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Microwave-Assisted Green Synthesis of Silver Nano-particles using *Pithecellobium dulce* (pulichinthakaya): Characterization and Detection of Hg^{+2} and Fe^{+3} metal Ions

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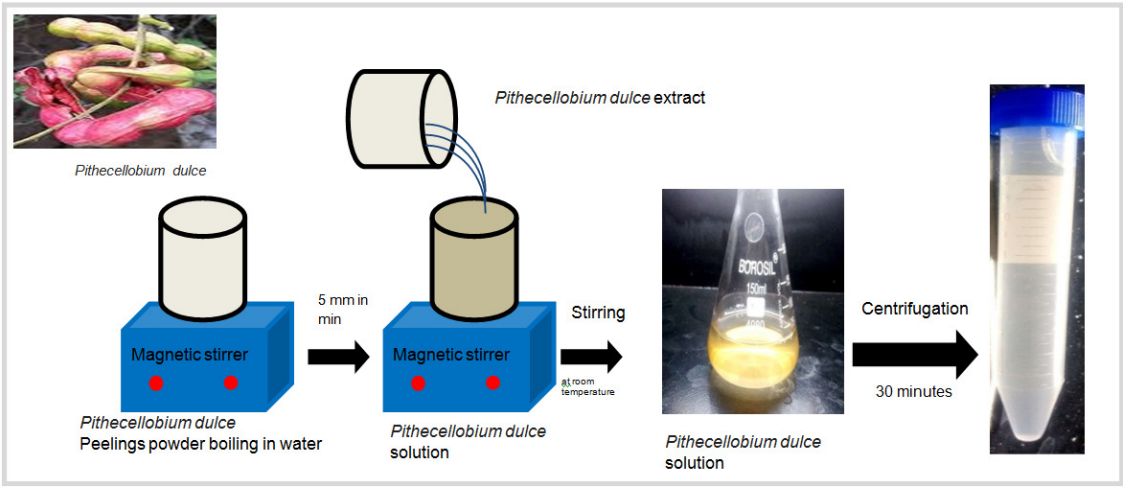
ABSTRACT: In conventional physical and chemical methods of synthesizing silver nano-particles the chemical stabilizers are used, which are toxic and causes environmental pollution. The need to develop rapid, non-dangerous, cost effective and eco-friendly methods for the synthesis of silver nano-particles using plants/plant parts in the green synthesis method, which is not required any additional stabilizing/capping agents. We have synthesized Silver nano-particles using the *Pithecellobium dulce* (pulichinthakaya) fruit peelings extract. The bands in FTIR spectroscopy at 3271.14, 2111.91 and 1637.82 cm^{-1}, are corresponding to N–H, –C=C and –CN and functional groups of the extract, which are responsible for the capping and stabilization of synthesized silver nano-particles. The morphology, size and structural properties of synthesized silver nano-particles were determined from the X–Ray Diffraction (XRD) pattern. The TEM analysis indicates that nano-particles are spherical in shape and mono-dispersed structure with an average size of 22.5 nm. Only Hg^{+2} and Fe^{+3} metal ions are detected by the synthesized AgNps In metal ions detection. The antibacterial activity of synthesized silver nano-particles was also investigated using six gram positive and gram negative bacteria. The *Pithecellobium dulce* (pulichinthakaya) fruit peelings extract is selected to synthesize the silver Nano-particles, because of its known medicinal properties.

Keywords: Microwave-Assisted Green Synthesis, Silver Nano-particles, *Pithecellobium dulce,* Hg^{+2} and Fe^{+3} metal Ions, FTIR spectroscopy,



Graphical abstract

I. INTRODUCTION

Nano-technology has become one of the most promising technologies with potentially effective application in all areas of science and potential effect on life [1]. Due to extremely small size and large surface to volume ratio the nano-particles are having great interest and importance [2-4]. Physic-chemical properties differ significantly from larger matter, due to extremely small size-of nano-particles.

Nano-particles of precious metals like, gold, silver, platinum and palladium are widely used in modern generation such as treatment of cancer, and synthesis of drugs and drug delivery systems [5], also in display boards, batteries, catalyst, sensors, food, agricultural and construction industries, cosmetic products [6, 7] and tooth paste, besides their applications in medical and

pharmaceutical products. In medicine and disease diagnostic systems, Silver nano-particles have been widely used [8, 9] Silver nano-particles are used in sensor technology, biological leveling and many other biomedical applications such as anti-bacterial, anti-cancer, and antifungal activity [10]. Synthesis of silver nano-particles can be synthesized using a number of routinely used chemical and physical methods, in which hazardous chemicals and some synthetic additive and capping/stabilizing agents are used [11, 12]. Silver nano-particles show potential application in various fields, such as biomedical, catalysis, optics & electronics, cancer therapy and synthesis of drugs and drug delivery systems [13-15], Soaps, detergents, paints, display board, batteries, catalyst, sensors, food, agricultural and construction industries cosmetic products [16, 17] and tooth paste. Silver nano-particles are formed by green synthesis are more effective in anti-bacterial activity and it known to be antifungal, anti-inflammatory and antiviral activity [18]. Silver nano-particles used in biological leveling and they have antibacterial activity against pathogenic bacteria. Silver nano-particles are also used in burns and open wounds treatment and silver has also long been recognized as having an inhibitory effect towards many bacterial strains [19].

