Phenolic Compounds and Antioxidant Activity of Rice Straw Extract

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Abstract. Agricultural wastes cause a serious environmental problem in Egypt. Utilization of these wastes by an environmentally friendly way is a very important issue. The objective of this study was to utilize rice straw into high-value products. Antioxidant activity, total soluble phenols, and flavonoids were evaluated in ethyl acetate extract prepared from rice straw after alkaline hydrolysis. Total phenols were found to be 221.6 mg gallic acid equivalents (GAE), while total flavonoids were 4.9 mg rutin equivalents (RE). The extract exhibited strong antioxidant activity measured by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method and its IC50 value was 0.4 mg/ml. Furthermore, the ethyl acetate extract possessed high antioxidant activity assayed by β-carotene bleaching method and its value of lipid peroxidation inhibition (LPI) was 75.4%. It also exhibited high reducing power and its IC50 value was equal to 0.06 mg/ml. HPLC analysis indicated that this extract contained seven phenolic acids including: protocatechuic, caffeic, syringic, p-coumaric, ferulic, rosmarinic and cinnamic, in addition to two flavonoids; quercetin and kaempferol. Results indicated that ferulic and p-coumaric acids were the major soluble phenolic acids in rice straw, and their concentrations were 3.9 and 2.9 mg/g DW. It can be summarized that the strong antioxidant activity of ethyl acetate extract, prepared from rice straw, was highly correlated with its high level of phenolic compounds.

Introduction

It was estimated that about 650-975 million tons of rice straw are produced every year worldwide [1]. In Egypt, about 26-35 million tons of agricultural wastes are produced every year [2], and only 11 million tons are utilized for animal feeding and organic fertilization [3].

As a lignocellulosic material, rice straw contains 32-47% cellulose, 19-27% hemicellulose, and 5-24% lignin [1]. Lignins are bound phenolic substances [4]; therefore, hydrolysis is needed to free soluble phenolic compounds from its bound form [5]. Released phenols showed high antioxidant activity [6, 7], and they might be utilized in food and pharmaceutical industry as antioxidant compounds [8, 9].

Although field burning practice has negative impacts on environment and public health [10], Egyptian farmers still use this procedure to get rid of rice straw. Previous reports showed that burning of rice straw would destroy soil microbial flora, and increase poisonous gas emissions causing air pollution [11].

The purpose of this study was to recover a phenolic-rich fraction from rice straw throughout converting lignin in rice straw into high value-added products, such as natural antioxidants. To attain this, phenolic compounds were removed from rice straw by pretreating them with sodium carbonate and then phenolic compounds were identified and quantified by using high-performance liquid chromatography (HPLC). In addition, their antioxidant activity was evaluated using different assay methods.
Theory

Lignin in rice straw consists of complex phenolic compounds, which can be converted to produce a phenolic-rich extract that can be utilized as a safe and natural antioxidants source in food industry.

Materials and Methods

Standards and Reagents

Standard phenolic acids (protocatechuic, caffeic, syringic, p-coumaric, ferulic, rosmarinic, gallic, and cinnamic) and flavonoids (quercetin, kaempferol, and rutin) were purchased from Sigma-Aldrich, Germany. Folin-Ciocalteu’s reagent, aluminium chloride, polyoxyethylene sorbitan monopalmitate (Tween-40), trichloroacetic acid, ferric chloride, potassium ferricyanide, sodium carbonate, sodium acetate, disodium hydrogen phosphate, sodium dihydrogen phosphate, hydrochloric acid were obtained from Loba Chemie Pvt. Ltd., India. Linoleic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and β-carotene were purchased from Sigma-Aldrich, Germany. All other solvents used were of analytical grade.

Plant Material

Straw of rice (Oryza sativa L.) cv. Sakha 104 was collected from rice fields in Tanta city. The straw was first washed with tap water, dried at 40 °C for 48 h, and finely ground to powder using a laboratory mill.

