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Original Research

Effect of salicylic acid on phenolic compounds, antioxidant and antihyperglycemic activity of *Lamiaceae* plants grown in a temperate climate

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Abstract

Background: The *Lamiaceae* family, one of the most important herbaceous and shrub plant families, includes a wide variety of plants with biological and medicinal uses. This study aimed to conduct a comparative analysis of phenolic compounds content and biological activity of extracts from eight species of *Lamiaceae* plants, cultivated in a temperate climate, and to study the effect of the foliar salicylic acid application on these parameters. **Methods**: *Lamiaceae* plants (*Lavandula angustifolia*, *Salvia officinalis*, *Hyssopus officinalis*, *Agastache foeniculum*, *Thymus serpyllum*, *Mentha × piperita*, *Origanum vulgare*, *Monarda didyma*) cultivated on field experimental sites. Plants were sprayed with salicylic acid at a concentration of 1 mM. The untreated with salicylic acid plants were used as control. **Results**: The highest contents of hydroxycinnamic acids and flavonoids in control plants were observed in *the Agastache foeniculum* (6.4 ± 0.6 mg g^{-1} and 6.5 ± 0.4 mg g^{-1} respectively). The highest content of phenolic compounds was found in *Monarda didyma* (13.8 ± 0.7 mg g^{-1}). Among the control plants, *Agastache foeniculum*, *Hyssopus officinalis,* and *Mentha × piperita* were characterized by the highest antioxidant activity. All the studied extracts had the ability to inhibit α-amylase and α-glucosidase. Significant positive correlations were revealed between the antioxidant activity and the contents of hydroxycinnamic acids, total phenolic compounds, and flavonoids. A high degree of correlation was found between the α-amylase inhibitory activity and the content of hydroxycinnamic acids ($r = 0.72, p \leq 0.05$), as well as between the α-glucosidase inhibitory activity and the content of flavonoids ($r = 0.83, p \leq 0.05$) and hydroxycinnamic acids ($r = 0.81, p \leq 0.05$). The foliar treatment with salicylic acid led to an increase in the contents of hydroxycinnamic acids (in 6 species), flavonoids (in 2 species), total phenolic compounds (in 7 species), antioxidant activity (in 5 species), as well as in α-amylase (in 4 species) and α-glucosidase (in 5 species) inhibitory activity compared to the control plants. *Lavandula angustifolia* was the most susceptible to foliar treatment with salicylic acid. In this plant species, a significant increase in all studied biochemical parameters was noted. **Conclusions**: The results obtained on the stimulating effects of salicylic acid can be used to increase the nutritional and pharmacological value of plants of the *Lamiaceae* family cultivated in temperate climates.

Keywords: Biostimulants; Hormone; Rosmarinic Acid; Amylase; Glucosidase; Diabet

1. Introduction

The *Lamiaceae* family, one of the most important herbaceous and shrub plant families, includes a wide variety of plants with biological and medicinal uses [1]. The family has significant economic value as it includes garden plants and culinary herbs [2]. The most famous members of this family are various aromatic spices such as thyme, mint, oregano, basil, sage, savory, rosemary, hyssop, lemon balm, and a few others which see more limited usage.

Plants of the *Lamiaceae* family are widely used in herbal medicine. Their main application is aimed at the treatment of the respiratory and cardiovascular systems and the gastrointestinal tract as well as; the prevention of skin diseases, nervous system disorders, allergies, and metabolic disorders [1–3]. Their value is inherent in the biosynthesis of a wide range of secondary metabolites with powerful antioxidant, antibacterial, anti-inflammatory, antiviral and antitumor effects [4]. Among the secondary metabolites of the plants of the *Lamiaceae* family which possess a wide spectrum of biological activity, the phytochemicals which are part of the essential oils isolated from these plants are of particular importance. Such components include: monoterpenes, namely α- and β-pinene; 1,8-cineole; menthol; thymol; carvacrol; limonene; γ-tetrinene and sesquiterpenes (germacrene D, caryophyllene, spatulenol) [1]. Another important class of secondary metabolites found in *Lamiaceae* plants are phenolic compounds (hydroxycinnamic acids, flavonoids, mainly flavonols, tannins) and iridoids [5,6]. It should be noted that, while the antioxidant and antibacterial effects of these phytochemicals have been studied extensively, there is much less research regarding their antidiabetic effects. However, as an example, it is known that flavonoids contained in various *Lamiaceae* plants (such as rutin, luteolin, apigenin) exhibit good antidiabetic qualities, inhibiting α-glucosidase and α-amylase due to the presence of hydroxyl groups in the C ring and hydroxyl groups in the C-3 position of the A ring of the flavone structure [2]. According to another mechanism, some hydroxycinnamic acids (rosmarinic, caffeic, and chlorogenic acids) exhibit the same activity [2].

