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A-GLUCOSIDASE AND α -AMYLASE INHIBITION OF SOME ETHANOLIC PROPOLIS SAMPLES

Bazı Etanolik Propolis Örneklerinin α -Glukosidaz ve α -Amilaz İnhibisyonu

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ABSTRACT

Propolis is a natural product, and it is of a great interest due to the possible uses of non-synthetic supplements in improving metabolic disorders. To support this claim, the current study was designed and presented. In this study, six propolis extracts obtained from different location of Turkey were investigated to prove the beneficial therapeutic properties such as inhibition potent against some enzymes and levels of antioxidant. IC₅₀ results of α -glucosidase (0.208-0.426 mg/mL) and α -amylase (0.487-0.938 mg/mL) were found the variable range. Moreover, antioxidant results of them were given to support the inhibition degrees. According to the total phenolic (TPC) and antioxidant data, S4 was noted as the most efficient sample. Future studies are needed to investigate the biological effects of propolis, but the ultimate evaluating showed that it could be a significant source thanks to its nutritional and clinical potential.

Keywords: α -glucosidase, α -amylase, Enzyme inhibition, Antioxidant, Propolis

ÖZ

Metabolik bozuklukların iyileştirilmesinde sentetik olmayan takviyelerin olası kullanımını nedeniyle, doğal bir ürün olan propolis büyük ilgi görmektedir. Bu iddiayı desteklemek için mevcut çalışma planlandı ve ortaya kondu. Bu çalışmada, Türkiye'nin farklı bölgelerinden elde edilen altı propolis ekstratının tedavi amacıyla kullanılabilmesi adına faydalı özelliklerini ortaya koymak için bazı enzimlere karşı inhibisyon etkileri ve antioksidan seviyeleri araştırıldı. α -glukozidaz IC₅₀ sonuçları 0,208-0,426 mg/mL ve α -amilaz IC₅₀ sonuçları 0,487-0,938 mg / mL aralığında bulundu. Ayrıca, inhibisyon etki derecelerini desteklemek için antioksidan aktivite sonuçları da verildi. Toplam fenolik (TPC) ve antioksidan verilerine göre, S4 en verimli örnek olarak kaydedildi. Propolisin biyolojik etkilerini araştırmak için ileride yapılacak yeni çalışmalara ihtiyaç vardır, ancak sonuç olarak, propolisin beslenme ve klinik potansiyeli olarak dikkate değer bir kaynak olabileceğini göstermektedir.

Anahtar Kelimeler: α -glukozidaz; α -amilaz; Enzim inhibisyonu; Antioksidan; Propolis

GENİŞLETİLMİŞ ÖZET

Amaç: Propolis bal arıları tarafından ağaçlardan, ve bitkilerin tomurcuk, yaprak, gövde ve salgılarından toplanan maddelerin başlarında bulunan salgı bezlerinden salgıladıkları enzimlerle işleyerek ürettiği çeşitli miktarlarda esansiyel ve aromatik yağlar, balmumu ve reçine karışımı içeren doğal bir

arı ürünüdür. Kovanı enfeksiyonlardan koruma amaçlı arıların ürettiği propolisin rengi reçinenin kaynağına bağlı olarak açık sarıdan koyu kahverengiye kadar değişebilir. Propolisin çok çeşitli fenolik ve flavanoid maddeler içermesi sebebiyle eski yıllardan beri geleneksel tıpta birçok hastalığın tedavisinde kullanıldığı bilinmektedir. Yapılan birçok

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bilimsel çalışmada propolisin antibakteriyel, antiviral, antioksidan, antienflamatuar, antifungal, antitümör ve antiülser gibi birçok biyolojik aktivitelere sahip olduğu gösterilmiştir. Bilinen birçok hastalığın tedavisi hastalık ile ilişkili enzimlerin inhibisyonu yada tam olarak aktivitesinin durdurulmasıyla mümkün olmaktadır. Bu çalışmada, propolisin sahip olduğu farmakolojik özelliklerinden yola çıkarak Tip-2 diyabet ile yakından ilişkili olan α -amilaz ve α -glukozidaz enzimleri üzerine inhibisyon etkisi ve antioksidan aktiviteleri *in-vitro* olarak incelendi.

