

Essential Oils of a Soft Coral (*Sinularia* sp) from Chabahar Bay of Iran

Mehdinia, Ali* ; Sheijooni Fumani, Neda; Rezaei, Hamid

Department of Marine Science, Iranian National Institute for Oceanography and
Atmospheric Science, Tehran, IR Iran

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Abstract

The essential oil extracted from the soft coral of *Sinularia* sp. have been extracted by soxhlet and analyzed by gas chromatography coupled to mass spectrometry (GC-MS). The principal compounds were found to be δ -Cadinene (12.4%), α -terpinene (10.7%) and α -Muurolene (8.3%). It is worth mentioning the presence of sesquiterpenes and diterpenes as main compounds of the essential oil. This is the first report on the essential oil of this species.

Keywords: *Soft corals; Sinularia sp.; Chabahar Bay; Essential oils.*

1. Introduction

Recent investigations of marine organisms as sources of new drugs have shown that marine environment is an exceptional reservoir of pharmacological active compounds (Anderson and Williams, 2000). A number of useful pharmaceutical metabolites bearing unique structural features have been isolated from marine biota in the last three decades (Faulkner, 2002; Blunt et al., 2003; Blunt et al., 2004).

Octocorals, particularly, soft corals belonging to the genus *Sinularia*, have been demonstrated to be the rich sources of bioactive natural products (Rocha et al., 2011). Marine soft corals of the genus *Sinularia* have attracted great attention in light of the structural diversity and wide range of biological

activities of their metabolites, including terpenoids and sterols (Blunt et al., 2012).

Sinularia comprises a group of soft corals belonging to the phylum Cnidaria, class Alcyonaria, and family Alcyoniidae. They are widely distributed from east Africa to the western Pacific, inhabiting the coral reefs or rocks in shallow water, but rarely forming large aggregates (Chen et al., 2012). Several species of *Sinularia* occur in the Persian Gulf and Gulf of Oman, however, so far only two of them have been identified and the rest are under further investigation.

They are *Sinularia compressa* Tixier-Durivault and *Sinularia erecta* Tixier-Durivault distributed commonly around Larak Island in the Strait of Hormuz and also recorded in Hengam and Farur Islands (Riegl and Dodge, 2012). However, to the best of our knowledge, there is no report about the

* E-mail: mehdinia@inio.ac.ir

species of *Sinularia* in Chabahar Bay.

Soft corals are marine invertebrates possessing a vast range of terpenoid metabolites. These terpenes, mainly cembranoids, represent the main chemical defense tools of animal against its natural predators (Hegazy et al., 2012).

A rich source of varied natural products are soft corals belong to the phylum Coelenterata (Cnidaria) i.e. marine invertebrates such as sea anemone, hydroids, corals, jelly fish and many other less distinguished animals.

The genus *Sinularia* consists of almost 90 species of which more than 50 have been chemically evaluated, including a hybrid species. Reported metabolites from the genus *Sinularia* displayed potential bioactivities such as antimicrobial, anti-inflammatory, and cytotoxic activities, closely related to the rich biodiversity of the marine environment.

These metabolites include sesquiterpenes, diterpenes, polyhydroxylated steroids, and polyamine compounds (Chen et al., 2012).

In our search, for bioactive compounds from marine sources, a soft coral, *Sinularia* sp., was collected from the Bay of Chabahar of Iran. In this work, preliminary screening was performed on the extracted materials from *Sinularia* soft corals of Chabahar Bay.

2. Materials and Methods

2.1. Collection of samples

The soft coral *Sinularia* sp. was collected by hand using scuba off the coast of the Chabahar Bay of Iran at a depth of 6 m in May 2010 and transferred to the laboratory in seawater. It was washed with double-distilled water three times and then, freeze-dried by Operon Freeze-dryer (FDB 5503, Korea) at -50 °C under vacuum and stored in

a freezer (-50°C) until extraction.

2.2. Extraction and Separation

The freeze-dried samples were milled. Ten grams of milled sample were extracted by Soxhlet extractor with 100 mL of dichloromethane-methanol (50:50) mixture. The extraction was performed by the method of Patra and Majumdar (2003) on a smaller scale. The sample was refluxed at 60 °C for 24 h. The extract was collected after 24h and concentrated to 1 mL with N₂. The extract was subjected to a C18 SPE cartridge.

The extracted material was eluted subsequently with n-hexane (5 mL) and ethyl acetate (5 mL). The extracts were combined and then, concentrated to 1 ml under N₂ stream. The final extracted material was injected to GC-MS instrument.

Gas chromatographic analysis was performed by an Agilent 6890 (Agilent, USA) Series N instrument equipped with a 5973 mass selective detector (Agilent, USA) and a split/splitless injector. The GC column was a HP-5 column (30 m× 0.25 mm i.d., 0.33 μm film thickness). Helium was used as a carrier gas at a flow of 1.3 mL min⁻¹. The oven temperature was programmed to increase from 50 °C to 300 °C at a rate of 10 °C min⁻¹. Injector and interface temperatures were set at 300 °C and 310 °C, respectively. The injection mode and volume were splitless and 1μL, respectively. The chemical compounds of extract were identified by Wiley 275 MS library.

