

# In vitro Therapeutic Effect of *Spirulina* Extract

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## ABSTRACT

Recent Research focused on bio therapeutic has wide range of application in medicinal field due to its activity and enormous nutritional values. The main objective of the study is to evaluate the blue green algae, *Spirulina* for their anti-bacterial, antioxidant, hemolytic, anti-diabetics and screening of phytochemical constituents. By disc diffusion method the four extracts (chloroform, hexane, petroleum ether and acetic acid) of *Spirulina* were screened for antibacterial activity against four pathogenic micro-organisms. The antioxidant activity was determined by DPPH method (free radical scavenging activity). The hemolytic activity was determined by using ELISA plate assay. *In-vitro* anti diabetic activity (Alpha amylase enzyme inhibition activity) of potential phytochemical constituents was determined qualitatively. The acetic acid extract of *Spirulina* showed significant anti-microbial activity among the different extracts tested. The most susceptible microorganism was found to be gram positive bacteria (*Staphylococcus sp*) and gram negative bacteria (*E. coli*, *Klebsiella sp* and *Pseudomonas sp*). DPPH free radical scavenging activity of *Spirulina* was found to be increase with its concentration. 2.303 ± 0.5 microgram of *Spirulina* was found in 20% concentration of ethanol extract. *Spirulina* was up to inhibit the activity of alpha-amylase that was dose dependent and also exhibits anti-inflammatory activity. Hence the present study shows that all the extracts of blue green algae of *Spirulina* have wide range of therapeutic effects.

Key words: *Spirulina*, Antimicrobial, Phytochemical constituents and antioxidant, Antidiabetic, Anti-inflammatory activity.

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## INTRODUCTION

*Spirulina* is an oxygenic photosynthetic cyanobacterium found in fresh and marine waters worldwide. It is a microscopic, filamentous, dried biomass of *Arthrospira plantensis*. Humans and animals consume this as a food supplement or as a whole food. *Spirulina* is rich in proteins, carbohydrates, polyunsaturated fatty acids, sterols and minerals such as calcium chromium, iron, zinc, magnesium, manganese and selenium because of which *Spirulina* has been labeled as super food. *Spirulina* proves to be a natural source for provitamin A and

Vitamin B<sub>12</sub>, E, C, phenolic acids, linoleic acid, and xanthophylls phytopigments.<sup>[1]</sup> The presence of chromium selenium, phenolic acids, zinc, linoleic acid, and xanthophylls phytopigments maybe potential contribution to the antidiabetic effect of *Spirulina*. *Spirulina's* Potential anti-inflammatory, anticancer, cholesterol lowering, and antiviral effect is gaining attention as a nutraceuticals and also as a source of a potential pharmaceutical.<sup>[2]</sup> The immunomodulation, anticancer, antioxidant, Anti-diabetic, antiviral and antibacterial activities, as well as positive effects against malnutrition, hyperlipidemia, obesity, diabetes, heavy metal/chemical-induced toxicity, inflammatory allergic reactions, radiation damage and anemia has been included as potential health effects.<sup>[3]</sup> The present study has been designed to observe the *in vitro* phytochemical, antibacterial, hemolytic activity, antioxidant, anti-inflammatory and anti-diabetic effects of *Spirulina*.

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## MATERIALS AND METHODS

### Sample Collection and Isolation

The microalgae water sample was collected from Aliyar Dam, Pollachi, Coimbatore, Tamil Nadu, India (10.4706°N and 76.979°E). Blue-green algae was isolated by serial dilution method which was developed in the microalgae Culture Laboratory, Department of Microbiology, Karpagam Academy of Higher Education (Deemed to be University), India (10.9177°N and 76.982°E). The species were identified using the morphological characters of blue green algae *Spirulina*.

