Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

2024

63

Print ISSN: 0367-4444
Online ISSN: 2232-7266

Original Scientific Article
DOI: 10.35666/2232-7266.2024.63.03
UDK:638.162:577.1(497.6)

19-24

Correlations of total phenolics and antioxidant activity in royal jelly from Bosnia and Herzegovina

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Article info
Received: 07/11/2023
Accepted: 06/05/2024

Keywords:
Phenolics
Antioxidant activity
Correlations
Royal jelly
Spectrophotometric method

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Abstract: In this study, the total phenolics content (TPC), and antioxidant activity against hydroxyl ($AA_{OH}^{\bullet}$) and peroxyl ($AA_{ROO}^{\bullet}$) free radicals in centrifuged (low molecular weight fractions, LM) and uncentrifuged (the bulk of low molecular weight and high molecular weight, LM+HM fractions) samples of fresh royal jelly (RJ) from Bosnia and Herzegovina (B&H), as well as correlations between these parameters were determined. For determination of the TPC, spectrophotometric method with gallic acid (GA) as the standard was used. For the determination of AA, the oxygen radical absorbance capacity (ORAC) assay with catechin as the standard was used. The highest TPC expressed in equivalent mass of GA per mass of RJ (LM+HM fractions) was found in the sample from Kalinovik, 5.54±0.76 mg GAE/g, and in the sample from Bosanska Krupa, 4.07±0.04 mg GAE/g for LM phenolics of RJ. The highest $AA_{OH}^{\bullet}$ expressed as mmol catechin equivalents (CE) per mass of LM+HM fraction of RJ was found in the sample from Cazin 58.15±1.55 mM CE/g, and in the sample from Ključ, 58.15±0.81 mM CE/g for LM fraction. The highest $AA_{ROO}^{\bullet}$ per mass of LM+HM fraction of RJ was found in the sample from Bosanska Krupa, 8.04±0.04 mM CE/g, in which also found the highest $AA_{ROO}^{\bullet}$ for LM fractions, 7.58±0.39 mM CE/g. There were very high positive correlations between $AA_{OH}^{\bullet}{}_{(LM+HM)}$, as well as $AA_{OH}^{\bullet}{}_{(LM)}$) and $TPC_{(LM+HM)}$, as well as $TPC_{(LM)}$ (Pearson correlations (P.c.)). It was moderate positive correlation $AA_{OH}^{\bullet}{}_{(HM)}$ to $TPC_{(HM)}$. Also, there were weak positive correlations between $AA_{(ROO^{\bullet})(LM+HM)}$, as well as $AA_{(ROO^{\bullet})LM}$ and $TPC_{(LM+HM)}$, as well as $TPC_{(LM)}$, and weak negative correlation $AA_{(ROO^{\bullet})(HM)}$ to $TPC_{(HM)}$. Based on a very high TPC and significant AA, it can be concluded that RJ from B&H is a good source of natural antioxidants and is an important dietary supplement.

INTRODUCTION

Royal jelly (RJ) is a secretion product of the cephalic glands of nurse bees that has been used for centuries for its extraordinary properties and health effects (Pavel *et al*., 2011). It is produced under partial digestion of pollen and nectar (Stocker, 2003). Due to its complex composition1(water, proteins, lipids, carbohydrates, amino acids, mineral salts, vitamins, enzymes, hormones, oligo-elements, natural antibiotics), RJ has a multitude of biological activities: antioxidant (Nagai and Inoue, 2004; Nagai *et al*., 2006), hypoglycemic (Pourmoradian *et al*., 2014; Maleki *et al*., 2019), hypocholesterolemic (Chiu *et al*., 2017; Pan *et al*., 2018; Petelin *et al*., 2019) and hepatoprotective (Almeer *et al*., 2018; Bilgic *et al*., 2018), hypotensive and blood pressure regulatory (Liang *et al*., 2018; Pan *et al*., 2019), antitumor (Albalawi *et al*., 2022), antibacterial (Alreshoodi and Sultanbawa, 2015; Gevorgyan *et al*., 2021; Guo *et al*., 2021), anti-inflammatory (Aslan and Aksoy, 2015) immunomodulatory and anti-allergic (Guendouz *et al*., 2017), general tonic, healthy aging and longevity (Kunugi and Ali, 2019) etc.

