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Review

Broad spectrum antiinfective potential of xanthohumol from hop (*Humulus lupulus* L.) in comparison with activities of other hop constituents and xanthohumol metabolites

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This review summarizes the capacity of xanthohumol (XN) in comparison with additional hop constituents and metabolites to act as an antiinfective agent against microorganisms including bacteria, viruses, fungi and malarial protozoa. XN was shown to inhibit the Gram-positive bacteria *Staphylococcus aureus* and *Streptococcus mutans.* Antiviral activity was demonstrated against bovine viral diarrhea virus, cytomegalovirus, herpes simplex virus type 1 and 2 and human immunodeficiency virus 1. Inhibition of two *Trichophyton* spp. was indicative of antifungal activity. Finally, XN potently inhibited the replication of *Plasmodium falciparum*, the causative agent of malaria. This effect was linked to the inhibition of glutathione-mediated degradation and detoxification of haemin, a by-product of the parasitic digestion of haemoglobin. Overall, these activities further contribute to the broad spectrum of biological effects observed with XN.

Keywords: Antibiotic / Antifungal / AntiHIV / Antiinfective / Antimalarial / Antiviral / *Humulus lupulus* L. / Review

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Contents

1 Introduction

Hop (*Humulus lupulus* L.) has been used since ancient times for brewing. The earliest references to hop cultivation were during the 8th and 9th century AD from the Hallertau district in Germany. Hop use as beer additive goes back to the German monks in the 12th century [1]. It was soon realized that hop not only added bitterness and aroma to beer, but also played an important role as a preservative. In the early 1900s, Brown and Clubb first described the antiseptic properties of hops [2]. Subsequently, hop *alpha*- and *beta*-acids (humulones and lupulones), constituents of the essential bitter resin of hop, were identified as strong antibiotics against Gram-positive bacteria ([3] and literature cited therein).

Hop is also an important source of phenolic constituents in beer, including (prenylated) flavonoids [4, 5]. In recent years, hop has attracted considerable interest because of the biological and potential cancer chemopreventive activities of some of its constituents [6–8]. As an example, the prenylated chalcone xanthohumol (XN) was identified as a broad-spectrum cancer chemopreventive agent with inhibitory mechanisms in the initiation, promotion and progression phase of carcinogenesis [9]. In this review, the capacity of XN to act as an antiinfective agent and to inhibit the replication of selected microorganisms including bacteria,

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Abbreviations: BVDV, bovine viral diarrhea virus; **HIV-1**, human immunodeficiency virus; **HSV-1 and -2**, herpes simplex virus type 1 and 2; **IC_{50}**, half-maximal inhibitory concentration; **IXN**, isoxanthohumol; **MIC**, minimal inhibitory concentration; **TC_{50}**, half-maximal toxic concentration; **XN**, xanthohumol

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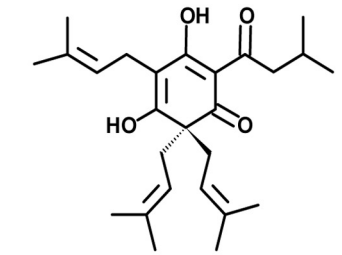

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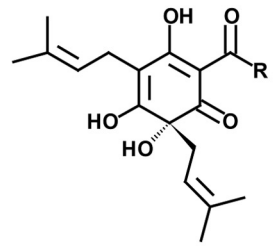

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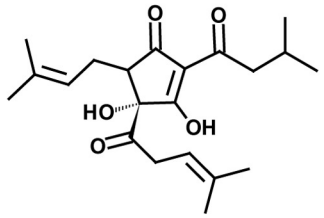

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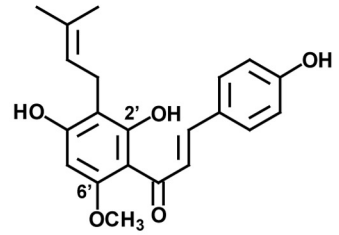

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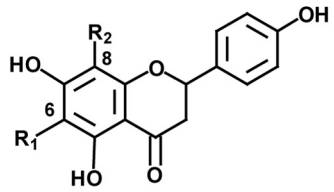

