



## Original Article

## Asian Pacific Journal of Tropical Biomedicine

journal homepage: [www.apjtb.org](http://www.apjtb.org)

doi: 10.4103/2221-1691.321129

Impact Factor: 1.55

## *In vitro* antimicrobial and synergistic effect of essential oil from the red macroalgae *Centroceras clavulatum* (C. Agardh) Montagne with conventional antibiotics

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### ABSTRACT

**Objective:** To study the chemical profile, antimicrobial properties, and synergistic effect with known antibiotics of essential oil extracted from the marine red macroalgae *Centroceras clavulatum* (C. Agardh) Montagne, collected in Morocco.

**Methods:** The chemical composition of the oil was analyzed by gas chromatography-mass spectrometry. The oil was evaluated for antibacterial (*Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*), and antifungal activity (*Candida albicans*, *Candida glabrata*, *Candida krusei*, and *Candida parapsilosis*), by the disc diffusion method. The minimum inhibitory and minimum microbicidal concentrations of the oil were determined, as well as the synergistic effects of its application combined with the antibiotics ciprofloxacin and fluconazole, by the checkerboard method.

**Results:** Thirty molecules were identified in the essential oil, comprising 96.27% of the total oil composition. Monoterpenes such as carvacrol (36.06%) were the most abundant compounds, followed by caryophyllene (14.67%), endo-borneol (9.04%), pyroterebic acid (3.23%) and caryophyllene oxide (3.13%). The oil exhibited a moderate antimicrobial activity with inhibition zone diameters ranging from 9.0 to 15.0 mm. The minimum inhibitory concentration values varied between 0.9 and 14.7 mg/mL, and *Bacillus subtilis* and *Escherichia coli* were the more sensitive bacteria with 0.9 and 1.9 mg/mL, respectively. The minimum

microbicidal concentration values ranged from 0.4 to 14.7 mg/mL. A significant synergic action was observed when the oil was applied in combination with ciprofloxacin and fluconazole, with fractional inhibitory concentration index values ranging from 0.31 to 0.50. Synergy was found in 80% of the combinations and a 2 to 16-fold reduction of antibiotics MIC was observed.

**Conclusions:** Our findings suggest that the essential oil of *Centroceras clavulatum* should be further appraised for its potential use in the management of multi-drug resistant microorganisms, with the purpose to restore the activity of standard antimicrobial drugs.

**KEYWORDS:** Macroalgae; *Centroceras clavulatum* (C. Agardh); Antimicrobial potential; Microbial drug resistance; Marine natural products; Synergism with antibiotics

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**How to cite this article:** Nafis A, El Khalloufi F, Aknaf A, Oudra B, Marraiki N, Al-Rashed S, et al. *In vitro* antimicrobial and synergistic effect of essential oil from the red macroalgae *Centroceras clavulatum* (C. Agardh) Montagne with conventional antibiotics. Asian Pac J Trop Biomed 2021; 11(9): 414-420.

**Article history:** Received 22 October 2020; Revision 17 November 2020; Accepted 1 June 2021; Available online 22 July 2021

## 1. Introduction

The increasing microbial drug resistance occurring worldwide is a major worry of public health[1]. Approximately 700 000 persons die each year due to resistant microorganisms, while about 10 million lives will be threatened per year as a result of the increase in pharmaco-resistant infections[2]. Therefore, the development of new strategies to overcome microbial drug resistance becomes mandatory, such as the identification of new antimicrobial agents from natural sources, increasing the efficacy of existing antibiotics, or the combination of both[1,3]. In particular, the assessment of possible synergy between natural products and existing antibiotics is considered an emerging area in phytomedicine's research[4].

Marine organisms are an exceptional source of secondary metabolites with unique chemical features[5]. Until now, more than 28 000 compounds were identified from different marine species, with potential applications in the energy, food, cosmetic, pharmaceutical, and agricultural areas[6]. Among marine organisms, seaweeds, *i.e.*, marine macroalgae, have a long history of traditional uses either for direct human consumption or for medicinal purposes[7,8]. These uses are due to their richness in a wide variety of bioactive compounds, which include carrageenans, alkaloids, polysaccharides, and quinones[6,9], displaying important biological properties, such as antioxidants, anticancer, antimicrobial, and antiviral[10–12].

The *Centroceras* genus contains 17 species distributed worldwide[13], but only one, *Centroceras clavulatum* (*C. clavulatum*) (C. Agardh) Montagne (Ceramiaceae, Rhodophyta), is found in Morocco[14]. *C. clavulatum* is a red macroalga and contains several bioactive molecules, such as (-)-loliolide, neophytadiene, phytol, phenolics, and phycobiliproteins[13,15]. This species shows relevant bioactivities, such as antioxidant, anti-trypanosomal, and antifungal[13,16]. In addition, Murugan *et al.*[16] reported that *C. clavulatum*-synthesized silver nanoparticles had significant activity against *Aedes aegypti*, the primary vector of dengue, with residual toxicity towards mammalian cells. Organic extracts of *C. clavulatum* also had significant inhibition against *Proteus mirabilis*[17] and *Staphylococcus aureus* (*S. aureus*)[18]. This work aimed to further explore an essential oil (EO) of *C. clavulatum* as a potential source of antimicrobial agents. For that purpose, EO was extracted in a Clevenger-type apparatus, and its chemical profile was established by gas chromatography coupled to mass spectroscopy (GC/MS). The EO was then evaluated for *in vitro* anti-bacterial and anti-fungal properties, and for synergistic effects with conventional antibiotics, namely ciprofloxacin and fluconazole.

