Spectrophotometric method for the study of the antioxidant activity applied on Ziziphus jujuba and Hydrangea paniculata aqueous extracts

Abstract: Antioxidant activity of water extracts from Ziziphus jujuba fruits and Hydrangea paniculata leaves, maintained in a refrigerator, was determined by the potassium permanganate method. The results presented in this paper indicate that the fruit extracts from Ziziphus jujuba had higher antioxidant activity than Hydrangea paniculata leaf extracts and that both fermentation processes had a strong effect on antioxidant activity.

Key Words: antioxidant activity, plant extract, spectrophotometric method

Introduction

It is a current trend in different domains, especially food production and medicine, to search for natural products that can replace the synthetic ones that can Ziziphus jujuba fruits and Hydrangea paniculata leaves are known to be rich in flavonoids (Pridham, 1965; Clement et al., 2004). and this is why we have studied their antioxidant properties versus ascorbic acid, a synthetic and water-soluble antioxidant.
MATERIAL AND METHODS

Material: Potassium permanganate and sulfuric acid were purchased from “Reactivul”, București, the ascorbic acid from Merck and the YPD agar medium from Teknova.

Apparatus: Spectrophotometer SPEKOL 10 (Carl Zeiss, Jena) fitted with 30 ml quartz vats and magnetic stirrer and coupled with an acquisition plate (Ai-chrom 727 computer interface Aion Chromatography, Inc., USA) and a Pentium II computer for the antioxidant activity determination.

Plant material: Fruits of Ziziphus jujuba (E1) and leaves of Hydrangea paniculata (E2) were collected in October 2004 and were dried at 20°C in the dark place. After that, 4 g of the dried sample was finely chopped into small parts and then extracted with 60 ml water for 70 h at 10°C, followed by filtration. The final volume of the extract was 40 ml.

Antioxidant properties screening by the potassium permanganate assay: The method is based on the redox reactions between the antioxidant sample and the potassium permanganate in sulfuric acid media, leading to sample discoloration. Previously done experiments (Ianculov et al., 2001) determined only the time until no color could be observed. We have improved this method by the spectrophotometric pursuit of the process, which allows a more accurate calculus. We have already published a series of results obtained by this method on various spices (Szabo et al., 2005; Caciog et al., 2005).

Variable amounts of samples (v mL), depending on the intensity of the antioxidant activity, were introduced in a 30 ml quartz vat containing an oxidative mixture of: 1.5 ml potassium permanganate 0.01M; 3.5 ml sulfuric acid 2M and (20-v) ml distilled water. That moment was considered the zero time. The spectrophotometer signal (mV) was then registered at 535 nm until constant value. Subsequent variations of the potassium permanganate concentration were afterwards determined based on a previously prepared calibration curve. The extracts were kept in the refrigerator (10°C) and the analyses were done within 40 days from the beginning of their storage.

RESULTS AND DISCUSSION

The spectrophotometer signal appearing on the monitor represents mV values. A calibration curve was determined by preparing a series of six solutions with different concentrations of potassium permanganate and registration of the electronic signal (mV) for each of them. Pairs of data were graphically presented and linearly fitted. The obtained graphic and the corresponding equation are presented in Figure 1.

Based on this, the potassium permanganate concentration (x) can be determined from the signal value (y) using the formula:

\[ x = \frac{y - 0.929}{381328} \]  

(1)
The corresponding curves for the extracts kept in the refrigerator, are shown in Figure 2a, b after 1, 2, 4, 8, 12, 18, 24, 32 and 40 days from their preparation.

Most curves show a decrease in the potassium permanganate concentration, as expected. Still, in some cases, the rapid decrease is followed by a false increase, due to the formation of MnO₂ particles, which afterwards precipitate.

It is probable that during the first step, corresponding to the rapid decrease of the concentration, reacts substances with higher antioxidant activity which are able to reduce Mn (VII) to Mn (II). After that, reacts substances with lower antioxidant activity which reduces Mn (VII) up only to Mn (IV), — precipitating MnO₂ leading to the precipitate formation. During the first phase of this phenomenon, the formed MnO₂ particles thus formed induces the light diffusion and temporarily increase total fake absorption. Later on, a part of the MnO₂ precipitates, and another part is probably dissolved by the sulfuric acid in the medium and this is why the absorption decreases again.

Fig. 1 — Calibration curve for the potassium permanganate in the oxidative mixture
Fig. 2a — Variation of the potassium permanganate concentration after adding 0.2 ml of *Ziziphus jujuba* fruits (E1), 1, 2, 4, 8, 12, 18, 24, 32 and 40 days from their sample preparation.

