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GREEN SYNTHESIS OF AGNPS BY USING LEAF EXTRACTS OF *SOLANUM NIGRUM* L. AND STUDY OF THEIR ANTIMICROBIAL ACTIVITY

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Abstract: Extracts of leaves of *Solanum nigrum* L. was extracted by distilled water, methanol, acetone and ethanol. These extracts were mixed in 5mM solution of silver nitrate ($AgNO_3$) for the synthesis of silver nanoparticles (AgNPs). Synthesized AgNPs were characterized by UV-Vis spectroscopy and Scanning electron microscopy (SEM). SEM analysis revealed that the synthesized AgNPs were having a size range between 8 to 18nm. AgNPs were studied for their antimicrobial activity against bacteria including *Klebsiella spp, E. coli* and fungi *Aspergillus niger*. Results showed that synthesized AgNPs were effective against these microbes but their activity was better against bacteria than fungi. Additionally, AgNPs preapared using methanolic leaf extracts exhibited better efficiency as compare to those were prepared with the other solvents used in the study.

Introduction

Pathogenic microbes like bacteria, viruses, fungi, etc. causes serious problems in humans (Gourama, 2020). To control pathogenic microbe's antimicrobial medicines are being synthesized for decades but antimicrobial resistance of harmful microbes is a major hazard to the people health. Resistance to antibiotics is a problem for world health challenge which involves transfer of genes and bacteria between environment, humans and animals. The capacity to withstand antibiotics has been developed in several bacterial species long before humans began mass-producing them to cure and prevent infectious illnesses (D'Costa et al., 2011; Maqsood et. al., 2014). Resistant pathogens resulting in 24 million hospitalizations and 700,000 deaths annually (Dadgostar, 2019).

The science of nanotechnology is still in its infancy, deals with the formation of nanoscale particles (Varadharaj et al., 2019). Nanoparticles (NPs) synthesized through nanotechnology simply incorporates the nano-size particles with sizes between one and one hundred nanometres. Nanoparticles possess special qualities because of their shape, structure, size, composition and arrangement. The characteristics of nanoparticles are very important in many biomedical applications like antioxidants. There are noble metal nanoparticles among them, such as silver, platinum, and gold. Because of their unique characteristics and chemical durability, silver nanoparticles are the most often used type of nanoparticles. These properties enable them to have wide range of applications like as catalyst agents, biosensors, and most importantly as antimicrobial agents. Silver nanoparticles have

applications in biomedicine and have high anti-inflammatory effectiveness. Apart from this, they can also be used in antiseptic sprays, topical lotions, and wound dressings (Ahmed et al., 2016; Gavade et al., 2015).

A multitude of physical and chemical techniques are available for preparation of nanoparticles. Nanoparticles (NPs) synthesis can be done by two ways: "top down" and "bottom up" (Ana-Alexandra et al., 2016). The green synthesis method refers to the process of preparing nanoparticles using plant and fruit extracts (Hubenthal, 2011). Because it does not require any particular preparation for culture or isolation procedures, this method is inexpensive. It is simple to scale up for the manufacturing of nanoparticles at the large scale. Most significantly, depending on the extract's quantity and kind, it generates crystalline nanoparticles that are non-toxic and environmentally benign in a range of sizes and forms. The green approach produces no hazardous by-product since it uses no chemicals or energy, in contrast to the physical and chemical procedures. There are several benefits of using plant extracts, including their accessibility and safety, as well as the fact that they include a range of metabolites that are crucial for reduction (Gavade et al., 2015). Plant extract possess biomolecules which helps in reduction and stabilizing process these biomolecules are amino acids, alkaloids, phenol, tannins, enzymes, proteins, saponins, vitamins, terpenoids and polysaccharides (Pand et al., 2018).

Garden nightshade is the common name for *Solanum nigrum* L., a member of the Solanaceae family and linked to a number of health advantages, such as its hepato-protective, immunomodulatory, anti-ulcer, calming, anti-convulsant, cardio defensive, antibacterial, antidiabetic, and pain-relieving qualities. *Solanum nigrum* has been used in traditional medicine in India and other areas of the world to cure a variety of conditions, including liver ailments, persistent skin illnesses, fevers, menstrual cramps, diarrhoea, eye infections, dizziness, and other conditions (Mani et al., 2022). Objectives of this research was to prepare AgNPs by using extract of *Solanum nigrum* L., to investigate its antimicrobial activity and to determine its properties.

