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## Morphological, Chemical and Protein Characterization of the Red Sea Soft Coral *Sarcophyton* Species, a Comparative Study

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**Abstract:** *Sarcophyton* species are soft corals abundant in the Red Sea. *Sarcophyton glaucum* produces sarcophine that has a well documented antitumor activity and inhibitory activity against tumor promoters. This study was established to test the accuracy of the morphological and sclerite based taxonomy against the chemical fingerprints and the protein profiles for the same samples. High performance thin layer chromatography and high performance liquid chromatography analyses were performed on the organic extract obtained from the samples. The analyses revealed that all *Sarcophyton* samples belonging to different species contained sarcophine with varying concentrations. Total cellular proteins were extracted and subjected to 10% Sodium dodecyl sulphate polyacrylamide gel electrophoresis. Characteristic protein profiles were obtained for the different species studied. The data obtained from the protein profiles and the chemical fingerprints were consistent and in some instances disagreed with the morphological and/or the sclerite based identification of the *Sarcophyton* species. Furthermore, one of the *Sarcophyton* samples from Sharm El-Sheikh could not be identified by sclerite or morphological methods. It gave chemical and protein profiles that were different from all the other samples in this study.

**Key words:** *Sarcophyton* species, sarcophine, SDS-PAGE, high performance thin layer chromatography (HPTLC), high performance liquid chromatography (HPLC)

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### Introduction

*Sarcophyton* species are a group of soft corals belonging to family *Alcyoniidae*, subclass *Octocorallia*, Class *Anthozoa* and phylum *Cnidaria*. These soft corals attracted great attention because they yield a group of cembranoids with wide spectrum of biological activities. Sarcophine is an abundant cembranolide diterpene isolated from the Red Sea soft coral, *Sarcophyton glaucum* (Fig. 1) (Bernstein *et al.*, 1974). Sarcophine constitutes the corals chemical defense mechanism against the natural predators. It has toxic effects on fish, mice and rats (Ne'eman *et al.*, 1974). It acts as an inhibitor of a number of vital enzymes including cholinesterase and phosphofructokinase by reacting with their thiol groups (Ne'eman *et al.*, 1974; Erman and Ne'eman, 1977). Sarcophine was also found to have a potential to inhibit tumorigenesis assessed by its ability to inhibit 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced transformation, a relevant model for investigating

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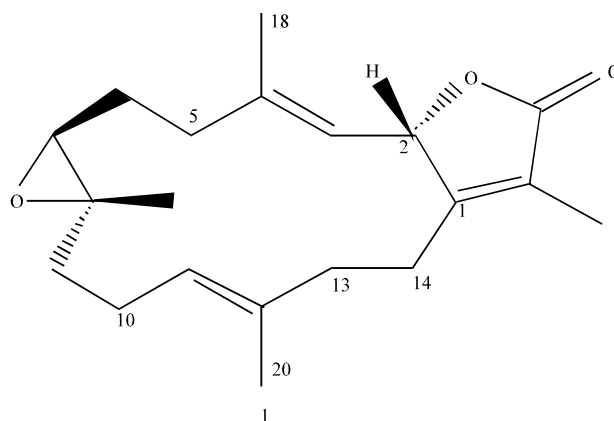


Fig. 1: Molecular structure of sarcophine isolated from *Sarcophyton glaucum*

neoplastic transformation (El Sayed *et al.*, 1998). The microbial transformation of sarcophine and its chemical oxidation with selenium dioxide yielded a group of compounds with cancer chemopreventive activity higher than sarcophine (El Sayed *et al.*, 1998; Katsuyama *et al.*, 2002; Fahmy *et al.*, 2004).

In addition to sarcophine, the Red Sea *Sarcophyton glaucum* was found to produce a lactone cembrane diterpene, sarcophytolide. It displayed a strong cytoprotective effect against glutamate-induced neurotoxicity and antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Saccharomyces cerevisiae* (Badria *et al.*, 1998).

An important issue that tends to be overlooked in the search for marine natural products and its associated research is that of fundamental taxonomy, that is accurate identification of the species. The identification of *Sarcophyton* species, as all octocorals, depends on the colony morphology and sclerite architecture, but these characters exhibit an extra-ordinary high degree of variability (Lohuis *et al.*, 1990). The phylogenetic relationships within the *Anthozoa* were re-evaluated based on the 41 morphological characters and the nuclear sequences of the 18S ribosomal DNA. The trees from the morphological data did not coincide closely with the molecular data (Won *et al.*, 2001). Furthermore, the phylogenetic analysis of the nuclear 18S rDNA from the *octocorallians* did not support either the historical or the current classification systems. Thus, the morphological characters used to derive the current classification system are not reflected consistently with the results revealed by the 18S rDNA performed (Brenston *et al.*, 2001).

