Journal of Biomedical and Pharmaceutical Research
Available Online at www.jbpr.in
CODEN: - JBPRAU (Source: - American Chemical Society)
NLM (National Library of Medicine): ID: (101671502)
Index Copernicus Value 2023: 85.86
Volume 14, Issue 1; 2025, 49-71

ISSN (Online): 2279-0594
ISSN (Print): 2589-8752

Original Research Article

Formulation and Evaluation of Silver Nanoparticles Containing Albizia Amara Leaf Extract

Lokesh Kumar Gautam

Professor, Department of Pharmaceutics, Jaipur College of Pharmacy, Jaipur, Rajasthan, India

Article Info: Received: 05-02-2024 / Revised: 20-02-2024 / Accepted: 25-02-2025
Corresponding Author: Lokesh Kumar Gautam
DOI: https://doi.org/10.32553/jbpr.v14i1.1319
Conflict of interest statement: No conflict of interest

Abstract:
The current study was investigated synthesizes silver nanoparticles from Albizia amara leavesextract and tests them in-vitro anti-microbial, antioxidant, and cytotoxic activities. Albizia amara aqueous leaf extract phytochemistry as previously noted, plant leaves extract was used to synthesise Ag NPs. In-vitro antibacterial, antioxidant, and anticytotoxicity investigations. Antimicrobial Albizia amara silver nanoparticles Antioxidant Albizia amara silver nanoparticles. Preparation of gel- silver nanoparticles containing Albizia amara will beincorporated in carbopol by cold mechanical method.
Keywords: Albizia amara, Spreadability**,** Extraction, anticytotoxicity, nanoparticles..

Introduction

Microorganisms, also known as microbes, are microscopic living organisms that canonly be seen with the aid of a microscope They are foundvirtually everywhere on Earth, including in soil, water, air, and even inside the bodiesof plants, animals, and humans. Bacteria, viruses, fungi, archaea, and protists are microorganisms. Microorganisms have significant ecological, environmental, and industrial importance. Antiseptics, antibiotics, and disinfectants are the three primary categories of antimicrobial agents. Here are some key points about antibacterials Mechanisms of Action Types of Antibacterials Broad-spectrum vs. Narrow-spectrum Prescription and Proper Use Antibiotic Resistance

Antivirals Antivirals are a class of medications used to treat viral infections. They work by targeting specific aspects of the viral life cycle to inhibit the virus's ability to replicate and spread in the body. Antiretrovirals Antivirals for influenza Antivirals for herpes viruses Antivirals for hepatitis viruses

Non-pharmaceutical

Copper Antimicrobial copper alloys are being added to some healthcare facilities' tube systems as a precaution for the general public's health.

Essential oils

Essential oils found in herbal pharmacopoeias are thought to have antibacterial properties they increase in research in this field, none of the 25 to 50 percent of therapeutic compounds that come from plants are used as antimicrobials.

Antimicrobial pesticides: Antimicrobial pesticides are used to prevent contamination from bacteria, viruses, fungi, protozoa, algae,

Disinfectants; Destroy or inactivate microorganisms (bacteria, fungus, viruses), but they are not permitted to act as sporicides (because they are the hardest to eradicate).

Ozone: Ozone has the power to eradicate microorganisms from the air, water

Antimicrobial scrub Antimicrobial scrubs can lengthen the life of scrubs by preventing the buildup of odours and stains

Cancer

Cancer is most deadly diseases typically there is an uncontrollable growth and multiplication of some cells of the body and spreads to other parts. The human bodyis made of trillions of cells, these cells grow and multiply by a process called cell division (Mitosis), and old cells become damaged and die, and the new cells take their place Tumors are of two types cancerous (Malignant) or Non-cancerous (Benign). The Benign tumor doesn't invade nearby tissue but, the cancerous tumors multiply by the process called metastasis and spreads and invade nearby tissue and also throughout the body to form the new tumors. Due to cancer cells exhibiting loss of control over cell growth, as result, there are cumulative irregularities in several cell regulatory systems

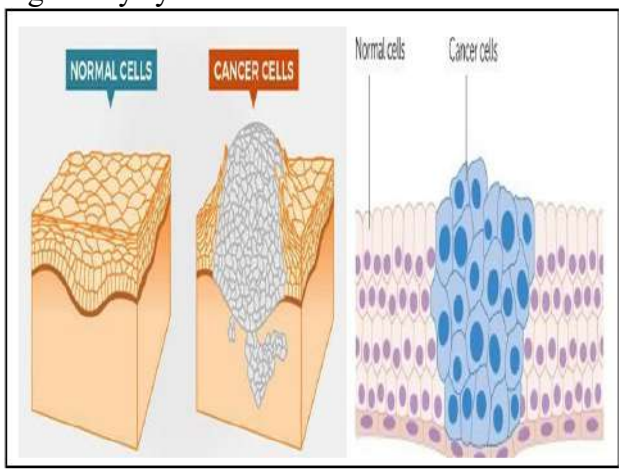


Figure 1: Structure of normal and cancer cells

Cancer pathogenesis also includes several gene mutations which are responsible for abnormal cell proliferation. Several factors directly or indirectly affect the gene mutation, these factors adversely affect the cell cycle and cause genetic disturbances, which make vital genes misfunctioning in the absence of tumor suppressor genes increasing abnormal cell multiplication and increasing cancerous cell development

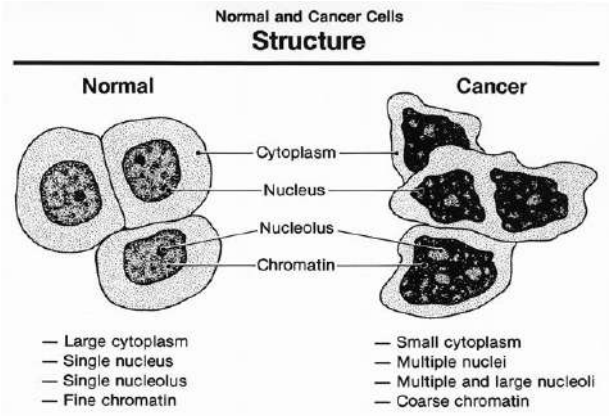


Figure 2: Difference between normal and cancer cells

Pathogenesis of Cancer

Molecular pathogenesis: The several pathogens are responsible for alter the gene mutation in the cell and thereby they activate the genes inappropriately and complete inactivation of the genes which are essential for normal cell life.

Chemical carcinogens Progression of cancer by chemical carcinogens in 3 series of steps namely i) Initiation ii) Promotion and iii) progression.

Physical carcinogens Radiations like UV rays (ultraviolet), ionizing radiation (x-rays and gamma rays) etc.

Treatment of cancer

The treatment of cancer becomes a challenging and complex process, due to there is increased incidence and death rate, hence the treatment of cancer disease involves a multidisciplinary approach, Chemo therapy, Radiation therapy, Surgery Targeted therapy Immunotherapy, Hormonal therapy, Hyperthermia Laser therapy.

Breast Cancer:

Breast cancer occurs when breast cells develop uncontrollably Breast cancer is varied. Breast cancer relies on which cells become cancerous. Uncontrollable alterations in breast tissue cells cause breast cancer. Blood and lymph vessels spread breast cancer. Breast cancer metastasizes when it spreads

Invasive ductal carcinoma

Cancer cells start in ducts and spread to other breast tissue

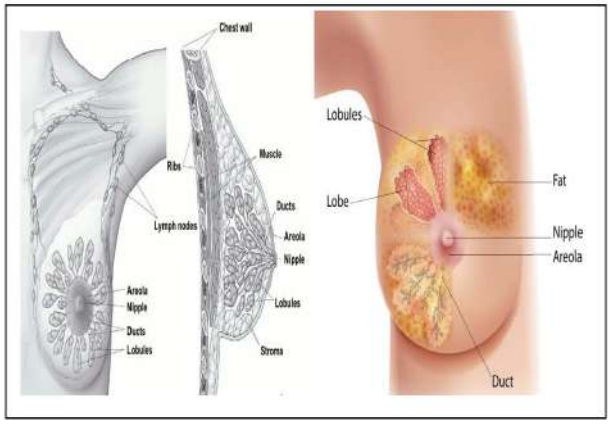


Figure 3: Normal Breast Cells

Your immune system includes the lymphatic system. It is a network of lymph nodes (small, bean-sized glands), ducts or veins, and organs If cancer cells have gone to your lymph nodes, they may have metastasized (spread) via the lymph system. Some women without lymph node cancer may develop Breast cancer cell lines are a valuable tool in cancer research as they provide a simplified model to study the characteristics, behavior, and response of breast cancer

Nanotechnology:

Nanotechnology studies atom and molecular matter control. Nanotechnology involves changing or producing materials between 1-100 nanometers in at least one dimension. Nanotechnology requires precision and molecular-sized components. Nanotechnology is the capacity to build things from the ground up utilizing tools and methods being developed to generate high-performance goods. Nanotechnology is frequently considered a general-purpose technology since its evolved form will affect practically all sectors of society and industry.

Branches of Nanotechnology

Nanotechnology has several study areas and applications because to its strong interpanel feature. These fields use nanotechnology

Nano engineering

Nanotechnology's nanoscale offshoot is nano engineering. Nano-engineering comes from nanometers, which are one billionth meters.

Green Nanotechnology

Green nanotechnology aims to reduce future environmental and health concerns from nanotechnology products and increase the usage of ecologically friendly nanoproducts.

Wet Nanotechnology

Wet nanotechnology emphasises pharmaceuticals and biology. Wet nanotechnology builds enormous masses from tiny ones. Drexler proposed dry nano-assemblers.

Nanoparticles:

Organic nanoparticles, organic nanoparticles, Inorganic nanoparticles, Carbon nanoparticles

Silver Nanoparticles:

Silver has a long history of medicinal use Silver nanoparticles' physical, chemical, optical, and antibacterial capabilities garnered interest.

