

Original Article : Open Access

Phytomediated fabrication of silver nanoparticles using *Syzygium cumini* L. aqueous leaf extract and evaluation of their antioxidant potential

Ritu Devi, Sushila Singh[♦], Seema Sangwan^{*}, Monika Moond, Simran Kakkar, Rajni Kant Sharma and Sandeep Kumar^{**}

Department of Chemistry, CCS Haryana Agricultural University, Hisar-125004, Haryana, India

^{*}Department of Microbiology, CCS Haryana Agricultural University, Hisar-125004, Haryana, India.^{**}PGT Chemistry, GMSSSS, Kuleri, Hisar-125001, Haryana, India.

Article Info

Article history

Received 26 January 2024

Revised 11 March 2024

Accepted 12 March 2024

Published Online 30 June 2024

Keywords

Phytochemicals

Stabilizing agent

Nanoparticles

Antioxidant activity

Biomedical applications

Abstract

Plant leaf extracts are a rich source of phytochemicals like polyphenols, flavonoids, and sugars that will serve as reducing, capping, and stabilising agents while reducing silver ions to silver, *Syzygium cumini* L. aqueous leaf extract was used to create silver nanoparticles (AgNPs). These phytochemicals were quantified in an aqueous leaf extract, and their potential to mediate AgNP biosynthesis was then assessed. Using UV-visible spectroscopy, a particle size analyzer (PSA), FTIR (fourier transform infrared spectroscopy), FESEM (field emission scanning electron microscopy), and HRTEM (high-resolution transmission electron microscopy), the optical, structural, and morphological characteristics of biosynthesized AgNPs were assessed. HRTEM analysis revealed the formation of AgNPs with a spherical shape and an average diameter of 15 nm. Using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, antioxidant potential was evaluated, biosynthesized AgNPs demonstrated increased antioxidant efficacy with $IC_{50} = 17.02 \mu\text{g/ml}$ compared to leaf extract with $IC_{50} = 21.09 \mu\text{g/ml}$. With the phosphomolybdenum assay, the AgNPs (74.26 mg AAE/g) showed higher total antioxidant capacity than the aqueous leaf extract (53.61 mg AAE/g) at a concentration of 100 $\mu\text{g/ml}$. Based on current research, AgNPs may prove as a potent antioxidant agent which enhances their biomedical applications in future.

1. Introduction

Globally, the research and development in the field of nanotechnology is expanding quickly (Moond *et al.*, 2022; Moond *et al.*, 2023). The primary outcome of this movement is the development of novel nanoscale products, such as nanoparticles. Synthesizing and effectively using nanoparticles of various sizes and shapes is known as nanotechnology (Marutikesavakumar *et al.*, 2014; Moond *et al.*, 2023). Special properties resulting from reducing the size and morphology of nanoparticles (size 1-100 nm) are the basic components of any nanomaterials. Because they fill the gap between bulk materials and atomic or molecular structures, they are extremely important to science (Thakore *et al.*, 2014; Dalal *et al.*, 2022). Particularly, due to their extensive applications in a variety of fields, silver nanoparticles have attracted a great deal of research attention. Presently, different chemical, physical and biological methods are developed to synthesize different nanoparticles (Liu *et al.*, 2011). Chemical methods involve chemical, photochemical reduction and sonochemical procedures (Sharma *et al.*, 2009). Three primary ingredients are needed for the chemical synthesis of AgNPs: silver salt (such as AgNO_3), a reducing agent (such as sodium citrate), and a capping or protecting agent (such as polyethylene glycol) in order to regulate the size of the produced nanoparticles and prevent their agglomeration. However, these traditional techniques are linked to

the use of excessively powerful hardware, high energy costs, extremely hazardous and poisonous chemical components that cause natural hazards, and are generally not environmentally friendly (Natsuki *et al.*, 2015).

In order to meet the demand for nanomaterials free of toxic contaminants, most researchers have chosen green synthesis methods. A lot of research has been done on the production of AgNPs using bacteria, fungi, and plant extracts (Vigneshwaran *et al.*, 2007). Plant extract-mediated synthesis of metallic nanoparticles is thought to be more suitable for large-scale production than microbial-based nanosynthesis because of its ease of handling and flexibility in reaction conditions.

In the field of nanotechnology, plant's capacity for bioassisted synthesis of metal nanoparticles (green synthesis) now plays a significant role. As the phytochemicals in plant extracts function as catalysts and stabilizers for the synthesis of nanoparticles without the need for additional stabilizing or capping agents, the biosynthesis of nanoparticles using plant products is economical, effective, and environmentally benign. In addition to forming a layer of nanoparticles, the amide and hydroxyl groups of proteins serve as a capping agent to keep the reaction medium stable and prevent agglomeration. The plant system's flavonoids and proteins act as reducing and stabilising agents during the synthesis of silver nanoparticles (Yugandhar *et al.*, 2015). AgNPs of various sizes and shapes are created using a variety of plant materials. For current nanotechnology however, there are still issues with developing suitable nanomaterial synthesis as well as complete control over size and monodispersity. The need to create clean, non-toxic,

Corresponding author: Dr. Sushila Singh

Assistant Professor, Department of Chemistry, CCS Haryana Agricultural University, Hisar-125004, Haryana, India

E-mail: singhsushila999@gmail.com

Tel.: +91-8199939339

Copyright © 2024Ukaaz Publications. All rights reserved.

Email: ukaaz@yahoo.com; Website: www.ukaazpublications.com

biocompatible, and environmentally friendly methods for synthesising nanoparticles is growing.

