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Phytochemical profiles, nutrient compositions, extraction yields and antioxidant activities of seven underground vegetable's peels

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ABSTRACT

A large number of studies on fruit's peels revealed the presence of important phytochemicals having diverse biological activities. Here we report the nutrient compositions, extraction yields, phenolic contents and antioxidants activities of the peel samples of seven underground vegetables namely *Beta vulgaris*, *Brassica rapa*, *Daucus carota*, *Ipomoea batatas*, *Raphanus sativus*, *Solanum tuberosum* and *Zingiber officinale*. Proximate analysis revealed that the peels are good sources of fibers, proteins and ash. The extraction yields of the peels ranged from 8 to 17%, 15 to 32% and 23 to 33% in ethyl acetate, water and methanol, respectively. The flavonoid, phenolic and carotenoid levels were in the ranges of 63.7 to 147.7 mg/100g, 930.0 to 1658.2 mg/100g and 44.0 to 188.3 mg/100g, respectively. The peels also showed good scavenging activities against DPPH radical and hydrogen peroxide.

Key words: Flavonoids, carotenoids, phenolics, proximate compositions, scavenging activities, DPPH˙, hydrogen peroxide

INTRODUCTION

Vegetables and fruits are considered essential part of diet due to their health promoting effects. Balanced and nutritious diets have plenty of plant ingredients and are strongly associated with reduced risk of ailments like cardiovascular disease, diabetes, arthritis, inflammation, various types of cancers, and aging [1-5]. Boeing *et al.* [6] in their review article reported a strong association between the high intake of vegetables and fruits and reduced risks of several chronic diseases. Consumption of fruits and vegetables is required on regular basis. Plants are rich sources of vitamins, minerals, proteins, sugars, lipids, dietary fibers and other beneficial phytochemicals such as flavonoids, phenolic, carotenoids etc. These constituents are present in almost all parts of plants viz. fruits, flowers, seeds, pods, leaves, barks, stalks, roots, stems, latex, hulls and fruit rinds [7-10].

There is increasing awareness among customers about the beneficial characteristics of plant-based food. Consequently, the global consumption of vegetables, fruits, plant extracts and teas has been enormously increased. Dieticians and health care professionals recommend regular and appropriate inclusion of fruits and vegetable in diets. Underground vegetables are readily consumed and available throughout the year in Pakistan. These vegetables have delicious taste and are consumed in various forms. These are eaten raw, cooked and pickled. Vegetable's peels are normally produced during household consumption and industrial processing. These agro wastes are thrown directly into the surroundings. These agro wastes are prone to microbial attacks, if not processed appropriately.

A sufficient number of scientific publications on fruit and vegetable peels [11-18] has revealed that these are precious source of various nutrients as well as they possess antimicrobial, anticancer and antioxidant activities. In the current study, the peels of seven underground vegetables were evaluated for nutrients, beneficial phytochemicals, antioxidant activity and extraction yields.

MATERIALS AND METHODS

Plant materials: Seven fresh underground vegetables were procured from the local market of Peshawar (**Table 1**). The vegetables were washed with distilled water and then peels were separated carefully, washed again, cut into small pieces and

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dried in shade. The dried peels were then pulverized in a grinder (Retch Muhle-Germany), passed through the 30 mesh sieve and packed in polyethylene pouches. The samples were sealed using an electric sealer and stored at 4 °C till further processing and analysis.

Preparation of extracts: The powder peels (25 g, each) of underground vegetables were separately extracted in methanol, water and ethyl acetate (3 × 200 ml). The samples were taken in conical flasks plugged with cotton wool and kept in an orbital shaker at 120 rpm for 16 hours. The extracts were combined and filtered through Whatman filter paper No. 41 for removal of peel particles and afterward the extracts were concentrated under vacuum at 45 °C. The dried extracts were stored in a refrigerator at 4°C. The weights of all dry extracts were determined using the following formula.

