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Green Synthesis Of Silver Nanoparticles Using Citrus Sinensis Peel Extract

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Abstract: *The ability of green nanotechnology to remove harmful chemicals while making the economical and efficient synthesis of desired products is making it increasingly important. The reduction of harmful heavy metals with aqueous plant extracts is a fast, economical and eco friendly way to create nanoparticles. In the present study, silver nanoparticles (AgNPs) were prepared through a chemical reduction process by using Citrus sinensis (orange) peel extracts and 1 mM silver nitrate ($AgNO_3$) solution. During the synthesis, orange peel extract acts as reducing agent as well as capping agent. Using UV–visible absorption spectroscopy, the resonance of surface plasmon phenomenon of AgNPs was found at 445 nm. Various physicochemical methods, such as Fourier transform infrared (FT-IR) spectroscopy, energy dispersive X-ray spectroscopy (EDS), transmission electron microscopy (TEM)and X-ray diffraction (XRD) were used to further characterize the biosynthesized AgNPs. Aldehydes were the main substances in charge of the successful production of AgNPs, according to GCMS spectrometry examination of the orange peel extract. In addition, the synthesized AgNPs displayed strong antibacterial activity against both Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli) microorganisms. This green synthesis method gives the added advantage of producing useful compounds from fruit waste that could have potential antibacterial activity.*
Key words: *Green nanotechnology, orange peel extract, FTIR, UV-Vis, antibacterial activity*

INTRODUCTION

Nanotechnology mainly focuses on creating nanoparticles with varying sizes and shapes while also managing their chemical and physical characteristics for possible human benefit [1], [2]. Among the areas of nanotechnology, which are expanding the fastest, is the creation of metal nanoparticles, usually between 1 and 100 nanometres (nm) in size.A material's intrinsic properties can vary as its size is scaled down to the nanoscale. For this reason, the properties of a nanostructured material may vary significantly from the bulk material, making it suitable for various applications in areas such as bioengineering, medical, and agriculture. It is also famous for its antibacterial and biocidal properties [3], [4]. Different techniques has been utilised to produce NPs, including chemical reduction [5], electrochemical, photochemical [6], [7], and physical techniques such as physical vapor condensation [8]. Currently, green synthesis has emerged as a promising approach for producing NPs [9], [10], [11], involving the combination of metal salts with natural agents such as sugars, vitamins, biodegradable polymers, microorganisms and plant extracts[12]. The silver nanoparticles synthesis process (AgNPs) refers to the chemical reduction of an $AgNO_3$ solution employing a plant extract. This process is divided into two stages:

Stage 1: The nucleation phase, where silver atoms aggregate to form small nuclei using high activation energy, and

Stage 2: The growth phase involves the clustering of small nuclei, resulting in the formation of nanoparticles (NPs) [13], [14].The properties of the resulting nanoparticles are determined by the types of biomolecules present in plant extracts [15], [16], [17]. Therefore, selection of the appropriate extract is crucial because it provides bio reductive agents for synthesis, such as phenolic chemicals, terpenoids, flavonoids, alkaloids, polysaccharides, proteins, enzymes, and amino acids [18].AgNPs developed by the above methods contain few or no harmful substances, qualifying them for pharmaceutical and medical uses [4], [5], [6]. Additionally, AgNP-based antimicrobial packaging is a potential category of active food packaging reducing the likelihood of pathogenic contamination and enhancing food storage life.

