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Comparative Phytochemical Studies and Antibacterial Activity of Green and Brown Seaweeds Extract of *Enteromorpha Compressa* and *Padina Pavonica*

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***Abstract*: Seaweeds are plant like organisms and potential renewable resources in the marine environment. The present study was performed to investigate the phytochemical constituents of seaweeds of Padina Pavonica and Enteromorpha Compressa such as alkaloids, flavonoids, steroids, terpenoids, phenol, saponins, xanthoproteins, amino acids, quinones, glycosides, sugar and coumarins. In this study we estimated phenol, alkaloids, flavonoids, steroids, terpenoids, saponins, anthraquinone, sugar and coumarins and determine the Quantitative and Physico chemical analysis of seaweed extracts of Padina Pavonica and Enteromorpha Compressa. This study shows that the different seaweed extract of Padina Pavonica and Enteromorpha Compressa against various human pathogens such as Klebsiella, Salmonella, S.aureus and Proteus.**

***Keywords*: Phytochemical, physico-chemical analysis, human pathogens.**

1. Introduction

Seaweed or microalgae refers to several species of macroscopic, multicellular, marine algae. The term includes some types of red, brown, and green macro algae. Brown algae are exclusively marine forms. They have different forms from simple, freely branched filaments to highly differentiated forms. They can be distinguished into blades, stipes and holdfast. Chlorophyta are microorganisms that are grouped in the kingdom called Protista. The microbes are plant-like, in that they are able to manufacture energy from sunlight. The microbes are also commonly known as green algae. Red algae are also used to produce agar that is used as a food additive. They are rich in calcium and also used in vitamin supplements. Padina pavonica, commonly known as the PEACOCKS TAIL, is a small brown alga found in the Indian Ocean, the Atlantic Ocean and the pacific zone. Enteromorpha Compressa is a species of seaweed in ulvaceae family that can be found in North America, Mediterranean Sea, and throughout Africa and Australia. Phytochemical analysis will help the identification of these bioactive molecules**.** *Enteromorpha and Padina pavonica* is green and brown algae which produces bioactive compounds that are useful for treatment of viral and bacterial infections, inflammation and cancer. *Enteromorpha compressa* is used as medicine and it is proved to be nontoxic and used in food.

2. Materials and Methods

A. Sample Collection

The seaweed Padina Pavonica and Entermorpha Compressa was collected from Central Salt & Marine Chemicals Research Institute in Mandapam, Ramanathapuram district. In the month of December 2018 and the seaweed was carefully identified the two seaweed were washed with water to remove the adhering dust particles. The two seaweed (Padina Pavonica and Enteromorpha Compressa) were dried under the shade and powdered. The powder has been used for the Phytochemicals analysis.

1) Preparation of seaweed Extract

The dried powdered (5g) was extracted with 100ml of different solvents such as petroleum ether, ethanol, and water. The extracts were used for Phytochemicals analysis.

2) Test for Xanthoproteins

Test solution + con nitric acid + excess of ammonia. The appearance of reddish orange precipitate. Presence of xanthoproteins

3) Test for Tannins

To 2ml of seaweed extract a few drops of 10% lead acetate were added. The appearance of white precipitate indicates the presence of tannins.

4) Test for Saponins

a) About 0.5g of the sample was shaken with water in a test tube and warming. Frothing which persisit on warming was taken as preliminary evidence. The presence of saponins.
b) To 1ml of extract taken in a measuring jar 9ml of distilled water was added and shaken vigorously for 15 seconds and extract were allowed to stand for 10

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min. formation of stable foam indicates the presence of saponins.

5) Test for Anthraquinone

About 0.5g of sample was taken and 5ml of chloroform was added and shaken for 5 minutes. The extract was filtered and filtrate was shaken with equal volume of 10% ammonia solution. The pink violet or red colour in ammonical layer indicates. The presence of anthraquinone.

6) Test for Flavonoids

About 0.5g of sample was treated with 2ml of 2% sodium hydroxide solution an intense yellow colour turned to colorless on the drop wise addition of dilute acid. An intense yellow colour turned to colorless. The presence of flavonoids.

7) Test for Phenol

Equal amount of ferric chloride was added to the sample. Deep bluish green colour indicates. The presence of phenol.

