

Green Synthesis of Silver Nanoparticles from *Salvia Aethiopis* L. and their Antioxidant Activity

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Research Article

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Abstract

Salvia aethiopsis L. was heated in distilled water for 2 hours. After filtration, water extract was treated with silver nitrate for 2 hours at 60°C to yield the silver nanoparticles (Sa-AgNPs). The structure of silver nanoparticles was elucidated by spectroscopic methods such as Ultraviolet-visible (UV-Vis), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and Scanning electron microscope (SEM). The maximum absorption in UV-Vis spectrum was observed at 508 nm. XRD pattern at (2θ) 38.1°, 44.3°, 64.4°, and 77.4° degrees can be assigned to the (111), (200), (220) and (311) Bragg's reflections of the face-centered cubic crystalline structure. The average size of Sa-AgNPs was found as 74.09 nm by SEM analysis. The characteristic hydroxyl vibration signal appeared at 3222 cm^{-1} . Antioxidant activity of extract and Sa-AgNPs were carried out using DPPH[•], ABTS^{•+} FRAP assay. The Sa-AgNPs revealed the considerable ABTS^{•+} scavenging effect with the value of 4.93 (IC_{50} , $\mu\text{g/mL}$) compared to BHT (IC_{50} , $\mu\text{g/mL}$, 8.34). However, Sa-AgNPs displayed the lower DPPH[•] activity (IC_{50} , $\mu\text{g/mL}$, 24.37) than that of the standard BHT (IC_{50} , $\mu\text{g/mL}$, 9.67). The reducing power activity of Sa-AgNPs was found as 4.52 ($\mu\text{mol TE/mg extract}$) while the standard BHT value was 488 ($\mu\text{mol TE/mg extract}$).

1 Introduction

Nanotechnology is a science that has developed rapidly in recent years and has a wide range of applications [1]. Nanoparticles are basic building blocks with different properties due to their large surface area/volume ratio. In recent years, nanoparticles have formed the basis of modern materials science [2]. Silver nanoparticles (AgNPs) gain great interest in biology, biomedical, drug delivery, medicine, agriculture, food industries, textile industries, and electronics [3].

Several synthetic routes have been developed to produce AgNPs including electrochemical, radiation technique [4], and photochemical [5]. However, these methods lead to environmental contamination, toxic residue, and high cost. Therefore, natural products gain great interest in the synthesis of nanoparticles due to their eco-friendly, low cost, high efficiency, and scale-up properties [6, 7].

Natural products such as plant extract, microorganisms, algae, oilcake, vegetable waste, seaweed, enzymes, arthropods have been used for the production of AgNPs. It has been accepted that plant-based materials are the promising substrate for the AgNPs synthesis due to the corresponding advantages [8]. The biological effects of AgNPs depend on some crucial factors such as surface chemistry, size, shape, particle morphology, particle composition, coating/capping, agglomeration, and dissolution rate, particle reactivity in solution, the efficiency of ion release, cell type, the type of reducing agent [9]. AgNPs synthesised from plants were reported to show significant biological activities such as antioxidant [10], antibacterial [11], anticancer [12, 13], antifungal [14], antiviral [15], anti-inflammatory [16].

Silver nanoparticles were synthesised from *Salvia leucantha* that revealed considerable antibacterial activity [17]. *Salvia officinalis* is well known of *Salvia* genus. Silver nanoparticles synthesised from *S.*

officinalis was reported to display significant antibacterial [18], antiplasmodial [19], antioxidant and anti-inflammatory [20], antileishmanial effects [21].

Free radicals are called reactive oxygen species including hydroxyl (OH[•]), peroxy (ROO[•]), superoxide (O₂^{•-}), peroxynitrite ([•]ONOO⁻) radicals that were produced throughout oxidation within the mammalian body [22]. The human body has many protection systems against oxidative stress. The natural antioxidants become insufficient in some situations and then, the excess radicals can damage to cell membrane resulting in diseases [23]. Therefore, food including antioxidants should be consumed to cope with this situation. An antioxidant is defined as a substance that inhibits the oxidation of the substrate [24]. Accordingly, phenolic compounds are produced from the secondary metabolism of plants and are considered natural antioxidants because they protect many organs from oxidation [25]. There has been an increase in the use of natural antioxidants due to the benefits provided by the aromatic herbs of extracts, essential oils, and spices [26]. Herbal-based products contain phenolic phytochemicals, one of the most powerful antioxidants, and contribute to body defense against oxidative damage. These compounds protect against deterioration and provide antioxidant substances to the human body because of their consumption [27–29].