Preparation of the Extract

Dried powder of rice straw (60 g) was pre-treated with 1L Na₂CO₃ (6.5%) at 90 °C with continuous stirring for 2 h as described previously [12] with some modifications. After hydrolysis, the sample was cooled at room temperature, centrifuged at 5000 rpm for 15 min and then filtered through two layers of cloth. This procedure was repeated again using the residue, then filtrates were compiled and pH was adjusted to 2.0 by HCl (6M). The acidic solution was extracted several times with ethyl acetate using a separating funnel. Ethyl acetate extracts were collected, filtered using Whatman No. 1, and then dried under vacuum at 40 °C. The ethyl acetate extract was re-dissolved in methanol for further experiments.

 Determination of Total Soluble Phenols

Total soluble phenols in the ethyl acetate extract was measured according to the Folin-Ciocalteu procedure described previously [13]. Briefly, 1.0 mL Folin-Ciocalteu’s reagent (10%) and 0.8 mL Na₂CO₃ (7.5% w/v) were added to 0.2 mL of methanolic solution of the ethyl acetate extract. After shaking, the mixture was incubated at room temperature for 30 min. Absorption was measured at 765 nm using Abbota SM 1200 UV-VIS spectrophotometer, New Jersey (USA). Total phenols was expressed as milligrams gallic acid equivalents (GAE) per gram extract.

 Determination of Total Flavonoids

Total soluble flavonoids in the ethyl acetate extract were determined using the method described by Djeridane et al. [14]. In brief, 1.0 mL of methanolic solution of the ethyl acetate extract was mixed with 1.0 mL aluminum chloride (2% in methanol). After shaking, the mixture was incubated at room temperature for 15 min, and then absorption was measured at 430 nm using Abbota SM 1200 UV-VIS spectrophotometer, New Jersey (USA). Total flavonoids were expressed as milligrams rutin equivalents (RE) per gram extract.

 Antioxidant Activity by DPPH Radical Scavenging Method

The radical scavenging activity was evaluated as described previously [15]. Two milliliters of the methanolic solution of the sample at (200, 400, 600, and 800 ppm) were mixed with 1 mL of 0.5 mM DPPH methanolic solution and 2 mL sodium acetate buffer (0.1 M, pH 5.5). After shaking,
the mixture was incubated at room temperature in the dark for 30 min, and then the absorbance was measured at 517 nm using Abbota SM 1200 UV-VIS spectrophotometer, New Jersey (USA). The percentage of DPPH radical scavenging was calculated using the following equation:

\[
\text{Radical scavenging (\%) = } \left( \frac{\text{abs}_{\text{control}} - \text{abs}_{\text{sample}}}{\text{abs}_{\text{control}}} \right) \times 100
\]

where \(\text{abs}_{\text{control}}\) is the absorbance of reaction without sample and \(\text{abs}_{\text{sample}}\) is the absorbance of reaction with sample. IC\(_{50}\) (inhibitory concentration) value was determined as inhibitory concentration of the extract necessary to decrease the initial DPPH radical concentration by 50\%, and it was expressed in mg/mL. Lower IC\(_{50}\) value indicates higher DPPH radical scavenging activity.

**Antioxidant Activity by \(\beta\)-Carotene Bleaching Method**

Antioxidant activity was evaluated according to \(\beta\)-carotene bleaching method as described previously by Elzaawely and Tawata [16]. \(\beta\)-Carotene (2.0 mg) was dissolved in 10 mL chloroform. One milliliter of the chloroform solution was mixed with 20 \(\mu\)L linoleic acid and 200 mg Tween-40. The chloroform was evaporated at 45\(^\circ\)C, then 50 mL oxygenated water was added, and the mixture was vigorously shaken. An aliquot (250 \(\mu\)L) of the \(\beta\)-carotene-linoleic acid emulsion was distributed in test tubes. Methanolic solution (30 \(\mu\)L) of the ethyl acetate extract at 1000 ppm was added. An equal amount of methanol was used as a control. The test tubes were incubated at 50\(^\circ\)C, and, absorbance was measured using Abbota SM 1200 UV-VIS spectrophotometer, New Jersey (USA) at 492 nm. Readings of the sample were performed immediately at zero time and every 15 min up to 180 min. Lipid peroxidation inhibition (LPI) percentage was calculated using the following equation:

\[
\text{LPI (\%) } = \left( \frac{A_0}{A_1} \right) \times 100
\]

where \(A_0\) is the absorbance value measured at zero time for the test sample, while \(A_1\) is the corresponding absorbance value measured after incubation for 180 min.

