Biosynthesis of Gold Nanoparticles by Medicinal Cyanobacterium *Spirulina platensis* Geitler

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Abstract

The biosynthesis of nanoparticles using microorganisms as emerging bionanotechnology has received considerable attention due to a growing need to develop environmentl-friendly technologies in materials synthesis. Nanoparticles produced by a biogenic enzymatic process are far superior in biomedical applications to those produced by chemical methods. This study explored the biosynthesis of gold nanoparticles by *Arthrospira platensis* Gomont. Two series of experiments hence the dose dependency (chloroauric acid solution with different concentrations) and the temperature dependency (room, 75º C, and 90º C temperature) of Au nanoparticles formation, were studied. Optimizing the synthesis of gold nanoparticles and gold nanoparticles concentration determination was done. The results showed that the gold nanoparticles' size is reduced by reducing the gold concentration and raising the reaction temperature. In addition, the size of spherical shape nanoparticles has decreased from 80 nm to 20 nm, and as the concentration increased, nanoparticles became more stable. Extracted nanoparticles solutions were examined by UV-visible Spectroscopy, scanning electron microscopy (SEM), dynamic Light Scattering (DLS), and EDAX or EDS (Energy-dispersive X-ray spectroscopy) analysis. Results indicated that algae extract is very suitable for biosynthesis and are more efficient than biomass. The maximum production efficiency with this method is 98%, which is excellent and economical.

Keywords: Cyanobacteria, Green Synthesis, Biological production, Optimization, Bionanotechnology

Introduction

The first report on the synthesis of gold nanoparticles by Alfalfa sprouts was done by Gardea-Torresdey (2002), who showed that at the beginning, Au (III) ions are reduced to Au (0) ions by the alfalfa plant, and then the metal atoms are absorbed by the plant, which leads to It will be revived later. Present observation lends support to a

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previous study where formation of triangular gold nanoparticles was reported using the *Spirulina* (Chandran et al., 2006). Shankar et al. (2003) demonstrated the neem leaf mediated synthesis of Ag, Au and bimetallic Au core–Ag shell nanoparticles. Further, silver and gold nanoparticles have been synthesized using bacteria, fungi, yeasts (Gericke and Pinches, 2006; Deendayal et al., 2006) and amino acids (Selvakannan et al., 2003). There are also reports on the microbes-mediated synthesis of alloy nanoparticles, both extra and intracellular (Nair and Pradeep, 2002; Ahmad et al., 2005). The synthesis of gold nanoparticles in algae has also been reported, including *Chlorella vulgaris* (Ting et al., 1995), *Sargassum wightii* (Singaravelu et al., 2007) and *Plectonema boryanum* (Lengke et al., 2006b). In addition, Chakraborty et al. (2006, 2009), Nayak et al. (2006) synthesized nanoparticles by cyanobacteria, green algae, and diatoms. Recently, cyanobacterium-mediated platinum nanoparticles' synthesis by the reaction of filamentous *Plectonema boryamum* with platinum (IV) chloride complex has also been reported. Biological production systems are of special interest due to their effectiveness and flexibility. One of the issues raised in connection with algae, especially blue-green algae, is the ability to use them for biological production. Today, microorganisms such as bacteria, fungi, yeasts, actinomycetes, and algae are used to produce gold nanoparticles. Green synthesis of nanoparticles using microorganisms as an emerging technology has received much attention due to the growing need to develop environmentally friendly technologies in material synthesis (Rai et al., 2011).Variant nanoparticles are produced by three physical, chemical and biological methods; the first two methods are problematic, expensive and cause environmental pollution, hence the use of biological production procedure due to not cause environmental pollution is of excessive significance (Faramarzi et al., 2010; Rastgar Farajzadeh et al., 2010). Biological production systems are of particular interest due to their effectiveness and flexibility. Nanoparticles produced by a biogenic enzymatic process (the product of the activity of livingorganisms) are far superior to those produced by chemical methods in biomedical applications (Li et al., 2011; Mandal et al., 2006).

