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Original Article

Phytochemical Screening And Antioxidant Activity Of Marine Alga, Gelidiella Acerosa

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Abstract:
Gelidiella acerosa (Forsskal) is a genus of red algae with high economic value found in sub tidal areas in many parts of India and other countries. The phytochemicals and antioxidant studies were carried out using DPPH and ABTS radical scavenging assay. Preliminary phytochemical screening of *Gelidiella acerosa* revealed the presence of various bioactive components like alkaloids, flavanoids, steroids, glycosides, phenols, saponins, terpenoids, resins, carbohydrates and tannins. All the three extracts of *Gelidiella acerosa* were also evaluated for the in-vitro DPPH free radical scavenging activity and the hexane, ethyl acetate and methanolic extract of the *Gelidiella acerosa* were found to exhibit 32.77%, 50.71% and 68.42% free radical scavenging activity as compared to the highest percentage free radical scavenging activity of ascorbic acid (standard) 76.55%. The result of the present study concluded that antioxidant activity due to the presence of significant amount of phenolic compounds which are the major contributors of antioxidant activity. The finding of this study suggests that the studied plant is a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing or slowing the progress of oxidative stress related degenerative diseases.

Introduction-

seaweeds are a collection of plant kingdom referred as algae. Seaweeds, also known as marine alga, are categorized as Rhodophyta (red algae), Phaeophyta (brown algae) or Chlorophyta (green algae) depending on their nutrient, pigments and chemical composition. Almost 841 species of marine algae are found in both inter-tidal and deep water regions of the Indian coast[1] . They contain innumerable inorganic and organic substances valuable for human health[2] . Recent reports have publicized that the seaweeds have been conventionally used for the treatment of many diseases. Seaweeds are measured as a source of bioactive compounds and a prodigious variety of secondary metabolites categorized by a broad spectrum of biological and pharmacological activities[3] . Green and brown algae have been classified with the compounds having antifungal and antimicrobial activities[4] .Seaweeds are of great interest in the biomedical area, mainly due to their contents of bioactive substrate which show great potential as antimicrobial, anti-inflammatory, antiviral[5] and anti-tumoral drugs[6,7] . The anti-cancerous, anti-obesity and anti-proliferative effects have been shown by the in vivo studies from the components of seaweeds[8, 9] . Many substances obtained from marine algae such as alginate, carrageenan and agar as phycocolliods have been used for decades as pharmaceutical products[10] . Gelidiella acerosa (Forsskal) is a genus of red algae with high economic value found in sub tidal areas in many parts of India and other countries.

MATERIALS AND METHODS:

The red marine alga *Gelidiella acerosa* was harvested in August from Erwadi coast at Gulf of Mannar, Ramanathapuram district of Tamil nadu during 2019. Adhered sand and salts were removed from the algae by washing with seawater. The formal authentication and identification was done by Dr. K. Eswaran, The Principal Scientist, CSIR- CSMCRI, Marine Algal Research Station, Mandapam camp.

Extraction of plant material:

The plants of *Gelidiella acerosa* were dried in shade and powdered. Dried algal powder (550 g) was extracted with n- Hexane, three times each by cold maceration at room temperature. In cold maceration whole powdered plant drug is kept in contact with the solvent in a stoppered container for 48-72 hours with frequent agitation until soluble matter is dissolved. This method is best suitable for use in case of the thermo labile drugs. For extraction, the powder was taken in round bottom flask and macerated for 72-48 hours at room temperature with all material defatted. The algae was extracted with three solvents with increasing polarity in order of n-hexane, ethyl acetate and methanol respectively at the room temperature three times each. The extracts thus obtained were concentrated by distilling off the solvent under reduced pressure by using Rota vacuum Evaporator. The defatted marc thus obtained was air dried and then the percentage yields of all three extracts i.e. n-hexane, ethyl acetate and methanol of *Gelidiella acerosa* was calculated.

IDENTIFICATION OF PHYTOCONSTITUENTS BY CHEMICAL TESTS

The individual extracts were subjected to qualitative chemical investigations for the identification of the phytoconstituents such as sterols, flavonoids, triterpenes, alkaloids, glycosides, tannins, proteins, carbohydrates. The Preliminary Phytochemical tests were performed for each extract.

DETERMINATION OF DPPH RADICAL SCAVENGING ACTIVITY

Principle:

To determine the antioxidant activity the stable 2, 2- diphenyl-1-picrylhydrazyl (DPPH) radical is extensively used. The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in colour. The colour turns from purple to yellow as the molar absorptive of DPPH radical at 517 nm reduces as the odd electron of DPPH radical undergoes pairing with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolourization is absorbance produced at 517 nm has been used as a measure of radical scavenging activity.

