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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2020 17(1): 110-115. **OPEN ACCESS**

Bioactive compounds from histological extracts of the marine sponge collected from Red Sea as a potential source for medical applications

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The present study focuses on the evaluation of biological activities in histological extracts of marine sponges on different microbial pathogens. The sponges were collected from Red Sea by SCUBA diving technique. The methanolic extract of each sponge was dissolved in water and extracted with ethyl acetate (EtOAc). The organic extract was dissolved in 60% aqueous methanol (MeOH) and successively fractioned with n-hexane and dichloromethane (CH_2Cl_2). The n-hexane and dichloromethane fractions of the organic extracts of the sponges of were evaluated for their *in-vitro* inhibitory activity using disc diffusion assay at 100 μg/disc against *E. coli*, *S. aureus* and *C. albicans*. From the screened fractions, four fractions showed moderate activities against *E. coli* with inhibition zones of 12-14 mm, while four were potently active against *S. aureus* with inhibition zones of 20-30 mm. Six different fractioned of sponges showed inhibitory activities against *C. albicans* with inhibition zones of 22-30 mm. In conclusion, the organic extracts of the Red Sea marine sponges possess lead compounds for the development of novel drugs to control infections.

Keywords: Red Sea sponge, biological activity, anti-candida, drugs

INTRODUCTION

Natural products from marine animals and their derivatives play an important role in the development of drugs for the treatment of human diseases (Newman and Cragg, 2013; Abdelmohsen et al., 2017). The marine-derived natural compounds could be considered as unexplored as a very small ratio not exceeding 1% were investigated for the biological activities (Wu et al., 2019). Many marine invertebrates such as sponges are sessile and accordingly develop tremendous number of secondary metabolites to attract food, block the growth of intruding neighbors or repel predators. Marine invertebrates including sponges contribute a considerable number of complex and unprecedented chemical entities (Blunt et al., 2015). Hundreds of research articles reported the biological activities of new metabolites isolated from sponges. It is clear that sponges have the potential to develop secondary metabolites possessing a number of activities including anticancer (Ruiz-Torres et al., 2017; Pfeffer and Singh, 2018), antiviral (Anjum et al., 2016) and anti-plasmodial (Badshah and Naeem, 2016). Additionally, a substantial number of sponge extracts and sponge-derived compounds

revealed diverse antimicrobial activities (Laport et al., 2009, Afifi and Khabour 2017).

The present study reported the findings of biological potential of Red Sea marine sponge extracts on microbial pathogens. The need for new drugs for controlling fungal pathogens has become a growing priority. Although effective drugs are relatively abundant, very few drugs are available which are therapeutically useful in the treatment of systemic fungal infections. It has recently pointed out that a potent, broad-spectrum drugs to control fungal infections, which lack significant side-effects, has not yet been discovered (El Amraoui et al., 2014). Fungal diseases have become increasingly in recent years, and there are certain important risk groups which are especially susceptible to fungal infections. For examples, patients undergoing treatment with immunosuppressive agents during organ transplantation, and those who have been immunocompromised by cancer chemotherapy (Eades and Armstrong-James 2019), or by Acquired Immune Deficiency Syndrome (AIDS), are particularly vulnerable to debilitating, sometimes fatal fungal infections (Limper et al., 2017). Novel and effective systemic drugs that control infections are urgently needed in order to combat these problems. Several microbial natural products (e.g., griseofulvin, nystatin, and amphotericin B) are useful for antifungal chemotherapy in some instances. Synthetic azoles are also available, and these are currently the most widely used drugs, especially against topical infections. However, the conspicuous lack of success in finding new families of systemic antifungal suggests that new approaches are needed (Roemer and Krysan, 2015). The present study presents a new approach to the exploration of Red Sea sponges for potential biological activity and to use as chemotherapeutic agents through screening of the organic extracts of these sponges using disc diffusion assay. Therefore the present study report the findings of biological potential of marine sponges collected from Red Sea against microbial pathogens.

MATERIALS AND METHODS

Collection of marine sponges

The Red Sea sponges were collected by hands using SCUBA at the Saudi Red Sea coast off Jazan and Al-Lith at 10-30 m depth during May 2014. The sponges were identified by Prof. Dr. van Soest. A voucher specimen for each sponge was kept in the collections of the Naturalis Biodiversity Center at Leiden, The Netherlands.

Extraction of the sponges and preparation of extracts

The collected sponges were freeze-dried. Ten grams of each dried sponge was extracted with methanol (3 x 100 mL) at room temperature. The methanolic extracts of the sponges were dried separately under vacuum to yield a dark residue. The resulting residue was dissolved in 60% methanol followed by successive fractionation with n-hexane and dichloromethane. The organic solvents of all fractions were dried under vacuum to obtain hexane (Hex) and dichloromethane (DCM) fractions from each collected samples.

