ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF PUNICA GRANATUM

A RESEARCH PROJECT

Submitted by

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VIVEKANAND COLLEGE, KOLHAPUR

(AN EMPOWERED AUTONOMOUS INSTITUTE)

YEAR 2024-2025

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Ms.Vrushali Misal

Research Project Guide

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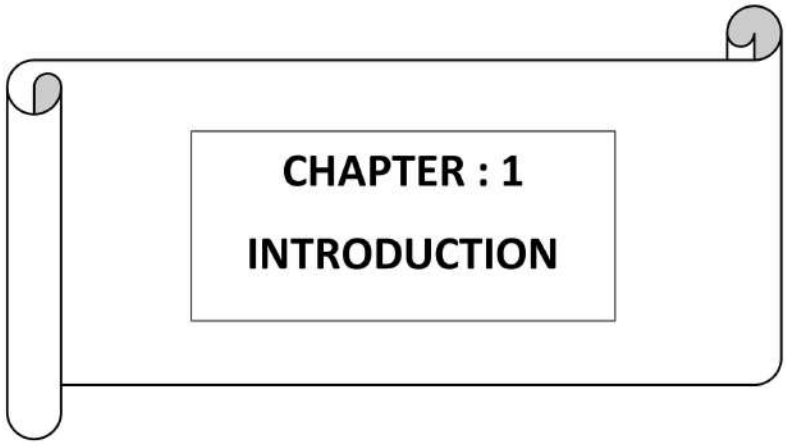


CHAPTER : 1

INTRODUCTION

1.0 INTRODUCTION

Pomegranate (*Punica granatum*) is a small deciduous tree or shrub known for its nutrient-rich, ruby-red arils and numerous health benefits. Native to Iran and northern India, it thrives in warm, arid climates and has been cultivated globally. The fruit holds immense significance for its nutritional value, medicinal properties, and cultural importance across various regions.

Fig.1 Punica granatum tree , fruit.

Pomegranate (*Punica granatum*) is classified under the

Kingdom : Plantae

Division : Angiosperms

Class : Eudicots

Order : Myrtales

Family : Lythraceae

Genus : Punica

Species: Punica granatum.

It is native to the Middle East (Iran and Iraq), Northern India, the Mediterranean Basin (Turkey, Spain, and Italy), parts of Southeast Asia, and North Africa. Over time, it has been cultivated in regions like California, Afghanistan, and China due to its adaptability to various climates. [*Prakash, 2011*]

The pomegranate (*Punica granatum L.*) is cultivated for its ornamental and medicinal value. Typically growing to a height of 12–16 feet, it can reach 20–30 feet under optimal conditions. The tree has a neat, rounded shape with stiff, angular branches that often exhibit spines and a tendency to sucker at the base. Its bark transitions from red-brown when young to gray as it matures. Although pomegranate trees are known for their longevity, their productivity and vigor generally decline after approximately 15 years. The glossy, narrow, lance-shaped leaves are leathery and remain vibrant green throughout the growing season.

Pomegranate flowers, a key feature of the tree, are large and vivid, appearing in scarlet, white, or variegated shades. Each flower exceeds an inch in width and features a distinctive tubular calyx that persists as the fruit develops. These flowers occur singly or in clusters of two to three and can self-pollinate or cross-pollinate, with the latter, facilitated primarily by insects, significantly enhancing fruit set. Wind pollination is relatively insignificant.

The fruit of the pomegranate is nearly round and ranges from 2½ to 5 inches in diameter. Its tough, leathery rind varies in color from yellow with a blush of pink to deep red, depending on the cultivar. A prominent calyx at the fruit's base, a remnant of the flower, often helps distinguish varieties. Inside, the fruit is divided by thin membranous walls and spongy, bitter tissues into compartments filled with juicy arils.

Each aril encases a seed, which may be soft or hard, depending on the cultivar. The arils range in color from pale pink to deep red or whitish. High temperatures during ripening are essential for the fruit to develop its optimal flavor and sweetness. The fruit matures approximately 5–7 months after flowering, and under favorable conditions, the tree begins bearing fruit within 2½ to 3 years of planting.

The pomegranate (*Punica granatum*) has a rich history that spans millennia, intertwined with both cultural symbolism and medicinal use. Revered for its associations with fertility, abundance,

and immortality, it was not only a nourishing fruit but also a key remedy in ancient medical traditions. *[Archana kumari,2012*]

In ancient Egypt, pomegranate was valued for its ability to expel intestinal parasites, a common health issue. The Egyptians also used its rind and peel in embalming, recognizing its antimicrobial properties, which were crucial for preserving bodies. The Greeks, heavily influenced by Egyptian practices, associated the fruit with life and rebirth. Greek physicians like Hippocrates and Galen recommended it for digestive ailments, including dysentery and intestinal worms. The Romans expanded upon these uses, applying pomegranate to treat gastrointestinal disorders, skin issues, and even in cosmetics. Pliny the Elder's Natural History documented its medicinal uses, emphasizing its versatility.

During the Islamic Golden Age, scholars like Avicenna (Ibn Sina) advanced the knowledge of pomegranate in their medical texts, particularly its use in treating digestive problems and promoting heart health. Its cooling properties were noted as beneficial in balancing the body's humors, especially for treating fevers and inflammation. As the Islamic Empire spread, this knowledge traveled across Europe and Asia, establishing pomegranate as a renowned medicinal fruit.

In medieval Europe, pomegranate was a staple in herbal medicine, regarded for its antiseptic and anti-inflammatory effects. During the Renaissance, European botanists continued to highlight its medicinal properties, and it was introduced to the Americas by Spanish settlers, where it was quickly embraced for its therapeutic potential.

By the 19th century, scientific validation of pomegranate's medicinal value grew, especially regarding its alkaloids, such as pelletierine, found in the peel, which proved effective in treating parasitic infections. In the modern era, pomegranate has been the focus of extensive pharmacological research, revealing its antioxidant, anti-inflammatory, and anticancer properties. Studies have demonstrated its ability to reduce blood pressure, improve heart health, and even help manage type 2 diabetes by enhancing insulin sensitivity.

Today, pomegranate is celebrated as a "superfood," valued for its rich nutrient content and continued use in health products like juices and skincare. Its enduring symbolism, representing health, vitality, and prosperity, continues to make it a powerful cultural and medicinal icon. From

ancient Egypt to modern science, the pomegranate's legacy remains strong as one of the most revered fruits in both traditional and contemporary medicine. [*G. S. Seeram, 2006*]

In Ayurveda, pomegranate holds significant therapeutic value. Its taste (Rasa) is sweet (Madhura) and sour (Amla), with light (Laghu) and unctuous (Snigdha) properties (Guna). Its potency (Virya) is cooling (Shita), and it balances Pitta and Kapha doshas. Therapeutic uses include improving gastrointestinal health by treating diarrhea and dysentery, promoting heart health by acting as a cardiac tonic, managing anemia by boosting hemoglobin levels, and supporting oral health by reducing inflammation and improving gum health. Additionally, it is used for skin care, promoting glowing skin and treating infections (*Prakash, 2011*).

Pomegranates are rich in phytochemicals that contribute to their extensive medicinal applications. The peel, in particular, is notable for its high concentration of bioactive compounds, including punicalagins, ellagic acid, gallic acid, and tannins, which exhibit potent antioxidant, anti-inflammatory, and antimicrobial properties. The seeds are rich in punicic acid, a unique omega-5 conjugated fatty acid with anti-inflammatory and cardioprotective effects, as well as isoflavones like genistein and daidzein, which further enhance their health benefits.

The roots and stems contain alkaloids such as isopelletierine, pseudopelletierine, and N-methylisopelletierine, traditionally used for their anthelmintic properties. Anthocyanidins such as pelargonidin, along with ellagitannins and other phenolic compounds, further augment the fruit's pharmacological potential.

The antimicrobial activity of pomegranate peel is particularly significant, owing to its high content of polyphenols, punicalagins, ellagic acid, and tannins. These compounds demonstrate broad-spectrum antibacterial effects against both Gram-positive and Gram-negative bacteria. They achieve this by disrupting bacterial cell walls, inhibiting quorum sensing, and suppressing enzymatic activities critical for bacterial survival. pomegranate peel extract effectively targets pathogens such as *Escherichia coli, Salmonella spp.*, and *Staphylococcus aureus.* By disrupting bacterial biofilm formation and cell membrane integrity, the extract inhibits bacterial proliferation and virulence. [*Negi,2003*]

Its antifungal activity, mediated by tannins and flavonoids, is equally potent, effectively inhibiting fungal spore germination and disrupting fungal cell membranes demonstrated that

fungal pathogens like *Candida albicans* and *Aspergillus niger* are particularly susceptible to pomegranate peel extract, which alters membrane permeability and suppresses essential metabolic pathways. [*Al-Zoreky ,2009*]

In addition to its antimicrobial and antifungal effects, pomegranate peel exhibits strong anti-helmintic properties due to alkaloids like pelletierine. These compounds induce neuromuscular paralysis in parasites and damage their protective layers, facilitating their elimination. Such activity has been demonstrated against gastrointestinal parasites like Taenia solium (tapeworm) and Ascaris lumbricoides (roundworm), making pomegranate peel an effective natural remedy for parasitic infections. The peel also shows antiviral potential by inhibiting viral replication and preventing viral entry into host cells, an area of growing interest in recent studies.

