



Prospects of the application of some species of the Lamiaceae family and some features of the development of their tinctures

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The search for new powerful herbal products with anti-inflammatory, antimicrobial and antinociceptive activities presents an important area of pharmaceutical research. Some plants of the Lamiaceae family are well-known for their significant antimicrobial, anti-inflammatory, and pain-relieving activities. Species of the Lamiaceae attract a great scientific interest mainly due to the diversity of terpenes and phenolic compounds, including phenolic acids and flavonoids. Essential oils possess certain antimicrobial activity. For experimental studies, we selected four herbs. Among them were *Monarda fistulosa* L., *Satureja hortensis* L., *Thymus vulgaris* L., and *Mentha piperita* L. Four tinctures of the above-mentioned herbs were elaborated and partly phytochemically evaluated. We established the coefficients of alcohol absorption for the tested raw materials and the maximum absorption for active markers and tinctures after adding aluminum chloride that is needed for the development and standardization of tinctures. The solutions of complexes aluminum chloride with quercetin (20 mg/L), rutin (50.2 mg/L), and chrysin (80 mg/L) had the maximum absorption at the wavelengths of 425.9 ± 0.3 nm at 77 min of the reaction, 412.3 ± 0.3 nm at 82 min, 388.4 ± 0.7 nm at 81 min, respectively. The tinctures of *Monarda fistulosa*, *Satureja hortensis*, *Thymus vulgaris*, and *Mentha piperita* had the maximum absorption at 391.2 ± 0.5 nm at 91 min, 389.9 ± 0.5 nm at 76 min, 391.8 nm at 83 min, 394.9 ± 1.1 nm at 78 min, respectively. The carried out spectrophotometric studies confirmed the prevalence of flavones in the tested tinctures, considering the proximity of the maximum absorption of the tested tinctures and chrysin. The next studies will be continued at the standardization of the developed tinctures and the establishment of their antimicrobial activity.

Keywords: *Monarda fistulosa*, *Satureja hortensis*, *Thymus vulgaris*, *Mentha piperita*, ethanolic extracts, essential oil

Introduction

The search for new powerful anti-inflammatory and antimicrobial herbal products with antinociceptive potential presents an important area of pharmaceutical research (Casian et al., 2020). Some plants of the family of Lamiaceae Lindl. are well-known for their significant antimicrobial, anti-inflammatory and pain-relieving properties (Ben-Arye et al., 2011; Hamidpour et al., 2014; Honcarenko et al., 2019; Casian et al., 2020;

Shanaida et al., 2021a, b). In this context, *Monarda fistulosa* L., *Satureja hortensis* L., *Thymus vulgaris* L., and *Mentha × piperita* L. are the subject of several studies in the field of pharmaceutical technology and pharmacology (Thompson et al., 2003; Hamidpour et al., 2014; Liu et al., 2014; Marwa et al., 2017; Fierascu et al., 2018; Shanaida, 2018; Casian et al., 2020; Shanaida et al., 2021a, b).

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Species of the Lamiaceae attract great attention mainly due to the diversity of monoterpenes (Thompson et al., 2003; Honcharenko et al., 2019; Hudz et al., 2020). It has been revealed that essential oils possess certain antimicrobial activity (Ben-Arye et al., 2011; Rezvanpanah et al., 2011; Honcharenko et al., 2019). Therefore, they could be considered as active substances of potential antimicrobial herbal preparations (Rezvanpanah et al., 2011). The essential oil of *Satureja hortensis* contains significant amounts of two phenolic ketones: carvacrol and thymol. They are isomeric compounds and contain a phenol group in their structures (Hamidpour et al., 2014). The thymol chemotype of *Thymus vulgaris* produces thymol (22.4–72.9 %) and carvacrol (0.8–26.8 %) in glandular trichomes on the surface of leaves (Thompson et al., 2003). Carvacrol and thymol have a strong inhibitory effect on the growth of a wide range of microorganisms, including fungi and bacteria (Hamidpour et al., 2014).

Rosmarinic acid is the major compound of the aqueous and ethanolic extracts of *Dracocephalum moldavica*, *Ocimum americanum*, *Satureja hortensis* and other herbs of the Lamiaceae family (Shanaida et al., 2018; Shanaida et al., 2021a). Rosmarinic acid is known for its antiviral, antioxidant, anti-inflammatory, and immunostimulating activities (Hamidpour et al., 2014; Shanaida et al., 2021a). It was established that administration of pure rosmarinic acid at a dose of 25 mg/kg decreased the carrageenin-induced paw oedema in rats at 6 h by over 60 %. The effect of rosmarinic acid (25 mg/kg) can be comparable with Trolox (30 mg/kg) and indomethacin (10 mg/kg), which are known as a strong antioxidant and anti-inflammatory substances, respectively (Rocha et al., 2015).

