Report on Characterization and Antimicrobial Activity of Biogenic Synthesis of Silver Nanoparticles from Amchur (Indian Spice) obtained from *Mangifera indica*: A Sustainable Resource

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Abstract
Rapid development and advancements in nanotechnology with its green synthesis approach for nanoparticle preparation have led to many opportunities for research. This study discusses the synthesis of silver nanoparticles (SNPs) from the aqueous extracts (AE) of dried mango fruit (Amchur), an Indian spice prepared from Mangifera indica. Green synthesis of SNP was done by preparing an AE of Amchur mixed with 1 mM $AgNO_3$. The characterization of the synthesized SNP was done by observing the color change from light yellow to deep brown and with UV-VIS spectrophotometry from the 300-700 nm range. Further characterizations were done using X-ray diffraction (XRD), Scanning electron microscopy (SEM), Energy-dispersive X-ray spectroscopy (EDX) and Fourier transform infrared spectroscopy (FTIR). The phytochemical tests were performed from the crude AE of amchur proving the presence of several plant secondary metabolites like phenolic compounds, saponins and sugars.

The antimicrobial activity (AMA) of Amchur SNP (ASNP) was detected using the disc-diffusion method against Gram-positive, Gram-negative bacteria and Candida species. Statistical analysis was done using a single-factor ANOVA.

t-tests were used to compare populations of interest and statistical significance was defined as $p < 0.05$. The average size of the green synthesized SNPs was ~10 nm and cubic. The absorbance spectrum was observed at the peak of 440 nm in UV-Vis spectroscopy. The antibacterial activity was found to be more potent than the antifungal activity provided by the SNPs. The inhibitory effect of the ASNP is satisfactory in comparison to the standard drugs. The current study sought to create a novel, economical, environmentally benign method for plant-mediated SNP production and its antimicrobial efficacy.

Keywords: Green synthesis, silver nanoparticles, dried mango powder, antimicrobial activity (AMA).

Introduction
Nanotechnology has numerous applications including agriculture[36], wastewater treatment, medicine, biosensing[33], medication delivery, phytopathology[37], textiles, cosmetics, plant stress[35] and the food sector[21]. Biological methods for nanoparticle synthesis are preferred due to their advantages over physical and chemical approaches[25]. The biological or green technique uses resources like plants, algae and microbes to synthesize metallic nanoparticles without the use of toxic chemicals[9,20,24]. The green approach uses plant parts and is simple, quick, cost-effective and environmentally beneficial offering numerous added benefits[10,29]. Plant parts contain various phytoconstituents including phenols, flavonoids, saponins, glycosides, tannins, alkaloids and anthocyanins[38] which act as reducing and stabilizing agents for nanoparticle synthesis, eliminating the need for toxic solvents and chemicals.

Phenolic acid, flavonoids, alkaloids and terpenoids are the phytochemicals used to manufacture green nanoparticles. These compounds are engaged in the reduction of metallic compounds to create nanosized metallic particles[5]. Silver nanoparticles or SNPs are produced using a variety of metals, but because they can be produced chemically or biologically, they are thought to be very important in the medical area. Silver (Ag) has a strong antibacterial activity and a very particular surface area for maximal environmental contact[16]. Since silver has special qualities that make it a wound healer and is employed in the biomedical area, it is also the most extensively used noble metal.

Dried mango powder has been utilized in Indian cuisine for thousands of years and acts as a taste enhancer. Mango has been shown in research to have anti-enteric[30], antibacterial[13], antidiarrheal[7] and antioxidant effects[22]. Silver nanoparticles were successfully biosynthesized from several mango sections, including the peel[41], leaves[34], kernel[31] and blossom[3]. However, silver nanoparticle synthesis utilizing dry mango powder is yet to be documented.

This study aims to investigate the potential of dried mango powder from *M. indica*, a traditional medicinal plant of India, in the green production of silver nanoparticles (SNPs). Biogenic SNPs were tested for antibacterial properties against pathogenic microbes such as *Staphylococcus*, *Streptococcus*, *E. coli* and *Candida spp*.

The generation of SNPs is attributed to the bioreductive capacity of *M. indica*[32]. Earlier research has shown that when exposed to metal salts, the phytochemicals included in plant extracts function as reductants to prepare the SNPs[14]. Owing to the swift advancement of nanostructured noble metals and their application in healthcare, this work focuses on creating SNPs using *M. indica* amchur extract and Indian spice, which possess numerous medicinal properties. The *M. indica* amchur extract's phytochemical content was examined qualitatively.

Material and Methods

Plant Material: The raw fruits of *Mangifera indica* (Anacardiaceae) were harvested in April 2022 from the Agricultural Research Station, Banswara (Rajasthan) with a latitude of 23.54ºN and a longitude of 74.433ºE. The identification of the plant material was done at the Herbarium of Botany Department, University of Rajasthan, Jaipur and a reference number was provided (RUBL 21398). The samples collected were thoroughly washed under the tap water followed by distilled water. The process was repeated at least three times for the sample collected to remove the dirt and dust properly. After washing the samples, they were cleaned and dried to remove the excess water by keeping them on muslin cloth. Later, the samples were shade-dried till they became suitable to be grounded to fine powder.

Plant Extract Preparation: According to Dudhane et al[12], 3 gm of dried mango powder was added to 100 ml of distilled water to prepare the aqueous extract (AE) in a conical flask. The solution was kept for heating at 60°C for 30 minutes on a water bath with continuous stirring and kept on shaker overnight for thorough dissolution at room temperature. Overnight filtration of the aqueous extract of the sample was done using Whatmann filter paper no. 1. The extract was then centrifuged at 5000 rpm for 5 minutes and the supernatant was collected. The filtrate was then adjusted to 100 ml with deionized water (DW) and the rest was stored in the refrigerator.

