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Phytochemical Analysis of Orange Peel Extract and Its Antioxidant, Anti-Inflammatory, and α-glucosidase Inhibitory Activities

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The valorization of orange peels (OP) through the extraction of bioactive compounds represents a sustainable strategy consistent with circular economy goals. In this study, ultrasound-assisted extraction (UAE) was applied to recover phenolic and flavonoid compounds from OP using various solvent systems. The 70% ethanol–water mixture proved to be the most efficient medium. High-performance liquid chromatography (HPLC) revealed high levels of gallic acid, quercetin, and rutin among other phenolic constituents. Total phenolic and flavonoid contents were determined using standard colorimetric methods. The extract exhibited moderate antioxidant capacity with an IC_{50} value of 1813.75 ppm in DPPH radical scavenging assays. It demonstrated anti-inflammatory activity by reducing TNF-α levels by 20.7% at 100 ppm in diabetic patients with cardiovascular disease. Additionally, significant α-glucosidase inhibition (IC_{50} = 617.88 ppm) was observed, suggesting its potential in managing postprandial hyperglycemia. Overall, the findings highlight orange peel as a renewable, low-cost source of natural bioactive compounds with promising therapeutic and industrial applications.

Keywords: Orange fruit, high-performance, antioxidant, anti-inflammatory, alpha-glucosidase

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Flavonoids are aromatic secondary metabolites classified as polyphenolic compounds and are broadly distributed across the plant kingdom. They occur abundantly in fruits (e.g., oranges, grapefruits, apples, and strawberries), vegetables (such as onions, broccoli, green peppers, and tomatoes), cereals (including soybeans and various herbs), as well as in common foodstuffs, herbal preparations, and some dairy products. Regular dietary sources include red wine, tea, dark chocolate, grapes, and apples. More than 5,000 naturally occurring flavonoids have been identified from botanical and related sources. In plants, they fulfill essential physiological functions, such as modulating growth by suppressing auxin (indole-3-acetic acid, IAA) exocytosis [1]. The sweet orange (Citrus sinensis) originated in China, with records dating back to at least 314 BC. Today, it is extensively cultivated in tropical and subtropical regions due to its sweet flavor. Oranges can be consumed fresh, processed into juice, or used for their fragrant peels. As of 2012, sweet oranges represented nearly 70% of total citrus production. Nevertheless, citrus peels—which account for about half of the fruit's fresh weight—remain largely underutilized, generating a significant waste-management concern. Industrially, the peel residue is commonly dried for pectin extraction or pelletized for animal feed [2]. Citrus fruits contain a wide range of bioactive constituents, including vitamin C, β-carotene, flavonoids, limonoids, essential oils, acridone alkaloids, dietary fibers, minerals, and B vitamins (thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, and folic acid [3, 4]. Several studies highlighted some characteristics of these compounds, including antioxidant, anti-inflammatory, anti-diabetic, and cardioprotective [5-8]. Research revealed that Hesperidin and naringenin decrease the inflammation and oxidative stress by blocking pro-inflammatory cytokines like tumor TNF-α and IL-6 and scavenging reactive oxygen species (ROS) [9,10]. Citrus polyphenols act by inhibiting NF-κB nuclear translocation and boosting endogenous antioxidant defenses by increasing the levels of enzymes such as superoxide dismutase and catalase to restore redox balance [11-13]. Moreover, Citrus components can inhibit α-glucosidase, an intestinal enzyme responsible for hydrolyzing carbohydrates [14, 15], leading to hyperglycemia associated with type 2 diabetes mellitus (T2DM) complications [16]. Because of this, the peels of C. sinensis provide a continued and promising source of bioactive chemicals. A cyclical bioeconomy and sustainable healthcare are in accordance with the value-adding of an industrial by-product into a possible therapeutic agent. So, this study aims to assess the inhibitory activity of α-glucosidase along with antioxidant/anti-inflammatory characteristics of

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a peel extract from Citrus sinensis to investigate its potential as a natural therapeutic product for the management of postprandial hyperglycemia along with associated metabolic diseases.

