Orginal Research Article
Sample Work

Biosynthesis, Antimicrobial, and Cytotoxic Effects of Silver Nanoparticles Using Acacia Concinna POD Extract and Kigelia Africa Leaf Extract

Abstract

Secondary metabolites found in plants include alkaloids, flavonoids, phenolic compounds, phytosterols, saponins, tannins, carbohydrates, proteins, lipids, and minerals. These secondary metabolites have a wide range of uses, including the production of nanoparticles. This effort involved the production of silver nanoparticles using Acacia Concinna pods and Kigelia Africana tree leaves. The production of silver nanoparticles was performed at room temperature, and their average size and shape were validated using the requisite experimental support, which included Fourier Transform Infrared (FT-IR), UV-visible spectroscopy, and a High-Resolution Scanning Electron Microscope (HRSEM). Furthermore, X-ray diffraction investigations (XRD), Atomic Force Microscopy (AFM), and Particle Size Analyzer (PAS) were performed and found to range from 20nm to 110nm. The N-Broth dilution approach was used to determine the Minimum Inhibitory Concentration (MIC) against Gram-positive (Bacillus subtilis) and Gram-negative (Escherichia coli) bacteria. MTT assay was used to test its potential Cytotoxicity against MCF-7 Human Breast cancer cell lines. The resulting silver nanoparticle was discovered to exhibit outstanding free radical scavenging action and extremely powerful antibacterial activity. The anticancer potential of AgNPs against (MCF-7) human breast cancer cell lines is highlighted in particular. According to the study findings, using biosynthesized silver nanoparticles provides tremendous advantages to humans.

Introduction

Nanotechnology is a fascinating field of study that is quickly expanding in modern material science. Because of their particular biological and physical features, nanoparticles play a major role in nanotechnology research in the domains of electronics, biology, and medicine [1]. Nanoparticle biosynthesis, particularly, has received increased interest in material synthesis due to its environmental friendliness. These nanoparticles come in a wide range of sizes and shapes. Various microbes created silver, gold, magnetic, and alloy and are widely employed in creating various chemicals such as pharmaceuticals, vitamins, steroids, proteins, flavours, etc. Silver, among nanoparticles, is crucial in medicine, biological systems, and living organisms [2-4]. In contrast, biosynthesized silver nanoparticles provide a variety of healthcare advantages.

Similarly, extracting medicinal plants and using silver nanoparticles is becoming more popular due to their different biological qualities. Antioxidant [5, 6], hepatoprotective [7, 8], anthelmintic [9, 10], anticancer [11], antibacterial [11], wound healing [12], and an antiproliferative agent are among its properties.

Despite the fact that significant original research article has been conducted using biosynthesis of silver nanoparticles to identify antimicrobial activity [13-17] and anticancer activity [18-20], only a small number of studies have focused on the combined effects of anticancer, antimicrobial, and antioxidant activity. Furthermore, from a health standpoint, there is a greater need for anticancer therapy and in vitro testing procedures [21]. Alkaloids, flavonoids, phenolics, and tannins are some of the bioactive elements found in plants [22-26]. Phytochemicals are regularly linked to a variety of health advantages [27]. As a result, two plants were chosen for our study:

Acacia concinna, a native tree, is one of the medicinal plants present in most regions of the Asian continent, and its body parts are utilized for various reasons. Their pods (for purgative, biliousness, skin ailments, and hair wash), leaves (for malaria), and barks are all utilized for medicinal purposes. Acacia concinna, sometimes known as 'Shikakai,' is a popular hair-washing plant in India and Sri Lanka [28].

Another tree in the Bignoniaceae family is Kigelia africana (Lam.) Beneath. This plant frequently treats various skin conditions, including leprosy, psoriasis, syphilis, fungal infections, cancer, eczema, and boils. The plant's leaves, wood, and roots contain luteolin, kigelinone, iridoids, kigelin, 6-hydroxyluteolin, and vernolic acid [29], which have antibacterial properties [30].

As a result, the study seeks to investigate the anticancer (biosynthesized silver nanoparticles against MCF-7 cell line), antioxidant (DPPH assay), and antimicrobial (against K.pneumoniae, E.coli, B.subtilis, and S.aureus) activity of silver nanoparticles synthesized by aqueous pod extracts of Acacia concinna and leaves of Kigelia africana. SEM, AFM, UV-Vis, FT-IR, and XRD methods characterize these silver nanoparticles.

Materials and Methods

Preparation of sample extracts:

Healthy plant samples of Acacia concinna pod extract and Kigelia africana leaves were acquired from the Chennai supermarket and the Anna University (CEG) campus, Guindy, respectively. To eliminate contamination, the pods and leaves of both plant samples were washed with tap water and surface sterilized with a 10% Sodium hypochlorite solution. It was then rinsed in sterile water and dried at room temperature. The samples are pulverized by using a pestle and mortar. 10 grams of crushed material were suspended in 50 mL of solvent (aqueous extracts) and incubated overnight in a rotatory shaker to remove fat compounds. Then, the incubated material was collected, the supernatant was discarded, and the leftover material was dried. After fractionating the residue into three parts, each component was suspended in a 250 ml conical flask and stored at 4°C overnight. Following incubation, the supernatant was filtered using Whatman No. 42 filter paper, and the filtrate was collected and used for further experimental study.

