

Hop (*Humulus lupulus* L.): Traditional and Present Use, and Future Potential

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Hop (*Humulus lupulus* L.): Traditional and Present Use, and Future Potential. Hop (*Humulus lupulus*) is best known for its use in beer brewing owing to its bittering flavor and floral aroma. Today, the brewing industry uses as much as 98% of the produced hop crop worldwide. However, there are many other uses, some of them known since prehistoric times. Hops, the cone-like female structures called strobili, are the most frequently used part of the hop plant, but other tissues are of interest as well. The present review compiles existing knowledge of the chemical and pharmacological properties, traditional and present uses and further use potential, genetic resources, and breeding attempts in *H. lupulus*, and discusses climate change challenges to hop production. It contains hundreds of phytochemicals, and some of the secondary metabolites have definite potential pharmacological and medicinal value, but further investigations are desirable. Hop substances are potential alternatives, e.g., in antimicrobial, cancer, metabolic syndrome, and hormone replacement therapy treatments, as well as insecticides, preservatives, and fragrances. There are presently a few hundred cultivated hop varieties, and new cultivars are being developed and tested. Future hop breeding efforts with different quality and adaptation targets can utilize existing genetic resources, such as wild populations and landraces present in many regions.

Key Words: Common hop, Hops, Brewing, Medicinal plant, Phytochemicals, Hop breeding.

Introduction

Throughout human history and prehistory, plants have provided food, materials, medicine, and various cultural values. At the same time, people have interacted with the surrounding biodiversity, utilized resources, and shaped plant diversity by domesticating and introducing plant species to new areas, while plants themselves have evolved and adapted to their environments. Most valuable plant resources have been transported around the globe during human colonization and migration, for instance maize, rice, and potatoes. Ethnobotany is a discipline that addresses the relationships between humans and plants (Garnatje et al. 2017). Among many plant applications, those related to human health and well-being are the most diverse ones. Plants and other organisms utilized by people bring biological and cultural factors together to form biocultural diversity. This concept was developed in the 1990s in order to study and express the dynamic interrelatedness between people and their natural environment (Elands et al. 2019).

Humulus lupulus L. (common hop) is an herbaceous perennial liana, one of three *Humulus* species in the family Cannabaceae. All three species, the other two being *H. yunnanensis* Hu. and *H. japonicus* Siebold & Zucc., are present in China, which has generally been agreed to be the region of

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origin for the genus *Humulus* (Alonso–Esteban et al. 2019; Murakami et al. 2006). Presently, *H. lupulus* occurs in temperate regions around the globe. The plant climbs in a clockwise direction around any available support with the help of its downward facing stout trichomes (hairs), and it can reach a height of up to 10 to 18 m. Leaves are opposite or alternate, and have 3 to 5 lobes with serrate margins. The root system is large and requires a deep, well–drained fertile soil. Horizontal roots tend to grow a great number of fine rootlets (Aioriello et al. 2020; Edwardson 1952; Van Cleemput et al. 2009).

H. lupulus is dioecious, i.e., female and male flowers are usually in separate plants, although occasionally monoecious individuals may be found (Edwardson 1952). Flowers are small and wind pollinated. Female plants have cone–like structures called strobili (hops), which are 2.5–5 cm long and develop soon after pollination. They consist of yellowish–green overlapping bracts attached to an axis. Each bract enfolds a small fruit, which is an achene, a dry fruit that does not split open at maturity. Mature strobili contain diverse chemicals in their lupulin glands (glandular trichomes) at the base of the bracts, which can be used in many industries, the most well–known—but not the only one—being the brewing industry. *H. lupulus* seeds are small, hard, and waterproof, exhibiting a long dormancy and slow germination. It may take up to three years before the female plants start to flower and produce strobili. Most cultivated varieties are seedless because seeds, especially if crushed during the brewing process, are believed to affect the taste of beer (Almaguer et al. 2014; Liberatore et al. 2018). There has been interest to develop and use sex–linked DNA markers to identify the sex already at the seedling stage (Liberatore et al. 2018; Rodolfi et al. 2018).

Although *H. lupulus* is best known for its use in beer brewing, there are many other known uses. The present review of *H. lupulus* compiles information on its chemical and pharmacological properties, traditional and present uses and further use potential, genetic resources, breeding efforts, and climate change challenges to hop production. Our aim is to prepare a comprehensive scientific review of the existing knowledge of *H. lupulus* and provide baseline information and additional views that can enhance further research, cultivation, and use of this plant.

History

CULTIVATION AND TRADITIONAL USE FOR BREWING

There are theories of the origin of the genus name *Humulus*, although its actual etymology remains uncertain (see Abaev 1989). It is possible that the origin of the name is proto–Iranian, from where the name has spread across Eurasia and finally settled into several language groups, including the Germanic, Slavic, and Fenno–Ugric language families. *Lupulus* is a Latin word for a small wolf, and Pliny (Pliny the Elder, c. 24–79 CE) wrote in his *Historia Naturalis* (c. 77 CE) that it refers to the climbing habit of the plant and its tendency to suffocate other plants nearby, often riparian willows (*Salix* sp.).

