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BIOSYNTHESIS AND DESIGN OF Ag-Fe BIMETALLIC NANOPARTICLES USING A MEDICINAL PLANT *Buchanania lanzan* AS ANTIMICROBIAL SYNERGISTIC COMBINATION THERAPIES AGAINST CLINICALLY RELEVANT PATHOGENS

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ABSTRACT

Buchanania Lanzan Indian tribes employ the dry deciduous forest tree Spreng, which belongs to the *Anacardiaceae* family, extensively to treat a variety of ailments. An organic leaf extract has been used to define three primary chemical constituents with strong therapeutic potential: vomicine, epinitol, and celidoniol. These extracts primarily show anti-inflammatory, anti-hyperlipidemic, antioxidant, wound-healing, anti-diarrheal, and antivenomic effects, among many other therapeutic qualities.Very recently, unique biomaterials and biofilms are being extracted from seeds, which promise to become a major contributor in pharmaceutical industry. This review attempts to present thorough updated account of ongoing and emerging areas of research of this plant, especially in the field of phytomedicnes and pharmaceuticals.

Keywords: Nanoparticles, Sliver nanoparticles, Iron Nanoparticles, Antimicrobial activity.

INTRODUCTION

A concern to global health is the rapid spread of infectious diseases that are resistant to treatments. 1By 2050, drug-resistant diseases will cause 10 million deaths annually if the current trend keeps on its current pace. [2]Metal nanoparticles (NPs) have great potential to be utilised as antimicrobial agents. [3]Noble metal NPs, such as silver (Ag) and gold (Au), have been shown to exhibit strong and sustainable antibacterial action against a wide array of microorganisms; therefore, They have been used in dental resin composites, food preservatives, medical instrument coatings, medical device coatings, and cosmetics." [4]Bimetallic NPs have gained specific attention in the last decade due to their optical, electronic, magnetic, and catalytic properties, which, in most cases, are significantly distinct from their monometallic counterparts. Two distinct metal elements are combined to create bimetallic nanoparticles (NPs), which can have a variety of shapes and forms.5 Because green synthesis is an easy, stable, quick, low-cost, and environmentally friendly process, it opens up new possibilities for NP synthesis. [6]In general, the synthesis of bimetallic NPs involves the mixing of two differcnt aqueous metal solutions with an environmentally friendly reducing agent, such as a plant extract.[7] The plant phytochemicals, with antioxidant reducing properties, are believed to reduce metal ions into metal nanoparticles.[8] Theoretically, metal ions with a stronger reduction potential are reduced faster than metal ions with a weaker reduction potential. One example of this is the well-known Au-Ag bimetallic nanoparticle system, in which Ag ions are reduced later and adsorbed onto the Au particles, generating a core-shell structure, whereas Au ions are reduced first, forming the nuclei.

PROPERTIES OF NANOPARTICLES:

Unlike bulk materials, nanoparticles have unique optical, thermal, electrical, chemical, and physical properties and, hence, they find a wide range of applications within the areas of drugs, chemistry, environment, energy, agriculture, information, and communication, heavy industry, and commodity.[1]

1. Physical Properties: Nanoparticles have large amount of surface atoms, large surface energy, and imperfections decreased and spatial confinement.

2. Optical Property: For the previous couple of decades, the colorful colloidal solutions have a desirable part in research in metallic nanoparticles. Electromagnetic light wave interacted in small metallic spheres which solve Maxwell's equation.

3. Magnetic Property: The nanostructured materials differ from bulk materials in magnetic properties. There is a good range of application of magnetic nanoparticles, like immobilization of enzymes and proteins, biosensors, drug delivery, bioseparation, and immunoassays.

4. Mechanical Property: When the dimensions are decreased mechanical properties is seen to be increased.

TYPES OF NANOPARTICLES:

Counting on dimensions the nanostructure is assessed into four types. They are
i. Zero dimensions
ii. One dimensional
iii.Two dimensional
iv. Three dimensional nanostructures.

Based on the materials which they are made: [2]Nanomaterials are often made from inorganic or carbon (fullerenes, nanotubes, etc.) based materials. The latter, include metal oxides (iron oxide, cerium oxide, etc.) metals (iron, silver, gold, etc.) and quantum dots (cadmium selenide and cadmium sulfide).

ETHNOMEDICINAL IMPORTANCE:

1. The tribal people often consume and sale the highly nutritious seeds to sustain and also to earn their livelihood. The seeds possess 3.0% moisture and are rich in lipid/fat (59.0%), protein (19.0-21.6%), starch/carbohydrate (12.1%), fibre (3.8%), minerals such as calcium (279.0 mg), phosphorus (528.0 mg), iron (8.5 mg) and vitamins such as thiamine (0.69 mg), ascorbic acid/vitamin C (5.0 mg), riboflavin (0.53 mg), niacin (1.50 mg) and also contain 34-47% fatty oil.