Herbal preparations of some species of the Lamiaceae family (thyme, mint, and oregano) are stated as safe and efficient for the symptomatic treatment of discomfort conditions related to strep throat (Ben-Arye et al., 2011; Wijesundara et al., 2019).

Therefore, the development of herbal preparations with antioxidant, anti-inflammation and antimicrobial activity for the prevention and treatment of inflammatory and infectious diseases of the oral cavity is a topical question of modern medicine and pharmaceutical technology.

Material and methodology

While carrying out the research, the following methods were used: analysis, synthesis, systematization and comparison for processing published scientific

data; technological method (maceration); spectrophotometric method for the development of the analytical procedure of the determination of the total flavonoid content.

Plant material

Aerial parts of *Monarda fistulosa* (wild bergamot, bee balm, or horse mint) were collected in 2019 and *Satureja hortensis* (summer savory), *Thymus vulgaris* (thyme), and *Mentha piperita* (peppermint, balm mint, lamb mint) were collected in 2017. All the raw materials were picked up in the flowering stage in the Kherson region (Ukraine). The voucher specimens were deposited at the Herbarium of the Sector of Mobilization and Conservation of Plant Resources of the Rice Institute of the NAAS (Plodove, Kherson region, Ukraine) and at the Department of Analytical and Ecological Chemistry of University of Opole (Poland). The aerial parts of the four herbs were dried and kept at room temperature (15–25 °C) in a dark place before the preparation of the tinctures.

Extraction

All the tinctures were obtained in a ratio of the herbal substance to a final product as approximately 1 to 10. As a solvent, 70 % ethanol was used. The herbal substance was reduced to pieces. The crushed herbal substance was sieved through suitable sieves with the size of holes of 0.5 and 5.0 mm. Then the ground herbal substance of the size in the range of 0.5–5.0 mm was mixed with 70 % ethanol. The mixtures stood in closed containers. Maceration was performed at room temperature for 7 days. After this period the residue was separated from the extraction solvent by means of filtration through a paper filter.

Determination of the maximum absorption in differential spectra

For the determination of the maximum absorption we used the analytical procedure of differential spectrometry provided by Hudz et al. (2017a) for the estimation of the TFC in bee bread and by Hudz et al. (2020) for *Satureja montana*. 50 µL of the developed tinctures were diluted with 50 % ethanol up to 1.0 mL and was mixed with 1.0 of 2 % solution of aluminum chloride hexahydrate. The mixture was mixed by vortex and incubation was done at room temperature for 70–90 min. The volume of 2 % solution of aluminum chloride was replaced by the same amount of 50 % ethanol in the blank. The measures of all the spectra were carried out for each tincture in triplicate in the range of 360–440 nm. Instead of 50 µL of a tincture,

we used the stock solutions of rutin trihydrate (1000 mg/L), quercetin dihydrate (400 mg/L) and 200 µL of chrysin (400 mg/L).

Results and discussion

Lamiaceae is a large plant family of mostly shrubs and herbs (Hamidpour et al., 2014; Hassanzadeh et al., 2016; Karpova et al., 2020). These plants are popular due to various biological activities, including antioxidant and antimicrobial ones (Rezvanpanah et al., 2011; Li et al., 2014; Karpova et al., 2020). These properties are closely related to a variety of secondary metabolites. Species of the Lamiaceae family are used in folk medicine for many years (Ben-Arye et al., 2011; Karpova et al., 2020). Currently, anti-inflammatory, antitussive, diuretic, anti-asthmatic, antiseptic, antispasmodic, and antipyretic activities of the herbs were revealed. Some species demonstrated even antiviral properties (Karpova et al., 2020; Shanaida et al., 2021a).

Monarda is a genus endemic to North America. This genus embraces annual and perennial flowering plants. Many species are grown as ornamentals in different countries because the flower color ranges from red to pink or light purple. *Monarda* plants produce a high quantity of essential oil. *Monarda fistulosa* and *M. didyma* (oswego tea) have a long history of use as medicinal plants by Native Americans (Francati and Gualandi, 2017).

