Hops (Humulus lupulus) and Methicillin-Resistant Staphylococcus aureus (MRSA): A Tale of Discovery

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American Herbalist Guild
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Staph infection sparks curiosity

• In 1997, I contracted a head-to-toe Staph infection
• MD diagnosed infection and prescribed antibiotics
• Leaving for Nepal one week later
• Concerned about effect of antibiotics on GI system
• Unwilling to take them
• Decided on a combination of Usnea (Usnea barbata) lichen and Hops (Humulus lupulus) strobile extracts externally and internally
• Infection gone within a week
• Traveled without any GI incidents

Skin infections and MRSA

• Herbalists frequently encounter skin infections in their practice
• Skin infections getting more challenging because of ever-increasing microbial multi-drug resistance
• Community-associated MRSA infections now the dominant cause of skin and soft tissue infections
• CA-MRSA infections represent the majority of patients with skin and soft tissue infections being treated at emergency departments
• MRSA-associated hospitalization have increased from 8% in 1993, to 44% in 1998, and to 65% in 2005

Evaluation of Selected Medicinal Plants Extracted in Different Ethanol Concentrations for Antibacterial Activity Against Human Pathogens

Chitra Wendakoon, PhD
Peter Calderon, PhD
Daniel Gagnon, MS


Initial in vitro study

• In 2007, Herbs, Etc. sponsored a study at New Mexico State University of eight herbs: boldo, buchu, Echinacea angustifolia, hops, licorice, Oregon grape, usnea, and yerba mansa

Aim of the study

• Perform a comparative study to evaluate the relative effectiveness of the antimicrobial properties of eight medicinal herb extracts

Study design

• Extracts were prepared with 3 different ethanol concentrations (50%, 70% and 90%)
• Screened for antibacterial activity against four Gram-positive and four Gram-negative pathogens
• Used standard well assay and micro-broth dilution method
• Determined minimum bactericidal concentrations (MBCs) for each extract
Plants and Bacterial Strains Used in the Study

**Table 1. Plants used in the study**

<table>
<thead>
<tr>
<th>Common name</th>
<th>Latin binomial</th>
<th>Family</th>
<th>Plant part</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boldo</td>
<td>Peumus boldus</td>
<td>Monimiaceae</td>
<td>Leaf (dry)</td>
</tr>
<tr>
<td>Buchu</td>
<td>Agathosma betulina</td>
<td>Rutaceae</td>
<td>Leaf (dry)</td>
</tr>
<tr>
<td>Echinacea angustifolia</td>
<td>Echinacea angustifolia</td>
<td>Asteraceae</td>
<td>Root (dry)</td>
</tr>
<tr>
<td>Hops</td>
<td>Humulus lupulus</td>
<td>Cannabaceae</td>
<td>Strobile (dry)</td>
</tr>
<tr>
<td>Licorice</td>
<td>Glycyrrhiza glabra</td>
<td>Fabaceae</td>
<td>Root (dry)</td>
</tr>
<tr>
<td>Oregon grape</td>
<td>Mahonia aquifolium</td>
<td>Berberidaceae</td>
<td>Root (dry)</td>
</tr>
<tr>
<td>Usnea</td>
<td>Usnea barbata</td>
<td>Usneaceae</td>
<td>Lichen (dry)</td>
</tr>
<tr>
<td>Yerba mansa</td>
<td>Anemopsis californica</td>
<td>Saururaceae</td>
<td>Root (dry)</td>
</tr>
</tbody>
</table>

**Table 2. Bacterial strains used in the study**

<table>
<thead>
<tr>
<th>Gram-positive:</th>
<th>Gram-negative:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus (ATCC 25923)</td>
<td>Escherichia coli (ATCC 25922)</td>
</tr>
<tr>
<td>Staphylococcus aureus (COL)*</td>
<td>Pseudomonas aeruginosa (ATCC 27853)</td>
</tr>
<tr>
<td>Staphylococcus epidermidis (ATCC 12228)</td>
<td>Salmonella enteritidis (ATCC 13076)</td>
</tr>
<tr>
<td>Streptococcus pyogenes (ATCC 19615)</td>
<td>Klebsiella pneumoniae (ATCC 13883)</td>
</tr>
</tbody>
</table>

