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Research Article

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Antitumor activity of silver nanoparticles biosynthesized by micro algae

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ABSTRACT

The purpose of this study is to evaluate the antitumor efficiency of different concentrations of silver nanoparticles (AgNPs) biosynthesized by The blue green algae (Anabaena oryzae, Nostoc muscorum and Calothrix marchica)on Ehrlich Ascites Carcinoma (EAC) in vitro. Silver nanoparticles were synthesized by the reduction of silver nitrate in the algal aqueous extracts . The green synthesis of AgNPs through algal extracts was monitored by colour change (from bale red to brown) and UV spectroscopy range from 400-450 nm^{-1}. Different concentrations of AgNPs were used to study their cytotoxic activity against EAC in vitro. Results of trypan blue exclusion test showed a decrease in EAC cells viability by increasing AgNPs concentration in all tested algae. The maximum inhibition percentage of EAC cells was 80% with 98µg/ml AgNPs biosynthesized by Calothrix marchi. Silver nanoparticles biosynthesized by Calothrix marchi were the most cytotoxic against EAC in vitro.

Keywords: The blue green algae; *Calothrix marchi*; Ehrlich Ascites Carcinoma (EAC); *in vitro*; silver nanoparticles (AgNPs).

INTRODUCTION

Micro-algae and their aqueous extracts generated an enormous amount of bioactive compounds with immense medicinal potential interest in the pharmaceutical industry. Micro algae were reported to contain various significant compounds with antibacterial, antiviral and antitumor activity [1].

Nanobiotechnology is used for producing nano-sized particles with unique biological or mechanical functions through mixture of organic, physical and chemical standards [2]. Diagnosis, treating and dealing with diseases such as human cancers, is one of the important biological application of nanobiotechnology [3]. Ultimately, the use of nanomedicine will allow simultaneous drug delivery, wound healing and tumor cell targeting in a unique manner [4].

Chemical and physical properties of silver metal reflux the important role of its nanoparticles in human health, so it is necessary to investigate formation, stability, and sedimentation of AgNPs[5]. Chemical synthesis of nanoparticles has many flaws in using toxic solvents and production hazard by-products, on the contrary, the biomolecules involved in the green synthesis of nanoparticles are less toxic and acting as functionalizing ligands, so green synthesis of nanoparticles is more suitable than chemical synthesis [6].

Several biological systems such as microorganisms, marine organisms, micro-fluids, and plant have been used as reducing agents for the green synthesis of silver nanoparticles[7]. Moreover Green synthesis of silver nanoparticles by micro-algae showed more advantageous over other biological processes by bacteria and fungi, because it is more suitable for large scale production of silver nanoparticle with various size and shapes and it eliminates the cell culture maintaining process [8].

This work aimed to study the cytotoxic effect of biologically synthesized AgNPs prepared by biological (green) techniques using *Anabaena oryzae, Nostocmuscorum and Calothrix marchica in vitro* against EAC.

EXPERIMENTAL SECTION

2.1.Algae identification

A drop of the blue green algae suspension was placed on an ordinary glass slide covered with a cover slip and examined by ocular lens. Blue green algal species were identified according to Desikachary [9]; diameters and lengths of 100 vegetative cells, 100 heterocyst and 100 akinetes were measured by ocular lens (40x magnification).

2.2. Growth conditions

Each isolate was added to 500 ml Erlenmeyer flasks containing 200 ml BG11 medium. The Erlenmeyer flasks were incubated at 25 ±1 ºC under constant light (80 µ mol^{-1}) for 15 days. The cultures were harvested by centrifugation at 1000 rpm for 10 minutes.

2.3.Rapid synthesis of AgNPs

Five mL of silver nitrate solution (560 mg/L) was added to 5 mL of washed cyanobacterial cultures. The mixture was incubated at 25°C for 28 days and maintained in the dark. The sample has been characterized for the synthesis of silver nanoparticles by colour change from bale red to brown and also by using Ultra Violet –Visible Spectrum (Uv-Vis) as reported by Mubarak [10].