### Stock Culture Maintenance

*Spirulina* strains were grown in 250 ml conical flasks containing 90 ml medium added with 10 ml (10%) of inoculum. All the cultures were maintained in an incubator shaker set at 100 rpm at 25°C±1. The cultures were illuminated with cool fluorescent lamps with the irradiance of 42 μ mol m<sup>-2</sup>s<sup>-1</sup> on 12:12 hr light dark- cycle. The stock cultures were maintained by sub culturing into new medium every 15 days.

### Preparation of Inoculum

The inoculum was prepared from the maintained stock culture for further cultivation. The inoculum was obtained from exponential phase cultures standardized at an optical density of 620 nm.

### Determination of Total Biomass

At the final day of growth, the biomass was harvested by flocculation using alum and it was filtered and allowed to dry under room temperature. The filtered biomass was kept in sterile dried petriplates were weighed initially and calculated the initial weight (fresh weight or wet biomass). After, it was allowed to dry under (sun) light and the dried biomass in the petriplate was weighed (dry weighed).

The total biomass can be calculated by using the formula as follows

$$\text{Total biomass} = \text{Dry weight} - \text{Initial weight} \\ (\text{wet biomass})$$

### Preparation of algal extract

Wet biomass were collected and dried at room temperature. Dried biomass was powdered mechanically. Powdered biomass was then extracted in Soxhlet apparatus.

### Preliminary phytochemical screening

The extracts were subjected to preliminary phytochemical testing to detect the presence of different chemical groups or compounds. *Spirulina* extract were

screened for the presence of different phytochemical compounds like Alkaloids, Carbohydrates, Reducing Sugar, Saponins, Phytosteroids, Cardiac Glycosides, Phenols, Tannins, Flavonoids, Protein, Amino acid, Terpenoids, Fixed oils/fats, Gums/mucilage and Steroids as reported in literature.<sup>[4-6]</sup>

### Microbial cultures

*E. coli*, *Staphylococcus*, *Klebsiella* and *Pseudomonas* were chosen based on their clinical and pharmacological importance. All microbial cultures were obtained from Department of Microbiology, Coimbatore Medical College and hospital, Tamil Nadu, India. The bacterial cultures were sub-cultured on nutrient agar plates/slants and incubated for 24 hr at 37°C. Sub-cultured plates and slants were maintained at 4°C.

### Antibacterial activity

*In vitro* antibacterial activities were examined for chloroform, hexane, petroleum ether and acetic acid extracts. Antibacterial activities of *Spirulina* extracts against four pathogenic bacteria (one Gram positive and three Gram negative) were investigated by the well and disk diffusion methods. Agar plates were inoculated with 100 μL of standardized inoculums (1.5 × 10<sup>8</sup> CFU/mL) of each selected bacterium (in triplicates) and spread with sterile swabs. Wells of 6 mm size were made with sterile borer into agar plates containing the bacterial inoculums.

### Antioxidant Activity

#### DPPH free radical scavenging assay

The antioxidant potency of the different algal extracts was determined using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radicals scavenging technique. 150 μl DPPH solutions were added to 3 ml methanol and the absorbance was taken immediately at 517 nm for control reading. Different volumes of test sample (20, 40, 60, 80 and 100 μl) were taken and diluted with 3 ml methanol. Then the mixture was vortexed and kept at room temperature for 5 min in the dark place. Absorbance was taken at 517 nm spectrophotometrically using methanol as a blank. The percentage of DPPH free radicals scavenging activity was calculated with the formula:

$$\% \text{ Inhibition} = \left[ \frac{(\text{Absorbance of the control} - \text{Absorbance of the test sample})}{\text{Absorbance of the control}} \right] \times 100$$

Entire reactions were carried out in duplicates and the points of purple color develop and decolorization indicates the free radical scavenging activity of the extracts. Ascorbic acid was maintained as a standard antioxidant.

