GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM BALANITES AEGYPTIACA AND THEIR ANTIBACTERIAL ACTIVITY

A RESEARCH PROJECT

Submitted by

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VIVEKANAND COLLEGE, KOLHAPUR

(AN EMPOWERED AUTONOMOUS INSTITUTE)

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"Dissemination of education for Knowledge, Science and culture"

-Shikshanmaharshi Dr. Bapuji Salunkhe

Shri Swami Vivekanand Shikshan Sanstha's

VIVEKANAND COLLEGE, KOLHAPUR
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PG DEPARTMENT OF MICROBIOLOGY

CERTIFICATE
OF
RESEARCH PROJECT COMPLETION

This is to certify that **MS. SANIKA GORAKHNATH CHAVAN** studying in M. Sc. part II Microbiology at Vivekanand College, Kolhapur (An Empowered Autonomous Institute) has sincerely completed research project work entitled " **GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM BALANITES AEGYPTIACA AND THEIR ANTIBACTERIAL ACTIVITY** " during academic year 2024-2025

Ms.Vrushali Misal

Research Project Guide

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INTRODUCTION

1.0 INTRODUCTION

The use of medicinal plants as antibacterial, antiviral and anti-cancer therapeutic agents had significant progress in the past few decades. However, the emergence of resistant strains for the antimicrobial and antiviral agents and the non-specific effect of the anticancer drugs, in addition to their severe side effects, limited absorption and poor bioavailability reduce the clinical efficacy of currently used therapies. Thus, the need to discover and to develop new, alternative, or synergistic drugs from natural or synthetic origin remains urgent. Recently, nanotechnology had an enormous impact on medical technology, significantly improving the activity, specificity, bioavailability and therapeutic index of various natural products. [Novel *Balanite aegyptiaca*]

The plant source used in the present study was *Balanites aegyptiaca* (Linn.) Del. Zygophyllaceae, commonly known as desert date. This prickly shrub grows into a tree and is widespread throughout Africa and southern Asia. The traditional or ethnobotanical uses of this plant include the treatment of diseases including jaundice, malaria, syphilis, asthma, epilepsy, hemorrhoids, abdominal pain, dysentery, constipation, and fever. Its phytocomponents also include coumarins, sinapic acid, ferulic acid, and organic acids [Al-Thobaiti and Abu Zeid, 2018; Murthy et al., 2021].

Balanites aegyptiaca (Del.); local name in Egypt is (Heglig or Laloub) and English name (Desert date, Egyptian balsam and Soapberry tree); is a plant belonging to the family Balanitaceae. It is one of the most common but neglected wild plant species of the dry land areas of Africa and South Asia (Hall and Waljer, 1991 and Hall, 1992). Beside its nutritive edible young succulent leaves, fruits and oil (Katende et al., 1999 and Teklehaimanot, 2008), it can be used for the preparation of a lot of cosmetics including shampoo, soap, lotion and lubricants (Charity et al., 2018); high grade biodiesel (Linda et al.,2018 and Naik and Balakrishna, 2018); a lot of therapeutic purposes (Montasser et al., 2017), it can be also used as animal feed supplement (Morkaz et al., 2011). According to Mutwali and Abdelgadir (2016), *Balanites aegyptiaca* is an important woody xerophytic tree.[Egypt, J. Biotechnol vol 58 2019]

The silver nanoparticles have various and important applications. Historically, silver has been known to have a disinfecting effect and has been found in applications ranging from traditional medicines to culinary items. It has been reported that silver nanoparticles are non-toxic to humans and most effective against bacteria, virus and other eukaryotic micro-organism at low concentrations and without any side effects. Moreover, several salts of silver and their derivatives are commercially manufactured as antimicrobial agents. In small concentrations, silver is safe for human cells, but lethal for microorganisms. Antimicrobial capability of AgNPs allows them to be suitably employed in numerous household products such as textiles, as well as disinfection in water treatment, food storage containers, home appliances and in medical devices.

Silver nitrate (AgNO3) plays a crucial role in the synthesis of silver nanoparticles (AgNPs) by acting as the source of silver ions, which are then reduced to form the nanoparticles. The concentration of AgNO3 can significantly influence the size and properties of the resulting AgNPs. AgNO3 is a common precursor used in various synthesis methods, including chemical and biological approaches, as it provides the necessary silver ions (Ag+) for the reduction process. AgNO3 is typically reduced by a reducing agent (e.g., sodium borohydride, glucose, plant extracts) to form silver nanoparticles (AgO3). The concentration of AgNO3 can affect the size of the AgNPs produced. Higher concentrations of AgNO3 may lead to larger particle sizes and even agglomeration. Stabilizing agents (e.g., polyvinyl pyrrolidone, gelatin, plant extracts) are often used in conjunction with AgNO3 to prevent the nanoparticles from clumping together and to control their growth and size. In chemical methods, reducing agents like borohydride are used, while in biological methods, plant extracts or other biological sources serve as reducing agents.

Figure 1 Tree of Balanite aegyptiaca

Nanotechnology is emerging as a rapidly growing field with its application in Science and Technology for the purpose of manufacturing new materials at the nanoscale level . The word "nano" is used to indicate one billionth of a meter or 10–9. The term Nanotechnology was coined by Professor Norio Taniguchi of Tokyo Science University in the year 1974 to describe precision manufacturing of materials at the nanometer level. The concept of Nanotechnology was given by physicist Professor Richard P. Feynman in his lecture There's plenty of room at the Bottom . Nanoparticles are clusters of atoms in the size range of 1–100 nm. "Nano" is a Greek word synonymous to dwarf meaning extremely small. The use of nanoparticles is gaining impetus in the present century as they possess defined Chemical, optical and mechanical properties.[Asian journal]

Nano technology is the branch of science which studies the fundamental principles of molecules and, deformation of materials by one atom or by one molecule. Nano materials are cornerstones of Nano science and nanotechnology. Nanostructure Science and technology is a broad and interdisciplinary area of research and development activity that has been growing explosively worldwide in the past few years. It has the potential for revolutionizing the ways in which materials and products are created and the range and nature of functionalities that can be accessed. It is already having a significant commercial impact, which will increase in the future.

