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Influence of jasmonic acid on the growth and antimicrobial and antioxidant activities of *Lavandula angustifolia* Mill. propagated *in vitro*

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ABSTRACT

The aim of the study was to determine the influence of jasmonic acid added to culture medium on the growth of plants and antioxidant properties of dry plant material, as well as on the antimicrobial properties of essential oils produced by the narrow-leaved lavender. For plant propagation, MS media supplemented with JA at concentrations of 0.2-1.5 mg dm^{-3} were used. The use of the lower JA concentrations did not influenced the growth parameters measured, whereas at the higher concentrations (1 and 1.5 mg dm^{-3}) JA caused growth inhibition and a decrease in plant weight. With increasing JA concentration, the number of secretory trichomes decreased. Addition of 0.5 mg dm^{-3} JA caused an increase in secretory trichome diameter on both the adaxial and abaxial surface of leaves (83.3 and 73.2 µm, respectively). The antioxidant activity of the lavender plants propagated on media with the addition of JA (regardless of the concentration used) was higher than that of the control plants. The plants from JA-supplemented media were used to isolate essential oils, the antimicrobial activity of which was tested using the disc diffusion method at the concentrations of 10 and 50%. All the oils tested exhibited activity towards *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Candida albicans*. The essential oils isolated from the plants propagated on the medium with 1 mg dm^{-3} JA were characterized by the highest antimicrobial activity against the majority of the tested microorganisms.

Keywords: ABTS, DPPH, FRAP, growth regulator, JA, Lamiaceae, trichomes/oil glands

Abbreviations:

ABTS – determination of free radical-scavenging ability by the use of a stable 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid radical cation, DPPH – determination of 2,2-diphenyl-1-picrylhydrazyl radical scavenging capacity, FRAP – determination of ferric reducing antioxidant power, GAE – gallic acid, JA – jasmonic acid, LSD – least significant difference, MS – Murashige and Skoog medium, TAA – total antioxidant activity, TE – trolox equivalent, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid

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INTRODUCTION

The narrow-leaved lavender is a herbaceous plant native to the Mediterranean (Basch et al., 2004), occurring also in India and on the Canary Islands (Upson and Andrews, 2004). It is used in medicine and pharmacy (Lis-Balchin and Hart, 1999), aromatherapy (Moss et al., 2003; Setzer, 2009), cosmetology (Erland and Mahmoud, 2016) and perfumery (Lahlou, 2004). The wide possibilities of making use of the plant are linked to its production of essential oils, which constitute a mixture of chemical compounds, primarily mono- and sesquiterpenes (Kennedy and Wightman, 2011; Sharopov et al., 2015). They exhibit strong antimicrobial (Moon et al., 2006), antifungal (D'Auria et al., 2005) and antioxidant properties (Hamad et al., 2013).

The composition of essential oils may vary due to environmental factors, including plant origin, insolation, plant age, growth conditions and even the method of collecting plants (Verma et al., 2010; Wesołowska et al., 2015). It has been determined that the composition and amount of oils isolated from tissues of lavender plants propagated *in vitro* differ from those of the oils isolated from plants growing in field conditions (Gonçalves and Romano, 2013).

Elicitors, which are stressors, are also the factors used in plant tissue cultures that significantly alter both the composition as well as the number of secondary metabolites produced, including essential oils. The most commonly used elicitors include jasmonates, in particular: jasmonic acid (JA) and jasmonic acid methyl ester, also known as methyl jasmonate (Me-JA) (Zhao et al., 2005). Under natural conditions, jasmonates regulate the growth and development of plants, and they also play an important role in plant responses to biotic and abiotic stresses, including the initiation of the mechanisms of induced systemic resistance (Ryu et al., 2004; Chung et al., 2008). The production of JA in plant cells is a strictly regulated process, yet its concentration is often very low. It is accumulated in damaged plant tissues or cells and acts as a signal activating the expression of various genes (Creelman and Mullet, 1997; Kant et al., 2004). Under natural conditions, JA is synthesized from α-linolenic acid, which is a fatty acid accumulated primarily in the leaves. It occurs mostly in an esterified form as glycerolipid (Browse and Somerville, 1991). Free fatty acids are not found at high concentrations in healthy, undisturbed plant cells and tissues. The release of α-linolenic acid from plant cell membranes is considered an important step in the control of JA synthesis. The increase in free α-linolenic acid has been observed in cultivated cells of different plant species, in damaged plant tissues and after treating them with fungal elicitors (Ryu and Wang, 1998).

