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Short communication

Anatomical characterization, chemical analyses and essential oil antimicrobial activity of *Hyssopus officinalis* L. (Lamiaceae)

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Introduction

Hyssopus officinalis L. (Lamiaceae) is a perennial aromatic subshrub, distributed mainly in the Mediterranean area. The aerial parts of the plant are used as a culinary and medicinal herb (*Hyssopi herba*) against various ailments, and its essential oil is used in the perfume industry. It contains essential oil, phenolic acids, and flavonoids. However, in a number of cases, certain differences arose between samples of different origin (Mićović et al., 2019). In this work, we investigated the anatomy and chemical composition of *H. officinalis* from Mt. Galičica, North Macedonia. Furthermore, based on an *a priori* knowledge on good antimicrobial activity of essential oils, we investigated its effects against selected microorganisms.

Materials and methods

Plant material

Aboveground shoots of *H. officinalis* were collected during flowering period on the Mt. Galičica, North Macedonia.

Anatomical analyses of stem and leaf

Permanent and native slides of stem and leaf were prepared by standard methods. Briefly, cross-sections were made on a Reichert sliding microtome, then cleared in Parazone and stained in safranin and alcian blue solutions. Native slides were stained by Tucakov reagent. Epidermal peels were prepared using Jefferson solution. All histological slides were examined and photographed using an Olympus BX41 light microscope.

Chemical analyses

Isolation and determination of the essential oil (EO) content in the plant material was done by hydrodistillation using Clevenger-type apparatus. The chemical composition of the EO was determined by gas chromatography with flame ionization and mass detectors (GC-FID/MS). Methanol extract, obtained by bimaceration, was analyzed by high pressure liquid chromatography (HPLC). The GC-FID/MS and HPLC analyses were conducted as described previously (Arsenijević et al., 2019). The content of hydroxycinnamic acid (HCA) derivatives in the extract was determined by the spectrophotometric method as described in the current Ph. Eur. Monograph of Ash leaf.

Antimicrobial activity

Antimicrobial activity was tested by the broth microdilution method against four strains of Gram (+) (*Staphylococcus aureus* ATCC25923, *Enterococcus faecalis* ATCC29212, and *Bacillus subtilis* ATCC6633) and four strains of Gram (–) bacteria (*Escherichia coli* ATCC25922, *Salmonella* Abony NCTC6017, *Pseudomonas aeruginosa* ATCC27853, *Klebsiella pneumoniae* ATCC13083), as well as against one standard (ATCC10231) and five clinically isolated strains of yeast *Candida albicans*. EO (1% in DMSO) was tested in the concentration range of 62.5-1000 μg/mL (Petrović et al., 2017). The synergism between the EO and ciprofloxacin or

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amikacin was investigated by the Checkerboard method (White et al., 1996), against the strains of *S. aureus*, *E. coli* and *P. aeruginosa*, and the results are evaluated using fractional inhibitory concentration (FIC) indices (FICI).

Results and discussion

Anatomical characterization

The stem is quadrangular, primary in structure, with vascular tissues in the form of cylinders. In the central cylinder, the pericycle is composed of fibres and parenchyma cells. The leaves are isobilateral, amphystomatic with diacytic stomata. Two-layered palisade tissue is on the adaxial and abaxial side of the leaf, with a narrow zone of spongy parenchyma in between.

The epidermis of the stem and the leaf is single layered with a thickened outer cell wall and dense indumentum. The indumentum is composed mainly of glandular trichomes (capitate, with an unicellular and bicellular glandular head, and peltate), as well as of non-glandular trichomes (short multicellular, uniseriate).

Results of chemical analyses

The investigated herb was rich in the EO, yielding 1.77% (*V/m*) of this pale yellow aromatic liquid. The most abundant class of compounds in the EO were oxygenated monoterpenes (82.4%), among which 1,8-cineole (72.3%) prevailed. The composition of the investigated oil is in accordance with the previously analyzed oils of aerial parts of wild growing *H. officinalis* from eastern Serbia (Mićović et al., 2021). HPLC analysis of the methanol extract of revealed the presence of phenolic acids, *i.e.* HCA derivatives, with chlorogenic acid as the dominant component. The content of total HCA derivatives was 6,2%, which is in accordance with the previous data (Mićović et al., 2021).

Antimicrobial potential of the essential oil

The investigated EO inhibited the growth of *S. aureus* and *B. subtilis* at the concentration of 1000 μg/mL, as well as of all tested strains of *C. albicans* at 250-500 μg/mL. The observed moderate activity is in accordance with the previously reported modest effects of the main EO's constituent 1,8-cineole (Dorman and Deans, 2000).

Concomitant application of the EO (0.5 μg/mL) with ciprofloxacin (0.062–1 μg/mL) inhibited the growth of *S. aureus*, as well as of *E. coli* and *P. aeruginosa* in the combination with amikacin (0.25–4 μg/mL). The calculated FICI value for the EO and ciprofloxacin was between 0.5 and 1, indicating the additive effect of these two substances on the *S. aureus* strain, whereas simultaneous presence of the EO and antibiotics did not have any benefits in comparison to the use of pure antibiotic against tested Gram (–) bacteria.

Conclusion

Important anatomical features of the examined herb of *H. officinalis* are: isobilateral leaves, diacytic stomata, dense indumentum, mainly of glandular trichomes. The herb was rich in the essential oil abundant with monoterpene ether 1,8-cineole, as well as with the derivatives of hydroxycinnamic acid. The essential oil exhibited moderate antimicrobial activity, but its application together with ciprofloxacin additively affected the growth of *Staphylococcus aureus*.

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