Comparative analysis of the Green Synthesis of Silver nanoparticles using *Azadirachta indica* (neem), *Punica granatum* (pomegranate), *Psidium guajava*, Clove buds extract and *Ocimum sanctum* (Tulsi) and their antimicrobial activity against pathogenic bacteria.

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ABSTRACT

Nanoparticles are the center of attraction in recent years due to their immense application in electronics along with biological sciences as antimicrobial agents. The metallic nanoparticles remarkably differ in their physical and chemical properties from their corresponding bulk form and can be used as an anti-microbial agent. Green synthesis of nanomaterials is the environmentally friendly approach and most emerging method of nanoparticle manufacture. The present work utilized leaves of Azadirachta indica (neem), Punica granatum (pomegranate), Psidium guajava, Clove buds extract, and Ocimum sanctum (Tulsi) for reduction of 3mM AgNO3 to silver nanoparticles and compare the antimicrobial effects. The synthesis of nanoparticles was confirmed by the change of color from a colorless to a brown-colored solution. Further characterization was done by UV-visible spectroscopy and FTIR analysis, and UV-vis spectrophotometer characterized AgNPs with a range between 450–500 nm. The cellular structure of silver nanoparticles was studied by Phase contrast which gives confirmation that silver nanoparticles are in pure form. In the FTIR study, the stretching was found to be 617.18,1521.95,1122.49 and 2337.57. Well diffusion method showed the antimicrobial effect of AgNPs on different microorganisms with the zone of inhibition of 0.7, 0.6, 0.5,1 mm for Staphylococcus aureus, 0.8,0.9, 1.2, 0.9mm for Bacillus sp.0,8,0.9,1.2,1.1mm for Streptococcus, 0.8,0.6,1.3, for Pseudomonas and 1.1,1.4,1 0.9 1.3mm for E. coli under MIC 5mg/ml Mode of action of antimicrobial activity of nanoparticles was investigated by determining leakage of reducing sugars and proteins, suggesting that AgNPs were able to destroy membrane permeability. Out of all plants screened Guava is the best-reducing agent in terms of synthesis rate. Guava-mediated AgNPs were found to be highly active against microbes based on the zone of inhibition and minimal inhibitory concentration.

Keyword: - *UV-visible, FTIR, , antibacterial activity, Silver Nanoparticles, SEM, MIC , ZOI….*

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1. INTRODUCTION.

Nanotechnology is a branch of science and technology which concerns with the development of process for the design, synthesis, and manipulation of particle structure, different shapes, sizes, and controlled disparity of Particles which having a diameter of less than 100 nm. Nanoparticles exhibit novel properties as compared to their larger particles of bulk materials. Nanoparticles have valuable applications in various fields like medicine, drug delivery, Xray imaging, agriculture and phphotothermalherapy biosensing, protein detection,DNA testing, imaging, tissue engineering, biological labeling, cosmetics, anti-aging drugs, surface-enhanced Raman scattering (SERS), and heat destruction of the tumor ((hyperthermia). etc. (Ejidike & Clayton, 2022). Metallic nanoparticles are mostly synthesized from precious metals viz., silver (Ag), gold (Au), platinum (Pt), and lead (Pb).but , silver is the most preferred metal in the area of the biological system due to its wide range of activities over other metals).due to its unique characters such as antimicrobial,anticancer,antiviral .and useful applications in the area of medicine and biology . Rigorous research has been done on silver nanoparticles (AgNPs) owing to their extensive variety of applications in medical, pharmacy and water decontamination due to their properties of antimicrobial action, adsorption sensing of food adulterants and DNA detection (Rautela, et al 2019)Nanoparticles can be synthesized by several methods , such as physical and chemical method but these methods are expensive and use of many toxic substances, which makes them difficult to scale these methods for mass production(Rakib-Uz-Zaman et al., 2022).Chemical reduction is the common method used for the synthesis of nanoparticles but due to lower yield and complex procedures for purification there was an strong need for the evolving a safe alternate method. This resulted in development of green synthesis which utilizes microorganisms and plant extracts for the reduction of silver to silver nanoparticles. In contrast to chemical method the synthesis of nanoparticles from plant extract using green route is now widely used due to its simplicity , cost effective , and environmental friendliness, as opposed to the conventional one such as chemical method. More so, biogenic reduction of metal precursors to generate corresponding nanoparticles is environmentally benign, cost effective, free from chemical contaminants and medical applications where purity of nanoparticles is of major concern(Yoro et al., 2022).so the present study was designed to screen potential of various plants leaves as reducing agent and screen the synthesized nanoparticle for antimicrobial activity against representative human pathogenic microorganisms *(Bacillus sp, Staphylococcus aureus, and Escherichia coli*). Minimum inhibitory concentration of silver nanoparticles against these microorganisms was determined in the present study.

2 . MATERIAL AND METHODS.

The following work was carried out at MIET Meerut in the department of Biotechnology and Microbiology ccs.For the synthesis of silver nanoparticles(Azadirachta indica (neem leaves), *Punica granatum* Leaves (pomegranate), *Psidium guajava*, clove buds extract and *Ocimum sanctum*(Tulsi) are collected from the herbal garden of MIET, Meerut (U.P.) (India).The Plant extract is used as a reducing and capping agent.

2.1 Medicinal properties:

2.1.1 .*Azadirachta indica*

Azadirachta indica A. *Juss.(Meliaceae)* (common name “neem”), is native to the Indian subcontinent and was introduced first to Africa and then to the rest of the world through the Middle East and Caribbean (Kumar and Navaratnam, 2013). A. indica possesses contraceptive properties (Patil et al., 2021). Use of Neem has been recommended by Ayurveda for a wide range of diseases. Such usage are attributed to its purification effect on blood. Scientific research on Neem demonstrates it to be a Panacea. It is suggested to be an antibacterial, anthelmintic, antiviral, anticancer and more importantly Immunomodulatory agent.

