Original Article

Oxidant/Antioxidant Status of Propolis Samples Obtained from Turkey

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ABSTRACT

Honeybees produce propolis by collecting resinous material from various plant parts, such as buds, sap flows, leaves, and bark. It is used in traditional medicine and health services due to its biological activity. The content of propolis affects by their geographical and botanical origins and changes in their contents cause changes in their biological effects. Turkey has a rich structure in geography, ecology and climate because it incorporates three different floristic areas in country. These different structures have led to a variety of plants that vary from region to region. Propolis samples for this study were collected from 11 regions across Turkey, including Artvin, Duzce, and Balikesir. Antioxidant measurements were made on propolis samples extracted with a specific method. Total oxidant status and oxidative index were calculated by in vitro analysis, and then compared. The in vitro analyses were performed using newly developed research measurement kits that were extremely sensitive and reliable. In the results of this study, antioxidant capacity has been linked to phenolic compounds. Statistical significance was determined to each propolis samples in different regions. As a result, the total antioxidant capacity of propolis was highest in the Artvin region ($P<0.01$). Antioxidant and oxidant capacities and oxidative stress indices of propolis samples of different regions were determined statistically. This research includes in vitro assays that include highly reliable tests based on very useful and precise measurements. In Turkey variable characteristics of the region were monitored. For this reason, differences were observed in the total antioxidant capacities of propolis samples by region. Honeybees produce propolis, a natural resinous substance, by collecting it from various plant parts, such as buds, sap flows, leaves, and bark. This substance has found application in traditional medicine and health services due to its biological activity. The chemical composition of propolis varies depending on its geographical and botanical origins, with alterations in the constituent components resulting in corresponding changes in biological effects. Turkey's unique geographical, ecological, and climatic characteristics are attributable to the presence of three distinct floristic regions within its borders. The diversity of these regions is reflected in the variety of plant species found in each area. For the present study, propolis samples were collected from 11 regions across Turkey, including Artvin, Duzce, and Balikesir. Antioxidant measurements were made on propolis samples extracted using a specific method. The antioxidant status and oxidative index of the samples were calculated using in vitro analysis and subsequently compared. The in vitro analyses were performed using research measurement kits that were newly developed and characterized by extreme sensitivity and reliability. The study's findings suggest a correlation between antioxidant capacity and phenolic compounds. Statistical significance was determined for each propolis sample from different regions. The Artvin region was found to have the highest total antioxidant capacity of the propolis samples ($P<0.01$). The study further examined the antioxidant and oxidant capacities, along with oxidative stress indices, of propolis samples from diverse regions. The research encompasses in vitro assays, incorporating highly reliable tests based on precise measurements. The study also monitored the variable characteristics of the region in Turkey. Consequently, variations in the total antioxidant capacities of propolis samples by region were observed.

Keywords: Antioxidant, In Vitro Analyses, Oxidant, Propolis, Turkey.

1. Introduction

Honeybees (Apis mellifera) are known to produce propolis, a natural substance composed of collected resinous exudates, flower buds, and various plant parts. The composition of propolis is approximately 50% resins (including flavonoids, phenolics, and their esters), 5% pollen, 30% wax, 10% essential oils, and 5% other organic compounds (e.g., vitamins) (1-5). A more detailed breakdown reveals the presence of polyphenols (flavonoid aglycones, phenolic aldehydes, phenolic acids and esters, ketones, and alcohols), sesquiterpene quinones, coumarins, steroids, amino acids, and inorganic compounds (1, 6). It is important to note that the content of propolis varies depending on its geographical and botanical origins (1, 7). Propolis is a substance with a wide range of biological activities, making it a valuable component in traditional medicine and healthcare. These activities include anticancer, anti-inflammatory, antibacterial, antibiotic, antioxidative, antiviral, and anesthetic properties (8-12). The anti-oxidative effects of propolis contribute to the reduction of cellular damage and oxidative stress (12-14). Erel (2004) proposed a novel Total Antioxidant Status (TAS) method, which has been shown to be more efficient than traditional methods for evaluating antioxidant capacity. These traditional methods are often time-consuming and expensive. This method utilizes an automatic measurement system to determine Total Oxidant Status (TOS) and derive the Oxidative Stress Index (OSI) as a novel indicator of oxidative stress. The OSI is derived from the TAS and TOS values (16). The recent surge in research underscores the mounting interest in the significance of natural antioxidants for human health (17). Numerous studies have demonstrated a consistent correlation between the phenolic content of propolis and its antioxidant properties (7-19). Honeybee-collected propolis demonstrates compositional variations influenced by several factors, including collection time, local vegetation, and collection area (17). These geographical and ecological factors contribute significantly to the overall composition of propolis. The present study investigates the total antioxidant capacity, total oxidant capacity, and oxidative stress index of propolis samples collected from 11 different cities in Turkey.

