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Synthesis, Characterization and Antidiabetic Potential of Bee Pollen Based Silver Nanoparticles

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Abstract: Bee pollen is both an important food source and a part of apitherapy applications because of its chemical components such as carbohydrate, lipid, vitamins and phenolics. In this study, environmentally friendly, biocompatible bee pollen-based silver nanoparticles (BP-AgNPs) were synthesized by utilizing the potential reducing powers of the components contained in bee pollen. The resulting nanoparticles were characterized using spectroscopic and microscopic techniques. It was determined that silver nanoparticles gave maximum absorbance at 348 nm. It was observed that the particle sizes ranged between 40-60 nm on average. The inhibition effect of the obtained BP-AgNPs on α-amylase and α-glucosidase enzymes was investigated for the first time. IC_{50} values were determined as 2.56 ± 0.10 and 2.13 ± 0.11 mg/mL for α-amylase and α-glucosidase enzymes, respectively. It was determined that obtained BP-AgNPs could be used as a support in the treatment of Diabetes mellitus.

Keywords: Green synthesis, enzyme inhibition, nanoparticle, Diabetes mellitus, eco-friendly

Arı Poleni Bazlı Gümüş Nanopartiküllerin Sentezi, Karakterizasyonu ve Antidiyabetik Potansiyeli

Öz:. Arı poleni içerdiği karhohidrat, lipit, vitaminler ve fenolik bileşenler sayesinde hem önemli bir besin kaynağı olmakta hem de apiterapi uygulamalarında yer almaktadır. Yapılan bu çalışma ile arı poleninin içerdiği bileşenlerin potansiyel indirgeme güçlerinden faydalanılarak çevre dostu, biyouyumlu arı poleni temelli gümüş nanopartiküller sentezlendi (BP- AgNPs). Elde edilen nanopartiküller spektroskopik ve mikroskobik teknikler kullanılarak karakterize edildi. Gümüş nanopartiküllerin 348 nm' de maksimum absorbans verdiği tespit edildi. Partikül boyutlanırınn ortalama 40-60 nm arasında değiştiği görüldü. Elde edilen BP-AgNPs' in α-amilaz ve α-glukozidaz enzimleri üzerine inhibisyon etkisi araştırıldı. IC_{50} değerleri α-amilaz ve α-glukozidaz enzimleri için sırasıyla 2.56 ± 0.10 ve 2.13 ± 0.11 mg/mL olarak tespit edildi. Elde edilen BP- AgNPs'in Diabetes mellitus tedavisinde destekleyici olarak kullanılabileceği belirlendi.

Anahtar Kelimeler: Yeşil sentez, enzim inhibisyonu, nanopartikül, çevre dostu

1. Introduction

Nanotechnology is a technology that allows studies such as processing, measurement, design, modeling and editing performed on materials in 1-100 nanometer sizes [1]. It is a new and rapidly developing field that aims to provide technologically advanced or completely new physical, chemical and biological properties at the atomic and molecular levels. Recently, the interest in nanotechnology has increased. Nanotechnological research is the most popular topic in nearly all of the scientific field, such as physics, electronics, communication, energy production, textile,

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medicine, food and health. Nanotechnology has promising new applications gaining a commercial dimension in industrial prototyping [2,3]. In addition, it is thought that nanotechnology will have an effect on the prevention of diseases in agriculture, the rapid elimination of existing diseases and the ability of plants to absorb nutrients from the soil [4]. Antibacterial and odor-repellent textile products, anti-scratch car paints, dirt-repellent coatings, transparent protective sunscreens, self-cleaning glasses are some of the products in which silver nanoparticles are used [5]. These developments indicate that nanotechnological products will take more place in our lives from now on.

There are different chemical and physical methods for the synthesis of silver nanoparticles [6]. Physical techniques such as vapor deposition and ball milling often require high pressure, high temperature and complex tools. In chemical synthesis methods, toxic chemicals and stabilizing agents that are harmful to human health are used during synthesis [5]. Although the controlled production of nanoparticles size and obtaining morphologically high quality nanoparticles are important in chemical synthesis methods, the obtained nanoparticles are not suitable for biological applications [7]. Therefore, it is important to obtain biocompatible and environmentally friendly nanoparticles [5-7].

