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Phytochemical screening of marine red alga *Botryocladia leptopoda* (J.Agardh) Kylin

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Abstract: **The present examination was planned to comprehend the phytochemical screenings of the methanol extract of marine red alga *Botryocladia leptopoda*. The methanol extract of *B. leptopoda* demonstrated the nearness of steroids, anthroquinones, alkaloids, triterpenoids, saponins, glycosides, flavonoids, phenols and tannins. In this way, *B. leptopoda* has significant lots of primary and secondary phytochemicals. Among the phytochemical contents the triterpenoids and glycosides are available in higher amounts. The present study on *B. leptopoda* exhibited various novel phytochemical, which can exploit and used for human well beings.**

Keywords: **Marine algae, *Botryocladia leptopoda*, Phytochemicals, Phenols.**

1. INTRODUCTION

Marine algae has been nearly connected with human life and has been comprehensively utilized as a part of various courses as a source of nourishment, feed, fertilizer and medicine, and mainly used for economically important phycocolloids (Chapman, 1970). In India, a significant number of the rough shorelines, mudflats, estuaries, coral reefs and tidal ponds along the drift give perfect habitats to the development of seaweed growth. In all, 271 genera and 1153 species of seaweeds have been counted till date from the Indian waters (Rao and Mantri, 2006).

Natural products are accepted to have the benefit of having huge structural and chemical diversity, expanded protein binding characteristics and specific biological activity. Bioactive compounds from marine algae are broad over a wide span of time use in the treatment of numerous diseases and serve as compounds of attention both in their normal form and as templates for synthetic change.

Herbal drugs may not only be valuable as remedies, as well as growth stimulators, stress resistance boosters and anti-infection agents (Bhavan *et al.*, 2014; Dhanalakshmi *et al.*, 2016). Consequently, herbal drugs are accomplishing success in disease management, since they are cheap, ecofriendly and have fewer side effects. A few hundred plant varieties are used as in pharmaceutical, and stand for fundamental sources for successful medicine (Pandey *et al.*, 2012).

Marine algae contain noteworthy pharmacological and biological active components such as flavonoids, terpenoids, carotenoids, dietary fiber, protein, essential fatty acids, vitamins and minerals. The attention in marine algae as a potential source of capable new bioactive natural agents has expanded late (Molinski *et al.*,2009). The phytochemicals from marine algae are widely used in different ventures such as food, confectionery, textile, pharmaceutical, dairy and paper, for the most part as gelling, stabilizing and thickening agents.

Natural products from marine algae are known for their strong and expansive range of bioactivities including antimicrobial, antiviral, anti-helminthic, antituberculosis, antimycobacterial, antioxidative, anticoagulant, anti-inflammatory, antipyretic, analgesic, anticancer, insecticidal, antidiabetic and antiprotozoan activities (Lauritano *et al.*, 2016). As far back as researchers faces an awesome test in distinguishing new successful medicines for numerous life intimidating diseases. In this way, everywhere on the planet, numerous researchers have an eye on the natural sources for novel molecule discovery. The current examination was carried to assess the phytochemical constituents of methanol extract of marine red alga *B. leptopoda*.

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2. MATERIALS AND METHODS

2.1 Seaweed collection

The red seaweed, *Botryocladia leptopoda* (J.Agardh) Kylin was collected from inter-tidal rocky shore of the Mandapam, Gulf of Mannar, South East coast of Tamil Nadu, India in September-December 2017. The seaweed species were distinguished by Dr.R.Veeragurunathan, Scientist, CSIR-Central Salt & Marine Chemicals and Research Institute, Mandapam, Tamilnadu, India. The fresh samples were washed with sea water, followed by fresh water to remove salts, epiphytes, microorganisms and other suspended materials, sponged up and extend to dry at room temperature for 2 weeks.

2.2 Preparation of sample for phytochemicals analysis

Shade dried *B. leptopoda* sample was ground to coarsely and powder was put away in sterilized containers for further treatment. The coarsely powdered sample was extracted using a soxhlet apparatus by methanol. The extract was filtered by using double layer muslin cloth, concentrated on 40-50°C using a rotary vacuum evaporator (ROTAVAP) joined with a vacuum pump and cryostat and dried at 40°C under hot air oven. The dark, gummy solid obtained was used for further investigations.

2.3 Phytochemical Analysis

Shade dried, powdered material of the *B. leptopoda* was extracted frequently for 6 hrs with 80% methanol (1:10 ratio) in a soxhlet apparatus. The extract was then dried over anhydrous calcium chloride in a desiccator and the deposit was redissolved in 100 mL of 80% ethanol. Tests for different phytochemicals were done using the residue extract (Peach and Tracoy, 1955).

