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RESEARCH ARTICLE

Investigation of phytochemical constituents in *Spirulina fusiformis* for antibacterial activity

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ABSTRACT

Background: *Spirulina fusiformis* provides a wide range of health benefits, so a major percentage of the population in developing countries depends on *S. fusiformis* due to its nutritional value. **Aim and Objective:** To examine the phytochemical constituents in *Spirulina fusiformis* for antibacterial activity. **Materials and Methods:** This study was a laboratory observational, phytochemical analysis, and antibacterial assay of *S. fusiformis* was carried out. **Results:** Ethanol and chloroform extract showed a maximum inhibitory effect against all species chosen for this study. **Conclusion:** *S. fusiformis* sample found to contain certain phytochemicals in higher ratio and a wider range of protein and amino acids. It also possesses good antibacterial activity against various organisms.

KEY WORDS: Anti-bacterial Assay; Cyanobacteria; Phytochemical Analysis; *Spirulina fusifomis*

INTRODUCTION

Among ancient civilization trend of medicine, the usage of medicinal plants extensively relies only in India amidst many countries India enfolds the richest sources of all medicinal plants, and many of these plants were extensively used in curing human ailments.[1,2] The preliminary methods of medicine such as Ayurveda, unani, sidda, and folk medicine play a tremendous role, and they were predominantly practiced all over India.[3,4] About two-third of the human population makes use of these traditional system of medicine for curing their ailments.[5] The development of these herbal plants results in the formulation of medicines, drugs, etc., for the welfare of human community.[6,7] Rather than gaining side effects with an inefficient system of medicine, ethnomedicine has unique properties for human population.[8,9] *Spirulina fusiformis* of Pseudomonadaceae family has an extensive medicinal property among all cyanobacteria.[10] This single-celled dense mat is found with richest source of proteins, amino acids, saponins, flavonoids, vitamins and minerals, and many other phytocomponents.[11,12] Spirulina serves as the best source of various vitamins and minerals.[13,14]

Spirulina generally serves as the best remedy for digestion, energy, depression, weight loss, healing of wounds, fatigue, anxiety, immunity gaining, cardiovascular diseases, premenstrual syndrome, and dietary supply of vitamins and minerals. This algal species showed extensive properties such as antibacterial, antioxidant, antifungal, antiviral, and antitumor properties.[15] They generally possess antimicrobial property by nature and prevent certain bacterial and fungal infections.[16] The present study reveals the basic phytochemical components, antibacterial activity against certain organisms.

Objectives

- The present study was done to investigate phytochemical and antibacterial activity in *S. fusiformis.*

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- This study was conducted to assess whether *S. fusiformis* is found to possess good antimicrobial action against the four selected organisms.

MATERIALS AND METHODS

Collection of Sample

The *Spirulina* sample was collected from SAB research center, Pudukkottai main road, Thanjavur Dt.

Sample Processing

The collected sample was completely washed in tap water and shade dried for about a week. Then, they were finely powdered by means of mechanical grinder. Approximately 10 g of the algal sample was dissolved in various solvents such as methanol, ethanol, chloroform, and petroleum ether, and aqueous, respectively. Them they were subjected for the process of continuous agitation for 3 consecutive days at 180–220 rpm. After the process of agitation they were filtered gently in no 42 filter paper and they were stored in an airtight container for future use.

Phytochemical Analysis

Alkaloids

To 1 ml of the concentrate few drops of Meyers reagent were included, creamy white precipitate demonstrates the nearness of alkaloids.

Steroids

To a small amount of the sample, 2 ml acetic anhydride was included, and they were layered with concentrated sulfuric acid. Appearance of reddish to yellow-green layer demonstrates the nearness of steroids.

Terpenoids

The concentrate was blended with 2 ml of chloroform followed by concentrated sulfuric acid showed the presence of terpenoids with the demonstration of reddish brown interface.