Green Synthesis of Silver Nanoparticles: Silver (II) nitrate ($AgNO_3$) of AR grade (Hi-Media) was used in this study as a starting material for nanoparticle synthesis. 1 mM (millimolar) of $AgNO_3$ was prepared using 100 ml DW with continuous stirring. 20 ml of the prepared plant extract were then added to 80 ml of 1mM $AgNO_3$. The mixture was continuously stirred on the magnetic stirrer for 10-15 mins and then incubated at room temperature until the color change was observed from light to dark brown.

The color change indicated the formation of SNP (Figure 1). The solution was filtered and poured into a wide glass tray for drying in a hot air oven at 50ºC. Once the solution got dried completely, it was scratched from the plate using a sterile scalpel blade and stored in the vials at 4ºC for the characterization.

Microbial strains used: The microbial strains used for detecting the antimicrobial activity (AMA) of SNPs were obtained from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh. The microbes were maintained on agar slants with time to time sub-culturing using Muller Hinton broth (MHB) and Muller Hinton agar (MHA) for bacteria and Sabouraud dextrose agar (SDA) for fungus at 37ºC for 24 hrs and 3-5 days respectively. The following microbes were used for the study:

1. Gram-positive Bacteria:
   a) *Staphylococcus aureus* (SA) MTCC 96
   b) *Streptococcus pyogenes* (SP) MTCC 442
2. Gram-negative Bacteria:
   a) *Pseudomonas aeruginosa* (PA) MTCC 3541
   b) *Escherichia coli (*EC*)* MTCC 729
3. Fungal Strains:
   a) *Candida albicans* (CA) MTCC 3017
   b) *Candida glabrata* (CG) MTCC 3019

Statistical Analysis: The biological experiments were conducted in triplicate and independently three times. The mean ± standard deviation (SD) represents the results. To find the equality of population means, statistical analysis was done using single-factor analysis of variance. t-tests were used to compare populations of interest and statistical significance was defined as $p < 0.05$.

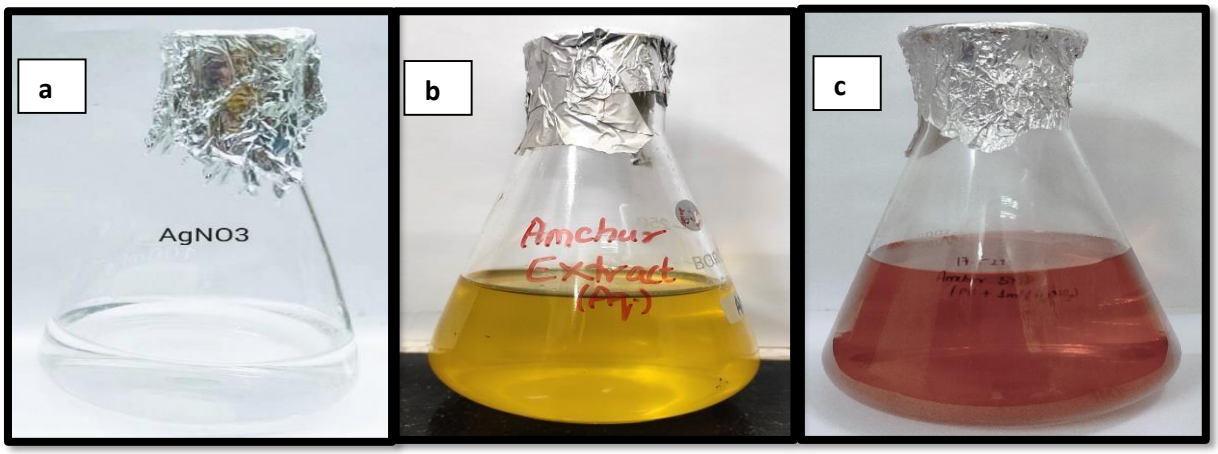

Figure 1: Color change after adding (a) $AgNO_3$ to (b) Amchur extract forming (c) ASNP (Amchur SNP).

Characterization: The green synthesized silver nanoparticles (SNPs) from the dried mango powder were subjected to morphological and structural studies through various characterizing techniques. The formation of nanoparticles was monitored by visual assessment of the color changes of the solutions. The reduction of metal ions to zero-valent nanoparticles was measured periodically at a wavelength range of 300 nm-700 nm using a UV-Vis spectrophotometer (Shimadzu-1900i series). The powder X-ray diffraction (XRD) was performed using a Bruker AXS D8 Advance X-ray diffractometer. The crystal structure and peak data were analyzed at a voltage of 40 kV and a current of 30 mA using radiation of wavelength 1.540 Å between 2θ° angles of 30°-80°. A Bruker FTIR Alpha spectrophotometer was used for the FTIR spectroscopy.

The FTIR spectra were observed at a spatial resolution of 4 cm^{-1} in the transmission mode between 4000–400 cm^{-1}. The KBr pellet technique was used for the sample preparation and the analysis was done for the bio-functional group present in the samples under examination and to determine the surface chemistry of the reduced silver ion. The morphological study of the green synthesized SNPs was analyzed using a Carl Zeiss Gemini field emission electron scanning electron microscope (FESEM) (Zeiss Gemini SEM300).

The elemental study was done using Energy-Dispersive X-ray (EDX) analysis embedded within the SEM instrument. Using the conventional disc-diffusion method, the antimicrobial properties of the biosynthesized silver nanoparticles were tested on Gram-positive (SA and SP), Gram-negative (EC and PA) and fungal strains of *Candida spp*. Fluconazole and ampicillin were used as the standard drug (positive control) for AMA and the zone of inhibition (ZI) during the sensitivity test was evaluated. The microbial culture was grown in a sterilized MH-agar and SDA medium respectively.

Antimicrobial Susceptibility Test: To cultivate newly acquired microbial strains, they were inoculated in nutrient broth (NB) (Hi-Media, Mumbai, India). The cultures were then incubated for 18 to 24 hours at 37°C. Additionally, the cultures were grown on nutrient agar (NA). SDA (Hi-Media, India) and MHA were used for overnight re-inoculation of cultures, yielding good growth. Following an overnight growth period in the NB, these cultures were examined for viability and microbial culture density by adjusting to the 0.5 McFarland standard[1].

