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Phenolic compounds, antiradical activity and antioxidant capacity of oil-bearing rose (*Rosa damascena* Mill.) extracts

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ABSTRACT

The main target in the study was to determine total phenolic content by the Folin–Ciocalteu method, total flavanols by the DMAC method, total flavonols with Neu's reagent solution, antiradical activity by the DPPH method, antioxidant capacity by ferric reducing antioxidant power (FRAP) method and phenolic compounds by HPLC system were analyzed and measured in the extracts from the fresh and spent flowers, and green leaves of oil-bearing-rose (*Rosa damascena* Mill.). After drying and powdering of the samples, hot and cold extractions with methanol were made. While hot extractions gave more extract yields, cold extractions gave more total phenolic, flavanol and flavonol contents. The highest values of total phenolics were obtained from the hot and cold methanolic extractions of leaf with 478.34 and 530.40 mg GAE/g, respectively. The relative concentrations of the various phenolic compounds were not noteworthy influenced by the extraction methods. Gallic acid in the fresh and spent flower extracts, and (+)-catechin and (−)-epicatechin in the leaf extract were the most abundant phenolic compounds. Leaf extracts exhibited more antiradical activity at concentration of 50 μg/ml even better than from synthetic antioxidants as Trolox, BHA and BHT. The leaf cold extractions had also the strongest antioxidant properties when measured with the absorbance was measured at 700 nm as 1.43 and 1.72 at the concentrations of 100 and 150 μg/ml, respectively. The strongest antioxidant properties measured with the FRAP assay had leaf cold extractions as 1.43 and 1.72 μg/ml at the concentrations of 100 and 150 μg/ml, respectively. As a conclusion, these results indicate that oil-bearing rose by-products, a large scale wastes, can be evaluated for natural antioxidant sources.

1. Introduction

Rosa damascena Mill. (Damask rose, oil-bearing rose, pink rose) is the most important rose species producing high-value essential oil (Lawrence, 1991). Bearing in mind that Turkey is one of the most important country growing industrial *R. damascena* in the world together with Bulgaria. From 6000 to 8000 tonnes of rose flowers are produced annually for essential oil, rose water, concrete and absolute production in Isparta province at the Southwest region of Turkey. The main flowering season in the region is a period with 40 days of May and June months. The flowers collected freshly in the early hours of the days in this period are hydro-distilled for rose oil and rose water production (Anaç, 1984; Başer, 1992), and also extracted with organic solvents for concrete and absolute production (Kürkçüoğlu and Başer, 2003; Aycı et al., 2005). Annual rose oil, concrete and absolute production of Turkey are 1.2–1.8, 6–9, and 2–2.5 tonnes, respectively with high exporting qualities. These high-price products for flavorings, fragrance and pharmaceutical industries are mainly imported by the USA and EU countries (Baydar, 2006).

Biochemical bioactive constituents with antioxidant activity were found in high concentrations in plants (Balasundram et al., 2006). It is well known that free radicals cause auto-oxidation and phenolic compounds whose antioxidant activity is based on the breaking of the free radical chain by donating a hydrogen atom. For example, one gram of hydro-distilled extract from laurel is as effective as about 212 mg of trolox in the prevention of lipid peroxidation (Hinneburg et al., 2006). The benefits resulting from the use of natural products rich in bioactive substances has promoted the growing interest of pharmaceutical, food and cosmetic industries (Capecka et al., 2005). Recently, interest in plant-derived natural products has grown, mainly because synthetic antioxidants suffer from several drawbacks. Many plants were screened for their antioxidant capacities, and some of them had strong activity (Pocorny, 1991; Chang et al., 1997; Zheng and Wang, 2001; Lee and Shibamoto, 2002; Göktürk Baydar et al., 2004; Capecka et al., 2005; Ivanova

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et al., 2005; Katalanic et al., 2006) as related to phenolics and flavanoids such as rosmarinic acid, carnosik acid, quercetin, luteolin, and rutin (Pizzale et al., 2002; Tunalıer et al., 2004; Vinokur et al., 2006; Baydar et al., 2009). Phenolic compounds, the major determinant of antioxidant potentials, are natural sources of antioxidants (Balasundram et al., 2006).

There have been some researches focused on antiradical and antioxidant activities of roses compared to the other plants. Vinokur et al. (2006) analyzed hot water infusions (teas) of air-dried petals of twelve rose cultivars for antioxidant activity, total phenolics, and total anthocyanins contents, and reported that rose-petal tea may serve as caffeine-free beverage with high antioxidant capacity, and may be consumed either separately or in combination with other herbal materials. They expressed that oil-bearing rose *R. damascena* exhibited the highest antioxidant activities (higher than green tea). Ng et al. (2004) linked the antioxidant activity in an aqueous extract of rose flowers (*R. rugosa*), primarily with the presence of a phenolic compound identified as a gallic acid derivate. Phenolic compounds were associated with radical scavenging activity in flower extracts of *R. rugosa* and *R. davurica* (Cho et al., 2003). Antioxidants of a polysaccharide structure were also found by Vanderjagt et al. (2002), but exhibited lower activity. Essential oils were also responsible for antioxidant activity of oil-bearing rose flowers and their extracts (Lee and Shibamoto, 2002; Achuthan et al., 2003; Yassa et al., 2009; Ulusoy et al., 2009). Wei and Shibamoto (2007) detected that rose oil contained a high percentage of citronellol (34.2%) had a high antioxidant capacity over 50% even at a low concentration of 50 μg/ml according to DPPH method. Ulusoy et al. (2009) suggested that rose absolute with high tocopherol and carotene levels could be used a potent natural antioxidant for commercial exploration. The other utilization of the rose oil and absolute was evaluated as antibacterial agent (Arıdogan et al., 2002; Basım and Basım, 2003).

