Phytochemical and Ethnopharmacological Review of *Humulus lupulus L.* on its Traditional Uses and Biomedical Potential

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Abstract

Background: Hops, derived from Humulus lupulus L. (Cannabinaceae), have been used in traditional medicine for treating various complaints. This review explores the morphological, phytochemical, and ethnopharmacological aspects of hops, summarizing key findings from preclinical and clinical research and providing a critical appraisal of their traditional use and pharmacologic characteristics. The primary aim is to evaluate the botanical, phytochemical, and pharmacological properties of hops and their traditional and modern applications, particularly in managing menopausal symptoms and potential chemopreventive activities. Methods: This review synthesizes data from various studies on hops, including preclinical and clinical research, in vitro and in vivo investigations, and traditional medicinal uses. It also examines the sedative activity and other pharmacological effects of hop extracts. Discussion: Hop extracts have demonstrated significant anti-inflammatory, antioxidant, and anti-lipoperoxidation activities. In vitro studies suggest that hops possess antiangiogenetic, antiproliferative, and apoptotic effects, indicating potential chemopreventive properties. Hop terpene phenolics, crucial for beer flavoring, have garnered interest in biomedical research. Notably, prenylated chalcones like xanthohumol exhibit cancer chemopreventive activity, and 8-prenylnaringenin is recognized as a potent phytoestrogen. Conclusion: Hops hold promise for various medicinal applications, supported by both traditional use and scientific research. While compounds like xanthohumol and 8-prenylnaringenin show significant potential, further studies are necessary to fully understand their biomedical applications and to harness their benefits effectively.

Keywords: Humulus lupulus, phytochemicals, traditional medicine, pharmacological properties, chemoprevention

Significance | This review describes hops' traditional medicinal uses and their emerging biomedical applications, emphasizing their anti-inflammatory, antioxidant, and chemopreventive potential.

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1. Introduction

The plant *Humulus lupulus L.* is well-known throughout the world as the raw material in the brewing industry. The female inflorescences (hop cones or "hops"), rich in polyphenolic compounds and acyl phloroglucinol are widely used to preserve beer and to give it a characteristic aroma and flavour. In addition, hop cones have long been used for medicinal purposes. In particular, hop preparations were mainly recommended for the treatment of sleeping disorders, as a mild sedative, and for the activation of gastric function as a bitter stomachic. In line with a growing interest in the health benefits of plants used in traditional medicine, *Humulus lupulus* has received particular attention by researchers and, as a result, a significant number of articles have been published. Starting from the second half of the 20th century,

several phytochemical studies were performed to investigate the composition of hop cones and other parts of the plant, leading to the isolation and identification of pharmacologically relevant compounds such as flavanones, chalcones, phloroglucinol derivatives.

During the past decade, many pharmacological investigations in vitro and in vivo tried to produce scientific evidence of the reported traditional uses. The effect of hop plant at the central nervous system (CNS) level and in particular its efficacy in sleeping disturbances has been repetitively studied in laboratory animals, but the results are sometimes contradictory and require a thorough reinvestigation.

Moreover, the number of clinical studies supporting the use of hops as a sedative is rather limited: therefore, the effectiveness of hops in the treatment of sleeplessness is still questionable. In recent years the estrogenic properties as well as the potential cancer chemopreventive activities of hops have been investigated and some active compounds from hops have received much attention. Among these, 8-prenylnaringenin is considered one of the most potent phytoestrogens currently known, while xanthohumol proved to possess a broad spectrum of cancer-inhibiting mechanisms.

Starting from the current knowledge about the traditional use of hops and its botanical, phytochemical, and pharmacological characteristics, the present review provides a critical appraisal of the ethnopharmacological issues. Particularly we focused our attention on the effect of the hop plant on CNS, comparing the results obtained in our laboratory and already published in this journal (Zanoli *et al.,* 2005, 2007) with those obtained by other authors. Other effects of the hop plant, such as the estrogenic and cancer-related bioactivities, are only briefly discussed since comprehensive reviews have been recently published (Stevens and Page, 2004; Gerhauser, 2005a; Chadwick *et al.,* 2006).

1.1 Phytogeography

The genus Humulus, belonging to the family of Cannabinaceae, consists of three species: *Humulus lupulus* Linneus, Humulus japonicus Siebold & Zucc. and Humulus yunnanensis Hu (Small, 1978; Neve, 1991). The origin of the genus has been suggested to be China because all Humulus species were found in this area (Small, 1980; Neve, 1991; Murakami *et al.,* 2006a). From China, an eastward migration to Japan and America and a westward migration to Europe should be responsible for the actual distribution of Humulus species (Figure 1).

Humulus lupulus (commonly named hops) is naturalized in central Europe and it is widely cultivated throughout the temperate regions in the world (North and South America, South Africa, Australia). The species *Humulus lupulus* has been classified by Small (1978) into five taxonomic varieties based on their morphological characteristics and geographical locations: the var. lupulus Small for European hops, the var. cordifolius Small for Japanese hops, the var.

neomexicanus Nelson & Cockerell, the var. pubescens Small and the var. lupuloides Small for North American hops.

The molecular phylogeny, based on the nucleotides of nuclear and chloroplast DNA, has demonstrated a clear difference among North American, Asian, and European hops, with the divergence of European hops from the others occurring approximately one million years ago, followed by the diversification of Asian and North American hops, which are genetically close (Murakami *et al.,* 2006a,b). The cultivation of *Humulus lupulus* for a long time caused the existence of hundreds of named cultivars and many recognized chemotypes (Neve, 1991). The major reason was the need to select specific organoleptic properties to improve the flavor and aroma of beer, besides producing different bitterness levels (Chadwick *et al.,* 2006). Therefore, cultivars with an increased content of volatile oil or bitter acids have been selected (Burgess, 1964; Neve, 1991).

2. History and ethnopharmacology

As reported by Behre (1999), in Europe there are only a few single findings of *Humulus lupulus* from prehistoric periods, while there is an increased number of sites and quantities of findings from the early Middle Ages, probably due to an increased utilization of the plant in the brewing process. The oldest sources of *Humulus lupulus* in Europe and its use in brewing were described by Wilson (1975). At the earliest, the plants were collected in the wild. The cultivation of hops

started from the middle of the ninth century, between A.D. 859 and 875, in Germany where it extended from north to south during the early and high Medieval period, as well as to other regions of central Europe.

In the beginning, *Humulus lupulus* was utilized as an alternative to Myrica gale which was the prevailing beer additive inside the European area where it was native. In the eighteenth century, the use of *Humulus lupulus* overcame that of Myrica gale due to its better-preserving property (Behre, 1999). Currently, the beer brewing industry accounts for 98% of the world's use of hops. Originally *Humulus lupulus* was used as a preservative for its antimicrobial activity; later it was additionally used to add a bitter flavour to beer (Moir, 2000).

Moreover, it can stabilize beer foam mainly due to the highly hydrophobic components iso--acids (Simpson and Hughes, 1994); also, xanthohumol and its derivative isoxanthohumol were found to have positive effects on foam stability (Smith *et al.,* 1998; Wilson *et al.,* 1998). *Humulus lupulus* was first mentioned by the naturalist Pliny the Elder (23–79 A.D.) who described the use of the young shoots as a vegetable by the Romans (Grieve, 1971). The leaves and flower heads were used to produce a fine brown dye (Grieve, 1971). Flowers are a natural source of food flavouring for cereals, spices, sauces, tobacco, and alcoholic beverages other than beer (Lawless, 1995; Barnes *et al.,* 2002). The fibrous stems, similar to hemp (Cannabis sativa), were used in the manufacture of a coarse kind of

cloth and the production of paper (Grieve, 1971). Hops were used in perfumes, especially the spicy and oriental types, in skin creams and lotions (Lawless, 1995). *Humulus lupulus* has a long history as a medicinal remedy to treat a wide range of complaints. It has been mainly recommended as a mild sedative useful to treat sleeplessness and nervousness (Blumenthal, 1998).

Traditionally hops were used to treat excitability and restlessness associated with tension headaches; to improve appetite and digestion; and to relieve toothache, earache, and neuralgia (Grieve, 1971; Barnes *et al.,* 2002). In addition, hops have been reputed to exert diuretic, antispasmodic, and anaphrodisiac effects (Duke, 1985; Weiss, 1988; Blumenthal, 1998). Native American tribes used hops as a sedative, antirheumatic, analgesic, and urinary aid for "gravel" and inflammation (Hamel and Chiltoskey, 1975; Blumenthal, 1998; Bown, 2001). Also, they used heated hops as a poultice in the treatment of pneumonia (Carr and Westey, 1945) and a decoction of hops was recommended for intestinal pain and fevers in Dakota (Bown, 2001). In India, the Ayurvedic Pharmacopoeia recommends hops to treat restlessness associated with nervous tension, headache, and indigestion (Karnick, 1994).

