Biogenic Synthesis, Characterization of Silver Nanoparticles Using *Multani mitti (Fullers Earth)*, Tomato (*Solanum lycopersicum*) seeds, Rice Husk (*Oryza sativa*) and Evaluation of their Potential Antimicrobial Activity

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Summary: The synthesis of silver nanoparticles of three different biogenic materials *Multani mitti (Fullers earth)*, Tomato (*Solanum lycopersicum*) seeds, Rice Husk (*Oryza sativa*) was carried out. The possible presence and variability of comprehensive biomolecules in these materials turned as capping and reducing agents which optimize the reduction rate and stabilization of silver nanoparticles. Characterizations were determined by using ultraviolet-visible (UV-Vis) spectroscopy, Scanning electron microscope (SEM), Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD). Stable silver nanoparticles of average size 4.6, 41.1 and 10.6 nm were obtained for *Multani mitti*, tomato seeds and rice husk respectively. Phenolic and carboxylic biomolecules were identified as active reducing agents of Ag^{+2} to Ag^0. The antimicrobial activity was carried out against *Klebsiella pneumonia, Salmonella enterica, Escherichia coliand Staphylococcus aureus* strains by using well diffusion method. Maximum zone of inhibition (ZOI) was found against *Staphylococcus aureus* by all of the three biogenic materials.

Keywords: Silver Nanoparticles; *Multani mitti*; Tomato seeds; Rice Husk; Biogenic Synthesis; Antibacterial activity.

Introduction

In chemical and material sciences, the synthesis of nano-sized particles is considered as a developing field of research in conformity with the advance of nanotechnology [1-3]. In recent years, due to variety of good effect and vast range of research applications, this versatile and emerging field has attained the attention of various scientific researchers. These tiny particles have a size characteristically below 100 nm and the application of these nanoparticles in the fields of biomedicine and bioscience is increasing day by day with various other commercial applications [4-7]. Nanoparticles (NPs) can be synthesized by a wide selection of chemical, physical and biological processes, some of these are rather common and others are novel [8].

Silver nanoparticles (AgNPs) have involved a significant attention of scientific researcher due to very unique properties like shape, size, optical, conducting, catalytic, magnetic and electrical and most importantly the incorporation of AgNPs in antimicrobial applications. Stable AgNPs can be synthesized by employing variety of chemical and physical methods [9-11]. Worldwide, it is estimated that five hundred tons of AgNPs are produced per year and this quantity is projected to increase [12, 13]. There are various positive sides like heat resistance, durability, stability and wide spectrum of antimicrobial activity that make AgNPs as superior antimicrobial agents. Silver nanoparticles which are small in size and have larger surface area are chosen because of their good bactericidal capacity [3, 14-19].

Green synthesis is a developing branch of nanotechnology in which environmentally benign or biogenic materials like plant extracts or powder, fungi, bacteria etc. are used in the synthesis of AgNPs. Aforesaid synthesis method does not require any toxic chemical thus; it bids versatile benefits like pharmaceutical compatibilities, agricultural and biomedical applications and most important eco-friendliness. Green method of nanoparticle synthesis by using biogenic materials is more innovativeover physical and chemical methods due to its cost effectiveness [8, 20]. In recent years, synthesis of metallic nanoparticles by employing green method has attained a huge attention because it is more eco-friendly and cost effective [21].

Rice (*Oryza sativa* L.) is considered as important human food crop intended for half of the world's population [22] and it is well-thought-out to be a model for research in monocot species [13, 23].

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Nearly 582 million tons of rice is produced annually in all over the world with its 145 million tons of production is recorded in form of rice husk. This husk is source of serious pollution so it is of very restricted commercial interest. Rice husk has 20-22 mass % ash contents and eminent low calorific value which are attracting the interests of scientific researchers to find various applications of this residue [1, 24, 25]. Tomato (*Solanum lycopersicum*) is very important vegetable and it is cultivated and consumed on a very large scale. Tomato is a good source of flavonoids, beta-carotene,lycopene, potassium, folate, vitamin C and vitamin E [26-28]. Tomatoes and its products are consumed to minimize the menace of various diseases [29]. Tomatoes skin and seeds are considered as one-third of total weight of tomatoes. In the formation of tomato paste this one-third part is discarded off. Study showed that skin and seeds of tomatoes are rich in ascorbic acid, flavonoid, lycopene and phenolic contents so, to attain maximum health benefits it in very significant to utilize tomatoes along with skin and seeds [30]. Seeds of tomatoes are also considered as rich source of edible oil [31].In earlier times, people used ethnic medications for the cure of various diseases. There was a traditional use of clay for the healing purposes [32]. Clays are mainly rockswhich are made by the amassing of infinite fine particles with size less than 2 mm [33]. Fullers Earth more commonly known as '*Multani mitti*' is used as healing purposes especially of skin. It is natural and has variety of effects on metabolism in our body [34]. *Multani mitti* is silty clay and in Pakistan it is found in plenteousdeposits, which are located in 'the Land of Spiritualists', Multan. Scientifically, *Multani mitti* is a mineral of montmorillonite: smectite and bentonite clay. From the ancient Chinese, Mesopotamians, Egyptian and Indian civilizations, one can trace down various beneficial and curative effects of clay [35]. Clay has a variety of application i.e. aesthetic medicine, dietary supplements, therapeutics, healing and cleaning agents [36, 37].

