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RESEARCH ARTICLE

Phytochemical Analysis of selected Seaweeds collected from Mandapam coast in Rameshwaram, Tamilnadu, India.

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Abstract

Seaweeds constitute a vital part of marine ecosystems. They are the reservoirs of carotenoids, pigments, polyphenols, enzymes, diverse functional polysaccharides. The secondary metabolites synthesized by seaweeds demonstrate a broad spectrum of bioactivity. Seaweeds samples such as (i) *Spathoglossum asperum* (ii) *Padina tetrasmatica* (iii) *Turbinaria conoides* were collected from Mandapam coast in Rameshwaram, Tamilnadu, India. Phytochemical screening of the seaweed extracts were carried out using standard methods. The present study screened the phytochemical properties of seaweeds with five extracts (aqueous, chloroform, ethanol, petroleum ether and acetone) and showed varied degree of phytochemicals present. The presence or absence of phytochemicals depends upon the solvent medium used for extraction and the physiological property of the seaweeds. From the results of the present study, it can be concluded that the seaweeds may be used as broad spectrum of antimicrobial and bioactive agent after extensive investigation.

INTRODUCTION

Seaweeds constitute a vital part of marine ecosystems. It was estimated that about 90% of the species of marine plant are algae and about 50% of the global photosynthesis is contributed from algae (Domettila *et al* 2013). Over the past decades, seaweeds have been used by humans as medicine and food and their extracts have generated an enormous amount of interest in the pharmaceutical industry as a fresh source of bioactive compounds with immense medicinal potential. Seaweeds are the reservoirs of carotenoids, pigments, polyphenols, enzymes, diverse functional polysaccharides. Seaweeds are excellent source of vitamin A, B1, B12, C, D and E (OM Skulberg, 2000).

The Southern Coast of India bears luxuriant growth of seaweeds. These vast varieties of seaweeds were potential reservoirs of biochemical compounds, which might be a potential source of drug discovery in the future (Tuney *et al*, 2006). More than 2400 marine natural products have been isolated from seaweeds. These natural products are known as secondary metabolites which possess a broad range of biological activity (E Taskin *et al*, 2006). From the findings it is well known that seaweeds contained antibacterial, antiviral, antifungal, cytotoxic and larvicidal potentials (Domettila *et al* 2013). The secondary metabolites synthesized by seaweeds demonstrate a broad spectrum of bioactivity. With this background, the present study was aimed to explore the bioactive potential of three major seaweeds collected from the south east coast of India as a potential source of marine bio prospecting.

Materials and Methods

Seaweed collection and processing

Three seaweed samples (i) *Spathoglossum asperum* (ii) *Padina tetrasmatica* (iii) *Turbinaria conoides* were collected from Mandapam coast in Rameshwaram, Tamilnadu, India. The algal sample was handpicked and washed thoroughly with seawater to remove all the impurities, sand particles and epiphytes. It was kept in icebox containing

slush ice, transported to the laboratory and washed thoroughly using tap water to remove the salt on the surface of the sample. The water was drained off and the algal material was spread on blotting paper to remove excess water. They were shade dried. The dried seaweeds were finally pulverized in the commercial grinder and the powdered seaweed samples were stored at 4°C and used for further analysis.

Solvent extraction

The samples were shade dried for 15 days and then pulverized into fine powder using pestle and mortar. The extraction was done by Soxhlet extraction. Different solvents were used successively with gradient polarity (aqueous, chloroform, petroleum ether, acetone and ethanol). The extracts were evaporated to complete dryness by vacuum distillation and stored in refrigerator for further use (Patra *et al*., 2008). Aqueous, methanol and ethanol solvents were prepared from the seaweed powder and used for the analysis.

Phytochemical Screening of given sample:

The phytochemical screening of given sample were assessed by standard method as described by Savithramma *et al.,* (2011). Phytochemical screening was carried out on the given sample using different solvents to identify the major natural chemical groups such as tannins, saponins, flavonoids, phenols, terpenoids, alkaloids, glycosides, cardiac glycosides**,** coumarins and steroids. General reactions in these analyses revealed the presence or absence of these compounds in the algal extracts tested.

Phytochemical analysis

1. Test for Tannins

For tannin identification, 1 mL of the algal extract, one mL of ferric chloride (5% $FeCl_3$) was added. Formation of dark blue or greenish black indicates the presence of tannins.

