Agrobiodiversity for Improving Nutrition, Health and Life Quality

Research Article

OPEN ACCESS

Influence of application forms of alginite on phytomass formation, bioactive contents and antioxidant activity of extracts from plants *Rosmarinus officinalis* L.

Jarmila Eftimová*, Natália Nociarová

University of Veterinary Medicine and Pharmacy in Kosice, Department of Pharmaceutical Technology, Pharmacognosy and Botany, Slovakia

Article Details:
Received: 2022-04-27
Accepted: 2022-05-23
Available online: 2022-05-31

DOI: https://doi.org/10.15414/ainhlq.2022.0011

In this study, we compared the effect of soil-climatic factors and application forms of alginite on the weight of vegetable drug, total content of polyphenols, flavonoids, phenolic acids and antioxidant activity of extracts from *Rosmarinus officinalis* L. The experiment was based on a site in Tornal (SR), in 2020. The plot was divided into three equal (1 m^2) parts (variants), on which 9 plants were planted and alginite was applied in two forms. On variant (V_1) alginite was incorporated in powder form, (V_2) alginite in the form of a top dressing (1 : 10) and control (V_K) without application. When the effect of alginite application on plant weight was evaluated, the highest increase (508.8 g) was observed in variant V_1, followed by V_2 (469.6 g) and the lowest value (251.2 g) in V_K. The highest total amount of flavonoids expressed as quercetin equivalent (QE) was determined in acetone extracts of *Rosmarinus officinalis* samples in variant V_1 (0.3225 mg QE.100 g^{-1} DW). In the extract of the drug from variant V_2 we determined the total amount of flavonoids to be 0.2922 mg QE.100 g^{-1} DW and in the control V_K – 0.2235 mg QE.100 g^{-1} DW. In the aqueous extracts of *Rosmarinus officinalis* samples, we determined the highest total polyphenol content converted to caffeic acid equivalent (CAE) per 100 g dry drug in variant V_1 (27.69 mg CAE.100 g^{-1} DW), followed by variant V_2 (26.13 mg CAE.100 g^{-1} DW) and in the control V_K it was 16.42 mg CAE.100 g^{-1} DW. In the ethanolic extracts of *Rosmarinus officinalis* samples, we determined the total phenolic acid content converted to caffeic acid equivalent (CAE) per 100 g of dried drug in variant V_1 (34.61 mg CAE.100 g^{-1} DW) >V_2 (33.41 mg CAE.100 g^{-1} DW) >V_K (29.32 mg CAE.100 g^{-1} DW). The antioxidant activity of methanol extracts of samples from variants V_1, V_2, V_K was expressed as % inhibition of DPPH radical. The highest percentage of inhibition (37.76%) was observed in the drug methanol extract V_1, followed by variant V_2 (30.77%) and control V_K (15.38%). The results showed that alginite as compared to control significantly affects the growth, weight of *Rosmarinus officinalis* and increases the content of total polyphenols, flavonoids, phenolic acids and antioxidant activity, which is crucial in terms of production of medicinal plants as well as their pharmaceutical uses.

Keywords: *Rosmarinus officinalis*, rosmarinic acid, alginite, antioxidant activity

*Corresponding Author: Jarmila Eftimová, University of Veterinary Medicine and Pharmacy in Kosice, Komenskeho 73, 041 81 Kosice, Slovakia
jarmila.eftimova@uvlf.sk

Introduction

Rosmarinus officinalis L. belongs to the order family Lamiaceae Martinov (URL1, 2022). The Lamiaceae are represented by species that have essential oils (aetheroleum) stored in the essential oil cells, secretory ducts, glandular trichomes, representing a mixture of heterogeneous, lipophilic, easily volatilized substances with a distinct fragrance (Košťálová et al., 2012; Suchý et al., 2013; Petruzzello, 2021). Essential oils have antibacterial, anti-inflammatory, carminative, antispasmodic, hyperacidifying, antiviral, antitussive, diuretic, sedative, granulating and deodorizing effects (Košťálová et al., 2012). Rosmarinic acid is antiviral, antibacterial, antifungal, and anti-inflammatory (Petersen and Simmonds, 2003). It eliminates inflammation in osteoarthritis, pancreatitis, hepatitis, colitis, atopic dermatitis, asthma, and other inflammatory diseases (Chunxu et. al., 2020). It has nociceptive, neuroprotective and neuroregenerative effects on the CNS (Ghasemzadeh and Rahbardar, 2020). It inhibits the aggregation of β-amyloid plaques and reduces the amount of reactive oxygen species, which may be used in the treatment of Alzheimer's disease (Mahboubi, 2019).

