1 Introduction

Flavonoids as a group of phenolic compounds are found in most plants and are part of the human diet. As part of the human diet, they are absorbed from the gastrointestinal tract and excreted unchanged or as metabolites of flavonoids in urine and faeces. These compounds are potent antioxidants, free radical scavengers, and lipid peroxidation inhibitors. Many studies have shown that flavonoids have good biological activities, including antiallergenic, antiviral, and anti-inflammatory. The onion (*Allium cepa* L.), as one of the better sources of flavonoids in the human diet, is common. Studies have shown total quercetin content in onion to be about 350 mg kg⁻¹, which is about 10 times more in comparison with other vegetables.

The contents of onions include protein, fat, minerals (Na, Ca, P, Fe, K, Zn), fibre, carbohydrates, vitamin C, unsaturated sulphurs, and other organic compounds like quercetin di- and triglycosides: 3,4ʹ-O-β-D-diglucoside, 7,4ʹ-O-β-D-diglucoside, 3,7-O-β-D-diglucoside, 3-O-sophoroside-7-O-β-D-glucuronide, 3,7,4ʹ-O-β-D-triglucoside and rutin.

Rutin, also known as rutoside, quercetin-3-rutinoside, and sophorin, is a bioflavonoid present in buckwheat, onion, garlic, and other plants. Rutin is a heteroside which upon hydrolysis gives quercetin, glucose, and rhamnose. Its structure is shown in Fig. 1. Being discovered for the first time in *Ruta graveolens* L., it got its name after the herbal species. This compound is widely present in plants, and exhibits many health effects upon consumption, such as strengthening capillary walls, reducing the harmful effects of LDL cholesterol, and reducing the risk of cardiovascular diseases.

Traditionally, onions (*Allium* species) have been used for the treatment of asthma, antipyretic, urinary, and respiratory tract infections, as well as cardiac complaints. Many studies indicate that garlic extract has broad-spectrum antimicrobial activity against Gram-positive and Gram-negative microorganisms and potent antifungal activity.

Onion (*Allium cepa* L.) is a biennial herbaceous plant of the family *Alliaceae*. It is related to garlic, leek, and chive, and is used in cooking and medicine. The onion has a spherical inflorescence, greenish-white flowers on one or several stems without leaves. The leaf basis swells to form the underground edible bulb.
proliferation, may even show antibacterial effects, and are a good source of flavonoids.5–10

Garlic (Allium sativum L.) is a bulbous plant and another member of the family Alliaceae. Acknowledged as a natural antiseptic and antibiotic, garlic has been widely used in traditional medicine especially in China, India, and Southern Europe. Garlic has strong antioxidant properties that originate from phenolic compounds including flavonoids.11

Onions are an important source of phenolic compounds that imply health benefits for consumers. However, garlic is known to be an important source of organosulphur compounds. The antioxidant activity in garlic cultivars is more related to organosulphur content than phenolic content.12 Its essential oil contains allin, a compound that decomposes to alllicin, which contributes to the specific smell of garlic, degrades fats, and shows antibiotic activity.8

Wood garlic, ramsons or bear leek (Allium ursinum L.) is a perennial herbaceous plant, 20–25 cm long, which throws up a flowering stem with a white to transparent cover. The leaves are flat and oblong. All parts of the plant are edible. It can be used to lower high blood pressure, while the other beneficial health effects are similar to those of garlic. Inexperienced pickers often do not distinguish wood garlic from poisonous plants such as crocus, lily of the valley, and hellebore. None of these plants has the specific smell of garlic, which is a useful characteristic.6–7 Because of their phytoconstituents, Allium species exhibit considerable antioxidant properties and could modulate the detoxification systems.9

The aim of this study was to determine rutin content in different species of onions by the HPLC-ECD method. The analysed onion species were: Allium sativum L., Allium cepa L., Allium ursinum L., grown in Bosnia and Herzegovina.

