WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Microwave Assisted Green Synthesis of Silver Nanoparticles from Plant Extract Mimosa Hamata.

[1]Miss. Jakkalwar Shital M., [2]Miss. Patil Komal S., [3]Miss. Suryawanshi Kanhopatra B., [4]Miss. Mazalkar Komal K., [5]Sawant Supriya D.

[1,2,3,4,5]Durgamata Institute of Pharmacy, Dharmapuri, Parbhani.

ABSTRACT

Natural products are the source of synthetic and traditional herbal medicine. Plant extracts have been in spot light for their extreme ability to synthesis nanoparticles, including silver and gold nanoparticles. Green synthesis of silver nanoparticle (AgNPs) has gained a drastic importance in the field of nanotechnology. The present study deals with an environment friendly and biosynthesis process of antibacterial silver nanoparticles using *Mimosa Hamata*leaf extract. The synthesized AgNPs using*Mimosa Hamata*leaf extract wasdetermined by UV-Visible spectroscopy, FT-IR, XRD,TEM, and DSC. Antibacterial efficacy of silver nanoparticles was also investigated by disc diffusion method and it was found that the antibacterial activity of silver nanoparticles is impressive in hampering the growth of *E. coli*.

Key words: *Mimosa Hamata*leaf extract, Silver Nano Particle, Antimicrobial, Disc diffusion.

INTRODUCTION

In recent trends, nanotechnology is gaining more attention among researchers due to its tremendous role in eliminating the use of toxic chemicals in efficient synthesis of nanomaterials

The synthesis of nanoparticles has shot into limelight because of its efficiency and minimal health and environmental hazards as compared to conventional chemical synthesis.[1] Synthesis of metal nanoparticles from bacteria and plants has initiated a new era in the history of nanoparticle synthesis. Moreover, fabricating nanomaterials by "green approach" has less impact on the environment and minimize health associated risks.[2] Even today compounds from plants continue to play a major role in primary healthcare as therapeutic remedies in many developing countries. Plants are a rich source of secondary metabolites with interesting biological activities. Secondary metabolites like flavonoids, phenols, phenolic glycosides, saponins and glycosides are an important source with a variety of structural arrangements and properties.[3] The improvement of health after herbal treatment, low cost of the drugs, non-availability of synthetic rugs particularly in the rural areas, where available were either fake or expired drugs and in some cases the people are more accustomed to and comfortable with traditional healing.[4]Plants are important source of phytochemicals and phytopharmaceuticals, used to prepare various herbal drugs. The numbers of plants are used as folk remedies in different countriesand are source ofmany potent and powerful drugs or natural product medicines (Srivastava et al., 1996; Raja et al., 2010).

A variety of secondary metabolites are found in plants which may have useful effect for mankind (Kamboj, 2000). The present study was focused to evaluate the bioactive compounds and beneficial uses of M. hamata. Plants play important role in Indian traditional system of medicines (Iwu et al., 1999). However, several secondary metabolites such as alkaloids, flavonoids, steroids, phenolics, terpenes, volatile oils etc., are usually present in plants and some phytocompounds are responsible for the pharmacological effects (Vyas et al., 2012). Herbal products have proved to be reliable source of large amount of drugs which are used in the treatment of numerous diseases. Synthetic drugs are effective but they fall behind the undesired properties and may generate frequent side effects. So, herbal drugs have no side effect, affordable and easily accessible than synthetic drugs (Jasuja et al., 2012a, b). However, the clinical study is mandatory to herbal drugs before being recommended for human being. According to the evaluation of WHO (2003), 80% people of the world still depend on traditional medication system for primary health care (Santos et al., 1995; Bizimenyera et al., 2007). Recently, researchers are emphasizing on valuation and description of phytoconstituents of plant against various diseases based on their traditional claim given in ayurveda. Isolation and identification of the bioactive compounds of plants have always been a challenging task for researchers (Bairwa et al., 2011). Mimosa hamata is an Ayurvedic plant which belongs to family Mimosaceae (Touch me not) which is used in several traditional medicines to cure various diseases. In Hindi, the plant is commonly known as chilati, Jinjani, jijni, ali, alaili, korindum, gulabi babul, liptti, bander kirakhi and hooked Mimosa.

The main objectives of this study was to synthesize the silver nanoparticles using aqueous extract of *Mimosa Hamata*. to characterize the AgNPs by using UV-Visible spectroscopy, FT-IR, DSC, XRD, TEM, and also to analyze antimicrobial properties against Gram-positive and Gram-negative bacteria.

MATERIALS AND METHOD

Collection and authentication of plant materials

The leaves of *Mimosa Hamata*were collected from S.R.T.M. University campus and authenticated in School of Life science, S.R.T.M.U. Nanded.

Preparation of plant extract: From *Mimosa Hamata*leaves

100gm of *Mimosa Hamata*leaves are washed with distilled water dried and chopped into small pieces and grinded to form powder .The powder was subjected to extraction using methanol as solvent in soxhlet apparatus for 6hrs at 400°C the resultant extract dried to get powder. The powdered extract was used for study.

