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Green Synthesis Of Silver NanoParticles From Terminalia Chebula Fruit And Its Bactericidal Activity Against Diabetes Wound Pathogens

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Abstract

In this study the antibacterial activity of eight medicinal plants (*Biophytum sensitivum, Senna auriculata, Centella asiatica, Aegle marmelos, Terminalia chebula, Cinnamomum , Azadirachta indica, Ficus benghalensis*) were tested against the diabetes wound pathogens such as *Pseudomonas aeruginosa, Staphylococcus aureus and Klebsiella pneumoniae*. Antimicrobial activity of extracts of prefered medicinal plants were tested by the Well diffusion method. Highly significant antimicrobial action was noted in aqueous extract of *Terminalia chebula* and was subjected to phytochemical studies and synthesis of silver nanoparticles. Depiction of silver nanoparticles can be confirmed by UV-Vis spectroscopy FT-IR analysis and XRD analysis. Antimicrobial activity of silver nanoparticles prepared from dried fruit extract of *Terminalia chebula* showed a highest zone of inhibition around 27mm in *Staphylococcus aureus* followed by 17mm in *Klebsiella pneumonia* and 14mm in *Pseudomonas*. In this work the in vitro antibacterial activity of silver nanoparticles produced from *Terminalia chebula* fruit showed highest activity against *staphylococcus aureus* when compared with, *klebsiella pneumonia* and *Pseudomonas aeruginosa.* On the basis of the result it could be judged that the silver nanoparticles synthesized from fruit extract of *Terminalia chebula* exhibited highest antimicrobial activity against diabetes wound pathogens.

Keywords: *Terminalia chebula*, Antibacterial activity, silver nanoparticle

1. INTRODUCTION

Diabetes could also be a worldwide health issue approximate to rise over 642 million by 2040.[Mangaoni et al. 2016] Mainly two types of diabetes are there: Diabetes type I and Diabetes type II.[Selvan Madhaiyan et al. 2019] Type I diabetes is insulin dependent and accounts for 10 % of total diabetes worldwide. Type II diabetes is no insulin dependent and it accounts for 90% of the world diabetic patients, which is about 150 million people. Diabetes mellitus commonly known as diabetes is a metabolic disease that cause high blood sugar.[Mangaoni et al. 2016] Diabetes is occur when the pancreas is not producing sufficient insulin or cells of the body not respond properly to the insulin product [Shoback et al. 2011].Bacteria are the foremost essential factors liable for causing wound infections in diabetic patients.[Uçkay et al. 2013] [Lorina Badger et al. 2015] Diabetic wounds are slow, non-healing wounds which will persist for weeks despite adequate and appropriate care. Such wounds are difficult and hard to manage. Though the precise pathogenesis of poor wound healing in diabetic wounds is not clearly understood, evidence from studies involving both human and animal reveal several abnormalities within the various phases of the wound healing process [Manish Pal Singh et al. 2010].

Plants are the main source of drugs for the treatment of diabetes mellitus in the Indian system of drugs and other ancient systems within the world. It is assumed that ingredients from medicinal plants are less toxic and have fewer side effects compared with orthodox therapeutic agents; hence, the increased and renewed interest within the use and application of medicinal plants within the wound healing process both in diabetic and non-diabetic conditions. [Mangaoni et al. 2016]. Some of the medicinal plants that shows wound healing properties are *Biophytum sensitivum, Senna auriculata, Centela asiatica, Aegle marmelos, Terminalia*

chebula, Cinnamomum, Azadirachta indica, Ficus benghalensis. The current study targeted the importance of in experienced synthesis of *Terminalia chebula* fruit against polygenic disorder wound pathogens. *Terminalia chebula* belongs to Plantae, Anthophyta, Magnoliopsida, order Myrtales, family combretum, Genus Terminalia, and Species chebula. They're called the king of medicines in Tibet. In India it is called "Kadukkai" [Oguntibeju 2019]. Terminalia chebula seeds are also a best alternative for the treatment of wound healing and anti diabetes. *Terminalia chebula* possesses a good style of activities like antimicrobial, antioxidant, antiviral, anticarcinogenic, hypocholesterolemic radioprotective, antispasmodic and anti purgative [Afshari et al. 2016]. The antibacterial property of these nanoparticles was studied against some strains of diabetes wound pathogens and the characterisation of silver nanoparticles were confirmed by UV-Vis Spectrophotometric analysis, FT-IR and XRD.