Materials and methods

Chemicals of analytical grade used in present study were purchased from BDH, sigma, Merck, etc.

2. Collection of plant material:

Plants *Solanum nigrum* L. was collected from the different villages of district Sialkot, Pakistan. The leaves of collected plant were washed with tap water thoroughly.

3. Preparation of leaf extracts:

To make plant extracts, collected leaves were dried under shade and then grinded into fine powder. Four different types of solvents (water, acetone, ethanol and methanol) separately were used for extraction. The dry powder of leaves was weighed on weighing balance (Pioneer, PA423) and was mixed in 100ml of each solvent. All the solutions were heated at 60°C in Water bath (JSR, KOREA/JSSB-50T) for 1h and then were kept at room temperature for 24h. After 24h these suspensions were filtered by Whatman filter paper no.1 and were kept at room temp until the solvent was evaporated. The left behind dried extracts in the petri plates were dissolved in freshly prepared Dimethyl Sulfoxide (DMSO). These DMSO solutions were stored at 4°C for further use (Sajid et al., 2012).

4. Preparation of Silver nitrate ($AgNO_3$) solution:

To prepare stock solution of silver nitrate (5mM), 0.9g $AgNO_3$ was dissolved in 1000ml of distilled water. The reaction mixture was incubated at room temp in the dark (El-Moslamy et al., 2018).

5. Synthesis of Nanoparticles:

Aqueous extract was added to 5mM $AgNO_3$ solution. Reaction mixture was shaken on shaker for 1h and was incubated in dark at room temp for 48h. A change in colour was observed that indicated the reduction of $AgNO_3$ to Ag^+. Synthesized AgNPs were harvested by centrifugation at 10,000 rpm for

25 min at 4°C in Centrifuge (Eppendorf, centrifuge 5810R). The obtained pellet was washed twice with distilled water. Particles were collected in the petri plates. Petri plates were dried in Hot air oven (MERMERT, B2162810) at 100°C for 2h. Dried particles were scratched and stored in Eppendorf tubes (1.5ml) at 4°C in Refrigerator (Panasonic, MPR-S163) (Ali et al., 2016).

6. Characterization of nanoparticles:
Synthesized silver nanoparticles were characterized by SEM and UV-visible spectroscopy (Nino-Martinez et al., 2008).

7. Preparation of Bacterial growth media:
About 5-7ml of media was poured in pre-sterilized petri plates in laminar air flow (Clean Bench). Media plates were placed at room temp to solidify. Petri plates was kept at 4°C for future use. About 3-5ml of media was poured into the test tubes and then cotton plugged test tubes were autoclaved. Then test tubes were placed in slanting position at room temp to solidify the media then after solidifying these slant tubes were be kept at 4°C for further uses (Khasi, 2023).

8. Bacterial strains:
Bacterial strains (*Klebsiella spp.* And *E. coli*) were isolated from local soil and were cultured in prepared agar slants.

9. Inoculum preparation for bacteria:
Nutrient broth 0.8g was dissolved in some amount of water and the final volume was raised to 100ml in conical flask. The media containing flask was autoclaved. A loop full of desired bacteria was inoculated in the media and was kept at 37°C in a shaking incubator for 24h. This inoculum (0.5ml) was used in further study (Patra et. al., 2020).

10. Determination of antibacterial activity of Silver nanoparticles (AgNPs):
Well diffusion method was used for the determination of antibacterial activity of green synthesized silver nanoparticles. Three wells were bordered in each petri plate one for extract which was as positive control, second was for silver nitrate solution (as negative control) and the third was for silver nanoparticles solution. Then plates were inoculated with bacterial inoculum (0.5ml) by spreading them on surface of media. Plates were incubated at 37°C for 24-48h for bacterial growth (Jabbar, et. al., 2023)

11. Measurement of zone of inhibition:
Zone of inhibition was recorded in millimetres by using a ruler from the bottom side of petri plates.

12. Preparation of Fungal growth media:
Potato dextrose agar 4% (PDA) was prepared and autoclaved. About 5-7ml of the prepared media was poured into pre-sterilized petri plates in laminar air flow. These plates were allowed to solidify at room temp. These PDA plates were kept at 4°C for future use. In test tubes about 3-5ml of media was poured and then cotton plugged test tubes were autoclaved and placed in slanting position at room temp for solidification. Prepared slants were kept at 4°C for further use (Khasi, 2023).

