ISSN: 2157-7439
Journal of Nanomedicine & Nanotechnology

OPEN ACCESS Freely available online

Research Article

Green Synthesis of Silver Nanoparticle using four Ethno Medicinal Plant *Kalanchoe pinnata, Artocarpus heterophyllus, Vitex negundo* and *Millettia pinnata* of Jharkhand

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ABSTRACT

In the present study, a plant-mediated green method of synthesizing AgNPs was successfully performed by employing the leaf extracts of ethnomedicinal plants used traditionally by ethnic people for curing various diseases *Artocarpus heterophyllus* and *Kalanchoe pinnata* (fresh), *Vitex negundo* and *Millettipinnata* as a source of reducing an

Stabilizin agents is reporte. Nanoparticles are particles having diameters below 100 nm. The nanoparticles formed were characterized using UV-Vis spectrophotometer, scanning FT-IR. A Surface Plasmon Resonance (SPR) peak was observed at 450 nm. UV-V is analysis. The FT-IR analysis spectra peak were observed at *Artocarpus heterophyllus* (1532.40 cm^{-1}, 1373.50 cm^{-1}, 1221.40 cm^{-1}, 961.31 cm^{-1} and 545.35 cm^{-1}) *Kalanchoe pinnata* (1738.60 cm, 1549.30 cm, 1370.10 cm, 1218.00 cm, 1005.00 cm) *Vitex negundo* (2,326 cm, 2921.7 cm, 1731.8 cm, 1626 cm, 1525.8 cm, 1366.8 cm, 1224.8 cm, 987.89 cm, 525.63 cm) and *Millettia pinnata* (2911.5 cm^{-1} , 1623.7 cm^{-1}, 1529 cm^{-1}, 1373.5 cm^{-1}, 1211.3 cm^{-1} and 947.6 cm^{-1}), which corresponds to the presence of capping agents such as primary and secondary amines, hydroxyl compounds, flavonoids, alcoholic and phenolic compounds the stability of the synthesized nanoparticles. The AgNPs synthesized in the present study displayed antibacterial activity against *E. coli* which suggested that they may play a role in new drug development The AgNPs synthesized in the present study displayed significant antibacterial activity against *E. coli* in comparision to antibiotics and crude extract.

Keywords: Nanotechnology; Nanoparticles; Capping agents; FT-IR; Nano scale; Surface plasmon resonance; Antibacterial activity; *E. coli*

INTRODUCTION

In recent years, appearance of nanometric scale technologies and biological techniques has given rise to a new field called green nanotechnology, which focuses on creation and use of materials of nano scale [1] Using plants. Nanoparticles are of great interest due to their novel physicochemical, magnetic and optoelectronic properties that are governed by their shape and size distribution [2, 3]. Nanotechnology concerns the size of matters in the range between one nm to 100 nm of size. The nano scale imparts ultra-small size, large surface to volume ratio, high reactivity [4]. Biology of plant-mediated nanoparticles is gaining grounds. The principle parameters of NPs are "size, shape, surface characteristics and inner structure" [5]. The special features and properties of nanoparticles like catalytic property, optical property, surface-enhanced Raman scattering [6] and chemical strength [7] are attributed to high fraction of surface atoms and quantum confinement [8, 9, 7] (Figure 1). The green method of synthesis of nanoparticles has several important applications in the field of biolabelling sensors, drug delivery system. The nanoparticles formed with help of plant extracts exhibit new physico-chemical properties, which are not observed in polar or non-polar extracts of plants [10]. In the last few years nanoparticles have been widely used in cosmetics, medicine and food preservatives that come in direct contact with humans. Especially, nanoparticles and metal oxide metals such as silver, gold, platinum, ruthenium, palladium, iron oxide, rare-earth oxides and silver nanoparticles are used for anti-bacterial, drug delivery and cancer treatment. As discussed earlier these nanoparticles can be synthesized using a physical method, a chemical method

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Receiv ed03-July-2023, Manuscript No: jnmnt-23-22204, **Edit or assigned** 05-July-2023, Pre QC No: jnmnt-23-22204 (PQ), **Reviewed:** 19-July-2023, QC No: jnmnt-23-22204, **Re vised**24-July-2023, Manuscript No: jnmnt-23-22204 (R), **Published:** 31-July-2023, DOI: 10.35248/2157-7439.23.14.692.