In traditional Chinese medicine, hops are used to treat insomnia, restlessness, dyspepsia, and lack of appetite. Alcoholic extracts of hops have been clinically used in China to treat leprosy, pulmonary tuberculosis, acute bacterial dysentery, silicosis, and asbestosis with positive outcomes (Blumenthal *et al.,* 2000). Topically hops were used to treat crural ulcers and skin injuries and to relieve muscle spasms and nerve pain (Lawless, 1995; Tyler and Foster, 1999; Wichtl and Brinckmann, 2004). In aromatherapy hops have been used for skin care, breathing conditions, nervousness, nerve pain, and stress-related conditions (Lawless, 1995). The Committee on Herbal Medicinal Products (HMPC) of the European Medicines Agency (EMEA) (2007) reports the traditional use of *Humulus lupulus* floss for the relief of mild symptoms of mental stress and insomnia. The German Commission E and European Scientific Cooperative on Phytotherapy (ESCOP, 2003) approved hops as a treatment for excitability, mood disturbances (restlessness, anxiety), and sleep disturbances (Blumenthal, 1998).

2.2 Botany

Humulus lupulus L. is a perennial plant that regrows each spring from the rhizomes of an underground rootstock. It is a vine producing stems annual, slender, climbing, growing up to 6–9 m in length, often with stout-hooked hairs (Burgess, 1964; Neve, 1991). The stems twist around their support in a clockwise direction. A reference to the plant's habit of climbing on other plants is reflected in its name Lupulus, which is derived from the Latin term lupus, a wolf climbing on a sheep (Grieve, 1971). In addition, the English common name hop comes from the Anglo-Saxon hoppan meaning to climb. The origin of the name *Humulus* is doubtful but it has been suggested to come from humus, the rich moist ground in

which the plant grows. The leaves are dark-green coloured, long petiolate, heart-shaped with 3–5 lobes, sharply toothed and they have a very rough surface. They are placed opposite one another on the stem, but sometimes the upper leaves are arranged singly on the stem. It is a dioecious plant with male and female flowers on separate plants, although individual monoecious plants are frequently found in some wild North American hop populations, instead rarely found among the European types (Haunold, 1991; Haunold *et al.,* 1993). Male and female plants are easily distinguished by their different flowers; no other morphological differences identify the sex of the plant. The male flowers are long racemes, 7.5–12.5 cm long, while the female inflorescences are cone-like catkins (called strobiles), 2.5–5 cm long, made up of overlapping membranaceous bracts. The external bracts are flattened and symmetrical. The internal bracts are longer and generally enfolding at the base of a small fruit (achene). A resinous substance, named lupulin, is secreted by yellow glandular trichomes found at the base of cone bracts and can be separated by shaking the strobiles. Lupulin-like glands are also present on the underside of hop leaves. Female strobiles are collected in August–September when they are ripe and their colour changes from pale greenish-yellow to yellow-brown. Only female individuals are present in hop-growing areas to maintain a genetically consistent product (Neve, 1991). Males are essential, however, in hop breeding programs to develop new varieties through controlled hybridization.

2.2 Phytochemistry

The main structural classes of chemical compounds identified from hop mature cones include terpenes, bitter acids, and chalcones. Hops are also rich in flavonol glycosides (kaempferol, quercetin, quercitrin, rutin) (Sagesser and Deinzer, 1996) and catechins (catechin gallate, epicatechin gallate) (Gorissen *et al.,* 1968). Hundreds of terpenoid components were identified in the volatile oil (0.3–1.0% of hop strobile weight): primarily - caryophyllene, farnesene, and humulene (sesquiterpenes) and myrcene (monoterpene) (Malizia *et al.,* 1999; Eri *et al.,* 2000). The bitter acids (5–20% of hop strobile weight) are phloroglucinol derivatives usually classified as - acids and acids. Both groups contain a 3-,4-,5-, or 6-carbon oxo-alkyl side chain: acids are structurally different from -acids for one more prenyl group. The bitter acids are present in hops as a complex mixture of variable composition and concentrations. The main -acids are humulone (35–70% of total -acids), cohumulone (20–65%), and adhumulone (10–15%); the corresponding - acids are lupulone (30–55% of total -acids), colupulone and adlupulone (Fig. 1). In addition to the two series of normal, co- and ad-homologs, there exist some minor bitter acids represented by post humulone/postlupulone, prehumulone/prelupulone, adprehumulone. The biosynthesis, isomerization, oxidation and degradation of hop bitter acids have been extensively studied (Verzele and De Keukeleire, 1991; Fung *et*

al., 1997; Goese *et al.,* 1999) (Figure 3). Acids are the crucial compounds for the quality of hops used in brewer industry, contributing to foam stability as well as exerting antibacterial activity (Verzele and De Keukeleire, 1991). At high pH value and high temperature, -acids isomerize to the corresponding iso--acids which are more soluble and more bitter than their parent compounds. Therefore, they are responsible mainly for the typical bitter taste of beer, in addition to eliciting foam stabilizing and antibacterial properties, like -acids (Verzele and De Keukeleire, 1991).

Besides to the volatile oil and the bitter acids, several prenylflavonoids were identified from hop cones (Stevens *et al.,* 1997) (Fig. 2). The most important compound is the chalcone xanthohumol (XH) (up to 1% in dry hop cones) (Stevens *et al.,* 1999), which can be converted to the prenylflavanone isoxanthohumol (IX) in consequence of thermal treatment and increased pH value (Stevens *et al.,* 1998, 1999). Therefore, IX is the main prenylflavonoid present in beer. Also, other chalcones, occurring at 10–100-fold lower concentrations than that of XH, isomerize to the corresponding flavanones. A chalcone named xanthogalenol (XG) has been identified only in some hop varieties growing in North America or East Asia (Stevens *et al.,* 2000). The compound 2 ,4 ,6 ,4-tetrahydroxy-3 - C-prenylchalcone commonly known as desmethylxanthohumol (DMX) is considered as the precursor of the most flavonoids present in hops (Chadwick *et al.,* 2006).

Through a chemical isomerization, it gives rise to the major estrogen of hops identified as the 1:1 racemate (±)-8-prenylnaringenin (8-PN), along with the racemic 6-prenylnaringenin (6-PN) (Hansel and Schulz, 1988). In humans 8-PN has been shown to derive from IX through activation by intestinal microflora (Possemiers *et al.,* 2006) or by liver cytochrome P450 enzymes (Guo *et al.,* 2006). Hence, the estrogenically inactive XH possesses an estrogenic potential through its conversion to IX and then to 8-PN. The chemistry, biological activity and biotechnological aspects of xanthohumol and other prenylated flavonoids from hops have been recently reviewed (Steven and Page, 2004).

During the development from female inflorescences to ripe cones, the levels of –acids. DMX and XH gradually increase, the accumulation rate depending on hop variety and climatological conditions (De Keukeleire *et al.,* 2003, 2007). The bitter acids and XH were also detected in male inflorescences: their concentrations are similar to those found during early female flowering (De Keukeleire *et al.,* 2003). The same authors demonstrated the presence of bitter acids and chalcones in leaves of fully grown hops even if their levels were found generally lower than in the hop cones and strictly related to the hop varieties (De Keukeleire *et al.,* 2003, 2007). The hop leaves contain also volatile compounds but in a much lesser amount than the hop cones. The European

Pharmacopoeia 5th ed. (2004) and the British Pharmacopoeia (2007) report the microscopical and chromatographical identification assays of hops (Lupuli flos). The thin-layer chromatogram of hop strobiles, examined in ultraviolet light at 254 nm, shows some quenching bands due to xanthohumol, humulones, and lupulones.

3. Chemical and Pharmacological Properties

H. lupulus contains hundreds of phytochemicals, and some of the secondary metabolites have definite potential pharmacological and medicinal value (reviewed by, e.g., Astray *et al.,* 2020; Iniguez and Zhu 2021; Tronina et al. 2020). Especially the female inforescences but also other parts of the plant (leaves, stems, and rhizomes) are rich in different biologically active molecules, which are responsible for a range of health-promoting effects and bioactivities (Muzykiewicz *et al.,* 2019; Zanoli and Zavatti 2008). Lupulin is yellowish–brown granular powder secreted from the lupulin glands of the mature female cone-like structures of the hop plant. It contains bitter resins and aroma substances that give the characteristic aroma and flavor of hops (Krottenthaler 2009). The secondary metabolites present in lupulin can be divided into three groups: the hop resins, the hop oils, and the hop polyphenols (Steenackers *et al.,* 2015).

