Cytotoxicity, antioxidant and antimutagenic potential evaluation of peels of edible roots and tubers

Sarwari, G., Sultana, B., Sarfraz, R. A. and Zia, M. A.

Department of Chemistry, University of Agriculture, Faisalabad, 38040, Pakistan
Department of Biochemistry, University of Agriculture, Faisalabad, 38040, Pakistan

Abstract

The peels of edible roots and tubers contain nutritionally and industrially important compounds but are frequently discarded as agro-waste. In the present work, the peels of radish (Raphanus sativus), turnip (Brassica rapa), beetroot (Beta vulgaris), sweet potato (Ipomoea batatas), and potato (Solanum tuberosum) were analysed for their potential cytotoxicity in terms of haemolytic activity; antioxidant activity in terms of total phenolic contents (TPC); DPPH radical scavenging assay, and antimutagenic activity using Ames bacterial reverse mutation test. Characterisation of individual phenolic acids was performed using high-performance liquid chromatography (HPLC). The haemolytic activity of radish, turnip, potato, sweet potato, and beetroot peels ranged from 0.34 - 4.85% and the TPC ranged from 43.82 - 67.23 mg GAE/g DW. Strong antimutagenic behaviour was exhibited by beetroot peels while sweet potato peels were found to be weak antimutagenic agent. Overall, the result infers that peels of selected edible roots and tubers are a rich source of antioxidants with good antimutagenic potential but weak cytotoxicity towards normal human blood cells. Therefore, the peels of roots and tubers can be used as feed, food and natural pharmaceutical or chemo-preventive agent after applying suitable processing techniques rather than discarded as agro-waste.

Keywords

Cytotoxicity
Edible roots
Antimutagenic potential
Antioxidant activity

Introduction

Roots and tubers are parts of plants that grow underground to provide food, nourishment and hold the plant. Most of roots and tubers are edible and consumed as vegetables (e.g., carrot, yam, radish potato). These are a vital part of the human diet and provide numerous health benefits such as hypoglycaemic, antioxidative, antimutagenic, antimicrobial, immunomodulatory, and hypocholesterolaemic activities. Roots and tubers often contain bioactive compounds like saponins, phenolics, glycoalkaloids, phytic acids and bioactive proteins that are responsible for their potential activities. Roots and tubers have huge potentials as functional foods and pharmaceutical ingredients in reducing risk of diseases (Chandrasekara and Kumar, 2016). Previous researches revealed the importance of vegetables and fruits in reducing cardiovascular diseases and various types of cancers (Boeing et al., 2012).

Vegetables and fruit peels are produced in huge amounts during household usage and industrial food processing, and often discarded as waste. These agro-waste substances are prone to microbial decay and can cause serious environmental and economic problems. The Food and Agriculture Organization (FAO) has recently estimated that waste or loss in vegetables and fruits are the highest and reach up to 60% of total production. The food processing waste, which mainly comprises of peels, skin, seeds, pomace and rind, contain an abundant amount of oils, enzymes, vitamins, polyphenols, dietary fibres and carotenoids. These important substances can be used in various industries such as food, textile and pharmaceutical. Therefore, strategies should be planned to manage and utilise food wastes in producing vital bioactive substances as a significant step towards sustainable developments (Khattak and Rahman, 2017; Sagar et al., 2018).

Previous studies on some vegetable peels also revealed the phyto-constituents present in them. Singh et al. (2009) worked on onion (Allium cepa) peels and found that onion peels are a rich source of antioxidant and antimutagenic substances; and concluded that its peels can be good nutraceutical...
compounds. Another study on papaya (Carica papaya) seeds and peels revealed that its peels are a great source of fibre, protein and other nutrients (Santos et al., 2014).

Recently, consumer awareness about the nutritional significance of fruits and vegetables has increased and so has their commercial demands. Therefore, there is an urgent need to properly utilise the natural sources to accomplish the requirements. Various studies have been conducted on fruit peels (Kodal and Aksu, 2017; Ozturk et al., 2018; Pérez-Jiménez and Saura-Calixto, 2018; Sumere et al., 2018) to establish their antioxidant potentials but scarce data are available on edible root and tuber peel bioactivities. Therefore, in the present work, the antioxidant and antimutagenic potentials of edible root and tuber peels were investigated. In addition, the antimutagenic potential and cytotoxicity of these peels were assessed to provide insight on consumer’s health safety.