Citation: Rani L, Verma R, Kashyap S, Mandal RK, Hembrom A, et al. (2023) Green Synthesis of Silver Nanoparticle using four Ethno Medicinal Plant *Kalanchoe pinnata, Artocarpus heterophyllus, Vitex negundo* and *Millettia pinnata* of Jharkhand. J Nanomed Nanotech. 14: 692.

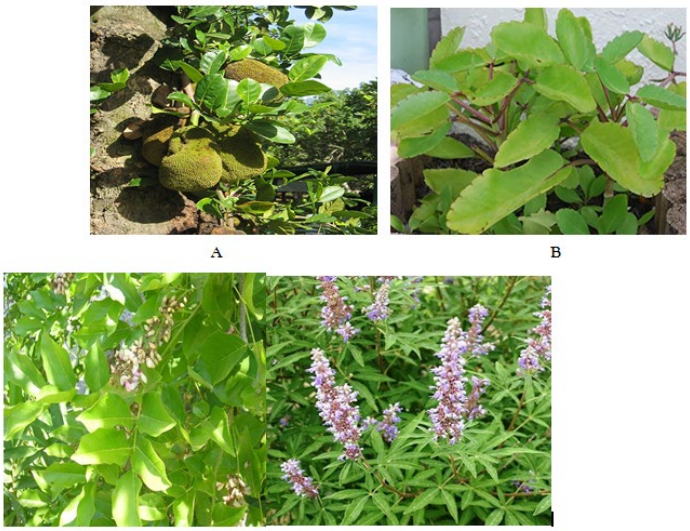

Figure 1: Sample collection of (a) A. heterophyllus (b) K. pinnata (c) M. pinnata (d) V. negundo leaves.

and a biological method. However, the physical method is more expensive method of producing Nano-particles - required the most sophisticated tools vacuum arrangement and continuous flow of gases such as argon, helium, neon, oxygen, nitrogen and hydrogen. Therefore the byproduct produced during compilation plays an important role in production cytotoxic effect this may increase the adverse effect on cosmetics and medicine applications. To avoid the use of toxic chemicals, organic solvents, several researchers are trying to develop metallic nanoparticles in ways that are environmentally friendly and biological perspective Currently the crude synthesis of metallic nanoparticles is an emerging science in the field of nanotechnology and nanomedicine. On the other hand, the method of extracting green plants is a non-toxic and safe procedure. The method used for this process is a process of reduction the plant due to its phytochemicals such as ketones, aldehydes, amides and carboxylic acids Due to its wide range of applications in various fields, "green chemistry" has been widely translated. Nanoparticles are composed of biological channels of raw materials using bacteria, fungi, algae enzyme and plant extracts instead of the usual chemical synthesis method. Recently, it has been reported that silver nanoparticles are more effective in biomedical applications, especially for antibacterial and antifungal purposes. Therefore, the synthesis of silver nanoparticles can be a major breakthrough in biomedical applications, especially in the field of antibacterial activity.

State of Jharkhand, India homes many tribal communities along with a dynamic floristic diversity. Many a rare and important plants economic, agricultural and therapeutic uses. There are all together 29 scheduled tribe in the state, but the largest tribal group are Munda, Oraon, Kharia, Ho and Santhal. Due to close association of forest, the tribes possess a unique knowledge about the medicinal uses of plant wealth of their surroundings from many generations. A some medicinal plants among kondhas around of Koraput district Orissa, Ancient Science Life 8:60-67. They depend mostly on ethno medicines for the treatment of different diseases, disorders and ailments. This traditional knowledge is now fast disappearing due to modernization, habitat destruction and tendency of the younger generation to discard traditional health system. Herbal medicines of Dongrias, Adibasi 26/1-4. In this study, we synthesized silver nanoparticles using a screened leaf Extract of *Artocarpus heterophyllus* (Jack Fruit) and *Kalanchoe pinnata* (Indian Patharchatta), 2 (5) 1478-1482 *Vitex negundo* and *Millettia pinnata*. Which are used here form ancient times as medicine to cure many infectious as well as non-communicable diseases (Figure 2).