The subject of this paper was to investigate the correlations between total phenolics content (TPC) and antioxidant activity against hydroxyl and peroxyl free radicals of fresh royal jelly from Bosnia and Herzegovina. There is no data on these correlations in the available literature. Therefore, the scientific significance of this

work could be in the direction of determining TPC in correlation with the antioxidant activity of Bosnian royal jelly.

MATERIAL AND METHODS

Chemicals - Fluorescein, Standard Fluka for fluorescence – free acid was obtained from Fluka Chemie GmbH, Steinheim, Germany. Catechin hydrate and 2,2'-azobis (2 amidino-propane) dihydrochloride (AAPH) were obtained from Sigma, cupric sulfate pentahydrate and hydrogen peroxide from Kemika, Zagreb, Croatia, and Folin-Ciocalteu reagent, sodium carbonate, sodium phosphate dibasic, potassium phosphate monobasic were purchased from Semikem, Sarajevo, Bosnia and Herzegovina. All chemicals were analytical grade.

Samples - The samples of royal jelly originating from the area of three cantons from the Federation of Bosnia and Herzegovina and two cities from Eastern Bosnia are listed in Table 1.

All samples were stored at -32 °C until analysis.

Instrumentations - All analyses were performed on UV/Vis spectrometer Lambda 25, and fluorescence spectrometer LS 55 (all by Perkin-Elmer). The samples were centrifuged on a Hettich Mikro 22R centrifuge. For weighing the samples, the balance Mettler Toledo AB 104 was used. Thermostate KP 20-Lauda was used for temperature regulation.

Sample preparation - Approximately 0.05 g of RJ sample dissolved in 1 ml of distilled water (concentrated RJ solution obtained: 50 mg/ml). This RJ solution was diluted[1] and they were analysed before and after centrifugation at 15000 rpm, 15 minutes, at 15 °C (after centrifugation, the supernatants were used for the analyses).

Determination of total phenolic content (TPC)
The total phenolics content (TPC) was determined by spectrophotometry, using gallic acid (GA) as a standard, according to the method described by Keskin-Šašić *et al*., (2012). All measurements were done in triplicate at 743 nm.

Determination of antioxidant activity – Oxygen Radical Absorbance Capacity (ORAC)
For the analyses of antioxidant activity (AA) of RJ the oxygen radical absorbance capacity (ORAC) assay was used (Cao and Prior, 1999) with catechin (C) as standard. For the analyses of AA of RJ, all samples were measured before [AA of the low-molecular (LM) + high-molecular (HM) antioxidants, $AA_{(LM+HM)}$] and after centrifugation [AA of the low-molecular antioxidants, $AA_{(LM)}$] (Tahirović *et al*., 2017).

Table 1. Royal jelly samples.

Sample label	Sample location	Sample label
1	Bjelašnica (Rakitnica)	1
2	Kalinovik (Jezero)	2
3	Bosanska Krupa 1 (Vranjska)	3
4	Bosanska Krupa 2 (Krčevine)	4
5	Milići (Johovača)	5
6	Cazin 1 (Stijena)	6
7	Bosanska Krupa 3 (Ljusina)	7
8	Bosanska Krupa 4 (Ljusina)	8
9a	Konjic 1 (Seonica)	9a
9b	Konjic 2 (Čuhovići)	9b
9c	Konjic 3 (Čuhovići)	9c
9d	Konjic 4 (Čuhovići)	9d
9e	Konjic 5 (Seonica)	9e
9f	Konjic 6 (Seonica)	9f
9g	Konjic 7 (Čuhovići)	9g
7	Bosanska Krupa 3 (Ljusina)	7
8	Bosanska Krupa 4 (Ljusina)	8
9a	Konjic 1 (Seonica)	9a
9b	Konjic 2 (Čuhovići)	9b
9c	Konjic 3 (Čuhovići)	9c
9d	Konjic 4 (Čuhovići)	9d
9e	Konjic 5 (Seonica)	9e
9f	Konjic 6 (Seonica)	9f
9g	Konjic 7 (Čuhovići)	9g
10	Ključ (Hanlovići)	10
11	Sanski Most 1 (Skucani Vakuf)	11
12	Sanski Most 2 (Čaplje)	12
13	Sanski Most 3 (Čaplje)	13
14	Bosanska Krupa 5 (Velika Jasenica)	14
15	Cazin 2 (Klisa)	15
16	Bratunac 1 (Suha)	16
17	Bratunac 2 (Suha)	17
18a	Modriča 1 (Svilaj)	18a
18b	Modriča 2 (Svilaj)	18b
18c	Modriča 3 (Svilaj)	18c

Statistical analysis - For the statistical analysis of the obtained results Kolmogorov-Smirnov and Shapiro-Wilk by SPSS 17 were used as normality tests. According to these tests, data were in normal distribution, thus average values with standard deviations, and the Student's t-test with p=0.05 as the statistical level of significance, were used.

