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Physical Properties of Extracts of Medicinal Plant *Melissa officinalis*

Abstract

Melissa officinalis is a medicinal plant, has been used in traditional folk medicine for thousands of years to treat various diseases. This study aimed to determine the physical parameters of extracts obtained from Melissa officinalis leaves. Extracts were prepared from dried leaves of Melissa officinalis by filtration with water and ethanol solvents, and as well as homogenate from fresh leaves. The physical parameters of the extracts, including alkalinity, surface tension, electrical conductivity, density and pH changes were determined using standard procedures. It was found that as the infusion time of the extract also increases, its alkalinity, density, and electrical conductivity increase. The alkalinity, surface tension coefficient, electrical conductivity, and density of the homogenate remain stable. The addition of homogenate and extracts to the AgNO3 salt solution changes the physical properties of the solution in which nanoparticles are synthesized. This change is more pronounced in the electrical conductivity and pH value. Therefore, when using these extracts, the values of physical parameters should be taken into account to regulate the synthesis process of nanoparticles.

Keywords: *medicinal plant, extracts, homogenate, nanoparticles, viscosity,density,surface tension, conductivity*

Introduction

Melissa officinalis - a medicinal plant rich in biologically active compounds, has been used in folk medicine for centuries to treat various diseases. The phytochemical composition, physiological properties, distribution and methods of use of extracts obtained from its various organs have always been studied in detail and have been the main subject of a number of scientific studies. Due to the numerous biologically active substances contained in this plant, it is still successfully used in folk medicine to treat various diseases. Melissa officinalis belongs to the family Lamiaseae in the plant kingdom. The leaf surface is coarse and deeply veined, and the leaf margin is wavy or toothed (Turhan, 2006).

Research

Melissa officinalis has been identified as containing terpenes (monoterpenes, triterpenes and sesquiterpenes), phenolic components (phenolic acid, tannins and flavonoids) (Allahverdiyev, et. al., 2004; Moradkhani, et. al., 2010). The main components of this plant are volatile compounds (geranial, neral, citronellal and geraniol), triterpenes (urosylic acid, oleanolic acid) and phenols (cis and trans Ra

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isomers, caffeic acid derivatives, luteolin, naringin, hesperidin) (Argyropoulos, Muller, 2014; Ibragic, et al., 2014). The phenolic acids (secondary metabolites) present in Melissa officinalis are grouped into two classes: benzoic acid derivatives - gallic acid, and cinnamic acid derivatives - caffeic acid. These compounds have antioxidant properties (Dai and Mumper, 2010).



Figure 1. *Melissa officinalis* plant (A), dried leaf (B), homogenate (C) and extract (D)

Aqueous extract of Melissa officinalis reduces the level of corticosterone in the blood plasma (Cases, et al., 2011). Ethanol extract, which enhances norepinephrine neurotransmission (Taiwo, et al., 2012), reduced the duration of immobility in mice (Takeda, et al., 2002a; Takeda, et al., 2002b). Both its aqueous and ethanolic extracts significantly reduce the production of intracellular ROS, exerting a neuroprotective effect (Lopez, et al., 2009). Melissa extract has been shown to remove [3H] nicotine from the membranes of neurons in the human brain containing acetylcholine receptors (Wake, et al., 2000). These experiments' results have allowed for the treatment of neurodegenerative diseases such as dementia, epilepsy, stroke and paralysis. *Melissa officinalis* is traditionally used to treat dementia, amnesia associated with Alzheimer's disease, and psychosis. It has been demonstrated that stimulating of acetylcholine receptors is a promising strategy for Alzheimer's disease treatment (Kihara, Shimohama, 2004). In vivo, the ethanolic extract of *Melissa officinalis* has been shown to improve scopolamine-induced learning and memory in rats. *Melissa officinalis* is a common traditional remedy for palpitations and has potent cardiotonic and cardioprotective effects. Its aqueous extract has shown moderate protection against lethal reperfusion-induced ventricular arrhythmias in rats through the stimulation of muscarinic receptors (Joukar, et al., 2014).