Extraction yield (%) = (weight of the residue)/(initial weight of the peel powder) × 100

Proximate analysis: The samples were assessed for moisture, ash, crude fat, crude protein and fiber contents on a dry weight basis, according to AOAC [19]. The moisture was measured in a drying oven at 105°C until constant weight. Analysis of crude fat was carried out using petroleum ether (bp. 40-60°C) in a Soxtec system (Tecator). Determination of crude protein (% N x 6.25) was done by employing micro-Kjeldahl method. The crude fiber was estimated using Fibertec system (Tecator) by treating the samples with acid and alkali, while the ash contents were estimated by heating the samples at 550°C. The NFE (%) contents were predicted by using difference method i.e. by deducting the sum of the percent of moisture, fat, protein, ash and fiber from 100.

Determination of phenolic contents: The phenolic contents of peels were appraised by using the Folin-Ciocalteau colorimetric assay [20]. In a test tube, 200µl of methanolic extract was mixed thoroughly with 4ml of 2% aqueous sodium carbonate solution. Later, 200µl of 50% Folin-Ciocalteau reagent was added to the mixture. The mixture was kept for 45 minutes and the absorbance of green-blue complex was recorded at 750 nm on a spectrophotometer. The results were expressed as milligram of gallic acid equivalents per 100 gram of the dry extract.

Estimation of flavonoids: Flavonoid contents were determined by employing the method of Khattak [21] with slight modifications. One ml of the methanolic extract of peel sample was mixed with 0.2ml of 10% aluminum chloride, 0.2ml of 1M potassium acetate, 3ml of methanol and 5.6ml of distilled water. The mixture was kept at room temperature for one hour. The absorbance of the mixture was noted on a spectrophotometer at 420 nm. Quercetin was used as standard. Concentration levels of flavonoids were calculated from the standard curve and shown as mg/100g quercetin equivalents.

Determination of carotenoids: Total carotenoids were determined by the method of Jensen [22]. One gram powdered sample was extracted in 30 ml of 80% methanolic solution and centrifuged for 25 minutes at 5000 rpm. The supernatant was carefully separated and concentrated under vacuum at 45 °C. The died residue was dissolved in 20 ml of diethyl ether and after addition of 20 ml of 10% methanolic potassium hydroxide, the mixture was washed with 5% ice-cold saline water to remove alkali. Afterward the ether extract was dried using anhydrous sodium sulphate for 3 hours. Lastly, the extract was filtered and its absorbance was recorded at 450 nm by using ether as blank.

Determination of DPPH radical scavenging activity: The scavenging activity of the peel samples was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical [21]. Two ml of DPPH radical in methanol solution (60µM) was mixed with 80 µl of the sample and shaken carefully. The solution was kept in dark at 37°C for two hours and subsequently its absorbance was measured at 517nm. The absorbance of corresponding blank (control) was also noted. Quercetin was used as positive control. The results were expressed as EC_{50} values. The EC_{50} value is defined as the extract concentration at which DPPH radicals were reduced by 50%.

Determination of Hydrogen peroxide scavenging activity: The **s**cavenging of hydrogen peroxide by the peel extracts were estimated using the method of Ruch *et al.* [23]. Plant extract (4 ml) prepared in distilled water at various concentration was mixed with 0.6 ml of 4 mM H_2O_2 solution prepared in phosphate buffer (0.1 M pH 7.4) and incubated for 20 minutes. The absorbance of the solution was noted at 230 nm against blank solution containing the plant extract without H_2O_2. Ascorbic acid was used as positive control. The results were expressed as EC_{50} values.

RESULTS AND DISCUSSION

Vegetables are considered as an important part of a diet in Pakistan. In the current study, the peels of seven underground vegetables were evaluated for their nutrients, phytochemicals, extraction yields and scavenging activity against DPPH radical and hydrogen peroxide.

