

Antimicrobial Activity of *Punica granatum*, *Citrus sinensis*, *Musa acuminata* Fruit Peel and Applications in Food Preservation

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ABSTRACT

This study evaluated the antimicrobial, antifungal, antioxidant, and phytochemical properties of ethanolic extracts derived from the peels of *Punica granatum* (pomegranate), *Citrus sinensis* (orange), and *Musa acuminata* (banana). The antimicrobial and antifungal activity against *E. coli* and *Aspergillus niger* was assayed using the agar well diffusion method. Antioxidant activity of the fruit peel extract was assessed using DPPH radical scavenging activity. The assay for total antioxidant capacity was utilized to verify the antioxidant potential and the phytochemical content, including total phenols and flavonoids. The study findings indicate that *Citrus sinensis* peel exhibited strong antibacterial and antifungal activity against *E. coli* and *Aspergillus niger* and facilitated shelf-life extension of tomato paste. The phytochemical analysis revealed that *Punica granatum* peels comprised bioactive compounds, thus supporting that fruit peels, often discarded as waste, can be effectively repurposed as natural preservatives in food systems as a sustainable alternative to chemical preservatives.

Keywords: Antimicrobial activity, Antioxidants, Fruit peels, Food preservation.

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INTRODUCTION

Fruit peels are a major component of agro-waste and are often overlooked despite being rich sources of bioactive compounds.^[1] These discarded peels are rich in phenolic compounds, proteins, lipids, fibre, polysaccharides, and calories, all of which contribute to their high antioxidant properties with higher nutritional value.^[2] Fruits and their peels are an excellent and abundant source of bioactive substances. Antioxidants, fibre, pectin, and starch occur as major components in pomegranate, citrus, mango, and fig peel. Due to their abundance in these major components, peels can be extremely important to the food, pharmaceutical, and packaging sectors.^[3] The Punicaceae family includes the pomegranate (*Punica granatum*), which is native to India and Iran. The pomegranate fruit has delicious, juicy seeds inside and a hard outer peel. It is renowned for being high in antioxidants and having a sweet-tart flavour.^[4,5] It is well known that the fruit and its peel contain high concentrations of several phytochemicals, such as tannins, flavonoids, and phenolic acids. It is believed that phytochemicals' varied properties account for their considerable potential as antioxidants and other health

advantages. Peels produce a significant number of by-products during processing, some of which are known to contain high levels of hydrolysable tannins.^[6] The scientific confirmation of Pomegranate Peel Extract (PPE) and its anti-inflammatory, anti-cancer, antibacterial, and antioxidant capabilities has drawn increased interest in pomegranate by-products.^[7]

Banana (*Musa acuminata*) belongs to the family Musaceae. A wholesome snack, bananas are high in potassium, vitamins, and dietary fiber.^[8] The banana peel comprises around 30% of the fruit; it is a significant by-product of the fruit due to its high nitrogen and phosphorus content. Banana peels are a good source of antioxidants and both soluble and insoluble fiber, and have several benefits.^[9] It can be used as a meat tenderizer, a tooth whitening agent, a polishing agent, and to prevent dyslipidemia and preserve vision. Nutrients, including potassium, dietary fiber, and antioxidants, can be found in banana peels. Moreover, they contain serotonin, which improves mood.^[10] The nutrients in banana peels are essential and decompose quickly; the yeast *Saccharomyces cerevisiae* has also utilized banana peels to make bioethanol.^[11] The citrus fruit orange is a member of the Rutaceae family,^[12] and an excellent source of dietary fiber, antioxidants, and vitamin C. Peels continue to be the largest byproduct, making up 50-65% of the fruit's overall weight. The plant material's antioxidant properties are a result of the existence of several active phytochemicals, such as plant sterols, flavonoids,



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terpenoids, carotenoids, coumarins, and curcumin, as well as vitamins and lignin.^[13] Bioactive substances found in citrus fruits and liquids include antioxidants, including ascorbic acid, flavonoids, phenolic compounds, and pectins, which are crucial for human nutrition.

The present study aims to identify the antimicrobial and antifungal activity in fruit peels. The research specifically examines determining the presence of antimicrobial and antifungal compounds in the fruit peels (*Citrus sinensis*, *Punica granatum*, *Musa acuminata*). It also aims to assess the effectiveness of these antimicrobial and antifungal properties against common pathogens such as bacteria and fungi. The study also envisages to assay and evaluate antioxidant activity and phytochemicals in fruit peels.

