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Original Article

Comparative Study of Silver Nanoparticles: Green Synthesis, Characterization and Biological Evaluation of Carica papaya and Andrographis paniculata Leaf Extract

C. Shunmugadevi [a], and P. Palanisamy[b*]

[a] Department of Animal Science, Manonmaniam Sundaranar University, Tirunelveli–627 012, TN, India
[b*]Department of Chemistry, Pioneer Kumaraswamy College, Nagercoil-629003, TN, India

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ABSTRACT

Green synthesized the silver nanoparticles from Carica papaya and Andrographis paniculata fresh and dried leaves extracts. The entire silver nanoparticles have been characterized by UV–visible spectrophotometer, SEM, FTIR, and X-ray diffraction method. The average size of fresh leaves *Carica papaya* and *Andrographis paniculata* silver nanoparticles between 5-30 and 40-60 nm and average size of dried leaves *Carica papaya* and *Andrographis paniculata* silver nanoparticles between 20-60 and 50-70 nm. The result showed that the particles were of spherical and cubic shape in case of fresh and dried leaves *Carica papaya* and *Andrographis paniculata* silver nanoparticles. Fresh leaves *Carica papaya* silver nanoparticles exhibited better antimicrobial and DPPH radical scavenging than the dried leaves *Carica papaya* silver nanoparticles. Similarly the fresh and dried leaves *Carica papaya* and *Andrographis paniculata* silver nanoparticles can cleave DNA completely compared to untreated DNA.

Keywords: Silver Nanoparticle, Carica papaya, Andrographis paniculata, and Biological evaluation.

INTRODUCTION

Nanobiotechnology is one of the most promising areas in modern nanoscience and technology. Nanoscience has been established recently as a new interdisciplinary science. It can be defined as a whole knowledge on fundamental properties of nano-size objects [3]. Size and shape of nanoparticles provide an efficient control over many of the physical and chemical properties [4], and their potential application in optoelectronics [5], recording media [6], sensing devices [7], medicine[8] and catalysis hence, synthesis and characterization of nanoparticles is now a day's an important area of research. Application of AgNPs is wide, such as in biomedical engineering, drug delivery, food industries, agriculture, textile industries, water treatment as an antioxidant, antimicrobial and anticancer agent, cosmetics, ointments, and as larvicides.[9-15] Different processes have been developed for AgNP synthesis such as chemical and photochemical reduction, thermal evaporation, sonoelectrochemical, and microwaveassisted process.[16-18] There is a growing need to introduce environmental-friendly methods for AgNPs without using hazardous chemicals. Biological synthesis has emerged as a green alternative and these approaches have numerous advantages over chemical and physical syntheses, because it is cost-effective, environment-friendly, and can be easily scaled up. It has great potential with environmentally benign materials such as plant extract,[19] and use of plant extracts has great advantage as they eliminate the elaborate process of maintaining cell cultures and can be suitably used for mass-scale production under no aseptic environments.

Carica papaya belongs to family Caricaceae and commonly known as Papaya, Paw Paw, Kates, and Papaw. The *Carica papaya* is one of the medicinal plants. The papaya fruits, bark, leaves are being used as medicine to treat various diseases [20] The literature suggests that C. papaya fruit and leaf extracts are being used to treat dengue fever [21] to increase RBC and platelet counts [22]. It is also reported that the C. papaya leaf extract works against sickling of RBC [23]. The extract of C. papaya leaves and fruit is rich in vitamins, phenols, proteolytic enzymes which acts as a good antioxidant and an excellent antimicrobial agent [24, 25]. A. paniculata is predominantly used as a constituent in various ayurvedic formulations [26]. A. paniculata contains major bioactive compounds

***Corresponding Author Address:** Dr. **P. Palanisamy,** Department of Chemistry, Pioneer Kumaraswamy College, Nagercoil-629003, TN, India*

such as diterpenoids, flavonoids and polyphenols [27] and shows multiple biological actions [28, 29]. In the present work comparative study of silver nanoparticles: green synthesis, characterization and biological evaluation of carica papaya and andrographis paniculata leaf extract.

MATERIALS AND METHODS

Materials: Freshly leaves of *Carica papaya* and *Andrographis paniculata* were collected from Kasilingapuram village at Thoothukudi Dist. Silver nitrate ($AgNO_3$) was obtained from Aldrich Chemicals. All glassware have been washed with sterile distilled water and dried in an oven before use.

Methods

Preparation of fresh *Carica papaya* and *Andrographis paniculata* leaf extract: The experimental work was carried out by preparing leaf extract with 10 g of fresh *Carica papaya* and *Andrographis paniculata* leaves collected and thoroughly washed the leaves with running tap water and distilled water for 3–4 times. The leaves were then chopped into fine pieces and boiled in a 250 ml Erlenmeyer flask by stirring at 60 ºC for 1h. After cooling, the extract was filtered using whatman No.1 filter paper and stored at 4 °C for further experimental work.

Preparation of dried *Carica papaya* and *Andrographis paniculata* leaf extract: The plant was washed with tap water, rinsed with distilled water and air dried for 2 h and cut into small pieces and allowed to dry at room temperature (37°C) for a week. The dried *Carica papaya* and *Andrographis paniculata* was ground to fine powder and stored at 37°C. About 10 g of *Carica papaya* and *Andrographis paniculata* powder was weighed and dissolved in 250 ml of distilled water and kept at 60°C for 1 h. The aqueous extract of *Carica papaya* and *Andrographis paniculata* was filtered and stored at 4 °C for further studies.

Bio synthesis of silver nanoparticles: About 10 ml of whole plant aqueous extract of *Carica papaya* and *Andrographis paniculata* was mixed with 40 ml of 1 mM silver nitrate solution (1:4) and kept in magnetic stirrer at 60 °C for 30 min and the color change was observed. The colour change indicated the formation of silver nanoparticles by *Carica papaya* and *Andrographis paniculata* leaf extract. The bio reduction of silver ions in the solution was monitored by sampling the aqueous component after incubation period and the absorption maxima was scanned at different wavelengths (420-500 nm) using a UV-Visible spectrophotometer [30].

