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A comparison of antioxidant and antimicrobial activity between hop leaves and hop cones

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ABSTRACT

The leaves of the hop plant (*Humulus lupulus* L.) are an agricultural by-product that is not currently being exploited. This study compared two hop cultivars cv. 'Aurora' and cv. 'Hallertauer Magnum' from four different hop-growing regions (Žalec, Slovenia; Leutschach (Kranach), Austria; Hüll, Germany; Žatec, Czech Republic). The leaves and cones were collected and their total phenolics and the antioxidative and antimicrobial activities of their ethanol extracts were determined. Samples were collected three years in succession (2008–2010). Total phenolics ranged from 0.099 to 0.542 mg CAE/mL for the leaf extract and from 0.738 to 1.734 mg CAE/mL for the cones, which had both higher levels and greater variability of phenolics. The leaves had much lower DPPH radical scavenging activity. Their IC_{50} of approximately 0.020 mg/mL was much higher than the cones' extracts (0.005 to 0.010 mg/mL) regardless of the year and of the growing location. The best reducer was the extract from the Aurora leaves collected in the Czech Republic in 2010, which reduced 0.117 mL/μg of ferric ions in 25 min. Antimicrobial activity against gram positive *Staphylococcus aureus* was extraordinary for all hop cones extracts (minimal inhibitory concentrations, MICs < 0.003 mg/mL), while moderate antimicrobial activity (MICs > 0.16 mg/mL) against gram negative *Escherichia coli* O157:H7 was observed for hop cones and leaves extracts. The results of the profile analysis showed the caffeic acid peak at t_r = 35.1 min for the leaves, while the cones had no such peak at that retention time.

1. Introduction

The hop, *Humulus lupulus* L. (Cannabaceae), is a widely grown perennial plant. The dry hop cones and other hop-cone preparations (e.g. pellets, extracts) are essential for the characteristic bitterness and aroma of beer, with these coming mostly from the bitter substances (i.e. alpha-acids and beta-acids) and the hop essential oil. More recently, other hop components (e.g. leaves, stems) have been receiving greater attention as antioxidants and potential antibacterial and antiviral agents, and even for cancer chemoprevention (Gerhauser, 2005). However, in beer making, the polyphenols from the hops (and also from the malt) are generally considered to be undesirable, as some of these can result in an irreversible haze in the beer (Forster, 2013).

The composition and amounts of secondary metabolites in hops depend primarily on the cultivar (i.e. variety, accession), which is related to their genetic potential to synthesise certain substances (Kralj et al., 1995; Green, 1997). These secondary metabolites depend on the hop growing area, as well as on the weather conditions during each growing season (Green, 1997; Čeh et al., 2007). As only the hop cones are used in the beer-making industry, large amounts of hop leaves remain as an agricultural by-product that is not being exploited at all. Hop leaves could also be used as a source of phenolic compounds. Some preliminary studies have shown that the concentrations of phenolic compounds in hop-leaf extracts correspond well to their antioxidant potential, giving these kinds of analysis high importance (Čeh et al., 2007).

In Slovenia, the whole aboveground portion of the hop plants is cut in the field and taken to the harvest machine when the

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hop cones are technologically mature. Hop cones are then harvested from the plants and represent the yield, while the remaining biomass is divided into leaves and stems inside the harvester and cut into smaller pieces. Both fractions (leaves and stems) represent residue; they can be composted and returned to the field or can be used for other purposes. Because the quantity of that biomass is approximately 10–15 t/ha annually (2.6 kg/plant), it was our aim to find a potential use for the leaves. There is no need for extra preparation of the material (no extra cost), because it already comes out of the harvester directly and can be easily packed and taken to a potential user.

In the present study we used two hop cultivars. Cultivar 'Super Styrian Aurora' ('Aurora') is known as an aroma cultivar, due to the noble, hoppy aroma, and has an alpha-acid content of 7.0% to 9.5%. This cultivar also contains about 3.9% polyphenols (determined using a modified Analytica-EBC 9.11 method). On the other hand, the second cultivar used, 'Hallertauer Magnum' ('H. Magnum') is a typical bitter hop cultivar, with 11.0% to 16.0% alpha-acid but with no outstanding aroma characteristics and a polyphenol content of 2.0% to 3.0% (Analytica-EBC 9.11) (Descriptive 2009; Global Hops, 2013; Slovenian Hops, 2013; Beer Legends, 2013; Hallertauer Magnum, 2013). Our aim was to investigate whether the leaves of these hop cultivars could be used in the food industry as antioxidants or as antimicrobial agents.

The leaves and cones of these two cultivars were collected in four different countries in Central Europe where they are grown, and the total phenolics and antioxidative and antimicrobial activities of their ethanol extracts were determined. The samples of cones and leaves were collected for three years in succession (2008–2010) and the extractions were performed using the dry leaves and dry cones of the 48 samples included in the study. Samples collected in 2008, 2009 and 2010 were used for the total phenolics and antioxidant activity assays. HPLC analysis of the ethanol extracts obtained from the cones of these two cultivars was performed with the samples collected in 2009, and the antimicrobial activities were determined for the ethanol extracts from the leaves and cones collected in 2010.

