of Abou-Quier and Stanleybay, Alexandria, Egypt. All samples were microscopically identified at the National Institute of Oceanography and Fisheries, Egypt. Freshly collected seaweeds samples were washed with distilled water then allowed to dry in the shade after that grinded to get about 100 g powder, then successively extracted at 40 ^{o}C with petroleum ether to extract non-polar constituents (3×400ml), then extracted with a mixture of CH_2Cl_2/MeOH (1:1) (0.4L × 3 times) until complete extraction to get petroleum ether extracts (P1-P6) and crude extracts (M1-M6), respectively. After filtration, both petroleum ether and crude extracts were concentrated under reduced pressure at 40 ^{o}C using a rotary evaporator to get about green to brown gummy extracts which is ready for different measurements.

2 .2. Identification of major volatile constituents of petroleum ether extracts

petroleum ether extract samples (P1-P6) were analysed using the GC-MS technique using Agilent 7890A Series instrument equipped with a multi-mode injector and a123-BD11 column (15 m × 320 μm × 0.1 μm) to compare their volatile chemical compositions [11, 12].

2 .3. Quantitative estimation of total flavonoids/phenolic content of different crude extracts

The total flavonoids content (TF) of crude extracts (M1-M6) was determined according to Herald *et al*. method by preparing different concentrations of standard. Also, a sample solution was prepared by dissolving 1mg of crude extract in 1ml methanol then the absorbance of each standard was measured spectrophotometrically using multi-mode microplate reader at 510 nm using (FLUO star Omega) [13]. Also, the total phenolic contents of crude extract (M1-M6) were determined spectrophotometrically according to Attard method by preparing a standard solution of gallic acid of different concentrations

and a solution of the sample was prepared by dissolving 5 mg in 1 ml methanol Then the absorbance is measured at 630 nm using (FLUO star Omega) [14].

2 .4. Quantitative estimation of total flavonoids/phenolic content of different crude extracts

HPLC analysis was performed using Agilent 1260 infinity HPLC Series (Agilent, USA) coupled with a quaternary pump and diode-array detector. The column uses Akinetex®1.7µm EVO C150 mm x 4.6 mm, (phenomenex, USA), operated at 30 ºC. The separation is achieved using a ternary linear gradient with (A) HPLC grade water 0.1% Trifluoro acetic acid (TFA), (B) acetonitrile, (C) methanol. Flow rate 1ml/min. The injected volume was 20μl. Detection at variable Wavelength detector (VWD) set at 280 nm.

2 .5. Assessment of antibacterial activity

The antibacterial activity of each obtained extract was carried out against Gram-negative bacteria; *Klebsiella pneumoniae (ATCC 10031*) and *Pseudomonas aeruginosa (ATCC 9027)* and against Gram-positive bacteria**;** *Staphylococcus aureus* ***(ATCC 6538)*** and *Staphylococcus epidermidis* using well-cut diffusion technique. All tests were performed in duplicates. After the incubation period, the zone of inhibition was measured and expressed in millimeter (mm) [15, 16].

3. Results

3.1. Major volatile constituents of petroleum ether (P1-P6) extracts via GC/MS

Twenty-five volatile compounds were identified from Twenty-five volatile compounds were identified from different petroleum ether extracts (**Table 1, Fig. 2 and Fig. 3**) using GC-MS analyses and accounting for various classes; hydrocarbons, fatty acid, fatty acid derivatives, sesquiterpenes, triterpene, steroid and sesterterpene. There were similarities in the contents of some extracts. For example, *n*-hexadecanoic acid **(8)** was identified in all extracts recording 18, 17.2, 17.2, 20.2, 19.2 and 16.8 for p1-p6, respectively. Also, *n*-dodecane **(3)** was identified in *Punctaria plantaginea*, *Colpomenia Sinuosea* and *Dictyota dichotoma*. The steroidal compound; brassicasterol acetate **(21)** was identified in all extracts. (*Z,Z,Z*)-9,12,15-octadecatrienoic acid **(12)** fatty acid and azafrin **(22)** sesterterpene were only identified in *Enteromorpha intestinalis*. linoleoyl chloride **(11)** fatty acid derivative was only identified in *Ulva lactuca.* cis-vaccenic acid **(13)**, timnodonic acid **(16)** fatty acids, squalene **(18)** triterpene and (*E*)-24-propylidenecholesterol **(25)** were only identified in *Punctaria plantaginea*. cholesta-3,5-diene **(19)** and lanosta-8,24-diene-3,22-diol **(24)** steroids were only identified in *Colpomenia Sinuosea*. *α*-cubebene **(6)**, D-germacrene **(7)** sesquiterpenes, linoleic acid **(14)** and arachidonic acid **(17)** fatty acids were only identified in *Dictyota dichotoma* petroleum ether extract.

