Available online at www.dergipark.org.tr/en

INTERNATIONAL ADVANCED RESEARCHES and ENGINEERING JOURNAL

Journal homepage: www.dergipark.org.tr/en/pub/iarej

e-ISSN: 2618-575X

International Open Access

Volume 07
Issue 01

April, 2023

Research Article

Green biosynthesis, characterization of silver nanoparticles using a green alga *Spirogyra* sp. and their antioxidant and enzyme activities

Aysel Basoglu [a,*] ***and Zeynep Akar*** [b]

[a]*Department of Occupational Health and Safety, Faculty of Health Sciences, Gümüşhane University, 29100 Gümüşhane, Turkey*
[b]*Department of Genetics and Bioengineering, Faculty of Engineering, Gümüşhane University, 29100 Gümüşhane, Turkey*

ARTICLE INFO

Article history:
Received 26 September 2022
Accepted 24 March 2023
Published 15 April 2023

Keywords:
Antioxidant activity
Green-biosynthesis
Silver nanoparticles
Spirogyra sp.
α-Glucosidase enzyme

ABSTRACT

A simple, environmentally friendly, inexpensive, and one-step alternative method was reported for the green biosynthesis of silver nanoparticles (AgNPs) operating the *Spirogyra* sp. extract as a reducing and stabilizing substance. Concentration of $AgNO_3$ and reaction time were optimized to prepare AgNPs under controlled conditions. The synthesized silver nanoparticles were characterized by UV-Vis absorption spectroscopy, fourier transform infrared spectroscopy (FT-IR), transmission electron microscopy (TEM), energy dispersive X-ray analysis (EDX), and elemental mapping. The TEM analysis showed that the average particle size of AgNPs was 18.3 nm. Structural details of silver nanoparticles elucidated by Selected Area Electron Diffraction (SAED) based on TEM images. In addition, biological activity tests were applied to nanoparticles and algal extracts to determine antioxidant activity (3 different tests: DPPH (1,1-diphenyl-2-picrylhydrazil) radical scavenging activity, total phenolic content (TPC) and total flavonoid content (TFC)) and α-glucosidase enzyme inhibition. Antioxidant activity and α-glucosidase enzyme inhibition values of silver nanoparticles are higher than the values of *Spirogyra* sp. extracts.

1. Introduction

High-tech materials advanced on the nano-scale, that have many benefits as compared with the research performed on the macro-scale, are actively used in many fields such as medicine, biotechnology, environment, energy, defense industry, textile, electronics and space research. Nanotechnology is typically well-known as a crucial branch of science that objectives natural and synthetic practical materials, that have a size distribution on the scale of nanometers (10^{-9} m) and feature attracted the eye of many scientists in the modern century. Nanotechnology involves understanding the basic physics, chemistry, biology, and technology of nanometer-scale materials. In recent decades, advances in nanotechnology have accelerated, with numerous engineered nanoparticles (NPs) having outstanding optical, magnetic, catalytic, and electrical properties being produced. Thanks to the development and diversification of nanoparticles, which are at the core of the field of nanotechnology, final products with pre-designed functional properties can be obtained. Nanostructures are the subject of all nanotechnological applications everywhere in nature and the dimension of nanoparticles determines their characteristic properties [1]. In the context of nanosciences and nanotechnologies, it is widely accepted to focus on units of size [2]. Nano-sized particles have a larger surface area/volume ratio and surface molecule fraction, making them unique materials because of superior physicochemical properties like optical property [3], magnetic property [4], catalytic property [5], and antimicrobial property [6] at the nano-size compared to the bulk materials with the same chemical composition [7, 8]. Among the nanoparticles, silver nanoparticles (AgNPs) are widely used in scientific research due to unique predictable properties. About 5000 years ago, people of many races such as Greeks and Egyptians used silver to keep meals products safe [9]. In many dynasties in ancient times, the use of silver ware and utensils for various purposes such as eating, drinking, and storage various foods were quite common all over the world, in all probability because of the information of antimicrobial action [10]. It is mentioned in the Indian Ayurvedic medicine book known as “Charak Samhita” in the medical

* Corresponding author. Tel.: *+90 (544) 210 61 60*; *Fax: +90 (456) 233 11 79*
E-mail addresses: abasoglu@gumushane.edu.tr *(BAŞOĞLU)*, ziskefiyeli@gumushane.edu.tr *(AKAR)*
ORCID: 0000-0002-2300-1554 *(BAŞOĞLU)*, 0000-0001-9262-8070 *(AKAR)*
DOI: 10.35860/iarej.1180456

literature that some metals such as silver have therapeutic potentials. As before, silver utensil is still used for the preparation of "panchamrit" in the worship of Hindu religion [11]. Also, silver was widely used as an antimicrobial agent until the determination of antibacterial effect of penicillin by Alexander Fleming 70 years ago.

Silver nanoparticles (AgNPs) commonly used in scientific research, are also preferred in different application areas of biomedical material [12], drug delivery [13], water treatment [14], agriculture (food storage-containers) [15], textile [16], energy [17], cosmetic [18] because of their particular properties such as good electrical conductivity, antifungal, antimicrobial, antiviral, antibacterial, anti-inflammatory, anticancer, photoelectrochemical, antibiofilm, and enzyme activity.

AgNPs are synthesized by numerous strategies, together with chemical reduction, impregnation, laser ablation, ultrasonic radiation, and explosion. Since the potent reducing and capping agents often utilized by the chemical methods are deadly to humans, and the environment, there is a desire to synthesize AgNPs in additional economical and eco-friendly manners. Some chemicals known to be toxic, such as sodium borohydride [19], citrate [20] or ascorbate [20], are widely used as reducing agents. In this classical preparation methods, the usage of several synthetic reactants as precursors, diverse of chemicals as reducing agent, large quantities of surfactants as stabilizing agents and, carcinogenic solvents firstly changed into dangerous to the living life, and then the future of the world. As a sub-branch of nanotechnology, the nanobiotechnology approach based on nanoparticle synthesis using herbal extracts, which has made significant progress in recent years, arouses interest as a popular research area. In this context, the procedures used have a green synthesis perspective, thus providing energy savings by bringing a very effective and environmentally friendly approach compared to classical methods, as well as being more cost-effective compared to conventional materials, offers a very interesting and innovative alternative to researchers working in this field. The production of silver nanoparticles by the green synthesis method has also paved the way for their use in biological systems.

The principle of green chemistry was first mentioned in the book "Green Chemistry" written by Anastas and Warner in 2000 [21]. The book "Green Chemistry" contains 12 principles that should be applied to limit the release of hazardous chemicals into the environment and human exposure to them. The headlines that stand out in principles of green chemistry are: -preventing the production of waste,- limiting energy usage - need materials in synthesis strategies have nominal or no toxicity to the surroundings or individual,- Organic solvents and auxiliary chemicals should not be used, - Using Renewable Raw Materials, - Performing Real-Time Analysis for Pollution Prevention. Moreover, the 12 of principles were explained in detail by Gałuszka et al. in 2012 [22]. The main issue emphasized by both working groups is the use of agricultural products as reducing and limiting agents. Biosynthesis of AgNPs consist of bacteria like Escherichia Coli (E. coli) [23], yeast like Saccharomyces cerevisiae [24], fungi like Coriolus versicolor [25] and Penicillium brevicompactum [26], Algal Species like Caulerpa racemose [27], Gracilaria corticata [28] and Laminaria japonica [29] and plant/plant extracts like tea [30], Artemisia quttensis [31], Gardenia Jasminoides Ellis [32] and P. granatum L. [33].

