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Research paper

Green synthesis of Silver Nanoparticles (AgNP's) from *Passiflorafoetida*Linn.and assessment of its antibacterial activity

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Abstract

In recent years, there has been an expandable interest in the development of novel drug delivery systems using nanoparticles. Among metallic nanoparticles, silver nanoparticles (AgNPs) are crucial due to their physiochemical and antimicrobial properties which help in several therapies. In the present study aqueous and ethanol extracts from leaf and fruit of *Passiflorafoetida* Linn. or commonly known as stinking passion flower plant were used for the synthesis of silver nanoparticle. Characterization studiesinvolvedUV-vis, peak absorption for extract followed by FTIR studies to understand structure and respective bonds of synthesized nanoparticle and Energy-dispersive X-ray spectroscopy-Scanning electron microscopy analysis of nanoparticle were done to understand the surface morphology and composition of the elements. Antimicrobial property was determined through agar well diffusion. The study showed Uv- vis absorption peak for leaf extract falling between 320 -490nm and fruit extract between 310- 390nm. FTIR studies revealed presence of aromatic groups, alkyl halide group, alcohol group, alkenes group, and halide groups resent in varying proportion denoting presence of biomolecule involved in capping & stabilizing nanoparticle.Energy-dispersive X-ray spectroscopy-Scanning electron microscopy analysis yielded major elemental constituents of leaf aqueous extract nano-particles are potassium, calcium, chlorine, oxygen, iron, magnesium, sodium and phosphate. Form the study it was found that leaf extracts showed significant activity due to the presence of varied elements and several functional groups.

Key words: silver nanoparticle *Passiflorafoetida*, pathogens, green synthesis, characterization, analysis and antimicrobial activity.

1. Introduction

In recent years interest in AgNps is growing increasingly due to exemplified defence against wide range of micro organisms and possibilities of using them to overcome antimicrobial resistance. The physiochemical chemical characteristics of AgNps make them to be used in vast industrial applications such as,in the preparation of adhesives, electronic devises, pastes etc . Extending benefits in biomedical, drug delivery to water treatment and agricultural [1].There are several physio-chemical methods available for synthesis of nanoparticle,

though the methods are cost effective in terms of yield, but they are associated with the limitations like use of toxic chemicals and high operational cost and energy needs [2-4]. Considering the drawbacks of physio-chemical methods, an alternative cost-effective and energy efficient synthesis of AgNPsfrom plant extracts is practised from decade [5]. leaf, bark, root, and stem parts of several medicinally important plants like *Boerhaaviadiffusa, Tinosporacordifolia*, *Aloe vera*,*TerminaliachebulaCatharanthusroseus*, *Ocimumtenuiflorum*, *Azadirachtaindica*, *Emblicaofficinalis*,*Cocosnucifera*, *common spices Piper nigrum*, *Cinnamon zeylanicum* are used for AgNps synthesis [6-10]. Few weeds which lack natural enemies like *Partheniumhysterophorus* have also been used for AgNps synthesis [11]. *Passiflora*species have also been reported with significant medicinal properties and are used in the treatment of several diseases, such as insomnia, anxiety, and hysteria. It is also used in the treatment of tuberculosis, worms, coughs and colds. Pressed fluid from leaves and stem is used to improve fertility in women. The leaves of this plant have the potential to heal wounds, snake bites and used in curing sleeplessness. In addition to this, they possess anti-inflammatory, antioxidant, anti-helminthic (intestinal nematodes and flatworms), analgesic and antibacterial potential. In recent days *Passiflora*species are contemplated for their antidiabetic activity [12]. Generally, plant extracts functions to play dual role as potential reducing and stabilizing agents with an exception in few cases where external chemical agents like sodium-do-decyl sulphate were used for stabilization of AgNPs[13]. Presence of metabolites, such as proteins [14-18] and chlorophyll in the extracts is known to act as capping agents for synthesized AgNPs. The preferred solvent for extracting reducing agents from the plant is water in most of the cases however, there are few reports regarding the use of organic solvents like methanol [19-22], ethanol [23, 24] and ethyl acetate [25]. Nanoparticles displays well defined shaped [26] when synthesized using extract, when compared to those obtained through utilization of bark, tissue and whole plant.

In present study leaf and fruit aqueous and ethanol extracts of *Passiflorafoetida* Linn. or commonly known as stinking passion flower plant were used for the synthesis of silver nanoparticle.

2. Materials and Methods

2.1 Collection of Sample:

The sample specimen (leaves and fruits) were collected from Koipady village, Kumbla post of Kasaragod district, Kerala during the month of December 2018 (**Figure 1 and 2**). The plant parts were sorted and allowed to shade dry for 4-5 days. These were then kept in hot air oven at 60^0C for 24-48 hours until the material dried completely. The obtained sample were then crushed and stored for further uses.

Figure1: Leaves of *Passiflorafoetida***Figure 2:** Fruits of *Passiflorafoetida*

2.2 Preparation of extracts:

The fine powders of leaves (28 grams)and fruits (48 grams)were extracted by soxhlation process. The extraction was carried out for about 1 to 1½hours with 150 ml of double distilled water and ethanol for aqueous extract and ethanol extract respectively, which was followed by distillation process. The extract obtained was dried in hot air oven at 50^{0}C for a week. Aqueous and ethanol extracts were prepared from the dried extract, followed by assessment of antibacterial activity.

2.3 Synthesis of silver nanoparticles (AgNP's): For biosynthesis of nanoparticles, 300 mL of 1mM $AgNO_3$ was taken in a conical flask. Nine grams of leaf and fruit extract powders were added into their respective conical flasks, followed by centrifugation at 2000 rpm for 30 minutes. The supernatants were collected and kept in boiling water bath at 40^0 C. Colour change of the solution was obtained within 1 hour (**Figure 3**) [27]. The extracts were stored at 4^{0}C for further usage.

