Phenolic composition and antioxidant, antimicrobial and cytotoxic properties of hop (Humulus lupulus L.) Seeds

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ABSTRACT

Hop (Humulus lupulus L.) is a perennial climbing plant cultivated for commercial use, mainly in brewing industry, since the female inflorescences (cones) contain lupulin glands that confer the characteristic bitterness, aroma and flavour to beer. However, the bioactive potential of the seeds found in these cones remains unexplored. In this study, the phenolic composition of hop seeds was analysed by LC-DAD-ESI/MSn and its antioxidant, cytotoxic and antimicrobial activities were screened against oxidizable substrates, human tumour and non-tumour cell lines, and different bacteria and fungi, respectively. (+)-Catechin and (-)-epicatechin were identified in the seeds, totalizing 17.6 ± 0.7 mg/g extract. The seed extract showed antioxidant activity mainly through the formation inhibition of thiobarbituric acid reactive substances (TBARS), and had cytotoxic effects against MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), HeLa (cervical carcinoma), and HepG2 (hepatocellular carcinoma) tumour cells, but no toxicity towards the normal PLP2 cell line was observed. The extract showed remarkable antimicrobial properties, being better than the positive controls against almost all tested bacteria and fungi of the genus Penicillium. These results highlight the potential of hop seeds as a source of functional ingredients with applications in the food and pharmaceutical sectors.

1. Introduction

Humulus lupulus L. (hop) is one of the three species of the genus Humulus that belongs to the family Cannabaceae. The origin of the genus has been suggested to be China, from where eastward and westward migrations to America and Europe, respectively, took place. The species has been classified into five taxonomic varieties based on their morphological characteristics and geographical locations (Small, 1980, 1978). The cultivation of this plant for long time caused the existence of different cultivars and chemotypes. The main reason was the need to choose specific organoleptic properties of hops, in order to improve the flavour and aroma of beer, one of the oldest and most widely consumed alcoholic drinks worldwide (Zanoli and Zavatti, 2008).

Hop is an industrial plant massively used in the brewing industry. The female hop inflorescences (cones or strobiles) have lupulin glands that contain different compounds, such as resins, bitter acids, essential oils, and flavonoids, which play an important role in the brewing process. These compounds improve the preservation of beer and provide bitterness (Karabin et al., 2015). Bitter acids are phloroglucinol prenylated derivatives and are generally classified as α-acids and β-acids; the most important are humulone and lupulone, respectively (Zanoli and Zavatti, 2008). Hop has different types of flavonoids, such as flavan-3-ols, proanthocyanidins, flavonols, flavanones, and prenylflavonoids, which have prenyl or geranyl chains on the flavonoid structure. The most important prenylflavonoid is xanthohumol, a chalcone that can be converted to the flavanone isoaxanthohumol in consequence of brewing conditions. Isoxanthohumol is therefore the main prenylflavonoid present in beer (Karabin et al., 2015).

Apart from brewing industry, hop cones has been used in traditional medicine to control anxiety, spasms, cough, fever, toothache, and inflammation; and hop resins have been applied as a mild sedative (Chattopadhyay, 2010). In fact, the health-promoting effects and bioactivities of hop flavonoids are numerous and the most outstanding are the cardioprotective effect and the antioxidant, anti-inflammatory, anticarcinogenic, and antimicrobial activities (Karabin et al., 2015;
Stevens and Page, 2004). Because of this, there is a growing interest in the possible use of the hop plant for non-brewing purposes, especially in the pharmaceutical and nutraceutical industries.

The hop cones are the most used part of the plant, but there are other parts that also have an interest as food. Hop shoots with little leaves are eaten stewed in a traditional way in Spain, whose nutritional composition and antioxidant properties have already been described (Tardío et al., 2016). In addition, there are also reports on the antioxidant and antimicrobial activities of hop cones and leaves (Abram et al., 2015). However, as far as we know, the bioactive potential of the seeds found in the cones of this plant remains unexplored. Therefore, this study aimed to characterize the phenolic composition and to evaluate the antioxidant, antimicrobial (antibacterial and antifungal), and cytotoxic properties of hop seeds.

