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Characteristic Volatile Composition of Seven Seaweeds from the Yellow Sea of China

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Abstract: Plant volatile organic compounds (VOCs) represent a relatively wide class of secondary metabolites. The VOC profiles of seven seaweeds (*Grateloupia filicina*, *Polysiphonia senticulosa*, *Callithamnion corymbosum*, *Sargassum thunbergii*, *Dictyota dichotoma*, *Enteromorpha prolifera* and *Ulva lactuca*) from the Yellow Sea of China were investigated using multifiber headspace solid phase microextraction coupled with gas chromatography–mass spectrometry (HS-SPME/GC–MS), among them, the VOCs of three red algae Grateloupia filicina, Polysiphonia senticulosa, and Callithamnion corymbosum were first reported. Principal component analysis (PCA) was used to disclose characteristic categories and molecules of VOCs and network pharmacology was performed to predict potential biomedical utilization of candidate seaweeds. Aldehyde was found to be the most abundant VOC category in the present study and (*E*)-*β*-ionone was the only compound found to exist in all seven seaweeds. The chemical diversity of aldehydes in *E. prolifera* suggest its potential application in chemotaxonomy and hinted that divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber is more suitable for aldehyde extraction. VOCs in *D. dichotoma* were characterized as sesquiterpenes and diterpenes and the most relevant pharmacological pathway was the neuroactive ligand–receptor interaction pathway, which suggests that *D. dichotoma* may have certain preventive and therapeutic values in cancer, especially in lung cancer, in addition to neuropsychiatric diseases.

Keywords: volatile organic compounds; seaweeds; characteristic VOCs; chemotaxonomy; biomedical utilization; headspace solid phase microextraction

1. Introduction

Plant volatile organic compounds (VOCs) are produced by a range of physiological processes in many different plant tissues and typically occur as a complex mixture of lipophilic compounds with extremely diverse structures [1]. Their low molecular weight and high vapor pressure under ambient conditions allow them to freely exit through cellular membranes and reach the surrounding environment [2].

Plant VOCs have been reported in different geographical conditions ranging from Mediterranean environments [3] and tropical rainforests [4] to various extreme environments [5]. In aquatic ecosystems, algae are the main emitter of VOCs and the extraction of VOCs using headspace solid phase microextraction (HS-SPME) technology has been reported from brown algae [6], red algae [7] and green algae [8]. Algae release an abundance of VOCs to increase their tolerance to abiotic stresses, transfer stress information to homogeneous algae to induce defense, play allelopathic roles on heterogeneous algae and aquatic macrophytes for competing nutrients, or protect against predators [9]. In addition, the volatile contaminant in algae caught the interest of environmentalists [10]. Most of the studies on marine algae VOCs have focused on their allelopathy effects, whereas their

contributions in other areas have been seldom investigated. VOCs are released under biotic or abiotic stresses and have great significance for plant survival and reproduction. In many cases, evolution has resulted in some plant VOCs acting as metabolic safety valves, protective or defense compounds, or communication cues [1]. VOCs can be divided chemically as aldehydes, alcohols, terpenoids, ketones, halogenated compounds, sulfur compounds and hydrocarbons and exhibit multitudinous functions, such as in being feeding attractants [11], pheromones [12] involved in chemical defenses [13–15] and acting as antimicrobial agents [16].

In the present study, VOC profiles of seven seaweeds blooming in the littoral area of Qingdao (the Yellow Sea of China) were investigated, including the first report of VOCs for three red algae species—*Grateloupia filicina*, *Polysiphonia senticulosa* and *Callithamnion corymbosum*. Characteristic categories and molecules of VOCs are discussed from the point of view of their chemotaxonomic significance and bioactivities are discussed in order to further expand these seaweeds' potential utilities in pharmaceutical areas. Our study provides new insights for the chemotaxonomy of algae and for the biomedical application of *D. dichotoma*.

2. Results and Discussion

2.1. Headspace VOC Composition of Seaweeds

VOC compositions were investigated in seven algae using headspace solid phase microextraction coupled with gas chromatography–mass spectrometry (HS-SPME/GC–MS) and are reported according to their phylum. The number of VOC categories identified in each alga are given in Figure 1.
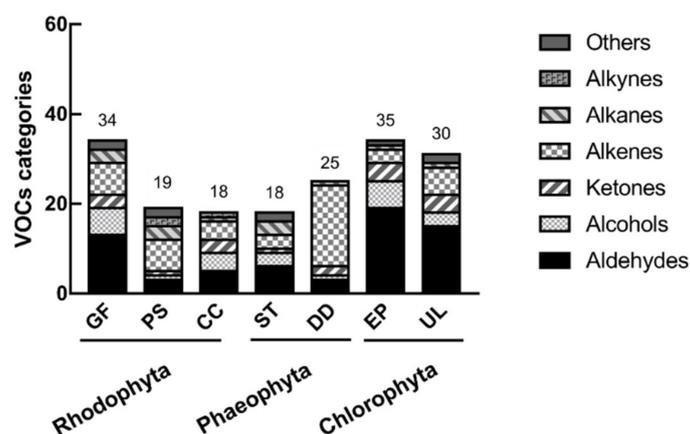

Figure 1. The number of volatile organic compound (VOC) categories identified in seaweeds GF—*Grateloupia filicina*, PS—*Polysiphonia senticulosa*, CC—*Callithamnion corymbosum*, ST—*Sargassum thunbergii*, DD—*Dictyota dichotoma*, EP—*Enteromorpha prolifera* and UL—*Ulva lactuca*.

2.1.1. Headspace VOC Composition of Rhodophyta

There were 34, 19 and 18 compounds identified from *Grateloupia filicina*, *Polysiphonia senticulosa* and *Callithamnion corymbosum*, respectively (Table 1), and, among them, four compounds were common to all three species, including two norisoprenoids (β-cyclocitral and (*E*)-β-ionone), one alkene (3,5,5-trimethyl-1-hexene) and one alkane (heptadecane). This is the first report on the headspace VOC composition for these three red algae species.

The VOCs of *G. filicina* exhibited the most chemical diversity, with the most abundant being pentadecanal (20.11% in polydimethylsiloxane (PDMS) fiber), tridecanal (19.20% in PDMS fiber), 1-hexen-3-ol (19.58% in divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber) and 1-octen-3-ol (18.72% in DVB/CAR/PDMS fiber). Wang Xiu-juan et al. [17] analyzed the semi-volatile organic compounds (SVOCs) of *G. filicina* using ethyl acetate as an extraction solvent and found that the main SVOCs were aldehydes, fatty acids and alcohols, with pentadecanal having the highest concentration. Our VOC

analysis results are consistent with the results for SVOCs in the literature, suggesting that aldehydes play an important role in the metabolism of *G. filicina*.

Table 1. VOC composition in Rhodophyta, determined by headspace solid phase microextraction coupled with gas chromatography–mass spectrometry (HS-SPME/GC–MS).

