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Green synthesis of silver nanoparticles using *Sorghum bicolor* var. *technicum* seed extract, their characterisation and investigation of biological activities

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ABSTRACT

Synthesis of nanoparticles can be long and costly processes using physical and chemical methods. Biological synthesis of nanoparticles is known to be cheaper and easier than other methods. In this study, silver nanoparticles (AgNP) were obtained by biological synthesis, also known as green synthesis, using *Sorghum bicolor* var. *technicum* (Körn) Stapf ex Holland seed extract, popularly known as sorghum. AgNPs were characterised by SEM, EDS, TEM, FT-IR, and UV-Vis Spectroscopy. SEM images confirmed that the shape of AgNPs was spherical. TEM analysis showed that the average sizes of AgNPs ranged from 51 to 56 nm. EDS analysis confirmed the presence of AgNPs by detecting a silver ion peak at 3 KeV. UV-Vis spectroscopy analyses indicated that the brown-burgundy colour of AgNPs exhibited maximum absorbance at 450 nm. The biological activities of the extract and AgNPs were investigated through antimicrobial, antibiofilm, antioxidant, mutagenic, and DNA cleavage activity analyses. The extract exhibited the highest MIC value against Gram-positive bacterium *Bacillus subtilis* (0.62 μg mL^{-1}), whereas AgNPs demonstrated the highest antimicrobial activity specifically against Gram-negative bacterium *Escherichia coli* (0.31 μg mL^{-1}). The antibiofilm results revealed that the extract displayed the highest percentage of biofilm inhibition against *B. subtilis*, while AgNPs exhibited notable efficacy against both *Candida*

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albicans yeast and *Pseudomonas aeruginosa* bacterium. The antioxidant activities were evaluated using $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ methods, and it was determined that both samples had high antioxidant activity. Mutagenicity of the extract and AgNPs were evaluated by the Ames/*Salmonella* test using two strains of *Salmonella typhimurium* (TA98 and TA100). The mutagenic activity of the extract increased depending on the concentration for both strains, while AgNP did not show mutagenicity at any concentration. The agarose gel electrophoresis method showed that the extract and AgNPs cleaved DNA in the presence of an oxidising agent.

KEYWORDS

silver nanoparticles, green synthesis, *Sorghum bicolor* var. *technicum*, characterisation, biological activity

1. INTRODUCTION

During recent years, interest in metallic nanoparticles (NPs) has been increasing rapidly. NPs have unique magnetic, physicochemical, and optical properties with high surface area-to-volume ratios and extremely small sizes compared to other materials (Patil and Kim, 2017). Nano-sized materials have significant biological activities compared to other materials. NPs can be produced with physical, chemical, and biological methods. Physical and chemical synthesis of NPs involve disadvantages such as the cost of the chemicals used for synthesis, low production speed, and toxic effects of reducing agents and solvents against nature and organisms. Biological synthesis, also called green synthesis, is considered the best alternative when compared to other methods. One of the reasons for the increasing demand for green synthesis is the need for environmentally non-toxic synthetic protocols (Ai et al., 2011). There is an increasing interest in the synthesis of NPs that do not produce toxic by-products, with biological methods that minimise the use of environmentally harmful chemicals. NP synthesis with the green synthesis method is cost-effective and environmentally friendly. It is preferred more than physical and chemical methods as there is no need to use high temperature, pressure, toxic chemicals, and energy (Yaqoob et al., 2020; Yassin et al., 2022).

Environmentally friendly bioreduction of metal ions is possible with a combination of biomolecules using biological materials such as microorganisms, enzymes, and plant extracts (Narayanan and Sakthyel, 2010). Plants, which play an important role in the ecosystem with their productivity, also have many advantages for biological synthesis. They are easy to obtain, affordable, repeatable, reproducible plant extracts contain reducing and coating agents (Iravani, 2011; Shreyash et al., 2021).