2. Materials and Methods

2.1. Collection of Propolis Products

Propolis samples were collected from eleven geographically distinct locations across Turkey, encompassing various ecological zones (Table 1). These locations represent diverse regions within the country. These locations are as follows: Artvin-A, Balikesir-B, Duzce-C, Edirne-D, Kahramanmaras-E, Mersin-F, Mugla-G, Nigde-H, Ordu-I, Sivas-J, and Van-K. The samples were collected by beekeepers from these locations throughout 2011. It is acknowledged that propolis production can vary seasonally due to factors like plant availability. The collection of samples in different months is attributed to variations in harvest time, ecological, geographical and climatic conditions. The samples were then stored at a temperature of 4°C until analysis.

2.2. Preparation Of the Ethanol Extract of Propolis (EEP)

The extraction of propolis samples was accomplished through the utilization of 25 mL of 70% ethanol. A sonicator was utilized for a period of 15 minutes in order to achieve a homogenate mixture (Selecta Ultrasons). Following filtration using a Whatman no. 4 paper filter, the mixture was subjected to a process of concentration under reduced pressure at 40°C in a rotary evaporator (Heildolph Heizbad HB Digit). The extracts were then stored for subsequent analysis.

2.3. Biochemical Analyses

This study employed commercially available Rel Assay kits to determine both Total Antioxidant Status (TAS) and Total Oxidant Status (TOS) of propolis samples. As delineated in Erel (2004) (15), the TAS assay utilizes the ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation and its color bleaching capacity. The assay's high sensitivity, with values below 3%, is a notable feature. The results of this assay are expressed in terms of mmol Trolox equivalent per liter (µmol Trolox Eq/L). The TOS determination process involves the utilization of the oxidation of ferrous ions by oxidants present within the sample, as elucidated in Erel (2005) (16). This method is reliant upon the formation of a coloured ferrous-odianisidine complex and its subsequent oxidation by sample oxidants. The oxidation of glycerol molecules also contributes to the reaction. The presence of an orange-coloured ferric ion within an acidic xylenol environment serves as an indicator for the total oxidant content in the sample. The concentration of oxidant molecules is then measured spectrophotometrically based on color intensity. Hydrogen peroxide was used for calibration, with results expressed as micromoles of hydrogen peroxide equivalent per litre (µmol H_2O_2 Eq/L). The oxidative stress index (OSI) is determined by calculating TAS and TOS values. The OSI is calculated using the following formula (16): OSI (optional unit) = TOS (µmol H_2O_2 equivalent / L) / TAC (µmol Trolox equivalent / L).

2.4. Statistical Analysis

The evaluation of all results was conducted by means of the one-way ANOVA method, with SPSS software (Chicago, IL, USA; version 16.0) being utilized for this purpose. The mean differences among the samples were then compared with Duncan's multiple tests and expressed as±SE.

3. Results

3.1. Total Antioxidative Status of Propolis Samples

As illustrated in Table 2, the antioxidant capacities of propolis samples collected from diverse geographical locations are presented. The TAS values of the propolis samples obtained from regions A to K were determined to be 4.53 ± 0.24, 3.32 ± 0.06, 3.19 ± 0.05, 3.19 ± 0.05, 3.17 ± 0.05, 3.19 ± 0.05, 3.77 ± 0.06, 3.18 ± 0.05, 3.32 ± 0.06,

Table 1. Geographic distribution of propolis samples.

Regions	City (Sample Code)
Northeastern Turkey	Artvin (A)
Marmara Region	Balikesir (B)
Black Sea Region	Duzce (C)
Black Sea Region	Ordu (I)
Thrace	Edirne (D)
Southeastern Anatolia Region	Kahramanmaras (E)
Mediterranean Region	Mersin (F)
Aegean Region	Mugla (G)
Central Anatolia Region	Nigde (H)
Central Anatolia Region	Sivas (J)
Eastern Anatolia Region	Van (K)

Table 2. TAS, TOS, and OSI values of propolis samples obtained from different regions of Turkey.

