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REVIEW PAPER

Antibacterial effects of biologically active ingredients in hop provide promising options to fight infections by pathogens including multi-drug resistant bacteria

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ABSTRACT

Antibiotic resistance constitutes a global threat to the health care systems. The number of infections due to multidrug-resistant (MDR) bacteria increases progressively resulting in an estimated annual number of 750,000 fatal cases worldwide. Additionally, the lack of novel antibiotic compounds worsens the dilemma. Hence, there is an urgent need for alternative ways to fight antibiotic resistance. One option may be natural compounds with antibacterial properties such as hop and its biologically active ingredients which are used in traditional medicine since ancient times. This prompted us to perform an actual literature survey regarding the antibacterial properties of biologically active ingredients in hop including humulone, lupulone and xanthohumol. The 20 included studies revealed that lupulone and xanthohumol do in fact inhibit the growth of Gram-positive bacteria *in vitro*. In combination with distinct antibiotic compounds the hop ingredients can even exert synergistic effects resulting in enhanced antibiotic activities against defined Gram-positive and Gram-negative bacteria. In conclusion, biologically active ingredients in hop including lupulone and xanthohumol may be potential antibiotic compounds which either alone or in combination with other antibacterial substances open novel avenues in the combat of infections caused by pathogenic including MDR bacteria.

KEYWORDS

hop, humulone, lupulone, xanthohumol, antibacterial effects, multidrug-resistant bacterial infections, antibiotic synergism

INTRODUCTION

Antibiotic resistance

Since the discovery of Arsphenamin (Salvarsan®) in 1909 by Paul Ehrlich antibiotics have been used to treat bacterial infections which are considered as one of the most important milestones in the history of medicine [1]. Distinguished as a miracle drug, antibiotics saved a countless number of lives, revolutionized medicine, and became a new weapon in the fight against life threatening infections. Antibiotic resistance exists since ancient times long before coexistence with mankind. However, the inappropriate use of antibiotic compounds since their discovery in the last century resulted in the emergence of resistant bacteria causing fatal infections [1]. Antibiotic resistance develops when bacteria are still able to survive or grow, even when they are exposed to antibiotics that are meant to kill them or at least limit their growth. The inappropriate use is among the major factors that have contributed to the development of antibiotic resistance. Examples of those factors are the use in treating viral

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infections in humans or as feed additives in livestock farming to prevent infections in otherwise healthy animals, for instance [2].

Since the common use of antibiotics, the interbacterial gene transfer and the development of resistant genes have accelerated tremendously and cannot be compared to the development of bacteria in several billions of years of evolution [1]. The main advantage of bacteria in the evolutionary challenge of the "survival of the fittest" is their immense genetic plasticity, allowing them to respond to environmental threats, including the threats through antibiotics. Bacteria have evolved two ancient mechanisms to defend those threats and to adapt to the antibiotic attack. One is the mutational resistance, the second is horizontal gene transfer [1]. Mutational resistance is a quite heterogenous, spontaneous phenomenon and affects the efficacy of the antibiotic compound through lowering the affinity to the antibiotic molecule, decreasing the drug uptake, or increasing the efflux activity. The transmission of antibiotic resistance genes among bacteria has been studied intensively. Horizontal gene transfer is performed by transformation, transduction, and conjugation in order to exchange genetic information in the same generation [3].

When Prof. Alexander Fleming and Prof. Howard Walter Florey were awarded the Nobel Prize in 1945, they already warned about upcoming antibiotic resistance. In 2014, the WHO declared that a post-antibiotic era, in which even rather minor infections might result in a fatal outcome, were a realistic scenario in the 21st century [4]. Nowadays, every year an estimated number of 214,000 newborns and 750,000 patients die from sepsis due to MDR bacterial infections [5], and the Union Internationale Contre le Cancer (UICC) forecasted that this fatality number could reach 10 million people in 2050 [2]. In consequence, antibiotic resistance is a matter of global concern and is regarded as a major health care challenge of this century. As bacterial resistances to conventional antibiotics are progressively increasing, there is an urgent need for the development of novel antibiotics. Furthermore, biologically active ingredients of natural compounds which have been used in traditional medicine since ancient times might be promising options to tackle this dilemma given that they might exert antibiotic effects by themselves or enhance the antimicrobial activities of conventional antibiotics when applied in combination.

Hop and its biologically active ingredients

Hop (*Humulus Lupulus* L.) is a species of the hemp family Cannabaceae and is cultivated since ancient times most exclusively for the brewing industry, especially in Germany and the United States of America [6]. For brewing purposes secondary metabolites, namely humulone, lupulone and xanthohumol, are extracted from the lupulin glands (specialized epithelial cells) of female hop plants, which are responsible for the typical bitter taste, for the stability, and for the froth on top of the beer. Other than that, lupulin glands secrete resins and essential oils as well as proteins, polyphenols, lipids, waxes, cellulose, and amino acids [6].

In 1153, abbess Hildegard von Bingen wrote in her "Physica sacra", Book 1, Chapter 61: "putredines prohibet in amaritudine sua", pointing out, that the bitterness of hop keeps some putrefactions from beverages, to which it may be added, so that they may last longer [7]. Hildegard von Bingen was the first person to detect antimicrobial effects exerted by hop, which since then became an important part in the brewing process. Before the use in the brewing industry, hops were traditionally applied for medicinal purposes, mainly for the treatment of sleeping disorders, for activation of gastric functions and as stomachic, antibacterial, and antifungal remedies [8]. In recent years hop has also been subject to research on numerous medical applications particularly due to its inhibitory effects on bone resorption, its tumor suppressive, anti-inflammatory [9, 10], and sedative properties, and its disease-alleviating effects in neurobiological diseases such as Alzheimer's disease, as well as cardiovascular morbidities, diabetes mellitus among others [11].