2. MATERIALS AND METHODS

2.1 Collection of material

Eight different plant species such *as Biophytum sensitivum, Senna auriculata, Centella asiatica, Aegle marmelos, Terminalia chebula, Cinnamomum , Azadirachta indica, Ficus benghalensis* were collected from the localities of Peroorkada ,Trivandrum Kerala and drug sellers in Trivandrum. Plant materials were washed thoroughly for 2-3 times with running H2O and surface sterilized with 1% mercuric chloride, shade dried, powdered and used for extraction.

2.2 COLLECTION AND IDENTIFICATION OF TEST ORGANISMS

The pathogenic bacteria like *Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumonia* were isolated from patients previously diagnosed with diabetes from an diagnostic laboratory Trivandrum. Pathogenic cultures were confirmed in our laboratory using selective media, by Gram's staining and biochemical tests like IMViC test, catalase test, oxidase test, urease test, TSI test and hydrolysis tests.

2.3 PREPARATION OF EXTRACTS

2.3.1 Aqueous extract

One gram of dried plant materials were mixed with 10 ml of sterile H2O and stored at temperature for 48 hours. After 48 hours it had been subjected to centrifugation and supernatant was used for further study.

2.3.2 Solvent extract

One gram of powdered samples were extracted with 10 ml of solvents like methanol, Ethanol, acetone, dichloro methane and petroleum ether successively up to 48 hours. Then the solvent extracts were subjected to antimicrobial activity tests by the well diffusion method.

2.4 Antimicrobial activity assay

Antimicrobial activity of aqueous extract and solvent extracts were determined by well diffusion method on Muller-Hinton agar medium. Wells were made on the Muller-Hinton agar plates using cork borer and therefore the inoculum containing 106 CFU / ml of bacteria were spread on the solid plates. Then 10 µl each of all aqueous, solvent extracts were placed within the wells made in inoculated plates. The treatments also included 50 µl of sterilized H2O and solvents separately which served as control. The plates were incubated for 24 h at 37ºC and zones of inhibition round the wells were measured in mm.

2.5 PHYTOCHEMICAL ANALYSIS OF ACTIVE PLANT EXTRACTS:

Phytochemical analysis for major phyto constituents of the plant extracts under taken using standard qualitative methods as described by various authors.The plant extracts were screened for the presence or absence of secondary metabolites like alkaloids, saponins, phenols, flavonoids, quinons, tannins, glycosides, steroids and terpenoids[,Singh et al. 2009][vogel 1958][Kapoor et al. 1969].

2.6 BIOSYNTHESIS OF SILVER NANOPARTICLES

For the biosynthesis and characterization of silver nanoparticles 40g of powdered sample were mixed with 200 ml distilled water then boiled for five min, with whatman no 1 paper, the extract was then purified. The

filtrate was treated with 1mM caustic solution and incubated at room temperature. After incubation the filtrate was centrifuged at 7000 rpm for 40 min and therefore the pellet was collected. The pellets were dissolved with ethanol and again centrifuged. Then the pellet was collected and dried in a hot air oven to produce a fine powder[Fedeyi et al. 1989]. Presence of silver nanoparticles were observed by changing the color of the filtrate[Ponarulselvam et al. 2012].

2.7 CHARACTERISATION OF SILVER NANOPARTICLES

2.7.1 Uv Vis Spectroscopy

The samples were subjected to optical measurements which were carried out by using a UV Vis Spectrophotometer and scanning the spectra between 200 and 1000 nm . UV Vis spectral analysis of the above said samples were done by using double beam spectrophotometer, Thermofisher, Aquamate8000[Bhat et al. 2015]

2.7.2 FTIR analysis

The FTIR analysis of the sample was done using Thermo Niolet, Avatar 370 with spectral range 4000 to 400 of resolution 8 cm-1 to identify the chemical constituents of the Ag-NPs from the sample[15].

