

ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF *MELISSA OFFICINALIS* ESSENTIAL OIL AGAINST SELECTED BACTERIAL STRAINS

Petru RAIN¹ (ORCID: 0009-0007-0837-9202), Ciprian BUZNA¹ (ORCID: 0009-0002-0183-2709), Nicolae Marinel HORABLAGA^{1,2} (ORCID: 0009-0002-3520-7362), Cristian BOSTAN¹ (ORCID: 0000-0002-7549-9454), Anca PANDA¹ (ORCID:0000- 0003-2785-7111), Mihai TABARA¹, Cristian CRISTEA¹ (ORCID: 0009-0003-7092-8533)

¹ Agricultural Research and Development Station Lovrin
² University of Life Sciences King Mihai I from Timisoara

Corresponding author: ciprian.buzna@scdalovrin.com

Abstract. The present study investigates the antioxidant and antimicrobial potential of *Melissa officinalis* essential oil (MOEO) as a natural bioactive compound. Antioxidant activity was evaluated using the DPPH radical scavenging assay, revealing a clear concentration-dependent increase in inhibition capacity. Antimicrobial activity was assessed by the broth microdilution method against selected Gram-negative bacterial strains, namely *Escherichia coli*, *Salmonella typhimurium*, and *Haemophilus influenzae*. The results demonstrated a dose-dependent inhibitory effect, with increasing concentrations of MOEO leading to a progressive reduction in bacterial growth. The minimum inhibitory concentration (MIC) values ranged between 0.85 and 2.50 mg/mL, indicating variable sensitivity among the tested strains, with *H. influenzae* being the most susceptible. The observed biological activities are associated with the presence of bioactive compounds, particularly oxygenated sesquiterpenoids and phenolic constituents, which contribute both to radical scavenging capacity and to the disruption of bacterial cell membranes. The correlation between antioxidant and antimicrobial effects suggests a common mechanism related to redox interactions and membrane destabilization. Overall, these findings highlight the multifunctional potential of *Melissa officinalis* essential oil as a natural antioxidant and antimicrobial agent, with promising applications in agriculture, food preservation, and alternative therapeutic strategies.

Keywords: *Melissa officinalis*; essential oil; antioxidant activity; antimicrobial activity; DPPH assay; microdilution method; Gram-negative bacteria; minimum inhibitory concentration (MIC); bioactive compounds.

INTRODUCTION

The rapid increase in antimicrobial resistance represents a major global health challenge, driving the need for alternative strategies based on natural compounds. Plant-derived bioactive molecules have gained increasing attention due to their broad-spectrum biological activities and reduced toxicity compared to synthetic agents (MORADKHANI et al., 2019).

Species belonging to the *Lamiaceae* family, particularly *Melissa officinalis*, are recognized for their rich phytochemical composition, including terpenoids and phenolic compounds with proven antimicrobial and antioxidant properties (DOGAN et al., 2019; MLADENOVIC et al., 2023). The biological activity of these compounds is strongly influenced by their chemical profile, which varies depending on environmental and technological factors (NOURI et al., 2020; WANG et al., 2020).

The aim of this study was to evaluate the chemical composition, antioxidant potential, and antimicrobial activity of *Melissa officinalis* essential oil against selected Gram-negative bacterial strains, in order to assess its potential as a natural bioactive agent.

MATERIAL AND METHODS

Antioxidant activity (DPPH assay)

The free radical neutralization activity was evaluated by the DPPH method (1,1-diphenyl-2-picrylhydrazil test), using a 0.3 mM DPPH solution prepared in 70 % ethanol. This method is commonly used to evaluate the antioxidant activity of samples, based on their ability to neutralize free radicals. The DPPH molecule (1,1-diphenyl-2-picrylhydrazil) is a free radical, purple in color, stable due to the possibility of unpaired electron transfer from its structure. When a solution of DPPH reacts with an antioxidant capable of donating a proton, the radical is reduced to the corresponding hydrazine and the color changes from purple to pale yellow (FLOARES et al., 2025).