Method of synthesis of silver nanoparticles

Silver nanoparticles were synthesised using "TOP-DOWN" and "BOTTOM- UP" methods. 1) Physical 2) Chemical and 3) biological approaches synthesise silver nanoparticles

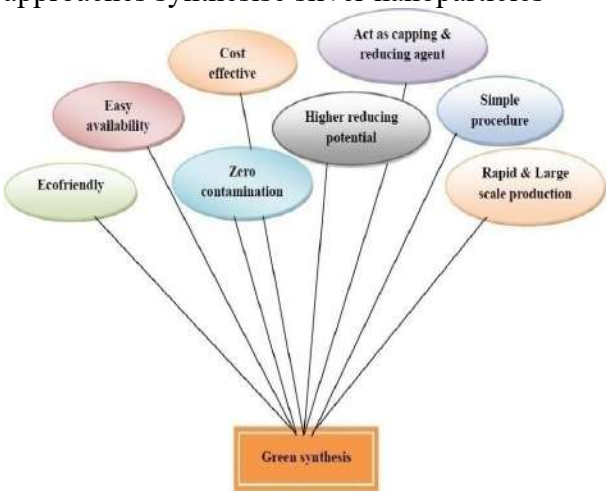


Figure 4: Advantages of green synthesis of AgNPs by plant extracts

Application of silver nanoparticles:

Silver nanoparticles are widely used nowadays because of their interdisciplinary properties. Silver nanoparticles are used in many medical implants, due to antiseptic nature of silver ion for example- Dental care implants, cardiovascular implants (prostheticsilicon heart valve), many orthopedic devices, etc

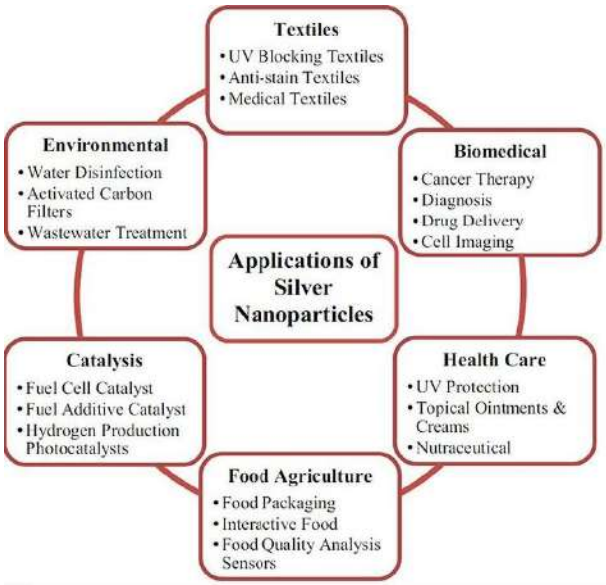


Figure 5:

Traditional herbal medicines:

Traditional herbal medicines employ medicinal plants and natural components for treatment. Medicinal plant chemicals are thought to heal the body. Alkaloids, flavonoids, terpenes, essential oils, and others are examples. Different plants cure digestion diseases, respiratory issues, skin issues, infections, and more. Researchers are exploring the active compounds in medicinal plants, conducting clinical trials, and investigating potential applications for various health conditions. This integration of traditional and modern medicine is known as integrative medicine and represents a growing field in healthcare.

Herbal medicines

Herbal medicines are medicinal preparations made from plants or plant extracts that are used to treat or prevent various health conditions

Figure 6:

Application of Herbal medicine

Facts the study is planned to prepare and characterize silver nanoparticles of Albizia amara leaf extract and their potential use on the microbial infection. Though Albizia amara reported to have a wide therapeutic activity, still their effectiveness increases by nanotechnology approaches.

Green synthesis method:

Green synthesis is the ecologically friendly synthesis of chemicals, nanoparticles, and materials. It eliminates harmful ingredients and energy- intensive procedures used in conventional synthesis techniques

Plant-mediated synthesis, Microbial synthesis, Solvent-free synthesis, Catalytic synthesis, Energy-efficient methods, Waste reduction and recycling

Topical Gels

Topical preparations are used for the localized effects at the site of their application as drug penetration into the underlying layers of skin or mucous membranes Topical gels are pharmaceutical formulations designed to be applied directly to the skin's surface. These gels typically contain active ingredients that are intended to provide local therapeutic effects. They are commonly used in dermatology and for thetreatment of various skin conditions

Problem Statement

Antimicrobial Study: Antimicrobial research investigates how various chemicals or treatments suppress or destroy microorganisms like bacteria, viruses, fungus, or parasites.

Antioxidant Study: Antioxidants are substances that can protect cells from damage caused by free radicals, which are unstable molecules that can lead to oxidative stress and contribute to various diseases, including cancer, cardiovascular disorders, and neurodegenerative conditions

Materials

Materials Used:

All the materials used were of either AR/LR grade or the best possible grade available as supplied by the manufacturer without any further purification

Table 1: List of chemicals

Sr. No	Materials	Manufacturer
1	Albizia amara leaves	Local area Belagavi
2	Silver Nitrate	Hi-Media Laboratories Pvt. Ltd. Mumbai.
3	Methyl paraben	Reachem laboratory chemicals pvt.ltd. Chennai
4	Triethanolamine	Nice Chemicals Pvt, Ltd, Kerala
5	Sodium Hydroxide pellets	S D Fine Chem Limited, Mumbai
6	Ascorbic acid	Himedia Laboratories Pvt. Ltd, Mumbai
7	Ethanol	Changshu Hongsheng Fine chem.Co. Ltd.
8	Pot. Ferricyanide	Molychem, Mumbai.
9	Trichloroacetic acid	Kemphasol, Mumbai.
10	Ferric chloride	Nice Chemicals Pvt, Ltd, Kerala
11	Ammonium Molybdate	John baker inc. Colorado USA
12	Sulphuric acid	Reachem laboratory chemicals pvt.ltd. Chennai
13	Sodium phosphate	Loba chemie, laboratory reagents and chemicals, Mumbai
14	Alcohols	Molychem, Mumbai.
15	Sodium hydroxide	Loba chemie, laboratory reagents and chemicals, Mumbai
16	Copper sulphate	Reachem laboratory chemicals pvt.ltd. Chennai

Collection and Authentication of Albizia amara leaves of plant:

Albizia amara (Fabaceae) leaves are collected from the Medicinal Garden of ICMR-National Institute of Traditional Medicine. RMRC ICMR layout, Nehru NagarBelagavi, Karnataka and authenticated (Accession number: RMRC-1739) by Dr. Harsha Hegde, Taxonomist ICMR Belagavi, Karnataka, India (Annexure 1).

Figure 7: Leaves of Albizia amara

Albizia amara, commonly known as the Indian Siris or Black Siris, is a species of tree belonging to the Fabaceae family The Albizia amara is a sizable deciduous tree that may grow up to 10 metres tall. Its branches are heavily pubescent in a yellowish or grey colour most significantly, some of the species most often used in Ayruvedic medicine are Albizia julibrissin, Albizia lebbeck, Albizia procera, and Albizia amara. Albizia amara, also known as Chigaraku or Allaregoo in Telugu.

Leaves: The leaves of Albizia amara are bipinnately compound, meaning they are divided into multiple pairs of leaflets. Flowers, Fruit, Distribution Habitat, Ecological Importance

Uses

Ulcer and cough remedies are made from the blossoms

Albizia amara is the most significant Sudanese traditional medicinal plant used to cure a number of illnesses; traditional healers utilise Albizia amara's aqueous leaf extracts to treat diarrhoea, epilepsy, excruciating back pain, loin pain, and other stomach issues

Albizia amara seeds are used as an astringent to treat gonorrhoea, piles, and diarrhoea.

Albizia amara has been reported to have anticancer, antimicrobial, antioxidant and anti-inflammatory properties

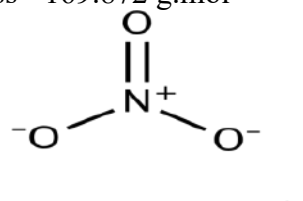
Mental health and neurological disorders, Digestive disorders

Albizia amara extracts. The plant extracts have shown cytotoxic effects against various cancer cell lines, inhibiting their growth and inducing apoptosis (programmed cell death). These findings suggest that Albizia amara may have promising applications in cancer treatment and prevention

Silver nitrate: An inorganic substance with the chemical formula AgNO3 is called silver nitrate. It serves as a flexible precursor for a variety of different silver compounds, including those used in photography. Compared to halides, it is far more light-sensitive

Description:

- Formula - AgNO3
- IUPAC name - silver nitrate
- Systemic IUPAC name - silver(I) nitrate
- Synonym - argentous nitrate, lapis infernalis, nitric acid silver (1+) salt.
- Molar mass - 169.872 $g.mol^{-1}$



- Structure -
- Appearance - colorless solid
- Odour - odourless
- Solubility - water, ammonia, acetone, glycerol, ether
- Density - 4.35g/cm^3 (24°c) and 3.97g/cm^3 (210°c)
- Melting point - 209.7°c
- Boiling point - 440°c
- Heat capacity - 93.1J/mol.K

Carbopol 934.

Carbopol 934 represents a proprietary variant within the carbomer category, a group of synthetic polymers with elevated molecular weight. Carbomers are cross-linked polyacrylic acid polymers that are highly hydrophilic (water-loving) and have the ability to absorb and retain large amounts of water

Methyl paraben.

Methyl paraben is a common preservative used in a variety of personal care and cosmetic products.

Triethanolamine.

Triethanolamine, also known as TEA, is a chemical compound with the formula COHOONOO. It is a viscous, colorless liquid that has a strong ammonia-like odor. Triethanolamine is produced by the reaction of ethylene oxide with aqueous ammonia.

Sodium hydroxide pellets.

Sodium hydroxide pellets, also known as caustic soda pellets or NaOH pellets, are solid form of sodium hydroxide

Ascorbic acid.

Vitamin C, commonly known as ascorbic acid, is a water-soluble vitamin essential to many physiological functions. It is a necessary nutrient that the body cannot synthesize and must be consumed.

Ethanol.

Ethanol, also known as ethyl alcohol or grain alcohol, is a volatile, colorless liquid that is commonly used as a fuel, solvent, and beverage. It is a type of alcohol produced through the fermentation of sugars by yeasts or through chemical processes, such as the hydration of ethylene.

Potassium ferricyanide.

Potassium ferricyanide, also known as potassium hexacyanoferrate (III), is a chemical compound with the formula $K_3[Fe(CN)_6]$. It is a deep red crystalline solid that is commonly used in various applications.

Trichloroacetic acid (TCA).

Trichloroacetic acid (TCA) is an organic compound with the chemical formula CCl3COOH. It is a strong acid and is commonly used in various industrial, medical, and cosmetic applications.

Ferric chloride, Ammonium molybdate, Sulfuric acid, Sodium phosphate, Alcohols, Sodium hydroxide, Copper sulphate, Chloroform, Glacial acetic acid, Silver nitrate solution, Ninhydrine reagent, Pyridine solution, Wagner's reagent were Also used in this Study.

Methodology:

Pre-Formulation Studies: Pre-formulation is a systematic approach made to analyze the samples and excipients to evaluate the physicochemical properties of drug substances. It is the initial step in the formulation of any dosage form.

The main objective of the pre-formulation study is to give the complete valuable knowledge about each ingredient to formulator for the production of safe and bio- available dosage forms.

Preparation of aqueous leaf extract:

Preparing a leaf extract involves extracting the bioactive compounds and substances from plant leaves using different methods.

Extraction Methods:

Extraction methods refer to a variety of techniques used to separate or isolate specific components from a mixture.

- Solvent Extraction:
- Steam Distillation
- Solid-Phase Extraction (SPE)
- Liquid-Liquid Extraction
- Soxhlet Extraction
- Supercritical Fluid Extraction
- Microwave-Assisted Extraction
- Ultrasound-Assisted Extraction
- Pressurized Liquid Extraction

Preparation of aqueous leaf extract:

Qualitative Phytochemical analysis of the plant extract was performed using the standard experimental procedure to identify the various phytoconstituents present in the extract

Statistical analysis:

In order to determine the significance of the test and standard samples, analysis of variance (ANOVA) was utilized. The test and standard samples were considered significant at a 5% significance threshold if the p-value (significant probability value) was less than 0.05. GraphPad Prism software version 9.0 (GraphPad software Inc., CA, USA) was used to do the statistical analysis of the in-vitro antioxidant and in- vitro cytotoxic test findings using two-way ANOVA and multiple comparison techniques. Using the GraphPad Prism programme, IC50 values were calculated by obtaining the inhibitor v/s response log. Statistical significance was defined as a p value less than 0.05.