While metallic NPs can be used in various applications, interest in the antimicrobial and cytotoxic effects of metallic NPs synthesised by green synthesis method with biological applications has increased considerably (Patil and Kim, 2017). Among metallic NPs, silver NPs are of great interest in research with wide applications in chemistry, pharmacology, and parasitology. In particular, silver NPs have come to the fore with unique properties that can be included in biosensor materials, antimicrobial applications, and catalytic applications (Azizi et al., 2017). Plant secondary metabolites, which can combine with nano-sized particles, can change the activities. Extracts from different plants are generally economical, environmentally friendly materials, and were investigated in the synthesis of silver NPs (Bhardwaj et al., 2020).

Oxygen-derived free radicals are reactive oxygen species (ROS), and an increase in production can cause protein denaturation, lipid peroxidation in cell membranes, and DNA damage. In addition, excessive ROS accumulation can lead to cell death and oxidative damage. Protection against diseases that can be caused by free radicals can be increased by the abundant intake of antioxidants in the diet. Due to this concern, intense interest is shifting to natural antioxidants that can be obtained from various natural materials. DNA damage can cause cancer, aging, mutation, and cell death. Hydrolytic cleavage occurs in the phosphodiester bond, while oxidative cleavage occurs in nucleobases or sugars of the DNA. Oxidative DNA cleavage begins with the formation of ROS. Free radicals, which are derivatives of ROS, cleave hydrogens in sugar and initiate DNA cleavage (Russo et al., 2001).

Permanent changes in the hereditary material of the cell that occur due to various factors or spontaneously are called mutations. Mutagens are the physical or chemical agents that cause mutagenicity. Spontaneous mutations are naturally occurring mutations that can be seen in all cells.

Sorghum (*Sorghum bicolor* L. Moench) is an important grain belonging to the Poaceae family. Condensed tannins are common in sorghum. With these compounds, sorghum has higher antioxidant levels than any other grains (Chung et al., 2011). Its basic components include starch, fat, protein, as well as B vitamins and fat-soluble vitamins (Przybylska-Balcerek et al., 2019). In Africa and India, it is used as an important source of protein, energy, minerals, and vitamins in food consumption of the majority of the population (Iyabo et al., 2018). Sorghum seeds have higher lipid levels than most grains. When compared to corn oil, sorghum seed oil contains higher levels of oleic and stearic acids, while exhibiting lower amounts of linoleic, myristic, and palmitoleic acids. This composition indicates a lower content of saturated fat in sorghum seed oil (Althwab et al., 2015). Sorghum seeds are known to possess antimicrobial, anti-cancer and anti-inflammatory activities (Rao et al., 2018).

S. bicolor var. *technicum* (Körn) Stapf ex Holland, also known as sorghum, used in the study is cultivated in Turkey, especially in the Western region. Silver nanoparticle formation has been reported from *S. bicolor* (Gilaki, 2010; Sreedevi et al., 2020), however there is no such studies about *S. bicolor* var *technicum*. In this study, we aimed to determine the effects of both the aqueous extract of this plant and silver NPs (AgNPs) synthesised from this extract with green synthesis method.

2. MATERIALS AND METHODS

2.1. Materials

Silver nitrate ($AgNO_3$), Mueller–Hinton Agar (MHA), Mueller–Hinton Broth (MHB), Tryptic Soy Broth (TSB), sodium azide, and tris(hydroxymethyl)aminomethane were purchased from Merck. 1,1-diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$), L-histidine, biotine, ampicillin trihydrate, 4-nitro-*o*-phenylenediamine, agarose, ethylenediaminetetraacetic acid (EDTA), and hydrogen peroxide were obtained from Sigma-Aldrich. pBR322 DNA and GeneRuler 1 kb DNA Ladder were purchased from Thermo Scientific. All other reagents were of analytical grade.

2.2. Preparation of seed extract and synthesis of AgNPs

Sorghum seeds were collected from Uzunköprü, Edirne, Turkiye. 5 g of powdered seed sample was mixed with 100 mL distilled water. The extract was obtained by continuous mixing at 70–80 °C for 1 h. The resulting extract was cooled and passed through Whatman No 1 filter paper. 1 mM silver nitrate ($AgNO_3$) and the extract were mixed in a ratio of 9:1. The colour change from yellow to brown indicated the formation of AgNPs. After the colour change, the mixture was centrifuged (Hettich Universal 320) at 7.000 r.p.m. for 1 h. Obtained pellet was centrifuged to be washed 3 times with water. The washed pellet was dried in an oven (Wisd WiseVen) at 60 °C for 2 days.

