Effect of various cooking processes on antioxidants of potato (*Solanum tuberosum*)

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ABSTRACT

Potato (*Solanum tuberosum*) has been used as a widely accepted food in different forms all over the world and is well known for deliciousness. Different forms involve different type of cooking process. Study from our laboratory had indicated potential ulcer preventive properties based on the inhibition of H⁺K⁺-ATPase and *Helicobacter pylori* (ulcer causing bacteria in humans) growth. In the current study we evaluated the antioxidant and antiulcer potentials in potato during various cooking processes. Crude extract, free and bound phenolic fractions as well as pectic polysaccharides were isolated and analysed for total phenolic content, antioxidant activity, H⁺K⁺-ATPase/*Helicobacter pylori* inhibitory activity and cytoprotective effect. PCE (Potato Crude Extract), PFP (Free phenolic fraction from potato), PBP (Bound phenolic fraction from potato) and PPP (Pectic polysaccharide from potato) showed antioxidant activity with an IC₅₀ of 4.1±0.25, 8.3±0.58, 8.9±0.61 and 9200±552 µg/mL and H⁺K⁺-ATPase inhibitory activity was also evidenced with an IC₅₀ of 3.13±0.28, 1.22±0.11, 0.69±0.05 and 420±30.0 µg/mL respectively, as opposed to the standard H⁺K⁺-ATPase inhibitor, lanzoprazole (19.3±2.2 µg/mL). Loss of activity was associated with loss of phenolic acids during cooking conditions as evidenced by HPLC quantification. *Helicobacter pylori* growth was also inhibited by various fractions of potato with an IC₅₀ of 19.12, 37.5, 0.73 and 81.8 µg by PCE, PFP, PBP and PPP respectively. DNA protection was retained in PBP and PPP even during cooking conditions. Stabilizing of bioactivity during cooking conditions thus become a key issue in Food and Bioprocess Technology.

KEYWORDS: *Solanum tuberosum*, free and bound phenolics, pectic polysaccharide, cooking processes, H⁺K⁺-ATPase, *Helicobacter pylori*

1. INTRODUCTION

For over a century, gastric ulcer disease has been one of the leading causes of gastrointestinal surgery, with high morbidity and mortality rates 1. The pathophysiology of the disease involves an imbalance between offensive (acid, pepsin, and *Helicobacter pylori*) and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide, and growth factors). The problem has compounding effect particularly when duodenum of the stomach is harboured with *Helicobacter pylori*. A gastric ulcer complication gets worsened with *Helicobacter pylori* and finally ends up with gastric cancer 3. There have been two main approaches for treating gastric ulcer. The first deals with reducing the production of gastric acid and the second with re-enforcing gastric mucosal protection. However, treatment is effective if the treating component comprises of both. Modern medicines have their own limitations, especially against ulcers, which has a complex pathology and currently there are only specific drugs to attack at different steps. Obviously, drug load becomes higher during the treatment and a number of anti-ulcer drugs including proton pump inhibitors, prostaglandin analogues, histamine receptor antagonists etc., pose side effects. These drugs are known to even alter biochemical mechanisms of the body upon chronic usage 4. Current approach therefore has been to use natural compounds from commonly used edible sources, such as fruits and vegetables, because of their presumed safety and potential nutritional and therapeutic effects 5.

Potato (*Solanum tuberosum*), has been selected in the study since it is grown in about 150 countries and is the world’s single most important non-cereal crop with a vital role in the global food system 6. Potatoes are one of the most important staple crops for human consumption, together with wheat, rice and corn. On the other hand, potato peels reported to contain an array of nutritionally and pharmacologically interesting compounds such as phenolic compounds, glycoalkaloids and cell wall polysaccharides, which may be used as natural antioxidants, precursors of steroid hormones and dietary fibres. Potato is a globally important crop producing high yields of nutritionally valuable food in the form of tubers. Further, potato has now gained importance because of exceptionally high-yielding carbohydrates in addition to delineating the type of starch as “Resistant starch”. Resistant starch has been documented with several health
beneficial effects. Although in general potato is not regarded as a high antioxidant food, one cannot ignore potato’s high daily consumption. As a staple food, potatoes have a dietary role unique from vegetables and foods consumed in much lower quantities. Previous studies indicate that potato tuber contains caffeic acid derivatives as the main phenolic constituents and that the biosynthesis of these compounds is affected by external factors such as bruising and wounding. Most of the vegetables are cooked before consumption. These cooking processes are known to bring about a number of changes in physical characteristics and chemical composition of vegetables. During vegetable processing, qualitative changes, antioxidant activity breakdown and their leaching into surrounding water may influence the antioxidant activity of the vegetables. The effects of thermal processing on the antioxidant properties would be therefore useful since potatoes are consumed in cooked forms. Common methods to cook potato include boiling, pressure cooking and microwave processing. The effect of technological treatments and processing in the content of these substances is a topic of major research interest, since the understanding of the same reveal the input required to avail their health beneficial properties.

