Journal of Agricultural and Marine Sciences 2024, 29(2): 17– 32
DOI: 10.53541/jams.vol29iss2pp17– 32
Received 12 August 2023
Accepted 21 April 2024

RESEARCH PAPER

Bioactive Compounds from Omani Soft Corals Belonging to Genera *Sarcophyton* and *Sinularia*

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المركبات النشطة بيولوجيا من الشعاب المرجانية العمانية الناعمة والتي تنتمي إلى جنس ساركوفايتون (*Sarcophyton*) و سينيولاريا (*Sinularia*)

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Abstract. Soft corals are marine invertebrates belonging to the class *Anthozoa* and are rich in bioactive substances that provide their defense and protection from predators, pathogens, and other unfavorable organisms. Seven soft coral species belonging to the genera *Sarcophyton* and *Sinularia* were collected from Omani water and extracted and their antibacterial and antifungal properties were tested using the agar-disk diffusion method and a MicroResp technique. Potential compounds that were present in the species were characterized using GC-MS analysis. The results showed that the soft coral species have anti-bacterial activity against Gram-negative bacteria *Pseudomonas putida* and *Pseudomonas tunicata*. The GC-MS analysis found the presence of antibacterial, antifungal, antiviral, anticancer, antioxidant agents, agrochemicals, and medicine chemicals. Three novel compounds were found in a *Sarcophyton* sp. In addition, the compound Andrographolide found in a *Sarcophyton* sp. has been recently studied as an antiviral agent against SAR-CoV-2. Moreover, the compound Decosane which is found in *Sinularia* sp. has been used as an anti-parasitic agent against the female *Anopheles* mosquito to treat malaria in Pakistan. This study highlighted the importance of Omani soft corals as a source of novel bioactive compounds.

Keywords: Soft coral; Bioactive compound; Biological activity; Antibiotics; Sea of Oman.

الخلاصة: المرجان الناعم عبارة عن لافقاريات بحرية تنتمي إلى فئة الأنثوزوا وهي غنية بالمواد النشطة بيولوجيًا والتي توفر لها الدفاع والحماية من الحيوانات المفترسة ومسببات الأمراض وغيرها من المواد غير المرغوبة. تم جمع سبعة أنواع من المرجان الناعم التي تنتمي إلى جنس *Sarcophyton* و *Sinularia* من المياه العمانية وتم استخلاصها واختبار خصائصها المضادة للبكتيريا والفطريات باستخدام طريقة انتشار قرص الأجار وتقنية MicroResp. تم تشخيص المركبات المحتملة في هذه الأنواع باستخدام تحليل GC-MS. أظهرت النتائج أن أنواع المرجان الناعمة لها نشاط مضاد للبكتيريا ضد الأنواع سالبة الجرام *Pseudomonas putida* و *Pseudomonas tunicata*. بينما أظهر تحليل GC-MS وجود مضادات للبكتيريا، ومضادات للفطريات، ومضادات للفيروسات، ومضادات للسرطان، وعوامل مضادة للأكسدة، وكذلك أظهر وجود مركبات كيميائية زراعية، ومواد كيميائية طبية. تم العثور على ثلاثة مركبات جديدة في المرجان *Sarcophyton sp*. بالإضافة إلى ذلك، تم العثور على مركب *Andrographolide* في المرجان *Sarcophyton sp*. هذا وقمت دراسته مؤخرًا كعامل مضاد للفيروسات ضد SAR-CoV-2. علاوة على ذلك، فإن مركب ديكوزان الموجود في المرجان *Sinularia sp*. تم استخدامه كعامل مضاد للطفيليات ضد أنثى بعوضة الأنوفيلة لعلاج الملاريا في باكستان. سلطت هذه الدراسة الضوء على أهمية الشعاب المرجانية العمانية الناعمة كمصدر لمركبات نشطة بيولوجياً جديدة

الكلمات الرئيسية: المرجان الناعم؛ مركب نشط بيولوجيا. النشاط البيولوجي؛ مضادات حيوية؛ بحر عمان.

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Introduction

Soft corals are marine invertebrates that belong to the phylum *Cnidaria*, class *Anthozoa*, subclass *Octocorallia* and to the order *Alcyonacea* which involves three families *Xeniidae*, *Nephtheidae*, and *Alcyoniidae;* many genera are included within these families (Aratake et al., 2012). Soft corals exist throughout the entire ocean, from shallow tropical waters to deep cold waters (Fabricius et al. 1995). In contrast to the hard corals which belong to the same class *Anthozoa*, soft corals do not have the calcium carbonate skeleton which acts as a defense mechanism for the hard corals. Therefore, soft corals use toxic and bioactive compounds to protect themselves against predators (Van et al., 1992; Dobretsov et al. 2016). Experiments with soft corals showed that compounds produced by soft corals lead to the death of nearby hard corals (Sammarco et al., 1987).

Antimicrobial, anti-cancer, antiviral, antifouling, and antifungal biological activities of soft corals have been intensively studied (Kim et al., 1994; Weinheimer et al., 1977; Seo et al., 1995; Su et al., 1993; Anthoni et al., 1991). A number of marine natural products have been isolated from soft corals. For example, a study of Red Sea marine organisms' cytotoxic and antiviral activities suggested that soft corals *Sarcophyton trochliophorum* and *Litophyton arboreum* have potent activity against HeLa and U-937 cancer cell lines (Ellithey et al., 2014). The antimicrobial activity of soft corals belonging to genera *Parerythropodium*, *Dendronephthya*, *Lobophytum,* and *Sarcophyton* have been studied (Kelman et al., 1998; Harder et al., 2003; Gomaa et al., 2016; Zhao et al., 2013). One of the studies investigated the antimicrobial effect of the Red Sea soft coral *Sarcophyton trocheliophorum* against bacteria *Bacillus cereus*, *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus,* and *Pseudomonas aeruginosa*. The results showed intense antimicrobial activity which was linked to the presence of terpenoid bioactive derivatives in the crude extracts (Gomaa et al., 2016). In addition, the potential antioxidant activity of the same soft coral species was studied using the DPPH radical scavenging method. The study linked the antioxidant activity of soft corals to the presence of vitamin E in their extracts (Kim 1994). Therefore, marine organisms that have defensive features, such as soft corals, could be used as antibiotic products against bacterial, fungal, cancerous, viral, and parasitic diseases.

