Therapeutic Potential of Hops

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Contributor: Katya Carbone , Fabio Gervasi

The medicinal potential of hop (*Humulus lupulus* L.) is widely cited in ancient literature and is also allowed in several official pharmacopoeias for the treatment of a variety of ailments, mainly related to anxiety states. This is due to the plethora of phytoconstituents (e.g., bitter acids, polyphenols, prenyl flavonoids) present in the female inflorescences, commonly known as cones or strobili, endowed with anti-inflammatory, antioxidant, antimicrobial, and phytoestrogen activities. Hop has attracted the interest of the scientific community due to the presence of xanthohumol, whose strong anti-cancer activity against various types of cancer cells has been well documented, and for the presence of 8-prenyl naringenin, the most potent known phytoestrogen. Studies in the literature have also shown that hop compounds can hinder numerous signalling pathways, including ERK1/2 phosphorylation, regulation of AP-1 activity, PI3K-Akt, and nuclear factor NF-κB, which are the main targets of the antiproliferative action of bitter acids and prenylflavonoids.

Humulus hops disease prevention

1. Origins of Hops and Early Official Uses

Among the medicinal plants with interesting biological properties is certainly the hop (*Humulus lupulus* L.,), known to most for its use in the brewing industry, but recently also finding increasing use in the medicinal sector. Its strong therapeutic potential is due to the presence, especially in the cones, of a wide range of bioactive molecules, mainly secondary metabolites, some of which characterise the plant itself, such as bitter acids and specific prenylflavonoids such as xanthohumol (XN) or 8-prenylnaringenin (8-PN) [1].

To date, the origin of hops is uncertain, but the presence of the three species of the genus *Humulus* (*H. lupulus, H. yunnanensis*, and *H. japonicus*) in China has suggested that the first hop species appeared in Asia and spread from there eastwards to North America and westwards to Europe [1][2]. Hop pollen has been discovered in archaeological sites in England dating back to 3000 B.C. (Stone Age) and it is known that since ancient Egyptian times, hops were used as a medicinal herb [1][3][4]. During Roman times, hops gained importance for their use in the treatment of liver diseases, digestive disorders, and as a blood purifier. When the Romans occupied Britain, they began to regard hops as a delicacy, using them to make infusions and adding them to cereal fermentations, along with other ingredients used at the time [1]. As tastes and customs changed, all the ingredients used in the fermentation process fell into disuse except for hops, which remained and became a key element in the preparation of beer. The oldest report on the medicinal uses of hops can be found in a book dating back to the Middle Ages, specifically to the 11th century, in which the Arabian physician Mesue described the anti-inflammatory properties of this perennial herb [5]. In the 13th century, the Arabian botanist Ibn al-Baytar highlighted the soothing

properties of hops [6]. Between 1300 and 1600, there was a widespread use of hops as a remedy for fevers, spleen disorders, as a diuretic, and for liver purging [5]. In North America, various indigenous tribes used hops as a remedy for various ailments [1]. The Delaware, for example, used it for earache and toothache; the Cherokee used it against sleeping disorders [7]; the Navajo against coughs and colds; and the Dakota used hop infusions as a cure for intestinal disorders and wound healing [8]. Hops were also used in Ayurvedic medicine, a traditional medicine used in India since ancient times and still widespread in the subcontinent today [9][10]. Although there was presumably no exchange of knowledge on the action of hops, the uses of the plant were common between the various continents. In this regard, one can consider this parallel use as indirect proof of the effectiveness of hops in medical applications. In 1820, the physician Ansel W. Ives examined the resin glands of hops and first proposed the name “lupulin”, also pointing out its peculiar sedative properties [11]. The name “*Humulus lupulus”* was given by Carl Linnaeus. The term *Humulus* is thought to derive from the Latin *humus* (earth), alluding to the plant’s flexible stems resting without a support on the ground; *lupulus*, on the other hand, may originate from the Latin word ”*lupus”*, justified, according to some, on the basis of a reference by Pliny the Elder (23–79 AD), who referred to the plant as the “wolf of the willows” [1]. In contrast, the common name “hop/hops” seems to be of Anglo-Saxon derivation (hoppan: to climb; [12])

2. Sedative and Neuroprotective Activities

Sleep disorders are quite frequent in the population worldwide and may lead to health problems. The use of complementary therapies based on botanical extracts and herbs are evidenced to be a powerful ally, in addition to standard drugs and psychological interventions, for treating insomnia and anxiety [13].

