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Antibacterial and insecticidal activity of volatile compounds of three algae species of Oman Sea

Ardalan Pasdaran[1, 2*], Azadeh Hamedi[2, 3] and Nazim Mamedov[4]

[1] *Department of Pharmacognosy, School of Pharmacy, Research and Development Center of Plants and Medicinal Chemistry, Guilan University of Medical Sciences, Rasht, Iran,*

[2] *Medicinal Plants Processing Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*

[3] *Department of Pharmacognosy, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran*

[4] *Medicinal Plants Program, Stockbridge School of Agriculture University of Massachusetts at Amherst, USA*

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Abstract: Many of the volatile oils showed important biological and pharmacological activities, these compounds as part of the traditional medicine in many cultures used as long time. Potencies of them caused these natural products gained many scientific researches in felid of natural products. The volatile oils of *Actinotrichia fragilis* (Forsskål) Børgesen, *Liagora ceranoides* J.V.Lamouroux and *Colpomenia sinuosa* (Mertens ex Roth) Derbes and. Solier were extracted by hydrodistillation. These volatile oils were analyzed by GC-MS and GC-FID techniques and tested for their toxicity against *Oryzeaphilus mercator* and *Tribolium castaneum*, antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* using by the disc diffusion method also free-radical-scavenging properties. The identified constituents of these volatile oils represented 92.7%, 99.9% and 93.8% of the total volatile oils, respectively, of *A. fragilis*, *L. ceranoides* and *C. sinuosa*. Ethyl cinnamate and Tetradecane were the main compounds in *L. ceranoides*, 1-dodecanol and caryophyllene oxide in *A. fragilis* whilst hexadecane and 7-pentadecanone were the principal components of *C. sinuosa* volatile oil. All three volatile oils showed 55-90% mortality of *O. mercator* and 60-80% mortality of *T. castaneum* at a dose of 12 µL/L air after 48h of exposure. Based on the observed contact toxicity of the essential oils of these species, it is fair to state that these volatile oils may have some potential as an insecticide against the crop pests, *O. mercator* and *T. castaneum.* Also antibacterial activity of *L. ceranoides* volatile oil against *Pseudomonas aeruginosa* and *Staphylococcus aureus* is significant.

Keywords: antibacterial activity; contact toxicity; *Actinotrichia fragilis; Liagora ceranoides; Colpomenia sinuosa*

1. Introduction

Secondary metabolites isolated from different alga are playing an important role as lead components, natural medicine or nutriceuticals in drug discovery researches and pharmaceutical industries [1-3]. Recently, due to the resistance of different pathogenic bacteria and pest to antibiotics and insecticidal agents [4-6], finding new active components against these health and environmental problems is one of the major areas of medical and agricultural researches. Peculiarity of the marine environment and high biological activity of marine natural products make algal metabolites fascinating source for finding new antimicrobial and insecticidal compound [6, 7]. The aim of this study is to investigate volatile

*Corresponding Author Tel.: +989127948891; Fax: +981313263670. E-mail address: Ardalan_pasdaran@gums.ac.ir (Ardalan Pasdaran) or mamedov@cas.umass.edu (Nazim Mamedov)

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components, insecticidal and antibacterial properties of three algae from Oman Sea (*Actinotrichia fragilis* (Forsskål) Børgesen, *Liagora ceranoides* J.V.Lamouroux and *Colpomenia sinuosa* (Mertens ex Roth) Derbes and. Solier). Red alga *A. fragilis* (Galaxauraceae, Nemaliales), is a small (c. 1±5–5 cm high) calcified, dichotomously divided multiaxial species, with an Indo-Pacific tropical distribution [8]. Some studies can be found on the biology and reproductive system of this alga [8-10]. Red alga, *L. ceranoides* J.V.Lamouroux is (Liagoraceae, Nemaliales) was previously reported to exhibit antioxidant properties [11]. Distribution and biological aspects of this alga were previously reported in literature [12-15]. Brown alga, *C. sinuosa* (Mertens ex Roth) Derbes and. Solier, which is known as the oyster thief or sinuous ballweed is found throughout South Africa and is widespread around Australia and some other tropical areas. Its distribution and history of life was investigated in some previous studies [16-18]. Methanol extract and body mass powder of this alga exhibited antibacterial effect against *Staphylococcus aureus* [6]. Despite some reports on biology and distribution of these three algae, there metabolite or biological activates have not been studied to date.