2.1.2 Punica granatum Leaves.

Punica granatum L. commonly known as pomegranate is a small tree of the family of *Lythraceae*. . It is native from Persia and has been cultivated extensively in the Mediterranean countries such as Tunisia, Turkey, Egypt, and Spain and to some extent also in California, China,Japan, and Russia .. pomegranate leaf extracts or active compounds isolated from those extracts could be used to improve human health especially the prevention and treatment of infectious diseases and to fight the emergence and spread of resistant bacterial strains. (Trabelsi et al., 2020

2.1.3 *Psidium guajava*

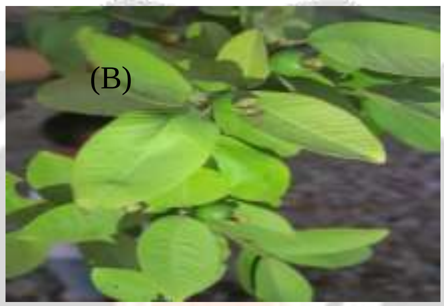
Psidium guajava, commonly known as the guava plant, is a small evergreen shrub belonging to the *Myrtaceae* family. The medicinal properties of the leaves and fruits of the P. guajava plant can be attributed to the high amount of carotenoids, essential oils, flavonoids, phenolic compounds, and vitamins. Due to their antibacterial and analgesic properties, these preparations were used to treat diabetes mellitus, hypertension, obesity, rheumatism, and gastrointestinal diseases like diarrhea and stomachache (Lok et al., 2023).

2.1.4 *Ocimum sanctum*

The Tulsi plant is a bushy shrub or small tree native to the tropics and subtropics. It smells and tastes completely different from anything else. *Ocimum* is used in cold distillation, vegetable or pulse soup, refreshing beverages, Ghrit (medicinal ghee), medicinal powders, medicinal oil, Sheeta jwarantak vati (anti-malaria pills), and tulsi tea . Tulsi has been studied for its extracts' potential to treat a wide range of illnesses, from the common cold to heart disease to headaches to gastrointestinal problems, so its medicinal uses are not limited to inflammatory disease.Mosquitoes and flies are just two Molecules of the many insects that can be repelled by tulsi (Hasan et al., 2023).

2.1.5 Clove buds.

Clove not only enhance the flavor, aroma, and color of food and beverages, but they can also protect from acute and chronic diseases. It possess antioxidant, anti-inflammatory, antitumorigenic, anticarcinogenic, and glucose- and cholesterol-lowering activities as well as properties that affect cognition and mood. Research over the past decade has reported on the diverse range of health properties that they possess via their bioactive constituents, including sulfur-containing compounds, tannins, alkaloids, phenolic diterpenes, and vitamins, especially flavonoids and polyphenols. (Jiang, 2019).



(A):Azadirachta indica ***(B): Psidium guajava*** ***(C): Punica granatum***

Botanical Name -*Azadirachta indica* .	Botanical Name- *Psidium guajava*	Botanical Name- *Punica granatum*
English Name -divine tree neem	English Name -Guava	English Name- pomegranate
Common Name -Neem	Common Name -Amrud	Common Name- Anar
Genus -*Azadirachta*	Genus -*Psidium*	Genus -*Punica*

(D): ***Ocimum sanctum*** ***(E) Syzygium aromaticum***

Botanical Name -*Ocimum sanctum*	Botanical Name- *Syzygium aromaticum*
EnglishName- Holy basil	EnglishName- Clove

Common Name- Tulsi	Common Name- laung
Genus -*Ocimum*	Genus- *Syzygium*

2.2. Preparation of extract.

Ocimum sanctum.

The Ocimum sanctum plant leaves were collected. About 20 gm of fresh leaves were thoroughly washed several times with tap water then washed again with distilled water and chopped into small pieces. Then 20 g finely incised leaves are added in100 ml of deionized water, stirred, and boiled for 30 min at 70∘ C. After boiling, the leaf broth was cooled and filtered with Whatman filter paper no. 1 and a pale yellow clear solution obtained was stored at 4 ∘ C(Khan et al., 2017). The plant material and leaf broth are showed in Figure1.

