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Research Article

IN VITRO ANTIMICROBIAL POTENTIAL EFFICIENCY OF CLATHRIA FRONDIFERA MARINE SPONGE

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ABSTRACT

In the present study, extract of *clathria frondifera* was examined for *in vitro* antimicrobial potency against three clinical human pathogens. The biologically active compounds were obtained by column chromatographed on siliga gel-60 through step gradient elution. *Clathria frondifera* yielded bioactive franctioned extract that efficiently repressed the growth of all kinds of pathogens. The chemical constituents of the *clathria frondifera* franctioned extract were analyzed by GC-MS which revealed the presence of major compounds such as 26,26-Dimethyl-5,24(28)-ergostadien-3a-ol, 2-tert-Butyl-4-isopropyl-5-methylphenol and E-15-Heptadecenal which might have a functional role in the chemical defense against microbial invasion. Based on the findings, marine sponge of *clathria frondifera* effectively represses the bacterial and fungal.

Keywords: *Clathria frondifera,* FT-IR, GC-MS, Antimicrobial activity.

1. INTRODUCTION

Marine sponges provide classic examples of microbial macro faunal partnerships that have been a productive source for the discovery of bioactive compounds[1]. There was a worldwide interest in marine natural products as one of the few de novo sources of drug discovery. Since the rich diversity of marine organisms and habitats, marine natural products encompass a wide variety of chemical class, including terpenes, shikimates, polyketides, acetogenins, sterols, lipids and peptides, alkaloids of varying structures and multitude of compounds of mixed synthesis[2]. Marine invertebrates, such as sponges, have proven to be a rich source of biologically active and pharmacologically valuable natural products, with a high potential to become effective drugs for therapeutic use[3].

The great interest in these chemically varied compounds has resulted in the development of novel therapeutic agents for the treatment of various diseases afflicted by human beings. Marine species studied in the recent years have yielded a variety of compounds which possess known or novel pharmacological activities in mammals and have exhibited antimicrobial, anti-inflammatory, anti-tumor, anti-viral and antineoplastic property[4]. However, the bioactive potential of compounds from Indian sponges has been little studied. Since marine natural products are becoming increasingly attractive due to their potential applications in the pharmaceutical industries, the identification of new sources of these materials could be extremely important[5]. In the past decade alone structures of over 5,000 marine natural products have been published[6]. Among them, *Clathria* was one of the genus of marine sponges which were an abundant source of novel secondary metabolites exhibiting various biological activities and unusual chemical structures.

Therefore, the present study comprises an initial effort to assess the bioactivity of secondary metabolites from the marine sponge of *clathria frondifera*. The *in vitro* antimicrobial efficiency of *clathria frondifera* was tested against human pathogenic bacteria, and fungal. The structure

and functional group have been elucidated by FT-IR, GC-MS.

2. MATERIALS AND METHODS

2.1. Collection of sample

Samples of *Clathria frondifera* were collected from lobster net operated in 20 to 25 feet depth in the Gulf of Mannar coast of India (Figure. 1). Immediately after collection, sponges kept frozen and transported to the laboratory. Specimens were identified based on their size, shape, pigmentation, consistency, oscule size, ostia distribution, spicule diversity and skeleton arrangement[7].

2.2. Bioactive compound extraction

Samples of *clathria frondifera* collected from Gulf of Mannar, southeast coast of India were stored in methanol. The methanol crude extract was concentrated under vacuum evaporator to yield a brown crude gum. The crude extract was chromatographed on siliga gel-60 through step gradient elution commencing with a nonpolar solvent (hexane) followed by addition of increasing amounts of a more polar solvent (EtOAc) to each successive solvent fraction. The mixture compound was isolated from the nonpolar fraction, which was eluted at a solvent ratio 95:5 (hexane/ EtOAc). The obtained mixture compound was stored for further studies.

