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An *in vitro* study on cholesterol degradation by *spirulina*

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Abstract
The aim of this study is to screen the *Cyanobacterium Spirulina* for cholesterol degradation. The *Spirulina* culture were collected and identified based on morphological appearance. *In vitro* study of cholesterol degradation by *Spirulina* was carried out by the cultivation of *Spirulina* in the medium containing 0.5% of two different concentrations of blood serum cholesterol (Hypocholesterol-142 mg/dl & Hypercholesterol-280 mg/dl). The cholesterol degrading activity of *Spirulina* was detected by the concentration of cholesterol in the medium. It was measured by enzymatic colorimetric method at 500 nm after 5 days. The *Spirulina* showed better degradation (89.2%) in the medium containing blood serum cholesterol in 5 days.

Keywords: *Spirulina*, Zarrouk medium, Serum cholesterol and enzymatic colorimetric method.

1. Introduction
Cholesterol circulates in the blood stream. It is an essential molecule for the human body. Cholesterol is a molecule from which hormones and steroids are made. It is also used maintain nerve cells. Between 75 and 80% of the cholesterol that circulates in a person's bloodstream is made in that person's liver. The remainder is acquired from outside source. Cholesterol is found in animal sources of food, not found in plants (Saile and Taki., 2007) [16]. The WHO has predicted that, by 2030, cardiovascular diseases will remain the leading causes of death, affecting approximately 23.6 million people around the world, the risk of heart attack is three times higher in those with hypercholesterolemia, compared to those who have normal blood lipid profiles (WHO., 2009) [18]. The *Spirulina* used in the treatment of many diseases, including Cholesterol degradation (Nakaya *et al*., 1988 and Ramamoorthy, Premakumari., 1996) [13, 14], as well as to reduce body weight in humans (Becker *et al*., 1986) [2]. *Arthrospira platensis*, also called *Spirulina platensis*, used in nutritional rehabilitation in undernourished / malnourished people with excellent results (Amha *et al*., 1993, Gatugel *et al*., 2000, Dia *et al*., 2009, Simpore *et al*., 2005 and Kulshreshtha *et al*., 2008) [1, 9, 7, 17, 12]. *Spirulina* possess other biological functions such as antiviral, antibacterial, antifungal and antiparasite activities (Khan *et al*., 2005) [11] and it boosts the immunity and increase resistance to various infections.
Spirulina is useful for human nutrition, because of the high quality and quantity (60-70% of its dry weight), (Ciferri., 1983) [6] of protein and amino acids (Dillon, Phan., 1993 and Richmond., 1992) [8, 15]. *Spirulina* contains essential aminoacids, especially leucine (10.9% of total amino acids), valine (7.5%) and isoleucine (6.8%) (Cohen., 1997) [5]. *Spirulina* has a relatively high provitamin A concentration (Belay., 1997) [4] and harmless b-carotene (Henrikson., 1994) [10]. *Spirulina* is a very rich source of vitamin B12, which is important for people who need supplements to treat pernicious anemia (Richmond., 1992, Belay., 1997 and Becker., 1984) [15, 4, 3].

2. Materials and methods
2.1. Collection of the culture
Spirulina culture 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. The pH of the medium was maintained by using NaOH solution.

2.3. Morphological Identification
The *Cyanobacterium Spirulina* was observed under microscope.

2.4. Cholesterol degradation by *Spirulina* using blood serum Cholesterol
2.4.1. Collection of Blood serum
Blood serum of hypocholesterolemic and hypercholesterolemic patients were collected from Clinical Laboratory.

2.4.2. Preparation of the *Spirulina* culture medium
10 ml of Zarrouk medium was prepared in screw cap tubes and it was closed with cotton plug. 0.5 ml of serum was added to the medium having the following amount of cholesterol such as 142 mg/dl (Hypercholesterol) and 280 mg/dl (Hypercholesterol) respectively. The pH of the medium is adjusted to 10 by using NaOH solution. Then 2% 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 after 5 days.

2.4.3. Analysis of Total Cholesterol *In vitro*
Total Cholesterol was analyzed on 5th day of incubation by using enzymatic colorimetric method.
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 ºC. 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
The aim of this work was to identify and screen the *Cyanobacterium Spirulina* for cholesterol degradation. 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.

3.1. Cholesterol degradation by *Spirulina* using blood serum cholesterol
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* after 5 days incubation. The *Spirulina* degrades the serum having the following amount of cholesterol such as 142 mg/dl (Hypo) and 280 mg/dl (Hyper) cholesterol in Zarrouk medium. The decreasing cholesterol level was measured by using the enzymatic colorimetric method.

Table 2: Cholesterol degradation by *Spirulina* using blood serum Cholesterol

Concentration of Blood serum cholesterol (0.5 ml)	Concentration of Cholesterol (mg/dl) (0th Day)	Concentration of Cholesterol (mg/dl) (5th Day)	Percentage of cholesterol degraded (%)
Hypo cholesterol	142	19.17	86.5
Hyper cholesterol	280	30.14	89.2

4. Conclusion
From this present study, it was concluded that the *Spirulina* showed the better degradation of cholesterol in the medium containing blood serum cholesterol. Two concentrations of cholesterol were taken for the degradation such as Hypercholesterol and Hypercholesterol. The hypercholesterol showed the 86.5% of degradation and hypercholesterol showed the 89.2% of degradation by *Spirulina*. Hence it was proved and suggested that the *Spirulina* is a better option for *In vivo* treatment of patient with hypercholesterolemia.

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