In parallel, previously established techniques were used to prepare the autoclaved or sterilized MHA and SDA (Hi-Media, India) plates for AMA[4,6,40]. Using a sterile cotton swab, the overnight cultures of the four strains of bacteria and two strains of fungus were spread out on the agar plate following the media's solidification. In this instance, the 5 mm sterile paper discs were labelled with the corresponding concentration and employed in the disc diffusion test. In the present investigation, paper discs were employed that were inoculated with 1 mM $AgNO_3$, 50 µl of ASNP1 and 100 µl of ASNP2 and the crude extract of Amchur respectively.

After being partially dried by air, the paper discs were evenly distributed throughout the infected plates. Paper discs soaked in autoclaved water (DW) were used as a negative control in these antimicrobial plates whereas ampicillin (10 mcg/disc for bacteria) and fluconazole (10mcg/disc for fungi) from Hi-Media Mumbai, India were used as a positive control. Antimicrobial activity was assessed after 24–36 hours by measuring the diameter of the clear zone of inhibition (ZI) surrounding the discs. The experiment was conducted in triplicate. The SNP's mean ZI diameters (mm) were used to quantify the antimicrobial activity.

Results and Discussion

UV-VIS Spectroscopy: A rare visual phenomenon observed at the nanoscale by silver particles is called surface plasmon resonance (SPR). It is caused by conducting metal surface electrons oscillating cumulatively when they are in resonance with non-particulate radiation. The type, size, shape and immediate chemical environment of the particles all have a significant impact on this characteristic. The SNP-containing fingerprint zone typically lies in the 400–500 nm range peculiar to silver nanoparticles[17,39]. The absorption spectra of amchur derived from the *M. indica* plant are displayed in figure 2. The sample's unique SPR zone, which falls within the specified range, is visible in the spectra at the range of 440nm. Additionally, the study shows that when the SNPs' color darkens over time, their absorbance rises and approaches the blue wavelength shift.

X-Ray Diffraction Analysis (XRD): After the amchur sample of *M. indica* was reduced, five strong peaks were observed at the 2θ angles of 32°, 38°, 46°, 64° and 77° respectively, in the XRD analysis of the silver nanoparticles (Figure 3). Four distinct ICDD cards—87-0718, 87-0719, 87-0717 and 03-0921—match the patterns. The dimension, hkl (Miller Indices) and 2θ values of the generated silver powders are displayed in table 1. As per the ICDD (International Centre for Diffraction Data) card standards, the resulting silver powders possess an FCC (face-centered cubic) lattice structure with a cubic system standard to the silver nanoparticles.

The development of nanoparticles is indicated by the broadening of Bragg's peaks. The Debye-Scherrer equation $D= 0.94 \lambda/\beta \cos \theta$ was utilized to determine the average size of silver nanoparticles where β is the full width at half maximum (FWHM), θ is the diffraction angle, λ is the X-ray wavelength and D is the average crystallite domain size perpendicular to the reflecting planes. The FWHM of the peaks indicates that the computed average size is 9.91 nm.

Fourier Transform Infrared Spectroscopy Analysis (FT-IR): The FTIR spectrum showed the peak at 3468/cm which indicates the presence of O-H stretching and H-bonding of

alcohols and phenols. The peaks at 2928/cm indicate the presence of the O-H stretch of carboxylic acids. The peak observed at 1734/cm represents the C=O stretch of the carbonyl functional group of aldehydes.

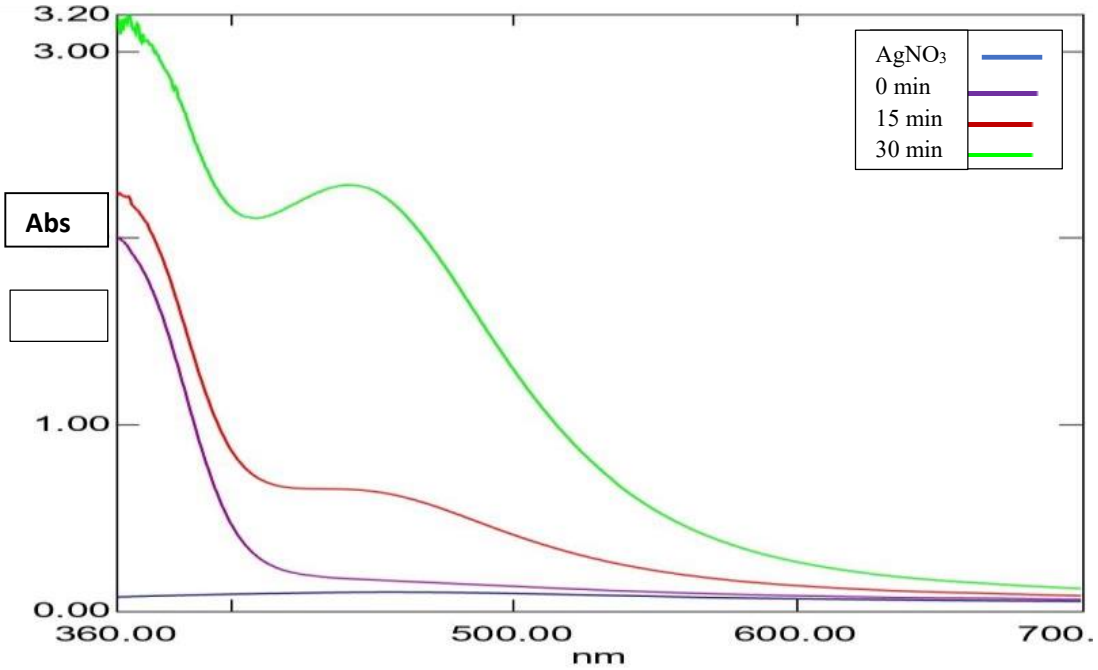

Figure 2: UV-vis spectroscopy of amchur SNP showing a peak at 440nm.