## 2. Materials and methods

### 2.1. Algal sample collection

Samples of *C. clavulatum* (4 kg) were collected in the Marchica

Mediterranean lagoon (35.156468° N; -2.90434200° W), in June of 2019. The alga was identified by one of the authors (Asmae AKNAF) and a voucher specimen (CECL-101) was deposited at the Laboratory of Microbial Biotechnologies, Agrosociences and Environment, Faculty of Sciences Semlalia, Cadi Ayyad University, Marrakech Morocco. The collected material was air-dried for one week at room temperature (circa 25 °C), and reduced to powder.

### 2.2. Extraction and chemical composition analysis of the EO

The dried biomass was subjected to steam-distillation for 3 h until total recovery of EO was achieved, using a Clevenger-type apparatus, and the obtained sample was stored at 4 °C in darkness until use. The obtained EO yield in relation to the dry plant material was 0.6% (w/v). The quantitative and qualitative analyses of the chemical components of the EO were performed by GC/MS, as described previously[19]. The volatile constituents were identified by comparing their retention indices and mass spectra with reference libraries[20].

### 2.3. Assessment of the antimicrobial activity

#### 2.3.1. Tested microorganisms

The following pathogenic bacterial species were used to evaluate the antibacterial potential of the EO: *Pseudomonas aeruginosa* (*P. aeruginosa*) (DSM 50090), *Escherichia coli* (*E. coli*) (ATCC 8739), *Bacillus subtilis* (*B. subtilis*) (ATCC 9524), *Micrococcus luteus* (ATCC 10240), *S. aureus* (CCMM B3) and *Klebsiella pneumoniae* (clinical isolate). The antifungal activity was tested against four pathogenic *Candida* strains, namely *Candida albicans* CCMM-L4, *Candida glabrata* CCMM-L7, *Candida krusei* CCMM-L10, and *Candida parapsilosis* CCMM-L18[21].

#### 2.3.2. Disc diffusion method

The disk diffusion method was used to test the antimicrobial activities of the EO as described previously by the National Committee for Clinical Laboratory Standards[22]. Briefly, Whatman sterile disks (6 mm) were soaked with 10 µL of the EO (at the concentration of 981 mg/mL) and placed on the surface of the inoculated agar media using a cell suspension of bacteria (at 10<sup>8</sup> CFU/mL) and yeasts (at 10<sup>6</sup> CFU/mL). The Petri dishes were incubated for 2 h at 4 °C to promote the diffusion, and then further kept at 28 °C for 48 h and 37 °C for 24 h for yeasts and bacteria, respectively. The antimicrobial activity was determined by measuring the diameters of the clear inhibition zone. Discs of ciprofloxacin (5 µg) and fluconazole (10 µg) were used as positive standards for antibacterial and antifungal activity, respectively.

### 2.3.3. Determination of minimum inhibitory concentration (MIC), minimum microbicidal concentration (MMC), and synergistic effects

The two-fold serial dilution method in 96-well microplates, known as the microdilution assay, was used to evaluate the MIC of the algae EO[23]. In brief, 100 µL of each dilution of the sample (from 40 mg/mL to 0.0195 mg/mL) were added to the same volume of an overnight microbial culture diluted at a ratio of 1/50. The plates were then incubated at the optimal conditions for each microorganism. The MMC was determined by spreading an inoculum, taken from each without visible growth microwell, on the agar media.

The synergistic effect of the EO, at a sub-inhibitory concentration (MIC/4), combined with the reference antibiotics (ciprofloxacin and fluconazole) was evaluated by the checkerboard method[24]. Aliquots (50 µL) of the EO were mixed with 50 µL of each antibiotic dilution and added to 100 µL of cell suspension. Then, the 96-microwell assay plates were incubated at the optimal temperature of each microorganism. The fractional inhibitory concentration (FIC) was calculated according to the following formula:

FIC of oil = MIC of EO in combination with antibiotic/MIC of EO alone; FIC of antibiotic = MIC of antibiotic in combination with EO/MIC of antibiotic alone; FIC index = FIC of EO + FIC of antibiotic

This assay allows for the determination of the fractional inhibitory concentration index (FICI) values which are then interpreted according to the following odds[25]: No interaction (FICI between 0.5 and 4), synergism (FICI ≤ 0.5), and antagonism (FICI ≥ 4).

The antibiotic MIC gains were calculated according to the following formula: MIC gain = MIC of antibiotic alone/MIC of antibiotic combined with EO.