EXPERIMENTAL

Materials and Methods

Preparation of the Sample and Extraction Method

Sweet orange fruits were purchased from a local market, peeled, and the edible portions were separated carefully. The peels were washed thoroughly with distilled water to eliminate surface impurities and then air-dried at room temperature (25 ± 2 °C) for several days until complete dryness was achieved. The dried peels were finely ground using an electric grinder and stored in airtight containers to prevent moisture absorption. Subsequently, 12.5 g of the powdered sample was extracted with 100 mL of various solvents, including ethanol, 70% ethanol, and an aqueous solution.

Approximately 12.5 g of powder of the peel was extracted with each of the three different solvents [distilled water, 70% ethanol, and absolute ethanol (100%)] in 100 mL. Ultrasonic-assisted extraction (UAE) was applied by using a sonication bath (50°C) for 30 min to achieve the maximum release of phytochemicals. The suspensions were then filtered with Whatman No. 1 filter paper, the filtrates were collected, poured into amber glass jars, and stored at 4 °C until analysis for chemical stability. The best solvent that gave the most extract was 70% ethanol.

Chemicals and Reagents

Solvents used in this work were distilled water and absolute ethanol. The Folin–Ciocalteu phenol reagent and sodium carbonate anhydrous were used to perform the Folin–Ciocalteu method to determine the flavonoid content in each essay.

Preliminary Phytochemical Test

The dried crude ethanoic extract was subjected to a preliminary phytochemical evaluation to identify the presence of major classes of secondary metabolites. Standard chemical assays were performed to detect polyphenols and flavonoids in the extract.

Test for Phenolic

One milliliter of the ethanoic extract was mixed with 1 ml of 5% ferric chloride solution, and the color change was monitored. The appearance of a deep green to black coloration confirmed the presence of phenolic acids [17].

Test for Flavonoids

To 1 ml of the ethanoic extract, 2 ml of 1% potassium hydroxide solution was added in a test tube. The formation of a yellow coloration indicated the presence of flavonoid compounds [18].

Determination of Total Phenolic Compound (TPC)

The total phenolic compound (TPC) was measured using the Folin–Ciocalteu colorimetric method, with gallic acid as the standard. A stock solution of Gallic acid (200 ppm) was prepared by dissolving 10 mg of Gallic acid in distilled water. The calibration curve was constructed using solutions at concentrations of 15, 25, 50, 100, and 200 ppm.

In all assays, 1 mL of standard (different concentration) from Gallic acid and 1mL orange extract was mixed with 2.5 mL of 10% Folin–Ciocalteu reagent. After 5 minutes, 2 mL of 10% sodium carbonate was added, and the total volume was adjusted to 10 mL with distilled water separately. After 30 minutes of incubation at room temperature in the dark, the absorbance was measured at 765 nm using a UV-visible spectrophotometer [17].

The total phenolic content of the extract 70% ethanol was calculated from the standard curve and expressed as mg gallic acid equivalents ppm.

The TPC values, expressed as mg of gallic acid equivalent (GAE) per g of OPW, were obtained from Eqn. 1:

$$TPC\ (mg\ GAE/g\ OP) = [GAE]m\ OP \cdot V\ solvent \quad (1)$$

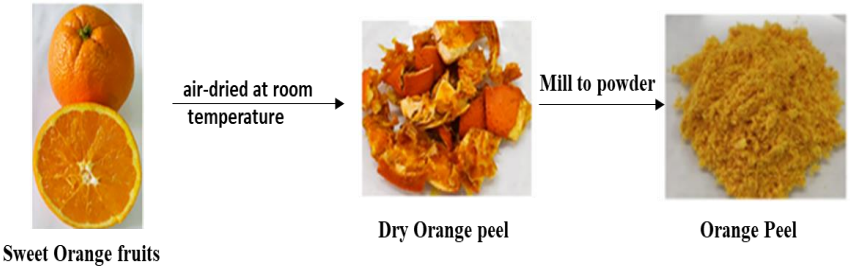


Figure 1. Showing the preparation steps of orange peel (OP).

