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Glandular trichomes, essential oil composition, anti-*Aspergillus* and antioxidative activities of *Lamium purpureum* L. ethanolic extracts

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†We are sad to report that the author Mihailo S. Ristić is deceased.

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Abstract: The aim of this study was to investigate the structure of glandular trichomes and essential oil composition of *Lamium purpureum* L. (Lamiaceae) and to determine potential biological activities of its different extracts. The micromorphology and distribution of trichomes on vegetative plant parts were investigated using scanning electron microscopy (SEM). Two types of trichomes were identified: non-glandular (simple, uniseriate, multicellular) and glandular (both peltate and capitate). The essential oil of *L. purpureum* was isolated by hydrodistillation and analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). The main compounds were *trans*-anethole (59.61%), fenchone (6.54%), *α*-pinene (6.14%) and *β*-pinene (4.98%). The total phenol and flavonoid contents of 10%, 30% and 50% ethanol extracts were determined spectrophotometrically. The free radical scavenging and reducing power activity of extracts were analyzed using the DPPH and ABTS radical scavenging methods, and the ferric reducing antioxidant power (FRAP) and total reducing power (TRP) assays. The 50% ethanolic extract exhibited the strongest antioxidative activity while the lowest was determined for the 10% ethanolic extract. Antifungal activity of *L. purpureum* hydroalcoholic extracts was evaluated against eight *Aspergillus* species by the microdilution method. Light microscopy (LM) demonstrated structural abnormalities of the tested *Aspergilli*. While the plant extracts did not exhibit fungicidal activity, morphophysiological changes (sporulation loss and depigmentation of conidial heads) were observed in all tested isolates.

Keywords: *Lamium purpureum*; trichomes; essential oil; ethanolic extract; phenol, flavonoid content; biological activity

INTRODUCTION

The genus *Lamium* L. (Lamiaceae) includes both annual and perennial herbaceous plants that are widely distributed throughout temperate Eurasia. *Lamium purpureum* L. is an annual species that grows on cultivated ground as a common weed [1]. It is a well-known although somewhat less popular herb that contains a small amount of essential oils (less than 0.1%) [2]. Different chemical compounds from different species of the genus *Lamium* are used for diuretic, purgative purposes and to treat epilepsy, abscesses and wounds [3]. *L. purpureum* aerial parts possess antirheumatic, hemostatic, astringent and emollient properties [4]. *Lamium* species ("dead nettles") have been used in folk medicine worldwide, as a remedy in the treatment of several disorders including scrofula, hypertension, paralysis, trauma, fracture, uterine hemorrhage and menorrhagia [5]. These species exhibit multiple biological actions, including antimicrobial [6], antifungal [7], antioxidant [8-12], antiinflammatory and nociceptive [13] as well as anticancer [14,15] activities. Literature data point to the presence of different classes of chemical compounds: including ecdysone and phenylpropanoid glucosides [16], iridoid glucosides [17,18] and oligosaccharides [19]. Interest in the medicinal properties of these plants has led to intensive phytochemical and pharmacological investigations [5,20,21].

Different trichome types and the essential oil produced by glandular trichomes are characteristics of

the Lamiaceae family [22]. Glandular trichomes are considered as the site of biosynthesis or accumulation of essential oils [23]. Plant species from the genus *Lamium* belonging to the subfamily Lamioideae contain a small amount of essential oils but possess the same type of trichomes as species of the subfamily Nepetoideae [2,24]. The glandular and non-glandular trichomes of Lamioideae representatives have not been sufficiently examined. Further investigation could clarify the ecological and taxonomical significance of the micro characteristics of trichomes. The essential oils produced by glandular trichomes act as a protectant of the plant aerial parts against herbivores, pathogens and UV radiation. The function of non-glandular trichomes depends on their morphology, the organ on which they are situated as well as on their direction and orientation. Non-glandular trichomes can serve as a mechanical barrier [25]. The great diversity of plant trichomes is very important because of their adaptive and taxonomic values. Investigation of trichome morphology, diversity and distribution was conducted on a small number of species of genus *Lamium* [26-28]. The essential oils obtained from *Lamium* species are scarcely studied. Some recent papers dealing with essential oils and chemical characteristics have been published [21,29-32].

The main goals of this work were to determine the micro morphology and distribution of the trichomes and to analyze the essential oil composition of *L. purpureum*. It should be noted that because *Lamium* species produce miniscule amounts of essential oils it is often arduous to isolate essential oil in sufficient yield to perform biological assays. Because of this we performed biological tests only on plant extracts. In this work we also describe the phenol contents, free radical, reducing power and anti-*Aspergillus* activity of different alcoholic extracts with the aim of improving the knowledge about the biological activity of this species.