### 2.4. Statistical analyses

Statistical analysis was made using one-way analysis of variance (ANOVA) with SNK test (Student, Newman, Keuls) of SPSS version 21.0. Data of all analyses, in triplicate, expressed as mean values ± standard deviations (SD) were calculated. Analysis of variance was performed on the basis of mean values to determine the significant difference among results at  $P < 0.05$ .

## 3. Results

### 3.1. Chemical composition of the EO

The steam distillation of the dried thallus of the red macroalgae *C. clavulatum* afforded a yellow liquid, EO, with an extraction yield of 0.6%. The chemical composition of the EO was established by GC/MS, and results are summarized in Table 1 and Figure 1. Thirty compounds were identified, representing 96.27% of the total oil

composition. Monoterpenes were the predominant compounds (56.67%), followed by sesquiterpenes (24.03%). Other compounds accounted for 15.57% of the total oil. The major constituents were carvacrol (36.06%), caryophyllene (14.67%), endo-borneol (9.04%) pyroterebic acid (3.23%) and caryophyllene oxide (3.13%).

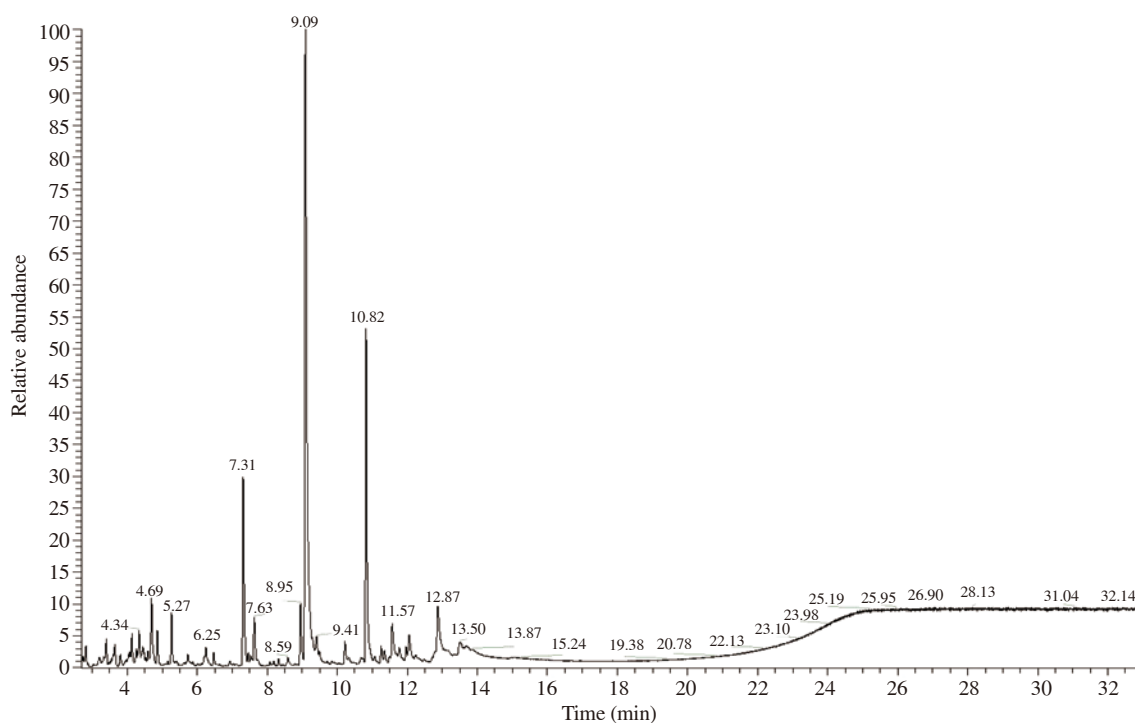
### 3.2. Antimicrobial activity

The antimicrobial activity of the EO was evaluated against pathogenic bacteria and clinical yeasts and obtained results are summarized in Table 2. The oil had a moderate antimicrobial activity with inhibition zone diameters ranging from 9.0 to 15.0 mm, and the MIC values ranged from 0.9 to 14.7 mg/mL. The bacteria *B. subtilis* and *E. coli* were more sensitive, with MIC values of 0.9 and 1.9 mg/mL, respectively. *S. aureus*, *Klebsiella pneumoniae*, and *P. aeruginosa* had the same sensitivity as *Candida albicans* and *Candida krusei*, with MIC values of 14.7 mg/mL. More importantly, the MIC values were often equal to the MMC values showing the microbicidal action of the tested oil, except for *B. subtilis*, *E. coli*, *Candida glabrata*, and *Candida krusei*.

**Table 1.** Volatile components identified in the essential oil from *Centroceras clavulatum* (C. Agardh) Montagne by gas chromatography coupled to mass spectrometry.