AgNPs, one type of metal nanoparticle, have drawn a lot of attention due to their diverse biological activities, including antioxidant, anticancer, catalytic, and antimicrobial properties (Kumara Swamy *et al.*, 2015; Vasanth *et al.*, 2014; Kohler *et al.*, 2008; Marutikesavakumar *et al.*, 2014). The primary process for the synthesis of silver nanoparticles by plants is the phytochemical-induced reduction of Ag^+ to Ag^0 . The phytochemicals mainly involved in reduction are terpenoids, flavones, carboxylic acids, ketones, aldehydes and amides. Various water soluble phytochemicals such as flavones and quinones are responsible for the instant reduction of silver ions and stabilization of synthesized nanoparticles (Park *et al.*, 2011). *Syzygium cumini* L. also known as *Syzygium jambolanum* and *Eugenia cumini* belongs to family Myrtaceae. This plant popularly known as “Jamun” is a traditional medicinal herb native to India. Besides India, it is also distributed in Eastern Africa and South-East Asia. (Modi *et al.*, 2010). *S. cumini* is an important indigenous commercial herb having a wide range of medicinal activities such as antibacterial, anti-inflammatory, antioxidant, anticancerous, antifungal, cardioprotective and hepatoprotective (Jain and Mehata, 2017). Its wide medicinal applications may be due to its ability to synthesize various phytochemicals (Aggarwal *et al.*, 2022; Devi *et al.*, 2023; Moond *et al.*, 2023). The plant possesses acetyl-oleanolic acid, ellagic acid, triterpenoids, quercetin, isoquercetin, myricetin and kaempferol in different concentrations (Rastogi and Mehrortra, 1990; Devi *et al.*, 2023; Moond *et al.*, 2023). Its leaves contain phenolic compounds such as catechin and ferulic acid, responsible for their antioxidant activity. The essential oils from Jamun leaves contains α -cadinol, geranyl acetone, α -pinene, α -terpineol, caryophyllene. The leaves of *S. cumini* are used in the treatment of various skin diseases.

In this work, we extracted the aqueous leaf extract of *S. cumini* and quantified its phytochemicals. Then, using aqueous leaf extract and optimal conditions, we observed the reduction of silver ions to enable simple and quick phytomediated synthesis of silver AgNPs. These biosynthesized AgNPs were then examined using FTIR, HRTEM, FESEM-EDX, PSA and UV-visible spectroscopy. Both the extract and the AgNPs sample's additional antioxidant activity was assessed using the phosphomolybdenum assay and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay.

2. Materials and Methods

2.1 Chemicals and collection of plant material

Himedia Private Limited provided the following chemicals: Copper sulphate, sodium hydroxide, sodium bicarbonate, sodium hydrogen arsenate, sodium phosphate, sodium carbonate, gallic acid, sodium sulphate, sodium potassium tartrate, Folin-Ciocalteu reagent, silver nitrate (AgNO_3), sodium nitrite, catechin, Conc. sulfuric acid, ammonium molybdate, aluminium chloride, phenol and 2,2-diphenyl-1-picrylhydrazyl (DPPH).

The leaves of *S. cumini* were collected from the campus of Chaudhary Charan Singh Haryana Agricultural University. Dr. Anita, Assistant Scientist, Department of Botany and Plant Physiology, CCS HAU, Hisar, India, verified the collected leaf samples using Tropicos and IPNI, an online platform.

2.2 Preparation of plant extract

The process of extraction plays a vital role in the recovery of bioactive compounds from plants. It can be done by using selective solvents or by leaving out the non-desired compounds (Dhanani *et al.*, 2017). *S. cumini* leaves were shade-dried at room temperature for this experiment and 5 g dried leaves and 50 ml of distilled water then placed in a flask and microwave-heated (Milestone, Start S Microwave, USA, 90 W) for 5 min before being allowed to cool. The cooled filtrate was collected and used to synthesize AgNPs.

2.3 Phytochemical analysis of *S. cumini* aqueous leaf extract

2.3.1 Total phenolic content

Using Folin-Ciocalteu method and gallic acid as the standard, the total phenolic content of the aqueous leaf extract was calculated. After adding 1 ml of the Folin-Ciocalteu reagent and 2 ml of Na_2CO_3 (20% w/v) to 1 ml of the extract, the final volume was adjusted to 10 ml using distilled water. After 10 min, this mixture was centrifuged for 12 min at 6000 rpm (Singleton and Rossi, 1965). The absorbance of the supernatant solution was measured at 730 nm against a blank using the UV-vis double beam spectrophotometer (Model UV 1900 Shimadzu). In a similar manner, the blank was made, but it contained the appropriate solvent in place of extract.

2.3.2 Total flavonoids

Using an aluminium chloride colorimetric assay and catechin as a standard, the total flavonoid content in aqueous leaf extract was ascertained (Marinova *et al.*, 2005). Add 4 ml of distilled water, 0.3 ml of 5% NaNO_2 , and 0.3 ml of 10% AlCl_3 to 1 ml of extract. Mix thoroughly and wait for 5 min. Add 2 ml of 1 M NaOH right away, and use distilled water to make the final volume reach 10 ml. Using a UV-vis double beam spectrophotometer (Model UV 1900 Shimadzu), the absorbance of the solution was measured at 510 nm against a blank after it had been thoroughly mixed. The blank was made in the same manner, but it has the matching solvent in place of the extract.

2.3.3 Total sugars

Using D-glucose as a standard and the Dubois method, the total sugar content of the leaf extract was ascertained (Dubois *et al.*, 1956). Add 2.0 ml of phenol solution and 5.0 ml of concentrated H_2SO_4 to the reaction mixture for every 1 ml of aqueous leaf extract. The solution was allowed to cool for 30 min after this procedure. Using a UV-vis double beam spectrophotometer, the absorbance of the reaction mixture was measured at 490 nm in comparison to a blank that was prepared identically but substituted the appropriate solvent for the plant extract.

2.3.4 Reducing sugars

Using D-glucose as a standard and the Nelson method, reducing sugars in leaf extract were ascertained (Nelson *et al.*, 1944). Add 1 ml of alkaline copper reagent to 1 ml of leaf extract. The solution was thoroughly mixed, covered with aluminium foil, and heated in a hot water bath for 20 to 25 min. It was then allowed to cool to room temperature. Following cooling of the solution, 1 ml of arsenomolybdate reagent was added, and 10 ml was the final volume of the reaction mixture after diluting it with distilled water. Using a UV-vis double beam spectrophotometer, the absorbance of the reaction mixture at 520 nm was measured against a blank made in a similar manner but with the appropriate solvent instead of extract.

