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Short communication

Phytochemical screening of the olive variety 'Istrska Belica' infected by fungus *Venturia oleaginea*

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Introduction

The olive tree (*Olea europaea* L.) is one of the most important agricultural plant of considerable ecological and socioeconomic importance. The fungus *Venturia oleaginea* (Castagne) Rossman & Crous represents the most widespread fungal pathogen of olive species in the world (Bernès 1923) causing foliar disease olive leaf spot - OLS (peacock's eye), and resulting in complete defoliation and consequently yield losses.

Many bioactive compounds can function as constitutive or inducible barriers against microbial pathogens, and their composition can change in response to microbial attack. Phenolic compounds often have role in plant defence against pathogens and their synthesis and accumulation are associated with plant host resistance (El Modafar & El Boustani, 2005).

The aim of our study was to perform phytochemical screening of symptomless and infected leaves of *Olea europaea* var. 'Istrska Belica' in order to get insight into the composition of mainly phenolic compounds.

Materials and methods

Plant material

Leaves (symptomless, leaves on a branch and fallen, both infected with *V. oleaginea* and showing symptoms of OLS) were collected in November 2018 after harvest from olive grove in Bonini, Slovenia from randomly selected olive trees, treated according to the integrated pest menagement practice. The voucher specimen (BEOU 17677) was deposited at the Herbarium of the University of Belgrade - Faculty of Biology.

Extraction of chemical compounds

Air-dried leaves (5 g of each sample) were grinded using a laboratory mill and submerged in 100 mL of dichloromethane:methanol (1:1). Samples were ultrasonicated for 30 minutes in the ultrasonic bath at 25 °C. After ultrasonication and filtration through filter paper (Whatman No. 1), extracts were evaporated to dryness using rotary vacuum evaporator. The obtained crude extracts were stored at 4 °C prior to analysis.

Chemical profiling

The chemical profile of the extracts was determined by UHPLC-DAD-ESI/MS2 (Dionex Ultimate 3000 UHPLC, Thermo Scientific, San Jose, CA, USA). Compounds were separated and identified as previously described (Bessada et al. 2016). The MS detection was performed in negative mode using a triple quadrupole (QqQ) mass spectrometer (Thermo Finnigan, San Jose, CA, USA) equipped with a heated electrospray ionisation (H-ESI) source. Phenolic compounds were identified based on their chromatographic behavior and mass spectra by comparison with standard compounds. Data acquisition was carried out with a Xcalibur® data system

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(Thermo Finnigan, San Jose, CA, USA). For quantitative analysis, a calibration curve for each available phenolic standard was constructed based on the UV-vis signal. The results were expressed as mg/g of the extract.

Results and discussion

In this study, in all three samples, 14 phenolics (protocatechuic acid, aesculin, chlorogenic acid, caffeic acid, rutin, isoquercetin, narcissin, quercitrin, apigetrin, luteolin, quercetin, apigenin, naringenin and hispidulin) and one cyclitol (quinic acid) were identified. There was no qualitative diferences among examined extractrs. The main compounds in symptomless leaves and leaves on a branch were quinic acid (4.12 mg/g and 1.97 mg/g, respectively) and rutin (0.80 mg/g and 0.70 mg/g, respectively), while in fallen leaves it was rutin (0.58 mg/g) and quinic acid (0.32 mg/g). It was documented decrease in amount of certain metabolites comparing symptomless leaves, leaves on branch and fallen leaves, e.g. amount of quinic acid decreased from 4.12 mg/g to 1.97 mg/g and to 0.32 mg/g, respectively. Also, amount of quercetin was the highest in symptomless leaves (0.54 mg/g), less in leaves on the branch (0.17 mg/g), and the lowest in fallen leaves (0.07 mg/g).

The high amounts of quinic acid and rutin in symptomless leaves may be responsible to their resistence. We may hypothesise that somehow attacked infected leaves signaling to the rest of plants parts the presence of fungus and as a response the synthesis and amount of quinic acid and rutin is increasing.