1 mL of extract (40 mg/mL) was used and 2.5 mL of 0.3mM DPPH solution was added. The resulting mixture was shaken vigorously and incubated for 30 minutes in the dark, at room temperature. After this rest period, absorption was measured at 518 nm with a UV-VIS spectrophotometer (Specord 205; Analytik Jena AG, Jena, Germany). 70% ethyl alcohol was used as a reference. The free radical inhibition activity (DPPH%) was calculated using equation (1):

$$\text{DPPH (\%)} = (A_{\text{control}} - A_{\text{sample}}) / (A_{\text{control}}) \times 100 \quad (1)$$

where A_{control} = absorbance value of the control sample

A_{sample} = absorbance value of sample

Antimicrobial activity

The antimicrobial activity of MOEO was evaluated in broth by the microdilution method against different ATCC strains. The ATCC strains tested which belong to the biological strain collection of the Microbiology Laboratory within the Interdisciplinary Research Platform of the "King Michael I of Romania" University of Life Sciences in Timisoara were *Escherichia coli* (ATCC 25922), *Salmonella typhimurium* (ATCC 14028) and *Haemophilus influenzae* type B (ATCC 10211). A stock solution in dimethyl sulfoxide (DMSO) was prepared by adding 50 μ L of essential oil in 1000 μ L of DMSO. The freshly grown bacterial suspension was diluted to an optical density (OD) of 0.5 McFarland standard (1.5×10^8 CFU \times mL).

Several dilutions were made in series. They final tested concentrations were 0.85 mg/mL, 1.70 mg/mL, 2.50 mg/mL, 5.15 mg/mL and 8.50 mg/mL.

The plates were covered and left for 24 hours at 37°C. After 24 hours, the OD was measured at 540 nm using an ELISA reader (BIORAD PR 1100, Hercules, CA, USA). Triple tests were carried out for all samples. Strain and BHI suspensions were used as a positive control.

The lowest concentration of the compound, MIC, which inhibits visible microbial growth, was determined by measuring OD using the spectrophotometric method.

The results are presented as three values, the mean value, the percentage of bacterial growth (PBG%) and the percentage of bacterial inhibition (PBI%), percentages calculated using the formulas (2), (3):

$$\text{PBG (\%)} = (\text{OD}_{\text{Sample}}) / (\text{OD}_{\text{control}}) \times 100 \quad (2)$$

$$\text{PBI (\%)} = 100 - \text{PBG (\%)} \quad (3)$$

where $\text{OD}_{\text{sample}}$ represents the optical density of the MOEO and contains a certain concentration of the sample that has been tested, as the average value of the triple readings at 540 nm. The negative control, represents the optical density at 540 nm as the mean value of the triplicate readings for the selected strains selected in the BHI.

The broth microdilution method is one of the most basic methods of antimicrobial susceptibility testing (OBISTIOIU et al., 2021).

RESULTS AND DISCUSSIONS

Antioxidant activity

The antioxidant activity of *Melissa officinalis* essential oil (MOEO), evaluated using the DPPH radical scavenging assay, demonstrated a clear concentration-dependent effect and is presented in Table 1. The inhibition percentage increased progressively from 10.47% at 4 mg/mL to 44.93% at 20 mg/mL, indicating a moderate but consistent antioxidant potential.

Table 1.

Antioxidant activity of MOEO		
MOEO concentration (mg/mL)	MOEO	Ascorbic acid
	Inhibition (%)	
4	10.47	48.94
6	15.82	54.12
12	29.93	78.59
20	44.93	96.35

As can be seen, the antioxidant activity of MOEO starts as early as 4 mg/mL, with an inhibition percentage of 10.47%, a percentage that increases successively with the increase in essential oil concentration, reaching 44.93% at 20 mg/mL.

The antioxidant activity of essential oils derives mainly from the presence of compounds with the potential to neutralize reactive oxygen species (ROS) and reduce cellular oxidative stress. In the case of *Melissa officinalis* essential oil analysed in this study, antioxidant activity may be correlated with the presence of oxygenated sesquiterpenes and phenolic compounds in significant concentrations.