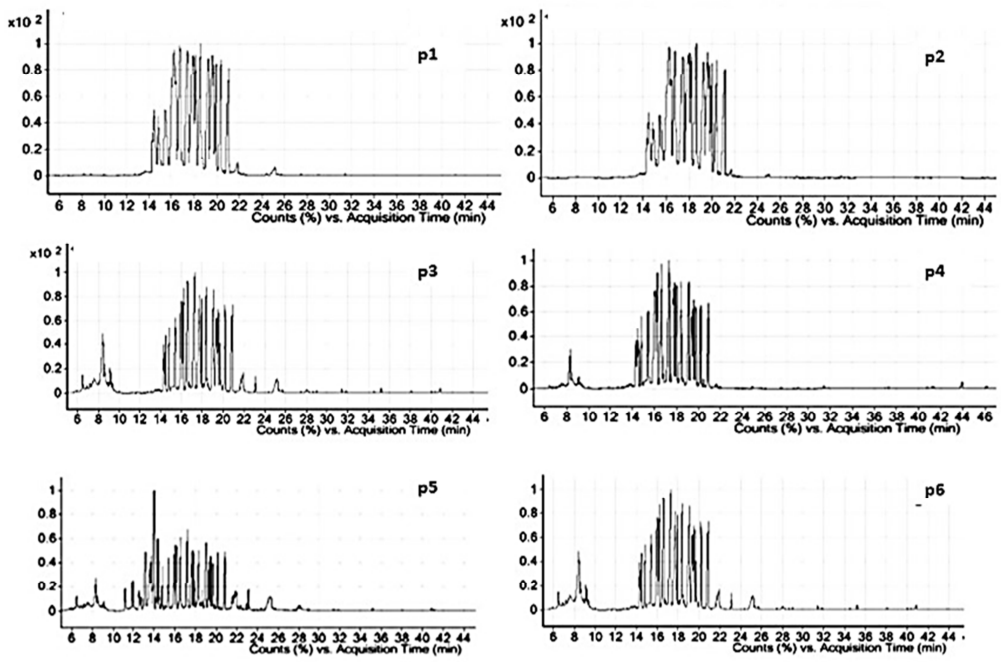

Figure 2: GC-MS chromatograms of the selected seaweeds petroleum ether extracts, P1: *E. intestinalis,* P2: *U. lactuca*, P3: *P. plantaginea,* P4: *C. sinuosea*, P5: *D. dichotoma* and P6: *C. officinalis*.

