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Synthesis and Characterization of Silver Nano Particles of Kaempferol and Their Application as Antibacterial and Against Prostate Cells (Pc-3)

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ABSTRACT

Background: Silver nanoparticles (AgNPs) have demonstrated a broad range of antibacterial & anti-cancer activities during the last 20 years.
Aim: The current endeavor seeks to produce kaempferol silver nanoparticles that are favourable to the environment.
Materials and Methods: Green synthesis of silver nanoparticles was prepared. Nano particles was subjected to different analysis such as FTIR, SEM, XRD and antimicrobial assay with *pseudomonas* and PC3 cell lines were used for the determination of anticancer potential of kaempferol silver nanoparticle.
Results: The n-H stretch of the amine was identified in the FTIR analysis of the nanoparticles as a peak at 3335 cm-1, whereas a tiny O-H stretch was seen at 3442 cm-1. At 2538 cm-1, the absorption peak of the C-H stretch alkyne was found. With its assistance, the C-O asymmetric stretch carbon compounds were synthesized. A band at 1598.00 cm-1 was used to express the C-O asymmetric stretch carbon compounds. Additionally expressed were the C-X alkyl halides and the C-O-C asymmetric stretch carbon compounds at bands of 551, 526, and 512 cm-1. According to our research, the several functional groups that kaempferol-coated AgNPs contain help to stabilize them. The sample exhibited inhibiting effects on *Pseudomonas*, and antimicrobial assays were conducted using test compounds against test organisms, the zone of inhibition seen against test chemicals and standard. The findings demonstrated that nanoparticles decreased cellular motility and PC 3 (prostate cancer cell) cell proliferation. AgNO3 was used in cell-based cytotoxic tests at different concentrations.
Conclusion: The results of the study show that kaempferol has more potent antibacterial and anticancer effects when it is present in the form of silver nanoparticles.

Keywords: Silver Nanoparticle; Kaempferol; Antibacterial; Prostate cancer cells; Cells-based cytotoxic assay.

INTRODUCTION

A nanoparticle of silver, measuring one to one hundred nanometers in size. For over 2,000 years, silver has been utilized both medicinally and as a preservative. The ancient Greek and Roman civilizations preserved drinkable water in silver pots. From the 1800s until the present, silver-
based materials have been used mostly as burn and wound treatment agents, bactericidal agents, etc. Silver has been polished into nanoparticles ranging in size from 1 to 100 nm throughout the last few decades. The nanoparticles' tiny size maximizes their overall surface area, resulting in the best activity-to-weight ratios. Many fields, including textile engineering, biotechnology, medicine, and bioengineering, have expressed interest in the usage of silver nanoparticles. This is because their characteristics differ greatly from those of the metal in bulk. The creation of nanoparticles by environmentally friendly and sustainable procedures is referred to as "green synthesis." This method's ability to lessen the environmental impact of nanoparticle synthesis has made it extremely relevant in the field of nanotechnology. An overview of the process used to create silver nanoparticles using the flavonoid kaempferol, which is found in many different plant species, will be provided in this essay. Silver nanoparticles have been utilized for millennia due to their distinct characteristics, including their antifungal and antibacterial actions. They are used in several industries, such as electronics, medicine, and catalysis. Nevertheless, hazardous substances that may be detrimental to both environment and human health are used in the traditional processes of creating silver nanoparticles. Thus, the development of environmentally friendly production techniques for silver nanoparticles is necessary. Our study's goal was to create silver nanoparticles with kaempferol and assess the pharmacodynamic qualities of those particles. To create the nanoparticles, we employed a straightforward and reasonably priced technique that entailed reducing silver nitrate with kaempferol in a mild reaction.

MATERIALS AND METHODS

The production of silver nanoparticles was accomplished for utilizing kaempferol as a green synthesis agent[1]. The manufacture process added the reduction of silver nitrate with kaempferol in the existence of sodium hydroxide[2]. Analyses of the generated nanoparticles included transmission electron microscopy, UV-visible spectroscopy, and X-ray diffraction[3]. Using disk diffusion method, the antibacterial characteristics of the nanoparticles were assessed[4]. With a variety of cell lines, the pharmacodynamic investigation was conducted in vitro.

