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Preliminary phytochemical investigation of brown sea weed *Sargassum wightii* and *Turbinaria conoides*

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Abstract
In the present investigation, *Sargassum wightii* and *Turbinaraia conoides* were analysed for the phytochemicals studies by qualitative and quantitatively. The samples of *Sargassum wightii* and *Turbinaria conoides* were collected from shores of Mandapam, (south east coast of India). The extracts were subjected to phytochemical analysis to detect the presence of following biomolecules using the standard qualitative procedures. Both sea weeds shows the different solvency in different extracts. Not a single solvent showing a complete result. A maximum of phytochemical yield by acetone extract of both sea weeds. The total phenolic and flavanoid content in the examined extracts ranged from 2.5 to 22.5 mg GA/g and 3.1 to 21.5 mg QE/g. respectively. The high concentrations of phenolics were obtained from ethanolic extract of *S. wightii*. The total phenolic content in the examined extracts ranged from 1.2 to 14.6 mg GA/g. The high concentrations of phenolics were obtained from Acetone extract of *T. conoides*. The total flavonoid content in the examined extract ranged from 1.1 to 22.5 mg QE/g. The highest concentration of flavonoid was measured in the acetone extract of *T. conoides*. In TLC analysis, the Rf values were recorded 0.58,0.78 and 0.68 in ethanol, petroleum and acetone respectively; 0.65, 0.69 and 0.74 in ethanol, petroleum ether and acetone extract respectively when a solvent phase of Benzene: Ethyl acetate (1:1) was used.

Keywords: *Sargassum wightii, Turbinaraia conoides,* phytochemical, seaweed

Introduction

The term seaweed refers to the large marine algae that grow almost exclusively in the shallow waters at the edge of the world's oceans. They provide home and food for many different sea animals, lend beauty to the underwater landscape, and are directly valuable to man as a food and industrial raw material (˘Kandale *et al.*, 2011).

Seaweeds are plants because they use the sun's energy to produce carbohydrates from carbon dioxide and water (this is called photosynthesis). They are simpler than the land plants mainly because they absorb the nutrients that they require from the surrounding water and have no need for roots or complex conducting tissues. Some large seaweeds such as the kelps have root-like parts called holdfasts, but these only serve to attach them to the rock. Most seaweeds have to be attached to something in order to survive, and only a few will grow while drifting loose in the sea (Gullon *et al*., 2020; Perez *et al.*, 2020).

Three groups of seaweeds are recognised, according to their pigments that absorb light of particular wavelengths and give them their characteristic colours of green, brown or red. Because they need light to survive, seaweeds are found only in the relatively shallow parts of the oceans, which means around the shores. Here they occur in a variety of shapes and sizes, from the large kelps (certain brown seaweeds) that form forests on temperate (cooler) coasts, to the hard "encrusting corallines" that look like pink icing, but are so important in building and cementing coral reefs in the tropics. Some seaweeds, especially many of the larger reds, are showy and attractive, while others may be small and inconspicuous, and grow in a low "turf" on the rocks (Wang *et al*., 2020) [29].

The Southern Coast of India bears luxuriant growth of seaweeds. More than 200 species of seaweeds have been found in this area. In coastal waters they grow almost like grass in large areas, extending over hundreds of kilometres. Indian seaweed industries depend on this coastline for raw materials for the production of Agar and Sodium Alginate.

At present, hundreds of fishermen are engaged in the harvest of seaweeds. Agar yielding Red Algae such as Gelidiella acerpsa, Oracilaria adulis, Gracilaria crassa and Gracilaria follifera are collected throughout the year while Algin yielding Brown Algae such as Sargassum and Turbinaria are collected seasonally from August to January. In coastal Tamil Nadu and in many other places seaweeds are made into halwa or used for making porridge.

Apart from the commonly available 200 species of seaweeds, about 680 species of marine algae belonging to Rhodophyta, Pheaophyta, Chlorophyta commonly known as red, brown and green seaweeds have also been identified in both inter-tidal and deep water regions. Among these seaweeds green algae are rare. Red algae are small and delicate, with a feathery appearance.

Seaweeds have many industrial uses. Brown algae yield a gummy substance called algin and red algae produce jellylike substances called agar and carrageenan. These substances are used as additives in food products and drugs to give them a smooth texture and help them retain moisture. They are also used in lipsticks, soaps, film, paint, varnish and buttons. The sea weeds were comprising of many metabolites and enrich in all minerals by up taking the marine minerals.