Salvia L. species have been used since ancient times due to their antioxidant, natural preservative, spice, aromatic substance, and medicinal properties [30]. *Salvia* is an important genus belonging to the Lamiaceae (formerly Labiatae) family. Around the world, 1000 species of *Salvia* are used as herbal tea and flavoring, as well as in the cosmetics and pharmaceutical industries. *Salvia* species have been used in the treatment of colds, coughs, toothache, gastrointestinal problems, coronary heart disease, hepatochirrosis, hepatitis, cerebrovascular disease, chronic renal failure, dysmenorrhea, and neurasthenic insomnia as traditional medicine [31]. In addition, *Salvia* herbs are known to have a wide variety of pharmacological activities such as antimicrobial, antioxidant, anti-inflammatory, anticancer, hypoglycemic, hypolipidemic, antinociceptive, memory-enhancing effects. *Salvia* genus is rich in polyphenols, especially phenolic acid, and flavonoids [32, 33].

In this study, *Salvia aethiopsis* mediated synthesis of silver nanoparticles was achieved and antioxidant activity of corresponding Sa-AgNPs was carried out.

2 Materials And Methods

2.1 Chemicals

Butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), silver nitrate (AgNO₃), 1,1-diphenyl-2-picryl-hydrazyl (DPPH), and solvents with analytical grade were purchased from Fluka and Sigma-Aldrich Chemicals.

2.2 Plant materials

Salvia aethiopsis L. was obtained from Tokat Gaziosmanpasa University Aromatic and Medicinal Plant Field.

2.3 Synthesis of silver nanoparticles

Salvia aethiopsis leaves were powdered by grinder and powder material (50 g) was heated with distilled water (200 mL) at 50°C for 2 hours. After filtration with Whatman filter paper, silver nitrate distilled water solution (0.037 mM, 200 mL) was added to the extract solution slowly. The reaction mixture was heated at 60°C for 2 hours. The color change from yellow to brown was observed. After completion of the reaction, Sa-AgNPs were obtained by repeated centrifugation at 5000 rpm for 20 minutes then washed thoroughly with distilled water. The Sa-AgNps were dried by lyophilisation [7].

2.4 Characterization of silver nanoparticles

The UV-vis spectra were recorded on Hitachi U-2900 spectrophotometer. The maximum absorption was detected at 508 nm. XRD measurement was carried out on an Empyrean, Malvern Panalytical diffractometer, the operation voltage of 45 kV at a 40-mA current strength. The crystallographic structure of Sa-AgNPs was determined by the XRD pattern. The diffracted intensity was carried out in the region of 2θ from 20° to 90° at 0.02°/ min. The particle size was calculated by dynamic light scattering (DLS) on a Delsa Nano C instrument. The Sa-AgNPs properties were determined by Scanning Electron Microscope (SEM) on Quanta Feg450. EDAX detector and EDX were used to determine the elemental analysis. quanta 450 FEG was used for surface and point analysis.

2.5 Antioxidant activity

2.5.1 DPPH[•] free radical scavenging assay

DPPH[•] free radical scavenging effect of *S. aethiopsis* mediated silver nanoparticles and the extract was carried out according to the procedure described in the literature [29]. DPPH[•] radical (1.0 mL, 0.26 mM) was treated with the Sa-AgNPs (3.0–30 µg/mL) at room temperature (rt) for 20 minutes. During the reduction, the solution color fades, and the absorbance decreases. BHT, BHA and Trolox were used as standard compounds. The equation was used for the calculation of DPPH[•] scavenging effect (1)

$$\text{DPPH}^{\bullet} \text{ scavenging effect (\%)} = [(A_1 - A_2) / A_1] \times 100 \quad (1)$$

A_1 is the absorbance of the control and A_2 is the absorbance of the sample.

2.5.2 ABTS^{•+} radical cation activity

ABTS^{•+} radical cation assay is based on the ability of antioxidants to reduce ABTS^{•+} (blue/green) to ABTS⁻² (colorless). ABTS radical cation solution was produced by the reacting of 7.0 mM ABTS with K₂S₂O₈ (2.45 mM) at a ratio of 2/1 (v/v), the mixture could stand in the dark at rt for 12 h. After adjusting pH by treatment of ABTS^{•+} solution with phosphate buffer (0.1 mM, pH 7.4), Sa-AgNPs were treated with ABTS^{•+} (1.0 mL) at several concentrations (3.0–30 µg/mL). The absorbance measurement was executed

at 734 nm and ABTS concentration was calculated by the calibration curve. ABTS^{•+} effect was calculated by the given Eq. (2):

$$\text{ABTS}^{\bullet+} \text{ scavenging effect (\%)} = [(A_1 - A_2) / A_1] \times 100 \quad (2)$$

in which, A_1 is ABTS^{•+} initial concentration and A_2 is ABTS^{•+} remaining concentration in the sample [30].