Gold nanoparticles play a significant role in nanotechnology due to their potential use in industry and medicine (Sadowski, 2010). When microorganisms take metal ions from the environment, nanoparticles are synthesized, and then they are converted into nanoscale particles by cell-produced intracellular or extracellular enzymes (Lengke et al., 2011). The particles produced by these processes have a higher catalytic reaction, more specific surface area, and improved contact between the enzyme and the desired metal salt due to the presence of the matrix (field) carrying the bacteria. They have been used in various applications such as targeted drug delivery, cancer treatment, gene therapy, and DNA analysis, antibacterial agents, biosensors,

increasing reaction speed, separation science (Khosravi-Darani et al., 2017). In many types of research, *Spirulina* has been used to produce nanoparticles. Cyanobacterial such as *Plectonema* can convert gold chloride solution into gold metal (Lengke et al., 2006 .(Lengke et al. (2006 a, b) studied the synthesis of gold nanostructures with different shapes (spherical, cubic, and octagonal) by filamentous cyanobacteria and analyzed their formation mechanism. Bakir et al.(2018) showed that the cyanobacterium *Lyngbya majuscula* in contact with a solution of 1500 mg/ml of gold chloride produces gold intracellular nanoparticles for a day and can produce gold extracellular nanoparticles after two months of incubation. Tikariha et al. (2012) showed that the cyanobacterium *Plectonema boryanum* and green algae *Chlorella* can produce gold nanoparticles, and *Sargassum* seaweed can produce gold nanoparticles with dimensions of 8 to 12 nm from gold chloride solution. Govindaraju et al. (2008) have studied the extracellular biosynthesis of silver, gold, and mixed metal nanoparticles using *Spirulina*. These researchers have studied the synthesis of silver and gold nanoparticles by *Spirulina* because of its nutritional and medicinal importance. *Spirulina* is a filamentous cyanobacterium or multicellular spiral filamentous algae. It is one of the most valuable natural nutritional sources known in the world. This microalga contains 60-70% vegetable protein, is perfectly balanced in terms of amino acids, rich in beta-carotene, iron, the richest natural source of vitamins, essential fatty acids, and other biologically active beneficial substances in the world (Doshi et al., 2007). *Spirulina* blue-green microalgae is widely used as a medicinal matrix and also as a food additive for humans and animals. The production of complexes that are easily absorbed by the human organism is one of the distinctive features of *Spirulina*. Considering that *Spirulina* is a valuable medicinal alga and can be used for the green synthesis of gold nanoparticles, this research is devoted to investigating the biosynthesis ability of gold nanoparticles by *Spirulina*. So far, no such research has been done in Iran, and it is a green approach in the field of synthesis of gold nanoparticles in the country.

In this research article, which is the result of the first author's postdoctoral research course, we report the use of *Spirulina platensis* biomass and extract for the biosynthesis of pure metallic gold nanoparticles by simultaneous reduction of aqueous $HAuCl_4$.

Materials and methods

Preparation of S. platensis stock and its cultivation

In order to conduct the experiments, the green stock of *Spirulina platensis* Geitler was purchased from Parsjolbak Company of Shiraz, then it was cultivated in BG11 medium. This blue-green algae culture medium containing (0.1 Na_2Mg EDTA, 0.6 Ferric ammonium citrate, 0.6 Citric acid. $1H_2O$, 3.6 $CaCl_2.2H_2O$, 1.5 $NaNO_3$, 7.49 $MgSO_4$. $7H_2O$, 0.02 Na_2CO_3, 4 $K_2HPO_4.3H_2O$, 2.86

H_3BO_3, 1.81 $MnCl_2.4H_2O$, 0.22 $ZnSO_4.7H_2O$, $CuSO_4.5H_2O$, 0.05 $CoCl_2.6H_2O$, 0.39 $NaMoO_4.2H_2O$ g/L) is used to produce abundant biomass (Stanier et al., 1971).

Gold nanoparticles production using S. platensis biomass

Before experimentation, the biomass was washed thrice in deionized water to remove the unwanted materials. For all the synthesis of gold nanoparticles, 613 mM chloroauric acid ($HAuCl_4$) was used as received. Gold nanoparticle formations were carried out by taking 0.1 gr of *S. platensis* biomass in a 50 mL Erlenmeyer flask with 0.613 mM aqueous $HAuCl_4$ and incubated at room temperature. The pH was checked during the course of the reaction, and it was found to be 3 (Kalabegishvili et al., 2012).

Spirulina extraction by methanol solvent

10 gr *S. platensis* powder in 20 ml methanol (100%) twice used to prepare methanolic extract. After 24 hours, the solution was filtered with filter paper and the extract was concentrated with a rotary device (Saad AM. et. al., 2020)

Biosynthesis of gold nanoparticles by extract of S. platensis with a high concentration of gold salt

After placing 0.1 g of fresh extract of *S. platensis* exposed to 100 micro liters of 613 mM gold solution for 24 hours, the color change of the solution and the biosynthesis of nanoparticles were investigated (Xie et al., 2007).

