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ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF *TUPIBORA MUSICA* (METHANOL EXTRACT)

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ABSTRACT

The recent appearance of a growing number of microbial resistant to conventional antibiotics has stimulated the search for novel antimicrobial agents or lead compounds from a variety of sources, including natural sources. The marine environment is an exceptional reservoir of a wide variety of organisms which are prolific producers of novel and bioactive secondary metabolites with diverse chemical structures and biological activities. This study screens methanol extract of a marine red-Sea coral *Tubipora miusica* (Tubiporidae) for in vitro antimicrobial activity against four gram (+/-) bacteria and four fungal strains using the broth macro dilution method to determine the minimum inhibitory concentration (MIC) of the extract against the standard microorganisms under the test followed by plating out to determine the minimum lethal concentration (MLC). The MIC was appeared at concentration 1.25 mg/ml against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus niger* and at 0.625 mg/ml against *Bacillus subtilis*, *Aspergillus flavus* and *Aspergillus fumigates*. The MLC of *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* was 2.5 mg/ml and 1.25 mg/ml for *Escherichia coli*, *Aspergillus niger* and *Aspergillus flavus* and 0.625 mg/ml against *Aspergillus fumigates*. The extract show a good bactericidal and fungicidal activities, All findings sugges that *Tubipora musica* is a quite promising as antibacterial and antifungal agent.

KEYWORDS: Antimicrobial activity, phytochemical screening, *Tupibora musica*, methanol extract.

INTRODUCTION

Although many communicable diseases have been effectively contained, bacterial infections remain a major cause of morbidity and mortality, particularly in developing countries. Moreover, in both developed and developing countries, the risk of some serious bacterial infections has increased because of treatments such as chemotherapy for cancer and the emergence of diseases such as human immunodeficiency virus (HIV) infection and the acquired immunodeficiency syndrome (AIDS), which impair the patient's defenses against infection[1], also fungal infections have assumed a much greater importance to the same reason.[2] Moreover the recent appearance of a growing number of bacteria resistant to conventional antibiotics has stimulated the search for novel antimicrobial agents or lead compounds from a variety of sources, including natural sources.[3] The marine environment is an exceptional reservoir of a wide variety of organisms which are prolific producers of novel and bioactive secondary metabolites with diverse chemical structures. This diversity of chemical compounds is believed to be a consequence of competition between organisms for space and resource in most marine habitats, due to antimicrobial actions some of the biologically active natural products have become excellent of new and effective drugs such as antimicrobial agents.[4] [5]

Tubipora miusica (Tubiporidae) colonies are composed of parallel arrays of pipes or tubes linked together at regular intervals along their length by connecting platforms The skeletons are a dull red color but the living colonies usually appear various pale shades of green, blue or purple due to the color of the expanded polyps. Generally any one colony is composed of polyps of a single color, although occasionally portions of large colonies were found to be composed of polyps of a different color from that of the remainder of the colony.[6] This species is widespread in the Indo-Pacific region. It can be found from the Red Sea and East Africa to Southeast Asia, including southern Japan, Australia and the Coral Sea. It has also been recorded off the coast of South Africa and Mozambique.[7] A relatively small number of marine plants, animals and microbes have already yielded more than 12,000 novel chemicals, marine has a broad spectrum of applications that range from biomedicine to the environment.[8] Organ- pipe coral (English name) is used traditionally in eye diseases and bleeding, strengthens the heart, headache, cough rheumatism and kills worms[9], in Sudan it is

called (Dm Al-akhwain) found in the Red sea state and used as poultices using Sesame oil as vehicle to treat some dermatitis and some dermal hypersensitivity problems.

MATERIAL AND METHOD

Chemicals, solvents, glassware, standard microorganisms were obtained according to standard requirements.

Marine material

The marine material was collected from Sawaken, Red sea state, Eastern Sudan and the botanical identification was authenticated by Dr. Mohammed Ali Omer Rahoom (The head of zoology department at Khartoum university, 2015.