13. Fungal strains:
Fungal strains (*Aspergillus niger* L.) were isolated from soil and were cultured on prepared PDA slants.

14. Inoculum preparation for fungi:
Saline water (0.98% NaCl) was added to the fungal slants and then shaken gently to make a fungal spore suspension. That suspension (1.0ml) was used as inoculum in further study.

15. Determination of antifungal activity of silver nanoparticles (AgNPs):
Well diffusion method was used for the determination of antifungal activity of green synthesized silver nanoparticles. Green synthesized AgNPs (0.5ml) was added in the wells to check the antifungal activity. Plant extract as a positive control and silver nitrate solution as a negative control were also run parallel. Then plates were inoculated fungal inoculum (0.5ml) by spreading them on surface of media. Petri plates were incubated at 28°C for 48-72h for fungal growth (Mekky, et. al., 2021).

16. Measurement of zone of inhibition:
Zone of inhibition was recorded from the bottom side of petri plates in millimetres by using a ruler.

Results
UV-visible spectroscopy:
UV- visible spectroscopy of AgNPs of *Solanum nigrum* L. in methanol, ethanol, acetone and Distilled water is shown in fig. 4.1. The figure reveals that AgNPs synthesized by methanolic extract showed maximum absorbance peak at 440nm, ethanol extract at 462nm, acetone extract at 432nm and distilled water extract at 442nm respectively.

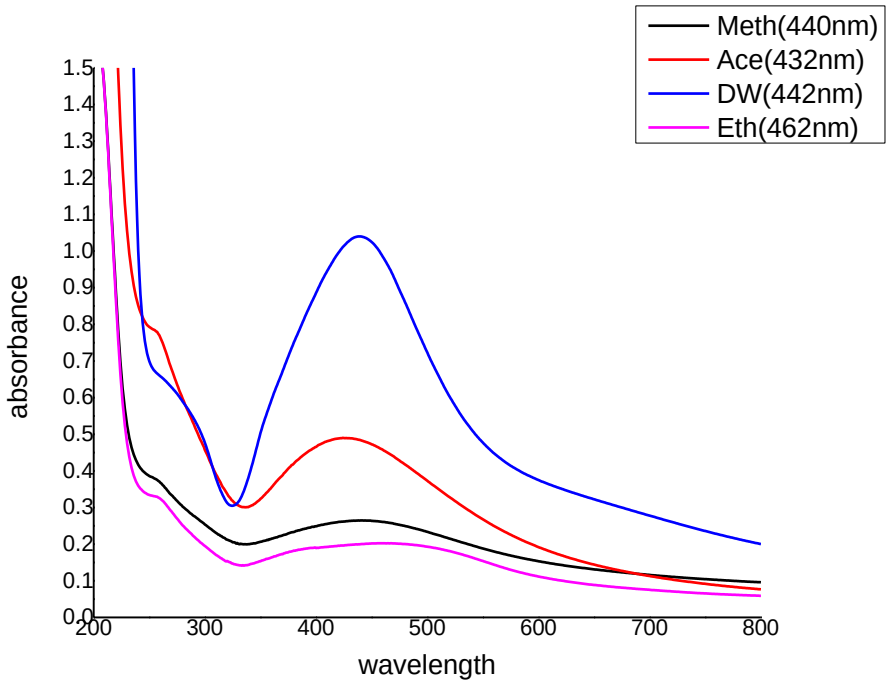
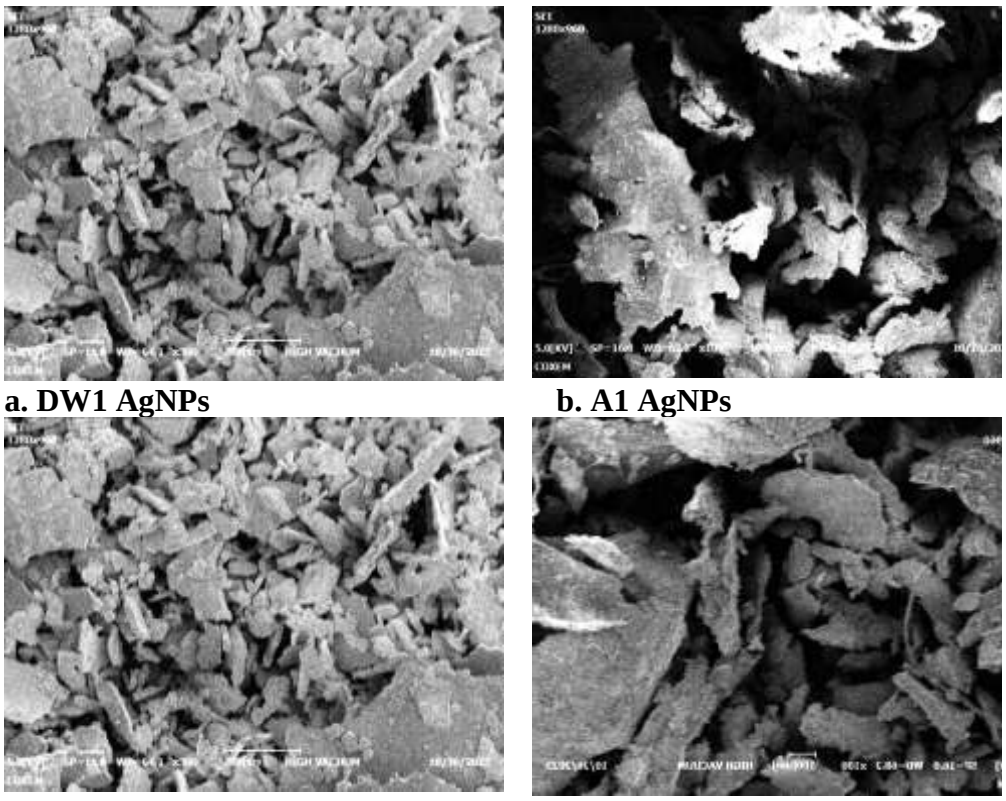


