Citation/Atıf: Keskin M, Kaya G, Keskin Ş. Green Synthesis and Biochemical Properties of Propolis Based Silver Nanoparticles (Extended abstract in Turkish). U. Arı D. / U. Bee J. 2022, 22:59-67. DOI: 10.31467/uluaricilik.1080096

ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

GREEN SYNTHESIS AND BIOCHEMICAL PROPERTIES OF PROPOLIS BASED SILVER NANOPARTICLES

Yeşil Sentez ile Propolis Temelli Gümüş Nanopartikül Sentezlenmesi ve Biyokimyasal Karakterizasyonu

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Geliş Tarihi / Received: 28.02.2022 Kabul Tarihi / Accepted: 25.03.2022 DOI: 10.31467/uluaricilik.1080096

ABSTRACT

Propolis is a substance collected by honey bees from different parts of plants. Honey bees store it in their hives in order to defend against different threats. Propolis is a bee-product rich in plant waxes, esters, fatty acids, volatile components and phenolic compounds. It`s rich phenolic content makes propolis a potential reducer for Ag^+ to Ag. In this study, propolis-based silver nanoparticles were obtained using the green synthesis technique. It was determined that the obtained silver nanoparticles had a maximum absorbance at 425 nm and their sizes ranged from 67 to 75 nm. When the FT-IR data of propolis extract is examined, the band at 3200 cm^{-1} represents functional -OH groups, the band at 2919-2850 cm^{-1} represents C-H stretching, the band at 1634 cm^{-1} represents C=C, C=O or NH stretching, the band at 1508 cm^{-1} represents aromatic C=C stretching and the band at 1451 cm^{-1} originate from the C-H stretching of CH_3, CH_2, flavonoids and aromatic rings. Total phenolic content of propolis extract and silver nanoparticles was determined as 176.42±0.18 and 122.63±0.23 mg GAE/mL, respectively. IC_{50} value of P-AgNPs for α-amylase and α-glycosidase enzyme inhibition was defined as 47.08 ± 0.002 and 52.18 ± 0.001 µg/mL, respectively. Inhibition of α-Amylase and α-glycosidase is still a valid approach in the treatment of diabetes. The high inhibition effect of the obtained nanoparticles on the related enzymes shows that they have diabetes treatment potential. In addition, showing that cheap and abundant nanoparticles can be obtained by using propolis, this study may contribute to the development of new products containing nanoparticles that can be used in apitherapy applications.

Key words: Green synthesis, Eco-friendly, Diabetes mellitus, Enzyme inhibition

ÖZ

Propolis; bal arılarının kovanlarını farklı tehditlere karşı savunmak amacıyla bitkilerin farklı kısımlarından topladıkları ve kovanlarında depoladıkları bir maddedir. Propolis, bitkisel mumlar, esterler, yağ asitleri, uçucu bileşenler ve fenolik bileşiklerce zengin bir üründür. Zengin fenolik içeriği propolisi potansiyel bir indirgen kılmaktadır. Yapılan bu çalışmada propolis temelli gümüş nanopartiküller yeşil sentez tekniği kullanılarak elde edildi. Elde edilen gümüş nanopartiküllerin 425 nm'de maksimum absorbansa sahip olduğu ve boyutlarının 67 ile 75 nm arasında değiştiği tespit edildi.

ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

Propolis ekstraktı FT-IR verileri incelendiğinde 3200 cm^{-1}'deki bant fonksiyonel -OH gruplarını temsil eder. 2919-2850 cm^{-1}'deki bant CH gerilmesinden, 1634 cm^{-1}'deki bant C=C, C=O veya NH gerilmesinden, 1508 cm^{-1}'deki bant aromatik C=C gerilmesinden ve 1451 cm^{-1} 'deki bant CH_3, CH_2, flavonoidler ve aromatik halkaların CH gerilmesinden kaynaklanır. Propolis ekstraktı ve gümüş nanopartiküllerin toplam fenolik madde miktarları sırasıyla 176,42±0,18 ve 122,63±0,23 mg GAE/mL olarak belirlenmiştir. P-AgNPs'lerin α-amilaz ve α-glukozidaz enzimleri IC_{50} değerleri sırasıyla 47,08 ± 0,002 ve 52,18 ± 0,001 µg/mL olarak tespit edildi. α-Amilaz ve α-glukozidaz inhibisyonu diyabet tedavisinde hala geçerli bir yaklaşımdır. Elde edilen nanopartiküllerin ilgili enzimler üzerine yüksek inhibisyon etkisi diyabet tedavisi potansiyelleri olduğunu göstermektedir. Ayrıca bu çalışmanın, propolis kullanılarak ucuz ve bol miktarda nanopartikül elde edilebildiğini göstermesi, apiterapi uygulamalarında kullanılabilecek nanopartikül içeren yeni ürünlerin geliştirilmesine de katkı sunacağı söylenebilir.

