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# ANTIOXIDANT PROPERTIES OF DMSO AND WATER EXTRACTS OF TURKISH BEE POLLEN

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## ABSTRACT

Bee Pollen is a bee product which has nutritive, anti-inflammatory, atioxidant, antitumoral, antibacterial, cardioprotective, hepatoprotective properties. This study was aimed at investigating antioxidant activity of dimethyl sulfoxide **(**DMSO) and water extracts of Turkish pollens. In this study, the Total Phenol Contents(TPC), Total Flavonoid Contents(TFC), Total Antioxidant Activity(TAA) and Total Antioxidant Capacity(TAC) of DMSO and water extracts of bee pollen which were collected Balıkesir, Bayburt, Erzurum and Trabzon in Turkey were measured using modified Folin-Ciocalteu, Aluminum Nitrate colorimetric method, FRAP assay and commercial kit, respectively. TPC, TFC, TAA and TAC of water and DMSO extract of pollen were found to be 5.29± 0.41 and 18.86 ± 7.98 mg Gallic acid Equivalent (GAE)/g pollen, 2.27 ± 0.14 and 5.66 ± 0.87 mg Quercetin Equivalent (QE)/g pollen, 15.96 ± 0.87 and 51.21 ± 15.23 mg Trolox Equivalent (TE)/g pollen and 0.49 ± 0.07 and 3.47 ± 0.33 mmol Trolox/100 g pollen, respectively. This results suggested that DMSO extracts of Turkish bee pollen have higher antioxidant activity than water extracts of Turkish bee pollen and both of extracts can be used as a good source of antioxidant power.

**KEYWORDS:** Antioxidant Activity, Bee Pollen, Phenol Contents.

## INTRODUCTION

During ancient times, people uses bee pollen as an astrigent, sedative tonic, aphrodisiac and supplementary food cause of nutritive value, and also they use it because of beneficial effects on stomach, bowels and heart (Crane, 1997; Linskens and Jorde, 1997). In 1991, the German Federal Board of Health has officially recognized the use of pollen as medicine. Pollen has been administered in cases of chronic prostatitis, bleeding stomach ulcers and some infectious diseases and has been reported to be helpful (Linskens and Jorde, 1997; Cornara et al., 2017). Because of nutrient and biological properties of bee pollen are used dietary supplements as capsules, tablets and granulates form, cosmetic and pharmaceutical material (Rzepecka-Stojko et al., 2015).

Pollen is used as a food by bees after collected from different plants and mixed it bee saliva (Rzepecka-Stojko et al., 2015). Bee pollen is composed of polyphenolic compounds, flavonoids, proteins (10-40 g/100g dry weight(dw)), essential free amino acids for humans, carbohydrates (13-55 g/100 g dry weight), lipids—especially unsaturated fatty acids (1-13 g/ dw), vitamins (expect Vitamins $B_{12}$, D and K), minerals, dietary fibre and pectins (0.3-20 g/100 g dw), ash (2-6 g/100 g dw) depending on geographic area and climate which was collected (Campos et al., 2008; Arruda et al., 2013; Rzepecka-Stojko et al., 2015; Sattler et al., 2015). Bee pollen has anti-atherosclerotic (Cornara et al., 2017), anti-neoplastic (Campos et al., 1997), antibiotic (Morais et al., 2011; Pascoal et al., 2014), anti-allergic (Medeiros et al., 2008; Ishikawa et al., 2008), anti inflammatory (Pascoal et al., 2014), antidiabetic Cornara et al., 2017), anti-tumoral effects (Omar et al., 2016). Also, it has anabolic effect on bone components (Yamaguchi et al., 2006), cytotoxic and apopitotic effects on different cancer cells (Wu and Lou, 2007; Uçar et al., 2016), antianaemic, hepatoprotective effect (Rzepecka-Stojko et al., 2015), and antioxidant activities (Wu and Lou, 2007; Kroyer and Hegedus, 2001; Campos et al., 2003; Feas et al., 2012). Antioxidant activities of pollen originate from their polyphenolic compounds and flavonoids. Pollen contains considerable amounts of poyphenolic substances such as quercetin, caffeic acid and caffeic acid phenethyl ester(CAPE), pinocembrin, galangin, p-coumaric acid, genistein, kaempherol, apigenin, chrysin etc. which may act as potent antioxidants (Rzepecka-Stojko, 2015; Pascoal et al., 2014; Zhou et al., 2015; Nurdianah et al., 2016).