2.3.4 Non-reducing sugars

The difference between total sugars and reducing sugars was used to calculate non-reducing sugars.

2.4 Biosynthesis of AgNPs

In the current experiment, 0.2 ml of an aqueous leaf extract solution and 20 ml of AgNO₃ solution (1 mM) were combined, and the mixture was then microwave-heated (Milestone, Start S Microwave, USA, 90 W) for 5 min. Without the use of an outside stabilising agent, the nanoparticles were synthesised. Initially, the colour shift in the flask holding the AgNO₃ solution and leaf extract was observed. Microwaving it turns the colourless solution into a light brown one. The typical sign that the AgNPs colloidal solution was formed is this change in colour. Centrifugation was used to extract AgNPs from the reaction mixture solution for 15 min at 10,000 rpm. The produced AgNPs were then dried at 37°C and put to use in additional research.

2.5 Characterization of AgNPs

A UV-vis double beam spectrophotometer (Model UV 1900, Shimadzu) was used to measure the UV-visible absorption spectrum of the biosynthesized AgNPs in the 350–550 nm wavelength range. The hydrodynamic size distributions, zeta potential, and polydispersity index (PDI) of nanoparticles were measured with the PSA Microtracnanotracer wave II apparatus. The surface morphology of biosynthesized AgNPs was examined using field emission scanning electron microscopy (JSM-7610FPlus) operating at an accelerating voltage of 0.1 to 30 kV. Using a JEM/2100 PLUS operating at 200 kV, HRTEM was successfully finished. For the HRTEM investigation, a drop of the biosynthesized AgNPs dissolved in ethanol was placed on a 400 mesh copper grid covered with a holey carbon film. The chemical composition of AgNPs and leaf extract was analysed using a Perkin Elmer FT-IR spectrophotometer.

2.6 Antioxidant activity

2.6.1 DPPH free radical scavenging activity

Using a DPPH free radical scavenging assay, the antioxidant activity of the biosynthesized AgNPs and the aqueous leaf extract was assessed (Hatano *et al.*, 1988). In a standard experiment, two millilitres of DPPH solution (0.1 mM in methanol) were combined with one millilitre of each sample (leaf extract, AgNPs) at varying concentrations (5–55 µg/ml). A UV-visible spectrophotometer was used to measure the absorbance at 517 nm following the reaction mixture's 30 min dark incubation period. Ascorbic acid was utilised as a standard and subjected to comparable analysis at various concentrations (10–60

µg/ml). To determine the percentage of scavenging activity, the following formula was used:

$$\% \text{ DPPH}^*_{\text{sc}} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

where,

A_{control} is the absorbance of control,

A_{sample} is the absorbance of the sample.

2.6.2 Total antioxidant capacity using phosphomolybdenum assay

Using a phosphomolybdenum assay and ascorbic acid as the reference, the total antioxidant capacity of the aqueous leaf extract and the biosynthesized AgNPs was assessed and expressed in milligrams of ascorbic acid equivalents per gram (mg AAE/g). In this experiment, glass vials containing 0.3 ml of each sample (leaf extract/AgNPs) at varying concentrations (5–55 µg/ml) were filled with phosphomolybdenum reagent (3 ml). The solution was thoroughly mixed and then sealed with lids. The absorbance of the reaction mixture was measured using a UV-visible spectrophotometer set to 695 nm after it had been incubated for 90 min at 95°C (Prieto *et al.*, 1999). The same procedure was used to create a blank, but instead of a sample, the appropriate solvent was added.

2.7 Statistical analysis

Each sample was run through three times for statistical analysis, and the results were reported as mean ± standard error (S.E.). In online Statistical Analysis, one way analysis of variances (ANOVA) was used to assess whether there were any noteworthy variations between the sample means (OPSTAT). The DPPH free radical scavenging activity's IC₅₀ values were determined using regression analysis in Microsoft Excel. Microsoft Excel 2016 was used to make all other measurements.

3. Results

3.1 Phytochemical estimation of *S. cumini* aqueous leaf extract

Table 1 summarises the various phytochemicals that were quantitatively reported in the aqueous leaf extract of *S. cumini*. The aqueous leaf extract was evaluated for total sugars (23.86 ± 0.49), reducing sugars (14.81 ± 0.40), non-reducing sugars (9.05 ± 0.39), total flavonoids (20.95 ± 1.48 mg Catechin equivalent (CE)/g), and total phenolic content (54.27 ± 3.42 mg GAE/g).

Table 1: Phytochemical estimation of *S. cumini* aqueous leaf extract

Sample	Total phenolics (mg GAE/g)	Total flavonoids (mg CE/g)	Total sugars (mg/g)	Reducing sugars (mg/g)	Non-reducing sugars (mg/g)
Leaf extract	54.27 ± 3.42	20.95 ± 1.48	23.86 ± 0.49	14.81 ± 0.40	9.05 ± 0.39

3.2 Biosynthesis of AgNPs

Generally speaking, phytochemicals present in plant extract (Table 1) have the ability to bind to metals in order to prevent nanoparticle agglomeration, which lowers Ag⁺ ions and stabilises the formed AgNPs. Using *S. cumini* leaf aqueous extract as a reducing and stabilising agent, we reported in this study the green synthesis of

AgNPs without the use of any external reducing or capping agents. According to Samari *et al.* (2018), the methodology employed was entirely safe, non-toxic, clean, and environmentally sustainable. Silver ions may be reduced to metallic ions by ascorbic acid, which is present in the leaf extract of *S. cumini*. The presence of proteins that can function as capping agents may have contributed to the stability

of these biosynthesized nanoparticles. Ag^+ can be converted to AgNPs due to the ascorbic acid molecule's isolated pair of hydroxyl group, electrons of the double bond, and carbonyl group of the lactone system. Ascorbic acid functions as a donor of protons and electrons during the suggested reduction, producing a radical known as semidehydroascorbic, which is subsequently transformed into dehydroascorbic acid. According to Jain and Mehata (2017), this dehydroascorbic acid and ascorbic acid combine to form a redox system with enough potential (-0.06 V) to convert Ag^+ to Ag^0 . Figure 1 illustrates the suggested process of silver nanoparticles synthesis by flavonoid reduction of silver ions to AgNPs.