Anahtar Kelimeler: Yeşil sentez, Çevre dostu, Diabetes mellitus, Enzim inhibisyonu

GENİŞLETİLMİŞ ÖZET

Amaç: Nanoteknoloji, tıp, diş hekimliği, ilaç salınım sistemleri, eczacılık gibi birçok biyomedikal uygulamalar ile çevre ve mühendislik alanlarında yaygın olarak kullanılan gelişmiş bir teknoloji dalıdır. Nanopartiküller (1-100 nm boyutunda), nanoteknolojinin temel yapı taşları olarak görülmektedir. Gümüş, altın, çinko gibi metallerin kullanılmasıyla hazırlanan metalik nanopartiküller geniş kullanım alanına sahiptir. Özellikle gümüş nanopartiküller, göstermiş oldukları antimikrobiyal aktiviteleri nedeniyle sağlık uygulamalarında en çok tercih edilen nanopartiküllerden biridir. Nanopartiküllerin sentezinde farklı fiziksel ve kimyasal teknikler kullanılmaktadır. Kullanılan kimyasal teknikler çok miktarda toksik ürün oluşturmaktadır. Bu nedenle çevre dostu üretim tekniklerine ihtiyaç duyulmaktadır. Yeşil sentez çevre dostu ve biyouyumlu nanopartiküllerin elde edilmesini sağlayan hızlı ve düşük maliyetli bir yöntemdir. Genellikle funguslar, bakteriler, algler ve bitkiler yeşil sentezde kullanılan doğal ürünlerdir. Bitkilerin içerdiği fitokimyasalllar da yeşil sentez ile daha kararlı, ekonomik ve geniş uygulama alanına sahip nanopartiküllerin elde edilmesine olanak sağlamaktadır. Bal, polen, propolis ve arı sütü gibi fitokimyasallarca zengin arı ürünleri de yeşil sentez ile nanopartikül elde edilmesine olanak sağlamaktadır.

Gereç ve Yöntem: Çalışmada kullanılan ham propolis örneği 2021 yılında Bilecik ilinden hasat edildi. Dondurulmuş ve öğütülmüş ham propolis örneği %70'lik glikol kullanılarak ekstrakte edildi. Propolis temelli gümüş nanopartikülleri (P-AgNPs) elde etmek amacıyla 5mM $AgNO_3$ ve eşit hacimli propolis ekstraktı karıştırıldı ve reaksiyonun gerçekleşmesi beklendi. Propolis bazlı gümüş nanopartiküller UV-Vis spektrofotometre kullanılarak karakterize edildi. Nanopartiküllerin maksimum absorbans verdiği dalga boyu değeri kaydedildi. Propolis ekstraktının içerdiği fonksiyonel gruplar FT-IR kullanılarak belirlendi. 4500 rpm'de santrifüjlenerek elde edilen nanopartiküller 50°C'de kurutuldu ve partikül boyutları SEM ile belirlendi.

Daha sonra propolis ekstraktı ve P-AgNPs'lerin toplam fenolik madde miktarı tespit edildi. Ayrıca propolis ekstraktı ve P-AgNPs'lerin α-amilaz ve α-glukozidaz enzimleri üzerine inhibisyon etkileri de incelendi.

