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Effects of Beech Bark Extract in the Sage (*Salvia Officinalis* L.) Plant Growth and Volatile Oil Profile

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Abstract: The use of bioactive compounds can act in growth stimulation and also influence the biosynthesis of the metabolites in plants. The aim of this paper is to assess the influence of the beech (*Fagus sylvatica* L.) bark crude extract (BBCE) on the growth and development of sage (*Salvia officinalis* L.) plants. Special attention was given to the analysis of volatile oil obtained from the sage treated plant. Thus, the biological activity of BBCE was assessed by determining the germination capacity, biomass accumulation, histo-anatomical aspects, and photoassimilatory pigment accumulation, quantitative, and qualitative sage volatile oil analysis. The results show stimulation of the biomass and photoassimilatory pigment accumulation. The mesophyll thickness and the vascular tissue surface are smaller in the treated variants, compared to the control. On the other hand, the amount of volatile oil was significantly higher in the treated plants. In the experimental variants, an increase in the quantity of eucalyptol, camphor, camphene, and α-caryophyllene is observed. The amount of eucalyptol increased in the experimental variant, with about 82%, compared to the control. BBCE could be properly used as natural bioregulators because according to our results seems to improve the yield of the sage crop. The results of this research have the potential to contribute greatly to ecological agricultural production.

Keywords: beech bark; bioregulators; bioactive compounds; camphor; eucalyptol; sage; volatile compounds

1. Introduction

Bioregulators have the same effect as phytohormones on plant growth and development [1]. Applied to plants in very small quantities in certain phases of their development, bioregulators, change the growth or resistance of organisms [1]. Thus, by inducing changes in the vital processes, an improvement in the quality and quantity of the crops appears, as well as an increase in production [2]. The literature data provides numerous information regarding the influence of polyphenolic compounds in seed germination and plant development, in the form of global crude extracts, fractions or individual compounds obtained from different plant sources [1,3,4].

Some studies have been performed to highlight the effect of polyphenolic compounds, capable of inducing rapid seed germination, harmonious seedling development, rapid growth in plant biomass, and improved plant metabolism [1,5,6]. Information regarding the effects of aromatic compounds on plant development is important in establishing the potential uses in agriculture as natural growth bioregulators.

In the literature research, there are a number of topics addressed concerning the separation and augmentation of polyphenolic compounds from waste resulted in the aftermath of manufacturing processes [1]. Thus, research was conducted by using the waste resulted from the manufacturing of timber as primary materials, such as the bark and nodes of spruce (*Picea abies* L.) or the wood (red heart) of the common beech (*Fagus sylvatica* L.) which contains import quantities of chlorogenic acid, derived from catechin, quercetin, kaempferol and apigenin [7–9].

Beech is used in the wood industry, and bark is regarded as a by-product. Using appropriate extraction methods bioactive compounds from bark tissue could be extracted and utilized [10].

Salvia officinalis L. (sage, *Lamiaceae* family) is one of the most important medicinal plants with many pharmacological properties. *Salvia officinalis* L. is a cultivated round shrub with the Mediterranean origin [11]. The sage leaves (*Salvia officinalis* folium) are used for medicinal purposes [12]. The *Salvia officinalis* leaves contain volatile oil 1–2.5% (*Salviae aetheroleum*) represented by terpenic substances. The main compounds are α-thujone (10–60%), β-thujone (4–36%), camphor (5–20%) and 1,8-cineole (1–15%) [11]. The sage volatile oil has bactericidal, antiseptic, fungicidal, antiviral, and anti-inflammatory action or buffers improvement caused by the menopause [11–13]. Antimicrobial properties are attributed, mainly to the presence of α-thujone [14]. The sage leaf infusions relieve pain in the throat or gums, an effect attributed to volatile compounds such as 1,8-cineole, borneol, camphor, and α-thujone [15].

As a consequence, the aim of this paper is to assess the influence of the beech (*Fagus sylvatica* L.) bark crude extract (BBCE) on the growth and development of sage (*Salvia officinalis* L.) plants, with special attention to quantitative and qualitative analysis of volatile oil obtained from the sage folium.

2. Materials and Methods

2.1. Plant Sample and Chemicals

The beech (*Fagus sylvatica* L.) bark was provided from the forest of Gurghiului Mountains, Mureș County, Romania. The beech trees were deforested (autumn 2018) for timber processing. Only the bark (multiple layers of periderms) was collected from the beech trees (15–20 years). The species was identified and authenticated by Dr. Corneliu Tanase from the Department of Pharmaceutical Botany. The beech bark was air-dried at room temperature (10.5% humidity) and milled in a GRINDOMIX GM 2000 mill (mean particle size diameter of <0.5 mm). The beech bark was used without any pre-treatments.