2.3. Characterisation of AgNPs

The morphology, size and chemical analysis of the synthesised AgNPs were examined using Scanning Electron Microscopy (SEM, Quanta FEG 250, FEI, USA), Transmission Electron Microscopy (TEM, Hitachi HT-7700), and Energy Dispersive Spectroscopy (EDS). The hydrodynamic size, polydispersity index (PDI), and zeta potential of the synthesised AgNP were investigated using the Dynamic Light Scattering (DLS) method with the Zeta Sizer Nano ZS instrument (Malvern Panalytical, Germany). Fourier Transform Infrared Spectroscopy (FT-IR) analysis was conducted to identify the reducing agent used in the synthesis of AgNPs. The FT-IR (KBr disk) spectra of the extract and AgNPs were recorded with a Perkin Elmer BX II FT spectrometer at a range of 4,000–400 cm^{-1} and resolution of 1 cm^{-1} with 16 scan. The biosynthesis of AgNPs in solution was monitored by measuring the UV-Vis spectra of the reaction mixture. Periodic scans of optical absorbance between 200 and 800 nm were performed with a dual-beam spectrophotometer (T80+ UV/VIS Spectrometer, PG Instruments Ltd).

2.4. Antimicrobial activity assay

Antimicrobial activities of the extract and AgNPs were investigated with the broth microdilution method. Minimum Inhibitory Concentration (MIC) values were evaluated for different microorganisms (*Staphylococcus haemolyticus* ATCC 43252, *Staphylococcus aureus* ATCC 6538P, *Bacillus subtilis* ATCC 6633, *Acinetobacter baumannii* ATCC 19606, *Escherichia coli* NRRL B-3704, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 13315, and *Candida albicans* ATCC 10231). The MIC values were identified as the lowest concentration that showed no visible growth (CLSI, 2006). All experiments were performed in triplicate.

2.5. Antibiofilm activity

Antibiofilm activities of the samples were studied using the microplate method (Merritt et al., 2005). A suitable environment for the growth of microorganisms was provided by incubation in Tryptic Soy Broth (TSB) medium. After incubation, planktonic cells were removed, and then 0.1% crystal violet was added to each well and left for 15 min. Repeatable washing was done after the dye was removed. Absorbance values were measured on a microplate reader at 620 nm.

2.6. Determination of antioxidant activities

2.6.1. DPPH radical scavenging activity. The free radical scavenging activities of the extract and AgNP were determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method with slight

modification (Blois, 1958). Synthetic antioxidant butylated hydroxytoluene (BHT) was used as the positive control. The inhibition % of the samples was calculated using the equation:

$$\text{DPPH Scavenging Activity Inhibition (\%)} : [(A_0 - A_1)/A_0] \times 100$$

A_0: The absorbance value of control; A_1: The absorbance value of sample

2.6.2. $ABTS^{\bullet+}$ radical cation scavenging activity. The 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) radical cation scavenging activity assay was determined according to Re et al. (1999) with minor modifications. The inhibition was calculated for the obtained values, and these results were evaluated by comparing them with the positive control (BHT).

$$ABTS^{\bullet+} \text{ Radical Cation Scavenging Activity Inhibition (\%)} : [(A_0 - A_1)/A_0] \times 100$$

A_0: $ABTS^{\bullet+}$ radical absorbance value of control, A_1: $ABTS^{\bullet+}$ radical absorbance value of sample.

2.7. Determination of mutagenicity

Ames/*Salmonella* test was used to determine mutagenic activity. In the test, the mutants were obtained as a result of *in vitro* mutations from *Salmonella typhimurium* LT2 ancestral strain with different types of mutations in various genes involved in the histidine operon. In the study TA98 and TA100 strains of *S. typhimurium* were used to determine the frameshift and base pair change mutations, respectively (Maron and Ames, 1983; Mortelmans and Zeiger, 2000). Experiments were carried out using the plate incorporation method developed by Maron and Ames (1983). All experiments in the research were carried out in triplicate.

