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PROJECT TITLE: COMPARATIVE STUDY OF ANTIMICROBIAL PROPERTIES OF BLACK CUMIN AND POMEGRANATE PEEL EXTRACT AGAINST DIFFERENT MICROORGANISMS

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2. INTRODUCTION

Microorganisms are present everywhere—in soil, water, air, and even inside our bodies. While some microorganisms are useful, others cause diseases and infections. For many years, antibiotics have been used to kill harmful bacteria and prevent infections. However, because of overuse and misuse of these antibiotics, many microorganisms have developed resistance. This means the medicines no longer work effectively. This growing problem, known as antibiotic resistance, is now one of the most serious global health issues.

This inspired me to think about safer, natural alternatives. Plants have been used in traditional medicine for thousands of years. They contain many active compounds that can fight harmful microbes naturally without harming the environment. These natural antimicrobial agents are biodegradable, renewable, and non-toxic.

In this project, I studied two easily available plant materials: black cumin seeds and pomegranate peels. Black cumin, with the scientific name *Nigella sativa*, is known for its strong medicinal properties. It contains thymoquinone, alkaloids, and flavonoids—compounds that can break down the cell walls of bacteria and stop their multiplication.

Pomegranate peel, *Punica granatum*, is usually thrown away as waste after eating the fruit. However, research has shown that the peel is very rich in antioxidants, tannins, flavonoids, and polyphenols. These compounds are known to have antimicrobial and anti-inflammatory properties. Instead of wasting it, we can use it to make natural antimicrobial agents.

The aim of this project is to compare the antimicrobial properties of black cumin and pomegranate peel extracts against different microorganisms to determine which one is more effective.

Purpose of the project:

The purpose of a project comparing the antibacterial properties of black cumin and pomegranate extract is to identify and evaluate the effectiveness of natural compounds as alternatives to synthetic antibiotics, which are increasingly becoming less effective due to widespread antimicrobial resistance. This comparative study would reveal which plant source offers superior antibacterial potential against various pathogens, paving the way for the development of new, naturally derived antimicrobial agents for healthcare and food safety applications.

Selection of Problem and Background Information:

Pomegranate Peel Extract (PPE)

- **Source:** The peel of the *Punica granatum* L. fruit, which accounts for up to 50% of the fruit's total weight.
- **Active Compounds:** The primary active compounds are hydrolyzable tannins, especially **punicalagin**, ellagic acid, and other phenolic compounds. The concentration of these compounds is typically higher in the peel than in the pulp or seeds.
- **Traditional Uses:** Historically used in Roman, Middle Eastern, and Ayurvedic medicine as an anthelmintic, anti-inflammatory agent, and for treating dysentery and oral infections.
- **Modern Research:** Confirmed to have potent, broad-spectrum activity against Gram-positive (e.g., *Staphylococcus aureus*, MRSA) and Gram-negative bacteria (e.g., *E. coli*, *P. aeruginosa*), as well as fungi (*Candida albicans*). Its antimicrobial action involves disrupting bacterial cell membranes and inhibiting enzyme activity.

Black Cumin Extract (BCE)

- **Source:** Seeds of the *Nigella sativa* plant.
- **Active Compounds:** The main active component is **thymoquinone** (TQ), along with other volatile oils like p-cymene and carvacrol.

- **Traditional Uses:** Widely used in various traditional systems to treat a wide range of conditions, including respiratory problems, inflammation, and infections.
- **Modern Research:** Exhibits significant antimicrobial, antioxidant, and anti-inflammatory properties. It is effective against several bacterial strains, though some studies suggest it may be less potent than PPE against certain Gram-negative bacteria when tested at similar concentrations. Its mechanism also involves damaging cell membranes and inhibiting virulence factors.

Research Question:

Which natural extract—black cumin or pomegranate peel—shows stronger antimicrobial activity against microorganisms such as *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*?

Hypothesis:

The pomegranate peel extract will show stronger antimicrobial properties because it contains a higher concentration of polyphenols and tannins, which can destroy microbial cells more efficiently.

This study also relates to Environmental Science because it encourages the use of natural and eco-friendly substances instead of harmful synthetic chemicals. By using plant-based extracts, we can reduce environmental pollution, make use of biodegradable materials, and promote sustainability.

