Content and Antimicrobial Activities of Bingol Royal Jelly

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Abstract

Royal jelly (RJ) is the special nutriment of the larva of queen honeybee (Apis mellifera) which is composed of several bioactive substances that include amino acids, proteins, carbohydrates, lipids, mineral salts, and vitamins. The content of RJ varies depending on genotype of the bees according to the flora species and climatic conditions and this affects biological activities of RJ. In recent years, literature has perceived an exponential growth in amount of drug (antibiotic)-resistant pathogenic bacteria. The main reasons of growing antibiotic resistance might be credited to the abuse of the antibiotic usage demonstrating prominence of examining other choices other than the communal antibiotics, such as bee products), to avoid a additional build-up in antibiotic resistance. To our knowledge, chemical content and antimicrobial activity of Bingol Royal Jelly (BRJ) has not been investigated to date. Therefore, the purpose of this study was to study composition and antimicrobial activities of BRJ. The results demonstrated that BRJ contains major flavonoids and phenolics such as apigenin, quercetin, naringenin, gallic acid, caffeic acid that contribute antimicrobial and antioxidant properties of BRJ. We have also shown that there are some middle and short chain fatty acids that include Linoleic acid and Propionic acid. BRJ also contain majority of trace elements and mineral. In addition to chemical content, antimicrobial activity of BRJ was also investigated towards pathogens. BRJ showed antimicrobial activity against Salmonella typhimurium (8.64 mm), Escherichia coli (9.1 mm) and Staphylococcus aureus (10.73 mm).

Keywords: Bingol royal jelly, antimicrobial, flavonoids, fatty acids, propionic acid

Bingöl Arı Sütünün İçeriği ve Antimikrobiyal Aktiviteleri

Özet

Arı sütü (AS), amino asitler, proteinler, karbohidratlar, lipitler, mineral tuzları ve vitaminleri içeren çeşitli bioaktif maddelerden oluşan kraliçe balınsı larvalarının (Apis mellifera) özel besleyicisidir. AS içerikleri, flora türlerine ve iklim koşullarına göre arıların genotipine bağlı olarak değişir ve bu, AS’nin biyolojik aktivitelerini etkiler. Son yıllarda, literatürde ilaca (antibiyotik) dirençli patojenik bakteri miktarında aşırı artışa rastlanmaktadır. Antibiyotik direncinin artmasını ana nedenleri, antibiyotik kullanımının kötüye kullanılmasına bağlı olabilir. Bu durum antibiyotik direncinde ek bir birikmeyi önleme için arı ürünleri gibi antibiyotik özellik taşıyan maddelerin diğer seçenekler olarak incelenmesinin önemi ortaya koymaktadır. Bingol AS’nin (BAS) kimyasal içeriği ve antimikrobiyal aktivitesi bugüne kadar araştırılmamıştır. Bu çalışma ile BAS’in içeriği ve antimikrobiyal aktivitelerinin araştırılması amaçlanmışdır. Sonuçlar BAS’nın, antimikrobiyal ve antioksidan özelliklerine katkıda bulunan apigenin, quercetin, naringenin, gallik asit, kafeik asit gibi büyük flavonoidler ve fenolikler içerdığı göstermiştir. Ayni zamanda BAS’nin içeriğinde linoleik asit ve propionik asit gibi bazı orta ve kısa zincirli yağ asitlerinin varlığı da gösterilmiştir. BAS ayrıca eser elementlerin ve mineralin birçoğunu içermektedir. Kimyasal içeriğe ek olarak, BAS’in antimikrobiyal aktivitesi de patojenlere karşı artırılmış ve Salmonella typhimurium (8.64 mm), Escherichia coli (9.1 mm) ve Staphylococcus aureus’a (10.73 mm) karşı antimikrobiyal aktivite göstermiştir.

Anahtar Kelimeler: Bingöl arı sütü, antimikrobiyal, flavonoidler, yağ asitleri, propionik asit
Introduction

Royal jelly (RJ) is a yellow milky sticky bee product which is produced from the mandibular and hypopharyngeal glands of the worker honeybee and is the special nutriment of the larva of queen honeybee (Apis melliífera) (Pavel et al. 2011). It is composed of bioactive compounds such as amino acids, carbohydrates, proteins, lipids, minerals and vitamins (Nagai and Inoue 2004). Considering its potent antioxidant activity, major components of the RJ are flavonoids and phenolic compounds (Yang et al., 2019; Šedivá et al., 2018; Kocot et al., 2018).