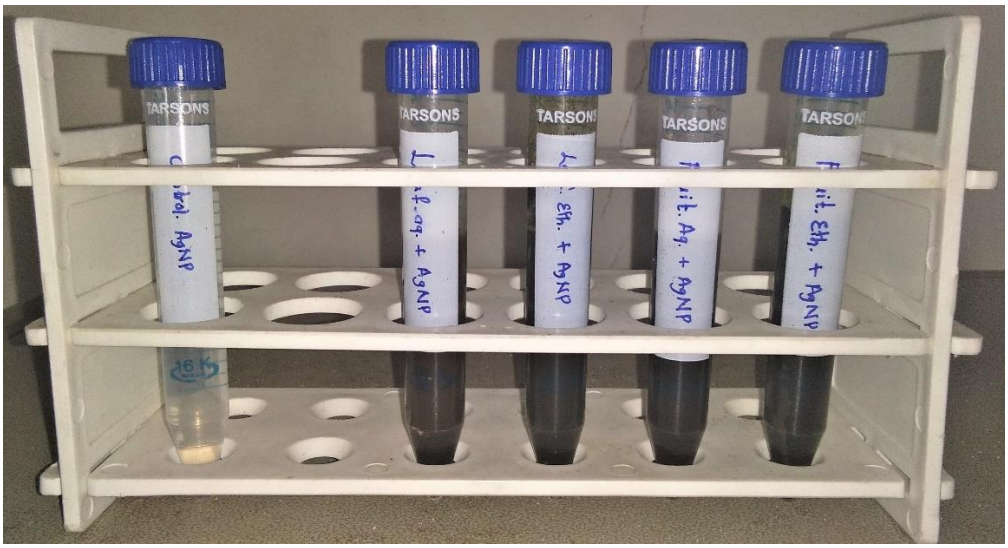

Figure 3: Synthesis of Silver nanoparticles

3. Characterization of silver nanoparticles (AgNP's):

3.1 UV-VIS spectra analysis: UV-VIS spectral analysis was done by using PC-Based Double beam UV-VIS spectrophotometer (Model 2202, make India). The reduction of Ag+ ions was monitored by measuring the UV-VIS spectrum of the sample [28] after 24 hrs.

3.2 ESD-SEM analysis of silver nanoparticles: Energy-dispersive X-ray Spectroscopy-Scanning Electron Microscope (ESEM EDAX XL-30, Make Philips, Netherland) analysis was done using SEM machine.

Thin film of the sample was prepared on a carbon coated copper grid by dropping an aliquot amount of the sample on the grid and then the film on the grid was dried dry by putting it under a mercury lamp for 5 minutes [29, 30].

3.3 Attenuated Total Reflection-Fourier Transform Infrared Spectroscopy (ATR-FTIR):

Fourier Transform Infrared Spectroscopy was used to identify the functional groups bound to the silver nanoparticles. The liquid sample was used and examined by Infrared (IR) Spectrum at the spectral range of 500-3500cm-1[30].

3.4 Determination of anti-bacterial activity of silvernano-particles (AgNP's):

For antibacterial studies *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa*and *Staphylococcusaureus*were used as test organisms. Microscopic examinations were done for the confirmation and were maintained as slants on nutrient agar.

3.5 Preparation of inoculums: A loop full of each culture was inoculated into100ml of respective nutrient broth and incubated at 37°C for 24 hours to obtain a bacterial culture.

3.6Antimicrobial activity test by agar well diffusion method: Petri dishes were plated with Muller Hinton Agar media and allowed to solidify. A lawn of each test organisms was then prepared by spreading on the surface of the media using sterile cotton buds.

Cork borer (4mm), procured from Durga laboratories, Mangalore was used to bore wells in media. The aqueous extract of different concentrations viz, 25µl, 50µl, 75µl and 100µl was dispensed into the respective wells using a micropipette (Eppendorf, PvtLtd.India). Similar method was followed for ethanol extract.

A negative control of double distilled water and ethanol was maintained followed by maintaining a positive control of Ampicillin and $AgNO_3$. The positive and negative samples along with extracts were allowed to diffuse for 30 minutes at room temperature. Then the plates were incubated at 37°C for 24 hours**.** Zone of inhibitions were measured and tabulated [31,32].

4. Results and Discussion

4.1 Synthesis of silver nano-particles: Synthesis of silver nano-particles was preliminary identified by the reduction silver ions during the exposure to fruit and leaf extracts of *P. foetida,* whichwas easily monitored by the color change in the reaction mixture from yellow to dark brown in case of fruit extract and green to dark brown in case of leaf extract.

After centrifugation the supernatants were collected and kept in boiling water bath at 40^0 C. Colour change of the solution was observed within 1 hour which indicates the formation of silver nanoparticle. In *Salvia spinosa*[34]synthesis of nanoparticle,reaction mixture was maintained at 27^0c held for 6 hours. Similarly*Berberis vulgaris* maintained high temperature and 1 hour incubation for silver nanoparticle synthesis[40].The present work with low temperature and incubation time indicates that reaction conditions are feasible allowing the silver ions in the reaction mixture to convert into elemental silver having the size of nanometer range.

4.2 Ultraviolet–visible (UV- VIS) spectra analysis of the leaf and fruit extracts:

Silver nanoparticles are known to exhibit UV-Visible absorption spectra with a peak in the range of 300-500 nm. In this study the formation of silver nano-particles was initially confirmed by color change followed by using UV-Visible spectroscopy. Surface Plasmon resonance band is depended on the particle size and refractive index of the solution. The flavenoids and terpenoids present in extract [35] acts like natural reducing agent which are responsible for reducing silver salts to silver nanoparticles. From several literatures, it was reported that the SPR peak of silver nanoparticles is

around 420 nm and in the present study it was centred at 430 nm According to ISO 2018, Z average size is a hydrodynamic parameter and predicts particle shape to be spherical or nearly spherical if we get a monomodal (i.e., only one peak), however, it has to be further confirmed with SEM analysis [36-40]. (NIR-UV Vis Spectroscopy).The absorption peaks were observed at 390 nm for leaf aqueous extract, 420nm, 450nm, 490nm for leaf ethanol extract, 320nm, 350nm, 430nm for fruit aqueous extract and 310nm, 390nm for fruit ethanol extract (**Figure 4 to Figure 7**)

