



Phytochemical Analysis, Antioxidant, and Antibacterial Properties of Waste Fruit Peels (Orange, Banana, Apple, and Lemon)

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Abstract

Fruit peels contain secondary metabolite compounds with potential antioxidant properties. This research aims to investigate the phytochemical composition and antioxidant properties of extracts from the peels of orange, banana, apple, and lemon. The peels were extracted using distilled water through cold percolation. The percentage yield was determined, followed by qualitative phytochemical screening. Total phenolic and flavonoid presence were quantified. Additionally, antioxidant properties were evaluated using the DPPH radical scavenging assay, and antibacterial activities were assessed. The yield percentage of fruit peel extracts followed the order: of banana > apple > orange > lemon. The phytochemical analysis illustrated the presence of the alkaloid, reducing and non-reducing sugars, coumarins, saponins, flavonoids, phenols, tannins, glycosides, terpenoids, and steroids. TPC values (mg GAE/g) were highest in lemon (132.22 ± 3.95) and lowest in banana (60.69 ± 2.005). TFC values (mg QE/g) were highest in orange (4.373 ± 1.667) and lowest in lemon (1.204 ± 0.277). Antioxidant activity (DPPH scavenging) was highest in oranges (56.29%) and lowest in bananas (17.11%). The antibacterial activity of fruit peel, tested using agar well diffusion, showed no inhibition against *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *S. aureus*. The research quantified TPC and TFC and evaluated antioxidant and antibacterial activities. While the extracts demonstrated moderate antioxidant potential, their antibacterial properties were negative, suggesting they may be more suitable as natural antioxidants rather than antimicrobial agents.

Keywords: Antioxidant activities, secondary metabolite, Total phenolic content, total flavonoid presence, Bioactive.

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Introduction

Peels of fruit are good sources of bioactive compounds with anticipated use in pharmaceutical industries. These peels contain significant amounts of phytochemicals, including phenolics, flavonoids, tannins, and terpenoids, which enhance their antioxidant properties[1]. Antioxidants make a significant contribution to balancing radicals, decreasing oxidative stress, and also help prevent chronic diseases like cancers[2], heart-related diseases, and neurodegenerative conditions[3][4]. Given the growing interest in natural antioxidants, the exploration of fruit peels as sustainable sources of bioactive compounds has gained significant attention.

Among various fruits, (*Citrus sinensis*) orange, (*Musa paradisiaca*) banana, (*Malus domestica*) apple, and (*Citrus limon*) lemon peels have demonstrated promising phytochemical profiles[5][6]. The qualitative and quantitative analysis of these phytochemicals is crucial for understanding their potential benefits for health and industrial applications[7].

Through qualitative and quantitative determination of the presence of flavonoid as well as phenolic content, this research seeks to highlight the capability of fruit peels as

a sustainable source of secondary metabolite components[8]. The findings could contribute to waste valorization strategies and explore the potential applications of natural antioxidants for commercial applications as well as their effectiveness against clinically significant bacteria.

Materials and Methods

This study follows an experimental and observational research design to evaluate the phytochemical composition of tested fruits, involving qualitative and quantitative phytochemical screening and antioxidant activities assessment to determine the bioactive ability of the extracts. Fruits (orange, banana, apple, and lemon) were collected, cleaned, peeled, dried at 35°C for a week, ground into powder, and stored for further processing. The cold percolation method was used to extract fruit peel extracts by agitating 17g of powdered peel with 100 mL sterile water, followed by filtration and concentration using a Rotary Evaporator[9]. After that, the resulting dried extracts were placed at 4°C in Eppendorf tubes, and stock suspensions of different concentrations were prepared at 100mg/mL and 200mg/mL. Aqueous



extraction was selected as an extracting solvent for its nontoxic, and suitable for food and pharmaceutical applications, despite the higher efficacy of methanol and ethanol[10][11].

Yield Calculation

The percentage of Yield was estimated using the given formula[12].

Yield (%) = (Extract weight / Total dry weight taken) × 100%

Qualitative Phytochemical Analysis

Phytochemical analysis was conducted, (n=3) on aqueous extracts to find out the key chemical constituents such as glycosides, alkaloids, flavonoids, and others, using color reactions with various reagents. The method for conducting these tests was by the literature[13, 14].