Gereç-Yöntem: Ağustos 2019 yılında Türkiye' nin 6 farklı ilindeki (Ankara, Kars, Giresun, Erzurum, Düzce, Zonguldak) Arı Yetiştiricileri Birliği'nden propolis örnekleri temin edildi. Propolis örneklerinin %70'lik etanol içerisinde ekstraktları hazırlandı. Hazırlanan propolis örneklerinin *diabetes mellitus* ile yakından ilişkili olan, özellikle ağız ve midede nişastanın sindiriminden sorumlu α -amilaz enzimine ve bağırsaklarda disakaritlerin sindirimini gerçekleştiren α -glukozidaz enzimine karşı inhibitör etkisi incelendi. Her iki enzim için IC_{50} değerleri (ortamda var olan enzim aktivitesini yarıya düşüren propolis konsantrasyonu) belirlendi. Pozitif kontrol (standart ilaç) olarak akarboz kullanıldı. Ayrıca, ekstraktların toplam fenolik madde miktarı Folin-Ciocalteu metodu kullanılarak gallik asit eşdeğeri cinsinden belirlendi. Propolis örneklerinin serbest radikal temizleme aktiviteleri ABTS [2,2 -azino-bis (3-etilbenzotiyazolin-6-sülfonik asit)] ve DPPH (2,2 difenil1-pikrilhidrazil) yöntemleri kullanılarak belirlendi ve örneklerin bu radikaller varlığında SC_{50} (ortamda var olan radikal miktarının yarısını temizlemek için gerekli olan propolis miktarı) değerleri tayin edildi

Bulgular: Etanolik propolis ekstraktlarının α -glukozidaz ve α -amilaz enzimi varlığında IC_{50} değerleri sırasıyla 0,208-0,426 mg/mL ve 0,487-0,938 mg/mL aralığında bulundu. Propolis örneğinin IC_{50} değeri ne kadar düşük ise enzim inhibisyonunda daha etkili olduğu anlamına gelmektedir. Propolis ekstraktlarının toplam fenolik madde miktarı 123,210 ile 258,815 mg GAE/g örnek aralığında bulundu. Örneklerin etkin derecede ABTS ve DPPH radikallerini temizlediği gözlemlendi. ABTS metodunda en aktif örnek olan S4'ün SC_{50} değeri 0,078±0,001 mg/mL olarak bulundu. DPPH radikal temizleme yönteminde ise örneklerin SC_{50} değerleri 0,412±0,005 ile 0,876±0,005 mg/mL aralığında hesaplandı.

Sonuç: Bu çalışmada, Türkiye'nin farklı illerinden temin edilen propolis örneklerinin yüksek antioksidan aktiviteye sahip olduğu, α -amilazın ve α -glukozidazın enzimatik aktivitesini engellediği gözlemlendi. Önemi her geçen gün daha da iyi anlaşılakta olan ve ender bulunan geniş spektrumlu bir antibiyotik sınıfında adından söz ettiren propolisin, antidiyabetik doğal bir ürün olabileceği söylenebilir.

INTRODUCTION

Propolis is a resinous substance produced by honeybees (*Apis mellifera*) from various leaf buds and plant exudates, which is used to seal and repair unwanted open spaces in the hive. Also, it is superior to other bee products because of its crucial bioactivity content such as antioxidant, antimicrobial, anticarcinogenic, antimutagenic etc. (Baltas et al. 2016, Miguel et al. 2014).

Nowadays and from ancient times, people have used complementary therapies to protect and cope with different diseases. *Diabetes mellitus* (DM) is a metabolic disorder containing multiple etiologies can be characterized by chronic postprandial hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism. The results of this metabolic disorder defects general imbalance between blood sugar absorption, insulin secretion, and insulin action. Two types are described, Type 2 diabetes (TD2) is much more common than Type 1 (TD1). According to the World Health Organization (WHO) and the International Diabetes Federation estimating, the number of total diabetic patients will reach approximately 440 million in 2030 (Mekonnen Abebe and Alemu Balcha 2012, Telagari and Hullatti 2015).

When we examine the reason for the diseases given such as two types, generally it is seen because of the abnormal activity of the relevant enzyme activity in metabolic pathways. These activities should be kept at a reasonable and desirable level. Furthermore, if it is possible, there should be a need to search for new sources from natural compounds.