In recent years, studies have motivated on the antimicrobial (Coutinho et al., 2018; Park et al., 2019), anti-inflammatory (Yang et al., 2018), anti-diabetic (Khazaei et al., 2018), anti-oxidant (Danis et al., 1994; Asadi et al., 2019; Gu et al., 2018), anti-tumor (Filipič et al., 2015) and anti-aging (Park et al., 2012) activities of RJ from different origins. The physical properties and chemical composition of the bee products vary with the genotype of the bees according to the flora species and climatic conditions and this affects the anticancer, antioxidant and antimicrobial activities of the bee products (Kocot et al., 2018). RJ has been utilised as an alternative therapy with outstanding antimicrobial activities and is presently used as a pharmacological substance (Fratini et al. 2016; Cornara et al. 2017). Accordingly, an enhanced comprehension of the constituents of RJ might further develop the pharmacological and medical usages of RJ as an unconventional medication.

Recent years have witnessed an exponential increase in amount of drug (antibiotic)-resistant pathogenic bacteria (Nugent et al., 2010; WHO, 2012), that slowed down the labours to preserve pathogen-free therapeutic abilities. This augmented the harshness of bacterial diseases and infections. The main reasons of growing antibiotic resistance might be credited to the abuse of the antibiotic usage (Nugent et al., 2010) revealing importance of investigating other choices other than the communal antibiotics, such as bee products, to prevent a further build-up in antibiotic resistance (Noori et al., 2013). To our knowledge, chemical content and antimicrobial activity of Bingol RJ (BRJ) has not been investigated to date. Therefore, the main goal of this study was to study composition and antimicrobial activities of BRJ.

Material And Method

Mineral Content Analysis by ICP-MS

In the study, ICP-MS NexiON® 2000 (PerkinElmer® Inc., USA) device with quartz nebulizer gasifier, cyclonic spray chamber and integrated auto-sampler was used for elemental analysis of the samples. The ICP-MS method was prepared by using a washing solution containing 1% hydrochloric acid-ultra-pure water and approximately 0.2 grams of RJs samples were weighed in the microwave oven. 10 ml HNO3 was added into the sample and burnt in microwave. The solution for ICP-MS calibration was made at the concentrations given in Table 1 by diluting them with commercially available multi-element standards of 1% (HNO3-ultra-pure water). Additionally, ICP-MS calibration experiments were carried out prior to the measurements. 100 ppb 45Sc, 89Y, 209Bi internal standard were used for control of elemental analysis.

Table 1. Calibration standards used in ICP-MS

|       |       |       |
|-------|-------|-------|
| 1. Std  | 0,1 (ppb) | 27Na |
| 2. Std  | 1 (ppb) | 24Mg |
| 3. Std  | 10 (ppb) | 41Ca |
| 4. Std  | 50 (ppb) | 52Cr |
| 5. Std  | 125 (ppb) | 55Mn |
| 6. Std  | 250 (ppb) | 59Co |
| 7. Std  | 500 (ppb) | 63Cu |
| Internal Std | 250 (ppb) | 45Sc |

Fatty Acids Analysis by GC-MS

Hara and Radin (1978) method was used for lipid extraction from RJ. For this purpose, 5 g of RJ was dissolved in 10 mL of hexane/isopropanol (with as ratio of 3:2) for 30 sec at 10 krpm in the homogenizer and centrifuged at 5 krpm for 10 minutes. The supernatant was taken and filtered and put in tubes. Fatty acids require to be derivatised in order to look at GC. Derivation with methyl esters is often preferred. For this aim, Christie (1990) method was preferred because it was practical and highly efficient. According to this method: the above-prepared lipid extract was taken into 30 mL capped tubes to prepare the methyl ester. 5 mL of 2% methanolic sulfuric acid was added and vortexed. This mixture was allowed to methylate in a 50 °C oven for 15 hours. After 15 hours the tubes were removed and chilled to ambient temperature and vortexed by adding 5 mL of 5% NaCl. The fatty acid methyl esters (FAME) formed in the tubes were extracted with 5 mL of hexane and the hexane phase was removed from the top with a pastor pipette and treated with 5 mL of 2% KHCO3 and allowed to stand for 1-2 hours. The solvent of the mixture containing the methyl esters was then evaporated under nitrogen at 45 °C and the fatty acids under the flasks were dissolved with 1 mL of hexane and analyzed on GC-MS by capping amber color GC vials.
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Salmonella typhimurium

Escherichia coli

Staphylococcus aureus

Saccharomyces cerevisiae

(baker's yeast)

The anti-microbial activity was tested using the spread plate agar method, in which 6 mm discs of the samples were placed on plates based on the number of trial agents (Kaya et al., 2018). Using a micropipette, 10 μl each of the RJ at 0.25, 0.5 and 1 mg/ml concentrations and other agents were absorbed into the discs on all plates. During this test, a negative control (sterile pure water), a positive control (penicillin-streptomycin), different concentrations of RJ were tested. After incubation at 37°C for 24 h, the diameter of the inhibition zone was measured. The activity of RJ was tested for antifungal activity against Saccharomyces cerevisiae and antibacterial capability against various pathogenic organisms; Salmonella typhimurium, Escherichia coli, Staphylococcus aureus.