The biosynthesis of secondary metabolites in plants is highly dependent on environmental factors. For example, the content of secondary metabolites is strongly influenced by such factors as temperature, light, water regime, the availability of mineral nutrients, and more [7]. Even though plants of the *Lamiaceae* family are found throughout the world, the area where they are most abundant is in the Mediterranean region, with the climate of this area being subtropical and characterized by hot dry summers and warm rainy winters [8]. Cultivation of these plants in a temperate climate, characterized by a cool summer, moderately cold winter, and sufficient levels of precipitation evenly distributed throughout the year, usually leads not only to a decrease in yield but to changes in the qualitative and quantitative profile of the biologically active plant compounds [9,10].

In addition, the cultivation of some plants of the *Lamiaceae* family, such as lavender, sage, anise hyssop, and Scarlet beebalm, outdoors in a temperate climate, is fraught with some difficulties due to the thermophilicity of these plants and their low resistance to frost. One of the ways that have been found to increase plant resistance to the actions of unfavorable abiotic factors is the use of plant growth regulators [11,12]. One of these growth regulators, which is widely used in agricultural practice, is salicylic acid (SA) [13–15]. It is known that the action of SA is associated with the regulation of dynamic physiological reactions such as photosynthesis, nitrogen and proline metabolism, the functioning of the antioxidant defense system, and the maintenance of water balance. Therefore SA is involved in the operational protection of plants from stress [14,16]. In addition, it is known that SA affects the accumulation of secondary metabolites, including compounds of the phenolic structure in various plant species [17–19]. Thus, the use of exogenous salicylic acid can not only increase the resistance of plants to the action of abiotic factors but also improve the nutritional value of crops.

As far as we know, no study of the effect of salicylic acid on the accumulation of phenolic compounds, antioxidant and *in vitro* antihyperglycemic activity of extracts of *Lamiaceae* plants cultivated in temperate climates has been carried out. In this regard, the purpose of this study was (i) to conduct a comparative analysis of the content of phenolic compounds in eight species of plants of the *Lamiaceae* family, cultivated in a temperate climate, and the biological activity of their extracts; (ii) to study the effect of foliar treatment with salicylic acid on the content of phenolic compounds, antioxidant and the *in vitro* antihyperglycemic activity of extracts of plants of the *Lamiaceae* family.

2. Materials and methods

2.1 Plant material and design of experiment

Plants of the *Lamiaceae* Martinov family used as research objects: lavender (*Lavandula angustifolia* Mill.), sage (*Salvia officinalis* L.), hyssop (*Hyssopus officinalis* L.), anise hyssop (*Agastache foeniculum* (Pursh) Kuntze), thyme (*Thymus serpyllum* L.), peppermint (*Mentha* × *piperita* L.), oregano (*Origanum vulgare* L.) and Scarlet beebalm (*Monarda didyma* L.) all cultivated on field experimental sites located on the northern part of the Vistula Spit, Baltiysk District, Kaliningrad Region, Russia (altitude 16 m above sea level, longitude 19°89′ E, latitude 54°60′ N). The plants were seeded in 2019. The size of each test plot was 1 m × 1 m, the distance between the sites on each side was 2 m. Planting was done at a spacing of 45 cm × 45 cm giving a plant population of 9 plants per 1 m^2. The soil characteristics were the following: soil texture – clay clam, pH — 6.9, organic matter — 2.71%, total N content — 0.25%, available P (P_2O_5) — 758 mg kg^{-1}, available K (K_2O) — 512 mg kg^{-1}. Basic meteorological data for 2019 (planting year) and 2020 (harvesting year) are presented in Fig. 1.

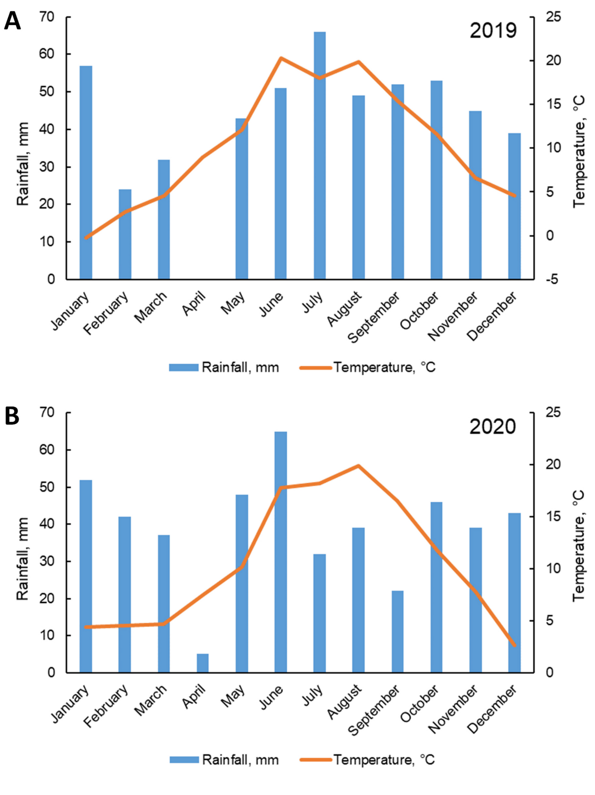

Fig. 1. Basic meteorological data for (A) 2019 (planting) year and (B) 2020 (harvesting) year at the experiment site.

