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# Microbial transformation and HPLC Quantitative Analysis of Sarcophine extracted from five Egyptian Red Sea soft corals *Sarcophyton sp*.

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**ABSTRACT**

A quantitative determination of the major compound, sarcophine, of ethyl acetate extracts in five species of the genus *Sarcophyton* determined by RP HPLC using ODS column. The HPLC analysis of *S. glaucum, S. acutum, S. regulare, S. convolutum, S. ehrenbergi* samples were collected from Hurghada in the Red Sea, as well as, microbial transformation of sarcophine was tested.

**Keywords:** HPLC, sarcophine, red sea, *Sarcophyton* sp.

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## INTRODUCTION

Indeed soft coral (Cnidaria: Anthozoa: Octocorallia), which are an important structural component of coral reef communities [1,2], are approximately 40% native to the Red Sea [3]. The Red Sea, in which extensive reef formation occurs, is arguably the world's warmest (up to 35 C in summer) and most saline habitat (ca. 40 psu in the northern Red Sea) [3]. Many terpenes isolated from the genus *Sarcophyton*, especially cemberene type, sarcophine is one of the major compounds isolated from the genus *sarcophyton*.

Most of The Red Sea soft coral *Sarcophyton* species have sarcophine as a major secondary metabolite with other cemberiond derivatives [4, 5]. Many biological studies about sarcophine have been studied [6,7]. Sarcophine was also found to have a potential to inhibit tumorigensis assessed by its ability to inhibit 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced transformation [8]. The microbial transformation of sarcophine and its chemical modification to afford number of synthesized with cancer chemopreventive activity higher than sarcophine [8-11].

Herein, a quantitative determination of sarcophine in five species of the genus *Sarcophyton* extracts to illustrate the best species and way for sarcophine production. Additionally, microbial transformation of sarcophine was tested by using different fungi to figure out the possibility of biotransformed products obtained from sarcophine as a substrate.

## MATERIAL AND METHODS

### General Experimental Procedures

$^{1}H$ and $^{13}C$ NMR spectra were recorded in $CDCl_3$ on a JEOL ECA-600 spectrometer (600 MHz for $^{1}H$ and 150 MHz for $^{13}C$, respectively). All chemical shifts (δ) are given in ppm units with reference to TMS as an internal standard and coupling constants (*J*) are reported in Hz. FAB-MS was performed on a Finnigan LCQ ion trap mass spectrometer and HR-FAB-MS experiments were performed on Fourier transform ion cyclotron mass spectrometer (Ion Spec, Varian, Walnut Creek, CA, USA). EI-MS experiments were performed using a Thermo ISQ Single Quadrupole system (Thermo Scientific, San Jose, CA, USA). High performance liquid chromatography (HPLC) was performed on an Agilent pump equipped with an Agilent-G1314 variable wavelength UV detector at 254 nm and a semi-preparative reverse-phase column (Econosphere™, RP-C18, 5 µm, 250 × 4.6 mm, Alltech, Deerfield, IL, USA). Optical rotation was determined at 589 nm (sodium D line) using a Perkin–Elmer-341 MC digital polarimeter (Wellesley, MA, USA); [α] D-values are given in units of 10 $deg^{-1}{\cdot}cm^2\,g^{-1}$. CD was measured with an OLIS, DSM-10 UV/Vis CD (Olis, Bogart, GA, US). Silica gel 60 (230–400 mesh, Merck, Darmstadt, Germany) and Sephadex LH-20 (Sigma, St. Louis, MO, USA) were used for column chromatography. Pre-coated silica gel plates (Merck, Darmstadt, Germany, Kieselgel 60 $F_{254}$, 0.25 mm) were used for TLC analyses. Spots were visualized by heating after spraying with 10% $H_2SO_4$.