[1] The dilution factor was taken into account in the calculation

RESULTS AND DISCUSSION

For analysis of TPC, all measurements were performed at 743 nm. The equation of the calibrated curve was: y = 0.0927x + 0.0129; R^2 = 0.9999. Obtained values of TPC for 26 samples of royal jelly (RJ), expressed as mass (in mg) of gallic acid equivalents per mass of RJ (mg GAE/g) are shown in the Table 2. Normality tests showed that values were in a normal distribution, and in that case, they are listed as mean from three determinations with standard deviations (±S.D.).

Table 2. Total phenolics in 26 analyzed samples of royal jelly.

Sample label	TPC[I] (mg GAE/g±S.D.)		
	TPC[II]	TPC[III]	TPC[IV]
2	**5.54±0.76**	3.80±0.05	1.75±0.78
8	5.47±0.24	**4.07±0.04**	1.40±0.20
3	5.30±0.01	3.55±0.02	1.76±0.02
4	5.27±0.28	3.50±0.01	1.65±0.27
9e	5.25±0.06	3.62±0.09	1.63±0.01
9a	5.14±0.42	2.97±0.05	**2.17±0.37**
9f	4.94±0.05	3.21±0.06	1.76±0.07
6	4.89±0.28	3.40±0.04	1.48±0.30
9b	4.77±0.11	3.15±0.14	1.62±0.03
9d	4.52±0.07	2.68±0.04	1.84±0.04
16	4.49±0.09	3.27±0.20	1.22±0.18
9c	4.46±0.04	2.84±0.25	1.62±0.23
9g	4.40±0.06	3.31±0.02	1.09±0.06
1	4.33±0.52	3.22±0.34	1.11±0.75
5	4.12±0.10	3.30±0.21	0.94±0.12
18c	3.95±0.03	2.78±0.01	1.16±0.03
7	3.89±0.12	2.87±0.06	1.03±0.09
11	3.70±0.14	2.99±0.07	0.71±0.19
10	3.65±0.04	2.88±0.07	0.76±0.09
18a	3.62±0.01	3.01±0.04	0.60±0.03
13	3.60±0.04	3.01±0.08	0.59±0.11
17	3.57±0.10	2.89±0.23	0.68±0.16
15	3.55±0.13	2.48±0.02	1.07±0.11
14	3.45±0.13	2.65±0.10	0.80±0.07
12	3.36±0.03	2.99±0.21	**0.37±0.19**
18b	**3.28±0.09**	**2.35±0.05**	0.99±0.12

[I]Mean from three determinations±standard deviations
[II]Total phenolics in the bulk (uncentrifuged samples)
[III]Low-molecular (LM) total phenolics (centrifuged samples)
[IV]High-molecular (HM) total phenolics (IV=II-III)

Obtained TPC values were slightly lower than those obtained by Nabas *et al*., (2014).

The TPC values of uncentrifuged samples of RJ, which are a mixture of low molecular weight (LM) and high molecular weight (HM) phenolics ($TPC_{(LM+HM)}$) were statistically significantly higher than the TPC values of centrifuged samples of RJ ($TPC_{(LM)}$) ($p^{***}<0.001$; Student's t-test) (mean values 4.42±0.72 GAE/g, and 3.16±0.38 mg GAE/g, respectively). The values of high molecular weight fractions of phenolics ($TPC_{(HM)}$) (mean value 1.26±0.48 mg GAE/g) were statistically significantly lower than $TPC_{(LM)}$ ($p^{***}<0.001$; Student's t-test).