Fresh sample will subjected to shade air drying, milling and homogenization.

Extraction

150 gm of the dried *Tubipora musica* was extracted by soxhlet with 500ml methanol (95%) for 48h until the solvent in the siphoning tube become colorless, the extraction process repeated many times to obtain a considerable extract quantity. The extract was evaporated of to dryness, the dried extract was dissolved in methanol and conducted for antimicrobial test.

Test microorganisms

Bacterial microorganisms

Bacillus subtilis (NCTC 823), *Staphylococcus aureus* (ATCC25923), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853).

National Collection of Type Culture (NCTC), Colindale, England.

American Type Culture Collection (ATCC) Rockville, Maryland, USA.

Fungal microorganisms

Candida albicans (ATCC 7596), *Aspergillus niger* (ATCC9763), *Aspergillus flavus* and *Aspergillus fumigatus* were isolated from Royal Care hospital- Khartoum.

Standard drugs

Ciprofloxacin tablet 500mg (ZYDUS CADILA- India) and Itraconazole tablet 100mg (GLENMARK- India).

Phytochemical Analysis

Qualitative analysis of the extract was carried out according to the method reported by Tiwari P. *et al.*[10]

Antimicrobial test

Preparation of Test Samples

The antibacterial and antifungal activities of *Tubipora musica* was studied by broth macro dilution method, which was carried out as described by (Wiegand *et al*, 2008)[11] with some modification, 2ml of nutrient broth media was added to eight numbered test tubes then 2ml containing 20mg of extract was added to the first test tube, well mixed and 2ml was transferred to the second test tube then to the third and so on until reach the seventh one to get two folds dilution with a net concentrations (5, 2.5, 1.25, o.625, 0.313, 0.156 and 0.078) mg/ml. A positive control tube was included for every test microorganism to demonstrate adequate microbial growth over the course of the incubation period, all tubes were inoculated with about 10^8- 10^9 C.F.U/ml (colony forming units per ml) of the specified microorganism. Growth is assessed after incubation for (24 h) at 37°C. and the MIC value was read (minimal inhibitory concentration, MIC) that, under defined test conditions, inhibits the visible growth of the bacterium (which indicated by turbidity) being investigated to evaluate the activity of the extract under the test, then the inhibited cultures were plated out onto nutrient agar to assess growth (bacteriostatic) or no growth (bactericidal) after incubation and therefore determine the MLC (the lowest concentration of which kill the organism).

Mentioned steps were carried out on all bacteria and fungi under the test (Sabouraud dextrose broth and agar media were used for fungi instead of nutrient broth and agar, *Aspergillus niger* took 48h at incubation.

RESULTS AND DISCUSSION

Phytochemical screening

The screened secondary metabolites in the tested marine are shown in table (1).

Table (1): phytochemical screening

Phytochemical group	Name of test	Reagents	Results
Alkaloids	Test of alkaloids	Mayer's	(+)
		Hager's	(+)
		Wagner's	(+)

		Dragendroff's	(+)
Glycosides	Modified Borntrager's test	Ferric chloride, benzene, ammonia solution	(+)
Phenols	Ferric chloride test	Ferric chloride test	(-)
Phytosterols	Salkowisk's test	Chloroform, conc. sulphuric acid	(-)
	Libermann test		(-)
Flavonoids	a-Alkaline reagent test	a-Sodium hydroxide	(-)
	b-Lead acetate test	Lead acetate	(-)
Diterpens	Copper acetate test	Copper acetate	(+)

(+): indicate the presence of phytochemical group, (-): indicate the absence of phytochemical group.

Antimicrobial activity results

The experimental results of the antibacterial, antifungal activities of the tested extract and standard drugs are presented in Tables (2), (3), (4) and (5).

Table (2): Minimum inhibitory concentration (MIC) of methanol extract and standard antibacterial drug (Ciprofloxacin).

