DEVELOPMENT, CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF SILVER NANOPARTICLES CONTAINING *ALBIZIA AMARA* LEAF EXTRACT

Lokesh Kumar Gautam[1*], Yuvraj Singh Sarangdevot[2]

Abstract

Silver nanoparticles (AA-AgNPs) were made from *Albizia amara* leaf extract using green synthesis. UV-Visible spectroscopy, Zeta Potential and Transmission electron microscope (TEM). Silver nanoparticles' -22.9 mV Zeta potential indicates stability. In-vitro antioxidant efficacy against DPPH radicals, reducing power test, and Phosphomolybdenum assay was shown using AA-AgNPs. IC_{50} value and statistical analysis were calculated using Graph pad prism software, ascorbic acid shows the lowest IC_{50} value of 6.076, and AA-AgNPs and AA-Extract have IC_{50} values of 27.19 and 77.33 respectively. IC_{50} value inversely proportional to antioxidant activity, the lower the IC_{50} value, the higher is the antioxidant activity and statistical analysis suggests ascorbic acid is seen as highly significant when compare to AA-Extract and moderate or non-significant nature when compare to AA-AgNPs.

Key words: Silver nanoparticle, green synthesis, Albizia amara, antioxidant activity.

[1*,2]B.N. College of Pharmacy, Bhupal Nobles' University, Udaipur, Rajasthan, India
[1]Email id: lokesh.gautam294@gmail.com

***Corresponding Author:** Lokesh Kumar Gautam
*B.N. College of Pharmacy, Bhupal Nobles' University, Udaipur, Rajasthan, India
Email id: lokesh.gautam294@gmail.com

DOI: 10.53555/ecb/2022.11.9.147

Introduction

Medicinal plants have been used for centuries by various cultures around the world for their therapeutic properties [1-2]. The historical use of medicinal plants dates back to ancient times, and the knowledge of these plants has been passed down through generations [3-5].

Traditional herbal medicines employ medicinal plants and natural components for treatment. Herbal medicines are derived from various parts of plants, including leaves, flowers, stems, roots, and seeds. These plant materials contain active compounds that have therapeutic properties [6-9].

The problem statement for an antioxidant study focuses on exploring the properties and effects of compounds or interventions with antioxidant activity. Antioxidants are substances that can protect cells from damage caused by free radicals, which are unstable molecules that can lead to oxidative stress and contribute to various diseases, including cancer, cardiovascular disorders, and neurodegenerative conditions [10-11]. The objective of an antioxidant study is to investigate natural or synthetic compounds, dietary interventions, or lifestyle factors that exhibit antioxidant properties and determine their potential in preventing or mitigating oxidative damage in biological systems [12-13].

Albizia amara is a tree in the family Fabaceae. Its range includes southern and Eastern Africa, from South Africa to Sudan and Ethiopia. It is also found in India and Sri Lanka. The leaves and flowers are used for treatment of boils and ulcers. The leaf is also used for treatment of erysipelas. Paste of leaf and rootbark is used to cure both skin diseases and poisonous bites. The seeds are regarded as astringent and used in the treatment of piles, diarrhea and gonorrhea. The flowers are used as a remedy for cough, ulcers, dandruff and malaria [14-17].

This research aims to uncover the mechanisms of action, assess the efficacy, and explore the therapeutic applications of antioxidants in promoting overall health and reducing the risk of oxidative stress-related diseases.

Material and Methods

Pre-Formulation Studies

Pre-formulation is a systematic approach made to analyze the samples and excipients to evaluate the physicochemical properties of drug substances.

Collection and Authentication of *Albizia amara leaves* of plant

Albizia amara (Fabaceae) leaves are collected from the Medicinal Garden of ICMR- National Institute of Traditional Medicine. RMRC ICMR layout, Nehru Nagar Belagavi, Karnataka and authenticated (Accession number: RMRC-1739) by Dr. Harsha Hegde, Taxonomist ICMR Belagavi, Karnataka, India

Preparation of aqueous leaf extract

Approximately 20 grams of freshly collected *Albizia amara* leaves were precisely weighed. Subsequently, the leaves underwenttwo thorough washes under flowing tap water to eliminate any surface mud and dust particles. Following this, the leaves were rinsed using Milli Q water. The leaves were then finely chopped into small fragments and subjected to boiling in 100 ml of MilliQ water at a temperature of 60 °C for a duration of 30 minutes. Boiling the mixture produced an extract, which was then filtered twice—once through normal filter paper and once through Whatman No. 1 filter paper—after cooling to room temperature. At a temperature of 4 °C, the filtered extract was properly preserved [18-21].

Qualitative Phytochemical analysis

Tests for Carbohydrates: Molish’s test, Fehling's test and Tannic acid test for starch were used.
Tests for Proteins: Biuret test and Million's test were used.

Tests for Amino acids: Ninhydrin test was used.
Tests for Steroid: Salkowski Reaction was used.
Tests for Glycosides: Keller-Killiani test, Legal's test and Borntrager’s test for anthraquinone glycosides were used.

Test for Flavonoids: Shinoda test and Sulphuric acid test were used.

Tests for Alkaloids: Wagner's test, Tannic acid test and Mayer’s test were used.

And the extract was also tested for Tannins, phenolic compounds and Saponins (Foam text) [18-21].

Synthesis of silvernanoparticles

The addition of 10 ml of aqueous AA-extract to 100 ml of 1 mM silver nitrate solution at 70 °C while stirring continuously on a magnetic stirrer caused the reduction of Ag+ ions to Ag0, which will result in the formation of silver nanoparticles. The pH of the reaction mixture was then adjusted to 8 by

adding 1% w/v NaOH solution, followed by its incubation (24 hr) in the dark at The obtained AA-AgNPs were then separated by centrifugation at 10000 rpm for 30 min (Kubota Corporation, Japan). Pellets were then washed three times by re-suspending them in Milli Q water and again subjected to centrifugation to remove any residue of the extract. Then optimised the effects of temperature, pH, concentration of silver nitrate, extract and silver nitrate ratio, and reaction incubation period [22-26].