2.3. Partial purification and structural elucidation

2.3.1. Fourier transmission infrared spectroscopy (FT-IR)

The FT-IR spectrum in the mid-infrared region (400-4000 cm^{-1}) was used for discriminating and identifying various functional groups present in *clathria frondifera* extract[8].

2.3.2. Gas chromatography-mass spectrometry (GC-MS) analysis

The fractioned extract was quantified using gas chromatograph (Shimadzu QP2010) equipped with a VF-5 ms column (diameter 0.25 mm, length 30.0m, film thickness 0.25 µm) mass spectrometer (ion source 2000 C; EI-70 eV), programmed at temperature 40-650 ^{o}C with a rate of 4 ^{o}C /min. Injector flow rate was 200 ^{o}C; carrier gas was He 99.9995%.Electron impact spectra in positive ionization mode were acquired between m/z 50 and 650.

2.4. Microbial analyses

2.4.1 Antibacterial assay

Antibacterial screening of the *Clathria frondifera* fractioned extract against three (*Staphylococcus aureus, Escherichia coli,* and *Bacillus subtilis*) bacterial strains in DMSO were carried out employing disc diffusion method[9,10]. The discs measuring 5 mm in diameter were prepared from Whatman No.1 filter paper sterilized by dry heat at 140 ºC for 1 h. The sterile discs previously soaked in concentration (10 µg/mL) of the test compounds were placed in a nutrient agar medium. The plates were inverted and kept in an incubator at 30 ±1 ºC. The inhibition zone thus formed was measured (in mm) after 24 h and amikacin was used as the standard. Minimum inhibitory concentrations (MICs) were determined by broth dilution technique. The stock solution (10^{-3} mol L^{-1}) was prepared by dissolving the compounds in DMSO and the solutions were serially diluted in order to find out the Minimum Inhibitory Concentration (MIC) values. The nutrient broth, which contained logarithmic serially twofold diluted amount of test compounds and controls, was inoculated within approximately 5×10^5 c.f.u. of actively dividing bacteria cells. The cultures were incubated for 24 h at 37 ºC and the growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria is regarded as minimum inhibitory concentration (MIC). The lowest drug concentration, at which 99.9% of the inoculum killed, is considered as minimum bacterial concentration (MBC). To obtain the MBC, 0.1 mL volume was taken from each tube and spread on agar plates. The number of c.f.u. was counted after 18-24 h of incubation at 35 ºC.

2.4.2 Antifungal assay

The newly prepared *Clathria frondifera* fractioned extract were also screened for their antifungal activity against *(Aspergillus niger, Rhizoctonia bataticola and Candida albicans)* in DMSO by agar diffusion method[11,12]. Agar media was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 mL). Normal saline water was used to make suspension spore of fungal strain lawning. A loopful of particular fungal strain was transferred to 3 mL saline to get suspension of corresponding species. Twenty milliliters of agar media were poured into each Petri dish. Excess of suspension was decanted and plates were dried by placing in an incubator at 37 ºC for 1 h using an agar punch. A control was also

prepared in triplicate and maintained at 37 ºC for 3-4 days. The fungal activity of each compound was compared with Greseofulvin as standard drug. Inhibition zones were measured and compared with controls. The cultures were incubated for 48 h at 35 ºC and the growth was monitored. The lowest concentration (highest dilution) required to arrest the growth of fungus was regarded as minimum inhibitory concentration (MIC). To obtain minimum fungicidal concentration (MFC), 0.1 mL volume was taken from each tube and spread on agar plates. The number of c.f.u. was counted as the lowest drug concentration at which 99% of the inoculum was killed.

3. RESULTS AND DISCUSSION

3.1 FT-IR Spectrum

The different peaks of the FT-IR spectrum revealed that, the broad peak at 3450 cm^{-1} was assigned to the O-H stretching group which is shown in Figure 2. The absorption peaks at 1639 and 1536 cm^{-1} were assigned to stretching and bending vibration of C=C group respectively. The absorption peak at 1743 cm^{-1} were attributed to the stretching vibration of C=O. The peak at 2850 cm^{-1} could be assigned to C-H stretching vibration mode.