2. Materials and methods

2.1. Plant material and extract preparation

Hop seed samples were purchased from Semillas Cantuesto, S.L., Spain, a certified seed company that collects, produces and commercialises seeds of the Iberian Peninsula flora with ecological certification. The seeds were reduced to a fine powder (20 mesh), which was subjected to a solid-liquid extraction with methanol:water (80:20, v/v) as previously described by Pinela et al. (2012). The extraction yield was calculated and expressed as g extract per 100 g hop seeds.

2.2. Analysis of phenolic compounds

The dry extract (10 mg) was dissolved in methanol:water (20:80, v/v; 2 mL) and filtered through a 0.22-μm syringe filter disk. The LC-DAD-ESI/MS² analysis was performed on a Dionex Ultimate 3000 UPLC with a diode array detector (DAD, 280, 330 and 370 nm) coupled to a Linear Ion Trap LTQ XL mass spectrometer (Thermo Scientific, San Jose, CA, USA). The chromatographic conditions and procedures used to identify and quantify the phenolic compounds were previously described by Bessada et al. (2016). The results were expressed as mg per g of dry extract.

2.3. Evaluation of bioactive properties

2.3.1. Antioxidant activity

The antioxidant activity of the extract (at concentrations from 2.5 to 0.039 mg/mL) was evaluated in vitro by the DPPH free radical scavenging capacity, reducing power, β-carotene bleaching inhibition, and thiobarbituric acid reactive substances (TBARS) formation inhibition assays, as described previously by Pinela et al. (2012). Trolox (Sigma-Aldrich, St. Louis, MO, USA) was used as positive control. Results were given as EC₅₀ values (μg/mL).

2.3.2. Cytotoxic and hepatotoxic activities

The sulforhabdine B assay was performed to evaluate the cytotoxic activity of the extract (at concentrations from 4 to 0.125 mg/mL) against the MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), HeLa (cervical carcinoma) and HepG2 (hepatocellular carcinoma) human tumour cell lines (Vaz et al., 2010). The assay was also performed to evaluate the hepatotoxicity of the extract against non-tumour primary cells (PLP2) (Pereira et al., 2013). The procedures and the origin of cell lines were previously described by the authors. Ellipticine (Sigma-Aldrich) was used as positive control. Results were given as GI₅₀ values (μg/mL).

2.3.3. Antimicrobial activity

The extract (at concentrations from 5 to 0.005 mg/mL) was tested against Bacillus cereus (food isolate), Staphylococcus aureus (ATCC 11,632), Listeria monocytogenes (NCTC 7973), and Enterococcus faecalis (ATCC 19,433) (Gram-positive bacteria) and Escherichia coli (ATCC 35,218) and Salmonella typhimurium (ATCC 13,311) (Gram-negative bacteria) (Soković et al., 2010). The fungi Aspergillus fumigatus (ATCC 1022), Aspergillus ochraceus (ATCC 12,066), Aspergillus niger (ATCC 6275), Penicillium ochrochloron (ATCC 9112), Penicillium funiculosum (ATCC 36,839), and Penicillium verrucosum var. cyclopium (food isolate) were also tested (Soković and van Griensven, 2006). The procedures and the origin of the microorganisms were previously described by the authors. As positive controls (Sigma-Aldrich), streptomycin and ampicillin were used for the antibacterial activity, while ketoconazole and bifonazole were used for the antifungal activity. The results were given as minimum inhibitory (MIC) and minimum bactericidal (MBC) or minimum fungicidal (MFC) concentrations (mg/mL).

2.4. Statistical analysis

The experiments were carried out in triplicate and the results were expressed as mean ± standard deviation. The SPSS Statistics software (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.) was used to analyse differences among hop seed extract and positive control in the antioxidant and cytotoxicity assays by applying a two-tailed paired Student’s t-test at a 5% significance level. Significant differences between the samples were considered when the p-value was lower than 0.05.

3. Results and discussion

3.1. Extraction yield

The solid-liquid extraction yielded 6.0 ± 0.3 g of extract per 100 g of hop seeds. This low yield may be justified by the hardness of the seeds, which also affects the germination. The value is within the range achieved by Chen et al. (2012) for hempseed (Cannabis sativa L.) kernel (0.45–14.97%). For extraction, the authors used methanol, ethanol and acetone at different concentrations, and verified that the higher yields were obtained when using the more polar solvent. They also reported a higher amount of soluble substances in the hempseed kernel than in the hull, which gave a lower yield (0.18–3.29%). In our study, the hop seed was analysed whole because of its very small size.