2. Test for Saponins

For tannin identification, 2mL algal extract, 2mL of distilled water was added and shaken in graduated cylinder for 15 min lengthwise. Formation of 1cm layer of foam indicates the presence of saponins

3. Test for Quinones

For Quinone identification, 1mL algal extract, 1mL of concentrated sulphuric acid (H_2SO_4) was added. Formation of red colour indicates the presence of Quinones

4. Test for Flavonoids

For flavonoid identification, 2mL of algal extract, 1mL of 2N sodium hydroxide (NaOH) was added. Formation of yellow colour indicates the presence of flavonoids.

5. Test for Alkaloids

For Alkaloid identification, 2mL algal extract, 2mL of concentrated Hydrochloric acid (HCl) was added. Then few drops Mayer's reagent was added**.** Presence of green colour or white precipitate indicates the presence of alkaloids.

6. Test for Glycosides

2mL of the algal extract, 3mL of chloroform and 10% ammonium solution was added. Formation of pink colour indicates the presence of glycosides.

7. Test for Cardiac glycosides

For Cardiac glycosides identification, 0.5 mL of the algal extract, 2 mL of glacial acetic acid and few drops of 5 % ferric chloride were added. This was under layered with 1 mL of concentrated sulphuric acid. Formation of brown ring at interface indicates the presence of cardiac glycosides.

8. Test for Terpenoids

For Terpenoids identification, 0.5 ml L of the algal extract, 2 mL of chloroform along with concentrated Sulphuric acid. Formation of reddish brown colour at the interface indicates the presence of Terpenoids.

9. Test for Phenols

For phenol identification, 1mL of the algal extract, 2mL of distilled water followed by few drops of 10 % ferric chloride was added. Formation of blue / green colour indicates the presence of phenols

11. Test for Steroids

For steroid identification, 0.5 mL of the algal extract, 2 mL of chloroform and 1 mL of Sulphuric acid ($H_2 SO_4$) were added. Formation of reddish brown ring at interface indicates the presence of steroids.

10. Test for Coumarins

For coumarins identification, 1 mL of algal extract, 1 mL of 10 % NaOH was added. Formation of yellow colour indicates the presence of coumarins.

12. Test for Anthocyanin and Beta cyanin

To 2mL of the algal extract, one mL of 2N sodium hydroxide (NaOH) was added and heated for 5 min at 100 °C. Formation of bluish green colour indicates the presence of anthocyanin and formation of yellow colour indicates the presence of betacyanin.

Result

Phytochemicals such as tannins, saponins, flavonoids, steroids, glycosides, alkaloids, anthraquinones glycosides, cardioactive glycosides of the different extracts of the seaweeds were carried out. In the present study the phytochemical screening was performed with aqueous, chloroform, ethanol, petroleum ether and acetone extracts of three different marine algae. The results were depicted in table -1.

Tannins were found only in the ethanol extract of *T.conoides*. Tannins did not show any positive result in any of the extract in *P.tetrasmatica* and *S.asperum*. Saponins showed its presence in ethanol extract of all three seaweeds tested. The chloroform extract of *S.asperum* and *P.tetrasmatica* and petroleum ether and acetone extract of *S.asperum*, saponins were present. Quiniones showed its presence in ethanol, chloroform and acetone extract of three seaweeds tested. Terpenoids and steroids showed its presence in ethanol, chloroform and acetone extract of the three seaweeds tested. Flavanoids and alkaloids showed its presence in ethanol and acetone extract of all the three seaweeds. Phenols were also found in all the extracts. Flavanoids also showed its presence in aqueous extract. Glycosoides and anthocyanin showed its negative result in all the extracts tested. Cardiac glycosides and coumarins showed a maximum presence in ethanol and acetone extract.

Among the five different extracts, ethanol extract showed the presence of maximum number (11) of compounds. Next to that, acetone extracts showed ten compounds. chloroform and aqueous extracts showed the presence of six compounds.