According to their characteristic chemical composition, caryophyllene oxide and α -cadinol are the oxygenated compounds with the highest concentrations identified in the literature. Recent studies have shown that these sesquiterpenoids possess a strong antioxidant potential, acting by capturing free radicals and protecting cell membranes against lipid peroxidation.

Caryophyllene oxide has been identified as one of the most effective compounds in DPPH and ABTS tests, two standard methods of evaluating antioxidant activity. Also, carvacrol, although present in a relatively low concentration in MOEO, contributes significantly to antioxidant activity due to its free phenolic group, which facilitates electron donation (SOUHI et al., 2020). The synergy between carvacrol and oxygenated sesquiterpenoids is highlighted in the literature as an enhancer of the oxidative stability of the essential oil (TUNG et al., 2022).

Studies by ILIEV et al. (2023) and EHSANI et al. (2017) on other chemotypes of *Melissa officinalis* reported high antioxidant activity, especially in citral-rich variants (neral and geranial) (ILIEV et al., 2023.; EHSANI et al., 2017) Although oxygenated monoterpenes (such as citral) are effective in capturing free radicals, their activity is less stable over time and under conditions of prolonged storage (NURZYŃSKA-WIERDAK et al., 2018).

In contrast, oxygenated sesquiterpenes and sesquiterpenoids are more thermostable and oxidatively resistant, which can give the oil analyzed here sustained and long-lasting antioxidant activity, even though its immediate radical-scavenging power may be lower than that of citral-rich oils (MIRAJ et al., 2017; TUNG et al., 2022).

Antimicrobial activity

The percentage of bacterial growth for strains of *E.coli*, *S. typhimurium* and *H. influenzae* in the presence of different concentrations of MOEO is shown in Figure 1.

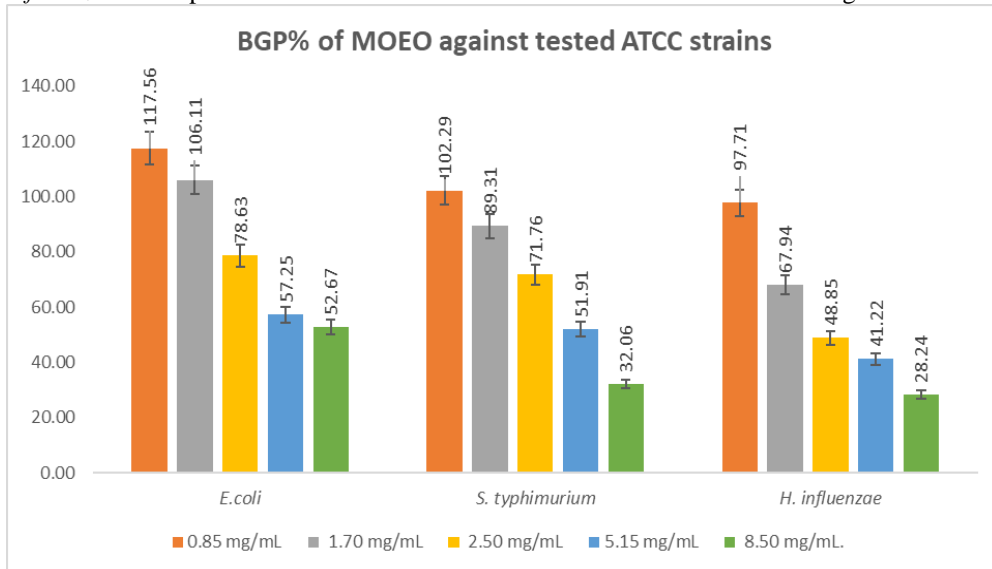


Figure 1. Percentage of bacterial growth (BGP%) of *E. coli*, *S. typhimurium*, and *H. influenzae* in the presence of different concentrations of *Melissa officinalis* essential oil (mg/mL)

The antimicrobial activity of MOEO against Gram-negative bacterial strains (*Escherichia coli*, *Salmonella typhimurium*, and *Haemophilus influenzae*) showed a concentration-dependent inhibitory effect. The percentage of bacterial inhibition is shown in Figure 2.