The use of hops in traditional medicine as a mild sedative arose from the experience of drowsiness and chronic forms of fatigue that the pickers and those who worked with or handled hop inflorescences manifested [14]. An ancient folk remedy is the sleeping drink of fresh hop cones, which are also sealed inside a pillow to be held under the head during the night in order to facilitate sleep. The European Medicine Agency classifies hop herbal preparations as “traditional herbal medicinal products” to be used for the relief of mild symptoms of mental stress and to aid sleep [Lupuli flos. European Medicines Agency. Available online: https://www.ema.europa.eu/en/medicines/herbal/lupuli-flos, accessed on 10 August 2022).

In 1980, Hänsel and his collaborators identified the compound to which to ascribe this activity, namely a degradation product formed during the storage process of humulone and lupulone by auto-oxidation: 2-methyl-3-buten-2-ol [15]. The molecule, which is a volatile tertiary alcohol, is also found in hop EO [16]. This molecule increases the activity of the neurotransmitter γ-aminobutyric acid (GABA), inhibiting the central nervous system. Franco et al. (2012) demonstrated in a diurnal animal model that the concentration of 2 mg of hop extract effectively decreased nocturnal activity in the circadian rhythm [17]. However, the concentration of 2-methyl-3-buten-2-ol in hops is too low to justify the plant’s sedative activity alone, suggesting synergy with other compounds present in the volatile fraction such as linalool [16]. Moreover, recent literature studies have highlighted the possible role of other phytocompounds in determining the sedative potential of hops [18]. Among phloroglucinol derivatives, α-acids are considered the main phytochemical class with significant pentobarbital properties, while lupolones

mainly express antidepressant and sedative activities [1][19]. It is hypothesised that these sedative effects are mediated by an increase in the function of $GABA_A$ receptors, which are responsible for fast-acting inhibitory synaptic transmission in the brain.

Other hop phytoconstituents that possess sedative effects include 8-PN. From a stereochemical point of view, this compound has a single chiral carbon centre in its structure, leading to two enantiomers, with enantiospecific bioactive properties that may significantly influence the pharmacological, toxicological, and pharmacokinetic profile of 8-PN [20]. Bagatin et al. (2014) investigated the effectiveness of a long-term administration (21 d) of a racemic mixture of 8-PN to rats submitted to the elevated T-maze (ETM) model of generalized anxiety and panic disorders. The pharmacological trials demonstrated no effects following an 8-PN acute treatment (10 mg/kg), confirming the existence of a latency period until the onset of the therapeutic action. Moreover, using docking simulations, the authors found that between the two enantiomers, (R)-8-PN had a greater affinity (lower inhibition constant) for all transporters tested (serotonin, norepinephrine, and dopamine ones) than enantiomer (S)-8-PN, indicating that the panicolytic effect observed in the animal model can be due mainly to the R enantiomer of the racemic mixture used [21].

Recently, Benkherouf et al. (2020) proposed that the neuroactivity of hops may involve more than one phytocompound, whose synergistic action leads to enhanced $GABA_A$ receptor function. Indeed, the researchers highlighted the role of IX and 6-PN in enhancing the effects exerted by humulone on this receptor [22]. The authors also found that a low dose of humulone (10 mg/kg) increased ethanol- but not pentobarbital-induced sleep duration, also pointing out a non-competitive synergy of humulone with ethanol at $GABA_A$ receptors, which could be responsible for further increased alcohol intoxication with high-hopped beers. The synergistic effect in promoting sedative activity, however, is not only observed in the additive action of hop phytoconstituents but has also in the combined action of plant essences such as hops and valerian. In this regard, Morin et al. (2005) reported that a valerian–hop combination and diphenhydramine might be useful adjuncts in the treatment of mild insomnia [23]. In addition, it has been observed that a mixture of valerian and hop (cv. ”Cascade”) demonstrated a higher binding ability on GABA receptor than valerenic acid or/and XN, which are estimated to be active compounds in the extract tested, improving sleep-related behaviours, including sleeping time, by modulating GABAergic/serotonergic signalling [24].