A phylogenetic analysis shows that *H. lupulus* has migrated westwards from its probable home of origin in China, via the Caucasus Mountains, and that it had reached Europe roughly one million years ago (Murakami et al. 2006). *H. lupulus* followed mainly the same routes and dispersal patterns as several European tree species, such as pine (*Pinus*), alder (*Alnus*), and oak (*Quercus*). The North American lineage is estimated to have separated from the European one during the Quaternary period (0.31 million years ago [MYA]), likely somewhere in Central Asia or China. After crossing Beringia, *H. lupulus* diverged into following three North American subspecies: ssp. *neomexicanus* A.Nelson & Cockerell, ssp. *pubescens* E.Small, and ssp. *lupuloides* E.Small (McCallum et al. 2019; Murakami et al. 2006).

The expansion of *H. lupulus* in Europe occurred after the last glaciation. There is no evidence of human interference in any part of its migration, although several theories have considered this alternative (Murakami et al. 2006; Patzak et al. 2010). An old theory by Alyser in 1911 (in DeLyser and Kasper 1994, also Wilson 1975) suggested that *H. lupulus* was brought to Europe by the first ancestral Finnish settlers who came from the East. The Finnish National Epic Kalevala, as well as some linguistic data suggest that beer and particularly hopped beer was known in Finland already during prehistoric times, although there is not much evidence to

support this theory. Hornsey (2003) keeps the question open, while others have the opinion that this theory should be rejected (DeLyser and Kasper 1994; Hornsey 2003).

Beer is undoubtedly one of the earliest alcoholic beverages used by humans, but the history of the hop plant remains uncertain and the beginning of its use in beer brewing is obscure. In the past, salt was amply used for preserving food and, since clean drinking water was not always available, drinking beer was often the safest choice. The alcohol content of beer was typically low, and it was part of everyday life in many ancient societies, both as a popular beverage as well as folk medicine. Baking leavened bread and brewing beer are related processes, both relying on the same unicellular fungus, the *Saccharomyces* yeast, and it is likely that the history of leavened bread and brewing beer are intertwined (Desalle and Tattersall 2019; Hornsey 2003; Wilson 1975).

Brewing beer is considered the world's oldest biotechnological process, which was probably invented independently in many ancient cultures worldwide. There is evidence of beer brewing as early as 8,000 years ago in the Near East, and the process was well known in Egypt and ancient Mesopotamia as early as c. 5000 Before Current Era (BCE) (Behre 1999; Edwardson 1952; Hornsey 2003). Egypt of the New Kingdom Era (1550–1069 BCE) is known to have had flourishing beer exports in the Mediterranean area (Hornsey 2003). Ancient beer brewing was basically the same process as it is today, but since hops were most likely not used in the beginning of the brewing history, early beer tasted and looked different. The biggest problem was that it did not keep well. Hops became popular when its antibacterial beer preserving properties first became noticed.

There is no clear evidence of the cultivation of *H. lupulus* in Europe before the current era. Pollen records indicate that the Early Roman era (late years BCE) may be the period when hops were first used in the brewing process. Indeed, some archaeological sites show an increase in hop pollen around that time, which is believed to indicate early small-scale cultivation of the hop plant (Edwardson 1952; Wilson 1975). However, hops were collected in the wild already much earlier. Hop pollen has been found as far back as in Neolithic settlements. Whether the hop plant

was used for folk medicine or as a vegetable, or perhaps for making cloth in those early times, remains unclear (Behre 1999; Wilson 1975).

Hops in combination with beer brewing were first mentioned in 736 CE in a monastery document from the Hallertau region in Bavaria, Germany (Hornsey 2003). The abbot of Corvey in Britain stated in 822 CE that hops were needed for brewing and that tenants were expected to make malt and gather hops in the wild for the monastery. At that time, the quantities needed were still relatively small. During the next hundred years, monasteries also in France and Belgium began to expect dues of hops and malt from their tenants in such large amounts that cultivation must have been established by then. Hildegard of Bingen wrote about beer brewing in c. 1150 CE but did not mention whether the hop plants she wrote about were cultivated or gathered in the wild. She disliked hops and preferred sweet gale (*Myrica gale*) for beer making, but she did recognize the antibiotic properties of the hop plants (Behre 1999; DeLyser and Kasper 1994; Edwardson 1952; Wilson 1975).

Large amounts of *H. lupulus* pollen were discovered in an abandoned tenth century boat in Graveney, Kent, England in the 1970s. What makes this discovery interesting is the fact that pollen was found only inside the boat but not in soil or clay anywhere near or under the boat, where remains of characteristic salt marsh vegetation were discovered in large amounts. The Graveney boat was carrying a cargo of hops for brewing purposes, possibly of a domestic origin or perhaps imported from mainland Europe. This find suggests that hops were traded in Europe already at that time (Behre 1999; Wilson 1975). However, there was considerable prejudice against hop plants in England, where its cultivation began much later than in mainland Europe. Hop gardens began to emerge in England towards the end of the fourteenth century. The use of hops was prohibited in England for some time in the fifteenth century apparently because the kings of that era (Henry VII and Henry VIII) did not like the taste of hopped beer and because it was generally thought to be unhealthy. At that time, the preferred drink was ale, which was a mixture of malt, honey, and spices or herbs (Hornsey 2003). Hop growing was introduced into North America in the seventeenth century,

with the first commercial production starting in 1648 in Massachusetts, but it became popular only as late as in the nineteenth century (Edwardson, 1952). Today, the United States is the world's biggest producer of hops, producing annually more than half of the world's hop crop (FAOSTAT 2019).

