SHORT COMMUNICATION

Antibacterial effect of the red sea soft coral Sarcophyton trocheliophorum

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The marine soft corals Sarcophyton trocheliophorum crude extracts possessed antimicrobial activity towards pathogenic bacterial strains, i.e. Bacillus cereus, Salmonella typhi, Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa. Bioassay-guided fractionation indicated that the antimicrobial effect was due to the presence of terpenoid bioactive derivatives. Further biological assays of the n-hexane fractions were carried out using turbidity assay, inhibition zone assay and minimum inhibitory concentration for investigating the growth-inhibition effect towards the Gram-positive and Gram-negative bacteria. The fractions were screened and the structure of the isolated compound was justified by interpretation of the spectroscopic data, mainly mass spectrometry (GC-MS). The structure was assigned as (5S)-3-[(3E,5S)-5-hydroxy-3-hepten-6-yn-1-yl]-5-methyl-2(5H)-furanone and was effective at concentrations as low as 0.20 mg/mL. The above findings, in the course of our ongoing research on marine products, may implicate that the profound antimicrobial activity of the S. trocheliophorum soft corals, inhabiting the red sea reefs, is attributed to the presence of growth-inhibiting secondary metabolites mainly terpenoids.

Keywords: soft coral; Sarcophyton trocheliophorum; terpenoid; in vitro; antimicrobial assays

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1. Introduction

Soft corals such as the Coelenterates (class: Anthozoa, subclass: Octocorallia, order: Alcyonacea, family: Alcyoniidae) have been studied extensively and produce a range of interesting compounds (Rézanka & Dembitsky 2001). Sarcophyton is one of the most commonly found soft corals in the tropical and sub-tropical oceans (Li et al. 2009). To date, around 30 species from this genus have been sampled and tested for bioactive secondary metabolites (Bie et al. 2008). Extracts from these species displayed a plethora of interesting pharmacological properties (Anjaneyulu & Rao 1997), including HIV inhibition, neuroprotective activity, cytotoxicity, anti-inflammatory activity (Lin et al. 2011a) and antimicrobial activity (Badria et al. 1997).

For this study, we investigated the effects of the soft coral Sarcophyton trocheliophorum as a potential natural bactericidal lead. The antimicrobial activity of the soft coral S. trocheliophorum was tested against the most common opportunistic bacteria. The Gram-positive (G+) and Gram-negative (G–) bacteria are amongst the medically important and emerging human pathogens and become a leading cause of morbidity and mortality especially in immune-compromised patients. The growth inhibitory effects on Bacillus cereus, Salmonella typhi, Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa were compared using turbidity assay, inhibition zone assay and minimum inhibitory concentration. In addition, the study identified a class of targeted antimicrobial compounds represented by (5S)-3-[(3E,5S)-5-hydroxy-3-hepten-6-yn-1-yl]-5-methyl-2(5H)-furanone as part of our ongoing project on chemical investigations of bioactive compound from natural sources (El-Seedi et al. 2010, 2013).

2. Results and discussion

The hexane and ethyl acetate (EtOAc) extracts were initially tested for antibacterial activity against E. coli 0157 H7 ATCC 51659 and S. aureus ATCC 13565, which were used as model G– and G+ bacteria, respectively. Inhibition of bacterial growth was detected for both types of bacteria after incubation at 37°C for 24 h with various extract concentrations (Figures 1 and 2). The effect of extracts on bacterial growth at different concentrations (0.5, 1, 2.5 and 5 mg/mL) was evaluated using turbidity measurements. The extent of the growth inhibition zone was dose dependent, and significant inhibition was observed at low concentrations of 0.5 and 1.0 mg/mL. The EtOAc extract was more potent, inhibiting G– bacterial growth at all tested concentrations (Figure 1), whereas the hexane extract was more potent against G+ bacteria (Figure 2).