Plants show biological activities because of their rich phenolic compounds content. Therefore, besides their use as food, plants are also widely used for medicinal purposes [34]. One of the applications used to more effective biological activity values of plant compounds is to form metal nanoparticles from plant compounds [35]. Biological activity determination studies on macroscopic terrestrial plants are more than other groups of organisms. However, algae, which contribute to the primary production of aquatic ecosystems, have an important place among organism groups in terms of biological activity [36].

Silver nanoparticles are usually formed in the presence of $AgNO_3$ salt solution. Ag^+ ions originating from the silver nitrate salt are first reduced to Ag atoms by reducing agents. Then, the reduced Ag atoms create several nucleates. Finally, the nucleates carried out in tiny clusters grow the particles. The size and shape of the nanoparticles can be controlled with the presence of atoms based on the concentration ratio of silver salt to reducing agent. The present study regarding the synthesis and characterization of AgNPs using the aqueous alga *Spirogyra* sp. and, their antioxidant and enzyme activities. This study purpose to form a new biological source for the synthesis of silver nanoparticles besides evaluating their antioxidant and enzyme activities of silver nanoparticles by comparing with that of the algal extract. There are limited studies in which algae are used as a source for silver nanoparticle production. [27-29, 37]. It should be especially noted that silver nanoparticles have been synthesized the usage of the *Spirogyra varians* in a study by Salari et al., and the antibacterial properties of was studied through measuring the inhibition zone, MIC and MBC [38]. In our study, the algal extracts could be available as reducing and stabilizing substance to make stable AgNPs operating a green chemistry road as a different method over other toxic chemical reducing substance.

2. Materials and Methods

2.1. Materials

All chemical compounds are of analytical grade and do not need any purification before using in the study. The

standart solution of silver nitrate ($AgNO_3$, 1000 mg/L)) was bought from Merck. The silver nitrate solutions (1mM, 2 mM and 3 mM) were prepared in deionized water. The deionized water was achieved by using the Sartorius Milli-Q system (arium 611UV; Sartorius AG, Göttingen, Germany). Also the filter paper is Whatman No. 1. The chemical for bioactivity tests were purchased from Sigma Aldrich (chemicals of DPPH, gallic acid and α-Glucosidase enzyme), Merck (chemicals of folin-ciocalteu and quercetin) and Biosynth Carbosynth (4-nitrophenyl-α-D-glucopyranoside).

The algal sample (*Spirogyra* sp.) was collected from a spring in Torul district of Gümüşhane province. The collected algal biomass were washed with deionized water a couple of times to clear from the dust and particles. Next procedure is an drying under the sun to remove the ultimate moisture from the algae *Spirogyra* sp.. The well-dried algae were easily pulverized by hand crumbling.

2.2. Preparation of Spirogyra sp. extract

The *Spirogyra sp.* extract was carried out according to the similar methods described earlier with modifications [29, 32]. The algal extract was obtained by placing 7.5 g of fine powders together with 100 mL of deionized water: For the preparation of the algal extract, at first, this *Spirogyra* sp. aqueous solution was heated until boiling. After boiling, it was incubated at 60ºC in water bath for 30 minutes. The mixture changed its color from watery to darkish brown. The algal extract was given time to cool down to room temperature, filtered using filter paper. Also, to take away the suspended particles, this extract was centrifuged at 10000 rpm for 10 minutes. The filtrate volume was finally maintained to 50 mL with deionized water. The stock filtrate of the Spirogyra sp. was stored further as a reducing substance for the biosynthesis of AgNPs in refrigerator at 4 ◦C.

2.3. Biosynthesis of silver nanoparticles (AgNPs)

The *Spirogyra* sp. extract was biologically served as reducing substance in the synthesis of silver nanoparticles. For the reduction of Ag^+ ions, the algal extract was added to aqueous solution of $AgNO_3$ (45 mL) and heated at 60 ºC under magnetic stirring at 500 rpm/min for 2 h (C-MAG HS7 digital magnetic stirrer, IKA Co., Staufen, Germany) [29]. The incubation of the reacting solution in a dark place protects against the excitation of nanoparticals' atoms by light energy at room temperature.

In many studies, the effect of different $AgNO_3$ concentrations on the biosynthesis of AgNPs has been studied [18, 23, 33].AgNPs were also synthesized with various concentration of $AgNO_3$ (1mM, 2 mM and 3 mM) by ensuring the concentration of the extract the same (5 mL, 7.5% w\v). For all $AgNO_3$ concentrations, the color of the reaction mixtures was changed shortly after the addition of the algae extract, demonstrating the presence of specified reduction reaction. As the reduction reaction continues, the initial slightly yellowish color of the solution mixture turned to gray, brown and finally reddish color. The strong absorbance created by the excitation of the nanoparticle surface plasmons causes the change in color which was the strongest evidence for the formation of the silver nanoparticle [39, 40]. The reduction reactions in the formation of silver nanoparticles were observed with the changes in their color of the reaction mixtures including different $AgNO_3$ concentrations and also were spectrophotometrically monitored as a function of time of reaction (1 h, 3 h, 24 h, 48 h and 72 h) on a spectrophotometer [29,32].

For the purification of AgNPs, the reaction mixture of AgNPs were centrifuged at 10000 rpm for 15 min: The aqueous silver nanoparticle solution was centrifuged and then the supernatant solution was decanted. The resulting pellet was redispersed in deionized water and centrifuged again. The process of centrifugation and redispersion was iterated three times for the supporting separation of AgNPs from the freely available proteins/enzymes.

2.4. Characterization of silver nanoparticles

UV–vis spectral analyzes were done by Shimadzu UV-vis spectrophotometer (UV-1800 UV, Japan). 4 mL of the aqueous solutions of AgNPs were pipetted into a 1 cm quartz cell and subsequently analysed using UV-Vis absorption spectrophotometer with a resolution of 1.0 nm between 200 and 800 nm at room temperature.

The surface functional groups of AgNPs were investigated by Fourier transform infrared spectroscopy. The spectral analyzes were done with Perkin-Elmer Spectrum Two FTIR spectrometer in a spectral range of 400–4000 cm^{-1} at room temperature. AgNPs was purified before FT-IR spectra of was recorded: 20 mL of bioreduced AgNPs solution using 1mM $AgNO_3$ after 24 h of reaction was centrifuged at 10000 rpm for 15 min. Subsequent to the centrifugation, a novel solution mixture was prepaired by dispersing the pellet in 20 mL of deionized water to remove any proteins/enzyme molecules that are freely found in the solution of the silver nanoparticles. Three replicates were performed in both the centrifugation and the dispersion. Furthermore, FTIR spectra of the *Spirogyra* sp. extract was recorded. The aqueous *Spirogyra* sp. solution was removed from water by rotary evaporation before FT-IR spectra of was recorded.

Transmission electron micrographs were performed at 200 kV by using a Talos F200S microscope (FEI, USA) in the Research Laboratory of Bayburt University. 1.5 mL of AgNPs solution after 24 h of reaction was removed from water by heating at 60ºC. Then, AgNPs was dried with N_2 gas and redispersed with alcohol treating by an ultrasonic probe for about 10 s. Two drops of the AgNPs solution was

poured on a carbon-coated copper grid. The grid was kept at room temperature until the solvent disappeared before the morphological images of AgNPs were acquired.

2.5. Antioxidant activity of extract and silver nanoparticles of Spirogyra sp.

2.5.1. DPPH radical scavenging activity

The DPPH antioxidant activities of extract and silver nanoparticle of the algae were measured in three parallel based on method of Brand-Williams [41], it was also determined sample reagent blanks. In the study, 750 µL of 100 µM methanolic DPPH radical solution was added onto 750 µL sample solutions. The mixture was vortexed and incubated at room temperature for 60 minutes. At the end of the period, it was determined at 517 nm absorbance with spectrophotometer.

The change of absorbance of DPPH radical in different sample concentration was measured. The graph was plotted based on absorbance corresponding to the concentrations. The results were expressed as the IC_{50} value. Lower IC_{50} values indicate higher radical scavenging potential.