Pomegranate peel's antioxidant, antidiabetic, and anti-inflammatory activities further underscore its therapeutic potential. Its antioxidant effects, attributed to punicalagins and ellagic acid, involve scavenging free radicals, reducing oxidative stress, and preventing cellular damage. These properties make it a valuable agent for addressing oxidative stress-related conditions such as aging, neurodegenerative disorders, and cardiovascular diseases. [*Archana kumari, 2012*]

The peel's antidiabetic activity is mediated through the inhibition of carbohydrate-hydrolyzing enzymes like alpha-amylase and alpha-glucosidase, which slow glucose absorption and regulate postprandial blood sugar levels. Furthermore, it enhances insulin sensitivity and regulates glucose transporter 4 (GLUT4), making it effective in managing type-2 diabetes and related complications. [*Banihani et al.,2013*]

Pomegranate peel's robust anti-inflammatory properties are mediated through the suppression of pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α). By inhibiting key inflammatory pathways like cyclooxygenase (COX) and lipoxygenase (LOX), the peel alleviates symptoms of chronic inflammatory conditions. Highlighted its potential in managing diseases such as rheumatoid arthritis and inflammatory bowel disease. [*Adams et al.,2006*]

Additionally, polyphenols in pomegranate peel have demonstrated anticancer potential by modulating signaling pathways, including nuclear factor kappa B (NF-κB) and phosphoinositide

3-kinase/protein kinase B (PI3K/Akt). These effects, observed in cancers like breast, prostate, and colon, involve inducing apoptosis, inhibiting proliferation, and suppressing metastasis.

In conclusion, pomegranate, particularly its peel, is a rich source of bioactive compounds with immense therapeutic potential. Its antimicrobial, antifungal, antioxidant, anti-diabetic, and anti-inflammatory properties position it as a valuable natural remedy for addressing a wide range of health issues, from infectious diseases to chronic inflammatory conditions. As research continues, the role of pomegranate in developing nutraceuticals and pharmaceuticals becomes increasingly evident, highlighting its significance as a natural agent with far-reaching health benefits.

CHAPTER : 2

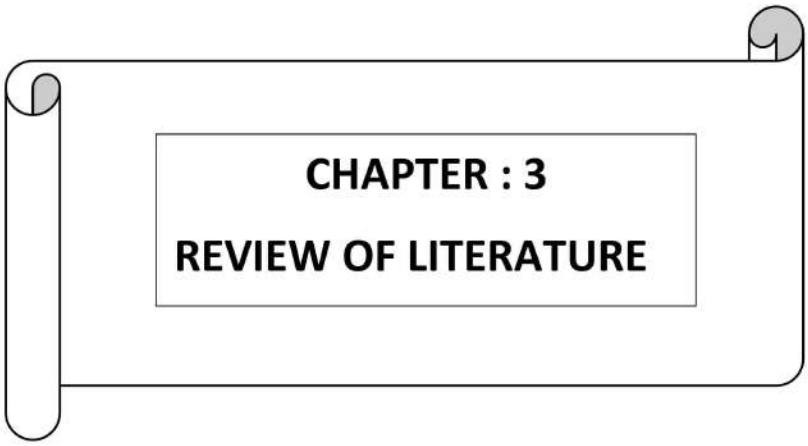
AIMS AND OBJECTIVES

2.0 AIMS AND OBJECTIVES

Aim :

To study the Anti-microbial activity and Phytochemicals analysis of *Punica granatum.*

- Following objectives under taken for present study-
  1. Phytochemical analysis of punica granatum.
     - Qualitative and Quantitative analysis.
  2. Determination of Biomedical Applications including :
     - Anti-microbial activity by well diffusion method using *Punica granatum* peel extract
     - Anti-diabetic assay by alpha amylase method using *Punica granatum* peels extract.
     - Anti-inflammatory assay by protein denaturation method using *Punica granatum* peels extract.
     - Anti-helminthic assay of *Punica granatum* peels extract.
     - Anti-fungal activity by well diffusion method using *Punica granatum* peels extract.



CHAPTER : 3

REVIEW OF LITERATURE

3.0 Review of Literature

Pomegranate cultivation is widespread in tropical and subtropical regions like India, Iran, and Mediterranean countries. Although it is not endangered, challenges such as rising demand and environmental changes threaten its sustainability. Climate change and overexploitation for industrial purposes can negatively impact cultivation, while monoculture farming reduces genetic diversity. Sustainable farming practices and full utilization of by-products like the peel are critical for maintaining environmental balance *(Levin, 2006)*. Conservation strategies include promoting agroforestry to protect wild varieties and establishing seed banks and germplasm collections to preserve genetic diversity *(Mars, 2000)*.

Nutritionally, pomegranate peel is a rich source of bioactive compounds, making it beneficial for both dietary and medicinal uses. It contains polyphenols like tannins, flavonoids, and ellagic acid, which offer strong antioxidant benefits. Dietary fiber constitutes 16–20% of the peel's weight, aiding digestion. It is also rich in minerals like potassium, magnesium, and calcium, which support heart and bone health, as well as Vitamin C, which strengthens immunity *(Ismail et al., 2012)*.

Economically, pomegranate peel has significant potential in various industries, including pharmaceuticals, cosmetics, and agriculture, transforming agricultural waste into a valuable resource. In the pharmaceutical and nutraceutical sectors, bioactive compounds from the peel are used in supplements that combat inflammation, infections, and oxidative stress *(Negi & Jayaprakasha, 2003)*. In the food industry, the peel serves as a natural preservative and enhances baked goods and beverages with fiber and antioxidants *(Al-Zoreky, 2009)*. In cosmetics, the phenolic compounds in the peel are key ingredients in anti-aging and skin-lightening products *(Faria et al., 2007)*. Moreover, in agriculture and environmental applications, the peel serves as organic fertilizer, livestock feed, and a tool for treating wastewater by removing heavy metals *(Reddy et al., 2014)*.

Antimicrobial resistance (AMR) has emerged as a major global health concern, making the treatment of infections more difficult and leading to increased mortality and morbidity. AMR occurs when microorganisms such as bacteria, fungi, and viruses evolve mechanisms to resist the effects of antimicrobial agents, reducing the effectiveness of treatments like antibiotics, antifungals, and antivirals. This issue is aggravated by the overuse and misuse of antibiotics in both medical and agricultural settings, underscoring the importance of finding alternative antimicrobial agents. [*Negi,2003*]

Plant-derived antimicrobial agents are gaining attention due to their diverse bioactive compounds, which often target multiple microbial functions and are less likely to contribute to resistance. Punica granatum, commonly known as pomegranate, has shown promise as a source of antimicrobial agents, particularly from its peels, which are often discarded as agricultural waste. Recent studies have highlighted the antimicrobial potential of pomegranate peel extracts, making them an attractive alternative to synthetic antibiotics.

Pomegranate peel has gained attention as a natural antimicrobial agent, offering a potential solution to the growing issues of antibiotic resistance and food safety. Rich in components like tannins and flavonoids, it works by damaging microbial membranes and inhibiting enzymes. Gallic acid, a key compound in the peel, acts as both a bacteriostatic (prevents bacterial growth) and bactericidal (kills bacteria). It has been shown to be effective against bacteria such as *E. coli* and *Staphylococcus aureus*, fungi including *Candida albicans* and other pathogenic species, and possibly viruses, although more research is needed to confirm its antiviral properties *(Al-Zoreky, 2009).*

Pomegranate peels are rich in bioactive compounds such as tannins, flavonoids, ellagic acid, and punicalagins, which exhibit antimicrobial, antioxidant, and anti-inflammatory properties. These compounds play a significant role in the antimicrobial activity of pomegranate peel extracts. Tannins, known for their astringent properties, can bind to microbial cell walls and proteins, disrupting microbial cell integrity and inhibiting growth.

Pomegranate peel extracts exhibit strong antimicrobial activity against both Gram-positive and Gram-negative bacteria. Gram-positive bacteria are generally more susceptible to these extracts due to their simpler cell wall structure, which allows for easier penetration by antimicrobial compounds. However, studies have shown that pomegranate peel extracts can also inhibit the growth of certain Gram-negative bacteria, including *Escherichia coli*, at higher concentrations. In addition to bacterial activity, pomegranate peel extracts also show significant antifungal properties, particularly against common fungal pathogens such as *Candida albicans and Aspergillus niger*. These extracts disrupt fungal cell membranes and inhibit enzymes responsible for cell wall synthesis, making them effective in combating fungal infections. *[Al-Zoreky,2009]*

They are effective against a range of bacterial and fungal strains, including Gram-positive bacteria like *Staphylococcus aureus* and Gram-negative bacteria like *Escherichia coli.* Flavonoids such as quercetin and kaempferol possess antioxidant properties and also exhibit antimicrobial activity by interfering with microbial metabolism and cell structures. Ellagic acid, a polyphenolic compound, disrupts microbial cell membranes and inhibits enzymes responsible for cell wall synthesis, further contributing to its antimicrobial effects.

Punicalagins, unique to pomegranate peels, significantly enhance the antimicrobial and antioxidant properties of the extracts by disrupting microbial membranes and preventing pathogen growth. These bioactive compounds provide a broad-spectrum antimicrobial effect, making pomegranate peels a valuable resource in combating microbial infections.

In comparison to standard antimicrobial agents like penicillin and Miconazole, pomegranate peel extracts have demonstrated comparable or even superior antimicrobial activity. Penicillin, widely used for treating Gram-positive bacterial infections, has become less effective due to the rise of penicillin-resistant strains like *Staphylococcus aureus*. Pomegranate peel extracts offer a promising alternative or complementary treatment, particularly against resistant strains. Similarly, Miconazole, a common antifungal, is becoming less effective due to the development of resistance in fungal strains like *Candida albicans*. Pomegranate peel extracts have demonstrated potent antifungal activity, potentially serving as an alternative or adjunct to conventional antifungal therapies.

The antimicrobial activity is usually assessed through laboratory techniques like the Disc Diffusion Method, where a filter paper disc impregnated with the extract is placed on an agar plate inoculated with the target microorganism. A clear zone of inhibition around the disc indicates antimicrobial activity. The Minimum Inhibitory Concentration (MIC) test, which determines the lowest concentration of the extract that inhibits visible microbial growth, is also used to assess the potency of the extracts.

To evaluate the antimicrobial potential of pomegranate peel extracts, researchers typically test them against a range of clinically relevant microbial strains, including both bacterial and fungal pathogens. Common bacterial strains used in testing include *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Klebsiella pneumoniae,* while fungal strains such as *Candida albicans and Aspergillus niger* are also commonly tested.