Treatment of plants with salicylic acid (SA) was carried out in the second year after planting using the foliar method. To prepare a solution of SA, salicylic acid (ACS reagent, ≥99.0%; Sigma-Aldrich Rus., Moscow, Russia) was diluted in a small volume of ethanol (up to 0.5 mL) and then with distilled water until a solution of the required concentration was obtained. Spraying was performed at the rate of 100 mL of 1 mM salicylic acid solution per 1 m^2. Plants were treated from the beginning of April to the end of July at two-week intervals. Plants used in the control group were sprayed on the same dates with distilled water to which was added the same amount of ethanol that was used to dissolve salicylic acid.

In this study, inorganic fertilizers and pesticides were not used during the entire experiment, and weed control was carried out manually. The experiment was carried out with three replicates for each treatment (SA and control) and each plant species. There were 48 test plots in total. The plots were arranged in a Randomized Complete Block Design.

The collection of plants was carried out at the flowering stage in the period from August to September 2020. After collection, the aboveground parts of the plants were dried at a temperature of 60 °C until a constant mass was reached and crushed to a particle size passing through a sieve with holes at a diameter of 1.0 mm.

2.2 Preparation of extracts for analysis

To obtain extracts, 10.0 mL of a 60% aqueous solution of methanol was added to a weighed portion of plant material (0.1 g). Extraction was carried out for 40 minutes at 1400 rpm (MPS-1, Biosan). The extraction conditions (concentration of methanol, solvent volume to plant mass ratio, and time of extraction) were preliminarily determined based on one-factor-at-a-time experiments (data not shown). The resulting extracts were centrifuged at 4500 rpm for 10 minutes. The extraction was repeated three times. The combined supernatants were used to further determine the content of phenolic compounds and antioxidant activity.

To determine the inhibitory activity of the extracts against α-amylase and α-glucosidase, the supernatant was dried on a vacuum rotary evaporator (EV311, Labtech) at a controlled temperature (40 $\pm$ 2 °C) and stored at –20 °C until further analysis was carried out.

2.3 Determination of phenolic compounds content

The total content of hydroxycinnamic acids was determined spectrophotometrically by reaction with Arno's reagent according to [20]. To construct a calibration graph, standard solutions of rosmarinic acid with a precisely known concentration were used. The total content of hydroxycinnamic acids was expressed in mg of rosmarinic acid equivalents per gram of dry mass of plant (mg RAE g^{-1}).

The flavonoid content was determined by the reaction with $AlCl_3$ according to the protocol described in [21]. Rutin was used as a standard. The total content of flavonoids was expressed in mg of rutin equivalents per gram of dry mass of plant (mg RE g^{-1}).

The total content of phenolic compounds was determined spectrophotometrically by using Folin–Ciocalteu phenol reagent [22]. Standard solutions of gallic acid with a precisely known concentration were used to construct a calibration graph. The total content of phenolic compounds was expressed in mg of gallic acid equivalents per gram of dry mass of plant (mg GAE g^{-1}).

2.4 Determination of the biological activity of extracts

Antioxidant activity (AOA) of the extracts was measured by the ability to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radicals, as well as by the reducing power when interacting with a complex of Fe (III)-2,4,6-tripyridyl-5-triazine (FRAP) [23]. Solutions of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) of known concentration were used as a standard solution. The analysis results were expressed in μmol Trolox equivalents per gram of dry mass of plant (μmol TE g^{-1}).

Antihyperglycemic activity of the extracts was measured *in vitro* by their ability to inhibit α-amylase and α-glucosidase according to the protocol described in [24]. Acarbose standard solutions of known concentration were used as a positive control. The results were expressed in mmol acarbose equivalents per gram of dry mass of plant (mmol AE g^{-1}).

Extractions and analyzes were carried out in triplicate.

2.5 Statistical analysis

The data obtained were statistically processed using the OriginPro 2019b software (OriginLab Corporation, USA). The graphs show mean values with standard deviation (n = 3). In the analysis, only biological replicates were taken into account, since technical and analytical errors made a small contribution to the variance of the data. To identify statistically significant differences between the experimental variants, the data was processed using one-way ANOVA for each factor separately (plant species, SA-treatment). The Tukey test was used as the criterion to determine the significance of differences at a significance level of $p \leq 0.05$. The degree of relationship between the studied parameters was assessed using the Pearson correlation coefficient.

3. Results

3.1 Phenolic compounds

Hydroxycinnamic acids are an important component of the phytochemical profile of the *Lamiaceae* plants. The total content of hydroxycinnamic acids in the studied plant species of the *Lamiaceae* family varied from 1.6 to 6.4 mg RAE g^{-1}. The maximum total content of hydroxycinnamic acids in control plants was observed in anise hyssop and hyssop and amounted to 6.4 $\pm$ 0.6 and 5.7 $\pm$ 0.6 mg RAE g^{-1}, respectively (Fig. 2).

