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ANTIBACTERIAL ACTIVITY OF AQUEOUS AND ACETONE IPS EXTRACTS OF *GRACILARIA CRASSA*

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ABSTRACT

Marine macroalgae are considered as an excellent source of bioactive compounds which has a broad range of biological activities including antibacterial and antioxidant. In the present study *G.crassa* was collected from Rameswaram coast Mandapam and amended in CHU 13 media for cultivation under suitable lab conditions. Crude aqueous and acetone extracts was used. The present study aims to test its effectiveness against five strains of bacteria and five strains of fungi. Three gram (+) ve bacteria namely *B.subtilis*, *S.aureus*, *S.typhi* and two gram (-) ve bacteria namely *E.coli* and *K.pneumoniae* and five fungal strains namely *C.albicans*, *C.tropicalis*, *E.floccusum*, *M. gypseum*, *T.rubrum* were carried out by well diffusion method. Results showed the IPS extracts were resisted by most bacterial and fungal strains used of which higher inhibitory effect in bacteria was showed in *B.subtilis* and *S.aureus* and in fungi *C.albicans* showed higher inhibitory effect.

KEYWORDS: *G.crassa*, IPS, antibacterial activity.

INTRODUCTION

Marine algae contain more than 60 trace elements in a concentration much higher than in terrestrial plants. They also contain protein, iodine, bromine, vitamins and substances of stimulatory and antibiotic nature. The phytochemicals from marine algae are extensively used in various industries such as food, confectionary, textile, pharmaceutical, dairy and paper mostly as gelling, stabilizing and thickening agents. Seaweeds or marine macroalgae are the renewable living resources which are also used as food, feed and fertilizer in many parts of the world. In addition to vitamins and minerals, seaweeds are also potentially good sources of

proteins, polysaccharides and fibres.[1,2] Indian seaweeds are of great food value and certain seaweeds contain 16 to 30% protein on dry weight and have all essential amino acids which are not available in vegetable food materials. The highest protein content is recorded for red seaweeds such as *Porphyra tenera* and *Palmaria palmata* which contain, respectively, up to 47 and 35% proteins expressed as dry weight.[3, 4]

Seaweeds are considered to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities. Compounds with cytostatic, antiviral, antihelminthic, antifungal and antibacterial activities have been detected in green, brown and red algae.[5,6] Extracts of marine algae were reported to exhibit antibacterial activity.[14,7,8]

Several workers have reported that the seaweed extracts exhibit inhibitory activity against a number of gram positive and gram negative bacterial pathogens. A number of seaweeds have been studied for their antibacterial activity both in India and abroad[9-13,15] found high protein content in three species of *Ulva.*[16] have estimated the protein, carbohydrate and organic carbon content in 43 marine algal species collected from different marine stations along the Maharashtra coast and observed more protein and carbohydrate content in Chlorophycean and Rhodophycean than Phaeophycean algae.

Among Rhodophyta, *Gracilaria* is one of the edible seaweed and major commercial source of agar and agarose.[17,18]

Numerous substances were identified as antimicrobial agents from algae such as Chlorellin derivatives, acrylic acid, halogenated aliphatic Phenolic inhibitors etc. Nowadays there is an increasing demand for biodiversity in screening programmes for selecting therapeutic drugs from natural products, the marine organisms; especially seaweeds are of immense interest since they have a broad range of biological activities such as antibacterial, antifungal, antiviral, antitumour, anti-inflammatory and antioxidants. Seaweeds have been recognized as potential sources of antibiotic substances. The production of antimicrobial activities is considered as an indicator of the bioactive secondary metabolites.[19-21] Fungi represent an immense source of bioactive substances with immuno-stimulating and anticancer properties that make them very potent natural supplements in cancer therapy. Thus, the activities of more and various fungal species are worth investigation in order to establish other, more potent fungal substances that can be reliable therapeutics for humans.[22]

The present study was undertaken with objectives: To evaluate the antibacterial and antifungal potentials of crude aqueous and acetone IPS extracts of *G. crassa* against 5 human pathogens.

MATERIALS AND METHODS

Collection of algae

G. crassa belonging to Rhodophyceae was collected from shore of mandapam, nearby Rameswaram coast Gulf of mannar, Tamilnadu.

Identification of algae

The collected algal species were identified and authenticated from Botanical Survey of India, Coimbatore.

Intracellular polysaccharide (Ips)

To 100 g of the tested alga culture in 100 mL of CHU13 were taken and centrifuged at 3000 rpm for 10 minutes. IPS was extracted by homogenizing the derived pellet in distilled water (50 mL). The homogenates were then heated in water bath at 95°C for 6 hours. The extracts were filtrated through Whatman No.2 filter paper, then precipitated with four volumes of 95% ethanol, stirred vigorously and left overnight at 4°C. The precipitate were washed with distilled water and subsequently lyophilized for quantitative assessment and analysis.