Sinularia and *Sarcophyton* are the most dominant soft coral genera under the family *Alcyoniidae* (Aratake et al. 2012). The species of these genera are resistant to predation and live for a long time (Mcfadden et al., 2006). These soft coral species are made up of tiny animals called polyps which connect to each other and act like a single organism. Each soft coral species varies from the others according to its colony-forming polyps and zooxanthellae type. *Sarcophyton* species have a special sterile stalk, a wide mushroom-shaped top known as the capitulum (Aratake et al., 2012). The capitulum of the *Sarcophyton* could change its shape into a cup or funnel-shaped one for preservation in case of touch and thereof (Verseveldt et al. 1982). On the other hand, *Sinularia* species are low and flat in shape and they do not have a stalk. The colony is dish-shaped (Verseveldt et al., 1980).

Species of soft corals *Sinularia* and *Sarcophyton* are rich in bioactive compounds. Some new compounds were isolated from these soft corals (Rodrigues et al., 2019). These compounds have potent biological properties. A new 18-oxygenated polyhydroxy steroid from the soft coral *Sarcophyton* sp. showed cytotoxic and antimicrobial activities (Sun et al., 2013). In addition, a new 9,11-secosteroid, 25(26)-dehydrosarcomilasterol, two new polyhydroxylated steroids, 7α-hydroxy-crassarosterol A and 11-acetoxy-7α-hydroxycrassarosterol A were isolated from the soft corals *Sarcophyton trocheliophorum* and *Sinularia flexibilis*, respectively.

The compounds were tested against the tumor cell lines K562 and HL-60 (Chen et al., 2014). Moreover, three new cembranoids: sarcophytolol, sarcophytolide B, and sarcophytolide have been isolated from the soft coral *Sarcophyton glaucum* which showed cytotoxic activity against human hepatocellular liver carcinoma, human breast adenocarcinoma and prostate cancer (Al-Lihaibi et al., 2014). Essential oils were extracted from the Iranian soft coral *Sinularia* sp. (Mehdinia et al., 2014). This was the initial report on the essential oil of this species.

Only a few studies of bioactive compounds from soft corals inhabiting the Arabian Gulf and the Sea of Oman have been published (Erfani et al., 2015; Dobretsov et al., 2016; Gozari et al., 2019). The extracted compounds from Omani marine organisms showed strong activity against the breast adenocarcinoma model (Dobretsov et al., 2016). Malformin A, kuanoniamine D, hymenialdisine, and gallic acid have strong anticancer effects. This indicates that, in particular, Omani organisms and soft corals can have high biotechnological potential.

This study was aiming to test the antimicrobial and antifungal activity of the soft corals *Sinularia* spp. and *Sarcophyton* spp. and to characterize the potential compounds that are present in these species.

Materials and Methods

Soft Coral Collection and Extraction

The seven species of soft corals belonging to the genera *Sinularia* and *Sarcophyton* were collected by SCUBA diving from the Bander Al-Khairan coast in the Sea of Oman (Figure 1, Supplementary Table 1). Immediately, specimens were placed individually in clean plastic bags and stored on ice on the boat. At the SQU Marine Science and Fisheries laboratory, the samples were stored at -20°C for further processes.

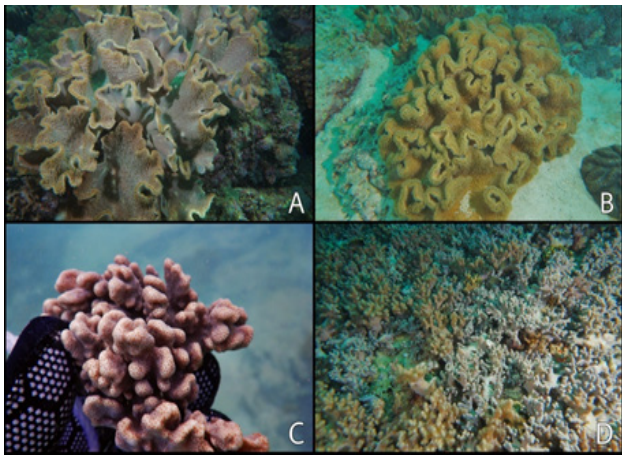

Figure 1. Photographs of the soft coral species belonging to the genera *Sarcophyton* and *Sinularia*: a) *Sarcophyton* BK40401; b) *Sarcophyton* BK40402; c) *Sinularia* BK1105191; d) *Sinularia* BK1105192.

After a few days, the stored soft corals were cleaned, chopped into small pieces, weighed, and placed in separate clean glass flasks for the extraction process. Soft coral specimens were extracted by an equal ratio (1:1) of the solvent mix (Methanol: Ethyl Acetate, Sigma). The solvent fully covered the soft coral material. Specimens were extracted for 5 days under shaking. This step was repeated twice in order to extract all compounds from the coral. Then, both extracts were combined and filtered using 9 cm Whatman filter paper. Finally, the samples were evaporated until dryness under reduced pressure using a rotary evaporator (Buchi) at the temperature of 40°C. The crude extract of each soft coral sample was weighed and then dissolved in the same solvent mix (Methanol: Ethyl acetate, 1:1). Different concentrations of the crude extracts (0.15 mg/ml, 0.5 mg/ml, and 0.8mg/ml) were prepared by diluting the extracts using Methanol: Ethyl acetate, 1:1. These concentrations of extracts were used as standard concentrations for the further tests (see below).

Anti-microbial Activity

An agar-disk diffusion method was used to investigate the antimicrobial activity of the soft corals' extracts. Species of Gram-positive and Gram-negative bacteria were chosen for the an-

Supplementary Table 1. Sampling information about the soft coral species.

