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ANTIMICROBIAL POTENTIAL ACTIVITY OF ONION (ALLIUM CEPA) WASTE EXTRACTS PREPARED IN DIFFERENT SOLVENT SYSTEMS

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Abstract

The antimicrobial properties of onion waste against common food-borne microorganisms were the subject of this study. Using polar (ethanol) and non-polar (hexane) solvents, onion waste samples were collected, dried, and removed. The concentrates were gone after for antimicrobial development against Staphylococcus aureus, Escherichia coli, and Aspergillusniger using the agar well scattering system. Hexane removes lacked antimicrobial properties, whereas ethanol separates displayed barrier zones against the two microscopic organisms and growths. Restraint zones of 1.4-1.6 cm were created for S. aureus, 1.4 cm for E. coli, and 1.0 cm for A. niger by the ethanol extricate. These results show that, particularly in polar concentrates, onion waste contains bioactive mixtures with antimicrobial properties. The revelations suggest expected applications for onion waste isolates as typical antimicrobial experts in food protection, medications, agribusiness, and various endeavours. Further investigation is legitimate to perceive and portray the specific antimicrobial combinations present in onion waste removers.

Keywords: Antimicrobial Potential, Onion Waste Extracts,Solvent Systems, Phenolic Compounds, Flavonoids, Antioxidants, Quercetin, Bacterial Infections.

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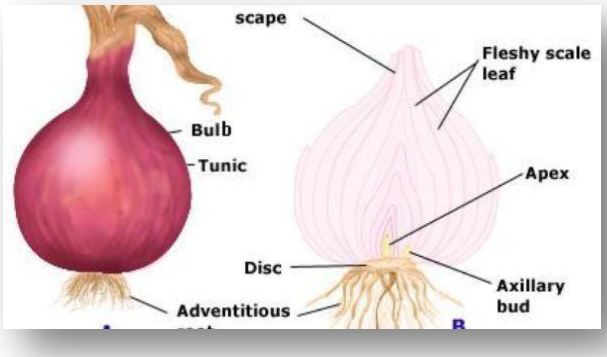


Introduction

Allium cepa L. (common Onion) is one of the most important vegetable crops. Onionis a richsource of flavonoids like quercetin and kaempferol. Onions contain high amounts of waste andthese wastes contain various useful and bioactive compounds. Red onion skin can be used toproduce value-added products due to its bioactive compounds such as phenolic and flavonoids.Redonionskinbioactivecompoundscontainbiological propertiessuchasantibacterial,antioxidant, and other biological properties and are also used to prevent many diseases. The mainpigmentin red onion skinis cyanidin3-glucoside(1,2).

During processing huge amount of onion peel waste is extracted and this waste containsa huge source of antioxidants especially quercetin which can be used to increase the phenolic and flavonoid content of the food products. Crude extract of red onion can be obtained separately with acetone, ethanol and a mixture of solvent with water. Isolated phenolic compounds and quercetin from onion skin can be 3 to 5 times higher as compared to onion edible parts.In vitro,the antibacterial activity of the onion extract has been investigated on both Gram-negative (Escherichia coli and Pseudomonas aeruginosa) and Gram-positive (Staphylococcus aureus and Bacillus cereus) by using the modified Kirby-Bauer disc diffusion

method. Onion wastes reported containa high content of phenolics and flavonoids mainly quercetin which generally have high antibacterial activity.(3–5).

Onion peel contains abundant phytochemicals contributing to its antioxidant characteristics which can be useful in the prevention of oxidative damage or free radical formation. It can be used to improve food quality, shelf-life extension and packaging. The antioxidant properties can be obtained by using the extraction method. There are two different techniques(Conventional and Non-conventional) that can be used to extract bioactive compounds and make suitable applications used in food industries.Aspergillusniger is a filamentous ascomycete fungus that is ubiquitous or found everywhere in the environment within soil and water, on vegetation, in faecal matter, on decomposing matter and suspended in the air. A. niger is used for the production of citric acid and currently it is used in a large amount in the industries for the production of citric acid. The genome sequences of Aspergillusniger will be used for basic applied research programs applicable to fermentation process development, and morphology.Aspergillusniger is one of the most important microorganisms used in biotechnology. It has been already used for a long period for the production of extracellular (food) enzymes and citric acid. It is also used for biotransformation and waste treatment. A few medical cases have been also noticed as lung infections but always in severely immunocompromised patients. A. niger is considered safe production microorganisms(5,6).