The sage (*Salvia officinalis* L.) seeds come from the seeds collection of the Botanical Garden of the University of Medicine, Pharmacy, Sciences and Technology "G.E. Palade" from Târgu Mureș, being collected in 2017. The seeds were sown after sterilization (immersion for 2 min in a 20% HClO solution, and well washed with water).

All chemicals and standards were provided by Sigma-Aldrich (St. Louis, MO, USA).

2.2. Extraction

Beech bark crude extract (BBCE) was obtained by applying a batch water extraction, using 10 g of the beech bark placed in an Erlenmeyer flask over which 300 mL of distilled water was added. The mixture was introduced in a water bath (45 min at 85 to 90 °C). Collected extracts were filtered, and the beech bark was subjected to a second extraction with fresh distilled water. This operation was repeated twice. The extract was collected in a 1000 mL volumetric flask and marked up to volume with distilled water.

The extraction of the volatile oil was performed by hydrodistillation, using a Neo-Clevenger device. The sage leaves were collected during the first year of flowering (July 2019). The leaves were collected from 100 plants/experimental variants. After weighing, the dried vegetal material (100 g) was introduced into the vessel of the device, then 1000 mL of water and 0.1 mL xylene/10 g of plant material were added. It was left for distillation for 2–3 h (moderate distillation speed). The oil volume was read in mL and reported to 100 g of the sage leaves. In order to calculate the amount of volatile oil obtained, the total volume of oil obtained was taken into account, from which the volume of xylene was subtracted and was compared to 100 g of sage leaves.

2.3. Working Protocol

The experiment was conducted between February 2018 and July 2019 in the Botanical Garden of University of the Medicine, Pharmacy, Sciences and Technology "G.E. Palade" from Târgu Mureș. The sage seeds were carefully selected and then immersed in the tested solutions for 12 h, at a constant temperature of 25 °C. The seeds were directly sown into pots according to the experimental plan (Table 1). After sowing the cultivated soils were wetted with 20 mL of tested solution/pot (tap water for control samples and beech bark extract for tested variants). After 45 days from the beginning of the experiments, the sage plants were transferred in the field. During the vegetative period, there were two applications, the first at the basal level (radicular absorption) and the second by spraying at the foliar level (foliar absorption at cuticle level). During the flowering period, one foliar application of the tested solutions was administered. The amount of solution was 10 mL/plant/application.

Table 1. Experimental plan for sowing sage (*Salvia officinalis* L.) seeds.

Tested Solutions	Pots Number	Seeds Number/Pot	Seeds Number
C [1]	66	3	198
BBCE0.5 [1]	66	3	198
BBCE1 [1]	66	3	198

[1] C—Control; BBCE0.5—beech bark crude extract 0.5 g bark/100 mL extract; BBCE1—beech bark crude extract 1 g bark/100 mL extract.

2.4. Plant Growth and Development Analysis

To determine the germination capacity, periodic counters were performed for 30 days. Germination was recorded when the radicle was at least 2 mm long. Germination capacity (CG) was calculated as follows: CG (%) = (Total number of seeds germinated/Total number of seeds tested) × 100 [16].

The biomass determination was done by gravimetric methods. During the first year of flowering (July 2019) all plants were collected. The results were expressed in g/plant. The effects were evaluated as a percentage compared with the control. The experiment was performed in triplicate.

For pigment quantification, 0.05 g of fresh vegetal material, collected in the first year of vegetation (5 months after sowing), was milled with quartz sand and extracted with acetone (80%). The chlorophyll a and b, and carotenoid contents were spectrophotometrically determined at wavelengths of 470, 646, and 663 nm and quantified [17]. The experiment was performed in triplicate.

2.5. Histo-Anatomical Analysis

For histo-anatomical analysis, the sage plants, collected in the first year of vegetation (5 months after sowing), were fixed and preserved in 70% alcohol. The cross-sections of the root, stem, and leaf were made. The sections obtained were double-stained using iodine green and ruthenium red [9]. Finally, the stained sections were mounted between slides in a few drops of water and analyzed with a Motic Microscope and photographed with a Nikon Coolpix L22 camera, Tokyo, Japan. 25 sections for each experimental variant/vegetative organ, were obtained. Analysis of microscopic images was

performed with ImageJ Image Processing and Analysis in Java Version 1.51j8 (National Institute of Mental Health, Bethesda, MD, USA).