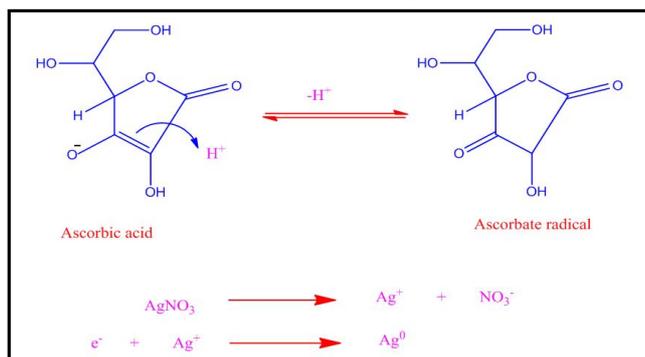


Figure 1: Ascorbic acid reduction mechanism of silver ions to obtain silver nanoparticles.

3.3 Characterization of AgNPs

UV-Visible spectroscopy can be used to examine the creation and stabilisation of AgNPs. As seen in Figure 2, the surface resonance plasmon (SPR) band in biosynthesized AgNPs was detected at 450 nm. The morphology, size, dielectric constant, and chemical environment of the synthesised nanoparticles would all have an

impact on the absorption spectra of AgNPs (Tomaszewska, 2013). The current study's results were consistent with those of earlier research, such as that conducted by Krishnaraj *et al.*, (2010), who synthesised AgNPs using *Acalypha indica* leaf extract and produced the distinctive 420 nm UV-VIS absorption peak.

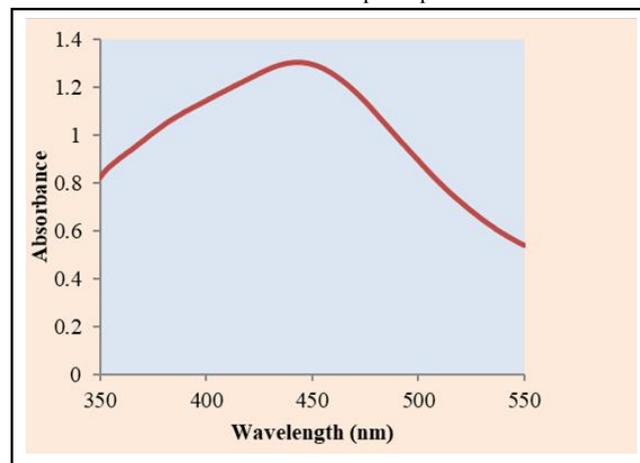


Figure 2: UV-vis absorption spectra of AgNPs.

The average size, size distribution profile, and polydispersity index of the nanoparticles in the colloidal suspension were ascertained using a particle size analyzer (PSA). The average particle size, polydispersity index (PDI), and zeta potential of the synthesised AgNPs were found to be 55 nm, 0.06, and 43.7 mV, respectively (Figure 3). The stability of AgNPs in an aqueous colloidal solution is determined by their surface charge, which can be assessed using the zeta potential method. The size determined by PSA was larger than that determined by microscopic methods (FESEM and HR-TEM) because PSA measures the hydrodynamic diameter of AgNPs, which includes the phytochemical layer coated on AgNPs surface.

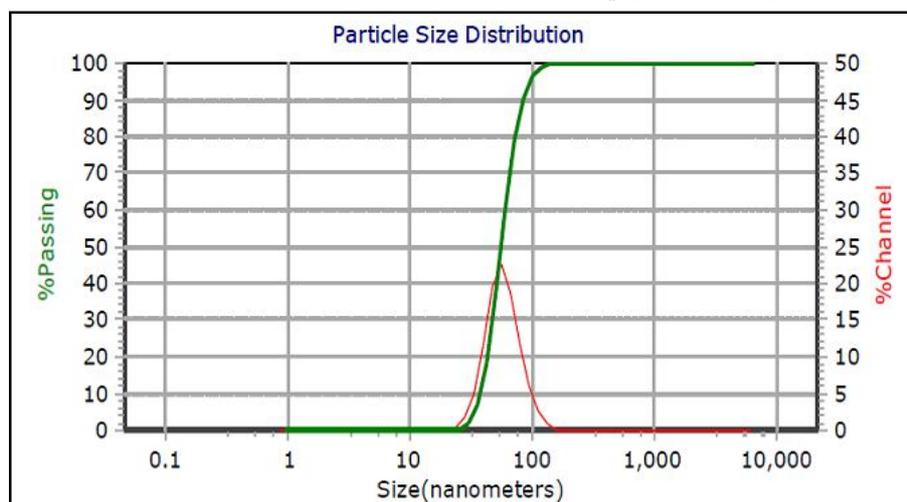


Figure 3: PSA of biosynthesized AgNPs.

Field emission scanning electron microscopy coupled to energy dispersive x-ray spectroscopy (FESEM-EDX) analysis was used to examine the surface morphology, size, and elemental composition of the biosynthesized AgNPs (Figure 4). The biosynthesised AgNPs were primarily spherical in shape and ranged in size from 30 to 40 nm. Due to a temporal variation in their formation during synthesis,

small and large-sized AgNPs coexisted; this suggests that the aggregation and formation of new nanoparticles happened simultaneously. Figure 4(b) depicts the elemental analysis of AgNPs. It shows a strong silver signal (48.41%) and weak signals of O (8.76) and Cl (0.79%).

