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PRELIMINARY PHYTOCHEMICAL PROFILE OF *HYPNEA FLAGELLIFORMIS* GREVILLE EX J.AG. IN KOOTHANKUZHI COAST, TIRUNELVELI DISTRICT, TAMIL NADU, INDIA

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ABSTRACT

The study was conducted to identify the preliminary phytochemical constituents present in *Hypnea flagelliformis* Greville ex J.Ag. from Koothankuzhi coast, Tirunelveli district, the south east coast of Tamil Nadu, India. The preliminary phytochemical analysis was carried out 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 anthocyanins, alkaloids, phytosteroids, tannins, saponins, flavonoids, quinones, glycosides, cardiac glycosides, terpenoids, phenols, anthraquinones, phlobatannins, coumarins and diterpenes. Among the various secondary metabolites studied, coumarins, phenols and tannins showed the maximum presence, being found in six different extracts and anthocyain, cardiac glycosides, glycosides and phlobatannins were observed only in three extracts. From the observation, it was recorded that the various extracts of *Hypnea flagelliformis* Greville ex J.Ag. was found to be the presence of a number of active secondary metabolites. This result will lead to the isolation and characterization of these active secondary metabolites for bioactivity.

KEY WORDS: Phytochemical, Metabolites, Seaweed extracts, *Hypnea*, Tamil Nadu

INTRODUCTION

The use of medicinal plants as treatments against various diseases can be traced back to early civilizations in China, India and the Near East, thus making phytomedicine doubtlessly one of mankind's oldest professions [1]. Medicinal plants have been used for centuries as remedies for human diseases and have provided new sources of chemical compounds with biological activity [2].

The World Health Organisation (WHO) has pointed it out that medicinal plants could be the best source to obtain a variety of natural drugs [3]. Therefore, there has been a global resurgence in the use of herbal preparations in disease management in all continents of the World and most developing countries are now integrating phytomedicine into their health care systems [4]. Seaweeds are marine macro algae, divided into three categories based on the pigments, reserved food materials and color, such as red (4,500 species), green (900 species) and brown (1,000 species). These plants constitute a total of around 30,000 species, with a great diversity of forms and sizes. Seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities. The environment in which seaweeds grow is harsh as they are exposed to a combination of light and high oxygen concentrations.

These factors can lead to the formation of various phytochemicals. This fact implies that seaweed cells have some protective mechanisms and compounds [5]. Extensive screening of seaweeds has led to the isolation and chemical determination of over 15, 000 compounds including fatty acids, sterols, phenolic compounds, terpenes, enzymes, polysaccharides, alkaloids and flavonoids.

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In this background, the present study indented to evaluate the preliminary phytochemical profile of *Hypnea flagelliformis* Greville ex J.Ag. in Koothankuzhi coast, Tirunelveli district, Tamil Nadu, India.

MATERIAL AND METHODS

Collection of plant materials

The collection of *Hypnea flagelliformis* Greville ex J.Ag. belonging to Rhodophyceae (Red algae) was made during the low tidal and subtidal regions (up to 1m depth) by hand picking. The collected plant 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.

Preliminary phytochemical analysis

The different extracts namely methanol, acetone, chloroform, ethyl acetate, petroleum ether, benzene and hexane of *Hypnea flagelliformis* Greville ex J.Ag. were analyzed for the presence of anthocyanins, alkaloids, phytosteroids, tannins, saponins, flavonoids, quinones, glycosides, cardiac glycosides, terpenoids, phenols, anthraquinones, phlobatannins, coumarins and diterpenes. The preliminary phytochemical screening of the various extracts was carried out according to the standard methods [6].

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 namely methanol, acetone, chloroform, ethyl acetate, petroleum ether, benzene and hexane for 8h separately.

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 anthraquinones

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 catechin: 2ml extract was mixed with Ehrlich's reagent and few drops of Conc. HCl. Formation of pink colour indicates the presence of catechin.