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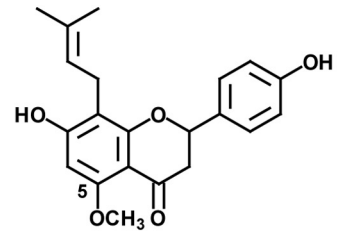

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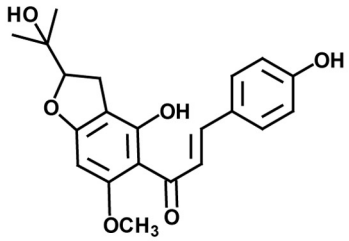

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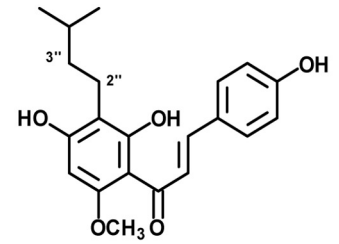

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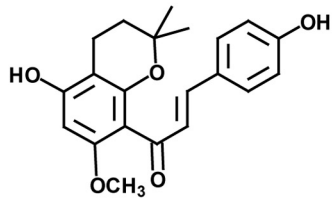

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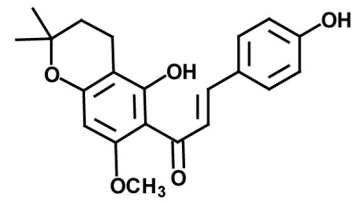

(**10**)

Figure 1. Chemical structures of antiinfective hop constituents and derivatives mentioned in the text. (**1**) Lupulone (*beta*-acid), (**2**) humulone (*alpha*-acid), (**3**) isohumulone (*iso-alpha*-acid), (**4**) XN, (**5a**) naringenin, (**5b**) 6-prenylnaringenin, (**5c**) 8-prenylnaringenin, (**6**) IXN, (**7**) microbial transformation product, (*E*)-2″-(2‴-hydroxyisopropyl)-dihydrofurano [2″,3″:4′,3′]-2′,4-dihydroxy-6′-methoxychalcone, (**8**) 2″,3″-dihydroxanthohumol, (**9**) pyrano-derivative of XN, 2″,2″-dimethyl-3″,4″-dihydropyrano[2″,3″:3′,4′]2′,4-dihydroxy-6′-methoxychalcone, (**10**) pyrano-derivative of XN, 2″,2″-dimethyl-3″,4″-dihydropyrano [5″,6″:2′,3′]4,4′-dihydroxy-6′-methoxychalcone.

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fungi, viruses and malarial protozoa will be summarized. Chemical structures of the hop constituents and derivatives that are mentioned in the text are shown in Fig. 1.

2 Antibacterial activity

Antibacterial activity is the best investigated antiinfective property of hop constituents. The hop bitter acids inhibit mainly Gram-positive bacteria, including some species of *Bacillus*, *Micrococcus*, *Staphylococcus*, *Mycobacterium* and *Streptomycetes*, whereas yeast (*Saccharomyces cerevisiae*) and *Escherichia coli* are not affected [10]. Teuber and Schmalreck postulated and later demonstrated that the lipophilic region of the cell membrane represents the target site for antibacterial action of hop bitter resins. Consequently, the antibiotic properties were shown to depend mainly on the hydrophobic parts of the molecules and increased with decreasing solubility [3, 10]. The water-insoluble *beta*-acid lupulone (**1**) was about twice as active as the *alpha*-acid humulone (**2**), whereas the soluble *iso-alpha* acid isohumulone (**3**) was 25-fold less active than lupulone (**1**) [11].

