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Extraction, chemical characterization and antibacterial potential of marine algae *Galaxaura oblongata* against clinically isolated human pathogens

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Abstract

This study looked at the antibacterial efficacy of a methanol extract produced coming from *G. Oblangata* towards clinical isolated human pathogens. The chemical constituents of an extracts from methanol was examined by using phytochemicals and FT-IR. Furthermore, methanol extract was tested for antibacterial activity against eleven human infections. At 100 µg/ml concentration, the methanol extract inhibited S. typhi with a maximal zone of 19mm. The MIC values for the methanolic extract were 100, 80, 100, 60, 100, 100, and 100 g/ml against infections caused by *S. aureus, K. pneumoniae K. oxytoca, S. typhi, S. paratyphi, P. mirabilis,* and *S. pyogenes*, respectively. The methanolic extract has MBC values of 100, 80, 100, 100, 60, 100, 100, and 100 µg/ml against strains of bacteria including *S. typhi*, *S. aureus*, *K. pneumonia*, *E. coli*, and *S. paratyphi*. These findings indicate that the extract of methanol from *G. Oblangata* is a good candidate for pharmaceutical research, opening the path for the development of powerful future antibiotics.

Keywords: *G. Oblangata*; FT-IR; Anti-bacterial; MIC; MBC

1. Introduction

Seaweeds are sometimes referred to as marine macroalgae is a complex group of multicellular photosynthetic organisms characterized by great diversity in morphology and size. They are mainly classified into two or three groups generally named *Rhodophyta* (red algae), *Phaeophyta* (brown algae), and the *Chlorophyta* (green algae) are classified by pigmentation (1). Marine creatures have been extensively used as an abundant source of sustenance for mankind from prehistoric times across various cultures(2). Seaweed cell walls have a structural complexity which may pose challenges in the extraction of bioactive substances since their polysaccharides are stiff, branched and sulfated polysaccharides that are typically associated with proteins and metal ions (3). It is known that the extraction method influences the biological activities of these compounds.

The common method is water based extraction, which is favorable because of its low costs, non-toxic and food-friendly properties. Nevertheless, this approach is usually characterized by low selectivity and extracted efficiency (4). In comparison, organic solvents, like benzene, acetonitrile, and diethyl ether, generally exhibit better extraction performance, although they are more unsustainable and cannot be used in foods because of their environmental and health risks (5). Seaweeds have been identified as sources of many natural bioactives, such as alkaloids, polyphenols, flavonoids, terpenes, carotenoids, tocopherols, peptides, and sulfated polysaccharides (6). Solvent selection and extraction methodology are essential and should be based on the polarity and solubility of compounds of interest with organic solvents (ethanol, methanol, and acetone) typically efficient with lipophilic compounds and aqueous systems (water) with hydrophilic substances. Moreover, a special inorganic solvent, e.g. supercritical carbon dioxide, can be used under a controlled set of conditions to

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improve extraction results. Effective isolation and purification procedures are necessary in the acquisition of bioactives that have favorable therapeutic, biological as well as environmental remedial properties. The various studies have established that the marine algae in classes, brown, red, and green algae, are reported to bind to a wide range of bioactivities, antioxidant, antimicrobial, anticancerous, cytotoxic, anti-inflammatory, and antiviral bioactivities (8). Some factors that affect the production of antioxidant and antibacterial agents extracted in the sea weeds like extraction temperature, time, solvent polarity and concentration plays an important role on the production of the yield and the potency of the extract that is produced. It is important to note the presence of compounds such as phlorotannins, fatty acids, phenolic substances, terpenoids, steroids, acrylic acid, and amino acids that confer the bactericidal or bacteriostatic effect of bacteria on a range of seaweed species (9). During the last 20 years, many studies have supported the antimicrobial potential of seaweed-derived extracts and their purified constituents (10,11). In this regard, the current work will investigate the antibacterial potency of a methanolic extract of *Galaxaura oblongata* against pathogenic bacteria of humans of clinical importance.

