"GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM PUNICA GRANATUM AND THEIR ANTIMICROBIAL ACTIVITY "

A RESEARCH PROJECT

Submitted by

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VIVEKANAND COLLEGE, KOLHAPUR

(AN EMPOWERED AUTONOMOUS INSTITUTE)

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Ms.Vrushali Misal

Research Project Guide

For

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Place: Kolhapur

Date: 07/05/2025

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Mr. Nikhil Anil Khatave

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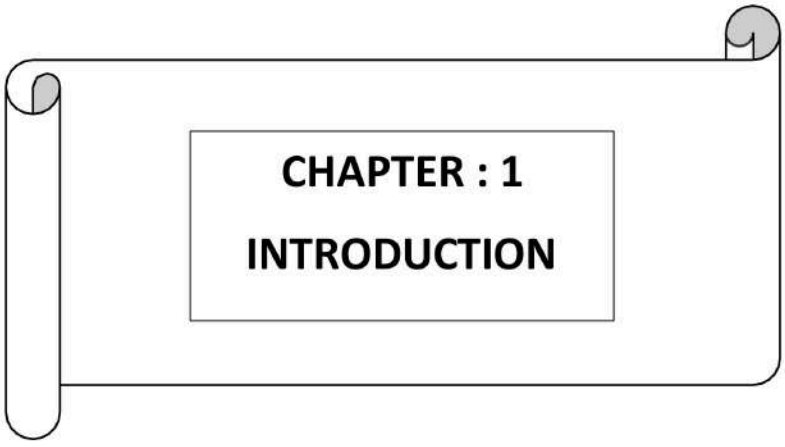

CHAPTER : 1
INTRODUCTION

1.0 INTRODUCTION

Pomegranate (Punica granatum) is a small deciduous tree or shrub, native to Iran and northern India, that thrives in warm, arid climates. The fruit is valued for its nutritional content, medicinal properties, and cultural significance (Prakash, 2011).

Fig.1 Punica_granatum tree , fruit.

Pomegranate is classified under the Kingdom Plantae, Division Angiosperms, Class Eudicots, Order Myrtales, Family Lythraceae, Genus Punica, and Species Punica granatum. It is native to the Middle East, Northern India, the Mediterranean Basin, Southeast Asia, and North Africa, and has been cultivated in regions like California, Afghanistan, and China (Prakash, 2011).

The tree typically grows 12–16 feet tall, with stiff, angular branches, glossy narrow leaves, and large flowers in shades of scarlet, white, or variegated colors (Prakash, 2011).

The fruit is nearly round, with a tough rind that varies in color. It contains compartments filled with juicy arils, each encasing a seed. The fruit matures 5–7 months after flowering (Prakash, 2011).

Pomegranate has been used for centuries, from ancient Egypt, where it was valued for its ability to expel intestinal parasites, to the Greeks and Romans who used it for digestive health and skin conditions (Archana Kumari, 2012).

During the Islamic Golden Age, scholars like Avicenna expanded on pomegranate's medicinal uses, and it became well-known across Europe and Asia (Archana Kumari, 2012).

By the 19th century, its medicinal benefits, particularly for parasitic infections, were scientifically validated, and in modern times, it is considered a "superfood" with various health applications (Seeram, 2006).

In Ayurveda, pomegranate is used to treat gastrointestinal issues, promote heart health, manage anemia, and improve skin and oral health (Prakash, 2011).

The peel is rich in bioactive compounds like punicalagins, ellagic acid, gallic acid, and tannins, which have antioxidant, anti-inflammatory, and antimicrobial effects (Prakash, 2011).

Pomegranate peel shows antimicrobial activity against bacteria like Escherichia coli, Salmonella, and Staphylococcus aureus by disrupting bacterial cell walls (Negi, 2003). It also exhibits antifungal activity against Candida albicans and Aspergillus niger (Al-Zoreky, 2009).

The peel contains alkaloids such as pelletierine, which are effective against gastrointestinal parasites like Taenia solium (tapeworm) and Ascaris lumbricoides (roundworm) (Negi, 2003).

Pomegranate peel's antioxidant effects help combat oxidative stress, benefiting conditions like aging, neurodegenerative disorders, and cardiovascular diseases (Archana Kumari, 2012). It also enhances insulin sensitivity, making it useful for managing type-2 diabetes (Banihani et al., 2013).

The peel's anti-inflammatory properties alleviate symptoms of chronic conditions such as rheumatoid arthritis and inflammatory bowel disease (Adams et al., 2006). Furthermore, it shows anticancer potential by modulating key cancer-related pathways (Adams et al., 2006).

In conclusion, pomegranate, especially its peel, is a rich source of bioactive compounds with broad therapeutic potential, supporting its use as a natural remedy for various health issues (Seeram, 2006).

Silver nanoparticles (AgNPs) have attracted considerable attention in recent decades due to their remarkable properties, such as a high surface area, chemical reactivity, and antimicrobial activity. These nanoscale particles, typically ranging in size from 1 to 100 nm, display unique physical, chemical, and biological characteristics that make them ideal for a wide variety of applications. AgNPs are particularly noteworthy for their interaction with biological systems, making them highly valuable in fields like medicine, electronics, and environmental science. The synthesis of AgNPs can be accomplished through a range of methods, each allowing for precise control over the size, shape, and distribution of the nanoparticles. Consequently, AgNPs have found use in several sectors, including the coatings of medical devices, drug delivery systems, food preservation, and environmental remediation. (Sharma et al., 2014)

