Macedonian Journal of Chemistry and Chemical Engineering, Vol. **41**, No. 2, pp. 243–250 (2022)

MJCCA9 – 865

ISSN 1857-5552
e-ISSN 1857-5625
DOI: 10.20450/mjcce.2022.2579

Received: September 15, 2022
Accepted: November 13, 2022

Original scientific paper

SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES USING *SPHAEROPHYSA KOTSCHYANA* FRUIT AND THE ASSESSMENT OF THEIR ANTIOXIDANT ACTIVITY

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Nanoparticles have attracted great interest recently due to their application in many fields. In this study, an eco-friendly, scalable, cost-effective method was used for the synthesis of silver nanoparticles (s-AgNPs) using the fruit extract of *Sphaerophysa kotschyana* as a reducing agent, and their structure was elucidated by extensive spectroscopic techniques. The color change from yellow to dark brown indicated the formation of s-AgNPs. In the UV-Vis spectrum, the maximum absorption was observed at 437 nm. Fourier transform infrared (FTIR) spectroscopy displayed the functional group of the natural compounds in the extract that capped and stabilized the s-AgNPs. The characteristic hydroxyl vibrational signal appeared at 3168 cm^{-1}. The X-ray diffraction (XRD) pattern indicated that the s-AgNPs were face-centered cubic crystalline structures. Scanning electron microscopy (SEM) exhibited the spherical-shaped nanoparticles with an average size of 67.37 nm. The antioxidant activity of the extract and s-AgNPs was established using the DPPH, ABTS, and FRAP assays. In the DPPH test, the effect of s-AgNPs was observed to be significantly higher than that of the extract, and the activity of s-AgNPs in the FRAP test was also reported to be higher than the extract. In the ABTS assay, the s-AgNPs displayed outstanding activity, which was even better than the standards. Consequently, s-AgNPs synthesized from *S. kotschyana* are promising drug products for diseases caused by oxidative stress.

Keywords: *Sphaerophysa kotschyana* fruit; silver nanoparticles; spectroscopy; natural products; green synthesis; antioxidant activity

СИНТЕЗА И КАРАКТЕРИЗАЦИЈА НА НАНОЧЕСТИЧКИТЕ ОД СРЕБРО СО УПОТРЕБА НА ПЛОД ОД *SPHAEROPHYSA KOTSCHYANA* И АНАЛИЗА НА НИВНАТА АНТИОКСИДАЦИСКА АКТИВНОСТ

Наночестичките од неодамна привлекуваат големо внимание поради нивната примена во многу области. Во ова истражување беше применет евтин еколошки метод што може да се користи за синтеза на наночестички од сребро (s-AgNPs) со примена на екстракт од плод на *Sphaerophysa kotschyana* како редукциско средство, а потоа беше одредена нивната структура со бројни спектроскопски техники. Промената на бојата од жолта до темнокафена укажува на образување на s-AgNP. Во UV-Vis спектарот максималната апсорпција изнесуваше 437 nm. Фуриеовата трансформна инфрацрвена (FTIR) спектроскопија ги прикажа функционалните групи на природните соединенија во екстрактот кои ги обложија и стабилизираа s-AgNP. Карактеристичниот хидроксилен вибрациски сигнал се манифестираше на 3168 cm^{-1}. Рендгенската дифракциска (XRD) слика покажа дека s-AgNP се странично центрирани кубни кристални структури. Скенирачката електронска микроскопија (SEM) ги покажа наночестичките со сферна форма и просечна големина од 67,37 nm. Антиоксидациската активност на екстрактот и на s-AgNP беше утврдена со примена на анализа со DPPH, ABTS и FRAP. Во тестот DPPH, беше забележано дека ефектот на s-AgNP е значително повисок од тој на екстрактот. Во анализата со ABTS, s-AgNP покажаа извонредна активност што беше подобра дури и од стандардите. Следствено, s-AgNP синтетизирани од *S. kotschyana* се потенцијални лекови за болести што предизвикуваат оксидациски стрес.

