

# Journal of Medicinally Active Plants

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Volume 1 | Issue 2

---

January 2012

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Wendakoon, Chitra; Peter Calderon; and Daniel Gagnon. 2012. "Evaluation of Selected Medicinal Plants Extracted in Different Ethanol Concentrations for Antibacterial Activity against Human Pathogens."

*Journal of Medicinally Active Plants* 1, (2):60-68.

DOI: <https://doi.org/10.7275/R5GH9FV2>

<https://scholarworks.umass.edu/jmap/vol1/iss2/4>

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

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Manuscript received: December 2, 2011

**Keywords:** Boldo, buchu, *Echinacea angustifolia*, hops, licorice, MRSA, Oregon grape, *Staphylococcus aureus*, usnea, yerba mansa.

### ABSTRACT

Medicinal plant extracts prepared with selected ethanol concentrations from eight species, *Peumus boldus* (boldo leaf), *Agathosma betulina* (buchu leaf), *Echinacea angustifolia* (echinacea root), *Humulus lupulus* (hops strobile), *Glycyrrhiza glabra* (licorice root), *Mahonia aquifolium* (Oregon grape root), *Usnea barbata* (usnea lichen), and *Anemopsis californica* (yerba mansa root), were screened for antibacterial activity against four Gram-positive and four Gram-negative pathogens. The antibacterial activity of the extracts (50, 70, and 90% ethanol) was evaluated using a standard well assay and micro-broth dilution method. Minimum bactericidal concentrations (MBCs) were also determined for each extract. Plant extracts showed strong antibacterial action against Gram-positive bacteria, *Staphylococcus aureus*, methicillin resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis* and *Streptococcus pyogenes*, while negligible to no inhibitory activity against Gram-negative bacteria; *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Salmonella enteritidis* was observed. Among the plant extracts, the boldo, hops, licorice and yerba mansa exhibited a strong antibacterial action at all three ethanol concentrations. Of these four, hops showed the strongest activity at 90% ethanol. *Echinacea angustifolia* extracts did not show any

considerable antibacterial activity, while usnea showed strong activity only at 90% against *S. epidermidis*. Except *Echinacea angustifolia* and usnea, the plant extracts were strongly inhibitory towards the MRSA strain. Buchu, yerba mansa and Oregon grape showed higher activity at 50% or 70% on MRSA. MBCs varied from 1/4 to >1/256 dilution levels and were in agreement with well assay results. The results suggest that the extracts of boldo, hops, licorice and yerba mansa could be considered as potentially effective antibacterial agents against Gram-positive bacteria including MRSA. For hops, buchu, Oregon grape and usnea, the activity is dependent on the concentration of ethanol used in the extraction procedure. The ratio of ethanol/water mixture used for extraction of medicinal plants is an important factor to obtain optimum antibacterial activity.

### INTRODUCTION

For centuries, the therapeutic properties of various medicinal plants have been used to treat human diseases. It has been estimated that between 60-90% of the populations of developing countries use traditional and botanical medicines almost exclusively and consider them to be a normal part of primary healthcare (WHO, 2002). Consumers are increasingly interested in complementary and

alternative medicines, including herbal medicine, as they perceive these forms of healing as being both safe and effective. This trend in use of alternative and complementary healthcare has prompted scientists to investigate the various biological activities of medicinal plants. In the US, a number of medicinal plants have been documented as important source of bioactive compounds (Balunas and Kinghorn, 2005).

In herbal medicine, crude plant extracts in the form of infusion, decoction, tincture or herbal extract are traditionally used by the population for the treatment of diseases, including infectious diseases. Although their efficacy and mechanisms of action have not been tested scientifically in most cases, these simple medicinal preparations often mediate beneficial responses due to their active chemical constituents (Barnes et al., 2007). Plant-derived products contain a great diversity of phytochemicals such as phenolic acids, flavonoids, tannins, lignin, and other small compounds (Cowan, 1999). These compounds possess numerous health-related effects such as antibacterial, antimutagenic, anticarcinogenic, antithrombotic and vasodilatory activities (Bidlack et al., 2000).

