

DETERMINATION OF ANTI-UREASE ACTIVITY OF PROPOLIS FROM THE MARMARA REGION OF TURKEY

Türkiye'nin Marmara Bölgesinden Elde Edilen Bazı Propolislerin Anti-ürez Aktivitesinin Belirlenmesi

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ABSTRACT

Propolis is a natural product collected by honeybees from plants especially flowers and buds by mixing with wax and resin and used for many purposes in hive. It is known that propolis has been used in the treatment of various diseases in traditional medicine for many years and has biological activities such as antioxidant, antimicrobial, antiulcer, antitumor, anti-inflammatory. There is increasing interest in alternative approaches to inhibit *Helicobacter pylori* (*H. pylori*) and thereby treat many gastric diseases. This study investigated various properties of raw propolis samples from the Marmara region of Turkey. Anti-urease activity was investigated in propolis samples. The anti-urease activity of IC₅₀ values ranged from 1.110 to 5.870 mg/mL. As a result, it can be said that propolis extract is a good inhibitor that can be used in the treatment of *H. pylori* to improve human health.

Keywords: Propolis, urease, enzyme inhibition

ÖZ

Propolis, bal arıları tarafından bitkilerden özellikle çiçek ve tomurcuklardan balmumu ve reçine ile karıştırılarak toplanan ve kovanda birçok amaç için kullanılan doğal bir üründür. Propolisin uzun yıllardır geleneksel tıpta çeşitli hastalıkların tedavisinde kullanıldığı ve antioksidan, antimikrobiyal, antiülser, antitümör, antiinflamatuvar gibi biyolojik aktiviteleri olduğu bilinmektedir. *Helicobacter pylori*'yi (*H. pylori*) inhibe etmeye ve böylece birçok mide hastalığını tedavi etmeye yönelik alternatif yaklaşımlara artan bir ilgi vardır. Bu çalışma, Türkiye'nin Marmara bölgesinden alınan ham propolis örneklerinin çeşitli özelliklerini araştırmıştır. Propolis örneklerinde anti-ürez aktivitesi araştırıldı. IC₅₀ değerlerinin anti-ürez aktivitesi 1.110 ila 5.870 mg/mL aralığındaydı. Sonuç olarak propolis ekstraktının *H. pylori* tedavisinde insan sağlığını iyileştirmek için kullanılabilecek iyi bir inhibitör olduğu söylenebilir.

Anahtar Kelimeler: Propolis, ürez, enzim inhibisyonu

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GENİŞLETİLMİŞ ÖZET

Giriş: Mide ülserine genellikle asidik ortamlarda yaşayan ve büyüyen *H. pylori*'nin neden olduğu bilinmektedir. *H. pylori* üreaz enzimi salgılar, üreyi amonyağa dönüştürür ve ürettiği amonyak ile mide asidinin etkilerinden kendini korur. *H. pylori* ve üreazı, kronik gastrit, peptik ülser, mide kanseri ile güçlü bir şekilde ilişkilidir. Üreaz enzimi (E.C. 3.5.1.5.), ürenin karbon dioksit ve amonyağa hidrolizini katalize eden çok spesifik bir enzimdir.

Amaç: Propolis, bal arılarının (*Apis mellifera* L.) ağaç, bitkilerin tomurcuklarından topladıkları öz sularını, kendisinde bulunan salgı bezlerinden salgılanan enzimlerle işleyerek oluşturdukları açık sarı renginden koyu rengine kadar değişim gösteren yapışkan doğal bir üründür. Yapılan araştırmalarda propolisin antioksidan, antibakteriyal, antienflamatuar, antifungal, antitumor ve antiülser gibi biyolojik aktiviteye sahip olduğu bildirilmiştir. Çoğu hastalığın tedavisi onunla ilişkili olan enzimlerin inhibisyonu ile mümkündür. Bu çalışmada, Marmara bölgesinin farklı illerindeki propolislerin potansiyel üreaz inhibitörü olup olamayacağı incelendi.

Gereç-Yöntem: 2018-2019 yılları arasında Türkiye'nin Marmara bölgesinin farklı yerlerinden (Bursa Uludağ Üniversitesi Kampüsü, Bursa (Merkez), Kapıdağ peninsula (Balıkesir), Bandırma, İznik, Marmara adası, İstanbul, Çanakkale, Tekirdağ, Yalova, Sakarya) Arı Yetiştiricileri Birliklerinden propolis örnekleri temin edildi. Herbir propolis örneği %70'lik etanol çözütüsünde ekstraktlar hazırlandı. Hazırlanan propolis örneklerinde üreaz enzim inhibisyonuna karşı inhibitor etkisi araştırıldı. Standart olarak tiyoüre kullanıldı.