Abstract

This comparative study investigated the antimicrobial properties of pomegranate peel extract (PPE) and black cumin extract (BCE) against various pathogenic bacteria and fungi. The objective was to evaluate and compare their efficacy as natural antimicrobial agents, particularly in the context of rising antibiotic resistance.

Both extracts demonstrated significant, broad-spectrum antimicrobial activity. PPE is rich in polyphenols and hydrolyzable tannins, especially **punicalagin**, which disrupt bacterial cell membranes and inhibit microbial enzymes. BCE is primarily effective due to its active component, **thymoquinone** (TQ), which also damages cell structure and interferes with essential cell processes.

Comparative analyses consistently indicated that **pomegranate peel extract exhibits more potent antimicrobial effects** than black cumin extract when tested at similar concentrations. For instance, in one study using methanolic extracts, PPE produced significantly larger inhibition zones against *Bacillus cereus* (20.5 mm vs. 12.2 mm) and *E. coli* (12.5 mm vs. 8.6 mm) compared to BCE. PPE was particularly effective against Gram-positive bacteria like *Staphylococcus aureus* (including MRSA strains) and *Listeria monocytogenes*, often showing superior activity to conventional antibiotics like tetracycline and chloramphenicol in *in vitro* tests. While BCE also showed good activity, particularly against Gram-positive bacteria, its overall efficacy was generally lower in direct comparisons.

The variation in results depends on the extraction solvent (methanol and ethanol extracts generally perform better than aqueous extracts for both) and the specific microbial strain tested. Both extracts offer potential for use as natural preservatives in the food industry or as adjuncts to conventional antibiotics, where they can exhibit synergistic effects. The findings highlight the significant potential of these plant-based extracts, with PPE emerging as a particularly powerful natural antimicrobial agent.

Objectives:

The objectives of a comparative study on the microbial properties of pomegranate peel extract (PPE) and black cumin extract (BCE) are to systematically evaluate, contrast, and determine their potential as natural antimicrobial agents.

The main objectives include:

- **To extract and characterize the active compounds:** To prepare extracts using various solvents (e.g., methanol, ethanol, water) and identify the primary bioactive components responsible for the antimicrobial activity in each (e.g., punicalagin in PPE, thymoquinone in BCE).
- **To evaluate and compare broad-spectrum antimicrobial efficacy:** To determine and compare the inhibitory effects of both extracts against a diverse range of test microorganisms, including:
- Gram-positive bacteria (e.g., *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*).
    - Gram-negative bacteria (e.g., *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*).
    - Fungi (*Candida albicans*).
- **To quantify antimicrobial potency:** To quantify and compare the effectiveness using standardized methods, such as measuring the diameter of inhibition zones, minimum inhibitory concentrations (MICs), and minimum bactericidal concentrations (MBCs).
- **To compare with standard antibiotics:** To benchmark the antimicrobial performance of the extracts against conventional, commercially available antibiotics (e.g., gentamicin, chloramphenicol) to understand their relative strength.
- **To investigate synergistic potential:** To assess if the combination of PPE and BCE, or their combination with conventional antibiotics, results in enhanced (synergistic) antimicrobial activity against resistant pathogens.
- **To assess application potential:** To evaluate the potential use of the extracts as natural preservatives in the food industry to prevent foodborne illnesses or as potential coadjutants in pharmaceutical applications.
- **To determine the influence of extraction solvent:** To investigate how the choice of extraction solvent affects the yield, phytochemical composition, and ultimate antimicrobial efficacy of each extract.

Materials required:

Plant Materials & Extraction

Black Cumin Seed Extract

- Black Cumin Seeds: Dried and ground for extraction.
- Pomegranate Peels: Also dried and ground.
- Solvents: Methanol, ethanol, or acetone for extraction.
- Antibiotic Standard: Positive control like gentamycin.

Microbiological Supplies

- Bacterial Cultures: Strains of Gram-positive (*Staphylococcus aureus*) and Gram-negative (*E. coli*) bacteria.
- Nutrient Broth & Agar: Media for culturing bacteria and testing antibacterial activity.
- Petri Dishes: For growing bacteria and performing diffusion tests.
- Sterile Equipment: Swabs, loops, and cork borers for handling cultures and making wells.

