Chemical and Pharmacological Characterization of Fire Coral from Eastern Red Sea

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Abstract

Two species of the fire coral, Millepora dichotoma and Millepora platytphylla, were found along the Eastern Red Sea Coast and collected. The chemical constituents as well as pharmacological screeing of the crude extracts were studied. Plant pigments content (chlorophyll a,b,c and phaeopigments) in the two species of fire coral were having more or less the same level (6.3 μgg^{-1} and 4.5 μgg^{-1}) respectively. It was observed that the content of carbohydrate in both species was lower than lipid content, whereas protein was the highest component in the two species (3.5 mgg^{-1} and 3.4 mgg^{-1} respectively). The fatty acid methyl esters were analyzed by gas chromatography mass spectrometry (GC-MS), and it was observed that in both species, the saturated fatty acids were abundant (63.5% in M. dichotoma and 68.9% in M. platyphylla). Saturated fatty acids are indicative of dominant mode of action of autotrophic feeding. The residual material (mostly calcite in nature) in the two species was 57% and 53% respectively. The chemical composition of the M. dichotoma and M. platyphylla was compared with the obtained composition from the corals (other than fire corals) from the same area. It has been found that the fire coral have lower protein, lipid and carbohydrate contents compared to other corals.

The preliminary screening; bactericidal and fungicidal activities of the crude extracts of M. dichotoma and M. platyphylla were tested. The bactericidal activity of the M. platyphylla was moderately active compared to the crude extracts of the M. dichotoma. Whereas no significance was observed on the phytotoxic bioassay tests of the crude extracts of the M. dichotoma and M. platyphylla..

Key Words: Fire Coral, Plant pigments, Chemical composition, Bioassay screening, Saudi Arabia, Red Sea.

Introduction

Red Sea contains a large variety of marine resources; both living and non-living. The Saudi Red Sea coast is also inhabitated by toxic plants and animals. It has been noticed that the contact with fire coral can cause a burning sensation and blistery rash. Two species, *Millepora dichotoma* and *Millepora platyphylla*, have been observed along the Eastern Red Sea coast.

Several studies were conducted on testing the isolated nematocyst, using different techniques from different species of fire coral[11, 13, 14, 16]. However, most of these studies showed lethal and hemolytic properties. Wittle et al[16] have studied two different species of fire coral i.e. *M. alicornis* and *M. tenera* and revealed that the toxic agent was proteinous in nature and 40 g/kg of body wt. was sufficient to cause hemolytic property.

This study is a continuity of work on the fire coral from the Eastern Red Sea (Jeddah coast) and some results were already published[2]. The chemical compostion and the bioassay tests of the crude extracts of the two species were investigated.

Material and Methods

M. dichotoma and *M. platyphylla* specimen were collected from central Eastern Red Sea area (10m deep). It was observed that *M. dichotoma* had a fan shape like structure, while *M. platyphylla* appeared like a cluster.

Water content was determined by drying the coral at 70° C for 72 hours until constant weight. Residual matter was determined by heating a known weight of the two species in muffle furnace at 650° C for 24 hours till constant weight. The fire coral (whole) was ground in a mortar to powder. Plant pigments and carbohydrate were determined using standard methods[7, 12]. Protein content was also determined with albumin as a standard and measuring the absorbance at 530 nm[8]. Lipids were extracted with chloroform:methanol (1:2, v/v) using modifying Folch method[5]. Methyl esters were prepared by hydrolyzing the lipid in 1M potassium hydroxide under reflux for one hour, it was partitioned between ether and water. The water layer was neutralized with 5M hydrochloric acid and repeatedly extracted with ether. The free fatty acids were dissolved in tetrahydrofuran and 5% methanolic solution of hydrogen chloride was added. It was refluxed for two hours and the esters were extracted with hexane. The hexane layer was washed with 2% potassium bicarbonate, dried over anhydrous sodium sulphate and evaporated in a stream of nitrogen. The methyl esters of fatty acids were analyzed using gas chromatography-mass spectrometry (Shimadzu QP-5000). Gas chromatography (GC) equipped with a spilt/splitless injector, and a DB fused silica column (25 m x 0.3 mm i.d., 0.17 µm DB-5% phenyl/ 95% methyl-silicone) using helium carrier gas. The GC conditions were 40-300° C at 5° C min^{-1} then isothermal for 5 min., and the