Phytochemical Screening:

1. Test for alkaloids:

3.0 mL of the extract was taken and dissolved in 5.0 mL of 2N HCl, which was then held in a boiling water bath to get the filtrate. This filtrate was separated into two parts, one treated with a few drops of Wagner's reagent and the other with Mayer's reagent. Precipitation was observed and used as an indication of the presence of alkaloids.

2. Test for tannin:

Similarly, tannin was detected when 10.0 mL of the extract was dissolved in 0.9% NaCl solution with 1.0% gelatin salt reagent. The development of precipitation indicates the presence of tannin.

3. Test for flavonoid:

5mL of the extract was obtained and mixed with a few drops of concentrated 2N HCl and magnesium turnings (0.5g). The presence of flavonoids is indicated by the emergence of pink or magenta red colour (within three minutes).

4. Test for steroids:

10 ML of the extract were combined with 10 ML of chloroform and 10 ML of sulfuric acid. The presence of steroids was detected, with the top layer becoming red and the acid layer turning yellow.

5. Test for carbohydrate:

10 ML of extract were combined with two drops of Molisch reagent. The solution is then carefully poured into a tube holding 2 mL of concentrated sulfuric acid. The formation of a purple ring between the surfaces shows the presence of carbohydrates.

Synthesis of silver nanoparticles:

Silver nanoparticles were synthesized using an aqueous solution (0.5mM) of silver nitrate (AgNO3). For silver ion reduction, 10 mL of extract was mixed with 40 mL of 0.5 mM AgNO3 solution. The nanoparticle production took 24 hours at room temperature. Later, the colour shift from yellow to dark brown indicates the creation of silver nanoparticles.

Characterization of silver nanoparticles:

The silver nanoparticles were determined by measuring the wavelength of the reaction mixture using the PerkinElmer spectrophotometer's UV-Vis spectrum at a 1 nm resolution (from 300 to 600 nm) in a 2 ml quartz cuvette with a 1 cm path length.

To explore plant extracts with the characterization of functional groups present on the surface of AgNPs, FTIR analysis (Shimadzu) was used. The spectral range used for scanning is 4000-400 cm-1 with a resolution of 4 cm-1. The sample is prepared by spreading the AgNPs uniformly into a matrix filled with dry KBr, which is then crushed to produce a clear disc. To examine, KBr is employed as a standard.

The study used an X-ray diffractometer and particle size analyzer to analyze silver nanoparticle performance under Cu K (α) radiation. The Malvern particle size analyzer was used to examine the size of synthesized silver nanoparticles. The High-Resolution Scanning Electron Microscope (HRSEM) was used to quantify the elemental composition of the prepared AgNPs. The sample was coated with platinum and then subjected to analysis. The Atomic Force Microscope (Veeco) was used to measure the surface morphology of the nanoparticles in normal atmospheric conditions. The SPM raster scans the probe over a small sample area to calculate local properties, such as probe, friction, height, and magnetism. The sample was poured on the small side and ranged over the contact mode of the instrument.

DPPH assay (2, 2-diphenyl-1-picrylhydrazyl):

The study aimed to determine the free radical scavenging activity of plant extracts and silver nanoparticles using an assay method based on Chang et al. (2002). The method involved measuring the decrease in absorption at 517 nm of DPPH solution after adding antioxidants. The experiment was conducted at room temperature, and absorption was observed after 20 minutes using α-Tocopherol as a reference. The assay method was measured using the following equation:

$$\% \text{ of DPPH Radical Scavenging Activity (\% RSA)} = \frac{\text{Abs. control} - \text{Abs. sample} * 100}{\text{Abs. control}}$$

Where Abs. The sample was referred to as the absorbance of DPPH radical + sample extract and Abs. Control is the absorbance of DPPH radical + ethanol. Measurements were performed in triplicates.

MTT Assay:

The MTT assay was used to determine the Cytotoxicity of biosynthesized silver nanoparticles using MCF-7 cell lines. The cells were plated in 96 microlitre well plates, washed twice, and starved at 37°C for an hour. After starvation, they were treated with a specific compound for 24 hours. After treatment, the medium was aspirated, and MTT (0.5 mg/ml) was added and incubated at 37°C for 4 hours. The MTT sample was then cleaned with PBS, and DMSO was added to dissolve the crystals. The sample was then measured using a purple and blue formazan dye spectrophotometer.

The below formula was used to measure the survival of the cell.

Viability% = (At/Ac) x100 Cytotoxicity % = 100 - % Viability.

Where Ac in the above formula refers to the control absorbance, At indicates the absorbance of the test sample. Absorbance value is essential to dilute the sample based on the value measured.

Antibacterial action of plant extracts and nanoparticles:

Well Diffusion Assay:

The assay method involves using Nutrient agar in petri dishes to solidify bacterial cultures. After 24 hours, five wells were made with test samples of different concentrations. The samples were mixed with DMSO/distilled water, and tetracycline was used as a control. The plates were incubated at 37 ºC for 24 hours, and the diameter of Inhibition was measured and calculated using the following equation.

% of Inhibition = I (diameter of the inhibited zone) / 90 (diameter of the Petri plates in mm) * 100 ------ (2)

Broth Dilution Method:

The method involved mixing nutrient broth with 0.1ml of growing cultures for 24 hours, then adding a specific plant extract concentration. The tubes were incubated at 37ºC for 24 hours, and the optical density at 600 nm was calculated using a spectrophotometer. The viable cell percentage was calculated using equation (3).