2.7.3 XRD

The particle size and nature of the silver nano particle were determined using XRD. This was carried out using the Shimadzu XRD-6000/6100 model with 30 kv, 30 mA with Cu kα radians at 2θ angle. X-ray powder optical phenomenon could be a fast analytical technique primarily used for part identification of a crystalline material and might give information on unit dimensions. The analyzed material is finely ground, and average bulk composition is decided. The particle or grain size of the particles on the silver nanoparticles was determined using Debye Sherrer's equation. $D = 0.94\lambda / B \cos\theta$ [Ponarulselvam et al. 2012].

2.8 ANTIMICROBIAL ACTIVITY ASSAY FOR SILVER NANOPARTICLES

The antimicrobial activity of silver nanoparticles was tested against the wound pathogens by using agar well diffusion method. 20 ml of sterilized Muller Hinton agar was poured into the sterile petri dishes and allowed for solidification. After solidification 24 h nutrient broth grown wound pathogen cultures were swabbed on the respective agar plates. Wells of 6 mm diameter were punched over the agar plates using a sterile gel puncher . 100µl of synthesized silver nanoparticles were poured into the wells. The plates were incubated at 370C for 24 h. After incubation, the diameter of inhibition zones formed around each well were measured and expressed in mm, to evaluate the antimicrobial activity[Gajbhiye et al. 2009].

2.9 RESULT AND DISCUSSION

The extracts of eight medicinal plants (Table 1) tested were found to have effective antibacterial activity against a group of microorganisms that were isolated from diabetes wound infections (*Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumonia*). Aqueous extract of eight different plants were shown in (Fig 1) .In well diffusion method aqueous extract of *Terminalia chebula* shows maximum zone of inhibition in *Pseudomonas aeruginosa* (2cm) and minimum zone of inhibition in (0.9cm). Among the five solvents tested in each plant, ethanolic and methanolic extract of *Terminalia chebula* showed high significant activity. *Klebsiella pneumonia* shows highest zone in ethanolic extraction (2.9cm) and *Pseudomonas aeruginosa* shows highest zone in methanol(2.9cm) .Antimicrobial activity of solvents such as acetone, methanol, ethanol, petroleum ether and dichloromethane against the eight medicinal plants were presented in Fig 2. Based on the results obtained, potential antimicrobial activity was noted in *Terminalia chebula.*

Table 1. Different types of plants used in this study

Sample No	Common name	Part used	Scientific Name	Family
N1	Mukkutti	Whole plant	*Biophytum sensitivum*	*Oxalidaceae*
N2	Koovalam	Leaf	*Aegle marmelos*	*Rutaceae*
N3	Neem	Leaf	*Azadirachta indica*	*Meliaceae*
N4	Peral	Bark	Ficus bengalensis	*Moraceae*

N5	Avaram	Leaf	*Senna auriculata*	Fabaceae
N6	Kudangal	Leaf	*Centella asiatica*	*Umbelliferae*
N7	Karuka patta	wood	*cinnamomum*	*Lauraceae*
N8	Kadukka	fruit	*Terminalia chebula*	*Combretaceae*

