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FORMULATION, CHARACTERIZATION AND EVALUATION OF SILVER NANO PARTICLES OF GREWIA ASIATICA L. FOR ANTIMICROBIAL ACTIVITY

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ABSTRACT

Green synthesis of nanoparticles is a novel way to synthesis nanoparticles by using biological sources. It is gaining attention due to its cost effective, eco-friendly and largescale production possibilities. In this present study ***grewia asiatica*** leaves extract was taken to investigate the potential for synthesizing silver nanoparticle. The silver nanoparticles synthesized were confirmed by their microscopy change of colour to dark brown due to the phenomenon of surface plasmon resonance. The characterization studied was done by UV-Vis spectroscopy, Scanning electron (SEM), Zeta potential studies and Fourier Transmission infrared spectroscopy (FTIR). The UV absorption peak at 448 nm clearly indicates the formation of AgNPs. The SEM characterization techniques confirmed successful formation of spherical AgNPs Particle size and Zeta potential studies validated the size and charge of the nanoparticles in the colloidal system without any aggregation. Moreover, the biological evaluation of AgNPs revealed good bactericidal and antifungal properties against *Staphylococcus aureus*, *Escherichia coli*, and *A. niger*, *C. albicans*, which are pathogens commonly involved in infectious of various diseases.

Keywords-Green synthesis, silver nanoparticles, *grewia asiatica*, SEM.

1. INTRODUCTION

Plants are fundamentally a divine endowment for the synthesis of medicinal compounds. The process of isolating these compounds from medicinal plants and characterizing their active ingredients plays a crucial role in the advancement of new pharmaceuticals aimed at addressing various diseases, thereby demonstrating considerable therapeutic value[1]. The extract derived from plants, commonly known as a natural product, has significantly contributed to novel findings in the field IRof chemical diversity, particularly due to the unpredictable availability of either standardized extracts or pure compounds[2]. Research in the pharmaceutical sector reveals that approximately 10 to 20% of plants are effectively utilized in healthcare to combat serious illnesses such as cancer[3]. A classic illustration is the bark of the yew tree, which is primarily composed of taxol and is utilized in the treatment of ovarian and breast cancer. The isolation or extraction process of medicinal plants generally results in one or more substances that are responsible for their therapeutic effects, which are intricately linked[4]. Plants are the principal source of medications in modern medicine, traditional practices, folk remedies, dietary supplements, and synthetic pharmaceuticals[5]. Recent studies have shown that medicinal plants predominantly exhibit antioxidant properties. Phenolic compounds, which include flavonoids, lignins, vitamins A, C, and E, as well as tannins, are all antioxidants that are primarily sourced from plants[6]. Individuals who are inclined towards conventional medicine often turn to plant-derived drugs for various reasons, including their effectiveness, favorable therapeutic outcomes, and minimal side effects, while ecological considerations suggest that natural products are harmless. In contrast, the misuse or overuse of synthetic drugs can result in numerous complications and adverse effects[7].

In recent times, nanotechnology has catalyzed significant scientific progress in the domains of research and technology. Nanotechnology encompasses the study and application of minuscule objects that can be utilized across various disciplines, including chemistry, biology, physics, material science, and engineering. A nanoparticle serves as a fundamental unit that operates cohesively in terms of transport and properties[8]. As suggested by its name, 'nano' denotes a billionth or 10^-9 unit. Its size typically ranges from 1 to 100 nm; due to this diminutive scale, it occupies a pivotal role in numerous areas of nanoscience and nanotechnology. Nano-sized particles exhibit unique characteristics because their small dimensions enhance the surface-to-volume ratio,

resulting in distinct physical, chemical, and biological properties compared to bulk materials. Therefore, the primary objective of studying these minute sizes is to stimulate chemical activity with unique crystallography that amplifies the surface area[9-10]. Consequently, in recent years, extensive research has been conducted on metallic nanoparticles and their properties, such as catalysis, sensing, optics, antibacterial activity, and data storage capacity[11]. The concept of nanotechnology can be traced back to the 9th century. For the first time in 1959, Richard Feynman delivered a lecture on the concept of nanotechnology, discussing molecular machines constructed with atomic precision and referring to nanoparticles, coining the phrase, 'There's plenty of space at the bottom.[12]' Professor Peter Paul Speiser and his research team were pioneers in investigating polyacrylic beads for oral administration and targeting microcapsules. In the 1960s, nanoparticles were developed for drug delivery and vaccine purposes, revolutionizing the medical landscape. The inaugural paper published in 1980 by K. Eric Drexler from the Space Systems Laboratory at the Massachusetts Institute of Technology was titled 'An approach to the development of general capabilities for molecular manipulation.' The term 'nanotechnology' was first introduced as a scientific field by Nario Taniguchi in 1974 in his paper titled 'Nanotechnology,' which primarily focused on the processing ofseparation, consolidation, and deformation of materials by one atom or one molecule[13].

