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The Antioxidant Activity of Water, DMSO and Methanol Extracts of Royal Jelly from Bursa Province in Turkey

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Research Article

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Abstract

Royal jelly is a bee product and has many benefical effects on human health. In this study, the antioxidant activity of water, Dimethy sulfoxide (DMSO) and methanol extracts of royal jelly that collected from Bursa province in Turkey were investigated. Total Phenol Contents (TPC), Total Flavonoid Contents (TFC), Total Antioxidant Activity (TAA) and Free Radical Scavenging Capacity were detected Folin-Ciocalteu method, Aluminum Nitrate colorimetric method, Ferric Reducing Antioxidant Power and DPPH Assay respectively. TPC and SEM values of water, DMSO and methanol extracts of royal jelly were 1.05 ± 0.38, 15.65 ± 7.36 and 1.65 ± 0.42. TFC and SEM values of water, DMSO and methanol extracts of royal jelly were 0.17 ± 0.09, 0.61 ± 0.02 and 0.06 ± 0.02. TAA and SEM values of water, DMSO and methanol extracts of royal jelly were 35.04 ± 17.07, 6.94 ± 1.36 and 13.74 ± 6.87. Finally percentage of DPPH inhibition and SEM values of water, DMSO and methanol extracts of royal jelly were 10.62 ± 2.61, 6.70 ± 2.47 and 10.39 ± 5.19. TPC and TFC results of DMSO extracts of royal jelly significantly higher than methanol and water extracts of royal jelly results. Also all extracts of royal jelly were shown TAA and free radical schavenger activity but results weren't significantly different eachother. In conclusion, royal jelly extracts of Bursa province in Turkey were shown antioxidant potential and it can be used as an antioxidant product.

Keywords: Royal Jelly; Antioxidant Activity

Abbreviations: DMSO: Dimethy Sulfoxide; TPC: Total Phenol Contents; TFC: Total Flavonoid Contents; TAA: Total Antioxidant Activity; MRJP: Major Royal Jelly Proteins; FRAP: Ferric Reducing Antioxidant Power; QE: Quercetin Equivalent; GAE: Gallic Acid Equivalent; SEM: Standart Error Meaning; TE: Trolox Equivalent; DPPH: 2,2-diphenyl-1-picrylhydrazyl; 10-HDA: Trans-10-hydroxy-2-decenoic acid.

Introduction

Royal Jelly is one of bee produced that has white-yellow colour and selected from the hypopharyngeal and

mandibular glands of nurse honey bees (5-15 days old). Royal Jelly is used to fed bee larvas and queen bee. Bee larva can consume royal jelly first 3 days while queen bee can consume it during the her life [1]. Royal jelly consist of 60-70% water, proteins 9-18%, total sugar 10-16%, small amounts of lipids, vitamins, salts and free amino acids [2]. Composition of royal jelly depend on race of honey bees, climate, ecological conditions and geografic area where were produced and harvested time [2-4]. Major Royal Jelly Proteins (MRJP) such as MRJP1-MRJP9, Apismin, Royalactina, Royalisin, Jelleines, Glucose oxidase, Apolipophorin II-like are member of Royal Jelly proteins [1]. Lipid compositions of royal jelly are occured phenols, waxes, steroids, phospholipids and faty acids such as trans-10-hydroxy-2-decenoic acid (10-HDA), gluconic acid, dicarboxylic acids. Carbohydrates composition of royal jelly is reported as Fructose, Glucose, Sucrose and oligosaccharides such as Maltose, Melibiose, Ribose [5]. Moreover, it contains minerals such as copper, zinc, calcium, sodium, potasium, iron, flavonoids and polyphenols such as hesperetin, naringenin, pigenin, kaempferol, genistein, vitamins such as folic acid, niacin, pantothenic acid, vitamin E and free amino acids lysine, proline, cystine, aspartic acid, phenylalanine, leucine [6].

Royal jelly has many biological and pharmaceutical properties such as antioxidant, anti-cancer, antibacterial, antifungal, wound-healing, antidiabetic, anti-inflammatory, immunomodulatory, antihypertension activity, estrogenic and neurotrophic effects, hepato-renal protective activity [1,6-8]. Royal jelly is used as cosmetics, food and diet supplements in case for its antiaging effect, nutritional value and benefical effects on human health [5,9,10].

This study was aimed to investigate antioxidant activity of water, Dimethyl sulfoxide and methanol extracts of royal jelly which is obtained from Bursa province of Turkey.

Material and Methods

Royal Jelly Origin

Royal jelly samples were produced by honey bee (*Apis Mellifera*) in Bursa province, Mustafakemalpaşa district of Turkey. Royal jelly samples were obtained via Fanus Food Company in Trabzon.