2. The seeds are also used as expectorant and tonic. The oil extracted from kernels is applied on skin diseases and also used to remove spots and blemishes from the face.[3] The root is used as expectorant, in biliousness and also for curing blood diseases. The leaf juice has purgative, aphrodisiac, expectorant, and digestive properties.

3. The gum after mixing with goat milk is used as an analgesic. [4]Seed collection should be done from 2nd to 3rd week of May for quality seed collection with respect to fruit weight, kernel weight, germination percent, and chemical content i.e. oil, protein and sugar contents.

4. Destructive harvesting could be checked by educating forest tribal population about collection of ripe fruits at proper time i.e. from 2nd to 3rd week of May without damaging the trees by organized collection .

5. This review is an attempt to present an updated comprehensive account about the ethnomedicinal and phytochemical investigation carried out on B. lanzan.[5]

PHYTOCHEMICAL PROFILE :

- This plant like many other forest plants is storehouse of important unknown phyto-medicines. Till now sporadic reports have been published that reveals that specially leaf, bark, and seed are the major source

of various important metabolites of great pharmaceutical value. [6]Of late, researchers are focusing their attention on various forest plants including Chironji.

- The leaves are reported to contain tannins, triterpenoids, saponins, flavonoids, kaempferol-7-o'glucosides, quercetin-3-rahmnoglucoside, quercetin, gallic acid, kaemferol, and reducing sugars, including a new glycoside, and myricetin-3'-rhmnoside-3-galactoside.[7]
- Three major chemical constituents isolated from the methanolic extract of leaves, characterized based on chemical tests and spectral analysis such as infrared, H nuclear magnetic resonance, mass spectroscopy were epinitol, vomicine, and celidoniol.
- The bark contains tannins, alkaloids, and saponins. The seed and seed oil contains fibres, carbohydrates, mineral, fats, vitamin B1 , B2 , B3 , C, calcium, chlorine, copper, iron, magnesium, phosphorus, potassium, sodium, sulfur, fatty oil, β-amyrin.[8]
- The fatty acid composition of B. lanzan seed oil, determined by urea complex formation and gas liquid chromatography is found to contain following: Myristic, 0.6%; palmitic, 33.4%; stearic, 6.3%; oleic, 53.7%.

NEED TO STUDY AND OBJECTIVES :

The synthesis of bimetallic nanoparticlcs using plant extracts is a promising method since it has proved to be cheaper, safer, simpler,quicker, and easier than conventional methods. The green synthesis of Au-Ag bimetallic nanoparticles and their biomedical applications have been extensively reported.[11] On the other hand, silver-iron (Ag-Fe) bimetallic nanoparticles have several applications in optical, medical, and the remediation fields. [12]However, there are synthesis methods and important properties and characteristics that still need to be investigated.

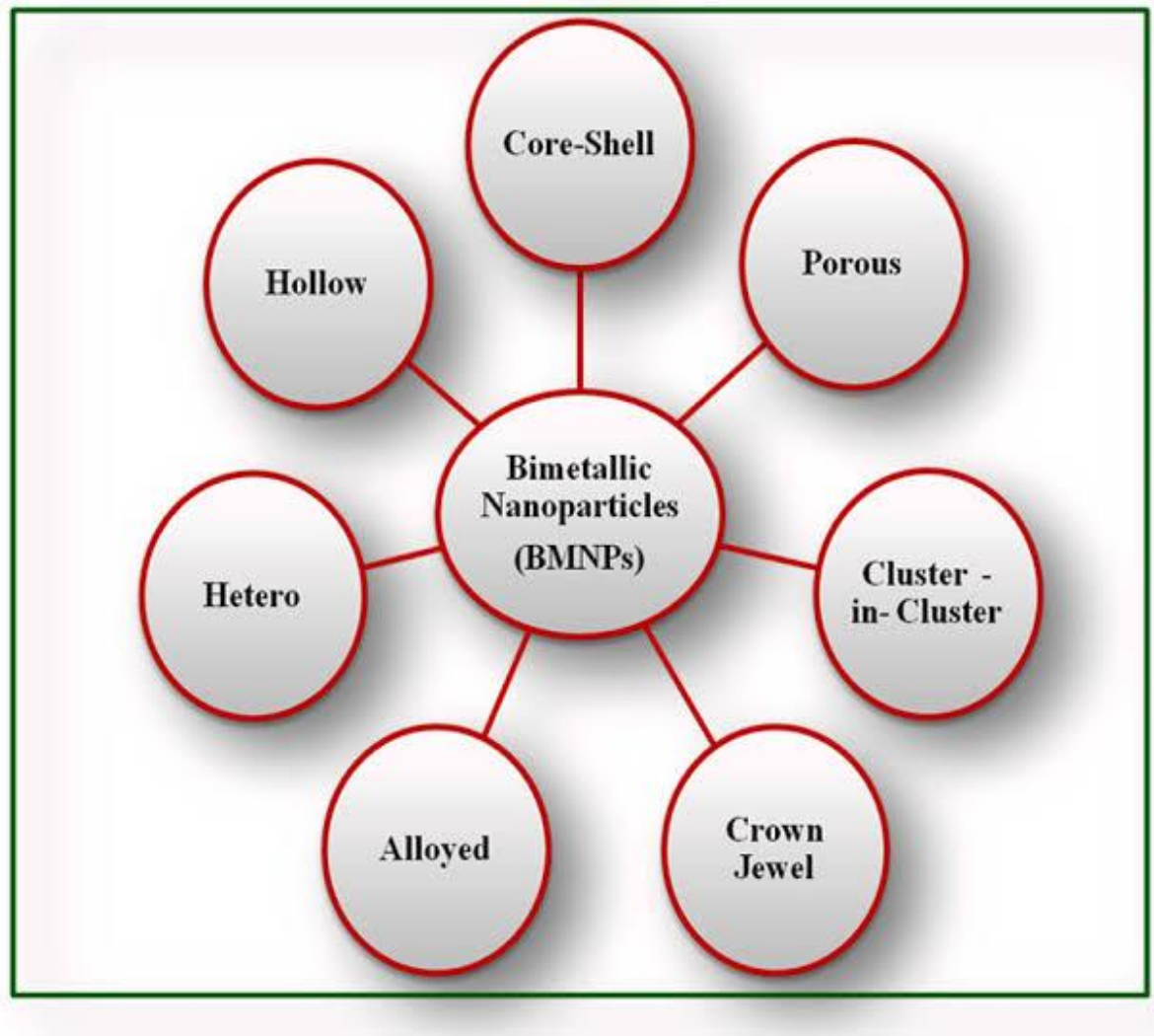
Therefore, in this study we planned to synthesis Ag,Fe, and Ag-Fe bimetallic nanoparticles using B. lanzan extract as a reducing agent in the redox synthesis of the nanomaterials, We will be characterizing the nanostructures by spectroscopic and microscopic analyses and study their magnetic properties as well as the minimal inhibitory concentration (MIC) against clinically relevant pathogenic strains.