The accurate identification is of enormous importance once the marine organism becomes a source for large scale production of a potential pharmaceutical drug. Hence, for the industrial purposes, the proper species, location and environmental conditions need to be predetermined to ensure a sufficient and steady supply of the drug. Consequently, this study focused on testing the reliability of the morphological and sclerite based taxonomy against the chemical fingerprint and the protein profile for the different species of the genus *Sarcophyton* from the Red Sea and hence offer new insights for a more precise classification of the genus. Also, the study aimed at determining whether the chemical diversity within the genus is due to the geographical location and/or is species specific.

## Materials and Methods

### General

The chemical fingerprint was performed using HPTLC plates 10×10 cm silica gel 60 F 254 (Merck). Fully automatic TLC sampler, horizontal developing chamber 10×10 cm and chromatogram immersion device, equipped with 20×20 cm dip tank (CAMAG, Switzerland).

HPLC Shimadzu instrument, equipped with a model series LC-10 ADVP pump, SCL-10 AVP system controller, DGU-12 A Degasser, Rheodyne 7725 injector with a 20  $\mu$ L loop and a SPD-10AVP UV-VIS detector and with a HPLC column TSK-Gel ODS-80 TM column, 5  $\mu$ m, with dimensions 150 $\times$ 4.6 mm were used to determine the quantity of sarcophine in the organic extract. The UV spectral analysis of sarcophine was measured in double-beam Shimadzu UV-Visible spectrophotometer, model UV-1601 PC equipped with 1 cm quartz cells. The bundled software was UVPC personal spectroscopy software version 3.7. EM science silica gel 60/230-400 mesh (flash) was used for column chromatography.

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) was performed in the SE 600 vertical slab gel casting unit (Amersham, Pharmacia, Sweden).

#### *Animal Material*

We collected 26 samples of the soft coral *Sarcophyton* species by hand using scuba diving from different locations in the Red Sea: Sharm El-Sheikh (SH), Ras Mohammed (RM), El-Gouna (GN), Hurghada (HU), Safaga (SA) and Marsa Alam (MA). Each sample was preserved in two portions. The first one of each individual was frozen immediately after collection in liquid nitrogen then at -80°C till manipulation. The second portion was preserved in 70% ethanol for morphological identification.

#### *Sclerite Examination*

The stalk and capitulum sclerites were prepared from the alcohol preserved samples following the method described by Fabricus and Aderslade (2001). Different *Sarcophyton* species were identified by the sclerite examination according to the keys described by Verseveldt (1982).

#### *Chemistry*

In order to obtain the organic extract, the frozen samples were freeze dried and then extracted with methanol, dichloromethane mixture (1:1) for five days. The solvent mixture was freshly changed daily to ensure extraction efficiency. The extract was dried under vacuum, dissolved in chloroform and partitioned against water to remove the sodium chloride traces from the extract then dried under vacuum.

Pure sarcophine standard was prepared by the fractionation of a portion of the organic extract of one sample on silica gel column using 30% ethyl acetate in hexane as an eluent. The fraction containing sarcophine was further purified by crystallization out of the cold hexane. The identity of sarcophine was confirmed by comparing the spectral data with the published one (Bernstein *et al.*, 1974).

HPTLC technique was applied in order to obtain a chemical fingerprint for the organic extract using a mobile phase made up of 30% ethyl acetate in n-Hexane. Samples were applied on HPTLC plates in the form of bands of 7 mm width leaving 5 mm in between. The sample volume and concentration was 1  $\mu$ L and 1  $\mu$ g/30  $\mu$ L methanol, respectively. The separated bands were detected using the anisaldehyde spray reagent after heating to 120° for 5 min.

HPLC was carried out to quantify the amount of sarcophine present in the organic extract of each *Sarcophyton* sample. The best separation was obtained using a mobile phase of 70% acetonitrile, 30% deionized water and pH adjusted to 3.5 by phosphoric acid. The detector was set to 220 nm and the flow rate was 1.5 mL min<sup>-1</sup> and the injection volume was 20  $\mu$ L. All determinations were performed at ambient temperature. Six standard solutions were prepared by diluting the stock with methanol to reach a concentration range of 10 to 80  $\mu$ g mL<sup>-1</sup>. The used concentrations were 10, 20, 40, 50, 60 and 80  $\mu$ g mL<sup>-1</sup>. Triplicate of 20  $\mu$ L injections were chromatographed under the conditions described above. The peak area of each concentration was plotted against the corresponding concentration to obtain the calibration graph. The samples were prepared by dissolving a specified weight of the extract in methanol so that the final concentration was 20 mg% and filtered through 0.45  $\mu$ m disposable filters and 20  $\mu$ L injection of each sample was assayed.

