Sea Weed Gel and Its Antimicrobial Effect against Bacteria

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***Abstract*: Now-a-days, medicinal agents involve the use of natural sources which helps in the extraction and use of secondary metabolites. Sea weeds pave way in this regard which are potential renewable sources that constitutes phytochemicals in them and conducts various activities using their extracts in order to identify the ability of seaweeds to be used as a medicinal agent. In this study, the sea weed gel was extracted using solvents and identified for the presence of phytochemicals, Antioxidant activity was performed to compare the efficiency of the available antioxidants in various solvents. Also, the ethanol extract of sea weed was subjected to antibacterial activity which showed a better inhibition rate than the other solvents against bacterial species such as Vibrio cholerae, Staphylococcus aureus and Staphylococcus flexnerii.**

***Index Terms:* Sea weed gel, Phytochemicals, Antioxidant activity, Antibacterial activity.**

I. INTRODUCTION

Seaweeds are primitive non-flowering plants without true root stem or leaves. They are one of the commercially important marine renewable sources. Seaweeds have been used as food stuff in Asia for centuries as it contains carotenoids, dietary fibres, proteins, essential fatty acids, vitamins and minerals. Seaweeds are of immense interest since they have a broad range of biological activities such as antibacterial, antifungal, antiviral, antitumour, anti-inflammatory and antioxidant activities.

The Seaweeds have been cultivated for the pharmaceutical industry as a fresh source of bioactive compounds with immense medicinal potential. Seaweeds are rich in antioxidants such as carotenoids, pigments, polyphenols, enzymes and diverse functional polysaccharides. Seaweeds are excellent source of vitamins, proteins and lipids and also they are more consumable compared to other vegetables mainly due to their high essential amino acid content and relatively high level of unsaturated fatty acids.

The seaweeds contain large amount of polysaccharides in their cell wall. A large number of these algae contain compounds such as carrageenans; alginates and agar which are widely used by food and cosmetic industries. Presence of metabolites such as fatty acids, steroids, carotenoids, lectin, and mycosporine like amino acids, halogenated compounds polypeptides and toxins as well as other sulphated polysaccharides make these organisms economically important. Seaweeds are the excellent source of bioactive compounds such as carotenoids, dietary fibres, protein and exhibit essential total antioxidant activity. [6]

Seaweeds have been recognized as potential sources of antibiotic compounds. The production of antimicrobial activities was considered to be an indicator of the bioactive secondary metabolites. Earlier research was also depicts the important functional activities of marine seaweeds, such as antioxidant, anti-mutagen and anticoagulant effect, antitumor activity, and an important role in the modification of lipid metabolism in the human body.

The chemical composition of seaweeds varies with species, habitat, maturity and environmental conditions. The extract thus obtained, after standardization, may be used as medicinal agent as a such in the form of tinctures or fluid extracts or further processed to be incorporated in any dosage form such as tablets and capsules. These products contain complex mixture of many medicinal plant metabolites, such as alkaloids, glycosides, terpenoids, flavonoids and lignans

Phytoactives and Antioxidants

The medicinal plants are useful for healing as well as for treating of human diseases because of the presence of phytochemical constituents. Phytochemicals are naturally occurring in medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases. Phytochemicals are primary and secondary compounds. Reactive oxygen species (ROS), such as superoxide anions, hydrogen peroxide, and hydroxyl, nitric oxide and peroxynitrite radicals, play an important role in oxidative stress related to the pathogenesis of various important diseases . In healthy individuals, the production of free radicals is balanced by antioxidative defense system.[1].

II. .MATERIALS AND METHODOLOGE

Collection of sample

The sea weed gel extract was procured from Research and Development wing of Genewin Biotech, Hosur, Tamilnadu.

Extraction

The sea weed gel was extracted using water, ethanol and acetone solvents in the ratio of 3:1 and refrigerated until further use.

Qualitative Phytochemical Analysis

Qualitative phytochemical analysis was fowolled by[4].

Test for Alkaloids

Wagner's test

1 ml of extract was added in 2 ml of Wagner's reagent (iodine in potassium iodide) which leads to formation of reddish brown precipitated and indicated the presence of alkaloids.

Test for Saponins

A small quantity of alcoholic and aqueous was extracted separately and added 20 ml of distilled water and shake in a graduated cylinder for 15 minutes lengthwise. Layer of foam indicated the presence of Saponins.

Test for Carbohydrates

Molisch's test

2ml of extract was added in 1ml of a-napthol solution, and then added concentrated sulphuric acid through the side of the test tube. Purple or reddish violet color at the junction of the two liquids reveals the presence of carbohydrates.

Fehling's test

1ml of extract was added in equal quantities of Fehling solution A and B, upon heating formation of a brick red precipitated indicates the presence of sugars.

Benedict's test

5ml of Benedict's reagent was added in 1ml of extracted solution and boiled for 2 minutes and cooled. It leads to formation of red precipitated shows the presence of sugars.