MATERIALS AND METHODS

Plant material

The aerial parts of *L. purpureum* (1500 g) were collected in 2016 at the flowering stage in the Botanical Garden "Jevremovac" Belgrade. A voucher specimen was deposited in the Herbarium of the Institute of Botany and Botanical Garden "Jevremovac", University of Belgrade, Faculty of Biology, Serbia (BEOU), voucher No 16348.

Chemicals and apparatus

All chemicals and reagents were of analytical grade and were purchased from Sigma Chemical Co. (St. Louis, MQ, USA), Aldrich Chemical Co. (Steinheim, Germany), AnalaR Normapur, VWR, Geldenaaksebaan, Leuven Belgium, Fluka Chemie AG, Buchs, Switzerland and TCI Europe NV, Boerenveldsweg, Belgium. All spectrophotometric measurements were performed on a Perkin Elmer LAMBDA Bio spectrophotometer.

Scanning electron microscopy (SEM)

For trichome morphology analysis small segments of the stem (middle part just beneath the first nodule), leaves (upper right part in proximity to the central nerve), and the whole calyx of *L. purpureum* were coated with a thin layer of gold in a BALTEC-SCD 005 Sputtering Device. Observations were carried out on a JEOL JSM 6460 LV scanning electron microscope at 20 kV. The type of trichomes and their distribution were described and classified as described [33].

Isolation and chemical analysis of essential oil

The aerial plant parts were dried at room temperature in the shade. Two hundred g were shredded and subjected to hydrodistillation in a Clevenger-type apparatus according to the described procedure [34]. Since essential oil was obtained in traces (<0.1% V/w), it was dissolved in *n*-hexane (Merck, Germany), dried over anhydrous sodium sulfate and stored in dark glass vials at 4°C until gas chromatography analysis. Hydrodistillation was performed in 3 replicates. Gas Chromatography (GC) analysis of the oil was carried out on a GC HP-5890 apparatus, equipped with a split-splitless injector, attached to an HP-5 column (25 m x 0.32 mm, 0.52 μm film thickness) and fitted to a flame ionization detector (FID). Carrier gas flow rate (H_2) was 1 mL/min, the split ratio was 1:30, injector temperature was 250°C, detector temperature was 300°C, the column temperature was linearly programmed from 40-240°C (at a rate of 4°C). The same analytical conditions were employed for gas chromatography/mass spectrometry (GC/MS) analysis, where an HP

G 1800C Series II GCD system was used. The transfer line was heated at 260°C. Mass spectra were obtained in EI mode (70 eV), at a m/e range of 40-400. Column HP-5MS (30 m x 0.25 mm, 0.25 μm film thickness) was used. Identification of the individual oil components was performed by comparison of the retention times with standard substances and by matching mass spectral data with the MS library (Wiley 275.L) using a computer search and literature [35]. The retention indices (RI) were in relation to a homologous series of n-alkanes (C_6–C_{28}) on the HP-5 column under the same chromatographic conditions. For the purpose of quantitative analysis, the percentage area obtained by FID were used as a base. The relative proportion of the essential oil constituents were expressed as percentages obtained by peak area normalization, with all relative response factors being taken as one.

Preparation of plant extracts

The air-dried and powdered aerial parts of *L. purpureum* (10 g) were extracted with 10%, 30% and 50% ethanol (100 mL) using an ultrasonic bath. The mixture was exposed to ultrasound during the first and the last hour of extraction. Extraction lasted 24 h in the dark, after which samples were filtered through Whatman filter paper No 1. The solvent was removed by evaporation under reduced pressure by a rotary evaporator (BÜCHI Rotavapor R-114). The crude extracts were stored in the fridge at +4°C. Extracts of different concentrations were prepared for further testing.

Determination of total phenol and flavonoid content

The concentration of phenol compounds in the ethanolic extracts of *L. purpureum* was estimated by colorimetric assay according to Singleton and Rossi [36]. The total phenol content was expressed as milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g). All measurements were repeated three times and expressed as mean values±standard deviations (SD). Determination of the total flavonoid content was carried out as described [37]. The total flavonoid content in different extracts was calculated using a quercetin hydrate calibration curve and expressed as milligrams of quercetin equivalents per gram of dry extract (mg QE/g).

