

## Antioxidant Activity and Total Polyphenol Content of Royal Jelly from Bulgaria

Ralitsa Balkanska<sup>1\*</sup>, Liviu-Alexandru Marghitas<sup>2</sup> and Crenguta Ioana Pavel<sup>2</sup>

<sup>1</sup>Department of Special Branches – Bees, Institute of Animal Science, Kostinbrod, Spirka  
Pochivka 1, 2232, Bulgaria

<sup>2</sup>Department of Beekeeping and Sericulture, University of Agricultural Sciences and Veterinary  
Medicine Cluj-Napoca, Calea Manastur 3-5, 400372, Romania

\*Corresponding author

### ABSTRACT

#### Keywords

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This study aimed to screen the antioxidant activity of royal jelly (RJ) collected from 2 regions of Bulgaria (Lovech and Veliko Tarnovo) and RJ samples from the market using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method for antiradical activity and the ferric reducing antioxidant power (FRAP) assay. Total phenolic content was determined according to Folin-Ciocalteu procedure. All RJ samples were able to scavenge free radicals in wide ranges (10.17 – 39.39% inhibition). The FRAP and total polyphenols also showed high variability and a correlation between them was found. For the all samples the following ranges were obtained: FRAP 0.44 – 8.49 mM Fe<sup>2+</sup>/g, total polyphenols 11.66 – 36.73 (mg GAE/g).

### Introduction

In the recent years, consumer and market demand are showing increased interest in natural products with health-promoting effects. Among them, the bee products as popular and traditional health foods have generated considerable interest. Royal jelly (RJ) is one of the most attractive bee products. RJ is the principal food of the honeybee queen and it is produced by hypopharyngeal and mandibular glands of worker honeybees (Isidorov *et al.*, 2009). It is composed mainly of proteins, sugars, lipids, free amino acids, vitamins and mineral elements. RJ has been demonstrated to

possess a number of pharmacological activities. For this reason, it is widely used in medical products, health foods and cosmetics in many countries (Guo *et al.*, 2009).

It has been reported that RJ has antitumor (Nakaya *et al.*, 2007; Izuta *et al.*, 2009), antibacterial (Fujiwara *et al.*, 1990), cardioprotective (Krylov *et al.*, 2006), hypotensive (Tokunaga *et al.*, 2004), anti-inflammatory (Kohno *et al.*, 2004), antifatigue (Kamakura *et al.*, 2001), hypoglycaemic activity (Fujii *et al.*, 1990), estrogenic effect (Mishima *et al.*, 2005) and

effects on fertility (Husein and Kridli, 2002). Furthermore, RJ has anti-inflammatory activity and wound-healing properties (Fujii *et al.*, 1990), insulin-like (Okuda *et al.*, 1998; Salazar-Olivo and Paz-Gonzales, 2005), liver-protecting activity (Karadeniz *et al.*, 2011; Kanbur *et al.*, 2009) and promotes collagen production (Koya-Miyata *et al.*, 2004).

The DPPH and FRAP assays are widely used to evaluate antioxidant activity in different natural products. The DPPH radical scavenging test is one of the simplest methods available to investigate the overall hydrogen/electron-donating activity of single antioxidants and health-promoting dietary antioxidant supplements (Kedare and Singh, 2011). The FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method, employing an easily reduced oxidant system present in stoichiometric excess.

There are a few reports about its antioxidative role connected to antiaging effects (Iannuzzi, 1990; Inoue *et al.*, 2003) and reduction of intracellular oxidation (Jamnik *et al.*, 2007). Royal jelly also prevents radiation-induced brain damage (Cihan *et al.*, 2011). Biological activities of royal jelly are mainly attributed to bioactive fatty acids, proteins and phenolic compounds (Jamnik *et al.*, 2012; Ramadan and Al-Ghamdi, 2012).

Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions (Velioglu *et al.*, 1998). The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals (Javanmardi *et al.*, 2003).

Several studies have been previously conducted on the antioxidant activity of RJ (Inoue *et al.*, 2003; Nagai *et al.*, 2006; Barnutiu, 2013). However, there is no study

to present the antioxidant activity and total polyphenolic content of Bulgarian RJ.

In this respect, the purpose of the present study is to evaluate the antioxidant activity and to determine the total polyphenol content in Bulgarian RJ samples.

## Materials and Methods

A total of 20 samples from Bulgaria were obtained from experimental apiaries (from 2 regions – Lovech (8 RJ samples), Veliko Tarnovo (7 RJ samples) and directly from market (5 RJ samples).

Antioxidant activity of RJ was estimated by determination of DPPH (2, 2-diphenyl-1-picrylhydrazyl), according to the procedure of Brand-Williams *et al.*, (1995) and modified after Liu *et al.*, (2008). FRAP assay (Ferric Reducing Antioxidant Power) (Benzie and Strain, 1996) was performed as described in Mohammadzadeh *et al.*, (2007) with modification of sample preparation. For both methods, 1 mM ascorbic acid was used as positive control (DPPH – 98.21% inhibition, FRAP – 14.77 mM Fe<sup>2+</sup>/g).