In the present work an effort is made by employing a solvent free method for synthesis of AgNPs by using *Multani mitti*,powdered tomato seeds (*Solanum lycopersicum*) and rice husk (*Oryza sativa* L.). Further the antimicrobial activity of these metallic AgNPs was studied against *Klebsiella pneumonia, Salmonella enterica, Escherichia coli*and *Staphylococcus aureus* strains.

Experimental

Collection of Biogenic Materials

Rice husk (*Oryza sativa* L.), Tomato (*Solanum lycopersicum*), Fullers earth or *Multani mitti* were collected from local market of Lahore and Gujranwala Districts, Pakistan. Rice husk was undergone grinding process and sieved through 60 ASTM to get uniform sized material. Tomatoes skin and paste was separated out from seeds manually. Tomatoes seed went through washing process and dried in oven for 18 hours at 80°C. *Multani mitti* was dried to eliminate any kind of moisture and ground manually to get fine particles. These all biogenic materials were used in the green synthesis of AgNPs.

Synthesis of Silver Nanoparticles

AgNPs of Rice husk, powdered tomato seeds and *Multani mitti* were synthesized by slightly modified method [38]. The reaction medium contains silver nitrate, well grind biogenic material (Rice husk, dried tomato seed powder and *Multani mitti*), and 10 gm of oleylamine (2.7 mg) in 1:1:10 ratios respectively (Table-1). The reaction medium is heated up to 165°C. This mixture is stirred constantly for 30 minutes and then cooled at room temperature. Black solid nanoparticles are obtained on precipitation of this reaction mixture into chloroform. Obtained nanoparticles are washed thrice with chloroform and were subjected to drying.

Table-1: Composition used for the preparation of AgNPs of different biogenic materials.

Composition of reaction medium	Silver nitrate (g)	Biogenic material (g)	Oleylamine (g)
For AgNPs of Rice husk	1g	1g	10g
For AgNPs of Tomato seeds	1g	1g	10g
For AgNPs of *Multani mitti*	1g	1g	10g

Characterization of Silver Nanoparticles

All of the synthesized AgNPs were characterized by UV-Visible Spectrophotometer (UV-1700 Shimadzu) using Labomed inc. Quartz cells. FT-IR spectroscopic study was done by using Thermo Nicolet iS10 Spectrophotometer. Microstructures are observed with Hitachi S-3400N Scanning Electron Microscopy. The composition is analyzed by X-ray diffraction (XRD) (X'pertPRO, PANalytical).

Antimicrobial Studies

AgNPs of rice husk (*Oryza sativa* L.), tomato seeds (*Solanum lycopersicum*) and *Multani mitti* were employed to study antimicrobial activity by slightly modified well diffusion method [38, 39]. 10-15 ml molten agar was decanted into the sterilized petri plates. At 121°C Agar was autoclaved for 30 minutes. Wells were developed in agar plates. A serial dilution of the microorganisms *Klebsiella* *pneumonia*, *Salmonella enterica*, *Escherichia coli*, *Staphylococcus aureus*was prepared in distill water. 1 ml of the dilution was decanted which yields 100 cfu/ml onto the agar plates. Microorganisms were spread on the agar plate with the help of sterile spreader. The dilution of the AgNPs sample was prepared in DMSO as 10 mg/ml. 20 µl of the sample dilution was loaded into each wells. The plates were incubated for 24 hours at 37°C for 48 hours. Diameter of the inhibition zones was recorded in mm. The experiment was repeated thrice and the average values were calculated for antibacterial activity.

