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FLUORESCENCE AND UV-VIS CHARACTERIZATION OF *MELISSA OFFICINALIS* EXTRACT AND FRESH LEAVES

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ABSTRACT

Melissa officinalis is a medicinal plant that has been studied for its medicinal value since classical times. It has been used to treat various diseases due to its wide range of pharmacological properties. Most traditional healer's primarily use aqueous extracts from the plant. Therefore, in the research presented, an aqueous extract of lemon balm as well as its green leaves were used. Fluorescence and UV-Vis characteristics were studied based on the time of infusion of the extract. The emission fluorescence spectra were measured by illuminating with an exciting light in the 337 – 400 nm wavelength range. The dependence of the emission spectra on the infusion time of the extract shows that as time increases, a shift occurs in the peak of the spectra. The intensity increases in the first 30 minutes but then begins to decrease. Generally, the peak wavelength of the emission spectrum is between 474 - 486 nm. A comparison of the fluorescence emission spectra recorded on the upper and lower sides of the Melissa leaf shows that the intensity of fluorescence radiation is higher on the upper side of the leaf than on the lower side. The emission fluorescence spectrum of the green leaf homogenate of Melissa at an excitation wavelength of 337- 400 nm shows blue, green and red fluorescence. Therefore, UV-Vis spectroscopic analysis of *Melissa officinalis* extract can contribute significantly to the further study of its pharmacological properties.

Keywords: medicinal plant, leaves, extracts, fluorescence spectra, excitation, emission, ultraviolet absorption.

Introduction

Medicinal plants are currently widely used in medical practice by approximately 80% of the world's population, especially in developing countries. This is due to their better tolerance, biocompatibility, and fewer side effects (WHO. Global Atlas of Traditional, Complementary and Alternative Medicine: World Health Organization, Geneva. 2005). Bioactive molecules from medicinal plants are also of great importance in the development of new therapeutic agents and the discovery of nanomedicines. Nanomedicines, which will be an alternative to traditional drugs in preventing or curing many diseases, have generated great interest worldwide in the use of plant-based pharmaceuticals (Mukherjee, 2002).

The study of bioactive molecules with pharmacological activity in medicinal plants has been ongoing for years, as researches explore their potential health benefits. Recently, there has been a surge of interest in the nanotechnology industry regarding these molecules, further increased the increasing research on their extraction. The main goal of many studies is to use these bioactive molecules as drugs for both conservation and health purposes. In general, bioactive molecules found in medicinal plants are natural components of plant-

based medicines, responsible for their color, taste and odor. Plant phytochemicals are diverse group of compounds synthesized by medicinal plants as secondary metabolites, including polyphenols. These compounds are classified into major classes such as flavonoids, phenolic acids, tannins, stilbenes and lignans (Ignat et al., 2011). Polyphenolic compounds, in particular, have been linked to health benefits due to their antioxidant, anti-inflammatory and antimicrobial properties (Thompson et al., 2009; Dziri et al., 2012). Research data suggests that consuming plant foods rich in polyphenols can reduce the risk of chronic diseases like cardiovascular diseases, type 2 diabetes and cancer (Thompson et al., 2009; Sancho & Pastore, 2012).

Other biological compounds such as carotenoids (Negi, & Namitha, 2010) and betalains (Strack et al., 2003) have also demonstrated antioxidant activity and have piqued the interest of researchers in the pharmaceutical industry. These compounds are valued for their anti-inflammatory properties (Porrini et al., 2008) and potential health benefits against diseases like cancer and cardiovascular diseases (Mordente et al., 2011; Perveen et al., 2015). Therefore, it is crucial to monitor the bioactive compounds present in medicinal plants, study their characteristics, and oversee their extraction

process to prevent any changes that could negatively impact their beneficial use.

