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Antibacterial activity of silver nanoparticles biosynthesized using *Spirulina platensis* microalgae extract against oral pathogens

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ABSTRACT

In the present investigation, *Spirulina platensis* extract was used for biosynthesis of silver nanoparticles (AgNPs) and the antibacterial activity of the biosynthesized nanoparticles against oral pathogens was assessed. The biosynthesis of AgNPs was successfully achieved using 0.5 ml of *Spirulina* extract and 50 ml of silver nitrate (1mM) after 5 minutes of heating at 50°C.

The formation of AgNPs was confirmed using UV- Visible spectrophotometer by the appearance of typical plasmon resonance band of AgNPs at 425nm. The average particle size of the biosynthesized AgNPs was 25.56 nm. Three Gram positive oral bacteria were used to investigate the antibacterial activity of biosynthesized silver nanoparticles. These bacteria were *Streptococcus mutans*, *Staphylococcus aureus* and *Enterococcus faecalis*. The antibacterial activity was studied by determining the inhibition zone diameter using the disc diffusion method. The results showed that the AgNPs biosynthesized using *Spirulina* extract have good antibacterial activity against the three tested bacteria. The inhibition zone diameters were 12 mm, 12 mm and 13 mm for *Streptococcus mutans*, *Staphylococcus aureus* and *Enterococcus faecalis*, respectively. In conclusion, AgNPs biosynthesized using *Spirulina platensis* extract could be employed as antibacterial materials in dentistry. Further work could be addressed towards the optimization of the preparation conditions and controlling the size of nanoparticles.

INTRODUCTION

Microalgae research is increasingly attracting researchers’ interest. Microalgae are infinite sources of various metabolites such as alkaloids, carbohydrates, flavanoids, pigments, phenols, saponins, steroids, tannins, terpenes and vitamins which possess several biotechnological and industrial applications (Guihéneuf *et al.*, 2016). They are also enriched with several pharmacologically active compounds with antibacterial, anticancer, antifungal and antiviral activities. Several authors reported many beneficial applications of microalgae especially cyanobacteria in different aspects (Semary and Fouda, 2015; Vijayakumar and Menakha, 2015; Singh *et al.*, 2017; Mostafa *et al.*, 2019; Rashad *et al.* 2019). *Spirulina platensis* is a photosynthetic cyanobacteria with a plenty of nutritious compounds, phytochemicals, nutraceuticals and probiotics (Soni *et al.*, 2017).

Nanoparticles have unique biological and optical properties which make them considered as efficient materials of the next technology generation with therapeutic and diagnostic applications. Metal nanoparticles are highly employed in a wide range of biomedical applications especially as antimicrobials (Saran *et al.,* 2017). Silver ions and silver based compounds are known bactericides and have been extensively used in nanoparticles as antibacterial agents. Due to their large surface area that comes into contact with the microbial cells, the silver nanoparticles show effective antibacterial activity and thus have a higher percentage of interaction than larger particles of the same parent material (Patel *et al.*, 2015; Salaheldin *et al.,* 2019).

Silver nanoparticles are usually synthesized by chemical techniques thought their biosynthesis using extracts offers a worthy alternate. Biosynthetic methods employing either microorganisms or plant extracts have emerged as a simple and feasible alternative to chemical and physical synthetic procedures (Merin *et al.*, 2010).

The application of nanoparticles in dentistry is of particular interest as the oral cavity frequently comes across a superfluity of microorganisms (Song and Ge, 2019). Among oral bacteria, *Streptococcus mutans* are Gram positive bacteria most frequently found in the oral cavity's normal flora (Azizi *et al.*, 2015). They has been reported as a primary cariogenic bacterial pathogen due to their ability to metabolize different carbohydrates of sugar (Zhou *et al.*, 2018). S*aphylococcus aureus* is also an abundant Gram-positive bacteria which has a strong connection to dental implant infections (Wang and Ren, 2017). *E*nterococcus *faecalis* has been connected to oral infections, such as caries, endodontic infections, periodontitis, and peri-implantitis (Komiyama *et al.,* 2016). Controlling these bacteria is of utmost importance in dentistry. Thus, the present work aims to use *Spirulina platensis* extract for the biosynthesis of silver nanoparticles and to investigate the antibacterial activity of these nanoparticles against some oral bacterial pathogens.

MATERIALS AND METHODS

Spirulina extract preparation

Spirulina platensis was obtained in its dried powder form from the National Research Center, Egypt.

The extract was prepared as previously given by El-Chaghaby *et al.,* (2019). Briefly, *Spirulina* powder was added to ethanol and the mixture was ultrasonicated for two hours at room temperature. The extract was then filtered using Wattman N^{o} 1 filter paper to obtain Spi-extract.

Preparation of silver nitrate (1mM) solution

Silver nitrate ($AgNO_3$) solution was prepared by dissolving 0.169 g of $AgNO_3$ (Sigma, 99%, 169.87g/mol) in 1 liter of deionized water.

Biosynthesis of silver nanoparticles AgNPs

The preparation of AgNPs using Spi-extract was achieved by drop wise addition of 0.5 ml of Spi-extract to 50 ml of 1mM $AgNO_3$ while stirring at 50°C. The formation of AgNPs was monitored by visual transformation of the silver nitrate solution from colorless to yellow.

Spectrophotometric measurements

The formation of Ag nanoparticles was monitored by UV/ Vis spectroph-otometer (SpecorD 250 plus, Analytik Jena).

Particle size distribution

Particles size distribution of Ag nanoparticles was determined by laser diffractometer using Zeta Sizer nano-series (Nano ZS).