The proximate compositions were determined using standard methods and results are given in **Table 2**. The vegetable peels were found to have appreciable quantities of fiber (11.6 to 17.4%), protein (6.9 to 8.3 %) and ash (5.3 to 9.6%). The NFE ranged from 57.5 to 63.7% and fat contents were between 2.1 to 3.5%. The *Zingiber officinale* plant showed the maximum fiber content, while the peels of *Beta vulgaris* exhibited maximum ash content. Maximum protein content were recorded for *Brassica rapa* peels. An earlier study conducted by Sharoba *et al.* [24] showed that carrot pomace has moisture 4.61%, ash 7.29%, fat 1.75% and protein 10.06%. The same study also revealed that potato peels contain 3.58% moisture, 6.92% ash, 2.25% crude fat and 12.16% crude protein. In 2014, Adewole *et al.* [25] worked on the orange peel and reported 10.0% moisture, 5.51% ash, 14.35% crude fat, 16.40% crude protein, 12.47% crude fiber and 40.47% carbohydrate. The results of another study accomplished by Ullah *et al.* [26] revealed that the peels of pomegranate have moisture (04 ± 0.22%), ash (05 ± 0.14%), fat (9.4 ± 0.1%), crude fiber (21 ± 0.6%) and protein (8.719 ± 0.10%) contents.

The extraction yields in three different solvents namely methanol, ethyl acetate and distilled water showed that the ability to extract phytochemicals was generally in the order of methanol > water > ethyle acetate (**Table 3**). The methoanlic peel extract of *Zingiber officinale* showed the highest extraction yield (33%). An investigation conducted by Khonkarn *et al.* [27] on fruit peel extracts showed that the yields of ethanolic peel extracts of coconut, rambutan and mangosteen were 5.8, 10.7 and 7.2%, respectively. Shiban *et al.* [28] carried a research study, which indicated 45.5%, 35% and 7% extraction yields for pomegranate fruit peels in 80% methanolic, water and ether extracts, respectively. Fruits and vegetables are the main dietary sources of flavonoids. There has been increasing interest in the research on flavonoids from plant sources because of their versatile health benefits. Many flavonoids are shown to have antioxidant, anti-inflammatory, anticancer, antidiabetic, antineoplastic, antithrombogenic, antiatherosclerotic, antiulcer and hepatoprotective activities (29-30]. The flavonoid contents of the plants ranged from 63.7 to 147.7 mg/100g (**Table 4**). The methanolic peel extract of *Daucus carota* showed maximum flavnoids. A recent study carried out by Janjua *et al.* [15] showed that the peel extract of the roots of *Raphanus sativus* L. carry important phytoconstituents like flavonoids, tannins, saponins, phlobatannins, anthraquinones, carbohydrates, reducing sugars, steroids, phytosterol, alkaloids, amino acids, terpenoids, cardiac glycosides and chalcones. The extract also showed in vitro antibacterial activity. Shiban *et al.* [28] conducted an investigation on pomegranate fruit peels. The results of their study indicated that methanolic peel extract contains 56.4 mg/g flavonoid contents. Another research carried out by Manzoor *et al.* [31] on five apple (*Malus domestica* Borkh.) cultivars from Pakistan, showed that the flavonoid contents in peel and pulp were in the ranges of 1214.3–1816.4 mg catechin equivalent/100 g and 711.8–999.3 mg CE/100 g on dry weight basis, respectively.

The phenolic content of the peel extracts ranged from 930.0 to 1658.2 mg/100g (**Table 4**). The peels of *Beta vulgaris* vegetable showed the maximum phenolic content. Singh and Immanuel [18] analysed the peels of pomegranate, lemon and orange. They reported highest phenolic content (249.41 mg/g) in pomegranate peel. Another study carried out by Shiban *et al.* [28] showed that total phenolics of pomegranate fruit peels in methanloic, water and ether extracts were 274.1, 91.2 and 8.5 mg/g, respectively. Manzoor *et al.* [31] worked on peel and pulp samples of five apple cultivars of Pakistan, namely Red Delicious, Golden Delicious, Kashmiri Amri, Kala Kulu and Sky Spur. The total phenolics in peel and pulp samples were in the ranges of 1907.5 to 2587.9 and 1185.2 to 1475.5 mg gallic acid equivalent/100 g, respectively. A scientific investigation conducted by Al-Juhaimi [32] showed that peels of orange, mandarin and lemon contain 178.90, 169.54 and 61.22 mg GAE/100g total phenolics, respectively.