Bulgular: Yapılan bu çalışmada propolis temelli gümüş nanopartiküller yeşil sentez tekniği kullanılarak sentezlendi. Elde edilen gümüş nanopartiküllerin 425 nm'de maksimum absorbans verdiği ve boyutlarının 67 ile 75nm arasında değiştiği tespit edildi. Propolis ekstraktı FT-IR verileri incelendiğinde 3200 cm^{-1}'deki bant fonksiyonel -OH gruplarını temsil eder. 2919-2850 cm^{-1}'deki bant CH gerilmesinden, 1634 cm^{-1}'deki bant C=C, C=O veya NH gerilmesinden, 1508 cm^{-1}'deki bant aromatik C=C gerilmesinden ve 1451 cm^{-1}'deki bant CH_3, CH_2, flavonoidler ve aromatik halkaların CH gerilmesinden kaynaklanır. Propolis ekstraktı ve gümüş nanopartiküllerin toplam fenolik madde miktarları sırasıyla 176,42±0,18 ve 122,63±0,23 mg GAE/mL olarak belirlendi. P-AgNPs'lerin α-amilaz ve α-glukozidaz enzimleri için IC_{50} değerleri sırasıyla 47,08±0,002 ve 52,18±0,001 µg/mL olarak tespit edildi.

Sonuç: Yapılan bu çalışma ile Bilecik ilinde hasat edilen ham propolis glikol ile ekstrakte edildi ve yeşil sentez tekniği kullanılarak propolis temelli gümüş nanopartiküller elde edildi. Kullanılan teknik nanopartiküllerin ekonomik, çevre dostu, kolay ve hızlı bir şekilde sentezlenebilmesine olanak sağlamaktadır. Elde edilen P-AgNPs'lerin α-amilaz ve α-glukozidaz enzimleri üzerine inhibisyon etkisi olması, partiküllerin Diabetes mellitus tedavisinde potansiyel bir ürün olabileceğini göstermektedir. Ayrıca elde edilen bulguların, nanopartikül temelli farklı ürünlerin geliştirilmesine imkân sağlayabileceği de ifade edilebilir.

INTRODUCTION

Nanotechnology is an innovative technology that is widely used in medicine, dentistry, drug delivery systems, many biomedical applications, environment and engineering. Nanoparticles (1-100 nm in size) are seen as the basics of the nanotechnology (Beykaya and Çağlar 2016). Metallic nanoparticles prepared by using metal ions such as silver, gold and zinc have a wide range of uses. Especially silver nanoparticles are one of the most preferred nanoparticles in health applications due to their antimicrobial activities (Rai et al. 2009). Different physical and chemical techniques are used in the synthesis of nanoparticles. The chemical techniques used create a large amount of toxic products. Therefore, environmentally friendly production techniques are needed (Ali et al. 2016). Green synthesis is a fast and low-cost method that provides environmentally friendly and biocompatible nanoparticles. Generally, fungi, bacteria, algae and plants are natural products used in green synthesis. The phytochemicals contained in plants enable the production of nanoparticles with green synthesis resulting more stable, economical and have a wide range of applications (Mohammadi et al. 2019). Bee products such as honey, pollen, propolis and royal jelly are rich in such phytochemicals and they also could be used to obtain nanoparticles by green synthesis.

Propolis is a resinous natural bee product that worker bees collect from various parts of plants and use to protect their hives against all kinds of dangers (Keskin and Kolaylı 2018). Although the phytochemical content of propolis varies depending on the flora of the region where it is collected, propolis is quite rich in these phytochemicals. Propolis has been found to contain more than 300 different components up to now and it is very rich in volatile (terpene, terpenoid, etc.) and phenolic components (Özkök et al. 2021). Therefore, propolis is a very good product that can be used to obtain nanoparticles.

In this study, silver nanoparticles were synthesized by green synthesis technique using raw propolis samples harvested in Bilecik province in 2021. The synthesized nanoparticles were characterized using UV-Vis spectrophotometer, FT-IR and SEM methods. In addition, inhibition effects of propolis-based nanoparticles on α-amylase and α-glycosidase enzymes, which are important to be inhibited in the treatment of Diabetes mellitus, were determined.

MATERIALS AND METHOD

Raw propolis sample was harvested from Bilecik province in 2021. Silver nitrate, α-amylase, α-glycosidase, glycol, Folin reagent and p-nitro phenyl-α-D-glucopyranoside were purchased from Sigma Aldrich. All other chemicals used were of analytical grade. FTIR (Perkin Elmer), UV spectrophotometer (GENESYS 150) and scanning electron microscope (SEM, EVO 40 LEQ) were used in the characterization processes.

Extraction of Raw Propolis

Propolis extraction was performed according to the method indicated by Yıldız (2020). The frozen and ground raw propolis sample was extracted by using 70% glycol. For this purpose, 20 g of crude propolis sample was mixed with 100 mL of 70% glycol. The mixture was stirred at room temperature under constant speed for 24 hours. At the end of the period mixture was filtered and the obtained extract was stored at +4°C until use.