Green Synthesis of *Mimosa Hamata*Silver Nanoparticles (Ag NPs*)*

For the green synthesis of Ag NPs, 10 ml of the aqueous leaf extract of *Mimosa Hamata*was allowed to react with 90 ml of $AgNO_3$ (1 mM) in an volumetric flask (100 ml) at room temperature. The conversion of colorless transparent solution into a characteristic reddish brown was observed within five minutes which clearly indicated the formation of Ag NPs. Then, the obtained solution was purified by centrifugation at 20,000 rpm for 20 minutes. After centrifugation, the sedimentation was re-dispersed in deionised water. Finally a powder form of AgNPs was obtained after It was then placed in a domestic microwave oven operating at a power of 800W and frequency 2450MHz for the time interval of 90 sec. powder dried and the same was further used for characterization.[15]

Phytochemical screening test:

All the preliminary phytochemical tests *Mimosa Hamata*were performed.[16]

1) Detection of alkaloids

Methanol extracts were dissolved individually in dil. hydrochloric acid (10 ml) and then filtered and referred as test solution.

- **Mayer's test:** To 1 ml of test solution of methanolic extract added few drops of Mayer's reagent (Potassium Mercuric Iodide Solution). Cream precipitate indicated the presence of alkaloids.
- **Wagner's test:** To 1 ml of test solution of methanolic extract added equal volumes of Wagner's reagent (Iodine in Potassium Iodide). Reddish precipitate indicated the presence of alkaloids.
- **Hager's test:** To 2 ml of test solution of methanolic extract added few drops of Hager's reagent (Saturated Picric Acid Solution). Bright yellow precipitate indicated the presence of alkaloids**.**
- **Dragendroff's test:** To 1 ml of test solution ofmethanolic extract added few drops of Dragendroff's reagent (Solution of Potassium Bismuth Iodide). Formation of red precipitate indicated the presence of alkaloids.

2) Detection of glycosides: Methanol extracts were treated individually with dil. hydrochloric acid (10 ml), and then filtered and referred as test solution.

- **Modified Borntrager's test:**Methanol extract were treated with ferric chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink color in the ammonical layer indicated the presence of anthranol glycosides.
- **Legal's test:**Methanol extract were treated with sodium nitropruside in pyridine and NaOH. Formation of pink to blood red color indicated the presence of cardiac glycosides.
- **Keller-killani test:** Methanol extrac (50 mg) were treated with 2 ml of glacial acetic acid containing one drop of 5% ferric chloride, followed by addition of 1 ml of concentrated sulphuric acid. A brown ring at the interface is the feature of cardenolidedeoxy sugar. Appearance of the violet ring below the brown ring and greenish ring in acetic acid layer indicated the presence of cardiac glycoside.

3) Detection of Saponins

- **Froth test:** Methanol extract were diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1cm layer of foam indicated the presence of saponins.
- **Foam test:** 0.5 gm of Methanol extract were shaken with 2 ml of water. If foam produced and persists for 10 minutes it indicates the presence of saponins.

4) Detection of phenols

- **Ferric chloride test:** Methanolic extract were treated with 3-4 drops of ferric chloride solution. Formationof bluish black color indicated the presence of phenols.

5) Detection of flavonoids

- **Alkaline reagent test:** Methanol extract weretreated with few drops of sodium hydroxidesolution. Formation of intense yellow color,which becomes colorless on addition of dilute acid, indicates the presence offlavonoids.
- **Lead acetate test:**Methanol extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicated the presence of flavonoids.

6) Detection of proteins and amino acids

- **Xanthoproteic test:** Methanol extract were treated with few drops of conc. nitric acid.Formation of yellow color indicated thepresence of proteins.
- **Ninhydrin test:** To Methanol extract 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue color indicated the presence of amino acid.

7) Detection of phytosterols

- **Salkowski's test:**Methanol extract were treated with chloroform and filtered. The filtrateswere treated with few drops of Conc.sulphuric acid, shaken and allowed to stand.Appearance of golden yellow colour indicatedthe presence of triterpenes.
- **Libermann Burchard's test:** Methanol extract were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride then boiled and cooled. After that conc. Sulphuric acid (0.5 ml) was added. Formation of brown ring at the junction indicated the presence of phytosterols.

8) Detection of tannins

- **Ferric chloride test:** Methanol extract were dissolved in 5 ml of distilled water and few drops of 5% ferric chloride were added. Bluish black color indicated the presence of tannins.

9) Detection of carbohydrates

Methanol extract were dissolved individually in 5 ml of distilled water then filtered and referred as test solution.

- **Benedict's test:** Filtrates were treated with Benedict's reagent and heated gently. Orangered precipitate indicated the presence ofreducing sugars.
- **Molisch's test:** Filtrates were treated with 2 drops of alcoholic a-naphthol solution in atest tube. Formation of the violet ring at thejunction indicated the presence of carbohydrates.