Addition of different concentrations of JA to media in plant tissue cultures may help to activate the transcription of defence genes, which are involved in the biosynthesis of secondary metabolites (Pirbalouti et al., 2014). JA also plays a significant role in the intracellular signal cascade that starts with the interaction of the elicitor molecule with the surface of a plant cell. JA induces the synthesis of specific proteins in polyribosomes and thus contributes to the accumulation of secondary metabolites (Yu et al., 2000). The addition of jasmonate to the media used in plant tissue cultures stimulates and increases the production of oleanolic acid in pot marigold (Wiktorowska et al., 2010), ginsenoside in ginseng (*Panax ginseng*) (Yu et al., 2000), and tropane in *Datura stramonium* L. (Amdoun et al., 2009).

Apart from regulating the defensive response and increasing the production of secondary metabolites, JA further influences the formation of secretory trichomes covering flower petals, both leaf sides, as well as the stem (Lane et al., 2010). Studies on tomato and *Arabidopsis thaliana* have shown a positive correlation between the level of endogenous JA produced in response to fungal infection and the number of trichomes formed on leaf blades (Traw et al., 2003; Kapoor, 2008).

In plant tissue cultures, the addition of JA to media also activates the transcription of the genes which are involved in plant defensive mechanisms, contributing to the increase in secondary metabolite biosynthesis (Aoyagi et al., 2001; Kim et al., 2004; Thanh et al., 2005). At the same time, the addition of jasmonates may have an inhibitory or even lethal effect on plant development. JA and Me-JA added to media at the propagation and rooting stages, depending on the concentration, have an inhibitory effect on the growth of shoots and roots, thus their concentration in the medium must be accurately determined (Martin-Closas et al., 2000; Maciejewska and Kopcewicz, 2002; Luo et al., 2009).

Many species of lavender have already been propagated by tissue culture, including *L. dentata* (Echeverrigaray et al., 2005), *L. vera* (Andrade et al., 1999), *L. pedunculata* (Zuzarte et al., 2010), *L. latifolia* (Al-Bakhit et al., 2007) and *L. angustifolia* (Al-Bakhit et al., 2007; Falk et al., 2009). However,

the influence of JA on the development of *Lavandula* sp. in *in vitro* cultures has not yet been determined.

Previous studies indicate that JA changes the qualitative composition of essential oils. According to the study by Andrys et al. (2017), the essential oils isolated from plants grown *in vitro* on media with the addition of JA differ in their chemical composition from the plants propagated on media without elicitors. The appearance of new compounds in the chemical composition of oils, i.e. *p*-cymene, cis β-terpineol and linalool, may influence the antioxidant and antimicrobial properties of plant tissues and essential oils isolated from them, hence the possibility of their use in industry, e.g. as an alternative to the harmful, synthetic preservatives in cosmetic products.

The aim of the study was to evaluate the influence of jasmonic acid (JA) added to culture medium on the growth and development of *L. angustifolia* plants, including secretory trichomes, and on the antimicrobial activity of essential oils and antioxidant activity of dry plant material obtained from the tissue cultures.

MATERIAL AND METHODS

Tissue cultures

The source material for the study consisted of plants of *Lavandula angustifolia* 'Munstead' grown *in vitro* on MS medium (Murashige and Skoog, 1962) without growth regulators. Explants for the establishment of the experiment were single-node shoot fragments, with leaf blade removed, which were placed onto MS medium supplemented with vitamins: nicotinic acid 0.5 mg dm^{-3}, pyridoxine-HCl 0.5 mg dm^{-3}, thiamine-HCl 0.1 mg dm^{-3}, glycine 2 mg dm^{-3}, with the addition of 0.2, 0.5, 1 or 1.5 mg dm^{-3} jasmonic acid (JA, Sigma-Aldrich). The pH of the media was established at 5.7 using 0.1 M concentrations of HCl and NaOH. The medium was supplemented with 30 g dm^{-3} sucrose, 100 mg dm^{-3} inositol and 7 g dm^{-3} agar, and sterilized in an autoclave at a temperature of 121°C and 1 atmosphere pressure for 20 minutes. JA was added to the warm medium directly after autoclaving. JA was sterilized by filtration using 0.22 µm filter. The control consisted of the above medium without the addition of growth regulators. To each 200 ml jar containing 25 ml of medium 8 explants were introduced in 20 replications. The cultures were placed in a phytotron for 21 days at a temperature of 23 ± 1°C and relative air humidity of 70-80%. The cultures were illuminated with fluorescent light at an intensity of 40 PAR (E m^{-2} s^{-1}) for 16 h per day. After 21 days of culturing, the height of plants, number of shoots and plant weight were evaluated.