AIM AND OBJECTIVES

2.0 AIM AND OBJECTIVE

Aim –

To study green synthesis of silver nanoparticles from *Balanites aegyptiaca* and their antibacterial activity

1) Silver Nanoparticles of *Balanite aegyptiaca*.

2) Determination of the antimicrobial activity of *Balanite aegyptiaca*.

REVIEW AND LITERATURE

3.0 Review of Literature

Medicinal plants are playing major role in the treatment of many disease the extract of this plants can act as anti-inflammatory, antioxidant, anti-allergic, anti-cancerous, analgesic and antidiabetic due to this medicinal properties this plants have been used since century to cure and prevention of different kinds of disease. Derivatives from the medicinal plants and extracts are effective in small amounts, economical and safe to use, with negligible side effects morever medicinal plants are easily accessible and have better compatibility *Balanites* aegyptiaca, also known as the Desert Date, is a species of tree that has been extensively studied for its medicinal, nutritional, and economic importance. (Abu-Al-Futuh, IM. 1983)

Balanites aegyptiaca is an important food source in many African countries, providing fruit, oil, and other edible products. The tree's wood is valued for its durability and resistance to termite damage, making it a valuable resource for timber products. The tree's extracts are used in traditional medicine to treat various ailments, including fever, rheumatism, and skin conditions. *Balanites aegyptiaca* is listed as "Least Concern" on the IUCN Red List, but its populations are declining due to over-exploitation for timber, fuelwood, and other products.

The oil remains stable when heated and has a high smoking point, and therefore its free fatty acid content is low. Its scent and taste are good. The leaves are eaten raw or cooked, the oily seed is boiled to make it less bitter and eaten mixed with sorghum, and the flowers can be eaten. The fruit can be fermented for alcoholic beverages. The seed contains seed oil used as cooking oil. The seed cake remaining after the oil is extracted is commonly used as animal fodder. 7. Medicinal Application of *B. aegyptiaca*. All the parts of *Balanites aegyptiaca* are traditionally used in several folk medicines across the globe. (Casamatta D. A. 2013)

This plant has got tremendous importance and being used in treatment of several diseases and disorders. According Wilson et al, the fruit used as oral hypoglycemic drug in the Sahara region of Africa. Furthermore, Hall and Walker reported that the fruits are also commonly used as purgative, antiparasitic and schistosomicide. According to Chapagain and Wiesman, reported that, the stem, root and leaf extracts of *B.aegyptiaca* have commonly been used as various

traditional folk medicines especially in the treatment of parasites, sore throat, constipation and eye irritation as reported by (Gaur et al).

Studies have shown that extracts from *Balanites aegyptiaca* possess antimicrobial activity against various bacteria, fungi, and viruses. The tree's extracts have been found to exhibit anti-inflammatory properties, making it a potential treatment for inflammatory diseases. *Balanites aegyptiaca* extracts have been shown to possess antioxidant activity, which can help protect against oxidative stress and related diseases. The fruit of *Balanites aegyptiaca* is rich in nutrients, including proteins, fibers, and minerals like potassium, magnesium, and iron. Oil nutritional content: The tree's oil is rich in unsaturated fatty acids, particularly oleic acid, which has been linked to several health benefits. (Casamatta D. A. 2013)

Therapeutic values of *B. aegyptiaca* shown anthelminthic, anticancer, antioxidant, mosquito larvicidal Chapagain and Wiesman, anti-inflammatory, anti-diabetic Motaal et al, wound healing, hepatoprotective, hypocholesterolemic, diuretic contraceptive and antiviral activities in various parts of Balanites extracts Gaur et al. Aqueous extract of fruits showed spermicidal activity as reported by Saperoni et al, without local vaginal irritation in human being antidiabetic, treatment of jaundice. Seed is used as expectorant, antibacterial, antifungal, febrifuge, anthelmintic and purgative as indicated by Mitra et al; Chothani and Vaghasiya. Fruit is used in whooping cough, also in leucoderma and other skin diseases. Bark is used as spasmolytic. The seed oil is used to treat tumors and wounds used as laxative, also used in treatment of hemorrhoid, stomach aches, jaundice, yellow fever, syphilis, and epilepsy as explained by Chapagain and Wiesman, the bark of this plant is used in the treatment of syphilis, round worm infections, and as a fish poison. (Green and Sustainable chemistry 6 (10), 34, 2016)

Nanotechnology is the process of synthesizing particles which are in the nano range ranging from approximately 1 to 100nm. They have large surface area to volume ratio due to which they possess optical properties as they are small enough to confine their electrons and produce quantum effects by which their detection becomes easy. Intensive research is being done, silver nano particle devices (He et al 2013), pharmaceutical. In 1700, silver nitrate was used for the treatment of venereal diseases, fistulae from salivary glands and bone and perianal abscesses. In 1881, Carl S.F Crede cured opthalmia neonatorum using silver nitrate eye drops. (Kumars et al 2011).