RI*	Abundance %	Compounds
854	0.89	2-Hexenal, (E)-
883	1.91	o-Xylene
897	1.37	Nonane
899	0.57	cis-1-Methyl-4-Ethylcyclohexane
939	1.53	α-Pinene
942	0.39	3,5-Dimethyl-3-heptene
953	1.49	Camphene
978	1.10	m-Methylethylbenzene
995	3.23	<b>Pyroterebic acid</b>
999	1.44	Decane
1011	2.01	o-Cymene
1062	0.78	ζ-Terpinene
1098	1.09	Linalool
1112	0.57	2,6-Dimethylcyclohexanol
1165	9.04	<b>endo-Borneol</b>
1189	2.39	α-Terpineol
1285	2.74	Bornyl acetate
1298	36.06	<b>Carvacrol</b>
1300	0.84	Ascaridole epoxide
1376	1.19	α-Copaene
1399	0.36	Tetradecane
1440	14.67	<b>Caryophyllene</b>
1444	0.76	Humulene
1464	0.68	Aromandendrene
1469	2.44	10-Methyltricyclo[4.3.1.1(2,5)] undecan-10-ol
1473	0.48	γ-Gurjunene
1477	0.58	ζ-Muuroolene
1538	1.64	α-Cadinene
1581	3.13	<b>Caryophyllene oxide</b>
1608	0.90	α-Muurolol

\*RI: Retention index measured relative to n-alkanes (C-9 to C-24) on a non-polar TG-5MS column. Entries in boldface are major components.



**Figure 1.** Gas chromatography/mass spectrometry chromatogram of *Centroceras clavulatum* (C. Agardh) Montagne essential oil.

**Table 2.** Inhibition zone diameters, minimum inhibitory concentration, and minimum microbicidal concentration of essential oil extracted from the algae *Centroceras clavulatum* (C. Agardh) Montagne against pathogenic bacteria and yeasts using the disc diffusion and micro-well dilution assays.

Microorganisms	EO			Ciprofloxacin		Fluconazole	
	IZ	MIC	MMC	IZ	MIC	IZ	MIC
<b>Gram-positive bacteria</b>							
<i>Staphylococcus aureus</i>	13.0 ± 0.6 <sup>a</sup>	14.7 ± 0.0 <sup>a</sup>	14.7 ± 0.0 <sup>a</sup>	26.0 ± 0.2 <sup>c</sup>	0.01 ± 0.00 <sup>a</sup>	-	-
<i>Micrococcus luteus</i>	12.0 ± 0.6 <sup>a</sup>	7.4 ± 0.0 <sup>b</sup>	7.4 ± 0.0 <sup>b</sup>	27.0 ± 0.1 <sup>b</sup>	0.03 ± 0.00 <sup>a</sup>	-	-
<i>Bacillus subtilis</i>	13.0 ± 0.0 <sup>a</sup>	0.9 ± 0.0 <sup>c</sup>	0.4 ± 0.0 <sup>c</sup>	35.0 ± 0.1 <sup>a</sup>	0.01 ± 0.00 <sup>a</sup>	-	-
<b>Gram-negative bacteria</b>							
<i>Escherichia coli</i>	9.0 ± 0.1 <sup>b</sup>	1.9 ± 0.0 <sup>b</sup>	0.9 ± 0.0 <sup>b</sup>	12.0 ± 0.1 <sup>a</sup>	0.06 ± 0.00 <sup>a</sup>	-	-
<i>Klebsiella pneumoniae</i>	9.0 ± 0.0 <sup>b</sup>	14.7 ± 0.0 <sup>a</sup>	14.7 ± 0.0 <sup>a</sup>	9.0 ± 0.2 <sup>b</sup>	1.00 ± 0.00 <sup>a</sup>	-	-
<i>Pseudomonas aeruginosa</i>	10.0 ± 0.2 <sup>a</sup>	14.7 ± 0.0 <sup>a</sup>	14.7 ± 0.0 <sup>a</sup>	8.0 ± 0.1 <sup>c</sup>	0.25 ± 0.00 <sup>a</sup>	-	-
<b>Yeasts</b>							
<i>Candida albicans</i>	15.0 ± 0.3 <sup>a</sup>	14.7 ± 0.0 <sup>a</sup>	14.7 ± 0.0 <sup>a</sup>	-	-	20.0 ± 0.2 <sup>c</sup>	1.0 ± 0.0 <sup>a</sup>
<i>Candida glabrata</i>	14.0 ± 0.2 <sup>b</sup>	7.4 ± 0.0 <sup>b</sup>	3.7 ± 0.0 <sup>c</sup>	-	-	13.0 ± 0.1 <sup>d</sup>	1.0 ± 0.0 <sup>a</sup>
<i>Candida krusei</i>	14.0 ± 0.1 <sup>b</sup>	14.7 ± 0.0 <sup>a</sup>	7.4 ± 0.0 <sup>b</sup>	-	-	24.0 ± 0.2 <sup>b</sup>	1.0 ± 0.0 <sup>a</sup>
<i>Candida parapsilosis</i>	15.0 ± 0.1 <sup>a</sup>	7.4 ± 0.0 <sup>b</sup>	7.4 ± 0.0 <sup>b</sup>	-	-	28.2 ± 0.2 <sup>a</sup>	1.0 ± 0.0 <sup>a</sup>

EO: essential oil; IZ: inhibition zones (mm); MIC: minimum inhibitory concentration (mg/mL); MMC: minimum microbicidal concentration (mg/mL); The data are expressed as mean ± SD of three replicates, different letters in column indicate significant difference ( $P < 0.05$ ).