3. Results

Figure 1 shows the chromatogram of extracted material from *Sinularia* sp. Identification of compounds was performed by Wiley 275 MS library.

Table 1 also shows the retention times and percent report of the identified compounds.

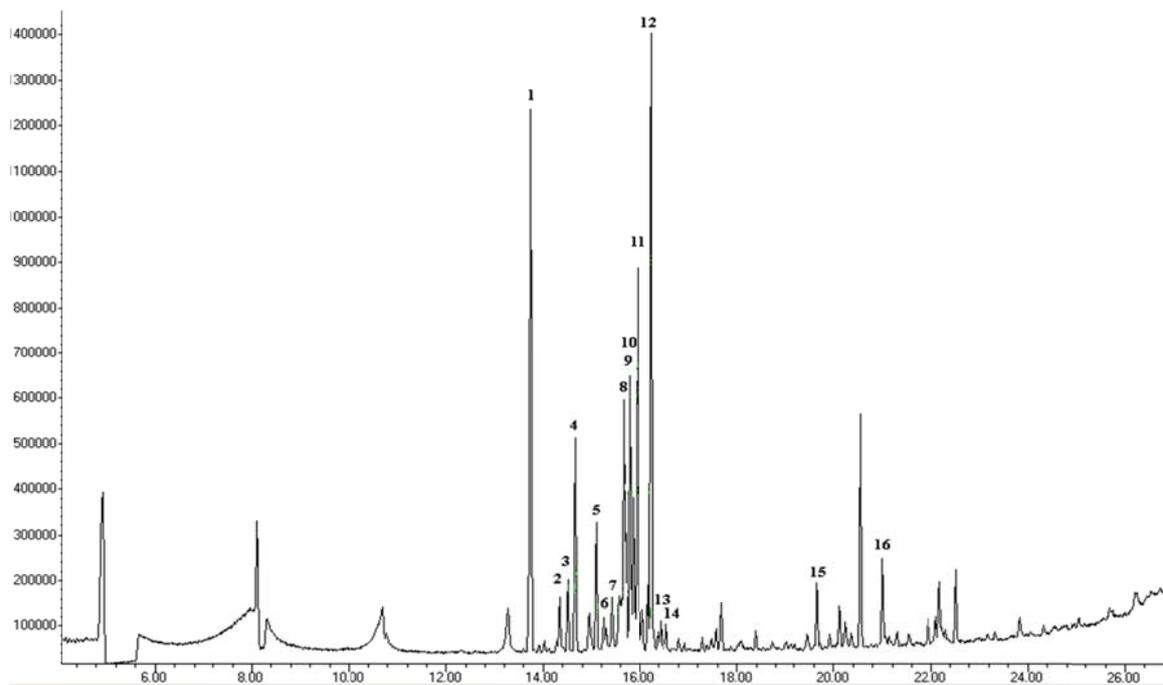


Fig 1: Chromatogram obtained from injection of extracted material from *Sinularia* Sp.

Table 1. Percent compositions and retention times of identified compounds by GC-MS

Compound No.	Retention time (min)	Compound name	Percent composition (%)
1	13.75	α -terpinene	10.75
2	14.34	γ -Cadinen	0.92
3	14.50	(-)-Sinularen	1.34
4	14.66	β -Cubebene	3.99
5	15.09	α -Copaene	2.45
6	15.25	(+)-Aromadendrene	0.78
7	15.42	Epizonarene	1.33
8	15.68	α -Amorphene	5.32
9	15.77	D-Germacrene	5.64
10	15.86	δ -Selinene	3.16
11	15.94	α -Muurolene	8.31
12	16.20	δ -Cadinene	12.41
13	16.45	α -Cadinene	0.38
14	15.54	α -Calacorene	0.37
15	19.67	Neophytadiene	1.11
16	20.99	Cembrenene	1.56

4. Discussions

As shown in Table 1, δ -Cadinene, α -terpinene and α -Muurolole represented about 52% of the total identified compounds in the studied coral. Cadinene was the trivial chemical name of a number of isomeric hydrocarbons that occur in a wide variety of essential oil-producing plants (Borg-Karlson et al., 1981). Chemically, the Cadinenes are bicyclic sesquiterpenes. Six new cadinene sesquiterpenoids were previously isolated from the Formosan Soft Coral *Xenia puertogalerae* (Duh et al., 2002). On the other hand, Cadinane was the most abundant sesquiterpene isolated from *T. atomariu* (a brown Alga), and it had been previously reported from the plant Piper cubeba (Andersen et al., 1973). δ -Cadinene is a bicycled sesquiterpene which have the stimulation effect on the testosterone hormone. Based on our study, δ -Cadinene comprized 12.4% of total identified compounds from *Sinularia* sp. Secondary metabolites with basic skeleton of cadinene type of sesquiterpenes had been previously isolated from the Red Sea soft coral *Heteroxenia fuscescens* (Mohammed et al., 2012).

