CODEN [USA]: IAJPBB **ISSN: 2349-7750**

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.2545646

Available online at: http://www.iajps.com ***Research Article***

SYNTHESIS, CHARACTERIZATION OF SILVER NANOPARTICLES FROM COLUTEA ARMATA AND ASSESSMENT OF INHIBITORY EFFECT ON THE GROWTH OF PYROFOMES DEMIDOFII

[1]Shagufta Fahmid (PhD Scholar), [2]Rukhsana Jabeen, [3]Saima Mehar, [1]Naheed Sajjad, [4]Jaffar Ali

[1]Department of Biotechnology, Sardar Bahadur Khan Women's University, Quetta, Pakistan, [2]Department of Botany, Sardar Bahadur Khan Women's University, Quetta, Pakistan, [3]Department of Chemistry, Sardar Bahadur Khan Women's University, Quetta, Pakistan, [4]Office of Agriculture Extension (Plant Protection), Barkhan, Balochistan, Pakistan

Abstract:

Introduction*: Nano biotechnology which involves modern research dealing with synthesis, strategy and manipulation of particles structure ranging from 1 to 100 nm in size. The silver at nano scale can be used in just about all fields, such as antimicrobial coating, ecological wellbeing, chemical industries, catalysis, drug delivery and bio sensing with tremendous unique and effective properties. Production of silver nanoparticles (AgNPs) from plant is a more advantageous route of green synthesis, over chemical and physical scheme as it is cost effective and environment friendly. The resistant wood rotor pathogenic fungus Pyrofomes demidofii found on juniper trees in Juniper forest around the globe together with Pakistan. The fungus is environmental threat and white heart rot disease caused in tree trunks. Keeping in view the above facts, the present study was designed to investigate the synthesis of AgNPs through green route with plant extract Colutea Armata; assessment of inhibitory potential of AgNPs on growth of forest fungi Pyrofomes demidofii to adjoin innovative approach for ecological wellbeing.*

Materials and method*: The synthesis of AgNPs was carried out through green route by utilization of methanolic extract of plant Colutea armata and 1mM solution of silver nitrate without addition of foreign stabilizing or capping agent. Assessment of inhibitory potential of plant extract and AgNPs on growth of fungi Pyrofomes demidofii was done using disk diffusion method. The manufactured AgNPs was characterized with UV-Vis spectroscopy, Infra red spectroscopy, X-ray diffraction and Atomic Force Microscopy.*

Results*: The study revealed that during synthesis of AgNPs, Colutea Armata, plant extract found as an effective reducing engine for the reduction of silver ions of silver salt solution to zero valent silver at nano scale dimension due to having potential bio active metabolites. Characterization revealed the small, stable and spherical shaped AgNPs with poly dispersion regarding size. Plant metabolites polyphenols, carbohydrates and carboxylic acids were involved in reduction, stabilization and capping of AgNPs in synthesis. Moreover synthesized AgNPs were found effective antifungal agent against selected wood rotor fungus.*

Key Words: *Synthesis, silver nanoparticles, Colute armata, Pyrofomes demidofii, Juniper tree, white heart rot disease.*

Corresponding author:

Shagufta Fahmid,

PhD Scholar,Department of Biotechnology,
Sardar Bahadur Khan Women's University, Quetta, Pakistan
fahmidhhh@yahoo.com

QR code

Please cite this article in press Shagufta Fahmid et al., ***Synthesis, Characterization Of Silver Nano Particles From Colutea Armata And Assessment Of Inhibitory Effect On The Growth Of Pyrofomes Demidofii.,*** *Indo Am. J. P. Sci, 2019; 06(01).*

INTRODUCTION:

Silver has long been recognized as anti microbial agent due to inhibitory consequences on the growth of microorganisms present in remedial and industrial practices along with other fields [1] and have antimicrobial properties with minor toxicity [2]. The use of silver has diminished or become less effective due to emergent of new and resistant strain of microorganisms. Now a day, by means of nano biotechnology, which involves modern research dealing with synthesis, strategy and manipulation of particles structure ranging from 1 to 100 nm in size [3], the silver at nano scale can be used in just about all fields, such as antimicrobial coating, ecological wellbeing, chemical industries, catalysis, drug delivery and bio sensing with tremendous unique and effective properties [4, 5]. At the nano-scale chemical, physical and biological properties changes in fundamental conduct of atoms, molecules along with corresponding bulk based on shape, size, their distribution and morphology more precisely surface to volume ratio. Surface Plasmon Resonance (SPR) effect is observable in all metallic nanoparticles. It arises due to oscillation of the free electrons in the nanoparticles conduction band, which oscillate in resonance with the incident light frequency. Wave length of SPR effect changes, depending on the shape, size and environment of nanoparticles. Intense colored displayed by NPs due to SPR and also affects the toxicity and bio compatibility of nanoparticles when applied in medicine and field of biology [6, 7].