Studies have shown that ethanol-based extracts exhibit superior antimicrobial activity compared to aqueous extracts due to the higher solubility of bioactive compounds in ethanol. Water-based extraction methods, while more eco-friendly, are less effective in extracting non-polar compounds like punicalagins. Proper storage of the extracts, such as through freeze-drying or refrigeration, is essential to preserve their bioactivity and prevent oxidative degradation.

The extraction of antimicrobial compounds from pomegranate peels depends on the efficiency of the extraction method used. Common methods include solvent extraction, supercritical fluid extraction, and cold pressing, with solvent extraction being the most widely used due to its simplicity and effectiveness. Polar solvents like ethanol and methanol are particularly effective in extracting phenolic compounds such as tannins, flavonoids, and ellagic acid.

The anti-inflammatory effects of pomegranate peel make it valuable in managing conditions like arthritis, heart disease, and metabolic disorders. The peel targets key pathways, such as inhibiting enzymes like COX-2 and lipoxygenase, thereby reducing inflammatory mediators like prostaglandins and leukotrienes. It also suppresses cytokines such as TNF-α and IL-6 and decreases reactive oxygen species (ROS) that exacerbate inflammation *(Shukla et al., 2008).*

Pomegranate peel also exhibits anticancer properties, thanks to its ability to inhibit tumor growth and promote the death of cancer cells. It induces apoptosis (programmed cell death), prevents angiogenesis (the formation of blood vessels that nourish tumors), and reduces metastasis, thereby limiting the spread of cancer cells *(Seeram et al., 2005).* Additionally, it has shown antidiabetic potential by lowering blood sugar levels and improving insulin sensitivity. It achieves this by inhibiting carbohydrate-digesting enzymes, protecting pancreatic beta cells from oxidative damage, and improving lipid profiles associated with diabetes *(Li et al., 2005).*

The antimicrobial properties of pomegranate peel extracts have diverse applications in both the food and healthcare industries. In the food industry, pomegranate peel extracts can be used as natural preservatives to extend the shelf life of perishable products by inhibiting microbial spoilage. They can also be incorporated into biodegradable packaging materials, providing an eco-friendly alternative to synthetic preservatives. In healthcare, pomegranate peel extracts have potential applications as topical treatments for skin infections, wounds, and fungal infections. Their anti-inflammatory properties make them useful for managing conditions like dermatitis and psoriasis. Oral supplements containing pomegranate peel extracts may also help prevent or treat gastrointestinal infections and promote gut health. [*Archana kumari, 2012*]

Although the antimicrobial potential of pomegranate peel extracts is promising, further research is needed to fully understand their clinical applications. Clinical trials are necessary to assess the safety, efficacy, and optimal dosing of pomegranate peel extracts in humans. In addition, more sustainable and environmentally friendly extraction methods, such as enzymatic or solvent-free techniques, should be developed to enhance the yield of bioactive compounds while reducing environmental impact. Investigating the synergistic effects of pomegranate peel extracts when combined with conventional antimicrobial agents could also help enhance their effectiveness and reduce the risk of resistance development.

In conclusion, pomegranate peels hold significant promise as a sustainable and effective alternative to conventional antimicrobial agents in the fight against antimicrobial resistance. With their rich content of bioactive compounds like tannins, flavonoids, ellagic acid, and punicalagins,

pomegranate peels offer broad-spectrum antimicrobial activity against both bacteria and fungi. Further research and optimization of extraction methods could enable pomegranate peel extracts to play a crucial role in combating AMR, contributing to the development of novel antimicrobial therapies in both healthcare and food industries. By integrating pomegranate peel extracts into pharmaceutical and industrial practices, we can reduce agricultural waste, promote sustainable healthcare solutions, and address the global challenge of antimicrobial resistance.

Future Directions:

- Synthesis of Silver Nanoparticles from *Punica granatum* peels extract.
- Antimicrobial testing of silver nanoparticles synthesized from *Punica granatum* peels extract.
- Applications of silver Nanoparticles from *Punica granatum* peels in Pharmaceutical and Cosmetics products.

CHAPTER : 4

METHODS AND MATERIAL

4.0 Methods and materials

4.1 Materials

Reagents :

1. Wagner's reagent:
    - Iodine: 1.27 g
    - Potassium iodide: 2 g
    - Distilled water: 100 ml
2. FeCl3 Solution:
    - 2gm Powder
    - Distilled water : 100 ml
3. Alkaline Reagent:
    - Sodium hydroxide solution: 10 %
    - Dilute acid (HCl) : Few drops
4. Lead acetate solution (10%):
    - Powder : 10 gm
    - Distilled water : 100 ml
5. Salkowski reagent :
    - Ferric chloride : 0.5 M
    - Perchloric acid : 35%
6. Molischs reagent :
    - Alpha naphthol : 5 gm
    - Ethanol : 100 ml
7. Benedict's reagent:
    - Sodium carbonate: 17.3 g
    - Sodium citrate: 17.3 g
    - Copper sulfate pentahydrate: 1.73 g
    - Distilled water: 100 ml
8. Ninhydrin reagent :
    - Ninhydrin : 0.2 gm
    - Acetone : 100 ml

9. Starch reagent :
    - Starch : 1 gm
    - Distilled water : 100 ml

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

Sabouraud's Dextrose Agar (SDA)

1. Peptone: 1.0 g

2. Dextrose (glucose): 4.0 g

3. Agar: 1.5 g

4. Distilled water: 100 mL

5. pH : Adjust to 5.6 ± 0.2

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.
2. Maceration.

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 two methods:

- Soxhlet Method
- Maceration 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:

The Soxhlet apparatus was first assembled according to the standard procedure. A powder column was prepared using filter paper, into which 10 g of Punica granatum peel powder was placed. Next, 160 ml of solvent (ethanol) was added to the thimble to dissolve the powder. 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. Approximately six cycles of the Soxhlet apparatus were run to ensure the proper extraction of phytochemicals.

- For Maceration Method we used Shaker or Shaker Incubator.

Procedure:

10 g of Punica granatum powder was weighed and dissolved in 100 ml of distilled water. The flask was covered with cotton and aluminium foil to prevent contamination. Efforts were made to ensure the powder was completely dissolved in the water. The flask was then incubated at room temperature for 2-3 days in a shaker.

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. The filtrate was collected and evaporated

using a boiling water bath. Once evaporation was complete, the extract was transferred to a closed container and stored at 4°C for further use.

Fig.4 Extraction of Punica granatum by Soxhlet method.

Fig.5 Extraction of Punica granatum by Maceration method.

Phytochemical Analysis

Qualitative analysis

1. Glycosides :
    - Killer killani test : (Cardiac glycosides) the test solution with few drops of glacial acetic acid in 2 ml of ferric chloride solution and concentrated sulphuric acid is added from the sides of the test tube which shows the separation between two layers, lower layer shows reddish brown and upper layer turns bluish green in colour.
2. Alkaloids:
    - **Wagners test :** The acidic test solution treated with Wagner's reagent (lodine in potassium iodide) gives brown precipitate.
3. Flavonoids:
    - **Ferric chloride test :** The test solution with few drops of ferric chloride solution shows intense green colour.
    - **Shinoda test:** Test solution with few fragments of magnesium ribbon and concentrated hydrochloric acid shows pink to magenta red colour.
    - **Zinc-hydrochloric Acid reduction test:** Test solution with zinc dust and few drops of hydrochloric acid shows magenta red colour.
    - **Alkaline reagent test:** Test solution when treated with sodium hydroxide solution shows increase in the intensity of yellow colour which becomes colourless on addition of few drops of dilute acid.
    - **Lead acetate test:** Test solution with few drops of lead acetate solution (10% w/v) gives yellow precipitate.
4. Steroid:
    - **Salkowskis test**: The second portion of solution above was mixed with concentrated sulphuric acid carefully so that the acid formed a lower layer and the interface was observed for a reddish-brown colour indicative of steroid ring.

5. Carbohydrate:
    - **Molishs test:** Test solution with few drops of Molisch's reagent and two ml of concentrated sulphuric acid added slowly from the sides of the test tube shows a purple ring at the junction of two liquids.
    - **Benedicts test:** Test solution treated with Benedict's reagent and boiling on a water bath shows reddish brown precipitate.
6. Protein:
    - **Xanthoproteic test:** Test solution treated with concentrated nitric acid and on boiling gives yellow precipitate.
    - **Ninhydrin test:** Test solution treated with ninhydrin reagent gives blue colour.
7. Starch:
    - **Starch reagent test:** Iml of of extract was added into 10ml of Nacl solution. After heating, starch reagent was added a blue purplish colour is a positive test for the presence of starch.

8. Tannins:
    - **Gelatin test:** Plant Extract is dissolved in 5ml of distilled water and 1% gelatin solution and 10% Nacl. Reaction gives a white precipitate.
    - **NaOH test:** 4 ml of 10% NaoH added into the 0.4ml of extract and shaken well formation of emulsion.

QUANTITATIVE ANALYSIS OF PHYTOCONSTITUENTS

Estimation of Total Phenol by FCR Method using Plant Punica granatum .

Aim:

To estimate the Total Phenolic content by FCR method.

Principle:

The estimation of total phenols typically involves using a colorimetric method, such as the Folin-Ciocalteu assay. In this method, phenolic compounds react with the Folin-Ciocalteu reagent, forming a blue complex. The intensity of the color is proportional to the concentration of phenols and can be measured spectrophotometrically. Calibration with known standards helps quantify the total phenolic content in the sample.