Nanotechnology represents a rapidly expanding domain within the scientific field, characterized by its interdisciplinary nature that bridges both science and technology. This field enhances the potential for investment and regulation at the cellular level, particularly concerning the interactions between synthetic materials and biological systems[14]. Nanotechnology advances through three primary processes: separation, consolidation, and deformation of materials at the atomic or molecular scale[15]. It is categorized into three distinct types: Wet nanotechnology, which focuses on biological systems such as enzymes, membranes, and cellular components; Dry nanotechnology, which emphasizes surface science and physical chemistry, particularly in the fabrication of structures from carbon, silicon, and inorganic materials; and Computational nanotechnology, which involves modeling and simulating complex structures at the nanometer scale[16]. These three areas are interdependent. There are two principal methods for synthesizing metallic nanoparticles: the chemical method and the physical method. The chemical approach encompasses techniques such as chemical reduction, electrochemical methods, and photochemical reduction[17]. The chemical process can be further divided into classical chemical methods, which utilize reducing agents like hydrazine, sodium borohydride, and hydrogen, and radiation chemical methods that are generated through ionizing radiation[18]. The physical approach includes methods such as condensation, evaporation, and laser ablation for the synthesis of metal nanoparticles. Biological synthesis of nanoparticles, often referred to as green synthesis, presents a challenging yet promising concept. This method of synthesizing nanomaterials can address environmental challenges, including solar energy conservation, agricultural production, catalysis, electronics, optics, and biotechnological applications. Green synthesis of nanoparticles is cost-effective, readily available, environmentally friendly, non-toxic, and suitable for large-scale production, serving as both reducing and capping agents. In contrast, the chemical method is considerably more expensive and produces hazardous by-products that can have detrimental effects on the environment.[19]

Table No:-1 Applications of Nanoparticles.

Metal Nanoparticle	Application
Silver	Anti-microbial, anti-cancer, anti-protozoal, anti- fungal
Gold	Anti-microbial
Palladium	Anti-bacterial

Copper	Anti-microbial, anti-cancer
Selenium	Anti-cancer[20-24]

2.0 MATERIAL AND METHODS

The Grewia aristica plant (AR grade) was collected in and around Shikaripura Taluk. Petroleum ether (AR grade), Chloroform (LR grade), 70% Ethanol (LR grade), Ethyl acetate (LR grade), n-butanol (LR grade), Distilled water (AR grade), Silver nitrate ($AgNO_3$, AR grade), Potassium bromide (KBr, AR grade), Nutrient broth (AR grade), Nutrient agar (LR grade), and Sodium hydroxide (AR grade) were all procured from S.D. Fine Chem. Ltd., Mumbai.

2.1 Preparation of crude extracts

The Fresh Plant was washed under running tap water, air dried in shade and then homogenized to make coarse powder. The coarse power was passed through mesh no.60. The powder obtained is subjected to successive Soxhlet extraction with the solvents with increasing order of polarity i.e. petroleum ether (60-80°), chloroform (59.5-61.5°) and 70% hydro alcohol (64.5-65.5°) the extracts were concentrated under reduced pressure and stored in desiccators until further use and the percentage yield of corresponding extracts were calculated[25].

2.2 Preliminary phytochemical investigation.

The extracts were concentrated and preliminary phytochemical investigation was carried out for pet. ether (60-80), chloroform and 70% alcoholic extracts of Grewia aristica Linn for the detection of various phytoconstituents by following standard methods.

2.3 Test for Carbohydrates

Molisch test: Two ml of extract solution was treated with few drops of 15 percent ethanolic α-napthol solution in a test tube and 2 ml of concentrated Sulphuric acid was added carefully along the side of tubes. The formation of reddish violet ring at the junction of two layers indicates the presence of carbohydrates.