Table 1: Major identified compounds using GC/MS of 100% petroleum ether extracts

Compound	RT	Area %						Class	Mol. Mas.	Mol. For.
		P1	P2	P3	P4	P5	P6			
Undecane **(1)**	6.43			6.4	0.86			Hydrocarbon	156.31	$C_{11}H_{24}$
2-Hexyldecan-1-ol **(2)**	7.58			5.6				Fatty alcohol	242.44	$C_{16}H_{34}O$
n-Dodecane **(3)**	8.35			22	8.7	3.5		Hydrocarbon	170.34	$C_{12}H_{26}$
2,6-Dimethylundecane **(4)**	8.54			3.2	5.6			Hydrocarbon	184.36	$C_{13}H_{28}$
n-Hexylcyclohexane **(5)**	9.15			5.2				Hydrocarbon	168.32	$C_{12}H_{24}$
α-Cubebene **(6)**	11.19					7.2		Sesquiterpene	204.35	$C_{15}H_{24}$
D-Germacrene **(7)**	11.93					6.4		Sesquiterpene	204.35	$C_{15}H_{24}$
n-Hexadecanoic acid **(8)**	21.6	18	17.2	17.2	20.2	19.2	16.8	Fatty acid	256.42	$C_{16}H_{32}O_2$
cis-5,8,11,14,17-Eicosapentaenoic acid, methyl ester **(9)**	23.1			2.4				Fatty ester	316.5	$C_{21}H_{32}O_2$
trans-13-Octadecenoic acid **(10)**	24.88		1.3		0.6			Fatty acid	282.46	$C_{18}H_{34}O_2$
Linoleoyl chloride **(11)**	25.03		1.6					Fatty acid der.	298.89	$C_{18}H_{31}OCl$
(Z,Z,Z)-9,12,15-Octadecatrienoic acid **(12)**	25.16	5.04						Fatty acid	292.45	$C_{19}H_{32}O$
cis-Vaccenic acid **(13)**	25.19			6				Fatty acid	282.46	$C_{18}H_{34}O_2$
Linoleic acid **(14)**	25.27					2.4		Fatty acid	280.45	$C_{18}H_{32}O_2$
2-Methylhexadecan-1-ol **(15)**	25.70			1.6				Fatty alcohol	256.46	$C_{17}H_{36}O$
Timnodonic acid **(16)**	28.0			1.2				Fatty acid	302.45	$C_{20}H_{30}O_2$
Arachidonic acid **(17)**	28.02					6.1		Fatty acid	304.47	$C_{20}H_{32}O_2$
Squalene **(18)**	35.19			0.56				Triterpene	410.73	$C_{30}H_{50}O$
Cholesta-3,5-diene **(19)**	36.99				0.74			Steroid	368.6	$C_{27}H_{44}$
Stigmasterol acetate **(20)**	38.0			0.84				Steroid	454.74	$C_{31}H_{50}O_2$
Brassicasterol acetate **(21)**	40.07	5.04	1.6	0.56	0.7	3.8	1.4	Steroid	440.70	$C_{30}H_{48}O_2$
Azafrin **(22)**	40.71	2.4						Sesterterpene	426.6	$C_{27}H_{38}O_2$
Vitamin E **(23)**	41.11			2.8			1.9	Fatty acid der.	430.7	$C_{29}H_{50}O_2$
Lanosta-8,24-diene-3,22-diol **(24)**	43.8			0.56	3.9			Steroid	442.38	$C_{30}H_{50}O_2$
(*E*)-24-Propylidenecholesterol **(25)**	46.8			1.2				Steroid	426.7	$C_{30}H_{50}O$

RT: retention time, **GC-MS:** gas chromatography-mass spectrometry**, P1:** petroleum ether extract of *E. intestinalis,* **P2:** petroleum ether extract of *U. lactuca,* **P3:** petroleum ether extract of *P. plantaginea,* **P4:** petroleum ether extract of *C. Sinuosea*, **P5:** petroleum ether extract of *D. dichotoma*, **P6:** petroleum ether extract of *C. officinalis,* **Mo. Mass:** molecular mass, **Mol. for.:** molecular formula.