Reagent preparation:

1. Plant extract.
2. Folin-Ciocalteu reagent.
3. 10% Sodium carbonate.
4. Standard-Gallic acid.

Procedure:

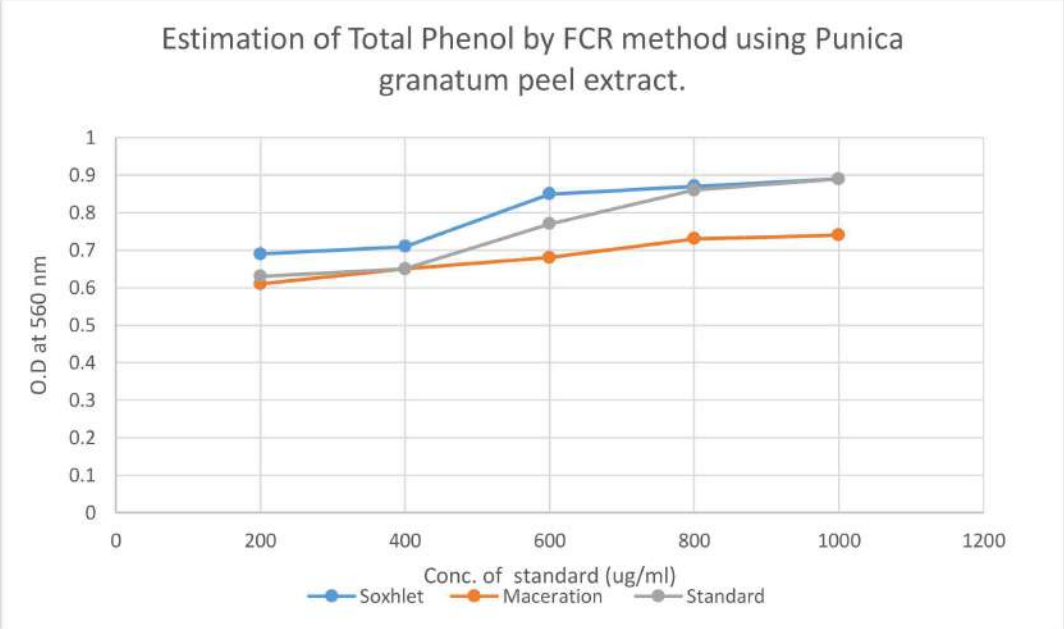
1. The reaction mixture was prepared by mixing 1 ml of fruit peel extract with 9 ml of distilled water.
2. 1 ml of Folin reagent was added to the reaction mixture.
3. The flasks were incubated for 5 minutes at room temperature.
4. After incubation, 10 ml of 10% sodium carbonate was added to the reaction mixture.
5. The flasks were further incubated for 90 minutes at room temperature.
6. After the incubation period, the absorbance was measured at 560 nm.

7. A standard curve was plotted on a graph.

OBSERVATION TABLE:

Concentration (microgram/ml)	**Maceration O.D. at 560nm**	**Soxhlet O.D at 560nm**	**Standard O.D at 560nm**
200	0.61	0.69	0.63
400	0.65	0.71	0.65
600	0.68	0.85	0.77
800	0.73	0.87	0.86
1000	0.74	0.89	0.89

Graph:



BIOMEDICAL APPLICATIONS:

Anti-Inflammatory assay by protein denaturation method using Plant

Punica granatum.

Aim:

To evaluate the anti-inflammatory activity of the given extract by protein denaturation method.

Principle:

The protein denaturation method is often used to assess anti-inflammatory activity. In this assay, the principle revolves around the prevention of protein denaturation, which is a key step in the inflammatory process. Proteins denature during inflammation, leading to structural changes.In the anti-inflammatory assay, a sample is tested to determine its ability to inhibit the denaturation of a protein, often using a standard protein like albumin. The degree of protein denaturation is assessed by measuring changes in turbidity or absorbance. A lower absorbance or turbidity in the presence of the sample indicates its potential anti-inflammatory activity, as it suggests protection against protein denaturation that occurs during inflammation.

Reagents preparation:

1) Egg albumin & PBS (Phosphate buffer saline)

2) Standard-Diclofenac sodium

Procedure:

1. The reaction mixture was prepared by combining 0.4 ml of egg albumin (collected from a fresh hen's egg) with 5.6% phosphate-buffered saline (PBS).

2. 1 ml of plant extract and standard Diclofenac sodium were then added to the reaction mixture.

3. The mixture was incubated for 30 minutes at 70°C.

4. After incubation, the absorbance of the reaction mixture was measured at 620 nm.

Observation Table :

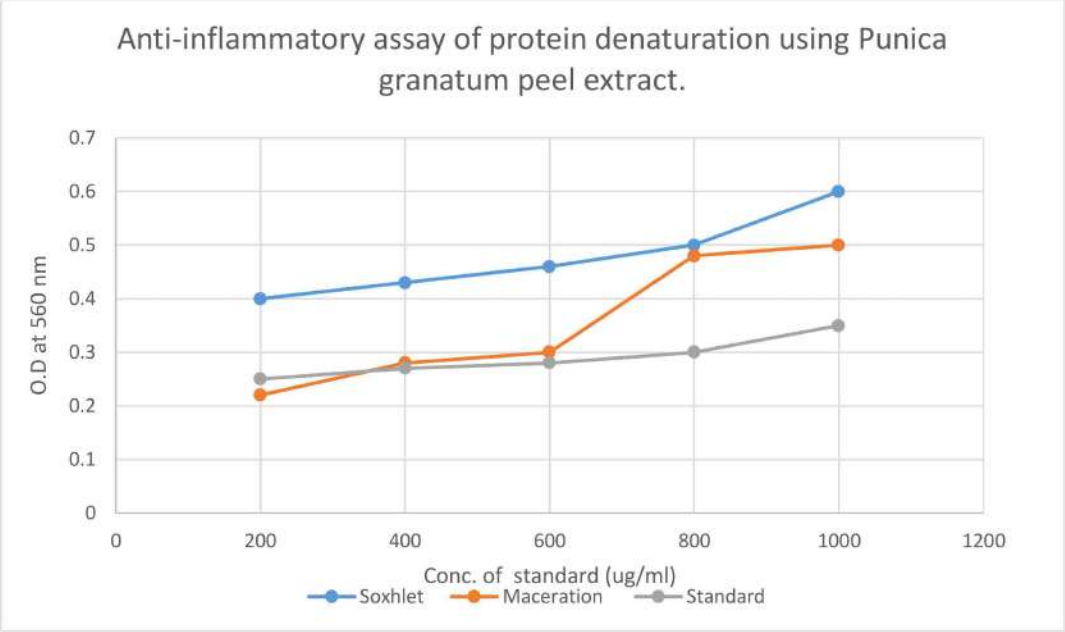
Concentration (mg/ml)	Maceration O.D at 620nm	Soxhlet O.D at 620nm	Standard O.D at 620nm
200	0.22	0.40	0.25
400	0.28	0.43	0.27
600	0.30	0.46	0.28
800	0.48	0.50	0.30
1000	0.50	0.60	0.35

Formula:

% OF INHIBITION =O.D. OF CONTROL-O.D. OF TEST/O.D. OF CONTROL*100

Concentration (mg/ml)	Maceration % of inhibition	Soxhlet % of inhibition	Standard % of inhibition
200	82.25%	67.74%	79.83%
400	77.41%	65.32%	78.22%
600	75.80%	62.90%	77.41%
800	61.29%	59.67%	75.80%
1000	59.67%	51.61%	71.77%

Graph:


Anti-inflammatory assay of protein denaturation using Punica granatum peel extract.
O.D at 560 nm
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
200
400
600
800
1000
1200
Conc. of standard (ug/ml)
Soxhlet
Maceration
Standard

Anti-diabetic Assay by Alpha- Amylase Method using Plant Punica granatum.

An antidiabetic assay using the alpha-amylase method is designed to evaluate the potential of substances to inhibit the enzyme alpha-amylase, crucial in carbohydrate digestion. Inhibition of alpha-amylase can help regulate glucose levels, making this assay valuable in identifying compounds with anti-diabetic properties. The assessment often involves measuring the inhibition's impact on the breakdown of starch, providing insights into a substance's ability to modulate postprandial glucose levels, a key aspect in diabetes management.

Aim:

To perform anti- diabetic activity by alpha amylase method.

Principle:

In the alpha-amylase inhibition assay, the principle lies in assessing the ability of a substance to inhibit the activity of the enzyme alpha-amylase. Alpha-amylase is involved in the breakdown of complex carbohydrates into simpler sugars, including glucose. Inhibition of this enzyme can help regulate postprandial glucose levels, making it relevant to anti-diabetic research.The degree of inhibition is often measured by assessing the reduced formation of reducing sugars, typically through colorimetric or spectrophotometric methods. A lower absorbance or color development indicates a higher level of inhibition, suggesting potential anti-diabetic properties by regulating carbohydrate digestion.

Reagent preparation:

1) Plant extract
2) DNSA
3) Test sample 0.5%Alpha amylase
4) 1% starch.

Procedure:

1. The reaction mixture was prepared by combining 0.5 ml of peel extract or standard Acarbose with 0.5 ml of α-amylase (0.5%).
2. 1% starch was then added to the prepared reaction mixture.

3. The mixture was incubated at room temperature for 30 minutes.
4. Subsequently, 2 ml of DNSA was added to the reaction mixture.
5. The mixture was incubated in a boiling water bath at 100°C for 15 minutes.
6. After incubation, the absorbance of the reaction mixture was measured at 620 nm.