8) Test for Alkaloids

a) 0.5g of seaweed extract (sample) was stirred with 5ml aqueous hydrochloric acid on steam bath and filtrate 1ml of each of the filtrate with few drops of Mayer's reagent, was taken. Precipitate was formed of the reagent. Presence of alkaloids.

b) 0.5g of seaweed extract (sample) was stirred with 5ml aqueous hydrochloric acid on steam bath and filtrate 1ml of each of the filtrate with few drops of Dragendrof reagent was taken. Precipitate was formed of the reagent. Presence of alkaloids.

9) Test for Steroids

About 200mg sample was boiled chloroform and mixture was filtrate was added 2ml of acetic anhydride and 2ml of conc. Sulphuric acid blue green ring indicates. Presence of steroids.

10) Test for Triterpenoids

The test for Triterpenoids is same as that for steroids the appearance of red, pink colour at the junction indicates the presence of Triterpenoids.

11) Salknowsky Test

0.5g of sample was dissolved in 2ml concentrated sulphuric acid was carefully added to form a lower layer (chloroform layer). Reddish brown colour at the interface indicates presence of Salknowsky test.

12) Test for Phytosterols

About 5g of sample was filtrate and add a few drops of acetic anhydride to filtrate then add conc. sulpuricacid. Through the walls of the test tube. The formation of the brown coloured ring shows. The presence of phytosterols.

13) Test for Proteins

To 2mg of the sample, 2ml millions reagent was added and observed for two minutes for the formation of white precipitate on gentle heating. White precipitate turned red indicates. The presence of proteins.

14) Test for amino acid

To 2mg of sample, 2ml of ninhydrin reagent was added. Violet colour indicates. Presence of amino acid.

15) Test for reducing sugar

To 0.5ml of extract solution 1ml of water and 5-8 drops of Fehling's solution was added to the test tube hot and observed for brick red precipitate. Presence of reducing sugar.

16) Test for Coumarins

For coumarine identified 1ml of 10% NaOH was added to 1ml of plant extract. Formation of yellow colour indicates. The presence of coumarine.

17) Test for Glycosides

For glycosides identification 3ml of chloroform and10% ammonium solution was added to 2ml of the plant extract. Formation of pink colour indicates. Presence of glycosides.

18) Physico chemical characters

Physicochemical characters such as the percentage of moisture content, total ash water soluble ash, acid insoluble ash of Padina Pavonica & Entermorpha Compressa have evaluated by the following method and weighted. The percentage of acid insoluble ash with reference to the air sample was calculated. Heat until ash was free froth carbon. The crucible was cooled and weighted the total ash with reference to the air dried sample was calculated.

19) Determination of total ash

Exactly 5g of dried powdered sample was taken in previously weighted silica crucible and ignited carefully not exceeding dull red head until the ash was free froth carbon. The crucible was cooled and weighted the total percentage of ash with reference to the air dried sample was calculated.

20) Determination of water soluble ash

Exactly 0.2g of the ash was boiled with 25ml of distilled water the insoluble matter was collected in a previously weighted sintered crucible washed with water and weighted the percentage of water soluble ash with reference to the air dried sample was calculated.

21) Determination of acid insoluble ash

Exactly 0.2g of the ash was boiled with 25 ml of dilute hydrochloric acid (2N). The insoluble matter was collected in a previously weighted sintered crucible, washed with hot water, dried constant weight.

3. Result and Discussion

A. Quantitative Determination of Physico chemical Analysis

Quantitative determination of physicochemical features such as percentage of loss of drying, total ash, acid insoluble ash. moisture content was determined. The percentage of extractive value in petroleum ether, water and ethanol, were also determined. These results were recorded in table.

Table 1

Particulars	Enteromorpha Compressa
Loss of weight on drying	**1.99%**
Total ash	**1.79%**
Acid in soluble ash	**0.82%**
Water soluble ash	**0.96%**

Table 2

Particulars	Padina Pavonica
Loss of weight on drying	**1.85%**
Total ash	**1.49%**
Acid in soluble ash	**0.62%**
Water soluble ash	**0.36%**

B. Fluorescence Analysis

Powdered seaweed materials of the powdered in various

solvents and powdered seaweed material treated with chemical reagents were examined for fluorescence analysis under ordinary light and UV light and the changes in colour was recorded. The results were recorded in table.

