Antimicrobial potential of chosen Indian south peninsular coast sponges

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Abstract : Marine sponge secondary metabolites paid more importance because of their unusual bioactive chemical compounds. In the present study aims to determine the antimicrobial potential of three marine sponges such as *Clathria (Clathria) gorgonoids, Callyspongia sp., Sigmadocia carnosa* collected from south peninsular coast of India. The antimicrobial effects of these sponge extracts were assayed against a test panel of pathogenic bacteria. The test panel consisted of 10 strains including *Bacillus subtilis, Eschirichia coli, Staphylococcus mutants, Streptococcus aureus, Proteus vulgaris, Shigella sp, Salmonella typhi, Mycobacterium sp, Klebsiella pneumonia* and *Enterobacterium sp.* Bioassay results revealed that *Sigmadocia carnosa* species (90%) exhibited antibiotic activity against most of the bacterial isolate followed by *Clathria (Clathria) gorgonoids*.

Key words: Chemical defence, Antimicrobial, Sponges, Secondary Metabolites,

I. INTRODUCTION

Sponges are simple, multicellular, sessile animals organisms inhabit in diversified marine environments, from polar seas (Dayton et al. 1974) to temperate and tropical waters (Reiswig 1973, Wenner et al. 1983), and are often more abundant and diverse in the tropics than stony and soft corals (Targett and Schmahl 1984). The sponge class Demospongiae is known to produce the largest number and diversity of secondary metabolites isolated from marine invertebrates (Faulkner 1998). Although the functions of these secondary metabolites are largely unknown, there is some evidence that they provide chemical defenses against predators (Pawlik et al. 1995, Chanas et al. 1996). It has also been suggested that sponge secondary metabolites may provide defenses against fouling and infection (Pawlik 1993), however, this possibility has not been adequately explored.

The first report of antimicrobial activity of sponge extracts was by Nigrelli et al. (1959). Since then, there has been a growing number of antimicrobial extracts reported from marine sponges (Amade et al. 1987, Uemera 1991). Some organic extracts from sponges have additionally yielded compounds with pharmacological properties such as antitumor, antifungal, and antiviral activity (Tachibana et al. 1981, Kashman et al. 1989). Despite the wealth of biologically active secondary metabolites isolated from marine sponges, the potential functions of these compounds in antimicrobial chemical defense have rarely been explored (Burkholder & Riitzler 1969, Mokashe et al. 1994). In this study, we tested the crude extracts of 3 species of sponges for antibacterial activity against a panel of 10 strains of pathogenic bacteria.

II. MATERIALS AND METHODS

Collection of sponges

A diverse variety of sponges were collected from the peninsular coast of India. Three species were collected off from Muttom and Poovar coast by netting process. In this process, the local fisher folks were arranged to operate a purse seine on the sponge abundant rocky substratum so that the nets were entangled with sponges. When the net was pulled back with force, the sponges, which get entangled in the nets, were dislodged and get accumulated in the net. Immediately after collection, they were immersed in methanol for extraction.

Preparation of crude extracts from sponges

The collected sponges in the methanol containers were squeezed/minced in a tissue homogenizer, depending up on the nature of sponge species, which was used for extraction. In the case of *Clathria gorgonoides*, it was cut into small pieces and minced through in a tissue homogenizer prior to the extraction. In the case of *Sigmadoicia* the extract was collected as such from the methanol container and filtered through a Whatman no.1 filter paper fitted with a Buchner funnel using suction. They were extracted thrice and the combined extract was concentrated in a rotary vacuum evaporator at room temperature. The concentrated crude extract was collected in airtight plastic containers and kept in the refrigerator.

In vitro antibacterial screening of sponge extracts

Antibacterial studies were carried out using the bacterial type cultures obtained form Microbial Type Culture Collections (MTCC), Chandigarh (Table -1). The MTCC type cultures were initially activated in nutrient broth and subsequently purified by agar streak plate method. Agar diffusion method was followed to evaluate the antibacterial test.

Table 1: Bacterial cultures used for the antibacterial screening

No	*Test organisms*	*Source*
1	*Bacillus subtilis*	MTCC *
2	*Eschirichia coli*	MTCC *
3	*Staphylococcus mutants*	MTCC *
4	*Streptococcus aureus*	MTCC *
5	*Proteus vulgaris*	MTCC *
6	*Shigella sp*	MTCC *
7	*Salmonella typhi*	MTCC *
8	*Mycobacterium sp*	MTCC *
9	*Klebsiella pneumonia*	MTCC *
10	*Enterobacterium sp*	MTCC *

* Microbial Type Culture Collections, Chandigarh

III. RESULT

Collection and identification of sponges

The successful development of anticancer drugs from the sponges completely relies on the continuous availability of the source organism and the cost effective collection methods. The major distributed species were: *Callispongia sp*. and *Sigmadocia sp*. followed by *Clathria gorgonoides*.

Identified species of sponges collected as bycatch in the fishing nets

Species	Colour pattern	Area of collection
Clathria (*Clathria*) *gorgonoids*	Straw yellow	Muttom
Callyspongia sp.	Pale yellow	”
Sigmadocia carnosa	Light brown	”

(Identified by the renowed sponge taxonomist, Dr. P.A. Thomas, Principal Scientist, CMFRI, Vizhinjam)

Preparation of crude extract from sponges

The colour of methanolic extract of sponges is presented in Table 3. The sponge extracts exhibited their characteristic colour. The recovery of extract was dependent on colour pattern and softness of the sponge body. The yield was high in *Callyspongia sp*. (4.6 g/kg) folloed by *Sigmadocia carnosa* and *C. gorgonoides*.