2.3.1 Test for Alkaloids

2 mL of concentrated Hydrochloric acid (HCl) was included to 2 mL algal extract. At that point include a couple drops Mayer's reagent. The nearness of green colour or white precipitate demonstrated the occurrence of alkaloids.

2.3.2 Test for triterpenoids

Roughly 2 mg of dry extract was shaken with 1 mL of chloroform and a couple drops of concentrated sulfuric acid were included on the edge of the test tube. A red brown colour produced at the border showed the test as positive for triterpenoids.

2.3.3 Test for Anthroquinones

Around 0.2 g of the extracts was boiled with 10% HCl for a couple of minutes in a water tub. It was separated and permitted to cool. Equal volumes of $CHCl_3$ were included to the filtrate. Scarcely any drops of 10% NH_3 were included in the blend and warmed. The appearance of pink colour shows the nearness of anthraquinones.

2.3.4 Test for Steroids

2 mL of chloroform and 1 mL of sulphuric acid was included to 0.5 mL of the algal extract. A development of the reddish brown ring at border shows the nearness of steroids.

2.3.5 Test for Tannins

One mL of ferric chloride (5% $FeCl_3$) was included to 1 mL of the algal extract. The appearance of dark blue or greenish black colour shows the nearness of tannins.

2.3.6 Test for Saponins

2 mL of distilled water was included to 2 mL algal extract and stunned in a graduated cylinder for 15 min lengthwise. The development of 1 cm layer of froth shows the presence of saponins.

2.3.7 Test for Flavonoids

1 mL of 2N sodium hydroxide (NaOH) was included to 2 mL of algal extract. The development of yellow colour shows the nearness of flavonoids.

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2.3.8 Test for Phenols

Mix 2 mL of distilled water, followed by a couple of drops of 10% ferric chloride was included to 1 mL of the algal extract. The development of blue/green colour shows the nearness of phenols.

2.3.9 Test for Glycosides

Mix 3 mL of chloroform and 10% ammonium solution was included to 2 mL of the algal extract. The development of pink colour shows the nearness of glycosides.

2.3.10 Quantitation ofphytochemicals

A liquid chromatograph from Shimadzu with an LC- 10 AT VP pump, an SCL – 10A VP, control system, an SIL – 10AD VP auto sampler, an SPD 10AV VP spectrophotometric detector, a DGU – 14A degasser and a computer system Class VP (version 5.0) were used. The analyses were carried out on a Luna C_{18} 250 x 4.6 mm, 5 µm. The mobile phase was composed of different proportions of (A) Acetonitrile (B) methanol and (C) acidified water. The initial mobile phase composition was 5% B and 90% C, followed by a linear gradient to 10% B and 85% C in 5 min; 5–30 min, from 85 to 80% C and B constant; 30–38 min, from 10 to 30% A and 80 to 70% C; 38–50 min, from 30 to 60% A and 70 to 40% C. The post-running time was 5 min. The flow rate was 1 mL/min, the column temperature was set at 25C, and the sample injection volume was 20 L. The acquisitions were performed in the range 190– 450 nm and the chromatograms were recorded. A stock solution of 1 mg/mL was prepared by dissolving each PA and FL standard in methanol. Working standard solutions were made by gradual dilution with the mixture of acidified water/ACN/MeOH (9:0.5:0.5, v/v/v) to the required concentration, which was based on the sensitivity of detection and the linearity range identified. Identification of PAs and FLs was performed by comparing retention times and absorption spectra of the unknown peaks with reference standards.

2.4 Statistical analysis

All the tests were carried out in triplicates and the data for phytochemical analysis subjected to analysis of variance (ANOVA) using SPSS version (17.0). The outcomes were expressed as mean ± standard error (SE).

3. RESULTS

In the preparatory phytochemical examination of crude methanol extract of *B. leptopoda* demonstrated the presence higher content of triterpenoids (0.45 ± 0.00 mg/g dry wt.), glycosides (0.33 ± 0.00 mg/g dry wt.), phenols (0.28 ± 0.00 mg/g dry wt.), saponins (0.24 ± 0.00 mg/g dry wt.), steroids (0.20 ± 0.00 mg/g dry wt.), and low level of alkaloids (0.15 ± 0.00 mg/g dry wt.), anthraquinones (0.13 ± 0.00 mg/g dry wt.), flavonoids (0.12 ± 0.00 mg/g dry wt.), and tannins (0.10 ± 0.00 mg/g dry wt.) (Table.1).