The expanding bacterial resistance to antibiotics has become a growing concern worldwide (Gardam, 2000). Intensive care physicians consider antibiotic-resistant bacteria a significant or major problem in the treatment of patients (Lepape et al., 2009). Increasing bacterial resistance is prompting a resurgence in research of the antimicrobial role of herbs against resistant strains (Alviano and Alviano, 2009; Hemaiswarya et al., 2008). A vast number of medicinal plants have been recognized as valuable resources of natural antimicrobial compounds (Mahady, 2005). Medicinal plant extracts offer considerable potential for the development of new agents effective against infections currently difficult to treat (Iwu et al., 1999).

A wide range of phytochemicals present in plants are known to inhibit bacterial pathogens (Cowan, 1999; Medina et al., 2005; Romero et al., 2005). Successful determination of such biologically active compounds from plant material is largely dependent on the type of solvent used in the

extraction procedure. Organic solvents such as ethanol, acetone, and methanol are often used to extract bioactive compounds (Eloff, 1998). Ethanol, however, is the most commonly used organic solvent by herbal medicine manufacturers because the finished products can be safely used internally by consumers of herbal extracts (Low Dog, 2009). Additionally, the bioactivity of plant extracts depends on the water and ethanol concentration used in the extraction process (Ganora, 2008). Although a great amount of research has been performed to determine the antibacterial activity of medicinal plants, optimal extraction of bioactive compounds has not been well established for most plants.

To maximize the recovery of plant antimicrobials for human consumption, establishing optimal and specific extraction condition using binary solvent system of ethanol and water is important. Therefore, the objective of this study was to determine the best ethanol to water ratio to prepare extracts from selected medicinal plants to optimize their antimicrobial activity against several human pathogenic bacteria. We report a comparative study on the antimicrobial properties of eight medicinally important plants extracted with three different concentrations of ethanol to assess the concentration that would produce the highest antimicrobial action on selected human pathogens.

## MATERIALS AND METHODS

**Plant material.** The medicinal plants used in this study were collected from the wild or cultivated on herb farms (Table 1). All collected plants were shade dried and then stored in their whole form until used in experiments. Voucher samples of the plant materials are stored at Herbs, Etc., Santa Fe, New Mexico.

All eight herbs were ground cryogenically, using a Fitzpatrick hammer mill (Fitzpatrick Co., Elmhurst, IL) and injecting liquid nitrogen into the grinding chamber while grinding to prevent heat build-up. After grinding, the collected ground plant tissue was passed through a 0.125 mesh sieve to remove debris and then reground. To check for particle size distribution, collected fraction following the second grinding was passed through a 0.033 mesh

sieve using a sieve shaker (Model RX-86, W.S. Tyler, Inc., St. Catharines, ON). Except for yerba mansa, three 50 g samples of each herb were passed through the sieve by shaking for 10 min. Yerba mansa samples were shaken for 20 min due to the oily nature of the tissue.

Table 1. Evaluated plant/lichen materials.

Common name	Latin binomial	Family	Plant part	Harvest Method	Location source
Boldo	<i>Peumus boldus</i>	Monimiaceae	Leaf (dry)	Wild-crafted	Chile
Buchu	<i>Agathosma betulina</i>	Rutaceae	Leaf (dry)	Cultivated	South Africa
Echinacea	<i>Echinacea angustifolia</i>	Asteraceae	Root (dry)	Cultivated	Washington, USA
Hops	<i>Humulus lupulus</i>	Cannabaceae	Strobile (dry)	Cultivated	Washington, USA
Licorice	<i>Glycyrrhiza glabra</i>	Fabaceae	Root (dry)	Cultivated	Washington, USA
Oregon grape	<i>Mahonia aquifolium</i>	Berberidaceae	Root (dry)	Wild-crafted	Oregon, USA
Usnea	<i>Usnea barbata</i>	Usneaceae	Lichen (dry)	Wild-crafted	Oregon, USA
Yerba mansa	<i>Anemopsis californica</i>	Saururaceae	Root (dry)	Wild-crafted	New Mexico, USA

