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Bark Assisted Green Synthesis of Silver Nanoparticles from *Walsura trifoliata* (A. Juss.) Harms. (Synonym: *Walsurapiscidia* Roxb.) Characterization and Antimicrobial Efficacy

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Abstract

Background: Silver nanoparticles are (AgNPs) are one of the most widely fascinating area and applicable particles whose application is enhancing in Nano world circadian. Silver nanoparticles have exposed significant applications various significant advantages give to wide range of applications in the area of bio-medical, catalysts, antimicrobials, sensors, electrons, optical fibers, bio-labeling, agriculture and other fields. Green synthesis is the safe and easiest method to produce silver nanoparticles. Due to the production of silver ions, SNPs were found to have antibacterial efficacy. **Aim/ objective:** The aim of the present study is the bio-synthesis of silver nanoparticles using from silver nitrate using the bark as bio-resource of the *walsura trifolita* (A. Juss.) Harms as cost-effective nonhazardous lessen and stabilizing admixtures. **Methods:** In this study silver nanoparticles prepared with adding 1mM silver nitrate to aqueous bark extract of *walsura trifoliata* (A. Juss.) Harms. Antibacterial activity assay with the disc diffusion method against *E. coli*, *K. pneumonia*, *B. subtilis* and *S. aureus*. Synthesized nanoparticles of AgNPs characterized by using with UV-VIS spectroscopy, DLS Zeta potential, XRD, FT-IR, SEM and TEM. **Results:** Produced synthesized AgNPs showed significant antibacterial activity against four bacteria i.e. *E. coli*, *K. pneumonia*, *B. subtilis* and *S. aureus* in comparison with Ag $(NO3)_2$ solution, aqueous bark extract and Streptomycin used as standard drug. **Conclusion:** The secondary metabolites of *Walsura trifoliata* will be helpful to the phytochemists and pharmacologyts for identification of new active principle and Bio-synthesized nanoparticles is the most enlarging research area due to the biomedical application to growth novel biotechnological study.

Keywords
Walsura trifoliata, Bio-synthesis of silver nanoparticles, antibacterial activity, characterization.

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INTRODUCTION:

Nanotechnology is an acclivitous technology with enormous applications in various fields in science. Nanoparticles (NPs) are extremely important because of their exceptionally small size and large surface area to volume ratio, which determines their properties such as electrical, mechanical, chemical, physical, optical, solubility and stability properties.

Generally, nanoparticles are classified into organic and inorganic nanoparticles. Nanoparticles that contain carbon are considered organic nanoparticles whereas metallic nanoparticles, such as noble metal (gold, silver and platinum) and semiconductor (titanium dioxide, zinc oxide and zinc sulfide) are considered inorganic nanoparticles [1–8]. For many years, scientists have uninterruptedly explored different synthetic methods to synthesize nanoparticles. On the contradictory, the green method of synthesis of nanoparticles is easy, efficient and eco-friendly in comparison to chemical mediated or microbe mediated synthesis. The chemical synthesis involves toxic solvents, high pressure, energy and high temperature conversion and microbe involved synthesis is not feasible industrially due to its lab maintenance. Since, green synthesis is the best option to opt for the synthesis of nanoparticles [9]. Production of nanoparticles can be succeeded through different chemical methods like thermal decomposition of silver compounds [10], radiation assisted [11] electro chemical [12] But these methods have developed environmental issues by involving use of hazardous or toxic chemical reagents. Hence to prevent these adverse consequences the use of plant extract has been explored to synthesize AgNPs and this biological method has found to be nontoxic, cost effective and eco-friendly approach which might pave the way for researchers across the globe to explore the potential of different herbs in order to synthesize nanoparticles [13] and biogenetic production is now more preferable due to simplicity of the procedures and versatility by docile [14]. Biologically synthesized Gold and AgNPs could be of abundance utilize in medical and biomedical textiles for their efficacious antibacterial and antimicrobial properties and also in other applications such as spectrally-selective coating for solar energy absorption and interaction material for electrical batteries and also useful as optical receptors and as catalysts in chemical reactions [15]. Biosynthesis of AgNPs using with the plant extracts may be influenced directly or indirectly by phytochemicals in extracts such as phenols, flavonoids and antioxidants as well as the physicochemical factors governing the kinetics of the reaction. Silver has long been known to have strong inhibitory and bactericidal effects as well as broad spectrum of antimicrobial activity even at low concentrations [16]. Moreover, it is a well-organize way of waste biomass utilization for the biosynthesis of AgNPs. Biosynthesis of silver nanoparticles has been carried out successfully of their potential biomedical applications. Studying different properties of the AgNPs is an important research field in nanotechnology. AgNPs can be used in various applications such as medicine, agriculture cosmetic and food industries (food packaging), bioengineering, catalysts, electrochemistry or disinfection in environmental applications [17]. Green synthesis of nanoparticles were successfully carried out in the recent past years through many researchers from plant materials as reducing agent like Iron oxide nanoparticles from *Medicago sativa* [18] Palladium nanoparticles from *Cinnamomum camphora* [19], calcium carbonate nanoparticles through *Vigna mungo* (L.). [20] Zinc oxide nanoparticles from *Catharanthus roseus* [21]. Copper nanoparticles from *Magnolia kobus* [22] gold nanoparticle formation by *Avena sativa* [23] among the synthesis of silver nanoparticles work has been done with *Syzygium Alternifolium* (Wt.) Walp. [24] *Plumbago zeylanica L*. [25] *Adansonia digitata L*. [26] *Ocimum tenuiflorum*, *Solanum tricobatum*, *Syzygium cumini*, *Centella asiatica*, and *Citrus sinensis* [27]. Hence the present study aimed to synthesis of silver nanoparticles from Stem bark extract of *walsura trifoliata* (A. Juss.) Harms and evaluation its antibacterial activity.