UV-visible spectrophotometer (UV) analysis

A potent technique for determining the optical characteristics of silver nanoparticles that can shed light on their behaviour and possible uses is UV-visible spectroscopy[5]. Through the interpretation of UV-visible spectroscopy results optical properties and behaviour of the silver nanoparticles can be understood. The extent and concentration of the optical characteristics of nanoparticles, such as absorbance and scattering, can be influenced by them and are relevant for a range of uses, such as imaging and sensing[6]. Depending on the particular experiment, the temperature at which the measurements are made may change, although it is typically room temperature, or about 25°C[7]. UV-visible spectroscopy is used in green synthesis of nanoparticles to track the nanoparticle formation process by monitoring the absorbance of the solution at specific wavelengths over time[8]. The concentration and size of nanoparticles being manufactured are ascertained by measuring the absorbance at a specific wavelength, which translates to the amount of light absorbed by the nanoparticles in the solution[10]. The UV-visible spectrophotometer calculates the sample's transmittance or absorption of light in the visible

and ultraviolet spectrums Since surface Plasmon resonance affects absorbance, the 400–450 nm range is often where silver nanoparticles exhibit their maximal absorbance. Through analysis of the UV-visible spectra collected from the material,size and concentration of the nanoparticles may be analysed. The size and concentration of the nanoparticles can be determined from the absorbance spectrum's peak position and intensity.

Preparation of the sample

There are a few methods for producing silver nanoparticles, such as electrochemical deposition and chemical reduction. After being manufactured, the nanoparticles must be dispersed in an appropriate solvent to prepare a sample for UV-visible spectroscopy.

Fourier Transform Infrared (FTIR) analysis

FTIR, or Fourier Transform Infrared Spectroscopy, is a potent method for characterizing nonmaterial materials, such as silver nanoparticles. The purpose of this test was to determine which functional groups contains kaempferol-coated AgNPs.

Here are some general steps that can be followed for FTIR analysis of silver nanoparticles: Preparation of the sample
For FTIR analysis, the silver nanoparticles must be distributed in an appropriate solvent (such as ethanol or water) and placed on an appropriate substrate (such as a quartz slide or KBr pellet). Before the analysis, the sample needs to be dried.

Background correction
Before examination, a background spectrum of the substrate should be captured To account for substrate interference, this spectrum will be subtracted from the sample spectrum[11].

Instrument setup

The FTIR instrument should be calibrated and optimized for the analysis of the silver nanoparticles. The instrument should be set up to acquire a high-quality spectrum with appropriate resolution and signal-to-noise ratio[12].

Recording the spectra

The optimal scanning range for the silver nanoparticle sample is mid-infrared (4000–400 cm−1). The FTIR spectrum obtained will contain the facts about functional groups present in the sample, including any surface coating or capping agents[13].

Data analysis

The FTIR spectrum can be analyzed using suitable software. The peaks noticed in the spectrum can be allocated to specific functional groups based on their characteristic frequencies. This information is used to identify the surface coating or capping agents on the silver nanoparticles[14].
Scanning Electron Microscope (SEM) analysis:

Using this technique, the size, shape, and morphology of the silver nanoparticles were remarked[15]. Silver nanoparticles' consistent size distribution and spherical shape were revealed by the SEM pictures[16]. It was found that the AgNPs were, on average, 50 nm in size[17]. Additionally, the scanning electron microscopy photos show that the AgNPs coated with kaempferol were evenly distributed and did not aggregate or cluster[18]. AgNPs' surface looked flawless and devoid of any imperfections or anomalies[19]. Overall, the SEM examination demonstrated that the AgNPs generated in this work were of excellent quality and possessed the appropriate morphological traits.

Preparation of the sample:

The silver nanoparticles need to be distributed in a suitable solvent (e.g. water, ethanol) and deposited on a suitable substrate (e.g. KBr pellet, quartz slide) for FTIR analysis. The sample should be dried before the analysis[20].

Background correction:

A background spectrum of the substrate should be recorded before the analysis. This spectrum will be subtracted from the sample spectrum to correct for any interference from the substrate[21].

Instrument setup:

The FTIR instrument should be calibrated and optimized for the analysis of the silver nanoparticles. The instrument should be set up to acquire a high-quality spectrum with appropriate resolution and signal-to-noise ratio[22].