Test for reducing sugars

Benedict's test: To 2 ml of Benedict's reagent, 1 ml of extract solution was added, warmed, and allowed to stand. Formation of red precipitate indicates presence of sugars.

Fehling's test: 5 ml of extract solution was mixed with 5 ml Fehling's solution (equal mixture of Fehling's solution A and B) and boiled. Development of brick redprecipitate indicates the presence of reducing sugars.

2.4 Test for Proteins

Biuret test: The extract was treated with 1 ml of 10 percent sodium hydroxide solution and heated. A drop of 0.7 percent copper sulphate solution was added to the above mixture. The formation of purple violet colour indicates the presence of proteins.

Million's test: The extract was treated with 2 ml of Million's reagent. Formation of white precipitate indicates the presence of proteins and amino acids.

2.5 Test for amino acids:

Ninhydrin test: The extract was treated with Ninhydrin reagent at pH range of 4-8 and boiled. Formation of purple colour indicates the presence of amino acids.

2.5 Test for Steroids:

Salkowski test: 1 ml of concentrated Sulphuric acid was added to 10 mg of extract dissolved in 1 ml of chloroform. A reddish brown colour exhibited by chloroform layer and green fluorescence by the acid layer suggests the presence of steroids.

Liebermann – Burchard reaction: 2 ml of extracts were mixed with chloroform. Add 1– 2 ml acetic anhydride and two drops of concentrated Sulphuric acid from the side of the test tube. First red, then blue and finally green colour suggested presence of steroid.

2.6 Test for Cardiac Glycosides

Test for deoxysugars (Keller-Killiani test): To 2 ml of extract, glacial acetic acid, one drop 5 % Ferric chloride and conc. Sulphuric acid was added. Presence of cardiac glycosides was indicated by formation of reddish brown colour at junction of the two liquid layers and upper layer appeared bluish green.

Legal's test (Test for cardenoloids): To the extract 1 ml pyridine and 1 ml sodium nitroprusside was added. Appearance of Pink to red colour indicated presence of cardiac glycosides.

2.7 Test for Anthraquinone Glycosides:

Borntrager's test: To 3 ml extract, dil.

H2SO4was added and the mixture was boiled and filtered. To cold filtrate, equal volume benzene or chloroform was added. The mixture was shaken. The organic layer was separated and ammonia was added to it. Ammonical layer turned pink or red.

Modified Borntrager's test: To 5 ml extract 5 ml, 5 % FeCl3 and 5 ml dil. HCl was added. It was heated by boiling in a water bath. It was cooled and benzene or any organic solvent was added and shaken well. Organic layer was separated and equal volume dilute ammonia was added to it. Appearance of pinkish red colour showed presence of glycosides.

2.8 Test for Alkaloids: To the extract, add dilute HCL. Shake well and filter. With filtrate perform following tests.

Mayer's test: To 2-3 ml filtrate few drops of Mayer's reagent was added to give precipitate.

Hager's test: 2-3 ml. filtrates with few drops Hager's reagent gives yellow ppt.

Wagner's test: 2-3 ml. filtrates with few drops Wagner's reagent gives reddish brown ppt.

2.9 Test for Tannins and Phenolic compounds:

Ferric Chloride test: 5 ml of extract solution was allowed to react with 1 ml of 5 % ferric chloride solution. Greenish black coloration indicates the presence of tannins.

Lead acetate test: 5 ml of extract solution was allowed to react with 1 ml of 10 percent aqueous lead acetate solution. Development of yellow colored precipitate indicates the presence of tannins.

2.10 Test for Flavonoids:

Lead acetate test: To the extract few drops of 10 percent lead acetate are added Development of yellow colored precipitate confirms the presence of flavonoids.

Sodium Hydroxide test: To the extract increasing amount of Sodium Hydroxide was added gives yellow colour, which disappeared after addition of acid.

Shinoda test (Magnesium Hydrochloride reduction test): To the test solution add few fragments of Magnesium turning and add cone. Hydrochloric acid dropwise, pink scarlet, crimson red or occasionally green to blue colour appears after few minutes.