The positive, negative, and spontaneous controls were applied in parallel to the studies. 4-nitro-*o*-phenylenediamine (NPD) and sodium azide (SA) were used as positive controls for *S. typhimurium* TA98 and TA100 strains, respectively; dH_2O was used as negative control for both strains. Data were collected as mean ± standart deviation of three plates ($n = 3$).

2.8. DNA cleavage activity

The pBR322 plasmid DNA was used to detect the DNA cleavage activities of the extract and AgNPs using agarose gel electrophoresis. Samples were prepared at different concentrations and mixed with DNA in Tris-HCl buffer (pH: 7.4). Afterwards, it was loaded into the agarose gel by mixing with 6X DNA loading dye. Obtained bands were photographed with a gel imaging system under UV light (DNR, Bio-Imaging system).

3. RESULTS AND DISCUSSION

3.1. Synthesis of AgNPs

When a clear $AgNO_3$ solution combines with an extract, the colour of the extract turns dark burgundy-brown, and this colour change indicates that the aqueous Ag ions are bioreduced to form highly stable AgNPs. Bioreduction is initiated with phytochemicals in extracts, and

phenolic compounds, one type of phytochemicals, act as strong reducing agents by donating electrons to Ag^{+} ions. The colour changes when Ag is reduced to form NPs (Ponarulselvam et al., 2012; Kalaiselvi et al., 2015) (Fig. 1).

3.2. Characterisation of AgNPs

Since the homogeneity of NPs is important for their application, they are generally characterised by their shape, size and surface area (Kumar et al., 2014). SEM and TEM studies confirmed the development of NPs with almost the same spherical shapes, and dimensions particle size ranged between 51 and 56 nm (Fig. 2A and C). The size and shape of the AgNPs depend on the extracts used for synthesis. In EDS analysis, silver ions produce strong signals at approximately 2.983 KeV, which was used as evidence of the formation of silver nanoparticles (Kumar et al., 2014). AgNPs typically showed an absorption peak at 3 KeV (Fig. 2B). Enzymes or proteins in the water extract of *S. bicolor* var. *technicum* can be cited as the possible cause of other weak element signals.

The synthesis of AgNPs was monitored using a UV-Vis spectrophotometer. The absorbance peak increased from 400 to 500 nm, and broadened in width due to the increase in particle size from 10 to 100 nm (Paramelle et al., 2014). The maximum absorbance peak for AgNPs was determined at 450 nm (Fig. 2D).

DLS method determined the hydrodynamic size as 89.91 ± 2.53 nm, PDI as 0.264, and zeta potential value of −25.7 mV (Fig. 2E and F). The significant negative zeta potential of the AgNPs indicates their strong stability. The observed difference in size between the TEM and DLS analysis may be attributed to the hydrodynamic diameter measured by the DLS method.

When the FT-IR spectrum of the extract is examined, the wide peak observed at 3,426 cm^{-1} showed that the polyphenols, cyanidins, flavonoids, and phenolic acids in the extract have characteristic OH, COOH, and aromatic-H vibrations. The peaks observed at 2,929 cm^{-1} belong to the aliphatic-H vibrations in the extract (Fig. 3).

3.3. Antimicrobial activity

The antimicrobial effects of the extracts and AgNPs were using the broth microdilution method against bacteria and *C. albicans* yeast. Streptomycin and nystatin were used as positive controls

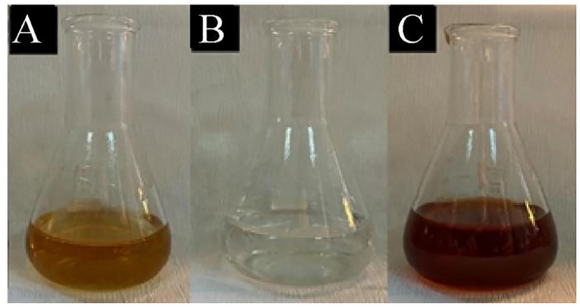

Fig. 1. A) Sorghum seed extract, B) $AgNO_3$, C) Synthesised silver nanoparticles (AgNP)