% of Inhibition = (Control O.D – Test O.D) / (Control O.D) * 100 ------ (3)

Results and Discussion

UV-Vis analysis:

The study used UV-Vis absorption spectra to examine the formation and stability of AgNPs synthesized from Acacia Concinna pod extract and Kigelia Africana leaf extract. Results showed a bioreductive formation of AgNPs, with a high-intensity surface Plasmon resonance band at 450 nm and 430 nm and a large number of weak absorption peaks at shorter wavelengths, indicating the presence of various organic compounds that can interact to reduce silver ions.

FTIR Analysis:

The FTIR spectrum of stabilized AgNPs shows narrower stretching vibrations due to aliphatic and aromatic –O.H. groups, shifting to 3425 cm-1 due to weakening intermolecular H-bonding. A new peak at 667 cm-1 is due to metal oxide formation. The FTIR spectrum of AgNPs from Acacia Concinna pod extract and Kigelia Africana leaf extract shows bands between 2000-2500 cm-1, corresponding to all samples.

The X-ray diffraction (XRD) pattern for AgNPs synthesized from pod extract of Acacia concinna and leaf extract of Kigelia Africana was obtained using a powder X-ray diffractometer instrument.

The major peaks at 77° (2θ), 64.45°, 44.32°, and 38.15° correspond to the reflections from the (311), (220), (200), and (111) planes, respectively. The XRD pattern indicates that silver nanoparticles formed by the reduction of Ag+ ions by Acacia Concinna and Kigelia Africana are crystalline in nature, with an average crystal size of 48 and 43, respectively.

$$D = 0.94 \lambda / \beta \, Cos\theta$$

Where θ is the reference peak width at an angle; β is the full width of maximum (FWHM), D is the average crystallite area size, and λ is the X-ray wavelength perpendicular to the planes. However, XRD is used to examine the crystal structure and chemical composition of material [35].

Particle size analyser:

The particle size analyzer (Malvern version 2.0) was used to assess the size distribution of particles in the 1-100 nm range using dynamic light scattering and three-dimensional photon correlation techniques. The upper limit number of particles was found to be 38nm. The mean particle size of silver nanoparticles synthesized via pod extract was found to be 37nm from the Acacia concinna plant and 70nm from the Kigelia africana plant, as shown in Figure 4.

Atomic force microscopy analysis:

The study uses AFM images to observe the morphology of prepared AgNPs in 2D and 3D forms. The silver nanoparticles were observed as needle-shaped, smooth surfaces and compacted structures in 3D images. The accumulation of nanoparticles was also observed in 3D frames. The AFM was primarily used for morphology observation, and the prepared AgNPs showed a singular propensity to form non-uniform-sized and shaped agglomerates.

HRSEM with EDAX analysis:

The study used Scanning Electron Microscopy (SEM) to study the size details and morphology of silver nanoparticles synthesized from pod extract of A. concinna and leaf extract of K. Africana. The nanoparticles were spherical, with a diameter of 20-110nm. EDAX analysis confirmed the presence of elemental silver, with a strong signal at 3 KeV. The study found that the nanoparticles were spherical, with a 20-110nm diameter. However, other studies showed a difference of 100-400nm.

Phytochemical Screening:

The study conducted phytochemical Screening on commercially available plant powder samples to detect flavonoids, tannins, steroids, and alkaloids. Silver nanoparticles were found in the plant extract, which changed from yellowish to bright yellow after incubation, indicating the presence of these nanoparticles.

APPLICATIONS OF SILVER NANOPARTICLES

Antibacterial activity:

Table 2 shows the differences in mean O.D. at 600nm and Inhibition of K.A. type between various microorganisms. The F-value for O.D. at 600nm is 3.662, with p-values less than 0.05. There is a significant difference in mean O.D. at 600nm between E.coli, B.subtilis, S.aureus, and K. pneumoniae. K. pneumoniae has a higher mean (1.38) in O.D. at 600nm, while the F-value for Inhibition is 4.308 and p-values less than 0.05. E. coli has a higher mean (34.50) in Inhibition. Similarly, there is a significant difference in mean O.D. at 600nm and Inhibition of A.C. type between E.coli, B.subtilis, S.aureus, and K.pneumoniae. S. aureus has a higher mean (1.40) in O.D. at 600nm but no significant difference in mean Inhibition.

The primary research used Tukey HSD for multiple comparisons to select the best pairwise comparisons when the sample size is unequal. Table 2 reveals significant differences in O.D. at 600nm for K.A. type between E. coli and K. pneumonia and E. coli and B. subtilis. However, no differences were found between E. coli and S. aureus, K. pneumoniae and S. aureus, K. pneumoniae and B. subtilis, and S. aureus and B. subtilis.

In Inhibition, there is a significant difference between E. coli and S. aureus ($p=0.033$), E. coli and K. pneumoniae ($p=0.027$). However, there were no differences between E. coli and B. subtilis ($p=0.052$), K. pneumoniae and S. aureus ($p=0.998$), K. pneumoniae and B. subtilis ($p=0.992$), S. aureus and B. subtilis ($p=1.000$). It shows that biosynthesized silver nanoparticles inhibited different microorganisms. Silver nanoparticles have a wide range of antibacterial activity and kill Gram-positive and Gram-negative microbes such as E. coli, B. subtilis, K. pneumonia and S. aureus.