Fig. 4.1: UV-Visible spectrum of AgNPs synthesized by using the leaf extract of *Solanum nigrum* L.

Scaning electron microscopy (SEM):
Scaning electron microscopy analysis revealed that the size of green synthesized nanoparticles was in the range of 8 to 16nm. AgNPs synthesized in methanol extracts of plants were the smallest in size. AgNPs synthesized in the methanol extract of *S. nigrum* L. were 8nm in diameter, AgNPs synthesized in the ethanol extract of *S. nigrum* were 14nm in diameter, AgNPs synthesized in the acetone extract of *S. nigrum* L. were 15nm in diameter and AgNPs synthesized in the distilled water extract of *S. nigrum* L. were 16nm in diameter. SEM images of AgNPs are shown in figure 4.2 in which alphabets M, E, A and DW represents the solvent in which extract was formed M represents methanol, E for ethanol, A for acetone, DW for distilled water.



a. DW1 AgNPs **b. A1 AgNPs**

c. M1 AgNPs **d. E1 AgNPs**

Fig 4.2. SEM analysis pictures of AgNPs synthesized by using leaf extracts formed by using (a) methanol (M), (b) ethanol (E), (c) acetone (A) and (d) distilled water (DW) as solvent for plant *Solanum nigrum* L.

Activity of green synthesized AgNPs synthesized by using leaf extracts of *Solanum nigrum* L. in different solvents on *Klebsiella spp*:

Silver nanoparticles (AgNPs) which were synthesized by leaf extracts of *solanum nigrum* L. showed a very good activity against *Klebsiella spp.* The average inhibition zone formed by silver nanoparticles synthesized by distilled water extract was 25.7mm, inhibition zone formed by negative control (silver nitrate $AgNO_3$) was 20.7mm and zone of inhibition formed by positive control (Distilled water extract) was 15.7mm. Silver nanoparticles synthesized by methanol extract showed a zone of inhibition of 31.7mm, zone of inhibition formed by $AgNO_3$ was 21.7mm and zone of inhibition formed by methanol extract was 20mm. Zone of inhibition of AgNPs synthesized by ethanol extract was 29.7mm, zone of inhibition formed by silver nitrate was 20.3mm and zone of inhibition formed by ethanol extract was 18.7mm. Furthermore, silver nanoparticles synthesized by acetone extract showed an average zone of inhibition of 27.3mm, silver nitrate formed a zone of inhibition of 20.3mm and zone of inhibition formed by acetone extract was 17.3mm. All the values were significant ($p < 0.05$) (Dunnett's multiple comparison Prism Graph Pad version 5.0, Graph pad software, USA). Activity of AgNPs prepared by leaf extract of *Solanum nigrum* L. are shown in figure 4.3.

