Chemical composition and antioxidant, antibacterial activity of *Cirsium rivulare* (Jacq) All. roots

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**Abstract**

The mixture of three phytosterols (campesterol, stigmasterol and β-sitosterol), β-sitosterol 3-O-glucoside and syringin were isolated from hexane and methanol extract of *Cirsium rivulare* roots after chromatographic separation. The main component of the source was syringin which was obtained with the yield 0.08% of the dry source. In hexane extract, the qualitative and quantitative composition of fatty acids was determined. The predominant component was linoleic acid (23.31 mg/g of extract). The extracts showed antioxidant activity. The ability to scavenge DPPH• free radical was in correlation with appointed total phenol content. The not-defatted methanolic extract was the most active. Hexane and defatted methanol extracts showed moderate antibacterial activity against G(+) and G(−) strains with MIC and MBC ranged from 25 to 200 μg/mL.

**1. Introduction**

*Cirsium rivulare* (Jacq) All. brook thistle is one of five common species in Polish flora. The above-ground parts of the plant are used in folk medicine as anxiolytic remedy, and this activity was confirmed in contemporary examination (Walesiuk et al. 2010). The extracts obtained from flowers and leaves showed antioxidant and antimicrobial properties (Nazaruk 2008; Nazaruk et al. 2008). The main group of components isolated from inflorescences...
are flavonoids (Nazaruk & Jakoniuk 2005). Roots are poorly examined, until now only the constituents of essential oil have been recognised. In volatile fraction, aplotaxene and its derivatives were predominant. The essential oil showed moderate cytotoxic activity against some cancer cell lines (Nazaruk et al. 2012). One of the components of essential oil influenced collagen biosynthesis (Karna et al. 2015). The further investigation of chemical composition and activity of the roots of *C. rivulare* is the subject of this article.

### 2. Results and discussion

#### 2.1. Identification and quantification of compounds, and DPPH• scavenging ability

The hexane and methanolic extracts of *C. rivulare* roots were obtained with the yield 0.77 and 13.86%, respectively. The first extract was analysed with GC-MS method according to the lipophilic components. The composition and content of fatty acids were determined after alkaline hydrolysis. The results of this analysis were presented in Table 1 (supplementary material). The main compound was linoleic acid (23.31 mg/g of extract), and total content was 51.43 mg/g, whereas total content of phytosterols equalled 7.85 mg/g of the dry extract.

The hexane and methanol extracts were subjected to chromatographic separations. GC-MS analysis of obtained residues R1 (from hexane extract) and R2 (from methanol extract) showed that these are the mixtures of phytosterols—campesterol, stigmasterol and β-sitosterol. The content of the components of these residues was 15.04, 41.34, 43.62% in R1 and 16.6, 35, 48.4% in R2, respectively. Two pure compounds were isolated from methanolic extract. Compound 1 was recognised as β-sitosterol 3-O-β-glucoside according to 1H, 13C NMR analysis and comparison with literature data (Faizi et al. 2001). Proton and carbon NMR analysis of compound 2 showed that this is syringin. The data were in accordance with literature (Syrchina et al. 1993). All compounds were identified in the roots of *C. rivulare* for the first time.

Total phenol content in methanolic extract prepared directly from the source and from the source after defatting with hexane equalled 108 and 81 mg/g of dry extract, respectively. In hexane extract, a small amount (30 mg/g) of phenolic compounds was detected. In correlation with these results, there was antioxidant activity of the extracts. The not-defatted methanolic extract which has the highest concentration of phenol compounds also showed the highest ability to scavenge DPPH• free radical. Correlation coefficient equalled 0.995 (Microsoft Office Excel Software). This activity was comparable with positive control caffeic acid used in concentration of 1 mg/mL. The results were presented in Table 2 (in supplement).

Syringin seems to be the main constituent of the roots of *C. rivulare*. Its amount obtained by us equalled 588 mg (0.08% of the dry source). This compound often occurs in the Asteraceae family and has chemotaxonomic importance (Cis et al. 2003). Previously, it was also isolated from some plants of the genus *Cirsium* such as *Cirsium setosum* (Syrchina et al. 2000) and *Cirsium setidens* (Lee et al. 2009). The content of syringin in leaves of the first plant was higher than in inflorescences, 0.18 and about 0.001%, respectively (Syrchina et al. 1998). The roots of *C. setidens* contained 0.305 μg/mg (0.003%) of this compound (Lee et al. 2009). Syringin exhibits multidirectional biological action. It showed, among others, hepatoprotective (Park et al. 1999), anti-allergic, (Cho et al. 2001) and antidiabetic activity (Krishnan et al. 2014).
2.2. Antibacterial activity

The antibacterial activity of hexane extract and defatted methanolic extract was tested in vitro using microdilution method. The experiment was carried out using nine different reference bacterial strains. The results were combined in Table 3 (supplementary material). Both examined extracts showed moderate antibacterial activity with MIC and MBC ranged from 25 to 200 μg/mL. They are slightly more active against G(−) bacteria. It is important, that almost in all cases, MIC and MBC values are similar. In general, methanolic extract was more active than hexane extract. The most sensitive bacteria were *Escherichia coli*.

Natural extracts and compounds are promising antibacterial agents in the age of increasing antibiotic resistance. Our examination is the first step in searching bioactive constituents of *C. rivulare* root. Further experiments are needed to recognise how these compounds interact with pathogens. Perhaps the mechanism of action is similar to this of synthetic monobactams, which are also more active against multiply resistant G(−) bacteria. They have high affinity to penicillin-binding protein 3 (PBP3) (Sykes et al. 1982). Another possible way is the inhibition of antibiotic efflux pump (Mahamoud et al. 2007).

3. Conclusion

This study is the first mention of phytosterols, fatty acids and syringin in *C. rivulare* roots. Methanolic extract is the good source of this last compound which potentially can influence on the activity of the extract and the whole source. Brook thistle roots showed antioxidant and moderate antibacterial properties. Further investigations are needed to recognise mechanisms of action and curative possibilities of compounds from this morphological part of *C. rivulare*.

Disclosure statement

No potential conflict of interest was reported by the authors.

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