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RESEARCH ARTICLE

Preliminary Phytochemical Analysis of the Crude extract of Marine Red and Brown Seaweeds

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ABSTRACT:
The seaweed is a large and diverse group of marine macro algae that can be found in intertidal and sub tidal coastal regions around the world.Seaweed are simpler in their structural composition because they take up the nutrients into their blades or fronds directly from the seawater, unlike more complex land plants which take up the nutrients through their roots.There are three different types of marine macro algae–red algae (Rhodophyta), brown algae (phaeophyta), green algae (chlorophyta). They are grouped according to their unique photosynthetic pigments, which give them their characteristics color and unique properties. Secondary metabolites from natural resources are a potential source that leads and drugs can exploited to combat antimicrobial resistance in microorganisms. The present study investigated to explore the preliminary phytochemical constituents of marine red and brown seaweed such as Gracilaria corticata, Gracilaria edulis, Saragussum wightii wherelse Methanol, Acetone and Aqueous were used as a solvent system for the preparation of the extract.

KEYWORDS: Seaweeds, *Gracilaria corticata*, *Gracilaria edulis*, *Saragussum wightii*, Phytochemical, solvents.

INTRODUCTION:
Seaweeds constitute a vital and important of the marine ecosystem. It was estimated that about 90% are algae, and over 50% of global photosynthesis were contributed from algae[1]. Seaweed has no formal definition. Seaweed may belong to one of several groups of multicellular algae such as the red algae, green algae and brown algae, but these three groups do not have a common multicellular ancestor. Seaweeds are commonly grown close to the littoral zone. The genera of Saragassum wightii and gracilaria are free floating and occupy a wide range of ecological niches and also used widely in the field of medicine[2].

Over the past decades seaweeds had been consumed by humans as a medicine, food and their extracts have generated enormous amount of interest in the pharmaceutical industry as fresh source of bioactive compounds with lots of massive medicnal potential[3]. Marine seaweed was used as the potent source of human health because of its active constituents which is responsible for its various pharmacological activities. Being a unique plant structure and its biochemical composition, it could be exploited for its multi-functional properties in the form of food and medicine[4]. The seaweeds offer more curative properties both externally and internally by intake of raw and dried seaweeds which may give more healthy benefits. Seaweeds are toxin free and also provide hundreds of organic compounds[5].

Among the coastal region of Tamilnadu, South India supports a rich vegetation of marine algae. Among macro algae brown and red algae were growing abundance in the shores of kanyakumari and Ramanathapuram districsts of Tamilnadu state, India.

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Seaweeds have the capacity to produce a huge diversity of derived metabolites characterized by wide range of biological activities[6]. It is already reported that seaweeds contain important phytochemical constituents which had the potential biological activities[7]. Phytochemicals are responsible for medicinal property of plants. They are non-nutritive chemicals that will protect humans from various kinds of diseases. Hence phytochemical analyis of the seaweeds will be a best preliminary approach to reveal its secondary metabolite constitutents and the resultant many medicnal values[8]. Phytochemicals will sudsides any overlapping mechanisms of action in our body, which including antioxidant effects, stimulation of the immune system, modulation of hormone metabolism, as well as antibacterial and antiviral effects[9]. The present study was carried out to estimate the preliminary phytochemical analysis of three diferent red and brown seaweeds *Gracilaria corticata, Gracilaria edulis, Saragassum wightii.*

MATERIALS AND METHODS:

Seaweed collection and Sample preparation:

The sample *Gracilaria corticata, Gracilaria edulis, Saragassum wightii* (Brown algae) were collected from intertidal zone of madapam coast (Lat. 9° 17‟N; Lon. 79° 19‟E) of Gulf of Mannar, south-east coast of Tamil Nadu, India. The seaweed were identified and authenticated by Dr.Ganesan, Senior Scientist, CSMCRI-Central Salt and Marine Chemicals Research Institute, Marine Algal Research Center, Mandapam. The collected sample was cleaned with seawater to remove the epiphytes, sand particle and the sample has been packed in polythene bag and brought them to laboratory. Then the sample was washed with fresh water and shade dried for about one week. The shade dried *Sargassum wightii* were pulverised to fine powder. One kilogram fresh seaweed yields approximately 100g dried powder.