Total polyphenol content was determined by Folin – Ciocalteu colorimetric method (Slinkard and Singleton, 1977) using gallic acid as a calibration standard as described in Liu *et al.*, (2008) with measuring the absorbance at 760 nm.

All measurements were performed from dilutions of 10% RJ in bidistilled water. All analysis was done in Laboratory of Quality Control for Bee Products, Cluj-Napoca.

The analyses were carried out in triplicate and the data were expressed as means ± standard deviations (SD). Statistical analyses of data were performed using IBM SPSS Statistics version 21 for Windows. Correlation analyses between the antioxidant parameters and total

polyphenols were done. The Student's t-test was used to determine the significance of the differences. Level of statistical significance was defined as  $p < 0.05$ .

### Results and Discussion

The results for DPPH and FRAP assays are presented in Table 1 which summarize the average values  $\pm$  standard deviations and ranges for all RJ samples. All tested RJ samples exhibit antioxidant activity.

A few articles deal with the antioxidant activity of RJ. Using the same equipment and technique for both DPPH and FRAP Pavel *et al.*, (2014) obtained higher DPPH values than in this study (an average of 37.23% inhibition in Romanian RJ). On the contrary Bulgarian samples have higher average values for FRAP ( $3.50 \pm 2.41$  mM  $\text{Fe}^{2+}/\text{g}$ ) than Romanian samples ( $2.20$  mM  $\text{Fe}^{2+}/\text{g}$ ). This parameter varies within a larger range (Table 1) than the results obtained by the same authors, ( $1.50 - 3.81$  mM  $\text{Fe}^{2+}/\text{g}$ ). Ceksteryte *et al.*, (2016) also studied the antioxidant activity of RJ samples but they used different scale for DPPH.

The total polyphenol contents of analysed RJ samples are shown in Table 1. The values varied in following range:  $11.66 - 36.73$  mg GAE/g RJ with an average of  $19.54$  mg GAE/g. Our results are similar to those presented by Pavel *et al.*, (2014) ( $14.56 - 39.90$  mg GAE/g RJ). Lui *et al.*, (2008) studied the antioxidant activity of RJ collected 24, 48 and 72 h after the larval transfer. RJ collected 72 h after the larval

transfer had  $168.0 \pm 5.9$   $\mu\text{g}/\text{g}$  total phenolic content. This value is much lower than the average value obtained in the present study (Table 1). Ceksteryte *et al.*, (2016) also received lower average value for this parameter ( $10.7 \pm 0.03$  GAE/g).

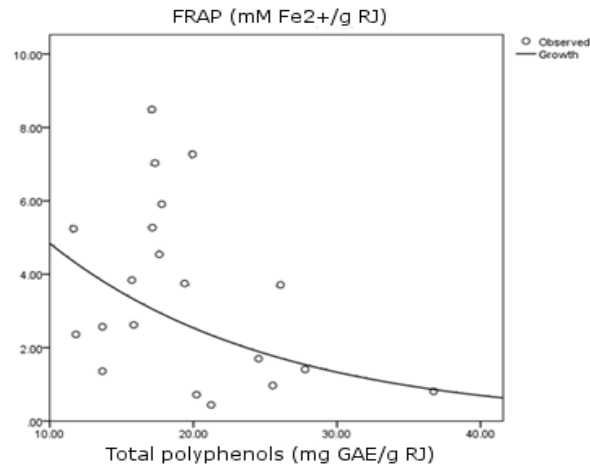
The phenolic compounds or polyphenols are one of the most important groups of compounds occurring in plants where they are widely distributed. The polyphenols are also products of the secondary metabolism of plants. These compounds are reported to exhibit anticarcinogenic, anti-inflammatory, anti-atherogenic, antithrombotic, immune modulating and analgesic activities, among others and exert these functions as antioxidants (Gomez-Caravaca *et al.*, 2006). The phenolic compounds present in royal jelly are known to possess antioxidant properties and these properties may play a key role in the pharmacological activities of RJ.

The spectrophotometric procedure with Folin–Ciocalteu reagent is widely used to determine total phenolic content in different bee products (Lachman *et al.*, 2010; Serra Bonvehi and Gutierrez, 2011). A positive correlation between the total antioxidant activity determined by the FRAP method and total phenolic content (Figure 1) for all RJ samples was observed. The moderate correlation coefficient ( $r=0.461$ ,  $p < 0.05$ ) indicates that the phenolics are one of the components responsible for the antioxidant behaviour of RJ. This result suggests that only 21% of the antioxidant capacity of the RJ samples accessions results from the contribution of the total polyphenols.