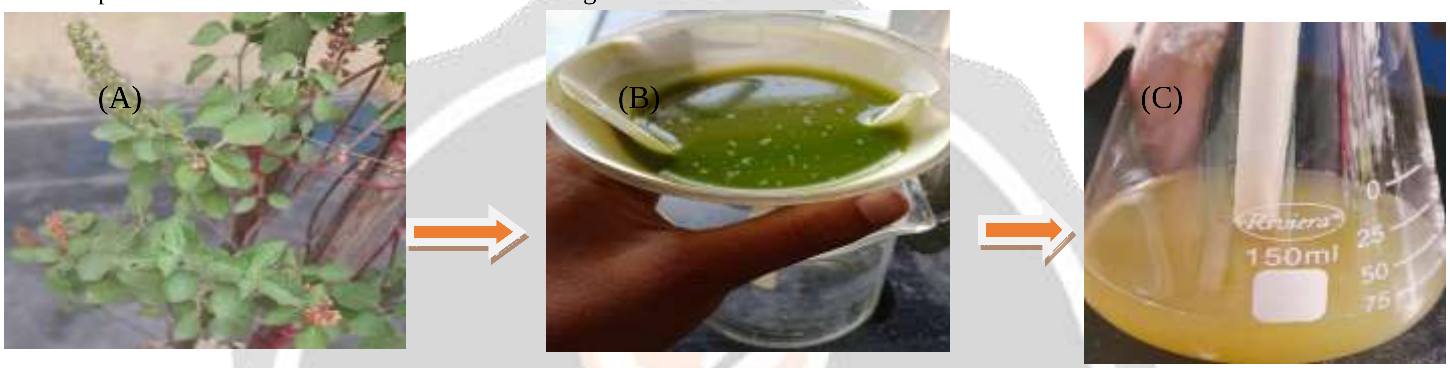


Figure.1 (a). *Ocimum sanctum*. Leaf used for synthesis, (b). filtration through Whatman filter paper no.1 (c). formation of leaf extract.

2.2.2 *Punica granatum*

Punica granatum leaves were freshly collected and washed multiple times with tap water then 2-5 times with double distilled water to remove dirt from the surface then surface sterilization is done with alcohol. Clean leaves were shade dried for 10 days to remove moisture content, followed by crushing with mortar and pestle. 10 g powder was added in 100 mL of deionized water and boiled at 90 0 Celsius for 15–20 min in a water bath. The solution was left at room temperature to cool. Whatman filter paper No. 1 was then used to filter the prepared extract and stored at 4 ∘ C till further requirement. (Singhal et al., 2021) seen in figure no. 2

Fig 2. Dried leaves of *Punica granatum* and (b) formation of extract.

2.2.3 *Psidium guajava*

Healthy plant samples were collected and were washed multiple times properly under running tap water to remove dust. The samples were shade dried for 15 days and homogenized to a fine powder using a mortar and pestle. 10g of powdered Psidium guajava Leaves was dissolved in 100ml of distilled water and heated for about 15 minutes at 60-70celsis in a water bath then stir the solution thoroughly on a magnetic stirrer for 20 -30 minutes cool. The extract was filtered using a Whatman No. 1 filter paper and kept the solution at 4 Celsius for further use. (Yoro et al., 2022)

Fig. 3 (a) Dried leaves of *Psidium guajava and (b) formation of extract.*

2.2.4 *Azadirachta indica*

Fresh green leaves of A. indica were selected and washed with distilled water. Dried for 5-6 days in air tight container to remove moisture. Dried leaves homogenized into fine powder using mortar pestle and weigh the 20 gram leaves with the help of weighing machine add into 100 ml deionized water and boiled for half an hour. After cooling to room temperature, the broth was filtered and stored at 4°C. (Bhat et al., 2019).

2.2.5 *Syzygium aromaticum*

First crushed the clove with the help of mortar pestle after that 2g of grounded clove bud powder was mixed with 300 mL of distilled water. Then the mixture was heated to boiling for 20 min in water bath to obtain its aqueous extract. After cooling at room temperature, the extract was filtered using Whatman No.1 filter paper and stored at 4°C for further experimental study. (Lakhan et al., 2020b).

Fig .4 (a) . Buds of *Syzygium aromaticum* and, (b). formation of clove extract**.**

2.2.5 synthesis of AgNPs

For the preparation, ofAgNP, 15 mL of aqueous of (A), 20ml of (T), 15ml of (C), 10ml of (G), and 18ml of (P) was added slowly to 100 mL of 3mM silver nitrate (AgNO3) solution. Drop by drop addition of silver aqueous extract for reduction of Ag + into Ag0.The resultant solution was continuously stirred for 60 min at 80°C. The color of the solution changed when the extract was added to the solution of AgNO3was pale-yellow the mixture solution was immediately placed in complete darkness placed for 24 hours. The formation of AgNPs is done by a color change from pale cream to dark brown after 24 hours. The appearance of darkish brown precipitation was a clear indication of AgNP formation a suggestive of surface plasmon resonance. The nanoparticle synthesis success was monitored via UV-vis spectroscopy. The AgNPs precipitates obtained were separated through repeated washing and centrifugation at 10000 rpm for 30 min then washed with distilled water and dried the sample with a rotary evaporator. (Ejidike & Clayton, 2022) (Singhal et al., 2021)