Mizobuchi and Sato [12] investigated the potential of several prenylated flavonoids from hop to inhibit the growth of Gram-positive *Staphylococcus aureus*, a pathogen often found in pneumonia and sepsis, in comparison with antibiotic activity against *E. coli*. None of the compounds inhibited *E. coli* proliferation, whereas XN (**4**) and 6-prenylnaringenin (**5b**) were the most potent inhibitors of *S. aureus*, with a minimal inhibitory concentration (MIC) of 6.25 μg/mL^{-1} (XN: 17.7 μM and 6-prenylnaringenin: 18.4 μM, respectively). *Streptococcus mutans* is the causative agent of dental caries. Bhattacharya *et al.* [13] tested a series of hop components including XN (**4**) against three strains of *Streptococcus*, and compared the activities with those of some essential oils often found in anticaries mouth washes. In a disc diffusion assay, all tested hop constituents demonstrated antimicrobial activity against *S. mutans*, *S. salivarius* and *S. sanguis*. At a dose of 50 μg *per* disc, XN (**4**, 140 nmol) produced similar zones of inhibition against all three strains as thymol (333 nmol), a well-known additive to a popular mouthwash. Since these results cannot be compared quantitatively due to differences in the diffusion rate of compounds, the lowest concentration to prevent visible bacterial growth (MIC) was determined in a turbidity assay. At a pH of 7.5, XN (**4**) inhibited the growth of *S. mutans* ATCC 25175 with an MIC of 12.5 μg/ml (35.3 μM) and was about sixfold less active than the most potent *beta*-acid (MIC 2.0 μg/ml, exact compound not specified). When the pH was lowered to 6.5 by addition of ascorbic acid or HCl, notably, the MIC of XN (**4**) and *beta*-acid decreased to 2.0 μg/ml (5.7 μM) and 0.5 μg/ml, respectively. Although both compounds were considerably more potent than thymol, the practical use of hop constituents to prevent caries might be limited by their bitter taste.

Recent *in vivo* investigations on bioavailability of XN (**4**) in rats demonstrated that the chalcone and its metabolites are excreted mainly in faeces within 24 h after oral or intravenous administration [14, 15]. Accordingly, it was of interest to investigate whether XN (**4**) might influence the intestinal microbiota. Hanske *et al.* [16, this issue] analyzed the composition of rat intestinal microbiota by PCR-DGGE. Daily applications of XN (**4**) (100 mg/kg body weight *per* day in drinking water) to male and female Sprague Dawley rats for 4 wk did not influence the diversity of the faecal microbial community in comparison with untreated controls.

3 Antiviral effects

Viral diseases, including viral respiratory diseases, herpes virus infections, and in particular retroviral infections, are an increasing worldwide health concern. As a consequence, the discovery of new antiviral agents from plants has gained increasing importance [17].

In a recent study by Buckwold *et al*. [18], XN (**4**) and further hop constituents were tested against a series of DNA and RNA viruses *in vitro*. Bovine viral diarrhea virus (BVDV) as a surrogate model of hepatitis C virus and human rhinovirus (HRV) were included as RNA viruses. Further, the DNA herpesviruses cytomegalovirus (CMV) as well as herpes simplex virus types 1 and 2 (HSV-1 and -2) were utilized to assess antiviral activity. Inhibitory effects of hop constituents against BVDV (NADL strain in MDBK cells), HRV (rhinovirus 14 strain in MRC-5 cells), HSV-1 (F strain in Vero cells) and HSV-2 (MS strain in Vero cells) were tested using cell-based assays designed to assess inhibition of cytopathic effects (CPE). CMV (strain AD169 in MRC-5 cells) was tested in a plaque reduction assay. XN (**4**) inhibited the growth of BVDV, CMV, HSV-1 and HSV-2 more potently than its isomerization product isoxanthohumol (IXN, **6**). Half-maximal inhibitory concentrations (IC_{50} values) of XN (**4**) to inhibit viral replication were in the range of 1.5–2.7 μg/ml (4.2–7.6 μM). Concomitantly, the half-maximal toxic concentrations (TC_{50}) to reduce the number of viable cells used to propagate the viruses were about 2.6- to 6.3-fold higher than IC_{50} values and ranged from 6.1 to 8.9 μg/ml (17.2–25.1 μM). XN (**4**) did not have any antiviral activity against HRV, whereas on the other hand, IXN (**6**) was found to possess weak antiviral activity against this viral species with an IC_{50} value of 6.6 μg/ml (18.6 μM) and a TC_{50} of 18.0 μg/ml (50.8 μM). In the same study, an XN-enriched extract was tested. XN (**4**) contained in the extract appeared to account for almost all of the antiviral activity of the extract, since the therapeutic indices TI

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(TC_{50}/IC_{50}) of XN (**4**) against BVDV, HSV-1 and HSV-2 were similar to those of the XN-enriched extract.