The previous studies on propolis have mainly focused on bioactivity. Besides the current bioactivity effects of ethanolic extract of some propolis samples; the manuscript at hand is prepared to show the capacity of inhibition degree of α -glucosidase and α -amylase.

MATERIAL AND METHODS

Samples

Propolis samples were supplied from the experienced Beekeepers Association Union in different geographical zones (Ankara, Kars, Giresun, Erzurum, Düzce, Zonguldak) of Turkey. For extraction, 5 g of the powdered propolis was placed with 50 mL 70% ethanol in a glass flask and stirred on a shaker (Heidolph Promax 2020, Schwabach, Germany) at room temperature for 24 h. The suspension was centrifuged at 10.000 g for 15 min, and then supernatants were evaporated. The residue was resolved in minimal volumes of 70% ethanol.

In vitro α -Glucosidase inhibition study

α -Glucosidase from *Saccharomyces cerevisiae* inhibition assay was determined spectrophotometrically (Özil et al. 2018). The enzyme solution 20 U/mL was prepared in phosphate buffer (pH 6.8, 50 mM). In test tubes, 200 μ L of test sample, 5 μ L of the enzyme (20 U/mL) and 1245 μ L of buffer were added and incubated for 15 min at 37°C. After incubation period, 250 μ L of *p*-nitrophenyl- α -D-glucopyranoside (2 mM) was added and change in absorbance was monitored for 20 min at 400 nm in the UV/VIS spectrophotometer (1601UV-Shimadzu, Australia). Acarbose was used as a standard inhibitor. The IC₅₀ value was determined as the concentration of compound that give 50% inhibition of maximal activity.

In vitro α -amylase inhibition study

The inhibition of α -amylase activity was performed according to a previously described method (Unnikrishnan et al. 2015). Briefly, 250 μ L of ethanolic propolis extracts with varying concentrations (20–0.625 mg/mL) and 250 μ L of 0.02 M sodium phosphate buffer (pH 6.9) containing alpha-amylase (porcine pancreatic alpha-amylase) solution (0.5 mg/mL) were incubated for 10 min at 25°C. After pre-incubation, 250 μ L of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) was added to each tube at 5s intervals. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 500 μ L dinitrosalicylic acid color reagent. The tubes were then incubated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was then diluted by adding 2 mL of distilled water, and absorbance

was measured at 540 nm in the UV/VIS spectrophotometer (1601UV-Shimadzu, Australia).

Total phenolic contents (TPC)

Total phenolic contents of the ethanolic extracts of propolis samples were determined following the Folin–Ciocalteu method using gallic acid as standard (Singleton and Rossi 1965). TPC was shown as mg of gallic acid equivalents per g samples (mg GAE/ g sample).

ABTS assay

The ABTS radical scavenging activity of the propolis extracts was measured using the actual method in the literature (Re et al., 1999). ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] was dissolved in water to a 7 mM concentration. To perform the radical cation (ABTS⁺), this stock solution reacted with 2.45 mM potassium persulfate and incubated in the dark for 16–18 h at room temperature. Before using this chemical, the ABTS solution was diluted to get an absorbance of 0.700 \pm 0.020 at 734 nm with phosphate-buffered at pH 7.4. Briefly, 1.8 mL of adjusting solution was mixed 0.2 mL of the sample extract at different concentrations. Test samples were allowed to react with stable free radicals, in the dark, at room temperature for 5 min. After the incubation period, the decrease in optical density (OD) at 734 nm was measured, using a UV–Visible spectrophotometer (1601UV-Shimadzu, Australia).

DPPH-free radical scavenging assay

For DPPH assay, the procedure followed the method of Brand-Williams et al. (1995) with minor modifications. Different concentration ranges of propolis extracts were used for calculation of 50% scavenging of DPPH radical (SC₅₀ – mg of sample per mL). Furthermore, the equal milliliter of propolis extracts and fresh DPPH solution was mixed, and its optical density (OD) was taken at 517 nm after 50 min using a spectrophotometer (1601UV-Shimadzu, Australia). The scavenging activity was calculated by the showing equation in DPPH assay.