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 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.

RESULT AND DISCUSSION

Since ancient times, seaweeds have been closely associated with human mankind and have been exhaustively used in numerous ways as a source of food, feed, fertilizer and medicine which are chiefly utilized for economically important phycocolloids [1,2]. It was noted that seaweeds possesses more than 60 trace elements in much concentration higher than in terrestrial plants. Seaweeds also contain carbohydrates, proteins, lipids, iodine, bromine, vitamins and substances of stimulatory and antibiotic nature. The phytochemicals from seaweeds are extensively used in various industries such as food, confectionary, textile, pharmaceutical, dairy and paper that mostly as gelling, stabilizing and thickening agents. In this context, the presence of secondary metabolites was analyzed in the present study.

In the preliminary phytochemical analysis of *Hypnea flagelliformis* Greville ex J.Ag. fifteen different types of secondary metabolites (anthocyanins, alkaloids, phytosteroids, tannins, saponins, flavonoids, quinones, glycosides, cardiac glycosides, terpenoids, phenols, anthraquinones, phlobatannins, coumarins and diterpenes) were tested in seven different extracts of *Hypnea flagelliformis* Greville ex J.Ag. Thus, out of (1x7x15) 105 tests for the presence or absence of the above compounds, 67 tests gave positive results and the remaining gave negative results. The 67 positive results showed the presence of anthocyanins, alkaloids, phytosteroids, tannins, saponins, flavonoids, quinones, glycosides, cardiac glycosides, terpenoids, phenols, anthraquinones, phlobatannins, coumarins and diterpenes. Coumarins, phenols and tannins show the maximum presence, being found in six different extracts. Followed by, alkaloids, anthraquinones, flavonoids, saponins and terpenoids showed the presence in the five extracts. Anthocyanins, cardiac glycosides, glycosides and phlabatannins showed the minimum of only in three extracts. Among the seven different extracts, the methanol extract showed the presence of the maximum number (12) of compounds. Next to methanol extract, hexane extract showed the presence of eleven compounds and the ethyl acetate extract showed the presence of minimum (6) compounds (Table-1).

Table1: Preliminary phytochemical analysis of *Hypnea flagelliformis* Greville ex J.Ag.

Tests	SOLVENTS						
	Met.	Ace.	Chl.	EA	PE	Ben.	Hex.
Alkaloids	+	+	+	-	+	-	+
Anthocyanin	-	-	-	+	-	+	+
Anthraquinone	+	+	+	-	-	+	+
Cardiac Glycosides	-	+	-	+	+	-	-
Coumarins	+	+	+	-	+	+	+
Diterpenes	+	+	+	-	-	-	+
Flavonoids	+	+	+	+	-	+	-
Glycosides	+	+	-	-	-	+	-
Phenols	+	+	-	+	+	+	+
Phlobatannins	+	-	-	-	-	+	+
Phyto steroids	+	-	+	+	+	-	-
Quinones	-	+	-	-	+	+	+
Saponins	+	+	-	+	+	-	+
Tannins	+	+	+	-	+	+	+
Terpenoids	+	+	+	-	+	-	+

CONCLUSION

In the present study, the preliminary phytochemical analysisvof *Hypnea flagelliformis* Greville ex J.Ag. from Koothankuzhi coast, Tirunelveli district, the south east coast of Tamil Nadu, India. was studied. The selected red seaweed was found to have the presence of a number of active secondary metabolites namely anthocyain, alkaloids, cardiac glycosides, coumarins, flavonoids, phenols, phlobatannins, quinones, saponins, steroid, tannins and terpenoids. Among the various secondary metabolites studied, coumarins, phenols and tannins showed the maximum presence, being found in six different extracts and anthocyain, cardiac glycosides, glycosides and phlobatannins were observed only in three extracts.

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CONFLICT OF INTEREST:
The authors declare that they have no conflict of interest.

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