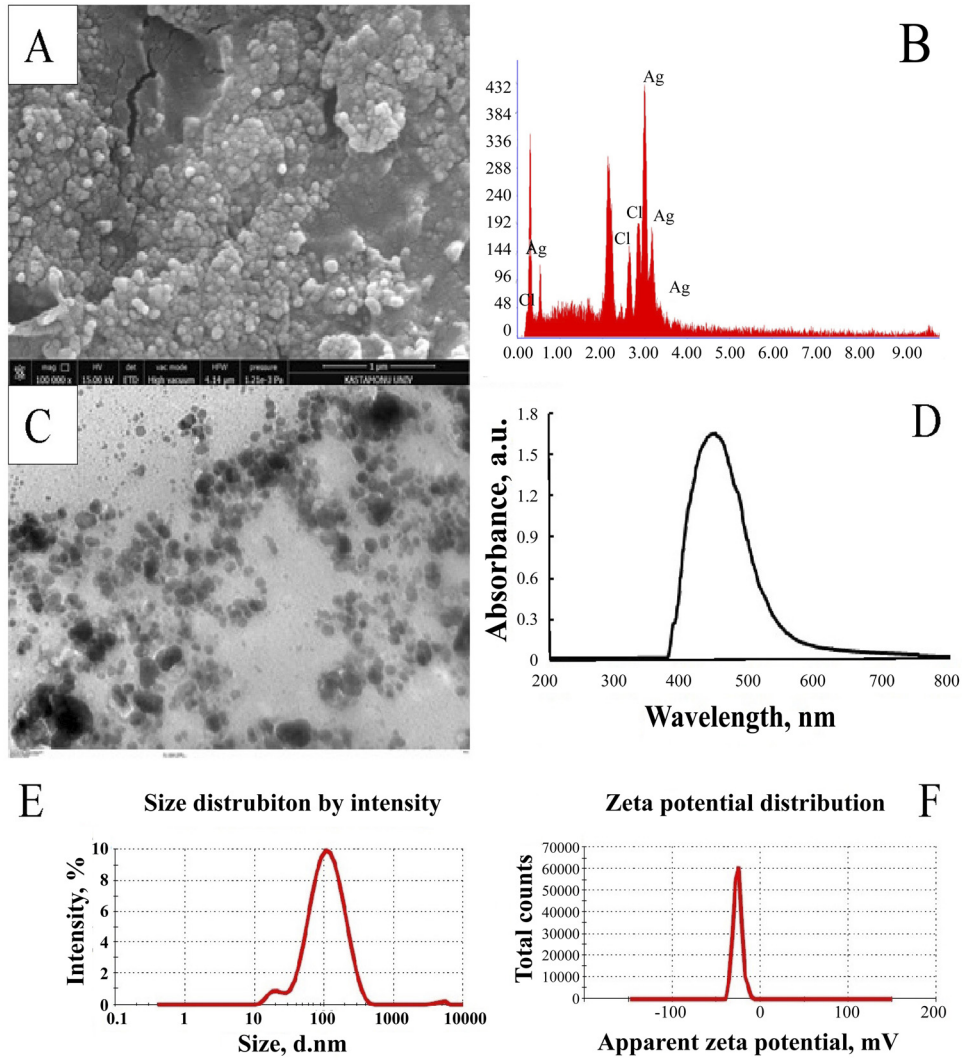

Fig. 2. Characterisation of AgNPs. A: SEM image of AgNP; B: EDS analysis; C: TEM image of AgNPs; D: UV-Vis spectrum; E: Size distribution analysis; F: Zeta potential distribution analysis of synthesised AgNPs

for bacteria and yeasts, respectively. The results showed that the highest MIC value for the extract was found for Gram positive bacterium *B. subtilis* (0.62 μg mL^{-1}), while the highest MIC for AgNP was against the Gram negative bacterium *E. coli* (0.31 μg mL^{-1}) (Table 1). Tippayawat et al. (2016) investigated the bactericidal effects of silver nanoparticles synthesised with *Aloe vera* against pathogenic Gram positive *Salmonella epidermis* and Gram negative