2.5.3 Reducing power

Reducing power of extract and Sa-AgNPs were measured according to previously published method.[22] Reducing power was calculated from the calibration curve of ascorbic acid and presented as $\mu\text{g/ml}$ of extract or silver nanoparticles. The samples (extract and Sa-AgNPs) were mixed with 200 mM of sodium phosphate and volume was adjusted to 1.25 mL with water, followed by the addition of potassium ferricyanide, $\text{K}_3\text{Fe}(\text{CN})_6$ (1.25 mL, 1%). Later, the mixture was incubated for 20 min at 50°C, and then 1.25 mL of 10% trichloroacetic acid was added and then thoroughly vortexed. An aliquot (1.0 mL) was mixed with water (1.0 mL) and ferric chloride (0.5 mL, 0.1%) and then vortexed. The absorbance was measured at 700 nm against a blank using a spectrophotometer [31]. The high absorbance value revealed the high reducing activity.

2.6 Statistical analysis

GraphPad Prism software (version 8.0.1), one-way ANOVA with Tukey's multiple comparisons test was used for statistical analysis. The results were stated as mean values \pm standard deviation ($P < 0.05$).

3 Results And Discussion

3.1 Synthesis of silver nanoparticles

The silver nanoparticles were synthesized using *Salvia aethiopsis* leaves. The plant material was heated in distilled water, after removal of the solid part, the extract solution was treated with the silver nitrate solution. The secondary metabolites in the water solution reduced the Ag^+ to Ag^0 . Afterward, Ag atoms were capped and stabilized by secondary metabolites that the plant synthesized. The color change of the reaction mixture from dark yellow to dark brown confirmed the formation of Sa-AgNPs (Fig. 1).

In the reaction mechanism (Fig. 2), the silver ions were reduced by bioactive compounds that oxidized. After the stage of ion reduction, clustering, and growth of nanoparticles, the silver nanoparticles formed. Since *Salvia* species include luteolin, the reaction mechanism was showed for this compound.

3.2 UV-Vis spectral analysis

The maximum absorption at 508 nm at UV-vis spectrum revealed the formation of the silver nanoparticles (Fig. 1). The UV-Vis spectroscopy is mostly used for the identification of silver nanoparticles. Free electrons in metal nanoparticles yield a surface plasmon resonance absorption band.

The peak revealed the typical surface plasmon resonance of Sa-AgNPs. The UV-Vis spectrum showed the presence of bioactive compounds in the *S. aethiopsis* leaves for the formation of silver nanoparticles. The particle size and shape have a significant effect on the wavelength shift. The various metal nanoparticles in the size range from 2 to 100 nm were identified at 300–800 nm wavelengths.

3.3 Fourier-transform infrared spectroscopy (FTIR)

FTIR spectrum showed the functional groups responsible for the silver ion reduction and stabilization of Sa-AgNPs. It was not observed the good absorption signal of Sa-AgNPs. So, FTIR spectrum of water extract was presented (Fig. 3). The signal that appeared at 3222 cm^{-1} could be attributed to the hydroxyl group and the peak at 2931 might be due to the CH stretching. The peak at 1567 cm^{-1} could be due to the NH bending. The other signals at 1370 cm^{-1} , 1259 cm^{-1} , and 1064 cm^{-1} belonged to the OH bending, C-O stretching, and C-O stretching, respectively.

3.4 X-ray diffraction (XRD)

X-ray diffraction (XRD) pattern was obtained by Empyrean, Malvern Panalytical diffractometer with highScore Plus software (Fig. 4). The nanoparticles synthesized were characterized by XRD. The pattern indicated that the main peaks at (2θ) 38.14, 44.29, 64.48, 77.38 corresponded to the (111), (200), (220), (311) planes, respectively. The pattern of green synthesized AgNPs was found to possess a face-centered cubic unit cell (fcc) structure by comparing JCPDS (File no: 89-3722) [34].