This work contributes to the advancement of knowledge on synthesis and antimicrobial properties of mono and bimetallic nanoparticles composed of Ag and Fe.

- Identification and authentication of potential medicinal plant possessing antimicrobial activity. Based on the literature, wehave selected Buchanania lanzan for our study.
- Preparation & Preliminary Phytochemical Screening of various Extracts of Buchanania lanzan.
- Biosynthesis of Silver and Iron Nanoparticles using Aqueous extract of Buchanania lanzan.
- Synthesis of Ag-Fe bimetallic nanoparticles.
- Nanoparticle characterization. By UV, FTIR, SEM and XRD analysis and Magnetic properties.

- Evaluation of antibacterial activity of Synthesized Bimetallic nanoparticles, Silver & Iron Nanoparticle from Buchanania lanzan and plant extract against infection causing bacteria such as Escherichia coli (E. coli), Staphylococcus aureus (S. aureus),Pseudononas aeruginosa.

Fig 1: Properties of Bimetallic Nanoparticles



REVIEW OF LITERATURE :

PLANT PROFILE:

- **PLANT NAME:** *Buchanania lanzan*
- **SYNONYM:** Charoli Nut,Chironji
- **TAXONOMY**

- **Scientific name:** *Buchanania lanzan*
- **Kingdom:** Plantae
- **Phylum:** Tracheophyta
- **Order:** Sapindales
- **Family:** Anacardiaceae
- **Genus:** Buchanania

➢ **VERNACULAR NAMES**

Hindi: Char,Chironji

English: Almondette

Gujrati: Charoli

Bengali: Piyal

Kannada: Kole Maava

Malayalam: Mungaappeezh

Tamil: Charam

DESCRIPTION

Tree height: approximately 20 meters (66 ft). The hairs on young branches are woolly, tangled, and dense. The broad oblong leaves measure 10–20 by 6–9 centimeters (3.9–7.9 by 2.4–3.5 inches), with rounded bases and emarginate (slightly indented) tips. The white flowers have a diameter of 0.3–0.4 centimeters (0.12–0.16 inches). The drupes are subglobose, or inflated but not quite spherical, and measure between 0.4 and 1 centimeters (0.16 and 0.39 inches) in diameter. They are reddish-purple in hue and quite firm when ripe. March through April is when the fruit flowers, and April through June is when it is usually harvested. The chemical compositions of B. lanzan include lipid/fat, protein, carbohydrate,

fiber, minerals such as calcium, phosphorus, iron, and vitamins such as thiamine, Vitamin C, riboflavin, niacin, and also contain fatty oil.