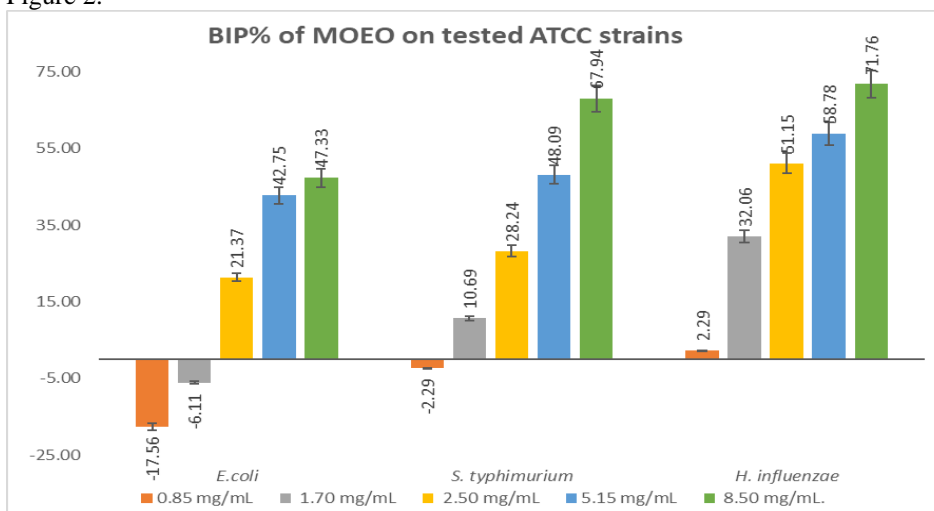


Figure 2. Percentage of bacterial inhibition (BIP%) of Gram-negative strains exposed to increasing concentrations of *Melissa officinalis* essential oil (mg/mL)

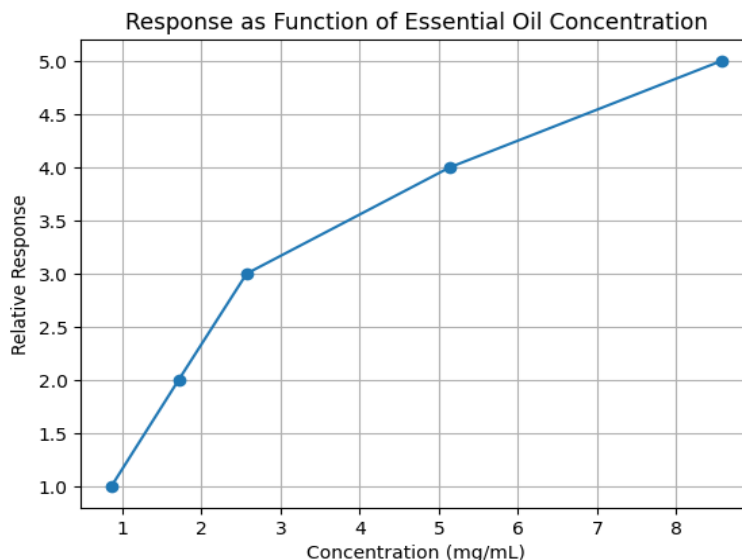


Figure 3. Linear relationship between essential oil concentration (mg/mL) and tested volume in the microdilution system

The graphical representation of the tested concentrations (Figure 3) expressed in mg/mL highlights a clear proportional relationship between the volume of essential oil added and the final concentration in the microdilution system. The concentrations ranged from 0.86 mg/mL to 8.57 mg/mL. This linear increase confirms the accuracy of the dilution approach and ensures a reliable basis for evaluating the biological activity of the essential oil. The use of standardized concentrations in mg/mL allows for better comparability with other studies and facilitates the interpretation of dose-dependent effects observed in both antioxidant and antimicrobial assays. Such representation is essential for correlating the intensity of biological responses with the actual amount of bioactive compounds present in the system.