Results and Discussion

When biogenic materials are used in green synthesis of metallic nanoparticles, there would be various biomolecules and secondary metabolites which are active candidates and control the reduction of metal ions [40-43]. The AgNPs synthesized by using rice husk, tomato seeds and *Multani mitti* were analyzed by UV-Visible spectroscopy, XRD, SEM and FT-IR spectrometer.

UV-Visible Spectroscopy

As the biogenic materials were mixed $AgNO_3$ and oleylamine, it underwent a change in color from yellowish brown to black due to silver ion reduction which showed formation of silver nanoparticles. Fig. 1 shows the UV-Visible spectra recorded for AgNPs of rice husk, tomato seeds and *Multani mitti.* Absorption spectra of AgNPs formed in the reaction media has absorbance peak at 400 nm for all of three biogenic materials.

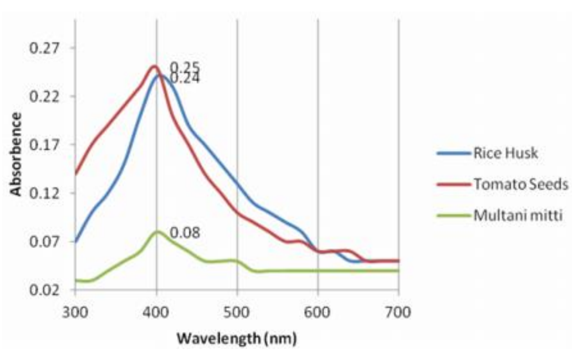

Fig. 1: UV-Visible spectra of AgNPs of Rice husk (*Oryza sativa* L.), Tomato seeds (*Solanum lycopersicum*) and *Multani mitti.*

X-ray Diffraction (XRD) Analysis

Structural characterization like the crystallite phase and size of nanoparticle are analyzed by X-ray diffraction (XRD). Diffraction pattern is calculated by Match software (CRYSTAL IMPACT, Bonn, Germany) and the particle size of AgNPs is calculated by Scherrer equation: D = Kλ / β CosΘ; Where, β = π/ 180*FWHM (FWHM = Full Width Half Maximum); λ = 1.540598 A°; Kλ= 0.94*1.540598 A°= 1.4482.

XRD pattern of AgNPs of Rice husk (Oryza sativa L.)

Fig. 2a shows the XRD pattern of AgNPs of Rice husk. The diffraction intensities were studied from 20° to 80° at 2Θ angles. There were four diffraction peaks (2-theta) at 38.16, 44.29, 64.30 and 77.91 corresponding to Bragg reflections (111), (200), (202) and (311) cubic planes of Ag are observed and compared with COD (Crystallography Open Database), silver file No. 96-110-0137 [45]. AgNPs of rice husk showed 2-theta values of 38.16, 44.29, 64.30 and 77.91 having particle size 9.6, 9.8, 10.7 and 11.6 nm respectively, calculated by Scherrer equation. An average calculated particle size of rise husk AgNPs is 10.4 nm.

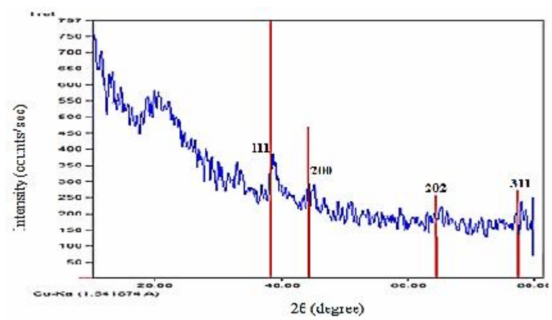

Fig. 2a: XRD pattern of AgNPs of Rice husk (*Oryza sativa L.*).

XRD pattern of AgNPs of Tomato seeds (Solanum lycopersicum)

Fig. 2b shows the XRD pattern of AgNPs of Tomato seeds. The diffraction intensities were studied from 20° to 80° at 2Θ angles. There were three diffraction peaks at 37.54, 43.55 and 76.16 corresponding to Bragg reflections from (111), (200) and (311) cubic planes of Ag are observed and compared with COD (Crystallography Open Database), silver file No. 96-901-3051 [46]. AgNPs of tomato seeds showed 2-theta values of 37.54, 43.55 and 76.16 having particle size 38.3, 39.0 and 46.1 nm respectively, calculated by Scherrer equation. An average calculated particle size of tomato seeds AgNPs was 41.1 nm.

