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Qualitative and Quantitative Analysis of Phytochemical Studies on Selected Seaweeds Acanthopora Spicifera and Sargassum Wightii

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Abstract:- The sample for the study constitutes acanthopora spicifera from red seaweed and sargassum wightii from brown seaweed. They were freshly obtained from mandapam and elatharavai coast of erwadi and were rinsed in seawater and the packed in aseptic bags and brought to the laboratory for further processing. The extracts were subjected to phytochemical analysis to secondary metabolites both qualitatively and quantitatively p. By preliminary phytochemical screening of eight different chemical compounds (alkaloids Teripioids, steroids, tannin, saponins, flavonoids, Polyphenols, glycosides) were tested in four different extracts. The present investigation revealed that Saponins did not show any positive result for their presence in any of the five extracts tested. Among the five different extracts, petroleum ether and methanol extract showed the presence of maximum number (8) of compounds. Next to that, Acetone extracts showed seven compounds. Water extracts showed five compounds and ethanol extracts showed only two compounds. The quantative phytochemical analysis revealed that the highest total phenol (216.65±17.38) and flavanoid (358.25±18.21) was in the brown seaweeds. *wightii*. The highest tannin compound was recorded in acanthopora spicifera seaweed. (28.54± 0.89).
Key words:- Seaweed, Phytochemicals, Phenol, Extraction, Pigmentation.

I. INTRODUCTION

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. They are not mandatory for humans to consume. However, it is proven that these chemicals protect plants as well as the humans eating them [(1)].

Seaweeds are scientifically termed macro algae literally meaning large algae. Algae are relatively simple photosynthetic plants with unicellular reproductive structure the range from unicellular organisms to non vascular filamentous are thalloid plants [(2).]Seaweeds can be classified into three broad groups based on pigmentation brown, red and green, these are fascinating and diverse group of organism living for the benefit of human race. Seaweed contains virtually all the minerals and it is useful in preventing free radical formation seaweed contain several vitamins. Red brown algae are rich carotenes [(3).]

Seaweeds also contain a range of unique phytochemicals not present in terrestrial plants. As such, edible seaweeds may be the only relevant dietary source of some of these factors. A wide range of studies have described the high antioxidant capacity of a range of edible seaweeds. This capacity is endowed by the presence of sulphated polysaccharides, polyphenolic compounds and antioxidant enzymes [(4)] Oxidative stress may play a key role in the development of cancers and cardiovascular disease [(5)]. Phytochemical-rich foods should clearly form part of a healthy balanced diet. However, the human body has a number of physiological, biochemical and enzymatic processes by which it can combat oxidative stress outside of dietary intake. The route by which the wide variety of phenolic compounds enters the circulation is not well characterized, nor is the bioavailability and half-life/distribution of such factors in the human body. Previous intervention studies where dietary antioxidant intake has increased have not evidenced a parallel change in the total antioxidant capacity of the body [(6)]. While this casts doubt on the benefit of increasing polyphenolic consumption from the perspective of reducing oxidative stress, it must be noted that such compounds may have other physiological effects. Previous studies in animal models and cell culture have suggested that seaweed phytochemicals have the potential to inhibit the progression of carcinoma formation [(7,8)]. Although thousands of bioactive compounds have been discovered, the need for novel therapeutic compounds is still urgent in concern of number of new diseases and resistant strains of microorganisms [(9).] In this present study revealed that screening the *Acanthopora spicifera and Sargassum wightii seaweed* the both quantitatively and qualitatively for the phytochemicals

II. MATERIAL AND METHODS

A. Selection of area and sample

In Tamilnadu, Thonithurai coast of mandapam, Elanthravai of erwadi coast in gulf of manner possess abundant wealth of seaweeds. The investigator had an easy access to this coast and also interested to study the nutritional composition of the seaweeds available in this area. The sample for the study constitutes acanthopora spicifera from red seaweed and sargassum wightii from brown seaweed.