Fig 1. Antimicrobial activity of medicinal plants with aqueous extract

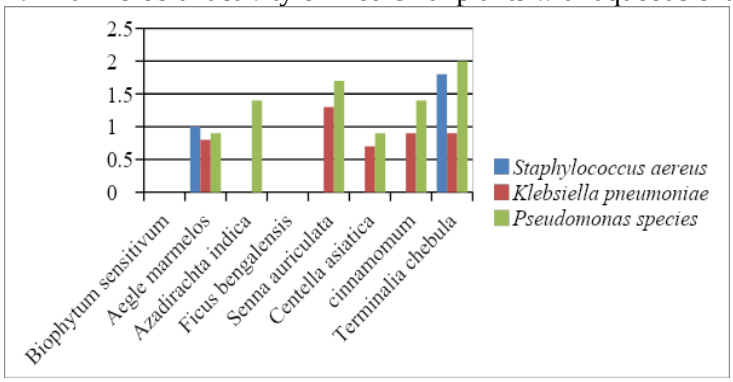
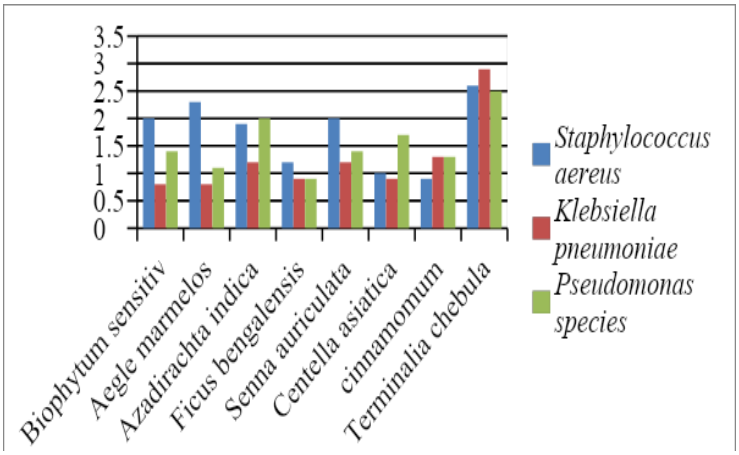


Fig 2 Antimicrobial activity of medicinal plants with solvent extracts

2.a Ethanol



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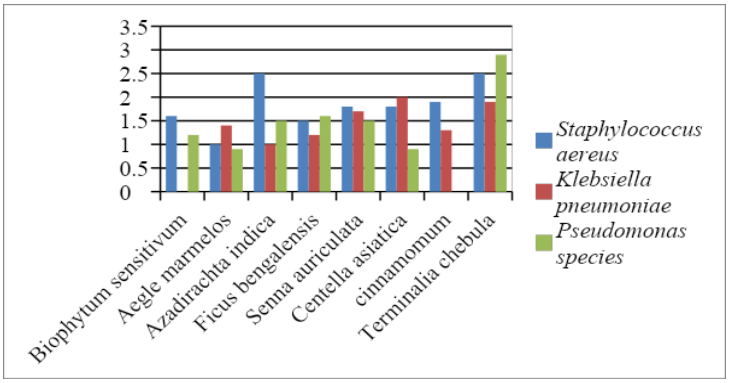
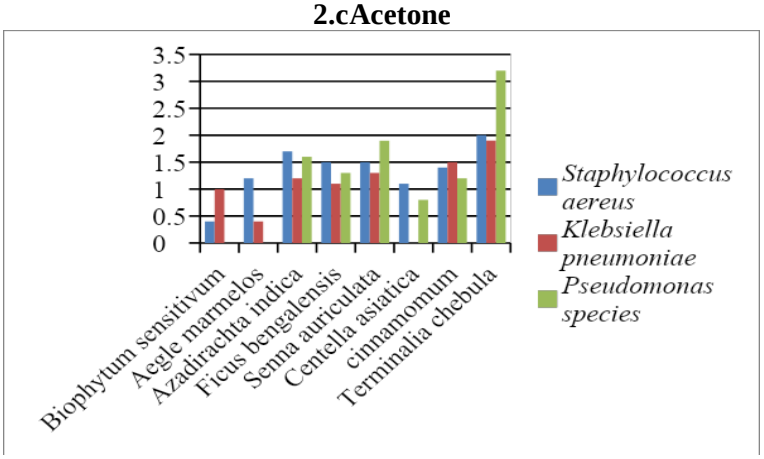
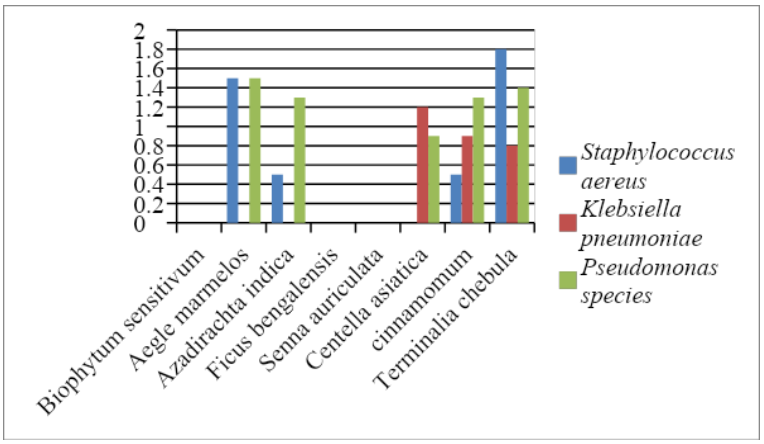
2.b Methanol