Fluorescence and UV-Vis spectrophotometry are methods that can be used to determine the properties of bioactive compounds contained in medicinal plant extracts (Nanna et al., 2013). These methods can be used to assess the ability of medicinal plant extracts to absorb and emit ultraviolet radiation. The principle of this analysis method is to measure the absorption of light energy at the excitation wavelength and the emission of light energy at the emission wavelength (Fardiyah et al., 2020). In the presented study, UV-Vis screening and fluorescence characterization of extracts from the medicinal plant *Melissa officinalis* were initially conducted. *Melissa officinalis* is one of the most extensively studied medicinal plants. Its homeland is considered to be the Mediterranean coastal regions and it is predominantly used in folk medicine in Europe, the Middle East, the Caucasus, Iran, and Central Asia. Studies of its pharmacological properties have revealed shown that the composition of this plant is rich in bioactive substances such as flavonoids, phenolic acids, volatile compounds, and triterpenoids. It has been

demonstrated that extracts and pure bioactive compounds isolated from *Melissa* have various pharmacological effects, including anxiolytic, antiviral, and spasmolytic activities, as well as effects on mood, cognition, and memory. Furthermore, *Melissa officinalis* has been identified as a potential source for the treatment of various diseases, particularly anxiety and other central nervous system disorders (Abolfazl Shakeri et al., 2016).

The mechanisms of action of this plant, pharmacokinetics, properties of its extracts, and various aspects of its potential interaction with modern, active compounds have created opportunities for obtaining nanomedicines from. This is considered the most promising area, especially in pharmacology.

2. Materials and methods

2.1 *Melissa officinalis* plant

Dried leaves of *Melissa officinalis* are produced by "Herba Flora" LLC in the Absheron region of the Republic of Azerbaijan and are freely sold in pharmacies. Fresh leaves of *Melissa officinalis* were collected from plants cultivated in a laboratory Plant Growth Chamber



Figure 1. *Melissa officinalis* plants: A-in vegetative pot, B-leaves, C-dried leaves, D- aquatic extract

2.2. Preparation of aqueous extract.

Approximately 2 g of dried *Melissa Officinalis* L. leaves were added to 100 mL of double distilled water in a 250 mL flask and stirred using a magnetic heater stirrer at 80 °C for 40 minutes to obtain the extract. The extract was then centrifuged at 6000 rpm and filtered. The filtrate was kept in a refrigerator for further use.

2.3. Spectral analysis

Fluorescence analysis of the *Melissa officinalis* extract and the solution of nanoparticles synthesized in this extract was performed on a Cary Eclipse (Varian) fluorescence spectrometer. UV-Vis analysis was conducted on a SPICORD -250 (Plus) UV-vis spectrometer.

3. Results and discussion

3.1 Fluorescence Spectra of *Melissa* Extract

The extract obtained from dry leaves of *Melissa officinalis* is rich in bioactive substances, making it highly suitable for the green synthesis of nanoparticles. The composition of the extract may vary depending on the solvent used (methanol, ethanol, acetone, distilled water, etc.). Therefore, it is important to prepare an optimal extract for the green synthesis of nanoparticles. In many studies, the aqueous extract of *Melissa officinalis* is preferred. In our experiments, we initially recorded the fluorescence excitation and emission spectra of the prepared aqueous extract of *Melissa*, and then studied both its fluorescence and UV-vis characteristics based on infusion time. In Figure 2, the fluorescence excitation and emission spectrum of the extract was recorded when irradiated with exciting light at a wavelength of 407.07 nm. The emission peak was observed at 477.01 nm in the range of 400-700 nm.

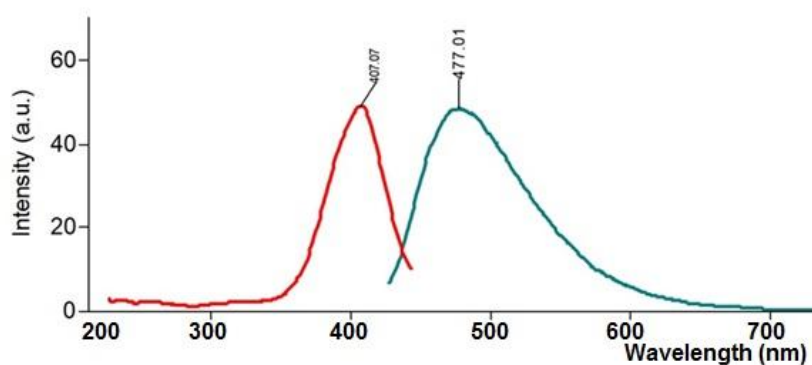


Figure 2.

