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Whole Orange Powder as A Rich Source of Polyphenols, Flavonoids and Antioxidants

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ABSTRACT

On a global scale, consumers' faith in dietary therapy for illness remediation has been bolstered by health claims for phytochemical-containing foods, including fruits and vegetables. Thanks to their antioxidant capabilities and chemical variety, polyphenols gave dietary supplements and nutraceuticals new life. **Objectives:** To investigate the antioxidant activity and phytochemicals (TPC and TFC) of the entire orange powder while extracted in water, ethanol and methanol. **Methods:** Each extract was tested for its total flavonoid composition using the aluminium chloride technique and its total polyphenolic content using the Folin reagent. The DPPH test was used to measure the antioxidant activity. **Results:** Results demonstrated that whole orange powder water extract had the lowest total phenolic content values (167.2 ± 3.3 mg GAE/g), flavonoids (35.8 ± 0.2 mg QE/g) whereas methanol extracts displayed the highest values (350.8 ± 6.3 mg GAE/g; 72.5 ± 2.2 mg QE/g) and ethanol extracts showed the moderate values (283.4 ± 5.2 mg GAE/g; 57.4 ± 1.8 mg QE/g) respectively. At a concentration of 20-100 µg/ml, the methanol extract had the greatest antioxidant% inhibition value, 38.50 ± 1.3 - 87.67 ± 2.4 %, followed by the ethanol extract (28.70 ± 1.1 - 65.40 ± 2.1 %) whereas the water extract had the lowest antioxidant% inhibition value, 17.95 ± 0.3 - 52.25 ± 1.6 % and it showed a statistically significant difference values ($p < 0.05$) among the extracts. **Conclusions:** It was concluded that the antioxidant levels, polyphenols and flavonoids in whole orange powder were strongly affected by the solvent type employed for extraction, with methanol being the solvent of choice.

INTRODUCTION

Every year, more than 100 million tons of citrus fruits are produced throughout the world, marking a remarkable increase in output. The fruit juice extraction process is the lifeblood of this booming sector, but it also produces copious amounts of waste items, including seeds, pulp, and peels. Half of the fruit is not fit for human consumption, leading to an annual waste of 60 million tons [1, 2]. The peels of citrus fruits, which make up more than half of the fruit itself, are often wasted and ignored, which

has serious consequences for the environment, the economy, and our nutrition. These problems are made worse in emerging nations like India due to their inadequate infrastructure for managing this massive amount of biomass. To encourage environmentally friendly production and consumption, the European Union and the United Nations have made efforts in the last decade to redistribute and valorize food waste under a hierarchical system. One of the biggest producers of citrus (Oranges),



which is aptly referred to as the king of all simple peeler kinds and surpasses the greatest varieties worldwide, is Pakistan. Furthermore, in Pakistan's overall fruit culture, citrus is the country's main crop in terms of both area and production. With a production level of 2.29 million tons, these fruits are cultivated on 210.47 thousand hectares in the Punjab province [3]. There is a lost chance to extract useful bioactive chemicals from citrus peels and entire trash oranges, which are now thrown away. The wide variety of chemicals that make up these compounds has made them famous for the positive effects they may have on health [4]. Citrus peel extracts have not been well investigated. There are several nutritional advantages of eating citrus fruits, which are common in the subcontinental diet. For example, oranges are a good source of flavonoids like narirutin 4'-glucoside, eriocitrin, narirutin, a compound called isosakuranetin, sinensetin, quercetagenin/gossypetin, a medication called no, 3,5,6,7,8,3',4'-heptamethoxyflavone, tangeritin/5-hydroxy-3,7,8,3',4'-pentamethoxy-flavone, Chrysoeriol, limocitrol, limocitrol, and limocitrol, among others, which are most commonly found in orange juice [5]. In addition to a wealth of nutrients, such as vitamins C, A, and B, micronutrients (calcium, phosphorus, potassium), and polyphenols (carotenoids, amino acids, which are triterpenes, phenolic acids, and flavonoids) found in orange peel powder, it is also an outstanding source of dietary fiber [6]. The presence of a group of flavonoids, including narirutin 4'-glucoside (1), eriocitrin (2), narirutin (3), hesperidin (4), isosakuranetin (5), sinensetin (6), quercetagenin/gossypetin (7), nobiletin (8), 3,5,6,7,8,3',4'-heptamethoxyflavone (9), tangeritin/5-hydroxy-3,7,8,3',4'-pentamethoxyflavone (10), Chrysoeriol (11), limocitrin (12), limocitrol (13) (Figure 1) [7].