Although AgNPs have been in use for several years as antibacterial agents for packaging food, but there may be a possibility of releasing Ag^+ ions into food and beverage. As a result of these issues, regulatory bodies overseeing food safety have adopted a more vigilant approach [7]. Plant extracts, similar to those from Murrayakoenigii leaves [8], mangosteen leaves [9], Mangifera indica leaves [10], Jatropha curcas [13], Cinnamomum zeylanicum leaves [19], Camellia sinensis [20], Aloe vera [21], mushrooms [22], and honey [23], are used in different experiments to synthesize AgNPs. There are fewer reports on the synthesis of

AgNP from fruit extracts, including papaya [16], tansy [18], pear [23], lemon [24], and gooseberry [25]. Utilizing plant and fruit extracts has the benefit of creating stable nanoparticles that do not aggregate, even when kept for a long period. While the literature includes numerous studies on the production of silver nanoparticles using plant extracts, most of these focus on plant species from Europe, Africa, America, and Asia. Investigation on the environmentally friendly preparation of nanoparticles using fruit extracts, particularly from Northeast India, remains limited [26].Citrus sinensis, the scientific name for oranges, are native to Southern China, Northeast India, and Myanmar. They were first recorded in Chinese literature as early as 314 BC. It is a cross between the mandarin orange and the pomelo, with the pomelo providing the maternal line. Oranges are extensively cultivated for their sweet and juicy fruit; Brazil leads the world in production, followed by China and India. Global production reached 76 million tons in 2022. With Assam contributing 3.34%, Arunachal Pradesh 1.08%, and Madhya Pradesh leading the pack at 32.89%, followed by Punjab, Maharashtra, Rajasthan, and Haryana, India's production is noteworthy. After the fruit pulp is eaten, the peels are usually thrown away. Very less number of reports on the use of peel extracts have been published for the manufacture of AgNPs [27], [28]. Further, peels are the main byproduct produced by the industrial citrus juice extraction process, which uses a large number of oranges and make up 50 to 65 percent of the fruit's weight. Proteins, soluble and insoluble fibres, and bioflavonoids are all prevalent in this biomass and have potential uses in nanobiotechnology, particularly in the creation of nanoparticles [29], [30].The objective of present study was to investigate the utilization of orange peel that are available in Northeast India, both as capping and reducing agent in the production of AgNPs through biosynthesis. Characterization of AgNPs was carried out by using several spectroscopic and microscopic techniques, including UV-Vis spectroscopy, FTIR, TEM, EDS and XRD. Moreover, the antibacterial activities of AgNPs against Staphylococcus aureus and Escherichia coli were studied.

MATERIALS AND METHODS

Plant material and extracts preparation

Oranges were obtained from the local market in Guwahati, Assam (19.47°N 72.8°E), in October 2022.Fresh orange peels were allowed to dry in the shade after being gathered and carefully cleansed with double-distilled water. Ten grams of the powdered peel were reflux extracted for one hour in a 500 mL beaker filled with 100 mL of double-distilled water. Filtration was done by using Whatman No. 1 filter paper and the filtrate was then stored in a different flask.

Green synthesis of AgNPs

An aqueous extract of orange peel and silver nitrate ($AgNO_3$) were used for the synthesis of nanoparticles in the presence of sunlight. The sunlight-driven NP synthesis was conducted following the method of Rizwana et al.[31]**.** A 1 mM aqueous solution of silver nitrate ($AgNO_3$) was prepared fresh by dissolving it in 1000 mL of distilled water and used for the synthesis of AgNPs (silver nanoparticles). To this solution, 9 mL of the 1 mM $AgNO_3$ solution and 1 mL of orange extract was added, and the mixture was then kept underneaththe sunlight for the reduction process. It was seen that the solution changed the color from yellow to reddish-brown, signifying the conversion of silver nitrate to silver nanoparticles, driven by the surface plasmon resonance phenomenon. This was further validated by the appearance of a peak in the UV-visible spectra.