Artocarpus heterophyllus (Jack Fruit) *Artocarpus heterophyllus* (Syn. Kathal) belonging to family Moraceae is an integral part of common Indian diet its medicinal properties are also mentioned in Ayurveda. The plant is reported to possess antibacterial, anti-inflammatory, antidiabetic, antioxidant and immunomodulatory properties. *Artocarpus heterophyllus* is an important source of compounds like morin, dihydromorin, cynomacurin, artocarpin, isoartocarpin, cyloartocarpin, artocarpesin, oxydihydroartocarpesin, artocarpetin, norartocarpetin, cycloartinone, betulinic acid, artocarpanone and heterophylol which are useful in fever, boils, wounds, skin diseases, convulsions, diuretic, constipation, ophthalmic disorders and snake bite etc..

Kalanchoe is a medicinal plant largely used in folk medicine for the treatment of kidney stones, gastric ulcer, pulmonary infection, rheumatoid arthritis etc. commonly known as 'air plant,' *Kalanchoe* is botanically classified with two main Latin names which refer to the same plant: *Bryophyllum pinnatum* and *Kalanchoe pinnatum* (as well as various synonyms of both). And *Kalanchoe pinnatum* (as well as various synonyms of both). The chemicals reported from the plant belong to different classes such as alkaloid, diterpenoidal lactones, glycosides, steroids, phenolics, aliphatic compounds, etc. The notable pharmacological properties include anti-diabetic, anti-neoplastic, antioxidant, immunomodulation, anti-lipidaemic, anti-allergic and many more activities which are yet to be explored

Vitex negundo is a shrub or a small tree growing throughout Jharkhand. A common yet very significant of them is *Vitex negundo*, locally called as "sandvar". It is grown on the boundaries of agriculture fields and houses in rural Jharkhand. The plant is an established source of drugs such as β-Sitosterol (Leaves and root) p-hydroxy benzoic and 5-hydroxylsophthalic acid (Leaves). It is widely used by the village folks as a repellent, a powerful discutient and fungicide.

Millettia pinnata

Its Botanical name is *Pongamia pinnata*, Pierre. In mundari it is

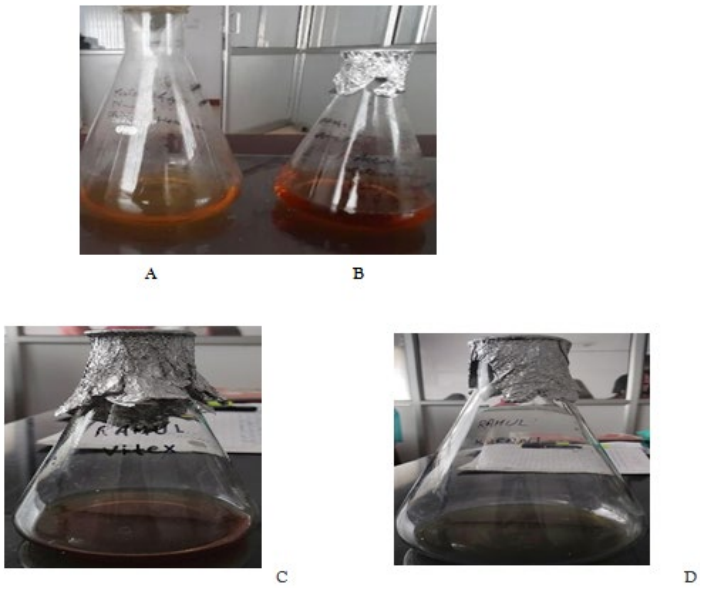

Figure 2: Leaf extract of (a) A. heterophyllus (b) K. pinnata (c) Vitex negundo (d) Millettia pinnata.

called 'koronjo' where as in sadri it is called 'karanj'. Karanj oil is used for burning, skin disease, rheumatism, sores, ulcer, bronchitis, chest pain, ear complaints, joint pain, whooping cough, cold, eczema, fever, itching and scabies. Flower is used for diabetes, and fruit is used for cough and leprosy. Bark is used for malaria, piles and tooth- brush. The traditional use of **Millettia** species includes antibacterial, anti-tumour, insecticidal, pesticidal, piscicidal antispasmodial, chemopreventive joint pain, rheumatoid arthritis, amenorrhea, tuberculosis, etc. Most of them are used in the production of biodiesel.

