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Humulus lupulus L. (hops) BASED SILVER NANOPARTICLES: SYNTHESIS, CHARACTERIZATION AND ENZYME INHIBITION EFFECTS

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ABSTRACT

Silver nanoparticles (AgNPs) are well known to have antimicrobial activity, but very little is known about the effect of AgNPs on various enzyme activities. They (AgNPs) are valuable metal nanoparticles that exhibit exceptional properties compared to their bulk materials. *Humulus lupulus* L. (hops) is an important medicinal aromatic plant used in industry. It has many compounds such as phenolic, flavonoids, tannins, etc. In this study, green syntheses of *Humulus lupulus* L. based silver nanoparticles were performed. Accordingly, it was determined that HL-AgNPs gave maximum absorbance at approximately 450 nm and nanoparticle sizes ranged from 30.60 nm to 36.72 nm. The potential peaks of the prepared aqueous extract and HL-AgNPs were determined using FTIR-ATR. It was determined that the synthesized nanoparticles gave 2296.89 cm^{-1}, 1161.05 cm^{-1}, 1112.34 cm^{-1} peaks. Total phenolic content of HL-AgNPs was determined as 30.62 ±0.02 mg GAE/mL, and DPPH· radical scavenging activity IC_{50} value was determined as 4.4 ±0.01 mg/mL. Inhibitory effects of HL-AgNPs on α-amylase, α-glycosidase and urease enzymes were studied and IC_{50} values were determined as 3.10 ±0.01 mg/mL, 9.42 ±0.02 mg/mL and 0.76 ±0.01 mg/mL, respectively. The synthesized *Humulus lupulus* L. based silver nanoparticles showed better biochemical activity than the prepared *Humulus lupulus* L. aqueous extract. It is clear that it is possible to use HL-AgNPs obtained by green synthesis in various biomedical applications.

Key words: green synthesis, nanoparticles, urease, α-amylase, α-glycosidase

INTRODUCTION

Humulus lupulus L. (hops) is an important perennial herb used in industry. This herb belongs to the *Cannabinaceae* family. It is winding and climbing as in kiwi and vine [Bocquet et al. 2018, Keskin et al. 2019]. Every year, winding branches grow and their length can reach up to 8–10 m. The flowers of the plant are in the form of cones (Fig. 1). These flowers contain glands that secrete bitter and aromatic substances. The length of the cone varies between 2–6 cm and its width varies between 1.5–3.5 cm. In its composition, there is a substance called lupulin, which consists of resin-essence mixture chemicals. This substance, found at the bottom of the mature cone leaflets, is in the form of yellow powders [Bocquet et al. 2018].

The perennial part of the plant is the roots and rhizomes under the ground. The underground part can live up to 100 years. Its wrapper stems, which can grow up to 8–10 m in height, die together with the leaves in winter, and new stems take their place the next year. Therefore, 3–4 weeks after the harvest, the shoots are removed from the plant by pruning from the soil. The root system can go down to a depth of 4 m. Studies show that hops have the potential to be used in different fields such as medicine, pharmacy,

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Fig. 1. *Humulus lupulus* L. [Bocquet et al. 2018]

nanotechnology, biotechnology, thanks to the valuable primary and secondary components it contains [Bocquet et al. 2018].

Nanotechnology is a scientific field for the characterization, production and application of nanoscale particles, 1 to 100 nm in size. Nanoparticles are widely used in physical, biological and pharmaceutical applications [Beykaya et al. 2016]. Especially, silver nanoparticles are used in many fields from weaving, textile and carpet industry to pharmaceutical industry due to their rapid penetration through the cell membrane and their antibacterial, antivirus and antifungal effects. Physical, chemical and biological synthesis methods of silver nanoparticles have been described in many literatures. The use of dangerous chemicals, which are quite expensive and may pose potential environmental and biological risks, are the most basic factors in not preferring physical and chemical methods in silver nanoparticle synthesis. Also, chemical synthesis methods can in most cases cause the absorption of some toxic substances on the surface, hindering its medicinal use. Obtaining silver nanoparticles by bio-based synthesis is an alternative to chemical production due to its green synthesis and stable structure [Chung et al. 2016]. Unlike the cost of silver nanoparticles synthesized by chemical and physical methods and the use of various toxic chemicals during production, their ease of application, non-toxicity and rapid synthesis make bio-based production a more preferable option. In order for biogenic silver to compete with chemically produced nano silver on the market, it must be produced at the same price or cheaper. This can be achieved by choosing the right natural source that can be used in production of biogenic silver with the best efficiency. Chosen natural source determines the functionality, shape and size and the antimicrobial, toxicological and catalytic properties of the produced nanoparticle. Therefore, microorganisms and plants are alternative natural sources for silver nanoparticle synthesis [Chung et al. 2016].