Characterization of silver nanoparticles

After the incubation period, the silver nitrate treated *Carica papaya* and *Andrographis paniculata* extract was centrifuged at 9,000 rpm for 15 min. The supernatant was taken for the analysis of size, shape and stability of the bioreduced silver nanoparticles using UV Spectroscopy, Scanning Electron Microscope (SEM), Transmission Electron Microscope (TEM), X-Ray Diffraction analysis and Fourier Transform Infra-Red Spectroscopy (FTIR).

UV-Visible spectrometric analysis of silver nanoparticles: An PERKIN ELEMER (Lambda 35 model) UV-Visible spectrophotometer was employed for the spectrometric analysis of biosynthesized silver nanoparticles. The reduction of silver was measured periodically at 200-800 nm. A spectrum of silver nanoparticles was plotted with wave length on x-axis and absorbance on y-axis.

Fourier transform infrared (FTIR) analysis of silver nanoparticles: The $AgNO_3$ treated *Carica papaya* and *Andrographis paniculata* extract was centrifuged at 9,000 rpm for 25 min. The pellet was washed thrice with 20 ml of deionized water to get rid of free proteins/enzymes. The residue was dried and mixed with potassium bromide (KBr). The pellet was used for FTIR analysis in the range of 400-4000 cm-1 at a resolution of 4 cm^{-1}.

SEM analysis of silver nanoparticles: Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid. The excess solution was removed using a blotting paper. The film on the SEM grid was dried under a mercury lamp for 5 min. The thin film on grid was examined using Scanning Electron Microscope.

X-Ray Diffraction analysis of silver nanoparticles: X-ray diffraction measurements of biologically reduced silver-nitrate solution drop coated onto glass slides were determined by an X'Pert Pro P Analytical X-ray diffractometer instrument with Xan'Pde high score plus software operating at a voltage of 40 kV and a current of 30 mA with Cu K radiation. The scanning of biosynthesized silver nanoparticles is exercised in 2θ region and the data is analyzed. The Debye–Scherrer equation was employed to calculate the average particle size of the silover nanoparticle [31].

$D = k\lambda/\beta 1/2 \cos\theta$

Biological Evaluation

Antimicrobial evaluation: Standard sterilized filter paper discs (5 mm diameter) impregnated with a solution of the test compound in DMSO

(1mg/mL) was placed on an agar plate seeded with the appropriate test organism in triplicates. Grampositive bacteria, *Staphylococcus aureus, Streptococcus pneumoniae, and Klebsiella pneumoniae* as well as Gram-negative bacteria, *Pseudomonas aeruginosa, and Escherichia coli* were used. They were also evaluated for their in vitro antifungal potential against *Candida albicans, Aspergillus flavus*, and *Aspergillus niger* strains. Ampicillin was used both as a standard antibacterial agent and antifungal agent. DMSO alone was used as control at the above-mentioned concentration. The plates were incubated at 37 ^{0}C for 24h for bacteria and 28 0C and 48h for fungi. Compounds that showed significant growth inhibition zones (>20 mm) using the twofold serial dilution technique were further evaluated for their minimal inhibitory concentrations (MICs).

Minimal inhibitory concentration (MIC) measurement: Broth dilution test is used to determine 'Minimum Inhibitory Concentration (MIC)' of the above mentioned samples. The micro dilution susceptibility test was used for the determination of antibacterial and antifungal activity, respectively. Stock solutions of the tested compounds, amikacin, chloroamphenicol, and clotrimazole were prepared in DMSO at concentrations of 1000 mg/mL followed by twofold dilution at concentrations of 500, 250, 3.125 mg/mL. All the plates were incubated at 37^{0}C for 24 h for bacteria and at 280C for 48 h for fungi and the minimal inhibitory concentrations (MIC) were determined. Control experiments were also done [32, 33].

DNA cleavages: For investigation of the DNA cleavage potential of the silver nanoparticle were used as the test candidate. Electrophoresis sample solutions were prepared by 4.0 µL of the solution (prepared by dissolving of 5 mg of each compound in 1 mL DMSO) and 4.0 µL DNA. The samples were incubated at 37 ˚C for 2h. After incubation, the samples were mixed with bromophenol blue dye and then were carefully loaded into the wells along with the standard DNA (alone), the DNAH2O2 mixture. Gel electrophoresis performance on the samples was carried out at constant 100 V of electricity for about 30 min. The resultant bands of electrophoresis were visualized by UV light and then photographed [34].

DPPH radical scavenging assay: Scavenging effect of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was measured according to the procedure described by Blois with a slight modification [26]. Different concentrations (5µg, 50µg, and 100µg) of samples in dimethyl sulfoxide (DMSO), were taken in a series of test tubes. The volume was adjusted to 500µl by adding methanol. Five milliliters of a 0.1 mM methanolic solution of DPPH (Sigma–Aldrich, Bangalore) was added to these tubes and shaken vigorously. A control without the test compound, but with an equivalent amount of methanol was maintained. The tubes were allowed to stand at room temperature for 20 min. The absorbance of the samples was measured at 517 nm. Ascorbic acid (AA) was used as the reference [35].

Free radical scavenging activity was calculated using the following formula:

$$\% \text{ Radical scavenching activity} = \frac{(\text{control OD} - \text{sample OD})}{\text{control OD}} \times 100$$

RESULTS AND DISCUSSION

Visible Observation: According to literature studies silver nanoparticle solution has dark brown or dark reddish colour. Plant extracts from fresh leaves of *Carica papaya* and *Andrographis paniculata* extracts before the addition of $AgNO_3$ its colour was red and greenish yellow, but after its treatment with $AgNO_3$ its colour changes to dark brown and dark reddish which indicated the formation of AgNPs. Likewise the dried leaves of *Carica papaya* and *Andrographis paniculata* extracts colour changed to dark brown and dark reddish after treatment with $AgNO_3$ (Fig. 1). This colour change is due to the property of quantum confinement, which is a size dependent property of nanoparticles, which affects the optical property of the nanoparticles.