Results and Discussion

In this current research, we successfully created silver nanoparticles through bio-fabrication, utilizing the aqueous leaf extract of Albizia amara. Subsequently, we assessed the potential antimicrobial and anticancer effects of these nanoparticles on various cancer cell lines.

Preliminary studies: The collected aqueous extract solution was used for further studies.

Visual observation:

AA-Extract to a 1mM silver nitrate solution, the reaction mixture changed colour from pale yellow to deep brown. Secondary metabolites and metal surfaces cause this change. Secondary metabolites bound to silver nuclei electrostatically. Thus, these silver nuclei's charge changes reduced silver ions. This complicated process causedthe colloidal fluid to change colour, indicating silver nanoparticle

formation. FIGURE15 UV-visible spectroscopy verified.

Figure 8: Color comparison of synthesized silver nanoparticles

AA-Extract, b) 1mM AgNO3solution c) Colloidal solution of AA-AgNPs

Table 2: Phytochemical analysis of aqueous leaf extract of Albizia amara

S.No.	Name of The Test	Aqueous extract of Albizia amara
1	Test for Carbohydrates:	
	Molish Test	+
	Fehling's Test	+
	Benedict's Test	-
	Iodine Test	-
	Tannic Acid Test	+
2	Test for Proteins:	
	Biuret Test	+
	Million's Test	+
3	Test for Amino Acids:	
	Ninhydrin Test	+
	Test for Tyrosine	+
4	Test for Steroid:	
	Salkowski Reaction	+
5	Test for Glycosides:	
	Keller-killiani Test	+
	Legal's Test	-
	Borntrager's test	+
6	Test for Flavonoids:	
	Shinoda Test	+
	H_2SO_4 Test	+
	NaOH Test	+
7	Test for Alkaloids:	
	Mayer's Test	-
	Wagner's Test	+

	Tannic Acid Test	+
8	Test for Tannins and Phenolic Compounds	
	5% $FeCl_3$ Solution	+
	Lead acetate	+
	Potassium dichromate	-
	Dilute Iodine Solution	+
9	Test for Saponin:	
	Foam Test	+
10	Test for Quinones:	
	H_2SO_4 Test	+
	HCl Test	+

The prepared silver nanoparticles were used to perform optimization:

The present study optimised temperature, pH, silver salt concentration, aqueous leaf extract-silver nitrate ratio, and reaction incubation time. These parameters influence silver nanoparticle production and particle size and shape. Green synthesis creates silver nanoparticles by reducing and capping silver ions with plant metabolites.

Effect of temperature:

In the present study an attempt has been made to investigate the effect temperature on synthesis of Albizia amara leaf extract mediated silver nanoparticles, because it is wellknown fact that temperature basically increase rate of reaction, hence effect of temperature is taken into consideration and synthesis was carried out at different temperature (Room temperature, 40 °C, 60 °C, 70 °C, 80 °C, 90 °C, and 100 °C), UV spectra of 24hr incubation time and we found that there is increase in peak intensity with increase in temperature and peak slightly shifted towards shorter wavelength from (445nm to 442nm), because at higher temperature kinetic energy of bio-molecules increased thus, complete consumption of silver nucleiby plant metabolites so complete reduction of silver ion takes place, results in better yield and small size of particles, But at 80 °C there is significant decrease in peak intensity seen, study by (Sunday Adewale et al 2021) reported specific suitable temperature is essential to maintain stability of plant metabolite for their better reactivity with silver ions. Also, we saw that asymmetry in the SPR band at 90 °C and100 °C may be due to the agglomeration of particles at high temperatures. Hence **60 °C** is selected as the optimum temperature for the synthesis of AA-AgNPs.

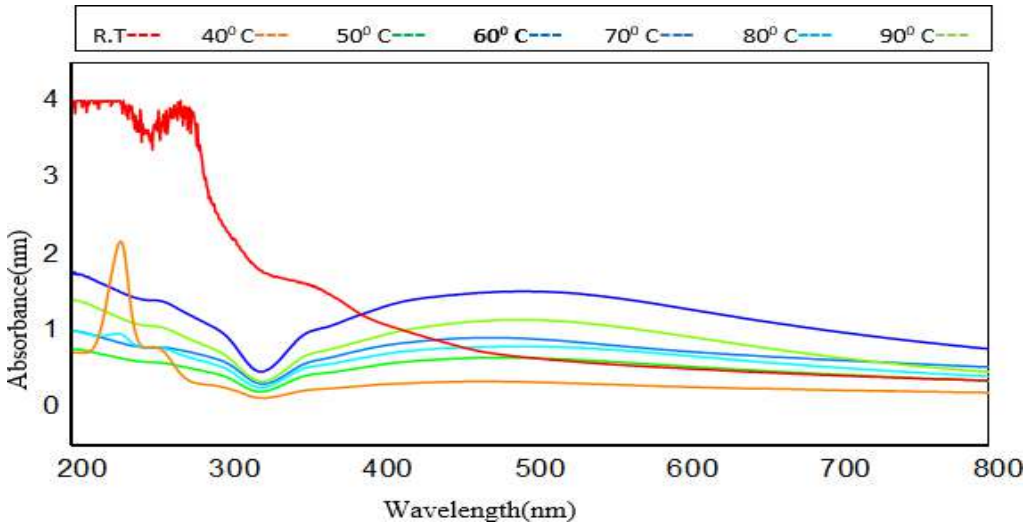


Figure 9: Effect of temperature on synthesis of AA-AgNPs

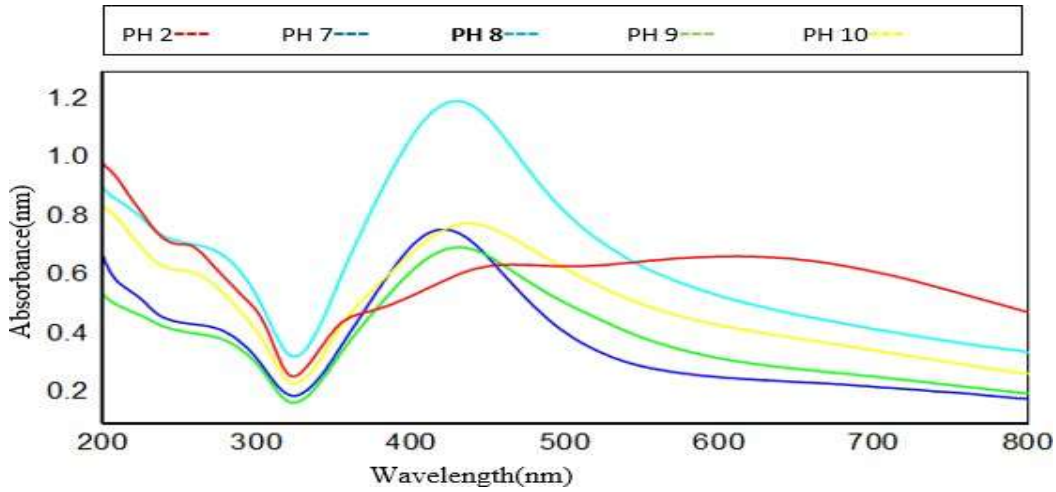


Figure 10: Effect of pH on synthesis of AA-AgNPs

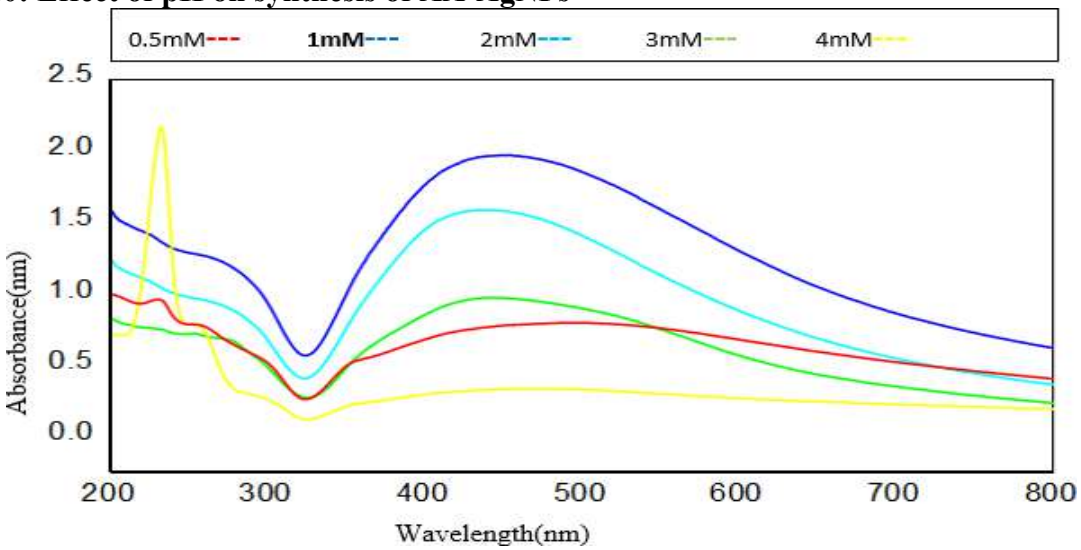


Figure 11: Effect of concentration of AgNO3 on the synthesis of AA-AgNPs

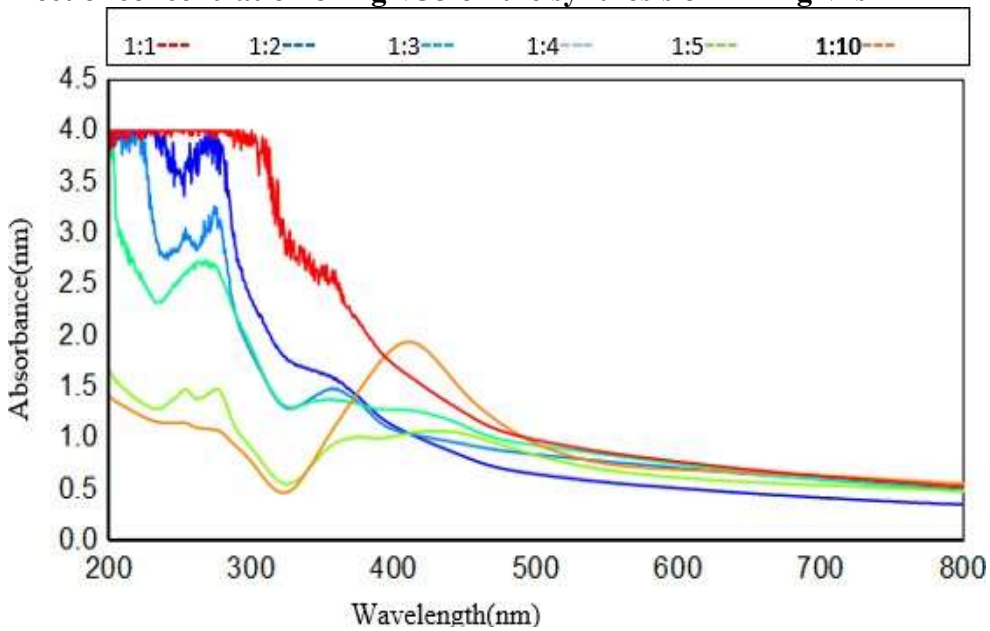


Figure 12: Effect of ratio of the reaction mixture on the synthesis of AA- AgNPs

Characterization of Albizia Amara Silver Nanoparticles:

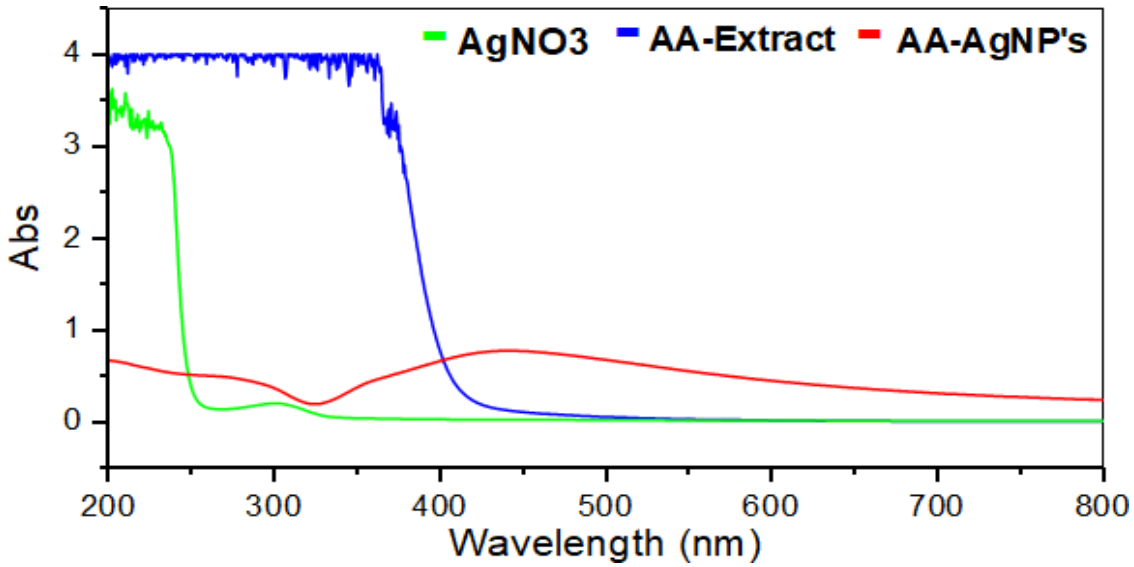


Figure 13: UV-visible spectra of silver nanopaticles synthesized from aqueous leaf extract of Albizia amara

Table 3: The IR frequencies of AA -extract and AA-AgNPs

Functional Groups	Standard IR Peaks (cm^{-1})	Observed IR Peaks of AA-Extract (cm^{-1})	Observed IR Peaks of AA-AgNPs (cm^{-1})
-OH	3500-2500	2921	3048-3376
C=O	1610-1550	1550	1599-1575
-C-N	1430-1250	1223	1383
-COC	1320-1050	1033	1110

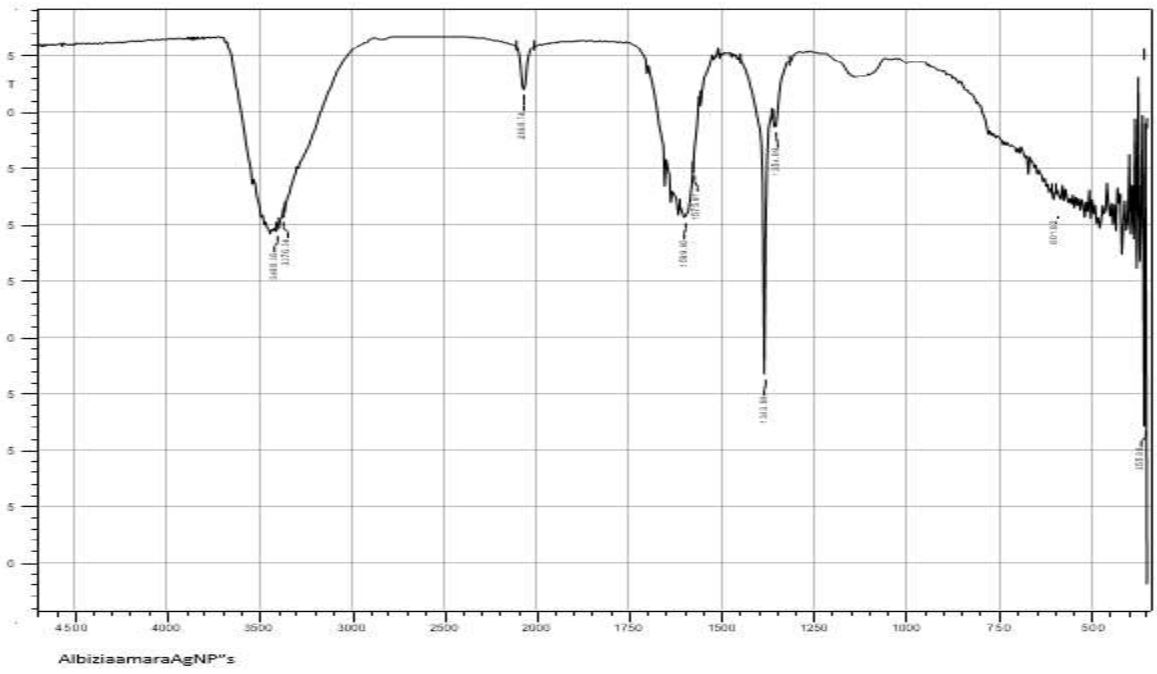


Figure 14: Fourier transform infrared spectra of AA-Extract

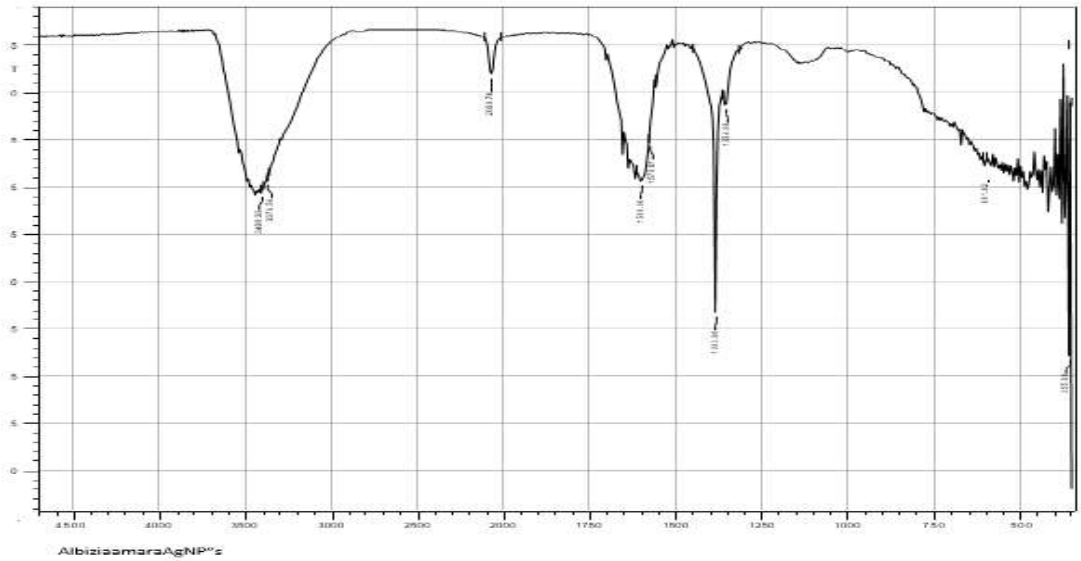


Figure 15: Fourier transform infrared spectra of AA-AgNPs

Particle size distribution and zeta potential of AA-AgNPs:

The stability of synthesized nanoparticles was tested by the zeta sizer instrument. Here the surface charge of silver nanoparticles was measured. In the currentstudy result of zeta, sizer revealed that synthesized silver nanoparticles fabricated from aqueous leaf extract of Albizia amara have the zeta potential -22.9, here the negative charge indicates particles have negative charge on their surface and higher the value higher repulsion between particles, which indicates less aggregation and high stability of nanoparticles. Average particle size AA-AgNPs of 95.4nm with poly dispersibility index of 0.314. Nanoparticles with very large size particle distribution and have poly dispersibility index values > 0.8 clearly indicates that the obtained nanoparticles are highly poly disperseble in nature.

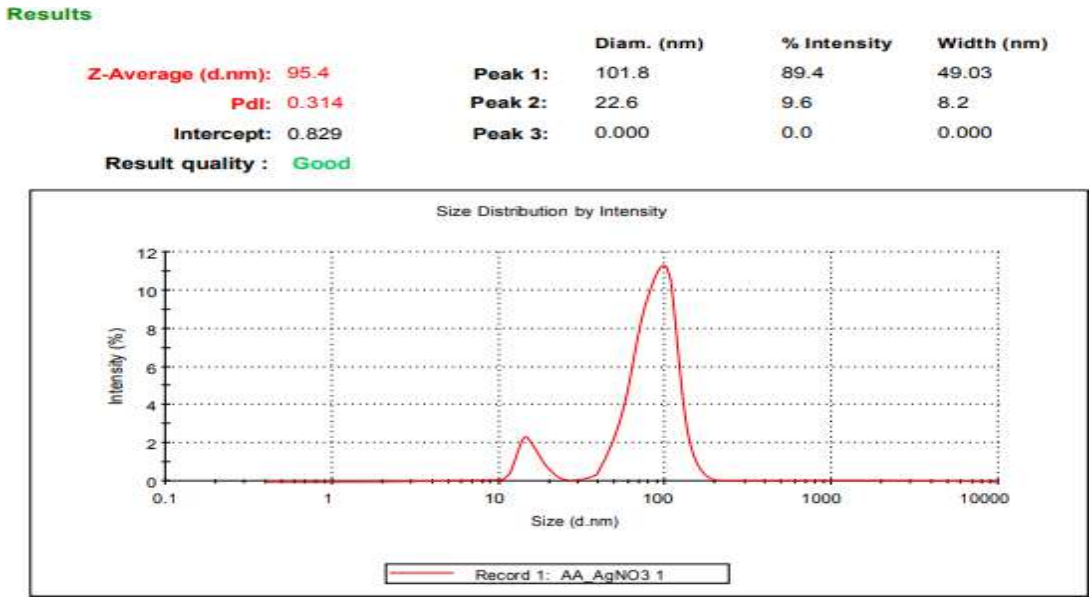


Figure 16: Particle size distribution of the AA-AgNPs

		Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -22.9	Peak 1:	-22.9	100.0	6.01
Zeta Deviation (mV): 6.01	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.0394	Peak 3:	0.00	0.0	0.00
Result quality : Good				