Fig 1: Area where the sea weeds grow

B. Collection of seaweed

The selected sample of acanthopora spicifera and sargassum wightii is a known species of edible green seaweed, which have good amount of nutrients. They were freshly obtained from mandapam and elatharavai coast of erwadi and were rinsed in seawater and the packed in aseptic bags and brought to the laboratory for further processing.

Fig 2: Selected sample of acanthopora spicifera and sargassum wightii

C. Development of dry seaweed powder

The collected seaweeds (acanthopora spicifera from red seaweed and sargassum wightii from brown seaweed).were rinsed in fresh water to remove dirt and dust and they were blanched at 100c remove impurities and salt. It was drained using sieve and shadow dried at room temperature for 3 days further the dried species were shunted again for removal of excess salt and powder in a blender.

D. Preparation of plant extracts

The seaweed powder was successively extracted using solvents of increasing polarity according to Arokiyaraj *et al* (2009) with some modifications. 15 g powder was initially soaked in 60ml of petroleum ether in air tight conical flask for two days and then it was first filtered through double layered muslin cloth and then filtered through Whatman no 1 filter paper and filtrate was collected into sterile air tight bottle. Similar process was repeated twice with fresh petroleum ether and the filtrate was collected together. After all, petroleum ether was removed from the filtrate at 40oC using oven and the extract was stored at the refrigerator for further studies. [(10)]

Likewise, the above dried residue was used for sequential extraction of acetone, ethanol, methanol and water.

E. Qualitative analysis of phytochemical substance

The extracts were subjected to phytochemical analysis to detect the presence of following biomolecules using the standard qualitative procedures as described by Trease and Evans (1989) [11].

1) *Test for Alkaloids:* 1ml of 1% HCl was added to 3ml of extract in a test tube and was treated with few drop of Meyer's reagent. A creamy white precipitate indicted the presence of alkaloids
2) *Test for terpenoids:* 5 ml of extract was mixed with 2 ml of CHCl3 in a test tube. 3 ml of concentrated H2SO4 was carefully added to the mixture to form a layer. An interface with a reddish brown coloration was formed for the presence of terpenoids.

3) *Test for saponins:* 5 ml of extract was shaken vigorously to obtain a stable persistent froth. The frothing was then mixed with 3 drops of olive oil and observed for the formation of emulsion, which indicated the presence of saponins.
4) *Test for flavonoids:* A few drops of 1% NH3 solution was added to the extract in a test tube. A yellow coloration was observed for the presence of flavonoids
5) *Test for tannins:* To 0.5 ml of extract solution, 1 ml of distilled water and 1-2 drops of ferric chloride solution were added and observed for brownish green or a blue black coloration.
6) *Test for Glycosides:* 10ml of 50% H2SO4 was added to 1ml of extract in a boiling tube. The mixture was heated in boiling water for 5min. 10ml of Fehling's solution (5ml of each solution A and B) was added and boiled. A brick red precipitate indicated presence of glycosides
7) *Test for phenols:* Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenol

F. Quantitative analysis of phyto chemicals

1) *Estimation of Phenol:* Total phenolic assay was determined by using Folin-Ciocalteu assay (Sadasivam and Manickam, 1992). A known amount of the sample was taken, ground well with 80% ethanol and was centrifuged at 4000 rpm. An aliquot (1ml) of extract or standard solution of caffic acid is added to 250ml of flask containing 9ml of distilled water. A reagent blank using double distilled water was prepared .1ml of folin-ciocalteu phenol reagent was added to mixture and shaken. After 5 minutes 10ml of 7%sodium bi carbonate was added. The solution was diluted to 25ml with distilled water and mixed. After incubation for 90 minutes at room temperature. The absorbency is determined by 750nm withUV spectrophotometer. Total phenolic content is expressed as mgcaffic acid equivalents mg/100 gm dry weight samples were analyzed in duplicates[(12).]
2) *Estimation of Flavonoids:* Total flavonoid content was measured by the Aluminum chloride calorimetric assay (Zhishen et al., 1999). A known amount of the sample was taken; ground well with 80% ethanol and was centrifuged at 4000 rpm. An aliquot 1ml of extracts or standard solution of catechin (20, 40, 60, 80 &100mg/l) was added to 10ml volumetric flask containing 4ml of double distilled water. To the flask was added 0.3ml 5% sodium nitrate, After 5 min.,0.3ml 10% aluminum chloride was added. At sixth minute, 2ml of1M NaoH was added and the total volume was made up to 10mlwith double distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at510nm. Total flavonoid content was expressed as mg catechin equivalents (CE)/100g dry mass samples were analyzed in duplicate[(13).]
3) *Estimation of tannin:* The tannin content in the samples investigated was assessed on the basis of modified methods by Price et al. [1978]. The vanillin method involves the reaction of an aromatic aldehyde, vanillin, with meta-substituted ring of flavanols to yield a red coloured adduct measured spectrophotometrically at 500 nm. The tannins content in extracts examined was calculated using a standard curve drawn up for methanol solutions of catechin and expressed as mg catechin × 100 g-1 dried weight [(14).]