Fluorescence excitation (red – 410 nm) and emission (green - 477) spectra of *Melissa officinalis* extract

The fluorescence spectra of the extract depending on the infusion time are presented in Figure 3. The emission fluorescence spectra were measured by illuminating with an exciting light at a wavelength of 398 nm. The infusion times of the extracts were 5, 10, 20, 30 and 40 minutes. Therefore, when preparing a standard extract with distilled water, samples were taken at these intervals and their emission spectra were recorded. As shown in Fig. 3, the peak wavelength of the emission spectrum of the 5-minute extract was 474.02 nm.

While the peak wavelength of the emission spectrum remained at 474.02 nm in the 10-minute extract sample, the intensity increased slightly. In the emission spectrum of the 20 minute sample, a shift in the peak wavelength occurred reaching 480 nm, with a slight increase in intensity. A shift was also observed in the peak of the emission spectrum of the 30-minute extract sample, reaching 486 nm, with a significantly higher intensity. In the emission spectrum of the 40-minute extract sample, the peak was at 477.01 nm with a slight decrease in intensity.

The correlation between the emission spectra and the infusion time of the extract indicates that as time increases, there is a shift in the peak of the spectra. The intensity increases in the first 30 minutes, but then starts to decrease. Generally, the peak wavelength of the emission spectrum falls within the range of 474 - 486 nm. The composition of the extract from the *Melissa officinalis* plant, is quite diverse containing rosmarinic acid, flavonoids, quercetin, rutin furilic acid, and other biologically active molecules.

Fluorescence emission spectra of some bioactive molecules are displayed in Figure 4. When the extract is exposed to an exciting light with a wavelength of 398 nm, the emission wavelength falls between 474-486 nm, which does not align with the peak wavelength of the standard emission spectra of bioactive molecules shown in Figure 4. While they are somewhat close to the peak of the standard emission spectrum of rutin molecules, it cannot be definitively stated that these spectra are the emission spectra of those molecules.

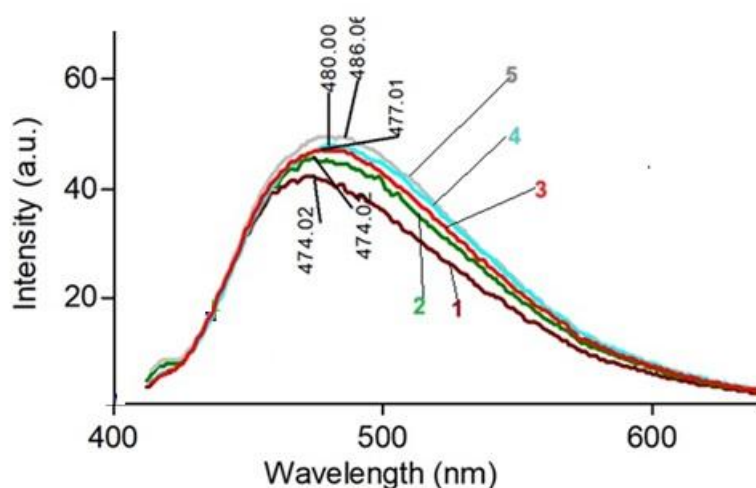


Figure 3. Fluorescence emission spectra of *Melissa officinalis* extract depending on the infusion time: 1- 5 min (excitation 398.03 nm), 2- 10 min (excitation 398.03 nm), 3 – 40 min ((excitation 400 nm), 4- 20 min (excitation 398.03 nm), 5- 30 min (excitation 398.93 nm),