Several studies have investigated the possible role of hop phytoconstituents in the treatment of age-related neurodegenerative diseases. Iso-α-acids have been found to activate peroxisome-proliferator-activated receptor-g (PPAR-g)^2, a known therapeutic target in Alzheimer’s disease (AD), highlighting a possible role of these compounds on the pathogenesis of AD [25][26]. Ano et al. (2017) demonstrated, in a primary microglia cell culture, that both isohumulone epimers were able to significantly increase β-amyloid phagocytosis by increasing CD36 expression, with trans-isohumulone being more active in this process. In contrast, trans-isoadhumulone and cis-isoadhumulone did not show any significant effect in *in vitro* experiments. Moreover, the authors demonstrated in experiments on an animal model of AD that iso-α-acids administered orally (0, 4, or 20 mg/kg iso-α-acids once a day for 3 days) could penetrate the rat’s brain and that cerebral microglia demonstrated increased anti-inflammatory capacity and β-amyloid phagocytosis [27]. In addition, a single intragastric administration of hop iso-α-

acids (0.02–2 mg/Kg), in a rat model, was shown to increase total and extracellular levels of dopamine and its metabolites in the hippocampus, though not in the frontal cortex, in a vagus-nerve-dependent manner, thereby improving hippocampus-dependent memory. The dose equivalent for humans, according to the authors, should be of 0.03–3 mg/Kg, i.e., for a 70 kg person, 2 and 20 mg of active constituents [28].

During storage, hop bitter acids undergo a series of oxidative processes that give rise to oxides generally referred to as matured hop bitter acids, which share with iso-α-acids the β-tricarbonyl moiety (2-acetyl-3-hydroxy-2-cyclopenten-1-one) believed to contribute to vagal activation, also by increasing the levels of noradrenaline [29][30]. These compounds suppressed the activation of microglia and memory impairment observed in AD mouse models, an effect mediated by the noradrenergic system. Albeit with obvious limitations, which will have to be overcome through increased research in this area, these results open up new scenarios towards the use of bioactive hop compounds in the prevention of neurodegenerative diseases.

3. Antimicrobial and Antiviral Activity

Since ancient Egypt, hops have been used for food preservation and later used for extended storage of beverages, such as beer [14]. The addition of hops reduces the growth of *Lactobacillus*, the main contaminant in beer, which causes losses in ethanol production and the formation of undesirable flavours [31]. Several studies reported an inhibitory activity of bitter acids towards Gram-positive bacteria, such as *Lactobacillus*, *Streptococcus*, *Staphylococcus*, *Micrococcus,* and *Bacillus*, and fungi, such as *Penicillium* and *Aspergillus* species [32][33]. It has been reported that hop bioactive compounds are also active against Gram-negative bacteria such as *Helicobacter pylori* and *Brucella* species [33][34].

Hop bioactive compounds can exhibit either bacteriostatic or bactericidal activity depending on the bacterial growth conditions [35]. In general, among bitter acids, lupulone has greater antimicrobial activity than humulone, which is, in turn, more active than isohumulone. Several molecular mechanisms of action have been proposed to explain these observed effects, the lipophilic region of the bacterial cell membrane being one of the main target sites of hop bitter resins [36][37]. Behr and Vogel (2010) proposed two different mechanisms of hop bacterial inhibition: proton-ionophore-induced and oxidative-stress-induced mechanisms [38]. Michiu et al. (2019) evaluated the inhibitory effects of hop iso-α and -β acids against the bacterium *Pediococcus pentosaceus* at both high (6.0–7.0) and low (4.0–5.0) pH values, testing whether the identified iso-α acid stress altered *S. cerevisiae boulardii* yeast activity and ethanol production [39]. Results pointed out an inhibitory effect of both iso-α and β-acids against *P. pentosaceus* at the pH values tested, opening the possibility of hops being used as a supplement to prevent beverage contamination with spoilage microorganisms.

Several other hop phytoconstituents have shown antimicrobial and antiviral activities. Cermak et al. (2017) tested the antimicrobial activity of purified hop constituents humulone, lupulone, and XN against some anaerobic bacteria of indigenous human flora (*Bacteroides fragilis, Clostridium perfringens, Clostridium difficile*) [35]. Results reported by the authors demonstrated that XN exhibited the highest antimicrobial activity against all three microorganisms,

followed by β-acids and α-acids; they also highlighted a different course of inhibitory effects between bitter acids and the prenylflavonoid studied.

Fahle et al. (2022) reviewed the antibacterial activities of humulone, lupulone, and XN, also underlining a synergistic effect when used in combination with antibiotic drugs, not only on Gram+ but also on Gram- bacteria. In addition, the authors pointed out the paucity of *in vivo* studies that could support a real application of these valuable phytochemicals from the laboratory to the clinical setting [40]. Terpenes from hops also demonstrated moderate antimicrobial effects against Gram-negative bacteria (e.g., *Proteus vulgaris*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* spp.), and Gram-positive bacteria (e.g., *Enterococcus faecalis* and *Staphylococcus aureus*) [32].

