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Original Article

ANTIBACTERIAL AND ANTIOBESITY ACTIVITIES OF MARINE ALGAE GRACILARIA CORTICATA AND SPIRULINA PLATENSIS

KANNAN M[1], DHEEBA B[2], NAGESHWARI K[2], KANNAN K[3], VENKATESAN S

[1]Research Department of Microbiology, VHNSN College, Virudhunagar 626001, Tamil Nadu, India, [2] Department of Chemistry and Biosciences, Srinivasa Ramanujan Centre, SASTRA University, Kumbakonam 612001, [3]Department of Mathematics, Srinivasa Ramanujan Centre, SASTRA University, Kumbakonam 612001, [4]Department of environmental sciences, Periyar University, Salem. Email: microkannan@gmail.com, deepabaskaran76@gmail.com

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ABSTRACT

Objective: Natural products of plants played an important function in treatment and prevention of human diseases during thousands of years. The major objective of the present study was to evaluate the usage of marine algae as medicine.

Methods: In the present study, the marine algae *Gracilaria corticata (G.cortica)* and *Spirulisna platensis (S.plantesis)* collected and shade dried. The dehydrated marine algae material was powdered and extracted with methanol by Soxhelet apparatus. The antibacterial activity of the marine algae was done by using agar cup plate method and MIC method. Lipase inhibitory action of various concentrations of methanolic extract of these marine algae was estimated using olive oil as substrate and there by antiobesity action was determined.

Results: The marine algae extracts of *S.platensis* showed better activity against *Staphylococcus aureus* but the *G. corticata* showed better activity against *Bacillus* compared with the other organisms. Minimal inhibitory concentration was observed at the concentration of 250µl for all micro-organisms.

Conclusion: The marine algae extracts of S. *platensis* showed better inhibition of lipase activity compared with *G. corticata* showed low lipase inhibition activity.

Keywords: Antibacterial, Antiobesity, Pancreatic lipase, Algae.

INTRODUCTION

Algae are the amazing sustainable resources in the marine ecology which have been used as a source of foodstuff and drug. It was estimated that the class of marine plant are algae about 90% and as regards 50% of the total photosynthesis is contributed from algae [1]. Microalgae make an extensive range of chemically active metabolites in their environs, potentially to protect themselves against the other organisms. These dynamic metabolites also identified as biogenic compounds, that are formed by numerous species of marine macro and microalgae and have antibacterial, antiacrofouling and antifungal activities which are efficient in the avoidance of fouling and have other likely uses in therapeutics [2 & 3]. Antimicrobial resistance is the chief crisis with a considerable impact on death, morbidity and healthcare-associated expenses. Immediately researches should be carried out for alternatives to synthetic antibiotics. The evaluation of the discovery of new variety of antimicrobial peptides makes accepted antibiotics as the basic element of making of new drugs for the management of fungal and bacterial infections [4 & 5]. Obesity is the sixth most important public health complications in both developed and developing countries because of a raise in entire fat accumulation. It happens since unilocular adipocytes have hyperplasia or hypertrophy and subsequent macrophage fat tissue infiltration [6]. A huge pool of pancreatic lipase inhibitors are present in natural products and offer possibility for being developed into clinical products. A variety of extracts and secondary metabolites, isolated from microorganism and plants that contain pancreatic lipase inhibitory activity was reviewed by Birari and Bhutani [7]. The present *invitro* investigation was undertaken to investigate the antimicrobial and antiobesity actions of methanol extracts of *Gracilaria corticata* and *Spirulina platensis*.

MATERIALS AND METHODS

Antibacterial testing

Selection of microorganisms

In vitro antibacterial analysis was performed against bacteria for instance *Proteus vulgaris* (MTCC 426), *Escherichia coli* (MTCC 1687), *Bacillus subtilis* (MTCC 8114), *Pseudomonas aeruginosa* (MTCC 4996) and *Staphylococcus aureus* (MTCC 2940). The bacteria were inoculated on a nutrient agar (M001), slant for 24 h at 37 ± 2°C.