Estimation of Total Flavonoid Content (TFC)

Total flavonoid content was determined by the aluminum chloride colorimetric method, using quercetin as the standard. A calibration curve was obtained using quercetin standard solutions (12.5–200 ppm) in 70% ethanol. For each reaction, a standard (different concentration) of quercetin and 1mL of extract were mixed with 1 mL of 2% aluminum chloride in a 10 mL volumetric tube, and the volume was made up to 10 mL with 70% ethanol. Reaction mixtures were incubated in the dark at room temperature for 30 minutes. The absorbance was measured at 415 nm on a UV/Vis spectrophotometer [18]. TFC was calculated from the calibration curve and expressed as mg quercetin equivalents per litre (mg QE/L). The results, expressed as quercetin equivalent ppm of OPW, were calculated using Eqn. 2: where [QE] is the quercetin equivalent present in the extract expressed as ppm.

TFC (mg QE/g OP) =[QE] / m OP·V solvent (2)

Analysis of HPLC of Phenolic and Flavonoid Compounds

Detection and Quantification of Special Phenolic and Flavonoid Compounds. The individual phenolic and flavonoid compounds in orange peel extracts were analyzed using high-performance liquid chromatography (HPLC). The analysis was carried out on a SYKAM HPLC system (Germany) fitted with a C18-ODS column (25 cm × 4.6 mm). The mobile phase consisted of methanol, water, and formic acid in a ratio of 70:25:5, with a flow rate of 1.0 mL/min. Detection was performed at 280 nm, using an injection volume of 0.1 mL and a run time of 20 minutes. Peaks were identified by comparing retention times with standard compounds, including gallic acid, ferulic acid, quercetin, rutin, and apigenin. Quantification was achieved by referencing calibration curves prepared from the pure standards. All analyses were performed in triplicate to ensure reproducibility.

Determination of the Anti-Inflammatory Activity of Orange Peel Extract

The anti-inflammatory effect of orange peel extract was tested in pooled serum collected from diabetic patients with cardiovascular illnesses by evaluating the extract's inhibitory effect on TNF-α. As directed by the manufacturer, the TNF-α concentration was first measured using a human ELISA kit (SunLong Biotech Co., Ltd., Catalogue Number: SL1761Hu_1) in pooled serum without extract. Different extract concentrations (50, 100, 200, 400, and 800 ppm) were then applied in duplicate to the pooled serum. For comparison, a blank control (extract without serum) was used. The same ELISA kit was used to test the TNF-α level again after incubation under standard conditions. The subsequent equation was employed to determine the proportion of TNF-α inhibition.

Investigating the Antioxidant Efficacy of Orange Peel Extract

The antioxidant efficacy was measured by the DPPH test [19]. Ten milliliters of methanol were used to make a 1.4 mg DPPH solution. Each test involved mixing (250 µL) of extract or ascorbic acid with (150) µL of DPPH solution, then adjusting the amount to 5 mL using 70% ethanol. The test was then incubated in the dark for half an hour at room temperature, and after that, the absorbance was determined at 517 nm. Ascorbic acid was tested at values between 50 and 800 ppm, and the extract was evaluated at concentrations between 50 and 2000 ppm.

Evaluation of the Inhibitory Activity of α Glucosidase

As directed by the manufacturer, the Alpha-Glucosidase Inhibitor Screening Kit (Elabscience®, Cat. No. E-BC-D017) was used to assess the orange peel extract's inhibitory efficacy against α-glucosidase. In summary, a 96-well microplate was filled with 80 µL of buffer solution for both sample(extract) and positive control(acarbose) wells, 120 µL for blank wells, and 100 µL for total enzyme wells (without extract). The complete enzyme, sample, and positive control wells were then filled with 20 µL of enzyme working solution. Following this, 20 µL of substrate working solution was added to these wells. Later, 20 µL of orange peel extract solution at different concentrations (50, 100, 200, 400, 800, 1000, and 3000 ppm) or acarbose working solution (positive control) were introduced. Next, the incubation of the mixture was done for 10 minutes at 37 °C, and the absorbance was determined. The inhibition rate (%) was calculated according to the kit formula:

Inhibition Rate (%) = (ΔA1 –ΔA2) ÷ ΔA1 × 100%

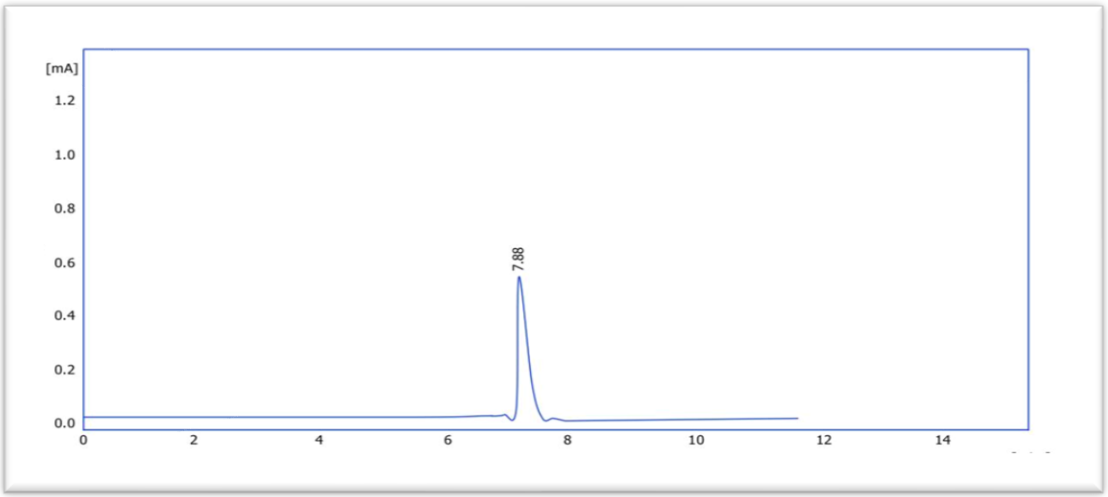
ΔA1 : ΔA1= ODtotal – ODblank. ΔA2 : ΔA2= ODsample – ODblank.

RESULTS AND DISCUSSION

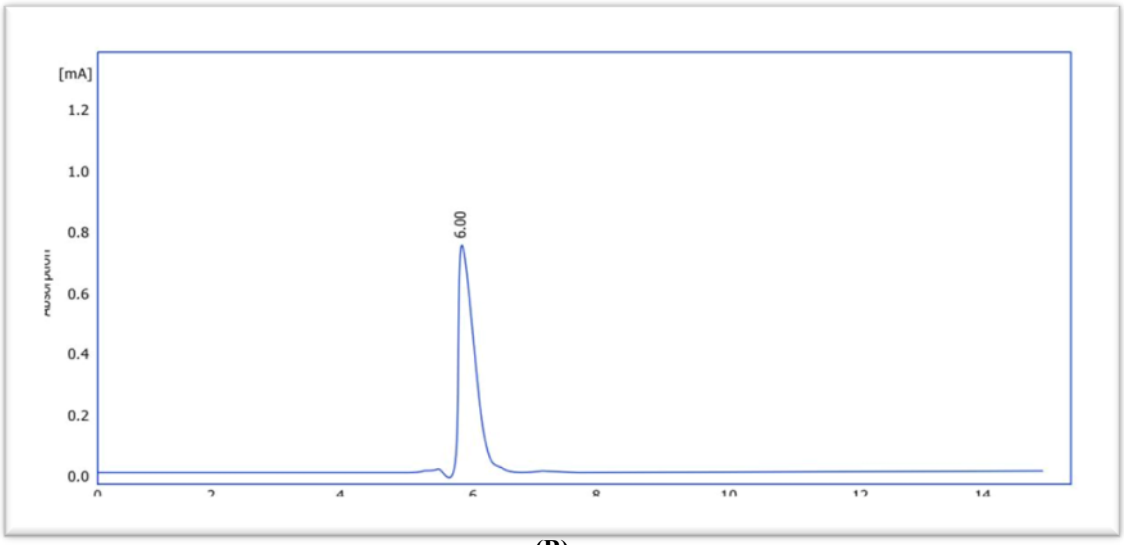
HPLC Profiling of Phenolic–Flavonoid Constituents in Mate Tea Extract

High-performance liquid chromatography (HPLC) was applied to characterize standard phenolic markers commonly encountered in medicinal plant extracts. The chromatographic fingerprints obtained under optimized conditions provide

reliable reference patterns for evaluating phenolic–flavonoid compounds in orange peels.