When addressing the molecular mechanisms underlying the antibacterial effects of hop, Teuber and Schmalreck hypothesized that the bacterial cell membrane was the main target of the biologically active ingredients. The authors were able to show that lupulone and humulone interfere with the phosphoenolpyruvate (PEP) system of Gram-positive bacteria resulting in a membrane leakage and a subsequent inhibition of respiration and synthesis of proteins, DNA and RNA. The integrity of Gram-negative bacteria, however, was not affected upon coincubation with the hop ingredients which was most likely due to the serum phosphatides present in the phospholipids containing outer membrane leading to the inactivation of lupulone and humulone [12].

Due to their hydrophilic as well as lipophilic properties hop compounds can also function as ionophores catalyzing transport of ions across biological membranes [13]. This effect traps protons at one membrane surface and exchanges them for Mn^{2+} or other cations. This lowers the intracellular pH, which inhibits other transporters and induces the starvation of the bacteria cell [14]. Another study revealed that the manganese-hop compound complexes inside the bacteria cell function as an electron acceptor/donator inducing oxidative stress [13]. All these antibacterial effects happen in parallel, but depend of the pH of the medium given that low pH favors the antibacterial activity [15].

Humulone

The total resins of the hop cone are divided into soft and hard resins. Humulone and lupulone are part of the soft resins, which can be further separated into α- and β-acids.

Humulone, belonging to the α-acids, is a phloroglucinol derivate with two prenyl groups and one isovaleryl group as a side chain and is responsible for the bitter flavor in hopped beer. At least 32 congeners of humulon like cohumulone, isohumulone and adhumulone have been isolated [16], of which around 5–13% can be extracted out of dried hop

cones [6, 17]. In this review all these derivatives will be comprised in the term "humulone". In nature, humulone are crystalline with a melting point at 64–65 °C [18].

Lupulone

Lupulone is a part of β-acids and all derivatives of lupulone like colupulone, adlupulone and prelupulone will subsumed in the term "lupulone". In dried hop the fraction of lupulone is approximately 3–8% depending on the hop variety and respective growth conditions [6, 17]. Lupulone and its derivatives are crystalline, exert a melting point between 82 and 92 °C, are less water soluble and possess a higher hydrophobicity when compared to humulone [17].

Xanthohumol

Hard resins are the portion of the total resin that are soluble in methanol and diethyl-ether. One small part of those hard resins is the so-called yellow compound xanthohumol (Greek: xantho = yellow), a polyphenol first isolated in 1913, which constitutes a fraction of 0.3–1.5% of weight in dried hop cones [6] and is the only known naturally occurring methylated hop resin. Upon oral administration the bioavailability of xanthohumol is very low, but relatively high concentrations could be achieved inside intestinal epithelial cells given specific binding of the molecule to cytosolic proteins [19]. Xanthohumol has gained medicinal attention due its anti-cancer properties. In fact, the molecule has been shown to inhibit virtually all stages of carcinogenesis such as initiation, promotion, and progression of a plethora of cancer types including intestinal, breast, ovary, prostate cancer as well as multiple myeloma, and lymphocytic leukemia [20].

Aim

In this literature review we summarize studies addressing antibacterial effects of biologically active ingredients in hop such as humulone, lupulone and xanthohumol which might be promising treatment options in the fight of infections caused by pathogens including MDR bacteria.

METHODS

Inclusion and exclusion criteria

Studies addressing antibacterial effects of hop and its derivatives in *in vitro* and *in vivo* experiments were included in this review, whereas studies on antiviral, antifungal and antiparasitic effects were excluded.

Search

The literature search was performed on the MEDLINE database PubMed between the 23rd of August and 3rd of September 2021. After initial screening search, it became obvious, that there were no existing MeSH terms covering hop in general which also held true for the hop compounds. Instead of using MeSH terms we experimented with the tag "Title/Abstract". This tag ensured that the key words were used in the title or abstract of articles. This search yielded only three results, of which only one covered an experiment with hop compounds. For the subsequent, less specific search we used the hop compounds "lupulone", "humulone" and "xanthohumol" divided by the Boolean operator "OR" in connection with the MeSH term "Anti-microbial agents" by the Boolean operator "AND", ensuring that articles would end up in the final search mentioning "Anti-microbial agents" and at least one of the biologically active ingredients in hop. From the 50 results yielded by the query one study had to be excluded because it was available in French language only. Another 22 studies were excluded since they were addressing different topics or antifungal, antiviral or antiparasitic properties of hop. This had to be expected by using the rather general term "antimicrobial agents" instead of using a term addressing exclusively antibacterial effects. Of the remaining 28 studies 8 were reviews. The remaining 20 studies were included in this review and will be summarized in the following.

RESULTS

Antibacterial effects of hop ingredients – the role of pH

In 1949 Chin et al. used lupulone soluted in ethyl alcohol and propylene glycol to a concentration of 1% and tested this agent against *Staphylococcus aureus* FDA 209, *Mycobacterium phlei* and *Mycobacterium tuberculosis* H37Rv. An inhibition of *S. aureus* was observed at a lupulone minimum inhibitory concentrations (MIC) (i.e., the lowest concentration of the tested agent that does not result in bacterial growth) of 1.56 µg ml^{-1} up to a concentration of 1:1,000 of the inoculum. Furthermore, a negative correlation between antibacterial effect and pH could be assessed given that the antibacterial properties of lupulone were enhanced at low pH. Whereas lupulone could inhibit the growth of *M. phlei* with an MIC of 50 µg ml^{-1} at pH 7 and 8, an MIC of 40 µg ml^{-1} was assessed at pH 5 and 6. Even lower MICs were obtained against *M. tuberculosis*, namely a lupulone MIC of 25 µg ml^{-1} at pH 7 and 8 and an MIC of 15 µg ml^{-1} at pH 6.

After adding 10% pooled human or horse serum to the medium, Seitz-filtering and heat inactivation (i.e., at 56 °C for 30 min), the antibacterial activity of lupulone against *M. tuberculosis* was not affected, whereas the same procedure revealed a decreased antibacterial activity against *M. phlei.* [21].