2. Experimental

2.1. Plant Materials

The *A. fragilis, L. ceranoides* J and *C. sinuosa* were collected from Chabahar coast wild populations growing in the Sistan and Baluchestan province, Iran. Voucher specimens (2558, 2559 and 2560, respectively), were deposited in the herbarium of pharmacognosy department, pharmacy faculty, Guilan University of Medical Sciences, Rasht, Iran.

2.2. Extraction of the Essential Oils

The air-dried ground of *A. fragilis, L. ceranoides and C. sinuosa* (500 g each) were subjected to hydrodistillation for 3h using a Clevenger type apparatus, yielding respectively, 1.3%, 0.5% and 0.8% v/w yellowish essential oils with distinct fragrance. The volatile oil samples were dried over anhydrous sodium sulphate (Na_2SO_4) and stored at 4°C in the dark until analyzed.

2.3. Analysis of the Essential Oils

The volatile oils were analyzed by Shimadzu GC-MS-QP5050A fitted with a fused methyl silicon DB-5 column (60 m × 0.25 mm i.d., 0.25 μm film thickness). Helium was used as carrier gas at a flow rate of 1.3 mL/min. The column temperature was kept at 50°C for 3 min, increased to 300°C at a rate of 5°C/min, and finally kept at 300°C for 5 min. The injector temperature was 270^{o}C and split ratio was adjusted at 1:33. The injection volume was 1 μL. The mass spectral (MS) data were obtained at the following conditions: ionization potential 70 eV; ion source temperature 200^{o}C; quadrupole temperature 100^{o}C; solvent delay 2 min; resolution 2000 amu/s and scan range 30-600 amu; EM voltage 3000 volts. Identification of compounds was based on direct comparison of the Kovats indices and MS data with those for standard compounds or by comparison of their relative Kovats indices to series of *n*-alkanes, and computer matching with the NIST NBS54K Library, by comparison with references. For quantization (area %), the GC analyses were also performed on an Agilent 6890 series apparatus fitted with a FID detector. The FID detector temperature was 300ºC. To obtain the same elution order as with GC-MS, simultaneous auto-injection was performed on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

2.4. Contact Toxicity Assay

The contact toxicity of the volatile oils of these algae was determined by previously insect toxicity assay model [19, 20]. All insect species were watched in controlled laboratory

conditions for about three weeks (25-29 ° C and relative humidity of 80%). The adult insect samples were collected of 1-3 week old and of mixed sex. All essential oils were applied with an automatic pipette on a paper strip (6 cm × 3 cm). The amounts of essential oils applied were 12, 24, 36 and 48 µL, corresponding to 3, 6, 9 and 12 µL/L air. Each dose was applied with automatic pipette as 100 µL acetone solution and acetone was used as a control. After evaporation of the acetone, twenty adults of *O. mercator* and *T. castaneum* were placed in Petri dishes (9 cm) (at 27±2°C, 12% moisture and 12h photoperiod). The experimental design was completely randomized, with three replicates. Mortality of the samples was evaluated after 12, 24, and 48h of exposure. Responses to treated sample versus control were converted to "percentage of mortality" [21].

2.5. Antibacterial Assay

Antibacterial activity assays of the volatile oils were carried out by the disk diffusion method. Bacteria were purchased in lyophilized form from the Institute of Pasture, Iran. Suspensions (100 µL) of the bacteria were adjusted to 10 cfu/mL final cell concentration after this 50 µL of bacterial suspension was poured by 25 mL of sterile normal saline into Petri dish flasks (spread by a sterile swab). Amounts of 60, 90, and 120 mg of the volatile oils were dissolved in 1 mL of methanol. Sterilized disks (5 mm) were impregnated with 10 µL of these volatile oil solutions, corresponding to 600, 900, and 1200 µg/disk, respectively, placed on the inoculated agar. Penicillin (1 mg of penicillin was added into 1 mL of sterilized and distilled water, and then the sterilized disk was soaked with 10 µL of this solution) was used as a positive control, corresponding to 10 µg/disk. At the end of 6 days, inhibition zones were measured in diameter (millimeter) around the disks. All of the tests were made in triplicate [22, 23].