The tropical genus *Walsura* (Meliaceae) is represented by 15 species in Indo-Malaysia [28] and by 10 in India [29] of which only one, *W. robusta* Roxb, commonly grown in Chittagong, is found in Bengal [30]. The species under investigation, *Walsura piscidia* Roxb. now known as *Walsura trifoliata* (A. Juss.). Harms are usually cultivated in the gardens of India for its ornamental and medicinal purposes. Plants play a vital role in existence and survival of man. Medicinal Plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value [31]. It is estimated that there are about 250,000 species of higher plants and the majority of them have not been examined for their pharmacological activities. The antimicrobial properties of certain Indian medicinal plants were reported based on folklore information [32-37] and a few attempts were made on inhibitory activity against certain pathogenic bacteria and fungi [38]. The plant is well reputed in traditional system of medicine and used by tribal peoples to treat various diseases i.e., skin allergies, astringent and diarrhoea [39].

MATERIALS AND METHODS:

Plant collection and extraction preparation

Fresh stem Stem bark was collected from the *Walsura trifoliata* and then washed thoroughly running tap water thrice and subsequently washed with distilled water to remove dust particles then this was shade dried for 15 days to evaporate moisture

and made this ground fine powder. 50 g of powdered Stem bark extracted with 200 ml Milli pore water boiling on water both 30 min with using 500mL Erlenmeyer flask. The aqueous extract was filtration through What man no.1 filter paper and stored at room temperature until the biosynthesis of AgNPs.

Characterization

UV-Vis absorption spectrum of SNPs was using with Nano drop 800nm spectrophotometer. Fourier-Transform Infra-Red (FT-IR) spectra of synthesized SNPs were analyzed in the range of 4000 to 500 cm-1 with an ALPHA interferometer (ECO-ART), Bruker, Ettlingen, Karlsruhe, Germany by KBr pellet method. Crystalline nature of metallic silver nanoparticles were monitored using with an X-ray diffractometer (XRD) from shimadzu, XRD-6000 equipped with Cu Ka radiation source using Ni as filter at a setting of 30 kV/30 mA. Scanning electron microscopy (SEM) and percentage of silver ions in synthesized sample was done by using FEI Quanta 200 FEG HR-SEM machine epuiped with EDAX instrument. Transmission electron microscopy (TEM) analysis was performed with the using HF-3300 advanced 300 kV TEM from Hitachi.

Synthesis of Silver nanoparticles

5 ml of aqueous bark extract were taken into 250 ml conical flask and titrate with 50 ml of silver nitrate with heating between 60-80^{o}C for 60 min. Color change from light brown to deep brown indicated formations of silver nanoparticles. Then were centrifuged at 20000 rpm for 20 min to remove the presence biological admixture, and were used for characterization and as well as antibacterial, anti-oxidant activities.

Antimicrobial studies of AgNPs

Stem bark extract of synthesized SNPs of *Walsura trifoliata* was analyzed for antimicrobial activity against two gram positive like *Bacillus subtilis* ATCC, *Staphylococcus aureus* ATCC, and two gram negative bacterial stains like *Escherichia coli* ATCC and *Klebsiella pneumonia* ATCC. Disc diffusion assay method was carried out using standard protocol [40]. For this 20 µl of plant extract, Ag (NO3)2 solutions, SNPs, Streptomycin was applied on each separate What man no. 1 filter paper discs (6 mm diameter), allowed to dry before being placed on agar pored petriplates. Each stain tested triplicate with each extract and incubated at 37 ^{0} C for 24 hours. In incubation chamber. Diameter of the zones was measured with the help of scale and results were tabulated.