Preparation of algal extract

Ten gram of IPS powder of *G.crassa* was extracted separately with 50 ml of acetone and aqueous for 5 days in dark at room temperature. The extract is then filtered through whatmann filter paper and condensed. The final condensed portion was transferred to a sample bottle and weighed.[23]

DETERMINATION OF ANTIBACTERIAL ACTIVITIES OF ACETONE AND AQUEOUS EXTRACTS

Stock solution preparation

The crude acetone extracts were dissolved with 100% Dimethyl sulfoxide (DMSO). The stock solutions were prepared as 5 µg/µL concentrations. From the stock solution, 25 µL, 50 µL and 75 µL of sample extracts were immediately dispensed into each agar wells (9 mm) of culture inoculated Nutrient Agar (NA) plates using sterilized micropipette.

Test organisms

Type of pathogens	Name of the pathogens
Gram -ve	*Escherichia coli*
Gram -ve	*Klebsiella pneumoniae*
Gram +ve	*Bacillus subtilis*
Gram +ve	*Staphylococcus aureus*
Gram -ve	*Salmonella typhi*

Preparation of Bacterial inoculum: The inoculum were prepared using two gram positive (*Bacillus subtilis, Staphyllococcus aureus,*) and three gram negative (*Escherichia coli, Klebsiella pneumoniae, Salmonella typhi*) bacterial pathogens from a 24 h old culture on Nutrient Agar (NA). With a sterile loop, the tops of four to five colonies were transferred to a tube containing 5 ml of Nutrient Broth (NB). The tube was then incubated at 35˚C for 24 h. The turbidity of the culture suspension was adjusted with broth on a sterile saline solution (0.85 – 0.9%). The density of this culture was adjusted with 0.5 McFarland standards and finally made the inoculum size approximately of 5 x 105 CFU/mL.

Well diffusion method: The well diffusion test[24-26] was performed using NA medium. The medium was prepared and autoclaved at 15 lbs pressure (121ºC) for 15 min immediately cooled in a 50-55ºC water bath after removed from the autoclave. The cooled medium was poured into sterile petriplates to a uniform depth of 4 mm; this is equivalent to approximately 25 ml in a 90 mm plate. Once the medium was solidified, then the culture was inoculated on the medium. Within 15 min of adjusting the density of the inoculum, a sterile cotton swab was dipped into the standardized bacterial suspension. The sterile swab was used to streak on the surface of the NA containing plates. The plates were allowed undisturbed for 3 to 5 min to absorb the excess moisture. Sterilized 9 mm cork borer was used to make agar wells, 25 µL, 50 µL, 75 µL of extract stock solutions were placed into each wells and 100% DMSO as a control. Positive control was made by streptomycin 30 µg which were suspended in 100% DMSO solvent. Zone of inhibition (ZI) were measured by 1mm accuracy scale prescribed method and calculated the zone of inhibition percentage also by the following formula

Percentage of inhibition = I/diameter of the petriplate in mm×100.

DETERMINATION OF ANTIFUNGAL ACTIVITIES OF ACETONE AND AQUEOUS EXTRACTS

Stock solution preparation: The crude acetone extracts were dissolved with 100% Dimethyl sulfoxide (DMSO). The stock solutions were prepared as 5µg/µL concentrations. From the

stock solution, 25 µL, 50 µL and 75 µL of sample extracts were immediately dispensed into each agar wells (9 mm) of culture inoculated Potato Dextrose Agar (PDA) plates using sterilized micropipette.

Test organisms

1.Candida albicans

2.Candida tropicalis

3.Epidermophyton floccusum

4.Mycosporum gypseum

5.Tinea rubrum

Preparation of fungal inoculum

The clinical fungal test organisms used are *Candida albicans,. Candida tropicalis, Epidermophyton floccusum, Mycosporum gypseum, Tinea rubrum.*

The fungal strains were inoculated separately in Potato Dextrose Broth for 6 h and the suspensions were checked to provide approximately 10^5 CFU/mL.

Well diffusion method: Antifungal activity was screened by agar well diffusion method.[27] The PDA medium was poured in to the sterile petriplates and allowed to solidify. The test fungal culture was evenly spread over the media by sterile cotton swabs. Then wells (6 mm) were made in the medium using sterile cork borer. 25µL, 50µL, 75µL of each extracts were transferred into the separate wells. The plates were incubated at 27°C for 48-72 hrs. After the incubation the plates were observed for formation of clear incubation zone around the well indicated the presence of antifungal activity. The zone of inhibition was recorded.

Statistical analysis: Results are presented as mean ± SD (standard deviation) for three replicates. Data obtained were subjected to one way Anova by Tukey HSD method.

