Synthesis and Characterization of Silver nanoparticles using Justicia adhatoda(leaf) and its Pharmacological Activity

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Abstract - **Nanotechnology is an emerging field in Biotechnology as the Green technology works with nanotechnology to develop significant and practical protocols when manufacturing nanotechnology-enabled products. By modifying the production process, the risks that are associated with nano-products are minimized and the green products can be used for environmental applications. Biomolecules present in herbal extracts can be used to reduce metal ions to nanoparticles in a single-step green synthesis process. This biogenic reduction of metal ion to base metal is quite rapid and they readily conduct at room temperature, pressure and can easily scale up. Silver nanoparticles are reported to have magnetic, catalytic, optical and anti-inflammatory activity. In this study, Silver nanoparticles were successfully synthesized by reduction of Silver nitrate solution using aqueous solution of Justicia adhatoda (leaf) extract. The method was non-lethal, simple, eco-accommodating and relatively inexpensive. The resulting Silver nanoparticles were characterized by physical color changes, UV-Vis spectroscopy, and Fourier-transform infrared (FTIR) spectroscopy. The reaction mixture of silver nitrate and Justicia adhatoda (leaf) extract exhibited black color confirming the synthesis of silver nanoparticles. UV–V is spectra showing an absorption band at 300 to 450 nm is the characteristic of the Silver nanoparticles. Also, indeed revealed the presence of nanoparticles. From the spectrum of the biosynthesized Silver nanoparticles at different magnifications from XRD and TEM showed that the average particle size of the nanoparticles is found to be 25 nm. FTIR spectra, the absorption band at 3887.9 cm−1 which is characteristic of the OH stretching of phenolic group. The absorption bands at 1173.8 and 1605 cm−1 correspond to carbonyl group present in the extract; synthesized Silver nanoparticles had significantly higher Anti- inflammatory activity by Albumen Denaturation method.**

Index Terms - **Justicia adhatoda (leaf), Silver nanoparticles, Silver nitrate, UV, XRD, FTIR, Antibacterial activity, etc.**

INTRODUCTION

Nanomaterials are cornerstones of nanoscience and nanotechnology. Nanostructure science and technology is a broad and interdisciplinary area of research and development activity that has been growing explosively worldwide in the past few years. It has the potential for revolutionizing the ways in which materials and products are created and the range and nature of functionalities that can be accessed. It is already having a significant commercial impact, which will assuredly increase in the future.

What are nanomaterials?

Fig. 1: Nanomaterial (For example: Carbon nanotube)

Nanoscale materials are defined as a set of substances where at least one dimension is less than approximately 100 nanometers. A nanometer is one millionth of a millimeter - approximately 100,000 times smaller than the diameter of a human hair. Nanomaterials are of interest because at this scale unique optical, magnetic, electrical, and other properties emerge. These emergent properties have the potential for great impacts in electronics, medicine, and other fields.

Where are nanomaterials found?

Some nanomaterials occur naturally, but of particular interest are engineered nanomaterials (EN), which are designed for, and already being used in many commercial products and processes. They can be found in such things as sunscreens, cosmetics, sporting goods, stain-resistant clothing, tires, electronics, as well as many other everyday items, and are used in medicine for purposes of diagnosis, imaging and drug delivery.

Engineered nanomaterials are resources designed at the molecular (nanometer) level to take advantage of their small size and novel properties which are generally not seen in their conventional, bulk counterparts. The two main reasons why materials at the nano scale can have different properties are increased relative surface area and new quantum effects. Nanomaterials have a much greater surface area to volume ratio than their conventional forms, which can lead to greater chemical reactivity and affect their strength. Also at the nano scale, quantum effects can become much more important in determining the materials properties and characteristics, leading to novel optical, electrical and magnetic behaviours.

The scope of this study is summarized below:

To synthesize Ag and Au NPs using aqueous flower extract of medicinally important plants.

To confirm the formation of nanoparticles, to study the rate of reactions and to examine the viable reaction parameters through UV-Visible spectroscopy.

To identify the possible biomolecules responsible for reduction and stabilization through FT-IR studies.

To ascertain the crystalline nature of the synthesized nanoparticles through XRD analysis.

To analyze the Anti- inflammatory activity, antioxidant and anti-diabetic activity.