NO.	Compound	Molecular Formula	Compound Class	RI [a]	Area Percentage (%) GF [b]	PS [b]	CC [b]	Identification
1	(*E,E,E*)-2,4,6-Octatriene [T]	C_8H_{12}	Alkene	<800	1.03 *	0.00	0.00	MS
2	3,5,5-Trimethyl-1-hexene [T]	C_9H_{18}	Alkene	<800	8.24 *	1.74 *	2.74 *	MS
3	3,5-Dimethyl-1-hexene [T]	C_8H_{16}	Alkene	<800	0.36 *	0.00	0.00	MS
4	1-Hexen-3-ol [T]	$C_6H_{12}O$	Alcohol	<800	19.58 *	0.00	4.44 *	MS
5	2-Propyl-furan [T]	$C_7H_{10}O$	Furan derivative	<800	0.30 *	1.02 *	0.00	MS
6	3-Ethyl-1,4-hexadiene [T]	C_8H_{14}	Alkene	846	1.16 *	1.25 *	0.00	MS
7	2-Methylpropylidene-Cyclopentane	C_9H_{16}	Alkene	908	1.48 *	0.00	0.00	MS, RI
8	(*Z*)-2-Octen-1-ol [T]	$C_8H_{16}O$	Unsaturated alcohol	927	8.5 #	0.00	0.00	MS
9	Isocumene	C_9H_{12}	Others	931	0.00	1.67 *	0.00	MS, RI
10	3-Cyclohexene-1-ethanol	$C_8H_{14}O$	Alcohol	935	2.90 #	0.00	0.00	MS.RI
11	(*E*)-2-Heptenal	$C_7H_{12}O$	Aldehyde	960	4.78 *	1.51 *	0.00	MS, RI
12	1-Octen-3-ol [S]	$C_8H_{16}O$	Alcohol	980	18.72 *	0.00	2.15 *	MS, RI
13	3,7-Dimethyl-1-octene [T]	$C_{10}H_{20}$	Alkene	985	0.00	0.00	7.81 *	MS
14	2,7-Octadien-1-ol [T]	$C_8H_{14}O$	Unsaturated alcohol	986	8.39 *	0.00	0.00	MS
15	4-Methyl-2-propyl-1-pentanol [T]	$C_9H_{20}O$	Alcohol	990	0.00	0.00	3.59 #	MS
16	5-Methyl-1-undecene [T]	$C_{12}H_{24}$	Alkene	1025	0.00	6.91 *	12.70 *	MS
17	(*E*)-2-Undecen-1-ol [T]	$C_{11}H_{22}O$	Unsaturated alcohol	1039	0.25 *	1.28 *	0.00	MS
18	(*9Z*)-1,9-Dodecadiene [T]	$C_{12}H_{22}$	Alkene	1092	0.00	0.00	1.77 #	MS
19	Ectocarpene [T]	$C_{11}H_{16}$	Alkene	1105	0.00	21.83 *, 14.71 #	0.00	MS
20	1-Undecyne	$C_{11}H_{20}$	Alkyne	1108	0.00	2.46 *	8.86 *	MS, RI
21	Dictyopterene D′ [T]	$C_{11}H_{18}$	Alkene	1112	0.00	1.74 *, 0.95 #	0.00	MS
22	(*Z*)-6-Nonenal	$C_9H_{16}O$	Aldehyde	1113	3.25 *	0.00	0.00	MS, RI
23	Decanal	$C_{10}H_{20}O$	Aldehyde	1173	0.07 *	0.00	0.00	MS, RI
24	β-Cyclocitral	$C_{10}H_{16}O$	C_{10}-Norisoprenoid	1192	0.79 *, 0.69 #	1.55 *	0.95 *	MS, RI
25	β-Cyclohomocitral	$C_{11}H_{18}O$	Aldehyde	1236	0.07 *	0.00	0.00	MS, RI
26	2,4-Decadienal	$C_{10}H_{16}O$	Unsaturated aldehyde	1275	0.33 *	0.00	0.00	MS, RI
27	Undecanal	$C_{11}H_{22}O$	Aldehyde	1290	0.47 *, 0.52 #	0.00	0.00	MS, RI
28	(*E,E*)-2,4-Decadienal	$C_{10}H_{16}O$	Unsaturated aldehyde	1298	0.98 *, 0.66 #	0.69 *	0.00	MS, RI
29	Dodecanal	$C_{12}H_{24}O$	Aldehyde	1400	0.37 *, 0.95 #	0.00	0.00	MS, RI
30	α-Ionone	$C_{13}H_{20}O$	C_{13}-Norisoprenoid	1420	1.50 *, 2.01 #	0.00	1.16 *, 1.60 #	MS, RI
31	Dihydropseudoionone	$C_{13}H_{22}O$	C_{13}-Norisoprenoid	1447	0.15 *, 0.40 #	0.00	0.00	MS, RI
32	(*E*)-β-Ionone [S]	$C_{13}H_{20}O$	C_{13}-Norisoprenoid	1481	2.65 *, 5.68 #	1.37 *	2.39 *, 4.01 #	MS, RI
33	1-Pentadecene	$C_{15}H_{30}$	Alkene	1484	3.84 *, 2.33 #	1.33 #	0.00	MS, RI
34	Pentadecane [S]	$C_{15}H_{32}$	Alkane	1492	0.57 *, 0.88 #	1.39 *, 11.19 #	0.00	MS.RI
35	Tridecanal [S]	$C_{13}H_{26}O$	Aldehyde	1503	5.55 *, 19.20 #	0.00	2.28 *, 5.60 #	MS, RI
36	Cyclopentadecane [T]	$C_{15}H_{30}$	Alkane	1508	0.31 *	1.16 #	0.00	MS
37	Dihydroactinidiolide	$C_{11}H_{16}O_2$	C_{11}-Norisoprenoid	1525	0.06 #	0.00	0.00	MS, RI
38	Tetradecanal [S]	$C_{14}H_{28}O$	Aldehyde	1608	1.75 #	0.00	1.16 #	MS, RI
39	8-Heptadecene	$C_{17}H_{34}$	Alkene	1681	0.10 *	2.85 #	0.00	MS, RI
40	Z-11-Pentadecenal [T]	$C_{15}H_{28}O$	Aldehyde	1689	0.26 *, 1.78 #	0.00	1.09 #	MS
41	Heptadecane [S]	$C_{17}H_{36}$	Alkane	1693	1.73 *, 5.76 #	3.19 *, 15.93 #	2.04 *, 3.15 #	MS, RI
42	Pentadecanal	$C_{15}H_{30}O$	Aldehyde	1711	3.23 *, 20.11 #	0.00	11.64 *, 35.43 #	MS, RI
43	Perhydrofarnesyl acetone	$C_{18}H_{36}O$	Ketone	1840	0.00	0.00	0.91 *, 3.14 #	MS, RI
44	1,11-Dodecadiyne [T]	$C_{12}H_{18}$	Alkyne	1845	0.00	0.81 #	0.00	MS
45	(*Z,Z*)-6,9-Pentadecadien-1-ol [T]	$C_{15}H_{28}O$	Unsaturated alcohol	1889	0.00	0.00	0.46 *, 1.92 #	MS
	Total Identified (%)				90.59 *, 74.10 #	49.61 *, 48.92 #	60.53 *,62.46 #	

[T] tentatively identified. [S] identified by mass spectra (MS) and retention index (RI) compared with authentic standard. [a] RI: retention index relative to C7-C30 alkanes. [b] GF—*Grateloupia filicina*; PS—*Polysiphonia senticulosa*; CC—*Callithamnion corymbosum*. * data from fiber DVB/CAR/PDMS. # data from fiber PDMS.

