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PHYTOCHEMICAL CONSTITUENTS AND BIOACTIVITY OF EXTRACT OBTAINED FROM ALGAE *GELIDIUM SPPS*

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ABSTRACT The *Gelidium acerosa* contain large number of valuable phytochemicals like sapononinis, flavinoids and alkaloids etc., which are known for its medicinal uses. The preparations of the seaweeds are also useful for the common ailments, includes dysentery, hypertension, urinary tract infection, and some other microbial infections among people. The extracts of seaweeds are prepared with methanol and tested against nine different test microbial strains of microorganisms namely; *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas* sp, *Streptococcus pyrogenes*, *Staphylococcus aurius*, *Proteus vulgaris*, *Klebsiella pneumonia*, *Serrratia marganii* and one fungal strain *Candida albicans* using paper disc plate method. Antioxidant assay was carried out for methanolic extract of the macroalgae using DPPH test, where ascorbic acid was used as standard. Results revealed the presence of Tannins, Cardiac glycosides, Terpenoides, Flavonoids, Phenols, Anthraquinones, Alkaloids. The methanol extracts had wide range of antibacterial activity against bacterial pathogens. This would lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial and antioxidant drugs of natural origin.

KEYWORDS : Phytochemicals, Gelidium spps, antioxidant activity, antimicrobial activity

INTRODUCTION

Seaweeds have been one of the richest and most promising sources of bioactive primary and secondary metabolites. These compounds have diverse simultaneous functions for the seaweeds and can act as antimicrobial, allelopathic, antifouling, and herbivore deterrents, or as ultraviolet- screening agents. They are also used by the pharmaceutical industry in drug development to treat diseases like cancer, acquired immune-deficiency syndrome (AIDS), infection from virus, bacteria and fungus, inflammation, pain, arthritis etc. Currently, algae represent about 9% of biomedical compounds obtained from the sea (Jirge *et al.,* 2011). Seaweeds are extensive and differing gatherings of that are wealthy in dynamic metabolites and a wellspring of novel elements for useful functional foods. Wholesome examinations on ocean growth demonstrate that darker, green and red Seaweeds have great nutritious quality and could be utilized as an elective wellspring of dietary fiber, protein, and minerals (Fitton, 2006). Also, seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites, characterized by a wide range of biological activities such as antimicrobial (Newman *et al.,* 2002; Stirk *et al.,* 2007; Alghazeer *et al.,* 20013), anti-inflammatory (Lindequist and Schweder, 2001), anti-viral; as well as anti-tumoral activities (Zandi *et al.,* 2010; Ale *et al.,* 2011). Moreover, many studies show that some algal extracts display substantial antioxidant activities (Zubia *et al.,* 2009; Lekameera *et al.,* 2008; Cox *et al.,* 2010 and Keyrouz, *et al.,* 2011).

Gelidium acerosa is the genus of red algae with a high economic value, found in subtidal area in many parts of Tamilnadu, so far Gelidium acerosa is known for its production of high-grade agar in food industries, and pharmaceutical industries. Recently secondary metabolites known as phytochemicals have been extensively investigated as a source of medicinal agents (Abet, 1980). These phytochemicals play an important role in antioxidant and antimicrobial activity and used as a treatment for many microbial infection (Okigbo and Omodamiro, 2006; Grouch et al., 1992). *Gelidium* is a polymorphic genus consisting mainly of highly branched algae with morphological differences in species ranging from terete (cylindrical) to compressed branches. Species of *Gelidium* can vary a lot in color within and between species, from red to purple to green-black.

MATERIALS AND METHODS

Sample collection:

Macroalgae *Gelidium spps*, *was* collected from the intertidal region of Ratnagiri located along the west coast of India. After the collection seaweeds were brought to the laboratory and washed thoroughly 3-4 times with fresh water. The samples were finally kept for drying. After drying they were put into small plastic bags, labeled properly and stored at -20^{0}C for further use.

Extraction:

In the laboratory collected algae was shed dried and powdered. About 250 g of algal powder was weighed and kept in a beaker. About one liter of methanol was added to it. The beaker was covered tightly and kept for three days. After three days it was filtered and extract was collected. Extract was concentrated using a rotary evaporator.

Microbial cultures

The standard pathogenic bacterial cultures were procured from Department of microbiology, Bhavan's College, Andheri, Mumbai, and used in the present study (Table 1). The bacterial cultures were rejuvenated in Mueller- Hinton broth (Hi-media laboratories, Mumbai, India) at 37^{0}C for 18h and then stocked at 40C in Mueller-Hinton Agar. The inoculum size of the bacterial culture was standardized according to the National committee for Clinical Laboratory Standards (NCCLS, 2002) guideline. The pathogenic bacterial culture was inoculated into sterile Nutrient broth and incubated at 37^{0}C for 3h until the culture attained a turbidity of 0.5 McFarland units. The fungal culture rejuvenated in Sabouraud dextrose media (Hi-media laboratories, Mumbai, India) at 37^{0}C for 18h and then stocked at 4^{0}C in SDA. Subcultures were prepared from the stock for bioassay. A loopful of culture was inoculated in 10 ml of sterile Potato Dextrose broth and incubated at 37^{0}C for 24h. Turbidity of the culture was standardized to 10^5 CFU with the help of SPC and turbidometer.

