Jour Pl Sci Res **37 (2)** 507-515 2021

Biosynthesis of Silver Nanoparticles using various botanicals and evaluation of its antimicrobial property

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The present study deals with biosynthesis of Silver Nanoparticles using various plant extract and evaluation of its antimicrobial property. Silver Nanoparticles (AgNPs) were biosynthesized using plants extract of *Azadirachta indica* (Neem), *Cymbopogan citratus* (Lemon grass), *Piper betle* (Betele leaf) and *Trachyspermum ammi* (Carom leaf) separately. Silver nitrate was used as substrate and botanical extract as biotemplate for biological synthesis of AgNPs. It was characterized by UV–vis spectrophotometer, X-ray diffractometer (XRD), and Fourier Transform Infrared Spectroscopy (FTIR). The antimicrobial property of the AgNPs were carried out by well diffusion method using gram positive bacteria, *Staphylococcus aureus* and gram negative bacteria, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The highest antimicrobial activity was observed against *K. pneumoniae* (12±0.26 mm) and *E. coli* (11±0.11 mm) for AgNPs synthesized using *P. betle* and *C. citratus* leafextracts. The AgNPs synthesized using *T. ammi* was showed highest antifungal activity against *Aspergillus niger*. The present study report, the enhanced antimicrobial property of AgNPs synthesized using medicinal plants as reducing and capping agents which would be a promising alternative drug for the treatment of wound infections and skin infections with reduced hazards.

Keywords: AgNPs, Antimicrobial, FTIR, Green Synthesis, XRD.

1. INTRODUCTION

The field of nanotechnology is the most dynamic region of research in material sciences and synthesis of nanoparticles is increasing exponentially because of its wide range of applications in the field of bio-nanotechnology, biomedicine, biosensors, optoelectronics etc. (Verma & Mehta 2015; Rafique et al., 2017). Earlier, conventional methods were used for the production of nanoparticles but it is expensive, toxic, and non-environment friendly (Rafique et al., 2017). Nowadays, environmental friendly, green synthesis of nanoparticles received huge consideration as an alternative approach to fulfill the growing need (Arumai Selvan et al., 2018; Soshnikova et al., 2018; Rana et al., 2020). Few years back, researchers were using microorganisms for the synthesis of various metal nanoparticles (Lee et al., 2004; Saxena et al., 2012; Konishi et al., 2007; Shahverdi et al., 2007; Ibrahim 2015). Recently, plant used nanoparticle synthesis attracted much attention and highly preferred because: Environmental friendly, Cheaper, Single step method (free from elaborated process of culture and maintenance) and also safe for human therapeutic use (Valli &Vaseeharan 2012; Soshnikova et al., 2018; Garibo et al., 2020). As it is cost effective, therefore