Rosmarinus officinalis requires stony soils, slopes, sunny, dry positions, is sensitive to frost and does not overwinter in the climatic conditions of the Slovak Republic (Bednářová, 2017). Medicinal plants for drug production and pharmaceutical use must have optimal conditions for growth and development to exploit their genetic potential. They must have optimum climatic, soil conditions, optimum doses of inorganic, organic fertilizers, organic-mineral substance (alginite). Alginite is an algal rock, formed by the process of decomposition of plants, has the properties of soil sorbent (water, heavy metals – Si) but is a suitable alternative to organic fertilizers (Kúšik et al., 2017, Brindza et al., 2021a; Elisovetcaia et al., 2021; Kovár et al., 2021). Natural organic fertilizers include alginite, which contains algae. Clay, humus (40%), mineral nutrients (aragonite, dolomite, smectite, quartz, siderite, and others). Smectite can retain water and provides long-term hydration. Of the biogenic elements, Ca and Mg are the most abundant, with less N, Fe, Zn, Cu, Mn, Ti, Cr, Li, Co, Ni. It does not contain any chemical additives and phytotoxins (Kulich-Valko-Obernauer, 2001; Brindza et al., 2021b).

The aim of the experiment was to evaluate the effect of soil-climatic factors and alginite application on the yield and content of *Rosmarinus officinalis* bioactive substances involved in antioxidant effects.

Material and methodology

Samples of the drug *Rosmarinus officinalis* folium were used in the experiment. Plants were planted on 20. 5. 2020 on the experimental plot in Tornaľa – part of Stárňa, region Gemer, SR. Brown soils are typical for the area (Tolmáči and Gajdoš, 2011). The soil is mechanically treated before planting to create suitable conditions for plant rooting. The area was not fertilized with organic or inorganic fertilizers in order not to affect the metabolism and synthesis of plant contents. The plot was divided into three equal 1 m^2 sections (variants), on which 9 plants were planted and alginite was applied in two forms. The first variant (V_1) – plants with incorporation of powdered form of alginite 1 $kg.m^{-2}$, the second variant (V_2) – plants with alginite in the form of 1 : 10 drench, the third variant (V_K) = control variant – plants without alginite application. The irrigation was in the form of rainwater sprinkling applied once a day in the morning hours. Harvesting of plants was divided on 27. 7. and 2. 9. 2020. The ratio of fresh sample to dried sample was 8 : 1.

Morphometric characteristics

When the aerial part of *Rosmarinus officinalis* the height of planted individuals was measured on V_1, V_2 and V_K variants. Then the above-ground part of each variant was harvested, and the mass was weighed. Leaves were then separated from the stems and their length measured. The vegetative drug from each variant was dried and then the dry weight of the drug was weighed. All the evaluated data were recorded in a table and standard deviations were calculated.

Total polyphenol content

The total polyphenol content in the aqueous plant extracts was measured using Folin-Ciocalteu reagent by the spectrophotometric method according to Singleton and Rossi (1965) modified by Suchý et al. (2013). We prepared a 250 mL aqueous extract of 0.5 g of homogenized dry drug. The extract was filtered, 5 mL of the filtrate was diluted with water to 25 mL. From the solution, we pipetted 2 mL for determination, added 1.0 mL of Foulin-Chicoulet reagent and 17.0 mL of 20% sodium carbonate Na_2CO_3 solution. We mixed the samples and after 2 min, we measured the absorbance at 750 nm against the blank. was measured using the spectrophotometer Jenway (6405 UV/Vis, England). The absorbance was measured in triplicate and the mean value of the samples, and their standard deviation were determined. The total polyphenol content in the aqueous extracts (as caffeic acid equivalent) was calculated from the caffeic acid

calibration curve (10–100 µg caffeic acid equivalents (CAE)/L; R^2 = 0.9991) and is expressed in mg CAE.100 g^{-1} DW.

Total flavonoid content

The total contetnt of flavonoids expressed as qvercetin was determined by the spectrophotometric method with aluminium chloride according to European Pharmacopoeia 7 (04/2013:1174). The total flavonoid content expressed as quercetin was determined by the spectrophotometric method with aluminium chloride, according to European Pharmacopoeia 7 (Article 04/2013:1174). Absorbance was measured with a Jenway spectrophotometer (6405 UV/Vis, England) at a wavelength of 425 nm. The absorbance was measured in triplicate and the mean value of the samples, and their standard deviation were determined. The total flavonoid content of the acetone extracts (as quercetin equivalent) was calculated from the quercetin calibration curve (2–100 µg.l^{-1} quercetin equivalent (QE)/L; (R^2 = 0.9997) and expressed in mg CAE.100 g^{-1} DW.

Total phenolic acids content

Total phenolic acid content in the ethanol plant extracts was carried out using a method of Farmakopea Polska (1999). Briefly, 0.5 mL of extract was mixed with 0.5 mL of 0.5 M hydrochloric acid, 0.5 mL of Arnova reagent, 0.5 mL of 1 M sodium hydroxide (w/v), and 0.5 mL of distilled water. Absorbance at 490 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). The absorbance was measured in triplicate and the mean value of the samples, and their standard deviation were determined. Caffeic acid was used as a standard for the calibration curve (0.01–1.0 mg caffeic acid equivalents (CAE)/L, R^2 = 0.9997) and the results are expressed in mg CAE.100 g^{-1} DW.