We have shown consistently from various dietary sources that phenolic acids are powerful antioxidants and possesses multi-health beneficial properties including antibacterial, antiviral, anticarionogenic, anti-inflammatory and vasodilatory actions etc. Present study focuses on isolation of crude water extract (PCE), free (PFP) and bound (PBP) phenolics from potato; their bioactive potential in terms of gastroprotective effect during various cooking conditions.

2. MATERIALS AND METHODS

2.1. Plant Material
Potato (Solanum tuberosum) was purchased from the local market at Mysore, India, and used for studies. One kilogram of fresh potato was cleaned, washed and powdered at a particle size of 20 mesh.

2.2. Chemicals
Agarose, calf thymus DNA, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2-thiobarbituric acid (TBA), phenolic acid standards, synthetic antioxidants such as butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT), 2-thiobarbituric acid (TBA) were obtained from Sigma (St.Louis,MO). All other chemicals were of the highest quality purchased from Qualigens Fine Chemicals (Mumbai, India). HPLC grade solvents employed for HPLC analyses were purchased from Spectrochem Biochemicals Pvt. Limited (Mumbai, India).

2.3. Preparation of aqueous extract of potato
Potato powder (10 g) was defatted using hexane in a soxhlet apparatus. One gram of defatted powder was taken in 10 mL distilled water and centrifuged at 1000 × g for 10 min. The clear supernatant was separated and referred as potato crude aqueous extract (PCE).

2.4. Isolation of Free and Bound Phenolic Fractions of Potato
Free phenolics were extracted according to the method of Ayumi et al. Briefly, 2 g of potato powder was extracted with 4×50 mL of 70% ethanol and centrifuged at 3000 × g for 10 min at room temperature. Clear supernatant was concentrated by flash evaporation (Buchi 011, Flawil, Switzerland), and the pH was adjusted to 2.0 with 4 N HCl followed by centrifugation and concentration. Phenolic acids were separated by ethyl acetate phase separation (5×50 mL), and the pooled fractions were treated with anhydrous disodium sulfate to remove moisture, filtered, evaporated to dryness, and taken in 2 mL of methanol (w/v); this is designated as potato free phenolic fraction (PFP). Bound phenolics were extracted according to the method of Nordkvist et al. Two grams of potato powder was extracted with 4×50 mL of 70% ethanol, followed by 4×50 mL of hexane to remove free phenolics and fat, respectively. The dried samples were extracted with 2 × 100 mL of 1 M sodium hydroxide containing 0.5% sodium borohydride under nitrogen atmosphere for 2 h, and the clear supernatant was collected followed by centrifugation at 3000 × g for 10 min. The combined supernatants were acidified with 4 N HCl to pH 1.5, and phenolic acids were processed as mentioned in the case of free phenolics; this is designated as potato bound phenolic fraction (PBP).

2.5. Isolation of pectic polysaccharide from potato
Pectic polysaccharide from potato was isolated following the ammonium oxalate extraction method. Briefly 100g of defatted potato powder was washed with 70% alcohol to remove free sugars and then centrifuged. Residue was then treated with protease, termamylase and glucoamylase respectively at their optimum temperatures to deplete proteins, amylase and amyllopectins and centrifuged. Further the residue was extracted with 200 mL of 0.25% (w/v) ammonium oxalate solution and filtered; the filtrate was precipitated by ethanol at 4°C. The precipitate was resuspended in 100 mL of water and lyophilised to obtain pectic polysaccharide and designated as PPP.

