Research Article

Bioprospection of Bioactive Compounds from *Spirulina platensis* and *in-vitro* Therapeutic Applications

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ABSTRACT

The objective of the present research work was to evaluate the phytochemical analysis, antioxidant, anti-inflammatory, antidiabetic, antiurolithiasis, and antimicrobial activities of different extracts obtained from Spirulina platensis. The Spirulina platensis was collected from Rankala Lake, Kolhapur, India and full-grown in CHU-10 media for 15 days. After 15 days the culture was recovered, dried, powdered and extracted using aqueous, methanol, chloroform, and ethyl acetate by solvent- solvent extraction method. Qualitative and quantitative phytochemical analysis was performed. Further extracts were submerged for different therapeutic applications. Potent crude extracts were purified by thin layer chromatography and Fourier Transfer Infra Red spectroscopy. The qualitative phytochemical analysis of all extracts revealed the presence of alkaloids, phenols, flavonoids, tannins, anthroquinones, glycosides, Lignins, sterols, and volatile oils while quantitative analysis revealed 27.0090±0.04129mg of highest phenolic content in the aqueous extract and 63.47± 0.88059mg of highest flavonoids content in the chloroform extract. Highest ferric ion reducing power was showed by the aqueous extract at the rate of 1.2407±0.00702 and chloroform extract showed better antioxidant capacity at the rate of 0.8983±0.00351 in Phosphomolybdenum assay. The methanol extract showed highest anti-inflammatory activity and alpha-amylase inhibition at the rate of 85.3200±1.63000 and 50.1907±0.33250 with IC50 value 99.62µg/ml respectively. The aqueous crude extract showed highest inhibition zone at the 100% concentration against Escherichia coli (16.3±0.57) and Aspergillus niger (20.5 ± 0.24). The maximum calcium oxalate inhibition shown by aqueous crude extract at the rate of 67.5±1.49 with 100% concentration.

Keywords: Spirulina platensis, phytochemical analysis, antioxidant activity, anti-inflammatory activity, antimicrobial activity, antiurolithiasis activity.

INTRODUCTION

Spirulina is a multicellular, filamentous *cyanobacterium*, belonging to Phormidiaceae family. Under the microscope it appears as blue-green filaments, composed of cylindrical cells arranged in unbranched helicoidal trichomes[1]. 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 LIV from the innermost to outward. All the layers are very weak except LII, which is made up of peptidoglycan, and responsible for the rigidity[2]. Spirulina is a non-heterocystous and a non-nitrogen fixer. The helical shape of the trichome is characteristic of the genus which is due to hydration/dehydration of oligopeptides in the peptidoglycan layer. Spirulina is natural food belongs to the Plantae kingdom which consists of different phytochemicals[3]. These phytochemicals are biologically significant and play vital role in medicinal applications. Mainly laboratory experiments revealed that phytochemicals from *Spirulina* and their use in cancer, tuberculosis, inflammation, and many other blood-related diseases. Free radicals are highly reactive particles also highly reactive byproducts with an unpaired electron, produced when cells are exposed to stress[4]. They instigate chain reactions, which lead to crumbling of cell membranes and cell compounds, including lipids, proteins, and nucleic acids. Oxidative stress generated from free radicals is the major implication in the pathogenesis of a wide variety of clinical disorders, such as cancer, cardiovascular disease, Alzheimer's disease, autoimmune disease, diabetes, multiple sclerosis and arthritis. Inflammation is an extremely complex process involves many systems which are closely associated with the process of repair[5]. Inflammation can be defined as localized response of the tissues to injury caused due to any agent and exhibit usually in form of painful swelling associated with some changes in skin. The typical signs of inflammation are local redness, swelling, pain, heat and loss of function. Inflammation can be either acute or chronic based on the occurrence[6]. Acute inflammation is the immediate response of the body to disparaging stimuli wherein the plasma and leucocytes of the blood infiltrate in to the site of injury. Chronic inflammation involves shifting of the cells which are present at the site of inflammation characterized by instantaneous destruction and curative effects of the tissue from the inflammatory process. Presently Non-steroidal anti-inflammatory drugs are being administered for the treatment of the orthopedic related injuries, arthritis and bone fractures[7].