Statistical Analyses

All measurements were repeated three times, and statistical analysis was performed with GraphPad Prism 5.01 software and comparable data sets were evaluated and the analyses were conducted using by one-way ANOVA (Analysis of variance) by Tukey’s multiple comparison test and one-way ANOVA Newman-Keuls Post-Hoc Test with; p<0.05 was considered as significant.

Results and Discussion

Analysis of BRJ Content

The physical properties and chemical composition of the RJ vary with the genotype of the bees according to the flora species, climate and geography (Kocot et al., 2018). RJ produced by Apis mellifera is a highly active biological compound and is likely one of the most interesting bee products (Stocker et al., 2005). The mineral content of Bingol RJ was determined by ICP-MS. The minerals in Bingol RJ and their concentration are given in Table 2. Na (98.525 ppm), Mg (287.237 ppm), Al (8773 ppm), K (2579.161 ppm), Ca (102.488 ppm), Cr (701 ppm), Mn (636 ppm), Fe (20.456 ppm), Cu (144 ppm), Zn (3.188 ppm), Se (76 ppm), Rb (2,960 ppm), Ag (588 ppm). Hg and Pb residues were not found in the contents of Bingol RJ both of which are heavy metals and produce toxicity in human cells and tissues. Trace elements possess a very important role in the biological properties associated with RJ, owing to their numerous biological activities. Detailed literature search revealed that concentrations of 28 trace (Mo, Ti, W, Sb, Sr, Bi, Cr, Al, Ba, Cd, Hg, Pb, Sn, Te, Ni, Ti, V and Co) and mineral (Cu, Mn, Ca, Mg, K, Na, Zn, Fe, P and S) elements were methodically explored in botanically and geographically distinct RJ samples (Stocker et al., 2005).

The vast amount of bee-derived compounds have been investigated in several variety of cancer cell lines and preclinical studies (Kocot et al., 2018). Flavonoids are a diverse chemical class having a widespread range of pharmacological properties some of which include anti-inflammatory, antidiabetic, antimicrobial and anticancer activities. A number of flavonoids particularly chrysin, hesperidin, quercetin, myricetin, rutin hydrate and kaempferol have been widely studied in cancer cell lines and animal models of tumorigenesis (Turk et al., 2019; Taslimi et al., 2019; Caglayan et al., 2019; Arshi et al., 2019; Imran et al., 2019; Demirel Sezer et al., 2019). The flavonoid content of RJ was analysed by HPLC. The major flavonoids determined in RJ are as follow: luteic acid, apigenin, gallic acid, ellagic acid, quercetin, vanillin, caffeic acid, rutin hydrate, catechin, kaempferol and chlorogenic acid (Table 3). In another study RJ was shown to contain
hesperetin, isosakuranetin, naringenin, acacetin, apigenin, and its glucoside, chrysin, luteolin glucoside, isorhamnetin and kaempferol (López-Gutiérrez et al., 2014).

Table 2. Mineral content of BRJ. Concentrations are given as ppm. ND: not detected.

| Element | Concentration (ppm) |
|---------|---------------------|
| Na      | 98.525              |
| Mg      | 287.237             |
| Al      | 8773                |
| K       | 2,579.161           |
| Ca      | 102.488             |
| Cr      | 701                 |
| Mn      | 636                 |
| Fe      | 20.456              |
| Ca      | 144                 |
| Cu      | 3.188               |
| Zn      | 14.145              |
| Se      | 76                  |
| Rb      | 2.96                |
| Ag      | 588                 |
| Hg      | ND                  |
| Pb      | ND                  |

In literature, fatty acids (FAs) have been categorised as long-chain (contain more than 12 C), medium-chain (between 6–12 C), and short-chain (less than 6 C) fatty acids, of which medium-chain fatty acids (MCFAs) exist mostly in the free form. Intracellular fatty acid metabolism possess an main role in regulation of inflammation and excessive amount of reactive oxygen species as oxidative stress causing agents might imitate a pathogen triggered inflammation and promote lipid peroxidation when antioxidant levels are decreased (Wang et al., 2016).