### Protein Profile

This is the first time to attempt isolating the total cellular proteins from the genus *Sarcophyton*. Therefore, different optimization steps were made so as to obtain maximal total cellular protein yield with minimal proteolysis and contamination. Protein were extracted from the capitulum tissue, where equal amounts of the capitulum sample and the protein extraction buffer were manually homogenized over ice. The protein extraction buffer was composed of 50 mM Tris-HCl (pH 8), 1 mM Dithiothreitol, 50 mM EDTA, 0.5 M NaCl, 1% v/v Triton×100, 1 mM Benzamidine and 1 mM Phenyl methyl sulfonyl fluoride. Proteins were separated by centrifugation for 15 min at 4°C. The extracted proteins were boiled for 3 min with the sample buffer. Equal protein concentration of each sample was loaded on 10% discontinuous SDS-PAGE prepared as described by Laemmli in 1970 (Walker, 2002). Protein bands were visualized by Coomassie blue R 250 staining. A volume of 20 µL of each sample was loaded per well. Molecular weight estimate was done by comparison to a full range marker of molecular weight from 205 to 14 KDa (Amersham Pharmacia Biotech). The molecular weight of the protein bands was determined by comparison to the marker using the software Gel Documentation, Advanced Software Version 2.0. The absence of contamination from the symbiotic algae *Zooxanthellae* proteins was verified by isolating the algal cells from the *Sarcophyton* tissue as described by Lin *et al.* (2000).

## Results

### Sclerite Examination

The sclerite examination indicated the presence of seven *Sarcophyton* species in the study, in addition to two unidentified samples from Safaga and Sharm El Sheikh that could not be identified by sclerite key. The similarities of sclerite shapes among the different species of the genus *Sarcophyton* made the unambiguous identification, a difficult task. In some instances, the variation in the shape of sclerites does not necessarily mean different species, instead it can reflect the age of the colony (Verseveldt, 1982). Species identification by gross morphology of the *Sarcophyton* colony was complicated by the fact that it harbors symbiotic algae that often show different colors within the same species. Also all the colonies capitulae showed different extension and contraction phases according to the water currents and the environmental conditions at the collection site. This further complicated the gross morphology based identification. According to sclerite examination, the identified species in our study were *S. auritum*, *S. digitatum*, *S. ehrenbergi*, *S. glaucum*, *S. gemmatum*, *S. pauciplactum* and *S. trocheliophorum*. Different sclerite shapes are shown in Fig. 2-4.

### Chemistry

#### High Performance Thin Layer Chromatography (HPTLC)

HPTLC analysis was used to obtain a chemical fingerprint of the organic extract obtained from different *Sarcophyton* species at different locations. Figures 5-9 show HPTLC plates with good separation of the sarcophine band in the extract. The plates revealed that all *Sarcophyton* species, identified according to the sclerite architecture, contained sarcophine; but in different concentrations.

*Sarcophyton auritum* produced a distinctive fingerprint that was consistent in almost all locations (Fig. 6-8) except for the one from Ras Mohamed (Fig. 5) that showed different pattern. *S. trocheliophorum* (Fig. 8) showed another pattern that was not found in any other species. The chemical fingerprints of *S. ehrenbergi* collected from Marsa Alam and from El-Gouna were not identical. Furthermore, *S. digitatum* collected from two different locations, Hurghada and



Fig. 2: Different shapes of the capitulum sclerites common in all *Sarcophyton* species. (100x magnification)

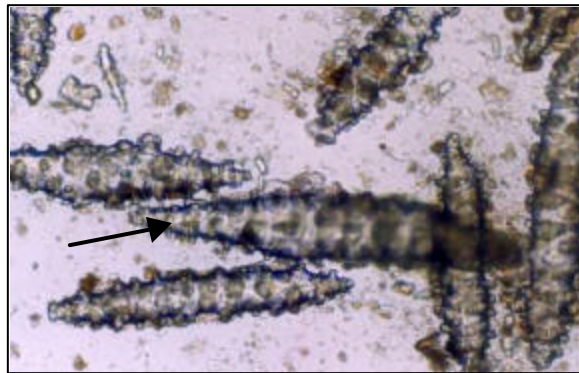


Fig. 3: The key sclerite of the interior stalk of *Sarcophyton glaucum* (100x)

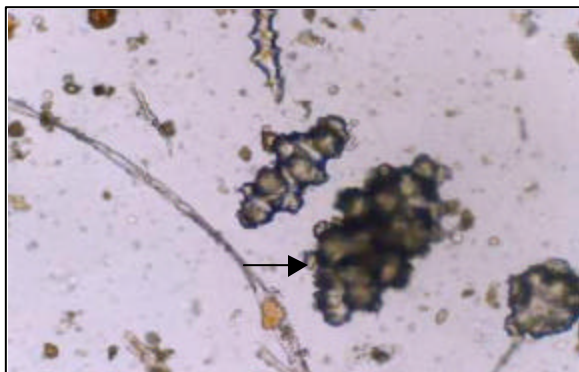


Fig. 4: The key sclerite of the interior stalk of *Sarcophyton trocheliophorum*. (100x)