2.6. Cooking treatments
1 gram of PCE and PBP were taken in 10 mL of warm distilled water and subjected to various cooking methods such as boiling (3–4min), pressure cooking (10min) and micro oven cooking (2min). 5mL of PFP and PBP obtained from above mentioned steps were dried under nitrogen and extracted in water and subjected to different cooking conditions and used for further analysis.

2.7. Estimation of total phenolic content; HPLC analysis of phenolic acids
The Folin-Ciocalteu assay method was used to determine the total phenolic content and expressed as Gallic acid equivalents (GAE) in milligram per gram sample. Phenolic acids of PCE, PFP and PBP with
cooking conditions were analysed by HPLC (model LC-10A, Shimadzu) using diode array UV-detector (operating at λ_max 280nm). A solvent system consisting of water/acetic acid/methanol (isocratic, 80:5:15 v/v/v) was used as mobile phase at a flow rate of 1 mL/min. Phenolic acid standards such as gallic, tannic, chlorogenic, p-coumaric, ferulic, protocatechuic, syringic, caffeic, gentisic, cinnamic and vanillic acids were employed for identification of phenolic acids present in PCE, PFP and PBP by comparing the retention time under similar experimental conditions.

2.8. Determination of H⁺, K⁺-ATPase inhibition (PPI)

Fresh sheep stomach was obtained from local slaughter house at Mysore, India. The mucosa of gastric fundus was cut off and the inner layer was scraped for parietal cells and homogenized in 16 mM Tris buffer (pH 7.4) containing 10% Triton-X 100 and centrifuged at 6000 x g for 10 min. The supernatant (enzyme extract) was used for the assay. Protein content was determined according to Bradford’s method using BSA as standard. The enzyme extract (350 µg/mL) was incubated with different fractions of potato phenolics, PCE, PFP, PBP and PPP in a reaction mixture containing 16 mM Tris buffer (pH 6.5) and the reaction was initiated by adding the substrate 2 mM ATP, in addition to 2 mM MgCl_2 and 10 mM KCl. After 30 min of incubation at 37°C, the reaction was stopped by the addition of assay mixture containing 4.5% ammonium molybdate and 60% perchloric acid. Inorganic phosphate formed was measured spectrophotometrically at 400 nm. Enzyme activity was calculated as micromoles of inorganic phosphate formed was measured spectrophotometrically at 400 nm. Enzyme activity was calculated as micromoles of inorganic phosphate (Pi) released/hour at various doses of PCE, PFP, PBP and PPP. Results were compared with known antulcer proton potassium ATPase inhibitor drug lanzoprazole and with standard phenolic acids, since the active fraction of potato contained phenolic acids.

2.9. Anti-Helicobacter pylori activity

Helicobacter pylori was obtained from endoscopic samples of ulcer patients from KCDC (Karnataka Cardio Diagnostic Centre, Mysore, India) and cultured on Ham’s F-12 nutrient agar medium with 5% FBS (Fetal Bovine Serum) at 37°C for 2–3 days in a microaerophilic condition. Helicobacter pylori culture was characterized by specific tests such as urease, catalase, oxidase, gram staining, colony characteristics and morphological appearance under scanning electron microscope and also confirmed by the growth of culture in the presence of susceptible and resistant antibiotics.

2.10. Minimal inhibitory concentration (MIC)

MIC values were determined by conventional broth dilution method. Serial dilutions (final volume of 1 mL) of PCE, PFP, PBP and PPP (5–100 µg/mL) were performed with 0.9% saline. Following this, 9 mL of Ham’s F-12 nutrient medium with 5% FBS was added. Broths were inoculated with 100 µL of Helicobacter pylori suspension (10^6 CFU) and incubated for 24 h at 37°C. Amoxicillin was used as a positive control since Helicobacter pylori is susceptible to amoxicillin and 0.9% saline as negative control. After 24 h, Helicobacter pylori growth was assayed by measuring absorbance at λ_max 570 nm.

