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BIOSYNTHESIS OF SILVER NANOPARTICLES USING EXTRACTS OF TWO SPECIES OF *PORTULACA* AND THEIR ANTIBACTERIAL ACTIVITY

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Keywords:

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ABSTRACT: *Portulaca oleraceae* commonly known as Purslane and *Portulaca quadrifida* commonly known as chicken weed are consumed in salads, pickles, and soup in Asia, Africa, and Mediterranean region. In the traditional systems of medicine in Asia and Africa, they have been attributed to several pharmacological properties. Phytochemical investigations point to the presence of various beneficial constituents including flavonoids, tannins, alkaloids, polysaccharides, vitamins, unsaturated fatty acids among others. In this study, ethanolic extracts of *P. oleraceae* and *P. quadrifida* were prepared using microwave-assisted extraction. These extracts were used separately to synthesize silver nanoparticles, and their characteristics were studied using SEM, TEM, and FTIR analysis. The antibacterial activity was tested on different gram-positive and gram-negative bacteria using agar diffusion technique and measuring the zone of inhibition. The analysis of biogenic nanoparticles indicated the presence of chemical bonds between the surface of nanoparticles and phytochemicals. The nanoparticles were found to be mostly spherical of diameters ranging from 140 nm to 1.1 µm present in clusters. All the nanoparticle samples exhibited antibacterial activity against the tested bacteria. The microwave assisted extracts obtained from both the species of *Portulaca* were thus used for the synthesis of biogenic nanoparticles with antibacterial activity. The obtained silver nanoparticles could further be used in medical or pharmacological applications requiring antibacterial agents after proper safety and efficacy tests are conducted.

INTRODUCTION: *Portulaca oleraceae*, commonly known as purslane, (Family: Portulacaceae) grows as turfgrass or weed. The plant is used in the preparation of pickles, soup or salad owing to its sour taste.

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The plant might have originated in Asia but is now found in Africa, Australia, Mediterranean region and Asia.

The plant is indicated for use as anticancer, antidiabetic, hypocholesteremic, neuroprotective, hepatoprotective, nephroprotective, anti-inflammatory, antiulcer, antimicrobial in traditional systems of medicine in Asia and Africa [1-4]. Phytochemical studies have indicated the presence of polyphenolic compounds such as flavonoids, alkaloids, tannins, steroids as well as minerals, vitamins, essential fatty acids.

The extracts of purslane have anti-oxidant properties owing to the phenolic compounds [5]. *Portulaca quadrifida* is another species of *Portulaca*, commonly known as a chicken weed which grows as a small plant with succulent leaves and woody stem bears yellow flowers. Just like *P. oleraceae*, *P. quadrifida* has been indicated to possess medicinal properties and is used in the treatment of asthma, cough, urinary discharges, inflammations and ulcers, hemorrhoids. These two species of *Portulaca* have nutritional value due to the presence of polyunsaturated fatty acids namely omega-3 and omega-6 fatty acids [6].

With the progress of nanotechnology, the application of metallic nanoparticles is being investigated in the various fields such as wound healing, radio imaging, and potential candidates for anti-viral, anti-cancer treatment [7-14]. The conventional methods employed for nanoparticle synthesis make use of chemicals, reducing agents, capping agents and stabilizing agents [12]. With the advent of 'green nanotechnology,' novel methods for synthesis of nanoparticles were proposed. One of them being the use of phytochemicals as reducing agents and capping agents in synthesis. There have been numerous reports, especially of silver nanoparticle synthesis using plant-derived phytochemicals and this technique is relatively easy, economical, faster, can be carried out at neutral pH, ambient temperature and is eco-friendly [15-18].

In the present study, microwave assisted extraction technique was employed which is known to be economical, time-saving, solvent saving, and leads to improvement in extraction yield [19]. The obtained extracts of *Portulaca oleraceae* and *Portulaca quadrifida* were used to biosynthesize silver nanoparticles in the presence of bright sunlight irradiation. The silver nanoparticles were then characterized using scanning electron microscopy, transmission electron microscopy, Fourier transform infrared spectroscopy, and their antibacterial activity was tested using Gram-negative and Gram-positive bacteria.