Simpson and Smith studied the influence of distinct environmental factors including pH on the antibacterial activity of hop *in vitro*. Therefore, the antibacterial activity of humulone and lupulone against *Lactobacillus brevis* IFO 3960 were determined over the pH range 4–7. Increases in pH resulted in less distinct antibacterial effects of both, humulone and lupulone including derivatives indicative for an inverse correlation between the pH and the antibacterial activity of respective hop ingredients. Furthermore, the

influence of cations on the antibacterial effects of hop constituents against *L. brevis* were determined. Results revealed that monovalent cations such as K^+, Na^+, NH_4^+, Rb^+ and Li^+ increased the antibacterial effect with H^+ resulting in most distinct enhanced antibacterial results. Bivalent cations such as Ca^{2+} and Mg^{2+}, however, decreased the antibacterial activity of humulone and lupulone against *L. brevis* [15].

Roehrer et al. tested the antibacterial properties of xanthohumol. The authors purchased a commercially available pre-concentrated xanthohumol extract (Xantho-Flav®) and extracted xanthohumol (purity 98.6%) as well as the derivative xanthohumol C (95% purity). Then, the xanthohumol extracts as well as Xantho-Flav® were tested at different concentrations (i.e., 100 and 1,000 µM) against *Bacillus subtilis* under varying pH conditions applying the disc diffusion assay. Whereas *B. subtilis* growth was not inhibited at a xanthohumol concentration of 100 µM (pH 7), a growth inhibition could be observed at 1,000 µM (pH 7). Even higher inhibition zone diameters were obtained for Xantho-Flav® (18.6 mm) and xanthohumol (18.2 mm) at pH 5, whereas at pH 6 the inhibition zone diameters were smaller (10.0–13.5 mm) for 1,000 µM of respective molecules. The authors concluded that the antibacterial effect of xanthohumol was pH dependent: the lower the pH, the higher the observed antimicrobial activity [22].

Another study assessed the MIC of hop compounds against foodborne pathogens such as *Escherichia coli*, *Salmonella enterica*, *Listeria monocytogenes* and *S. aureus*. Therefore, humulone and lupulone were dissolved in propylene glycol to a final concentration of 40% and 20%, respectively, whereas xanthohumol was dissolved in DMSO and further diluted in sterile deionized water. Results revealed that no relevant inhibition of the Gram-negative bacteria *E. coli* and *S. enterica* could be observed by the hop compounds. Humulone was able to inhibit the growth of the two Gram-positive bacteria strains at MICs from 6.3 up to 200 ppm. In case of lupulone the MICs were lower (ranging from 1.6 up to 12.5 ppm), whereas the xanthohumol MICs were slightly higher and ranged from 3.1 to 12.5 ppm. The authors also observed that growth inhibition was pH dependent given that all measured MICs were lower at pH values of 5.0 when compared to pH 7.2 [23].

Antibacterial effects of hop ingredients against Gram-positive versus Gram-negative bacteria

In an early study from 1949, Salle and colleagues tested the inhibitory activity of lupulone against a plethora of bacterial species. Therefore, 1 g lupulone was dissolved in 25 ml of propylene glycol and then added to distilled water for a 1:500 emulsion. Using the penicylinder method the emulsion was then added to a medium of 1,000 ml containing NaCl (5 g), beef extract (5 g), peptone (10 g), agar (20 g) and distilled water. Lupulone (1:10,000 concentration) inhibited the growth of all 12 tested Gram-positive bacterial isolates (*Micrococcus lysodeikticus, Bacillus anthracis, Streptococcus* (i.e., *Enterococcus*) *faecalis*), whereas the 1:500 emulsion did not inhibit any of the 18 Gram-negative bacteria. Remarkably, lupulone was able to inhibit the growth of *M. tuberculosis* strain H37Rv in a 1:300,000 emulsion indicative for a strong *in vitro* antibiotic activity.

Furthermore, the authors expanded their study to *in vivo* tests. Therefore, mice were challenged with *Streptococcus pyogenes* and subsequently treated with lupulone (2 mg three times daily via the intraperitoneal route), but all mice died within 24 h. The authors concluded that the antibiotic effect of lupulone was completely inactivated *in vivo* [24].

The antibacterial activity of xanthohumol against *S. aureus* and *E. coli* was tested in another study. The polyphenol had been isolated from superficial carbon-dioxide-extracted hops, with a purity of more than 95% and was diluted to a final concentration of 100 µg ml^{-1}. Using the disc diffusion method *E. coli* was resistant, whereas the *S. aureus* was tested susceptible with an inhibition zone diameter of 3.57 mm [25].

In 1970 Teuber investigated the inhibitory activity of hop compounds against both, Gram-positive and Gram-negative bacterial species such as *B. subtilis*, *S. aureus*, and *M. lysodeikticus* and furthermore, against *E. coli* and *Proteus mirabilis*. Teuber aimed for the inhibitory concentrations resulting in a 50% bacterial growth reduction. The Gram-negative bacteria *E. coli* and *P. mirabilis* were neither inhibited by humulone nor by lupulone. Whereas a concentration of 32 µg ml^{-1} humulone was needed to achieve a 50% reduction in bacterial growth of *M. lysodeikticus* (MIC_{50}), the humulone MIC_{50} against *B. subtilis* and *S. aureus* were even lower (i.e., 16 µg ml^{-1}). Overall, the MIC_{50} values of lupulone were slightly higher, with concentrations ranging from 20 to 62 µg ml^{-1} against the three Gram-positive bacterial strains. Of note, this has been the only study to date describing more pronounced antibacterial efficacies of humulone versus lupulone [26].