2.11. Scanning Electron Microscopic (SEM) studies

SEM has been used as a tool to understand and analyse the characteristic features of the bacteria. Lysis if any was determined by SEM studies. 500 µL of overnight grown culture of Helicobacter pylori (10^6 cells/mL) was washed and resuspended in PBS, incubated with equal volume of different concentrations of PCE, PFP, PBP and PPP (10–100 µg) in PBS for 1 h at 37°C. Amoxicillin was used as standard Helicobacter pylori inhibitor and the cells without inhibitor were taken as control. After incubation, cells were fixed with 2% glutaraldehyde and dehydrated with series of ethanol treatments and examined by SEM (Model No. LEO 425 VP, Electron microscopy LTD, Cambridge, UK) with an acceleration voltage of 20 kV. Multiple fields of visions were viewed at different magnifications.

2.12. Measurement of antioxidant activity

The antioxidant activity was determined by the method described by Braca et al. An aliquot of 100 µL of PCE, PFP, PBP and PPP at various concentrations (5–25 µg/mL) were added to 1 mL of 250 µM DPPH solution. The mixture was shaken vigorously and left to stand for 20 min at room temperature in the dark. The absorbance of the resulting solution was measured spectrophotometrically at 517 nm. The capability to scavenge the DPPH radical was calculated using the following equation:

\[
\text{FRS} (%) = \frac{[\text{Absorbance of control at 517nm} - \text{Absorbance of sample at 517nm}]}{\text{Absorbance of control at 517nm}} \times 100
\]

2.13. DNA protection assay

The DNA protective effect of phenolic fractions was determined electrophoretically (Submarine Electrophoresis System, Bangalore Genei, Bangalore, India) using calf thymus DNA. Calf thymus DNA (1 µg) was subjected to oxidation by Fenton’s reagent (30 mM H_2O_2, 50 mM ascorbic acid and 80 mM FeCl_3). Relative difference in the migration between the native and oxidized DNA was ensured on 1% agarose gel electrophoresis after staining with ethidium bromide. Gels were documented (Herolab, Germany) and the intensity of the bands were determined (Easywin software). Protection to DNA was calculated based on the DNA band corresponding to that of the native in the presence and absence of 4 µg of PCE, PFP, PBP and PPP.

2.14. Cytoprotection assay

Buccal cells were obtained from gentle scraping of the inner cheek layer of healthy person, washed 3 times and suspended in sterile PBS. Incubated with PCE and PPP fractions (1mg/mL) at 37°C for 1h and indomethacin (1mg/mL) was induced. The cells were then stained with acridine orange and observed under fluorescence microscope.

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2.15. Statistical analysis
Data are expressed as mean ± SD and analysed by one way analysis of variance (ANOVA) and Tukey-Kramer multiple comparisons test for significance at p<0.05 using graph pad instat statistical software.

3. RESULTS AND DISCUSSION
Potatoes in the diet contribute significantly to antioxidant daily intake worldwide. Extensive research indicates that foods rich in antioxidants have been shown to play an essential role in the prevention of various lifestyle diseases including the lowering of risks of a number of chronic diseases, such as atherosclerosis, cancers, cardiovascular complications, diabetes, ulcers etc.

Generally all fruits and vegetables contain different proportions of health beneficial components including phenolics, flavonoids, saccharides etc. Our previous studies clearly indicated that although consumed in deep fried form as well as in cooked form such as boiling, pressure cooking and microwave cooking, the effect of cooking conditions on these phenolic bioactives was not clearly understood. Current study was undertaken to understand the effect of these processes on phenolic acids that contributes significantly to antioxidant, DNA/Cytoprotective, \( H^+K^+\)-ATPase/\textit{Helicobacter pylori} inhibitory properties. Main goal of the current study was to identify phenolic acids which are vulnerable for destruction during cooking conditions, so that such components can be added after cooking, before consumption or to consume fruits which are rich in such phenolic acids. In food science, food although are known to be consumed for health beneficial purposes, they are also used for satiety; thus cooking or processed conditions cannot be avoided. Understanding the components that are lost during cooking conditions however provide insights on extra adjunct one has to use in order to compensate the loss. The fact that phenolic acids bind to different components of the physiological system, function of phenol bound protein also differs as evidenced by our previous studies.