(A)



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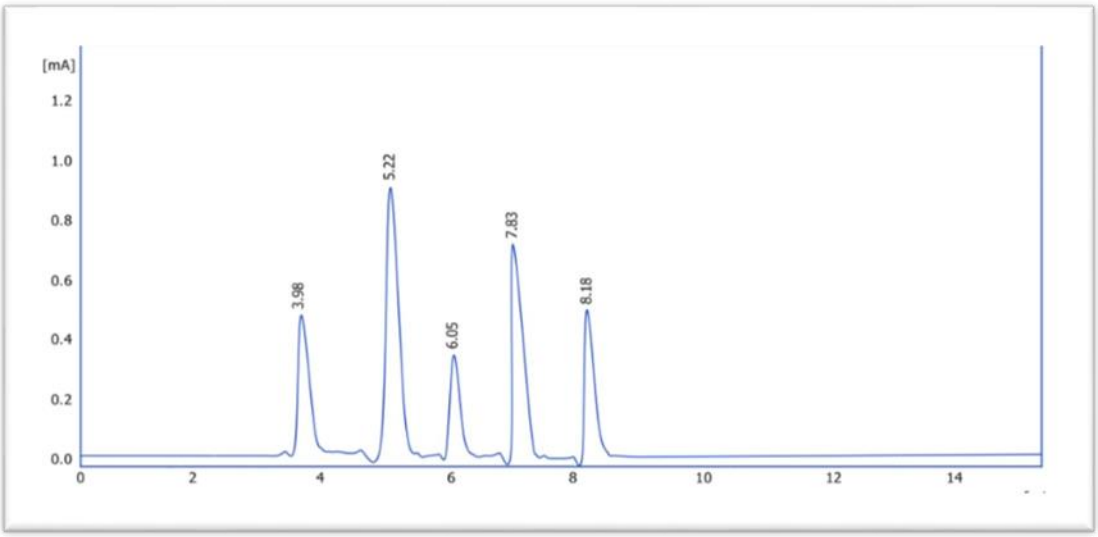

(C)

Figure 2. HPLC profile of Phenolic-flavonoid compounds A (Gallic acid, B Quercetin, Orange peels extract).

Table 1. HPLC retention times of major phenolic–flavonoid constituents in orange peel extract.

No.	Reten. Time (min)	Amount(mg)	Height (%)	W 0.5(min)	Compound Name
1	3.98	92.6	17.00	0.03	apigenin
2	5.22	139.7	28.00	0.10	Rutin
3	6.05	65.8	12.00	0.01	Quercetin
4	7.83	114.0	25.00	0.05	Gallic acid
5	8.18	94.7	18.00	0.03	Ferulic acid

When combined, the chromatogram of the five reference phenolic compounds demonstrated an identical elution order to the individual profiles. The overlaid fingerprint resembled a synchronized sequence of peaks, with retention times closely matching previously reported values (Figure 2, Table 1). The compounds identified included Gallic acid (7.83 min), apigenin (3.98 min), Ferulic acid (8.18 min), quercetin (6.05 min), and Rutin (5.22 min).

Flavonoid bioactivity is generally linked to the structural presence of such compounds, contributing to antioxidant and therapeutic potential. However, preclinical assessments remain indispensable for confirming the pharmacognostical, phytochemical, toxicological, and biological properties of herbal remedies. Thus, systematic qualitative and quantitative profiling of plant-derived phenolics, alongside comparison with authenticated standards, is fundamental for establishing their effectiveness and safety.

Anti-Inflammatory Efficacy of Orange Peel Extract

According to numerous studies, it was reported that flavonoids and phenolic compounds abundant in Citrus sinensis (orange) peels have strong antioxidant and anti-inflammatory qualities. These characteristics are crucial in the fight against inflammation-related disorders, including cardiovascular and metabolic diseases. In this regard, TNF-α is regarded as a quintessential pro-inflammatory cytokine that has a significant function in the appearance of various clinical illnesses.

The effectiveness of the orange peel ethanolic extract in lowering TNF-α levels was assessed. Incubation of serum samples with the extract led to an increase the levels of TNF-α, indicating that the extract has anti-inflammatory properties. The current study revealed that 100 ppm is the only concentration that exhibits inhibition equal to 20.7%. This suggests that this concentration is the optimal to reveal the inhibitory effect.