Monarda fistulosa is commonly known as an annual or perennial medicinal plant. It produces monoterpenes in trichomes located on leaves, calyces, and even flower petals. When these trichomes are broken, the scent of escaping monoterpenes appears almost immediately. Monoterpenes have been used for thousands of years as fragrances and flavors. However, plants use them for a variety of functions, including suppression of plant competitors, repelling herbivores, or attracting pollinators and seed dispersers (Harborne, 1993).

Monarda fistulosa is known for its strong therapeutic effects. Its essential oil is characterized by high antibacterial, antimycotic, and anti-inflammatory activities (Zhilyakova et al., 2009). Wild bergamot is mentioned among plants with a high content of thymol and carvacrol up to 60–70 % in the essential oil. At the same time, it also produces significant amounts of thymoquinone – a substance with antimycotic, anticancerous and antituberculous activity (Casian et al., 2020). For this reason, it could be proposed for the treatment of the throat. Shanaida et al. showed that the major constituent of the methylene chloride

extract of *Monarda fistulosa* was thymol (23.73 %), followed by carvacrol (10.09 %), *p*-cymene (9.74 %), and thymoquinone (8.52 %) (Shanaida et al., 2021b). Casian et al. (2020) stated about a yield of 12.5–14.5 g from 1 kg of the dried plant material of *Monarda fistulosa* and content of 20–32 % of thymoquinone and 23–32 % of thymol and carvacrol.

The genus *Satureja* L. (savory) embraces about 200 species of herbs and shrubs which are grown mostly in the Europe, Mediterranean region, North Africa, the Canary Islands, South America, and West Asia (Hamidpour et al., 2014). *Satureja hortensis* is an annual herbaceous crop species, strongly branched, with linear leaves.

Dried summer savory contains approximately 0.2–3.0 % of volatile oil (Hamidpour et al., 2014; Hassanzadeh et al., 2016). The main compounds found in extracts and essential oils of *Satureja hortensis* are terpenoids, phenolic compounds, flavonoids, tannins, steroids, acids, gums, mucilage, and pyrocatechols (Hamidpour et al., 2014). According to different studies, the main components of the volatile oil of *Satureja hortensis* are thymol (0.3–28.2 %), γ -terpinene (15.30–39 %), carvacrol (11–67 %), *p*-cymene (3.5–19.6 %), α -pinene (2.91 %) (Hamidpour et al., 2014; Fierascu et al., 2018). For instance, 18 compounds were identified in summer savory collected in Timis County (western region of Romania) during the growing season of the year 2017. Among them were: γ -terpinene (37.862 %), *o*-cymene (15.113 %), thymol (13.491 %), carvacrol (13.225 %), (+)-4-carene (6.086 %), β -myrcene (3.931 %), α -thujene (3.695 %), β -caryophyllene (1.496 %), β -pinene (1.374 %), isothymol (0.645 %), D-limonene (0.558 %), α -thujone (0.546 %) and camphor (0.521 %) (Popovici et al., 2019). Rezvanpanah et al. (2011) identified 31 compounds in summer savory of Iranian origin. Among them were: γ -terpinene (31.95 %), *p*-cymene (2.69 %), thymol (1.11 %), carvacrol (48.69 %), (+)-4-carene (6.086 %), β -myrcene (1.78 %) (Rezvanpanah et al., 2011).

The leaves of summer savory are rich in phenolic compounds, particularly rosmarinic acid and flavonoids, which provide a high antioxidant capacity of the leaves (Hamidpour et al., 2014; Shanaida et al., 2018).

The methanolic extract obtained by maceration contained rosmarinic acid (24.9 mg/g), caffeic acid (1.3 mg/g), naringenin (1.1 mg/g), isoferulic acid (220 µg/g), and apigenin (165 µg/g) (Fierascu et al., 2018). The high pressure liquid chromatography analysis confirmed the presence of gallic acid, caffeic

acid, chlorogenic acid, ferulic acid, rosmarinic acid, and flavonoids (rutin, hyperoside, quercitrin, apigenin, quercetin, catechin, and apigenin-7-glucoside) in aqueous extracts of *Satureja hortensis* herb of Ukrainian origin (Shanaida et al., 2018).

The herbal products obtained from summer savory have antioxidant, antimicrobial, antiparasitic, pesticidal, anti-inflammatory, analgesic, hepatoprotective and anticancer properties (Hamidpour et al., 2014; Fierascu et al., 2018).