DOI:-https://doi.org/10.32381/JPSR.2021.37.02.19

can be used as an economic and valuable alternative for the large-scale production of nanoparticles (Dakal et al., 2016). Also, different compounds (primary and secondary metabolites) present in the plant which could play an essential role in the mechanism of metal ions uptake, reduction of precursor salt as well as capping agents and some of them with inherent antimicrobial properties (Garibo et al., 2020). Thorough literature survey found maximum advantages of plant used NPs, leading researcher were focused to develop green synthesis of NPs by using different parts of the plant such as from leaf (Prabu and Johnson 2015; Shakeel et al., 2015; Khatoon et al., 2018), peel (Annu, et al., 2018), flower (Padalia et al., 2015), fruit (Lakshmanan et al., 2018) and root (Benakashani et al., 2017).Generally the different metal precursor were used for synthesis of NPs are iron (Fe) aluminum (Al), copper (Cu), silver (Ag), gold (Au), lead (Pb), cadmium (Cd), zinc (Zn) and cobalt (Co) (Elkodous et al., 2019; Attia et al., 2019; El-Batal et al., 2019). Out of these, AgNPs have received particular attention due to their unique morphologies, stability, and controlled geometry (Grace and Pandian 2007). Also the scientific community is exploiting the use of silver nanoparticles (AgNPs) in nano-medicine for diagnosis and treatments of various diseases (Khan et al., 2014). Particularly, AgNPs found to have excellent antibacterial and antifungal activities against several microorganisms which are responsible for several infectious diseases (Garibo et al., 2020). With this, AgNPs used in different medical products, such as in coating materials of catheters for cerebrospinal fluid drainage (Bayston et al., 2007; Galiano et al., 2008), bone cement (Alt et al., 2004), surgical masks, impregnated textile fabrics, nanogels, nanolotions (Furno et al., 2004; Ip et al., 2006; Leaper 2006), Indeed, a majority of the Ag-based products developed have been commercialized and are approved by global regulatory bodies (Shaik et al., 2018). In view of this contemplate of advantages of green synthesis (plant based) of NPs over other methods, in the present work, we report in vitro antimicrobial activity of AgNPs synthesized by a green method using highly important medicinal plant: *A. indica*, *C. citratus*, *P. betle* and *T. ammi* leaves extract. The AgNPs were tested against four bacterial strains (*E. coli*, *P.aeruginosa, K. pneumonia* and *S. aureus*) and fungal strain (*A. niger*). The combination of AgNPs with leaf extract of different plantscan then make a promising alternative against infectious diseases with reduced cytotoxicity.

2. MATERIALS AND METHODS

2.1 Collection of sample and Preparation of plant extract

Fresh leaf of *A. indica*, *C. citratus*, *P. betle* and *T. ammi* were collected from Botanical garden, Savitribai Phule Pune University, Maharashtra, India. The leaves were rinsed under running tap water for 5 min followed by rinsing with sterile distilled water. Five grams of finely chopped fresh leaves were crushed in mortar and pestle by adding 5 ml of distilled water. Further, it was centrifuged at 10000 rpm for 15 min. The supernatant was filtered using Whatman (grade 1) filter paper and stored in amber glass bottle at 4 °C for further experimental use.

2.2 Synthesis of biogenic nanoparticles

Biosynthesis of silver nanoparticles performed using aqueous leaf extract of *A. indica*, *C. citratus*, *P. betle* and *T. ammi.* In the reaction solution containing 20 ml $AgNO_3$ (1×10^{-3} M), 10 ml of leaf extract was added drop wise with stirring using glass rod and further it was incubated at ambient conditions for 2 min. The change in color of reaction solution was observed and synthesized AgNPs were pellet down by centrifuging reaction solution at 15000 rpm for 25 min. The supernatant was removed and pellet washed using 10 ml of Milli-Q water two times. Furthermore, The AgNPs were stored in powdered form after drying at room temperature in desiccator. This setup was incubated in dark (to minimize the photo-activation of silver nitrate), at 37°C under static condition. A control setup was also maintained without plant extract.

2.3 Characterization of AgNPs

In the reaction mixture biosynthesis of AgNPs were monitored at wavelength of 300–700 nm with regular intervals using UV–visible spectrophotometer (Model:Shimadzu UV-1000) after 24 h. The functional group of biomolecules of AgNPs was detected by FTIR spectroscopy (Shimadzu FT-IR Prestige, Corporation, Kyoto, Japan). The XRD pattern of

AgNPs were identified and qualitative structure of AgNPs were collected using NPs Ultima IV (Rigaku).The mean particle diameter of silver nanoparticles was calculated from the XRD pattern according to the line width of the plane, refraction peak using thefollowing Scherrer's equation (Balaji et al., 2009).

2.4 Antimicrobial sensitivity testing

Antibacterial susceptibility of AgNPs was evaluated on *E. coli*, *P. aeruginosa*, *S. aureus, K. pneumoniae* and *A. niger.* The bacterial cultures were obtained from Biochemical Division, National Chemical Laboratory, Pune. Antibacterial susceptibility test was performed according to method reported Alsalhi et al., (2016) and Hamelian et al., (2018). The *A. niger* suspension cultures was prepared as per the method of Fiedler et al., (2018). All the experimental media were purchased from Sigma Aldrich, India.