In this study, the TPC and TFC, the TAA by FRAP and TAC of water and DMSO extracts of Turkish bee pollen were investgated to identify the antioxidant properties of Turkish bee pollen.

## MATERIALS AND METHODS

### Pollen origins

Pollen samples were produced by honey-bees (Apis mellifera L.) in Turkey. Pollen samples were collected from four different cities (Balıkesir, Bayburt, Erzurum and Trabzon in Turkey). Turkey is rich in Picea orientalis, Fagus orientalis, Castanea sativa, Rhodendron ponticum, Rhododendron luteum, Rubus caucasicus (Davis, 1965-1985).

### Chemicals

Dimethyl sulfoxide (DMSO), Sodium carbonate ($Na_2CO_3$), Sodium dihydrogen phosphate dihydrate ($NaH_2PO_4.2H_2O$), Disodium phosphate dihydrate ($Na_2HPO_4.2H_2O$), Potassium acetate ($KCH_3COO$), were supplied from Merck (Berlin, Germany),Iron(III) chloride ($FeCl_3$), Trichloroacetic acid (TCA), gallic acid, quercetin dihydrate, Folin Ciocalteu reagent from Sigma (St. Louis, MO, USA). Ethanol was obtained from Carlo Erba (Milano, Italy). Trolox, Aluminium nitrate nonahydrate ($Al(NO_3)_3.9H_2O$) were supplied from Fluka (Steinheim, Germany) and potassium ferricyanide ($K_3Fe(CN)_6$) was supplied from Lancaster (Morecambe, England).

### Preparation of DMSO and water extracts of pollen

Each natural pollen samples were mixed, grinded (Retsch, ZM 200) and bottled in 5 g portion. Portions were dissolved in 20 mL of DMSO (100%) and pure water by continuous shaking in incubator (Shelleb/Sheldon Mod: 514, USA) at 150 rpm and 60C$^o$ for 24 h. Extracts which were 250 mg/mL concentration obtained by centrifuging at 4000 rpm for 10 min. Working solutions at concentrations of 0, 100, 150 and 200 µg pollen-water/mL and µg pollen-DMSO/mL prepared by diluating stock water and DMSO extracts of pollen with pure water and DMSO.

### Determination of TPC of water and DMSO extracts of pollen

TPC of water and DMSO extracts of pollen were determined spectrophotometrically according to the modified Folin-Ciocalteu method (Singleton and Rossi, 1965). The method is based on the reduction of phosphotungstic acid ($H_3[W_3O_{10}]_4$) in alkaline solution to phosphotungstic blue. The absorbance of formed phosphotungstic blue is proportional to the

number of aromatic phenolic groups and is used for their quantification, with gallic acid as the standard. Briefly, to a volume of 12.5µL of pollen extracts, 62.5µL Folin-Ciocalteu's reagent(which was diluted to 1:10 with pure water before used), and 125µL of 20% $Na_2CO_3$ was added and vortexed. After incubated 30 min in dark and room temperature, the absorbance was mesured three times at 700 nm with Tunable VERSAmax microplate reader (US) against blank. TPC in pollen extracts was expressed as mg GAE/g pollen.

**Determination of TFC of water and DMSO extracts of pollen**

TFC of water and DMSO extracts of pollen were determined spectrophotometrically according to the modified Aluminum Nitrate colorimetric Method which reported by Moreno (Moreno et al., 2000). Quercetin was used to make the standart curve. Ten miligrams of quercetin was dissolved in 80% ethanol and then diluted to 100, 75, 50, 25, 12.5, 6.25, 3.125 ve 1.562µg/mL. Briefly, to a volume of 20 µL of pollen extracts 172µL of ethanol, 4µL of 10% aluminum nitrate and 4 µL of 1M potassium acetate was added and vortexed. After incubation at room temperature for 40 min, the absorbance was measured three times at 415 nm with Tunable VERSAmax microplate reader (US) against blank and TFC of pollen extracts were expressed as mg QE/g pollen.