Parameters / Locations	N	TAS (mmol/L)	TOS (μmol/L)	OSI (TOS/TAS)
Artvin(A)	5	4.53±0.038	95.02±1.917	2.10±0.045
Balikesir(B)	5	3.32±0.041	45.99±0.622	1.39±0.026
Duzce(C)	5	3.19±0.042	37.29±0.512	1.17±0.009
Edirne(D)	5	3.19±0.044	21.68±0.216	0.68±0.008
Kahramanmaras(E)	5	3.17±0.032	42.49±0.422	1.34±0.018
Mersin(F)	5	3.19±0.024	45.83±0.670	1.44±0.017
Mugla(G)	5	3.77±0.049	71.69±0.747	1.90±0.039
Nigde(H)	5	3.18±0.038	12.07±0.286	0.38±0.005
Ordu(I)	5	3.32±0.044	59.53±0.918	1.79±0.039
Sivas(J)	5	3.18±0.050	29.08±0.854	0.91±0.026
Van(K)	5	3.22±0.045	18.44±0,586	0.57±0.020
OVERALL	55	3.39±0.055	43.55±3.201	1.24±0.073

3.18 ± 0.05 and 3.22 ± 0.06 mmol Trolox Equivalent/L. In comparison with the TAS data of propolis samples from eleven different regions, the highest TAS level was observed in region A. The TAS value of the propolis sample obtained from the G region was found to be statistically significantly lower than the TAS data of the propolis sample collected from the A region, and it was also found to be statistically significantly higher than the TAS values of the other propolis samples ($P<0.01$). However, no statistically significant differences were observed between the TAS values of the propolis samples from B, C, D, E, F, H, I, J and K ($P>0.01$).

3.2. Total Oxidative Status of Propolis Samples

The TOS values of the propolis samples obtained from regions A to K are presented in Table 2. These values range from 95.02±5.99 μmol H2O2 Equivalent/L in region A to 18.44±5.88 μmol H2O2 Equivalent/L in region K. In comparison with the established TOS data of propolis samples from diverse geographical locations, it was observed that the A region propolis sample exhibited the highest statistically significant TOS value ($P<0.01$). Conversely, the lowest TOS values ($P<0.01$) were detected in the E, H and K propolis samples. There was not a statistically significant difference between the TOS results of the G and I propolis samples ($P>0.01$). Furthermore, no statistically significant difference was observed between the TOS results of propolis samples obtained from the B, E, and F regions ($P>0.01$). However, a statistically significant difference was observed between the TOS results of the propolis samples collected from C and J ($P>0.01$). The TOS data of the G and I propolis samples were found to be statistically significantly higher than the TOS data of the B, E, and F propolis samples ($P<0.01$). Finally, TOS values of propolis were found to be statistically significantly higher in B, E and F regions than in C and J regions ($P<0.01$).

3.3. Oxidative Stress Indexes Status of Propolis Samples

The OSI values of propolis samples collected from different regions were investigated; the OSI results of the A to K propolis samples were found to be 2.10±0.22, 1.39±0.17, 1.17±0.16, 0.68±0.12, 1.34±0.17, 1.44±0.17, 1.90±0.21, 0.38±0.11, 1.79±0.20, 0.91±0.14 and 0.57±0.12 (see Table 2 for details). Upon comparison of the data, it was ascertained that the highest OSI data was observed in A and G ($P<0.01$), while the lowest OSI data was identified in H

(P<0.01). No statistically significant differences (P>0.01) were observed in the OSI values of the propolis samples from B, E and F. However, propolis samples from B, E and F exhibited statistically significantly lower OSI levels in comparison to the propolis sample from I (P<0.01). No statistically significant difference was observed between the OSI values of C and J (P>0.01). Once more, OSI values for the B, E and F propolis samples were higher (P<0.01) than those observed for the C and J propolis samples. No statistically significant difference was observed between the OSI values of samples D and K (P>0.01). However, a statistically significant difference was observed between the OSI values of C and J and those of the D and K samples (P<0.01).