2.5 Culture media and Zone of inhibition

The well diffusion method was used to determine antimicrobial susceptibility of AgNPs and counted in the form of zone of inhibition. Aliquots of 0.1 ml of each test organism were spread on LB agar for bacteria and PDA agar for *A. niger*. Each Petri plates were dried. 10 ìl of biogenic AgNPs, *A. indica*, *C. citratus*, *P. betle* and *T. ammi*aqueous extract were placed on the surface of agar plates and incubated at 37 °C during 24 h after which diameters of inhibition zones were measured.

2.6 Data analysis

The experiment of antimicrobial analysis were replicated three times and the results are presented as mean±SE. Data were calculated by using 't-test' and analyzed using in SPSS software

3. RESULTS AND DISCUSSIONS

3.1 NPs Synthesis and UV-Vis spectral analysis

The process of synthesis of silver nanoparticle of different plant extracts from $AgNO_3$ was traced after incubating $AgNO_3$ with prepared extract of different plants. The syntheses of silver nanoparticles were confirmed by observing the color change in leaf extract. Initially, the mixture was pale white in color. Pale white turned dark brown after completion of synthesis (Fig. 1). The possible reason behind the change in color of solution (Colorless to dark brown)

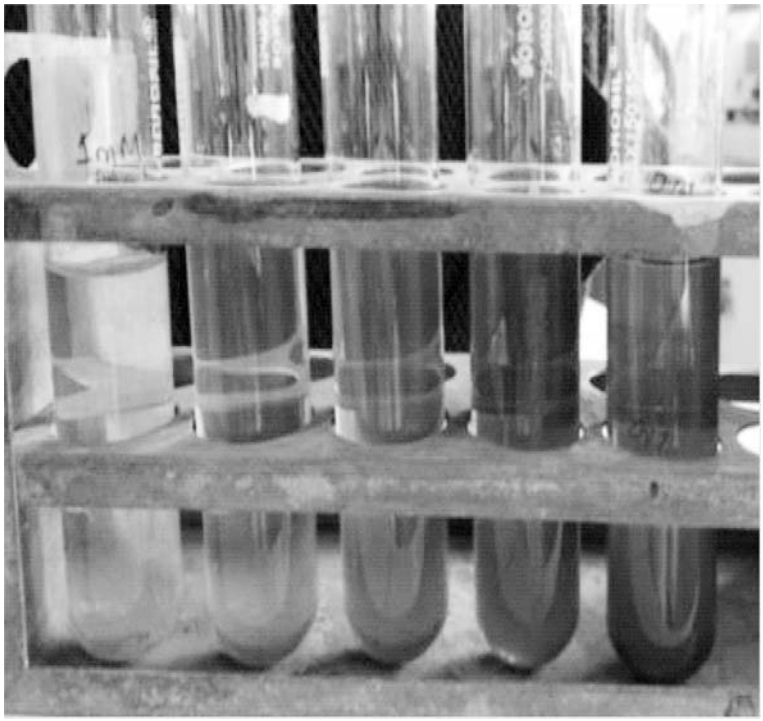

Fig. 1. Biosynthesis of Silver Nanoparticle using *Trachyspermum ammi* leaf

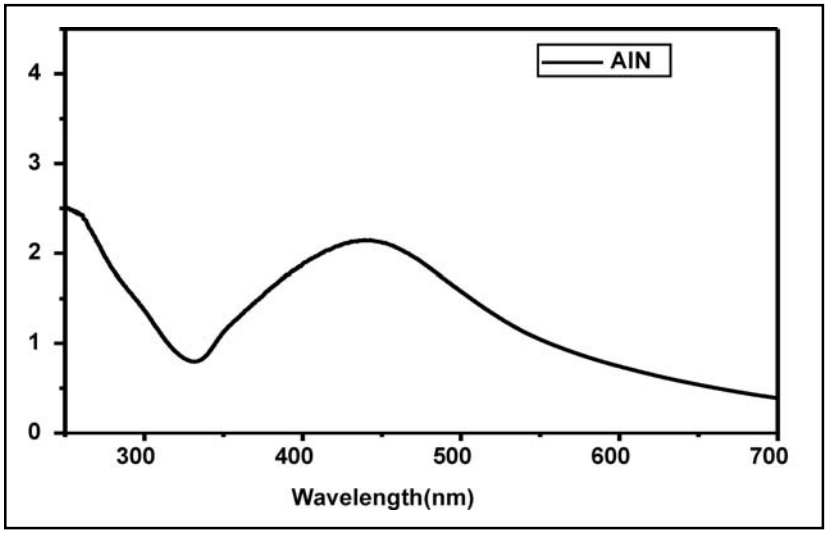

Fig. 2. UV-Vis absorption spectrum of silver nanoparticles synthesized from *A. indica*