In the present work, we have synthesized silver nano-particles by using renewable green synthetic method. Since the chemical stabilizers are used in conventional physical, chemical methods for the synthesize of silver nano-particles, are toxic and pollute the environment. In this green synthesis of Silver nano-particles, the DD H_2O as a solvent and the *Pithecellobium dulce* fruits peelings extract, ecofriendly renewable and non-toxic material [20, 21] is used as reducing as well as stabilizing agent (without adding any external chemicals). To avoid explosive and harsh reaction conditions and for the rapid production of nano-particles [22] microwave irradiation method is selected [23]. Optical and physicochemical properties of synthesized silver nano-particles are thoroughly characterized using various analytical techniques such as UV-Vis spectroscopy (SPR), Fourier Transform Infrared spectroscopy (FTIR), X-Ray Diffraction spectroscopy (XRD), Scanning Electron Microscope and Energy Dispersive spectroscopy (SEM-EDX), high resolution of synthesis Transmission Electron Microscopy (HR-TEM) and Zeta analyser. The antibacterial activity of silver nano-particles was evaluated using six gram positive and gram negative pathogens (a) *Staphylococcus aureous* (Gram-positive), (b) *Escherichia coli* (Gram-negative), (c) *Klebsiella pneumoniae*, (Gram-negative), (d) *Bacillus* subtitles (Gram-positive), (e) *Enterococcus faecalis* (Gram-positive) and (f) *Proteus mirabilis*. (Gram-nagative) The antibacterial activity of silver is nanoparticles depend on the size, shape, volume of the synthesized AgNps and stabilizing agent.

II. MATERIALS AND METHODS

Fresh and healthy of *Pithecellobium dulce* fruits were collected from local market area near the Osmania University, Hyderabad, TS, India. A.R Grade (99.9%) of Silver Nitrate ($AgNO_3$) was purchased from Sigma Aldrich (merk), Mumbai. The DD water is used as solvent for further sample analysis in this study.

A. Methods

Preparation of *Pithecellobium dulce* fruits peelings extract. Nearly 5 gms of *Pithecellobium dulce* fruits peelings were collected and cleaned with tap water followed by DD water. The washed fruits peelings are thoroughly dried under sun light to remove water from the fruits peelings. The dried peelings are grinded to get the fine powder. Nearly 5 gms of fine powder is added to 500 ml of DD water and heated with continuous stirring at 160℃ for 60 min by using magnetic stirrer. Then the solution was filtered with what man no. 1 filter paper and centrifuged at 6000 rpm. The filtrate is stored at 15℃ for further usage.

Synthesis of AgNPs. We have prepared different solutions of AgNPs by mixing 5ml $AgNO_3$ solutions (0.1mM, 0.2mM, 0.3mM, 0.4mM, 0.5mM) each mixed with 50 ml of *Pithecellobium dulce* fruits peelings extract and 15ml peelings extract solutions (l%, 2%, 3%, 4%, 5%) each mixed with 50ml of $AgNO_3$ solution. The reaction mixture also prepared by mixing 5mM of $AgNO_3$, 5% peelings extract and metal salt solution (1M NaCl) to study colloidal stability of silver nano-particles. The colloidal stability of nano-particles has also been studied at different time durations of 5min, 10min, 30min, 60min; 2hrs, 5hrs, 10hrs, 18hrs, 24hrs.

Characterization. The characterization of peelings extract capped AgNPs solution was carried out in a Dual Beam UV– visible spectrophotometer (Shimadzu-3600 Japan). Fourier Transforms Infrared (FTIR) spectra for peelings extract alone and peelings extract capped AgNPs were recorded separately using an FT-IR spectrophotometer (Bruker Optics-TENSOR 27, Germany). The scan was performed in the wave number range of 400–4000 cm^{-1}. Powder X-ray Diffraction (XRD) measurements of peelings capped AgNPs were carried out in X'pert Pro-powder X-ray diffractometer (PAN analytical BV, Netherlands) operating at 40 kV and a current of 30 mA at a scan rate of 0.366 min^{-1}. The morphology and size distribution measurements of the peelings capped AgNPs were carried out by HR-TEM, {FEI Company, USA} TECHNAI G2 F30 S-TWIN Transmission Electron Microscope) operated at an accelerating voltage of 200 kV, by casting nanoparticle dispersion on carbon-coated copper grids and allowing for drying at 27℃ temperature.

Antibacterial activity test. The AgNps solution samples were taken and are made into aliquots of 10 µl, 25µl, 50µl, 75µl, 100µl by dissolving them in DMSO for MIC assay in case of powdered samples and use liquid samples directly.

Antibacterial assay: The antibacterial assay was carried out by performing pour plate method, in which 1ml bacterial active cultures per plate were mixed into agar media before solidifying temperature and poured into plates. Wells were made by using sterile well borer and samples loaded with 100µl of each respectively, in Gram positive and Gram negative plates. Plates were incubated at 37℃ degrees for 48 hours. Six Gram positive and Gram negative bacteria (a) *Staphylococcus aureous* (Gram-positive), (b) *Escherichia coli* (Gram-negative), (c) *Klebsiella pneumoniae*, (Gram-negative) (d) *Bacillus subtitles* (Gram-positive), (e) *Enterococcus faecalis* (Gram-positive) and (f) *Proteus mirabilis* (Gram-negative) are used in antibacterial study.

