International Journal of Pharmaceutical research and Applications
Volume 11, Issue 5, Sep.-Oct.2026, pp:854-860 www.ijprajournal.com ISSN:2456-4494

Comparative Estimation of Total Phenolic Content in Selected Fruit Juices and Their Peel Extracts Using UV–Visible Spectrophotometry

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Date of Submission: 01-10-2026 Date of Acceptance: 11-10-2026

ABSTRACT

Background: Fruits that are contain a variety of naturally occurring phenolic compounds that contributes to their chemical and nutritional properties. The fruit peels which are frequently discarded as waste but it also also contain phenolic constituents of interest. Quantitative estimation of these compounds can help assess the potential value of fruit peels as sources of naturally occurring bioactive substances.

Objective: The present study aimed to Quantify and correlate the total phenolic content of lemon (Citrus limon), orange (Citrus sinensis), and pomegranate (Punicagranatum) juices and their corresponding peel extracts using the Folin–Ciocalteu colorimetric method and UV–Visible spectrophotometry.

Methods: Fresh fruits were obtained from a local market. The fruit juices were collected and filtered, while the peels were shade-dried, powdered and extracted using ethanol by maceration. Tannic acid was used as the reference standard for preparing a calibration curve. The Folin–Ciocalteu reagent and sodium carbonate were used to develop the colour reaction, and absorbance was measured at 760 nm. Sample concentrations were calculated using the reported calibration equation.

Results: The reported calibration curve had equation of $y = 0.0079x + 0.0319$ and R^2 value of 0.9952. The calculated concentrations for lemon, orange, and pomegranate juices was 11.65, 13.93, and 10.26 µg/mL, respectively. The corresponding peel extracts showed calculated concentrations of 22.92, 19.25, and 15.83 µg/mL, respectively. The peel extracts showed higher concentrations than their corresponding juices.

Conclusion: The selected fruit-peel extracts showed higher apparent total phenolic concentrations than their corresponding juices. The further validation of the analytical procedure, sample calculations and measurement reproducibility is necessary before the findings can be used to establish quantitative differences with the total phenolic content**.**

Keywords: Total phenolic content, Folin–Ciocalteu method, UV–Visible spectrophotometry, tannic acid, fruit juice, fruit peel, *Citrus limon*, *Citrus sinensis*, *Punica granatum*.

I. INTRODUCTION

The Phenolic compounds were naturally occurred plant constituents that contain one or more aromatic rings bearing hydroxyl groups. They were distributed in fruits, vegetables, and other plant materials. These compounds include phenolic acids, flavonoids, tannins and several other structurally diverse groups. The chemical properties contribute to the color, flavor and other characteristics.

Phenolic compounds have attracted considerable scientific interest because of the ability to participate in oxidation–reduction reactions and interact with the reactive chemical species. The concentration in fruits and vegetables has been analyzed in relation to antioxidant activity and other biological properties. The concentration and composition of phenolic constituents may vary with the plant species, maturity, growing conditions, processing and extraction method.[1]

Fruit juices are consumed for human diet and it contain different naturally occurring compounds. The corresponding peels may also contain phenolic constituents that contribute to the protective functions of plant tissues. The peels from commonly consumed fruits are often discarded during food preparation and processing. The analysis of chemical composition helps to identify opportunities for the more effective utilization of fruit-processing by-products.

Lemon (Citrus limon), orange (Citrus sinensis) and pomegranate (Punicagranatum) are widely consumed fruits. Their juices and peels have been investigated for the presence of various phytochemicals. However, the measured phenolic content may differ between the juice and peel portions of the same fruit because of differences in tissue composition and extraction characteristics.[2]

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Several analytical techniques are available for investigating phenolic compounds, including high-performance liquid chromatography, mass spectrometry, and spectrophotometric methods. Among these, the Folin–Ciocalteu method is widely used for the estimation of apparent total phenolic content because it is relatively simple and requires commonly available laboratory equipment.[3]

The Folin–Ciocalteu method is based on the reduction of phosphomolybdic and phosphotungstic acid complexes under alkaline conditions, producing a blue-coloured reaction mixture. The intensity of the colour is measured spectrophotometrically and related to the response obtained from a reference standard. Nevertheless, the reagent can also react with certain non-phenolic reducing substances, meaning that the method does not provide a compound-specific measurement of polyphenols.[5]

The present study was undertaken to estimate the apparent total phenolic content of lemon, orange, and pomegranate juices and their corresponding peel extracts using the Folin–Ciocalteu method. The study also aimed to compare the calculated concentrations between the juice and peel samples to investigate their relative analytical responses under the experimental conditions employed[4-6].