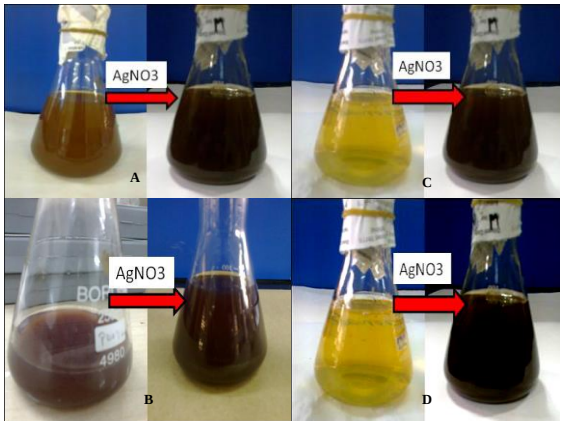

Fig. 1 Colour change of plant extract before after addition of AgNO3, A and B Fresh and dried leaves of *Carica papaya*, C and D. Fresh and dried leaves of *Andrographis paniculata*

UV-Vis spectrophotometer analysis: Reduction of silver ions into silver nanoparticles during exposure to plant extracts was observed as a result of the color change. The color change is due to the Surface Plasmon Resonance phenomenon. The metal nanoparticles have free electrons, which give the SPR absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with light wave. The sharp bands of silver nanoparticles were observed around 421 and 419 nm in case of fresh and dried leaves of *Carica papaya* Fig. 2 whereas the band for fresh and dried leaves of *Andrographis paniculata* were observed around 418 and 416 nm (Fig. 2). From different literatures it was found that the silver nanoparticles show SPR peak at around 420 nm [36]. So we confirmed that *Carica papaya* leaf extract has more potential to reduce Ag ions into Ag nanoparticles than *Andrographis paniculata* extract. The reduction of the metal ions occurs fairly rapidly; more than 90% of reduction of Ag^{+} ions is complete within 1 Hrs. after addition of the metal ions to the plant extract. The metal particles were observed to be stable in solution even 4 weeks after their synthesis. By stability, we mean that there was no observable variation in the optical properties of the nanoparticle solutions with time.

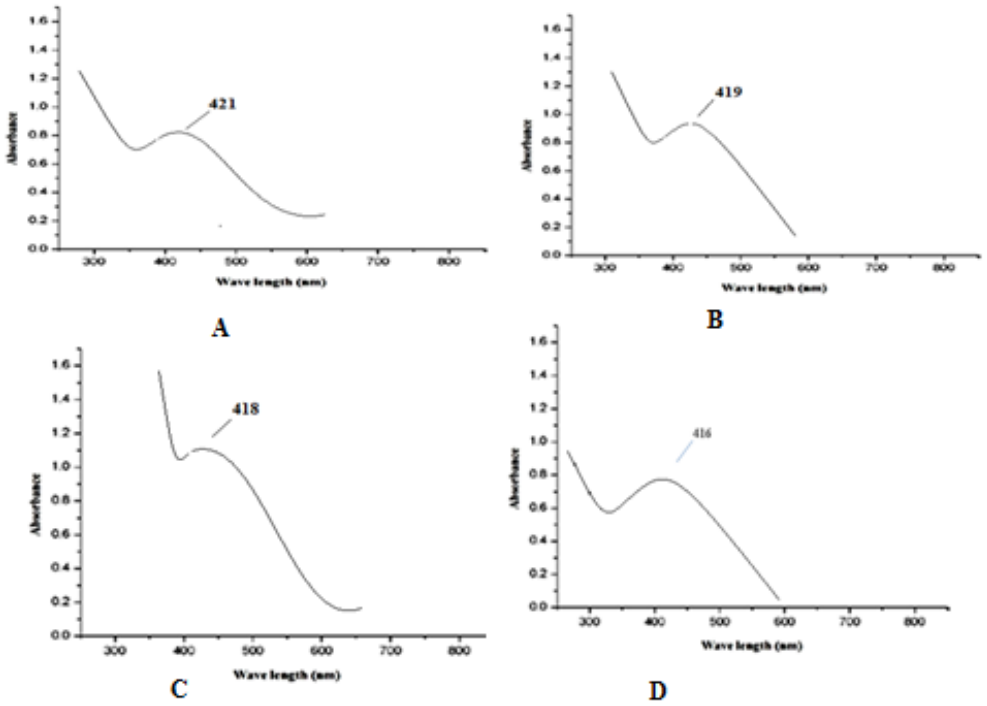

Fig. 2 UV-visible absorption peaks A and B fresh and dried leaves - *Carica papaya*, C. fresh leaves - *Andrographis paniculata*, *D.* Dried leaves - *Andrographis paniculata*

FTIR spectrophotometer analysis

Carica papaya: FTIR measurements were carried out to identify the biomolecules for capping and efficient stabilization of the metal nanoparticles synthesized. The FTIR spectrum of silver nanoparticles in case both of fresh and dried leaves Carica papaya showed sharp absorbance between 400 and 4000 cms-1 (Fig. 3). The IR peaks in the spectrum at 3186 & 3201, 1796 & 1686, 1596 & 1569, 1398 & 1384, 1256 & 1292, 986 & 889, 798 & 800, which could be the amide, carbonyl (polyol's) esters, heterocyclic compounds, nitro compounds, ethers and chloro substituted compounds. The absorption peak at 1686 & 1796 cm-1 could be due to the amide bond coming from the carbonyl group of a protein and the peak at 3186 & 3201 cm-1 may be because of OH groups present in alcohols and phenolic groups [37].