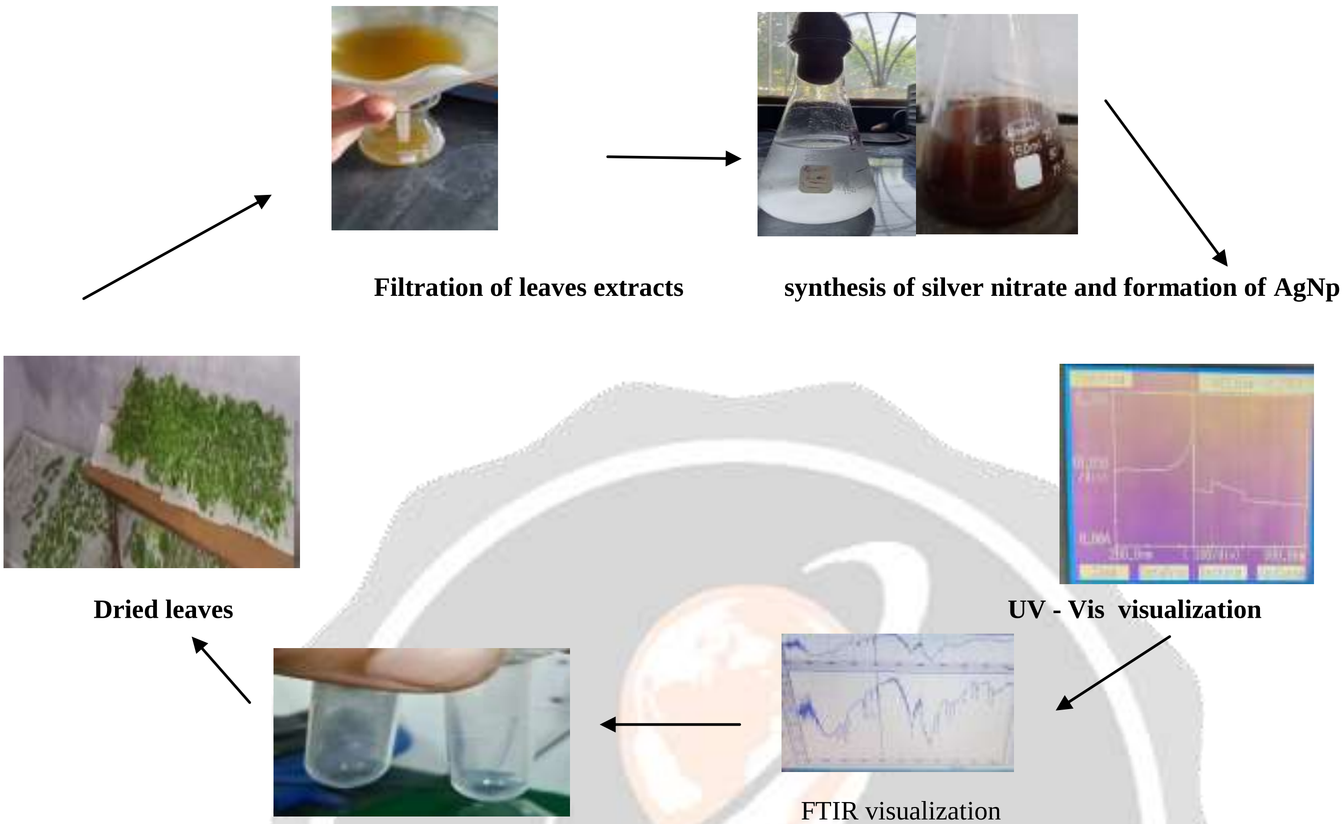


Fig.5: Schematic Representation of the production of silver Nanoparticles.

3. CHARACTERISATION OF SILVER NANOPARTICLES

3.1: UV-visible spectra analysis

The silver nanoparticles were confirmed by measuring the wavelength of reaction mixture in the UV-vis spectrum at a resolution of 1 nm (from 300 to 800 nm).

3.2: FTIR analysis:

The characterization of the active functional groups on the surface of silver nanoparticles (AgNPs) synthesized from *Azadirachta indica* (neem leaves), *Punica granatum* Leaves (pomegranate), *Psidium guajava*, clove buds extract and *Ocimum sanctum*(Tulsi) was investigated by FTIR analysis and the spectra was scanned in the range of 4000–400 cm^{-1} at a resolution of $4cm^{-1}$. The sample was prepared by dispersing the silver nanoparticles uniformly in distilled water as a matrix.

3.3. Antimicrobial analysis of synthesized silver nanoparticles

The synthesized silver nanoparticles using plant extracts were examined for antibacterial potential by agar well diffusion method against some selected gram-positive and gram-negative bacteria. The microbial culture was Collection Miet Meerut (U.P) India.

4. RESULT AND DISCUSSION

4.1 Visual examination.

The color change is the preliminary conformation for the formation of silver nanoparticles. Usually, unique optical property of silver nanoparticles exhibit variety of color based upon their size and shape. The color change is attained due to the effect of SPR of the nanoparticles (Kanniah et al., 2020). Neem ,pomegranate , Guava, Tulsi leaves and clove buds Aqueous Extracts were used to synthesized silver nanoparticles (AgNPs) In the experiments, addition of plant extract into the beakers containing aqueous solution of silver nitrate led to the change in the color of the solution to yellowish to reddish brown after 40 min due to excitation of surface plasmon vibrations in silver nanoparticles constant, the color of the solution changed from faint light to yellowish brown and finally to colloidal brown indicating formation of silver nanoparticles). The color exhibited by metallic nanoparticles is due to the coherent excitation of all the “free” electrons within the conduction band, leading to a phase oscillation. There is no significant change beyond 180 min, therefore indicating the completion of the reduction reaction. The color change in the solution is presented in Figs. This was further confirmed by UV vis spectroscopic analysis.