Serkani et al. (2012) evaluated the antimycobacterial effect of ethanolic hop extracts (4 and 8 mg/mL) on rifampin-sensitive and -resistant strains of *Mycobacterium tuberculosis*. The results demonstrated that hop extracts completely inhibited all Mycobacteria strains tested, comparably to rifampin, with minimum inhibitory concentration (MIC) values ranging from 400 to 800 µg/mL, indicating the effectiveness of hop phytocompounds to control tuberculosis *in vitro* [41].

Recently, Blaxland et al. (2022) evaluated fifty aqueous hop extracts from different hop varieties provided both whole and pelleted against *Mycobacterium bovis* BCG, showing that all extracts tested exerted inhibitory activity ranging from 1.2 mm to 15.7 mm depending on the hop cultivar. ”Citra” was the most active, with a MIC and a minimum bactericidal concentration (MBC) of 16% *v*/*v* [42]. An increasing amount of scientific research is focusing on the study of natural compounds as alternative agents to manage the treatment of infections caused by multidrug-resistant bacteria, such as methicillin-resistant *Staphylococcus aureus* and multidrug-resistant *Staphylococcus epidermidis*, which, together with *Cutibacterium acnes*, are the main strains involved in skin diseases. Recently, Di Lodovico et al. (2020) investigated the antimicrobial and antibiofilm properties of an hydroalcoholic hop extract (cv. “Cascade”) against staphylococcal strains and *C. acnes,* including multiresistant isolates. The results highlighted a strong antibacterial action of the extract against the anaerobic *C. acnes* (MIC:1 µg/mL) and a significant reduction in biofilms formed in the presence of subinhibitory concentrations of hop extract [43]. The susceptibility of *P. acnes* and *S. aureus* to a hop CO_2-extract with 50% humulone and lupulone was tested *in vitro* (MIC values: 3.1 and 9.4 µg/mL, respectively). The extract also exhibited an additional anti-inflammatory effect by reducing the IL-6 expression (IC_{50}: 0.8 µg/mL). The authors also demonstrated the efficacy of a gel formulation with 0.3% of the extract (*w*/*w*) as an antibacterial agent against the two strains tested, significantly superior to the placebo gel [44].

Hop extracts are also reported to inhibit some fungal strains such as *Candida*, *Fusarium*, *Trichophyton,* etc. [34][45]. Yan et al. (2021) reported a moderate antifungal activity of an ethanolic extract of *H. lupulus* against five phytopathogenic fungi (*Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Botrytis cinerea*, *Fusarium graminearum*, and *Magnaporthe oryzae*; inhibition rate: 37–51% at 500 µg/mL) [46]. Moreover, the authors reported that IX was effective against *S. sclerotiorum*, *F. graminarum*, and *B. cinerea*, for the latter both *in vivo* and *in vitro*. The prenylflavonoid inhibited the spore germination of *B. cinerea* in a dose-dependent manner, causing the destruction

of the cell membrane by membrane lipid peroxidation, which caused final damage to the fungal mycelia. Among the hop prenylflavoinoids, 8-PN has been shown to exert an important antifungal activity against *Tricophyton mentagrophytes*, the most common fungal agent in mice, which can also be transmitted to humans, with a MIC equivalent to that of griseofulvin (6.25 mg/L), a natural antibiotic extracted from a species of *Penicillium*, whose antifungal effect is achieved through inhibition of the mitotic process. That being said, the prenylflavonoid demonstrated no inhibitory activity against *Candida* and *Fusarium* strains tested. Similar results were reported by Macchioni et al. (2021), who did not observe any growth inhibition when testing green hop extracts against different *Saccharomyces cerevisiae* (non-pathogenic yeast) strains and against *Candida albicans* (the most common human fungal pathogen), indicating that the effect of these extracts was specific for prokaryotic cells [33].

