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Original Article

In vitro Antioxidant and Antiglycemic Effects of *Malus domestica, Musa acuminata* Colla, *Mangifera indica, Citrus reticulata* and *Punica granatum* Fruits Peel Extracts

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ABSTRACT

Objectives: This study aims to evaluate the anti-glycemic and antioxidant qualities of *Malus domestica* fruit (apple), *Musa acuminata Colla* fruit (banana), *Mangifera indica* fruit (mango), *Citrus reticulata* fruit (orange), and *Punica granatum* (pomegranate) fruit peels. **Materials and Methods:** In this study we used DDPH assay as per standard protocols to test the anti-oxidant activity of different peel extract of this study. Antiglycemic activity was tested using glucose diffusion inhibition assay. **Results:** According to the DDPH test, the antioxidant activity of various fruit peels was found to vary. Pomegranate showed the highest activity at 77%, followed by orange at 71%, and mango peel at 66%. Results showed that mango peel inhibited glucose diffusion by 37%, pomegranate by 35%, and orange by 30%. **Conclusion:** According to these findings, the peels of pomegranates and mangoes have an abundance of chemicals that have antioxidant and anti-glycemic properties. The research backs up the idea that the usually wasted fruit peels can be a source of natural compounds that can help with type 2 diabetes and oxidative stress. This provides encouraging evidence that these waste products can play a part in the creation of phytopharmaceuticals and functional foods, all while contributing to more environmentally friendly methods of trash management.

Keywords: Antidiabetic, Apple, Banana, Free Radical Scavenging Activity, Mango, Orange, Pomegranate.

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INTRODUCTION

Diabetes is a growing global concern among the chronic diseases with significant prevalence upshift. Additionally, there exists enormous research gap in treatment outcomes. Natural, plant-based treatments are gaining popularity worldwide as chronic diseases like diabetes and oxidative stress disorders become increasingly widespread (Modak *et al*., 2007; Chenchula *et al*., 2024; Weller *et al*., 2017). Fruit peels, a major source of agro-industrial waste, include bioactive chemicals and other botanicals. Even though their peels were antioxidant-rich and antidiabetic, they are now edible. Growing awareness of food waste's environmental impact and the need for long-term, cost-effective health solutions are driving this paradigm shift. Fruit peels including pomegranate, orange, banana, mango, and apple have shown antioxidant and blood sugar-regulating properties (Hussain *et al*., 2022).

Fruit peels contain essential oils, phenolic acids, tannins, carotenoids, dietary fibers, and flavonoids. Health benefits include lower inflammation and diabetes risk from these substances. Research shows that fruit peels contain more polyphenols than pulp. The peel accumulates defensive chemicals to protect against UV radiation, pests, and microbiological attacks. Therapeutic fruit peel improves value by reducing food waste. Bioactive chemical recovery was another benefit. These peels include antioxidant chemicals that boost the body's antioxidant defenses, scavenge free radicals, and protect cellular macromolecules from oxidative stress (Prior *et al*., 2005). Patel and Patel, (2016) discovered that phytochemicals enhance insulin sensitivity, protect pancreatic β-cells, and impact glucose metabolism (Patel *et al*., 2016).

Apple peels contain high levels of quercetin, catechins, chlorogenic acid, and phloridzin, all of which have powerful antioxidant and antidiabetic properties. Quercetin improves insulin sensitivity and inhibits α-glucosidase (Ansari *et al*., 2022). while other study found that phlorizin resembles SGLT2 inhibitors like dapagliflozin,

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a constituent in apple peel polyphenols with powerful radical scavenging activity (Choi *et al*., 2016; Leontowicz *et al*., 2003; Sun *et al*., 2002). Dopamine, catecholamines, as well as gallic and ferulic acids in banana peels exhibited antioxidant activity (Someya *et al*., 2002). Inhibiting α-amylase and α-glucosidase helps manage postprandial glucose levels, whereas dietary fiber enhances glycemic control (Gong *et al*., 2020). Ajila *et al*., and Imran *et al*., found antioxidant and hypoglycemic properties in mango peels' xanthonoids, mangiferin, and flavonoids (Ajila *et al*., 2007; Imran *et al*., 2017). Mango peel extracts mildly inhibit α-amylase and inhibit α-glucosidase (Pal *et al*., 2014). Limonoids, hesperidin, and ascorbic acid in orange peels reduce oxidative stress and insulin resistance (Manthey *et al*., 2002). Citrus peel extracts reduce α-glucosidase and free radicals, while its constituent hesperidin protected pancreatic β-cells (Gorinstein *et al*., 2001; Mahmoud *et al*., 2012). Punicalagin, ellagitannins, and ellagic acid in pomegranate peels have high antioxidant activity and protect β-cells from oxidative damage by inhibiting carbohydrate-digesting enzymes (Jurenka *et al*., 2008).