Both *P. senticulosa* and *C. corymbosum* are from the same taxonomical order, Ceramiales Oltmanns and were found to contain two common compounds, 5-methyl-1-undecene and 1-undecyne, which were not found in *G. filicina*. Moreover, ectocarpene (21.83% in DVB/CAR/PDMS fiber; 14.71% in PDMS fiber) was a uniquely abundant compound in *P. senticulosa*.

2.1.2. Headspace VOC Composition of Phaeophyta

The VOC compositions of brown algae *Sargassum thunbergia* and *Dictyota dichotoma* are reported in Table 2. Four compounds were commonly found, including one norisoprenoid ((*E*)-β-Ionone), one alkane (pentadecane) and two aldehydes (tridecanal and pentadecanal).

Table 2. VOC composition in brown algae, determined by headspace solid phase microextraction coupled with gas chromatography–mass spectrometry (HS-SPME/GC–MS).

NO.	Compound	Molecular Formula	Compound Class	RI [a]	Area Percentage (%) ST [b]	Area Percentage (%) DD [b]	Identification
1	2-Propyl-furan [T]	$C_7H_{10}O$	Furan derivative	<800	1.37 *	0.00	MS
2	(*E*)-2-Hepten-1-ol [T]	$C_7H_{14}O$	Alcohol	830	0.00	4.62 *	MS
3	Sulcatone [T]	$C_8H_{14}O$	C_{13}-Norisoprenoid	852	0.00	5.21 *	MS
4	2-Propyl-1-pentanol [T]	$C_8H_{18}O$	Alcohol	896	0.82 *	0.00	MS
5	Isocumene	C_9H_{12}	Others	931	1.14 *	0.00	MS, RI
6	2,7-Dimethyl-1-octanol [T]	$C_{10}H_{22}O$	Alcohol	1023	0.94 *	0.00	MS
7	(*E*)-2-Undecen-1-ol [T]	$C_{11}H_{22}O$	Alcohol	1039	1.48 *	0.00	MS
8	Ectocarpene [T]	$C_{11}H_{16}$	Alkene	1105	3.40 *	0.00	MS
9	β-Cyclocitral	$C_{10}H_{16}O$	C_{10}-Norisoprenoid	1192	1.05 *	0.00	MS, RI
10	2,4-Decadienal	$C_{10}H_{16}O$	Unsaturated aldehyde	1275	0.00	0.72 *	MS, RI
11	α-Cubebene	$C_{15}H_{24}$	Sesquiterpene	1338	0.00	0.81 *	MS, RI
12	β-Bourbonene	$C_{15}H_{24}$	Sesquiterpene	1376	0.00	1.07 *, 1.39 #	MS, RI
13	Cedrene	$C_{15}H_{24}$	Sesquiterpene	1418	0.00	0.28 #	MS, RI
14	β-Copaene	$C_{15}H_{24}$	Sesquiterpene	1423	0.00	0.57 *	MS, RI
15	cis-Muurola-3,5-diene	$C_{15}H_{24}$	Sesquiterpene	1441	0.00	0.48 *	MS, RI
16	β-Gurjunene	$C_{15}H_{24}$	Sesquiterpene	1458	0.00	1.72 *	MS, RI
17	γ-Muurolene	$C_{15}H_{24}$	Sesquiterpene	1468	0.00	2.82 *, 0.62 #	MS, RI
18	Germacrene D [S]	$C_{15}H_{24}$	Sesquiterpene	1477	0.00	34.83 *, 62.00 #	MS, RI
19	(*E*)-β-Ionone [S]	$C_{13}H_{20}O$	C_{13}-Norisoprenoid	1481	1.22 *, 0.93 #	0.52 *, 0.44 #	MS, RI
20	1-Pentadecene	$C_{15}H_{30}$	Alkene	1484	4.31 *, 5.46 #	0.00	MS, RI
21	α-Selinene	$C_{15}H_{24}$	Sesquiterpene	1488	0.00	0.38 #	MS, RI
22	Pentadecane [S]	$C_{15}H_{32}$	Alkane	1492	9.24 *, 13.87 #	5.94 *, 7.98 #	MS.RI
23	Tridecanal [S]	$C_{13}H_{26}O$	Aldehyde	1503	11.46 *, 11.02 #	2.51 *, 1.74 #	MS, RI
24	Cyclopentadecane [T]	$C_{15}H_{30}$	Alkane	1508	2.94 *, 1.51 #	0.00	MS
25	Muurola-4,9-diene	$C_{15}H_{24}$	Sesquiterpene	1510	0.00	3.37 *	MS, RI
26	β-Cadinene	$C_{15}H_{24}$	Sesquiterpene	1518	0.00	0.52 #	MS, RI
27	δ-Cadinene	$C_{15}H_{24}$	Sesquiterpene	1520	0.00	5.28 *	MS, RI
28	1,4-Cadinadiene	$C_{15}H_{24}$	Sesquiterpene	1529	0.00	0.44 *	MS, RI
29	α-Muurolene [T]	$C_{15}H_{24}$	Sesquiterpene	1535	0.00	0.82 *	MS
30	Tetradecanal [S]	$C_{14}H_{28}O$	Aldehyde	1608	0.62 *, 0.48 #	0.00	MS, RI
31	Cubenol	$C_{15}H_{26}O$	Sesquiterpene	1637	0.00	3.69 *, 4.70 #	MS, RI
32	8-Heptadecene	$C_{17}H_{34}$	Alkene	1681	5.97 *, 14.82 #	0.00	MS, RI
33	Heptadecane [S]	$C_{17}H_{36}$	Alkane	1693	1.63 *, 1.59 #	0.00	MS, RI
34	(*Z*)-11-Pentadecenal [T]	$C_{15}H_{28}O$	Aldehyde	1689	5.49 *, 5.85 #	0.00	MS
35	Pentadecanal	$C_{15}H_{30}O$	Aldehyde	1711	6.86 *, 11.33 #	0.46 #	MS, RI
36	(*Z,Z,Z*)-7,10,13-Hexadecatrienal [T]	$C_{16}H_{26}O$	Unsaturated aldehyde	1890	0.91 #	0.00	MS
37	1,5,9-Trimethyl-12-(1-methylethyl)-4,8,13-Cyclotetradecatriene-1,3-diol [T]	$C_{20}H_{34}O_2$	Diterpene	1989	0.00	5.70 #	MS
38	Thunbergol	$C_{20}H_{34}O_2$	Diterpene	2089	0.00	3.30 #	MS, RI
39	Geranyl-α-terpinene [T]	$C_{20}H_{32}$	Diterpene	>2100	0.00	1.04 *, 0.99 #	MS
	Total Identified (%)				59.94 *, 67.77 #	76.46 *, 90.51 #	

[T] tentatively identified. [S] identified by mass spectra (MS) and retention index (RI) compared with authentic standard. [a] RI: retention index relative to C7–C30 alkanes. [b] ST—Sargassum thunbergii; DD—Dictyota dichotoma. * data from fiber DVB/CAR/PDMS. # data from fiber PDMS.