Anti-staphylococcal and anti-biofilm activities of hop ingredient

In their study Bogdanova and colleagues assessed the antibacterial activities of humulone, lupulone and xanthohumol against different *Staphylococcus* species including strains causing life-threatening biofilm-associated infections of artificial heart valves, for instance. The tested hop extracts contained between 83.2% and 90.0% of the hop ingredients. Methicillin-susceptible *Staphylococcus epidermidis* and *S. aureus* strains, three methicillin-resistant strains, namely *S. epidermidis*, *Staphylococcus capitis subspecies ureolyticus*, as well as *S. aureus* which had been isolated from an intravenous catheter of a diseased patient were included. The analyses revealed that relatively high MICs between 7.5 and 30.0 µg ml^{-1} were obtained for humulone, whereas the MICs of xanthohumol were significantly lower (<4.0 µg ml^{-1}). The lowest MICs of 0.5 µg ml^{-1}, however, were obtained for lupulone. In addition, the authors also reported minimum bactericidal concentrations (MBC) ranging from 1.0 to 15.0 µg ml^{-1} for lupulone and xanthohumol on all tested strains.

The study also assessed the ability of the tested hop compounds to penetrate formed biofilms. Particularly xanthohumol could effectively reduce bacterial biofilm release. Collectively, this study showed potent anti-staphylococcal and biofilm-reducing effects of distinct bioactive hop ingredients [27].

In order to test the antibacterial activity of xanthohumol against *S. aureus* and *S. epidermidis*, Bartmanska et al. used spent hops that had been obtained as waste material from brewing facilities containing many valuable flavonoids that were not extracted in the brewing process. The content of xanthohumol, the main flavonoid found in spent hops, ranged from 0.1 to 1% of the cone dry mass. Six xanthohumol derivatives were obtained by microbial transformation, by chemical modifications or by extraction from the waste hops, and the antibacterial activities were assessed by measurements of the MIC_{80} (defined as the concentration inhibiting 80% of the bacterial growth). The analyses revealed that the xanthohumol derivatives showed antibacterial activity against methicillin sensitive and resistant strains of *S. aureus* and *S. epidermidis* with MIC_{80} values ranging from 5 to 50 μg ml^{-1}, whereas ampicillin as antibacterial control yielded MIC_{80} values between 2.5 and 5 μg ml^{-1}, respectively. The authors suggested further investigations addressing some compounds from spent hops and their derivatives as potential treatment options of staphylococcal infections [28].

Antimicrobial effects of hop ingredients against bacteria involved in the immunopathogenesis of acne vulgaris

Weber et al. investigated the potential of humulone and lupulone against *Propionibacterium acnes* and *S. aureus* strains involved in hyperkeratosis and inflammation during pathogenesis of acne vulgaris and used hop–CO_2–extract with 50% humulone and 50% lupulone. For MIC determinations the authors applied the broth microdilution assay and tested four different strains of *P. acnes*, which were inhibited by a concentration of 3.1–6.2 μg ml^{-1}. As a positive control the lincosamide antibiotic clindamycin was used resulting in MICs of less than 0.2 up to 0.8 μg ml^{-1} against respective *P. acnes* isolates. Against *S. aureus* the obtained MIC results ranged between 6.25 and 12.5 μg ml^{-1} of lupulone, whereas clindamycin inhibited the bacterial growth with 0.002–0.25 μg ml^{-1}. Of note, the hop extract was able to inhibit the growth of the methicillin-resistant *S. aureus* (MRSA) at 12.5 μg ml^{-1}, whereas the clindamycin MIC was 50 μg ml^{-1}. Hence, lupulone could effectively inhibit growth of acne vulgaris associated pathogens such as *P. acnes* and *S. aureus* including MRSA isolates [29].

Yamaguchi et al. tested hop compounds against various bacterial species that are discussed to be involved in the immunopathogenesis of acne vulgaris. Humulone exerted MICs ranging from 3 to 30 μg ml^{-1} against *P. acnes*, *S. aureus*, *S. epidermidis*, *S. pyogenes* and *Kocuria rhizophila*, whereas lupulone could inhibit bacterial growth at relatively low MICs of between 0.1 and 1 μg ml^{-1}, and with an MIC of 10 μg ml^{-1} against *S. epidermidis*. For xanthohumol MICs between 1 and 3 μg ml^{-1} were measured against respective acne associated bacterial strains. Notably, the authors hypothesized that certain lactobacilli involved in the beer brewing process might develop a hop resistance suggesting that the potential use of hop components may be associated with evolving hop-resistant acne associated bacteria [30].

Further antibacterial effects of hop ingredients

Teuber and Schmalreck, the group that described the bacterial membrane-leakage following application of hop ingredients, tested humulone and lupulone against *B. subtilis*. The pure hop compounds were dissolved in 96% ethanol and diluted to the final concentration with 64% ethanol. Whereas ethanol alone had no antibacterial effect, lupulone exerted a more pronounced growth inhibition of *B. subtilis* (with an MIC of 1 μg ml^{-1}) as compared to humulone (MIC of 2 μg ml^{-1}). The authors postulated a correlation between hydrophobicity and the antibiotic potential of the hop compounds due to the fact that the solubility of lupulone in distilled water is lower than humulone [12].

In another study humulone, lupulone and xanthohumol were tested against several obligate anaerobic gut bacterial isolates including *Bacteroides fragilis*, *Clostridium perfringens* and *Clostridioides difficile*.

The MIC of humulone ranged from 160 to 1,540 μg ml^{-1} against *B. fragilis* and from 680 to 1,370 μg ml^{-1} against the four tested *C. perfringens* strains. Lower MICs were measured for lupulone ranging from 50 to 430 μg ml^{-1} for *B. fragilis* and 150–260 μg ml^{-1} for respective *C. perfringens* strains. The lowest MICs and thus the most effective antibacterial results were obtained for xanthohumol ranging from 10 to 56 μg ml^{-1} in case of *B. fragilis* and *C. perfringens*, respectively, whereas the MBCs were slightly higher (up to 80 μg ml^{-1}).