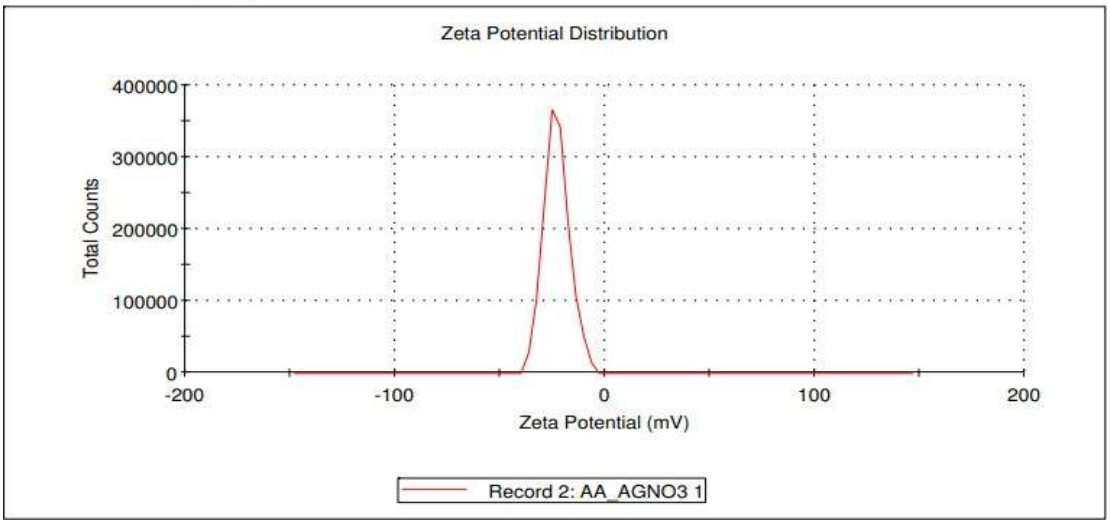


Figure 17: The Zeta potential of the AA-AgNPs

Transmission electron microscopy (TEM): In the present study, the morphological characteristics of silver nanoparticles, including their size and shape, were examined using the Transmission Electron Microscopy (TEM) method. As shown by TEM images in Figure No. 25, the results support the polydisperse character of the synthesised AA-AgNPs, which display a range of forms, namely spherical, triangular, pentagonal, and hexagonal. The average particle size, determined by plotting a histogram of particle diameters (nm) against their respective counts, utilizing Origin Pro software as depicted in Figure 26, was established to fall within the range of 80-85 nm

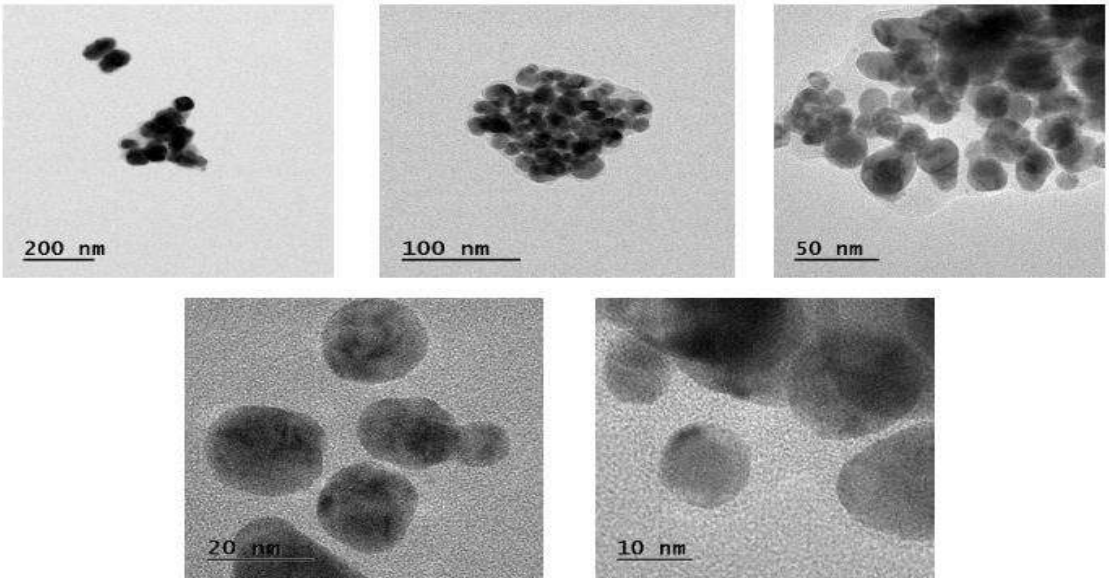


Figure 18: TEM images of Albizia amaraAgNPs at different magnification scalesof (A) 200 nm, (B) 100 nm, (C) 50 nm, (D) 20 nm (E) 10nm

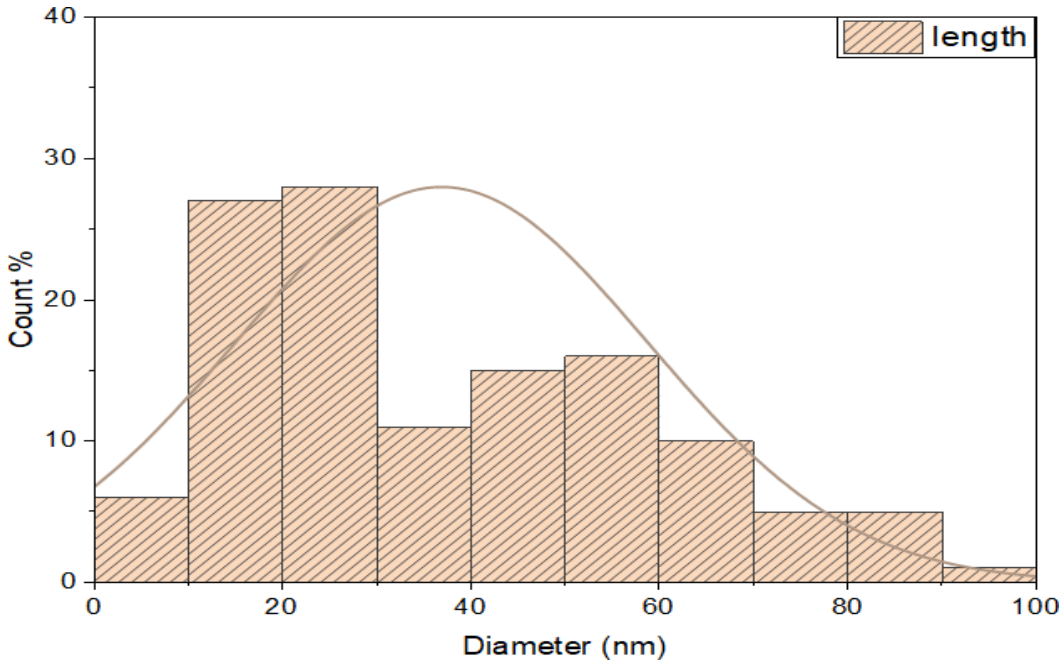


Figure 19: Histogram showing size distribution of AA-AgNPs

Selected area electron diffraction (SAED) analysis:

One of the crucial characterisation methods for figuring out the crystal structure of the silver nanoparticles is the selected area electron diffraction (SAED) pattern. As illustrated in Figure No. 28, the four brilliant circular rings of light spots corresponding to the Bragg's reflection angles at (111), (200), (220), and (222) on the black field were exposed by the SAED pattern silver nanoparticles created utilisingthe aqueous leaf extract of Albizia amara. These rings at the appropriate planes serve as a sign of the produced nanoparticles' crystalline character.

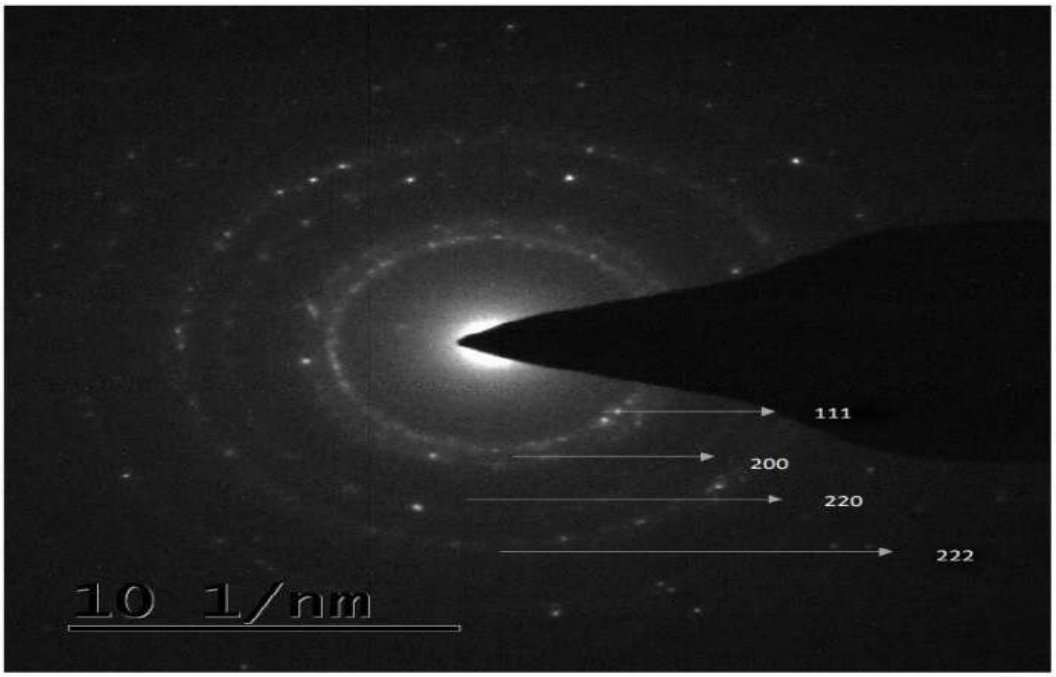


Figure 20: SAED spectrum of AA-AgNPs

X-ray diffraction analysis (XRD):

Another method used to identify the crystalline/amorphous nature of synthesised silver nanoparticles is X-ray diffraction spectroscopy (XRD). Figure 29 depicts the AA-AgNPs' XRD pattern. According to the XRD pattern, the (111), (200), (220), and (222) planes have Bragg's diffraction peaks at 38.05°, 44.17°, 64.44°, and 77.32°, respectively. The face-centered cubic lattice structure of nanoparticles is thus connected to these angles. Data from an XRD study were used to further validate the SAED findings.