**Table 3.** Synergistic interaction of *Centroceras clavulatum* (C. Agardh) Montagne essential oil and ciprofloxacin against resistant bacteria.

Samples	<i>Micrococcus luteus</i>			<i>Staphylococcus aureus</i>			<i>Bacillus subtilis</i>			<i>Escherichia coli</i>			<i>Pseudomonas aeruginosa</i>			<i>Klebsiella pneumoniae</i>		
	FIC	FICI	Gain	FIC	FICI	Gain	FIC	FICI	Gain	FIC	FICI	Gain	FIC	FICI	Gain	FIC	FICI	Gain
EO	0.25	-	-	0.25	-	-	0.25	-	-	0.25	-	-	0.25	-	-	0.25	-	-
Ciprofloxacin	0.50	0.75 <sup>b</sup>	2	0.12	0.37 <sup>a</sup>	8	0.06	0.31 <sup>a</sup>	16	0.12	0.37 <sup>a</sup>	8	0.50	0.75 <sup>b</sup>	2	0.13	0.38 <sup>a</sup>	8

FIC: fractional inhibitory concentration; FICI: fractional inhibitor concentration index; <sup>a</sup>Synergism; <sup>b</sup>No interaction.

**Table 4.** Synergistic interaction of *Centroceras clavulatum* (C. Agardh) Montagne essential oil and fluconazole against clinical pathogenic yeasts.

Samples	<i>Candida albicans</i>			<i>Candida glabrata</i>			<i>Candida krusei</i>			<i>Candida parapsilosis</i>		
	FIC	FICI	Gain	FIC	FICI	Gain	FIC	FICI	Gain	FIC	FICI	Gain
EO	0.25	-	-	0.25	-	-	0.25	-	-	0.25	-	-
Fluconazole	0.25	0.50 <sup>a</sup>	4	0.25	0.50 <sup>a</sup>	4	0.25	0.50 <sup>a</sup>	4	0.25	0.50 <sup>a</sup>	4

FIC: fractional inhibitory concentration; FICI: fractional inhibitor concentration index; <sup>a</sup>Synergism.

### 3.3. Synergistic effects with antimicrobial drugs

The results obtained on the synergistic effects of *C. clavulatum* EO at a sub-inhibitory concentration (MIC/4) in combination with two standard antimicrobial drugs, ciprofloxacin, and fluconazole, are presented in Tables 3 and 4. Eighty percent of the tested combinations showed synergistic interaction regarding all tested strains with a considerable reduction of the antibiotic's MICs, with FICI values varying between 0.31 and 0.50. No interaction was observed for the combination of ciprofloxacin and EO against *Micrococcus luteus* and *P. aeruginosa*, with a FICI value of 0.75. The EO reduced the MICs of the antibiotics by 2 to 16-fold, as gains. The highest synergism was observed in *B. subtilis*, with a 16-fold gain. The EO significantly reduced the sensitivity of *S. aureus*, *E. coli* and *Klebsiella pneumoniae* to ciprofloxacin, with an 8-fold gain. Concerning the yeast strains, a 4-fold gain was obtained for all tested *Candida* species.

## 4. Discussion

Few investigations have focused on EO from macroalgae. The obtained yield (0.6%) from the EO in this study is higher than that obtained from the red macroalgae *Laurencia dendroidea* J. Agardh (0.066%)[26]. Our results greatly differ from those previously obtained with other macroalgae species. For example, the main terpene components of the EO extracted from the cultivated red macroalgae *Ochtodes secundiramea* (Montagne) M. Howe were myrcene, 10Z-bromomyrcene, 10E-bromo-3-chloromyrcene, apakaoctodene B, and acyclic 1,3-dibromoadamantane[27]. Moreover, Gressler *et al.*[26] identified two major tricyclic sesquiterpenes (SPF-1 and SPF-2) in EO from the red seaweed *Laurencia dendroidea* collected on the Brazilian coast. In another work, Jerković *et al.*[28] reported the volatile oil composition of the brown macroalgae *Taonia atomaria* (Woodward) J. Agardh and *Padina pavonica* (Linnaeus) Lamouroux, sampled from the central Adriatic Sea. The major compounds identified in the oil from *Taonia atomaria* were germacrene D, epi-bicyclosesquiphellandrene,  $\beta$ -cubebene, and gleenol, while higher aliphatic alcohols, such as trans-phytol and pachydictol A, were the chief molecules in *Padina pavonica*[28]. In another study, Patra *et al.*[29,30] identified high levels of tetradecanoic and hexadecanoic acids in the oil extracted from the edible seaweed species, namely *Laminaria japonica* L. (kombu, brown algae) and *Undaria pinnatifida* (Harvey) Suringar (wakame, green algae).