Today, the synthesis process of advanced materials from the atomic or molecular scale to the macroscopic scale emphasizes the ordered formation of atoms and molecules. Nanostructured materials, which are generally characterized by physical size, surface and interface between 1 and 100 nm; it is attracting a lot of attention because of its proven or hoped for unique properties compared to known materials [Chung et al. 2016].

In this study, *Humulus lupulus* L. based silver nanoparticles were obtained by using cheap and environmentally friendly green synthesis technique. Physicochemical characterization of the obtained nanoparticles was performed using UV spectrophotometer,

FTIR (Fouirer Transform Infrared Spectrophotometer) and SEM (Scanning Electron Microscope). Biochemical activity of obtained nanoparticles on some medicinally important enzymes such as α- amylase, α- glycosidase and urease enzymes was also performed.

MATERIAL AND METHODS

Humulus lupulus L. plant was supplied from local producer in Bilecik city, Turkey in 2020. Silver nitrate, α-amylase, α-glycosidase and urease were purchased from sigma Aldrich, USA. All other reagents were analytical grade.

Preparation of *Humulus lupulus* L. extract. Dried *Humulus lupulus* L. plant 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°C.

Green synthesis of *Humulus lupulus* L. silver nanoparticles (HL-AgNP). HL-AgNPs were synthesized according to Al-Yousef et al. [2020] with minor modifications. For this purpose, *Humulus lupulus* L. water extract was mixed with 5 mM silver nitrate ($AgNO_3$) solution in a dark flask (1 : 1). The mixture was stirred for ~2 h at room temperature. The changes of color to dark brown was noted. Formation of silver nanoparticles was affirmed by scanning of UV absorption between 250 nm and 750 nm.

Characterization of HL-AgNPs. The formation and presence of AgNPs were determined by UV-vis spectroscopy. The absorbance of the sample was recorded by scanning the wavelength in the spectrophotometer device. FTIR-ATR device data were analyzed in order to evaluate the functional groups of the bioactive components responsible for 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.

Total phenolic content of *Humulus lupulus* L. water extract and HL-AgNPs. The Folin method is based on the formation of colored complexes of phenolic substances with the Folin and Ciocâlteu reagent and is the most widely used method for the measurement of total phenolic content in natural products [Singleton and Rossi 1965, Singleton et al. 1999]. The blue colored complex formed by phenolic substances with the Folin reagent has a maximum absorbance at 765 nm. A calibration curve was prepared by using gallic acid (GA) as the standard phenolic compound [Singleton and Rossi 1965, Singleton et al. 1999]. The results were expressed in milligrams of GA equivalent per milliliter (mg GAE/mL).

DPPH free radical scavenging assay. The DPPH· free radical scavenging activity was determined using the method described by Molyneux [2004]. Using a spectrophotometer, a decrease in light absorbance was determined at a wavelength of 517 nm. The IC_{50} results were expressed in milligrams per milliliter (mg/mL).

Phytochemical analysis. Amount of each extract was dissolved in methanol solution. Then the dissolved extracts were used to detect the presence of alkaloids, phenols, tannins, flavonoids and saponins according to standard methods [Lefahal et al. 2020]. The last solution was observed for color change and/or precipitate formation to indicate a positive test result.

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 (3,5-dinitrosalicylic acid) method described [Bernfeld 1955]. Equal volume of 1% soluble starch and enzyme solution was incubated for 30 min at 35°C. Equal volume of DNS reagent was added into tubes and kept in a boiling water bath. After reaching the room temperature, absorbance of the tubes was recorded at 550 nm [Keskin and Saglam Ertunga 2017]. The analyses was performed in triplicate. IC_{50} value of the extract and HL-AgNPs were determined at five different concentrations at standard assay condition and dose response curve was generated. Acarbose was used as reference inhibitor.