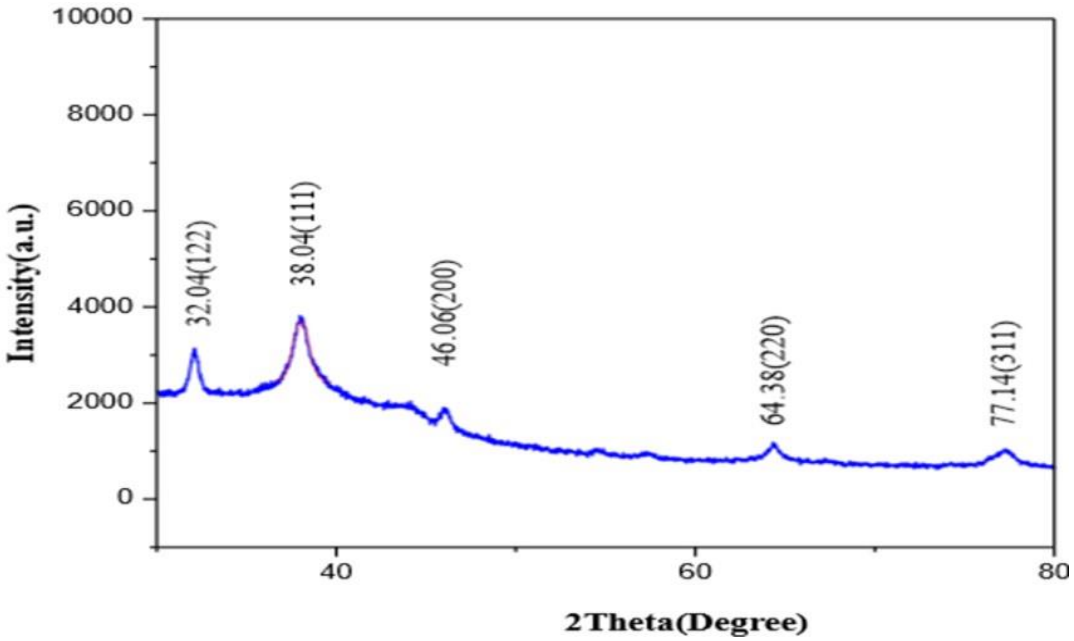

Figure 3: XRD pattern of amchur SNP

Table 1
XRD Details for amchur SNP (ASNP)

S.N.	PEAK VALUE (2 THETA)	FWHM	hkl VALUE (MI)	Crystal Size nm (D)	Average Size (D)
1	32.04	0.9387	122	9.44	9.91nm
2	38.04	0.9837	111	9.4	
3	46.06	0.8975	200	10.05	
4	64.38	0.9532	311	10.29	
5	77.14	0.9785	222	10.4	

The N-H bend of primary amines and the C-H bend of alkanes occurred at 1639/cm and 1385/cm respectively (Figure 4, Table 2). This N-H bend is responsible for SNP stability as reported in previous studies[27]. The C-O stretching at 1262/cm and 1079/cm corresponds to aromatic and primary alcohol respectively. The C-H bend of alkene and the C-Br stretch of alkyl halide occurred as a weak band at 800/cm and 607/cm. O-H stretching of polyphenolic chemicals and C-H stretching of proteins respectively, were attributed to the peaks at 3469/cm and 2928/cm[8,26].

The *M. indica* Amchur powder showed the presence of tannins, phenolics, saponins and sugars[11]. The synthesis, reduction, capping and stabilization of SNPs may be significantly influenced by the presence of these crucial secondary metabolites. The reduction of Ag-to-Ag nanoparticles by *Prunus cerasifera* fruit extract is facilitated by proteins and phenolic compounds[15], including alkyl halides, alkynes, carboxylic acid, ketones, amines, aromatics, aliphatic amines and alcohol or phenol as functional groups. This is consistent with our research.

Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray (EDX) Analysis: A few irregularly granulated compact/fused agglomerates of powder with brighter facets may be seen in the consequent green synthesized silver sample image, along with polymorphic morphologies including rocky, flake type, spherical and cubical. Also visible are the slack connections at the extremities of each cluster. Usually, the sizes of the agglomerates vary from 100 nm to 200 nm (Figure 5a and b).

To ascertain the elemental composition of the biosynthesized SNPs which primarily include elemental silver in significant quantities with negligible levels of oxygen as a contaminant, Energy dispersive X-ray analysis, which is housed inside the SEM apparatus, was employed. Furthermore, throughout the entire scan rate method, no additional element was discovered. Surface plasmon resonance causes metallic silver nanocrystals to display a significant signal at 3 keV, as seen in the EDX (Figure 5c)[23].