The levels of aromatic hop oils and other biochemicals depend on several factors, such as the variety, ripening stage, climatological conditions, soil composition, and storage (Almaguer *et al.,* 2014; Alonso–Esteban *et al.,* 2019; Bedini *et al.,* 2016; Čermák *et al.,* 2015; Edwardson 1952; Gerhäuser 2005; Sanz *et al.,* 2019; Van Cleemput *et al.,* 2009; Zanoli and Zavatti 2008). The concentration and accumulation of many biochemicals increase when the cones mature, at a rate depending on several variables. The chemistry of hop substances is complex and has been studied for over a century, because of their importance for the brewing industry, but new aspects and uses continue to be discovered. Hop resins consist of a hard and soft resin fraction, where the soft resins (bitter acids) are those used in brewing beer and have marked pharmaceutical potential. The bitter acids consist of alpha–acids (humulones) and beta–acids (lupulones). Alpha acids are the most important bitter acids and they isomerize easily into iso–alpha–acids, whereas the beta–acids do not isomerize and they generally become destroyed during beer brewing processes. Beta–acids are hydrophobic and not soluble in water (especially at low pH), which gives them antibacterial value and a high potential for pharmaceutical applications (see below, Ban *et al.,* 2018; Čermák *et al.,* 2015).

Hop essential oils form a small portion of the dried hop strobile (0.5–3.0% v/w) but many of their different aromatic compounds are of interest to the brewing industry, as well as to the perfume and favor industry. Myrcene, linalool, and geraniol are the most important aroma compounds of the oil, with myrcene being the

most abundant one (Almaguer *et al.,* 2014; Bedini *et al.,* 2016; Eyres *et al.,* 2007; Van Opstaele *et al.,* 2012).

Some aromatic compounds and their concentrations are specific to certain cultivated varieties, and some components are found mainly in American varieties. The North American *H. lupulus* ssp. neomexicanus plants possess a different scent compared to the European subspecies ssp. lupulus. Myrcene seems to be the main aromatic component in both of them and the monoterpene composition is similar to in other varieties, but there are differences in the sesquiterpene fraction of the oil (Almaguer *et al.,* 2014; Brendel *et al.,* 2019; Knobloch *et al.,* 1982; McCallum *et al.,* 2019). Besides hop resins and oils, lupulin contains polyphenols, such as kaempferol, quercetin, catechins, and estrogenically active 8–PN (8–prenylnaringenin, a derivative of xanthohumol) (Almaguer et al. 2014; Astray et al. 2020; Zanoli and Zavatti 2008). Depending on the temperature and pH, xanthohumol isomerizes to isoxanthohumol, a prenylfavanone, which is the form found in beer (Ban et al 2018; Gerhäuser 2005). Since hop polyphenols show multiple antioxidant, antimicrobial, and other effects, and have a possible therapeutic use, numerous efforts have been developed to produce hop extracts with a high polyphenolic content (e.g., Astray *et al.,* 2020; Tronina *et al.,* 2020). Bitter acids, volatile oils, and xanthohumol are present also in male inforescences, though in much smaller amounts. The leaves of hop plants do not contain bitter acids, while volatile oils, e.g., myrcene, are present in small quantities (Langezaal 1992). Recently, the chemical compounds of the hop seeds have been studied and found to be rich in catechins (catechin, epicatechin), which are products widely used in pharmaceutical, cosmetic, and nutraceutical industries (Alonso–Esteban *et al.,* 2019).

3.1 Hop extracts

Immediately after harvesting, hop cones are carefully dried by artificial heat: water content must be reduced from 65–80% to 8–10% for storage. In the early nineteenth century extraction of hops was first attempted in water and ethanol (Gardner, 1987), but other methods have been also reported, such as the use of steam or carbon disulfide (Moir, 2000). The production of hop extracts has been improved in the last century when the chemical structure and reactivity of the resin compounds have been elucidated. Owing to their lipophilic nature, a wide range of effective solvents, including alcohols, chloroform, acetone, and hexane, has been used to dissolve the resin constituents.

There was however a growing concern about the possible harmful effect of even small amounts of solvent residues in the extract. Therefore, the technique of extraction using liquid or supercritical carbon dioxide was applied to hop cones. Carbon dioxide is a rather selective and non-polar solvent that is particularly suited to dissolve hop-soft resin and oil, but it does not extract polar components or only traces of them. Some extracts obtained by supercritical carbon

dioxide extraction of hop cones at four different combinations of temperature (40–60 ∘C) and pressure (125–275 bar) were analyzed using HPLC and 1H NMR spectroscopy (Langezaal *et al.,* 1990). The authors observed that the extraction parameters influenced the yield and the composition of the mixture of the bitter compounds as well as the presence of volatile components. Among the different combinations of parameters tested, of 40 ∘C and 200 bar were found the best conditions for the extraction of both the bitter compounds and the volatiles. It is important to underline that the knowledge of the active principles and the influence of the extraction procedure on extract composition can give clues to standardization and quality control.

4. Pharmacology

4.1 Sedative activity

The traditional use of hops as a mild sedative stems from the observation of sleepiness and fatigue in the hop-pickers, apparently due to the transfer of hop resin from their hands to their mouths (Tyler, 1987). The German Commission E approved hops for the treatment of "mood disturbances, such as restlessness and anxiety, sleep disturbances" (Blumenthal, 1998). Nevertheless, the sedative activity of hops was poorly investigated in experimental and clinical studies. The first investigation carried out in rodents was published by Hansel and Wagener (1967). The authors did not observe an alteration in locomotor activity and in hexobarbital-induced sleeping time in mice orally treated with three types of hop extracts, two produced with ethanol and the third with methylisobutyl-ketone, at doses up to 500 mg/kg b.w.

In addition, neither antagonistic effect against methamphetamine-induced stimulation nor muscle relaxation was found. The lack of a clear sedative effect was also reported in human subjects treated with 250 mg/day of a lipophilic hop extract for 5 days (Stocker, 1967). The tranquilizing property of different extracts of *Humulus lupulus*, intraperitoneally (i.p.) injected in mice, was investigated by Bravo *et al.,* (1974). The authors observed a reduction in spontaneous motor activity, related to the type of solvent used in the extraction procedure. The ether extract was the most active in comparison with the aqueous and alcoholic ones. It must be underlined that a high dose, 1 ml of *Humulus lupulus* extract 10%/20 g b.w. was needed to elicit the reduction in motility. None of the tested extracts exerted a myorelaxant effect. The neuropharmacological effect of an undefined hop extract, dosed from 100 to 500 mg/kg, was evaluated in mice by Lee *et al.,* (1993): hypothermic, analgesic, and anticonvulsant activities were observed after i.p. injection. In addition, sedative and hypnotic properties were ascribed to the hop extract following the observation of a dose-dependent reduction in spontaneous locomotor activity and a dose-dependent increase in pentobarbital-induced sleeping time. The above-mentioned studies do not demonstrate the sedative effect of

Humulus lupulus. First of all the oral administration was applied only in the study of Hansel and Wagener (1967) and they did not observe a sedative effect. The finding of this effect after the i.p. injection of hop extracts (Bravo *et al.,* 1974; Lee *et al.,* 1993) opens up a problem of bioavailability.

Moreover, the different extraction procedures and the undefined composition of the administered preparations make questionable the neuropharmacological activity of hops as well the identity of the active sedative principle/s. About the last issue, Hansel *et al.,* (1980, 1982) attributed the sedative effect of hops to 2-methyl-3-butene-2-ol, deriving from hop constituents during storage at room temperature. This compound caused a 50% reduction of spontaneous motility without inducing a myorelaxant effect, when i.p. injected in rats at the dose of 206.5 mg/kg (Wohlfart *et al.,* 1983a,b). A high dose (800 mg/kg) of the same compound was needed to induce narcosis in mice (Hansel *et al.,* 1980). It must be underlined that the hop extracts commercially available were found to contain small amounts of 2-methyl-3- butene-2-ol (Hansel *et al.,* 1982), therefore it cannot be considered the major responsible constituent for the sedative effect of hop extract.

