Antioxidant components and activity in the peel of Ziziphus jujuba Mill.
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ABSTRACT
The aim of the present study was to compare the antioxidant components and antioxidant activity of the raw and cooked peel of Ziziphus Jujuba Mill. The peel was analyzed for polyphenols, glutathione and tannin contents. In addition methanol and aqueous extracts were analyzed for flavonoid, saponin contents and antioxidant activity. The antioxidant activity of extracts was determined by DPPH and reducing power assay. Polyphenol and tannin contents were significantly (p<0.05) higher in the raw peel (1.67±0.07 and 7.69±0.09 g/100g respectively), while glutathione (GSH) content of cooked peel (125.75±5.04 µMol/100g) was significantly (p<0.05) higher than raw peel (99.49±8.84 µMol/100g). The methanolic extract of raw peel had the highest flavonoid and total saponin content (3.52±0.02 and 83.07±3.39 g/100g respectively). The steroidal saponins content (6.13±0.63 g /100g) was higher in the hot aqueous extract (HA) of cooked peel. It was found that antioxidant activities of all the extracts increased with increasing concentration except in HA of raw peel. In the raw peel, methanolic extract with highest antioxidant capacity revealed the same antioxidant activity as compared to standard of butylated hydroxytoluene (BHT). The three extracts of cooked peel were found to be significantly (p<0.01) inferior in antioxidant efficacy than BHT. Reducing power assay indicated varying degrees of efficacy in a dose-dependent manner in all peel extracts. HA extract was the most potent antioxidant in the raw and cooked extracts. Data indicates the raw and cooked peel of Ziziphus Jujuba Mill. have a great potential for utilization as a source of natural antioxidant.

KEYWORDS: Ziziphus Jujuba Mill., Peel, Antioxidant Components, Antioxidant activity, Chinese date

INTRODUCTION
Antioxidants have been shown to protect cellular membranes and organelles from the damaging effects of reactive oxygen species (ROS). [1] Ziziphus jujuba Mill. (Chinese date, Annab in Iran and ber in India) belongs to Rhamnaceae family. [2] Ziziphus jujuba Mill. is commonly used in folklore medicine for healing of various diseases such as digestive disorders, weakness, liver complaints, urinary troubles, diabetes, pharyngitis, bronchitis and insomnia. [3-5] Due to the ill effects of pesticides, whole fruits and therefore fruit peel consumption has currently decreased. In the present study, the antioxidant components and activity of the raw and cooked peel of Ziziphus jujuba Mill. fruits were compared.

MATERIALS AND METHODS
Chemicals
1. 1-Diphenyl-2-picrylhydrazyl (DPPH), 5, 5-dithio (bis) nitro benzoic acid (DTNB) and quercetin were purchased from Sigma Aldrich, India. Glutathione was obtained from Sisco Research Lab. Pvt. Ltd. (Bombay, India) and Butylated hydroxytoluene (BHT) from Qualigens Fine Chemicals, Mumbai, India. All other solvents and chemicals used were of analytical grade.

Preparation of plant peel
Ziziphus jujuba Mill. fruits were obtained in bulk from local market in Birjand city of Iran. The specimen was identified and authenticated by Dr. Sudarshana Department of Botany and the voucher specimen was deposited at the herbarium of Department of Studies in Botany, University of Mysore, Mysore, India. To obtain raw peel, fruits (300g) were washed and peeled in order to separate the peels from the pulps. To prepare cooked peel, washed fruit (300g) was soaked in water for 18 h followed by heat treatment for 45 minutes at <90°C. The smashed whole fruits were passed through a 60-mesh screen and the peels separated and washed. Both raw and cooked peel were separately dried in an air oven at <50°C then made as a fine powder.

Preparation of the extracts
Raw and cooked peel materials were extracted using methanol (MeOH), cold (CA) and hot (HA) aqueous separately. To each of the powdered samples (20g), 100 ml of the solvents (Methanol or distilled water) were added and allowed to shake for 6 h on a mechanical shaker. For hot water extraction, the samples were extracted using hot water (70°C) on a hot plate with occasional shaking for 45 minutes. Each of these preparations was centrifuged at 4000 rpm for 10 minutes. Afterward the slurry was separated and evaporated to dryness in a steady air current at <50°C in a previously weighed crucible. The obtained extracts were stored in air-tight containers at 4°C, until further use.