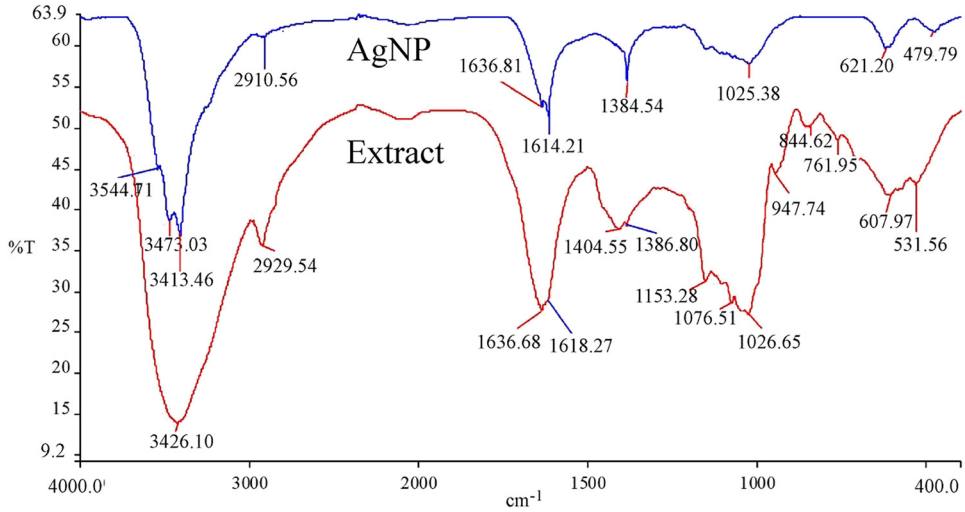

Fig. 3. FT-IR spectra for extract and AgNP

Table 1. Minimum Inhibitory Concentration (MIC, μg mL^{-1}) values of sorghum seed extract and silver nanoparticles (AgNPs)

Microorganisms	Extract	AgNP	Streptomycin	Nystatin
S. haemolyticus (ATCC 43252)	1.25	2.50	5.00	–
S. aureus (ATCC 6538P)	2.50	2.50	5.00	–
B. subtilis (ATCC 6633)	0.62	2.50	5.00	–
A. baumannii (ATCC 19606)	1.25	2.50	2.50	–
E. coli (NRRL B-3704)	2.50	0.31	5.00	–
P. aeruginosa (ATCC 27853)	2.50	1.25	5.00	–
P. vulgaris (ATCC 13315)	2.50	2.50	5.00	–
C. albicans (ATCC 10231)	2.50	1.25	–	2.50

bacterium *P. aeruginosa*. They reported high activity against Gram negative bacteria at much lower concentrations but high activity against Gram positive bacteria at high concentrations.

3.4. Antibiofilm activity

In the study, the antibiofilm activities of the extract and AgNPs were investigated against Gram positive and Gram negative bacteria and *C. albicans* yeast. The highest biofilm inhibition percentage was 64.12 ± 0.80 with the extract against *B. subtilis*. For AgNP, highest inhibition was obtained against *C. albicans* yeast with a percentage of 53.87 ± 0.15 and against *P. aeruginosa* with a percentage of 53.34 ± 0.32 (Table 2). Previous studies have reported that green-synthesised silver nanoparticles have strong antibiofilm activity against microorganisms with primary biofilm formation such as *S. aureus* and *P. aeruginosa* (Ramalingam et al., 2014).