III. RESULTS AND DISSCUSION

A. Qualitative analysis of phytochemical substance screening in seaweed

By preliminary phytochemical screening of eight different chemical compounds (alkaloids Teripioids, steroids, tannin, saponins, flavonoids, Polyphenols, glycosides) were tested in four different extracts. Saponins did not show any positive result for their presence in any of the five extracts tested. Poly phenol, teripheoids, flavoids, glycosides, tannins showed the maximum presence in five different extracts. Alkaloids compound only present in acetone and petroleum ether extract. Teripheoids compound showed the positive result presence in acetone extract.

Among the five different extracts, petroleum ether extract showed the presence of maximum number (7) of compounds. Next to that, Acetone extracts showed six compounds. Methanol and water extracts showed five compounds each and ethanol extracts showed only four compounds.

TABLE-1: Qualitative analysis of phytochemical constituents for different extraction of Acanthopora spicifera seaweed

S.No	Name of the compound	Petroleum ether	acetone	methanol	ethanol	water
1	Alkaloids	+	+	-	-	-
2	Teripioids	+	+	+	-	-
3	Steroids	+	+	+	-	+
4	Tannin	+	+	+	+	+

5	Saponin	-	-	-	-	-
6	Flavanoids	+	+	+	+	+
7	Polyphenols	+	-	-	+	+
8	Glycosides	+	+	+	+	+

The preliminary phytochemical studies on acetone, ethanol, petroleum ether, methanol and water extracts of S. wightii revealed the presence of 30 gave positive results out of 40. The 30 positive results showed the presence of steroids, phenolic groups, saponins, tannin, flavonoids, By preliminary phytochemical screening of eight different chemical compounds (alkaloids Teripioids, steroids, tannin, saponins, flavonoids, Polyphenols, glycosides) were tested in four different extracts. Alkaloids Teripioids, steroids, tannin, saponins, flavonoids, Polyphenols, glycosides showed the maximum presence in petroleum ether, methanol and acetone extracts. Tannin and poly phenol compound showed the presence in ethanol extract. (Steroids, tannin, flavonoids, Polyphenols, glycosides) compound showed the positive result presence in water extract. Among the five different extracts, petroleum ether and methanol extract showed the presence of maximum number (8) of compounds. Next to that, Acetone extracts showed seven compounds. Water extracts showed five compounds and ethanol extracts showed only two compounds.

TABLE -2: Qualitative analysis of phytochemical constituents' different extraction of sargassum wightii

S.No	**Name of the compound**	**Petroleum ether**	**acetone**	**methanol**	**ethanol**	**water**
1	Alkaloids	+	+	+	-	-
2	Teripioids	+	+	+	-	-
3	Steroids	+	+	+	-	+
4	Tannin	+	+	+	+	+
5	Saponin	+	-	+	-	-
6	Flavanoids	+	+	+	-	+
7	Polyphenols	+	-	+	+	+
8	Glycosides	+	+	+	-	+

B. Quantitative analysis of phytochemical substance in acanthopora spicifera and sargassum wightii

The total phenol and flavanoid content ranged between (127.24 ±6.541) to (195.39± 15.27) and (190.12±14.24) to respectively (**Table-2**).The highest total phenol (216.65±17.38) and flavanoid (358.25±18.21) was in the brown seaweeds. *wightii*. The highest tannin compound was recorded in acanthopora spicifera seaweed. (28.54± 0.89).