II. MATERIALS AND METHODS

2.1 Chemicals and reagents

Tannic acid was used as the reference standard. Folin–Ciocalteu reagent and sodium carbonate were used for the colour-development reaction. Ethanol was used for peel extraction and sample preparation, while distilled water was used for dilution and reagent preparation. According to the project record, the chemicals were of analytical-reagent grade.[3,11]

2.2 Instruments

Absorbance measurements were performed using a UV–Visible spectrophotometer (UV-1800, Shimadzu, Japan). A digital balance (BL-200H, Shimadzu, Japan) was used for weighing the materials. Whatman filter paper was used for filtration.

2.3 Collection and preparation of fruit samples

Fresh lemon, orange, and pomegranate fruits were obtained from a local fruit market. The fruits were washed with tap water to remove surface dirt and other visible impurities.

The fruit pulp was taken and the juice was collected and filtered through Whatman filter paper. The filtered juice samples were retained for subsequent analysis.

2.4 Preparation of fruit-peel powder

The peels of lemon, orange, and pomegranate were separated from the fruits and cut into small pieces. The peel pieces were shade-dried at room temperature for approximately 8–10 days. The dried peels were powdered using a grinder and passed through a sieve.

The resulting peel powders were transferred into airtight polyethylene bags and stored under refrigerated conditions until extraction.

Figure.1 Extraction and extracts of Peel Sample

2.5 Extraction of phenolic constituents from fruit peels

Maceration was used to extract constituents from the fruit-peel powders.

Initially, the solubility of the peel powders was investigated using different solvents, including ethanol, ethyl acetate, acetone, methanol, and water. Ethanol was subsequently used for the reported extraction procedure.

Approximately 5 g of each peel powder was mixed with 50 mL of 70% ethanol, corresponding to a nominal powder-to-solvent ratio of 1:10 (w/v). The mixtures were transferred into suitable flasks and maintained in a dark place for one week. During this period, the mixtures were monitored and stirred periodically.

Following maceration, the mixtures were filtered through Whatman filter paper. The filtrates were collected, and the solvent was evaporated to obtain the crude extracts. The resulting extracts were stored at room temperatureuntil analysis.[5]

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Figure.2(a,b) Extraction and extracts of Peel Sample

2.6 Preliminary phytochemical tests

Preliminary tests were performed to investigate the presence of phenolic constituents in the prepared samples.

2.6.1 Ferric chloride test

The sample extract was treated with 2 mL of 1% ferric chloride solution. The development of a characteristic colour was recorded as a preliminary indication of phenolic constituents.

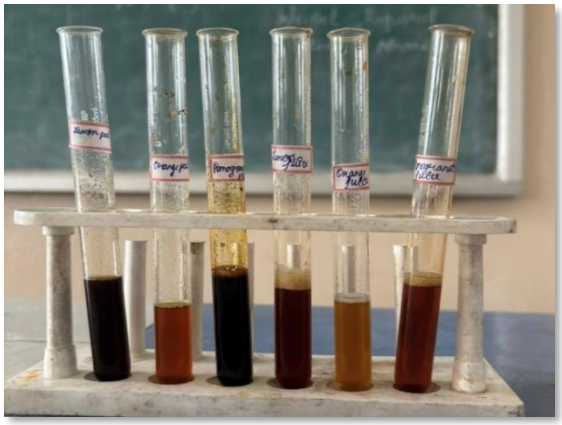

Figure.3 Ferric Chloride Test

2.6.2 Alkaline reagent test

Approximately 1 mL of sample was treated with 1 mL of 2 N sodium hydroxide solution. The development of a yellow or orange colour was recorded as a preliminary reaction.

