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Evaluation of rose roots, a post-harvest plantation residue as a source of phytochemicals with radical scavenging, cytotoxic, and antimicrobial activity

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ABSTRACT

Roots of rugosa rose are often treated as by-products obtained in industrial amounts during renovation of cultivations. In this work, we attempted to examine their chemical composition and biological activity, to evaluate whether they can provide an interesting material for further use. As a result, the first such a detailed report on *Rosa rugosa* roots is presented. Total phenolic, flavonoid, phenolic acid, tannin, and carotenoid contents were determined using spectrophotometric methods. Additionally, LC–ESI–MS/MS was used for identification and quantification of phenolic acids and flavonoid glycosides in the methanolic extract and its fractions. Moreover, the antiradical, cytotoxic, and antimicrobial potential of *R. rugosa* root was evaluated. A significant decrease (up to 90%) in the number of viable cells in cervical (HeLa) and breast cancer (T47D) cell lines was demonstrated. Extremely high antiradical potential of extracts (comparable with the activity of Trolox and ascorbic acid) was revealed (EC_{50} 0.28–0.85 mg/mg DPPH). Furthermore, moderate antimicrobial activity against eight bacterial (i.e., *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus luteus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Proteus mirabilis*) and two yeast strains (i.e., *Candida albicans* and *Candida parapsilosis*) was showed. According to our results, rugosa rose roots constitute a potential source of phytochemicals of multidirectional biological potential, which are easily available in industrial amounts.

1. Introduction

Different rose species are widely cultivated for obtaining products for flavorings, fragrance, as well as food and pharmaceutical industries. For these purposes, petals and hips are extensively used. Huge plantations of these plants can be found in Bulgaria, Turkey, Cyprus, Saudi Arabia, India, and China (Baydar and Baydar, 2013). A growing interest in such cultivations has also been observed in Poland, especially in the case of *Rosa rugosa* Thunb. (rugosa rose, Japanese rose), since it produces fragrant petals and large hips used for culinary and medicinal purposes. Moreover, *R. rugosa* is easy to grow and disease resistant.

Rugosa rose plantations need to be renewed periodically. Elimination of old cultivations results in obtaining significant amounts of post-harvest plantation residues, such as stems and roots. In the majority of cases, this biomass is not further used. Meanwhile, *R. rugosa* roots have been used in Asian folk medicine, e.g., for the treatment of diabetes mellitus, pain, and chronic inflammatory diseases. Moreover, *R. rugosa* roots have been reported to possess anti-hepatotoxic and anti-HIV protease activity (Park et al., 2004, 2005; Thao et al., 2014). According to some literature data, extracts, and compounds isolated from rose roots exert antinociceptive and/or anti-inflammatory effects (Jung et al., 2005; Kim et al., 2012). In spite of these reports, the knowledge of roots outside Asia is marginal. This plant material still seems to be underestimated and underused, particularly in Europe.

Numerous studies have demonstrated the therapeutic value and interesting chemical composition of the above ground parts of *R.*

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rugosa (Jeon et al., 2009; Nagai et al., 2007; Nowak et al., 2013; Yoshizawa et al., 2000). However, to date there are no comprehensive studies investigating the composition as well as antiradical, antimicrobial, and cytotoxic activities of the underground organs of this species.

Therefore, the goal of the present study was to examine the chemical composition and activity of *R. rugosa* roots in detail. We have attempted to determine the total phenolic, flavonoid, phenolic acid, and tannin contents. Moreover, identification and quantification of phenolic acids and flavonoid glycosides in the root extract have been scheduled. Several tests to evaluate antioxidant activity, antimicrobial, and antiproliferative properties of root extracts have been performed. We have also attempted to identify the group of compounds responsible for the biological activity of the plant material.

2. Materials and methods

2.1. Plant material

Roots of *R. rugosa* were collected from the 8-year-old plantation in Lublin (Poland) in October, 2011. The raw material was spread in thin layers and dried at 25 °C using an own construction laboratory air-flow dryer (Krzykowski et al., 2011) with an air flow rate of 1.0 m s^{-1}. Dried plant material was powdered according to the European Pharmacopoeia (2006).

2.2. Chemicals

Standards of gallic, protocatechuic, gentisic, salicylic, caffeic, *p*-coumaric, 3-hydroxybenzoic, 4-hydroxybenzoic, pyrogallol, ascorbic acid, Trolox, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•), hide powder, and quercetin were purchased from Sigma–Aldrich Fine Chemicals (St. Louis, MO, USA). Flavonoids: quercetin-3-L-arabinofuranoside (avicularin), quercetin-3-*O*-rutinoside (rutin), quercetin-3-*O*-galactoside (hyperoside), quercetin-3-*O*-glucoside (isoquercitrin), kaempferol-3-*O*-(6″-*O*-(*E*)-*p*-coumaroyl)-glucoside (tiliroside), kaempferol-3-*O*-glucoside (astragalin), and quercetin-3-*O*-rhamnoside (quercitrin) were obtained from ChromaDex (Irvine, USA). Ethanol, methanol, hexane, diethyl ether, ethyl acetate, sodium molybdate, sodium nitrite, dimethyl sulfoxide (DMSO), and Folin–Ciocalteu reagent were from POCh (Gliwice, Poland), β-carotene was from Fluka (Buchs, Switzerland).