As noted, BGP% ranged from 117.25% to 62.67% for *E. coli* depending on the concentration of oil used. For *S. typhimurium*, the BGP% ranged from 102.29 to 32.06, and for *H. influenzae* from 97.71% to 28.24%. Notably, for each strain taken in the study, the BGP% value decreased with the increase in the concentration of the oil tested, with a minimum value at the concentration of 8.5 mg/mL.

In the case of the *E. coli* strain, the BIP% had negative values at a concentration of 0.85 mg/mL (-17.56) and 1.70 mg/mL (-6.11), respectively, becoming positive at 2.50 mg/mL, this concentration representing the minimum inhibitory concentration. At this concentration of the essential oil tested, the BIP% value was 21.37%.

It is worth mentioning that the BIP% values have an upward trend, with a maximum value of 47.33% at a concentration of 8.50 mg/mL MOEO. Similarly, the BIP% values for *S. typhimurium* are on an upward trend, starting from -2.29% at the concentration of 0.85 mg/mL and reaching 67.94% at the concentration of 8.50 mg/mL. The MIC value against *S. typhimurium* is 1.70 mg/mL.

Of the three Gram-negative strains, *H. influenzae* is the most sensitive to MOEO, with a positive BIP% value from the concentration of 0.85 mg/mL, respectively 21.37%, and reaching 71.76% at the concentration of 8.50 mg/mL.

Determination of MIC values (minimum inhibitory concentrations) showed significant variability in the sensitivity of Gram-negative bacteria to the oil tested. The lowest MIC value was observed for *H. influenzae* (0.85 mg/mL), followed by *S. typhimurium* (1.70 mg/mL) and *E. coli* (2.50 mg/mL). This difference in activity can be attributed to the structural peculiarities of each bacterium. *E. coli* is recognized for the presence of efficient efflux pumps and a complex cell wall, which contributes to increased resistance to antimicrobial agents. On the other hand, *H. influenzae*, although still Gram-negative, has a cellular structure that is more sensitive to the action of volatile compounds in the essential oil (CHOUHAN et al., 2017; OBISTIOIU et al., 2021).

The results obtained are supported by the chemical composition of MOEO, dominated by the sesquiterpenes and oxygenated sesquiterpenoid classes, with a reduced presence of phenols derived from monoterpenes and monoterpenes (ABDELLATIF et al., 2014; AHL et al. 2018).

As for monoterpenes, the composition includes β -cis-ocimene and β -citral, compounds with recognized antimicrobial activity. Citral, in particular, can affect bacterial membrane permeability and intracellular osmotic balance, but at small concentrations, its role is likely complementary, contributing synergistically rather than directly.

Phenols derived from monoterpenes are compounds with strong antibacterial potential even at low concentrations, such as: carvacrol – which act on the bacterial membrane, causing proton losses and membrane depolarization; p-Menthane-3,8-diol – an alcohol with documented antimicrobial activity, involved in the breakdown of bacterial walls (WANG, et al., 2020).

Sesquiterpenes are the main chemical class responsible for the overall antibacterial effect. The most abundant compounds are: β -caryophyllene, β -Cubebene, α -Caryophyllene, α -Copaene and β -Elemene.

These molecules are lipophilic, with the ability to penetrate bacterial cell membranes, leading to ion leakage, loss of membrane integrity, and cell death. The activity of these sesquiterpenes is more pronounced against Gram-positive bacteria, but at high concentrations and in the presence of synergistic compounds, they can also act on Gram-negative (EHSANI et al., 2017; MORADKHANI et al., 2019).

The lower activity observed against *E. coli* can be explained by the complex structure of the outer membrane and the efflux mechanisms, which limit the penetration of hydrophobic compounds. In contrast, *S. typhimurium* has an intermediate sensitivity, and *H. influenzae*, with a more permeable capsule, is the most susceptible to the action of volatile and oxygenated compounds (HULEA et al., 2026; OBISTIOIU et al., 2020).

A direct relationship between the antioxidant and antimicrobial activities of MOEO can be observed, both being strongly influenced by its phytochemical composition. The compounds responsible for radical scavenging activity, particularly oxygenated sesquiterpenoids such as caryophyllene oxide and α -cadinol, also contribute to antimicrobial effects through mechanisms involving membrane destabilization and oxidative imbalance in microbial cells (HULEA et al., 2026).