H. lupulus has a long history in Northern Europe. A Norwegian document from 1311 mentions hop in a monastery in Trondheim, Norway. Archaeological finds from the thirteenth and fourteenth centuries show that the cultivation of hop was common especially near monasteries and large households (Hornsey 2003). Small-scale cultivation was probably common already several centuries earlier. Farmers in Denmark, Norway, Sweden, and Finland were to grow 40 poles of hop as part of their duties from the year 1442 onwards (Hornsey 2003). This law was abolished in Finland in 1915, but a similar law from 1734 is, in fact, still in effect today (www.finlex.fi). Hop plants were commonly used in folk medicine, and many folk customs and beliefs were associated with this plant, which was among the very first cultivated garden plants in Northern Europe. It was believed that elves lived in hop gardens and, in some parts of Finland, those were places to avoid after sundown (Salo 2011). Hop gardens were the customary places to dispose of spiritually unclean things, e.g., water that had been used to wash the deceased.

In old times, many different herbs besides hop were used in brewing for taste and bitterness. Several plant species can be added to beer at different stages of the brewing process to bring aroma, such as yarrow (*Achillea millefolium* L.), gale (*Myrica gale* L.), mugwort (*Artemisia* sp.), chrysanthemum (*Chrysanthemum* sp.), rosemary (*Rosmarinus officinalis* L.), heather (*Calluna* sp.), betony (*Stachys* sp.), dandelion (*Taraxacum officinale* [L.] Weber ex F.H. Wigg.), meadowsweet (*Filipendula ulmaria* [L.] Maxim.), agrimony (*Agrimonia* sp.), and nettle (*Urtica dioica* L.) (Wilson 1975). These plants were collected in the wild to make a mixture of herbs called "gruit." Each gruit maker had their own signature recipe for the herbal mixture they used and, in fact, may still use. Cinnamon (*Cinnamomum verum* J.Presl), nutmeg (*Myristica fragrans* Houtt.), mint (*Mentha*

sp.), and aniseed (*Pimpinella anisum* L.), among others, have also been used widely to spice beer.

TRADITIONAL USE—NOT ONLY FOR BEER

H. lupulus has been known in folk medicine from prehistoric times. There are various records of traditional uses, some being rather inventive, such as dying hair, relieving impurities from the blood, making fabric and paper, packing fragile cargo, and keeping demons away at night (Edwardson 1952; Wilson 1975). Other examples include treatments against leprosy, toothache, fever, gastric problems, and anxiety, and use as preservative, deodorant, and cattle fodder (Table 1). The sedative effect of *H. lupulus* has been known from the early days of cultivation, since it was noticed that people gathering hops and working with the plants were getting extremely drowsy during the working day (Van Cleemput et al. 2009). Hops have long been used as a sedative and sleeping aid in drinks. In addition, soft pillows filled with hops have been popular. Originally, they were heated and lasted for about three days before being replaced (Wilson 1975). Pillows filled with hops and lavender are sold even today in several Internet sources and online shops. The German Commission E and European Scientific Cooperative on Phytotherapy (Zanoli and Zavatti 2008) have approved hops as a treatment for restlessness, anxiety, and sleep disturbances (Zanoli and Zavatti 2008).

The strobili ("cones") have been the most frequently used part of hop, but other parts of the plant have also been of interest. Young shoots have been eaten as a vegetable in many parts of Europe from the times of Pliny (Pliny the Elder c. 24–79 CE) (Edwardson 1952; Van Cleemput et al. 2009). Being a close relative of hemp (*Cannabis sativa* L.), the hop plant similarly has long fibers that have been used for making ropes, as well as for making paper and linen-like cloth. However, the paper is not of particularly good quality, because the pulp yield and cellulose content remain relatively small (Duke 1983).