EXPERIMENTAL:

Collection of Plant: The plants *Portulaca oleraceae* and *Portulaca quadrifida* were collected from a local farm of Walva taluka, District Sangli, State Maharashtra where they were growing like a weed. The plant specimens were authenticated by Dr. Dhanaji S. Pawar, Associate Professor, Department of Botany, M. H. Shinde Mahavidyalaya, Tisangi, State Maharashtra and specimen vouchers were deposited for *P. oleraceae* L. (V03) and *P. quadrifida* L. (V04). Some of the *P. oleraceae* plants were dried in the shade, and their seeds were separated and stored for further use.

Extraction: Microwave-Assisted Extraction was carried out in a controlled Catalyst microwave system having maximum power output 800 Watt, 50 gram (g) sample and 120 milliliters (ml) solvent for 20 minutes (min) [19]. The extracts obtained were as follows-

1. Ethanolic extract of fresh *Portulaca oleraceae* whole plant.
2. Ethanolic extract *Portulaca quadrifida* fresh whole plant.
3. Ethanolic extract *Portulaca oleraceae* dry whole plant.
4. Ethanolic extract *Portulaca oleraceae* seed.

The extracts were evaporated to dryness and stored at minus 20 degree Celsius (°C) deep freezer until required.

Green Synthesis: 2 milliliter (ml) each of extract mentioned above was separately transferred to 48 ml of 0.1 molar (M) silver nitrate solution in a beaker while constantly stirring the reaction mixture. The reaction mixture was then exposed to direct sunlight and change in color from colorless to brown was observed. The beaker was then left overnight at room temperature in the dark. Following day, the biogenic silver nanoparticles (AgNP) were separated by centrifugation and stored in a suitable container.

Physicochemical Characterization: Silver nano-particles biosynthesized by using the above-motioned procedure were characterized in Fourier Transform Infra-Red Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), and Transmission Electron Microscopy (TEM).

Fourier Transform Infra-Red Spectroscopy (FTIR): FTIR analysis of the silver nanoparticles

was performed using Nicklet 380 Thermo, US Fourier Transform Infra Red Spectroscopy.

Scanning Electron Microscopy (SEM): For SEM analysis, the colloidal solutions containing biogenic silver nanoparticles were separated by centrifugation. The supernatant was discarded, and final pellets were re-suspended in de-ionized water and air dried. The samples were then analyzed using INSTRUMENT JSM-6390.

Transmission Electron Microscopy (TEM): The measurement of the size of the biogenic silver nanoparticles was performed using Philips CM 200 transmission electron microscopy.

Antibacterial Activity: In antibacterial studies, standard agar well diffusion method as described in the Clinical Laboratory Institute (CLI) guidelines were used to test the anti-bacterial efficacy of biogenic silver nanoparticles. The organisms used for this study were gram-positive bacteria *Staphylococcus aureus*, *Streptococcus pyogenes*, and gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*. The organisms were clinical isolates obtained from Krishna Hospital, Karad. The efficacy of samples was measured in terms of the zone of inhibition.

The pure cultures of bacteria were subcultured appropriately on Muller Hinton Agar and Blood agar. *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* were cultured using Muller Hinton agar, and blood agar were used for *Streptococcus pyogenes*. Each strain was spread uniformly onto the individual plates. Wells of 10 mm diameter were made on nutrient agar plates using sterile micropipettes tips. 25 microliter (µl) of the nanoparticles solution was added to each well. The plates were incubated at 37 ºC for 24 h following which the diameter of the zone of inhibition was measured millimeter and results were recorded as the mean ± standard error of the mean (SEM) [20].