Determination of Antioxidant Activity:

Table 4: Determination of percentage inhibition of 2, 2-diphenyl-1- picrylhydrazyl radical scavenging activity of Albizia amara–AgNP's

S No.	Concentration	Percentage of inhibition		
		Albizia amra Extract	Albizia amra silver nanoparticles	Ascorbic acid
1	80µg/ml	52.041±0.641	69.326±0.996	81.2±0.976
2	40µg/ml	35.121±0.657	54.358±0.318	75.21±1.415
3	20µg/ml	27.414±1.324	42.195±1.744	67.76±0.985
4	10µg/ml	17.497±1.315	34.453±0.647	59.471±1.215
5	5µg/ml	11.459±0.976	29.36±1.415	49.798±1.325
6	2.5µg/ml	5.421±0.667	22.662±0.338	33.676±0.656

Note: The data presented are Arithmetic mean ± S.D of 3 trials.

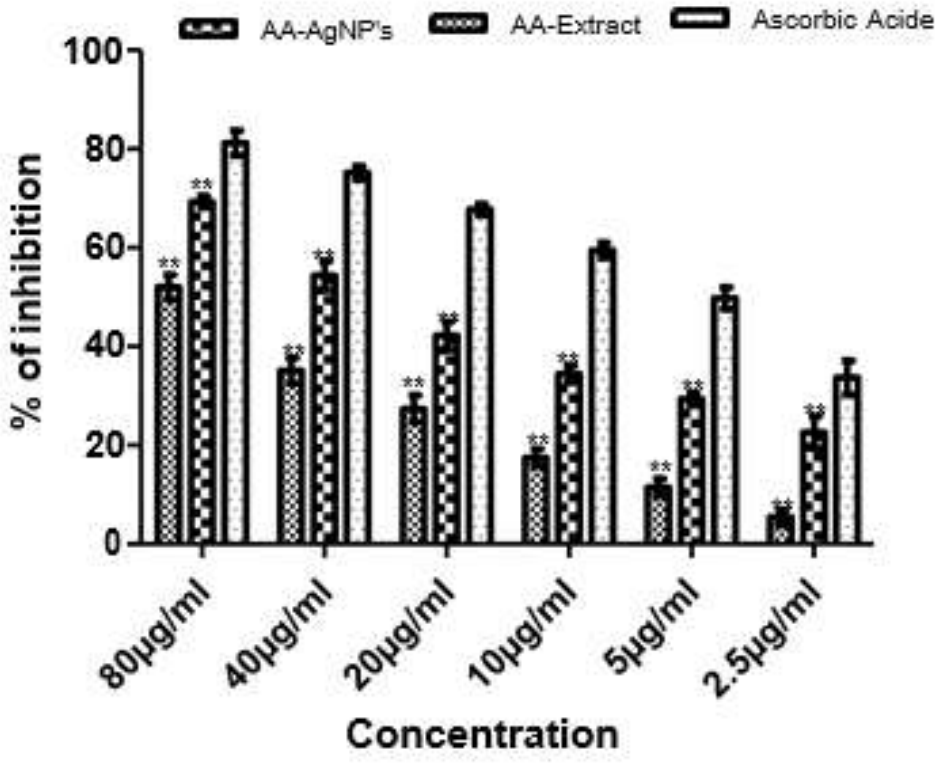


Figure 21: DPPH free radical scavenging activity of AA-extract, AA- AgNPs and ascorbic acid at different concentrations. Statistical significance was found to be *p≤0.05, **p≤0.01, ***p≤0.001 between AA-extract, AA- AgNPs v/s ascorbic acid

Table 5: Ferric reducing antioxidant power assay for Albizia amara extract, Albizia amara AgNP's and Ascorbic acid

S No.	Concentration	Percentage of inhibition		
		Albizia amra Extract	Albizia amara silver nanoparticles	Ascorbic acid
1	80µg/ml	45.041±0.0013	70.219±0.0013	82.41±0.131
2	40µg/ml	14.621±0.0014	53.216±0.0060	73.21±0.0041
3	20µg/ml	10.414±0.0060	42.195±0.0049	63.53±0.0028
4	10µg/ml	7.4975±0.0046	36.113±0.0021	58.91±0.0041
5	5µg/ml	6.459±0.0031	29.344±0.0034	49.59±0.0049
6	2.5µg/ml	4.921±0.0034	22.156±0.0041	33.17±0.0041

Note: The data presented are Arithmetic mean ± S.D of 3 trials

Table 6: Phosphomolybdenum assay for Albizia amara extract, Albizia amara AgNPs and ascorbic acid

Sr No.	Concentration	Percentage of inhibition		
		Albizia amra Extract	Albizia amra silver nanoparticles	Ascorbic acid
1	80µg/ml	56.4295±2.138	73.413±2.149	89.933±3.612
2	40µg/ml	41.889±2.618	57.591±0.426	83.031±0.461
3	20µg/ml	29.659±1.491	51.517±1.390	78.261±0.698
4	10µg/ml	25.04±1.816	45.31±0.614	67.714±1.754
5	5µg/ml	19.339±0.213	33.719±0.841	60.701±1.266
6	2.5µg/ml	13.838±1.816	30.739±0.423	51.683±2.602

Note: The data presented are Arithmetic mean ± S.D of 3 trials

In-Vitro assessment of Antibacterial Activity of AA-AGNPs:

Table 7: Antibacterial properties of samples in MIC and MBC

Sl.	Sample name	Staphylococcus aureus		Escherichia coli	
		MIC (µg/ml)	MBC(µg/ml)	MIC(µg/ml)	MBC(µg/ml)
1	Albiziaamara Extract	250	300	250	300
2	Albizia amara silver nanoparticles	100	140	80	120
3	Std. kanamycin	30	60	30	70

MIC STUDY

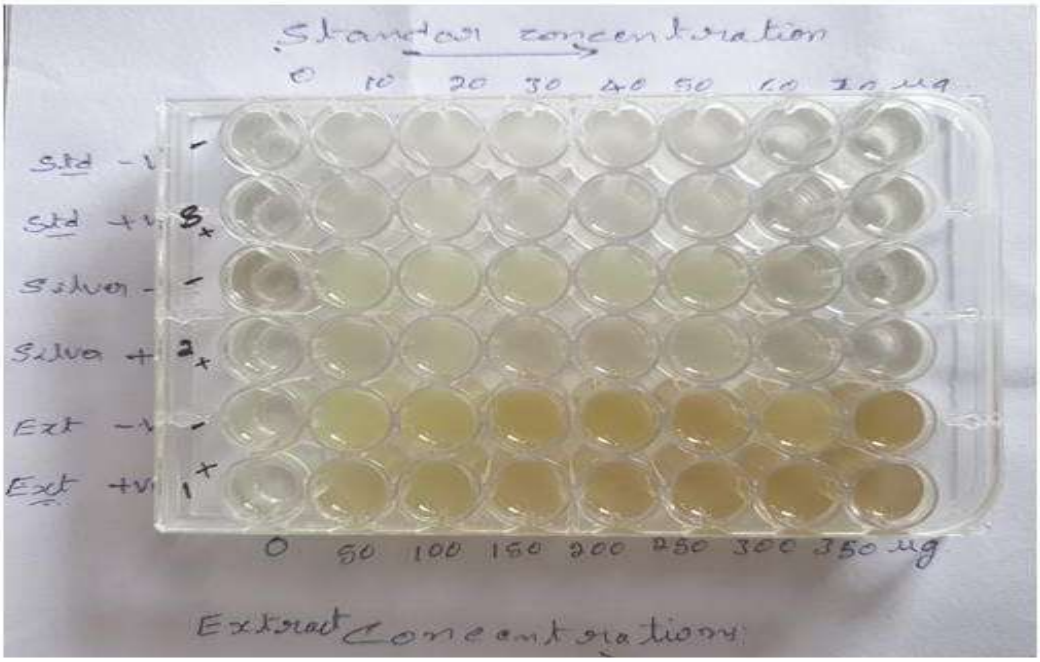


Figure 22: MIC of Albizia amara AgNPs, Albizia amara extract, standard Std. kanamycin against bacterial strains

MBC STUDY

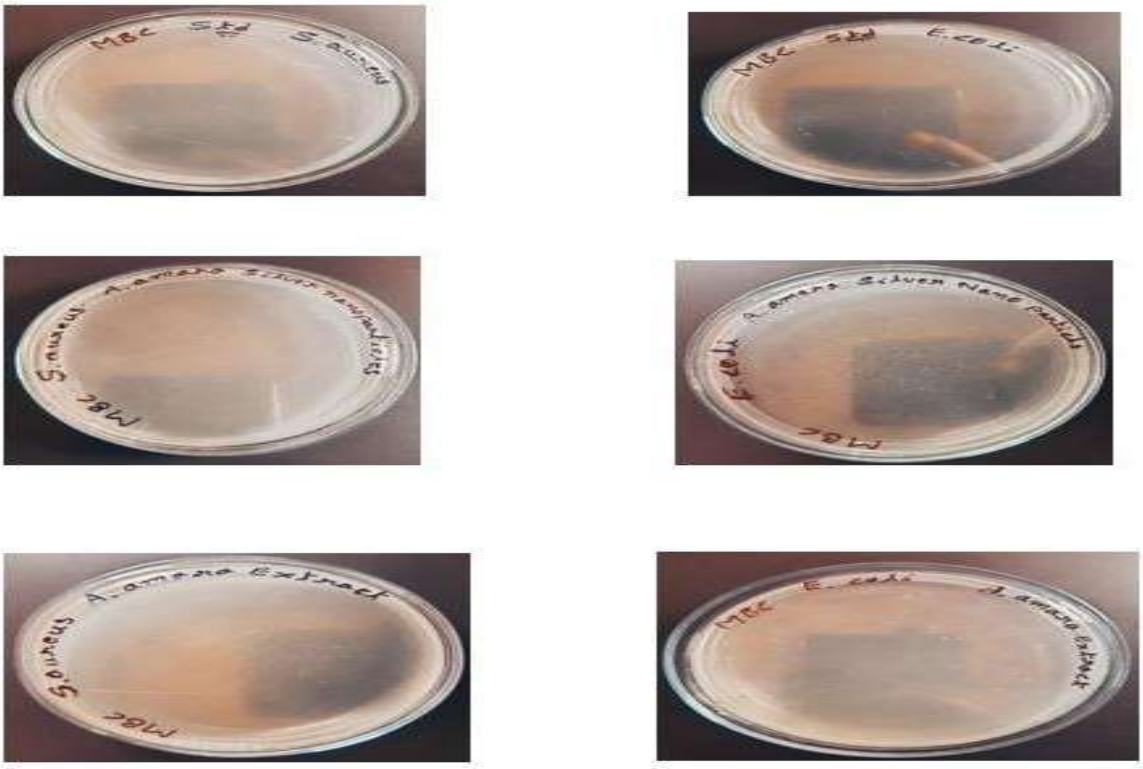


Figure 23: MBC of standard Std. kanamycin, Albizia amara AgNPs, Albizia amara extract against bacterial strains