### Anti-hemolytic assay

The hemolytic activities of crude extracts of *Spirulina* were assayed on chick erythrocyte which was reported by.<sup>[7]</sup>

### Determination of thrombolytic activity

The thrombolytic activity of *Spirulina* extract was investigated by the following method. To the 0.8 ml of *Spirulina* extract solution (1%), 0.8 ml of saline solution was added. Then 1 ml of plasma and 0.2 ml of calcium chloride solution (1%) were added to the tubes. The tubes were closed immediately and inverted three times for mixing of the contents until the entire inner surface of the tube become wet. Then the time required for clotting was recorded.

### *In vitro* anti diabetes activity

#### Alpha-amylase enzyme inhibition activity

Alpha amylase enzyme (Sigma, USA) was performed according to the method.<sup>[8]</sup> The reaction mixture contained 1 mL of *Spirulina* at concentration ranges of 20–100 µg/mL and 1 mL of alpha amylase solution. The mixture was pre incubated for 30 min and then 1 mL of starch solution was added to reaction and incubated at 37°C for 10 min. The reaction mixture was stopped by addition of 1 mL of DNS solution and the mixture was boiled for 5 min. The negative control was prepared without sample and alpha amylase enzyme solution and acarbose was used as positive control. The absorbance was measured at 540 nm.

#### β-glucosidase inhibitory assay

The *in vitro* β-glucosidase inhibitory activity of *Spirulina* extract was done by following method. 1 mg of glucosidase was dissolved in 100 ml of phosphate buffer (pH 6.8). To 100µl of buffer different concentrations (50, 100, 150, 200 and 250µg/ml) of sample, 200µl glucosidase were added and the mixture was incubated at 37°C for 20 min and the same concentrations of Acarbose (Standard drug) was taken for comparison. To the reaction mixture 100µl of 3mM Nitrophenyl p-Dglucopyranoside (p-NPG) was added and incubated at 37°C for 10 min. The reaction was terminated by the addition of 2ml of 0.1 M Na<sub>2</sub>CO<sub>3</sub> and the β-glucosidase activity was determined spectrophotometrically at 405 nm on UV-VIS spectrophotometer (Shimadzu UV -1800) by measuring the quantity of nitrophenol released from p-NPG. Acarbose was used as positive control for amylase and β-glucosidase inhibitor.

### *In vitro* anti-inflammatory assays

The *in-vitro* anti-inflammatory activity of *Spirulina* extract was estimated by inhibition of albumin denaturation,

antiproteinase, hypotonicity induced haemolysis and anti-lipoxygenase activities.

### Inhibition of Albumin (proteinase) activity

The mixtures of 0.5ml Trypsin, 1.0ml 25 mM tris - Hcl buffer (pH 7.4) and 1.0ml of *Spirulina* extract was added and mixed. Aspirin (50-100µg/ml) was maintained as a standard. The mixtures were incubated at 37°C for 5 min. Then 1.0ml of 0.87% (w/v) Bovine serum albumin was added. The mixtures were incubated for additional 20 min. 2.0ml of 70% perchloric acid was added to terminate the reaction. Cloudy suspension formed was centrifuged. The supernatant was measured at 214 nm against water as a blank. Protein inhibitory activity (in %) is calculated as follows.

### Inhibition of proteinase denaturation

About 450µl of 5% aqueous bovine serum albumin was mixed with 50µl of *Spirulina* extract then the pH was adjusted to 6.3 using 1N HCl. The samples were incubated at 37°C for 20 min followed by heating at 57°C for 3 min. This mixture was then brought to room temperature and 2.5ml phosphate buffer saline (pH 6.3) was added. Turbidity formed was measured at 660 nm, 50µl distilled water was used as a control. The percentage inhibition of protein denaturation was calculated as follows.

$$\text{Inhibition \%} = \frac{\text{Control OD} - \text{Test OD}}{\text{Control OD}} \times 100$$

### *In vitro* anti-lipoxygenase (LOX) activity

The crude extract of LOX enzyme was prepared from pre-soaked soybeans by homogenizing in phosphate buffer (pH 6.8) for 20 min at 4°C. Then the mixture was centrifuged at 10,000 rpm for 10 min at 4°C and supernatant was taken. Aliquots of crude LOX enzyme extract were incubated for 5 min (in 50 mM Tris buffer, pH 7.4) with samples at different concentrations (10, 20, and 40 µl). Simultaneously aliquots of enzyme were incubated with indomethacin (standard) and the *Spirulina* samples separately. The enzyme reaction was started by adding 1 ml of linoleic acid (50 µM). Increase in absorbance was recorded at 234 nm using a spectrophotometer against buffer blank.