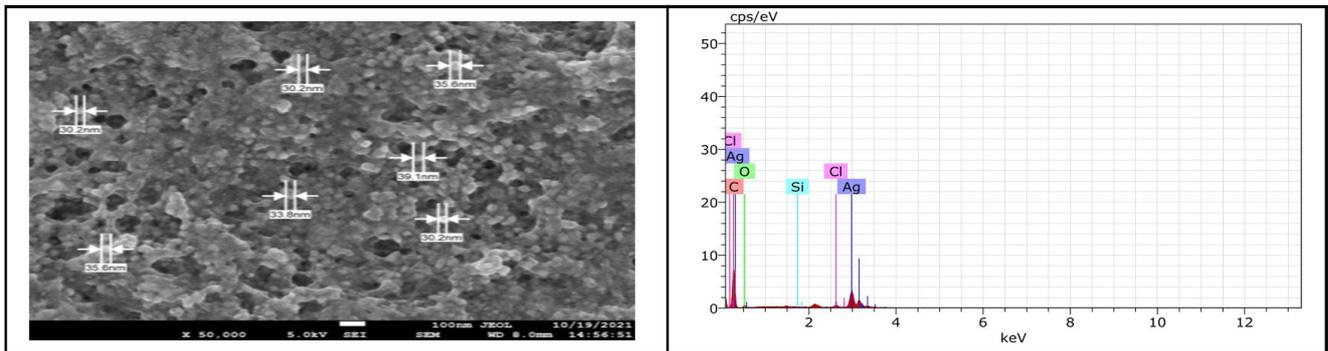


Figure 4: (a) FESEM micrograph of bio-synthesized AgNPs at 100 nm scale; (b) elemental mapping of AgNPs.

Better spatial resolution and additional analytical measurements of nanoparticles are two benefits that HRTEM offers over FESEM. It is unique in that it can identify and measure each nanoparticle's unique

chemical and electrical structure. The majority of the biosynthesized AgNPs were monodispersed, with a maximum average size of 15 nm (Figure 5).

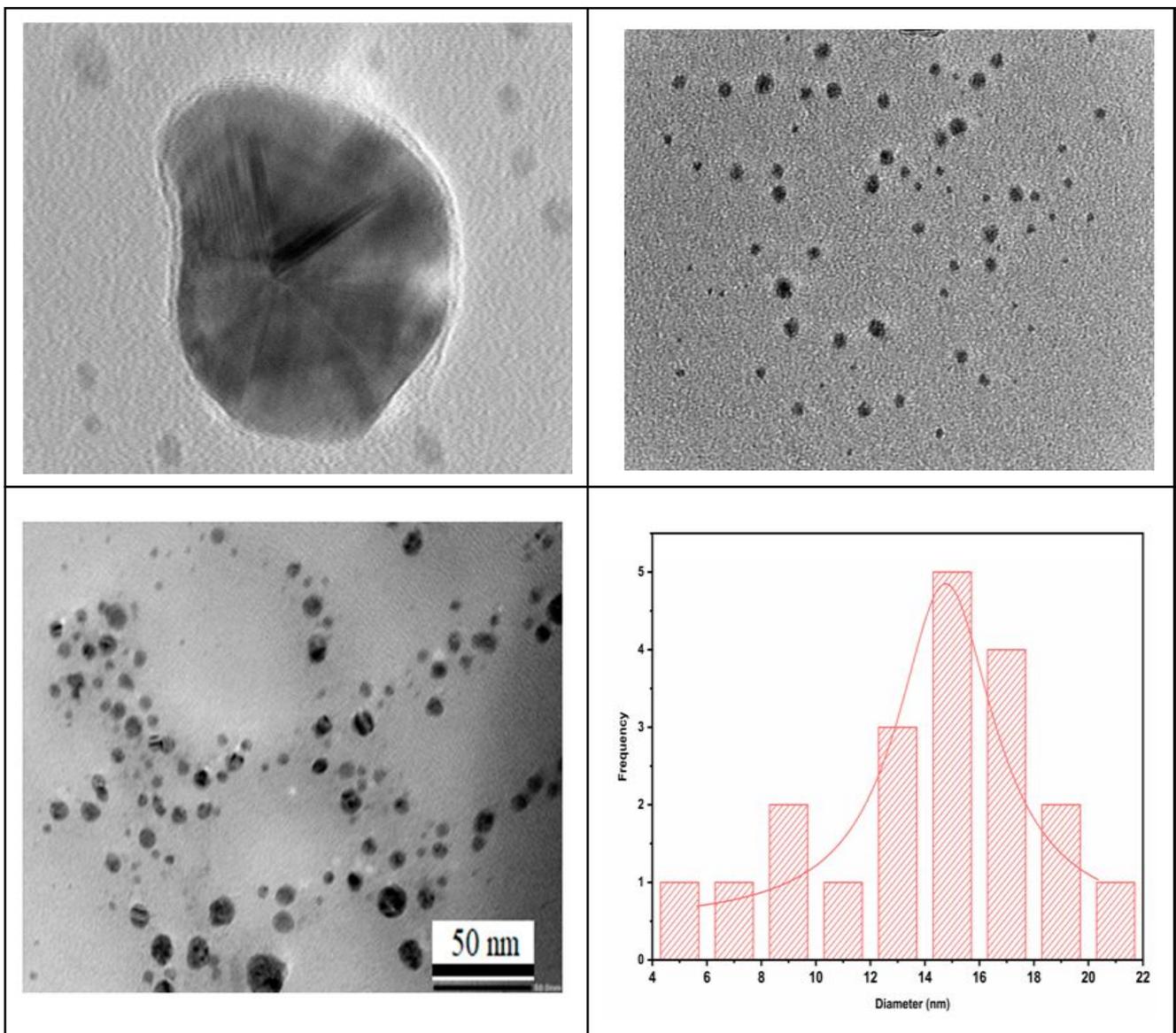


Figure 5: Images of HR-TEM showing the presence of AgNPs recorded at different magnification levels and particle size histogram.