Green synthesis of nanoparticles is the technique that provides the most environmentally friendly and biocompatible products. Many biological resources are used in the synthesis of metallic nanoparticles as an environmentally friendly method. Recently, the synthesis of metallic nanoparticles using biological sources has attracted attention due to its ease of application, environmental friendliness and low cost [5-7]. Generally, natural products such as fungi, algae, bacteria and plants are some materials used in green synthesis [8-10]. In green synthesis more and faster nanoparticles could be obtained when compared to other techniques. In addition, these particles are biocompatible for medical applications [11, 12]. At the same time, it has been reported that nanoparticles obtained by extracting natural products are stable, economical and have wider biological activity thanks to their phytochemicals [13]. These phytochemicals are not only increase the bioavailability of the biomaterial but also enable the synthesized nanoparticles to bind to different biomolecules and biosurfaces [11]. Since bee products such as pollen, royal jelly, beeswax and honey obtained from honey bees are rich in phenolics, they are thought to be promising for obtaining nanoparticles in green synthesis [14,15].

Pollen, which is the reproductive cell of flowering plants, plays an important role in the nutrition of honey bees. It has high protein content so it provides the development of brood, muscle and digestive systems of young bees and the production of royal jelly [16]. Plant pollen is collected by honey bees, after adding digestive enzymes, it is stored in the third pair of legs and brought to the hive [17]. Bee collected pollen has a rich content in terms of proteins, carbohydrates, amino acids, lipids, sterols, terpenes, phenolic substances and vitamins, [18]. Studies have shown that bee pollen has antimicrobial, anti-inflammatory, antitumoral, antifungal and antioxidant properties. It has also been reported to have therapeutic properties such as antiprostatic, antianemic, antiatherosclerotic, antiosteoporosis, antiallergic and liver protective [19].

Free oxygen radicals produced in cells as a result of cellular metabolism cause aging of cells and slowdown of metabolic activities [19]. Antioxidants, on the other hand, reduce free oxygen radicals, prevent and delay oxidation in cells and protect the organism against degenerative diseases [20]. Natural antioxidants and especially flavonoids, play an important role in these therapeutic effects [21]. The main biological effect of bee pollen is due to phenolic acid derivatives and polyphenolic compounds as well as nutritional substances in its structure [22]. Because of these properties, bee pollen is collected with the help of pollen traps attached to the hive entrances and offered for human consumption as a food supplement [19,23]. Total phenolic content is an important parameter for the

characterization of the bee pollen extract. The total phenolic content of bee pollen collected from different geographical regions also differs. For this reason, it is seen that total phenolic content of pollen samples varies on a wide scale [14-18].

Figure 1. Bee pollen samples

Diabetes mellitus is a metabolic and degenerative disease with long-term effects. Blindness, heart attack, lower limb amputation and kidney failure complications are the main cause of Diabetes mellitus [24]. Type-2 diabetes is the dominant form and inhibition of pancreatic amylase and glucosidase can be effective in controlling postprandial sugar levels in patients with type-2 diabetes [24]. There are many studies showing that bee pollen can be used as a supplement in the treatment of Diabetes mellitus [19, 23, 24].

In this study, an aqueous extract of bee pollen was prepared and silver nanoparticles were obtained from the prepared extract by green synthesis (BP-AgNP). The characterization of the formed nanoparticles was characterized by UV spectrophotometer, FT-IR and SEM imaging techniques. Then, the effects of BP-AgNPs on α-amylase and α-glucosidase enzymes, which have an important role in the treatment of Diabetes mellitus, were investigated for the first time.

2. Experimental Methods

2.1. Materials

Bee pollen samples were supplied from local beekeepers in Amasya city, Turkey in 2021. Silver nitrate, α-amylase and α- glucosidase were purchased from Sigma Aldrich, USA. All other reagents were analytical grade. Perkin Elmer one branded Fourier Transform Infrared spectroscopy (FT-IR), Thermoscientific GENESYS 150 branded UV-visible spectrophotometer (UV-Vis.) and EVO 40 LEQ Scanning Electron Microscope (SEM) were used for the characterization of BP- AgNPs.

2.2. Methods

2.2.1. Preparation of Bee Pollen Extract

Frozen bee pollen sample was powdered by grinding and 20 g of this fine powder was mixed with 200 mL of distilled water. Extraction was carried out for 72 h on a magnetic stirrer under constant stirring at 150 rpm. Finally, mixture was filtered and filtrate was stored at +4 $^{\circ}$C.

2.2.2. Green Synthesis of Bee Pollen Based Silver Nanoparticles (BP- AgNP)

BP-AgNPs were synthesized according to reference [25] with minor modifications. For this purpose pollen extract and 0.1 M silver nitrate ($AgNO_3$) solution was mixed in a dark flask at a 1:1 ratio (v/v).
The mixture was stirred for ~2 h at room temperature. The changes of color to dark brown was noted and confirmation of nanoparticle synthesis was applied by UV absorption spectroscopy between 250 and 550 nm.