RESULTS AND DISCUSSION:

When the 1 mM Ag(NO3)2 solution was added to aqueous stem bark extract of *Walsura trifoiata,* the color changed from light brown to deep brown which is primary method to confirm that the synthesized nanopaticles are silver (fig.1). The color change is because of the reduction of silver ions with the help of bio active molecules present in the sample [41]. Due to NAD and ascorbic acid present in plant parts at higher levels act as strong reducing agents by donating electrons to Ag+ to form Ag^{0} nanoparticles [42]. This may be main reason behind the reduction and color change pattern of Silver nanoparticles.

UV-Vis

Reduction of the silver ions was monitored with the help of UV- Visible Nano drop range from 190 to 750nm. The AgNPs exhibit color change light brown to deep brown at the 400 to 500nm range. The obsorption peak were obtained at 434 nm, which further confirm the reduction nanoparticles are silver. This respective peak shown due to the surface Plasmon resonance (SPR) phenomenon in the reaction mixture. Same results were found in Leaf Assisted Green Synthesis of Silver Nanoparticles from Syzygium Alternifolium (Wt.) Walp.[43] Fig (1).

FT-IR

FT-IR spectrum of silver nanoparticles carried out to identify the possible bio-molecules responsible for the capping and stabilization of nanoparticles. For this sample analysed in the IR spectra range from 4000 to 500 cm-1 by the FT-IR. Fig. 2. (a) Indicates only extracts and Fig.2. (b) Belongs to biosynthesized nanoparticles. The broad peaks were obtained majorly at 3396/cm medium bond N-H stretching indicates aliphatic primary amine, 2928/cm-1 strong and broad peak assigned for N=C=O stretching isocyanate, 1614/cm-1 correspond to C=C strong bond stretch α, β- unsaturated ketone, 1523/cm-1 strong bond indicates N-O stretching nitro compound, 1444/cm-1 and 1404/cm-1 medium correspond to O-H bending carboxylic acid, 895/cm-1 strong C=C bending alkene vinylidene, 667/cm-1 strong bond belongs C=C bending alkene fig. (b). And towards biosynthesis of SNPs peaks were also obtained at 3406/cm strong bond belongs to N-H primary amines, 2923/cm-1 medium showed C-H stretching alkane whereas 2853/cm-1 medium C-H stretching aldehyde, 2551/cm-1 weak bond indicates S-H stretch thiol, 2274/cm-1 strong broad bond assigned N=C=O isocyanate, 1903/cm-1 medium bond stretch indicates allene, 1384/ cm-1 medium bending aldehyde, 1204/cm-1,1098/cm-1,1068 and 1044/cm-1 assigned often overlapped bonds were aliphatic amines, and 666/cm-1 bending indicates C=C alkene respectively fig (c). This suggests that all group of compounds presence in the reaction medium formation of complex layer or combinations around the nanoparticles that act as stabilizing

agents, prevent the agglomeration and precipitation. AgNO3 dissociates in to silver (Ag+) and nitrate ions (NO3-). After releasing the protons from flavonoids silver ions are reduced. These ions are grouped to form AgNPs [44] same results were found in *Syzygium alternifolim* (Wt.) Walp. [45].

Zetapotential and particle size –DLS

In this basic parameter revealed stability of aqueous AgNPs. Among the techniques for the characterization of nanoparticles, the most commonly used is DLS [46–48]. DLS is the advanced tool used to analyze the size distribution of fabricated nanoparticles. DLS measures the light scattered from a laser that passes through a colloid, and mostly relies on Rayleigh scattering from the suspended nanoparticles [49]. Therefore; DLS is mainly used to determine particle size and size distributions in aqueous or physiological solutions [50]. Synthesized AgNPs acquired negative value and the zeta potential of bio-synthesized nanoparticles sharp peak shown in the Fig. (3), that's indicatively of that the surface of the nanoparticles negatively charged. Generally the zetapotential of the NPs should be highest than +30mV [51]. Average size and poly disparity index of SNPs were measured by DLS (Malvern-Zeta analyzer) operated with a He-Ne laser. The result shown in the Fig. (3). *Walsura trifoliata* revealed particle size and Zeta potential values 15 nm average sizes with -4.4 mV Zeta potential value. Same results were found in Nothapodytes nimmoniana (Graham) Mabb. [52]

SEM

By the SEM analysis revealed that the results of synthesized silver nanoparticles are polydispersed and size range from 26-50 nm at the 500 nm resolution spherical shape and without any agglomeration. EDAX analysis synthesized sample showed 00.30 weight percentage of Ag metal along with 13.79 % of carbon, 27.28% of oxygen, 08.20% of sodium, 02.71 Magnesium, 05.58 of Calcium, 42.15 of Silicon and this was fatherly confirmed presence of silver nanoparticles in the sample and also presence of carbon and oxygen suggesting that the silver nanoparticles must be capped by the organic components present in the plant extract. (Fig.4).