Table.1 Quantitative phytochemical analysis of *B. leptopoda*

S.No	Phytochemicals	mg/g dry wt
1	Steroids	0.20 ± 0.00
2	Glycosides	0.33 ± 0.00
3	Antheraquinones	0.13 ± 0.00
4	Phenols	0.28 ± 0.00
5	Alkaloids	0.15 ± 0.00
6	Triterpenoids	0.45 ± 0.00
7	Tannins	0.10 ± 0.00
8	Saponins	0.24 ± 0.00
9	Flavonoids	0.12 ± 0.00
P-Value		0.000
F-Value		4295

Values are expressed as Mean ± SEM, n=3 as ANOVA test $p < 0.05\%$ level.

4. DISCUSSION

The active metabolites are of immense medicinal value and have been broadly used as a part of the drug and pharmaceutical industry. Therefore the present examination on phytochemical investigation can help the manufacturers for identification and an assortment of raw materials for drug manufacture. So these biochemical characteristics make the seaweed nutraceutical in nature and in this way they are vital as nourishment supplement, keeping in mind the end goal to give greater wellbeing and oppose diseases.

The presence of phytoconstituents make the seaweeds helpful for treating various diseases and have a potential of providing useful drugs of human use. These phytoconstituents posses antibacterial (Richards *et al.,* 1978), antiviral (Oumaskour *et al.,* 2012), antifungal (Athukorala, *et al.,* 2007*)*, anticoagulant, antitumor (Abirami and Kowsalya, 2012) and anti-inflammatory (Boonchum *et al.*, 2011) activities.

The steroid content of the marine red alga *B. leptopoda* was 0.20 ± 0.00 mg/g dry wt. Algal steroids are enormously varied and can be biosynthesized by two diverse pathways (Lopes *et al.*, 2011). Steroids can be used to care for circumstances resulting from a steroid hormone shortage, such as postponed puberty, as well as diseases that result in the loss of lean muscle mass, such as cancer and AIDS. Steroids are useful in the human body's adrenal glands to fight stress related illnesses and injuries. They diminish inflammation and lift up the immune system. Steroids are used to treat an assortment conditions where the body's defense system malfunctions and causes tissue injury (Shahidi and Zhong, 2015)

Glycosides are produced when the hydroxyl group on the anomeric carbon of a sugar and the hydroxyl group of another particle concentrates to form an acetal or metal linkage, known as a glycosidic bond. In the present study, the methanol extract of marine red alga *B. leptopoda* showed the presence of glycosides (0.33 ± 0.00 mg/g in dry wt). The nearness of glycosides in the investigated alga may discover its applications in the antioxidant, anti-inflammatory properties and could be used as a supplement in the treatment of cancer. Yang *et al.,* (1992) reported the occurrence of glycosides in the macro algae *Zostera* sp., *Zhongguo Haiyang Yaowu* and *H. porphyroides* (Vishnukiran *et al.,* 2014) demonstrated lesser amounts of glycoside content contrasted to that of *B. leptopoda*.

Anthraquinone is an aromatic organic compound. In the current examination, the anthroquinone content of *B. leptopoda* was 0.13 ± 0.00 mg/g. The anthroquinone content of the present examination was observed to be the like to that of Thinakaran and Sivakumar (2012), Shankhadarwar (2015) from the marine red alga *Acanthophora spicifera* and higher than the red alga *Kappaphycus alvarezii* (Paul and Shri Devi, 2015). Quinones are regularly used in the treatment of malaria and tumors (Al-Khalil *et al*., 1998). They have antiinflammatory, anti-bacterial and immunomodulating potentials (Bergh *et al.,* 1997). The use of anthraquinone in the manufacture in different substances is a protection for most producers because of the exceedingly burnable properties of the substance. The methanol extract of extracts of *B. leptopoda* demonstrated likewise the nearness of anthraquinones.

Phenols are found to assume a more function in the preservation of the human body, Latha and Daniel (2001). Seaweed extracts are measured to be a rich source of phenolic compounds (Heo *et al.,* 2005). Phenolic compounds are one of the best antioxidants in brown algae (Huang *et al.,* 2008). Depending on their formation and concentration, phenol compounds, chemical components of algal cells, could have an activating or inhibiting effect on microbial activity (Alberto *et al.,* 2001). Moreover, seaweeds have been reported to act as inhibitors of the oxidative phosphorylation and factor cell lysis owing to their capacity to attach with bacterial proteins such as enzymes and those of cell membranes (Perez *et al.,* 2016). In the current examination, methanol extracts of *B. leptopoda* demonstrated the presence of phenols. Phenolic compounds have biological and pharmacological properties, particularly antimicrobial, anti-viral, anti-inflammatory, cytotoxic, anti-mutagenic, and anticarcinogenic activities (Mungole *et al.,* 2010).