Micromorphological analysis

The leaves of the narrow-leaved lavender 'Munstead' propagated on media with different JA content were subjected to a micromorphological analysis using a scanning electron microscope (SEM). The material was dried in a Quorum Technologies Critical Point Dryer, then sprayed with a gold layer in a Quorum Technologies Sputter Coater. Observations were conducted using a Carl Zeiss EVO LS 10 SEM with an accelerating voltage 1 or 15 kV.

Determination of antimicrobial activity of essential oils

The samples of plant material micropropagated in tissue cultures on MS medium supplemented with JA (0.2, 0.5, 1.0 or 1.5 mg dm^{-3}) and on the control medium in the amount of 20 g each were subjected to hydrodistillation in the Deryng apparatus for three hours, according to the European Pharmacopoeia (2014). The material was treated with distilled water, heated to boiling temperature and left to cool down. Next, the distillate isolated in a condenser was dried with the use of anhydrous sodium sulphate (VI). The thus obtained essential oil was used for the study. The disc diffusion method was used to evaluate the antimicrobial activity. The activity was determined against the bacteria: *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027), *Staphylococcus aureus* (ATCC 6538), *Staphylococcus epidermidis* (ATCC 12228), and the fungi *Candida albicans* (ATCC 10231). Nystatin (*Candida albicans*), piperacillin (*Escherichia coli* and *Pseudomonas aeruginosa*), cefoxitin (*Staphylococcus aureus* and *Staphylococcus epidermidis*) and 96% ethyl alcohol constituted the positive control. DMSO constituted the negative control. Mueller Hinton media were inoculated with 100 µl of bacteria (106 CFU colony-forming unit ml^{-1}). Filter paper discs (diameter 6 mm) were soaked with 10 µl of essential oil at a concentration of 10% and 50% (soaking time 1-2 min) and immediately transferred symmetrically onto the inoculated plate. The plates were incubated at 37°C for 24 h (bacteria) or 48 h (*C. albicans*), then readout was performed. Zones of growth inhibition were measured (as diameter given in millimeters). The analyses were performed in three replications.

Preparation of plant extracts

The material for the study consisted of dried narrow-leaved lavender plants 'Munstead' propagated *in vitro* on media with different JA contents (0.2, 0.5, 1.0 or 1.5 mg dm^{-3}) and on the control medium. Dried lavender herb samples were crushed using a laboratory mill. The ground dry plant material (1 g) was treated with 80% aqueous methanol (MeOH) to 100 ml volume. The mixtures were placed in an ultrasonic bath at room temperature and sonicated continuously for two periods: 10 and 15 minutes, and then left for 24 hours at room temperature. The obtained extracts were filtered through Whatman No. 1 filter paper. The filtrates were diluted 10 times with 80% MeOH and then centrifuged at 252 RCF for 10 minutes. All the extractions were carried out in three replications. The extracts were kept at 4°C and used for analyses within 24 hours.

Determination of total polyphenol content

Total polyphenol content was determined spectrophotometrically using the Folin-Ciocalteu colorimetric method as described by Wojdyło et al. (2007) with some modifications. Plant extract (100 µl) was mixed with 0.2 ml of the Folin-Ciocalteu reagent, 2 ml of distilled water and 1 ml of 20% sodium carbonate. The samples were allowed to stand for 1 hour at room temperature in darkness. Then absorbance was measured at 760 nm. Gallic acid (GAE) was used to calculate the standard curve, and the results were expressed as milligrams GAE per g of dry weight (DW).

Determination of DPPH radical scavenging capacity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging capacity was evaluated according to the procedure of Kumaran and Karunakaran (2007), and Wojdyło et al. (2007). DPPH (0.3 mM) was dissolved in pure ethanol (99.8%). Plant extract (1 ml) or 1 ml of 80% MeOH (control) was added to 3 ml of pure ethanol (EtOH) and 1 ml of the DPPH solution. The samples were incubated at room temperature for 10 minutes in the dark. The reduction of the DPPH radical was determined spectrophotometrically by measuring absorption at 517 nm. The percentage of DPPH scavenging activity was calculated using the equation: $[A_0 - A_1 /A_0] \times 100$, where A_0 is the absorbance of the control, and A_1 is the absorbance of the extract. Moreover, trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) was used to calibrate the standard curve and the results were expressed as mg of trolox equivalent (TE) antioxidant capacity per g of dry weight sample (mg TE g^{-1} DW).