Bacteria / MIC of	***B.s***	***S.a***	***Ps***	***E.c***
Methanol extract (mg/ ml)	2.5	1.25	1.25	1.25
Ciprofloxacin (mg/ ml)	0.625	1.25	1.25	0.625

Con: concentration, B.s: Bacillus subtilis, S.a: Staphylococcus aureus, E.c: Escherichia coli, Ps: Pseudomonas aeruginosa.

Table (3): Minimum inhibitory concentration (MIC) of methanol extract and standard antifungal drug (Itraconazole).

Fungi / MIC of	***C.a***	***A.n***	***A.fl***	***A.fu***
Methanol extract (mg/ ml)	1.25	1.25	0.625	0.625
Itraconazole (mg/ ml)	0.625	1.25	0.625	0.625

Ca:candida albicans, A.n: Aspergillus niger, A.fl: Aspergillus flavus, A.fu: Aspergillus fumigates.

Table (4): Minimum bactericidal concentration (MBC) of (*Tubipora musica)* MeOH extract and standard antibacterial drug (Ciprofloxacin).

Bacteria / MBC of	***B.s***	***S.a***	***Ps***	***E.c***
Methanol extract (mg/ ml)	2.5	2.5	2.5	1.25

Ciprofloxacin (mg/ ml)	1.25	1.25	5	2.5

Con: concentration, B.s: Bacillus subtilis, S.a : Staphylococcus aureus, E.c: Escherichia coli, Ps: Pseudomonas aeruginosa.

Table (5): Minimum fungicidal concentration (MFC) of (*Tubipora musica)* MeOH extract and standard antifungal drug (Itraconazole).

Fungi / MFC of	*C.a*	*A.n*	*A.fl*	*A.fu*
Methanol extract (mg/ ml)	2.5	1.25	1.25	0.625
Itraconazole (mg/ ml)	0.625	2.5	1.25	0.625

Ca: Candida albicans, A.n: Aspergillus niger, A.fl: Aspergillus flavus, A.fu: Aspergillus fumigates.

DISCUSSION

The marine *Tubipora musica* is mainly used in Sudan for some dermal infections and hypersensitivity this traditional use should be scientifically explored. So the study is mainly focus to evaluate its antimicrobial activity, the result of this study show that the methanol extract from the marine *Tubipora musica* have various degrees of antimicrobial activities against two gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), two gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and four fungal strains (*candida albicans, Aspergillus niger, Aspergillus flavus* and *Aspergillus fumigatus*) using the broth macro dilution method. The MIC is the lowest concentration of antimicrobial agent that completely inhibits growth of the organism in tubes as detected by the mere eye[12], both the extract under the test and the standard drug Ciproloxacin give the same activity and inhibit the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* at concentration of 1.25 mg/ ml, the extract inhibit the growth of *Bacillus subtilis* at 2.5 mg/ ml showing lesser activity than Ciproloxacin (0.625 mg/ ml). *Escherichia coli* was inhibited at 2.5 mg/ ml by extract and 0.625 mg/ ml by Ciprofloxacin (better result). Both the extract and Itraconazole show the same MIC against *Aspergillus niger* at concentration of 1.25 mg/ ml and against *Aspergillus flavus* and *Aspergillus fumigatus* at concentration of 0.625mg/ ml while Itraconazole give a better result againt *candida albicans* at (0.625 mg/ ml) than the extract at 1.25 mg/ ml. This suggests that the marine under the test has a good and broad spectrum of antimicrobial activity. It was active against both the Gram-positive and Gram-negative organisms in addition to fungi, this justifies the traditional use of *Tubipora musica* against antimicrobial infections like skin infections.