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Urolithiasis is a third prevalent urological disorder of humans formed usually within the kidney in the form of stones from ancient times[8]. There are quite a few types of kidney stones but the most common are calcium oxalate which represents 80% of analyzed stones. At present, kidney stone affects extra in industrialized countries owing to the change in lifestyle. Formation of the stone is a multifaceted process and involves several physicochemical actions which start with crystal nucleation, supersaturation, aggregation, and ending with retention inside the urinary tract[9]. Another focused area is microbial infections. Infection is the attack of an organism's body tissues by disease-causing agents, their multiplication, and the reaction of host tissues to the communicable agents and the toxins they produce. Infections are caused by pathogens and these pathogens are bacteria, fungi, viruses, actinomycetes, and protists[10]. The greatest concern about these drugs is toxicity and reappearance of the symptoms after the discontinuation of the drugs. So it is the need of the day to look into alternative medicines for the treatment of the inflammatory responses[11]. The objectives of the present research study were (I) Selection of study area and collection of *Spirulina platensis* (II) Extraction and phytochemical analysis of bioactive compounds (III) Therapeutic applications.

MATERIALS AND METHODS

Study area and sample collection

Rankala Lake is located in Kolhapur District, Maharashtra, India and was selected as the study area. 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. *Spirulina platensis* was collected from the Rankala Lake Kolhapur (MH), India, identified, and used as the experimental algae to that biodiversity. Samples were thoroughly washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles in refrigerator[12].

Cultivation, extraction, and phytochemical analysis of *spirulina platensis* crude extract

Spirulina platensis was axenically grown in CHU-10 medium and incubated in a culture room at temperature of 25°C ± 2°C and illuminated with day- light fluorescent tubes. During the process of growth the flask was shaken at every half hour per day. The experiments were run in triplicates. All culturing were carried out under aseptic conditions in a laminar flow. 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 different selected solvents such aqueous, methanol, chloroform, and ethyl acetate 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. Qualitative phytochemical analysis of all extracts of *Spirulina platensis* was employed for the detection of different bioactive compounds by preferring standard protocols[13]. The flavonoids and phenol content were estimated by using Folin-Ciocalteu colorimetric method[13]. Quantification was done on the basis of the standard curve of gallic acid and results were expressed as gallic acid equivalent.

In-vitro therapeutic applications

Antioxidant activity

Antioxidant activity assay of all extracts were performed by Ferric ion reducing power assay and Phosphomolybdenum assay by the following standard protocols[13].

Anti-inflammatory activity

Anti-inflammatory activity of all extracts was performed by following protein denaturation assay. This activity was performed in the accordance with the standard method[14].

The percentage inhibition was measured by the formula

$$\% \text{ Inhibition} = A_t\text{-}A_c/A_c \times 100$$

Where A_c is the absorbance of the control and A_t is the absorbance of the test.

Antidiabetic activity

Alpha -amylase inhibitory assay

The Alpha-amylase inhibitory assay for different solvent extracts of *Spirulina platensis* were evaluated according to a previously described method[13-17]. The inhibition of α-amylase was calculated using the following equation:

$$\% \text{ inhibition of } \alpha\text{-Amylase} = (\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}) / (\text{Abs}_{\text{control}}) \times 100$$

Where

$\text{Abs}_{\text{control}}$ = to the absorbance of the solution without extract (buffer instead of extract) and with α -amylase solution $\text{Abs}_{\text{sample}}$ = to the solution with extract and α -amylase solution.

Antimicrobial activity

Microorganisms

The crude extract was screened against different pathogens by using well diffusion method[13]. The bacteria such as *Escherichia coli, Salmonella Typhi, Staphylococcus aureus, Bacillus spp.* and *Pseudomonas aeruginosa* and fungi such as *Aspergillus niger, Aspergillus fumigatus*, and *Candida albicans* were used.

Well Diffusion Method

The bacterial and fungal inoculum were prepared to the concentration of 1.0×10^4 CFU/ml adjusted with saline. The culture suspension was prepared and used as a stock culture for the experiment purpose. The culture

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suspension was spread on nutrient agar medium for verification of other microbial contamination. Fluconazole and Telithromycin (10mg/mL) were used as positive control and solvent DMSO was used as the negative control. The verified microbial culture suspensions were spread on Muller-Hinton agar medium plates and purified extract samples were added in the wells with standard antibiotics. Plates were incubated at 37°C for 24 hrs or 72 hrs and the zone of inhibition was recorded with the help of zone reader. All experiments were performed in triplicates.