Antimicrobial activity of silver nanoparticles has been reported in many research papers. The mechanism of antibacterial action of silver nanoparticles is a topic of debate and is not well understood. But many assumption and theories are there. Different types of nanoparticles/nanomaterial are there like copper zinc titanium magnesium gold alginate and silver have come up but silver nanoparticles have proved to be most effective as it has good antimicrobial efficacy against bacteria viruses and other eukaryotic microorganism. (Green and Sustainable chemistry 6 (10), 34, 2016)

The ethanol, Petroleum ether and chloroform extracts of *B. aegyptiaca* was tested against four standard bacteria i.e.: two Gram positive (*Bacillus subtilis* and *Staphylococcus aureus*), two Gram negative (Escherichia coli and *Pseudomonas aeruginosa*) and against two standard fungi species i.e. *Aspergillus niger* and *Candida albicans* using the disc diffusion method. The microbial activities were provide that most of the extracts ethanol, petroleum ether, and chloroform extract of B. aegyptiaca (Leafs). The ethanol, petroleum ether, and chloroform extract exhibited inhibitory effects against most of the tested organisms with the zone of inhibition ranging from (11 to 14 mm), (15-16 mm) and (13-15mm) respectively. (Shams Eldien koko et.al 2017)

Balanites aegyptiaca popularly known as desert date, various parts of the plant are used in Ayurvedic for the treatment of syphilis, jaundice, livers and spleen problems, epilepsy, yellow fever and the plant also has insecticidal, anthelmintic. Nanoparticles have been shown to exhibit various novel properties and these properties on other hand rely upon the size, shape and morphology of these particles. Moreover, these physical characteristics enable them interact with microbes, plants and animals. Smaller-sized particles have shown more toxicity than larger-sized particles.

AgNPs have shown growth inhibition of many fungi like *candida albicans*, bacteria like *E. coli, S. aureous*. Therefore, this investigation is focused on the formulation and evaluation of silver nanoparticles of *Balanite aegyptiaca* plant extract (leaf, pulp, stem) the effective treatment of Bacterial and fungal disease. (Green and Sustainable chemistry 6 (10), 34, 2016)

METHOD AND MATERIALS

4.0Materials and methods.

4.1 Materials

Media

1. **Nutrient agar**

Composition:

Beef extract	- 1 gm.
Yeast extract	- 2 gm.
Peptone	- 5 gm.
Sodium chloride (Nacl)	- 5 gm.
Agar	- 1.5 gm.
D/W	- 100 ml

2. **Potato Dextrose agar**

Composition:

Potato (infusion form)	- 20 gm.
Dextrose	- 2 gm.
Agar-Agar	- 1.5 gm.
D/W	- 100 ml

2. AgNO3: (0.5 mM)

1. Silver nitrate(AgNO3): 8.49 mg
2. Distilled water : 100 ml

4.2 Methods:

Collection of Plant material

Extraction of *Balanites aegyptiaca* .

Extraction is a common technique used in organic chemistry to isolate a target compound from biological samples such as cells, tissues, or fluids. In the two main types of extraction, which are liquid-liquid extraction and liquid-solid extraction, the separation is based on solubility. After extraction is complete the solvent can be removed and the desired product collected. The process of separating or extracting particular compounds or components, is referred to as extraction. Steps involved in extraction process

1) Find the source-

E.g. Plant (*Balanites aegyptiaca*)

2) Selection of plant part-

E.g. Fruit Coat, Fruit Peel, Fruit Leaf, Fruit Pulp.

3) Collection -

Collect *Balanites aegyptiaca* fruit dry coat, Peel, Leaf, Pulp, stem.

4) Washing-

Tap water is used for washing.

5) Cutting of fruit dry Coat, Fruit Leaf, stem pulp.

6) Drying-

In Hot Air Oven at 40°c. For 3-4 days.

7) Powdering-

Dry peels can be powder by use of mortar.

8) Methods of extraction-

1. Maceration.

It can be found in many kinds of habitat, tolerating a wide variety of soil types, from sand to heavy clay, and climatic moisture levels, from arid to subhumid. It is relatively tolerant of flooding, livestock activity and wildfire. The plants were collected from their natural habitat, form different parts of Solapur District.

Chemicals: The entire chemicals used in the present study are of analytical grade.

Figure 2 Fruits and Fruit part of Balanites aegyptiaca

Figure 3 Powder of Balanites aegyptiaca

Preparation of plant (fruit pulp, leaf, Stem) extract

The collected plant material was carefully washed under running tap water followed by sterilized distilled water, and air dried at room temperature in laboratory for 3-5 days. These dried plant materials were then homogenized to a fine coarse powder using a mortar pestle and then stored in air tight containers until further use. Various organic solvents viz. water and ethanol were used for extractions.

Extraction methods: Maceration Method

A biological process in which the phytoconstituents are highly concentrated with gradual shaking is referred as maceration. Maceration process is influenced by numerous factors such as temperature, duration, solvent used, etc. It is an easy and popular technique that uses the concepts of diffusional solubility to extract active ingredients. Maceration is a method of extraction where plant material is soaked in a solvent, typically a liquid like ethanol to extract desired compounds. The principle involves allowing the solvent to penetrate the plant material, facilitating the dissolution of soluble compounds. This process occurs over an extended period, allowing for a comprehensive extraction. After maceration, the solvent is separated, leaving behind the extracted substances, often in the form of a liquid extract

For Maceration Method we used Shaker or Shaker Incubator.

Procedure:

1. 3-4 g of *Balanite aegyptiaca* powder was weighed and dissolved in 30-40 ml Ethanol.
2. The flask was covered with cotton and aluminum foil to prevent contamination. Efforts were made to ensure the powder was completely dissolved in the water.
3. The flask was then incubated at room temperature for 2-3 days in a shaker.
4. After two days, when a separated layer between the extract and distilled water was observed, the mixture was filtered using filter paper or muslin cloth.
5. The filtrate was collected and evaporated using a boiling water bath.
6. Once evaporation was complete, the extract was transferred to a closed container and stored at 4°C for further use.