Citric acid produced by microorganisms has very high economic importance and is widely used in beverage, food, detergents, cosmetics, and pharmaceutical industries. The filamentous fungus Aspergillusniger is a very important factor in the industries for the production of citric acid. A fewstrains of Aspergillusniger produce potent mycotoxins we also call ochratoxins.The cell wall composition of Aspergillusniger has been investigated and it is composed of six sugar, glucose, galactose, mannose, arabinose, glucosamine and galactosamine all in the d-configuration, except that a small amount of l-galactose may be present. The wall of A. nigerconsists chiefly of neutral carbohydrates and hexosamine with small amounts of lipid, protein and phosphorous.Bacteria are ubiquitous, found everywhere in the environment and they have extensive power of adaptation means bacteria can adaptto environmental changes. Bacterial infections can cause disease but bacterial infections are easy to treat than viral infections. Bacteria can transmit to humans through air, water, food, or some living vectors. Bacteria are prokaryotic and unicellular organisms that carry their genetic information in a double-stranded circular molecule of DNA. It is classified as Gram-positive and Gram-negative bacteria in the presence and absence of air it is of two types that is Aerobic and Anaerobic.(7,8).

The cell wall of bacteria is made up of peptidoglycan. The cell wall has many functions, the most important is to protect the wall from bursting by osmotic forces. Bacteria are considered the scavengers of the biotic environment and play an important role in the biological cycle of elements. Bacteria help in the degradation of organic substances in both the soil and water.Communications between bacteria occur by using chemical signalling molecules in the form of words. The bacteria release chemical signals detect these signals and then respond to the accumulation of these molecules, which are called autoinducers. The autoinducers detection allows bacteria to differentiate between low and high cell population density and helps to control gene expression in response to changes in cell number. This process is known as quorum sensing which allows bacteria to control the entire community's gene expression.(9).

Antibiotics are among the most successful drugs ever developed. An increase in antibiotic resistance can impact human health at two levels. Firstly, it has a direct effect on the possibility of treating infections. Secondly, it can compromise treatments that require immunosuppression, for example, transplantation, as well as procedures such as intubation or catheterisation, advanced surgical procedures or anticancer chemotherapy, which require the use of antibiotics to prevent or treat the associated infection.Generally, bacteria are used as an anticancer agent to treat the cancer. It is used as an oncolytic agent for malignant tumours. Bacteria work as sensing agents for chemotherapy, as delivery agents for anticancer drugs, and as vectors for gene therapy. The toxin of bacteria can be used for the destruction of tumours and cancer vaccines are based on immunotoxins of bacterial origin. The bioactive components found in agro-industrial residues, particularly the phenolic compounds in fruit and vegetable waste, are receiving increased attention in the food industry. The outer layers of fruits and vegetables, such as husks, peels, and shells, are often discarded, despite containing higher concentrations of phenolic compounds compared to the inner parts.The extraction of antioxidant compounds from industrial waste such as peanut shells, tomato skins, lemon skins, grape seeds, etc. has identified important antioxidants. Waste from onions is a source of antioxidants and compounds that work to prevent the blackening caused by enzymatic reactions(10,11).

The study focused on examining the antimicrobial and antioxidant properties of onion peel extracts. The aim was to assess whether this by-product could be a potential source of natural bioactive compounds. The hypothesis guiding the study was that onion peel extracts have antioxidant and antimicrobial effects on gastrointestinal microorganisms, and that the type of solvent used for extraction would influence these properties. To test this hypothesis, dried onion peels were extracted using methanol, ethanol, acetone, or ethyl acetate. The resulting extracts were then analyzed to compare their antioxidant potential, chemical composition, and effectiveness against

nine species of gastrointestinal microorganisms, including eight bacterial species and one fungal species.Antibiotics are chemical substances generally produced by microorganisms that inhibit the growth of bacteria and other microorganisms. Antibiotic substances such as penicillin, streptomycin and many other drugs are used use in the medical field for the treatment of different types of infectious diseases. Antibiotics are used for the treatment of bacterial infections and it is one of the most important drugs found in clinics that are becoming increasingly resistant to available treatment.Antibiotics are the drugs that are most commonly used by surgical patients. These antibiotics kill bacteria and prevent them from spreading. The study of the antibiotics to the effector site is known as the Pharmacokinetics. The elements in the delivery of drugs to the effective sites involve drug absorption, distribution, biotransformation and excretion.Antibiotics resistant are used to treat infectious disease in humans and animals. Generally, it inhibits the growth of bacteria by the inhibition of cell wall or protein synthesis, interference with DNA (or RNA) replication and break-down metabolic pathways or cell membranes.It is very necessary to instruct patients and the general community on the suitable use of antibiotics, the importance of infection preventive measures and illness behaviour. People should avoid antibiotics on self-medication. In hospitals, management should ensure the establishment of an infection control programme with the help of antibiotic resistance control and they should have to also develop continuously updated antibiotic treatments and prevention guidelines, The Government should also fund research in the universities and research institutes for the discovery of new form forms of antibiotics.Microbes are found everywhere and they are capable of producing antibiotics in all instances like in the air, in the water, in sewage disposal systems and in the plants and animal residues but mainly in the soil under our feet. The organisms that produce antibiotics are found in every group of microbes but the primary production of antibiotics is by bacteria, actinomycetes, and fungi. The antibiotics produced by these three groups are bacillus among the bacteria, Streptomyces among the actinomycetes and Penicillium and Aspergillus among the fungi.(6,12,13).