Antoxidant activity

The radical scavenging activity in the methanol plant extracts of the samples was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Ahmad et al., 2014). The extracts (0.5 mL) were mixed with 3.6 mL of DPPH medium (0.025 g of DPPH in 100 mL of ethanol). Methanol was used as blank. The absorbance of the sample extract was determined sing the spectrophotometer Jenway (6405 UV/Vis, England) at 515 nm (Varényi, Chrenová, Lukáč 2019). The absorbance was measured in triplicate and the mean value of the samples, and their standard deviation were determined. Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) (10–100 mg Trolox/L; R^2 = 0.9997) was used as the standard for the calibration and the results were expressed in mg Trolox equivalent antioxidant capacity (TEAC).100 g^{-1} DW.

Statistical analysis

Data were analyzed with ANOVA test and differences between means were compared through the Tukey-Kramer test ($p < 0.05$). The variability of all these parameters was evaluated using descriptive statistics.

Results and discussion

Plants of the Lamiacae chaeliad are nowadays intensively investigated and studied mainly for analgesic, antiphlogistic, antioxidant, antimicrobial, gastroprotective, hypoglycemic, anticancer, and hypolipidemic effects (Costea et al., 2020). Investigating the polyphenolic profile of natural *Rosmarinus officinalis* populations may reveal essential compounds that have biological activities (Elansary et al., 2020).

Morphometric characteristics of *Rosmarinus officinalis*

Cultivation of medicinal plants on arable land requires knowledge of the requirements of individual species for soil, climatic conditions, cultivation method, fertilization, protection with respect to the environment (Eftimová and Habán, 2012).

Effect of soil-climatic factors and application forms of alginite on plant height, leaf length as well as weight

Table 1 Comparison of the effect of applied alginite in different forms (V_1 and V_2) on the evaluated traits on plants *Rosmarinus officinalis* L. in comparison with the control variant (V_K)

Variants	Traits evaluated		
	height (cm)	leaf length (cm)	total dry weight of the drug (g)
V_K	52.4 ±0.34^b	1.8 ±0.18^b	95.6 ±0.42^c
V_1	63.4 ±0.33^a	2.3 ±0.16^a	182.4 ±0.81^a
V_2	57.8 ±0.21^b	2.1 ±0.16^a	162.8 ±0.78^b

Notes: different superscripts in each row indicate the significant differences in the mean at $p < 0.05$

of the plant drug *Rosmarinus officinalis* is shown in Table 1.

Table 1 shows that the best growth of *Rosmarinus officinalis* was recorded in V_1. The height of the plants was 63.4 cm, and the leaf length was 2.3 cm. The total dry weight of all two harvests was 182.4 g. In V_2, plant height was 57.8 cm, leaf length 2.1 cm and total dry weight 162.8 g. In V_K, plant height was 52.4 cm, leaf length 1.8 cm and total dry weight 95.6 g. In V_K, plant height was 52.4 cm, leaf length 1.8 cm and total dry weight 95.6 g.

Total polyphenol content

Among the constituents present in *Rosmarinus officinalis* are essential oils (1,8-cineole, α-pinene, camphor, borneol, bornyl acetate, camphene, α-terpinol and (+)-verbenone), diterpenes (carnosol, carnosic acid, rosin, rosadiol, rosadiol), triterpenes (betulin, betulinic acid, ursolic acid, oleanoic acid), flavonoids (diosmin, hesperidin, luteolin glycosides, cirsimaritin), and cinnamic acid derivatives with rosmarinic acid being the most prominent (Nagy, Mučaji, and Grančai 2017). Major polyphenols confirmed in *Rosmarinus officinalis* leaf extracts, are flavonoids: apigenin, luteolin, nepetin, nepitrin and phenolic acids: rosmarinic acid (c.a. 23%), chlorogenic acid, caffeic acid, and other organic acids like: ursolicacid, betulinic acid, carnosic acid, and carnosol (Begum et al., 2013). Polyphenols as secondary metabolites contain one or more hydroxyl groups bound to one or more aromatic rings (Stagos, 2020). Bourhia et al. (2019) confirmed that polyphenols, carnosol and carnosic acid are responsible for the antioxidant activity of ethanolic extracts of *R. officinalis* leaves from Morocco. The contents of total polyphenols in the aqueous extract of the *Rosmarinus officinalis* treated by the differet aplication of alginite are presented in Table 2.

After fitting the measured absorbance values of the samples to the calibration equation, we calculated the total polyphenol content as caffeic acid equivalent. Table 2 shows that the highest polyphenol content was observed in V_1 (27.69 mg CAE.100 g^{-1} DW). Slightly lower values were exhibited by V_2 (26.13 mg CAE.100 g^{-1} DW). And the lowest values were recorded in V_K (16.42 mg CAE.100 g^{-1} DW).

