Evaluating Bioactivity and Bioaccessibility Properties of Turkish Propolis Extracts Prepared with Various Solvents

Propolis is rich in polyphenols with a large number of biological activities. Many researchers currently focusing on propolis attributing to its broad spectrum of biological activities and thus considered as a functional food. In vitro propolis digestibility is an important factor on evaluation of biological activity. This study was designed to assess bioaccessibility alterations of water, ethanol, and monopropylene glycol extracts of Turkish propolis by in vitro gastrointestinal digestion. The total content of polyphenols was investigated by Folin–Ciocalteu colorimetric method. Antioxidant activities of extracts were estimated by ferric reducing antioxidant power (FRAP) assays. Significant decreases were founding the total phenolic and antioxidant capacities in the digested fractions when compared to the undigested extracts. Approximately 87 and 91% losses were determined in the total phenolic content and antioxidant activity of propolis extracts after in vitro post gastric digestion, respectively. For extracting bioactive compounds of propolis samples in intestinal digestion stage including dissolved polyphenolic compounds in the absorbed fraction (IN), the most favorable solvent was determined as water (with 1.95±0.08 GAE/g and 6.28±0.55 μmol of Trolox /g propolis).

Keywords: Propolis, Phenolic, Antioxidant activity, in vitro digestion

Propolis is a common biologically active substance, with a wide range of biological activities. Many researchers are currently focusing on propolis due to its broad spectrum of biological activities and considering it as a functional food. In vitro propolis digestibility is an important factor in evaluating biological activity. This study was designed to assess the bioaccessibility alterations of water, ethanol, and monopropylene glycol extracts of Turkish propolis by in vitro gastrointestinal digestion. The total content of polyphenols was investigated using the Folin–Ciocalteu colorimetric method. Antioxidant activities of extracts were estimated using the ferric reducing antioxidant power (FRAP) assay. Significant decreases were observed in the total phenolic and antioxidant capacities in the digested fractions when compared to the undigested extracts. Approximately 87% and 91% losses were determined in the total phenolic content and antioxidant activity of propolis extracts after in vitro post gastric digestion, respectively. For extracting bioactive compounds of propolis samples in the intestinal digestion stage, including dissolved polyphenolic compounds in the absorbed fraction (IN), water was determined as the most suitable solvent (with 1.95±0.08 GAE/g and 6.28±0.55 μmol of Trolox /g propolis).

Keywords: Propolis, Phenolic, Antioxidant activity, in vitro digestion

Abbreviations: TPC, Total phenolic content; GAE, gallic acid equivalent; WE, water extract; ET, ethanol extract; MPG, monopropylene glycol extract; FRAP, ferric reducing antioxidant power.
1. INTRODUCTION

Propolis including resin, wax and essential oils is a resinous substance that bees collect from bud and exudates of plants and transform with enzymes (Viuda-Martos, Ruiz-Navajas, Fernández-López & Pérez-Álvarez, 2008). Bees benefit from propolis as a protective against predators and microorganisms and thermal insulator in the hive. Concurrently, it is used to repair the hive damage and to create aseptic regions to prevent the microbial infection of the larvae (Huang, Zhang, Wang, Li & Hu, 2014). The chemical composition of propolis contains many components which are 50% resin and vegetable balsam (including flavonoids and phenolic acids), 30% waxes, 10% essential and aromatic oils, 5% pollen and other organic compounds (Burdock, 1998; Popova, Graikou, Chinou & Bankova, 2010). It also includes proteins, amino acids, carbohydrates, and vitamins (B1, B2, B6, C, E) and mineral elements.

Propolis, as a healthy and therapeutic product, has been shown to possess antioxidant, anti-fungal, antibacterial, anti-inflammatory, immuno-stimulating, and many other activities regarding its aforementioned nutritional and bioactive properties (Ristivojevic et al., 2018; Torres et al., 2018; Sforcin & Bankova, 2011).