Materials and Methods

1. Sample collection & preparation

- We have collected the onion wastes from the vegetable market, Munshi Pulia Lucknow.
- Then, wash it with tap water three times.
- Spread the washed sample on the blotting paper for drying and also use the hot air oven for complete dryness.
- After that, we have used the grinder to grind the sample into the fine powder form.
- And Store the sample in powder form to perform further use.Sample & Location of Sample

Sample	Location
Onion waste	Munshi Pulia, Lucknow

1. Selection of solvent:

Hexane and Ethanol are used for the study and utilization of onion waste as antimicrobial activity. These solvents show the polar and non-polar properties are as follows:

Selection of Solvent

Polar	Non–polar
Ethanol3e	Hexane

- Ethanolis aclear, colourless liquid with a characteristic pleasant odor and burning taste. It is highly flammable. Ethanol isused to dissolve other chemical substances and mixes readily with water and many organic liquids. Pure ethanolisa flammable, colorless liquid with a boiling point of 78.37°C.
- Hexanes colourless liquid, odourless when pure, and with boiling points of approximately69°C (156°F). It is widely used as a cheap, relatively safe.
- Largelyunre active and easily evaporated non-polar solvent.

Fig1(i): Ethano

Fig2 (ii):Hexane

2. Extract preparation:

We have prepared the extracts by using different solvents like polar (methanol, ethanol)andnon-polar(hexane) with the help of Soxhlet extract or techniques.

Soxhlet: Anapparatus for use in extracting fatty or other material (thermostable) compounds, oil, lipid) with a volatile solvent.

Principleof SoxhletApparatus: Reflux&Siphoning

Partsof Soxhlet Apparatus:

Round bottom flask: In it, we put thesolvent & give the heatat the bottom through the heating mantle.

1. Soxhlet extractor: It is a cylindrical tube consisting of an evaporator tube, siphoningtube and thimble. A thimble (sample packed in **Whatman filter paper**) is placed inthe extractor chamber.

2. Condenser: It consists of a water inlet and water outlet to condense the vaporized solvents.

The procedure of extraction:

- Weigh the sample on a digital weighing machine i.e., 3 grams.

- Properly wash and wipe all the parts of the Soxhlet apparatus.
- Fill the sample in the Whatman filter paper and prepare a pack called a thimble.
- Put the thimble in the chamber of the Soxhlet extractor.
- Round bottom flask filled with solvents measured to 120 ml.
- Join the water inlet of the condenser with tap water.
- Heating starts at the bottom of round flasks, the solvent is boiling.
- After some time, the extractor chamber is filled with a condensed solvent with extract colour.

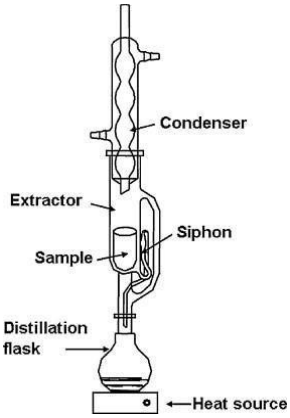


Fig 3. Parts of Soxhlet Apparatus.

Fig 4. Soxhlet apparatus.

Then the process is called siphoning (percolation of the sample), the condensed solvent transfers back into the round flask through the siphoning tube and the chamber becomes empty.

- After siphoning, one cycle completes.
- Repeat the cycle till the condensed solvent in the extractor chamber becomes colourless.
- Collect the extracts for further use.

4. Selection of Bacterial strain

To evaluate the antimicrobial activity of onion extract against spoilage and pathogenic food there are two foods spoiling microorganisms are used for the experiment.

These are of two bacteria one is gram-positive and the other one is gram-negative bacteria these are as follows:

Selection Bacterial Strain

Gram-positive

Gram Negative

Staphylococcus aureus

Escherichia coli

Selection of antibiotic (Streptomycin):

Streptomycin is an Antibiotic.

Used for Gram-positive and Gram-negative Bacteria.

According to the literature, 10 mg/ ml concentration of Streptomycin has been used. We purchased the antibiotic (streptomycin) of 0.75mg from the market.

And we prepared the working solution of this antibiotic (10mg in 1ml autoclaved DDW).