Recording the spectra:

It is recommended to scan the sample of silver nanoparticles in the mid-infrared range (4000-400 cm−1). Statistics regarding the functional groups present in the sample, including any surface coating or capping agents, can be found in the Fourier transform infrared spectroscopy spectrum that is acquired[23].

Data analysis:

With the appropriate VEGA 3 TESCAN software, the FTIR spectrum can be examined at an accelerating voltage of 10 kV. Based on their distinctive frequencies, the peaks in spectrum can be attributed to particular functional groupings. The surface coating or capping agent on the silver nanoparticles can be identified using this information.

X-ray diffraction (XRD) analysis:

As a prominent method for characterizing nanoparticles, X-ray diffraction (XRD) has gained major popularity[24]. The crystalline grain size, lattice parameters, phase, and crystalline structure are often shown by XRD[25]. By using the Schere equation to the X-ray diffraction data, the sharpest peak can be enlarged to get the lattice parameter for a given sample. The XRD technique is commonly performed to powdered sample forms after drying the matching colloidal solutions, and volume-averaged data are used to statistically describe the results. The database of To compare position and strength of the peaks with reference patterns, utilise the International Centre for Diffraction Data (ICDD; formerly known as the Joint Committee on Powder Diffraction Standards, JCPD It is not appropriate for amorphous materials, though.

Particle Size Distribution:

Using a Nanotrac Instrument (Model Nanotrac, USA) nanoparticle size analyzer, size of the nanoparticles was discovered by dynamic light scattering[26,27]. Finding the nanoparticles' mean hydrodynamic diameter and particle size distribution is a routine process[28]. A temperature of 250˚C was used for the dynamic light scattering measurement[29].

Antimicrobial Assay:

An antimicrobial assay is a laboratory technique used to assess a substance's effectiveness against microorganisms such as bacteria, fungus, viruses, and parasites[30]. This test is used to find an antibacterial agent's minimum inhibitory concentration (MIC) or minimum bactericidal concentration (MBC)[31]. Numerous tools can be used for the antimicrobial assay, such as the following: A microplate reader is used to measure optical density or fluorescence signals from microplates containing bacteria or fungi when antimicrobial medicines are present[32]. Disk diffusion assay: an easy-to-use, reasonably priced technique that measures the zone of inhibition by placing a disk impregnated with an antimicrobial agent on a bacterial culture plate[33]. Agar dilution assay: a technique where various antimicrobial doses are used[34]. The antimicrobial assay temperature differs depending on the microorganisms being tested. A typical bacterial assay temperature, for instance, is 37°C, which is also the temperature of the human body However, the temperature is often 25°C or 30°C for fungal test[35].

Anti-cancer activity:

Materials & Methods:

1. MTT Powder (the solution is filtered through a 0.2µM filter and stored at 2–8°C for frequent use or frozen for extended periods)[36].
2. Dimethyl sulfoxide
3. SpectraMaxi3

Cell lines and culture medium

PC3 cell line obtained from ATCC[37]. A 5% CO_2 humidified atmosphere at 37 °C was used to cultivate stock cells in RPMI[38], supplemented with 10% Foetal Bovine Serum (FBS)[39], penicillin (100 IU/ml), and streptomycin (100µg/ml) until confluent. To dissociate the cell, cell dissociating solution[40] (0.2 percent trypsin, 0.02 percent EDTA, and 0.05 percent glucose in PBS) was utilized. The cells underwent centrifugation once their viability was evaluated. After seeding a 96-well plate with an extra 50,000 cells, the plate was incubated for 24 hours at 37 °C and 5% CO_2.

Preparation of standard solution (standard: Vinblastine):

Stock Solution:

10 mM Vinblastine
Prepare by adding 30 µl of 10 mM Vinblastine to 270 µl of plain RPMI media.
Serial Dilutions:

1 mM Vinblastine
Prepare by adding 30 µl of 1 mM Vinblastine to 270 µl of plain RPMI media.

100 µM Vinblastine
Prepare by adding 150 µl of 100 µM Vinblastine to 150 µl of plain RPMI media.

50 µM Vinblastine
Prepare by adding 150 µl of 50 µM Vinblastine to 150 µl of plain RPMI media.

25 µM Vinblastine
Prepare by adding 150 µl of 25 µM Vinblastine to 150 µl of plain RPMI media.