Characterization Techniques

Silver ion concentration in the solution was measured at regular intervals using UV-Vis spectroscopy (Shimadzu UV spectrophotometer, model UV-1240). The reaction mixture was diluted a little and the absorption spectra were taken. The measuring range of the wavelengths varied between 200 and 800 nm with an integration time of 1 sec and gap of 1 nm for each measurement. An Advance Powder X-ray diffractometer, model D8 (Bruker, Germany), was employed to get further characterization. For this analysis, an X-ray source with Cu Kα (λ = 1.54056 Å), at 40 kV, and a current of 40 mA was applied. The scanning rate used here was 3°/minute. The scanning was conducted over 2θ between 5° and 100°. A PerkinElmer FTIR spectrometer, model FTIR System Spectrum BX, was utilized for FTIR analysis. The KBr method was adopt to create the samples pellet and used to identify the functional groups present on the surface of the reduced AgNPs. Transmission mode was used to gather spectra, covering a wavelength

range of 4000 to 400 cm^{-1}, with a spatial resolution of 4 cm^{-1}. TEM investigation was carried out with a JEM-2100 PLUS (HR). The analysis was carried out in a JEOL apparatus coupled with an EDS energy dispersive system run at 200 kV. A small amount of the specimen was deposited onto carbon tape mounted on a copper stub to form thin films; these were then dried out before measurements. The distribution of size for AgNPs was assessed by analyzing TEM images using specialized imaging software. The chemical composition of the fruit peel aqueous extract was determined by GC-MS analysis (Hewlett-Packard 6890 Plus gas chromatograph equipped with a 5975 mass selective detector).

Antimicrobial assessment

Antibacterial efficacy of the AgNPs solution was assessed against the Staphylococcus aureus, a gram-positive bacteria and Escherichia coli, a gram-negative using the conventional agar well diffusion method. Fresh overnight-grown suspensions of S. aureus and E. coli were evenly swabbed onto sterile nutrient agar plates. The antimicrobial compounds utilized in this method are extracted from holes drilled in agar plates that have been treated with specific bacterial strains. The appropriate bacteria were seeded onto agar plates prepared accordance with the manufacturer's instructions. Using a sterile cork borer, 8 mm diameter holes were cut after the agar had time to solidify. Using sterilized tools, a precise amount of AgNPs solution was cautiously decant into each hole. Following a 15-minute pre-diffusion period at room temperature, the plates were incubated for 48 hours at 25 ± 1°C. Bacterial growth was measured after incubation and the diameter of each inhibitory zone was measured.

RESULTS AND DISCUSSION

An eco-friendly synthesis of AgNPs was achieved by adding orange peel extract to an aqueous solution of silver nitrate. Initial color shifts indicated that the AgNPs' creation was successfulfrom yellowish to brown due to excitation of surface plasmon vibrations.UV-Vis spectroscopy was used to observe the biosynthesis of AgNPs. Figure 1 showhow orange peel extract is used to create silver nanoparticles. The formation of silver nanoparticles is confirmed by the color shift. In this procedure, electronswith a reducing agent involved in the process. (orange peel extract) are transferred to silver ions (Ag^{+}) in aqueous solution, reducing them from a positive valence to a zero-valent state (Ag^{0}). After this reduction, nucleation and growth occur, forming colloidal AgNPs that first clump together into tiny clusters.Numerous variables, including reaction temperature, pH, precursor concentrationtypes of stabilizing and lowering agents, and the ratio of $AgNO_3$ to orange peel extract, might affect the nucleation and development of AgNPs [32].