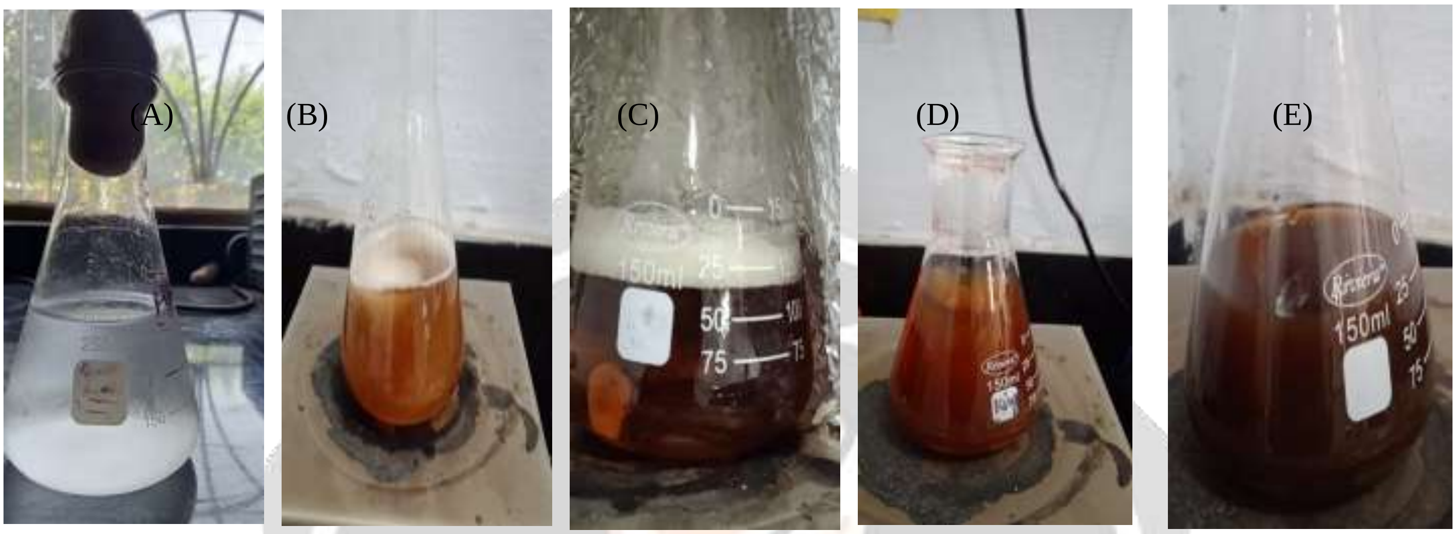


Figure 6. Synthesis of AgNPs at different time intervals (A) 15 min, (B) 30 min, (C) 1 h, (D) 1.5 h, and (E) 2 h. (A E), the color of the solution turns from a pale yellow to color to a dark brown color. The color is darkest at (E), indicating the presence of AgNP

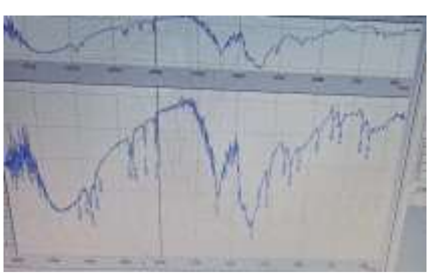
4.2 UV-vis absorption spectroscopy analysis.

UV-vis spectroscopy is the preliminary prominent tool to characterize the nanoparticles based on their size, shape and distribution. It was used to confirm the formation and stability of AgNPs in an aqueous solution.. Herein, UV–Vis spectrum of AgNPs revealed a surface plasmon resonance absorbance band peak between the ranges of 450–500 nm. The shifting of the absorbance peak from a shorter wavelength to a bigger wavelength revealed the formation of smaller to bigger nanoparticles, respectively(Lakhan et al., 2020). The absorption band intensity of the clove bud silver nanoparticle synthesized was identified 446nm. The produced AgNPs with plant extract of Punica granatum Leaves (pomegranate) Azadirachta indica (neem leaves), Ocimum sanctum(Tulsi), and Psidium guajava show a extreme absorption band of 443 nm,447nm,455nm, 448 nm. Initially, no SPR absorbance was observed in the UV-vis spectra, after that (10 mins) the surface plasmon resonance peak increases when increase the time of incubation. The SPR peak was monitored from 300–780 nm and it and the peak was centered at 460 nm. After 30 min incubation, the decreased SPR peak (40 mins) indicates that the formation of silver nanoparticles from silver metal ions was achieved maximum within 30 mins of reaction. (Kanniah et al. (2020**).**

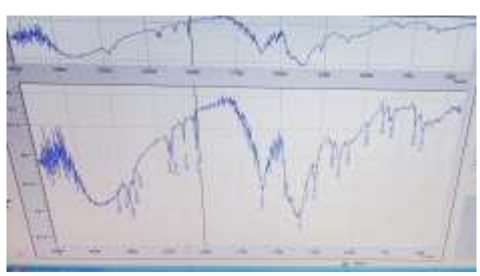
Graphical Representation of UV-Vis Spectrophotometer

4.2 FTIR Analysis.

FTIR used for the identification of possible biomolecules present in the plant extract responsible for the reduction of Ag+ into Ago. The spectrum of Extract/Ago shows different main peaks positioned at be 617.18,1521.95,1122.49 and 3402 The intense and wide peak appeared at 3402 cm−1 denoting the presence of hydrogen-bonded N-H stretching vibrations of amide groups respectively and 800 cm−1. AgNPs are capped with different bio moieties. The peak that appeared at 1122 cm−1 can be relevant to the C-N stretching vibration of aliphatic amine.



(A)



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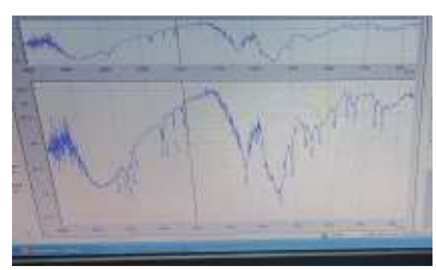
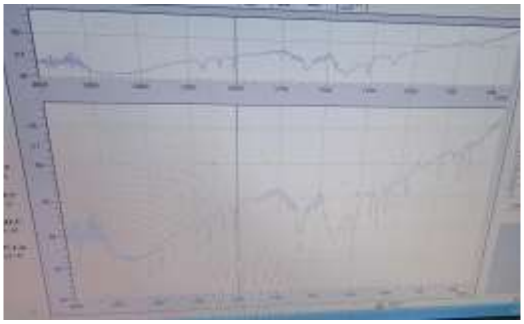
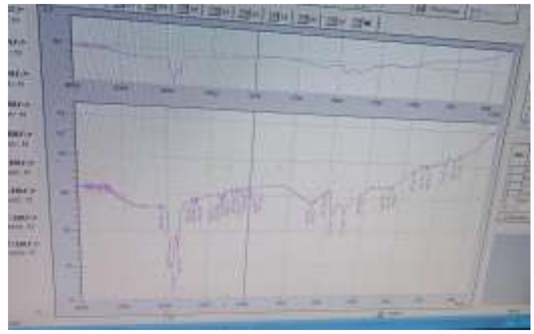