Chemicals

Sodium dihydrogen phosphate dihydrate, Disodium phosphate dihydrate, Dimethyl sulfoxide, Sodium carbonate, Potassium acetate were obtained from Merck (Berlin, Germany), Iron(III) chloride, Trichloroacetic acid, Folin Ciocalteu reagent, quercetin dihydrate, gallic acid from Sigma (St. Louis, MO, USA). Ethanol was supplied from Carlo Erba (Milano, Italy). Trolox, Aluminium nitrate nonahydrate were received from Fluka (Steinheim, Germany) and potassium ferricyanide was receieved from Lancaster (Morecambe, England).

Extract Preperation of Royal Jelly

A weight of 1.25g royal jelly samples were dissolved in 5ml 100% ratio of DMSO to water. After vortexing of royal jelly extract solutions they incubated by shaking during the 12 hour at 60°C and 150rpm. All extracts were centrifuged at 4000rpm at 10minutes. All supernatants are collected and diluted suitable solvents ratio of 1:10, 1:20, 1:100, 1:200. Water, DMSO and methanol extracts of royal jelly were stored +4C^{o} in a dark place until they were used.

The Total Phenol Contents (TPC)

Total Phenol Contents of water, DMSO and methanol extracts of royal jelly were evaluated spectrophotometrically with modified Folin-Ciocalteu method that was described by Singleton and Rossi in 1965 [11]. In this method gallic acid was used as a standard and all royal jelly extracts diluted 1:100 and 1:200 ratio. Seperately royal jelly extracts, gallic acid were pipetted 12.5µL in 96 well ELISA plate, 62.5µL fresh 1:10 diluted Folin-Ciocalteu's reagent and 125µL 20% Sodium Carbonate were added and incubated 30minutes in a dark place at 25°C. All absorbance were measured against deionized water, DMSO and methanol blanks at 700nm with Tunable Versamax microplate reader (US). Total Phenol Contents of royal jelly extracts were expressed as mg Gallic Acid Equivalent (GAE)/g royal jelly. Experiments repated three times and results were given mean ± Standart Error Meaning (SEM).

Total Flavonoid Contents (TFC)

Total Flavonoid Contents of water, DMSO and methanol extracts of royal jelly were evaluated with spectrophotometrically modified Aluminum Nitrate colorimetric method by Park, et al. 1997 [12]. Quercetin was used as standard in determination of total flavonoid contents of extracts of royal jelly. Quercetin standards and royal jelly extracts that were diluted 1:10 and 1:20 ratio were pipetted at 20µL, 80% ethanol was added at 172µL, 10% aluminium nitrate and 1M potassium acetate were added at 4µL volume in 96 well plate. After mixing plate incubated 40minutes at 25°C and absorbances were measured at 415nm against blanks wih Tunable

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Versamax microplate reader (US). Total Flavonoid Contents of water, DMSO and methanol extracts of royal jelly were expressed as mg Quercetin Equivalent (QE)/g royal jelly. Experiments were repeated four times and results were shown mean ± SEM.

Total Antioxidant Activity (TAA)

Total Antioxidant Activity of water, DMSO and methanol extracts of royal jelly were determinated by Ferric Reducing Antioxidant Power (FRAP) assay which was described in Oyaizu in 1986 [13]. In FRAP assay Trolox was used as an standard and 40µL extracts of royal jelly that diluated 1:10, 1:20, 1:100 and 1:200 ratio and Trolox standards were mixed with 100µL of 0.2M phospate buffer (pH 6.6) and 100µL 1% potassium ferricyanide in test tubes. After incubation at 50°C for 20minutes, 100µL 10% trichloroacetic acid was added and centrifuged at 3000g for 5minutes. Supernatants at 100µL volume were mixed with 100µL distilled water and 20µL 1% Iron (III) chloride in 96 well ELISA plate and all absorbance were measured against blank at 700 nm with Tunable Versamax microplate reader (US). Total Antioxidant Activity results of water and DMSO extracts of royal jelly were expressed as mg Trolox Equivalent (TE)/g royal jelly. TAA experiments of royal jelly were done four times and results were given mean ± SEM.

Free Radical Scavenging Capacity

Free Radical Scavenging Capacity of water, DMSO and methanol extracts of royal jelly were determineted by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay accordance with the method of Blois in 1958 with a slight modification [14]. 0.04% Methanol solution of DPPH were mixed with same volume of extracts of royal jelly and incubated 30minutes in dark and than absorbance was recorded at 595nm with microplate reader. All experiments repeated four times and results were expressed as percentage of inhibition with mean and SEM values.

Statistical Analysis

Kruskal Wallis and Mann Whitney U tests were used for comparating the TPC, TFC, TAA and Free Radical Scanvenging Capacity results of water, DMSO and methanol extracts of royal jelly. Differences were considered significant if $p < 0.05$.