2.6. GC-MS Qualitative Analysis of Volatile Compounds

The volatile profiles of the sage essential oils samples were determined by gas chromatography-mass spectrometry (GC-MS) using a GC-MS Shimadzu model QP-2010 (Shimadzu Scientific Instruments, Kyoto, Japan) equipped with a Combi-PAL AOC-5000 autosampler (CTC Analytics, Zwingen, Switzerland) and a capillary column (ZB-5 ms, 30 m × 0.25 mm i.d. × 0.25 µm, Phenomenex, Torrance, USA). The essential oil samples were properly diluted before injection into the GC-MS injector. The separation of volatile compounds was performed using the following column temperature program: from 50 °C (held for 2 min) to 160 °C with a 4°/min rate and then to 250 °C with a 15°/min rate and held for 10 min. The carrier gas was helium at a flow rate of 1 mL/min, and the split ration 1:100. The injector temperature and that of ionic source and interface were set at 250 °C. The MS detection was performed on a quadrupole mass spectrometer operating in the full scan (40–500 m/z) electron impact (EI) at ionization energy of 70 eV. The volatile constituents of the essential oils were identified based on their mass spectra, by comparison with those of reference compounds from NIST27 and NIST147 mass spectra libraries (considering a minimum similarity of 85%) and verified with retention indices drawn from www.pherobase.com or www.flavornet.org for columns with a similar stationary phase. This technique offers a qualitative assessment of volatile compounds, so the relative percentage of each compound was estimated as a fraction of its integrated ion area from the total ion chromatograms (TIC) area (100%).

2.7. Statistical Analysis

The experiment was performed in triplicate and the results are expressed as means ± standard deviations. For histo-anatomical analysis, the statistical tests used were the Kruskal–Wallis test for including a sample in a specific distribution and Mann–Whitney U test (non-parametric) for comparing two population means. The tests were applied in the Past 2.17.

3. Results and Discussions

3.1. Extract Characterization

The characteristics of BBCE were summarized in previous work [18]. The total phenolic and tannin content in the BBCE was 36.66 and 1.47 mg GAE/g, respectively [18]. The results showed that the BBCE contain (+)-catechin, (−)-epicatechin, quercetin-O-hexoside, taxifolin-O-hexosides, taxifolin-O-pentosides, B-type and C-type procyanidins, syringic acid, and coumaric acid-di-O-glycosides, coniferyl alcohol and sinapyl alcohol-glycosides, and (+) and (−) glucodistylin [15–17].

3.2. Seed Germination

Applying the BBCE, enzymatic changes were induced, influencing the seeds germination capacity [19]. The recorded data show that the tested solutions (BBCE0.5 and BBCE1) have a significant stimulatory influence on the sage seed's germination capacity compared to the control (Figure 1). The higher germination stimulating effect was recorded for BBCE0.5 (with 73% higher compared with the control). For BBCE1 the stimulation percentage, compared to the control was about 40%.

3.3. Biomass Accumulation

Figure 2 shows that at the stem level, there are no significant differences in biomass accumulation between BBCE solutions and control. However, the amount of leaves biomass is significantly ($p \leq 0.05$) higher at experimental variants compared to the control. The higher stimulation percentage was for BBCE1 (61%). At a lower concentration (BBCE0.5) the percentage of stimulation is 27%. These results showed the bioregulator effect of BBCE on leaves biomass. These findings are important because the

sage leaves (Salviae officinalis folium) are the main source for essential oil [11]. Similar results have been reported in other previous studies [20,21]. For example, it was shown that aqueous crude extracts from chestnuts stimulate the growth and development of rape plantlets and the development of the oat root system [20]. The stimulating effect of biomass accumulation in rape plants was observed in the presence of aqueous crude extracts obtained from the *Asclepias syriaca* plant and grape seeds [20,21].

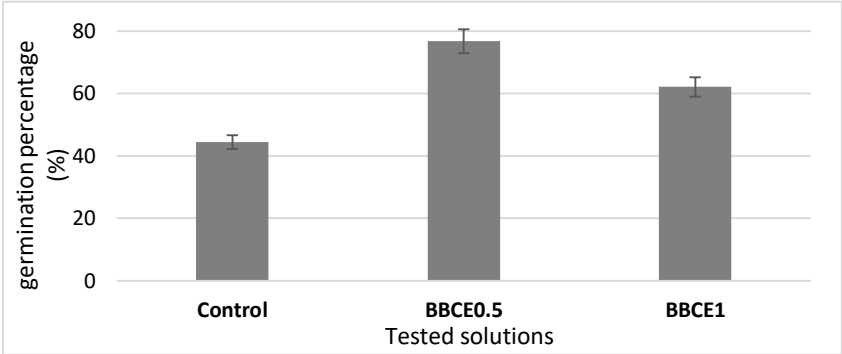

Figure 1. The influence of beech (*Fagus sylvatica* L.) bark crude extract (BBCE) on *Salvia officinalis* L. seed germination capacity. Error bars represent the standard deviations of means. BBCE0.5—beech bark crude extract 0.5 g bark/100 mL extract; BBCE1—beech bark crude extract 1 g bark/100 mL extract.