2.5.2. Total phenolic content (TPC)

Total phenolic contents of the extract and the silver nanoparticle were determined according to the method of Slinkard and Singleton [42] using Folin-Ciocalteu reagent. Firstly, 50 µL of the sample (the solution of extract and synthesized silver nanoparticle) was diluted with 2.5 mL of distilled water. Then 250 µL of 0.2 N Folin-Ciocalteu reagent and 750 µL of Na_2CO_3 (7.5%) was on the mixture, respectively. It was vortexed. After the tubes were kept at room temperature for 2 h, absorbance values were determined at 765 nm.

The antioxidant standard of gallic acid (500-250-125-62.5-31.25 µg/mL) was used to draw the standard calibration graphic. The amounts of phenolic compounds in the samples were calculated in gallic acid equivalents (GAE µg/mL).

2.5.3. Total flavonoid content (TFC)

Method developed by Fukumoto and Mazza [43] was applied to determine the flavonoid contents of extracts and the nanoparticle. As in the other tests, the sample measurments were carried out in three parallels. In addition, measurements were also made for sample and reagent blanks. First of all, 250 µL equal amounts of samples were pipetted into the tubes. Then 2.1 mL of methanol was added to all tubes. Finally, 50 µL of 1M ammonium acetate (CH_3COONH_4) and 10% aluminum nitrate ($Al(NO_3)_3{\cdot}9H_2O$) were transferred to the tubes, respectively, except the sample blank and vortexed. At the end of the 40 minutes incubation period, absorbance values were read at 415 nm.

Quercetin was used as the antioxidant standard. Quercetin (0.25 mg/mL). It was prepared at six different concentrations and absorbance values-concentration graphs were drawn. According to the graphic, the total amount of flavonoid substance in the samples was determined as quercetin equivalent (QAE µg/mL).

2.6. % α-Glucosidase enzyme inhibition

The α-glucosidase inhibitory activities of extract and silver nanoparticle of the *Spirogyra* sp. were determined with modified method which developed by Zhipeng Yu et al. [44]. Firstly, 650 µL of phosphate buffer (pH: 6.8 and 0.1 M) was transferred onto 20 µL sample in test tubes. Then, 30 µL of α-glucosidase enzyme (Saccharomyces cerevisiae, lyophilized powder, ≥ 10 units/mg protein) prepared in phosphate buffer was added to mixture. After that it was incubated at 37 °C for 10 minutes, 75 µL of substrate (4-Nitrophenyl-α-D-glucopyranoside) was added. This time, the mixture was kept at 37 °C for 20 minutes, then 650 µL of 1M Na_2CO_3 was added and the reaction was stopped. As in α-glucosidase, the control solution was also prepared. Absorbance values of the mixture were measured at 405 nm in a UV-vis spectrophotometer. The % inhibition values of the samples were calculated according to the following Equation (1)

$$\%\, inhibition \;=\; \left[\left(\frac{A_{control} - A_{sample}}{A_{control}}\right)\right] \times 100 \qquad (1)$$

$A_{control}$: The absorbance value of the control solution
A_{sample}: Absorbance value of the sample extract

3. Results and Discussion

3.1. Synthesis and characterization of the AgNPs

A one-step green chemistry-based approach involving the algal (*Spirogyra* sp.) biosynthesis method was performed to synthesis silver nanoparticles (Figure 1). When the algal extract was placed in Ag^+ solution, the colorless mixture became reddish color as Ag^+ was reduced to silver metal (Ag^0) through the algal extract.

In this study, the prepared AgNPs was characterized by UV-Vis spectroscopy, FT-IR spectroscopy, and transmission electron microscopy (TEM).

After adding the algal extract to the Ag^+ solution, the formation of AgNPs was visually noticed by the color change of the reaction mixtures. At the end of the reaction, the transparent color of the silver nitrate solution changed to reddish color forming a colloidal dispersion, verifying the growth of silver particles. The formation of colloidal AgNPs was also confirmed by UV−vis spectroscopy: Figure 2 consists of the UV-Vis absorption spectra for both the algal extract solution and AgNPs solution.

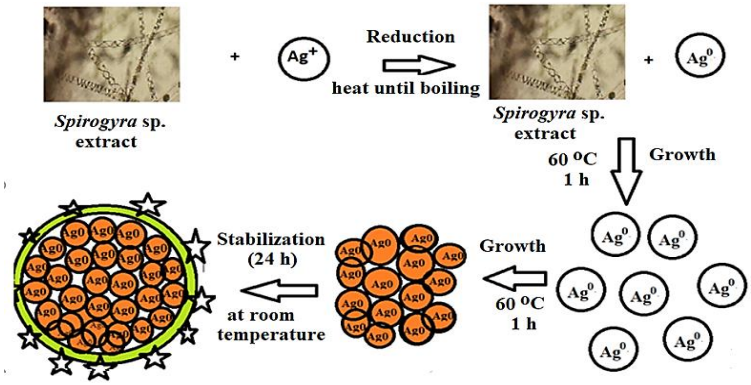

Figure 1. Schematic representation for the green biosynthesis of AgNPs from *Spirogyra* sp.

The spectra of the algal extract solution indicated the absence of an SPR peak. The location of SPR peaks are particularly attached to the size and shape of the nanoparticles, surface charge and, environmental medium conditions. The free electrons belonging to groups such as -OH and -COOH as well as -NH_2 on the surface of silver nanoparticles interact with the incident light in UV-Vis spectrophotometry is the most important factor for the decision of the SPR particularities of AgNPs. SPR emerged when the wavelength of incident light combined with the vibration frequency of free electrons, and intense absorption peaks were exhibited in UV-Vis spectra. Surface plasmon resonance, SPR, to be briefly expressed, is a physical phenomenon that develops due to the vibrational movements that occur on the metal surface when plane polarized light hits a metal surface and is reflected. As seen in Figure 2, AgNPs has a SPR absorption peak at about 488 nm which has been documented for various silver nanoparticles [1, 45, 46]. It is well known that Ag nanospheres have only one SPR peak. In the study, the synthesized-AgNPs consist of spherical- and oval-shaped. In addition to the surface plasmon resonance, the other peak at 378 nm may attributed to the presence of contaminating proteins in the AgNPs solution. In the typical algal (*Spirogyra* sp.) synthesis,three different concentrations of aqueous $AgNO_3$ solutions (1 mM, 2 mM and 3 mM) were used to prepare AgNPs keeping the algal extract concentration constant (5 mL, 7.5% w\v). The reduction of the Ag^+ at different concentrations by the constant amounts of aqueous the algal extract were also monitored as a function of time of reaction by UV–Vis spectroscopy measurements and the spectra obtained are shown in Figure 3.

So, the changes of SPR absorption peak with the concentration of $AgNO_3$ were examined as a function of time from Figure 3. In Figure 3a, it was observed that for 1mM of $AgNO_3$ a broad peak recorded at about 480 nm appeared as a shoulder in the UV-Vis spectra after 24 h of reaction and increased in intensity and shifted from 480 nm to 488 nm until 72 h of reaction.