Melissa officinalis is widely used in the treatment of various types of cancer with its main component, citral, shown to induce apoptosis of GBM cell lines expressing active MRP1. *Melissa officinalis* has also been used to treat inflammatory conditions, like asthma and arthritis and as a painkiller. Pharmacological experiments have demonstrated that Melissa officinalis extract exhibits significant antimicrobial activity in vitro against a range of gram-negative pathogenic bacteria, against multidrug-resistant strains. The most potent activity was observed against multidrug-resistant strains of E. coli and Shigella sonnei (Mimica-Dukic, et al., 2004). Aqueous extracts of this plant also shown to exhibit significant anti-HSV-1 and anti-HSV-2 activity in vitro (Mazzanti et al., 2008). Methanolic and aqueous extracts of Melissa officinalis have been reported to have powerful antiepileptic activity in an animal model of epilepsy (Bhat, et al., 2012).

The diverse effects of *Melissa officinalis* extracts depend not only on their composition but also on their physical parameters. It has been established that the solubility, density, electrical conductivity, surface tension coefficient and pH of the extracts of this plant play a crucial role in the pharmacological effects they produce. Additionally, the synthesis of nanoparticles in these extracts in influenced by their physical parameters. Therefore, studing of the physical parameters of *Melissa officinalis* extracts is essential for experiments conducted in this field (Zargari, 1990).

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Materials and Methods

Homogenate and its preparation. Homogenate is obtained from fresh leaves of the plant in the form of juice. It is a physical extraction method that extracts biocomponents from fresh leaves through mechanical cutting, chopping, crushing and mixing with a knife without the use of heat or pressure.Various methods can be used to for obtain homogenate. Such as beating in a bag, crushing with a mortar and pestle in liquid nitrogen, grinding with blenders and rotors, extraction with focused ultrasound, and crushing with a traditional mortar and pestle. For our experiments, fresh leaves of the melissa plant grown in the laboratory were cut, washed with distilled water, and dried. 1 gram of dried leaves was weighed and crushed with a porcelain mortar and pestle to extract the juice. After adding 50 ml of distilled water to the obtained juice, the mixture was processed in a centrifuge for 10 minutes. The resulting solution was carefully cleared of sediment and filtered.

Extraction and its preparation are crucial steps in the study of bioactive compounds in plant. The results of both qualitative and quantitative studies on various plant organs (such as leaves, stems, flowers, roots and fruits) heavily rely on the selection of the appropriate extraction method (Sasidharan, et al., 2011). In research involving medicinal plants,extraction serves as the inital step and the proper preparation of the extract plays significantly impacts the final outcome. Extraction methods are offen reffered to as “sample preparation methods”. Medicinal extracts ,whether oily or aqueous,have been utilized in medical practices centuries. In extracts, the constituents of plant organs are concentrated, and their concentration can be precisely adjusted. Extraction methods usually depend on the type of plant material used. These methods include mechanical, traditional and solvent extraction methods. An extract of a sample taken from a plant is obtained with a solvent that will dissolve the solutes of interest. The solvent can be vapor, supercritical fluid or liquid, and the sample can be gas, liquid or solid (Bekman, 2009). In our experiments, extracts were obtained from the dried leaves of the Melissa officinalis plant. To obtain the extract, 2 g of dry leaves are weighed, mixed with 200 ml of distilled water in a flask, heated to 80ºC and kept for 24 hours. It is cooled and then filtered through Whatman No. 1 filter paper. 50 ml of 70% ethyl alcohol and 2 g of dry leaves are added to another flask and covered. After 24 hours, it is filtered through Whatman No. 1 filter paper. 50 ml of distilled water is added to it, then centrifuged for 10 minutes and the extract is separated. The determination of biologically active substances contained in the Melissa officinalis plant is of great importance as these substances perform a number of important functions. Therefore, the methods for determining these compounds vary. During the experiments, saponins, flavonoids, tannins, phenolic compounds and glucosides were identified (Ibragic, Salihovic, Tahirovic, et al., 2014).