Genera	Label	Coordinates	Depth (m)	Sampling date
Sarcophyton	BK40401	23.520457N 58.747446E	10	04/04/2019
Sarcophyton	BK40402	23.520457N 58.747446E	10	04/04/2019
Sarcophyton	BK40403	23.520457N 58.747446E	10	04/04/2019
Sarcophyton	BK40404	23.520457N 58.747446E	10	04/04/2019
Sinularia	BK1105191	23.518834N 58.755429E	5	11/05/2019
Sinularia	BK1105192	23.518834N 58.755429E	5	11/05/2019
Sinularia	BK1105193	23.518834N 58.755429E	5	11/05/2019

timicrobial activity test. The test was carried out on separate days for each bacterial species. First, freshly cultured bacteria were prepared by inoculating pure bacterial colonies of *Lysinibacillus fusiformis* (Gram-positive) and *Pseudomonas putida* (Gram-negative) to freshly autoclaved marine broth (Sigma, USA). Both bacteria were isolated previously from seawater. The broth with the bacterial inoculum was incubated at 37 ℃ for 24 hours. Second, freshly prepared Marine agar (MA, Sigma, USA) was inoculated with a bacterial culture of *L. fusiformis* and *P. putida* (OD620: 0.08 ABS for *L. fusiformis* and OD620: 0.03 ABS for *P. putida*). Third, 20µL of each concentration (0.15, 0.5, and 0.8 mg/ml) of extracts were added individually to sterile paper disks (6mm in diameter). Then, the extracts were evaporated until fully dry. Disks immersed in 20% gentamicin (Sigma) were used as a positive control and sterile paper disks were used as a negative control. Forth, the disks with extracts or control ones were placed onto the inoculated MA plates. The MA plates were incubated at 37 ℃ for 24 hours. Finally, the plates were observed, and the diameters of the growth inhibition zones were measured using a ruler and recorded. The test was carried out in 3 replicates for each soft coral extract of each bacterial species.

Microbial Respiration Rate

The MicroResp™ technique was used to analyze the microbial respiration rate of the same species of Gram-positive *L. fusiformis* and Gram-negative bacteria *P. putida* in the presence of soft coral extracts. This method is based on measuring the amount of CO_2 produced by microbes and absorbed by an indicator stock solution during its growth phases for a period of time. The principle of this method is by exposing an indicator stock solution to the CO_2 emitted by microbes in a closed controlled ambient environment. The amount of CO_2 emitted by the microbes is greater when microbes are growing and respiring.

The MicroResp colorimetric method contains several stages. First, 3% purified agar and indicator stock solution using a 1:2 ratio

(agar: indicator) was mixed and warmed in the 60°C water bath. Second, each well of an autoclaved multi-well plate (96 wells, Nunc, Denmark) was filled with 150 µL of this solution. Third, the detection plate was kept under the desiccator in a dark place for at least 4 days in order to equalize the agar color. Then, freshly-cultured bacteria were prepared one day before the test by inoculating pure bacterial colonies of *Lysinibacillus fusiformis* (Gram-positive), *Pseudomonas putida,* and *Pseudomonas tunicata* (Gram-negative) to freshly autoclaved marine broth (Sigma, USA). The cultures were incubated at 37 ℃ for 24 h. On the day of the test, the detection plate was read at 570 nm by an Emax MultiScan microplate reader (Molecular Devices, USA) and the time was recorded. Each well of sterile 96-well 1.2 mL and deep-well plate (James Hutton Limited, UK) was filled with 100µL of [0.5mg/mL] soft coral extracts and 500 µL of standard inoculum of bacteria (OD620: 0.03 ABC). Methanol: Ethyl Acetate (1:1) was used as a control. There were 4 replicates for each inoculum. Fourth, the detection plate was assembled with the deep-well plate using the MicroResp seal (James Hutton Limited, UK). Finally, the assembled device was fixed using a metal clamp (James Hutton Limited, UK), and kept under a desiccator at room temperature (25°C) in a dark place. The plate was read twice using a microplate reader (MultiScan) after 3 h and 22 h. The data were analyzed by MicroResp equations using Microsoft Excel software.

As a first step, we exported and sorted the data from the spectrometer program into an Excel spreadsheet. Second, the normalization of Absorbance data (Ai) was done by the following equation:

$$Ai = (At22/At0) \times Mean\ (At0) \qquad (1)$$

Third, the following formula used to convert the (Ai) into $\%CO_2$:

$$\%CO_2 = A + B/\ (1 + D \times Ai), \qquad (2)$$

where A = -0.2265, B = -1.606 and D = -6.771, Finally, the CO_2 rate (µgC/ml/h) was calculated as following:

$$(((\%CO_2/100) \times volume\ (\mu L) \times (44/22.4) \times (12/44) \times (273/\ (273 + T(°C))))/\ (Specimen\ volume\ (mL/well)))/Incubation\ Time(hour) \qquad (3)$$

where the headspace volume is normally 945 µL for the standard set-up adjusted according to manufactures specifications for the microplate and deep-well plate. The specimen weight is 1 mL/well, the temperature is 25°C, and the incubation time was 22.33 h. The results were represented through Excel graphs and ANOVA statistical analysis with a LSD test was applied for the whole set of extracts and three different bacteria species.

Anti-fungal Activity

The antifungal activity of soft coral extracts was tested against 3 species of plant and human pathogenic fungi including *Fusarium* sp., *Pythium* sp., and *Rhizoctonia* sp. (Munkvold, 2017; Del, 2016; Ajayi, 2017). An agar disk diffusion method was used to test the anti-fungal activity of extracts (Kim, 2018). Before the experiment, three dilutions (0.15, 0.5, and 0.8 mg/mL) of soft coral extracts were filtered by Whatman® Nylon 0.2µm filters. The three species of fungi were cultured on PDA (potato dextrose agar, Sigma) agar plates prior to the study. The fungal cultures were incubated at 30°C for several days depending on the fungal species: *Fusarium* sp. was incubated for 6 days; *Pythium* sp. was incubated for one day and *Rhizoctonia* sp. for two days. Then, fungal mycelia were cut using a 6 mm cork. Finally, each fungal cut was placed in the middle of the freshly prepared PDA agar plate. Three paper disks (6 mm in diameter) with extracts (concentrations 0.15, 0.5 and 0.8 mg/mL) were placed 1 cm away from the fungal inoculum. A fourth disk containing the solvent mix was used as a control. The plates were kept

at room temperature and were daily observed for a week. There were 2 replicates for each treatment.

