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Anti-Microbial Potential of Onion Peel Extracts

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Abstract

Onion (Allium cepa L.) is a source of bioactive compounds, particularly flavonoids, such as quercetin, and possesses antioxidant and beneficial properties to health. Onion skin, which is normally considered as waste, has more of these compounds compared to the edible portion. This research sought to isolate and analyze the antioxidant and antimicrobial activity of onion skin with ethanol and hexane using Soxhlet and column chromatography techniques. Antioxidant activity was maximum with 80% ethanol for shorter extraction times. While antimicrobial activity was restricted, the extracts improved the antioxidant activity and shelf life of food items, such as bread and olive oil. These findings indicate that onion skin can be a useful, eco-friendly component of functional foods, promoting both nutrition and peel minimization.

Keywords: Onion (*Allium cepa* L.), antioxidant, isorhamnetin, column chromatography, Staphylococcus aureus

INTRODUCTION

Onion (*Allium cepa* L.) is a common vegetable that is highly prized for its high content of phytonutrients, especially flavonoids and phenolic compounds. These bioactive are responsible for various health benefits including antioxidant, anticancer, anti-inflammatory, and antimicrobial activities. Red onions have the highest level of these compounds among onion varieties and constitute an important functional food.

PHYTOCHEMICAL COMPOSITION OF ONION

Onions are one of the richest dietary sources of flavonoids, mainly aglycone and glucosides (3,4′-diglucoside and 4′-glucoside). Isorhamnetin, kaempferol, and luteolin are other flavonoids. Red onions also have anthocyanins cyanidin, peonidin, and pelargonidin responsible for their color as well as their strong antioxidant activity. Phenolic acids, like ferulic and caffeic acids, occur in free and bound forms, contributing to flavor and aroma [1–5].

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BIOLOGICAL ACTIVITIES OF ONION BIOACTIVE

Antioxidant Activity

Flavonoids, like quercetin and kaempferol, are very effective antioxidants that scavenge ROS and metal ions. Quercetin, present in more than 85% of onion flavonoids, is more effective in stabilizing ROS than sulphur-containing amino acids. They also activate endogenous antioxidant enzymes, and their applications in preventing oxidative stress-linked diseases are significant.

Antimicrobial Activity

Onion extracts exhibit antimicrobial activity against gram-positive and gram-negative bacteria, such as *Bacillus cereus*, *Escherichia coli*, and

Staphylococcus aureus, and fungi, such as *Aspergillus niger* and *Fusarium oxysporum*. Some of the major antimicrobial compounds are quercetin, kaempferol, and organosulfur compounds. The antimicrobial activity may, however, be affected by the extraction conditions, with some ethanol extracts possessing limited activity despite having high levels of antioxidant activity [5–12].

Extraction Methods and Optimization

Conventional solid–liquid extraction employing organic solvents, like ethanol and methanol, has succeeded in recovering bioactive onion but is environmentally and health-hazardous. New methods, such as ultrasound-assisted extraction, microwave-assisted extraction, pressurized liquid extraction, and subcritical water extraction (SWE) enhance yield, lower solvent consumption, and preserve bioactive integrity. For instance, SWE assisted by the cosolvent ethanol is capable of doubling quercetin yields up to eight times [13–24].

A recent paper on red onion skin identified that 80% ethanol extraction at certain times (165 minutes for phenolics, 60 minutes for flavonoids, and 120 minutes for anthocyanins) maximizes bioactive recovery. Antioxidant assays (FRAP, DPPH, IC50) supported these results, although antimicrobial effects were not evident under these conditions [25–28].

Valorization of Onion By-Products

Onion skins, which are one of the largest processing by-products, have more flavonoids and phenolics than their edible bulbs. Because they play a protective role in the plant, the skins accumulate, such as quercetin and kaempferol compounds that have significant antioxidant, anti-inflammatory, and antimicrobial effects. Onion skin valorization is one way of overcoming sustainability issues by converting peel into value-added products.

MATERIALS AND METHODOLOGY

Sample Collection and Preparation

Red onion (*Allium cepa* L.) skins were obtained from vegetable markets in Munshipuliya, Lucknow, India. The peel material was thoroughly washed with tap water to remove debris and dirt. Then, after washing, the skins were kept at room temperature for 24 hours and then in a hot air oven at 40°C until they were completely moisture-free. The dried sample was pulverized into fine powder by employing a laboratory blender and was kept in sealed containers at room temperature until use.

Choice of Solvents

Two solvents of different polarities were chosen for the extraction.