In this scenario it is important to understand the effect of these bioactive ingredients during processed conditions particularly during cooking conditions. Phenolic acids, flavonoids and anthocyanidins were found to be the commonly attributed components for bioactivity/antioxidant activity in potatoes. Potatoes although consumed in deep fried form as well as in cooked form such as boiling, pressure cooking and microwave cooking, the effect of cooking conditions on these phenolic bioactives was not clearly understood. Current study was undertaken to understand the effect of these processes on phenolic acids that contribute significantly to antioxidant, DNA/Cytoprotective, \( H^+K^+\)-ATPase/\textit{Helicobacter pylori} inhibitory properties. Main goal of the current study was to identify phenolic acids which are vulnerable for destruction during cooking conditions, so that such components can be added after cooking, before consumption or to consume fruits which are rich in such phenolic acids. In food science, food although are known to be consumed for health beneficial purposes, they are also used for satiety; thus cooking or processed conditions cannot be avoided. Understanding the components that are lost during cooking conditions however provide insights on extra adjunct one has to use in order to compensate the loss. The fact that phenolic acids bind to different components of the physiological system, function of phenol bound protein also differs as evidenced by our previous studies.

Levels of phenolics present in PCE, PFP, PBP and PPP during untreated and various cooking conditions are presented in Table-1. As indicated in the results, they exhibited potent antioxidant properties, with \( IC_{50} \) of 4.1, 8.3, 8.9 and 9200µg/mL in PCE, PFP, PBP and PPP respectively. Potent \( H^+K^+\)-ATPase activity was also evidenced with good \( IC_{50} \) in the range of 0.69 to 420 µg/mL as opposed to the standard \( H^+K^+\)-ATPase inhibitor – Lanzoprazole (\( IC_{50} \) – 19 µg/mL). Differential losses were observed in different fractions and results are summarised as percent contribution by each phenolic acids for antioxidant (Table – 2) and \( H^+K^+\)-ATPase activity (Table – 3).

Table 1.Total phenolic content, antioxidant activity and \( H^+K^+\)-ATPase inhibitory activity in potato fractions with various cooking conditions.

<table>
<thead>
<tr>
<th>Phenolic content (mg/g)</th>
<th>% Yield</th>
<th>Control</th>
<th>Boiling</th>
<th>Pressure cooking</th>
<th>Micro oven cooking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potato crude extract</td>
<td>7.0 ± 0.55</td>
<td>3.12 ± 0.177</td>
<td>2.20 ± 0.013</td>
<td>2.91 ± 0.174</td>
<td>2.66 ± 0.186</td>
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<tr>
<td>Potato free phenolics</td>
<td>3.25 ± 0.23</td>
<td>0.41 ± 0.020</td>
<td>0.33 ± 0.016</td>
<td>0.41 ± 0.015</td>
<td>0.41 ± 0.024</td>
</tr>
<tr>
<td>Potato bound phenolics</td>
<td>17.25 ± 1.03</td>
<td>0.11 ± 0.007</td>
<td>0.08 ± 0.008</td>
<td>0.13 ± 0.007</td>
<td>0.09 ± 0.004</td>
</tr>
<tr>
<td>Potato pectic polysaccharide</td>
<td>6.0 ± 0.46</td>
<td>0.85 ± 0.074</td>
<td>0.58 ± 0.028</td>
<td>0.52 ± 0.042</td>
<td>0.55 ± 0.049</td>
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</table>

Antioxidant activity (\( IC_{50}\)µg/mL)

<table>
<thead>
<tr>
<th>Phenolic content</th>
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<th>Boiling</th>
<th>Pressure cooking</th>
<th>Micro oven cooking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potato crude extract</td>
<td>4.1 ± 0.248</td>
<td>5.73 ± 0.346</td>
<td>20.9 ± 1.065</td>
<td>19.1 ± 1.128</td>
<td></td>
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<tr>
<td>Potato free phenolics</td>
<td>8.3 ± 0.581</td>
<td>7.13 ± 0.499</td>
<td>5.53 ± 0.345</td>
<td>7.20 ± 0.548</td>
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<tr>
<td>Potato bound phenolics</td>
<td>8.9 ± 0.612</td>
<td>7.80 ± 0.604</td>
<td>5.20 ± 0.448</td>
<td>3.40 ± 0.210</td>
<td></td>
</tr>
<tr>
<td>Potato pectic polysaccharide</td>
<td>9200.0 ± 552</td>
<td>7760.0 ± 620</td>
<td>9340.0 ± 732</td>
<td>10690.0 ± 750</td>
<td></td>
</tr>
</tbody>
</table>