Evaluation of DPPH and ABTS radical scavenging activities

The free radical scavenging activity of different ethanolic extracts of *L. purpureum* was evaluated by the 2,2-diphenyl-1-picrylhydrazil (DPPH) radical scavenging method described by Blois [38]. The extract concentration providing 50% of free radical scavenging activity (EC_{50}) was calculated from the graph of radical scavenging activity (RSA) percentage against extracts concentration. The decrease of absorption at 517 nm is calculated as follows:

DPPH radical scavenging effect (%)=$(A_0\text{-}A_1)x100/A_0$,

with A_0 the absorbance of the negative control and A_1 the absorbance of the reaction mixture. Butylated hydroxyanisole (BHA), a commercial antioxidant, was used as a positive control.

The ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging method [39] was used. Absorbance of the mixture was measured spectrophotometrically at 734 nm. The radical scavenging activity for each extract was determined on the basis of the linear calibration curve of ascorbic acid and was expressed as milligrams of ascorbic acid per gram of dry extract (mg AA/g). Three replicates were performed for each extract concentration.

Evaluation of the total reducing power (TRP)

TRP in this experimental procedure was evaluated as suggested [40] and expressed as RP_{50} (μg/mL). The TRP assay measures the electron donating capacity of an antioxidant. The presence of reducers causes the conversion of the Fe^{3+}/ferricyanide complex to the Fe^{2+}/ferrous form, which serves as an indicator of its antioxidant capacity. The reducing capacity of different extracts was compared with ascorbic acid and expressed as milligrams of ascorbic acid equivalents per gram of dry extract (mg AA/g). An increase in the absorbance indicates a higher antioxidant potential.

Evaluation of the ferric reducing antioxidant power assay (FRAP)

The reducing power of different ethanolic extracts of *L. purpureum* was determined using the ferric reducing

ability of the FRAP assay [41]. This assay is based on the reducing power of a compound (antioxidant). A potential antioxidant will reduce the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}); the latter forms a blue complex (Fe^{2+}/TPTZ), which increases the absorption at 593 nm. A standard calibration curve was prepared using different concentrations of $FeSO_4 \times 7H_2O$. The results were expressed in μmol of Fe^{2+} ion equivalents per milligrams of dried extract (μmol Fe/mg).

Anti-*Aspergillus* activity

Fungal isolates

Eight *Aspergillus* species used in this study were isolated from different substrata *A. flavus* BEOFB317m and *A. nidulans* BEOFB332m (stone), *A. parasiticus* BEOFB3122m (brick), *A. ochraceus* BEOFB3280m and *A. niger* BEOFB349m (textile), *A. terreus* BEOFB353m and *A. fumigatus* BEOFB322m (paper), and *A. chevalieri* BEOFB3290m (indoor air). Isolates were identified to the species level based on colony macromorphology and micromorphology of reproductive structures using identification keys [42,43]. Isolates were deposited in the culture collection of the University of Belgrade, Faculty of Biology (BEOFB), and maintained on Malt extract agar (MEA).

Micro-well dilution assay

A modified microdilution technique was used to determine the antifungal activity of *L. purpureum* ethanolic extracts [44,45]. Conidia were washed from the surface of the agar slants with sterile 0.85% saline containing 0.1% Tween 20. The conidia suspension was adjusted with sterile 0.85% saline to a concentration of about 1.0×10^7 in a final volume of 100 μL per well. The inocula were stored at -20°C until further use. Dilutions of the inocula were cultured on MEA to verify the absence of contamination and to check the validity of the inocula. Determination of the minimal inhibitory concentrations (MICs) was performed by a serial dilution technique using 96-well microtiter plates. Different volumes of the investigated ethanol extracts were dissolved in malt extract broth (MEB) with fungal inocula (10 μL) to make final concentrations (0.62-20 mg/mL). Ten μL of the biocide ketoconazole were used as a positive control. The microplates were incubated for 72 h at 28°C. After 24 h, 10 μL of the redox indicator, resazurin, was added to each microwell in order to assess the absence of respiration. After the incubation period, the microwells were examined and the lowest concentrations without visible growth (under a binocular microscope) were defined as the concentrations that completely inhibited fungal growth (MICs).

Statistical analysis

All measurements were carried out in triplicate and the results are expressed as the means±SD. Pearson's coefficients of correlation (R^2) were also obtained. All statistical analyses were performed using Microsoft Office Excel 2007.