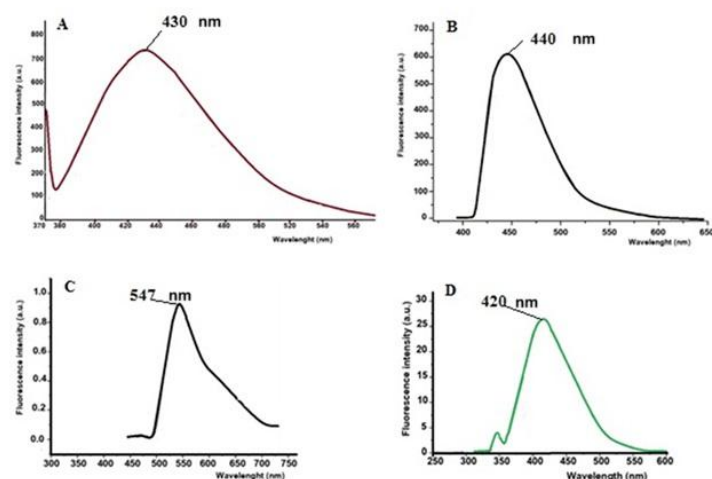


Figure 4. Standard fluorescence emission spectra were obtained for several bioactive molecules present in *Melissa officinalis* extract: A) 50 μM rosmarinic acid, B) quercetin: C) rutin and D) 10^{-5}molL^{-1} furilic acid at pH6.23. The spectra were measured for excitation wavelengths of 345 nm.

Also UV-Vis analysis was performed to identify phytochemicals in *Melissa* extract. UV-Vis analysis is a useful method to identify σ -bonds, π -bonds and unshared pairs of electrons in biologically active substances in the extract. The peaks observed in the UV-Vis spectra in the 200–400 nm range mainly indicate the presence of heteroatoms such as S, N and O as well as unsaturated groups (Saini et al., 2020). Figure 5 shows the UV-Vis spectra of *Melissa* extract depending on the infusion time.

The infusion times of the extracts were 5,10,20,30,40 minutes, 1 hour, 2 hours and 24 hours. Thus, when preparing a normal extract with distilled water, samples were taken at these times and their UV-Vis spectra were recorded. It was clear that two peaks are observed in the spectra in the 250–400 nm range. The first of these peaks is observed in the 284–285 nm range, depending on the to infuse time, and the second is observed in the 320–323 nm range. The absorption intensity in the UV-Vis spectra also depends on the to infuse time.

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Thus, the maximum absorption is observed in the 1-hour extract. Although it is difficult to clarify which molecules the peaks observed in the 250–400 nm range in the UV-vis spectrum of *Melissa officinalis* extract correspond to using this method, it is possible to make an opinion by comparing them with the UV-vis spectra of standard molecules. Figure 6 shows the standard UV-vis spectra of gallic acid, quercetin, rosmarinic acid at pH 7.4, and apigenin ($9.10 \times 10^{-5} \text{ mol L}^{-1}$) molecules. As can be seen from figure 6, the peak in the UV-Vis spectrum of gallic acid is observed at 270 nm, the peaks at 255 and 275 nm in the quercetin molecule, the peaks at 270 and 330 nm in rosmarinic acid, and the peaks at 250 nm, 270 nm and 350 nm in apigenin.

Since the 325 nm peak observed in the *Melissa* extract is close to the peaks of rosmarinic acid, and the 284, 323 nm peaks of apigenin and quercetin, it can be said that these molecules are in the composition of the *Melissa* extract. Of course, although the exact identification of the molecules contained in the extract cannot be determined by UV-vis analysis, it is possible to perform a certain qualitative analysis with this method. For precise determination, other methods, such as HPLC, GC/MS methods, should be used.