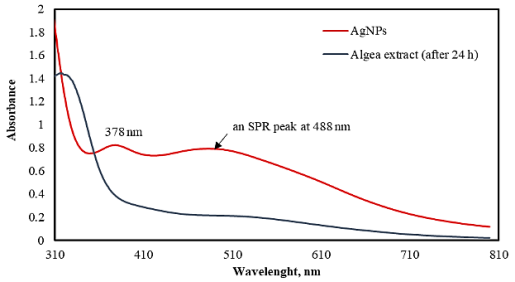

Figure 2. UV−vis spectra of the water-diluted *Spirogyra* sp. extract solution and the synthesized AgNPs solution using 1mM $AgNO_3$ at the end of 24 h of reaction

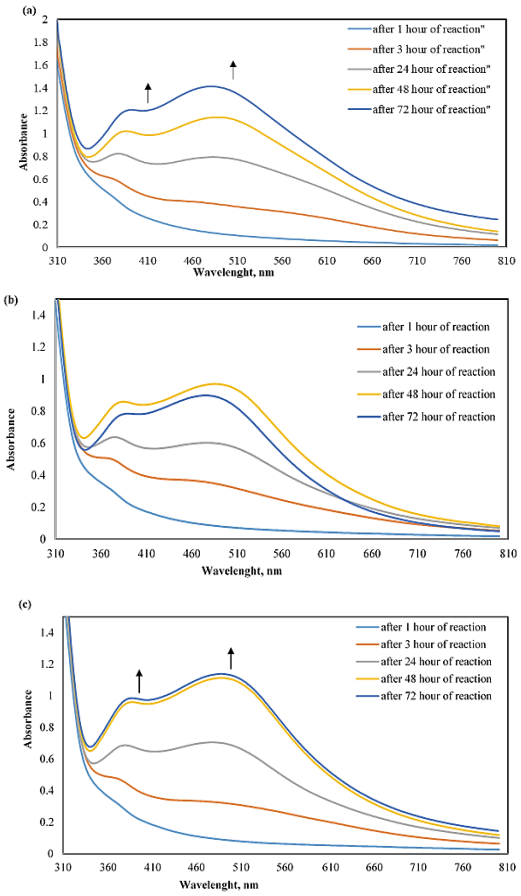

Figure 3. The reduction of the Ag^+ at different concentrations by the constant amounts of aqueous Spirogyra sp. extract were also monitored as a function of time of reaction by UV–vis spectroscopy measurements. The spectra obtained as a function of time in the presence of 1mM (a) 2 mM (b) and 3 mM (c) of $AgNO_3$.

In addition to the SPR absorption peak corresponding to the excitation of longitudinal plasmon vibrations, another peak at about 370 nm was also seen due to probably contaminating proteins in the presence of 1mM of $AgNO_3$ that increased in intensity with 8 nm of shift until 72 h of reaction. Similar changes in the absorbance band, such as increase in intensity and band-shifted, were also observed in the presence of 2 mM and 3 mM of $AgNO_3$ (Figure 3b,c).

In order to observe which concentration (1 mM, 2 mM and 3 mM of $AgNO_3$) would be more appropriate for the reduction of Ag^+ ion, the absorbance values of synthesized AgNPs solutions at about 488 nm were plotted against the time of reaction (in Figure 4). From the graph, it could be clearly seen that the greatest increase in absorbance of the SPR peak were obtained with $AgNO_3$ at 1 mM concentration. Choosing the lowest concentration of $AgNO_3$ will also be much more useful in terms of less silver accumulation in the environment.

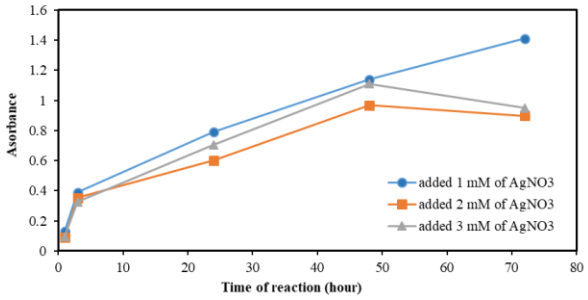

Figure 4. Time dependent-absorption for the reduction of the Spirogyra sp. extract with different values (1.0 mM, 2.0 mM and 3 mM) of $AgNO_3$ concentrations at the SPR peak of 488 nm

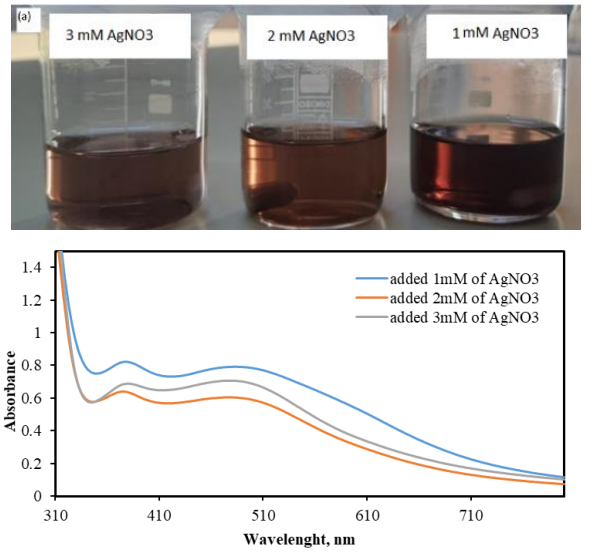

Figure 5. a) Visual appearance of the beaker containing the *Spirogyra* sp. extract and $AgNO_3$ added the *Spirogyra* sp. extract solution (1mM, 2mM and 3mM). b) UV-Vis spectra showing the effect of different conc. of $AgNO_3$ concentration on the AgNPs. The reaction time was 24 h

Furthermore, the color change of AgNPs solution to reddish color visually noticed was completed at the end of 24 h (in Figure 5a) which was determined as ideal reaction time. At the end of 24 h of reaction time, the UV-Vis spectra showing the effect of different concantration of $AgNO_3$ concentration on the AgNPs, could be seen from Figure 5b.

The size, shape, morphology and distribution of AgNPs was determined by using the High Resolution Transmission Electron Microscopy (HRTEM) technique. TEM images of AgNPs reveals that, the nanoparticles have two different shapes as spherical or oval shaped (Figure 6). Also, the dispersed silver nanoparticles consist of spherical- and oval-shaped in variable size. The size is substantially ranged between 8.9 and 23.0 nm as shown in Figure 6a. The histogram in Figure 6a inset reveals the particle size of AgNPs. The average particle size determined from 123 dark shaded areas considered to be particles in the image in Figure 6a is 18.3 nm.

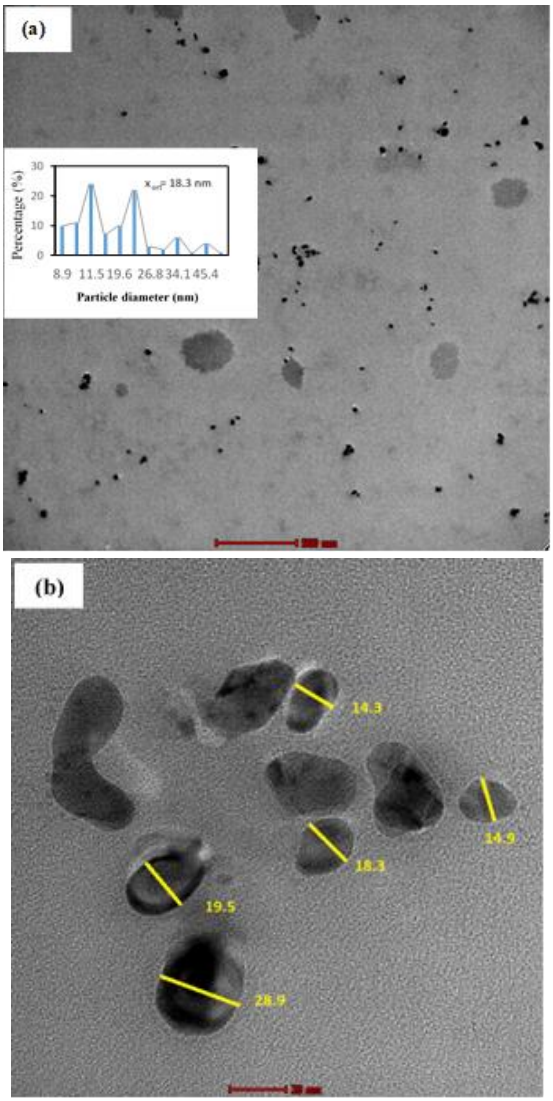

Figure 6. TEM image of spherically- and oval- shaped AgNPs at 500 nm (a) and 20 nm (b). Inset in (a) is the histogram related to the diameter of AgNPs in (a)