Flavonoids

To 1 ml of the sample, few ml of ammonia solution with the layer of concentrated hydrochloric acid were added, appearance of yellow color demonstrates the nearness of flavonoids.

Saponin

2 g of the powdered sample was blended with 5 ml of the distilled water shaken vigorously for froth formation.

Phenols

To 1 ml of sample, 2ml of gelatine solution was included; the presence of white precipitate demonstrates the nearness of phenols.

Tannins

To a few drops of the sample few drops of lead acetate solution was added to produce the white precipitate for the nearness of tannins.

Cardiac glycosides

To 2 ml of sample, 1ml glacial acetic acid was added with few drops of ferric chloride solution by the layering of concentrated sulfuric acid for the presence of bluish green upper layer and reddish brown layer at the junction.

Proteins

Few ml of sample was gently added with nitric acid and heated and then cooled for about a minute, few ml of sodium hydroxide solution were mixed. Appearance of orange color shows the nearness of proteins.

Amino acids

Few ml samples were blended with ninhydrin solution then it is boiled for a few minutes. Appearance of purple color shading shows the nearness of amino acid.

Carbohydrates

To a few drops of the sample, molisch reagent was included followed by the layering of concentrated sulfuric acid shows the presence of reddish-brown ring indicates the nearness of carbohydrates.

Test for Reducing Sugars

Fehling's test

1 ml of the extract is mixed with few drops of Fehling's solution and boiled for few minutes; reddish brown precipitate shows the nearness of reducing sugars.

Benedicts test

About 5 ml of Benedict's reagent was assorted with few ml of the sample and then boiled, orange red precipitate shows the nearness of reducing sugar.

Test Organisms

Certain microbial culture is prepared in the microbiology laboratory of Cauvery College for Women under proper screening. Among isolated microorganisms, four species were chosen for monitoring their antibacterial activity. These microorganisms were carefully isolated and identified in an

efficient way, and they were stored under proper refrigeration for future use.

Antibacterial Assay

The crude extracts thus prepared from certain solvents such as methanol, ethanol, chloroform, petroleum ether, and aqueous suspended with algal sample were performed for antibacterial activity for analyzing their inhibitory effect. Varying concentrations range from 10 ml, 20 ml, and 30 ml for this activity by disc diffusion method. The selected microorganisms were made as a lawn on Mueller-Hinton agar plates by means of cotton swab, the algal sample in various solvents was impregnated in varying concentrations on sterile disc and was placed gently on agar plates. The plates were kept at 37°C for 24 h. After the process, the plates were observed for the presence of a zone of inhibition. Trials were made, and the appropriate measurement was tabulated. Antibiotics such as gentamycin and ampicillin were used as a control.

RESULTS

Table 1 clearly shows the presence of certain phytochemicals in a higher ratio such as alkaloids, saponins, flavonoids, phenols, tannins, glycosides, and wider range of protein and amino acids.

Table 2 and Figure 1 reveal the antibacterial activity of the algal sample against different organisms which includes *Escherichia coli, Staphylococcus species, Klebsiella species*, and *Bacillus species*. It has been seen that ethanol and chloroform extract showed a maximum inhibitory effect against all species chosen and little effect was found in methanol extract against the test organisms and it was proved that no effect was found in petroleum ether and aqueous extract.

DISCUSSION

The synopsis of this work exposed that *S. fusiformis* has substantiated itself extremely powerful against

Table 1: Phytochemical analysis of *S. fusiformis*

Test	Methanol	Ethanol	Chloroform	Pet Ether	Aqueous
Alkaloids	+++	+++	-	-	-
Steroids	+	-	+++	++	-
Terpenoids	++	+++	++	-	-
Flavonoids	+++	+++	-	-	-
Saponins	++	+++	+	-	+
Phenols	+++	+++	-	-	-
Tannins	+++	+++	+++	+	+
Glycosides	++	++	++	+	-
Carbohydrates	-	++	++	+	+
Reducing sugars	-	-	-	-	-
Proteins	++	+++	+++	++	++
Amino acids	++	+++	+++	++	++