The average particle size was found as 74.09 nm by Debye-Scherrer's Eq. (1)

$$D = 0.9\lambda / \beta \cos \theta (1)$$

in which D is the crystal size, λ is the wavelength of x-ray, θ is the Braggs angle in radians and β is full of half maximum of the peak in radians.

3.5 Scanning Electron Microscope (SEM) Analysis

The surface morphology was found a spherical nature with an average size of 74.09 nm (Fig. 5). The energy dispersive analysis of x-rays (EDX) indicated the formation of AgNPs. The intense peak of AgNPs in the EDX spectrum at 3.2 keV confirmed the synthesis of AgNPs. The metallic silver nanoparticles show the typical strong signal peak at 3.0-3.3 keV due to the surface plasmon resonance [35].

3.6 Antioxidant activity

Aromatic and medicinal plants have been used extensively for medicinal purposes for years due to their bioactive contents [36–39]. After the development of spectroscopy in the 19th century, the isolation and identification of secondary metabolites from corresponding plants gained great interest and it became the focus of science [33, 40]. Therefore, these plants have been used for the synthesis of nanoparticles that exhibited a broad spectrum of biological activities [41]. Antioxidant activity of Sa-AgNPs was carried

out using DPPH[•] (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay, ABTS^{•+} [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt radical cation] scavenging assay, and ferric reducing antioxidant power (FRAP) assay (Fig. 6). In DPPH[•] assay, Sa-AgNPs revealed a significantly higher activity (IC₅₀, µg/mL, 24.37) than the extract (IC₅₀, µg/mL, 45.41). But the activities of both extract and Sa-AgNPs were lower than that of the standards. In ABTS^{•+} assay, Sa-AgNPs displayed excellent activity with the value of 4.93 (IC₅₀, µg/mL) compared to the BHT (8.34) and Trolox (5.71).

In concern to the reducing power assay, the Sa-AgNPs possessed a higher activity than extract but lower than BHA. Consequently, the silver nanoparticles synthesised from *S. aethiopsis* revealed good antioxidant activity. Hence, they have the potential to be used in the pharmaceutical and food industries. Further scientific research should be carried out to present the medicinal usage such as anticancer activity invitro and invivo.

4 Conclusion

Due to the increasing interest in natural products in the food and pharmaceutical industry, the silver nanoparticles green synthesised from *S. aethiopsis* could be an antioxidant agent. The synthesis of silver nanoparticles using *S. aethiopsis* was an easy, eco-friendly, and economical process. This synthesis did not require the use of any hazardous chemicals and reagents for reducing and processing. The XRD data of AgNPs had a good agreement with the standard JCPDS cards as reported. The XRD pattern displayed that the AgNPs have a face-centered cubic (fcc) lattice structure. The nature of AgNPs was found to be highly granulated. The further scientific study should be executed to show the anticancer potential of Sa-AgNPs.

5 Declarations

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Conflict of interest

The author has no conflict of interest to declare.

Data availability

Data are given in manuscript and they are confidential.

Ethics approval

Ethical rules are followed.