***Fig 2 : Buchanania lanzan* plant**

***Fig 3: Buchanania lanzan* leaves**

MEDICINAL USES:

- *Antioxidant activities*
- *Anti-diabetic activities*
- *Anti-microbial activity*
- *Anti-inflammatory*
- *Antipyretic activity*
- *Analgesic activity*
- *Antidiarrhoeal activity*

METHODOLOGY :

Buchanania lanzan extract preparation:

Leaves from the plant B.lanzan will be collected in and surroundings of Bangalore. Leaves will be washed using deionized water to remove dust and will be dried at room temperature (22-26 °C) for 24 h before being powdered. An aqueous extract will be prepared as follows: two grams of powdered leaves will be mixed with 60 mL of deionized water at room temperature, stirred for 2h, and filtered immediately through Whatmann filter paper. The aqueous extract of B. lanzan was stored at 4 °C until their use in the synthesis of NPs.

Synthesis of Ag-Fe bimetallic nanoparticles:[13]

The synthesis of Ag-Fe bimetallic nanoparticles will be performed by adding 12.5 mL of 0.02M $AgNO^3$, and 12.5 mL of 0.01M $Fe(NO^3)3$ in a Falcon tube. Then, the tube will be mixed and heated until reaching 60°C. When this temperature reached, 25 mL of the filtered B. lanzan extract will be added to the mixture (2:1:1 proportion), kept at constant stir, and heated at 80°C for 1 h. The formation of the nanoparticles will be monitored and confirmed by a change of colour of the mixer that changed from transparent to brown (for silver) or black (for iron). The reaction will be cooled at room temperature, centrifuged at 14,000 rpm for 5 min, and the supernatant will be discharged. The product will be then washed with 70% ethanol, left to dry at room temperature for 24h.

Characterization of Nanoparticle:[14]

The formation of Bimetallic nanoparticles and silver & Iron nanoparticles using plant extract is monitored by various analytical techniques like UV-Visible Spectroscopy,UV-Vis, Fourier-Transform,Infrared Spectroscopy,FT-IR, X-Ray DiffractometerXRD, Scanning Electron Microscopy SEM and Magnetic properties also will be analysed.

Anti-Bacterial Activity:

Preparation of inoculum:

For evaluation of antibacterial activity, 24h fresh culture of bacteria such as Escherichia coli (E. coli),Staphylococcus aureus (S. aureus) Pseudomonas aeruginosa), will be suspended in sterile water to obtain a uniform suspension of microorganisım.

Determination of zone of inhibition:[15]

Antibacterial activity will be checked by agar well diffusion method. In this method a previously liquefied medium will be inoculated with 0.1 mL bacterial suspension having a uniform turbidity at tempcrature of 40°C. In a sterile petri dish having an internal diameter of 8.5 cm will be taken, 20 ml of culture medium was poured into it. Care was taken to form a uniform thickness of the medium in different plates.Wells are made aseptically with cork borer having 6mm diameter after complete solidification of liquefied inoculated medium. In each of these Bimetallic nanoparticles, plant extract, Silver and Iron nanoparticle be placed carefully. Plates will be kept for pre diffusion for 30min at room temperature; Then the plates will be incubated at 37°C for 24h and the zones of inhibition wil be measured.

RESULT AND DISCUSSION:

Drug-resistant bacteria have emerged as a result of improper antibiotic use and insufficient infection management. Because of their broad-spectrum antibacterial capabilities, metallic nanoparticles and metallo-pharmaceutics have been suggested as viable substitute antimicrobials in recent years. Furthermore, new research has demonstrated that mixtures of transition metal compounds can have combined antibacterial effects. In order to harness the interactions between metal groups and the organic complex structures present in various microbial targets, it is worthwhile to investigate the synthesis and design of bimetallic nanoparticles. This will help develop more effective combinatorial antimicrobials made of synergistic metals.

SYNTHESIS OF BIMETALLIC NANOPARTICLES

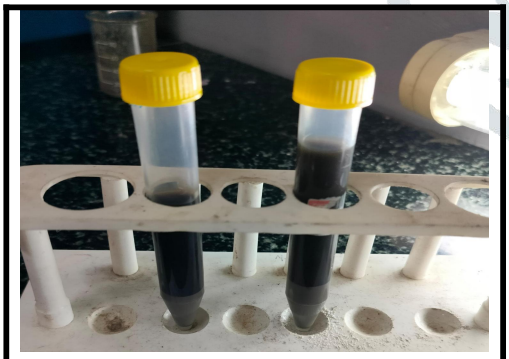
0.24g silver nitrate & 0.19g ferric nitrate