Antiurolithiasis activity

This assay was chosen for the study of oxalate crystallization because of its satisfactory results simplicity and reproducibility in order to study inhibitory capacity of crude extract. The solutions of $CaCl_2.2H_2O$ (9mM) and $Na_2C_2O_4$ (3mM) were prepared using a buffer containing 0.15 M NaCl and 0.05 M Tris HCl at pH 6.5. The inhibitory solution was prepared by adding 0.25 gm of crude extracts in 100 mL of 0.15 M NaCl solution. 1 mL of $CaCl_2$ solution was mixed with 1 mL of inhibitory solution of crude extract with different concentration (25%, 50%, 75%, 100%). Blank reading was taken and 1 mL of sodium oxalate was added and the absorbance was measured at 620nm with the help of UV spectrophotometer at different time intervals[13].

Statistical Analysis

All the results (triplicates) were represented as Mean ± Standard Deviation (SD). One way ANOVA was carried out to check the variation between the samples using SPSS software version 20.0. IBM. The levels of significance was considered as $p< 0.01$ and $p <0.05$ for the comparison.

RESULTS

The qualitative phytochemical analysis revealed the presence of alkaloids, phenols, flavonoids, tannins, anthroquinones, glycosides, lignins, sterols, volatile oils in various extracts like chloroform, ethyl acetate, methanol, ethanol and aqueous. The chloroform extract showed the presence of flavonoids, glycosides and sterols. Ethyl acetate extract showed the presence of alkaloids, phenols, glycosides and sterols. Methanol extract revealed the presence of flavonoids, glycosides, lignins, sterols and tannins. The aqueous extract unveiled the presence of alkaloids, flavonoids, glycosides, phenols, sterols, tannins, anthroquinones and volatile oils. The results are depicted in Table 1. The total phenolic content of the selected extracts was estimated for the selected extracts with gallic acid as a reference standard. The aqueous extract showed high phenolic content (27.0090±0.04129 mg) when compared to ethyl acetate extract (8.40±0.05000 mg). The results were expressed as mg/g GAE per gram of plant extract. The total flavonoids content was performed using $AlCl_3$ method with quercetin as a standard. The chloroform extract showed high flavonoids content (63.47± 0.88059 mg) whereas methanol extract showed moderate flavonoids content of (51.9221±0.07300 mg) and aqueous extract (20.3568±0.05064 mg). The results are expressed as mg/g of quercetin equivalent. Ferric ion reducing power assay was performed with chloroform, ethyl acetate, methanol and aqueous extracts by keeping ascorbic acid as reference standard. In this assay, aqueous extract (1.2407±0.00702) showed highest antioxidant activity among the extracts used for the assay which was comparable to standard. The results are depicted in figure 1. PM assay was performed for the four different extracts using ascorbic acid as reference standard. The results revealed that the chloroform extract showed better antioxidant capacity (0.8983±0.00351) than the other extracts and was comparable to the standard ascorbic acid. The results are depicted in figure 2. The anti-inflammatory activity results are shown in table 2. In the Alpha-amylase inhibitory assay the known concentration (100µg) of different solvent extracts of *Spirulina platensis* were subjected to α-amylase inhibitory assay along with Acarbose as a standard. Methanol extract *Spirulina platensis* showed very good antidiabetic activity on comparison with other tested extracts with Alpha-amylase inhibitory percentage 50.1907±0.33250 with IC50 value 99.62 µg/ml other remaining extracts showed percentage of inhibition 8.8481±0.46397, 13.3486±0.40362, 43.5545±0.80724 and 28.0701±1.05970 respectively (Table 3).