Клучни зборови: плод од *Sphaerophysa kotschyana*; наночестички на сребро; спектроскопија; природни производи; зелена синтеза; антиоксидациска активност

1. INTRODUCTION

Natural products have been employed for food and medicinal purposes since ancient times.[1-3] Following the discovery of bioactive compounds in plants, due to the advancement of spectroscopy, plants (especially natural products) have gained great interest in scientific fields.[4] Nanotechnology has become a rapidly developing science in recent years due to its wide application areas, such as medicine, optics, water purification, sensors, agriculture,[5] *etc*. Many synthetic methods have been developed for the synthesis of nanoparticles, including physicochemical techniques.[6] The corresponding methods contain toxic chemicals and are harmful to the environment and living things. Natural products have emerged and become of great interest in nanoparticle synthesis, particularly with respect to green chemistry.[7,8] Recently, natural products such as vegetables, seaweed, algae, enzymes, arthropods, and plants have begun to be used in the synthesis of nanoparticles. Among them, plant extracts are widely used due to their abundance and bioactive compound content.[9] Nanoparticles have been reported to reveal considerable biological activity, such as antioxidant,[10] anticancer,[11] antibacterial, and antifungal.[12]

The *Sphaerophysa* genus belongs to the Fabaceae family, distributed throughout Asia and north China, and includes two species in the world, *S. kotschyana* and *S. salsula*.[13] The species of this genus have been used in traditional medicine to treat hypertension. Phytochemical investigation of this genus revealed the isolation and identification of alkaloids, coumarins, and isoflavans.[14, 15] Due to the significant bioactive compounds of this genus, silver nanoparticles (s-AgNPs) capped, stabilized, and reduced by the corresponding compounds could be effective for food and pharmaceutical application.

Antioxidants are compounds that can inhibit, delay, and scavenge radicals formed in the human body during metabolism.[16] The human body develops enzymes to combat free radicals, but these enzymes can be insufficient under some conditions, such as bad living habits, cigarette smoking, stress, *etc*. Natural products attract great attention because they contain bioactive compounds that will remove free radicals in the body. In addition, drug formulations that include natural products have recently been used effectively.[17, 18]

In this study, s-AgNPs were synthesized from *Sphaerophysa kotschyana* fruit and investigated for their antioxidant activity.

2. MATERIALS AND METHODS

2.1. Plant material

S. kotschyana fruit was provided from Konya, Turkey, and identified by Prof. Dr. Ozgur Eminagaoglu, a specialist on plant taxonomy at the Artvin Coruh University, Faculty of Forestry, Forest Engineering Department. A voucher specimen was deposited at the herbarium of the same Faculty (No: 16788).

2.2. *Synthesis of silver nanoparticles (s-AgNPs)*

The fruit of *S. kotschyana* was collected, dried, and powdered (10.0 g) and then heated with deionized water (200 ml) for 3 h at 55 °C. After filtration with Whatman paper (180 µm thickness, 25 mm diameter), the solution was treated with silver nitrate (0.035 M, 100 ml) at 60 °C for 1 h, centrifugated at 5000 rpm for 15 minutes, and lyophilized to yield the black product.[19]

2.3. *Identification of nanostructure*

Extensive spectroscopic study revealed the proposed nanostructure. A Fourier transform infrared (FTIR) spectrometer (FT/IR-4700 Jasco) supplied information about functional group changes during the bio-reduction. UV-Vis analysis provided the maximum absorbance of the nanoparticles at a wavelength of 200–800 nm, using a UV-2600 Shimadzu spectrophotometer. The X-ray diffraction (XRD) pattern was recorded by an Empyrean, Malvern Panalytical diffractometer using CuKα radiation. The morphology of the synthesized s-AgNPs was determined by scanning electron microscopy (SEM) (Quanta Feg450) and the EDAX detector was used for elemental analysis.[20]

2.4. *ABTS$^{\bullet+}$ radical cation scavenging assay*

First of all, stock solutions of the s-AgNPs (0.25 mg/ml in DMSO) and extract (1.0 mg/ml in MeOH) were prepared and the phosphate buffer was produced (500 ml, pH 7.4, 0.1 mM). The reaction was then carried out in this buffer solution. The ABTS$^{\bullet+}$ radical cation solution was prepared by treating ABTS (2.0 M, 100 ml) with sodium persulfate (2.45 mM, 200 ml) in the dark for 6 h at room temperature. Different concentrations of s-AgNPs (2–40 μg/ml) was reacted with the ABTS$^{\bullet+}$ solution. The measurement was executed by a spectrophotometer (734 nm). A calibration curve of Trolox was obtained and the results were then calculated in terms of the Trolox equivalent (TE).[21]

2.5. *DPPH free radical scavenging assay*

Samples of the stock solution at various concentrations (2–40 μg/ml, 3.0 ml) were treated with DPPH$^{\bullet}$ solution in ethanol (1.0 ml, 0.26 mM) and then vortexed. The mixture was incubated at room temperature for 30 min. An absorbance measurement was performed with a spectrophotometer (517 nm). The results were calculated as IC_{50}.[22]