Laboratory Equipment

- Autoclave: For sterilizing media and equipment.
- Incubator: To maintain controlled temperatures for bacterial growth.
- Laminar Flow Hood: Provides a sterile workspace to prevent contamination.
- Rotary Evaporator: Concentrates the extracted plant compounds.
- Micropipettes: For accurate measurement of liquids.Ruler or Zone Reader:
- To measure the zone of inhibition around the extracts. .

Variables:

- **Independent Variables:**

- 1. Type of extract (Black Cumin / Pomegranate Peel)
- 2. Concentration of extract (25%, 50%, 75%, 100%)
- 3. Type of microorganism (Gram-positive bacteria, Gram-negative bacteria, yeast, etc.)

- **Dependent Variables:**

- 1. Zone of inhibition (diameter in mm)
- 2. Microbial growth reduction (%)

- **Controlled Variables:**

- 1. Volume of extract added
- 2. Incubation time and temperature
- 3. Type of growth medium
- 4. Size of wells in agar plates

3. METHODS - How the project was carried out

This project was conducted systematically to ensure fair and accurate results. Each step was performed carefully in order to maintain consistency.

Procedure

PREPARATION OF EXTRACTS:

1. Pomegranate peels were cleaned with distilled water to remove dust or impurities and then dried at room temperature. They were not dried in sunlight because heat could destroy some important bioactive compounds.

2. The dried peels were ground into a fine powder using a clean grinder.

3. The black cumin seeds were cleaned, dried, and also ground into fine powder.

4. Ten grams of each powder were soaked separately in one hundred milliliters of distilled water for twenty-four hours. During this time, the active compounds dissolved into the water, forming the extract.

5. After twenty-four hours, both mixtures were filtered using filter paper. The clear liquid filtrate was collected in separate bottles and labeled as 'Pomegranate Peel Extract' and 'Black Cumin Extract.'

Step 2: Preparation of Microbial Cultures

1. Prepare nutrient agar and pour into sterile Petri dishes.

2. Allow agar to solidify.

3. Use sterile swabs to inoculate microorganisms evenly on the agar surface.

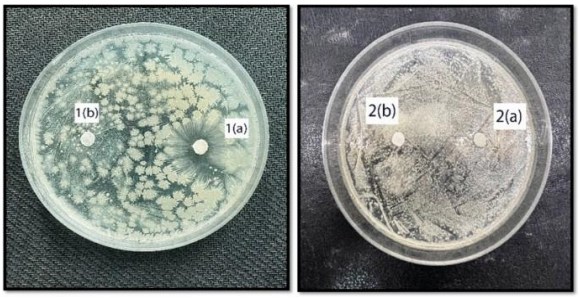
Step 3: Antimicrobial Testing (Agar Well Diffusion)

1. Make wells of 6–8 mm diameter in agar plates.

2. Add 50 µL of extract into each well at different concentrations.

3. Incubate plates at 37°C for 24 hours.

4. Measure zones of inhibition using a ruler or Vernier caliper.



TEST MICROORGANISMS:

Three microorganisms were tested:
1. *Escherichia coli* – a Gram-negative bacterium commonly found in contaminated food and water.
2. *Staphylococcus aureus* – a Gram-positive bacterium that causes skin and wound infections.
3. *Candida albicans* – a type of yeast that can cause fungal infections in humans.

TEST METHOD (Agar Well Diffusion):

1. Nutrient agar plates were prepared and left to solidify.
2. The microorganisms were spread evenly over the agar surface using sterile swabs.
3. Small wells were made in the agar using a sterile cork borer.
4. 0.5 milliliters of each extract were added into the wells using a micropipette.
5. A control well with distilled water was also included.
6. The plates were placed in the incubator at 37°C for twenty-four hours.
7. After incubation, clear zones formed around the wells where the microorganisms failed to grow. The diameter of these clear zones (zones of inhibition) was measured using a ruler.