Against the clinical *C. difficile* isolates included, lupulone was shown to be the most effective compound with MICs ranging from 12 to 96 μg ml^{-1} and MBCs between 16 and 212 μg ml^{-1}. For xanthohumol, MICs from 32 μg ml^{-1} to 107 μg ml^{-1} and MBCs between 40 and 107 μg ml^{-1} were obtained, whereas the respective humulone MICs were significantly lower. The authors concluded that these results were close to the MICs and MBCs when testing conventional synthetic antibiotics against respective bacterial isolates indicating potent antibacterial effects of defined hop ingredients against anaerobic bacteria and pathogens [31].

In a study from China, the authors analyzed the antibacterial activity of lupulone against *M. tuberculosis* and determined an MIC of 10 μg ml^{-1} against *M. tuberculosis* H37Rv (ATCC 27294) strain. Furthermore, the authors analyzed the transcriptional responses of *M. tuberculosis* upon lupulone coincubation. The applied genome-wide transcription analyses revealed a total of 540 genes that were differentially regulated by lupulone of which several were associated with heterogenous molecules and pathways such as surface-exposed lipids, cytochrome P450 enzymes, proline-glutamate/proline-proline-

glutamate (PE/PPE) multigene families, ABC transporters and protein synthesis, respectively [32].

Synergistic antibacterial effects of hop ingredients

In a study from 1964, lupulone and its derivative hexahydrolupulone were diluted in ethanol and ethylene glycol in solutions ranging from 1:10,000 to 1:400,000. Two strains of *S. aureus* were tested of which the first one had been isolated from a patient with recurrent furunculosis and tested resistant against multiple antibiotics such as penicillin, erythromycin, streptomycin, tetracycline and oxytetracycline, but was susceptible towards bacitracin, novobiocin, neomycin, methicillin, vancomycin, kanamycin, ristocetin and chloramphenicol. The latter *S. aureus* strain was obtained from the U.S. Department of Agriculture in Washington D.C. and was susceptible to penicillin. The antistaphylococcal activities of lupulone and its derivative were determined by a two-fold tube dilution test. Both molecules inhibited the *S. aureus* growth at 10 μg ml^{-1}. In combination with penicillin, hexahydrolupulon could inhibit staphylococcal growth at an MIC as low as 1 μg ml^{-1}. In addition, synergistic antibacterial effects were assessed upon coincubation of respective lupulone compound with erythromycin, tetracycline or oxytetracycline (100 μg ml^{-1} each) with resulting MIC ranging from 2.5 to 5.0 μg ml^{-1} [33].

In another study, Rozalski et al. evaluated the antibacterial properties of three different hop extracts on distinct *S. aureus* strains and on one *Enterococcus faecalis* strain by using the microdilution assay. Therefore, the hop cones extract (derived from wasting material of the brewing industry containing no xanthohumol) was purified with a final xanthohumol yield of 51% of the dry matter as determined by high-pressure liquid chromatography. The hop had been defatted, freeze-dried and dissolved in DMSO. The other tested compounds were pure xanthohumol and a natural hop cone extract containing xanthohumol. The authors observed that the antibacterial activities were dependent on the xanthohumol content since the xanthohumol extract from the wasting material of the brewing process were slightly less active than the other xanthohumol containing agents. When tested against two *S. aureus* strains (namely strains 29213, D5 and A7), the MICs for pure xanthohumol were between 0.015 and 0.125 μg ml^{-1}, whereas the *E. faecalis* 29212 strain was inhibited at a concentration of 0.062 μg ml^{-1} xanthohumol. The results for the xanthohumol containing hop cones extracts were comparable, whereas the MICs for the xanthohumol-free spent hops extract the MICs ranged between 1 and 2 μg ml^{-1} and were thus higher, but still indicative for pronounced antibiotic effects.

The authors further addressed potential synergistic effects of respective extracts in combination with oxacillin, vancomycin, and linezolid against *S. aureus* strain ATCC 29213. Whereas the antimicrobial activity of vancomycin could not be further increased by either extract, coincubation with respective extracts enhanced the antibiotic activity of oxacillin, which also held true, but to a lesser extent, for linezolid.

Finally, the authors analyzed the effect of xanthohumol extracts on bacterial biofilm formation. Therefore, *S. aureus* ATCC 29213 strain was cocultured with the hop extracts at their 1/2, 1/4 and 1/8 MICs for 24 h and the biofilm formation assessed by using a LIVE/DEAD detection assay. In comparison to a positive control, the xanthohumol containing compounds inhibited the biofilm formation by up to more than 80% at 1/2 of their MICs and between 40% and 60% at a concentration of 1/4 of their MIC [34].

Natarajan et al. investigated potential synergistic antibacterial effects of hop compounds and synthetic antibiotics. Therefore, lupulone and xanthohumol (with purities between 98% and 100%), were added to commercially prepared antibiotic creams and tested against Gram-positive and Gram-negative bacteria by using the disc diffusion assay. The obtained results revealed synergistic effects of both hop compounds in combination with the synthetic antibiotics polymyxin B, ciprofloxacin, and tobramycin against a wide spectrum of bacteria (including *Pseudomonas fluorescens, P. mirabilis, Bacillus megaterium, Staphylococcus saprophyticus, Proteus vulgaris, Pseudomonas aeruginosa* and *B. subtilis*). Besides the known mechanism underlying the distinct antibacterial effects of the three compounds from different antibiotic classes, the authors hypothesized that an enhanced bacterial permeability caused by lupulone and xanthohumol might overcome the resistance of the bacterial membrane to the permeation of the antibiotic. Furthermore, polymyxin which usually does not exert antimicrobial effects against Gram-positive bacteria, was able to synergize with the hop compounds by inhibiting the tested bacterial isolates including *B. megaterium, B. subtilis* and *S. saprophyticus*. Moreover, lupulone was able to synergize with polymyxin on Gram-negative bacteria which was rather unexpected given lupulone's lack of antimicrobial effect against Gram-negative bacteria. The authors concluded that the synergistic effects observed with lupulone in combination with synthetic antibiotics was independent of the bacterial cell wall architecture [35].