Bulgular: Etanolik propolis ekstraktlarının üreaz enzim varlığında IC₅₀ 1.110 to 5.870 mg/mL değerleri arasında tespit edildi. Propolis ekstraktlarının IC₅₀ değeri rakamsal olarak ne kadar düşük ise enzimi inhibe etme oranı da o derece yüksektir.

Tartışma ve Sonuç: Bu çalışmada polifenoller açısından zengin propolis örnekleri için inhibisyon etkileri oldukça güçlü olduğu görülmektedir. Önceki çalışmada, çeşitli Türk propolis numunelerinin anti-üreaz aktivitesi 0.080 ile 1.560 mg/mL arasında değişmekteydi ve yüksek fenolik içerikli numuneler daha yüksek anti-üreaz aktiviteleri sergiledi (Baltaş ve ark., 2016a). Başka bir çalışmada Baltaş ve ark.

(2016b), tüm EPE'lerin *H. pylori* üreazını 0.260 ila 1.525 mg/mL gibi geniş bir inhibitör aralıkla inhibe ettiğini bildirmiştir. Literatürdeki veriler mevcut çalışmadaki verilerden daha farklı görülmektedir. Bunun nedeni propolis örneklerinin alındığı bölgeden ve toplanma şekliinden kaynaklandığını söyleyebiliriz.

H. pylori'ye karşı ortaya çıkan antibiyotik direnci, araştırmacıları ülser iyileşmesini hızlandırmak ve enfeksiyonu ortadan kaldırmak için bir ajan olarak propolisi keşfetmeye teşvik ediyor. Yapılan çalışmalar 15 farklı propolisin üreaz enziminin aktivitesini önemli ölçüde inhibe ettiğini göstermiştir (Baltaş ve ark., 2016a). Üreaz inhibitörlerinin bazı toksik etkileri göz önüne alındığında, doğal bir ürün olan propolis, *H. pylori* ile ilişkili mide-duodenal enfeksiyonu baskılamak ve kontrol etmek için çok güvenli bir kaynak olma potansiyeline sahiptir.

Bu çalışmada, Türkiye'nin Marmara Bölgesinin farklı illerinden temin edilen propolis örneklerinin farklı oranlarda üreaz enziminin aktivitesini engellediği tespit edildi. Hergeçen gün önemi daha çok artan propolisin, *Helikobakteri pilori* bakterisinin sebep olduğu enflamasyon hastalıklarına karşı doğal bir inhibitör olarak kullanılarak ilaçların oluşturmuş olduğu yan etkilerini ortadan kaldırdığına inanılmaktadır.

INTRODUCTION

Bee products such as honey, pollen, propolis have been used for traditional and complementary treatment since ancient times (Sahin, 2016). In particular, propolis, one of the bee products, has antioxidant, antimicrobial, anti-inflammatory and anticancer properties due to the presence of phenolic acids, flavonoids (Catchpole et al. 2015). The chemical composition of propolis varies depending on the season, geographical and botanical origins, and the mechanisms used to collect the material (Bankova, 2005, Ulloa et al. 2017). Until this time, the chemical contents of propolis samples from different parts of the world have been investigated and most of them have been found to have a unique and region-specific phytochemical profile (Stavropoulou et al. 2021). The characteristic constituents of propolis are flavonoids such as chrysin, galangin, pinocembrin, and pinobanksin. Caffeic acid phenethyl ester is a major constituent of propolis with broad biological activities (Alanazi et al. 2021). There are about 250

and even more chemicals in propolis and and most of them are not known sufficiently.

It is now known that gastric ulcer is generally caused by *H. pylori*, which survives and grows in acidic environments (Dunn et al. 1997, Mégraud et al. 1999, Amin et al. 2013). *H. pylori* secretes urease enzyme, converts urea into ammonia and protects itself from the effects of stomach acid with the ammonia it produces. *H. pylori* and its urease are strongly associated with chronic gastritis, peptic ulcers, gastric cancer. The urease enzyme (E.C. 3.5.1.5.) is a very specific enzyme that catalyzes the hydrolysis of urea to carbon dioxide and ammonia (Mareska et al. 2013, Modakh et al. 2015).