RESULTS

Antibacterial activity of aqueous and acetone extracts

Herbal remedies play an essential role in traditional medicine in rural areas of South Africa, where these are often the therapeutic treatment of choice. The preparation of herbal medicine which depends on a cultural context may be obtained from healers as already prepared mixtures, or as unprepared raw materials. Although South Africa possesses a rich tradition in the use of medicinal plants and an outstanding floral diversity estimated at 251 220 species of

vascular plants.[28] Many of the infectious diseases are still a major challenge to health issues all over the world. The emergence of resistance to antibiotics has further compounded the problem.[29] They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects of synthetic antimicrobials.[30] They may act as lead compounds for the pharmaceutical industry or as the base for the development of new antimicrobials.[31,32]

In the present study the antibacterial activity of aqueous and acetone IPS extracts of *G. crassa* were tested against five human pathogens namely *B.subtilis*, *E.coli*, *S.aureus*, *S. typhi* and *K.pneumoniae* using well diffusion method. Both IPS extracts was resisted by most bacterial strains used, of which *B.subtilis* and *S.aureus* showed higher inhibitory effect with 20.3mm and 13.2 mm respectively. Ampicillin was used as positive control. *B.subtilis* was more inhibitory to acetone extract than aqueous.

Antifungal activity of aqueous and acetone extracts

Infectious diseases are one of the main causes of high morbidity and mortality in human beings around the world, especially in developing countries.[33] The severity of the diseases have increased in recent years and the emergence of multidrug resistant strains.[34]

In the present study the antifungal activity of aqueous and acetone IPS extracts of *G. crassa* were tested against five fungal pathogens namely *C. albicans*, *C. tropicalis*, *E. floccusum*, *M. gypseum*, *T. rubrum* using well diffusion method. In aqueous and acetone IPS extracts, *C. albicans* showed highest inhibitory effect with 27.3mm and 23.5mm respectively. Fluconazole was used as positive control. *C. albicans* showed best result in positive control with 16mm and 21mm respectively.

Minimum Inhibitory Concentration of aqueous and acetone extracts against five pathogenic bacteria: MIC represents the lowest concentration of crude or purified algal extracts that inhibits the bacterial or fungal growth. The concentration serial broth (micro) dilution assay has been used in several studies.[35-37] Minimum inhibition concentration (MIC) in aqueous and acetone IPS extracts of *G. crassa* was tested against five pathogenic bacteria. In acetone extracts MIC was found to be 1.0 mg/mL against *E.coli*, *S.typhi*, *S.aureus* and 2 mg/mL in *K.pneumoniae*, *B. subtilis*. In case of aqueous extracts MIC value was found to be 1.0 mg/mL in *E.coli*, *S.typhi*, *S.aureus*, *B.subtilis* and 2mg/mL in *K. pneumonia*.

Table 1. Antibacterial activity of acetone IPS extracts of *G. crassa*

Algal species	Bacterial strains	Concentration of acetone extracts (µg/mL)						Positive Control Ampicillin
		25		50		75		
		ZI	% I	ZI	% I	ZI	% I	
G.crassa	*E. coli*	15.49± 1.15	17.21±1.15	13.22±1.15	14.68±0.57	11.18±1.00	12.48±1.00	20.22± 4.33
	K.pneumoniae	11.12± 1.52	12.35±2.08	13.18±1.00	14.64±0.57	14.22±0.17	15.8±0.57	30.86± 0.57
	B. subtilis	17.32±1.05	19.24±3.05	18.18±7.37	20.2±9.81	15.43±0.50	17.15±0.17	29.46± 0.50
	S. aureus	13.66± 0.57	15.17±0.57	14.22±2.08	15.8±0.76	18.33±0.57	20.26±9.81	15.94± 1.50
	S. typhi	15.84±0.57	17.6± 0.57	16.29±0.28	18.1±0.76	17.11±0.76	19.01±0.00	24.33± 0.51

Values are mean ± standard deviation of triplicates. P≤ 0.05.

Table 2. Antibacterial activity of aqueous IPS extracts of *G. crassa*

Algal species	Bacterial strains	Concentration of aqueous extracts (µg/mL)						Positive Control Ampicillin
		25		50		75		
		ZI	% I	ZI	% I	ZI	% I	
G.crassa	*E. coli*	12.34±2.08	13.71± 0.26	11.72± 1.03	13.02± 1.00	10.06± 0.00	11.17± 0.23	13.38± 0.57
	K.pneumoniae	12.10±0.86	13.44± 0.75	11.21± 1.59	12.45± 0.50	11.07± 0.00	12.3± 1.52	26.76± 0.51
	B. subtilis	13.36±1.15	14.84± 0.35	12.94± 1.65	14.37± 0.50	11.93± 1.20	13.25± 0.30	26.42± 0.44
	S. aureus	13.24±1.07	14.71± 0.36	13.88± 0.97	15.42± 0.50	11.22± 0.40	12.46± 1.33	24.04± 0.50
	S. typhi	11.22±1.60	12.46± 1.31	10.94± 0.90	12.15± 0.76	10.98± 1.67	12.2± 0.57	28.11± 0.46

Values are mean ± standard deviation of triplicates. P≤ 0.05.