Observation Table:

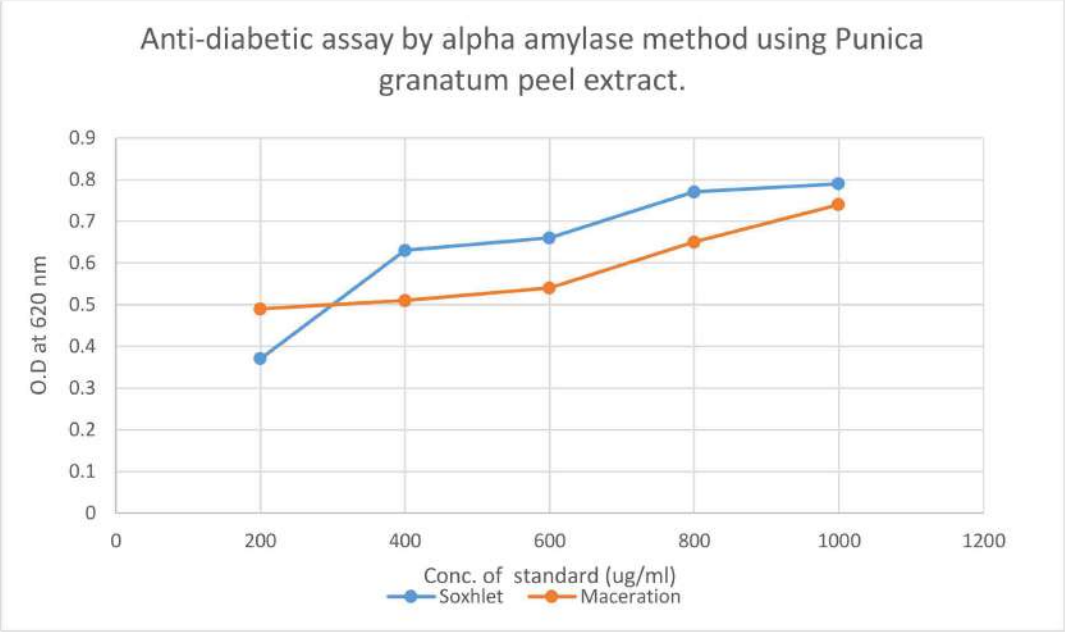
Concentraion (mg/ml)	Maceration O.D at 620nm	Soxhlet O.D at 620nm
200	0.49	0.37
400	0.51	0.63
600	0.54	0.66
800	0.65	0.77
1000	0.74	0.79

Formula:

% OF INHIBITION =O.D. OF CONTROL-O.D. OF TEST/O.D. OF CONTROL*100

Concentraion (mg/ml)	Maceration % of inhibition	Soxhlet % of inhibition
200	68.18%	75.97%
400	66.88%	59.09%
600	64.93%	57.14%
800	57.79%	50.00%
1000	51.94%	48.70%

Graph:


Anti-diabetic assay by alpha amylase method using Punica granatum peel extract.
O.D at 620 nm
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
200
400
600
800
1000
1200
Conc. of standard (ug/ml)
Soxhlet
Maceration

Anti-Helmenthic Assay of Punica granatum.

An antihelminthic assay is a laboratory test conducted to evaluate the effectiveness of substances in combating parasitic worm infections. This assay typically involves exposing the worms to the test substance and assessing parameters such as viability, motility, and reproductive capacity. The results aid in identifying potential agents with anti-parasitic properties, crucial for developing treatments against helminthic infections in both animals and humans.

Aim :

To estimate the anti-helmenthic activity of *Punica granatum.*

Principle :

The principle of an antihelminthic assay involves evaluating the ability of a substance to inhibit the survival, growth, or reproduction of parasitic worms, known as helminths. This assay is designed to identify potential agents with anti-parasitic properties. Typically, the process includes exposing the worms to the test substance and assessing parameters like mortality, motility, and reproductive capabilities. The degree of inhibition or damage to the helminths provides valuable insights into the efficacy of the tested compound as a potential treatment for parasitic worm infections.

Reagents :

1. Plant extract
2. Standard – Albendazole

Procedure:

1. Take two petri plates. Clean the petri plates and air dry them.

2. Collect the earthworms and wash them with water to remove any unwanted soil and dust.

3. Place five earthworms in each petri plate and close the plates using the petri lid.

4. Load the drug (Albendazole) and sample in separate petri plates and note the time of paralysis and the time of death.

Observation Table :

	Maceration	Soxhlet	Standard
Paralysis Time	30 sec.	28 sec.	10min.
Death Time	37 sec.	35 sec .	13min.

Anti-Bacterial Acitvity by Well Diffusion Method using Plant Punica granatum.

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) Plant extract

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)		
		Soxhlet	**Maceration**	**Standard(Penicillin)**
1	*Klebsiella pneumoniae*	18 mm	20 mm	-
2	*Pseudomonas aeruginosa*	-	-	-
3	*Escherichia coli*	-	-	17 mm
4	*Staphylococcus aureus*	15 mm	13 mm	-

Anti-Fungal Activity by Well Diffusion Method using Plant Punica granatum.

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) Plant extract

2) Standard – Miconazole

Procedure:

1. Prepare the Sabourad's 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 Sabourad's 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)		
		Soxhlet	**Maceration**	**Standard(Miconazole)**
1	*Aspergillus niger*	-	-	15 mm
2	*Candida albicans*	-	-	-

CHAPTER : 5

RESULT AND DISCUSSION

5.0 RESULTS AND DISCUSSION

5.1 Phytochemicals analysis : 1.0 Qualitative analysis.

Sr.no	Name of compound	Name of Test	Observation	Present or Absent	
				Soxhlet	Maceration
1.	Glycosides	1)Killer killani test	Brown and green colour present	Present	Present
2.	Alkaloids	1)Wagners test	Brown precipitate formed	Present	Present
3.	Flavonoids	1)Ferric chloride test	Intense green colour formed	Present	Present
		2)Shinoda test	No colour formation	Absent	Absent
		3)Zinc hydrochloric acid reduction test	No colour formation	Absent	Absent
		4)Alkaline reagent test	Yellow colour formed	Present	Present
		5)Lead acetate test	Yellow precipitate formed	Present	Present
4.	Steroids	1)Salkowski's test	Reddish brown colour formed	Present	Present
5.	Carbohydrates	1)Molisch test	Purple ring appeared	Present	Present
		2)Benedict's test	Reddish brown precipitate formed	Present	Present
6.	Protein	1)Xantho proteic test	No precipitation	Absent	Absent
		2)Ninhydrin test	No colour formation	Absent	Absent
7.	Starch	1)Starch reagent test	No colour formation	Absent	Absent
8.	Tannin	1)Gelatin test	No precipitation	Absent	Absent
		2)NaOH test	Formation of emulsion	Present	Present

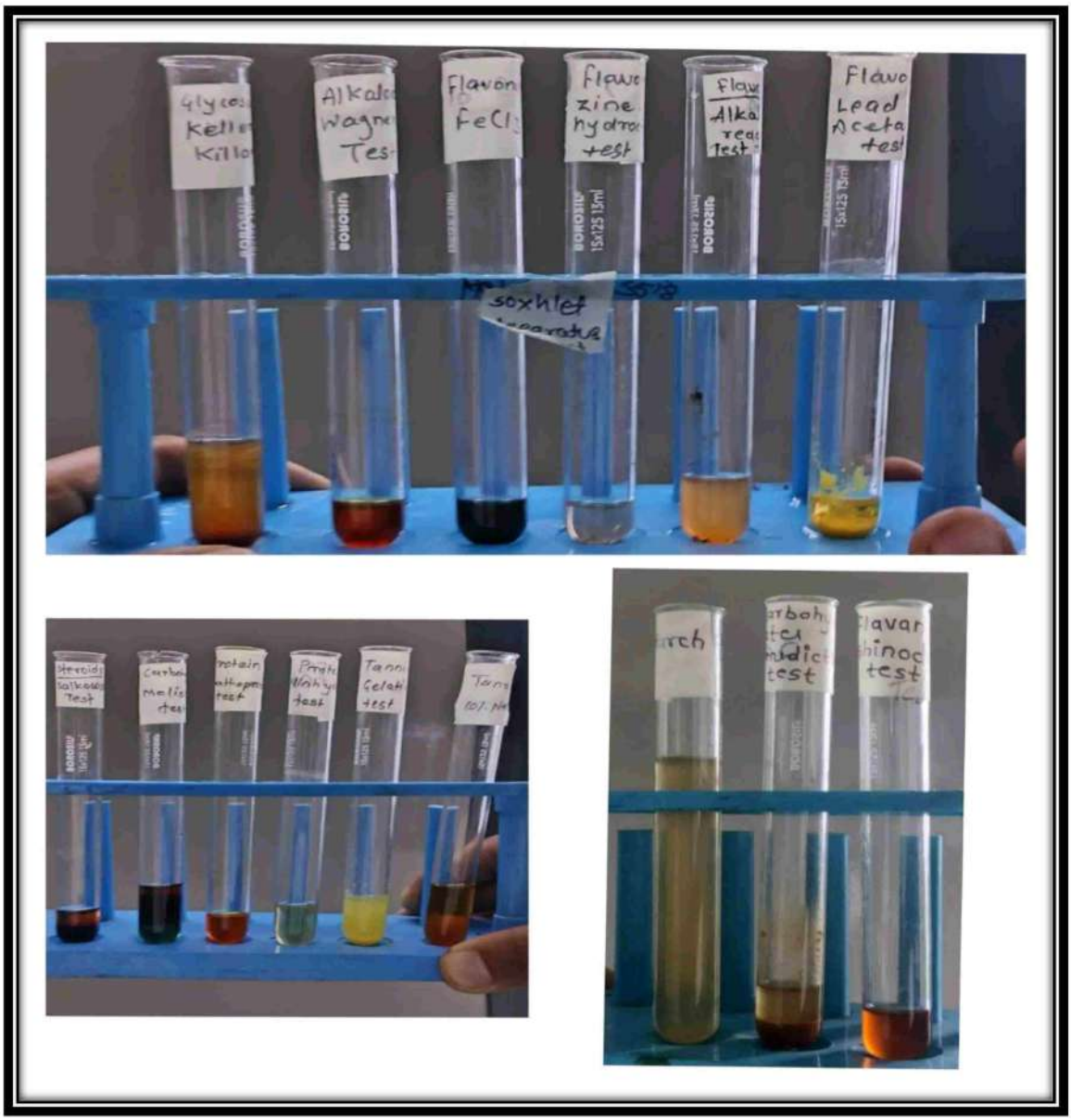


Fig.6 Phytochemical analysis by Soxhlet method using Punica granatum peels extract.