Fig.1: Change of colour from extract to nanoparticles

UV-Vis char

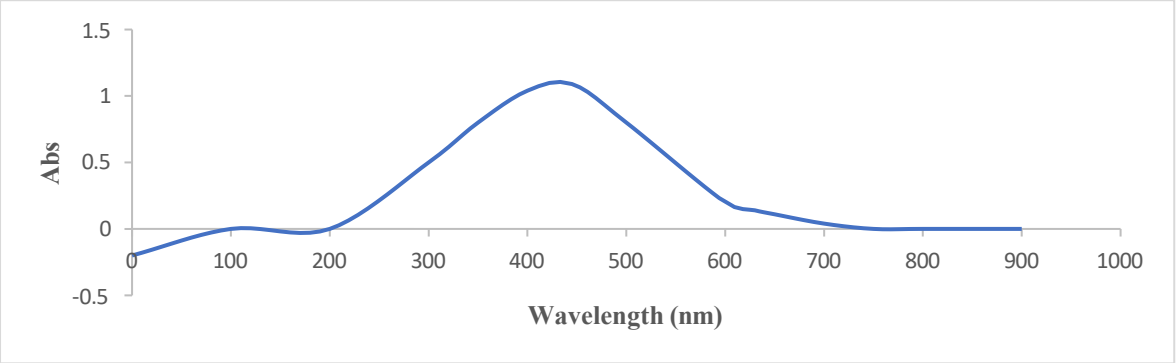

Fig. 2: UV-vis spectra of reduced Ag ions to AgNPs

Figure 2 shows the UV-Vis spectrum of AgNPs synthesized by utilizing the fruit peel extract. AgNPs' absorption spectra showed a clear peak at 445 nm, through a broad range of wavelength 200-800 nm. Since, it is inside the AgNPs' surface plasmon resonance (SPR) band, this signal indicates the production of AgNPs [33]. Moreover, the distribution of size of AgNPs fabricated through orange peel-mediated reduction may be accountable for the broad plasmon band that extends with an absorption tail at higher wavelengths (Figure 2)[34].

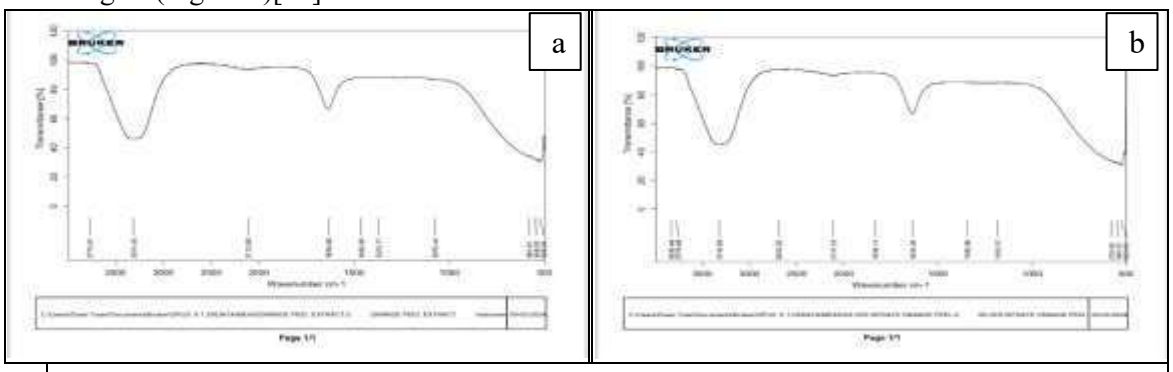

Fig. 3: (a) FTIR spectra of orange peel extract; (b) FTIR spectra of AgNPs

The extract's functional groups and their function in reducing AgNO3 to generate AgNPs were investigated using FT-IR experiments (Fig. 3a-b). FT-IR spectrum of orange peel extract and colloidalAgNPsare similar with a minor shift in the arrangement of the bands (Shown in Fig. 3a and 3b respectively). Peaks for AgNPs were found at 3319, 2111, 1634, 1345, 1183 cm^{-1} stretching vibration frequencies. For orange peel extract it were found at 3314, 2112, 1634, 1370, 1075 cm^{-1} stretching vibration frequencies. The extract's carboxylic groups and phenols' OH stretching vibrations are represented by the broad band at 3319 cm^{-1}. The phytoconstituents of the extract are thought to contain alkyne groups, which are accountable for the absorbance peak at 2111 cm^{-1}. The strong band at 1634 cm^{-1} corresponds to the C=C stretching of the aromatic ring. This stretch may be because of the C=O vibration of the ketones found in flavonoids. The peaks at 1183 and 1075 cm^{-1} are indicative of the C–N stretching vibrations of aliphatic amides [35].