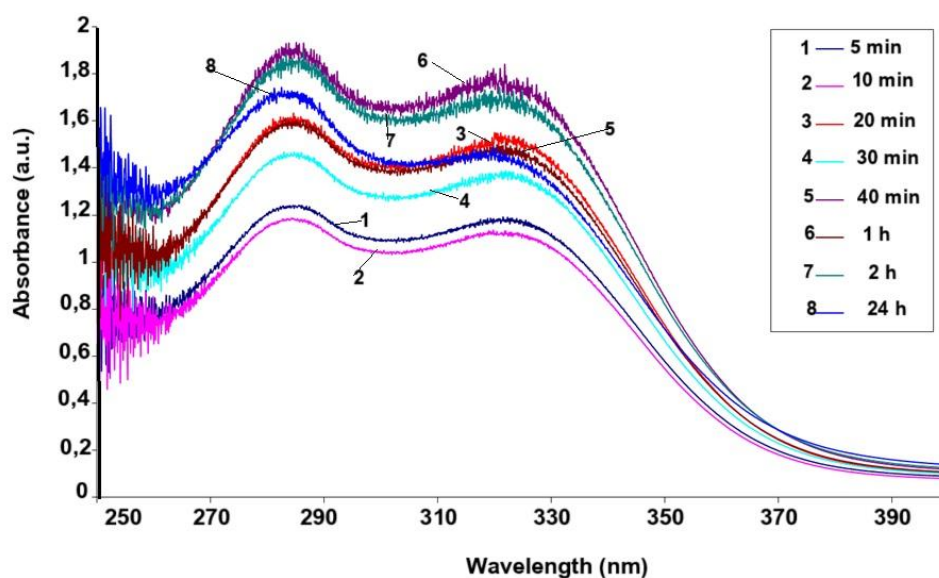


Figure 5. UV-Vis spectra of *Melissa officinalis* extract depending on the infusion time

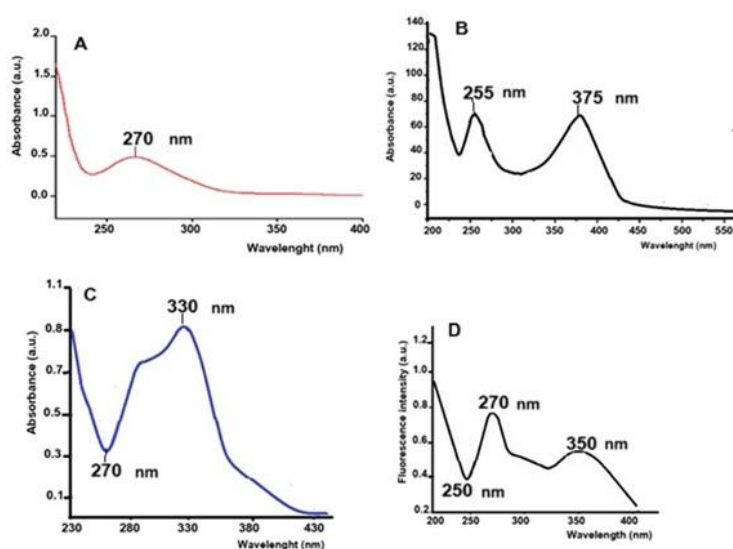


Figure 6. Standard UV-Vis spectra of some bioactive molecules contained in *Melissa officinalis* extract: A) gallic acid, B) quercetin: C) rosmarinic acid at pH 7.4, and D) apigenin ($9.10 \times 10^{-5} \text{ mol L}^{-1}$)

Phytochemical analysis of dried leaf extracts of *Melissa* showed the presence of various bioactive compounds. The quality and quantity of phytochemicals is depend on the selection of the solvent system and the extraction procedure used. The

phytoconstituents in different *Melissa* extracts could be beneficial in treating various diseases. Additionally, fluorescent analysis of the powdered drug plays crucial role in determining the quality and purity of the drug.

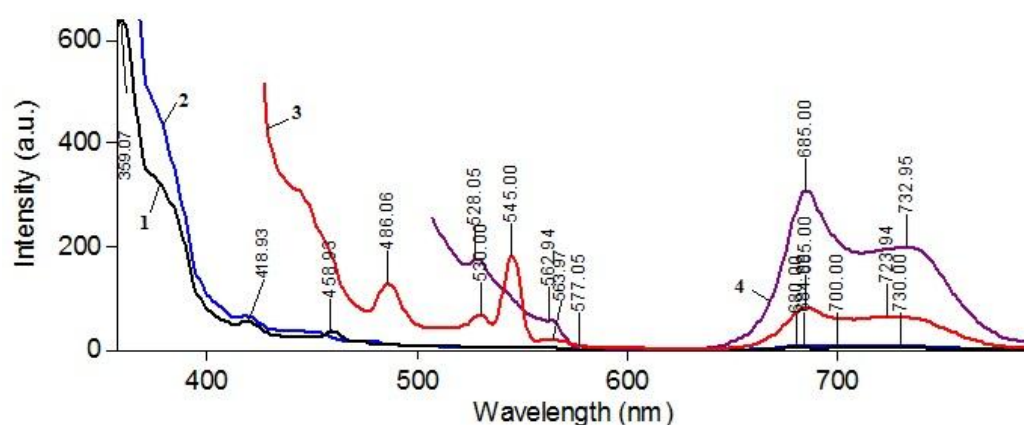


Figure 6. Fluorescence emission spectra from the upper side of *Melissa* leaf are as follows:
 1- excitation wavelength 337 nm, 2 - excitation wavelength 350 nm?,
 3 – excitation wavelength 400 nm, and 4 – excitation wavelength 487 nm