1. Ethanol (polar solvent) – employed for effective extraction of flavonoid and phenolic compounds.
2. Hexane (nonpolar solvent) – employed for comparison with a nonpolar system.

Bioactive Compound Extraction

Soxhlet Extraction

Three grams powdered onion skin were loaded into a cellulose thimble and extracted with 120 mL of each solvent (hexane and ethanol) individually under Soxhlet extraction conditions. The extraction process took about 6 hours, or until the siphoning of the solvent back into the flask turned colorless, showing exhaustive extraction. The resulting extracts were concentrated by a rotary evaporator at 40°C and stored at 4°C in sterile vials for future use.

Column Chromatography (Purification)

The hexane and ethanol crude extracts were then column chromatographed to isolate and concentrate active fractions. A glass column was filled with silica gel (60–120 mesh) as stationary material. Elution was carried out with a solvent gradient of increasing polarity beginning with hexane, then ethyl acetate, and culminating with ethanol. Fractions were taken in individual test tubes, combined according to similar TLC patterns, concentrated by evaporation, and kept at 4°C.

Microorganisms and Culture Preservation

Test Organisms

The antimicrobial activity was determined against the following microbial strains:

- *S. aureus* (gram-positive).
- *E. coli* (gram-negative).
- *A. niger* (fungus).

All microbial strains were procured from the Microbiology Laboratory, Department of Biosciences, and preserved on nutrient agar (NA) slants.

Revival of Culture and Inoculum Preparation

Bacterial cultures were revitalized by inoculating into nutrient broth and incubating at 37°C for 24 hours. Fungal cultures were subculture on potato dextrose agar (PDA) and incubated at 28°C for 72 hours. The microbial inoculum was standardized to 0.5 McFarland standard (around 1.5×10^8 CFU/mL).

Preparation of Culture Media

- Nutrient agar (NA) for bacterial growth.
- Potato dextrose agar (PDA) for fungal growth.

Media were filled according to manufacturer directions and sterilized by autoclaving at 121°C for 15 min at 15 psi. Sterilized media were filled in sterile Petri plates after sterilization and kept on solidifying in an aseptic environment.

Antimicrobial Activity Assay

Agar Well Diffusion Method

The antimicrobial activity of the respective extract and purified fraction was assessed by agar well diffusion method:

- Sterile Petri plates were set up with solidified agar medium.
- Each plate was inoculated with 50 µL of standardized microbial suspension by a sterile spreader.
- Wells of 6 mm diameter were made in the agar by a sterile cork borer.
- An amount of 50 µL of all extracts (crude and column purified), solvent control (hexane or ethanol), and positive control (streptomycin against bacteria) were added into specified wells.
- Plates were parafilm-sealed and incubated:
  - *Bacteria:* 24 hours at 37°C.
  - *Fungi:* 72 hours at 28°C.

After incubation, inhibition zones around all wells were taken in millimeters by using a digital Vernier calliper.

CONTROLS

- *Positive Control:* Streptomycin (10 mg/mL) for bacteria; an antifungal agent known for fungi.
- *Negative Control:* Matching pure solvents (hexane or ethanol) without any extract.

RESULT

Preparation of Crude Extract and Visual Appearance

Red onion skin extraction with Soxhlet apparatus using both polar (ethanol) and nonpolar (hexane) solvents yielded crude extracts with different physical characteristics. The ethanol extract was dark brown in color with a typical pungent smell, while the hexane extract was pale in color with little odor. Ethanol was more efficient at dissolving the bioactive constituents than hexane, giving a denser, more concentrated extract (Figures 1 and 2).

Figure 1. Ethanol extract.

Figure 2. Hexane extract.

Antimicrobial Activity of Crude Extracts

Crude ethanol and hexane extracts were screened for their antimicrobial potential employing the standard agar well diffusion method. Inhibition zones were determined after overnight incubation at 37°C.

Bacterial Strains

S. aureus (Gram-Positive)

The crude ethanol extract demonstrated weak antimicrobial activity toward *S. aureus*, with inhibition zones of 7–9 mm. On the other hand, no inhibition zone was detected for hexane extract as proof that it is unable to extract active antibacterial compounds (Figure 3).

Figure 3. Antimicrobial testing (50% ethanol & 50% extract + ethanol + antibiotic + *S. aures*).

E. coli (Gram-Negative)

No appreciable zone of inhibition was observed for either hexane or ethanol crude extracts. This implies that the chemicals in crude extracts were not effective enough to diffuse through the outer membrane of *E. coli*, which tends to be more resistant to plant antimicrobials owing to its intricate cell wall structure (Figure 4).