Characterization of *Albizia amara* AgNPs
Ultraviolet-visible spectral analysis
The reaction mixture's first colour shift signals the creation of silver nanoparticles. The reading of absorbance in the visible 200-800 nm region further supports it. To verify the creation of silver nanoparticles, the dried AA-AgNPs pellets were sonicated in a bath of Milli Q water. A UV-visible spectrophotometer (LABINDIA ANALYTICAL UV 3200) was then used to measure the absorbance throughout the wavelength range of 200 to 800 nm [27-28].

Principle of Particle size and Zeta Potential analysis
By using the DLS approach and a Zeta-sizer device (Malvern Instruments Ltd., UK), synthetic AA-AgNPs' particle size distribution, size, and zeta potential (ZP) were measured. Using an ultrasonic bath run at 40 kHz for 5 minutes at room temperature, the AA- AgNPs were equally disseminated in Milli Q water [29-30].

Transmission electron microscopy (TEM)
A powerful imaging technique, transmission electron microscopy (TEM) enables the visualisation and investigation of minute features inside specimens

Antioxidant Activity of *Albizia amara* AgNPs
DPPH radical scavenging assay
A working stock solution of AA-Extract and AA-AgNPs was prepared at 10mg/ml concentration using Milli Q water. From the above stock solution aliquots of 2, 4, 6, 8, and 10µl were pipette out into effendroff tubes and make up the volume upto 1ml to make concentrations of 20, 40, 60, 80, and 100µg/ml, to this various concentration of extract and nanoparticles 500µl of DPPH reagent of concentration 20µg/ml added, followed by incubation at a dark place for 30min. Then the 250µl of supernatant was placed into 96 well micro plate and absorbance was measured at 517nm using ELISA reader. Control was identically prepared without sample. DPPH was replaced by ethanol in case of the blank. Ascorbic acid was used as standard. The percentage scavenging was calculated using the formula [(Control-Test) /Control] x 100.

A graph was constructed by plotting concentration versus percentage inhibition. The concentration of the sample required for a 50% reduction in absorbance (IC50) was calculated using Graph pad prism software. A triplicate reading was taken and the average was calculated [31].

Reducing power assay (FRAP)
A working stock solution of 10mg/ml concentration of AA-Extract and AA-AgNPs was prepared. From the above stock different concentrations were made 20, 40, 60, 80, and 100 µg/ml then make up the volume up to 1.25 ml with Milli Q water, followed by the addition of 1% pot ferriccynide [$K_3Fe(CN_6)$] solution and incubate the tubes for 20min at 50 °C. After allowing the solution to cool to room temperature, add 250 l of 10% w/v trichloroacetic acid and 250 ml of 0.1% w/v ferrous chloride. Next, use a UV-Visible Spectrophotometer to measure the absorbance at 700 nm. Ascorbic acid served as the benchmark while phosphate buffer served as the control. The absorbance of the final reaction mixture from three parallel experiments was reported as mean standard error of the mean [32].

Phosphate molybdenum assay
The working stock solution of AA-Extract and AA-AgNPs was prepared at 10 mg/ml concentration. From the above stock solution, different concentrations were made 20, 40, 60, 80, and 100 µg/ml. (Pipette out 2, 4, 6, 8, and 10µl of aliquots from working stock and make up the volume to 1ml with Milli Q water), followed by the addition of 1ml of Phosphomolybdenum reagent and tubes were incubated in a water bath at 90 °C for 90min. Allow tubes to cool to room temperature and absorbance was read using (Labindia Analytical UV 3200) at a wavelength range of 765nm. Ascorbic acid is used as standard [33].
The % inhibition was calculated using the formula:
[(Control absorbance-Sample absorbance)/ control absorbance] X 100.

Results and Discussion

Phytochemical analysis of aqueous leaf extract of *Albizia amara*

Table 1: Phytochemical analysis of aqueous leaf extract of *Albizia amara*

S. No.	Name of the test		Aqueous extract of *Albizia amara*
1	Test for Carbohydrates:	Molish test	+
		Fehling's test	+
		Benedict's test	-
		Iodine test	-
		Tannic acid test	+
2	Test for Proteins:	Biuret test	+
		Million's test	+
3	Test for Amino acids:	Ninhydrin test	+
		Test for tyrosine	+
4	Test for steroid:	Salkowski reaction	+
5	Test for Glycosides:	Keller-killiani test	+
		Legal's test	-
		Borntrager's test	+
6	Test for Flavonoids:	Shinoda test	+
		H_2SO_4 test	+
		NaOH test	+
7	Test for Alkaloids:	Mayer's test	-
		Wagner's test	+
		Tannic acid test	+
8	Test for Tannins and Phenolic compounds	5% $FeCl_3$ Solution	+
		Lead acetate	+
		Potassium dichromate	-

'+'=Present; '-' = Absent

The phyto-constituents analytical profile indicated that the Albiziaamara aqueous leaf extract contains carbohydrates, proteins, amino acids, steroids, glycosides, flavonoids, alkaloids, tannins, phenolic compounds, saponin, and quinones. Table 1 shows they can cap and stabilise silver nanoparticles.

The prepared silver nanoparticles were used to perform optimization:

The present study optimised temperature, pH, silver salt concentration, aqueous leaf extract-silver nitrate ratio, and reaction incubation time. These parameters influence silver nanoparticle production and particle size and shape. Green synthesis creates silver nanoparticles by reducing and capping silver ions with plant metabolites. However, each plant species exhibits distinct morphology and contains unique secondary metabolites in varying types and concentrations. These variations in metabolites influence their effectiveness in reducing and capping, thereby significantly affecting the formation and morphology of the resulting silver nanoparticles. Hence optimization of these parameters becomes necessary inthe fabrication of silver nanoparticles.