RESULTS

Morphology and distribution of the trichomes

The indumentum of the vegetative parts of *L. purpureum* was composed of two different trichome types, non-glandular and glandular. Non-glandular trichomes were simple, uniseriate, multicellular, pointed and erect with cuticular micropapillae on the surface (Fig. 1B, D, G). They varied in length (100-600 μm) and consisted of 1, 3 or 6 cells. Simple, non-glandular trichomes were found on both leaf sides and on the calyx. Glandular trichomes were peltate and capitate. Peltate glandular trichomes (Fig. 1B, D, F, G) were typical sessile trichomes and consisted of a basal epidermal cell, a very short unicellular stalk, and a broad, round multicellular secretory head consisting of 4 cells in a single shield of various diameters (20-24 μm). Capitate glandular trichomes possessed a long stalk (2-3-celled) and a bulb-shaped head (Fig. 1D, E, H). They were not found on the abaxial leaf surface.

The simple non-glandular trichomes were found on both leaf sides and the calyx (Fig. 1A-D, G). This type of trichome was situated predominantly along the veins on the abaxial side of leaves (Fig. 1A). The peltate glandular ones with a 4-celled head were present on both sides of the lamina (Fig. 1B, D, F). Capitate glandular trichomes were present on the adaxial leaf surface together with peltate and non-glandular trichomes while these were absent on the abaxial leaf surface (Fig. 1D, E). Trichome distribution on the

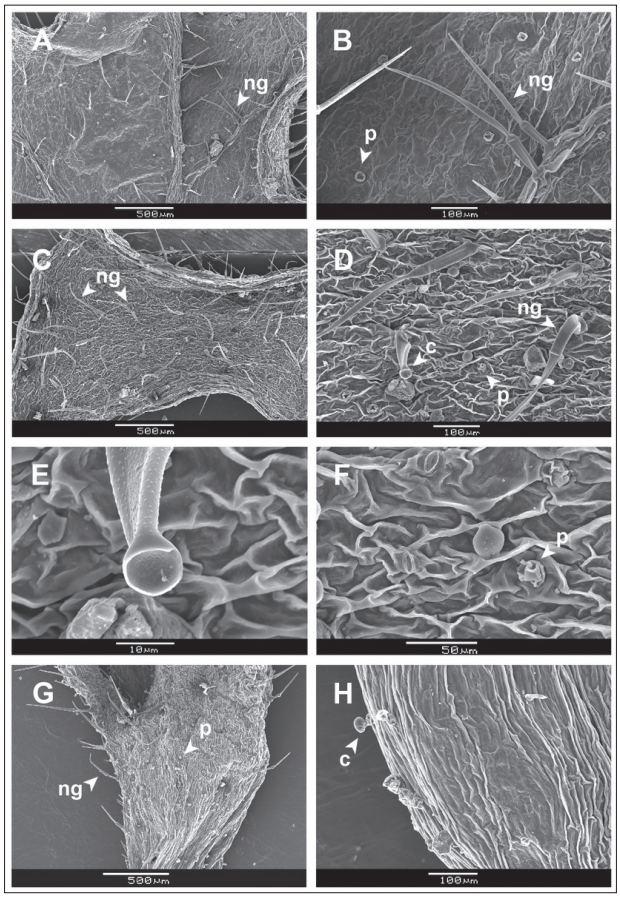

Fig. 1. SEM micrographs of *L. purpureum* leaf and calyx. **A**, **B** – abaxial leaf surface: simple non-glandular trichomes (ng) and peltate glandular trichomes (p); **C**, **D** – adaxial leaf surface: simple non-glandular trichomes (ng), peltate (p) and capitate glandular trichomes (c); **E** – adaxial leaf surface: capitate glandular trichome; **F** – adaxial leaf surface: peltate glandular trichomes (p); **G**, **H** – calyx: simple non-glandular (ng), peltate (p) and capitate (c) glandular trichomes.

calyx was similar to that on the adaxial leaf surface. Thus, the calyx was very hairy, covered with simple, uniseriate non-glandular trichomes. Additionally, the calyx was covered with both peltate and capitate glandular trichomes (Fig. 1G, H).

Qualitative and quantitative analyses of the essential oil

The average yield of essential oil was <0.1% (V/w). GC/MS analysis showed 46 well separated compounds, including 39 identified components which represent 98.29% of the total oil (Table 1). The main components were *trans*-anethole (59.61%), fenchone (6.54%), α-pinene (6.14%) and β-pinene (4.98%). The essential oil of *L. purpureum* contains monoterpene hydrocarbons (13.83%), oxygenated monoterpenes (6.99%), sesquiterpene hydrocarbons (6.36%), oxygenated sesquiterpenes (0.42%), phenylpropanoids (62.69%), diterpenes (1.04%), other compounds (6.96%) and compounds that were not identified (1.71%).