RESULT AND DISCUSSION:

Fourier Transform Infra-Red Spectroscopy (FTIR): The results for Fourier Transform Infra-Red Spectroscopy were as follows. The FTIR results and analysis of nanoparticles synthesized using the ethanolic extract of *Portulaca oleraceae* whole plant are as stated below **Fig. 1**, **Table 1**.

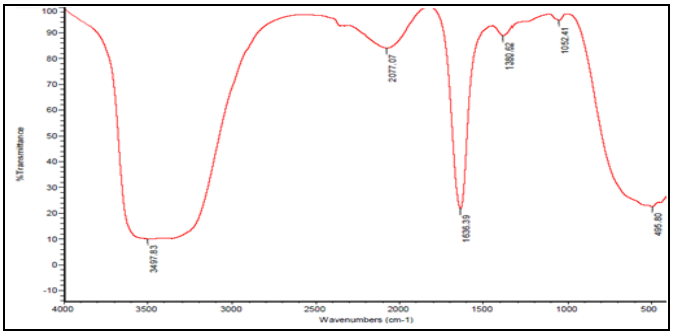

FIG. 1: FTIR RESULT FOR SILVER NANOPARTICLES SYNTHESIZED USING ETHANOLIC EXTRACT OF *PORTULACA OLERACEAE* WHOLE PLANT

TABLE 1: ANALYSIS OF FTIR RESULT FOR SILVER NANOPARTICLES SYNTHESIZED USING ETHANOLIC EXTRACT OF *PORTULACA OLERACEAE* WHOLE PLANT

Wavenumbers range (cm^{-1})	Functional group
3497.83	Amine NH-Strech
2077.07	Protein bond
1636.39	Aromatic C=C bending
1380.62	C-C Alkane
1052.41	Polysaccharide
495.80	Ammonia

As can be seen from the results obtained, the phytochemicals are bonded onto the surface of the silver nanoparticles via chemical bonds indicating that these proteins, aromatic compounds, poly-saccharides act as capping agents during the green synthesis of nanoparticles. The FTIR results for silver nanoparticles synthesized using the ethanolic extract of *Portulaca quadrifida* whole plant were as follows **Fig. 2**, **Table 2**.

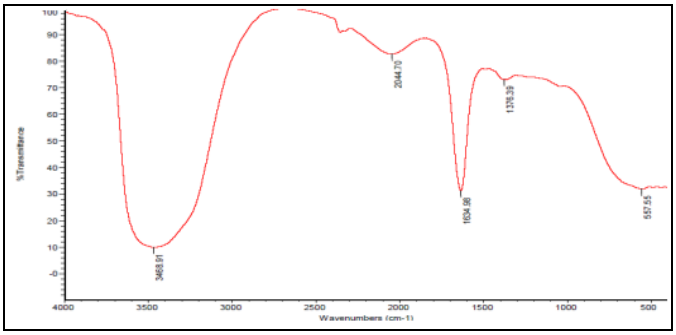

FIG. 2: FTIR RESULT FOR SILVER NANOPARTICLES SYNTHESIZED USING ETHANOLIC EXTRACT OF *PORTULACA QUADRIFIDA* WHOLE PLANT

TABLE 2: ANALYSIS OF FTIR RESULT FOR SILVER NANOPARTICLES SYNTHESIZED USING ETHANOLIC EXTRACT OF *PORTULACA QUADRIFIDA* WHOLE PLANT

Wave numbers range (cm^{-1})	Functional group
3468.91	N-H Stretching amides and amines
2044.70	Carbonyl group aldehyde = O
1634.98	C=O amides, Aromatic six-member rings
1376.39	C-N amines
557.55	Halogen compound

The above results indicate the presence of chemical bonds between the surface of silver nanoparticles and phytochemicals *via* an amide bond, carbonyl group indicating the involvement of amino acid moieties, aromatic compounds during nanoparticle synthesis. The FTIR results for silver nanoparticles synthesized using the ethanolic extract of *Portulaca oleraceae* dry whole plant were as follows **Fig. 3**, **Table 3**.