For each herb, separate 10 g dry samples were subsequently extracted at room temperature by a cold percolation process using 50 mL of 50%, 70%, and 90% ethyl alcohol solutions prepared with deionized water. After completion of the percolation, the remaining liquid was pressed from the extracted herbs using a hydraulic press (20.7 MPa). The pressed extract was filtered through Whatman No. 1 paper to remove debris and then combined with the percolated extract. All extracts were stored in amber glass bottles at 4°C throughout the study period.

**Bacterial susceptibility tests.** Except for MRSA, all bacterial species used in this study were sourced from the American Type Culture Collection (ATCC) (Manassas, VA) (Table 2). The MRSA culture was obtained from Dr. J. Gustafson at New Mexico State University, Las Cruces, NM. The organisms were maintained at -70°C until prepared for experiments by transferring to Brain-heart infusion broth (BHI) (Difco Laboratories, Detroit, MI) for 18-20 h growth at 37°C. Other growth media sourced from Difco and used in the current study included Mueller-Hinton agar (MHA), Mueller-Hinton broth (MHB), and tryptic soy agar with yeast extract (TSA-YE). Ampicillin (10 µg) Sensi-Disc™ (Becton Dickinson & Co.) was used as an antimicrobial positive control.

The susceptibility of the test bacteria to plant extracts was determined using a well diffusion assay on Mueller-Hinton agar plates, following the method

described in NCCLS manual (NCCLS, 2003). Diluted bacterial cultures were adjusted to a 0.5 McFarland turbidity ( $1-2 \times 10^6$  CFU mL<sup>-1</sup>) and spread evenly over the entire surface of the agar plates using a sterile cotton swab. The plates were allowed to air-dry for approximately 10 min before wells (6 mm holes) were cut into the agar using sterile plastic straws.

Individual wells were filled with plant extracts (150 µL). Additional wells were filled with the same concentrations of ethanol, but no plant extracts as a negative control or Ampicillin (10 µg) as a positive control. Each extract test was replicated three times. The plates were incubated at 37°C for 22-24h period. For each microorganism tested, zones of inhibition of growth were examined, and the diameter of each zone was measured and recorded.

Table 2. Bacteria test organisms.

Gram-positive:
<i>Staphylococcus aureus</i> (ATCC 25923)
<i>Staphylococcus aureus</i> COL (MRSA)*
<i>Staphylococcus epidermidis</i> (ATCC 12228)
<i>Streptococcus pyogenes</i> (ATCC 19615)
Gram-negative:
<i>Escherichia coli</i> (ATCC 25922)
<i>Pseudomonas aeruginosa</i> (ATCC 27853)
<i>Salmonella enteritidis</i> (ATCC 13076)

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

Minimum bactericidal concentrations of the extracts were determined by a broth assay using sterile 96-well (round bottom) micro titer plates (Woods and Washington, 1995) filled with a 150 µl aliquot of Mueller-Hinton broth. To the first column of wells 150 µl of the original extracts were added and mixed with the broth in the wells using a pipette tip.

After mixing, 150 µl of the mixture was transferred to the next well in each row and this process was continued making 2 fold dilutions until column #8. Each well was inoculated with 30 µl of inoculum ( $1-2 \times 10^4-10^5$  CFU/ml) and incubated at 37°C for 24h. After incubation, a small volume of the culture from each well was streaked on TSA-YE agar plates. The plates were then incubated at 37°C overnight and the lowest dilution that yielded complete inhibition of growth was taken as the minimal bactericidal concentration (MBC).

Each of the extract was tested in triplicate and the average values were obtained for two repeated experiments. Ampicillin (10 µg) was used throughout the study as the positive control.