Carvacrol, cymene and thymol in the essential oil provide antimicrobial activities against food, plants, and human pathogens (Hassanzadeh et al., 2016). The evaluation of the essential oil obtained from Iranian plants showed good antimicrobial activity against several types of microorganisms, with minimum inhibitory concentration values ranging from 0.06 $\mu\text{L}/\text{mL}$ for *Candida glabrata* to 8 $\mu\text{L}/\text{mL}$ for *Pseudomonas aeruginosa* and minimal lethal concentration values ranging from 0.06 $\mu\text{L}/\text{mL}$ for *Candida glabrata* to 16 $\mu\text{L}/\text{mL}$ for *Pseudomonas aeruginosa*. The results were superior to those obtained for the used reference substances (vancomycin, gentamicin and amphotericin) for all the studied microorganisms, with exception of *Pseudomonas aeruginosa* (Fierascu et al., 2018).

The antimicrobial mechanism is related to damage in membrane integrity, causing leakage of ions and other cell compounds and eventually death of a microbial cell (Fierascu et al., 2018).

Thymus vulgaris is an aromatic plant, which is used for medicinal and spice purposes almost everywhere in the world (Morales, 2002; Honcharenko et al., 2019). *Thymus vulgaris* shows a polymorphic variation in

monoterpene production, the presence of intraspecific chemotype variation being common in the genus *Thymus*. The wild thymus grown in southern France had six chemotypes (geraniol, α -terpineol, thuyanol-4, linalool, carvacrol, and thymol). Each of these six chemotypes is named after its dominant monoterpene (Thompson et al., 2003).

The chemical structure of the most important compounds of the essential oil of *Satureja hortensis* and *Thymus vulgaris* (thymol, carvacrol and *p*-cymene) is presented in Figure 1.

Mentha piperita is a hybrid mint – a cross-species between watermint and spearmint. It is one of the most economically important medicinal and aromatic plants (Shah and Mello, 2004; Liu et al., 2014). The essential oil of this plant possesses antimicrobial, anti-inflammatory, antitussive, local anesthetic activities (Shah and Mello, 2004; Ben-Arye et al., 2011).

Marwa et al. (2017) established that the essential oil of *Mentha piperita* contained menthol (46.32 %), menthofuran (13.18 %), menthyl acetate (12.10 %), menthone (7.42 %), and 1,8-cineole (6.06 %) as the principal constituents. The tested essential oil demonstrated strong inhibitory activity against the tested microorganisms (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Micrococcus luteus* ATCC 14452, *Staphylococcus aureus* ATCC 29213, *Bacillus subtilis* ATCC 6633, *Salmonella typhimurium*, *Bacillus cereus*, *Candida albicans*, and *Candida tropicalis*). The minimum inhibitory concentrations ranged from 0.062 to 0.5 % (v/v), except for *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa* was the least sensitive and was only inhibited by concentrations as high as 0.5 % (v/v) (Marwa et al., 2017).