2.4. *UV–Vis spectra analysis*

The reduction of silver ions Ag^{+} in aqueous extractsof micro algae and the formation of AgNPswasmonitoredby measuring the UV-Vis spectra. UV-Vis spectroscopy analysis of silver nanoparticles produced were carried out as a function of bioreduction time at a wavelength of 100- 700 nm^{-1}on Ultra violet-Visible spectroscopy (T80+UV/VIS Spectrometer) at Genetic Engineering and Biotechnology Research Institute (GEBRI), Egypt.

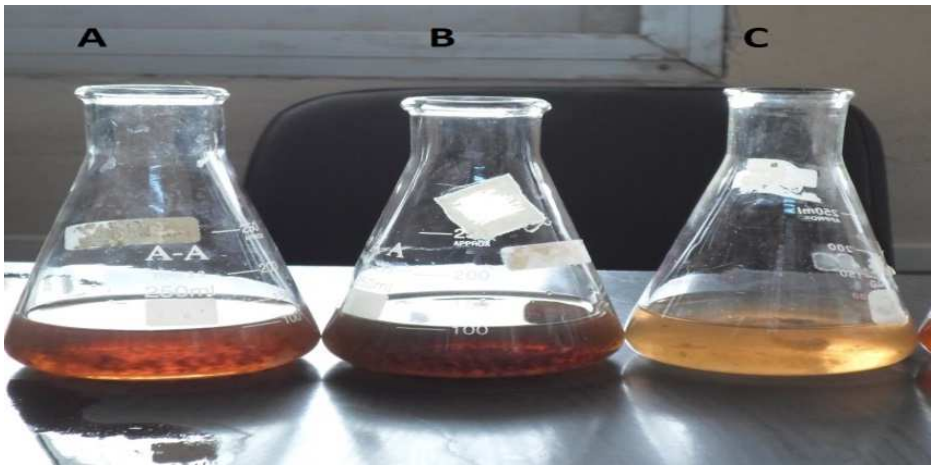
2.5. In vitro assessment of anti tumor activity of green synthesized silver nanoparticles

The antitumor activity of AgNPs synthesized by algal extracts was determined *in vitro* against Ehrlich Ascites Carcinoma (EAC) cell line which was kindly purchased from the National Cancer Institute, Cairo University, Egypt. 1ml of freshly ascitic fluid which was drawn from an albino mice bearing 7-14 days-old ascitic tumor(EAC) was diluted with 9ml normal saline in sterile test tube. EAC cells were thereafter propagated in GEBRI laboratories by weekly intraperitoneal injection of 0.2 ml ($1x10^{6}$EAC cells) of EAC suspension into three mices to ensure that the ascitic fluid would still propagated.

Appropriates dilutions of Ag nanoparticles were tested as antitumor activity *in vitro* by trypan blue exclusion method reported by Freshney[11]and calculated as followed:

(No. of non viable cells x100)/total cells

This method determines the effect of different concentrations of AgNPs for regression of tumors cells.



RESULTS AND DISCUSSION

3.1. Isolation and purification of cyanophyta

The blue green algae (*Anabaena oryzae*, *Nostocmus corum* and *Calothrix marchica*) were characterized by Light microscope (**Figure 1**) at GEBRI using diagnostic keys from cyanophyta book [9]**.**