Determination of antioxidant components
Total polyphenols content (TPC) was analyzed by Folin-Ciocalteu micro Method. [6] Results were expressed as g /100 g of dry weight as Gallic Acid Equivalent of dried plant material. Reduced glutathione was determined based on the progress of a yellow compound due to reaction of 5, 5-Dithio (bis) nitro benzoic acid with compounds including sulphydryll groups. [7] Estimation of tannins was based on the AOAC Methods. [8] The content of flavonoids was determined by a pharmacopoeia method using quercetin as a reference. [9] Saponins were estimated according to the method of Makkar et al. [10]

Evaluation of antioxidant activity
Free radical scavenging activity of various extract was measured according to the method of Blois. [11] This spectrophotometric assay uses stable radical 1,1-Diphenyl-2-picrylhydrazyl (DPPH) as a reagent. The reducing power capacity of extracts was determined by the method of Yildirim et al. [11] All tests were carried out in triplicates.

STATISTICAL ANALYSIS
Results were expressed as mean ± standard deviation. Descriptive statistical analysis, one-way analysis of variance (ANOVA), scheffe multiple range test, student ‘t’ test and Pearson correlation coefficients were performed using SPSS for windows version 18 program.

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RESULTS AND DISCUSSION

The current study reports the antioxidant components and activity of raw and cooked peel of *Zizyphus jujuba Mill.* in vitro. The raw peel contained significantly higher amounts of tannins (7.69 g/100g) and flavonoids (3.52 g/100g in MtOH extract) followed by total polyphenols (1.67 g/100g), total and steroidal saponins (83.07 and 4.75 g/100g of MtOH extract respectively) and glutathione (99.49 µMol /100g). In the cooked peel, the above components were observed in the following decreasing order; tannins (3.62 g/100g), polyphenol (0.90 g/100g), flavonoids (0.79 g/100g), total and steroidal saponins (20.90 and 6.13 g/100g respectively) and glutathione (125.75 µMol/MtOH/100g).

Polyphenols are the secondary metabolites in the plants with a wide range from simple molecules, such as phenolic acids, to highly polymerized constituents such as tannins. These constituents are a class of free radical scavengers which might be responsible for antioxidant activity. [14-18] The data regarding the TPC of extracts is presented in Table 1. The total phenolic content was found to be significantly (p<0.01) higher in raw compared to cooked extract of peel (1.67±0.07 and 0.90±0.05 g/100g GAE respectively). Zhang et al. [17] has reported total phenolic content ranging from 607.93 to 3280.29 mg/100g GAE in raw peel of three jujube varieties. It may be inferred from the reported and current observations that the TPC varies according to the jujube species. In the present study, higher level of total phenolic content was observed in both raw and cooked jujube peel than the values reported by Ruzlan et al. in dragon fruits [19] and by Leontowicz et al. in apple and pear peel. [19] However, lower TPC were found in jujube peel in comparison with the findings obtained by Jung et al. [20] and Ghasemi et al. [21] on the peel of eggplant and 13 citrus species respectively. There are some researches which have shown heat treatment might induce the changes in extractabilities of the phenolics and flavonoids due to the disturbance of the plant cell wall. [22-23] In the present study, heat treatment might have caused degradation of phenolic compounds hence the reduction in TPC of cooked peel. Similar results have been reported on heat treatment of red kidney beans, broad beans, red cabbage and broccoli. [24-27]

Tannins are water-soluble plant-derived polyphenolic compounds that precipitate proteins. In relation to their chemical structure they can be divided from simple molecules, such as phenolic acids, to highly polymerized constituents such as tannins. These constituents are a class of free radical scavengers which might be responsible for antioxidant activity. [14-18] The data regarding the TPC of extracts is presented in Table 1. The total phenolic content was found to be significantly (p<0.01) higher in raw compared to cooked extract of peel (1.67±0.07 and 0.90±0.05 g/100g GAE respectively). Zhang et al. [17] has reported total phenolic content ranging from 607.93 to 3280.29 mg/100g GAE in raw peel of three jujube varieties. It may be inferred from the reported and current observations that the TPC varies according to the jujube species. In the present study, higher level of total phenolic content was observed in both raw and cooked jujube peel than the values reported by Ruzlan et al. in dragon fruits [19] and by Leontowicz et al. in apple and pear peel. [19] However, lower TPC were found in jujube peel in comparison with the findings obtained by Jung et al. [20] and Ghasemi et al. [21] on the peel of eggplant and 13 citrus species respectively. There are some researches which have shown heat treatment might induce the changes in extractabilities of the phenolics and flavonoids due to the disturbance of the plant cell wall. [22-23] In the present study, heat treatment might have caused degradation of phenolic compounds hence the reduction in TPC of cooked peel. Similar results have been reported on heat treatment of red kidney beans, broad beans, red cabbage and broccoli. [24-27]