We recently investigated the neuropharmacological activity of *Humulus lupulus* using a CO2 hop extract and single fractions containing alpha-acids and beta-acids (Zanoli *et al.,* 2005, 2007). CO2 hop extract orally administered in rats exerted a pentobarbital sleep-enhancing effect in a dose-dependent manner, starting from a minimal effective dose of 10 mg/kg. The extract failed to affect the locomotor activity in the open field test and the anxious behavior of rats submitted to the elevated plus-maze test.

At our knowledge for the first time, we showed that hop extract, administered at the dose of 5–10 mg/kg b.w. three times (24, 5, and 1 h) before the test, reduced immobility time during the behavioural despair test, suggesting hence an antidepressant-like activity. The same pharmacological effects were elicited by the administration of hop fraction containing -acids. On the other hand, the fraction containing alpha-acids orally administered in rats (5–10 mg/kg) produced an increased exploratory activity in the open field, a reduction in the pentobarbital hypnotic activity, and a worsening of picrotoxin-induced seizures. In the elevated plus maze, the increased exploratory activity in the open arms showed by alpha-acid treated rats, in comparison with controls, suggested a modest anxiolytic-like activity.

In the forced swimming test, a significant reduction in the immobility time was observed in rats three times treated with alpha-acids fraction (5 mg/kg b.w., 24, 5, and 1 h before the test). Electrophysiological studies performed on cerebellar granule cells in culture showed that the alpha-acids fraction decreased GABA-evoked current in a dose-dependent manner. In conclusion, the -acids fraction can be considered the major responsible constituent for the enhanced pentobarbital effect and the antidepressant

properties observed after the administration of CO2 hop extract. The alpha-acids fraction exerted an antidepressant activity as well but reduced pentobarbital hypnotic activity.

In this context the behavioural (picrotoxin seizure) and electrophysiological results seem to suggest the ability of - acids to reduce GABAergic activity. The CO2 extract and the two fractions of bitter acids share an antidepressant-like effect: this property could be particularly interesting taking in consideration the poor availability of medicinal plants useful for the treatment of depressive disorders. A further study describing the sedative property of *Humulus lupulus* has been recently published by Schiller *et al.,* (2006). The authors found reduced locomotor activity, increased ketamine-induced sleeping time, and reduced body temperature in mice treated with different dosages, from 200 to 500 mg/kg, of ethanolic and CO2 hop extracts by oral gavage. These preparations were devoid of anxiolytic activity, thus confirming our previous results (Zanoli *et al.,* 2005). In the same experimental conditions, the authors tested also the effects of different fractions of hop extracts. Both fractions containing -acids and -acids were able to prolong ketamine-induced sleeping time, but the fraction containing -acids needed a dosage approximately 6- times higher (200 mg/kg) than that of -acids (25 mg/kg) to significantly potentiate the narcotic event. This last result seems to suggest a contribution of -acids to the sedative activity of *Humulus lupulus*. The discrepancy between these results and our findings (Zanoli *et al.,* 2007) should be elucidated taking in account several factors (raw material, storage condition, extraction procedure, type of solvent), besides the different applied dosages (Schiller *et al.,* 2006).

A recent study showed that myrcene, which is produced from myrcene during boiling hops, was able to prolong pentobarbital-induced sleeping time in mice and potentiate GABAa receptor response in vitro (Aoshima *et al.,* 2006). Taking into account the particular condition leading to the production of the tested compound, it is unlikely that myrcenol could play a role in the sedative effect of a hop extract. On the other hand, myrcenol could represent a positive modulator of GABAa receptor response as a component of beer. A study aimed to clarify the interaction of sedative herbs with selected central nervous system receptors demonstrated the capacity of a hop-dried extract to bind serotoninergic 5-HT6 receptors as well as melatoninergic ML1 receptors (Abourashed *et al.,* 2004). The involvement of 5-HT receptors in depression and sleep disturbances has been demonstrated (Shen *et al.,* 1993) and the role of melatonin in the regulation of circadian rhythm is well-known (Pickering and Niles, 1990). It must be underlined that the tested extract contained 0.48% flavonoids, but not bitter acids, owing to the utilization of a hydrophilic solvent in the extraction procedure (Abourashed *et al.,* 2004). The involvement of the melatoninergic system in the sedative effect of hops could be confirmed by the ability of luzindole,

a melatonin receptor antagonist, to counteract the hypothermic effect of a hop methanolic extract (250 mg/kg) as well as that of melatonin (50 mg/kg) in BL6/C57J mice (Butterweck *et al.,* 2007). In this study -acids were excluded to be responsible for the hypothermic activity of hops because they were not present in the hydrophilic extract used in the experiments. This finding is not in accordance with those by other authors (Schiller *et al.,* 2006) and by us (Zanoli *et al.,* 2005), if the hypnotic event is really mediated by the hypothermic effect, as suggested by Gilbert *et al.,* (1999). An agonistic activity of hops at adenosine A1 receptors was excluded in a study aimed to investigate the mechanism of action of a valerian–hop combination dried extract (Muller *et al.,* 2002). The authors suggested an alternative mechanism for the sedative effect of hops, probably involving GABA receptors (Muller et al., 2002). Both the authors of the reported studies (Muller et al., 2002; Abourashed *et al.,* 2004) agree on the fact that in vitro activities need to be further substantiated by in vivo models.

The clinical investigations on the efficacy of hops in sleep disturbances were generally performed using preparations containing a combination of hops and other sedative herbs, particularly valerian. A randomized, double-blind, controlled trial in patients suffering from sleep disorders showed equivalent efficacy and tolerability between a hop–valerian preparation and a benzodiazepine drug (Schmitz and Jackel, 1998). Sleep quality was determined by psychometric tests, psychopathologic scales, and sleep questionnaires. This study pointed out that the hop–valerian treatment for 2 weeks did not elicit the withdrawal symptoms, that normally occur with benzodiazepine therapy. The pharmacodynamic effects of a commercially available mixture of valerian and hops (Ze 91019) were studied in young adult patients using quantitative topographical electroencephalography (Vonderheid-Guth *et al.,* 2000). A clear effect at the central nervous system level was observed 4 h after the intake of a high dosage of the mixture (1500 mg valerian plus 360 mg hops).

A multicenter, randomized, and placebo-controlled study was performed in 184 patients with mild insomnia, nightly administered for 28 days with a combination of standardized extracts of hops (83.8 mg) and valerian (374 mg) (Morin *et al.,* 2005). Sleep parameters were measured by daily diaries and polysomnographic assays. The combination of hops–valerian showed a modest hypnotic effect, improving sleep without producing significant residual effects and rebound insomnia. The lack of residual sedative effects was previously stressed by Gerhard *et al.,* (1996) in healthy volunteers, receiving a hop–valerian combination or flunitrazepam, used as a reference drug. The objective measurement of cognitive psychomotor performance and the subjective questionnaires on well-being led to an emphasis on the impairment of vigilance in the morning after the ingestion of the benzodiazepine drug, while more alertness and activity were observed in patients treated with the herbal remedy. Therefore, the valerian–hop combination can be considered a useful and safe alternative to classic sedative drugs (Gerhard *et al.,* 1996; Schmitz and Jackel, 1998; Kubish *et al.,* 2004; Morin *et al.,* 2005).

A herbal preparation, containing lavender oil, lemon balm, and oat extracts besides hops, exhibited a relaxing effect, documented by electroencephalographic analysis, in healthy volunteers (Dimpfel *et al.,* 2004). However, the presence of valerian or other medicinal herbs in the clinical formulations does not allow us to assess the potential clinical efficacy of hops administered alone.