The synthesis of silver nanoparticles can be carried out using physical, chemical, or biological methods. Physical methods, such as laser ablation and evaporation-condensation, depend on the manipulation of silver atoms or ions. Chemical methods involve reducing silver salts in the presence of reducing agents, with one commonly used method being the chemical reduction of silver nitrate, typically employing agents like sodium borohydride. The size and distribution of the

nanoparticles can be controlled by adjusting factors like pH, temperature, and concentration during the reaction. Biological or "green synthesis" methods, using plant extracts, fungi, and microorganisms, offer an eco-friendly alternative to the traditional chemical processes, reducing silver ions to nanoparticles while maintaining sustainability. (Kumar et al., 2016)

Titration, though a classical approach, is a reliable technique for estimating the concentration of silver nanoparticles in a solution. The process involves adding a titrant, such as sodium chloride, to a sample containing silver ions. The silver ions react with chloride ions to form a precipitate, and by observing the endpoint of the reaction, one can deduce the concentration of the nanoparticles in the solution. While titration does not directly provide information on the size or shape of nanoparticles, it remains valuable in determining nanoparticle concentration, particularly when combined with complementary techniques like UV-Vis spectroscopy. (Rajiv et al., 2017)

X-ray diffraction (XRD) is an essential method for analyzing the crystal structure and phase composition of materials, including silver nanoparticles. By examining the diffraction of X-rays passing through a sample, XRD reveals the arrangement of atoms within the material. For AgNPs, XRD patterns often show distinct peaks corresponding to the crystalline structure of silver, with common peaks found at the (111), (200), (220), and (311) planes. By evaluating the width of these peaks, the crystallite size of the silver nanoparticles can be determined using the Scherrer equation. XRD is invaluable for confirming the purity and crystalline quality of AgNPs and distinguishing between amorphous and crystalline forms. (Singh et al., 2015)

Fourier Transform Infrared Spectroscopy (FTIR) is another crucial technique for the characterization of AgNPs, particularly for identifying the functional groups present on their surface. FTIR measures the absorption of infrared light by a sample, allowing for the identification of various molecular vibrations. In AgNPs, FTIR is used to detect the presence of capping agents—substances like polyvinyl alcohol or plant biomolecules that stabilize the nanoparticles and prevent aggregation. FTIR spectra of AgNPs typically display peaks corresponding to functional groups such as hydroxyl, carbonyl, and amine, which are indicative of the stabilizers used in the synthesis process. (Patel et al., 2018)

The unique properties of AgNPs have led to their widespread application across multiple industries. In the medical field, silver has long been recognized for its antimicrobial properties, and these properties are significantly enhanced when silver is reduced to the nanoscale. AgNPs are commonly used in wound healing, where they prevent infections by killing bacteria, fungi, and viruses at the site of injury. They are also being explored as drug delivery systems, as their size and surface characteristics allow for targeted delivery of therapeutic agents, particularly in cancer treatments. AgNPs are frequently incorporated into coatings for medical devices like catheters, implants, and surgical tools to reduce the risk of hospital-acquired infections. Their ability to exert

cytotoxic effects against cancer cells while remaining non-toxic to healthy cells makes them an exciting candidate for targeted cancer therapies. (Yadav et al., 2020)

In addition to their medical uses, AgNPs hold promise in environmental remediation. Thanks to their high surface area and chemical reactivity, AgNPs can effectively remove contaminants from water, such as heavy metals, organic pollutants, and bacteria. Their antimicrobial properties are particularly useful in water purification systems, where they help disinfect water and prevent the spread of waterborne diseases. AgNPs are also being incorporated into air purification technologies, embedded in filters that trap airborne pathogens. Furthermore, AgNPs are finding use in agricultural products to prevent plant diseases and promote healthier crop growth. (Singh et al., 2019)

The food industry also benefits from the antimicrobial properties of silver nanoparticles. AgNPs are integrated into food packaging materials to extend the shelf life of products by inhibiting the growth of bacteria and molds. These nanoparticles can either be incorporated into packaging films or applied directly to the food itself. This not only helps preserve the freshness of the food but also enhances its safety by preventing contamination. Additionally, AgNPs are being explored as preservatives to minimize microbial spoilage. However, their inclusion in food products raises regulatory concerns, necessitating careful evaluation of their safety and toxicity. (Zhang et al., 2021)

Silver nanoparticles are increasingly used in the textile industry as well, where they impart antimicrobial properties to fabrics. AgNPs are added to textiles such as sportswear, medical uniforms, and upholstery to prevent bacterial growth, reduce odor, and improve hygiene. This is especially important in healthcare settings, where the risk of infection is high. AgNPs are also used in textiles for their ability to control fungal growth, which can degrade fabrics and create unpleasant odors. These antimicrobial properties help create high-performance textiles that maintain their functionality and integrity over longer periods. (Agarwal et al., 2018)

Despite the many benefits of AgNPs, concerns have arisen regarding their potential toxicity to both human health and the environment. Due to their small size, nanoparticles can penetrate biological membranes and enter cells, potentially leading to cytotoxic effects, including oxidative stress, inflammation, and damage to cellular structures. In the environment, AgNPs may accumulate in aquatic ecosystems, posing risks to aquatic organisms and disrupting ecosystems. As their use becomes more widespread in consumer products and industrial applications, the safety and environmental risks associated with AgNPs are becoming significant areas of research. This highlights the need for careful monitoring and regulation to ensure safe use. (Patel et al., 2020)

In conclusion, silver nanoparticles are versatile nanomaterials with a broad range of applications in medicine, environmental science, food packaging, and textiles. Their synthesis can be achieved using physical, chemical, or biological methods, with various techniques such as titration, XRD, and FTIR playing vital roles in their characterization. While AgNPs offer significant advantages, particularly in antimicrobial and medical fields, their potential toxicity and environmental impact warrant careful evaluation to ensure their safe usage. Continued research into their biocompatibility, biodegradability, and ecological effects will be essential for their safe and sustainable integration into commercial products. (Sharma et al., 2021)