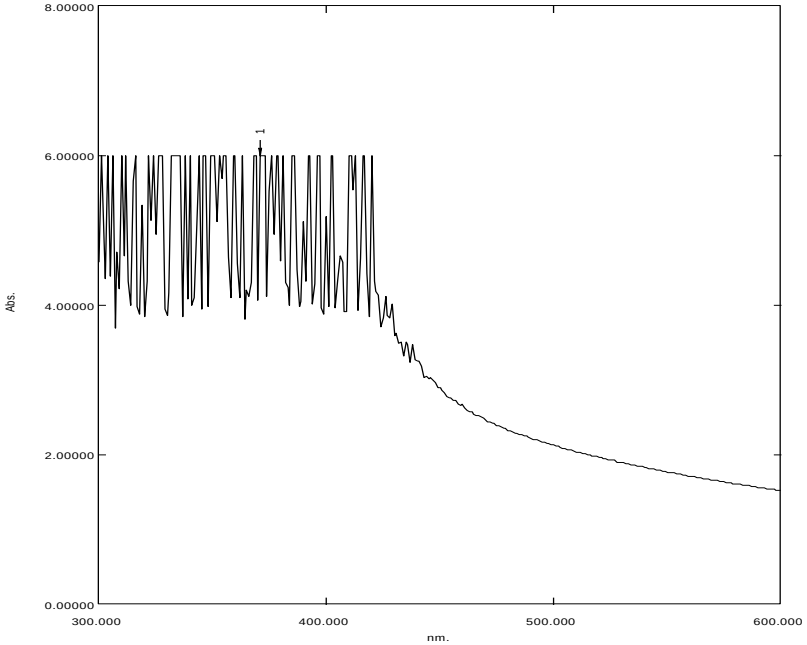

Figure 4: UV vis spectrum for leaf aqueous extract of *P. foetida*

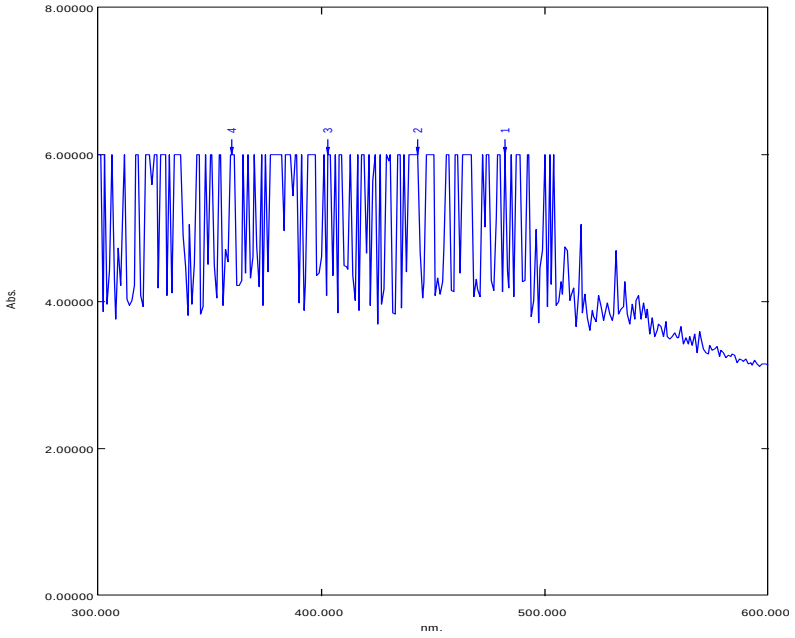

Figure 5: UV vis spectrum for leaf ethanol extract of *P. foetida*

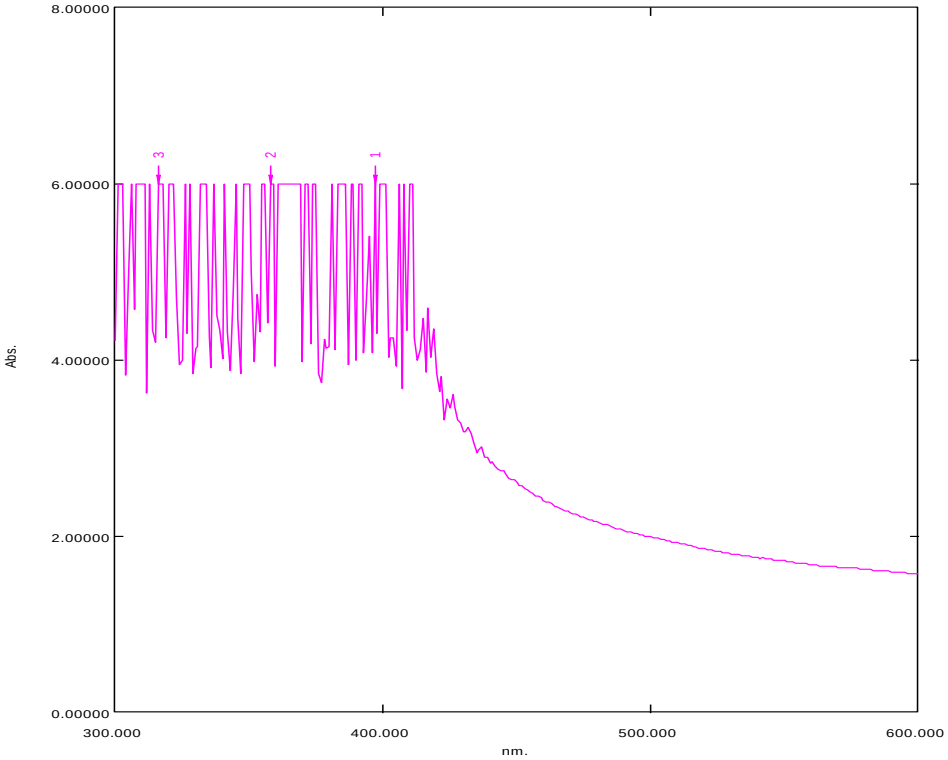

Figure 6: UV vis spectrum for fruit aqueous extract of *P. foetida*

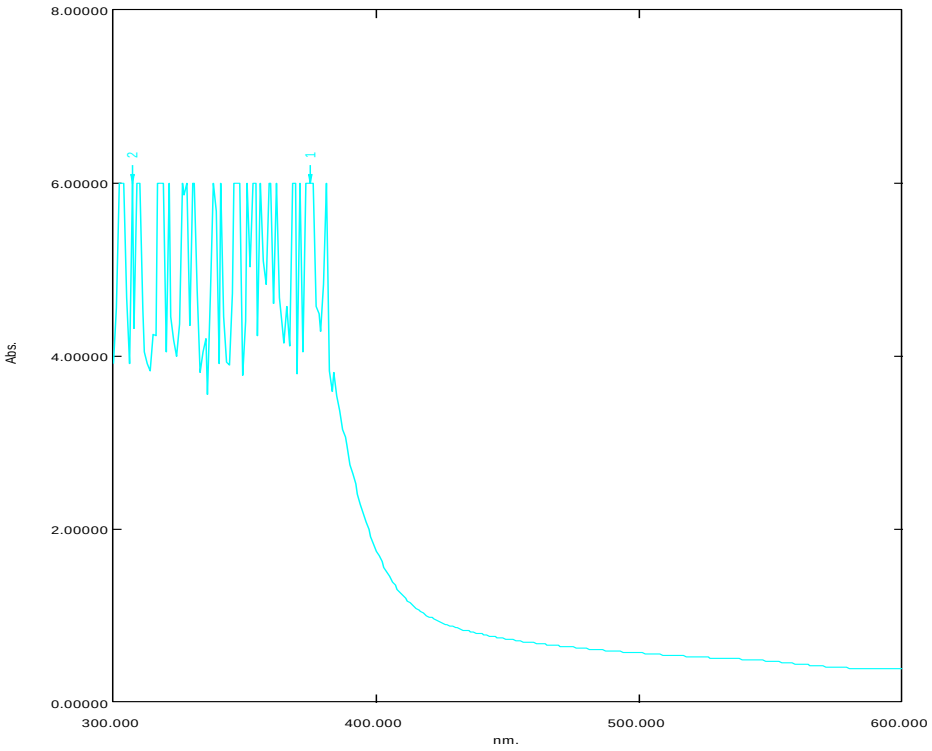

Figure 7: UV vis spectrum for fruit ethanol extract of *P. foetida*

4.3 EDS-SEM analysis of silver nanoparticles:

The scanning electron microscopic image has been employed to characterize the shape and size of synthesized silver nano-particles [16]. From the SEM image of synthesized silver nano-particles **(Figure 8-11**), it is evident that the shape of the synthesized silver nano-particles is cubic shaped [30, 31].

