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In Vitro Study on Hypocholesterolemic Effect of *Spirulina*

KEYWORDS: *Spirulina*, Cholesterol, Zarrouk medium, Enzymatic colorimetric method.

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ABSTRACT *The aim of this work was made to screen the Cyanobacterium Spirulina for cholesterol degradation. The Spirulina culture were collected and identified based on morphological appearance. Then the culture was subjected for cholesterol degradation by using the standard water-soluble cholesterol (200mg/dl) at various concentrations such as 200µl, 400µl, 600µl. The cholesterol degrading effect of Spirulina was frequently increased by increasing the time and the concentration of water-soluble cholesterol.*

1. Introduction

Coronary Heart Disease (CHD) is currently a leading cause of death worldwide, this disease is still increasing and has become a true pandemic that respects no borders [18]. Elevated blood cholesterol (hypercholesterolemia) is an important risk factor associated with atherosclerosis and coronary heart diseases [6]. Cholesterol is a lipid, produced by the liver that is required for various functions, found in some foods. Cholesterol is parent compound of hormone, bile acid and vitamin D [9]. A normal or desirable cholesterol level is defined as less than 200 mg of cholesterol per deciliter of blood (mg/dL). Blood cholesterol is considered to be border line when it is in the range of 200 to 239 mg/dL. Elevated cholesterol level is 240 mg/dL or above is considered to be hypercholesterolemia. Hypercholesterolemia indicates that, HDL is decreased and LDL is increased [13]. Excess of cholesterol is risk factor, cause of death worldwide, each year more than 17 million people or 30% of all death worldwide and 25 million death are expected in 2020 [17]. Recent modalities for lowering blood cholesterol levels involve dietary management, behavior modification, regular exercise and drug therapy [10]. Pharmacological agents that effectively reduce cholesterol levels are available. But they are expensive and known to have severe side effects [5].

Spirulina is blue-green microalgae, which contain high-anti-oxidant components, abundant amino acids, high-quality proteins, Fe and Ca, unsaturated fatty acids and many types of vitamins, including A, B2, B6, B8, B12, E and K. *Spirulina* have anti-viral, anti-inflammatory and anti-tumor effects and reduce blood lipid profile, blood sugar, body weight and wound healing time. Therefore, they are known as therapeutic and functional food [8]. *Spirulina* plays an important role in metabolic diseases like diabetes, hypertension, anemia and others [1]. A blue protein called phycocyanin, belonging to the photosynthetic apparatus of *Spirulina platensis* has antioxidant and radical scavenging properties both *in vitro* and *in vivo* models [4].

Recently more attention has been given to study its therapeutic effects, which include reduction of cholesterol and nephrotoxicity by heavy metals, anticancer properties, protection against radiation, and enhancement of the immune system [3]. *Spirulina* have also exhibited metabolic (hypoglycemic), cholesterol regulatory properties, anti-viral, liver-protecting and blood-vessel relaxing effects, anti-cancer, anti-inflammatory and anti-oxidant properties [11, 12,16]. *Spirulina* has the beneficial effects on blood pressure through its full content in anti-oxidant combined with vitamin A, B12, E, proteins and mineral salt and also in building immunity of patients with HIV infection and multiple cardiovascular risk factors [2,7,14,15]

2. Materials and Methods

2.1 Collection of the Culture:

Algal culture of *Cyanobacterium Spirulina* was collected from the Department of Microbiology, Ayya Nadar Janakiammal College, Sivakasi.

2.2 Maintenance of the Culture

The *Cyanobacterium Spirulina*, was cultivated in Zarrouk medium at 25±2°C, pH 10 under photoautotrophic condition by continuous illumination using white fluorescent tubes and thrice daily shaking by hand for 15 days. The pH of the medium was maintained by using NaOH solution.

Composition of the Zarrouk medium

One liter of Zarrouk's medium consists of (part A) $NaHCO_3$ 16.80 g and K_2HPO_4 0.50 g; (part B) $NaNO_3$ 2.50 g, K_2SO_4 1.00 g, NaCl 1.00 g, $MgSO_4.7H_2O$ 0.20g, EDTA-$Na_2.2H_2O$ 0.08 g, $CaCl_2.2H_2O$ 0.04g, and $FeSO_4.2H_2O$ 0.01 g; trace elements mixture A (part C 10 mL/l): 1.00 mL, trace elements mixture B (part D 1.0 mL/l): 1.00 mL; part C mg/l: H_3BO_3 2.86, $MnCl_2.4H_2O$ 1.810 g, $ZnSO_4.7H_2O$ 0.222 MoO_3 0.015, and $CuSO_4.5H_2O$ 0.074 (the used amount is 10 mL/l); part D mg/l: NH_4VO_3 22.9, $NiSO_4.7H_2O$ 47.8, $NaWO_2$ 17.9, $Ti_2(SO_4)_3.6H_2O$, and $Co(NO_3)_2.6H_2O$ 4.4 (the amount used was 1.0 mL/l) [19].