Interpretation of results. The bacterial plates were observed after 48 hours and results were noted.

III. RESULTS AND DISCUSSION

Microwave assisted green method, eco-friendly method, is used for the synthesis of AgNPs by using $AgNO_3$ - solution and *Pithecellobium dulce* peelings extract [24], which can act as reducing/stabilizing agent (Without adding of any external reagents/chemicals). Different samples of AgNPs prepared by mixing of $AgNO_3$ solutions (from 0.1mM to 0.5mM) each with 5% peelings extract and peelings extract solutions (1% to 5%) mixed each with 5mM solution of $AgNO_3$. The reaction mixture containing 5mM of $AgNO_3$ [25], 5% peelings extract and metal salt solution (1M NaCl) [26, 27] is prepared to study the colloidal stability of silver nano-particles. The stability of silver nano-particles are also studied at different time durations of 5 min, 10 min, 30 min, 60 min, 2 hrs, 5 hrs, 10 hrs, 18 hrs and 24 hrs. The microwave, which is environmentally safe, is used for the rapid production of nano-particles within few minutes by using heat energy [28].

A. UV-visible spectroscopic analysis

The microwave assisted green synthesis of silver nano-particles were carried out using *Pithecellobium dulce* peelings extract. The absorption peak in UV–Vis absorption spectrum for synthesized Silver nano-particles was observed at 421 nm. The prominent SPR peaks between 410 and 430 nm are ascribed to synthesis of AgNPs. And also indicates that nano-particles are spherical in shape with an average size ranges from 15 to 25 nm.

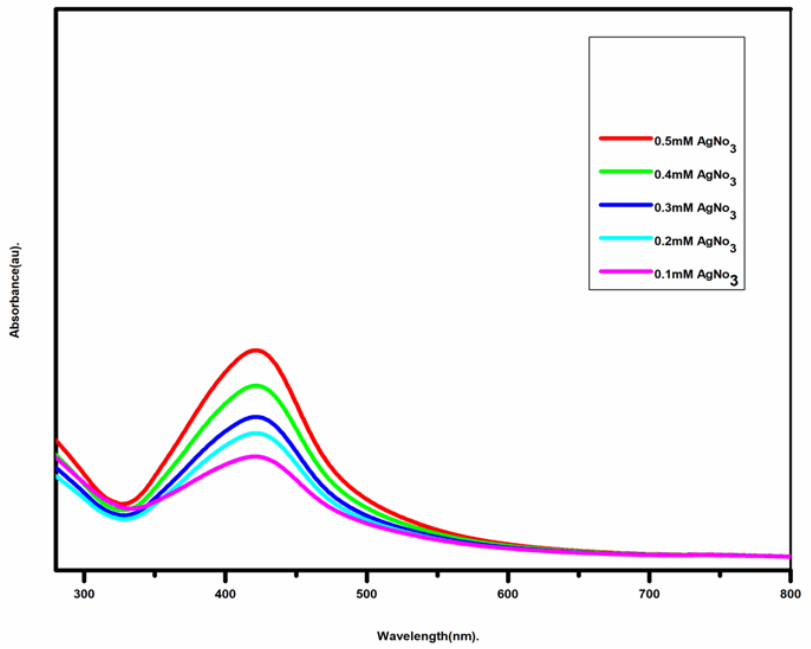


Fig. 1. (a) Different concentration of $AgNO_3$.

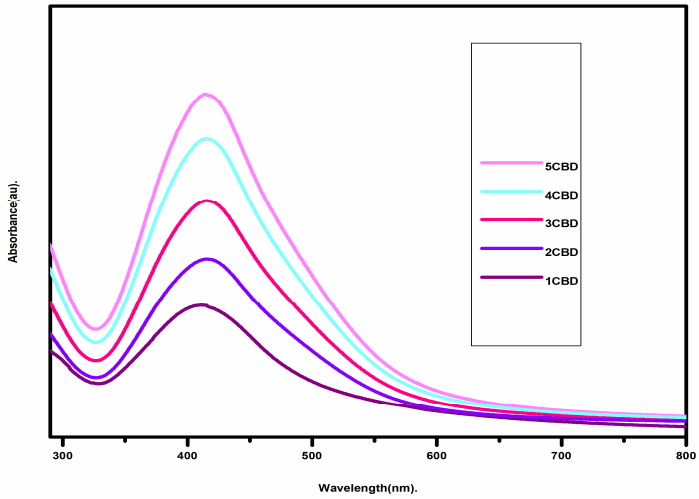


Fig. 1. (b) Different % of *Pithecellobium dulce* extract.

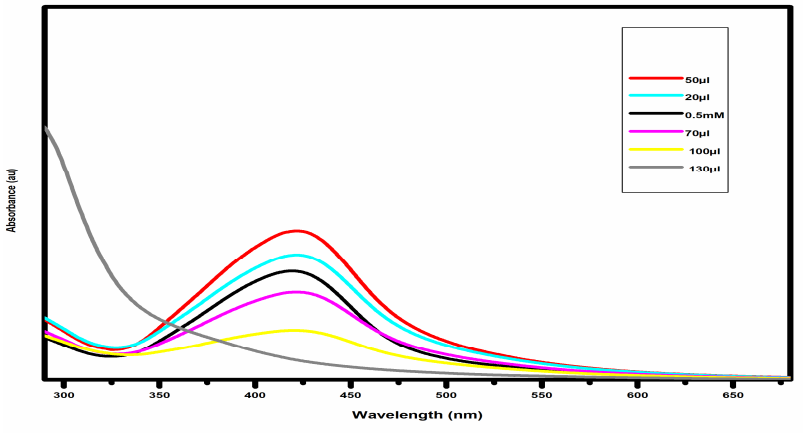


Fig. 1. (c) *Colloidal* stability of AgNps with NaCl salt solution.