Jiang et al. (2023) found that hop EO nano emulsion (d < 145 nm) displayed antifungal activity against *F. graminearum* growth and demonstrated mycotoxin-inhibitory activity, suppressing the production of deoxynivalenol. According to the authors, IX exerted its action by altering the total lipid and chitin content in the outer cell membrane and by impairing the permeability of the cytoplasmic membrane [47]. Bocquet et al. (2018) evaluated *in vitro* the antifungal activity of crude extracts of different hop tissues (cones, leaves, rhizomes, and stems; cv. “Nugget”), as well as the EO from the strobile, against *Zymoseptoria tritici,* responsible for Septoria tritici blotch, a devastating foliar disease of wheat. All tested samples demonstrated antifungal activity against *Z. tritici*, relevant only in the case of crude extract and EO, with a decrease of 85% and 100% in colony diameter at 1.25 g/L, respectively. Among purified phytochemicals from crude extract, only co-humulone and DMX demonstrated an inhibitory effect against *Z. tritici* in a dose-dependent manner. In addition, the authors highlighted a synergistic effect against the pathogen by using hop essential oil in combination with the synthetic fungicide bixafen, the antifungal activity of which was improved up to eight times in combination with hop oil [48].

Finally, hop phytochemicals have been demonstrated to also be active against some viruses, such as oral herpes virus infections, influenza, hepatitis C, HIV-1, and SARS-CoV-2 [34][49][50].

4. Antitumour Activity

Hop bitter acids affect cancer through the induction of controlled cell death in malignant cells [19]. This was first reported by Tobe and coauthors in 1997 when they observed the ability of humulone to induce apoptosis in leukaemia cells [51]. Lupulone, colupulone, and especially hexahydrocolupulone, a semysinthetic derivative of colupulone, inhibit the growth of several human cancer cell lines [52]. Saugspier et al. (2012) analysed the effects of bitter acids on tumorigenicity of hepatocellular carcinoma cells *in vitro*, pointing out that different cellular pathways, such as ERK1/2 phosphorylation, regulation of AP-1 activity, and nuclear factor NF-κB, are the main targets of the antiproliferative action of bitter acids, with β-acids more active than α-acids [53]. Furthermore, Lin et al. (2019) examined the antitumour potential of tetrahydro-, hexahydro-iso-α-acids, and rho-iso-acids (prepared from a modified hop extract), highlighting the ability of the latter to interfere with the prostaglandin E2 metabolic pathway by inhibiting its biosynthesis [54].

As already pointed out in the previous section, bitter acid oxidation compounds, present in greater quantities in hop pellets as they are more exposed to oxygen during the production process, may also potentially play an important role in cancer treatment, as adjuvants in chemotherapeutic protocols. In this regard, Salviati et al. (2019) demonstrated that a subfraction of a hexane extract of hop pellets, consisting mainly of humulinones and cohulupone derivatives, can act as a stimulator of natural killer (NK) cells, inducing, at a dose of 0.1 mg/mL, the selective activation of the NKp44 receptor, while simultaneously enhancing the cytolytic activity of NK cells against the leukemic K562 cell line. The authors also pointed out that the observed effect was dose-dependent, as higher-dose treatment (1 mg/mL) significantly reduced NKp44 expression [55].

Over the years, attention has been focused on the antitumour and chemo-preventive potential of XN as a multi-target compound able to modulate in a different way several signalling pathways and compounds involved in tumorigenesis [4][56]. Its action is dose-dependent, inducing a cytoprotective mechanism at low concentrations (0.01 µM), while leading to apoptosis and cellular cytotoxicity at higher concentrations (5 µM) [57].

Hsieh et al. (2022) investigated the apoptotic effect and anticancer properties of XN in human nasopharyngeal carcinoma cell (NPC) lines. Results indicated that XN effectively induced the upregulation of the c-Jun N-terminal kinase in the mitogen-activated protein kinase, promoting the apoptosis of NPC [58]. Recently, several authors have also suggested that the anticancer properties of XN may be due to its prooxidant activity, as it is able to induce ROS generation through NADPH oxidase in a dose-dependent manner [56][59][60]. Blanquer-Rossellò et al. (2013) found that XN increased ROS levels in breast cancer cells by improving mitochondrial function at high concentrations [57]. Moreover, Stevens (2020) found that XN can inhibit cytochrome P450 enzymes, which metabolically activate procarcinogens and induce the carcinogen-detoxifying quinone reductase and pro-angiogenic pathways [61]. Cancer cells are characterized by a higher level of ROS than healthy ones and, consequently, their ROS threshold for apoptotic induction is higher; in this regard, XN may be a promising chemotherapeutic agent, especially if used in combination therapy, since malignant cells cannot develop resistance against the mitochondrial uncoupling effects of prenylated flavonoids [61].