H. pylori infection remains a worldwide public health problem, particularly due to current treatment inadequacies. This bacterium is an important cause of ulcer and gastric cancer. Antibiotic treatment is widely used in the treatment of this disease. However, some drugs used in the treatment of this disease have a toxic effect. Increasing antibiotic resistance is one of the reasons why *H. pylori* eradication has failed in most countries (Baltas et al. 2016a). Therefore, it becomes necessary to search for new *H. pylori* urease inhibitors with improved stability and low toxicity in natural products such as plant extracts, honey, pollen, and propolis.

This study intended to survey the potential inhibitor activity of the Marmara Region propolis against urease enzyme inhibition, expanding the therapeutic use of propolis and thus consolidating the medicinal properties of propolis type as a possible new therapy for *H. pylori* eradication. Future studies should be focusing on propolis from different regions to treat different health problems particularly microbial infection problems.

MATERIAL AND METHODS

Chemicals

Jack bean urease, urea, acetohydroxamic acid, sodium nitroprusside and organic solvent were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Merck.

Propolis Samples

Propolis samples from 11 different areas in the Marmara region of Turkey were obtained from experienced beekeepers between 2018 and 2019. The samples, sample codes, and their geographic origin are given in Table 1. Extraction, approximately 3 g each propolis sample was taken and onto 30 mL 70% ethanol was added and stirred on a shaker (Heidolph Promax 2020, Schwabach). After 24 hours of extraction at room temperature, filtration was performed.

Urease enzyme inhibition assay

Urease enzyme inhibition of propolis samples was determined in UV-Vis spectrophotometer. (Weatherburn 1967). In summary, 500 µL of buffer solution (pH 8.2), 200 µL urease enzyme solution and 100 µL sample were incubated for 15 min in test tube. Then a phenol reagent 500 µL, and alkali reagent 600 µL were added to each tube absorbance reading was performed at 625 nm than incubated 50 min. Thiourea was used as standard inhibitor. The IC₅₀ results are given in mg/mL.

RESULTS

Anti-Urease Activity

The urease inhibition potential of propolis extracts was evaluated *in vitro* by Jack bean urease assay. The result of urease inhibitor activity of the test samples was shown as IC₅₀ (mg/mL). The propolis samples totaling 11 belonged to 10 different locations. (Figure 1). Exhibited inhibition effects with IC₅₀ values ranged from 1.110 to 5.870 mg/mL in all samples, and with a mean value of 2.493±1.678 mg/mL (Table 1).

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Table 1. Code, collected region of propolis samples and enzyme inhibition IC50 values.

Code	Regions	Anti-Urease IC ₅₀ mg/mL
Pr1	Bursa Uludag University Campus	1.420±0.090
Pr2	Bursa (Central)	2.230±0.110
Pr3	Kapidag peninsula (Balikesir)	1.542±0.100
Pr4	Bandırma	1.534±0.080
Pr5	İzmit	1.460±0.130
Pr6	Marmara island	1.900±0.070
Pr7	Istanbul	4.600±0.050
Pr8	Canakkale	5.870±0.700
Pr9	Tekirdag	1.200±0.010
Pr10	Yalova	1.110±0.020
Pr11	Sakarya	4.560±0.130
Mean±SD		2.494±1.678
Range		1.110-5.870
Thioure (µg/mL)		12.110±0.090