The hexane (H1–H7) and EtOAc (E1–E5) extract fractions were subjected to antibacterial screening against a wide range of bacterial species (S. aureus, B. cereus, S. typhi, E. coli 0157 H7 and P. aeruginosa) using the disc diffusion assay. The hexane extract fraction H2 produced the largest inhibition zones in experiments using the G+ bacteria S. aureus (16 mm) and B. cereus (17 mm). Moreover, H3 induced activity against all the tested bacteria except B. cereus, with inhibition zones of 10, 9 and 8 mm, respectively, for the G– bacteria S. typhi, E. coli 0157 H7 and P. aeruginosa. This fraction was the most active against E. coli (9 mm), followed by H2 (7 mm); other fractions had no effect on these bacterial strains growth. All fractions barring H1 showed moderate to high activity against S. typhi. Fractions H4 and H7 exhibited no activity against P. aeruginosa; and the other fractions showed modest activity against this species (10–7 mm) (Table 1). In turn, the E4 fraction was the most active of the EtOAc fractions against all tested species barring P. aeruginosa. It produced a particularly large inhibition zone in tests using E. coli 0157 H7 (17 mm). E2 and E1 exhibited intermediate activity, and E5 was the least active of the EtOAc fractions (Table 2). The most active fraction (H3) was further investigated to isolate the bioactive compound(s) and the active principle was characterised. The structure was confirmed based on spectroscopic tools and comparison with literature data (Figure 1).
Terpenoid compound was identified as (5S)-3-[(3E,5S)-5-hydroxy-3-hepten-6-yn-1-yl]-5-methyl-2(5H)-furanone (Figure 3).

(5S)-3-[(3E,5S)-5-Hydroxy-3-hepten-6-yn-1-yl]-5-methyl-2(5H)-furanone: The molecular formula was obtained as C_{12}H_{14}O_{3} with molecular ion peak at \( m/z \) 206.09 (M + H)^+ using HR-FAB-MS (positive mode). \(^1\)H NMR (CD$_3$OD): 1.39 (3H, d, \( J = 6.9 \) Hz, H-12), 1.99 (2H, m, H-6), 2.19 (2H, m, H-5), 2.48 (1H, d, \( J = 1.8 \) Hz, H-11), 4.90 (1H, dd, \( J = 1.5 \) and 6.6 Hz, H-9), 5.20 (1H, dq, \( J = 1.6 \) and 6.6 Hz, H-4), 5.60 (1H, dd, \( J = 6.2 \) and 14.6 Hz, H-8), 5.82 (1H, ddd, \( J = 1.3, 8.6 \) and 14.3 Hz, H-7), 7.00 (1H, d, \( J = 1.5 \) Hz, H-3), \(^{13}\)C NMR (CD$_3$OD): C-12 (18.9 q), C-6 (31.8 t), C-5 (34.0 t), C-9 (63.1 d), C-11 (74.0 d), C-4 (77.9 d), C-10 (83.9 s), C-8...
Table 1. Antibacterial activity in terms of the diameter of the inhibition zone in millimetre as produced by S. trocheliophorum hexane extracts fractions $H_1$–$H_7$ produced by S. trocheliophorum inhibited the growth of G+ and G– bacteria in a large zone of millimetres scales.

| Test microorganisms | $H_1$ | $H_2$ | $H_3$ | $H_4$ | $H_5$ | $H_6$ | $H_7$ | Cefo-perazone (100 μg/mL) |
|---------------------|-------|-------|-------|-------|-------|-------|-------|--------------------------|
| Gram + ve bacteria  |       |       |       |       |       |       |       |                          |
| S. aureus           | (9)   | (16)  | (8)   | (9)   | –     | –     | –     | (12)                     |
| B. cereus           | (7)   | (17)  | –     | –     | –     | –     | –     | (12)                     |
| Gram –ve bacteria   |       |       |       |       |       |       |       |                          |
| E. coli             | –     | (7)   | (9)   | –     | –     | –     | –     | (11)                     |
| S. typhi            | –     | (7)   | (10)  | (8)   | (11)  | (9)   | (8)   | (11)                     |
| P. aeruginosa       | –     | (7)   | (8)   | (8)   | (9)   | (10)  | –     | (15)                     |