From a critical analysis of the literature, it appears that the mechanisms underlying the anticarcinogenic action of XN are related to the inhibition of two signalling pathways implicated in the onset of malignancy and the metastatic process: Akt (or PI3K-Akt) signalling pathway that promotes a cell's survival and growth in response to extracellular signals, and NF-κB (nuclear factor kappa light chain enhancer of activated B cells), a family of highly conserved transcription factors that regulate many important cellular behaviours, in particular inflammatory responses, cellular growth, and apoptosis [62]. In addition, the antimetastatic effect of XN also appears to be due to the inhibition of the MAPK/ERK pathway (also known as the Ras-Raf-MEK-ERK pathway) [63]. The anticancer action of XN has also been reported for several tumour types such as gastric and colorectal cancer [64]. In this regard, Turdo et al. (2021) highlighted the synergistic effect of XN in enhancing the efficacy of nobiletin, a polymethoxyflavone derived from *Citrus sinensis*, in suppressing colorectal cancer stem cells, underlining the adjuvant potential of this mixture in cancer therapies. The authors found that this mixture of bioactive compounds was able to suppress the migration of cancer stem cells, reducing the expression of CD44v6 and inducing apoptosis and cycle arrest in the G2/M phase [65].

Among hop prenylflavonoids, not only XN has attracted the attention of scientists for anticancer potential. Wang et al. (2016) tested an enriched hop extract containing 1.2% 6-PN, 0.33% 8-PN, 0.99% IX, and 32% XH and its pure bioactive compounds for their effects on oestrogen metabolism in breast cancer cells (MCF-10A and MCF-7). Interestingly, the authors found that only 8-PN demonstrated slight up-regulation of metabolism in MCF-7 cells, whereas IX and XN did not have significant effects in either cell line [66]. Krajnovic et al. (2022) evaluated the anticancer potential of XN (5% *w*/*w*) and IX (3.5% *w*/*w*) loaded into mesoporous silica nanoparticles (as delivery/targeting system; pore diameter: 5.44 nm) against malignant mouse melanoma B16F10 cells, underlining that the main mechanism against tumour cells includes inhibition of proliferation and autophagic cell death [67]. Hajirahimkhan et al. (2022), in their preprint, demonstrated that 8-PN suppresses aromatase expression in postmenopausal women's breast tissue, thus standing for a key role in breast cancer prevention for high-risk postmenopausal women [68].

Recently, Ramazzina et al. (2022) also investigated the potential antitumour effects of hop bioactive compounds on cell viability, intracellular ROS production, and phase II enzyme activation, comparing the results with those obtained by treating Caco2 cells with Polyphenon E, a standardized green tea extract approved by the Food and Drug Administration (FDA). The authors highlighted the role of the extraction process and the chosen solvent on the biological properties of hop extracts towards target cells, explaining the observed in terms of the inhibitor–promoter pair using chemometric models. The results demonstrated the crucial role of molecules such as ferulic acid (promoting)–adlupolone (inhibitor), and coumaric acid (promoting) + protocatechuic acid (inhibitor), respectively [60].

In addition, Rutnik et al. (2021) examined the antitumour potential of hop EO, highlighting the most intense activity of b-caryophyllene and ß-caryophyllene oxide [16]. EO and hydrolate from hop cones (cv. "Chinook") were also tested *in vitro* to investigate their apoptotic potential against several cancer cell lines (human acute promyelocytic leukaemia cells, human neuroblastoma cells, human metastatic mammary adenocarcinoma cells, human mammary adenocarcinoma cells, and a normal mammary epithelial cell). The results demonstrated that the hydrolate was less active than the corresponding EO, which demonstrated greater selectivity against the HL60 leukemic cell line than the other cancer cell lines tested, while also showing cytotoxicity on the normal cell line. In addition, the results demonstrated the lower cytotoxic activity of hop extracts compared with hemp extracts on virtually all cell lines tested [69].

5. Antioxidant Activity

Hops contain several biomolecules with high antioxidant potential such as flavonols, found in the strobili mainly in glycosidic form, such as rutin (quercetin-3-rutinoside) and astragalin (kaempferol-3-glucoside) [6].