2 Experimental

2.1 Herbal material (onion samples)
The samples of onions were collected at the location of “Svrake”, Sarajevo, Bosnia and Herzegovina. Svrake is one of eight local communities of the municipality of Vogošća that belongs to the Sarajevo Canton. In the Vogošća area, there is a sub-mountainous, moderately continental type of climate, with a favourable geographical position, climatic conditions, abundance of water, fertility of land and forest resources. Most of the population of Svrake settlement are engaged in agriculture. Some of the products are marketed.

Samples collected: parts of onion (bulbs and leaves), garlic (bulbs and leaves), and wood garlic (leaves) were stored at −20 °C until preparation of samples and analysis.

2.2 Chemicals
The standard of rutin was obtained from Sigma (Germany). Methanol of HPLC analytical grade was purchased from Merck (Germany). Acetonitrile and glacial acetic acid were of analytical grade and purchased from Panreac (Spain).

2.3 Preparation of onion extracts
A weighed amount of 1.0 g of fresh bulbs and leaves of onion and garlic, and roots of wood garlic was macerated in a mortar and pestle with 9 ml methanol added. After 30 min of extraction with magnetic stirring at room temperature, the extracts were obtained by filtering through gauze. Additionally, the extracts were centrifuged for 20 min, at 15 000 rpm and at 4 °C (Microcentrifuge, Hettich; Micro 22R). The supernatants were evaporated to dryness, and stored at −20 °C until analysis. Prior to analysis by the HPLC system, the dry sample extract was weighed and methanol added to prepare a concentration of 1 mg ml−1. All three extracts were prepared in parallel the same way.

The content of methanolic extract per 1 g fresh sample of Allium species is listed in Table 1.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Content (mg g−1) ± SD</th>
</tr>
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<tbody>
<tr>
<td>garlic leaves</td>
<td>39.2 ± 2.2</td>
</tr>
<tr>
<td>garlic bulbs</td>
<td>33.0 ± 1.65</td>
</tr>
<tr>
<td>onion leaves</td>
<td>46.9 ± 1.36</td>
</tr>
<tr>
<td>onion bulbs</td>
<td>37.6 ± 1.90</td>
</tr>
<tr>
<td>wood garlic leaves</td>
<td>58.9 ± 0.96</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± standard deviations (SD) of three measurements.

2.4 Chromatographic conditions
HPLC, Shimadzu LC-SOL SINGLE-LC EN, Made in Kyoto, Japan; Detector: ECD, BAS Liquid Chromatography CC-5E, LC-4C; Pump: LC-20AT; Degasser: DGU-20A3; Oven: CTO-10Asvp; Working Electrode: Glassy carbon; model MF-1000; BASS; Reference electrode: Ag/AgCl; model MW-2030; BASS; Columns: ODS Hypersil 5 μm 250 × 4.6 mm, Phenomenex (the column was thermostat at 22 °C during the analysis); Isocratic elution, mobile phase: methanol-acetonitrile-water-acetic acid (volume ratio 20:10:70:10); Flow: 1 ml min−1; Injection volume was 20 μl; Run time: 40 min; Potential: +0.84 V.

2.5 Identification and quantitative determination of rutin
The identification and quantitation of rutin in onion samples after HPLC-ED analysis were done using a Lab-Solution program of Shimadzu (Kyoto, Japan).13,14 Rutin in the analysed samples of onion was identified by comparison of retention times with retention times of rutin’s standard. The retention times of rutin in standard solution was 15.317 ± 0.16 min., and in onion samples
15.380 ± 0.20 min. The concentrations of rutin in onion samples were determined based on the calibration curve of a standard solution of rutin, shown in Fig. 2. Firstly, the standard stock solution of rutin was prepared in methanol, at a concentration of 1 mg ml⁻¹. From the stock solution were then prepared standard solutions of concentrations: 0.1, 0.2, and 0.5 mg ml⁻¹, which were used in this study.