Data Analysis

Statistical analysis of the data was done using SPSS 10.0 software package. The results were showed significant difference at $p < 0.05$ levels.

3. Results and Discussion

This study investigated volatile constituents of *A. fragilis*, *L. ceranoides* and *C. sinuosa* using GC-MS apparatus and the identified components are shown in Table 1. The identified constituents of these volatile oils represented 92.7%, 99.9% and 93.8% of the total volatile oils, respectively. Different aliphatic alcohols and long chain hydrocarbon were the major components of the extracted essential oil. We could not find any report on volatile components of the selected alga to compare the result but these components were previously from other alga [24-26].

In this study, *O.mercator* and *T. castaneum* were selected as model insects to evaluate insecticidal activities of the extracted volatile components. The insect, *O. mercator* (merchant grain beetle, Coleoptera) feeds from food stuff with high oil content such as oatmeal, bran, brown rice and processed foods, cereals, dried fruit, nuts and seeds. They can spread as a chronic pest and contaminate and damage food quality. The *T. castaneum* (red flour beetle, Tenebrionidae), is another pest of stored products, such as food grains. This beetle is used as a model organism for ethological and food safety research. Both selected insects are worldwide most common pests and food contaminants. They also can cause allergic responses in human. These two insect can cause serious financial damages to food industry and routinely used in insecticides development researches.

Table 1. Major constituents of the volatile oils of *A. fragilis, L. ceranoides and C. sinuosa*

			Kovats' Indices	(%)		
	Chemical compounds	Chemical formula		*A. fragilis*	*L. ceranoides*	*C. sinuosa*
1	2-iodo-3-methyl-Butane	$C_5H_{11}I$	983	-	-	1.1
2	2-nonanone	$C_9 H_{18} O$	1096	1.1	-	-
3	Alpha-terpinolene	$C_{10} H_{16}$	1207	2.0	-	-
4	Citronellal	$C_{10} H_{18} O$	1161	1.9	-	-
5	2-Undecanone	$C_{11}H_{22}O$	1291	2.3	-	1.4
6	Tetradecane	$C_{11}H_{12}O_2$	1399	-	24.4	-
7	1-Dodecene	$C_{12} H_{24}$	1193	3.1	-	-
8	1-Dodecanol	$C_{12}H_{26}O$	1474	39.6	-	2.3
9	2-Tridecene	$C_{13}H_{26}$	1315	-	-	2.8
10	Tridecane	$C_{13}H_{28}$	1299	0.9	-	4.8
11	Beta- Ionone	$C_{13} H_{20}O$	1485	0.8	-	-
12	Neryl acetone	$C_{13} H_{20}O$	1428	-	-	1.5
13	Edulan I	$C_{13} H_{20}O$	1469	-	-	0.9
14	Ethyl cinnamate	$C_{13} H_{26}O$	1374	-	33.8	-
15	Pseudoionone	$C_{13} H_{26}O$	1469	-	20.7	0.8
16	1-Tridecanol	$C_{13}H_{28}O$	1572	0.6	-	3.6
17	1-Tetradecene	$C_{14}H_{28}$	1393	2.8	-	1.2
18	1-Tetradecanol	$C_{14}H_{30}O$	1680	1.7	-	3.4
19	Tridecanoic acid methyl ester	$C_{14}H_{28}O_2$	1625	-	21.0	-
20	Germacrene D	$C_{15} H_{24}$	1598	0.5	-	-
21	Beta-elemene	$C_{15} H_{24}$	1384	1.6		-
22	1-Pentadecene	$C_{15} H_{30}$	1492	1.0	-	-
23	Pentadecane	$C_{15} H_{32}$	1510	-	-	2.5
24	Caryophyllene oxide	$C_{15} H_{24}O$	1590	16.7	-	-
25	7-Pentadecanone	$C_{15}H_{30}O$	1699	-	-	35.8
26	(8S,14) Cedran-diol	$C_{15}H_{26}O_2$	1876	2.0	-	-
27	Hexadecane	$C_{16}H_{34}$	1589	3.8	-	28.5
28	1-Hexadecanal	$C_{16}H_{32}O$	1819	1.3	-	-
29	1-Hexadecanol	$C_{16} H_{34}O$	1880	1.5	-	2.1
30	1-Heptadecanol	$C_{17}H_{36}O$	1958	5.6	-	-
31	8-Heptadecene, 1-chloro	$C_{17}H_{33}$ CL	1992	1.4	-	1.1
	Grouped components					
	Terpenoids compounds			21.6	-	0.9
	Alcoholic hydrocarbons and derivatives			52.3	-	11.4
	Aldehydes and ketones hydrocarbons			6.6	20.7	39.5
	Long chain hydrocarbons			11.6	24.4	39.8
	Others			6	54.8	2.2
	Total identified			**92.7**	**99.9**	**93.8**