Agar cup plate method (ACPM)

The crude methanolic *Gracilaria corticata* and *Spirulina platensis* were analysed for their antibacterial activity by the agar cup plate technique [8].

Minimal inhibitory concentration

Minimum inhibitory concentrations (MIC) were measured by the micro dilution broth technique. The marine algae extracts were dissolved in methanol and successively diluted with Muller-Hinton broth to attain the preferred concentrations. For control Muller-Hinton broth with methanol (4%) and bacteria were used. Sample measuring 25µl of each bacterial suspension were added to the plant extract containing different concentrations of plant extract such as 250µl, 500µl, 750µl and 1000µl and they were incubated under aerobic conditions at 37±2°C. After 24hrs, the turbidity was measured. The MIC can be defined as the lowest antimicrobial concentration of the test samples that inhibits complete bacterial growth.

Antiobesity testing

Anti- lipase action of methanolic extract of *G. corticata* and *S.platensis* were analysed for antiobesity studies.

Freshly slaughtered chicken were selected and pancreas of that chicken were dissected. Collected pancreas was cleaned and stored in 0.01M ice cold sucrose. The pancreas was grind using sucrose (0.01M), centrifuged and supernatant was taken to precipitate with 50% saturated ammonium sulphate. After centrifugation, the pellets were mixed in sucrose and repeated the procedure. Formed pellet was then mixed in phosphate buffer and taken further as enzyme for analysis.

Estimation of chicken pancreatic lipase activity

The activity of pancreatic lipase was examined through incubating a mixture of olive oil (8ml), 0.4ml of phosphate buffer and chicken

pancreatic lipase (1ml) for an hour in rotary shaker. After that, response was terminated by way of adding 1.5ml of a combination comprising acetone and ethanol (95%) in 1:1 ratio. The fatty acids liberated were measured through titration of solution with 0.02M NaOH which is regularized by oxalic acid (0.01M) and phenolphthalein was used by means of an indicator [9].

Lipase inhibitory action of methanol excerpts of *G. corticata* and *S. platensis*

Lipase inhibitory action of various amount of methanol excerpt was analyzed by mingling oil emulsion (8ml), 1ml of chicken pancreatic lipase and 100µl of extract and it was incubated for 60 minutes for the reaction to carry out. The response was terminated by adding 1.5ml of acetone mixture and 95% ethanol with 1:1 ratio. The liberated fatty acids were estimated through titration of solution as mentioned above [10].

$$\text{Inhibition of Lipase} = \frac{M - N}{M} \times 100,$$

Where; M, N are the lipase activity without and with the extract respectively.

RESULTS

Antimicrobial study

Agar cup plate method (ACPM)

The antimicrobial activities of the methanolic extract of *Gracilaria corticata* and *Spirulina platensis* were studied for strains of 5 bacteria. The results were analyzed with that of regular antibiotic Gentamycin, and Tetracycline. The results got for the sensitivity are given in the table 1. *Bacillus* showed 10mm of zone when the marine algae extracts of *Gracilaria corticata* was loaded on the well like that, *S.aures, E.coli*, *Pseudomonas, P.vulgaris* showed the zone of about 9,2,3,1 mm, respectively. *Spirulina platensis is* the zone of inhibition of about 8,10,7,5 and 3mm by *Bacillus subtilis, Staphylococcus aureus, E.coli, Pseudomonas, Proteus vulgaris* respectively (Fig. 1 & 2).

To study the minimum inhibitory concentration, the marine algae extract were treated with the specific microorganism and the results were observed. From that it was observed that 250µl of the marine algae extract was enough to inhibit the microbial growth. The concentration dependant variation was observed in the results (Table 2 & 3).