### Hemolysis inhibition activity

Hemolysis is the damage of red blood cells membrane and it is an indicator of free radical damage to RBC. In the assay AAPH is a free radical generator which could attack the RBC membrane and eventually cause hemolysis. Whole blood specimens were collected from physiologically normal volunteers. Then the hole blood

samples were centrifuged at 5000 rpm for 10 min to separate packed red cell samples. The 5% hematocrit of RBC suspension was prepared in PBS solution at pH 7.4. The cell suspension was pre-incubated with various concentrations of *Spirulina* extracts at 37°C for 1 h. Diclofenac was used as a standard reference. Then, the treated cells were incubated with 50 mM AAPH solution at 37°C for 3 hr and the degree of hemolysis was analyzed by measuring optical density (OD) at 540 nm. The reaction without the extract was used as a control sample. The percentage of hemolysis inhibition was calculated by the formula given below.

$$\% \text{Inhibition} = 100 \times (1 - \text{OD sample}) / \text{OD control}$$

## RESULTS

### Phytochemical investigations

*Spirulina*-the blue green algae was extracted with six different solvents namely Distilled water, Ethanol, Ethyl acetate, Petroleum ether, Hexane and Chloroform. The phytochemicals that were present in *Spirulina* were identified as Alkaloids, Carbohydrates, Reducing sugar, Saponins, Phytosteroids, Cardiac glycosidase, Phenols, Tannins, Flavonoids, Proteins, Amino acid, Terpenoids, Fixed oils and fat, gums and mucilage and Steroids respectively. Maximum phytochemical compounds were found in the extract of petroleum ether which contains the presence of alkaloids, carbohydrates, reducing sugar, saponins, phyto steroids, cardiac glycosidase,

phenols, tannins, flavonoids, proteins, amino acids, terpenoids, fixed oils and fat, gums and mucilage and steroids. Hexane extract of *Spirulina* showed the presence of alkaloids, carbohydrates, reducing sugar, saponins, phyto steroids, cardiac glycosidase, phenols, tannins, proteins, amino acids and fixed oils and fats respectively. The minimum constituents were observed in the distilled water, ethanol and ethyl acetate extracts. (Table 1) represents the phytochemical screening of different extracts prepared from *Spirulina*.

### Antibacterial Activity

The acetic acid, chloroform, hexane and petroleum ether extracts were subjected to antibacterial activity against four pathogens at three different concentrations of *Spirulina* extracts. The extracts showed varying degree of antibacterial activity against tested bacterial pathogens. (Table 2) represents the antibacterial activity of the *Spirulina* extract. Maximum zone of inhibition was observed in the acetic acid extract compared to other extracts. Minimum zone of inhibition was found in the chloroform extract. The anti-bacterial activity and inhibitory effects were compared with the standard antibiotic cefotaxime.