Experiments and Discussion

Determination of Viscosity. The physical properties of plant extracts are crucial importance,as they impact on the choice of processes for application, such as the green synthesis of nanoparticles. One important parameter is the viscosity of the extract. The viscosity significantly influences the size of synthesized nanoparticles, their synthesis time, stability and efficiency. Since the synthesis process is temperature-dependent,the effect of temperature on the extracts viscosity is significant. This is because the viscosity of most liquids decreasesas temperatura increases. In our experiments, we determined the basicity of M. officinalis homogenate (HM), extracts with infusion time of 5 (EM5) and 24 (EM24) and solutions in which Ag nanoparticles were synthesized in extracts with a 5-minute infusion time (EAgNP) and homogenate (HAgNP).The capillary viscometer method was utilized for this purpose. In this method, the extract is filled into a capillary tube, and the time taken for a specific volume of it to pass through the length of the tube is measured.The viscometers known as Ostwald or Ubbelohde viscometers have been in existence since the early 20th century. The acidity of the extracts was measured at a temperature of T = 18.50C.

The parameters in the capillary viscometer were as follows**:** r = 0,99 mm, η_0 (18,5^{0}C) = 1,002 mPa.s, ρ_0 (18,5^{0}C) = 0,9986 g/cm^3, τ_0(18,5^{0}C) = 14,5 sec.

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Here, η_0 – represents the aciticy of the standard substance (distilled water), ρ_0 – is the density of the standard, τ_0 – is the time it takes for water to fall and τ - the time it takes for the extract to fall.

The acidicy of the extract (η) was calculated using the following formula,

$$\eta = \eta_0 . \frac{\rho}{\rho_0} . \frac{\tau}{\tau_0}, \text{ (mPa.s)}$$

The results of the measurements are presented in Table 1 and Figure 2. It is evident from the results that the alkalinity of the homogenate is higher than that of the aqueous extract. The alkalinity of the extract is also influenced by its brewing time. Specifically, the alkalinity of the extract brewed for 24 hours was 0.0582 mPa.s higher than that of the extract brewed for 5 minutes. Interestingly, that during the synthesis of Ag nanoparticles using an extract brewed for 24 hours, the alkalinity of the solution decreases significantly upon adding the extract to the AgNO3 salt solution. This decrease is also observed when the homogenate is added

This decrease is also observed when the homogenate is added. The decrease in the alkalinity of the Ag synthesized solution may be attributed to the low alkalinity of the AgNO3 salt solution, as well as the temperature (Moradkhani, Sargsyan, Bibak, et al., 2010).

Table 1. Viscosity of Melissa officinalis leaf extracts and homogenate.

Extracts and AgNP solutions	The time of the extract falling in the capillary – τ (sec.)	Density (mPa.S)
Homogenate	14,95 ± 0,20 sec.	1,0242
Lemon balm extract -5 minutes	14,195 ± 0,02 sec.	0,9527
Lemon balm extract -24 hours	14,5775 ± 0,08 sec.	1,0109
AgNP with 5-minute extract	13,44 ± 0,01 sec.	0,92075
AgNP with homogenate	13,884 ± 0,04 sec.	0,9528