Production of AgNPs from plant is a more advantageous route of green synthesis, over chemical and physical scheme as it is cost effective and environment friendly [8, 9]. Diverse approaches for synthesis of NPs are accessible like reverse micelles, thermal decomposition of silver compounds, sono chemical, electrochemical, radiation and microwave assisted processes [10]. Methods involve chemical synthesis lead to the association of toxic chemical over the surface of nanoparticles that cause adverse effect during different application particularly in medical [11]. To overcome such problems biosynthesis of nanoparticles are favored by the researchers.

Empirical knowledge of bio active components presents in plants play an imperative part in discovery of new-fangled anti fungal agents. Plant *Colutea armata* (Hemsely & Lace) belongs to the family *Fabaceae* found in Pakistan, Iran and Afghanistan including other countries of world. Bio active metabolites of plants are alkaloids, flavonoids, terpenoids, saponins, glycosides, tannins, proteins and carbohydrates. The significant Isofalavin, of the plant are Colutin, Sativan, Isovestitol and Cinnamic acid. Colutin put on displayed a momentous anti fungal effect. Colucin, a flavonoid and chalcon, a colucone derivative, Luteolin, Leutolin 7-O-β-D-glucoside, trans-Caffeic acid, Stigmasterol and Isoliquiritigenin has been isolated from this species and shown promising anti microbial properties [12]. The green growth of nanoparticles primarily depends on several bio active components especially poly phenols. Due to the bioactive component *of Colutea armata* its extract can serve as effective reducing engine for the reduction of silver ions to zero valent silver during synthesis of AgNPs. Plant metabolites could also serve as stabilizing and capping agent for AgNPs. Thus synthesis can be accomplished with no addition of external stabilizing and capping agents.

The resistant pathogenic fungus *Pyrofomes demidofii* (Lév.) Kotl. and Pouzar [13] found on juniper trees in Juniper forest around the world including Pakistan. It belongs to family *Polyporaceae*, division of *Basidiomycota.* The fungus is environmental threat and white heart rot disease is caused in tree trunks by this fungus. The 33% of trees of forest (*Juniperus excelsa* M. Bieb) in Ziarat, Balochistan are destroyed due to pathogenic fungi and is listed as a threatened tree by IUCN Red List in Pakistan. Juniper woodlands provide wood for fuel, construction material and grazing pasture, accumulation of humus and inorganic nutrients increases the soil fertility, it also conserve soil erosion, oxygen produced from trees purify the air and improve micro climate of the area. It recharges the underground water, increases water table as rainfall and snowfall penetrate into the soil and trim down the aridity of the area by transpiration [14, 15].

Keeping in view the above facts the present research was designed to carry out synthesis of AgNPs through green route with *Colutea armata* plant extract and estimation of inhibitory potential on growth of forest fungi *Pyrofomes demidofii* was made to adjoin innovative approach for ecological wellbeing.

General objective

The aim of this research was to explore the possible role of synthesized AgNPs for eradication of white heart rot disease of Juniper trees in Ziarat, Pakistan.

Specific objectives

Following were the specific objectives of the study:

1. To synthesize the environmentall friendly phytomediated silver Nanoparticles
2. To characterize the synthesized silver nanoparticles with UV-Vis spectroscopy,

Infra red spectroscopy, X-ray diffraction and Atomic Force Microscopy.

3. To conduct survey of Juniper forest, Ziarat to collect fungal strain for isolation and purification.
4. To assess the inhibitory potential of silver nanoparticles and plant extract against growth of *Pyrofomes demidoffii*, fungus causing white heart rot disease in juniper trees of Ziarat, Pakistan.

MATERIALS AND METHODS:

Synthesis of silver nanoparticles

Colutea armata plant was collected from Ziarat, Pakistan and identified with taxonomist. It was shed dried in order to avoid degradation of plant metabolites. The 50g of plant in powder form was extracted thrice with 100ml methanol and double filtered with whatman filter paper no. 1 and muslin cloth. The silver nitrate (Merck, Germany) solution of 1mM (0.1689g/1000ml H_2O) and plant extract in 1:1 ratio by volume was taken in Erlenmeyer flask covered with aluminum foil to avoid oxidation and stirred on hot plate until the reddish brown color appeared which indicated the manifestation of AgNPs. The pH of reaction mixture was maintained with addition of NaOH (0.1N) solution. Synthesis was also monitored continuously with UV-Vis spectrophotometer to record the SPR band, an evidence and confirmation of success full synthesis of AgNPs. The optimized volume ratio of plant extract and silver nitrate solution, time, pH and temperature were recorded at successful synthesis of nanoparticles.