Effect of temperature:

In the present study an attempt has been made to investigate the effect temperature on synthesis of *Albiziaamara* leaf extract mediated silver nanoparticles, because it is well known fact that temperature basically increase rate of reaction, hence effect of temperature is taken into consideration and synthesis was carried out at different temperature (Room temperature, 40 °C, 60 °C, 70 °C, 80 °C, 90 °C, and 100 °C), UV spectra of 24 hr incubation time is display in **Figure No 1** and we found that there is increase in peak intensity with increase in temperature and peak slightly shifted towards shorter wavelength from (445nm to 442nm), because at higher temperature kinetic energy of bio-molecules increased thus, complete consumption of silver nuclei by plant metabolites so complete reduction of silver ion takes place, results in better yield and small size of particles, But at 80 °C there is significant decrease in peak intensity seen, study by (Sunday Adewale *et al* 2021) reported specific suitable temperature is essential to maintain stability of plant metabolite for their better reactivity with silver ions. Also, we saw that asymmetry in the SPR band at 90 °C and 100 °C may be due to the agglomeration of particles at high temperatures. Hence **60 °C** is selected as the optimum temperature for the synthesis of AA-AgNPs.

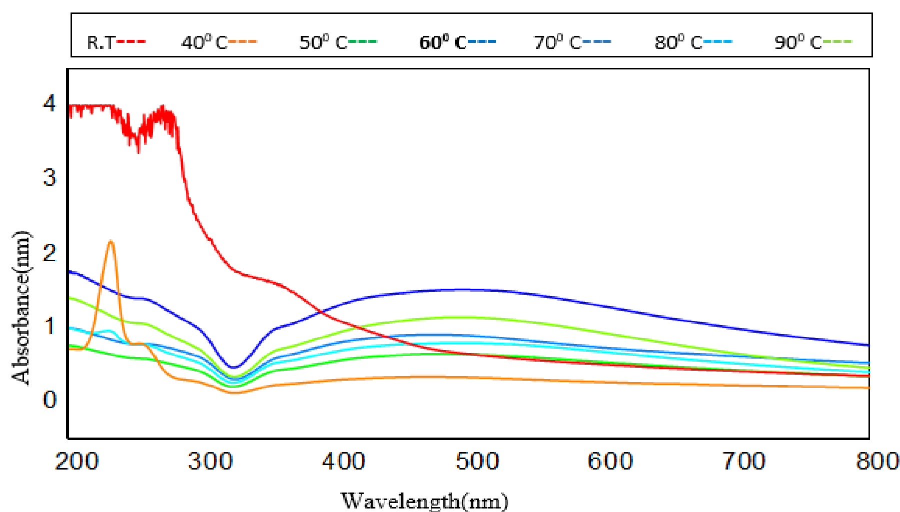

Figure 1: *Effect of temperature on synthesis of AA-AgNPs.*

In the similar way, the effect of pH, concentration of silver nitrate solution, ratio of plant extract and silver nitrate concentration were studied.

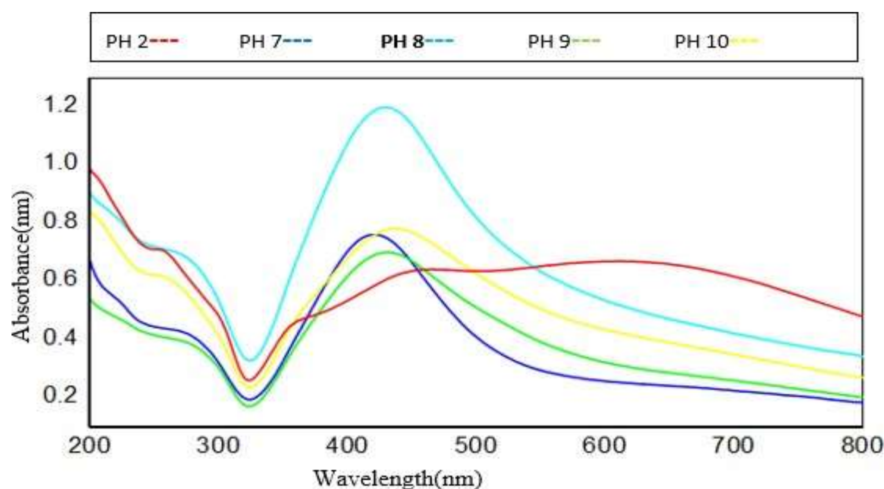

Figure 2: *Effect of pH on synthesis of AA-AgNPs*

pH 8 is selected as the optimized pH for the synthesis of AA-AgNPs.

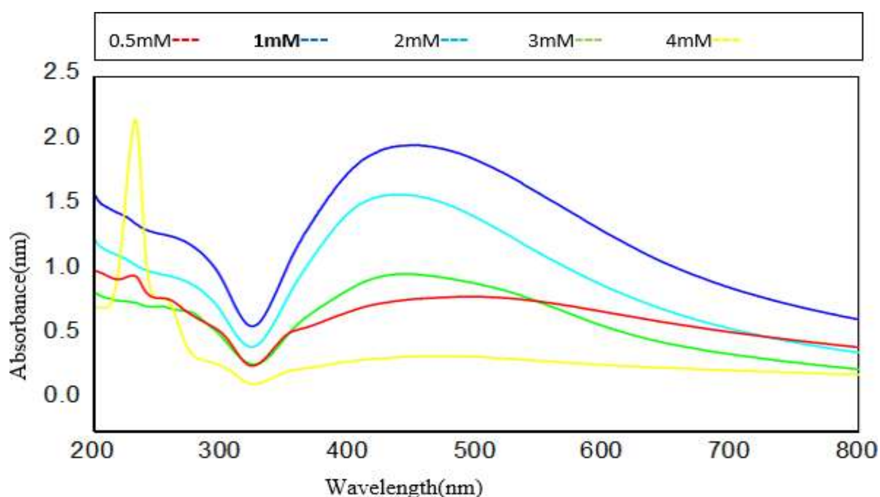

Figure 3: *Effect of concentration of $AgNO_3$ on the synthesis of AA-AgNPs.*

1mM concentration is considered as optimum concentration for the synthesis of AA-AgNPs.