MATERIALS AND METHODS

Characterization of Silver nano particles from Justicia adhatoda leaf extracts:

Preparation of flower extract

The fresh and young leaf Justicia adhatoda was collected and washed thoroughly with sterile double distilled water (DDW). Twenty gram of sterilized flower samples were taken and cut into small pieces. Finely cut leaves were placed in a 500 ml Erlenmeyer flask containing 100 ml of sterile DDW After that, the mixture was boiled for 5 minutes and then filtered. The extract was stored in 40oC.

Synthesis of silver nanoparticles

Silver nitrate was used as precursor in the synthesis of silver nanoparticles. 100 ml flower extract was added to 100 ml of 0.1N $AgNO_3$ aqueous solution in conical flask of 250 ml content at room temperature. The flask was thereafter put into shaker (100 rpm) at 500 ^oC and reaction was carried out for a period of 12 hrs. Then the mixture is kept in microwave oven for exposure of heat. The mixture was completely dried after a period of 20 minutes and hence nanoparticles in form of powders were obtained.

Fig.2. Visual identification of nanoparticles and Color change of the solution mixture with an increase in exposure

RESULTS AND DISCUSSION

UV Visible spectroscopy analysis

The Synthesize of silver nanoparticles was confirmed by color changes followed by UV–Visible spectrophotometer analysis. The transmittance of a sample (T) is defined as the fraction of photons that pass through the sample over the incident number of photons, i.e., T = I/I0. In a typical UV-vis spectroscopy measurement, we are measuring those photons that are not absorbed or scattered by the sample. It is common to report the absorbance (A) of the sample, which is related to the transmittance by A = -log10(T). Figure 2 illustrates the relationship between transmittance and absorbance.

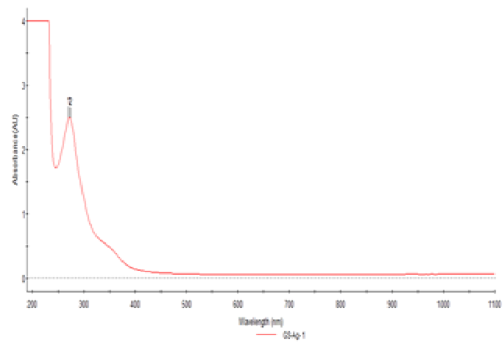


Figure 1: UV-Visible spectrum of synthesized Silver nanoparticles using Justicia adhatoda leaf extracts

The UV–Visible spectrophotometer has proved to be a very useful technique for the analysis of some metal nanoparticles and is a significant technique to authenticate the formation and stability of AgNPs in aqueous solution. It is renowned that AgNPs exhibit dark brown colors, depending on the intensity and the size of nanoparticles. The colors arise due to the excitation of surface Plasmon resonance (SPR) of the AgNPs. It is generally recognized that UV- vis spectroscopy could be used to examine size and shape-controlled nanoparticles in aqueous suspension. It is one of the most widely used methods for structural characterization of silver nanoparticles. The UV–vis spectra were recorded for aqueous leaf extract of plants. The absorption peaks were from 271.20 to 273.65nm. The maximum range of silver nanoparticles in UV-Vis spectrometer is 200 to 500 nm. In this study the results of UV-Vis spectrometer were from 271.20 to 273.65 nm. It confirms that the synthesized particles are silver

FT-IR Measurement

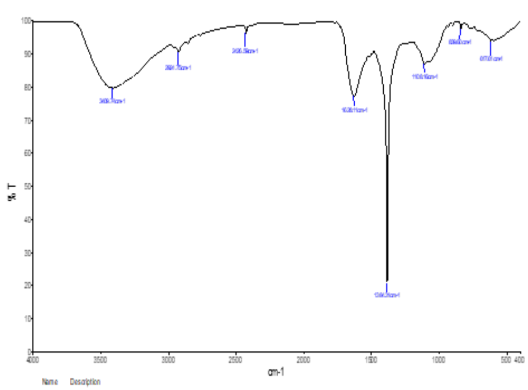


Figure 2:FT-IR Spectrum of synthesized Silver nanoparticles using Justicia adhatoda leaf extracts

The related functional groups of Justicia adhatoda leaf were identified using the peak assignments. FTIR spectroscopic analysis revealed the photochemical groups that acted as a capping agent for the synthesis and stabilization of nanoparticles like phenolic groups, amines, ether, carboxylic acid and a hydroxyl group. The spectrum was recorded in the wavelength region between 400 cm-1 to 4000 cm^{-}1.