FTIR spectroscopy is an effective method that can be used to assess functional atoms on the surface of nanoparticles, surface chemistry of metal nanoparticles, chemical bonds in surface atoms, and identification of the biomolecules that are responsible for the synthesis of metal nanoparticles. The extract of Jamun leaves, when subjected to FT-IR spectra analysis, displayed a range of characteristic peaks at 3298, 2950, 2844, 1648, 1451, and 1016 cm^{-1} , respectively. Moreover, the biosynthesized AgNPs exhibited peaks at 3026, 2952, 2836, 1657, 1447, and 1012 cm^{-1} (Figure 6). AgNPs were found to contain two peaks (1657 and 1012 cm^{-1}), which suggested that proteins and amino acids were involved in the reduction of Ag^+ to AgNPs and their complexation onto the AgNP surface.

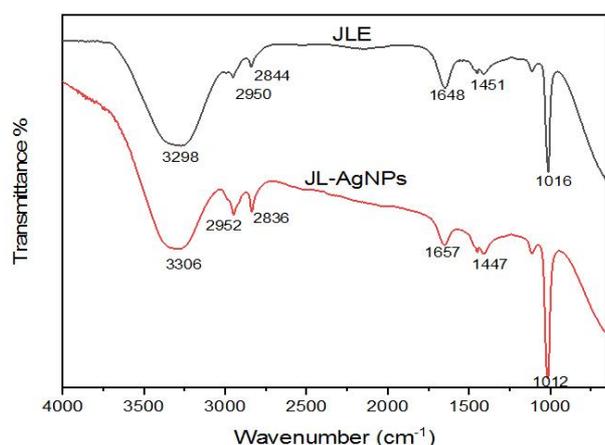


Figure 6: Comparative FTIR spectra of *S. cumini* leaf extract and their biosynthesized AgNPs.

3.4 Antioxidant activity

3.4.1 DPPH

By comparing the percentage of the synthesised AgNPs and the *S. cumini* leaf extract that scavenged DPPH free radicals, the antioxidant activity was ascertained using ascorbic acid as the standard. When the sample solution was added, it was noticed that the DPPH solution's colour changed from purple to yellow. This alteration resulted from the DPPH molecule being scavenged by hydrogen atoms that were donated to stabilize it. The DPPH free radical scavenging activity of ascorbic acid was 96.21, 82.18, 70.42, 56.12, and 32.54% at 45, 35, 25, 15 and 5 $\mu\text{g}/\text{ml}$ concentrations, respectively. It is generally acknowledged that the DPPH free radical, a stable free radical, can be used to calculate the antioxidant's capacity to scavenge free radicals. In aqueous extract DPPH free radical scavenging activity (%) of *S. cumini* leaves was high (92.6%) at 55 $\mu\text{g}/\text{ml}$, followed by 85.9, 72.3, 56.83, 33.99 and 11.6 % at 45, 35, 25, 15 and 5 $\mu\text{g}/\text{ml}$, respectively. AgNPs showed highest DPPH free radical scavenging activity (%), which was 86.1% at 45 $\mu\text{g}/\text{ml}$, followed by 78.4, 63.8, 46.05, 13.7 % at 35, 25, 15 and 5 $\mu\text{g}/\text{ml}$ concentration levels (Figure 7). Ascorbic acid had an IC_{50} of 14.33 $\mu\text{g}/\text{ml}$, while AgNPs had an IC_{50} of 17.02 $\mu\text{g}/\text{ml}$, and aqueous leaf extract had an IC_{50} of 21.09 $\mu\text{g}/\text{ml}$, indicating that ascorbic acid had the highest antioxidant efficacy. AgNPs exhibited greater antioxidant efficacy compared to the aqueous leaf extract. These current findings might be due to the presence of phytochemicals on the surface of biosynthesized AgNPs, that facilitate rapid single electron and hydrogen atom transfer and consequently stabilizes the DPPH molecule (Hernandez *et al.*, 2004).

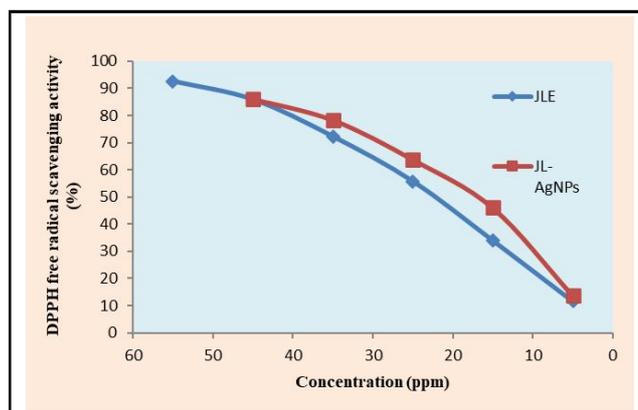


Figure 7: Quadratic regression equation for the IC_{50} ($\mu\text{g}/\text{ml}$) value by DPPH free radical scavenging activity.

3.4.2 Total antioxidant capacity using phosphomolybdenum assay

The total antioxidant potential of the biosynthesized AgNPs and the aqueous leaf extract was assessed using a standard curve that included ascorbic acid. The aqueous leaf extract and the biosynthesized AgNPs, which are antioxidants, were able to convert molybdenum (VI) into a green phosphomolybdate (V) complex. At a concentration of 100 $\mu\text{g}/\text{ml}$, the AgNPs (74.26 mg AAE/g) demonstrated higher antioxidant activity than the aqueous leaf extract (53.61 mg AAE/g). The binding of flavonoids and phenolics on the surface of biosynthesized AgNPs may be the cause of the increased antioxidant potential.