Similarly, the result shows that there is a significant difference in O.D. at 600nm for A.C. type between the E. coli and S. aureus (p = 0.004), E. coli and B. subtilis (p=0.005), K. pneumoniae and S. aureus (p=0.025), K. pneumoniae and B. subtilis (p=0.031). However, there were no differences between E. coli and K. pneumoniae (p=0.895), S. aureus and B. subtilis (p=1.000).

In Inhibition, there were no differences between E. coli and K. pneumoniae (p=0.907), E. coli and S. aureus (p=0.229), E. coli and B. subtilis (p=0.186), K. pneumoniae and S. aureus (p=0.674), K. pneumoniae and B. subtilis (p=0.578). It shows that a biosynthesized silver nanoparticle does not inhibit different microorganisms.

Free radical scavenging activity:

Table 4 compares the RSA (%) between various plant extracts, including AGNP+AC and AGNP+ KA. The finding shows that the t-value for RSA (%) of both AGNP+ AC and AGNP+KA extract shows a significant difference in free radical scavenging activity (Figure 8)

Anticancer activity:

Table 5 presents the difference in MTT assay (AC/AGNPS and KA/AGNPS) between trials. Since the p-value is greater than 0.05 for AC/AGNPS and KA/AGNPS, there is no significant difference in mean MTT assay between trials. However, the figure representing Cell proliferation of trials 1 and 2 is almost similar. The silver nanoparticles synthesized from the pods of Acacia Concinna show better anticancer activity between the two extracts (Figure 9).

Conclusion

The study demonstrates a cost-effective and simple method for synthesizing silver nanoparticles using plant extracts. The resulting stable nanoparticles, crystalline and ranging from 37-75 nm in size, exhibit excellent antimicrobial activity, antioxidant properties, and cytotoxic effects on MCF-7 cells. These nanoparticles have potential applications in medicine.

The study also found differences in mean O.D. at 600nm and Inhibition of K.A. type between various microorganisms, including E. coli, B. subtilis, S. aureus, and K. pneumoniae. K. pneumoniae showed higher mean O.D. and Inhibition, while E. coli had higher Inhibition. S. aureus had a higher mean O.D. at 600nm but no significant difference in mean Inhibition. The study demonstrates that biosynthesized silver nanoparticles inhibit various microorganisms, showing a wide range of antibacterial activity.

Therefore, it is concluded that the biosynthesized silver nanoparticles would be most effective in drug delivery. Future research is essential to completely characterize the molecular mechanisms and toxicity of silver nanoparticles' anticancer and antimicrobial activity.

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TABLE 1: PHYTOCHEMICAL INVESTIGATION OF ACTIVE CONSTITUENTS IN PLANTS POWDERS

S.No	Phytochemicals	Acacia concinna	Kigelia africana
1.	Alkaloids	+	+
2.	Flavonoids	+	+
3.	Phytosterols	+	+
4.	Saponin	+	-
5.	Tannin	+	-
6.	Phenolic compounds	-	-
7.	Gums and Mucilage	-	-
8.	Carbohydrates	-	+
9.	Steroids	-	+

TABLE 2: DIFFERENCE IN MEAN OD AT 600NM, INHIBITION BETWEEN VARIOUS MICROORGANISMS

Type			Mean±SD	F-value	p-value
KA	OD at 600nm	E.coli	1.07±0.33	3.662	0.022*
		K.pneumoniae	1.38±0.14		
		S.aureus	1.31±0.17		
		B.subtilis	1.36±0.21		
	Inhibition (%)	E.coli	34.50±19.22	4.308	0.014**
		K.pneumoniae	13.42±8.01		
		S.aureus	14.71±10.62		
		B.subtilis	15.47±12.73		
A.C.	O.D. at 600nm	E.coli	1.17±0.18	7.474	0.001**
		K.pneumoniae	1.21±0.13		
		S.aureus	1.40±0.08		
		B.subtilis	1.39±0.11		
	Inhibition (%)	E.coli	16.08±12.64	2.075	0.136
		K.pneumoniae	12.70±9.73		
		S.aureus	6.62±3.31		
		B.subtilis	5.46±2.08		

**p<0.01, *p<0.05

TABLE 3: MULTIPLE COMPARISONS USING TUKEY HSD

Type	Dependent Variable	(I) Group	(J) Group	Mean Difference (I-J)	S.E.	p-value
KA	Inhibition (%)	E. coli	K. pneumoniae	-.306033*	.105749	.032
			S. aureus	-.235144	.105749	.138
			B. subtilis	-.288033*	.105749	.048
		K. pneumoniae	E. coli	.306033*	.105749	.032
			S. aureus	.070889	.108496	.914
			B. subtilis	.018000	.108496	.998
		S. aureus	E. coli	.235144	.105749	.138
			K. pneumoniae	-.070889	.108496	.914
			B. subtilis	-.052889	.108496	.961
		B. subtilis	E. coli	.288033*	.105749	.048
			K. pneumoniae	-.018000	.108496	.998
			S. aureus	.052889	.108496	.961
		E. coli	K. pneumoniae	21.07571*	6.98177	.027
			S. aureus	19.79375*	6.74503	.033
			B. subtilis	19.03000	6.98177	.052
		K. pneumoniae	E. coli	-21.07571*	6.98177	.027
			S. aureus	-1.28196	6.98177	.998