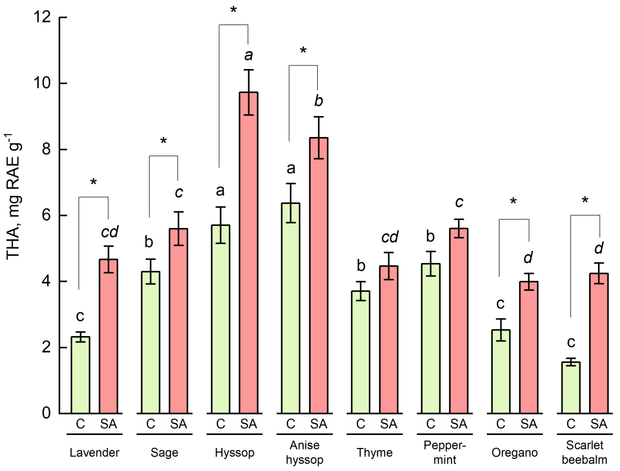

Fig. 2. Effect of salicylic acid on the hydrocinnamic acids content in plants of *Lamiaceae* family. THA, hydrocinnamic acids; RAE, rosmarinic acid equivalents; C, control; SA, salicylic acid.

Foliar treatment of plants with 1 mM salicylic acid solution stimulated the accumulation of hydroxycinnamic cinnamon in lavender (2.0 times), sage (1.3 times), hyssop (1.7 times), oregano (1.6 times), and Scarlet beebalm (2.7 times) (Fig. 2).

Among the control plants that were not treated with a salicylic acid solution, the maximal content of flavonoids was found in anise hyssop (Fig. 3). The total content of flavonoids in it was 6.5 $\pm$ 0.4 mg RE g^{-1}. The lowest content of flavonoids was found in the Scarlet beebalm and amounted to 2.3 $\pm$ 0.2 mg RE g^{-1}.

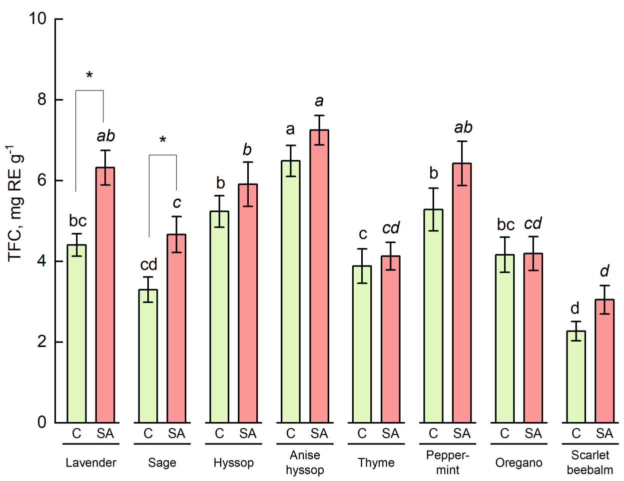

Fig. 3. Effect of salicylic acid on the flavonoids content in plants of *Lamiaceae* family. TFC, total flavonoids content; RE, rutin equivalents; C, control; SA, salicylic acid.

Even though almost all species showed an increase in the content of flavonoids at the foliar treatment of plants with salicylic acid, statistically significant differences were found only for two species namely lavender and sage. In plants of these species, the content of flavonoids was 1.4 times higher than in the control plants (Fig. 3).

Hyssop, anise hyssop, and Scarlet beebalm were distinguished by a high total content of phenolic compounds among the control plants (Fig. 4). The total content of phenolic compounds in them was 13.5 $\pm$ 0.7, 12.5 $\pm$ 0.6, and 13.8 $\pm$ 0.7 mg GAE g^{-1}, respectively (Fig. 4).

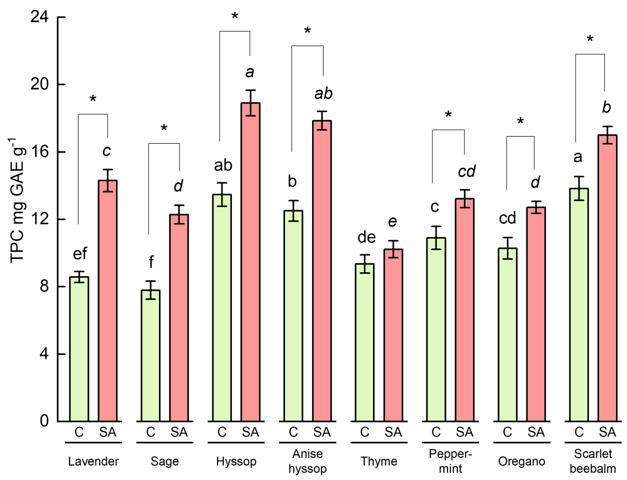

Fig. 4. Effect of salicylic acid on the total phenolic compounds content in plants of *Lamiaceae* family. TPC, total phenolic compounds content; GAE, gallic acid equivalents; C, control; SA, salicylic acid.

Foliar treatment with salicylic acid stimulated a significant increase in the content of phenolic compounds in all studied species, except thyme. In thyme, no significant differences were found between the control samples and the treated plants (Fig. 4). The greatest changes in the content of phenolic compounds were found in lavender and sage plants (1.7 and 1.6 times, respectively). In other species, the increase in phenolic compounds ranged from 1.2 to 1.4 times.