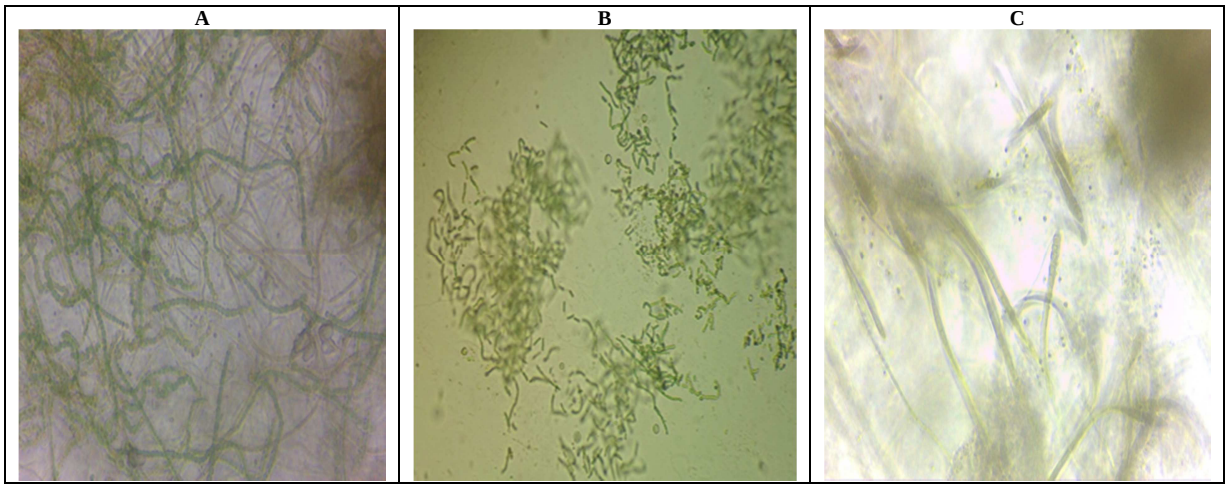


Figure 1. Microscopic pictures of: A) ***Anabaena oryzae*** **B)** ***Nostocmus corum*** **C)** ***Calothrix marchica*** **(X 40)**

3.2.Rapid Synthesis of silver nanoparticles (AgNPs) using algal aqueous extracts

The green synthesis of silver nanoparticles through algal extracts was carried out **(Figure 2).** It is well known that, silver nanoparticles exhibit yellowish – brown colour in aqueous solution due to the excitation of surface plasmon vibrations in silver nanoparticles [12].

Figure 2.Colour change of silver nanoparticles biosynthesized by micro algae :
A)*Anabaena oryzae* *B) Nostocmus corum* C) *Calothrix marchica*

3.3.UV-Vis spectroscopy of biosynthesized AgNPs by different cyanophyta

UV-Vis spectroscopy of silver nanoparticles were characterized by one of the most widely used Jain technique [13]. Peaks were observed from 400-450 nm^{-1} , which corresponded to plasmon excitation of silver nanoparticles (Figures 3-5), indicating the presence of silver nanoparticles. Several researchers have observed absorption of a broad peak of colloidal silver in solution between 400 and 450 nm, which is assigned to surface plasmon excitation of the metal nanoparticles [4].

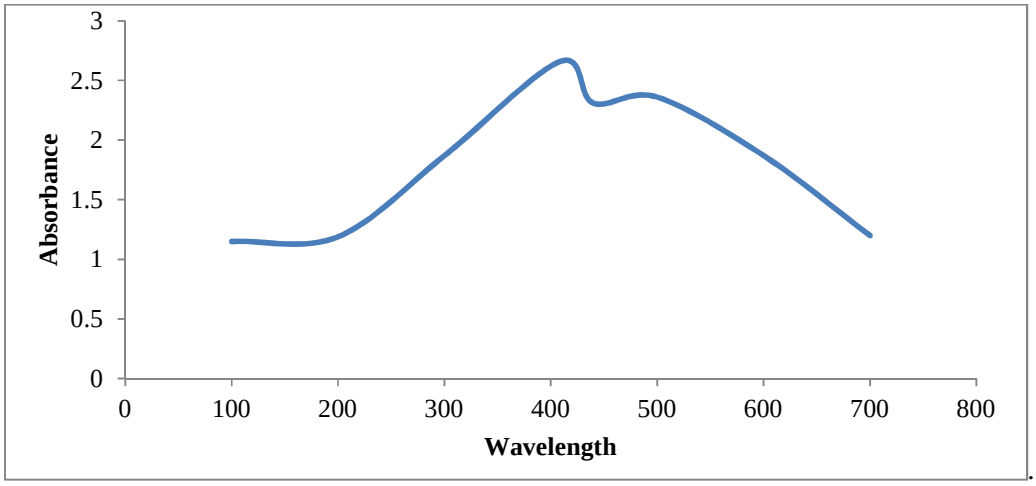


Figure 3.UV-Vis absorption spectra of silver nanoparticles biosynthesized from *Anabaena oryzae*