2.3 Morphological Identification

Morphology of the *Spirulina* was observed under mi-

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croscope. A drop of *Spirulina* culture was placed on the clean concavity slide and then the slide was observed under microscope. *Spirulina* was recognized mainly by morphological features.

2.4 Hypocholesterolemic effect of *Spirulina*

2.4.1 Preparation of the *Spirulina* culture medium

Zarrouk medium was prepared and standard water-soluble cholesterol (200mg/dl) was added to the medium at various concentrations such as 200µl (S1), 400µl (S2), 600µl (S3) respectively. The pH of the medium is adjusted to 10 by using NaOH solution. Then 1% of *Spirulina* culture was inoculated to the medium and the tubes were maintained under photoautotrophic condition by continuous illumination using white fluorescent tubes and thrice daily shaking by hand.

Concentration of cholesterol in the medium was measured at different times [after 5 days (T1), 10 days (T2), and 15 days (T3)].

2.4.2 Analysis of Total Cholesterol *in Vitro*

Total Cholesterol was analyzed by using enzymatic colorimetric method. The series of the reactions were involved in the assay system is as follows:

1. Cholesterol esters are enzymatically hydrolyzed by cholesterol esterase to cholesterol and free fatty acids.

2. Free cholesterol is then oxidized by cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide.

3. The hydrogen peroxide combines with phenol and 4-amino-antipyrine in the presence of peroxidase to form a chromophore (quinoneimine dye), which will be quantified at 500-550nm.

1 ml of culture from each tube was removed and centrifuged at 3000 towers/minute for 10 minutes. The supernatants were collected for the determination of total cholesterol. Prepare the test to be analyzed as indicated in Table-1.

Table-1: Method of analysis of total cholesterol *in vitro*

	Blank	Standard	Sample
Standard Cholesterol	-	10µl	-
S1 (200 µl)	-	-	10µl
S2 (400 µl)	-	-	10µl
S3 (600 µl)	-	-	10µl
Reagent	1ml	1ml	1ml

After the preparation of the tests, the tubes are well shaken and incubated for 5 minutes at 37° C. The absorbance of the standard and samples were observed in comparison with the reagent at 500 nm.

Cholesterol concentration of the sample is calculated as follows:

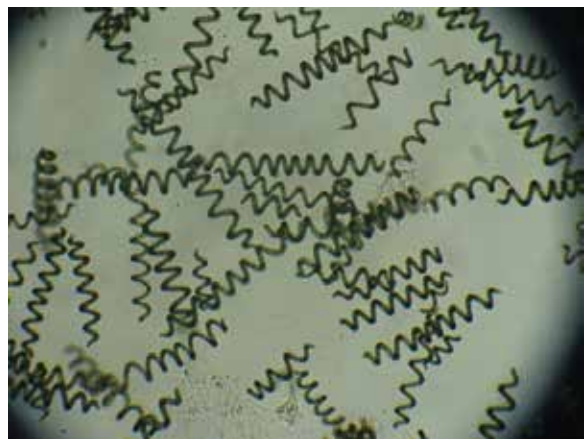
$$C_{Ch} \text{ of the Sample} = A_{Sample} / A_{Standard} \times C_{Standard}$$

3. Results and Discussion

3.1 Microscopic appearance of *Spirulina*

Morphology of the *Spirulina* was observed under microscope. These are filamentous *Cyanobacterium* with the arrangement of the multi cellular cylindrical trichomes in an open left-hand helix along the entire length. (Fig-1).

Fig-1: Morphological identification of *Spirulina*



3.2 Hypocholesterolemic effect of *Spirulina*

In this study, an attempt was made to screen the *Spirulina* for the cholesterol degradation. Table-2 indicates the results of the assay of total cholesterol *in vitro* at different times of incubation. The *Spirulina* degrades the water soluble cholesterol at various concentrations (200 µl, 400 µl, 600 µl) in Zarrouk medium. The decreasing cholesterol level was measured by using the enzymatic colorimetric method.

Table – 2: The hypocholesterolemic effect of *Spirulina*

Concentration of Standard Water-soluble Cholesterol	Concentration of Cholesterol on 5^{th} Day (T1) (mg/dl)	Concentration of Cholesterol on 10^{th} Day (T2) (mg/dl)	Concentration of Cholesterol on 15^{th} Day (T3) (mg/dl)
S1 (200 µl)	158.9	112.3	5.47
S2 (400 µl)	260.3	142.4	10.9
S3 (600 µl)	342.5	200	38.4

4. Conclusion

From this present study, it was concluded that the cholesterol added in the Zarrouk medium was degraded by the activity of *Spirulina*. The cholesterol degrading effect of *Spirulina* was frequently increased by increasing the time. Hence it was proved and suggested that the *Spirulina* is a better option for the treatment of hypercholesterolemic patients.

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