Soft Coral Extracts as Plant Growth Promoters

The effect of two soft coral extracts (BK1105191 and BK40404, Supplementary Table 1) was tested on the growth of radish seeds. We proposed that soft coral extracts could contain supplementary compounds for plant growth. In order to do the experiment, a 9 cm Whatman® filter paper was placed onto an empty Petri dish and 15 mL of 0.1 mg/mL soft coral extracts were added. About 5 radish seeds were placed at equal intervals onto the Petri dish and covered by a cover. The plate was sealed with Parafilm®, and the three holes were made in the cover. The test was carried out in 3 replicates. Petri dishes inoculated with tap water were used as a control. The plates were kept at room temperature and seeds were observed for 5 days.

Chemical Composition of Extracts

Gas Chromatography coupled with Mass Spectrometry was used for separating and detecting the organic compounds in the extracts of seven species of soft corals. GC-MS analysis was performed on a Perkin Elmer Clarus 600 GC System, fitted with an Rtx-5MS capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness; maximum temperature, 350°C), coupled to a Perkin Elmer Clarus 600C MS. Ultrahigh purity helium (99.9999%) was used as carrier gas at a constant flow of 1.0 mL/min. The injection, transfer line, and ion source temperatures were 280, 270, and 270°C, respectively. The ionizing energy was 70 eV. Electron multiplier (EM) voltage was obtained from autotune. All data were obtained by collecting the full-scan mass spectra within the scan range of 40-550 amu. The injected sample

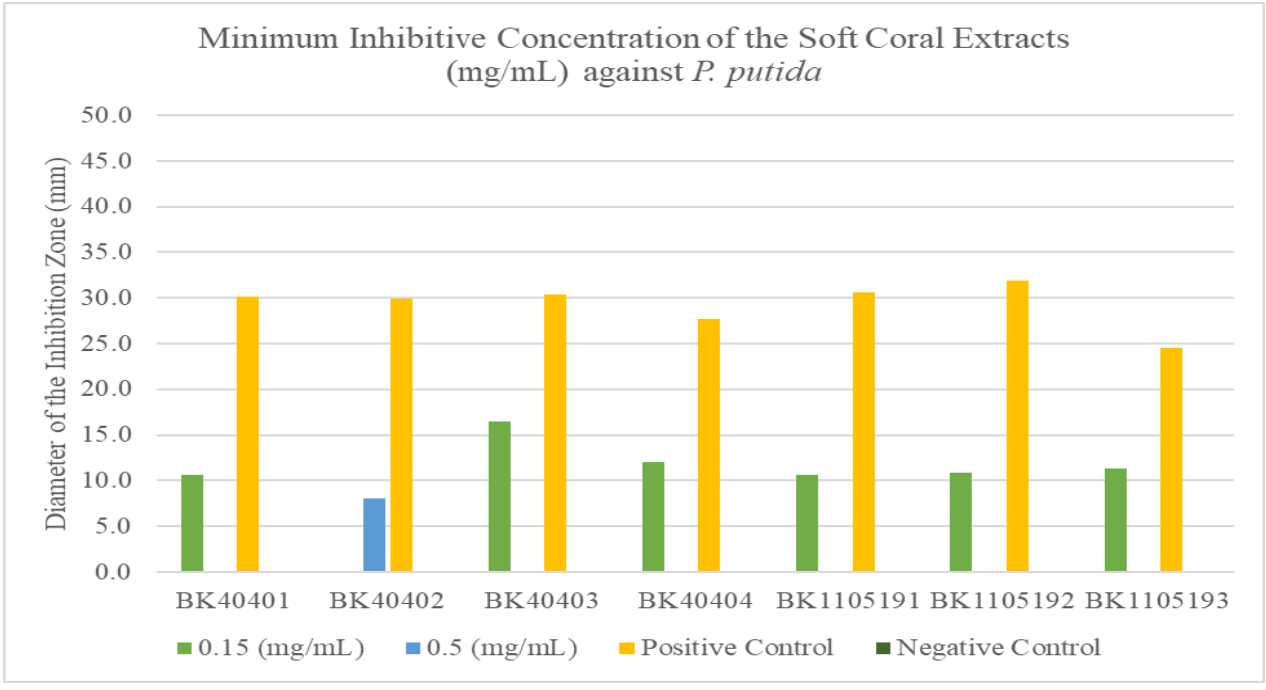

Figure 2. Minimum Inhibitive Concentrations (MIC, mg/mL) of the soft coral extracts of BK40401, BK40402, BK40403, BK40404, BK1105191, BK1105192, and BK1105193 against *P. putida*. Positive control – gentamicin, negative control – solvent.sitive control – gentamicin, negative control – solvent.

volume was 1 µL with a split ratio of 10:1. The oven temperature program was 60°C at a rate of 8°C/min and 280°C hold for 25 minutes. The run time was 53.5 minutes. The unknown compounds were identified by comparing the spectra obtained with mass spectrum libraries (NIST 2011 v.2.3 and Wiley, 9th edition). Finally, all chemical compounds that were listed for each soft coral extract were analyzed using the PubChem website (https://pubchem.ncbi.nlm.nih.gov/, National Library of Medicine, 2004), and then the most dominant compounds were listed. The composition of extracts from different species was clustered and ordinated using Excel and PAST software; the ordination was applied by a Non-Metric MDS statistical technique. NMDs is an indirect gradient analysis approach that produces an ordination based on a distance or dissimilarity matrix (Buttigieg et al., 2014).

