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**FILE: ▪Hops (*Humulus lupulus*)**
**▪*Streptococcus mutans***
**▪Ascorbic Acid**

**HC 110132-254**

**Date: April 15, 2004**

**RE: In vitro Antimicrobial Activity of Hops Extract**

Bhattacharya S, Virani S, Zavro M, Haas GJ. Inhibition of *Streptococcus mutans* and other oral streptococci by hop (*Humulus lupulus* L.) constituents. *Economic Botany* 2003;57:118–125.

Dental plaque and caries are primarily caused by the bacterium *Streptococcus mutans*. Efforts have been underway to reduce the incidence of dental plaque and caries with an antimicrobial agent that is not associated with adverse side effects and/or the development of antibiotic resistance. Extracts of the hops plant (*Humulus lupulus* L.) have known bacteriostatic antimicrobial activity. The yellow lupulin glands within the hops cones contain the essential oils and resins that give beer its characteristic aroma and bitter taste, respectively. The resins also help preserve beer because of their antimicrobial properties. The objectives of this study were to compare the antimicrobial activities of hops extracts (i.e., beta acid, xanthohumol, iso-alpha acid, and tetra iso-alpha acid) against *S. mutans* with those of some essential oils used in commercially available mouthwashes (i.e., thymol, cinnamon oil, oil of clove, nerol, citral, eucalyptol, and methanol) to determine whether ascorbic acid would enhance the activity of the hops extracts and to assess the likelihood of resistance development of *S. mutans* to the test compounds.

The disc diffusion assay and the fluid turbidity assay, two complementary assays, were used to evaluate the antimicrobial activities of the test compounds against four strains of *S. mutans* and one strain each of *S. sanguis* and *S. salivarius*. The minimum inhibitory concentration (MIC) (i.e, the lowest concentration of the test substance that shows no bacterial growth) of each test compound was determined with a turbidity assay. Changes in pH with the addition of ascorbic acid to the test compounds, to a concentration of 0.25% (weight/volume) of BHI (brain heart infusion) medium, were determined. The growth of *S. mutans* was monitored after its repeated passage through cultures containing beta acid or no hops extract to determine the microorganism's level of resistance to the hops extract.

All of the hops extracts tested showed antimicrobial activity against *S. mutans*, *S. sanguis*, and *S. salivarius*. The MIC at a pH of 7.5 ranged from 2 to 50 μg/mL. The size of the zone of inhibition was dependent on the rate of diffusion of the compounds; xanthohumol had a low diffusion rate. Cinnamon oil, nerol, and oil of clove had zones of inhibition, whereas citral did not. Tetra iso-alpha acid had the largest zone of inhibition. Of the four hops compounds tested, the MIC of beta acid against *S. mutans* 25175 was the lowest and was superior to that of thymol. The essential oils tested were much less powerful than were the hops extracts. Ascorbic acid enhanced the antimicrobial activity of the hops extracts but not over and above the enhancement due to pH lowering. No resistance development of *S. mutans* to beta acid was observed, even after nine sequential passages through 10-μg/mL beta acid cultures.

The results of the current study indicate that the hops constituents tested "can contribute to the overall antimicrobial activity against oral Streptococci." However, "definite conclusions can only be reached after experiments in vivo." Although the hops extracts tested all exhibited antimicrobial activity, the range of sensitivity was about 25-fold. Before hops extracts can be successfully used in oral applications (e.g., mouthwashes) to reduce the incidence of dental plaque and caries, their bitter flavor will need to be masked or they will be unacceptable to consumers.

*—Brenda Milot, ELS*

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