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Phytochemical study on *Melilotus officinalis* leaves: Green synthesis of silver nanoparticles and quantitative analysis of phenolic compounds

Melilotus officinalis yaprakları üzerinde fitokimyasal çalışma:
Gümüş nanopartiküllerin yeşil sentezi ve fenolik bileşiklerin kantitatif analizi

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ABSTRACT

Since ancient times, people have used plants as food and medicine. Bioactive chemicals in plants have been identified and used in medicinal development since the discovery of spectroscopy. Nanotechnology is a branch of science that has developed rapidly in recent years due to its widespread application. In this study, silver nanoparticles (AgNPs@Mo) were synthesized by a green approach using the *Melilotus officinalis* leaves. The structure of the nanoparticle was identified by analytical techniques. The maximum absorption at 483 nm confirmed the formation of silver nanoparticles. The TEM image presented the morphology and particle size of nanoparticles (36.3 nm). The XRD spectrum determined the shape of nanoparticles as a face-centered cubic structure (fcc). The particle size was also determined by the Scherrer equation as 32.4 nm. The zeta potential (-31.1 mV) revealed the high stability of nanoparticles. Quantitative analysis of phenolic compounds in *M. officinalis* leaves was determined by LC-MS/MS, and coumarin (2.167 µg/g extract), syringic acid (0.027), salicylic acid (0.025), and rutin (0.066) were defined as major compounds. *Melilotus officinalis* leaves may be a valuable source for silver nanoparticle synthesis.

Keywords: *Melilotus officinalis*, nanoparticles, spectroscopy, bioactive compounds

ÖZET

Antik çağlardan beri insanlar bitkileri gıda ve ilaç olarak kullanmıştır. Spektroskopinin keşfinden bu yana bitkilerdeki biyoaktif kimyasallar tanımlanmakta ve ilaç geliştirmede kullanılmaktadır. Nanoteknoloji, yaygın kullanım alanları nedeniyle son yıllarda hızla gelişen bir bilim dalıdır. Bu çalışmada, *Melilotus officinalis* yaprakları kullanılarak yeşil yaklaşımla gümüş nanopartiküller (AgNPs@Mo) sentezlenmiştir. Nanopartikülün yapısı analitik tekniklerle tanımlanmıştır. 483 nm'de maksimum absorbsiyon, gümüş nanopartiküllerin oluşumunu doğrulamıştır. TEM görüntüsü, nanopartiküllerin morfolojisini ve parçacık boyutunu (36,3 nm) belirlemiştir. XRD spektrumu, nanopartiküllerin şeklini yüzey merkezli kübik yapı (fcc) olarak belirlemiştir. Parçacık boyutu da Scherrer denklemi ile 32,4 nm olarak belirlenmiştir. Zeta potansiyeli (-31,1 mV) nanopartiküllerin yüksek kararlılığını ortaya koymuştur. *M. officinalis* yapraklarındaki fenolik bileşiklerin kantitatif analizi LC-MS/MS ile yapıldı ve kumarin (2,167 µg/g ekstrakt), siringik asit (0,027), salisilik asit (0,025) ve rutin (0.066) başlıca bileşikler olarak tanımlandı. *Melilotus officinalis* yaprakları, gümüş nanopartikül sentezi için değerli bir kaynak olabilir.

Anahtar kelimeler: *Melilotus officinalis*, nanopartikül, spektroskopi, biyoaktif bileşikler

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Although people have used plants for food and medicine throughout human history, spectroscopic advancements in the 19th century made plants a scientific field (Dede et al., 2019; Demirtas et al., 2013; Elmastas et al., 2004; Topçu et al., 1999). After that, many bioactive compounds have been isolated and identified from plants, and their biological effects have been investigated (Aksit et al., 2014; Bayir et al., 2014; Erenler et al., 2014; Sahin Yaglioglu et al., 2013). Plants contain fascinating compounds that exhibit high biological activity (Elmastaş et al., 2015; Kaya et al., 2014; Türkmen et al., 2014). Thus, they have attracted the attention of synthetic chemists, and many natural compounds have been synthesized (Cakmak et al., 2006; Erenler et al., 2007; Erenler et al., 2006; Erenler et al., 2009). In addition, semi-synthetic compounds with high activity were synthesized by bonding functional groups to natural compounds (Çelik et al., 2015; Lu et al., 2014; Okten et al., 2015). Due to the cancerogenic effects of synthetic antioxidants, natural antioxidants have gained considerable interest as a food additive. Moreover, many scientific studies have been conducted to discover antioxidant-based medicine (Elmastas et al., 2016; Elmastaş et al., 2015; Erenler et al., 2016).