injector temperature was 250° C. The end of the GC column was introduced into the electron impact (EI) source of a Shimadzu QP-5000 Quadrupole mass spectrometer. Typical mass spectrometer operating conditions were as follows: transfer line 230° C, ion source 250° C, electron energy 70 eV. All samples were analyzed in full data acquisition (SCAN) mode by scanning from 50-500 daltons at 1 cycle/s. Identification of the methyl esters of fatty acids was based on comparison of retention indices and mass spectra of the analytes with literature data.

Bioassay : The bioassay is appraised of the biological activity of a substance by testing the effect on an organism and comparing the results with some agreed standard. The solvent extracts of the two species were examined for their phytotoxic activity, antifungal and antibacterial properties. The phytotoxic bioassay was carried out following the protocol of McLaughlin[10], the plant selected was *Lemna aequinoctialis Welv* and the standard inhibitor used was Paraquat (1,1-dimethyl 4,4-bipyridyl). In the bioassay testing three different concentrations of test samples were used. These were incubated at 28° C for 12 hours and an average of 3 experiments was taken. The growth chamber was Fission Fi-torn 600 H Growth Cabinet. The light intensity was 9.00 lux kept at day light for 12 hours, incubated at 28° C (±1° C) with an average of rosette of 3 brands being taken. Paraquat standard was used in a concentration of 50 μg ml^{-1}. The insecticidal properties were aslo studied with respect to *Tribolium castaneum* and *Callosobruchus aralis*. The standard insecticide chosen was coopes, incubation temperature was maintained at 30° C, humidity was maintained at 60% and an average of two experiments was taken. The antifungal property was determined by Agar Dilution Method. Test function cultures were inoculated in the slant and growth inhibitions were observed after incubation period of 7 days[6]. Myconazole and Ketoconazole were used as standard antifungal drugs. MIC values were determined by the same procedure. The bactericidal activity was studied on 4 different human pathogens. The bactericidal properties were compared with standard drugs like Ampicillin, Amoxicillin and Cephalexin. A protocol of Well Agar Diffusion Method of Carron et al[4] was adopted. Agar Dilution Method was followed for this purpose.. It was incubated at 29° C for 7 days. Standard drugs used were Miconazole and Ketoconazole.

Results and Discussion

Chemical Composition : The chemical composition of *M. dichotoma and M. platyphylla* is presented in Table 1. Plant pigments (chlorophyll *a,b,c* and phaeopigments) in the two species of fire coral have more or less the same level (Fig. 1). The level of chlorophyll *a* was the highest followed by chlorophyll *c*, chlorophyll *b* and phaeopigments. In the present study chlorophyll *a* was found to be maximum as compared to chlorophyll *b, c* or phaeopigments, indicating a supporting factor operating for the photosystem. The colour of the coral is mainly arrtibuted to the presence of the symbiotic algae. Burkholder and Burkholder[3] found that the ratio of chlorophyll *c* to *a* in a number of corals from Caribbean Sea was 0.5:1. It is worthnoting that the ratio in *M. platyphylla* was in agreement with our finding, while the ratio in *M. dichotoma* was slightly higher (*i.e.* 0.5 :0.25). The distinct green colour of both species indicate that the effect of the bleaching is negligible. The chemical composition revealed some interesting points. The carbohydrate content in both species was lower compared to protein and lipid contents (Fig. 2), whereas the protein content was the highest. Both of the two species had a lipid content similar to the corals *S. pistillata* & *E. gemmacea*, but it was seven times lower than the lipid content of *L. corymbosa*. The difference may be attributed to the secretion on the coral body; it is cognizable in the sense that the latter in its general appearance contains viscous secretions on the coral body thereby explaining a high protein and lipid content. The higher protein content, compared to lipids and carbohydrates indicate these *Millepores* voracious plankton feeders, in addition to their autotrophic mode of feeding. The fatty acid methyl esters in *M. dichotoma*, included mainly saturated fatty acids 16:0, 18:0 (43.5% and 12.7% respectively) and among unsaturated fatty acids 16:1 (28.5%) was observed. In *M. platyphylla* the abundant fatty acids were 20:0, 18:0 and 16:0 (14.8%, 29.8% and 15.5%, respectively). The major unsaturated fatty acid was found to be 17:1 (12.3%). An overall picture indicated the saturated fatty acids to be dominant in both the species. Meyer[9] has stated that the abundance of saturated fatty acids indicates a greater reliance of coral on autotrophic feeding.