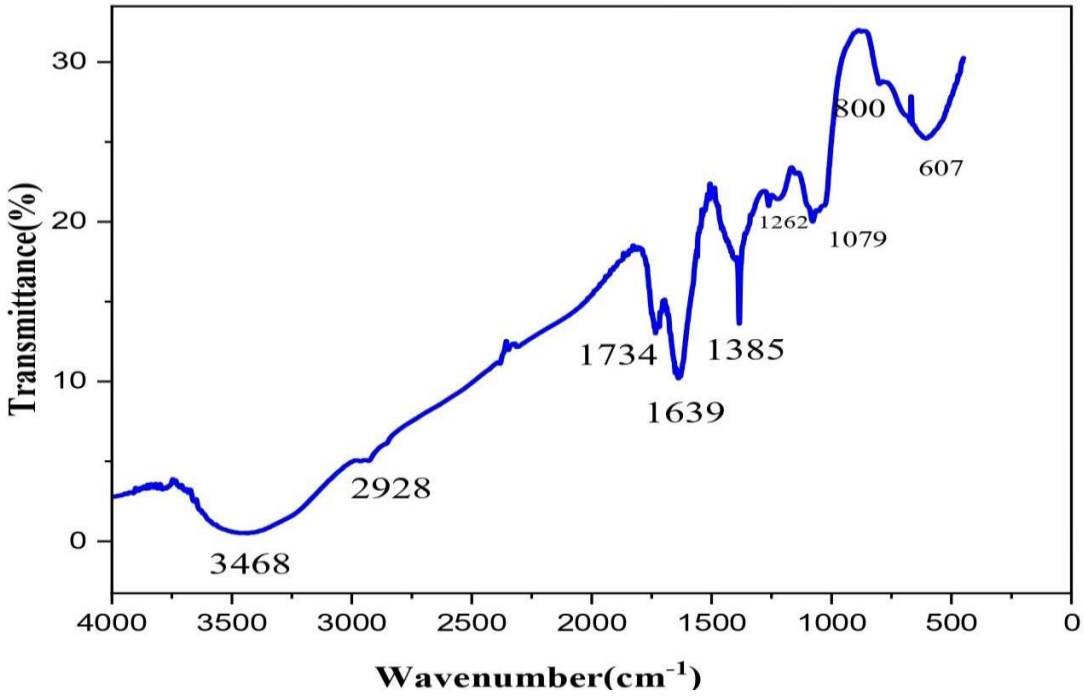

Figure 4: FTIR spectrum of *Mangifera indica* silver nanoparticles amchur extract

Table 2
FTIR data representing the functional groups in amchur SNP (ASNP)

S.N.	Chemical Bonding	Vibration Mode	Peak Value (cm^{-1})
1	O-H (Alcohol)	Stretching	3469
2	C-H (Alkane)	Stretching	2928
3	C=O (Aldehyde)	Stretching	1734
4	N-H (Primary Amines)	Bending	1639
5	C-H (Alkanes)	Bending	1385
6	C-O (Aromatic Ester)	Stretching	1262
7	C-O (Primary Alcohol)	Stretching	1079
8	C-H	Bending	800

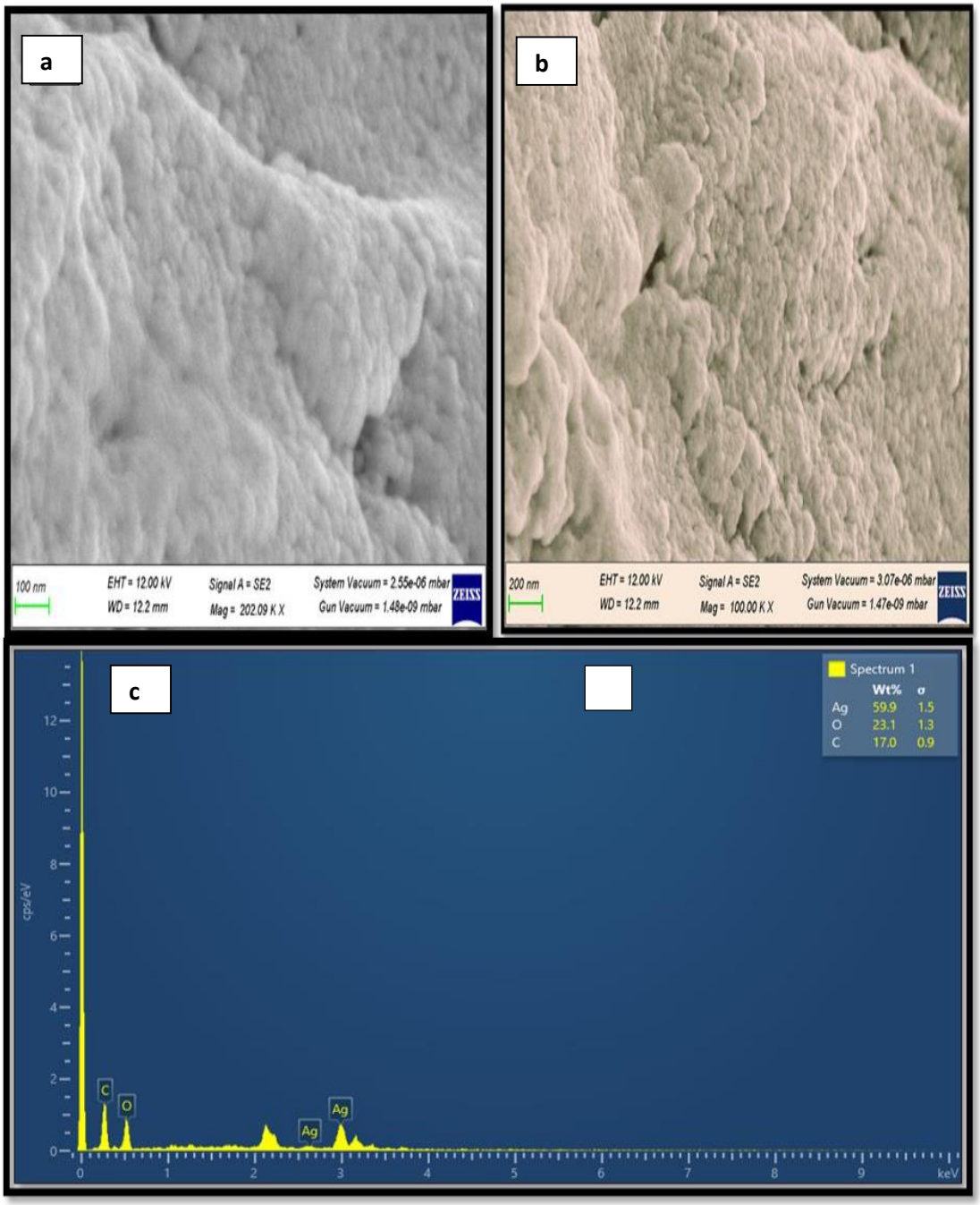

Figure 5: Scanning electron microscope images (a and b) at different magnifications and (c) EDX analysis of *Mangifera indica* silver nanoparticles from amchur extract