### Animal Material

Using SCUBA diving equipments and snorkeling, soft corals of *Sarcophyton glucum, S. acutum, S. regulare, S. convolutum, S. ehrenbergi* were collected from the Egyptian Red Sea off the coast of Hurghada in March 2013. Fragents of 5-7 $cm^2$ from separate colonies were cut and collected out from the disk of the large colonies that were tagged to recur it more one time according to Benayahu and Loya [12]. On the deck, water was removed from the bags and immediately transferred to foam box filled with ice waiting for transportation to National Institute of Oceanography and Fisheries laboratories within 6 hours. In laboratories, the collected samples was stored in at – 4 $C^o$ till to be transported to National Research Centre laboratories.

Soft corals were identified as possible to the nearest species according to the Great Barrier Reef Expedition [13], the SIBOGA Expedition [14], Xeniidae of Red Sea [15], Des Roten Meeres [16], revision of the genus *Sarcophyton* [17], and Soft corals and sea fans [18]. On the other hand, hard corals were identified by photographing in the field, as possible, and some samples were brought to the laboratory to be identified according to Sheppard and Sheppard [19]and Veron [20]

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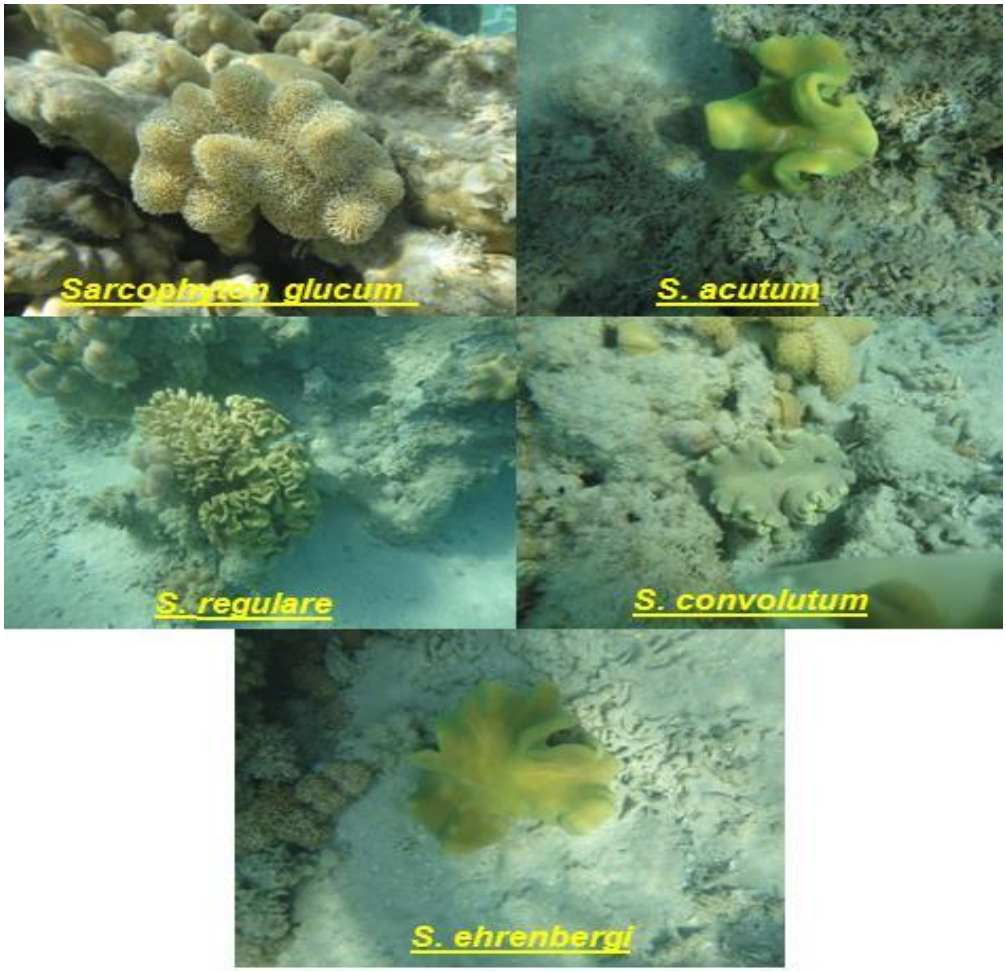


**Extraction**

Frozen soft coral (100 g, total wet weight) was cut into small pieces and extracted with ethyl acetate at room temperature (500mL × 3). The combined ethyl acetate extracts were concentrated *in vacuo* to a brown gum.