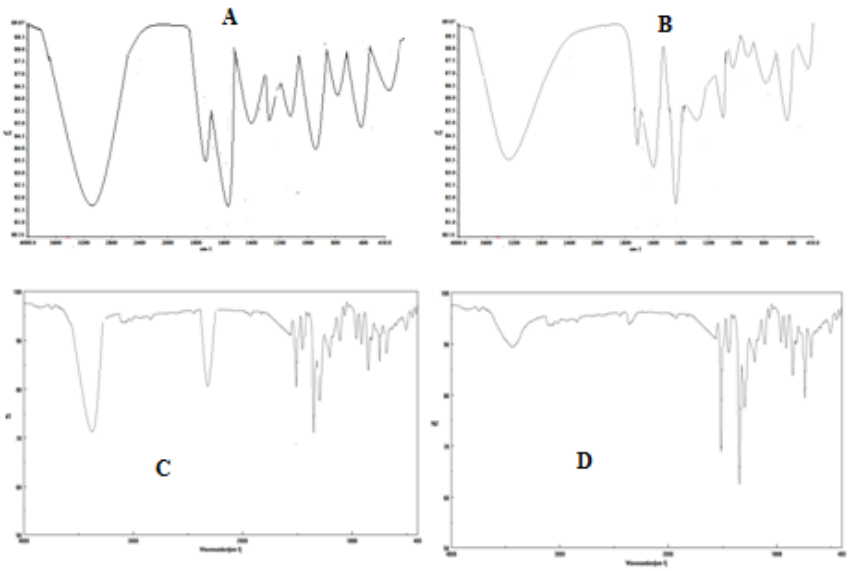

Fig. 3 FTIR spectra A and B fresh and dried leaves - *Carica papaya*, C. fresh leaves - *Andrographis paniculata, D.* Dried leaves - *Andrographis paniculata*

Andrographis paniculata: The FTIR spectroscopy measurement was studied to identify the possible biomolecules responsible as capping and reducing agents for the AgNPs synthesized by extract (Fig. 4.11 and 4. 12). The peaks near 2392 & 2396 cm-1 could be due to the aldehydic N-H stretching correlating to a amino group. The peak observed for AgNPs at 1796 & 1812 cm-1 C=O groups of aldehyde and carboxylic group. It was also possible that terpenoid played a role in the reduction of metal ions by oxidation of aldehydic groups in the molecules to carboxylic acids. The presence of peaks at 1203 & 1198 cm-1 indicated that the AgNPs might be surrounded by ester compounds and 1054 & 1086 cm-1 showed a sulphonic compounds. Based on the physical state of the extracts and the characteristic features of the infrared vibrational peaks in the spectra, terpenoids and secondary amide derivatives were found to be the possible compounds in the obtained nanoparticle [38].

Scanning Electron Microscopy (SEM)

Carica papaya: SEM provided further insight into the morphology and size details of the silver nanoparticles. Comparison of experimental results showed that the diameters of prepared nanoparticles in the solution have sizes of several µm in case of fresh and dried leaves *Carica papaya*. (Fig.4). The size of the prepared nanoparticles was more than the size of the nanoparticle between 1 and 100 nm. The average size of fresh leaves *Carica papaya* silver nanoparticles between 5 and 30 nm and average size of dried leaves *Carica papaya* silver nanoparticles between 20 and 60 nm. The size was more than the desired size as a result of the proteins which were bound on the surface of the nanoparticles. The result showed that the particles were of spherical shape in case of fresh and dried leaves *Carica papaya*.

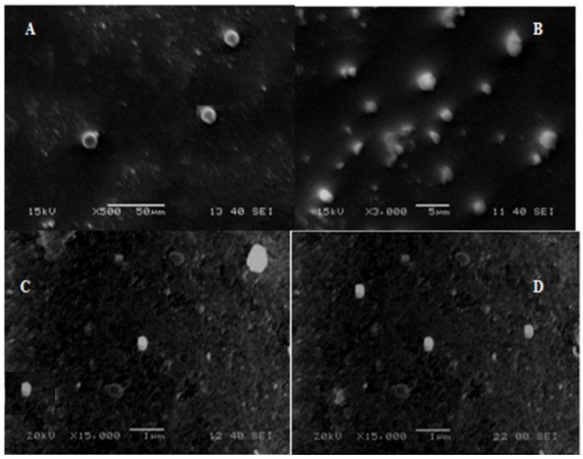

Fig. 4. 16 Scanning electron microscopic images of silver AgNPs A and B fresh and dried leaves - *Carica papaya*, C. fresh leaves - *Andrographis paniculata, D.* Dried leaves - *Andrographis paniculata*

Andrographis paniculata: A scanning electron microscope was employed to analyze the shape of the silver nanoparticles that were synthesised by fresh and dried leaves of *Andrographis paniculata* . SEM technique was employed to visualize the size and shape of the synthesized AgNPs (Fig. 4.). The The formation of nanoparticles as well as their

morphological dimensions in the SEM study demonstrated that the fresh leaves *Andrographis paniculata* average size was between 40 and 60 nm and fresh dried leaves *Andrographis paniculata* average size was between 50 and 70 nm. The result showed that the particles were of cubic shape in case of fresh and dried leaves *Andrographis paniculata* [39].

XRD analysis (*Carica papaya*): The fresh and dried leaves *Carica papaya* silver nanoparticles XRD data show diffraction peaks at 2θ= 38.0-38.3º, 44.1-44.4 º, 64.1-64.6 º, 77.2-77.5 º, and can be indexed to (111), (200), (220), and (222) planes of pure silver ions indicating the biosynthesis of silver nanoparticles (Fig. 5). The diffraction peaks obtained in XRD corresponded to face centred cubic structure of metallic silver ions in its purest form and the size of particle ranging between size of fresh leaves *Carica papaya* silver nanoparticles 5-30 nm and dried leaves *Carica papaya* silver nanoparticles between 20 and 60 nm.