Tests for alkaloids

A small quantity of the pure extract was mixed with some drops of oil. HCL. After filtering the mixture, the filtrate was analyzed using Dragendroff reagent (potassium bismuth iodide solution), Wagner reagent, and Myer reagent. Formation of orange color in the Dragendroff test, a reddish-brown precipitate in the Wagner test, and turbidity occurred in the Myer test, indicating the availability of alkaloids[15].

Test for glycosides

Killer-Killani test: To hydrolyzate, 2 ml glacial acetic acid with a single drop of ferric chloride was added[16]. The formation of a brown ring at the interference of two layers indicated the presence of deoxy sugar, and the greenish ring layer indicated cardiac glycoside[17].

Test for Coumarin: 1 ml of 10% NaOH was mixed with 1 ml extract. The formation of a yellow-colored solution confirmed the availability of coumarin.

Test for flavonoids

Shinoda test: A small amount of the extract was agitated with a few ml of water and the mixture was dissolved in 5 ml of alcohol (95%) and reacted with a few drops of conc. HCl and 0.5gm of magnesium metal. The formation of a pink color within a solution demonstrated the detection of flavonoids [15].

Test for phenol

Ferric chloride test: 3mL of the filtrate was subjected to newly prepared neutral ferric chloride suspension. The appearance of violet coloration illustrated the presence of phenols[18, 15].

Test for tannins

An amount of the extracted substance was diluted in water, warmed, and filtered. The resulting liquid was

performed for a Lead acetate test when 3mL of the filtrate was treated with 10% lead acetate solution. A white precipitate suggested the existence of phenolic compounds[15].

Tests for carbohydrate

Benedict's test: A small quantity of the peel extract was subjected to 4mL of distilled water and filtered. The filtrate was added to Benedict's test for the detection of carbohydrates. The observation of a brick-red colored precipitate confirmed the presence of carbohydrates.

Fehling test: To detect the presence of carbohydrates, a small amount of the alkaline hydrolyzed peel extract was subsequently subjected to an equal mixture of Fehling's solution A and B. The formation of a brick-red precipitate of cuprous oxide confirmed their presence[15].

Test for saponins

Foam test: A 1 gm extract sample was thoroughly mixed by shaking with 20 distilled water in a cylinder for up to 15 minutes. The formation froth was kept for an hour conforming to the presence of saponin glycoside[15].

Detection of terpenoids

Salkowski test: 2 mL of chloroform and 3 mL of conc. H₂SO₄ were mixed with 5ml of aqueous extracts[19]. A reddish-brown color forming at the interface verified the presence of terpenoids [20].

Detection of steroids

The crude extract was first combined with 2 mL of chloroform. Concentrated sulfuric acid was then gently added along the inner wall of the test tube, resulting in a red color in the lower chloroform layer, confirming the presence of steroids [21].

Quantitative Phytochemical Screening

The Folin-Ciocalteu method was used to assess the total phenolic content, where plant samples and standard Gallic acid solutions were reacted with Folin-Ciocalteu reagent and sodium carbonate (Na₂CO₃)[22], followed by UV spectrometric analysis at 765 nm. The concentration of polyphenolic compounds was written as milligrams of Gallic acid equivalent per gram of dry weight (mg GAE/gm) using a Gallic acid standard curve[23, 24]. The total flavonoid content (TFC) was measured using the aluminum chloride colorimetric method[24], with Quercetin as the standard for calibration[13, 25]. To prepare a 10% aluminum chloride (AlCl₃) solution, 1 g of AlCl₃ was diluted in distilled water. In addition, a 1 M potassium acetate solution was prepared by diluting 0.98 g of potassium acetate in 10 mL of distilled water. A stock solution (0.1 mg/mL) of Quercetin was prepared by

treating 1 mg in 10 mL of methanol, and working solutions (10–80 µg/mL) were obtained by dilution. In the modified UV spectrometric method, different concentrations of standard Quercetin and fruit peel samples were reacted with ethanol, AlCl_3 , and potassium acetate, incubated in the dark for half an hour, and evaluated at 415 nm[26]. The Quercetin standard curve was used to outline the total flavonoid content as mg QE per gram of dry weight[27].

