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Phenolic compounds of common hop (*Humulus lupulus* L.) – composition and content in Polish and foreign cultivars

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Common hop (*Humulus lupulus* L.) is cultivated industrially for its cones in more than 30 countries of the temperate zone, all over the world, including Poland (mainly in the Lublin province). Despite the well-known chemical composition of hop raw material (cones), confirmed by numerous scientific publications, relatively little has been known about the occurrence of secondary metabolites in other parts of the plant, such as leaves and roots, as well as the male form of the hop plant. Moreover, up to now, in the comparative analysis of cones of hop cultivars, almost exclusively major secondary metabolites (bitter acids and terpenes) were taken into account, neglecting the qualitative and quantitative composition of the remaining secondary metabolites of hops. The author's research was aimed at filling the gaps in knowledge about the phytochemical composition of *H. lupulus*.

The objective of the conducted study was phytochemical analysis (qualitative and quantitative) of secondary polyphenolic compounds present in generative (inflorescences and fruits), and vegetative (roots and rhizomes, stem and leaves) organs of Polish hop cultivar 'Marynka' and European hop subspecies (*H. lupulus* var. *lupulus*). In addition, secondary phenolics were determined in cones of wild *H. lupulus* var. *lupulus*, and those of different European cultivars.

The theoretical part of the thesis provides an updated overview of the taxonomic classification of the genus *Humulus*, with particular reference to *H. lupulus* species, botanical characteristics of hop plant, historical and current use of hops, and by-products of hop cultivation, chemical composition of hop cones, as well as biological activity of the main occurring classes of phenolic compounds.

In the experimental section, in the course of the performed study, 42 phenolic compounds were isolated altogether, from the extracts of hop female cones and male inflorescences. The chemical structures of these compounds were established by spectral analysis methods: NMR and mass spectrometry (LC-PDA-ESI-MS/MS), as well as chemical methods (acid hydrolysis and analysis of its products). Among the isolated hop secondary polyphenols were: 35 flavonoids (including 16 flavonol glycosides, 9 chalcones, 4 flavanones, 4 flavanocoumarins,

and 2 flavan-3-ols) and 7 non-flavonoid phenolic compounds (including 4 multifidol glycosides and 3 hydroxycinnamic acid amides). All identified compounds represent already known plant substances; however, the presence of 16 compounds was demonstrated for the first time in the *Humulus* genus, including: 6 flavonol glycosides (tamarixetin 3-*O*-glucoside; 3-*O*-sophorosides of kaempferol, 8-metoxykaempferol and 8-metoxyquercetin; 3,4'-di-*O*-glucosides of kaempferol and quercetin), 4 chalcones (xanthohumol J, xanthohumol L, 3-hydroxyxanthohumol and chalconaringenin), 4 flavanocoumarins (phyllocoumarin, isophyllocoumarin, epiphyllocoumarin and isoepiphillocoumarin) and 2 hydroxycinnamic acid amides (*N*-feruloyltyramine and N_1,N_5,N_{10}-tricaffeoylspermidine). In addition, 48 other polyphenolic compounds were tentatively identified, on the basis of LC-PDA-MS/MS analyzes and literature data, in the generative and vegetative organs of the hop plant. Among them were also substances which have never been reported in the *Humulus* genus (spermidine- or tyramine-phenolic acid derivatives, and metoxykaempferol 3-*O*-hexoside), and most probably even in the plant kingdom (oligomers of hydroxycinnamic acid amides and co-multifidol malonyl-hexoside).

Quantitative levels of 39 phenolic compounds, belonging to six groups of hops secondary polyphenols (hydroxycinnamic acids, multifidols, flavan-3-ols, flavonols, chalcones, and flavanones) were measured, by means of a developed UPLC-PDA-MS method, in extracts from hop cones of six cultivars (Hallertau Magnum, Lubelski, Marynka, Oktawia, Sybilla, Wye Challenger). The test plant material showed significant differences in the concentration of either individual compounds or polyphenol classes in the two growing seasons (2012 and 2014). The two main groups of polyphenols in the cones of all studied hop varieties were flavonols and chalcones. Xanthohumol was the dominant secondary phenolic constituent in all extracts (2-5 mg/g DW). The hops of *H. lupulus* var. *lupulus* were characterized by the highest total polyphenol content (13.5 mg/g DW), followed by cultivars Lubelski (13 mg/g DW) and Oktawia (12 mg/g DW), whereas the lowest level was observed in Hallertau Magnum variety (7.5 mg/g DW). Comparative phytochemical analysis of cone extracts, using chemometric methods (Cluster Analysis and Principal Cluster Analysis), allowed for grouping hop varieties into clusters and finding relationship (positive and/or negative correlation) between clusters and secondary polyphenols. In general, cones of the studied hop cultivars were grouped in the same clusters in terms of the growing season, but not entirely in accordance with the distinguished utility types (aromatic, dual, bitter).

Quantitative levels of 57 phenolic compounds, belonging to seven groups of hops secondary polyphenols (hydroxycinnamic acids, phenolic acid amides, multifidols, flavan-3-

ols, flavonols, chalcones, and flavanones) were determined in extracts from generative (inflorescences and cones) and vegetative organs (roots and rhizomes, stems and leaves) of the Marynka cultivar and the male and female form of *H. lupulus* var. *lupulus*. Significant differences (qualitative and quantitative) were observed in the phytochemical profiles of underground (roots and rhizomes) and aboveground parts, as well as generative (inflorescences and cones) and aboveground vegetative organs (stems and leaves), as well as female and male forms of the hop plant. The extracts from hop roots and rhizomes were characterized by the presence of only two groups of hop secondary polyphenols (flavan-3-ols and phenolic acid amides), and the lowest overall polyphenol content (3 mg/g DW). In turn, in the two extracts from aboveground vegetative organs (leaves and stems) three groups of phenolic compounds were the dominant constituents, i.e. flavonols, hydroxycinnamic acids, and flavan-3-ols. Nevertheless, the observed total polyphenol content was several times higher in leaves (30 mg/g DW), compared to stems (4.5 mg/g DW).

Hop male inflorescences were characterized by the presence of phenolic compounds absent in the female hop generative organs, including compounds belonging to the group of phenolic acid amides, flavonols, and chalcones. On the other hand, no multifidols were detected in the male hop. Moreover, male inflorescences were characterized by the highest levels of hydroxycinnamic acids (3.5 mg/g DW), phenolic acid amides (2 mg/g DW), flavonols (25 mg/g DW), and total polyphenols (35 mg/g DW) among all analyzed extracts from *Humulus* generative organs.

The female generative organs of both European and cultivated varieties of hop plant showed a very similar composition of secondary phenolic compounds. Moreover, in both varieties, significant quantitative differences were found between inflorescences and cones. The extracts from immature cones (inflorescences) were characterized by higher levels of hydroxycinnamic acids, flavan-3-ols, and total polyphenols, compared to mature cones, in which, in turn, higher concentrations of chalcones and multifidols were observed. The main secondary phenolic compound in the female hop inflorescences was (+)catechin (3 mg/g DW), while in the cones it was xanthohumol (3.5 mg/g DW).