Determination of ferric reducing antioxidant power (FRAP)

The total antioxidant potential of the samples was determined using the ferric reducing ability of plasma (FRAP) assay by Wojdyło et al. (2007) as a measure of antioxidant power. FRAP reagent was prepared by mixing acetate buffer (300 mM, pH 3.6), a solution of 10 mM TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) in 40 mM HCl, and 20 mM $FeCl_3 6H_2O$ (iron(III) chloride hexahydrate) at 10:1:1 (v/v/v), and warmed at 37°C before use. For the spectrophotometric assay, 2.7 ml of the reagent and 0.3 ml of the sample solution were mixed. The absorbance was taken at 593 nm after 4 minutes. The standard curve was prepared using different concentrations of trolox. The results were expressed in mg TE per g DW.

Determination of free radical-scavenging ability by the use of a stable ABTS radical cation

The free radical-scavenging activity was determined by ABTS radical cation decolorization procedures described by Re et al. (1999), Chew et al. (2011) and Wojdyło et al. (2007) with some modifications. ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt was dissolved in distilled water to a 7 mM concentration. ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting ABTS stock solution with 2.45 mM potassium peroxodisulfate and kept in the dark at room temperature for 16 h before use. The $ABTS^{\bullet+}$ solution was diluted with PBS (phosphate buffered saline, pH 7.4) until its absorbance was equilibrated to 0.7 (± 0.02) at 734 nm before usage. After the addition of 3.0 ml of diluted $ABTS^{\bullet+}$ solution ($A_{734} = 0.7 \pm 0.02$) to 300 µl of methanolic plant extracts, the absorbance reading was performed exactly 6 min. after the initial mixing. Trolox was used for calibrating the standard curve and the results were expressed as mg TE per g of sample dry weight. Moreover, the percentage of ABTS radical scavenging capacity (Total Antioxidant Activity, % TAA) was calculated using the formula: $[1 - (A_s /A_c)] \times 100$ (A_s – absorbance of plant extract; A_c – absorbance of control).

Statistical analysis

The experiment with plant tissue cultures was performed in 20 replications with 8 explants each. Data for antimicrobial activity, polyphenol content,

DPPH, FRAP and ABTS assays are presented as the mean ± SD for three replicates. The results of all the experiments were statistically analyzed using one-way analysis of variance. To evaluate the significance of the differences between treatments, Tukey's test was performed at $p = 0.05$.

RESULTS AND DISCUSSION

In vitro cultures

Our results showed that JA at the lower concentrations (0.2 and 0.5 mg dm^{-3}) did not influence the height or weight of the plants compared to the control (Tab. 1). At the higher concentrations (1 and 1.5 mg dm^{-3}), JA significantly decreased lavender shoot height (2.2 and 1.8 cm, respectively), in comparison with the control (3.3 cm). At the higher concentrations, a decrease in the weight of the propagated plants was also observed (0.2 and 0.1 g, respectively) in comparison with the control (0.2 g), and a decrease in the number of shoots (1.5 and 1.4, respectively). A lower number of shoots in comparison with the control medium (1.9) was also recorded for the medium with the addition of 0.5 mg dm^{-3} JA (1.6). So far, the literature concerning the influence of JA on lavender plants has been lacking in data. Jasmonic acid and its methyl ester have been used for the propagation of other plant species, e.g. *Solanum tuberosum* (Martín-Closas et al. 2000), *Gloriosa rothschildiana* (Weryszko-Chmielewska and Kozak 2002), rice 'Nipponbare' (Cho et al. 2007) and *Cymbidium* (Shimasaki et al. 2009). Both jasmonates, depending on their concentration in the medium, inhibited the formation and growth of shoots and roots (Ružic et al. 2015).

Micromorphological analysis

The addition of 0.5 mg dm^{-3} JA resulted in an increase in the secretory trichome diameter on both the adaxial and abaxial surface of the leaves (83.3 and 73.2 µm, respectively) (Fig. 1). JA the number of secretory trichomes on both sides of the leaf. The highest number of trichomes per 1 mm^2 was observed in the plants propagated on the control medium without the addition of JA, on both the adaxial (6.2) and abaxial (7.0) surface of the leaf. With the increase in jasmonic acid concentration, the number of trichomes decreased (Tab. 2). Naidu and Shah (1981) were among the first to conduct

Table 1. Morphological properties of *L. angustifolia* propagated on media with different JA concentrations. The values represent the mean of three replicates ± SD

JA content (mg dm^{-3})	Plant height (cm)	Plant weight (g)	Number of shoots
Control	3.4 ± 1.17	0.2 ± 0.18	1.9 ± 1.27
0.2	3.2 ± 0.65	0.2 ± 0.13	1.9 ± 0.86
0.5	2.4 ± 0.53	0.2 ± 0.13	1.6 ± 0.71
1.0	2.2 ± 0.37	0.2 ± 0.08	1.5 ± 0.63
1.5	1.8 ± 0.35	0.1 ±0 .13	1.4 ± 0.59
$LSD_{0.05}$	1.02	0.04	0.27