Total phenol and flavonoid contents of ethanolic extracts

The content of phenols varied from 82.00 to 146.30 mg GAE/g of dried extract for the different extracts of *L. purpureum*. The measured values of phenol compounds depended on the volume contribution of ethanol in the mixture. The highest phenol content was found in the 50% ethanol extract (146.33±1.60 mg GAE/g), a lower content in the 30% ethanol (120.00±1.20 mg GAE/g), and the lowest in the 10% ethanol extract (82.00±3.50 mg GAE/g). The total flavonoid content, evaluated using aluminum nitrate nonahydrate, was in the range from 16.53 to 31.93 mg QE/g, increasing with the concentration of ethanol in the solvent (16.53±0.13 for 10%; 27.13±0.06 for 30%, and 31.93±0.13 for 50%).

Antioxidative activity of ethanolic extracts

The free radical scavenging capacity of the studied hydroalcoholic extracts was measured using DPPH and ABTS assays (Table 2). Thirty and 50% ethanol extracts were able to reduce the stable, purple-colored radical DPPH into yellow-colored DPPH-H. The measured values for these extracts were equal (EC_{50}=0.06 mg/mL) while the 10% ethanol extracts exhibited a significantly lower free radical scavenging potential (EC_{50}=0.23 mg/mL). The EC_{50} values of the synthetic antioxidants BHA was 0.093 mg/mL. The values of ABTS antiradical activity ranged from 0.16 to 0.89 mg AA/g. The highest antioxidant potential was observed for the 50% ethanol extract.

The reduction potential of hydroalcoholic extracts was tested using the TRP and FRAP assays (Table 2). The reducing power assay is often used to evaluate the ability of an antioxidant to donate electrons. The results for the investigated extracts, measured by the TRP method, were within the range of 0.1242-0.1281 mg AA/g. The highest value was obtained for the 50% ethanol extract. The reducing capacity of the different

Table 1. Composition of *L. purpureum* essential oil

No	Constituents	KIE	KIL	%
1	α-Thujene	926	924	0.11
2	α-Pinene	930	932	6.14
3	Isovaleraldehyde diethyl acetate	955	959	0.18
4	n.i.	958	n.a.	0.36
5	Sabinene	970	969	1.06
6	β-Pinene	973	974	4.98
7	1-Octen-3-ol	980	974	0.25
8	Mesitylene	991	994	0.10
9	*p*-Cymene	1022	1020	0.52
10	Limonene	1026	1024	0.79
11	cis-β-Ocimene	1037	1032	0.23
12	n.i.	n.a.	n.a.	0.10
13	n.i.	n.a.	n.a.	0.28
14	Fenchone	1086	1083	6.54
15	*n*-Undecane	1099	1100	3.52
16	*n*-Nonanal	1104	1100	0.10
17	Camphor	1143	1141	0.20
18	Terpinene-4-ol	n.a.	1174	0.10
19	*p*-Cymene-8-ol	1184	1179	0.15
20	Estragole	1196	1195	2.75
21	n.i.	n.a.	n.a.	0.39
22	*p*-Anisaldehyde	1253	1247	0.92
23	*trans*-Anethole	1289	1282	59.61
24	*n*-Tridecane	1299	1300	0.27
25	δ-Elemene	1337	1335	0.08
26	Anisyl methyl ketone	1383	1380	0.33
27	β-Elemene	1391	1389	1.45
28	Dodecanal	1408	1408	0.10
29	*trans*-Caryophyllene	1417	1417	0.19
30	*trans*-β-Farnesene	1457	1454	0.37
31	*trans*-Prenyl limonene	1459	1457	0.12
32	2-Methyltetradecane	1461	1467	0.11
33	γ-Muurolene	1480	1478	4.12
34	Germacrene D	1485	1484	0.06
35	Bicyclogermacrene	1504	1500	0.09
36	n.i.	n.a	n.a	0.14
37	Muurola-4,10(14)-dien-1-β-ol	1626	1630	0.15
38	Hinesol	1647	1640	0.27
39	n.i.	1657	n.a.	0.17
40	Hexahydrofarnesyl acetone	1844	1846	0.18
41	*n*-Octadecanol	2082	2077	0.26
42	Phytol	2112	2114	1.04
43	Tricosane	2298	2300	0.12
44	n.i.	2472	n.a.	0.27
45	Pentacosane	2499	2500	0.14
46	Octacosane	-	2800	0.59
Grouped constituents				
Monoterpene hydrocarbons [1,2,5,6,9-11]				13.83%
Oxygenated monoterpenes [14,17-19]				6.99%
Sesquiterpene hydrocarbons [25,27,29,30,33,34,35]				6.36%
Oxygenated sesquiterpenes [37,38]				0.42%
Phenylpropanoids [20,23,26]				62.69%
Diterpenes [42]				1.04%
Other compounds [3,7,8,15,16,22,24,28,31,32,40,41,43,45,46]				6.96%
not identified [4,12,13,21,36,39,44]				1.71%

KIE – Kovats (retention index experimentally determined (AMDIS)); KIL – Kovats (retention) index literature data; n.i. – not identified; n.a. – not available

Table 2. Free radical scavenging and reducing power activity of *L. purpureum* hydroalcoholic extracts.