Media preparation:

Nutrient Agar:

- We have prepared the nutrient agar media to culture the bacterial strains.
- We measured the nutrient agar 0.84 grams by digital weighing machine for 30 ml, two times, one for gram-positive and another one for gram-negative bacteria.
- We dissolved the nutrient agar with DDW in a conical flask & then autoclaved the media.
- After that, we poured the media into the Petri dishes in the laminar airflow (LAF).

Nutrient Broth:

- We have prepared the nutrient broth for the cultivation and maintenance of bacterial strains.
- We measured the nutrient broth 0.065 grams by digital weighing machine for 5 ml, two times, one for gram-positive and another one for gram-negative bacteria.
- We dissolved the nutrient broth with DDW in a test tube & then autoclaved the media.
- After that, we put the test tube into the test tube stand in the laminar airflow (LAF).

Culture of bacterial strain:

First of all, we broke the ampoule of gram-positive (Staphylococcus aureus) & gram-negative (Escherichia coli) bacteria which is present in the lyophilization form.

Mixed the individual ampoule bacteria by 500µl DDW respectively.

Then the bacteria by the streak plate method through an inoculation loop on the nutrient agar media to make the master plate.

And remaining bacteria of the ampoule were mixed with the nutrient broth in the test tube. All these processes were carried out in the laminar airflow.

After that, we kept the streak plate in the B.O.D. incubator at 37°C for the over-night. We kept the nutrient broth test tube in the incubator shaker at 37°C for over-night

After the overnight, we saw the growth of bacteria on the streak plate and kept that plate in the deep freezer for further use.

And we kept the nutrient broth test tube in the deep freezer for further use. We filled the seven Eppendorf tubes with glycerol (80%) and cultured broth of gram-positive and another seven with glycerol (80%) and cultured broth of

gram-negative bacteria in the same amount (750µl).

We kept each Eppendorf tube in the deep freezer for stock preparation and storage.

Antimicrobial testing:

- We prepared the nutrient agar media (2.24 grams), for 80 ml in a conical flask.

- Then, autoclave the media.
- After that, pour the media into Petri dishes (4 Petri dishes), 20 ml in each in the laminar airflow.
- After the solidification of the media, we spread the microbes (broth) on the media by spread plate method, 50µl in each.
- After that, we created the wells on media of Petri dishes, 3 wells in each by well puncturing machine or by the tips.
- Then we nominated each well, the first well as positive control (for an antibiotic), the second as negative control (for solvent) and the third one as sample control (crude extract).
- We poured the 50µl antibiotic (streptomycin) in positive control, 50µl solvent in negative control and 50µl crude extract in sample control.
- We used the same solvent, which was used in extract preparation in a petri plate. Packed the Petri plates with the parafilm tape.
- Then we incubated our petri plates in a B.O.D. incubator at 37°C overnight.
- After overnight, we checked our plates to see the results as a zone of inhibition in the plates.

4. Standardization of antimicrobial testing:

- Due to the low concentration of a substance, results are not visualized properly.
- To modify the technology to get the best results, we have used column chromatography.
- Extract preparation:

By soaking

Weighed the 50 grams of onion waste sample in the digital weighing machine, mixed with 400ml of the solvent of polar(ethanol) and non-polar(hexane) measured by measuring cylinder (50 grams in each) respectively.

After that, we kept this in an incubator shaker at 28°C.

After some time, the sample solution remained at 175 ml both polar and non-polar. Then we prepared pure extract by column chromatography.

After that, we kept our extract in the B.O.D. incubator at 37°C to evaporate the solvent.

So that our extract became concentrated. Finally extract solution remained 5ml.

After that, we stored our sample in the vials and kept that in the deep freezer for further use.

Column chromatography

Column chromatography is described as the useful technique in which the substances to be isolated are presented onto the highest point of a column loaded with an adsorbent (stationary phase), and go through the column at various rates that rely upon the affinity of every substance for the adsorbent and the solvent or solvent mixture, and are typically gathered in solution as they pass from the column at various time. The two most common examples of stationary phases for column chromatography are silica gel and alumina while organic solvents are regarded as the most common mobile phases (14).

Column Chromatography principle

The main principle involved in column chromatography is the adsorption of the solutes of the solution with the help of a stationary phase and afterwards separates the mixture into independent components. At the point when the mobile phase together with the mixture that requires to be isolated is brought in from the top of the column, the movement of the individual components of the mixture is at various rates. The components with lower adsorption and affinity to the stationary phase head out quicker when contrasted with the greater adsorption and affinity to the stationary phase. The components that move rapidly are taken out first through the components that move slowly are eluted out last. The adsorption of solute molecules to the column happens reversibly (15). The pace of the movement of the components is communicated as:

Rf =The distance travelled by the solute/ distance travelled by the solvent

Where,

Rf is called the retardation factor.