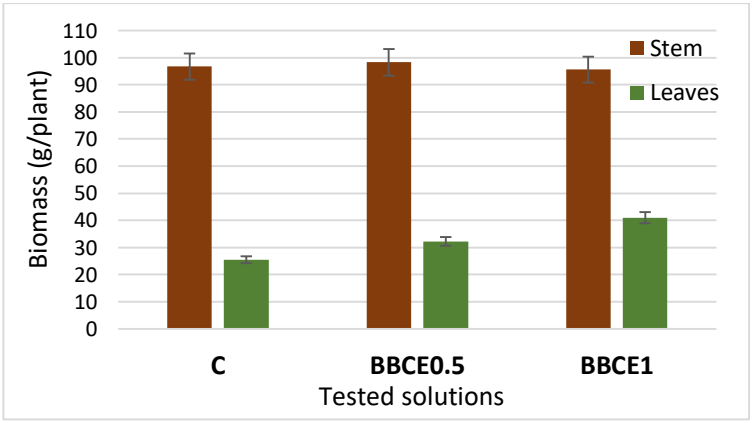

Figure 2. The influence of BBCE on *Salvia officinalis* L biomass accumulation. Error bars represent the standard deviations of means. C—Control; BBCE0.5—beech bark crude extract 0.5 g bark/100 mL extract; BBCE1—beech bark crude extract 1 g bark/100 mL extract.

3.4. Photo-Assimilating Pigment Content in Sage (Salvia officinalis L.) Leaves

To assess the metabolic activity of plants it is important to determine the quantitative photo-assimilating pigments [22]. Analyzing the results from Table 2, it is observed a stimulating effect of the accumulation of the photo-assimilating pigment in sage plants, for the BBCE1 experimental variant, compared to the control. The results show that the applied treatment does not negatively affect the accumulation of chlorophyll pigments.

Table 2. Content of photo-assimilating pigments synthesized (µg/g) in sage leaves.

Experimental Variant	Chl a	Chl b	Chl a + Chl b	Chl a/Chl b	Carotens
C [1]	64.3 ± 2.08	29.9 ± 1.82	94.28	2.15	0.072 ± 0.01
BBCE0.5 [1]	74.1 ± 1.10	22.2 ± 1.09	96.32	3.32	0.024 ± 0.02
BBCE1 [1]	248.7 ± 15.06	61.5 ± 2.03	310.2	4.04	1.135 ± 0.05

[1] C—Control; BBCE0.5—beech bark crude extract 0.5 g bark/100 mL extract; BBCE1—beech bark crude extract 1 g bark/100 mL extract; Chl a—chlorophyll a; Chl b—chlorophyll b.

3.5. Histo-Anatomical Aspects of the Sage (*Salvia officinalis L.*)

Taking into consideration the internal structure of the sage root (Figure 3a), the rhizodermis exfoliates in the early stage of the development process. The vascular tissue's origin is mostly cambial and forms two concentric rings, which includes very narrow phloem and very thick xylem with many vascular bundles. Analyzing the internal structure of the roots, there are no significant differences between the samples (Table 3).

The contour of the stem cross-section is quadratic due to the angular collenchym, present in the four ribs (Figure 3b). The epidermis has isodiametric cells that have a very thick and cutinized outer wall. The cortical tissue is well developed, being differentiated into collenchym tissue, multilayered, forming cordons near the ribs. The central cylinder contains the vascular tissues arranged in the shape of rings. In the section center, can be seen cellulose parenchymatic pith. Following the measurements and the statistical analysis, no significant differences were observed between the experimental variants and the control (Table 3).