The library of instrument proposed the percent probability of 98 % for α -terpinene. The monoterpene, α -terpinene, is used as a fragrance compound and is present in different essential oils. It is one of the components responsible for the antioxidant activity. α -Terpinene is structurally similar to other monoterpenes, e.g., limonene, known to autoxidize on air exposure and form allergenic compounds (Rudbäck et al., 2012).

The presence of α -Muurolole in marine organisms like the soft-coral *Heteroxenia fuscescens* (Kashman et al., 1978) and the sponge *Acanthella cavernosa* (Hirota et al., 1996) has also been reported. Analysis of *Heterogenic fuscescens*, the soft coral of Red Sea, led to the isolation of 6-hydroxy- α -muurolole from the alcoholic extract of the coral. α -Muurolole is a sesquiterpene that is active against *Staphylococcus aureus* and *Escherichia coli* with MIC of 19 μ g/mL (Mohammad et al., 2012).

D-Germacrene and α -Amorphene are the next compounds as a major group of metabolites obtained in the studied coral. Germacrene-D is sesquiterpene which have been obtained from the marine octocoral *Muricea austere* (Fraga, 2006).

Amorphenes are stereo-isomers of cadinenes and muurololes, and are biosynthetically related to β -bourbonene and germacrene D (Snajberk and Zavarin, 1975). α -Amorphene has previously extracted from essential oils of different plants (Muanda et al., 2011; Phutdhawong et al., 2007).

β -Cubebene, δ -Selinene and α -Copaene are the next groups of abundant compounds. β -Cubebene is a cadinane-type sesquiterpenes which is first isolated from plant of Cubeb (Suzuki et al., 1981).

The frequent close associations of germacrenes and selinenes suggests that they may be biosynthetically closely related, either both being derived from a common intermediate or the germacrenes being biosynthetic intermediates from which the selinenes or other compounds are formed (Martdp and Langenheim, 1976). Selinenes are a group of closely related isomeric chemical compounds which are classified as sesquiterpenes. They have been isolated from a variety of plant sources (Attokaran, 2011).

α -Copaene, is the common chemical name of an oily liquid hydrocarbon that is found in a number of essential oil-producing plants. The name is derived from that of the resin-producing tropical copaiba tree. Chemically, the copaenes are tricyclic sesquiterpenes. α -Copaene is strongly attracting to an agricultural pest (Murugesan et al., 2012).

(-)-Sinularen, Epizonarene, Neophytadiene and Cembrenene are the components that totally form about 9% of the identified compounds. Therefore, their concentrations were low in the studied soft coral. The sesquiterpene (-)-sinularene with a new non-famesolic skeleton (i.e., cannot be derived by direct cyclization of a farnesol precursor) was previously isolated from the soft coral *Sinularia*

mayi (Beechan et al., 1978). Epizonarene is a cadalenic sesquiterpene. Neophytadiene (3-methylene-7,11,15-trimethylhexadecen-1-ene) is a branched hydrocarbon belonging to the group of compounds known as phytanes, and is abundant in plant cuticular waxes (Hernandez-Matamoros et al., 2013). It is also reported in Tobacco smoke (Guerin and Olerich, 1975).

Cembrene is the most widespread representative of cembrane type diterpenoids. Of the chemical properties of this natural compound are electrophilic cyclizations and oxidative transformations (Tkachev and Vorobjev, 2000). Cembrane-type diterpenoids are a large and structurally varied group of natural products isolated from both terrestrial and marine organisms (Ortega et al., 2008). The parent hydrocarbon cembrene was reported from *S. mayi* along with cembrenene (Uchio et al., 1981). Cembrenes are the most frequent secondary metabolites isolated from various *Sinularia* species (Chen et al., 2012). Lin et al. (2012) identified two new Cembrane-based diterpenoids from the marine soft coral *Sinularia crassa*. Five new cembranoids, grandilobatins were isolated from the soft coral *S. grandilobata* (Ahmed et al., 2008).

Qin et al., (2008) found a new cembrane-based diterpene, diepoxycembrene A, from the Hainan soft coral *Sinularia* sp.

Finally, γ -Cadinene, (+)- Aromadendrene, α -Cadinene and α -Calacorene were obtained as the lowest values. Aromadendrene was previously identified from *S. mayi* (Beechan et al., 1978). Several aromadendrane diterpenoids have also been isolated from *Sinularia* species (Kamel and Slattery, 2005). α -Calacorene is a sesquiterpene which have originated from fruit essential oils. It was previously found in the Essential Oil of the *Pycnocycla spinosa* Decne. Exboiss (Asghari et al., 2002) and *Teucrium montanum* (Vukovic et al., 2007).

5. Conclusions

Among the various classes of secondary metabolites

that are produced by soft corals, sesqui- and diterpenes were major divisions produced by *Sinularia* sp., collected from the waters of Chabahar Bay. Monoterpenes come next as a major group of metabolites characterizing corals.

The major identified constituents in *Sinularia* sp. were terpenoids, firstly oxidized terpenes, which include α -terpinen as one of the major constituents. Cembrane-type diterpenoids are the other compounds of the organic extract of the soft coral, *Sinularia* sp., collected from the waters of Chabahar, and compounds of this type have been shown to have the potential for use in medical applications.

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