Measurement of an absorption spectrum of nanoparticles by UV-Visible Spectrophotometer

After the biosynthesis of gold nanoparticles, the absorption spectrum of the solution at the wavelength of 531 nm, which is the absorption wavelength of gold nanoparticles, was determined by the UV-Visible spectrophotometer.

Determining the shape and size of gold nanoparticles by electron microscopy

The size of nanoparticles was checked by SEM electron microscope. In order to determine the shape and size of the samples, the biosynthesized sample was centrifuged at 5000 rpm for ten minutes and after being concentrated and placed in a 60° C oven, it was delivered to the electron microscope room of the physics department of Yazd University.

EDAX analysis or EDS

EDAX analysis or EDS (X-ray Energy Diffraction Spectroscopy) is an add-on to SEM devices to detect elements in solid samples. This analysis can detect the type of element and its weight or atomic percentage by using the unique X-ray energy emitted from the sample. In this study, biosynthetic gold nanoparticles were transferred to Beam Goster Taban material analysis laboratory for EDAX analysis.

Biosynthesis by S. platensis extract with a low concentration of gold salt and estimate the hydrodynamic size (DLS)

One of the samples of *S. platensis* extract, which was exposed to less gold salt for the biosynthesis of nanoparticles, was subjected to DLS analysis to determine the size and size distribution. Sample size and distribution of biosynthesized particles were checked by

the DLS device. Gold nanoparticles were analyzed by Dynamic Light Scattering (DLS). Extracted gold nanoparticles were analyzed using Malvern Nano ZS to estimate the hydrodynamic size of the particles.

Gold nanoparticles concentration determination

The concentration of gold nanoparticles was determined by an atomic absorption spectrophotometer (AAS) (Analyst 400; Perkin Elmer, Waltham, MA, USA).

Sample stability test

The stability test of biosynthetic gold nanoparticles was also performed. In this way, the absorption of the sample was recorded during biosynthesis and after one month after biosynthesis, and if the absorption number has not changed, it indicates the stability of the nanoparticles and is one of the most critical indicators that determine the quality of the gold nanoparticles sample are the stability index.

Results

Solution color change and synthesis of gold nanoparticles

The biosynthesis of nanoparticles by biomass and *S. platensis* extract can be detected only by changing the color of the solution from yellow to red. Synthesis of gold nanoparticles at room temperature showed that after 24 hours, the extract solution and wet biomass changed from yellow to red, and red gold nanoparticles were made.

The result of the measurement of an absorption spectrum of gold nanoparticles

The addition of *S. platensis* biomass to 0.613 mM aqueous $HAuCl_4$ solutions led to the appearance of red color in biomass after 48 h of reaction, indicating the formation of gold nanoparticles. These colors arise due to the excitation of surface plasmon vibrations in the metal nanoparticles (Singaravelu et al., 2007). Figures 1 shows the UV–Vis spectra recorded from the aqueous auric chloride and *S. platensis* reaction medium as a function of the reaction time. The gold surface plasmon resonance (SPR) band occurred at 530 nm. Function of the time of reaction was recorded on a UV–Vis 1601 Schimadzu spectrophotometer which was operated at a resolution of 1 nm. The gold nanoparticles produced did not show an absorption peak at the wavelength of 531 nm, which is the absorption wavelength of gold nanoparticles in the biosynthesis section with biomass. This indicates that the intracellular gold nanoparticles are synthesized and are not in free form, and no extracellular nanoparticles have been synthesized. In the biosynthesis section with extract, the sharpie absorption peak at 530 nm is observed, which indicates the presence of extracellular biosynthetic nanoparticles in free form.

SEM electron microscopy

The size of nanoparticles was checked by SEM electron microscope. The shape of biosynthesized gold nanoparticles is spherical between 20- 80 nm (Figure 2).