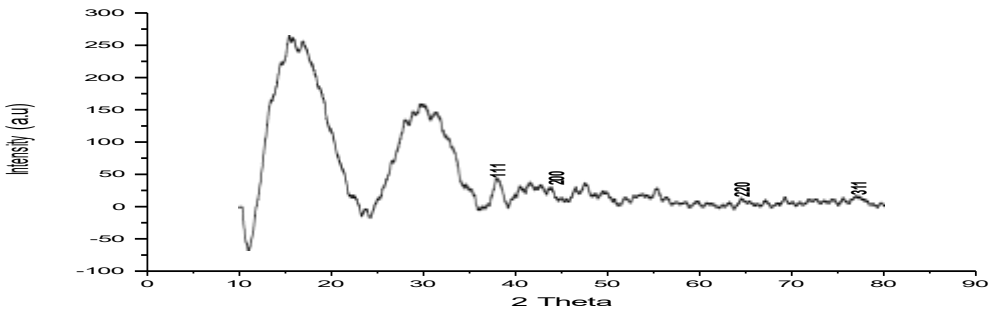

Fig. 4: XRD pattern of AgNPs by orange peel extract

Figure 4, the FCC (face-centered cubic) lattice structure of metallic silver is connected with the diffraction peaks at 2θ = 37.2°, 44.08°, 64.44°, and 77.28° in the XRD pattern of the biosynthesized AgNPs. These peaks correspond to the (111), (200), (220), and (311) planes, respectively. Figure 4 shows additional peaks at 2θ = 27.98°, 31.24°, 46.52°, 54.96°, 57.36°, and 76.92°, which may be related to the crystalline and amorphous organic phases that coexist with the crystallized AgNPs.Further, information about the shape and size of the synthesized AgNPs was given by the TEM analysis. TEM images at various magnifications are shown in Figures 5 (a), (b) and (c) along with the selected area electron diffraction (SAED) patterns that are shown in Figure 5 (d). The average diameter showed by the TEM images was about 8 to 20 nmwhich resembles the pattern identified in the SPR band of the UV-visible spectrum [36]. Furthermore, as seen in Figure 5, it was revealed that the nanoparticles were evenly distributed and hardly aggregated.