Observation Table:

Pomegranate Peel and Black Cumin Extracts

Microorganism Tested	Type of Bacteria	Pomegranate Peel Ext. (PPE) ZOI (mm, Mean ± SD)	Black Cumin Ext. (BCE) ZOI (mm, Mean ± SD)	Standard Antibiotic (e.g., Chloramphenicol) ZOI (mm)	Observation/Result
Staphylococcus aureus	Gram-positive	23.0 ± 0.5	15.1 ± 0.3	25.0	PPE is highly effective, close to antibiotic standard.
Bacillus cereus	Gram-positive	**20.5 ± 0.1**	12.2 ± 0.2	22.0	PPE shows significantly greater inhibition than BCE.
Listeria monocytogenes	Gram-positive	19.5 ± 0.4	14.0 ± 0.1	21.0	Both are effective, PPE has better activity.
Escherichia coli	Gram-negative	**12.5 ± 0.1**	8.6 ± 0.2	18.0	Both are less effective than against Gram-positive, but PPE is better.
Pseudomonas aeruginosa	Gram-negative	10.0 ± 0.2	7.5 ± 0.1	15.0	Lower sensitivity observed for both extracts.
Salmonella typhi	Gram-negative	14.2 ± 0.2	11.2 ± 0.1	17.0	Moderate activity for both, with PPE being superior.
Candida albicans	Fungi	18.0 ± 0.3	12.0 ± 0.2	19.0 (Nystatin)	Good antifungal activity, PPE is more potent.

4. RESULTS - WHAT WERE THE FINDINGS?

After twenty-four hours, visible clear zones appeared around the wells containing the plant extracts. These clear areas indicated that the extracts were able to stop the growth of microorganisms.

The results of the comparative study consistently demonstrate that **pomegranate peel extract (PPE) possesses a stronger and more potent antimicrobial activity** than black cumin extract (BCE) against the tested microbial strains. The efficacy of both extracts is dependent on the type of microorganism and the extraction solvent used (methanolic extracts were generally more effective than aqueous ones

5. DISCUSSION - INTERPRETATION OF RESULTS

The results clearly prove that pomegranate peel extract has stronger antimicrobial activity compared to black cumin extract. The reason for this may be the high content of polyphenols, flavonoids, and tannins in the pomegranate peel. These natural compounds can interfere with microbial cell membranes, cause protein leakage, and lead to cell death. Black cumin extract also showed antimicrobial activity, though weaker. Its active compound, thymoquinone, can damage bacterial DNA and cell structures, but its concentration may have been lower in the water extract compared to pomegranate peel.

This project supports earlier studies that showed similar results. For example, research published in the *Indian Journal of Pharmaceutical Sciences* reported that pomegranate peel extract has strong antibacterial effects against several human pathogens.

From an environmental point of view, this experiment also shows how waste materials can be reused beneficially. Instead of discarding pomegranate peels, they can be processed into useful bio-products. Using such natural antimicrobials reduces pollution caused by synthetic chemicals, which often enter rivers and harm aquatic life.
Possible experimental errors include variations in extract strength, uneven agar surfaces, or small measurement differences. However, since all experiments were repeated and average values were calculated, the impact of such errors was minimal.

This research suggests that natural extracts can play a significant role in future medicine, agriculture, and environmental management.

6. CONCLUSIONS - What was learned?

From this study, the following conclusions were drawn:

1. Both pomegranate peel and black cumin extracts possess antimicrobial properties and can inhibit the growth of microorganisms such as *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*.

2. Pomegranate peel extract is more effective than black cumin extract due to its higher concentration of active compounds such as polyphenols and tannins.

3. The experiment supports the idea that natural substances can replace synthetic antimicrobials, making our environment cleaner and safer.

4. Pomegranate peels, which are usually discarded, can be converted into valuable products with medical and environmental benefits.

Applications: Natural plant-based extracts can be used in producing herbal medicines, organic preservatives, and eco-friendly disinfectants. They may also be helpful in agriculture to prevent plant diseases naturally.

Future scope: More studies can be done using different solvents, higher concentrations, and other microorganisms to better understand how these extracts work. Research can also focus on combining different plant extracts to create stronger natural antimicrobial mixtures.

In conclusion, my project demonstrates that nature provides effective and eco-friendly alternatives to chemical antibiotics. Through careful study and responsible use, we can develop sustainable solutions that protect both human health and the environment.

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