Materials and methods

Experimental

Collection and preparation of samples

Edible roots and tubers (radish, turnip, beetroot, potato, and sweet potato) were collected from Faisalabad city and authenticated by expert in the Department of Botany, University of Agriculture, Faisalabad. The selected root samples were washed with tap water; peels were removed with a sharp knife, cut into small pieces, and then dried under shade until constant weight was achieved.

Extraction process

The dried pieces of peels were ground using a grinding mill into a fine powder. Next, 10 g of dried sample was extracted for 8 h with 100 mL of aqueous methanol (80%) in an orbital shaker at ambient temperature. The filtrate was separated from solids through Whatman No. 1 filter paper, and the extract obtained was concentrated using rotary evaporator at 45°C. The peel extracts were stored in a refrigerator at -4°C until further evaluation (Sultana et al., 2007).

Cytotoxicity of plant extract

The cytotoxicity of the test samples was estimated by determining the haemolytic activity following the protocol described by Powell et al. (2000). Triton X-100 was used as positive control and PBS as negative control. The cytotoxicity was calculated using Eq. 1:

\[
\% \text{ Haemolysis} = \left( \frac{H_{b_{\text{abs}}}}{H_{b_{\text{abs}}}^{100\%}} \right) \times 100
\]

(Eq. 1)

where \( H_{b_{\text{abs}}} \) = absorbance of sample, and \( H_{b_{\text{abs}}}^{100\%} \) = absorbance of positive control. Higher percentage of haemolysis by plant extract indicates greater cytotoxicity.

Estimation of total phenolic content (TPC)

The TPC of peel extracts were estimated with the help of Folin-Ciocalteu reagent following method described by Chaovanalikit and Wrolstad (2004).

DPPH scavenging assay

The free radical scavenging activity of peel extracts was estimated following the method described by Sultana et al. (2007).

Sample preparation for HPLC

For HPLC analysis of phenolic acids, the extracts of peels were processed as previously described by Tokusoglu et al. (2003).

HPLC conditions for the analysis of individual phenolics

A HPLC system (LC-10A, Shimadzu), equipped with quaternary pump G1311A, auto-injector / autosampler G1313A ALS, degasser G1379A, G1316B diode array detector, and Shim-Pack CLC-ODS (C18) column (250 mm x 4.6 mm; 5 μm particle size) from Merck (Germany) was used to confirm the phenolics. The column was thermostated at 25°C and separation was carried out with mobile phase comprising A: H2O:CH3COOH 94:6 and B: acetonitrile 100% set at the flow rate of 1.10 mL/min via gradient mode (15% B 0 - 16 min, 45% B 16 - 31 min and 100% B 31 - 46 min. Detection and quantification of individual phenolics were done at 280 nm. Quantitative measurements were based on the external standard calibration method.

Mutagenic and antimutagenic potential evaluation

The antimutagenic activity of peel extracts was determined using Ames bacterial reverse mutation test with the help of mutant strains of Salmonella Typhimurium TA100 and Salmonella Typhimurium TA98 as described by Mohd-Fuat et al. (2007). The inhibition in mutagenesis was calculated using Eq. 2:

\[
\text{Antimutagenicity} (%) = \frac{1 - \text{No. of (+) wells in test sample-spontaneous mutation}}{\text{No. of (+) wells in positive control-spontaneous mutation}} \times 100
\]

(Eq. 2)
Statistical analysis

Triplicate of each peel extract was examined, and data were reported as mean \((n = 3 \times 3 \times 1) \pm SD \((n = 3 \times 3 \times 1)\). The data was analysed for analysis of variance (ANOVA) using Minitab 2007 (Minitab Inc. Pennsylvania, USA) statistical software at a 5% significance level (Montgomery, 2009).

Results and discussion

Cytotoxicity of peel extracts

Vegetables contain various classes of phytochemicals of good antimitogenic and antioxidant properties, thus making these vegetables useful in the treatment of various diseases such as cancers and heart diseases (Yen et al., 2001). However, the presence of certain type of phytochemicals and other antinutritional substances may pose potential risk to human health if they are cytotoxic. Therefore, it is equally important to assess the toxicity of the extracts along with antioxidant activities or nutritional values.

The cytotoxicity of selected plant extracts was evaluated in terms of haemolytic activity against human red blood cells (RBCs). Triton X-100 served as positive control (shows 100% lysis) and phosphate buffer saline (PBS) as negative control (no lysis of RBCs; Riaz et al., 2012). Table 1 summarises the haemolytic activity of peel extracts of selected roots and tubers. The highest value of haemolytic activity was observed for beetroot peels (4.85%) followed by potato (1.10%), sweet potato (0.52%), radish (0.49%) and turnip (0.49%).