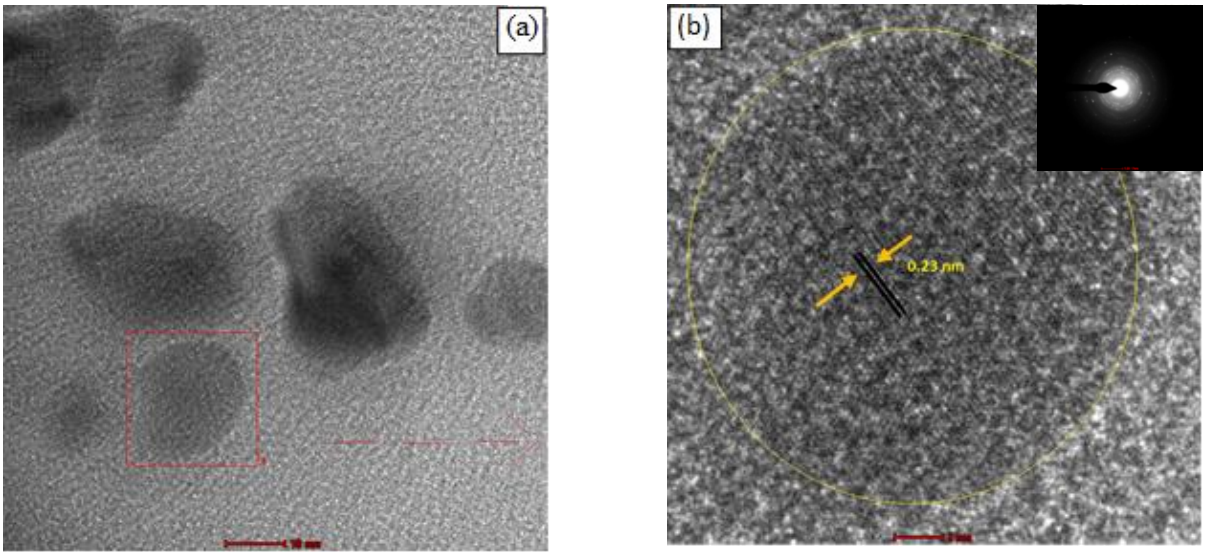

Figure 7. HRTEM images of AgNPs at 10 nm (a) and of spherically-shaped AgNPs at 2 nm which is marked in red in (a) showing fringer spacing of 0.23 nm (b). Inset in (b) is its corresponding SAED

Selected-area electron diffraction (SAED) and high resolution (HR) TEM studies were used to explain the crystal structure of the AgNPs. Figure 7a-b showed HRTEM images of AgNPs at 10 nm and 2 nm magnifications. Well-resolved lattice fringes with an interplanar spacing of 0.23 nm of spherical silver were verificated by HRTEM measurement (in Figure 7b) obtained from the particle which was red-marked in the image at 10 nm in Figure 7a. Also, SAED pattern of the particle was given in the inset of Figure 7b. The SAED pattern with bright spots on the rings clearly supported the crystalline structure of AgNPs.

In the energy dispersive X-ray analysis (EDX) analysis of AgNPs given in Figure 8 showed a peak of silver which was observed at 3 keV from the silver atoms in AgNPs. The peak proved the presence of silver nanoparticles. Because, metallic silver nanocrystals have a characteristic optical absorption peak at about 3 keV [47]. The other peaks based on carbon and copper arose because of the carbon coated copper grid of TEM. Furthermore, AgNPs were also characterized by elemental mapping results represent the distribution of elements.

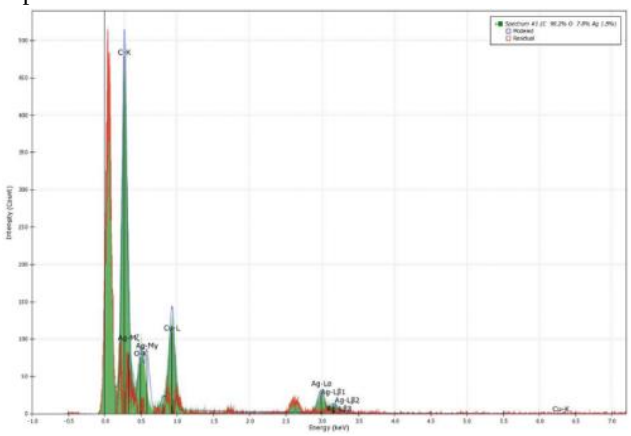

Figure 8. EDX spectrum of AgNPs. Inset the TEM micrograph of silver nanoparticles pellet solution for silver elements

According to elemental mapping results given in Figure 8 inset, the bright spot in the electron micrograph region of synthesized AgNPs proved the elemental silver atom.

FT-IR spectroscopy measurements were performed to reveale the functional groups that bound on the silver surface and involved in the biosynthesis of silver nanoparticles. The *Spirogyra* sp. extract displays a number of absorption peaks, stating its complex nature. In the FT-IR spectra of the *Spirogyra* sp. extract (Figure 9a), the intense and broad band in the range of 3500 – 3200 cm^{-1} belongs to the O–H stretching, which is usually characteristically observed in phenols and hydrogen bonded alcohols. Therefore, the peak of 3264 cm^{-1} stands for the stretching vibrations of –OH [48].

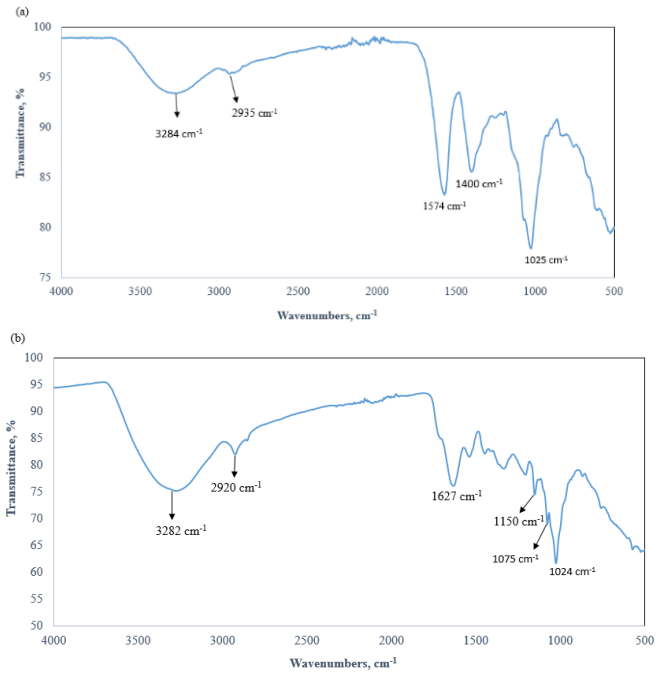

Figure 9. The FT-IR spectra of the *Spirogyra* sp. extract (a) and AgNPs (b)

The band of 2935 cm^{-1} is assigned to the stretching vibration of aliphatic C–H bonds from $–CH_3$ and $–CH_2$ functional groups. The absorption peak at around 1574 cm^{-1} could be attributed to the stretching vibration of C=O, C=N, and C=C. The stretching vibration of C–N appears at 1401 cm^{-1}. The peaks in the 1000-1200 cm^{-1} region are assigned to the stretching vibration of C–O–C group. So, the sharp and broad absorption peak of 1025 cm^{-1} could be contributed to the stretching vibration bands of symmetric C–O groups such as O=C–O, C–O–C and epoxide. The FT-IR study of the algal extract demonstrates the presence of –C=O/–C=C/–C=N, –OH (hydroxyl), C–O/C–N and –N–H (amine) groups in the algal extract. It is well known that this functional groups especially carboxy, hydroxy and amide groups take part in the reduction of Ag^+ ion to metallic silver.