CHAPTER : 2

AIMS AND OBJECTIVES

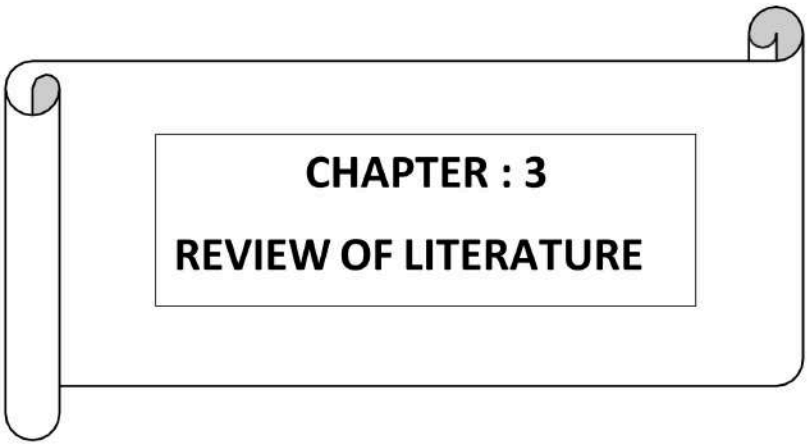
2.0 AIMS AND OBJECTIVES

Aim :

To study Green synthesis of silver nanoparticles from Punica granatum and their antibacterial activity.

Determination of Biomedical Applications including :

- Anti-microbial activity by well diffusion method using *Punica granatum* peel extract.
- Anti-fungal activity by well diffusion method using *Punica granatum* peels extract.



CHAPTER : 3

REVIEW OF LITERATURE

3.0 Review of Literature

Pomegranate peel has attracted attention as a natural antimicrobial agent, offering a solution to antibiotic resistance and food safety issues. Rich in tannins, flavonoids, and gallic acid, it damages microbial membranes and inhibits enzymes. It is effective against E. coli, Staphylococcus aureus, Candida albicans, and possibly some viruses (Al-Zoreky, 2009).

Its anti-inflammatory properties are linked to the inhibition of enzymes like COX-2 and lipoxygenase, reducing prostaglandins, leukotrienes, TNF-α, IL-6, and ROS. These actions help manage arthritis, heart disease, and metabolic disorders (Shukla et al., 2008).

The peel also shows anticancer effects by inducing apoptosis, inhibiting angiogenesis, and limiting metastasis. It has antidiabetic potential by lowering blood sugar, protecting beta cells, and improving insulin sensitivity (Seeram et al., 2005; Li et al., 2005).

Nutritionally, it is a rich source of antioxidants like ellagic acid and dietary fiber (16–20%), and contains minerals such as potassium and calcium, along with Vitamin C, enhancing its value in both diet and medicine (Ismail et al., 2012).

Economically, pomegranate peel is used in pharmaceuticals, cosmetics, and agriculture. Its bioactive compounds combat inflammation and infections, and it serves as a natural preservative and cosmetic ingredient. It also functions as fertilizer, livestock feed, and in wastewater treatment (Negi & Jayaprakasha, 2003; Al-Zoreky, 2009; Faria et al., 2007; Reddy et al., 2014).

Although widely cultivated, pomegranate faces threats from climate change and overuse. Sustainability depends on eco-friendly farming and full utilization of by-products. Conservation efforts like agroforestry and seed banks are essential (Levin, 2006; Mars, 2000).

With antimicrobial resistance rising, plant-derived alternatives are crucial. Pomegranate peel, often discarded, contains potent antimicrobial agents such as tannins, flavonoids, ellagic acid, and punicalagins (Al-Zoreky, 2009).

Tannins disrupt microbial cell walls; flavonoids interfere with microbial metabolism; ellagic acid damages membranes and inhibits cell wall synthesis; and punicalagins enhance antimicrobial action through membrane disruption (Ismail et al., 2012).

Effective extraction depends on the method used. Ethanol-based solvent extraction is most effective, preserving the activity of phenolics. Proper storage such as freeze-drying is vital for maintaining bioactivity (Negi & Jayaprakasha, 2003).

Extracts are tested against pathogens like E. coli, P. aeruginosa, S. aureus, K. pneumoniae, Candida albicans, and Aspergillus niger. Techniques include Disc Diffusion and MIC tests to assess antimicrobial potency (Reddy et al., 2014).

Compared to drugs like penicillin and Miconazole, pomegranate peel extracts show comparable or superior activity, especially against resistant strains like S. aureus and C. albicans (Al-Zoreky, 2009).

These extracts are more effective against Gram-positive bacteria but also inhibit Gram-negative strains at higher concentrations. They also show significant antifungal action (Shukla et al., 2008).

Applications span food preservation and healthcare. They are used in packaging and as topical treatments for infections, with potential for oral supplements supporting gut health (Faria et al., 2007).

However, clinical trials are needed to determine safe dosing. Eco-friendly extraction techniques and synergistic studies with conventional drugs are key areas for future research (Seeram et al., 2005).

Silver nanoparticles (AgNPs) have garnered significant interest due to their distinctive properties, including optical, electrical, and antimicrobial characteristics attributed to their high surface area-to-volume ratio. Historically, silver has been used for its therapeutic benefits, but with the advent of nanotechnology, its applications have expanded into various sectors such as medicine, agriculture, and environmental science (Ullah et al.).

AgNPs typically range in size from 1 to 100 nanometers and exhibit unique physical and chemical properties. These features have facilitated their integration into different scientific and industrial fields. A prominent application of AgNPs lies in their antimicrobial activity, which stems from their ability to disrupt microbial membranes and interact with essential cellular components like thiol groups, often generating reactive oxygen species that inhibit microbial growth and viability (Ullah et al.).