Our results confirm the anti-inflammatory activity of orange peel and are consistent with a previous study reported by Abou Baker 20. Abou Baker, D. H., Ibrahim, B. M., Abdel-Latif, Y., Hassan, N. S., Hassan, E. M., & El Gengaihi, S. [20]. Moreover, further research demonstrates that this extract acts as an inflammatory substance by lowering C-reactive protein (CRP) and interleukin-6 (IL-6); these results confirm the activity of the component of the extract.

Other important active flavonoids in orange peel are Hesperidin, limonene, and polymethoxylated flavones (PMFs), which exhibit anti -anti-inflammatory characteristics by influencing the synthesis of several proinflammatory cytokines, including TNF-α and IL-6 [20, 21].

These results suggest that orange peel extract has anti-inflammatory properties that may help to reduce inflammation, but other studies are needed to understand the mechanism of action of the extract and to investigate its role in food and pharmaceutical applications.

Antioxidant Efficacy by DPPH Test

The antioxidant properties of Citrus sinensis peel extract were evaluated by the DPPH radical scavenging test. Within the studied concentration range, the scavenging ability rose with the method, depending on the dose. From 1.38% at 50 ppm to 59.63% at 2000 ppm, the ethanoic extract's scavenging activity increased gradually, suggesting a moderate level of antioxidant capability.

As a reference standard, ascorbic acid, on the other hand, showed noticeably greater activity, achieving 94.96% inhibition at 800 ppm. Ascorbic acid demonstrated an IC_{50} of roughly 66.6 ppm, showing its outstanding antioxidant capability, whereas the extract's IC_{50} value was estimated at 1813.75 ppm based on the computed regression model. As shown in Table 2.

The current study observed that the prepared ethanoic peel has moderate antioxidant activity by its activity in DPPH scavenging (IC_{50} = 1813.75 ppm), less than that of ascorbic acid and some formerly

studies for orange peel extracts. The use of basic ethanoic extraction, along with a lower phenolic concentration in the starting point, may reduce the scavenging activity of the extract.

The difference in antioxidant efficiency may be due to one or more of the following reasons: the difference in the phenolic content of the prepared extract, the type of extraction solvent, the type and age of the orange peels used, and the extraction method used. The use of methods such as ultrasound assistance and subcritical water extraction increases the antioxidant capacity of the extract as a result of increasing its phenolic content [22].

Significantly, nutraceutical or functional food products may benefit from even moderate antioxidant activity, especially when linked with other extracts that are high in bioactive chemical components or when more advanced extraction methods are employed. Similar patterns have been noted by other researchers. For example, the total antioxidant capacity (TAC) of Vitex grandifolia's ethanolic root extracts was 183.9 mg AAE/g DW with TPC 57.2 mg GAE/g DW, while the TAC of the bark extracts was 158.7 mg AAE/g DW (TPC: 50.9 mg GAE/g DW), which was slightly greater than the results of this study [23].

Another study by 24. Umdale, S., Mahadik, R., Otari, P., Gore, N., Mundada, P., & Ahire, M. [24] found that TFC, TPC, and TAC were 6.67 mg RE/g, 4.64 mg GAE/g, and 0.67 mg AAE/g, respectively, in methanolic extracts of Frerea indica stems similarly, da Cruz, J. D., Mpalantinos, M. A., Ramos, A. D. S., Ferreira, J. L. P., de Oliveira, A. A., Júnior, N. L. N., & Amaral, A. C. F. [25] found that hydroethanolic extracts of Alpinia zerumbet have TAC of 243 mg AAE/g, while 26. Bahadori, M. B., Sarikurkcu, C., Kocak, M. S., Calapoglu, M., Uren, M. C., & Ceylan, O. [26] observed that Plantago lanceolata extract have TAC of 145 mg AAE/gIn contrast to total antioxidant capacity, in contrast to TAC, the highest activity of DPPH radical scavenging among the relevant works was 1.02 mg AAE/g DW. This hole may be clarified by the analysis of thermostable polyphenols and the formation of new chemicals, such as Maillard reaction products, at high extraction temperatures. Some of these ingredients may have antiradical activity, while others may be toxic, carcinogenic, or mutagenic [27, 28, 29]. The kind and amount of polyphenols that are extracted can also be influenced by the extraction temperature, as was previously mentioned. While antioxidant activity and phenolic content often rise with extraction temperature (up to 180–200 °C), polyphenol oxidation and degradation at high temperatures might result in a decrease of antioxidant ability [30]. Furthermore, other antioxidants that are found in the extract, such as vitamin C or carotenoids, both of which are present naturally in orange peel, may interact with the DPPH in the assay [31]. This may partly explain the differences in activity observed between our extract and the standard ascorbic acid.