Energy-dispersive X-ray spectroscopy (EDS) is an analytical technique used for the chemical characterization of a sample [40-42]. From the EDS graph of synthesised silver nano-particle (**Figure 8-11**) it is evident that the major elemental constituents of leaf aqueous extract nano-particles are potassium, calcium, chlorine, oxygen, iron, magnesium, sodium and phosphate. (**Figure 12-15**) [32]

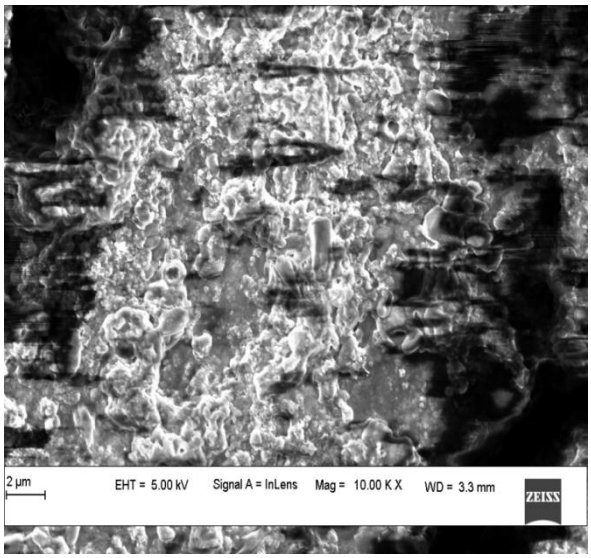

Figure 8: SEM image of nanoparticles synthesized from leaf aqueous extract of *P. foetida*

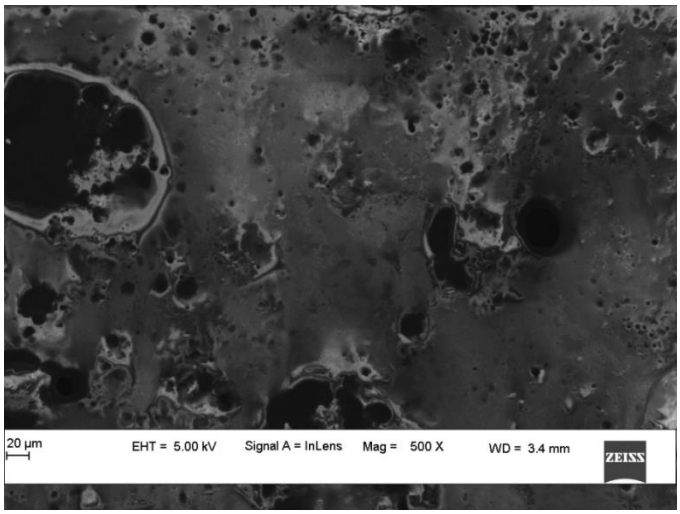

Figure 9: SI *oetida*

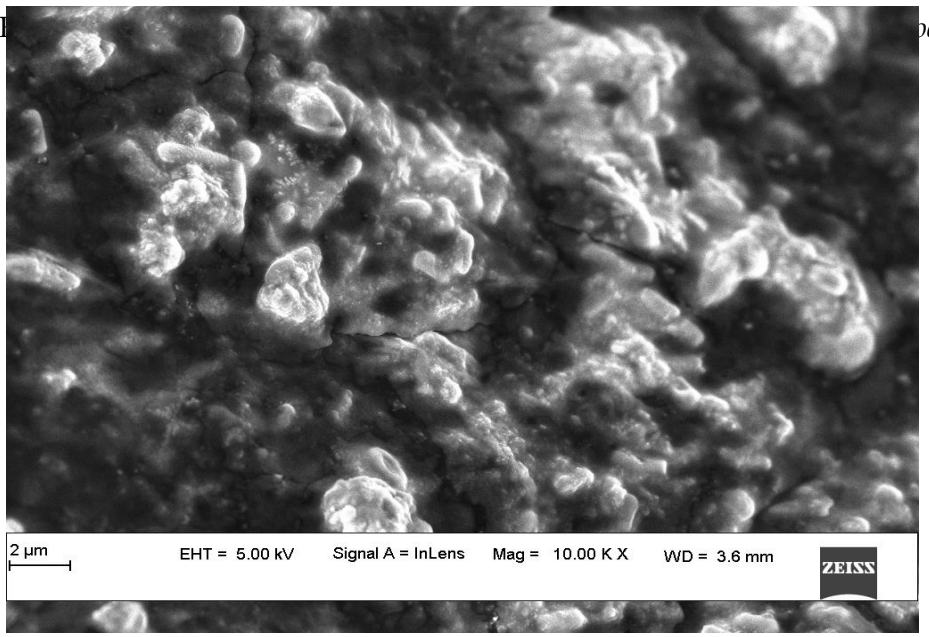

Figure 10: SEM image of nanoparticles synthesized from fruit aqueous extract of *P. foetida*

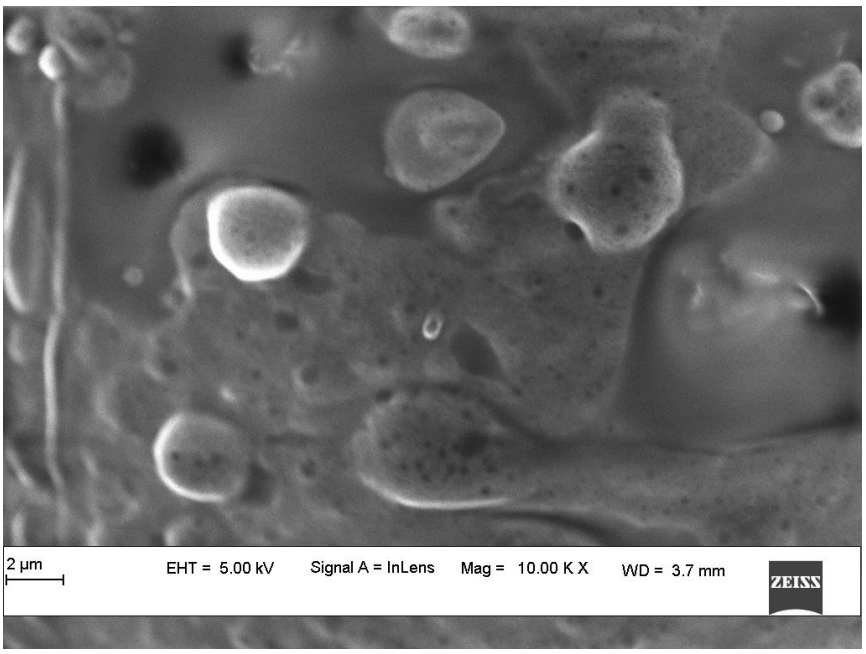

Figure 11: SEM image of nanoparticles synthesized from fruit ethanol extract of *P. foetida*