## RESULTS

**Well assay.** All four Gram-positive bacteria were sensitive to the plant extracts (Table 3), but none of the Gram-negative organisms were sensitive (data not shown). Ethanol alone (at any concentration) did not exhibit any inhibitory action for Gram-positive bacteria. Among the extracts, boldo, hops, licorice and yerba mansa demonstrated relatively strong antibacterial activity at all three ethanol levels of the extracts, with hops showing the highest activity. Of the three ethanol extracts of hops, the 90% ethanol extract had the highest activity against *S. aureus* strains.

Table 3. Antimicrobial activity of ethanol extracts.

Plant material	<i>Staphylococcus</i>									<i>Streptococcus</i>		
	<i>aureus</i>			<i>aureus</i> <sup>1</sup>			<i>epidermidis</i>			<i>pyogenes</i>		
	Ethanol %			Ethanol %			Ethanol %			Ethanol %		
	50	70	90	50	70	90	50	70	90	50	70	90
Growth inhibitory zone (mm) <sup>3</sup>												
Boldo	18	19	17	22	23	20	22	23	23	20	22	21
Buchu	14	12	10	21	14	9	N	11	10	18	20	18
<i>Echinacea</i>	N	N	N	N	N	N	N	N	N	12	10	11
Hops	32	32	36	31	31	37	35	34	34	24	24	25
Licorice	18	18	20	21	21	18	19	19	18	20	25	25
Oregon grape	13	13	12	15	16	12	20	19	17	28	28	25
Usnea	N	N	N	N	N	16	11	18	32	16	13	16
Yerba mansa	14	15	14	18	19	15	19	19	17	19	19	20
Ethanol	N	N	N	N	N	N	N	N	N	N	N	N
Ampicillin	34			12			25			35		

<sup>1</sup>MRSA strain of *S. aureus*. <sup>2</sup>*Echinacea* sample is *E. angustifolia*. <sup>3</sup>N= No inhibition

Overall, extracts of buchu, usnea, and *Echinacea* demonstrated lower inhibitory activity as compared with extracts of the other plants. The 50% ethanol extract of buchu showed relatively high activity against MRSA strains of *S. aureus* as compared with the other ethanol extracts of buchu. Usnea extracts were not active against *S. aureus* except for the 90% ethanol extract against the MRSA strain. Usnea was active against *S. epidermidis*, with the 90% ethanol extract, which produced the second highest inhibition of all the extracts. Both usnea and *E. angustifolia* were mildly inhibitory to *S. pyogenes*. Oregon grape root extracts at 50% and 70% levels were more inhibitory than at 90% for the organisms. *Echinacea angustifolia* did not show any antibacterial activity, except for a slight inhibition of *S. pyogenes*.

The MRSA strain was very sensitive to most of the extracts except *E. angustifolia* and two out of three usnea extracts. The activities of the other six extracts were stronger than ampicillin (10 µg) against MRSA. Among the plants, the inhibitory activity of hops against MRSA more than any other plant extract at all three levels of ethanol and more than the antibiotic ampicillin. All the plant extracts showed some inhibitory action on *S. pyogenes*, but the inhibition by the plant extracts was less than the inhibitory action of ampicillin.

**Broth Assay** Minimum bactericidal concentrations (MBCs) of each extract were expressed as the lowest dilution level of the extract needed to completely inhibit bacterial growth. Because the extracts were diluted only to 1/256 level, the inhibitory values expressed at this level may be greater, but were not determined in this study. The measured MBCs of the extracts ranged from 1/4 to 1/256 (Tables 4 & 5).

The MBCs of the extracts from boldo, hops, licorice and yerba mansa showed the highest antibacterial activity as compared with the other plant extracts. Results of the broth assay agree with the results obtained from the well assay, indicating Gram-positive bacteria were susceptible to the extracts, but Gram-negative bacteria were mostly resistant. Interestingly, the *S. aureus* MRSA strain was more susceptible to the plant extracts than the normal *S. aureus* (ATCC 25923) strain. *Echinacea*

*angustifolia* exhibited only mild inhibition towards *S. pyogenes*, similar to that of Oregon grape.

Table 4. Minimum bactericidal concentration of Gram positives.

