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Short communication

Phenolic profile and antifungal activity of leaf extract of *Artemisia absinthium* L. (Asteraceae)

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Introduction

Genus *Artemisia* L. is one of the largest genera from the Asteraceae family, mainly distributed in the northern hemisphere (Tan et al., 1998). *Artemisia absinthium* L., wormwood, is a perennial, aromatic herb, traditionally used for medicinal purposes (Sargin et al., 2013).

Wormwood contains different specialized metabolites including phenols and flavonoids (Singh et al., 1970). These natural compounds can be responsible for some biological activities, so the aim of this study is to determine the phenols, and to investigate their antifungal potential.

Materials and methods

Plant material

The leaves of *A. absinthium* were collected from Subovac (Serbia, 44°09'19"N, 22°03' 26"E) in 2020. The voucher specimen (BEOU17802) was deposited at the Herbarium of the University of Belgrade – Faculty of Biology, Institute of Botany and Botanical Garden "Jevremovac".

Preparation of leaf extract

Dried leaves (10g) were milled into powder, and then 150 ml of a solvent mixture of methanol and dichloromethane (MeOH: DCM = 1:1) was added. The sample was ultrasonicated for 30 min in the ultrasonic bath at 25 °C, filtered, evaporated to dryness using a rotary vacuum evaporator, and stored at 4 °C before the analyses. For the analysis of phenolic compounds extract was dissolved in MeOH at concentration of 30 mg/ml. For antifungal activity, extract was dissolved in 5% dimethyl sulfoxide (DMSO) at concentration of 40 mg/ml.

Chemical profiling of extract

The phenolic profile of the extract was determined by UHPLC-DAD-ESI/MS^2. Compounds were separated and identified as previously described (Mišić et al. ,2015). Phenolic compounds were identified based on their chromatographic behavior and mass spectra by comparison with standard compounds. Data acquisition was carried out with Xcalibur software. For quantitative analysis, a calibration curve for each available phenolic standard was constructed based on its MS spectra.

Tested fungal isolates

Isolated fungi used in this study were: *Alternaria alstromeriae, Aspergillus parasiticus*, *Botrytis cinerea*, *Cladosporium cladosporioides*, *Culvularia spicifera*, *Fusarium graminearum* B1, *F. graminearum* CIK, *F. oxysporum*, *F. verticillioides, Monillinia laxa, Penicillium citreonigrum* and *P. expansum*, The fungal isolates were deposited in the official culture colection of the Department for Algology, mycology and lichenology, Institute of Botany and Botanical Garden "Jevremovac", Faculty of Biology, University of Belgrade.

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Antifungal activity

Antifungal activity was tested against selected phytopathogenic fungi using two different methods: microdilution assay, and mycelial growth assay. Microdilution was used to determine the minimum inhibitory concentration (MIC), and minimal fungicidal concentration (MFC) using 96-well microtiter plates (Hanel & Rather, 1998). Mycelial growth dynamics were analyzed utilizing colony diameter measurement after treatment with *A. absinthium* extract (Unković et al., 2015), and percent growth inhibition (%) was calculated per formula described in Pandey et al., 1982.

Results and discussion

In this study seven phenolics and one cyclitol (quinic acid) were identified. The main compound was chlorogenic acid (0.71 mg/g) followed by quinic acid (0.47 mg/g). Other compounds were present in smaller quantities: rutin (0.08 mg/g), quercetin (0.07 mg/g), caffeic acid (0.05 mg/g), narcissin (0.04 mg/g), naringenin (0.04 mg/g), apigenin (0.04 mg/g), hispidulin (0.03 mg/g), aesculin (0.03 mg/g), isoquercetin (0.02 mg/g), apigetrin (0.02 mg/g) and vitexin (0.01 mg/g). These results are congruent with literature data about the phenolic profile of plant extracts (Lee et al., 2013). Chlorogenic acid can be responsible for antifungal activity, especially against *B. cinerea*. This phenolic acid has the possibility to inhibit spore germination and mycelial growth of plant pathogenic fungi (Martínez et al., 2017). The high antifungal activity was against *B. cinerea* (41.17% inhibition of mycelial growth). The extract also caused a significant reduction in the growth of *A. alstromeriae* (25.92%), and *M. laxa* (22.58%). Mycelial growth assay showed that the most resistant fungus was *F. graminearum* B1, since tested extract didn't inhibit mycelial growth at all (0%). High resistance was also documented for *Cu. spicifera* (3.22%), *Cl. cladosporioides* (5.71%), and *F. graminearum* CIK (7.69%).

MIC assay indicate that also *B. cinerea* was the most sensitive (MIC 2.5 mg/ml, MFC 20 mg/ml). In addition, extract exhibited high activity against *F. verticillioides* (MIC 5 mg/ml, MFC 10 mg/ml). Moderate antifungal activity was noted against *F. oxysporum* (MIC 10 mg/ml, MFC 20 mg/ml), and *P. citreonigrum* (equal MIC and MFC value of 10 mg/ml). The low antifungal activity was against: *A. parasiticus*, *C. cladosporioides*, and *P. expansum* (equal MIC and MFC values >20 mg/ml).

Conclusion

Plants synthesize different specialized metabolites, and some of them have a role in plant protection against phytopathogenes. This study showed the antifungal activity of wormwood against phytopathogenic fungi, so traditional use of this herb was confirmed.

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