The potential biomolecules were identified using FTIR measurements and are responsible for reduction and capping of the bioreduced silver nanoparticles. The major phytoconstituents present in the myroblan fruit are hydrolysable tannins, Gallic acid, chebulic acid, chebulic ellagitannins and gallate esters. The presence of three bands at about 3409.74 cm^{-1}, 2924.75 cm^{-1}, 2426.59 cm^{-1}, 1628.11 cm^{-1}, 1384.31 cm^{-1}, 1108.16 cm^{-1}, 839.60 cm^{-1} and 617.61 cm^{-1}(Figure 3).

3409.74 cm^{-1}	strong, broad	O-H stretching	Alcohol
2924.75 cm^{-1}	amine salt	amine salt	amine salt
2426.59 cm^{-1}	strong	O=C=O stretching	carbon dioxide
1628.11 cm^{-1}	strong	C=C stretching	α,β-unsaturated ketone
1384.31 cm^{-1}	medium	O-H bending	Phenol
1108.16 cm^{-1}	strong	C-O stretching	primary alcohol
839.60 cm^{-1}	medium	C=C bending	Alkene
617.61 cm^{-1}	strong	C-Br stretching	halo compound

The absorption bands that appear in the IR spectrum of the aqueous extract could also be seen in the IR spectra of phytocapped AgNPs. This shows that the phytoconstituents (mostly tannins) protect the AgNPs from aggregation.

XRD Measurement

X-ray powder diffraction (XRD) is a rapid analytical technique mainly used for phase identification of a crystalline material and can provide information on unit cell dimensions. Also it is commonly used for determining the chemical composition and crystal structure of a material therefore, detecting the presence of silver nanoparticles in plants tissues can be achieved by using XRD to examine the diffraction

peaks of the plant. The crystalline nature of Ag nanoparticles was further confirmed from X-ray diffraction (XRD) analysis shows the XRD pattern of the dried nanoparticles obtained from colloid samples. Four peaks were observed at 27.72, 32.14, 37.98, 44.13, 46.08, 54.73, 57.34, 64.42, 67.47 and 77.07 in the 2θ range. The X-ray diffraction results clearly show that the silver nanoparticles formed by the reduction of Ag (Figure 4). The observed peak broadening and noise were probably macromolecules present in the plant extract which may be responsible for the reduction of silver ions. Hence XRD pattern thus clearly illustrated that the silver nanoparticles formed in this present synthesis are crystalline in nature. In addition to the Scherrer Formula peaks representative of fcc silver nanocrystals, additional as yet unassigned peaks are also observed suggesting that the crystallization of bio-organic phase occurs on the surface of the silver nanoparticles. The line broadening of the peaks is primarily due to small particle size.
The sizes of synthesized ZnNPs are approximately 32.9 nm.

Scherrer Formula:
$D_p = (0.94 \times \lambda) / (\beta \times \cos\theta)$Where, D_p = Average Crystallite size, β = Line broadening in radians, θ = Bragg angle, λ = X-Ray wavelength

Peak List:

Pos. [°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]
27.7285	142.85	0.1968	3.21729	54.73
32.1457	260.99	0.1968	2.78458	100.00
37.9830	143.99	0.2952	2.36899	55.17
44.1362	39.44	0.4920	2.05196	15.11
46.0888	113.65	0.2952	1.96947	43.55
54.7376	41.28	0.1968	1.67698	15.82
57.3463	45.31	0.1968	1.60675	17.36
64.4220	36.81	0.2952	1.44631	14.11
67.4754	13.56	0.3936	1.38810	5.19
77.0731	31.70	1.1808	1.23743	12.15

Table 1: XRD Spectra peak of synthesized Silver nanoparticles using Justicia adhatoda leaf extracts determination of crystalline size

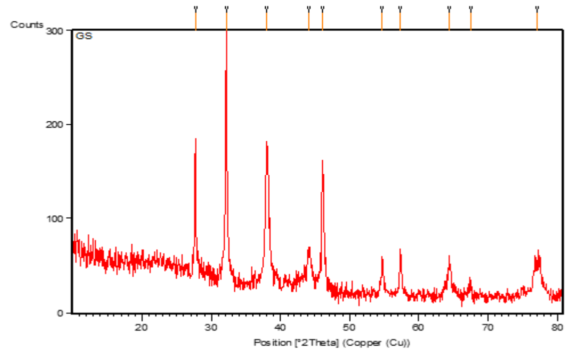
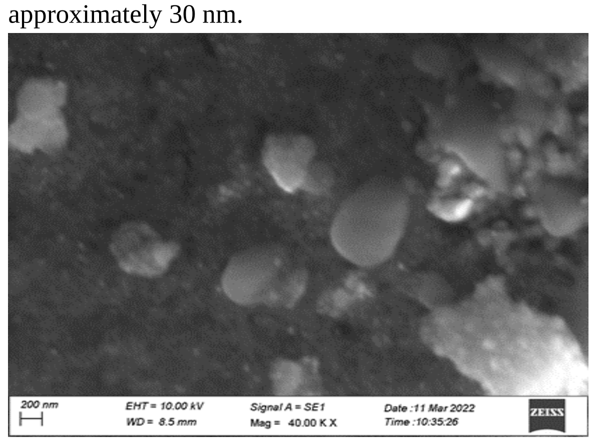