3.2 Gas chromatography-mass spectrometry

The chemical profiles of the fractioned extract, the amount (%) of the individual components, and gas chromatographic and mass spectral data were summarized in (Table 1). The presence of seven major constituents were identified in *clathria frondifera* fractioned extract such as 1-DODECENE, 2-tert-Butyl-4-isopropyl-5-methylphenol, E-15-Heptadecenal, 1-Heptadecanol, n-Nonadecanol-1, Cholesterol, 26,26-Dimethyl-5,24(28)-ergostadien-3a-ol which might have a functional role in the chemical defense against microbial invasion. GC-MS chromatogram of *clathria frondifera* fractioned extract gives one prominent peak (Figure 3) with retention time 35.33 indicating the presence of compound such as 26,26-Dimethyl-5,24(28)-ergostadien-3a-ol (Figure 4). The structure of 26,26-Dimethyl-5,24(28)-ergostadien-3a-ol present in figure 4 (inset).

3.3 Antimicrobial Screening

Nature has good source of medicinal agents for thousands of years and an immersive number of modern drugs have been isolated from natural sources based on the traditional information[13]. In the search for pharmaceutical or agrochemical lead structures, cytotoxic and other antimicrobial metabolites have received increasing attention at a rate much faster than those of other unicellular organisms[14]. The isolated compound has been evaluated for its antimicrobial screening. The minimum inhibitory concentration (MICs) and minimum bacterial/fungicidal concentration (MBC/MFC) values are given in (Tables 2 and 3). The MIC was the lowest concentration of an antibacterial or antifungal compound that will inhibit the visible growth of microorganisms after period of incubation, and the minimum inhibitory concentrations were important in diagnostic laboratories to confirm the resistance of microorganisms to biologically active compounds. The experimental result indicates that, the compound exhibits significant inhibition efficiency. But, the activity was lower than the standards. The present study has added strength to the biological properties of *clathria frondifera* fractioned extract as it was showed strong antimicrobial activity against few human pathogens. The mode of actions of antimicrobials may involve various targets in microorganisms. (i) Interference with the cell wall synthesis, damage as a result of which cell permeability may be altered or they may disorganize the lipoprotein leading to cell death. (ii) Deactivation of various cellular enzymes, which plays a vital role in different metabolic pathways of these microorganisms[15]. (iii) Denaturations of one or more proteins of the cell, as a result of which the normal cellular processes are impaired. Based on the above observations, the isolated compound was more sensitive in gram-positive bacteria compared to gram negative bacteria. This difference may be due to several possible reasons such as permeability barrier provided by the presence of cell wall with multilayer structure in gram-negative bacteria or the membrane accumulation mechanisms or presence of enzymes in periplasmic space which were able to break down foreign molecules introduced from outside.