The most abundant VOCs in *S. thunbergia* are 8-heptadecene (5.97% in DVB/CAR/PDMS fiber; 14.82% in PDMS fiber), pentadecane (9.24% in DVB/CAR/PDMS fiber; 13.87% in PDMS fiber), pentadecanal (11.33% in PDMS fiber) and tridecanal (11.46% in DVB/CAR/PDMS fiber; 11.02% in PDMS fiber). A low relative content of 8-heptadecene (1.70%) has been reported in *S. thunbergia*, also using the SPME method though the fiber type was not mentioned [18]. Our data suggest that the choice of fiber type in the HS-SPME method has a decisive influence on the study's conclusion. In addition, two types of volatile polyenes were reported to have been found in an essential oil prepared by a simultaneous distillation extraction method from *S. thunbergia* [19], although no polyenes were found in our study.

Fifteen sesquiterpenes were found in *D. dichotoma*, with the predominant one being germacrene D (34.83% in DVB/CAR/PDMS fiber; 62% in PDMS fiber) together with minor ones such as cadinene, muurolene and bourbonene. Compared with the VOC results (PDMS/DVB fiber) from *D. dichotoma* collected from the Adriatic Sea [20], we identified a lower number of sesquiterpenes. The reason for this difference may be that the profile of plant secondary metabolites may vary in different environments, or that different extract fibers have differing abilities to capture sesquiterpenes.

It has been reported that a total of 233 diterpenes were isolated from *Dictyota* species, most of which were from *D. dichotoma* [21]. The present study firstly reports three volatile diterpenes—1,5,9-trimethyl-12-(1-methylethyl)-4,8,13-cyclotetradecatriene-1,3-diol, thunbergol and geranyl-α-terpinene in *Dictyota*. 1,5,9-Trimethyl-12-(1-methylethyl)-4,8,13-cyclotetradecatriene-1,3-diol is a macrocyclic diterpene and is used as a representative flavor compound in tobacco [22]. Geranyl-α-terpinene is distributed in some Compositae plants, as well as existing in the volatiles of beech buds, which act as semiochemical attractants for the beech leaf-mining weevil, *Orchestes fagi* [23].

2.1.3. Headspace VOC Composition of Chlorophyta

The results for two green algae, *Enteromorpha prolifera and Ulva lactuca*, are presented in Table 3. In total, 40 VOCs were identified and, among these, 21 were aldehydes.

Table 3. VOC composition in green algae determined by headspace solid phase microextraction coupled with gas chromatography–mass spectrometry (HS-SPME/GC–MS).

NO.	Compound	Molecular Formula	Compound Class	RI [a]	Area Percentage (%)		Identification
					EP [b]	UL [b]	
1	3,5,5-Trimethyl-1-hexene [T]	C_9H_{18}	Alkene	<800	0.00	1.34 *	MS
2	3,5-Dimethyl-1-hexene [T]	C_8H_{16}	Alkene	<800	3.75 *	1.27 *	MS
3	2-Propyl-furan [T]	$C_7H_{10}O$	Furan derivatives	<800	4.61 *	4.74 *, 3.82 #	MS
4	2,4-Octadiene	C_8H_{14}	Alkene	805	0.00	3.06 *	MS, RI
5	3-Ethyl-1,4-hexadiene [T]	C_8H_{14}	Alkene	846	3.59 *	10.17 *	MS
6	(*E*)-2-Heptenal	$C_7H_{12}O$	Aldehyde	960	9.64 *	4.60 *	MS, RI
7	1,2-Dimethyl-cycloheptene [T]	C_9H_{16}	Alkene	988	3.32 *	3.40 *	MS
8	4-Heptenal [T]	$C_7H_{12}O$	Aldehyde	1001	2.94 *	0.00	MS
9	(4*E*)-2-Methyl-4-hexen-3-ol [T]	$C_7H_{14}O$	Alcohol	1025	3.08 *	0.00	MS
10	(*E*)-2-Undecen-1-ol [T]	$C_{11}H_{22}O$	Unsaturated alcohol	1039	3.15 *	1.27 *	MS
11	2,4-Dimethyl-Cyclohexanol	$C_8H_{16}O$	Alcohol	1045	0.80 *	1.90 *	MS, RI
12	3-Cyclohexene-1-carboxaldehyde [T]	$C_8H_{12}O$	Aldehyde	1056	0.50 *	0.00	MS
13	(*Z*)-6-Nonenal	$C_9H_{16}O$	Aldehyde	1113	0.00	1.94 *	MS, RI
14	2,4-Nonadienal	$C_9H_{14}O$	Unsaturated aldehyde	1180	0.95 *	0.52 *	MS, RI
15	Decanal	$C_{10}H_{20}O$	Aldehyde	1173	1.36 *	0.58 *	MS, RI
16	9-Oxabicyclo [6.1.0]nonan-4-ol [T]	$C_8H_{14}O_2$	Alcohol	1186	0.70 *	0.00	MS
17	β-Cyclocitral	$C_{10}H_{16}O$	C_{10}-Norisoprenoid	1192	0.89 *, 1.05 #	3.05 *, 3.17 #	MS, RI
18	(*Z*)-2-Decenal	$C_{10}H_{18}O$	Aldehyde	1243	0.70 *	0.00	MS, RI
19	Citral	$C_{10}H_{16}O$	C_{10}-Norisoprenoid	1253	0.32 *	0.00	MS, RI
20	1-Butenylidene-cyclohexane [T]	$C_{10}H_{16}$	Alkane	1260	0.81 *	1.80 *, 1.80 #	MS
21	Undecanal	$C_{11}H_{22}O$	Aldehyde	1290	0.31 *	0.15 *	MS, RI
22	2,4-Decadienal	$C_{10}H_{16}O$	Unsaturated aldehyde	1275	0.00	0.96 *, 0.48 #	MS, RI
23	(*E,E*)-2,4-Decadienal	$C_{10}H_{16}O$	Unsaturated aldehyde	1298	0.77 *, 1.61 #	3.02 *, 2.59 #	MS, RI
24	4,4,6-Trimethyl-cyclohex-2-en-1-ol	$C_9H_{16}O$	Alcohol	1330	0.28 *	0.00	MS, RI
25	2-Undecenal	$C_{11}H_{20}O$	Aldehyde	1353	0.65 *	0.00	MS, RI
26	(*E*)-4,5-Epoxydec-2-enal	$C_{10}H_{16}O_2$	Unsaturated Aldehyde	1368	1.70 *, 0.85 #	0.28 *	MS, RI
27	6,10-Dimethyl-2-undecanone	$C_{13}H_{26}O$	Ketone	1395	0.56 *	0.19 *	MS, RI
28	Dodecanal	$C_{12}H_{24}O$	Aldehyde	1400	0.43 *	0.00	MS, RI
29	α-Ionone	$C_{13}H_{20}O$	C_{13}-Norisoprenoid	1420	0.21 *, 0.45 #	2.59 *, 4.24 #	MS, RI
30	(*E*)-Geranylacetone	$C_{13}H_{22}O$	C_{13}-Norisoprenoid	1447	0.93 *, 1.74 #	0.29 *, 0.48 #	MS, RI
31	(*E*)-β-Ionone [S]	$C_{13}H_{20}O$	C_{13}-Norisoprenoid	1481	3.53 *, 5.97 #	8.69 *, 18.13 #	MS, RI
32	Tridecanal [S]	$C_{13}H_{26}O$	Aldehyde	1503	1.31 *, 2.37 #	0.37 *, 0.65 #	MS, RI