*The MRSA culture was kindly provided by Dr. J. Gustafson, Department Biology, New Mexico State University

**Analysis**

- Boldo, hops, licorice, and yerba mansa possess strong inhibitory activities on all four Gram-positive organisms tested at all levels of ethanol
- No inhibitory action detected against the four Gram-negative bacteria tested in this study
- Antibacterial activity of the other plant extracts varied depending on the ethanol level (Table 3)
- Buchu at 50% ethanol, Oregon grape at 70% ethanol were more inhibitory towards MRSA than either at 90% ethanol level
- Hops showed the strongest antibacterial activity and, further, its activity was highest at 90% ethanol level
- The results from the broth dilution study were in agreement with the well assay, confirming that the extracts of boldo, hops, licorice, and yerba mansa are potentially effective antibacterial agents

**Conclusions**

- Thousands of scientific papers describe the antimicrobial activities of plant extracts
- However, no systematic studies have been conducted on the effects of ethanol concentration on the antimicrobial activity of specific herbs
- Due to the complex nature of the phytochemicals present in herbs, extraction solvent system should be taken into consideration
- This study provides data on the importance of various ethanol concentration on the antimicrobial activity of herbs
- It also demonstrated that the ethanol extracts of Hops offered significant potential for the development of novel antibacterial therapies
- More studies on the antimicrobial activity of Hops against specific bacteria are needed

**Second study**

The potential antibacterial properties of Hops (*Humulus lupulus*) extracts against methicillin-resistant *Staphylococcus aureus* (MRSA)

Daniel Gagnon, MS, Herbs, Etc.
Chitra Wendakoon, PhD, Independent Consultant
Bob Smith, BS, HopSteiner
Jeremy Leker, BS, HopSteiner

Primary aim of the study

- Investigate the effectiveness of the *in vitro* antibacterial activity of five Hops (*Humulus lupulus*) strobile ethanolic extracts containing 10%, 30%, 50%, 70%, and 95% ethanol and three *H. lupulus* isolated constituents, alpha acids (α-acids), beta-acids (β-acids), and xanthohumol against methicillin-resistant *Staphylococcus aureus* (MRSA)

Secondary aims of the study

- Chemically analyze whole and ground strobiles as well as the extracts by quantifying the amounts of three constituents: α-acids, β-acids, and xanthohumol immediately after making the extracts as well as one year later and two years later
- Compare and contrast the constituents in the five ethanolic extracts and the three isolated constituents as to their relative MRSA antimicrobial activity

Results of HPLC analysis:

Chemical characterization of *H. lupulus* extracts

- Performed a HPLC analysis of five *H. lupulus* ethanolic extracts, as well as whole and ground strobiles after one and two year storage
- Evaluated the amount of constituents loss during cryogenic grinding process of whole *H. lupulus*
- Calculated the difference in amount of constituents immediately after extraction, as well as after one year and two years of storage

HPLC analysis of *Humulus lupulus* ethanolic extracts and strobiles at the beginning of the experiment

<table>
<thead>
<tr>
<th>Constituents</th>
<th>10% Ethanol</th>
<th>30% Ethanol</th>
<th>50% Ethanol</th>
<th>70% Ethanol</th>
<th>95% Ethanol</th>
<th>Whole Hops</th>
<th>Ground Hops</th>
</tr>
</thead>
</table>

HPLC analysis was performed by Iacosa & Co., 2013
HPLC analysis of *Humulus lupulus* ethanolic extracts and strobiles stored for one year

Constituents (g/100 ml)  

<table>
<thead>
<tr>
<th>Ethanol (%)</th>
<th>Alpha acids</th>
<th>Beta acids</th>
<th>Xanthohumol</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Y1</td>
<td>Y2</td>
<td><em>N.D.</em></td>
</tr>
<tr>
<td>30</td>
<td>0.003</td>
<td>0.0008</td>
<td>0.0004</td>
</tr>
<tr>
<td>50</td>
<td>0.016</td>
<td>0.0068</td>
<td>0.0011</td>
</tr>
<tr>
<td>70</td>
<td>0.040</td>
<td>0.0130</td>
<td>0.0020</td>
</tr>
<tr>
<td>95</td>
<td>0.081</td>
<td>0.043</td>
<td></td>
</tr>
</tbody>
</table>