TEM

Morphological structure and distribution of synthesized silver nanoparticles monitored at high magnifications (20nm) were done by TEM. TEM micrographs shows AgNPs signify that the synthesized nanoparticles are polydispersed, predominantly spherical in shape, owing 25.81-29.43 nm size and no physical contact each other i.e. no agglomeration of nanoparticles were seen. For TEM analysis the SNPs are coated on copper grids and analyzed by Hitachi HF3300 advanced with 300kV. (Fig. 5).

XRD diffraction analysis

The nature of the nanoparticles analysed from synthesized bark extract by X-ray diffraction method. The XRD pattern of plant mediated SNPS appears four peaks on 2θ on X-axis like 38, 44, 66 and 78 corresponding to the 111,200,220 and 311 Bragg reflections of Y- axis, respectively (fig. 6). These Bragg reflections confirm the face- centered cubic structure os silver nanoparticles. The mean particle diameter of SNPs 38.01 nm, calculating to according Debye- Scherer equation ($D = k\lambda/\beta \cos\theta$). The Full Width at Half Maximum (FWHM) values i.e., k=0.38 were derived from 38, 44, 64 and Bragg reflections of X- axis. [53]. This was carried out using with the Shimadzu XRD- 6000/6100 model with 30 kV, 30 mA with Cuk α radians at 2θ angle. This was carried out using Shimadzu XRD-6000/6100 model with 30 kV, 30 mA with Cuk radians at 2h angle.

Antimicrobial studies

Antibacterial activity of Bio- synthesized Silver nanoparticles from bark extract was analyzed against two positive and two negative bacteria like *B. subtilis*, *S. aureus*, *E. coli* and *K. pneumoniae* growing on nutrient agar medium. Zone of inhibition of different extracts were obtained with streptomycin standard drug. The diameter of inhibition zone around each disc measured and each disc contains 20µl SNPs solution. Maximum inhibition of zones were observed with synthesized SNPs of bacteria in *B. subtilis*, *S. aureus*, *E. coli* and *K. pneumonia*. The 1mM $Ag(NO_3)_2$ as negative control and the aqueous extract of bark without silver nanoparticles served as positive control and it was show broad spectrum of antibacterial activity. Gram positive species showed low inhibitory zones when compare to gram negative species due to containing thick layer of peptidoglycons (together with polypeptide contains proteins). Because of this, the penetration of SNPS through cell membrane is not easy in case of inhibition growth not possible than gram negative stains it leads to cell death. Antimicrobial activities of SNPs are dependent on size and shape of the particles. Small size nanoparticles have higher antimicrobial activity than larger particles because they large surface area to interact bacteria efficiently [54]. Synthesized AgNPs Stem bark extract of C. religiosum showed highest antibacterial activity *S. aureus* followed by *B. subtilis, E. coli* and *K. pneumonia.* Spherical-shaped SNPs, 20–60 nm in size, synthesised from stem bark of Syzygium cumini show good antibacterial activity [55]. Spherical shaped 20–35 nm sized SNPs synthesized from *Cochlospermum religiosum* stem bark show

significant antimicrobial activity on different bacterial species [56].

CONCLUSION:

The present study we have reported an uncomplicated, low- cost and eco-friendly process to produce bio-genic stable silver nanoparticles from *walsura trifoliata* aqueous extract a traditional medicinal plant as reducing agent. The color change pattern and surface Plasmon resonance (SPR) spectra UV- Vis data (433 nm) confirms the presence of silver nanoparticles in the sample. Phenols and Proteins are mainly responsible for reduction and stabilization of these SNPs revealed by FT-IR. Very exemplary small- sized nanoparticles were recognized through the SEM micrograph 26.3 nm at the higher resolution of 500nm magnification. In TEM recognized 25 – 29nm sized spherical shape nanoparticles were recognized at 50nm resolution without any agglomeration. Due to higher resolution and high magnification possible in TEM revealed below 30nm. Whereas TEM is advanced tool than SEM and other microscopy. All above microscopic studies reveal that the nanoparticles small size, well dispersed without any agglomeration mostly spherical in shape size range from 26 – 48nm (SEM), 25 – 29nm (TEM). The synthesized SNPs of *W.trifoliata* show magnificent antibacterial activity against two gram positive and two gram negative bacteria. Consequently, these biogenic synthesized nanoparticles are eco- friendly. Antimicrobial agent and best quality production of nanoparticles with small quantity plant extract. Hence this plant is well reputed in traditional system of medicine and used by tribal peoples to treat various diseases like skin allergies, astringent and diarrhoea [57]. The bark of the plant is reported to possess stimulant, expectorant, emmenagogue and emetic properties. The fruit pulp is used as fish poison [58]. The bark extract of *Walsura trifoliata* showed the activity against pathogenic microorganisms [59].