PPA inhibitory activity (\( IC_{50}\)µg/mL)

<table>
<thead>
<tr>
<th>Phenolic content</th>
<th>% Yield</th>
<th>Control</th>
<th>Boiling</th>
<th>Pressure cooking</th>
<th>Micro oven cooking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potato crude extract</td>
<td>3.13 ± 0.280</td>
<td>8.08 ± 0.503</td>
<td>5.97 ± 0.422</td>
<td>8.55 ± 0.602</td>
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</tr>
<tr>
<td>Potato free phenolics</td>
<td>1.22 ± 0.112</td>
<td>1.10 ± 0.650</td>
<td>0.90 ± 0.072</td>
<td>-</td>
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<tr>
<td>Potato bound phenolics</td>
<td>0.69 ± 0.050</td>
<td>8.20 ± 0.464</td>
<td>0.78 ± 0.0564</td>
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<td></td>
</tr>
<tr>
<td>Potato pectic polysaccharide</td>
<td>420.0 ± 30.0</td>
<td>1970.0 ± 100</td>
<td>340.0 ± 27.5</td>
<td>2370.0 ± 156</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as GAE mg/g for phenolics and \( IC_{50}\) (µg/mL) for antioxidant activity and PPA inhibitory activity. Antioxidant activity measured as free radical scavenging activity. All data are the mean ± SD of five replicates; mean values followed by different letters in the same column differ significantly (p <0.05). Different letters ‘a to o’ in the column represents that values are extremely significantly when compared with cooked and uncooked conditions.
Table 2. Relative percentage contribution of individual phenolic acids towards antioxidant activity.

<table>
<thead>
<tr>
<th>Standard phenolic acids</th>
<th>Structures</th>
<th>Potato crude extract</th>
<th>Potato free phenolics</th>
<th>Potato bound phenolics</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>Boiling</td>
<td>Pressure</td>
</tr>
<tr>
<td>Tannic/gallic acid</td>
<td></td>
<td>13.5</td>
<td>7</td>
<td>6.7</td>
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<tr>
<td>Protocatechuic acid</td>
<td></td>
<td>4.64</td>
<td>4.36</td>
<td>2.19</td>
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<tr>
<td>Chlorogenic acid</td>
<td></td>
<td>2.15</td>
<td>1.35</td>
<td>1.08</td>
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<tr>
<td>Gentisic acid</td>
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<tr>
<td>Caffeic acid</td>
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<td>-</td>
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<tr>
<td>Syringic acid</td>
<td></td>
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<tr>
<td>p-coumaric acid</td>
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<td>-</td>
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<td>-</td>
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<tr>
<td>Ferulic acid</td>
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</table>

PCE, PFP and PBP containing different phenolic acids are given with their yield and structure. Percent difference indicates the loss (↓) and gain (↑) in the phenolic acids contribution towards antioxidant activity during different cooking conditions.

Table 3. Relative percentage contribution of individual phenolic acids towards PPA inhibitory activity.