The antioxidant capacity reflects the ability of these compounds to donate electrons and neutralize reactive oxygen species (ROS), while in microbial systems, similar redox interactions can disrupt cellular homeostasis, leading to protein denaturation, lipid

peroxidation, and ultimately cell death. In addition, phenolic compounds such as carvacrol exhibit a dual mode of action, acting both as efficient radical scavengers and as membrane-active antimicrobial agents by altering permeability and inducing leakage of intracellular components (FLOARES et al., 2025, OBISTIOIU et al., 2021).

Therefore, the dose-dependent increase observed in both antioxidant and antimicrobial assays suggests a common underlying mechanism driven by the redox properties and lipophilic nature of the bioactive constituents. This dual functionality enhances the biological relevance of *Melissa officinalis* essential oil and supports its potential use as a multifunctional natural agent with both protective (antioxidant) and inhibitory (antimicrobial) effects.

CONCLUSIONS

The essential oil of *Melissa officinalis* analyzed in this study demonstrated both antioxidant and antimicrobial activities, which are closely interconnected and primarily determined by its phytochemical composition. The antioxidant capacity, evidenced by the DPPH assay, showed a clear concentration-dependent increase, reflecting the ability of the oil to neutralize free radicals through electron donation mechanisms associated with oxygenated sesquiterpenoids and phenolic compounds.

At the same time, the antimicrobial activity exhibited a dose-dependent inhibitory effect against all tested Gram-negative bacterial strains, with *Haemophilus influenzae* showing the highest sensitivity, followed by *Salmonella typhimurium*, while *Escherichia coli* displayed the greatest resistance. The determined MIC values (0.85–2.50 mg/mL) confirm a moderate but consistent antibacterial effect.

The dual biological activity of the essential oil can be explained by a common mechanistic basis involving redox interactions and membrane disruption. Compounds responsible for antioxidant activity, such as caryophyllene oxide, α -cadinol, and carvacrol, are also capable of altering bacterial membrane integrity, inducing oxidative imbalance, and interfering with essential cellular processes. Thus, the antioxidant potential is not only a protective property but also contributes to antimicrobial effectiveness through the modulation of oxidative stress within microbial cells.

Overall, the results highlight the multifunctional nature of *Melissa officinalis* essential oil, supporting its potential application as a natural agent with both antioxidant and antimicrobial properties. Further investigations, including in vivo studies and toxicity assessments, are required to validate its practical applicability in food preservation, agriculture, and alternative therapeutic strategies.

BIBLIOGRAPHY

- ABDELLATIF, F., BOUDJELLA, H., ZITOUNI, A., HASSANI, A., 2014 - Chemical composition and antimicrobial activity of the essential oil from leaves of Algerian *Melissa officinalis* L. EXCLI J., 13: 772; Germany.
- ABDEL-NAIME, W.A., FAHIM, J.R., FOUAD, M.A., KAMEL, M.S. 2019 - Antibacterial, antifungal, and GC-MS studies of *Melissa officinalis*. S. Afr. J. Bot., 124: 228–234; South Africa.
- AHL, H.A.S.-A., SABRA, A.S., GENDY, A.S., ASTATKIE, T., 2018 - Essential Oil Content and Concentration of Constituents of Lemon Balm (*Melissa officinalis* L.) at Different Harvest Dates. J. Essent. Oil Bear. Plants, 21: 1410–1417; India.
- CHOUHAN, S, SHARMA, K, GULERIA, S., 2017 - Antimicrobial Activity of Some Essential Oils-Present Status and Future Perspectives. Medicines (Basel), 4(3): 58; Switzerland.
- DOĞAN, H., 2021 - Phytochemical composition of wild lemon balm (*Melissa officinalis* L.). Anatolian Journal of Botany, 5(2): 112–119; Turkey.