These qualitative tests were considered preliminary screening procedures and were not used to quantify total phenolic content.

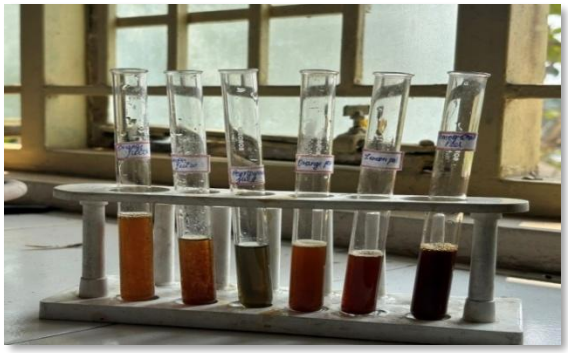

Figure.4 Alkaline Reagent Test

2.7 Preparation of tannic acid standard solutions

Tannic acid was selected as the reference standard for preparing the calibration curve. A stock solution was prepared in ethanol, and working standards were intended to be prepared at concentrations of 10, 20, 30, 40, and 50 µg/mL.

For the colour-development reaction, 1 mL of each standard solution was transferred into a separate test tube. Subsequently, 5 mL of Folin–Ciocalteu reagent diluted 1:10 with distilled water was added and 4 mL of sodium carbonate solution.

The reaction mixtures were shaken and allowed to stand at room temperature for 90 minutes to permit colour development. Absorbance was measured at 760 nm using a UV–Visible spectrophotometer.

The standard concentrations were plotted against their corresponding absorbance values to obtain the calibration curve.[3,4,11]

Figure 5: Dilution of Standard Solution

2.8 Estimation of apparent total phenolic content in fruit juices

The 1 mL of filtered fruit juice was collected and diluted with ethanol for the sample-preparation. The colour-development reaction, 1 mL of the prepared juice sample was mixed with 5 mL of Folin–Ciocalteu reagent diluted 1:10 with distilled water and 4 mL of sodium carbonate solution.

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The mixtures were shaken and allowed to stand at room temperature for 90 minutes. Absorbance was measured at 760 nm.[3,11]

Figure.6 Filtration of fruit

Figure.7 Homogeneous fruit juices

2.9 Estimation of apparent total phenolic content in fruit-peel extracts

A solution containing 10 mg of crude peel extract was prepared in ethanol. The 1 mL was transferred into a test tube, followed by the addition of 5 mL of Folin–Ciocalteu reagent diluted ratio of 1:10 with distilled water and 4 mL of sodium carbonate solution.

The reaction mixture were shaken and maintained at room temperature for 90 minutes for the colour development. Absorbance was measured at 760 nm using a UV–Visible spectrophotometer.[3,11]

2.10 Calculation of sample concentrations

The concentrations of the samples were calculated using the reported calibration equation:

y = 0.0079x + 0.0319

Where:

y = measured absorbance

x = concentration corresponding to the calibration curve

0.0079 = reported slope

0.0319 = reported intercept

Rearranging the equation:

x = (y − 0.0319)/0.0079

The reported sample absorbances were substituted into this equation to calculate the corresponding concentrations.

The calculated values were then compared between the juice and peel samples of each fruit.

III. RESULTS

3.1 Calibration curve of tannic acid

The calibration data reported in the project are presented in Table 1.

Table 1. Calibration data for tannic acid

S. No.	Concentration (µg/mL)	Absorbance
1	0	0
2	10	0.100
3	20	0.198
4	30	0.275
5	40	0.349
6	50	0.418

The reported calibration equation was y = 0.0079x + 0.0319, with an R^2 value of 0.9952.