Evaluating the composition and concentration of phenolic compounds that are available in the human gastrointestinal system has critical importance for utilizing bioactive properties and health benefits of propolis. Nowadays, there is a strong tendency to studies about digestibility of bee products in literature. This study is one of the preliminary studies for gain data about relative potential bioaccessibility of different propolis extracts. Although the results obtained with simulated in vitro gastrointestinal digestion do not directly predict the human in vivo conditions, this model is still considered as helpful for investigating the bioaccessibility of polyphenols present in propolis (Ozdal, Ceylan, Eroglu, Kaplan, Olgun & Capanoglu, 2019). This study demonstrates the effect of in vitro digestibility of various propolis extracts i.e. water (WE), ethanol (ET) and monopropylene glycol (MPG) in terms of total phenolic content by using Folin–Ciocalteu colorimetric method and antioxidant activity by ferric reducing antioxidant power (FRAP) assays.

2. MATERIALS AND METHODS

2.1. Chemicals

All chemicals were analytical grade and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), TPTZ (2,4,6-tripyridyl-s-triazine) and Folin–Ciocalteau’s phenol reagent were obtained from Sigma (St Louis, MO, USA) to use in the analysis of antioxidant capacity and total phenolic content.

2.2. Preparation of propolis extracts

All propolis were collected from beekeepers in Trabzon, Turkey and samples were stored in a refrigerator at +4 °C until use. The concentrations of the samples were 8 g of propolis/ 60 mL of solvent. Water, ethanol and monopropylene glycol were used for extraction and this process was carried out on the shaker (Heidolph Promax 2020, Schwabach, Germany) for 24 hours at room temperature.

2.3. Determination of total phenolic content (TPC)

The total phenolic content was determined using the Folin-Ciocalteu method following Singleton, Orthofer & Lamuela-Raventos (1999) and the results were expressed as mg gallic acid/g propolis. In briefly, 680 µL of distilled water, 20 µL of propolis extract and 400 µL of the Folin–Ciocalteu reagent were added in test tube, respectively. The mixture was vortexed for 2 min. After that, 400 µL of sodium carbonate solution (7.5%) was added to the mixture and the absorbance was read after 2 hours at 760 nm in dark. Gallic acid (0-1 mg/ml) was used as a standard to derive the calibration curve.

2.4. Ferric reducing antioxidant power (FRAP) assay

The ferric reducing power of propolis samples was determined based on the method described by Benzie & Strain (1999) with some modifications. The reduction of a ferric 2,4,6-tripyridyl-s-triazine complex (Fe³⁺-TPTZ) to its ferrous, colored form (Fe²⁺-TPTZ) in the
The presence of antioxidants is known as principle of this method. Briefly, The FRAP reagent was prepared by mixing 2.5 mL of a 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl, 2.5 mL of 20 mM FeCl₃, and 25 mL of 0.3 M acetate buffer (pH of 3.6) freshly prepared. An aliquot of 100 μL of propolis extract was mixed with 3 mL of FRAP reagent, and the absorbance of the reagent mixture was measured at 595 nm after incubation for 4 min at 37°C. Methanolic solutions of known Fe(II) concentrations in the range of 31.25-1000 μmol/L (FeSO₄·7H₂O) were used for calibration and the results were expressed as μmol Trolox/ g propolis.

2.5. In vitro digestion assay

In vitro digestion assay of propolis samples was performed based on McDougall, Dobson, Smith, Blake & Stewart (2005). For mechanical digestion in the mouth, 2.5 mL extract and 20 mL with distilled water were mixed. After that different stages of digestion as post gastric digestion (Pg), the dialyzable fraction of intestinal digestion (In) and undialyzable fraction of intestinal digestion (Out) were evaluated. All fractions (Pg, In and Out) were stored at −20°C until analysis.

2.6. Statistical analysis

Statistical significant differences between total phenolic content and antioxidant activity propolis samples were analyzed by one-way analysis of variance (ANOVA) by Duncan’s multiple range test. All analyses were carried out in triplicate, and data were shown as mean ± standard deviation (SD). Differences between means at the 95% (p < 0.05) confidence level were considered statistically significant.