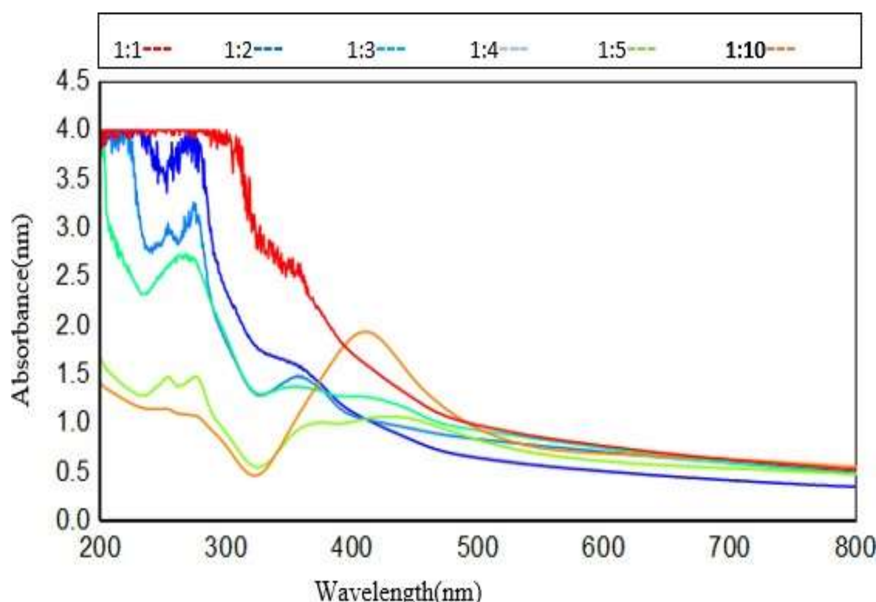

Figure 4: *Effect of ratio of the reaction mixture on the synthesis of AA- AgNPs*

1mM is considered as optimum concentration for the synthesis of AA-AgNPs.

Characterization of *Albizia amara* Silver Nanoparticles

UV-Visible spectral analysis

The size, shape, and quantity of the particles affect the SPR peak's form and absorption intensity. The little spherical particles' increasing intensity, as seen by the single narrow SPR peak, shows that there are more of them than before. After a 24-hour incubation period, AA-AgNPs displayed a single, strong, and somewhat wide peak, which supports the development of moderately large, polydispersed nanoparticles.

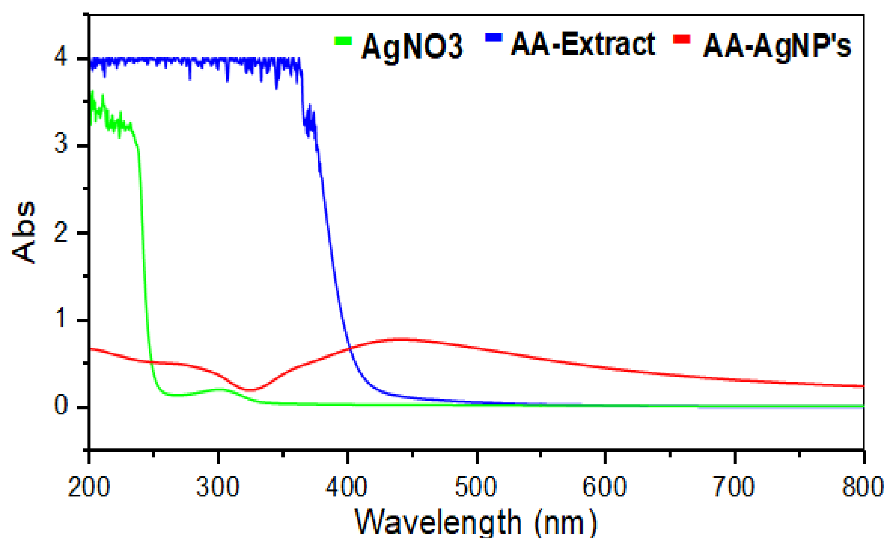

Figure 5: *UV-visible spectra of silver nanopaticles synthesized from aqueous leaf extract of Albizia amara.*

Particle Size Distribution and zeta Potential of AA-AgNPs

The stability of synthesized nanoparticles was tested by the zeta sizer instrument. Here the surface charge of silver nanoparticles was measured. In the current study result of zeta, sizer revealed that synthesized silver nanoparticles fabricated from aqueous leaf extract of *Albiziaamara*have the zeta potential -22.9, here the negative charge indicates particles have negative charge on their surface and higher the value higher repulsion between particles, which indicates less aggregation and high stability of nanoparticles. Average particle size AA-AgNPs of 95.4nm with polydispersibility index of 0.314. Nanoparticles with very large size particle distribution and have polydispersibility index values > 0.8 clearly indicates that the obtained nanoparticles are highly polydisperseble in nature.

Results

			Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm):	95.4	Peak 1:	101.8	89.4	49.03
PdI:	0.314	Peak 2:	22.6	9.6	8.2
Intercept:	0.829	Peak 3:	0.000	0.0	0.000
Result quality :	Good				