RESULTS

In the current study at hand, the results of α -Glucosidase and α -Amylase inhibitory activities of the tested samples were shown as IC₅₀ (mg/mL). The IC₅₀ values of these enzyme activities of analyzed propolis show the efficient different concentration ranges as 0.208-0.426 mg/mL and

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0.542-0.938 mg/mL, respectively (**Table 1**). Although it was even worse than the α -glucosidase inhibition value of acarbose (8.504 ± 0.086 μ g/mL) known as a standard drug, S4 sample showed a significant α -glucosidase inhibition activity as shown in **Table 1**. Furthermore, nearly the same situation with α -glucosidase was seen in α -Amylase results,

even though S2 was the best. This α -Amylase inhibition results could be seen as exciting for delaying the degradation of polysaccharides because its inhibition would decrease the absorption of glucose thus the postprandial blood sugar level would be reduced (Ramnath and Venkataramgowda 2017).

Table 1. IC₅₀ values of the ethanolic propolis extracts for the analyzed enzymes*

Tablo 1. Analiz edilen enzimler için etanolik propolis ekstraktlarının IC₅₀ değerleri *

| Samples | Inhibition of α -glucosidase | Inhibition of α -amylase |
|-----------------------------|-------------------------------------|-----------------------------------|
| | IC ₅₀ (mg/mL) | IC ₅₀ (mg/mL) |
| S1 | 0.334 \pm 0.002 | 0.542 \pm 0.008 |
| S2 | 0.214 \pm 0.005 | 0.487\pm0.006 |
| S3 | 0.292 \pm 0.001 | 0.696 \pm 0.010 |
| S4 | 0.208\pm0.009 | 0.635 \pm 0.008 |
| S5 | 0.328 \pm 0.002 | 0.754 \pm 0.010 |
| S6 | 0.426 \pm 0.002 | 0.938 \pm 0.007 |
| Min-Max. (Min.-Mak.) | 0.208-0.426 | 0.487-0.938 |
| Acarbose | 8.504 \pm 0.086 | 8.504 \pm 0.086 |

*The assays were done in triplicate. Means \pm standard deviations. IC₅₀ value of acarbose was given in terms of μ g/mL.

According to the obtained antioxidant activity results, the total phenolic content of studied propolis samples ranged from 123.210 to 258.815 mg GAE/g sample. Socha et al. (2015) evaluated the antioxidant activity of ethanol extracts of propolis from different regions of Poland. They reported slight differences in their total phenolic content ranged from 150.05 to 197.14 mg GAE/g.

The ABTS and DPPH are the synthetic compounds that involve a proton free radical with a characteristic absorption that decreases significantly upon exposure to radical scavengers (Lee et al. 2015). Although their application, which is based on the reduction of free radicals by an antioxidant resembles each other for the determination of

antioxidant capacity, each of them has different advantages. While the ABTS assay is more sensitive to identifying the antioxidant activity since it has faster reaction kinetics and a heightened response to antioxidants, DPPH may be applied in polar and nonpolar organic solvents, thus hydrophilic and lipophilic antioxidants can be examined (Kedare and Singh 2011, Lee et al. 2015). After shed light on this reality, **Table 2** was summarized as 0.078-0.524 mg/mL and 0.412-0.876 mg/mL for the ethanolic propolis extraction results of the half maximal scavenging concentration (SC₅₀), respectively, in the ABTS and DPPH methods. The SC₅₀ value of S4 propolis was nearly twice lower than that of the nearest values of the other propolis ethanol extracts.

Table 2. Antioxidant properties of the ethanolic propolis extracts*

Tablo 2. Etanolik propolis ekstraktlarının antioksidan özellikleri *

| Samples | Total Phenolic Contents (mg GAE/g) | ABTS Method SC ₅₀ (mg/mL) | DPPH Method SC ₅₀ (mg/mL) |
|-----------|-------------------------------------|--------------------------------------|--------------------------------------|
| S1 | 156.548 \pm 2.392 | 0.288 \pm 0.001 | 0.512 \pm 0.004 |
| S2 | 184.278 \pm 1.086 | 0.347 \pm 0.004 | 0.689 \pm 0.002 |
| S3 | 147.763 \pm 1.540 | 0.315 \pm 0.002 | 0.582 \pm 0.003 |
| S4 | 258.815\pm6.122 | 0.078\pm0.001 | 0.412\pm0.005 |
| S5 | 146.214 \pm 4.016 | 0.314 \pm 0.002 | 0.589 \pm 0.006 |
| S6 | 123.210 \pm 0.895 | 0.524 \pm 0.001 | 0.876 \pm 0.005 |

* The assays were done in triplicate. Means \pm standard deviations.