DPPH Free Radical Scavenging Assay

To prepare a 0.1 mM DPPH suspension, 3.94 mg of DPPH was dissolved in methanol, and the final volume was adjusted to 100 mL [28]. A gallic acid solution was prepared by dissolving 2 mg of gallic acid in 2 mL of methanol. The free radical scavenging activity of the extracted substance was tested using the DPPH assay, where equal volumes of DPPH and samples of different concentrations were kept in the dark for 15 minutes. Absorbance was measured at 517 nm, and the scavenging capability was determined based on the reduction in absorbance.

% scavenging = $(A_0 - A_1) / A_0 \times 100\%$ [29].

Where,

A_0 = absorbance of distilled water.

A_1 = absorbance of DPPH with test or reference sample

The % scavenging was calculated for each fruit peel extract. Lower absorbance of the reaction mixture denotes greater free radical scavenging properties[30].

Antibacterial Activity Assay

Four fruit peel extracts were tested[31]. Bacterial cultures were cultured overnight in Nutrient Broth, adjusted to 0.5 McFarland turbidity[32], and spread on Mueller-Hinton Agar using the carpet culture method. Sterile cork was used to form four 6mm wells per plate. Each well was loaded with 50µL of fruit peel extract and positive control broad-spectrum antibiotics of the same volume. Ofloxacin (25µg/mL) served as a positive control, whereas sterile water was the negative control.

Statistical analysis

All experiments were carried out in triplicates ($n = 3$) using independent sample preparation for each fruit peel. Each measurement was taken three times to ensure accuracy and reproducibility. The obtained results were expressed as mean $\pm$ standard error of the mean, statistical analysis was conducted using one-way ANOVA followed by Tukey's post hoc test, with a significant level of $p < 0.05$. Data were analyzed using IBM SPSS (version 21.0).

Result

Yield percentage of various extracts

About 17 grams of dried powder was weighed and extracted by distilled water. The percentage yield of different extracts was calculated.

The extraction yield percentage of fruit peel extracts using distilled water was highest in banana (23.5%), followed by apple (23.11%) and orange (16.76%), while lemon had the lowest yield (10.05%), mean $\pm$ SEM ($n = 3$); $p < 0.05$ considered significant. This variation in yield may be influenced by the differences in the chemical composition and water solubility of compounds present in each fruit peel.

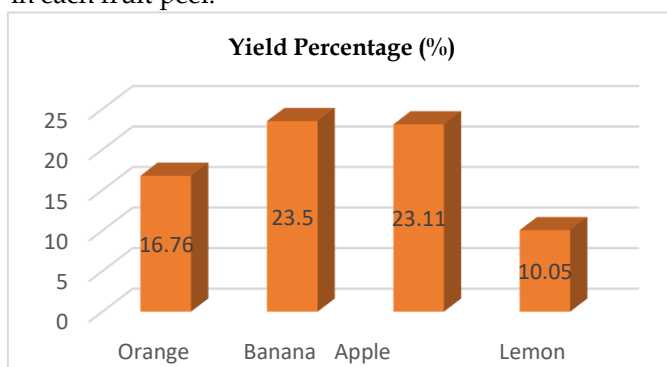


Figure 1. Percentage yield of different extracts of fruit peels (orange, banana, apple, and lemon). mean $\pm$ SEM ($n = 3$).

Qualitative Assessment of Secondary Metabolites in Plants

Qualitative phytochemical analysis was performed, ($n = 3$) using chemical reagents, based on color changes and precipitate formation with the sample.

Table 1. phytochemical analysis of different extracts, of fruit peels (orange, banana, apple, lemon).