Green Synthesis of Propolis-Based Silver Nanoparticles (P-AgNPs)

Propolis based nanoparticles were synthesized using the method described by Keskin (2022) with minor modifications. For this purpose, 500 mL of $AgNO_3$ (5 mM) solution and an equal volume of propolis extract were mixed. The mixture was stirred in the dark under constant speed for 24 hours. A color change from light yellow to dark brown was observed.

Characterization of P-AgNPs

Propolis based silver nanoparticles were characterized using UV-Vis spectrophotometer. The wavelength at which the nanoparticles gave maximum absorbance was recorded. The functional groups contained in the propolis extract were determined using FT-IR. The nanoparticles obtained by centrifugation at 4500 rpm were dried at 50°C and particle sizes were determined by SEM.

Determination of Total Phenolic Content of Propolis extract and P-AgNPs

The total phenolic content of propolis extract and obtained nanoparticles were determined by the Folin-Ciocalteu method (Singleton and Rossi 1965, Singleton et al. 1999). The blue colored complex formed by the phenolic components with the Folin reagent gives maximum absorbance at 765 nm. The calibration curve was prepared using gallic acid. Solutions of gallic acid at varying concentrations (0.03125-1.0 mg/mL) were prepared and a graph of absorbance versus concentration was drawn. The total phenolic content of propolis extract and P-AgNPs was calculated using this graph and the results were expressed in mg GAE/mL

Inhibition Properties of P-AgNPs on α-Amylase

α-Amylase enzyme activity was determined by DNS method in the presence of soluble starch as substrate. 300 µL of 1% soluble starch and 300 µL of enzyme were incubated at 35ºC for 30 minutes. After adding an equal volume of DNS reagent, the mixture was kept in a boiling water bath. At the end of the reaction, tubes were cooled to room temperature and the absorbance values were recorded at 550 nm (Bernfeld 1955). The IC_{50} values of propolis extract and P-AgNPs were determined in triplicate with concentration between 10 to 200 µg/mL under the above-mentioned analysis conditions. Acarbose was used as a reference inhibitor.

Inhibition Properties of P-AgNPs on **α-Glycosidase**

α-glycosidase enzyme activity was determined by using the method specified in the Gholamhoseinian et al. (2008). *p*-nitro phenyl-α-D-glucopyranoside was used as substrate. 5 µL of substrate, enzyme solution (0.1 U) and 900 µL of phosphate buffer 6.8 (50 mM) were mixed. The mixture was incubated at 37 °C and absorbance values at 405 nm were recorded. The IC_{50} values of propolis extract and P-AgNPs were determined in triplicate with concentration between 10 to 200 µg/mL under the above-mentioned analysis conditions. Acarbose was used as a reference inhibitor.

RESULTS

In this study, propolis-based silver nanoparticles were synthesized using green synthesis technique. It was determined that the obtained silver nanoparticles gave maximum absorbance at 425 nm. The size of the beads varied between 67 and 75nm.