Various synthesis methods have been employed to produce silver nanoparticles. Chemical methods involve the use of reducing agents like sodium citrate and sodium borohydride, although these can sometimes result in environmental and health concerns due to the involvement of hazardous chemicals. Physical methods such as laser ablation and evaporation-condensation require sophisticated equipment and high energy input, making them less practical on a larger scale. On the other hand, biological or green synthesis methods are gaining traction due to their eco-friendly and cost-effective nature. These utilize plant extracts, fungi, or bacteria to mediate the reduction of silver ions into nanoparticles (Ullah et al.).

An example of green synthesis includes the use of Punica granatum (pomegranate) extracts, which facilitate the eco-friendly synthesis of AgNPs. This process has been validated using analytical techniques like UV-visible spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR) is used to identify the functional groups and chemical bonds present in a material , X-ray Diffraction (XRD) is performed to determine the crystalline structure, phase identification, crystallite size, and purity of materials. confirming the formation and stability of the nanoparticles (Kumar et al., 2018).

The applications of AgNPs are vast and diverse. In the medical field, they are utilized for drug delivery systems, antimicrobial coatings on medical devices, wound healing agents, and as components in cancer treatment due to their biocompatibility and potent antimicrobial effects.

Environmentally, AgNPs contribute to water purification systems and wastewater treatment by neutralizing pathogenic microorganisms. In animal husbandry and fisheries, these nanoparticles serve as feed additives to enhance animal immunity, control microbial infections, and reduce disease transmission in aquatic environments (Ullah et al.).

Despite their wide applicability, AgNPs also present certain toxicity and safety concerns. The high surface reactivity of small-sized nanoparticles can lead to cytotoxic effects, oxidative stress, DNA damage, apoptosis, and bioaccumulation in living organisms. These issues raise significant concerns regarding their safe use in clinical and environmental settings, underscoring the need for thorough evaluation of their long-term effects (Ullah et al.).

Several studies have demonstrated the successful green synthesis of AgNPs using different parts of the pomegranate plant. One study utilized leaf extract and produced AgNPs with an average size of approximately 398.33 nm. The process, confirmed by UV-visible spectroscopy and SEM, proved to be an environmentally sustainable method of nanoparticle synthesis (Kumar et al., 2018).

Another study employed pomegranate peel waste to synthesize spherical AgNPs with a plasmon resonance peak at 440 nm. These nanoparticles exhibited strong antimicrobial activity against common foodborne pathogens, highlighting their potential application in food safety (Farouk et al.).

Additionally, AgNPs synthesized using pomegranate extract were tested for antioxidant and antimicrobial activity. These particles, ranging from 5 to 25 nm, demonstrated 91.6% antioxidant efficiency and strong antibacterial properties, particularly against gram-negative bacteria (Aygunet al., 2021).

In conclusion, the green synthesis of AgNPs using plant-based materials such as Punica granatum represents a sustainable approach to nanotechnology. These biologically synthesized nanoparticles offer valuable biological activities including antimicrobial and antioxidant effects, making them highly suitable for use in nanomedicine, agriculture, and biotechnology. Nevertheless, further research is essential to optimize synthesis protocols, understand the mechanisms of action, and assess the potential risks associated with long-term exposure to AgNPs to ensure safe and effective applications (Ullah et al.; Kumar et al., 2018; Farouk et al.; Aygun et al., 2021).

CHAPTER : 4

METHODS AND MATERIAL

4.0 Methods and materials

4.1 Materials

Media :

Nutrient Agar :

1. Peptone: 0.5 g
2. Beef extract: 0.3 g
3. Sodium chloride (NaCl) : 0.5 g
4. Agar: 1.5 g
5. Distilled water: 100 mL
6. pH : Adjust to 7.0 ± 0.2

Potato Dextrose Agar (PDA)

1. Potato extract : 20 g
2. Dextrose (glucose): 2.0 g
3. Agar: 1.5 g
4. Distilled water: 100 mL
5. pH : Adjust to ~5.6

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 Punica granatum (Pomegranate).

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 (*Punica granatum*)

2) Selection of plant part-

E.g. Fruit Coat

3) Collection -

Collect *Punica granatum* fruit coat.

4) Washing-

Tap water is used for washing.

5) Cutting of Coat (Peels).

6) Drying-

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

7) Powdering-

Dry peels can be powdered by use of mortar.

8) Methods of extraction-

1. Soxhlet method.

Fig.2 Fruit and Dry peels of Punica granatum.

Fig.3 Powder of dry peels of Punica granatum.

Preparation of plant (fruit peel) 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 done by Soxhlet method:

- For Soxhlet Method we used Soxhlet apparatus.

Soxhlet Method was developed by Franz von Soxhlet, in 1879. Soxhlet extraction is a Continuous solid/liquid extraction in which active phytoconstituents are concentrated by use of organic solvents in Soxhlet apparatus.

Procedure:

1. The Soxhlet apparatus was first assembled according to the standard procedure.
2. A powder column was prepared using filter paper, into which 10 g of Punica granatum peel powder was placed.
3. Next, 160 ml of solvent (ethanol) was added to the thimble to dissolve the powder.
4. The heating mantle was initially started at 30°C and, after 15-20 minutes, the temperature was gradually increased to reach the boiling point of ethanol.
5. Approximately six cycles of the Soxhlet apparatus were run to ensure the proper extraction of phytochemicals.