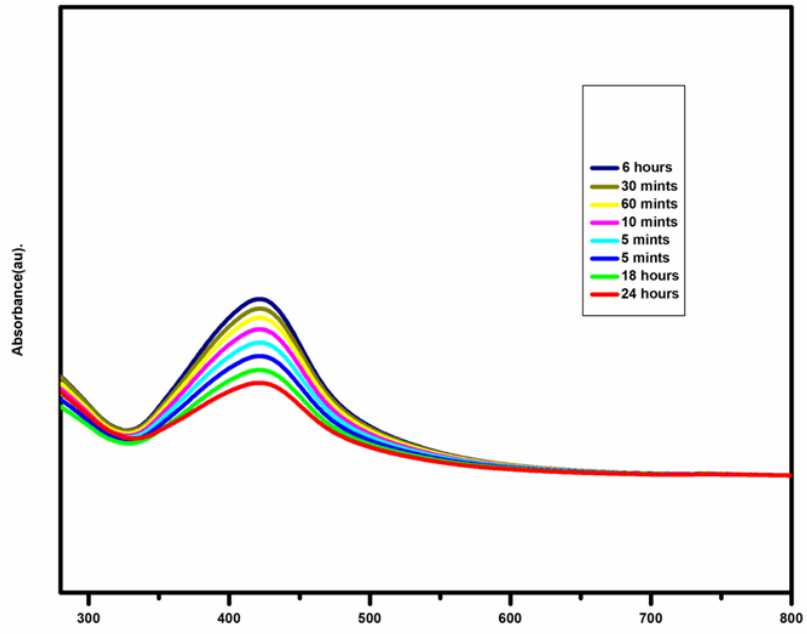


Fig. 1. (d) Different time durations.

Fig. 1(a) Shows the SPR peaks for the synthesized silver nano-particles formed from solutions prepared using $AgNO_3$ solution from 0.1mM to 0.5 mM, each mixed with 5% peelings extract. The increase in SPR peaks indicates the increase the number of AgNps, which are increased with the increase in the concentration of $AgNO_3$ [29]. Because the increase in number of silver ions with the increase concentration of $AgNO_3$ are reduced to AgNps by biomolecules of Extract.

Fig. 1(b) The increase in SPR peaks indicates the increase in number of AgNPs formation with the increase in extract concentration from 1% to 5% [30, 31]. Because with the increase in concentration of extract, the number of poly-phenols increased. Polyphenols and functional groups are important for the reduction of silver ions of $AgNO_3$ and formation of AgNPs. The formation of silver nano-particles is depends on poly-phenols and plant extract [32], containing to –N–H, –C=C, and –C–N functional groups, which are responsible for reducing and capping/ stabilization of synthesized silver nano-particles [21].

Fig. 1 (c). SPR peaks for the formation of AgNPs from $AgNO_3$ solution and peelings extract with addition of different volumes of NaCl solution [33]. The colloidal stability was studied by the addition of ionic charge containing salts solution such as sodium chloride (NaCl) to the AgNPs solution. Colloidal stability was studied in terms of binding of ions of sodium chloride (0.1 Mm NaCl) on AgNps surface, which are having highly active charge. With the increase in addition of salt solution from 20 microliters to 50 microliters to AgNPs solution the SPR peaks are increased [34]. Once the sodium chloride volume reaches to 70µl, SPR peaks starts decreases gradually and at 130µl the SPR peaks totally disappeared. SPR peaks increased due increase in number of nano-particles, which are formed due to reduction of unreacted silver ions of $AgNO_3$ by the charge of NaCl ions. But at 70 microliters NaCl solution, the ions of NaCl are binding on surface of the silver ions of $AgNO_3$, which leads to decrease in the formation of nano-particles. At 130 µl of $AgNO_3$ of NaCl solution, all silver ions of $AgNO_3$ solution are completely binded with the ions of NaCl, resulting in disappearance of SPR peak.

Fig. 1 (d) In the SPR peaks are belongs to a different time durations. As the time duration increases the SPR peaks also increased up to 5 hours [35]. SPR Peaks are increased up to 5 hrs, because colloidal dispersion is maximum up to 5 hours. After 5 hours SPR peaks are gradually decreasing due to formation of suspension in the solution and the peaks leads to zero stage after 24 hours.

B. Metal ion detection

10 microliters of different metal Fe^{+2}, Hg^{+2}, Ba^{+2}, Na^{+}, Zn^{+2}, Mg^{+2}, Cu^{+2}, Cd^{+2}, Sn^{+2}, Ni^{+2}, Pb^{+2}, Sr^{+2}, $Mn+^{2}$, K^{+}, Fe^{+3}, Ca^{+2}. Ion solutions, are mixed each with 2ml of AgNPs solution. For the detection of metal ion by AgNps. Only Mercurus (Hg^{+2}) and Ferric ($Fe^{+3)}$) metal ions are decolorizing the yellow color of AgNps solutions [36]. This may be due to oxidation of silver nano-particles to silver ions by mercurous and ferric ions and simultaneously Hg^{+2} ion reduced to Hg^{+1} and ferric ion reduced to Fe^{+2}. Hence, it is possible to detect the Hg^{+2} and Fe^{+3} present in waste water/polluted water by the addition of AgNPs solution [37, 38].