Fruit peels have been extensively studied for their therapeutic properties, but many impediments prevent their use (Hussain *et al*., 2022). Lack of clinical validation, non-standard extraction methods, and fruit variety, ripeness, and location affecting phytochemical content were some of these issues. Many questions remain about polyphenol absorption and metabolism. However, there is hope. Technology in extraction, nanoencapsulation, and formulation can improve phytochemical stability, bioavailability, and targeted distribution (Quesada-Vázquez *et al*., 2024). Long-term and convenient diabetes and oxidative stress control can be achieved with functional meals, nutritional supplements, and pharmaceutical formulations using fruit peel extracts. This study was designed to examine the anti-glycemic and antioxidant properties of water-extracted peels from several fruits, including apple, banana, mango, orange, and pomegranate.

MATERIALS AND METHODS

Extract Preparation

Farm fresh *Malus domestica* fruit (apple), *Musa acuminata* Colla fruit (banana), *Mangifera indica* fruit (mango), *Citrus reticulata* fruit (orange), and *Punica granatum* (pomegranate) were acquired and authenticated. Once the peels were extracted, they were thoroughly washed with distilled water, they were left to air dry in order to eliminate any leftover residues or debris. 10 g of peel samples were weighed and macerated in 100 mL of water from distillation first. The mixture was agitated continuously at 60°C for 30 min to help to simplify the extraction of bioactive compounds. According to Ajila *et al*., and Li *et al*., the filtration with Whatman No. 1 filter paper retained the filtration temperature at 4°C until further use (Li *et al*., 2006).

DDPH Radical Scavenging assay

Using the 2,2-Diphenyl-1-Picrylhydrazyl (DDPH) free radical scavenging experiment, the antioxidant activity of peel extracts was determined (Brand-Williams *et al*., 1995; Gallagher *et al*., 2003). The spectrophotometric detection of a shift in hue from deep violet to yellow was predicated on the idea that antioxidants can contribute electrons from hydrogen to the DDPH radical. Different volumes of the sample extract (5-20 μL) were mixed with 250 μL of DDPH solution in a 96-well microplate. Mixtures were shaken well before being set aside to incubate at room temperature for 20 min in the dark. The absorbance at 517 nm then was found using a UV-vis spectrophotometer. Ascorbic acid was the reference criteria. Reducing the absorbance increases the radical scavenging activity. The DDPH inhibition % could be found by applying the following formula:

The inhibiting formula:

DDPH scavenging effect (% inhibition) = [(absorbance of control-absorbance of reaction mixture)/absorbance of control] X 100

Glucose Diffusion Inhibition Assay

The modified Gallagher *et al*., (2003). approach was employed to test the peels of fruit extracts antiglycemic efficacy utilizing a dialysis membrane within a glucose diffusion experiment. After the dialysis membrane was cut into 10-cm sections and overnight immersed in distilled water, one end of it was knotted securely. 2 mL of 50 mM glucose solution and 2 mL of 400 mg/mL plant extract filled the dialysis bag then. Once the opening on one side was tightly shut, the bag was set in a beaker containing 40 mL of PBS. The beaker was lightly stirred on a magnetic stirrer at 37°C. Glucose at 540 nm was calculated using a Glucose Oxidase-Peroxidase (GOD-POD) assay kit. Removing 1 mL of the external solution at 0, 30-, 60-, 90-, and 180-min times in a control sample, a dialysis bag filled just with glucose solution free of plant extract was used. The % obstruction of glucose diffusion was calculated with this formula:

Inhibition (%) = 100– ((Glucose concentration in test sample)/(Glucose concentration in control)) ×100

Statistical Analysis

All experimental data were expressed as mean±Standard Deviation (SD) based on triplicate determinations. The statistical significance of differences between fruit peel extracts was analyzed using one-way Analysis of Variance (ANOVA), followed by Tukey's *post hoc* test to compare mean values among groups. A p-value less than 0.05 was considered statistically significant. Microsoft Excel and GraphPad Prism were used for data computation and graphical representation. Results were interpreted to determine variation in antioxidant activity through DDPH radical scavenging and antiglycemic potential via glucose diffusion inhibition assays. The dose-dependent response of each extract was plotted, and comparative analysis revealed significant differences among fruit peels. Pomegranate and mango extracts demonstrated the highest mean inhibition percentages in antioxidant and antiglycemic assays, indicating superior bioactive compound content and efficacy compared with other fruit peels tested.