Funding

Not applicable

Consent to participate

Not applicable

Author contribution

All research was carried out by ENG

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Figures

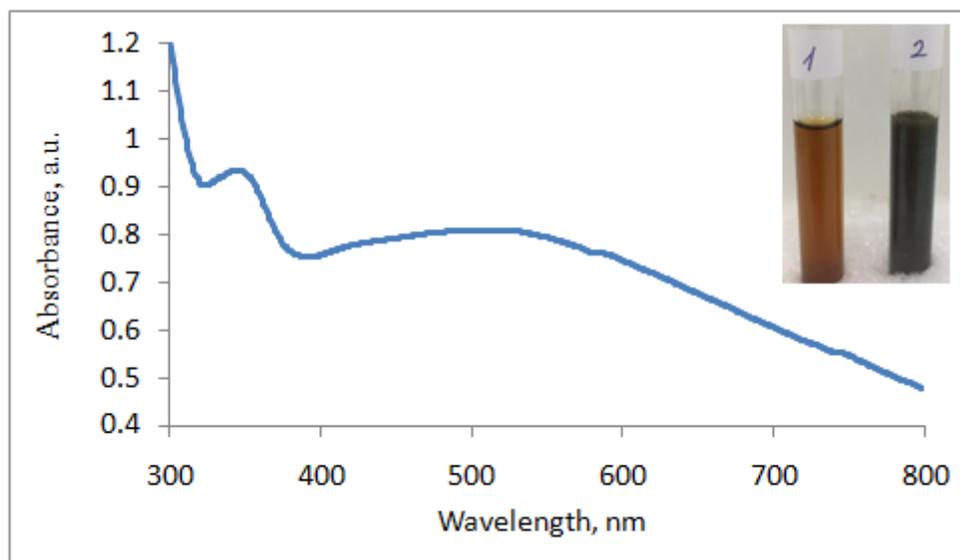


Figure 1

Aqueous solutions of plant extract (1) and AgNPs (2) and UV-Vis absorption

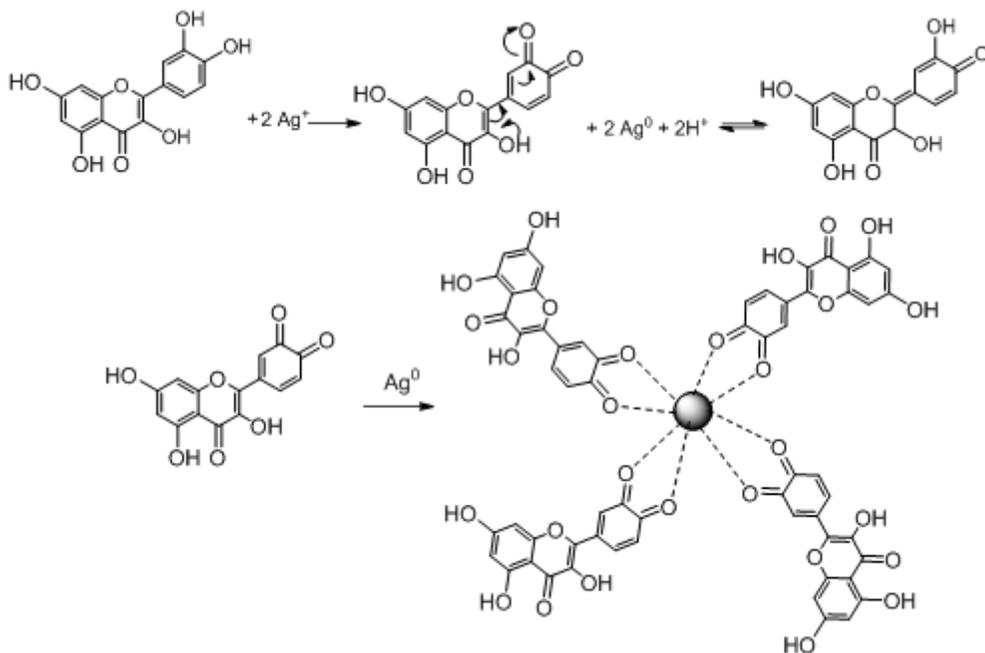


Figure 2

Plausible reaction mechanism of synthesis of Sa-AgNPs.