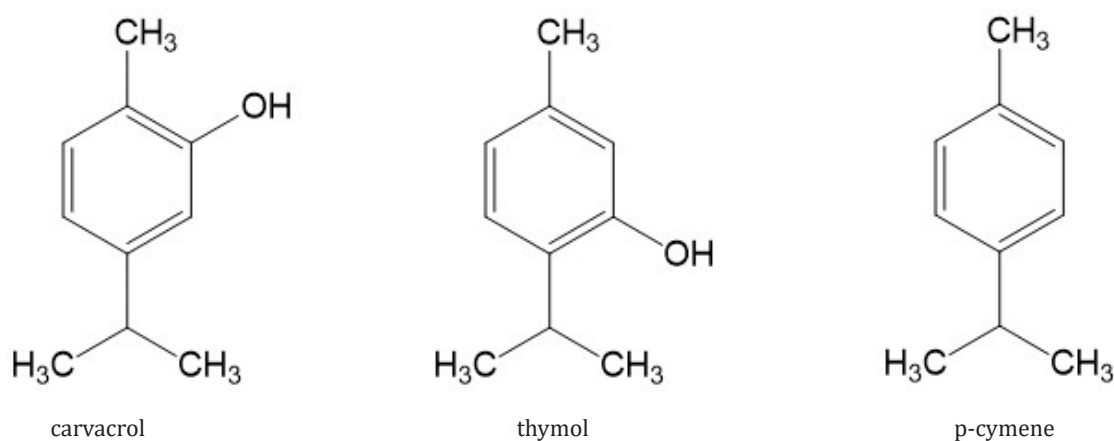


Figure 1 Chemical structure of carvacrol, thymol, and p-cymene

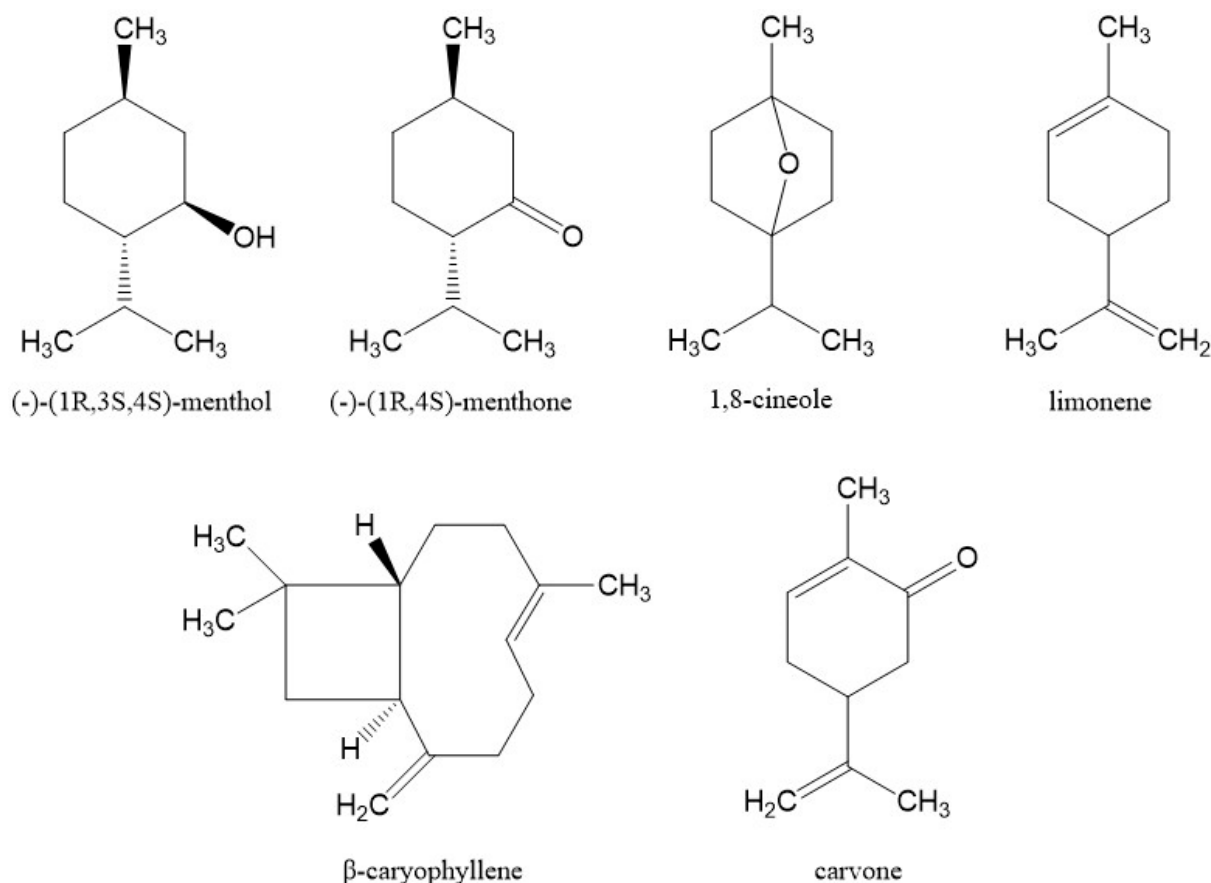


Figure 2 Active compounds of peppermint essential oil

The chemical structures of the major compounds of the essential oil of *Mentha piperita* are depicted in Figure 2.

Peppermint is famous for its flavoring and medicinal properties and is used in food, cosmetics, and medicines. It is helpful in symptomatic relief from illnesses such as colds, cramps, indigestion, nausea, sore throat, toothache, or even cancer. Many pharmacologic studies also have shown that peppermint possesses antioxidant, cytotoxic, antiallergenic, antiviral, and antibacterial activities with few side effects (Shah and Mello, 2014).