3 Preparation of Silver Nanoparticles (AgNPs)

Preparation of 1mM AgNO3 solution by One milimolar (mM) solution of AgNO3 (0.085gms) was prepared by dissolving in 500 ml distilled water (DW) and stored in amber colored bottle in cool and dry place. 500 mL of 1mM aqueous solution of AgNO3 solution was taken in Erlenmeyer flask and 75 mL of aqueous stem extract was added drop wise into it for bio reduction process at room temperature. The reaction mixture was allowed to stir at 200 rpm using magnetic stirrer till the solution was turned from yellow to dark brown indicating the formation of AgNPs. The reduced solution was centrifuged at 5000 rpm for 30 min to get clear supernatant. The supernatant was discarded and the particles obtained were centrifuged with water repeatedly to get pure nanoparticles. Various formulations of silver nanoparticles (NP1, NP2, NP3 and NP4) were prepared by using 70% alcoholic extracts of Grewia Hirusta Linn and 01 nM silver nitrate solution. Different amount of extract from plant Grewia Hirusta Linn has been used to obtain the best morphology and size of nanoparticles[26].

Characterization Techniques

UV-vis spectra analysis

The silver nanoparticles were confirmed by measuring the wave length of reaction mixture in the UV-vis spectrum of the Shimadzu spectrophotometer at a resolution of 1 nm (from 300 to 600 nm) in 2 ml quartz cuvette with 1 cm path length[26].

SEM analysis

The Morphological characterization of the samples was done using JEOL Jsm-6480 LV for SEM analysis. The samples were dispersed on a slide and then coated with platinum in an auto fine coater. After that the material was subjected to analysis.

Particle size and zeta potential analysis

The size distribution or average size of the synthesized AgNPs were determined by dynamic light scattering (DLS) and zeta potential measurements were carried out using DLS (Malvern, UK). For DLS analysis the samples were diluted 10 folds using 0.15M PBS (pH 7.4) and the measurements were taken in the range between 0.1 and 10,000 nm.

FT-IR analysis

The characterization of functional groups on the surface of AgNPs by plant extracts were investigated by FTIR analysis (Shimadzu) and the spectra was scanned in the range of 4000–400 cm−1 range at a resolution of 4 cm−1. The sample were prepared by dispersing the AgNPs

uniformly in a matrix of dry KBr, compressed to form an almost transparent disc. KBr was used as a standard analyze the samples.

4 Anti-microbial activity

Anti-bacterial activity.

The antibacterial activity f-or pure extract and prepared AgNPs was determined by well diffusion methods (Holder and Boyce 1994). About 25 mL of molten Mueller Hinton agar was poured into a sterile Petri plate (Himedia, Mumbai, India). The plates were allowed to solidify, after which 18 h grown (OD adjusted to 0.6) 100 µl of Escherichiacoli (Gram -Ve, MTCC443), Staphylococcus aureus (Gram +Ve, MTCC 96) were transferred onto a plate and made culture lawn by using a sterile L-rod spreader. After five min setting of the aforesaid microbes, a sterile cork-borer was used to make a 6 mm well on the agar. The test samples were loaded into wells with different concentrations of 50 µg/well, 100 µg/well, 150 µg/well, and 200 µg/well. The azithromycin (30 µg/ml) served as a positive control. The plates were incubated at 37°C in a bacteriological incubator for 24 h. The antimicrobial activity was Determined by measuring the diameter of the zone of inhibition around the well using the antibiotic zone scale (Himedia, Mumbai, India) [27].

Antifungal activity.

The antifungal activity for pure extract and prepared AgNPs was determined by well diffusion methods. About 25 mL of potato dextrose agar was poured into a sterile Petri plate (Himedia, Mumbai, India). The plates were allowed to solidify, after which three days grew 100 µl fungal spore suspension or 24 h grown yeast culture (105 cfu/mL) of fungal strain (Candida albicans (MTCC 227) and Aspergillus niger (MTCC 404) was swabbed using a sterile cotton swab on the agar plate and wells were made. The test samples were loaded into wells with different concentrations of 50 µg/well, 100 µg/well, 150 µg/well, and 200 µg/well. The clotrimazole (30 µg/ml) served as a positive control. All the drug-loaded plates were kept for 24-72 h. The antifungal activity was determined by measuring the diameter of the zone of inhibition around the well using the antibiotic zone scale (Himedia, Mumbai, India).