Primary metabolism in a plant comprises all metabolic pathways that are essential to the plant's survival. Primary metabolites are compounds that are directly involved in the growth and development of a plant whereas secondary metabolites are compounds produced in other metabolic pathways that, although important, are not essential to the functioning of the plant. However, secondary plant metabolites are useful in the long term, often for defense purposes, and give plants characteristics such as color. Secondary plant metabolites are also used in signalling and regulation of primary metabolic pathways. Plant hormones, which are secondary metabolites, are often used to regulate the metabolic activity within cells and oversee the overall development of the plant. As mentioned above in the History tab, secondary plant metabolites help the plant maintain an intricate balance with the environment, often adapting to match the environmental needs. Plant metabolites that color the plant are a good example of this, as the coloring of a plant can attract pollinators and also defend against attack by animals.

Research into secondary plant metabolism primarily took off in the latter half of the 19th century, however, there was still much confusion over what the exact function and usefulness of these compounds were. All that was known was that secondary plant metabolites were "by-products" of the primary metabolism and were not crucial to the plant's survival. Early research only succeeded as far as categorizing the secondary plant metabolites but did not give real insight into the actual function of the secondary plant metabolites. The study of plant metabolites is thought to have started in the early 1800s when Friedrich Willhelm Serturner isolated morphine from opium poppy, and after that new discoveries were made rapidly. In the early half of the 1900s, the main research around secondary plant metabolism was dedicated to the formation of secondary metabolites in plants, and this research was compounded by the use of tracer techniques which made deducing metabolic pathways much easier. However, there was still not much research being conducted into the functions of secondary plant metabolites until around the 1980s. Before then, secondary plant metabolites were thought of as simply waste products. In the 1970s, however, new research showed that secondary plant metabolites play an indispensable role in the survival of the plant in its environment. One of the most ground breaking ideas of this time argued that plant secondary metabolites evolved in relation to environmental conditions, and this indicated the high gene plasticity of secondary metabolites, but this theory was ignored for about half a century before gaining acceptance. Recently, the research around secondary plant metabolites is focused around the gene level and the genetic diversity of plant metabolites. Biologists are now trying to trace back genes to their origin and re-construct evolutionary pathways. In the present investigation, *Sargassum wightii* and *Turbinaraia conoides* were analysed for the phytochemicals studies by qualitative and quantitatively.

Materials and Methods

Collection of samples

The samples of *Sargassum wightii* and *Turbinaria conoides* were collected from shores of Mandapam, (south east coast of India). The collections were made during the low tidal and subtidal regions (up to 1m depth) by hand picking. The collected materials were washed thoroughly with marine water in the field itself to remove the epiphytes and sediment particles. Then the samples were packed separately in polythene bags in wet conditions and brought to the laboratory, then thoroughly washed in tap water followed by distilled water to remove the salt on the surface of the thalli. They were stored in 5% formalin solution. For drying, washed specimens were placed on blotting paper and spread out at room temperature in the shade. The shade dried samples were ground to fine powder using a tissue blender. The powdered samples were then stored in the refrigerator for further use.

Plate 1: Shows that experimental sea weeds *Sargassum wightii* and *Turbinaria conoides*

Qualitative Analysis of Phytochemical Substance

The extracts were subjected to phytochemical analysis to detect the presence of following biomolecules using the standard qualitative procedures (Trease *et al.,* 1989) [26].

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.
8. **Test for steroids:** 2 ml of acetic anhydride was added to extract and then 2ml of H_2SO_4 was added, the colour changes from violet to blue or green in samples extract indicates the presence of steroids.

9. **Test for reducing sugars:** A small fraction of extract was added vigorously with 5 ml of distilled water and filtered to the filtrates while equal volume of Fehling's solution was added and were shaken vigorously. A brick red precipitation indicates the presence of reducing sugars.

Quantitative Analysis of Phytochemicals

1. **Estimation of phenols**

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 was made by using double distilled water. 1ml of folin-ciocalteu phenol reagent was added to mixture and shaken. After 5 minutes 10ml of 7% sodium bicarbonate 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 with UV spectrophotometer. Total phenolic content is expressed as mgcaffic acid equivalents mg/100 gm dry weight samples were analysed in duplicated.

2. **Estimation of flavonoids**

Total flavonoid content was measured by the Aluminium 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/ml) was added to 10ml colorimetric flask containing 4ml of double distilled water. 0.3ml 5% sodium nitrate of 5%sodium nitrate, after 5minutes, 0.3ml of 10% aluminium chloride was added. At 6th minute, 2ml of 1M NaoH was added and the total volume was made up to 10ml with double distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510nm. Total flavonoid content was expressed as mg Catechin Equivalent (CE) per 100gm dry mass sample were analysed in duplicate.