Figure 3: XRD Spectrum of synthesized Silver nanoparticles using Justicia adhatoda leaf extracts determination of crystalline size
The sample was drop- coated onto Nickel plate by just dropping a small amount of sample on the plate frequently, allowed to dry and finally thick coat of sample was prepared. The particle size and nature of the silver nanoparticle was determined using X-ray diffraction (XRD). This was carried out using Rigaku miniflex-3 model with 30kv, 30mA with Cukα radians at 2θ angle.

SEM analysis of AgNPs
The morphology and size of the synthesized nanoparticles were also determined by SEM images. Justicia adhatoda leaf mediated AgNPs images shown. Images reveal that the AgNPs are predominantly spherical in shape and are not in physical contact with each other. Lower magnification image reveals the nanoparticles are embedded in a dense matrix which may be the organic stabilizing components of Justicia adhatoda leaf. The presence of organic content associated with AgNPs can be further confirmed by observing the sharp Scherrer Formula reflection in XRD spectrum. The sizes of synthesized AgNPs are approximately 30 nm.



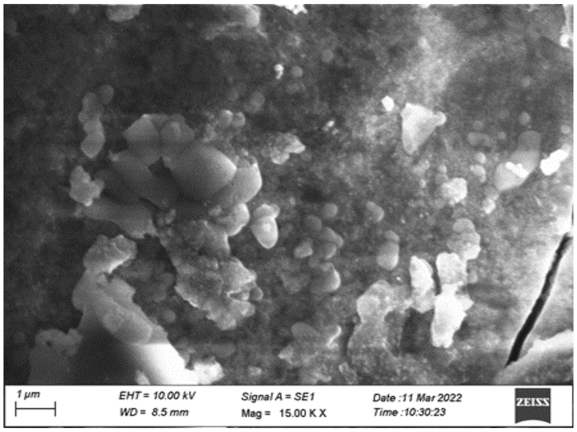


Figure 4: SEM images of synthesized Silver nanoparticles using Justicia adhatoda leaf extracts

Scanning Electron Microscopic (SEM) analysis was done using Quanta 200 FEG scanning electron microscope. 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, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

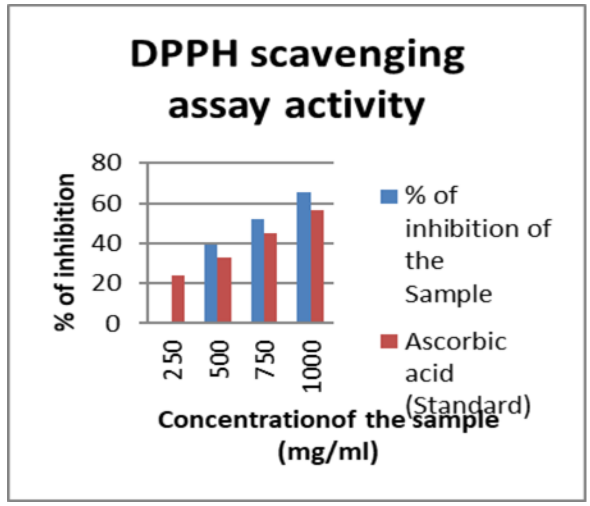
Anti-Oxidant Studies

DPPH scavenging assay

There are several methods available to assess the antioxidant activity of compounds. DPPH free radical scavenging assay is an easy, rapid, and sensitive method for the antioxidant screening of plant extracts. In the presence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases.