Table 1

| Compound        | Rt (min) | λmax (nm) | Pseudomolecular ion [M-H]⁻ (m/z) | MS² fragments (m/z) | Content (mg/g extract)³ |
|-----------------|---------|-----------|---------------------------------|--------------------|-------------------------|
| (−)-Catechin    | 7.1     | 278       | 289                             | 245(100), 205(37), 179(13), 165(5), 137(3) | 13.7 ± 0.5              |
| (−)-Epicatechin | 9.7     | 279       | 289                             | 245(100), 205(35), 179(15), 165(8), 137(3) | 3.9 ± 0.2               |
| L               |         |           |                                 |                    | 17.6 ± 0.7              |

Calibration curves: (−)-catechin (y = 84950x – 23,200; r² = 0.9999; LOD = 0.17 μg/mL; LOQ = 0.68 μg/mL); (−)-epicatechin (y = 10314x + 147,331; r² = 0.9994; LOD = 0.21 μg/mL; LOQ = 0.84 μg/mL).

³ The results are presented as mean ± standard deviation.
It should also be noted that cocoa and grape seeds contain complex compounds derived from flavan-3-ols, such as procyanidins (Fayeull et al., 2018; Ma et al., 2018), whereas hop seeds only contained the monomers.

(+)-Catechin and (-)-epicatechin are compounds of great importance in different industrial sectors. These are widely used as functional ingredients in functional foods, dietary supplements, and nutraceuticals to promote consumers health, prevent various diseases, and extend the product shelf-life, but also in different pharmaceutical and cosmetic formulations (Gadkari and Balaraman, 2015; Yilmaz, 2006). Given the high added value of these compounds, several studies have focused on novel methods to optimized their extraction from plant matrices (Albuquerque et al., 2017a) and techniques to promote their stability (Albuquerque et al., 2017b), before being incorporated into final products (such as food, pharmaceuticals, or active food packaging films) (Arrieta et al., 2017; Takwa et al., 2018). The present study showed for the first time that hop seeds are an interesting source of (+)-catechin and (-)-epicatechin, compared with other matrices described in the literature (Gadkari and Balaraman, 2015).

3.3. Antioxidant activity

The results of the antioxidant activity of the hop seed extract are given in Table 2. The lower the EC50 values, the higher the antioxidant activity. The TBARS assay gave the lowest EC50 value, being required 128 ± 2 μg/mL to provide 50% of antioxidant activity. This assay measures the extract capacity to inhibit the formation of malondialdehyde and other low-molecular weight end products that are generated from the ex vivo decomposition of certain lipid peroxidation products (Alonso et al., 2009). Porcine brain cells were used for this purpose because they are biological substrates rich in polyunsaturated fatty acids and, therefore, good models for lipid peroxidation studies.

The hop extract had comparable EC50 values for the DPPH free radical scavenging capacity (505 ± 16 μg/mL) and reducing power (530 ± 16 μg/mL) (Table 2). DPPH was used as a source of free radicals, since it simulates in vitro the reactive oxygen and nitrogen species (ROS and RNS) that affect biological systems. Furthermore, it is considered that the mechanism of ROS/RNS scavenging by antioxidants is involved in the inhibition of oxidative-stress-induced cellular damage and lipid peroxidation (Panat et al., 2016). On the other hand, the reducing power or ferricyanide/Prussian blue assay allowed to estimate the reductant compounds in the hop extract.

The highest EC50 value (1330 ± 74 μg/mL) was achieved with the β-carotene/linoleic acid antioxidant activity assay (Table 2). This result may indicate that the hop extract is less efficient in neutralizing the linoleic hydroperoxyl radicals (and also lipid hydroperoxides, Table 2)