In a study from Poland, a two-step supercritical fluid extraction was applied in order to obtain two hop samples. The first one, called "crude extract" contained humulone (42%), lupulone (19%), terpene derivatives and xanthohumol, whereas the other extract was pure xanthohumol. Both extracts were tested against *S. aureus, S. epidermidis*, *Streptococcus mutans*, *Streptococcus sanguinis* and *P. acnes*. Against *S. epidermidis* both extracts were shown to be very effective with MICs of 0.098 μg ml^{-1}. Similar antimicrobial activities were obtained for the synthetic antibiotics gentamicin and sparfloxacin against *S. epidermidis*. Slightly higher extract MICs were measured against *S. aureus* (0.195 μg ml^{-1}) and *S. mutans* (0.391 μg ml^{-1}). Xanthohumol did not exert potent growth inhibition of *S. sanguinis*, but the "crude extract" inhibited the growth at an MIC of 0.781 μg ml^{-1}, similar to ciprofloxacin and even more effective than the third generation cephalosporine ceftriaxone. Moreover, the hop extracts displayed relatively high MICs of 15.6–62.5 μg ml^{-1} against two *P. acnes* strains.

Another aim of this study was to assess potential synergistic antibacterial interactions of hop compounds and distinct synthetic antibiotics. For synergy assessment, the fractional inhibitory concentration index (FICI) using the MIC of tested agents was applied. Results demonstrated that the "crude extract" showed synergistic or at least additive effects in combination with cefepime, ceftriaxone, ciprofloxacin and sparfloxacin against all tested Gram-positive strains. Xanthohumol, however, did not exhibit as potent synergistic antibacterial effects in combination with respective antibiotics when compared with the "crude extract". The hop compounds did neither show synergistic effects when combined with gentamicin, nor did they exert antagonistic effects in combination with the applied antibiotics [20].

In another study, the authors tested chitosan nanoparticles, a biopolymer with antibacterial activity as a carrier for lupulone and xanthohumol. Chitosan was obtained from shrimp and then prepared by the ionotropic gelation method. Lupulone/xanthohumol in chitosan solutions (comparing 2:1 and 3:1 concentrations) were then tested against *S. aureus* and *P. aeruginosa*. As expected, the activity against *S. aureus* was higher than the antibacterial activity against *P. aeruginosa*. Remarkably, the encapsulation of lupulone with chitosan nanoparticles resulted in synergistic antibacterial effects against both Gram-positive and Gram-negative bacteria. Lupulone and xanthohumol showed comparably pronounced antibacterial activities against *S. aureus* and *S. epidermidis*, given that both staphylococcal strains were inhibited by the hop compounds at 93 µg ml^{-1} in a 3:1 lupulone/xanthohumol to chitosan solution. When tested against *P. aeruginosa*, however, the MICs of 187 µg ml^{-1} were higher for both hop compounds. In summary, all tested solutions increased the antibacterial effect against a broad spectrum of bacteria and showed remarkable synergistic interactions between chitosan and hop compounds. The authors concluded that respective compounds might be a promising preventive measure of contact dermatitis reported in hop pickers and the ability from chitosan masking the strong bitter taste of hop [36].

In vivo antibacterial effects of hop ingredients

In their *in vivo* study, Tillman et al. evaluated the effect of lupulone on the chicken intestinal microbiota. The intestinal microbiota of broilers was quantified after adding lupulone to the drinking water (final lupulone concentration of 125 µg ml^{-1}). 20 newly hatched chicken were separated into five groups. Groups 3 and 5 received lupulone on days 13–22, whereas groups 4 and 5 were perorally administered 0.1 ml *C. perfringens* on days 14–16. Birds from group 1 were sacrificed on day 14 and the remaining ones eight days later. Cecal and midgut sections were removed from the chicken gastrointestinal tracts for further investigations using quantitative real-time PCR. The results did not reveal any statistically significant effects of lupulone on the overall gut microbiota composition, irrespective of the section within the bird's intestinal tract. However, two bacterial strains showed a significant reduction after the treatment with lupulone since *C. perfringens* counts were significantly lower in the cecum and midgut of the lupulone treated cohort as compared to respective control birds, which also held true for the *Lactobacillus* counts in the midgut [37].

DISCUSSION

Findings of the research

Our literature survey revealed that hop compounds can inhibit bacterial growth in general. Lupulone and xanthohumol in particular [23, 27, 31] were shown effective against Gram-positive, but not Gram-negative bacterial species, however [23, 24, 25]. In case of humulone if at all, rather minor antibacterial effects could be assessed, whereas one paper described antibacterial effects directed against *B. subtilis* and *S. aureus* [26]. Remarkably, lupulone and xanthohumol were shown to be effective also against multidrug-resistant bacteria including *S. epidermidis*, *S. capitis subspecies ureolyticus*, and notably, *S. aureus* (MRSA) [27, 28, 33]. Furthermore, studies revealed that the antibacterial activities of hop constituents were highly pH dependent given that more pronounced antibacterial effects could be observed upon lower pH in the applied media [15, 21–23].

Regarding the molecular mechanism underlying the antibacterial effects of hop compounds, Teuber and Schmalreck hypothesized that the antibacterial activities of lupulone and humulone against *B. subtilis*, for instance, were due to induced bacterial membrane leakage [12]. Another study found transcriptional changes in genes responsible for surface-exposed lipids, cytochrome P450 enzymes, PE/PPE multigene families, ABC transporters and protein synthesis in hop-treated Mycobacteria [32].

Two studies demonstrated that the antibacterial efficacies of the tested hop compounds were virtually comparable to those of conventional synthetic antibiotics [20, 31], whereas other papers described even more enhanced antibacterial effects [28, 29]. The differences in observed antibacterial effects most likely depend on the respective compound, the applied tests as well as bacteria that have been used.