Table 8: Antibacterial properties of samples ininhibition zone

Sl.	Sample Name	Conc. (µg)	Staphylococcus aureus	Escherichia coli
1	Albizia amara Extract	100	5.33± 0.57	5.66± 0.57
		200	5.66± 0.57	6.66± 0.57
		300	6.66± 0.57	7.66± 0.57
		400	8.33± 0.57	9.33± 0.57
2	Albizia amara silver nanoparticles	100	5.33± 1.15	5.33± 0.57
		200	6.66± 0.57	6.66± 0.57
		300	7.33± 1.15	7.33± 0.57
		400	11.33± 1.15	13.33± 0.57
3	Rifampicin	30	10.33 ± 0.57	10.66± 0.57
4	Ampicillin	25	14.66± 0.57	14.33± 0.57

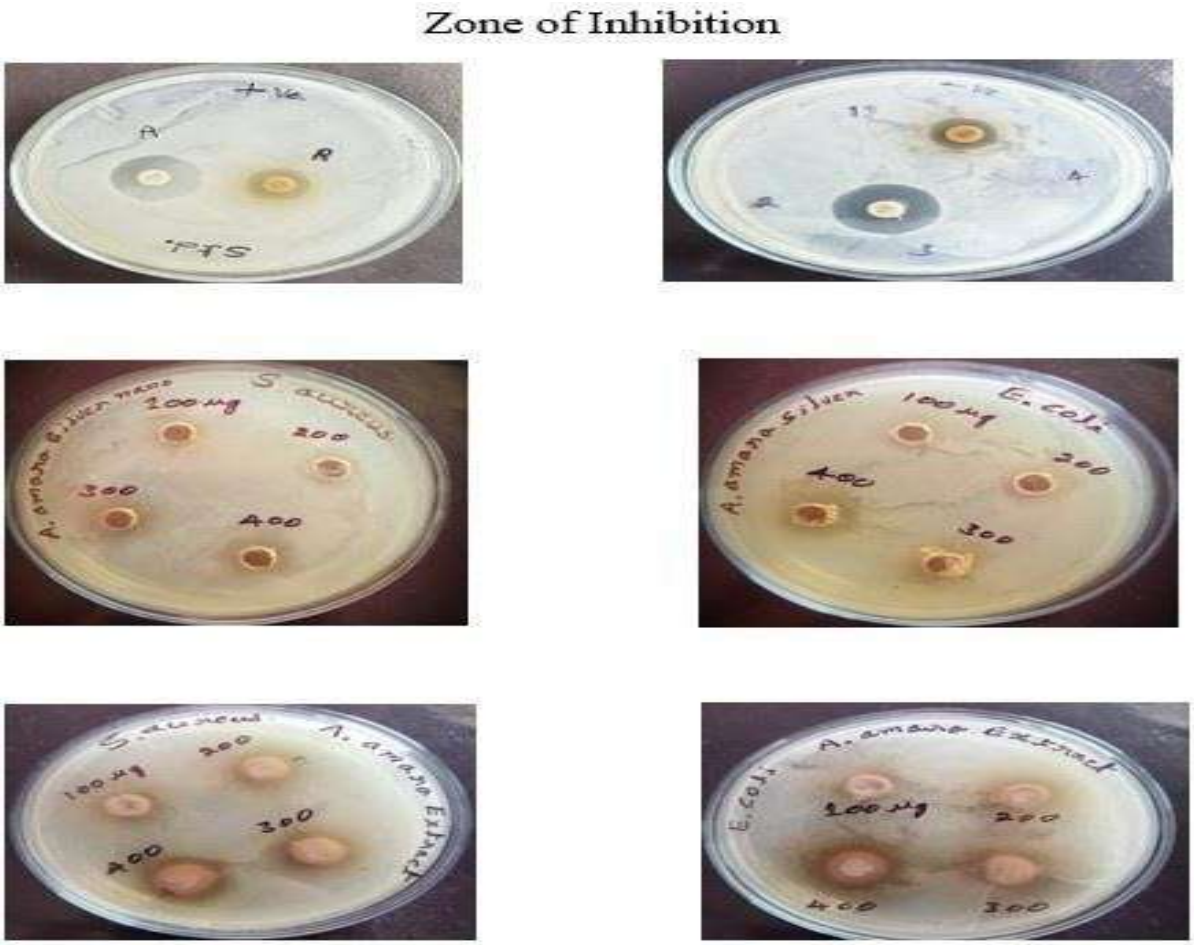


Figure 24: Zone of inhibition of standard Rifampicin and Ampicillin, Albizia amara AgNPs, Albizia amara extract against bacterial strains

Cell Viability and Cytotoxic Effect By Mtt AssayMethod:

By measuring the transformation of yellow-colored MTT into violet-colored formazan crystals by mitochondrial dehydrogenase enzyme of viable cells at different concentrations (3.125 to 100g/ml) on cell lines normal Breast and cancer cell lines, the biocompatibility and in-vitro anti-cancer activity of synthesised AA-AgNPs and AA-Extract were assessed.

Table 9: In-vitro Anti-Cancer and Cytotoxic assay: Cell line study (MDA MB 231)

SR. NO	Concentration (µg/ml)	AA-Extract	AA-AgNPs	Doxorubicin
1	100µg/ml	24.92 ±2.138	20.155±2.149	16.88±3.612
2	50µg/ml	43.62±2.618	35.720±0.426	24.77±0.461
3	25µg/ml	52.51±1.491	45.570±1.390	31.91±0.698
4	12.5µg/ml	62.71±1.816	49.990±0.614	34.85±1.754
5	6.25µg/ml	77.74±0.213	48.060±0.841	41.00±1.266
6	3.125µg/ml	85.20±1.816	49.125±0.423	44.00±2.602

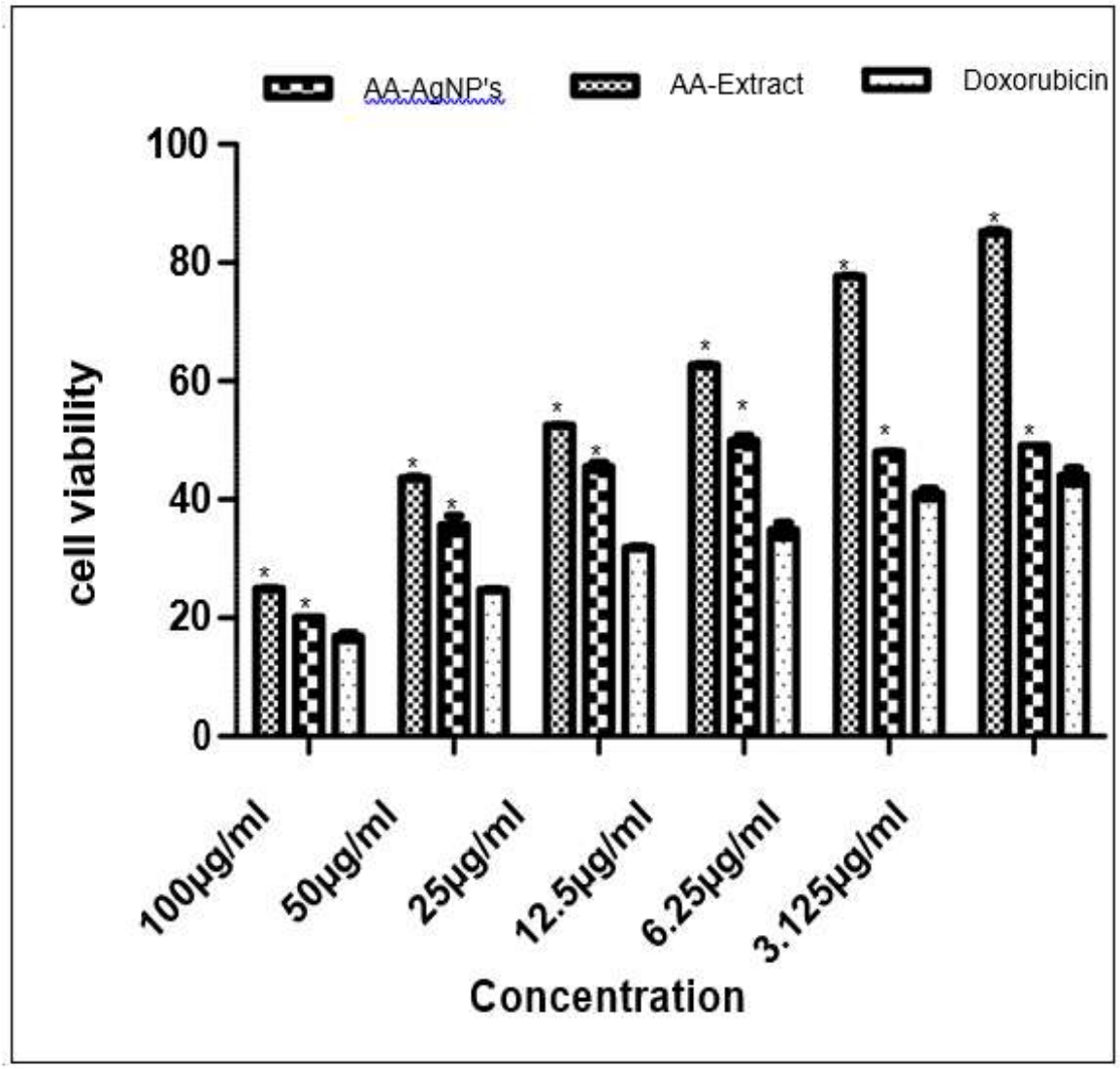


Figure 25: Doxorubicin treated MDA MB 231 cell, Significance difference was specified as **p≤0.01,**p≤0.0001 between AA-Extract, AA-AgNPs v/s Doxorubicin**