SN	Phytoconstituent	Orange	Banana	Apple	Lemon
Alkaloids					
1	Dragendroff Test	+	+	-	+
2	Wagner Test	+	+	-	+
3	Mayer's Test	+	+	-	+
Carbohydrate Test					
4	Benedict Test	-	-	+	-
5	Fehling Test	+	+	+	+
6	Coumarin	-	-	-	+
7	Saponin	-	-	-	-
8	Flavonoid	-	-	-	+
9	Phenol	-	-	-	-
10	Tannin	+	+	+	+
Glycoside					
11	Killer-killing test	+	+	+	+
12	Terpenoid	+	+	-	-
13	Steroid	+	+	+	-

Note: The '+' sign denotes the presence and the '-' sign denotes the absence of the respective phytochemical, ($n = 3$)

Phytochemical screening showed alkaloids in oranges, bananas, and lemons, while carbohydrates were present in all peels except in Benedict's test. Coumarin and

flavonoids appeared only in lemon, while tannins and glycosides were found in all. Terpenoids were detected in orange and banana, and steroids in all except lemon.

Quantitative Phytochemical Screening

Table 2: Quantitative measure of TPC and TFC of fruit peel extract [orange, banana, apple, and lemon]. Values are expressed as mean $\pm$ SEM (n = 3). Different superscript asterisk denotes a significant difference at $p < 0.05$, Turkey post hoc test

Fruit peel Extracts	TPC (mg GAE/gm)	TFC (mg QE/gm)
Apple	73.53 $\pm$ 2.333	1.639 $\pm$ 0.663
Orange	118.68 $\pm$ 9.375**	4.373 $\pm$ 1.667*
Banana	60.69 $\pm$ 2.005	2.1706 $\pm$ 0.481
Lemon	132.22 $\pm$ 3.950**	1.204 $\pm$ 0.277

Figure 2. Calibration curve of total phenolic content

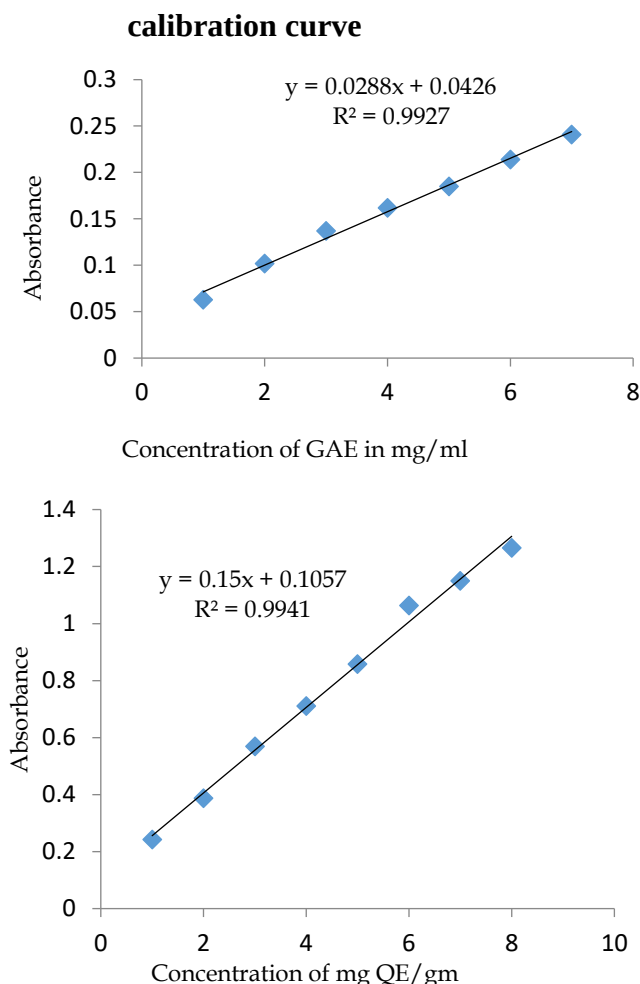


Figure 3. Calibration curve of total flavonoid content

The TFC of various fruit peel extracts was analyzed considering mean $\pm$ SEM (n = 3); $p < 0.05$ as a level of significance, in milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g), based on a calibration curve prepared with gallic acid. Among the extracts, lemon peel had the highest TPC (132.22 $\pm$ 3.950 mg

GAE/g), followed by orange (118.68 $\pm$ 9.375 mg GAE/g), apple (73.53 $\pm$ 2.333 mg GAE/g), and banana (60.69 $\pm$ 2.005 mg GAE/g). Similarly, the total flavonoid content was indicated as milligrams of Quercetin equivalent per gram (mg QE/g). Orange peel extract had the greatest TFC (4.373 $\pm$ 1.667 mg QE/g), and then by banana (2.1706 $\pm$ 0.481 mg QE/g), apple (1.639 $\pm$ 0.663 mg QE/g), and lemon (1.204 $\pm$ 0.277 mg QE/g).