Dynamic Light Scattering test result

DLS study supports the presence of gold nanoparticles of different sizes in the extracted solution. The average size distribution of

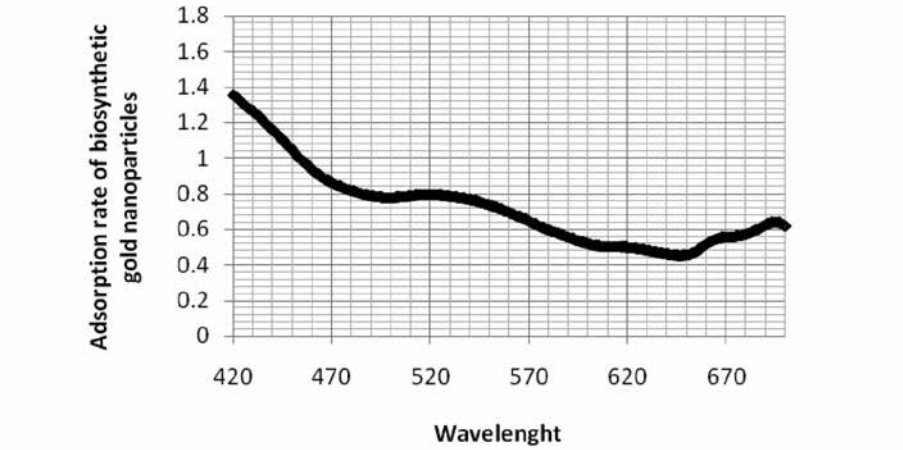
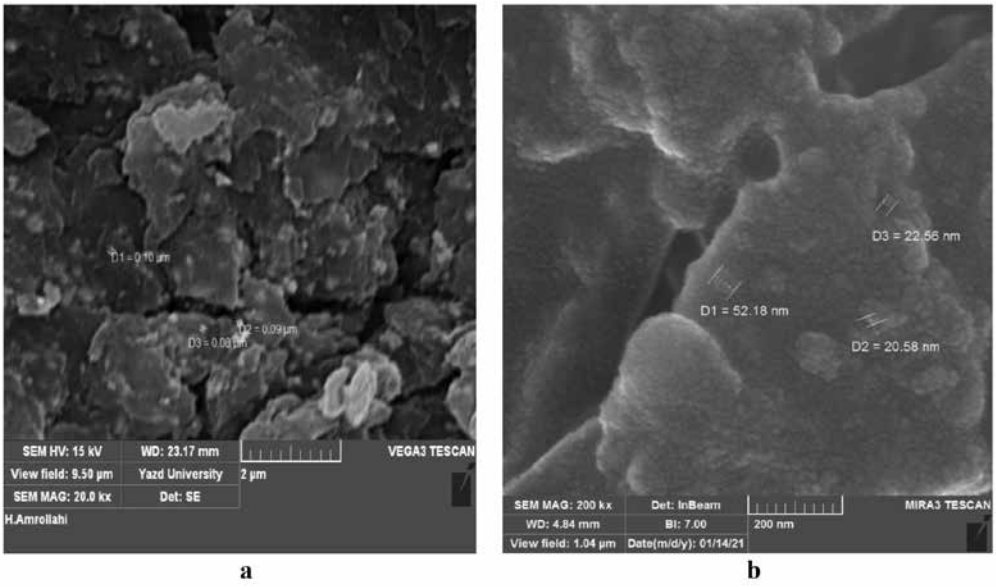


Fig. 1. UV–Vis spectra recorded as a function of time of reaction of the aqueous solution of chloroauric acid with *S.*



a b

Fig. 2. SEM images of gold nanoparticles formed by reduction of Au ions using *S. platensis* biomass and extract; (a) intracellular nanoparticles (with a high concentration of gold) and (b) extracellular nanoparticles (with a low concentration of gold)

biosynthesized gold nanoparticles is about 56 nm (Figure 3).

Optimization of the synthesis of gold nanoparticles

Three factors in temperature, the amount of gold sample in the environment, and the amount of algae biomass are significant in optimizing the synthesis of gold nanoparticles in different dimensions. The results show that the temperature is very effective in the duration of the synthesis of nanoparticles and significantly reduces the time required for the synthesis; also, the temperature affects the size of the nanoparticles, and as the temperature increases, the size of the nanoparticles decreases. In addition, with the reduction of gold concentration, the size of gold nanoparticles becomes smaller. In the