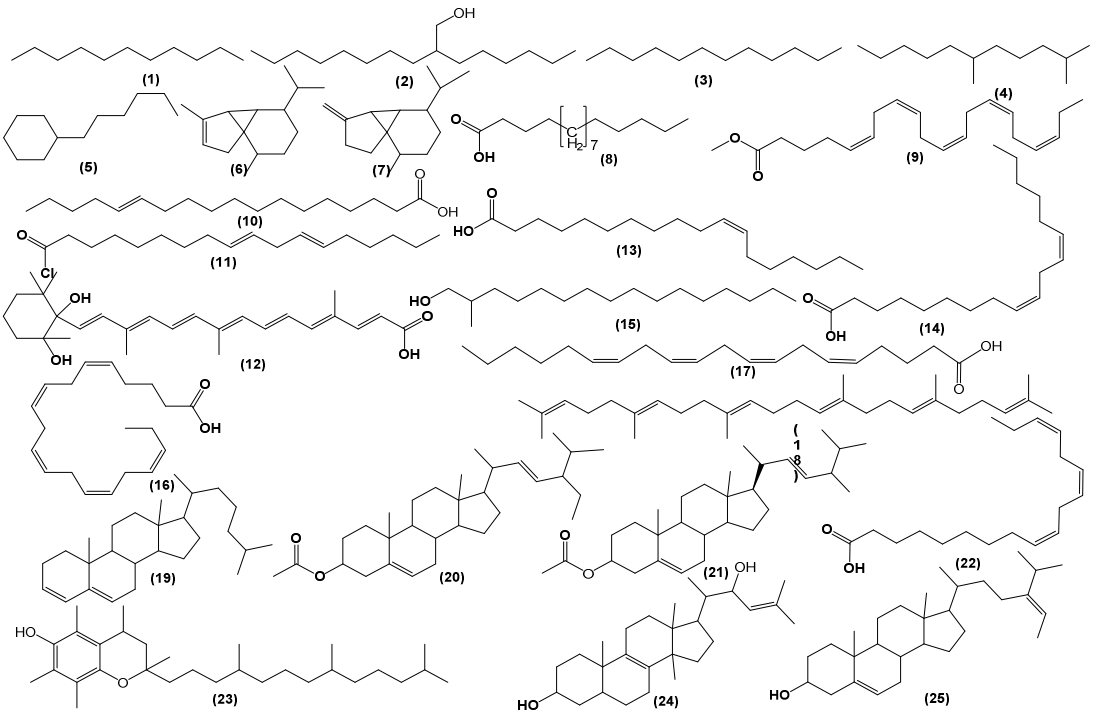

Figure 3: Identified compounds from petroleum ether extracts using GC-MS analysis.

3.2. Total phenolic& flavonoids content of algae crude extracts (M1-M6)

As shown in **(**table 2), the total phenolic content of brown seaweed; *D. dichotoma*, green seaweed; *E. intestinalis* and red seaweed; *C. Officinalis* is 31.42 mg/g, 25.98 mg/g and 23.42 mg/g, respectively which is higher than other crude extracts of *P. plantaginea*, *U. lactuca* and *C. Sinuosea* which recorded 16.9 mg/g, 6 mg/g and 8.975 mg/g. Total flavonoid content of brown seaweed; *D. dichotoma* and red seaweed; *C. Officinalis is*173.9 mg/g and 165.4 mg/g, respectively which is much higher than other extracts. While total flavonoid content of *C. Sinuosea* and *U. lactuca* is the lowest value recording 34.267 mg/g and 24.53 mg/g, respectively *E. intestinalis* and *P. plantaginea* recorded 71.77 mg/g 66.36 mg/g.

Table 2: Phenolic and flavonoids content of algae crude extracts

Extract	M1		M2		M3		M4		M5		M6	
	SD	T	SD	T	SD	T	SD	T	SD	T	SD	T
Phenolic **Ct**	1.85	25.98 mg / g	0.175	6 mg / g	0.64	16.9 mg / g	0.723	8.975 mg / g	2.44	31.42 mg / g	1.44	23.42 mg / g
Flavonoid **Ct**	3.94	71.77 mg / g	0.95	24.53 mg / g	6.16	66.36 mg / g	3.03	34.267 mg / g	13.94	173.9 mg / g	12.5	165.4 mg / g

Ct: content, SD: Standard Deviation**, T:** Total content compared with standard deviation curve, **M1:** crude extract of *E. intestinalis*, **M2:** crude extract of *U. lactuca,* **M3:** crude extract of *P. plantaginea,* **M4:** crude extract of *C. Sinuosea*, **M5:** crude extract of *D. dichotoma,* **M6:** crude extract of *C. officinalis.*