Results

Soft Coral Extracts

Table 1 shows the weight (g) of soft corals and their extracts. Among *Sarcophyton* species, the highest weight of extracts was recorded for BK40403, while the lowest was found for BK40402. Among *Sinularia* species the highest weight of extracts was recorded for BK1105193, while the lowest was found for BK1105192. The weight of extracts from *Sinularia* was higher than for *Sarcophyton* species (Table 1). Generally, the color of extracts from *Sarcophyton* species was darker than from *Sinularia* spp.

Anti-microbial Activity

The agar-disk diffusion method demonstrated that there was no antimicrobial activity of coral extracts against the Gram-positive *L. fusiformis* bacterium (data are not shown). On the other hand, the extracts of soft corals inhibited the growth of the Gram-negative *P. putida* as shown in Figure 2. The growth of the bacterium was inhibited by the concentration of 0.15mg/mL except BK40402 (Figure 2). The inhibition of growth was concentration depended; higher concentrations resulted in bigger inhibition zones. However, the diameter of the inhibition zone was smaller compared to the positive control (gentamicin).

Microbial Respiration Rate

In order to confirm the antibacterial activity of the extracts, the MicroResp test was conducted. The result of the MicroResp test showed that

Table 1. The weights before and after the extraction of crude oils from soft coral species. Me – methanol, EA – ethyl acetate.

Sample	Genera	Weight of coral (g)	Weight of extract (g)	Volume of Solvents used (ml) (Me:EA)	Color tone of extract
BK40401	Sarcophyton	434	335	500:500	Dark brown
BK40402	Sarcophyton	321	308	250:250	Light olive
BK40403	Sarcophyton	787	712	500:500	Olive
BK40404	Sarcophyton	928	753	300:300	Lime
BK1105191	Sinularia	857	746	350:350	Light lime
BK1105192	Sinularia	714	619	350:350	Light brown
BK1105193	Sinularia	921	787	300:300	Pale lime

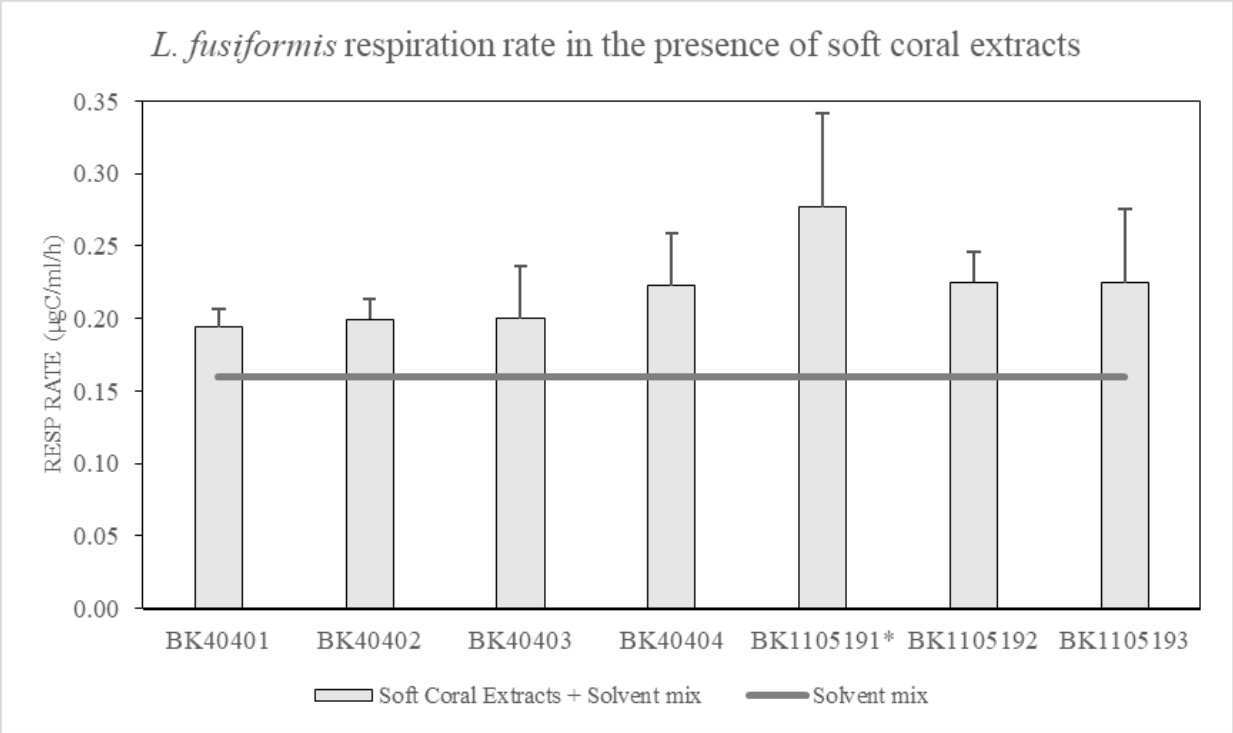

Figure 3. *L. fusiformis* respiration rate (µgC/mL/h) in the presence of soft coral extracts BK40401, BK40402, BK40403, BK40404, BK1105191, BK1105192 and BK1105193. The straight line represents the control respiration rate. *BK1105191 has a significantly higher (P<0.05) respiration rate.