2.2.3. Characterization of BP- AgNPs

The formation and presence of AgNPs were determined by a UV-Vis spectrophotometer. The absorbances were read by scanning the wavelength in the spectrophotometer device. FT-IR device data were analyzed in order to evaluate the functional groups of the bioactive components responsible for the reduction. At the end of the synthesis, centrifugation was performed at 9000 rpm with a high-speed centrifuge device to precipitate AgNPs from the aqueous medium. The resulting particles were dried at 75°C. Morphological appearances and particle sizes were determined by SEM.

2.2.4. Total phenolic content of pollen extract and BP-AgNPs

The Folin method is based on the formation of colored complexes of phenolic substances with the Folin Ciocalteu reagent and is the most widely used method for the measurement of total phenolic substances in natural products [26, 27]. The blue colored complex formed by phenolic substances with the foline reagent has a maximum absorbance at 765 nm. A calibration curve was prepared by using gallic acid (GA) as the standard phenolic compound [26, 27]. A standard graph was drawn with the measured absorbance values of gallic acid against methanol solutions at different concentrations (1.0; 0.5; 0.25; 0.125; 0.0625 and 0.03125 mg/ mL). The total phenolic content of the extracts was calculated according to the drawn graph and the results were expressed in mg GAE/ mL.

2.2.5. Inhibition Properties of α-amylase

α-amylase activity was assayed in the presence of soluble starch as substrate. Reducing ends were determined according to DNS method described in reference [28]. 300 µL of 1% soluble starch and 300 µL of enzyme solution were incubated for 30 min at 35ºC. An equal volume of DNS reagent was added into tubes and kept in a boiling water bath. The absorbance of the tubes was recorded at 550 nm. The analyse was performed in triplicate. IC_{50} value of the extract and BP-AgNPs were determined at five different concentrations under standard assay conditions and dose response curve was generated. Acarbose was used as reference inhibitor [28].

2.2.6. Inhibition properties of α- glucosidase

α- glucosidase enzyme activity was performed according to the method described by reference [29]. Enzyme reaction was carried out in 0.1 M pH 6.8 phosphate buffer using p-nitrophenyl-α-D-glucopyranoside as substrate. 5 µL of substrate, enzyme solution (0.1 U) and 900 µL of phosphate buffer (50mM) were mixed. The mixture was incubated at 37 °C and the absorbance values at 405 nm were recorded [29]. For the inhibition study, extracts in five different concentrations were added into the reaction medium and enzyme activity was calculated and dose response curve was generated for IC_{50} determination.

3. Results and Discussion

3.1. Synthesis and Characterization

After mixing the bee pollen extract and silver nitrate solution, it was observed that the color changed from light yellow to dark brown after about 2 hours. During the formation of AgNPs, color change occurs with vibrations occurring on the plasma surface due to reduction [30]. Maximum absorbance values were found at 348 nm wavelength (Figure 2). In similar studies, the maximum wavelengths at 350 nm [31], 390 nm [32] and 430 nm [33] and the color change from yellow to brown [34] were associated with the formation and presence of AgNPs.

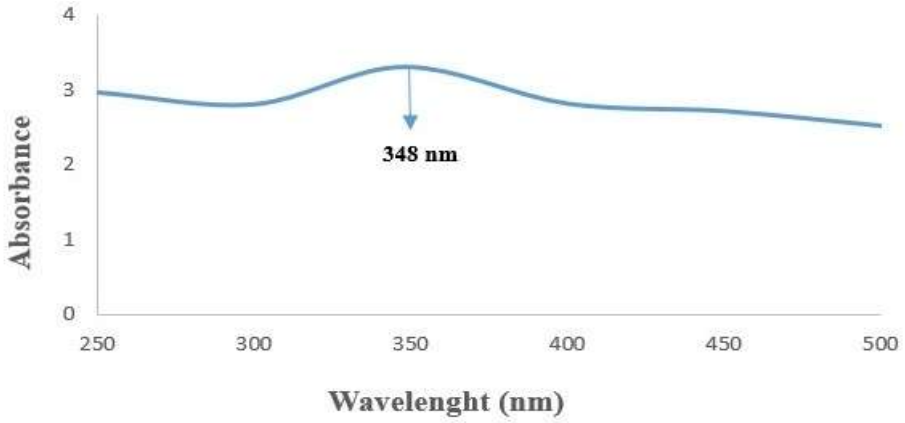

Figure 2. UV spectrum of BP- AgNPs.