2. Materials and methods

2.1. Collection and marine algae

The marine algae *Galaxaura oblongata* was collected from Thiruchendur, located in Tamil Nadu, India. The precise coordinates are provided (Latitude. 09°44' N; Longitude. 079°02' E), ensuring accurate documentation of the collection site. The collected algae samples were identified using Umamaheswara Rao's manual (1). This manual likely contains detailed descriptions, images, and taxonomic keys that aid in the accurate identification of marine algae species. The algal samples were washed using a sequence of three different types of water such as seawater, freshwater, and distilled water. This step helps remove debris, salts, and other impurities from the algae surface, ensuring cleaner samples for further processing. The washed algae samples were shade-dried at room temperature. Shade drying is a gentle method that preserves the integrity of the algae and prevents degradation of bioactive compounds due to exposure to excessive heat or sunlight. Once dried, the algae samples were chopped into small pieces to increase the surface area for drying and subsequent extraction. Then, they were powdered using a mixer grinder to achieve a fine, homogeneous powder suitable for various applications.

2.2. Extract preparation

500g of algal powder was extracted with 1000ml of methanol by soaking overnight at room temperature, after soaking, the mixture was then subjected to centrifuged for 30 minutes (2). The algal residue obtained after the first extraction was further subjected to three additional extraction rounds using the same method. This sequential extraction increases the yield of bioactive compounds from the algal material. All the collected methanolic extracts from the various extraction rounds were pooled together. The pooled methanolic extract was concentrated under reduced pressure using a (VC100A Lark Rota vapour®) rotary evaporator at 40ºC. The concentrated methanolic extract was further dried in a vacuum desiccator. Finally obtained the methanolic extract.

2.3. Quantitative phytochemical analysis

A number of significant phytochemical components, including flavonoids, alkaloids, tannins, saponins, anthraquinones, steroids, and terpenoids, were quantitatively determined from the methanolic extract. The normal procedures outlined in previous published work were followed for conducting these experiments.

2.4. FT-IR studies

A Thermo Fischer FT-IR-40 spectrophotometer (USA) was utilized to analyze 10 mg of methanolic extracts. Spectral data were collected between 4000 cm^{-1} and 500 cm^{-1} of wave numbers.

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2.5 Antibacterial activity

Bacterial strains

The study participated in the assessment of antibacterial activity on a panel of clinically relevant human pathogens which included such as *Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Proteus mirabilis, Salmonella typhi, Staphylococcus aureus, Streptococcus pyogenes, Vibrio cholerae,* and *Vibrio parahaemolyticus*.

2.6 Inoculum Preparation

Test tubes containing nutrient broth were autoclaved for 15 minutes at 15 pounds of pressure to sterilise it. After being separately injected into nutritional broth that had been sterilised, each of the 10 bacterial strains was incubated for twenty-four hours at 37°C.

2.7. Agar well diffusion technique.

The antibacterial activity of the test compounds was revealed using an agar well diffusion technique, as reported by Seedevi et al. (15). After culturing bacterial strains over a period of 24 hours, the strains were spread uniformly onto the nutrient agar plates by using sterile cotton swabs in aseptic conditions. The agar was gently punched with circular wells of a size 5 mm in diameter using sterile borer. After making a stock solution of seaweed-methanolic extract and diluting it 1:1000 (1 mg/mL) with 10% dimethyl sulfoxide (DMSO), tests were conducted at four different concentration levels: 25, 100, 50, 75, and 100 µg/mL. The positive control was tetracycline (1 mg/mL in 10% DMSO), while the negative control was 10% DMSO by itself. The content of each solution was poured into the wells individually and the plates placed upright at 37°c and incubated on a shaker table over a period of 24 hours. As an estimate of antibacterial activity, the diameters of the several distinct zones of inhibition surrounding the wells were measured in millimetres following incubation.

2.8 Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) of methanolic extract was determined according to the methods reported by Seedevi et al. (15). A stock solution at 1 mg/mL was prepared and serially diluted to give a realistic range of concentration of 20 to 100 ug/mL. Pipette 0.5 mL of the solution of each dilution into a test tube containing 2 milliliters of nutrient broth and 0.5 milliliters of an overnight bacterial culture. Control tubes containing nourishing a base without extracts were also prepared. All of the tubes had been incubating at 37 degrees Celsius for a period of 24 hours. The MIC was defined as the quantity of extracts with the smallest concentration that inhibited bacterial growth after incubation.

2.9 Minimum Bactericidal Concentrations (MBC)

The Minimum Bactericide Concentrations (MBC) was determined by following the procedure provided by Seedevi et al.(15) The procedure is described as follows. A loop full of culture was streaked in nutrient agar plates after MIC determination of each MIC tube that had no visible growth. This was followed by 24 hours incubation at 37°C. The minimum extract concentration where no colony developed on the agar surface was marked as MBC that displayed total bactericidal activity.