There was no statistically significant difference between both: the $TPC_{(LM+HM)}$ values for the samples of RJ from Una-Sana Canton to the $TPC_{(LM+HM)}$ values for the samples of Herzegovina-Neretva Canton (mean values 4.37±0.84 mg GAE/g, and 4.88±0.41 mg GAE/g, respectively), and between $TPC_{(LM)}$ values for the samples of RJ from these locations ($p>0.05$; Student's t-test) (mean values 3.13±0.46 mg GAE/g, and 3.24±0.33 mg GAE/g, respectively). The results showed that the $TPC_{(HM)}$ values for the samples of RJ from Herzegovina-Neretva Canton were statistically significantly higher than the $TPC_{(HM)}$ values for the samples of RJ from Una-Sana Canton ($p^{**}<0.01$; Student's t-test), (mean values 1.69±0.30 mg GAE/g, and 1.06±0.46 mg GAE/g, respectively). There were no statistically significant differences between the TPC values for RJ from Federation of Bosnia and Herzegovina to the TPC values of RJ in another entity of Bosnia and Herzegovina ($p>0.05$; Student's t-test, in all analyzed fractions).

Analysis of antioxidant activity

The antioxidant activity of royal jelly was examined using several methods, but data obtained using the ORAC method are not available (Liu *et al*., 2008; Park *et al*., 2020).

Antioxidant activity against hydroxyl free radicals

The equation of the calibrated curve for antioxidant activity (AA) of royal jelly (RJ) against hydroxyl free radicals ($OH^{\bullet}$) was: y = 346.78x + 486.68, and the obtained results, expressed as mmol catechin equivalents per mass of RJ (mM CE/g) are shown in Table 3.

Normality tests show that values for $AA_{OH^{\bullet}(LM+HM)}$, $AA_{OH^{\bullet}(LM)}$, and $AA_{OH^{\bullet}(HM)}$ were in a normal distribution, and in that case they are listed as mean from three determinations with ±S.D. The AA values for 26 uncentrifuged samples of RJ, which are a mixture of low molecular weight (LM) and high molecular weight (HM) antioxidants ($AA_{OH^{\bullet}(LM+HM)}$) were statistically significantly higher than the AA values for 26 centrifuged samples - low molecular weight (LM) fractions of antioxidants ($AA_{OH^{\bullet}(LM)}$) ($p^{**}<0.01$; Student's t-test) (mean values 33.91±11.51 mM CE/g, and 28.01±11.32 mM CE/g, respectively). The values of both: $AA_{OH^{\bullet}(HM+LM)}$ and $AA_{OH^{\bullet}(LM)}$ were statistically significantly higher than the $AA_{OH^{\bullet}(HM)}$ values ($p^{***}<0.001$; Student's t-test).

The results showed that there were no statistically significant differences between $AA_{OH^{\bullet}}$ values for the samples of RJ from Una-Sana Canton to the $AA_{OH^{\bullet}}$ values of RJ from Herzegovina-Neretva Canton in all analyzed fractions ($p>0.05$; Student's t-test).

Also, the values for $AA_{OH^{\bullet}(LM)}$ for the samples of RJ from the area of Bosanska Krupa, where natural meadow food for bees prevailed, were statistically significantly higher than the $AA_{OH^{\bullet}(LM)}$ values for the samples of RJ from the area of Konjic, where the same diet for bees prevailed

($p^{*}<0.05$; Student's t-test) (mean values 30.90±5.99 mM CE/g, and 21.90±1.87 mM CE/g, respectively).

Table 3. Antioxidant activity of RJ against hydroxyl free radicals.

Sample label	$AA_{OH^{\bullet}(HM+LM)}$	$AA_{OH^{\bullet}(LM)}$	$AA_{OH^{\bullet}(HM)}$ *
	$mM_{(CE)}$/g±S.D.		
6	**58.15±1.55**	47.39±1.90	10.76
10	53.69±3.55	**58.15±0.81**	-
13	49.64±0.08	37.58±1.17	12.06
11	48.60±0.37	30.09±0.53	**18.51**
3	47.30±3.51	39.67±1.70	7.63
9f	45.28±1.97	34.18±2.06	11.10
12	43.46±3.32	45.29±0.85	-
9a	40.02±4.38	33.35±0.37	6.67
1	39.10±3.81	24.40±0.79	14.70
9e	38.77±2.68	23.13±0.94	15.64
9b	36.62±0.31	21.44±1.59	15.18
14	35.55±8.25	28.83±1.07	6.72
15	35.36±1.84	39.54±1.92	-
5	27.15±0.01	14.71±1.52	12.44
9d	26.88±0.75	23.95±0.05	2.93
9c	25.90±1.19	22.64±0.33	3.26
7	25.11±4.52	22.71±0.29	2.40
2	24.62±0.58	19.25±0.48	5.37
18b	24.41±1.20	18.53±0.91	5.88
16	24.13±1.24	**10.71±1.09**	13.42
18c	23.73±1.65	12.16±0.67	11.57
8	23.29±2.11	26.17±1.50	-
4	22.32±1.81	28.92±0.19	-
9g	21.47±1.35	19.56±3.12	1.91
17	20.96±3.21	20.42±4.60	**0.54**
18a	**20.18±0.69**	25.44±1.03	-

*Values obtained from the difference $AA_{OH^{\bullet}(HM+LM)}$ - $AA_{OH^{\bullet}(LM)}$.