+

50ml B. lanzan extract

BOTH ARE MIXED AND KEPT IN MAGNETIC STIRRER HOT PLATE FOR 1 HOUR

AFTER THAT THE SOLUTION IS CENTRIFUSED



THE FORMATION OF NANOPARTICLES CAN BE SEEN BELOW THE TUBE

Fig 4: FLOW CHART FOR THE SYNTHESIS OF BIMETALLIC NANOPARTICLES

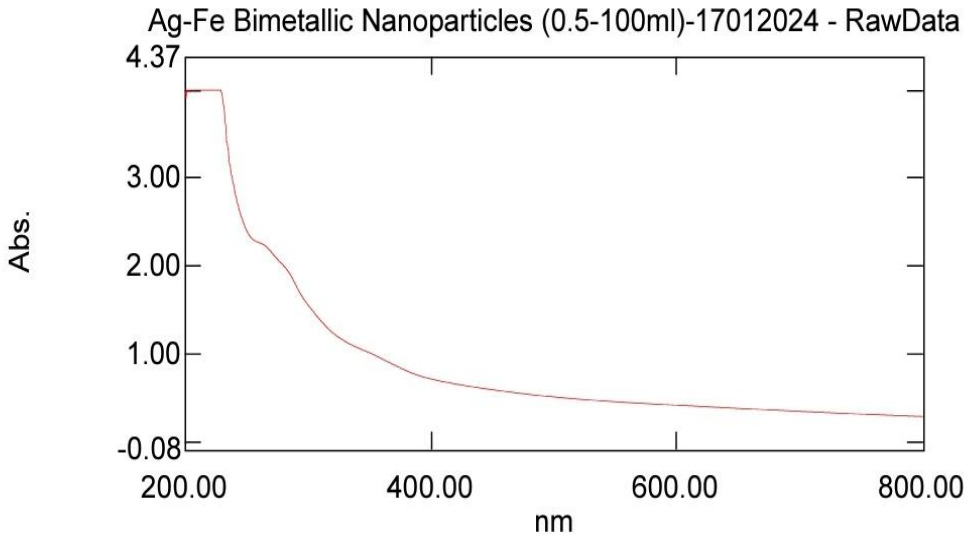
CHARACTERIZATION OF BIOSYNTHESIZED SILVER AND IRON NANOPARTICLES

Characterizations of silver and iron nanoparticles were done using UV-Vis spectrophotometer, Fourier Transform Infrared Spectroscopy FT-IR, X-ray diffraction XRD, Scanning Electron Microscope SEM and Anti-microbial analysis.

- **UV -Visible spectrophotometer (Fig 5)**

  UV-Visible spectroscopy (UV-Vis) measures the extinction (scatter + absorption) of light passing through a sample. UV-Vis is an invaluable instrument for the identification, characterization, and research of nanomaterials because of the special optical properties of nanoparticles, which are sensitive to the size, shape, concentration, aggregation state, and refractive index near the nanoparticle surface.

File Name: Ag-Fe Bimetallic Nanoparticles (0.5-100ml)-17012024 - RawData



[Summary]

Data Information		Software Information	
Data is:	Measurement Data	Software Name:	LabSolutions UV-Vis
Data Set Name:	RawData	Version:	1.14
Sample Name:	Ag-Fe Bimetallic Nanoparticles (0.5-100ml)	Instrument Information	
Sample ID:	Ag-Fe Bimetallic Nanoparticles (0.5-100ml)	Instrument Name:	UV
Option:		Instrument Type:	UV-1900 Series
Analyst:		Model (S/N):	UV 1900i (A12535780084)
Date/Time:	01/17/2024 02:24:49 PM		
Comments:			
Parameter File Name:	E:\Ag-Fe Bimetallic Nanoparticles-17012024.vspm		

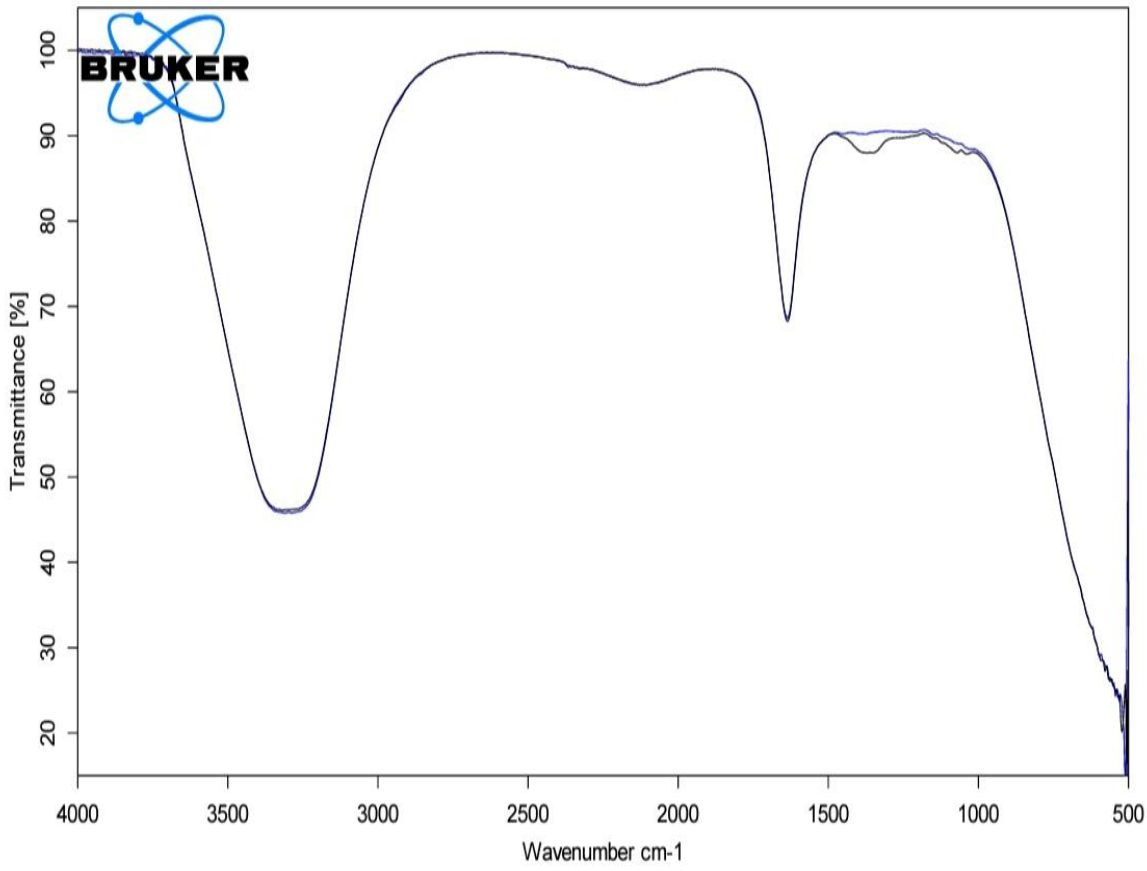
[Peak Pick Table]