(C)



(D)

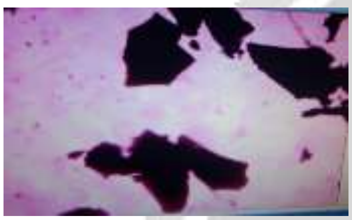


(E)

Fig 8 . FTIR analysis of AgNPs : (a) C . (b) G . (c) T . (d) N . (e) P

4.3 Phase contrast.

Phase-contrast microscopy is particularly used to reveals cellular structures of nanoparticles that are invisible with a naked eyes



AgNPs-G

AgNPs-C

AgNPs-T

s

AgNPs-P

AgNPs-N

Fig: .9 Morphology of AgNPs: Fig: Morphology of AgNPs:

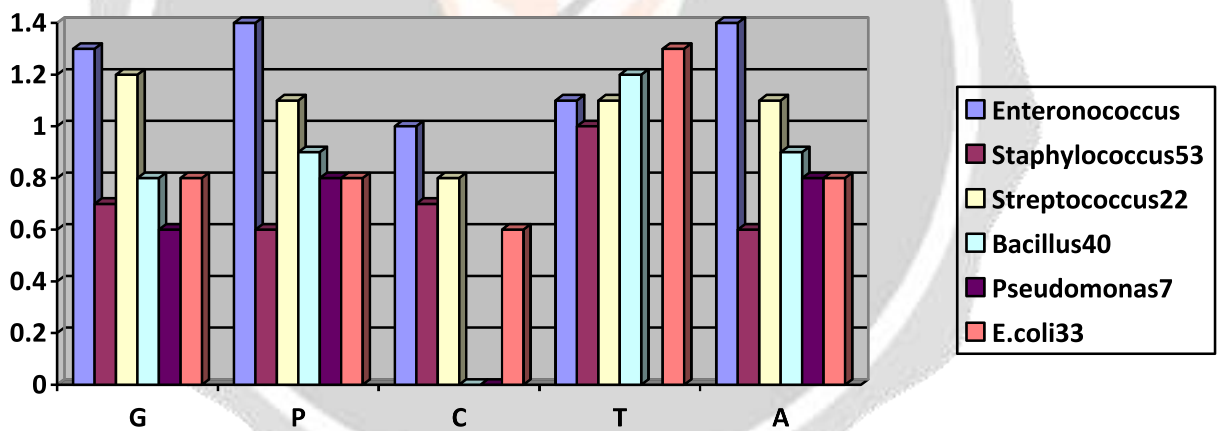
3.3 Antimicrobial action of Ag NP using well diffusion technique.

The antimicrobial action of Ag NP produced using plant extract was confirmed using well diffusion method, which is considered as one of the fastest and reliable method (Vinodhini et al., 2022). The antibacterial efficiency of the silver nanoparticle is analyzed with the help of microbial species like Pseudomonas sp.,*Staphylococcus sp., Streptococcus sp.,Bacillus sp., E.coli, and Enterococcus* The antimicrobial activity of silver particles in the presence of plant extracts in 24 h cultures is presented as MIC values. A control test was also performed on the extracts themselves in order to determine their effect on the specified microorganisms (Wasilewska et al., 2023)..The sample silver nanoparticle synthesized using *Azadirachta indica, Ocimum sanctum(Tulsi), Psidium guajava , Punica granatum* and clove bud is represented as N, T, G, P, and C Ag nanoparticle against the bacterial culture. In the sample G(Ag) and P(Ag) show highest zone of inhibition is observed while treating with *Pseudomonas sp(8)., Staphylococcus* then least is found with *Bacillus sp , E.coli* respectively. Similarly in case of sample- T(Ag) the greatest zone of inhibition was identified while treating through *Enterococcus* , *Bacillus, Streptococcus* and no zone of inhibition was found in the case of Escherichia coli respectively.Similarly, in the case of sample C(Ag) and N(Ag)show highest zone of inhibition is observed while treating *Enterococcus* , *Streptococcus* then least is found in case *Staphylococcus, E.coli*

.and no zone of inhibition is observed in case N(Ag) in *Pseudomonas* , in case of C (Ag). The comparison of the MIC results with the physicochemical data of nanoparticles synthesized in the presence of neem, pomegranate, and Tulsi, guava and clove extracts shows that the smaller the nano-particles, the stronger the antibacterial potential (Wasilewska et al., 2023). For Gram-positive bacteria, the strongest antibacterial activity is demonstrated by nanoparticles synthesized in the presence of Guvava extract. The nanoparticles show lower activity against Gram-negative bacteria,e.g. E. coli, which is linked to an outer membrane present in these bacteria (Wasilewska et al., 2023) .gram positive have thicker layer peptidoglycan layer so they are more sensitive towards silver nanoparticles then gram-negative bacteria. AgNPs could enhance the permeability of the membrane, and thus leakage of reducing sugars and proteins. There was an increase in the release of sugar and proteins in all the microorganisms as the incubation time increased.