Table 3. *Cont.*

NO.	Compound	Molecular Formula	Compound Class	RI [a]	Area Percentage (%)		Identification
					EP [b]	UL [b]	
33	Dihydroactinidiolide	$C_{11}H_{16}O_2$	C_{11}-Norisoprenoid	1525	0.00	0.29 *	MS, RI
34	Tetradecanal [S]	$C_{14}H_{28}O$	Aldehyde	1608	3.34 *, 6.32 #	0.79 #	MS, RI
35	8-Heptadecene	$C_{17}H_{34}$	Alkene	1681	2.72 #	5.32 *, 19.70 #	MS, RI
36	(Z)-11-Pentadecenal [T]	$C_{15}H_{28}O$	Aldehyde	1689	0.40 *, 0.81 #	0.77 *	MS
37	Pentadecanal	$C_{15}H_{30}O$	Aldehyde	1711	8.49 *, 24.40 #	3.83 *, 7.36 #	MS, RI
38	(Z)-11-Hexadecenal	$C_{16}H_{30}O$	Aldehyde	1793	0.59 *, 1.18 #	0.00	MS, RI
39	(Z,Z)-6,9-Pentadecadien-1-ol [T]	$C_{15}H_{28}O$	Unsaturated alcohol	1889	0.83 #	0.54 *, 0.93 #	MS
40	(Z,Z,Z)-7,10,13-Hexadecatrienal [T]	$C_{16}H_{26}O$	Unsaturated aldehyde	1890	0.46 *, 6.13 #	2.83 *, 4.96 #	MS
	Total Identified (%)				65.07 *, 56.43 #	69.79 *, 69.10 #	

[T] tentatively identified. [S] identified by mass spectra (MS) and retention index (RI) compared with authentic standard. [a] RI: retention index relative to C7–C30 alkanes. [b] EP—*Enteromorpha prolifera*; UL—*Ulva lactuca*. * data from fiber DVB/CAR/PDMS. # data from fiber PDMS.

The top three compounds with the highest content in *E. prolifera* were aldehydes, consisting of pentadecanal (8.49% in DVB/CAR/PDMS fiber; 24.4% in PDMS fiber), (*E*)-2-heptenal (9.64% in DVB/CAR/PDMS fiber) and tetradecanal (3.34% in DVB/CAR/PDMS fiber; 6.32% in PDMS fiber). It has been reported that significant differences were observed in the composition and types of VOCs in different harvest seasons for *E. prolifera* [24]. Differently from *E. prolifera*, the top three compounds with the highest content found in *U. lactuca* were not aldehydes, but alkenes and a ketone, including 8-heptadecene (5.32% in DVB/CAR/PDMS fiber; 19.70% in PDMS fiber), (*E*)-β-ionone (8.69% in DVB/CAR/PDMS fiber; 18.13% in PDMS fiber) and 3-ethyl-1,4-hexadiene (10.17% in DVB/CAR/PDMS fiber).

For *U. lactuca*, 30 VOCs were identified. Among them, 25 compounds also existed in *E. prolifera*, including 11 aldehydes ((*E*)-2-heptenal, 2,4-nonadienal, decanal, (*E,E*)-2,4-decadienal, undecanal, (*E*)-4,5-epoxydec-2-enal, tridecanal, tetradecanal, *Z*-11-pentadecenal, pentadecanal, (Z,Z,Z)-7,10,13-hexadecatrienal); four norisoprenoids (β-cyclocitral, α-ionone, (*E*)-geranylacetone, (*E*)-β-ionone); four alkenes (3,5-dimethyl-1-hexene, 3-ethyl-1,4-hexadiene, 1,2-dimethyl-cycloheptene, 8-heptadecene); two alkanes (1-butenylidene-cyclohexane, heptadecane); three alcohols ((*E*)-2-undecen-1-ol, 2,4-dimethyl-cyclohexanol, (Z,Z)-6,9-pentadecadien-1-ol); one ketone (6,10-dimethyl-2-undecanone) and one furan derivative (2-propyl-furan). The above common constituents in the two green algae covered almost all the VOC categories, differently from the red and brown algae.

(E)-β-Ionone was the only compound found to existing in all seven algae characterized in this study and it is widely distributed in the plant kingdom. As a C_{13} norisoprenoid, (E)-β-ionone can be produced by secondary metabolism of β-carotene and can also be produced by thermal degradation and photooxidation of carotenoids [25,26]. It has been reported that (E)-β-ionone has a wide range of biological activities, including having a strong anticancer effect [27].

2.2. Characteristic VOC Molecules and Potential Application in Chemotaxonomy

2.2.1. PCA on Total VOCs Variables

Principal component analysis (PCA) on data from fiber DVB/CAR/PDMS exhibited that, except for *D. dichotoma* and *E. prolifera*, the other five seaweeds were clustered together (Figure 2a). The PCA results for fiber PDMS show that *D. dichotoma* was distinct from the other six seaweeds (Figure 2b).
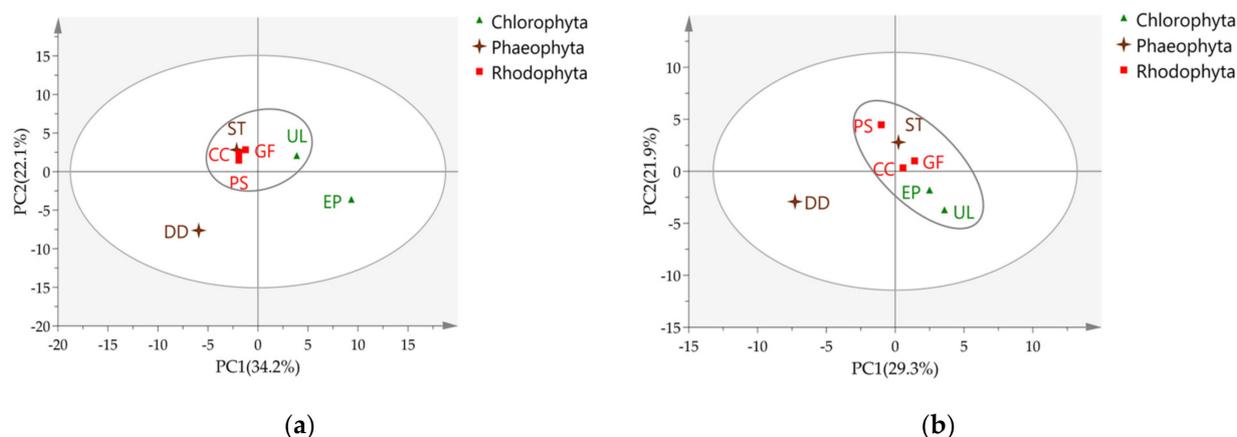
Figure 2. Principal component analysis (PCA) of VOC compositions in seven seaweeds determined by HS-SPME/GC–MS; score plot for (**a**) divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber; (**b**) polydimethylsiloxane (PDMS) fiber.