**Sample Preparation**

Samples were prepared by dissolving a specified weight of the extract in methanol to a final concentration of 20mg/2 mL and filtered through 0.45 μm filters (13 mm diameter), consequently, 20 μl injection of each sample was assayed.

**Isolation and Identification**

Pure isolated compound was confirmed by comparing the spectral data with the published data.

**(+)-Sarcophine** (**1**): $[\alpha]_D^{25}$ = +95.0 (*c* 0.5, $CHCl_3$); lit . $[\alpha]_D^{25}$: +92 (*c* 1.0, $CHCl_3$). $^1$H-NMR (600 MHz, $CDCl_3$): 4.97 (dd, *J* = 10.0, 1.0, H-2), 2.43 (m, H-5), 1.62 (m, H-6), 1.79 (m, H-6), 3.33 (dd, *J* = 10.0, 1.5, H-7), 1.68 (m, H-9), 2.13 (m, H-10), 5.26 (td, *J* = 7.5, 1.0, H-11), 2.39 (m, H-12), 2.13 (m, H-14), 2.62 (m, H-14), 1.87 (brt, *J* = 1.5, H-17), 1.91 (d, *J* = 1.0, H-18), 1.22 (s, H-19), 1.66 (s, H-20). $^{13}$C-NMR (150 MHz, $CDCl_3$): 163.2 (C-1), 80.2 (C-2), 118.6 (C-3), 146.1 (C-4), 36.4 (C-5), 29.2 (C-6), 73.8 (C-7), 75.1 (C-8), 38.5 (C-9), 22.6 (C-10), 126.7 (C-11), 135.1 (C-12), 36.7 (C-13), 26.1 (C-14), 123.0 (C-15), 175.2 (C-16), 9.5 (C-17), 17.8 (C-18), 24.3 (C-19), 16.2 (C-20). [21]

**2*R*,7*R*,8*R*-Dihydroxydeepoxysarcophine** (**2**) Colorless crystals. : $[\alpha]_D^{25}$ –21.2 (*c* 1.86, MeOH). HRCIMS: *m/z* [M + Na]+calcd for $C_{20}H_{29}O_4Na$: 357.20363; found: 357.20371. $^1$H-NMR (600 MHz, $CDCl_3$): 5.03 (dd, *J* = 10.0, 1.0, H-2), 2.39 (ddd, *J* = 10.5, 10.5, 6.0, H-5), 1.68 (m, H-6), 1.92 (m, H-6), 2.67 (t, *J* = 4.5, H-7), 1.10 (dt, *J* = 13.0, 2.5, H-9), 2.00 (m, H-9), 2.04 (m, H-10), 2.18 (m, H-10), 5.16 (dd, *J* = 9.5, 5.5, H-11), 2.09 (m, H-13), 2.26 (m, H-13), 1.93 (m, H-14), 2.73 (m, H-14), 1.86 (brt, *J* = 1.5, H-17), 1.88 (d, *J* = 1.0, H-18), 1.29 (s, H-19), 1.61 (s, H-20). $^{13}$C-NMR (150 MHz, $CDCl_3$): 162.3 (C-1), 78.7 (C-2), 120.6 (C-3), 144.2 (C-4), 37.6 (C-5), 25.2 (C-6), 61.5 (C-7), 60.0 (C-8), 39.1 (C-9), 23.6 (C-10), 124.8 (C-11), 135.3 (C-12), 36.4 (C-13), 27.5 (C-14), 123.0 (C-15), 175.5 (C-16), 9.0 (C-17), 16.1 (C-18), 17.3 (C-19), 15.5 (C-20). [21]

**Microbial transformation of Sarcophine**

**Microorganisms**

*Rhizopus nigricans*, *Fusarium culmorum* and *Aspergillus terreus* were from the stock culture of the Department of Chemistry of Natural and Microbial products, National Research Center, Cairo, Egypt. These fungi were maintained on potato dextrose agar slants and stored at 4 ºC.

