

PHENOLIC PROFILE, ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF *PELARGONIUM GRAVEOLENS* LEAVES' EXTRACTS

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Abstract

Pelargonium graveolens, commonly known as rose geranium, is an aromatic and medicinal plant belonging to the Geraniaceae family. The aim of the present study was to evaluate the *in vitro* antioxidant and antibacterial activity of *Pelargonium graveolens*, as well as to determine the total phenolic content of the studied extracts. Four spectrophotometric assays were used for the radical scavenging ability analysis, namely ABTS, DPPH, CUPRAC, and FRAP. The inhibitory activity of the leaves' extracts was tested against *S. aureus*, *L. monocytogenes*, *E. coli* and *Salmonella*.

The total phenolics ranged from 1.65 to 8.23 mg GAE/g FW. Antibacterial activity, whose zone of inhibition varied from 15 mm to 19 mm depending on the extract quantity, was shown only against *L. monocytogenes*.

Key words: *Pelargonium graveolens*, antioxidants, antimicrobial activity, phenolics.

INTRODUCTION

Antioxidants are compounds that can delay or prevent the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions (Velioglu et al., 1998). There are two basic categories of antioxidants, namely, synthetic and natural. Natural antioxidants are more readily acceptable than synthetic ones. They inhibit the oxidative damage of food products and may prevent inflammatory conditions and neurodegenerative disease (Mahdiah et al., 2013). The antioxidant activity is a fundamental property important for life. Many of the biological functions, such as anti-mutagenicity, anticarcinogenicity, and anti-aging, among others, originate from this property (Cook and Samman, 1996; Huang et al., 1992).

Plants are potential sources of natural antioxidants, and certain species are particularly significant because they may be used for the production of raw materials or preparations containing phytochemicals with significant antioxidant capacities and health benefits (Exarchou et al., 2002). The

antioxidative effect is mainly due to phenolic compounds, such as flavonoids, phenolic acids, tannins, and phenolic diterpenes (Shahidi et al., 1992; Chung et al., 1998; Pietta, 2000). Flavonoids and other polyphenolic compounds are a much investigated group of antioxidants in plants exerting bio-protective effects and having strongly positive influence on human health. Many studies have reported that phenolic compounds in spices and herbs significantly contributed to their antioxidant and pharmaceutical properties (Cai et al., 2004; Shan et al., 2005; Wu et al., 2006). Their effect in reducing many chronic, cardiovascular and carcinogenic diseases is remarkable (Slezák et al., 2007). Some studies claim that the phenolic compounds present in spices and herbs might also play a major role in their antimicrobial effects (Hara-Kudo et al., 2004).

The demand of producing high-quality, safe (pathogen-free) food relies increasingly on natural sources of antimicrobials to inhibit food-spoilage organisms and food-borne pathogens and toxins. The discovery and development of new antimicrobials from natural sources for a wide range of applications requires that knowledge of traditional sources

for food antimicrobials is combined with the latest technologies in identification, characterization and application.

Some plants have been added to food since ancient times, not only as flavoring agents, but also as folk medicine and food preservatives (Beuchat, 1994; Nakatani, 1994; Cutler, 1995). Spices and herbs are well known for their antimicrobial and antioxidant properties and have the ability to produce multidimensional flavors in food (Uhl, 2000).

Rose geranium is a species which belongs to the *Pelargonium* genus, *Geraniaceae* family. It has a woody, straight stem with branches; its leaves are usually alternate, palmately lobed or pinnate, often on long stalks, and sometimes with light or dark pattern; covered with short, rough hairs, which give the plant a strong, pleasant rose-like scent (Balchin et al., 1995).

It had been brought in Europe from South Africa in the beginning of the eighteenth century (Miller, 2002). At present days, it grows in different parts of the world and is cultivated, mostly for its repellent activity against mosquitoes (Pohlit et al., 2011). It is also widely used in cosmetic industry and as flavoring for foods (Lis-balchin, 2006). Rose-scented geranium (*P. graveolens* L'Hér.) is also widely known as one of the medicinal herbs with the highest antioxidant activity (Newman et al., 2007). In herbal medicine its leaves are used for the treatment of gastrointestinal diseases, throat infections, and bleeding (Saraswathi et al., 2011).