Macroalgae have a high potential to provide biomedical compounds, for example, antimicrobials to tackle antimicrobial resistance issues. The detected antimicrobial activity in the *C. clavulatum* EO may be

due to the presence of carvacrol, which was identified as the major oil component. Essential oils from plants containing high levels of carvacrol presented high antimicrobial activity towards numerous pathogens[31]. In addition, several studies showed that carvacrol has antimicrobial properties and stated that it is extensively used to control pathogens by causing damage to the microbial membrane, particularly the reduction of energy-dependent cellular processes due to the subsequent reduction in adenosine triphosphate synthesis and dissolution of the proton motive force[32,33]. Furthermore, the antimicrobial activity might be also related to the presence of other components in the EO, such as caryophyllene and caryophyllene oxide that also exhibit antimicrobial properties[34,35].

Several studies reported the inhibitory activities of seaweed extracts against bacteria and yeasts, but few focused on EO[27,29,30,36]. The EO from *Laminaria japonica* was tested against three foodborne pathogens and displayed potent antibacterial activity, especially against *S. aureus* ATCC49444[29]. In another study, the EO from *Undaria pinnatifida* had an interesting activity against *Salmonella typhimurium* ATCC 43174 with a MIC value of 25 mg/mL[30]. Furthermore, Patra and Baek[37] demonstrated the effectiveness of the EO extracted by microwave hydro-distillation method from *Enteromorpha linza* (Linnaeus) J. Agardh and *Porphyra tenera* Kjellman against *Listeria monocytogenes*, with a MIC value of 12.5 mg/mL for both species.

However, several recent works reported the interest in mixing carvacrol, which was the major volatile identified in the *C. clavulatum* EO, with several antibiotics[33]. For example, the combination of carvacrol with erythromycin was assessed by the checkerboard assay against several bacteria, and a 2 to 2048-fold reduction of the MIC value of erythromycin was observed[31]. Carvacrol could also significantly reduce the MIC values of tetracycline, ampicillin, novobiocin, penicillin, and erythromycin by 1/4 to 1/8[38]. However, many investigations have shown the synergistic effect of the combination of macroalgae extracts with antibiotics. The combination of a dichloromethane extract from *Bifurcaria bifurcata* R.Ross with rifampicin, ampicillin, gentamicin, and tetracycline, resulted in a significant decrease in the MIC values of these antibiotics against some bacterial strains[39]. Additionally, Eom *et al.*[40] evaluated the interaction between the hexane fraction of a methanol extract from *Ecklonia cava* Kjellman and two commercial antimicrobials, namely oxytetracycline and erythromycin, and observed that the antibiotic MICs were markedly reduced by 64-fold against the resistant strain *Streptococcus parauberis*. He *et al.*[41] also evaluated the synergistic effect of combining azithromycin and marine alginate-derived oligosaccharide against the resistant *P. aeruginosa* and a significant reduction factor of 2.5 was observed. A significant average reduction with a factor of four was obtained when fucoidan, a sulphated polysaccharide extracted from brown

seaweeds, was combined with ampicillin and gentamicin against some oral pathogenic bacteria[42].

In conclusion, 30 components were found in the EO, with a predominance of carvacrol and caryophyllene. The oil displayed moderate antimicrobial activity towards tested bacteria and pathogenic yeasts, but a significant synergistic interaction was observed regarding most of tested strains, with a considerable reduction of the MICs of the used antibiotics. The antimicrobial properties of the EO may be likely due to the presence of carvacrol and caryophyllene, as their major compounds. Our findings suggest that the antimicrobial properties of the EO extracted from *C. clavulatum* should be further explored and clarified for its possible use to restore the activity of used drugs and to aid to the management of multi-drug resistant microorganisms.

### Conflict of interest statement

The authors declare no conflict of interest.

### Acknowledgments

The authors extend their appreciation to the Researchers Supporting Project number (RSP-2021/56), King Saud University, Riyadh, Saudi Arabia, and the Foundation for Science and Technology (FCT) and the Portuguese National Budget for the UIDB/04326/2019 project, and the FCT Scientific Employment Stimulus (CEECIND/00425/2017).

### Funding

This study is supported by the Researchers Supporting Project number (RSP-2021/56), King Saud University, Riyadh, Saudi Arabia; the Foundation for Science and Technology (FCT) and the Portuguese National Budget for the UIDB/04326/2019 project; and the FCT Scientific Employment Stimulus (CEECIND/00425/2017).

### Authors' contributions

For the current article, individual author's contributions are as follows: conceptualization, AN and FEK; sampling and taxonomy, AA and BO; methodology, AN; statistic and validation, SAR and AME; review of the article draft, NM; investigation, LH and LC; writing—original draft preparation, AN; writing—review and editing, LC; resources and funding acquisition, AME and LC.