3.2. Phenolic composition

The phenolic compounds (+)-catechin and (-)-epicatechin were identified in the hop seed extract. Data related to the identification of these two compounds are given in Table 1 and their chemical structure is shown in Fig. 1. Both compounds were identified based on their chromatographic characteristics and UV–vis and mass spectra as compared with commercial standards. (+)-Catechin was found as the most abundant compound (13.7 ± 0.5 mg/g extract) in hop seeds, followed by (-)-epicatechin (3.9 ± 0.2 mg/g extract). Thus, 1 g of hop seed hydroethanolic extract contained 17.6 ± 0.7 mg of total flavan-3-ols. These compounds have been previously described in hops (Keskin et al., 2018) found catechin in hop cones (0.37 mg/g of methanolic extract) and epicatechin in hop leaves (0.83 mg/g of methanolic extract). According to Inui et al. (2017), the content of these polyphenols in hop cones can change depending on the time and year of harvest. The authors reported a decrease in late harvest samples and a catechin/epicatechin ratio ranging from 5 to 11 (in the hop seeds, the ratio is 3.5). Karabin et al. (2015) report in a research review that hop flavonoids (with the exception of prenylflavonoids) are present in all parts of hop cones, especially in seeds and bracts, and that the contents can be affected by several factors including variety, edaphoclimatic conditions, cultivation techniques, and storage conditions, among others. Additionally, some studies report that (+)-catechin is absent in beers (Arts et al., 2000), while others describe low amounts, close to 0.5 mg/L (Bartolomé et al., 2000).

(+)-Catechin and (-)-epicatechin have been also identified in hemeed, a plant of the same family than hop, but in a much lower quantity. Smeriglio et al. (2016) quantified 5.3 μg/100 g and 10.2 μg/100 g of (+)-catechin and (-)-epicatechin, respectively, in the methanolic extract of hemeed oil. Multari et al. (2016), on the other hand, described 166 μg/100 g of (+)-catechin and 46 μg/100 g of (-)-epicatechin in commercial hemp flour.

Catechins are found in other seeds, such as cocoa and grape, which are responsible for the high catechin content found in dark chocolate (46–61 mg/100 g) and red wine (8–30 mg/100 g), respectively; although green tea from Camellia sinensis L. leaves is the main source (10–80 mg/100 g) of these compounds (Gadkari and Balaraman, 2015).

Table 2

Antioxidant, anti-proliferative and hepatotoxic properties of hop seed extract and positive controls (trotlox or ellipticine).

| Antioxidant activity (EC50 values, μg/mL) | Hop seed extract | Positive control |
|-----------------------------------------|-----------------|------------------|
| DPPH free radical scavenging activity   | 505 ± 16        | 42 ± 1           |
| Reduction power                         | 530 ± 16        | 41 ± 1           |
| β-Carotene bleaching inhibition capacity | 1330 ± 74      | 18 ± 1           |
| TRABSS formation inhibition capacity    | 128 ± 2         | 23 ± 1           |
| Cytotoxic activity (GI50 values, μg/mL)  | 278 ± 4         | 13 ± 1           |
| HepG2 (hepatocellular carcinoma)        | 184 ± 6         | 8.0 ± 0.2        |
| NCI-H460 (non-small cell lung carcinoma) | 244 ± 21        | 4.75 ± 0.05      |
| HeLa (cervical carcinoma)               | 251 ± 9         | 3.7 ± 0.2        |
| Hepatotoxicity (Glut values, μg/mL)     | 400             | 8.6 ± 0.1        |

* a The results are presented as mean ± standard deviation. Statistical differences were found between hop seed extract and positive control when using a two-tailed paired Student’s t-test.
conjugated dienes and volatile by-products), which are generated in the in vitro system from the oxidation of limonene acid, since these radicals attack β-carotene and cause the bleaching of the reaction emulsion (Frankel, 2005; Sowndhararajan and Kang, 2013).

Table 2 also shows that a lower concentration of trolox (the used positive control) was required to induce antioxidant effects (EC50 values ≤ 42 ± 1 μg/mL). However, it should be noted that trolox is a pure antioxidant compound, whereas hop extract is a complex mixture of different phytochemicals with or without bioactive properties. Furthermore, according to the literature, catechins have remarkable antioxidant properties, being able to neutralize ROS, reduce metal ions, and inhibit lipoperoxidation (Grzesik et al., 2018). In fact, the antioxidant activity of (±)-catechin and (−)-epicatechin is much higher than that of prenylflavonoids (measured via DPPH assay), compounds also reported in hop (Inui et al., 2017).

In a previous study, Morales et al. (2012) evaluated the antioxidant activity of methanolic extracts of hop young shoots and also achieved the lowest EC50 value (30 μg/mL) with the TBARS assay. This extract was more effective in inhibiting the discoloration of β-carotene than the seed extract (with an EC50 value of 480 μg/mL), but it had a worse performance for the DPPH free radical scavenging and reducing power assays (with EC50 values of 1360 and 800 μg/mL, respectively). The authors measured 9.56 ± 0.65 mg of (±)-catechin equivalents per g extract (total flavonoids), a content lower than that quantified in the seeds, which may justify the variations in antioxidant capacity.