Total flavonoid content

Flavonoids are among the most widely consumed phenolic compounds. Many studies refer to their strong antioxidant activity, which is manifested by their ability to destroy free radicals and thus eliminate their adverse effect on DNA damage and lipid peroxidation. The number of hydroxyl OH-groups and their position in the molecule are important for the antioxidant activity of flavonoids. One of the most studied flavonoids is quercetin, which we used as a standard in our determinations (Stratil and Kuban, 2018). Michalak et al. (2021) reported that extracts of *Rosmarinus officinalis* contain polyphenols mainly flavonoids, which are involved in antioxidant activity and recommend them in free radical scavenging.

From Table 3 showed that the highest flavonoid content reached V_1 (0.3255 mg QE.100 g^{-1} DW). The lowest flavonoid content was found in V_2 (0.2922 mg QE.100 g^{-1} DW). V_K reached the lowest flavonoid content of 0.2235 mg QE.100 g^{-1} DW. Based on the measured values of flavonoid content, we can conclude that the best effect is shown by powdered

Table 2 Content of total polyphenols in the samples of plants of *Rosmarinus officinalis* L. treated by the different application of alginite (V_1, V_2) in comparison of control variant (V_K)

Variants	Average absorbance (nm)	Total polyphenols (mg CAE.100 g^{-1} DW
V_K	0.159 ±0.00115^b	16.42 ±0.112129^c
V_1	0.275 ±0.01159^a	27.69 ±0.1125483^a
V_2	0.259 ±0.00379^a	26.13 ±0.367638^b

Notes: different superscripts in each row indicate the significant differences in the mean at $p < 0.05$

Table 3 Content of total flavonoid in the samples of plants of *Rosmarinus officinalis* L. treated by the different application of alginite (V_1, V_2) in comparison of control variant (V_K)

Variants	Average absorbance (nm)	Total flavonoid content (mg QE.100 g^{-1} DW)
V_K	0.123 ±0.0098^b	0.22 ±0.0192^b
V_1	0.175 ±0.0005^a	0.32 ±0.0011^a
V_2	0.158 ±0.0020^a	0.29 ±0.0040^b

Notes: different superscripts in each row indicate the significant differences in the mean at $p < 0.05$

alginite. This is followed by alginite in the form of top dressing. This confirms the positive effect of alginite on the growth and the amount of content in *Rosmarinus officinalis*. Citrjáková (2019) confirmed that the application of alginite increases the content of total flavonoids in *Origanum vulgare* L. Her results showed a twofold increase in flavonoid content in samples grown with alginite.

Total phenolic acids content

Phenolic acids are a group of substances derived from cinnamic acid. The best known are caffeic acid and p-coumaric acid. It also includes depsides – rosmarinic acid. It is an ester of caffeic acid and 3,4-dihydroxyphenyl lactic acid. The synthesis is based on two amino acids: L-PHE and L-TYR (Blažević et al., 2021). Rosmarinic acid was the major polyphenol in both of *R. officinalis*. *R. officinalis* methanolic leaf extracts contained other phenols such as gentisic acid (Elansary et al., 2020). Many studies have confirmed that rosmarinic acid alone has potent antioxidant activities (Swisłocka et al., 2019). Gentisic acid, exhibited antioxidant activity and have as antioxidant agents in the food industry (Elsary et al., 2020).

The results of the determination of the total phenolic acid content expressed as caffeic acid equivalents showed that alginite was positive. After substituting the measured absorbance values of the samples into the calibration equation, the total phenolic acid content was determined for each sample. From Table 4, it can be seen that V_1 showed the highest content of 34.61 mg CAE.100 g^{-1} DW. V_2 showed the lowest content of 33.4 mg CAE.100 g^{-1} DW. The lowest content was observed in V_K (29.32 mg CAE.100 g^{-1} DW). Rosmarinic acid is responsible for the higher levels of phenolic acids. Rosmarinic acid encompasses a wide range of effects that are still the subject of research today. It is antiviral, antibacterial, antifungal, anti-inflammatory (Petersen and Simmonds, 2003). In the *Rosmarinus officinalis* methanolic leaf extract, the HPLC-DAD qualitative and quantitative analyses of selected phenolic compounds confirmed very high amount of rosmarinic acid (4040.00 mg.100 g^{-1} DW) (Elansary et al., 2020).

With its antioxidant effect, it shows high potential in the treatment of cancer. In the food industry, the antioxidant activity of rosemary is used as a preservative (Bednářová, 2017). Phenolic acids inhibit ACE, thereby exerting an antihypertensive effect. Caffeic acid contributes to this effect by inhibiting the enzyme renin. The antiphlogistic effect is provided by inhibition of COX-2, PGE-2, NF-κB and decrease in NO production. They have strong antioxidant effects, thereby scavenging free radicals. P-coumaric acid has anxiolytic effect at a dose of 30 mg.kg^{-1} similar to diazepam at a dose of 3 mg.kg^{-1} (Nagy et al., 2015).