*The values (average of triplicate) are diameter of zone of inhibition at 1 mg/mL.*

Table 2. Antibacterial activity in terms of the diameter of the inhibition zone in millimetre produced by S. trocheliophorum EtOAc fractions (E$_1$–E$_5$) against G+ and G– bacteria.

| Test microorganisms | E$_1$ | E$_2$ | E$_3$ | E$_4$ | E$_5$ | Cefo-perazone (100 μg/mL) | DMSO |
|---------------------|-------|-------|-------|-------|-------|--------------------------|------|
| Gram + ve bacteria  |       |       |       |       |       |                          |      |
| S. aureus           | (11)  | (12)  | (8)   | (13)  | (7)   | (12)                     | –    |
| B. cereus           | (8)   | (11)  | (11)  | (13)  | (8)   | (12)                     | –    |
| Gram –ve bacteria   |       |       |       |       |       |                          |      |
| E. coli             | (9)   | (12)  | (7)   | (17)  | –     | (11)                     | –    |
| S. typhi            | (12)  | (9)   | (8)   | (11)  | (8)   | (11)                     | –    |
| P. aeruginosa       | –     | –     | –     | –     | –     | (15)                     | –    |

*The values (average of triplicate) are diameter of zone of inhibition at 1 mg/mL.*

(128.8 d), C-7 (132.9 d), C-2 (135.6 s), C-3 (148.8 d), C-1 (174.7 s) the division of NMR values due to solvent effect (Rezanka & Dembitsky 2001).

Soft corals are the most widely distributed soft corals and thus attributed to a big portion of the reef biomass. This abundance necessitates an advanced level of defence mechanism to evade the organism against predators and competitors. The up-to-date research showed that the soft corals yield a range of biological activities such as antimicrobial, antifungal, anti-inflammatory, cytotoxic and anti-cancer activities. The great variation of the biological properties implies the chemical distinction of Sarcophyton’s natural derivatives and secondary metabolites such as sesquiterpenes, diterpenes, polyhydroxylated steroids and polyamine. Ring-based cembranoids were isolated earlier from the Red Sea and the South China Sea soft coral S. trocheliophorum and extracted by chloroform/methanol and EtOAc extracts, respectively (Liu et al. 2014; Al-Footy

Figure 3. Structure of the isolated compound.
et al. 2015). Moreover, Lin et al and Zhao et al reported the closely related 14-membered carbocyclic diterpenoids cembranoids from the soft coral Lobophytum crassum (Lin et al. 2011b; Zhao et al. 2013). Taken together, there is a growing body of evidence suggesting that the antibacterial activity is largely due to terpenoid compounds and fatty acids produced by the soft corals (Blunt et al. 2012; Vinothkumar & Parameswaran 2013). In agreement, S. trocheliopharm was amongst the potential marine organisms that possess new structures and novel molecules, and in our study, we report the identification of the new bioactive terpenoid, namely (5S)-3-[(3E,5S)-5-hydroxy-3-hepten-6-yn-1-yl]-5-methyl-2(5H)-furanone.

3. Conclusions
The hexane extract of Sarcophyton genus soft corals showed a potent antibacterial effect. Bioassay-guided fractionation strategy leads to isolation of terpenoid from the most active fraction. The structure was elucidated by NMR tools and compared with the literature data. Corals Sarcophyton displayed potential antibacterial activity that could be implemented as a therapeutic lead.

Supplementary material
Experimental details relating to this paper are available online at http://dx.doi.org/10.1080/14786419.2015.1040991.

Disclosure statement
No potential conflict of interest was reported by the authors.

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