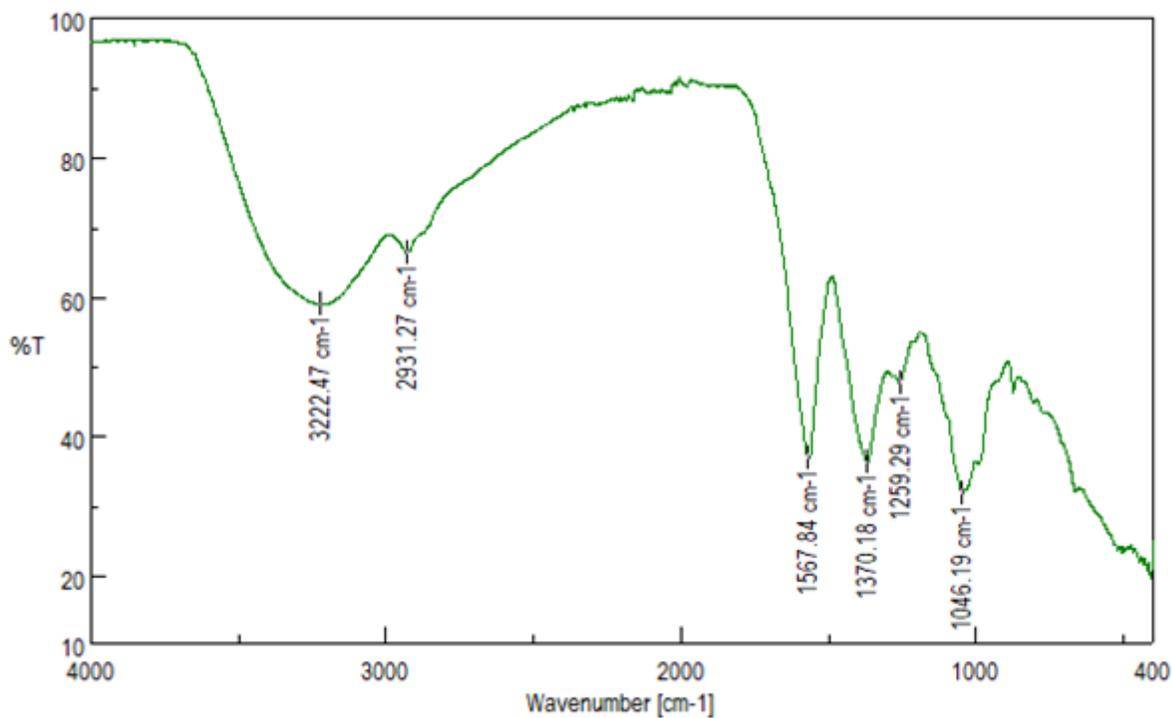


Figure 3

FTIR spectrum of water extract of *S. aethiopsis*

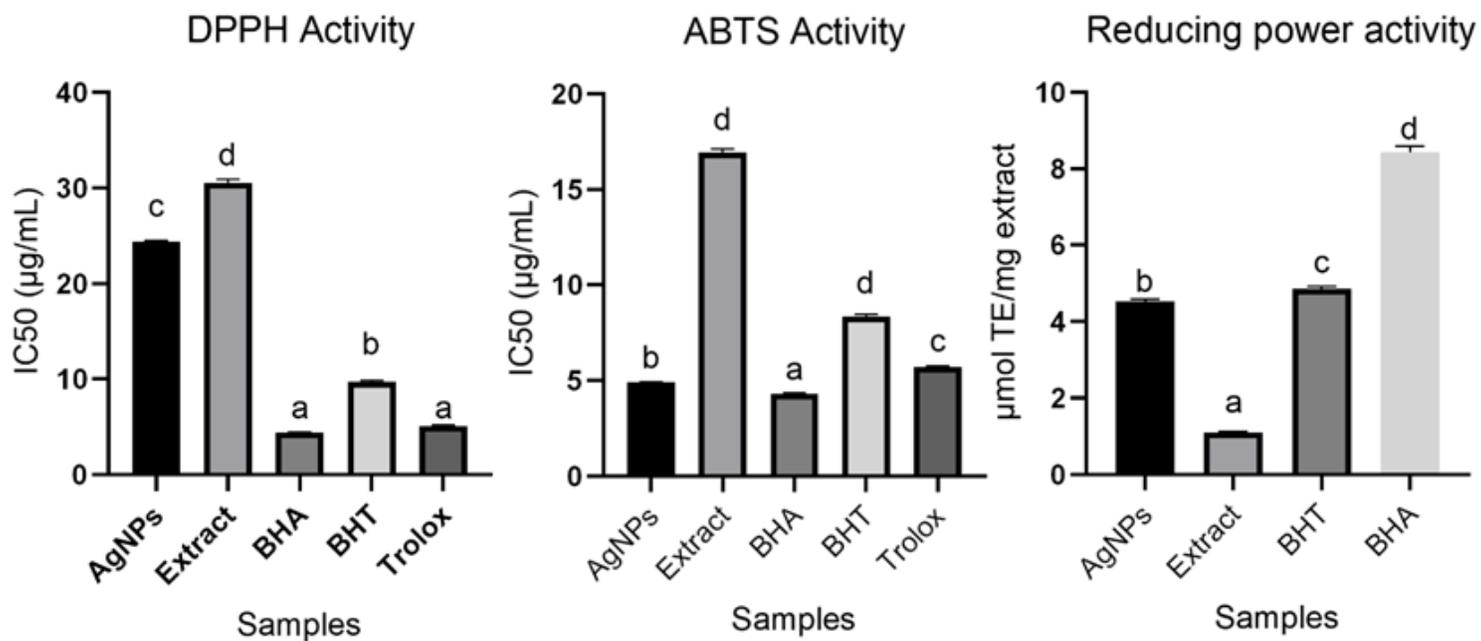


Figure 6

Antioxidant activity of Sa-AgNPs and extract. The results were reported as mean values \pm SDs of three independent assays ($P < 0.05$). Values followed by the same letter are not significantly different.