3.3. Major flavonoids and phenolic components of crude extracts

A The HPLC analysis for six seaweed species **(Table 3, Fig. 4 and Fig. 5)** represents the presence of eight main flavonoid compounds and eleven phenolic compounds differing in their amount in each crude compound are *ρ*-hydroxybenzoic **(28)**, catechin **(29)**, *o*-cumaric **(36)**, quercetin **(42)** and kaempferol **(43)** recording 26.8, 25.4, 36.6, 73.1 and 25.4 mg/kg. For *Ulva lactuca* crude extract (M2) the major phenolic and flavonoid compounds are *ρ*-catechin **(29)**, caffeic acid **(31)**, chlorogenic acid **(32)**, rutin **(37)** resveratrol **(39)**, rosemarinic acid **(41)** and quercetin **(42)** recording 95.6, 71.9, 513.5, 115.29, 79.68, 26.63 and 27.52mg/k, respectively. For *Punctaria plantaginea* crude extract (M3) the major phenolic and flavonoid compounds are catechol **(27)**, *ρ*-hydroxybenzoic **(28)**, catechin **(29)**, caffeic acid **(31)** *ρ*-coumaric **(34)** rutin **(37)**, resveratrol **(39)**, rosemarinic acid **(41)**, quercetin **(42)** and kaempferol **(43)** recording 43.63, 33.88, 118.86, 26.40, 40.39, 57.52, 88.30, 26.06, 72.09 and 83.43mg/k, respectively. For *Colpomenia Sinuosea* crude extract (M4) the major phenolic and flavonoid compounds are *ρ*-coumaric **(34)**, resveratrol **(39)**, rosemarinic acid **(41)** quercetin **(42)** kaempferol **(43)** recording 26.46, 40.74, 55.8, 75.14 and 66.9 mg/k, respectively. For *Dictyota dichotoma* crude extract (M5) the major phenolic and flavonoid compounds are ferulic **(35)**, rutin **(37)**, myricetin **(40)**, rosemarinic acid **(41)**, quercetin **(42)** and kaempferol **(43)** recording 43.63, 32.87, 34.61, 34.15, 66.89 and 47.92 mg/k, respectively. For *Corallina Officinalis* crude extract (M6) the major phenolic and flavonoid compounds are gallic acid (26) and quercetin (42) recording 88.97 and 27.28 mg/k, respectively. The results show that the mean value of (+)-catechin level was 7.786%.

3.4. Antibacterial activity

Data presented in Table 4 revealed that the crude extracts (M1-M6) and petroleum ether extracts (P1-P6) of the tested algae had potent activities against four common bacterial pathogens. On focus, considerable values ranged between 10 mm and 38 mm for crude extracts, while the petroleum ether crude extracts exhibited values ranged from 10 mm to 20 mm. However, the most effective extract was the *E. intestinalis* crude extract (M1), while the lowest effective one was of *C. officinalis* extract (M6). Moreover, the most affected microbe was *S. epidermidis*, while *P. aeruginosa* was the lowest affected one at all. Indeed, *P. aeruginosa* ATCC 9027 was not affected by either M4 or M5. Observably, petroleum ether extracts showed lower activity than those methanolic extracts. Furthermore, the total averages of inhibition zones confirmed that M1 and M5 recorded the highest antibacterial value at 20.5 mm for both, while the lowest value was 11.75 mm for M6.