The compounds have been sorted according to their Kovats retention indices on a DB-5 capillary column

Although, all three volatile oils showed 55-90% mortality of *O. mercator* and 60-80% mortality of *T. castaneum* at a dose of 12 µL/L air after 48h of exposure (Table 2), among the selected algae, *A. fragilis* volatile oil had the best insecticidal activity with mortality rate of 80-90% for both *T. castaneum* and *O. Mercator*. Interestingly, this oil was consisted of 49% aliphatic alcohols particularly 1-didacanol. Different aliphatic alcohols (C2 to C18) have been reported to exhibit insecticidal properties against different insects such as *Pediculus*

humanus capitis [1] *Rhodnius prolixus*, *Triatoma infestans* [2] and Aedes mosquitoes [3]. They proposed to have ovicide and larvicide effect against the mosquitoes *Aedes aegypti* Linneo and *Aedes scutellaris* Walker. The highest activity was reported for the 1-dodecanol and the lowest for 1-octanol. In addition, co-exposure of head lice to 1-dodecanol and d-phenothrin lotions, was made it more susceptible to insecticidal activity of pyrethroid [27]. The insecticidal activity of 1-dodecanol is suggested to be a result of interruption in the cuticular tanning process and thus interruption in the development of the physiological properties. [28]

Table 2. The toxicity of the volatile oils of *A. fragilis, L. ceranoides and C. sinuosa* against *O. Mercator* and *T. castaneum*

Essential oils	Dose (µL/L air)	% Mortality[a]					
		O. mercator			*T. castaneum*		
		Exposure time (h)			Exposure time (h)		
		12	24	48	12	24	48
A. fragilis	3	15.7± 3.3*	25.3± 3.3*	45.3± 3.3*	0.0± 0.0*	0.0± 0.0*	3.3± 3.3*
	6	30.0± 3.3*	40.0± 3.3*	50.7± 0.0*	0.0± 0.0*	15.7± 3.3*	20.3± 3.3*
	9	45.0± 3.3*	51.7± 0.0*	61.0± 3.3*	21.0± 3.3*	30.0± 0.0*	50.0± 0.0*
	12	50.3± 0.0*	86.3± 0.0*	90.0± 0.0*	45.3± 3.3*	51.3± 3.3*	80.0± 3.3*
L. ceranoide	3	0.0± 0.0*	0.0± 0.0*	0.0± 0.0*	0.0± 0.0*	0.0± 0.0*	0.0± 3.3*
	6	0.0± 0.0*	0.0± 3.3*	3.3± 3.3*	0.0± 0.0*	0.0± 0.0*	3.3± 0.0*
	9	3.3± 3.3*	18.7± 3.3*	23.3± 5.8*	0.0± 0.0*	3.3± 3.3*	15.3± 3.3*
	12	25.0± 5.8*	35.0± 5.8*	55.0± 0.0*	18.5± 0.0*	28.7± 0.0*	45.0± 3.3*
C. sinuosa	3	0.0± 0.0*	0.0± 0.0*	3.3± 3.3*	0.0± 0.0*	0.0± 0.0*	3.3± 0.0*
	6	6.7± 0.0*	25.0± 0.0*	35.0± 0.0*	0.0± 0.0*	6.0± 0.0*	18.0± 0.0*
	9	20.0± 0.0*	36.7± 3.3*	45.0± 3.3*	20.7± 0.0*	21.3± 3.3*	35.7± 0.0*
	12	35.0± 0.0*	45.7± 0.0*	55.0± 0.0*	30.3± 0.0*	35.3± 3.3*	60.0± 0.0*

[a] Mean±S.E. of three replicates, each set-up with 20 adults.
* There were no significant differences among treatments.