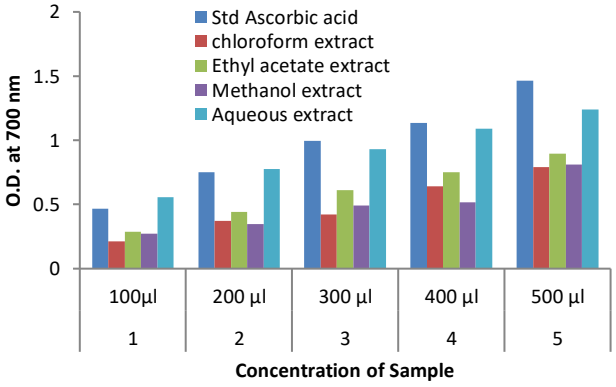

Figure 1: FRAP Assay of Spirulina platensis Extracts

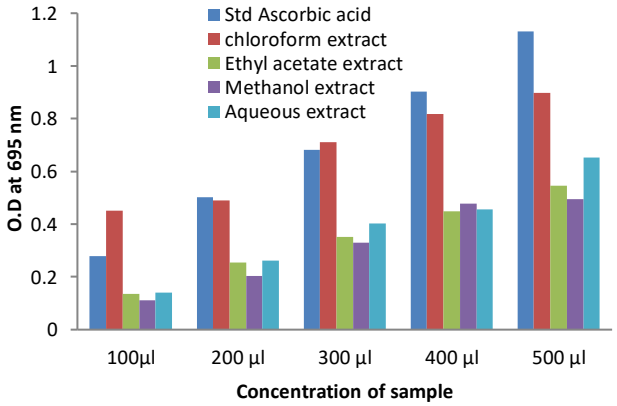

Figure 2: PM Assay of Spirulina platensis Extracts

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Table 1: Qualitative phytochemical analysis of Spirulina platensis

	Inference			
	Chloroform	**Ethyl acetate**	**Methanol**	**Aqueous**
A] Alkaloids				
Iodine	-	-	-	-
Waganer's	-	+	-	+
Dragendroff's	-	-	-	+
B] Flavonoids				
Pew's Test	+	-	-	+
Shinoda Test	++	-	+	-
NaoH Test	+	-	+	-
C] Glycosides				
K-K Test	+	+	+	+
Glycoside Test	-	+	-	-
Molish Test	+	+	++	
D] Phenols				
Ellagic acid Test	-	+	-	+
Phenols Test	-	+	-	+
E] Lignin				
Lobat Test	-	-	++	-
F] Saponins				
Foam Test	-	-	-	-
G] Sterols				
L-B Test	-	++	+	++
Salkowsk Test	++	++	+	++
H] Tannins				
Gelatin Test	-	-	+	+
Lead acetate Test	-	-	+	+
I] Anthraquinone				
Borntrager's Test	-	-	-	+
J] Phlobatanin	-	-	-	-
K] Reducing Sugar	-	-	-	-
L] Volatile oil	-	-	-	+

Table 2: Anti- inflammatory activity of Spirulina platensis Extracts

Sl. No.	Concentration	Treatment	% Inhibition
1	500 µg	Standard	94.2467±1.90148
2	500 µg	Chloroform extract	33.6900±1.43816
3	500 µg	Ethyl acetate extract	10.1367±2.26341
4	500 µg	Methanol Extract	85.3200±1.63000
5	500 µg	Aqueous extract	71.9167±1.12970

Table 3: Antidiabetic activity of *Spirulina platensis extracts*

Samples	Concentration	Inhibition (I %)	IC50 (µg/ml)
Chloroform extract	100µg/ml	8.8481±0.46397	565.09 µg
Ethyl acetate extract	100µg/ml	13.3486±0.40362	374.57 µg
Methanol extract	100µg/ml	50.1907±0.33250	99.62 µg
Aqueous extract	100µg/ml	28.0701±1.05970	178.12 µg
Standard (Acarbose)	100µg/ml	71.0907±0.67796	70.33 µg

All crude extracts of *Spirulina platensis* were investigated to evaluate their antimicrobial activity against food poisoning bacteria including *Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus species, Aspergillus fumigatus, Candida albicans,* and *Aspergillus niger* using disc diffusion method. The results revealed that all crude extracts were potentially effective in suppressing microbial growth of food poisoning bacteria with variable potency. The aqueous crude extract was most effective against *Escherichia coli* to retard their growth at concentration of 100mg/ml with the rate of 16.3±0.57 while in the case of fungal pathogens; aqueous crude extract was effective against *Aspergillus niger* at the concentration of 100mg/ml at the rate of 20.5 ± 0.24. In antiurolithiasis activity, all extracts actively inhibited the calcium oxalate at different concentrations. The 100% concentrations of aqueous crude extract were active and strongly inhibited calcium oxalate at the rate of 67.5±1.49.