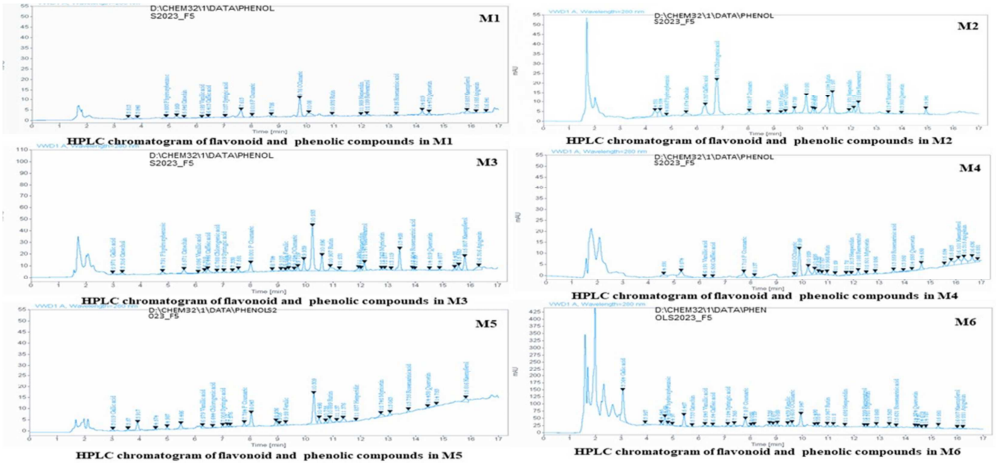

Figure 4: HPLC chromatograms of the selected seaweeds crude extracts

Table 3: Identified Compounds by HPLC of algae different crude extracts

Compound	RT [min]	class	Mo. mass	Mol. for.	M1		M2		M3		M4		M5		M6	
					A%	U	A%	U	A%	U	A%	U	A%	U	A%	U
Gallic acid (**26**)	3.00	Phenolic	170.12	$C_7H_6O_5$	----	----	----	----	9.20	9.15	----	----	4.10	8.16	671.55	88.97
Catechol (**27**)	3.23	Phenolic	110.1	$C_6H_6O_2$	----		----		13.25	43.63	----	----	----	----	----	
p-Hydroxybenzoic (**28**)	4.89	Phenolic	138.12	$C_7H_6O_3$	8.41	26.8	3.74	9.5	13.30	33.88	----	----	----	----	29.48	10.01
Catechin (**29**)	5.54	flavonoid	290.27	$C_{15}H_{14}O_6$	4.13	25.1	19.68	95.6	24.47	118.86	----	----	----	----	7.86	5.09
Vanillic acid (**30**)	6.18	Phenolic	168.14	$C_8H_8O_4$	2.82	5.8	----	----	2.49	4.14	2.72	6.48	6.21	20.64	52.39	11.59
Caffeic acid (**31**)	6.41	Phenolic	180.16	$C_9H_8O_4$	3.23	4.7	61.86	71.9	22.69	26.40	2.17	3.61	----	----	27.62	4.28
Chlorogenic acid (**32**)	6.72	Phenolic	354.31	$C_{16}H_{18}O_9$	----	----	164.3	513.5	6.43	20.10	----	----	2.42	15.14	----	
Syringic acid (**33**)	7.04	Phenolic	198.17	$C_9H_{10}O_5$	2.58	3.07	----	----	9.09	8.65	----	----	2.74	5.22	35.05	4.45
p-Coumaric (**34**)	8.01	Phenolic	164.04	$C_9H_8O_3$	3.60	3.3	15.79	11.69	54.54	40.39	24.99	26.46	6.29	9.33	157.33	15.53
Ferulic (**35**)	9.27	Phenolic	194.1	$C_{10}H_{10}O_4$	----	----	4.69	17.76	3.84	14.55	----	----	5.70	43.19	31.93	16.124
o-Cumaric (**36**)	9.76	Phenolic	164.16	$C_9H_8O_3$	49.74	36.6	5.26	3.09	4.09	2.41	4.03	3.39	----	----	49.59	3.89
Rutin (**37**)	10.93	flavonoid	610.52	$C_{27}H_{30}O_{16}$	7.03	12.6	80.07	115.29	39.94	57.52	4.85	9.99	11.41	32.87	37.99	7.29
Hesperidin (**38**)	11.99	flavonoid	610.56	$C_{28}H_{34}O_{15}$	4.74	11.9	7.29	14.74	6.96	14.05	4.33	12.52	2.58	10.46	13.83	3.72
Resveratrol (**39**)	12.20	flavonoid	228.24	$C_{14}H_{12}O_3$	5.72	20.7	27.45	79.68	30.42	88.30	9.81	40.74	----	----	13.12	5.07
Myricetin (**40**)	12.6	flavonoid	318.23	$C_{15}H_{10}O_8$	----	----	----	----	3.78	4.51	4.67	7.98	14.50	34.61	29.84	4.74
Rosemarinic acid (**41**)	13.26	Phenolic	360.31	$C_{18}H_{16}O_8$	3.45	18.3	6.27	26.63	6.13	26.06	9.19	55.8	4.02	34.15	27.00	15.29
Quercetin (**42**)	14.47	flavonoid	302.23	$C_{15}H_{10}O_7$	5.49	73.1	2.58	27.52	6.76	72.09	4.93	75.14	3.13	66.89	19.20	27.28
Kaempferol (**43**)	15.85	flavonoid	286.23	$C_{15}H_{10}O_6$	14.56	25.4	----	----	59.69	83.43	33.48	66.9	17.14	47.92	28.62	5.33
Apigenin (**44**)	16.19	flavonoid	270.24	$C_{15}H_{10}O_5$	2.02	0.38	----	----	37.04	5.55	28.61	**6.13**	----	----	21.97	0.439

Mo. Mass: molecular mass, **Mol. for.:** molecular formula, **RT**: retention time, **HPLC:** high-performance liquid chromatography**, U:** amount (mg/kg of dry extract), **A%:** Area percentage, **M1:** crude extract of *E. intestinalis*, **M2:** crude extract of *U. lactuca,* **M3:** crude extract of *P. plantaginea,* **M4:** crude extract of *C.sinuosea*, **M5:** crude extract of *D. dichotoma,* **M6:** crude extract of *C. officinalis*.