Carotenoids comprise a large family of C40 polyenes and have large number of applications in agriculture, food, health and cosmetic industries [33-34]. The carotenoid contents of the underground vegetable were recorded between 44.0 and 188.3 mg/100g (**Table 4**). The peel extracts of *Daucus carota* showed the maximum carotenoids.

The results regarding the scavenging activity against DPPH and hydrogen peroxide were reported in **Table 5**. The peel samples of *Beta vulgaris*, *Brassica rapa*, *Daucus carota*, *Ipomoea batatas*, *Raphanus sativus*, *Solanum tuberosum* and *Zingiber officinale* showed promising scavenging activity against hydrogen peroxide. The EC_{50} values were 164 ± 6.3, 279 ± 11.2, 126 ± 2.0, 169 ± 5.6, 91 ± 4.5, 124 ± 4.0 and 67 ± 1.8 µg/ml, respectively. The low EC_{50} value reflects higher scavenging activity. The data about DPPH scavenging activity revealed that the peel samples of *Beta vulgaris*, *Brassica rapa*, *Daucus carota*, *Ipomoea batatas*, *Raphanus sativus*, *Solanum tuberosum* and *Zingiber officinale* exhibit EC_{50} values at 62 ± 2.2, 103 ± 3.5, 71 ± 0.6, 92 ± 1.2, 67 ± 2.0, 92 ± 1.9 and 33 ± 1.9µg/ml, respectively. Earlier, Shiban *et al.* [28] checked the antioxidant

activity of pomegranate fruit peels and found that the DPPH radical scavenging activity of methanolic extract was stronger than that of α-catechin, the standard. They also reported that the reducing power of 80% methanolic extract was stronger than that of water and ether extracts. Al-Juhaimi [32] worked on the peels of orange, mandarin and lemon and reported that they exhibit 67.58, 68.57 and 46.98% DPPH radical scavenging activities, respectively. A study accomplished by Manzoor *et al.* [31] on peel and pulp samples of five apple (*Malus domestica* Borkh) cultivars, revealed that methanolic, water and ether extracts inhibit the activity of DPPH free radical by 66.6–80.8% in peel and 42.9–51.1% in pulp. Singh and Immanuel [18] used peels of pomegranate, lemon and orange as sources of natural antioxidants in the preparation of Paneer samples. The pomegranate extract exhibited high antioxidant activity 92.7% in comparison to those of lemon and orange peel extracts. The ability to prevent peroxide formation in paneer sample was in the order of pomegranate peel > lemon peel > orange peel.

Peels can serve as safe and cheap natural raw materials for food, poultry and livestock feed. These can be utilized beneficially to develop health products, nutritional supplements and food. Their utilization in product formulation may also lead to reduces pollution.

CONCLUSION

The antioxidant activities, phytochemical profiles and nutrient compositions of the underground vegetables showed the medicinal and nutritional importance of peels. The study suggests that these should not be wasted, but rather incorporated in feed for livestock and poultry. Variety of nutritional products for human consumption can also be prepared from peels after appropriate processing. Furthermore, plant based agro wastes can be utilized for therapeutic purpose. More studies are required to evaluate their anti-nutritional constituents and other biological activities.