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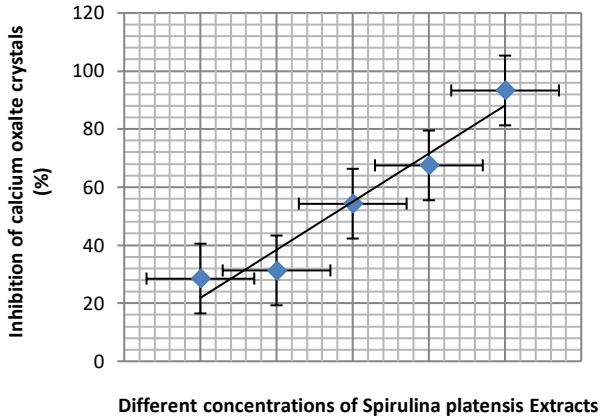

Figure 3: Antiurolithiasis Activity

DISCUSSION

The present study documented the segregation of a variety of bioactive compounds from *Spirulina platensis* which are known to possess different protective mechanisms to dreadful diseases[1]. In the recent years, there is an increased demand for traditional medicines which are based on these phytochemical principles[2]. The major secondary metabolites which can potentially inhibit the oxidative stress are mainly alkaloids, flavonoids, phenols, tannins, sterols, glycosides and terpenoids[3]. Flavonoids are involved in the free radical scavenging, anticancer and antimicrobial properties. These flavonoids are also involved in improving the blood circulation to the brain in alzheimer's disease. Alkaloids are mostly used in the treatment of alzheimer's disease, parkinson's disease, as antimicrobial and antimalarial agents. Phenols are a kind of natural products and antioxidant substances which are competent scavengers of free superoxide radicals, anti-aging and reducing the risk of cancer[4]. Tannins may be employed medicinally in antidiarrheal, haemostatic, and anti hemorrhoidal compounds. The anti-inflammatory effects of tannins help to control all indications of gastritis, esophagitis, enteritis, and irritating bowel disorders[5-7]. Sterols are the biological molecules which are mainly used in the treatment of cardiovascular disease, in lowering the LDL-cholesterol levels, in the treatment of breast cancer and prostate cancer. Terpenoids are having various medicinal properties such as antimalarial, anti-ulcer, hepaticidal, antimicrobial, diuretic activity and anticancer activity. The antioxidant defense mechanism is an in-built mechanism found in humans[8]. But now days due to life style changes and varied food habits the antioxidant balance to the oxidants produced has been reduced[9]. So there is an immediate need of potent antioxidant molecules which can stabilize the reactive oxygen species (ROS) and free radicals generated during the metabolic processes. In order to search for potent bioactive compounds, the present study aimed to evaluate the antioxidant, anti-inflammatory, and antidiabetic potential of *Spirulina platensis* extracts. FRAP, and Phosphomolybdenum assay were conducted to check the antioxidant power of the leaf extracts[10-12]. In FRAP assay Fe^{+3} ions donate an electron and converted into Fe^{+2} in the presence of *Spirulina platensis* extracts as reducing agents. This assay has become matter of interest due to its ease in performance and its accuracy. In the present study, aqueous extract showed highest antioxidant activity (1.2407±0.00702) when compared to other extracts. Phosphomolybdenum assay is a very useful method in predicting the antioxidant activity of crude extracts[13-15]. This assay is based on the principle of reduction of phosphate-molybdenum (VI) to phosphate-molybdenum (V). In the present study chloroform, ethyl acetate, methanol and aqueous extracts were subjected to PM assay. Out of all four extracts, the chloroform extract showed potent antioxidant activity (0.8983±0.00351). Further, it was observed that methanol extract showed high anti-inflammatory activity (85.3200±1.63000) which was comparable with standard. The aqueous extract also showed better anti-inflammatory activity (71.9167±1.12970) when compared to the remaining extracts inhibiting the heat induced albumin denaturation. The antidiabetic activities were checked by alpha amylase assay and glucose uptake in yeast model. In the alpha amylase assay, only methanol extract showed very good antidiabetic activity on comparison with other tested extracts. Till date no reports were available on bioactive compounds from *Spirulina platensis* in urolithiasis hence this study revealed new approach in urolithiasis. Crude extract showed effective inhibition of Calcium oxalate under controlled conditions.

CONCLUSION

In the present study *Spirulina platensis* extracts showed effective phytochemical analysis, antioxidant, anti-inflammatory and antidiabetic activities. Based on the present study results it can be used for the progress of new pharmaceutical drugs for treatment and curing of inflammation, diabetes, urolithiasis and also this study shows that these extracts offer a safe method or addition conduct strategy to manage diabetes through its alpha amylase inhibition. However further complete chemical and pharmacological study be supposed to be performed to separate the lively compounds and suitable clarification of its tool of exploit and it helps in the growth of novel pharmaceuticals to treat diabetes mellitus, pathogens, and urolithiasis.

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