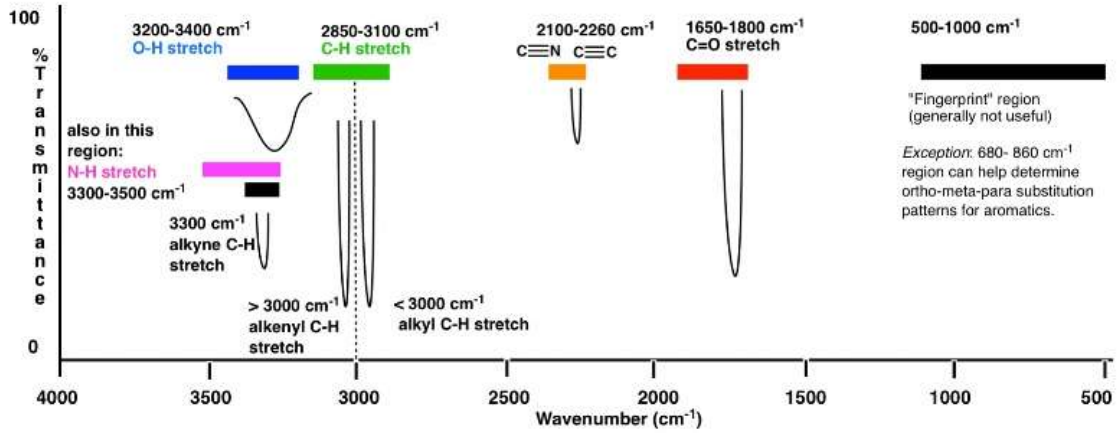

Figure 3. General infrared absorption for different types of bonds [35]

The FT-IR spectrum analysis of silver nanoparticles shows absorption peaks. The possible potential functional groups responsible for the reduction of silver ions to silver nanoparticles were identified using FT-IR analysis. IR absorption of different types of bonds were summarized in Figure 3. The band at 3200 to 3400 cm^{-1} represents O─H stretching groups and the band at 3300 to 3500 cm^{-1} presents H bonded NH stretching. The band at 1650 to 1800 cm^{-1} assigned to be C=O stretching group. It could be said that secondary metabolites such as phenolic compounds could possibly play a major role in the synthesis and stabilization of the metal nanoparticles.

As seen in Figure 4, the pollen extract has a widespread peak around 2160 cm^{-1}, while in the presence of nanoparticles it has branched peaks between 2350-1990 cm^{-1}. Also it is seen that there are peak shifts around the 1176.24 and 1080.92 cm^{-1}. These differences are indicative of the formation and presence of BP-AgNPs and support the UV data.