4. Discussion

The primary redox characteristics of plant phenolics, including their ability to scavenge free radicals, donate hydrogen, and quench singlet oxygen, are what make them superior antioxidants. The total phenolic and flavonoid content of plant extracts is frequently used to explain their antioxidant potential. Flavanoids providing health benefits through cell signaling pathway and antioxidant effects are secondary metabolites of plants and is a large family of polyphenolic plant compounds including flavones, flavonone, isoflavone, flavonols and anthocyanins. Therefore, present research was conducted to evaluate total phenolics and flavonoid content in *S. cumini* leaves aqueous extract. Although, there are many plants that are used as herbs worldwide, *S. cumini* is regarded as the queen of herbs because of its numerous therapeutic benefits. In present investigation, aqueous extract of *S. cumini* leaves contains total sugar content (mg/g), *i.e.*, 23.86 mg/g. Three main steps make up the mechanism of nanoparticle formation: Ag^+ reduction, clustering, and the subsequent growth of the nanoparticles. The type and concentration of the reducing agent determine each phase's properties (Makarov *et al.*, 2014). Alkaloids, terpenoids, flavanoids, and phenolic acid are a few of the secondary metabolites found in plant extracts that are responsible for lowering the amount of ionic metals in bulk metal nanoparticles (Aromal and Phillip 2012). Moond *et al.*, 2023 synthesized silver nanoparticles using *Trigonella foenum-graceum* L. leaf extract and concluded that quercetin, belongs to a group of plant pigments called flavonoids, was the active constituent of *T. foenum-graceum* leaf extract and is responsible for the AgNPs synthesis. Phytochemical analysis as discussed in previous section revealed that *S. cumini* leaves extract is a rich source of phenolic and flavonoid content. Carbonyl and hydroxyl found in phenolics, terpenoids, carbohydrates, and flavanoids are

strong reducing agents for the bioreduction of Ag⁺ ion, according to Park *et al.* (2011).

5. Conclusion

The synthesis of AgNPs using leaf extract from *S cumini* was reported as green in the current findings. Before silver nanoparticles were synthesised *via* phytomediated means, the phytochemicals serving as capping and reducing agents were quantified. Using a variety of spectroscopic methods, biosynthesized AgNPs were successfully characterized. Comparing AgNPs to the leaf extract, they showed increased antioxidant potential. Due to the special characteristics of the nanoparticles and the phytochemicals of the leaf extract adsorbed on their surface, they exhibit excellent biological activity. Additionally, the traditional physical/chemical methods for the synthesis of AgNPs will face competition from this environmentally friendly method.

Acknowledgements

For providing the required facilities, the authors are grateful to the Department of Chemistry at Chaudhary Charan Singh Haryana Agricultural University, Hisar.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

References

- Aggarwal, P.; Singh, S.; Sangwan, S.; Moond, M. and Devi, P. (2022). Effect of extraction solvents on antioxidant potential of *Prosopis cineraria*(L.) leaves. *Ann. Phytomed.*, **11**(1):426-431.
- Aromal, S.A. and Philip, D. (2012). Green synthesis of gold nanoparticles using *Trigonella foenum-graecum* and its size-dependent catalytic activity. *Spectrochimica acta Part A: Molecular and Biomolecular Spectroscopy*, **97**:1-5.
- Dalal, R.; Singh, S.; Sangwan, S.; Moond, M. and Beniwal, R. (2022). Biochemical and molecular mechanism of plant-mediated synthesis of silver nanoparticles : A review. *Mini-Reviews in Organic Chemistry*, **19**(8): 939-954.
- Devi, D.; Singh, S.; Moond, M.; Matoria, P.; Kumari, S.; Saini, K. and Sharma, R.K. (2023). Phytochemical analysis and antioxidant potential of *Albizia lebbek* (L.) seeds. *Ann. Phytomed.*, **12**:502-507.
- Devi, R.; Singh, S.; Moond, M.; Beniwal, R.; Kumar, S.; Kumari, S. and Sharma, R.K. (2023). Proximate composition, phenolic-flavonoid content and antioxidant potential of *Syzygium cumini* (L.) Skeels seed powder. *Ann. Phytomed.*, **12**(1):508-513.
- Dhanani, T.; Shah, S.; Gajbhiye, N.A. and Kumar, S. (2017). Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of *Withania somnifera*. *Arabian Journal of Chemistry*, **10**:S1193-S1199.
- Dubois, M.; Gilles, K.A.; Hamilton, J.K.; Rubers, P.A. and Smith, F. (1956). Colorimetric method for determination of sugar and related substances. *Analytical Chemistry*, **28**(3):350-356.
- Hatano, T.; Kagawa, H.; Yasuhara, T. and Okuda, T. (1988). Two new flavonoids and other constituents in licorice root, their relative astringency and radical scavenging effects. *Chemical and Pharmaceutical Bulletin*, **36**:2090-2097.
- Hernandez, I.; Alegre, L.; Van Breusegem, F.; Munne-Bosch, S. (2004). How relevant are flavonoids as antioxidants in plants? *Trends Plant Sci.*, **14**: 125-132.
- Jain, S. and Mehata, M.S. (2017). Medicinal plant leaf extract and pure flavonoid mediated green synthesis of silver nanoparticles and their enhanced antibacterial property. *Scientific reports*, **7**(1):1-3.
- Kohler, J.M.; Abahmane, L.; Wagner, J.; Albert, J. and Mayer, G. (2008). Preparation of metal nanoparticles with varied composition for catalytic application in microreactors. *Chemical Engineering Science*, **63**:5048-5055.
- Krishnaraj, C.; Jagan, E.G.; Rajasekar, S.; Selvakumar, P.; Kalaichelvan, P.T. and Mohan N.J. (2010). Synthesis of silver nanoparticles using *Acalypha indica* leaf extracts and its antibacterial activity against water borne pathogens. *Colloids and Surfaces B: Biointerfaces*, **76**(1):50-56.
- Kumara Swamy, M.; Sudipta, K.M.; Jayanta, K. and Balasubramanya, S. (2015). The green synthesis, characterization, and evaluation of the biological activities of silver nanoparticles synthesized from *Leptadenia reticulata* leaf extract. *Appl Nanosci.*, **5**:73-81
- Liu, Z.; Bucknall, D.G. and Allen, M.G. (2011). Inclined nanoimprinting lithography for 3D nanopatterning. *Nanotechnology*, **22**(22):225-302.
- Makarov, V.V.; Love, A.J.; Sinitsyna, O.V.; Makarova, S.S.; Yaminsky, I.V.; Taliansky, M.E. and Kalinina, N.O. (2014). "Green" nanotechnologies: Synthesis of metal nanoparticles using plants. *Acta Naturae*, **6**(1):20-27.
- Marinova, D.; Ribarova, F. and Atanassova, M. (2005). Total Phenolics and total flavanoids in Bulgarian fruits and vegetables. *Journal of the University of Chemical Technology and Metallurgy*, **40**:255-260.
- Marutikesavakumar, C.H.; Yugandhar, P.; Suhulatha, D. and Savitharamma, N. (2014). Synthesis, characterization and antimicrobial studies of stem bark mediated synthesis of silver nanoparticles from *Adansonia digitata* (L.). *Journal of Pharmacology Science and Research*, **7**: 76-82.
- Modi, D.C.; Patel, J.K.; Shah, B.N. and Nayak, B.S. (2010). Anti-inflammatory activity of seeds of *Syzygium cumini* linn. *Journal of Pharmaceutical Education and Research*, **1**(1):68-70.
- Moond, M.; Singh, S.; Sangwan, S.; Devi, P.; Beniwal, A.; Rani, J.; Kumari, A. and Rani, S. (2023). Biosynthesis of Silver Nanoparticles Utilizing Leaf Extract of *Trigonella foenum-graecum* L. for Catalytic Dyes Degradation and Colorimetric Sensing of Fe³⁺/Hg²⁺. *Molecules*, **28**:951.
- Moond, M.; Singh, S.; Sangwan, S.; Devi, R.; Beniwal, R. (2022). Green synthesis and applications of silver nanoparticles: A systematic review. *AATCC Journal of Research*, **9**(6):272-285.
- Moond, M.; Singh, S.; Devi, R.; Beniwal, R.; Matoria, P.; Kumari, S. and Sharma, R.K. (2023). Proximate and mineral analysis of *Trigonella foenum-graecum* (Fenugreek) seeds and leaves of variety HM444. *Ann. Phytomed.*, **12**(1):546-552.
- Moond, M.; Singh, S.; Sangwan, S.; Rani, S.; Beniwal, A.; Rani, J.; Kumari, A.; Rani, I.; Devi, P. (2023). Phytofabrication of Silver Nanoparticles using *Trigonella foenum-graecum* L. Leaf and Evaluation of its Antimicrobial and Antioxidant Activities. *Int. J. Mol. Sci.*, **24**:3480.
- Natsuki, J.; Natsuki, T. and Hashimoto Y. (2015). A review of silver nanoparticles: synthesis methods, properties and applications. *International Journal of Material Science*, **4**(5):325-332.
- Nelson, N. (1944). A photometric adaptation of the Somogyi method for determination of glucose. *Journal of Biological Chemistry*, **153**: 375-380.