Threshold: 0.001000
Number of Points: 4

No.	P/V	Wavelength nm.	Abs.	Description
1	↑	218.0	4.000	
2	↓	207.0	3.988	

- **Fourier Transform Infrared Spectroscopy FT-IR(Fig 6)**

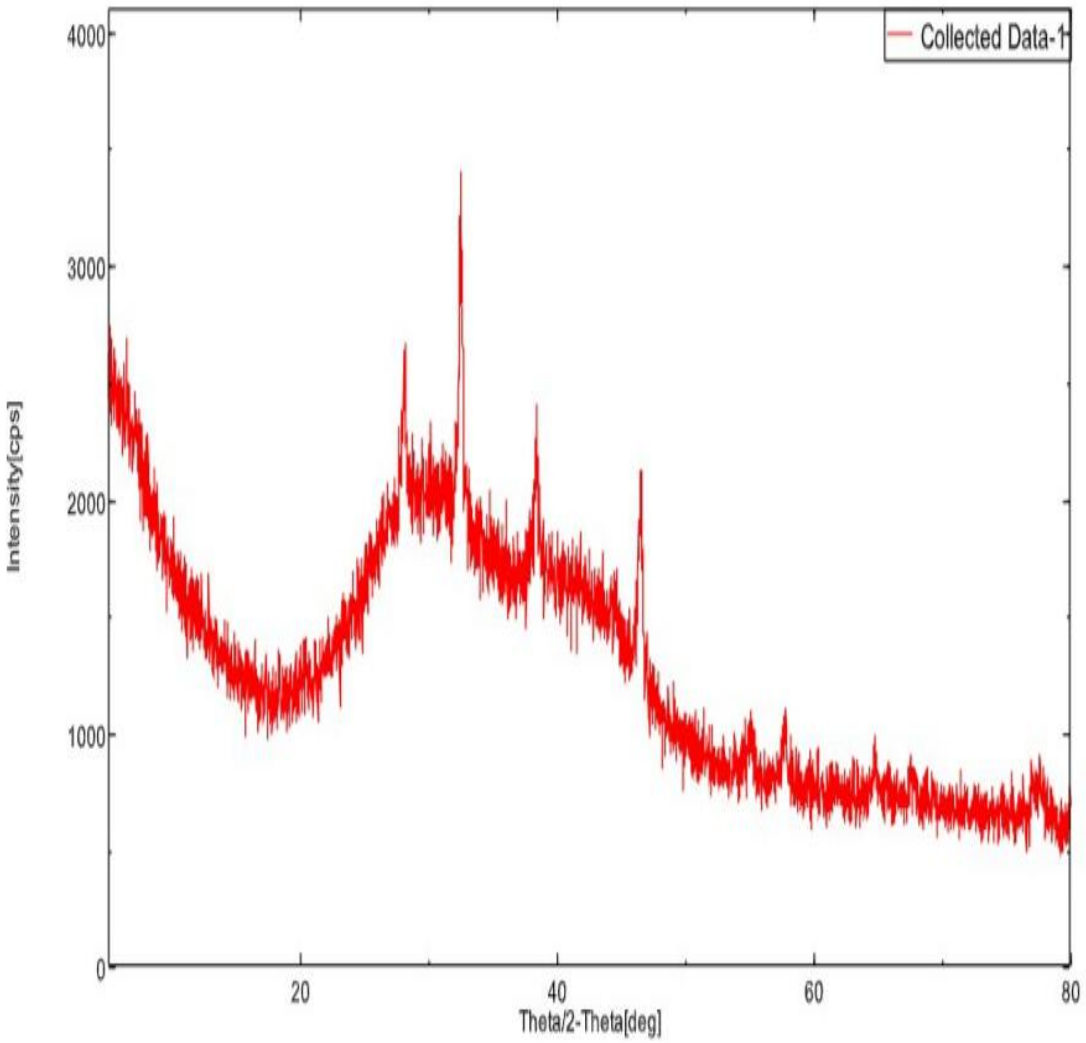
The Ag–Fe bimetallic nanoparticle FTIR spectra produced by the aqueous extract of S. officinalis leaves showed two major peaks at 3309 cm−1 and 1698 cm−1 which corresponds to OH functional groups of polyphenolic compounds and C–H stretching vibrations, respectively.