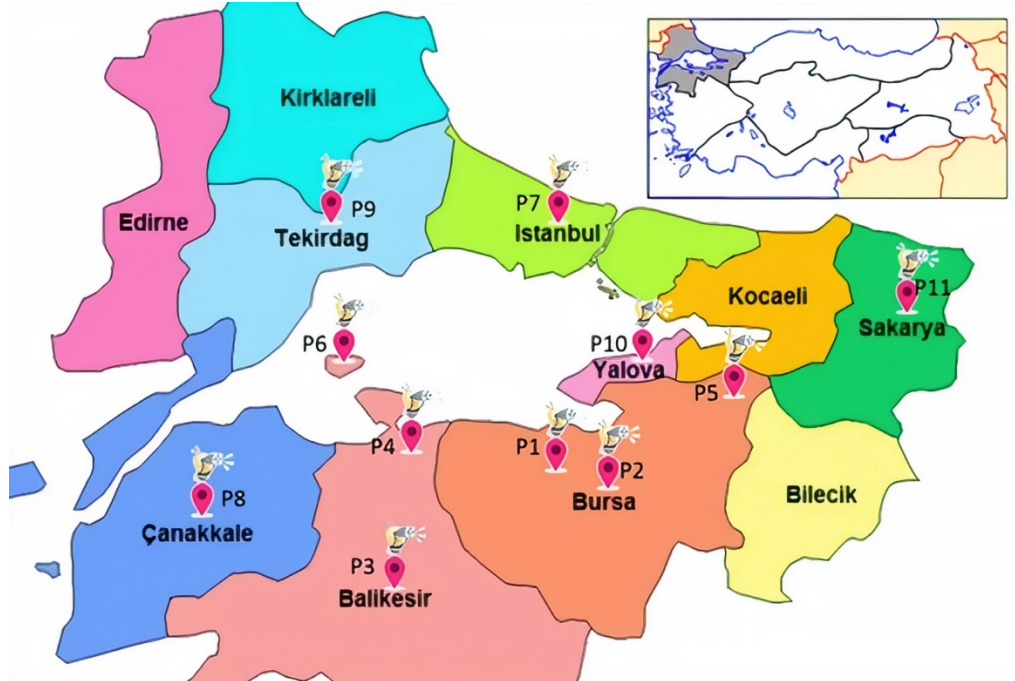


Figure 1. Distribution of propolis samples locations.

(<https://www.alfayapimuhendislik.com/bolge/marmara-bolgesi.html>)

DISCUSSION

The enzyme urease is a nickel-containing metalloenzyme belonging to the hydrolase class. Ammonia released as a result of the breakdown of urea by urease assists the colonization of *H. pylori* by shifting stomach acid to neutral pH (Baltas et al. 2016a). Urease provides *H. pylori* with the opportunity to live in the stomach at low pH, causing gastritis peptic ulceritus, and especially cancer (Morishita et al. 2008). A disease caused by *Helicobacter pylori* can be prevented through urease inhibition (Baltas et al. 2016a). Numerous urease enzyme inhibitors are used as medical drugs, but many are costly and have side-effects (Romeno et al. 2019, Song et al. 2020). In recent years, many investigations have indicated that polyphenol-rich extracts such as bee products (especially propolis) have substantial inhibitory effects (Manyi- Loh et al. 2012).

The inhibition effects are quite strong for propolis samples which are rich in polyphenols in this study. In previous study, the anti-urease activity of various Turkish propolis samples ranged from 0.080 to 1.560 mg/mL, and samples with high phenolic contents exhibited higher anti-urease activities (Baltas et al. 2016a). In another study, Baltas et al. (2016b) reported that all of the EPEs inhibited *H. pylori* urease with wide inhibitory ranges that is from 0.260 to 1.525 mg/mL. This data is a little different from ours. The reason for this, we can say that it is caused by the region where the propolis samples were taken and the way it was collected.

Şahin, (2016) reported that was investigate oak, chestnut and polyfloral honeys the inhibition effect of oak honey was found to be higher than other honeys. Another study, it was reported that chestnut honey inhibition urease with 12.36– 34.20 mg/mL inhibition values (Kolayli et al. 2016). Another study reported that the urease enzyme inhibition IC₅₀ result of buckwheat polen ana chesnut polen 7.41-5.23mg/mL respectively (Can, 2018). It is clearly seen that, propolis a bee product is more effective in urease inhibition compared to other bee products.

The emerging antibiotic resistance to *H. pylori* prompts researchers to explore propolis as an agent to speed up ulcer healing, and eradicate the infection. Studies have shown that 15 different propolis significantly inhibits the activity of urease enzyme (Baltas et al. 2016a). Considering some of the toxic effects of urease inhibitors, propolis, a natural product has the potential to be a very safe

source for suppressing and controlling gastro-duodenal infection associated with *H. pylori*. The future studies should focus on urease inhibitors of propolis from different regions.

Conflict of Interest: The author declares no possible conflicts of interest.

Author contribution: Zehra Can: Laboratory part of the study, evaluation of the results, manuscript writing, submitting to the journal, Yakup Kara: Laboratory part of the study, evaluation of the results İbrahim Çakmak: Design and planning of the study, site selection and sample collection, manuscript editing, and correction, Sevgi Kolaylı: Design and planning of the study

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