UV-Vis Absorbance Spectroscopy

The reduction of silver ions to Ag NPs was observed by investigating with UV-Vis spectrometer (UV-Visible spectrophotometer,Shimadzu UV-1800 Japan), by sampling 3 ml of aliquots of the reaction medium in the wavelength ranging from 200 – 800 nm, so as to confirm the formation of Ag NPs.[17]

Fourier Transform Infrared Spectrophotometer (FTIR)

Fourier Transform Infrared Spectrophotometer (FTIR) is the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. Dried powders of different solvent extracts of each plant material were used for FTIR analysis. 10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of each plant specimen was loaded in FTIR spectroscope (Shimadzu, IR, Japan), with a scan range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}

Differential Scanning Calorimetry Analysis:

The DSC was used to measure the occurrence of exothermic or endothermic changes with increase in temperature. The DSC because of its sensitivity and accuracy has been extensively used to study the phase transition of polymer. Differential Scanning Calorimetry (DSC) measures the temperature and heat in the material. It determines time function and temperature in a controlled atmosphere. These measurements provide quantitative and qualitative information about physical and chemical changes that involve during endothermic or exothermic processes, or changes in heat capacity. The onset, peak and conclusion temperatures of base transition were observed to be moderate. The knowledge of glass transition temperature is essential in production processes and storage glass transition temperature is affected by moisture and other additives, facilitating conversion to the rubbery state and hence fasciltating crystallization through molecular rearrangement.

Procedure:

To carry out DSC testing a required quantity of sample was taken. Then this sample was placed in the pan. This pan is carefully handled by using forceps. This was covered by using cap and pressed well. After this crucible is kept in the instrument where it comes in between length path and graph is obtained. The x-axis shows the time and energy change is given on y-axis. The DSC of synthesized silver nanoparticles in are shows in figure.

Scanning Electron Microscopy analysis:

The scanning electron microscope (SEM) uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens. The signals that derive from electron reveal information about the sample including external morphology (texture), chemical composition, and crystalline structure and orientation of materials making up the sample.

Preparation of sample:

Scanning Electron Microscope gives morphological features of synthesized silver Nanoparticles. SEM slides were prepared by making a smear of the solutions on slides. After 24Hrs of the addition of $AgNO_3$ the Samples were made conductive by coating a thin layer of platinum. Then the samples were characterized in the SEM at an accelerating voltage of 20KV.The SEM of synthesized silver nanoparticles in are shows in figure.

Transmission electron microscopy analysis:

TEM analysis of Ag-Nanoparticles was prepared by placing a drop of the Nanoparticles suspension on the carbon-coated copper grid and allowing the water to evaporate inside a vacuum dryer. Scanning under TEM revealed that the average mean size of silver nanoparticles was 18-20 nm and the tiny particles were seemed to be spherical in morphology. The show the existence of nano-crystalline structure in the particles.The TEM of silver nanoparticles are shows in figure.

X-ray diffraction spectroscopy Analysis:

X-ray diffraction spectroscopy used to identify phase diversity and particle size of synthesized silver nanoparticles was determined. Nanoparticles were studied with CUKα radiation at voltage of 30kV and current of 20MA with scan rate of 0.030/s. Scherer's equation used to determine particle size.

$D \approx 0.9\boldsymbol{\lambda}\boldsymbol{\beta}\cos\theta$

Where D is the crystal size, λ is the wavelength of X-ray, Θ is the Braggs angle in radians and is the full width at half maximum of the peak in radians. The XRD of silver nanoparticles are shows in figure.

Antimicrobial Study

Microorganisms used for Antimicrobial Activity

The strains used for screening antibacterial activity were performed from S.R.T.U.Nanded. The bacterial strains like *Staphylococcus aureus, Becillus subtilis, Escherichia coli* were obtained from school of life science.

Antimicrobial Activity by Disc Diffusion Method

Test pathogens were spread on the test plates- Nutrient Agar medium. Sterile antibiotic disc (in 5 mm diameter)-impregnated and the Medicament was loaded in the disc. The test plates were incubated for 24h. The zone of inhibition (mm in diameter) were read and taken as the activity against the test pathogen.[18]

RESULT AND DISCUSSION:

Phytochemical Analysis

The aqueous leaf extract of *Mimosa Hamata*is reported to contain glycoside, alkaloids, saponins, flavonoids, tannins, carbohydrates, proteins, phenolic compounds, phytosterols, and amino acids.The aqueous leaf extract of *Mimosa Hamata*was found to contain major phytochemicals. Phenolic compounds, flavonoid and tannin were present. The preliminary phytochemical tests that the leaves of the plant possess alkaloids, glycosides, flavonoids,[19] tannins etc. The flavonoids are known to reduce lipid peroxidation not only by preventing or slowing the onset of cell necrosis but also by improving the vascularity. Hence any drug that inhibits lipid peroxidation is believed to increase the viability of collagen fibrils by increasing the strength of collagen fibers, increasing the circulation, preventing the cell damage and by promoting the DNA synthesis.[20] Flavonoids[21] and triterpenoids [22] are also known to promote the wound healing property which seems to be responsible for wound contraction and increased rate of epithelialization.Tannins the main component of many plant extract acts as free radical scavenger.[23]*Mimosa Hamata*has many alkaloids such as glaucin and annonaine in different part of theplants.

Table 1 : Phytochemicals in aqueous leaf extract of *Mimosa Hamata*.