Antoxidant activity

Clinical studies investigating the effects of free radicals and oxidative stress have confirmed the effect of these irritants in the development of age-related degenerative diseases including atherosclerosis, cancer, asthma, heart attack, stroke and others (Packer and Weber, 2001). Free radicals can be characterized as unstable, highly reactive and energy-rich molecules capable of independent existence. Peroxidative enzyme systems, inflammation in the body, smoking, lipid oxidation, and others have been implicated in their formation (Stief, 2003). Studies have confirmed that polyphenols, rosmarinic acid, hesperidin and rosmanol are responsible for the antioxidant activity of *R. officinalis* leaf extracts (Nieto et al., 2018). Elansary et al. (2020) investigated the content of total polyphenols, phenolic acids, and antioxidant activity of two natural populations of *R. officinalis* in northern Riyadh using HPLC-DAD assays. They found that higher antioxidant activity was associated with higher rosmarinic acid content in the leaf extracts. For the determination of antiradical or antioxidant activity we chose the DPPH method. The antiradical activity determined as % reduction of DPPH was determined in the methane extract of *Rosmarinus officinalis* sample presented in Figure 1.

Table 4 Content of phenolic acids in the samples of plants of *Rosmarinus officinalis* L. treated by the different applications of alginite (V_1, V_2) in comparison of control variant (V_K)

Variants	Average absorbance (nm)	Total phenolic acids content (mg CAE.100 g^{-1} DW)
V_K	0.510 ±0.001^b	29.32 ±0.066^b
V_1	0.602 ±0.033^a	34.61 ±1.892^a
V_2	0.581 ±0.010^b	33.41 ±0.607^a

Notes: different superscripts in each row indicate the significant differences in the mean at $p < 0.05$

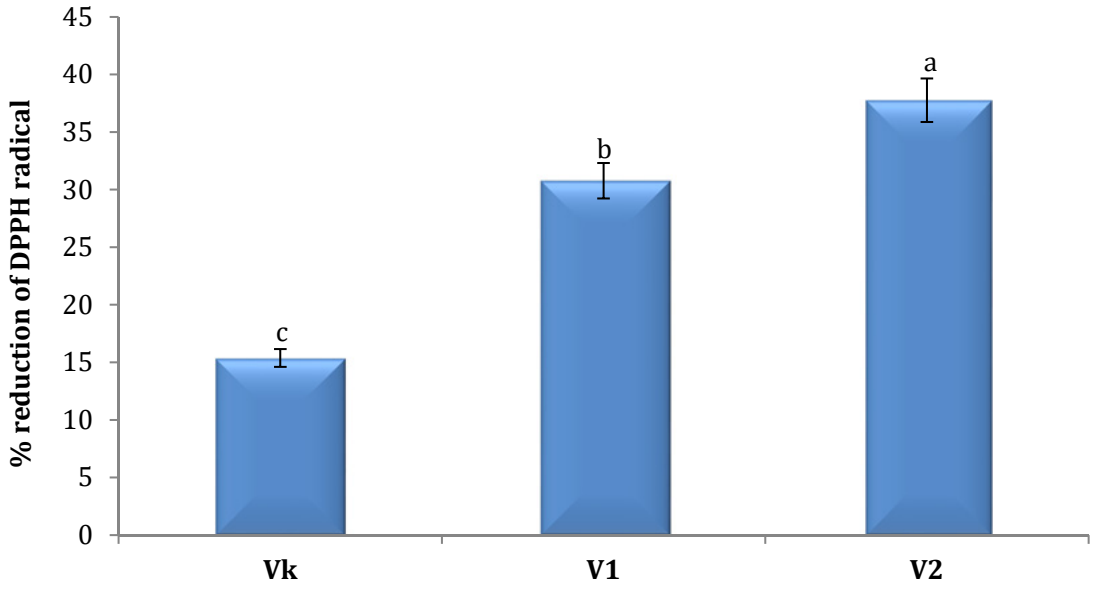

Figure 1 Comparison of the effect of applied alginite in different forms (V_1 and V_2) on antiradical activity determined as % reduction of DPPH in samples from plants of *Rosmarinus officinalis* L. in comparison with the control variant (V_K)
The different superscripts in each column indicate the significant differences in the mean at $p < 0.05$

V_1 showed the highest antioxidant activity of 37.76 ±1.47%. In V_2 variant, 30.77 ±1.69% inhibition of DPPH radical was observed, and the lowest inhibition was exhibited by V_K (15.38 ±0.31%). The antioxidant activity for 25-fold diluted extract corresponds to 0.04 g of dry sample.