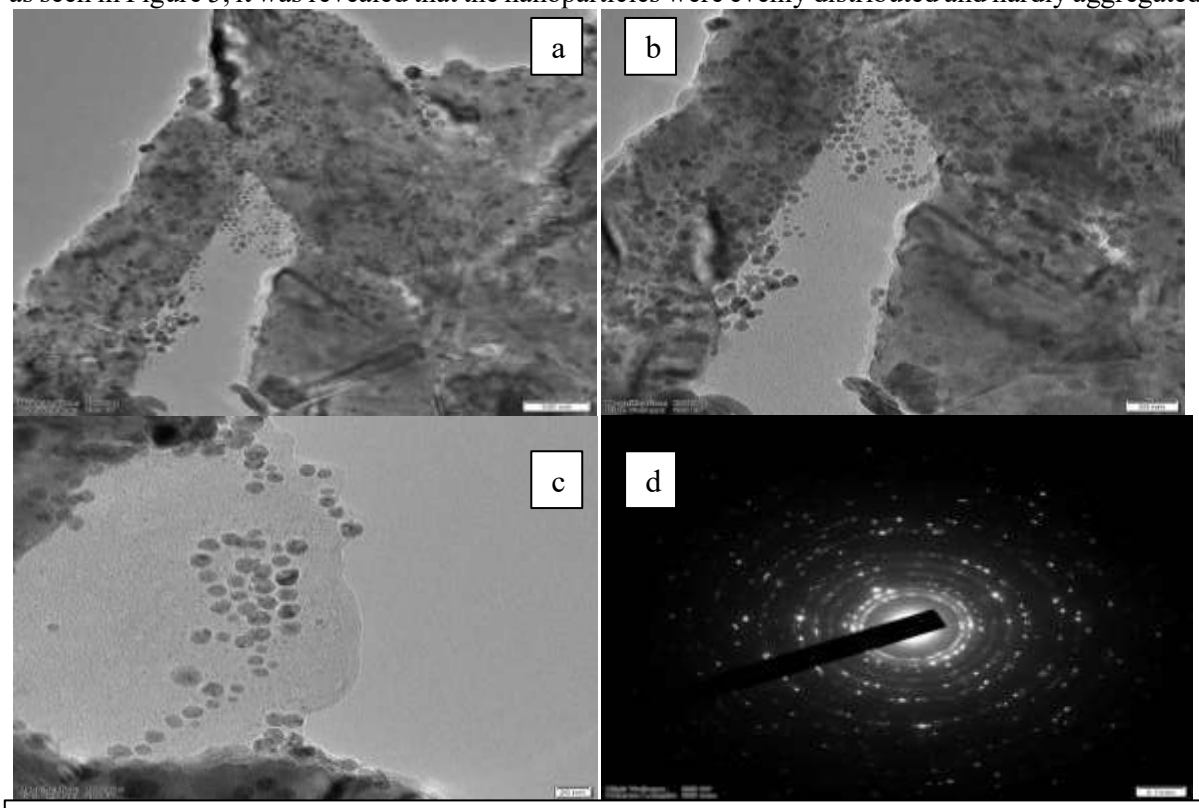

Fig. 5 : The images of AgNPs produced using orange peel extracts, which were captured by a transmission electron microscope (TEM), are: (a) a random field view of AgNPs (scale bar = 100 nm), (b) a high magnification image of spherical AgNPs (scale bar = 50 nm), (c) another high magnification image of spherical AgNPs (scale bar = 20 nm), and (d) the electron diffraction pattern (SAED) of several silver NPs.

analysis of the constituents or composition at specific locations. The spectra identified elements such as O, C, Ag, and Au. Peaks from Cu and C came from the carbon grid that had been used to get ready the sample. FTIR analysis verifies that the two carbonyl groups present in the sample are probably due to the origin of the elemental oxygen peak. AgNP production is confirmed by a signal in the silver portion of the spectrum. Surface plasmon resonance is linked to the optical absorption peak that metallic silver nanocrystals generally exhibit at about 3 keV [37].

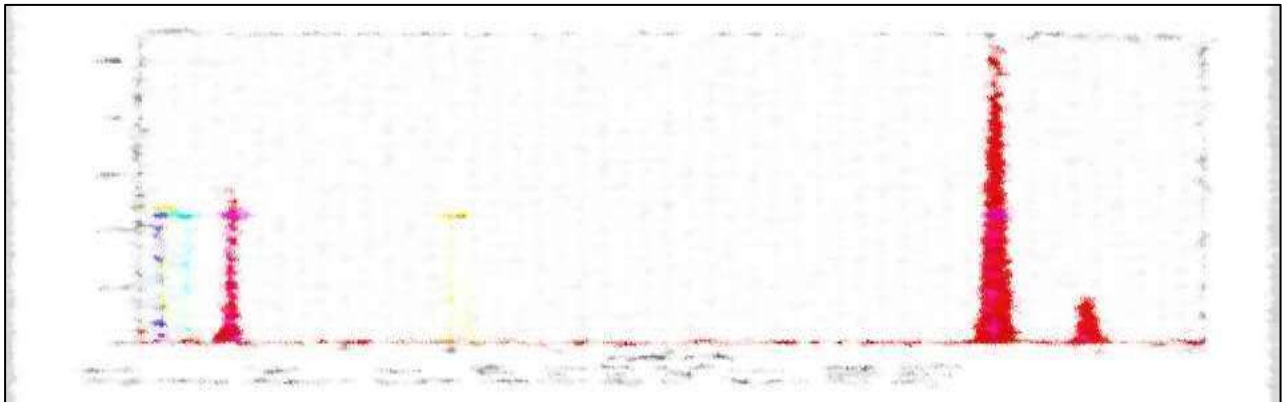

Fig. 6: Energy dispersion X-ray (EDS) spectra of AgNPs synthesized with *orange peel* extract