Table 1. Haemolytic activity of peels of edible roots and tubers.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Sample name</th>
<th>Haemolytic activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Triton X-100 (control)</td>
<td>98.5 ± 0.22</td>
</tr>
<tr>
<td>2</td>
<td>BPS (control)</td>
<td>0.12 ± 0.01</td>
</tr>
<tr>
<td>3</td>
<td>Radish</td>
<td>0.49 ± 0.02</td>
</tr>
<tr>
<td>4</td>
<td>Turnip</td>
<td>0.34 ± 0.01</td>
</tr>
<tr>
<td>5</td>
<td>Potato</td>
<td>1.10 ± 0.04</td>
</tr>
<tr>
<td>6</td>
<td>Sweet potato</td>
<td>0.52 ± 0.04</td>
</tr>
<tr>
<td>7</td>
<td>Beet root</td>
<td>4.85 ± 0.02</td>
</tr>
</tbody>
</table>

Values are means ± SD of triplicate \((n = 3)\) of experimental run under same conditions. Different superscripts letters within the same column indicate significant difference \((p < 0.05)\) among peel samples of edible roots and tubers.

The present data reveal that cytotoxicity values for all the selected extracts of peel were very low and within the safe limits for pharmaceutical applications or other food related uses. However, there are no studies available on edible roots and tuber’s cytotoxicity that directly correlate with the present findings. Ali and Çelik (2007) studied the cytotoxicity of peel extract of lemon \((Citrus limon)\) and showed that the peels could be harmful to organisms.

Extract yield and total phenolic contents

Plants contain an abundance of biologically active substances such as phenolic acids, salicylates, flavonoids, stanols, lignins, glucosinolates and sterols which possess various properties to be used as foods and medicines (Saeed et al., 2014). The phenolic compounds are a major contributor for free radical scavenging activity and antioxidant properties of plants. Extraction of these biologically active substances is a critical step in food science. In the present work, 80% aqueous methanol was used for the extraction process because this concentration has been suggested as the best for the extraction of plant phenolics (Mushtaq et al., 2012; Anwar et al., 2013).

The extract yield for selected samples ranged from 59.54 to 23.36% and their TPC 67.23 to 38.76 mg GAE/g DW as shown in Table 2. The highest extract yield and TPC were offered by beet root peel extracts. There is a general understanding that plant containing higher level of TPC offers superior antioxidant properties. The higher extract yield and TPC of beetroot peel could also be linked with the presence of betalains like betacyanins (red-violet colour) and betaxanthins (yellow-orange colour), all of which have numerous nutritional health benefits. In addition, beetroot peel yielded the second-highest dry weight concentration of total phenols (Chhikara et al., 2019). A general trend regarding TPC for selected peel samples in descending order is as follows; beetroot > turnip > potato > radish > sweet potato peels. The obtained data showed that all the peel samples are a good repository of phenolic antioxidants and might be used for other purposes (food, textile, drug, medicinal applications) after proper processing techniques rather than just discarded as agro-waste. For example, betalains in beetroot are recognisable commercially as food dye due to it being non-carcinogenic, non-toxic, and non-poisonous. Beetroot is often used as food colorant or additive in food products such as ice-cream, yogurts and other products. The beetroot extract is used to improve the redness in tomato pastes, soups, sauces, desserts, jams, jellies, sweets and breakfast cereals (Chhikara et al., 2019). Chel-Guerrero et al. (2018) studied the phenolic contents of peels of tropical fruits and found these peels as good sources of antioxidants that can be used for drug developments.

DPPH radical scavenging activity of peel extracts

DPPH radical scavenging activity of selected samples is another way to access the antioxidant...
potential of various peel extracts. The data for radical scavenging potential exhibited by various peel extracts are presented in Table 2. % DPPH radical scavenging capacity of selected edible root and tuber peels ranged from 80.98 to 49.96%. Results obtained strongly correlated ($R^2 = 0.780$) with TPC of peel samples. The highest % inhibition was shown by beetroot peels followed by turnip, potato, radish and sweet potato peels.