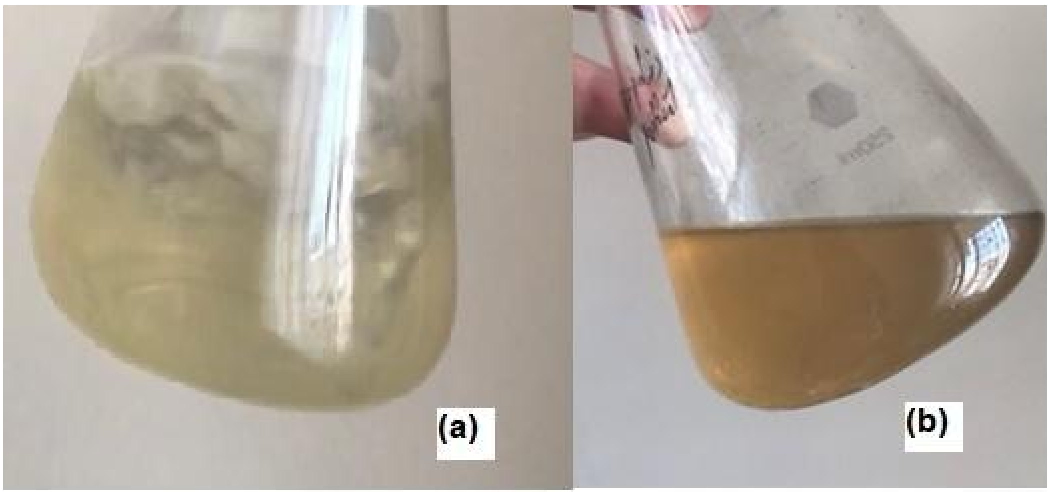

Figure 1. a) Propolis extract b) P-AgNPs synthesis

Şekil 1. a) Propolis ekstraktı b) P-AgNPs sentezi

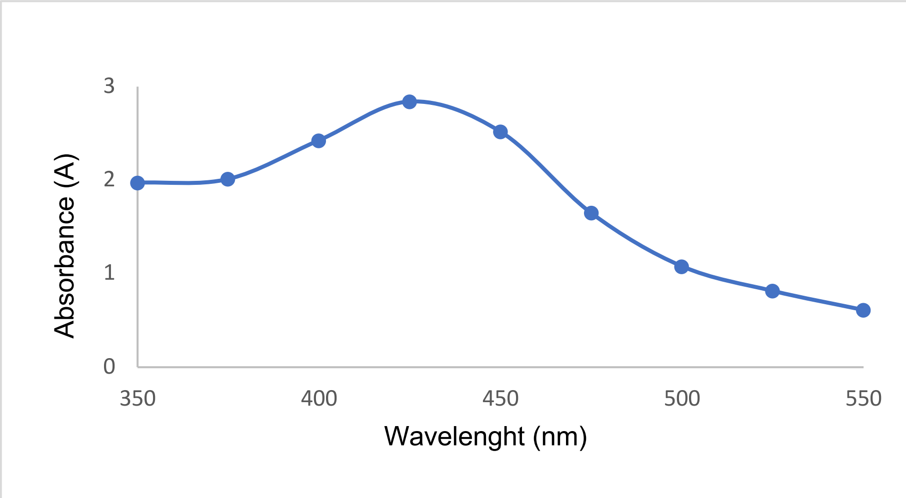

Figure 2. UV-VIS spectrum of P-AgNPs

Şekil 2. P-AgNPs UV-Vis spektrumu

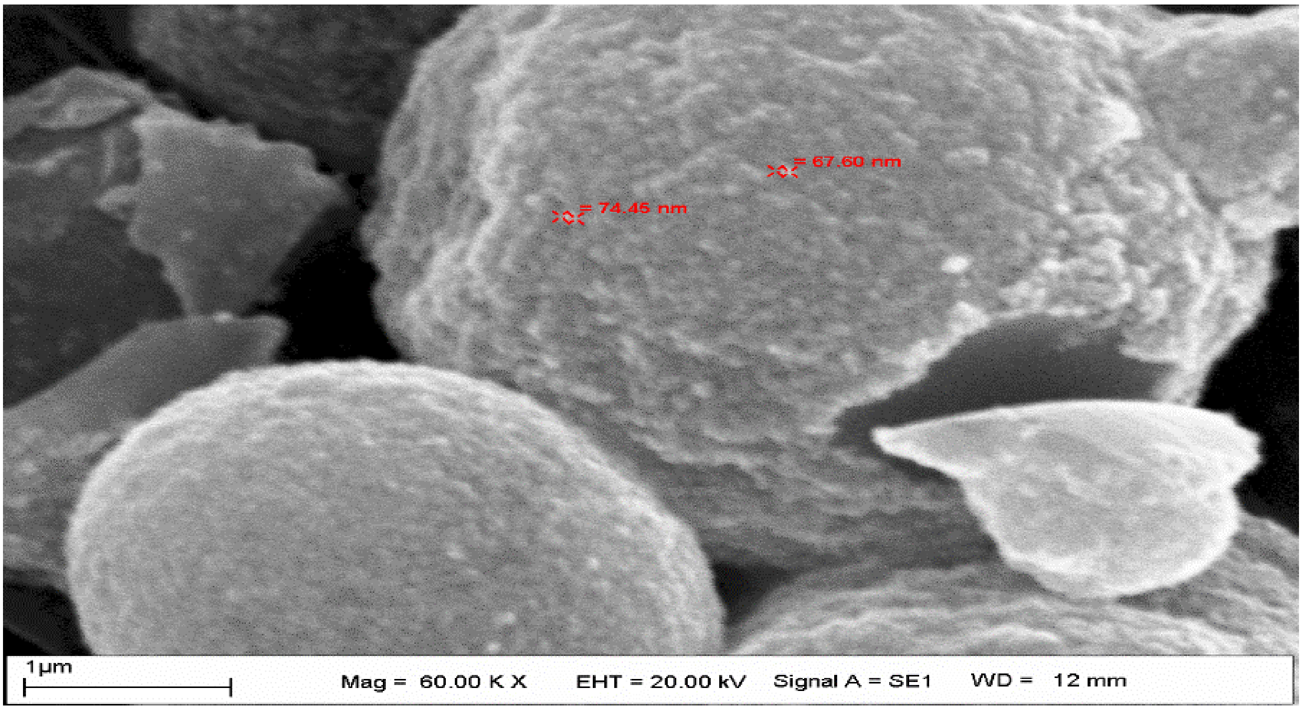

Figure 3. SEM data of P-AgNPs

Şekil 3. P-AgNPs SEM verisi

When the FT-IR data of propolis extract was examined, the bands could be explained with the presence of functional -OH groups at 3200 cm^{-1}, the C-H groups at 2919-2850 cm^{-1}, the C=C, C=O or NH groups at 1634 cm^{-1}, the