A number of long-chain fatty acid esters have also been detected which occur as waxy in nature and probably serve as a coating for the protection of the animal against environmental variations[2]. It is also postulated that they serve as energy reserves during starvation.

Corals can absorb calcium from seawater since it is abundant, the animal does not necessarily depend on food as a source of calcium. Both metabolic and environmental bicarbonates provide a source of carbonate for skeletogenesis. Light, warm water and the presence of zooxanthellae significantly favors the rate of calcification, explaining a reasonably good percentage of calcium carbonate in the corals studied (53-57%). This may explain the fact that corals in the dark with algae, calcify significantly faster than those in the dark without algae.

Bioassay Activities : Since the two species of fire coral are producing burning sensation upon direct skin contact, it was hypothesized that due to this powerful toxin, these species might have antimicrobial properties. The extracts were also examined for fungicidal bioassay. The results are summarized in Table 2 and 3. It is well documented that penetration and colonization of plant tissues by phytopathogenic fungi can

involve fungal production and release of host-selective toxins. The overall picture indicated that both the species did not exhibit any significant phytotoxic activity. Bleaching of fronds resulted with *M. dichotoma* whereas with *M. platyphylla* this phenomena was not observed. The growth inhibition was observed to be 66.6% compared to the standard whereas in *M. dichotoma* it was found to be 33%, the inhibitory effect was much less. The bactericidal activity of the coral extract was approximately 30% compared to Amoxicillin and Ampicillin drug standards. *M. dichotoma* did not show any significant activity against *K. pneumonia*, *P. mirabilis* and *S. pyogenes* while *M. platyphylla* showed moderate activity particularly against *Shigella* and *Coli* form bacteria. The fungicidal activity was studied on 8 different species of fungi. Both the species showed moderate activity, but the activity of *M. platyphylla* was more distinct as compared to *M. dichotoma*. Three other species of fire coral (*M. alicornis, M. complanata* and *M. squarrosa*) near shore of Dominican Republic were tested for antibacterial and antifungal activity[6]. The bacteria tested were *Ps. aurigenosa* and *B. cereus*; the fungus examined was *C. albicans*. Their findings were also more or less similar to our work; the three species of the coral showed a weak antibacterial property. The antifungal property against *C. albicans* was also not very pronounced. It is postulated that the fire coral had developed this antimicrobial activity just sufficient enough for their survival against foreign aggression, but it is not so pronounced or distinct that one can not speculate to isolate or identify the individual components to be exploited as pharmcologically active marine product.

Conclusion

The plant pigments content represented the same quantity in both species. The protein content represented the highest amount in the two species followed by the lipids while, the carbohydrate in both species were found to be lowest compared to other biomolecules. Greater clacification rate and abundance of saturated fatty acids indicate a greater reliance of these corals on autotrophic feeding.

The phytotoxic bioassay tests of the crude extracts of the *M. dichotoma* and *M. platyphylla* showed that against the fungi *T. simii*, the intensity of the activity was identical i.e the crude extracts were half as active as the standard antifungal drugs, whereas *M. platyphylla* was more active against *A. niger* than *M. dichotoma*. On the contrary, it was observed that against *Ps. boydii, M. dichotoma* was twice as active as *M. platyphylla*. The bactericidal activity of the crude extract of the *M. platyphylla* was moderately active compared to the crude extracts of the *M. dichotoma*. The indication of the antibacterial and antifungal activity leads to the assumption that they appear to be immune to common diseases. Additional studies are needed to isolate and identify the bioactive componantes and determine the chemical and ecological relationship and to further investigate the ecological role that *Millepora* plays in the reef ecosystem.