Inhibition properties of α-glycosidase. α-glycosidase activity was performed according to the method described by 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 (50 mM) were mixed. The mixture was incubated at 37°C and the absorbance values at 405 nm were recorded [Gholamhoseinian et al. 2008]. IC_{50} value of the extract and HL-AgNPs were determined at five different concentrations at standard

assay condition and dose response curve was generated. Acarbose was used as reference inhibitor [Saglam Ertunga et al. 2014].

Inhibition properties of urease. The inhibition properties of urease activity was performed according to Weatherburn [1967] method. Shortly, reaction mixture containing 5 µL of urea solution (100 mM), 40 µL of jack bean urease and 5 µL of buffer (0.01 M KH_2PO_4, 1 mM EDTA and 0.01 M LiCl; pH 8.2) was prepared. After incubation at 35°C 15 min, 750 µL of phenol reagent was added, vortexed and then, 750 µL of alkali reagent (0.5% w/v NaOH and 0.1% v/v NaOCl) was added and vortexed. This mixture was incubated for 15 min more at 35°C and absorbance was recorded at 625 nm. For inhibition study, activity assays were conducted at five different concentrations and dose response curve was generated. Thiourea was used as standard inhibitor [Keskin et al. 2019].

RESULTS

Humulus lupulus L. is an important plant with the potential to be used in traditional and complementary medicine applications with its active ingredients such as phenols, hydroxycinnamic acid, flavonoids, and proanthocyanidin. Anticancer, antibacterial, anti-inflammatory, antiallergic, antiviral, hepatoprotective, antithrombogenic and many other effects of hops have been reported. In this study, silver nanoparticles were obtained from the aqueous extract of *Humulus lupulus* L. by green synthesis. Antioxidant properties of the obtained nanoparticles and inhibition properties on *α*-amylase, *α*-glycosidase and urease enzymes were determined. Inhibition of these enzymes has an important place in the treatment of certain diseases namely diabetes mellitus and gastric ulcer.

Obtained results showed that *Humulus lupulus* L. extract contains flavonoids, phenols, tannins, alkaloids and glycosides (Tab. 1). It was determined that HL-AgNPs gave maximum absorbance at approximately 450 nm (Fig. 2). The reduction of Ag^+ ions into silver nanoparticles during the reaction of *Humulus lupulus* L. extract could be determined by color changes. The water extract of *Humulus lupulus* L. was light brown (Fig. 3). After the reaction of extract with $AgNO_3$, the color changed to dark brown. The water extract of *Humulus lupulus* L. is a good source for compounds such as phenolics. As a result of this, it has a good potential for reducing Ag^+ ions. The possible reaction is presented below [Salari et al. 2019].

Table 1. Phytochemical screening of *Humulus lupulus* L. extract

Phytochemicals	Metanol extract
Anthraquinones	–
Flavonoids	+
Saponins	+
Tannins	+
Alkaloids	+
Glycosides	+
Phenols	+

$$Ag^{+}_{(aq)} + \textit{H. lupulus}\ L._{(Aq)} \rightarrow \rightarrow [Ag\ (\textit{H. lupulus}\ L.)]^{+} \quad \text{(Equ. 1)}$$

$$[Ag\ (\textit{H. lupulus}\ L.)]^{+} + R\text{-}CHO \rightarrow \rightarrow [Ag\ (\textit{H. lupulus}\ L.)] + R\text{-}COOH \quad \text{(Equ. 2)}$$

SEM analyses revealed that size of obtained nanoparticles ranged from 30.60 nm to 36.72 nm (Fig. 4). The potential peaks of the prepared aqueous extract and HL-AgNPs were determined using FTIR-ATR (Fig. 5). It was determined that the synthesized nanoparticles, unlike the aqueous extract, gave 2296.89 cm^{-1}, 1161.05 cm^{-1}, 1112.34 cm^{-1} peaks and there were shifts in other peaks (Fig. 6). The band at 3200 to 3400 cm^{-1} represents O─H stretching groups of amides plane bonding respectively. The band at 1650 to 1800 cm^{-1} assigned to be C = O stretching. The general infrared absorption of different types of bonds summarized in Figure 5. Total phenolic content of HL-AgNPs was determined as 30.62 ±0.02 mg GAE/mL and IC_{50} value for DPPH· radical scavenging activity was determined as 4.4 ±0.01 mg/mL (Fig. 7). The synthesized *Humulus lupulus* L. based silver nanoparticles showed better inhibitory activity on tested enzymes than the prepared *Humulus lupulus* L. aqueous extract. Accordingly, inhibitory effects of HL-AgNPs