Gnipova (2021) and pointed out the beneficial effect of alginite on the antioxidant activity of *Thymus serpyllum* L. The highest antiradical activity as % reduction of DPPH radical 39.29% was determined in diluted (1 : 19) methanolic extract of the sample grown on soil treated with powdered form of fertilizer (V_3) The antiradical activity was 411.2 mg TEAC. kg^{-1} DW. The methanolic extract of diluted (1 : 19) sample grown with alginite in suspension form (V_2) showed antiradical activity of 32.86% and that of diluted (1 : 19) control without alginite (V_1) 25%. The antiradical activity converted to Trolox (TEAC) in the sample extract V_2 was 341.78 mg TEAC.kg^{-1} and in the control was 256.92 mg TEAC.kg^{-1} DW.

The results of the experiment confirmed that alginite increased the content of bioactive compounds in *Rosmarinus officinalis*, which exhibit antioxidant activity and can be used in the prevention and adjunctive therapy of oxidative stress-induced diseases. Based on the results obtained from the experiment, we can conclude that both application forms of alginite had a beneficial effect on the growth, leaf size as well as weight of the vegetative drug *Rosmarinus officinalis* compared to the control. They also positively influenced the total polyphenols, flavonoids, phenolic acids, and antiradical activity of the extracts. When *Rosmarinus officinalis* is grown, incorporation of powdered form of alginite into the soil before ploughing may be recommended as the most suitable form. Application by drench in the form of alginite dissolved in water is also suitable. Both applied forms achieved significantly higher favourable results compared to the control.

The effect of alginite on the contents contained in Lamiaceae plants was investigated by Plichta (2016), Horný (2017), Citrjáková (2019), Eftimova et al. (2021), Gnipova (2021), Horčinová Sedláčková et al. (2021) and Janovská (2021). Their results confirm our conclusions that alginite is beneficial for plants, which is manifested by an increase in aboveground mass and an increase in the contents of the plant.

Conclusion

Alginite is generally recommended for use on soils to maintain and efficiently use water and improve soil quality and fertility. Alginite as a natural bituminous rock contains more than 10% fossil organic matter, all macroelements (except nitrogen) and a significant content of microelements and other known and unknown organic components. For this reason, the application of alginite in the cultivation of individual plant species effect as a specific natural stimulator in the formation of phytomass and increases the content of some biologically active components. In our experiment, it was confirmed that the application

of alginite significantly increased the plant height, leaf length, total dry weight and simultaneously the content of polyphenols, flavonoids, phenolic acids and antioxidant activity in *Rosmarinus officinalis* plants. The increase in the given morphological and biochemical traits significantly improved the economic value of the raw material for pharmaceutical and agronomic use. It follows that the use of the right dose and form of alginite allows farmers and producers of medicinal plants to improve their socio-economic conditions.

Conflicts of interest

The authors declare no conflict of interest.

Ethical statement

This article does not contain any studies that would require an ethical statement.

Acknowledgments

The publication was prepared with the active participation of researchers in International Network Agrobio*Net*.

References

Blažević. T., Reznicek, G., Ding, L., Yang, G., Haiss, P., Heiss, EH., Dirsch, VM., & Liu, R. 2021. Short Chain (≤C4) Esterification Increases Bioavailability of Rosmarinic Acid and Its Potency to Inhibit Vascular Smooth Muscle Cell Proliferation. In *Frontiers in Pharmacology*, *11*, 609756. https://doi.org/10.3389/fphar.2020.609756

Begum, A., Sandhya, S., Ali, S., Ravindran, V., Reddy, S., & Banji, D. 2013. An in-depth review on the medicinal flora *Rosmarinus officinalis* (Lamiaceae). In *Acta Scientiarum Polonorum, Technologia Alimentaria*, *12*(1), 61–73.

Bourhia, M., Laasri, FE., Aourik, H., Boukhris, A., Ullah. R., Bari, A., Ali, SS., El Mzibri, M., Benbacer, L., & Gmouh, S. 2019. Antioxidant and antiproliferative activities of bioactive compounds contained in *Rosmarinus officinalis* used in the Mediterranean diet. In *Evidence-Based Complementary and Alternative Medicine*, *2019*, 7623830. https://doi.org/10.1155/2019/7623830

Brindza, J., Horčinová Sedláčková, V., & Grygorieva, O. 2021a. Active effects of less known bituminous rock Alginite on the biological processes of *Solanum lycopersicum* L. In *International Scientific Conference Genetics, Physiology and Plant Breeding*. 1st ed. 364 p.

Brindza, J., Vozár, Ľ., Miko, M., Gažo, J., Kovár, P., Horčinová Sedláčková, V., & Grygorieva, O. 2021b. Unique effects of alginite as a bituminous rock on soil, water, plants and animal organisms. In *Agrobiodiversity for Improving Nutrition, Health and Life Quality*, *5*(1), 169–184. https://doi.org/10.15414/Ainhlq.2021.0016

Citrjaková, J. 2019. *Vplyv aplikácie alginitu na množstvo a kvalitu obsahových látok v Origanum vulgare* L. *a možnosti využitia pre produkciu drogy* [Effect of alginite application on the quantity and quality of contents in *Origanum vulgare* L. and possibilities of utilization for drug production]. Thesis. Košice. University of Veterinary Medicine and Pharmacy in Košice. 59 p. [In Slovak].