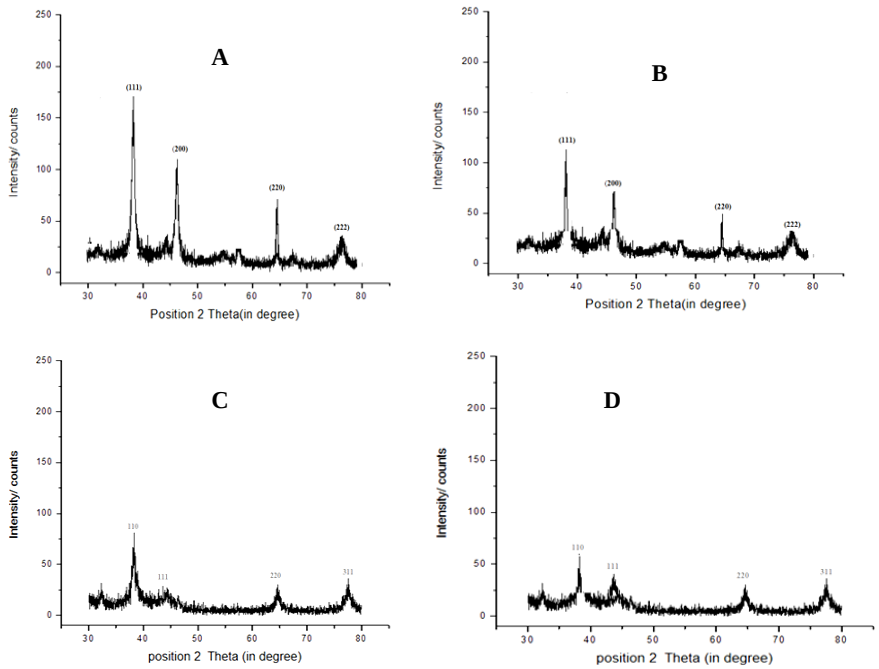

Fig. 5 X-ray diffraction pattern of synthesized silver nanoparticles, A and B fresh and dried leaves - *Carica papaya*, C. fresh leaves - *Andrographis paniculata, D.* Dried leaves - *Andrographis paniculata*

Andrographis paniculata: The fresh and dried leaves *Andrographis paniculata* silver nanoparticles XRD data show diffraction peaks at 2θ= 38.2-38.6º, 44.5-44.7 º, 64.5-64.8 º, 77.5-77.7 º, and can be indexed to (110), (111), (220), and (311) planes of pure silver ions indicating the biosynthesis of silver nanoparticles (Fig. 5). The diffraction peaks obtained in XRD corresponded to face centred cubic structure of metallic silver ions in its purest form and the size of particle ranging between size of fresh leaves *Andrographis paniculata* silver nanoparticles 40-60 nm and dried leaves *Andrographis paniculata* silver nanoparticles between 50 and 70 nm.

Biological Evaluation: The silver nanoparticles showed efficient biological property compared to other salts due to their extremely large surface area, which provides better contact with microorganisms. The nanoparticles get attached to the cell membrane and also penetrated inside the bacteria. The bacterial membrane contains sulfur containing proteins and the silver nanoparticles interact with these proteins in the cell as well as with the phosphorus containing compounds like DNA. When silver nanoparticles enter the bacterial cell it forms a low molecular weight region in the center of the bacteria to which the bacteria conglomerates thus, protecting the DNA from the silver ions. The nanoparticles preferably attack the respiratory chain, cell division finally leading to cell death. The nanoparticles release silver ions in the bacterial cells, which enhance their biological activity [40, 41].

Antimicrobial evaluation: The fresh and dried leaves *Carica papaya* silver nanoparticles were

evaluated for their *in vitro* antibacterial activity against *Staphylococcus aureus*, and *Streptococcus pneumoniae*, as examples of Gram-positive bacteria and *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli* as examples of Gram-negative bacteria. They were also evaluated for their *in vitro* antifungal potential against *Candida albicans*, *Aspergillus flavus*, and *Aspergillus niger* fungal strains. Agar-diffusion method was used for the determination of the preliminary antibacterial- and antifungal activity. Amikacin, and clotrimazole were used as reference drugs. For each tested compound, the results were recorded as the average diameter of inhibition zones (IZ) of bacterial- or fungal growth around the discs in mm^3 (Table 1).

Carica papaya: The fresh and dried leaves *Carica papaya* silver nanoparticles results depicted in Table 4. 1 revealed that most of the tested compounds displayed variable inhibitory effects on the growth of the tested bacterial strains, and also against antifungal strains. It would also be noticed that the fresh leaves *Carica papaya* silver nanoparticles exhibited better antimicrobial potentials than the dried leaves *Carica papaya* silver nanoparticles**.** In this view, The fresh leaves *Carica papaya* silver nanoparticles was found to exhibit higher activity (26-29 mm, 23-26 mm, and 27-30 mm) than that of amikacin (19-22 mm and 18-21 mm), and clotrimazole (20-22 mm) against *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*, and *Candida albicans* (Fig. 6-9).