For experimental studies, we selected four herbs. Among them were *Monarda fistulosa*, *Satureja hortensis*, *Thymus vulgaris*, and *Mentha piperita*. Four tinctures of the *Thymus vulgaris*, *Satureja hortensis*, *Mentha piperita*, and *Monarda fistulosa* herb were elaborated. Ethanol absorption coefficient is an important technological parameter in the tincture manufacture. The results of the technological research are presented in Table 1.

It was established that the coefficient of alcohol absorption of the crushed raw material with the size of particles in the range of 0.5 to 5.0 mm was 2.8 ml/g,

Table 1 Calculations of the experimental determination of the absorption coefficient of 70 % ethanol

Name of herb, year of the collection	Mass of a crushed raw material (g)	Volume of 70 % ethanol for the extraction (ml)	The amount of the tincture obtained after absorption (ml)	Calculations
<i>Thymus vulgaris</i> , 2017	7.5	100	79.0	$X_1 = (100-79):7.5 = 2.80 \text{ ml/g}$
<i>Satureja hortensis</i> , 2017	4.1	58	40.5	$X_2 = (58-40.5):4.1 = 4.30 \text{ ml/g}$
	5.5	78	55.0	$X_2 = (78-55):5.5 = 4.20 \text{ ml/g}$
<i>Mentha piperita</i> , 2017	6.6	93	66.5	$X_3 = (93-66.5):6.6 = 4.00 \text{ ml/g}$
<i>Monarda fistulosa</i> , 2019	4.2	59	39.0	$X_4 = (59-39):4.2 = 4.80 \text{ ml/g}$

4.0 ml/g, 4.25 ml/g, and 4.8 ml/g, respectively, for the herb of *Thymus vulgaris*, *Satureja montana*, *Mentha piperita* and *Monarda fistulosa* for 70 % ethanol.

In our studies, we also elaborated the analytical procedure for the determination of the total flavonoid content in the tinctures. We adopted the elaborated analytical procedure for the determination of the total flavonoid content in bee bread tinctures for the tinctures of the tested four herbs (Hudz et al., 2017a,b; Hudz et al., 2020).

The study of the total flavonoid content should be carried out by identifying the dominant group of flavonoids by determining the maximum absorption in the differential spectrum after adding aluminum chloride. The maximum absorption is necessary to select an analytical marker with identical or close maximum absorption of its complex with aluminum chloride in an identical solvent. Spectrometric studies should be supplemented by further chromatographic ones to confirm the correct choice of analytical marker to recalculate the amount of flavonoids (Hudz et al., 2017a; Hudz 2020).

As a result of our study we established that the solutions of quercetin (20 mg/L), rutin (50.2 mg/L), and chrysin (80 mg/L) had the maximum absorption at the wavelengths of 425.9 ± 0.3 nm at 77 min, 412.3 ± 0.3 nm at 82 min, 388.4 ± 0.7 nm at 81 min, respectively.

The tinctures of *Monarda fistulosa*, *Satureja hortensis*, *Thymus vulgaris*, and *Mentha piperita* had one maximum absorption at 391.2 ± 0.5 nm at 91 min, 389.9 ± 0.5 nm at 76 min, 391.8 ± 0.3 nm at 83 min, 394.9 ± 1.1 nm at 78 min, respectively, in the range of 360–440 nm. The differential spectra are provided in Figure 3–5.

Therefore, the conducted spectrophotometric studies confirmed the domination of flavones in the tested tinctures. Considering the proximity of the maximum absorption of the tested tinctures and chrysin, this reference substance could be used for the determination and calculations of the total flavonoid content in tinctures.

Conclusions

The present study sets the basis for future research into the development of antimicrobial and anti-inflammatory herbal products. The knowledge of the performed review about the chemical profile of essential oil and extracts of the studied species will aid in explaining the observed biological activity. The aqueous and ethanolic extracts and essential oil could be considered as cheap, easily accessible, and a potential source of