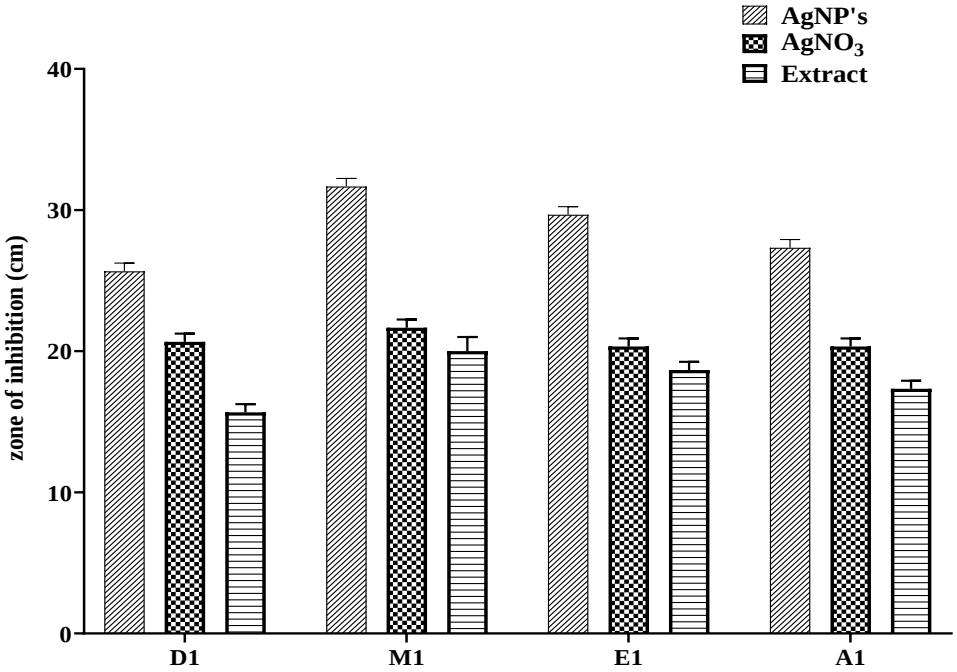


Fig 4.3. Activity of green synthesized AgNPs synthesized by using leaf extracts of *Solanum nigrum* L. in different solvents on *Klebsiella spp. (*D1 is for distilled water, M1 for methanol, E1 for ethanol and A1 for acetone.

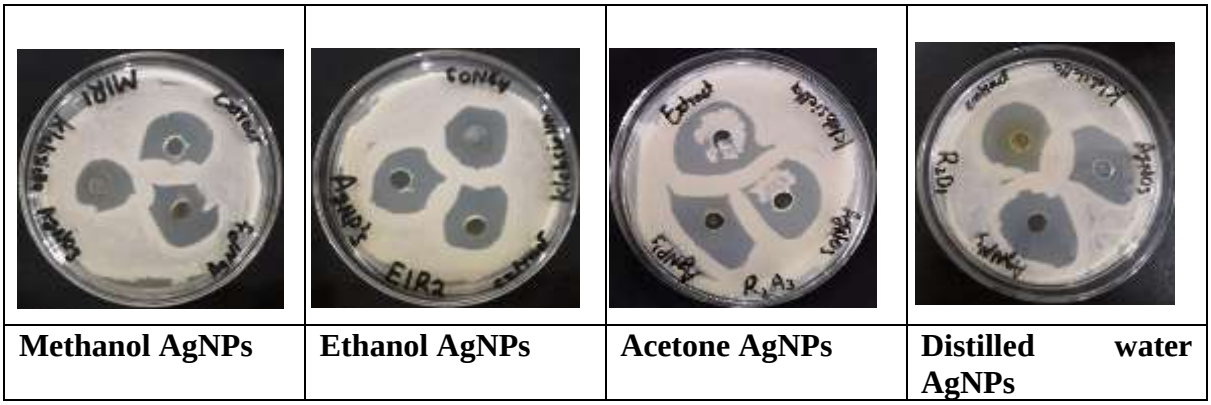


Fig 4.4. Activity of *Solanum nigrum* L. AgNPs against *Klebsiella spp.*

Activity of green synthesized AgNPs synthesized by using leaf extracts of *Solanum nigrum* L. in different solvents on *Escherichia coli*:

Activity of AgNPs of *Solanum nigrum* L. leaf extract was higher than simple leaf extract and silver nitrate against *E. coli* as shown in fig. 4.5. Inhibition zone shown by AgNPs synthesized by distilled water extract was 26mm in average, zone of inhibition formed by $AgNO_3$ was 20.7mm and zone of inhibition formed by distilled water extract was 15.3mm. Inhibition zone formed by AgNPs synthesized by methanol extract was 32mm, zone of inhibition formed by $AgNO_3$ was 21.7mm and zone of inhibition formed by methanol extract was 21.7mm. Silver nanoparticles synthesized by ethanol extract formed a zone of inhibition of 29.7mm, zone of inhibition formed by $AgNO_3$ was 20.7mm and zone of inhibition formed by ethanol extract was 19mm. And AgNPs synthesized by acetone extract showed a zone of inhibition of 27.3mm, zone of inhibition formed by $AgNO_3$ was 21mm and zone of inhibition formed by acetone extract was 17.3mm. All values were significant $p<0.05$. (Dunnett's multiple comparison Prism Graph Pad version 5.0, Graph pad software, USA).

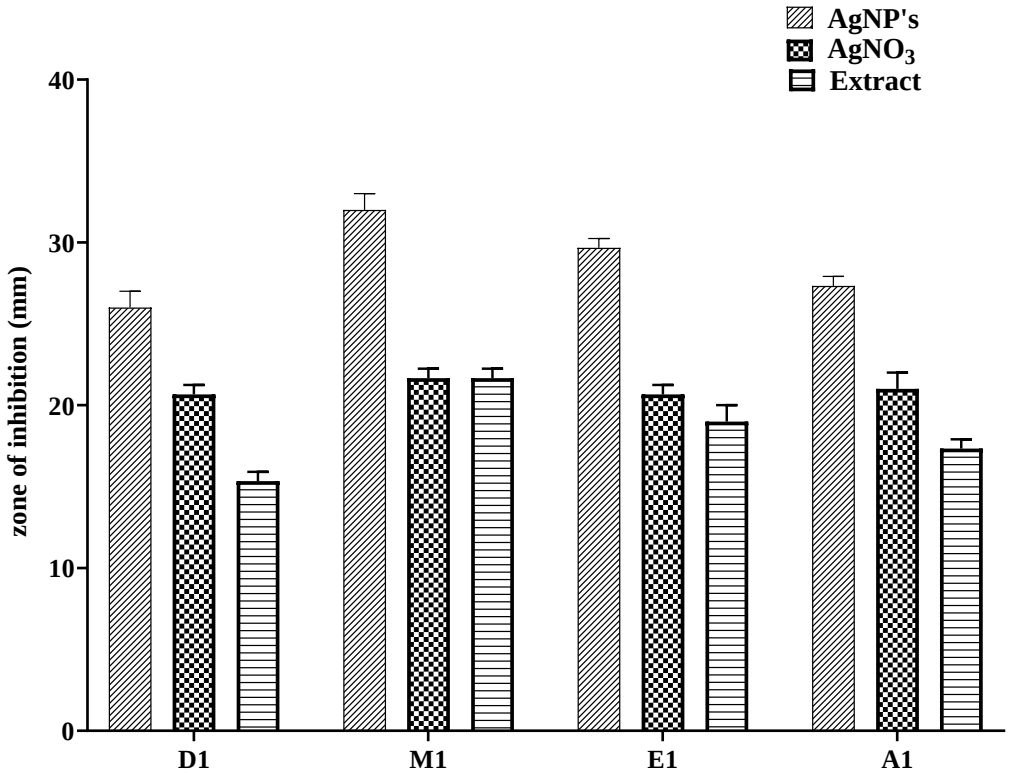
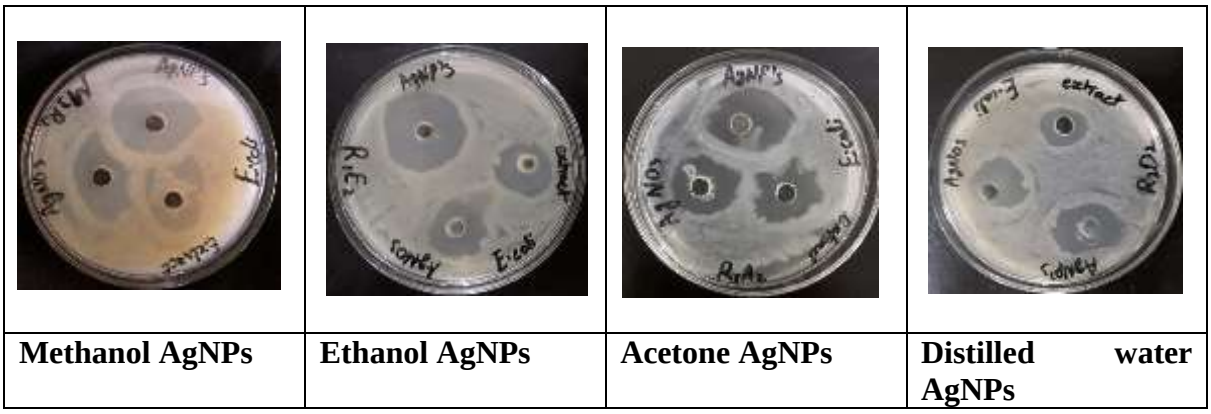