It was also already demonstrated that ethanolic extracts of hop cones have better DPPH radical scavenging properties and a higher phenolic content than the leaves (Abram et al., 2015).

For hopped extracts obtained by using different solvent systems, better scavenging activities for DPPH and 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical were reported for those obtained from hulls (IC50 values around 90–2210 μg/mL and 12–138 μg/mL, respectively) than kernels (IC50 values around 580–5630 μg/mL and 52–485 μg/mL, respectively) (Chen et al., 2012). This result evidenced that hopped hull, a usually discarded waste, could be used as source of antioxidants.

3.4. Cytotoxic and hepatotoxic activities

The cytotoxic effects of the hop seed extract against MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), HeLa (cervical carcinoma), and HepG2 (hepatocellular carcinoma) are shown in Table 2. The aqueous extract had cytotoxicity against all these tumour cell lines, with GI50 values ≤ 278 ± 4 μg/mL. The best result was against the NCI-H460 tumour cells, with 184 ± 6.9 μg/mL extract inhibiting 50% of the cell growth. The observed cytotoxic effects could be related to the high catechins content found in the extract. According to Kampa et al. (2000), (±)-catechin and (−)-epicatechin had a good effect on the proliferation inhibition of human prostate cancer cells. Interestingly, the hop extract had no hepatotoxic effects, up to the tested concentration (GI50 values > 400 μg/mL), against the normal cell line CaCo-2, was also investigated (Machado et al., 2017). Isoxanthohumol inhibited cell growth, but xanthohumol did not have anti-proliferative activity.

Previous studies on anti-proliferative and cytotoxic effects of hop compounds reported that xanthohumol (the most abundant prenylated flavonoid in hop cones) has a broad spectrum of chemopreventive and anticancer activities. Miranda et al. (1999) showed that it is highly effective in suppressing the growth of human breast (MCF-7) and ovarian (A-2780) cancer cells. The growth inhibition of MCF-7 cells by xanthohumol and isoaxanthohumol has been associated with inhibition of DNA synthesis (Miranda et al., 1999). Xanthohumol also has strong anti-proliferative activity against A549 lung adenocarcinoma cells and RK33 laryngeal cancer cells via modulation of the extracellular signal regulated kinase (ERK) signalling pathway and induction of apoptosis (Stawińska-Brych et al., 2016, 2015). In turn, Brunelli et al. (2009) showed that 8-prenylharmine inhibits epidermal growth factor-induced MCF-7 breast cancer cell proliferation by inhibiting activation of the PI3K/Akt signalling pathway, which is involved in the control of cell proliferation (Brunelli et al., 2009).

Prostate cancer has also been studied with PC-3 and DU145 cell lines; several prenylflavonoids had growth inhibition activity, and the most promising was xanthohumol (Delmule et al., 2006). Hop prenylflavonoids also inhibit breast cancer Sk-Br-3 cell line proliferation by aromatase inhibition (Monteiro et al., 2007). The anti-proliferative activity of hop compounds in cell lines of colon cancer, HT-29 and CaCo-2, was also investigated (Machado et al., 2017). Isoxanthohumol inhibited cell growth, but xanthohumol did not have anti-proliferative activity.

3.5. Antimicrobial activity

The results of the antimicrobial activity of the hop seed extract against the tested Gram-positive and Gram-negative bacteria are presented in Table 3. The MIC and MBC values obtained for the hop seed extract were better than those of the positive controls in all tested bacteria, except for S. aureus (MIC of 0.075 mg/mL and MBC of 0.15 mg/mL), which were better than ampicillin (MIC of 0.25 mg/mL and MBC of 0.45 mg/mL), but worse than streptomycin (MIC of 0.04 mg/mL and MBC of 0.10 mg/mL). In a previous study, Bogdanova et al. (2018) evaluated the antibiofilm activity of hop cone extracts in different Staphylococcus spp., including methicillin-susceptible and resistant strains, and achieved quite good results, except for S. epidermidis. Lupulone and xanthohumol were identified as the compounds responsible for such activity. A positive antibacterial co-action has also been observed between these two compounds and some antibiotics, such as polymyxin B sulfate, ciprofloxacin and tobramycin (Natarajan et al., 2008). In turn, Abram et al. (2015) reported that hop cone extracts have a powerful antimicrobial activity against S. aureus (MIC < 0.003 mg/mL). However, these hop compounds were not detected in the seeds, which were particularly rich in catechins. According to the literature, these compounds also display antibacterial activity, being able to precipitate bacterial cell proteins and, consequently, to interfere in the