Flavonoids belong to a group of natural materials with variable phenolic structures and over 4000 flavonoids have been discovered in plants. [14] Table 2 presents the total flavonoid content (TFC) in different solvents of raw and cooked peel. In all raw extracts, TFC is higher than cooked peel. The highest flavonoid content (3.52±0.02 g/100 g of dry weight as Quercetin equivalent) was in methanolic extract of raw peel. In cooked peel, total phenolic content was more than the flavonoid content while in methanolic and hot aqueous of the raw peel, flavonoid was higher (Table 1-2). Our results are comparable with the finding of Dietrych-Szostak et al. who reported dehulling buckwheat had drastic reductions of the total flavonoid concentration by using different temperature regimes. [30] A similar observation is reported by Khatun et al. [31] The results of this study contradict some investigations. Thermal processing of banana peel has shown retention of polyphenols and flavonoids. [32] A similar result was noted in the study of Khatun et al. [31] In the study conducted by Sukrasno et al. [33] flavonoid content of Cosmos caudatus leaves substantially increased during boiling. However, heating of fresh leaves in an air oven at elevated temperature from 30 to 100°C decreased the total flavonoid content except at 40°C. These findings indicate that heat processing is a major determinant of flavonoid content in plant foods.

Saponins, a group of natural products widespread throughout the plant kingdom, are glycosides of triterpenoid or steroidal aglycons and the corresponding aglycons are termed sapogenins. Saponins are believed to form the major components of many plant drugs and folk medicines, and are considered responsible for numerous pharmacological properties. [30-31] The findings of saponins in present work are given in Table 2. The methanolic extract of raw peel had the highest total saponins content (83.07±3.39 g/100g, p<0.01). The steroidal saponin content in HA of raw peel was lower (2.70±0.38 g/100g) than in cooked peel HA extract (6.13±0.63 g/100g). Solubility of saponins is affected by the properties of the solvent, temperature, composi-

could be concluded that heat treatment diminished almost 50 percent of tannins content of jujuba peel. This finding is in accordance with the data reported by Makkar and Becker. [29]

Glutathione (GSH) is an antioxidant and anticarcinogenic that is present in plant and animal tissues. [30] The results indicate, significant increase (p<0.01) in GSH content in cooked peel (125.75±5.04 µMol/100g d.w.b) compared to raw peel (99.49±8.84 µMol/100g d.w.b). It is reported that processing treat-ments including cooking and canning of potatoes, green peas, carrots, peaches and spinach resulted in the reduction of GSH. [30-31] Several factors related to conditions growth, seasonal and geographic situations, plant variety, length and condition of storage, processing and preparation are reported to affect the GSH content in food. [31] Chaitanya et al. have investigated in three mulberry cultivars by maintaining the plants at 40°C. Antioxidant enzyme activities such as glutathione reductase were high in all cultivars in response to high temperature treatment. [31] In the study conducted by Nieto-Sotelo et al. treatment of maize roots to heat shock temperatures of 40°C resulted in an increase of GSH levels. It is possible that a temperature above the optimum for cell growth damages a great variety of enzymic and nonenzymic reactions. Therefore, increasing GSH level in the prevention or repair of damage is important under stress conditions [33].

Table 1. Antioxidant Components in Raw and Cooked Peel of Jujuba

<table>
<thead>
<tr>
<th>Peel</th>
<th>Polyphenol a</th>
<th>Glutathione b</th>
<th>Tannin c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw</td>
<td>1.67±0.07*</td>
<td>99.49±8.84*</td>
<td>7.69±0.09*</td>
</tr>
<tr>
<td>Cooked</td>
<td>0.90±0.05*</td>
<td>125.75±5.04*</td>
<td>3.62±0.10*</td>
</tr>
<tr>
<td>t Test</td>
<td>0.001</td>
<td>0.01</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SD. Different superscripts in the same column indicate significant difference at 1% level as shown by post hoc Scheffe. A: Total polyphenol content is expressed as µMol /100g d.w.b. B: Glutathione content is expressed as µMol /100g d.w.b. C: Tannin content is expressed as g TAE/100 d.w.b.