Table 3. Antifungal activity of acetone IPS extracts of *G. crassa*

Algal species	Fungal strains	Concentration of acetone extracts (µg/mL)						Positive Control Flucanozole
		25		50		75		
		ZI	% I	ZI	% I	ZI	% I	
G.crassa	*C. albicans*	20.46±4.33	22.73±1.21	26.34±0.51	29.26±0.57	24.62±0.5	27.35±1.12	16.07± 1.12
	C. tropicalis	12.91±1.64	14.34±0.50	14.94±1.00	16.6±1.55	14.61±0.57	16.23±1.38	10.21±0.41

	E. floccusum	16.03±0.28	17.81±0.72	15.86±1.41	17.62±0.55	21.04±1.02	23.37±1.34	11.23±0.40
	M. gypseum	15.83±1.50	17.58±0.50	13.39±1.18	14.87±0.35	11.15±1.52	12.38±0.55	10.05±0.00
	T. rubrum	10.88±1.38	12.08±1.00	10.72±1.32	11.91±1.20	10.31±0.52	11.45±0.5	16.32± 0.34

Values are mean ± standard deviation of triplicates. P ≤ 0.05.

Table 4. Antifungal activity of aqueous IPS extracts of *G. crassa*

Algal species	**Fungal strains**	**Concentration of aqueous extracts (µg/mL)**						**Positive Control Flucanozole**
		25		**50**		**75**		
		ZI	**% I**	**ZI**	**% I**	**ZI**	**% I**	
G.crassa	*C. albicans*	20.43±0.76	22.7±1.21	19.88±0.28	22.08±0.05	19.76±0.28	21.95± 1.32	21.11±1.02
	C. tropicalis	13.62±1.02	15.13±0.11	14.08±0.00	15.64±1.15	14.39±0.5	15.98± 1.44	13.57±1.15
	E. floccusum	17.94±0.83	19.93±0.40	18.16±1.37	20.17±4.10	21.22±1.05	23.57± 1.37	17.33±1.17
	M. gypseum	17.36±0.52	19.28±0.25	16.48±1.32	18.31±0.57	14.31± 0.46	15.9±1.44	15.55±0.57
	T. rubrum	10.72±0.55	11.91±0.95	10.65±1.15	11.83±0.26	11.11± 1.52	12.34± 0.51	14.04±1.98

Values are mean ± standard deviation of triplicates. P ≤ 0.05.

Table 5. Minimum Inhibitory Concentration of aqueous and acetone extracts against five pathogenic bacteria

Algal species	**Name of the organisms**	**Minimum inhibitory Concentration of aqueous extracts (mg)**	**Minimum inhibitory Concentration of acetone extracts (mg)**
G.crassa	*E. coli*	1.0	1.0
	K.pneumoniae	2.0	2.0
	S. aureus	1.0	1.0
	B. subtilis	1.0	2.0
	S. typhi	1.0	1.0

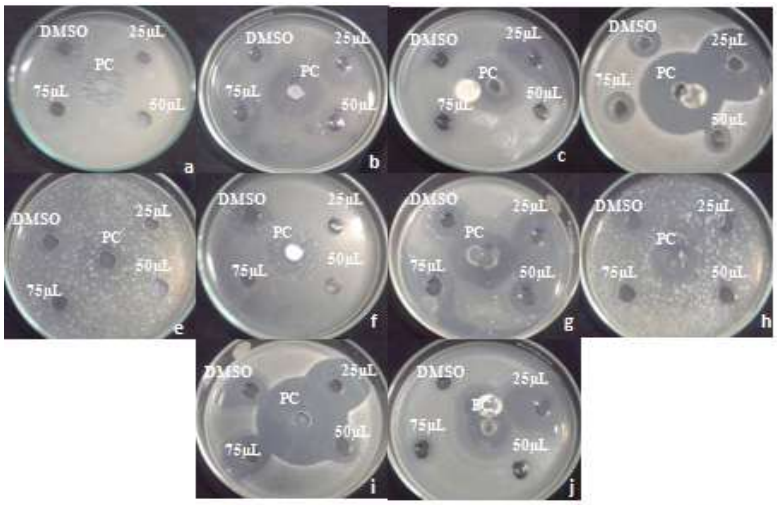

Fig. 1. Antibacterial activity of IPS aqueous and acetone extract of *G. crassa* a, b, c, d, e showing aqueous extract of *G. crassa*, f,g,h,I,j showing acetone extract of *G. crassa*. a – *K. pneumoniae*, b- *S. typhi*, c – *S. aureus*, d- *B. subtilis*, e- *E. coli*, f - *K. pneumoniae*, g- *S. typhi*, h – *E. coli*, i – *B. subtilis*, j – *S. aureus*.