Melilotus officinalis, belonging to the Fabaceae family, is used as an anti-aggregation drug and has antioxidant and hepatoprotective effects. *M. officinalis* contains coumarins, flavonoids, steroids, saponins, phenolic acids, and volatile components. This plant has anti-inflammatory, anti-swelling, and anti-tumor properties, as well as therapeutic effects against hemorrhoids, thrombophlebitis, and varicose veins (Liu et al., 2018).

Nanotechnology is the engineering and art of developing new materials at the nanometer scale (1–100 nm) (Erenler et al., 2021; Gecer, 2021; Genc, 2021). Due to their unique properties, the applications of nanoparticles in diverse fields such as medicine, electronics, optics, agriculture, textiles, and water treatment have increased significantly, leading to a growing demand for nanoparticles made from various metals (Dag, 2022; Genc et al., 2021). Silver is one of the most widely utilized metallic nanoparticles, particularly in medical and scientific applications (Erenler & Dag, 2022). Numerous methods, both chemical and physical, have been developed for the synthesis of silver nanoparticles. However, large-scale production often involves reagents that can harm the environment. Hence, there is increasing interest in designing eco-friendly synthesis approaches that avoid the use of toxic substances (Erenler & Gecer, 2022a, 2022b; Gecer & Erenler, 2022a). Effective biological substrates for AgNP production include a variety of substances, such as plant extracts, microorganisms, algae, plant wastes, seaweeds, enzymes, and arthropod metabolites. Plant-based materials have generally been suggested as promising substrates for AgNP synthesis because the process is easy to scale up (Gecer & Erenler, 2022b; Gecer, Erenler, et al., 2022; Karan et al., 2022).

Herein, silver nanoparticles were synthesized using *Melilotus officinalis* leaves by an eco-friendly, cost-effective, and scalable approach. Moreover, quantification of phenolics in *Melilotus officinalis* leaves was determined by LC-MS/MS analysis.

Materials and methods

Plant material

Melilotus officinalis (L.) Desr. was collected from the Iğdır University campus and identified by Dr. Belkıs Muca Yiğit. A voucher specimen was deposited at the herbarium of Iğdır University (INWM00000115).

Extraction

Melilotus officinalis leaves (6.0 g) were extracted with water (120 mL) for 3 hours at 40℃. After filtration, the solvent was evaporated under reduced pressure to yield the crude extract (0.4 g) to be used for further study.

Synthesis of silver nanoparticles

Melilotus officinalis leaves-mediated synthesis of AgNPs@Mo was achieved. The water extract of leaves (0.5 g) was dissolved in deionized water (120 mL), and this solution was treated with the silver nitrate solution (1.0 mM, 120 mL) for 3 hours at 55℃. Afterward, the centrifugation was applied for 15 min at 10000 rpm. Then, the nanomaterial in the Eppendorf was combined and washed thoroughly with deionized water and dried with freeze-drying (Sahin Yaglioglu et al., 2022).

Characteristics of silver nanoparticles

The characterisation of green-synthesized AgNPs@Mo was carried out using the spectroscopic techniques. UV-Vis spectroscopic analysis determined the maximum absorption. Zetasizer Nano ZSP measured the zeta potential to reveal the stability of AgNPs@Mo. X-Ray diffraction (XRD) analysis defined the crystal structure of the nanoparticles. Transmission electron microscopy (TEM) determined the structure, size distribution, and morphology of nanostructures (Erenler, Chaoui, et al., 2023).