			B. subtilis	-2.04571	7.21074	.992
			E. coli	-19.79375*	6.74503	.033
			K. pneumoniae	1.28196	6.98177	.998
		S. aureus	B. subtilis	-.76375	6.98177	1.000
			E. coli	-19.03000	6.98177	.052
			K. pneumoniae	2.04571	7.21074	.992
		B. subtilis	S. aureus	.76375	6.98177	1.000
			K. pneumoniae	-.043111	.061334	.895
			S. aureus	-.227111*	.061334	.004
		E. coli	B. subtilis	-.222111*	.061334	.005
			E. coli	.043111	.061334	.895
			S. aureus	-.184000*	.061334	.025
		K. pneumoniae	B. subtilis	-.179000*	.061334	.031
			E. coli	.227111*	.061334	.004
			K. pneumoniae	.184000*	.061334	.025
		S. aureus	B. subtilis	.005000	.061334	1.000
			E. coli	.222111*	.061334	.005
			K. pneumoniae	.179000*	.061334	.031
AC	OD at 600nm	B. subtilis	S. aureus	-.005000	.061334	1.000

			K. pneumoniae	3.38050	5.04981	.907
			S. aureus	9.45917	4.78384	.229
		E. coli	B. subtilis	10.61850	5.04981	.186
			E. coli	-3.38050	5.04981	.907
			S. aureus	6.07867	5.36375	.674
		K. pneumoniae	B. subtilis	7.23800	5.60226	.578
			E. coli	-9.45917	4.78384	.229
			K. pneumoniae	-6.07867	5.36375	.674
		S. aureus	B. subtilis	1.15933	5.36375	.996
			E. coli	-10.61850	5.04981	.186
			K. pneumoniae	-7.23800	5.60226	.578
	Inhibition (%)	B. subtilis	S. aureus	-1.15933	5.36375	.996

**p<0.01, *p<0.05

TABLE 4: DIFFERENCE IN MEAN RSA BETWEEN VARIOUS SOLVENT EXTRACTS

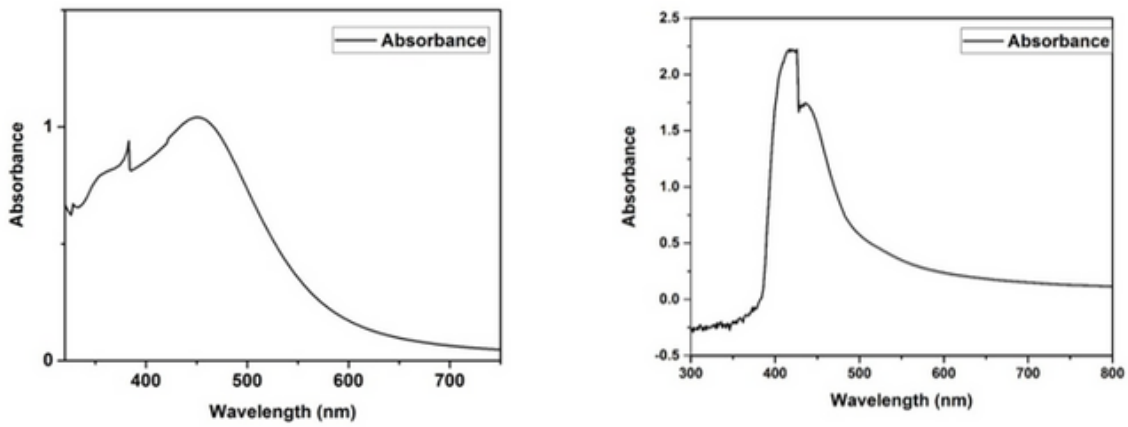
Group	RSA (%)	t-value	p-value
	Mean±SD		
AGNP	20.15±7.42	-2.370	0.045*
A.C.	35.21±12.13		
AGNP	22.05±6.10	-2.281	0.052*
K.A.	34.11±10.13		