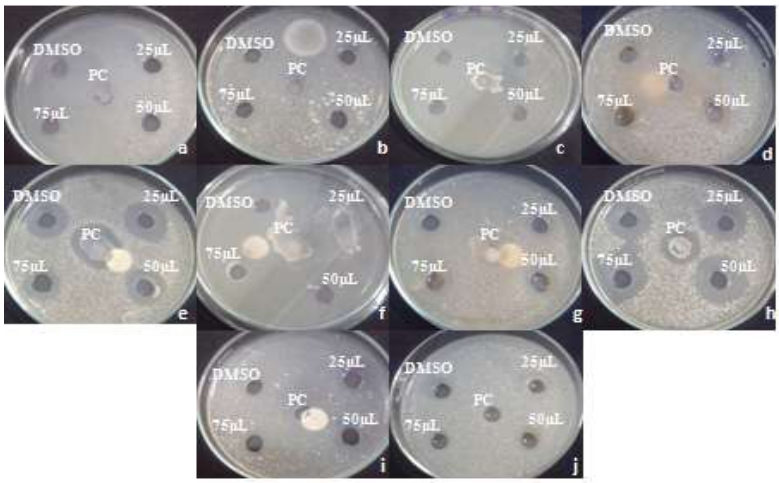

Fig.2. Antifungal activity of IPS aqueous and acetone extract of *G. crassa* a, b, c, d, e showing aqueous extract of *G. crassa*, f,g,h,I,j showing acetone extract of *G. crassa*. a – *M.gypseum* b- *E. floccusum*, c – *T. rubrum*, d- *C. tropicalis*, e- *C. albicans*, f - *T. rubrum*, g- *C. tropicalis*, h- *C. albicans*, i- *E. floccusum* , j – *M.gypseum*

DISCUSSION

The past decade have witnessed a significant increase in the resistance of pathogenic bacteria to antibacterial agents –with direct implications in human morbidity and mortality. Hence, attention has been paid to a more detailed understanding of the mechanisms underlying antimicrobial resistance – as well as to improved methods to detect resistance, new antimicrobial options for treatment of infections caused by resistant microorganisms, and methods to prevent emergence and spreading of resistance in the first place. Most efforts were devoted to the study of antibiotic resistance in bacteria for several reasons: (i) bacterial infections are responsible for most community-acquired and nosocomial infections; (ii) the large and expanding number of antibacterial classes offers a more diverse range of resistance mechanisms; and (iii) the ability to move bacterial resistance determinants into standard,

well-characterized bacterial strains facilitates more detailed studies of the underlying molecular mechanisms.

As per authors knowledge there are no previous reports dealing with antibacterial activity of aqueous and acetone IPS extracts of *G. crassa* According to[38] antibacterial activity of IPS extracts of *Pleurotus tuber regium* was resisted by most bacterial strains used, of which *E.coli* and *S. aureus* shows some sensitivity to IPS extracts (10mm and 0.75mm dia respectively). Vancomycin was acted as positive control with approximately 30µg/mL. *S. aureus* was more resistant to IPS compared to *E.coli* as 80µg/mL.

One another study of[39] of IPS extracts from *C. cicadae* were tested against *E. coli*, *K. pneumoniae*, *V. cholerae*, *P. aeruginosa*, *V. alginolyticus*, *S. aureus*, *V. parahaemolyticus*, *S. pneumoniae* respectively. From various bacterial strains *E.coli*, *K. pneumoniae* shows best inhibitory effect at 100% concentration with 11.9 and 12.9mm respectively. Tetracycline was used as positive control. Maximum inhibitory effect was observed in *K. pneumoniae* with 23mm. Polysaccharide from *C. cicadae* showed activity against gram positive and gram negative bacteria. The sensitivity of gram positive bacteria to polysaccharide extracts is in confirmatory with the previous studies.[40,41] This is due to the membrane composition of the bacterial strains.[42][43] reported that S. platensis of hot water extract, crude polysaccharide and cell free polysaccharide were tested against various bacterial strains namely *S. flexneri*, *K. pneumoniae*, *S. typhi* and *S. paratyphi*. From these extracts crude polysaccharide tested under *S. typhi* showed remarkable result with 13mm zone of inhibition. The zone of inhibition produced by crude polysaccharide was maximum followed by hot water extract and cell free polysaccharide against *Salmonella*.