Statistical analysis

After doing a single-way analysis of variance (also known as ANOVA) on all experimental data, Dunnett's numerous comparison test was performed to compare group mean differences. Statistical analysis was performed by GraphPad Prism statistics software (version 5.0; the Graph Pad Software, USA), with a significant level of $p < 0.05$.

3. Results and Discussion

3.1 Yield and phytochemical analysis

Methanol extract of the *Galaxaura oblongata* had a yield of 1.822 grams as a dry weight. As shown in Figure 1, quantitative phytochemical profiling indicated that it contained some bioactive components, such as tannins (24.8 mg/g), flavonoids (6.89

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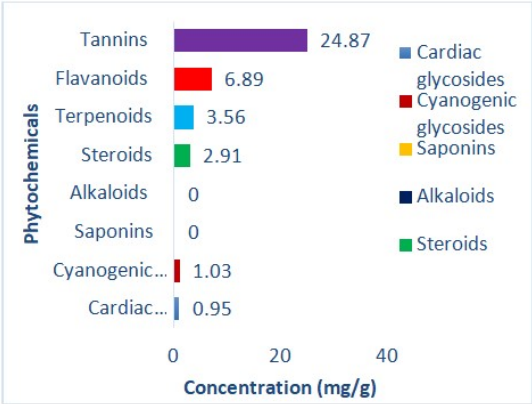

Fig. 1: Quantitative phytochemical makeup of G. *Oblangata* methanolic extract

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mg/g), terpenoids (3.56 mg/g), steroids (2.91 mg/g), cyanogenic glycosides (1.03 mg/g), and cardiac glycosides (0.95 mg/g). These chemicals have also been shown to exhibit a wide range of biological activities, including antioxidant, anti-inflammatory, antibacterial, and anticancer effects (16), highlighting the extract's potential for the nutritional supplements and natural health product sectors.

Nagaraj and Osborne (14) corroborated the foregoing, confirming the presence of compounds such as terpenoids, alkaloids, and saponins are present in the methanol extracted from of *Caulerpa racemosa*. These secondary metabolites are of great significance in pharmaceutical and medical sciences owing to their therapeutic properties. An example is flavonoids and polyphenols, which are powerful antioxidants that can neutralize free radicals, which may reduce cancer rate and mitigate menopause symptoms (17,19). Saponins and tannins are very potent antibiotics which makes them beneficial in treating diseases of antimicrobials. Alkaloids, which are compounds that contain nitrogen, also come with some pharmacological effects, which include antibacterial, anti-fungal, and anti-inflammatory activities (20). Collectively, the pharmacological importance of seaweed bioactives is highlighted by these findings.

3.2 FT-IR analysis

Fourier Transform Infrared (FTIR) spectroscopy has been identified as a sensitive, yet expansively employed analytical technology that is used in the characterization of functional groups present in biomolecules. FTIR spectroscopy used in this study produced clear absorption peaks of 3354.518 cm^{-1}, 1600.820 cm^{-1}, 1262.307 cm^{-1}, 1159.478, 1118.570 cm^{-1}, 1016.757 cm^{-1}, and 421.879 cm^{-1} using the methanolic extract of *G. Oblangata* (Fig. 2). The strong peak for absorption at 3354.518 cm^{-1} is related with O-H stretching vibrations, indicating the existence of hydroxyl groups. The occurrence