### AntiOxidant Activity

The ethanol, ethyl acetate and chloroform extracts of *Spirulina* of different concentrations ranges from 20-100 µg/ml was subjected to DPPH radical scavenging assay. It was observed that free radical was scavenged by test compounds at varying different concentrations. The

**Table 1: Phytochemicals screening of different extracts of *Spirulina* extract.**

Phytochemicals	Test performed	Extracts					
		Distilled water	Ethanol	Ethyl Acetate	Petroleum Ether	Hexane	Chloroform
Alkaloids	Mayer's Test	-	+	-	+	+	+
Carbohydrates	Molisch's test	-	-	+	+	+	+
Reducing sugars	Benedict's Test	-	-	-	+	+	+
Saponins	Foam Test	+	+	+	+	+	+
Phytosteroids	Salkowski's Test	-	+	-	+	+	+
Cardiac Glycosidase	Keller kallani Test	+	-	-	+	+	-
Phenols	Ferric chloride test	-	-	+	+	+	+
Tannins	Ferric chloride Test	-	-	+	+	+	-
Flavonoids	Lead Acetate Test	-	-	-	+	-	+
Proteins	Biruret's Test	+	-	-	+	+	+
Amino acid	Ninhydrin Test	-	-	+	+	+	-
Terpenoids	Salkowskitest	-	+	-	-	-	+
Fixed oils and fats	Spot Test	+	+	+	+	+	+
Gums and mucilage	-	+	-	+	-	-	+
Steroids	-	-	+	-	-	-	+

Table 2: Antibacterial activity of *Spirulina* extracts.

Micro-organisms	Zone of Inhibitions (mm)															
	Acetic acid				Chloroform				Hexane				Petroleum ether			
	20	40	60	+ve	20	40	60	+ve	20	40	60	+ve	20	40	60	+ve
<i>E. coli</i>	24±0.4	10±0.3	34±0.1	14±0.1	10±0.0	NA	15±0.3	21±0.1	NA	10±0.2	NA	27±0.7	NA	NA	7±0.6	24±0.3
<i>Staphylococcus</i> sp.	9±0.2	18±0.4	30±0.3	19±0.5	NA	24±0.1	17±0.5	24±0.0	NA	NA	NA	24±0.1	7±	NA	12±0.3	27±0.2
<i>Klebsiella</i> sp.	10±0.3	19±0.2	34±0.4	21±0.1	NA	NA	12±0.2	27±0.3	NA	18±0.5	NA	21±0.2	NA	NA	19±0.0	14±0.3
<i>Pseudomonas</i> sp.	12±0.5	10±0.1	34±0.2	12±0.3	NA	10±0.3	19±0.1	20±0.5	NA	7±0.3	NA	24±0.3	NA	8±	11±0.1	19±0.3

Results represented as means ± standard deviation (n = 3); +ve control: cefotaxime (µg/ml); NA: No activity

maximum inhibitory concentration (IC<sub>50</sub>) was found to be 4.46 ± 0.2, 4.12 ± 0.5 and 6.54 ± 0.4 µg/ml respectively. Figure 1 represents the scavenging percentage of different concentration of *spirulina* extracts in different solvents.

**Haemolytic Activity**

Figure 2 represents the haemolytic activity of the *Spirulina* extract in various concentrations. The zone of haemolysis was directly proportional to the concentration of the extract. Ethanolic extract of *Spirulina* showed maximum haemolytic activity than ethyl acetate and chloroform. The activity of the extract to lyse the blood cell can be linked with the antimicrobial factors like flavonoids and phenolic compounds which have been distributed in *Spirulina*.

**Thrombolytic Activity**

Figure 3 represents the thrombolytic activity of the *Spirulina* extract analyzed by measuring the lysis of a human red blood cells suspension in a spectrophotometric assay. Ethanol, chloroform, ethyl acetate, petroleum ether and aqueous solution extracts showed clot lysis at the range of 39.39 ± 0.5, 34.92 ± 0.3, 21.5 ± 0.2, 37.6 ± 0.5 and 19.04 ± 0.1 respectively.