Column Chromatography Components:

Components of a typical chromatographic system using a gas or liquid mobile phase include:

- Stationary phase – Generally, it is a solid material having a good adsorption property and should be suitable for the analytes to be separate. It should not cause any hindrance in the flow of the mobile phase.
- Mobile phase and delivery system – This phase is made up of solvents that complement the Stationary phase.

The mobile phase acts as a solvent, a developing agent (promotes separation of components in the sample to form bands), and an eluting agent (removes the components from the column that are separated during the experiment) (16).

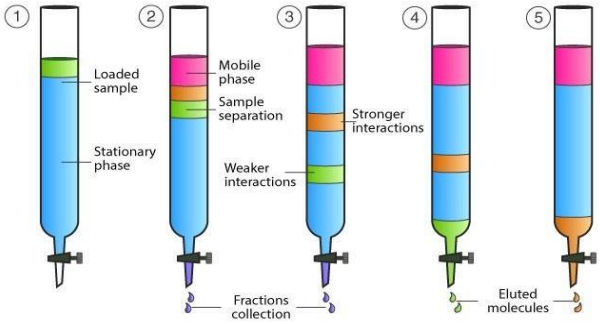


Fig 5. Process of Column Chromatography.

Column chromatography Procedure:

The steps included in the column chromatography are:

1. Preparation of the column:

- Mostly the column is comprised of a glass tube with an appropriate stationary phase.
- The bottom end of the column is packed with a glass wool/ cotton wool or an asbestos pad after which the stationary phase is packed.

- After packing the column, a paper disc is placed on the top to avoid the disturbance of the stationary phase during the introduction of the sample or mobile phase.
- The disturbance in the stationary phase (adsorbent layer) leads to irregular bands of separation (17).

Two types of preparing the column, known as packing techniques namely:

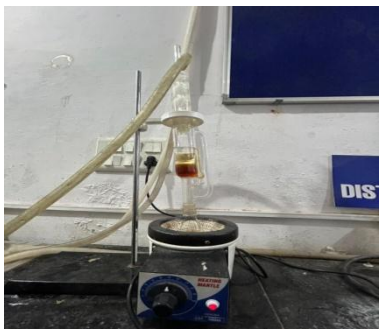
i. Dry packing technique – The amount of absorbent needed is added as a fine dry powder in the column and the solvent flows freely through the column until equilibrium is achieved.

ii. Wet packing technique – The slurry of adsorbent is prepared along with the mobile phase and is poured into the column.

It is regarded as the ideal technique for packaging. (17).

2. Introduction of the sample

- The sample (a mixture of components) is **dissolved in the minimum amount of the mobile phase.**
- At one instant, the sample is introduced into the column and on the top portion of the column,it is absorbed.
- Through the elution process, the individual sample can be isolated from this zone (17).



3. Elution technique:

3. Elution Technique:

The column should be properly washed and completely dried before use.

Through this technique, the individual components are separated from the column. The process of elution can be carried out by employing two techniques:

i. Isocratic elution technique – Throughout the procedure, a solvent of the same polarity or solvent composition is utilized.

Example: Use of chloroform alone.

ii. Gradient elution technique – Throughout the separation procedure, solvents of gradually increased polarity or increased elution strength are utilized

Example: Benzene → Chloroform → Ethyl acetate → Chloroform

4. Detection of Components

In case the mixture separated in a column chromatography procedure is coloured compounds, then monitoring the separation progress is simple.

In case the compounds undergoing separation are colourless, then small fractions of the eluent are sequentially collected in tubes that are labelled through TLC, and the composition of each fraction is determined (18).

Column chromatography uses:

Column chromatography is one of the versatile methods for purifying and separating both solids and liquids.

Major applications:

- To isolate active constituents.
- To separate compound mixtures.
- To remove impurities or carry out a purification process.
- To isolate metabolites from biological fluids.
- To estimate drugs in drug formulations or crude extracts (19).

Antimicrobial testing of Bacteria:

We prepared the nutrient agar media (2.24 gram), for 80 ml in a conical flask. Then, autoclave the media.

After that, pour the media into Petri dishes (4 Petri dishes), 20 ml in each in the laminar airflow.

After the solidification of the media, we spread the microbes (broth) on the media by spread plate method, 20µl in each.

After that, we created the wells on media of Petri dishes, 3 wells in each by well puncturing machine.

Then we nominated each well, the first well as positive control (for an antibiotic), the second as negative control (for solvent) and the third one as sample control (crude extract).

We poured the 50µl antibiotic (streptomycin) in positive control, 50µl solvent in negative control and 50µl crude extract in sample control.

We used the same solvent, which was used in extract preparation in a petri plate. Packed the Petri plates with the parafilm tape.

Then we incubated our petri plates in a BOD incubator at 37°C overnight.