Chen et al. (2013) reported antifungal activity of quinic acid isolated from *Araucaria cunninghamii* that was able to inhibit the growth of phytopatogenic fungus *Helminthosporium sativum*. In addition Tadych et al. (2015) showed that quinic acid, when added to the medium, completely inhibited H_2O_2 production and its secretion by phytopatogenic fungi into the medium.

Several studies have shown that oleuropein, hydroxytyrosol and rutin have a fungitoxic effect (Baidez et al., 2007). The oxidation of these o-diphenols, the preferential substrates of polyphenol oxidases (PPO), produces o-quinones and melanins, highly reactive metabolites whose secondary reactions are responsible for much of the oxidative browning that accompanies plant senescence, wounding, and responses to pathogens, and which appear in the form of brown pigments during the hypersensitive response (El Modafar & El Boustani, 2005). Also, El Aabidine et al. (2010) observed differences in the phenolic compounds between susceptible and resistant cultivars after the infection. The tyrosol and its derivatives were in relation to constitutive resistance, whereas the oleuropein and rutin were in relation to induced resistance.

Lanza et al. (2017) investigated the response of *Olea europaea* (cv. Conservolea) leaves to attack by the same fungal pathogen and found no significant increase in overall polyphenol oxidase (PPO) activity in infected leaves; only a limited local PPO activation occurred in a few upper epidermal cells of the leaf, indicating a feeble induction of a plant response.

Conclusion

We documented new phytochemical data of leaves of olive variety in relation to infection by *Venturia oleaginea*. However, this work should be confirmed by in-depth biochemical studies. Also, a deeper molecular insight into this plant–fungus interaction is still required.

References

Báidez, A.G., Gómez, P., Del Río, J.A., Ortuño, A., 2007. Dysfunctionality of the xylem in *Olea europaea* L. plants associated with the infection process by *Verticillium dahliae* Kleb. Role of phenolic compounds in plant defense mechanism. J. Agric. Food Chem. 55, 3373–3377. https://doi.org/10.1021/jf063166d.

Bernès, J., 1923. Les parasites de l'olivier au Congrès oléicole de Nice. Prog. Agr. et Vitic. 50, 518–524.

Bessada, S. M., Barreira, J. C., Barros, L., Ferreira, I. C., Oliveira, M.B.P., 2016. Phenolic profile and antioxidant activity of *Coleostephus myconis* (L.) Rchb. f.: An underexploited and highly disseminated species. Ind. Crops. Prod. 89, 45–51. https://doi.org/10.1016/j.indcrop.2016.04.065.

Chen, J., Yang, M.L., Zeng, J., Gao, K., 2013. Antimicrobial activity of *Araucaria cunninghamii* Sweet and the chemical constituents of its twigs and leaves. Phytochem. Lett. 6, 41–45. https://doi.org/10.1016/j.phytol.2012.10.013.

El Aabidine, A.Z., Moukhli, A., Jay-Allemand, C., Khadari, B., El Modafar, C., 2010. Resistance of olive tree to *Spilocaea oleagina* is mediated by the synthesis of phenolic compounds. Int. J. Agric. Biol., 12: 61–67. https://hal.inrae.fr/hal-02665560.

El Modafar, C., El Boustani, E., 2005. The role of phenolics in plant defense mechanisms, in: Regnault-Roger, C., Philogène, B.J.R., Vincent, C. (Eds.), Biopesticides of Plant Origin. Intercept, Andover, U.K, Springer-Verlag, pp. 157–172.

Lanza, B., Ragnelli, A. M., Priore, M., Aimola, P., 2017. Morphological and histochemical investigation of the response of *Olea europaea* leaves to fungal attack by *Spilocaea oleagina*. Plant Pathol. 66, 1239–1247. https://doi.org/10.1111/ppa.12671.

Tadych, M., Vorsa, N., Wang, Y., Bergen, M. S., Johnson-Cicalese, J., Polashock, J.J., White Jr, J.F., 2015. Interactions between cranberries and fungi: the proposed function of organic acids in virulence suppression of fruit rot fungi. Front. Microbiol. 6, 835. https://doi.org/10.3389/fmicb.2015.00835.