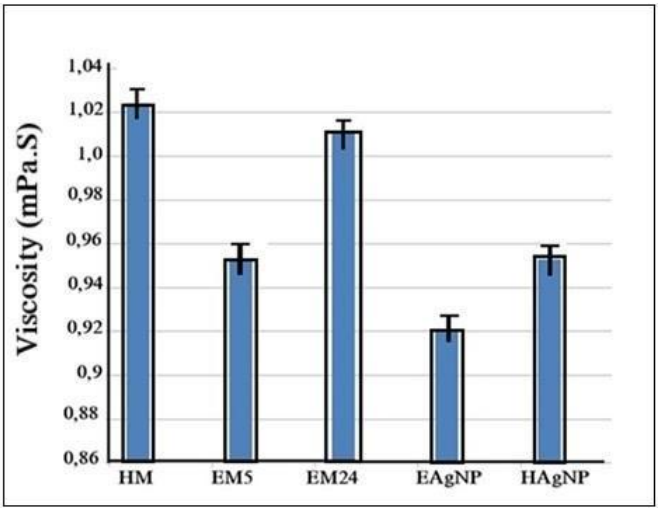


Figure 2. Desity of Melissa officinalis leaf extracts and homogenate

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Density Determination. The density of the extract and homogenate is crucial in the green synthesis of nanoparticles. The extract contains biologically active compounds in varying concentrations, which are reflected in its density. Therefore, knowing the density of the extract and homogenate is essential for controlling the nanoparticle synthesis process. Density is typically measured expressed in grams per milliliter (g/ml). To determine the density of extracts and homogenate from Melissa officinalis leaves, a known volume was measured and weighed. A 10 ml sample of the extract and homogenate was taken, and its mass was accurately measured. Once the mass is known, the density can be calculated using the formula [$\rho_b = m_b/V$**].**

where: ρ_b – density of the extract or homogenate, m_b - mass of extract, **V**-volume, the unit of density will be **g/ml**.

The results of the measurements are presented in Table 2 and Figure 3. It is evident from the results that the density of the extract after a brewing for 5 minutes is slightly higher than that of the homogenate.Additionally, the density increases as the brewing time is extendet. Hoüever, the density of the solution decreases after the synthesis process of nanoparticles, which involves adding the extract and homogenate to the AgNO3 salt solution.

Table 2. Density of Melissa officinalis leaf extracts and homogenate.

Extracts and AgNP solutions	Density of solutions (g/ml)
Homogenate	0,99 ± 0,001
Lemon balm extract -5 minutes	0,971 ± 0,002
Lemon balm extract -24 hours	1,002 ± 0,003
AgNP with 5-minute extract	0,99 ± 0,001
AgNP with homogenate	0,992± 0,002

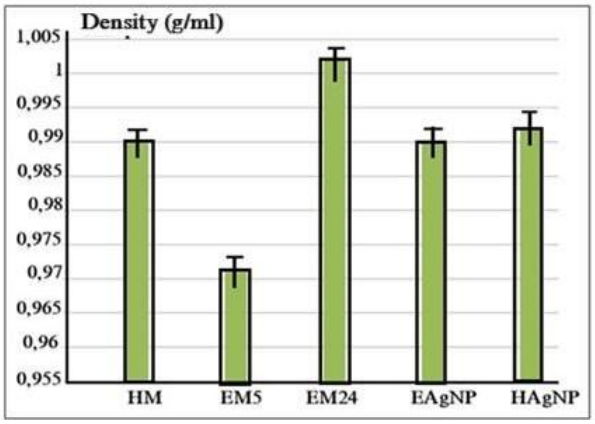


Figure 3. Density of Melissa officinalis leaf extracts and homogenate

Surface Tension Measurement. The surface tension of extracts and homogenates was determined using on the droplet hanging method. Before conducting the measurements, the density of the analyzed extracts and homogenates was measured at the dispensing glass tube's diameter (outer diameter 0.14 mm). To ensure accuracy, 60 drops were measured for each solution were repeated three times. The

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compined mass of the 60 drops and in three repetitions was 39.8 g, 39.5g and 39.7 g, respectively. The mass of the drops was calculated determined by subtracting the mass of the empty glass tube (36.7 g) from the total mass. The surface tension was then calculated at room temperature (23 ^{0}C) using the provided formula, with the unit represented as N/m.

G = mg/1,24 π r

Here, **G** is the surface tension, **m** is the mass of the droplet, **g** is the acceleration of free fall, and **r** is the radius of the glass tube (Wake, Court, Pickering, et al., 2000).

Table 3. Surface tension values of Melissa officinalis leaf extracts and homogenate.