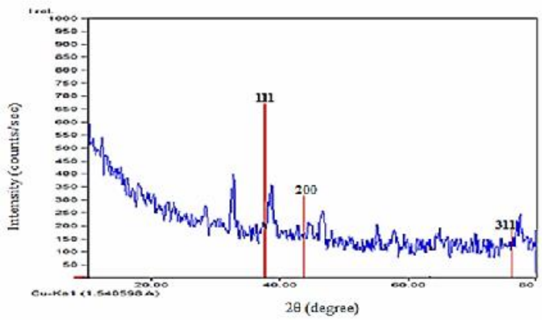

Fig. 2b: XRD pattern of AgNPs of Tomato seeds (*Solanum lycopersicum*).

XRD Pattern of AgNPs of Multani mitti

Fig 2c shows the XRD pattern of AgNPs of *Multani mitti*. The diffraction intensities were studied from 20° to 80° at 2ϴ angles. There were four diffraction peaks (2-theta) at 39.19, 44.42, 64.03 and 76.38 corresponding to Bragg reflections (111), (200), (202) and (311) cubic planes of Ag are observed and compared with COD (Crystallography Open Database), silver file No. 96-110-0137 [45]. AgNPs of rice husk showed 2-theta values of 39.19, 44.42, 64.03 and 76.38 having particle size 4.3, 4.3, 4.7 and 5.1 nm respectively, calculated by Scherrer equation. An average calculated particle size of tomato seeds AgNPs was 4.6 nm.

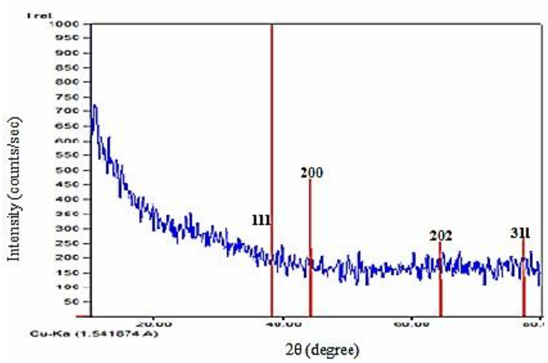

Fig. 2c: XRD pattern of AgNPs of *Multani mitti*.

Scanning Electron Microscopic Observation

The morphology of AgNPs of rice husk, tomato seeds and *Multani mitti* were put into characterize by SEM analysis as shown in Figs 3a-3c. The shape of the AgNPs of rice husk was rock and rod pebbles like while for AgNPs of Tomato seeds it is *agglomerated* and for AgNPs of *Multani mitti* it is coral reef rock like shape.

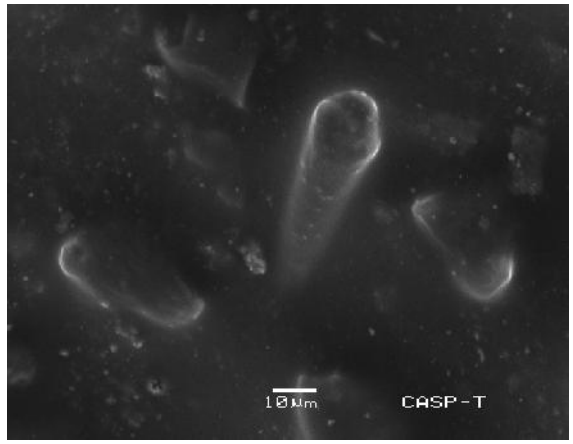

Fig. 3a: SEM image of AgNPs of Rice husk (*Oryza sativa* L.).

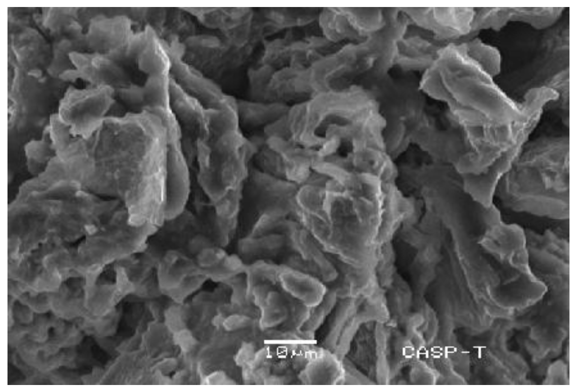

Fig. 3b: SEM image of AgNPs of Tomato seeds (*Solanum lycopersicum*).