RESULTS

Extraction yield

Figure 1 shows the percent yield of tested fruit peel extracts. The highest (12.44%) and lowest (1.71%) yields of extraction were obtained from the peels of orange, and apple respectively.

Effects of peel extracts against DDPH assay

The antioxidant activity of fruit peel aqueous extracts was determined using the DDPH radical scavenging test. Figures 2 and 3 demonstrate that the DDPH radical scavenging activity of all fruit peel extracts. The antioxidant activity of pomegranate peel extract was the highest at 71% at 5 µL and slightly increased to 77% at 20 µL, exceeding the other peel varieties evaluated (apple, banana, mango, and pomegranate).

Mango peel has the second highest antioxidant activity, increasing from 45% at 5 µL and 66% at 20 µL. This activity with orange peel extracts exhibited dose dependent outcomes with concentration, from 39% to 71%.

Effects of peel extracts against glucose diffusion inhibition assay

The same fruit peel extracts were investigated for antiglycemic properties utilizing a glucose diffusion inhibition experiment. This test mimics the intestinal glucose absorption barrier by using a dialysis membrane model. Over the duration of 150 min, the samples exhibited varying amounts of glucose diffusion inhibition (Figure 4).

Mango peel extract showed the strongest antiglycemic activity, with inhibition values ranging from 25% at 30 min to 37% at 150 min. The Antiglycemic activity of pomegranate peel was very strong, reaching 35% inhibition after 150 min. At 150 min, apple peel extract's inhibitory activity had increased to 29%.

DISCUSSION

The present invitro study aimed to evaluate antioxidant and Antiglycemic effect of apple, banana, mango, orange and pomegranate peel extracts. Highest yield was noticed in orange peel while least in apple peel. In DDPH assay the peel extract of fruits in study have exhibited dose dependent percent inhibition of free radical scavenging activity. The pomegranate peel extract has exhibited highest scavenging activity.

In glucose diffusion inhibition experiment mango peel extract has exhibited highest inhibition activity at 150 min the percent inhibition was above 12% for all peel extracts of the study.

The pomegranate fruit peel extract produced potent antioxidant activity in our study Earlier research revealed high level of