Natural products have repeatedly been screened for retroviral human immunodeficiency virus 1 (HIV-1)-inhibitory effects [19]. As an example, flavonoids showed antiHIV-1 activity with emphasis on inhibiting HIV-1 reverse transcriptase, the RNA-dependent DNA polymerase required for transcription of the viral RNA genome to proviral DNA [20]. In a recent study by Wang *et al*. [21], the potential of XN (**4**) to inhibit various steps essential for the replication of HIV-1 was tested. During replication, many viruses destroy not only the host cells that they infect but also neighbouring uninfected cells by CPE. XN (**4**) was able to inhibit HIV-1-induced CPE, as well as the production of viral p24 antigen and reverse transcriptase activity as measures of active retroviral replication, with IC_{50} values of 2.3, 3.6 and 1.4 μM, respectively, in C8166 lymphocytes infected with HIV-1_{IIIB}. XN (**4**) also inhibited HIV-1 replication in peripheral blood mononuclear cells with an IC_{50} value of 58.5 μM. The activity of recombinant HIV-1 reverse transcriptase and HIV-1 entry into cells were not inhibited by XN (**4**) [21]. From these results it was concluded that mechanisms postreverse transcription might be the target of XN action.

4 Antifungal properties

Investigations on antifungal activity of hop constituents are limited. Mizobuchi and Sato [12] tested five hop ingredients and related compounds, including XN (**4**), naringenin (**5a**), 6-prenylnaringenin (**5b**), 8-prenylnaringenin (**5c**), and IXN (**6**) against five human pathogenic fungi, *i.e. Trichophyton mentagrophytes, Trichophyton rubrum*, *Candida albicans*, *Fusarium oxysporum* and *Mucor rouxianus*. XN (**4**) and 6-prenylnaringenin (**5b**) were identified as the most potent antifungal agents. With MIC of 3.13 μg/ml (XN: 8.8 μM and 6-prenylnaringenin: 9.2 μM, respectively), both compounds inhibited the growth of the dermatophytic fungi *T. mentagrophytes* and *T. rubrum* more efficiently than the positive control griseofulvin (MIC: 6.25 μg/ml, equivalent to 17.8 μM), whereas IXN (**6**) was basically inactive. Also, weak inhibition of *M. rouxianus* was observed with XN (**4**) and 6-prenylnaringenin (**5b**) (MIC: 50 μg/ml, corresponding to 141.2 μM XN and 147.0 μM 6-prenylnaringenin, respectively). *C. albicans* and the opportunistic human pathogen *F. oxysporum* were not responsive to either XN (**4**) or 6-prenylnaringenin (**5b**) (MIC > 200 μg/ml). By comparison of the antifungal activities of naringenin (**5a**), its prenylated derivatives 6- and 8-prenylnaringenin (**5b**, **5c**) and IXN (**6**), it was concluded that introduction of the prenyl moiety increased the antifungal potency of naringenin (**5a**), and that methylation of the hydroxyl group in position 5 as in IXN (**6**) significantly reduced the activity.