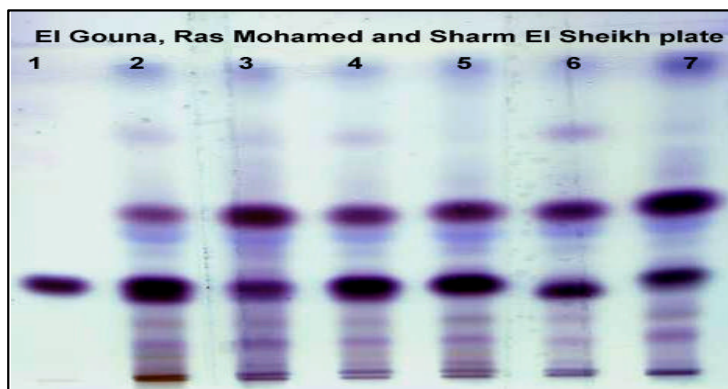


Fig. 5: HPTLC fingerprint for *Sarcophyton* species from El Gouna (GN), Ras Mohamed (RM) and Sharm El Sheikh (SH). Lane 1: Sarcophine standard. Lanes 2 and 3: *S. glaucum* from GN and 6 from RM. Lane 4: *S. auritum* from RM. Lane 5: *S. pauciplactum* from RM. Lane 7: *S. gemmatum* from SH

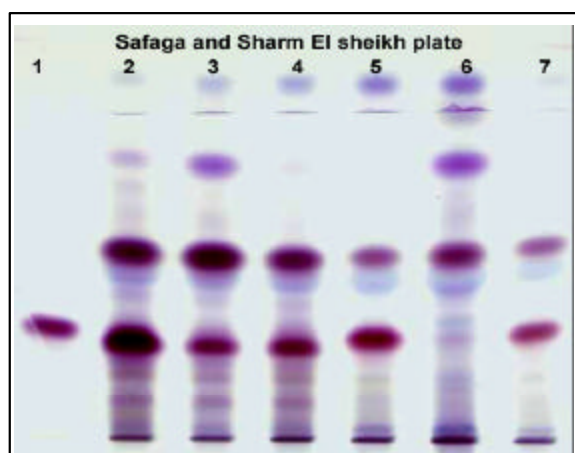


Fig. 6: HPTLC fingerprint for *Sarcophyton* species from Safaga (SA) and Sharm El Sheikh (SH). Lane 1: Sarcophine standard. Lane 2 and 5: *S. pauciplactum* from SH and SA, respectively. Lane 3: *S. gemmatum* from SH. Lane 4: unidentified sample from SA. Lane 6: *S. auritum* from SA. Lane 7: *S. glaucum* from SA

Sharm El-Sheikh (Fig. 8 and 9, respectively), had different pattern. *S. glaucum*, *S. gemmatum* and *S. Pauciplactum* collected from different locations (Fig. 5-9) and the unidentified species collected from Safaga (Fig. 6) showed similar chemical fingerprint. Another species collected from Sharm El Sheikh (Fig. 9), that we were not able to identify it by sclerite architecture, showed a distinctive chemical profile that was not found in any other species. The heterogeneity in the chemical fingerprints that was obtained from the same species collected at different locations might be attributed to the variation of the type and concentration of the complementary metabolites with the environmental conditions at the site of collection (Fabricius and Alderslade, 2001). Using HPTLC, it was possible to identify the sarcophine band using short UV when compared to sarcophine standard. Interestingly, it was found that all the *Sarcophyton* species studied contained sarcophine except for *S. auritum* and *S. trocheliophorum*.

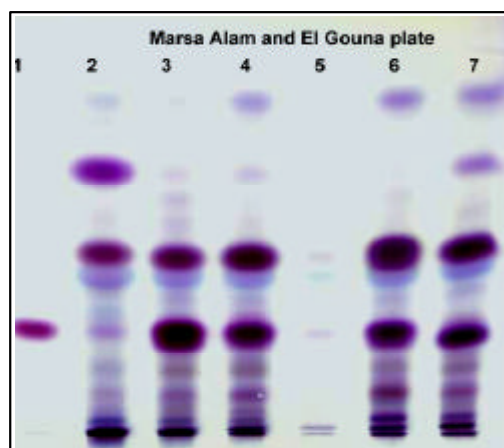


Fig. 7: HPTLC fingerprint for *Sarcophyton* species from Marsa Alam (MA) and El-Gouna (GN). Lane 1: Sarcophine standard. Lane 2: *S. auritum* from MA. Lanes 3 and 4: *S. glaucum* from MA. Lane 5: *S. ehrenbergi* from MA. 6 and 7: *S. ehrenbergi* from GN

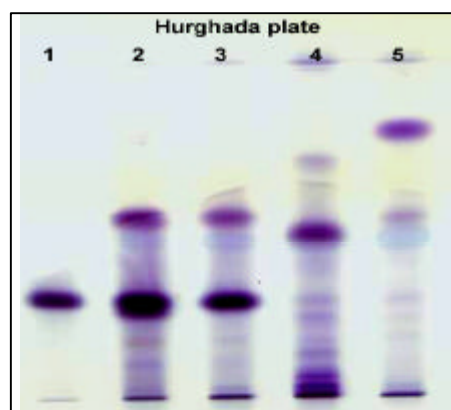


Fig. 8: HPTLC fingerprint for *Sarcophyton* species from Hurghada (HU). Lane 1: *Sarcophine* standard. Lane 2: *S. glaucum*. Lane 3: *S. digitatum*. Lane 4: *S. trocheliophorum*. Lane 5: *S. auritum*