The $AA_{OH^{\bullet}(LM+HM)}$ and $AA_{OH^{\bullet}(LM)}$ values of RJ samples from Federation of Bosnia and Herzegovina were statistically significantly higher than the $AA_{OH^{\bullet}(LM+HM)}$ and $AA_{OH^{\bullet}(LM)}$ values of RJ samples from another entity of Bosnia and Herzegovina ($p^{**}<0.01$; Student's t-test) (mean values 37.71±11.21 mM CE/g, and 23.60±2.35 mM CE/g for $AA_{OH^{\bullet}(LM+HM)}$, 34.93±9.90 mM CE/g, and 20.36±4.73 mM CE/g for $AA_{OH^{\bullet}(LM)}$, respectively). It was no statistically significant differences between $AA_{OH^{\bullet}(HM)}$ fractions in these two entities.

Antioxidant activity against peroxyl free radicals

The equation of the calibrated curve for AA of RJ against ROO• was: y = 691.38x + 212.9, and the obtained results, expressed as mmol catechin equivalents per mass of RJ (mM CE/g) are shown in Table 4.

Normality tests show that values for $AA_{ROO^{\bullet}(LM+HM)}$, $AA_{ROO^{\bullet}(LM)}$, and $AA_{ROO^{\bullet}(HM)}$ were in a normal distribution, and in that case, they are listed as mean from three determinations with ±S.D.

The $AA_{ROO^{\bullet}(LM+HM)}$ values for 26 samples of RJ were statistically significantly higher than the values of both: $AA_{ROO^{\bullet}(LM)}$ and $AA_{ROO^{\bullet}(HM)}$ ($p^{***}<0.001$ in both cases; Student's t-test). Also, the values of $AA_{ROO^{\bullet}(LM)}$ were statistically significantly higher than the values of $AA_{ROO^{\bullet}(HM)}$ ($p^{***}<0.001$; Student's t-test).

There were no statistically significant differences between the $AA_{ROO^{\bullet}}$ values of RJ from Una-Sana Canton and from Herzegovina-Neretva Canton in all analyzed fractions ($p>0.05$; Student's t-test).

Also, the $AA_{ROO^{\bullet}(LM)}$ values for the samples of RJ from the area of Bosanska Krupa, where natural meadow food for bees prevailed, were statistically significantly higher than the $AA_{ROO^{\bullet}(LM)}$ values for the samples of RJ from the area of Konjic, where the same diet for bees prevailed ($p^{**}<0.01$; Student's t-test), (mean values 5.82±1.91 mM CE/g, and 2.31±1.06 mM CE/g, respectively), while the $AA_{ROO^{\bullet}(HM)}$ values from the area of Konjic were statistically significantly higher than the $AA_{ROO^{\bullet}(HM)}$ values from the area of Bosanska Krupa (mean values 1.94±1.17 mM CE/g, and 0.34±0.20 mM CE/g, respectively) ($p^{**}<0.01$; Student's t-test).

Table 4. Antioxidant activity of RJ against peroxyl free radicals.