Remarkably, when combined with conventional antibiotics such as penicillin, erythromycin, tetracycline, oxytetracycline, oxacillin, vancomycin, linezolid, cefepime, ceftriaxone, ciprofloxacin, sparfloxacin, polymyxin B, and tobramycin additive or even synergistic effects could be assessed when tested against Gram-positive bacteria indicative for antibiotic enhancing properties of hop ingredient [20, 33–35]. Strikingly, one study found out, that these synergistic effects were not only effective against Gram-positive, but also distinct Gram-negative bacteria including *P. vulgaris, Serratia marcescens and P. aeruginosa* [35]. In line, synergistic effects could be shown of lupulone and xanthohumol in combination with chitosan nanoparticles, which were used as carrier, against both Gram-positive and Gram-negative bacteria including *S. aureus, S. epidermidis, and P. aeruginosa*, respectively [36].

One needs to take into consideration that the vast majority of the here reviewed papers reported *in vitro* results, whereas *in vivo* studies were, however, scarce and even conflictive. For instance, lupulone was described to be inactivated when injected into mice via the intraperitoneal route [24], whereas in another study the authors hypothesized, that lupulone would retain its antibacterial effects active under physiological conditions *in vivo* [21].

One study addressed the gut microbiota changes in chicken following peroral treatment with a hop ingredient. Overall, the changes in intestinal microbiota compositions following lupulone application was rather minor given that only *Lactobacillus* and *C. perfringens* counts were lower as compared to placebo treated counterparts [37].

Conclusion and outlook

In summary, biologically active ingredients in hop such as lupulone and xanthohumol and its derivatives constitute valuable natural compounds which alone or in combination with synthetic antibiotics might be considered as promising options to combat infection by pathogens including MDR bacteria. However, a lot more research, both *in vitro* and especially *in vivo,* is needed to provide more evidence for a translational application from bench to bedside.

Limitations

This literature survey aimed to explore antibacterial properties of biologically active hop ingredients, aiming at a search query both, as sensitive and broad as possible. But there might be relevant studies that have not been included in this review. Our search yielded three different compounds in hop with antibacterial properties. Through the research process we decided to search for antibacterial properties in all three compounds (instead of focusing on a single one only) in order to assure a broader overview of the antibacterial activity exerted by hop. In addition, the research process was impeded since there are no MeSH Terms for humulone, lupulone and xanthohumol on the MEDLINE database PubMed.

Furthermore, the comparability of results between the reviewed studies was limited due to differences in compounds and solvents used, in order to prepare the compounds and the test conditions, for instance. From the 20 studies in this review only two conducted *in vivo* experiments testing lupulone in mice and chicken. The other two compounds were only tested *in vitro* which leaves the question regarding translation of results to humans ("from bench to bedside") unanswered.

DECLARATIONS

Ethics statement: Not applicable (literature survey).

Conflict of interests: SB and MMH are Editorial Board members.

Authors' contributions: AF conceived and designed the survey, wrote the paper. SB provided critical advice in design of the survey, edited paper. MMH supervised the survey, co-wrote the paper.

REFERENCES

1. Davies J, Davies D. Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev 2010;74(3):417–33.
2. Antimicrobial resistance and its impact on cancer care. Available from: https://www.uicc.org/what-we-do/thematic-areas-work/antimicrobial-resistance-and-its-impact-cancer-care?gclid=Cj0KCQiAzMGNBhCyARIsANpUkzM64n4V6mpUS4pj4SP2Q4aiSS7bRfMZm2yEpuW_3vZ2cMDKZf3RpVgaAsSEEALw_wcB [Accessed 9 Jan 2022].
3. Munita JM, Arias CA. Mechanisms of antibiotic resistance. Microbiol Spectr 2016;4:1–37.
4. Nathan C, Cars O. Antibiotic resistance- problems, progress, and prospects. New Engl J Med 2014;371(19):1761–3.
5. Avafia T, Cars O. Antimicrobial resistance; an emerging crisis. Available from: https://www.undp.org/blog/antimicrobial-resistance-emerging-crisis?utm_source=EN&utm_medium=GSR&utm_content=US_UNDP_PaidSearch_Brand_English&utm_campaign=CENTRAL&c_src=CENTRAL&c_src2=GSR&gclid=Cj0KCQiAzMGNBhCyARIsANpUkzOJ_KSdow_VVsGxBS3s4qJRkn8b1_Ud_2CIKNAKyXEgXFbn3Ta0XkUaAn0QEALw_wcB [Accessed 9 Jan 2022].
6. Patzak J, Krofta K, Henychova A, Nesvadba V. Number and size of lupulin glands, glandular trichomes of hop (Humulus lupulus L.), play a key role in contents of bitter acids and polyphenols in hop cone. Int J Food Sci Technol 2015;50(8).
7. Zythophile UK. A short history of hops. Available from: https://zythophile.co.uk/2009/11/20/a-short-history-of-hops/ [Accessed 9 Jan 2022].
8. Bartmanska A, Walecka-Zacharska E, Tronina T, Poplonski J, Sordon S, Brzezowska E, Bania J, Huszcza E. Antimicrobial properties of spent hops extracts, flavonoids isolated therefrom, and their derivatives. Molecules 2018;23:2059.
9. Tobe H. Biological activities of humulone. Beer in Health and Disease Prevention; 2009. pp. 695–702.
10. Magalhaes PJ, Carvalho DO, Cruz JM, Guido LF, Barros AA. Fundamentals and health benefits of xanthohumol, a natural product derived from hops and beer. Nat Prod Commun 2009;4(5):591–610.
11. Muresan IE, Paucean A, Man S, Muste S, Beldean BV, Chis MS, Bordean ME, Mateescu MA, Fostoc G, Szanto L. The importance of biologically active compounds of hop (Humulus Lupulus) in pharmaceutical use increase impact of hop industry in the performances of US economy. Hop Med Plants 2020:99–113.
12. Teuber M, Schmalreck AF. Membrane leakage in Bacillus subtilis 168 induced by the hop consitients lupulone, humulone, isohumulone and humulinic acid. Arch Microbiol 1973;94:159–71.
13. Behr J, Vogel RF. Mechanisms of hop inhibition include the transmembrane redox reaction. ASM Journals 2020;76.