**Determination of TAA by Ferric Reducing Antioxidant Power (FRAP) in water and DMSO extracts of pollen**

FRAP assay as an easy to use and inexpensive method, which is based on ferric to ferrous ion reduction at low pH. The FRAP values were compared against Trolox, a water soluble analog of vitamin E, as an antioxidant standard compound. FRAP of water and DMSO extracts of Pollen was determined by the method of Oyaizu (Oyaizu, 1986). 40 µL of different concentrations of extracts were mixed with 100 µL of phospate buffer (0.2M, pH 6.6) and 100 µL of 1% potassium ferricyanide $[K_3Fe(CN)_6]$. The mixture was incubated at 50 $C^o$ for 20 min. After the incubation 100 µL of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged for 5 min at 3000 g. 100 µL of upper layer of solution was mixed with 100 µL of distilled water and 100 µL of 0.1% $FeCl_3$, and the absorbance was measured three times at 700 nm with Tunable VERSAmax microplate reader (US) aginst blank and FRAP assay results of pollen extracts expressed as mg TE/g pollen.

**Determination of TAC of water and DMSO extracts of pollen**

TAC of pollen extracts were measured using a colorimetric commercial kit (Rel Assay Diagnostics, Cat no: RL001, Gaziantep, Turkey) produced by Erel, a novel automated

colorimetric measurement method (Erel, 2004). In this method, reduced ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) is oxidized to a characteristic blue-green $ABTSS^{+}$. When the colored $ABTS^{\cdot+}$ is mixed with any substance that can be oxidized, it is reduced to its original colorless ABTS form again; in contrast, the reacted substance is oxidized. The reaction rate is calibrated with Trolox, which is widely used as a traditional standard for TAC measurement assays. The absorbance was measured three times at 660 nm with Tunable VERSAmax microplate reader (US) against blank and TAC assay results of pollen extracts expressed as mmol Troloks/100 g pollen.

## RESULTS

### TPC, TFC, TAA and TAC of water and DMSO extracts of pollen

The amount of TPC, mesured by modifed Folin-Ciolteu method, varied widely and ranged from 5.29 to 18.86 mg GAE/g dw of pollen (Mean ± 1SD). Data are shown Table I. The highest level of phenolics was found in DMSO extracts of pollen and lowest level was found in water extracts of pollen.

**TABLE I: TPC, TFC, TAA and TAC of water and DMSO extracts of pollen. Results were shown orderly mg GAE/g pollen, mg Quercetin/g pollen, mg Trolox/g pollen and mmol Trolox/100 g pollen. Each value represents the mean ±SD (n=3) of three independent experiments.**

| Parameters | Water Extracts of Pollen | DMSO Extracts of Pollen |
|---|---|---|
| TPC (mg GAE/ g pollen) | 5.29 ± 0.41 | 18.86 ±7.98 |
| TFC (mg QE/ g pollen) | 2.27 ± 0.14 | 5.66 ± 0.87 |
| TAA (mg TE/ g pollen) | 15.96 ± 0.87 | 51.21 ± 15.23 |
| TAC (mmol Trolox/ 100g pollen) | 0.49 ±0.07 | 3.47 ± 0.33 |

GAE: Gallic acid equivalents, QE: Quercetin Equivalents, TE: Trolox Equivalents

The amount of TFC, mesured by modified Aluminum Nitrate Colorimetric Method, varied and ranged from 2.27 to 5.66 mg QE/g pollen (Mean ±1 SD) as shown Table I.

DMSO extracts of pollen had higher TFC than water extracts of pollen.

The mesure of TAA of pollen extracts was determinated with FRAP assay, and results was different from 15.96 to 51.21 mg Trolox /g pollen (Mean ± 1SD) in Table I. Likely TFC, TAA results was higher in DMSO extracts than water extracts of pollen.