Sample label	$AA_{ROO^{\bullet}(LM+HM)}$	$AA_{ROO^{\bullet}(LM)}$	$AA_{ROO^{\bullet}(HM)}$ *
	$mM_{(CE)}$/g±S.D.		
8	**8.04±0.04**	**7.58±0.39**	0.46
9e	7.83±0.25	7.13±0.10	0.70
3	7.07±0.30	6.96±0.38	**0.11**
1	6.76±0.13	6.58±0.47	0.18
11	6.49±0.11	2.68±0.32	**3.81**
9b	6.18±0.17	3.04±0.17	3.14
15	5.70±1.36	3.22±0.53	2.48
6	5.64±0.38	5.32±0.24	0.32
7	5.09±0.02	4.06±0.02	1.03
9d	4.90±0.71	3.39±0.31	1.51
5	4.72±0.70	4.03±0.67	0.69
13	4.69±0.24	3.53±0.03	1.16
9c	4.02±0.33	1.44±0.02	2.58
4	3.96±0.20	5.43±0.76	-
14	3.75±0.01	3.29±0.30	0.46
2	3.71±0.01	4.42±0.32	-
10	3.62±0.19	1.44±0.09	2.18
12	3.48±0.18	3.05±0.12	0.43
9a	3.05±0.17	2.40±0.34	0.65
9f	2.59±0.23	1.75±0.08	0.84
18b	2.37±0.15	1.55±0.03	0.82
16	2.20±0.11	1.62±0.02	0.58
18a	2.13±0.07	1.49±0.15	0.64
17	1.94±0.29	**1.22±0.06**	0.72
9g	1.87±0.05	1.36±0.12	0.51
18c	**1.66±0.14**	1.41±0.10	0.25

*Values obtained from the difference $AA_{ROO^{\bullet}(HM+LM)}$ - $AA_{ROO^{\bullet}(LM)}$.

The $AA_{ROO^{\bullet}(LM+HM)}$ and $AA_{ROO^{\bullet}(LM)}$ values of RJ samples from the Federation of Bosnia and Herzegovina were statistically significantly higher than the $AA_{ROO^{\bullet}(LM+HM)}$ and $AA_{ROO^{\bullet}(LM)}$ values of RJ samples from another entity of Bosnia and Herzegovina ($p^{**}<0.01$, and $p^{*}< 0.05$, respectively, by Student's t-test) (mean values 4.99±1.77 mM CE/g, and 2.68±1.11 mM CE/g for $AA_{ROO^{\bullet}(LM+HM)}$, 3.88±1.98 mM CE/g, and 2.25±1.26 mM CE/g for $AA_{ROO^{\bullet}(LM)}$, respectively). It was no statistically

significant differences between $AA_{ROO^{\bullet}(HM)}$ fractions in these two entities.

Correlations between total phenolics and antioxidant activity

There were very high positive correlations between $AA_{OH^{\bullet}(LM+HM)}$, as well as $AA_{OH^{\bullet}(LM)}$) and $TPC_{(LM+HM)}$, as well as $TPC_{(LM)}$ (Pearson correlations (P.c.): 0.880, and 0.879 respectively, $p^{**}<0.01$ in both cases). It was moderate positive correlation $AA_{OH^{\bullet}(HM)}$ to $TPC_{(HM)}$ (P.c. 0.684, $p^{**}<0.01$). Also, there were weak positive correlations between $AA_{(ROO^{\bullet})(LM+HM)}$, as well as $AA_{(ROO^{\bullet})LM}$ and $TPC_{(LM+HM)}$, as well as $TPC_{(LM)}$ (P.c. 0.013, and 0.116 respectively, $p>0.05$ in both cases), and weak negative correlation $AA_{(ROO^{\bullet})(HM)}$ to $TPC_{(HM)}$ (P.c. -0.058).

Considering that most of the samples were from the area of two Bosnian-Herzegovinian cantons (Una-Sana Canton and Herzegovina-Neretva Canton), which are spatially separated from each other, a statistical analysis of these two groups of data was also performed. The results of this analysis showed that there were very high positive correlations between $AA_{OH^{\bullet}(LM+HM)}$ - $TPC_{(OH^{\bullet})(LM+HM)}$, $AA_{OH^{\bullet}(LM)}$ - $TPC_{(OH^{\bullet})(LM)}$, (P.c. 0.903, and 0.919 respectively, $p^{**}<0.01$ in both cases), and moderate positive correlation $AA_{OH^{\bullet}(HM)}$ - $TPC_{(OH^{\bullet})(HM)}$ (P.c. 0.770, $p^{**}<0.01$) for Una-Sana Canton, but there was very high correlation only between $AA_{OH^{\bullet}(LM)}$ - $TPC_{(OH^{\bullet})(LM)}$ for Herzegovina-Neretva Canton (P.c. 0.935; $p^{**}<0.01$). Also, the results showed that there was a moderate positive correlation between $AA_{(ROO^{\bullet})(LM)}$ and $TPC_{(LM)}$ of RJ samples from Una-Sana Canton (P.c. 0.390; $p^{**}<0.01$), and a very weak negative correlation between $AA_{(ROO^{\bullet})(LM)}$ and $TPC_{(LM)}$ of RJs from Herzegovina-Neretva Canton (P.c. -0.068; $p>0.05$).