aromatic C=C bonds at 1508 cm^{-1} and the CH_3, CH_2, flavonoids and aromatic ring C-H groups at 1451 cm^{-1} (Corciova et al. 2019).

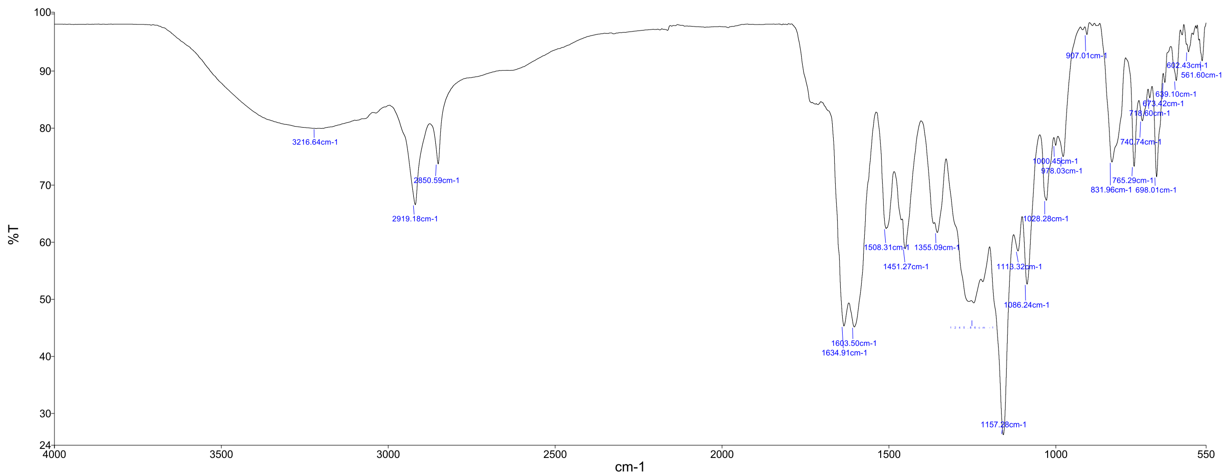

Figure 4. FT-IR data of propolis extract

Şekil 4. Propolis ekstraktı FT-IR verileri

Results for the inhibition effects of P-AgNPs on α-amylase and α-glycosidase enzymes and total phenolic content of propolis extract and P-AgNPs were summarized at Table 1. Lower IC_{50} values obtained for P-AgNPs indicating better inhibition activity than both propolis extract and acarbose, a standard inhibitor.