is, Surface Plasmon Resonance (SPR) at room temperature. Here, it clearly indicates that the reduction of silver ions into AgNPs of plant extracts due to numerous alternations of free electrons (Arokiyaraj et al., 2015; Yakout and Mostafa et al., 2015; AlSalhi et al., 2016; Omidi et al., 2018). However, no color transformation was noticed in the absence of plant extract. The formation of AgNPs was further assessed by UV–Vis spectroscopy. In Fig. 2, the absorption spectrum of different plant extract was exhibited with 24 h of time period. Theabsorption spectra of AgNPs formed in the reaction media has absorption maxima in the range of 450 to 475 nm due to Surface Plasmon Resonance of AgNPs. The UV-visible spectra recorded, implies that the most rapid bioreduction was achieved by using *A. indica* followed by *P. betle, T. ammi*, and *C. citratus* respectively.

3.2 X-ray diffraction analysis

The X-ray diffraction method was used further to confirm the formation of AgNPs formation from *A. indica*, *C. citratus*, *P. betle* and *T. ammi* plant extract.The XRD study of the AgNPs synthesized using different plant extracts indicated a crystalline nature. In *P. betle,* three main peaks were found at 2θ ranges of 27.86°, 32.14°, and 37.96° can be labeled to the 220, 122, and 111, which is indication silver in cubic structure (Fig. 3). While in *T. Ammi,* there were four main peaks at 2θ value 27.90°, 32.11°, 37.92°,

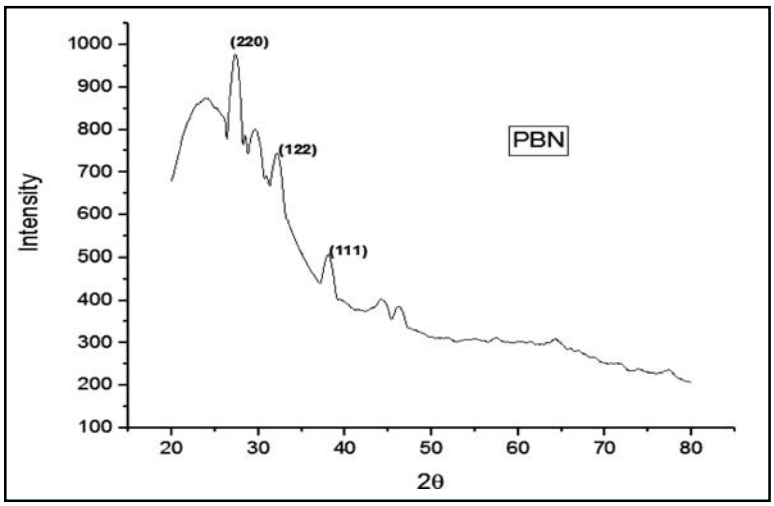

Fig. 3. X- ray diffraction analysis of silver nanoparticles synthesized from *P. betle*