<table>
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<tr>
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<th>Structures</th>
<th>Potato crude extract</th>
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PCE, PFP and PBP containing different phenolic acids are given with their yield and structure. Percent difference indicates the loss (↓) and gain (↑) in the phenolic acids contribution towards PPA inhibitory activity during different cooking conditions.
H⁺,K⁺-ATPase is an enzyme which is known to get up-regulated during stress/ulceration conditions. Inhibitor of H⁺,K⁺-ATPase is thus found to be an effective ulcer inhibitory or preventive compound. In this perspective it is interesting to observe that although phenolic content varies, phenolic acids contributing to antioxidant activity (Table-2) such as gallic/tannic acid (48%), protocatechuic acid (53%), and chlorogenic acid (53%) are affected the most; while those phenolic acids which are potent in H⁺,K⁺-ATPase inhibition such as caffeic acid and ferulic acid, as indicated in our earlier paper, are not affected (Table – 3). Therefore, although antioxidant activity is reduced drastically during cooking conditions, H⁺,K⁺-ATPase inhibition was affected only up to 21%, which means, a 3.8 folds less affected (Table – 3) when compared to that of antioxidant activity. In other words, the total H⁺,K⁺-ATPase inhibition activity by potato is not affected by cooking or processing conditions and all activity may not be driven by antioxidant potency only. *Helicobacter pylori* growth was also inhibited by various fractions of potato with IC₅₀ of 19.12, 37.5, 0.73 and 81.8µg for PCE, PFP, PBP and PPP respectively (Figure-1). Potatoes thus can be used in either raw form or cooked form to avail H⁺, K⁺-ATPase inhibition. Since our previous studies had indicated that phenolic acids inhibiting H⁺, K⁺-ATPase are also more potent in inhibiting *Helicobacter pylori* via interaction between hydrophobicity of phenolic acids and membrane domain of H⁺,K⁺-ATPase or *Helicobacter pylori* membrane, *Helicobacter pylori* inhibitory activity is also retained during cooking conditions (Figure- 1). Scanning electron microscopic data supported this observation. Potent inhibition by bound phenolic fractions is justified by observing creation of vacuoles in them. No or very little loss of *Helicobacter pylori* inhibitory phenolic acids during cooking conditions were noted.

![Control H.pylori](image1)

![Amoxicillin-IC₅₀ 16.0µg](image2)

![Ferulic acid-IC₅₀ 26.7µg](image3)

![Chlorogenic acid -IC₅₀ 30.8 µg](image4)

![PFP-IC₅₀ 37.5 µg](image5)

![PBP-IC₅₀ 0.73 µg](image6)

![PCE-IC₅₀ 19.12 µg](image7)

![PPP-IC₅₀ 81.8 µg](image8)

Figure 1. Scanning Electron Microscopic pictures of *H.pylori* with control(A), treated with 10-100µg/mL of *H.pylori* suspension and samples with least concentration (10µg/mL) was photographed at 20K magnification. Data showed maximum lysis of *H.pylori* by PBP. In a separate experiment growth inhibition was studied by MTT assay and concentration required for inhibition of 50% growth of *H.pylori* cells (IC₅₀) is indicated.
DNA (Figure-2) and cytoprotective effect (Figure-3) of potato phenolic fractions and polysaccharides were also conducted in native and processed fractions. DNA and cytoprotective abilities were lost only in the free phenolic fraction and the crude extract, while activity was retained in the PBP and PPP suggesting that potato even in cooked form possesses disease preventive properties, since disease conditions like cancer or ulcer result in DNA and Cellular damage by oxidative stress conditions.

![Figure 2. Lane 1 DNA(untreated); Lane 2- DNA oxidized; Lane 3-5 – DNA preincubated with BHA (3), PFP (4) and PBP (5) Lane 6-9 treated with uncooked PCE (6), boiled (7), pressure cooked (8) and micro oven cooked (9) PCE. Lane 10-13 treated with uncooked PPP (10), boiled (11), pressure cooked (12) and micro oven cooked (13) PPP. Data showed significant DNA protection activity in PFP and PPP. Further loss of activity was observed in PCE upon exposure to cooking conditions. PPP however retained DNA protection ability during all cooking conditions. *Control (C)- Uncooked , B-Boiling, PC- Pressure cooking , MC- Micro oven cooking](image)

Losses in the phenolic acids were evident by HPLC analysis (Figure-5) and subsequently it resulted in reduction in the antioxidant as well as H⁺, K⁺-ATPase inhibition. Various cooking conditions affected these activities differently suggesting that cooking has effect on different phenolic acids differentially. Since different phenolic acids are involved in different bioactivity such as antioxidant and H⁺,K⁺-ATPase activity; they were also affected differently. It is also interesting to observe that cinnamate derivatives such as caffeic acid (3,4-dihydroxy cinnamic acid), chlorogenic acid (3-(3,4-dihydroxycinnamoyl) quinate), ferulic acid (4-hydroxy-3-methoxy cinnamic acid) are not much affected by cooking conditions when compared to that of hydroxy benzoic acids such as gallic acid (3,4,5-trihydroxy benzoic acid), gentisic acid (2,5-dihydroxy benzoic acid) and protocatechuic acid (3,4-dihydroxy benzoic acid). Hydroxy cinnamates may be more stable due to conjugated side chains, since they have the option to stabilize themselves by accommodating the delocalized electrons by virtue of conjugated side chains. Loss of structural changes in pectic polysaccharides is also evidenced in Figure-4.