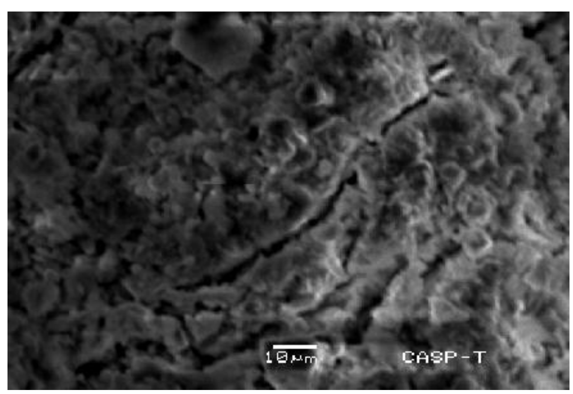

Fig. 3c: SEM image of AgNPs of *Multani mitti*.

Fourier Transform Infrared Spectroscopic study

Surrounded by the non-destructive characterization techniques, Fourier transform infrared (FTIR) spectroscopy is a multipurpose instrument for surface characterization of nanoparticles. FTIR analysis was carried out to independently identify the possible biomolecules which are part of nanoparticles surface and which worked for efficient stabilization and capping of the silver nanoparticles.

The FTIR spectrum (Fig. 4a) of AgNPs of Rice husk shows bands at 2919.56, 2851.10, 1708.43, 1015.99, 963.75, 787.95 and 719.24 cm^{-1}. The peak at 2919.56 cm^{-1} is predicting the O–H stretch of carboxylic acids [44]. The peak at 2851.10 cm^{-1} is characterizing the aldehyde C-H stretching [45, 47]. The peak at 1708.43 cm^{-1} is characterizing carbonyl functional group (C=O) stretching vibrations in aldehydes, ketones and carboxylic acids [45, 46]. The peak at 1015.78 is corresponding the C–O stretching in alcohols, phenols, acids, esters and ethers [47, 48]. The peak at 963.75 cm^{-1} is characteristic of stretching of P-OR esters, whereas two adjacent peaks 787.95 and 719.24 cm^{-1} are corresponding the S-OR esters stretching in given sample [49]. These spectral peaks are showing the presence of various biomolecules with discrete functional groups that are responsible for reduction of Ag^{2+} to Ag^{0}.

The FTIR spectrum (Fig. 4b)of AgNPs of Tomato seeds shows bands at 2916.16, 2848.42, 1514.97, 1469.82, 1411.88, 961.89 and 717.81 cm^{-1}. The peak at 2916.16 $cm^{-1}$is predicting the O–H stretch of carboxylic acids [44, 47]. The peak at 2848.42 cm^{-1} is characterizing the alkane and aldehyde C-H stretching [47]. The peak at 1514.97 cm^{-1} is characterizing C=O (amide) stretching in carboxylic acids and derivatives [45, 46]. The peak at 1469.82 cm^{-1} is corresponding the C-H scissoring of alkanes [49]. The peak at 1411.88 cm^{-1} is corresponding the C=O stretching of saturated ketones [49]. The peak at 961.89 cm^{-1} is characteristic of stretching of P-OR esters, whereas peak at 717.81 cm^{-1} is corresponding the S-OR esters stretching in given sample [49]. These spectral peaks are showing the presence of various biomolecules like fatty acids and sugars [53, 54] with discrete functional groups that are responsible for reduction of Ag^{2+} to Ag^{0}.

The FTIR spectrum (Fig 4c)of AgNPs of *Multani mitti* shows bands at 2916.06, 2848.60, 1514.62, 1469.84, 1411.14, 961.88 and 717.74 cm^{-1}. The peak at 2916.06 $cm^{-1}$is predicting the O–H stretch of carboxylic acids [44, 47]. The peak at 2848.60 cm^{-1} is characterizing the alkane and aldehyde C-H stretching [45, 47]. The peak at 1514.62 cm^{-1} is characterizing C=O (amide) stretching in carboxylic acids and derivatives [45, 46]. The peak at 1469.84 cm^{-1} is corresponding the C-H scissoring of alkanes [49]. The peak at 1411.14 cm^{-1} is corresponding the C=O stretching of saturated ketones [49]. The peak at 961.88 cm^{-1} is characteristic of stretching of P-OR esters, whereas peak at 717.74 cm^{-1} is corresponding the S-OR esters stretching in given sample [46, 49]. These spectral peaks are showing the presence of various biomolecules with discrete functional groups that are responsible for reduction of Ag^{2+} to Ag^{0}.