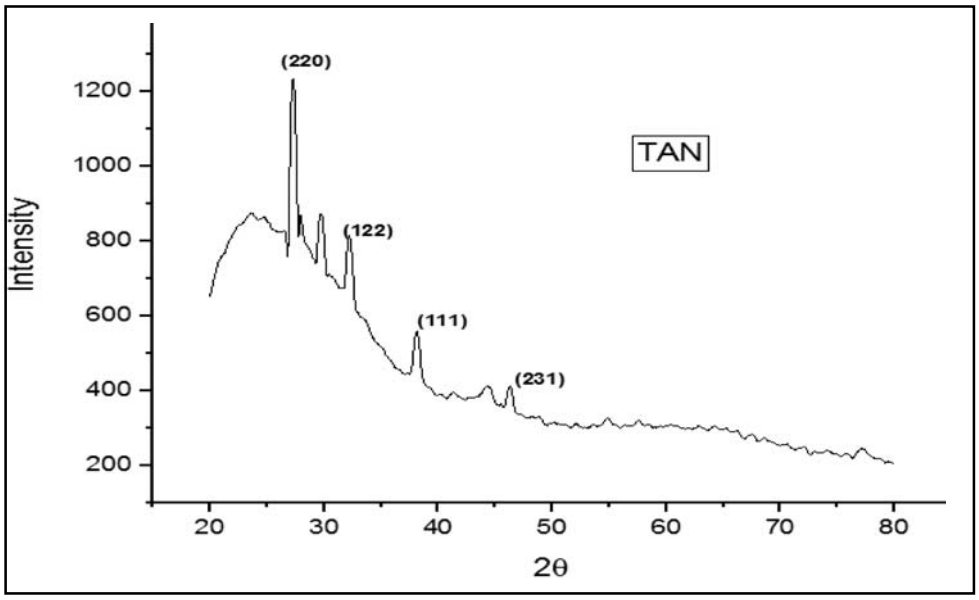

Fig. 4. X ray diffraction analysis of silver nanoparticles synthesized from *T. ammi*

and 57. 03° can be labeled to the 220, 122, 111, and 231 which is also indication silver in cubic structure (Fig. 4). From natural plants extract synthesized silver nanoparticles the calculated average particle size of the silver was found to be 19.95 nm and 12.67 nm, corresponding to *P. betele* and *T. ammi* respectively. Similar type of results was reported by shaik et al., 2018 when studying with *O. vulgare* plant extracts. However, nanoparticles synthesized from *A. indica* and *C. citratus* showed weak peaks in XRD analysis.

3.3 FT-IR Analysis

FTIR spectroscopy is important method to study the various functional groups present in the plant extract and group is analyzed from the peak position in the IR spectrum (Omidi et al., 2018). In present study, FTIR analysis was carried with different plant extracts. Various absorption bands in the FTIR spectra showed different chemical groups in the *C.citratus* extract containing biosynthesized AgNPs.