Table 1 Inhibition zone (mm) of Fresh and dried leaves *Carica papaya* and Fresh and dried leaves *Andrographis paniculata* silver nanoparticles

Compound	**Zone of inhibition (mm)**							
	Bacteria					**Fungi**		
	Gram-positive bacteria		**Gram-negative bacteria**					
	Staphylococcus aureus	***Streptococcus pneumoniae***	***Klebsiella pneumoniae***	***Pseudomonas aeruginosa***	***Escherichia coli***	***Candida albicans***	***Aspergillus flavus***	***Aspergillus niger***
Fresh leaves *Carica papaya* silver nanoparticles	10-13	**26-29**	09-12	**23-26**	14-17	**27-30**	12-15	07-10
Dried leaves *Carica papaya* silver nanoparticles	08-10	**20-22**	10-13	**19-21**	12-15	**21-13**	14-17	08-11
Fresh leaves *Andrographis paniculata* silver nanoparticles	**22-24**	17-19	**21-23**	15-17	**24-26**	17-19	**26-28**	**27-29**
Dried leaves *Andrographis paniculata* silver nanoparticles	**18-20**	16-18	**17-19**	14-16	**20-22**	16-18	**20-22**	**22-24**
Amikacin (Standard)	**15-18**	**19-22**	**14-17**	**18-21**	**18-21**	NT	NT	NT
Clotrimazole (Standard)	NT	NT	NT	NT	NT	**20-22**	**17-20**	**21-24**

NT- Not Tested

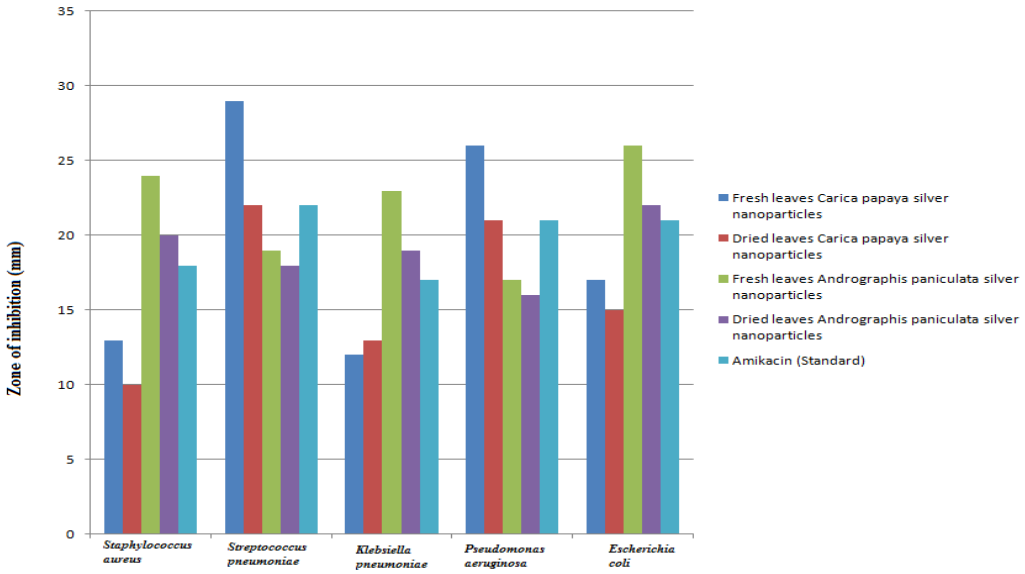

Fig. 6 Antibactirial activity of Fresh and dried leaves *Carica papaya* and Fresh and dried leaves *Andrographis paniculata* silver nanoparticles

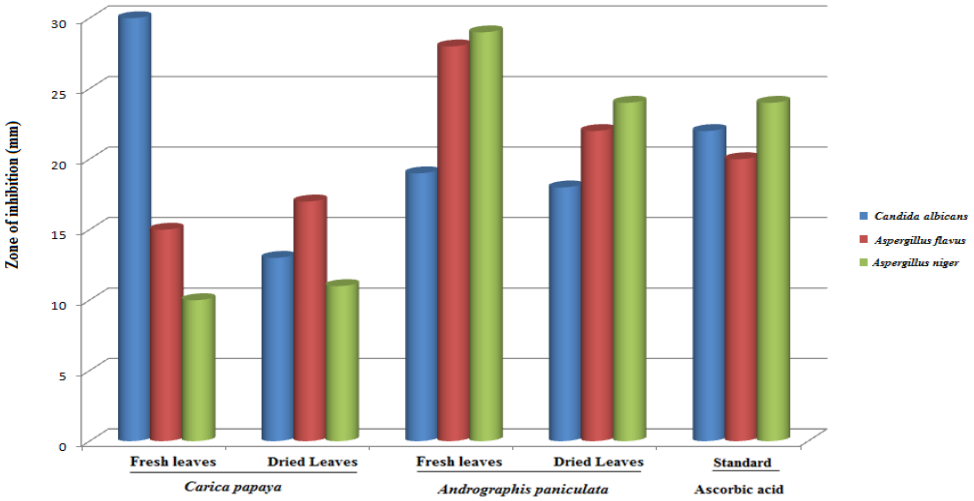

Fig. 7 Antifungal activity of Fresh and dried leaves *Carica papaya* and Fresh and dried leaves *Andrographis paniculata* silver nanoparticles