Table 1. List of underground vegetables used in the study

Botanical name	Local name	English name	Family
Beta vulgaris	Chukandar	Sugarbeet	Chenopodiaceae
Brassica rapa	Shaljam	Turnip	Brassicaceae
Daucus carota	Gajar	Carrot	Apiaceae
Ipomoea batatas	Shakarkandi	Sweet potato	Convolvulaceae
Raphanus sativus	Moli	Radish	Brassicaceae
Solanum tuberosum	Aaloo	Potato	Solanaceae
Zingiber officinale	Adrak	Ginger	Zingiberaceae

Table 2: **Proximate composition of peel samples of underground vegetables**

Plants	Moisture %)	Protein (%)	Fat (%)	Ash (%)	Fiber (%)	NFE (%)
Beta vulgaris	7.6±0.6	7.1±0.5	3.2±0.0	9.6±0.4	15.0±1.3	57.5±2.8
Brassica rapa	6.9±0.7	8.3±0.2	2.1±0.1	7.9±0.3	16.2±0.7	60.6±2.0
Daucus carota	7.9±0.0	7.6±0.9	2.7±0.0	8.3±0.1	14.4±0.4	59.1±1.4
Ipomoea batatas	9.0±0.2	6.9±0.8	3.5±0.2	5.3±0.3	11.6±0.5	63.7±2.0
Raphanus sativus	7.2±0.6	8.2±0.4	2.9±0.1	6.6±0.2	16.4±0.3	58.7±1.6
Solanum tuberosum	7.3±0.3	7.5±0.2	2.6±0.1	7.9±0.0	12.4±0.4	62.3±1.0
Zingiber officinale	6.6±0.2	7.9±0.8	2.7±0.1	7.6±0.6	17.4±1.1	57.8±2.8

The values presented in this table are on dry weight basis. Values are means ± standard deviations of three determinations.

Table 3. Extraction yields of underground vegetables peels in various solvents

Plants	Yield (%)		
	Methanol	Ethyl acetate	Water
Beta vulgaris	29 ± 0.4	13 ± 0.5	32 ± 0.5
Brassica rapa	31 ± 0.3	13 ± 0.5	15 ± 0.5
Daucus carota	29 ± 0.2	15 ± 0.5	23 ± 0.5
Ipomoea batatas	27 ± 0.5	8 ± 0.5	19 ± 0.5
raphanus sativus	23 ± 0.5	12 ± 0.5	17 ± 0.5
Solanum tuberosum	29 ± 0.5	15 ± 0.5	21 ± 0.5
Zingiber officinale	33 ± 0.5	17 ± 0.5	22 ± 0.5

Each value is the average of three measurements ± standard deviation.

Table 4: Phytochemical profile of peel samples of selected underground vegetables

Phytochemicals	Phenolics (mg/100g)	Flavonoid (mg/100g)	Carotenoids (mg/100g)
Beta vulgaris	1658.2±34.2	63.7±3.8	44.0±1.4
Brassica rapa	995.7±15.7	135.0±5.9	71.7±1.9
Daucus carota	1053.7±10.6	147.7±8.9	188.3±7.0
Ipomoea batatas	890.4±12.5	93.3±6.3	52.0±3.3
Raphanus sativus	1595.6±22.7	111.0±6.0	71.7±2.1
Solanum tuberosum	930.0±5.6	97.7±4.4	48.3±1.0
Zingiber officinale	1290.7±15.5	133.4±7.5	87.0±3.7

Values are means ± standard deviations of three determinations.

Table 5. The EC_{50} values of the hydrogen peroxide and DPPH scavenging activity of methanolic peel extracts of underground vegetables

Plants	H_2O_2 (µg/ml)	DPPH (µg/ml)
Beta vulgaris	164 ± 6.3	62 ± 2.2
Brassica rapa	279 ± 11.2	103 ± 3.5
Daucus carota	126 ± 2.0	71 ± 0.6
Ipomoea batatas	169 ± 5.6	92 ± 1.2
Raphanus sativus	91 ± 4.5	67 ± 2.0
Solanum tuberosum	124 ± 4.0	92 ± 1.9
Zingiber officinale	67 ± 1.8	33 ± 1.9
Vitamin C	18 ± 0.9	-
Quercetin	-	8 ± 0.5

Values are means ± standard deviations of three determinations.

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