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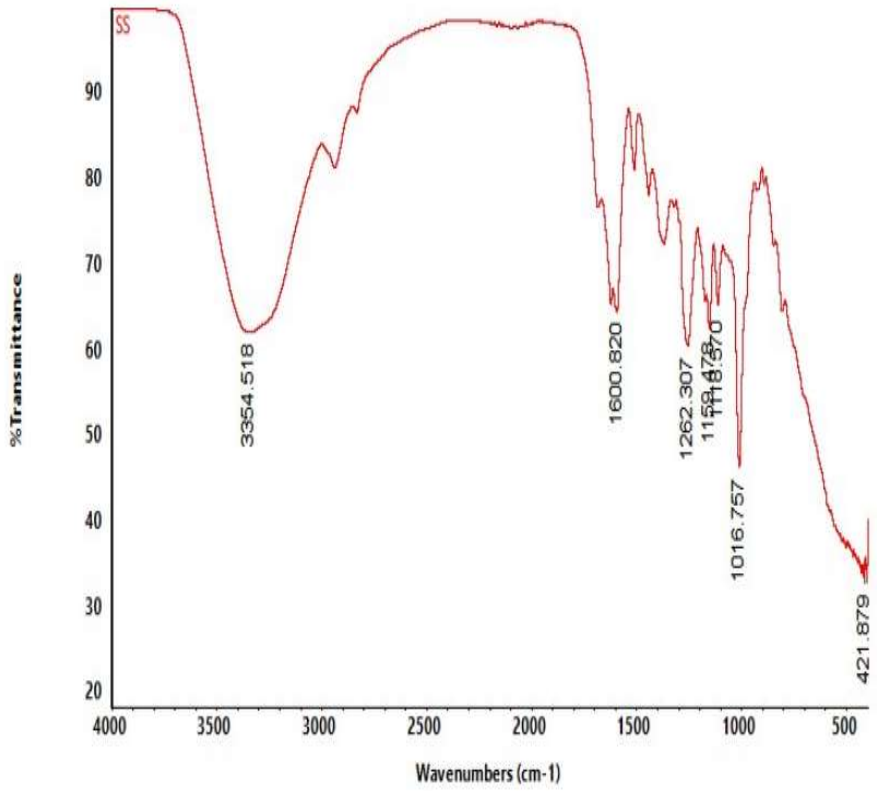

Fig. 2: FTIR spectrum of methanolic extract from *G. Oblangata*

of the carbonyl or amino acids is indicated by a prominent band at 1600.820 cm^{-1} (21), which correspondence to the C = O stretching. The highest value at 1262.307 cm^{-1} is a band that represents C-H bent vibrations of carboxylic acids. The values found at 1159.478 and 1118.570 cm^{-1} correspond to C-O stretching band, while the peak at 1016.757 cm1 indicates the existence of complex oxygen functional groups (22).

The 421.879 cm^{-1} band belongs to the methyl alpha groups and the proximity peak of 409.867 cm^{-1} indicates the existence of hexanedioic acid. These spectrum features indicate that the extract contains a range of functional groups, including carboxyl, hydroxyl, and amino acid functional groups. They are typical in FTIR spectra of seaweed-derived compounds and are associated with their variable biological activities (23,24).

3.3 Antibacterial activity

In the present study, the antibacterial activity of methanolic extract showed maximum inhibition zone 19mm against *S. typhi*, 14mm (*K. pneumonia* and *S. paratyphi*), 13mm (*K. oxytoca* and *P. mirabilis*), 12mm (*S. aureus* and *S. pyogenes*), 11mm (*E. coli* and *V. cholera*) and 10mm (*V. parahaemolyticus*) at maximum concentration of 100 µg/ml. In 75 µg/ml concentration showed 16mm against *S. typhi*, 13mm (*K. pneumonia*), 12mm (*S. paratyphi, P. mirabilis* and *S. pyogenes*), 11mm (*S. aureus*), 9mm (*E. coli* and *V. cholera*). At 75 µg/ml concentration showed 14mm against *S. typhi,* 10mm (*K. oxytoca, S. paratyphi, P. mirabilis* and *S. pyogenes* (24). On 50 µg/ml concentration showed 12mm against *S. typhi,* 10mm (*K. oxytoca* and *S. paratyphi*), 9mm (*S. aureus, P. mirabilis* and *S. pyogenes*) respectively (3). Among the antibacterial activity of seaweed

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Table 1: The methanol extract of (*G. Oblangata*) species has antibacterial activity against clinically acquired human infections

S. No	Name of the strains	Zone of inhibition (mm)					
		25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml	+ve	-ve
1	*S. aureus*	9	9	11	12	17	-
2	*K. pneumonia*	11	12	13	14	26	-
3	*K. oxytoca*	10	10	12	13	25	-
4	*V. parahaemolyticus*	-	-	-	10	22	-
5	*E. coli*	-	-	9	11	21	-
6	*S. typhi*	12	14	16	19	28	-
7	*S. paratyphi*	10	10	12	14	25	-
8	*V. cholera*	-	-	9	11	24	-
9	*P. mirabilis*	9	10	12	13	25	-
10	*S. pyogenes*	9	10	12	12	26	-

Table 2: MIC of the methanolic extract from *G. Oblangata* against bacterial pathogens