In this work, we report the synthesis of silver nanoparticles mediated by aqueous leaf extract of and its anti bacterial activity against resistant *E.coli*. The size of the AgNPs were characterized by UV-Visible spectroscopy. The functional group present in the AgNPs were analysed by FTIR spectroscopy [Figure 3].

MATERIAL AND METHODS

Preparation of plant extract: Fresh leaves of *Artocarpus heterophyllus* and *Kalanchoe pinnat* were collected from Bahubazar and Namkum Titritoli, Jharkhand, India. The collected leaves were washed several times with water to remove the dust particles and then sun dried to remove the residual moisture. The fresh leaves are dried in the oven at 50 °C for 48 hours and stored for 5 days at room temperature. Apply leaf powder using mud and pestle. 10 g of leaf powder is added 200 ml of distilled water and boil at 80 °C for 60 minutes. solution was filtered. The filtrate here is called *Artocarpus heterophyllus* and *Kalanchoe pinnata* leaf extract. Fresh leaves of *Vitex negundo* and *Millettia pinnata* were collected from Morabadi, Ranchi, Jharkhand. The leaves were thoroughly washed under the tap water to remove the adhered dust particles present on the surface and then rinsed with distilled water. Cleaned leaves were completely dried at room temperature. Dried leaves were finely chopped into small pieces. 10 gram of chopped leaves of both the plant samples were mixed with 100 ml of distilled water in two different conical flasks. Contents were boiled for 10 minutes at 100 °C. Contents were cooled to room temperature. Extracts were filtered through Whatman No. 1 filter paper in two different conical flasks and the clear leaf extract of *Vitex negundo* and *Millettia pinnata* thus obtained were used for synthesis of silver nanoparticles (AgNPs) (Figure 4).

Figure 3: Addition of leaf extract with 0.05M AgNO3 solution (2:1). AgNPs was prepared AgNPs synthesized by (a) A. heterophyllus (b) K. pinnata (c) Vitex negundo (d) Millettia pinnata leaf extract.

Synthesis of silver nanoparticles: An aqueous solution of 0.05 M silver nitrate ($AgNO_3$) was prepared using 100 ml deionized water used for the fusion of silver nanoparticles. Extracts of Artocarpus heterophylus leaves mixed with freshly diluted 0.05 M $AgNO_3$ solution in a conical flask, the mixture was heated continuously at 60 °C for 1 hour. It is noteworthy that the solution of the mixture heated until the colour of the mixture changes from brown to black. The altered color of the mixture, which promotes the formation of silver nanoparticles. Synthesized silver nanoparticles are centrifuged at 5,000 rpm for 20 min so that the nanoparticles are collected after extracting the supernatant. As a result, a pellets was obtained that was washed with distilled water repeated to remove impurities and untreated parts. After that, the pellets is collected, dried in the presence of 70% ethanol at 60 °C in the oven and finally stored in a desiccator for various analyzes. 10 ml of leaf extracts was mixed with 90 ml of 1mM silver (Figure 5).

CHARACTERIZATION OF SILVER NANOPARTICLES

UV-Vis analysis: The reduction of b + Ag + ions in the solution was monitored by measuring the UV-VIS spectrum of the reaction medium. Visual analysis of UV-VIS of solar solids of *Kalanchoe pinnata* mediated silver nanoparticles is performed using a Shimadzu UV- 1800 spectrophotometer at room temperature operating with a resolution of 1 nm and a diameter between 300-700 nm for *A. heterophyllus* and *K. pinnata* and 220-800 nm for *V. negundo* and *M. pinnata*.