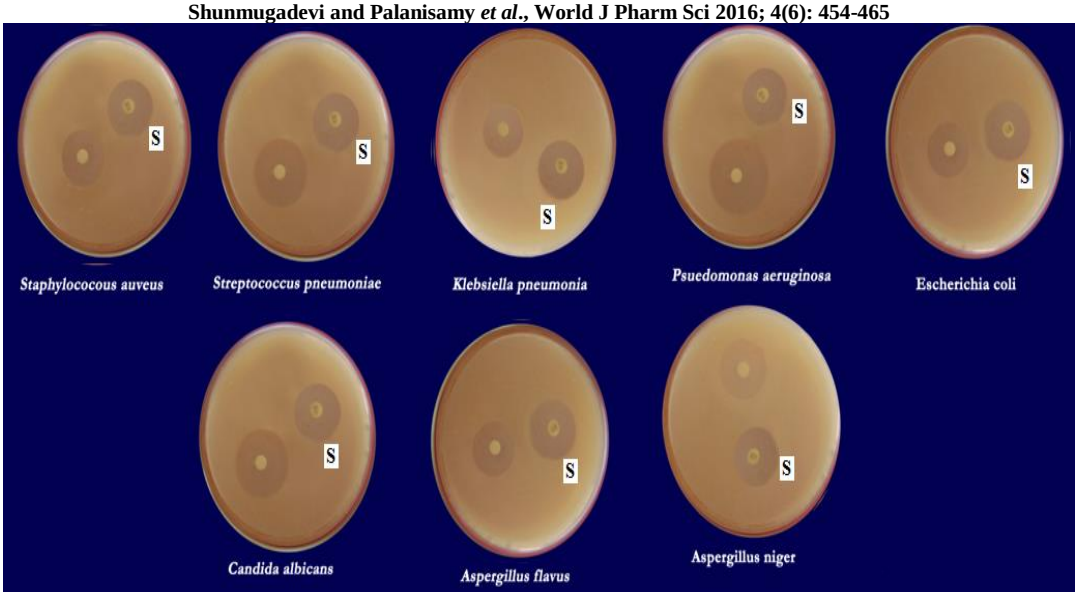

Fig. 8 Inhibition zone (mm) of Fresh leaves *Carica papaya* silver nanoparticles

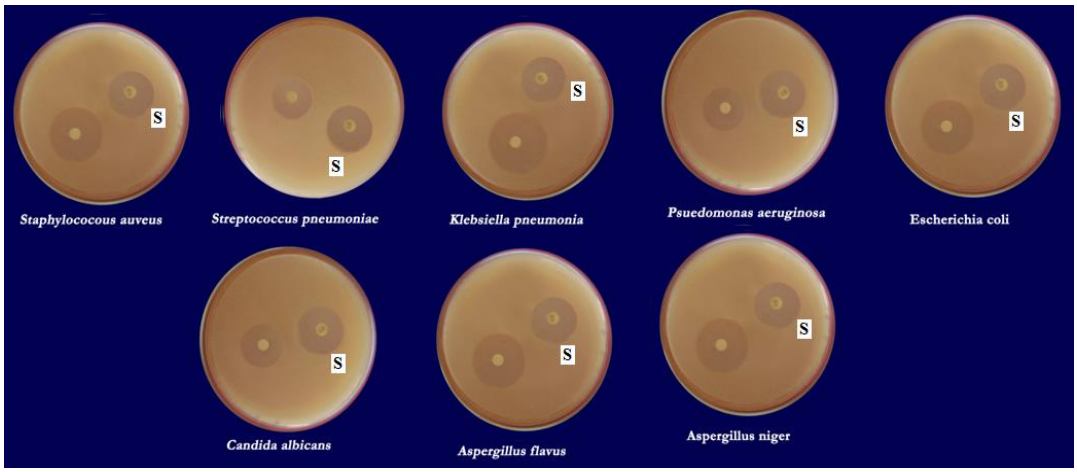

Fig. 9 Inhibition zone (mm) of Fresh leaves *Andrographis paniculata* silver nanoparticles

Andrographis paniculata: The fresh and dried leaves *Andrographis paniculata* silver nanoparticles results depicted in Table 4. 1 revealed that most of the tested compounds displayed variable inhibitory effects on the growth of the tested bacterial strains, and also against antifungal strains. It would also be noticed that the The fresh leaves *Andrographis paniculata* silver nanoparticles exhibited better antimicrobial potentials than the dried leaves *Andrographis paniculata* silver nanoparticles**.** In this view, The fresh leaves *Andrographis paniculata* silver nanoparticles was found to exhibit higher activity (22-24 mm, 21-23 mm, 24-26 mm and 26-28 mm, 27-29) than that of amikacin (15-18, 14-17, and 18-21 mm), and clotrimazole (17-20, & 21-24 mm) against *Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli,* and *Aspergillus flavus & Aspergillus niger* (Fig. 6-9). . They can also comparable activity of *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*, and *Candida albicans.*

DNA cleavage: The DNA cleavage (*E. coli*) potential of fresh and dried leaves *Carica papaya* and *Andrographis paniculata* silver nanoparticles were investigated using agarose gel electrophoresis method. In the gel electrophoresis, the changes are manifested by the change in the intensity of each band assigned to a particular form of DNA. The results (Fig. 10) indicate that all silver nanoparticle can interact with *E. coli* DNA in the presence of H_2O_2. The fresh and dried leaves *Carica papaya* and *Andrographis paniculata* silver nanoparticles can cleave DNA completely compared to untreated DNA. As a result, it can be said that the ability of these compounds for the DNA cleavage may be considered as a major reason for the inhibitory effect of them on the growth of the pathogenic organisms.

Free radical-scavenging activity (anti-oxidant analysis): The free radical-scavenging activity for the fresh and dried leaves *Carica papaya* and *Andrographis paniculata* silver nanoparticles were evaluated using the DPPH model system and the results are presented in Table 2. From the results it is clear that the free radical-scavenging activities of the fresh and dried leaves *Carica papaya* silver nanoparticles are greater than that of the fresh and dried leaves *Andrographis paniculata* silver nanoparticles **(Table 2).**