3.2 Antioxidant activity

Among the control plants, the anise hyssop and hyssop were characterized by the highest antioxidant activity against the DPPH and ABTS radicals (Figs. 5 and 6). The antioxidant activity measured by the DPPH assay was 64.2 $\pm$ 1.2 and 67.3 $\pm$ 1.4 μmol TE g^{-1} for anise hyssop and hyssop extracts respectively. The antioxidant activity measured by the ABTS assay was 67.3 $\pm$ 1.9 and 61.3 $\pm$ 1.5 μmol TE g^{-1}, respectively.

Extracts from hyssop plants were also characterized by high ferric reduced power activity (FRAP), which was 44.7 $\pm$ 1.8 μmol TE g^{-1} (Fig. 7). The maximum antioxidant activity, measured by the FRAP assay, was determined for peppermint extracts and amounted to 46.2 $\pm$ 1.5 μmol TE g^{-1}.

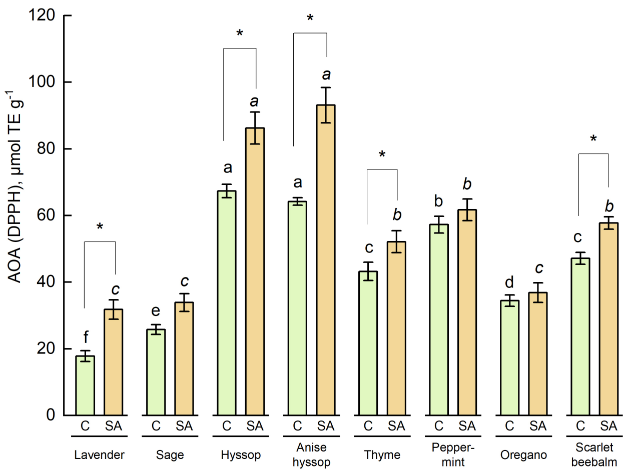

Fig. 5. Effect of salicylic acid on the antioxidants activity measured by DPPH assay of extracts from plants of *Lamiaceae* family. AOA (DPPH), antioxidants activity measured by DPPH (2,2-diphenyl-1-picrylhydrazyl) assay; TE, Trolox equivalents; C, control; SA, salicylic acid.

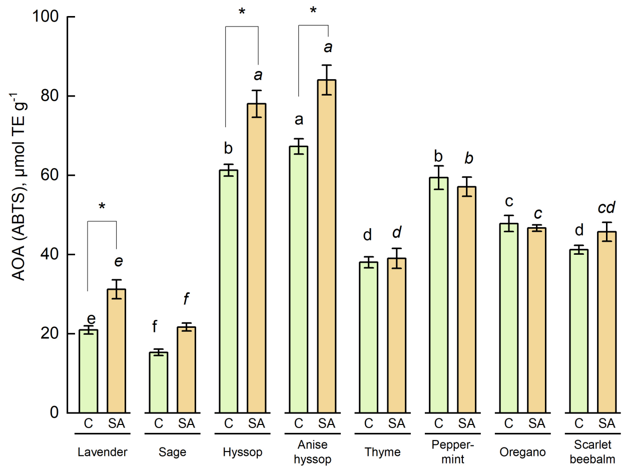

Fig. 6. Effect of salicylic acid on the antioxidants activity measured by ABTS assay of extracts from plants of *Lamiaceae* family. AOA (ABTS), antioxidants activity measured by ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay; TE, Trolox equivalents; C, control; SA, salicylic acid.

After foliar treatment of plants with salicylic acid, the antioxidant activity of the extracts of experimental plants increased or was not significantly different in comparison with control plants. Significantly higher values of antioxidant activity (according to the DPPH assay) were found for lavender (1.8 times), hyssop (1.3 times), anise hyssop (1.4 times), thyme (1.2 times), Scarlet beebalm (1.2 times) treated with salicylic acid in comparison with control plants of the same species (Fig. 5). Significantly higher values of antioxidant activity (according to the ABTS assay) were found only for three species, in particular, for lavender (1.5 times), hyssop (1.3 times), and anise hyssop (1.2 times)

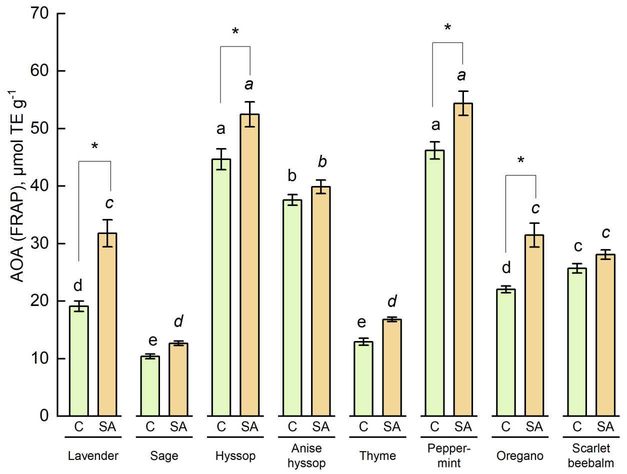

Fig. 7. Effect of salicylic acid on the antioxidants activity measured by FRAP assay of extracts from plants of *Lamiaceae* family. AOA(FRAP), antioxidants activity measured by FRAP (ferric reducing antioxidant power) assay; TE, Trolox equivalents; C, control; SA, salicylic acid.