After overnight, we checked our plates to see the results as a zone of inhibition in the plates (20–22).

Antimicrobial testing of Fungi:

We prepared the nutrient agar media (2.24 gram), for 80 ml in a conical flask. Then, autoclave the media.

After that, pour the media into Petri dishes (4 Petri dishes), 20 ml in each in the laminar airflow.

We have inoculated the fungus by dipping some portion of the fungi in Eppendorf from the fungi plate.

After that, we created the wells on media of Petri dishes, 3 wells in each by well puncturing machine.

Then we nominated each well, the first well as positive control (for antibiotic), the second as negative control (for solvent) and the third one as sample control (crude extract).

We poured the 50µl antibiotic (streptomycin) in positive control, 50µl solvent in negative control and 50µl crude extract in sample control.

We used the same solvent, which was used in extract preparation in a petri plate. Packed the Petri plates with the parafilm tape. Then we incubated our petri plates in a BOD incubator at 37°C for at least 3 nights. After 3 nights, we checked our plate to see the results as a zone of inhibition in the plate (23–25).

Results and Discussion

Sample collection:

We have collected the onion waste from the Vegetable market, in Banthara Lucknow.

Fig 7. (i): Washing.

Fig 8. (ii): Drying.

Fig 9. (iii): Tray Drying.

Fig 10. (iv): Onion waste sample.

Extract preparation:

We have prepared the extracts by using different solvents like polar (methanol, ethanol ethyl acetate) and non-polar (hexane) with the help of soxhlet extractor techniques.

Fig 11. (i): Ethanol extract.

Fig 12. (ii): Hexane extract.

Extract preparation by Column chromatography:

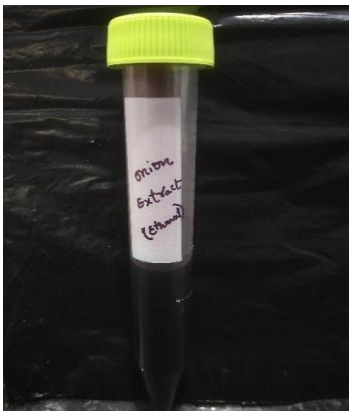


Fig 13. Ethanol extract.

Fig 14. (ii): Hexane extract.

Culture of bacterial strain:

Master streak plate of Gram positive and Gram negative and broth culture prepared by Masterplate.

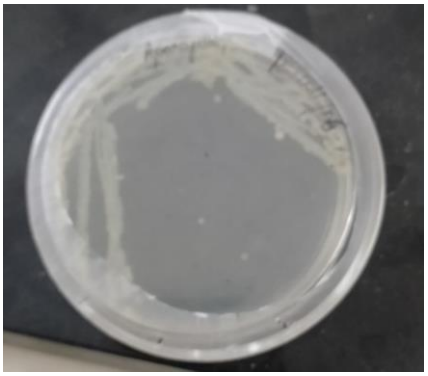


Fig 15. *Staphylococcus aureus.*

Fig 16. (ii): *Escherichia coli.*

Inoculum of the bacterial strains:

Fig 17. *Staphylococcus aureus.*

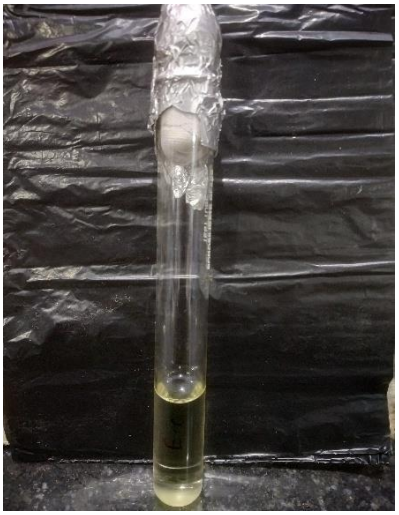


Fig 18. (ii)*: Escherichia coli*.

Antimicrobial testing:

Fig 19. (i): Antimicrobial testing (50% ethanol & 50% extract+ Ethanol+ Antibiotic+ *S.aures*).

Fig 20. (ii): Antimicrobial Testing (50% ethanol & 50% extract + Ethanol+ Antibiotic+ *E.coli*).