Table 1. Phytochemical screening from algal extracts

Phytochemicals Tested	Seaweed tested	Extracts				
		Aqueous	Ethanol	Chloroform	Petroleum ether	Acetone
Tannins	***P.tetrasmatica***	-	-	-	-	-
	S.asperum	-	-	-	-	-
	T.conoides	-	+	-	-	-
Saponins	***P.tetrasmatica***	-	+	-	-	-
	S.asperum	-	+	+	+	++
	T.conoides	-	+	+	-	-
Quinones	***P.tetrasmatica***	-	+	+	-	++
	S.asperum	+	+	+	-	++
	T.conoides	-	+	+	-	++
Terpenoids	***P.tetrasmatica***	++	+	+	-	+
	S.asperum	+	+	+	-	++
	T.conoides	-	+	+	-	+
Steroids	***P.tetrasmatica***	++	+	+	-	+
	S.asperum	+	+	+	-	++
	T.conoides	-	+	++	-	+
Flavonoids	***P.tetrasmatica***	+	+	+	-	+
	S.asperum	+	+	-	-	++
	T.conoides	-	+	-	-	-
Phenol	***P.tetrasmatica***	+	++	+	+	+
	S.asperum	++	++	+	+	+
	T.conoides	-	+	+	-	+

Alkaloids	***P.tetrasmatica***	-	+	-	-	+
	S.asperum	+	+	-	-	+
	T.conoides	-	+	-	-	+
Glycosides	***P.tetrasmatica***	-	-	-	-	-
	S.asperum	-	-	-	-	-
	T.conoides	-	-	-	-	-
Cardiac glycosides	***P.tetrasmatica***	+	+	-	-	+
	S.asperum	-	+	-	+	++
	T.conoides	+	+	-	+	++
Coumarins	***P.tetrasmatica***	-	+	+	-	+
	S.asperum	+	+	+	-	++
	T.conoides	-	+	-	-	-
Antho cyanin	***P.tetrasmatica***	-	-	-	-	-
	S.asperum	-	-	-	-	-
	T.conoides	-	-	-	-	-
Beta cyanin	***P.tetrasmatica***	+	+	+	+	+
	S.asperum	+	+	+	+	+
	T.conoides	+	+	-	-	-

++ = strong positive; + = positive; - = negative

DISCUSSION

The present study screened the phytochemical properties of three marine algae (*S.asperum*, *P.tetrasmatica* and *T.conoides*) with five extracts and showed varied degree of phytochemicals present. The presence or absence of the phytochemicals depends upon the solvent medium used for extraction and the physiological aspect of the sea weeds selected.

In the present study phenolic compounds were noticed in all the extracts of *S.asperum* and *P.tetrasmatica* . In general, phenolic compounds possessed specific physical, chemical and biological activities that make them useful as drugs. Phenolics were also responsible for the antimicrobial, anti-inflammatory, anti-feedant, anti-viral, anticancer and vasodilatory actions (Aliyu *et al*, 2009). Saponins are considered as a key ingredient in traditional chinese medicine and are responsible for most of the observed biological effects. Saponins are known to produce effect on inflammation and it is commercially exported as dietary supplements (BK Manjunatha, 2006) In the present study Saponins showed its presence in ethanol extract of all three seaweeds tested. Saponins also showed its presence in chloroform extract of *S.asperum* and *P.tetrasmatica* and petroleum ether and acetone extract of *S.asperum*.

Tanins were found only in the ethanol extract of *T.conoides.* Tannins did not show any positive result in any of the extract in *P.tetrasmatica* and *S.asperum*.. Tannins were used therapeutically as antiviral, antibacterial, antiulcer and antioxidant agents. Many tannin containing drugs are used in the treatment of piles, inflammation, burns and as astringent (H Kolodziej *et al* , 2005). Steroids of plant origin are known to be important for insecticidal, antimicrobial, antiparasitic and cardiotonic properties.

Steroids also play an important role in nutrition, herbal medicine and cosmetics (DE Okwu, 2001). Coumarins were noticed in chloroform, acetone and ethanol extracts. Coumarins have been used as anti-coagulant to treat lymphedema. The various phytochemicals detected from the seaweeds are known to have beneficial importance in industrial and medicinal sciences (S Jeeva *et al*, 2012). Seaweeds with secondary metabolites have enormous therapeutical potential and from these results it can be concluded that the selected seaweed extracts may be used as broad-spectrum as antimicrobial, bioactive agent after extensive investigation. Further work will emphasize the isolation and characterization of active principles responsible for bio-efficacy and bioactivity.

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