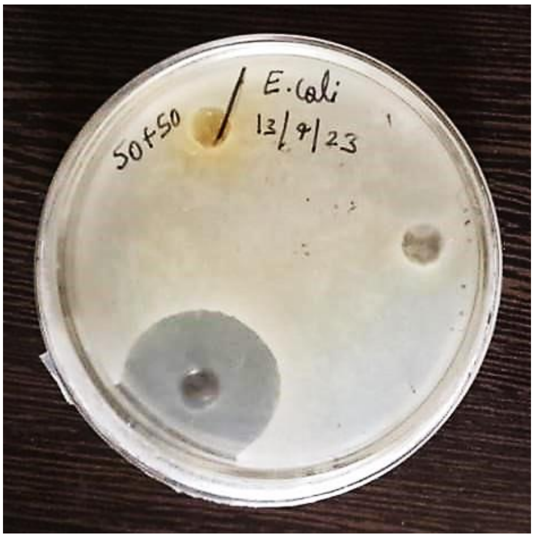

Figure 4. Antimicrobial testing (50% ethanol & 50% extract + ethanol + antibiotic + *E. coli*).

Fungal Strains

A. niger, Penicillium cyclopium, and Trichoderma viride

The crude extracts did not display any observable antifungal action in the initial screening. No inhibition zones were found around the wells. The lack of antifungal activity could be attributed to low concentrations of phenolics or organosulfur compounds in the unpurified extracts (Figure 5).

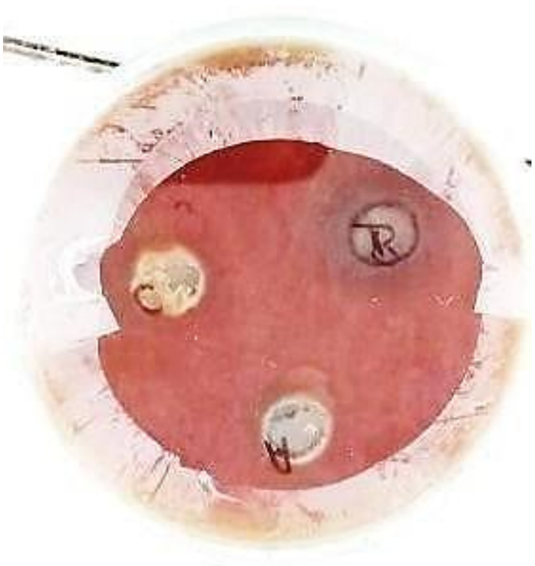

Figure 5. Antimicrobial testing for fungal strain.

Potentiation Through Column Chromatography

To enhance extract strength, crude ethanol extracts were purified by column chromatography. Fractions were concentrated and collected, thereafter, retested for antimicrobial activities. Column chromatography ethanol fractions showed enhanced zones of inhibition between 11 and 13 mm. This indicates that fractionation served to concentrate the active flavonoids (quercetin and kaempferol) with antibacterial activity (Figure 6).

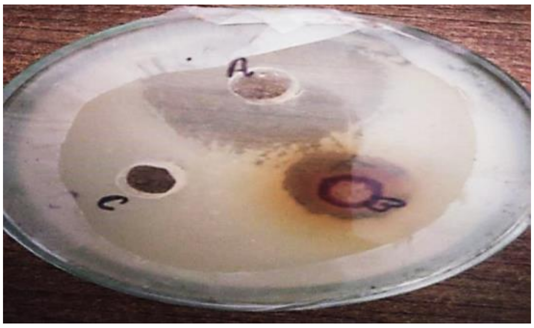

Figure 6. Antimicrobial testing (column extract + ethanol + antibiotic + *S. aureus).*

Against E. Coli

A slight improvement was noticed, with the ethanol column extract presenting a 6–7 mm zone of inhibition. While still weak by comparison, this activity was not detected in the crude state, proving that purification enhanced effectiveness (Figure 7).

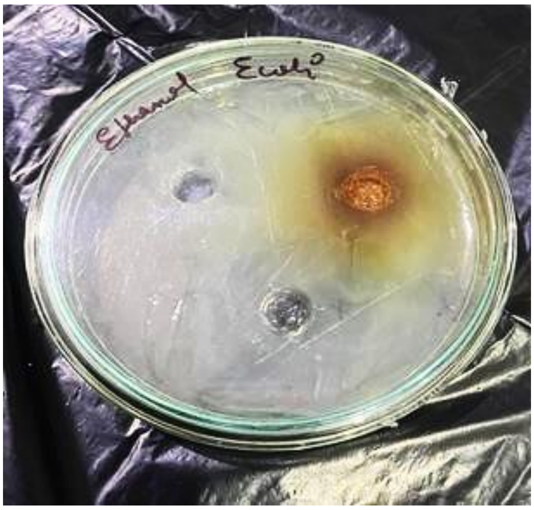

Figure 7. Antimicrobial testing (column extract + ethanol + antibiotic + *E. coli*).