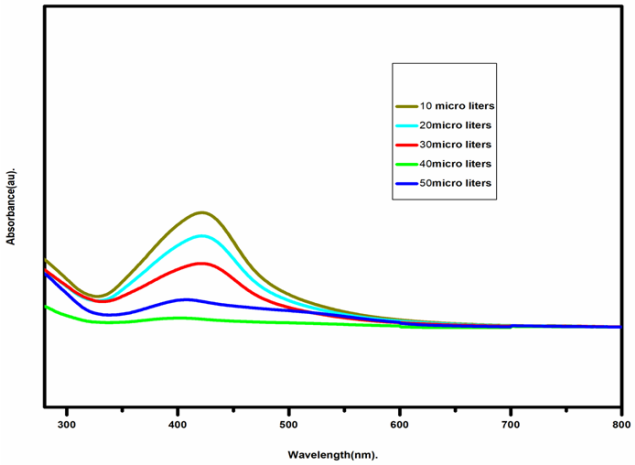


Fig. 2. (a) Metal ions detections Hg^{+2}

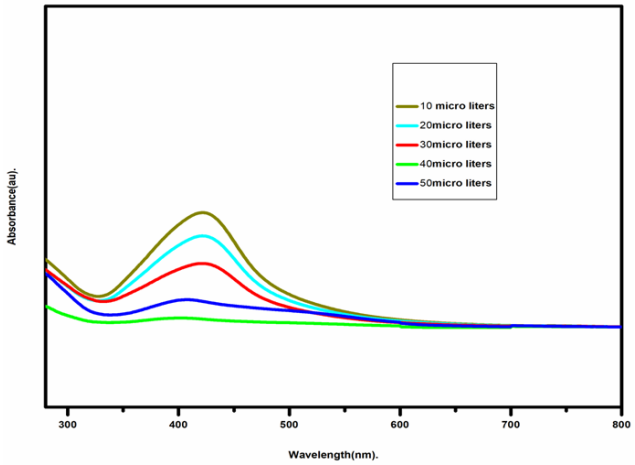


Fig. 2. (b) Metal ions detections Fe^{+3}.

C. FTIR

FTIR analysis was carried out to study the *Pithecellobium dulce* fruit peelings extract alone and reduction of silver ions of $AgNO_3$, by the biomolecules present in the *Pithecellobium dulce* fruit peelings extract and the stabilization of AgNPs [29]. The FTIR spectra of *Pithecellobium dulce* fruit peeling extract alone (Fig. 3 b) exhibited stretching vibrations at 3400, 2937, 1727, 1348 and 1050 cm^{-1}, while the *Pithecellobium dulce* fruit peelings extract capped/stabilized AgNPs (Fig. 3 a) shows the characteristic stretching frequencies at 3240, 2943, 1756, 1642, 1310, 1020 cm^{-1}. The broad peak at around 3240 cm^{-1} in Fig. 3 (a) corresponds to the -OH stretching vibrations of polyphenols. The peak at around 2943 cm^{-1} corresponds to -CH stretching, and strong peak at around 1756 cm^{-1} can be assigned to the carbonyl stretching. Further the peaks at around 1020 cm^{-1} can ascribe to the C–O stretching. FTIR spectra of *Pithecellobium dulce* fruit peelings extract capped/stabilized AgNPs shows some clear distinctions from that of FTIR spectra of *Pithecellobium dulce* fruit peelings extract alone. Most importantly, the intensity of -OH stretching vibration is reduced and the intensity of carbonyl stretching got increased, which suggest that the hydroxyl groups get oxidized to carbonyl groups. This may be due to the reduction of Ag^{+} ions by the OH groups of polyphenol of extract, which in turn undergo oxidation. Furthermore, clear shifts in the peak positions are also observed, which confirms the binding of these functional groups onto the AgNPs.

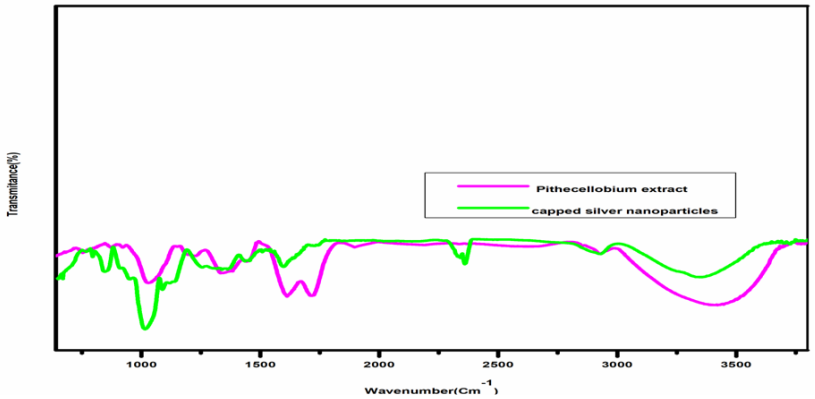


Fig. 3. FTIR spectra of (a) *pithecellobium* capped AgNPs and (b) *pithecellobium* alone.