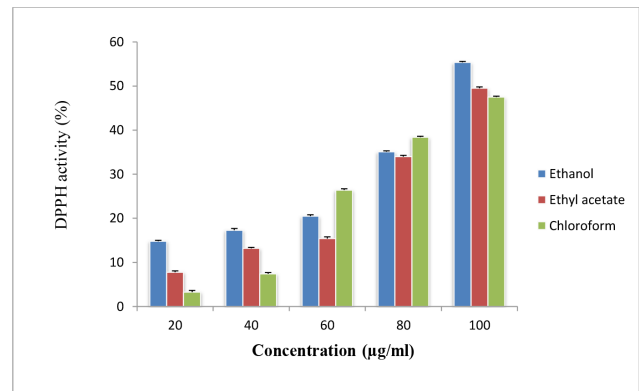


Figure 1: Antioxidant activity of *Spirulina* extracts.

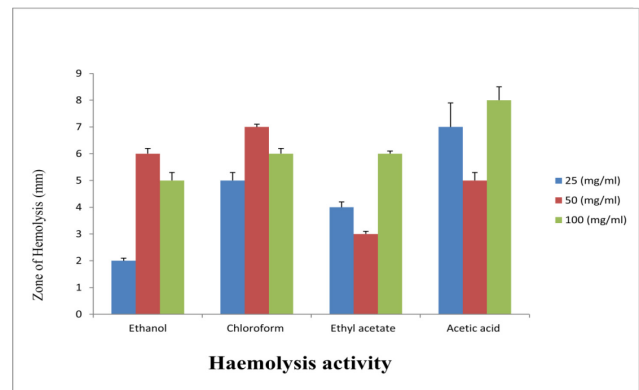


Figure 2: Zones of haemolysis (mm) of *Spirulina* extracts at different concentration.

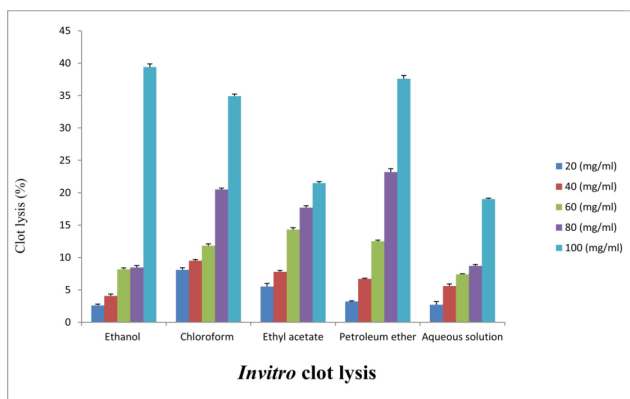


Figure 3: Effect of Spirulina extracts on in vitro clot lysis.

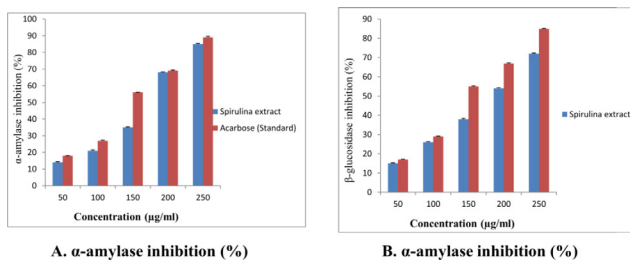


Figure 4: In vitro Anti-Diabetic activity.

### Anti-Diabetic Activity

In vitro anti diabetic activity of Spirulina was estimated by α-amylase and β-glucosidase enzyme inhibition assay through spectroscopy methods. In this present study, the α-amylase activity was recorded as concentration dependent and increasing of activity with increasing concentration of Spirulina. The maximum α- amylase enzyme inhibition activity was recorded in Spirulina and Acarbose at the concentration of 250µg/ml for 85% followed by 200µg/ml for 68% respectively and the standard acarbose showed maximum inhibition in a lower concentration than the Spirulina Figure 4.

By spectroscopic methods β-glucosidase enzyme inhibition activity of Spirulina was screened. B-glucosidase enzyme inhibition action was recorded as concentration dependent and increasing of activity with increasing concentration of Spirulina. The maximum activity of Spirulina was found to be 54% and 72% at the concentration of 250µg/ml. And 54% and 67% at the concentration of 200µg respectively. The standard acarbose have showed maximum inhibition in lower concentration than Spirulina Figure 4.