Against A. Niger

The ethanol column extract presented a very slight, indistinct zone (0–1 mm) of inhibition, which was not reliably reproducible. Thus, no definite antifungal effect was established, even in the purified extracts.

CONTROLS AND VALIDATION

Positive Control (Streptomycin)

Streptomycin generated the expected large zones of inhibition (20–25 mm) with both bacterial strains, validating the assay conditions (Figure 8).

Negative Control (Solvent only)

There were no inhibition zones for ethanol or hexane alone, thus guaranteeing that the antimicrobial activity was due to the onion skin extracts (Figures 9–11).

COMPARATIVE ANALYSIS

These findings concur with existing research that highlights the high antibacterial and antioxidant effects of quercetin containing red onion peels. Literature is in agreement with the increased efficiency of ethanol when used as a solvent for phenolic extraction due to their increased polarity. Flavonoids, like quercetin, kaempferol, and luteolin, possibly concentrated on column chromatography, inhibit microbial cell wall formation and impair membrane permeability, especially in gram-positive bacteria like *S. aureus*.

Figure 8. Antibacterial testing (hexane extract + *E. coli* + antibiotic) (hexane extract + *S. aureus* + antibiotic).

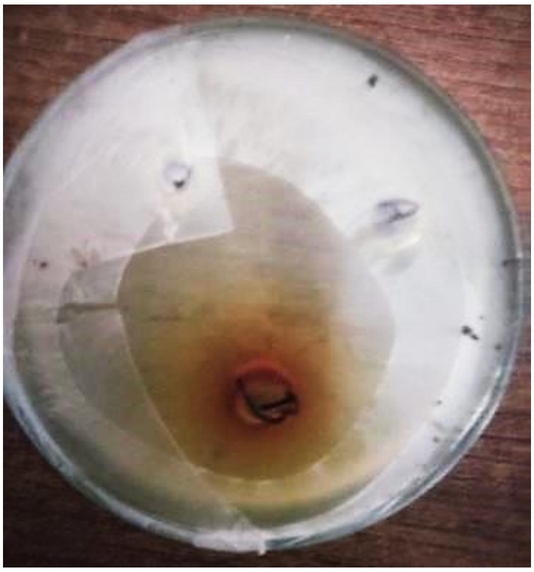

Figure 9. Antimicrobial testing (Soxhlet extract + ethanol+ antibiotic+ *E. coli*).

Figure 10. Antimicrobial testing (Soxhlet extract + ethanol + antibiotic + *S. aureus).*