- Park, Y.; Hong, Y.N.; Weyers, A.; Kim, Y.S. & Linhardt, R.J. (2011). Polysaccharides and phytochemicals: A natural reservoir for the green synthesis of gold and silver nanoparticles. *IET Nanobiotechnology*, **5**(3):69-78.
- Prieto, P.; Pineda, M. and Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphor molybdenum complex: Specific application to the determination of vitamin E. *Analytical Biochemistry*, **269**:337-41.
- Rastogi, R.M. and Mehrotra, B.N. (1990). *Compendium of Indian Medicinal Plants*, Central Drug Research Institute: Lucknow, India, **1**:388-389.
- Samari, F.; Salehipoor, H.; Eftekhari, E.; Yousefinejad, S. (2018). Low-temperature biosynthesis of silver nanoparticles using mango leaf extract: Catalytic effect, antioxidant properties, anticancer activity and application for colorimetric sensing. *New J. Chem.*, **42**:15905-15916.
- Sharma, V.K.; Yngard, R.A. and Lin, Y. (2009). Silver nanoparticles: Green synthesis and their antimicrobial activities. *Advances in Colloid and Interface Science*, **145**(1-2):83-96.
- Singleton, V.L. and Rossi, J.A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.*, **16**:144-158.
- Thakore, S.; Rathore, P.S.; Jadeja, R.N.; Thounaojam, M. and Devkar, R.V. (2014). Sunflower oil mediated biomimetic synthesis and cytotoxicity of monodisperse hexagonal silver nanoparticles. *Materials Science and Engineering: C*, **44**:209-215.
- Tomaszewska, E.; Soliwoda, K.; Kadziola, K.; Tkacz-Szczesna, B.; Celichowski, G.; Cichowski, M.; Szmaja, W. and Grobelny J. (2013). Detection limits of DLS and UV-Vis spectroscopy in characterization of polydisperse nanoparticles colloids. *Journal of Nanomaterials*, **12**:404-416.
- Vasanth, K.; Ilango, K.; Mohan Kumar, R.; Agarwal, A. and Dubey, G.P. (2014). Anticancer activity of *Moringa oleifera* mediated silver nanoparticles on human cervical carcinoma cells by apoptosis induction. *Colloids and Surfaces B*, **1**:354-359.
- Vigneshwaran, N.; Kathe, A.A.; Varadarajan, P.V.; Nachane, R.P. and Balasubramanya, R.H. (2007). Functional finishing of cotton fabrics using silver nanoparticles. *Journal of Nanoscience and Nanotechnology*, **7**(6):1893-1897.
- Yugandhar, P.; Haribabis, R. and savithramma, N. (2015). Synthesis, characterization and antimicrobial properties of green-synthesised silver nanoparticles from stem bark extract of *Syzygium alternifolium* (Wt.) Walp. *Biotechnology*, **5**:1031-1039.

Citation

Ritu Devi, Sushila Singh, Seema Sangwan, Monika Moond, Simran Kakkar, Rajni Kant Sharma and Sandeep Kumar (2024). Phytomediated fabrication of silver nanoparticles using *Syzygium cumini* L. aqueous leaf extract and evaluation of their antioxidant potential. *Ann. Phytomed.*, **13**(1):1054-1061. <http://dx.doi.org/10.54085/ap.2024.13.1.113>.