(Fig. 6). A significant increase in ferric reduced power activity (FRAP) after treatment with salicylic acid was observed in lavender (1.7 times), hyssop (1.2 times), peppermint (1.3 times), and oregano (1.4 times) (Fig. 7).

3.3 α-amylase and α-glucosidase inhibitory activity

All the studied extracts had the ability to inhibit α-amylase and α-glucosidase. Among the control plants, the maximum inhibitory activity concerning α-amylase was exhibited by extracts of hyssop and peppermint, and concerning α-glucosidase, extracts of anise hyssop (Figs. 8 and 9).

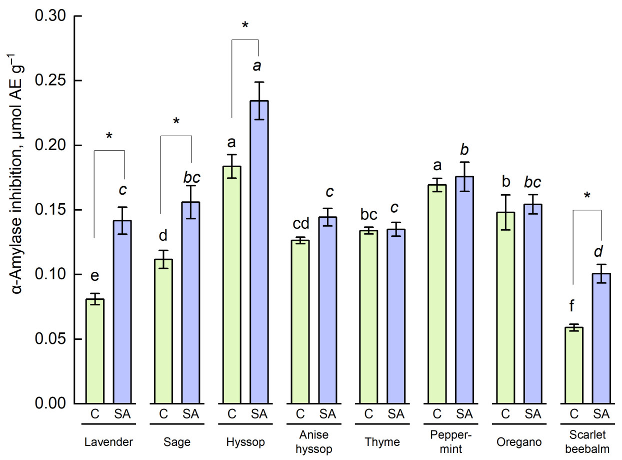

Fig. 8. Effect of salicylic acid on the α-amylase inhibitory activity of extracts from plants of *Lamiaceae* family. AE, acarbose equivalents; C, control; SA, salicylic acid.

Table 1. A correlation matrix with the Pearson coefficient values for the phenolic compounds, antioxidant and antihyperglycemic activities of the extracts.

Parameters	THA[1]	TFC	TPC	AOA (DPPH)	AOA (ABTS)	AOA (FRAP)	α-Amylase IA	α-Glucosidase IA
THA	1	0.71*	0.66*	0.77*	0.66*	0.58*	0.72*	0.81*
TFC		1	0.38	0.52*	0.58*	0.63*	0.56*	0.83*
TPC			1	0.74*	0.67*	0.62*	0.36	0.4
AOA (DPPH)				1	0.91*	0.71*	0.50*	0.68*
AOA (ABTS)					1	0.80*	0.53*	0.71*
AOA (FRAP)						1	0.61*	0.70*
α-Amylase IA							1	0.81*
α-Glucosidase IA								1

1: THA, total hydroxycinnamic acids content; TFC, total flavonoids content; TPC, total phenolics content; AOA, antioxidant activity according to the DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) and FRAP (ferric reducing antioxidant power) assays; IA, inhibitory activity. *: correlation is significant at $p \leq 0.05$.

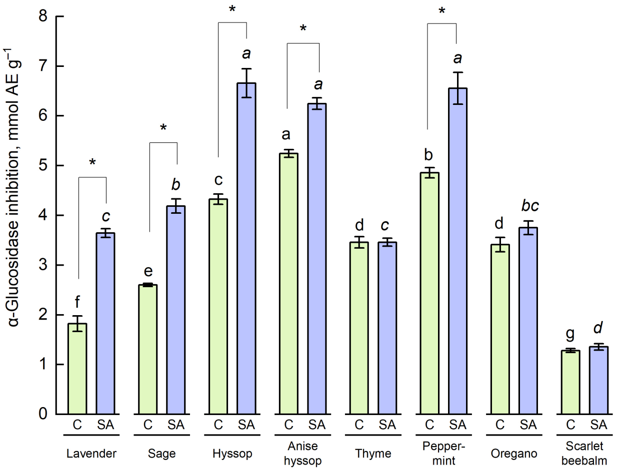

Fig. 9. Effect of salicylic acid on the α-glucosidase inhibitory activity of extracts from plants of *Lamiaceae* family. AE, acarbose equivalents; C, control; SA, salicylic acid.

After foliar treatment with salicylic acid, the inhibitory activity against α-amylase significantly increased for extracts of lavender (1.7 times), sage (1.2 times), hyssop (1.2 times), and Scarlet beebalm (1.7 times). For the rest of the studied species, there were no significant differences compared to the control groups (Fig. 8).

The inhibitory activity against α-glucosidase of extracts of some plants treated with salicylic acid was also higher compared to the control group. The maximum differences were found for lavender (2 times) and sage (1.7 times) and a significant increase was also found for hyssop, anise hyssop, and peppermint (Fig. 9).