C:\PRL\Ag-Fe Bimetallic Nanoparticles.0	Ag-Fe Bimetallic Nanoparticles	Liquid	17-01-2024
C:\PRL\B lanza plant extract.0	B lanza plant extract	Liquid	17-01-2024

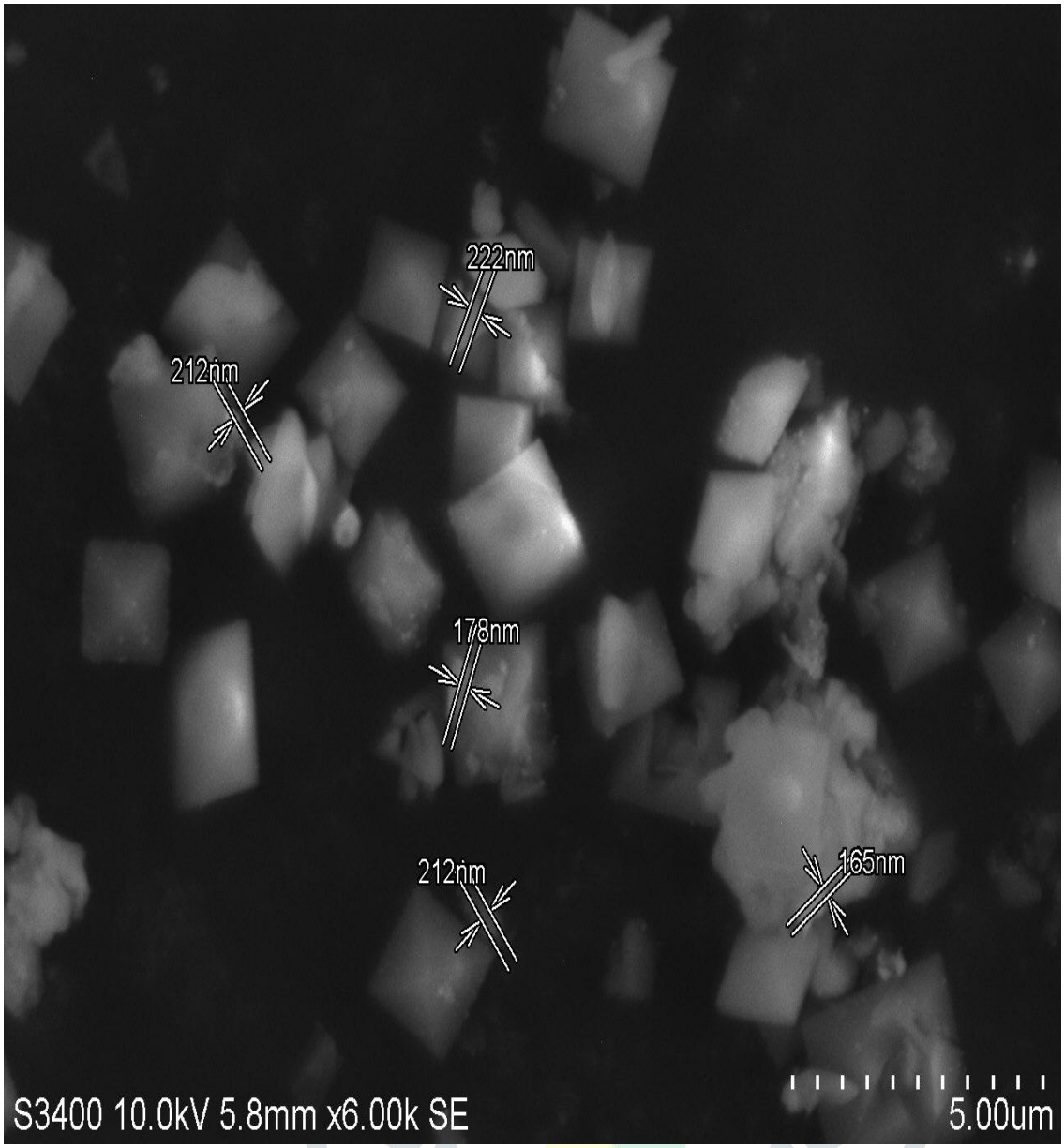
- **X-ray diffraction XRD(Fig 7)**

  XRD is used for characterization of nanopowders of any sizes, and the observed changes in positions of diffraction peaks are used to make conclusions on how crystal structure and cell parameters changes with the change in nanoparticles size and shape.