### References

- [1] Perron GG, Inglis RF, Pennings PS, Cobey S. Fighting microbial drug resistance: A primer on the role of evolutionary biology in public health. *Evol Appl* 2015; **8**(3): 211-222.
- [2] Saskia N, Ellen N, Bernd R. Antimicrobial resistance - The role of public health organizations in addressing public health problems in Europe. [Online] Available from: <https://www.ncbi.nlm.nih.gov/books/NBK536190/> [Accessed 2021 April 29].
- [3] Veras HNH, Rodrigues FFG, Colares A, Menezes IRA, Coutinho HDM, Botelho MA, et al. Synergistic antibiotic activity of volatile compounds from the essential oil of *Lippia sidoides* and thymol. *Fitoterapia* 2012; **83**(3): 508-512.
- [4] Wagner H, Ulrich-Merzenich G. Synergy research: Approaching a new generation of phytopharmaceuticals. *Phytomedicine* 2009; **16**(2-3): 97-110.
- [5] Kosanic M, Stanojkovic T, Rankovic B. Biological activities of two macroalgae from Adriatic coast of Montenegro. *Saudi J Biol Sci* 2015; **22**(4): 390-397.
- [6] Lauritano C, Andersen JH, Hansen E, Albrigtsen M, Romano G, Ianora A. Bioactivity screening of microalgae for antioxidant, anti-inflammatory, antibacterial activities. *Front Mar Sci* 2016; **3**: 68.
- [7] Cardoso S, Pereira O, Seca A, Pinto D, Silva A. Seaweeds as preventive agents for cardiovascular diseases: From nutrients to functional foods. *Mar Drugs* 2015; **13**(11): 6838-6865.
- [8] Vazirian M, Hamidian K, Noorollah M, Manayi A, Samadi N. Enhancement of antibiotic activity and reversal of resistance in clinically isolated methicillin-resistant *Staphylococcus aureus* by *Trachyspermum ammi* essential oil. *Res J Pharmacogn* 2019; **6**(1): 1-10.
- [9] Al-Saif SSA, Abdel-Raouf N, El-Wazanani HA, Aref IA. Antibacterial substances from marine algae isolated from Jeddah coast of Red sea, Saudi Arabia. *Saudi J Biol Sci* 2014; **21**(1): 57-64.
- [10] Mohamed S, Hashim SN, Rahman A. Seaweeds : A sustainable functional food for complementary and alternative therapy. *Trends Food Sci Technol* 2012; **23**(2): 83-96.
- [11] Silva M, Vieira LMM, Almeida AP, Silva AMS, Seca AML, Barreto MC, et al. Oceanography and marine research chemical study and biological activity evaluation of two Azorean macroalgae: *Ulva rigida* and *Gelidium microdon*. *J Oceanogr Mar Res* 2013; **1**(1): 1-7.
- [12] Catarino MD, Silva AMS. Phytochemical constituents and biological activities of *Fucus* spp. *Mar Drugs* 2018; **16**: 1-34.
- [13] Rocha OP, Felício R De, Rodrigues AHB, Ambrósio DL, Cicarelli RMB, Albuquerque S De, et al. Chemical profile and biological potential of non-polar fractions from *Centroceras clavulatum* (C. Agardh) Montagne (Ceramiales, Rhodophyta). *Molecules* 2011; **16**(8): 7105-7114.
- [14] Hassoun M, Wynne MJ, Moussa H, Salhi G, Zbakh H, Riadi H, et al. An investigation of members of the tribe *Ceramieae* (Ceramiales, Rhodophyta) occurring on both the Mediterranean and Atlantic shores of Morocco. *ALGAE* 2018; **33**(3): 243-267.