- EHSANI, M., AHARIZAD, S., GHAFFARI, M., 2017 - Chemical composition and antimicrobial activity of essential oil of *Melissa officinalis* L. from Iran, Journal of Essential Oil Research, 29(5): 405–410; United Kingdom.
- FLOARES, D., OBISTIOIU, D., HULEA, A., SULEIMAN, M. A., POPESCU, I., BERBECEA, A., SAMFIRA, I., RADULOV, I., 2025 - Antimicrobial and Antioxidant Properties of Sambucus nigra L. (Elderflower) Oil: A Molecular Docking and Biochemical Study. Agronomy, 15(2), 310; Switzerland.
- HULEA, A., IMBREA, F., FLOARES, D., POPESCU, I., SULEIMAN, M. A., HULEA, C., IMBREA, I. M., NEACȘU, A.-G., HORABLAGA, M., POPESCU, C. A., OBISTIOIU, D. 2026 - Chemical Profiling, Ampicillin Interaction Patterns, and Exploratory Molecular Docking of Lauraceae Essential Oils. International Journal of Molecular Sciences, 27(3), 1447; Switzerland.
- ILIEV, I., NEDELICHEV, V., GANDOVA, V., 2023 - Chemical composition of lemon balm essential oil from Bulgaria depending on harvest time. Journal of Botany, 5(10): 112-121; United States.
- MIRAJ, S., RAFIEIAN-KOPAEI, M., KIANI, S., 2017 - *Melissa officinalis* L: A Review Study with an Antioxidant Prospective, J. Evid.-Based Integr. Med., 22:385–394; United States.
- MLADENOVIĆ, M. Z., 2023 - The influence of sesquiterpenes on antioxidant activity of selected essential oils. Journal of Applied Botany and Food Quality, 96: 117–123; Germany.
- MORADKHANI, H., SARGSYAN, E., BIBAK, H., NASERI, B., SADAT-HOSSEINI, M., FAYAZI-BARJIN, A., MEFTAHIZADE, H., 2019 - *Melissa officinalis* L., a valuable medicine plant: A review. J. Med. Plants Res., 4: 2753–2759; Nigeria.
- NOURI, A., MIRABZADEH, M., SAFARI, N., EBADI, M.T., 2020 - Evaluation of Essential Oil Composition and Rosmarinic Acid Content in Lemon Balm (*Melissa officinalis* L.) Cultivated in South of Iran, J. Med. Plants By-Prod., 9: 159–166; Iran.
- NURZYŃSKA-WIERDAK, R., BOGUĆKA-KOCKA, A., SZYMCZAK, G., 2018, - Volatile Constituents of *Melissa officinalis* Leaves Determined by Plant Age. Nat. Prod. Commun., 9: 703–706; United States.
- OBISTIOIU, D., COCAN, I., TÎRZIU, E., HERMAN, V., NEGREA, M., CUCERZAN, A., NEACȘU, A.-G., COZMA, A.L., NICHITA, I., HULEA, A., RADULOV, I., ALEXA, E., 2021 - Phytochemical Profile and Microbiological Activity of Some Plants Belonging to the *Fabaceae* Family, Antibiotics, 10: 662; Switzerland.
- SOUIHI, M., AMRI, I., SOUISSI, A., HOSNI, K., BEN-BRAHIM, N., ANNABI, M., 2020, Essential oil and fatty acid composition of *Melissa officinalis* L., Prog. Nutr., 22: 253–258; Italy.
- STOJANOVIĆ, N. M., MLADENOVIĆ, M. Z., RANDJELOVIĆ, P. J., RADULOVIĆ, N. S., 2023 - Potential of lemon balm essential oil as an anti-anxiety agent – is citronellal the activity carrier?, Journal of Ethnopharmacology, 314: 116661; Netherlands.
- TUNG, Y. T., CHUA, M. T., WANG, S. Y., CHANG, S. T., 2022 - Antioxidant and anticancer activities of sesquiterpenoids from essential oils, Bioresource Technology, 344: 126204; Netherlands.
- WANG, W., XU, J., FANG, H., LI, Z., LI, M., 2020 - Advances and challenges in medicinal plant breeding, Plant Sci., 298: 110573; Netherlands.