Sr. No.	Name of the phytochemical test	*Annona squamosa* leaf extract
1	Alkaloid test	+
2	Carbohydrate test	-
3	Saponin test	+
4	Flavonoid test	+
5	Tannins test	+
6	Phenol test	+
7	Glycosides test	+

8	Protein and amino acid test	-

UV-Vis Absorbance Spectroscopy

The synthesized Ag NPs was determined by UV-Vis absorbance spectroscopy. The silver nanostructure has the optical properties directly related to surface plasmon resonance (SPR); it depends on the morphology of the samples. Bio reduction of silver ions to AgNPs mediated by *Mimosa Hamata*leaf extract was observed by recording the absorption spectra. The yellowishbrown color of silver nanoparticles became visible due to the excitation in surface Plasmon vibrations by absorbance in 582 nm. The spectrum shown in Figure 1 has strong broad peak located between 500-580 nm, the nanoparticles were dispersed in the aqueous solution with no evidence for aggregation. The aqueous leaf extract of *Mimosa Hamata*is reported to contain glycoside, alkaloids, saponins, flavonoids, tannins, carbohydrates, proteins, phenolic compounds, phytosterols. Flavonoids may play an important role in the reduction process for biosynthesis AgNPs .[24] Accordingly, the high content source of flavonoids and phenolic acids in aqueous leaf extract of *Mimosa Hamata*supports the potential bioreduction of Ag+ to Ag. Optical properties of synthesized nanoparticles was determine by UV-Visible spectrophotometer. The formation of silver ion to silver nanoparticles was reflected in 580 nm.

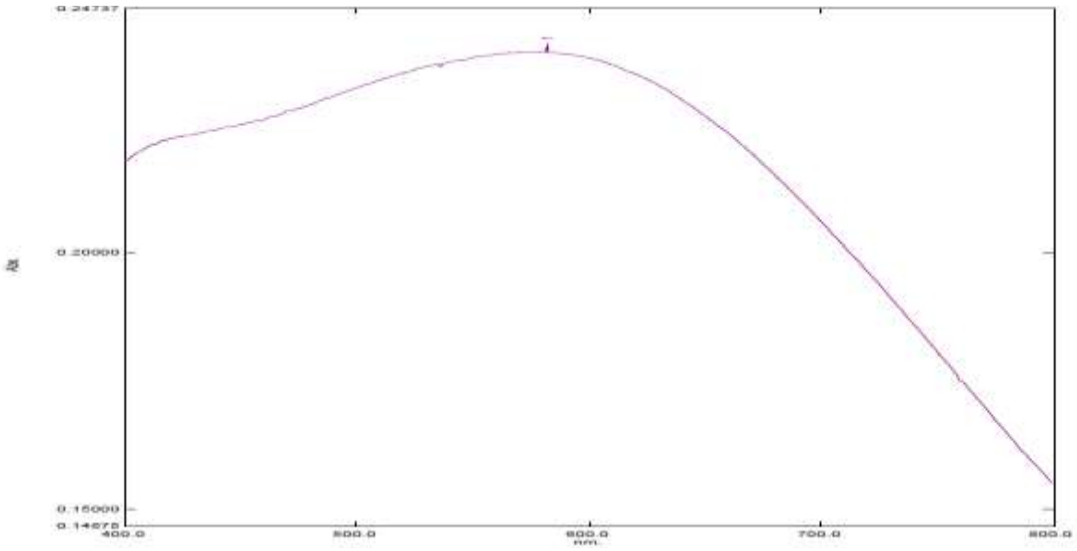

Fig .1: UV Spectrum of silver nanoparticles

Table 2. Data for Calibration curve of *Mimosa Hamata*leaf Extract

conc.(µg/ml)	Absorbance(nm)
2	0.028
4	0.061
6	0.096
8	0.125
10	0.158

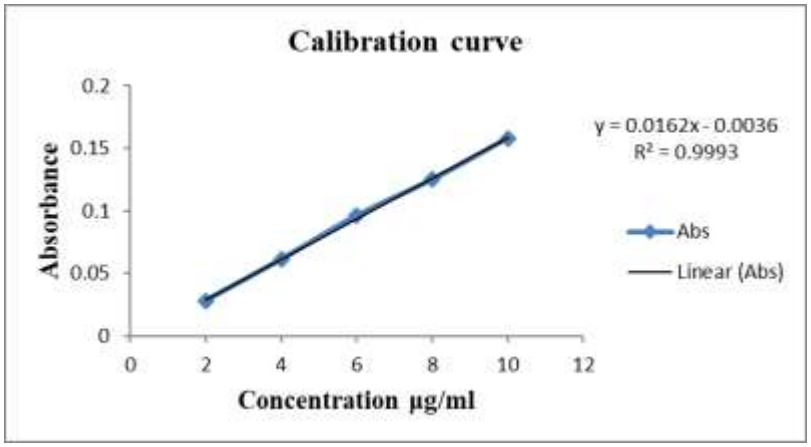

Fig .2: UV Calibration curve of *Mimosa Hamata*leaf Extract

FTIR ANALYSIS:

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 extract and SNPs showed the band between 3490-3500 cm-1 corresponds to O-H stretching H-bonded alcohols and phenols. The peak found around 1500-1550 cm-1 showed a stretch for C-H bond, peak around 1450-1500 cm-1 showed the bond stretch for N-H. Whereas the stretch for Ag-NPs were found around 500-550 cm-1. From the analysis of FTIR studies we confirmed that the carbonyl groups from the amino acid residues and proteins has the stronger ability to bind metal indicating that nanoparticles) to prevent agglomeration and thereby stabilize the medium. This suggests that the biological molecules could possibly perform dual functions of formation and stabilization of silver nanoparticles in the aqueous medium. Carbonyl groups proved that flavanones or terpenoids absorbed on the surface of metal nanoparticles. Flavanones or terpenoids could be adsorbed on the surface of metal nanoparticles, possibly by interaction through carbonyl groupsorπ-electrons in the absence of other strong legating agents in sufficient concentration. The presence of reducing sugars in the solution could be responsible for the reduction of metal ions and formation of the corresponding metal nanoparticles. These issues can be addressed once the various fractions of the *Mimosa Hamata*leaf extract are separated, identified and individually assayed for reduction of the metal ions.