Costea, T., Radu, C. F., Popescu, ML., Dutu, LE., & Gird, CE. 2020. Chemical composition and antioxidant activity of some Lamiaceae spices. In *Proceedings*, *57*(1), 12. https://doi.org/10.3390/proceedings2020057012

Eftimová, J., & Habán, M. 2012. *Produkcia liečivých rastlín* [Production of medicinal plants]. Košice. UVLF [In Slovak].

Eftimová, J., Petrovič, V., & Vodhanel, V. 2021. Effect of alginite on some antioxidant indexes in extracts of two variants of *Mentha* and their toxicity. In *Agrobiodiversity for improving nutrition, health and life quality*, *5*(2), 315–321. https://doi.org/10.15414/ainhlq.2021.0030

Elansary, H.O., Szopa, A., Kubica, P., El-Ansary, D.O., Ekiert, H., & Al-Mana, F.A. 2020. *Malus baccata* var. *gracilis* and *Malus toringoides* bark polyphenol studies and antioxidant, antimicrobial and anticancer activities. In *Processes*, *8*(3), 283. https://doi.org/10.3390/pr8030283

Elansary, O.H., Szopa, A., Kubica, P., Ekiert,H., El-Ansary, D., Al-Mana, F. & Mahmoud, E. 2020. Saudi *Rosmarinus officinalis* and *Ocimum basilicum* L. polyphenols and biological activities. In *Processes*, *8*(4), 446. https://doi.org/10.3390/pr8040446

Elisovetcaia, D., Ivanova, R., Šimková, J., & Brindza, J. 2021. Biological effects of alginite on tomato plants (*Lycopersicon esculentum*) and some insects (*Leptinotarsa decemlineata*, *Galleria mellonella* and *Halyomorpha halys*). In *Agrobiodiversity for Improving Nutrition, Health and Life Quality*, *5*(2), 339–347. https://doi.org/10.15414/ainhlq.2021.0033

European Pharmacopoeia Commission (2012). European Pharmacopoeia 7th ed.: Supplement 7.8. Strasbourg: Council of Europe.

Farmakopea Polska (1999). In Wyd. P.T. Farm. Warszawa

Ghasemzade, H., & R.M.-Hosseinzadeh, H. 2020. Effects of rosmarinic acid on nervous system disorders. In *Iranian Journal of Basic Medical Sciences*, *23*(9). https://doi.org/10.22038/ijbms.2020.45269.10541

Gnipová, A. 2021. *Inovatívne a agrotechnické opatrenia na zvýšenie produkcie a obsahových látok v Thymus serpyllum* L. [Innovative and agrotechnical measures to increase the production and content of *Thymus serpyllum* L.]. Thesis. Košice. University of Veterinary Medicine and Pharmacy in Košice. 50 p. [In Slovak].

Horčinová Sedláčková, V., Šimková, J., Mňahončáková, E., Hrúzová, M., Kovár, P. Vozár, Ľ., & Hric, P. 2021. Effect of alginite in the form of ALGEXr 6 preparation on the biomass formation and antioxidant activity of some medicinal plants. In *Agrobiodiversity for Improving Nutrition, Health and Life Quality*, *5*(1), 80–94. https://doi.org/10.15414/ainhlq.2021.0009

Horný, M. 2017. *Využitie alginitu pri pestovaní liečivých rastlín a jeho vplyv na obsahové látky v droge – ich toxicita na vodné organizmy* [The use of alginite in the cultivation of medicinal plants and its effect on the contents of the drug – their toxicity to aquatic organisms]. Thesis. Košice. University of Veterinary Medicine and Pharmacy in Košice. 82 p. [In Slovak].

Janovská, Z. 2021. *Vplyv rôznych aplikačných foriem alginitu na obsahové látky v Ocimum basilicum* L. [Effect of different application forms of alginite on the contents of *Ocimum basilicum* L.]. Thesis. Košice. University of Veterinary Medicine and Pharmacy in Košice. 59 p. [In Slovak].

Košťálová, D., Fialová, S., & Račková, L. 2012. *Fytoterapia v súčasnej medicíne* [Phytotherapy in the current medicine]. Martin: Osveta. 384 p. [In Slovak].

Kovár, P., Vozár, Ľ., Hric, P., Brindza, J., & Verešová, P. 2021. Vplyv alginitu a extraktov z neho na klíčenie lipnice lúčnej (*Poa pratensis* L.) [Influence of alginite and extracts from it on germination of Kentucky bluegrass (*Poa pratensis* L.)]. In *Proceedings*: *XV. národní odborný a vedecký seminár "Osivo a sadba",* February 4th. [In Slovak].

Kulich, J., Valko, J., & Obernauer, D. 2002. Perspective of exploitation of alginit in plant nutrition. In *Journal of Central European Agriculture*, *2*(3), 200–205.