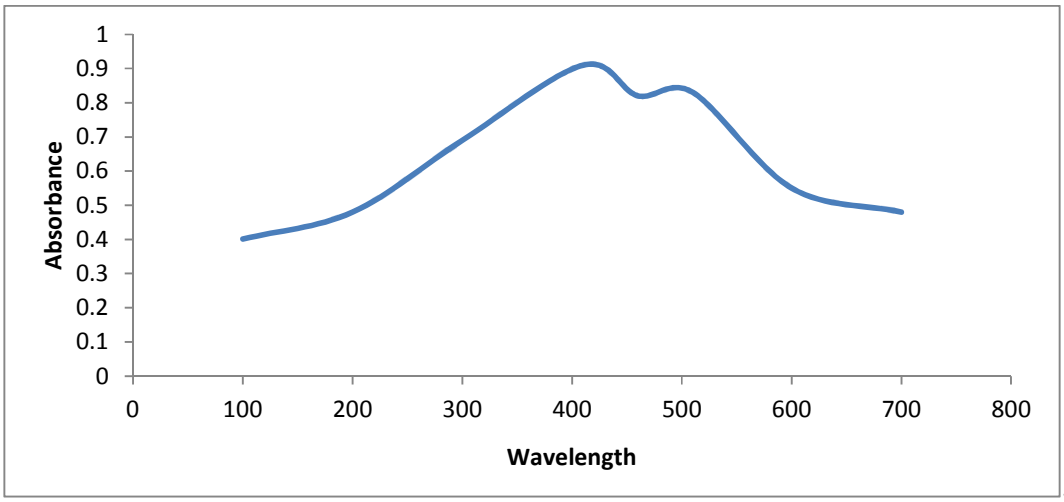


Figure 4. UV-Vis absorption spectra of silver nanoparticles biosynthesized from *Nostocmus corum*

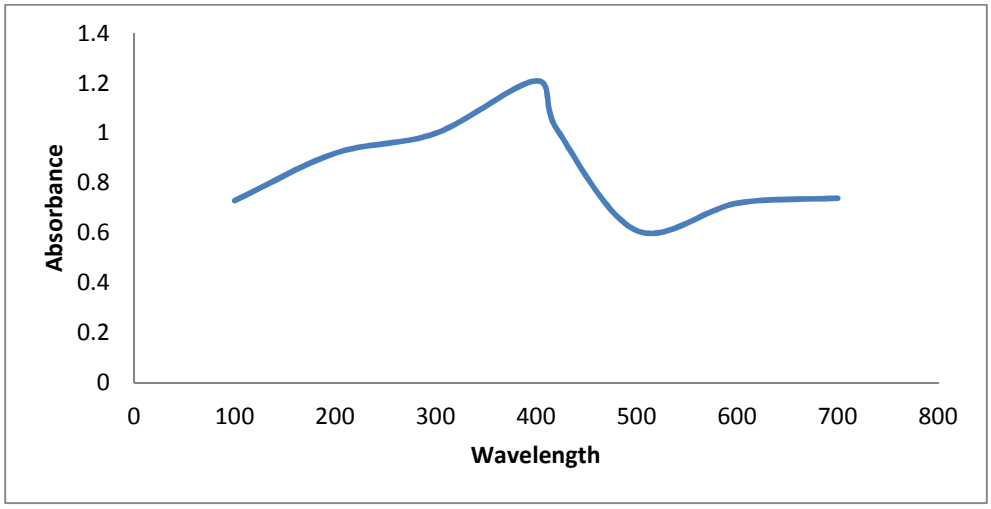


Figure 5. UV-Vis absorption spectra of silver nanoparticles biosynthesized from *Calothrix marchica*