12.5 µM Vinblastine
Prepare by adding 150 µl of 12.5 µM Vinblastine to 150 µl of plain RPMI media.

6.25 µM Vinblastine
Prepare by adding 150 µl of 6.25 µM Vinblastine to 150 µl of plain RPMI media.

3.125 µM Vinblastine
Prepared from the final serial dilution.

Sample Preparation for Cytotoxicity Studies:

Stock Solution:

32 mg/ml
Prepare by adding 20 µl of 32 mg/ml stock to 180 µl of plain RPMI media.
Serial Dilutions:

3.2 mg/ml
Prepare by adding 30 µl of 3.2 mg/ml to 270 µl of plain RPMI media.

320 µg/ml
Prepare by adding 150 µl of 320 µg/ml to 150 µl of plain RPMI media.

160 µg/ml
Prepare by adding 150 µl of 160 µg/ml to 150 µl of plain RPMI media.

80 µg/ml
Prepare by adding 150 µl of 80 µg/ml to 150 µl of plain RPMI media.

40 µg/ml
Prepare by adding 150 µl of 40 µg/ml to 150 µl of plain RPMI media.

20 µg/ml
Prepare by adding 150 µl of 20 µg/ml to 150 µl of plain RPMI media.

10 µg/ml
Prepared from the final serial dilution. RESULTS

UV spectra

Using a Shimadzu (UV-1900i) spectrophotometer, a solution of silver nanoparticles containing kaempferols was produced, and a UV spectrum was obtained. A 300–600 nm range was used to scan the solution (table 1). The UV analysis results for the kaempferol-coated AgNPs showed strong evidence of the silver nanoparticles' plasmon resonance peak, which is situated at roughly 420 nm (figure 1). Mie theory was employed to assess the size of nanoparticles based on intensity of this peak.[41]

nm	Absorbance Of AgNPs
310	1.0015
320	0.9138
330	0.8202
340	0.7624
350	0.7586
360	0.7545
370	0.766
380	0.7931
390	0.8195
400	0.8465
410	0.8841
420	0.9234
430	0.9457
440	0.9374
450	0.9009
460	0.8401
470	0.7775
480	0.707
490	0.6301
500	0.5581
510	0.5581
520	0.4871
530	0.3576
540	0.3001
550	0.252
560	0.2055
570	0.165
580	0.1308
590	0.0978
600	0.0689

TABLE 1:- Range of the UV spectrum for silver nanoparticles

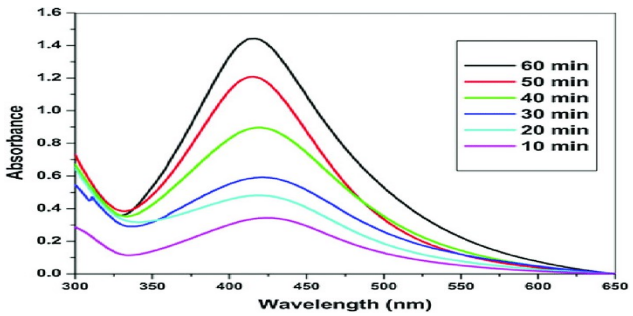
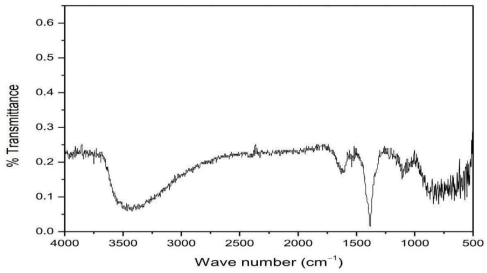


Figure 1: UV-Visible spectrum of AgNPs synthesized in 10 min time interval

Fourier Transform Infrared (FTIR) analysis

The convenient application of FTLR spectroscopy allows for the investigation of both surface composition of the silver nanoparticles and the local molecular environment of the capping agents on nanoparticles. FTLR spectrum of nanopowder silver is viewed in Figure 8. It was possible to observe three main bands with this figure. When a broad band appears at 3450 cm1, which is assigned to the OH stretching vibration, it indicates that the reducing agents contain hydroxy groups. Strong, bright peaks at 1379 and 1625 cm1 are correlated with the C-N stretch vibrations and the amide I bands of the proteins in the apple fruit extract. Red apple fruit extract has the capability to carry out both reduction and stabilization of silver nanoparticles, according to the results of this FTIR spectroscopy study.