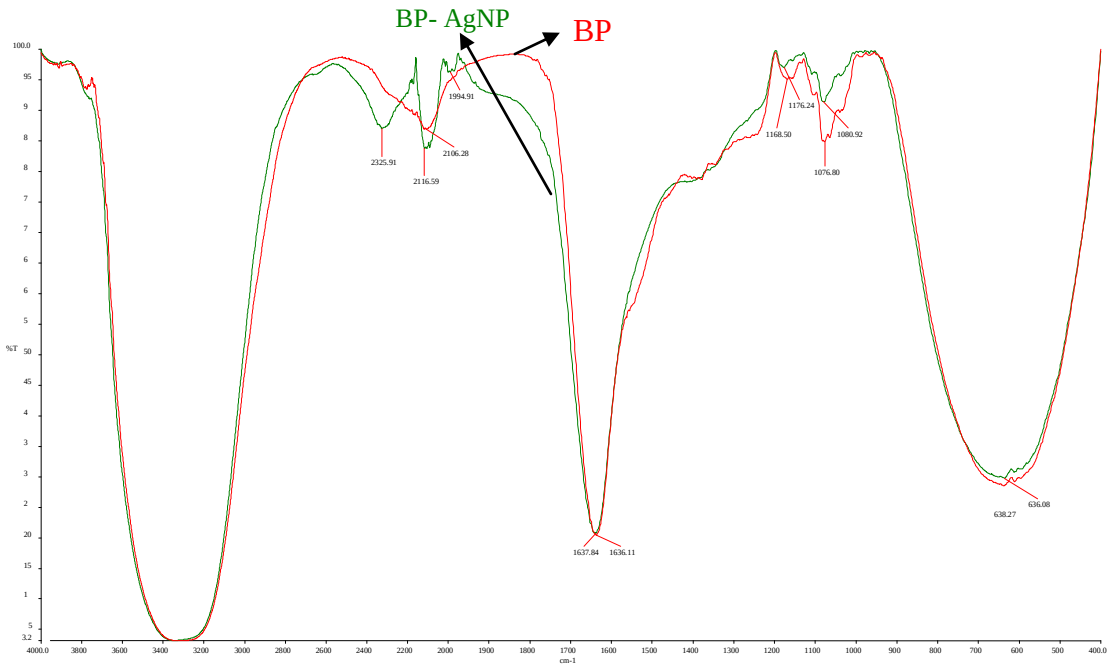

Figure 4. FT-IR spectrum of pollen extract and BP- AgNPs

The shape and size of BP-AgNPs were determined by using SEM and obtained image was given in Figure 5. The obtained nanoparticles were found mostly spherical and they had an average size of 40-60 nm. The size should be ranged in 1-100 nm for nanoparticles. It was seen that the obtained findings were compatible with the literature data [36- 41].

Figure 5. SEM images of BP- AgNPs

3.2. Total Phenolic Content and Enzyme Inhibition Properties

In this study, the effect of BP-AgNPs on Diabetes mellitus related enzymes was investigated. Results were summarized in Table 1. It is clearly seen that promising inhibition effect of BP-AgNPs on tested enzymes was obtained. The effect of bee pollen on Diabetes mellitus has been shown by in vivo and in vitro studies [37, 38]. The findings of the present study are compatible with these literature data.

Table 1. Total phenolic content and enzyme inhibition properties of BP- AgNPs

	Total Phenolic Content mg GAE/mL	**α-amylase IC50(mg/mL)**	**α-glucosidase IC50 (mg/mL)**
Bee pollen extract	8.18 ± 0.02	6.32 ± 0.10	6.83 ± 0.10
BP- AgNPs	12.07 ± 0.03	2.56 ± 0.10	2.13 ± 0.11
Acarbose		7.32 ± 0.12	7.32 ± 0.12

In the literature, there are studies showing that silver nanoparticles obtained by using different herbal sources are effective on Diabetes mellitus. In a study, antidiabetic activity of silver nanoparticles obtained with *Allium cepa* extract was reported. According to findings of that study, α-amylase and α-glucosidase enzymes were highly inhibited by silver nanoparticles [32]. In another study, *Musa paradisiaca*-based silver nanoparticles were synthesized and Rats with controlled diabetes were fed with these nanoparticles. Authors concluded that silver nanoparticles were effective on diabetes [39]. Alkhalaf et. al.; reported the green synthesis and antidiabetic activity of *Nigella sativa*-based silver nanoparticles. It was stated that nanoparticles were very effective on mice with Diabetes mellitus [31]. In another study, silver nanoparticles were obtained using *Ocimum basilicum* and *Ocimum sanctum* water extract. Antidiabetic activity of silver nanoparticles obtained was compared and inhibition properties of α-amylase and α-glucosidase enzymes were determined in vitro. Accordingly, it was stated that AgNps obtained by using *O. sanctum* and *O. Basilicum* water extract respectively displayed an inhibitory effect at 89.31%, and 79 %, respectively [40]. The effect of *Solanum nigrum*-based AgNPs on diabetic Rats was investigated. It was stated by the authors that dyslipidemia was significantly controlled with the obtained nanoparticles [41]. In a study, silver nanoparticles were obtained in the presence of *Halymenia poryphyroides*. The effect of nanoparticles on α-amylase and α-glucosidase enzymes was determined in vitro. Accordingly, it was declared that nanoparticles had a significant effect on these enzymes. It was also stated that inhibition of both enzymes increased with the increased amount of nanoparticles [42]. It is clear that AgNPs have promising potential in the treatment of Diabetes mellitus.

4. Conclusions

Bee pollen is an important bee product that bees collect from plants and treat with their own secretions. This product, which is very rich in protein, lipid, essential fatty acids, amino acids and phenolic components, has an important place in apitherapy applications. BP-AgNPs were synthesized with environmentally friendly green synthesis in order to better benefit from the active ingredients of this product, which is widely used as a food supplement in the treatment of diabetes mellitus, and to increase its effect. Accordingly, it was observed that the obtained particles inhibited α-amylase and α-glucosidase enzymes better than bee pollen extract. These enzymes have an important place in the treatment of Diabetes mellitus. The findings of both present study and literature data can be concluded that AgNPs have promising potential in the treatment of Diabetes mellitus.

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Authors' Contributions

MK was designed and performed all studies. MK also wrote the article. The author read and approved the final manuscript.

Competing Interests

The author declares that she has no competing interests.

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