L. purpureum extracts	DPPH EC_{50} (mg/mL)	ABTS (mg AA/mg)	TRP (mg AA/g)	FRAP (μmol Fe/mg)
10% ethanol	0.23±0.05	0.16±0.01	0.12±0.00	0.38±0.01
30% ethanol	0.06±0.02	0.59±0.02	0.12±0.00	0.81±0.05
50% ethanol	0.06±0.02	0.89±0.03	0.13±0.00	0.91±0.01

extracts was compared with ascorbic acid for the reduction of Fe^{3+} to Fe^{2+}. The reducing capacity of a compound can serve as a significant indicator of its potential reducing power activity. The results of reducing power capacity of investigated extracts measured by the FRAP method varied between 0.38-0.91 μmol Fe^{2+}/mg, where the highest value was obtained for the 50% ethanol extracts. The lowest value was documented for the 10% ethanol extract.

Correlation between antioxidant capacity and the total phenol and flavonoid contents of ethanolic extracts

Our results revealed that there was a significant correlation between the total phenol and free radical scavenging activity of *L. purpureum* ethanolic extracts. It is well established that the free radical scavenging activity of plant extracts is mainly due to phenol compounds. In the present study, the coefficient of correlation (R^2) between total phenol content and the DPPH test was found to be 0.836 while the obtained R^2 value between the flavonoid content and DPPH was 0.907. Furthermore, the R^2 values obtained between the total phenol content and ABTS assay, as well as between flavonoids and the ABTS were 1.00 and 0.987, respectively.

Anti-*Aspergillus* activity

The results for anti-*Aspergillus* activity are presented in Table 3. Plant extracts in the tested concentrations did not exhibit fungicidal activity although mycelial growth inhibition and morphophysiological changes were observed. Morphophysiological alterations were documented in all fungal isolates for each tested plant extract and they included sporulation loss or depigmentation of conidial heads. It should be noted that alterations were more intense as the concentration of the tested plant extracts increased. Regarding the obtained data, the strongest antifungal activity was documented for the 10% ethanolic extract, where both sporulation and pigmentation loss were observed at the highest applied concentration for *A. parasiticus*, *A. ochraceus* and *A. chevalieri* isolates. Conidial head depigmentation to a more or less extent was subsequently found at the higher tested concentrations of all examined *L. purpureum* extracts. On the other hand, the 30% ethanolic extract exhibited the weakest antifungal activity as the observed morphophysiological alterations were least frequent. It should be emphasized that *A. niger* did not exhibit any reaction to any of the applied extract concentrations since morphophysiological changes were absent. On the contrary, mycelial growth of *A. chevalieri* was completely inhibited by lower concentrations while positive growth was observed with higher concentrations. This isolate was, in general, most susceptible in all tested cases.

Table 3. Effect of *L. purpureum* extracts on mycelial growth and sporulation of tested *Aspergillus* species