MATERIALS AND METHODS

The study comprised steps that involved sample collection, microbial isolation, extract preparation, and antimicrobial testing. Fruits (*Citrus sinensis*, *Musa acuminata*, *Punica granatum*) and *Solanum lycopersicum* (fresh, rotten) were purchased from the local markets. Appropriate culture media to grow a wide variety of microbes were prepared. Four different types of culture media were prepared: nutrient agar, MacConkey agar, EMB agar, and XLD agar, respectively. Serial dilution and spread plating techniques were adopted to isolate bacteria from the sample by pouring the prepared agar medium into sixteen petri plates. The plates were incubated at 37°C for 24 hr. From the incubated plate, based on the structure, pigmentation, and appearance, colonies were identified as *Escherichia coli* bacteria and *Aspergillus niger* on the morphology and pigmentation described by Kanatt^[14] and Smaoui.^[15] This was followed by streak plating to obtain pure isolates. The fruit peels were washed, cleaned, crushed, and ethanol was added, mixed well, and kept aside. The peels were extracted using the Clevenger apparatus method. After 8 hr, *Citrus sinensis* oil was formed from the *Citrus sinensis* peel, and the extract used for the study. *Musa acuminata* peel, *Punica granatum* peel, and ethanol were all mixed and left to dry for 24 hours by the evaporation method.^[16] Subsequently, the fruit peel content was scraped using sterilized water and transferred to a centrifuge tube and stored in the refrigerator.

The antibacterial activity of the fruit peel extract was evaluated by using the agar well diffusion assay. The sterile MHA (Muller Hinton agar) plate and sterile SDA (Sabouraud Dextrose Agar) plates were inoculated with *Escherichia coli* and *Aspergillus niger*, respectively. Using a sterile pipette tip, wells were made in the inoculated agar medium. 1 mL of fruit peel extract was added to the wells in the plates using a micropipette. The antifungal activity of fruit peel extracts was evaluated by using an agar well diffusion assay by means of a sterile cotton swab. Using sterile pipette tips, wells were made in the inoculated agar medium. 1 mL of the extracts were added to the wells in the plates using

a micropipette. Positive control (fluconazole) as well as negative control (distilled water) were added to the respective wells. Then the plates were incubated at 37°C for 24 hr.^[17] To evaluate the shelf life, tomato paste was prepared and treated with peel extracts, stored at various conditions (room temperature, refrigerated, and humidity-controlled), and monitored at 0, 3, and 7 days. Microbial load on paste samples was assessed using serial dilution followed by plating on PCA (Plate Count Agar) and DRBCA (Dichloran Rose Bengal Chloramphenicol Agar). Plates were incubated at 37°C for 24 hr until the presence or absence of colonies appeared.

In addition to the antimicrobial assays, the DPPH (2,2-Diphenyl-1-Picrylhydrazyl) assay at 517 nm was employed to assay the antioxidant activity of the peel extracts.^[18] Ascorbic acid and distilled water served as positive and negative controls, respectively. For the isolation of bioactive constituents such as alkaloids (Wagner's test), glycosides (brown ring test), saponins (foam test), terpenoids (Salkowski's test), phenols (ferric chloride test), tannins (gelatine test), and flavonoids (alkaline reagent test), phytochemical screening was conducted using standard qualitative methods.^[19] The overall antibacterial and antioxidant activity of the extracts was augmented by the existence of these constituents.

RESULTS AND DISCUSSION

To isolate spoilage microorganisms, samples of *Solanum lycopersicum* (tomato) were purchased and allowed to rot at ambient temperature. Serial dilution up to 10⁻⁸ was performed to isolate both spoilage bacteria and fungi. The bacterial and fungal isolate obtained from plating was identified as *Escherichia coli*, *Aspergillus niger*, respectively. The antibacterial activity of *Citrus sinensis*, *Musa acuminata*, and *Punica granatum* peel extracts were evaluated by using agar well diffusion assay, and the diameters of clear zones were measured after 24 hr of incubation at 37°C.