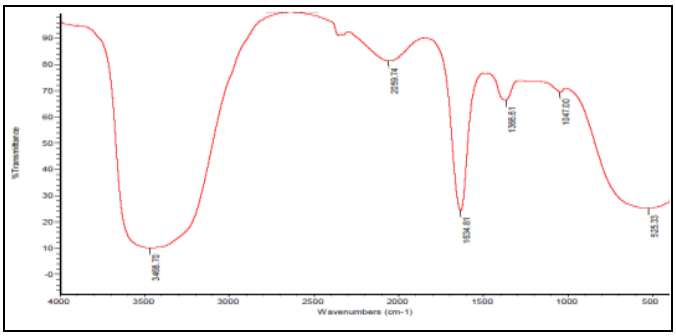

FIG. 3: FTIR RESULT FOR SILVER NANOPARTICLES SYNTHESIZED USING ETHANOLIC EXTRACT OF *PORTULACA OLERACEAE* DRY WHOLE PLANT

TABLE 3: ANALYSIS OF FTIR RESULT FOR SILVER NANOPARTICLES SYNTHESIZED USING ETHANOLIC EXTRACT OF *PORTULACA OLERACEAE* DRY WHOLE PLANT

Wave numbers range (cm^{-1})	Functional group
3468.70	N-H Stretching amides
2069.74	C-H Alkane
1634.81	Amide N-H stretching
1366.61	C-O
525.33	C-O

The results obtained indicate the presence of amide, carbonyl linkages between phytochemicals and surface of silver nanoparticles. The FTIR results for silver nanoparticles synthesized using an ethanolic extract of *Portulaca oleraceae* seeds were as follows **Fig. 4**, **Table 4**.

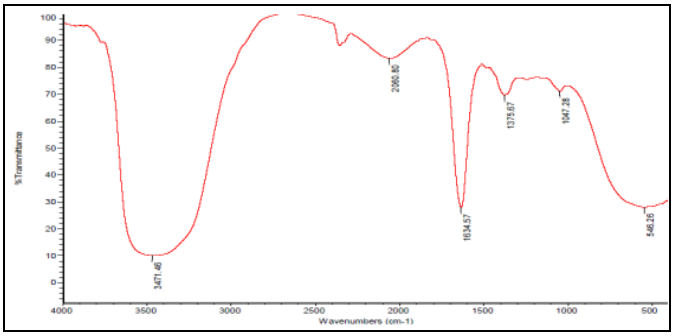

FIG. 4: FTIR RESULT FOR SILVER NANOPARTICLES SYNTHESIZED USING ETHANOLIC EXTRACT OF *PORTULACA OLERACEAE* SEEDS

TABLE 4: ANALYSIS OF FTIR RESULT FOR SILVER NANOPARTICLES SYNTHESIZED USING ETHANOLIC EXTRACT OF *PORTULACA OLERACEAE* SEEDS

Wavenumbers range (cm^{-1})	Functional group
3471.46	Carbonyl group
2060.80	Silicon compound
1634.57	Carbonyl group ester c=o alkene
1375.67	Carbonyl group ester c=o alkene
1047.28	Alkylamine
546.26	Halogen c-c

The above results indicate the presence of chemical bond between nanoparticles and carbonyl group-containing compounds which might be unsaturated fatty acids, in addition to amide linkages.