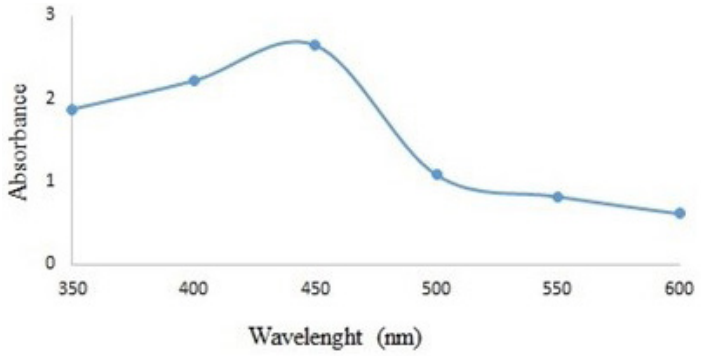

Fig. 2. UV absorbance of HL-AgNPs

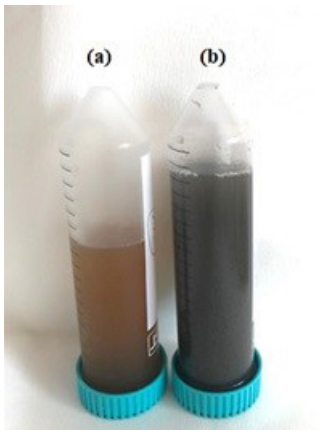

Fig. 3. a) Water extract of *Humulus lupulus* L., b) HL-AgNPs

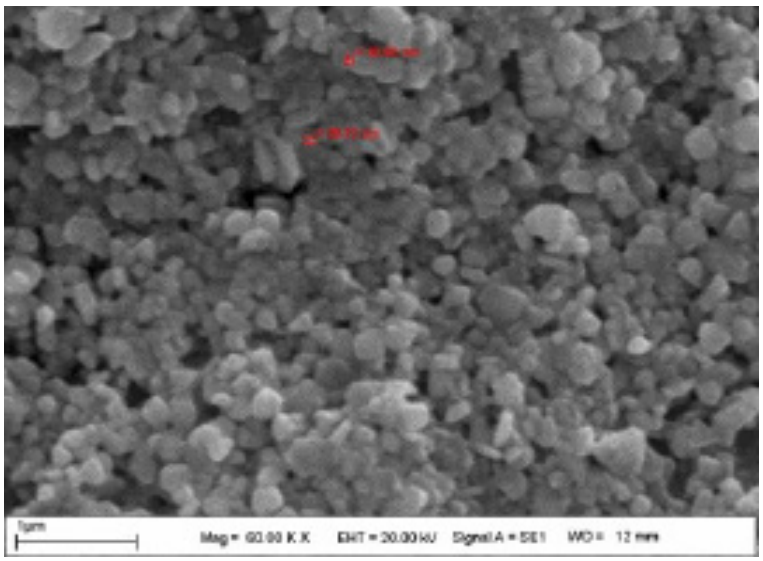

Fig. 4. SEM images of HL-AgNPs