Plant material	<i>Staphylococcus</i>						<i>Streptococcus pyogenes</i>		
	<i>aureus</i>		<i>aureus</i> <sup>1</sup>		<i>epidermidis</i>				
	Ethanol % 50 70 90	Ethanol % 50 70 90	Ethanol % 50 70 90	Ethanol % 50 70 90	Ethanol % 50 70 90	Ethanol % 50 70 90	Ethanol % 50 70 90	Ethanol % 50 70 90	Ethanol % 50 70 90
	(Dilution level, 1 to 256) <sup>2</sup>								
Boldo	64 64 32	256 256 256	128 128 128	128 128 128	128 128 128	128 128 128	128 128 128	128 128 128	128 128 128
Buchu	32 8 8	64 32 16	4 8 8	4 8 8	4 8 8	4 8 8	4 8 8	4 8 8	4 8 8
<i>Echinacea</i> <sup>3</sup>	8 8 8	4 4 8	4 4 8	4 4 8	4 4 8	4 4 8	4 4 8	4 4 8	4 4 8
Hops	64 64 64	256 256 256	128 128 128	128 128 128	128 128 128	128 128 128	128 128 128	128 128 128	128 128 128
Licorice	64 64 64	256 256 256	128 128 128	128 128 128	128 128 128	128 128 128	128 128 128	128 128 128	128 128 128
Oregon grape	16 16 16	16 32 32	16 32 16	16 32 16	16 32 16	16 32 16	16 32 16	16 32 16	16 32 16
Usnea	4 8 16	16 16 32	16 32 16	16 32 16	16 32 16	16 32 16	16 32 16	16 32 16	16 32 16
Yerba mansa	32 32 32	128 128 128	128 128 128	128 128 128	128 128 128	128 128 128	128 128 128	128 128 128	128 128 128
Ethanol	4 4 4	2 2 2	4 4 4	4 4 4	4 4 4	4 4 4	4 4 4	4 4 4	4 4 4
Ampicillin	128	16	256	256	256	256	256	256	256

<sup>1</sup>MRSA strain of *S. aureus*.

<sup>2</sup>The larger the number the more active the extract; dilutions labeled 256 represent the maximum dilution tested.

<sup>3</sup>*Echinacea* sample is *E. angustifolia*.

Table 5. Minimum bactericidal concentration of Gram negatives.

Plant material	<i>Escherichia coli</i>			<i>Klebsiella pneumoniae</i>			<i>Pseudomonas aeruginosa</i>			<i>Salmonella enteritidis</i>		
	Ethanol %			Ethanol %			Ethanol %			Ethanol %		
	50	70	90	50	70	90	50	70	90	50	70	90
	(Dilution level, 1 to 256) <sup>1</sup>											
Boldo	8 8 8	32 32 64	16 16 16	8 16 16	8 16 16	8 16 16	8 16 16	8 16 16	8 16 16	8 16 16	8 16 16	8 16 16
Buchu	4 8 8	64 64 64	16 16 8	8 8 4	8 8 4	8 8 4	8 8 4	8 8 4	8 8 4	8 8 4	8 8 4	8 8 4
<i>Echinacea</i> <sup>2</sup>	4 8 4	16 32 64	2 2 4	8 8 8	8 8 8	8 8 8	8 8 8	8 8 8	8 8 8	8 8 8	8 8 8	8 8 8
Hops	8 8 16	16 64 64	8 8 16	8 16 8	8 16 8	8 16 8	8 16 8	8 16 8	8 16 8	8 16 8	8 16 8	8 16 8
Licorice	4 8 8	32 32 64	4 8 8	2 4 8	2 4 8	2 4 8	2 4 8	2 4 8	2 4 8	2 4 8	2 4 8	2 4 8
Oregon grape	4 4 4	32 32 32	4 8 4	4 8 8	4 8 8	4 8 8	4 8 8	4 8 8	4 8 8	4 8 8	4 8 8	4 8 8
Usnea	4 4 4	64 64 64	2 4 2	4 8 8	4 8 8	4 8 8	4 8 8	4 8 8	4 8 8	4 8 8	4 8 8	4 8 8
Yerba mansa	16 16 16	32 64 32	16 16 16	16 16 16	16 16 16	16 16 16	16 16 16	16 16 16	16 16 16	16 16 16	16 16 16	16 16 16
Ethanol	4 4 4	32 32 32	4 4 4	4 4 4	4 4 4	4 4 4	4 4 4	4 4 4	4 4 4	4 4 4	4 4 4	4 4 4
Ampicillin	4	16	2	32	32	32	32	32	32	32	32	32

