SHORT COMMUNICATION

Multidirectional characterisation of chemical composition and health-promoting potential of *Rosa rugosa* hips

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**ABSTRACT**

Rugosa rose provides one of the largest hips frequently used in the preparation of pharmaceutical and food products. The aim of work was to conduct multidirectional study of biological activity and chemical composition of *Rosa rugosa* hips. Antiradical, cytotoxic (against cervical and breast cancer cell lines), antibacterial (against eight bacterial strains) and antifungal potential of the species in question was evaluated. Total contents of phenolics, phenolic acids, flavonoids, tannins, carotenoids and ascorbic acid were determined. LC–ESI–MS/MS analysis was performed in order to investigate closely phenolic acids and flavonoid glycosides. As a result, interesting selective cytotoxic effects on cervical (HeLa) and breast cancer (T47D) cell lines, significant antiradical activity (EC\(_{50}\) 2.45 mg mg\(^{-1}\) DPPH\(^•\)) and moderate antimicrobial potential (MIC 0.625–1.25 mg mL\(^{-1}\)) were observed. Nine phenolic acids and 11 flavonoid glycosides were qualitatively and quantitatively determined, including 7 compounds previously not reported in *R. rugosa* hips.

**1. Introduction**

Hips (pseudofruits) of different rose species are widely used for food purposes. They are also known and valued medicinal raw material. Amongst different species, rugosa rose (*Rosa*...
rugosa Thunb.) provides one of the largest and heaviest hips, making the aforementioned species one of the best sources of the plant material. R. rugosa has been cultivated on an industrial scale in many countries all over the world. However, there are only few studies dealing with hips chemical composition and biological activity. Therefore, the aim of our study is multidirectional analysis of biological potential and chemical composition of R. rugosa hips. Special attention is paid to their antiproliferative, antimicrobial and antiradical activity, as previous studies indicated that these effects are presented by different parts of roses (Olech et al. 2012, 2015; Ma et al. 2013; Yang et al. 2013; He et al. 2014). Moreover, the content of major active ingredients (phenolics, carotenoids and ascorbic acid) and detailed analysis of phenolics is scheduled.

2. Results and discussion

2.1. Content of the major groups of active ingredients in plant material

Total phenolic, flavonoid, phenolic acid, tannin and carotenoid contents were determined using spectrophotometric assays. Studies revealed considerable amount of phenolic constituents in dry plant material (48.68 ± 2.45 mg of gallic acid g⁻¹), with large portions of tannins and phenolic acids (19.44 ± 1.12 and 8.25 ± 0.47 mg g⁻¹, respectively). Slightly lower amount (3.11 ± 0.11 mg g⁻¹ of dry plant material) of flavonoids was also found. The carotenoid content was shown to be very high (1.40 ± 0.11 mg g⁻¹ of dry plant material). Traditionally, rose hips are particularly valued for high natural vitamin C content. Growing wild varieties contain 0.5–1.7% of ascorbic acid in dry weight. In our study, ascorbic acid was shown to be present in an amount of 10.45 mg g⁻¹ of raw material, which constitutes 1.045% of the dry matter. This result is comparable with the amount reported by Bozan et al. (1998) in Rosa canina. However, it should be noted that both the results were obtained using different methods.

Although the presence of aforementioned groups of biologically active compounds was previously reported, the comprehensive analysis of their total contents has been quantitatively evaluated for the first time in the present study.

2.2. LC–ESI–MS/MS analysis of phenolic acids and flavonoid glycosides

LC–ESI–MS/MS analysis of phenolic acids and flavonoid glycosides was performed due to the confirmation of the presence of polyphenolic compounds through spectrophotometric assays. Analysis was conducted on the basic methanolic extract (M) and its three fractions of different polarity which were subsequently investigated for biological activity (FE – diethyl ether fraction, OE – ethyl acetate fraction and W – water fraction; please refer to the Supplementary material for details). Nine phenolic acids and 11 flavonoid glycosides were qualitatively and quantitatively determined (Tables S1 and S2).

In the basic methanolic extract (M), five phenolic acids were determined, including larger amount of gallic acid (0.13 μg mg⁻¹ of dry extract) followed by smaller amount of p-coumaric and protocatechuic acid and trace amount of caffeic and salicylic acid.

Analysis of the fractions obtained by separation of the basic extract provided additional information about the raw material. The largest amount of phenolic acids passed to the ether fraction (FE), in which four other phenolic acids were determined i.e. gentisic, sinapic,
3-hydroxybenzoic and 4-hydroxybenzoic. In FE, a particularly high level of gallic acid (8.30 μg mg⁻¹ of dry extract) and large amount of p-coumaric, p-hydroxybenzoic and protocatechuic acid were observed. Relatively lower quantities of phenolic acids passed to the OE fraction, where gallic acid was also the dominant one (4.01 μg mg⁻¹). The aqueous fraction (W), in turn, contained only small amount of gallic acid.