N.D.: Not Detectable

Percent of constituents from *Humulus lupulus* ethanol extracts remaining when stored for one or two years

<table>
<thead>
<tr>
<th>Ethanol concentration (%)</th>
<th>One year old extract</th>
<th>Two years old extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha acids</td>
<td>Beta acids</td>
<td>Xanthohumol</td>
</tr>
<tr>
<td>10%</td>
<td>7.5</td>
<td>0.2</td>
</tr>
<tr>
<td>30%</td>
<td>26.7</td>
<td>2.9</td>
</tr>
<tr>
<td>50%</td>
<td>82.5</td>
<td>67.9</td>
</tr>
<tr>
<td>70%</td>
<td>90.1</td>
<td>95.2</td>
</tr>
<tr>
<td>95%</td>
<td>95.8</td>
<td>95.1</td>
</tr>
</tbody>
</table>

Whole Strobiles  |
| 86.8 | 75.4 | 95.6 |

Ground Strobiles  |
| 92.9 | 86.9 | 95.6 |

Antibacterial activity of *H. lupulus* strobile extracts against methicillin-resistant *S. aureus* (MRSA)

<table>
<thead>
<tr>
<th>Ethanol concentration (%)</th>
<th>Zone Diameter (mm)</th>
<th>Zone Diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus (MRSA)</td>
<td>E. coli</td>
<td></td>
</tr>
<tr>
<td>10%</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>30%</td>
<td>8</td>
<td>N</td>
</tr>
<tr>
<td>50%</td>
<td>8</td>
<td>N</td>
</tr>
<tr>
<td>70%</td>
<td>12</td>
<td>N</td>
</tr>
<tr>
<td>95%</td>
<td>14</td>
<td>N</td>
</tr>
</tbody>
</table>

Ampicillin (µg)  |
| 10 | 1 |

Ethanol (10, 30, 70, 95%)  |
| N | N |
Antibacterial activity of *H. lupulus* ethanolic extracts against methicillin-resistant *S. aureus* ATCC 43300

Minimum bactericidal concentrations (MBC) of *Humulus lupulus* ethanolic extracts

Minimum bactericidal concentrations (MBC) of diluted *H. lupulus* isolated constituents

Antibacterial activity of diluted *H. lupulus* isolated constituents against methicillin-resistant *S. aureus* (MRSA)
Bactericidal activity of *H. lupulus* extract (95% ethanol) against methicillin-resistant *S. aureus* (MRSA)

Growth was monitored for 24h in the presence of 100µl of the extract (●), 200µl of the extract (▲) and in the absence of the extract (●).  

**Discussion**

- Plant metabolites that display super potent biological activity are relatively rare
- Very large amounts of active constituents, essential oils, and other compounds are needed to act effectively as antibiotics
- The minimum inhibitory concentration for most herbs are in the range of 100 to 1,000µg/ml of active constituents or higher
- However, the *Humulus lupulus*’ constituent beta-acids was active in the 0.09µg/ml range
- *In vitro* *Hops (Humulus lupulus)* is active against MRSA and is comparable to the few antibiotics that are still active against this bacteria

**Clinical notes: MRSA skin infection**

- 24 years old man, mechanic by trade, diagnosed with MRSA skin infection under his nail bed
- Second MRSA infection affecting a finger in six years
- Doctors cut the nail bed to drain the infection
- First infection took two rounds of antibiotics to stop it. This time, on third round of antibiotic
- MD told him he would need to cut his finger at the first joint if unable to stop the infection – kept getting worse
- Applied Hops (*Humulus lupulus*) 95% ethanol extract compresses on the infection
- Within two weeks the infection was gone
- Six years have now elapsed since that incident and he has remained free from MRSA infections

**Conclusions**

- Much work needed to be done to elucidate the secrets of the antibacterial properties of Hops (*Humulus lupulus*)
- The research presented here points toward the usefulness of the strobile against methicillin-resistant *S. aureus* (MRSA)
- Initial clinical results suggest that Hops may be useful against MRSA
- Keep Hops strobiles frozen until use
- High ethanol level essential to make a strong and effective Hops extract
- High ethanol level is essential to safeguard the potency of Hops extracts over time

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