igure 2: FTIR of silver nanoparticles synthesize by treating 5ml AFE with 0.1 M aqueous $AgNO_3$ solution

Scanning electron microscope (SEM) analysis:

A 41.9 nm mean size of silver nanoparticle was observed through scanning electron microscopy. A face-centered cubic (FCC) structure and a crystalline nature are indicated by the X-ray diffraction study. In contrast to Gram-positive and Gram-negative bacteria, silver nanoparticles demonstrated biological activity.

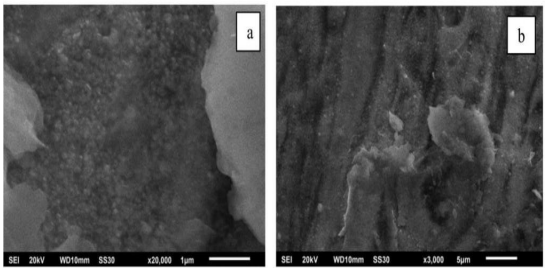


Figure 3: The image of SEM on AgNPs (a) and the size of AgNPs (b)

X-ray diffraction (XRD) analysis

Artificial silver nanoparticles were studied using XRD to learn more about their crystalline nature. The XRD data display features of nanoparticles and crystalline planes with peaks[43]. The XRD pattern of AgNO3 is definitively linked to the

kaempferol development of recognizable crystalline peaks of Ag nanoparticles at 2 θ=26.11.

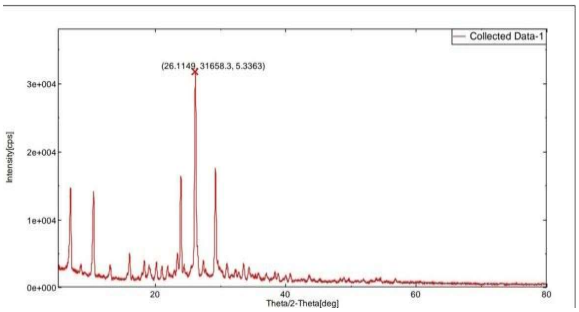


Figure 4: XRD Data for AgNPs

Antimicrobial assay

An antimicrobial assay is the process of testing the efficacy of silver nanoparticles containing kaempferol against microorganisms such as bacteria, fungi, viruses, and parasites. This experiment determines the minimum bactericidal concentration (MBC) or minimum inhibitory concentration (MIC) of an antimicrobial agent.

Minimum inhibitory Zone		
Micro Organism	Flavonoids (mg/ml)	
	1	2
Escherichiacoli	5	7.5
Pseudomonas	7.5	10
Candida Albicans	15	15
Aspergillus Niger	5	
*1. Kaempferol *2. Rhamonisorobin		

Table 2 : Antimicrobial assay of kaempferol

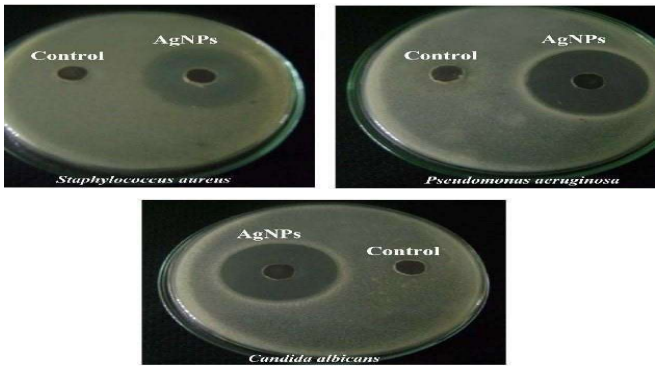


Figure 5: MIC test of standard and test sample of silver nano particle

Anti-cancer activity

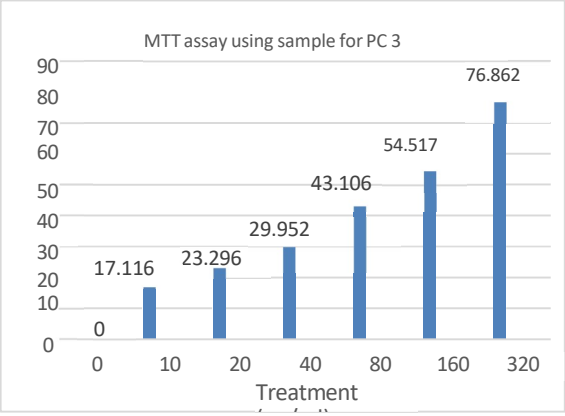
Cells based cytotoxic MTT (multi troop transport) assay using sample or standard for PC 3 cells was performed at different