3. RESULTS AND DISCUSSION

Total phenolic content of propolis samples were given in Table 1. The obtained results were varied to be between 168.59 and 753.75 mg GAE/g (Table 1) for undigested samples. The monopropylene glycol extract of propolis showed the highest (p < 0.05) contents of total phenolics. More phenolic compounds were released from ethanolic extracts compared with water extracts of propolis. A large number of existing studies showed that the amount of phenolic compounds in propolis extracts depended on solvent type used in extraction process (Laskar, Sk, Roy & Begum, 2010; Silva, Rodrigues, Feás & Estevinho, 2012; Sun, Wu, Wang & Zhang, 2015).

Table 1 also epitomized the total phenolics in different digestive products of propolis samples. The maximum amounts of total phenolics were noted in post gastric digestion (PG). Total phenolic content losses in post gastric digestion were 84.72, 85.72, and 92.11% for WE, ET, and MPG, respectively. Yesiltas, Capanoglu, Firatligil-Durmus, Sunay, Samanci & Boyacioglu (2014) found 98% loss for post gastric (PG) fraction of different ethanolic propolis extracts. To utilize bioactive properties of propolis, phenolic compounds have to be bioaccessible. Therefore, this in vitro gastrointestinal digestion procedure was also used to evaluate the stabilities and bioaccessibility of phenolics. The bioaccessibility of phenolics calculated as percentage of total phenolic content of in vitro digested sample and total phenolic content of undigested sample ratio. The bioaccessibility results were 10.33, 8.98, and 3.93% for WE, ET and MPG samples, respectively. The low bioaccessibility of propolis in its intact form has been reported in the literature by Yesiltas, Capanoglu, Firatligil-Durmus, Sunay, Samanci & Boyacioglu (2014) and Ozdal, Ceylan, Eroglu, Kaplan, Olgun & Capanoglu (2019).
propolis samples were found almost eight – ten fold more than their samples. Seregliot et al. (2017) demonstrated similar result in comparison to our results.

Table 2 shows total antioxidant capacity of different propolis extracts before and after in vitro digestion. Total antioxidant capacities were statistically different from each other for undigested samples. This difference is related with solvent types. Digestion process has remarkable effect on antioxidant activity likely to total phenolic content. The highest antioxidant activity belongs to MPG post gastric (PG) fraction for ingested fraction (343.34 μmol of Trolox/g). The FRAP values change dramatically as total phenolic content. Those dramatical decreases were attracted the attention of intestinal digestion. Retention percentage of the antioxidant activity in gastric system were lower than 2.0. According to a study conducted by Yen et al. (2017), antioxidant activities losses of total intestinal digestion fractions were found range from 50 to 90%. This study evaluated dialyzable fraction and undialyzable fraction together.

Table 2. The total antioxidant capacity in different digestive products of propolis extracts.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Initial</th>
<th>Post gastric (PG)</th>
<th>Intestinal digestion</th>
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<tbody>
<tr>
<td>WE</td>
<td>915.66±39.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>71.17±0.54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.28±0.55&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ET</td>
<td>1229.33±15.23&lt;sup&gt;d&lt;/sup&gt;</td>
<td>101.57±1.98&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.44±0.87&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MPG</td>
<td>3610.80±66.75&lt;sup&gt;d&lt;/sup&gt;</td>
<td>343.34±6.58&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.87±0.44&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* FRAP value expressed as μmol of Trolox /g propolis. Values were expressed as means ± SD.
Different letters (a-c) in the same columns are significantly different at the 5% level (p < 0.05)
Different letters (A-D) in the same line are significantly different at the 5% level (p < 0.05)

4. CONCLUSION

In conclusion, significant decreases in phenolic content and antioxidant activity were determined for all digestion fractions of propolis extracts. Alcohol derivatives solvents usually extract better bioactive components than other solvents. But, aqueous extracts had the highest these components in intestinal phase in our study. The present low bioaccessibility can be associated with the degree of exposure to acidic conditions, matrix of food containing phenolic compounds, and possible isomerization reactions in phenolic components due to the digestion process. Moreover, in terms of bioaccessibility of phenolic compounds, it is thought that propolis samples have higher potential than other bee products such as pollen, honey.

REFERENCES


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