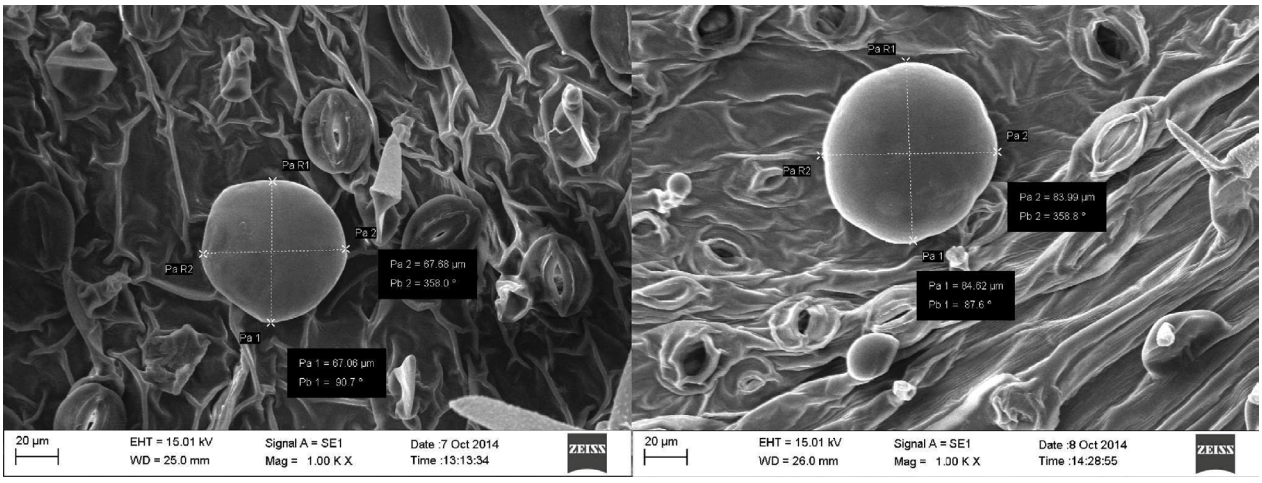

Figure 1. Trichome on the abaxial side of lavender leaf on control medium (on the left) and propagated on medium with 0.5 mg dm^{-3} JA (on the right)

Table 2. Size and number of secretory trichomes of *L. angustifolia* propagated on media with different JA concentrations. The values represent the mean of three replicates ± SD

JA content (mg dm^{-3})	Trichome size (µm)		Number of trichomes (per mm^2)	
	Adaxial surface of leaf	Abaxial surface of leaf	Adaxial surface of leaf	Abaxial surface of leaf
Control	66.7 ± 8.96	68.1 ± 6.08	6.2 ± 1.26	7.0 ± 1.41
0.2	69.3 ± 6.50	68.3 ± 5.43	6.0 ± 1.41	6.5 ± 0.71
0.5	83.3 ± 7.94	73.2 ± 6.14	5.0 ± 0.00	5.5 ± 0.71
1.0	64.5 ± 5.98	59.9 ± 7.25	3.3 ± 0.58	3.7 ± 0.58
1.5	68.8 ± 6.20	67.2 ± 5.79	3.0 ± 0.00	3.3 ± 0.58
$LSD_{0.05}$	3.10	1.80	1.12	0.98

observations of trichomes in *L. angustifolia*. In their classification, which included other genera of the Lamiaceae, they divided trichomes into five groups; in *L. angustifolia*, however, four groups were observed. Type I are the trichomes in which the foot consists of one cell or very rarely of 2 cells, polygonal, trapezoidal or square. The body is uniseriate, straight or slightly curved, consists of 1-3 elongated stem cells, 1-2 neck cells, and a spherical elongated or ovoid head. In type I trichomes, the cells of the stem are rectangular, trapezoidal or square, and typically longer than wider, with thin contents or without contents. The head is unicellular or bicellular, and the cells of the neck are subterminal, rectangular or trapezoid. On the other hand, type II trichomes have a simpler structure, consisting of a unicellular foot without contents. The body of a trichome consists of a short stem made of a rectangular, trapezoidal or polygonal cell and a spherical head (numerous cells). The cells of the stem and the head are smooth,