#### High performance liquid chromatography (HPLC)

The developed reversed phase high performance liquid chromatography (RP HPLC) method was applied to determine the sarcophine concentration in the organic extract. The chromatographic conditions were studied and optimized as a function of the pH and the acetonitrile concentration in the mobile phase. Figure 10 shows the chromatogram of  $40 \mu\text{g mL}^{-1}$  of sarcophine standard. The average retention time $\pm$ standard deviation (SD) was found to be  $4.6\pm 0.012$  min. The HPLC method was specific to sarcophine, hence we used short UV (220 nm) at which only sarcophine has maximum absorbance with minimum interference from other compounds in the extract. The specificity of the RP HPLC is shown in Fig. 11 where there is a good separation of sarcophine from the rest of the analyzed matrix. This validated HPLC method provides a simple, accurate and reproducible quantitative analysis for the determination of sarcophine in *Sarcophyton* natural extracts without interference from other compounds in the extract (unpublished data).



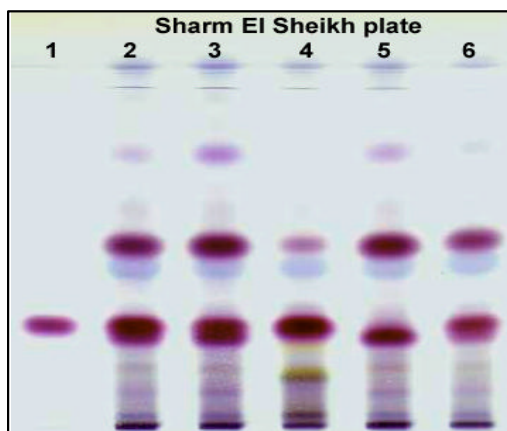


Fig. 9: HPTLC fingerprint for *Sarcophyton* species from Sharm El-Sheikh. Lane 1: Sarcophine standard. Lanes: 2, 3 and 6: *S. glaucum*. Lane 5: *S. digitatum*. Lane 4: unidentified sample

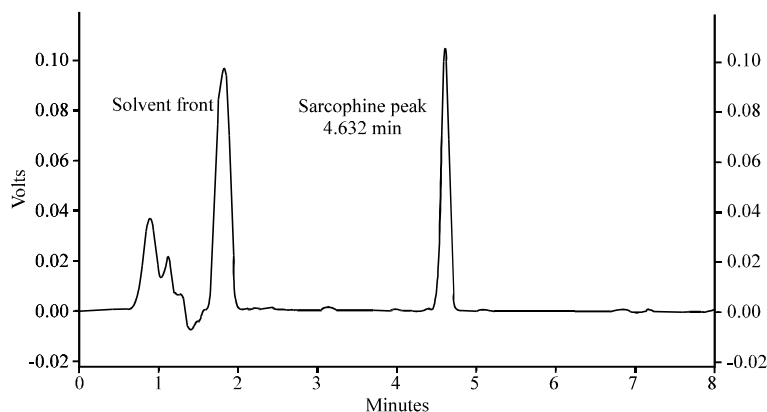


Fig. 10: A typical chromatogram of 20  $\mu\text{L}$  injection of  $40 \mu\text{g mL}^{-1}$  of sarcophine standard

The HPLC results indicated that all *Sarcophyton* species contained sarcophine but with variable quantities that ranged from 0.05 and 0.1% for *S. auritum* and *S. trocheliophorum*, respectively and increased from 0.2 to 8.2% for the *S. digitatum*, *S. ehrenbergi*, *S. gemmatum* *S. pauciplactum* and *S. glaucum* (Table 1). This may explain the low rate of mortality in *Sarcophyton* colonies and their long term stability despite having slow growth rates and being poor colonizers (Cornish and Didonato, 2004). Of the Red Sea species and locations tested using the described HPLC method; *Sarcophyton glaucum* from Hurghada location produced the highest concentrations of sarcophine (Table 1). The HPLC results revealed that the sarcophine concentration is variable among the different species and within the individual species collected from different locations. This finding agrees with another report (Koh *et al.*, 2000) on the distribution of sarcophytol A in the genus *Sarcophyton*. Therefore, sarcophine concentration is influenced by both, genetic factors and environmental conditions.