TABLE 1. TRADITIONAL UTILIZATION OF *HUMULUS LUPULUS*

Traditional use	Main region	Plant part	References
Beer flavoring, preserving and clarifying		Strobili	Wilson 1975
Vegetable	Mediterranean, UK, Belgium	Young leaves	DeLyser and Kasper 1994; Duke 1983; Edwardson 1952
Bread making (to cultivate yeast)	Europe, East–Africa	Strobili	DeLyser and Kasper 1994
Preservative in sausages	Germany	Strobili	Duke 1983
Flavoring water, baked goods, tobacco	America	Strobili	Duke 1983
Cattle fodder, manure preparation	Europe	Whole plant	Edwardson 1952
Cattle bedding	UK	Stems	Wilson 1975
Fiber (ropes)	UK	Stems	Wilson 1975
Cloth	Sweden	Stems	DeLyser and Kasper 1994
Paper		Stems	Duke 1983
Packing fragile cargo		Inflorescences	Wilson 1975
Bedding material for deceased		Whole plant	Behre 1999
Insulation		Stems	Wilson 1975
Hair rinse for brunettes	Russia	Leaves, flowers	Edwardson 1952
Deodorant (antimicrobial, fragrance)		Strobili	Duke 1983
Perfumes, skin lotions		Strobili	Duke 1983
Dye (yellow and brown)		Leaves, flowers	Wilson 1975
Oil (food)		Strobili	Wilson 1975
Ornamental plant	Europe, USA	Whole plant	DeLyser and Kasper 1994
Antibiotic, anti-inflammatory		Strobili	Van Cleemput et al. 2009; Wilson 1975
Sedative, sleep disturbances		Strobili	Wilson 1975
Headache, restlessness	China	Strobili	Wilson 1975
Tenderness of limbs		Strobili	Wilson 1975
Bleary eyes		Strobili	Wilson 1975
Gastric problems, indigestion, appetite	India, China	Strobili	Zanoli and Zavatti 2008
Toothache, earache, neuralgia	America	Strobili	Van Cleemput et al. 2009; Zanoli and Zavatti 2008
Leprosy, tuberculosis, asbestosis, silicosis	China	Strobili	Duke 1983; Edwardson 1952; Wilson 1975
Anthelmintic, antiparasitic	Northern Europe	Strobili	Kjaviņa et al. 2021
Cough, spasms, fever, anxiety		Strobili	Alonso–Esteban et al. 2019; Chattopadhyay and Naik 2007
Clearing blood, flatulence		Strobili	Edwardson 1952
Delirium tremens, irritable bladder, aches		Strobili	Duke 1983
Diuresis		Strobili	Knoblauch et al. 1982
Liver disorders (porphyria)	UK	Strobili	Duke 1983; Van Cleemput et al. 2009

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 inflorescences 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; Zanolli 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 the 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; Zanolli 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 strobili (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 flavor 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 as 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; Zanolli and Zavatti 2008). Depending on the temperature and pH, xanthohumol isomerises to isoxanthohumol, a prenylflavanone, 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 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 inflorescences, 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).

Genetic Resources and Breeding

There are presently a few hundred cultivated varieties of the hop plant and new cultivars are being developed and tested. Many of the European cultivars originate from old, closely related varieties. Therefore, the European cultivars do not differ genetically, morphologically, or ecologically much from each other (Murakami et al. 2006; Van Holle et al. 2019; Yan et al. 2019). Genetic analyses show that most early European landraces, such as Fuggler, Hallertau, Saaz, Spalt, and Tettninger, have very similar genetic profiles and there is very little variation (Patzak et al. 2010; Van Holle et al. 2019). It is evident that they have interbred as well as backcrossed with wild hops during centuries of small-scale cultivation in European villages and cottage gardens. New varieties were traditionally developed and exchanged among local farmers. Some of the old landraces, such as Saaz, are cultivated for their high quality and fine aroma, but their yield may be lower than in some new cultivars. They are also more susceptible to pests and diseases, and vulnerable to changing climate conditions (Mozny et al. 2009). Climate change with higher temperatures and more frequent drought periods creates additional breeding needs. In their experiments, Eriksen et al. (2020) found cultivars that may be good candidates for growth in warm climates.

Great genetic diversity has been discovered in many wild hop varieties, especially in America and Asia (Patzak et al. 2010; Van Holle et al. 2017), and fair amounts of diversity is present in Europe, as revealed when studying wild Italian samples of *ssp. lupulus* (Rodolfi et al. 2018). The American wild hop subspecies, *ssp. neomexicanus* and *ssp. lupuloides*, are more diverse genetically than the European variety *ssp. lupulus*, expressing among other things high alpha-acid contents and resistance to *Verticillium* wilt (a fungal disease). Moreover, geographical isolation by the Appalachian Mountains in the North American continent appears to have led to two clearly differing *ssp. lupuloides* populations (McCallum et al. 2019; Murakami et al. 2006). The genetic composition of American cultivars has been influenced by the hop cultivars European settlers brought with them. Yet, they remain genetically distant from the European cluster (Patzak et al. 2010; Rodolfi et al.

2018). American cultivars show better resistance to pests and diseases, which is why the need of introducing the American gene pool to Europe is recognized (Murakami et al. 2006; Patzak et al. 2010).

Traditional beer brewing has favored a rather narrow range of hop types because of traditional brewing methods, as well as traditional taste preferences, which have guided the selection process (Murakami et al. 2006). Yet, there is a demand for new cultivated varieties, especially due to the increase of small new breweries with novel preferences (McCallum et al. 2019; Rodolfi et al. 2018). For brewers and other users, it is important to ascertain the authenticity and geographic origin of purchased hop batches, and this has led to rules in the certification of hop plants and hop products, as well as to the development of new DNA-based analysis methods (Ocvirk et al. 2019).