As evident from Table 1, all the fruit peels exhibited inhibitory activity against the bacterial pathogen *Escherichia coli* by the zone of inhibition assay, with *Citrus sinensis* showing the highest inhibition zone (26 mm). Thus, it was the most effective among the tested peels. The antifungal activity of *Citrus sinensis*, *Musa acuminata*, and *Punica granatum* peel extracts was evaluated by using agar well diffusion assay, and the diameters of clear zones were measured after 24 hr of incubation at 37°C. The findings concur with Rather,^[20] who reported that fruit peels have high anti-microbial capacity, especially citrus and pomegranate peels, which showed 21 mm inhibition zone against *E. coli*. The researchers emphasized that citrus peels have significant inhibitory activity against a vast range of bacterial species, such as *E. coli*, *S. aureus*, and *P. aeruginosa*.

As evident from Table 2, all the fruit peels exhibited inhibitory activity against the fungal pathogen *Aspergillus niger* by the zone of inhibition assay. *Citrus sinensis* again exhibited the strongest antifungal activity, showing the greatest inhibition zone (20 mm)

Table 1: Inhibition zone diameter (mm) of fruit peel extract against *E. coli*.

Fruit Peel Sample	Test Control (mm)	Positive Control (mm)
<i>Citrus sinensis</i>	26	31
<i>Musa acuminata</i>	19	31
<i>Punica granatum</i>	14	31

Table 2: Inhibition zone diameter (mm) of fruit peel extract against *Aspergillus niger*.

Fruit peel Sample	Test Control (mm)	Positive Control (mm)
<i>Citrus sinensis</i>	20	31
<i>Musa acuminata</i>	14	31
<i>Punica granatum</i>	14	31

Table 3: Shelf-life study of *Solanum lycopersicum* – (Day Zero).

Sample	Plate Count Agar (PCA)		Dichloran Rose Bengal Chloramphenicol Agar (DRBC)	
	10 ⁻²	10 ⁻³	10 ⁻²	10 ⁻³
<i>Citrus sinensis</i>	9	3	60	5
<i>Musa acuminata</i>	10	6	80	10
<i>Punica granatum</i>	15	6	90	15
Control	18	10	110	30

Table 4: Shelf-life study of *Solanum lycopersicum* (Day three).

Sample	Plate Count Agar (PCA)		Dichloran Rose Bengal Chloramphenicol Agar (DRBC)	
	10 ⁻²	10 ⁻⁴	10 ⁻²	10 ⁻⁴
Room temperature	18	10	TNTC	TNTC
Room temperature - Control	TNTC	TNTC	TNTC	10
Refrigerator	-	-	-	-
Refrigerator - control	TNTC	TNTC	TNTC	40
Accelerated	18	9	TNTC	20
Accelerated - control	TNTC	TNTC	TNTC	TNTC

among the tested samples. To assess the shelf-life, tomato paste samples were treated with the three peel extracts and stored under various conditions: room temperature, refrigerated, and humidity-controlled. Microbial load counts were monitored on Day Zero, Day Three, and Day Seven.

As evident from Table 3, when the shelf life of *Solanum lycopersicum* paste was tested, *Citrus sinensis* showed the lowest microbial count, indicating effective shelf-life as the number of colonies formed were few in number.

When the shelf-life of *Solanum lycopersicum* paste was tested with *Citrus sinensis* peel extract, significant antimicrobial activity was observed. Therefore, only *Citrus sinensis* peel extract was considered for the third-day shelf-life test of *Solanum lycopersicum* paste. Room temperature, room temperature control, accelerated, accelerated control, refrigerator, and refrigerated control conditions were the different conditions of observation. On Day three, refrigerated samples with *Citrus sinensis* extract showed no microbial growth, highlighting the benefit of low temperature storage. Therefore, it can be recommended that refrigerated

condition is best to increase the shelf life of *Solanum lycopersicum* paste with the addition of *Citrus sinensis* peel extract (Table 4).

When the shelf-life of *Solanum lycopersicum* paste was tested with *Citrus sinensis* peel extract, significant antimicrobial activity was observed. Therefore, only *Citrus sinensis* peel extract was taken for the seventh-day shelf-life tester of *Solanum lycopersicum* paste under different conditions, as depicted in Table 5. Again, *Citrus sinensis* extract preserved the tomato paste best under refrigeration, suggesting a synergistic effect of temperature and natural antimicrobial compounds. Therefore, refrigerated conditions are best to increase the shelf life of tomato paste with added *Citrus sinensis* peel extract. The findings concur with Sharma^[21] who also reported that citrus peels can be effectively used in meat and bakery products to enhance shelf-life.