2.cAcetone



2.d Petroleum ether



Ethanol, Methanol and Aqueous extract of *Terminalia chebula*, were subjected to phytochemical evaluation (Table 2). Phytochemical analysis of these solvents revealed the presence of secondary metabolites such as saponins, tannins, flavonoids, quinones, terpenoids, and glycosides.The authors[Kathirvel, et al. 2012]studied the phytochemical analysis of Terminalia chebula and concluded that, it contains classes of compounds like phenol, flavonol, flavonoid, ascorbic acid, and proteins, carbohydrates.

Table 2. Phytochemical screening tests for *Terminalia chebula*

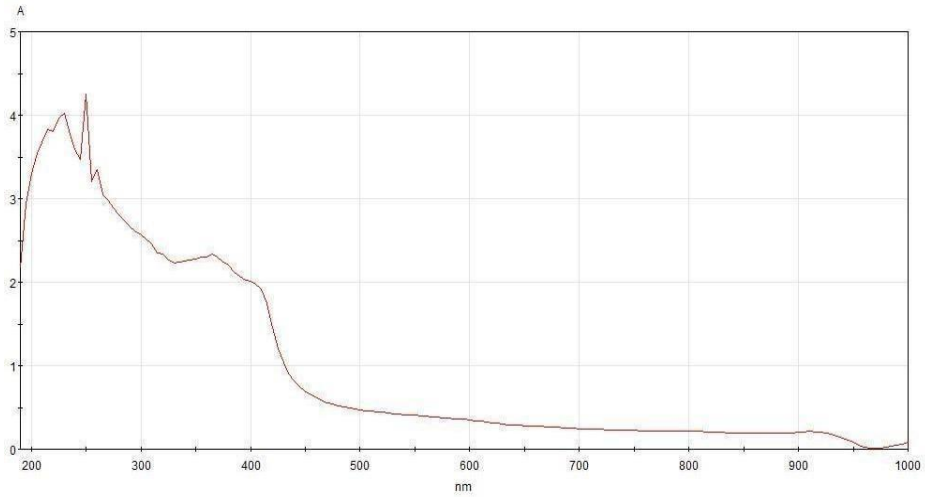
Sl. No	Phytochemical tests	*Dis water*	Ethanol	Methanol
1	Saponins	+	-	-
2	Tannins	+	+	+
3	Alkaloids	+	+	+
4	Flavonoids	+	+	-
5	Steroids	-	-	+
6	Triterpanoids	-	-	+
7	Quinones	+	+	+

For the synthesis of Silver nanoparticles, it utilizes a non-toxic agent ,which functions as both reducing and stabilizing agent during synthesis. The mechanism of the reaction involves the reduction of aqueous metal ions with plant fruit extract. The color changed from pale yellow to dark brown[Ankanna et al. 2010] .In the present study formations of silver nanoparticles by reduction of silver nitrate during exposure to *Terminalia chebula* fruit extract was easily monitored from the changes in color within 2 hours (Plate 1).