5 .RESULTS AND DISCUSSION

Extraction

The above-ground portion of Grewia aristica L was subjected to extraction using three distinct solvents. The characteristics of the plant extracts are detailed below. All extracts obtained from Grewia aristica L produced slightly sticky to crystalline green-colored extracts.

Table no:- 02 Properties of extracts and percentage yield

Name of Plant	Type of extract	Color of extract	Appearance	%Yield
Grewia aristica	Pet. Ether	Dark green	sticky	8.9
	Chloroform	Greenish	Crystalline	9.3
	70%Alcohol	Reddish brown	Crystalline	23.23

Phytochemical investigation

Plant bioactive compounds have played a vital role worldwide in preventing and curing numerous human ailments. It is because of their broad spectrum of chemical and biological activities. All medicinal plants require a detailed investigation before their exploitation as medicine because the therapeutic potential entirely depends on the quality of plant material used and the study of any crude sample material of natural origin is beneficial only if it contains the active constituents which have to be recognized to valid ateitsreal value. Moreover, information about different phyto-constituents of plants is a very important and advantageous as it is much valuable in the production of complex

chemical compounds as well as screening of their biological activities.

The result of preliminary phytochemical screening is compiled in table No.5.The preliminary phytochemical screening of petroleum ether (60-80), chloroform and70% alcoholic extracts of bark of Grewia aristica L shown that they contain proteins, amino acids, cardio glycoside, alkaloids, flavonoids, tannins, and phenolic compounds. The amounts of flavonoids, tannins and phenolic compounds resulted visibly higherin the alcoholic extracts of leaves of Grewia aristica L than in those of the pet. ether, chloroform extracts.

Table No:- 03 Preliminary phytochemical investigation

Phytochemical constituents	Petroleum ether extract	Chloroform extract	70%ethanolic extract
Fats and oils	++	--	--
Proteins	+	--	++
Alkaloids	++	--	++
Chlorophyll	+	++	--
Carbohydrates	--	--	+
Flavonoids	--	--	+++
Tannins	--	--	+++
Phenolic compounds	--	--	+++

Preparation of silver nanoparticles

Visible Observation on

According to literature studies silver nanoparticle solution has dark brown or dark reddish in colour. In Grewia aristica bark extract before addition of AgNO3 its colour was yellow but after its treatment with AgNO3 its colour changes to dark brown which indicated the formation of AgNPs.This colour change is due to the property of quantum confinement which is a size dependent property of nanoparticles which affects the optical property of the nanoparticles.