Table 3

Particulars of treatment	Under ordinary light	Under UV light
Powder as	Green	Dark green
Powder ethanol	Brown	Pale brown
Powder conc. HCL	Pale green	Dark brown
Powder conc.H_2SO_4	Dark brown	Dark green
Powder conc .HNO_3	Yellow	Light green
dil. HCL	Pale yellow	Pale green
dil. HNO_3	Light brown	Dark green
dil. H_2SO_4	Pale yellow	Pale green

Table 4

Particular of treatment	Under ordinary light	Under UV light
Powder as	Brown	Dark green
powder ethanol	Dark green	Light green
Powder conc. HCl	Dark green	Pale green
Powder conc. H_2SO_4	Dark brown	Dark green
Powder conc. HNO_3	Reddish orange	Pale green
Dil. HCl	Pale green	Dark green
Dil. H_2SO_4	Pale brown	Dark green
Dil. HNO_3	Pale green	Dark green

Many drugs give fluorescence when the cut of surface on the powder is exposed to uv radiation and it is a useful routine procedure to examine in uv light. The Padina Pavonica and Enteromorpha Compressa powderd and its extracts in various solvents such as petroleum ether (60^0c -80^0 c) chloroform and ethanol were examined under ordinary light and uv light. the powder was also treated in various chemical frequents such as IN aqueous NAOH, conc. Hcl, dilute Hcl, conc. H_2SO_4, conc. HNO_3 and the changes in colour were recorded.

The bioactive compounds present in seaweed extract of Enteromorpha Compressa and Padina Pavonica were tested for its antimicrobial activity against human pathogens such as Klebsiella, Salmonella, S.aureus and Proteus among four pathogens were tested. Salmonella was found to be more susceptible to the bioactive compounds of the seaweed extract of Enteromorpha Compressa with zone size of 15mm.The inhibitory activity of the bioactive compounds was less against Klebsiella (10mm), S.aureus (5mm) and Proteus (5mm) when compared to Padina Pavonica.

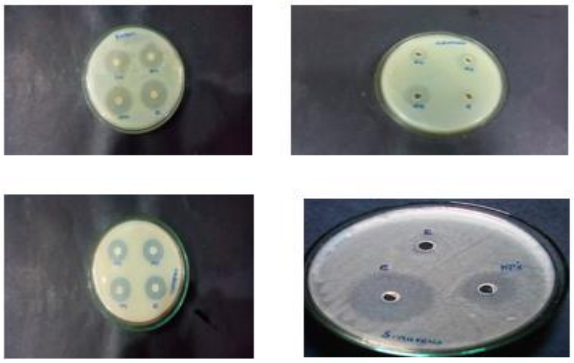

Fig. 1. Antimicrobial activity

Table 6

Pathogens	Zone of Inhibition (mm)	
	E. Compressa	P. Pavonica
Salmonella	15	5
Klebsiella	10	10
Proteus	5	5
S. aureus	5	5

4. Conclusion

The phytochemical screening of seaweeds showed the presence of alkaloids, flavanoids, phenol, protein and amino acids, sterols, sugar and terpenoids in two seaweed samples tested. Quantitative determination such as loss of weight on drying, total ash, insoluble ash and water soluble ash and residue on ignition and extractive values in petroleum ether, water and ethanol have been carried out and the results are present. Physico chemical characters, preliminary phytochemical analysis can be used as a diagnostic tool for the correct identification of the plants. The presence of phytochemicals (secondary metabolite) is responsible for their therapeutic effects.

Table 5

S. No.	Tests	Padina Pavonica			Enteromorpha Compressa		
		Water	Ethanol	Petroleum Ether	Water	Ethanol	Petroleum Ether
1	Xantho proteins	–	–	–	–	–	–
2	Tannins	+	+	+	+	+	+
3	Saponins	+	+	+	+	+	+
4	Anthraquinone	+	–	–	+	–	–
5	Flavonoids	–	+	+	+	+	+
6	Phenol	–	–	–	+	+	+
7	Alkaloids	–	–	–	–	–	–
8	Steroids & Terpenoids	–	–	–	–	–	–
9	Salknowsky Test	–	–	–	–	–	–
10	Phytosterols	–	–	–	–	–	–
11	Proteins	–	–	–	–	–	–
12	Amino acid	+	+	–	–	+	–
13	Sugar	–	–	–	+	+	+
14	Reducing Sugar	–	–	–	–	–	–
15	Coumarins	+	–	+	–	–	–
16	Quinons	–	–	–	–	–	–
17	Glycosides	–	–	–	–	–	–

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