Extracts and AgNP solutions	Mass of extract and homogenate droplets (g)	Surface tension (N/m)
Homogenate	2,97 ± 0,01 g	53,3953± 0,03
Lemon balm extract -5 minutes	6,37 ± 0,01 g	114,5213± 0,05
Lemon balm extract -24 hours	5,13 ± 0,01 g	92,2283± 0,03
AgNP with 5-minute extract	5,37 ± 0,47 g	96,5431± 0,02
AgNP with homogenate	5,07 ± 0,01 g	91,1496± 0,04

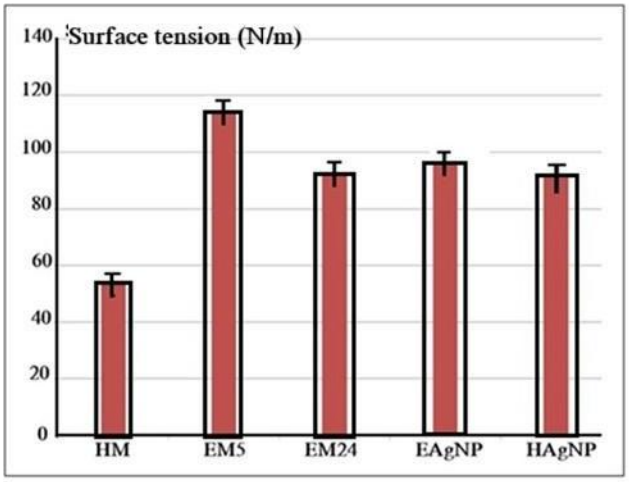


Figure 4. Surface tension values of Melissa officinalis leaf extracts and homogenate.

The results of the measurements are provided in Table 3 and Figure 4. It is evident from the measurement results that the surface tension coefficient of the homogenate is lower than that of the extracts. The highest surface tension value was observed in the extract brewed for 5 minutes. As the brewing time increases, the surface tension value of the extract decreases. It is important to note that no significant change in surface tension is observed in the solution where nanoparticles are synthesized by adding the extracts and homogenate to the AgNO3 salt solution (Allahverdiyev, Duran, Ozguven, et al., 2004).

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pH Determination. The pH values of extracts and homogenates crusial factors that affect the nanoparticle synthesis process. To achieve successful green synthesis of nanoparticles, it is essential to determine the pH values of the extracts and homogenates before adding them to the AgNO3 salt solution. In our experiments, we determined the pH values of the extracts and homogenates prior to synthesizing Ag nanoparticles. The results are presented in Table 4 and Figure 5. We observed that the pH of the solutions influenced by temperature, and the pH of the extracts and homogenates is similar. However, when added to the AgNO3 salt solution,the pH decreases significantly. For instance, the pH of the solution containing nanoparticles synthesized with the extract brewed for 5 minutes is 3.4, much lower than that pH of distilled water. This pH decrease also occurs when homogenate is added.

Table 4. pH values of Melissa officinalis leaf extracts and homogenate.

Extracts and AgNP solutions	Temperature	pH
Distilled water	22,9^{0}C	6,58± 0,2
Homogenate	15,8^{0}C	6,75± 0,1
Lemon balm extract -5 minutes	13,3^{0}C	6,39± 0,2
Lemon balm extract -24 hours	23,6^{0}C	6,1± 0,3
AgNP with 5-minute extract	21^{0}C	3,4± 0,6
AgNP with homogenate	22,4^{0}C	4,62± 0,1