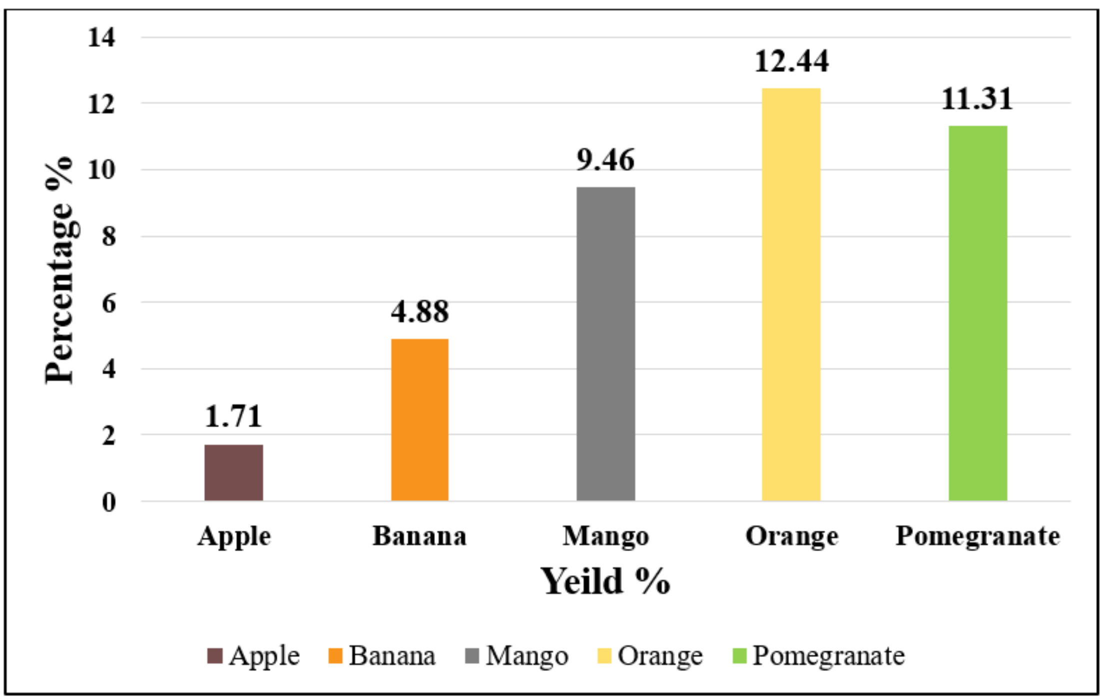

Figure 1: Percent yield of tested fruit peel extracts. Values are expressed in percentage.
A bar chart showing percent yields for Apple (1.71%), Banana (4.88%), Mango (9.46%), Orange (12.44%), and Pomegranate (11.31%).

antioxidant/free radical scavenging activity attributed to the presence of polyphenols such as ellagitannins, punicalagin, and ellagic acid (Jurenka *et al.*, 2008; Quesada-Vázquez *et al.*, 2024).

Similarly, mango also exhibited potent activity inferior to pomegranate. In concordance previous studies showed mango peels possess a high antioxidant capacity because they include bioactive components such as carotenoids, mangiferin, and gallotannins (Ajila *et al.*, 2007; Imran *et al.*, 2017). This activity with orange peel extracts also exhibited dose dependent outcomes with concentration, from 39% to 71%. Citrus peel includes flavonoids such as hesperidin and naringin, which have antioxidant capabilities by scavenging free radicals and reducing lipid peroxidation (Patel *et al.*, 2016; Pal *et al.*, 2014). Moderate antioxidant activity demonstrated with banana and apple peel extracts can be contributed to presence of polyphenols including quercetin, chlorogenic acid, and phlorizin increased apple peel extract activity as suggested by previous studies. Because of low phenolic content, banana peels demonstrated poor antioxidant activity (Choi *et al.*, 2016; Sun *et al.*, 2002). Someya *et al.* and Anhwange *et al.*, suggest that gallic acid, catecholamines, and dopamine may still contribute to its measurable activity (Li *et al.*, 2006).

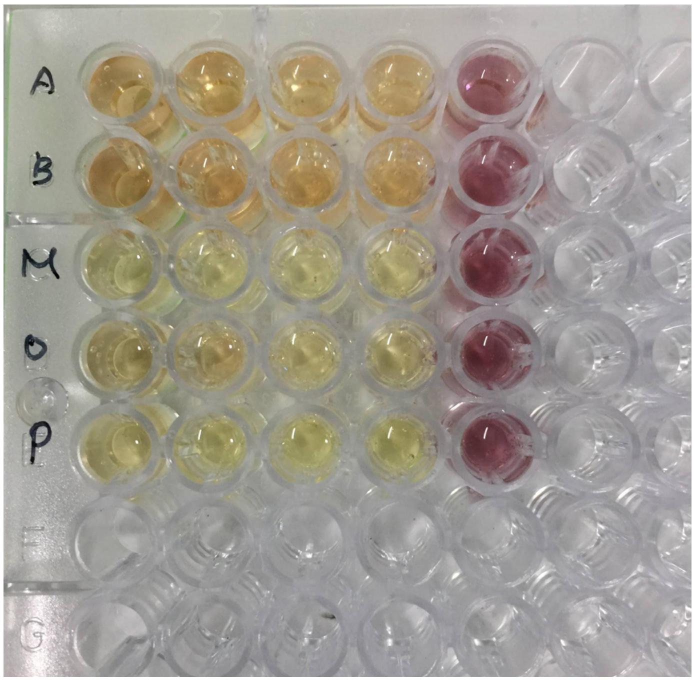

Figure 2: DPPH assay absorbance of different fruit peels using the ELISA method. A-Apple, B-Banana, M-Mango, O-Orange, P-Pomegranate.

Consistent with earlier research highlighting the use of fruit by products for nutritional purposes, our findings suggest that pomegranate, mango, and orange tree peels have significant antioxidant potential and can be used as natural antioxidant sources (Modak *et al.*, 2007; Li *et al.*, 2006).

The free radical scavenging activity exhibited by the peel extracts in our study can be linked to antioxidant activity which is crucial in management of type 2 diabetes. To link this activity, we have also demonstrated the antiglycemic activity of these extracts using *in vitro* glucose diffusion inhibition assay.