Characterization

Various characteristics of synthesized AgNPs were evaluated as illustrated beneath.

UV–Vis spectroscopic analysis

Double beam UV-Vis spectrophotometer (Shimadzu-UV-1800) was used to witness the SPR band, an attribute of silver nanoparticles. The measurement was done by taking aliquot of reaction mixture in quartz cuvette during synthesis after each 10 minutes. Subsequent to base line correction and auto zero the spectrum was recorded over the entire range of 800-300nm at resolution of 1nm with methanol as blank.

FTIR-ATR analysis

FTIR-ATR (Bruker, Germany) equipped with OPUS soft ware was used to scrutinize the plant metabolites associated in production of AgNPs. The centrifuged (4000 rpm/15min), washed and oven dried pellets of nanoparticles was kept over the ZnSe crystals of instrument and spectrum was recorded after background subtraction over the entire wave length range 4000-500 cm^{-1}. Washing of AgNPs with distilled water was done to get rid of un conjugated metabolites and oven dried to eliminate the associated water.

X-Ray Diffraction analysis

The dried pellet of AgNPs was used for X-Ray Diffraction (XRD) measurement to characterize the crystalline nature and crystallite size of AgNPs. XRD analysis was done by an X-ray diffractometer using CuKα radiation. The crystallite size of AgNPs was calculated from the X- ray diffraction patterns using Scherer equation.

$$D = 0.9\lambda / \beta \cos \theta$$

D is crystallite size, K is shape factor (at this juncture, 0.9), λ is wave length of X-ray (at this juncture, 0.1541), β is FWHM (full width half maximum) and θ is diffraction angel.

Atomic Force Microscopic analysis

Height parameters, 2D and 3D topographic images and histogram of size distribution of AgNPs was measured with equipment Atomic Force Microscope (AFM Agilent Technologies-5500 USA) equipped with software Pico View 1.12.2 for processing of data and using tapping dynamic alternating current mode (ACAFM) with probe silicon nitride on resonance frequency of 298.563 kHz on mica sheets as sample substrate.

Anti fungal assay

The antifungal assay was carried out by means of disc diffusion methods with some modifications [16].

Culturing for pure strain of fungus

Fungal strain *Pyrofomes demidofii* was collected from juniper trees of Ziarat, Pakistan. The collected wood pieces of infected juniper tree carrying spores of forest fungi was cut in to approximately 01 inch in length, washed, dried and incubated on Muller Hinton agar Petri plate at room temperature for several weeks in a safety hood level-2. After the sizeable growth of fungus on the juniper wood holding in Petri plates, the fungus was isolated and purified with traditional culturing and sub culturing technique with streak plate method.

Disk diffusion method

Agar disk diffusion method was used to evaluate the antifungal activity of AgNPs. Filter paper disks of 5mm were loaded with 50, 100, 150, 250, 500 and 1000 ppm solution of AgNPs and incubated on the Petri plates inoculated with fungal colony suspension

equivalent to a 0.5 McFarland (1×10^8 cfu/ml) standard. Same concentration of plant extract and fungicide, thiophenate methyl as standard was also loaded on disks for comparison in agar plates. After 48 hours of incubation at room temperature, zones of inhibition were measured to ensure fungus susceptibility of AgNPs, plant extract and standard fungicide.

During experiment sterilization was done by autoclaving at 15lbs pressure at 121ºC for 15 minutes and 70% ethanol. Stock solutions was prepared by dissolving 1 gm of AgNPs, plant extract and standard fungicide thiophenate methyl in 1000ml of water in three different volumetric flasks, other concentration was made with dilution formula $M_1V_1=M_2V_2$.

Statistical analysis

The data was analyzed by using computer software i.e. Statistical Package for Social Sciences (SPSS). Analysis of Variance (ANOVA) was applied to measure the significance of differences between two groups (Plants extract and AgNPs), within groups and with standard fungicide at all tested concentrations (50, 100, 150, 250, 500, 500 and 1000ppm) at 0.05 level of significance.