Fourier transform infra-red spectroscopy (FTIR) analysis: FTIR analysis for dried silver nanoparticles was performed using the Nicklet 380 Thermo, a US Fourier Transform Infrared Spectrometer

Assay of anti-bacterial activity: The antibacterial activity of silver nanoparticles was determined using a well diffusion method. In this way, 20 ml of purified Mueller Hinton Agar was poured into sterile petri plates, after solidification, 100 µl of bacterial culture was poured into the plates and the culture was spread on plates using a spreader. Then, a hole with diameter of 6mmis punched aseptically with a sterile cork borer. Various concentrations of AgNPs were loaded on a 6 mm well. The loaded plates were stored to be placed at 37 ° C for 24 hours. Chloramphenicol was used as a controller along with solvents. At the end of the incubation, the built-in block areas around the well are measured with a light

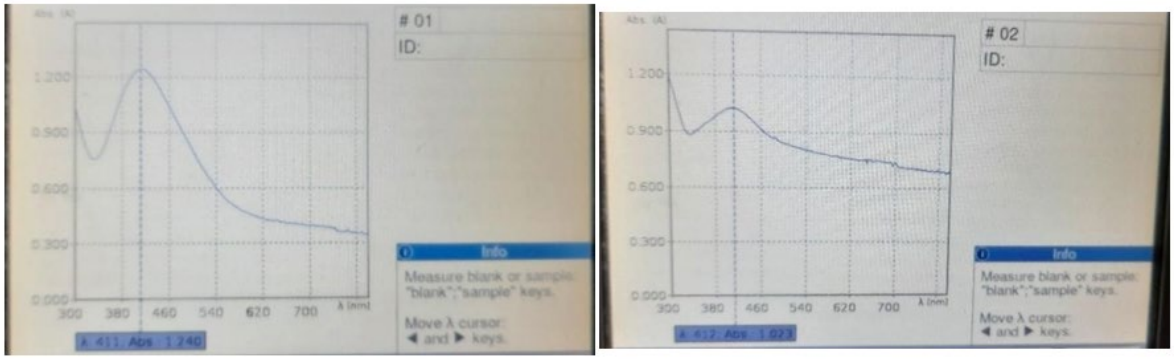

Figure 4: UV-Vis spectrum of AgNPs produced by the reduction of AgNO3 solution (0.05M) with (1) kalanchoepinnata and (2) Artocarpus heterophyllus.

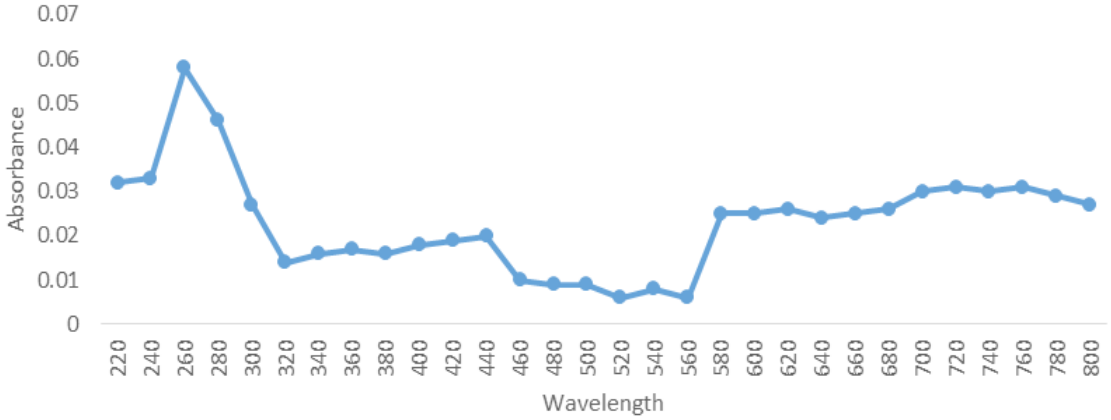

Figure 5: UV-Visible spectra of AgNPs synthesized by Vitex negundo leaf extract.