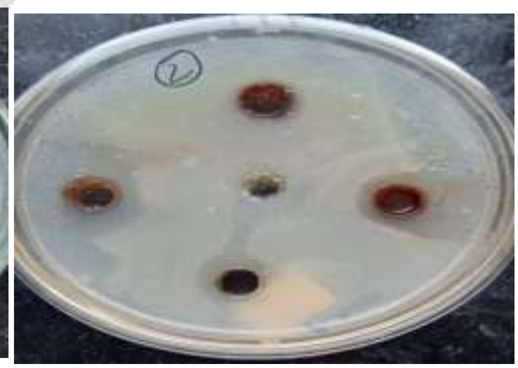
Table :1 Antibacterial efficiency of silver nanoparticles using well diffusion method.

S.no Name of microorganisms	Name of Plant extract to synthesis silver nanoparticles					
	Zone of inhibition in mm					
	Gram-positive bacteria					
	CONTROL	**G**	**P**	**C**	**T**	**A**
1. *Enterococcus*	-	1.3	1.4	1	1.1	1.4
2. *Staphylococcus*	-	0.7	0.6	0.7	1	0.6
3. *Streptococcus*	-	1.2	1.1	0.8	1.1	1.1
4 *Bacillus*	-	0.8	0.9	-	1.2	0.9
	Gram-negative bacteria-					
5. *Pseudomonas*	-	0.6	0.8	-	-	0.8
6. *E.coli*	-	0.8	0.8	0.6	1.3	0.8



Control.

Enterococcus



Staphylococcus

Streptococcus . *Pseudomonas* . *E.coli*

Fig 9: Graph of antibacterial activity of different AgNps

4. CONCLUSIONS .

UV-vis spectroscopy is the preliminary prominent tools to characterize the nanoparticles based on their size, shape and distribution. It was used to confirm the formation and stability of AgNPs in aqueous solution.. Herein, UV–Vis spectrum of AgNPs revealed a surface plasmon resonance absorbance band peak between the ranges of 450–500 nm. The shifting of the absorbance peak from a shorter wavelength to bigger wavelength revealed the formation of smaller to bigger nanoparticles, respectively(Lakhan et al., 2020). The absorption band intensity of clove bud silver nanoparticle synthesized was identified 446nm. The produced AgNPs with plant extract of Punica granatum Leaves (pomegranate) Azadirachta indica (neem leaves), *Ocimum sanctum*(Tulsi*), Psidium guajava* shows a extreme absorption band of 443 nm,447nm,445nm, 448 nm. Initially, no SPR absorbance was observed in the UV-vis spectra, after that (10 mins) the surface plasmon resonance peak increases when increase the time of incubation. The SPR peak was monitored from 300–780 nm and it and the peak was centered at 460 nm. After 30 min incubation, the decreased SPR peak (40 mins) indicates that the formation of silver nanoparticles from silver metal ions was achieved maximum within 30 mins of reaction. (Kanniah et al. (2020).

6. REFERENCES

1 .Lakhan, M. N., Chen, R., Shar, A. H., Chand, K., Shah, A. H., Ahmed, M., Ali, I., Ahmed, R. R., Liu, J., & Wang,
J. (2020). Eco-friendly green synthesis of clove buds extracts functionalized silver nanoparticles and evaluation of antibacterial and anti diatom activity. Journal of Microbiological Methods, 173, 105934. https://doi.org/10.1016/j.mimet.2020.105934

2. Kanniah, P., Radhamani, J., Chelliah, P., Muthusamy, N., Thangapandi, E. J. J. S. B., Thangapandi, J. R., Balakrishnan, S., & Shanmugam, R. (2020). Green Synthesis of Multifaceted Silver Nanoparticles Using the Flower

3.Extract of Aerva lanata and Evaluation of Its Biological and Environmental Applications. ChemistrySelect, 5(7), 2322–2331. https://doi.org/10.1002/slct.201903228

4 Vinodhini, S., Vithiya, B. S. M., & Prasad, T. a. A. (2022). Green synthesis of silver nanoparticles by employing the Allium fistulosum, Tabernaemontana divaricate and Basella alba leaf extracts for antimicrobial applications. Journal of King Saud University - Science, 34(4), 101939. https://doi.org/10.1016/j.jksus.2022.101939

5. Patil, S. M., Shirahatti, P. S., B, C. K. V., Ramu, R., & N, N. P. M. (2021). Azadirachta indica A. Juss (neem) as a contraceptive: An evidence-based review on its pharmacological efficiency. Phytomedicine, 88, 153596. https://doi.org/10.1016/j.phymed.2021.153596

6 .Trabelsi, A., Kaibi, M. a. E., Abbassi, A., Horchani, A., Chekir-Ghedira, L., & Ghedira, K. (2020). Phytochemical Study and Antibacterial and Antibiotic Modulation Activity of Punica granatum (Pomegranate) Leaves. Scientifica, 2020, 1–7. https://doi.org/10.1155/2020/8271203

7. Lok, B., Babu, D., Tabana, Y. M., Dahham, S. S., Adam, M. a. A., Barakat, K., & Sandai, D. (2023). The Anticancer Potential of Psidium guajava (Guava) Extracts. Life, 13(2), 346.
https://doi.org/10.3390/life13020346

8. Hasan, M. K., Alotaibi, B. S., Althafar, Z. M., Mujamammi, A. H., & Jameela, J. (2023). An Update on the Therapeutic Anticancer Potential of Ocimum sanctum L.: "Elixir of Life." Molecules, 28(3), 1193.
https://doi.org/10.3390/molecules28031193