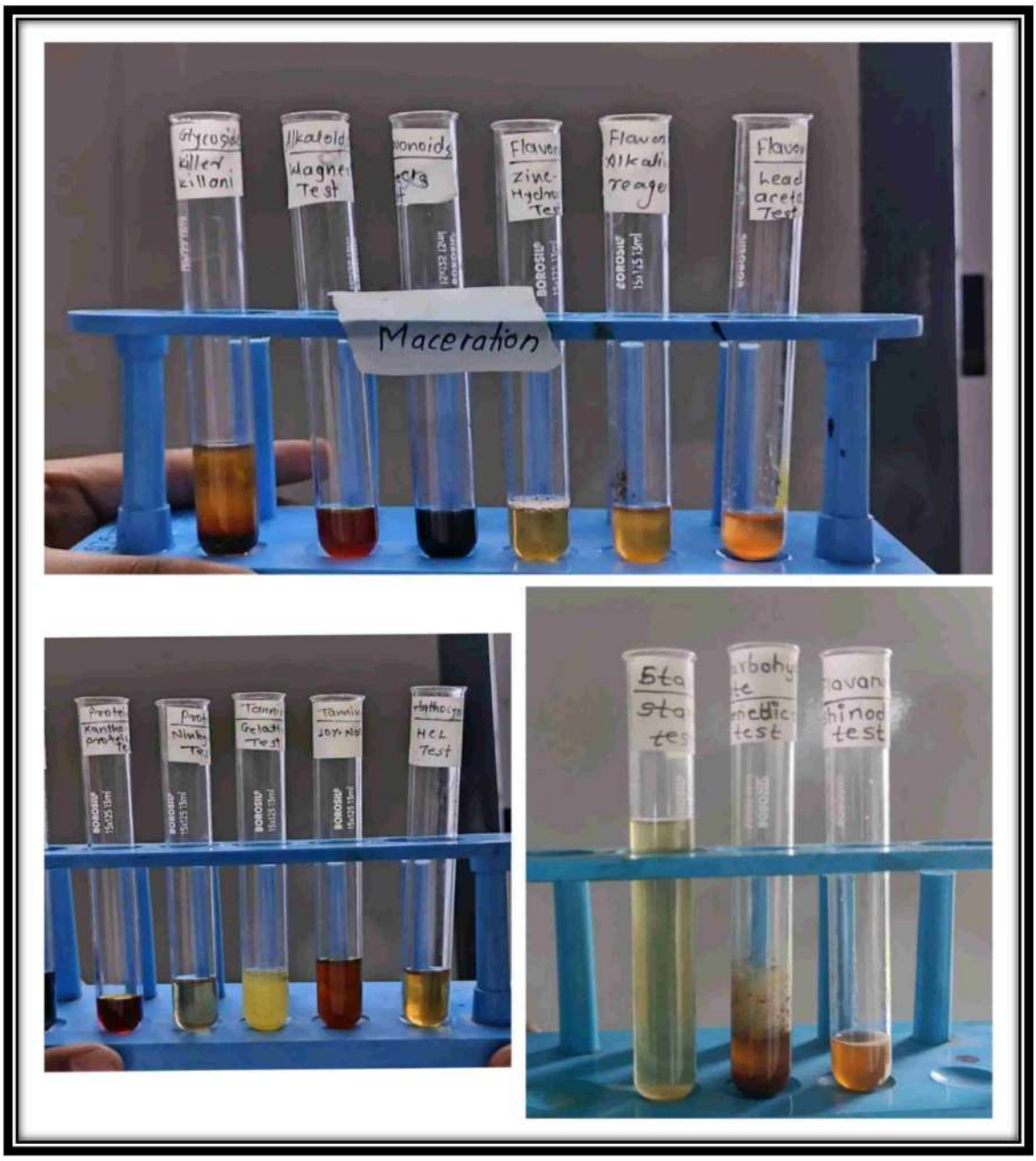


Fig.7 Phytochemical analysis by Maceration method using Punica granatum peels extract.

1.1 Quantitative analysis:

Estimation of Total Phenol by FCR method using *Punica granatum* peels extract.

Result:

Total Phenol by FCR method using *Punica granatum* peels extract was estimated.

Fig. 8 Estimation of total phenol of Punica granatum peel extract by using soxhlet apparatus.

Fig.9 Estimation of total phenol of Punica granatum peel extract by using Maceration method.

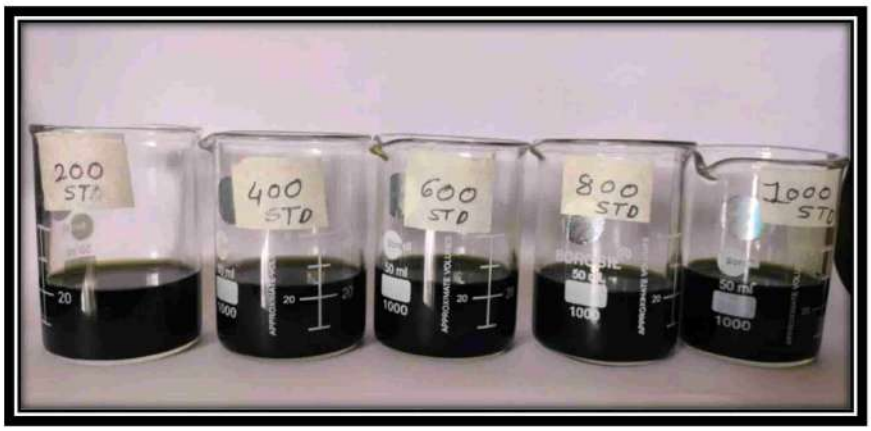


Fig.10 estimation of total phenol of Punica granatum peel extract by using standard Gallic acid.

5.2 Biomedical applications:

1.1 Anti-inflammatory assay by protein denaturation method using *Punica granatum* peels extract.

Result:

Anti-inflammatory assay of *Punica granatum* peel extract by protein denaturation method was performed.

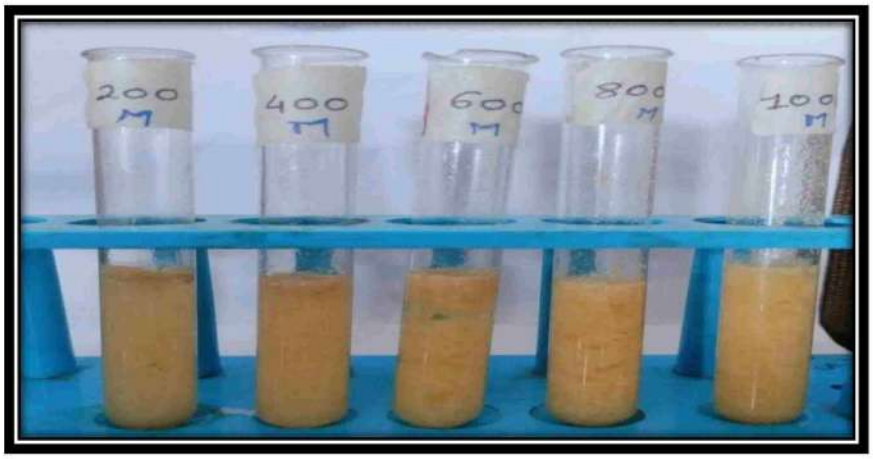


Fig.11 Anti-inflammatory assay by protein denaturation method of Punica granatum peel extract by using Maceration method.

Fig.12 Anti-inflammatory assay by protein denaturation method of Punica granatum peel extract by using Soxhlet method.

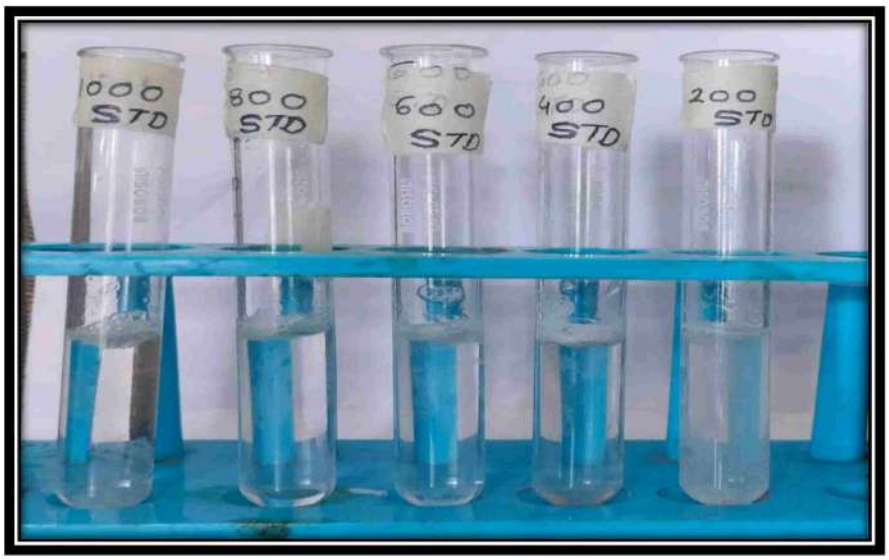


Fig.13 Anti-inflammatory assay by protein denaturation method of Punica granatum peel extract using standard Diclofenac sodium.

1.2 Anti-diabetic assay by alpha amylase method using Punica granatum peels extract.

Result :

Anti-diabetic assay of *Punica granatum* peel extract by alpha amylase method was performed.