Antimicrobial Activity by Disc Diffusion Method: The SNP's antimicrobial activity was tested using the disc diffusion method, following the protocol outlined in the previous section. As shown in figure 6 (a and b), it reveals that SNPs have strong antibacterial effects against both Gram-negative and Gram-positive bacteria, but poor antifungal activity. The negative control DW also showed a zero zone of inhibition. SNPs exhibit superior antibacterial efficacy against Gram-negative (PA and EC) than Gram-positive (SA and SP) bacteria on the plates. ASNP1 (100 µl) was shown to be more effective than ASNP2 (50 µl) and comparable to the standard drug (ampicillin) in terms of inhibitory zone. Figure 7 displays the zone of inhibition for the antibacterial activity of Gram-positive (SA and SP) and Gram-negative (EC and PA) bacteria whereas figure 8 shows the antifungal activity of the green synthesized SNP from Amchur extract against *C. albicans* (CA) and *C. glabrata* (CB).

Both the figures depict the ZI by the crude amchur extract, ASNP1 and ASNP2, $AgNO_3$ and antibiotic. *C. glabrata* displayed more antifungal activity than *C. albicans.* Table 3 represents the measurement of the ZI (mm) calculated with Vernier calipers and associated controls. The control DW showed no zone of inhibition, confirming the proper execution of the procedure. Nanoparticles exhibited higher

antibacterial action against Gram-negative bacteria than Gram-positive bacteria. The distinction between Gram-positive and Gram-negative bacteria stems from their distinct cell wall structures. Gram-negative bacteria have a thin peptidoglycan layer in their cell walls whereas Gram-positive bacteria have a thicker layer[19].

Antimicrobial efficacy varies based on nanoparticle concentration and bacterial species[28]. As per the study reported, mango core nanoparticles demonstrated better antifungal properties against *C. glabrata* than fluconazole[31]. The outcomes are consistent with the research by Khatoon et al[18] on fungal pathogens of *C. albicans, C. glabrata and C. tropicalis*. In another study, the researchers examined the antifungal properties of silver nanoparticle spray (15 ppm) with an applicator against *Candida vaginitis* in a clinical trial. This study found that silver nanoparticles in the form of a spray gave better antifungal action against *Candida* in comparison to 1% clotrimazole[2].

Conclusion and Future Aspects

This study demonstrated using dried mango fruit (amchur), an Indian spice and its aqueous extract to synthesize silver nanoparticles utilizing a green method. This green approach to SNP synthesis has several advantages for bulk production including 1) ease of use, low energy consumption, eco-friendliness and cost-effectiveness; 2) absence of hazardous reagents and chemicals for reduction and processing and 3) absence of the need for heating or sophisticated synthesis equipment. The powder XRD data on silver nanoparticles is consistent with conventional ICDD cards, as reported by researchers. The XRD pattern revealed that the sample was made up of crystalline face-centered cubic (FCC) lattice structures of elemental silver.