+++: Highly present, ++: Fairly present, +Poorly present, -: Nil

Table 2: Antibacterial activity of *S. fusiformis*

Extract	Concentration	*E. coli*	*Bacillus sp.*	*Klebsiella sp.*	*Staphylococcus sp.*
Methanol	10	0.8	1	0.4	0.3
	20	1	1.4	0.7	0.5
	30	1.2	2	1	0.7
	40	1.4	2.4	1.2	0.9
Ethanol	10	1	1	0.8	1.0
	20	1.2	1.2	1.0	1.1
	30	1.3	1.4	1.2	1.3
	40	1.4	1.6	1.3	1.4
Chloroform	10	1	1	1.2	1.1
	20	1.5	1.4	1.4	1.3
	30	2	2	1.5	1.5
	40	2.3	2.2	1.7	1.7

S. fusiformis: Spirulina fusiformis, E. coli: Escherichia coli

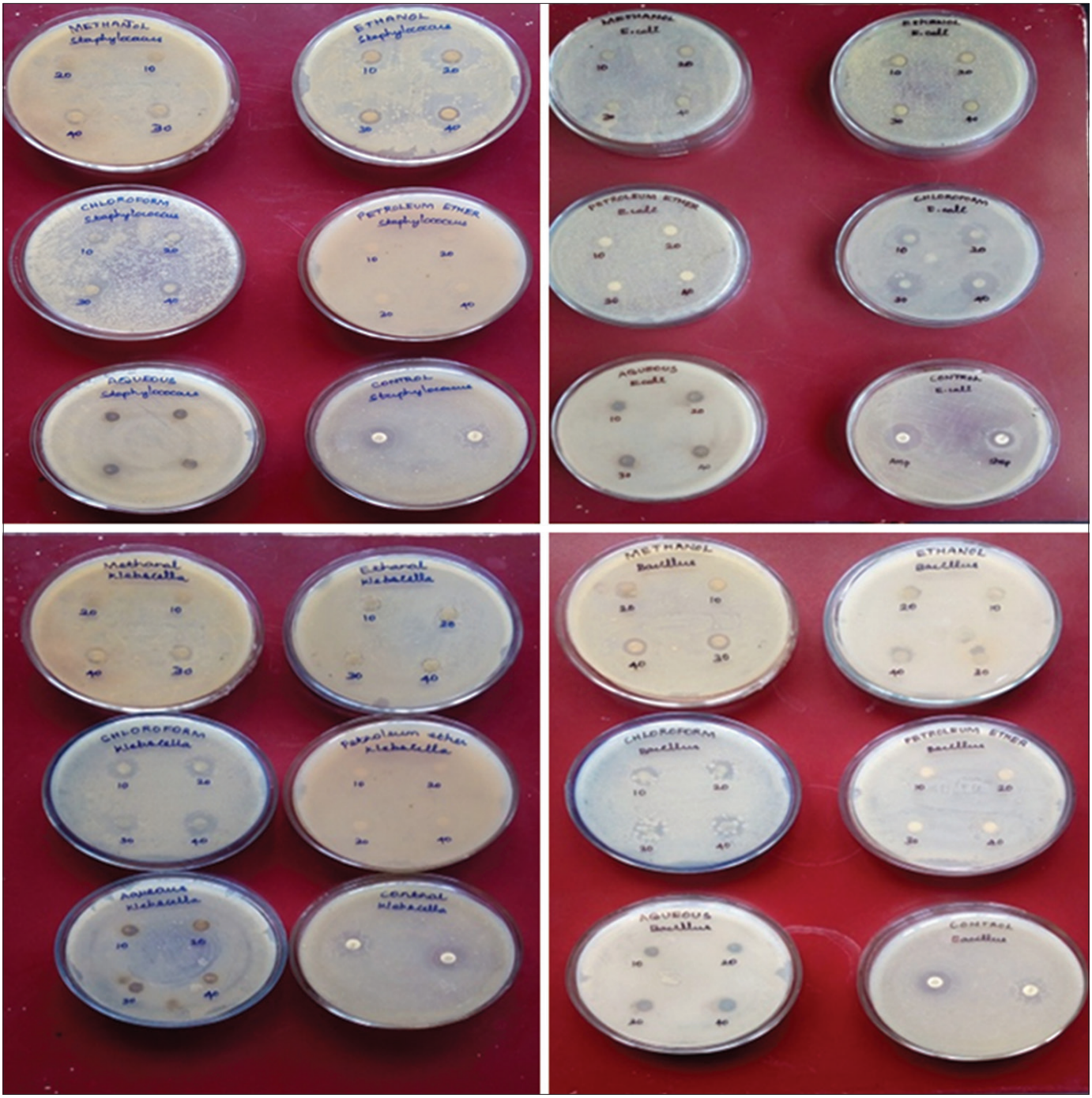

Figure 1: Antibacterial activity of *Escherichia coli, Staphylococcus species, Bacillus species, and Klebsiella species*

Staphylococcus sp., *Klebsiella* sp., *Bacillus* sp., and *E. coli*. It has, additionally, found to contain certain phytochemicals in higher proportion and more extensive source of protein and amino acids.

Sharma *et al.* recommended that *S. fusiformis* can essentially alter the renal deformities against mercuric chloride prompted lethality.[2] Simon *et al.* demonstrated that *S. fusiformis* has noteworthy antidiabetic and antihyperlipidemic impacts in streptozotocin-induced diabetic rats by viably lessening the ascent in blood glucose levels and lipid profile.[6] Martin *et al.* explored *in silico* investigations which demonstrated that chosen segments of *S. fusiformis* cooperate with LXR and FXR and could be a conceivable component of the activity. *S. fusiformis* rendered activity against tuberculosis drugs-induced oxidative stress in kidney tissues of rats.[10]

Kuhad *et al.* recommended that *S. fusiformis* treatment essentially and dosage conditionally re-established renal deformities, decreased lipid peroxidation, and upgraded diminished glutathione levels, superoxide dismutase, and catalase activity. The consequences of the present investigation obviously exhibit the vital part of reactive oxygen species and their connection to renal dysfunction