Plate 1.synthesis of silver nanoparticles

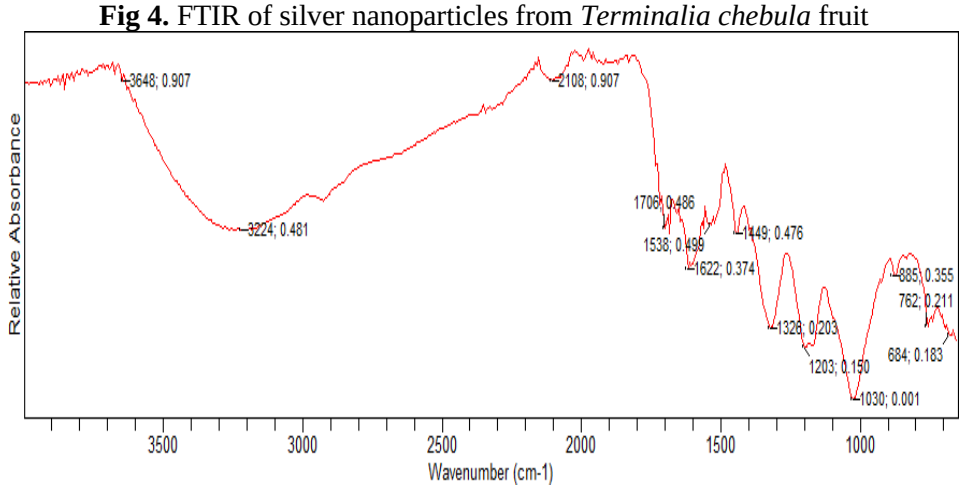
FT-IR , XRD and SEM analysis are the methods used for the characterisation of silver nanoparticles . The UV–Visible spectrophotometer is a very useful technique for the analysis of some metal nanoparticles and is a significant technique to authenticate the formation and stability of silver nanoparticles in aqueous solution. UV spectrum is essentially used for determination of size and form of nanoparticles once increase of silver nanoparticles reactivity would be less. The maximum range of silver nanoparticles in UV-Vis spectrometers is 300 to 500 nm. In this study the result of UV-Vis spectrometer synthesized from Terminalia chebula ranges from 370 to 400 nm. It confirms that the synthesized particles are silver (Fig. 3).

Fig 3. UV-spectroscopic analysis of silver nanoparticles from *Terminalia chebula* fruit



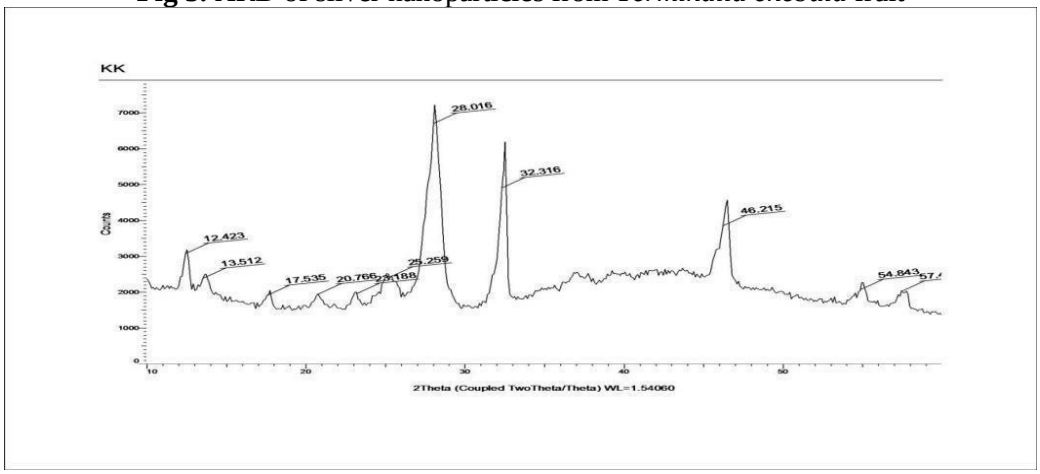
FT-IR analysis of nanoparticles from *Terminalia chebula* shows different bonds at different peaks (Fig 4) at 3684 cm-1, 3224 cm-1, 2108 cm-1, 1706 cm-1, 1622 cm-1,
1538 cm-1, 1449 cm-1, 1326 cm-1, 1203 cm-1, 1030 cm-1, 885 cm-1, 762 cm-1, 684 cm-1
respectively. The bands at 3684 cm-1 and 3224 cm-1 revealed the O-H alcohol group, , 2108 cm-1 revealed the C=C alkyne group, 1706 cm- revealed the C=O carboxylic acid, 1622 cm-1 revealed the C==C conjugated alkene group, 1538 cm-1 revealed the N-O nitro compounds, 1326 cm-1 O-H phenol group, 1203 cm-1 revealed the C-O ester group, 1030 cm-1 revealed the S=O sulfoxide group, 885 cm-1 and 684 cm-1 revealed the C=C alkene group, 762 cm-1 revealed the C-H.(figure 4) FT-IR measurements were carried out to identify the potential functional group of the biomolecules in the aqueous extract of *Terminalia chebula* for the reduction of the silver ions into silver nanoparticles .The present work reveals that the FTIR analysis supported the reducing property of silver nanoparticles synthesized by *Terminalia chebula* fruit extract in which different functional groups were observed[jain et al. 2009].