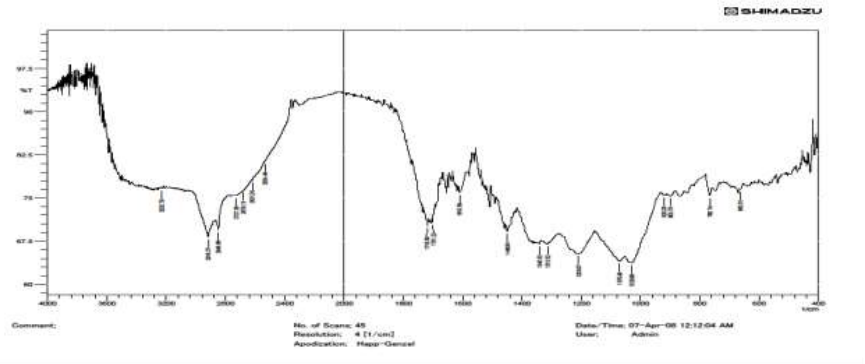

Fig.3: FT-IR of *Mimosa Hamata*leaf Extract

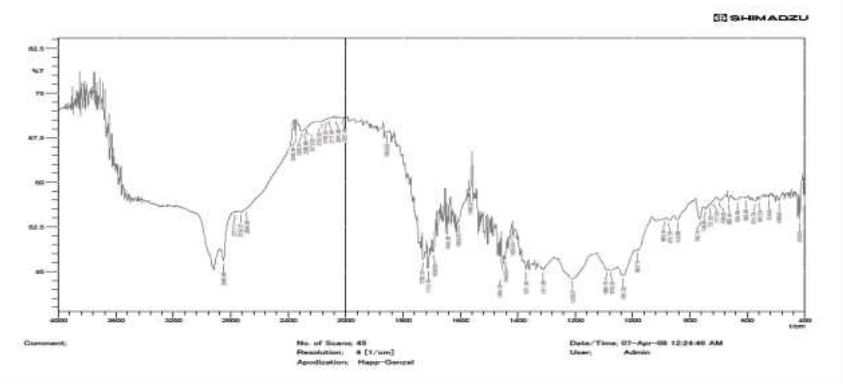

Fig.4: FT-IR of 1:6 Ratio Ag NPs *Mimosa Hamata*leaf Extract

DSC ANALYSIS

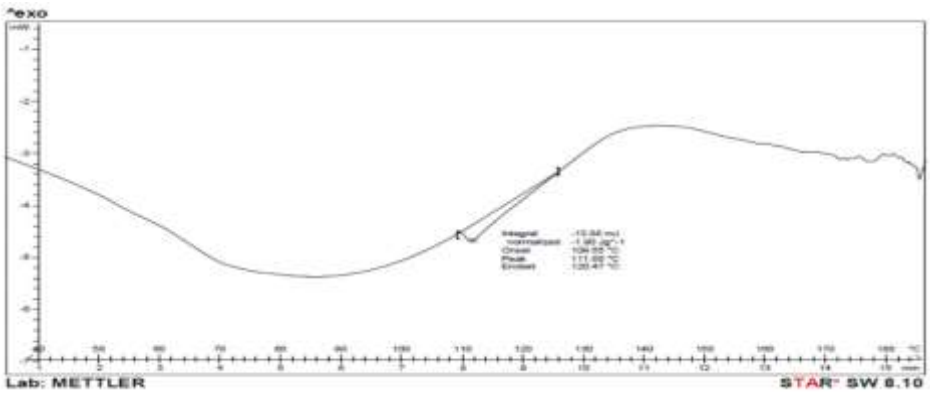

Fig.5: DSC of *Mimosa Hamata*leaf extract

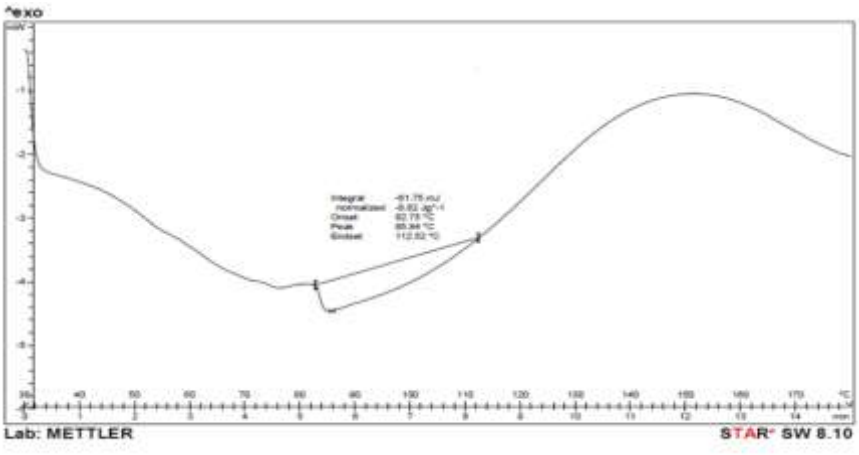

Fig.6: DSC of 1:6 Ratio Ag NPs of *Mimosa Hamata*leaf extract

In the above fig.5 DSC of *Annona Squamosa* plant extract graph shows melting temperature i.e. onset-109.65˚C, Peak-111.65˚C & Endset-120.47˚C which is maximum temperature at which extract degraded. And in the fig.6 DSC graph 1:6 Ag NPs of *Annona Squamosa* plant extract shows melting temperature i.e. onset-82.75˚C, Peak-85.84˚C & Endset-112.52˚C which is maximum temperature at which Ag NPs degraded.

Sr. No.	**Parameter**	***Mimosa Hamata* Plant Extract**	**SNP of *Mimosa Hamata* Plant Extract**
1.	Onset Temperature(˚C)	109.65˚C	82.75˚C
2.	Peak Temperature(˚C)	111.65˚C	85.84˚C
3.	Endset Temperature(˚C)	120.47˚C	112.52˚C

XRD ANALYSIS:

The suspension of silver nanoparticles was dried inside a vacuum chamber for 24 hours sothat a small amount of dry silver nanoparticles can be obtained for X-ray diffraction (XRD) analysis. The XRD curve (Fig 8) confirmed that the nanoparticles are nothing but silver.Interpretation of this XRD pattern reveals the existence of diffraction lines at low angles (5°to 75°). The silver nanoparticles showed the two peaks of silver at 2θ = 38° and 44° that canbe assigned to the (111) and (200) facets of silver, respectively, which go very well with thevalues manipulated for face centered cubic structure of silver nano-crystals.

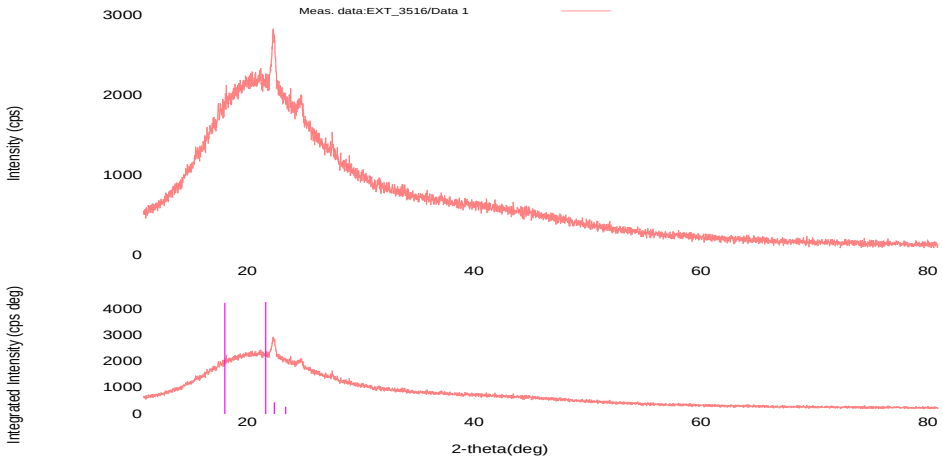

Fig.7: XRD of *Mimosa Hamata* leaf extract

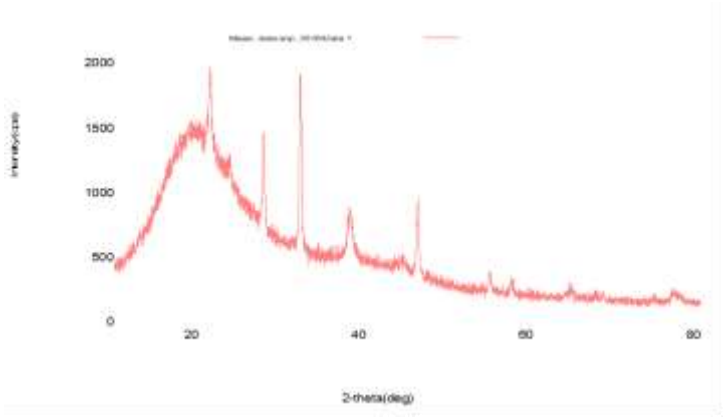

Fig.8: XRDof 1:6 ratio Silver Nanoparticles

TEM ANALYSIS

The grid for the TEM analysis of Ag-nanoparticles was prepared by placing a drop of the nanoparticles suspension on the carbon-coated copper grid and allowing the water to evaporate inside a vacuum dryer. Scanning under TEM (JEOL/JEM-2100) revealed that the average mean size of silver nanoparticles was 5-20 nm and the tiny particles were seemed to be spherical in morphology as shown in the following images (Fig10). The images also show the existence of nano-crystalline structure in the particles.