![Fig. 2 – Calibration curve of standard rutin solutions](image)

The concentrations of rutin in samples were determined with the HPLC-ED method. The standard linear calibration curve was run to obtain the linear range of sample analysis. The correlation factor for rutin was with accepted value \( R^2 = 0.9915 \), and the standard calibration curve was linear over the range (0.1–1.0 mg ml⁻¹). The limit of detection (LOD) and limit of quantification (LOQ) were calculated from equations. \(^{15}\) LOD for rutin was 0.0111 mg ml⁻¹ and LOQ was 0.037 mg ml⁻¹. Representative HPLC chromatograms of the leaves and bulbs of garlic (Allium sativum L.) are presented in Fig. 3; leaves and bulbs of onion (Allium cepa L.) in Fig. 4, and in the wood garlic (Allium ursinum L.) leaves in Fig. 5.

![Fig. 3 – HPLC chromatograms of the methanolic extracts of garlic leaves (a) (rutin – 15.276 min), and garlic bulbs (b) (rutin – 15.075 min)](image)

![Fig. 4 – HPLC chromatograms of the methanolic extracts of onion leaves (a) (rutin – 15.426 min), and onion bulbs (b) (rutin – 15.702 min)](image)

![Fig. 5 – HPLC chromatogram of the methanolic extract of wood garlic leaves (rutin – 15.423 min)](image)

One can notice that retention times of rutin among all samples are different. Since the retention time is the only iden-
Table 2 – Content of rutin in Allium species

<table>
<thead>
<tr>
<th></th>
<th>Garlic leaves</th>
<th>Garlic bulbs</th>
<th>Onion leaves</th>
<th>Onion bulbs</th>
<th>Wood garlic leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>rutin / mg g⁻¹</td>
<td>0.78 ± 0.09</td>
<td>0.33 ± 0.13</td>
<td>0.19 ± 0.21</td>
<td>0.04 ± 0.10</td>
<td>0.46 ± 0.17</td>
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</tbody>
</table>

Results are expressed as mean ± standard deviations (SD) of three determinations.

4 Conclusions

We determined the contents of rutin in Allium sativum L., Allium cepa L., Allium ursinum L. collected in Bosnia and Herzegovina. The contents of rutin were obtained using the HPLC-ED method. It was observed that leaves of onion and garlic contained higher concentration values in comparison to bulbs, while leaves of wood onion had a high content of rutin. Our study has shown that rutin content of leaves was higher compared that in the bulbs of Allium species. Rutin is a powerful antioxidant and has beneficial health effects, such as strengthening capillary walls, reducing the risk of cardiovascular diseases. Because our results have shown that Allium species contain more rutin, and due to rutin’s aforementioned health benefits, they are recommended as a better natural source of this glycoside.

References

Analiza rutina tekućinskom kromatografijom visoke učinkovitosti u vrstama Allium sakupljenim u Bosni i Hercegovini

Mirsada Salihića* i Emin Sofićb

Rutin je heterozid kvarcetin široko rasprostranjen u biljkama i pokazuje brojne pozitivne učinke na zdravlje, poput jačanja stijenki kapilara, smanjenja štetnih učinaka LDL kolesterola i smanjenja rizika od bolesti krvožilnog sustava. Udio rutina određen je u tri vrste roda Allium (A. cepa, A. sativum i A. ursinum) koje rastu u Bosni i Hercegovini primjenom tekućinske kromatografije visoke učinkovitosti s elektrokemijskom detekcijom (HPLC-ECD). Analiza je provedena u metanolnim ekstraktima lukovice i lista češnjaka, lukovice i lista crvenog luka, lista medvjeđeg luka. Rutin je određen u svim analiziranim uzorcima vrste Allium. Najveći sadržaj rutina određen je u listovima češnjaka (0.78 ± 0.09 mg g⁻¹), a najmanji u crvenom luku (0.04 ± 0.10 mg g⁻¹). Udjeli rutina bili su veći u uzorcima lišća, što sugerira da se listovi luka i češnjaka preporučuju kao bolji prirodni izvor ovog glikozida.

Ključne riječi
Vrste Allium, rutin, metoda HPLC-ECD

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