RESULTS AND DISCUSSION:

Synthesis

The rapid synthesis of stable AgNPs at 62ºC within 25 minutes revealed the potential of active constituents of plant extract *Colutea armata*. The recorded optimized volume ratio (ml) of silver nitrate and plant extract was 2:9 with pH 10.9. Without addition of stabilizing agent, synthesis of AgNPs revealed the reducing capability of plant metabolites. The production of AgNPS which are homogenous in shape, extremely small in size and stable in solution depend on the presence of organic compounds in plant which can potentially offer important biological activity for creation of such nanoparticles.

Characterization

UV–Vis spectroscopy

Recorded UV-Vis spectrum (Fig. 1) of AgNPs depicted the single, narrow, smooth SPR peak at 403nm which is the characteristics of AgNPs. It was established that the synthesized nanoparticles are spherical in shape and within the nano scale range. Position of SPR band is fine in concord with various previously reported studies. In a study, AgNPs synthesized from plant powder exhibit SPR band at 420 nm [17] and biosynthesis of AgNPs using bark extract of *Zizyphus xylopyrus* exhibit SPR peaks in the range 413–420 nm [18]. In another study, root bark extract of *Berberis lycium* was used for the synthesis of AgNPs, and found spherical in shape, crystalline in nature with size ranged from 10-100 nm and capped by bio molecules [19].

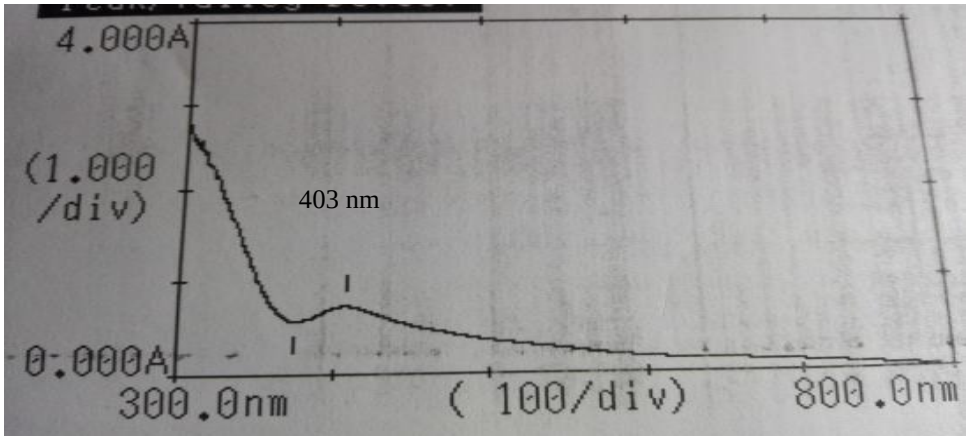

Fig. 1: UV-VISIBLE Spectrum of AgNPS synthesized from Colutea. Armata plant extract, showing SPR at 403 nm

FTIR-ATR analysis

The FTIR spectrum was interpreted according to information given in book [20]. The broad absorption band appeared at 3294.82 cm^{-1} in the recorded FTIR–ATR spectrum (Fig. 2) of fabricated AgNPs from the methanolic extract of *Colutea armata* depicted the association of plant metabolites containing hydroxyl functional group (hydrogen bonded) and N-H functional moieties. While non hydrogen bonded –OH indicated by 3020.96 cm^{-1} band. The two bands at positions 2925.11 and 2842.95 cm^{-1} revealed the C-H stretches of an aldehyde functional group of reducing

sugar present in plant. Vibration of triple and cumulative bonds appeared at 2368.26 cm^{-1}. The bands at position 1554.25 cm^{-1} and 1499.48 cm^{-1}, indicated the association of nitro group. Higher frequencies 1866.91 cm^{-1} and 1739.10 cm^{-1} of carbonyl group indicated the attachment of electronegative atom. Bands at position 833.08 cm^{-1} and 746.62 cm^{-1} indicated the out of plane C-H bending vibration frequencies of mono and di substituted aromatic rings. The present data strongly suggested the involvement of -OH, -CO and –NH groups. This indicated the various plant metabolites like poly phenols, carboxylic acid, carbohydrates used in reduction, capping and stabilization of AgNPs during biosynthesis of AgNPs from *Colutea armata*. The results are in agreement with other studies which conclude that biological molecules in plants undergo very controlled assemblage for making them suitable for the metal nanoparticle manufacture [21, 22].