D. dichotoma is an outlier in PCA, being very different from the other six seaweeds. The molecules that contributed most to this difference were revealed in the loading plot and are reported in the Supplementary Materials (Figure S1 and Table S1). As expected, the main differences were regarding sesquiterpenes and diterpenes.

Based on the revised biogenetic scheme that is widely cited, *Dictyota* diterpenes can be divided into three groups (I–III), resulting from the first formal cyclization of the geranylgeraniol precursor, which makes them potentially useful as chemotaxonomic and phylogenetic markers [28]. Unfortunately, the number of diterpenes available in *Dictyota* VOCs is very limited, making it difficult for them to be used in chemotaxonomy.

On the other hand, *Dictyota* contains large numbers of sesquiterpenes, distinguishing them from other seaweeds in this study. It is still not clear whether volatile sesquiterpenes can be used as chemotaxonomic markers for species identification of genus *Dictyota*, but it is worth exploring this through the VOC approach.

2.2.2. PCA on Aldehyde Variables

Using all the VOC data as PCA variables, *E. prolifera* was found to be an outlier, as shown in Figure 2a (DVB/CAR/PDMS fiber), but not in Figure 2b (PDMS fiber). Considering that aldehydes are the most abundant VOC categories (Figure 1), aldehydes were used as variables in PCA to reveal chemical differences between species. The results show that *E. prolifera* was separated well from other seaweeds (Figure 3). The aldehydes that contribute the most to species differences are shown in Table 4. There are six aldehydes characteristically distributed in *E. prolifera*.
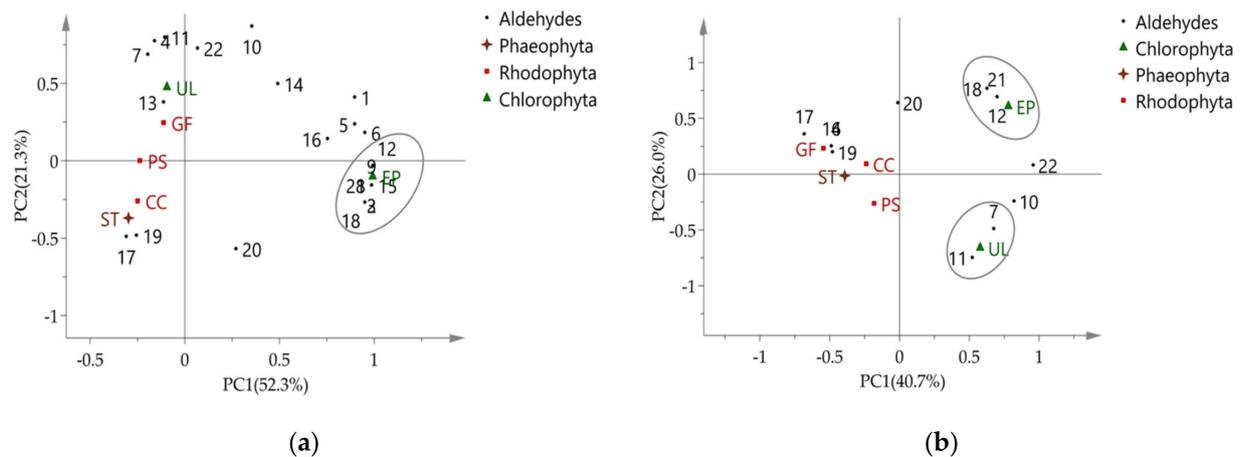
(**a**) (**b**)

Figure 3. Bi-plot of PCA using aldehydes: (**a**) DVB/CAR/PDMS fiber; (**b**) PDMS fiber.

Table 4. Various aldehydes in PCA loading plot, as shown in Figure 3.

No.	Aldehydes	Molecular Formula	Related Fiber	Algae
2	4-Heptenal	$C_7H_{12}O$	DVB/CAR/PDMS	EP
3	3-Cyclohexene-1-carboxaldehyde	$C_8H_{12}O$	DVB/CAR/PDMS	EP
7	*β*-Cyclocitral	$C_{10}H_{16}O$	PDMS	EP, UL [Δ], GF, PS, CC, ST
8	(*Z*)-2-Decenal	$C_{10}H_{18}O$	DVB/CAR/PDMS	EP
9	Citral	$C_{10}H_{16}O$	DVB/CAR/PDMS	EP
11	2,4-Decadienal	$C_{10}H_{16}O$	PDMS	UL [Δ], GF, DD
12	(*E*)-4,5-Epoxydec-2-enal	$C_{10}H_{16}O_2$	DVB/CAR/PDMS, PDMS	EP [Δ], UL
15	2-Undecenal	$C_{11}H_{20}O$	DVB/CAR/PDMS	EP
18	Tetradecanal	$C_{14}H_{28}O$	DVB/CAR/PDMS, PDMS	EP [Δ], UL, GF, CC, ST
21	(*Z*)-11-Hexadecenal	$C_{16}H_{30}O$	DVB/CAR/PDMS, PDMS	EP

[Δ] with the highest content.

Aldehydes are substances released by algae under biotic or abiotic stress and some aldehydes may induce the synthesis of a series of oxylipins in algae and therefore act as inducers of metabolic responses [29]. In view of the limited species and numbers of sea-weeds in the present study, our data can only suggest that aldehydes may be of important value for the chemotaxonomic significance of *E. prolifera* and that the DVB/CAR/PDMS fiber is more suitable for headspace solid phase microextraction for aldehydes.

The volatile components of red algae *Bangia fuscopurpurea*, *Gelidium latifolium*, *Callithamnion granulatum*, *Ceramium elegans*, *Laurencia papillosa* and *Laurencia coronopus* from Black sea was detected and found that hydrocarbons can be used as chemotaxonomic markers of the two classes Bangiophyceae and Florideophyceae [30]. The chemotaxonomic significance was also discussed according to VOCs detection in two brown algae: *Taonia atomaria* and *Padina pavonica* [6]. The VOCs were used in evolutionary relationship discussion between the algae and liverwort *Fossombronia angulosa* due to the similarity [31].

