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SCREENING OF PRELIMINARY PHYTOCHEMICALS OF *GRACILARIA CYLINDRICA* BOERGESEN IN KOOTHANKUZHI, TIRUNELVELI DISTRICT, TAMIL NADU, INDIA

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Abstract:

In the present study, the preliminary phytochemical constituents were screened from Gracilaria cylindrica Boergesen from Koothankuzhi, Tirunelveli district in the south east coast of Tamil Nadu, India. The preliminary phytochemical analysis was conducted in seven extracts namely methanol, acetone, chloroform, ethyl acetate, petroleum ether, benzene and hexane by Harborne method. The preliminary phytochemical analysis showed the presence of alkaloids, anthocyanin, anthraquinones, cardiac glycosides, coumarins, diterpenes, flavonoids, glycosides, phenols, phlobatannins, steroids, quinones, saponins, tannins and terpenoids. Alkaloids showed the maximum presence being found in seven different extracts, followed by cardiac glycosides, coumarins and flavonoids in six extracts, phenols, saponins and steroids in five extracts, glycosides and tannins in four extracts, anthocyanin and diterpenes in three extracts, anthraquinones, phlobatannins, quinines and terpenoids in two extracts. From the results, it was concluded that the extracts of Gracilaria cylindrica Boergesen was found to be the presence of a number of active secondary metabolites. This report will lead to the isolation and characterization of these active secondary metabolites for bioefficacy and bioactivity.

Keywords: *Phytochemical, Seaweed extracts, Gracilaria cylindrica, Tamil Nadu*

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INTRODUCTION:

Marine organisms are not generally used in traditional medicine, but recently they represent a massive resource for the development of potential therapeutic agents in the field of medicine [1]. Most of the marine organisms are soft-bodied and they are not able to use mechanical defense mechanisms like shelter or ability to escape, thus they need chemical defense mechanisms to survive [2]. Therefore, they have created an efficient defense mechanism that helps them to survive during evolution and to avoid extinction [3]. This mechanism encompasses the ability to synthesize or accumulate toxic metabolites and the secretion of highly toxic metabolites as they are rapidly diluted in the ocean water [4]. The metabolites secreted by marine organisms are characterized by the presence of halogen unlike the terrestrial secondary metabolites [5]. For these reasons, and because of the high biological diversity in the sea [6, 7], marine organisms have attracted researchers to find useful drugs for mankind [8]. Therefore, the aim of the present investigation is to screen the presence of secondary metabolites in *Gracilaria cylindrica* Boergesen collected from Koothankuzhi, Tirunelveli district in the south east coast of Tamil Nadu, India.

MATERIALS AND METHODS:

Collection of plant materials

The collection of *Gracilaria cylindrica* Boergesen belonging to Rhodophyceae (Red algae) was made during the low tidal and subtidal regions (up to 1m depth) by hand picking. *Gracilaria cylindrica* Boergesen was collected from Koothankuzhi, Tirunelveli district in the south east coast of Tamil Nadu, India during the month of January 2017. 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 grounded to fine powder using a tissue blender. The powdered samples were then stored in the refrigerator for further use 30g powdered samples were packed in Soxhlet apparatus and extracted with methanol, acetone, chloroform, ethyl acetate, petroleum ether, benzene and hexane for 8h separately [9].

Preliminary Phytochemical analysis

The different extracts (methanol, acetone, chloroform, ethyl acetate, petroleum ether, benzene and hexane) of *Gracilaria cylindrica* were tested for alkaloids, anthocyanin, anthraquinone, cardiac glycosides, coumarins, diterpenes, flavonoids, glycosides, phenols, phlobatannins, steroids, quinones, saponins, tannins and terpenoids. Phytochemical screening of the extracts was carried out according to the standard methods.

Preparation of extracts

For the preparation of different extracts, the plant specimens were washed thoroughly and placed on blotting paper and spread out at room temperature in the shade condition for drying. The shade dried samples were grounded to fine powder using a tissue blender. The powdered samples were then stored in the refrigerator for further use. 30g powdered samples were packed in Soxhlet apparatus and extracted with methanol, acetone, chloroform, ethyl acetate, petroleum ether, benzene and hexane for 8h separately.

In the preliminary phytochemical analysis of the selected red seaweeds, fifteen different types of secondary metabolites (alkaloids, anthocyanin, anthraquinone, cardiac glycosides, coumarins, diterpenes, flavonoids, glycosides, phenols, phlobatannins, steroids, quinones, saponins, tannins and terpenoids) were tested in seven different extracts using the standard procedure of Harborne [10].

Test for alkaloids

1ml of 1% HCl was added to the 2ml of extract in a test tube and was treated with few drops of Mayer's reagent. A creamy white precipitate indicates the presence of alkaloids.

Test for anthocyanin

1ml of 2N HCl was added to the 1ml of extract and was treated with NH3. Pink red colour turns blue violet.

Test for anthraquinone

2ml of extract was mixed with 1ml of benzene and 1ml of 10% ammonia solution was added. The presence of a pink, red or violet color indicates the anthraquinones.

Test for cardiac glycosides

0.4ml of glacial acetic acid was added with 1ml extract and trace amount of FeCl3 and 0.5ml Conc. H_2SO_4. Blue colour indicates the presence of cardiac glycosides.

Test for coumarins
1ml of seaweed extract was added with 1ml of 10% NaOH. Formation of yellow colour indicates the presence of coumarins.