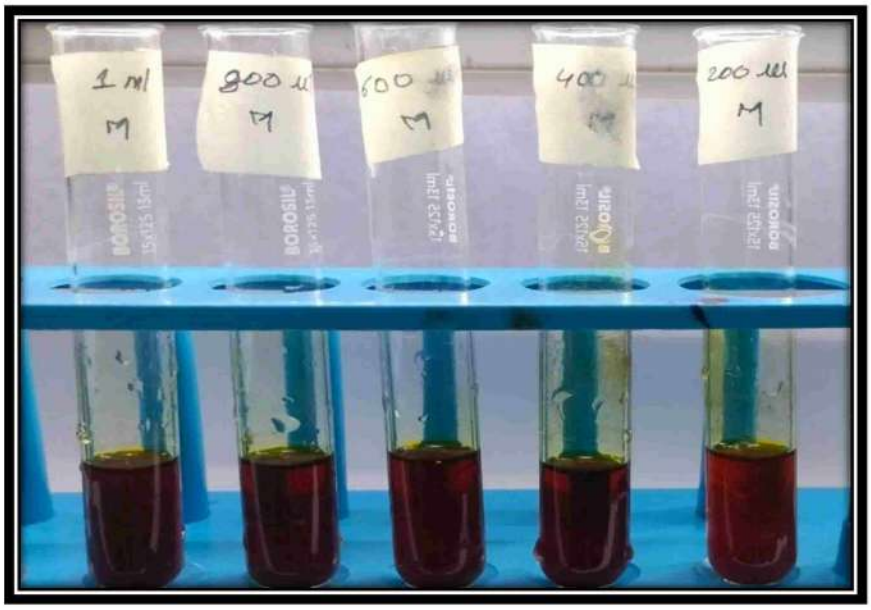


Fig.14 Anti-diabetic Assay by Alpha- Amylase Method using Punica granatum peel extract done by Maceration method.

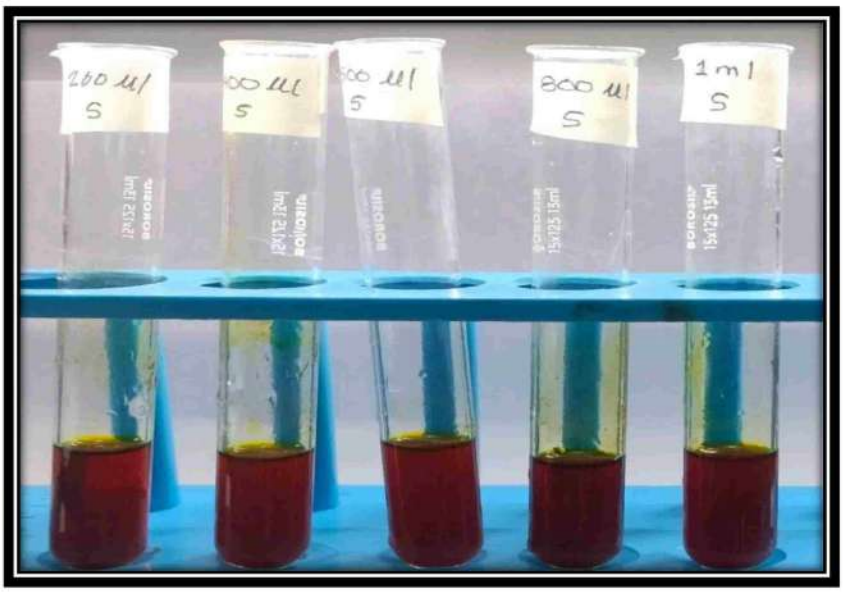


Fig.15 Anti-diabetic Assay by Alpha- Amylase Method using Punica granatum peel extract done by Soxhlet method.

1.3 Anti-helminthic assay by Punica granatum peel extract.

Result :

Anti-helminthic activity of *Punica granatum* peel extract was estimated.

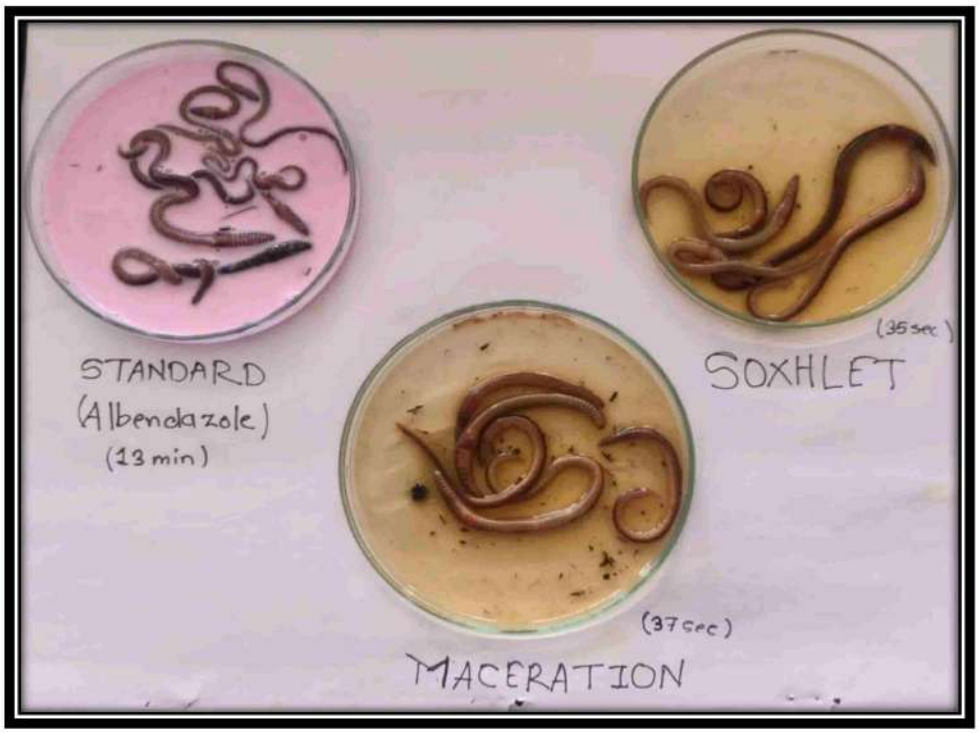


Fig16. Anti-helminthic assay by Punica granatum peels extract.

1.4 Anti-bacterial activity by well diffusion method using *Punica granatum* peels extract.

Result :

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

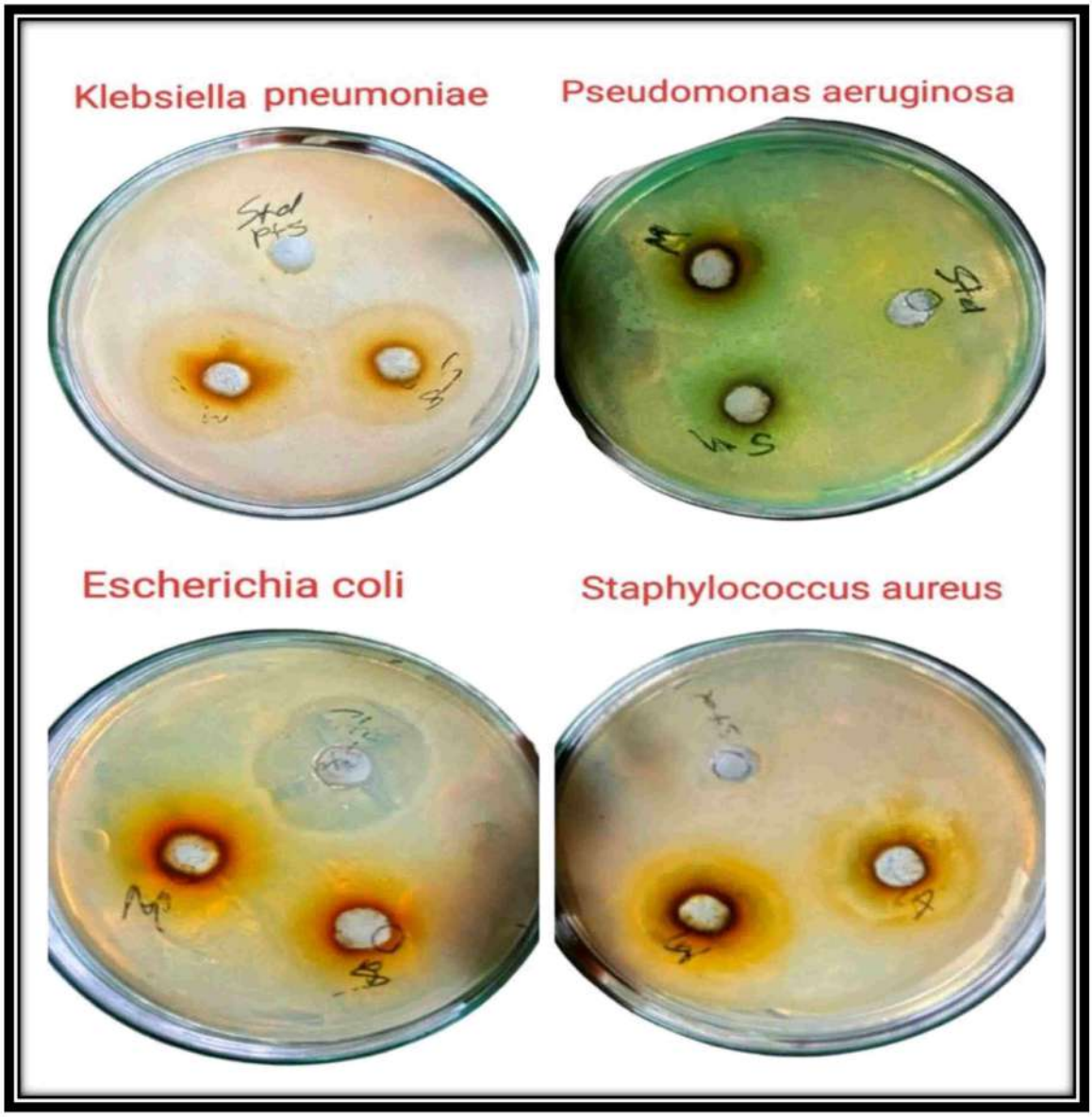


Fig.17 Anti-Bacterial Acitvity by Well Diffusion Method using Punica granatum peel extract.