The determination of minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFC), also known as the minimum lethal concentration (MLC), is the most common estimation of bactericidal or fungicidal activity. The MBC is defined as the lowest concentration of antimicrobial agent needed to kill 99.9% of the final inoculum after incubation for 24 h under a standardized set of conditions. in which the MBC can be determined after broth macro dilution by sub-culturing a sample from tubes, MFC is also defined as the lowest concentration of the drug that yields 98%–99.9% killing effect as compared to the initial inoculums[12], at 2.5 mg/ ml *Tubipora musica* methanol extract show the MBC against *Staphylococcus aureus, Bacillus subtilis* and at 2.5 mg/ml while Ciprofloxacin MBC was 1.25 mg/ ml against the same microorganisms, Against *Pseudomonas aeruginosa* the extract MBC was 2.5 mg/ ml better than the drug (5 mg/ ml) the extract MBC was 1.25 mg/ ml against *Escherichia coli* also better than Ciprofloxacin (2.5 mg/ ml). The MFC of both the extract and Itraconazole was 1.25 mg/ml against *Aspergillus flavus*. *Candida albicans* was killed at concentration 2.5 mg/ml by extract and 0.625 mg/ml by the drug, *Aspergillus niger* was killed at concentration 1.25 mg/ml by extract and 2.5mg/ml by the drug indicating that the extract shows abetter activity than Itraconazole. Finally, Itraconazole MFC against *Aspergillus fumigatus* was 0.625 mg/ml same to the methanol extract.

From this results the methanol extract of *Tubipora musica* shows a variable (better, lesser and same) activities with the standard drug against tested organisms meaning that it is an interested area of research as an antimicrobial agent.

CONCLUSSION

Microorganisms used in the research study were causative agents of many infectious disease, while *Tubipora musica* inhibit the growth of these microbes, so it can be used for the treatment of infections which caused by them, sugges that *Tubipora miusica* contains a quite promising as antibacterial and antifungal compound(s).

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REFERENCES

1. World Health Organization. WHO Model Prescribing Information: Drugs used in Bacterial Infections. Geneva. Malta. 2001; 3-3.
2. Malcolm D, Richardson, David W, Warnock. Fungal infection, diagnosis and treatment. 4ed, Wiley– Blackwell, 2011; 1-1.
3. 3.Abubakar L, Mwangi C, Uku J, Ndirangu S. (Antimicrobial activity of various extracts of the sea urchin *Tripneustes gratilla* (Echinoidea). African Journal of Pharmacology and Therapeutics, 2012; 1(1): 19-23.
4. Choi DH, Shin S, Park IK. (Characterization of antimicrobial agent extracted from Asterina pectinifera). Int. J. Antimicrob agents. 1999; 11(1): 65-68.
5. Devi P, Wahidulla S, Kamat T, Souza LD. (Screeninig marine organisms for antimicrobial activity against clinical pathogens). Indian J. of Geo-Marine sciences, 2011; 40(3): 338-338.
6. Anderson P.A. (The electrophysiology of the organ- pipe coral, *Tubipora musica*). The biological bulletin, 1976; 150(3): 338-339.
7. Obura, D., Fenner, D., Hoeksema, B., Devantier, L., Sheppard, C. 2008. *Tubipora musica*. The IUCN Red List of Threatened Species 2008:.http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T133065A3589084.en.
8. National Research Council (US) Committee on Marine Biotechnology: Biomedical Applications of Marine Natural Products. Marine Biotechnology in the Twenty-First Century, Problems, Promise and Products, Washington (DC): 2002.
9. Lev E. Healing with animals in the Levant from the 10th to the 18th century. J Ethnobiol Ethnomed., 2006; 2(11): 4-4.
10. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. (Phytochemical screening and extraction (A Review). Journal of International Pharmaceutica Scinica, 2011; 1(1): 96-104.
11. Wiegand I, Hilpert K, Hancock RE. (Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances). Nature protocols, 2008; 3(2): 163-75.
12. Balouiri M, Sadiki M, Ibnsouda S K. (Methods for in vitro evaluating antimicrobial activity: A review). Journal of Pharmaceutical Analysis, 2016; 6(2): 71-9.