Table 4: Antibacterial activity of different algal extracts

Bacterial species	I(mm)											
	E. intestinalis		*U. lactuca*		*P. plantaginea*		*C. sinuosea*		*D. dichotoma*		*C. officinalis*	
	M1	P1	M2	P2	M3	P3	M4	P4	M5	P5	M6	P6
K. pneumoniae	14	15	11	17	22	20	23	19	29	20	10	17
P. aeruginosa	19	16	11	15	10	13	-	10	11	10	-	15
S. aureus	14	19	10	18	13	18	15	15	13	17	10	16
S. epidermidis	35	17	38	18	26	18	32	16	29	12	27	14
total averages of inhibition zones	20.5	16.75	17.5	17	17.75	17.25	17.5	15	20.5	14.75	11.75	15.5

I(mm): Inhibition zone diameters (mm)**, *K. pneumoniae:*** *Klebsiella pneumoniae (ATCC 10031)* ***Gram-negative*****, *P. aeruginosa:*** *Pseudomonas aeruginosa (ATCC 9027)* ***Gram-negative***, ***S. aureus:*** *Staphylococcus aureus* ***(ATCC 6538) Gram-positive, S. epidermidis:*** *Staphylococcus epidermidis* ***Gram-positive*****, M1:** crude extract of *E. intestinalis*, **M2:** crude extract of *U. lactuca,* **M3:** crude extract of *P. plantaginea,* **M4:** crude extract of *C.sinuosea*, **M5:** crude extract of *D. dichotoma,* **M6:** crude extract of *C. officinalis,* **P1:** petroleum ether extract of *E. intestinalis,* **P2:** petroleum ether extract of *U. lactuca,* **P3:** petroleum ether extract of *P. plantaginea,* **P4:** petroleum ether extract of *C. Sinuosea*, **P5:** petroleum ether extract of *D. dichotoma*, **P6:** petroleum ether extract of *C. officinalis,*.