According to the data on chemical composition the dominant volatiles of the *P. graveolens* essential oil were citronellol, geraniol and citronellyl formate (Verma et al., 2010; Ghannadi et al., 2012).

Through several studies it was shown that extracts of *Pelargonium graveolens* possess antibacterial and antifungal activity (Baratta et al., 1998; Dorman and Deans, 2000). The antimicrobial and antimalarial activity of *P. graveolens* extracts was also studied by Lalli (2006). In addition to that, antioxidant and antitermitic activity of *P. graveolens* has also been reported (Zheng and Wang, 2001; Fayed, 2009; Seo et al., 2009; Čavar and Maksimović, 2012).

The objective of the present work is to provide much needed information concerning the antioxidant and antimicrobial activity of

extracts of Bulgarian *Pelargonium graveolens* which have not yet been extensively studied and evaluated.

MATERIALS AND METHODS

Extract preparation

Fresh plant material of *Pelargonium graveolens* was subjected of three different types of extractions:

- *decoction* – extraction by boiling of the plant material for 30min with distilled water;

- *infusion* - extraction by boiling water and then pouring it over the herb, which is then allowed to steep in the liquid for 30 min

- *heat reflux extraction* - alcoholic extraction (70 % ethanol as solvent) for 30min;

The resulting extracts solutions were filtered before analyzed.

Determination of total phenolics

A modified Kujala et al. (2010) method with Folin - Ciocalteu's reagent was used for the determination of the total polyphenolic content (TPC). Gallic acid was employed as a calibration standard and the results were expressed as mg gallic acid equivalents (mg GAE) per gram of plant fresh weight.

Determination of antioxidant activity

DPPH radical scavenging activity

The ability of the extracts to donate an electron and scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was determined by the slightly modified method of Brand-Williams, Cuvelier, and Berset (1995). Freshly prepared 4×10^{-4} M methanolic solution of DPPH was mixed with the samples and a standard solution in a ratio of 2:0.5 (v/v). The light absorption was measured at 515 nm and the percentage of inhibition of DPPH[•] by the obtained extracts was calculated for each sample using the following formula:

$$\% \text{ Inhibition} = [(A_B - A_E) / A_B] \times 100$$

Where: A_B = absorbance of the control without sample; A_E = absorbance of the test sample with DPPH[•]

The DPPH radical scavenging activity was presented as a function of the concentration of Trolox. The unit of Trolox equivalent antioxidant capacity (TEAC) was defined by the concentration of Trolox having equivalent antioxidant activity expressed as $\mu\text{M TE/g FW}$.

ABTS radical scavenging assay

The radicals scavenging activity of the extracts against radical cation (ABTS^{•+}) was estimated according to Re et al. (1999) with some modifications. ABTS^{•+} was produced by reacting 7 mM of ABTS solution with 2.45 mM potassium persulphate, and the mixture was kept in the dark at room temperature (20 - 22°C) for 12-16 h. At the moment of use, the ABTS solution was diluted with ethanol to an absorbance of 0.7 ± 0.02 at 734 nm and equilibrated at 30°C. Each sample (0.01 ml) was added to 1 ml of ABTS diluted (working) solution and mixed vigorously. After reaction at 30°C for 6 min, the absorbance at 734 nm was measured. The percentage of inhibition of ABTS^{•+} by the obtained extracts was calculated for each sample using the following formula:

$$\% \text{ Inhibition} = [(AB-AE)/AB] \times 100,$$

Where: A_B = absorbance of the control without sample; A_E = absorbance of the test sample with ABTS^{•+}.

The TEAC value was defined as the concentration of Trolox having equivalent antioxidant activity expressed as $\mu\text{M TE}$ per gram fresh weight ($\mu\text{M TE/g FW}$).

CUPRAC assay

The CUPRAC assay was carried out according to the procedure of Apak et al., 2008. To a test tube were added 1 mL of CuCl_2 solution ($1.0 \times 10^{-2}\text{M}$), 1 mL of neocuproine methanolic solution ($7.5 \times 10^{-3}\text{M}$), and 1 mL NH_4Ac buffer solution (pH 7.0), and mixed; 0.1 mL of herbal extract (sample) followed by 1 mL of water were added (total volume = 4.1 mL), and mixed well. Absorbance against a blank reagent was measured at 450 nm after 30 min. Trolox was used as standard and total antioxidant capacity of extracts was expressed as $\mu\text{M TE/g FW}$.