In case of the silver containing the *Spirogyra* sp. extract (AgNPs), the spectrum (Figure 8b) show medium or strong absorption peaks at 3282, 2920, 1627 and 1150/1075/1024 cm^{-1} suggesting –OH [48], aliphatic –CH stretching [49], C=O stretching frequency [50] and C–O stretching of ethers, phenols and epoxide [51]. Comparison of the spectra of *Spirogyra* sp. extract and AgNPs suggested that in silver containing the *Spirogyra* sp. extract (AgNPs) O–H, C=H stretching frequency shifted from 1574 to 1627 cm^{-1} to produce nanoparticles. In case of AgNPs, the stretching frequencies of –OH, aliphatic –CH and C=O shifted from 3284, 2935 and 1574 cm^{-1} to 3282, 2920 and 1627 cm^{-1}, respectively. Otherwise, clearly more absorption peaks were formed on broad spectral range of 1400–1025 cm^{-1} on AgNPs indicating interactions between the silver nanoparticles.

3.2. Antioxidant activity and α-Glucosidase Enzyme inhibition of the extract and AgNP of Spirogyra sp.

Determination of antioxidant activity (or capacity) of samples of various is based on different methodologies and assays. In the study were used three antioxidant activity methods with different mechanisms. The antioxidant activity values of the *Spirogyra* sp. extracts for DPPH TPC, TFC tests were determined as 21.78 (mg/mL IC_{50}), 80.03 (GAE µg/mL) and 0.015 (QAE µg/mL), respectively. The values in the silver nanoparticles (AgNPs) were also measured as 17.20 (mg/mL IC50), 97.61 (GAE µg/mL) 0.027 (QAE µg/mL). In addition, values of % α-glucosidase enzyme inhibition were determined as 44.70 in the algal extract and 63.50 in silver nanoparticules (Table 1).

Antioxidant activity and α-glucosidase enzyme inhibition values of silver nanoparticles are higher than algal extracts. Bedlovičová et al. [52] pointed out that antioxidant activity values of silver nanoparticles were always not higher than plant extracts. The higher antioxidant activity of silver nanoparticle than plant extracts may be associated with content and the amount and variety of chemical compounds of the plant. In one study, silver nanoparticles were synthesized using various phenolic compounds (such as flavonoids, benzoic acids, cinnamic acids). The antioxidant capacities of structurally different phenolic compounds were evaluated. The hydroxylation of the aromatic ring appeared to play an important role in forming silver NPs. The high degree of hydroxylation in the chemical structures of phenolic compounds showed a high radical scavenging capacity and a tendency to reduce Ag^+ to AgNPs [53]. α-glucosidase enzyme is a key enzyme for non-insulin treatments of diabetes because of that it catalyzes the final step in the digestion process of carbohydrates [54]. Some studies indicated that plant silver nanoparticles have remerkable α-glucosidase enzyme inhibition [55, 56].

4. Conclusion

Silver nanoparticle synthesis is based on the reduction procedure performed by a chemical or biological reducing agent. Biosynthesis of AgNPs consist of bacteria, yeast, fungi, algal species and plant/plant extracts. In this study, the *Spirogyra* sp. extract was considered as an appropriate substance for the green biosynthesis of AgNPs. The algal extract acted as both a reducing and stabilizing agent. The green approach, based on silver nanoparticle synthesis using the *Spirogyra* sp. extracts, is highly remarkable in terms of being energy-saving (heated at only 60 ºC for 2 h), more cost-effective (not require any commercial chemicals other than $AgNO_3$) and environmentally friendly (carry out in an aqueous medium without the use of any toxic chemicals and organic solvents). The UV-vis spectra primarily showed that AgNPs has a SPR absorption peak at about 488 nm. The concentration of $AgNO_3$ and reaction time were optimized to prepare AgNPs. So, the greatest increase in absorbance of the SPR peak of AgNPs were obtained with $AgNO_3$ at 1mM concentration. Choosing the lowest concentration of $AgNO_3$ is much more useful in terms of less silver accumulation in the environment. The color change of AgNPs solution to reddish color visually noticed was completed at the end of 24 h which was determined as ideal reaction time. FTIR spectroscopy confirmed the surface modification of the *Spirogyra* sp. extract and AgNPs by water-soluble biomolecules.

The FT-IR study of the *Spirogyra* sp. extract demonstrates the presence of –C=O/–C=C/–C=N, –OH (hydroxyl), C-O/C-N and –N-H (amine) groups in the *Spirogyra* sp. Extract [17, 23, 26]. This functional groups take part in the reduction of Ag^+ ion to metallic silver. TEM images of AgNPs reveals that, the nanoparticles have two different shapes as spherical or oval shaped. The analysis of TEM confirmed that the average particle size of AgNPs was 18.3 nm.

Table 1. Enzyme inhibition and antioxidant activity of the *Spirogyra* sp. extract and AgNP

Samples	Enzyme inhibition	Antioxidant activity		
	% α-Glucosidase	DPPH (mg/mLIC_{50})	TPC (GAE µg/mL)	TFC (QAE µg/mL)
***Spirogyra* sp. extract**	44.70 ± 0.12	21.78 ± 0.11	80.03 ± 0.19	0.015 ± 0.01
AgNPs	63.50 ± 0.23	17.20 ± 0.07	97.61 ± 0.27	0.027 ± 0.03

Well-resolved lattice fringes with an interplanar spacing of 0.23 nm of spherical silver were verificated by HRTEM measurement. The SAED pattern with bright spots on the rings clearly supported the crystalline structure of AgNPs. EDX analysis of AgNPs showed that the peak at 3 keV from the silver atoms proved the presence of silver nanoparticles. Furthermore, the elemental mapping results of AgNPs represented the distribution of elements. According to this results, the bright spots in the electron micrograph region of AgNPs proved the elemental silver atom.

The antioxidant activity values of the algal extracts for DPPH TPC, TFC tests were determined as 21.78 (mg/mL IC_{50}), 80.03 (GAE µg/mL) and 0.015 (QAE µg/mL), respectively. In addition, the values of the same tests were measured as 17.20 (mg/mL IC_{50}), 97.61 (GAE µg/mL) 0.027 (QAE µg/mL) for AgNPs. Furthermore, values of % α-glucosidase enzyme inhibition were determined as 44.70 in the *Spirogyra* sp. extract and 63.50 in AgNPs. Antioxidant activity and α-glucosidase enzyme inhibition values of silver nanoparticles are higher than the values of Spirogyra sp. extracts.

Declaration

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. The authors also declared that this article is original, was prepared in accordance with international publication and research ethics, and ethical committee permission or any special permission is not required.

Author Contributions

Aysel BAŞOĞLU (AB) and Zeynep AKAR (ZA) developed the methodology. AB and ZA performed biosynthesis and characterization of silver nanoparticles (AgNPs). AB wrote the Biosynthesis of silver nanoparticles (AgNPs) and Characterization of silver nanoparticles. ZA wrote the Antioxidant activity of extract and silver nanoparticles of *Spirogyra* sp. AB and ZA supervised and improved the study.

Acknowledgment

The authors did not receive support from any organization for the submitted work. We would like to thank Bülent AKAR, who is responsible for the collection and identification of the algal sample (*Spirogyra* sp.).