LC-ESI-MS/MS analysis

To measure the bioactive components in *Melilotus officinalis* leaves, LC-MS/MS analysis was performed (Agilent Technologies 1260 Infinity II). Methanol (1.0 mL) was combined with 50 mg of *Melilotus officinalis* leaf extract in an Eppendorf. After adding the hexane, the mixture was centrifuged for 15 minutes at 10.000 rpm. Water (450 µL) and methanol (450 µL) were added to dilute the methanol phase (100 µL). The solution was filtered using a 0.22 µm filter before being put into the apparatus (Başar et al., 2024).

Results and discussion

UV-Vis analysis of silver nanoparticles

Silver nanoparticles were synthesized using *Melilotus officinalis* leaves. The natural compounds in plant leaves are responsible for reducing, capping, and stabilizing agents. Because plant materials are eco-friendly, economical, and biocompatible, they are a good choice for creating nanoparticles. One essential method for verifying the presence of nanomaterials is UV-Vis. A particular peak appears in the UV-Vis spectrum when silver ions are reduced to form nanoparticles; for silver nanoparticles, this peak is usually detected between 400 and 500 nm (Erenler, Gecer, et al., 2023). The maximum absorption of AgNPs@Mo was observed at 483 nm, proving the nanoparticle formation (Figure 1).

Zeta potential

Zeta potential is a crucial indicator of the stability of AgNPs in suspension. It represents the surface charge of the particles, which affects their colloidal stability, tendency to aggregate, and interactions with biological or environmental systems. Zeta potential values exceeding ±30 mV indicate strong electrostatic repulsion between particles, which helps prevent aggregation and promotes a stable dispersion (Erenler & Hosaflioglu, 2023). In this study, the zeta potential value of -31.1 mV presented high stability (Figure 2).

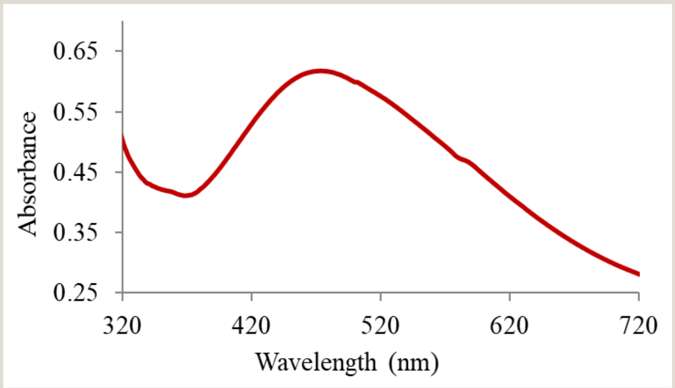

Figure 1. UV-Vis spectrum of AgNPs@Mo

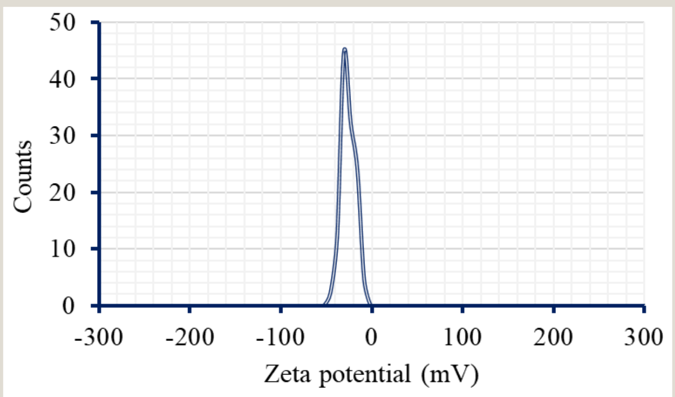

Figure 2. Zeta potential of AgNPs@Mo

TEM analysis

Understanding the size and size distribution of individual AgNPs at the nanometer scale is made possible by TEM, which is essential for comprehending their chemical and physical characteristics (Erenler, Ojelade, et al., 2023). The particles were found to be spherical, and an aggregate was not observed. The particle size was calculated to be 36.3 nm (Figure 3).