In 1995, Tagashira et al. demonstrated that hop bitter acids possess a high antioxidant potential and also a lipid peroxidation inhibitory activity [70]. Recently, Wang et al. (2022) found that the antioxidant activity of β-acid extracts with different contents of colupulone (30%, 50%, 70%, 90%, and 100%) differed in a dose-dependent manner [71]. In addition, Yang et al. (2020) demonstrated that a mixture of colupulone and n-lupulone + ad-lupulone (1:4)

possessed greater antioxidant activity than colupolone alone [72]. In general, α-acids exhibit higher scavenging potential than the corresponding iso-α-acids towards both OH radicals and lipid peroxidation [73]. Wang et al. (2014) evaluated the antioxidant activity of a high purity hop polyphenol extract (total phenolic content: 887 mg/g) both *in vitro* and *in vivo*, comparing their results with the effects produced by green tea polyphenols. The authors found that the scavenging effects of hop polyphenols on DPPH, •OH, and $O2^{\bullet-}$ radicals were superior to those of tea ones and that hop polyphenols were able to counteract oxidative DNA damage in a dose–response manner over the range of concentrations tested (0.1–0.3 mg/mL). *In vivo* trials on mice pointed out that a hop polyphenol dose of 400 mg/kg body weight demonstrated the most beneficial effects in terms of protection from bromobenzene-induced lipid peroxidation in mouse liver, highlighting a protective effect of these polyphenols on antioxidant enzymes (i.e., superoxide dismutase and glutathione peroxidase) from damage by reactive oxygen species *in vivo* [74].

Recently, Santarelli et al. (2022) demonstrated that ultrasound-assisted extractions of hop cones (cv. “Cascade”) provided samples characterized by the highest antiradical capacity, assessed using both ABTS and DPPH assays [75]. The authors demonstrated that not only the type of extraction technique applied, but also the process parameters (i.e., temperature, extraction time, ultrasound power) statistically influenced the antiradical capacity of the extracts produced. Similarly, Macchioni et al. (2021) found that tailor-made natural deep eutectic solvents profoundly affected the antiradical capacity of hop extracts. In the study, the authors found that the eutectic solvent mixture lactic acid: glycine (molar ratio 3:1) was able to produce hop extracts with the highest values of antiradical capacity compared to all the samples tested [33].

Interestingly, other plant tissues besides cones also help in defining the antioxidant potential of hops. In this regard, Maietti et al. (2017) evaluated the antioxidant potential of wild hop shoot samples from different locations. Samples analysed demonstrated an antiradical capacity, expressed as mg of Trolox equivalents per g of shoots (fresh weight), ranging from 0.68 to 1.07 mg/g, depending on the location considered [76].

Bitter acids and polyphenols are not the only ones responsible for the antioxidant potential of hop cones. Maliar et al. (2016) analysed the antioxidant capacity of methanol extracts of eight Czech hop cultivars and found that DPPH• did not correlate with any compounds in the cone extracts, while ABTS radical scavenging correlated highly with α-bitter acid content, DMX, and hop EO, but not with total phenolics and total flavonoid content. The latter families of compounds, however, were well correlated with the results from the FRAP assay. Among the cultivars analysed, the best scavenging ability was exerted by Saaz Late, while Agnus was the best in reducing ions of the transition metals tested [77]. Regarding the antioxidant potential of prenylflavonoids, in their very interesting review of the biological activity on 8-PN, Pohjanvirta and Nasri (2022) pointed out some discordant literature data. The main critical issues seem to be related to the type of analytical assay used, particularly the nature of the radical employed. The authors also pointed out a dose-dependent antioxidant activity of 8-PN, on average lower than that of XN [78]. Finally, Kontek et al. (2021), in their recent study, fractionated the powder of the freeze-dried hop cone (cv. “Marynka”), obtaining a fraction (A) rich in bitter acids, both humulones and lupulones, and a second fraction (B) dominated by XN and α-acids, characterised by a different antiradical potential, which was, however, rather moderate for both fractions. Fraction A demonstrated an overall higher antioxidant potential than fraction B;

however, both fractions were able to attenuate oxidative stress by 65–95% in lipid peroxidation and protein carbonylation tests [79].

6. Estrogenic Activity

Research on the estrogenic activity of hops arose from the observation on the occurrence of menstrual disorders commonly contracted by hop cone pickers, to the extent that they had the onset of their cycle two days after the start of inflorescence harvesting, regardless of the time of the cycle they were in [80]. The World Health Organisation reports that the component showing estrogenic activity is 8-PN, the most potent phytoestrogen known to date [81]. It has been reported that the estrogenic activity of hops corresponds to the presence of the equivalent of 20–300 g 17-β-estradiol/g [82]. Particular attention has also been paid to IX, which is a methylated derivative of 8-PN. This molecule has no estrogenic activity; however, *in vivo,* it is demethylated by bacteria in the intestinal flora, leading to 8-PN, which implies that hop products may be sources of more 8-PN than that present in the cone itself, thus increasing its estrogenic potential [78].