14. Simpson WJ. Cambridge prize lecture. Studies on the sensitivity of lactic acid bacteria to hop bitter acids. J Inst Brewing Distilling 1993;99:405–11.
15. Simpson WJ, Smith ARW. Factors affecting antibacterial activity of hop compounds and their derivatives. J Appl Bacteriol 1991;72: 327–34.
16. Urban J, Dahlberg CJ, Carroll BJ, Kaminsky W. Absolute configuration of Beer′ bitter compounds. Angew Chem 2012;52:1553–5.
17. Almgauer C, Schönberger C, Gastl M, Arendt EK, Becker T. Humulus lupulus – a story that begs to be told. A review. J Inst Brewing 2014;120(4):289–314.
18. Gabel L. Zur Effizienz der Zerschäumungsanalyse im halbtechnischen Maßstab am Beispiel von Humulus lupulus L. und Rosmarinus officinalis. L. Technische Universität München 2008: 39–42.
19. Pang Y, Nikolic D, Dongwei Z, Chadwick LR, Pauli GF, Farnsworth NR, van Breemen RB. Binding of the hop (Humulus Lupulus L.) chalcone xanthohumol to cytosolic proteins in Caco-2 intestinal epithelial cells. Mol Nutr Food Res 2007;51(7):872–9.
20. Klimek K, Tyskiewicz K, Miazga-Karska M, Debczak A, Roj E, Ginalska G. Bioactive compounds obtained from polish "Marynka" hop variety using efficient two-step supercritical fluid extraction and comparison of their antibacterial, cytotoxic, and anti-proliferative activities in vitro. Molecules 2021;21:2366.
21. Chin YC, Chang NC, Anderson HH. Factors influencing the antibiotic activity of Lupulon. J Clin Invest 1949;5:909–15.
22. Roehrer S, Behr J, Stork V, Ramires M, Médard G, Frank O, Kleigrewe K, Hofmann T, Minceva M, Xanthohumol C. A minor bioactive hop compound: production, purification strategies and antimicrobial test. J Chromatogr B 2018;105:39–49.
23. Kramer B, Thielmann J, Hickisch A, Muranyi P, Wunderlich J, Hauser C. Antimicrobial activity of hop extracts against foodborne pathogens for meat applications. J Appl Microbiol 2014;118: 648–57.
24. Salle AJ, Jann GJ, Ordanik M. Lupulon- an antibiotic extracted from the strobiles of Humulus lupulus. Proc Soc Exp Biol Med 1949;70:409–11.
25. Stompor M, Zarowska B. Antimicrobial activity of Xanthohumol and its selected structural analogues. Molecules 2016;21:608.
26. Teuber M. Low antibiotic potency of Isohumulone. Appl Microbiol 1970;19(5):871.
27. Bogdanova K, Röderova M, Kolar M, Langova K, Dusek M, Jost P, Kubelkova K, Bostik P, Olsovska J. Antibiofilm activity of bioactive hop compounds humulone, lupulone and xanthohumol toward susceptible and resistant staphylococci. Res Microbiol 2018:127–34.
28. Bartmanska A, Walecka-Zacharska E, Tronina T, Poplonski J, Sordon S, Brzezowska E, Bania J, Huszcza E. Antimicrobial properties of spent hops extracts, flavonoids isolated therefrom, and their derivatives. Molecules 2018;23:2059.
29. Weber N, Biehler K, Schwabe K, Haarhaus B, Quirin KW, Frank U, Schempp CM, Wölfle U. Hop extract as an antioxidant with antimicrobial effects against Propionibacterium Acnes and Staphylococcus Aureus. Molecules 2019;24:223.
30. Yamaguchi N, Satoh-Yamaguchi K, Ono M. In vitro evaluation of antibacterial, anticollagenase, and antioxidant activities of hop components (Humulus lupulus) addressing acne vulgaris. Phytomedicine 2009;16:369–76.
31. Cermak P, Olsovska J, Mikyska A, Dusek M, Kadleckova Z, Vanicek J, Otakar NYC, Sigler K, Bostikova V, Bostik P. Strong antimicrobial activity of xanthohumol and other derivatives from hops (Humulus lupulus L.) in gut anaerobic bacteria. APMIS 2017;125:1033–8.
32. Wei J, Liang J, Shi Q, Yuan P, Meng R, Tang X, Yu L, Guo N. Genome-wide transcription analyses in Mycobacterium tuberculosis treated with lupulone. Braz J Microbiol 2014;45:333–41.
33. Barboriak JJ, Koch ML. Effect of antibiotic drugs in anti-staphylococcal activity of Lupulon and Hexahydrolupulon. Clin Med 1964;71:1211–5.
34. Rozalski M, Micota B, Sadowska B, Stochmal A, Jedrejek D, Wieckowska-Szakiel M, Rozalska B. Antiadherent and antibiofilm activity of Humulus lupulus L. derived products: new pharmacological properties. Biomed Res Int 2013;2013. Article ID 101089.
35. Natarajan P, Katta S, Andrei I, Babu Rao Ambati V, Leonida M, Haas GJ. Positive antibacterial co-action between hop (Humulus lupulus) constituents and selected antibiotics. Phytomedicine 2008; 15:194–201.
36. Leonida MD, Belbekhouche S, Benzecry A, Peddineni M, Suria A, Carbonnier B. Antibacterial hop extracts encapsulated in nanochitosan matrices. Int J Biol Macromolecules 2018;120:1335–43.
37. Tillman GE, Haas GJ, Wise MG, Oakley B, Smith MA, Siragusa GR. Chicken intestine microbiota following the administration of lupulone, a hop-based antimicrobial. FEMIS Microbiol Ecol 2011; 77:395–403.