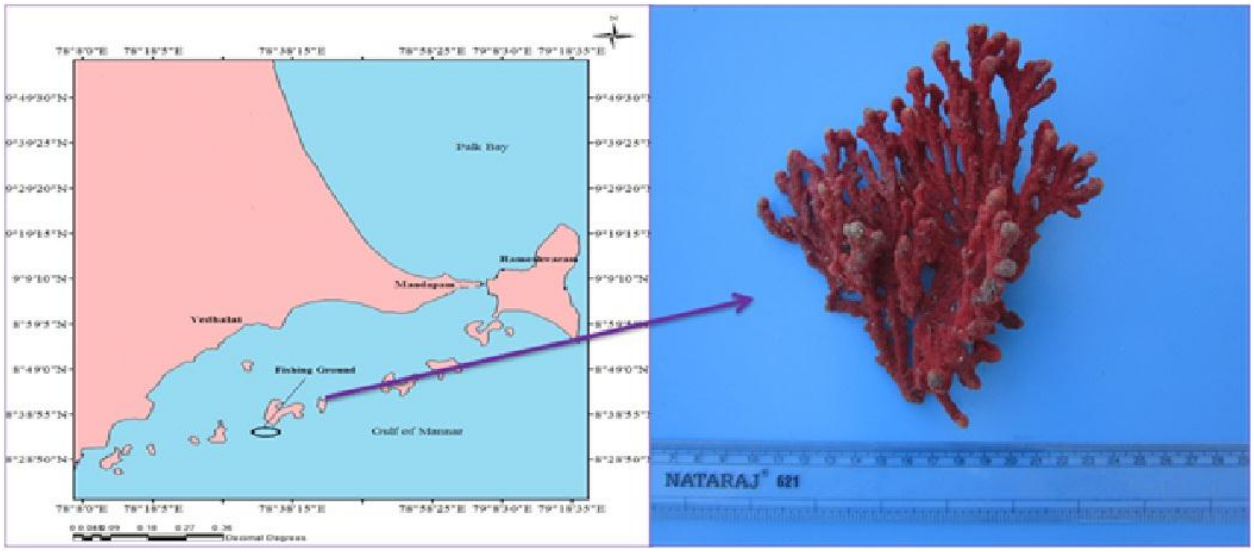

Fig. 1: Details of the sponge collected location in Gulf of Mannar, southeast coast of India