In this study, fatty acid content of the RJ was analysed by GC-MS. Methyl octanoate, Methyl tetradecanoate, Methyl hexadecanoate, Methyl octadecanoate, Methyl 7-octadecenoate, Linoleic acid and Propionic acid are some of the fatty acids present in BRJ (Table 4). The total content of fats and fatty acids in the RJ has been predicted to be between 7–18%. Instead of carboxylic acids between 14–20 C atoms generally identified in animals and plants, the RJ includes short hydroxy FA with 8–12 carbon atoms in the chain and dicarboxylic acids (Nabas et al., 2014; Kocot et al., 2018).

Table 3. Flavonoid and phenolic content of BRJ

| Flavonoid | Concentration (ppm) |
|-----------|---------------------|
| Lutein    |                     |
| Apigenin  |                     |
| Gallic Acid|                    |
| Ellagic Acid|                  |
| Epicatechin|                     |
| Quercetin |                     |
| Vanillin  |                     |
| Caffeic acid|                    |
| Rutin hydrate |                  |
| Catechin  |                     |
| Kaempferol|                     |
| Chlorogenic acid|              |

Table 4. Fatty acid content of BRJ

| Fatty Acid     | Concentration (ppm) |
|----------------|---------------------|
| Methyl octanoate|                   |
| Methyl tetradecanoate|         |
| Methyl hexadecanoate|       |
| Methyl octadecanoate|       |
| Methyl 7-octadecenoate|     |
| Linoleic acid   |                     |
| Propionic acid  |                     |

Antimicrobial Properties of BRJ

Protein and peptides found in RJ has been reported to contribute in defence machinery of honeybee towards pathogenic microorganisms through straight inactivation of microorganism occurring RJ, as well as through stimulation of cytokines involving in regulation of transcription of defensive proteins and/or peptides (Bârnutiu et al. 2011; Al-Abbad 2019). In addition to peptides, flavonoids also contribute microbiological activity of RJ. In one study, some flavonoids that include apigenin, gallic acid, quercetin (which is also found in BRJ) were demonstrated to have antimicrobial effects towards Enterobacter cloaceae, E. aerogenes and Pseudomonas aeruginosa (Basile et al. 1999; Chanwitheesuk et al., 2007; Nitiema et al., 2012). To evaluate antimicrobial activity of BRJ, different concentrations of it were assessed toward different Gram negative/positive bacteria and yeast by disc diffusion method. 1 mg/mL BRJ showed antimicrobial activity against Salmonella typhimurium (8.64 mm), Escherichia coli (9.1 mm) and Staphylococcus aureus (10.73 mm) while antibiotic mixture revealed an inhibition zone of approximately 20 mm for each bacteria (Table 5).
Table 5. Antimicrobial activity of BRJ toward different Gram negative/positive bacteria and yeast by disc diffusion method. Antibiotic zone was measured as cm.

| Bacteria                             | BRJ (1 mg/ml) | BRJ (0.5 mg/ml) | BRJ (0.25 mg/ml) | Negative control (Water) (4) | Antibiotic zone diameter (cm) (5) |
|--------------------------------------|---------------|-----------------|-----------------|-------------------------------|----------------------------------|
| *Salmonella typhimurium* NRRL 4413 (Gr -) | 0.864         | 0.784           | 0.638           | 0                             | 1.918                            |
| *Escherichia coli* ATCC 25922 (Gr -)     | 0.91          | 0.884           | 0.774           | 0                             | 2.024                            |
| *Staphylococcus aureus* ATCC 6538 P (Gr +) | 1.073         | 1.06            | 0.934           | 0                             | 2.072                            |
| *Sacharomyces cerevisiae* (baker's yeast) | 0.658         | 0.685           | 0               | 0                             | 1.803                            |

Royal jelly, secreted from the salivary glands of worker bees, is a special food that influences the development of female bee larvae, where a diet low in royal jelly allows the development of larvae into worker bee adults, but larvae feed sufficient royal jelly instead develop into queen bees. It has been reported that royal jelly has a potential antitumor activity in mice. Studies have demonstrated that RJ possesses anticancer activity by inducing apoptotic and anti-proliferative pathways (Kocot et al., 2018).

Conclusion
Best of our knowledge, chemical content and antimicrobial activity of BRJ has not been studied to date. The aim of this study was to examine composition and antimicrobial activities of BRJ. The results demonstrated that BRJ contains major flavonoids and phenolics such as apigenin, quercetin, naringenin, gallic acid, caffeic acid that contribute antioxidant properties of BRJ. We have also shown that there are some middle and short chain fatty acids that include Linoleic acid and Propionic acid. BRJ also contain majority of trace elements and mineral. In addition to chemical content, antimicrobial activity of BRJ was also investigated towards pathogens indicating antimicrobial properties of BRJ.

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