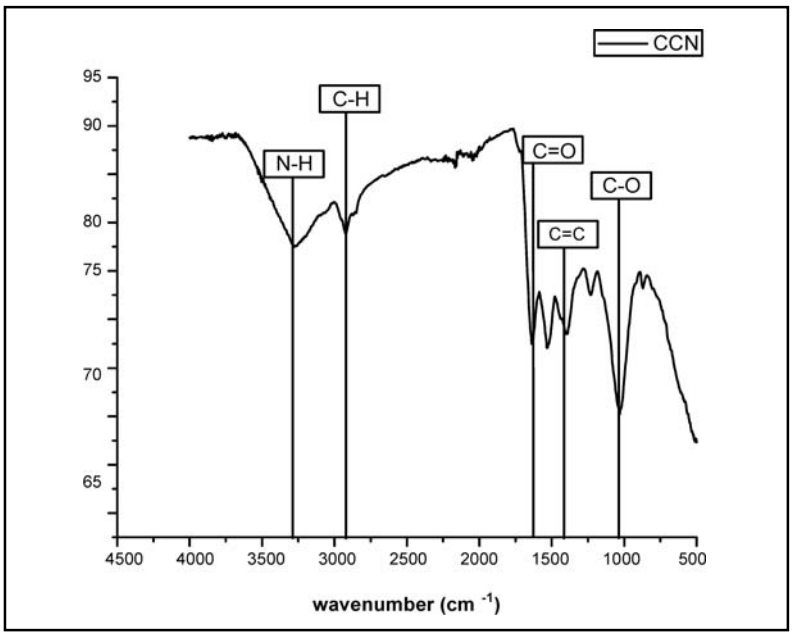

Fig. 5. FTIR spectroscopy of silver nanoparticles synthesized from *C. citratus*

While a broad band at 3272 cm^{-1} showed the-N-H and –O-H groups, the absorption bands at 2920, 1635,1404 and 1036 cm^{-1} corresponded to –C-H, C=O, C=C and C-O- groups, respectively. The presence of -N-H, -O-H, C=C AND –C-H groups in the FTIR spectra suggests that the *C. citratus* extract contained the hydroxyl and amino groups substituted flavonoids. The flavonoids can act as reducing agents (which reduce Ag+ to Ag0), whereas the amino group serves as a stabilizing agent in the green synthesis of AgNPs. Thus, the FTIR spectra obtained in this study may explain the interaction of the leaf biomolecules of *C. citratus* with silver nitrate, leading to biosynthesis of AgNPs (Fig. 5).

FTIR spectrum of the *P. betel* leaf extract mediated silver nanoparticles shown band at 3262 cm^{-1} which corresponds to the O-H stretching for alcohols and phenols, 2924 cm^{-1} corresponds to the C-H stretching for alkanes, 1595 cm^{-1} corresponds to the C=O stretching of amides, and 1363 cm^{-1} corresponds to the C-N stretching of the aromatic amino group, 1037 cm^{-1} corresponds to the C-O stretching of alcohols and ethers (Fig. 6). FTIR spectrum of the *T.ammi* leaf extract mediated silver nanoparticles shown band at 3240 cm^{-1} which corresponds to the O-H stretching for alcohols and phenols, 2916 cm^{-1} corresponds to the C-H stretching for alkanes, 1597 cm^{-1} corresponds to the C=O stretching of