3.4 Correlation between phenolic compounds, antioxidant activity, and antihyperglycemic activity

Antioxidant activity depends on the content of various classes of phytochemicals in plants. Usually, phenolic compounds contribute significantly to the antioxidant activity of extracts. The correlation analysis carried out by us revealed a positive relationship between the content of various groups of phenolic compounds and the antioxidant activity of extracts of the plants of the *Lamiaceae* family (Table 1). A high degree of correlation was noted between the antioxidant activity, measured using the DPPH assay, and the content of hydroxycinnamic acids (r = 0.77, $p \leq$ 0.05) as well as the total content of phenolic compounds (r = 0.74, $p \leq$ 0.05). A high degree of correlation was also found between antioxidant activity (DPPH assay) and flavonoid content (r = 0.58, $p \leq$ 0.05). The correlation coefficients between the content of phenolic compounds and antioxidant activity measured by the ABTS and FRAP assays were slightly lower but also statistically significant. As the antioxidant activity, measured by the ABTS assay, a stronger correlation was also established between this parameter and the total contents of hydroxycinnamic acids and phenolic compounds. For antioxidant activity, measured by the FRAP assay, the maximum correlation coefficient was established for flavonoids (r = 0.63, $p \leq$ 0.05) (Table 1).

There was a high degree of correlation between the α-amylase inhibitory activity and the content of hydroxycinnamic acids (r = 0.72, $p \leq$ 0.05) (Table 1). A moderate degree of correlation was found between the α-amylase inhibitory activity and the content of flavonoids (r = 0.56, p $\leq$ 0.05).

A high degree of correlation was also found between the α-glucosidase inhibitory activity and the content of flavonoids (r = 0.83, $p \leq$ 0.05) and hydroxycinnamic acids (r = 0.81, $p \leq$ 0.05) (Table 1).

4. Discussion

Plants of the *Lamiaceae* family are promising potential sources of natural pharmaceuticals due to their high content of phenolic compounds. The scientific and epidemiological data available today link the consumption of foods rich in phenolic compounds with health benefits, primarily in such areas as reducing the risk of cardiovascular disease, which is mediated by the anti-inflammatory effects of these compounds [25,26]. The main classes of phenolic compounds represented in the *Lamiaceae* plants are phenolic acids, mainly consisting of hydroxycinnamic acids (rosmarinic, chlorogenic, and caffeic), and flavonoids (naringenin, quercetin, rutin, apigenin, luteolin, and their glycoside derivatives) [2,5]. Depending on the type of plant, the proportion of hydroxycinnamic acids and flavonoids in the total content of phenolic compounds in plants of the *Lamiaceae* family can vary greatly. So, for example, in [5], it was shown that the proportion of phenolic acids and their derivatives was 8% to 24% of the total content of phenolic compounds in *Origanum vulgar ssp. hirtum*, *Thymus capitatus*, and *Satureja thymbra* and 64% and 69% in *Melissa officinalis* and *Rosmarinus officinalis*. In our study, the proportion of hydroxycinnamic acids in the total content of phenolic compounds ranged from 11% (in Scarlet beebalm) to 55% (in sage), which is consistent with the data available in the literature. The proportion of flavonoids in the total content of phenolic compounds ranged from 16% (in Scarlet beebalm) to 52% (in lavender and anise hyssop). According to the total content of phenolic compounds, the studied species of plants untreated with salicylic acid can be arranged in the following order (in decreasing order): Scarlet beebalm $>$ hyssop $>$ anise hyssop $>$ peppermint $>$ oregano $>$ thyme $>$ lavender. The high total content of phenolic compounds in Scarlet beebalm is consistent with the previously obtained results of a comparative analysis of various medicinal plants presented in [6,27].

Phenolic compounds are known for their antioxidant properties and are among the most important sources of antioxidants in the diets of humans. Due to the potentially harmful effects of synthetic antioxidants such as butylated hydroxyanisole and butylated hydroxytoluene, new natural antioxidants have become the focus of attention for protecting food, beauty products, and reducing oxidative stress *in vivo* [28,29]. In addition to phenolic compounds, other compounds that differ in the structure and mechanisms of antioxidant action also have antioxidant properties in plant extracts [30]. In this regard, in the study of antioxidant activity *in vitro*, it is necessary to use several methods for determining antioxidant activity. In this work, we used three assays for determining the antioxidant activity: assays based on the reaction with radicals (DPPH and ABTS), as well as the FRAP assay which allows for the determination of the reduction power of the extracts. To compare the data obtained in all three assays, Trolox was used as a standard. Despite some differences in absolute values, in general, the results of all three assays correlated well with each other (r = 0.71–0.91, $p \leq 0.05$). Extracts of hyssop, anise hyssop, and peppermint were distinguished by high antioxidant activity. It should be noted that the result of a study of the antioxidant activity of Scarlet beebalm turned out to be somewhat surprising. Even though this species was the leader in the total content of phenolic compounds, the antioxidant activity of its extracts was at only an average level. It can thus be assumed that the extracts of hyssop, anise hyssop, and peppermint, in addition to phenolic compounds, had a higher content of other biological components with antioxidant properties compared to Scarlet beebalm. However, for more correct conclusions on this issue, additional studies of the phytochemical composition of the above plants using HPLC/LC-MS and GC-MS methods are required.