2.6. *Reducing power*

A reducing power assay was executed according to the given literature. Briefly, each extract and s-AgNPs (100 μl) were mixed with phosphate buffer (1.15 ml, 0.20 M, pH 6.7) and potassium ferric cyanide (1.0 %, 1.25 ml) and the mixture was then incubated for 30 min at 50 °C. Trifluoroacetic acid (1.25 ml, 10 %) and $FeCl_3$ (0.25 ml, 0.1 %) were added to the reaction flask and vortexed. UV-Vis measurement was then carried out at 700 nm. The calibration curve of Trolox at various concentrations was constructed and the results of the samples were calculated in terms of the Trolox equivalent (TE).[23]

2.7. *Statistical analysis*

The statistical analysis of this work was performed by GraphPad prism (8.00 version). One-way ANOVA with Tukey test was carried out for the comparison test. The results were stated as mean values ± standard deviation ($P < 0.05$).

3. RESULTS AND DISCUSSION

3.1. *UV-Vis spectral analysis of s-AgNPs*

The observed color change of the s-AgNPs solution from yellow to dark brown was a significant indication of nanoparticle formation. The maximum absorption was obtained at 437 nm, which proved the desired product, since the characteristic absorption peak of green-synthesized s-AgNPs appears to range from 350 to 550 nm (Fig. 1). The plant secondary metabolites have a functional group that can reduce the silver ions to silver metal. After the oxidation and reduction reaction in the solution of plant extract and silver ions, the silver nanoparticle formed in the following stages: reduction, clustering, and growth of nanostructures.

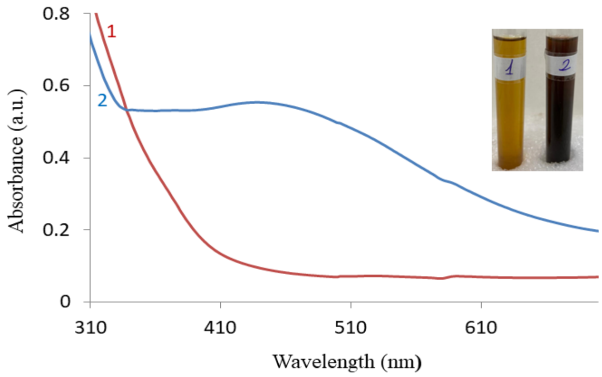

Fig. 1. UV-Vis spectra of extract (1) and s-AgNPs (2). Inset – the solution of extract (1) and s-AgNPs (2)

3.2. *Fourier transform infrared (FTIR) spectroscopy*

FTIR analysis revealed that the functional groups of the secondary metabolites capped and stabilized the silver ions. The slight differences between the extract and s-AgNPs proved the formation of nanostructures (Fig. 2). The characteristic hydroxyl vibrational signal appeared at 3168 cm^{-1}. The peak observed at 2916 cm^{-1} belonged to the C-H stretching of the alkane. The absorption at 1539 cm^{-1} and 1506 cm^{-1} could be due to the N-O stretching of the nitro group. The OH bending of phenol was observed at 1361 cm^{-1}. The signals at 1239 cm^{-1} and 1031 cm^{-1} belonged to C-N stretching and S=O stretching, respectively. The signal of C-H bending appeared at 756 cm^{-1}, and the signals at 504 cm^{-1} and 420 cm^{-1} could be due to the silver oxide, in agreement with the literature.[24]