The hot water extract, crude and cell free polysaccharide did exhibit antimicrobial property as has been reported by [44] in case of crude polysaccharide. The zone exhibited by the hot water extract was wider than that of the crude and cell free polysaccharide. Potent antibacterial activity of methanol extract has been already reported by.[45]

Antifungal activity

According to[46] the antimicrobial activity of four extracts from two isolated soil fungi against ten different bacterial isolates comprising of both Gram negative and Gram positive organisms. *C. albicans* was sensitive to the intracellular extract of *A. flavus* var. *columinaris* only. The study shows that some of the soil fungi may be used in medicine as potentially

effective antimicrobial agents. This study is similar to other studies,[47-53] aimed to test the antimicrobial and anticancer effects of some soil fungi extracts, but differ from them in the type of isolated fungi. In the study of[54], they studied the antifungal activity of intra- and extra-cellular metabolites of *Aspergillus terreus* cultured on two types of media against some unicellular and filamentous fungi and they concluded that the extracts of *Aspergillus terreus* which were cultured on yeast extract sucrose medium remarkably inhibited the growth of *Aspergillus fumigatus.*[55] reported the antifungal activity of crude endopolysaccharide of hot water extracts of various mushroom was tested against *C. albicans, C. tropicalis* compared with two fungal species *C. albicans* showed test result in *Flammulina velutipes* with 20mm respectively. In PIMG % with various mushroom species was tested against various fungal species. The results showed that *Rhizoctonia* species had best inhibitory effect of 17mm.

Minimal inhibitory concentration (MIC)

According to[57] revealed the inhibitory activity of leaves of *Arbutus unedo* leaves ethanol extract MICs for the mycobacterial species studied are then determined. The MIC was 5.59 ± 0.69 mg/mL for *M. aurum* A + and 6.02 ± 0.76 mg/ ml for *M. smegmatis* MC2 and *M. bovis.* The results obtained with the method of discs, the MIC study showed that the ethanol extract part I has approximately the same inhibitory effect against growth of the three bacterial species tested: *M. smegmatis*, *M. aurum* A+ and *M. bovis* PPI ($p > 0.05$).[56] reported the effect on bacterial viability and growth of *S. mutans* The minimum inhibitory concentration (MIC) of Quercitrin and Deoxynojirimycin (DNJ) against *S. mutans* was found to be 64 mg/mL and 16 mg/mL respectively. Hence, they were found to have significant antimicrobial activity.

The results from our findings showed that *G.crassa* have best inhibitory antibacterial activity in *B. subtilis* and *S.aureus* with 20.3mm and 13.2mm and best inhibitory antifungal activity in *C. albicans* with 27.3mm and 23.5mm respectively.

CONCLUSION

These findings will lead the way for large scale industrial fermentations and commercial uses of IPS from *G. crassa* as antibacterial constituents. Present studies will open up the scope for large scale industrial fermentation of *G. crassa* culture for the production of biologically active polysaccharides and clinical trials of IPS on animal models.

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REFERENCES

1. Lahaye M. Marine algae as a source of dietary fibers: Determination of soluble and insoluble dietary fiber contents in some 'sea vegetable'. J Sci Food Agric. 1991; 54: 587-594.
2. Darcy-Vrillon B. Nutritional aspects of the developing use of marine macroalgae for the human food industry. Int. J. Food Sci. Nutr. 1993; 44: 523-535.
3. Fujiwara-Arasaki T, Mino N, Kuroda M. The protein value in human nutrition of edible marine algae in Japan. Hydrobiol. 1984; 116/117: 513–516.
4. Morgan KC, Wright JLC, Simpson FJ. Review of chemical constituents of the red algae. Palmaria palmata. Econ. Bot. 1980; 34: 27–50.
5. Lindequist U, Schweder T. Marine Biotechnology. In: Rehm, H.J, Reed G. (Eds.), Biotechnology, vol. 10. Wiley-VCH, Weinheim, 2001; 441-484.
6. Newman DJ, Cragg GM, Snader KM. Natural products as source of new drugs over the period 1981-2002. J. Nat. Prod. 2003; 66: 1022-1037.
7. Mahasneh I, Jamal M, Kashashneh M, Zibdeh M. Antibiotic activity of marine algae against multi antibiotic resistant bacteria. Microbios. 1995; 83: 23-26.
8. Sachithananthan K, Sivapalan A. Antibacterial properties of some marine algae of Sri Lanka. Bulletin of Fisheries Research Station, Sri Lanka. 1975; 26: 5-9.
9. Pratt R, Mautner R, Gardener GM, Sha Y, Dufrenoy J. Report on antibiotic activity of seaweed extracts. J. Amer. Pharm. Asst. Sci. 1951; 40: 579-579.
10. Chester CG, Stott JA. The production of antibiotic substances by seaweeds. In: (T. Braarud and N.A. Soressen,eds) Second International Seaweed Symp. Pergamon, Newyork. 1956; 49-53.
11. Burkholder PR, Burkholder LM, Almodovar LR. Antibiotic activity of some marine algae of Puerto Rico. Bot. Mar. 1960; 2: 149- 156.
12. Allen MB, Dawson NY. Production of antibacterial substances by benthic tropical marine algae. J.Bacteriol. 1960; 79: 459-460.
13. Nunez G, Sanabria LMS. Effects of products by extracts of macroscopic marine algae on bacterial growth. Acta. Biol. Venez. 1975; 9: 121-134.

