STUDIES ON THE OPTIMAL EXTRACTION OF FLAVONOIDS FROM THE FRUIT OF JUNIPERUS VIRGINIANA L.

ABSTRACT: The isolation and quantitative determination of flavonoid compounds in fruit of Juniperus virginiana L. (Cupressaceae) are described. A method for the detection of those flavonoids was high performance liquid chromatography (HPLC). Rutin and kaempferol were determined in accordingly extracts and quercetin only in hydrolysated extracts.

KEY WORDS: flavonoids, extract, Soxhlet extraction, Juniperus virginiana L.

INTRODUCTION

Juniperus virginiana L. (red cedar, juniper) which belongs to genus Juniperus of family to Cupressaceae, is a species of juniper native to eastern North America, from southeastern Canada to the Gulf of Mexico, east of the Great Plains.

Juniper is used in the treatment of arthritis, asthma, colds, cough, cystitis, diabetes, diarrhea, sore throat, tuberculosis. The berries are being studied for their anticancer and anti-tumor properties. Topical use is applied for acne, burns, dandruff, hemorrhoids, herpes, rheumatism, and warts. In food industry the berries are used in jams, pepper substitutes, beer and gin, and made into a coffee substitute, also are used in composition of bitter and other energetics.

Juniper fruit contains volatile oil (myrcene, cineole, terpineol, camphen), resin, saccharides, organic acids, ascorbic acid, tannins, vitamins and minerals. Apart from these, juniper fruit contain flavonoids (Leung et al., 1996). It was reported that flavonoids could remove $O_2^\cdot$ in human bodies, improve blood circulation, and lower blood pressure (Fang, 1998; Liu et al., 2002; Wang et al., 1996).
At present, studies on the extraction of flavonoids from juniper fruits have not been reported. In this study, optimum conditions to extract flavonoids from red cedar fruits were studied in order to achieve scientific evidence for the processing and utilizing of juniper fruits.

MATERIAL AND METHODS

Plant Material: The juniper fruits were collected from The Macea Botanical Garden of West University Vasile Goldiș (Arad, Romania) and were dried at 20°C in dark place.

Solvents and reagents: Quercetin, rutin, and kaempferol were purchased from Sigma-Aldrich and all HPLC-grade solvents were purchased from Merck (Germany). All other chemicals were of analytical grade and were purchased from Chimopar Bucharest.

Extraction and preparation of extracts: The dried and finely ground samples of juniper fruits (10 g each) were extracted with 100 mL solvent for 4 h in a Soxhlet apparatus (Ca cig, 2007). The extracts were concentrated at 15 mL and then stored at 4°C for further analysis. The solvents used were methanol, ethanol, dichloromethane, tetrachloromethane, benzene, and toluene.

HPLC analysis (Ca cig et al., 2006): Flavonoids were measured at 365 nm by a HPLC Agilent 1100. Separation was carried out on a Lichrospher 100-RP-18 column (5 μm, 250 x 4 mm). A gradient elution was performed with eluent acetonitril: water = 1:1. The flow rate was 1 mL/min and the injection volume was 20 mL. Identification of the flavonoids was carried out by comparing their retention times to those of standards.

Hydrolysis conditions: The total amount of each flavonoid in the extracts was determined after hydrolysis of its glycosides by refluxing samples of extract in HCl 25% for 30 min. According procedures (Hasler et al., 1990) and its injection into the HPLC. The analytical data of each flavonoid detected were compared with datum of an authentic standard.

RESULTS AND DISCUSSION

Figure 1 shows the structures of the compounds under study.

The content of flavonoid according to HPLC method was calculated as rutin type compound and kaempferol. Results given as rutin varied from 0.2364 mg/mL extract in tetrachloromethane extract to 11.7365 mg/mL extract in methanolic extract of juniper fruit.

Flavonoid content was the higher for methanolic and ethanolic extracts (Table 1).
Tab. 1. — Samples and results of the determination of flavonoids in Juniperus virginiana L. by using the HPLC method

<table>
<thead>
<tr>
<th>Sample</th>
<th>Solvent for extraction</th>
<th>Content mg/mL extract</th>
<th>Rutin</th>
<th>Kaempferol</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>Methanol 94%</td>
<td>11.7365</td>
<td>11.7365</td>
<td>0.7892</td>
</tr>
<tr>
<td>E2</td>
<td>Ethanol 96%</td>
<td>9.0525</td>
<td>9.0525</td>
<td>trace</td>
</tr>
<tr>
<td>E3</td>
<td>Dichloromethane</td>
<td>5.0885</td>
<td>5.0885</td>
<td>trace</td>
</tr>
<tr>
<td>E4</td>
<td>Benzene</td>
<td>4.6880</td>
<td>4.6880</td>
<td>trace</td>
</tr>
<tr>
<td>E5</td>
<td>Toluene</td>
<td>1.8335</td>
<td>1.8335</td>
<td>trace</td>
</tr>
<tr>
<td>E6</td>
<td>Tetrachloromethane</td>
<td>0.2364</td>
<td>0.2364</td>
<td>trace</td>
</tr>
</tbody>
</table>

At same time flavonoid compound was determined after acid hydrolysis, frequently applied to standardize flavonoid material. Kampferol, rutin and quercetin were used as standards.

Quercetin was found to dominate in alcoholic extracts after acid hydrolysis (Table 2).

Tab. 2. — Results of the quantitative determination of flavonoids in Juniperus virginiana L. by using the HPLC method after acid hydrolysis

<table>
<thead>
<tr>
<th>Sample</th>
<th>Content mg/mL extract</th>
<th>Rutin</th>
<th>Quercetin</th>
</tr>
</thead>
<tbody>
<tr>
<td>E7*</td>
<td>0.2460</td>
<td>0.2460</td>
<td>5.2356</td>
</tr>
<tr>
<td>E8**</td>
<td>0.0841</td>
<td>0.0841</td>
<td>5.6630</td>
</tr>
</tbody>
</table>

* methanolic extract after hydrolysis

** Ethanolic extract after hydrolysis

Fig. 1. — The structure of flavonoids analyzed in Juniperus virginiana extracts
The content of quercetin in other extracts is a much lower quantity than flavonol type compound.

HPLC chromatograms are presented in Figures 2, 3, and 4.

Fig. 2. — Chromatograms of standards: rutin (a), kaemferol (b) and quercetin (c)
Fig. 3. — Chromatograms of alcoholic extracts (E1, E2)
Fig. 4. — Chromatograms of hydrolised extracts (E7, E8)
CONCLUSION

The optimum process to extract flavonoids from junipers fruit were obtained, namely extracted for 4 h by using 94% methanol solution in a Soxhlet apparatus with the material ratio of 1:10 (w:v).

REFERENCES