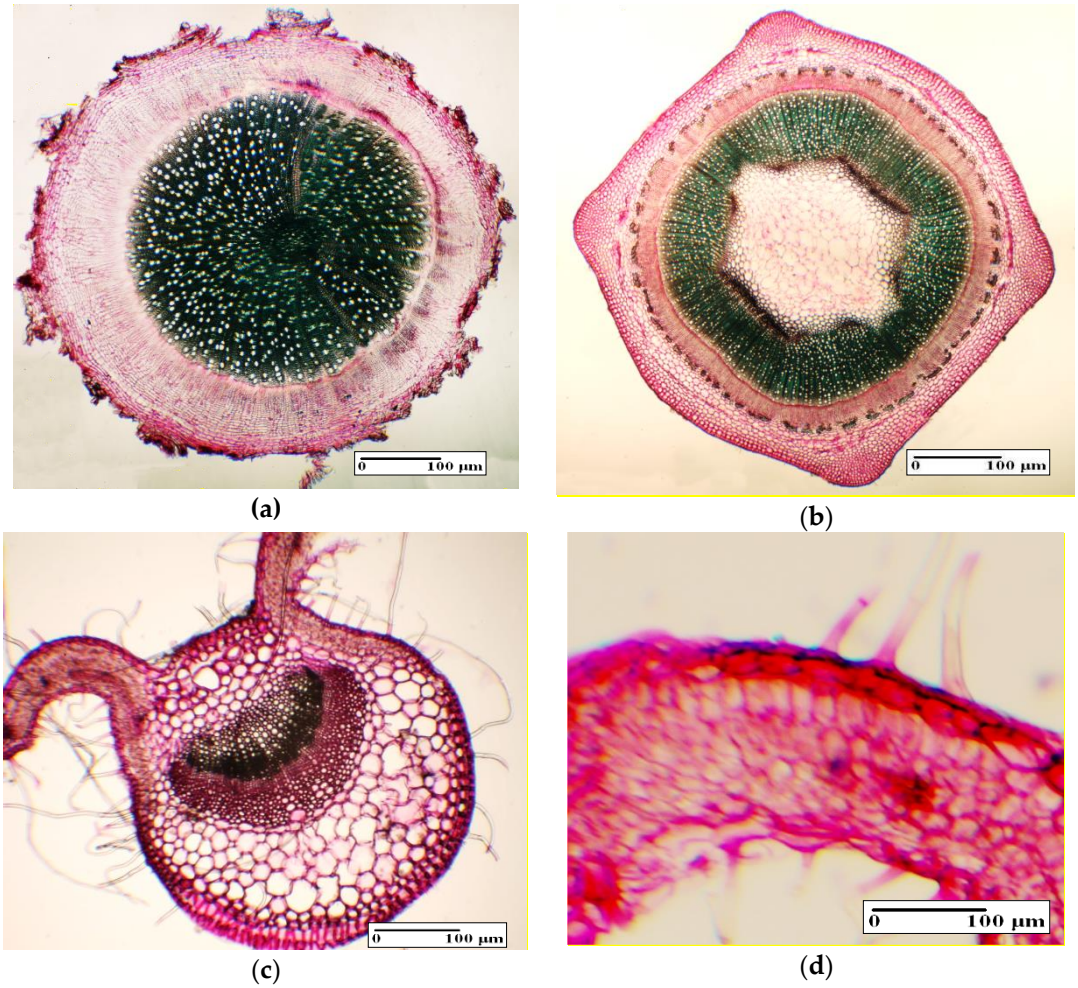

Figure 3. The general aspects of internal structure of sage (*Salvia officinalis* L.) vegetative organs: Root (**a**), stem (**b**), main string of leaf (**c**), and leaf lamina (**d**).

Table 3. The microscopic characteristics of vegetative organs in case of treated and control sage plants.

Vegetative Organs	Microscopic Characteristics	Treated Plants (Mean ± SD)		Control Plants (Mean ± SD)
		BBCE0.5 [1]	BBCE1 [1]	
		n = 5	*n* = 5	*n* = 5
Root	Central cylinder area (%)	31.56 ± 5.22	35.56 ± 5.33	33.02 ± 3.71
	Cortex area (%)	68.44 ± 5.22	64.44 ± 5.33	66.97 ± 3.71
Stem	Cortex area (%)	32.62 ± 3.76	34.05 ± 1.64	36.84 ± 3.22
	Floem area (%)	14.61 ± 1.15	13.35 ± 1.31	15.37 ± 1.69
	Xylem area (%)	35.96 ± 4.31	34.31 ± 2.51	29.09 ± 2.81
	Pith area (%)	16.82 ± 3.25	18.31 ± 2.37	18.69 ± 1.99
	Colenchim area (%)	5.85 ± 0.84	5.09 ± 0.83	4.71 ± 1.48
	Sclerenchyma sheath area (%)	1.69 ± 0.19	1.49 ± 0.17	1.38 ± 0.33
Leaf	Leaf lamina thickness (mm)	0.062 ± 0.009	0.054 ± 0.005	0.072 ± 0.006
	Mesophyll thickness (mm)	0.045 ± 0.006	0.039 ± 0.004	0.052 ± 0.004
	Vascular bundles area in the main string (%)	20.37 ± 2.03	18.75 ± 3.02	26.84 ± 2.64
	Cortex area in the main string (%)	79.62 ± 2.03	81.29 ± 3.02	73.61 ± 2.64

[1] BBCE0.5—beech bark crude extract 0.5 g bark/100 mL extract; BBCE1—beech bark crude extract 1 g bark/100 mL extract; ± SD (standard deviation); *n*—samples number.