4.2 Estrogenic activity

The frequent menstrual disturbances observed in female hop-pickers, during the early days of hop cone harvesting, suggested a potential hormonal activity of hops. In Germany, hop baths were traditionally used to treat gynecological disorders. The presence of estrogenic substances in hops ("equivalent of 20–300 g estradiol/g") was first suggested by Koch and Heim (1953). On the contrary other authors did not find estrogenic activity in hop essential oil, hop extracts, -acids, - acids, and hop resin (Fenselau and Talalay, 1973). The discrepancy could be due to the different nature of extracts as well as to the variety of the specific assays used to determine estrogenic properties. In the study of Liu *et al.,* (2001) the estrogenic activity of a methanol hop extract was demonstrated by: (a) the significant binding capacity to both estrogen receptors (ER and ER); (b) the induction of alkaline phosphatase activity in Ishikawa cells (human endometrial adenocarcinoma epithelial cell line); (c) the up-regulation of progesterone receptor mRNA in Ishikawa cells; (d) the up-regulation of presenelin-2, an estrogen-inducible gene in S30 cells (breast cancer cell line transfected with ER). These results were confirmed by Overk *et al.,* (2005) using a chloroform partition of a methanol extract from a previously CO_2-extracted Nugget hops cultivar. The extract showed an estrogenic potency equivalent to that of a red clover (Trifolium pratense L.) ethanol extract: both demonstrated significant activities in the ER competitive binding, activation of transiently transfected ERE-luciferase, quantitative real-time PCR of an estrogen-inducible gene, and alkaline phosphatase enzyme induction assays. Several phytochemical investigations were performed to identify the estrogenic principle, firstly named "hopsproestrogen" by Nastainczyk (1972) and subsequently recognized as a mixture of 8-PN and 6-PN (Hansel and Schulz, 1988). Among the different compounds (XH, IX, 6-PN, 8- PN) of a hop polyphenolic fraction showing estrogenic property, 8-PN displayed the major activity, measured in vitro using a sensitive bioassay based on the ability of estrogenic compounds to stimulate alkaline phosphatase activity in Ishikawa cells. In the same study, the high estrogenic potency of 8-PN was confirmed by its ability to interact with estrogen receptors in a radioligand binding assay on rat uterine cytosol. On the other hand 6- PN showed a very weak estrogenic activity as isoxanthoumol did, while

xanthoumol was inactive. These findings were subsequently confirmed in a yeast screen expressing the human estrogen receptor (Milligan *et al.,* 2000). Using a mammalian cell-based transient transactivation assay, 8- PN was demonstrated to be approximately 100 times more potent than genistein, but unlike genistein, 8-PN displayed twofold higher affinity for ER than measured by in vitro competitive binding assay (Schaefer *et al.,* 2003).

The high estrogenic activity of 8-PN was also confirmed in different in vivo experiments. The subcutaneous administration of 8-PN (30 mg/kg/day) for 2 weeks was reported to suppress the decrease in bone mineral density and the reduction in uterine weight, induced in rats by ovariectomy (Miyamoto *et al.,* 1998). 8-PN induced a characteristic estrogenic response in an acute in vivo test using uterine vascular permeability as an endpoint (Milligan *et al.,* 2002) as well as in a 3-day uterotrophic assay in ovariectomized female rats (Diel *et al.,* 2004). Recent studies performed in vivo demonstrated the capacity of 8-PN: (a) to reduce serum-luteinizing hormone (LH) and follicle-stimulating hormone (FSH); (b) to increase serum prolactin level and uterine weight; (c) to induce vaginal hyperplastic epithelium; (d) to cause secretion in the mammary glands of ovariectomized rats, after a 3-month treatment with a high dose (68.4 mg/kg) (Christofell *et al.,* 2006; Rimoldi *et al.,* 2006). These effects on the hypothalamo-pituitary-uterine axis are very similar (though milder) to the ones elicited by estradiol. A lower dose of 8-PN (18 mg/kg) daily administered in rats for 28 days was reported to prevent ovariectomy-induced trabecular bone loss (Humpel *et al.,* 2005). In these animals it was observed a minimal and dose-independent stimulatory effect on uterine cells; it was approximately 10-fold lesser than that of an equivalent bone protective dose of estradiol. This finding demonstrated a remarkable tissue specificity of 8-PN, which was confirmed in a transgenic reporter mouse model (Humpel *et al.,* 2005).

The capacity of 8-PN to reduce menopausal hot flushes was recently assessed by Bowe *et al.,* (2006), by measuring the tail skin temperature (TST) in ovariectomized rats. The subcutaneous daily administration of 400 g/kg of 8-PN for 2 days resulted in a significant decrease in TST similar to that induced by estradiol (4g/kg). The effect of both substances was completely blocked by the peripheral estrogen receptor antagonist, ICI 182,780, thus demonstrating that peripheral mechanisms are involved in the regulation of the vasomotor response by estrogens and phytoestrogens. In the study performed by Milligan *et al.,* (2000) on the endocrine activity of hop flavonoids, none of the tested compounds (XH, IX, 6-PN, 8-PN) showed progestogenic or androgenic bioactivity. On the other hand, 8-PN was shown to possess anti-androgenic activity in a yeast-based androgen receptor assay (Zierau *et al.,* 2003). From the clinical point of view, the first randomized, double-blind, placebo-controlled study on the use of a standardized (on 8-PN) hop extract in menopausal women has

recently been published by Heyerick *et al.,* (2006). The daily administration of the extract, at a dose corresponding to 100 g 8-PN for 6 weeks, to postmenopausal women decreased the incidence of hot flushes and other discomforts associated to estrogen deficiency (sweating, insomnia, heart palpitation, irritability).

The efficacy of hop extracts in reducing hot flushes in menopausal women was previously suggested by Goetz (1990) and recently confirmed by the same author (Goetz, 2007) in a few patients treated with different types of non-standardized hop preparations. Vaginal dryness in postmenopausal women was significantly reduced by the topical application of a gel containing hyaluronic acid, liposomes, vitamin E, and hop extract (Morali *et al.,* 2006). Single doses, from 50 to 750 mg, of 8-PN were orally given to healthy menopausal women in a randomized, double-blind, placebo-controlled study performed by Rad *et al.,* (2006). The decrease in LH serum levels found after the highest dose demonstrated the ability of 8-PN to exert endocrine effects in menopausal women.

4.3 Cancer-related bioactivities

Over the past 10 years, several in vitro studies have been carried out to evaluate the potential activity of hop components as chemopreventive agents (Figure 4). Among hop components, xanthohumol (XH) has received major attention because it seems to inhibit in vitro initiation, promotion, and progression stages of carcinogenesis, hence appearing as a broad-spectrum chemopreventive agent (Stevens and Page, 2004; Gerhauser, 2005a; Colgate *et al.,* 2007). A recent study performed in vivo showed the ability of XH to induce significant inhibition of angiogenesis in mice implanted with a matrigel sponge when administered in drinking water at a concentration of 2M. At higher concentrations (200M) XH displayed a marked angiogenesis inhibition without adverse effects on animal health parameters (Albini *et al.,* 2006). In the same study, the oral administration of XH at the concentration of 20 M significantly inhibited the growth rate of KS-IMM tumors (Kaposi's sarcoma cell line) in male nude mice, starting from the 20th day of treatment.

The inhibition of tumor angiogenesis and growth (33% and 83%, respectively, in comparison with controls) was observed by Gerhauser (2005a) in female immuno-deficient mice implanted with human breast tumor xenograft and treated with XH subcutaneously injected at the dose of 1000 mg/kg b.w./day for 14 days. The *H. lupulus* extract and its components have been shown to have direct inhibitive effects on carcinogenesis by regulating different biochemical pathways of cancer cells at various key stages of their development (Zanoli and Zavatti, 2008) (Figure 5 to 8).

Numerous in vitro and *in vivo* studies have shown the inhibitive effects of hop extracts on several types of cancer, e.g., colon, skin, and bone cancer, as well as promyelocytic and monoblastic leukemia. Several cancer-inducing pathways and processes related

to the aging of cells, such as apoptosis, increased extracellular matrix, angiogenesis, diminishing vitality, and oxidative damage to DNA, proteins, and lipids.

Hop extracts can intervene with the biochemical pathways of these processes in multiple ways. Some of the cancer chemopreventive effects of *H. lupulus* are relatively well known and have received attention, whereas other mechanisms remain under investigation (Akazawa *et al.,* 2012; Bolton *et al.,* 2019; Gerhäuser 2005; Lee *et al.,* 2007; Lempereur at el. 2016; Philips *et al.,* 2017; Van Cleemput *et al.,* 2009; Yasukawa *et al.,* 1995). Humulone inhibits mouse skin cancer growth by diminishing the amounts of the DNA–binding nuclear factor (NF–κB) and activator protein AP–1 with subsequent effects on upstream pathways (Lee *et al.,* 2007). Several studies have described the effects of alpha acids (humulone), while beta–acids (lupulone) being less soluble in water have been less in focus. Among other effects, lupulone seems to induce apoptosis in prostate cancer cells. Similarly, a study by Lamy *et al.,* (2007) showed that colon cancer was inhibited by apoptosis after exposure to hop lupulones in laboratory rats. Interestingly, lupulones were also able to overcome resistance to apoptosis in mutated cancer cells (Lamy *et al.,* 2007). Later, Philips *et al.,* (2017) discovered that the hop extract and its components (xanthohumol, isoxanthohumol, and bitter acids) exhibit direct antioxidant activities and inhibit melanoma cell growth in vitro. The cancer-inducing effects of alcohol is well known and a high alcohol intake is connected to many types of cancer, especially cancers of the alimentary tract and lung cancer, as well as breast cancer in women (Gerhäuser 2005). Some of the most beneficial hop constituents are present in beer only in very small quantities and some (prenylated flavonoids) are not heat-stable during the brewing process. Iso–alpha acids are heat stable but present only in low quantities. Thus, no therapeutic effect can be achieved by drinking beer.