S. No	Name of the strains	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml	+ve	-ve
1	*S. aureus*	+++	+++	++	+	*	-	+++
2	*K. pneumonia*	+++	++	+	*	-	-	+++
3	*K. oxytoca*	+++	+++	++	+	*	-	+++
4	*V. parahaemolyticus*	+++	+++	+++	++	+	-	+++
5	*E. coli*	+++	+++	+++	++	+	-	+++
6	*S. typhi*	++	+	*	-	-	-	+++
7	*S. paratyphi*	+++	+++	++	+	*	-	+++
8	*V. cholera*	+++	+++	+++	++	+	-	+++
9	*P. mirabilis*	+++	+++	++	+	*	-	+++
10	*S. pyogenes*	+++	+++	++	+	*	-	+++

+ - Cloudy solution; ++ - Turbid solution; +++ - Highly turbid solution; - MIC concentration; - No growth; * - Significantly arrest

extracts may reduce bacterial pathogen growth by a variety of processes, including RNA, DNA and protein synthesis suppression, cell wall breakdown, bacterial membrane lysis, and metabolic pathway interference (4). These bioactive chemicals' antibacterial capabilities are strongly controlled by their interactions with bacterial strains' hydrophobic structures (5). Seaweed's antibacterial effect is mostly due to the presence of essential fatty acids such as ifurcateacid and dl-4- amino-3-hydroxybutyric acid (Table 1).

3.4 MIC of methanolic extract from *G. Oblangata* against bacterial pathogens

The lowest concentration of an antimicrobial agent that may prevent a microbe's apparent growth is known as the Minimum Inhibitory Concentration, or MIC. In this study, the methanolic extract of Gracilaria oblongata had a minimum inhibitory concentration (MIC) of 100 mcg/ml against *Staphylococcus aureus, Salmonella typhi, Salmonella paratyphi, Proteus mirabilis,* and *streptococcus pyogenes*, with the exception of S. typhi, which had a lower MIC of 60 to 100 mg/ml (Table 2).

Comparatively, the extracts of methanol, ethanol and acetone of Caulerpa racemosa proved to possess the same minimum inhibitory concentration or MIC value of 100 ug/mL across all of the given strains of Aeromonas species (26). Moreover, non-polar solvents like hexane and petroleum ether were used to prepare extracts of

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Table 3: MBC of *G. oblangata's* crude polysaccharides against human pathogens that have been clinically isolated

S. No	Name of the strains	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml
1	*S. aureus*	+++	+++	++	+	-
2	*K. pneumonia*	+++	++	++	-	-
3	*K. oxytoca*	+++	+++	++	+	-
4	*V. parahaemolyticus*	+++	+++	+++	++	+
5	*E. coli*	+++	+++	+++	+	-
6	*S. typhi*	++	+	-	-	-
7	*S. paratyphi*	+++	+++	+++	+	-
8	*V. cholera*	+++	+++	+++	+++	++
9	*P. mirabilis*	+++	+++	++	+	-
10	*S. pyogenes*	+++	+++	++	+	-

C. racemosa which showed, comparatively, lower antibacterial potential, compared to other projections, with minimum of inhibitory concentration (MIC) of 400 gg/mL against P. aeruginosa, S. aureus, and K. pneumoniae (23,28). The broad antimicrobial activity was also observed in another brown seaweed, *Bifurcaria bifurcata*, whose MIC levels are between 0.11 and 1.87 mg/mL. The greatest inhibitory force was registered with Bacillus subtilis (0.11 mg/mL), E. coli (0.23 mg/mL), S. aureus (0.46 mg/mL) and P. aeruginosa (1.87 mg/mL) (25). These results point to the different antibacterial activity of seaweed extracts regarding species, solvent type, and specific microorganism.