All the chemicals were of analytical grade. LC grade methanol (MeOH), acetonitrile (ACN) were purchased from Merck (Darmstadt, Germany). LC grade water was prepared using a Millipore Milli-Q purification system (Millipore, Bedford, MA, USA).

2.3. Analysis of the major groups of active ingredients in plant material

Content of the major groups of secondary metabolites was evaluated spectrophotometrically in Thermo Scientific Evolution 300 (Lafayette, CO, USA).

2.3.1. Total phenolic, phenolic acid, flavonoid, and tannin content

The total phenolic content in plant material was determined according to method described by Singleton and Rossi Jr. (1965), with some previously described modifications (Olech et al., 2012). The results were expressed as milligram of gallic acid per gram of dry plant material.

In order to evaluate the content of phenolic acid, the method described by Nichiforesco and Coucou (1965) was used. The phenolic acid content was calculated using a reference curve plotted for caffeic acid and expressed in milligram of caffeic acid per gram of dry plant material.

Total flavonoid content in plant material was determined colorimetrically according to the method described by Lamaison and Carret (1990). Result was expressed in milligram of quercetin per gram of dry plant material.

Determination of tannin content in *R. rugosa* roots was performed according to the method described in the Polish Pharmacopoeia (2005) with some modifications (Olech et al., 2012). Tannins were estimated indirectly after adsorption on and precipitation with insoluble hide powder. Result was expressed in milligram of pyrogallol per gram of dry plant material.

2.3.2. Total carotenoid content

The carotenoid content in *R. rugosa* roots was determined spectrophotometrically by means of the modified method described in Current Protocols in Food Analytical Chemistry (Olech et al., 2012; Scott, 2000) and expressed as milligram of β-carotene (reference standard) per gram of dry plant material.

2.4. LC–ESI–MS/MS analysis of phenolic composition of R. rugosa roots extracts

2.4.1. Preparation of samples

Twenty-five grams of dried and pulverized roots were extracted four times (for 24 h each), at 25 °C with 150 mL of 80% (v/v) aqueous methanol in SBS40 water bath (Stuart, Staffordshire, UK). During the last extraction, a sonication step at 50 °C (30 min) was applied in order to achieve exhaustive extraction. Extracts were combined, filtered, and evaporated to dryness under vacuum. The residue was weighted and redissolved in 80% (v/v) methanol at a concentration of 100 mg/mL (M). Part of this solution (20 mL) was lyophilized in a Free Zone 1 apparatus (Labconco, Kansas City, KS, USA) and stored at 4 °C in the refrigerator prior to further analysis.

The remaining amount of methanolic extract (M) was fractionated into diethyl ether, ethyl acetate, and water fractions. Briefly, 80 mL of the M solution was evaporated to dryness under vacuum. Syrupy residue was dissolved in hot water, left to cool (12 h, 4 °C), filtered, and filled up with distilled water to the volume of 100 mL. Subsequently elution with solvents with increasing polarity was carried out. Initially, the aqueous fraction was extracted with diethyl ether (50 mL) (six times). Collected ether extracts were combined, the solvent was evaporated and the residue was lyophilized to give the ether fraction (FE).

Aqueous solution after extraction with diethyl ether was further washed six times with ethyl acetate (50 mL). Eluates were combined, concentrated in a vacuum evaporator and the residue was lyophilized to give the acetate fraction (OE).

The aqueous solution remaining after the extractions was also evaporated, freeze-dried and used for further analysis as the water fraction (W).

Prior to LC–MS analysis, methanolic extract and its fractions were cleansed of ballast substances using solid phase extraction on SPE octadecyl columns (500 mg, J.T. Baker Inc., Philipsburg, USA). All extracts were prepared in duplicate.

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

Phenolic acids and flavonoid glycosides contents in methanolic extract (M), diethyl ether (FE), ethyl acetate (OE), and water (W) fraction were determined by reversed-phase ultra-high-pressure liquid chromatography–mass spectrometry, performed on an ACQUITY UPLC System (Waters Corporation, Milford, MA, USA) according to the previously described methods (Nowak et al., 2014). For the specific detection of analytes a Waters DAD and TQ detector in negative electrospray ionization mode with Metastable