Fig 4. FTIR of silver nanoparticles from *Terminalia chebula* fruit



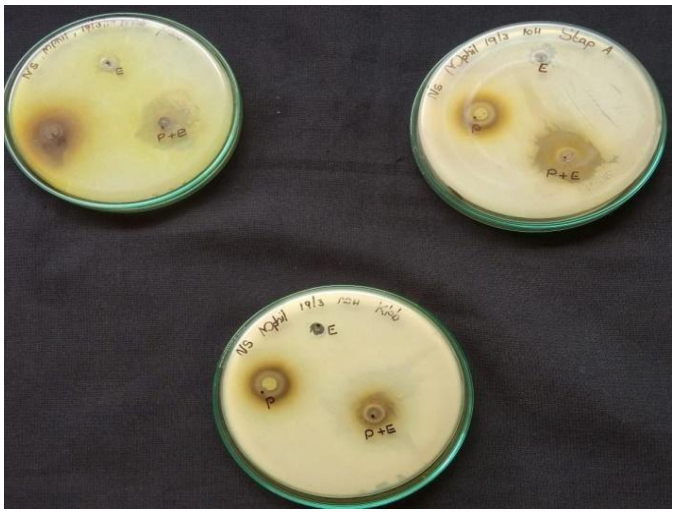
Structural characterization has been performed using XRD analysis. Average size of the silver nanoparticle formed in the bioreduction process was determined by scherrer formula d =0.9 × λ / β × cos and was estimated as 10-15 nm. XRD can be confirmed by the existence of silver colloids in the sample fig 5.In this the Diffraction peaks are observed at 28.016, 32.316, 46.216, 45.259, and 20.766. **(Fig 5)**. XRD results confirms the presence of silver colloidal in the sample[Rama Bhat et al 2015]

Fig 5. XRD of silver nanoparticles from *Terminalia chebula* fruit



Antimicrobial study revealed that the synthesized nanoparticles from *Terminalia chebula* was found to be very efficient against test pathogens such as *Staphylococcus aureus, Pseudomonas sp and Klebsiella pneumonia.*The silver nanoparticles prepared from dried fruit extract of *Terminalia chebula* showed a highest zone of inhibition around 27mm in *Staphylococcus aureus* followed by 17mm in *Klebsiella pneumoniae* and 14mm in *Pseudomonas sp* (Plate 2).which was co-relating the earlier works[Savithramma et al. 2012]. From this work it was concluded that silver nanoparticles synthesized from the fruit of Terminalia chebula had higher activity than the chemical constituent present in the fruit against diabetes wound pathogens.

Plate 2. Antimicrobial activity of silver nanoparticles synthesized from *Terminalia chebula* fruits



CONCLUSION

In conclusion, the synthesis of silver nanoparticles by using the *Terminalia chebula* fruit extract acts as a reducing agent for nanoparticles synthesis.In vitro antibacterial activity of silver nanoparticles produced from *Terminalia chebula* fruit showed highest activity against *staphylococcus aureus* When compared with, *klebsiella pneumonia* and *Pseudomonas sp.* Based on the result of the present study, we concluded that the silver nanoparticles synthesized from fruit extract of *Terminalia chebula* showed highest antimicrobial activity against diabetes wound pathogen

ABBREVIATIONS

XRD – X ray diffraction
SEM –Scanning electron microscope
FT-IR -Fourier transform infrared spectroscopy mm -Millimeter

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