Table 3. Nature of methanolic sponge extracts

Species	Colour of extract	Quantity of yield (g/kg)
Clathria gorgonoides	Straw yellow	3.8
Sigmadocia carnosa	Light brown	4.2
Callyspongia sp.	Straw yellow	4.6

Screening for in vitro antibacterial activity

The extracts were initially used for primary antibacterial screening using different MTCC cultures (Table 4). The antibacterial activity was done by disc diffusion method.The result is presented in the tabular column (Table 4)

Table1- 4: Antimicrobial activity of chosen marine sponges

Sl.No	*Test Organisms*	*Sigmadocia carnosa*	*Callyspongia* sp.	*Clathria gorgonoides*
1	*Bacillus subtilis*	14.1±0.08	-	-
2	*Escherichia coli*	05.9±0.02	05.0±0.70	06.8±0.80
3	*Staphylococcus mutants*	11.8±0.05	07.4±0.54	-
4	*Streptococcus aureus*	14.6±0.04	-	-
5	*Proteus vulgaris*	08.6±0.04	-	-
6	*Shigella sp*	14.1±0.08	06.2±0.44	04.6±0.08
7	*Salmonella typhi*	-	-	-
8.	*Mycobacterium sp*	06.9±0.02	-	07.6±0.54
9	*Klebsiella pneumonia*	07.9±0.02	04.6±0.54	-
10	*Enterobacterium sp*	08.6±0.48	04.2±0.44	-

IV. DISCUSSION

Nearly all cultures from ancient times to the present day have used sea as a source of medicines. Current strategies to overcome the global problem of antimicrobial resistance include research in finding new and innovative antimicrobial from natural origin. Sponges provide as with an enormous array of chemicals which help them to flourish and survive. Among them, the secondary metabolites are more specialized and are usually peculiar to only one organisms or species. Some of these metabolites are defensive compounds designed to deter or kill disease causing organisms, potential predators or competitors. The use of natural medicines play a vital role in covering the basic health needs in developing countries and these plants may offer a new source of antimicrobial with significant activity against infective microorganism. Many plant extracts have been used as a source of medicinal agents to cure urinary tract infections, cervicities, vagnities gastrointestinal disorders, respiratory disorders, cuteneous affections, parasitic protozoan's diseases and inflammatory diseases. Likwise, the antimicrobial activity, Brine shrimp activity, Larvicidal activity and Ichthyo toxicity studies were utilized for the preliminary screening of selected marine sponges.

The natural products of one organism are toxic to other organism, the substance are said to be antibiotic (against life). The term antibiosis was originally conceived as the destruction of one creature's life by another in order to preserve its own. The majority of antimicrobial agents display antimetabolic activity rather than chemical degradation. Agents that affect metabolic activity act on growing cells. The result of action may be either bacteriostatic (reversible) or Bactericidal (irreversible). In general, agents that damage the cell wall, cell membrane or DNA are bactericidal, and those that inhibit enzyme action are bacteriostatic. Phytoconstituents present in plants namely flavanoids, alkaloid, tannins and triterpenoids are producing exciting opportunity for the expansion of modern chemotherapies against wide range of microorganisms (Lutterodt *et al.*, 1999; Majorie, 1999). The concentration of an antimicrobial agent is of paramount importance. Agents may produce many changes in microorganisms and different changes may be related to varying concentrations of the agent present. As soon as the antibiotic impregnated disc comes in contact with the moist agar surfaces it absorbs moisture from the agar and the antibiotic diffuses in to the surrounding medium. The rate of extraction of the antibiotic out of the disc is greater than out ward diffusion in to the medium. The extent of antibiotic diffusion is also affected by the depth of the agar. Visible growth of the bacteria occurs in the surface of the agar, where the concentration of the antibiotic has fallen below its inhibitory level for the test organism.

In the present study a variety of gram positive, Gram negetive bacterial strains were selected for the screening of antimicrobial effect of three selected sponge methanolic extracts to perceive the antimicrobial spectrum as well as to authenticate ethanomedicinal claims. In the light of antibiogram, high antibiotic broad spectrum activity could be ascribed to the sponge *Sigmadocia sp*., *Clathria gorgonoides* and *Callyspongia sp*. had more potent larvicidal activity followed by and effectively. Moreover all the three species appeared to control all the pathogenic organisms tested. Lot of reports were indicated the antibacterial property of *Sigmadocia sp*., was reported by many authers. The *Clatheria* was displayed the moderate antibacterial activity and *Callyspongia* showed very least antibacterial activities over all gram positive and gram negetive bacteria. The growth media also play an important role in the determination of antibacterial activity. Methanolic extract of *S. cordifolia* showed antibacterial activity against both gram positive and gram negative bacteria. The present result was in accordance with the previous result of Balakrishnan *et al*., (2006). Among the gram positive and gram negative bacteria, gram positive bacteria were

more susceptible to the extracts. This is an agreement with previous reports that plant extracts are more active against gram - positive strains.

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