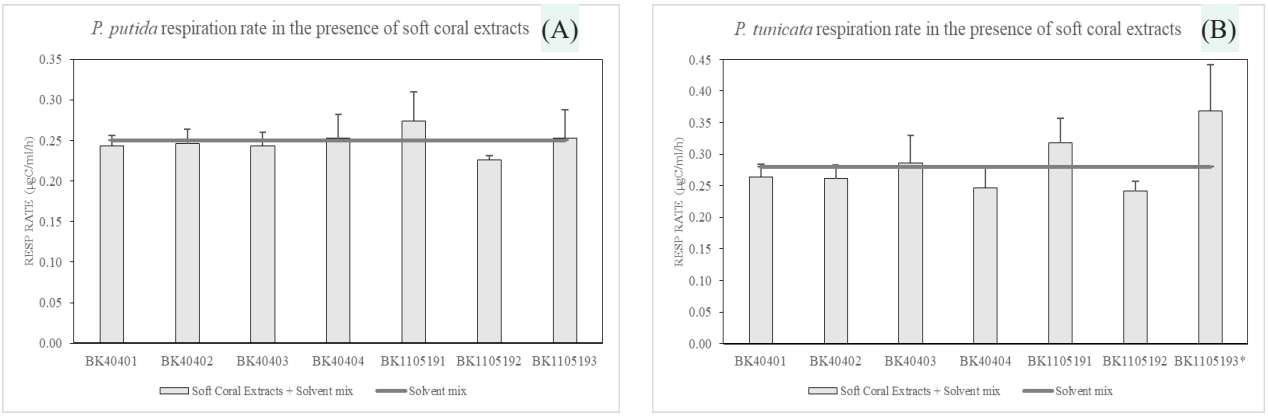

Figure 4. The respiration rates (µgC/mL/h) of *Pseudomonas putida* (A) and *Pseudomonas tunicata* (B) species in the presence of soft coral extracts. Only BK1105192 significantly (P<0.05) reduced respiration rate. The straight line represents the control respiration rate.

there was no antimicrobial effect of soft coral extracts on the Gram-positive bacterium *L. fusiformis* (Figure 3). On the opposite, the microbial respiration rate for BK1105191 was significantly higher (ANOVA, P<0.05) than that of the control (solvent mix). This suggests that coral extracts enhanced the growth of the bacterium possibly by providing some nutrients.

On the other hand, the Gram-negative bacteria (*P. putida* and *P. tunicata*) showed a lower respiration rate in the presence of some soft coral extracts compared to the control (Figure 4). The respiration rate of *P. putida* in the control treatment was 0.25µgC/ml/h on ave-

rage (Figure 4a). Only the extract of soft coral BK1105192 significantly (ANOVA, P<0.05) reduced the microbial respiration rate compare to the solvent control (Figure 4a). The bacterium *P. tunicata* had a respiration rate of 0.28 µgC/ml/h on average in the control (Figure 4b). The extract of BK1105192 significantly (ANOVA, P<0.05) reduced the microbial respiration rate.

Table 2. The most dominant chemical compounds (>10%) in each of the seven soft coral species which were investigated by the GC-MS process.

Soft coral code/ species	Chemical compound	Proportion (%)
Sarcophyton spp.		
BK40401	Fucosterol	21.5
	Hexadecanoic acid, hexadecyl ester	11.9
	22,23-Dibromostigmasterol acetate	12.4
BK40402	Unidentified at RT 26.888min	47.8
	Unidentified at RT 27.148min	30.2
	Unidentified at RT 27.560min	12.9
BK40403	Heptadecanoic acid, methyl ester	15.1
	Retinol, acetate	16.4
	Methyl arachidonate	17.3
BK40404	22,23-Dibromostigmasterol acetate	12.5
***Sinularia* spp.**		
BK1105191	Octadecanoic acid, methyl ester	13.5
	Heptadecanoic acid, 16-methyl-, methyl ester	15.0
	Fucosterol	30.4
BK1105192	.(-)-Alloaromadendrene	21.0
	22,23-Dibromostigmasterol acetate	12.6
BK1105193	Alloaromadendrene	11..2
	Germacrene D	15.6
	Palmitic acid, methyl ester	26.3

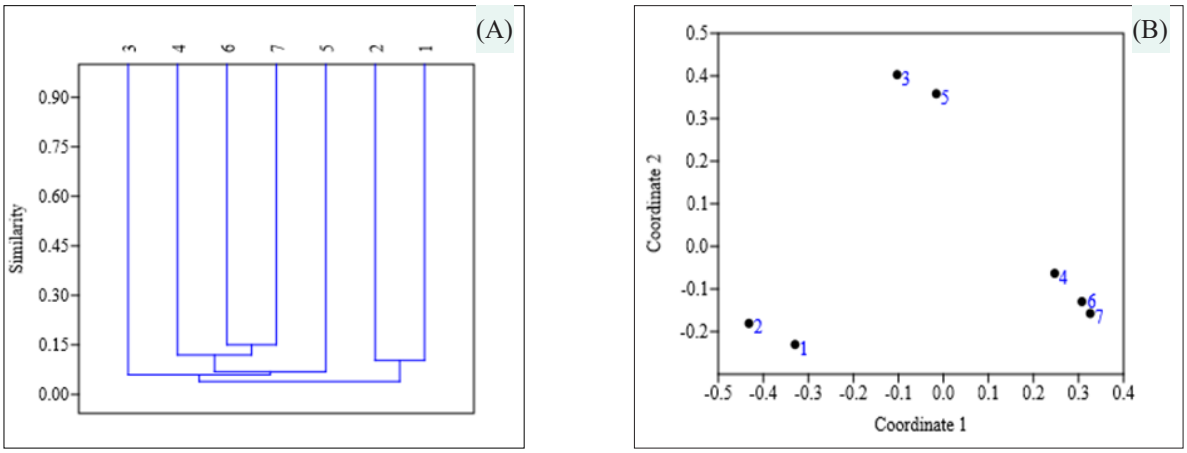

Figure 5. The comparison of chemical compounds in soft corals and similarity between extracts: (A) The cluster analysis between extracts in soft corals: (1) BK40401; (2) BK40402; (3) BK40403; (4) BK40404, (5) BK1105191; (6) BK1105192; (7) BK1105193. (B) The NMDs analysis of chemical compounds in soft corals.

On the opposite, the extract BK1105193 showed a significant increase (ANOVA. $P<0.05$) in the respiration rate of the bacterium up to 0.37µg ± 0.07(SD) C/ml/h compared to the control.

Anti-fungal Activity

No antifungal activity was observed for tested soft coral extracts in the disk diffusion method against the hazardous fungal species *Fusarium* sp., *Pythium* sp., and *Rhizoctonia* sp. (results are not shown). After 48h, the fungi overgrown the disks with the extracts.