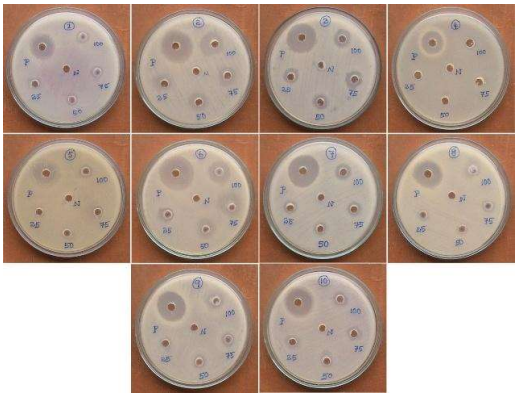

Fig. 3: Antibacterial activity of the Methanolic extract from the *G. Oblangata* against clinically isolated human pathogens

3.5 MBC of the methanolic extract from *G. Oblangata* against bacterial pathogens

The methanolic extract obtained using the root of *Gracilaria oblongata* showed Minimum Bactericidal Concentrations (MBCs) of 100, 80, 100, 100 and 60, 100, 100 and 100 mu/ml against various bacteria pathogens such as *Salmonella Typhi, Staphylococcus aureus Klebsiella pneumoniae Escherichia coli (E. coli),* and *Salmonella paratyphi* (Figs. 3-5 and Table 3) Similarly, methanolic extract of the brown seaweed *Bifurcaria bifurcata* obtained similarly higher values of MBC with *Bacillus subtilis* having an MBC over 7. 50 mg/mL and *Pseudomonas aeruginosa* having MBC above 30 mg/mL, being the most resistant. The observed high antibacterial activity of methanolic extracts could be attributed to the effectiveness of methanol in solubilizing broad range of hydrophilic and lipophilic bioactive compounds which have been documented to have antimicrobial activity (29). Even though several organic solvents have been used in extraction of the seaweeds, methanol is often cited as the most excellent solvent with the capacity to isolate high potent bioactives that exhibit high antibacterial activities (28). Diversity in the results of MBC could be impacted by variations in the extraction methods, environmental and seasonal conditions during collection of algae, and natural variability in the formation of the secondary metabolites within individual species (27,22). These

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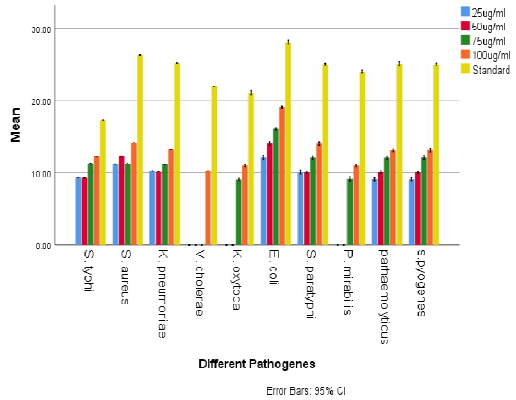

Fig. 4: Methanolic extract from *G. Oblangata* has antibacterial action against clinically collected human pathogens

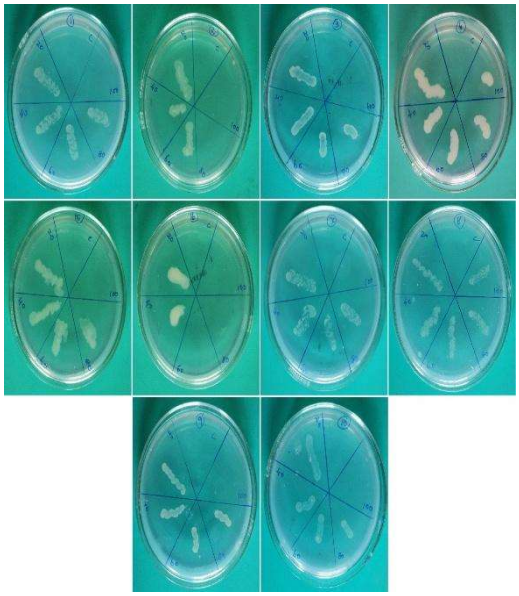

Fig. 5: MBC of a methanolic extract from *G. Oblangata* against clinically isolated human pathogens

results further validate the practice of utilizing methanol based extraction in order to maximize the output of antimicrobial activities in seaweed studies.

Conclusion

The current work indicates the immense antimicrobial activity of *Galaxaura oblongata* methanol extract against some pathogenic microorganisms of clinical origin. The extract showed a significant inhibition diameter of 19 mm against *Salmonella typhi* and good MIC and MBC values of many bacterial strains, especially against *S. aureus, K. pneumoniae, S. typhi* as well as *S. paratyphi*. Phytochemical analysis and FT-IR spectral technique also determined the presence of active constituents in the antimicrobial activity. These results confirm the possible value of *G. Oblangata* methanol extract as a resource to the development of alternative antibacterial agents that can be used to provide natural sources to curb any antibiotic-resistant diseases in the pharmaceutical industry and in clinic.

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