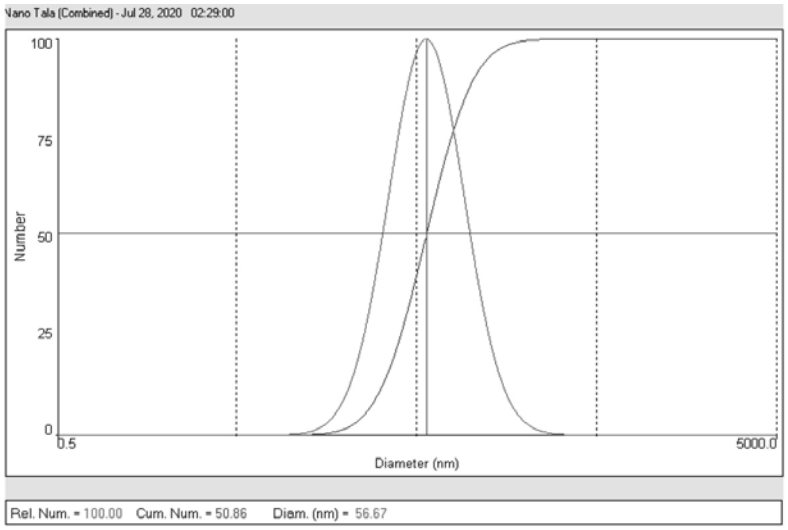


Fig. 3. Dynamic light scattering test result

present experiment, the size of nanoparticles has decreased from 80 nm to 20 nm by halving the gold concentration compared to previous experiments.

Gold nanoparticles concentration determination

An atomic absorption spectrophotometer analysis determined the concentration of gold nanoparticles. The results show that more biosynthetic nanoparticles are synthesized with *S. platensis* algae extract at high temperatures and low volumes of gold solution. The maximum production efficiency with this method is 98%, which is excellent and economical.

Table 1. Determining the concentration of biosynthetic gold nanoparticles by the gold standard

Number	Test temperature	Initial concentration of gold solution (ppm)	sample	pity	Concentration of gold nanoparticles (ppm)	Production efficiency (%)
1	25° c	71.2	*Spirulina platensis* 1	1	A: 28.60 O: 28.60	47.35
2	75° c	71.2	*Spirulina platensis* 2	25	A: 2.368 O: 59.19	98
3	90° c	85.6	*Spirulina platensis* 3	25	A: 2.755 O: 68.88	95.14

(A: The concentration value of the diluted solution, O: The concentration value of the original solution)

(A: The concentration value of the diluted solution, O: The concentration value of the original solution)

EDAX analysis or EDS (Energy-dispersive X-ray spectroscopy)

an EDAX (energy dispersive X-ray analysis) pattern of gold nanoparticles synthesized by treating *S. platensis* with chloroauric acid aqueous solution is proved the presence of gold nanoparticles in Figure 4. The energy of X-rays is characteristic of the elements from which these X-rays are emitted. A spectrum of the energy versus relative counts of the detected X-rays is showed in Figure 4. Two peaks of Au were observed for biomass of *S. platensis*, Edax analysis confirmed the particles only with gold.

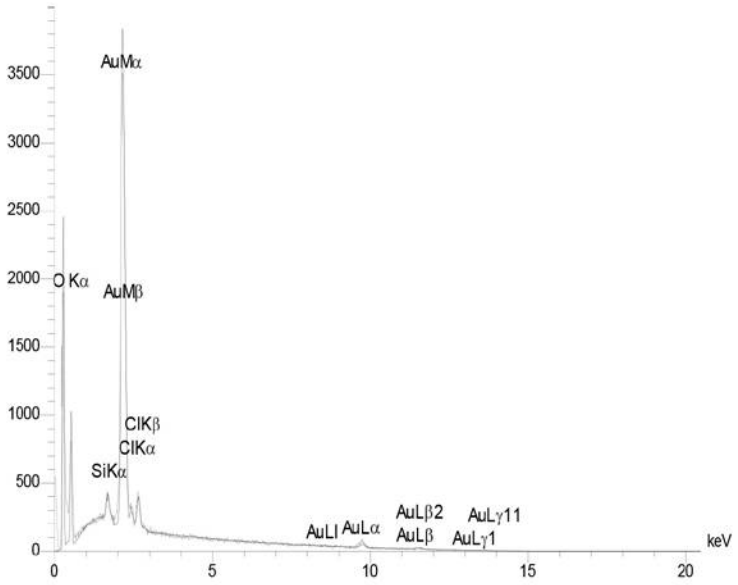


Fig. 4. Edax analysis of biosynthetic gold nanoparticles confirmed the particles only with gold