Determination of the antimicrobial activities using the disc diffusion assay

The *in-vitro* antimicrobial activity was evaluated using the disc diffusion method as previously described (Kiehlbauch et al., 2000). This method is based on diffusion of drug component from the impregnated paper disc to the surrounding inoculated nutrient agar and Sabouraud's dextrose agar medium, so that the growth of tested microorganism is inhibited as a zone around the paper disc impregnated with the extract. Varieties of test microorganisms were used, including a Gram-positive bacterium (*Staphylococcus aureus* ATCC 25923), a Gram-negative bacterium (*Escherichia coli* ATCC 25922), and yeast (*Candida albicans* ATCC 14053). The adjusted inoculum of each microorganism, equivalent to a turbidity of 0.5 McFarland standards, were streaked separately using sterile swabs over the surface of Muller-Hinton agar plates. Sterile filter paper discs (6 mm diameter) were impregnated with 100 μg of each extract and applied to the inoculated plates. The plates were incubated at 37 °C for 24 h. Solvent control discs were used to determine the effect of solvents on bacteria and fungi as control. Ciprofloxacin (5 μg/disc) was used as an antibacterial standard, while ketoconazole (50 μg/disc) was used as an antifungal standard. The activity of each compound was determined by measuring the diameter of the inhibition zone in mm. The technique was performed in triplicate, and the mean diameter of each inhibition zone was recorded. Ketoconazole and ciprofloxacin were used as positive controls.

RESULTS AND DISCUSSION

The aim of the present study was to

determine the biological activities of sponge samples collected from the Red Sea. In the present study 20 sponges were collected and extracted as described under the experimental section. The hexane and dichloromethane fractions of each sponge were evaluated for their antimicrobial effects against *E. coli*, *S. aureus* and *C. albicans*. From the 40 fractions, only four fractions showed moderate activities against *E. coli* with inhibition zones of 12-16 mm at 100 μg/disc. These fractions belong to the sponges *Amphimedon chloros* (S-1), *Theonella swinhoei* (S-15), *Fascaplysinopsis reticulata* (Jazan) (S-17) and *Fascaplysinopsis reticulate* (Al-Lith) (S-18) (Table 1). In addition, four fractions displayed significant effect against *S. aureus* with inhibition zones of 20-30 mm at 100 μg/disc. The fractions belong to the marine sponges *Carteriospongia foliascens* (Jazan) (S-2), *Carteriospongia folia* (Al-Lith) (S-3), *Dactylospongia metachromia* (S-5), *Dactylospongia foliascens* (S-6), *Fascaplysinopsis reticulata* (Jazan) (S-17) and *Fascaplysinopsis reticulata* (Al-Lith) (S-18). The activity of hexane fractions against the tested pathogens were given in Table 2.

Table 1. Antibacterial activities dichloromethane fractions isolated from of the Red Sea sponge by disc diffusion assay

Sponge code	Sponge	Collection Site	Inhibition zone (mm)	
			E. coli	*S. aureus*
S 1	*Amphimedon chloros*	Jazan	16	-
S 2	*Carteriospongia foliascens*	Jazan	-	21
S 3	*Carteriospongia foliascens*	Al-Lith	-	20
S 5	*Dactylospongia metachromia*	Jazan	-	30
S 6	*Dactylospongia foliascens*	Jazan	-	19
S 7	*Hyrtios erectus*	Jazan	-	15
S 8	*Hyrtios erectus*	Al-Lith	-	16
S 10	*Hyrtios erectus*	Al-Lith	-	15
S 15	*Theonella swinhoer*	Jazan	12	-
S 17	*Fascaplysinopsis reticulata*	Jazan	12	28
S 18	*Fascaplysinopsis reticulata*	Al-Lith	13	30
S 19	*Suberea mollis*	Jazan	8	-
S 20	*Pseudoceratina arabica*	Jazan	7	-
Positive control	Ciprofloxacin		30	22

Table 2. Antibacterial activities hexane fractions isolated from of the Red Sea sponge by disc diffusion assay

Sponge code	Sponge Name	Collection Site	Inhibition zone (mm)	
			E.coli	*S. aureus*
S 1	*Amphimedon chloros*	Jazan	6	-
S 2	*Carteriospongia foliascens*	Jazan	-	14
S 3	*Carteriospongia foliascens*	Al-Lith	-	14
S 5	*Dactylospongia metachromia*	Jazan	-	11
S 6	*Dactylospongia foliascens*	Jazan	-	12
S 7	*Hyrtios erectus*	Jazan	-	15
S 8	*Hyrtios erectus*	Al-Lith	-	13
S 9	*Hyrtios erectus*	Jazan	-	14
S 10	*Hyrtios erectus*	Al-Lith	-	14
S 15	*Theonella swinhoer*	Jazan	6	-
S 17	*Fascaplysinopsis reticulata*	Jazan	10	17
S 18	*Fascaplysinopsis reticulata*	Al-Lith	8	16
Positive control	Ciprofloxacin		30	22