Analyzing the internal structure of the leaf, two unistratified epidermis was observed. The mesophyll is thick, differentiated into palisadic tissue, and relatively compact lacunar tissue on the lower face (Figure 3d). The vascular bundles have a primary structure. At the leaf lamina and mesophyll level, there are significant differences in the BBCE1 variant (Table 3), where the thickness is smaller, compared with control. At the main string level (Figure 3c), the vascular tissue has a smaller surface area in BBCE variants, compared to the control. On the other hand, the cortex area is higher compared with the control (Table 3). The histo-anatomical results show that the BBCE does not negatively affect the growth and development of sage.

3.6. Volatile Oil Content Analysis

The total volatile oil content of aromatic plants indicates their quality [23]. Thus, the lower limit of the volatile oil content is one of the specifications of medicinal plants. The volatile oil from sage has a yellow color and aromatic odor. According to PhEur sage leaves contain at least 1.5% volatile oil [24]. Significant differences, compared with control, appear for BBCE1, where the highest amount of volatile oil was obtained (Table 4). Thus, the results regarding the quantity of volatile oil are correlated positively with those for the leaves biomass accumulation.

Table 4. Total volatile oil content (mL/100 g) from sage (*Salvia officinalis* L.) plants.

	C [1] (Mean ± SD)	BBCE0.5 [1] (Mean ± SD)	BBCE1 [1] (Mean ± SD)
Salviae officinalis herba	1.26 ± 0.08	1.29 ± 0.05	1.43 ± 0.09
Salviae officinalis folium	1.69 ± 0.13	1.70 ± 0.06	1.79 ± 0.11

[1] C—Control; BBCE0.5—beech bark crude extract 0.5 g bark/100 mL extract; BBCE1—beech bark crude extract 1 g bark/100 mL extract; ± SD (standard deviation).

The working hypothesis for this experiment stage was that BBCE applied to sage plants will influence the chemical composition of the essential oil produced. Thus, the GC-MS chromatograms were obtained (Figure 4), and the data were processed, comparing the experimental variant with the control results.

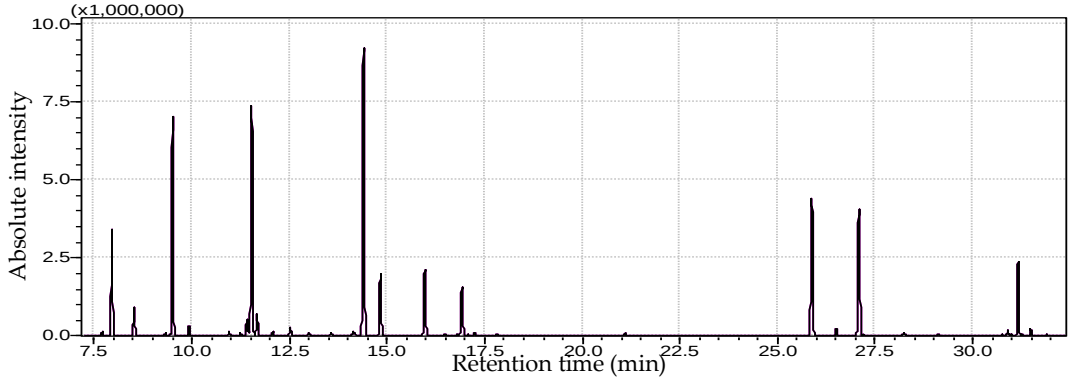

Figure 4. GC-MS chromatogram of the essential oil obtained from Salviae folium, by classical extraction with Neo-Cleavenger.

The qualitative and semi-quantitative composition of the sage essential oil is presented in Table 5, where the compounds are listed by order of their elution. In total, 25 compounds were identified accounting for 99.87–99.99%. The standardized sage essential oil contains the following percentages of compounds: Eucalyptol (5.5–13%), α-thujone (18–43%), β-thujone (3–8.5%), camphor (4.5–24.5%), α-caryophyllene (0–12%), α-pinene (1–6.5%), camphene (1.5–7%), and limonene (0.5–3%) [25] In our samples, in accordance with literature data, the volatile compounds that were found in high quantities are: α-thujone (16.21%), β-pinene (12.86%), α-pinene (11.72%), eucalyptol (11.13%), β-thujone (7.15%), limonene (6.72%) β-myrcene (5.31%), and α-caryophyllene (3.46%).