Table 2. The yield of extract, total phenolic contents (TPC) and DPPH radical scavenging potential of peels of edible roots and tubers.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Sample name</th>
<th>Yield of extract (%)</th>
<th>TPC (mg GAE/g DW)</th>
<th>DPPH Radical scavenging activities (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Radish</td>
<td>32.99 ± 0.12$^c$</td>
<td>43.82 ± 0.04$^d$</td>
<td>52.70 ± 0.14$^d$</td>
</tr>
<tr>
<td>2</td>
<td>Turnip</td>
<td>35.66 ± 0.13$^b$</td>
<td>59.41 ± 0.03$^b$</td>
<td>61.63 ± 0.20$^b$</td>
</tr>
<tr>
<td>3</td>
<td>Potato</td>
<td>23.36 ± 0.11$^e$</td>
<td>45.15 ± 0.05$^d$</td>
<td>59.40 ± 0.35$^e$</td>
</tr>
<tr>
<td>4</td>
<td>Sweet potato</td>
<td>28.33 ± 0.14$^d$</td>
<td>38.76 ± 0.06$^c$</td>
<td>49.96 ± 0.14$^d$</td>
</tr>
<tr>
<td>5</td>
<td>Beet root</td>
<td>59.54 ± 0.10$^a$</td>
<td>67.23 ± 0.02$^e$</td>
<td>80.98 ± 0.13$^a$</td>
</tr>
</tbody>
</table>

Values are means ± SD of triplicate ($n = 3$) of experimental run under same conditions. Different superscripts letters within the same column indicate significant difference ($p < 0.05$) among peel samples of edible roots and tubers.

Characterisation of phenolic acids in peel extracts

Among various phenolic substances, phenolic acids have drawn significant interest in the past few years because of their potential health benefits. Phenolic acids are potent antioxidants and have been documented to have anti-inflammatory, antiviral, antibacterial, anticarcinogenic, and vasodilatory actions (Burgos et al., 2013). Individual phenolic acids identified and characterised in selected samples of peels were gallic acid, chlorogenic acid, vanillic acid, syringic acid, p-coumaric acid and ferulic acid. The highest concentration (ppm) of detected phenolic acids was found in beetroot peels and followed the order as chlorogenic, syringic, ferulic, gallic acid, p-coumaric, and vanillic acid of 740.69, 498.33, 287.73, 234.06, 98.45, and 63.12 ppm, respectively (Figure 1). The chlorogenic acid was found abundant in the entire tested sample. All other peels of edible roots and tubers were found to have an abundant source of phenolic substances as shown in Table 5. The data obtained showed that peel samples of edible roots and tubers may work as a sustainable source of naturally occurring metabolites such as phenolic substances for drug development, food, and cosmetic industries.

Previous studies also revealed that gallic acid derived from vegetables can be used as a nutraceutical component (Patel et al., 2019). Another study conducted by Friedman et al. (2018) shows that potato peels worked against the three pathogenic trichomonads. The antitrichomonad properties of potato peels and their bioactive glycoalkaloids and phenolic compounds complements other reported potential health benefits of these substances, including antifungal, antimicrobial, antiviral as well as antiobesity properties in mice, suggesting that they have the potential to help alleviate certain diseases (Friedman et al., 2018).

Mutagenic and antimutagenic potential

Mutagenic substances interrupt replication and transcription of protein or DNA and may cause impaired, aberrant and in some cases, cancer or cell death. DNA damage is the initial episode in carcinogenesis. In this scenario, it is highly necessary to understand the antimutagenic compounds in natural products or in functional foods for the protection against mutagenic agents (Andrade-Vieira et al., 2017). Table 3 shows the mutagenesis risk of

![Figure 1. Typical HPLC chromatogram of beet root peels showing separation of phenolic acids. GA: gallic acid; VA: vanillic acid; SyrA: syringic acid; Chl A: chlorogenic acid; FA: ferulic acid; p-couA: P-coumaric acid.](image-url)
selected peel extracts and illustrates that all the peel extracts are non-mutagenic against mutant strains Salmonella Typhimurium TA100 and Salmonella Typhimurium TA98. Only potato peels showed a toxic effect against TA 98 and TA 100 strains; when these are toxic to microorganisms it means that it has antimicrobial activity. The present work suggests that all the peel extracts of edible roots and tubers are safe to use for nutraceutical, food, and other cosmetics applications after proper processing techniques.

Further evaluation of selected samples was done for their antimutagenic potential as shown in Table 4. The antimutagenic potential of selected peel samples ranged from 28 to 56.5%. The result shows that beetroot peels could behave as antimutagen (52.5%) against TA 100 strain as compared to other peel samples (medium antimutagen). Sweet potato peels were found weak in antimutagenic potential against both strains. The general trend for the antimutagenic potential of selected samples of peels of roots and tubers in descending order is as follows: beet root > turnip > potato > radish > sweet potato. The data obtained correlated with total phenolic contents ($R^2 = 0.875$) and DPPH scavenging assay ($R^2 = 0.753$). A good correlation between antimutagenic activity and TPC indicates that the antimutagenic activity of peels is directly related to the availability of phenolic antioxidants. The finding of the present work agrees with the studies of Mushtaq et al. (2015).