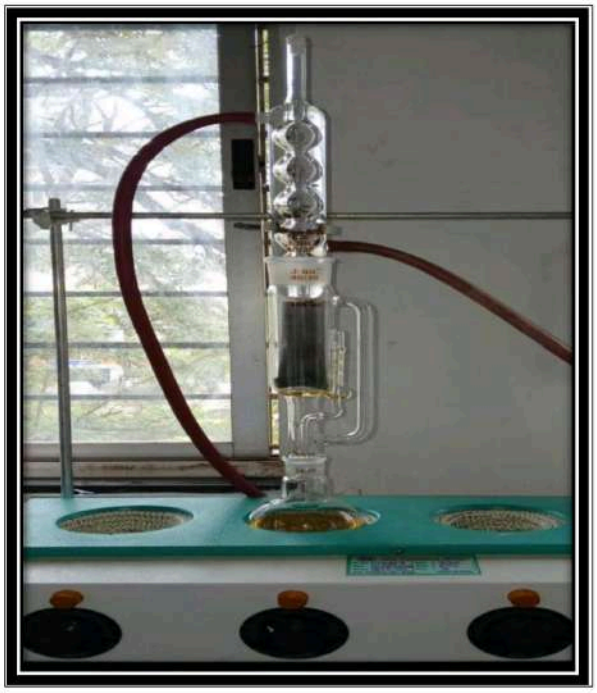


Fig.4 Extraction of Punica granatum by Soxhlet method.

Preparation of AgNO3 :

Silver nitrate ($AgNO_3$) 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 $AgNO_3$.
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.

Fig.5 Preperation of AgNO3

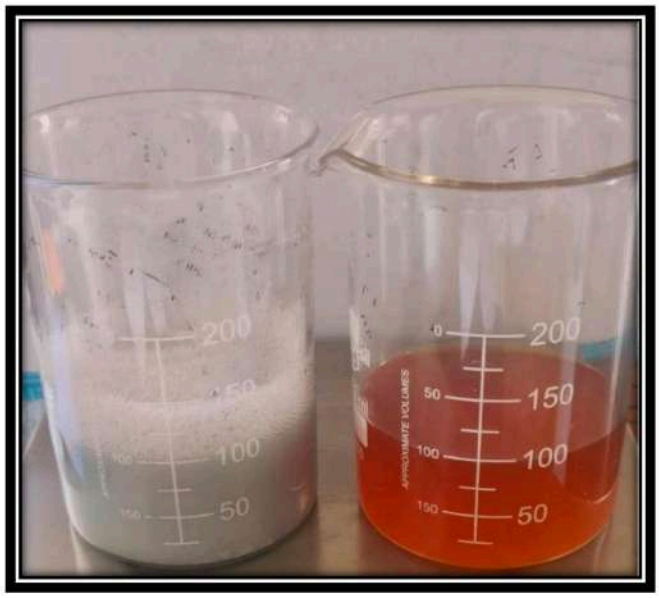


Fig.6 Titered AgNO3 with peel extract and Peel extract.

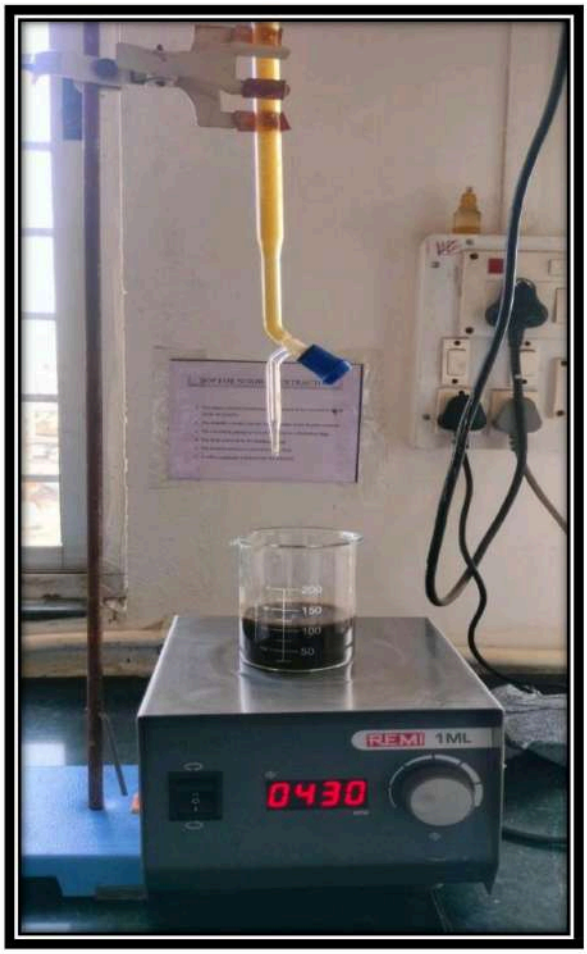


Fig.7 Preperation of AgNO3 by Titration method.

Titration Procedure:

1. Fill the burette with 50 ml of the peel extract.

2. Take 100 ml of silver nitrate ($AgNO_3$) 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 $A = \varepsilon \times c \times l$, where A is absorbance, ε is a constant for the substance, c is concentration, and l 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.

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:

Sr. No	Name of Organisms	Zone of inhibition (Diameter in mm)		
		AgNO3 Solution.	**Standard : penicillin**	**Control**
1.	***Escherichia coli***	**16mm**	**32mm**	**-**
2.	***Staphylococcus aureus***	**17mm**	**31mm**	**-**

Anti-Fungal Activity by Well Diffusion Method using AgNO3 Solution.

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 assessing 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) AgNO3 Solution.

2) Standard – Penicillin

Procedure:

1. Prepare the Potato Dextrose Agar media and autoclave it.
2. Pour the media into the respective petri dishes and allow it to solidify.
3. Prepare the inoculum and spread it on the top of the Potato Dextrose agar plate media.
4. After preparing the plates, make wells using a cork borer.
5. Inoculate the sample into the particular wells.
6. After inoculation, incubate the plates at room temperature for 24 hours.