Table 2. Antibiofilm activity of sorghum seed extract and silver nanoparticles (AgNPs)

Microorganisms	Extract (μg mL^{-1})	AgNPs (μg mL^{-1})
S. haemolyticus (ATCC 43252)	42.12 ± 0.10	32.18 ± 0.54
S. aureus (ATCC 6538P)	36.12 ± 0.10	38.72 ± 0.15
B. subtilis (ATCC 6633)	64.12 ± 0.80	45.45 ± 0.61
A. baumanii (ATCC 19606)	46.43 ± 0.20	44.12 ± 0.26
E. coli (NRRL B-3704)	42.75 ± 0.22	48.12 ± 0.42
P. aeruginosa (ATCC 27853)	30.78 ± 1.20	53.34 ± 0.32
P. vulgaris (ATCC 13315)	38.12 ± 1.12	41.03 ± 0.16
C. albicans (ATCC 10231)	27.12 ± 0.15	53.87 ± 0.15

3.5. Antioxidant activities

3.5.1. DPPH radical scavenging activity. The basis of DPPH analysis is the transfer of a hydrogen atom to DPPH. When an antioxidant sample is mixed with DPPH solution, the solution reduces and absorbance decreases; hence, the purple-coloured DPPH turns yellow. AgNPs had higher antioxidant activity than the extract. A concentration-dependent increase was observed in both samples (Fig. 4A). The inhibition value calculated at the highest concentration of 800 μg mL^{-1} was 52.89 ± 0.84% and 61.28 ± 3.26% for the extract and AgNP, respectively. Wang et al. (2021) investigated the antioxidant activity of *Zingiber officinale* leaf water extracts and silver nanoparticles synthesised by green synthesis using the DPPH method. In their results, the IC_{50} values of *Z. officinale* extract, BHT and silver nanoparticles were 275, 203, and 172 μg mL^{-1}, respectively. They stated that both the extract and AgNPs may be caused by the conversion of electrons and hydrogen ions to DPPH radical and the formation of a controlled complex by the connection of silver nanoparticles with DPPH.

3.5.2. ABTS$^{\bullet+}$ radical cation scavenging activity. Since the evaluation of a single antioxidant property makes it difficult to understand the antioxidant capacity of the samples, the antioxidant activities of extract and AgNPs were also investigated with the ABTS$^{\bullet+}$ radical cation scavenging

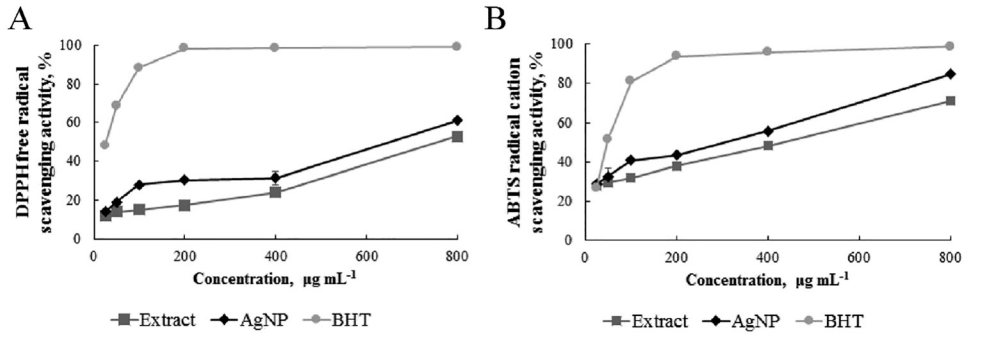

Fig. 4. Antioxidant activites of extract and AgNP. A: DPPH free radical scavenging activity, B: ABTS radical cation scavenging activity

activity method. AgNPs had significant ABTS radical cation scavenging activity compared to the extract. At the highest concentration of 800 μg mL^{-1}, the inhibition rates for the extract and AgNP were calculated as 71.30 ± 0.60% and 84.72 ± 0.65%, respectively (Fig. 4B). Fafal et al. (2017) synthesised silver nanoparticle with *Asphodelus aestivus* extract, and they observed 39.62 ± 0.02% and 79.94 ± 0.02% inhibition for the extract and silver nanoparticles, respectively. They stated that silver nanoparticles have stronger $ABTS^{\bullet+}$ cation removal activity than the extract.