Diabetes is one of the most dangerous metabolic disorders from which there is a high death rate worldwide. In 2017 diabetes accounted for 425 million cases and its prevalence will rise to 629 million by 2040. Type 2 Diabetes mellitus (T2DM) is the most common type of diabetes, accounting for approximately 90% of all cases [31]. An important role in carbohydrate metabolism and the flow of glucose into the blood is played by such enzymes as α-amylase and α-glucosidase. Pancreatic α-amylase is an important enzyme in the initial stage of starch hydrolysis to smaller carbohydrates, including maltose and maltotriose. Then α-glucosidase breaks down these products into glucose which enters the bloodstream. Therefore, inhibition of α-amylase and α-glucosidase activity is necessary to slow down the release and absorption of glucose, which in turn lowers blood glucose levels and thus suppresses hyperglycemia and diabetes [32]. In our study, it was found that extracts of hyssop, peppermint, and anise hyssop showed the maximum α-amylase and α-glucosidase inhibitory activities. The effects of extracts from the plants of the *Lamiaceae* family have also been confirmed in previously published studies [32–36]. The antihyperglycemic effect of their extracts is associated primarily with the presence of flavonoids, hydroxycinnamic acids, as well as diterpenes (forskolin and marrubiin), and triterpenes (ursolic and oleanolic acids) [2].

Salicylic acid (SA) is one of the most important simple phenolic compounds that have attracted a maximum level of attention by scientists around the world. Intensive studies related to the role of SA in plants have revealed its active role in various physiological processes, such as seed germination, stomatal movements, accumulation of pigments, photosynthesis, ethylene biosynthesis, heat production, enzyme activity, nutrient absorption, flow induction, membrane functions, nodulation and more [17]. It is also known that SA exhibits the functions of a hormone and a signaling molecule and is involved in the formation of systemic acquired resistance (SAR) [37,38]. In many respects, the protective function of SA through the action of abiotic and biotic factors on plants is associated with its role in the regulation of the biosynthesis of secondary metabolites [17,39]. In our study, it was found that foliar treatment with salicylic acid led to an increase in the contents of hydroxycinnamic acids (in 6 species), flavonoids (in 2 species), total phenolic compounds (in 7 species), antioxidant activity (in 5, 3 and 4 species according to DPPH, ABTS, and FRAP assays, respectively), as well as α-amylase and α-glucosidase inhibitory activity (in 4 and 5 species, respectively), compared to the untreated plants. It should be noted that no significant decrease in the studied parameters was found in any of the studied species at the foliar treatment with salicylic acid. Since the correlation analysis revealed a high or medium positive correlation between the content of phenolic compounds and the biological activity of the extracts, it can be assumed that an increase in biological activity is associated precisely with an increase in the biosynthesis of phenolic secondary metabolites under the action of salicylic acid. The results of the positive effect of foliar SA treatment on the total content of phenolic compounds, phenolic acid, and flavonoids are in agreement with previously published data [40]. It is known that salicylic acid increases the activity of phenylalanine ammonia-lyase (PAL), the most important enzyme involved in the initial stages of the biosynthesis of phenolic compounds [41]. For plants of the genus Sage (*Salvia officinalis, Salvia virgate, Salvia miltiorrhiza*), it was shown that the use of exogenous salicylic acid led to the activation of PAL expression [42,43]. For some plants of other families, the activation of genes involved in stilbene and flavonoid biosynthesis was shown [44,45].

Of all the plant species we studied, lavender was the most susceptible to foliar treatment with salicylic acid. In this plant species, a significant increase in all studied indicators was noted. In contrast, in thyme plants, none of the studied parameters changed when treated with salicylic acid. Our results are consistent with the results of previous studies which also noted the species-specific reaction of plants to exogenous salicylic acid [42]. In addition, the result obtained in our study may be due to the fact that the same concentration of salicylic acid was used for all plant species. While at the same time there is evidence of a dose-dependent plant response to the use of salicylic acid [41,46,47]. Thus, for a more accurate interpretation of the data on the species differences established by us, it is necessary to carry out additional studies, including the selection of individual optimal concentrations of salicylic acid for each species.

5. Conclusions

Plants of the *Lamiaceae* family are a valuable source of biologically active substances. Cultivation of 8 species of this family in a temperate climate showed that the most promising for use in the pharmaceutical and food industries are the following species: Scarlet beebalm, hyssop, anise hyssop, and peppermint which were all characterized as possessing the highest content of phenolic compounds. The last three species also exhibited maximum antioxidant activity. All studied plant species exhibited α-amylase and α-glucosidase inhibitory effects which allows considering these plants as potential antihyperglycemic agents. Foliar treatment with salicylic acid led to an increase in the content of phenolic compounds, the antioxidant and the antihyperglycemic activity of extracts of some of the plant species studied. The most pronounced effect of salicylic acid was observed in lavender in which all of the studied parameters increased in comparison with untreated plants. The results obtained can be used to increase the nutritional and pharmacological value of plants of the *Lamiaceae* family cultivated in temperate climates.

Author contributions

LS designed the research study. AG and TS performed the research. TS and AG analyzed the data. LS and AG wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

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Conflict of interest

The authors declare no conflict of interest.

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