4. Discussion

Turkey's distinctive geography, encompassing three discrete floristic regions, engenders a rich tapestry of plant life that varies regionally. This study explores the potential correlation between this botanical diversity and the antioxidant capacity of propolis samples collected from various regions throughout the country. The findings of the study suggest a correlation between regional variations and the observed differences in propolis antioxidant properties, which may be influenced by the phenolic compounds present in the bees' plant sources. The TAS value of propolis obtained from the A region was observed to be higher than that of the other regions. Conversely, the OSI (TOS/TAS) and TOS values of the propolis sample collected from the H region were found to be the lowest. Consequently, it can be deduced that the antioxidant activity of the propolis sample obtained from the H region is superior to the others. Phenolic acids represent the most prevalent plant metabolites on the planet. Recent research has increasingly focused on the potential protective role of phenolic acids against damage caused by oxidative stress. The analysis of the propolis sample revealed the presence of cinnamic acid, amino acids, terpenes, phenolic acid, phenolic acid esters, flavonoids and caffeic acid. The biological activities of propolis, including its antiviral, anti-inflammatory, and antibacterial properties, are contingent upon the presence of these substances (20, 21). Given the potential for variation in chemical constituents among diverse propolis samples, standardization is imperative for effective comparison and interpretation of biological analysis results (22). A study exploring the antioxidant properties of propolis samples collected from the Erzurum region of Turkey employed various in vitro assays to evaluate phenolic content and antioxidant capacity. These assays encompassed the determination of total antioxidant activity, ferric reducing antioxidant power (FRAP) assay, cupric ion reducing antioxidant capacity (CUPRAC) assay, scavenging activity against superoxide anion radical ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2), and metal chelating activity. Consistent with previous reports (e.g., [22]), our study observed a variation in the antioxidant activity of propolis samples, potentially linked to their phenolic content. Consistent with these observations, other researchers have documented high levels of antioxidant, antibacterial, and antifungal activity in propolis samples from Greece and Cyprus (23). The TAS values of propolis samples collected from 11 different regions in Turkey exhibited significant variations, ranging from 3.17 ± 0.05 to 4.53 ± 0.24 mmol Trolox Equivalent/L. This observation is consistent with the findings of Oses et al. (24), who reported a range of 1184.66 to 1400.86 mmol Trolox/L for the antioxidant activity of undiluted soft propolis extracts. Extensive research has underscored the multifaceted biological activities of propolis. These include its capacity to scavenge free radicals, inhibit tumor cell growth, protect cells against oxidative stress (as evidenced in germinal cells) (20, 21), and reduce the viability of cancer cells by increasing DNA damage. In addition, Watanabe et al. investigated the tumor-inhibiting properties of propolis extracts derived from diverse geographical regions and obtained using various solvents, including ethanol, methanol, and water (25). The present study employed the commercially available Rel Assay kits to assess the Total Oxidant Status (TOS) and Oxidative Stress Index (OSI) of propolis samples collected from eleven distinct regions across Turkey. Statistical analysis was performed to evaluate potential regional variations in the antioxidant capacity (TOS), oxidant capacity (OSI), and overall oxidative stress index of the propolis samples. It is noteworthy that the Rel Assay kits utilize in vitro assays that are recognized for their high reliability and precision in measuring these parameters. The present study investigated the relationship between regional variations in Turkey and the antioxidant capacity of propolis samples. The findings of this study revealed a significant correlation, suggesting that the diverse plant communities across Turkey influence the composition and, consequently, the antioxidant properties of propolis collected by honeybees. These results underscore the pivotal role of geographical origin in shaping the biological activity of propolis.

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Authors' Contribution

Study concept and design: Zeliha Selamoglu and Hasan Akgul

Acquisition of data: Zeliha Selamoglu, Hasan Akgul and Hamide Dogan.

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Critical revision of the manuscript: Muhammad Yasir Naeem, Hamdia Yousif Issa, Betul Ozdemir and Zeliha Selamoglu.
Statistical analysis: Hasan Akgul and Hamide Dogan.

Ethics
It is hereby asserted that all ethical considerations were duly considered during the preparation of the submitted manuscript.

Conflict of Interest
The authors declare no conflict of interest.

Data Availability
The data that support the findings of this study are available on request from the corresponding author.

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