Observation Table:

Sr. No	Name of Organisms	Zone of inhibition (Diameter in mm)		
		AgNO3 Solution.	**Standard: Penicillin**	**Control**
1.	***Candida albicans***	**15mm**	**-**	**-**

CHAPTER : 5

RESULT AND DISSCUSSION

5.0 RESULTS AND DISSCUSSION

5.1 Anti-bacterial activity by well diffusion method using AgNO3 Solution.

Result :

Antimicrobial activities by well difusion method was assesed using AgNO3 Solution.

Fig.8 Anti-Bacterial Acitvity by Well Diffusion Method using AgNO3 Solution.

(Staphylococcus aureus, Escherchia coli.)

Observation Table:

Sr. No	Name of Organisms	Zone of inhibition (mm)		
		AgNO3 Solution.	**Standard : penicillin**	**Control**
1.	***Escherichia coli***	**16mm**	**32mm**	**-**
2.	***Staphylococcus aureus***	**17mm**	**31mm**	**-**

5.2 Anti-fungal activity by well diffusion method using *Punica granatum* peels extract.

Result :

Antifungal activities by well difusion method was assesed using *Punica granatum* peel extract.

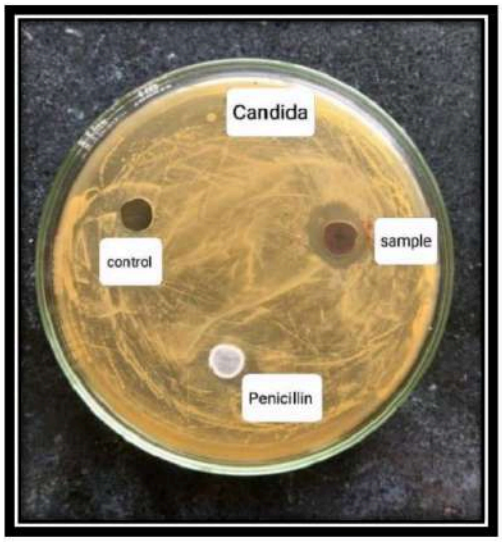


Fig.9 Anti-Fungal Activity by Well Diffusion Method using AgNO3 solution.

(Candida albicans.)

Observation Table:

Sr. No	Name of Organisms	Zone of inhibition (mm)		
		AgNO3 Solution.	**Standard: Penicillin**	**Control**
1.	***Candida albicans***	**15mm**	**-**	**-**

5.3 UV-Visible Spectroscopy

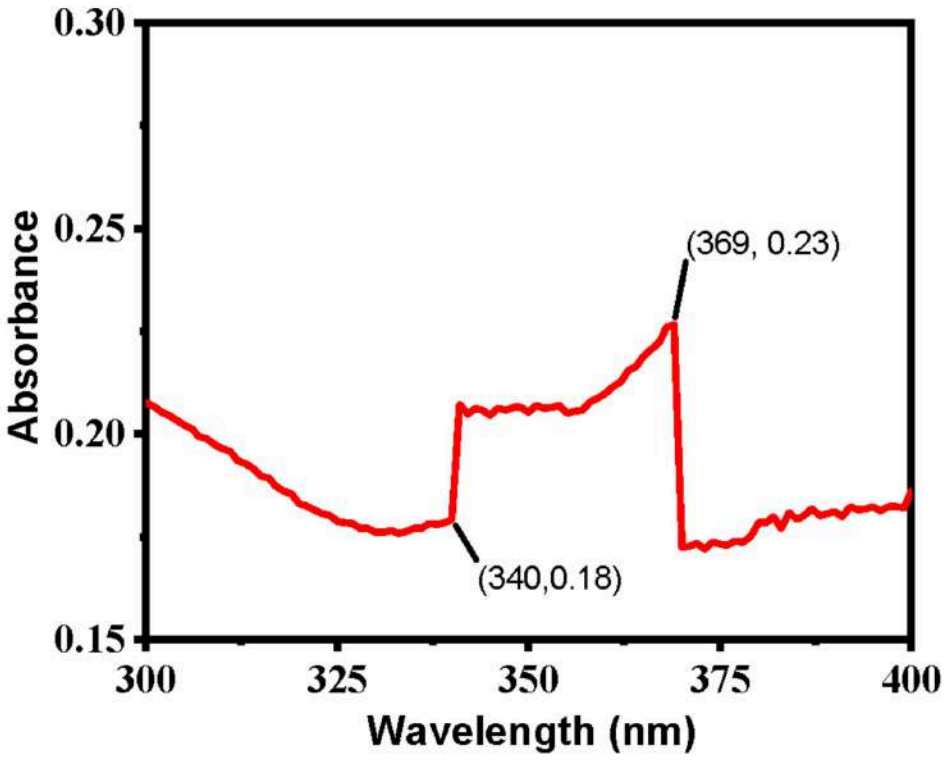


Fig. 10 UV-Visible Spectroscopy Analysis AgNPs from Punica granatum.