Antioxidant Activity

DPPH radical scavenging activity test

Mostly phenolic compounds, carotenoids, tocopherol, and ascorbic acid-containing antioxidant substances possess free radical scavenging activities because of the presence of hydroxyl groups[33]. Therefore, extracts that have significant amounts of phenol and flavonoids can be the strongest indicator of antioxidant properties [26].

The percentage inhibition of apple, orange, banana, and lemon was obtained at absorbance 517nm.

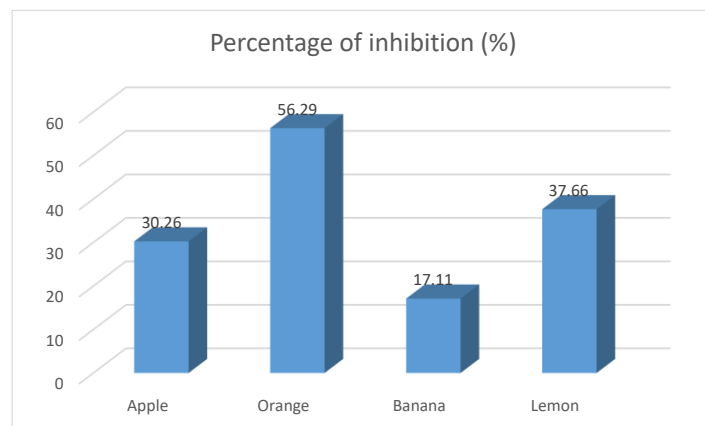


Figure 4. Result of DPPH scavenging

Antibacterial activity

Table 3. Antibacterial activity of different fruit peels with a concentration of 100mg/ml 200mg/ml

Bacteria (ATCC Culture)	Fruit Peel Extract	Positive Control (25 μ g/mL)	Negative Control (Distilled Water)	Concentrations	
				100 Mg/mL	200 Mg/mL
<i>Escherichia coli</i> ATCC 25922	Orange	50 mm	-	-	-
<i>Staphylococcus aureus</i> ATCC 25923	Banana	55 mm	-	-	-
<i>Pseudomonas aeruginosa</i> ATCC 9027	Apple	40 mm	-	-	-
<i>Klebsiella pneumoniae</i> ATCC 700603	Lemon	30 mm	-	-	-

The positive control showed significant antibacterial activity with inhibition zones ranging from 30mm to 55mm. However, the fruit peel extracts (orange, banana, and apple) and the negative control (distilled water)

revealed negative antibacterial activity against any tested bacteria.

Discussion

Based on the literature review and traditional medicinal uses of given fruit peel extracts, they were selected for the study as they have good antimicrobial properties and flavoring activity[35]. The fruit peel powders were extracted using distilled water, which was more suitable than other solvents like methanol, ethanol, ethyl acetate, and chloroform due to their higher polarity. The cold percolation method, involving filtration and evaporation at 50°C, was used for extraction. Distilled water's higher boiling point (100°C) compared to methanol (80°C) resulted in a longer extraction time, leading to a lower yield compared to ethanol, which has a higher concentration and faster extraction rate[36][37].

The phytochemical analysis of fruit peel extracts was conducted to determine pharmacologically active components, serving as a preliminary method for detection. The four fruit peel samples contained alkaloids, carbohydrates, coumarin, saponin, flavonoids, phenolic compounds, deoxy sugar, cardiac glycosides, terpenoids, and steroids. Phytochemical screening reveals the predominant components of plant extracts as well and it also helps identify bioactive agents for potential drug synthesis, playing a vital role in the discovery of new therapeutically and industrially valuable compounds such as saponins, steroids, tannins, and terpenoids[38].