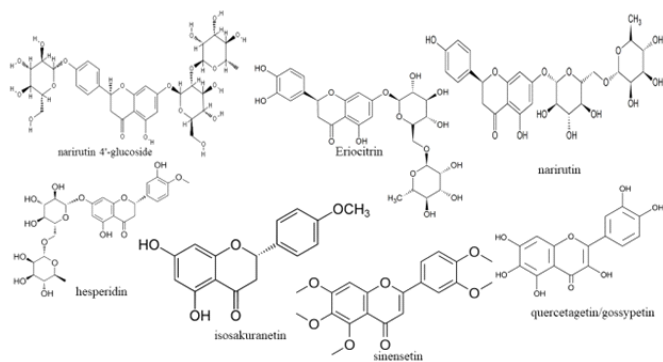


Figure 1: The Presence of a Group of Flavonoids

Using HPLC-DAD, a research by researcher [8] identified nine peaks of polyphenolic compounds, including catechin, caffeic acid, naringin, epicatechin, rutin, quercitrin, quercetin, kaempferol, and luteolin. According to [9], these phenolic compounds can be incorporated into food products or, upon extraction, can be used as natural

protectors to shield certain foods from oxidation. Scientific research has shown that orange by-products, which contain a high concentration of bioactive compounds, may have unique and boosted therapeutic effects against oxidative stress in cancer cells, Type 2 diabetes, and cardiovascular diseases [10]. Catechin (peak 1), caffeic acid (peak 2), naringin (peak 3), epicatechin (peak 4), rutin (peak 5), quercitrin (peak 6), quercetin (peak 7), kaempferol (peak 8) and luteolin (peak 9) (Figure 2) [8].

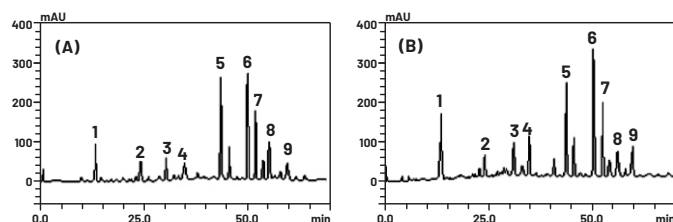


Figure 2: Representative High Performance Liquid Chromatography Profile of Unripe and Ripe Orange Peel Powder

Internally produced reactive oxygen species (ROS) like hydrogen peroxide, superoxide anion radicals, and hydroxyl radicals can cause oxidative stress, which in turn can lead to several diseases, such as cancer, cardiovascular disease, ageing, and neurodegenerative disorders [11, 12]. Exogenous antioxidants from polyphenol-rich foods, such as fresh produce, play a key role in regulating reactive oxygen species (ROS), which in turn has a good effect on human health [13].

Despite the recognized nutritional value of citrus by-products, particularly orange peels, their systematic evaluation as whole orange powder remains limited, especially under local agro-climatic conditions of Pakistan. Most previous studies have focused on peel extracts alone or employed advanced analytical profiling, with insufficient comparative assessment of different extraction solvents on total polyphenols, flavonoids, and antioxidant activity. Furthermore, limited region-specific data exist on valorizing whole citrus waste into functional antioxidant-rich products. Therefore, comprehensive evaluation of whole orange powder using conventional phytochemical and antioxidant assays is needed to validate its nutraceutical potential. This study aims to determine the whole orange powder's polyphenols, flavonoids, its antioxidant activity, and the phytochemical bioactive components. This research has the potential to reveal these byproducts' untapped potential and confirm their status as an affordable, all-natural antioxidant source.