Table 5. Concentrations (peak area percent) of the volatile compounds from Salviae folium.

Compounds	Retention Time	Concentration (% of Total Surface Area of Peaks)		
		C [1]	BBCE0.5 [1]	BBCE1 [1]
α-Tricyclene	7.519	n.i.	0.17 ± 0.03	n.i.
α-Thujene	7.639	0.71 ± 0.04	0.37 ± 0.06	0.43 ± 0.05
α-Pinene	7.887	11.72 ± 0.06	11.38 ± 0.11	13.93 ± 0.20a
Camphene	8.466	1.61 ± 0.05	6.9 ± 0.13	2.51 ± 0.11
β-Pinene	9.458	12.86 ± 0.11	6.88 ± 0.31	4.97 ± 0.10
β-Myrcene	9.88	5.31 ± 0.14	3.55 ± 0.17	4.89 ± 0.13
3-Carene *	10.512	0.35 ± 0.04	0.11 ± 0.01	n.i.
α-Terpinene	10.91	1.65 ± 0.14	0.47 ± 0.02	0.75 ± 0.08
p-Cymene	11.207	3.58 ± 0.11	1.46 ± 0.13	1.96 ± 0.04
d-Limonene	11.381	6.72 ± 0.11	5.05 ± 0.13	6.08 ± 0.14
Eucalyptol	11.503	11.13 ± 0.22	20.28 ± 0.35	16.9 ± 0.12
β-*trans*-Ocimene	11.639	5.74 ± 0.03	3.49 ± 0.12	4.93 ± 0.11
β-*cis*-Ocimene	12.042	1.55 ± 0.09	0.99 ± 0.06	1.09 ± 0.14
γ-Terpinene	12.494	2.15 ± 0.54	0.68 ± 0.03	1.01 ± 0.07
Terpinolene	13.537	0.58 ± 0.08	0.29 ± 0.17	0.49 ± 0.04
β-Linalool *	14.119	0.39 ± 0.01	0.22 ± 0.02	0.39 ± 0.05
α-Thujone	14.383	16.21 ± 0.16	16.66 ± 0.12	20.36 ± 0.18
β-Thujone	14.818	7.15 ± 0.03	2.31 ± 0.14	2.2 ± 0.10
allo-Ocimene	15.189	2.12 ± 0.08	1.24 ± 0.09	1.71 ± 0.08
Camphor	15.953	1.59 ± 0.34	5.18 ± 0.22	4.19 ± 0.08
Pinocamphone	16.462	0.3 ± 0.03	n.i.	0.18 ± 0.04
Borneol	16.907	0.4 ± 0.05	2.98 ± 0.07	0.77 ± 0.06
Isocamphopinone *	17.061	0.17 ±0.04	n.i.	n.i.
Isobornyl acetate *	21.091	n.i.	0.38 ± 0.04	0.63 ± 0.03
Copaene *	24.354	n.i.	n.i.	0.12 ± 0.02
Caryophyllene	25.872	2.42 ± 0.04	3.22 ± 0.09	2.08 ± 0.06
Spatulenol *	26.502	0.17 ± 0.01	0.6 ± 0.11	n.i.
α-Caryophyllene	27.083	3.46 ± 0.04	5.05 ± 0.05	7.3 ± 0.18
Unknown	31.159	n.i.	0.1 ± 0.04	0.12 ± 0.03

[1] C—Control; BBCE0.5—beech bark crude extract 0.5 g bark/100 mL extract; BBCE1—beech bark crude extract 1 g bark/100 mL extract; ± SD (standard deviation); *—tentative identification; n.i.—not identified.

In the BBCE variants, an increase in the quantity of eucalyptol, camphor, camphene, and α-caryophyllene is observed (Figure 5). Noteworthy, that the concentration of eucalyptol increases with about 82% (BBCE0.5) and 51% (BBCE1) compared to the control. In the literature, it is reported that the antibacterial activity of sage oil has been attributed to the presence of eucalyptol and camphor [26,27]. Pinto et al. [27] suggest that eucalyptol and camphor are the main compounds responsible for the antifungal activity in the tested strains. Thus, the experimental variants are very promising as they possess a high amount of camphor and eucalyptol.

In the experimental variant, the total amount of thujones (α- and β-diastereoisomers) decreases, compared to the control. In literature data, thujone has been reported to be toxic to brain and liver cells [28,29]. The experimental variants are promising as they possess a low amount of β-thujone, thereby evidencing their safety profile. The α-caryophyllene exhibited high cytotoxic activity in the murine macrophage cells, colorectal adenocarcinoma cells, and breast melanoma cells [30]. In BBCE variants, the α-caryophyllene amount was higher, compared to the control.