Plants produce phenolic compounds through the shikimic acid and pentose phosphate pathways[39] and play a key role in antimicrobial activity due to their structure of benzene rings with hydroxyl substituents[40]. Phenolic compounds accumulate at infection sites in plants to inhibit microbial pathogen growth and enhance plant resistance through an induced hypersensitive response. Common phenolic compounds found in fruit peel extracts include hydrobenzoic acids, hydroxyl cinnamic acids, and simple phenols. These compounds are widely used in food preservation for their antioxidant properties and in the development of photographic film. In this study, lemon peel exhibited a higher phenolic content than orange, banana, and apple, the result is consistent with the study conducted by [41]. In comparison to other studies, the flavonoid content in fruit peels has been found to vary significantly. [42] reported higher flavonoid concentrations in citrus fruits, which aligns with the higher TFC value observed in orange peel in this study. Similarly, [43] found that flavonoids contribute significantly to the antimicrobial

properties of fruit peels, which supports the findings that flavonoids in the studied peels may play a crucial role in combating microbial threats.

Antioxidants prevent cells from damaging effects caused by free radicals, which are produced by the body in response to various environmental and physiological pressures[44]. The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was used to calculate the antioxidant property of fruit peel extracts[45], where antioxidants provide hydrogen atoms to DPPH free radicals, converting their violet color to yellow.

These results indicate that orange peel exhibited the highest antioxidant activity, which is consistent with findings from previous studies[46], where DPPH scavenging increased with the concentration of extracts. While, the opposite result was observed by [47], where Bananas showed the greatest antioxidant activity rather than orange, using the ferric reducing antioxidant power method, which indicates that the analyzing technique also plays a crucial role. Additionally, the combined action of vitamins, carotenoids, and pigments may have a significant role in the outcome through synergistic interactions[48]. Synergistic effects from other substances, like vitamins, carotenoids, and pigments, could have amplified the higher antioxidant activity observed in orange peel, aligning with the results [49].

The fruit peel extracts showed no antibacterial activity, however [50] observed significant response and gram-negative bacteria were more susceptible than gram-positive. Negative results in our research are likely due to low active compound concentration or poor agar diffusion. While [31] observed inhibition, possibly due to differences in extraction methods and solvent used. Further studies using optimized extraction techniques or higher concentrations may improve efficacy[51].

One of the major limitations of the research is the absence of dose-response analysis and determination of IC₅₀ for the antioxidant properties of the fruit peel extracts. The DPPH assay was analyzed at a single concentration of each extract because of the limited availability of standard gallic acid and DPPH reagent to run triple replicates across multiple concentrations. Given the limitations of single-dose testing, upcoming work should focus on a concentration gradient approach with appropriate curve-fitting models to determine IC₅₀ and comparative analysis with standard compounds for benchmarking extract efficacy.

Conclusion

In conclusion, extracts of the fruit peel revealed measurable antioxidant activities at a single tested

concentration, among them, orange peel showed the highest antioxidant ability. Phytochemical screening tests detected the presence of secondary metabolite compounds, including phenolics, flavonoids, and saponins, which are generally linked with antioxidant and antimicrobial activities. In contrast, moderate antioxidant properties and no antibacterial activities were observed. These outcomes may be due to the lack of appropriate concentration and limited diffusion of aqueous solvents. As a result, while orange reveals relative potential in this regard, its practical applications require further verification by testing in multiple concentrations. Upcoming research should focus on isolating specific constituents, optimizing necessary techniques for extraction and purification, broader solvent comparison in multiple extract concentrations, and quantitative phytochemical validations are recommended. Increasing phytochemical concentration and synergistic study with conventional antimicrobial agents may enhance the therapeutic effects.

Author Contributions

Anjali Kumari Sah¹, Kripa Pandey¹, Aaraju Khadka¹, Surjina Shrestha¹: Proposal preparation, laboratory experiments, and manuscript preparation.

Sagar Kandel^{1*}: Manuscript writing, visual representation, manuscript submission, and communication.

Sudip Bhandari¹, Era Tuladhar¹: Supervision, theoretical and practical guidance, and intellectual contributions to study design and data interpretation.

Conflict of Interest

No conflicts of interest were disclosed.

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Ethical Approval and Consent

Not applicable

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