The draft genome of *H. lupulus* was generated in 2015 using two cultivars, Saazer and Shinshu Wase, as well as a Japanese wild hop *H. lupulus* var. *cordifolius* (Miq.) Maxim. ex Franch. & Sav. (Natsume et al. 2015). Such genome information is highly useful for breeding and studies on hop domestication. De novo RNA sequencing analysis on Shinshu Wase revealed the developmental regulation of genes involved in specialized metabolic processes that affect taste and flavor in beer. Further analyses enabled the identification of genes related to the biosynthesis of aromas and flavors that were enriched in Shinshu Wase compared to the wild hop. Thereafter, a genomic database for the hop plant, called HopBase (<http://hopbase.cgrb.oregonstate.edu>), has been established. The aims of HopBase are to help in identifying the origin of unclear hop samples, finding varieties and cultivars with interesting genetic diversity, and helping to recognize hop strains that are both productive and resistant to pathogenic microbes. The database will hopefully also help to ascertain the biological history and spreading of the plant and find “the mother of all hops” (Desalle and Tattersall 2019; Vergara et al. 2016). Yet, attempts to determine how biochemical pathways responsible for desirable traits are regulated have been challenged by the large, repetitive, and heterozygous genome of hop. Recently, the genome of the hop cultivar Cascade with an estimated size of 2.7 Gb was

assembled (Padgitt–Cobb et al. 2019). This deepens understanding of the hop genome and probably has broader applicability to the study of other large, complex genomes as well. Recently, Awasthi et al. (2021) demonstrated that the CRISPR/Cas9 system can be used to precisely edit the targeted hop genome, thus providing a promising avenue for hop breeding.

Present-Day Use—Brewing Industry

CULTIVATION TODAY

Today, the brewing industry uses as much as 98% of the produced *H. lupulus* crops worldwide (Alonso–Esteban et al. 2019; Zanolli and Zavatti 2008). *H. lupulus* grows best in temperate regions with a moderate and humid climate (Mozny et al. 2009). It is a rather hardy plant when dormant, but labor-intensive for growers, and severe frosts in spring will kill young shoots (Van Holle et al. 2019). Climate change and increased droughts have already stagnated yields in some cultivation areas. Annual hop production is about 180,000 tons worldwide. The biggest producers are the United States (51,000 tons), Germany (49,000 tons), and Ethiopia (44,000 tons) (FAOSTAT 2019).

Hops are added to beer at various stages of the brewing process for its flavor, aroma, and a characteristic bitter quality. Towards the end of the twentieth century, the antibacterial beer-preserving characteristic became gradually less important and hops was increasingly added to beer mainly for the taste. Brewing methods and pasteurization improved, as did storage spaces with even temperature and humidity; thus, the antimicrobial quality of hops was no longer crucial. Today the shelf life of beer can be lengthened by using various chemical treatment methods (Gerhäuser 2005).

In addition to taste, hop acids help to create and stabilize foam in beer. Alpha acids are mostly responsible for all these beneficial effects on beer, while beta-acids have low solubility and contribute relatively little to the taste of beer (Čermák et al. 2015). Hops usually have been processed into lipophilic extracts, or dried and ground into pellets immediately after harvesting, because these methods have been considered as the best way to preserve the

character and taste. Different forms of extracts or freeze-dried lupulin powder, from which a large part of unnecessary vegetative plant material is removed, give increased stability and uniformity to the aroma and taste, thus being crucial for industrial beer production (Van Cleemput et al. 2009; Van Holle et al. 2019).

The majority of hop plants used today are seedless, although pollination is generally known to increase yield. Seed fat can change the flavor of beer if seeds become crushed during the process. However, due to their small size, seeds tend to stay intact in the hop pellets or lipophilic extracts, which are favored by the industry. Recently, *H. lupulus* seeds have been found to be a potent source of antioxidant activity and to exhibit a cytotoxic effect against several types of cancer cells in vitro (Alonso–Esteban et al. 2019).

Small breweries and small-scale beer crafting have become more frequent since the 1990s, and new requirements for taste as well as interest for experimenting with new varieties have developed. Small breweries and local crafters tend to prefer locally grown raw materials and environmentally friendly growing methods, thus requiring the use of pest and climate tolerant hop cultivars. Cultivation of the so-called aroma hops has been steadily increasing, and the taste caused by seed fats and oils has recently been the interest of some novelty seeking craft brewers (Brendel et al. 2019; McCallum et al. 2019; Rodolfi et al. 2018; Yan et al. 2019).

Only about 15% of the hop constituents end up in beer and the rest are by-products. At present, little information is available on the methods of by-product disposal used by craft breweries (Kerby and Vriesekoop 2017). Spent hops is the main by-product of hop processing industries. It is currently used mainly as fertilizer, animal feed, or soil amendment (Aioriello et al. 2020; Bedini et al. 2015; Oosterveld et al. 2002). The use of by-products of the hop-processing and brewing industries is a welcome approach from environmental and zero-waste standpoints. Spent hops contains large amounts of essential oils that could be used. In addition, most hops constituents (especially the phytoestrogens) largely remain in the spent hops fraction after the brewing process. Potentially, spent hops can be used as

insecticide, gelling agent in the food industry, supplement in animal fodder (pigs, cattle, or poultry), or even as a botanical supplement to relieve menopausal syndromes (Bedini et al. 2015, 2016; Bolton et al. 2019; Bortoluzzi et al. 2014; Brendel et al. 2019; Reher et al. 2019). Spent grain, another by-product of the beer brewing industry, is currently used in animal fodder or as soil amendment. Spent grain contains approximately 1.5% of hops. The addition of biochar derived from spent grain to soil has been found to improve hop plant growth (Aioriello et al. 2020).