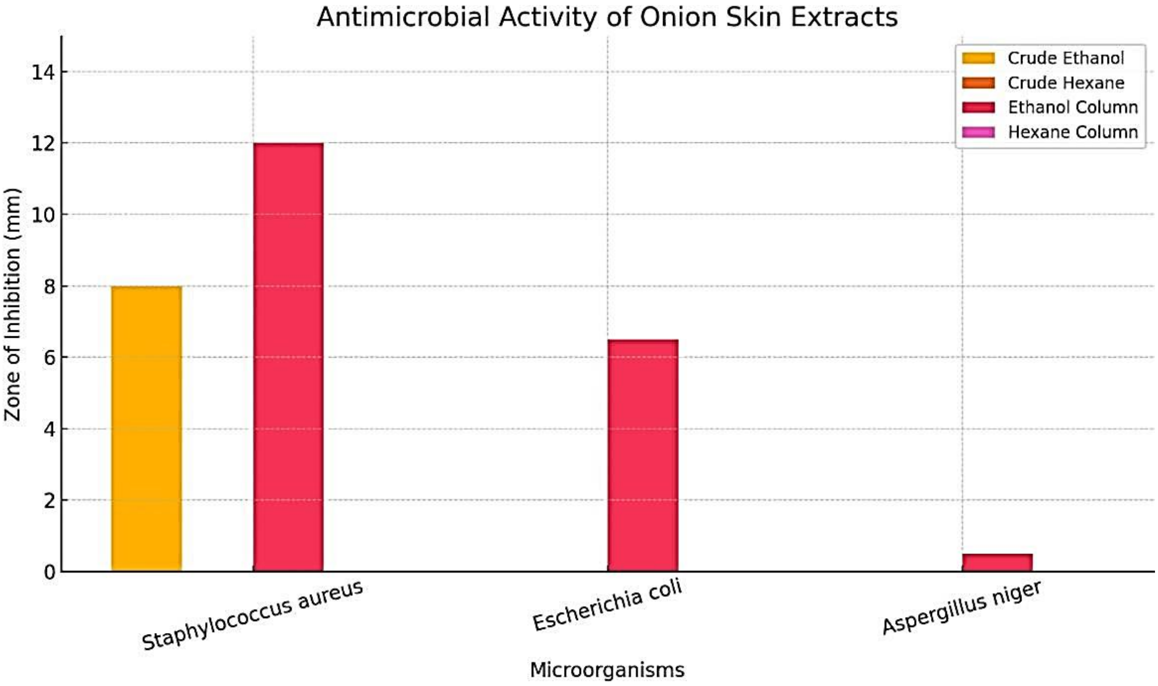

Figure 11. Zone of inhibition of onion skin extracts on *S. aureus*, *E. coli*, and *A. niger*.

DISCUSSION

This research examined the antimicrobial prospect of onion skin as a source of bioactive compounds, in conformity with increased interest in sustainable agro-food systems and agro-industrial peel valorization. Onion skin is a rich source of phenolic and flavonoid compounds, which are antioxidants, antimicrobial, anti-inflammatory, and anticancer in nature. Soxhlet extraction using ethanol followed by hexane, and column chromatography were employed to evaluate the antimicrobial activity of the extracts against various bacterial and fungal pathogens [29–34].

The findings showed that ethanol extracts possessed weak antimicrobial activity against *S. aureus*, but hexane extracts were characterized by weak or no antimicrobial activities. This is in accordance with the findings of earlier research that phenolic and flavonoid compounds are better extracted using polar solvents, such as ethanol. Ethanol supports the solubilization of hydrophilic bioactive compounds, like quercetin and kaempferol, because it is polar in nature. Conversely, hexane, being apolar, is efficient in recovering lipophilic constituents like essential oils but is poor at recovering polyphenols, accounting for the negligible antimicrobial activity reported [35–39].

The partial inhibition of *S. aureus* is attributed to the rather straightforward structure of gram-positive bacterial cell walls, which consist predominantly of a thick peptidoglycan layer, rendering them more vulnerable to damage by flavonoids and phenolics. Conversely, gram-negative bacteria, such as *E. coli*, have an outer membrane highly composed of lipopolysaccharides that prevent compound penetration. There was no notable antifungal activity against *A. niger*, *Trichoderma viride*, and *Penicillium cyclopium*. This can be explained by the low level of antifungal constituents in the crude extracts or the fungal strains' resistance to the compounds extracted. While sulfur compounds, such as diallyl disulfide, have demonstrated antifungal activity, they are optimally extracted using conditions suited for volatile sulfur constituents, not those for phenolics. Extraction efficiency is affected by several factors, such as solvent, temperature, and duration of extraction. Although Soxhlet extraction had ensured the recovery of thermally stable compounds effectively, the process might not have been suitable for thermolabile or volatile bioactive [40–44].

Column chromatography facilitated the separation of active fractions from crude extracts, demonstrating increased antimicrobial activity after purification. This supports the need for purification and standardization in phytochemical studies, as crude extracts may not accurately portray a material's bioactive potential. Microbial variability in susceptibility could have also had an effect. Laboratory strains may not represent the more general resistance patterns observed with clinical isolates, especially with *E. coli*, which tends to be multidrug resistant.

While direct antioxidant activity was not measured, the respective flavonoid and phenolic content of onion skin implies further functional uses. Previous works have successfully integrated onion skin extracts into foodstuffs, improving shelf life and antioxidant activity [45–48].

CONCLUSIONS

This work brings to light the high antimicrobial and antioxidant activity of onion extracts, particularly from the outer peels. Flavonoids, such as quercetin and kaempferol, are rich in onions, which had higher activity against gram-positive bacteria with nonaqueous extracts registering more activities compared to aqueous extracts. The extracts were also capable of inhibiting the growth of the usual foodborne pathogens including *S. aureus* and *E. coli*, which makes onions a good candidate for a natural source in creating safe and efficient antimicrobial agents. Although pure quercetin was not very active, the synergistic effect of several compounds in the extract seems to improve its overall activity. Such findings imply that onion extracts, especially from red onion skins, may be useful in food preservation, traditional medicine, and even cosmetics.

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