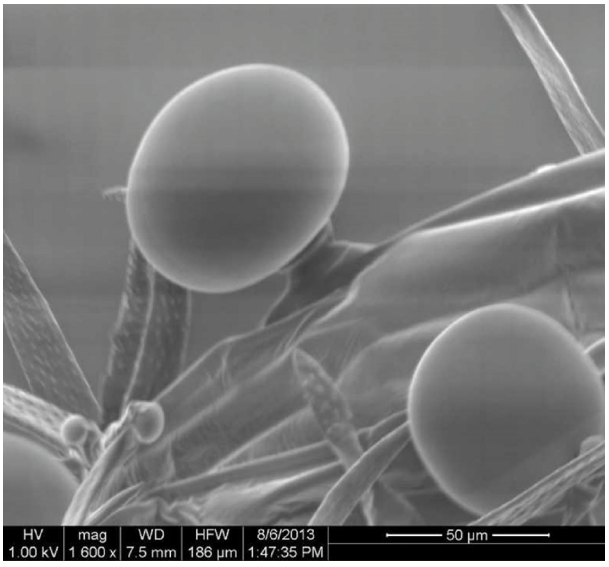

Figure 2. Fully developed peltate trichome with a swollen head

with thin walls, cuticularized, and each contains a thick cytoplasm and nucleus. Type III trichomes are cone-shaped, with an acute or subacute tip, which is straight or curved. In contrast, type IV trichomes, in the form of non-pointed, cylindrical and branched hairs on both leaf sides, were observed by Naidu and Shah (1981) only in the genus *Lavandula*. Type IV trichome body is cylindrical, rounded, or open at the ends. Following the classification by Naidu and Shah (1981), type I trichomes were identified in the present study. They were fully developed peltate trichomes with a swollen head (Fig. 2).

Antimicrobial activity

The antimicrobial activity of *Lavandula* essential oils has been commonly tested. It has been determined that essential oils inhibit the propagation of *Proteus vulgaris* (Prabuseenivasan et al., 2006), *Escherichia coli* (Abroomand Azar et al., 2011; Criste et al., 2014), *Bacillus subtilis* (Prabuseenivasan et al., 2006; Abroomand Azar et al., 2011), *Bacillus cereus* (De Martino et al., 2009), *Staphylococcus aureus* (De Martino et al., 2009; Criste et al., 2014), *Shigella flexneri* (Rota et al., 2004; Jianu et al., 2013), *Enterococcus faecalis* (Stanojević et al., 2011; Adaszyńska-Skwirzyńska et al., 2014), *Pseudomonas aeruginosa* (Niculae et al., 2009; Criste et al., 2014), *Listeria monocytogenes* (Rota et al., 2004; Valizadeh et al., 2015), and of the fungi: *Candida albicans* (Rapper et al., 2013; Thosar et al., 2013), *Aspergilloma* and *Aspergillosis* (Uniyal et al., 2012).

A commonly used method of assessing the antimicrobial activity of essential oils is the disc diffusion method (Benkeblia, 2004; Kordali et al., 2005). By using this method, Şerban et al. (2011) determined that the essential oil of *L. hybrida* had the weakest effect (8 mm) towards *S. aureus*. A slightly larger inhibition zone was observed for *E. coli* (10 mm) and for the fungi *C. albicans* (13 mm). The antibacterial activity of essential oils

of *L. angustifolia* was also determined by Jianu et al. (2013). The essential oils showed antimicrobial activity against *S. flexneri, S. aureus, E. coli* and *S. typhimurium*, but showed no activity against *S. pyogenes*.

In the present study, all the tested essential oils exhibited antimicrobial activity towards *P. aeruginosa, S. aureus, S. epidermidis, E. coli* and *C. albicans*, commonly found on human skin (Tab. 3). At 10% concentration, the oils isolated from the plants propagated on the media supplemented with 0.2-1 mg dm^{-3} JA exhibited stronger antimicrobial activity towards the tested bacteria in comparison with the control, yet at the concentration of 1.5 mg dm^{-3} JA their activity decreased and was lower than or at the level of the control. *E. coli* was an exception, as in that case only the essential oil isolated from the plants propagated on the medium with the addition of 1 mg dm^{-3} JA was characterized by higher activity than in the control. In the case of *C. albicans*, the antimicrobial activity was lower than in the control for the essential oil isolated from the plants growing on the media with the addition of 0.2 mg dm^{-3} JA, but it increased at a higher concentration (1 mg dm^{-3} JA).