FRAP assay

The FRAP assay was carried out according to the procedure of Benzie & Strain (1996) with slight modification. FRAP assay measures the change in absorbance at 593 nm owing to the formation of a blue coloured Fe (II)-triipyridyltriazine compound from colourless oxidized Fe (III) form by the action of electron donating antioxidants. Briefly, the FRAP reagent was prepared from 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl, and 20 mM iron (III) chloride solution in proportions of 10:1:1 (v/v),

respectively. The FRAP reagent was prepared fresh daily and was warmed to 37°C in a water bath prior to use. One hundred and fifty microliters of plant extracts were allowed to react with 2850 μL of the FRAP reagent solution for 4 min at 37°C. The absorbance of the reaction mixture was recorded at 593 nm. The results were expressed as $\mu\text{M TE/g FW}$.

Antimicrobial analysis

Antibacterial activity was tested against Gram-positive bacteria - *Listeria monocytogenes* NCTC 11994 and *Staphylococcus aureus* ATCC 25093, and Gram-negative bacteria – *Escherichia coli* ATCC 8739 and *Salmonella enterica* subsp. *Enterica* serovar *Abony* NCTC 6017. The selective growth media, used in the analyses respectively were: *Listeria* Oxford Agar Base with cycloheximide supplement /Biolife/; ENDO agar /Merck/; LEIFSON Agar /Merck/; Baird Parker Agar Base with Egg Yolk Tellurite emulsion supplement /Biolife/. The media were inoculated with 24-hour suspension of the corresponding bacterial species.

For the microbial analyses a crude extract of rose geranium leaves was obtained under aseptic surroundings by mashing fresh leaves after preliminary washing under tap water, sterilized distilled water and soaking for 5 min with ethanol and exposing under the influence of ultraviolet illumination for 20 min.

Antimicrobial assay by agar diffusion method

The agar diffusion test was used to determine the antibacterial activity of crude extract of *P. graveolens*. Melted and cooled to the temperature at about 45°C selective media were inoculated with the tested microorganisms and after setting of media, small amount of crude extract, respectively 0.05; 0.10 and 0.15 cm^3 , was placed into sterile metal rings ($\varnothing$ 6 mm). Plates were incubated at 37°C for required incubation periods (24h or 48h) according to the strain type and then the distinct zone of growth inhibition around the rings was measured.

Statistical analysis

All measurements were carried out triplicates. The results were expressed as mean $\pm$ SD and statistically analysed using MS-Excel software.

RESULTS AND DISCUSSIONS

Since there have been numerous studies suggesting a direct correlation between phenolic substances and antioxidant activity, the estimation of the total phenolics is very important. The phenolic content could be an indicator of the antioxidant capacity of the studied extracts. The total content of phenolics was determined using the FC reagent, which is sensitive to many classes of phenolic compounds. The results are given on Fig. 1.

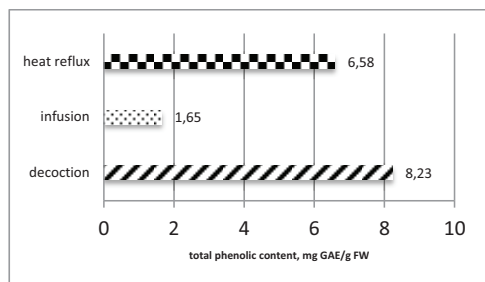


Figure 1. Total phenolic content of extracts of fresh *R. geranium* leaves, mg GAE/g FW

The decoction extract contained 1.2 times more phenolic substances than the heat reflux extract, while the infusion led to the lowest phenolic values. Water was also proven to be more suitable in other herb extracts (Alexieva et al., 2014). Bichra et al. (2013) reported significantly lower TPC values (0.12 mg/g EGA) from an aqueous extract.

Measuring the antioxidant activity of food products such as natural compounds began to present a great interest in recent years. There are several methods to determine the antioxidant capacity of plant extracts. However, the chemical complexity of extracts could lead to scattered results obtained from different techniques, depending on the test employed. Therefore, an approach with multiple assays in the screening work is highly advisable.