In the present study, the Silver nanoparticles using leaf extracts of Justicia adhatoda have high DPPH scavenging capacity, which increased with increasing concentration [Table 2 and Figure 6] The DPPH assay was carried out at different concentrations of Cadmium nanoparticles using root extracts of Justicia adhatoda samples, namely 250 mg/ml, 500 mg/ml 750 mg/ml and 1000 mg/ml. DPPH assay did not show any significant difference at 250 mg/ml Concentrations in Justicia adhatoda, however, it was significant for 500 mg/ml 750 mg/ml and 1000 mg/ml for the nanoparticles, all the values are compared with standard drug of Ascorbic acid. DPPH is a relatively stable free radical. DPPH radical react with suitable reducing agents, the electrons become paired off, and the solution losses color stoichiometrically depending on the number of electrons taken up. Hence, this assay provided information on the reactivity of test samples with a stable free radical. The decrease in the absorbance of the DPPH radical caused by test samples was due to the scavenging of radical by electron donation.

Anti oxidant activity of Silver nanoparticles using Justicia adhatoda leaf extracts by DPPH scavenging assay activity



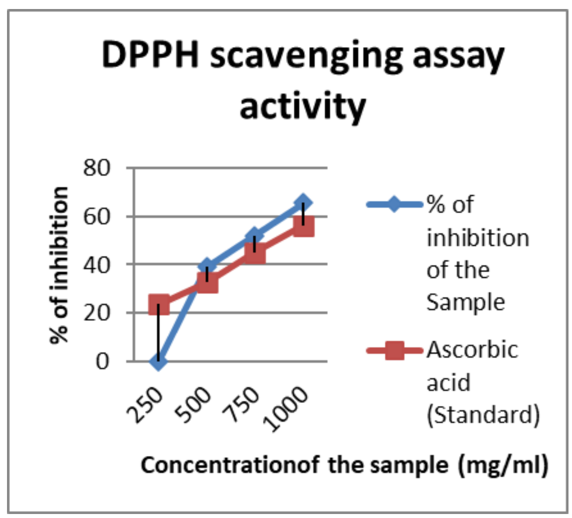


Figure 5: Graphical representation of Anti oxidant activity of Silver nanoparticles using Justicia adhatoda leaf extracts by DPPH scavenging assay activity

Anti-inflammation activity

Inhibition of Albumen Denaturation

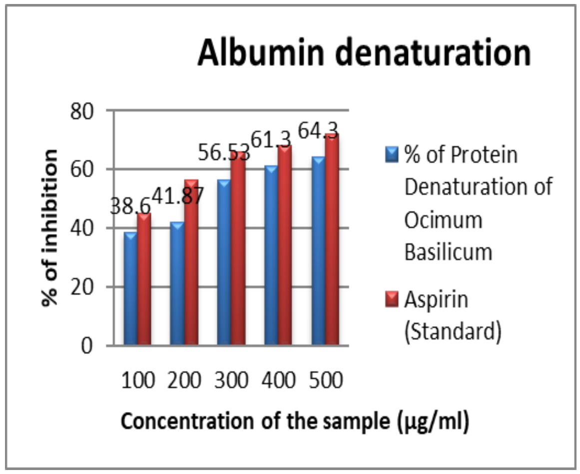
Problems still exist in continuing pharmacological research on animals. Such as ethical issues and the lack of rationale for their use when other suitable methods are available. Henceforward, in the present study, the protein denaturation bioassay was selected for in vitro assessment of the anti-inflammatory property of Silver nanoparticles synthesized Justicia adhatoda. The Albumen Denaturation is a well-documented cause of inflammation. Most biological proteins lose their biological functions when denatured. Production of auto antigen in certain arthritic disease is due to denaturation of protein. The mechanism of denaturation involves an alteration in electrostatic hydrogen, hydrophobic, and disulfide bonding. In the presence study, denaturation of proteins is the main cause of inflammation. As part of the examination on the mechanism of the anti-inflammatory activity, ability of the extract to inhibit protein denaturation was studied. Selected extracts were effective in inhibiting heat-induced albumin denaturation. Aspirin was used as a standard anti inflammation drug as shown in Figure [Table 3 and Figure 7].