ANTIOXIDANT ACTIVITY OF FRUIT PEEL EXTRACTS

The fruit peel of *Punica granatum* and *Musa acuminata* were assayed for antioxidant activity. As the *Citrus sinensis* peel is extracted with oil. The antioxidant activity could not be tested.

Table 5: Shelf-life study of *Solanum lycopersicum* – Day seven.

Sample	Plate Count Agar (PCA)		Dichloran Rose Bengal Chloramphenicol Agar (DRBC)	
	10 ⁻²	10 ⁻³	10 ⁻²	10 ⁻³
Room temperature	12	10	80	70
Room temperature - control	Too Numerous To Count	TNTC	TNTC	TNTC
Refrigerator	1	-	35	27
Refrigerator - control	TNTC	TNTC	TNTC	TNTC
Accelerated	18	15	200	130
Accelerated - control	TNTC	TNTC	TNTC	TNTC

Table 6: DPPH free radical scavenging activity (%) of *Musa acuminata* peel extract.

Sample	OD value	% of free radical scavenging
Negative control	1.44	
Positive control	0.23	84.5
0.1 g of sample	0.33	77.8
0.2 g of sample	0.22	85.2
0.3 g of sample	0.48	67.7
0.4 g of sample	0.26	82.5
0.5 g of sample	0.38	74.4

Table 7: DPPH free radical scavenging activity (%) of *Punica granatum* peel extract.

Sample	OD value	% of free radical scavenging
Negative control	0.59	
Positive control	0.02	66.1
0.1 g of sample	0.17	71.1
0.2 g of sample	0.13	77.9
0.3 g of sample	0.15	74.5
0.4 g of sample	0.22	62.7
0.5 g of sample	0.21	64.4

As evident from Tables 6 and 7, both extracts possessed antioxidant activity, with *Musa acuminata* depicting up to 85.2% scavenging activity, though the relationship between concentration and activity was not strictly linear. It can also be inferred that *Punica granatum* peel extract exhibited free radical scavenging activity, although it was not proportional to the concentration. These results are in alignment with the review by Vu,^[22] which reported that banana peel is rich in catecholamines, dopamine, and flavonoid compounds with known antioxidant properties. Similarly, pomegranate peel is highlighted for its high content of punicalagin, ellagic acid, and gallic acid compounds with powerful antioxidant activity. The strong DPPH scavenging ability reflected in the current study suggests the high bioactivity of these peels.^[23]

PHYTOCHEMICAL ANALYSIS OF EXTRACTS

Phytochemical evaluation was carried out using *Punica granatum* and *Musa acuminata* peel extracts.

As evident from Table 8, the results showed that *Punica granatum* peel extract was rich in various phytochemicals, while *Musa acuminata* extract tested negative for all compounds. This aligns with the observations in the review by Selahvarzi,^[24] which detailed that pomegranate peel contains a diverse range of bioactive phytochemicals, including punicalin, catechin,

Table 8: Phytochemical analysis of fruit peel extract.

Phytochemicals	<i>Punica granatum</i> Peel Extract	<i>Musa acuminata</i> peel Extract
Alkaloids	+	-
Glycosides	+	-
Saponin	+	-
Terpenoids	+	-
Phenols	+	-
Tannins	+	-
Flavonoids	+	-

+ indicate presence, - indicate absence.

kaempferol, and various phenolic acids, which contribute to its therapeutic and antioxidant properties. While banana peel does contain many phenolic compounds, its composition can vary significantly based on extraction methods and maturity.

CONCLUSION

The findings of the study indicate ethanolic peel extracts of *Musa acuminata*, *Punica granatum*, and *Citrus sinensis* possess potential antibacterial, antifungal, antioxidant, and phytochemical properties. In the qualitative test, *Musa acuminata* indicated negative for bioactive compounds, whereas *Punica granatum* peel

indicated positive trends, but of all, *Citrus sinensis* peel extract was observed to possess the highest antibacterial and antifungal activity, particularly against *Aspergillus niger* and *Escherichia coli*. Its use in tomato paste notably enhanced shelf life under different storage conditions, particularly under refrigeration, where microbial load was low. *Musa acuminata* and *Punica granatum* peel extracts possessed high antioxidant activity, with *Musa acuminata* achieving as much as 85.2% DPPH radical scavenging efficiency. These findings justify the valorisation of fruit peels, which were earlier discarded as waste, and were introduced as green and inexpensive natural preservatives with a high possibility of application to minimize microbial contamination and enhance product shelf-life.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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