Fig. 2b — Variation of the potassium permanganate concentration after adding 0.2 ml of *Hydrangea paniculata* leaves (E2), 1, 2, 4, 8, 12, 18, 24, 32 and 40 days from their sample preparation.
In order to quantitatively compare the antioxidant activities, we proposed the following formula:

$$A_{50} = \frac{l_{\text{standard}}}{l_{\text{plant sample}}} \cdot \frac{C_{\text{standard}}}{m_{\text{plant}}} \cdot \frac{V_{\text{standard}}}{V_{\text{plant sample}}} \cdot V_{\text{extract}}$$

(2)

where:

- $A_{50}$ — antioxidant activity expressed, reflected in the time until the sample induces a decrease of the oxidizing agent [potassium permanganate] concentration up to one half, compared against a standard [ascorbic acid] (mmol equivalent standard / g plant)
- $l_{\text{plant sample}}$ — the time until the sample induces a decrease of the permanganate concentration up to one half (min)
- $l_{\text{standard}}$ — the time until the standard (ascorbic acid) induces a decrease of the permanganate concentration up to one half (min) [0.65 minutes as seen in Figure 3]
- $C_{\text{standard}}$ — standard (ascorbic acid) concentration (mmol/ml) [0.01 mmol/ml]
- $m_{\text{plant}}$ — weight (g) of the plant sample submitted to extraction [4g]
- $V_{\text{plant sample}}$ — volume of the plant extract submitted to the analysis [0.2 ml]
- $V_{\text{standard}}$ — volume of the standard submitted to the analysis [1 ml]
- $V_{\text{extract}}$ — volume (ml) of the obtained extract [40 ml]

The variations in the $A_{50}$ values are given in Table 1.

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Fig. 3 — Variation of the potassium permanganate concentration after adding 1 ml of ascorbic acid 0.01 mol/l (mmol/ml)
Tab. 1 — $A_{50}$ values for the studied extracts

<table>
<thead>
<tr>
<th>Day</th>
<th>$A_{50}$ (mmol equivalent ascorbic acid/g plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ziziphus jujuba</td>
</tr>
<tr>
<td>1</td>
<td>0.1935</td>
</tr>
<tr>
<td>2</td>
<td>0.1231</td>
</tr>
<tr>
<td>4</td>
<td>0.1401</td>
</tr>
<tr>
<td>8</td>
<td>0.1563</td>
</tr>
<tr>
<td>12</td>
<td>0.1935</td>
</tr>
<tr>
<td>18</td>
<td>0.2902</td>
</tr>
<tr>
<td>24</td>
<td>0.1195</td>
</tr>
<tr>
<td>32</td>
<td>0.0813</td>
</tr>
<tr>
<td>40</td>
<td>0.0903</td>
</tr>
</tbody>
</table>

The differences in the $A_{50}$ values were related to the observed fermentation processes. It previously noticed (Caçiga, 2005) that the samples maintained at the room temperature, produce different patterns (Caçiga, 2005), compared to those maintained in the refrigerator. Temperature has influenced, not only on the reaction kinetics, but also on the microorganisms present. Our assumption that there were at least two types of microorganisms was proved correct after sowing on YPD (yeast peptone dextrose) agar medium (Figure 4).

Fig. 4 — Results of the YPD agar medium sowings, 4 days later

CONCLUSION

The spectrophotometric method we propose allows the quantitative appreciation of the antioxidant activity for the tested aqueous plant extracts.

With a single exception, Ziziphus fruit extracts seemed to have higher antioxidant activity than Hydrangea leaf extracts.
Fermentative processes seem to improve the antioxidant activity of the aqueous extracts, but further studies are necessary in order to determine the influence of ethanol, which is probably produced during the fermentation processes, on the obtained results.

REFERENCES


СПЕКТРОФОТОМЕТРИЈСКА МЕТОДА ЗА ОДРЕЂИВАЊЕ АНТИОКСИДАТИВНЕ АКТИВНОСТИ ВОДЕНИХ ЕКСТРАКТА ЗИЗИФУС ЈУЈУБА И HYDRANGEA PANICULATA

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Резиме

У раду је изучавана антиоксидативна активност воденог екстракта Ziziphus jujuba (плод) и Hydrangea paniculata (лист).

Активност је одређена на бази реакције антиоксидативних узорака са калијум перманганатом у киселом средину. Резултати испитивања показују да екстракти добијени из плода Ziziphus jujuba имају већу антиоксидативну активност него екстракти из листа Hydrangea paniculata.

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