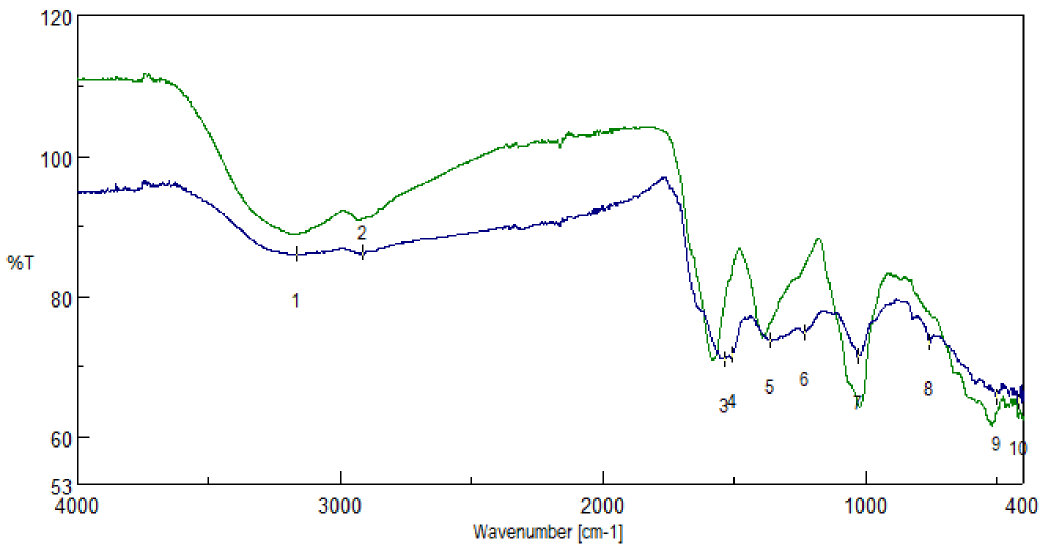

Fig. 2. Fourier transform infrared spectra of the extract (green) and s-AgNPs (blue).
1: 3168, 2: 2916, 3: 1539, 4: 1506, 5: 1361, 6: 1239, 7: 1031, 8: 756, 9: 504, 10: 420

3.3. *X-ray diffraction (XRD)*

The XRD pattern revealed the crystalline nature of the s-AgNPs (Fig. 3). The characteristic signals observed at 38.23°, 46.27°, 64.89°, and 77.13° could correspond to the 111, 200, 220, and 311 facets of the face-centered cubic structure, respectively. The planes were consistent with the standard diffraction pattern.[25] The crystal structure was presented by the intense peak (200). The impurity signals in the XRD spectrum could be due to the silver chloride.

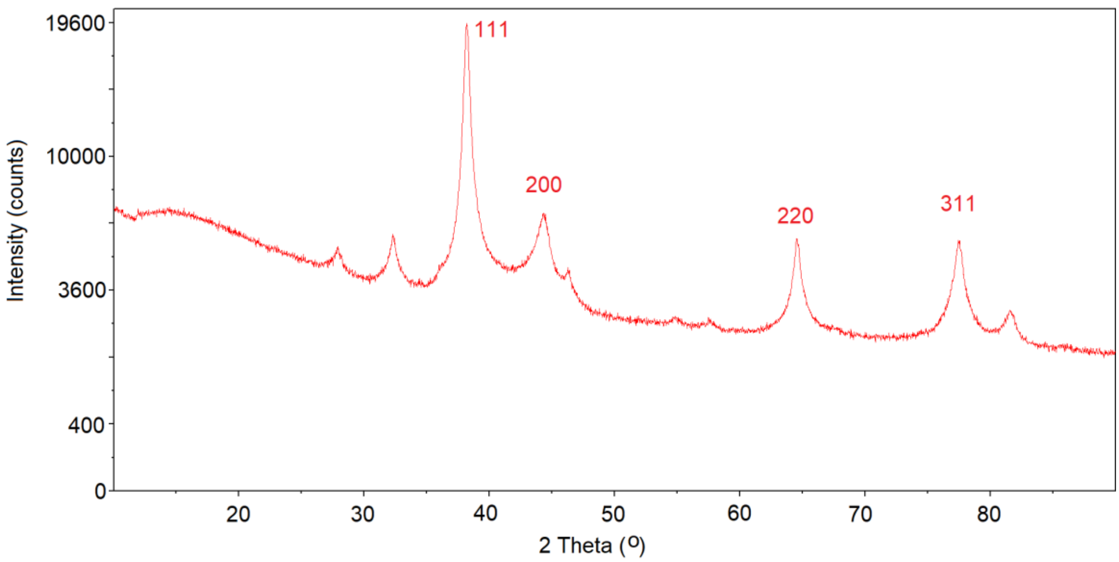

Fig. 3. X-ray diffraction pattern of s-AgNPs

3.4. *Scanning electron microscopy-energy-dispersive X-ray spectroscopy (SEM-EDX) analysis*

The SEM image indicated the morphology of uniformly distributed s-AgNPs (Fig. 4). The agglomerated clusters were distributed over the surface. The particle size was found to be 67.37 nm. The energy-dispersive X-ray (EDX) spectrum showed a strong silver signal at 2.5–3.5 keV, verifying the s-AgNPs, as well as weak carbon and oxygen peaks (Fig. 5). The atomic percentage of Ag was found to be 32.73 %.