Preparation of crude extract (methanol, acetone and aqueous):

Methanol extraction:

The acetone extract of *Gracilaria corticata, Gracilaria edulis was extracted by using* 50g of the power sample with 150ml of acetone. The mixture was placed in the orbital shaker for 24hrs at 32^0 C in room temperature. After squeezing, the solvent was taken out and extraction liquid was filtered by using Whattman filter paper. The extracted sample was condensed by using Soxhlet extractor at 50^0 C. This process will separate the metabolities like flavanoids, phenols, etc., which is further stored as crude acetone extract.

Acetone extraction:

The acetone extract of *Sargassum wightii was extracted by using* 50g of the power sample with 150ml of acetone. The mixture was placed in the orbital shaker for 24hrs at 32^0 C in room temperature. After squeezing, the solvent was taken out and extraction liquid was filtered by using Whattman filter paper. The extracted sample was condensed by using Soxhlet extractor at 50^0 C. This process will separate the metabolities like flavanoids, phenols, etc., which is further stored as crude acetone extract.

Aqueous extraction:

The aqueous extract seaweed *Gracilaria corticata, Gracilaria edulis, Sargassum wightii* was dried. After drying, 3g of seaweed is measured and pulverize it gently. Then add 50ml of distilled water to the added seaweed in the conical flask. The solution was filtered by using Whattman filter paper and the filtered solution was condensed by using Soxhlet extractor. The solution was stored in a refrigerator for further use as crude extract of aqueous.

Phytochemical analysis:

The various qualitative chemical tests can be performed in finding a profile of a given extract for its bio-active compounds. The prepared extracts using Methanol, Acetone and Aqueous were analyzed for the occurrence of alkaloids, sapo-nins, tannins, steroids, flavonoids, glycosides, proteins, amino acids and reducing sugars by using the protocols offered in the literature[11].

Detection of Alkaloids:

Wagner's test:

Extract was added with 2ml of Wagner's reagent by sides of the test tube. Result of a reddish brown colour precipitate confirms the test as positive.

Detection of Carbohydrates:

Fehling's test:

1ml of filtrate was boiled on water bath with 1ml each of Fehling solution. Red precipitate indicates the presence of sugar.

Detection of Glycosides:

Borntrager's test:

To 2ml of filtrate, 3ml of chloroform was added and shaken.chloroform layer was separted and 10% ammania solution wass added to it. Pink colour indicates the presence of Glycosides.

Detection of Saponins:

Foam test:

1ml extract as dissolved in 2 ml of ditilled water and filtered through Whatmann No.1 filtre papper and the filtrate was subjected to test.

Detection of Proteins:
Biuret test:
To 2ml of filtrate was treated with few ml of 2% of copper sulphate solution.To this,1ml of ethanol 95% was added followed by excess of potassium hydroxide pellets. Pink colour in the ethanolic layer indicated the presence of protein.

Detection of Amino acids:
Ninhydrin test:
Two drops of ninhydrin solution (10mg of ninhydrin in 200 ml of acetone) were added with 2ml of extract. A characteristic purple colour indicated the presence of amino acid.

Detection of phenolic compounds:
Ferric chloride test:
1ml of extract was dissolved in 2ml of distilled water. To this,few drops of neutral ferric chloride solution were added. A drak green colour indicated the presence of phenolic compounds.

Detection of Terpenoids:
To 2ml of extract, 1ml of chloroform was added and mixed well. Add a little of concentrated H_2SO_4 was carefully added to form a reddish brown layer.

Detection of Steroids:
Two microlitre of chloroform was added to the extract and a few drop of acetic acid were poured. Followed by concentrated H_2SO_4. The mixture of blue and green colour showed the presence of steroids.

Detection of flavanoid:
1ml extract with 2ml ammonia solution. A mixture of yellow colour showed the presence of flavanoid.

Detection of tannin:
1ml extract with 2ml water and 2 drop of ferric chloride. A green colour indicated the presence of tannin compound.

RESULTS AND DISCUSSION:

Phytochemical substances such as alkaloids, carbohydrates, glycosides, saponins, Proteins, amino acids, phenolic compounds, terpenoids, steroids, flavanoid and tannin were determined in various extracts (methanol, acetone, and aqueous) in species of algae *G. corticata, Gracilaria edulis, Saragassum wightii* (Table 1).