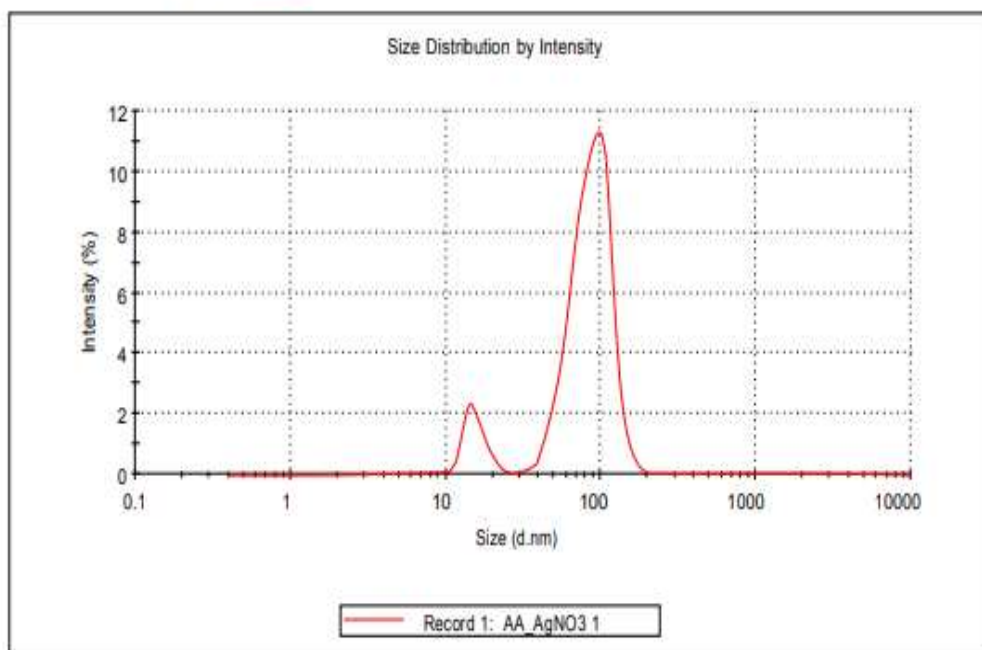

Figure 6: *Particle size distribution of the AA-AgNPs*

			Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV):	-22.9	Peak 1:	-22.9	100.0	6.01
Zeta Deviation (mV):	6.01	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm):	0.0394	Peak 3:	0.00	0.0	0.00
Result quality :	Good				

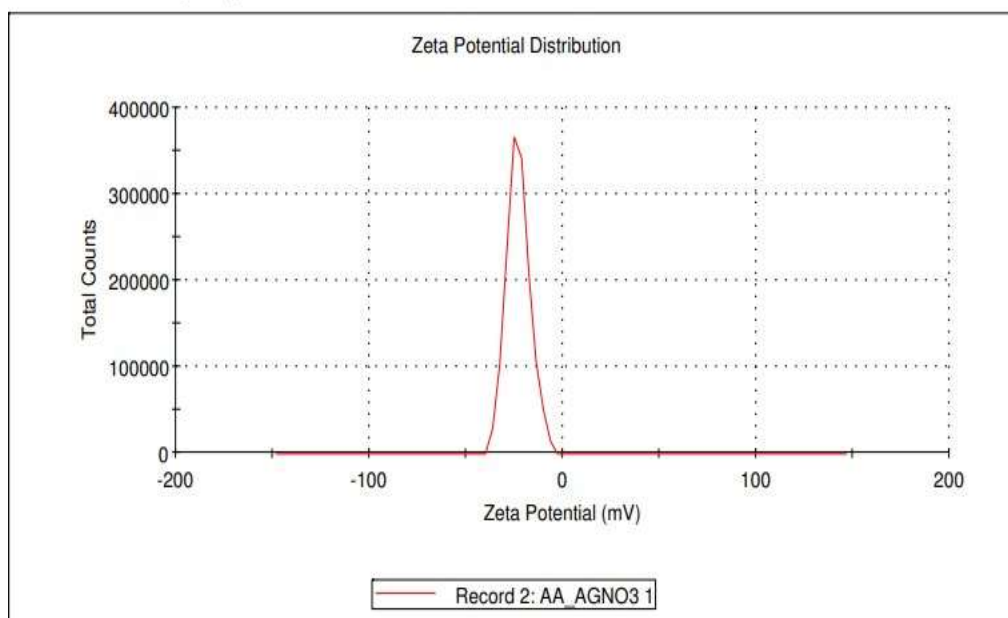

Figure 7: *The Zeta potential of the AA-AgNPs*

Transmission electron microscopy (TEM)
The morphological characteristics of silver nanoparticles, including their size and shape, were examined using the Transmission Electron Microscopy (TEM) method. As shown by TEM images in Figure No. 8, the results support the polydisperse character of the synthesised AA-AgNPs, which display a range of forms, namely spherical, triangular, pentagonal, and hexagonal. The average particle size, determined by plotting a histogram of particle diameters (nm) against their respective counts, utilizing Origin Pro software as depicted in Figure 9, was established to fall within the range of 80-85 nm.

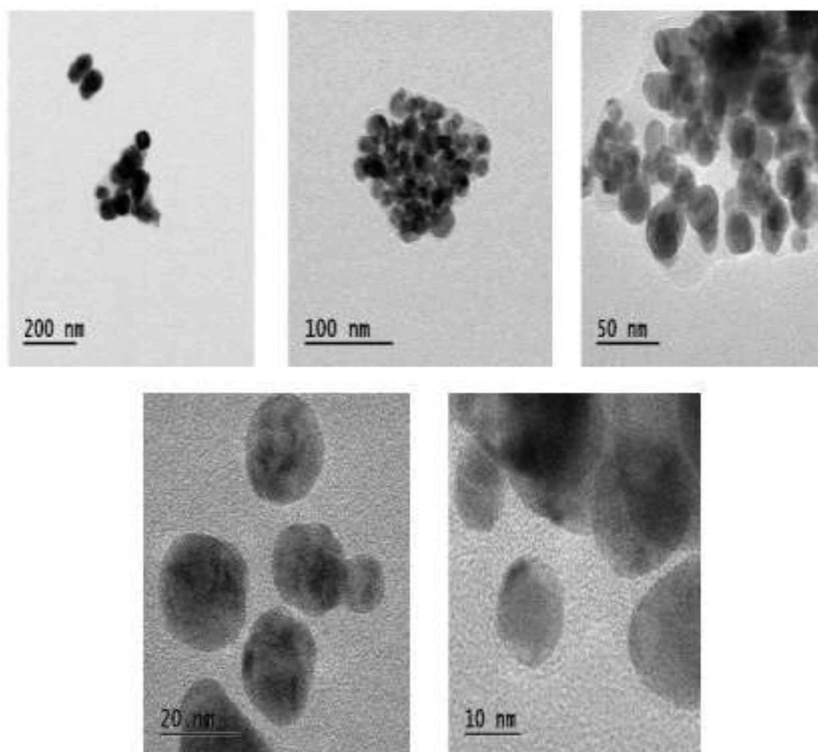

Figure 8: *TEM images of Albizia amara AgNPs at different magnification scales of (A) 200 nm, (B)100 nm, (C) 50 nm, (D) 20 nm (E) 10nm.*