Results and Discussion

Total Phenol Content and Total Flavonoid Content results of DMSO extracts of royal jelly were higher than water and methanol extracts of royal jelly significantly $p<0.05$. The lowest value of TPC and TFC results were obtinated from water and methanol extracts of royal jelly respectively. Total Antioxidand Activity and Free Radical Scavenging Capacity (DPPH % Inhibition) results of water extracts of royal jelly were higher than DMSO and Methanol extracts of royal jelly but results were not significant. All results were shown Table 1 with mean and SEM values.

Type of Extracts	TPC (mg GAE/g royal jelly)	TFC (mg QE/g royal jelly)	TAA (mg TE/g royal jelly)	DPPH (% Inhibition)
Water Extracts of Royal Jelly	1.05 ± 0.38	0.17 ± 0.09	35.04 ± 17.07	10.62 ± 2.61
DMSO Extracts of Royal Jelly	15.65 ± 7.36*	0.61 ± 0.02*	6.94 ± 1.36	6.70 ± 2.47
Methanol Extracts of Royal Jelly	1.65 ± 0.42	0.06 ± 0.02	13.74 ± 6.87	10.39 ± 5.19

GAE: Gallic acid equivalents, QE: Quercetin Equivalents, TE: Trolox Equivalents, * $p<0.05$.
Table 1: TPC, TFC, TAA and percentage of DPPH Inhibition of water, DMSO and methanol extracts of royal jelly. Results were given mean and SEM values, (n=4).

Balkanska, et al. in 2017 researched 2 different royal jelly from Bulgaria and TPC value of royal jelly samples that diluted 10% in bidistilled water were measured range of 11.66 - 36.73mg GAE/g. This results was higher than TPC value of water and methanol extracts of Bursa royal jelly but similar with DMSO extracts of Bursa royal jelly [15]. Ceksteryte, et al. in 2016 investigated Lithuania royal jelly for antioxidant activity. TPC value of Lithuania royal jelly were determined as 10.7 ± 0.03mg GAE/g royal jelly [16]. This results were lower than TPC value of Bursa royal jelly. Pavel, et al. in 2014 determined TPC value of commercial and local Romanian royal jelly and they found that range of TPC were 14.56 - 39.90mg GAE/g for local and 15.42 - 32.51mg GAE/g for commercial samples [17]. This results similar with TPC value of DMSO extracts of Bursa royal jelly but higher than water and methanol extracts of royal jelly. Özkök and Silici investigated royal jelly samples in Turkey and they found that TPC value of Turkey royal jelly was 59.16 ± 1.94mg GAE/100g (0.5916 ± 0.0194mg GAE/g) [18]. Kolayli, et al. in 2016 investigated lyophilized Anatolian/Turkey royal jelly samples for TPC. They used methanol extracts of royal

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jelly and found that mean and SD value of were 163.9 ± 57.1mg GAE/kg (0.01639 ± 0.00571mg GAE/g) [19]. Both of results of research were lower than TPC value of Bursa royal jelly.

Juszczak, et al. in 2016 suggested that royal jelly couldn't increased antioxidant activity of honey when they used together with royal jelly. Total Flavonoid Contents of royal jelly and honey mixture was 3.48 ± 0.83mg QE/100g (0.0348 ± 0.0083mg QE/g) [20]. This result was lower than water, DMSO and methanol extracts of Bursa royal jelly.

TAA of Anatolian, Bulgarian and Romanian royal jellies were determined as 566.4 ± 238.6µmol Trolox/kg, 3.50 ± 2.41mM Fe^{2+}/g and [19,17,15]. TAA values of local and commercial Romanian royal jellies were measured as 2.20 ± 0.47mM Fe^{2+}/g royal jelly and 1.83 ± 0.24mM Fe^{2+}/g royal jelly. These results were not conformable for comparing TAA results of Bursa royal jelly.

DPPH(% Inhibition) results of Bulgarian royal jelly were found range of 10.17 - 39.39 and average ± SD at 24.23 ± 8.19 [15]. These results were smilare with DPPH results of water and methanol extracts of Bursa royal jelly. DPPH (% Inhibition) results of local and commercial royal jelly of Romania were detected as 32.23 ± 7.59 and 35.94 ± 4.11 [17]. These results were higher than DPPH results of Bursa royal jelly. DPPH value of Lithuania royal jelly was measured 0.82 ± 0.28mg TE/g royal jelly but we couldn't compare it with DPPH results of Bursa royal jelly [16].

Antioxidant activity of royal jelly may originate from its phenolic compounds, small peptids, organic acids and 10-HDA and other fatty acid [19]. Mechanism of antioxidant activity of royal jelly may be illustrated by future investigations.

Conclusion

In conclusion, this study total antioxidant activity of water, DMSO and methanol extracts of royal jelly that obtinated from Bursa were determined. Royal jelly may be a good source as an natural antioxidant product for humans. Composition and antioxidant mechanism of royal jelly extracts can be investigated with futher research.

Conflict of Interest

No conflict of interest associated with this work.

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