D. XRD Analysis of AgNPs

Powdered x-ray diffraction studies of synthesized AgNPs using *Pithecellobium dulce* fruit peelings extract and peelings extract capped AgNPs were carried out by coating the colloidal samples on a glass slide. These slides are placed in hot air and reduced pressure to remove volatile products. The characteristic refractions at 2θ = 39.2°, 44.5°, 64.6° and 79.8° for 111, 200, 220,311 planes were identified the crystalinity of *Pithecellobium dulce* fruit peelings extract capped AgNPs (Fig. 4). These values agreed with reported fcc structure crystalline of silver nano-particles [27]. The additional peaks are also observed at 2θ = 33.5°, 48.2° and 56°. The X-ray diffraction studies indicate the presence of phytochemicals in the *Pithecellobium dulce* fruit peelings extract solution.

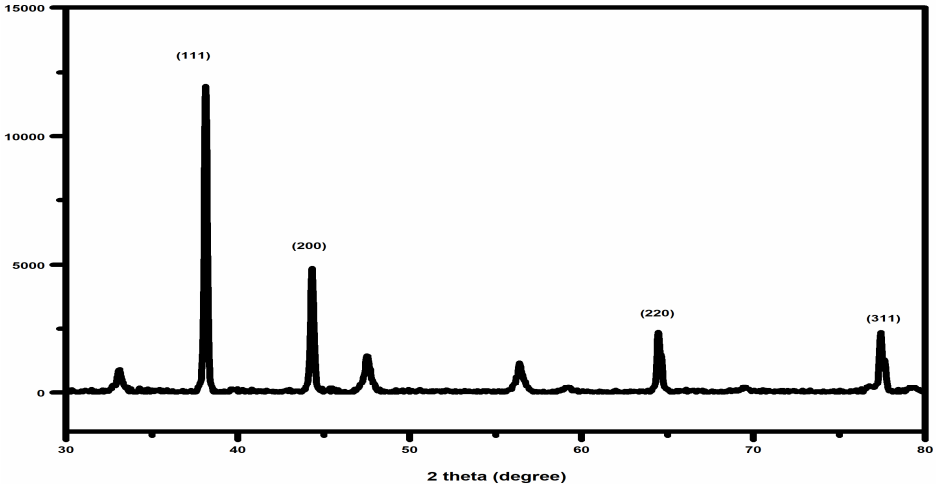
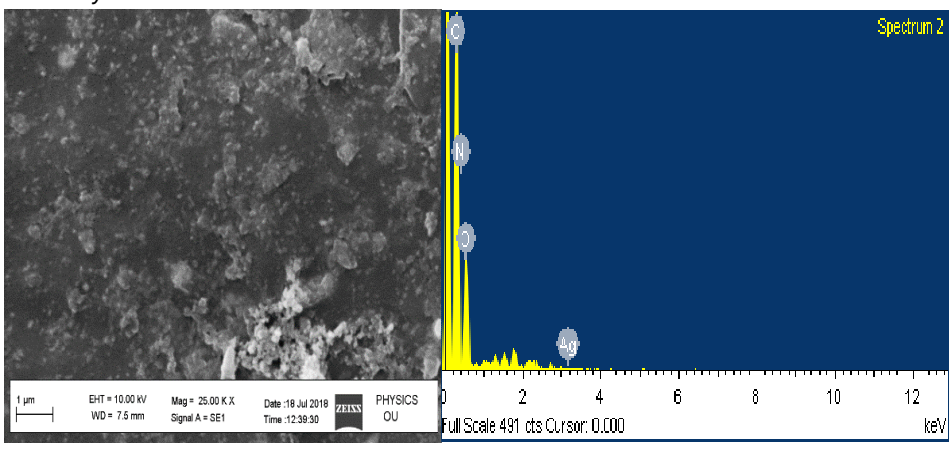


Fig. 4. Powder XRD pattern of *Pithecellobium* capped AgNPs.

E. SEM and EDX Analysis

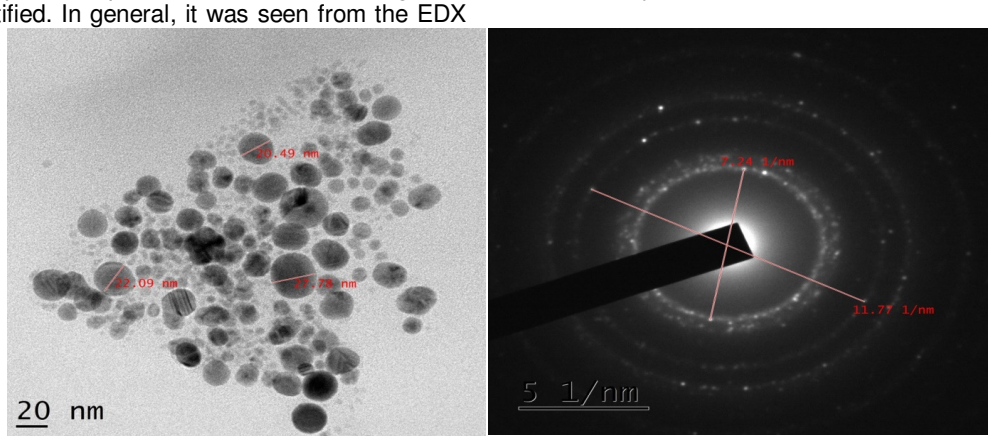


(a) (b)

Fig. 5. (a) SEM image of *pithecellobium* capped AgNPs (b) corresponding EDX spectrum.