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Table I

Chemical composition of different corals (fire and non-fire) from Eastern Red Sea (Jeddah Coast) n = 3

Parameter	*M. dichotoma*[1]	*M. platyphylla*[1]	*S. pistillata*[2]	*L. corymbosa*[2]	*E. gemmacea*[2]
Water content % By wt.	17.55 ±0.21	16.50 ±0.25	—	—	—
Residual matter % dry wt. of total coral	57.08 ± 0.14	53.85 ± 1.5	42.5 ± 2.5	32.8 ± 3.03	41.4 ± 3.04
Chlorophyll *a* :g g^{-1}	3.10 ± 0.35	2.16 ± 0.25	0.9 ± 0.44	2.6 ± 1.36	5.0 ± 2.51
Chlorophyll *b* :g g^{-1}	0.56 ± 0.30	0.75 ± 0.15	0.4 ± 0.15	1.0 ± 0.71	3.1 ± 0.77
Chlorophyll *c* :g g^{-1}	2.08 ± 0.42	1.20 ± 0.21	2.9 ± 1.1	4.9 ± 3.73	11.5 ± 4.86
Carotenoids :g g^{-1}	0.65 ± 0.23	0.44 ± 0.22	0.3 ± 0.04	1.4 ± 0.39	1.5 ± 0.86
Carbohydrate :g g^{-1}	39.57 ± 0.63	45.75 ± 0.80	559 ± 83	425 ± 49	859 ± 0.22
Protein mg g^{-1}	3.50 ± 0.5	3.45 ± 0.45	9.4 ± 1.44	21.4 ± 5.21	3.1 ± 1.06
Lipid mg g^{-1}	1.10 ± 0.47	1.13 ± 0.33	1.9 ± 0.27	8.6 ± 1.46	1.3 ± 0.22

[1] present study, [2]Al-Lihaibi et al (1998).

Table II

***In-Vitro* Fungicidal Bioassay of the two fire coral extracts from Eastern Red Sea (Jeddah Coast).**

Name of Fungi	*M. dichotoma* Inhibition Zone %	*M. platyphylla* Inhibition zone %	Standard Drugs[1] Inhibition %
Human Pathogens			
Trichophyton schoenleinii	31.1	40	100
Pseudallescheria boydii	50	25	100
Candida albicans	0	7	100
Aspergillus niger	40.6	50	100
Animal Pathogens			
Micosporum canis	39.3	43	100
Tricophyton simii	54.5	50	100
Plant Pathogens			
Fusarium oxysporum var.	25	14	100
Macrophomina phaseolina	7.0	33	100

[1]Miconazole, Ketoconazole Benlate, Nabam Concentration was :g/ml of media SDA for 7-10 days at 29° C (28 ± 1° C) .

Table III

In-Vitro Bactericidal Bioassay of the two fire coral extracts from Saudi Red Sea Coast.

Name of Bacteria	*M. dichotoma* Inhibition zone %	*M. platyphylla* Inhibition zone %	Standard drugs Inhibition zone %	Reference Drugs
Escherichiae coli	8	7.5	19	Amoxicillin
ETEC			19	Ampicillin
Klebsella	7	---	16	Amoxicillin
pneumoniae			17	Ampicillin
Proteus mirabills	6	---	19	Amoxicillin
			20	Ampicillin
Pseudomonas	6.5	6	17	Amoxicillin
aeroginosa			17.5	Ampicillin
Salmonella typhi	6.5	6	15	Amoxicillin
			14.5	Ampicillin
Shigella boydii	8	6.5	23	Amoxicillin
			24	Ampicillin
Staphylococcus	7	6.5	20	Amoxicillin
aureus			19.5	Ampicillin
Streptococcus	---	---	---	Amoxicillin
pyogenes				Ampicillin

Concentration was 200 µg/100ml (ul) of DMSO. Colony Forming unit (CFU)m. = 10^4–10^6 Size of well = 5 mm (radius).

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