![Figure 3. Fluorescent microscopic pictures of buccal cells treated with PCE and PPP fractions with cooking conditions. Control buccal cells (A), Indomethacin induced buccal cells (B). Untreated PCE (C), boiled PCE (D), pressure cooked PCE (E), micro oven cooked PCE (F) and untreated PPP (G), boiled PPP (H), pressure cooked PPP (I), and micro oven cooked PPP (J). Cytoprotective ability was lost in PCE upon exposure to cooking conditions; while activity is retained in PPP despite exposure to cooking conditions.](image)
Current lifestyle practices have been the cause of stress and in turn are the causative factor for various diseases. Ulcer is one such condition. Invariably any emergency hospitalizations have been shown to lead to gastric ulcerations. Besides the use of non steroidal anti-inflammatory drugs, infection by *Helicobacter pylori* is the key contributors to ulcer incidences 3. Several H+, K+-ATPase blockers and *Helicobacter pylori* inhibiting antibiotics are known and are being prescribed to patients. However, they pose lots of side effects and also increase the drug burden due to inefficacies to even triple antibiotics that is practiced commonly to eliminate the *Helicobacter pylori* 31.

![Figure 4. SEM pictures of Potato powder (A) and PPP (B) at 200 X magnification. PPP uncooked (C), PPP after boiling (D), PPP after pressure cooking (E) and PPP after micro oven cooking conditions (F) at 5 K magnification. Expansion and changes in PPP structures are evidenced during cooking conditions.](image)

![Figure 5. HPLC analysis of potato fractions with cooking conditions. Total percent of phenolic acids in PCE, PFP and PBP with various cooking conditions.](image)
hydroxyl groups seems more important than their number for antioxidant capacity. For example antioxidant capacity of gentisic acid and protocatechuic acids are the same; however, their position in para position (PCA), enhanced antioxidant activity by 2.2 folds, while decreased PPAI by 1.2 folds. The dominant phenolic compounds identified in this study - gallic/tannic acid, chlorogenic and ferulic acids contain a variety of phenolic hydroxyl groups with differences in their hydrophilicity and hydrophobicity and hence may interact differently in the assay system involved in antioxidant, H⁺,K⁺-ATPase/Helicobacter pylori inhibitory and DNA/Cytotoxic properties.

4. CONCLUSIONS

Potato (Solanum tuberosum) is one of the popular and abundant source in many parts of the globe. It is also a presumed notion that potato is not good for health because of its high starch content. Existed traditional knowledge however indicated that it has got tremendous gastroprotective potential. Current study therefore is an attempt to provide evidence for gastroprotective potential in terms of antioxidant, H⁺,K⁺-ATPase/Helicobacter pylori inhibitory and DNA/Cytotoxic abilities. Studies also identified the components responsible for the activity and their effect during cooking conditions which has a greater impact since potato is consumed in processed forms. Results of the study indicated that phenolic and pectic polysaccharide fractions play a significant role in antioxidant, DNA/Cytotoxic and H⁺,K⁺-ATPase/Helicobacter pylori inhibitory activity in cooked and uncooked conditions. Underlying basis for the loss of activity during cooking conditions indicated differential losses of phenolic acids. It is thus suggested that although potato dishes are taken for satiety, its gastroprotective effect can be availed by taking raw potato juice in water apart from continuing its use with processed conditions. Pectic polysaccharide, in other words dietary fibre from potato will be useful in offering health beneficial properties no matter how it is used, either in the cooked form or raw. Though phenolic losses were observed during cooking conditions, hydroxy cinnamate derivatives were found to be stable than hydroxy benzoate phenolic acids. Retention of different levels of phenolics in different cooking conditions indicates the stability of the molecule in different compositional environment. Understanding of contribution to activity by individual phenolic acids indeed reveals that the activity is also dictated by the components present in higher abundance.

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