9 .Jiang, T. A. (2019). Health Benefits of Culinary Herbs and Spices. Journal of AOAC International, 102(2), 395–411. https://doi.org/10.5740/jaoacint.18-0418

10 .Wasilewska, A., Klekotka, U., Zambrzycka, M., Zambrowski, G., Święcicka, I., & Kalska-Szostko, B. (2023).Physico-chemical properties and antimicrobial activity of silver nanoparticles fabricated by green synthesis. Food Chemistry, 400, 133960. https://doi.org/10.1016/j.foodchem.2022.133960

11. Dua, T. K., Giri, S., Nandi, G., Sahu, R., Shaw, T., & Paul, P. (2023). Green synthesis of silver nanoparticles using Eupatorium adenophorum leaf extract: characterizations, antioxidant, antibacterial and photocatalytic activities. Chemical Papers, 77(6), 2947–2956. https://doi.org/10.1007/s11696-023-02676-9

12. Khan, M. I., Tarek, F. K., Nuzat, M., Momin, M. A., & Hasan, M. Z. (2017). Rapid Biological Synthesis of Silver Nanoparticles from Ocimum sanctum and Their Characterization. Journal of Nanoscience, 2017, 1–6.
https://doi.org/10.1155/2017/1693416

13. Singhal, M., Chatterjee, S., Kumar, A., Syed, A., Bahkali, A. H., Gupta, N., & Nimesh, S. (2021). Exploring theAntibacterial and Antibiofilm Efficacy of Silver Nanoparticles Biosynthesized Using Punica granatum Leaves. Molecules, 26(19), 5762. https://doi.org/10.3390/molecules26195762

14 .Yoro, M., Garba, A., Joshua, J., & Isiyaku, A. (2022). Green Synthesis, Characterization and Antimicrobial Potency of Silver Nanoparticles from <i>Psidium</i><i> </i><i>guajava</i> Leaf Extract. Online Journal of Chemistry, 2(1), 14–22. https://doi.org/10.31586/ojc.2022.255

15. Bhat, R. S., Almusallam, J., Daihan, S. A., & Al-Dbass, A. (2019). Biosynthesis of silver nanoparticles using Azadirachta indica leaves: characterisation and impact on Staphylococcus aureus growth and glutathione-S-transferase activity. Iet Nanobiotechnology, 13(5), 498–502. https://doi.org/10.1049/iet-nbt.2018.5133

16 .silver nanoparticles and evaluation of antibacterial and antidiatom activity. Journal of Microbiological Methods, 173, 105934. https://doi Lakhan, M. N., Chen, R., Shar, A. H., Chand, K., Shah, A. H., Ahmed, M., Ali, I., Ahmed, R. R., Liu, J., & Wang, J. (2020b). Eco-friendly green synthesis of clove buds extract functionalized.org/10.1016/j.mimet.2020.105934

17. Rakib-Uz-Zaman, S. M., Apu, E. H., Muntasir, M., Mowna, S. A., Khanom, M. M., Jahan, S., Akter, N., Khan, M. I., Shuborna, N. S., Shams, S., & Khan, K. (2022). Biosynthesis of Silver Nanoparticles from Cymbopogon citratus Leaf Extract and Evaluation of Their Antimicrobial Properties. Challenges, 13(1),

18. https://doi.org/10.3390/challe13010018 18 Mohamed, S., & Mohamed, L. (2022). ECO-FRIENDLY, NON-TOXIC, AND HIGHLY ANTIMICROBIALBIOSYNTHESIZED OF Silver NANOPARTICLES (PAgNPs) FROM POMEGRANATE(PUNICA GRANATUM) PEEL (WASTE) EXTRACT (PPE) WITH MANY APPLICATIONS IN NANO INDUSTRIES AND PATHOGEN ELIMINATION FROM WATER. Egyptian Journal of Chemistry, 0(0), 0. https://doi.org/10.21608/ejchem.2022.156513.6782

19. Ejidike, I. P., & Clayton, H. S. (2022). Green synthesis of silver nanoparticles mediated by Daucus carota L.:antiradical, antimicrobial potentials, in vitro cytotoxicity against brain glioblastoma cells. Green Chemistry Letters and Reviews, 15(2), 298–311. https://doi.org/10.1080/17518253.2022.2054290

20 .Wasilewska, A., Klekotka, U., Zambrzycka, M., Zambrowski, G., Święcicka, I., & Kalska-Szostko, B. (2023).Physico-chemical properties and antimicrobial activity of silver nanoparticles fabricated by green synthesis. Food Chemistry, 400, 133960. https://doi.org/10.1016/j.foodchem.2022.133960

21. Ejidike, I. P., & Clayton, H. S. (2022b). Green synthesis of silver nanoparticles mediated by Daucus carota L.:antiradical, antimicrobial potentials, in vitro cytotoxicity against brain glioblastoma cells. Green Chemistry Letters and Reviews, 15(2), 298–311. https://doi.org/10.1080/17518253.2022.2054290

22. Singhal, M., Chatterjee, S., Kumar, A., Syed, A., Bahkali, A. H., Gupta, N., & Nimesh, S. (2021). Exploring the Antibacterial and Antibiofilm Efficacy of Silver Nanoparticles Biosynthesized Using Punica granatum Leaves. Molecules, 26(19), 5762. https://doi.org/10.3390/molecules26195762

23. Rautela, A., Rani, J. & Debnath (Das), M. Green synthesis of silver nanoparticles from Tectona grandis seeds extract: characterization and mechanism of antimicrobial action on different microorganisms. J Anal Sci Technol 10, 5 (2019)