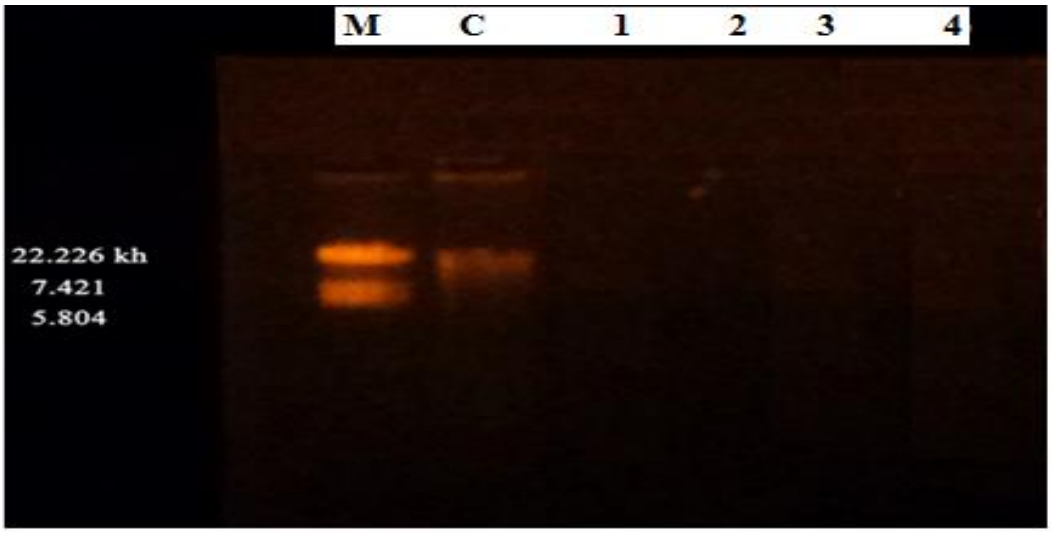

Fig.10 Gel electrophoresis pattern of fresh and dried leaves *Carica papaya* and *Andrographis paniculata* silver nanoparticles (1-4) showing the effect on *E.coli* DNA. Lane M: DNA marker. Lane C: untreated DNA.

Table 2 DPPH radical-scavenging activity of fresh and dried leaves *Carica papaya* and *Andrographis paniculata* silver nanoparticles

Concentration	DPPH radical scavenging (%)				
	Carica papaya		*Andrographis paniculata*		Standard
	Fresh leaves	Dried Leaves	Fresh leaves	Dried Leaves	Ascorbic acid
10µg/mL	22.36	19.36	13.62	10.48	21.62
50 µg/mL	76.35	72.52	49.68	46.92	75.34
100µg/mL	97.13	95.24	67.36	64.28	96.15

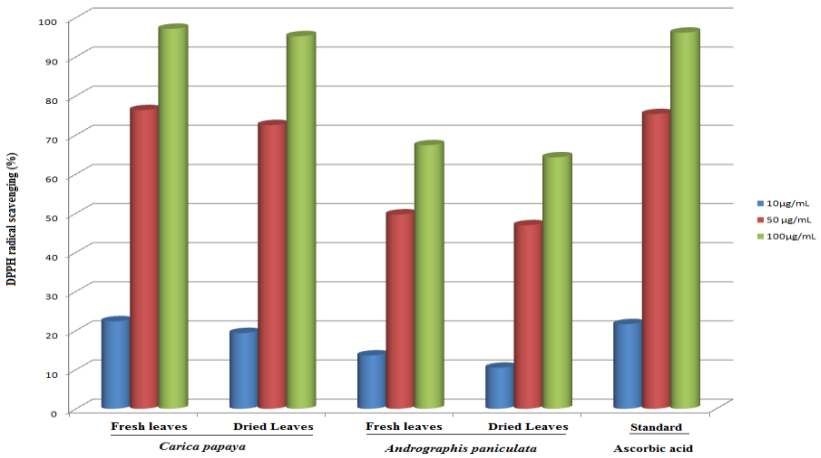

Fig. 11 DPPH radical-scavenging activity of fresh and dried leaves *Carica papaya* and *Andrographis paniculata* silver nanoparticles

The fresh and dried leaves *Carica papaya* silver nanoparticles results depicted in Table 4. 2. It would also be noticed that the fresh leaves *Carica papaya* silver nanoparticles exhibited better DPPH radical scavenging than the dried leaves *Carica papaya* silver nanoparticles**.** In this view, The fresh leaves *Carica papaya* silver nanoparticles was found to exhibit higher activity 22.36 % (10µg/mL), 76.35 % (50 µg/mL) , and 97.13 % (100µg/mL) than that of Ascorbic acid.

CONCLUSION

Green synthesized the silver nanoparticles from *Carica papaya* and *Andrographis paniculata* fresh and dried leaves extracts. The entire silver nanoparticles have been characterized by UV–visible spectrophotometer, SEM, FTIR, and X-ray diffraction method. The average size of fresh leaves *Carica papaya* and *Andrographis paniculata* silver nanoparticles between 5-30 and 40-60 nm and average size of dried leaves *Carica papaya* and *Andrographis paniculata* silver nanoparticles between 20-60 and 50-70 nm. The result showed that the particles were of spherical and cubic shape in case of fresh and dried leaves *Carica papaya* and *Andrographis paniculata* silver nanoparticles. Antibacterial and antifungal studies for the silver nanoparticle from *Carica papaya* and *Andrographis paniculata* fresh and dried leaves extract. The fresh leaves *Carica papaya* silver nanoparticles was found to exhibit higher activity (26-29 mm, 23-26 mm, and 27-30 mm) than that of amikacin (19-22 mm and 18-21 mm), and clotrimazole (20-22 mm) against *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*, and *Candida albicans.*The fresh leaves *Andrographis paniculata* silver nanoparticles was found to exhibit higher activity (22-24 mm, 21-23 mm, 24-26 mm and 26-28 mm, 27-29) than that of amikacin (15-18, 14-17, and 18-21 mm), and clotrimazole (17-20, & 21-24 mm) against *Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli*, and *Aspergillus flavus & Aspergillus niger*. They can also comparable activity of *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*, and *Candida albicans.*The fresh and dried leaves *Carica papaya* and *Andrographis paniculata* silver nanoparticles can complete DNA cleaved. The free radical-scavenging activity for the fresh and dried leaves *Carica papaya* and *Andrographis paniculata* silver nanoparticles were evaluated using the DPPH model system. The free radical-scavenging activities of the fresh and dried leaves *Carica papaya* silver nanoparticles are greater than that of the fresh and dried leaves *Andrographis paniculata* silver nanoparticles. These results agree with the experimental observations that fresh leaves *Carica papaya* silver nanoparticles shows better biological activity.

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