The morphology of the AgNPs was studied from FE-SEM images. The FE-SEM images indicates that Ag nano-particle are spherical in shape. There were a few Ag nano-particles having oval shape as well. The FE SEM analysis also indicates that the maximum number of nano-particles are having an average diameter and size is less than 50 nm. It indicates that maximum nano-particles are uniform in nature [39] and these Nano-particles were formed by microwave assisted green method. The size distribution of 25-40 nm and an average diameter about 11.2 nm of silver nano-particles, indicates that AgNPs were bio-synthesized by using *Pithecellobium dulce* fruit peelings extract. According to EDX spectrometer analysis the presence of the elemental Ag signals indicates the conformation of Ag nano-particles presence. Furthermore, Ag was exactly identified. In general, it was seen from the EDX spectrometer that the impurity and presence of different elements [40]. In the form of nano-particles were relatively low. Moreover, the structures of the nano-particles were rich in Ag element (Fig. 5 a & b).

TEM and SAED Pattern of AgNPs. Transmission Electron Microscope (TEM) was used to determine the size, shape and distribution of synthesized AgNPs formed with *Pithecellobium dulce* fruit peelings extract [8]. The TEM analysis indicates that the size of AgNPs ranges from 10 to 50nm. TEM analysis also suggests that synthesized AgNPs are spherical in shape with an average size of the AgNPs was found to be 11 ± 2nm. The SAED pattern (Fig. 6 a & b) exhibited concentric rings with intermittent bright dots, it indicates that the Nano-particles are highly crystalline in nature, and their size is nearly 50 nm in size.



(a) (b)

Fig. 6. (a) TEM image of *pithecellobium* capped AgNPs and (b) corresponding SAED pattern.

Antibacterial activity synthesized AgNPs. The antibacterial activity of *Pithecellobium dulce* fruit peelings extract capped AgNPs carried out by using (a) *Staphylococcus aureous* (Gram-positive), whose zone of inhibitions are (1.0), (1.3), (1.3), (0.4) cm (b) *Escherichia coli* (Gram-negative), whose zone of inhibitions are (1.0), (1.0), (0.9), (1.2) cm, (c) *Klebsiella pneumoniae* (Gram-negative), whose zone of inhibitions are (0.3), (0.2), (0.2), (0.3), (d) *Bacillus subtitles* (Gram-positive) whose zone of inhibitions are (0.3), (0.5), (0.5), (0.2) cm. The zone of inhibition of AgNPs for the bacteria, indicates the positive antibacterial activity against *Staphylococcus aureous, Escherichia coli, Klebsiella pneumoniae*, *Bacillus subtitles* and negative antibacterial activity against *Enterococcus facalis*, *Protious mirabilis* (Fig. 7).

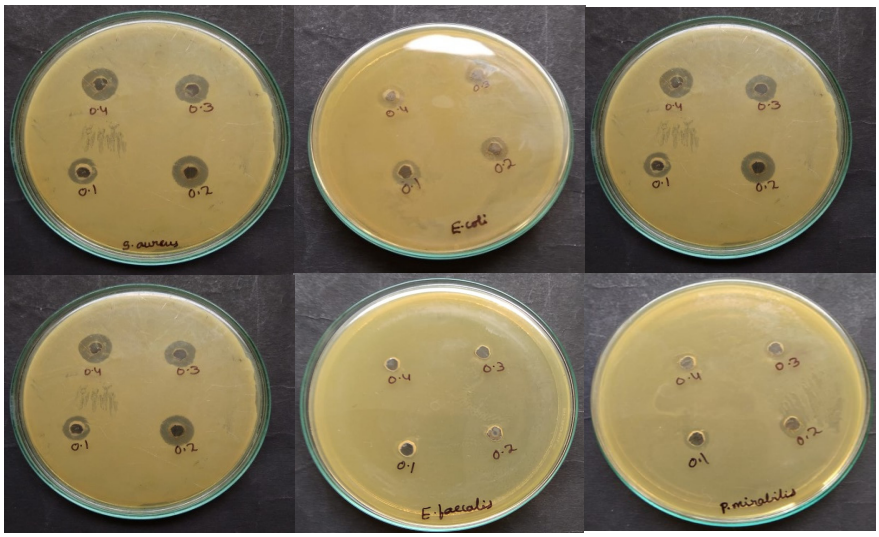


Fig. 7. Antibacterial activity of *Pithecellobium dulce* fruit extract capped AgNPs using (a) *Stayphylococus aureoous* (b) *Escharesiae coli* (c) *Klebisella pneumonia* (d) *Basilusb tilis,* (e) *Enterococcus facalis* (f) *Protious mirabilis*.