2.6. Antitumor activity of AgNPs biosynthesized by different micro algae

Different concentrations of AgNPs synthesized by Cyanophyta (*Anabaena oryzae, Nostocmus corum and Calothrix marchica*) showed significant reduction on Ehrlich Ascites Carcinoma (EAC) cell line viability as shown in **Table (1)**. The result showed that AgNPs biosynthesized by *Calothrix marchic* had the most cytotoxic activity in comparing to that biosynthesized by other tested micro algae. The results showed decrease in EAC cells viability by increasing AgNPs concentration in studied algae. Different concentrations (42, 56, 69, 85 and 98 µg/ml) of green synthesized AgNPs by micro algae inhibited the proliferation of EAC cells.

Table 1: Percentage of *In vitro* cytotoxic effect of different concentrations of AgNPs synthesized by the isolated cyanophyta on the viability of EAC

Conc. of AgNPs µg/ml	Percentage of cytotoxic activity against EAC %		
	Calothrix marchica	*Nostocmus corum*	*Anabaena oryzae*
42	49	40	38
56	55	48	39
69	59	52	44
85	73	60	49
98	80	69	62

Micro- algae contain several biologically active molecules which are used as source of food, feed and medicine (as anticancer agents). Until now, more than 2400 marine natural products have been isolated from microalgae[14].

Silver nanoparticles are being extensively used in medicine for its therapeutic values. Recently, AgNPs have been reported to be efficient anti-tumor agents because silver nanoparticles had the ability to induce apoptosis by caspase signaling[15].

Synthesis of metal and semiconductor nanoparticles through biological route offers a few advantages over the common chemical and physical procedures as it is an easy, fast and eco-friendly alternative that doesn't involve any costly instruments and hazardous chemicals as well.

The present study was presented to evaluate the synthesis of AgNPs by micro-algae and their cytotoxic potential against EAC cell line. The organic compounds in the micro algae are responsible for reduction of silver ions into nanoparticles [16]. After exposure of the aqueous extract of cyanobacteria to silver nitrate solution (keeping the whole reaction in a dark place to avoid excitation energy)color changed to red, thus is an indication for the synthesis of silver nanoparticles [1].

UV spectrum showed that peaks wavelength varying from 400-450 nm^{-1} indicated the synthesis of AgNPs. At the beginning of the reaction the band recorded low wavelength and the reaction was carried out hasty. After 48hrs of the reaction the band was at high wavelength due to aggregation of nanoparticles forming large size of nanoparticles that needed less energy and hence longer wavelength due to poly dispersion of the nanoparticles [14]. So the reaction rate is directly proportional to reaction time till 48hrs of synthesis because after 48hrs, the activity of AgNPs in the solution were stable for a period of 2 months [17].

The maximum inhibition percentage of EAC cells was 80% with 98µg/ml AgNPs biosynthesized by *Calothrix marchi*. Silver nanoparticles biosynthesized by *Calothrix marchi* were the most cytotoxic against EAC *in vitro*. The biologically synthesized AgNPs showed excellent antioxidant potential, antimicrobial activity and possessed considerable cytotoxic effect on MCF-7 cell [18]. Devi and Bhimba[19] showed that Hep2 cells proliferation were significantly inhibited by AgNPs synthesized from *Ulva lactucain vitro*.

The cytotoxic activity of biosynthesized AgNPs were discussed by *in vitro* study so in the near future we are going to study the anti-proliferation activity of different concentrations of silver nanoparticles biosynthesized by micro algae *in vivo*, and evaluate the toxicity of AgNPs *in vivo*.

CONCLUSION

In the present study silver nanoparticles were biosynthesized by micro-algae, *Anabaena oryzae, Nostocmus corum and Calothrix marchica* .Silver nanoparticles biosynthesized by *Calothrix marchica* were the most cytotoxic against EAC.

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