Currently, beer is the only source of iso-alpha acids for humans, but Mahli *et al.,* (2018) have calculated that to gain the beneficial effects reported in their study, one would have to consume 100 L of beer. However, hop extracts and xanthohumol–enriched beer would be a potential solution for producers and consumers alike (Gerhäuser 2005; Magalhães *et al.,* 2009). Particularly the terpenophenolics (prenylated flavonoids xanthohumol and its further constituents isoxanthohumol and 8–PN), as well as the alpha–acids seem to exhibit great pharmaceutical potential (Ban et al 2018). Xanthohumol is abundant, particularly in European hop varieties (Figure 2). It activates several biochemical pathways that result in detoxification, anti-inflammatory effects, and enzyme inhibition, for example, in rat hepatoma cells (Gerhäuser 2005).

5. Hormone Replacement Therapy (HRT)

Plant-based estrogen mimics (phytoestrogens) have gained popularity as alternatives for treating menopause-related symptoms. Loss of estrogen during menopause has multiple effects,

among the most harmful ones being weight gain, increased visceral fat, and the risk of a metabolic syndrome (non–alcoholic fatty liver disease, NAFLD). There has been interest in hops due to hop biomolecules showing extraordinary estrogen activities (e.g., Tronina *et al.,* 2020).

The hop extract could be a useful alternative for treating menopausal triglyceride accumulation in the liver, visceral adiposity, and weight gain by modulating lipid metabolism and inflammatory cytokines (Gerhäuser 2005). Investigations have shown that the *H. lupulus* extract contains phytoestrogen precursors (flavonoids, especially xanthohumol, and isoxanthohumol) that can transform into estrogenic forms (8–prenylnaringenin) by the activation of intestinal fora or by liver cytochrome P450 enzymes. Hop bioactive compounds show slow absorption through the intestinal epithelium, as well as a tendency to enter the enterohepatic circulation. Estrogenic activities of xanthohumol and isomerized isoxanthohumol are weak or non–existent, but their derivative, demethylated 8– prenylnaringenin, is the most potent phytoestrogen known in the plant kingdom (Almaguer *et al.,* 2014; Hamm *et al.,* 2019; Zanoli and Zavatti 2008). Animal testing shows that 8–8-prenylnaringenin has estrogenic activity in mammals, and it may thus exhibit symptom relief in women as well. This hop extract has been shown to aid in treating some vasomotor side effects caused by estrogen deficiency, such as hot flashes, as well as menopause–connected insomnia and mood swings.

In addition, it seems to enhance bone-protecting mechanisms through its estrogen-mimicking activity (Bolton *et al.,* 2019; Erkkola *et al.,* 2010). Bolton *et al.,* (2019) have proposed spent hops as a source for hormone replacement preparations to relieve menopausal syndromes. Spent hops are rich in pharmacologically active prenylated phenols, especially chalcones and flavanones (6– prenylnaringenin, 8–prenylnaringenin, 8–prenylnaringenin, isoxanthohumol). Chalcones are end–products of biochemical pathways, whereas flavanones arise through the isomerization of chalcone precursors. Spent hop extracts exhibit effects via multiple biochemical pathways and it is possible to modify hop extracts selectively with specific targets in mind. As an example, the most beneficial concentration of 8–prenylnaringenin differs depending on whether treating premenopausal and postmenopausal women using either small or large amounts of 8– prenylnaringenin, respectively (Bolton *et al.,* 2019; Hamm *et al.,* 2019).

5.1 Antimicrobial Effects

New alternatives for antibiotics are being investigated given the spread of antibiotic resistance. Since the antimicrobial potential of *H. lupulus* has long been known in folk medicine, it has recently been in focus as a phytotherapeutic alternative. Several studies have shown the potential of hop extracts to interfere with and inhibit bacterial, fungal, and protozoan growth, and act as antiviral agents (e.g., Şener 2020). There are multiple related biochemical pathways,

but one of the most important characteristics of hop extracts seems to be their ability to affect the function of microbial plasma membranes. Particularly, hop terpene phenolics have a high potential for antimicrobial applications (Ban et al 2018; Srinivasan *et al.,* 2004). Bitter acids (primarily alpha and beta acids) of *H. lupulus* have been shown to exhibit strong antimicrobial activities by permeating bacterial cell membranes and, thus, causing leakage. Membrane transport, enzyme functions, and nutrient intake of especially gram-positive bacteria are effectively inhibited. Alpha acids isomerize into iso-alpha acids, which have been found to inhibit the growth of several gram-positive bacteria, such as *Propionibacterium acnes, Staphylococcus aureus, S. epidermitis, Bacillus anthracis, B. subtilis, Corynebacterium diphtheriae, Sarcina lutea, Streptococcus faecalis,* and *Lactobacillus brevis* (Bhattacharya *et al.,* 2002; Čermák *et al.,* 2015). In addition, the growth of some species of *Micrococcus, Mycobacterium, Streptomycetes, Listeria,* and *Clostridium* is inhibited by hop extracts (Langezaal 1992; Weber et al. 2019; Yamaguchi et al. 2009). Beta acids are effective mainly because of their hydrophobic character and following ability to disrupt bacterial cell membranes, particularly in gram-negative bacteria. Several oral bacterial species are inhibited by *H. lupulus* constituents: dental caries caused by *Streptococcus mutans* as well as S. sanguinis and *S. salivarius* are more effectively inhibited by beta acids (lupulone) than by common mouthwashes. The bitter taste due to beta acids, low pH, and ethanol needed for dilution form the main problem in developing commercial applications (Bhattacharya *et al.,* 2002; Čermák *et al.,* 2015). Chalcones (bitter acids lupulone and humulone) do not generally inhibit the growth of gram-negative pathogens, such as Escherichia coli, but some exceptions have been discovered. In addition, growth is inhibited in gastritis and gastric ulcers caused by Helicobacter pylori and in some brucellosis caused by Brucella species via cell membrane disruption (Čermák *et al.,* 2015; Oshugi *et al.,* 1997; Wendakoon *et al.,* 2018). Antimycobacterial activity of hop acids was found by Stavri *et al.,* (2004) when studying Mycobacterium fortuitum (in vitro) as an alternative model for M. tuberculosis to evaluate the effects of hop strobile extracts. Hop terpenophenolics are important for beer favoring and are of interest in biomedical research. Their inhibitory activity towards the malaria protozoa Plasmodium falciparum has also been reported. Among terpene phenolics, both beta acids and iso-alpha acids are effective but xanthohumol has the most potent lethal activity against the protozoa (Čermák et al. 2015; Gerhäuser 2005; Srinivasan *et al.,* 2004). Methicillin-resistant Staphylococcus aureus (MRSA) is one of the microbes that increasingly causes serious and costly problems in healthcare. It results in infections that can lead to sepsis and death, and it is the main cause of soft–tissue and skin infections. The resistance of MRSA to most antibiotics calls for new and effective treatment strategies. The *H. lupulus* strobile extract was tested by Wendakoon et al., (2018). All tested constituents (alpha acids, beta acids, and xanthohumol, but especially the beta acid extract) were shown to be effective in inhibiting the growth of methicillin-resistant S. aureus. Bacterial biofilms are often problematic in antibiotic treatments since they resist many antimicrobials. However, bacterial biofilms were not able to hinder the effects of *H. lupulus* constituents (Bogdanova *et al.,* 2018). Acne is the most common inflammatory skin disease among teenagers. The typical pathogens linked to the disease are Propionibacterium acnes and Staphylococcus aureus, which are both gram-positive bacteria and found to react well to the hop extract (Weber *et al.,* 2019). It was concluded that using effective phytochemicals, such as hop alpha acids, when treating particularly the milder forms of acne would be a comparable alternative to antibiotics (Weber *et al.,* 2019). *H. lupulus* feed, processed from spent hops, has been investigated as an alternative to antibiotic growth enhancers in animal husbandry. Bortoluzzi *et al.,* (2014) studied the effects of hop beta acid feed additives in poultry and found beneficial effects. Beta acids seem to control the proliferation of Clostridium perfringens in the small intestine and ceca of broilers. The effects of hop feed on piglets and cattle have been in focus as well, but the results have been ambiguous (Lavrenčič *et al.,* 2018; Williams 2007).