METHODS

After being thoroughly cleaned and chopped, the waste, approximately 40 kg of oranges 500 (*Citrus aurantium* L.) from the citrus orchard in Sargodha, Pakistan, were dried in a hot air oven dryer for six to eight hours, and the samples were kept in airtight containers [14]. For effective

extraction, 20 grams of powdered material was extracted in 200 milliliters of distilled water, methanol, and ethanol, then left at room temperature for 24 hours. After passing the extract through Whatman filter paper No.1, it was kept at 4°C until needed. Following the recommendation of Singleton [15], the total polyphenolic content was determined. To summarize, 2.5 mL of 10% Folin-Ciocalteu's reagent (v/v) was used to oxidize the proper dilutions of the extracts and 2.0 mL of 7.5% sodium carbonate was used to neutralize the reaction. In a spectrophotometer (UV-700 Shimadzu Japan), the intensity of absorption was detected at 765 nm after 40 minutes of incubation at 45°C. After that, the gallic acid equivalent was used to determine the total phenol level. As reported earlier, the colorimetric approach was modified to estimate the total flavonoid makeup of both extracts. For this experiment, we mixed half a milliliter of the extract solution with half a milliliter of an ethanol solution containing 20 milligrams per milliliter of $AlCl_3$. The amount of flavonoids was determined as mg QE/g of the extract mixture using the absorbance at 420 nm reading from a wavelength analyzer (UV-1700, Shimadzu Japan) after an hour of incubation at 25°C. The DPPH, which (2,2-diphenyl-1-picrylhydrazyl) scavenging technique, was used to assess the anti-radical activity of the extracts and was slightly modified. A purple DPPH solution (20 mg/L in methanol) was added to the extracts (50 µg/mL at the finish throughout the well), and the mixture was left to incubate at a comfortable temperature for 15 minutes. With the use of an ultraviolet (UV)-visible spectrophotometer (UV-1700, Shimadzu Japan), the DPPH radical's decolonization was seen at 517 nm. The result was reported as DPPH antiradical activity as a percentage compared to the control group that received just DPPH and solvent, without the extracts. The mean \pm standard deviation (SD) was used to express the results. Analysis of variance unidirectional (ANOVA) was used to identify significant differences ($p < 0.05$), and the Tukey test for multiple comparisons was then used.

RESULTS

Results demonstrated that whole orange water extracts had the lowest total phenolic content values (167.2 ± 3.3 mg GAE/g), flavonoids (35.8 ± 1.2 mg QE/g) whereas methanol extracts displayed the highest values (350.8 ± 6.3 mg GAE/g; 72.5 ± 2.2 mg QE/g) and ethanol extracts showed the moderate values (283.4 ± 5.2 mg GAE/g; 57.4 ± 1.8 mg QE/g) respectively. At a concentration of 20-100 µg/ml, the methanol extract had the greatest antioxidant % inhibition value, 38.50 ± 0.3 - $87.67 \pm 2.4\%$, followed by the ethanol extract (28.70 ± 1.1 - $65.40 \pm 2.1\%$), and the water extract had the lowest, 17.95 ± 0.3 - $52.25 \pm 1.6\%$ (Table 1).

Table 1: Total Polyphenols and Flavonoids of Various Extracts of Whole Orange Powder

Extracts	TPC (mg GAE/g)	TFC (mg QE/g)
Methanol (Me-OH)	350.8 ± 6.3	72.5 ± 0.1
Ethanol (Et-OH)	283.4 ± 5.2	57.4 ± 0.4
Water (H ₂ O)	167.2 ± 3.3	35.8 ± 1.2

Data are represented as mean \pm SD

The DPPH radical was used to assess the extracts' free radical scavenging capabilities. At the concentration 20-100 µg/ml, the methanol extract exhibited the highest antioxidant DPPH (% inhibiting) value, ranging from 38.50 ± 1.3 - $87.67 \pm 2.4\%$, while the ethanol extract came in second with a value ranging from 28.70 ± 1.1 - $65.40 \pm 2.1\%$. The water extraction procedure of whole dried orange peel had the lowest value, ranging from 17.95 ± 0.3 - $52.25 \pm 1.6\%$, and it showed statistically significant differences in values ($p < 0.05$) among the extracts. The availability of several bioactive components, including the flavonoids that greatly determines the antioxidant potential of whole orange powder peel extracts. Preventing cellular damage and chronic illnesses relies on these chemicals' ability to scavenge free radicals and alleviate oxidative stress. Because these bioactive chemicals have variable distributions and solubility, the antioxidant capabilities of the various plant sections and solvents used in this investigation varied significantly (Figure 3).