CONCLUSIONS

The obtained results showed that royal jelly from Bosnia and Herzegovina is rich in phenolics. Also, royal jelly from Bosnia and Herzegovina has very high antioxidant activity against both: hydroxyl and peroxyl free radicals. Because the results for the antioxidant activity of royal jelly against these free radicals showed significant statistical differences, it is necessary to investigate the effects of these parameters on a larger group of samples. There were observed very strong positive correlations between total phenolics and antioxidant activity against hydroxyl free radicals, and a moderate positive correlation between total phenolics and antioxidant activity against peroxyl free radicals. The obtained results support the recognition of the quality of royal jelly from Bosnia and Herzegovina.

ACKNOWLEDGEMENT

This work was supported by the Federal Ministry of Education and Science, Bosnia and Herzegovina (Grant No 05-39-2603-1/18).

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Summary/Sažetak

U ovom istraživanju, određen je sadržaj ukupnih fenola (SUF) i antioksidativna aktivnost protiv hidroksilnih ($AA_{OH}^{\bullet}$) i peroksilnih ($AA_{ROO}^{\bullet}$) slobodnih radikala, kao i korelacije između ovih parametara u uzorcima svježe matične mliječi (MM) porijeklom iz Bosne i Hercegovine. Za određivanje SUF korištena je spektrofotometrijska metoda sa Folin-Ciocalteu reagensom i galnom kiselinom (GA) kao standardom. Za određivanje AA korištena je metoda apsorbirajućeg kapaciteta kisikovih radikala (ORAC) sa katehinom kao standardom i fluoresceinom kao fluorescentnom metom. Najviši SUF od ukupno 26 uzoraka MM izražen u ekvivalentnoj masi GA po masi svježe necentrifugirane MM nađen je u uzorku iz Kalinovika, 5,54±0,76 mg GAE/g, dok je među centrifugiranim uzorcima MM najviši SUF nađen u uzorku iz Bosanske Krupe, 4,07±0,04 mg GAE/g. Najviša $AA_{OH}^{\bullet}$ izražena u mmol katehin ekvivalenata (KE) po masi svježe necentrifugirane MM (frakcija nisko- i visokomolekularnih antioksidanasa u otopini, $AA_{OH}^{\bullet}{}_{(n.m.+v.m.)}$) nađena je u uzorku iz Cazina, 58,15±1,55 mmol KE/g, dok je najviša $AA_{OH}^{\bullet}$ od centrifugiranih uzoraka MM (niskomolekularna frakcija antioksidanasa, $AA_{OH}^{\bullet}{}_{(n.m.)}$) nađena u uzorku iz Ključa 58,15±0,81 mmol KE/g. Najviša $AA_{ROO}^{\bullet}{}_{(n.m.+v.m.)}$ nađena je u uzorku MM iz Bosanske Krupe 8,04±0,04 mM KE/g, u kojem je nađena i najviša $AA_{ROO}^{\bullet}{}_{(n.m.)}$, 7,58±0,39 mmol KE/g.Uočena je vrlo visoka pozitivna korelacija između $AA_{OH}^{\bullet}{}_{(n.m.+v.m.)}$ prema $SUF_{(n.m.+v.m.)}$, (Pearson-ova korelacija (P.k.)), kao i između $AA_{OH}^{\bullet}{}_{(n.m..)}$ prema $SUF_{(n.m.)}$. Između $AA_{OH}^{\bullet}{}_{(v.m..)}$ i $SUF_{(v.m.)}$ postojala je umjerena P.k.. Također, postojala je slaba pozitivna P.k. između $AA_{ROO}^{\bullet}{}_{(n.m.+v.m.)}$ prema $SUF_{(n.m.+v.m.)}$, kao i između $AA_{ROO}^{\bullet}{}_{(n.m..)}$ i $SUF_{(n.m.)}$. Između $AA_{ROO}^{\bullet}{}_{(v.m..)}$ i $SUF_{(v.m.)}$ postojala je slaba negativna P.k. Na osnovu vrlo visokog SUF i značajne AA može se zaključiti da je MM sa prostora Bosne i Hercegovine dobar izvor prirodnih antioksidanasa i značajan dodatak prehrani.