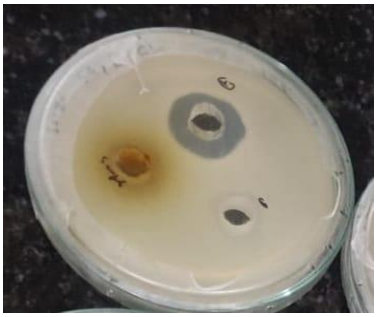


Fig 21. (iii): Antimicrobial testing (40% ethanol & 60% extract+ Ethanol+ Antibiotic+ *S.aures).*

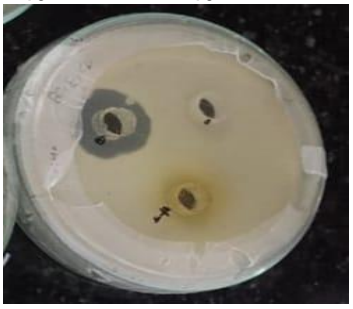
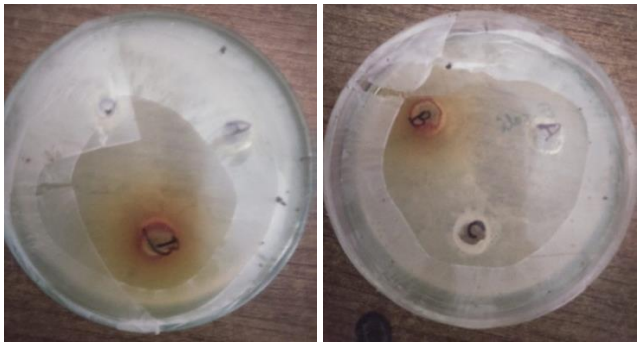


Fig 22. (iv): Antimicrobial Testing (40% ethanol & 60% extract + Ethanol+ Antibiotic+ *E. coli*).

a. E. coli. ***b. S. aureus.***

Fig 23. (v): Antibacterial testing (Hexane extract+ *Escherichia coli+* Antibiotic) &(Hexane extract + *Staphylococcus aureus* + Antibiotic).



E.coli plate. b. *S.aureus* plate.

Fig 24. (vi): Antimicrobial Testing

a. (Soxhlet extract + Ethanol+ Antibiotic+ *E. coli*) &

a. (Soxhlet extract + Ethanol+ Antibiotic+ *S. aureus*)

Final Result:

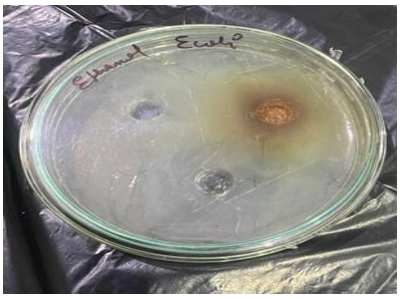


Fig 25. (vii): Antimicrobial Testing (Column extract + Ethanol+ Antibiotic+ *E. coli*)Results shown in the table as Zone of inhibition (ZOI): In centimetre (cm.) Zone of inhibition (ZOI) in *E. coli* plate

S.no	Ethanol extract + *Escherichia coli*
4	1.4 ± 0.1

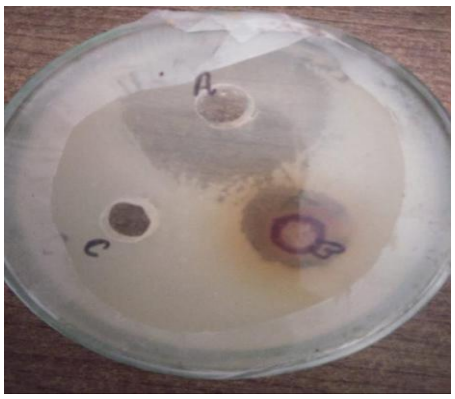


Fig 26. (vii): Antimicrobial Testing
(Column extract + Ethanol+ Antibiotic+ *S. aureus).*

Zone of inhibition (ZOI) in *S. aureus* plate:

S.no	Ethanol extract + *Staphylococcus aureus*
1	1.5 ± 0.2
2	1.5 ± 0.2
3	1.6 ± 0.2

In antibacterial testing, we got the results as a zone of inhibition.

All results were observed only in the ethanol extract with Gram-positive and Gram-negative bacteria butnot in the hexane extract.

Zone of inhibition of antibiotic (Streptomycin) = 3.5 ± 0.2 cm. Well, diameter = 0.8 cm.

Inoculum of the fungal strains:

Fig 27. Fungal Inoculum.

Culture of Fungal Strain and Staining:

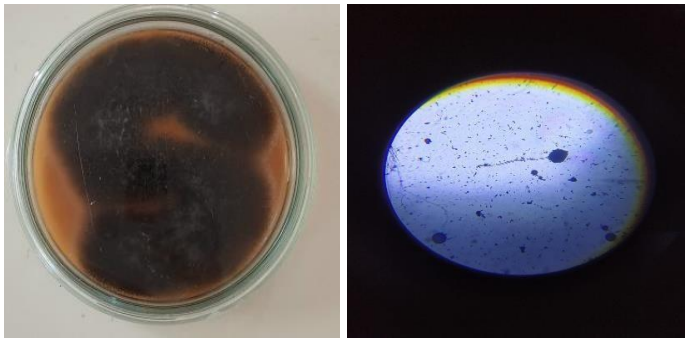


Fig 28. Fungus Culture.