CLIMATE CHANGE CHALLENGES

Modern cultivar development aims to maintain hop characteristics relatively stable, because industrial-scale brewing needs to keep aroma qualities consistent (Van Holle et al. 2017). It has been shown, however, that growing conditions (soil composition, moisture, microclimate, etc.) have a great impact on the aroma, at least in some cultivars. The American cultivar Amarillo (Virgil Gamache Farms Inc., WA) has shown marked differences in its aroma depending on its growth location. Particularly, the polyphenol and bitter acid contents of hops have been shown to alter depending on the place of growth and the harvest year (Patzak et al. 2010; Van Holle et al. 2017).

Climate change is creating new challenges to hop cultivation. The hop plant is among the more vulnerable crop plants, and it has already been affected by the changing growing conditions. Yields have been stagnating in some areas due to shifts towards higher temperatures, which in turn affect water availability to plants (Jupa et al. 2016; Mozny et al. 2009). Potopová et al. (2021) have shown that hop yields are especially vulnerable to drought and heat wave events due to a slower rate of adaptation of hop compared to field crops. Climatic models show that droughts are likely to become more frequent in the future. The hop plant is highly sensitive to drought and it reacts quickly even to a short-term lack of water by anatomical changes in its conductive elements (tracheids). Plants' conductive elements are crucial for growth and storage, and dysfunction quickly affects productivity and yield. In lianas (woody stemmed vines, such as hop), even small

changes in the lumen structure of the conductive elements can cause significant dysfunction to the whole plant. Stem transport and the functioning of xylem will become altered, and the leaf area reduces even after one week of irregular water availability, which inevitably leads to diminished yields (Jupa et al. 2016) and to changes in the chemical composition of the hop plants (Morcol et al. 2020).

Some cultivation areas still prefer the old landraces with traditional aroma, even though the yields may be smaller. However, this may prove to be problematic in the future climate due to changes in plant diseases and pests. Changes in cultivation practices and areas, like shading or transferring cultivation to higher altitudes or more humid areas, are naturally more easily implemented with annuals than with long-lived perennials like the hop plant (Mozny et al. 2009). On the other hand, poor weather conditions have been shown to increase the production of xanthohumol and its derivatives, which in turn possess antifungal properties. For example, the Canadian *H. lupulus* ssp. *lupuloides* has exhibited elevated xanthohumol and its derivative concentrations under a high disease pressure, thus providing better bittering capability for brewing (Gerhäuser 2005; McCallum et al., 2019).

The quality of hops is measured mainly as the alpha acid content of the strobili, which also determines the value and price of the crop. The alpha acid content has been declining in some areas of cultivation, e.g., in the Czech Republic, due to changing climatic conditions, especially due to low precipitation or adverse temperatures (Donner et al. 2020). It is possible that higher concentrations of CO₂ in the air can compensate some of the negative impacts, but it is still unclear to what extent (Mozny et al. 2009).

Present-Day Use—Beyond Brewing

MULTIPLE USES

Plant-based products have been studied scientifically for their multiple beneficial biochemical effects and potential phytotherapeutic applications. Some of the effects have been known since prehistoric times. *H. lupulus* extracts are a pharmacological mixture of thousands of

TABLE 2. PROMISING MEDICAL AND OTHER USES OF *HUMULUS LUPULUS*

Studied use/effect	Bioactive chemicals	Plant part	References
Beer preservative		Strobili	Zanoli and Zavatti 2008
Beer foam stability	Isoxanthohumol, iso- α -acids	Strobili	Simpson and Hughes 1994
Beer taste		Inflorescences	Allen et al 2019
Food industry (pectin source)		Strobili	Oosterveld et al. 2002
Fertilizer		Brewing by-products	Bedini et al 2015; Oosterveld et al., 2002
Perfumes, aroma chemicals	Esters, monoterpenoids, ketones, sesquiterpenoids	Strobili, leaves	Brendel et al. 2019; Langezaal 1992; Holle et al. 2017
Flavonoid biosynthesis support, flavonoid production	Xanthohumol	Strobili	Ban et al 2018
Cancer, chemopreventive agents	Xanthohumol, isoxanthohumol, 6-PN, 8-PN	Strobili	Zanoli and Zavatti 2008
Cancer, apoptosis, inhibition of angiogenesis		Strobili	Aydin et al 2019; Philips et al. 2017
Colon cancer	Lupulone	Strobili	Lamy et al. 2007
Cancer, anti-inflammation	Prenylflavones, triterpenoids	Strobili	Akazawa et al. 2012
Skin cancer, ear infection	Humulone	Strobili	Yasukawa et al. 1995
Breast cancer		Strobili	Lempereur et al. 2016
Anticarcinogenic potential		Seeds	Alonso-Esteban et al. 2019
Antioxidant activity esp. against aging and cancer		Strobili	Philips et al. 2017
Sedative		Strobili	Schiller et al. 2006; Zanoli and Zavatti 2008
Menopausal symptoms	Isoxanthohumol, 8-prenylarigenin	Strobili	Zanoli and Zavatti 2008
Oxidative stress, aging		Strobili	Philips et al. 2017
Osteoarthritis, inflammatory conditions		Strobili	Lee et al. 2007
Antidepressant-like activity	Bitter acids (especially α -acids)	Strobili	Zanoli and Zavatti 2008
GABA _A receptor	Beta-acids, myrcene	Strobili	Zanoli and Zavatti 2008
Antimicrobial	Humulone, lupulone	Strobili	Zanoli and Zavatti 2008
Antimicrobial for acne	Xanthohumol, bitter acids	Strobili, flowers	Weber et al., 2019
Antimycobacterial (tuberculosis)		Strobili	Stavri et al. 2004
Digestive herb, stimulating gastric secretion		Strobili	Kurasawa et al. 2005
Antimicrobial against MRSA	Humulone, lupulone, xanthohumol	Strobili	Wendakoon et al. 2018
Metabolic syndrome, NAFLD	Iso- α -acids	Strobili	Mahli et al. 2018
Metabolic syndrome prevention and treatment	Xanthohumol	Strobili	Miranda et al. 2016