Evaluation of α-Glucosidase Inhibitory Activity

In contrast to the typical inhibitor utilized in this investigation, acarbose, orange peel extract showed inhibitory efficacy against α-glucosidase. The orange peel extract's IC_{50} value was 617.88 ppm, as shown in Table 1, whereas acarbose's IC_{50} was 300 nmol/L. This suggests that the extract has a significant inhibitory effect despite being less powerful than acarbose. Some previous studies revealed comparable results relative to acarbose (IC_{50} = 999.31 mg/L). Sheng et al. [32] reported IC_{50} values of 2004.58, 1258.35, 283.67, 247.35, and 3.86 mg/L for five Bacillus stearothermophilus compounds isolated from Musa spp. Flowers, in which compounds 3, 4, and 5 demonstrate a substantial α-glucosidase inhibitor effect.

Table 2. Comparison of DPPH radical scavenging activity (%) of Citrus sinensis ethanoic peel extract and ascorbic acid at different concentrations.

Concentration (ppm)	Ascorbic Acid (% Inhibition)	Citrussinensis Extract (% Inhibition)
50	31.09	1.38
100	88	2.77
200	94.6	5.42
400	94.84	11.19
800	94.96	18.11
1200	-	32.41
1600	-	42.33
2000	-	59.63

Table 3. Inhibitory effects of orange peel Extract on α-glucosidase.

Concentration (ppm)	Orange peel Extract (% Inhibition)
50	43.58
100	43.96
200	44.33
400	45.97
800	49.25
1000	56.42
2000	69.4
3000	83

Recently, 33. Indrianingsih, A. W., Asari, S. M., & Pratiwi, S. I. [33] found that epicatechin, a positive control, only exhibited 32.0% antidiabetic activity at 625 ppm, while orange and jade lemon peel oils obtained 89.7% and 89.0%, respectively, at 1000 ppm. Jade lemon peel oil had a higher phenolic content (433.3 mg GAE/g) than orange peel oil in the same sample. These results are consistent with other studies that used the α-glucosidase inhibition test to demonstrate the promising antidiabetic potential of extracts from navel orange peel [34]. Numerous bioactive flavonoids may contribute to the observed inhibitory effects [35]. Additional investigations have shown that lemon peel extract has a high TPC and strong antidiabetic properties [36]. These differences show that α-glucosidase inhibition is greatly affected by the amount of substrate, enzyme supply, and incubation conditions [37]. These results reinforce the idea that citrus peel extracts may be natural α-glucosidase inhibitors and illustrate how their phenolic and flavonoid components contribute to their antidiabetic effects.

CONCLUSION

The findings of the present study demonstrated that both the choice of solvent and the extraction method played a crucial role in determining the extraction yield, as well as the total phenolic and flavonoid contents, in addition to their antioxidant properties. The results further revealed that orange peel extract contained appreciable levels of phenolic and flavonoid compounds, accompanied by strong antioxidant activity, highlighting its potential as a valuable source of natural antioxidants. Moreover, a. The extract produced revealed a significant α-glucosidase inhibitory potential, moderate, and a marked decrease in TNF-α levels in diabetic patients with cardiovascular disease. These results highlight its potential as a natural treatment for postprandial hyperglycemia, oxidative stress, and inflammation. In addition, this study supports the evaluation of agricultural byproducts as viable. In order to clarify the underlying molecular oxidative stress, inflammation, mechanisms, and to authenticate the extract's efficiency and safety in clinical settings, more research is necessary.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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None

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