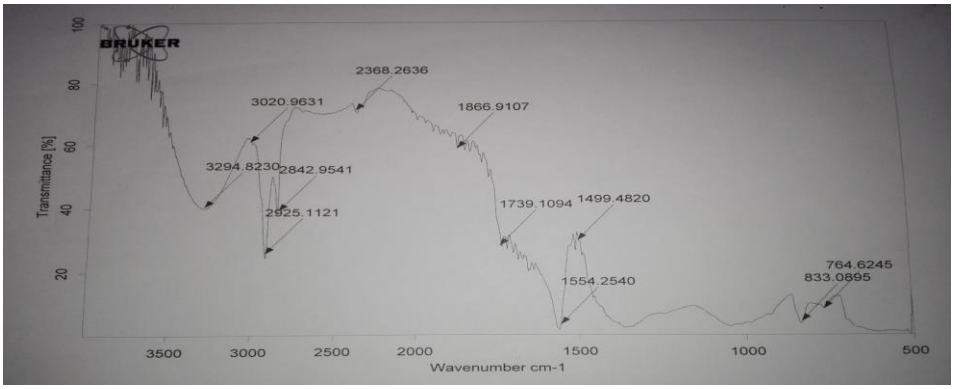

***Fig. 2*: FTIR-ATR Spectrum of AgNPS synthesized from Colutea. Armata plant extract**

XRD analysis

Recorded crystallographic Bragg reflection planes (1,1,1), (2,0,0), (2,2,0) and (3,1,1) demonstrated by the peaks 38.14, 44.26, 64.46 and 77.49 appeared in X-ray diffractogram of synthesized AgNPs (Fig. 3). These investigational diffraction angels (2 Θ in degree) correspond fine with standard diffraction angels of silver (38.11, 44.27, 64.42 and 77.48) specified in Joint Committee on Powder Diffraction Standard (JCPDS) (file 04-0783).

All the reflection confirmed the face centered cubic structure with high grade of crystalline nature of AgNPs. No crystallographic impurities were found which recognized by the nonappearance of fallacious peaks. The broad diffraction peaks appeared in X-ray diffractogram indicated the diminutive size of AgNPs. The crystallite size of synthesized AgNPs was found 19.06 nm, calculated from XRD data using Scherrer equation. Position of Crystallographic Bragg reflection planes is fine in concord with the previous study in which AgNps were prepared with mulberry leaves extract and calculated crystallite size was found 20 nm [23].

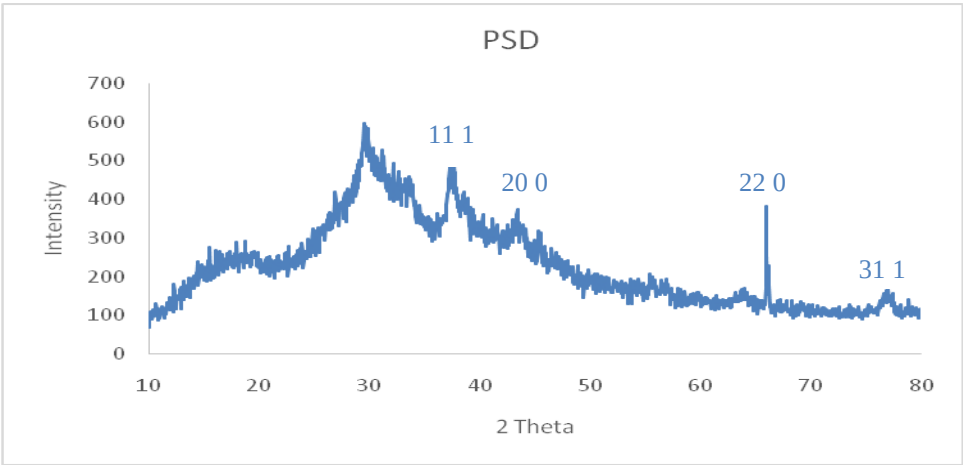

***Fig. 3*: XRD pattern of AgNPS synthesized from *Colutea. Armata* plant extract**

AFM analysis

The dimension and superficial structure of nanoparticles were evaluated with atomic force microscopy. The topographical (2D & 3D) images (resolution 2 X 2µm) (Fig. 4) disclosed the traditional spherical shape of AgNPs with maximum height of 65 nm.