Test for diterpenes
1ml extract was added with 1ml dis. H2O and 10 drops of copper acetate solution. Emerald green colour indicates the presence of diterpenes.

Test for flavonoids
A few drops of 1% NH_3 solution was added to 2 ml of extract in a test tube. Yellow
coloration indicates the presence of flavonoids.

Test for glycosides
2ml of 50% H_2SO_4 was added to 2ml of extract in a boiling tube. The mixture was heated in boiling water bath for 5 min. 10ml of Fehling's solution was added and boiled. A brick red precipitate indicates the presence of glycosides.

Test for phenolic groups
To 1ml extract, add 2ml distilled water followed by few drops of 10% Ferric chloride. The formation of blue or black colour indicates the presence of phenolic groups.

Test for phlobatannins
1ml extract was added with 1% aqueous HCl and then boiled. Red precipitate indicates the presence of phlobatannins.

Test for steroids
1ml of extract added to 1ml $CHCl_3$ and few drops of Conc. H_2SO_4. Golden red colour or Brown colour indicates the presence of phytosteroids.

Test for quinones
1ml seaweed extract added with 1ml of alcoholic KOH. Red to blue colour indicates the presence of quinones.

Test for saponins
2ml of extract was shaken vigorously with 5ml distilled water to obtain stable persistent foam. The formation of emulsion indicates the presence of saponins.

Test for tannins
To 2ml extract, 1ml of distilled water and 1-2 drops of ferric chloride solution was added and observed for brownish green or a blue black coloration indicates the presence of tannins.

Test for terpenoids
2ml extract was mixed with 2ml of $CHCl_3$ in a test tube. 3ml Conc. H_2SO_4 was added carefully along the wall of the test tube to form a layer. An interface with a reddish brown coloration confirms the presence of terpenoids.

RESULTS AND DISCUSSION:

Seaweeds are the richest resource of structurally novel and biologically active metabolites. So far many chemically unique compounds of seaweeds with different biological activities have been isolated and a number of them are under investigation and being developed as new pharmaceuticals. Seaweeds are potentially excellent sources of highly bioactive secondary metabolites that could lead to the development of new functional ingredients (Pelegrin *et al*., 2008). Seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites.

In the preliminary phytochemical analysis of *Gracilaria cylindrica* Boergesen, fifteen different types of secondary metabolites (alkaloids, anthocyanin, anthraquinone, cardiac glycosides, coumarins, diterpenes, flavonoids, glycosides, phenols, phlobatannins, steroids, quinones, saponins, tannins and terpenoids) were tested in seven different extracts of *Gracilaria cylindrica* Boergesen. Thus, out of (1x7x15) 105 tests for the presence or absence of the above compounds, 62 tests gave positive results and the remaining gave negative results.

Table 1: Preliminary phytochemical analysis of *Gracilaria cylindrica* Boergesen

Tests	Solvents						
	Methanol	Acetone	Chloroform	Ethyl Acetate	Petroleum Ether	Benzene	Hexane
Alkaloids	+	+	+	+	+	+	+
Anthocyanin	+	+	+	-	-	-	-
Anthraquinones	+	-	+	-	-	-	-
Cardiac glycosides	+	-	+	+	+	+	+
Coumarins	+	+	+	+	-	+	+
Diterpenes	+	+	-	-	-	-	+
Flavonoids	+	+	+	+	-	+	+
Glycosides	+	+	+	+	-	-	-
Phenols	+	+	+	-	-	+	+
Phlobatannins	+	-	+	-	-	-	-
Quinones	+	-	+	-	-	-	-
Saponins	+	-	+	+	+	+	+
Steroids	-	+	+	+	-	+	+
Tannins	+	+	+	+	+	+	+
Terpenoids	+	+	-	-	-	-	-

The 62 positive results showed the presence of alkaloids, anthocyanin, anthraquinones, cardiac glycosides, coumarins, diterpenes, flavonoids, glycosides, phenols, phlobatannins, steroids, quinones, saponins, tannins and terpenoids. Alkaloids showed the maximum presence being found in seven different extracts, followed by cardiac glycosides, coumarins and flavonoids in six extracts, phenols, saponins and steroids in five extracts, glycosides and tannins in four extracts, anthocyanin and diterpenes in three extracts, anthraquinones, phlobatannins, quinines and terpenoids in two extracts. Among the various solvent extracts studied, methanol showed the presence of maximum number of fourteen secondary metabolites, followed by chloroform with thirteen compounds, acetone with ten compounds, hexane with nine compounds, ethyl acetate and benzene with eight compounds each. The minimum number of four compounds was present in petroleum ether extract (Table 1).

CONCLUSION:

Based on the results obtained from the present study, it can be concluded that *Gracilaria cylindrica* Boergesen was found to be the presence of a number of active secondary metabolites namely alkaloids, anthocyanin, anthraquinones, cardiac glycosides, coumarins, diterpenes, flavonoids, glycosides, phenols, phlobatannins, steroids, quinones, saponins, tannins and terpenoids. Alkaloids showed the

maximum presence being found in seven different extracts, followed by cardiac glycosides, coumarins and flavonoids in six extracts, phenols, saponins and steroids in five extracts, glycosides and tannins in four extracts, anthocyanin and diterpenes in three extracts, anthraquinones, phlobatannins, quinines and terpenoids in two extracts. This report will lead to the isolation and characterization of these active secondary metabolites for bioefficacy and bioactivity.

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