14. Siddddhanta AK, Mody KH, Ramavat BK, Chauhan VD, Garg HS, Goel AK, Jinandra Doss M, Srivastava MN, Patnaik GK, Kamboj VP. Bioactivity of marine organisms: Part VIII Screening of some marine flora of Western coast of India. Indian Journal Experimental Biology. 1997; 35: 638-643.
15. Sitakara Rao V, Tipnis VK. Protein content of marine algae from Gujarat coast. Curr. Sci. 1964; 33: 16-17.
16. Dhargalkar VK, Jagtap TG, Untawale AG. Biochemical constituents of seaweeds along the Maharastra coast. Indian J. Mar. Sci. 1980; 9(4): 297-299.
17. McDermid KJ, Stuercke B. Nutritional composition of edible Hawaiian seaweeds. J Appl Phycol 2003; 15: 513-524.
18. Baghel RS, Kumari P, Bijo AJ, Gupta V, Reddy CRK, Jha B. Genetic analysis and marker assisted identification of life phases of red alga Gracilaria corticata (J. Agardh). Mol Biol Rep 2011; 38: 4211– 4218.
19. Gonzalez Del Val A., Platas A, Basilio J, Gorrochategui I,. Suay F, Vicente E, Portillo M, Jimenez Del Rio R.G, Garcia and Pelaez F. Screening of antimicrobial activities in red, green and brown macroalgae from Gran Canaria (Canary Islands, Spain). Int. Microbial., 2001; 4: 35-40.
20. Chiheb I, Hassane R, Martinez- L. José D.S.J, Francisco G.V.J, Antonio B, Hassan and Mohamed K. Screening of antibacterial activity in marine green and brown macroalgae from the coast of Morocco. Afr. J. Biotechnol., 2009; 8(7): 1258-1262.
21. Srivastava N, Saurav K, Mohanasrinivasan V, Kannabiran K and Singh M. Antibacterial Potential of Macroalgae Collected from the Mandapam Coast, India British J. Pharmacol. And Toxicol, 2010; 1(2): 72-76.
22. Hu H, Ahn N.S, Yang X. Ganoderma lucidum extracts induce cell cycle arrest and apoptosis in MCF-7 human breast cancer cell. Int J Cancer, 2002; 102: 250-253.
23. Raaman N. Phytochemical Techniques.New India Publishing Agency, New Delhi, India. 2006.
24. Bennet J.V, Brodie J.L, Benner J.L, Kirby W.M.M. Simplified accurate method for antibiotic assay of clinical specimens. Applied Microbiology, 1966; 14: 2170– 2177.
25. Janssen A.M, Sheffer J.J.C, Baerheim-Svendsen. A. Antimicrobial activity of essential oils: A 1976 1986 literature review: Aspects of the test methods. Planta Med., 1987; 53: 395- 398.
26. Magaldi S, Mata-Essayag S, Hartung C, Perez C, Colella M.T, Olaizola C, Ontiveros Y. Well diffusion for antifungal susceptibility testing. Int. J. Infect. Dis., 2004; 8: 39-45.

27. Perez C, Pauli M, Bazerque, P. An antibiotic assay by agar-well diffusion method. Acta Biologiaeet Medecine Experimentaalis, 1990; 15: 113-115.
28. Cracraft J, Grifo F. The living planet in crisis: Biodiversity science and policy, 1st ed. Columbia University Press, New York. 1999; 139-172.
29. Alli A.I, Ehinmidu JO, Ibrahim Y.K.E. Preliminary phytochemical screening and antimicrobial activities of some medicinal plants used in Ebiraland. Bayero J. Pure Appl. Sci. 2011; 4: 10-16.
30. Iwu MW, Duncan AR, Okunji CO. New antimicrobials of plant origin. In: Janick, J. (Ed.), Perspectives on New Crops and New Uses. ASHS Press, Alexandria, VA. 1999; 457-62.
31. Aiyelaagbe OO. Antibacterial activity of Jatropha multifida roots. Fitoterapia. 2001; 72: 544-546.
32. Aiyegoro OA, Akinpelu DA, Afolayan AJ, Okoh AI. Antibacterial activities of crude stem bark extracts of Distemonanthus benthamianus Baill; J. Biol. Sci. 2008; 8(2): 356-361.
33. Waldvogel FA. Infectious diseases in the 21st century: Old challenges and new opportunities International. J Infec Dis 2004; 8(1): 5-12
34. Martinez-Donate AP, Hovell Melbourne F, Blumberg EJ, Zellner JA, Sipan CL, Shillington AM, Carrizosa C. Gender differences in condom-related behaviors and attitudes among Mexican adolescents living on the U.S.-Mexico border. AIDS Education and prevention: official publication of the International society for AIDS Education 2004; 16(2): 172-186.
35. Boisvert C, Beaulieu L, Bonnet C, Pelletier E. Assessment of the antioxidant and antibacterial activities of three species of edible seaweeds. J. Food Biochem. 2015; 39: 377–387.
36. Bazes S, Silkina A, Defer D, Bernède-Bauduin C, Quéméner E, Braud J.P, Bourgougnon N. Active substances from Ceramium botryocarpum used as antifouling products in aquaculture. Aquaculture 2006; 258: 664–674.
37. Hellio C, De La Broise D, Dufossé L, Le Gal Y, Bourgougnon N. Inhibition of marine bacteria by extract of macroalgae: Potential use for environmentally friendly paints. Mar. Environ. Res. 2001; 52: 231–247.
38. Comfort Olukemi Bamigboye, Julius Kola Oloke, Joanna Felicity Dames. Biological Activity of Extracellular and Intracellular Polysaccharides from Pleurotus tuber-regium Hybrid and Mutant Strains, 2016; 4(7): 422-428.