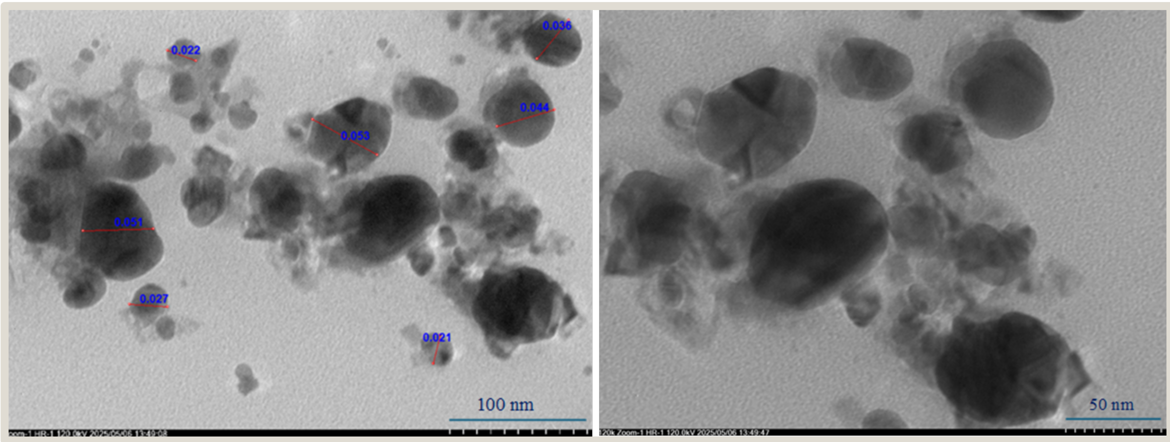

Figure 3. TEM image of AgNPs@Mo (36.3 nm)

XRD analysis

A crucial technique for describing AgNPs is X-ray diffraction (XRD), which yields details about the particles' crystalline structure, phase composition, crystallite size, and potential contaminants (Gecer, 2023; Gecer et al., 2023). The facets were represented as [1 1 1, 2 0 0, 2 2 0, 3 1 1] by the angles at 38.14^0, 44.26^0, 64.75^0, and 77.63^0 degrees, indicating the face-centered cubic structure (Figure 4). The crystallite size (D) of AgNPs can be estimated using Scherrer's equation (1):

$D = K\lambda/\beta\ cos\theta$ (1)

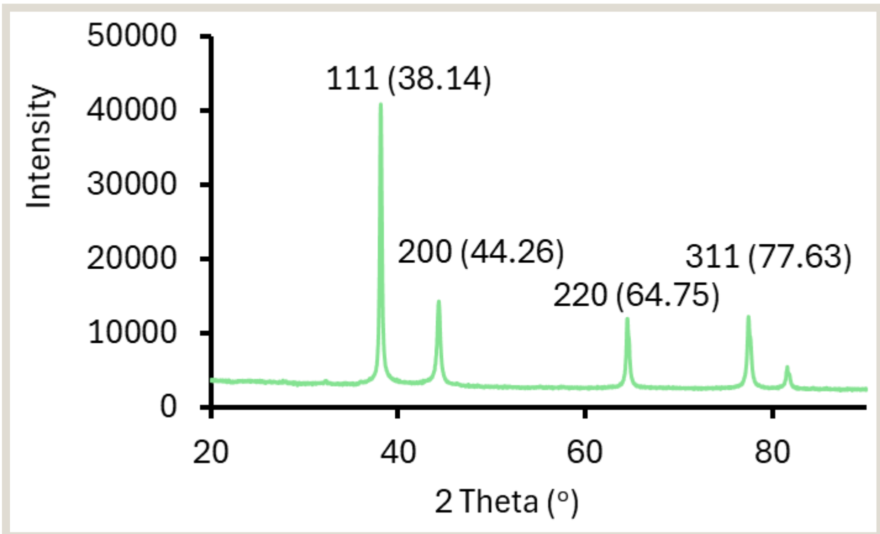

Figure 4. XRD pattern of AgNPs@Mo

Quantitative analysis

In many different fields, but especially in pharmaceutical, food, agricultural, and biomedical research, the quantitative analysis of phenolic compounds using LC-MS/MS is vital. Quantitative analysis of phenolics provides the opportunity to assess the nutritional value of plant-based foods, identify bioactive compounds responsible for health benefits, and standardize herbal formulations and functional foods. In this study, the quantification of phenolic compounds was determined by LC-MS/MS analysis.