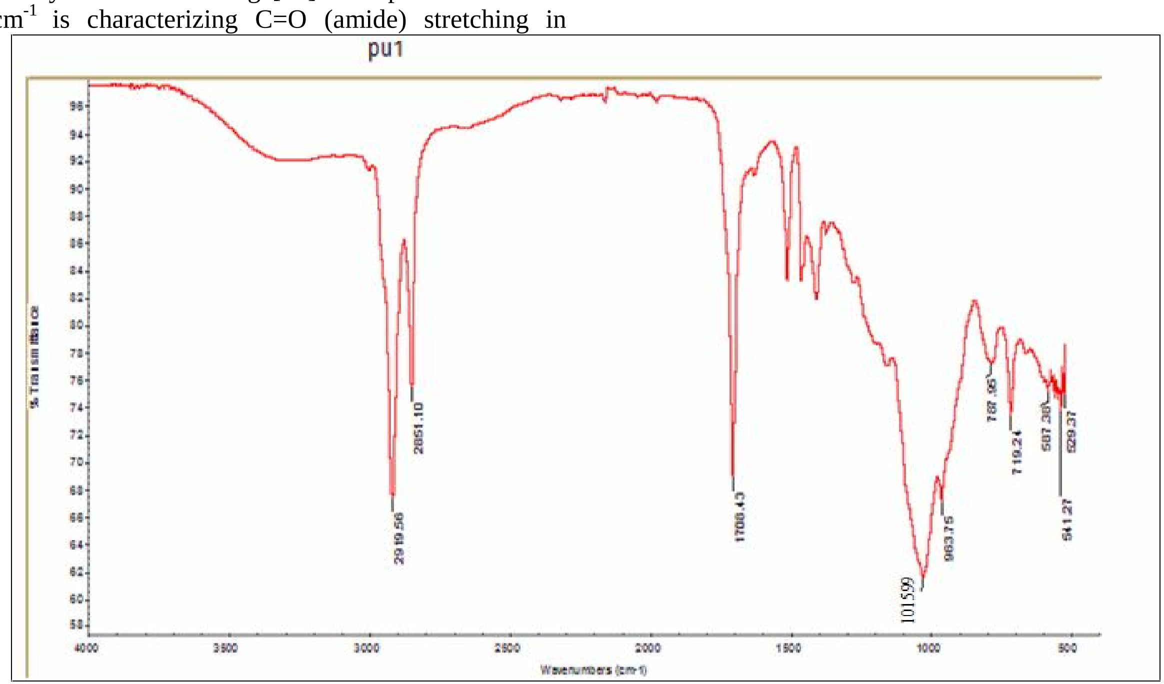

Fig. 4a: FTIR spectrum of AgNPs of Rice husk (*Oryza sativa* L.)

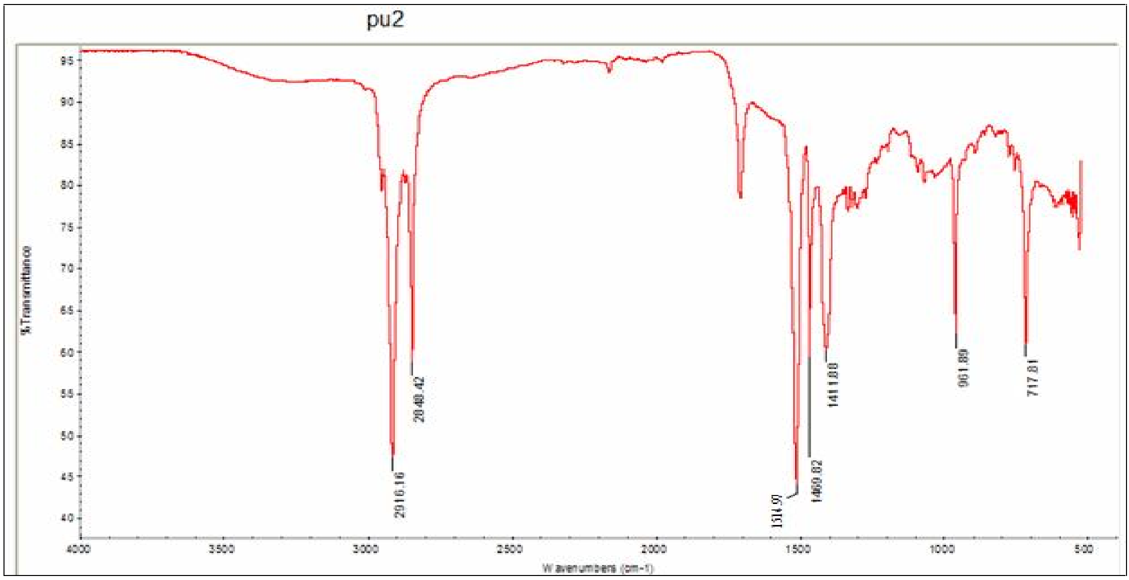

Fig. 4b: FTIR spectrumof AgNPs of Tomato seeds (*Solanum lycopersicum*).