Table 1: Microbial cultures used in study

Microbial Pathogens
Bacillus subtilis
Escherichia coli
Pseudomonas sp.
Streptococcus pyrogenes
Proteus vulgaris
Klebsiella pneumonia
Serrratia marganii
Candida albicans

Phytochemical screening: Preliminary qualitative phytochemical screening was carried out with the following methods (Khandelwal, 2001).

Test for Tannins: To 0.5 ml of extract solution, 1 ml of distilled water and 1 to 2 drops of ferric chloride solution was added, observed for blue or green black coloration.

Test for Saponins: Two ml of distilled water was added to 2 ml of the test solution shaken well and observed for frothing.

Test for Flavonoids: A volume of 1.5 ml of 50 % methanol was added to 4 ml of the extracts. The solution and magnesium metal were added and warmed. Then, 5 to 6 drops of concentrated hydrochloric acid were added to the solution and observed for red coloration.

Test for Steroids (Salkwoski's test): Five drops of concentrated sulphuric acid (H2SO4) was added to 2 ml of each extract and observed for red coloration.

Test for Glycosides: To 4 ml of extract solution and add few drops of glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid and observed for a reddish-brown coloration at the

junction of 2 layers and bluish green color in upper layer.

Test for Alkaloids: To 4 ml of extract filtrate, a drop of Mayer's reagent was added along the sides of test tube. Creamy yellow or white precipitate indicates that the test is positive.

Test for Anthraquinones: One gram of powdered plant material was taken and extracted with 10 ml of hot water for five minutes and filtered. Filtrate was extracted with 10 ml of CCl4 then CCl4 layer was taken off. Five ml water and 5 ml dilute ammonia solution was added. No free anthraquinones were revealed as absence of appearance of pink to cherry red color. One gram of second sample of the same plant material was extracted with 10 ml of ferric chloride solution and 5 ml of hydrochloric acid then it was heated on water bath for 10 minutes and filtered. Filtrate was cooled and treated as mentioned above.

Test for phenolic compounds: Two ml of extract was diluted to 5 ml with distilled water. To this a few drops of neutral 5 % ferric chloride solution was added. A dark green color indicates the presence of phenolic compounds

Bioactivity of Extract:

Evaluation of antioxidant properties

Several methods have been developed to measure the antioxidant capacity of pure compounds and plant extracts. One of the most frequently used techniques for measuring antiradical capacity is depletion of the free radicals 2,2-diphenyl-1-picrylhydrazyl (DPPH) by addition of scavenger compounds. It determines the ability of crude extracts for trapping this unpaired-electron to disappearance of radical color. This will lead to the formation of less reactive species derived from the antioxidant (Brand-Williams *et al.,* 1995). There are many assays which have been used to assess the total antioxidant content of foods, e.g. the ferric-reducing ability of plasma (FRAP). The FRAP assay directly measures antioxidants with a reduction potential below the reduction potential of the Fe3+/Fe2+ couple (Halvorsen *et al.,* 2002). Therefore, the antioxidant properties of extracts were determined using two methods, DPPH. These methods seem to be rapid and accurate methods for assessing the antioxidant activity of Pomegranate seed extracts.

Antioxidant activity

DPPH Assay

The percentage of antioxidant activity of the aqueous peel was assessed by DPPH free radical assay. The samples were reacted with the stable DPPH radical in a methanol solution. The reaction mixture consisted of 3.7mL of absolute methanol in all test tubes along with blank. The blank tube was added with 100µL of absolute methanol and 100µL of respective samples to all other tubes marked as tests27. Finally, 200µL of DPPH reagent were added to all the test tubes including blank. The test tubes were incubated in dark condition for 30minutes to the reaction to take place. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in color (from deep violet to light yellow) were read (absorbance) at 517nm. The scavenging activity percentage was determined according;

% Antioxidant activity= {(absorbance at blank) -(absorbance at test)/absorbance at blank)} X100

Antimicrobial Assay:

The Kerby Bauer or paper disc plate method is the most commonly used technique for determining susceptibility of different microorganisms. Small paper discs inoculated with bioactive agents were placed upon the surface of plate and incubated for 12 hours, the plates were then observed for any zones of inhibition surrounding the discs. A zone of inhibition around the disc indicated that the growth of organism was hindered by the compound diffusing in the agar plate. An organism was considered sensitive to a drug only if diameter of inhibition zone exceeded 17 mm. The Kerby Bauer Test (Paper disc plate method) was used to check the anti-microbial activity in the present investigation.