Antimicrobial testing:

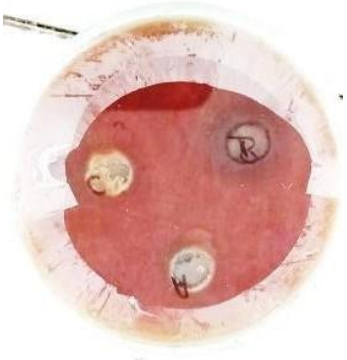


Fig 29. Result of Fungus.

Zone of Inhibition in Fungus Plate

SN	Ethanol extract + *Aspergillus niger*
1	1 ± 0.1
2	1 ± 0.2
3	1 ± 0.2

4. **Application**:

1. Pharmaceuticals: Onion waste extracts could be utilized in the development of novel antimicrobial drugs. Compounds extracted from onion waste have shown promising antimicrobial activity against a wide range of pathogens, including bacteria, fungi, and even some viruses (26).

2. Food Preservation: The antimicrobial properties of onion waste extracts make them ideal candidates for natural food preservatives. Incorporating these extracts into food packaging materials or directly into food products could help extend shelf life

by inhibiting the growth of spoilage bacteria and fungi (27).

3. Agriculture: Onion waste extracts could be utilized in agriculture as eco-friendly alternatives to synthetic pesticides and fungicides. By harnessing the antimicrobial properties of these extracts, farmers can effectively control plant pathogens while minimizing the environmental impact (28).

4. Waste Management: Onion waste is generated in large quantities by food processing industries, and finding valuable applications for this waste material can contribute to waste reduction efforts. Extracting bioactive compounds from onion waste for antimicrobial applications not only adds value to the waste material but also reduces the environmental burden associated with its disposal (29).

5. Health and Wellness Products: Consumer interest in natural health and wellness products continues to grow. Onion waste extracts could be incorporated into various personal care products such as soaps, lotions, and oral care products for their antimicrobial properties (30,31).

Future Prospects:

- Where seasoning is desired, essential oil extracts of onions can be used as natural antimicrobial additives for incorporation in various food products.
- Most of the herbal products.
- Example: Onion hair oil.
- Absorbent of Pollution.
- Can be used in cosmetics as it protects against UV radiation.
- Soil incorporation or other types of utilization where they could be used in a truly circular manner (32).

6. Conclusion

Onions are known as a good source of flavonoids, mainly quercetin and its glycosylated derivatives. The flavonols quercetin and kaempferol were efficient inhibitors of gram-positive bacteria while gram-negative bacteria were more resistant. So, onions are miracle vegetables that can be used to make new therapeutic medicines as it has high antimicrobial and antioxidant activities that are considered to be a boon for the treatment of many diseases. Onion also contains flavonoids like kaempferol and quercetin that are beneficial as they exhibit anti-allergenic, anti-inflammatory, cardioprotective, vasodilatory, anti-carcinogenic and antioxidant properties antibacterial and antifungal properties. Onion consumption prevents DNA damage and breakage due to the presence of quercetin which acts as a good antioxidant. Onion extracts have effectively inhibited gram-positive bacteria than gram-negative bacteria. The non-aqueous extracts of onion have a greater tendency to inhibit bacterial growth than aqueous extracts. There is no evidence of drug interaction by the consumption of onion thus onion can also be used as a safe therapeutic drug in addition to a natural remedy. (33).

The results from this study indicate that onion skin extracts possess high activity against the growth of food poisoning bacteria such as S. aureus and E. coli for all tested concentrations and extraction solvents. On the other hand, onion edible part extracts showed lower or no inhibition activities against tested bacteria. High inhibition activities were observed also against T. viride for all skin and edible part extracts obtained with 60% solvent mixtures with water. (34,35).

Antifungal and antibacterial activities of pure quercetin were generally much lower than that of extracts, or not even observed at concentrations investigated. Nevertheless, future studies are required to determine the synergistic effects of bioactive compounds of onion extracts. The results indicate that red onion skin and edible part extracts have the potential to be used as antioxidants and antimicrobial agents in the food, cosmetics and pharmaceutical industries. (36–38).

Ethical Statement

A pharmacist ought to act with integrity and sincerity. A pharmacist abstains from behaviours that could undermine their commitment to acting in their patient's best interests, such as prejudiced acts or behaviours and unfavourable working environments that impair their judgment. A pharmacist upholds their reputation in the industry.

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Conflict of Interest

The authors attest that they are free of any known financial or personal conflicts of interest that would taint the findings of this study.

Informed Consent

Using websites, review articles, and other sources to produce research content.

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