2.3. Network Pharmacology and Potential Biomedical Application of D. Dichotoma

VOCs in *D. dichotoma* are characterized by sesquiterpenes and diterpenes. It has been reported that a total of 78 structurally diverse diterpenes have been isolated from

D. dichotoma and exhibit multi-biological properties, such as cytotoxic, antitumor, antiviral, antifouling, antioxidant, antibacterial and antifungal activities [21]. In the present study, a network pharmacology method was performed to verify and discover new bioactivities of *D. dichotoma*.

First, the VOCs of *D. dichotoma* were entered into the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) in the "chemical name" search box to obtain relevant targets. The "compound–target" relationships are shown in Figure 4, with 31 nodes (1 alga, 10 compounds and 20 targes). Comparing with the average degree score, 6.07, from network topology analysis, six key compounds were disclosed as cedrene, *α*-selinene, cubenol, *α*-muurolene, 2,4-decadienal and *γ*-muurolene, of which most are sesquiterpenes. The first three compounds are cedrene, *α*-selinene and cubenol, each of which can act on 10 targets.
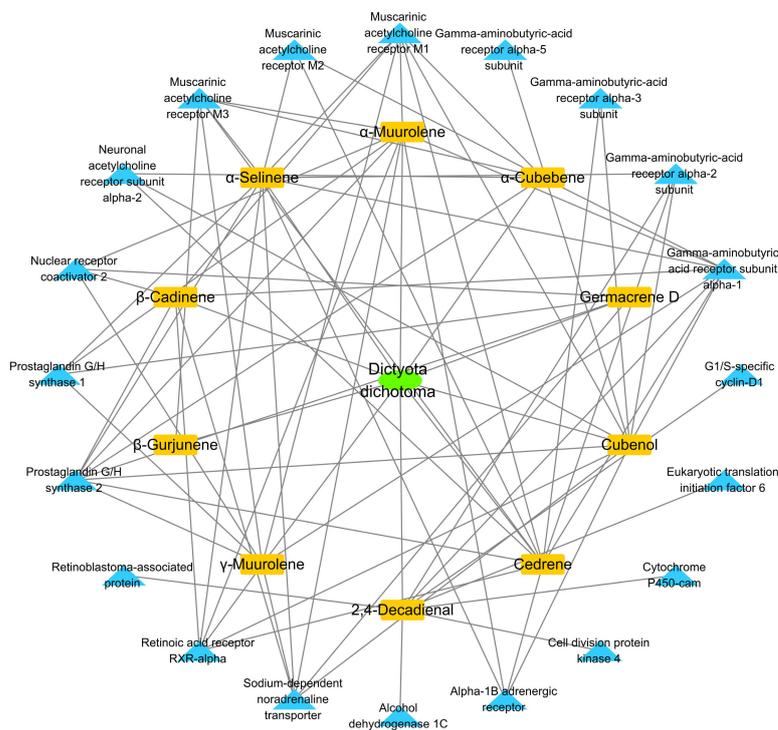
Figure 4. Compound–target interaction network of Dictyota dichotoma.

Next, the Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis was used to obtain the potential pathway in which VOCs might play a role. The 10 identified pathways ($p < 0.01$) are shown in Figure 5: neuroactive ligand–receptor interaction, small cell lung cancer, retrograde endocannabinoid signaling, nicotine addiction, non-small cell lung cancer, GABAergic synapse, morphine addiction, bladder cancer, PI3K–Akt signaling pathway and the calcium signaling pathway.

According to the literature, the neuroactive ligand–receptor interaction pathway plays an important role in lung cancer etiology [32]. Considering other pathways such as small cell lung cancer, non-small cell lung cancer, retrograde endocannabinoid signaling and PI3K–Akt signaling, we supposed that *D. dichotoma* may have certain preventive and therapeutic value in cancer, especially in lung cancer. The non-polar fractions of *D. dichotoma* exhibit anticancer activity in vitro, including the lung adenocarcinoma cell line (A-549) [33].
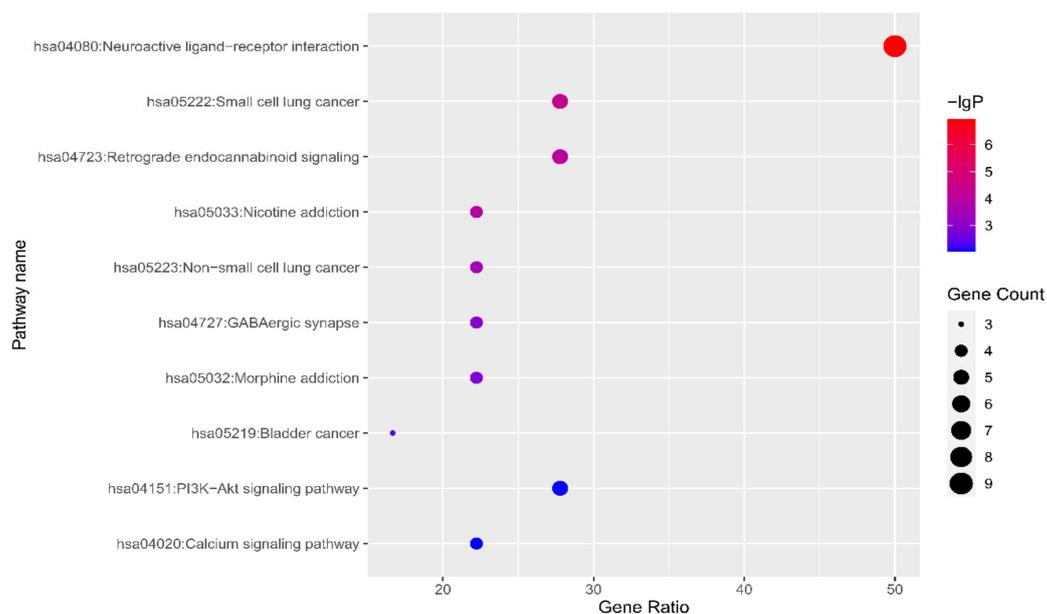
Figure 5. Bubble chart of pathway enrichment information.

In addition, it should be noted that the neuroactive ligand–receptor interaction pathway is the most relevant pathway for VOCs in *D. dichotoma*, with nine target genes involved—CHRM1, CHRM2, CHRM3, CHRNA2, GABRA1, GABRA2, GABRA3, GABRA5 and ADRA1B. The neuroactive ligand–receptor interaction pathway is involved in environmental information processing as well as signaling molecules and interactions and an association has been found with certain neuropsychiatric disorders [34]. Therefore, *D. dichotoma* may have some potential use in the study of some neuropsychiatric diseases.