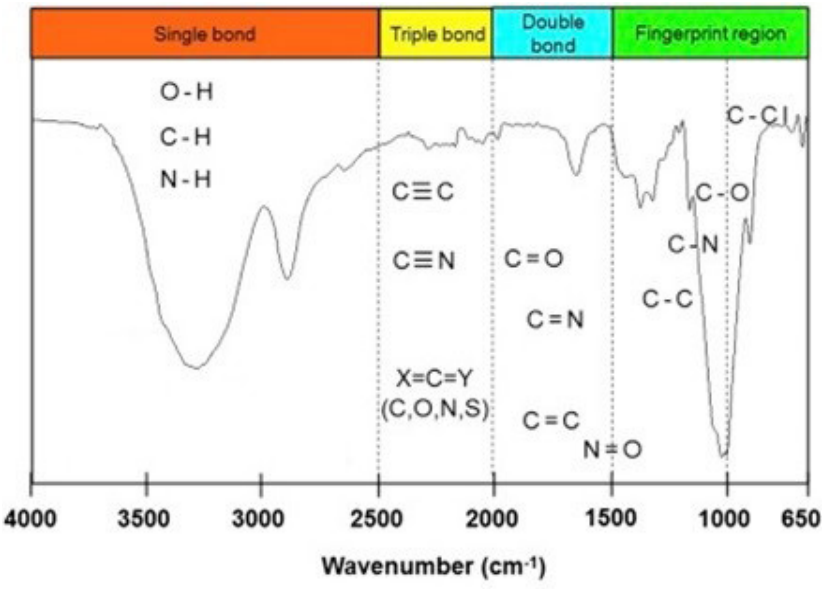

Fig. 5. General infrared absorption of different types of bonds

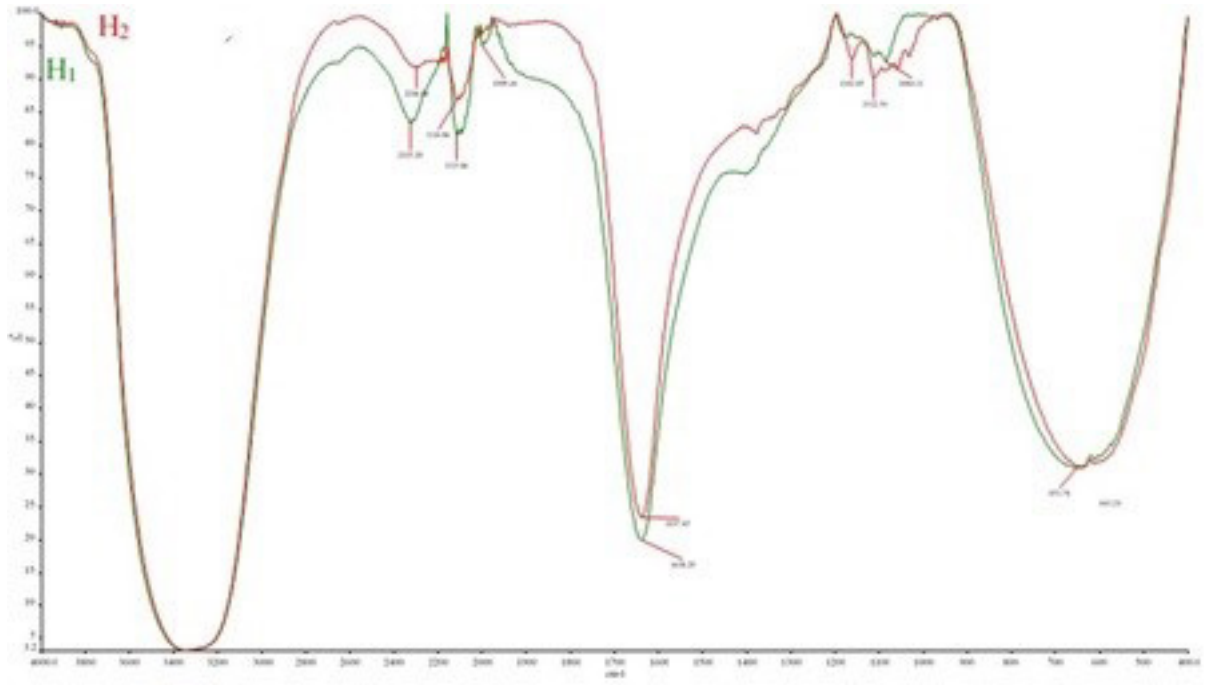

Fig. 6. FTIR-ATR data of *Humulus lupulus* L. aqueous extract (green) and HL-AgNPs (red)