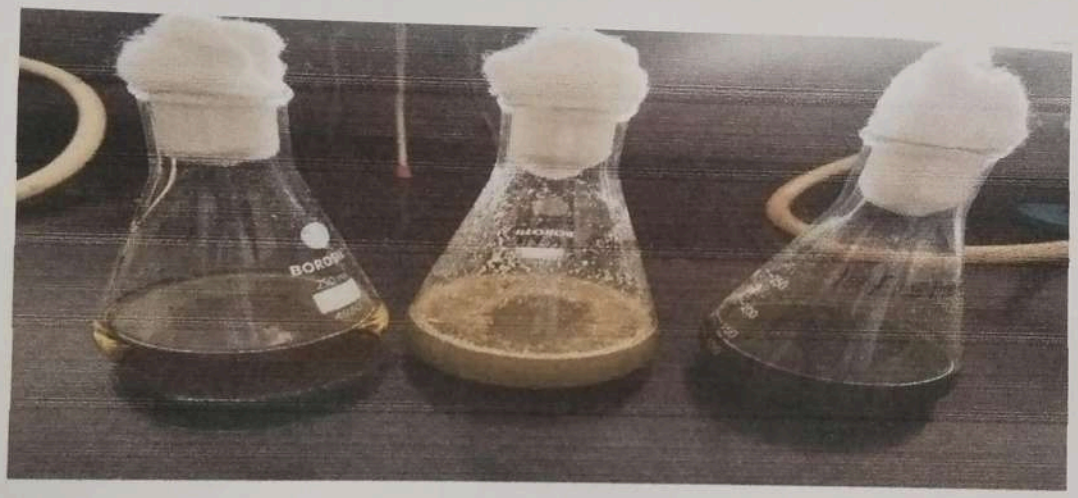


Fig 4. Extraction of *Balanite aegyptiaca* by Maceration Method using VDRL shaker.

Extraction of plant *Balanite aegyptiaca* by maceration was performed by using 3 gm powder *Balanite aegyptiaca* of given plant sample dissolved it in 100 ml Ethanol then covered it in the flask by using cotton or aluminum foil. After preparing the flask, the Flask were placed on VDRL shaker for 2 to 3 days to separate out two layers real extract and crude extract by using filtrations.

In Maceration method, shaking and its duration time had a positive effect on yield of the extract. As compared to other method maceration method is very simple.

Preparation of AgNO3:

Silver nitrate (AgNO) is a pretty handy chemical that shows up in a lot of labs and industries. It looks like a white powder and dissolves easily in water, but if it's left in the light, it can turn dark because it reacts and forms silver. It's made by mixing silver with nitric acid a simple but powerful reaction. People use it to test for things like chloride in water, and it's also been used as a disinfectant. Just be careful with it. It can burn, stain your skin black, and with too much exposure, it can cause a bluish-gray tint to the skin.

Procedure:

1. Take a dry, clean 250 ml beaker and clean it with alcohol.
2. Weigh 8.5 g of AgNO.
3. Wrap the beaker with black paper (to protect from light).
4. Add approximately 20 ml of distilled water (DIW).
5. Mix using a magnetic stirrer for 15 minutes.
6. Keep the beaker in an oven for 15-20 minutes.
7. After heating, mix again with the magnetic stirrer for 10 minutes.
8. Adjust the final volume to 100 ml with distilled water and mix properly.

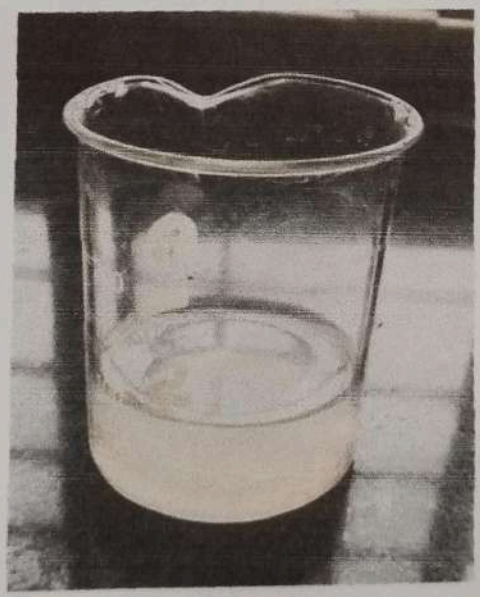


Figure 5 Praperation of AgNO3

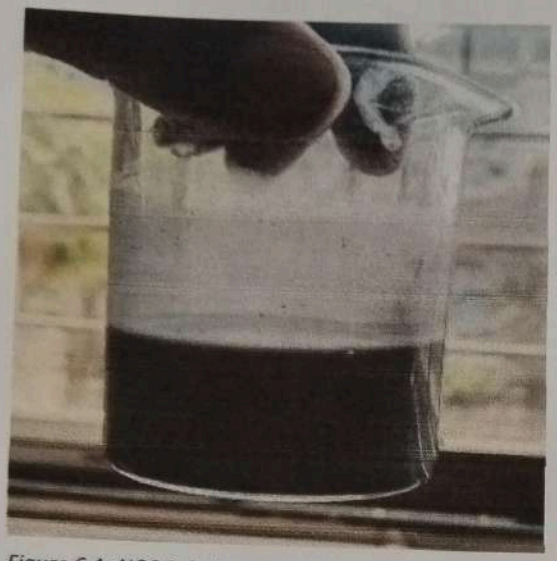


Figure 6 AgNO3 Solution

Figure 7 Preperation of AgNO3 by Titration Method

Titration Procedure:

1. Fill the burette with 50 ml of the peel extract.
2. Take 100 ml of silver nitrate (AgNOs) solution into a clean beaker.
3. Place the beaker on a magnetic stirrer and start stirring gently.
4. Slowly add the peel extract from the burette into the beaker, drop by drop.
5. Continue the addition until a bluish-grey color appears, indicating the reaction between the peel extract and silver nitrate.