The phytochemical analysis of methanol and aqueous extract of *G. corticata* contained six primary compounds such as alkaloids, saponins, phenolic compounds, flavanoids, tannins, amino acids and rest of which carbohydrates, proteins, glycosides, amino acids, terpeniods were luxuriantly absent. The crude methanolic and aqueous extract of *G. edulis* shows that the presence of six primary compounds such as alkaloids, saponins, Phenolic compounds, terpenoids, steroids, tannins while others such as carbohydrates, glycosides, proteins, amino acids, flavanoids were luxurinantly absent. The crude acetone and aqueous extract of *S. wightii* showed the presence of alkaloids, phenolic compounds, terpenoids, flavanoids, steroids, and tannins. The algal extraction was mostly done with polar solvents. More yield was depending upon the solvent type which dissolves more of a particular compound. Hence, the methanolic extraction of *G. corticata* were contains more yields followed by other solvents[11]**.** The seaweed *S. wightii* may have a lot of potential chemical constituents in acetone compared to aqueous extract. The study of phytochemical properties of brown seaweed *S. wightii* helps to set up a basic standard to do any future research on this species[12].

The results of the phytochemical investigation of various solvents revealed the presence of various secondary metabolites like alkaloids, saponins, phenolic compounds, steriods, flavonoids, tannins. Flavonoids are known as nature's tender drug on which it possesses numerous biological and pharmacological activities. Phenolic compounds are widely distributed in the plant kingdom and have been reported to possess various biological activities including antioxidant properties. Earlier reports revealed that marine seaweed extracts, especially polyphenols have strong antioxidant activity[13,14]. Saponins has enormous amount of biological properties which includes antimicrobial, anti-inflammatory, anti-feedent and hemolytic effects. Saponins possess numerous biological properties which include antimicrobial, anti-inflammatory, anti-feedent and haemolytic effects[15]. Steriods are believed to be a biosynthetic precursor for cardenolides in plants. Marine algae have shown to be best source of unsaponifiable, non-toxic sterols which will have high medicinal values[16,17.] Hence the presence of these above secondary metabolites in the seaweeds suggest that, it can be used as antimicrobial, anti-parasitic, antifeedent,antioxidant, antiallergenic, antithrombic, anticarcinaogenic and anti-ulcer agents, that have great medicinal values and extensively used in the drug and pharmaceutical industry.

The obtained results can be used as an initial step for further identification of bioactive compounds from the methanol, acetone, aqueous extract of seaweed *G. corticata, G. edulis, S. wightii.*

Table 1: Phytochemical analysis of the extracts of *Gracilaria corticata, Gracilaria edulis (Red seaweed), Saragassum wightii* (Brown seaweed) (Presence +, absence -)

S.NO.	Name of the test	*Gracilaria corticata* in methanol	*Gracilaria corticata* in *aqueous*	*Gracilaria edulis* in methanol	*Gracilaria edulis* in Aqueous	*Saragassum wightii* in *Acetone*	*Saragassum wightii* in Aqueous
1.	Test for Alkaloids	+	+	+	+	+	+
2.	Test for Carbohydrates	-	-	-	-	-	-
3.	Test for Glycosides	-	-	-	-	-	-
4.	Test for Saponins	+	+	+	+	-	-
5.	Test for Proteins	-	-	-	-	-	-
6.	Test for Amino acids	-	+	-	-	-	+
7.	Test for phenolic compounds	+	+	-	+	+	+
8.	Test for Terpenoids	-	-	+	-	+	+
9.	Test for Steroids	+	-	+	-	+	-
10.	Test for flavanoid	+	+	-	+	+	+
11.	Test for tannin	+	-	+	+	+	+

CONCLUSIONS:

The present study revealed that *G. corticata G.edulis, S.wightii.* Contained significant amount of primary phytochemical constituents. Therefore, it is suggested that isolation, purification and characterization of individual bioactive compounds from these three diferent seaweeds, may able to study their unique pharmaceutical active principles.

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CONFLICTS OF INTERESTS:

There is no conflict of interests.

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