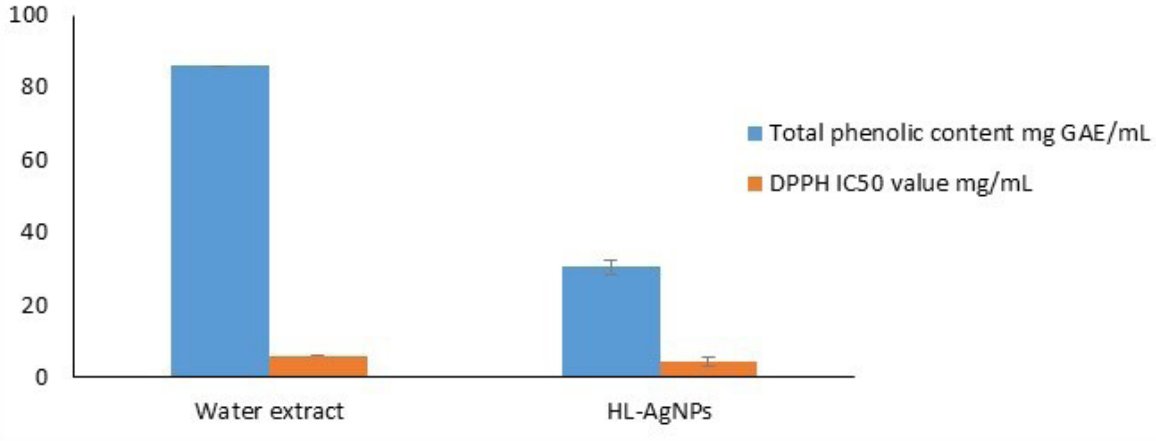

Fig. 7. Total phenolic content and antioxidant activity of *Humulus lupulus* L. extract and HL-AgNPs

Table 2. Enzyme inhibition properties of *Humulus lupulus* L. and HL-AgNPs

	α-amylase (mg/mL)	*α*-glucosidase (mg/mL)	Urease (mg/mL)
Water extract	4.41 ±0.02	13.65 ±0.02	4.52 ±0.14
HL-AgNPs	3.10 ±0.01	9.42 ±0.02	0.76 ±0.01
Acarbose	5.82 ±0.01	5.82 ±0.01	–
Tiourea	–	–	10.92 ±0.01

on *α*-amylase, *α*-glycosidase and urease enzymes were found as 3.10 ±0.01 mg/mL, 9.42 ±0.02 mg/mL and 0.76 ±0.01 mg/mL, respectively (Tab. 2).

DISCUSSION

In a study, berberine based silver nanoparticles was evaluated and it was reported that silver nanoparticles gave absorbance at 424 nm. They had spherical construction and their size was nearly 35 nm [Hosseini Bafghi et al. 2021]. Poor et al. [2017] were synthesized *Plantago major* based silver nanoparticles and determined their effects on human breast cancer cell line. The nanoparticles were characterized by using a spectrophotometer, FTIR and TEM (Transmission Electron Microscopy). It was reported that the nanoparticles had maximum absorbance at 432 nm. Obtained silver nanoparticles were mentioned as spherical in shape with an average size ranging from 1 to 30 nm [Poor et al. 2017]. In another study, silver nanoparticles were synthesized by using of *Ocimum basilicum* and *Ocimum sanctum* L. plant extracts. It is stated that the size of particles obtained vary between 3–25 nm. It was stated that nanoparticles gave maximum absorbance at 439 nm and 433 nm. Inhibition properties of nanoparticles on *α*-amylase and *α*-glycosidase enzymes was reported as 89.31 ±5.32%, and 79.74 ±9.51%, respectively [Malapermal et al. 2017]. Govindappa et al. [2018] synthesized silver nanoparticles from *Calophyllum tomentosum* plant extract. They stated that the obtained particles gave maximum absorbance at 438 nm. Antioxidant capacity, total phenolic content and antidiabetic activity of silver nanoparticles were also reported. It was stated that

the DPPH· scavenging activity was more than 90%. Inhibition properties of obtained silver nanoparticles on *α*-amylase and *β*-glycosidase enzymes was reported as 20% and 50% respectively. Jini and Sharmila [2020] investigated the antidiabetic potential of *Allium cepa* based silver nanoparticles. They stated that the size of obtained particles varied between 49.27 nm and 72.84 nm. Inhibitory activity of silver nanoparticles on *α*-amylase enzyme by 75% and *α*-glycosidase enzyme by 65% was also mentioned. Antioxidant activity (DPPH) of nanoparticles was stated as 60%. Ali et al. [2020] synthesized *Crataegus oxyacantha* based silver nanoparticles and examined the inhibition effect of nanoparticles on urease enzyme. They stated that the dimensions of the obtained particles were 85 nm and that the nanoparticles obtained using different solvents gave maximum absorbance between 400–450 nm. They stated that obtained nanoparticles showed inhibition activity on urease enzyme by 99.25% and the IC_{50} value was declared to be 1.38 ±0.3 mg/mL.