FORMULA:

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

Where:

L1 = 6.53

L2 = 0.0216

λSPR = 369 nm (maximum absorbance point)

λ_0 = 340 nm (minimum absorbance dip)

Calculation:

- λSPR=369nm
- λ0=340 nm

$$\mathbf{d} = \frac{log(\frac{369-340}{6.53})}{0.0216}$$

$$\mathbf{d} = \frac{log(\frac{29}{6.53})}{0.0216}$$

$$\mathbf{d} = \frac{log(4.44)}{0.0216}$$

∴ log(4.44) = 0.647

$$\mathbf{d} = \frac{0.647}{0.0216} = \mathbf{29.95nm}$$

d = 30nm

Result:

Estimated nanoparticle size = ~30 nm

5.4 FTIR (Fourier Transform Infrared Spectroscopy)

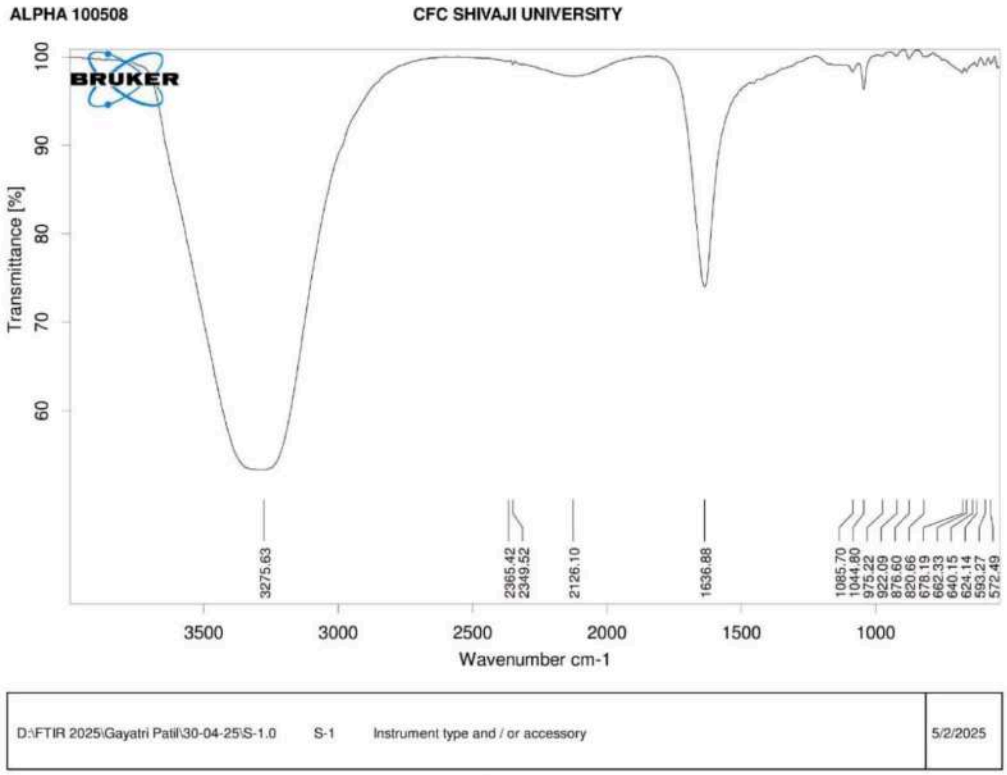


Fig. 11 FTIR analysis of biosynthesized PPE-AgNPs from Punica granatum.

IR Spectrum Peak Analysis

Peak (cm^{-1})	Intensity	Identified Bond (from Chart)	Functional Group	Compound
3275.63	3272.25	3333–3267 cm^{-1}	C–H stretch	Alkyne
2365.42	2192.50	2360–2190 cm^{-1}	C≡C stretch	Alkyne
2349.52	2192.50	2360-2190cm^{-1}	C≡C stretch	Alkyne
2126.10	2135.48	2145-2120	N=C=S stretch	Carbodiimide
1636.88	1618.32	1620–1610 cm^{-1}	C=C stretch	α,β-unsaturated ketone
1085.70	1065.15	1400–1000 cm^{-1}	C–F stretch	Alkyl & Aryl halides
1044.80	1065.15	1400-1000 cm^{-1}	C–F stretch	Alkyl & Aryl halides
975.22	895.63	900–680 cm^{-1} range	C–H bend	Aromatic compound
922.09	895.63	900–680 cm^{-1} range	C–H bend	Aromatic compound
876.60	895.63	900–680 cm^{-1} range	C–H bend	Aromatic compound
820.66	895.63	900–680 cm^{-1} range	C–H bend	Aromatic compound
678.19	689.20	900–680 cm^{-1}	C–H bend	Aromatic compound
662.33	619.20	840-600 cm^{-1}	C-Cl bend	Alkyl & Aryl halides
640.50	619.20	840–600 cm^{-1}	C–Cl bend	Alkyl & Aryl halides
624.14	619.20	840-600 cm^{-1}	C-Cl bend	Alkyl & Aryl halides
593.27	500.14	600–500 cm^{-1}	C–I stretch	Aryl halides
572.49	500.14	600–500 cm^{-1}	C–I stretch	Aryl halides

1. 3275.63 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. 2365.42 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. 2349.52 cm^{-1}, Intensity 2192.50:

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

4. 2126.10 cm^{-1}, Intensity 2135.48:

Within the 2145–2120 cm^{-1} range, associated with N=C=S stretch, indicating a carbodiimide functional group.

5. 1636.88 cm^{-1}, Intensity 1618.32:

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–H bending in aromatic compounds.

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

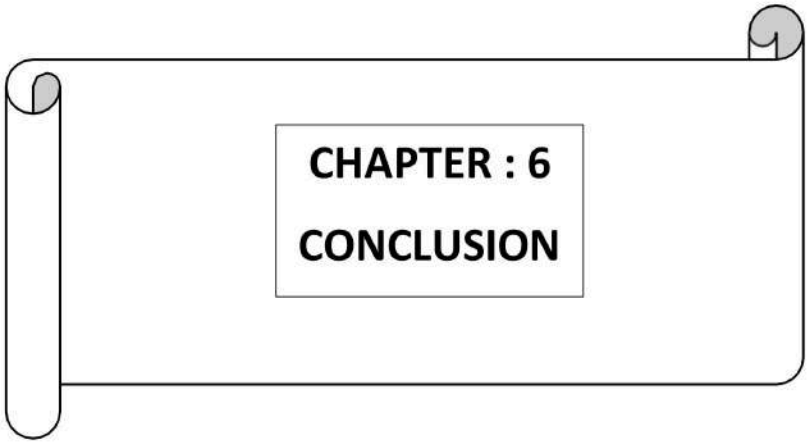
Same region and meaning—C–H bend in aromatic compounds.