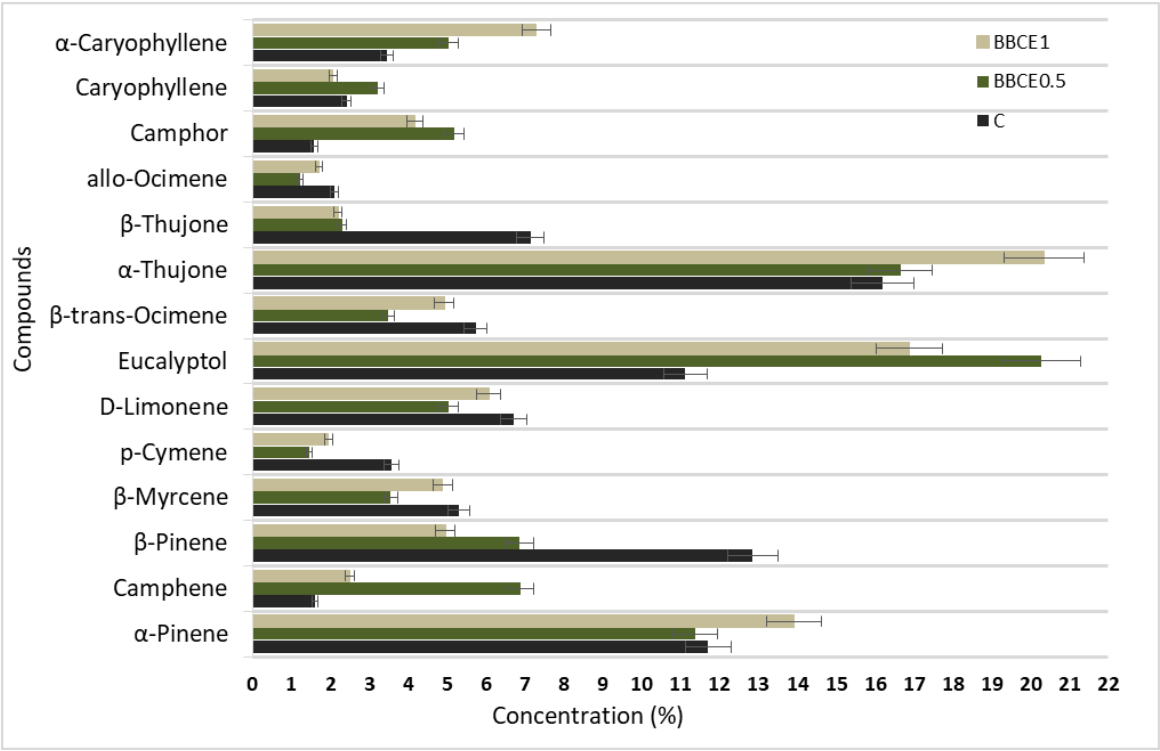

Figure 5. Representative graphic for concentrations (peak area percent) of the main volatile compounds from Salviae folium, obtained by GC-MS method. Error bars represent the standard deviations of means. C—Control; BBCE0.5—beech bark crude extract 0.5 g bark/100 mL extract; BBCE1—beech bark crude extract 1 g bark/100 mL extract.

Compared with the control group, in the BBCE1 variant, α-pinene was in a significantly higher concentration, while β-pinene was in a significantly lower concentration. It seems that the treatment stimulates the preferential formation of α-pinene from the pinyl cation in the monoterpene biosynthesis, by influencing the three pinene cyclases found in *Salvia officinalis* [31,32]. This outcome could have a great therapeutically value, as it was shown that α-pinene has a higher microbicidal activity than the β-isomer [33,34].

The formation of many volatile compounds has different biosynthetic pathways which employ special enzymes in the processes [35]. These suggest that the differences observed in this study could be related to the regulation of the enzymes responsible for the formation of these compounds.

4. Conclusions

Taking into account the results, beech bark aqueous extract can be recommended for the bioregulator properties given by its polyphenolic composition. Although beech bark extract has demonstrated efficiency in germination and in leaves biomass accumulation process and did not significantly affect the histo-anatomical structure of the sage vegetative organs. Thus, the highest total amount of volatile oil was obtained in the experimental variant (beech bark crude extract 1 g bark/100 mL extract), compared to the control and an increase in the quantity of eucalyptol, camphor, camphene, and α-caryophyllene is also observed.

These results reveal the potential of beech bark crude extract to contribute to the ecological agricultural production of sage plants. Beech bark aqueous extract can be further studied to prove bioregulator potential in order to improve the quantity and quality of the sage volatile compounds with pharmaceutical use.

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