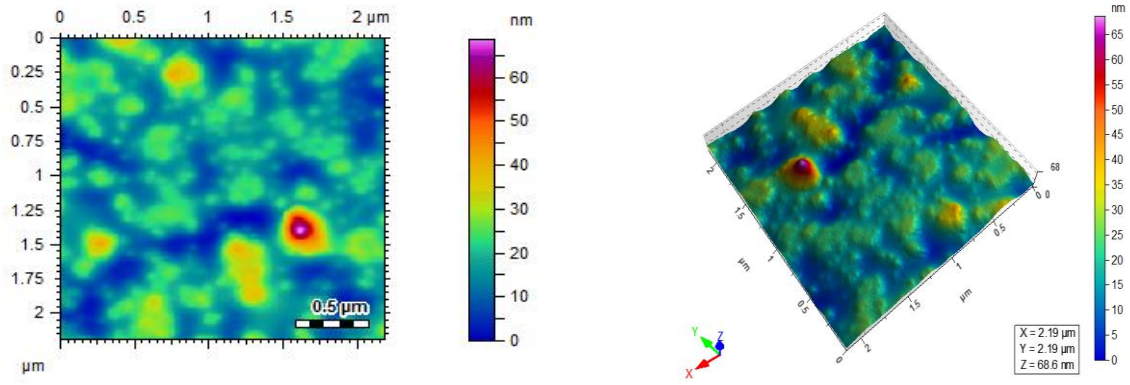

Fig. 4: Topographical (2D, 3D) images of AFM analysis of AgNPs synthesized from *Colutea armata* plant extract

Height parameters calculated from AFM data depicted the maximum size (*Sz)* of 139 nm and arithmetic mean size (*Sa)* 24.4 nm. The calculated root mean square height (Sq) was 28.7 nm. Percentage of scope distribution of synthesized AgNPs determined with Histogram (Fig. 5) revealed the poly dispersion regarding size range of nanopartciles. The maximum particles are established in the size range of 13.9-139 nm. While 100% includes the size range less than 83.4 nm, out of which 84% of AgNPs was 27.8 nm in size. The size of AgNPs measured with AFM is in agreement with another study conducted on Silver nanoparticles-disk diffusion test against *Escherichia coli* isolates in which, AgNPs of 85.07nm in size with mono dispersion regarding size and spherical shape was found [24].

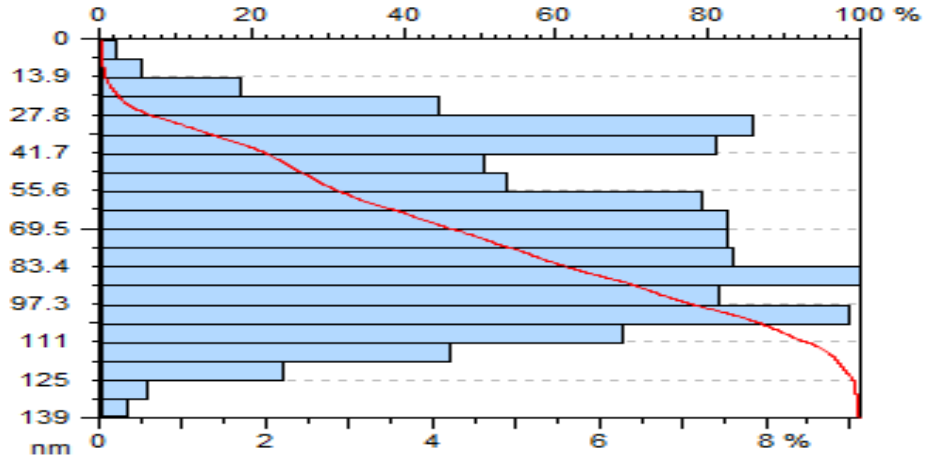

***Fig. 5*:** Size distribution histogram of AFM analysis of AgNPs synthesized from *Colutea armata* plant extract

Antifungal assay

The anti fungal activity of synthesized AgNPs was investigated against wood rotor fungi *Pyrofomes demidofii* (Fig: 6) and found efficient compared to plant extract due to the extremely large surface area of AgNPs which provide better contact with cell membrane of microorganism and penetration in cell.

***Fig. 6* : *Pyrofomes demidofii* growing on piece of juniper's wood in an agar plate**

The nanoparticles interact with sulfur containing protein and phosphorus containing compounds like DNA and cause cell death. Silver nanoparticles also released silver ion in cell which enhance the antimicrobial activity [25, 26]. Highly Significant difference between two groups (Plants extract and AgNPs), within groups and with standard fungicide at all tested concentrations (50, 100, 150, 250, 500, 500 and 1000ppm) was observed. The calculated zones of inhibition are given in Table: 1.