Thin Layer Chromatography Analysis

Preparation of TLC plates

30gms of silica gel 'G' was weighed and made to a homogenous suspension with 60ml distilled water for two minutes. The suspension was distributed over the plate which was air dried until the transparency of the layer disappeared. The plates were dried in hot air oven at 110°C for 30 mins and then stored in a dry atmosphere and used whenever required.

Preparation of sample solution

Prior to TLC, samples are dissolved in a suitable solvent and then applied usually in 1-10µl volumes to the origins of a TLC sheet or plate.

Formula for calculating Rf value

Rf = (Distance traveled by the solute/Distance travelled by the solvent) 100; Application of the Substance Mixture for separation: The solutions of the different samples were taken in capillary tubes and were spotted on a TLC plate 2cm above its bottom.

Development of the chromatogram: After the application of the sample on the adsorbent the TLC plate was kept in the solvent in TLC glass chamber and allowed the mobile phase to move through adsorbent phase upto 3/4th of the plate. The separation took place and the colored spots were obtained.

Results and Discussion

Qualitative analysis of phytochemicals

The phytochemical analysis of nine different chemical compounds (Alkaloids Terpenoids, Saponins, Flavonoids, Tannin, Glycosides, Phenols, Steroids and Reducing sugars) were tested in different extracts such as aqueous, petroleum ether, actone and ethanol extracts respectively. However, all these chemicals were not extractable in one solvent. In *S. wightii* Alkaloid, Flavonoid and Saponins only present in Aqueous extract. For ethanol, Flavonoid, saponin and steroid; For petroleum ether flavonoid, saponin and phenol; In Acetone extracts tannin, terpenoid and phenol were absent rest of all the components were present (Table. 1). In *T. conoides* flavonoid, saponin, glycosides were present. In ethanol extracts alkaloids and glycosides only present. In petroleum ether extract alkaloids and saponins only present. For acetone, alkaloid, saponin, glycosides and steroids were present. Both sea weeds shows the different solvency in different extracts. Not a single solvent showing a complete result. A maximum of phytochemical yield by acetone extract of both sea weeds.

Sargassum weightii and *Turbinaria conoides* extract by using different solvents such as aqueous, petroleum ether, acetone and ethanol extracts were prepared to study the total phenolic, flavonoid and tannin content. The yield of extract obtained from 15gm of dry plant material was measured for each extract (Table: 2). The total phenolic content in the plant extracts was examined using Folin-Ciocalteus reagent and is expressed in terms of Gallic acid equivalent. The values obtained for the concentration of total phenolics are expressed as mg of GA/g of extract.

Table 1: Shows that qualitative analysis phytochemicals in *Sargassum wightii* and *Turbinaria conoides*

		Sargassum				***Turbinaria***			
S.no	**Test**	**AQ**	**EE**	**PE**	**AE**	**AQ**	**EE**	**PE**	**AE**
1.	Alkaloids	+	-	-	+	-	+	+	+
2.	Flavonoids	+	+	+	+	+	-	-	-
3.	Tannin	-	-	-	-	-	-	-	-
4.	Saponin	+	+	+	+	+	-	++	+
5.	Glycosides	-	-	-	+	+	+	-	+
6.	Phenols	-	-	+++	-	-	-	-	-
7.	Terpenoids	-	-	-	-	-	-	++	-
8.	Steroids	-	++	-	+	-	-	-	+

Table 2: Shows that O.D. value of Total phenolic and flavonoid content of *Sargassum wightii* and *Turbinaria conoides*

Sample	Extract	Phenolic	Flavonoid
S. wightii	Aqueous	2.5	3.1
	Petroleum ether	8.8	10.6
	Ethanol	21.2	20.5
	Acetone	22.5	21.5
T. conoides	Aqueous	1.2	1.1
	Petroleum ether	2.8	6.5
	Ethanol	7.1	15.5
	Acetone	14.6	22.5

The total phenolic content in the examined extracts ranged from 2.5 to 22.5 mg GA/g. The high concentrations of phenolics were obtained from ethanolic extract of *S. wightii*. The total flavonoid content in the examined extract ranged from 3.1 to 21.5 mg QE/g. The highest concentration of flavonoid was measured in the acetone extract of *S. wightii*. The total phenolic content in the examined extracts ranged from 1.2 to 14.6 mg GA/g. The high concentrations of phenolics were obtained from Acetone extract of *T. conoides*. The total flavonoid content in the examined extract ranged from 1.1 to 22.5 mg QE/g. The highest concentration of flavonoid was measured in the acetone extract of *T. conoides*.