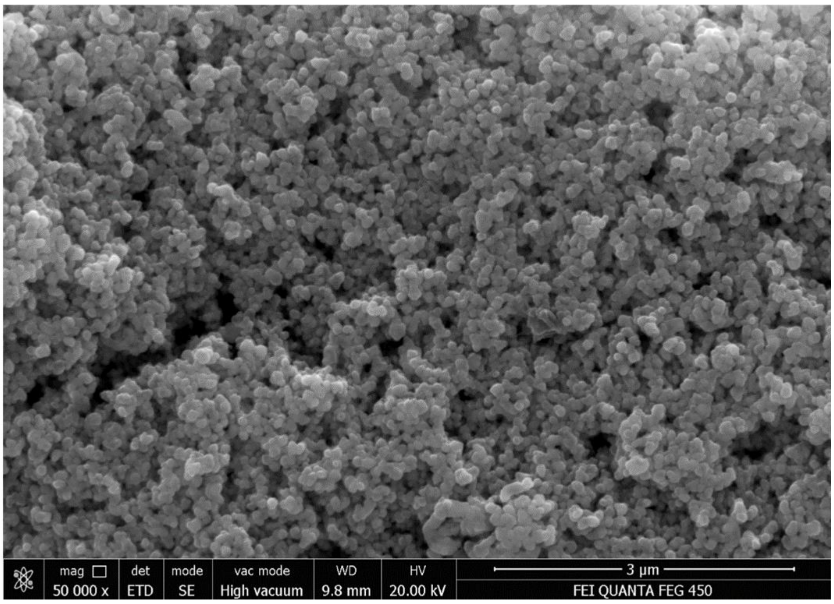

Fig. 4. Scanning electron microscopy image of s-AgNPs

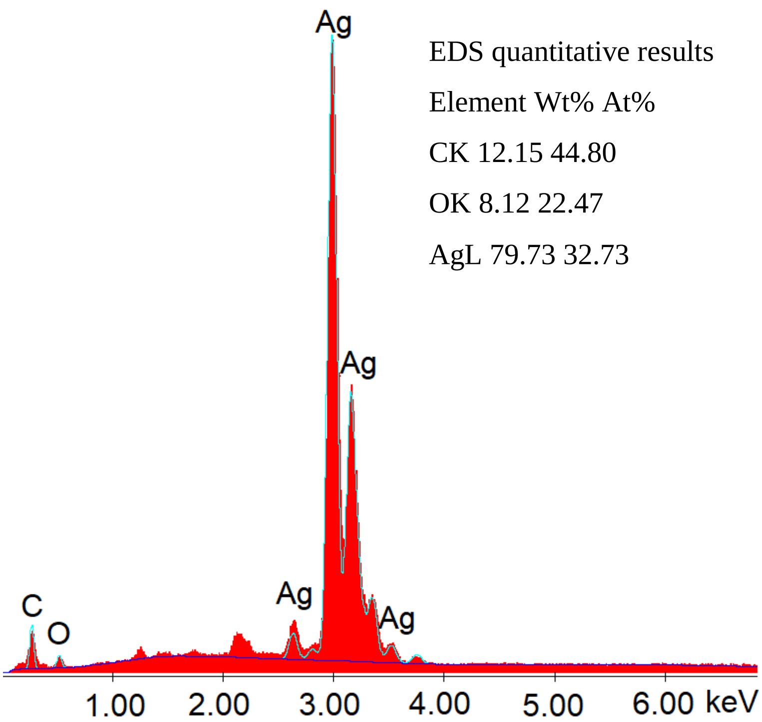

Fig. 5. Energy-dispersive X-ray spectrum and elemental analysis of s-AgNPs

3.5. *Antioxidant activity*

The discovery and development of antioxidant agents are of the highest importance in the food and pharmaceutical industry. In this study, s-AgNPs were synthesized from an aromatic and medicinal plant, *S. kotschyana*. Due to the bioactive compound content of *S. kotschyana*, s-AgNPs that are capped and stabilized by the corresponding compounds would be expected to exhibit considerable activity. In the DPPH assay, the s-AgNPs revealed significantly higher activity (IC_{50} 8.90 ± 0.16 µg/ml) than the standard BHT (IC_{50} 10.78 ± 0.10 µg/ml) and extract (IC_{50} 14.15 ± 0.12 µg/ml). In the ABTS assay, the s-AgNPs exhibited an outstanding effect (IC_{50} 3.33 ± 0.058 µg/ml) compared to the standard BHT (IC_{50} 7.14 ± 0.18 µg/ml) and extract (IC_{50} 6.34 ± 0.34 µg/ml). The reducing power of the s-AgNPs (µmol TE/mg extract, 1.44 ± 0.016 µg/ml) was also found to be higher than the extract (µmol TE/mg extract, 1.44 ± 0.016 µg/ml), but lower than the standard BHT (µmol TE/mg extract, 5.34 ± 0.12 µg/ml). The results of the three assays are shown in Fig. 6. There is an agreement in the reported study in which s-AgNPs synthesized from some aromatic and medicinal plants displayed considerable antioxidant activity.[26-30] Moreover, s-AgNPs were synthesized using *Atrocarpus altilis* leaf extract, and the nanoparticles and extract revealed antioxidant and antimicrobial activity.