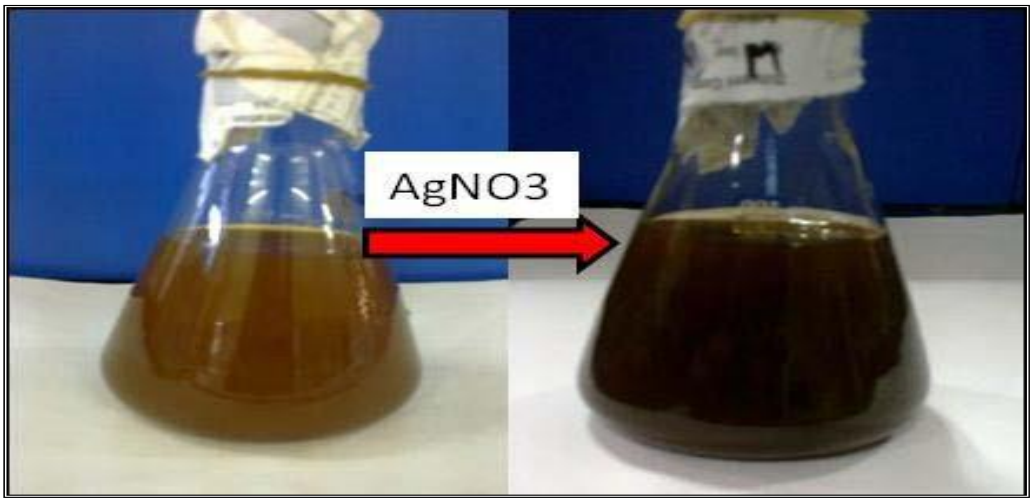


Fig 1 : Colour change of plant extract before and after addition ofAgNO3

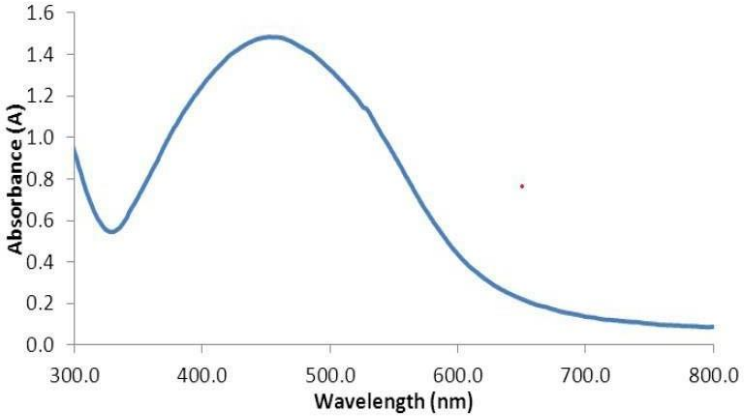
Characterization of silver nanoparticles

UV-Visible spectroscopy

The successful synthesis of AgNPs using the investigated Grewia aristica Lbark extract was confirmed by colour change and spectroscopic analysis of the reaction medium and isolated nanoparticles. Indeed, after stirring the mixture of AgNO3 and plant extracts for 10 min, an obvious colour change was observed for the plant studied. The colour of the reaction mixture changed from yellowish to brown,suggesting the conversion of ionic silver (Ag+) to metallic silver (Ag0) that self- ensembles into colloidal particles (AgNPs). This observation is consistent with the established literature, which stipulates that silver ions are reduced in the presence of plant extract due to the reducing properties of some secondary metabolites (i.e. polyphenols, alkaloids, terpenoids, proteins, etc.

In fact, the brown colour of AgNPs arises from the concomitant vibration of free electrons of the metallic silver that are in resonance with the light wave. This explains the origin of the surface plasmon resonance (SPR) absorption often observed with metallic nanoparticles, which is commonly verified using UV-Vis spectroscopy to complement the visual observation (colour change) in establishing AgNPs formation. As shown in Figure 1, the synthesized AgNPs exhibited distinctive UV-Vis absorption bands with maximum absorbances at 443 nm. The observed UV-Vis bands are due to the SPR absorption and confirm the presence of AgNPs, alike the colour change.

UV-Visible spectrum of synthesized AgNPs



SEM Analysis

A scanning electron microscope was employed to analyze the shape of the silver nanoparticles that were synthesized by green method. SEM analysis shows that the five plants have tremendous capability to synthesize silver nanoparticles which were roughly sphericalin shape and were uniformly distributed.

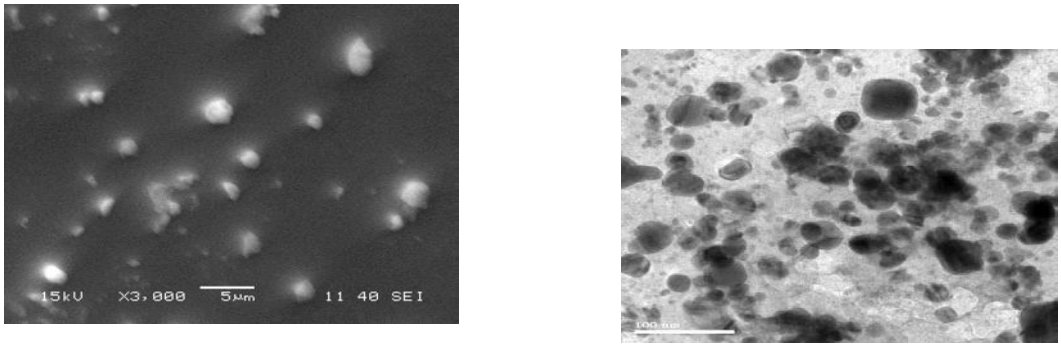
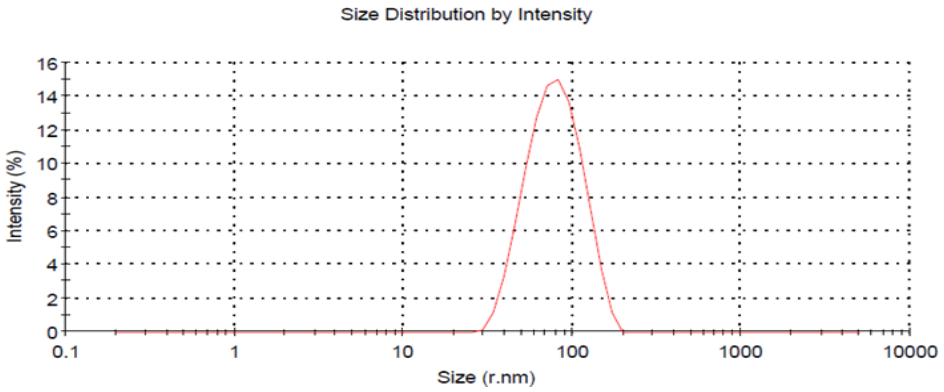