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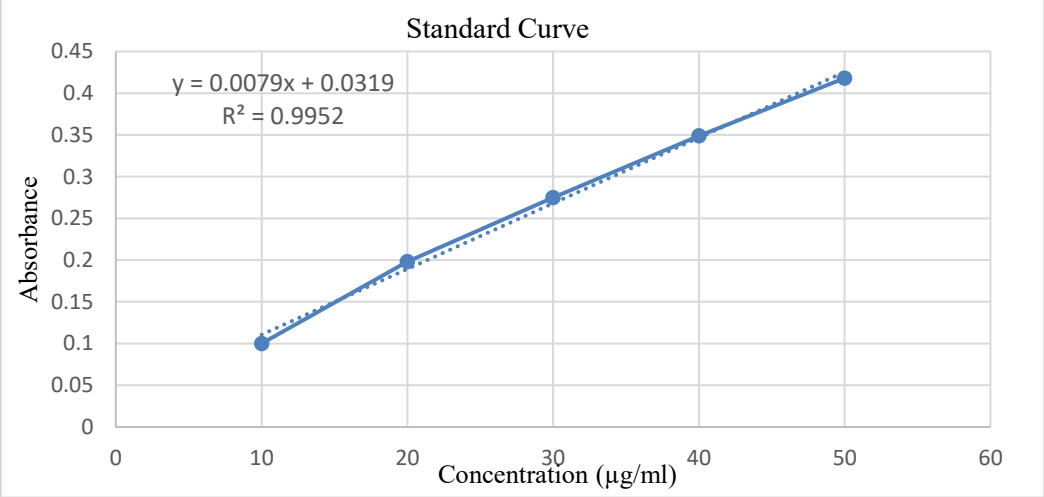

Figure.8 Standard Calibration Curve

The reported coefficient of determination indicated a strong linear relationship according to the calibration analysis recorded in the project. However, the calibration data and regression equation should be verified before final submission.

3.2 Absorbance of fruit-juice samples

The absorbance values reported for the fruit juices at 760 nm are presented in Table 2.

Table 2. Absorbance values of fruit juices

S. No.	Fruit juice	Absorbance at 760 nm
1	Lemon	0.124
2	Orange	0.142
3	Pomegranate	0.113

Among the three juice samples, orange juice showed the highest reported absorbance, followed by lemon juice and pomegranate juice.

3.3 Absorbance of fruit-peel extracts

The absorbance values recorded for the fruit-peel extracts are presented in Table 3.

Table 3. Absorbance values of fruit-peel extracts

S. No.	Fruit-peel extract	Absorbance at 760 nm
1	Lemon peel	0.213
2	Orange peel	0.184
3	Pomegranate peel	0.157

Among the peel extracts, lemon peel showed the highest reported absorbance, followed by orange peel and pomegranate peel.

3.4 Calculated concentrations of fruit juices and peel extracts

The concentrations calculated using the reported calibration equation are summarized in Table 4.

Table 4. Concentrations of fruit juices and peel extracts

Fruit	Juice concentration (µg/mL)	Peel-extract concentration (µg/mL)	Difference (µg/mL)
Lemon	11.65	22.92	11.27
Orange	13.93	19.25	5.32

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Pomegranate	10.26	15.83	5.57

The calculated values indicated that each peel extract had a higher apparent concentration than its corresponding juice sample.
The largest reported difference between the juice and peel values was observed for lemon, followed by pomegranate and orange.