The value of TAC of pollen extracts determined by commercial kit produced by Erel, varied and ranged from 0.49 to 3.47 mmol Trolox/100 g pollen (Mean ± 1 SD) as shown in Table 1.

## DISCUSSION

In recent years, there has been considerable interest in natural products because of protective and benefical effects on human heath. Bee pollen is another important bee product like propolis or royal jelly. People can use bee pollen as additive food for nutritional value, antioxidative, cardioprotective, hepatoprotective, anti-inflammatory, antibacterial, anticarcinogenic, antianaemic effect and positive influence on osseous tissue (Rzepecka-Stojko et al., 2015). This study is the first which use DMSO for extraction of bee pollen in Turkey and it can give opportunity for comparing data with water extract of bee pollen.

In this study TPC of Water and DMSO extract of pollen were mesuredat 5.29 ± 0.41 and 18.86 ±7.98 mg GAE/g pollen using the Folin-Ciocalteu method, respectively. TPC in water extracts of several samples of Turkish honeybee pollen have been reported different range between at 500.9-1746 mg GAE/100g of bee pollen (5.09-17.46 mg GAE/g pollen) (Kalaycıoğlu et al., 2017). Also, TPC in methanolic extracs of Portuguese bee pollen have been reported different values between at 10.5 - 16.8 mg GAE/g of extract (Morais et al., 2011). Pascoal *et al*, 2014 ,reported that TPC in methanolic extract of eight different commercial bee pollen which collected Portugal and Spain varied from 18.55 - 32.15 mg GAEs/g pollen. Also, Freire *et al.* 2012, reported that TPC in ethanolic extracts of harversted bee pollens which were collected during the nine mount were determined values between 41,5 – 213,2 mg GAE/g.

TFC of water and DMSO extract of pollen were determined at 2.27 ± 0.14 and 5.66 ± 0.87 mg QE/g pollen using the Aluminum Nitrate colorimetric method. Almeida *et al.* 2017, reported that TFC of lyophilized Brazilian bee pollen was 6,81 ± 0,08 mg QE/g. Also, TFC of methanol extracts of Romania bee pollen were determined by Cosmulescu *et al. 2015,* between 7.32-7.95 mg QE/g.

TAA of water and DMSO extract of pollen were mesured at 15.96 ± 0.87 and 51.21 ±15.23 mg TE/g pollen using the FRAP assay. Ulusoy and Kolaylı 2014, determined that TAA of Anzer pollen from Turkey varied from 11.77-105.06 µmol Trolox/g pollen (2.95 – 26.30 mg TE/g pollen). Also, Zuluaga *et al.* 2015, reported that TAA of fresh bee pollen was 75.71 ± 12.74 µmol Trolox /g bee pollen (18,95 ± 3.19 mg TE/g pollen).

TAC of water and DMSO extract of pollen were determined at 0.49 ± 0.07 and 3.47 ± 0.33 mmol Trolox/100 g pollen using commercial kit. Zuluaga *et al.* 2015, measured that TAC of fresh bee pollen which were collected from Colombia was 75.13 ± 16.82 µmol Trolox/g bee pollen (7.51 ± 1.68 mmol Trolox/100 g bee pollen).

DMSO extract of Turkey bee pollen had higher TPC, TFC, TAA and TAC rather than water extract of Turkey bee pollen. It may be related some coumpounds which may be solved in DMSO solved but not in water. Also it is hard to compare of data of other scientists who used different extraction procedure and solvent for antioxidant activity of bee pollen. Bee pollen can not be standardized because of different chemical compositions and biological proporties as for propolis or other bee products. All chemical composition and biological activity of bee pollen may be changed as depend on geographical area and climate which were collected.

In conclusion, this finding shows that both water and DMSO extracts of Turkish bee pollen have clear antioxtidant activity. Bee pollen may be used as an alternative antioxidant natural product. Futher investigation is required to identify chemical compositions, the molecular mechanisms for biological proporties and quality of bee pollen.

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**CONFLICT OF INTEREST**

No conflict of interest associated with this work.

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