It is evident from the fluorescence and UV-Vis spectra of *Melissa* extract that the emission and absorption peaks of the bioactive molecules it contains are primarily observed in the 250-480 nm range. Therefore, in our experiments, we found it intriguing to investigate both blue and red fluorescence, in living leaves of *Melissa*. To capture blue fluorescence, we recorded emission and excitation spectra on both the upper and lower parts of the green leaves of *Melissa*. It is well known that the upper and lower parts of leaves of higher plants have a chloroplast-free epidermis layer. The vacuoles of the cells in this layer contain various phenolic compounds, such as cinnamic acids, stilbenes, coumarins, and various types of flavonoids, which can vary from plant to plant. Phenolic substances, alkaloids, catechins and other secondary plant products, can be localized not only in the vacuole, but also in the cell walls (Harborne & Turner, 1984; Hegnauer (1989 and 1990). The epidermal cells of the leaf serve to protect the mesophyll cells from the harmful effects of UV rays (Caldwell, 1981)

The fluorescence emission spectra were recorded from the upper and lower sides of *Melissa* leaves at different excitation wavelengths (337 nm, 350 nm, 400 nm and 487 nm). On the upper side of *Melissa* leaves, two maxima were recorded at 418.93 nm and 458.93

nm (blue fluorescence) and 680 nm and 730 nm (red fluorescence of chlorophyll) in the emission fluorescence spectrum at the excitation wavelength of 337 nm (Figure 6- spectrum 1). At the excitation wavelength of 400 nm, one maximum was recorded at 486.06 nm (blue fluorescence), 530 nm, 545 nm (green fluorescence) and 680 nm and 723 nm (red fluorescence of chlorophyll) (Figure 6- spectrum 3). At an excitation wavelength of 487 nm, the emission fluorescence spectrum shows a maximum at 528 nm (green fluorescence) and at 685 nm and 732 nm (red fluorescence of chlorophyll) (Figure 6- spectrum 4).

A comparison of the fluorescence emission spectra recorded on the upper and lower sides of the *Melissa* leaf shows that the intensity of fluorescence radiation is higher on the upper side of the leaf than on the lower side. Also, the F486/F685 ratio of blue and red fluorescence is higher on the upper side of the leaf (2.56) than on the lower side (1.22). Due to the structure of the leaf, there are fewer photosynthetic pigments on its lower side, resulting in higher the intensity of fluorescence emission in the blue-green and red spectral regions. The F486/F685 ratio and the blue and red fluorescence ratio are also 2 times higher on the upper side of the leaf than on the lower side.

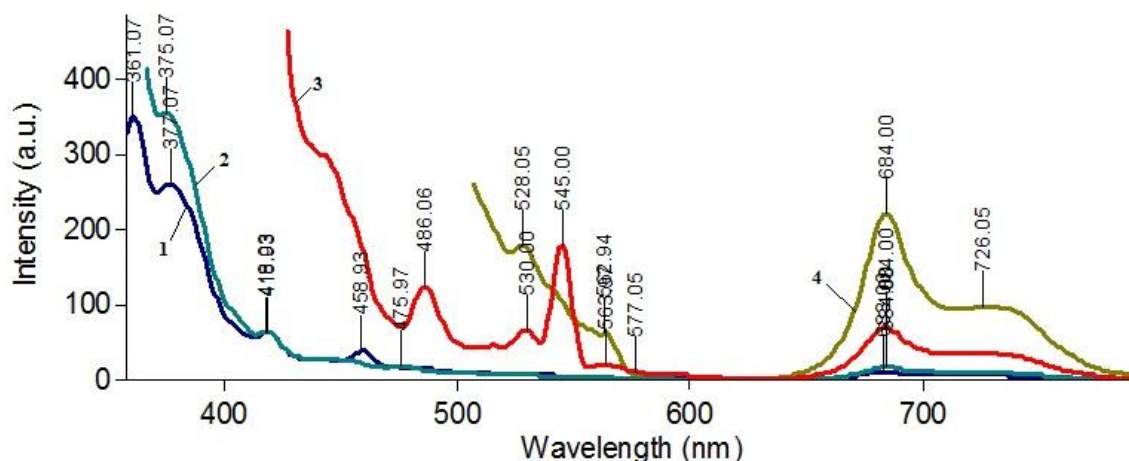


Figure 7. Fluorescence emission spectra were obtained from the underside of a *Melissa* leaf at four different excitation wavelengths: 1) 337 nm, 2) 350 nm, 3) 400 nm, 4) 487 nm