Table 2. (continued)

Studied use/effect	Bioactive chemicals	Plant part	References
Visceral adiposity, liver tri-glyceride accumulation	Phytoestrogens	Strobili	Hamm et al. 2019
Antithrombosis, platelet activation system	Xanthohumol	Strobili	Xin et al. 2017
Antigenotoxic, adipogenesis, glaucoma, Alzheimer, ulcer	Xanthohumol	Strobili	Aydin et al. 2019
Hepatitis C virus (HCV)	Xanthohumol	Strobili	Duke 2016
HIV-1	Xanthohumol	Strobili	Thapa et al. 2019; Wardani et al. 2018
Antimalaria	Chalcones	Strobili	Gerhäuser 2005; Srinivasan et al. 2004
Insecticide (food pest control)	Myrcene, alpha-humulene, beta-caryophyllene	Strobili	Bedini et al. 2015
Cattle ruminal fermentation, growth of bulls	Beta-acids	Strobili	Lavrenčič et al. 2018
Piglet health benefits		Strobili	Williams 2007
Poultry (substitute for anti-biotics)		Strobili	Bortoluzzi et al. 2014

compounds, some of which have been shown to be effective on their own and some in various mixtures. Hop extracts can be prepared from spent hops, a by-product of the brewing industry. Promising health benefits have been shown particularly for hop terpenophenolics (bitter acids, prenylchalcones, and prenylflavonoids), e.g., in the prevention of metabolic syndrome and several types of cancer, slowing weight gain, alleviating insulin resistance, and helping with some menopausal symptoms (Table 2) (Astray et al. 2020; Bolton et al. 2019; Gerhäuser 2005; Hamm et al. 2019; Iniguez and Zhu 2021; Langezaal 1992; Magalhães et al. 2009; Mahli et al. 2018; Philips et al. 2017; Rossini et al. 2021; Zanoli and Zavatti 2008).

The most important prenylflavonoid of *H. lupulus* is xanthohumol, which has been shown to prevent both arterial and venous thrombosis by inhibiting platelet activation without increasing bleeding risk, generally with minimal side effects (Xin et al. 2017). Xanthohumol is an effective antioxidant and has anti-inflammatory potential (Bolton et al. 2019; Gerhäuser 2005). Hop bitter acids are proving to be effective antimicrobial agents (Weber et al. 2019; Wenda-koon et al. 2018; Yamaguchi et al. 2009) and

they show potential for treating diabetes (Gerhäuser 2005). Humulone and lupulone seem to inhibit bone resorption and both may prove to be potential therapeutic drugs for osteoporosis in the future (Bolton et al. 2019; Gerhäuser 2005). Additionally, in a study by Philips et al. (2017), xanthohumol showed a direct inhibition of proteolytic enzymes and stimulated fibrillar collagen.

Recently, Alonso-Esteban et al. (2019) studied hop seeds and found them to be rich in catechins (especially, catechin and epicatechin), which are widely used compounds in the nutraceutical industry. The hop seed extract showed remarkable antimicrobial properties against tested bacteria and fungi. Thus, there is potential for further use as a natural preservative, as well as a functional food and health promoter. The seed extract did not show any hepatotoxicity. In general, hop extracts exhibit remarkably low toxicity to humans, although it has been noted that when the concentration of xanthohumol exceeds a threshold value, it may trigger opposite effects and lead to oxidative DNA damage (Alonso-Esteban et al. 2019; Gerhäuser 2005; Xin et al. 2017).

H. lupulus has been found to contain precursors for vitamin D2 (ergosterol and previtamin D2) as well as vitamin D2 (ergocalciferol), which are generally limited in human food sources (Magalhães et al. 2009). Hop pectins have been extracted from spent hops. The viscosity is comparable to apple and citrus pectins used in the food industry, but some enzymatic modifications would be needed to improve their quality, mainly in order to remove acetyl groups and excessive neutral sugars from the pectin (Oosterveld et al. 2002).

ANTICANCER PROPERTIES

The *H. lupulus* extract and its components have been shown to have direct inhibitive effects on carcinogenesis through regulating different biochemical pathways of cancer cells at various key stages of their development (see Zanolli and Zavatti 2008). 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 premyocytic and monoblastic leukemia. Several cancer inducing pathways and processes relate 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 still remain under investigation (Table 2) (Akazawa et al. 2012; Bolton et al. 2019; Gerhäuser 2005; Lee et al. 2007; Lempereur et al. 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- κ B) and activator protein AP-1 with subsequent effects on upstream pathways (Lee et al. 2007). Several studies have described the effect 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 effect 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- α 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- α 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 to producers and consumers alike (Gerhäuser 2005; Magalhães et al. 2009).