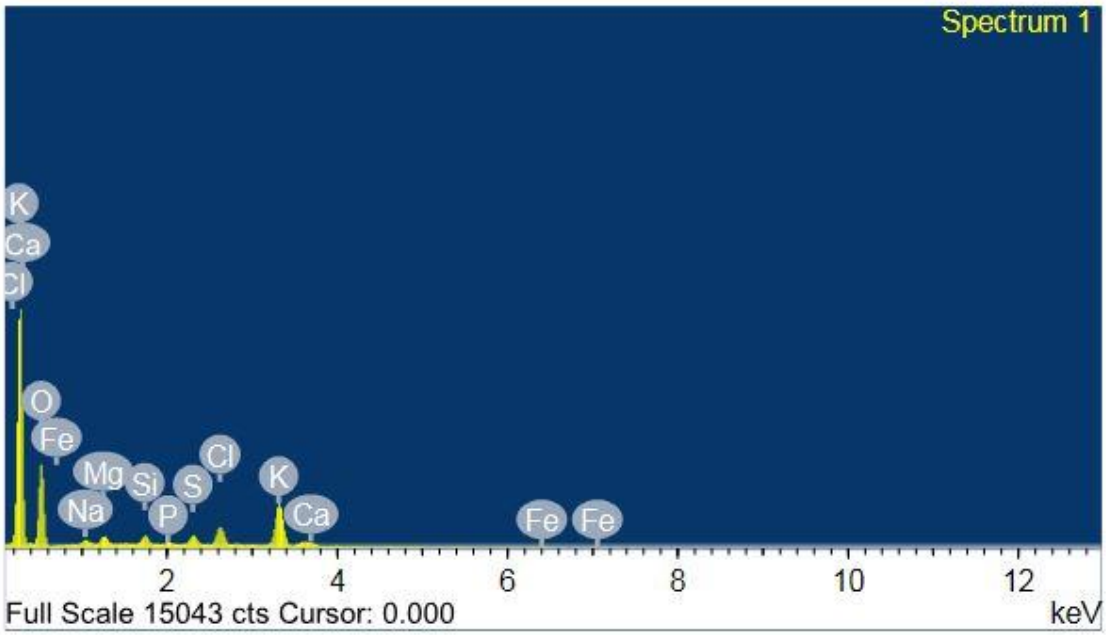

Figure 12: EDS graph of nanoparticles synthesized from leaf aqueous extract of *P foetida*

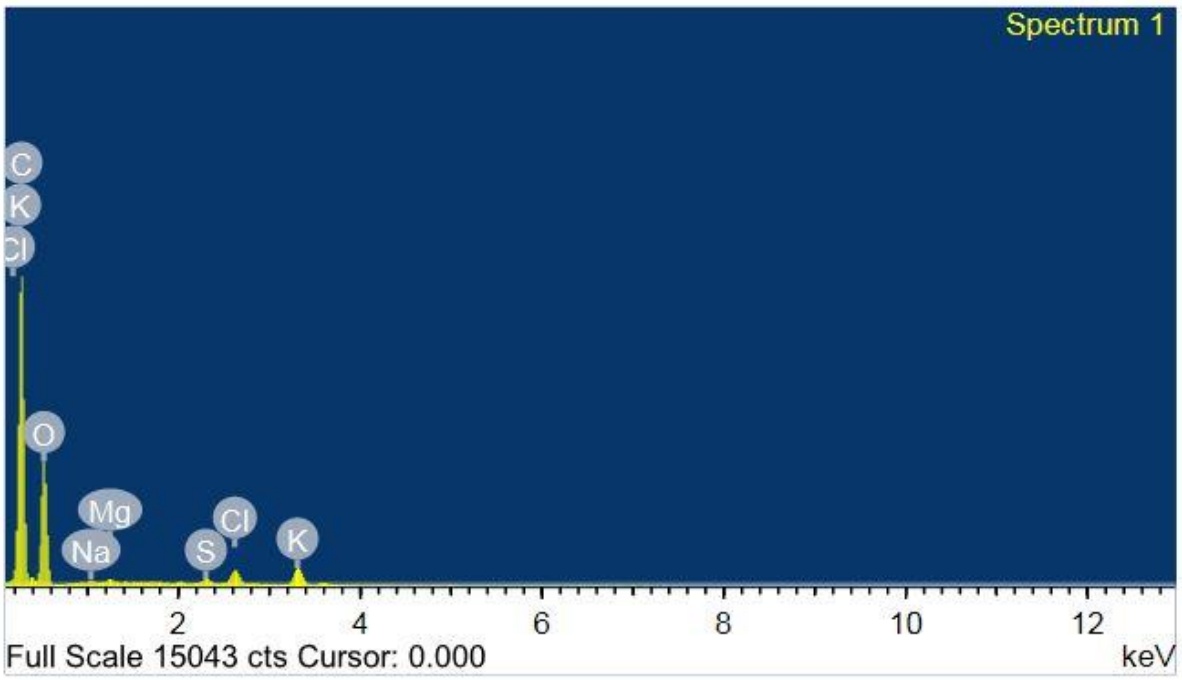

Figure 13:EDS graph of nanoparticles synthesized from leaf ethanol extract of *P. foetida*