### Anti-inflammatory activity

An excessive activation of phagocytic cells during inflammatory disorders leads to the production of O<sub>2</sub><sup>-</sup>,

OH and other non-free radicals which can damage the surrounding tissue either directly by the oxidizing action or indirectly with the hydrogen peroxide. The production of free radical results in membrane destruction hence the tissue gets damaged. The production of mediators and chemotactic factors induce the damaged tissue to provoke inflammatory response. The anti-inflammatory activity parameters were investigated in the current research work is enclosed as follows.

The Spirulina extract has maximum protein inhibition of 43% in the concentration of 500 µg/ml whereas aspirin has 95 % inhibition in the concentration of 500 µg/ml respectively. Represents the protein inhibition (%) of Spirulina extract. The proteinase inhibition of Spirulina extract has maximum inhibition at 47% in the concentration of 500 µg/ml whereas aspirin has 110% inhibition in the concentration of 500 µg/ml respectively. Represents the proteinase inhibition (%) of Spirulina extract. Hemolysis inhibition effect was performed in which Spirulina extract has maximum inhibitory effect of 65% in the concentration of 500 µg/ml. Enzymes like LOX actively participate in the inflammatory reactions in vivo. Lipoxygenase are autocatalytic enzymes which are activated by numerous factors due to inflammatory response. The Spirulina extract has maximum lipoxygenase activity of 85 % in the concentration 500 µg/ml. Hence the results provide evidence that the Spirulina exhibits maximum in-vitro anti-inflammatory response Figure 5.

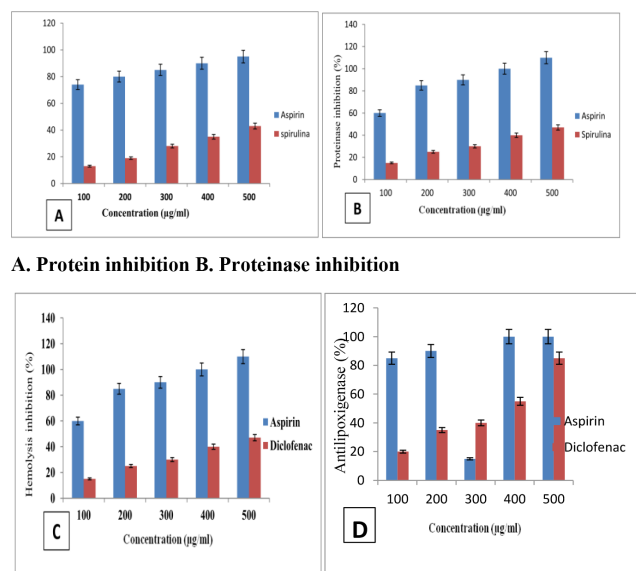


Figure 5: Anti-inflammatory activity.