[31] In another study, *Thymus kotschyanus* extract was used for the synthesis of s-AgNPs, and the extract and nanoparticles were reported to display antioxidant, antibacterial, and cytotoxic effects.[32] In addition, s-AgNPs synthesized using *Carissa carandas* revealed considerable antioxidant and antibacterial activity.[33] In a phytochemical study, pinitol and sucrose were isolated from *S. kotschyana*.[34]

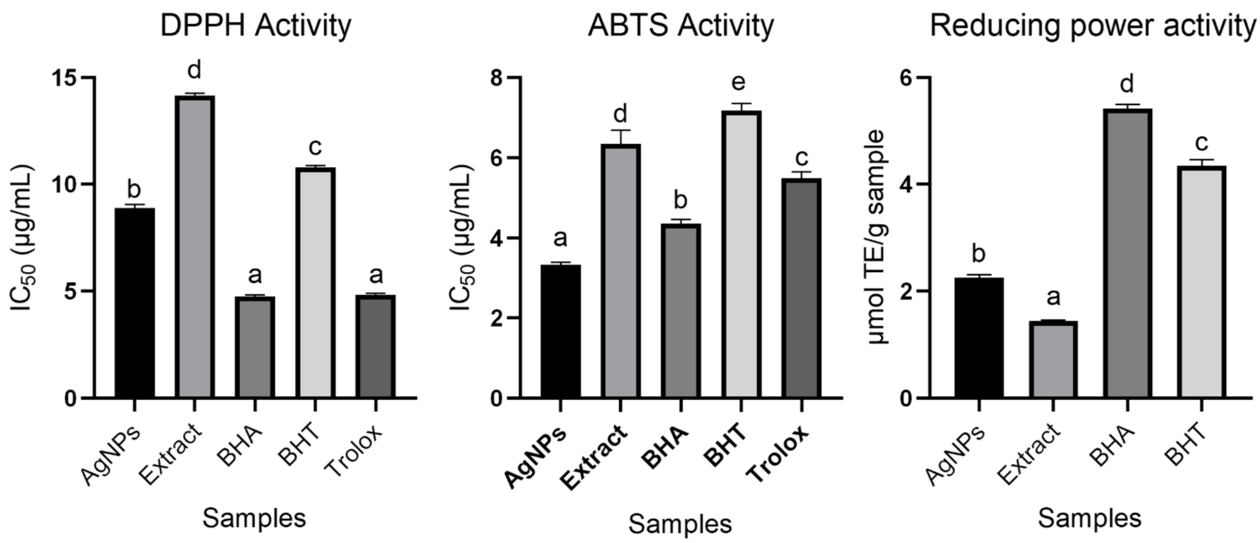

Fig. 6. Antioxidant activity of extract and s-AgNPs

4. CONCLUSION

s-AgNPs were synthesized using *S. kotschyana* fruit extract in an eco-friendly, low-cost, easy manner. *S. kotschyana* contains significant bioactive compounds, such as alkaloids and coumarins. Hence, the nanostructures capped, stabilized, and reduced by the corresponding compounds could be raw materials for the food and pharmaceutical industries. Due to the environmental pollution caused by the chemical industry, the use of eco-friendly methods in nanoparticle synthesis has been an important field of study in recent years. Since the nanoparticles revealed a higher antioxidant activity than that of the plant extract, nanoparticles synthesized from *S. kotschyana* fruit can be used in food and pharmaceutical production. Alkaloids are an important class of secondary metabolites and at least sixty plant-derived alkaloids have been approved as drugs in various countries. Therefore, nanoparticles capped by alkaloids may have potential for the drug development process. Further studies should be carried out using s-AgNPs, such as *in vivo* studies, studies of the antiproliferative effect, *etc*., to establish the compatibility for drug development.

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