Table 1: Antimicrobial activity of methanol excerpts from marine algae of *Gracilaria corticata* and *Spirulina platensis*

Test organism	Diameter of zone (mm)		
	Gracilaria corticata	***Spirulina platensis***	**Standard Antibiotic**
E.coli	7±0.15	3±0.11	Gentamycin (13mm)
P.vulgaris	3±0.09	2±0.14	Gentamycin (14mm)
B.subtillis	8±0.17	10±0.13	Terayclin (16mm)
Pseudomonas aeruginosa	5±0.04	2±0.03	Terayclin (14mm)
S.aureus	10±0.12	8±0.14	Terayclin (12mm)

Fig. 1: Antimicrobial activity of methanol excerpts of *S.platensis* with positive control

Fig. 2: Antimicrobial activity of methanol excerpts of *G. Cortica* with positive control Minimal inhibitory concentration

Table 2: Minimum inhibitory concentration of *Spirulina platensis at* 540nm

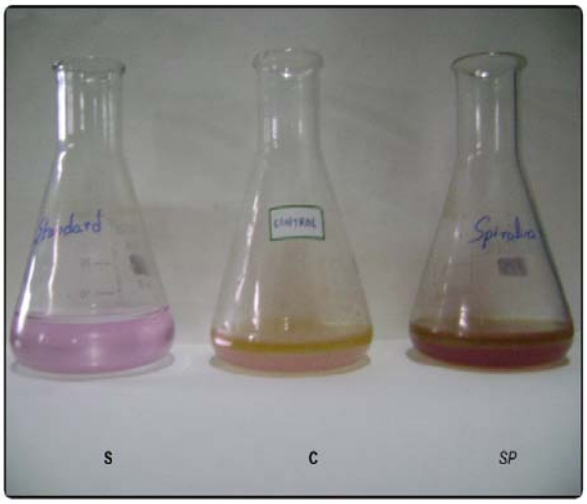
Test Organism	Control	Marine algae extract of various concentration			
		250 µl	500 µl	750 µl	1000 µl
E.coli	2.73	2.58±0.15	2.23±0.12	1.99±0.14	1.87±0.11
P.vulgaris	2.18	2.28±0.02	1.48±0.11	1.36±0.13	1.25±0.09
B.subtillis	2.43	2.28±0.03	2.03±0.08	1.89±0.05	1.68±0.01
Pseudomonas aeruginosa	1.88	1.80±0.04	1.64±0.11	1.53±0.12	1.08±0.12
S.aureus	2.24	2.04±0.08	1.88±0.12	1.67±0.14	1.44±0.11

Table 3: Minimum inhibitory concentration of *Gracilaria corticata* at 540nm

Test Organism	Control	Marine algae extract of various concentration			
		250 µl	500 µl	750 µl	1000 µl
E.coli	1.24	1.08±0.12	0.95±0.13	0.837±0.13	0.72±0.09
P.vulgaris	1.08	0.98±0.12	0.86±0.16	0.66±0.12	0.34±0.08
B.subtillis	1.22	1.06±0.11	0.92±0.18	0.80±0.14	0.69±0.10
Pseudomonas aeruginosa	1.64	1.48±0.12	1.23±0.15	1.08±0.15	0.98±0.10
S.aureus	1.11	0.97±0.14	0.87±0.13	0.78±0.12	0.65±0.07

Table 4: Lipase inhibitory activity of methanol extract of *G.corticata* and *S.platensis*

Concentration	Inhibition of lipase activity of *G.corticata* and *S.platensis*	
	Gracilaria corticata	*Spirulina platensis*
0.1	25±0.12	30±0.18
0.25	25±0.14	32±0.07
0.5	20±0.11	25±0.06
1	30±0.11	35±0.07
25	27±0.13	32±0.16
5	30±0.16	33±0.17
10	35±0.18	38±0.15
15	40±0.17	50±0.18
20	45±0.21	55±0.23



S-Standard, C-Control, SP-*Spirullina platensis*

Fig. 3: Lipase inhibitory activity of methanol excerpt of *S.platensis*

S-Standard, C-Control, GC-*Gracilaria cortica*

Fig. 4: Lipase inhibitory activity of methanol excerpt of *G.corticata*

Antiobesity study

Inhibitory activity of chicken pancreatic lipase for various amounts of methanol extracts of *G. corticata* and *S. platensis* were examined by taking olive oil as the substrate (Table 4). Pancreatic lipase activity was analyzed. It is clear that the lipase activity was changed when treated with methanolic extract. The activity of extracts was on the dose dependant manner. Marked inhibition of enzyme was noticed by raising extract concentration. A noticeable inhibition of enzyme activity was seen having 5mg/ml extract and higher (Fig. 3 & 4).