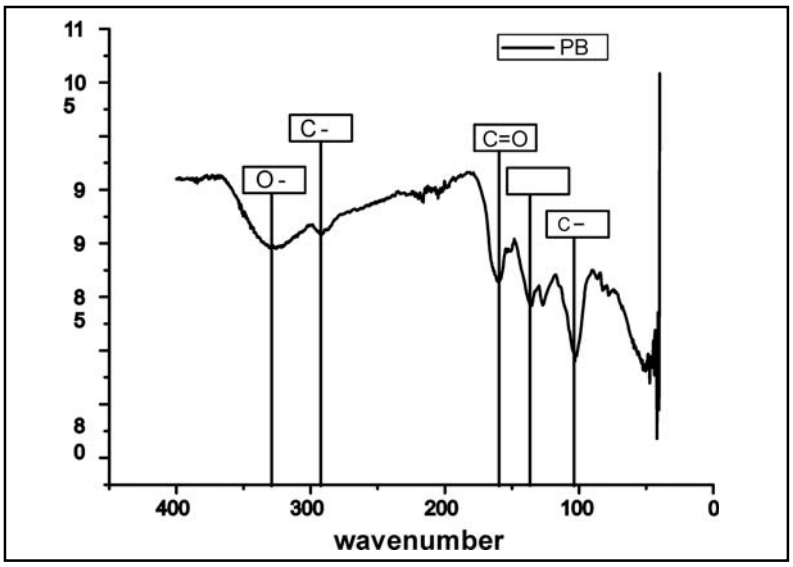

Fig. 6. FTIR spectroscopy of silver nanoparticles synthesized from *P. betle*

amides,1017 cm^{-1} corresponds to the C-O stretching of alcohols and ethers. FTIR spectrum of the *A. indica* leaf extract mediated silver nanoparticles shown band at 3249 cm^{-1} which corresponds to the O-H stretching for alcohols and phenols, 2918 cm^{-1} corresponds to the C-H stretching for alkanes, 1639 cm^{-1} and 1521 cm^{-1} corresponds to the C=O stretching of amides,1020 cm^{-1} corresponds to the C-O stretching of alcohols and ethers. These FTIR spectrum of silver nanoparticles suggested that synthesized silver nanoparticles were surrounded by various bioactive compounds such as phenols, terpenoids, alcohols, ketones, aldehydes and carboxylic acids. These bioactive compounds would act as reducing, stabilizing and capping agents.

3.4 Antimicrobial Activity of synthesized silver nanoparticle

In the present study, the efficiency of AgNPs synthesized using different plant extracts were tested for antibacterial and antifungal activities. The result showed the higher potential of synthesized AgNPs from different plant extracts when compared with control. AgNPs prepared with various concentrations of different plant extract, were subjected to antimicrobial analysis against various gram-positive and gram-negative bacteriaand fungus. In present study, it was found that all the plant extract prepared NPs showed significant antimicrobial activity against both gram positive (*S. aureus*) and gram negative (*E. coli*, *P. aeruginosa* and *K. pneumoniae*)