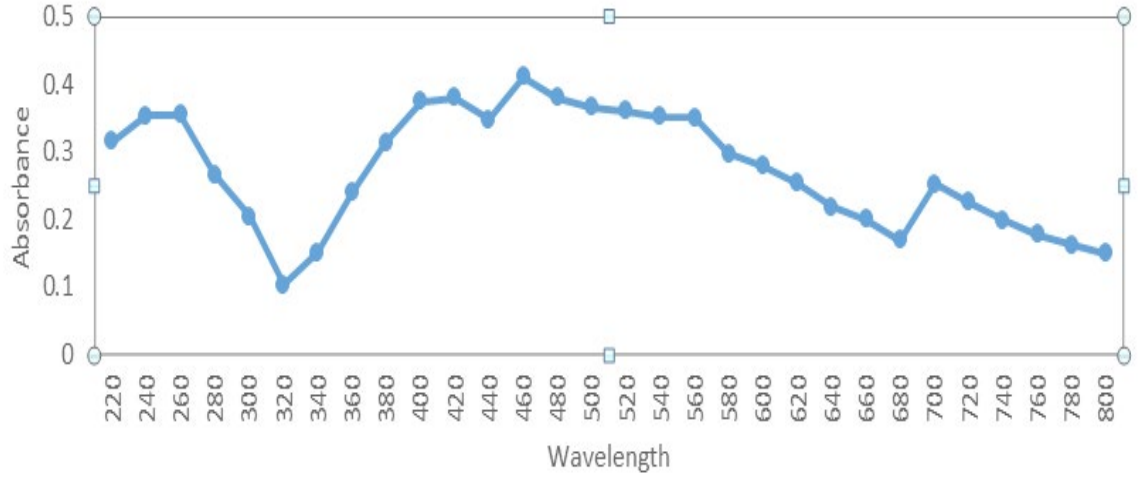

Figure 6: UV-Visible spectra of AgNPs synthesized by Millettia pinnata leaf extract.

ruler by a millimeter. A pure culture of *E. coli* was sub-cultured in nutrient broth. Strain was uniformly spread on sterilized petri plates. 4 circular wells, A, B, C and D, of 6 mm diameter were made using a sterile cork-borer. The well A was loaded with a chemical antibiotics, 50 µL chloramphenicol (2 mg/ml concentration) as a positive control. The well B was loaded with 50 µL SNPs (100 % concentration). The well C was loaded with 50 µL SNPs (50 % concentration). The well D was loaded with 50 µL distilled water. The plates were incubated at 37 °C overnight and the zones of inhibition were observed.

RESULTS AND DISCUSSION

UV-Vis spectroscopic studies UV spectroscopy is an effective, first-class method of separating Ag nanoparticles based on a visual field called Surface Plasmon Resonance (SPR). The addition of *Kalanchoe pinnata*, *Artocarpus heterophylus*, *Vitex negundo* and *Millettia pinnata* sundried leaves aqueous extract has led to a change in color changes from brown to black. The color changes are due to excitation SPR with silver nanoparticles. The SPR of silver nanoparticles produced a high concentration rate of around 420 nm. During the green synthesizing, the reaction process is monitored by UV. The bio-reduction reaction of the silver nitrate solution in contact with the extracted leaf was evaluated by UV-Vis spectroscopy ranging from 300 nm to 700 nm in the present study. High absorption was observed around 412 nm and 411nm indicating the successful conversion of silver nitrate (Ag^+) to silver nanoparticles (Ag^0) shown in (Figure 6). 260 nm, 720 nm and 760 nm for *Vitex negundo* and at wavelength of 260 nm and 460 nm for *Millettia pinnata* because of surface plasmon resonance (SPR). The reduction of silver nitrate to silver nanoparticles by the leaf extract of *Vitex negundo* and *Millettia pinnata* was confirmed by measuring the UV–Vis spectrum of the colloidal solution. The colour change to yellowish confirmed the formation of silver nanoparticles.

This color change was due to the bioreduction of Ag^+ to Ag^0 by various biomolecules present in the leaf extract. The absorption spectra of the silver nanoparticles were recorded. The SNPs showed a characteristic absorption peak at wavelength of 260 nm, 720 nm and 760 nm for *Vitex negundo* and at wavelength of 260 nm and 460 nm for *Millettia pinnata* because of surface plasmon resonance (SPR). This SPR peak is very sensitive to the size and shape of the nanoparticles, amount of extract, silver nitrate concentration and the type of biomolecules present in the leaf extract.