	20	10	5	2.50	1.25	0.62	GC	EtC	PC
Extracts (10% ethanol) concentration (mg/mL)									
A. flavus	S_{AP}	S_{AP}	S_{AP}	S_{AP}	S_P	S_P	S_P	M	-
A. parasiticus	M	M	S_{AP}	S_{AP}	S_P	S_P	S_P	M	-
A. ochraceus	M	S_A	S_A	S_{AP}	S_{AP}	S_{AP}	S_P	M	-
A. niger	S_{AP}	S_{AP}	S_{AP}	S_P	S_P	S_P	S_P	M	-
A. terreus	S_A	S_{AP}	S_{AP}	S_{AP}	S_{AP}	S_{AP}	S_P	M	-
A. fumigatus	S_A	S_{AP}	S_{AP}	S_{AP}	S_P	S_P	S_P	M	-
A. nidulans	S_A	S_A	S_{AP}	S_{AP}	S_P	S_{AP}	S_P	M	-
A. chevalieri	M	-	S_A	-	-	-	S_P	M	-
Extracts (30% ethanol) concentration (mg/mL)									
A. flavus	S_{AP}	S_{AP}	S_{AP}	S_P	S_P	S_P	S_P	M	-
A. parasiticus	S_{AP}	S_{AP}	S_{AP}	S_{AP}	S_P	S_P	S_P	M	-
A. ochraceus	S_A	S_A	S_{AP}	S_{AP}	S_{AP}	S_P	S_P	M	-
A. niger	S_P	S_P	S_P	S_P	S_P	S_P	S_P	M	-
A. terreus	S_A	S_{AP}	S_{AP}	S_P	S_{AP}	S_P	S_P	M	-
A. fumigatus	S_A	S_{AP}	S_{AP}	S_{AP}	S_P	S_P	S_P	M	-
A. nidulans	S_{AP}	S_{AP}	S_{AP}	-	S_{AP}	S_P	S_P	M	-
A. chevalieri	S_A	S_A	-	-	-	S_P	S_P	M	-
Extracts (50% ethanol) concentration (mg/mL)									
A. flavus	S_{AP}	S_{AP}	S_{AP}	S_P	S_P	S_P	S_P	M	-
A. parasiticus	S_{AP}	S_{AP}	S_{AP}	S_{AP}	S_P	S_P	S_P	M	-
A. ochraceus	S_A	S_A	S_A	S_{AP}	S_{AP}	S_P	S_P	M	-
A. niger	S_{AP}	S_{AP}	S_{AP}	S_{AP}	S_{AP}	S_P	S_P	M	-
A. terreus	S_A	S_A	S_A	-	S_A	S_{AP}	S_P	M	-
A. fumigatus	S_{AP}	S_A	S_A	M	S_P	S_P	S_P	M	-
A. nidulans	S_A	S_A	S_A	-	S_{AP}	-	S_P	M	-
A. chevalieri	S_A	M	-	-	-	S_{AP}	S_P	M	-

GC – growth control; **EtC** – Solvent control (ethanol); **PC** – positive control (ketoconazole); **M** – mycelial growth; $\mathbf{S_A}$ – sporulation present, conidial heads depigmented ("albino"); $\mathbf{S_{AP}}$ – sporulation present, some conidial heads pigmented; $\mathbf{S_P}$ – sporulation present, most conidial heads pigmented; (–) no growth

DISCUSSION

Analysis of SEM micrographs gave insight into the presence of *L. purpureum* (which belongs to the Lamioideae subfamily) trichomes that were the same type as those reported in the subfamily Nepetoideae [2,24]. The indumentum of *L. purpureum* vegetative parts was composed of two different trichome types, non-glandular and glandular. Glandular trichomes were peltate and capitate. Peltate trichomes were 4-celled (subsessile) while capitate glandular trichomes were more variable in stalk length (2-3 celled). Previous research [27] points to the presence of simple multicellular uniseriate non-glandular trichomes, which are present on the leaves and calyx. The same authors ascertained that trichomes have micropapillae on the surface which are regarded as a valid taxonomic character. These observations are in agreement with our findings. On the other hand, the same authors reported the presence of glandular trichomes only on the calyx, which does not correspond with our data. Peltate trichomes with 4-celled heads, reported in our work, were also observed in *L. truncatum* and *L. orientale* [26,28]. Additionally, capitate glandular trichomes were documented on *L. truncatum* calyx and midrip. On the other hand, peltate and capitate trichomes are absent on the adaxial leaf surface [26]. In the case of *L. orientale*, 4-celled peltate trichomes were documented on the surface of both leaf sides and calyx, which corresponds with our results [28]. Contrary to the mentioned data, capitate glandular trichomes with a long stalk and bulb-shaped head were reported.

Essential oil analysis of *L. purpureum* was characterized by a high amount of the aromatic ether *trans*-anethole (59.61%), similar amounts of monoterpene hydrocarbons α-pinene (6.14%) and β-pinene (4.98%) and oxygenated monoterpene fenchone (6.54%). According to previously reported data [32], the essential oil from *L. purpureum* was characterized by a high content of hydrocarbons (91%), nearly equally divided between mono (43.7%) and sesquiterpene hydrocarbons (47.1%). Germacrene D (35.4%), β-pinene (26.8%) and α-pinene (13.4%) were the main constituents. The same compounds (α-pinene, β-pinene) were found to be the main compounds of the essential oil examined in this study, which was also characterized by the domination of *trans*-anethole (59.61%). Furthermore, α- and β-pinene, 1-octen-3-ol, β-elemene and germacrene D were identified as the main compounds in *L. purpureum* oil [29]. In this case, the predominant components were sesquiterpene hydrocarbons and oxygenated sesquiterpenes (60-70%). This was not the case in our study where the mentioned components were reported in lower amounts, i.e. 6.36% and 0.42%, respectively. The differences in essential oil composition found by different authors may be explained by different ecological conditions or harvesting time or the investigated plant parts [46]. It should be noted that the essential oil composition of *L. purpureum* differs from that of some other investigated *Lamium* species. For example, Sajjadi and Ghannadi [30] investigated the essential oil composition of *L. amplexicaule* which contained nearly 60% of alcohols with *trans*-phytol as a dominant compound (44.8%). The same authors reported germacrene D in minor quantities (less than 1%), similar to our findings. On the other hand, germacrene D was documented as the main compound of *L. hybridum* (39.0%) and *L. bifidum* (34.9%), as well as the second most abundant constituent in *L. amplexicaule* (28.9%) [32]. Furthermore, 6,10,14-trimethyl-2-pentadecanone (10.2%) and 4-hydroxy-4-methyl-2-pentanone (9.1%) were observed as the major compounds of the *L. album* essential oil obtained from the dried flowering aerial parts [31], which is to be expected since different plant parts possess characteristic compounds.