**Methodology**

The fungi were maintained in potato dextrose agar (PDA) medium slants purchased from Lab M Limited 1 Quest Park, Moss Hall Road, Heywood, Lancashire BL97JJ, United Kingdom. Medium used for biotransformation was Czapek Dox broth medium composed of $NaNO_3$, 2.0 g ; $K_2HPO_4$, 1.0 g ; KCl, 0.5 g ; $MgSO_4.7H_2O$, 0.5 g ; $FeSO_4.7H2O$, 0.01 g ; sucrose and 20.0 g. The pH was adjusted at 6.5-7.0, sterilized at 121°C for 17 minutes. Medium without fungi was also prepared to act as a control. No metabolites were detected in the control.

Fungal cultures were preincubated on PDA for one week at 28ºC and then a spore suspension was inoculated into a 250 ml flask containing 50 ml of Czapek dox broth medium. After three days of incubation at 28 ºC on a rotatory shaker at 120 rpm, sarcophine (20 mg dissolved on 200 µL methanol) was added to each flask. The mixture was shaken (120 rpm) at 28 ºC for 14 days. Control flasks were used containing inoculated medium without addition of sarcophine. The cultures were filtered and extracted thrice with ethyl acetate (EtOAc). The residues were spotted on TLC with sarcophine as a standard to detect biotransformation products using n-hexane– EtOAc, 2:1 as developing solvent.

**RESULT AND DISCUSSION**

The extract yields data for the ethyl acetate of five *Sarcophyton* species extracts are shown in Table 1. 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 by MeOH / $H_2O$ (65:35) as the mobile phase.

**Table 1: HPLC quantitative analysis of sarcophine in five species of the genus sarcophyton collected from the Red Sea.**

| Species name | (%) of Sarcophine |
|---|---|
| *Sarcophyton glucum* | 53.5 |
| *S. acutum* | 48.5 |
| *S. regulare* | 38.4 |
| *S. convolutum* | 21.4 |
| *S. ehrenbergi* | 35.0 |

Figure 2 shows the chromatogram of 60 µg/ml of standard sarcophine. The average retention time ± standard deviation (S.D.) was found to be 4.6±0.012 min. The HPLC method was specific to sarcophine as standard, hence we used RID. The specificity of the RP HPLC is illustrated in Fig. 2 where there is a good separation of sarcophine in five *sarcophyton* species.