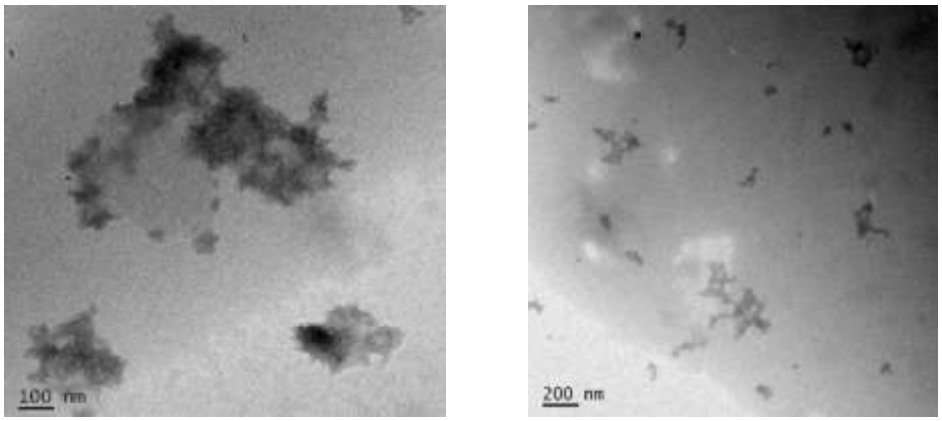

Fig.9 : TEM of *Mimosa Hamata* leaf extract

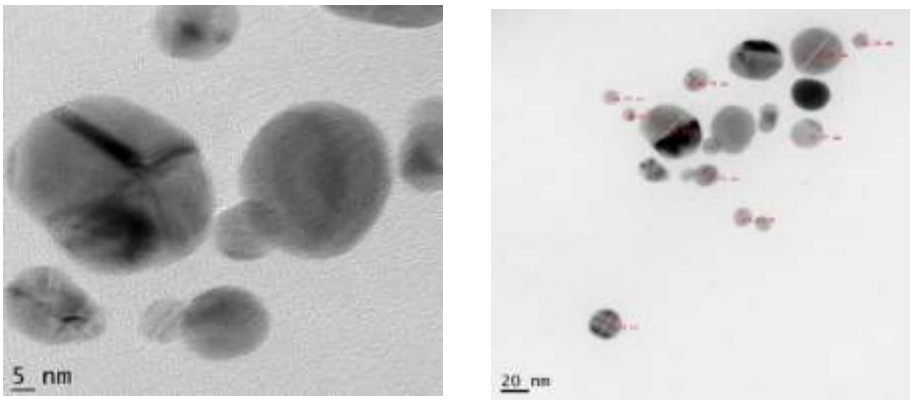

Fig.10: TEM of 1:6 ratio silver nanoparticles

Antimicrobial Activity of Synthesized *Mimosa Hamata*Ag-NPs

The Antimicrobial effects of silver salts have been noticed since many times. But with the advent of nanotechnology, the use of silver in nanoparticle has contributed to the new treatment avenues. Here antimicrobial activity of *Mimosa Hamata*Ag-NPs have been investigated against *staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*. The growth of organisms was tested in both the plates containing prepared *Mimosa Hamata*Ag-NPs and aqueous extract of *Mimosa Hamata*.Table 3 shows the zone of inhibitions of prepared *Mimosa Hamata*Ag-NPs and *Annona squamosa* leaf extract along with Positive control. Among the three organisms *E. coli* showed a zone of inhibition of about 30 mm in diameter in plates containing prepared *Mimosa*

*Hamata*Ag-NPs when compared to the plates containing*Mimosa Hamata*leaf extract.*S. aureus* shows a zone of inhibition of about 20mm and *B. subtilis* with a zone of inhibition of about 15 mm inplates containing Ag-NPs. The susceptibility of the microbes to the plant extract and the synthesised Ag-NPs were compared with the antibiotic ciprofloxacin.

The result are shown in table 3.

Table 3: Zone of inhibition of the extract

Name of the Micro organism	Zone of inhibition in diameter in mm			
Sr.	Ag-NPs (20 µg/disc)	Prepared *Mimosa Hamata*Ag-NPs (20 µg/disc)	*Mimosa Hamata*leaf extract	Positive control Ciprofloxacin (5 µg/disc)
B. Subtilis	8	15	8	22
E. coli	8	30	15	30
S. aureus	6	20	11	35

Minimum Inhibitory Concentration

Disc diffusion method (MIC).

Disc diffusion method was followed by taking ciprofloxacin as standard antibiotic for bacteria. High potency Nutrient agar were prepared and placed on the lawn spreaded agar. After 2 days incubation at 26 ± 2º C for. 24h of incubation at a specific temperature 36 ± 2 º C for B. subtilis, E. coli, S. aureus the plates were examined and the minimum inhibitory concentrations were measured.