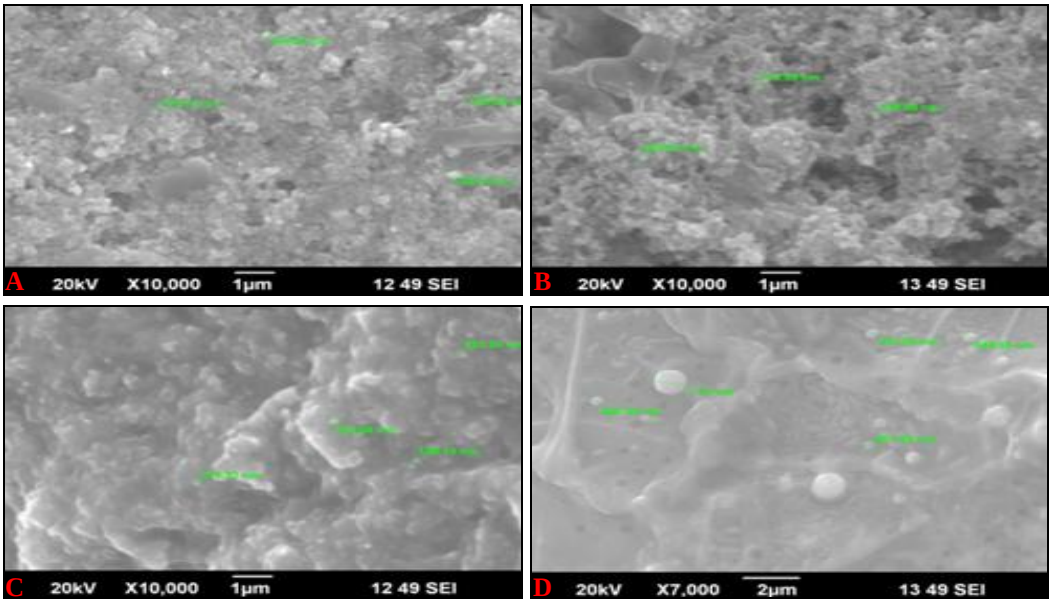

FIG. 5: THE RESULTS OF SCANNING ELECTRON MICROSCOPY OF BIOGENIC SILVER NANOPARTICLES SYNTHESIZED USING (A) ETHANOLIC EXTRACT OF *P. OLERACEAE* FRESH WHOLE PLANT; (B) ETHANOLIC EXTRACT OF *P. QUADRIFIDA* FRESH WHOLE PLANT; (C) ETHANOLIC EXTRACT OF *P. OLERACEAE* DRY WHOLE PLANT; (D) ETHANOLIC EXTRACT OF *P. OLERACEAE* SEEDS

TABLE 5: THE DIAMETER OF SILVER NANOPARTICLES STUDIED USING SCANNING ELECTRON MICROSCOPY

S. no.	Sample	Diameter
1	AgNP using the ethanolic extract of fresh *P. oleraceae*	140-189 nm
2	AgNP using the ethanolic extract of fresh *P. quadrifida*	178-228 nm
3	AgNP using the ethanolic extract of dry *P. oleraceae*	189-233 nm
4	AgNP using the ethanolic extract of *P. oleraceae*seeds	1.13 µm, 344-446 nm

Scanning Electron Microscopy (SEM): Biogenic silver nanoparticles as polycrystalline structure were analyzed using scanning electron microscopy. The analysis indicated that all four nanoparticle samples were presented as clusters **Fig. 5A-D**. The biogenic silver nanoparticles were found to be uniform and relatively globular. The diameters for silver nanoparticle samples were as follows in **Table 5**.

Transmission Electron Microscopy (TEM): Morphology of the biogenic silver nanoparticles of was investigated by TEM ad the results were as follows **Fig. 6**.