Fourier transforms infra - red spectroscopy (FTIR): In the present study, the FTIR spectra were used to identify potential biomolecules in the product responsible for the reduction and synthesis of silver nanoparticles. The FTIR spectrum is useful in examining the chemical composition on the surface of silver nanoparticles. In, FTIR spectrum of synthesized silver nanoparticles showed two strong IR hydroxyl bands and phenols (3453.06 cm^{-1}), and a simple C = C benzene and amide-I linkage (1637.76 cm^{-1}) and other IR bands of (2076.95 cm^{-1}) and (559.46 cm^{-1}). A broad band from (3453.06 cm^{-1} and 3452.81 cm^{-1}) was assigned to a simple O – H vibration indicating the presence of hydroxyl and phenolic

hydroxyl groups, responsible for the reduction and capping agents. The absorption peak (1631.39 cm^{-1} and 1631.27 cm^{-1}) in the infrared area of the electromagnetic spectrum shows the binding binding of amide and silver nanoparticles that may be assigned to the carbonyl enlargement of proteins and clearly demonstrate the presence of protein as an agent - capping with silver nanoparticles. Proteins have a strong binding properties of silver nanoparticles that increase the stability of synthetic nanoparticles. These results confirmed that the carbonyl group of amino acid residues has a strong binding effect on the metal leading to the formation of a layer that is advertised over the metal nanoparticles as a capping agent to prevent bonding.

Antibacterial activity: The antibacterial activity of synthetic silver nanoparticles was investigated against a pathogen selected as *Escherichia coli* in a well-propagated way and the effect were demonstrated in (Table 1) and (Figure 7). The width of the block area is measured in mm. In the process of dispersing the source silver nanoparticles show an important anti-bacterial activity in the bacterium. The results of the antibacterial study clearly showed that antibacterial activity (according to the Zone of inhibition) increases by increasing concentration of silver nanoparticles (5, 2.5 mg / ml) which may be due to increased concentration of H_2O_2 in the surface of silver nanoparticles *Escherichia coli* (18, 16 mm) for *K. Pinnate* and (16, 15 mm) for *A. Heterophylus* as shown in (Figure 8) (Table 2).

For Chloramphenicol (20mm for *K. Pinnata* and 25mm for *A. Heterophylus*).For deionized water (0 mm). demonstrated the antibacterial activity of synthesized silver nanoparticles against bacteria. Normally compacted metal oxide nanoparticles carry a positive charge and micro-organism carries a negative charge thus this interaction creates an "electromagnetic" attraction between the microorganism and the host as a result of this pleasurable concentration of singlet oxygen incre ases. Singlet oxygen's are highly active and enhance antimicrobial activity.

CONCLUSION

Blending of medically useful nanoparticles using chemical synthesis techniques produces more toxins in the environment. Thus in the present paper the production of Ag nanoparticles is effected by green synthesis. *Kalanchoe pinnata, Atrocarpus Heterophyllus, Vitex negundo* and *Millettia pinnata* leaves has been used successfully in the above compilation. The biological formulation of silver nanoparticles using the using *Kalanchoe pinnata, Atrocarpus heterophyllus, Vitex negundo* and *Millettia pinnata* leaf provides a natural, simple and effective method for the synthesis of nanoparticles. The use of plant material avoids the use of toxic and detoxifying substances. The incorporation of Ag nanoparticles is done using different techniques such as, FTIR and UV-Vis etc. To analyze the antimicrobial activity of the sample, the samples are

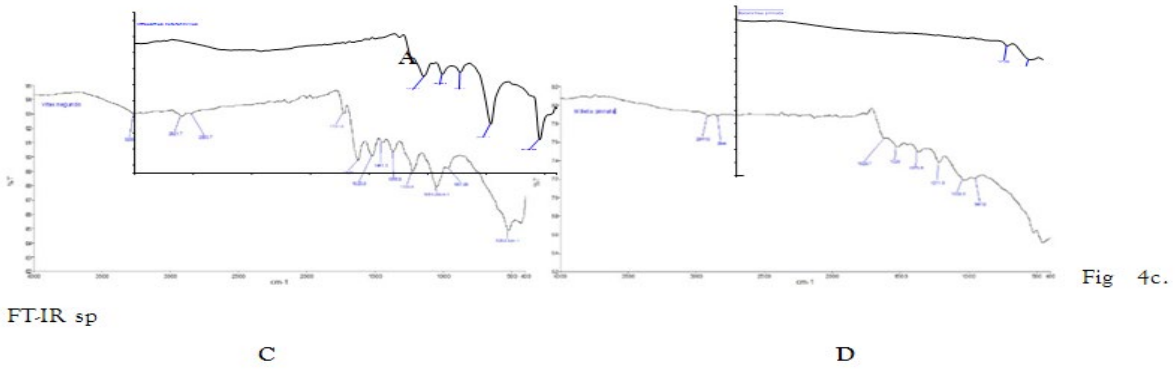

Figure7: FTIR spectra of A) A. heterophyllus B) K. pinnata C) Vitex negundo D) Millettia pinnata leaf extract.