Fig 02 SEM photograph of synthesized AgNPs

Particle size distribution

The average size of the particles distribution of AgNPs was determined by a particle size analyzer and the obtained results are shown in Figure 3. The average particle diameter of AgNPs is 96 nm. The same particle size of AgNPs with the highly mono dispersed. These

particles were in the range of 50 to 100 nm. Bio-active molecules in the Grewia aristica Linn extract were also responsible for the stability of the Ag-NPs for a prolonged exposure. Similarly, Observe in gmicro graph, AgNPs were also non-agglomerated.

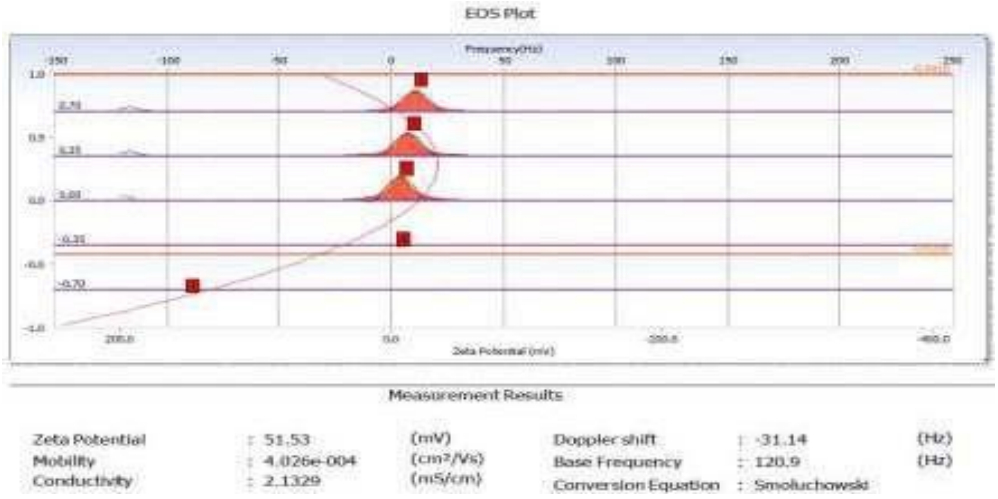


Particle size distribution of biologically prepared Silver nanoparticles

Zeta Potential Analysis

The Zeta potential of prepared silver nanoparticles was measured using Beckman Coulter Delsa™ Nano and it was found to be 51.53 mV from which we considered it to be Stable.

Zeta potential (Surface potential) has direct relation with the stability of a form/structure as mentioned below



A table showing the stability of the NPs according to the potential charge

Zeta potential[mV]	Stability behavior of the colloid
from0 to ±5	Rapid coagulation or flocculation
from±10 to ±30	Incipient in stability
from±30 to ±40	Moderate stability
from±40 to ±60	Good stability
morethan±61	Excellent stability

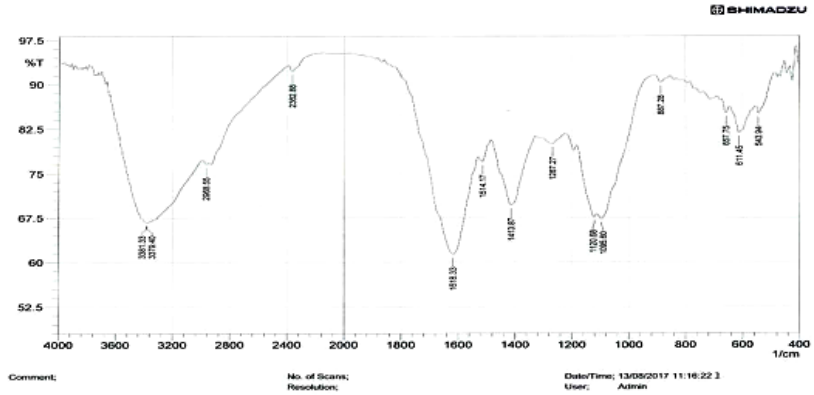
Fourier transformation infrared spectroscopy (FTIR)