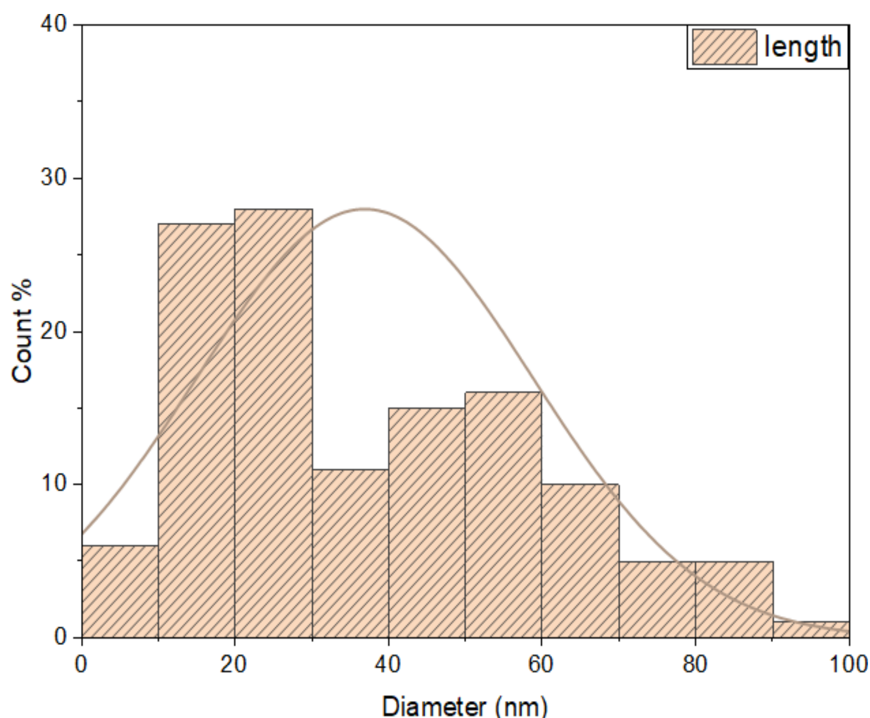

Figure 9: *Histogram showing size distribution of AA-AgNPs*

Results of Antioxidant Activity:
(DPPH) 2,2-diphenyl -1-picrylhydrazyl assay:

Table 2: Determination of percentage inhibition of 2, 2-diphenyl-1- picrylhydrazyl radical scavenging activity of *Albiziaamara*–AgNP's.

S. No.	Concentration	Percentage of inhibition		
		Albizia amra Extract	*Albizia amra* silver nanoparticles	Ascorbic acid
1	80µg/ml	52.041±0.641	69.326±0.996	81.2±0.976
2	40µg/ml	35.121±0.657	54.358±0.318	75.21±1.415
3	20µg/ml	27.414±1.324	42.195±1.744	67.76±0.985
4	10µg/ml	17.497±1.315	34.453±0.647	59.471±1.215
5	5µg/ml	11.459±0.976	29.36±1.415	49.798±1.325
6	2.5µg/ml	5.421±0.667	22.662±0.338	33.676±0.656

Note: The data presented are arithmetic mean ± S.D of 3 trials.

The antioxidant potential of AA-Extract and AA-AgNPs were evaluated in different concentrations (2.5 µg/ml to 80 µg/ml) against the standard ascorbic acid using DPPH free and results revealed that ascorbic acid, AA-AgNPs and AA-Extract show antioxidant activity in a dose-dependent manner, we found increased % of inhibition at higher concentration (80µg/ml) by Ascorbic acid, AA-AgNPs and AA-Extract 81.2±0.976%, 69.326±0.996%, and 52.041±0.641% respectively shown in Table 2. IC_{50} value and statistical analysis were calculated using Graph pad prism software, ascorbic acid shows the lowest IC_{50} value of 6.076, and AA-AgNPs and AA-Extract have IC_{50} values of 27.19 and 77.33 respectively. IC50 value inversely proportional to antioxidant activity, the lower the IC_{50} value, the higher is the antioxidant activity and statistical analysis suggests ascorbic acid is seen as highly significant when compare to AA-Extract and moderate or non-significant nature when compare to AA-AgNPs. Generally, DPPH is HAT based assay, which measures hydrogen atom transferring capacity, DPPH is one of the most frequently used stable free radicals having purple color, when it reacts with antioxidants or hydrogen donating group converts into reduced (hydrazine form) of DPPH and purple color changes to pale yellow color. The disappearance of the purple color is based on the concentration of antioxidants, compounds with better antioxidant activity cause the complete disappearance of the purple color.

Ferric reducing antioxidant power assay (FRAB):

Table 3: Ferric reducing antioxidant power assay for *Albizia amara* extract, *Albizia amara* AgNP's and Ascorbic acid

S. No.	Concentration	Percentage of inhibition		
		Albizia amra Extract	*Albizia amara* silver nanoparticles	Ascorbic acid
1	**80µg/ml**	45.041±0.0013	70.219±0.0013	82.41±0.131
2	**40µg/ml**	14.621±0.0014	53.216±0.0060	73.21±0.0041
3	**20µg/ml**	10.414±0.0060	42.195±0.0049	63.53±0.0028
4	**10µg/ml**	7.4975±0.0046	36.113±0.0021	58.91±0.0041
5	**5µg/ml**	6.459±0.0031	29.344±0.0034	49.59±0.0049
6	**2.5µg/ml**	4.921±0.0034	22.156±0.0041	33.17±0.0041

Note: The data presented are arithmetic mean ± S.D of 3 trials.