UV-Visible Spectroscopy

UV-Visible Spectroscopy is a method used in labs to find out how much of a substance is present in a solution by shining light on it. The light used comes from the ultraviolet (UV) and visible parts of the light spectrum. When this light passes through a liquid sample, some of it is absorbed by the molecules in the solution. This absorbed light makes the electrons in those molecules jump from a lower energy level to a higher one. The more molecules there are, the more light is absorbed.

This process follows a rule called Beer-Lambert's Law, which says: the amount of light absorbed depends on how concentrated the solution is, how far the light travels through the liquid (path length), and the type of substance. The formula is AECI, where A is absorbance, & is a constant for the substance, e is concentration, and I is the path length.

Procedure:

1. Prepared the sample by dissolving it in a suitable liquid usually ethanol.

2. Then, turn on the spectrophotometer (the instrument used for this test) and allow it to stabilize.

3. After that, pick the right wavelength (where our sample absorbs light the most),

4. Before testing, use a blank solution just the solvent (Ethanol) without the sample to set the machine to zero. place the sample in the machine.

5. The spectrophotometer shows us how much light is absorbed.

6. Finally, use this reading to figure out how much of the substance is in the solution, either by calculation or comparing with known samples.

RD (X-ray Diffraction)

Principle:

XRD works on the principle of Bragg's Law, which states that constructive interference of X-rays occurs when the path difference between rays reflected from successive crystal planes equals an integer multiple of the wavelength.

Bragg's Law:

Where:

n= order of reflection (usually 1)

= X-ray wavelength

d= distance between atomic layers in a crystal

angle of incidence

Mechanism:

- A sample is bombarded with X-rays.
- X-rays are diffracted by the crystal lattice.
- The diffraction pattern (intensity vs. angle) is recorded.
- The pattern is used to determine crystal structure, lattice parameters, and phase composition.

Applications:

- Identification of crystalline phases
- Determination of crystal structure
- Particle size and strain analysis

FTIR (Fourier Transform Infrared Spectroscopy)

Principle:

FTIR is based on the absorption of infrared radiation by molecules, which causes them to vibrate. Each molecular bond absorbs a specific IR frequency depending on its bond strength and atomic mass.

Mechanism:

- An IR light beam passes through the sample.
- Molecules absorb specific frequencies and transmit the rest.
- The resulting signal is an interferogram (all frequencies at once).
- A Fourier Transform converts the interferogram into an IR spectrum (intensity vs. wavenumber).
- Peaks correspond to functional groups in the molecule.

Applications:

- Identification of functional groups
- Determination of molecular structure
- Quality control in pharmaceuticals and polymers

Anti-Bacterial Acitvity by Well Diffusion Method using AgNO3 Solution.

The term "anti-bacterial activity" refers to the ability of a substance to inhibit the growth or kill bacteria. This activity is of significant interest in various fields, including medicine, food preservation, and agriculture. There are numerous natural and synthetic compounds that exhibit antibacterial activity, and understanding their mechanisms and effectiveness is crucial for developing new antibiotics, disinfectants, and antimicrobial agents.

Aim:

To study the Anti-Bacterial activity by well diffusion method.

Principle:

The well diffusion method assesses antibacterial activity by introducing a substance into wells on an agar plate inoculated with bacteria. The principle involves the diffusion of the substance through the agar, creating a concentration gradient. The zone around the well where bacterial growth is inhibited indicates the effectiveness of the substance against the tested bacteria. A larger zone of inhibition suggests stronger antibacterial activity, providing valuable information about the substance's potential as an antibacterial agent.

Reagents:

1) AgNO3 Solution.

2) Standard-Peniciline

Procedure:

1. Prepare the nutrient agar media and autoclave it.
2. Pour the media into the respective petri dishes and allow it to solidify.
3. Prepare the bacterial inoculum and spread it on the top of the nutrient agar media.
4. After preparing the plates, use a cork borer to make wells.
5. Inoculate the sample into the particular wells.
6. After inoculation, incubate the plates at 37°C for 24 hours.
7. After successful incubation, measure the zone of inhibition.

Observation Table:

- Anti-Bacterial activity by well diffusion method using *Balanite aegyptiaca* pulp, leaf, Stem extract in ethanol (*E.coli*)

Sr. no.	Plant Extract Sample	Zone of Inhibition (diameter in mm)		
		Standard (Penicillin)	Control (Ethanol)	AgNO3 Solution
1	Leaf	24mm	-	34 mm
2	Stem	17 mm	-	31 mm
3	Pulp	15mm	-	31mm

- Anti-Bcterial activity by well diffusion method using *Balanite aegyptiaca* pulp, leaf, Stem extract in ethanol (*s. aureus*)

Sr. no.	Plant Extract Sample	Zone of Inhibition (diameter in mm)		
		Standard (Penicillin)	Control (Ethanol)	AgNO3 Solution
1	Leaf	31mm	-	25 mm
2	Stem	32 mm	-	16 mm
3	Pulp	30mm	-	30 mm

Antifungal Test

Anti-Fungal Activity by Well Diffusion Method using Plant *Balanite aegyptiaca*

Antifungal activity refers to the ability of a substance to inhibit or kill the growth of fungi. This activity is of particular interest in various fields, including medicine, agriculture, and industry, where fungal infections or contamination can have significant implications. Antifungal agents can be natural or synthetic compounds that target specific fungal structures or functions.