*p<0.05

TABLE 5: DIFFERENCE IN MEAN MTT ASSAY BETWEEN TRIALS

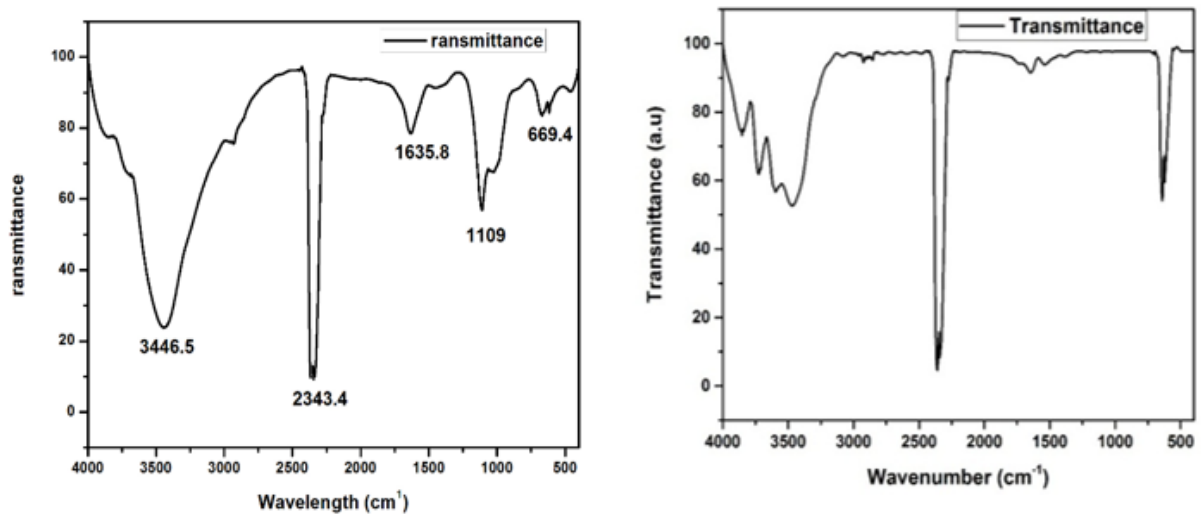
MTT Assay	Trial		t-value	p-value
	Trial 1	Trial 2		
	Mean±SD			
AC/AGNPS	54.13±26.48	53.83±27.07	0.029	0.977
KA/AGNPS	46.03±29.61	46.08±29.66	-0.005	0.996

LIST OF FIGURE



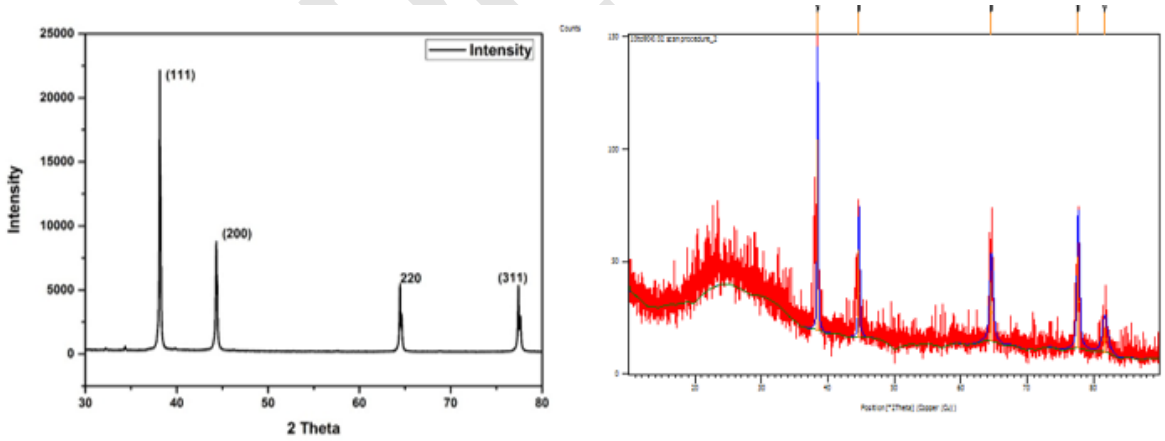
(a) (b)

Fig. 1: UV-Vis spectra of silver nanoparticles synthesized from two plants (a) Acacia concinna and (b) Kigelia africana.



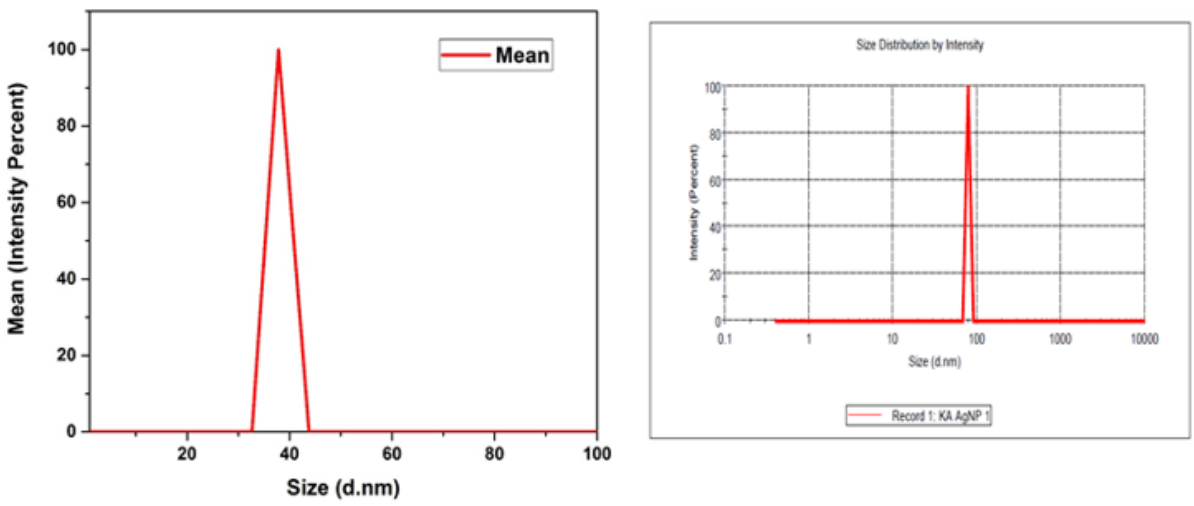
(a) (b)

Fig. 2: FTIR spectra analysis of silver nanoparticles synthesized from two plants (a) Acacia concinna (b) Kigelia africana.