At 50% concentration, the essential oils isolated from the plants propagated on the media with the addition of 0.2-1 mg dm^{-3} JA exhibited stronger antimicrobial activity towards *S. aureus* (14.0-14.3 mm) and *S. epidermidis* (14.8-18.5 mm) than the essential oils isolated from the plants grown on the control medium. However, at the concentration of 1.5 mg dm^{-3} JA their activity decreased and was 8.0 mm for *S. aureus* and 10.7 mm for *S. epidermidis*. The largest inhibition zone was observed in *S. epidermidis* (18.5 mm) in the case of the oil isolated from the plants propagated on the medium with 1 mg dm^{-3} JA. At the concentration of 50%, all the essential oils isolated from the plants grown on the media with the addition of JA, regardless of its concentration, exhibited stronger activity towards *C. albicans* (9.7-12.3 mm) in comparison with the control. In our previous research (Andrys et al. 2017), we had observed that the essential oils isolated from plants grown *in vitro* on media with the addition of JA had a different chemical composition from plants propagated on media without an elicitor. The addition of jasmonic acid to the medium resulted in an increase in the amount of compounds such as borneol – its concentration, obtained by using a medium with JA, amounted to 12.4-17.5%, with 6.7% in the control (medium without JA). The occurrence of borneol could have an impact on the antimicrobial activity of essential oils.

Polyphenol content

The addition of JA to culture medium increased the polyphenol content in plant material compared to the control. The amount of phenolic compounds (Tab. 4) was generally the highest in the analyzed extracts from lavender plants propagated on the medium with the addition of 1.5 mg dm^{-3} JA (43.9 mg GAE g^{-1} DW), whereas a lower polyphenol

Table 3. Antimicrobial activity of essential oil (EO) isolated from *L. angustifolia* propagated on media with different JA concentrations. The values represent the mean of three replicates ± SD

JA content (mg dm^{-3})		Zone of bacterial/fungal growth inhibition (mm)				
		S. aureus	*S. epidermidis*	*E. coli*	*P. aeruginosa*	*C. albicans*
10% EO	Control	9.1 ± 0.14	10.2 ± 0.29	7.7 ± 0.21	6.7 ± 0.30	7.4 ± 0.36
	0.2 JA	9.9 ± 0.04	11.1 ± 0.12	7.4 ± 0.11	8.2 ± 0.26	6.8 ± 0.17
	0.5 JA	10.5 ± 0.11	10.9 ± 0.22	7.8 ± 0.26	7.1 ± 0.40	7.5 ± 0.08
	1.0 JA	9.9 ± 0.04	11.0 ± 0.40	8.2 ± 0.27	7.3 ± 0.17	8.1 ± 0.30
	1.5 JA	8.0 ± 0.09	7.9 ± 0.26	6.9 ± 0.32	6.6 ± 0.19	7.5 ± 0.10
50% EO	Control	10.2 ± 0.02	13.3 ± 0.07	6.9 ± 0.13	8.7 ± 0.07	10.2 ± 0.02
	0.2 JA	14.3 ± 0.01	18.1 ± 0.18	7.6 ± 0.21	6.9 ± 0.20	12.3 ± 0.31
	0.5 JA	14.0 ± 0.30	14.8 ± 0.20	7.4 ± 0.03	6.5 ± 0.30	9.7 ± 0.07
	1.0 JA	14.2 ± 0.17	18.5 ± 0.23	8.3 ± 0.07	7.4 ± 0.23	11.8 ± 0.32
	1.5 JA	8.0 ± 0.40	10.7 ± 0.33	7.8 ± 0.21	7.1 ± 0.12	10.9 ± 0.14
Control	Antibiotic	23.7 ± 0.10	19.3 ± 0.26	21.2 ± 0.26	24.5 ± 0.17	21.1 ± 0.44
	DMSO	6.0 ± 0.00	6.0 ± 0.00	6.0 ± 0.00	6.0 ± 0.00	6.0 ± 0.00
	96% EtOH	11.4 ± 0.35	9.9 ± 0.20	10.9 ± 0.21	9.7 ± 0.20	11.1 ± 0.17
$LSD_{0.05}$		0.14	0.18	0.17	0.18	0.14

Table 4. Antioxidant activity of *L. angustifolia* propagated on media with different JA concentrations. The values represent the mean of three replicates ± SD

JA content (mg dm^{-3})	Polyphenols (mg GAE g^{-1} DW)	DPPH		FRAP	ABTS	
		mg TE g^{-1} DW	DPPH inhibition (%)	mg TE g^{-1} DW	mg TE g^{-1} DW	% TAA
Control	33.3 ± 1.38	35.1 ± 0.81	83.3 ± 1.65	42.8 ± 1.90	78.5 ± 1.35	95.0 ± 1.89
0.2	40.6 ± 1.12	37.8 ± 0.14	88.6 ± 0.21	71.5 ± 4.97	82.6 ± 0.08	99.7 ± 0.09
0.5	39.4 ± 1.00	37.4 ± 0.06	88.4 ± 0.46	61.7 ± 3.56	82.7 ± 0.08	99.8 ± 0.09
1.0	36.9 ± 0.89	37.3 ± 0.12	88.4 ± 0.14	63.1 ± 4.33	81.7 ± 1.65	98.7 ± 1.92
1.5	43.9 ± 1.64	37.5 ± 0.03	89.0 ± 0.21	80.1 ± 5.38	82.7 ± 0.08	99.8 ± 0.09
$LSD_{0.05}$	1.52	0.82	1.22	4.19	1.69	1.96

content (although still higher in comparison with the control) was in the dried material from the plants propagated on MS medium supplemented with 1 mg dm^{-3} JA (36.9 mg GAE g^{-1} DW). Duda et al. (2015) had examined the amount of phenolic compounds in extracts from 3-year-old *L. angustifolia* plants and it was considerably lower at 12.4-18.2 mg GAE g^{-1} DW.