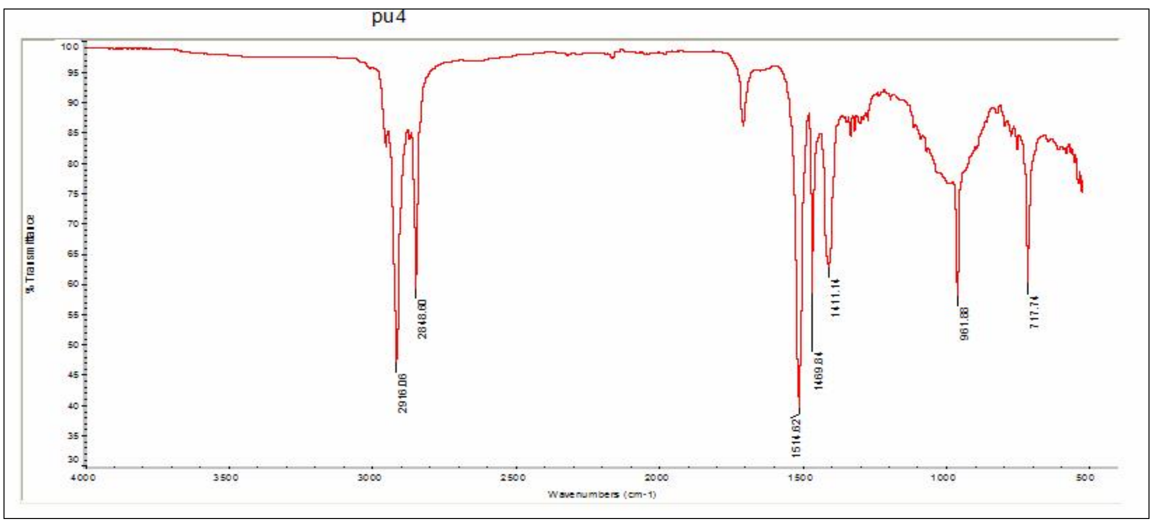

Fig. 4c: FTIR spectrumof AgNPs of *Multani mitti*.

Antibacterial Activity Results

The antimicrobial activity was carried out against *Klebsiella pneumonia, Salmonella enterica, Escherichia coliand Staphylococcus aureus* bacterial strains (Table-1). All samples displayed good inhibition activity against all bacterial strains as shown in Fig 6a-6d. AgNPs of *Multani mitti* showed maximum zone of inhibition (ZOI) *15* ± 1.414214 mm, *14* ± 0.707107 mm, *15* ± 2.828427 mm and *15* ± 0.00 mm against *Klebsiella pneumonia, Salmonella enterica, Escherichia coli* and *Staphylococcus aureus* respectively, whereas AgNPs of rice husk is more active against *Staphylococcus aureus* with zone of inhibition *15* ± 4.949747 mm. AgNPs of tomato seed were more inhabitant against *Staphylococcus aureus with zone of inhibition 15* ± 0.00 mm. Eminent inhibition of AgNPs of under study biogenic materials was recorded against *Staphylococcus aureus* as comparison is given in Fig 5.

Silver nanoparticles of biogenic materials inhibited the bacterial growth by giving clear inhibition zone. Different sizes of cubic AgNPs were estimated in which small cubic AgNPs of *Multani mitti* (mean size 4.6 nm) showed the strongest antibacterial activity, followed by AgNPs of rice husk (mean size 10.4 nm), compared to larger cubic AgNPS of tomato seed (mean size 41.1) (Fig 5). From the aforesaid results, it was proven that small size AgNPs has greater antibacterial potential, as it is easy for such small sized particles to easily penetrate into the cell wall and readily react with the cell components [55, 56].

Table-1: Antibacterial activity of silver nanoparticles of Rice husk (*Oryza sativa* L.), Tomato seeds (*Solanum lycopersicum*) and *Multani mitti* (20 µL/mL).