Discussion

Green synthesis of AuNPs has become of great interest using bacteria, fungi, and algae because of its numerous benefits, such as low-cost medium for microbial growth, efficiently handling process, and its ability to absorb gold ions (Lee et al., 2020; Akintelu and Folorunso et al., 2021). Several material scientists have synthesized various types of gold nanoparticles through physical and chemical methods (Hu et al., 2007; Panigrahi et al., 2005; Wang and Shi, 2007). Ahmad et al. (2005) envisaged that chemical synthesis may still lead to the presence of some toxic chemical species adsorbed on the surface that may have adverse effects in medical applications. On this basis, the present study has its essentials. Although the therapeutic potential of *S. platensis* is promising, its bio-reduction property of inorganic materials is yet to be exploited. Kalabegishvili et al. (2012) studied the synthesis of gold nanoparticles by *S. platensis* at different doses and at different time intervals. This study showed that after 1.5-2 days of exposure to gold salt, spherical

intracellular nanoparticles were formed, and the peak size of the particles was between 20-30 nm.

The results showed that the concentration of gold accumulated by the *S. platensis* biomass was proliferating at the beginning, followed by some increase over the next few days. In the UV-visible absorption spectrum of *S. platensis* suspension after the addition of gold chlorate solution at different concentrations (dose-dependent), it has been shown that a broad gold Surface Plasmon Resonance (SPR) peak appears at 530 nm for its concentrations (10^{-3}-10^{-4} M). At higher concentrations of $HAuCl_4$ 10^{-2} M such a peak was not observed. The results show that at the concentration of $HAuCl_4$ 10^{-3} M, the size of gold nanoparticles is ≈ 14 nm, at 10^{-3} M ≈ 20 nm, and at 10^{-2} M ≈ 100 nm. In the present research, the size of gold nanoparticles becomes smaller with the reduction of gold concentration. In the current experiment, the size of nanoparticles decreased from 80 nm to 20 nm by halving the gold concentration compared to previous experiments, which is consistent with the results of Kalabegishvili et al. (2012). In the current research, by increasing the concentration of *S. platensis* extract and decreasing the attention of the gold solution used for biosynthesis, as well as increasing the temperature, nanoparticles with a smaller size and higher concentration are synthesized, as seen in Table 3, which indicates The results are similar to the research of Kalabegishvili et al. (2012) on the synthesis of gold nanoparticles by the cyanobacterium *S. platensis*.

The results of the studies by Sharma et al. (2009) show that the color of gold nanoparticles changes from red to blue and this color change depends on the shape and size of the nanoparticles. The nanoization of gold particles by microorganisms is caused by the reduction of Au(III) to Au(0) and its production in intracellular and extracellular form, which is entirely consistent with the present research; *S. platensis* makes intracellular and extracellular nanoparticles brown to red depending on the size by absorbing gold solution in different temperature and concentration conditions (Duff et al., 1987; Chow and Zukoski, 1994; Lujan et al., 1994; Chakraborty et al., 2009; Parial et al., 2012).

Gerick and Pinches (2006) reported that the shape of particles is spherical if the amount of regeneration (reduction) is low. If the amount of regeneration is high, the form of particles becomes nanorods and nanoplates. In addition, their investigation showed that the high amount of regeneration at low pH might lead to the production of nanorods. Therefore, we find that according to the results of this research, which shows that biosynthetic nanoparticles are spherical, so the amount of reduction is low.

According to the Miefis Theory, only one SPR band is expected in the absorption spectrum of spherical nanoparticles. At the same time, anisotropic particles can produce two or more SPR bands depending on the particle shape (Sosa et al., 2003). In the present case, a single band was observed, which shows evidence of the presence of

spherical gold nanoparticles, which was confirmed by TEM and SEM images. In the present research, the synthesis of spherical nanoparticles is proved by having a sharp band. As all the reports state that biosynthesis with algae extracts is better than biomass, this research also shows that algae extract very suitable for biosynthesis and has more efficiency (Shankar et al., 2016). The maximum production efficiency with this method is 98%, which is excellent and economical.

The "green route" of biosynthesis of extracellular gold nanoparticles in *S. platensis* is a very simple, economically viable, and environmentally friendly process, which has a significant advantage over the intracellular synthesis process in terms of applications in medicine, pharmaceuticals, and other technological fields and has a chemical synthesis process.

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