Preparation of Reagents

DPPH Solution (100 mM)

DPPH solution was prepared by dissolving 33 mg of DPPH in 100 ml of methanol. From this stock solution, 10 ml was taken and diluted to 100 ml using methanol to obtain 100 mM DPPH solution and kept in a test tube covered with the aluminium foil to protect from sunlight.

Ascorbic Acid Standard Solution

100 µg /ml stock solution was prepared by dissolving 10 mg of ascorbic acid in 100 ml of distilled water, from this 10, 20, 40, 60, 80, 100 µg /ml of ascorbic acid solution was prepared.

Preparation of test solutions

A stock solution of concentration 1 mg/ml was prepared by adding 10 mg of n- Hexane, ethyl acetate and methanol extracts in 10 ml methanol and solutions of various concentrations of different extracts such as 50, 100, 200, 400, 800, 1000 µg /ml were prepared.

Procedure

100 µl of various concentrations (50-1000 µg /ml) of different extracts and 100 µl solution of DPPH (100 mM in methanol) was incubated at 37^0 C for 30 minutes and change in absorbance of reaction mixture was read at 517 nm. An equal amount of methanol and DPPH was served as control. The experiment was performed in triplicate and percentage radical scavenging activity was calculated by formula given below: % of Inhibition = (A of control – A of Test)A of control×100

Statistical Analysis: The experiments were conducted in triplicates and data were expressed as mean ±SD. It was analyzed using mega stat model 23.

RESULTS AND DISCUSSION:

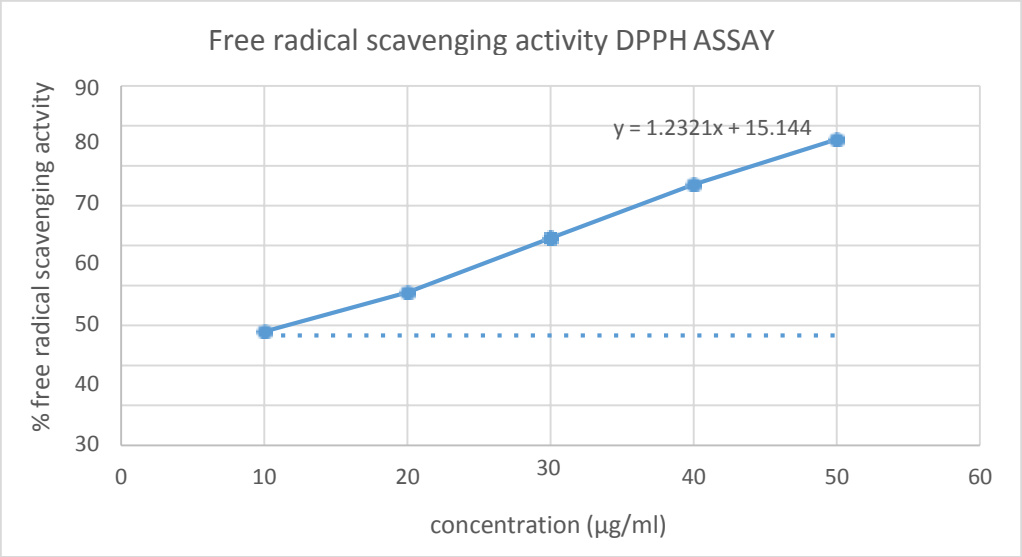
Preliminary phytochemical qualitative analysis of different extracts of *Gelidiella acerosa* indicated the presence of alkaloids, saponins, flavonoids, tannins, phenol compounds in the extract

Table 1 : Phytochemical screening of the extracts of *Gelidiella acerosa*.

S. no.	PHYTOCONSTITUENTS PRESENT	Hexane extract of *Gelidiellaacerosa*	Ethyl acetate extract of *Gelidiellaacerosa*	Methanol extract of *Gelidiellaacerosa*
1	Alkaloids	(++)	(++)	(++)
2	Cardiac glycosides	(+)	(++)	(+)
3	Tannins	(+)	(+)	(++)
5	Reducing Sugars	(--)	(--)	(+)
6	Anthraquinones	(--)	(--)	(+)
7	Steroids	(--)	(--)	(+)
8	Proteins	(--)	(+)	(+)
9	Flavonoids	(+)	(+)	(++)
10	Saponins	(+)	(+)	(+)
11	Phenols	(+)	(+)	(++)

DPPH Free radical scavenging activity

All the extracts produced significant DPPH radical scavenging activity. Antioxidant activity of *Gelidiella acerosa* was found to be increase with increasing concentration of hexane, ethyl acetate and methanol extracts. DPPH antioxidant assay is based on the ability of DPPH, a stable free radical to decolorize in the presence of antioxidant. The antioxidant activity of *Gelidiella acerosa* was compared with standard (ascorbic acid). The obtained results (Shown in fig: 10) indicated that methanol extract has better antioxidant activity than the other two extracts.