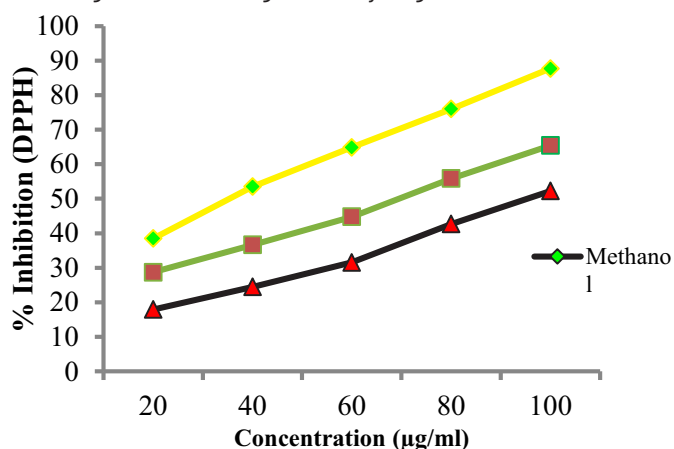


Figure 3: % Inhibition (DPPH) Various Extracts of Whole Orange Peel Powder

DISCUSSION

It was necessary to identify phenolics in food due to the health claims linked with them. The Folin-Ciocalteu technique, which is based on reducing phosphor-tungstic acid to phosphor-tungstic blue, was used for phenolic analysis. This led to a rise in absorbance because there were more aromatic phenolics [16]. If the blue hue becomes more intense, it means that there are more antioxidants in the sample. The orange peel extracts in methanol had the highest concentrations of phenol (350.8

± 6.3 mg GAE/100 g), and the ethanol extract had TPC (283.4 ± 5.2 mg GAE/g), while the water extract from the whole orange powder showed the lowest concentration of TPC (167.0 ± 3.3 mg GAE/g). The anti-inflammatory, flavor-enhancing, colour-enhancing and phenolic-rich β -carotene found in abundance in orange peels has been linked to the alleviation of several ailments [17]. Carotenoids present in orange peel powder are phytonutrients that are soluble in fat and have effects that prevent cancer, oxidative stress and mutations [18]. The unique citrus scent that has a profound effect on human existence is believed to be mostly produced by flavonoids, which are secondary metabolic product components often present in sweet orange peels [19]. Anticancer, antibacterial, antioxidant, anti-inflammatory and anti-allergic are only a few of the many biological effects shown by polyphenols, terpenes and terpenoids [20]. The antioxidant activity of the extracts in this investigation was excellent, with methanol showing the greatest results. Additionally, compared to the ethanol as well as water extracts, the methanol extracts exhibited somewhat greater antioxidant activity. This could be because different solvents extract antioxidant molecules to different degrees. Research has shown that orange peel extracts have strong antioxidant properties; our findings are in line with the literature cited [21].

This study was limited to in vitro antioxidant assays and did not include advanced compound identification techniques such as HPLC or LC-MS for precise phytochemical profiling. Additionally, only three extraction solvents were evaluated, and the bioavailability or in vivo therapeutic potential of the extracts was not assessed. Future research should focus on detailed compound characterization, optimization of green extraction methods, and biological efficacy studies in cell or animal models. Expanding this research could support the development of functional foods and sustainable valorization strategies for citrus waste.

CONCLUSIONS

It was concluded that whole orange (*Citrus aurantium* L.) powder is a great option due to its high phenolic, flavonoid and antioxidant content. Since they contain bioactive compounds or therapeutic candidates that show considerable action against oxidative stress. It is therefore a good choice for either creating new products or treating or aiding in the therapy of various illnesses. Furthermore, the whole orange powder's antioxidant content was significantly impacted by the kind of extraction solvent used.

Authors' Contribution

Conceptualization: MKS

Methodology: MKS, NZ, AS, KS

Formal analysis: SS

Writing and Drafting: SN, AAR, SHIA, QUAS

Review and Editing: SN, AAR, SHIA, QUAS, SS, MKS, NZ, AS, KS

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

All the authors declare no conflict of interest.

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