We could not find any report on insecticidal activity of *A. fragilis* volatile oil or 1-dodecanol on *T. castaneum* and *O. mercator* thus it is not possible to compare the efficacy or the applied doses. We believe that this is the first report on insecticidal effects of *A. fragilis* volatile oil or 1-dodecanol on these two crop pest. On the other hand the highest applied lethal dose was 12 (µL/L) is not a high dose and did not need topical exposure which can be considered as a bonus for its application as in insecticide. Despite the non-polar narcotic toxicity of 1-dodecanol to aquatic organisms of about 1 mg/l, the aliphatic alcohols especially 1-dodecanol are readily degradable and do not give rise to environmental concerns. 1-Dodecanol also considered non-toxic to human health, and is a permitted as a food additive.

The algae *C. sinuosa* volatile oil also showed some degree of insecticidal activity with the mortality rate of 55-60% within 48h. The major constituents of *C. sinuosa* essential oil were 7-pentadecanone and hexadecane. We could not find any reports on insecticidal activity for these two components but Long chain aliphatic methyl ketone series of C_{11}-C_{15} particularly 2-pentadecanone was reported to exhibit insect repellency [29]. Although the algae *C. sinuosa* volatile oil contained 2.3% of 1-dodecanol, but the observed insecticidal activity could not be just related to 1-dodecanol and it may worth the potential insecticidal activity of 7-pentadecanone in future works.

Despite the considerable amount of ethyl cinnamate (33.8%) in *L. ceranoides* volatile oil and the previous reports on potent insecticidal activity of ethyl cinnamate [30] against *S.*

littoralis (LD_{50} = 0.37 µg/larva), this oil did not showed a significant insecticidal properties especially at low doses or in shorter exposure time (12-24h). The observed different results might be due to the differences in selected insects' type which was applied in these two studies.

Antibacterial activities of these volatile oils were investigated against *E. coli*, *P. aeruginosa* and *S. aureus*. The selected bacteria are among the most common causes of infectious diseases. As it seen in Table 3, only *A. fragilis* showed significant antimicrobial properties. The three investigated algae showed some degrees of inhibition on microbial growth. But among them, *L. ceranoides* volatile oil had the highest antimicrobial activity (Table 3). As it was mentioned above, ethyl cinnamate is the major volatile constituents of this alga and may have an important role in the observed result. Several reports have demonstrated that essential oils containing cinnamate derivatives as one of the major constituents, exhibit antibacterial activity [31-34]. Also, Stefanović et al. reported antimicrobial activity of different synthetic derivatives of cinnamate particularly against *S. aureus* agent with minimum inhibitory concentration of 62.5 µg/ml [35].

Table 3. Antibacterial activities of the volatile oils of *A. fragilis, L. ceranoides and C. sinuosa*

Bacterial strain	*L. ceranoides* 600-1200 µg/disk	*A. fragilis* 600-1200 µg/disk	*C. sinuosa* 600-1200 µg/disk	Penicillin 10 µg/disk
E. coli	10-12	6-8	- [b]	-
P. aeruginosa	15-18	10-12	7-11	42
S. aureus	17-19	8-11	9-12	25

[a] Inhibition zones are given as minimum and maximum inhibition zones in diameter (mm) around the disks impregnated at 600, 900, and 1200 µg/disk doses. [b] Not active.

The result of present study is consistence with the previous reports. All three tested bacterium in this study are Gram-negative bacteria and has been developing resistance to common antibiotics. The outer membrane of these bacteria inhibits permeation of antibacterial compounds. Although the activity of tested essential oils is lower than the pure penicillin. But, considering the results of previous studies about antibacterial activity of naturally occurring or synthesized cinnamates, it can be concluded that essential oil of *L. ceranoides* and ethyl cinnamate indicate significant activity and may possess potential application as antibacterial agents [35-37]. The observed activity of A. *fragilis* might be due to the high concentration of long chain aliphatic alcohol including 1- dodecanol and 1-tridecanol. These two components have been previously reported to exhibit bactericidal effects [38]. Despite the previous reports on antibacterial effects of methanolic extract of *C. sinuosa* [6], we did not observe a significant antimicrobial effects. This might be due to application of volatile components in the present study instead of methanolic extract which can contain volatile and non-volatile metabolites of this alga.

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