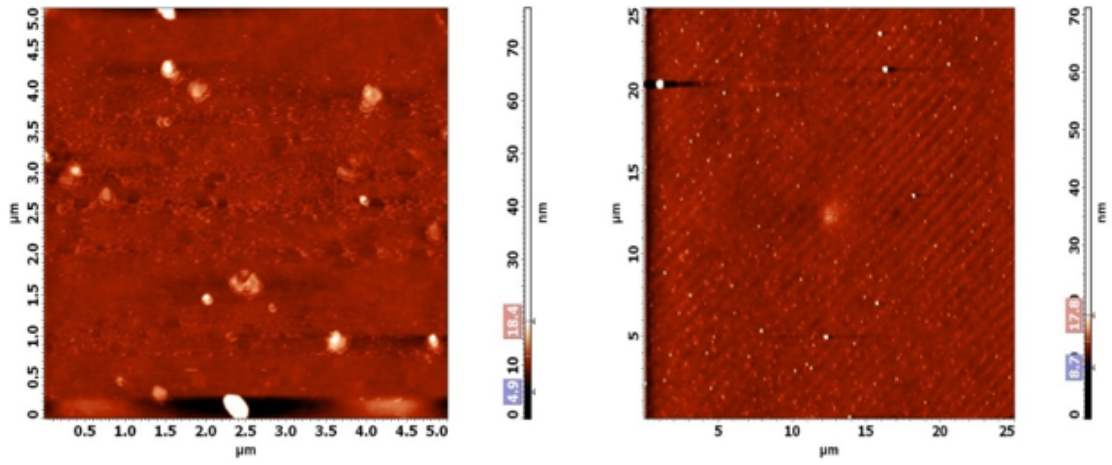
(a) (b)

Fig. 3: X-ray diffraction pattern of silver nanoparticles synthesized from two plant powders (a) Acacia concinna (b) Kigelia africana.



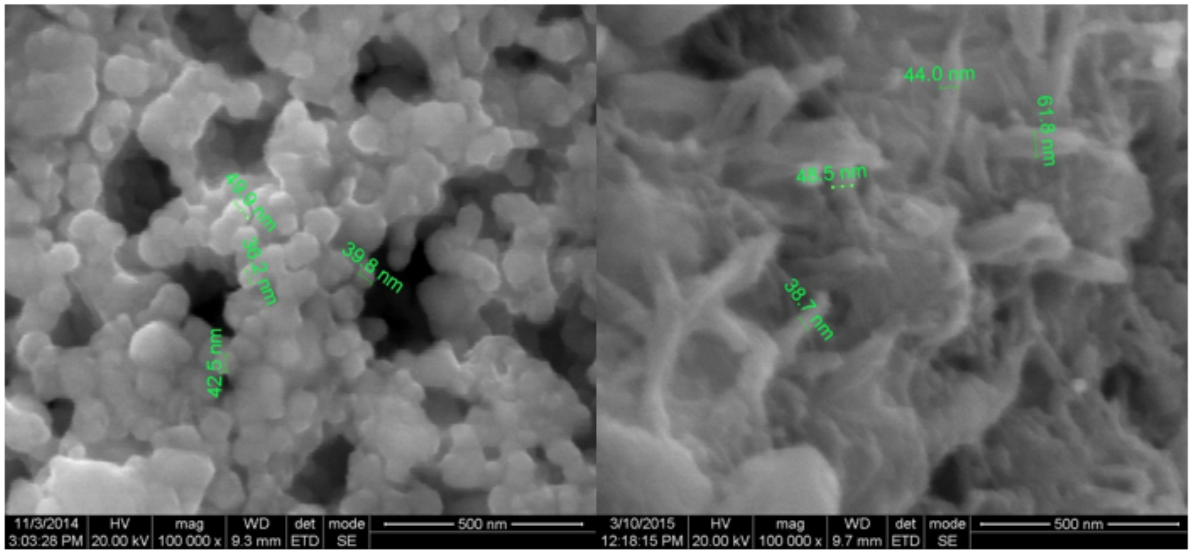
(a) (b)

Fig. 4: PSA of silver nanoparticles synthesized from two plants (a) Acacia concinna (b) Kigelia Africana



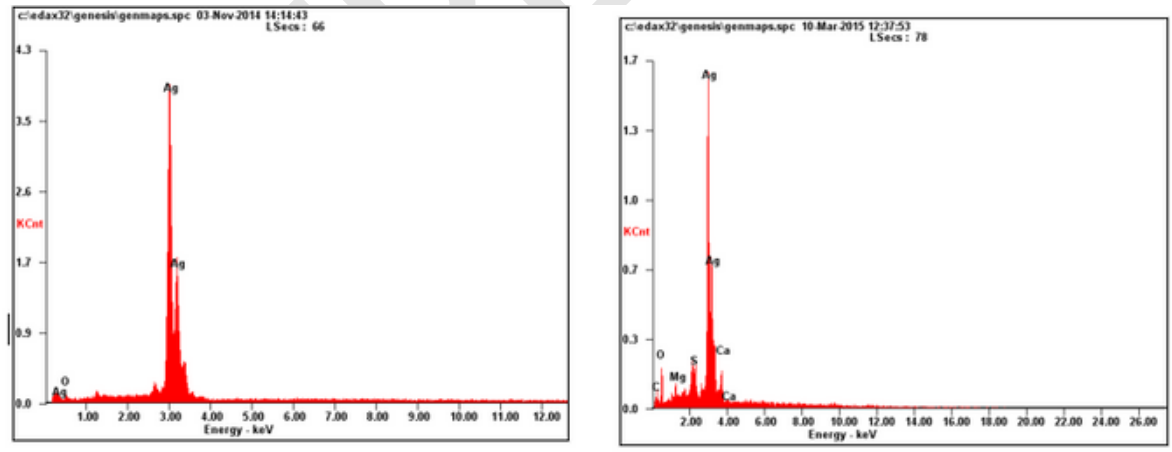
(a) (b)

Fig. 5: AFM images of silver nanoparticles synthesized from two plant powders (a) Acacia concinna (b) Kigelia africana.