(Klebsiella pneumoniae , Pseudomonas aeruginosa , Escherichia coli , Staphylococcus aureus.)

Observation Table:

Sr. No	Name of Organisms	Zone of inhibition (diameter in mm)		
		Soxhlet	**Maceration**	**Standard(Penicillin)**
1	*Klebsiella pneumoniae*	18 mm	20 mm	-
2	*Pseudomonas aeruginosa*	-	-	-
3	*Escherichia coli*	-	-	17 mm
4	*Staphylococcus aureus*	15 mm	13 mm	-

1.5 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.

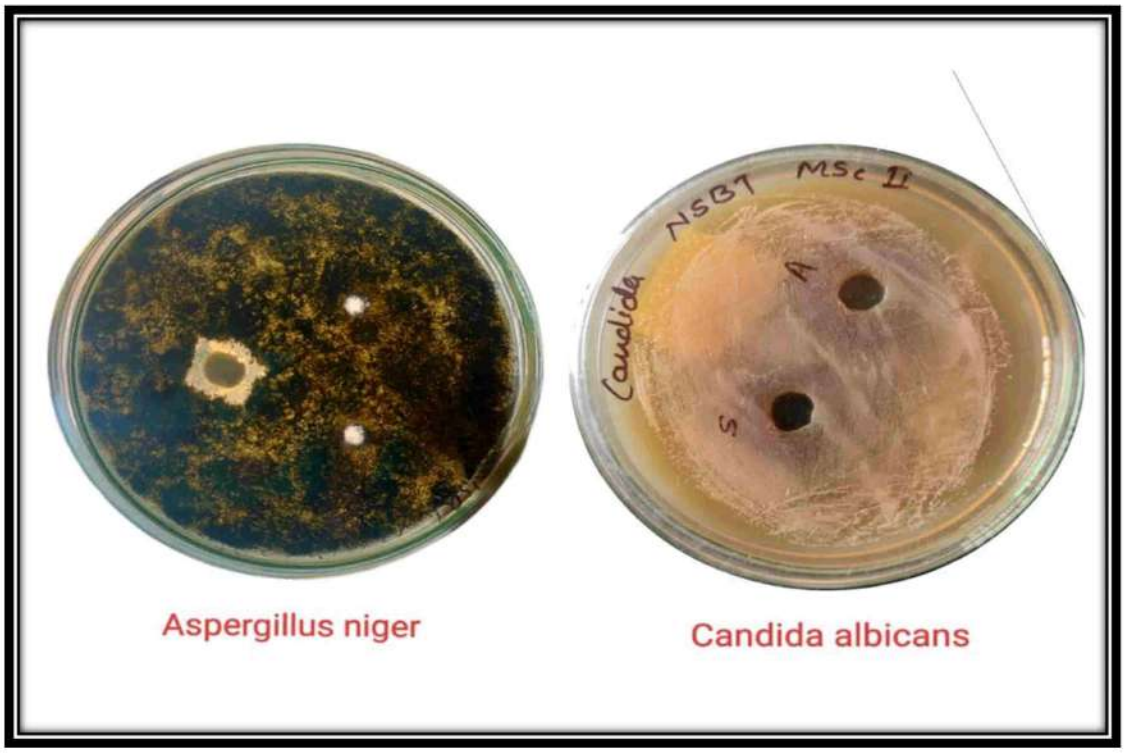
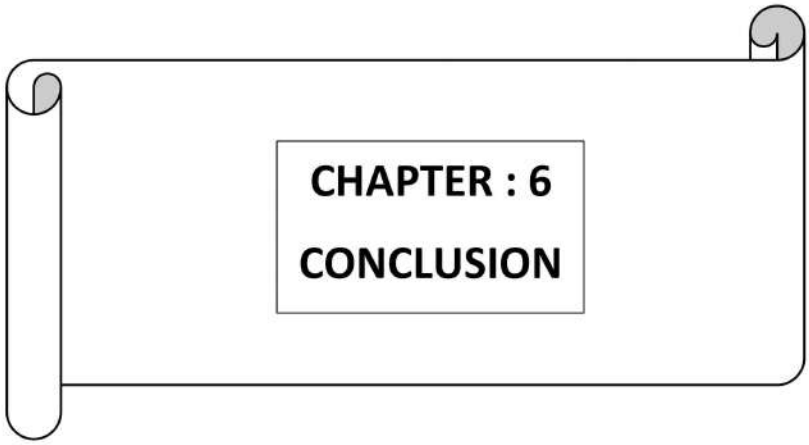


Fig.18 Anti-Fungal Activity by Well Diffusion Method using Punica granatum peel extract.

(Aspergillus niger , Candida albicans.)

Observation Table:

Sr. No	Name of Organisms	Zone of inhibition (diameter in mm)		
		Soxhlet	**Maceration**	**Standard(Miconazole)**
1	***Aspergillus niger***	-	-	***15 mm***
2	***Candida albicans***	-	-	-



CHAPTER : 6

CONCLUSION

6.0 CONCLUSION

The research on Punica granatum (pomegranate) focused on its medicinal and nutritional significance, particularly the bioactive properties of its peel. Often discarded as a by-product, pomegranate peel contains high levels of phytochemicals such as glycosides, flavonoids, tannins, and polyphenols, known for their biological activities. The study explored its potential to address contemporary health challenges, including antimicrobial resistance, inflammatory disorders, diabetes, parasitic infections, and fungal diseases. Through qualitative and quantitative analyses, the peel was found to be rich in phenolic compounds, as confirmed by the Folin-Ciocalteu assay. These compounds were effectively extracted using Soxhlet and maceration methods, with some differences in yield and concentration.

The phytochemical analysis identified glycosides, alkaloids, flavonoids, tannins, carbohydrates, and steroids, each contributing to the peel's bioactivity. Glycosides were identified via the Keller-Killani test, while alkaloids and flavonoids were detected using Wagner's and other chemical assays. These compounds exhibited significant antioxidant, anti-inflammatory, and antimicrobial properties. The biomedical applications of the peel extract were highlighted through various assays. Anti-inflammatory potential was assessed using the protein denaturation method, where the extract showed inhibition comparable to Diclofenac sodium, indicating its efficacy in managing arthritis and chronic inflammatory diseases. The alpha-amylase inhibition assay demonstrated its anti-diabetic properties, as the extract reduced postprandial glucose spikes, making it a potential natural alternative to synthetic anti-diabetic agents. The anti-helminthic activity of pomegranate peel extract, demonstrated by its ability to induce rapid paralysis and death in earthworms, highlights its efficacy as a natural remedy for gastrointestinal parasitic infections. The presence of tannins and alkaloids, which disrupt neuromuscular functions and energy production in worms, underscores its potential as an eco-friendly and sustainable alternative to synthetic anti-parasitic agents.

The study also evaluated the antimicrobial activity of the peel extract through the well-diffusion method. It exhibited significant activity against bacterial pathogens like *Klebsiella pneumoniae*

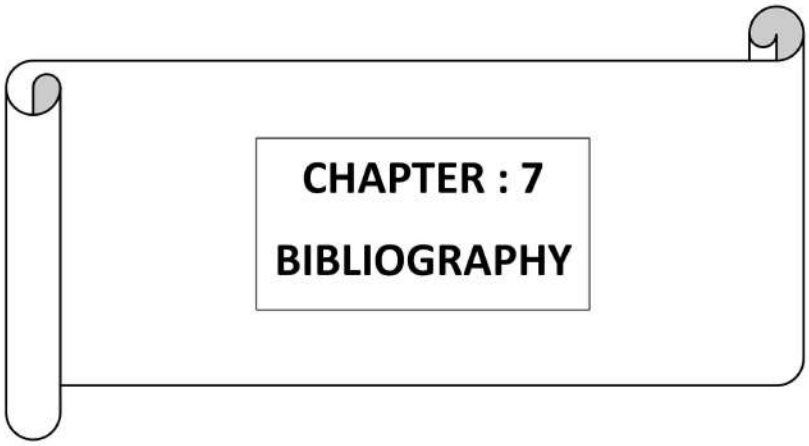
and Staphylococcus aureus, with inhibition zones comparable to standard antibiotics, though its effect on *Escherichia coli and Pseudomonas aeruginosa* was limited. Antifungal activity against *Aspergillus niger and Candida albicans* was less pronounced, pointing to a need for further optimization. These antimicrobial effects were attributed to compounds like punicalagins, ellagic acid, and flavonoids, which disrupt microbial cell walls and inhibit enzyme activity.

The extraction methods used—Soxhlet and maceration—demonstrated the versatility of the peel extract. Soxhlet extraction yielded a higher concentration of bioactive compounds due to continuous solvent reflux, while maceration was simpler and more cost-effective. The findings emphasize the peel's potential as a natural antimicrobial agent, anti-inflammatory and anti-diabetic supplement, food preservative, and cosmetic ingredient, leveraging its antioxidant and bioactive properties. However, the research also highlighted challenges and future directions. Optimizing extraction techniques, conducting clinical trials, exploring synergistic effects with standard drugs, and investigating nanoparticle synthesis could enhance the extract's applications in pharmaceuticals and cosmetics.

In conclusion, *Punica granatum* peel extracts demonstrate significant potential for various biomedical and industrial uses. Rich in diverse bioactive compounds, they offer sustainable and effective alternatives to synthetic drugs for managing infections, inflammation, and metabolic disorders. By repurposing agricultural waste, the study provides an eco-friendly approach while adding economic value to pomegranate cultivation. With further research and development, pomegranate peel extracts could play a crucial role in addressing global health challenges, particularly antimicrobial resistance and chronic disease management

Future Directions:

- Synthesis of Silver Nanoparticles from Punica granatum peels extract.
- Antimicrobial testing of silver nanoparticles synthesized from Punica granatum peels extract.
- Applications of silver Nanoparticles from Punica granatum peels in Pharmaceutical and Cosmetics products.



CHAPTER : 7

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