Lamioideae species have a low polyphenolic content in extracts, and exhibit weak or no antioxidant activity [47]. The content of total phenols depends on the polarity of solvents, methods used for extraction and extraction temperature [48]. The results of the phenol and flavonoid contents of *L. purpureum* hydroalcoholic extracts depended on solvent concentration. The flavonoid content depended on the extraction solvent used, as was recently reported [48]. These results are in accordance with earlier studies of different extracts of *Lamium* species [49]. The antioxidant activity of flavonoids also depends on the extraction method as well as on solvent polarity and the presence of hydroxyl group or its substitution pattern [50].

L. purpureum hydroalcoholic (ethanolic) extracts possessed free radical scavenging activity, as shown by the DPPH and ABTS assays. These data are in agreement with reported results of other authors [51,52]. The results of our study revealed that the extracts from *L. purpureum* possessed moderate free radical scavenging and reducing power activities. The significant effects of phenol compounds and their antioxidant activity depend on solvent characteristics (polarity, concentration) as well as on the extraction procedure, time of extraction and extraction temperature [53]. Antioxidant activity depends on solvent polarity, thus differences between the observed antioxidant activities among extracts could be attributed to different extracting solvents. The most efficient solvent for polyphenolic extraction was 50% ethanol. The existing data is not enough to explain the mode of antioxidant action, but it enriches the comprehensive data of *L. purpureum* antioxidant activity. Ethanol extracts have been proven as good scavengers, estimated by both antiradical methods and reducing power methods. Furthermore, butanol extracts of *L. purpureum* exhibited high scavenging effects when compared to reference substances [11]. Our results showed that *L. purpureum* could be a new potential source of relatively mild natural antioxidants. We found that biological activity of *Lamium* extracts would be sufficiently informative since in traditional folk medicine whole aerial plant parts (as decoctions or as an ingredient of mild alcoholic beverages) were used rather than the essential oil itself. Further research is needed to identify the individual compounds that possess free radical scavenging and reducing power activities.

There are a few papers examining the antifungal activity of extracts or volatile secondary metabolites from *Lamium* species [7,54]. The hydroalcoholic extracts of *L. purpureum* did not show fungicidal activity against *Aspergillus* species although morphophysiological changes were documented for all fungal isolates

and they included sporulation loss or depigmentation of conidial heads. This is significant since sporulation is an essential part of fungal life cycle and melanin is responsible for the survival and endurance of fungal spores [55]. Depigmentation of conidial heads is probably due to the inhibition of melanin synthesis and its loss can significantly reduce pathogen virulence [56]. It is known that the essential oils of some plants can inhibit sporulation of certain fungal species [57] and cause depigmentation of conidial heads [58]. This may be of great importance when *Aspergillus* species are considered, since some of them are well known human and animal pathogens, allergens and producers of mycotoxins [43]. Certain data nonconformity observed for *A. chevalieri,* which exhibited growth at higher but did not grow at lower concentrations of the applied extracts, could be explained by the fact that this species is osmophilic and therefore high concentrations of *L. purpureum* extracts could have promoted its growth.

CONCLUSIONS

The results of our study showed that the indumentum of the vegetative organs of *L. purpureum* is composed of non-glandular and glandular trichomes (peltate and capitate). The main compound in the essential oil was *trans*-anethole. Data about ethanolic extracts confirmed a high correlation between total phenol content and antioxidant activity, and morphophysiological alterations in tested *Aspergillus* species were observed at higher concentrations of the applied plant extracts. Better insight into the roles of specific compounds in different antioxidant mechanisms requires deeper phytochemical and pharmacological investigations.

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