IV. DISCUSSION

The present study investigated the apparent total phenolic content of three fruit juices and their corresponding peel extracts using the Folin–Ciocalteu method and UV–Visible spectrophotometry. The reported absorbance values differed among the three juice samples. Orange juice exhibited the highest absorbance at 0.142, lemon juice at 0.124 and pomegranate juice at 0.113. Based on reported calibration equation, the calculated concentrations were 13.93, 11.65, and 10.26 µg/mL.Differences in the measured responses may be associated with the chemical composition of the fruits and the characteristics of the sample preparation. Nevertheless, the observed absorbance values alone do not establish the concentrations of individual phenolic compounds. The peel extracts exhibited higher reported absorbance values than their corresponding juice samples. Lemon peel showed absorbance of 0.213, orange peel 0.184, and pomegranate peel 0.157. The calculated concentrations were 22.92, 19.25, and 15.83 µg/mL, according to the reported calibration equation. The comparison suggests that the prepared peel extracts produced greater Folin–Ciocalteu responses than the corresponding juice samples under the conditions employed. This observation is consistent with the possibility that fruit peels contain appreciable quantities of extractable phenolic constituents. Phenolic compounds can contribute to plant-tissue protection against environmental and biological stress but the distribution varies between fruit tissues and species. The Folin–Ciocalteu method offers practical advantages for preliminary quantitative investigations because it is comparatively simple and can be performed using a UV–Visible spectrophotometer. However, the reaction is based on reducing capacity rather than a reaction exclusively specific to phenolic compounds. Other reducing substances in fruit juices and peel extracts may contribute to the observed colour response. Consequently, the results should be interpreted as apparent total phenolic estimates rather than measurements of individual phenolic compounds. Several analytical considerations must be addressed when interpreting the present results. The standard preparation and calibration equation required verification to confirm that the reported concentrations and absorbances are internally consistent. The juice and peel samples must be prepared and diluted using clearly defined procedures so that their calculated concentrations can be compared. And the repeated measurements and suitable statistical analysis would helps to establish the reproducibility and uncertainty of the results. The reported concentration values should also be linked clearly to the relevant sample basis. In particular, concentrations expressed per millilitre of prepared solution cannot automatically be interpreted as equivalent amounts of phenolic compounds per gram of fruit or dry peel. Such comparisons require appropriate consideration of sample mass, extraction volume, dilution factors, and extraction yield. Despite these considerations, the reported findings provide a preliminary comparison of the Folin–Ciocalteu responses of selected fruit juices and their peel extracts. Further analytical validation and characterization would help establish the reliability of the estimates and support a more detailed investigation of the potential value of fruit-peel materials.[5,6,7,9,10]

V. CONCLUSION

The present study was evaluated the total phenolic content of lemon, orange, and pomegranate juices and the corresponding peel extracts using the Folin–Ciocalteu method and UV–Visible spectrophotometry at 760 nm. Based on the reported calculations, the juice samples concentrations of 11.65 µg/mL for lemon, 13.93 µg/mL for orange and 10.26 µg/mL for pomegranate. The corresponding peel extracts showed calculated concentrations of 22.92, 19.25 and 15.83 µg/mL.The results suggest that the prepared peel extracts produced highest total phenolic concentrations than the juice samples under the reported experimental conditions. These findings are the potential value of investigating fruit peels as sources of extractable plant constituents. The verification of the standard-solution preparation, calibration calculations, dilution factors and measurements is necessary before the quantitative conclusions. Further studies using validated analytical procedures and complementary methods were helped to characterize the phenolic composition of these samples.

REFERENCES

[1]. Singla RK, Dubey AK, Garg A, Sharma R, Fiorino M, Ameen M, Haddad MA. Natural polyphenols: chemical classification, definition of classes, subcategories, and structures. *Journal of AOAC International*. 2019;102:1397–1400.
[2]. Ebrahimi P, Lante A. Polyphenols: a comprehensive review of their nutritional properties. *The Open Biotechnology Journal*. 2021;15:164–167.
[3]. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. *Methods in Enzymology*. 1999;299:152–178.
[4]. Prior RL, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*. 2005;53(10):4290–4302.
[5]. Dai J, Mumper RJ. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules*. 2010;15(10):7313–7352.
[6]. Kähkönen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen M. Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agricultural and Food Chemistry*. 1999;47(10):3954–3962.
[7]. Gardner PT, White TAC, McPhail DB, Duthie GG. The relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices. *Food Chemistry*. 2000;68(4):471–474.
[8]. Ajila CM, Naidu KA, Bhat SG, PrasadaRao UJS. Bioactive compounds and antioxidant potential of mango peel extract. *Food Chemistry*. 2007;105(3):982–988.
[9]. Ghasemi K, Ghasemi Y, Ebrahimzadeh MA. Antioxidant activity, phenol and flavonoid contents of citrus species peels. *Pakistan Journal of Pharmaceutical Sciences*. 2009;22(3):277–281.
[10]. Barros L, Ferreira MJ, Queirós B, Ferreira ICFR, Baptista P. Total phenols and antioxidant activity of fruit by-products. *Food Chemistry*. 2007;103(2):413–419.
[11]. Pérez M, Domínguez-López I, Lamuela-Raventós RM. The chemistry behind the Folin–Ciocalteu method for the estimation of polyphenol content in food. *Journal of Agricultural and Food Chemistry*. 2023;71:17543–17545.