Our results confirmed previous findings where the presence of some phenolic acids in the plant material was reported (Nowak 2006). Additionally, the present study is the first one to report sinapic and 3-hydroxybenzoic acid in *Rosa rugosa* pseudofruits.

LC–ESI–MS/MS analysis of flavonoid glycosides revealed the presence of quercetin, kaempferol and apigenin derivatives in the examined extracts (Table S2). In M, one kaempferol glycoside (tiliroside) and seven quercetin derivatives (mostly 3-O-glycosides) were determined. Avicularin and isoquercitrin were found in the highest amount (0.18 and 0.17 μg mg⁻¹ of dry extract), followed by slightly lower amount of hyperoside and tiliroside. The amount of the remaining glycosides in M was lower than 0.10 μg mg⁻¹. OE was the richest in flavonoids amongst the fractions obtained. The sum of flavonoids in OE was more than 20 times higher than in the initial extract (18.56 μg mg⁻¹). The quantities of avicularin, isoquercitrin and hyperoside were particularly high. Additionally, the highest concentrations of rutin, quercitrin and quercetin-3-O-glucopyranosyl-6″-acetate were estimated in ethyl acetate fraction. Quite a large amount (11.51 μg mg⁻¹) of flavonoids passed also to the FE fraction, including the entire amount of tiliroside and the majority of spireoside. Contents of tiliroside and avicularin were predominant there (4.36 and 3.08 μg mg⁻¹, respectively), followed by relatively large amounts of isoquercitrin and hyperoside. The concentrations of other flavonoids were significantly lower. After successive partitioning, only small quantities of flavonoid glycosides remained in the water fraction (W).

The presence of isoquercitrin, quercitrin, hyperoside, astragalin and rutin was previously reported in the plant material (Hvattum 2002; Nowak 2007; Kawakami et al. 2009) whereas avicularin, tiliroside, apigenin-7-glucoside, spireoside and quercetin-3-O-glucopyranosyl-6″-acetate were qualitatively and quantitatively determined for the first time.

### 2.3. Antiradical activity analysis

Earlier reports and our findings suggested that rose pseudofruits could be of potential antiradical value (Olech et al. 2012). Therefore, *in vitro* free radical potential of *R. rugosa* hips methanolic extract against DPPH• was determined (Table S3). As a result, a significant antiradical activity of M was observed (EC₅₀ = 2.45 mg mg⁻¹ DPPH•).

### 2.4. Cytotoxic activity

Cytotoxic activity of methanolic extract against cervical (HeLa), breast (T47D) cancer cell lines and normal human skin fibroblasts was evaluated. The conditions of analysis are given in Supplementary material. A dose–dependent cytotoxic effect was revealed (Table S4). After 48 h treatment with M added at the lowest concentration, no growth inhibition was observed. At a concentration of 75 μg mL⁻¹, significant growth inhibition on the T47D line (20% of dead cells) occurred; no influence on HeLa and fibroblasts was observed. After exposure to the highest M concentration, a reduced number of viable cells on both cancer lines (20 and 40% of dead cells for HeLa and T47D, respectively) without any toxic effect on skin fibroblasts
was revealed. It can be concluded that the methanolic extract obtained from *R. rugosa* pseudofruit was shown to possess considerable and selective cytotoxic effects. Its lack of harmful effects on normal human skin fibroblasts is particularly valuable since hips are a common component of natural remedies and food products.

### 2.5. Antimicrobial activity

In the present study, methanolic extract from hips and its two fractions (OE, W) were tested against eight reference bacterial strains, including Gram-positive and Gram-negative bacteria. Our findings revealed that the examined samples were active against all the micro-organisms used (Table S5). However, their activity was moderate (MIC 0.625–2.5 mg mL\(^{-1}\)) or slight (MIC >2.5 mg mL\(^{-1}\)). Methanolic extract was also investigated for its antifungal activity against two *Candida* species. This preliminary study showed moderate growth inhibition (MICs 1.25 mg mL\(^{-1}\)). This is the first report on the *R. rugosa* hip antifungal and antibacterial properties.

### 3. Conclusion

The chemical composition and biological potential of rose hips obtained from commercially available and used species were reported for the first time. Besides being a great source of vitamin C, *R. rugosa* hips were shown to provide significant quantities of different groups of bioactive phenolics and carotenoids. We found that hips demonstrate significant antioxidant effect. Moreover, considerable and selective cytotoxic activity and moderate antimicrobial potential was reported. In this respect, our paper expands the knowledge about pro-health properties of rose hips and suggests other potential applications of the plant material. It indicates that not only aqueous or lipophilic extracts, but also the fraction of higher polarity can be interesting and valuable e.g. for the production of dietary supplements.

### Supplementary material

Includes experimental section and Tables S1–S5.

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### Disclosure statement

No potential conflict of interest was reported by the authors.

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