concentration of As-Ag Nanoparticles. The findings, which are shown in Tables 3 and 4, indicate that nanoparticles inhibited the growth of A549 (lung cancer) cells and decreased their motility at an IC50 of 100.9 µg/ml.

IC 50 Value :

Sample	Conc. in µg/ml	OD at 590 nm	% inhibition	IC 50 µg/ml
control	0	0.63	0.000	
Nano particles	1 0	0.52	17.116	100.9
	20	0.48	23.296	
	40	0.44	29.952	
	80	0.35	43.106	
	160	0.28	54.517	
	320	0.14	76.862	

Table 3 : Statistical evaluation of sample



Standard				
Compound Name	Conc in µg/ml	OD at 590 nm	% Inhibition	IC 50 µg/ml
Control	0	0.698	0	
Vinblastine	3.12	0.616	16.6	10.81
	6.25	0.541	31.6	
	12.5	0.452	49.48	
	25	0.376	64.8	
	50	0.321	75.74	
	100	0.264	87.17	

Table 4 : Statistical evaluation of standard DISCUSSION

The antibacterial action of silver nanoparticles is diverse. Their biological and physicochemical characteristics can be changed thanks to silver nanoparticles. Natural flavanol kaempferol is found in many plants, is harmless, nontoxic, and has a variety of bioactivities. Because of its wide range of pharmacological properties, such as its anti-inflammatory, anti-oxidant, anticancer, and antidiabetic properties, kaempferol is widely employed in medical research. In these investigations, a kaempferol-containing silver nanoparticle solution was examined in the 300–60 nm range using a Shimadzu (UV-1900i) spectrophotometer Analyzing the Fourier transform infrared spectroscopy (FTIR) test, a peak at 3335 cm−1 represented the N-H stretch of the amine, and a minor O-H stretch was observed at 3442 cm−1 C-H. Peak absorption for stretch alkynes was measured at 2538 cm−1 A band at 1598 cm−1 is an expression of the C-O asymmetric stretch carbon compound. A cytotoxic experiment based on cells was conducted at varying concentrations of AgNO3. This finding indicates that PC3 growth was hampered by nanoparticles. For compositional analysis, the synthesized silver nanoparticles were assessed using an energy dispersive X-ray analyzer (Hitachi 3400, Japan). The purpose of this experiment is to find an antimicrobial agent's minimum inhibitory concentration (MIC) or minimum bactericidal concentration (MBC). The biological activity of the silver nanoparticles against both Gram positive and Gram-negative bacteria was shown by SEM experiments.

CONCLUSION

Kaempferol was effectively conjugated with silver nanoparticles, and this stabilizer proved to be an appropriate one for improving both the antibacterial and anticancer properties. The anti-bacterial and anti-cancer properties of the silver nanoparticles were examined by a variety of tests, such as FTIR analysis, SEM imaging, MTT testing, microbiological assays, and XRD data. These findings suggest that the many functional groups present in kaempferol-coated silver nanoparticles enhance their stability.

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well as Vidya Siri College of Pharmacy, Bangalore.

CONFLICT OF INTEREST

Authors Declare non

ABBREVIATIONS

Ag: Silver; **AgNO3:** Silver nitrate; **NP:** Nanoparticle **TEM:** Transmission Electron Microscopy; **SEM:** Scanning Electron Microscopy; **nm:** Nanometer; **MTT:** (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide); **ATR:** Attenuated Total Reflection; **PC3:** Prostate cancer cell line; **AgNPs:** Silver Nanoparticles; **XRD:** X-Rays deflection ; **EDXA:** Energy-dispersive X-ray analysis; **OD:** Optical Density; **ATCC:** American Type Culture Collection; **FBS:** Fetal Bovine Serum; **PBS:** Phosphate Buffered Saline; **ºC:** Degree Celsius; **GPP:** Graph Pad Prism;

REFERENCES

Kumar, V., Yadav, S. K. Plant-mediated synthesis of silver and gold nanoparticles and their applications.J of Chem Tech and Biotechnol. 2008; 82(2): 151-157.