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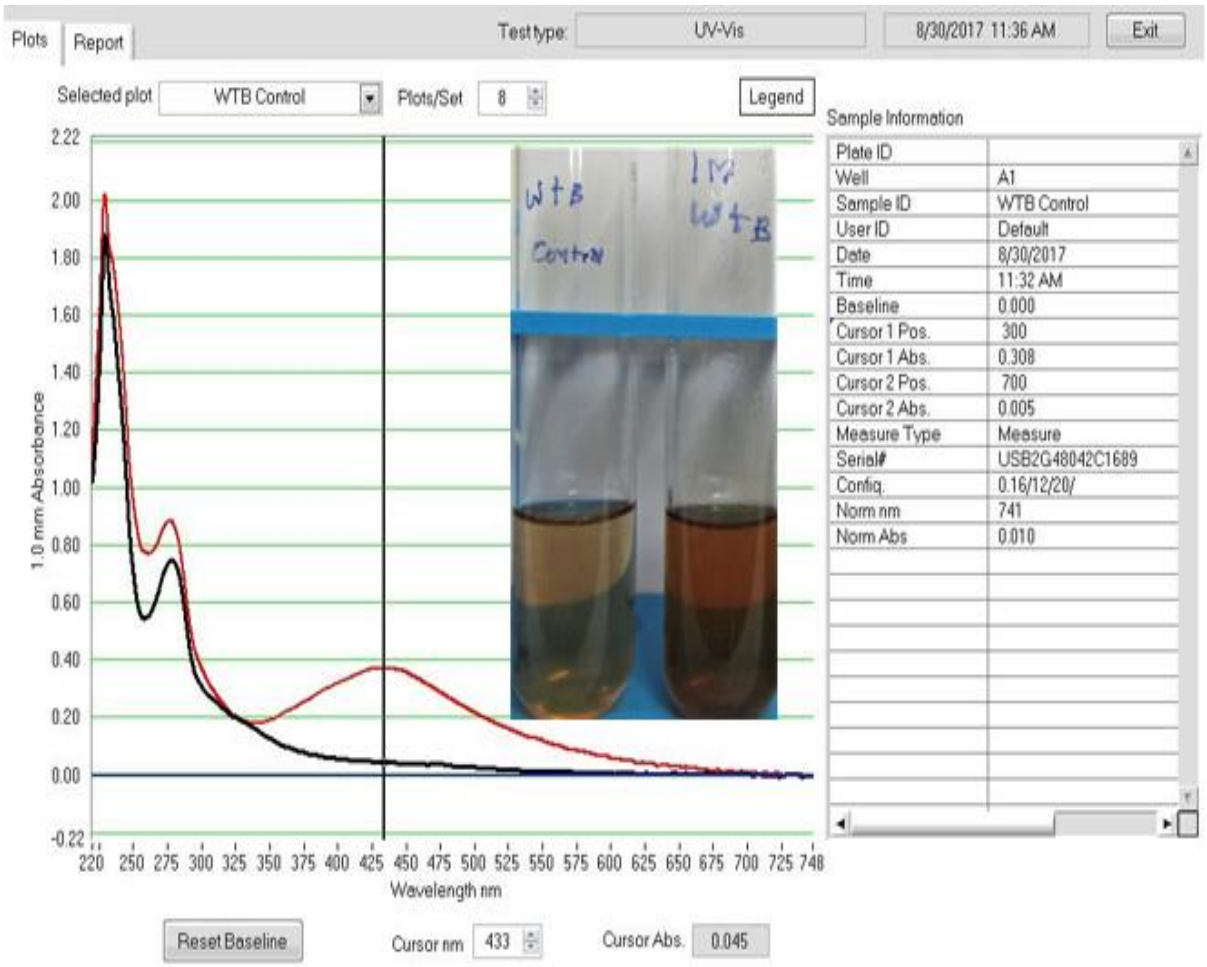


Fig. 1 Visible Color change of SNPs with UV- Vis absorbance peak at 433nm.