Fig 4.5. Activity of green synthesized AgNPs synthesized by using leaf extracts of *Solanum nigrum* L. in different solvents on *Escherichia coli (*D is for distilled water, M for methanol, E for ethanol and A for acetone.



Methanol AgNPs	Ethanol AgNPs	Acetone AgNPs	Distilled water AgNPs

Fig 4.6. Activity of *Solanum nigrum* L. AgNPs against *E. coli*

Activity of green synthesized AgNPs synthesized by using leaf extracts of *Solanum nigrum* L. in different solvents on *Aspergillus niger*:

Activity of silver nanoparticles of *solanum nigrum* L. leaf extract was higher than simple leaf extract and silver nitrate against *Aspergillus niger* as shown in figure no 4.7. Inhibition zone shown by silver nanoparticles which were synthesized by distilled water extract was 21.3mm in average, zone of inhibition formed by $AgNO_3$ was 18.7mm and zone of inhibition formed by distilled water extract was 13.7mm. Inhibition zone shown by the AgNPs synthesized by methanol extract was 29mm, zone of inhibition formed by $AgNO_3$ was 19mm and zone of inhibition formed by methanol extract was 20mm. Silver nanoparticles synthesized by ethanol extract formed a zone of inhibition of 26.7mm, zone of inhibition formed by $AgNO_3$ was 18.7mm and zone of inhibition formed by ethanol extract was 17.7mm. And AgNPs synthesized by acetone leaf extract showed a zone of inhibition of 24.3mm, zone of inhibition formed by $AgNO_3$ was 19mm and zone of inhibition formed by acetone extract was

16.3mm. All values were significant $p<0.05$. (Dunnett's multiple comparison Prism Graph Pad version 5.0, Graph pad software, USA)

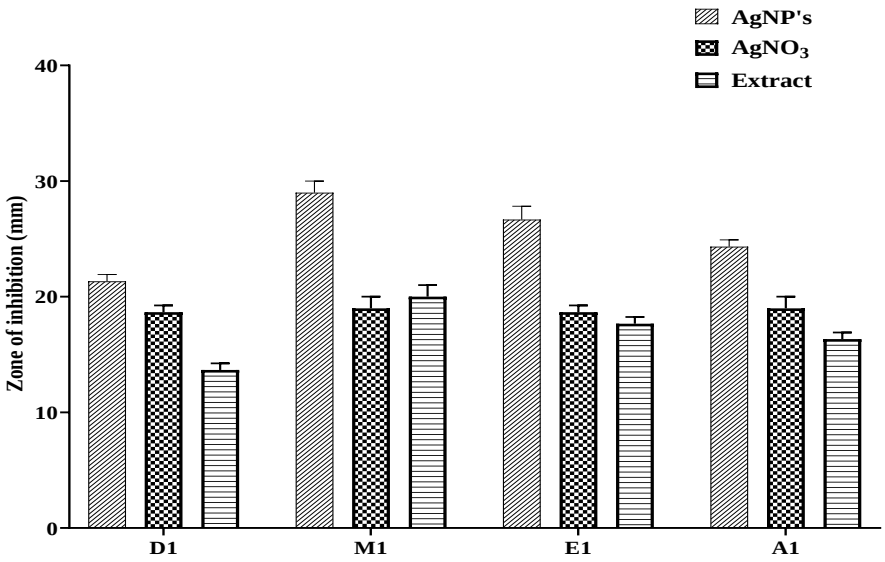


Fig 4.7. Activity of green synthesized AgNPs synthesized by using leaf extracts of *Solanum nigrum* L. in different solvents on *Aspergillus niger*, D is for distilled water, M for methanol, E for ethanol and A for acetone.