On the underside of the *Melissa* leaf, the emission fluorescence spectrum at an excitation wavelength of 337 nm shows three maxima at 337.07 nm, 418.03 nm, and 458.93 nm (blue fluorescence) and 680 nm of low intensity (red fluorescence of chlorophyll) (Figure 7- spectrum 1). At an excitation wavelength of 400 nm, the emission fluorescence spectrum shows one maximum at 486.06 nm (blue fluorescence), 530 nm,

545 nm (green fluorescence) and 680 nm and 723 nm (red fluorescence of chlorophyll) (Figure 7- spectrum 3). At an excitation wavelength of 487 nm, the emission fluorescence spectrum shows one maximum at 528,05 nm (green fluorescence) and 684 nm and 726.05 nm (red fluorescence of chlorophyll) (Figure 7- spectrum 4).

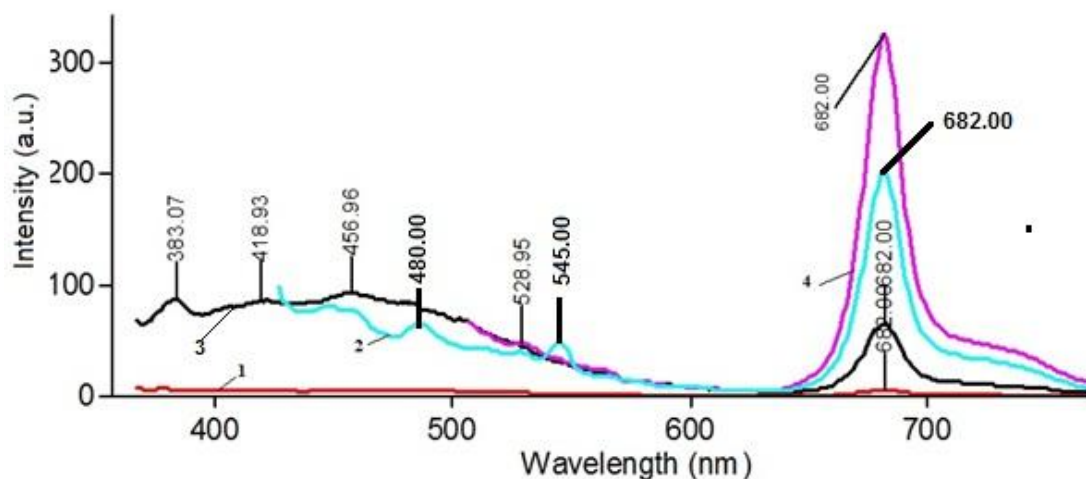


Figure 8. Fluorescence emission spectra of *Melissa* leaf homogenate: 1- excitation wavelength 337 nm, 2 - excitation wavelength 350 nm, 3 – excitation wavelength 400 nm, 4 – excitation wavelength 487 nm

The emission fluorescence spectrum of the green leaf homogenate of *Melissa* at an excitation wavelength of 337 nm shows only one maximum at 682 nm (red fluorescence of chlorophyll) (Figure 8- spectrum 1). At an excitation wavelength of 350 nm, the emission fluorescence spectrum shows one maximum at 480.00 nm (blue fluorescence), one at 545 nm (green fluorescence), and one at 682 nm (red fluorescence of chlorophyll) (Figure 8- spectrum 2). At an excitation wavelength of 400 nm, the emission fluorescence spectrum shows three maxima at 383.07 nm, 418.93 nm and 456.96 nm (blue fluorescence), and 682 nm (red fluorescence of chlorophyll) (Figure 8- spectrum 3). At an excitation wavelength of 487 nm, the emission fluorescence spectrum shows a maximum at 528.95 nm (green fluorescence) and 682 nm (red fluorescence of chlorophyll) (Figure 8- spectrum 4).