GC/MS analysis was done for orange peel extract of which the TIC diagram is shown in Figure 7. In Table 2 the identified components along with retention time, compound names and area percentage are listed. Briefly, several compounds were found in the orange peel extract (5-Bromopentanoyl chloride, retention time (RT) 6.97 min) or were found in higher abundance in the orange peel extract (2(3H)-Furanone, 5- methyl-, RT 7.381 min; 2,6-dimethyl-2,6-octadiene-1,8-dial, RT20.33 min; and 4-isopropenyl- 1-methyl-1,2-cyclohexanedial, RT 20.92 min). Alcohols D-limonene (RT 10.123 min), β-linalool (RT 16.67 min), 1-nonanol (RT 17.98 min), 2-(4-methylenecyclohexyl)-2-propen-1-ol (RT 20.10 min), perilla alcohol (RT 20.25 min), and 8-hydroxylinalool (RT 21.03 min) were identified to exist in the higher amount from orange peel.

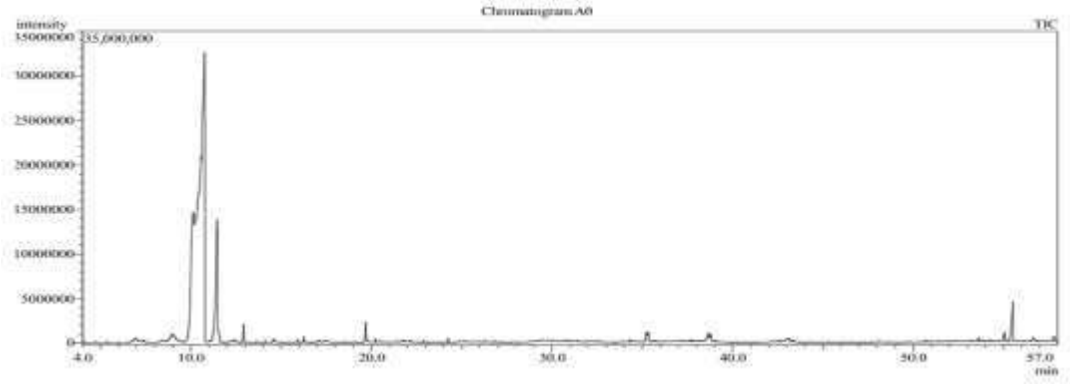

Fig. 7: GC/MS analysis of orange peel extract

Table 2-: Compounds present in orange peel extract

S. No.	RT. Time (min)	Compound name	Area Percentage
1	6.97	5-Bromopentanoyl chloride	0.25
2	6.915	.alpha.-Pinene	0.29
3	7.167	.beta.-Pinene	0.12
4	7.381	2(3H)-Furanone, 5-methyl-	0.14
4	8.972	.beta.-Myrcene	1.39
5	9.145	.beta.-Myrcene	0.31
6	9.27	Pentanoic acid, 4-oxo-, methyl ester	0.21
7	10.123	D-Limonene	17.08
8	10.577	D-Limonene	33.46

9	10.764	D-Limonene	26.52
10	11.457	.gamma.-Terpinene	9.41
11	12.4	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	0.19
12	12.929	Linalool	0.63
13	14.592	Citronellal	0.21
14	16.253	Decanal	0.16
15	19.685	2-Methoxy-4-vinylphenol	0.72
16	35.229	Dibutyl phthalate	0.41
17	38.656	Linoelaidic acid	0.59
18	38.799	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	0.35
19	42.558	3',4',5,6,7,8-Hexamethoxyflavone	0.15
20	42.995	3',4',5,6,7,8-Hexamethoxyflavone	0.18
21	43.119	3',4',5,6,7,8-Hexamethoxyflavone	0.12
22	43.347	3',4',5,6,7,8-Hexamethoxyflavone	0.13
23	53.633	4H-1-Benzopyran-4-one, 5-hydroxy-6,7-dimethoxy-2-(4-methoxyphenyl)-	0.16
24	55.056	5-O-Desmethyltangeretin	0.43
25	56.659	Stigmasterol	0.22
26	57.798	.gamma.-Sitosterol	0.26
27	55.504	4',5,6,7,8-Pentamethoxyflavone	2.01