5 Antimalarial action

Malaria caused by infection with *Plasmodium falciparum* is one of the most deadly diseases, with more than 280 million people infected worldwide and more than one million deaths annually. The most commonly used drug to fight malaria is chloroquine. Unfortunately, the emergence of drug-resistant strains has reduced its efficacy, and there is an urgent need for the identification of potent novel and cost-effective inhibitors. Mechanisms to inhibit the blood stages of *P. falciparum* include steps in the detoxification of haemin, a by-product of the parasites digestion of haemoglobin [22]. Haemoglobin degradation is mediated by malarial aspartyl and cysteine proteases, which represent targets for the development of antimalarial compounds [23]. Chalcones are among the structural classes for which antiplasmodial activity has been reported and are thought to act against malarial cysteine protease [23, 24]. Herath *et al.* [25] determined the antimalarial activity of XN (**4**) against a chloroquine-sensitive strain D6 and a chloroquine-resistant strain W2. Plasmodial lactate dehydrogenase activity was measured as an indicator of the number of parasites remaining in infected red blood cells. XN (**4**) as well as a microbial transformation product (**7**), obtained by incubation of XN (**4**) with cultures of the fungus *Pichia membranifaciens* (ATCC 2254), were active against both strains, with IC_{50} values of 3.3 μg/ml (9.3 μM) and 4.1 μg/ml (11.1 μM) against strain D6, and 1.0 μg/ml (2.8 μM) and 1.8 μg/ml (4.9 μM) against strain W2, respectively. Antimalarial activity was confirmed in a recent study by Frölich *et al.* [26]. *In vitro* antiplasmodial activity of XN (**4**) and seven prenylated chalcones was evaluated against the chloroquine-sensitive strain poW and the multiresistant clone Dd2, using a [^{3}H]-hypoxanthine incorporation assay. In addition, the influence of the compounds on glutathione (GSH)-dependent haemin degradation was analyzed to determine its contribution to the antimalarial effect of chalcones [26]. Of the eight compounds tested, four compounds, *i.e.* XN (**4**), 2″,3″-dihydro-XN (**8**), and two pyrano-derivatives (**9**, **10**), which were previously identified as XN-metabolites in faecal samples of XN-treated rats (compare structures 22 and 21 in Ref. [15]), possessed activity with IC_{50} values < 25 μM against at least one of the two strains of *P. falciparum*. XN (**4**) was most active with IC_{50} values of 8.2 μM (poW) and 24.0 μM (Dd2). For comparison, chloroquine was tested as a positive control, and IC_{50} values of 0.015 and 0.14 μM, respectively, were determined in the two strains. Structure-activity relationship analyses of the tested XN derivatives revealed that the double bond in the prenyl side chain is unrelated to the antiplasmodial activity,

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since XN (**4**) and the 2″,3″-dihydro derivative (**8**) displayed nearly identical inhibitory potencies. Demethylation of XN (**4**) at position 6′ resulted in about fourfold to fivefold reduced inhibitory activity in comparison with XN (**4**). This was attributed to a higher hydrophilicity of the compound, hindering the entrance of the compound to the site of action inside the parasite. Semisynthetic methylation of XN (**4**) and 6′-demethyl-XN completely abrogated the inhibitory potential.

The exact mechanism of the antiplasmodial activity of chalcones is not known, although they are often considered to be cysteine protease inhibitors [24]. Frölich *et al.* hypothesized that chalcones might be able to form complexes with haemin owing to their carbonyl moiety, especially in combination with a free hydroxyl group in position 2′, and therefore might interfere with haemin degradation. Interestingly, the authors subsequently demonstrated that the most potent inhibitors of parasite replication were also potent inhibitors of GSH-dependent haemin degradation. At a concentration of 11 μM, XN (**4**), 2″,3″-dihydro-XN (**8**) and one pyrano-derivative (**10**) inhibited haemin degradation by more than 60%. The 6′-demethyl-derivative of XN (**4**) was weakly active (36% inhibition), whereas the semisynthetic methylated derivatives, which were unable to inhibit the replication of *P. falciparum*, were also inactive in the haemin degradation assay. Noteworthy the pyrano-derivative (**9**) lacking the free OH-group in position 2′ was unable to interfere with haemin degradation, although it potently inhibited replication of *P. falciparum*. Therefore, other modes of action including the inhibition of cysteine proteases have to be considered [26].

6 Summary

This review summarizes antiinfective properties of XN (**4**) and related hop components against various microorganisms (viruses, bacteria, fungi and malarial protozoa). Although some reports provide data on structure-activity relationship analyses, detailed information on inhibitory mechanisms are often still missing. With hop bitter acids, high lipophilicity contributes to good antibacterial activity against Gram-positive bacteria due to a facilitated interaction with the bacterial cell wall. So far, it is unclear whether a similar conclusion can be drawn for hop flavonoids. Prenylation increased the antibiotic potential of naringenin against *S. aureus*; however, methylation of 8-prenylnaringenin (**5c**) at position 5 as seen in IXN (**6**) decreased the activity. With respect to antimalarial effects, XN (**4**) and related chalcones were shown to interfere with GSH-dependent haemin degradation and to inhibit parasite replication by the subsequent accumulation of toxic by-products. Since this mechanism could not explain the inhibitory potential of all active agents, additional mechanisms are discussed. In view of the emerging interest in XN (**4**) and hop products for health-beneficial preparations, the presented activities should be further explored.

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