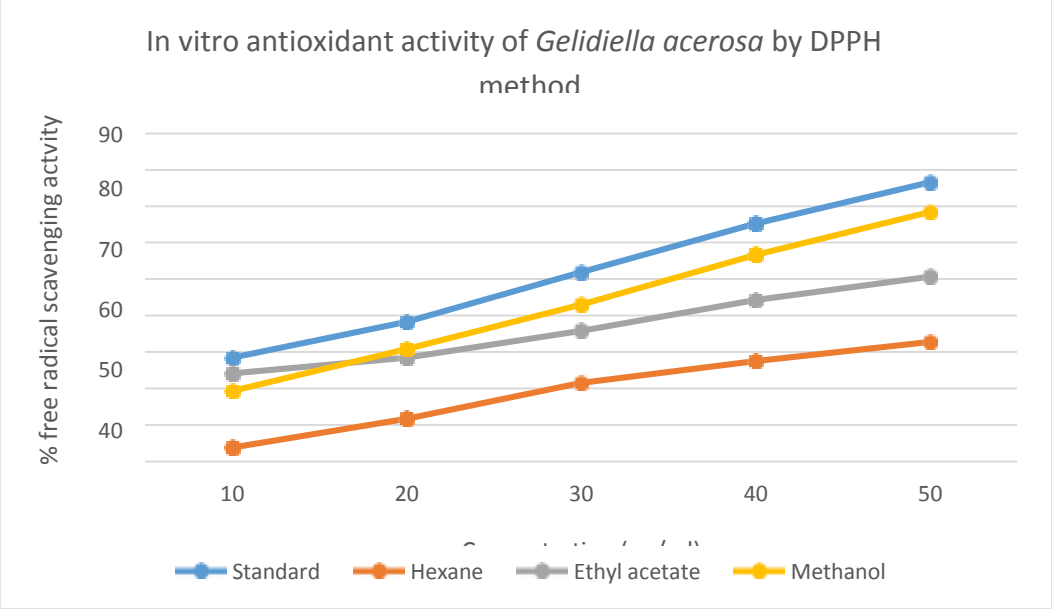
Percentage free radical-scavenging capacity DPPH Assay

DPPH percentage free radical-scavenging capacity for Ascorbic acid

Concentration(µg/ml)	Absorbanceof Standard(As)	Absorbanceof control(Ac)	(Ac-As)/Ac	{(Ac-As)/Ac}*100
10	0.299	0.418	0.284689	28.46889952
20	0.258	0.418	0.3827751	38.27751196
30	0.201	0.418	0.5191388	51.9138756
40	0.145	0.418	0.65311	65.31100478
50	0.098	0.418	0.7655502	76.55502392

DPPH %age free radical-scavenging capacity for standard & extracts of *Gelidiella acerosa*

Concentration(µg/ml)	Ascorbic acid	Hexane extract of *Gelidiellaacerosa*	Ethyl acetateextract of *Gelidiella acerosa*	Methanolextract of *Gelidiellaacerosa*
10	28.46889952	3.8277512	24.16267943	19.37799
20	38.27751196	11.722488	28.46889952	30.86124
30	51.9138756	21.5311	35.88516746	43.0622
40	65.31100478	27.511962	44.25837321	56.69856
50	76.55502392	32.77512	50.71770335	68.42105



In vitro antioxidant activity of *Gelidiella acerosa* by DPPH method

CONCLUSIONS

The phytochemicals present in the extracts like alkaloids, glycosides, phenolic compounds, flavonoids and tannins. The methanolic extract of *Gelidiella acerosa* can be concluded to possess highest amounts of phenolics, flavonoids and DPPH free radical scavenging activities from the present studies. It also possess the highest anti- inflammatory potential followed by the ethyl acetate and hexane respectively. Therefore the antioxidant and anti-inflammatory activity of the extracts of *Gelidiellaacerosa* can be attributed to its phytochemical compounds present in the extracts.

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