DISCUSSION

Nowadays, sometimes, the drugs could badly be mentioned due to some reasons such as biological side effects or drug resistance. Especially, drug resistance can be seen in the order: unconscious drug consumption or inadequate resistance against to form-changing diseases. But natural compounds could come to the help of these belligerent effects as drug potentials. For example, the drug potentials of propolis extracts for Type 2 diabetes disease could be controlled with their inhibition effects against α -glucosidase and α -amylase enzymes (Mekonnen Abebe & Alemu Balcha, 2012; Telagari & Hullatti, 2015).

The enzyme inhibition results were in agreement with the study on α -glucosidase and α -amylase inhibitory activities of previous propolis studies. Three of them mentioned that different types of propolis extracts acted as a significant enzyme inhibitor agent (Ramnath and Venkataramgowda 2017, Salah et al. 2017, Vongsak et al. 2015,).

Propolis variably contains some constituents such as flavonoids, coumarins, simple phenols (e.g., thymol and eugenol), and their derivatives (Izuta et al. 2009, Popova et al. 2015). This variation in propolis content is due to direct and/or indirect different conditions such as the collection region, climate, floral origin, processing techniques, storage conditions, seasonal variations, and collection methods (Souza et al. 2016, Popova et al. 2015, Vongsak et al. 2015). It has been demonstrated that the efficiency of the studied enzyme inhibitions related to the amount of polyphenolic constituent which was extracted from the source material.

So that, this prevailing idea could be elaborated, enzyme inhibition effects of phenolic compounds were supported by previous studies. *Gynura medica* leaf was studied for the purpose of isolation and characterization of phenolic compounds which were thought to be an α -glucosidase inhibitory agent. Kaempferol, quercetin, kaempferol-3-O- β -d-glucopyranoside, kaempferol-3-O-rutinoside, rutin, chlorogenic acid, and 3,5-dicaffeoylquinic acid methyl ester were isolated from the leaf of *G. medica*. All the compounds were showed the α -glucosidase inhibitory activity (Tan et al., 2013). Rasouli et al. (2017) evaluated the α -amylase and α -glucosidase inhibitory activity of 26 polyphenols using molecular docking and virtual screening studies. They speculated that caffeic acid, curcumin, cyanidin, daidzein, epicatechin, eridictiol, ferulic

acid, hesperetin, naringenin, pinoselinol, quercetin, resveratrol, and syringic acid were the potent α -glucosidase inhibitors, while catechin, hesperetin, kaempferol, silibinin and pelargonidin were dominant for α -amylase inhibition (Rasouli et al. 2017).

After giving a general opinion about phenolics, the next section where was reported the findings of our study based upon the actual methodologies it was detailed to gather information, was about the antioxidant characterization of ethanolic propolis samples.

Antioxidant activity results could be correlated when the previous researches are considered (Izuta et al. 2009, Ramnath and Venkataramgowda 2016, Popova et al. 2015). Ramnath and Venkataramgowda (2016) employed to assess the ABTS and DPPH radical scavenging potential of ethanol extract of propolis collected from 10 different locations of India successfully and they presented the ABTS and DPPH data in the range of 0.298-0.860 mg/mL and 0.333-0.600 mg/mL, respectively.

CONCLUSION

Complementary medicine, the study of natural products, is one of the major fields of therapeutic approaches, together with phototherapy, aromatherapy, apitherapy, and has been around for an exceptionally long time. We wanted to touch upon the reality of apitherapy because it is known as a virgin scientific area of these therapeutic approaches. Moreover, Turkey has one of the richest sources of apitherapy products in the world.

The current propolis samples demonstrated the ability of antioxidant activity, which were correlated with the assessment of some enzyme inhibition degrees like α -glucosidase, and α -amylase. Obtained results emphasize that this natural compound has massive potential in nutrition and complementary medicine. Further investigations are needed to increase the scientific value of the current results. Namely, potentially phytoactive compounds from propolis can be purified to chemical homogeneity. Then, these potential natural substances can be compared with well-known standard drugs. But the most important way to verify the current reports of *in vitro* inhibitory activities is preclinical studies, particularly using animal models.

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Conflict of Interest

The author declares no possible conflicts of interest.

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