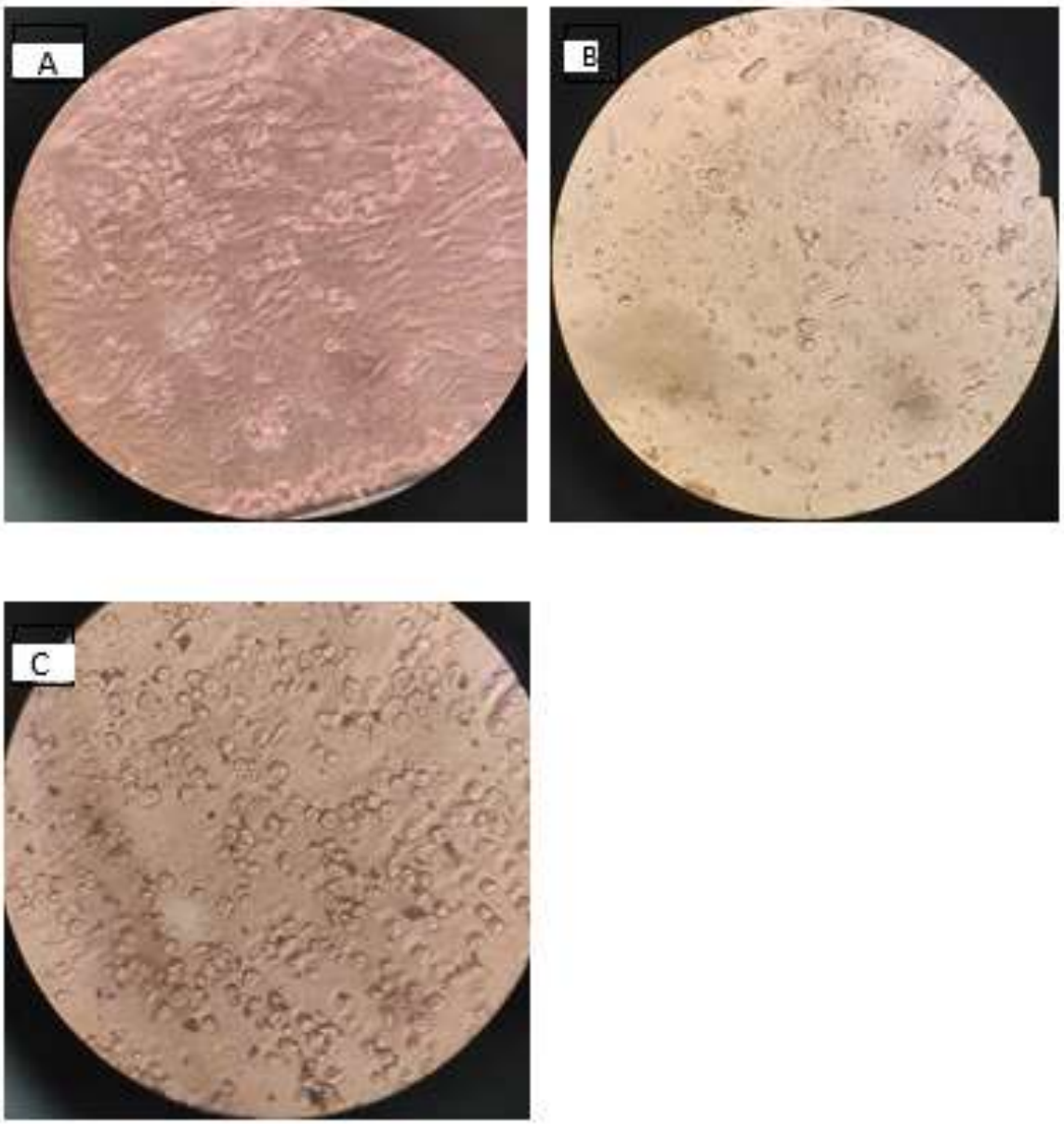


Figure 26: Cytotoxic activity of A) untreated MDA MB 231 cells B) MDA MB 231 cells treated with AA-extract and C) MDA MB 231 cells treated with AA- AgNPs

Evaluation Of Gel.

- **Measurement of pH:** The pH of the formulated gel was determined by using the digital pH meter. The pH of the gel was in the range of 6.9±0.1 to which confirms that the formulation has pH similar to the saliva. The pH of the gel was tabulated in**.**
- **Viscosity determination:** The viscosity of the gel generally reflects its consistency. The results obtained are shown in**.**
- **Spreadability:** The therapeutic potency of a formulation also depends upon its spreading value. Spreadability of all the formulation was in the range of 23.52±0.155 g.cm/sec. lesser the time is taken for separation of gel from slide better the spreadability. Thus, the results obtained shows better spreadability of allthe formulations. The result for spreadability was shown in **Table No 13.**

Table 10: Study of pH, viscosity and spreadability

Formulation	pH	Viscosity (cps)	Spreadability g.cm/sec)
AA AgNPs gel	6.8±0.1	248	23.12±0.145

Table 11: Study of pH, viscosity and spreadability of stability

Formulation	Time	Temperature(°C)	RH%	pH	Viscosity (cps)	Spreadability g.cm/sec)
AA AgNPs gel	90 days	4	40±5	6.8±0.1	247	23.129±0.155
		25±2	60±5	6.8±0.21	246	22.120±0.675

Summary and Conclusion

Albizia amara leaf plant is a popular traditional medicine. Guava leaves with high phytoconstituents may treat chronic illnesses including diabetes, cancer, liver disease, and cardiovascular disease. Several researches discovered that the aqueous leaf extract of Albizia amara leaf is extremely efficient against several cancer cell lines in in-vitro and in-vivo settings. No medication delivery mechanism or dosage form of Albizia amara leaf extract for cancer therapy has been established. Metallic nanoparticles have become a popular cancer treatment delivery technique over traditional therapy. This work synthesises silver nanoparticles from Albizia amara leaf aqueous extract.

Recommendations:

- As all of the intended objectives were achieved, it is advised to use this research for further development and to create a strong correlation between in-vitro and in-vivo in order to use the formulations for the treatment of various type of cancers so that the research work may be continued with new developments.
- It is also advised to analyze the effectiveness of silver nanoparticles on certain disease.
- To boost the antimicrobial activity and encourage more metallic nano particles can be added to the preparation of Albizia amara.

Formulating any further innovative dosage form for simple application on cancer, such as ointment, tablets and injections.

Future Scope:

The work was aimed to develop silver nano particles by using simple, easily available, nontoxic, biocompatible, biomaterials which are the essential needs in present scenario, still successive attempts are required to prove the effectiveness of the infectious disease and treatment for cancer disease.

Limitations of research work:

A satisfactory attempt has been made to prepare and evaluate silver nanoparticles composite to nanogel for cancer treatment. However, the following limitations can be made.

- In the present study the in-vitro anti-microbial, anti-oxidant activity and cytotoxicity activity will be evaluated. However, the potential of Albizia amara loaded nano gel as an anti-oxidant activity and cytotoxicity in humans will not be included.
- There was no attempt to develop the formulations on a broad scale or to market it.
- The effectiveness of natural medications can be enhanced by preparing them in the form of nanoparticles. However, a herbal medicine usually direct towards aiding the body's own healing process.

Bibliography

1. Agostinetto, E., Gligorov, J., & Piccart, M. (2022). Systemic therapy for early- stage breast cancer: Learning from the past to build the future. Nature Reviews Clinical Oncology, 19(12), 763-774.
2. Alobaid, H. M., Alzhrani, A. H., Majrashi, N. A., Alkhuriji, A. F., Alajmi, R. A., Yehia, H. M., ... & Elkhadragy, M. F. (2022). Effect of biosynthesized silver nanoparticles by

Garcinia mangostana extract against human breast cancer cell line MCF-7. Food Science and Technology, 42, e41622.
3. Biparva, A. J., Raoofi, S., Rafiei, S., Kan, F. P., Kazerooni, M., Bagheribayati, F., & Ghashghaee, A. (2022). Global quality of life in breast cancer: systematic review and meta-analysis. BMJ Supportive & Palliative Care.
4. Elhawary, S., Hala, E. H., Mokhtar, F. A., Sobeh, M., Mostafa, E., Osman, S., & El-Raey, M. (2022). Green Synthesis of Silver Nanoparticles Using Extract of Jasminum officinal L. Leaves and Evaluation of Cytotoxic Activity Towards Bladder (5637) and Breast Cancer (MCF-7) Cell Lines [Retraction]. International Journal of Nanomedicine, 17, 2805-2806.
5. Hong, R., & Xu, B. (2022). Breast cancer: an up-to-date review and future perspectives. Cancer Communications, 42(10), 913-936.
6. Kovács, D., Igaz, N., Gopisetty, M. K., & Kiricsi, M. (2022). Cancer therapy by silver nanoparticles: fiction or reality?. International journal of molecular sciences, 23(2), 839.
7. Mao, X. D., Wei, X., Xu, T., Li, T. P., & Liu, K. S. (2022). Research progress in breast cancer stem cells: Characterization and future perspectives. American Journal of Cancer Research, 12(7), 3208.
8. Smolarz, B., Nowak, A. Z., & Romanowicz, H. (2022). Breast cancer— epidemiology, classification, pathogenesis and treatment (review of literature). Cancers, 14(10), 2569.
9. Akintelu, S. A., Olugbeko, S. C., Folorunso, A. S., Oyebamiji, A. K., & Folorunso, F. A. (2021). Potentials of phytosynthesized silver nanoparticles in biomedical fields: a review. International Nano Letters, 11(3), 273-293.
10. Anand, B., Kalra, R., Shree, S., Chauhan, H., & Sharma, H. (2021). Association of physical activity and quality of life among cancer patients. Indian Journal of Health Sciences and Care, 8(spls), 64-64.
11. Govindappa, M., Tejashree, S., Thanuja, V., Hemashekhar, B., Srinivas, C., Nasif, O., ... & Raghavendra, V. B. (2021). Pomegranate fruit fleshy pericarp mediated silver nanoparticles possessing antimicrobial, antibiofilm formation, antioxidant, biocompatibility and anticancer activity. Journal of Drug Delivery Science and Technology, 61, 102289.
12. Kumavat, S. R., & Mishra, S. (2021). Green synthesis of silver nanoparticles using Borago officinalis leaves extract and screening its antimicrobial and antifungal activity. International Nano Letters, 11(4), 355-370.
13. Łukasiewicz, S., Czeczelewski, M., Forma, A., Baj, J., Sitarz, R., & Stanisławek, A. (2021). Breast cancer—epidemiology, risk factors, classification, prognostic markers, and current treatment strategies—an updated review. Cancers, 13(17), 4287.
14. Lava, M. B., Muddapur, U. M., Basavegowda, N., More, S. S., & More, V. S. (2021). Characterization, anticancer, antibacterial, anti-diabetic and anti-inflammatory activities of green synthesized silver nanoparticles using Justica wynaadensis leaves extract. Materials Today: Proceedings, 46, 5942-5947.
15. Nallappan, D., Fauzi, A. N., Krishna, B. S., Kumar, B. P., Reddy, A. V. K., Syed, T., ... & Rao, P. V. (2021). Green biosynthesis, antioxidant, antibacterial, and anticancer activities of silver nanoparticles of Luffa acutangula leaf extract. BioMed research international, 2021, 1-28.
16. Pallavi, S. S., Bhat, M. P., & Nayaka, S. (2021). Microbial synthesis of silver nanoparticles using Streptomyces sp. PG12 and their characterization, antimicrobial activity and cytotoxicity assessment against human lung (A549) and breast (MCF-7) cancer cell lines. Int J Pharm Pharm Sci, 13, 94-102.
17. Reddy, N. V., Li, H., Hou, T., Bethu, M. S., Ren, Z., & Zhang, Z. (2021). Phytosynthesis of silver nanoparticles using Perilla frutescens leaf extract: Characterization and evaluation of antibacterial, antioxidant, and anticancer activities. International journal of nanomedicine, 15-29.

18. Singh, R., Hano, C., Nath, G., & Sharma, B. (2021). Green biosynthesis of silver nanoparticles using leaf extract of Carissa carandas L. and their antioxidant and antimicrobial activity against human pathogenic bacteria. Biomolecules, 11(2), 299.
19. Sedahmed, A. A., AL-NOUR, M. Y., MIRGHANI, M. H., ABUALGASIM, H. E., ALTIB, F. A. A., Ahmed, A. D. İ. L., ... & ARBAB, A. H. (2021).