Nomenclature

AgNPs : Silver nanoparticles
FT-IR : Fourier transform infrared spectroscopy
TEM : Transmission electron microscopy
EDX : Energy dispersive *X*-ray analysis
SAED : Selected area electron diffraction
DPPH : 1,1-diphenyl-2-picrylhydrazil
TPC : Total phenolic content
TFC : Total flavonoid content
UV-Vis : Ultraviolet–visible
IC_{50} : Half-maximal inhibitory concentration
GAE : Gallic acid equivalent
MIC : Minimum inhibitory concentration
MBC : Minimum bactericidal concentration
QAE : Quercetin antioxidant equivalent

References

1. Elias, E., E. Elemike, D. Olugbenga, D. Inyang, J. Samuel, and O. Chinedu, *2-Imino-(3,4-dimethoxybenzyl) ethanesulfonic acid Schiff base anchored silver nano-complex mediated by sugar cane juice and their antibacterial activities.* J Appl Res Technol, 2016. **14**: p. 38-46,
2. Husen, A., and K. S. Siddiq, *Phytosynthesis of nanoparticles: concept, controversy and application.* Nanoscale Res Lett, 2014. **9**(1): p. 9-229.
3. Sakar, M., S. Balakumar, P. Saravanan, and S. N. Jaisankar, *Annealing temperature mediated physical properties of bismuth ferrite ($BiFeO_3$) nanostructures synthesized by a novel wet chemical method.* Mater Res Bull, 2013. **48**: p. 2878–2885.
4. Tang B, L. Yuan, T. Shi, L. Yu, Y. Zhu, *Preparation of nano-sized magnetic particles from spent pickling liquors by ultrasonic-assisted chemical co-precipitation.* J Hazard Mater, 2009. **163**: p. 1173–1178.
5. Chen. X., T. Todorova, A. Vimont, V. Ruaux, Z. Qin, J. P. Gilson, and V. Valtchev. *In situ and post-synthesis control of physicochemical properties of FER-type crystals*. Microporous Mesoporous Mater, 2014. **200**: p. 334–342.
6. Annamalai, A., V. L. P. Christina, D. Sudha, M. Kalpana, and P. T. V. Lakshmi, *Green synthesis, characterization and antimicrobial activity of Au NPs using Euphorbia hirta L. leaf extract.* Colloids Surf. B: Biointerfaces, 2013. **108**: p: 60–65.
7. Das, S. K., and E. Marsili "*Bioinspired metal nanoparticle: synthesis, properties and application*," *in Nanotechnology and Nanomaterials.* InTech, 2011. **11**: p. 253–278.

8. Faraday M., *Experimental relations of gold (and other metals) to light.* Philos Trans R Soc Lond B Biol Sci, 1857. **147** : p. 145–181.
9. Grier N., *Silver and Its Compounds. In: Block, S.S., Ed., Disinfection, Sterilization and Preservation*. Lee and Febiger, Philadelphia, 1968. p. 375-398.
10. Hill, W. R. and D. M. Pillsbury, *Argyria—The Pharmacology of Silver.* Williams and Williams, Baltimore 1939.
11. Galib, M. Barve, M. Mashru, C. Jagtap, B. J. Patgiri and P. K. Prajapati, *Therapeutic potentials of metals in ancient India: A review through Charaka Samhita.* J Ayurveda Integr Med, 2011. **2**(2): p. 55-63.
12. Stevens, K. N. J., O. Crespo-Biel, E. E. M. van den Bosch, A. A. Dias, M. L. W. Knetsch, Y. B. J. Aldenhoff, F. H. van der Veen, J. G. Maessen, E. E. Stobberingh, and L. H. Koole *The relationship between the antimicrobial effect of catheter coatings containing silver nanoparticles and the coagulation of contacting blood*. Biomaterials, 2009. **30**: p. 3682–3690.
13. Austin, L. A., M. A. Mackey, E. C. Dreaden, and M. A. El-Sayed. *The optical, photothermal, and facile surface chemical properties of gold and silver nanoparticles in biodiagnostics, therapy, and drug delivery*. Arch Toxicol, 2014. **88**(7): p. 1391–1417.
14. Lv, Y., H. Liu, Z. Wang, S. Liu, L. Hao, Y. Sang, D. Liu, J. Wang, and R. I. *Boughton Silver nanoparticle-decorated porous ceramic composite for water treatment.* J. Membr. Sci, 2009. **331**: p. 50–56.
15. Martinez-Abad, A., J. M. Lagaron, and M. J. Ocio, *Development and characterization of silver-based antimicrobial ethylene-vinyl alcohol copolymer (EVOH) films for food-packaging applications*. J Agric Food Chem, 2012. **60**: p. 5350-5359.
16. Zhang, F., X. Wu, Y. Chen and Lin H, *Application of silver nanoparticles to cotton fabric as an antibacterial textile finish.* Fibers Polym, 2009. **10**(4): p. 496-501.
17. Sani, E., P. D. Ninni, L. Colla, S. Barison and F. Agresti, *Optical Properties of Mixed Nanofluids Containing Carbon Nanohorns and Silver Nanoparticles for Solar Energy Applications.* J. Nanosci Nanotechnol, 2015. **15**(5): p. 3568-3573.
18. Arroyo, G. V., A. T. Madrid, A. F. Gavilanes, B. Naranjo, A. Debut, M. T. Arias and Y. Angulo, *Green synthesis of silver nanoparticles for application in cosmetic.* J Environ Sci Health Part A, 2020. **55**(11): p. 1304-1320.
19. Song, K. C., S. M. Lee, T. S. Park, and B. S. Lee, *Preparation of colloidal silver nanoparticles by chemical reduction method.* Korean J. Chem. Eng, 2009. **26**(1): p. 153-155.
20. Steinigeweg, D., and S. Schlücker, *Monodispersity and size control in the synthesis of 20–100 nm quasi-spherical silver nanoparticles by citrate and ascorbic acid reduction in glycerol–water mixtures*. Chem Commun, 2012. **48**: p. 8682-8684.
21. Anastas, P. T. and J. C. Warner, Green Chemistry; Oxford University Press: Oxford, UK, 2000.
22. Gałuszka, A., Z. Migaszewski, J. Namies´nik, *The 12 principles of green analytical chemistry and the significance mnemonic of green analytical practices.* Trends Anal Chem Trends, 2013. **50**: p. 78–84.
23. Gurunathan, S., K. Kalishwaralal, R. Vaidyanathan, V. Deepak, S. R. K. Pandian, J. Muniyandi, N. Hariharan, S. H. Eom, *Biosynthesis, purification and characterization of silver nanoparticles using Escherichia coli*. Colloids Surf. B Biointerfaces, 2009. **74**: p. 328–335.
24. Sivaraj, A., V. Kumar, R. Sunder, K. Parthasarathy, and G. Kasivelu, *Commercial Yeast Extracts Mediated Green Synthesis of Silver Chloride Nanoparticles and their Anti-mycobacterial*. Activity J Clust Sci, 2020. **31**: p. 287–291.
25. Sanghi, R., and P. Verma, *Biomimetic synthesis and characterisation of protein capped silver nanoparticles*. Bioresour Technol, 2009. **100**: p. 501–504.
26. Shaligram, N. S., M. Bule, R. Bhambure, R. S. Singhal, S. K. Singh, G. Szakacs and A. Pandey, *Biosynthesis of silver nanoparticles using aqueous extract from the compactin producing fungal strain*. Process Biochem, 2009. **44**: p. 939–943.
27. Pugazhendhi, A., D. Prabakar, J. M. Jacob, I. Karuppusamy, R. G. Saratale, *Synthesis and characterization of silver nanoparticles using Gelidium amansii and its antimicrobial property against various pathogenic bacteria*. Microb Pathog, 2018. **114**: p. 41–45.
28. Kathiraven, T., A. Sundaramanickam, N. Shanmugam, T. Balasubramanian, *Green synthesis of silver nanoparticles using marine algae Caulerpa racemosa and their antibacterial activity against some human pathogens.* Appl Nanosci, 2015. **5**: p. 499–504.
29. Kumar, P., S. S. Selvi, A. L. Prabha, K. P. Kumar, R. S. Ganeshkumar, and M. Govindaraju, *Synthesis of silver nanoparticles from Sargassum tenerrimum and screening phytochemicals for its anti-bacterial activity*. Nano Biomed. Eng, 2012. **4**(1): p. 12–16.
30. Rolim W. R., M. T. Pelegrino, B. de Araújo Lima, L. S. Ferraz, F. N. Costa, , J. S. Bernardes, T. Rodigues and M. Brocchic, *Green tea extract mediated biogenic synthesis of silver nanoparticles: Characterization, cytotoxicity evaluation and antibacterial activity*. Appl Surf Sci, 2019. **463**: p. 66−74.
31. Ghanbar, F., A. Mirzaie, F. Ashrafi, H. Noorbazargan, M. D. Jalali, S. Salehi, S. A. S. Shandiz, *Antioxidant, antibacterial and anticancer properties of phyto-synthesised Artemisia quttensis Podlech extract mediated AgNPs*. IET Nanobiotechnol, 2017. **11**(4): p. 485–492.
32. Saravanakumar, K., R. Chelliah, S. Shanmugam, N.B. Varukattu, D. H. Oh, K. Kathiresan, and M.-H. Wang, *Green synthesis and characterization of biologically active nanosilver from seed extract of Gardenia jasminoides Ellis*. J Photochem Photobiol B, 2018. **185**: p. 126–135.
33. Islam, S.-U., B. S. Butola, A. Gupta, and A. Roy, *Multifunctional finishing of cellulosic fabric via facile, rapid in-situ green synthesis of AgNPs using pomegranate peel extract biomolecules.* Sustain Chem Pharm, 2019. **12**: p. 100-135.
34. Li, A. N., S. Li, Y. J. Zhang, X. R. Xu, Y. M. Chen, and H. B. Li, *Resources and biological activities of natural polyphenols*. Nutrients, 2014. **6**: p. 6020-6047.
35. Majumdar, M., S. Shivalkar, A. Pal, M. L. Verma, A. K. Sahoo, and D. N. Roy, *Chapter 15 - Nanotechnology for enhanced bioactivity of bioactive compounds.* Biotechnological Production of Bioactive Compounds, Elsevier, 2020. p. 433-466.
36. Akar, B., Z. Akar and B. Sahin. *Identification of Antioxidant Activity by Different Methods of a Freshwater Alga Microspora Sp. Collected From a High Mountain Lake*. Hittite J of Sci and Eng, 2019. **6**(1): p. 25-29.
37. Elgamouz, A., H. Idriss, C. Nassab, A. Bihi, K. Bajou, K. Hasan, M. A. Haija, and S. P. Patole, *Green Synthesis,*