Microorganisms	AgNPs of		
	Rice husk (RHNP)	**Tomato seeds (TNP)**	***Multani mitti* (CYNP)**
	Zone of Inhibition (mm)		
Bacterial Strains			
***K. pneumonia* (-)**	*13* ± 1.414214	*14* ± 0.00	*15* ± 1.414214
***S. enterica* (-)**	*10* ± 0.00	-	*14* ± 0.707107
***E. coli* (-)**	*13* ± 3.535534	*11* ± 2.12132	*15* ± 2.828427
***S. aureus* (+)**	*15* ± 4.949747	*15* ± 0.00	*15* ± 0.00

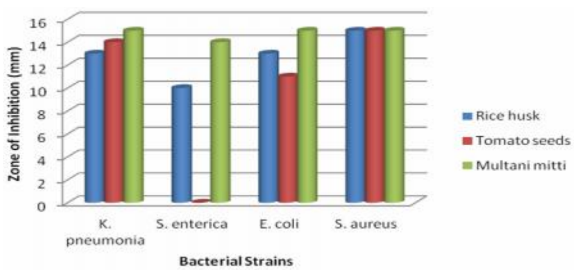

Fig. 5: Comparison of antibacterial activity of silver nanoparticles of Rice husk (*Oryza sativa* L.), Tomato seeds (*Solanum lycopersicum*) and *Multani mitti.*

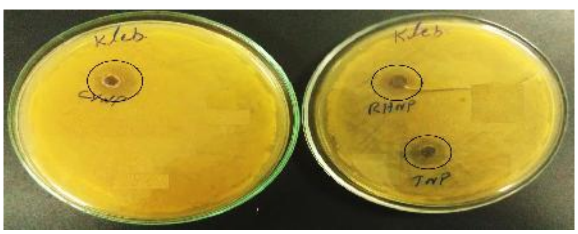

Fig 6a: Zones of inhibition of AgNPs of Rice husk (RHNP), Tomato seeds (TNP) and *Multani mitti (CYNP)* against *Klebsiella pneumonia*

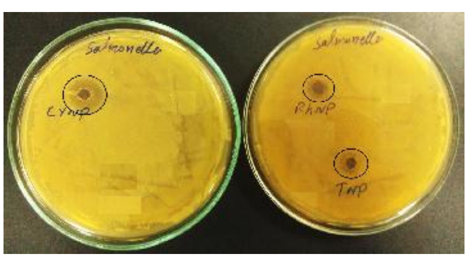

Fig 6b: Zones of inhibition of AgNPs of Rice husk (RHNP), Tomato seeds (TNP) and *Multani mitti (CYNP)* against *Salmonella enterica*

Fig. 6c: Zones of inhibition of AgNPs of Rice husk (RHNP), Tomato seeds (TNP) and *Multani mitti (CYNP)* against *Escherichia coli.*

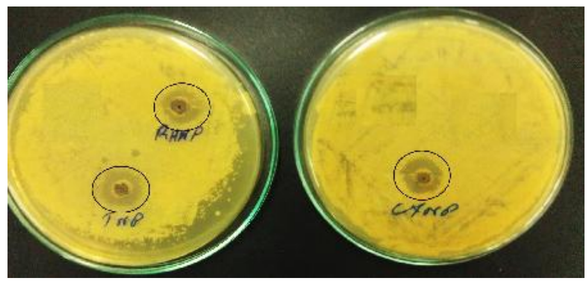

Fig. 6d: Zones of inhibition of AgNPs of Rice husk (RHNP), Tomato seeds (TNP) and *Multani mitti (CYNP)* against *Staphylococcus aureus*

Conclusion

A simple solvent free approach was attempted for the green synthesis of environmental friendly AgNPs of rice husk, tomato seeds and *Multani mitti having size of* 10.4 nm, 41.1 nm and 4.6 nm respectively. Further the synthesis of AgNPs was confirmed by UV-Visible spectroscopy, XRD, SEM and FT-IR analysis. FT-IR study revealed that acidic and phenolic functional groups were responsible for the reduction of silver ions. These AgNPs exhibited toxic effect on various disease causing bacteria as they underwent antibacterial study against *Klebsiella pneumonia, Salmonella enterica and Escherichia coli* but good inhibition was recorded against *Staphylococcus aureus.* As *Solanum lycopersicum* and *Multani mitti* has been traditionally utilized as antioxidant and for skin care respectively so, the silver nanoparticles of these biogenic materials would potentially be used as future biomedical applicants.

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