Table 1: Zone of inhibition (mm) at different ppm solutions against *Pyrofomes demidofii*

Anti fungal agents	Zone of inhibition , means (mm) against *Pyrofomes demidofii*					
	50ppm	**100ppm**	**150ppm**	**250ppm**	**500ppm**	**1000ppm**
Colutea. armata extract	4.13	6.08	7.13	8.07	9.13	10.13
Silver nanoparticels	6.50	6.30	9.25	10.15	11.25	12.30
Standard (thiophenat methyl)	.00	.00	5.00	6.00	7.00	7.50

Overall, AgNPs was found most effective against fungus growth. Extremely small size and root mean square values confirmed with AFM exhibit the large surface area and strong adhesion power of AgNPs which boost the antimicrobial activity of AgNPs. The zones of inhibition of plant extract and AgNPs at all concentrations around the test fungus are shown in fig. 7 and 8.

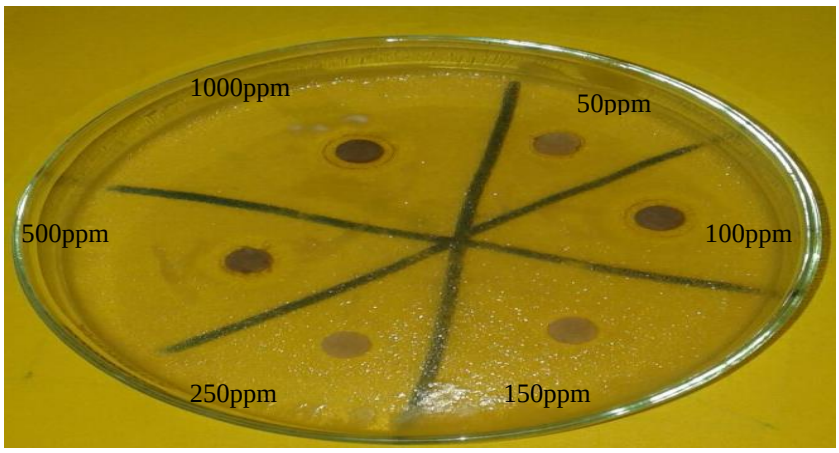

Fig. 7*: Agar Petri plates showing Zone of inhibition against *P. demidofii* due to AgNPs synthesized from *Colutea armata

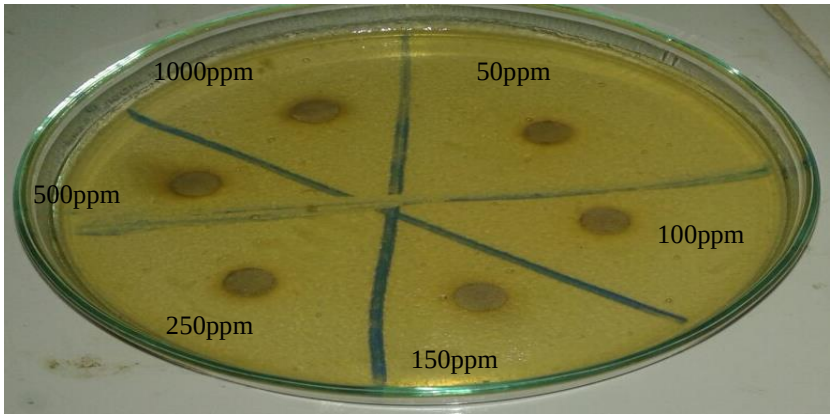

***Fig. 8*:** Agar Petri plates showing Zone of inhibition against *P. demidofii* due to *Colutea armata* plant extract

CONCLUSION:

The study outcome proved that the chosen plant has better reducing abilities for the manufacture of AgNPs and estimated inhibitory potential of AgNPs on growth of *Pyrofomes demidofii* could adjoin innovative approach for ecological wellbeing. The environment friendly synthesis of AgNPs from extract of *Colutea armata* is an economical and efficient process. It is sure that the susceptibility of manufactured AgNPs from selected plant was first time evaluated against fungus *P. demidofii* in this study.

REFERENCES:

1. El-Shishtawy RM, Asiri AM, Abdelwahed NAM, Al-Qtaibi MM. In situ production of silver nanoparticles on cotton fabric and its antimicrobial evaluation. Cellulose, 2011; 18:75-82.
2. Jain J, Arora S, Rajwade JM, Omray P, Khandelwal S, Paknikar KM. Silver nanoparticles in therapeutics: development of an antimicrobial gel formulation for topical use. Mol Pharmaceutics, 2009; 5:1388-1401.
3. Albrecht MA, Evans CW, Raston CL. Green chemistry and the health implications of nanoparticles. Green Chem, 2006; 8:417-432.
4. De Jong WH, Born PJA. Drug delivery and nanoparticles: Applications and hazards. International Journal of Nanomedicine, 2008; 2:133-149.
5. Hall WP, Ngatia SN, Van Duyne RP. LSPR biosensor signal enhancement using nanoparticles-antibody conjugate. Journal of Physical Chemistry, 2011; 5:1410-1414.
6. Noguez C. Surface Plasmons on Metal Nanoparticles: The Influence of Shape and Physical Environment. Journal of Physical Chemistry, 2007; 10:3806-3819.
7. Alkilany AM, Murphy CJ. Toxicity and cellular uptake of gold nanoparticles: what we have learned so far?. J nanopart Res, 2010; 7:2313-2333.
8. Kowshik M, Ashtaputre S, Kharazzi S, Vogel W, Urban J, Kulkarni SK, Paknikar KM. Extracellular synthesis of silver tolerant strain MKY3. Nanotechnology, 2003; 14:95-100.
9. Nabikhan A, Kandasamy K, Raj A, Alikunhi NM. Synthesis of antimicrobial silver nanoparticles by callus and leaf extracts from saltmarsh plant, *Sesuvium portulacastrum* L. Colloids Surf B Biointerfaces, 2010; 2:488-493.
10. Parashar V, Parashar R, Sharma B, Pandey A. Parthenium leaf extract mediated synthesis of silver nanoparticles: A novel approach toward weed utilization. Digest Journal of Nanomaterials and Biostructures, 2009; 1**:**45-50.
11. Jain D, Daima HK, Kachhwaha S, Kothari SL. Synthesis of silver nanoparticles using papaya fruit extract and evaluation of their antimicrobial activities. Digest Journal of Nanomaterials and Biostructures, 2009; 3:557-563.
12. Inamullah F, Fatima I, Khan S, Kazmi MH, Malik A, Tareen RB, Abbas T. New antimicrobial flavonoids and chalcone from Colutea armata. Arch Pharm Res, 2017; 8: 915-920.
13. Kotlaba F, Pouzar Z. Staining spores of Homobasidiomycetes in cotton blue and its importance for taxonomy. Feddes Repertorium Specierum Novarum Regni Vegetabilis, 1964; 69:131-142.
14. Atta MS. Population Structure and Regeneration Potential of Juniper forests in Baluchistan Province, Pakistan. Ph.D thesis submitted to the University of Balochistan Quetta, Pakistan, 2000a.

15. Zakaullah. Decay in Ziarat Juniper forests of Baluchistan. Pakistan Journal of Forestry, 1978; 1:28-34.
16. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol, 1966; 4:493-496.
17. Logeswari P, Silambarasan S, Abraham J. Synthesis of silver nanoparticles using plants extract and analysis of their antimicrobial property. Journal of Saudi Chemical Society, 2015; 19:311-317.
18. Maria BS, Devadiga, A, Kodialbail KV, Saidutta MB. Synthesis of silver nanoparticles using medicinal Zizyphus xylopyrus bark extract. Applied Nanoscience, 2015; 6:755–762.
19. Tufail A, Din MI, Bashir S, Qadir MA, Rashid F. Green synthesis and characterization of silver nanoparticles using *Ferocactus echidne* extract as a reducing agent. Nanotechnology, 2014; 7:1180-1189.
20. Rosaleen J, Andersons RJ, David J, Bendell DJ, Groundwater PW. 2004. Organic Spectroscopic Analysis. The Royal Society of Chemistry, Thomas Graham House, Science Park, Milton Road Cambridge, UK, 28-45.
21. Ahmed S., Ahmad M, Swami BL, Ikram S. A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise. Journal of Advanced Research, 2016; 1:17-28.
22. Mehmood A, Murtaza G, Bhatti TM, Kausar R, Ahmed MJ. Biosynthesis, characterization and antimicrobial action of silver nanoparticles from root bark extract of *Berberis lyceum. Royle*. Pak J Pharm Sci, 2016; 1:131-137.
23. Awwad AM, Salem NM. 2012. Green Synthesis of Silver Nanoparticles by Mulberry Leaves Extract. Nanoscience and Nanotechnology, 2012; 4:125-128.
24. Cunha FA, Maia KR, Mallman EJ, Cunha MD, Maciel AA, Souza IP, Menezes EA, Fechine PB. Silver nanoparticles-disk diffusion test against *Escherichia coli* isolates. Rev Inst Med Trop Sao Paulo, 2016; 58:73.
25. Sondi I, Salopek-Sondi B, 2004. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. J Colloids Interface Sci, 2004; 1:177-182.
26. Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramfrez JT, Yacaman MJ. The bactericidal effect of silver nanoparticles. Nanotechnology, 2005; 10:2346-2353.