39. Shrama SK, Gautam N, Atri NS. Optimization, composition and antioxidant activities of exo and intracellular polysaccharides in submerged culture of Cordyceps gracilis (Grev.) Durieu & Mont. Evid Based Complement Alternat Med. 2015; 1–8.
40. Venturini ME, Rivera CS, Gonzalez C, Blanco D. Antimicrobial activity of extracts of edible wild and cultivated mushrooms against food borne bacterial strains. J Food Protect. 2008; 71: 1701–6.
41. Yamac M, Bilgili F. Antimicrobial activities of fruit bodies and/or mycelial cultures of some mushroom isolates. Pharm Biol. 2006; 44: 660–7.
42. Holst O, M ller-Loennies S. Microbial polysaccharides structures. In: Kamerling JP, Boons GJ, Lee YC, Suzuki A, Taniguchi N, Voragen AGJ, editors. Comprehensive glycoscience. From chemistry to systems biology. Amsterdam: Elsevier Inc; 2007; 123–79.
43. Raj Kishori Xalxo, Shweta Sao, Pankaj K. Sahu. Effect of antibacterial properties of cyanobacterial Spirulina platensis 2013; 4(9).
44. Singh, S, Ganguly A., Baisya S. Antibacterial properties of polysaccharide from Spirulina platensis. 46th annual conference of association of microbiologist of India Department of Microbiology Osmania University Hyderabad (AP) India 2005; Dec. 8 – 10.
45. Ozdemir G, Karabay N, Dolay M, Pazarbasi B. Antibacterial activity of volatile extracts of Spirulina platensis. Phytother. Res. 2004; 18(9): 754-757.
46. Ghada A. Fawzy, Areej M. Al-Taweel, Nahla A. Melake. In Vitro Antimicrobial and Anti-Tumor Activities of Intracellular and Extracellular extracts of Aspergillus niger and Aspergillus flavus var. columinaris. 2011; 3(1): 980-987
47. Johannes C, Liermann H.K, Till O, Eckhard T, Heidrun A. Xanthepinone an Antimicrobial Polyketide from a Soil Fungus Closely Related to Phoma medicaginis. J. Nat. Prod., 2009; 72(10): 1905– 1907
48. Preecha P, Jakaphan R, Pongpun S, Santi T.P.9-epi-Viridiol, a novel cytotoxic furanosteroid from soil fungus Trichoderma virens. Natural Product Research, Vol.20, Issue 14 December, 2006; 1321 – 1325.
49. Ranieri R.L, Calton G.J. Tetrahedron Lett. 1978; (6): 499.
50. Glasby J.S. Encyclopaedia of Antibiotics 1976.
51. Beard R.L, Walton G.S. Kojic acid as an insecticidal mycotoxin. J. Invertebr. Pathol. 1969; 14: 53– 59.
52. Ning L, Qu G, Ye M, Guo H, Bi K, Guo D. Cytotoxic biotransformed products from triptonide by Aspergillus niger. Planta-Med. Sep, 2003; 69(9): 804- 8.

53. Saito S. Japan, J. Bot., 1907; 21: 7.
54. Zain M.E, Awaad A.S, Al-Jaber NA, Maitland D.J. New phenolic compounds with antifungal activity from Aspergillus terreus isolated from desert soil. J. Saudi Chem. Soc., 2008; 12(1): 107-114.
55. Mohamed Osman FRH, Hassan Ahmed WA, Soad Nady, Heba El-sayed. Growth and antimicrobial activity of different mushroom strains grown in submerged culture 2015; 5(1): 545-564.
56. Asmae El ouarti, Amina Cherif Haouat, Hakima Sqalli, Abdellatif Haggoud, Abdeslam Ennabili, Saad Ibnsouda, Mohamed lachgar, Mohammed Iraqui. Extra- and intracellular antimycobacterial activity of Arbutus unedo L. 2012; 6(6): 1283-1290.
57. Sadaf Hasan, Kunal Singh, Mohd Danisuddin, Praveen K. Verma, Asad U. Khan. Inhibition of Major Virulence Pathways of Streptococcus mutans by Quercitrin and Deoxynojirimycin: A Synergistic Approach of Infection Control 2014; 9(3): e91736.