Table 1: Percentage of sarcophine in the dry weight of the Red Sea soft coral *Sarcophyton* species from different locations.  
\*SH: Sharm El sheikh, HU: Hurghada, SA: Safaga, MA: Marsa Alam, GN: Gouna and RM: Ras Mohammed

Sarcophyton species	Locations *	% sarcophine in dry weight
<i>S. auritum</i>	HU	0.05
<i>S. auritum</i>	SA	0.05
<i>S. auritum</i>	MA	0.04
<i>S. auritum</i>	RM	3.96
<i>S. digitatum</i>	SH	0.20
<i>S. digitatum</i>	HU	5.70
<i>S. ehrenbergi</i>	MA	0.10
<i>S. ehrenbergi</i>	GN1	1.00
<i>S. ehrenbergi</i>	GN2	0.07
<i>S. gemmatum</i>	SH1	1.50
<i>S. gemmatum</i>	SH2	0.25
<i>S. glaucum</i>	SH1	1.10
<i>S. glaucum</i>	SH2	2.70
<i>S. glaucum</i>	SH3	2.20
<i>S. glaucum</i>	HU	8.20
<i>S. glaucum</i>	SA	3.80
<i>S. glaucum</i>	MA1	2.30
<i>S. glaucum</i>	MA2	1.30
<i>S. glaucum</i>	GN1	1.90
<i>S. glaucum</i>	GN2	0.60
<i>S. glaucum</i>	RM	0.35
<i>S. pauciplactum</i>	SA	5.52
<i>S. pauciplactum</i>	RM	4.60
<i>S. pauciplactum</i>	SH	0.29
<i>S. trocheliophorum</i>	HU	0.10
Unidentified species	SA	0.24
Unidentified species	SH	4.60

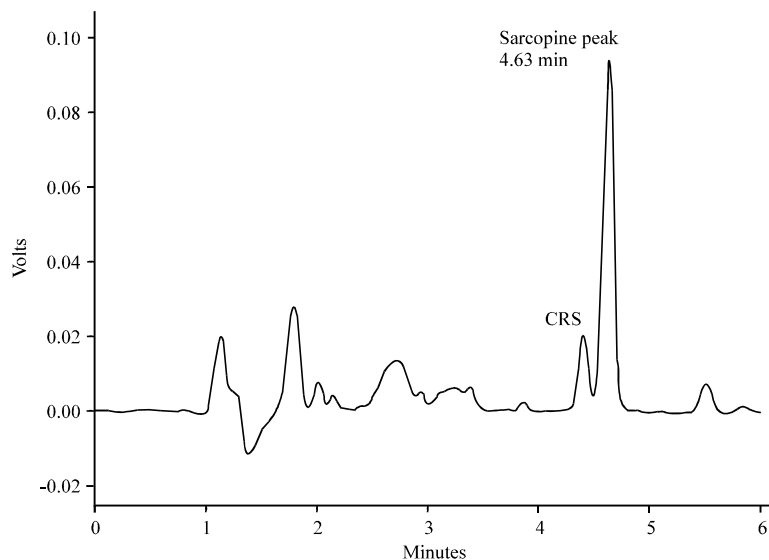


Fig. 11: Chromatogram showing the chromatographic separation of sarcophine from other organic compounds of *S. glaucum* from Marsa Alam. The sarcophine concentration was 2.3% of the dry weight. CRS is a closely related substance

The HPLC and HPTLC analyses showed the presence of a compound that elutes very close to sarcophine. It appears that this compound is closely related to sarcophine (designated CRS). It was shown by HPLC (Fig. 11) that the concentration of sarcophine is inversely proportional to the concentration of CRS.

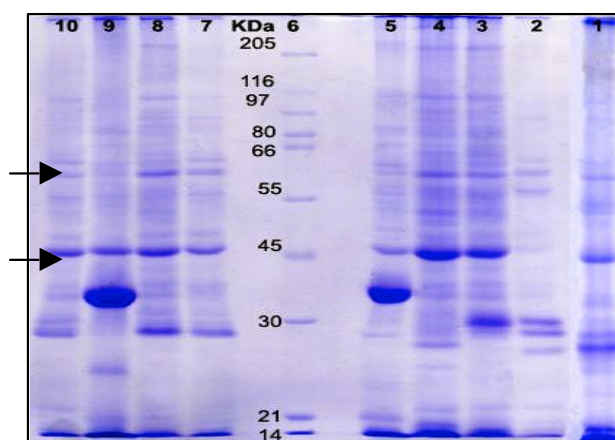


Fig. 12: 10% SDS-PAGE of different *Sarcophyton* species proteins from Hurghada (HU) and Safaga (SA) in the presence of *Simularia* as an out group, the arrows indicate the common bands in *Sarcophyton* species. Lane 1: *Simularia* species as an out group collected from HU. Lane 2 and 10: *S. glaucum* from HU and SA, respectively. Lane 3: *S. digitatum* from HU. Lane 4: *S. trocheliophorum* from HU. Lane 5 and 9: *S. auritum* from HU and SA, respectively. Lane 6: molecular weight marker. Lane 7: unidentified sample collected from SA. Lane 8: *S. pauciplactum* collected from SA.