5.2 Insecticide

Plant-derived essential oils have recently gained popularity as eco–friendly alternatives to chemical pesticides. Consumers favor such solutions, since aromatic plant volatiles are generally low in toxicity to most mammals, unlike many of the current commercial chemical pesticides. New potent alternatives for chemical insecticides are needed also for the increasingly common genetic resistance of pests to various chemicals. Plant oil-based alternatives to chemical pesticides have not been common, because often they have not been effective enough. However, hop oil seems to have the potential to control several insect pests. Although the functional role of essential oils and their various scents in hop plants is not yet fully understood, they are most likely functioning as a repellent against various herbivores and pathogens (Aydin et al. 2017; Bedini et al. 2015, 2016; Brendel *et al.,* 2019; Gerhäuser 2005; Naraine and Small 2017; Reher *et al.,* 2019; Wang and Dixon 2009). Using spent hops as a source of hop oil would be an easy and economically convenient solution due to the large number of spent hops available as a by-product of the brewing industry. Hop oil from spent hops appears to affect the larval infestation of the expanding fruit storage pest, the spotted wing drosophila Drosophila suzukii (Matsumura) (Reher *et al.,* 2019). Hop oil works well against several harmful animal species, e.g., storage pest beetles. Bedini *et al.,* (2015) tested the effects of spent hops' essential oil on two major storage–food pests, the lesser grain borer *Rhyzopertha dominica* (Fabrizius) and the granary weevil (*Sitophilus granarius* (Linnaeus), and apparent repelling

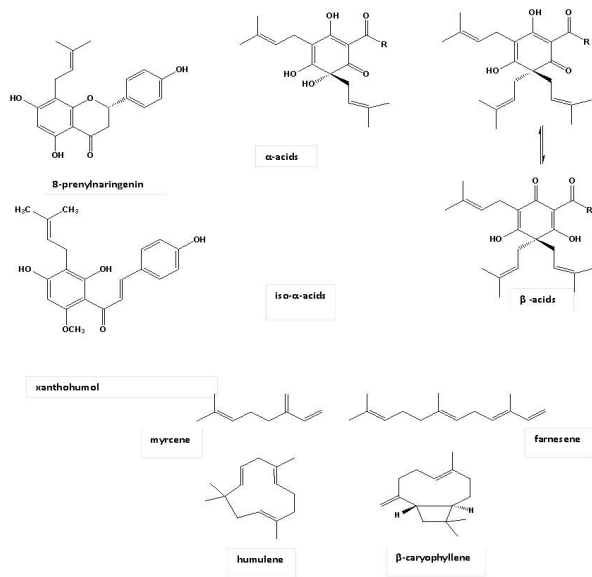**Figure 1**. A picture of *Humulus lupulus.*

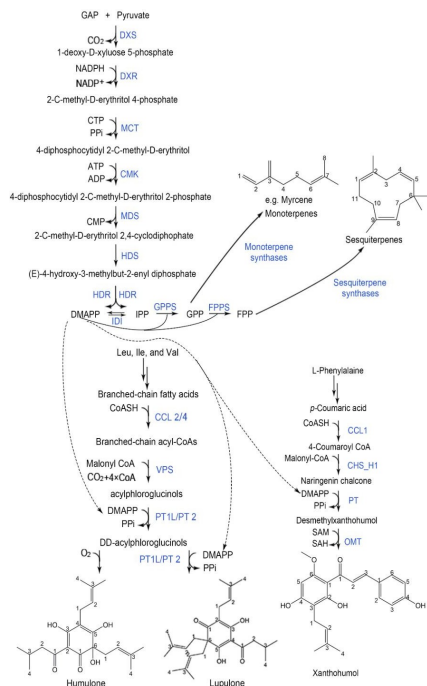**Figure 2.** Major Constituents of *Humulus lupulus.*

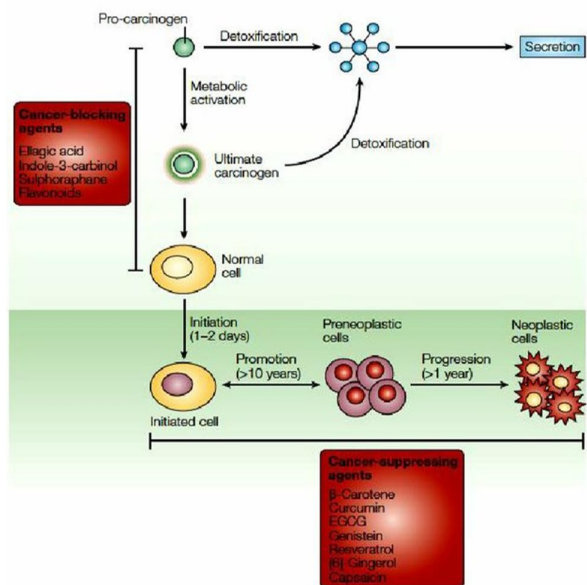**Figure 3**. Pathway Showing the Synthesis of Hop's Phytochemical.

Figure 4. Classification of Chemopreventive Agents based on their Mechanism of Action on Cancer Multistage.
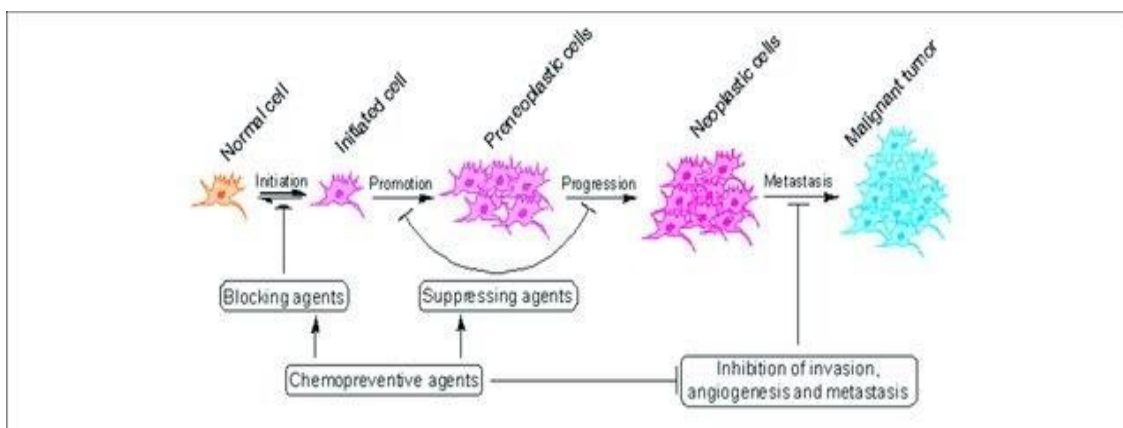

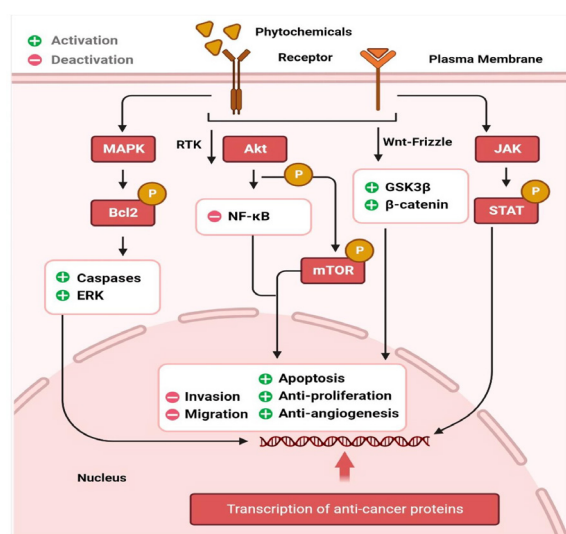**Figure 5.** Critical Steps in the Process of Cancer Development.

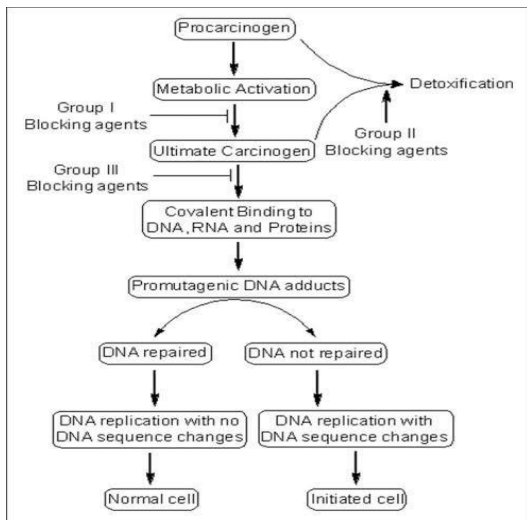**Figure 6.** Mechanism of Cancer Induction

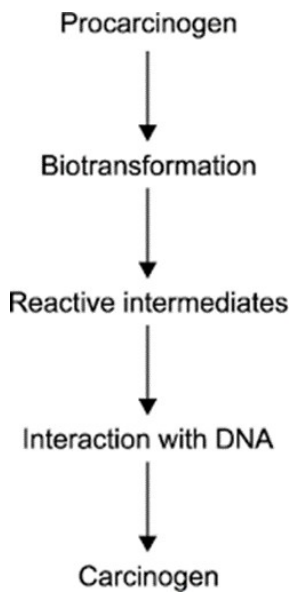**Figure 7.** Procarcinogen to a Normal Cell and Initiated Cell.