The antioxidant potential of AA-Extract and AA-AgNPs was evaluated by measuring the ferric ion reducing capacity at different concentrations (2.5 µg/ml to 80 µg/ml) and compared with standard ascorbic acid. It was observed that increase in the concentration of antioxidant, absorption intensity also increases, and ascorbic acid show absorption intensity at their higher concentration (80µg/ml) at 82.41±0.131 followed by AA- AgNPsat 70.219±0.0013 and AA-Extract at 45.041±0.0013 respectively, confirms the better activity of nanoparticles when compared to extract shown in Table 3. Results of statistical analysis done by using Graph pad prism software suggest non- significant nature of AA-Extract and AA-AgNPs compared to ascorbic acid. Generally, FRAP assay is ET based assay, which involves reduction of colorless Fe^{+3} (-2,4,6-tripyridyl-s-triazine complex) to intensively blue Fe^{+2} (-2,4,6-tripyridyl-s- triazine complex). Higher antioxidant activity higher the intensity of the blue color results in high absorption intensity.

Phosphomolybdenum assay (PM)

Table 4: Phosphomolybdenum assay for *Albizia amara* extract, *Albizia amara* AgNPs and ascorbic acid

Se No.	Concentration	Percentage of inhibition		
		Albizia amra Extract	*Albizia amra* silver nanoparticles	Ascorbic acid
1	80µg/ml	56.4295±2.138	73.413±2.149	89.933±3.612
2	40µg/ml	41.889±2.618	57.591±0.426	83.031±0.461
3	20µg/ml	29.659±1.491	51.517±1.390	78.261±0.698
4	10µg/ml	25.04±1.816	45.31±0.614	67.714±1.754
5	5µg/ml	19.339±0.213	33.719±0.841	60.701±1.266
6	2.5µg/ml	13.838±1.816	30.739±0.423	51.683±2.602

Note: The data presented are arithmetic mean ± S.D of 3 trials.

It is another approach to measure the antioxidant potential, it is the colorimetric method that usually measures the reduction of phosphate legend by antioxidants. In the present study, the antioxidant capacity of AA-AgNPs and AA-extract were tested compared with standard ascorbic acid. The antioxidant activity is in a dose-dependent manner ascorbic acid shows absorption intensity at (80µg/ml) concentration is 89.933±3.612 followed by AA-AgNPs 73.413±2.149 and AA-extract 56.4295±2.138 respectively which shown in Table 4, which indicates the good antioxidant property of nanoparticles compared to aqueous extract. Statistical analysis done by using Graph pad prism suggests ascorbic acid is non-significant compared AA-AgNPs, but highly significant compared to AA-extract. It is also an ET-based assay here by transferring electron Mo6+legend gets converted to green colored Mo5+, antioxidant measured in terms of intensity of color produced.

Conclusion

Metallic nanoparticles have become a popular cancer treatment delivery technique over traditional therapy. This work synthesizes silver nanoparticles from *Albizia amara* leaf aqueous extract. Aqueous *Albizia amara* leaf extract was the reducing, capping, and stabilising agent in the ecologically friendly bio-fabrication of AA-AgNPs. UV-spectroscopy of *Albizia amara* leaf silver nanoparticles showed a somewhat wide peak at 442 nm, confirming the development of tiny poly- dispersed nanoparticles. Synthesised silver nanoparticles' zeta potential value of -22.9 indicated good stability. Transmission electron microscopy shows *Albizia amara* leaf extract-mediated silver nanoparticles are substantially spherical, triangular, pentagonal, and hexagonal. The AA-AgNPs showed effective *in-vitro* antioxidant activity against DPPH radicals, reducing power assay and Phosphomolybdenum assay. IC_{50} value and statistical analysis were calculated using Graph pad prism software, ascorbic acid shows the lowest IC_{50} value of 6.076, and AA-AgNPs and AA- Extract have IC_{50} values of 27.19 and 77.33 respectively. IC50 value inversely proportional to antioxidant activity, the lower the IC_{50} value, the higher is the antioxidant activity and statistical analysis suggests ascorbic acid is seen as highly significant when compare to AA-Extract and moderate or non- significant nature when compare to AA-AgNPs. The exposure of AgNPs on RBC cells and normal breast cancer cells suggesting its biocompatible nature of AA-AgNPs**.**

References

1. Halberstein, R. A. (2005). Medicinal plants: historical and cross-cultural usage patterns. *Annals of epidemiology*, *15*(9), 686-699.
2. Jamshidi-Kia, F., Lorigooini, Z., & Amini-Khoei, H. (2017). Medicinal plants: Past history and future perspective. *Journal of herbmed pharmacology*, *7*(1), 1-7.
3. Jamshidi-Kia, F., Lorigooini, Z., & Amini-Khoei, H. (2017). Medicinal plants: Past history and future perspective. *Journal of herbmed pharmacology*, *7*(1), 1-7.
4. Petrovska, B. B. (2012). Historical review of medicinal plants' usage. *Pharmacognosy reviews*, *6*(11), 1.
5. Gurib-Fakim, A. (2006). Medicinal plants: traditions of yesterday and drugs of tomorrow. *Molecular aspects of Medicine*, *27*(1), 1-93.
6. Houghton, P. J. (1995). The role of plants in traditional medicine and current therapy. *The Journal of Alternative and Complementary Medicine*, *1*(2), 131-143.
7. Gurib-Fakim, A. (2006). Medicinal plants: traditions of yesterday and drugs of tomorrow. *Molecular aspects of Medicine*, *27*(1), 1-93.
8. Meena, A. K., Bansal, P., & Kumar, S. (2009). Plants-herbal wealth as a potential source of ayurvedic drugs. *Asian J Tradit Med*, *4*(4), 152-70.
9. Alamgir, A. N. M., & Alamgir, A. N. M. (2017). Cultivation of herbal drugs, biotechnology, and in vitro production of secondary metabolites, high-value medicinal plants, herbal wealth, and herbal trade.