Antioxidant activity (DPPH, FRAP and ABTS)

Parejo et al. (2002) assessed the antioxidant effect of lavender extracts using the NBT/Hypoxanthine superoxide and OH/Luminol chemiluminescence methods. However, the most commonly used methods are DPPH, FRAP and ABTS (Blažeković et al., 2010; Hamad et al., 2013; Proestos et al., 2013), and those methods were used in the present study. All of them showed that the antioxidant activity of the plants propagated on the media with the addition of JA (regardless of the concentration used) was increased in comparison with the control (Tab. 4). The antioxidant activity of lavender plants propagated on the control medium tested using DPPH was 35.1 mg TE g^{-1} DW, whereas the activity of the plants propagated on the media with the addition of JA was higher (regardless of the concentration used), ranging from 37.3 mg TE g^{-1} DW (1 mg dm^{-3} JA) to 37.8 mg TE g^{-1} DW (0.2 mg dm^{-3} JA). Examinations of the inhibition percentage of DPPH free radicals ranged from 88.4% (0.5-1 mg dm^{-3} JA) to 89.0% (1.5 mg dm^{-3} JA), compared to 83.3% for the control. Using the same method, Blažeković et al. (2010) had tested the antioxidant activity of dried material from *L. angustifolia* growing in field conditions – the DPPH values obtained by them were lower: 33.95 mg TE g^{-1}DW for the stems, 11.4 mg TE g^{-1} DW for the flowers, and 10.6 mg TE g^{-1} DW for the leaves.

The antioxidant activity of the plants propagated on the media with the addition of JA tested using the FRAP method ranged from 61.7 mg TE g^{-1} DW (0.5 mg dm^{-3} JA) to 80.1 mg TE g^{-1} DW (1.5 mg dm^{-3} JA), and was higher than the activity of plant extracts obtained from the plants grown on the control medium (42.8 mg TE g^{-1} DW). High antioxidant activity of tissues of plants propagated on the media with the addition of JA (regardless of the concentration used) were also found using the ABTS method. It ranged from 81.7 mg TE g^{-1} DW (1 mg dm^{-3} JA) to 82.7 mg TE g^{-1} DW (0.5 and 1.5 mg dm^{-3} JA), and was higher than in the control (78.5 mg TE g^{-1} DW); this corresponds to the TAA, which was 94.98% for the control and ranged from 98.7% (1 mg dm^{-3} JA) to 99.8% (0.5 and 1.5 mg dm^{-3} JA) (Tab. 4). Previous research had demonstrated that the addition of JA to the medium affected the chemical composition of essential oils and was responsible for the emergence of new compounds such as *p*-cymene (0.35-0.56%), cis β-terpineol (0.21-0.26%), linalool (0.36-0.60%), compared to the control (Andrys et al. 2017). Those compounds may be partly responsible for the higher antioxidant activity of the extract isolated from plants grown on media with the addition of JA compared to the extract isolated from plants grown on the control medium.

CONCLUSIONS

1. In the present study, elicitation of tissue cultures of the narrow-leaved lavender with 0.2-0.5 mg dm^{-3} JA did not have a negative impact on plant growth.
2. The increase in JA concentration to 1-1.5 mg dm^{-3} had a negative influence on the development of lavender plants, further leading to a decrease in the number of secretory trichomes.
3. An addition of JA to culture medium may contribute to the production of plant tissues with higher antioxidant activity.
4. Essential oils isolated from the lavender plants propagated on the media supplemented with 0.2-

1 mg dm^{-3} JA were characterized by elevated antimicrobial activity towards the majority of the tested microorganisms.

5. Essential oils with such properties may be used as an alternative to the harmful, synthetic preservatives commonly used in industry, in particular by the cosmetics and pharmaceutical industries.

AUTHOR CONTRIBUTIONS

D.A. and D.K. – developed the concept and designed the experiment; D.A., M.G. and B.B. – collected data and performed analyses; D.A. and D.K. – analysed the data and wrote the paper.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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