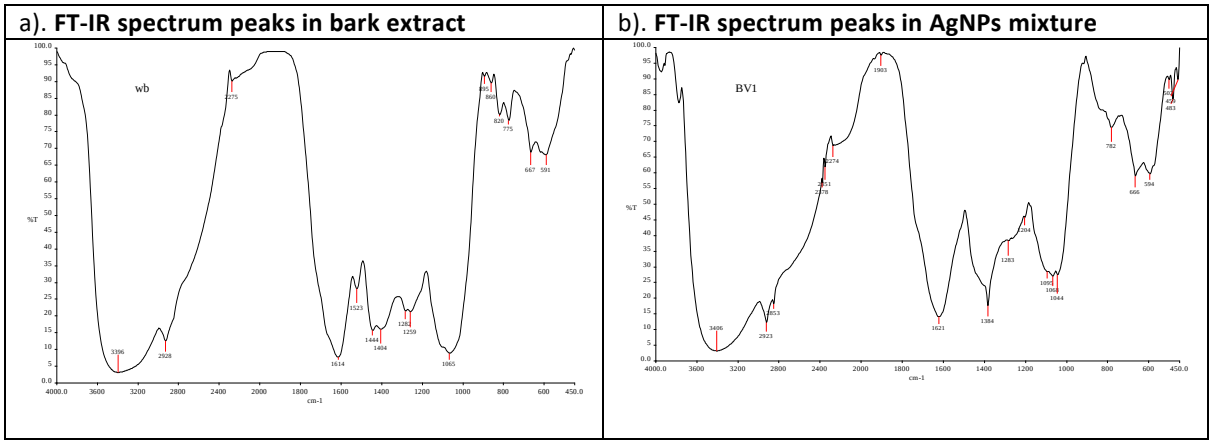


Fig 2 a) *Walsura trifoliate* bark - FT-IR spectrum peaks in bark extract and b) BV1 - FT-IR spectrum peaks in SNPs mixture.