Antimicrobial Activity

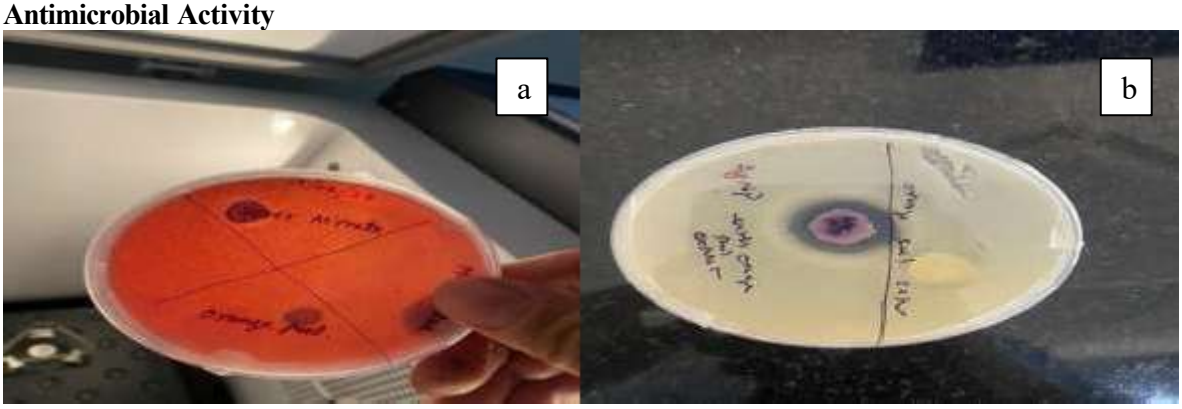

Fig. 8(a): *E. coli*

Fig. 8(b): *S. aureus*

The well-diffusion method was used to evaluate the antibacterial activity of orange peel-derived silver nanoparticles against Gram-negative Escherichia coli and Gram-positive Staphylococcus aureus bacteria (in Figures 8(a) and 8(b) respectively). The antibacterial activities of orange peel extract were also tested.AgNPs had inhibitory zones of 5 mm against E. coli and 7 mm against S. aureus. The values here represent the mean of three experimental trails. The inhibitory zone of AgNPs proved to be marginally more effective against the bacterial strains than the raw orange peel extract.According to certain theories, respiratory inhibition and eventual cell death result from interactions between silver nanoparticles and thiol groups present on cell membrane proteins. Moreover, interactions between the cell wall and silver nanoparticles may improve membrane permeability by forming pits or pores, which would aid in the killing of bacteria [38].

CONCLUSION:

In the present study, Citrus sinensis peel extract can effectively synthesize AgNPs in an eco-friendly manner. The fruit extract acts as both capping and reducing agent, providing a simple, cost-effective method without using any harmful chemicals. A change in color of the solution served as a visual indication for the synthesis of AgNPs. Further, confirmation was obtained by using UV-Vis spectroscopy,

where a surface plasmon absorption peak was observed at 466 nm in the UV-Vis spectrum. The FT-IR spectra of orange peel extract and colloidal AgNPs showed a minor difference in their frequency stretching. In TEM images, spherical or almost spherical nanoparticles with mean diameter between 8–20 nm were observed. EDS analysis revealed considerable indications of the silver element in the nanoparticles confirming the fact that there existed silver content. The XRD pattern confirms that the biosynthesized AgNPs have a face-centered cubic (FCC) crystalline structure. The biogenic AgNPs exhibited strong action against the E. coli and S. aureus. It may find interesting uses in hygiene and medicine by further researching on the preparation of AgNPs using orange peel extract.

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