Sankar, R., Manikandan, P., Malarvizhi, V., Fathima, T., & Shivashangari, K. S. Green synthesis of colloidalsilver nanoparticles using natural rubber latex extracted from *Hevea brasiliensis*. Spectrochemicia. Acta. 2011; 82: 140-145.

Kharissova, O. V.; Kharisov, B. I.; Dias, H. V. R.; and Pérez, B. O. J. environmentally friendly nanoparticle synthesis. Trends Biotechnol.2013; 31(4):240-248.
Gurunathan, S., Han, J. W., & Eppakayala, V. Antibacterial activity of dithiothreitol reduced graphene oxide.J of Industrial and Engineering Chemistry. 2013; 19(4): 1280-1288.
Zhu, X., & Radovic-Moreno, A. F. An overview of nanoparticle characterization using UV-visiblespectroscopy. Nanoscience and Nanotechnology Letters. 2014; 6(7): 649-655.

Zamani, M., Darroudi, M., and Mirzaei, H. creation, evaluation, and antimicrobial efficacy of silver nanoparticles utilizing eucalyptus leaf extract. Biotechnol nanomedicine, and artificial cells. 2017; 45(1): 1–7.

Anwar, F., and Amin, M. Effect of temperature on the synthesis of silver nanoparticles in aqueous solution by a microwave-assisted method. J of Nanostructure in Chem. 2015; 3(1):84.

Yang, D. C., Zhang, D., Kim, Y. J., and Singh, P. nanoparticles made by microbes and plants through biological synthesis. Biotechnol Trends. 2016; 34(7): 588–599.

Shamsi, S. F., Singh, V. N., Ahmad, N., Sharma, S., Alam, M. K., & Mehta, B. R. Qick production of silver nanoparticles with dried basil, a medicinal plant. Colloids and Surfaces B: Biointerfaces. 2010; 81(1): 81-86.

Singh, Sinha, Prasad, and Pant, A. B. Preparation of silver nanoparticles: A green approach with Kaempferol as reducing and capping agent. J of Nanomaterials. 2016; 6(1): 1-6.

C. H. Park, C. R. Park, and S. W. Lee. Fourier transform infrared (FTIR) spectroscopy analysis of protein adsorption onto ceramic membrane surfaces. J of Colloid and Interface Science. 2003; 263:190-195.

D. Pradhan, N. N. Sharma, S. Das, S. Baliarsingh, and M. R. Nayak. Synthesis and characterization of polyvinyl alcohol (PVA) coated silver nanoparticles by chemical method. Int. J of Sci Res in Science, Engineering and Technol. 2017; 3(2):524-528.

N. N. Arslanoglu, F. U. Altiparmak. Fourier transform infrared spectroscopy and thermogravimetric analysis of organoclays. J of Applied Polymer Science. 2006; 100(3): 1856–1861.

S. M. Gorji, M. M. Mozafari, S. Fathi-Roudsari, M. R. Ejtehadi, and H. R. Khalesi. Characterization and evaluation of silver nanoparticles-coated polyurethane foam as a new antibacterial wound dressing. J of Biomaterials Applications. 2001; 32(7): 896-906.

A. Ghosh. Silver nanoparticles as antimicrobial agent a case study on E. coli as a model for Gram-negative bacteria. J of Applied Biomedicine. 2011; 9(2): 97-105.