S. No	Test	Concentration of the sample (mg/ml)	% of inhibition of the Sample	Ascorbic acid (Standard)
1	DPPH	250	28.14	23.63
2		500	38.96	29 .03
3		750	52.04	45.25
4		1000	65.56	56.44

S. No	Test	Concentration of the sample (µg/ml)	% of Protein Denaturation of Ocimum Basilicum	Aspirin (Standard)
1	Albumin denaturation	100	38.6	45
2		200	41.87	56.25
3		300	56.53	66.2
4		400	61.3	68.02
5		500	64.3	72.05

Table 3: Anti- inflammatory activity of Silver nanoparticles using Anti Justicia adhatodaaleaf extracts by of Albumen Denaturation activity

The albumin denaturation method was carried out at different concentrations of Silver nanoparticles using root extracts of Justicia adhatoda samples, albumin denaturation, 100µg/ml 200 µg/ml, 300 µg/ml, 400 µg/ml and 500 µg/ml. Albumen Denaturation shows significant effect at 400 µg/ml and 500 µg/ml. Concentrations in Justicia adhatoda, however, it was significant for 400mg/ml for the nanoparticles, all the values are compared with standard drug of Aspirin.



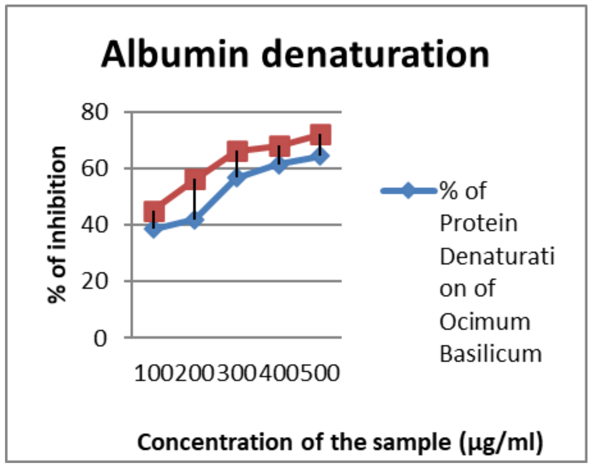


Figure 6: Graphical representation of Anti-inflammatory activity of Silver nanoparticles using Justicia adhatoda leaf extracts by Albumen Denaturation activity.

SUMMARY AND CONCLUSION

Nano silver has many important applications. It is used as an antimicrobial agent. It is applied in textiles, home water purification systems, medical devices, cosmetics, electronics and household appliances. The chemically synthesized metal nanoparticles are expensive, hazardous to environment and require high energy consumption. Biological approaches using plant extracts for metal nanoparticles synthesis have been suggested as valuable alternative tool towards chemical methods.

The characterization of synthesized AgNPs was carried out by different methods such as UV-Vis

Spectra, XRD and FTIR. The UV wavelength of AgNPs is from from 271.20 to 273.65 nm.

The FTIR results show that AgNPs contains the functional groups. FTIR spectra, the absorption band peak and functional groups are tabulated for tested sample.

3409.74 cm^{-1}	strong, broad	O-H stretching	Alcohol
2426.59 cm^{-1}	strong	O=C=O stretching	carbon dioxide
1628.11 cm^{-1}	strong	C=C stretching	α,β-unsaturated ketone
1384.31 cm^{-1}	medium	O-H bending	Phenol
1108.16 cm^{-1}	strong	C-O stretching	primary alcohol
839.60 cm^{-1}	medium	C=C bending	Alkene

The XRD pattern clearly confirmed that the synthesized silver nanoparticles are crystalline in nature. SEM results shows that synthesized silver nanoparticles are round in shape. The presence of organic content associated with AgNPs can be further confirmed by observing the Scherrer reflection in XRD spectrum. The size of synthesized AgNPs is approximately 32.9 nm.

The green synthesis of nanoparticles shows that cost-effective, environmentally friendly, and safe for human therapeutic use. Color change, UV-Vis spectra, SEM and XRD analysis confirmed the stability of synthesized AgNPs.

The ability of the synthesized silver nanoparticles to inhibit albumin denaturation has been investigated for potential anti-inflammatory action mechanism. It is well documented that protein denaturation is involved in arthritic reactions and development of tissue damage during inflammation. Results reveal that synthesized silver nanoparticles were effective in inhibiting thermally induced albumin denaturation at all tested concentrations, indicating their capability of controlling protein denaturation involved in the inflammatory process.

The maximum was obtained at the dose of 400 µg/ml and 500 µg/ml. This result suggests that nanoparticles consisting of silver and silver chloride may interfere with the release of acute inflammatory mediators or antagonize their action. Furthermore, the persistent anti-inflammatory activity might be due to enhanced permeability and retaining effect of silver nanoparticles in the edema region that has been reported.

Thus, the inhibition of edema formation and albumin denaturation activities of silver nanoparticles from Justicia adhatoda leaf extract clearly establish their anti-inflammatory potential and therefore could be considered as potential source of the anti-inflammatory drug.

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