Antibacterial activity of AgNPs

The antibacterial activity of biosynthesized AgNPs was done using the disc diffusion method following the procedure described by Malabadi *et al.*, (2012). The studied bacteria stains were *Enterococcus faecalis, Staphylococcus aureus* and *Streptococcus mutans*. The antibacterial tests were carried out at the Microbiology unit in the Microanalytical center, Cairo University.

RESULTS AND DISCUSSION

Visual observations and UV–visible spectroscopy

After 5 minutes of the addition of 0.5 ml of spi-extract to the silver nitrate (1mM) solution and heating to 50°C, the solution color changed from clear to yellow-brownish (Figure 1). This color change of the solution was taken as a first sign for the formation of silver nanoparticles.

The formation of silver nanoparticles (AgNPs) was further confirmed by spectral scanning over the wavelength range from 300 to 500 nm using UV-Vis spectrophotometer. As noted from Figure 2, the biosynthesized nanoparticles showed a maximum absorption peak at a wavelength of 425 nm. Silver nanoparticles reveal remarkable optical properties directly connected to Localized Surface Plasmon Resonance (LSPR) which is highly dependent on the morphology of the nanoparticles (Veerasamy *et al.*, 2011; Huang and Yang 2004).The typical plasmon resonance of AgNPs is confirmed by an absorption band in the range of 400- 450 nm.

Fig. 1: Visual observation for the change in color of the reaction mixture

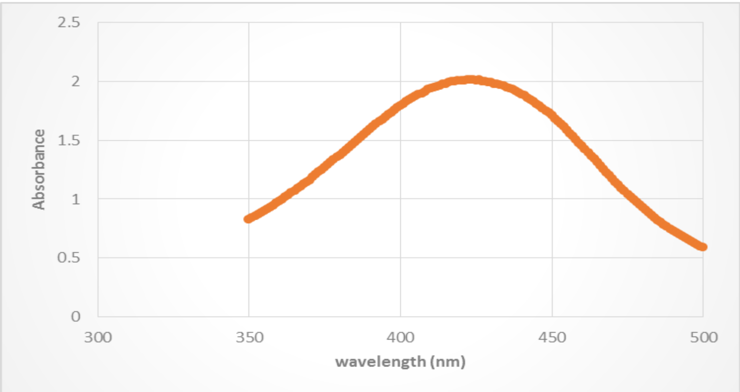

Fig. 2: UV-Vis absorption spectra of Spi-extract silver nanoparticles

Particle size distribution

It is generally agreed that he size of nanoparticles has a major role affecting their different bioactivities. Figure 3 shows the particle size distribution of AgNPs biosynthesized using *Spirulina* extract. The average particle size was found to be equal to 25.56 nm. The bioactivity of AgNPs is size-dependent and favors smaller particle size which is attributed to the relative increase of the surface area to volume ratio of nanoparticles and the contact surface area (Skandalis *et al.*, 2017).

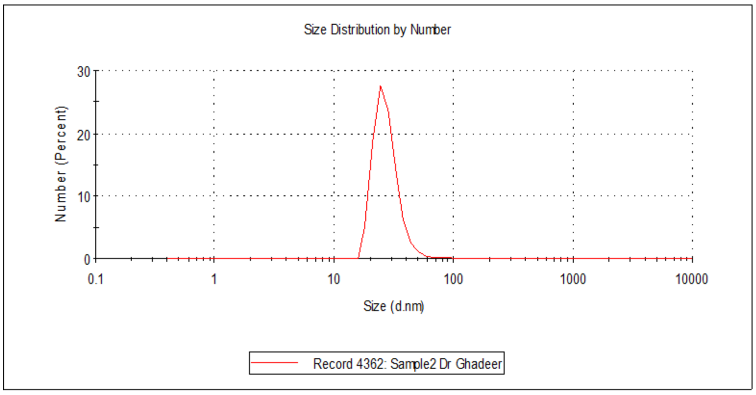

Fig. 3: Particle size distribution of biosynthesized silver nanoparticles

Antimicrobial efficacies of biosynthesized nanoparticles

Table (1) shows the results of antibacterial activity of silver nanoparticles biosynthesized using *Spirulina* extract. *Streptococcus mutans* and *Staphylococcus aureus* are key pathogens that cause infections concomitant with dental caries and dental implants (Wang and Ren, 2017). *Enterococcus faecalis* has been also related to oral diseases, such as caries, endodontic infections, periodontitis, and peri-implantitis (Komiyama *et al.*, 2016). Thus, it is imperative to find ways for decreasing the bacterial burden of the oral cavity to prevent dental caries and other oral diseases (Wassel and Khattab, 2017). The results indicate that the AgNPs biosynthesized using *Spirulina* extract have good antibacterial activity against the three Gram positive oral pathogens *Streptococcus mutans, Enterococcus faecalis* and *Staphylococcus aureus*. The efficiency of the biosynthesized AgNPs against the tested bacteria could be attributed to the adherence of small sized AgNPs to the bacterial cell membrane surface and thus disturbing its permeability and respiration functions (Jagtap and Bapat, 2013).

Table 1: Antibacterial activity of AgNPs

Bacteria	**Inhibition Zone diameter (mm)**
Streptococcus mutans	12
Staphylococcus aureus	12
Enterococcus faecalis	13

CONCLUSION

Spirulina platensis extract was used as a biomaterial for the biosynthesis of silver nanoparticles. The biosynthesized silver nanoparticles showed good inhibitory efficiency against three oral pathogens. These results propose the possibility of using such biosynthesized nanomaterial as antibacterial agent for dental applications. Further work could be addressed for investigating the mechanism of biosynthesis and to optimize it.

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