## DISCUSSION

*Spirulina* is proposed as a good source for nutritional supplements and has a major role in nutraceuticals and pharmaceutical applications. It plays a vital role in the absorption of nutrients due to the presence of various phytoconstituents.<sup>[9]</sup> Phytoconstituents profile are the important factor which determines the nutritional value of a particular product which serves energy to the cells for several biological functions based on the environmental conditions. The present work investigated and reported various phytochemicals present in *Spirulina* extract. Similarly<sup>[10]</sup> reported that the preliminary phytochemical analysis of *Spirulina plantensis* extract showed positive for various phytochemicals. There is an increasing attentiveness towards isolation of anti-microbial substance from algae. *Cyanobacteria*, *Spirulina* and *Nostoc* were reported to have higher antimicrobial activity against the major wound causing pathogens.<sup>[11]</sup> In the present study acetic acid extract of *Spirulina* had maximum inhibitory action against *Escherichia coli*, *Staphylococcus* sp. *Klebsiella* sp. and *Pseudomonas* sp respectively. DPPH is a stable free radical with the absorption band, loss of absorption takes place when there is a presence of antioxidant or a free radical species. Hence for the determination of antiradical or antioxidant activity DPPH method was widely used. The antioxidant activity (%) was performed using the *spirulina* extract and the result reveals that the anti-oxidant activities go parallel with the concentration of the extract. The high radical scavenging activity was found in the ethanol extract. The highest anti-radical activity of *Spirulina plantensis* water extract has 0.963 AU was reported.<sup>[12]</sup> Similarly<sup>[13]</sup> reported that the high absorbance was an indication of high degree of peroxidation of linoleic acid and low antioxidant activity of antioxidants. The result reveals that the extracts and antioxidants showed high inhibition activity towards peroxidation of linoleic acid. *Spirulina* was able to inhibit the alpha amylase and beta glucosidase enzyme; the activities were dose dependent was reported.<sup>[14]</sup> Acarbose a potent drug was used as a standard. The inhibition of alpha amylase and beta glucosidase enzyme as reported. The anti-inflammatory effects of the *Spirulina* extracts have been investigated by the inhibition of albumin denaturation, antiproteinase, hypotonicity-induced haemolysis and Anti-lipoxygenase activities. Neutrophils are known to be a rich source of proteinase which has been implicated in arthritic reactions hence the proteinase present in the lysosomal granules were reported to be serine proteinases. It was previously reported that leukocytes proteinase plays significant role in the inflammatory reactions by the development of tissue damage. Hence the

protection was provided by proteinase inhibitors.<sup>[15]</sup> The denaturation of proteins was also documented to be the cause of inflammation. Several anti-inflammatory drugs such as salicylic acid, phenylbutazone and flufenamic acid exhibit protein denaturation thermally which are said to be dose dependent. In the results of the present study, the *Spirulina* extract has showed the maximum and concentration dependent protein denaturation effect and it was found to be 43% at 500 µg/ml. Stabilization and inhibition effect of the RBC was studied to further establish the mechanism of anti-inflammatory action of *Spirulina* extract. The extract was effective in inhibiting the hemolysis at different concentrations. Thus the result provides evidence for membrane stabilization as an additional mechanism of their anti-inflammatory effect. The extract with hemolysis inhibition release the lysosomal content of neutrophils at the site of inflammation. These neutrophil lysosomal consists of enzymes such as bactericidal enzyme and protease, which upon extracellularly released and cause tissue inflammation and damage.<sup>[16]</sup> *Spirulina* extract 100-500 µg/ml inhibited the hemolysis of RBCs to varying degree. It showed the maximum inhibition 27.2±0.065% at 250µg/ml. Aspirin, standard drug showed the maximum inhibition of 65% at 500µg/ml. Enzymes like lipoxygenase actively participate in the inflammatory reactions hence they are autocatalytic enzymes which are activated by numerous factors. Therefore studying the inhibitory effects on the enzyme reveals a better picture of the biological activity. For this purpose, the crude extract of *Spirulina* was prepared and compared with standard Indomethacin significantly inhibited the lipoxygenase activity in a concentration-dependent manner (up to 85%).

## CONCLUSION AND SUMMARY

*Spirulina* a unicellular algae possess wide range of phytochemical compounds. *Spirulina* exhibits broad spectrum antibacterial activity against gram positive and gram negative pathogens. Antioxidant effect of *Spirulina* may be because of the presence of significant amount of phyto compounds such as saponins, phytosteroids, tannins, phenolic compounds etc. Also the extract exhibits anti-diabetic activity thereby providing the inhibition of alpha amylase and beta glucosidase enzyme respectively. *Spirulina* extract provides anti-inflammatory responses by denaturation of albumin and proteinase, inhibition of lipoxygenase and inhibition to hemolysis. Based on the findings *Spirulina* can be a very good potent natural alternative in therapeutics thus it is a bio-compatible, non-toxic and chemical free.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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