2. Materials and methods

2.1. Materials

2.1.1. Hop cultivars

Samples of two hop cultivars, cv. 'Aurora' and cv. 'H. Magnum', were collected from four different hop-growing regions: Žalec in Slovenia, Leutschach (Kranach) in Austria, Hüll in Germany, and Žatec in the Czech Republic. The 'Aurora' cultivar was planted in Slovenia in 2005, in Austria in 2003, in Germany in 1996, and in the Czech Republic in 2006. The 'H. Magnum' cultivar was planted in Slovenia in 2001, in Austria in 2007, in Germany in 1999, and in the Czech Republic in 2006.

2.1.2. Chemicals

Folin–Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), coumaric acid, caffeic acid, rutin and catechin were bought from Sigma, Germany. Potassium hexacyanoferrate(II) was from Kemika, Croatia, and $FeCl_3$ was from Carlo Erba, Italy. Xanthohumol (90% pure) was from Hop Steiner, Germany. All other solvents, buffers and reagents were analytically pure.

2.1.3. Sampling method

At the time of technological maturity of both of the hop cultivars and at all four of the locations, equal amounts of leaves and cones from the lower, middle and upper parts of the plants were collected, with approximately 5 L of leaves/cones for each sample. The collected material was air dried at 50–55 °C, and kept at room temperature in the dark until analysis. The collection and coding details of the samples is given in Table 1.

2.1.4. Collection locations and soil characteristics

The hop field in Žalec in the Savinja Valley in Slovenia is on a medium deep eutric brown soil on a sandy–gravel base. The upper soil layer is classified in the texture class of clay loam to sandy clay loamy (medium to heavy soil). In the hop field in Leutschach, Austria, the soil is heavy sandy loam, while in the hop field in Hüll in Germany it is heavy loamy. In the experimental hop field at Žatec, in the Czech Republic, the soil is light, loamy sand. The chemical characteristics of the soil at each of these collection locations are given in Table 2. The humus content at most of the locations was good, although it was low for the Czech Republic. The pH values of the soil varied from 6.0 to 7.2. The phosphorus content was lowest in the Austrian field for cv. 'Aurora' and in the Czech Republic (supply class B), with a good phosphorus supply for the Slovenian field and Austrian field with cv. 'H. Magnum'. The field in Slovenia that was planted with cv. 'Aurora' had soil that was oversupplied with phosphorus, while in Germany the phosphorous supply was excessive.

2.2. Methods

2.2.1. Extraction of phenolics

The dried leaves (L) and cones (C) from the hops were homogenised with a mortar and pestle to a fine powder. Two parallel extractions were prepared using ca. 400 mg of each homogenised sample and 20 mL 96% ethanol. The extractions were carried out at 60 °C for 24 h in a water bath (Kambič, Slovenia) with constant mixing at 170 rpm (Stout et al., 1999). The cooled extracts were then centrifuged at 2470 × *g* for 10 min (Centric 322B, Tehtnica, Slovenia) and the supernatants thus obtained were stored at −20 °C until analysis. The supernatants from the samples collected in 2008, 2009 and 2010 were used for the total phenolics and antioxidant activity assays, and only those from the samples collected in 2010 were used for the antimicrobial tests too.

2.2.2. Total phenolics

The total phenolics were determined by the reduction of phosphotungstic acid and phosphomolybdic acid (i.e. Folin–Ciocalteu reagent) to blue pigments (Singleton and Rossi, 1965), with the phenolics in alkaline solutions. A suitable volume of an extract was diluted with deionised water to 2.75 mL. Then 0.5 mL Folin–Ciocalteu reagent (previously diluted 1:2 with deionised water) was added and, after exactly 5 min, 0.5 mL 20% Na_2CO_3 was added. After 90 min at room temperature, the absorbance of the samples was read against a blank (ethanol) at 746 nm in a spectrophotometer (Hewlett Packard, HP DAD UV/vis, 8453; Agilent, USA). All of the supernatants were analysed in three parallel batches and the total phenolics were expressed as equivalents of chlorogenic acid in mg per gram dry sample (mg CAE/g dry sample). For this purpose, a calibration curve was constructed in the range from 0 μg to 80 μg chlorogenic acid (dissolved in ethanol) and a linear correlation ($y = 0.0181x$) with a correlation coefficient of 0.9993 was obtained.

2.2.3. DPPH antioxidant activity

The 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) scavenging assay was used to evaluate the antioxidative activities of the extracts obtained. DPPH is characterised as the stable free radical DPPH•. When the violet-coloured solution of DPPH• is mixed with an antioxidant that can donate an electron or a hydrogen atom, this gives rise to the reduced form of the DPPH•, which is accompanied by the loss of the violet colour. The reduction in DPPH• can be