Nine different test microbial strains (3 gram-positive and 3 gram-negative) of microorganisms namely; *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas* sp, *Streptococcus pyrogenes*, *Staphylococcus aurius*, *Proteus vulgaris*, *Klebsiella pneumonia*, *Serrratia marganii* and one fungal strain *Candida albicans* were used for the assay. Discs of 5 mm were made by punching the Whattman filter paper no. 1. These discs were sterilized by autoclaving at 121°C, 15 PSI for 20 minutes and then left to cool. Nutrient Broth was autoclaved, dispensed in test tubes and inoculated with loop full of organism to obtain an 18hr old culture. Nutrient Agar was autoclaved, poured in sterile petri plates and allowed to solidify. 0.1 ml of 18hr old bacterial culture was spread on it under sterile conditions (3 replicates of each bacterium). Sterilized filter paper discs (5 mm diameter) loaded with 10µl of seaweed extract (10 mg/100µl). One disc loaded with 10µl (0.01 mg/ml) of Gentamycin (positive control) and another with 10µl of sterilized DW / methanol (negative control) were also placed on the nutrient agar. Discs were picked up by forceps, and allowed to air dry. Discs then placed on the inoculated nutrient agar plates. These plates were incubated at room temperature for 24 hours and were observed for the zone of inhibition.

RESULTS AND DISCUSSION

Phytochemicals are increasing acceptor as health promoting and maintaining and repairing agents in cells, tissues or the whole human body. The phytochemicals that are frequently associated with human health are carotenoids, polyphenols and tocopherols.

In the present investigation methanol extracts of *Gelidium acerosa* are used for the phytochemicals analysis was summarized in the above Table1. Methanol extracts shows the minimum Presence of compounds. Preliminary phytochemical analysis revels the presence of Cardiac glycosides, Flavonoids and alkaloids in abundant quantity. Phytochemicals play a vital role against number of diseases such as asthma, arthritis, cancer etc. unlike pharmaceutical chemical these phytochemicals do not have any side effects.

Table 1: Phytochemicals analysis of Gelidium acerosa methanol extract

Sr. No.	Phytochemical constituents	Quantitively results
1	Tannins	++
2	Saponins	-
3	Cardiac glycosides	+++
4	Terpenoides	++
5	Flavonoids	+++
6	Phenols	+
7	Anthraquinones	++
8	Alkaloids	+++

(- absent, + traces, ++ moderate, +++ abundance)

Antioxidant Assay

The DPPH· scavenging activity has been commonly used to detect antioxidant activity of different samples sources, due to its sensitivity to lower concentrations of active standards from natural sources. The steady radical, DPPH, has a maximum absorbance at 517nm and could swiftly undergo scavenging by antioxidants. Complex free radical scavenging activities of samples are indicated by lower absorbance at 517nm. It was found that the highest concentration of aqueous extract at around 500µL had the highest percentage of antioxidant activity Antioxidant assay was carried out for methanolic extract of the macroalgae. Ascorbic acid was used as standard. In the DPPH tests the extract was able to reduce the stable radical DPPH to the yellow colored diphenylpicrylhydrazine. Six concentrations were tested. Those were 50, 100, 200, 300, 400 and 500µg/ml. The results are given in following table.

Table 2. Antioxidant activity of methanolic extract of Gelidium Sp.

Concentrations	Percentage radical scavenging activity
50 µg/ml	12%
100 µg/ml	27%
200 µg/ml	32%
300 µg/ml	41%
400 µg/ml	54%
500 µg/ml	72%
Standard	98%

Antimicrobial activity

The antibacterial activity has been attributed to the presence of some active constituents in the extracts. Most plants are able to produce a diverse range of bioactive molecules which become a rich source of different types of medicines. An interesting feature of plants in the present is focused on phytochemical compounds as potential sources of functional substances such as antioxidant and antimicrobial substances (Dahikar, 2018). As far as antimicrobial activity is concerned, this extract was effective against *Bacillus subtilis, Streptococcus pyrogenes, Staphylococcus aurius and Candida albicans*

Table 3. Antimicrobial activity of methanolic extract of Gelidium sp.

Concentrations	Zone of inhibition (in mm)
Bacillus subtilis	10
Escherichia coli	7
Pseudomonas sp.	-
Streptococcus pyrogenes	11
Proteus vulgaris	-
Klebsiella pneumonia	-
Serrratia marganii	8
Candida albicans	13

CONCLUSION: *the extract obtained from Gelidium species of macroalgae, collected from intetidal region was found to have* Cardiac glycosides, Flavonoids and alkaloids in abundant quantity. Interestingly this extract showed Antioxidant activity and also antimicrobial activity against broad range of microbes.

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