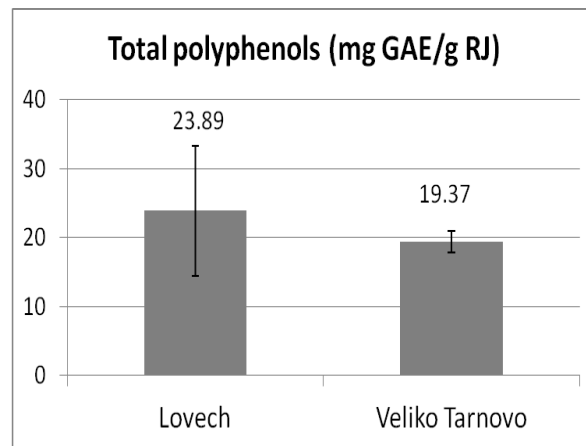
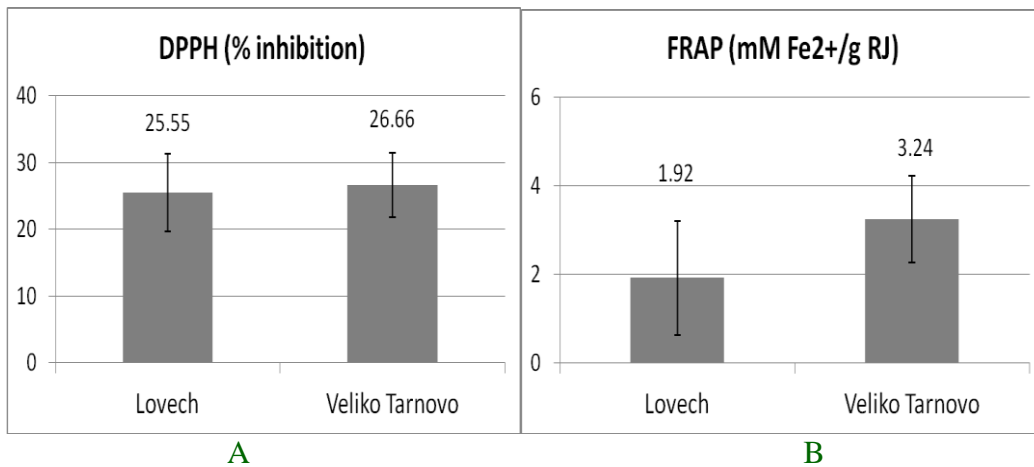
**Table.1** Antioxidant activity expressed by DPPH and FRAP assays and total polyphenols in Bulgarian RJ samples, n=20

	DPPH (% inhibition)	FRAP (mM $\text{Fe}^{2+}/\text{g}$ )	Total polyphenols (mg GAE/g)
Average $\pm$ SD	$24.23 \pm 8.19$	$3.50 \pm 2.41$	$19.54 \pm 6.12$
Range	$10.17 - 39.39$	$0.44 - 8.49$	$11.66 - 36.73$

**Fig.1** Correlation between FRAP assay and total polyphenols of Bulgarian RJ samples  $R^2=0.213$ , ( $p<0.05$ ),  $y=e^{2.2-0.65.x}$



**Fig.2** Average values and standard deviation of DPPH, FRAP and total polyphenols between RJ samples from different regions of Bulgaria, (A-C), n=15



It can be concluded that antioxidant activity of RJ is not due to total polyphenols exclusively. Activity may also result from the presence of other antioxidant secondary metabolites from pollen grains in RJ. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers. The unique feature of RJ is 10-hydroxy-2-decenoic known to possess various pharmacological effects, including antioxidative action (Nagai *et al.*, 2001; Nagai *et al.*, 2006; Isidorov *et al.*, 2011). It has also been suggested that organic acids present in RJ contribute to antioxidant activity through metal chelation and increase the effect of total polyphenols. These findings show that other components which are not analysed in our study should also play an important role for antioxidant activity of RJ. As previously mentioned the antioxidant activity is basically due to the presence of different compounds, although the exact action mechanism is unknown.

As a first step in characterization of Bulgarian RJ samples for antioxidant activity and total polyphenols the results are presented more comprehensively for 2 Bulgarian regions (Lovech and Veliko Tarnovo). The values obtained for DPPH, FRAP and total polyphenols do not vary greatly among these 2 regions, as is showed in Figure 2.

It should be mentioned that these 2 regions are not identical in floral sources. The DPPH values between the two regions are almost identical. The RJ samples from Veliko Tarnovo have higher values for FRAP analysis. The total polyphenols in the samples increased in the following order: RJ from Veliko Tarnovo < RJ from Lovech region. The differences for all analysed parameters are not significant. The composition of RJ and its antioxidant capacity can be influenced by a

large number of factors, such as the flower source of the nectar, environmental factors and processing methods. Therefore, it cannot be fully evaluated by one single method. Moreover, it is necessary to perform more than one type of antioxidant capacity method for taking into account the various mechanisms of antioxidant action.

To the best of our knowledge, this is the first report of the antioxidant properties of Bulgarian RJ samples. For the all samples the following ranges were obtained: DPPH 10.17 – 39.39 % inhibition, FRAP 0.44 – 8.49 mM Fe<sup>2+</sup>/g, total polyphenols 11.66 – 36.73 (mg GAE/g). The concentration of individual phenolic compounds and antioxidant properties are rather complex and further investigations are needed to determine possible correlations between DPPH, FRAP, total polyphenols and other quality parameters.

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