Test for Tannins

i) 1ml of extract was added in ferric chloride solution, formation of dark blue or greenish black color product shows the presence of tannins.

ii) ii) A little quantity of extracted was treated with potassium ferric cyanide and ammonia solution. A deep red color was indicates the presence of tannins.

iii) Strong potassium dichromate solution, was added to extract and a yellow color precipitated indicates the presence of tannins and Phenolic compounds.

Test for Flavonoids

Shinoda's Test

i) The extracted was treated with sodium hydroxide; formation of yellow color indicated the presence of flavones.

ii) The extracted treated with concentrated H_2SO_4, formation of yellow or orange color indicated flavones.

Test for Fats and Oils

i) Little amount of drug sample was placed on the filter paper and allowed it to stand for 15 minutes. A greasy spot is observed due to the presence of fats.

Qualitative Analysis

Determination of Protein

The dried and powdered samples were extracted by stirring with 50 ml of 50% methanol (1:5 w/v) at 25 °C for 24 h and centrifuged at 7,000 rpm for 10 minutes. 0.2 ml of extract was pipetted out and the volume was made to 1.0 ml with distilled water. 5.0 ml of alkaline copper reagent was added to all the tubes and allowed to stand for 10 minutes. Then 0.5 ml of **Folin's Ciocalteau reagent** was added and it was incubated in dark for 30 minutes. The intensity of the colour developed was read at 660 nm[3].

Total Antioxidant Capacity Determination

The total antioxidant capacity of the extracted is determined with phosphomolybdenum using Ascorbic acid was standard. An aliquot of 0.2 ml of the extracted is combined with 2.0 ml of the reagent (0.6 M sulphuric acid, 28.0 mM sodium phosphate and 4.0 mM ammonium molybdate). The blank solution is made by mixing 2.0 ml of the reagent solution with the appropriate volume of the same solvent used to dissolve the sample. The tubes are capped and incubated in water bath at 95 °C for a period of 90 minutes. The sample and blank are allowed to stand for half an hour to cool down to room temperature. The absorbance of samples is measured spectrophotometrically at 695 nm. The total antioxidant activity presents in plant extracted can be determined by plotting a graph using ascorbic acid was standard with Y = Absorbance at 695 nm and X = Concentration[7].

Antimicrobial and Antibacterial Activity

Pure bacterial cultures such as S*taphylococcus flexnerii, Staphylococcus aureus, Vibrio cholera,* were collected from Genewin Biotech, Hosur. Bacterial strains to be tested were streaked in nutrient agar plates to obtain pure culture. The pure cultures were streaked on Luria agar slant and stored at 4°C.
The agar well diffusion method was used to determine growth inhibition. Sterile Muller Hinton agar plates were prepared. Three wells of 6mm diameter were prepared with the help of a sterile well puncher. The 6 hour culture broth was taken and swabbed over the plate using sterile cotton swab to obtain a uniform lawn. Diameters of the inhibition zones were measured and tabulated. The ethanol extract was subjected to microbiological screening using different concentrations like 20 µl, 30 µl, 40 µl, 50 µl employing disc diffusion method, Diameter was measured (mm) & recorded.

IV. Results and Discussion

Phytochemical analysis was carried out using the selected plant parts such as leaves, fruits are as follows and seeds using water and acetone solvents and the results

Table 1: (Phytochemical analysis)

TEST	Leaves – Water	Leaves – Acetone	Leaves – Ethanol
SAPONINS	+	+	-
FATS AND OILS	-	+	+
ALKALOIDS Wagner's Test	 +	 +	 +
CARBOHYDRATES Fehling's Test Benedict's Test Molisch's Test	 - - -	 - - -	 - - -
TANNINS Lead acetate Ferric chloride	 - -	 - -	 - -
FLAVONOIDS Shinoda's Test NaOH H_2SO_4	 + +	 - +	 - -

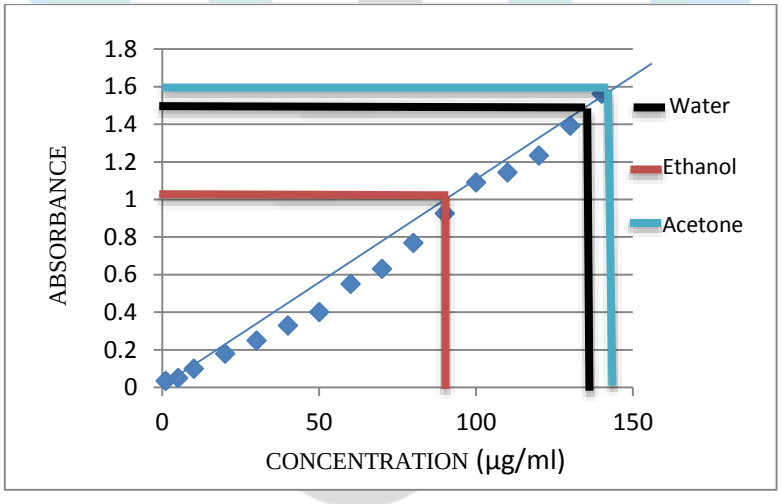
It can be suggested that alkaloids were presented in all the samples extracted in leaves followed by Saponins present in the water and acetone extracts except ethanol extracts. Negative results were also recorded for the presence of carbohydrates

Tannins were observed to be nil in all the samples. Presence of flavonoids was observed in the water extract and acetone extract nil in the ethanol extract. Fats and oils were found in all the samples.