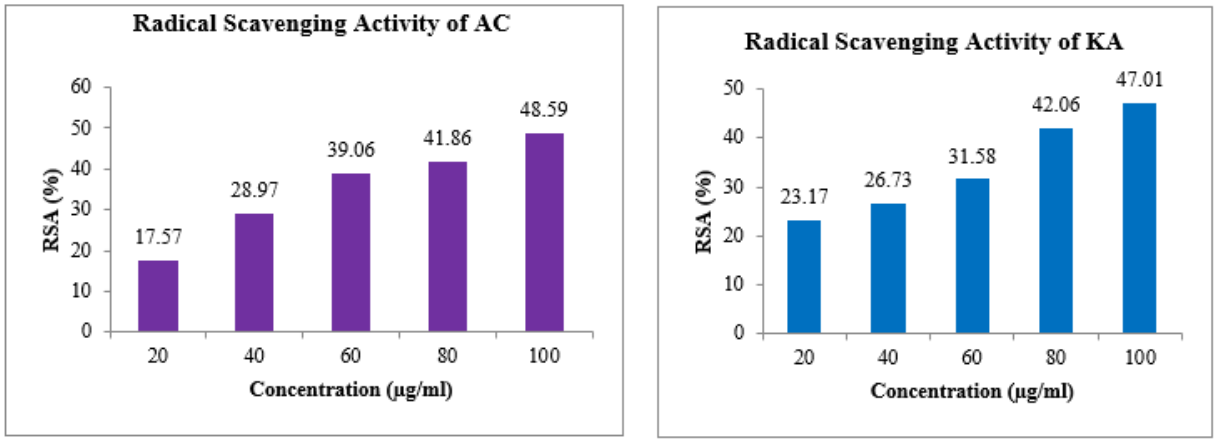
(a) (b)

Fig. 6: SEM images of silver nanoparticles synthesized from two plant powders (a) Acacia concinna (b) Kigelia Africana



(a) (b)

Fig. 7: EDAX images of silver nanoparticles synthesized from two plant powders (a) Acacia concinna (b) Kigelia africana.



(a) (b)

Fig. 8: Radical Scavenging Activity of two plants (a) Acacia concinna (b) Kigelia africana.

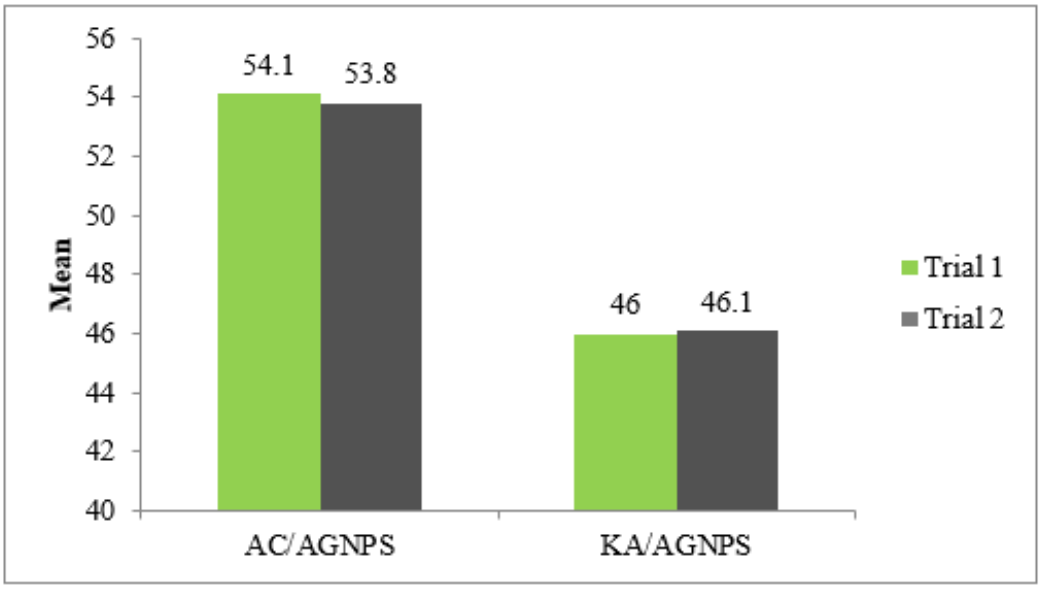


Fig. 9: Mean MTT assay for trial 1 and trial 2.

For further details, please see our website blog and Original Research Article.

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https://pubrica.com/academy/original-research-article/suggestions-given-by-peer-reviewers-in-the-introduction-section-of-the-original-research-article/

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