Aim:

To study the Anti-Fungal activity by well diffusion method.

Principle:

The principle of antifungal activity by the well diffusion method involves, assisting the ability of a substance to inhibit fungal growth. In this method, the substance is placed in wells on an agar plate inoculated with fungal cultures. As the substance diffuses into the agar, it creates a concentration gradient, leading to inhibition of fungal growth around the well. The size of the resulting zone of inhibition is indicative of the substance's effectiveness against the tested fungi, providing valuable information about its antifungal activity.

Reagents:

1) $AgNO_3$ Solution

2) Standard-Benzathine Penicillin

Procedure:

1) Prepare the Nutrient Agar and Potato Dextrose Agar media and autoclave it.

2) Pour the media into respective petri dishes and allow to solidify it.

3) Prepare the inoculum and spread it on the top of Nutrient Agar and Potato Dextrose Agar plate media.

4) After preparation of plates make the well with the help of cork borer.

5) Inoculate the sample in particular wells.

6) After the inoculation incubate the plates at room temperature for 24 hrs.

Observation Table

- Anti-fungal activity by well diffusion method using *Balanite aegyptiaca* pulp, leaf, Stem extract in ethanol (*Candida*)

Sr. no.	Plant Extract Sample	Zone of Inhibition (diameter in mm)		
		Standard (Penicillin)	Control (Ethanol)	AgNO3 Solution
1	Leaf	15 mm	-	-
2	Stem	11 mm	-	-
3	Pulp	14 mm	-	-

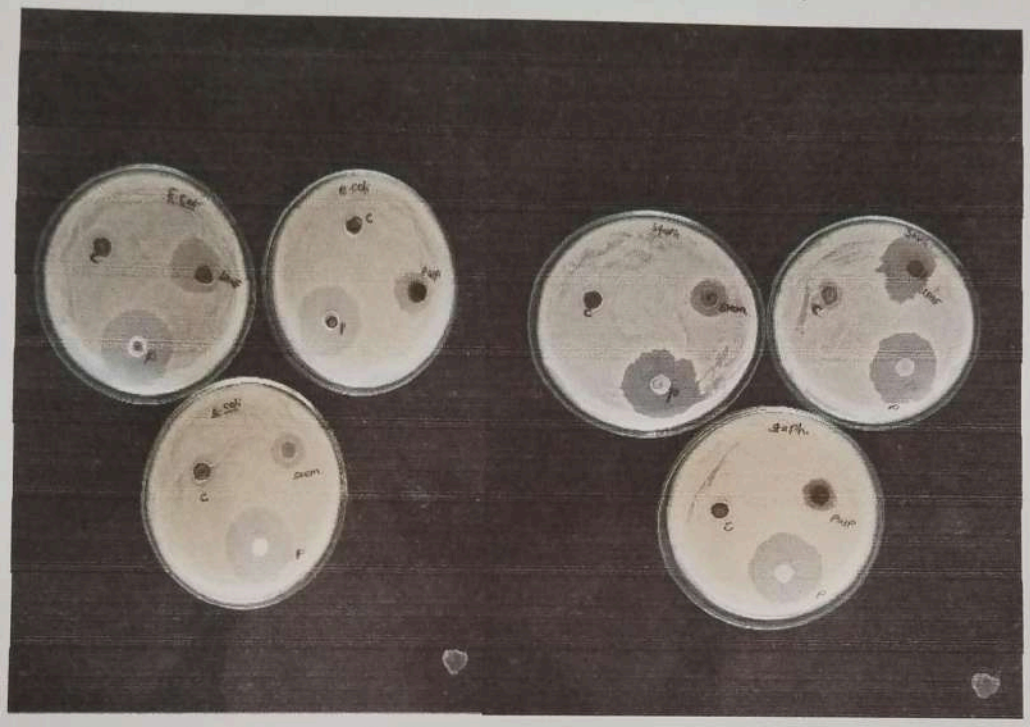
RESULT AND CONCLUSION

5.0Result and Conclusion

Anti Bacterial Activity by well Difusion Method Using AgNO3 Solution

Results:

Antimicrobial activity by well diffusion method was assessed using AgNO3 Solution



Anti-Bacterial activity by well diffusion method using AgNO3 Solution (Staphylococcus aureus, Escherchia coli)

Observation Table:

- Anti-Bacterial activity by well diffusion method using *Balanite aegyptiaca* pulp, leaf, Stem extract in ethanol (*E.coli)*

Sr. no.	Plant Extract Sample	Zone of Inhibition (diameter in mm)		
		Standard (Penicillin)	Control (Ethanol)	AgNO3 Solution
1	Leaf	24mm	-	34 mm
2	Stem	17 mm	-	31 mm
3	Pulp	15mm	-	31mm

- Anti-Bacterial activity by well diffusion method using *Balanite aegyptiaca* pulp, leaf, Stem extract in ethanol (*s.aureus*)

Sr. no.	Plant Extract Sample	Zone of Inhibition (diameter in mm)		
		Standard (Penicillin)	Control (Ethanol)	AgNO3 Solution
1	Leaf	31mm	-	25 mm
2	Stem	32 mm	-	16 mm
3	Pulp	30mm	-	30 mm

5.2 Anti-fungal activity by well diffusion method using *Balanite aegyptiaca* pulp, leaf, Stem extract in ethanol.

Result:

Anti-fungal activity by well diffusion method Was assessed using *Balanite aegyptiaca* pulp, leaf, Stem extract.