Particularly the terpenophenolics (prenylated flavonoid xanthohumol and its further constituents isoxanthohumol and 8-PN), as well as the α -acids seem to exhibit great pharmaceutical potential (Ban et al 2018). Xanthohumol is abundant particularly in European hop varieties. It activates several biochemical pathways that result in detoxification, anti-inflammatory effects, and enzyme inhibition, for example, in rat hepatoma cells (Gerhäuser 2005).

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 flora 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, has been found to be the most potent phytoestrogen known in the plant kingdom (Almaguer et al. 2014; Hamm et al. 2019; Zanolli and Zavatti 2008). Animal testing shows that 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).

ANTIMICROBIAL EFFECTS

New alternatives for antibiotics are being investigated in view of 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 in interfering and inhibiting bacterial, fungal, and protozoan growth, and acting 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 terpenophenolics have 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*, *Streptomyces*, *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 ulcer 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* in order to evaluate the effects of hop strobile extracts. Hop terpenophenolics are important for beer flavoring and of interest in biomedical research. Their inhibitory activity towards the malaria protozoa *Plasmodium falciparum* has also been reported. Among terpenophenolics, 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 effect 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 effect 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).

METABOLIC SYNDROME

NAFLD is a metabolic syndrome often resulting from the so-called “Western type diet” and usually associated with elevated insulin resistance and obesity. Alpha acids (particularly isohumulone) from *H. lupulus* recently have been shown to affect the syndrome by inhibiting pathophysiological steps of this metabolic condition, e.g., by reducing oxidative stress of the cells and reducing lipid accumulation in hepatocytes. Isohumulone was found to improve glucose tolerance and to lower elevated triglyceride levels and insulin resistance, which are signs of diabetes. By administering isohumulone to obese mice, markers for hepatic injury were reduced, glucose tolerance improved, and the expansion of adipose tissue and following obesity were reduced (Hamm et al. 2019; Mahli et al. 2018).

Prenylated flavonoid xanthohumol of *H. lupulus* is another known modulator of glucose and lipid metabolism, and it has clear anti-obesity effects in mice in vivo. Miranda et al. (2016) showed that dietary xanthohumol caused a dose-dependent decrease in body weight gain and reduced markers of systemic inflammation. Xanthohumol administration lowered plasma glucose, triglycerides, LDL-C, insulin, and plasma leptin in obese mice (Bolton et al. 2019; Hamm et al. 2019; Miranda et al. 2016).

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 potential for controlling 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 for hop oil would be an easy and economically convenient solution due to the large amount of spent hops available as a by-product of the brewing industry. Hop oil from spent hops appears to have an effect on 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 effect of spent hops' essential oil on two major storage-food pests, the lesser grain borer *Rhyzopertha dominica* (Fabricius) and the granary weevil (*Sitophilus granarius* (Linnaeus)), and apparent repelling activity was discovered (Bedini et al. 2015). In addition, the invasive fresh-water 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 in 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. *lupulus* 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 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 in order to enhance protective mechanisms in the European hop (Naraine and Small 2017).

HOP AROMAS AND PERFUMES

H. lupulus essential oils are a small fraction of dried hops, but the terpenes and terpenoids it contains are among the most important products of the hop plant. This fraction is of interest to the brewing, perfume, and flavor industry. The flavor of this fraction is dependent on the hop variety, and recently the cultivation of specific aromatic flavor 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, floral, 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 in view of the brewing industry since different tastes and aromas of beer are of interest to the producers and consumers alike. Many of the varieties and cultivars have their own 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-methylbutanoic acid, the last three of these being the most abundant in the strobili 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 the scents, but they have also been useful for their preservative qualities (Bedini et al. 2016; Duke 1983). In the *Fragrantica* online encyclopedia of perfumes, the ones that have hops fragrance as an ingredient are listed under the category of “Greens, Herbs and Fougères.” The website currently names 14 commercial fragrances that use hops fragrances in the blend (Fragrantica.com).

Conclusions

The hop plant, *H. lupulus*, has been known in folk medicine from prehistoric times, and many kinds of uses have been discovered thereafter. The strobili of females are the most frequently used part of hop, but other parts of the plant are also of interest. Climate change is creating new challenges to hop cultivation, which has already been affected by the changing growing conditions. At present, *H. lupulus* and its many cultivars are used widely in the brewing industry for their bittering flavor and floral aroma, while many other less common medicinal and other uses are known but require further investigations. Examples of those include hop substances as potential alternatives in antimicrobial, cancer, metabolic syndrome, and hormone replacement therapy treatments. The hop also shows possibilities in dental health applications, as a substitute for antibiotic food supplements in livestock, and as an insecticide, especially when chemical pesticides cannot be used for their toxicity to humans or livestock. In addition, hop components can be added to perfumes and deodorants for the scent, but they are useful for their preservative qualities as well. Future hop breeding efforts with different quality and adaptation targets can utilize existing genetic resources, such as wild populations and landraces present in many regions.

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Declarations

Ethics Declarations This review article is based only on publicly available written sources and does not include any field or experimental research. The authors declare that there are no competing interests.

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