Discussion

It should be noted that the application of UV-Vis spectrophotometry for studying on the composition of complex media such as plant extracts is limited. Therefore, it is difficult to characterize the absorption peaks for any specific component in the extract. To accurately characterize the extract components, it is necessary to use other analytical methods such as more advanced HPLC, GC/MS, etc. in addition to UV-Vis analysis (Jain et al, 2016; Sathish, 2012). To obtain high-quality and accurate peaks in the UV-Vis spectrum of the aqueous extract of the medicinal plant *Melissa officinalis*, spectra were recorded in the wavelength range from 200 nm to 800 nm. The profile of the UV-Vis spectra of the extract showed that the absorption peaks correspond to the absorption bands of flavonols, chrysin,

hesperetin, eriodictyol and taxifolin (270-288 nm), rosmarinic acid, apigenin (350 nm). Thus, UV-Vis spectroscopic analysis of *Melissa officinalis* extract can play a certain role in further studying its pharmacological properties.

Initial spectral screening of *Melissa* extract and green leaves revealed the presence of secondary metabolites, alkaloids, saponins, terpenoids, steroids, tannins and flavonoids. The results of the fluorescence properties study of the extract showed that ultraviolet radiation was absorbed in the excitation region (200-400 nm). The intensity of fluorescence indicates the amount of component compounds present in the extract that can absorb or emit ultraviolet radiation. As presented by Chapelle et al. (1984), it is possible to simultaneously measure the full fluorescence spectrum of plant leaves from 400 to 800 nm with UV-B excitation at 337 nm. The relative intensities of blue (BF), green (GF) and red fluorescence (RF) radiation can vary depending on the type of plant and even the structure of the leaves (Chapelle, et al., 1984).

The relative intensity of BF, GF and RF radiation varies depending on the structure of the leaf, so that the BF/RF ratio (F486/F685) of the upper surface of the leaf (2.56) is higher than the BF/RF ratio (1.22) of the lower surface. As mentioned, this is due to the differences in the chlorophyll content of the upper and lower parts of the leaf. Since the number of cells on the lower sides of the leaf is lower and therefore there is a lower amount of chlorophyll and carotenoids, the intensity of BF and GF radiation depends on the amount of chlorophyll and carotenoids. Since these pigments have a wide absorption band in the blue and green region, they can reabsorb most of the emitted blue and

green fluorescence. Since there are air spaces between the cells on the lower side of the leaf, the emitted BF and GF radiation have a greater chance of being reflected from the cell walls and passing through the leaf than from the upper layer of the leaf, which has a densely packed and pigment-rich palisade parenchyma.

Thus, the characterization of the fluorescence spectra of *Melissa* extract and green leaves showed that this medicinal plant has a specific fluorescence spectrum when absorbing (exciting) and emitting (emitting) ultraviolet rays. The results of this study indicate that UV-B emission induced by blue and green fluorescence appears to be a general feature of plant leaves and can be detected not only in green leaves but also in chlorophyll-free yellow leaves.

Conclusion

The blue fluorescence emission of *Melissa* extract is theoretically assumed to be derived from biologically active molecules as rosmarinic acid, flavonoids, quercetin, rutin and furilic acid. The main source of the blue-green fluorescence emission in green leaves is believed to be substances in the epidermal layer. However, fluorescence emission from compounds in green mesophyll cells, contributes less to the overall fluorescence of leaves due to reabsorption processes. The emission spectra of the extract shows that as time increases, a shift in the peak as infusion time increases with intensity initially increasing the first 30 minutes before decreasing. Generally, the peak wavelength of the emission spectrum falls within the range of 474 - 486 nm. A comparison of fluorescence emission spectra from the upper and lower sides of the *Melissa* leaf reveals higher intensity on the upper side. The fluorescence emission spectrum of the green leaf homogenate of *Melissa* when excited within the 337- 400 nm range, displays blue, green and red fluorescence. Therefore, UV-Vis spectroscopic analysis of *Melissa officinalis* extract could be valuable for further exploration of its pharmacological properties.

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Ethical approval

There was no need for ethics approval for the present research.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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