3. Materials and Methods

3.1. Sample Collection

The samples of seven algae were single-point collected from the coast of Qingdao city (120°20′30″ E, 36°3′43″ N, the Yellow Sea), Shandong Province, China, in July 2019. All of the studied algae are local common species, including *Grateloupia filicina* (Wulfen) C. Agardh, 1822 (Halymeniaceae Bory, Gigartinales Schmitz, Rhodophyceae), *Polysiphonia senticulosa* Harvey,1862 (Rhodomelaceae Areschoug, Ceramiales Oltmanns, Rhodophyceae), *Callithamnion corymbosum* (Smith) Lyngbye, 1819 (Ceramiaccae Dumoritier, Ceramiales Oltmanns, Rhodophyceae), *Sargassum thunbergii* (Mertens ex Roth) O'Kuntze, 1893 (Sargassaceae Kuetzing, Fucales Kylin, Phaeophyta), *Dictyota dichotoma* (Hudson) J. V. Lamouroux, 1809a (Dictyotaceae Lamourous ex Dumortier, Dictyotales Kjellman in Engler et Prantl, Phaeophyta), *Enteromorpha prolifera* (O.F.Müller) J. Agardh, 1883 (Ulvales Blackman et Tansley, Ulvaceae Lamourous ex Dumortier, Chlorophyta), *Ulva lactuca* Linneaeus, 1753 (Ulvales Blackman et Tansley, Ulvaceae Lamourous ex Dumortier, Chlorophyta). The voucher specimens have been deposited in the Key Laboratory of Marine Drugs, Chinese Ministry of Education, School of Medicine and Pharmacy, Ocean University of China.

The samples were separately collected and placed in an air-tight plastic bag containing surrounding seawater and were immediately transported to the laboratory. Before extraction, each sample were cut into small pieces and excess water was removed using filter paper.

3.2. Headspace Solid Phase Microextraction (HS-SPME)

HS-SPME was performed with a manual SPME holder. Four types of fibers obtained from Supelco Co. (Bellefonte, PA, USA) were compared for their adsorption properties in pilot experiments and two fibers, divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 50/30 μm) and polydimethylsiloxane (PDMS, 100 μm) were finally,

chosen. Fibers polyacrylate (PA, 85 µm) and 7 µm PDMS were not used in the present study. The fibers were activated in advance, according to the instructions.

A 1 g amount of prepared sample was placed into 4 mL glass vials and sealed using a cover with a Teflon gasket to provide a closed environment. The vials were placed at 60 °C for 15 min to equilibrate and the fiber was then pushed into the vial and maintained there for 20 min to adsorb the VOCs of the sample. After absorption, the fiber was removed from the vial and inserted into the injector (250 °C) of the GC–MS and kept for 6 min (DVB/CAR/PDMS fiber) or 4 min (PDMS fiber) to realize desorption. HS-SPME was performed in triplicate for each sample.

3.3. Gas Chromatography–Mass Spectrometry (GC–MS) Analyses

GC–MS analyses was performed on a Thermo Trace 1300 ISQ gas chromatography-mass spectrometer (Thermo Fisher Scientific Inc., San Jose, CA, USA) equipped with a TG-5MS capillary column (5% phenyl-methylpolysiloxane, 30 m × 0.25 mm, 0.25 µm, Thermo Fisher Scientific Inc.). For DVB/CAR/PDMS fiber, the oven temperature was initially set at 70 °C and maintained for 1 min, increased at a rate of 3 °C/min to 100 °C and continually increased at a rate of 5 °C/min to 200 °C, followed by a final hold at this temperature for 2 min. For PDMS fiber, the oven temperature was initially maintained at 70 °C for 2 min, increased from 70 to 200 °C at 3 °C/min and then held at 200 °C for 6 min. Helium (99.9% purity) was used as the carrier gas at a flow rate of 1 mL/min. The temperature of the injector, detector transfer line and ion source were 250, 270 and 230 °C, respectively. The MS detector was operated in the full scan mode and the electron energy was 70 eV and the scan range was from *m/z* 30 to 350 amu. All analyses were performed in triplicate.

Visualization, calibration and normalization of the GC–MS data were performed using Xcalibur 2.1 (Thermo Fisher Scientific Inc.). The VOC peaks were identified by comparison of their retention index (RI) and MS data with data in the NIST Chemistry WebBook [35] and NIST 11 MS Data Library, respectively. The RI values of the VOCs were calculated by analyzing the C7–C30 *n*-alkanes (Sigma-Aldrich, St. Louis, MO, USA) under the same GC–MS conditions as samples. Authentic standards included trans-2-nonenal (Shanghai Macklin Biochemical Co., Ltd., Shanghai, China), tridecanal (Shanghai Macklin Biochemical Co., Ltd.), 1-octen-3-ol (Shanghai Macklin Biochemical Co., Ltd.), tetradecanal (Shanghai Aladdin Biochemical Technology Co., Ltd., Shanghai, China), germacrene D (Toronto Research Chemicals, Toronto, ON, Canada) and (*E*)-*β*-ionone (Xiya Chemical Technology Co., Ltd., Chengdu, China), which were used for confirming VOC identity. All VOC peaks were quantified by area normalization with consistent peak parameters (baseline, area noise and peak noise) prior to data analysis and statistics.

3.4. Chemometrics and Network Pharmacology Analysis

The principal components analysis (PCA) of all VOCs was conducted using SIMCA 14.1. The targets of identified volatile components were obtained from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, https://tcmspw.com/tcmsp.php, accessed on 21 December 2020) [36], the gene information of the related targets was taken from the Uniport database (https://www.Unitprot.org/, accessed on 21 December 2020) and the String database (https://string-db.org/, accessed on 21 December 2020) [37,38] and KEGG pathway enrichment analysis were accessed through DAVID software (https://david.ncifcrf.gon/, accessed on 21 December 2020) [39] with the corresponding bubble chart realized using the R program. The interactive component–target relationships in algae were visualized using Cytoscape 3.7.2 software.

4. Conclusions

The volatile composition of seven algae from Yellow Sea of China was detected by HS-SPME/GC-MS, the VOCs of three red algae *Grateloupia filicina*, *Polysiphonia senticulosa* and *Callithamnion corymbosum* among them are first reported. The PCA analysis of VOCs

reveals the chemotaxonomy significance of aldehydes in green algae *Enteromorpha prolifera* and sesquiterpenes in brown algae *Dictyota dichotoma*, as well as the applicability of DVB/CAR/PDMS fiber in volatile aldehydes. The network pharmacology analysis of *Dictyota dichotoma* indicate the potential biomedical application in fields of lung cancer and neuropsychiatric diseases. Our study exploits the new research value of VOCs in algae from the perspective of chemotaxonomy and network pharmacology.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/md19040192/s1, Figure S1: PCA of VOC compositions in seven seaweeds determined by HS-SPME/GC–MS, loading plot with: (a) DVB/CAR/PDMS fiber; (b) PDMS fiber. Table S1: The compounds corresponding to dots with coordinates in the loading plot.

Author Contributions: Conceptualization, H.L. and P.W.; methodology, P.W.; software, P.W., J.C. and L.C.; validation, L.C. and L.S.; formal analysis, P.W.; investigation, P.W.; data curation, P.W.; writing—original draft preparation, P.W.; writing—review and editing, H.L.; project administration, H.L.; funding acquisition, H.L. and P.W. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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