The antioxidant activity of the rose oil was previously studied, and high antioxidant action was observed on the basis of its antiradical activity and inhibition of the lipid oxidation cycle (Wei and Shibamoto, 2007; Yassa et al., 2009). Although its high antioxidant activity, rose oil is one of the most expensive essential oils distilled from the aromatic plants. Due to the high level interest in usage of natural and healthy products of oil-bearing rose, it is important to research the alternative utilization from other parts and by-products oil-bearing rose for more effective and economical antioxidant source. In this respect, we aimed in this study to analyze the total phenolic, flavanol and flavonol contents, phenolic compounds, antiradical activity and antioxidant capacity of the cold and hot methanolic extracts obtained from the fresh flowers, spent flowers and green leaves of oil-bearing-rose.

2. Materials and methods

2.1. Materials

In this study, dried and powdered fresh and spent flowers, and leaves of *R. damascena* Mill. were used as plant materials. The flowers with pink color and leaves with green color were collected freshly in the early hours of the days from the rose garden of Rose and Rose Products Research and Implementation Centre (GÜLAR) at Süleyman Demirel University, Isparta, Turkey in May 2009. Spent flowers (distillation residues) were obtained as a by-product after water distillation of fresh rose flowers by using a Clevenger type hydro-distillation apparatus in the laboratory. Fresh flowers, spent flowers and green leaves were dried in the shade at room temperature until constant weights. The air-dried samples were powdered in a grinder (<1 mm), and then stored in sealed plastic bags in a desiccator at 25 °C until use.

2.2. Methods

2.2.1. Phenolic extraction

Phenolic extraction in the samples made of two different ways, including hot and cold extractions. For hot extraction, 10 g of powdered samples was extracted in a Soxhlet extractor for 4 h with 200 ml of methanol at 60 °C. In cold extraction, 10 g of powdered materials was extracted with 100 ml methanol for 1 min using an Ultra Turrax mixer (24,000 rpm) and soaked overnight at room temperature as the combination of the methods used by Pizzale et al. (2002) and Lu and Foo (2001). The hot and cold extracts were then filtered through Whatman No. 1 paper in a Buchner funnel. After filtration, the extracts were concentrated by rotary evaporation under vacuum at 40 °C to get crude extracts. Then these extracts were used the analyses.

2.2.2. Determination of total phenolic content

Spectrophotometric measurements were performed by a PG Instruments T70 Plus Dual Beam Spectrophotometer (Arlington, MA, USA). Total phenolic contents of the extracts were determined by the Folin–Ciocalteu colorimetric method (Singleton and Rossi, 1965). The estimation of phenolic compounds in the extracts was carried out in five replications and calculated by a calibration curve obtained with gallic acid. Total phenolics were expressed as gallic acid equivalents (mg GAE/g dw).

2.2.3. Determination of total flavanol content

Total flavanols were assayed colorimetrically by the DMAC method (Arnous et al., 2001). The absorbance of the extracts was measured at 640 nm and the contents of total flavanols in the extracts were expressed as catechin equivalents (mg CE/g dw). The data presented are the average of five measurements given as mean ± standard deviation.

2.2.4. Determination of total flavonol content

Total flavonols were determined with Neu's reagent solution by the method of Dai et al. (1995). The absorbance of the extracts was measured at 410 nm and the flavonols were expressed as mg rutin equivalent (mg RE/g dw). The data presented are the average of five measurements given as mean ± standard deviation.

2.2.5. HPLC determination of phenolic compounds

HPLC (high performance liquid chromatography) separation of phenolics was performed by the modified method of Caponio et al. (1999). The HPLC system (Shimadzu Corp., Kyoto, Japan) was equipped with a pump (LC 10AD), auto-sampler (SIL 10 AD), column oven (CTO 10A) and diode-array UV/VIS detector (DAD-λ_{max} = 278). The separation was executed on a Agilent Eclipse XB C-18 column (5 μm, 4.6 mm × 250 mm, Wallborn, Germany). The flow rate was 0.8 ml/min, the injection volume was 20 μl, and the column temperature was set at 30 °C. For gradient elution, as a mobile phase, solvent A contained 3% acetic acid in water and solvent B contained methanol (99%) (analytical grade). The following gradient programme was used: 0–3 min, from 100% A to 95% A, 5% B; 3–20 min, from 95% A, 5% B to 80% A, 20% B; 20–30 min, from 80% A, 20% B to 75% A, 25% B; 30–40 min, from 75% A, 25% B to 70% A, 30% B; 40–50 min 70% A, 30% B to 60% A, 40% B; 50–55 min, 60% A, 40% B to 50% A, 50% B; 55–65 min, 50% A, 50% B to 100% B. Samples, standard solutions and mobile phases were filtered by a 0.45 μm pore size membrane filter (Millipore Co. Bedford, MA). The data were integrated and analyzed using the Shimadzu Class-VP Chromatography Laboratory Automated Software system. The amounts of phenolic compounds in the extracts were calculated as mg/g dw, using external calibration curves obtained for each phenolic standard. Caffeic acid, (+)-catechin, chlorogenic acid, *p*-coumaric acid, (−)-epicatechin, ferulic acid, gallic acid, syringic acid and quercetin