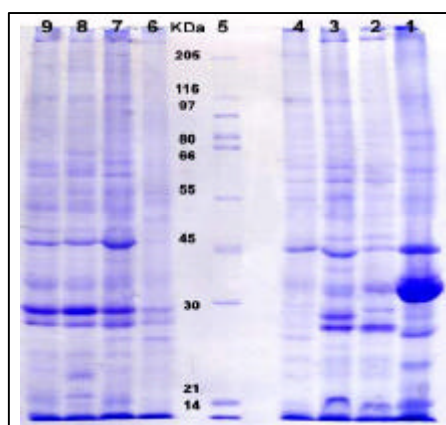


Fig. 13: 10% SDS-PAGE of different *Sarcophyton* species proteins from Marsa Alam (MA) and El Gouna (GN). Lane 1: *S. auritum* collected from MA. Lanes 2 and 3: *S. glaucum* obtained from MA and 8 and 9 from GN. Lanes 4: *S. ehrenbergi* from MA and 6 and 7 from GN. Lane 5: Molecular weight marker

#### Protein Profile

The SDS-PAGE of proteins indicated that there was distinctive protein pattern for the *Sarcophyton* species, when compared to other members of the same family Alcyoniidea, *Simularia*, (Fig. 12, lane 1). This was apparent in the high molecular weight proteins of molecular weight characteristic for *Sarcophyton*. The results also revealed that a 60 and 45 KDa protein bands are common in all *Sarcophyton* species. Figure 12- 14 show the 10% SDS-PAGE of different *Sarcophyton* species collected from different locations.

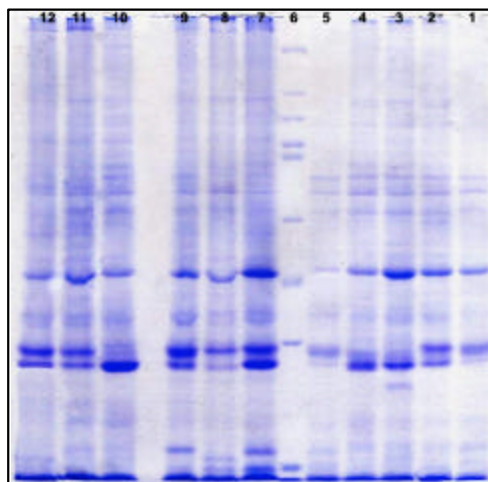


Fig. 14: 10% SDS-PAGE of different *Sarcophyton* species proteins from Sharm El Sheikh (SH) and Ras Mohammed (RM). Lanes 1, 2, 5: *S. glaucum* from SH and 9 from RM. Lane 3: unidentified species from SH. Lane 4: *S. digitatum* from SH. Lane 6: Molecular weight marker. Lane 7: *S. auritum* from RM. Lanes 8 and 11: *S. pauciplactum* from RM and SH. Lanes 10 and 12: *S. gemmatum* from SH

In all locations *S. glaucum* can be characterized by the presence of two consecutive protein bands of 27 and 29 KDa, in addition to the common protein bands. The protein profile coincides with high sarcophine content in almost all samples, except for the *S. glaucum* collected from El- Gouna and Ras Mohammed that showed low sarcophine concentration, 0.6 and 0.35%, respectively along with high closely related substance (CRS) concentration (Fig. 11). The highest sarcophine concentration was found in *S. glaucum* from Hurghada (8.2%). This particular sample had an additional protein band of molecular weight of 26 KDa. Whether this band is characteristic to the *S. glaucum* with the highest sarcophine concentration requires further investigation.

The slight individual differences in the low molecular weight proteins of *S. glaucum* collected from different locations might be due to environmental conditions that may induce the production of heat-shock protein reported in other soft corals (Tom *et al.*, 1999). Heat-shock proteins are induced by stressful factors such as extremes of temperature, UV radiation, the presence of heavy metals and amino acids analogs (Tom *et al.*, 1999). Moreover, the difference in the low molecular weight proteins might be attributed to the growth state of the colony and its sexual maturity. Sexual maturity is after six to seven years for male colonies and ten years for female colonies (Benayahu and Loya, 1986). In addition, the variation in the low molecular weight proteins may also be due to the altered gene expression as well as posttranslational modification of polypeptides, which may be due to environmental stress and/or aging (Soti and Csermely, 2003).

*S. auritum* showed a unique pattern of a 35 KDa protein band in addition to the characteristic protein bands found in the genus. This protein can be used to characterize the *auritum* species. Interestingly, the protein profile was similar in the three *S. auritum* collected from Hurghada, Safaga and Marsa Alam samples. These three specimens contained small amount of sarcophine between 0.04 and 0.05%. However, the *S. auritum* that was identified by sclerite architecture and collected from Ras Mohammed had a different protein profile of 27 and 29 KDa proteins without the unique 35 KDa band. In addition, the HPLC analysis revealed that it contained high sarcophine content (4%) and the HPTLC indicated that it has a different chemical fingerprint not identical to the other *S. auritum* samples. Taking together the protein profile and the chemical fingerprint along with the high

concentration of sarcophine found in the Ras Mohammed sample, it could be suggested that this sample might be *S. glaucum* due to the resemblance between the two.