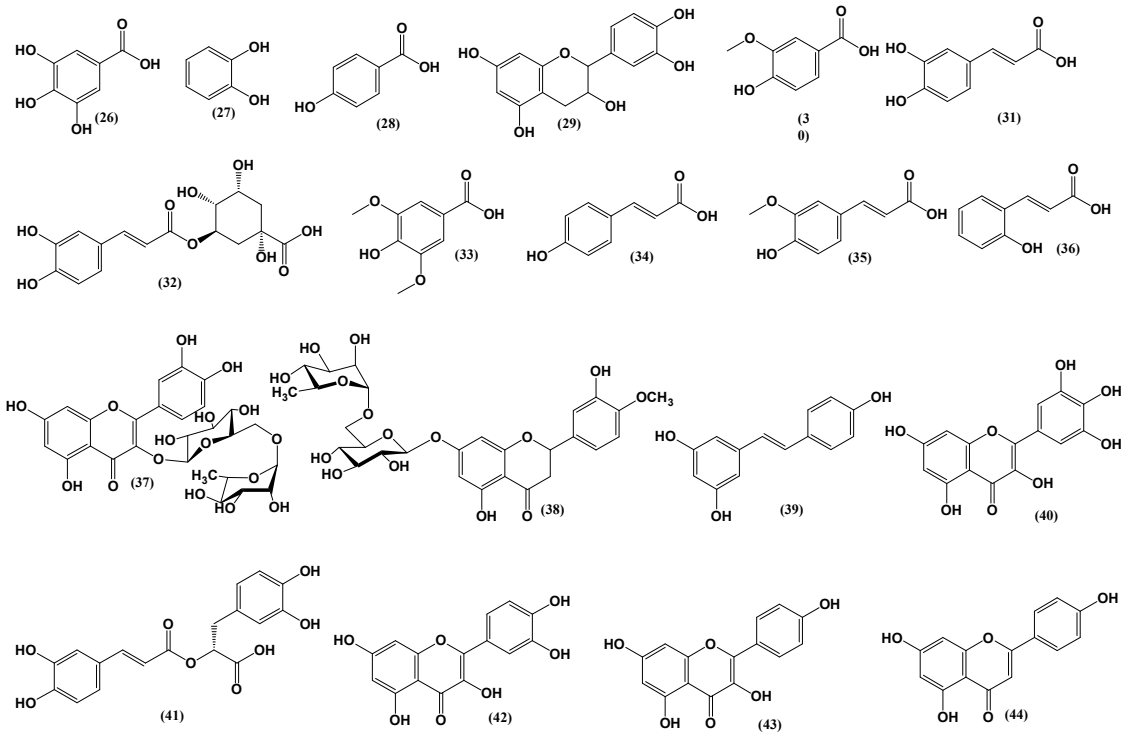

Figure 5: Identified compounds from seaweed crude extracts using HPLC analysis.

4. Discussion

Natural products possess an unparalleled range of chemical diversity, rendering them a promising source for distinctive and permissible additives and pharmacological interventions [17].

Many studies have reported findings that support the presented results; from the GC-MS chemical profile of the non-polar petroleum ether extracts leads to the identification of 25 compounds among these identified compounds n-hexadecanoic acid **(8)** which was reported before as is a strong antibacterial agent[18]. The percentage of n-hexadecanoic acid **(8)** recorded 18%, 17.2%, 17.2%, 20.2%, 19.2% and 16.8% for p1-p6, respectively which is nearly the same but the highest percentage detected in *C. Sinuosea* and *D. dichotoma*. *n*-Dodecane **(3)** which has antimicrobial activity in volatile oil mixtures [19] and was identified in p3, p4, p5 with 22, 8.7 and 3.5%. Also, α-cubebene **(6)** was identified in P5 with a percentage of 7.2%, and was previously well-known to have antimicrobial activity in volatile oil mixture [20]. Based on the above results, the potent antimicrobial activities of petroleum ether extracts (p1-p5) could be attributed to the synergetic effect arisen from the bioactive compounds mixture.

Also, total phenolic and flavonoid contents were evaluated for the selected six Mediterranean Sea seaweed species. *D. dichotoma* and *C. officinalis* recorded the highest phenolic and flavonoid contents among all selected species, 31.42 mg/g, 23.42 mg/g for phenolic and 173.9 mg/g, 165.4 mg/g for flavonoid, respectively. These findings are compatible with HPLC analyses as *D. dichotoma* contains eight flavonoids and eleven phenolic constituents, in addition to *C. officinalis* also contains eight flavonoids and ten phenolic compounds.

Quantitative and qualitative HPLC analysis of major flavonoids and phenolic components of species under investigation; *E. intestinalis, U. lactuca, P. plantaginea, C. sinuosea*, *D. dichotoma* and *C. officinalis* was carried out and led to the identification of several bioactive phenolic and flavonoids. Gallic acid **(26)** was reported as strong anti-oxidant activity as it protects skin from the harmful effects of UV radiation, anti-cancer, anti-HIV, anti-ulcer, anti-inflammatory, anti-microbial, and anti-fungal [21–25]. Catechol **(27)** is a key constituent in mussel adhesive proteins and crosslinking formation, antifouling coatings, drug carriers, antimicrobial polymers [21, 22, 31, 23–30]and is known as a strong antimicrobial agent [32]. *ρ*-Hydroxybenzoic **(28)** is a strong antimicrobial agent [33]. Of the identified compounds which well-known as strong antimicrobial agent, the flavonoids based on the flavan structure (C6-C3-C6); catechin **(29)** [34], rutin **(37)** [35]; hesperidin **(38)** [36]; resveratrol **(39)** [37] and quercetin **(42)** [37]. Syringic acid **(33)** was also reported as an antimicrobial agent and has a fungi-toxic effect [38]. Ferulic **(35)** has anti-inflammatory, antioxidant, antimicrobial, anticancer, and antidiabetic activities [39]. Also, rosemarinic acid **(41)** which found to be dynamic antimicrobial agent [40]. The antimicrobial activity of (M1-M6) extracts was evaluated and *E. intestinalis* crude extract (M1) was the most effective antimicrobial agent. Based on the above results and literature survey, the potent antimicrobial activities of all crude extracts (M1-M6) could be concluded

from the synergism effect between the bioactive blend agents identified in each extract leading to the development of antimicrobial activities.

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Not applicable

6. Conflict of interest
The authors have declared no conflict of interest.

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