TABLE-3: Quantitative analysis of phytochemical substance Acanthopora spicifera and Sargassium wightii seaweed

Seaweed	**Phenol**	**Flavonoids**	**Tannin**
Acanthopora spicifera	127.24 ±6.541	190.12±14.24	28.54± 0.89
Sargassium wightii	195.39± 15.27	358.25±18.21	27.54± 0.54

Note:*mg/caffic acid equivalence; ** mg/catechin equivalence

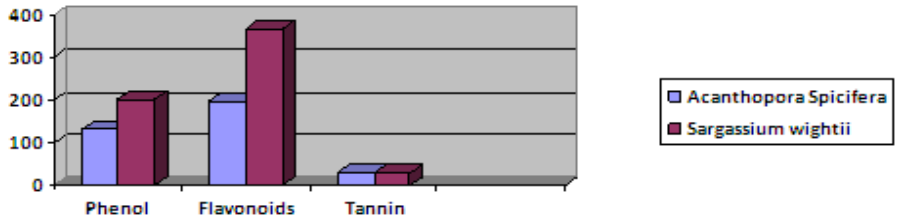

Fig 3: Quantitative analysis of phytochemical substance Acanthopora spicifera and Sargassium wightii seaweed

IV. CONCLUSION

The seaweeds known as medicinal are rich in secondary metabolites which include alkaloids, glycosides, flavonoids, saponins, tannins, steroids, related active metabolites, which are of great medicinal value and have been extensively used in the drug and pharmaceutical industry [15].The result of the qualitative analysis of phytochemical screening is petroleum ether, acetone, methanol, Ethanol and water extracts of acanthopora

spicifera and sargassum wightii seaweed. Extract of sargassum wightii showed the presence of all phytochemical were present in petroleum ether and methanol except acetone, ethanol and water extract. Whereas all the organic extract of acanthopora spicifera did not answer for sapoins. Alkaloids was absent in the methanol, ethanol and water extract of acanthopora spicifera. Teripioids compound was absent in the ethanol and water extract of acanthopora spicifera and sargassum wightii seaweed. Tannins were present in the all organic extract of acanthopora spicifera and sargassum wightii seaweed. Steroids were present in the petroleum ether, acetone, methanol and water extracts of acanthopora spicifera and sargassum wightii seaweed except ethanol extract. Flavnoids was present in the all organic extract of acanthopora spicifera and sargassum wightii seaweed except methanol extract. Poly phenols was absent in the acetone and methanol extract of acanthopora spicifera. Poly phenols was absent in the acetone extract of sargassum wightii. Glycoside was present in all the organic extract of acanthopora spicifera. Glycoside was absent in ethanol extract of sargassum wightii.
The quantative analysis of phytochemical substance among two seaweed. Highest amount of polypheol ad flavnoids was present in brown seaweed sargassum wightii than acanthopora spicifera. The highest amount tannin substance present in acanthopora spicifera.

The phenolic compounds such as phenol, tannin and flavniods have been found to be present in maximum amount in red and brow seaweed. The polyphenols includes a large subgroup of chemicals called flavonoids. Flavonoids are plant chemicals found in a broad range of fruits, grains, and vegetables. They are being studied to find out whether they can prevent chronic diseases such as cancer and heart disease. Other polyphenols (including some flavonoids) act as antioxidants. These are thought to rid the body of harmful molecules known as free radicals, which can damage a cell's DNA and may trigger some forms of cancer and other diseases. Flavonoids that are thought to act as antioxidants and may protect against some cancers and heart disease. Quercetin, is another flavonoid that appears to have antioxidant properties [16].

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