- [15]Nair D, Krishna JG, Panikkar MVN, Nair BG, Pai JG, Nair SS. Identification, purification, biochemical and mass spectrometric characterization of novel phycobiliproteins from a marine red alga, *Centroceras clavulatum*. *Int J Biol Macromol* 2018; **114**: 679-691.
- [16]Murugan K, Aruna P, Panneerselvam C, Madhiyazhagan P, Paulpandi M, Subramaniam J, et al. Fighting arboviral diseases: Low toxicity on mammalian cells, dengue growth inhibition (*in vitro*), and mosquitocidal activity of *Centroceras clavulatum*-synthesized silver nanoparticles. *Parasitol Res* 2016; **115**(2): 651-662.
- [17]Villarreal-Gómez LJ, Soria-Mercado IE, Guerra-Rivas G, Ayala-Sánchez NE. Antibacterial and anticancer activity of seaweeds and bacteria associated with their surface. *Rev Biol Mar Oceanogr* 2010; **45**(2): 267-275.
- [18]Rhimou B, Hassane R, José M, Nathalie B. The antibacterial potential of the seaweeds (Rhodophyceae) of the Strait of Gibraltar and the Mediterranean coast of Morocco. *Afr J Biotechnol* 2010; **9**(38): 6365-6372.
- [19]Nafis A, Kasrati A, Jamali CA, Mezrioui N, Setzer W, Abbad A, et al. Antioxidant activity and evidence for synergism of *Cannabis sativa* (L.) essential oil with antimicrobial standards. *Ind Crops Prod* 2019; **137**: 396-400.
- [20]Adams RP. *Identification of essential oil components by gas chromatography/mass spectrometry*. 4th ed. Allured Pub Corp; 2007.
- [21]Nafis A, Oubaha B, Elhidar N, Ortlieb N, Kulik A, Niedermeyer T, et al. Novel production of two new nonpolyenic antifungal macrolide derivatives by *Streptomyces* Z26 isolated from Moroccan rhizospheric soil. *Online J Biol Sci* 2018; **18**(2): 176-185.
- [22]Clinical and Laboratory Standards Institute. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*. 4th ed. Approved standard. M7-A4. Wayne, PA, USA; 1997.
- [23]Clinical and Laboratory Standards Institute. *Method for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*. 10th ed. Approved standard. M07-A10. Wayne, PA, USA; 2015.
- [24]Fadli M, Bolla JM, Mezrioui NE, Pagès JM, Hassani L. First evidence of antibacterial and synergistic effects of *Thymus riatarum* essential oil with conventional antibiotics. *Ind Crops Prod* 2014; **61**: 370-376.
- [25]Odds FC. Synergy, antagonism, and what the checkerboard puts between them. *J Antimicrob Chemother* 2003; **52**(1): 1.
- [26]Gressler V, Stein ÉM, Dörr F, Fujii MT, Colepicolo P, Pinto E. Sesquiterpenes from the essential oil of *Laurencia dendroidea* (Ceramiales, Rhodophyta): Isolation, biological activities and distribution among seaweeds. *Rev Bras Farmacogn* 2011; **21**(2): 248-254.
- [27]Pérez-López P, Jeffries C, Agathos SN, Feijoo G, Rorrer G, Moreira MT. Environmental life cycle optimization of essential terpene oils produced by the macroalga *Ochtodes secundiramea*. *Sci Total Environ* 2016; **542**: 292-305.
- [28]Jerković I, Kranjac M, Marijanović Z, Roje M, Jokić S. Chemical diversity of headspace and volatile oil composition of two brown algae (*Taonia atomaria* and *Padina pavonica*) from the Adriatic Sea. *Molecules* 2019; **24**(3): 495.
- [29]Patra J, Das G, Baek KH. Chemical Composition and antioxidant and antibacterial activities of an essential oil extracted from an edible seaweed, *Laminaria japonica* L. *Molecules* 2015; **20**(7): 12093-12113.
- [30]Patra JK, Lee SW, Park JG, Baek KH. Antioxidant and antibacterial properties of essential oil extracted from an edible seaweed *Undaria pinnatifida*. *J Food Biochem* 2017; **41**(1): e12278.
- [31]Magi G, Marini E, Facinelli B. Antimicrobial activity of essential oils and carvacrol, and synergy of carvacrol and erythromycin, against clinical, erythromycin-resistant Group A *Streptococci*. *Front Microbiol* 2015; **6**: 165.
- [32]Hyldgaard M, Mygind T, Meyer RL. Essential oils in food preservation: Mode of action, synergies, and interactions with food matrix components. *Front Microbiol* 2012; **3**: 12.
- [33]Langeveld WT, Veldhuizen EJA, Burt SA. Synergy between essential oil components and antibiotics: A review. *Crit Rev Microbiol* 2014; **40**(1): 76-94.
- [34]Dahham S, Tabana Y, Iqbal M, Ahamed M, Ezzat M, Majid A, et al. The anticancer, antioxidant and antimicrobial properties of the sesquiterpene  $\beta$ -caryophyllene from the essential oil of *Aquilaria crassna*. *Molecules* 2015; **20**(7): 11808-11829.
- [35]Francomano F, Caruso A, Barbarossa A, Fazio A, La Torre C, Ceramella J, et al.  $\beta$ -caryophyllene: A sesquiterpene with countless biological properties. *Appl Sci* 2019; **9**(24): 5420.
- [36]Kolanjinathan K, Kolanjinathan K, Ganesh P, Saranraj P. Pharmacological importance of seaweeds: A review. *World J Fish Mar Sci* 2014; **6**(1): 1-15.
- [37]Patra JK, Baek KH. Anti-listerial activity of four seaweed essential oils against *Listeria monocytogenes*. *Jundishapur J Microbiol* 2016; **9**(7): e31784.
- [38]Palaniappan K, Holley RA. Use of natural antimicrobials to increase antibiotic susceptibility of drug resistant bacteria. *Int J Food Microbiol* 2010; **140**(2-3): 164-168.
- [39]Santos S, Trindade S, Oliveira C, Parreira P, Rosa D, Duarte M, et al. Lipophilic fraction of cultivated *Bifurcaria bifurcata* R. Ross: Detailed composition and *in vitro* prospection of current challenging bioactive properties. *Mar Drugs* 2017; **15**(11): 340.
- [40]Eom SH, Santos JA, Kim JH, Jung WK, Kim DH, Kim YM. *In vitro* antibacterial and synergistic activity of an *Ecklonia cava* extract against antibiotic-resistant *Streptococcus parauberis*. *Fish Aquat Sci* 2015; **18**(3): 241-247.
- [41]He X, Hwang H, Aker WG, Wang P, Lin Y, Jiang X, et al. Synergistic combination of marine oligosaccharides and azithromycin against *Pseudomonas aeruginosa*. *Microbiol Res* 2014; **169**(9-10): 759-767.
- [42]Lee KY, Jeong MR, Choi SM, Na SS, Cha JD. Synergistic effect of fucoidan with antibiotics against oral pathogenic bacteria. *Arch Oral Biol* 2013; **58**(5): 482-492.