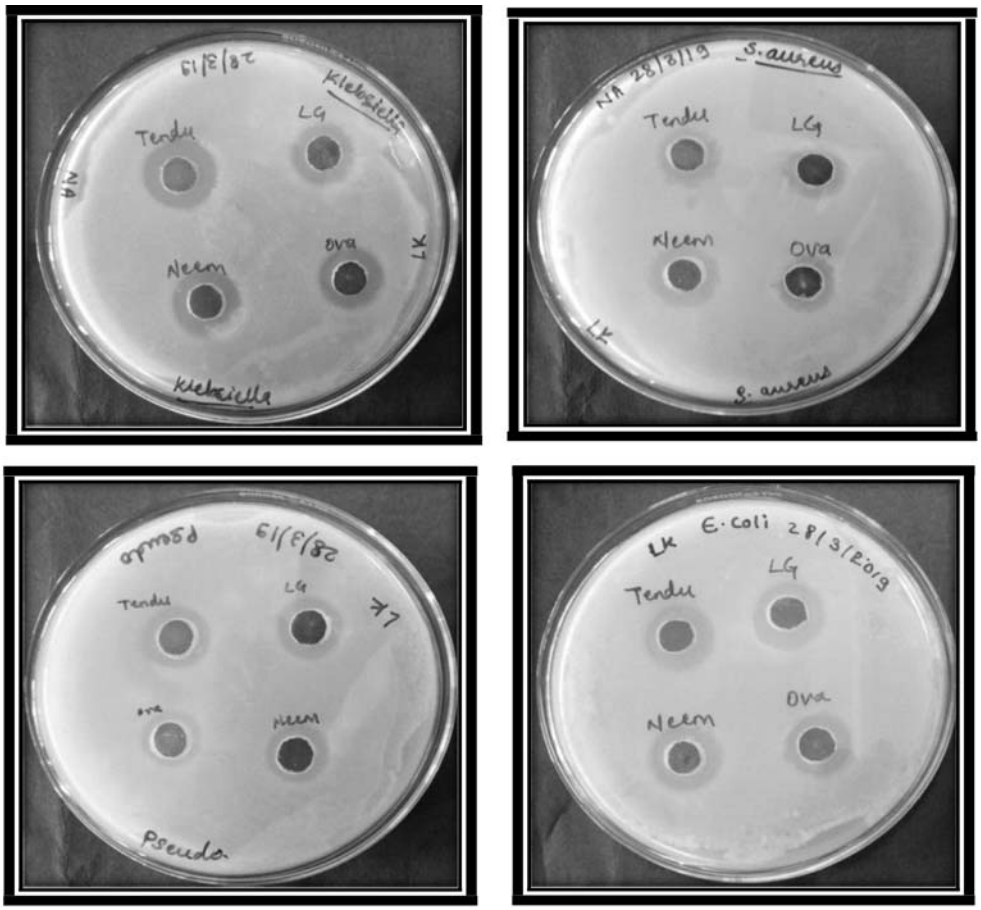

Fig. 7: Zone of inhibition against: (a) ***Klebshiella sp.*** (b) ***S. aureus*** (c) ***Pseudomonas sp.*** (d) ***E. coli***

bacteria and pathogenic fungi (*A. niger*). It is represented as a zone of inhibition (diameter) in Fig. 7 and summarized in Tables 1 and 2. The highest antimicrobial activity was found with silver nanoparticles synthesized from *P. betle* and *C. citratus* extracts against *K. pneumoniae* (12±0.26 mm) and *E. coli* (11±0.11 mm) respectively. The antifungal activity was checked against *A.niger* where *T. ammi* (6.2±0.20 mm) showed higher zone of inhibition.In the present study AgNPs prepared using different plant extracts were showed efficient antimicrobial property. The antimicrobial property may be due to the nature of nanoparticles get attached to the cell membrane and get penetrated inside the bacteria and attack the respiratory chain, cell division finally leading to cell death (Sondi and Salopek-Sondi 2007; Morones et al., 2005; Logeswari et al., 2015; Al-Dhabi et al., 2018).

Table 1. Antibacterial activity of different plant synthesized silver nanoparticles

Sr. No.	Nanoparticles	Zone of inhibition (mm)			
		E. coli	*Klebsiella sp.*	*Pseudomonassp.*	*S. aureus*
1.	Control	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
2.	*Piper betle*	10.5±0.28	12±0.26	8±0.23	10±0.28
3.	*Cymbopogancitratus*	11±0.11	9±0.33	9.1±0.25	7.1±0.18
4.	*Azadirachtaindica*	9±0.17	10.2±0.3	7.1±0.5	9±0.12
5	*Trachyspermumammi*	9.1±0.17	10±0.08	6.8±0.66	10±0.18

Table 2. Antifungal activity of different plant synthesized silver nanoparticles

Sr. No.	Silver nanoparticles	Zone of inhibition against *A. niger* (mm)
1	Control	0.0±0.0
2	*Piper betle*	5±0.11
3	*Cymbopogan citratus*	4.1±0.32
4	*Trachyspermum ammi*	6.2±0.20
5	*Azadirachta indica*	0.0±0.0

4. CONCLUSIONS

In present work, the silver nanoparticles have been produced from *A. indica*, *C. citratus*, *P. betle*, and *T. ammi* extracts,which is a cheaper, effective, and eco-friendly process.Used techniques; UV–vis spectrophotometer, XRD, and FTIR techniques have confirmed the formation of silver nanoparticles from different plant extract.The observed zones of inhibition in the antimicrobial screening test indicated, that the AgNPs synthesized in this process has the efficient antimicrobial activity against different pathogenic bacteria and fungus. The biologically synthesized silver nanoparticles can be of immense use in medical field due to their efficient antimicrobial function which could be used to cure different infectious diseases.

5. Acknowledgments

We are thankful to Head, Department of Botany, Savitribai Phule Pune University for providing the plants for present research work.

6. Conflict of Interest

There is no any conflict of interest.

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Received : 20-01-2021

Accepted : 22-01-2021