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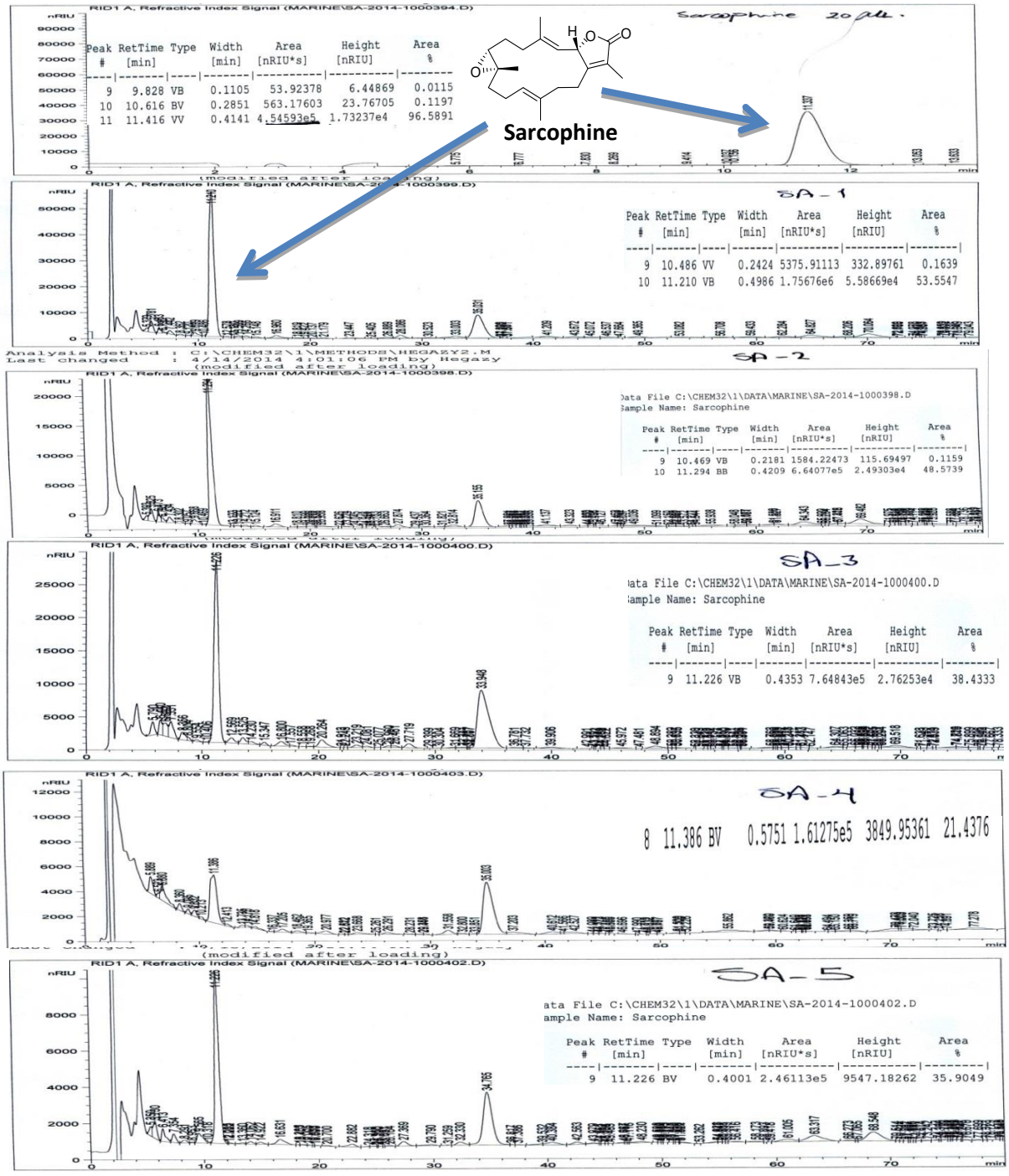


**Figure 2: Determination of sarcophine in different *Sarcophyton* species by HPLC**

A quantitative determination of the major compound, sarcophine of ethyl acetate extracts in five species of the genus *Sarcophyton* determined by RP HPLC using ODS column showed that sarcophine is the major compound in *S. glucum* with 53.3 % in comparison with other species under study.

Microbial transformation of sarcophine was tested with three microorganisms *Rhizopus nigricans*, *Fusarium culmorum* and *Aspergillus terreus*, as substrate. *R. nigricans* transforme sarcophine to dihydroxydeepoxysarcophine as a biotransformed product as described in Scheme 1.

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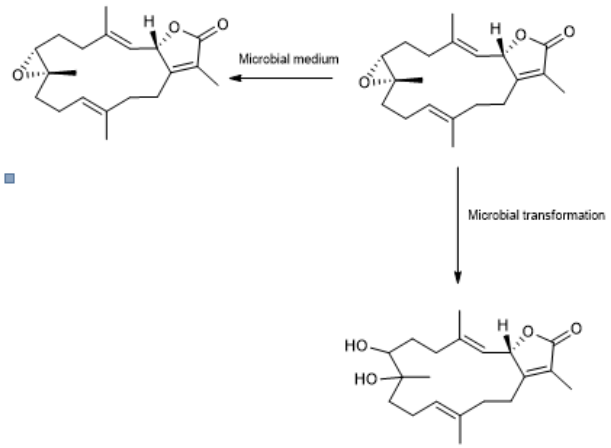


**Scheme 1: Microbial transformation of sarcophine**

**ACKNOWLEDGEMENTS**

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