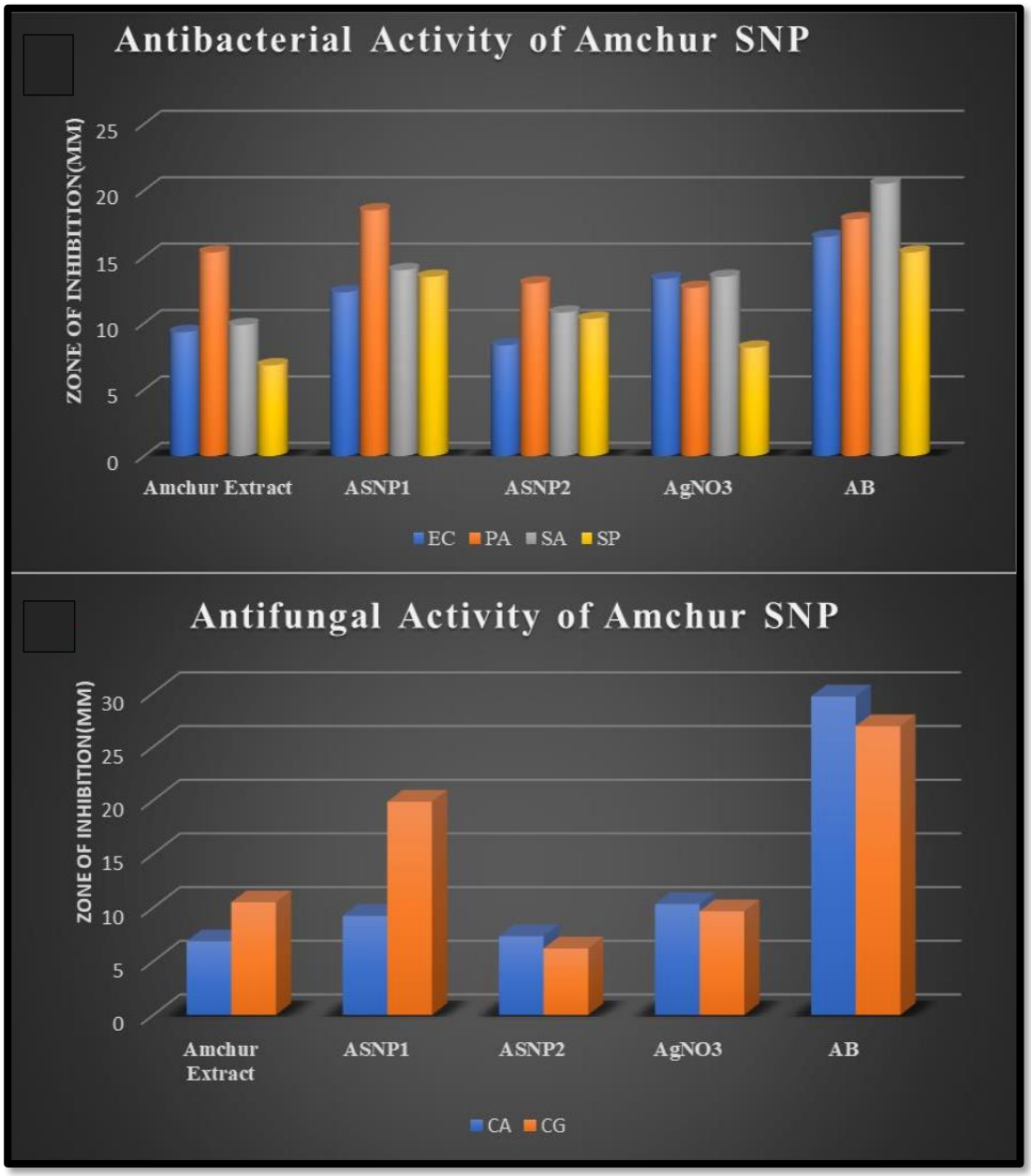

Figure 6: Antimicrobial activity of amchur SNP (a) against bacteria and (b) fungus

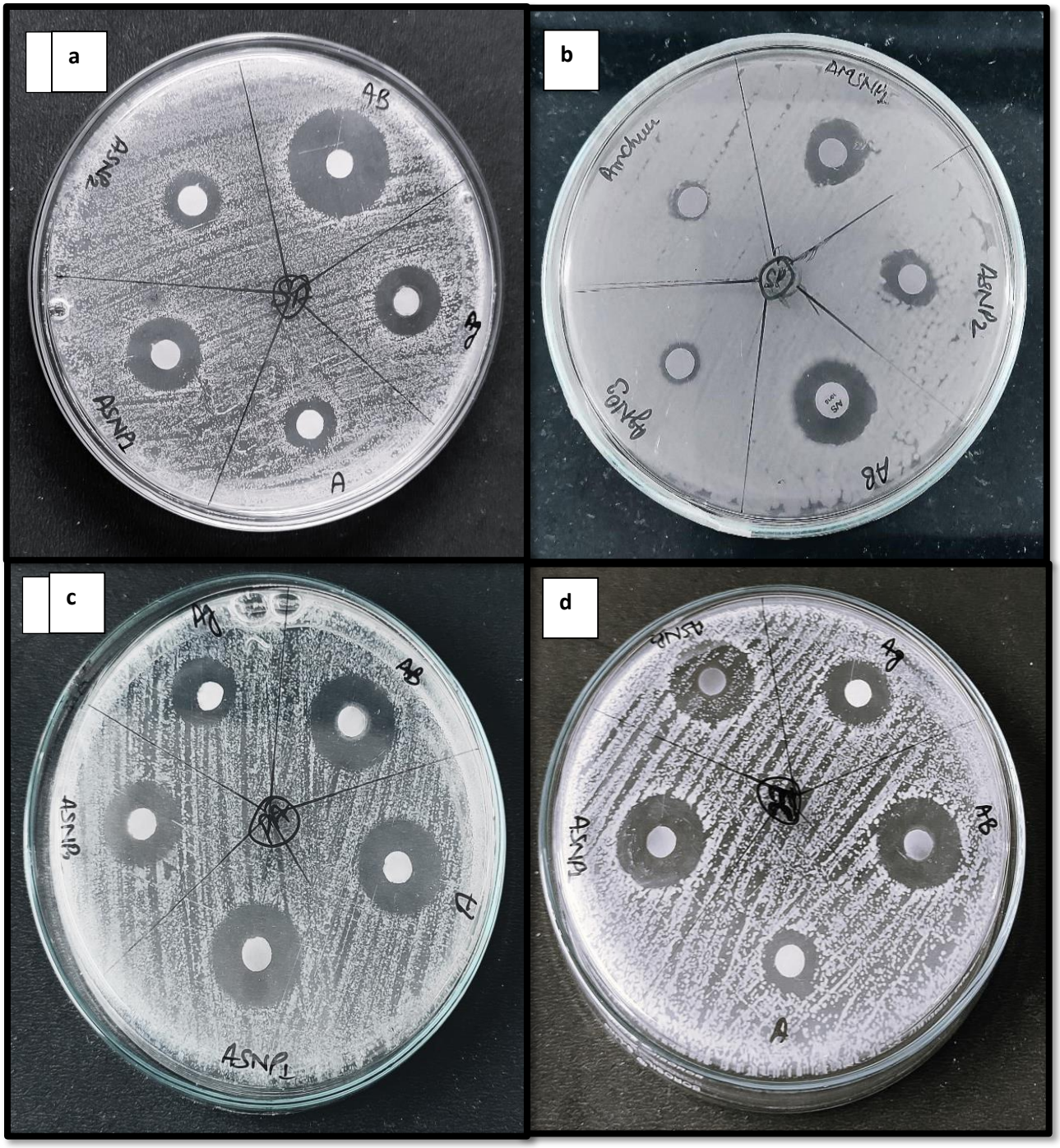

Figure 7: Antibacterial Activity of Amchur SNP against Gram-positive (a= *Staphylococcus aureus* and b= *Streptococcus pyogenes*) and Gram-negative (c= *Pseudomonas aeruginosa* and d= *Escherichia coli*) bacteria (ASNP1=100µl, ASNP2=50µl, $AgNO_3$=1mM, A=Amchur Extract crude, AB=Ampicillin (10µg disc)]

UV-Visible spectroscopy studies further revealed that the absorbance spectrum is unique to SNP. The SEM scan revealed dense agglomerates of silver particles. SNPs were seen to be highly granular with an average aggregate size of 100-200 nm. The photos revealed a variety of agglomerated formations including rocky, flake, spherical, ellipsoidal and irregularly granulated compact/fused agglomerates. Elemental analysis of SNPs revealed the presence of silver and trace amounts of oxygen as impurities. The EDX analysis demonstrated no additional elements, confirming that the manufactured sample is pure. FT-IR analysis revealed that amchur phenolics reduced silver nitrate solution to SNPs, indicating a positive bio-reduction fingerprint.