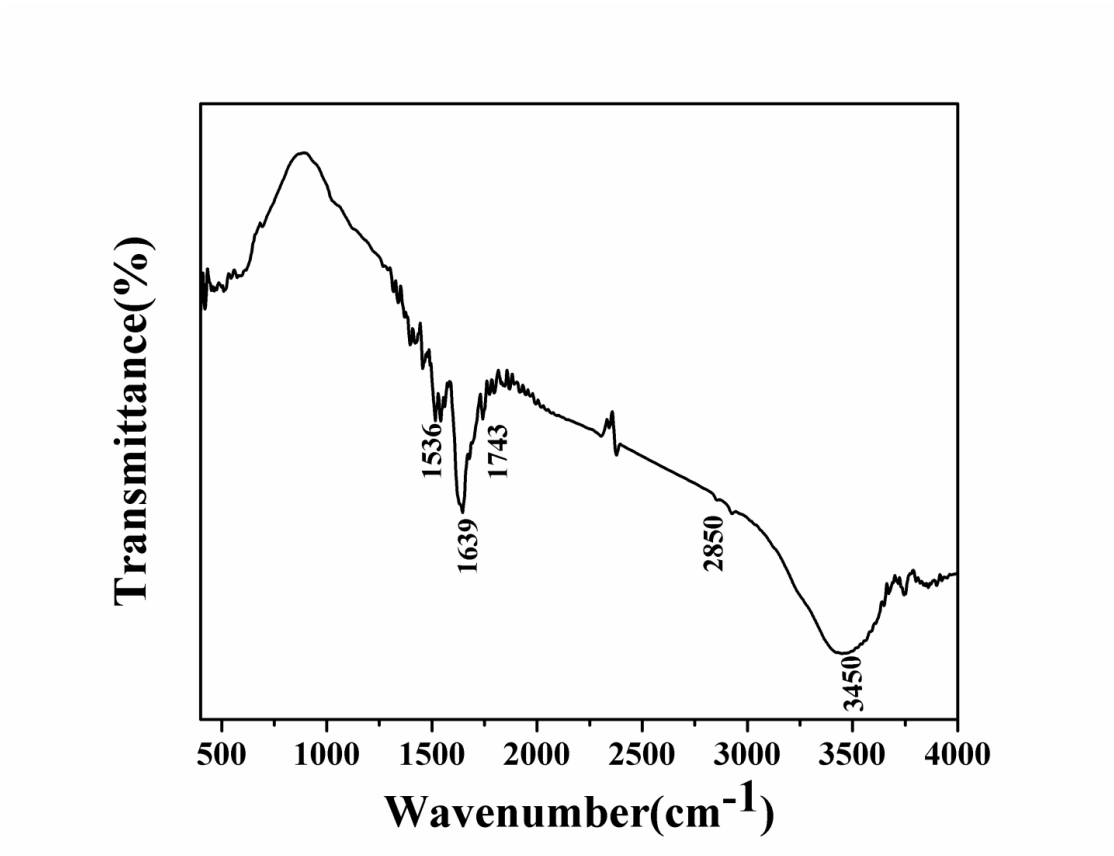

Fig. 2: FT-IR analysis for the *clathria frondifera* extract

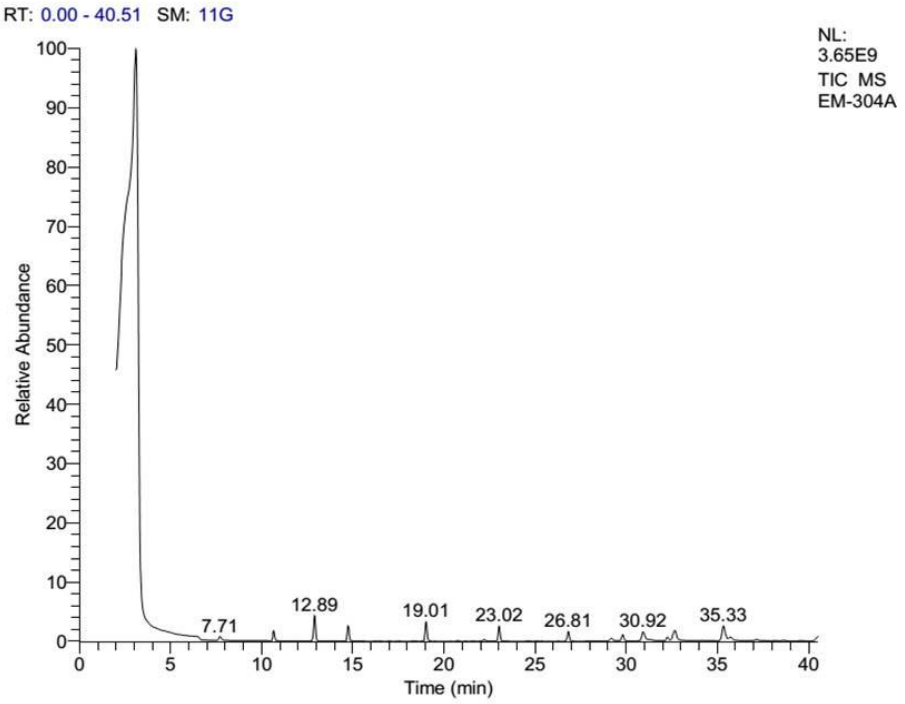

Fig. 3:. GC-MS chromatogram of extract of *clathria frondifera*

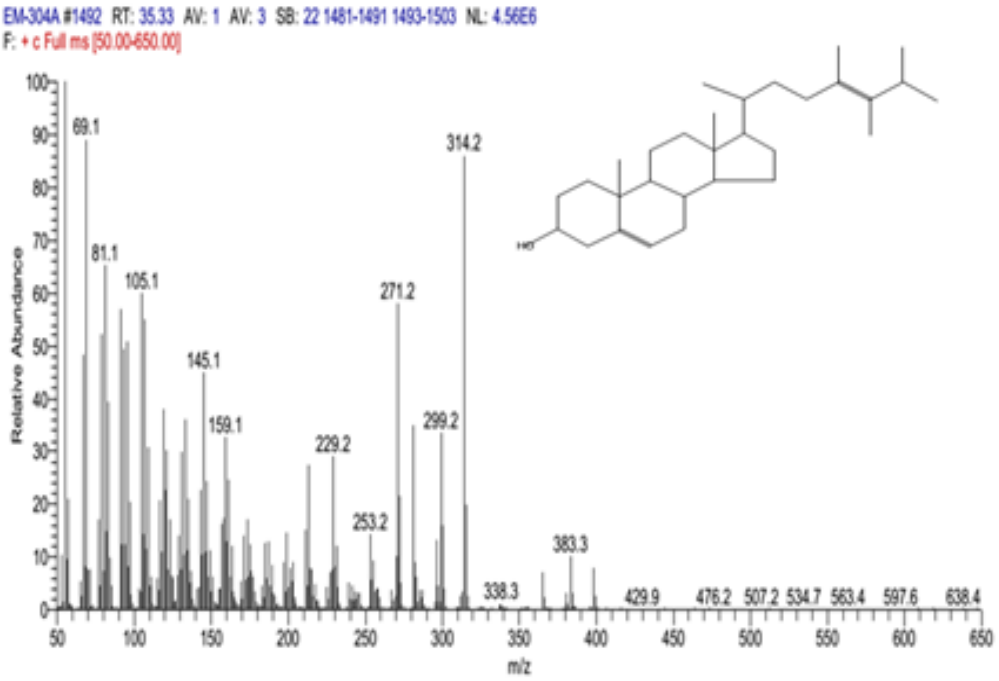

Fig. 4: Mass spectrum of extract of *clathria frondifera*

Table 1: Components identified in fractioned extract of *clathria frondifera*

R. T	Name of compound	M.F	M.W	Area %
7.71	1-DODECENE	$C_{12}H_{24}$	168	0.20
12.89	2-tert-Butyl-4-isopropyl-5-methylphenol	$C_{14}H_{22}O$	206	1.04
19.01	E-15-Heptadecenal	$C_{17}H_{32}O$	252	0.72
23.02	1-Heptadecanol	$C_{17}H_{36}O$	256	0.64
26.81	n-Nonadecanol-1	$C_{19}H_{40}O$	284	0.43
30.92	Cholesterol	$C_{27}H_{46}O$	386	0.64
35.33	26,26-Dimethyl-5,24(28)-ergostadien-3a-ol	$C_{30}H_{50}O$	426	1.18

Table 2: MIC and MBC results of the test compound on three bacterial strains

Complex	*Staphylococcus aureus*		*Escherichia coli*		*Bacillus subtilis*	
	MIC	MBC	MIC	MBC	MIC	MBC
Compound	10.2	13.6	13.9	13.2	11.7	10.8
Standard	1.25	2.5	1.10	2.50	1.00	2.50

Amikacin is used as the standard. MIC (µM) = minimum inhibitory concentration, i.e. the lowest concentration to completely inhibit bacterial growth; MBC (µM) = minimum bactericidal concentration, i.e., the lowest concentration to completely kill bacteria. Average of 3 determinations and 3 replicates.

Table 3: MIC and MFC results of the test compound on three fungal strains

Complex	*Aspergillus niger*		*Rhizoctonia bataticola*		*Candida albicans*	
	MIC	MFC	MIC	MFC	MIC	MFC
Compound	16.1	15.3	16.5	16.3	16.0	15.1
Standard	2.25	2.50	2.00	2.65	1.25	2.50

Greseofulvin is used as the standard. MIC (µM) = minimum inhibitory concentration, i.e. the lowest concentration to completely inhibit fungal growth; MFC (µM) = minimum fungicidal concentration, i.e., the lowest concentration to completely kill fungus. Average of 3 determinations and 3 replicates.

4. CONCLUSION

In this study, the bioactive fractioned extract of *clathria frondifera* was characterized by FTIR and GCMS. The fractioned extract contained seven major constituents such as 1-DODECENE, 2-tert-Butyl-4-isopropyl-5-methylphenol, E-15-Heptadecenal, 1-Heptadecanol, n-Nonadecanol-1, Cholesterol, 26,26-Dimethyl-5,24(28)-ergostadien-3a-ol. The *clathria frondifera* fractioned extract may be suggested as potentially good source of antimicrobial activity.

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