The secondary metabolites are the main factors for the biosynthesis of silver nanoparticles, the

plant extract contain phenol, alcohol, amine, carboxylic acid, alkaloids and terpenoids that responsible for reduction and stabilizing silver nanoparticles (Jha et al., 2009).

FTIR spectroscopy showed that photochemical analysis of leaf extract of Grewia aristica Linn, it shows prominent bands of absorbance at peaks 3381.33, 2968.55, 1618.33, 1514.17, 1413.87, 1120.68, 1095.60, 887.28 cm-1.

The comparative study of the FTIR spectrum of leaf extract of Grewia aristica (Linn.) figure (20) and the resulted silver nanoparticles figure (21). Predicts that a shift in the band from 3381.33 to 3315.74 cm-1 which attributed to the stretching vibration of O-H of alcohols and phenols, 2968.55 to 2924.18 cm-1 band is attributed to the stretching vibration of C-H aliphatic, 1618.33 to 1610.61 cm-1 band is attributed to the vibration of C-N, 1120.68 to 1118.75 cm-1 band is attributed to the vibration asymmetric of C-O,1095.60 to 1091.75cm-1b an distributed to the vibration symmetric of C-O, 887.28 to 827.49 cm-1 band is attributed to the vibration of C-C skeletal



FTIR spectrum of leaf extract of Grewia aristica (Linn.)

FTIR spectrum of synthesized silver nanoparticles of leaf extract of Grewia aristica (Linn.)Anti-microbial activity

The different spectrum of antibacterial and antifungal activity of the test samples are given The test drug at 50,100,150 and 200mg/ml concentrations showed very light and similar activity for E. coli but showed higher activity for S. aureus. Test drug did not show significant activity against E. coli. The test drug did not show any activity against A. niger but showed very low activity against the C. albicans.

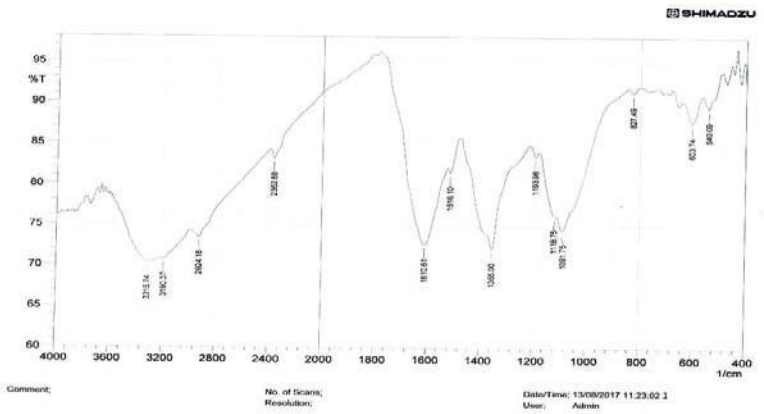


Table No:- 05 Antimicrobial activity of sample

Name of the Sample	0 µg/mL	50 µg/mL	100 µg/mL	150 µg/mL	200 µg/mL	Standard (30 µg/well)
E. coli	-	10	11	11	13	14
S. aureus	-	12	14	15	17	17

A. niger	-	-	-	-	-	16
C. albicans	-	7	7	8	9	16

Fig 03 Antimicrobial activity of sample

CONCLUSION

Despite the growing interest in both medical and nonmedical uses of metal nanoparticles, significant concerns persist regarding their safety and toxicity for humans and the environment. In this study, a very mild and solvent-free procedure was utilized. The method for synthesizing AgNPs demonstrates considerable potential as a straightforward, cost-effective, and environmentally sustainable approach for generating value-added products.

The extract of Grewia aristica was shown to be highly effective in producing AgNPs with advantageous physicochemical and biological characteristics. The synthesized AgNPs exhibited enhanced antimicrobial properties. These results are quite encouraging for the application of the biological effects of AgNPs produced using Grewia aristica extract.

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