**Antioxidant Activity by Reducing Power Method**

Reducing power was determined as described previously by Yildirim et al. [17]. One milliliter of the ethyl acetate extract at 25, 50, 100, and 250 ppm in methanol was mixed with 2.5 mL phosphate buffer (0.2 M, pH 6.6) and 2.5 mL potassium ferricyanide \([K_3Fe(CN)_6]\) (10 g/L), then the mixture was incubated at 50\(^\circ\)C for 30 min. Afterwards, 2.5 mL trichloroacetic acid (100 g/L) was added to the mixture, which was subsequently centrifuged at 4000 rpm for 10 min. Finally, 2.5 mL of the supernatant solution was mixed with 2.5 mL distilled water and 0.5 mL FeCl\(_3\) (1 g/L) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. The IC\(_{50}\) value was calculated as the sample concentration at which the absorbance was 0.5 [18]. Lower IC\(_{50}\) value indicates higher reducing power.

**Identification and Quantification of Phenolic Compounds by HPLC**

HPLC analysis was carried out using Agilent Technologies 1100 series liquid chromatograph equipped with an auto sampler and a diode-array detector. The analytical column was ZORBAX Eclipse XDB-C18 (I.D. 4.6 mm, length 150 mm, particle size 5 \(\mu\)m) with a C18 guard column (Phenomenex, Torrance, CA). The mobile phase consisted of acetonitrile (solvent A) and 2\% acetic acid in water (v/v) (solvent B). The flow rate was kept at 0.8 mL/min for a total run time of 70 min. The gradient system was as follows: 100\% B to 85\% B in 30 min, 85\% B to 50\% B in 20 min, 50\% B to 0\% B in 5 min and 0\% B to 100\% B in 5 min. The injection volume was 50 \(\mu\)L and peaks were monitored simultaneously at 280, 320 and 360 nm. All samples were filtered through a 0.45 \(\mu\)m Acrodisc syringe filter (Gelman Laboratory, MI) before injection. Peaks were identified by congruent retention times and UV spectra and compared with those of the standards.
Results and Discussion

Total Soluble Phenols and Flavonoids

Phenolic compounds include phenolic acids, polyphenols and flavonoids. Plant phenols and flavonoids have several healthy-beneficial roles due to their antioxidant, anticancer, antimicrobial, antiviral and anti-inflammatory properties [19, 20]. Contents of total soluble phenols and flavonoids of the ethyl acetate extract prepared from rice straw are shown in Fig. 1. The results indicated that ethyl acetate extract contained high amount of phenolic compounds (221.6 mg GAE/g extract) as illustrated in Fig. 1A. In addition, it has low amount of total flavonoids (4.9 mg RE/g extract) as presented in Fig. 1B.

![Figure 1. Total soluble phenols (A) and total soluble flavonoids (B) of rice straw extract.](image)

**Antioxidant Activity**

Antioxidant activity was measured by several methods, which are based on a different reaction. It is recommended to use more than test models to test antioxidant activity of a molecule [21]. Therefore, three different methods were used in this study to measure antioxidant activity of ethyl acetate extract of rice straw.

The ethyl acetate extract of rice straw exhibited strong DPPH radical scavenging activity that was dose dependent (Fig. 2). The IC$_{50}$ value of the extract was 0.45 mg/g extract. DPPH is a stable radical showing a maximum absorbance at 515 nm. In the presence of the antioxidant molecule, it can be reduced to uncolored solution [22]. It is a fast and an easy-common method to evaluate the antioxidant activity of plant extracts and natural products [23, 24]. A positive correlation has been
found between DPPH radical scavenging activity of this extract and its phenolic content ($R^2 = 0.9883$); therefore, this strong DPPH scavenging activity may be due to the presence of high amounts of phenolic compounds in this extract.

The ethyl acetate extract of rice straw inhibited the oxidation of $\beta$-carotene/linoleic acid emulsion (Fig. 3). It can be noticed that $\beta$-carotene bleaching activity increased with increasing concentration of the extract. Furthermore, the LPI value of the ethyl acetate extract was measured at 75.2%. This antioxidant activity may be correlated to the phenolic content of the extract ($R^2 = 0.9945$). The plant extracts or natural products that have antioxidant activity can neutralize the free radicals formed in the $\beta$-carotene- linoleic acid emulsion and it can hinder the extent of $\beta$-carotene-bleaching caused by oxidation [25]; thus, the discoloration and degradation rate of $\beta$-carotene was slowed down over time in the presence of antioxidant compounds.