Characterization, Antimicrobial, Anti-Cancer, and Optimization of Colorimetric Sensing of Hydrogen Peroxide of Algae Extract Capped Silver Nanoparticles. Nanomater, 2020. **10**: p. 1861-1880.

38. Salari, Z., F. Danafar, S. Dabaghi, and S. A. Ataei, *Sustainable synthesis of silver nanoparticles using macroalgae Spirogyra varians and analysis of their antibacterial activity*. Journal of Saudi Chemical Society 2016. **20(4)**: p. 459–464.
39. Sivaraman, S. K., I. Elango, S. Kumar, and V. A. Santhanam, *A green protocol for room temperature synthesis of silver nanoparticles in seconds.* Curr Sci, 2009. **97**(10): p. 1055–1059.
40. Hartland, G. V., *Optical studies of dynamics in noble metal nanostructures.* Chem Rev, 2011. **111**: p. 3858–3887.
41. Brand-Williams, W., M. E. Cuvelier, and C. Berset, *Use of a free radical method to evaluate antioxidant activity*. Food Sci Technol_LEB, 1995. **28**: p. 25–30.
42. Slinkard, K. and V. L. Singleton, *Total phenol analysis: Automation and comparison with manual methods*. Am J Enol Vitic, 1977. **28**(1): p. 49–55.
43. Fukumoto L. R., and G. Mazza, *Assessing antioxidant and prooxidant activities of phenolic compounds.* J Agric Food Chem, 2000. **48**: p. 3597–3604.
44. Yu Z, Y. Yin, W. Zhao, J. Liu, and F. Chen, *Anti-diabetic activity peptides from albumin against a-glucosidase and a-amylase*, Food Chemistry, 2012. **135**: p. 2078–2085.
45. Henglein, A. *Physicochemical properties of small metal particles in solution: microelectrode reactions, chemisorption, composite metal particles, and the atom-to-metal transition.* J Phys Chem, 1993. **97**(21): p. 5457–5471.
46. Soliman, H., A. Elsayed, and A. Dyaa, *Antimicrobial activity of silver nanoparticles biosynthesised by Rhodotorula sp. strain ATL72*. Egypt J basic Appl Sci, 2018. **5**(3): p. 228–233.
47. Kalimuthu, K., R. S. Babu, D. Venkataraman, M. Bilal, S. Gurunathan, *Biosynthesis of silver nanocrystals by Bacillus licheniformis.* Colloids Surf. B Biointerfaces, 2008. **65**: p. 150–153.
48. Ayo, R. G., J. O. Amupitan, I. G. Ndukwe, and O. T. Audu, *Some chemical constituents of the leaves of Cassia nigricans*. Vahl. Afr J Pure Appl Chem, 2009. **3**(11): p. 208–11.
49. Kaswar, S. M. A., G. Mostafa, E. Huq, N. Nahar, Y. Ozeki, *Chemical constituents and hemolytic activity of Macrotyloma uniflorum L.* Int J Biol Chem (2009) **3**(1): p. 42–8.
50. Selvi, J. A., S. Rajendran, V. G. Sri, A. J. Amalraj, and B Narayanasamy, *Corrosion inhibition by beet root extract*. Port Electrochimica Acta, 2009. 27(1): p. 1-11.
51. Chang Chien, S. W., M. C. Wang, C. C. Huang, and K. Seshaiah, *Characterization of humic substances derived from swine manure-based compost and correlation of their characteristics with reactivities with heavy metals*. J Agric Food Chem, 2007. **55**: p. 4820–7.
52. Bedlovičová, Z., I. Strapáč, M. Baláž, and A. Salayová, *A brief overview on antioxidant activity determination of silver nanoparticles.* Mol 2020. **25**:3191-3215.
53. Docea, A. O., D. Calina, A. M. Buga, O. Zlatian, M.M.B. Paoliello, G. D. Mogosanu, C. T. Streba, E. L. Popescu, A. E. Stoica, A. C., Bîrcă, B. Ş. Vasile, A. M. Grumezescu and L. Mogoanta, *The effect of silver nanoparticles on antioxidant/pro-oxidant balance in a Murine Model.* International Journal of Molecular Sciences, 2021. **21**: p. 1233-1239.
54. Kumar, S., S. Narwal, V. Kumar, and O. Prakash, *α-glucosidase inhibitors from plants: A natural approach to treat diabetes.* Pharmacogn. Rev, 2011. **5(**9):19-29.
55. Balan, K., W. Qing, Y. Wang, X. Liu, T. Palvannan, Y. Wang and, Y. Zhang, *Antidiabetic activity of silver nanoparticles from green synthesis using Lonicera japonica leaf extract*. RSC Adv, 2016. **6**(46): p. 40162-40168.
56. Jini, D., and S. Sharmila, *Green synthesis of silver nanoparticles from Allium cepa and its in vitro antidiabetic activity.* Mater Today Proc, 2020. **22**: p. 432–438