V. Thakkar. Biological synthesis of metallic nanoparticles. Nanotechnology, Biology, and Medicine. 2010; 6(2): 257–262.

Sharma, S. Green synthesis of silver nanoparticles from medicinal plants and evaluation of their antiviral activities. Advances in Virology. 2019; 6(2):11-15.
Huang J. Green synthesis of silver nanoparticles using tea leaf extract and evaluation of their stability and antibacterial activity, in Colloids and Surfaces. Physicochemical and Engineering Aspects. 2015;485: 330-335.
S. Saxena. Green synthesis of silver nanoparticles: a review in Green Chemistry Letters and Reviews. 2016; 9 (1): 31-42.
P. Kumar. Synthesis and characterization of silver nanoparticles from leaf extract of *Azadirachta indica*," in Colloids and Surfaces. Physicochemical and Engineering Aspects.2010; 368(1-3): 58-63.
S. Prabhu. Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications,and toxicity effects, in International Nano Letters.2018; 8(3): 201-210.
S. Singh. A review on green synthesis of silver nanoparticles and their applications, in Artificial Cells, Nanomedicine, and Biotechnology. 2018; 46(4): 855-872.

Pérez-Hernández. Characterization of Nanoparticles by X-ray Diffraction. SpringerLink. (2018).

Scardi. The Role of X-ray Diffraction in the Characterization of Nanoparticles. SpringerLink. (2017).

Puntes. Synthesis, Characterization, and Optical Response of Uniform-Sized Iron Oxide Nanoparticles. Chemistry of Materials. 2001;

Microtrac Inc. Nanotrac Wave User Manual. (2013)

Koppel, D. E. Analysis of Macromolecular Polydispersity in Intensity Correlation Spectroscopy: The Method of Cumulants. The Journal of Chemical Physics. 1972

Mendoza Guzmá. Characterization of Silver Nanoparticles Synthesized Using Stenocereus Pruinosus (Cactaceae) Mucilage and Their Antibacterial Activity. nanomaterials. 2019.

Hashim, R., Ali, A. M., Abdul-Rahman, P. S., and Lim, Y. Z. Antimicrobial assays: a summary of procedures and techniques. J of Biomolecular Techniques 2018; 29(2): 39–48.

A. P. Fraise testing for antibiotic and antimicrobial susceptibility. In A. P. Fraise, P.A Lambert, & J. F. S. Spencer (Eds.), Exeter Clinical Laborator International Practice and Procedures.2019;

(33–44). Elsevier.

Miranda, C. D., and Rojas, R. Creation of a high-throughput microplate screening technique for assessing bacterial viability in J of Microbiological Methods. 2007; 70(3): 484-490.

Clinical Laboratory Standards National Committee. 1997. Antimicrobial Disk Susceptibility Test Performance Standards, Sixth Ed. NCCLS.

Pfaller, M. A., & Yeast Antifungal Susceptibility Testing Performance Standards. The approved standard third edition of the reference method for testing yeasts' susceptibility to antifungals through broth dilution is M27-A3. The Institute for Clinical and Laboratory Standards. 2017.

Polonini, H. C., de Faria, A. F., and Rodrigues, C. M. Portuguese. A review of general principles and current practices for antimicrobial susceptibility testing was conducted by Microbiological. Brazalian J of Microbiology. 2015; 46 (3):661-671.

T. Mosmann. Rapid colorimetric assay for cellular growth and survival: Use in assays for cytotoxicity and proliferation. Immunological Methods Journal. 1983; 65(1-2): 55–63.

ATCC® CRL-1435TM, PC-3. The product is accessible at https://www.atcc.org/products/all/CRL-1435.aspx.

Schalken JA, Smit FP, Pfeiffer MJ, and Sedelaar JP. In prostate cancer, androgen deprivation leads to an upregulation of stem cell markers and steroidogenic enzymes. Mol Med 2011; 17(7-8):667–664.

Singh B, Singh A, Saini S, and others. An analysis comparing the effects of gold and silver nanoparticles on the human prostate cancer cell lines DU-145 and PC-3. J Drug Delivery Sci 2020; 58:101830.

Kaur, A., Kumar, M., and Dhawan, G Green synthesis of kaempferol-coated silver nanoparticles with strong anticancer activity against human lung cancer cell line by. Applied Physics A, 2020; 126 (9): 1–9.

Chandran, S.P., Sastry, M., Ahmed, A., and Chaudhary, M. Pasricha, R. Synthesis of Aloe Vera plant extract-based gold nanoparticles. Progress in Biotechnology, 2006; 22(2): 577–583.

H. M. Pathan, N. L. Tarafder, and M. Aslam's X-Ray Diffraction Analysis of Nanoparticles (Nanoparticles: Properties, Classification, Characterization, and Fabrication, 2021)