Table 2. Total Flavonoid Content, Total and Steroidal Saponins in Different Solvents of Peel

<table>
<thead>
<tr>
<th>Peel</th>
<th>Solvent</th>
<th>Flavonoid ± E.V.</th>
<th>Total Saponin ± E.V.</th>
<th>Steroidal Saponin ± E.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw</td>
<td>MtOH</td>
<td>3.52±0.02*</td>
<td>83.07±3.39*</td>
<td>4.75±0.41*</td>
</tr>
<tr>
<td>CA</td>
<td>1.59±0.08*</td>
<td>165.32±10.28</td>
<td>3.33±0.31*</td>
<td>29.58±0.82*</td>
</tr>
<tr>
<td>HA</td>
<td>2.32±0.21*</td>
<td>21.31±1.50</td>
<td>2.70±0.38*</td>
<td></td>
</tr>
<tr>
<td>MtOH</td>
<td>0.79±0.07*</td>
<td>17.75±0.87</td>
<td></td>
<td>3.46±0.30*</td>
</tr>
<tr>
<td>Cooked</td>
<td>0.40±0.05*</td>
<td>44.07±2.13</td>
<td>3.25±0.45*</td>
<td>33.58±0.56*</td>
</tr>
<tr>
<td>HA</td>
<td>0.48±0.03*</td>
<td>20.90±0.33</td>
<td>6.13±0.63*</td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as mean ± SD. Different superscripts in the same column indicate significant difference at 1% level as shown by post hoc Scheffe. * Significant difference at 1% level respectively. MtOH: Methanol, CA: Cold Aqueous Extract, HA: Hot Aqueous Extract. † Total flavonoid content is expressed as g QE/100 g d.w.b.
The DPPH is usually used as a substrate to assess the antioxidative action of antioxidants by determining the free radical-scavenging ability of various samples. Figure 1-2 show the DPPH radical scavenging activity of jujube peel extracts is dependent on the solvent used for extraction. Three extracts of raw and cooked peel showed increasing trends with increase in extract concentration except in hot aqueous extract of raw peel. The highest DPPH radical scavenging activity was found in methanolic extract of raw peel similar to that of standard BHT. The three extracts of cooked peel were found to be significantly (p<0.01) inferior in antioxidant efficacy compared to BHT. Present results are consistent with the data reported by Khalaf et al. [44], Sasikumar et al. [45], Ghasemzadeh et al. [46] and Al-Reza et al. [47]. The higher antioxidant activity is attributed to the presence of bioactive compounds such as polyphenols including tannins, flavonoid existed in the polar extracts.

<table>
<thead>
<tr>
<th>Antioxidant Content</th>
<th>DPPH Raw</th>
<th>DPPH Cooked</th>
<th>Reducing Power Raw</th>
<th>Reducing Power Cooked</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyphenol</td>
<td>0.988</td>
<td>0.975</td>
<td>0.822</td>
<td>0.995</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>0.702*</td>
<td>0.42</td>
<td>-0.633</td>
<td>0.409</td>
</tr>
<tr>
<td>Tannin</td>
<td>-0.359</td>
<td>-0.997*</td>
<td>-0.904</td>
<td>-0.774</td>
</tr>
<tr>
<td>Glutathione</td>
<td>-0.945</td>
<td>-0.934</td>
<td>-0.91</td>
<td>-0.774</td>
</tr>
</tbody>
</table>

* Correlation is significant at the 0.05 level

Figure 3: Reducing Power (RP) of Raw Peel Extracts

Figure 4: Reducing Power (RP) of Cooked Peel Extracts
A significant (p<0.05) correlation between free radical scavenging and flavonoid contents in the raw peel (R² = 0.702) as well as the tannin content (R² = 0.997) in the cooked peel were observed in the present study (Table 4). Correlation coefficient between total polyphenol content and DPPH in raw and cooked peel was R² = 0.988 and 0.975 respectively.

In the reducing power assay (RP), the presence of reductants in the antioxidant sample causes the reduction of the Fe³+/ferricyanide complex to the Fe²+/ferrous form. The results (Fig. 3-4) indicated that three extracts of raw and cooked peel showed varying degree of reducing efficacy in a dose-dependent manner. Hot aqueous extract in the raw peel, hot and methanolic extracts of cooked peel exhibited highest antioxidant activity. These results are in agreement with findings reported by Oboh et al. Therefore, it is suggested that hot aqueous of raw peel, methanol and hot extracts of cooked peel might have reducing power which may be due to the presence of the similar reductants (i.e., antioxidants) responsible for the reduction of the Fe³+/ferricyanide complex to the ferrous form.

CONCLUSION

The results obtained in this investigation indicate that *Ziziphus Jujuba Mill.* peel is a rich source of many antioxidant compounds. Heat treatment leads to reductions in the antioxidant capacity of cooked peel as analyzed by DPPH and RP assay. The reduction might be caused by degradation of phenolic compounds during cooking. In conclusion, preliminary study suggests that food industry waste by-products could be a good source of cheap and natural antioxidants with significant amount of antioxidant with a beneficial health effect in human. Therefore, re-utilization and re-cycling of this waste could be used as a natural antioxidants compounds in food processing and preservation. The overall evidence, however, is limited and much more research is needed.

REFERENCES


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