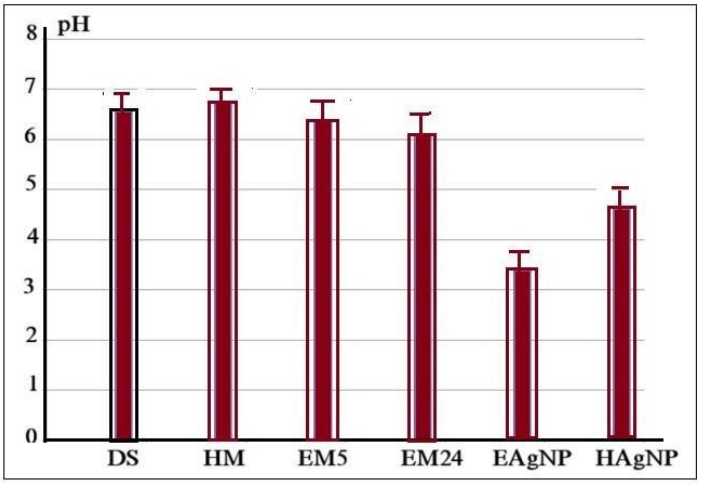


Figure 5. pH values of Melissa officinalis leaf extracts and homogenate

Determination of Electrical Conductivity. The electrical conductivity of Melissa officinalis plant extracts and homogenate was measured using a SevenCompact conductometer (Mettler Toledo, Switzerland) by immersing Electrode – LE703 electrode in the extract. Measurements were conducted in triplicate, and the results were expressed as the mean (n = 3) ± SD, with the unit of conductivity being µS/cm.

The results of the measurements are presented in Table 5 and Figure 6. It was discovered that the lowest electrical conductivity was observed in the extract brewed for 5 minutes. As the brewing time increased, the electrical conductivity of the extract also increased. The addition of extracts and

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homogenate to the AgNO3 salt solution did not significantly alter the electrical conductivity of the solution in which the nanoparticles were synthesized. However, it is important to note that despite the low electrical conductivity of the homogenate, its addition to the AgNO3 salt solution actually increased the electrical conductivity of the solution in which the nanoparticles were synthesized. This could be atributed to the high conductivity of the solution during synthesis, incomplete reduction of the AgNO3 salt ions of the presence of NO3 anions.

Table 5. Electrical conductivity values of Melissa officinalis leaf extracts and homogenates.

Extracts and AgNP solutions ı	**Electrical conductivity (**μ_s/sm
Homogenate	51,7
Lemon balm extract -5 minutes	10,39
Lemon balm extract -24 hours	140,5
AgNP with 5-minute extract	145,3
AgNP with homogenate	116,8

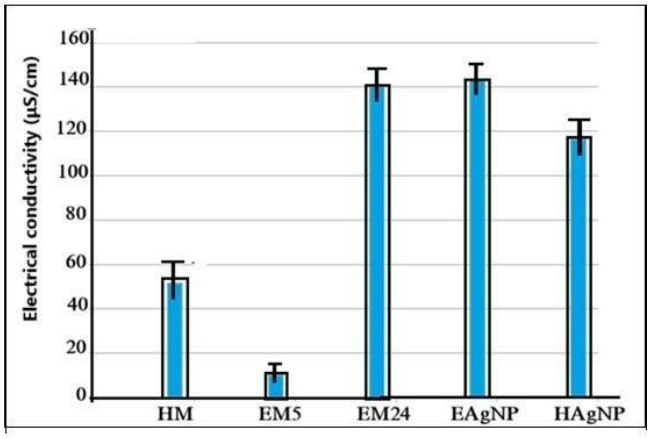


Figure 6. Electrical conductivity values of Melissa officinalis leaf extracts and homogenates

Conclusion

The conducted measurements have proven the importance of understanding the physical properties of plant extracts during their preparation and use. The results have shown that the physical parameters of extracts obtained from Melissa leaves depend on the composition of solvents, methods of extract preparation, temperature and application methods. It was observed that the physical parameters of the homogenate obtained from fresh leaves differ from those of extracts obtained from dry leaves. This distinction is particularly noticeable in the electrical conductivity and surface tension coefficient of the homogenate. Interestingly, the physical parameters also vary based on the infusion time of the extract. For instance, the electrical conductivity of the extract after 5 minutes on infusion is very low, but after 24 hours, it increases significantly. Another intriguing finding is that the physical parameters of the solution in which nanoparticles are synthesized change notably when extracts are added to the $AgNO_3$ salt solution. Consequently,this alteration is most evident in the values of basicity and surface tension. Therefore, based on these experiments, it is important to consider and adjust the physical properties of

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homogenates and extracts from leaves when utilizing them Melissa officinalis in the green synthesis of nanoparticles.

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