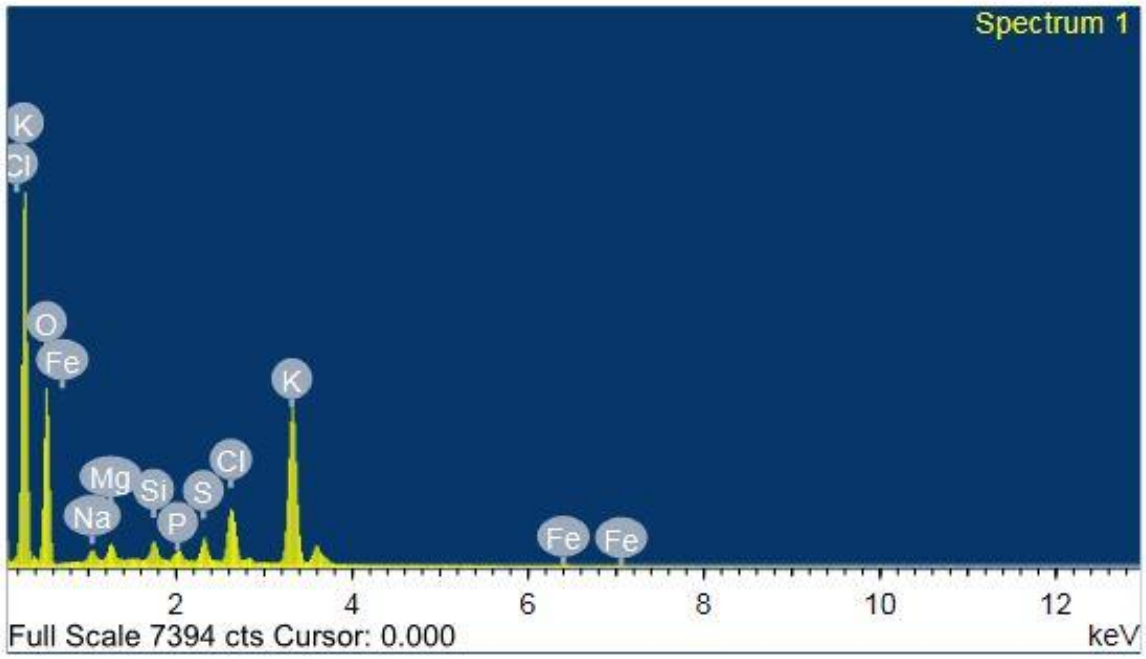

Figure 14:EDS graph of nanoparticles synthesized from fruit aqueous extract of *P. foetida*

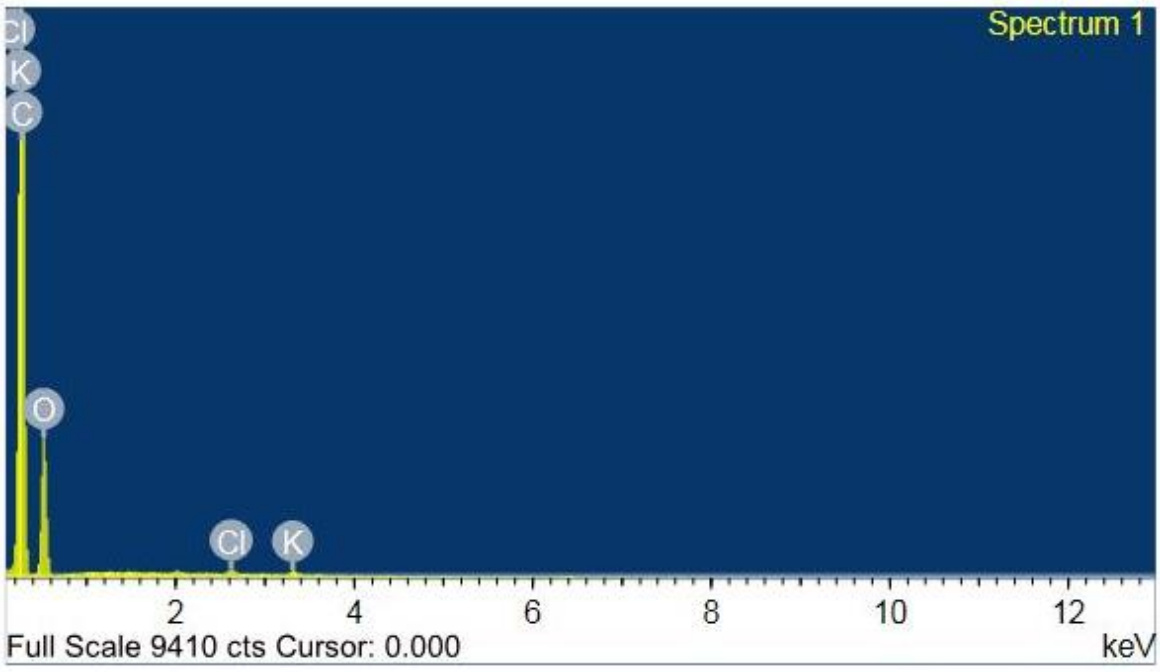

Figure 15:EDS graph of nanoparticles synthesized from fruit ethanol extract of *P. foetida*

4.4 Attenuated Total Reflection-Fourier Transform Infrared Spectroscopy (ATR-FTIR):

FTIR analysis was used for the characterization of the extract and the resulting nano-particles. Samples were

analyzed in ATR-FTIR to identify the possible bio-molecules responsible for the reduction of the silver ions by cell filtrate [40-43]. The representative spectra of nanoparticles obtained manifests absorption peaks using the spectral range between 500 to 3500cm^{-1}.

The absorption peaks for fruit ethanol extracts were observed at 3008.80cm-1, 2926.92cm-1, 2853.86cm-1, 2665.14cm-1, 1713.70cm-1, 1463.62cm-1, 1167.52cm-1, 1096.85cm-1, 970.14cm-1, 915.34cm-1 and 722.58cm-1.(**Figure 16**)

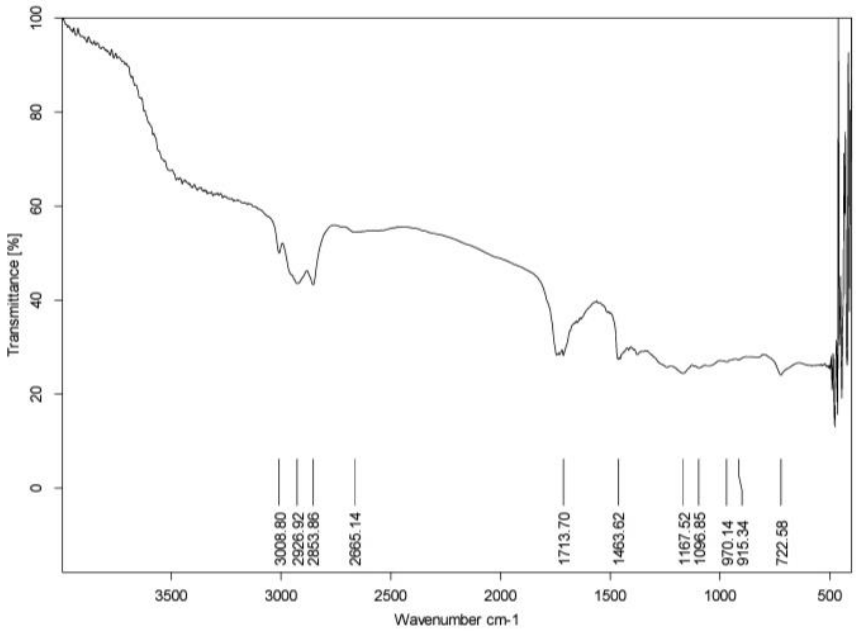

Figure 16: FT IR graph of fruit ethanol silver nanoparticle

The absorbance peaks for the leaf ethanol extracts were observed at 3625.72cm-1,3442.26cm-1, 2026.48cm-1, 1632.58cm-1, 1504.31cm-1,1504.31cm-1, 1402.83cm-1, 1384.54cm-1, 1120.15cm-1, 929.90cm-1, 712.23cm-1, 675.58cm-1, 651.83cm-1, 619cm-1, 567.69cm-1, 541.45cm-1, 534.41cm-1 and 514.45cm-1.(**Figure 17**)