Adegboye *et al.*, (2008) reported that the medicinal values of medicinal plant lies in these phytochemical compounds and as such procedure a definite physiological action on the human body. Saxena, (1989) Manicka25m also reported these phytochemical compounds were bioactive, easily that biodegradable and of narrow-spectrum activity against plant diseases. The anti-inflammatory effects of alkaloids and flavonoids were reported by (Hodek *et al.,* 2002) [9]. The effectiveness of glycosides in the treatment of congestive heart failure was reported by (Yakari *et al.,* 1995) [32]; while tannins and steroids were found to be used in the treatment of inflamed or ulcerated tissues.

TLC results

The Rf values for different spots for different extracts were determined and results have been tabulated in Table, along with the photographs of the few TLC plates

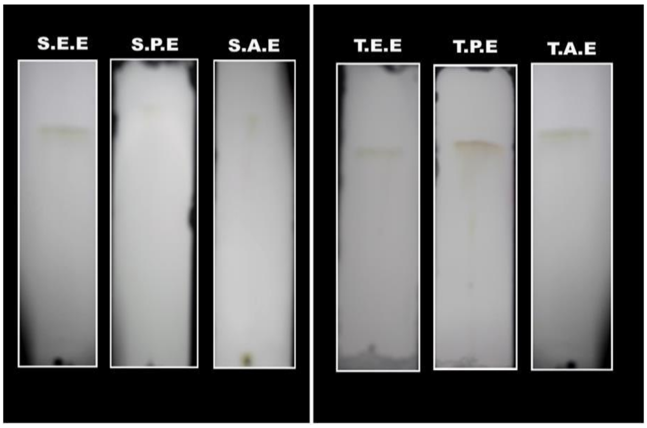

Plate 2: Shows that Thin layer chromatography of Ethanol, Petroleum ether and Acetone extracts of *S. wightii* and *T. conoides.*

The Rf values for different spots for different extracts were determined and results have been shown in the photographs in Plate.2. The Rf values were recorded 0.58,0.78 and 0.68 in ethanol, petroleum and acetone respectively; 0.65, 0.69 and 0.74 in ethanol, petroleum ether and acetone extract respectively when a solvent phase of Benzene: Ethyl acetate (1:1) was used.

By choosing the sea weeds in this study, investigation has found that it contained diverse groups of chemical compound as reported by Ibrahim *et al.* (2004). In related study by Tsui *et al.* (1996) [28], Makino *et al.* (1996), Satake *et al.* (1999), Nor Azah *et al.* (2010), Krishnamoorthy *et al.,* (2014a & b) [12, 13] that there were varieties of chemical elements of several plants are also reported.

Physicochemical and phytochemical analysis are used to check the genuine nature of the crude drug, thus it plays an important role in preventing the possible steps of adulteration (Mohan *et al.,* 2010) [18]. These phytoconstituents have been reported to have multiple biological effects such as anti-inflammatory, anti-allergic, antioxidant, antidiabetic, analgesic, antispasmodic, antibacterial, anti-viral, anti-cancer and aldose reductase inhibitory activities. It is also used for the treatment of diarrhea and dysentery (Patel *et al.,* 2011a and 2011b) [20, 21]. Phytoconstituents obtained from natural sources have been gaining importance in the day by day due to the health promoting activity. So it is necessary to check the quality safety and efficacy of herbal drugs before its consumption. Phytochemical standardization plays an important role to ensure the quality safety and efficacy of the herbal drug. In the last few decades, an HPTLC technique has gained much popularity for standardization of the herbal drugs and formulations. Analysis of several samples simultaneously using a small quantity of marker compound and mobile phase with very less time is the major advantage of HPTLC (Kshirsagar *et al.,* 2008; Krishnamoorthy *et al.,* 2014c) [15, 14].

Conclusion

In this present study, conclude that the secondary metabolites like phenols, flavonoids, alkaloids, saponins and tannins etc., are present in the experimental sea weed *S. wightii* and *T. conoides* qualitatively as well as quantitatively. The quantification of phytochemicals was proved by the method of GC analysis and spectrometry analysis. These bio-analytical parameters can be utilizes for the simultaneous analysis of different phyto-constituents present in the specimens. The different extracts of *Sargassum wightii* and *Turbinaria conoides* showed a number of active secondary metabolites. This report will lead to the isolation and characterization of these active secondary metabolites for bioefficacy and bioactivity. In future, this information may be useful as a standard to identify and elucidate the structural compound present in the plant species by GC-MS analysis.

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