Sr. no.	Plant Extract Sample	Zone of Inhibition (diameter in mm)		
		Standard (Penicillin)	Control (Ethanol)	AgNO3 Solution
1	Leaf	15 mm	-	-
2	Stem	11 mm	-	-
3	Pulp	14 mm	-	-

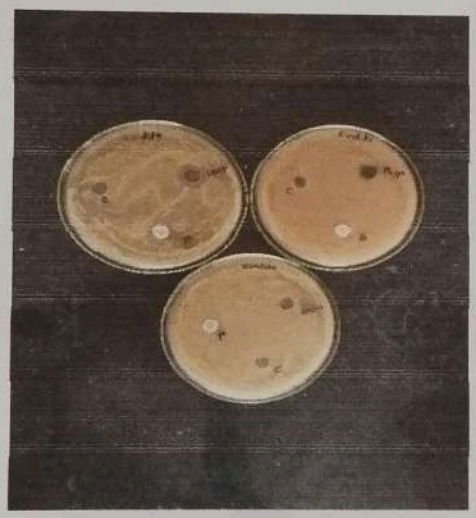
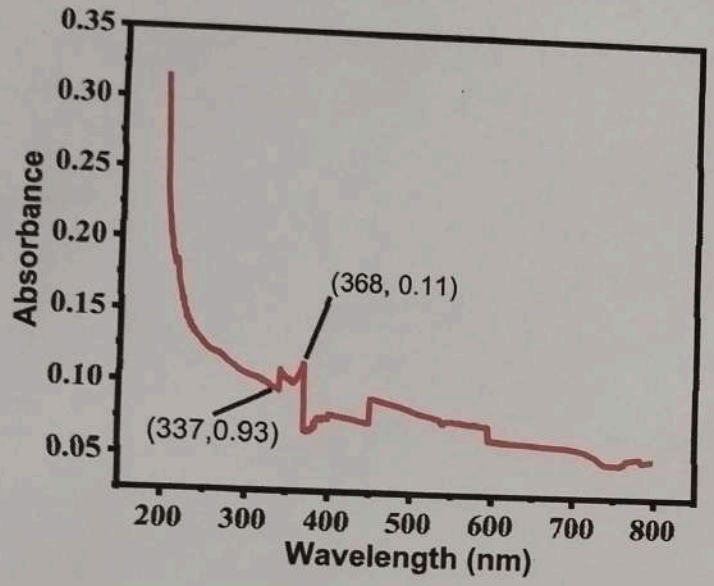


Figure 10 Anti fungal Activity by well Difusion Method Using AgNO3 Solution(Candida)

5.3 UV-Visible Spectroscopy



FORMULA:

$$d = log \frac{\frac{\lambda SPR - \lambda 0}{L1}}{L2}$$

Where:

L1 = 6.53

L2 = 0.0216

λSPR = 368nm(maximum absorbance point)

λ0 = 337nm (minimum absorbance dip)

Calculation:

λSPR = 368nm

λ0 = 337nm

$$d = log \frac{\frac{368-337}{6.53}}{L2}$$

$$d = log \frac{\frac{31}{6.53}}{0.0216}$$

$$d = log \frac{31}{6.53}$$

log(4.75)=0.676

$$d = \frac{0.676}{0.0216}$$

d=31.3

Result:

Estimated nanoparticle size~31.3 nm

- **Fourier transform infrared spectroscopy analysis and Raman spectroscopy. [FTIR]**

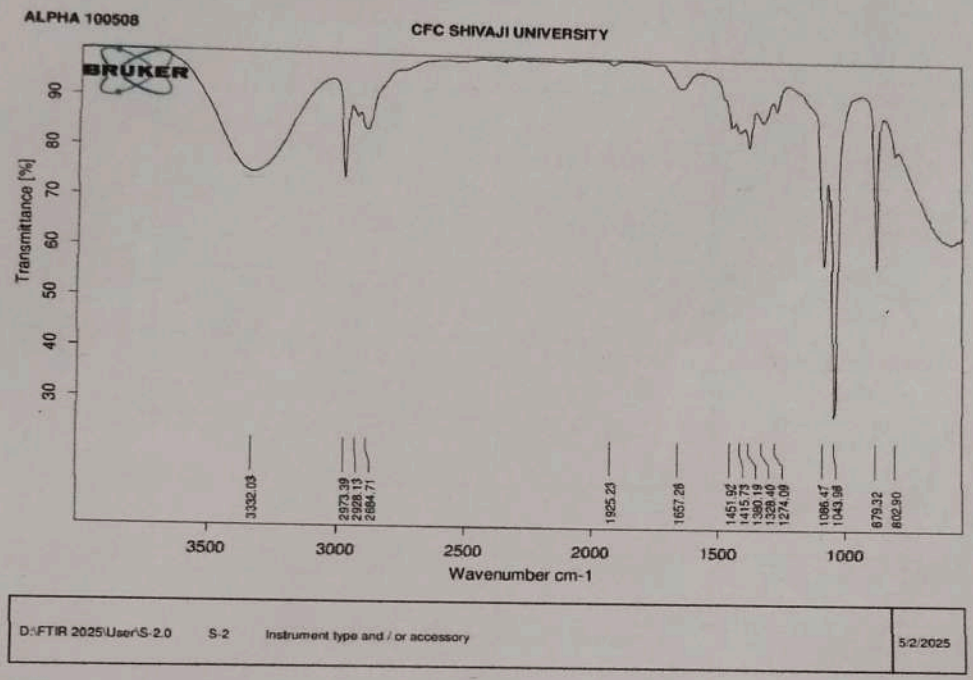


Figure 12 FTIR analysis of biosynthesized PPE-AgNPs Balanite aegyptiaca

- **FTIR Peak analysis Comparison**

Observed Peak (cm-1)	Matched Peak Range from Table (cm-1)	Functional Group	Compound Type
3332.03	3333-3267	C-H stretch	Alkyne
2973.39	3300-2500	O-H stretch	Carboxylic acid
2928.13	3300-2500	O-H stretch	Carboxylic acid
2884.71	3300-2500	O-H Stretch	Carboxylic acid
1925.23	2000-1900	C=C=C stretch	Alkene
1657.28	1620-1610	C-C stretch	Alpha - beta unsaturated ketone
1451.92	1400-1000	C-F stretch	Alkyl and aryl halides
1415.73	1400-1000	C-F stretch	Alkyl and aryl halides
1380.19	1400-1000	C-F stretch	Alkyl and aryl halides
1328.40	1400-1000	C-F stretch	Alkyl and aryl halides
1043.98	1400-1000	C-F stretch	Alkyl and aryl halides
879.32	900-680	C-H bend	Aromatic Compound
802.90	840-600	C-Cl bend	Alkyl and aryl halides