Figure 8. The Conversion of Procarcinogen to Carcinogen.

activity was discovered (Bedini *et al.,* 2015). In addition, the invasive freshwater snail *Physella acuta (Draparnaud),* as well as the mosquito Aedes albopictus (Skuse) were effectively repelled by spent hops (Bedini *et al.,* 2016). Bitter acids, especially the beta–acids, seem to have some effect on invertebrate pest control, but they have been studied much less because beta–acids are not present in beer (Naraine and Small 2017). It has been reported that native North American hop *H. lupulus* ssp. lupuloides effectively repels insect pests, including the aphid *Phorodon humuli* (Schrank) and mite (*Tetranychus urticae* (C.L. Koch), as well as several pathotypes of powdery mildew *Sphaerotheca humuli* (DC.) Burrill (Hampton et al. 2002). This American subspecies is a closer relative to the European ssp. lupus than the other two American subspecies, and a valuable genetic resource for hop breeding purposes (Hampton *et al.,* 2002). All three native North American taxonomic varieties have small protective glands on the abaxial sides of their leaf blades that protect the basal region of the leaves from insect damage. European hop subspecies and varieties have much fewer glands, if any, which gives an added reason for using American hop plants for breeding to enhance protective mechanisms in the European hop (Naraine and Small 2017).

5.3 Hop Aromas and Perfumes

H. lupulus essential oils are a small fraction of dried hops, but the terpenes and terpenoids it contain are among the most important products of the hop plant. This fraction is of interest to the brewing, perfume, and favor industry. The favor of this fraction is dependent on the hop variety, and recently the cultivation of specific aromatic favor hop cultivars has been increasing. In addition to the cultivar, the climatic conditions as well as the cultivation area and soil all influence the flavor of the oil (Allen *et al.,* 2019; Almaguer *et al.,* 2014; Bedini *et al.,* 2016; Eyres *et al.,* 2007; Holle *et al.,* 2019; Van Opstaele *et al.,* 2012). Most of the aromatics are accumulated in the strobili of the female plant, but all parts of both female and male plants contain essential oils. The odor–active fractions of the volatile oils have been analyzed, but since the most abundant compounds may not be the most important odorants, additional assessments are needed when evaluating the scents (Eyres *et al.,* 2007; Van Opstaele *et al.,* 2012). The most important odor compounds of *H. lupulus* belong to several different chemical classes, such as esters, terpenes, ketones, aldehydes, and furanes. The most important volatile in hop oil is beta–myrcene (monoterpene), which constitutes up to 50% (depending on the cultivar) of the volatile oil fraction, and 2–undercanone (ketone). Most of the esters are described as fruity, foral, or green, and the ketones (predominantly 2–undercanone) are generally citrusy or fruity (Van Opstaele *et al.,* 2012). *H. lupulus* essential oils have been studied particularly because of the brewing industry since different tastes and aromas of beer are of interest to producers and consumers alike. Many of the varieties and cultivars have their characteristic

bouquet of flavoring agents. For example, *H. lupulus* ssp. neomexicanus has a markedly different scent and oil spectrum (especially the sesquiterpene fraction) than ssp. lupulus (Knobloch et al. 1982). Some of the aroma–active compounds in *H. lupulus* ssp. lupulus include vanillin, geraniol, nonanal, methional, myrcene, linalool, and 3–methyl–butanonic acid, the last three of these being the most abundant in the strobila and commercially the most important ones (Brendel *et al.,* 2019). Some of the odors and bouquets are described as fruity, citrusy, herbal, flowery, caramel-like, coconut-like, honey-like, and vanilla-like in quality, while others are described as musty, earthy, woody, or cedarwood in character (Eyres *et al.,* 2007). Traditionally, *H. lupulus* essential oils have been added to perfumes and deodorants mainly for scents, but they have also been useful for their preservative qualities (Bedini *et al.,* 2016; Duke 1983).

SIDE EFFECTS

Humulus lupulus can be responsible for allergic reactions in sensitive individuals. Pronounced signs of bronchial irritation, dry cough, and dyspnea were observed in hop processing workers (Meznar and Kajba, 1990; Skorska *et al.,* 2003). Respiratory impairment, together with immunological reactions (increased serum level of total IgE) was confirmed in brewery workers exposed to organic dusts such as hops, barley, and brewery yeast (Godnic-Cvar *et al.,* 1999). Contact dermatitis from hops was first described by Badham in 1834 (cited by Cookson and Lawton, 1953); subsequently several cases of occupational dermatitis to fresh and dried hops were reported by different authors (Cookson and Lawton, 1953; Raith and Jager, 1984; Spiewak and Dutkiewicz, 2002). Nevertheless, to our knowledge, no clinical case of allergy or anaphylaxis resulting from the therapeutic use of hops has been published. Toxicological studies in animals stated that LD50 for orally administered hop extract in mice ranges from 500 to 3500 mg/kg (Hansel *et al.,* 1993). The oral administration of xanthohumol ($5 \times 10{-4}$ M ad libitum) to laboratory mice for 4 weeks did not affect major organ functions and protein, lipid, and carbohydrate metabolism (Vanhoecke *et al.,* 2005b). Furthermore, the sub-chronic oral administration of humulone derivatives in dogs was not associated with specific signs of toxicity demonstrating the wide safety margins of these substances (Chappel *et al.,* 1998).

6. Conclusion

In the last century and even more in recent years several researchers focused an increasing interest on *Humulus lupulus* and its components for their biological activities. A traditional application of hops in humans consisted in the treatment of sleep disturbances: recent studies performed in rodents evidenced the sedative properties of CO2 hop extract and some of its fractions (Zanoli *et al.,* 2005, 2007; Schiller *et al.,* 2006). However different or even

contradictory findings obtained by the authors concerning the activity of -acids fraction require a detailed reinvestigation. Experimental evidence of hop sedating properties has been produced in laboratory animals, but no randomized, double-blind, placebo-controlled clinical trial utilizing hop extract alone has been performed till now. No meaningful information regarding the potential clinical efficacy of hops can be extrapolated by using clinical formulations containing hops in combination with another medicinal plant, particularly valerian. Therefore, the real efficacy of hops in sleep disturbances remains to be ascertained. Today hop extracts are the major constituents of many food and dietary supplements with claims of "breast enhancement" (Coldham and Sauer, 2001) but also in this case, properly controlled clinical trials supporting the use of hops for their estrogenic properties are still lacking. In addition, no officially recognized standardization exists yet for estrogenic formulations of hops, even if the key compounds should be 8-PN for its estrogenic property, DMX as proestrogen, and IX for its possible conversion in vivo to 8-PN. The experimental studies performed *in vitro* and *in vivo*, suggesting estrogenic properties for hop extract or its single compounds, reasonably support the hypothesis that properly formulated hop preparations could represent an alternative to the classic hormone replacement therapy in the management of menopausal symptoms. However further research is required to assess the safety aspect, in particular, the potential risk of breast or uterine cancer as a consequence of high-dose supplements. Hop research performed in the last decade has been largely dedicated to the biological activities of single hop components, particularly xanthohumol, and isoxanthohumol among prenylflavonoids and humulone among bitter acids. Anti-inflammatory, antioxidant, and lipoperoxidative activities as well as antiangiogenetic, antiproliferative, and apoptotic effects, mainly assessed in in vitro studies, reasonably suggest a potential chemopreventive activity. In addition, these compounds proved to possess a broad-spectrum anti-infective activity against several microorganisms. However, in *vivo* studies to assess their bioavailability, distribution, efficacy, and safety in animal models are strongly recommended, before their application in humans.

Author contributions

O.S.B and A.O.M were instrumental in the conceptualization and methodology of this study. They were actively involved in data collection and played a crucial role in the preparation of the original draft.

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Competing financial interests

The authors have no conflict of interest.

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