Mango and pomegranate peel extracts showed the potent and comparable (p=0.3426) antiglycemic activity at 150 min. Previous literature showed mango peel polyphenols, such as mangiferin to increase insulin sensitivity and inhibit carbohydrate hydrolysis

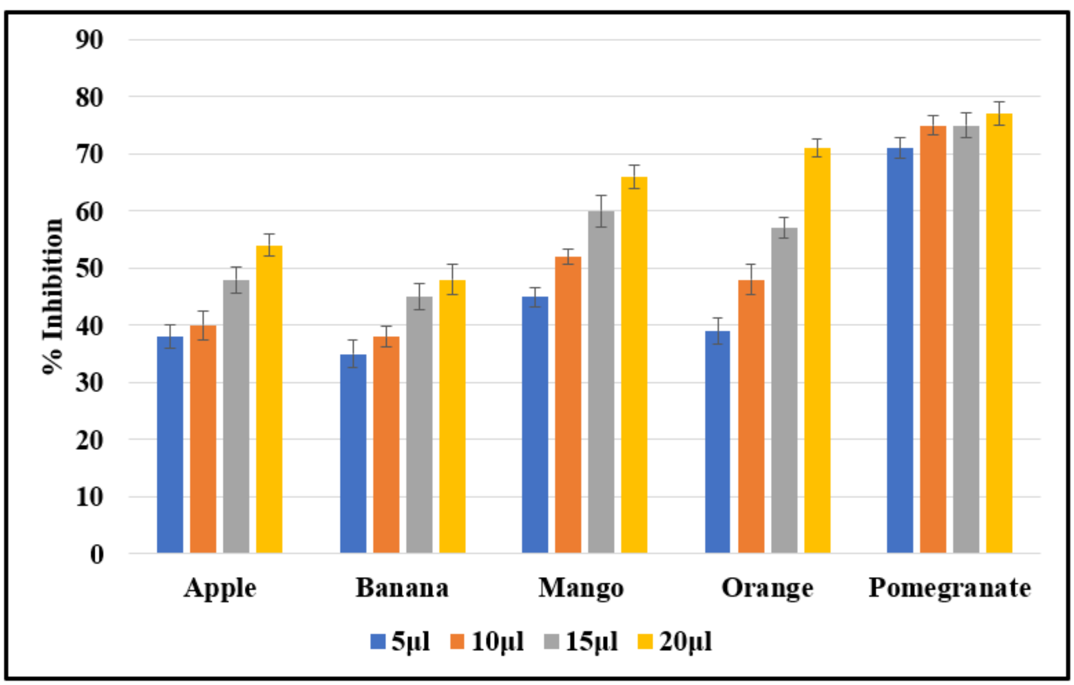

Figure 3: DPPH antioxidant activity of different fruit peels. Values expressed as Mean±Standard Deviation. A multi-bar chart showing % inhibition of DPPH activity at various concentrations (5 µL, 10 µL, 15 µL, 20 µL) for the five fruit peels.