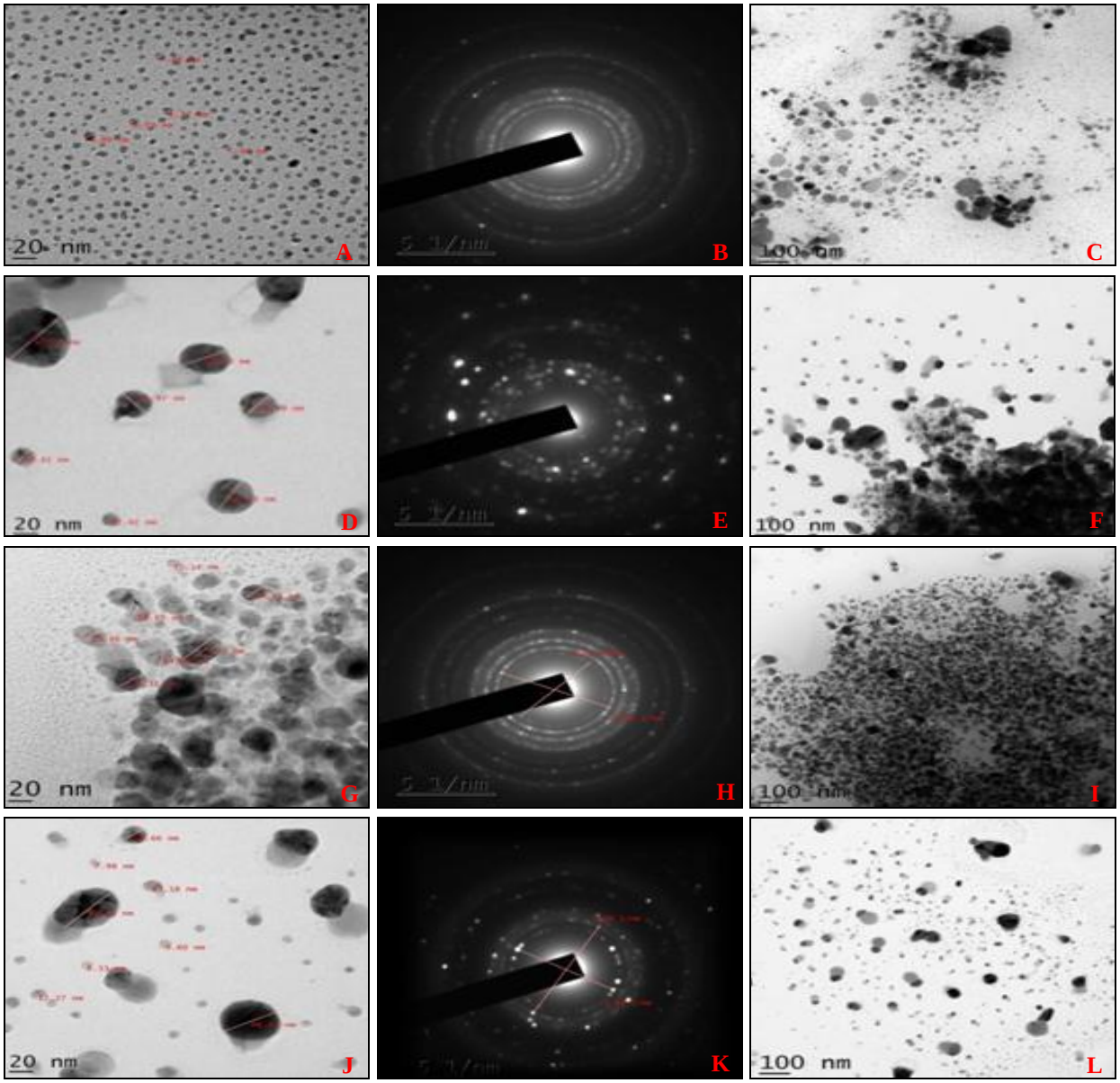

FIG. 6: THE RESULTS OF TRANSMISSION ELECTRON MICROSCOPY OF BIOGENIC SILVER NANOPARTICLES SYNTHESIZED USING (A, B, C) ETHANOLIC EXTRACT OF *P. OLERACEAE* FRESH WHOLE PLANT; (D, E, F) ETHANOLIC EXTRACT OF *P. QUADRIFIDA* FRESH WHOLE PLANT; (G, H, I) ETHANOLIC EXTRACT OF *P. OLERACEAE* DRY WHOLE PLANT; (J, K, L) ETHANOLIC EXTRACT OF *P. OLERACEAE* SEEDS