The antibacterial activity results suggest that AgNPs synthesized from *Pithecellobium dulce* fruit peelings extract shows the effective antibacterial activity against gram positive than in Gram-negative bacteria [25]. AgNps make holes in the cell wall, resulting in the fact that the AgNps disrupt the cell contents that leads to death of bacteria. The silver nano-particle can bind with the DNA of bacteria and inhibit the DNA transcription. Because of AgNPs strongly bind to the surface of the bacteria causing visible damage to the cell walls; thereby it can minimize treatment duration and side effects of drugs [41, 42]. It is reported that silver nano-particles can penetrate and disrupt the membranes of bacteria. Based on these results, it can be concluded that the green synthesized AgNPs had significant anti-bacterial activity.

Performing assay for Minimum inhibitory concentration of given samples (MIC). AgNps samples solutions assayed above were checked for their minimum inhibitory concentrations by loading 10 µl, 25 µl, 50 µl, 75 µl, 100 µl of samples in each well of the same plate respectively [43] (Fig. 8 a & b).

MIC against bacteria.

MIC of samples in Gram positive Bacteria.

Sample	Gram positive Bacteria- *Bacillus*			
Concentration	25 µl	50 µl	75 µl	100 µl
Ag-0.1	0	0	0	0.2
Ag-0.2	0	0	0	0.3
Ag-0.3	0	0	0	0.1
Ag-0.4	0	0	0	0.2

MIC of samples in Gram negative Bacteria.

Sample	Gram negative Bacteria- *E. coli*			
Concentration	25 µl	50 µl	75 µl	100 µl
Ag-0.1	0	0	0	0
Ag-0.2	0	0	0	0.1
Ag-0.3	0	0	0	0.1
Ag-0.4	0	0	0	0

Zeta potential.

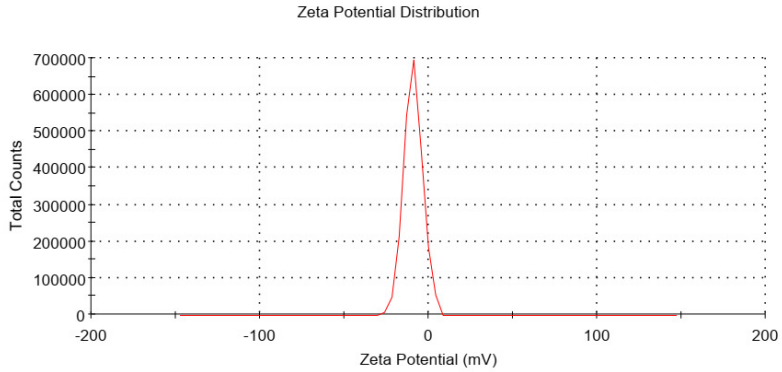


Fig. 8. (a) Zeta potential distribution of synthesized silver nano-particles.

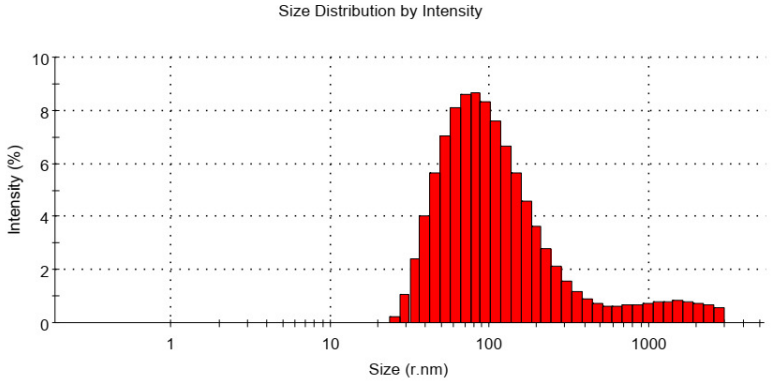


Fig. 8. (b) Size distribution by intensity of synthesized silver nano-particles.

The average hydrodynamic diameter of the silver nano-particles was found around 45 nm with 0.423 PDI. The aqueous stability of silver nanoparticles were tested by zeta potential analyser. Zeta potentials of the nano-particles obtained by DLS measurements were -14.67 mv, indicates the stability and size of nanoparticles is less than 100nm [44].

IV. CONCLUSIONS

The synthesis of stable AgNPs was achieved without adding any external agent(s). It is efficient and eco-friendly renewable "green" method has been established for the green synthesis of AgNPs. The synthesis is carried out using *Pithecellobium dulce* fruit peelings extrac, DD H_2O and microwave irradiation. The *Pithecellobium dulce* fruit peelings extract used as a reducing/stabilizing agent without using any hazardous chemicals and synthetic reducing agents. The concentration of *Pithecellobium dulce* fruit peelings extract and microwave irradiation time affected the formation of number of AgNPs. The study shows that microwave irradiation can enhance the number of AgNPs formation. Only Hg^{+2} and Fe^{+3} metal ions are detected in metal ions detection by the synthesized AgNps. The antibacterial activity studies of AgNPs reveals that the formed AgNPs shows positive antibacterial activity against staphylococcus aureus, *Staphylococcus aureous, Escherichia coli, Klebsiella pneumonia* and *Bacillus* subtitles. And also shows negative antibacterial activity against *Enterococcus faecalis* and *Enterococcus faecalis* bacteria. Such cheap source of material gives an opportunity to a cost-effective and ecofriendly preparation of stable AgNPs having various potential applications to be used.

V. FUTURE SCOPE

Further we can study the applications like anti-cancer, anti fungal, anti viral and how these the *Pithecellobium dulce* fruit peelings extract capped silver nano-particles are useful in water purification.

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