DISCUSSION

Most of the compounds of marine algae show anti-bacterial activities [11], used as direct and indirect human food sources [12 & 13] and used also in new pharmaceutical industries [14, 15 & 16] and recently showed antimicrobial activities [17, 18, 19 & 20]

The Antibacterial function of the marine algae *G. edulis* connected epiphytic bacteria against human bacterial pathogens from Indian waters and as well from west coast of India [21 & 22]. Marine algae have been known as vital sources of antibiotic substances. The production of antimicrobial activities was measured to be a sign of the marine algae to produce bioactive secondary metabolites [23, 24 & 25].

Antibacterial activity has been proposed in a number of marine algae which are collected from the coast of Mandapam to Kanyakumari. The maximum antibacterial activity was reported in the class *Rhodophyceae* (80%) followed by the *Chlorophyceae* (62.5%) and the *Phaeophyceae* (61.9%) [26].

The antibacterial screening of chloroform hexane and alcoholic leaves extracts of *Finlaysonia obovata* was conceded out for fresh water fish pathogenic bacteria viz*, Aermonas hytrophila*, *Vibrio alginolyticus, Escherichia coli,Staphylococcus aureus, Pseudomonas aeruginosa*, *Edwadsiella tarda* and *Micrococcus Sp*. by disc-assay technique [27]. Extracts of marine algae and sponge were analysed for various bacterial pathogens by well-cut agar diffusion method. The brown algae *Cytosoria compressa* had broad spectrum antimicrobial effect against different bacterial pathogens [28].

The antibacterial activities of four vital marine algae specifically *Ulva lactuca*, *Sargassum wightii, Padina gymnospora* and *Gracilaria edulis* were examined for the human bacterial pathogens *Vibrio cholerae Staphylococcus aureus, Salmonella paratyphi, Shigella dysentriae, P. seudomonas aeruginosa, Shigella bodii,* and *Klebsiella pneumonia.* The greatest activity (8.8 mm) was noted in *G. edulis* compared to *S. aureus* and minimum by *U. lactuca* (1.2 mm) compared to *P. aeruginosa*. The 1H-NMR analysis exposed the signals present concerning with poly unsaturated esters in *Gracilaria edulis, Sargassum wightii* and poly saturated alcohols in *Padina gymnospora* [29].

To date, in spite of the availability of numerous reviews are outstanding for anti-obesity agents in the literature, there is no reviews regarding summarizing actual, natural-product information on anti-obesity action, dynamic compound varieties, and way of action. In 2000, the The use of some renowned medicinal marine algae that had claimed to be helpful in treating obesity was reported by Moro and Basile [30].

The pancreatic lipase inhibitory action of 54 marine algae was reported and lipase inhibitory activity in their methanol or ethyl acetate extracts was showed [31]. Various amount of different extracts of *Gracilaria* sps and *Spirulina* sps were examined for their medicinal property and spirulina was reported to have antiarthritic activity [32,33].

CONCLUSION

Microbicidal activities observed in the crude methonolic extracts of *gracilaria sps* from the southwest coast of India provide good evidence that algae maintain effective antimicrobial chemical resistance, and this antibacterial property is due to the presence of active bio molecules. From the present study, it can be concluded that the red alga *Gracilaria corticata* is a potential source of bioactive compounds. These compounds maybe utilized for the development of natural antibiotic against multidrug resistant bacteria. The results of the antiobesity study again have revealed that medicinal marine algae still play vital role in the primary healthcare of the people.

Further ethanopharmacological and phytochemical of these algae may be investigated to explore possible agents in the marine algae.

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