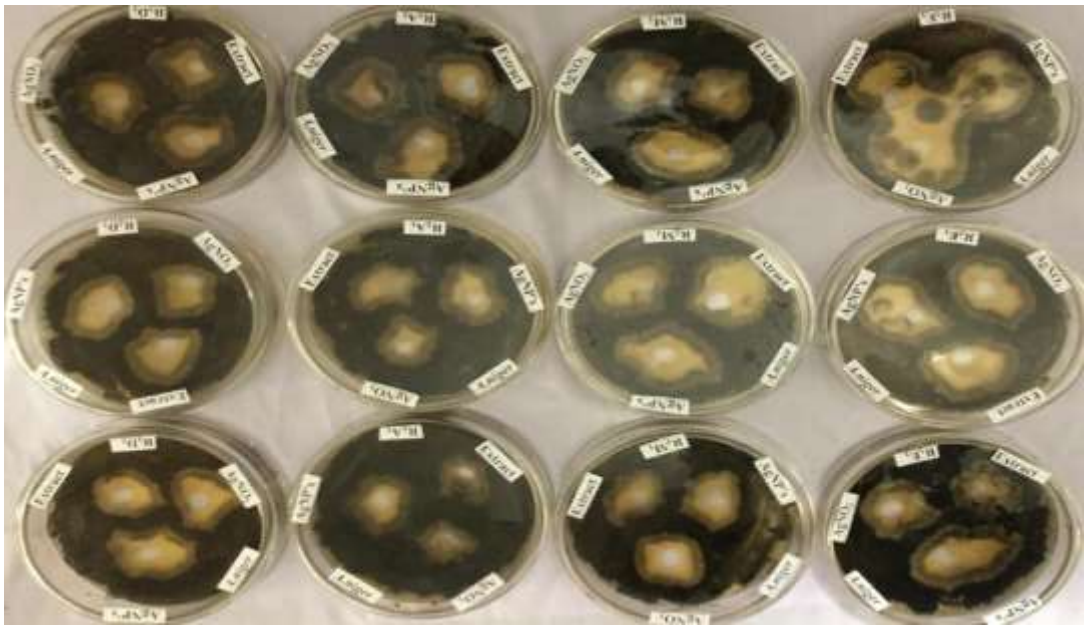


Fig 4.8. Activity of *Solanum nigrum* L. AgNPs against *A. niger*

Discussion

Silver itself have the ability to kill or inhibit the growth of microorganisms. This is the reason that in 19th century diluted solution of silver nitrate was used to treat bacterial infection due to its antimicrobial activity (Lui et al., 2006). Plant extracts also possess antimicrobial activity because of the presence phytochemicals and bio-active chemicals which are actually the secondary metabolites. These phytochemicals are responsible for antimicrobial activity of plant extracts. In comparison to the all extracted solvents, methanol extracts showed higher (as compare to nanoparticles made by ethanol, acetone and distilled water extracts) antimicrobial activity against microorganisms, the reason behind this difference may be the quality of extraction of different solvents. Studies revealed that, the extracts formed by the methanol extracts are more effective because they contain high intensity of phytochemicals, 16 bioactive compounds were found in the methanol extract, highest free

radical scavenging was also present in methanol extract. This is may be due to the high polarity of methanol. So, the combination of plant extract and $AgNO_3$ solution synthesize a better product with high antimicrobial activity, higher than both silver nitrate and plant extract. Flavonoids and phenolic acids are important to reduce silver salts for the synthesis of AgNPs. The studies have reported that methanol have better extraction efficiency for phenolic acids as well as flavonoids (Salehi et al., 2016; Dhawan and Gupta, 2018). This might be the reason that, nanoparticles made by methanol extract have high antimicrobial activity in comparison to the other solvent extracts.

Conclusion

Antibiotics resistance of microorganisms is a major threat of the present era. The need of the hour is to synthesize new antibiotics. Studies on antimicrobial activity of metallic silver nanoparticles are being conducted for last few years, to acquire knowledge about the activity of these nanoparticles, so one can use them as antibiotics in medical and pharmaceutical sectors. In the current research leaf extracts of *Solanum nigrum* L. was used for silver nanoparticles synthesis. Results shows that, the green synthesized nanoparticles have a very good antimicrobial activity, so they have the potential to be used in pharmaceutical sector for the synthesis of new antibiotics.

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