Balan et al. [2016] synthesized silver nanoparticles from *Lonicera japonica* extract by green synthesis. In the study, it was stated that nanoparticles gave maximum absorbance at 435 nm, IC_{50} values for *α*-amylase and *α*-glycosidase enzyme inhibition were declared to be 54.56 and 37.86 mg/mL, respectively. IC_{50} value for DPPH· radical scavenging activity of particles was reported as 46.70 mg/mL. Gul et al. [2021] synthesized silver nanoparticles from *Ricinus communis* extract by green synthesis. It was stated that the nanoparticles synthesized separately from the stem and leaves gave maximum absorbance at 418 and 424 nm, respectively. It was determined that the nanoparticles synthesized from the roots inhibited the urease enzyme by 94.2% (IC_{50} value 36.81 ±0.05 μg/mL^{-1}), while the nanoparticles synthesized from the leaves inhibited 92.1% (IC_{50} value 38.15 ±0.02 μg/mL^{-1}). Debnath et al. [2019] synthesized silver nanoparticles with green synthesis using *Pleurotus giganteus* mushroom and determined the inhibition effect of nanoparticles on *α*-amylase enzyme. It was stated that the size of the nanoparticles varied between 2–20 nm and the nanoparticles gave maximum absorbance at 420 nm. It was stated that the synthesized nanoparticles inhibited *α*-amylase enzyme at a rate of 70%. Cao et al. [2017] investigated the effects of synthesized silver nanoparticles on urease enzyme. According to this study, it was stated that increasing the amount of silver nanoparticles also increased the degree of enzyme inhibition. It was stated that the IC_{50} values of nanoparticles synthesized from different sources vary between 0.03672 mg/g dry soil and 4.666 mg/g dry soil. Amin et al. [2012] synthesized silver nanoparticles from *Solanum xanthocarpum* L. berry extract by green synthesis. It was stated that the average size of the synthesized nanoparticles was 10 nm and they gave maximum absorbance at 433 nm. It was determined that the obtained nanoparticles inhibited the urease enzyme by 64%. Chinnasamy et al. [2019] synthesized silver nanoparticles using *Melia azedarach* and tested the antidiabetic and antioxidant activities of nanoparticles. It was stated in the study that nanoparticles gave maximum absorbance at 420 nm and the size of nanoparticles vary between 14–20 nm. It was also mentioned in this study that nanoparticles scavenged DPPH· radical by 63.83% and inhibited *α*-amylase and *α*-glycosidase enzymes by 85.75% and 80.33%, respectively. In a study, synthesis of Black cumin (*Bunium persicum*) seeds-based silver nanoparticles was reported. It was stated that obtained nanoparticles gave maximum absorbance at 430 nm. Inhibition of urease enzyme by obtained nanoparticles as 90.3% was also declared [Khan et al. 2022]. Balciunaitiene et al. [2021] obtained different silver nanoparticles by using *Artemisia absinthium* L., *Humulus lupulus* L. and *Thymus vulgaris* L. extracts. Physicochemical characterization of the obtained nanoparticles was performed and their determined antioxidant capacities were reported. It was determined by the authors that total phenolic content of plant extract was higher than the corresponding silver nanoparticle sample. The DPPH· radical scavenging activity of synthesized silver nanoparticles were reported to be 0.14, 0.11 and 0.14 TE, mmol/g DW for *Artemisia absinthium* L., *Humulus lupulus* L. and *Thymus vulgaris* L, respectively. It was seen that the obtained data were compatible with the literature data.

CONCLUSIONS

In this study, environmentally friendly, fast and economical silver nanoparticles were synthesized using the aqueous extract of *Humulus lupulus* L. with green synthesis. The synthesized nanoparticles were characterized using UV-vis spectrophotometer, FTIR

and SEM. By determining the antioxidant properties of the characterized nanoparticles, the inhibition properties of enzymes (α-amylase, α-glucosidase and urease) were determined. The obtained data were compared with the aqueous *Humulus lupulus* L. data and it was determined that the biochemical activities of HL-AgNPs were better than the aqueous *Humulus lupulus* L. extract. It can be concluded that it is possible to use HL-AgNPs obtained by green synthesis in the treatment of gastric ulcer and diabetes mellitus and in various biomedical applications.

CONFLICT OF INTEREST

The author declare no competing interests.

Merve Keskin conceptualized and designed, performed the research and drafted the manuscript. All data generated or analyzed during this study are included in this published article. Ethics approval and consent to participate – not applicable. Consent for publication – not applicable.

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