Soft Coral Extracts as Plant Growth Promoters

The test demonstrated that all extracts completely stopped the growth of the seeds compared to the control (water only, data are not shown). This suggests that soft coral extracts are toxic to seeds and cannot be used as nutrient supplements.

Table 3. Varied chemical compounds were investigated by GC-MS technique from seven species of soft corals and their contents in percentages range (5% ~ 10%). The compounds were classified according to their possible uses.

Activity	Chemical compounds	Proportion%	Soft coral code
Anti-bacterial agents	Chlorocyanopropyldimethylsilane	5.1	BK40403
	2-Methyl-Z,Z-3,13-octadecadienol	9.3	BK1105191
	Methyl linoleate	5.1	BK1105193
	Andrographolide	6.7	BK40403
Anti-parasitic agent	Docosane	7.1	BK1105191
Anti-fungal agent	trans-Farnesol	6.1	BK40403
Anti-viral agents	Andrographolide	6.7	BK40403
	Cyclopentane, heneicosyl-	6.9	BK1105192
	Methyl linoleate	5.1	BK1105193
Anti-cancer agent	Andrographolide	6.7	BK40403
Human health uses	Palmitic acid, methyl ester	9.4	BK40404
	Methyl stearate	6.5 and 5.2	BK40404 and BK1105193
	Z-8-Octadecen-1-ol acetate	9.8	BK40404
	Methyl arachidonate	10	
	Lanolin	8.9	
	Arachidonic acid	7.1	BK1105191
	β-Cadinene	5.8 and 5.2	BK1105192 and BK1105193
	Methyl linoleate	5.1	BK1105193
Anti-oxidant agent	β-Cadinene	5.8 and 5.2	BK1105192 and BK1105193
Agrochemicals	β-Cadinene	5.8 and 5.2	BK1105192 and BK1105193
	(Z)-9-(E)-12-Tetradecadien-1-ol acetate	BK1105193	BK1105193

GC-MS Analysis

Table 2 is showing the most dominant compounds and their proportion in extracts of soft coral species investigated by GC-MS. The most dominant chemical compounds in the soft coral BK40401 (*Sarcophyton* sp.) are Fucosterol (21.5%), 22,23-Dibromostigmasterol acetate (12.4%) and Hexa-decanoic acid hexadecyl ester (11.9%). Compare to other soft corals, BK40402 has three unidentified compounds that were present in large quantities (47.8%- 12.9%) (Table 2). These could be novel compounds. BK40402 (*Sarcophyton* sp.) it is the only extract that has un-identified compounds in large quantities. In addition, there are only 2 compounds presented at less than 3%. The soft coral BK40403 (*Sarcophyton* sp.) contains the following dominant compounds: Heptadecanoic acid, methyl ester (15.1%), Retinol, acetate (16.4%), and Methyl arachidonate (17.3%) (Table 2). The soft coral BK40404 (*Sarcophyton* sp.) has only one compound dominant compound 22,23-Dibromostigmasterol acetate that presents at >10%. This compound is also present in the soft coral BK40401 (12.4%), as well as in the soft coral BK1105192 (12.6%). However, in BK40402 and BK40403 this compound was present at lower quantities (1-0.7%) (Table 2).

Different compounds were found in *Sinularia* spp. The soft coral BK1105191 contains the following dominant compounds: Octadecanoic acid, methyl ester (13%), Heptadecanoic acid, 16-methyl-, methyl ester (15%), and Fucosterol (30.4%) (Table 2). Fucosterol was found in *Sarcophyton* sp. BK40401 (21.5%). The soft coral BK1105192 contained mainly 22,23-Dibromostigmasterol acetate (12.6%) and (-)-Alloaromadendrene (21%). Finally, Alloaromadendrene (11.2%), Germacrene D (15.6%), and Palmitic acid, methyl ester (26.3%) were found in the soft coral BK1105193 (Table 2).

Other compounds were found at lower percentages (about 5%, Table 3). By screening the literature, we classified them according to their possible way of utilization. The soft coral compounds include antibacterial, anti-parasitic, anti-fungal, anti-viral, anti-oxidant, anti-cancer and agrochemical agents. In this regard, *Sarcophyton* sp. BK40403, and *Sinularia* spp. BK1105191, BK1105192 and BK1105193 contain potential antibacterial and anti-viral agents.

The chemical composition of soft coral extracts was analyzed by a cluster analysis (Figure 5a). The analysis has shown that soft coral extracts can be divided into four separate clusters: cluster 1 – extracts of BK40401 and BK40402, cluster 2 – extracts of BK1105191, cluster 3 – extracts of BK40404, BK1105192 and BK1105193 and cluster 4 – extracts of BK40403. It is interesting that in all cases the similarity between different extracts was lower (>15%). In order to further investigate dissimilarity between extracts, a Non-metric Multidimensional scaling (NMDs) analysis was used (Figure 5b). It showed several distinct groups of extracts: BK40401 and BK40402, BK40403 and BK1105191, and BK40404, BK1105192 and BK1105193.

Discussion

In this study, the antibacterial and antifungal activity of seven soft coral species from the genera *Sarcophyton* and *Sinularia* inhabiting the Sea of Oman were investigated. These species were selected due to the fact that they are highly abundant (Aratake et al. 2012) and according to previous studies produce many bioactive compounds (Dobretsov et al., 2016; Gozari et al., 2019; Rodrigues et al., 2019).

The results of the agar disk-diffusion method showed that all soft coral extracts have antibacterial effects only against Gram-negative (*P. putida)* but not Gram-positive bacteria (*L.fusiformis*). This might be due to the structural differences between Gram-positive and Gram-negative bacteria. Gram-positive bacteria have thick

cell walls consisting of peptidoglycan, while in Gram-negative bacteria peptidoglycan layer is thin and surrounded by lipopolysaccharides (Silhavy et al., 2010). On the opposite, previous studies demonstrated that Red Sea soft corals, *Sarcophyton* spp. and *Sinularia polydactyla* were active against Gram-positive but not Gram-negative bacteria, such as *E.coli* (Afifi et al., 2016). The differences between the results could be because different bacteria were used for testing in these experiments.