Kúšik, D., Mižák, J., & Šoltés, S. 2017. *Nerastné suroviny slovenskej republiky* [Mineral resources of the Slovak Republic]. State Geological Institute of Dionýz Štúr, Bratislava. [In Slovak].

Luo, C., Zou, L., Sun, H., Peng, J., Gao, C., Bao, L., Ji, R., Jin, Y., & Sun, S. 2020. A Review of the anti-inflammatory effects of rosmarinic acid on inflammatory diseases. In *Frontiers in pharmacology*, *11*, 153. https://doi.org/10.3389/fphar.2020.00153

Mahboubi, M. 2019. *Melissa officinalis* and rosmarinic acid in management of memory functions and Alzheimer disease. In *Asian Pacific Journal of Tropical Biomedicine*, *9*(2), 47. https://doi.org/10.4103/2221-1691.250849

Michalak, M., Zielinska A., & Paradowska K. 2021. Phenolic content, antioxidant activity and pharmaceutical availability of hydrogels with extracts of *Rosmarinus officinalis* L. and *Sambucus nigra* L. In *Acta Poloniae Pharmaceutica – Drug Research*, *78*(2), 219–226. https://doi.org/10.32383/appdr/134000

Martiš, M., & Eftimová, J. 2019. Vplyv intenzifikačných faktorov na kvalitu drogy rodu *Mentha* L. a možnosti jej kontaminácie [Influence of intensification factors on the quality of the drug from the genus *Mentha* L. and the possibility of its contamination]. In *Folia pharmaceutica cassoviensia*, *1*(3), 91–97. [In Slovak].

Nagy, M., Mučaji, P., & Grančai, D. 2015. *Farmakognózia: Biologicky aktívne rastlinné metabolity a ich zdroje* [Pharmacognosy: Biologically active plant metabolites and their sources]. 2nd ed. Bratislava : Herba, 128 p.

Nagy, M., MučajI, P., & Grančai, D. 2011. *Farmakognózia: Biogenéza prírodných látok* [Pharmacognosy: Biogenesis of natural substances]. 1st ed. Martin : Osveta, 42–95 p.

Nieto, G., Ros, G., & Castillo, J. 2018. Antioxidant and antimicrobial properties of rosemary (*Rosmarinus officinalis* L.). In *Medicines* (*Basel*), *5*(3), 98. https://doi.org/10.3390/medicines5030098

Packer, L., & Weber, S.U. 2001. The role of vitamin E in the energing field of nutraceuticals. In *Nutraceuticals in health and disease prevention*. New York: Marcel Dekker, 27–43 p. https://doi.org/10.5772/intechopen.83797

Petersen, M., & Simmonds, M.S.J. 2003. Rosmarinic acid. In *Phytochemistry*, *62*(2), 121–125. https://doi.org/10.1016/s0031-9422(02)00513-7

Plichta, T. (2017). *Štúdium antioxidačnej aktivity v extraktoch dúšky materinej* (*Thymus serpyllum* L.) *po jej pestovaní v substráte obohatenom o alginit* [Study of antioxidant activity in extracts of Motherwort (*Thymus serpyllum* L.) after its cultivation in alginite-enriched substrate]. Thesis. Košice. University of Veterinary Medicine and Pharmacy in Košice. 59 p. [In Slovak].

Singleton, V.L., & Rossi, J.A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. In *American Journal of Enology and Viticulture*, *16*, 144–158.

Suchý, V., Daňková, I., Dvorská, M., Hrazdilová, E., Kubinová, R., Šmejkal, K., Špačková, V., & Žemlička, M. 2013. *Praktická cvičení z farmakognozie* [Practical exercises in pharmacognosy]. University of Veterinary and Pharmaceutical Sciences in Brno. [In Czech].

Stief, T.W. 2003. The physiology and pharmacology of singlet oxygen. In *Medical Hypotheses*, *60*(4), 567–572. https://doi.org/10.1016/s0306-9877(03)00026-4

Stratil, P., & Kubáň, V. 2018. *Reaktivní kyslíkové radikály, přírodní antioxidanty a jejich zdravotní účinky* [Reactive oxygen radicals, natural antioxidants, and their health effects]. Český Těšín: 2 Theta. 190 p. [In Czech].

Stagos, D. 2020. Antioxidant activity of polyphenolic plant extracts. In *Molecules*, *9*(1), 19. https://doi.org/10.3390/antiox9010019

Varényi, S., Chrenová, S., & Lukáč, M. 2019. Analýza antioxidačnej aktivity polyfenolových zlúčenín lišajníkov a pečeňoviek metódou chromatografie na tenkej vrstve detekciou 2,2-difenyl-1-pikrylhydrazidom [Analysis of the antioxidant activity of lichen and liverwort polyphenolic compounds by thin layer chromatography detection with 2,2-diphenyl-1-picrylhydrazide]. In *Chemické listy*, *113*(1), 53–57.

URL1. 2022. WFO (2022): Lamiales Bromhead. http://www.worldfloraonline.org/taxon/wfo-9000000283