3.6. Ames/*Salmonella* mutagenicity test

In order to determine whether the extract and AgNPs used in the study were with mutagenic activity, the number of histidine revertant colonies was compared with the negative control group. The sample which increased the number of colonies observed in the negative control by 2-fold was considered mutagenic (Mortelmans and Zeiger, 2000). The extract had mutagenic activity for both strains from 50 μg mL^{-1}, while AgNPs did not have mutagenic activity for either strains (Table 3). De Cássia Proença-Assunção et al. (2021) investigated the mutagenic activities of AgNPs synthesised with curcumin against *S. typhimurium* TA97a, TA98, TA100, and TA102 strains with the Ames/*Salmonella* test. They determined that silver nanoparticles synthesised with curcumin did not show mutagenic activity against any strains tested. In our study, we concluded that AgNPs did not show mutagenic activity. This finding reveals the possible antimutagenic effects of AgNPs with certain physicochemical properties that should be considered for future studies on certain mutagenic agents.

3.7. DNA cleavage activity

Oxygen free radicals can cause damage to genomic DNA. Therefore, studies on DNA cleavage activity by synthetic materials such as NPs have been of great interest to researchers. DNA

Table 3. Mutagenic activity of sorghum seed extract and silver nanoparticles (AgNPs)

Treatment		Concentration (μg mL^{-1})	His^+ Revertant Colony Count/Plate	
			TA98 Mean ± SD	TA100 Mean ± SD
PC	NPD		800.00 ± 17.35	
	SA			1,232.67 ± 62.07
Extract		5	16.67 ± 1.53	52.00 ± 3.61
		25	71.33 ± 12.34	111.33 ± 31.94
		50	242.67 ± 28.36	307.67 ± 14.22
		100	404.33 ± 17.01	528.33 ± 45.62
AgNP		5	13.67 ± 1.53	59.33 ± 3.51
		25	12.33 ± 1.53	46.00 ± 9.64
		50	19.00 ± 5.00	69.67 ± 14.29
		100	18.33 ± 3.06	66.67 ± 5.13
NC		dH_2O	44.00 ± 6.24	109.33 ± 13.32
SC			28.33 ± 2.31	85.33 ± 11.37

PC: Positive control; NC: Negative control; SC: Spontaneous control; NPD: 4-nitro-*o*-phenylenediamine (10^{-2} μg/plate); SA: Sodium azide (10^{-3} μg/plate).

Fig. 5. DNA cleavage activity of extract (A) and AgNP (B). 1–6: Hydrolytic cleavage M: Marker; lane 1. DNA; lane 2. DNA + 50 μg mL^{-1}; lane 3. DNA + 100 μg mL^{-1}; lane 4. DNA + 200 μg mL^{-1}; lane 5. DNA + 400 μg mL^{-1}; lane 6. DNA + 800 μg mL^{-1}. 7–12 Oxidative cleavage M: Marker; lane 7. DNA + H_2O_2; lane 8. DNA + 50 μg mL^{-1}+ H_2O_2; lane 9. DNA + 100 μg mL^{-1}+H_2O_2; lane 10. DNA + 200 μg mL^{-1}+ H_2O_2; lane 11. DNA + 400 μg mL^{-1} + H_2O_2; lane 12. DNA + 800 μg mL^{-1} + H_2O_2

cleavage activities of the extract and AgNP were investigated using the agarose gel electrophoresis method. Both samples cleaved DNA in the presence of H_2O_2, which was used as an oxidising agent (Fig. 5A and B). Parvanak Boroujeni et al. (2018) synthesised copper-silver-nickel biometallic nanoparticles and examined the interactions of nanoparticles with DNA by agarose gel electrophoresis. As a result of their study with pET-28 plasmid DNA, they stated that the nanoparticles did not cause cleavage activity on DNA.

4. CONCLUSIONS

Metallic nanoparticles are getting more and more attention every day. One of the reasons for this is that they can be used in various fields and have important effects when synthesised for biological applications. Green synthesis is becoming increasingly important because biological synthesis of nanoparticles is environmentally friendly and does not require toxic chemicals. In the study, we report that when AgNPs were synthesised with seed extract, their biological activities generally increased significantly. We predict that in the future it will be possible to use nanomaterials in the medical industry together with *in vivo* and *in vitro* tests.

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