Determination of Antioxidant Activity content

Table 2(Total Antioxidant activity)

Concentration (µg/ml)	Absorbance of standard (Ascorbic acid) (nm)	Total Antioxidant Activity (µg/ml)		
		water	Acetone	Ethanol
1	0.034	142	148	98
5	0.050			
10	0.1011			
20	0.1810			
30	0.2503			
40	0.3322			
50	0.4064			
60	0.552			
70	0.6316			
80	0.7677			
90	0.9256			
100	1.0901			
110	1.1432			
120	1.2330			
130	1.3909			
140	1.5613			



Graph 1: The Antioxidant activity content

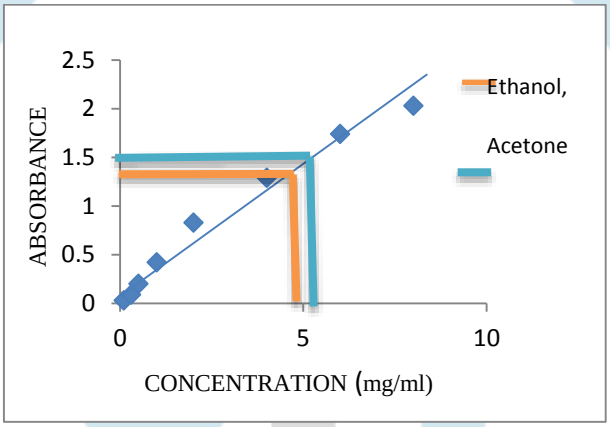
From the standard graph, it can be inferred the antioxidant activity content was higher only in the acetone extracts of leaves about a 148 µg/ml and followed by 142 µg/ml in the acetone extract and 98 µg/ml in ethanol extract which indicates the increased antioxidants in the leaves [2].

Test for Proteins

Proteins are compared in the collected sample extracted to know the amount of proteins present in the Sea weed extracts using BSA (Bovine Serum albumin) as standard.

Table 3:(Total Proteins)

Concentration (mg/ml)	Absorbance of standard (BSA) (nm)	TEST FOR PROTEINS (mg/ml)		
		Water	Acetone	Ethanol
0.1	0.03	5.6	5	5
0.3	0.09			
0.5	0.20			
1	0.42			
2	0.83			
4	1.29			
6	1.74			
8	2.03			



Graph 2: The Protein content

From the graph, higher protein content was measured higher in the water extract of leaves of about 5.6 mg/ml followed by 5 mg/ml in the acetone and ethanol extracts of leaves.

Antimicrobial and Antibacterial Activity

The bacterial species such as S*taphylococcus flexnerii, Staphylococcus aureus and Vibrio cholera*, were selected and the antibacterial activity was conducted using the specific media for specific species. The pour plate technique was followed for testing the microbial activity where the wells were punctured and the targeted extract was added at different concentrations such as 20, 30, 40, 50 µl and observed for the zone of inhibition was observed during 24-48 hrs [8].

Table:4(Antibacterial Activity)

Concentration of ethanol extract (µg/Disc) / Zone of inhibition(in mm)					
S. No	Bacteria	20 µl/ Disc	30 µl/ Disc	40 µl/ Disc	50 µl/ Disc
1	S*taphylococcus flexnerii*	0.7 mm	1 mm	2 mm	3.1 mm
2	*Staphylococcus aureus*	0.2 mm	0.3 mm	1.5 mm	0.3 mm
3	*Vibrio cholerae*	0.2 mm	0.4 mm	0.6 mm	2.3 mm

The ethanol extract showed moderate inhibition with bacteria as compared to the microbes, ethanol extract of about 50 µl proved to show higher resistance with zone of inhibition of 3mm against S*taphylococcus flexnerii* followed by 40 µl against S*taphylococcus flexnerii* and 0.7 mm against *Vibrio cholerae* . *Staphylococcus aureus* was inhibited with minimum zone of 0.2, 0.3 mm at 20, 30, 50 µl. There was no resistance seen against *Listeria* species [8].

Figure 1: Antibiotic sensitivity test

1.Staphylococcus aureus 2. Staphylococcus flexnerii 3. Vibrio cholerae

V. CONCLUSION

The extracts of the collected seaweeds were screened for phytochemicals, antioxidants, analysis with antimicrobial activity. The extracts showed presence of in phytochemicals and showed the highest antioxidant activity. These seaweed extracts and their active components could emerge as natural and alternative antioxidants and may serve as starting points for synthesizing more effective cytotoxic drugs of the future.

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