*S. ehrenbergi* collected from El Gouna, Fig. 13, lane 6 had a relatively high sarcophine concentration of 1% and the protein bands of 27 and 29 KDa characteristic to *S. glaucum* and a chemical fingerprint as *S. glaucum*. Therefore, these findings suggest that this species may be better identified as *S. glaucum*. Moreover, the *S. Pauciplactum* collected from Ras Mohammed, Fig. 14, lane 8 had the same pattern of high sarcophine concentration of 4.6% as well as the protein bands and the chemical fingerprint characteristic for *S. glaucum* which also suggest that it might be *S. glaucum*.

Furthermore, the *S. ehrenbergi* from El-Gouna, Fig. 13, lane 7, *S. Pauciplactum* and *S. gemmatum* collected from Sharm El-Sheikh, Fig. 14 lanes 11 and 12, respectively, all have the protein bands characteristic to *S. glaucum*, but the sarcophine concentration was low (0.07, 0.25 and 0.29%, respectively). Present findings also indicate the presence of an inverse relation between the concentration of sarcophine and its CRS (Fig. 11). Therefore, these samples appear to belong to *S. glaucum* which biosynthesized more of the CRS at the expense of sarcophine.

*S. ehrenbergi* collected from Marsa Alam, as was identified by sclerites, showed protein bands of molecular weight of 34, 26 and 24 KDa. Furthermore, the *S. trocheliophorum* sample collected from Hurghada showed an interesting protein profile with protein bands of molecular weight of 25 and 27 KDa. These protein profiles can be used to characterize the *S. ehrenbergi* and the *S. trocheliophorum* samples.

*S. digitatum* samples were collected from two locations, Hurghada and Sharm El Sheikh (Fig. 12 and 14, lanes 3 and 4, respectively). The Hurghada sample followed the sclerite keys described by Verseveldt (1982) for the identification of *S. digitatum*. It had a characteristic protein profile of the common bands of the genus *Sarcophyton* in addition to a 30 KDa protein band and with high sarcophine concentration of 5.7%. On the other hand, the Sharm El Sheikh sample was not well identified according to the sclerite keys. It had also the common bands of the genus *Sarcophyton* in addition to a band of molecular weight of 28 KDa and not of 30 KDa band, its sarcophine concentration was 0.2% along with high CRS concentration. Furthermore, the HPTLC chemical fingerprints of the two samples were not identical. This might indicate that the two samples are different and the Hurghada sample is the only *S. digitatum* included in the study.

The protein profile of the *S. digitatum* from Sharm El Sheikh is identical to the protein profile of the one of the *S. gemmatum* collected from Sharm El Sheikh as well as the unidentified sample and *S. pauciplactum* collected from Safaga (Fig. 14, lanes 4 and 10 and Fig. 12, lanes 7 and 8, respectively). Because the current taxonomy is based on the colony morphology and the sclerite architecture and these characters exhibit an extra-ordinary high degree of variability and were proven to be inaccurate tools for identification when compared to genetic based taxonomy (Won *et al.*, 2001; Brenston *et al.*, 2001), the above samples were misidentified. Moreover, the sarcophine concentration was variable among these samples, ranging from 0.189 to 5.52%. Therefore, further investigation is needed to correctly identify these samples.

The unidentified species collected from Sharm El Sheikh (Fig. 14, lane 3) had a unique protein pattern and chemical fingerprint on HPTLC that was different from the other species studied along with high sarcophine concentration (4.4%). Further studies are needed before this sample can be accurately and correctly identified.

## Discussion

As far as we are aware, this is the first study of the genus *Sarcophyton* that has correlated morphological, chemical and protein profiling for each sample. We hope that it will stimulate a broader approach to the delimitation of species identification in the Red Sea.

The results of this work indicate that the chemical fingerprint and the protein profile were in agreement but in some instances did not support the morphological or the sclerite based identification. Based on sclerite architecture, seven morphologically different *Sarcophyton* species were identified in the study, in addition to two unidentified species collected from Safaga and Sharm El Sheikh. The chemical fingerprint obtained by HPTLC and the protein profiles obtained by 10% SDS-PAGE supported the presence of five species only. These species are *S. auritum*, *S. digitatum*, *S. ehrenbergi*, *S. glaucum* and *S. trocheliophorum*. The chemical and protein fingerprints were instrumental in confirming the identification of the collected species. They were also important tools in correcting the sclerite based misidentification in some species. The identity of *S. gemmatum* and *S. pauciplactum* was uncertain because the samples collected had chemical and protein fingerprints similar to either *S. glaucum* or to the unidentified sample from Safaga indicating problems with the sclerite identification. Furthermore, the unidentified Sharm El Sheikh sample showed a unique protein profile and chemical fingerprint that was not found in any other species included in this study.

It is clear from the current study that depending solely on the sclerite based taxonomy, which is the widely used technique for all soft corals identification, is unreliable. Therefore, additional techniques such as the chemical and protein fingerprints should be used to support and ensure the reliability of identification. The accurate identification is of enormous importance once the organism becomes a source for large-scale production of a potential pharmaceutical drug. Hence, for the industrial purposes, the proper species, location and environmental conditions need to be predetermined to ensure a sufficient and steady supply of the drug.

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