Therapeutic Use of Medicinal Plants and Their Extracts: Volume 1: Pharmacognosy, 379-452.
10. Ratnam, D. V., Ankola, D. D., Bhardwaj, V., Sahana, D. K., & Kumar, M. R. (2006). Role of antioxidants in prophylaxis and therapy: A pharmaceutical perspective. *Journal of controlled release*, *113*(3), 189-207.
11. Taverna, M., Marie, A. L., Mira, J. P., & Guidet, B. (2013). Specific antioxidant properties of human serum albumin. *Annals of intensive care*, *3*, 1-7.
12. Gulcin, İ. (2020). Antioxidants and antioxidant methods: An updated overview. *Archives of toxicology*, *94*(3), 651-715.
13. Brewer, M. S. (2011). Natural antioxidants: sources, compounds, mechanisms of action, and potential applications. *Comprehensive reviews in food science and food safety*, *10*(4), 221-247.
14. Indravathi, G., Reddy, R. S., & Babu, P. S. (2016). Albizia amara-A potential medicinal plant: a. *International journal of Science and Research*, *5*(3), 621-7.
15. Balasubramani, G., Ramkumar, R., Raja, R. K., Aiswarya, D., Rajthilak, C., & Perumal, P. (2017). *Albizia amara* Roxb. mediated gold nanoparticles and evaluation of their antioxidant, antibacterial and cytotoxic properties. *Journal of Cluster Science*, *28*, 259-275.
16. Kokila, K., Priyadharshini, S. D., & Sujatha, V. (2013). Phytopharmacological properties of Albizia species: a review. *Int J Pharm Pharm Sci*, *5*(3), 70-73.
17. Singab, A. N., Bahgat, D., Al-Sayed, E., & Eldahshan, O. (2015). Saponins from genus Albizia: phytochemical and biological review. *Med Aromat Plants S*, *3*(001).
18. Santhi, K., & Sengottuvel, R. (2016). Qualitative and quantitative phytochemical analysis of Moringa concanensis Nimmo. *International Journal of Current Microbiology and Applied Sciences*, *5*(1), 633-640.
19. Shaikh, J. R., & Patil, M. (2020). Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies*, *8*(2), 603-608.
20. Egbuna, C., Ifemeje, J. C., Maduako, M. C., Tijjani, H., Udedi, S. C., Nwaka, A. C., & Ifemeje, M. O. (2018). Phytochemical test methods: qualitative, quantitative and proximate analysis. In *Phytochemistry* (pp. 381-426). Apple Academic Press.
21. Mumtaz, F., Raza, S. M., Ahmad, Z., Iftikhar, A., & Hussain, M. (2014). Qualitative phytochemical analysis of some selected medicinal plants occurring in local area of Faisalabad, Pakistan. *Journal of Pharmacy and Alternative Medicine*, *3*(3), 5-10.
22. Logeswari, P., Silambarasan, S., & Abraham, J. (2015). Synthesis of silver nanoparticles using plants extract and analysis of their antimicrobial property. *Journal of Saudi Chemical Society*, *19*(3), 311-317.
23. Tran, Q. H., & Le, A. T. (2013). Silver nanoparticles: synthesis, properties, toxicology, applications and perspectives. *Advances in natural sciences: nanoscience and nanotechnology*, *4*(3), 033001.
24. Ghaffari-Moghaddam, M., Hadi-Dabanlou, R., Khajeh, M., Rakhshanipour, M., & Shameli, K. (2014). Green synthesis of silver nanoparticles using plant extracts. *Korean Journal of Chemical Engineering*, *31*, 548-557.
25. Mashwani, Z. U. R., Khan, T., Khan, M. A., & Nadhman, A. (2015). Synthesis in plants and plant extracts of silver nanoparticles with potent antimicrobial properties: current status and future prospects. *Applied microbiology and biotechnology*, *99*, 9923-9934.
26. Das, G., Patra, J. K., Debnath, T., Ansari, A., & Shin, H. S. (2019). Investigation of antioxidant, antibacterial, antidiabetic, and cytotoxicity potential of silver nanoparticles synthesized using the outer peel extract of Ananas comosus (L.). *PloS one*, *14*(8), e0220950.
27. Babu Maddinedi, S., Mandal, B. K., & Maddili, S. K. (2017). Biofabrication of size controllable silver nanoparticles–a green approach. *Journal of Photochemistry and Photobiology B: Biology*, *167*, 236-241.
28. Jadhav, K., Dhamecha, D., Bhattacharya, D., & Patil, M. (2016). Green and ecofriendly synthesis of silver nanoparticles: characterization, biocompatibility studies and gel formulation for treatment of infections in burns. *Journal of Photochemistry and Photobiology B: Biology*, *155*, 109-115.
29. Sharmila, C., Ranjith Kumar, R., & Chandar Shekar, B. (2018). Psidium guajava: a novel plant in the synthesis of silver nanoparticles for biomedical applications. *Asian J Pharm Clin Res*, *11*(1), 341-345.
30. Kumavat, S. R., & Mishra, S. (2021). Green synthesis of silver nanoparticles using Borago officinalis leaves extract and screening its antimicrobial and antifungal activity. *International Nano Letters*, *11*(4), 355-370.
31. Bhakya, S., Muthukrishnan, S., Sukumaran, M., Muthukumar, M., Kumar, S. T., & Rao, M. V. (2015). Catalytic degradation of organic dyes using synthesized silver nanoparticles: a

green approach. *Journal of Bioremediation & Biodegredation*, *6*(5), 1.

32. Sankar, R., Karthik, A., Prabu, A., Karthik, S., Shivashangari, K. S., & Ravikumar, V. (2013). Origanum vulgare mediated biosynthesis of silver nanoparticles for its antibacterial and anticancer activity. *Colloids and Surfaces B: Biointerfaces*, *108*, 80-84.
33. Sahreen, S., Khan, M. R., & Khan, R. A. (2010). Evaluation of antioxidant activities of various solvent extracts of Carissa opaca fruits. *Food chemistry*, *122*(4), 1205-1211.