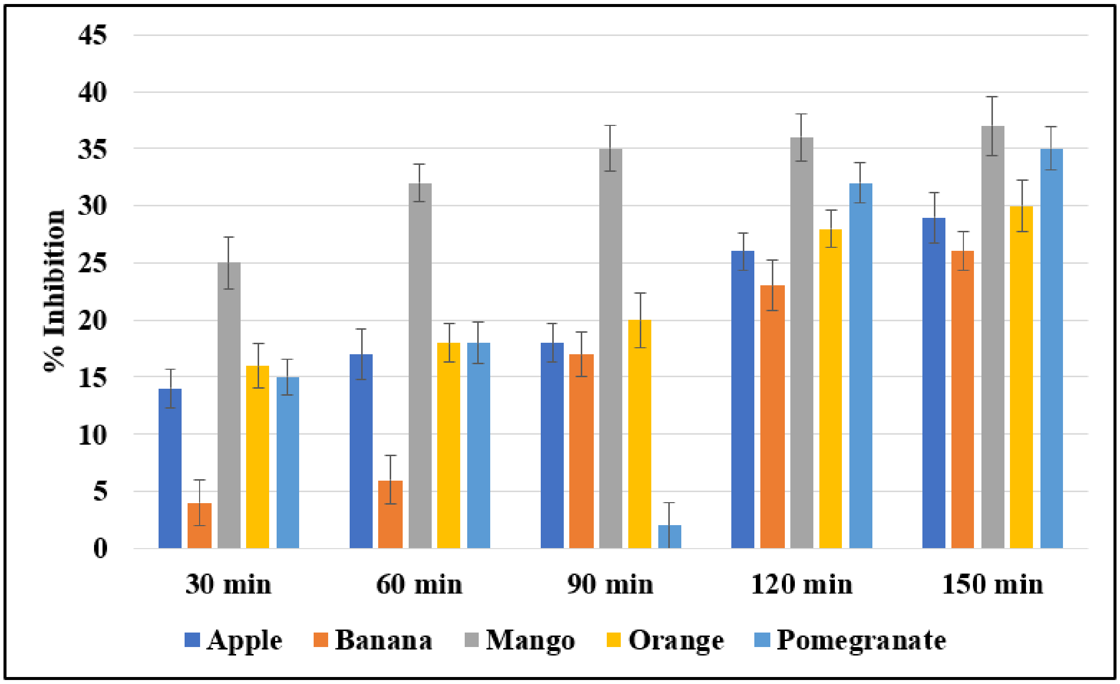

Figure 4: Antiglycemic activity of different fruit peels. Values expressed as Mean±Standard Deviation. A multi-bar chart showing % inhibition of glucose diffusion at different time points (30, 60, 90, 120, 150 min) for the five fruit peels.

enzymes such α-glucosidase (Gondi *et al*., 2015). Past studies with pomegranate demonstrated punicalagin and ellagic acid inhibiting α-amylase and α-glucosidase which regulate glucose transporter function, that reduce glucose transfer. Orange peel extract's mild inhibition (30% at 150 min) in our study can be linked to the activity of hesperidin and naringin, which were connected to enhance insulin sensitivity and β-cell protection, of previous study (Nazir *et al*., 2022).

At 150 min, apple peel extract's inhibitory activity had increased to 29%. According to Choi, Phlorizin, an anti-diabetic chemical found in apple peel, lowers glucose reabsorption in the kidneys by inhibiting Sodium-Glucose Cotransporter 2 (SGLT2) (Choi *et al*., 2016). Banana peel extract exhibited the lowest Antiglycemic activity (4-26%). However, the fruit's catecholamines and fiber may still help to regulate postprandial glucose levels as suggested by previous investigations (Wang *et al*., 2022).

According to earlier Antiglycemic research, polyphenol-rich fruit peels, like hypoglycemic medications and dietary fibers, can delay glucose absorption and diffusion when it comes to regulating postprandial hyperglycemia. Previous research has shown that polyphenols found in plants can inhibit the action of digestive and glucose transport activities (Gallagher *et al*., 2003; Seymenska *et al*., 2023). These findings were consistent with previous research.

CONCLUSION

According to the study, there are numerous antioxidants and anti-glycaemic compounds in fruit peels, particularly those of pomegranate, mango, and orange fruits. Their ability to neutralize free radicals and inhibit glucose transit suggests they may be effective in the treatment of oxidative stress and type 2 diabetes.

This study investigated the antioxidant and anti-glycemic properties of aqueous peel extracts from *Malus domestica* (apple), *Musa acuminata* (banana), *Mangifera indica* (mango), *Citrus reticulata* (orange), and *Punica granatum* (pomegranate). Antioxidant potential was assessed using the DDPH radical scavenging assay, while anti-glycemic activity was determined by glucose diffusion inhibition. Among the tested samples, pomegranate peel demonstrated the highest antioxidant activity (77%), followed by orange (71%) and mango (66%). Mango peel exhibited the strongest glucose diffusion inhibition (37%), followed by pomegranate (35%) and orange (30%). These findings indicate that fruit peels, particularly those of pomegranate and mango, contain abundant bioactive compounds with potent antioxidant and anti-glycemic capacities. The study highlights the therapeutic potential of these natural by-products in mitigating oxidative stress and type 2 diabetes, while also emphasizing their value in developing phytopharmaceuticals, functional foods, and sustainable waste-management approaches.

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ABBREVIATIONS

PBS: Phosphate-Buffered Saline; **UV-vis:** Ultraviolet-visible; **SIMATS:** Saveetha Institute of Medical and Technical Sciences; **DDPH:** 2,2-Diphenyl-1-Picrylhydrazyl; **MVJ:** M. V. Jayaram Medical College and Research Hospital; **GOD-POD:** Glucose Oxidase-Peroxidase; **SGLT2:** Sodium-Glucose Cotransporter 2.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION STATEMENT

RPC - Conceptualization, study designing, data collection, manuscript drafting and resources. BU - Conceptualization and study design. AKAK - Data analysis and manuscript drafting. YR, SRN - Data collection, data analysis and manuscript drafting. TJ - Supervision and approval of final draft of manuscript.

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