Table 3. Antifungal activities dichloromethane fractions isolated from of the Red Sea sponge against *C. albicans* by disc diffusion assay (inhibition zone in mm)

Sponge code	Sponge	Collection site	*C. albicans*
S 1	*Amphimedon chloros*	Jazan	20
S 6	*Dactylospongia foliascens*	Jazan	12
S 12	*Dragmacidon durissimum*	Jazan	16
S 13	*Stylissa carteri*	Jazan	7
S 15	*Theonella swinhoer*	Jazan	30
S 17	*Fascaplysinopsis reticulata*	Jazan	8
S 18	*Fascaplysinopsis reticulata*	Al-Lith	8
S 19	*Suberea mollis*	Jazan	28
S 20	*Pseudoceratina arabica*	Jazan	30
Positive control	Ketoconazole		30

Table 4. Antifungal activities hexane fractions isolated from of the Red Sea sponge against *C. albicans* by disc diffusion assay (inhibition zone in mm)

Sponge code	Sponge	Collection Site	*C. albicans*
S 1	*Amphimedon chloros*	Jazan	10
S 2	*Carteriospongia foliascens*	Jazan	25
S 3	*Carteriospongia foliascens*	Al-Lith	28
S 6	*Dactylospongia foliascens*	Jazan	16
S 14	*Callyspongia (Euplacella) densa*	Jazan	9
S 15	*Theonella swinhoer*	Jazan	16
S 17	*Fascaplysinopsis reticulata*	Jazan	6
S 18	*Fascaplysinopsis reticulata*	Al-Lith	7
S 19	*Suberea mollis*	Jazan	16
S 20	*Pseudoceratina arabica*	Jazan	16
Positive control	Ketoconazole		30

Six fractions of both the extracts showed significant antifungal activities against *C. albicans* with inhibition zones of 22-30 mm at 100 μg/disc (Table 3). These fractions belong to the marine sponges *Amphimedon chloros* (S-1), *Carteriospongia foliascens* (Jazan) (S-2), *Carteriospongia foliascens* (Al-Lith) (S-3), *Theonella swinhoei* (S-15), *Suberea mollis* (S-19) and *Pseudoceratina arabica* (S-20) (Table 4). The sponge samples collected from the Red Sea showed biological activity in one or the other fraction against bacteria and fungi. Many of the metabolic components isolated from sponges have been reported for biological activities (Agrawal et al., 2016, Andersen, 2017, Malve, 2016). The sponges produce variety of chemical substances for protecting themselves from the marine pathogenic bacteria and fungi. There are many reports on symbiotic and competitive interactions of marine sponges with marine microorganisms (Blockley et al., 2017). This interfaces might have stimulated the sponges to produce bioactive substances including components active against pathogens.

The remaining of the fractions are either inactive or weakly active against the three pathogens (data not shown). These results are considered as significant, as there is an urgent need to discover novel drugs active against pathogens especially from natural origin. Since *S. aureus* represent a most common cause of hospital-acquired bacteremia, the results of the current study should be taken in consideration and the active extracts are considered for further investigation to identify the active compound that could play a role in treatment of patients with nosocomial infection (Melzer et al., 2003). Furthermore, *C. albicans* is considered to be unique among pathogens as being a part of the normal microbial flora of the host and can be found in the oral cavity and the digestive and vaginal tracts. With the novel drugs and therapies clinical outcomes of fungal infections are very distant from the real approach. However, the newly emerged strains of many fungal pathogens are becoming resistant to commonly used drugs (Roemer and Krysan, 2014). Recently, an increased prevalence of candidiasis could be noticed and attributed to the widespread use of antibiotics and immunosuppressive agent *C. albicans* plays an important role in oral

candidiasis, denture stomatitis and severe periodontitis (Hirasawa and Takada, 2004). Several marine-derived animal products showed significant biological activities (Donia and Hamann, 2003; Afifi and Khabour 2017). The results in this study encourage us to proceed with the identification of biologically active compound in the extracts of the Red Sea sponges.

CONCLUSION

It is concluded from the present study that the collected sponges are potential source for bioactive components. Marine sponges collected from the Red Sea prove as a massive source to come across novel drugs or compounds with biological activities. Investigation is in progress to identify the many more bioactive compounds in the extracts using a bioassay-directed fractionation.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEDGEMENT

We would like to thank Prof. Rob van Soest for the taxonomic identification of the sponge specimens.

AUTHOR CONTRIBUTIONS

Dr. AAIY designed the work and reviewed the manuscript; Prof. HMH and Prof. MSA carried out the laboratory works; Dr. MIA contributed for preparation and revision of the manuscript

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