Antioxidant activity was measured by four different assays DPPH, ABTS, FRAP and CUPRAC using Trolox equivalents to express the results. The systems DPPH and ABTS are excellent tools for determining the antioxidant activity of hydrogen donating and chain breaking antioxidants (Thaipong et al., 2006). The FRAP and CUPRAC methods are based on the measurement of the ferric and cupric

reducing ability. Both methods are based on electron transfer and are considered to be a good indicator for total antioxidant power because total reducing power is the sum of the reducing powers of individual compounds presented in a sample (Tezcan et al., 2011).

The antioxidant activity of the tested samples is given in Table 1.

Table 1. *In vitro* antioxidant activity ($\mu\text{M TE/g FW}$) of *P. graveolens* extracts

Method/ Plant sample	Decoction	Infusion	Heat reflux
TEAC _{ABTS}	198.91 $\pm$ 10.71	23.15 $\pm$ 0.59	223.76 $\pm$ 1.26
TEAC _{DPPH}	120.26 $\pm$ 1.37	36.81 $\pm$ 0.46	121.26 $\pm$ 1.10
TEAC _{FRAP}	218.16 $\pm$ 0.71	55.77 $\pm$ 0.46	231.64 $\pm$ 3.57
TEAC _{CUPRAC}	122.96 $\pm$ 7.67	166.74 $\pm$ 7.82	176.98 $\pm$ 0.01

The ABTS scavenging capacity ranged from 23.15 (infusion) to 223.76 (heat reflux) $\mu\text{M TE/g FW}$. The highest DPPH values were found to be 121.26 $\mu\text{M TE/g FW}$. Čavar and Maksimović (2012) published the radical scavenging activity of extracts and essential oils of *P. graveolens*. They measured the DPPH antioxidant capacity and reported values of 63.70 mg/ml for the leaves. In another study the antiradical activity of the geraniol oil was found to be ranging from 14.49 mg/ml to 66.45 $\mu\text{g/ml EC}_{50}$ value (Fayed, 2009). Džamić et al. (2014) reported that the oil exhibited antioxidant activity and reduced DPPH to 50% at EC_{50} value of 0.802 mg/ml of oil solution.

Significant FRAP activity was evident in the heat reflux extract of *P. graveolens* leaves. In accordance with the FRAP, ABTS and DPPH assays, the highest values in the CUPRAC assay were also found in the heat reflux extract. Ethanol appeared to be a better extractant as far as antioxidant activity is being measured. This is in disagreement with the TPC values, where water was the most suitable medium, and is probably due to the different mechanism of contribution of each individual component to the total radical scavenging activity of the studied samples.

Many herbs and plants are known to have therapeutic and antimicrobial properties, and their biological activity is currently the subject of renewed interest (Okigbo et al., 2008).

However, only few of them have been characterized for their antibacterial activities (Halcon and Milkus, 2004). The antimicrobial activity of the crude extract of *Pelargonium graveolens* leaves were evaluated against four bacteria species. The results of the antibacterial activity are presented in Table 2.

Table 2. Zones of growth inhibition (mm) of tested pathogenic bacteria by crude extract of *Pelargonium graveolens* leaves

Plant sample / Bacteria	<i>P. graveolens</i>		
	0.05cm3	0.10cm3	0.15cm3
St. aureus	0,6	0,6	0,6
L. monocytogenes	1,5	1,7	1,9
E. coli	0,6	0,6	0,6
S. enterica	0,6	0,6	0,6

The tested extract possessed antibacterial activity against Gram (+) bacteria - *Listeria monocytogenes* NCTC 11994. On the basis of inhibition zone diameters, *Listeria monocytogenes* NCTC 11994 was more sensitive to the extract than the other bacterial species. Recent research on *P. graveolens* oil revealed that it manifests a strong inhibitory effect on Gram-positive bacterial the extract than the other bacterial species. Recent research on *P. graveolens* oil revealed that it manifests a strong inhibitory effect on Gram-positive bacterial strains such as *Staphylococcus aureus* (Silva and Fernandes, 2010).

CONCLUSIONS

The outcomes of the current investigation prompt the necessity for further studies of the *P. graveolens*, focusing on the isolation and structure elucidation of its antioxidant compounds, since they have potential use as therapeutic agents in managing diseases associated with free radicals and also have the potential to be employed as additives in the food industry.

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