Table 1. Biochemical properties of propolis extract and P-AgNPs

Tablo 1. Propolis ekstraktı ve P-AgNPs'lerin biyokimyasal karakterizasyonu

	Total Phenolic Content mg GAE/mL	**α-amylase IC_{50}(µg/mL)**	**α-glucosidase IC_{50} (µg/mL)**
Propolis Extract	176.42±0.18	59.78±0.001	67.43±0.001
P-AgNPs	122.63±0.23	47.08±0.002	52.18±0.001
Acarbose		96.7±0.42	96.7±0.42

DISCUSSION

Propolis is a natural substance rich in phenolic compounds especially as flavonoids. Since phenolic compounds and flavonoids are high-capacity natural reducing agents, they play a role in AgNPs green synthesis ($Ag^+ \rightarrow Ag^0$). In this study, it was observed that the inhibition effect of P-AgNPs on α-amylase and α-glycosidase enzymes was achieved with a lower IC_{50} value than propolis extract. Two possible reasons for this situation can be expressed as the easy ability of silver to transfer electrons and the high abundance of biomolecules on the AgNPs surface (Corciova et al. 2019). Al-Fakeh et al. (2021)

synthesized silver nanoparticles with green synthesis from propolis sample harvested from Saudi Arabia. It was stated that the synthesized nanoparticles gave maximum absorbance at 437 nm and had a higher total phenolic substance content than raw propolis. Corciova et al. (2019) stated that the silver nanoparticles they synthesized gave a maximum absorbance of around 480 nm and the sizes of the nanoparticles ranged from 10 nm to 50 nm. A quite low total phenolic content of the supernatant remaining after the silver nanoparticle production was expressed by the authors because of the fact that the components in the propolis extract take part in the reduction of silver. Priyadarshini et al. (2018) obtained propolis-based silver nanoparticles by green synthesis. It was stated that the particle size of the obtained nanoparticles, which gave maximum absorbance at 420 nm, was smaller than 100 nm but highly variable. In a study, Al-Yousef et al. (2020) reported the synthesis of bee pollen-based silver nanoparticles. It was stated that the synthesized nanoparticles gave maximum absorbance at approximately 440 nm and the particle sizes vary between 10-30 nm on average. Debnath et al. (2019) synthesized silver nanoparticles using mushroom extract and investigated the effects of obtained particles on α-amylase. They stated that the obtained nanoparticles gave maximum absorbance at 420 nm and the particle sizes ranged between 2-20 nm. It was also stated that silver nanoparticles have an inhibitory effect on α-amylase enzyme and the percentage of α-amylase inhibition increases with the use of nanoparticles with increasing concentration. Ramkumar et al. (2010) synthesized Gymnema Montanum-based silver nanoparticles and investigated the effects of nanoparticles on α-amylase and α-glycosidase. It was declared by the authors that the obtained nanoparticles inhibited α-amylase and α-glycosidase enzymes with IC_{50} values as 5 µg/mL and 7 µg/mL, respectively. Johnson et al. (2018) obtained Bauhinia variegate-based silver nanoparticles and determined their inhibition properties on α-amylase enzyme. It was stated that the nanoparticles obtained in that study gave maximum absorbance at 430 nm and the particle size was reported to be ranged between 5-15 nm. The IC_{50} value for α-amylase enzyme was declared to be 4.64 µg/mL. Synthesis of silver nanoparticles based on *Enhalus acoroides* and effects of the particles on the α-glycosidase enzyme was reported by Senthilkumar et al. (2016). It was stated that the obtained particles gave maximum absorbance at 419 nm, their sizes varied between 2-100 nm, and the IC_{50} value for the α-glycosidase enzyme was 47 µg/mL. Variable IC_{50} values of AgNPs on α-amylase and α-glycosidase enzymes have been reported in literature. This variation might be the result of used enzyme type. It is clear that our results are compatible with the literature data's.

Conclusion

In this study, crude propolis harvested in Bilecik province was extracted with glycol and propolis-based silver nanoparticles were obtained by using green synthesis technique. The technique used enables nanoparticles to be synthesized economically, environmentally friendly, easily and quickly. The inhibition effect of the obtained P-AgNPs on α-amylase and α-glycosidase enzymes shows that the particles have a potential in the treatment of Diabetes mellitus. It can be concluded that synthesis of silver nanoparticles by using propolis extract is a suitable way for the development of different nanoparticle-based products.

Authors Contributions: MK: Analyzed data, wrote the manuscript and organized the study **GK**: Analyzed data and wrote the manuscript **ŞK**: Analyzed data and edited the manuscript.

Conflicts of Interest: There is no conflicts of interest.

Ethics Approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Funding: There is no funding for this study.

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