The highest antibacterial activity in the agar disk-diffusion experiment was observed for the soft coral *Sarcophyton* sp. BK40403. The estimated reason could be due to the presence of the antimicrobial compound Andrographolide that was detected by GC-MS. It has been shown that Andrographolide has a strong antibacterial activity against *Pseudomonas* spp. (Zhang et al., 2022). The most interesting part is that Andrographolide can act against SARCoV-2 (COVID-19) (Shi et al., 2020; Sudeep et al., 2020; Enmozhi et al., 2021). A recent study showed that Andrographolide from the plant *Andrographis paniculate* has different biological activities involved in immunomodulation (Banerjee et al., 2020). In addition, Andrographolide has anti-cancer properties against different cancer cell lines (Zhang et al., 2022). However, the diameter of the inhibition zone for *Sarcophyton* sp. BK40403 was smaller compared to an antibiotic in our study. This could be due to the fact that gentamicin is a pure chemical compound, while soft coral extracts compose many compounds. Thus, the actual concentration of active compound(s) in soft coral extracts could be lower than that of gentamicin. In our study, the minimal inhibitory concentration (MIC) of soft coral extracts was 5-10-fold lower than previously reported for *Sinularia* and *Sarcophyton* species from Oman waters (Dobretsov et al., 2015). In that study, only *Cladiella* sp. inhibited the growth of *Bacillus* sp. from biofouling bacteria. The active compound was identified as a mixture of hexadecyl palmitate and hexadecyl stearate. The differences between our results and the work of Dobretsov et al. (2015) can be attributed to differences in chemical extracts of soft corals as well as differences in tested bacterial species.

In order to confirm our antibacterial results, we used the MicroResp® method. This method was successfully used to investigate the effect of pollutants on lotic biofilms in aquatic systems (Tlili et al. 2011). The method is measuring the production of CO_2 by bacteria during the short-term incubation of biofilms with chemical compounds or pollutants. Thus, this method allows an indirect estimate of the growth of bacteria. Results of the MicroResp® method confirmed that only Gram-negative bacteria were affected by soft coral extracts. Extracts of the soft coral *Sinularia* sp. BK1105192 had the best result for reducing the respiration rate of *P. putida* and *P. tunicata*. The possible reason could be the presence of 22,23-Dibromostigmasterol acetate recorded by GC-MS. This compound inhibited the growth of *Pseudomonas pvocyanea* and other Gram-negative bacteria (Sharma et al., 1993).

On the opposite, extracts of *Sinularia* sp. BK1105193 increased the respiration rate of the bacterium *P. tunicata* in our experiments. This surprising result could be explained by the presence of compounds that could be used as nutrients or growth promoters that enhance the growth of bacteria. To our knowledge, this is the first report of the enhancement of bacterial growth by soft coral extracts. However, there are some reports that some bacteria live in close association with soft corals (Harder et al., 2003), which could indirectly support our hypothesis.

The GC-MS analysis indicated the presence of many bioactive compounds in soft coral extracts, including antibacterial, antiparasitic, antiviral, antioxidant, anticancer, and agrochemical compounds. For example, one of the dominant soft coral compounds Fucosterol has potential antioxidant properties (Zhao et al., 2013),

and it is an important source of novel anticancer drugs (Jiang et al., 2018; Mao et al., 2019). In addition, it has anti-obesity activity (Maeda et al., 2018) and anti-diabetic properties (Jung et al., 2016). Fucosterol has been used as an antimicrobial agent (Zeb et al., 2017) against *Staphylococcus aureus*, *Staphylococcus albus*, and *Streptocorus viridans* (Gram-positive bacteria) and *Escherichia coli*, *Pseudomonas pvocyanea*, and *Klebsiella* (Gram-negative bacteria) (Sharma et al., 1993). While Trans-Farnesol found in *Sarcophyton* sp. BK40403 has antifungal properties against *Candida parapsilosis* CLIB214 and *Candida albicans* (Nurrachma et al., 2021), but we were not able to find anti-antifungal activity. This could be explained either by different fungal species that were used in previous studies or by different bioactive compounds from tested species.

The chemical composition of soft corals was compared using cluster and NMDs analysis. NMD is an indirect gradient analysis approach that produces an ordination based on a distance or dissimilarity matrix (Buttigieg et al., 2014). Opposite to our expectation, extracts were grouped not based on the genera of the soft corals. This could suggest two possible scenarios. First, the identification of soft corals was not correct. The identification was done by a coral taxonomist based on the morphology of the corals. In the future, genetic analysis of soft corals is needed to be done in order to confirm the morphological classification. Second, most of compounds could be produced not by soft corals but by their symbionts, which could be similar between different species. The production of bioactive compounds by symbionts of soft corals has been shown before (Bengen et al., 2015; Sulistiyani et al., 2010). Thus, this hypothesis most likely explains the observed differences.

There are a number of unidentified and possibly novel compounds detected by GC-MS in studied soft corals. Compare to other investigated species *Sarcophyton* sp.BK40402 has three unidentified compounds present in large quantities from 13% to 48%. This indicates that soft corals *Sarcophyton* spp. and *Sinularia* spp. could be a good source of novel secondary metabolites. Thus, in future studies, anti-cancer, anti-viral, anti-parasitic, and anti-oxidant activities of soft corals from Oman waters should be investigated.

Acknowledgements

The authors thank Prof. Al-Saadi, A., Dr. Al-Saadi, N., Mr. Al-Balushi, A.H., Mr. Al-Busaidi, H.K., and Mr. Al-Belushi, M. for their help with this study. The help of Dr. D. Blackburn in MicroResp is acknowledged. The authors thank Dr. Al-Sabahi, J. for his help with GC-MS analysis and Prof. Claereboudt M. for his help with collection of the samples, some ideas and fruitful comments. The authors acknowledge financial support of SQU (IG/DVC/CEMB/19/01) and TRC (CR/DVC/CEMB/20/01).

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