- **Scanning Electron Microscope SEM(Fig 8)**

Advances in scanning electron microscopy (SEM) enable the high-resolution imaging of single nanoparticles (NPs) with sizes well below 10 nm. The SEM analysis in transmission mode (T-SEM) of NPs on thin film supports has many benefits when compared to the analysis of NPs on bulk substrates.



- **Anti-microbial analysis**

Sl.No	Particulars	Source	Catalogue No

1	Agar Base	Himedia	90935
2	Petri plates		0
3	96 well plate		
4	L-spreader		
5	1000µl tips		0C
6	200µl tips		C
7	Micro centrifuge tubes		

Test organisms: ***E coli***

Test compound as standard: Tetracycline

Inoculum:

Staphylococcus mutans cell suspension were prepared and grown on media and cultures were incubated for 24hrs at 37°C. All of the cultures' cell suspensions were tuned to 1-2x 106 cells/ml.

Test compound:

Sample : Plant extract

S-Standard: Tetracycline 25 µg/ml

Procedure:

Determination of Antimicrobial activity

E coli were inoculated on media (90 mm)

Test compounds: Sample (25µl), Standard Tetracycline (25µl) ***E coli*** were added to the 5mm well on agar plates. The treated plates with ***E coli*** were incubated in aerobic chamber at 37°C for 24hrs. The treated plates were observed for zone of inhibition around the wells.

Fig 9 : Inhibitory activity of test sample against *E coli*

Std-Standard (Tetracycline). 1 -Agno3, 2 -Plant Extract

Results for antimicrobial studies:

Inhibitory activity of test compounds against test organisms(table 1)

	Test Compounds	**Conc. per well**	**Zone of inhibition (mm)**
E coli	Tetracycline (Standard)	25 µg/ml	18
	Agno3	-	10
	Palnat extract	-	8

Conclusion:

The zone of inhibition observed against test compound and standard are summarized in Table 1 and Figure 9. Sample has shown inhibitory activity against ***E coli*** in well diffusion.

SUMMARY

1. Biosynthesis and design of Ag-Fe bimetallic nanoparticles was done using a medical plant *Buchanania lanzan* as antimicrobial synergistic combination therapies against clinically relevant pathogens.
2. *Buchanania lanzan* Spreng, a dry deciduous forest tree of family Anacardiaceae is widely used by Indian tribes for treating various diseases.
3. This review attempts to present thorough updated account of ongoing and emerging areas of research of this plant, especially in the field of phytomedicnes and pharmaceuticals.
4. Two distinct metal elements are combined to create bimetallic nanoparticles (NPs), which can have a variety of shapes and forms
5. This plant like many other forest plants is storehouse of important unknown phyto-medicines. Till now sporadic reports have been published that reveals that specially leaf, bark, and seed are the major source of

various important metabolites of great pharmaceutical value. Researchers have recently turned their attention to chironji and other forest plants.

6. The formation of Bimetallic nanoparticles and silver & Iron nanoparticles using plant extract is monitored by various analytical techniques like UV-Visible Spectroscopy,UV-Vis, Fourier-Transform,Infrared Spectroscopy,FT-IR, X-Ray DiffractometerXRD, Scanning Electron Microscopy SEM and Magnetic properties also will be analysed.

7. UV-Visible spectroscopy (UV-Vis) measures the extinction (scatter + absorption) of light passing through a sample. UV-Vis is a useful technique for identifying, characterizing, and researching nanomaterials because nanoparticles have special optical properties that are sensitive to the size, shape, concentration, aggregation state, and refractive index near the nanoparticle surface.

8. The FTIR spectra of Ag–Fe bimetallic nanoparticles synthesized by the aqueous extract of S. officinalis leaves showed two major peaks at 3309 cm−1 and 1698 cm−1 which corresponds to OH functional groups of polyphenolic compounds and C–H stretching vibrations, respectively.

9. XRD is used for characterization of nanopowders of any sizes, and the observed changes in positions of diffraction peaks are used to make conclusions on how crystal structure and cell parameters changes with the change in nanoparticles size and shape.

10. Advances in scanning electron microscopy (SEM) enable the high-resolution imaging of single nanoparticles (NPs) with sizes well below 10 nm. When NPs on thin film supports are subjected to SEM analysis in transmission mode (T-SEM), it offers numerous advantages over NPs on bulk substrates.

11. Anti microbial properties are found in plant extract. The zone of inhibition observed against test compound and standard are summarized in Table 1 and Figure 9. Sample has shown inhibitory activity against *E coli* in well diffusion.

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