and point to the remedial capability of *S. fusiformis* in cisplatin-induced nephrotoxicity.[15] Rasool *et al.* examined that *Spirulina* has increased consideration from medicinal researchers as a nutraceutical and a wellspring of potential pharmaceuticals.[17]

Rasool *et al.* explained that oral dosage of *S. fusiformis* (800 mg/kg/b.wt) altogether altered the physical and biochemical properties in the arthritic animal. Henceforth, consequences of this investigation plainly show that *S. fusiformis* has promising calming movement against adjuvant-initiated arthritic animals.[18] Thus, *S. fusiformis* has substantiated itself exceptionally powerful against different pathogenic microbes and infections. This present paper elaborates about the phytochemical action and antibacterial action of *S. fusiformis*.

Strengths and Limitations of the Study

Quality of this investigation is that *S. fusiformis* serves as a superior source of protein and is a decent origin of iron and copper. Restrictions of this examination incorporate that it might contain substantial metals and harming poisons. Iodine content is another potential issue that we experience on the grounds that *Spirulina* contains more iodine content.

CONCLUSION

In this present study, *S. fusiformis* sample found to contain certain phytochemicals in higher ratio and wider range of protein and amino acids. It also possesses good antibacterial action against *Staphylococcus sp., Klebsiella sp., Bacillus* sp., and *E. coli*. Future research can be carried out in *S. fusiformis* for strain improvement and development process.

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