### Table 3

| Antibacterial activity | Hop seed extract | Positive control 1 | Positive control 2 |
|------------------------|------------------|-------------------|-------------------|
|                        | MIC (μg/mL)      | MBC (μg/mL)       | MIC (μg/mL)       |
| Bacillus cereus         | 0.03             | 0.02              | 0.10              | 0.20 |
| Staphylococcus aureus   | 0.075            | 0.15              | 0.04              | 0.10 |
| Listeria monocytogenes  | 0.15             | 0.30              | 0.20              | 0.30 |
| Enterococcus faecalis   | 0.037            | 0.075             | 0.20              | 0.30 |
| Escherichia coli        | 0.15             | 0.30              | 0.20              | 0.30 |
| Salmonella typhimurium  | 0.15             | 0.30              | 0.20              | 0.30 |
| Antifungal activity     | MIC (μg/mL)      | MFC (μg/mL)       | MFC (μg/mL)       |
| Aspergillus fumigatus   | 0.60             | 1.20              | 0.25              | 0.50 |
| Aspergillus ochraceus   | 0.15             | 0.30              | 0.20              | 0.50 |
| Aspergillus niger       | 0.30             | 0.60              | 0.20              | 0.50 |
| Penicillium ochrochloron| 0.15             | 0.30              | 0.20              | 0.50 |
| Penicillium funiculosum | 0.075            | 0.15              | 2.5               | 3.5  |
| Penicillium vernicosum  | 0.15             | 0.30              | 0.20              | 0.30 |

* As positive controls, streptomycin and ampicillin (positive control 1 and 2, respectively) were used for the antibacterial activity, while ketoconazole and bifonazole (positive control 1 and 2, respectively) were used for the antifungal activity.

b Minimal inhibitory concentration.
c Minimal bactericidal concentration.
d Minimal fungicidal concentration.

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formation of the cell wall (Gomes et al., 2018). Table 3 also shows the results of the antifungal activity of the hop seed extract. For P. funiculosum, the MIC and MFC values of the extract (0.075 mg/mL and 0.15 mg/mL, respectively) were lower than those of the positive controls ketoconazole (2.5 mg/mL and 3.5 mg/mL, respectively) and bifonazole (0.20 mg/mL and 0.25 mg/mL, respectively), evidencing the high activity of the extract against this pathogen. The extract also had a MIC value lower than the positive controls against P. ochrochloron (0.15 mg/mL vs. 0.20 mg/mL, respectively), while the MFC (0.30 mg/mL) was only lower than bifonazole (0.50 mg/mL). It is also interesting to note the low MIC and MFC values against A. ochraceus (0.15 mg/mL and 0.30 mg/mL, respectively), which were even better than ketoconazole (MIC of 0.20 mg/mL and MFC of 0.50 mg/mL and 0.30 mg/mL, respectively). Therefore, the extract was more efficient against fungi of the genus Penicillium than Aspergillus. The antifungal activity of a hop cone extract against different fungi, including Penicillium and Aspergillus strains, was demonstrated by Nionelli et al. (2018), which used it as a natural preservative to considerably extend the shelf-life of bread.

4. Conclusions

The recognized antioxidant flavan-3-ols, (+)-catechin and (-)-epicatechin were identified in hop seeds. The extract showed antioxidental activity mainly through the formation inhibition of TBARS generated from the ex vivo decomposition of lipid peroxidation products, and had cytotoxic effects against the tested tumour cells (MCF-7, NCi-H460, HeLa and HepG2), but it was not hepatotoxic against the normal cell line PLP2. The extract showed remarkable antimicrobial properties, being better than the positive controls against all tested bacteria (namely B. cereus, L. monocytogenes, E. faecalis, E. coli, and S. typhymurium) and fungi of the genus Penicillium. These results highlight the potential of hop seeds as a source of functional ingredients (natural extract or isolated compounds) with applications in the pharmaceutical and food sectors (e.g. as natural preservatives, antimicrobials, and health promoters).

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