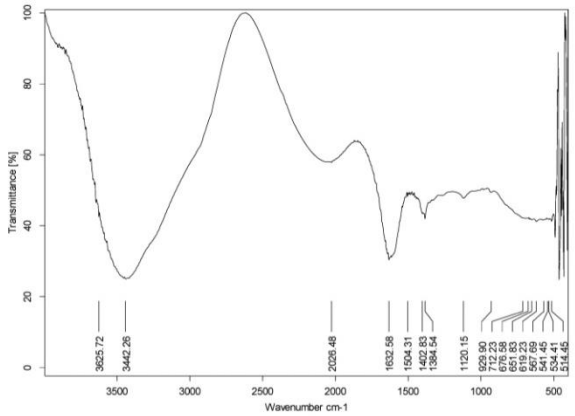

Figure 17: FT IR graph of leaf ethanol silver nanoparticle

The absorbance peak for the leaf aqueous extracts were observed at 3452.55cm-1, 2074.19cm-1,1637.98cm-1, 1404.18cm-1, 1384.55cm-1, 1113.38cm-1,1017.96cm-1, 613.69cm-1, 589.46cm-1, 557.53cm-1, 539.87cm-1 and 520.07-1. (**Figure 18**)

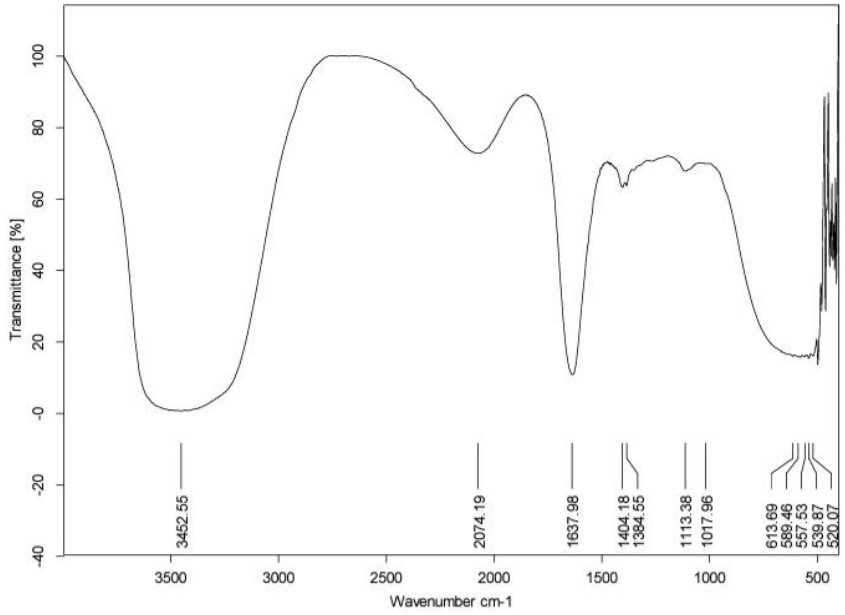

Figure 18: FT IR graph of leaf aqueous silver nanoparticle

The absorbance peak for the fruit aqueous extracts were observed at 3415.35cm-1, 2027.29cm-1,1633.01cm-1, 1460.27cm-1, 1118.99cm-1, 630.55cm-1,598.86cm-1and 530.45cm-1. (**Figure 19**)

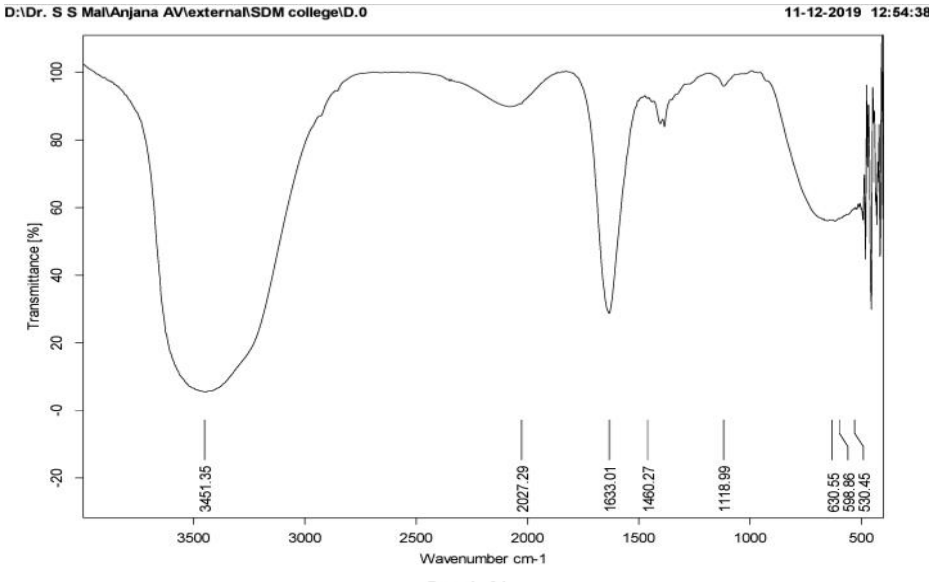

Figure 19: FT IR graph of fruit aqueous silver nanoparticle

These peaks can be assigned as absorption bands of C-H aromatic stretch of groups, C=H alkyl halide stretch of group, OH group of alcohol,C=C aromatic stretch of group, C-O alcohol stretch of group, C-Hbending of alkenes group, and C-Cl halide stretch of alkyl functional groups. (**Table 1**)

Reduction and bio-molecules which are involved in the silver ions can be identified by absorption peaks in the spectral range of 1000-4000 cm^{-1}. It can be observed through FTIR that, measurements to recognize the possible bio-molecules responsible for the capping and efficient stabilization of the metal nano-particles synthesized [20, 24, 32, 42].