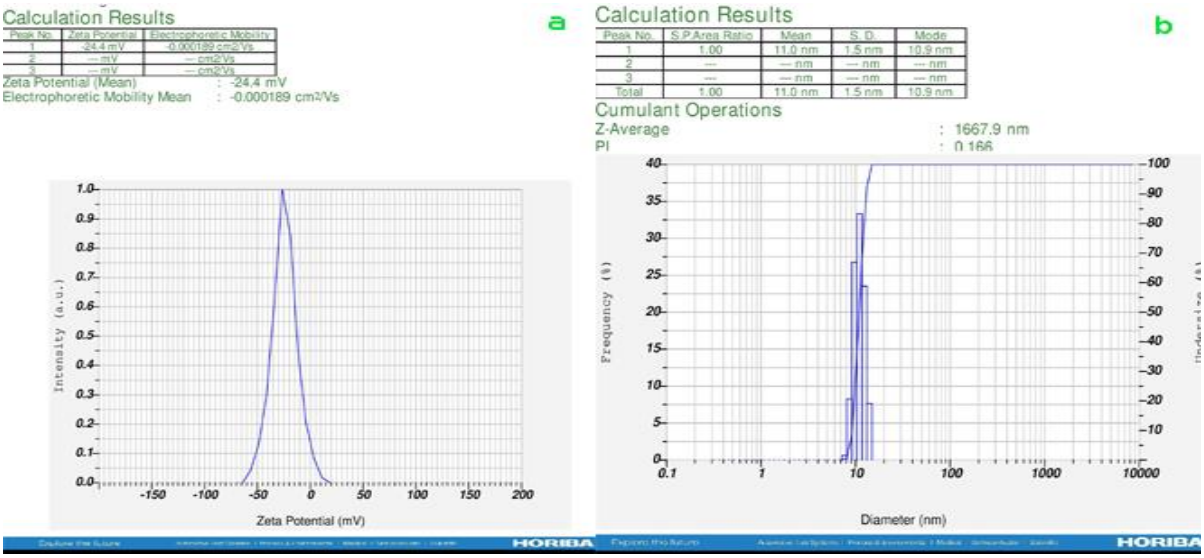


Fig. 3 *Walsura trifoliata*- Bark SNPs a). Zeta potential b). particle size

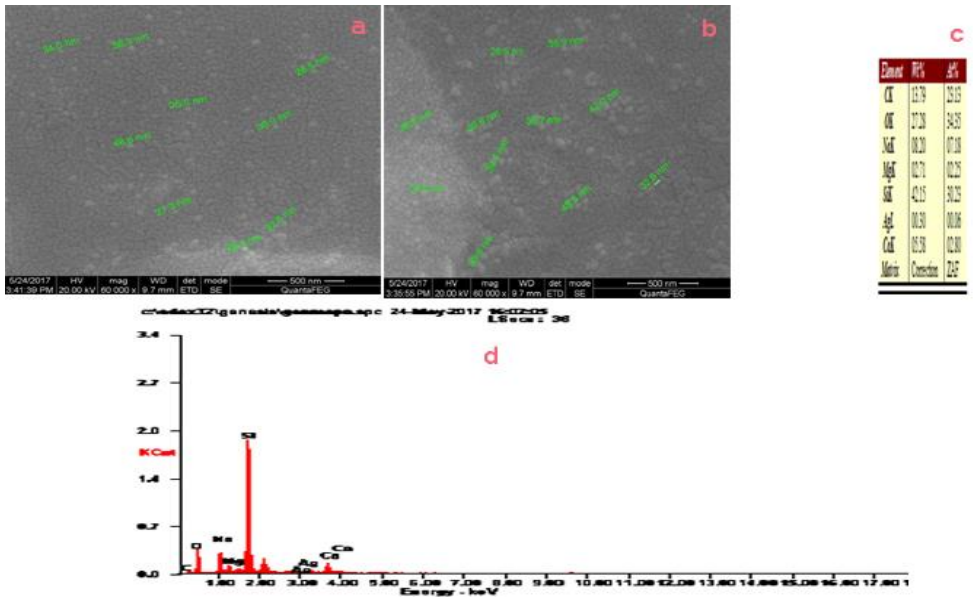


Fig. 4 SEM images: a) and b) average size of the AgNPs 33.85nm from Walsura trifoliata Stem bark from *Walsura trifoliata* bark, c) and d) EDAX analysis in the weight percentage of silver in mixture.

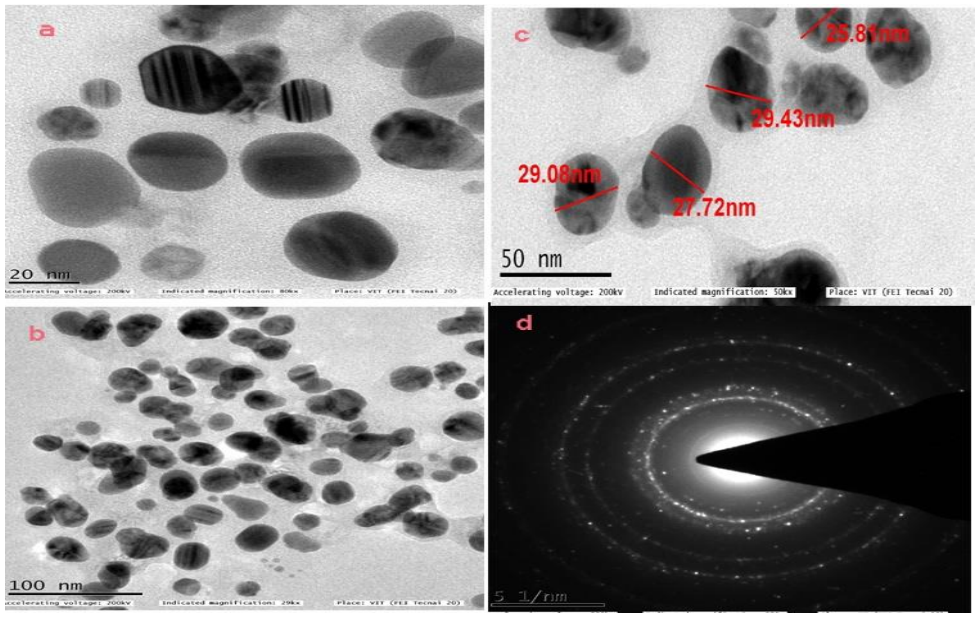


Fig. 5 TEM Images: a) Sphericle shape of SNPs at 20 nm, b). Dispertion of the nanoparticles at 100 nm, c). Average size of AgNPs at 50 nm 28.01nm and d). Imaging at 51 nm.