1. 3332 in.63 cm^{-1}, Intensity 3272.25:

The IR peak at 3275.63 cm^{-1} matches the range 3333–3267 cm^{-1}, which indicates a C–H stretch from a terminal alkyne.

2. 2973.39 cm^{-1}, Intensity 2192.50:

Falls in the range of 2360–2190 cm^{-1}, characteristic of C≡C triple bond stretching seen in alkynes.

3. 2928.71 cm^{-1}, Intensity 3300:

Also in the C≡H stretch range (alkyne type bond), confirming presence of alkyne.

4. 2884.71 cm^{-1}, Intensity 2135.48:

Within the 3300–2500 cm^{-1} range, associated with O=H stretch, indicating a carbodiamide functional group.

5. 1925.23 cm^{-1}, Intensity 1657.32:

Matches 1620–1610 cm^{-1}, which corresponds to a C=C stretch in α,β-unsaturated ketones (like enones).

6. 1085.70 cm^{-1} & 1044.80 cm^{-1}, Intensity ~1065:

Found in the 1400–1000 cm^{-1} region, typical of C–F stretches in alkyl and aryl halides.

7. 975.22, 922.09, 876.60, 820.66 cm^{-1} (Intensity ~895.63):

All lie in 900–680 cm^{-1} range, corresponding to C–F Stretch in aromatic compounds.

8. 678.19 cm^{-1}, Intensity 689.20:

Same region and meaning—C–H bend in aromatic compounds.

9. 662.33, 640.50, 624.14 cm^{-1}, Intensity 619.20:

Fall in the 840–600 cm^{-1} region, characteristic of C–Cl bending, indicating presence of alkyl or aryl halides.

10. 593.27 & 572.49 cm^{-1}, Intensity 500.14:

Fall in 840-600 cm^{-1} region, which corresponds to C–Cl bend, seen in aryl halides.

5.2 Conclusion :

This is a simple and non-toxic process used for the synthesis of nanoparticles. It is a green synthesis of silver nanoparticles, where plant extract acts as a reducing agent for nanoparticle synthesis.

This process utilizes plant-based components instead of potentially harmful chemical reducing agents. *Balanites aegyptiaca* leaf extract contains phytochemicals like sugars, terpenoids, alkaloids, and polyphenols, which can donate electrons to silver ions, causing their reduction to silver nanoparticles. Under alkaline conditions, a color change to brown was observed, and after the addition of the plant extract, the resultant AgNPs were purified by centrifugation and characterized using UV-visible spectroscopy. The UV-visible spectroscopic analysis of the colloidal AgNPs dispersion showed a distinct peak.

In a major step forward, one project explored an eco-friendly way to make silver nanoparticles (AgNPs) using pomegranate peel extract. This green method avoids the use of harmful synthetic chemicals and instead uses the natural reducing agents found in the peel. These natural substances help turn silver ions into silver nanoparticles in a safe and sustainable way.

The silver nanoparticles that were made were tested to see how well they could fight harmful microbes. The results were very promising. The nanoparticles showed strong antimicrobial activity. They created a 16 mm zone of inhibition against *Escherichia coli (E. coli*), a 17 mm zone against *Staphylococcus aureus (S. aureus*), and a 15 mm zone against *Candida albicans (C. albicans)*. These zones show how effectively the nanoparticles can stop the growth of bacteria and fungi, proving their potential as antimicrobial agents.

To make sure the nanoparticles were properly formed and to study their structure, the researchers used several advanced techniques. UV-Visible Spectroscopy was used to study the optical properties, confirming the presence of the nanoparticles. Based on UV-Vis spectroscopy and the Haiss equation, the synthesized nanoparticles showed a surface plasmon resonance peak at 368 nm and a baseline dip at 337nm. Using these values, the estimated particle size was calculated to be approximately 31.3 nm, confirming the successful formation of nanosized particles.

Fourier Transform Infrared Spectroscopy (FTIR) was used to identify the functional groups in the peel extract, which played a role in reducing and stabilizing the nanoparticles. The FTIR spectral analysis of *Balanite aegyptiaca* leaf, pulp, peel revealed the presence of multiple functional groups that strongly correlate with its known phytochemical constituents. Peaks at 3375.63 cm^{21} and 2349-2365 cm-indicate terminal alkyne and CBC triple bond stretches, which can be linked to complex alkaloids known for their bioactivity. The peak at 2126.10 cm suggests the presence of carbodiimide groups, possibly associated with bioactive nitrogen-containing compounds like alkaloids or glycosides. The sharp peak at 1636.88 cm corresponds to C=C stretches in a,ß-unsaturated ketones, commanly found in flavonoids and polyphenols, which are well-documented antioxidants. Peaks in the 1085-1044 cm^3 range represent C-F stretching, while those between 662-624 cm^{21} and 593-572 cm^{21} indicate C-Cl and C-1 bonds, respectively suggesting the presence of

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6.0 BIBLIOGRAPHY

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