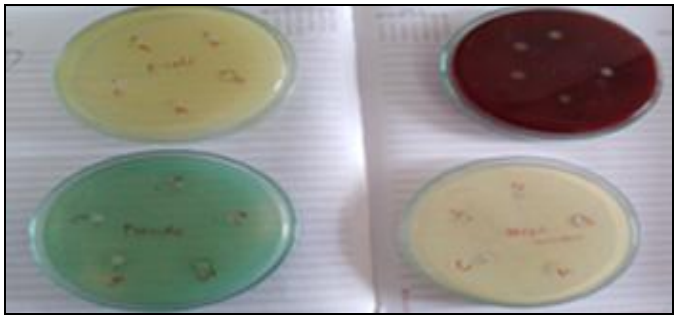

FIG. 7: THE ZONE OF INHIBITION OBSERVED DUE TO ANTI-BACTERIAL ACTIVITY OF SILVER NANO-PARTICLES ON AGAR PLATES OF THE RESPECTIVE CULTURED BACTERIA

Antibacterial Activity: The anti-bacterial activity of biogenic silver nanoparticles was studied using agar well diffusion method. The zone of inhibition for each sample performed in triplicate was measured, and the results were as follows **Fig. 7**.

Antibacterial activity has measured the efficacy of biogenic silver nanoparticles against bacterial strains was found in ascending order of *Escherichia coli* > *Pseudomonas aeruginosa* > *Staphylococcus aureus* > *Streptococcus pyogenes* **Table 6**.

TABLE 6: THE ZONE OF INHIBITION OF TESTED BACTERIA AFTER EXPOSURE TO BIOGENIC SILVER NANOPARTICLES

S. no.	Bacterial Strains	Nutrient media used	Zone of inhibition (mm), Mean ± S.E.M.			
			AgNP *P. oleraceae* whole plant	AgNP *P. quadrifida* whole plant	AgNP *P. oleraceae* dry plant	AgNP *P. oleraceae* seed
1	*Escherichia coli*	Muller Hinton agar	26.3 ± 0.2	27.3 ± 0.1	25.7 ± 0.1	27.7 ± 0.1
2	*Pseudomonas aeruginosa*	Muller Hinton agar	24.3 ± 0.1	23.3 ± 0.1	24.7 ± 0.2	26.7 ± 0.2
3	*Staphylococcus aureus*	Muller Hinton agar	15.7 ± 0.2	16.7 ± 0.2	19.3 ± 0.1	22.3 ± 0.1
4	*Streptococcus pyogenes*	Blood agar	12.3 ± 0.1	11.3 ± 0.1	15.3 ± 0.1	17.3 ± 0.1

All the biogenic silver nanoparticles exhibited antibacterial activity against strains of Gram-negative and gram-positive bacteria after 24 h exposure. The relatively greater zone of inhibition against gram-negative bacteria (*E. coli*, *P. aeruginosa*) may be attributed to the absence of the peptidoglycan layer of the cell wall which is otherwise present in Gram-positive bacteria (*S. aureus*, *S. pyogenes*) making them more susceptible to nanoparticles.

Silver nanoparticles synthesized using plant extract have been reported to exhibit similar antibacterial activity and physical characteristics studied using SEM, TEM, and FTIR [14, 16, 20]. The antibacterial activity was reported to be greater for Gram-negative bacteria than Gram-positive bacteria. The biogenic silver nanoparticles can thus be synthesized in a relatively economical, safer way and require less instrumentation as compared to conventional chemical methods of nanoparticle synthesis.

CONCLUSION: The described method of 'green nanotechnology' was successfully employed to synthesize silver nanoparticles using four separate plant extracts. The synthesis of nanoparticles was relatively simple, convenient and required a low level of instrumentation. The characteristics of nanoparticles were studied using SEM, TEM, and FTIR. The nanoparticles also exhibited antibacterial activity against all the tested bacterial strains.

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CONFLICT OF INTEREST: None to be declared.

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