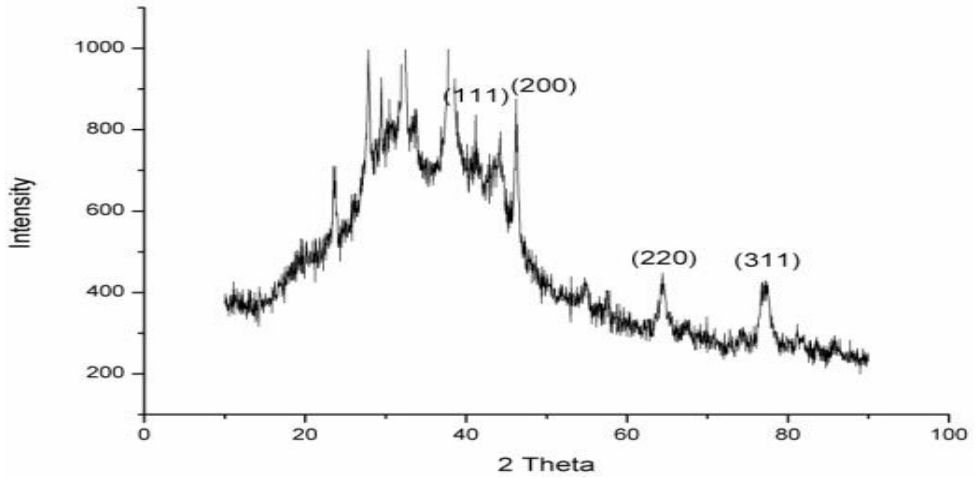


Fig. 6 XRD pattern of AgNPs shows Bragg reflections

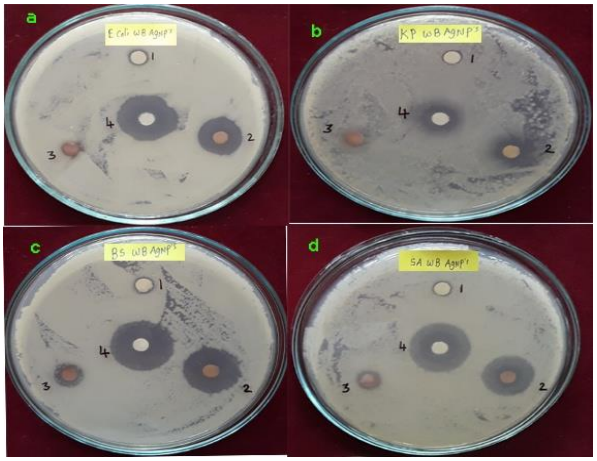
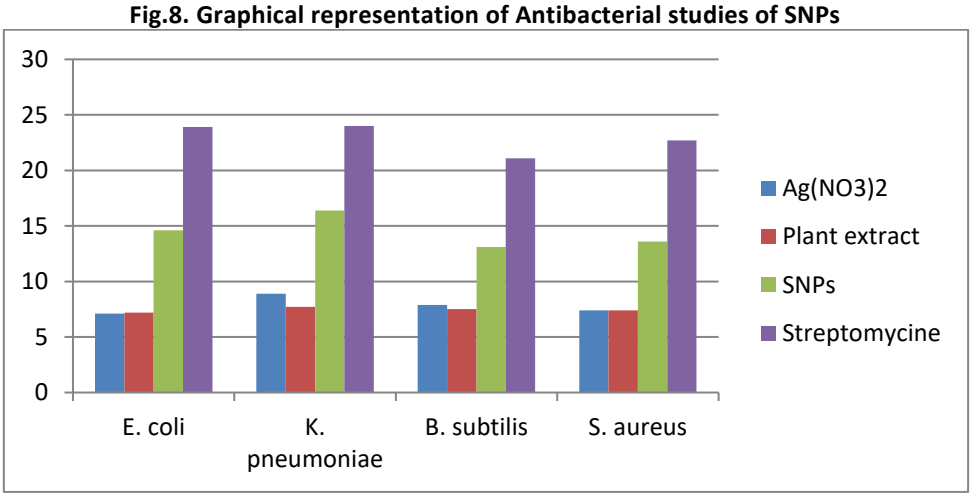


Fig. 7 Antibacterial studies of SNPs AgNPs
a) *E. coli* b) *K. pneumonia* c) *B. subtilis* d) *S. aureus*
1. AgNO3 solution 2. SNPs 3. Plant extract 4. Streptomycine.

Table. 1 Zone of inhibition (mm) of AgNPs on four bacteria with Ag(NO3)2, Plant extract and Streptomycine.

S. no	Name of the organism	Zone of inhibition (mm)			
		$Ag(NO3)_2$ (mm)	Plant extract (mm)	SNPs (mm)	Streptomycine (mm)
1	*E. coli*	7.1 ± 0.088	7.2 ± 0.088	14.6 ± 0.880	23.9 ± 0.057
2	*K. pneumoniae*	8.9± 0.033	7.7 ± 0.066	16.4 ± 0.120	23.9 ± 0.066
3	*B. subtilis*	7.9 ± 0.057	7.5 ± 0.057	13.1 ± 0.587	21.1 ± 0.115
4	*S. aureus*	7.4 ± 0.033	7.4 ± 0.088	13.6 ± 0.066	22.7 ± 0.133

Fig.8. Graphical representation of Antibacterial studies of SNPs



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