Table 4: Minimum Inhibitory Concentration

Conc. of Extract (ug/ml)	*B.subtilis*	*E.coli*	*S. aureus*
5	-	-	-
10	5mm	5mm	4mm
15	5mm	7mm	5mm
20	6mm	7mm	6mm

REFERENCE

1. Konwarh R, Gogoi B, Philip R, Laskar M.A, Karak N. Biomimetic preparation of polymer-supported free radical scavenging, cytocompatible and antimicrobial "green" silver nanoparticles using aqueous extract of *Citrus sinensis* peel. Colloids Surf B Biointerf. 2011, 84:338-345.
2. Ramanathan R,O"Mullane A P, Parikh R.Y,Smooker P M,Bhargava S K,Bansal V.Bacterial Kinetics controlled shapedirected biosynthesis of silver nanoplates using Morganellapsychrotolerans. Langmuir, 2010, 27:714-719.
3. de-Fatima A, Modolo LV, Conegero LS, Pilli RA, Ferreira CV,Kohn LK, de-Carvalho JE. Lactones and their derivatives:biological activities, mechanisms of action and potential leads fordrug design. Curr. Med. Chem. 2006; 13: 3371-3384.
4. Audu JA. Studies on the effective of medicinal Herbs Used asAntihelminitic by traditional medical Practitioners in South ofBauchi State Two. Journal of EconamicTaxanomic Botany.1995; 19: 653-661.
5. Suresh K; Mamoharan S; Panjamurthy K and Kavitak., "Chemopreventive and antilipidperoxidative efficiency of *Annonasquamosa* bark extract", Pakistan journal of Biological sciences;2006, 9(14); .2600- 2605.
6. Raj Sobiya. D, VennilaJannet. J, Aiyavu. C, Panneerselvam. "The heapatoprotective effect of alcoholic extract of *Annona squamosa*leves on experimentally induced liver injury in swiss albino mice". International Journal of Integrative Biology; 2009, 5(3); 182-186.
7. Kirtikar, K R.; Basu, B.D. Indian Medicinal Plants; International Book Distributors: Deharadun,India, 1987; 68–69.
8. Anonymous. The Useful Plants of India; Council of Scientific and Industrial Research: New Delhi,India, 1994, 43.
9. Chang, F R, Chen, J L, Chiu, H F, Wu, M J, Wu, Y C. Acetogenins from seeds of *Annonareticulata.* Phytochemistry1998, 47, 1057–1061.
10. Maeda,U, Hara, N, Fujimoto, Y,Shrivastava, A, Gupta, Y K,Sahai, M. N-fatty acyltryptamines from *Annonareticulata.* Phytochemistry1993, 34, 1633–1635.
11. Marjorie M C. (1999). Plant products as antimicrobial agents, Clinic microbiology reviews; 12:54
12. Hisham, A, Sunitha, C, Sreekala, U, Pieters, L, De Bruyne, T.; Van den Heuvel, H.; Claeys, M.Reticulacinone, an acetogenin from *Annonareticulata.* Phytochemistry1994, 35, 1325–1329.

13. Etse, J.T.; Waterman, P.G. Chemistry in the Annonaceae, XXII. 14-Hydroxy-25-desoxyrollinicinfrom the stem bark of *Annonareticulata.* J. Nat. Prod. 1986, 49, 684– 686.Molecules 2013, 18 4485.

14. Saad, J.M.; Hui, Y.H.; Rupprecht, J.K.; Anderson, J.E.; Kozlowski, J.F.; Zhao, G.X.; Wood, K.V.;McLaughlin, J.L. Reticulatacin: A new bioactive acetogenin from *Annonareticulate(Annonaceae).* Tetrahedron 1991, 47, 2751–2756.

15. U. B. Jagtap, V. A. Bapat: 2012. Biosynthesis, characterization and antibacterial activity of silver nanoparticles by aqueous *Annonasquamosa L*. leaf extract at room temperature. Journal of Plant Biochemistry and Biotechnology.

16. Trease& Evans WC. (1989). Pharmacognasy 11th edition BrailliaeTridal Can. M acmill and Publishers.

17. Sosa, I. O, C. Noguez, and R. G. Barrera. 2003. Optical properties of metal nanoparticles with arbitrary shapes. J. Phys. Chem. B 107:6269-6275.

18. Ashok kumar, B S,Lakshman, K,Tirupathi, S M,Jayaveera, K N,Narayanasamy, V B,Sabemulla&K,Nandeesh, R .(2009). Free radical scavenging and antibacterial activities of army card power (An Ayurvedic formulation). European Bulletien of DrugResearch: 17: 5-9

19. Getie, M,Gebre Mariam, T, Reitz, R. &Neubert, R H. (2002). Evaluation of the release profiles of flavonoids from topical formulations of the crude extract of the leaves of Dodonea viscose (Sapindaceae). Pharmazie. 57:320-322.

20. Tsuchiya, H,sato, M, Miyazaki, T,Fujiwaras,Tanigaki, S,Ohmyama, M, Tanaka, T &linuma, M. (1996). Comparative study on the antibacterial activity of phytochemical flavones against methicillin resistant staphytococcusaureus; J Ethanopharmacl; 50: 27

21. Scortichini, M,Pia, Rm. (1991). Preliminary invitro evaluation of antimicrobial activity of terpenes and terpenoids towards Erwiniaamylovora (burrill). J ApplBacteriol; 71:109-112.

22. Bekerecioglu, M,Tercan, M. &ozyazanl.(1998). The effect of Ginkagobiloba (Egb 761) as free radical scavenger on the survival of skin flaps in rats. Scand J plastReconstr Hand surg; 32:135 – 139.