Reducing power is considered a strong indicator of the antioxidant activity, as it measures the ability of the antioxidant molecules to reduce the Fe$^{3+}$/ferricyanide complex to the ferrous form through electron transfer [24, 26]. In this study, the high reducing power of the ethyl acetate extract of rice straw increased with increasing the concentration of the extract (Fig. 4) and its IC$_{50}$ value was measured at 0.06 mg/ml. The results presented in Fig. 4 also indicated that the reducing activity of the ethyl acetate extract might be revealed to the presence of phenolic compounds in the extract ($R^2 = 0.9955$). These phenols may act as reducing agents by donating electrons and converting the free radicals generated in the solution to more stable products and terminate radical chain reaction [27].

**Figure 2.** Antioxidant activity of rice straw extract measured by DPPH radical scavenging assay.
Identification and quantification of phenolic compounds by HPLC

Rice straw consists of cellulose, hemicellulose and lignin. Lignin is a mixture of phenolic compounds in a bound complex, such as esters and glycosides; therefore, using alkaline, acidic or enzymatic hydrolysis is needed to release free phenols [28]. The alkaline pretreatment of rice straw with sodium carbonate appeared to be sufficient to break down the bonds between cell-wall polymers; subsequently, lignin can be released [29, 30]. Plant phenolic compounds are mainly found in the form of benzoic acid or cinnamic acid derivatives as well as glycosidic esters [31]. They possessed multiple biological activities such as antioxidant [32], antimicrobial [33], or anti-inflammatory activity [34]. In this study, alkaline hydrolysis with Na$_2$CO$_3$ at 90°C was used to release conjugated phenols. Accordingly; nine phenolic compounds were identified and quantified in the ethyl acetate extract of rice straw by HPLC (Table 1). These compounds are seven phenolic acids including protocatechuic, caffeic, syringic, p-coumaric, ferulic, rosmarinic and cinnamic, in addition to two flavonoids including quercetin and kaempferol. Ferulic and p-coumaric acids were the predominant phenols (3.9 and 2.9 mg/g DW, respectively), while caffeic acid was the lowest one (0.03 mg/g DW). Previous studies reported that rice straw contained a variety of phenolic acids such as caffeic, chlorogenic, syringic, p-coumaric and ferulic [35-39].
Table 1. Phenolic compounds (mg/g DW) in the ethyl acetate extract of rice straw.

<table>
<thead>
<tr>
<th>No.</th>
<th>Compound</th>
<th>Retention time (min)</th>
<th>Amount (mg/g DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Protocatechuic acid</td>
<td>9.6</td>
<td>0.08</td>
</tr>
<tr>
<td>2</td>
<td>Caffeic acid</td>
<td>21.1</td>
<td>0.03</td>
</tr>
<tr>
<td>3</td>
<td>Syringic acid</td>
<td>22.4</td>
<td>0.13</td>
</tr>
<tr>
<td>4</td>
<td>p-Coumaric acid</td>
<td>27.9</td>
<td>2.90</td>
</tr>
<tr>
<td>5</td>
<td>Ferulic acid</td>
<td>31.7</td>
<td>3.90</td>
</tr>
<tr>
<td>6</td>
<td>Rosmarinic acid</td>
<td>39.7</td>
<td>0.39</td>
</tr>
<tr>
<td>7</td>
<td>Cinnamic acid</td>
<td>42.2</td>
<td>0.09</td>
</tr>
<tr>
<td>8</td>
<td>Qurecetin</td>
<td>42.8</td>
<td>0.58</td>
</tr>
<tr>
<td>9</td>
<td>Kaempferol</td>
<td>45.8</td>
<td>0.23</td>
</tr>
</tbody>
</table>

Conclusions
In this study, alkaline pretreatment with sodium carbonate was carried out to produce a phenolic-rich extract from rice straw. This extract contained high amount of phenolic compounds and exhibited strong antioxidant activity. Rice straw could be utilized as a safe natural antioxidant source.

Conflict of Interest
No potential conflict of interest was reported.

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References