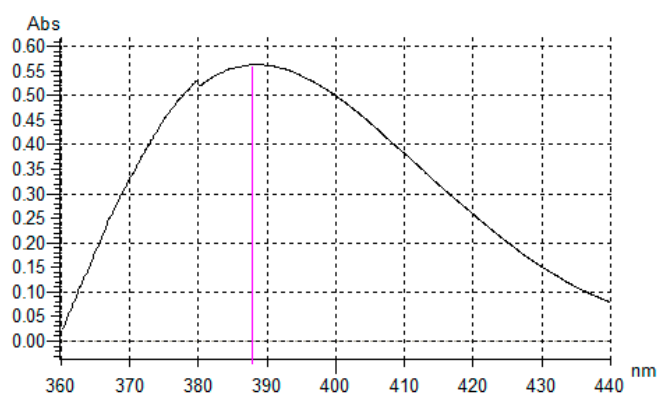


Figure 3 Differential spectrum of chrysin, concentration of chrysin 80 mg/L, $A = 0.604 \pm 0.038$, time of the reaction 81 min, $\lambda_{\max} = 388.4 \pm 0.7$ nm

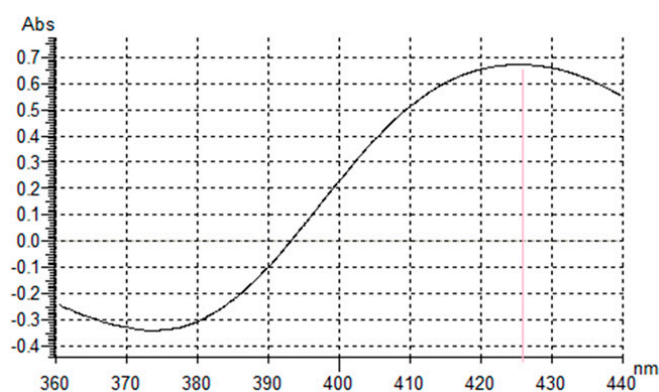


Figure 4 Differential spectrum of quercetin dihydrate, concentration of quercetin dihydrate 20 mg/L, $A = 0.619 \pm 0.045$, time of the reaction 77 min, $\lambda_{\max} = 425.9 \pm 0.3$ nm

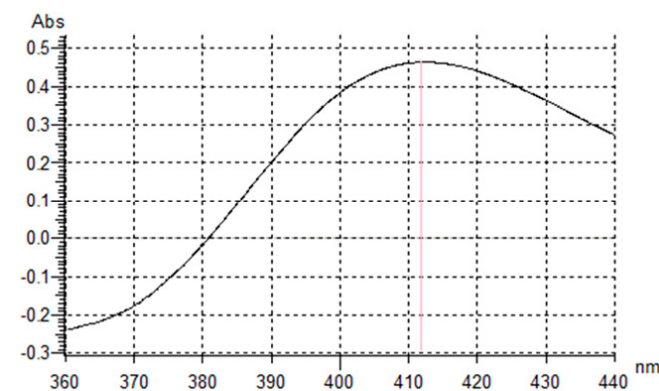


Figure 5 Differential spectrum of rutin trihydrate, concentration of rutin trihydrate 50.2 mg/L, $A = 0.458 \pm 0.010$, time of the reaction 82 min, $\lambda_{\max} = 412.3 \pm 0.3$ nm

natural antioxidants and antimicrobial compounds for the food and pharmaceutical industry. We established the coefficients of alcohol absorption as important technological parameters for the tested raw material with the purpose of the preparation of tinctures. The coefficient of alcohol absorption of the crushed raw material with the size of particles in the range of 0.5 to 5.0 mm was 2.8 ml/g, 4.0 ml/g, 4.25 ml/g, and 4.8 ml/g, respectively, for the herb of *Thymus vulgaris*, *Mentha piperita*, *Satureja hortensis*, and *Monarda fistulosa* for 70 % ethanol. The solutions of quercetin (20 mg/L), rutin (50.2 mg/L), and chrysin (80 mg/L) had the maximum absorption at the wavelengths of 425.9 ± 0.3 nm at 77 min, 412.3 ± 0.3 nm at 82 min, 388.4 ± 0.7 nm at 81 min, respectively, after adding aluminum chloride. The tinctures of *Monarda fistulosa*, *Satureja hortensis*, *Thymus vulgaris*, and *Mentha piperita* had the maximum absorption at 391.2 \pm 0.5 nm at 91 min, 389.9 \pm 0.5 nm at 76 min, 391.8 nm at 83 min, 394.9 \pm 1.1 nm at 78 min, respectively. Therefore, the performed spectrophotometric studies confirmed the domination of flavones in the tested tinctures, considering the proximity of the maximum absorption of the tested tinctures and chrysin. Future studies will be directed at the standardization of the developed tinctures and the establishment of their antimicrobial activity.

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