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 600–500 cm^{-1} region, which corresponds to C–I stretch, seen in aryl halides.


CHAPTER : 6
CONCLUSION

6.0 CONCLUSION

Pomegranate (Punica granatum) is well known for its health benefits, but its peel often thrown away as waste has recently gained attention for its medicinal value. Research has shown that pomegranate peel is packed with powerful natural compounds like glycosides, flavonoids, tannins, polyphenols, alkaloids, and more. These bioactive compounds have shown potential in treating various health problems such as infections, inflammation, diabetes, parasitic diseases, and fungal infections.

To explore these benefits, scientists carried out both qualitative and quantitative experiments. Using the Folin-Ciocalteu method, they confirmed that the peel contains a high amount of phenolic compounds, which are known for their antioxidant properties. Two extraction methods were used in the study: Soxhlet extraction, which provides a higher yield of active compounds through continuous heating and solvent use, and maceration, a simpler and more cost-effective method.

Through phytochemical screening, researchers found important compounds such as glycosides (identified using the Keller-Killani test) and alkaloids (detected with Wagner's reagent), among others. These compounds play a key role in the extract's healing potential. The extract showed strong antioxidant, anti-inflammatory, and antimicrobial activities. In anti-inflammatory tests, it was able to stop protein denaturation a common sign of inflammation almost as effectively as Diclofenac, a widely used drug for arthritis. Its anti-diabetic effect was demonstrated through alpha-amylase inhibition, meaning it could help control blood sugar levels after meals.

In anti-parasitic tests using earthworms, the extract caused quick paralysis and death, likely due to the action of tannins and alkaloids, which may interfere with the parasites' nervous system and energy supply.

The extract's antimicrobial activity was tested using the well-diffusion method. It worked well against bacteria like Klebsiella pneumoniae and Staphylococcus aureus, though it was less effective against E. coli and Pseudomonas aeruginosa. Its antifungal effects were seen against Aspergillus niger and Candida albicans, though the results were moderate, suggesting that further improvements could enhance its performance.

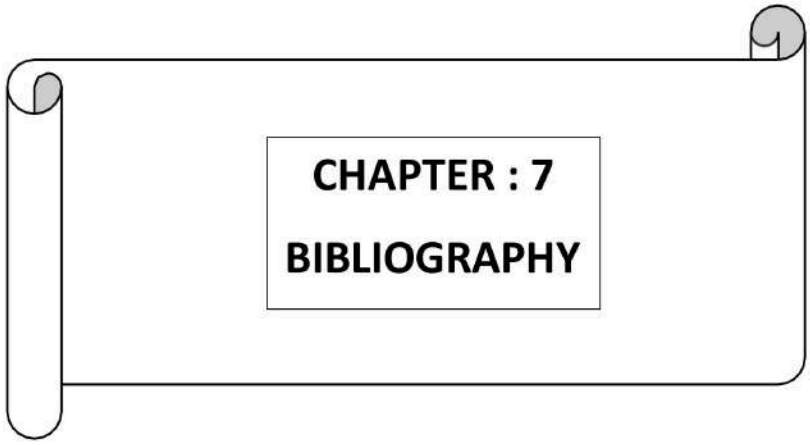
In a major step forward, one project explored an eco-friendly way to make silver nanoparticles (AgNPs) using pomegranate peel extract. This green method avoids the use of harmful synthetic chemicals and instead uses the natural reducing agents found in the peel. These natural substances help turn silver 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 369 nm and a baseline dip at 340 nm. Using these values, the estimated particle size was calculated to be approximately **30 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 pomegranate (Punica granatum) peel revealed the presence of multiple functional groups that strongly correlate with its known phytochemical constituents. Peaks at 3275.63 cm^{-1} and 2349–2365 cm^{-1} indicate **terminal alkyne and C≡C triple bond stretches**, which can be linked to complex **alkaloids** known for their bioactivity. The peak at 2126.10 cm^{-1} suggests the presence of **carbodiimide groups**, possibly associated with **bioactive nitrogen-containing compounds** like alkaloids or glycosides. The sharp peak at 1636.88 cm^{-1} corresponds to **C=C stretches** in α,β-unsaturated ketones, commonly found in **flavonoids** and **polyphenols**, which are well-documented antioxidants. Peaks in the 1085–1044 cm^{-1} range represent **C–F stretching**, while those between 662–624 cm^{-1} and 593–572 cm^{-1} indicate **C–Cl** and **C–I bonds**, respectively suggesting the presence of **halogenated aromatic compounds**, which are often part of **phenolic or tannin structures**. Additionally, multiple bands in the 975–820 cm^{-1} region confirm **aromatic C–H bending**, supporting the presence of **polyphenols and tannins**. Altogether, the FTIR profile substantiates the chemical presence of several key **phytochemicals** such as **flavonoids, glycosides, tannins, alkaloids**, and **polyphenols** which align with the documented medicinal properties of pomegranate peel. This integrated evidence supports its potential use in **pharmaceutical and nutraceutical applications**.

All of these results together give a clear message: pomegranate peel, which is usually thrown away as waste, is actually full of useful natural compounds. This study shows that the peel has great potential not only in creating green, safe, and sustainable antimicrobial products but also in replacing harmful synthetic chemicals. With more studies, including clinical trials and combined effect research, pomegranate peel extract could become an important natural resource in fields like medicine, food safety, cosmetics, and even environmental clean-up.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