Coumarin (2.167 μg/g extract), syringic acid (0.027 μg/g extract), and salicylic acid (0.025 μg/g extract) were defined as major compounds (Table 1, Figure 5).

Table 1. Quantitative analysis of phenolic compounds by LC-MS/MS (μg/g extract)

Compound	RT	Conc.
Shikimic acid	1.41	nd
Gallic acid	3.23	0.0041
Protocatechuic acid	6.08	nd
Epigallocatechin	6.83	nd
Catechin	6.86	nd
Chlorogenic acid	7.11	0.0032
Hydroxybenzaldeyde	7.60	0.0043
Vanillic acid	7.82	nd
Caffeic Acid	7.77	0.0025
Syringic acid	8.41	0.027
Caffein	8.40	nd
Vanillin	8.66	0.0046
o-coumaric acid	9.39	0.0048
Salicylic Acid	9.54	0.025
Morin	10.00	nd
Resveratrol	9.66	nd
Polydatine	9.83	nd
Trans-ferulic acid	10.12	0.0062
Sinapic acid	10.77	0.0059
Scutellarin	11.16	nd
p-coumaric acid	11.54	0.0026
Coumarin	11.57	2.167
Protocatechuic ethyl ester	11.57	nd
Hesperidin	11.84	0.016
Isoquercitrin	11.81	0.0064
Rutin	12.39	0.066
Quercetin-3-Ksilozid	12.25	nd
Kaempferol-3-glucoside	13.29	nd
Fisetin	13.44	0.0022
Chrysin	14.44	nd
Trans-cinnamic acid	14.34	nd
Quercetin	14.85	nd
Naringenin	15.07	0.0045
Hesperetin	15.87	0.0047
Catechin	15.81	nd
Kaempferol	16.12	0.015
Baicalein	17.12	nd
Luteolin	17.88	nd
Biochanin A	17.88	nd
Capcaicin	18.25	nd
Dihydrocapcaicin	18.70	nd
Diosgenin	23.56	nd

RT: Retention Time; **nd:** not detected

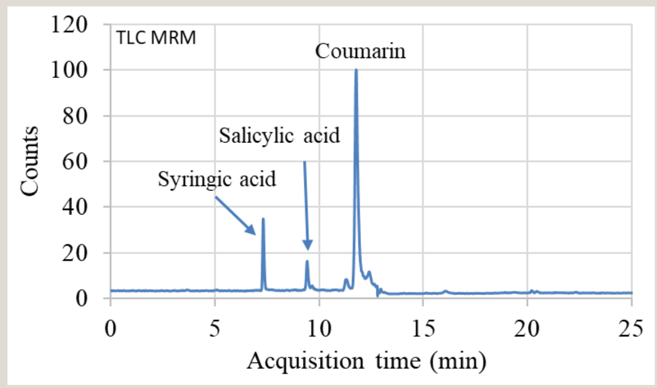

Figure 5. LC-MS/MS chromatogram of *Melilotus officinalis* leaves

Conclusion

Silver nanoparticles were synthesized using the *Melilotus officinalis* leaves using an eco-friendly, cost-effective, scalable, and fast approach. The major compounds were described. These compounds are essential for the food and pharmaceutical industries. Scientists will therefore be motivated by this work to separate these bioactive compounds, ascertain their structures, and carry out additional research on them. Every plant produces distinct secondary metabolites, both in terms of quantity and quality. Consequently, *Melilotus officinalis* may be a valuable source for coumarin, syringic acid, salicylic acid, hesperidin, and rutin. Moreover, these flowers have the potential to be an essential material for silver nanoparticle synthesis in large-scale production.

Author contributions

RE: Supervision, project administration; İY: LC-MS analyses; ENG: Nanoparticle synthesis; İH: Collection of plant material and extraction.

Declaration of interests

The authors declare no conflict of interest.

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