The degree of estrogenic activity of a molecule is generally determined by the affinity of the compound for the estrogenic receptors. Most phytoestrogens show a preference for ERβ; in contrast, 8-PN binds predominantly to ERα and is classified as a selective natural estrogenic receptor modulator [83][84]. Moreover, it has been reported that both 8-PN enantiomers displayed high affinity and selectivity for ERα, but S-8-PN exhibited an overall higher affinity for both receptors than R-8-PN, using recombinant human oestrogen receptor (ER)-α and ER-β from cytosolic SF9-cell extracts [85]. Other structurally related hop flavonoids, such as 6-PN, have little estrogenic activity [86]. DMX, on the other hand, is considered a pro-estrogenic because it can isomerise, giving 8-PN, and, unlike it, does not activate hormone receptors [87]. Due to their like-oestrogen activity, hop prenylflavonoids may be suitable to compensate for the reduced levels of 17-β-estradiol during menopause [88]. However, Zanardi et al. (2022) suggested caution in the use of hop preparations as an alternative to hormone replacement therapy to alleviate postmenopausal symptoms, due to the role of oestrogen compounds in the development of endometrial cancer. In this regard, the authors demonstrated, through *in vitro* experiments on Ishikawa cell lines, that 6-PN and hop extract activate the ERa receptor and aryl hydrocarbon (AHR) signalling pathways, with 6-PN being able to increase tumour suppressor gene expression and the expression of genes involved in oestrogen metabolism, by upregulating the expression of cytochrome P450 1A1 (CYP1A1), which is involved in the oestrogen detoxification mechanism, to a greater extent. Of the samples tested, neither hop extract nor 8-PN were able to act on the detoxification pathway, but only on the genotoxic pathway, highlighting the key role of 6-PN as a potential modulator of oestrogen metabolism by virtue of its ERa and AHR agonistic activity [89].

7. Other Bioactivities

Thanks to its plethora of bioactive constituents, hops have been evaluated for their bioactive potential in the treatment of metabolic disorders [90]. It has been shown through experiments on insulin-deficient diabetic mice that 8-PN can act as an ERa agonist in the regulation of glucose homeostasis and also protect, with an effect

comparable to that of naringenin, the pancreas from cell apoptosis and inflammatory responses [91]. Furthermore, XN and its hydrogenated derivatives can be considered a drug candidate for the treatment of metabolic syndrome by having a good anti-obesity activity, inhibiting differentiation of preadipocytes and inducing mature adipocyte apoptosis, thereby decreasing the risk of hypercholesterolemia and dyslipidaemia [92]. Improvements in metabolic syndrome by XN derivatives are linked to altered gut microbiota and bile acid metabolism [93]. Recently, Ponticelli et al. (2021) reviewed the potential of iso-α-acids as adjuvant in metabolic syndrome treatment, acting as agonists on peroxisome proliferator-activated receptors, which play a regulatory role in energy homeostasis and metabolic function [94]. Hop bitter acids stimulate salivation and secretion of gastric juice, as well as secretion of mucopolysaccharide-rich mucus, thereby facilitating digestion and absorption of food, consequently increasing appetite [95].

Treatment with crude hop extract, enriched in bitter acids, may modulate early satiety, which is associated with impaired gastric accommodation and gastric emptying, as these substances are potent ligands for human bitter taste receptors (T2R), with activation thresholds as low as 3 nM [96].

In addition, *in vitro* and *in vivo* studies highlight important biological properties of hop bitter acids and derivatives, including inhibition of bone resorption and anti-inflammatory (COX-2 inhibitory activity) activities [1][90].

Regarding prenylflavonoids, XN has been evidenced to be a drug candidate for the treatment of diabetic skin ulcers by increasing Nrf2 activation. The authors observed that, in a diabetic animal model, 2.5 mM XN was able to increase collagen deposition, promoting the activation of Nrf2 both by AMPKa activation and Keap1 cystein modification [97]. Finally, Frackowiak et al. (2010) found that hop extracts rich in sugar alcohols (mainly *myo*-inositol) and organic acids (i.e., glucuronic and malic acids) were effective in dissolving kidney stones, while also being non-mutagenic and non-cytotoxic [98].

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