Table 1: Details of FTIR spectrum A. heterophyllus, K. pinnata, Vitex negundo and Millettia pinnata.

Frequency range (cm-1)	A. heterophyllus	K. pinnata	V. negundo	M. pinnata	Functional group
3870-3550					O-H stretch alcohol
3500-3200			3263		O-H stretch vibration, presence of alcohol, phenol
3300-2850			2850.7	2911.5	O-H stretch vibration, carboxylic acids
			2921.7		
2500-2300					C-H stretch alkenes
2260-2100					C=C stretch vibration, alkynes
1990-1739		1738.6	1731.8		Ester C=O stretch, lipid, triglycerides
1700-1600			1626	1623.7	C=C stretch vibration, alkenes
1550-1475	1532.4	1549.3	1525.8	1529	N-O asymmetric stretch, nitro compound
1470-1400			1451.3		C-C stretch vibration, aromatics
1400-1320	1373.5	1370.1	1366.8	1373.5	N-O stretch vibration, nitro compound
1275-1150	1221.4	1218	1224.8	1211.3	C-H wag stretch vibration, alkyl halide
1020-1000		1005			C-N stretch vibrastion, aliphatic amines
990-800	961.13		987.89	947.6	N-H wag stretch vibration, primary and secondary amines
680-510	545.35		525.63		C-Br stretch vibration, alkyl halide, glycogen
490-400					Halogen compound

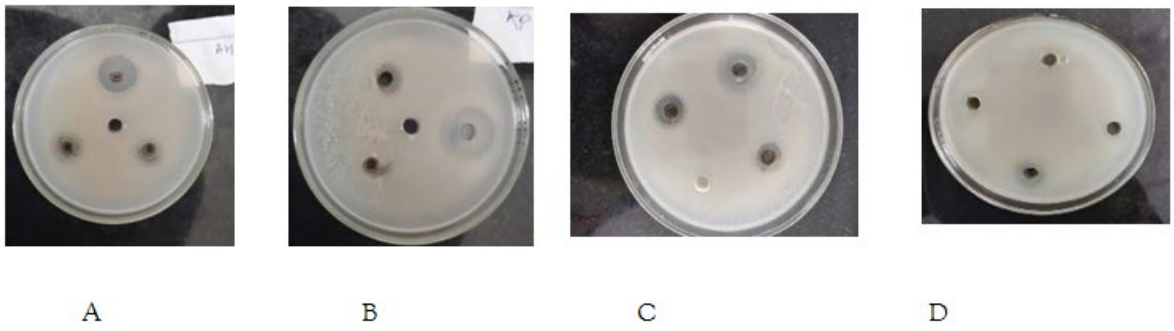

Figure 8: Antibacterial result of AgNPs synthesized by (a) A. heterophyllus (b) K. pinnata (c) Vitex negundo (d) Millettia pinnata leaf extract.

Table 2: Measurement of zone of inhibition of test samples against bacteria *E. coli*.

Test samples	Zone of inhibition (in mm)		
	Chloramphenicol	100% concentration	50% concentration
A. heterophyllus	25	16	15
K. pinnata	20	18	16
V. negundo	20	15	13
M. pinnata	11	11	9

subject to well diffusion method. Samples have been tested against *E. Coli*. As the production of AgNPs is cost effective, fast and harmless to the environment this process is problematic, one step using *Kalanchoe pinnata, Artocarpus Heterophyllus, Vitex negundo* and *Millettia pinnata* extracts mediated synthesis appears to be suitable for large-scale production. It is therefore concluded that the AgNPs incorporated into the present study could be potential for a variety of therapeutic and biological disciplines.

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