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Phytochemical screening of *Spirulina platensis* extracts from Rankala Lake Kolhapur, India.

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Abstract

Spirulina platensis algae are the source of bioactive compounds therefore they are used in various applications. Their use in traditional medicine has been reported since time immemorial. In the present study, *Spirulina platensis* algae were collected from the Rankala Lake Kolhapur, Maharashtra, India. Samples were washed, air dried and homogenized into fine powder. The methanol, ethanol, petroleum ether, acetone and aqueous extracts *Spirulina platensis* were subjected to phytochemical analysis to know the secondary metabolites present in the extracts.The phytochemical screening of the *Spirulina platensis* revealed that metabolites with higher medicinal activities such as Alkaloids, Terpenoids, Steroids, Saponins, Phenols and Flavonoids were present in all the five extracts while Tannins, Coumarins, Quinones and glycosides were absent in few extracts of *Spirulina platensis*.

Key words: *Spirulina platensis,* secondary metabolites, phytochemical analysis,

Introduction

Spirulina has been produced commercially for the last 20 years and the current manufacture universal is estimated to be about 3,000 metric tonnes. The chief commercial large-scale culture of *Spirulina* was started in the early 1970's at Lake Texcoco, Mexico [1-3]. Commercial production of *Spirulina* in man-made ponds was pioneered by Dainippon Ink and Chemicals Inc. (DIC) in 1978 in Bangkok, Thailand [4,7]. Earthrise farms was found in 1981, by the Proteus Corporation of the USA and later incorporated with DIC of Japan in 1982 [4,7]. Commercial production of *Spirulina* at Earthrise farms in California started in 1983. It is being sold as a health drink as well as in tablet form for more than ten years without undesirable effect on humans [1,4,7]. *Spirulina* is marketed and consumed in several countries, including, U.S.A, Thailand, Taiwan, Vietnam, China, India and Cuba [1-7].

Spirulina is a multicellular, filamentous cyanobacterium, belonging to Phormidiaceae family which under microscope, appears as blue green filaments composed of cylindrical cells arranged in unbranched helicoidal trichomes [1-5]. The trichomes are arranged in open left handed helix pattern along the entire length. The cell wall is made of four numbered layers, LI, III, LIU and L IV from the innermost to outward [4,7,8]. All the layers are very weak except LII, which is made up of peptidoglycan, which is responsible for its rigidity. *Spirulina* is a non- heterocystous and a non-nitrogen fixer [5,8,10]. The helical shape of the trichome is characteristic of the genus which is due to hydration / dehydration of oligopeptides in the peptidoglycan layer [2,4,8].

Spirulina is natural food belongs to plantae kingdom which consists different phytochemicals [1,2,4,8]. This all phytochemicals are biologically significant and plays a vital role in medicinal applications. Mainly laboratory experiments revealed phytochemicals from *Spirulina* and their use in cancer, tuberculosis, inflammation and many other blood related diseases [4]. But each and every *Spirulina* is varying from each other in the production of these compounds. Somehow these production is depends on environmental conditions such as temperature, pH, nutrients, metal ions and other chemicals [4,8]. The phytochemical research loom is measured effective in discovering novel bioactive compounds from *Spirulina*. There are two main methods for the analysis of phytochemical screening such as qualitative and quantitative analysis. The qualitative tests are used to identify the constituents [4,6,9]. The quantitative tests are used to quantify or determine the amount of active constituents present.

In the present study *Spirulina platensis* was used for qualitative phytochemical analysis.

Materials and Methods

Study area

Rankala Lake was selected as a study area. It belongs to Kolhapur District, Maharashtra, India. It is 16042" North 74015" East on the North West plateau of Maharashtra. The district is bordered by the steep ridges of Sahyadri to the west, the Deccan plateau on the east, and boundaries of Goa on the south and Karnataka on east. The area of the district is 7746 sq. km. and it is 2-5% of the state area.

Sample Collection

Spirulina platensis were collected from the Rankala Lake Kolhapur, Maharashtra, India. They were used as the experimental algae to their biodiversity. Samples were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles in refrigerator.

Preparation of Extract

Sample extracts were prepared by Soxhlet extraction method. 20 g of powdered material was uniformly packed into a thimble and extracted with 250 ml of methanol, ethanol, petroleum ether and acetone extract separately. The process of extraction has to be continued for 24 hours or till the solvent in siphon tube of extractor become colorless. After that the extract was taken in a beaker and kept on hot plate and heated at 30-40ºC till all the solvent got evaporated. Dried extract was kept in refrigerator at 4ºC till future use.

Phytochemical analysis

Test for Alkaloids

2 mL of concentrated Hydrochloric acid (HCl) was added to 2 mL *Spirulina platensis* extract. Then few drops Mayer's reagent was added. Presence of green color or white precipitate indicates the presence of alkaloids.

Test for Terpenoids

2 mL of chloroform along with concentrated Sulphuric acid were added to 0.5 ml of the *Spirulina platensis* extract. Formation of reddish brown color at the interface indicates the presence of Terpenoids.

Test for Steroids

2 mL of chloroform and 1 mL of sulphuric acid (H2SO4) were added to 0.5 mL of the *Spirulina platensis* extract. Formation of reddish brown ring at interface indicates the presence of steroids.

Test for Tannins

1 mL of ferric chloride (5% FeCl3) was added to 1 mL of the *Spirulina platensis* extract. Formation of dark blue or greenish black color indicates the presence of tannins.

Test for Saponins

2 mL of distilled water was added to 2 mL *Spirulina platensis* extract and shaken in graduated cylinder for 15 min lengthwise. Formation of 1 cm layer of foam indicates the presence of saponins.