Functional Group	Frequency (cm-1)	intensity
water OH Stretch	3700-3100	strong
alcohol OH stretch	3600-3200	strong
carboxylic acid OH stretch	3600-2500	strong
N-H stretch	3500-3350	strong
≡C–H stretch	~3300	strong
=C-H stretch	3100-3000	weak
-C-H stretch	2950-2840	weak
-C-H aldehydic	2900-2800	variable
C≡N stretch	~2250	strong
C≡C stretch	2260-2100	variable
C=O aldehyde	1740-1720	strong
C=O anhydride	1840-1800, 1780-1740	weak, strong
C=O ester	1750-1720	strong
C=O ketone	1745-1715	strong
C=O amide	1700-1500	strong
C=C alkene	1680-1600	weak
C=C aromatic	1600-1400	weak
CH_2 bend	1480-1440	medium
CH_3 bend	1465-1440, 1390-1365	medium
C-O-C stretch	1250-1050 several	strong
C-OH stretch	1200-1020	strong
NO_2 stretch	1600-1500 and 1400-1300	strong
C-F	1400-1000	strong
C-Cl	800-600	strong
C-Br	750-500	strong
C-I	~500	strong

Table 1: Representing absorbance for the respective groups

4.5 Determination of anti-bacterial activity of silver nano-particles:

The bacterial strains used in the present work showed varied levels of sensitivity towards different concentrations of aqueous and ethanolic silver nanoparticle extracts. Two positive controls were used including ampicillin and ethanol along with sole negative control, double distilled water. The tests were performed in triplicates and the

average zone of inhibition was recorded [21, 22, 26, 28, 29, 34, 35, 39, 41]. Tables 2-4 shows both positive and negative controls used. Tables 5-8 show the anti-bacterial activity of different silver nanoparticles.

Test Microorganism	Zone of inhibition (mm) for different concentration of Ampicillin			
	25µl	**50µl**	**75µl**	**100µl**
Bacillus subtilis	27.0	29.0	29.0	31.0
Pseudomonas aeruginosa	24.0	26.0	26.0	28.0
Escherichia coli	26.0	28.0	28.0	31.0
Staphylococcus aureus	17.0	18.0	18.0	19.0

Table 2: Anti-bacterial Activity of Ampicillin at Various Concentrations (Positive control)

Test Microorganism	Zone of inhibition (mm) for different concentration of silver nitrate			
	25µl	**50µl**	**75µl**	**100µl**
Bacillus subtilis	10.0	12.0	18.0	20.0
Pseudomonas aeruginosa	13.0	16.0	20.0	21.0
Escherichia coli	15.0	18.0	22.0	22.0
Staphylococcus aureus	16.0	19.0	20.0	23.0

Table 3: Anti-bacterial Activity of Ethanol at Various Concentrations (Positive control)

Test Microorganism	Zone of inhibition (mm) for different concentration of silver nitrate			
	25µl	**50µl**	**75µl**	**100µl**
Bacillus subtilis	16.0	19.0	21.0	22.0
Pseudomonas aeruginosa	17.0	20.0	22.0	23.0
Escherichia coli	17.0	18.0	22.0	25.0
Staphylococcus aureus	17.0	19.0	20.0	23.0

Table 4: Anti-bacterial Activity of Silver Nitrate at various Concentrations

Test Microorganism	Zone of inhibition (mm) for different concentration of Leaf Aqueous AgNP's			
	25µl	**50µl**	**75µl**	**100µl**
Bacillus subtilis	18.3	19.0	20.3	22.0
Pseudomonas aeruginosa	16.3	18.2	22.6	23.3
Escherichia coli	13.6	14.3	15.3	22.3
Staphylococcus aureus	14.0	14.8	18.6	20.3

Table 5: Anti-bacterial activity of leaf aqueous nano particle at various concentrations

Test Microorganism	Zone of inhibition (mm) for different concentration of Leaf Ethanol AgNP's			
	25µl	50µl	75µl	100µl
Bacillus subtilis	13.6	18.3	19.0	21.0
Pseudomonas aeruginosa	15.6	16.6	17.3	21.1
Escherichia coli	13.0	13.0	13.5	15.0
Staphylococcus aureus	16.0	16.8	17.3	20.3

Table 6: Anti-bacterial activity of leaf ethanol nano particle at various concentrations

Test Microorganism	Zone of inhibition (mm) for different concentration of Fruit Aqueous AgNP's			
	25µl	50µl	75µl	100µl
Bacillus subtilis	17.3	21.0	23.0	23.0
Pseudomonas aeruginosa	17.3	19.3	23.3	22.3
Escherichia coli	14.3	14.9	18.0	18.3
Staphylococcus aureus	14.3	14.3	15.6	17.6

Table 7: Anti-bacterial activity of fruit aqueous nano particle at various concentrations

Test Microorganism	Zone of inhibition (mm) for different concentration of Fruit Ethanol AgNP's			
	25µl	50µl	75µl	100µl
Bacillus subtilis	22.3	25.6	21.0	25.6
Pseudomonas aeruginosa	17.3	23.3	18.6	20.3
Escherichia coli	14.6	19.6	13.0	20.6
Staphylococcus aureus	16.3	23.3	22.6	19.6

Table 8: Anti-bacterial activity of fruit ethanol nano particle at various concentrations

Among different plant part extracts aqueous extract showed good response. The antibacterial activity of plant leaf and fruits in aqueous and ethanolic silver nanoparticles varied at different concentrations (**Tables 5 - 8**).The mechanism of bacterial activity of Ag NPs is most likely due to the attachment of AgNPs to the cell wall and generation of free radicals. Moreover, the presence of Ag NP in the cell membrane disturbs membrane permeabilityand causes intracellular ATP leakage, reported in earlier studies [41]. Silver ions released from AgNPs acting as reservoir causes antibacterial activity of AgNPs[42].The positively charged ions such as Ag+ shows tendency to attack phosphorus and sulfur present in biomolecule such as DNA and RNA, resulting in the loss of functionality of biomolecules[43].

In the present work, the antimicrobial effects were determined by evaluating its inhibitory effects against some microorganisms like *Bacillussubtilis, Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa,* against green synthesized silver nanoparticles. *P foetida* leaf and fruit extract exerted a significant antibacterial activity compared with positive control and bacterial strains which represented that the extract has potential anti-bacterial activity. Each varying concentration of the extract had varying inhibitory zone.

Conclusion

The study explores the possibilities of using green synthesized AgNPs as drug delivery systems to overcome antimicrobial resistance. As Bio AgNPs they have shown bactericidal activity against both gram positive and gram-negativepopulation.Theseeco-friendly green silver nanoparticles could be used as synergistic drug with convectional antibiotics to minimise antimicrobial resistance. Though *Passiflorafoetida* has already reported with biological property but green synthesis of fruit ethanol extract has yielded profound antimicrobial property. The study uncovers stability of nanoparticles but still requiresto expedite role as anticancer drug.

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