UV-Vis spectroscopy revealed the existence of SNPs by detecting elevated surface plasmon resonance values between 400-460 nm. SNPs' antibacterial potential was tested against a panel of microorganisms including Gram-positive and Gram-negative bacteria as well as fungal strains. Hence, it is concluded that the green synthesis of silver nanoparticles from Amchur powder, a well-known

Indian spice of *M. indica* has been done. The study found that SNPs have nearly as efficient antibacterial action as common drugs like ampicillin and fluconazole. Rather than relying on harmful conventional medications, they can provide a valuable supply of natural antibacterial agents that can satisfy the demand for sustainable resource use. It is necessary to do more research on the molecular mechanism and *in vivo* investigations.

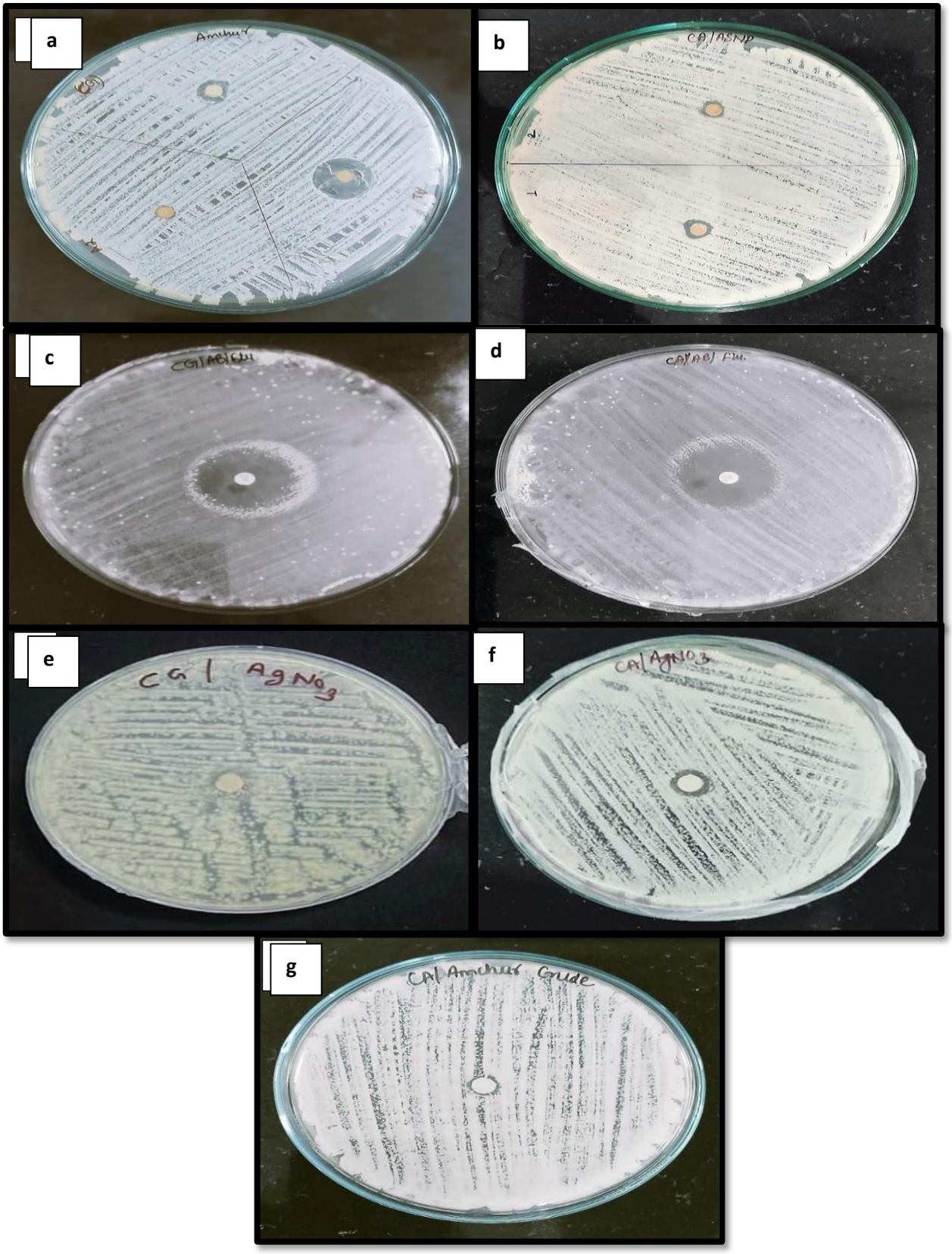

Figure 8: Antifungal Activity of Amchur SNP against *Candida glabrata* (CG=A, C, E) and *Candida albicans* (CA=B, D, F, G) [ASNP1=100µl, ASNP2=50µl, $AgNO_3$=1mM, A=Amchur Extract crude, AB=Fluconazole (10µg disc)]

Table 3
Zone of inhibition (mm) (Represented as Mean±SD)

S.N.	Microbes	Amchur Extract	ASNP1 (100µl)	ASNP2 (50µl)	AgNO3 (1 mM)	AB (10µg)
1	EC	9.33±0.57	12.33±0.28	8.33±0.57	13.33±0.57	16.5±0.5
2	PA	15.33±0.57	18.5±0.5	13±1	12.66±0.57	17.83±0.28
3	SA	9.86±0.23	14±1	10.8±0.34	13.5±0.5	20.5±0.5
4	SP	6.83±0.28	13.5±0.5	10.33±0.57	8.16±0.28	15.33±0.57
5	CA	6.87±0.23	9.23±0.46	7.33±0.28	10.33±0.57	29.67±0.57
6	CG	10.5 ±0.5	19.87±0.23	6.2±0.2	9.67±0.57	26.9±0.45

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