Test for Flavonoids

1 mL of 2N sodium hydroxide (NaOH) was added to 2 mL of *Spirulina platensis* extract. Formation of yellow color indicates the presence of flavonoids.

Test for Phenols

2 mL of distilled water followed by few drops of 10 % ferric chloride was added to 1 mL of the *Spirulina platensis* extract. Formation of blue or green color indicates the presence of phenols

Test for Coumarins

1 mL of 10 % NaOH was added to 1 mL of *Spirulina platensis* extract. Formation of yellow color indicates the presence of coumarins.

Test for Quinones

1 mL of concentrated sulphuric acid (H_2SO_4) was added to 1 mL *Spirulina platensis* extract. Formation of red color indicates the presence of quinones.

Test for Glycosides

3mL of chloroform and 10% ammonium solution was added to 2 mL of the *Spirulina platensis* extract. Formation of pink color indicates the presence of glycosides.

Results and Discussion

Phytochemical screening of ten different chemical compounds (alkaloids, terpenoids, steroids, tannins, saponins, flavonoids, phenols, coumarins, quinones and glycosides) were tested in five different extracts such as methanol, ethanol, petroleum ether, acetone and aqueous extracts of *Spirulina platensis.*
The qualitative phytochemical analysis of methanol, ethanol, petroleum ether, acetone and aqueous extracts of *Spirulina platensis* revealed that methanol extract had better activity. Alkaloids, Terpenoids, Steroids, Saponins and Flavonoids were present in powder and all the five extracts of *Spirulina platensis*. Tanins and glycosides were present in powder but only in the extract of methanol and acetone whereas these were absent in ethanol, petroleum ether and aqueous extracts. Coumarins were present only in the extract of methanol and ethanol while it was absent in petroleum ether, acetone and aqueous extracts. Quinones were present in the extract of methanol, petroleum ether, acetone and aqueous while it was absent in ethanol extract.
From the study, it was observed that the algae *Spirulina platensis* possesses medicinally important phytochemicals shown as in table I.

Table I. Phytochemical analysis of *Spirulina platensis* extracts (Here Methanol, ethanol, Petroleum Ether and acetone were used as a solvent for extraction of bioactive compounds at room temperature 25 ±2^{O}C, [+] = Presence, [-] = Absence).

Sr. No	Tests	Sample extracts					
		Powder	Methanol	Ethanol	Petroleum Ether	Acetone	Aqueous
1	Alkaloids	+	+	+	+	+	+
2	Terpenoids	+	+	+	+	+	+
3	Steroids	+	+	+	+	+	+
4	Tannins	+	+	-	-	+	-
5	Saponins	+	+	+	+	+	+
6	Flavonoids	+	+	+	+	+	+
7	Phenols	+	+	+	+	+	+
8	Coumarins	+	+	+	-	-	-
9	Quinones	+	+	-	+	+	+
10	Glycosides	+	+	-	-	+	-

Conclusion

Spirulina platensis is also used as raw material for the production industrially important products. It is consumed as food in many Asian countries. From the present research *Spirulina platensis* revealed with different ten metabolites with higher medicinal activities such as Alkaloids, Terpenoids, Steroids, Phenols, Saponins and Flavonoids in five extracts while Tannins, Coumarins, Quinones and glycosides were also present in few extracts of *Spirulina platensis.*

Thus, the algae *Spirulina platensis* can be a significant source of important compounds which can be used in formulation of drugs by the pharmaceutical industries.

Refeences

Anderson NS, Dolan TCS, Rees DA. Carrageenan. Part–VII. Polysaccharides from *Eucheuma spinosum* and *Eucheuma cottonii*. The covalent structure of i-carrageenan. Journal of the chemical Society, Perkin Transactions 1973; 1:2173-2176.

Balakrishnan CP, Venkataraman K, Mohan VR, Louis JL, Athiperumal ST. A general survey of the common agarophytes in the Gulf of Mannar in relation to agar ecology. Seaweed Research and Utilisation 2009; 31(1&2):33–46.

Bhat VB, Madyastha KM. Scavenging of peroxynitrite by phycocyanin and phycocyanobilin from Spirulinaplatensis: protection against oxidative damage to DNA. Biochem Biophys Res Commun, 2001; 285: 262-266.

Dawes, C. (1998). *Marine Botany*. New York: John Wiley and Sons, Inc, 480.

Lordan, S., Ross, R. and Stanton, C. (2011). Marine Bioactives as Functional Food Ingredients: Potential to Reduce the Incidence of Chronic Diseases. *Marine Drugs*, 9(12), 1056-1100.

Rizvi, M.A. and Shameel, M. (2004). Biological activity and elementology of benthic algae from Karachi coast. *Pakistan Journal of Botany*, 35(5; SPI), pp.717-730.

Shanmugam M, Mody KH. (2000) Heparinoid-active sulfated polysaccharides from marine algae as potential blood anticoagulant agents. Current Science; 79:1672-1683

Shyamala, V and N. Thangaraju. (2013) "Screening of Phytochemical and Antibacteriial activity of three different seaweeds from Gulf of Mannar, Tamil nadu". *Phykos.,*Vol. 43(1), pp. 32-38.

Wijesekara I, Pangestuti R, Kim SK. (2011). Biological activities and potential health benefits of sulfated polysaccharides derived from marine algae. Carb Polymer. 84: 14–21.

Wu, X.J. and Hansen, C. (2008). Antioxidant capacity, phenolic content, and polysaccharide content of *Lentinus edodes* grown in whey permeate-based submerged culture. *Journal of food science*, 73(1), M1-M8.