Alkaloids are heterocyclic nitrogen compounds, naturally taking place in plants, microbes, animals and marine organisms (Alghazeer *et al.,* 2013). Alkaloids are generally found to have antimicrobial properties against both Gram-positive and Gram-negative bacteria (Cowan, 1999). But, the differences in antibacterial activity may be due to the process of extraction, solvent used in the extraction and season at which samples were collected (Kandhasamy and Arunachalam, 2008).

Alkaloids from marine algae are moderately uncommon, when contrasted with terrestrial plants and their biological potential is not completely known (Guven *et al*., 2010). The greater parts of the alkaloids of the indole group are concentrated in Rhodophyta (Guven *et al*., 2013). A portion of these alkaloids displays various pharmacological effects

like neuromodulation, neurotransmission, growth regulation, cytotoxicity, angiogenesis, antioxidant, as well as antibacterial, antifungal and larvicidal activities (Kaleağasıoğlu *et al*., 2013). Macroalgae, give various distinctive and interesting bisindole alkaloids, which showed excellent bioactivities, along these lines drawing in awesome enthusiasm from scientists (Liu *et al*., 2012).

Triterpenoids are active metabolites, known as biogenic compounds (Smit, 2004). In the current investigation, the triterpenoid content of the marine brown alga *B. leptopoda* was 0.45 ± 0.00 mg/g. It was in lesser amount when contrasted with the marine red alga *Tolypiocladia glomerulata* (Mohanapriya and Murugesan, 2017). Recent investigations have featured the huge assorted variety of triterpene there in the marine algae (Kalinin *et al*., 2008). Ghazala and Shameel (2005) distinguished triterpene phytol from marine algae whereas, Gupta and Abu Ghannam (2011) affirmed, and that few types of triterpenoids and sesquiterpenoids have been established to be the major secondary metabolites of the marine algae. Among the macroalgae, red seaweeds are measured as one of the richest source of biologically and ecologically applicable terpenoids mostly diterpenes and meroditerpenes (Gaysinski *et al*., 2015).

Tannins are polyphenolic compounds and are extensive between terrestrial and marine plants (Waterman and Mole, 1994). At low concentration, tannins can reduce the growth of microorganisms and proceed as an antifungal agent at higher concentration by coagulating the protoplasm of the microorganism. The amount the tannin content was maximum in *B. leptopoda* and low amount in *Halymenia poryphyroides* (Vishnukiran and Murugesan, 2014). In the current investigation, the methanol extract of *B. leptopoda* indicated reasonable amount of tannins. The presence of tannins, polyphenols and cardiac glycosides was reported in the methanol extract of *S. polycystem* and *T. ornata* (Asha Kanimozhi *et al*., 2012; Rajkumar and Bhavan, 2017).

Saponins are viewed as a key ingredient in established Chinese medicine and are accountable for most of the experiential biological effects. Saponins have many biological properties which comprise antimicrobial, anti-inflammatory, anti-feedent and haemolytic effects (Xia *et al*., 2002). Saponins are concerned with different biological effects, including hemolytic and antibacterial activities (Sparg *et al*., 2004). Saponins are used in hypercholesterolemia, hyperglycemia, antioxidant, anticancer, anti-inflammatory and weight loss (Setty *et al*., 2011). Saponins are also having some tough antitumor effects and found helpful in targeting and inhibiting the tumor angiogenesis by suppressing its inducer in the endothelial cells in the blood vessels and preventing and adhering, attack of metastasis of tumor cells (Syed *et al*., 2015). In the current examination, the methanol extract of *B. leptopoda* demonstrated the rich in saponins.

Flavonoids are known as nature's delicate drug which has various biological and pharmacological activities. Modern information of antiviral, antifungal, antioxidant, anti-inflammatory, antiallergenic, antithrombic, anticarcenogenic, hepatoprotective and cytotoxic activities of flavonoids have generated interest in studies of flavonoid containing plants. In the current investigation the higher amount of flavonoids were present in the marine red alga *B.leptopoda.* Flavonoids, as antioxidants may keep the dynamic impedance of pancreatic beta cell because of the function due to oxidative stress and may, along these lines decrease the incidence of type 2 diabetes (Song *et al*., 2005).

5. CONCLUSION

These outcomes were contrasted with the lately reported phytochemical content which was used to identify the presence of alkaloids, tannins, saponins, flavonoids, glycosides and phenols from the seaweeds. Marine genus synthesizes active constituents which are used in traditional and complementary medicine. Diverse assortment of marine algae was reported to contain active ingredients that can cure diseases. These days, higher percentage of the people prefer to use remedies of natural source for therapeutic illness as these claimed to produce less side effects.

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