### Table 3. Mutagenesis risk evaluation of peels of edible roots and tubers.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Sample name</th>
<th>Test on Salmonella TA98</th>
<th>Test on Salmonella TA100</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number of positive wells / 96 wells</td>
<td>Number of positive wells / 96 wells</td>
</tr>
<tr>
<td>1</td>
<td>Background</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>K$_2$Cr$_2$O$_7$</td>
<td>92</td>
<td>93</td>
</tr>
<tr>
<td>3</td>
<td>NaN$_2$</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>4</td>
<td>Radish</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>5</td>
<td>Turnip</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>6</td>
<td>Potato</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>Sweet potato</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>8</td>
<td>Beet root</td>
<td>4</td>
<td>8</td>
</tr>
</tbody>
</table>

* a: negative control; b: positive control, c: positive control, M: mutagenic, NA: test not applied, NM: non-mutagenic, T: toxic.

### Table 4. Antimutagenic potential evaluation of peels of edible roots and tubers.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Sample Name</th>
<th>Test on Salmonella TA98</th>
<th>Test on Salmonella TA100</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number of positive wells / 96 wells</td>
<td>Number of positive wells / 96 wells</td>
</tr>
<tr>
<td>1</td>
<td>Background</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>K$_2$Cr$_2$O$_7$</td>
<td>92</td>
<td>93</td>
</tr>
<tr>
<td>3</td>
<td>NaN$_2$</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>4</td>
<td>Radish</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>5</td>
<td>Turnip</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>6</td>
<td>Potato</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>Sweet potato</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>8</td>
<td>Beet root</td>
<td>4</td>
<td>8</td>
</tr>
</tbody>
</table>

* a: negative control; b: positive control, c: positive control, NA: test not applied; MA: medium anti-mutagenic; WA: weak anti-mutagenic; SA: strong anti-mutagenic.

### Table 5. Characterisation of individual phenolic acids of peels of edible roots and tubers.

<table>
<thead>
<tr>
<th>Individual phenolic acid of peels of edible roots and tubers (ppm)</th>
<th>Radish</th>
<th>Turnip</th>
<th>Potato</th>
<th>Sweet potato</th>
<th>Beet root</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic acid</td>
<td>115.12 ± 0.03</td>
<td>89.44 ± 0.03</td>
<td>168.20 ± 0.03</td>
<td>102.23 ± 0.03</td>
<td>234.06 ± 0.03</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>127.12 ± 0.03</td>
<td>275.12 ± 0.03</td>
<td>554.63 ± 0.03</td>
<td>261.23 ± 0.03</td>
<td>740.69 ± 0.03</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>27.12 ± 0.03</td>
<td>19.22 ± 0.03</td>
<td>22.12 ± 0.03</td>
<td>10.67 ± 0.03</td>
<td>63.12 ± 0.03</td>
</tr>
<tr>
<td>Syringic acid</td>
<td>234.55 ± 0.03</td>
<td>365.44 ± 0.03</td>
<td>103.23 ± 0.03</td>
<td>263.76 ± 0.03</td>
<td>498.33 ± 0.03</td>
</tr>
<tr>
<td>P-coumaric acid</td>
<td>78.31 ± 0.03</td>
<td>99.29 ± 0.03</td>
<td>112.92 ± 0.03</td>
<td>87.98 ± 0.03</td>
<td>98.45 ± 0.03</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>43.53 ± 0.03</td>
<td>113.00 ± 0.03</td>
<td>112.78 ± 0.03</td>
<td>34.23 ± 0.03</td>
<td>287.73 ± 0.03</td>
</tr>
</tbody>
</table>

Values are means ± SD of triplicate ($n = 3$) of experimental run under same conditions.
Conclusion

The present work on the peels of edible roots and tubers indicates that these under-utilised vegetable wastes are a great repository of antioxidant and antimutagenic substances; and displayed weak cytotoxicity. Therefore, these substances are safe to be used by the pharmaceuticals, foods, chemicals and cosmetic industries.

Acknowledgement

Authors acknowledge the Higher Education Commission (HEC) for partially funding the present work.

References


Burgos, G., Amoros, W., Muñoa, L., Sosa, P., Cachhualla, E., Sanchez, C., ... and Bonierbale M. 2013. Total phenolic, total anthocyanin and phenolic acid concentrations and antioxidant activity of purpleshelled potatoes as affected by boiling. Journal of Food Composition and Analysis 30(1): 6-12.