<sup>1</sup>The larger the number the more active the extract; dilutions labeled 256 represent the maximum dilution tested.

<sup>2</sup>*Echinacea* sample is *E. angustifolia*.

In the broth assay, ethanol (at all concentrations) used in the extraction process was inhibitory for the bacteria with MBC ranging from 1/4 to 1/64 dilution level. For the Gram-negative bacteria, the MBC levels were, to some extent, similar to the values of ethanol alone, suggesting the inhibitory action observed against Gram-negative

bacteria for the plant extracts in the broth assay is primarily due to the action of ethanol.

## DISCUSSION

The type and level of biological activity exhibited by any plant material depends on many factors, including the plant part, geographical source, soil conditions, harvest time, moisture content, drying method, storage conditions, and post-harvest processing. For example, the relatively high temperatures that can be generated during tissue grinding can denature chemical constituents and the extraction solvent, time period, and temperature can affect the level and composition of secondary metabolites extracted from plant tissues.

Methanol, ethanol and acetone separately or mixed with water are commonly used to extract bioactive compounds from plant materials, depending on the intended use of the extract. In this study ethanol and water were chosen as extraction solvents because ethanol/water formulations are relatively safe for human consumption as compared with other organic solvents, such as acetone or methanol frequently used by researchers. Further, ethanol extraction is widely used to obtain crude extracts of phytochemicals from plant materials in the herbal medicine industry for therapeutic applications.

Due to the variation in composition of active compounds, different plant types may require different concentrations of ethanol to achieve maximum recovery of bioactive components. No standardized extraction protocol has been developed for preparation of herbal extracts, but 20-95% of ethanol-water mixture is frequently used by the herbal medicine industry to prepare ethanolic extracts (Ganora, 2008). In the present study, differences in the effectiveness among the extracts from three concentrations (50%, 70% and 90%) of ethanol in water demonstrated that the concentration of ethanol could affect the antibacterial activity.

The eight plants chosen for this study are commonly used for treating infectious diseases in herbal therapy and are known to produce a wide range of bioactive compounds, including antimicrobials (Bent and Ko, 2004; Mahady, 2005). Among the plant extracts, boldo, hops, licorice, and yerba mansa exhibited relatively strong inhibitory activities to-

wards all four Gram-positive organisms tested at all levels of ethanol used in the extraction. In contrast, the antibacterial activity of the other plant extracts varied depending on the level of ethanol used in the extraction.

The potentially effective antibacterial agents of ethanol extracts from boldo, hops, licorice and yerba mansa were confirmed by demonstrated bioactivity in both the well and broth dilution study. Of all the plants tested in the study, hops showed the strongest antibacterial activity with the maximum activity in the 90% ethanol extract. Previous studies have indicated that boldo (Vila et al., 1999), hops (Langezaal et al., 1992; Haas and Barsoumian, 1994), licorice (Fukai et al., 2002), and yerba mansa (Medina et al., 2005) are sources of antimicrobial compounds. Most of the data available, however, are for plant essential oils or for acetone and methanol-based extracts, not for ethanol-based extracts.

No inhibitory action of the extracts was observed against the four Gram-negative bacteria tested in this study. These differences in sensitivity between Gram-positive and Gram-negative bacteria to the extract can probably be attributed to the structural and compositional differences in membranes between the two groups (Lambert, 2002). The Gram-negative bacteria have an outer membrane that serves as an impermeable barrier for many small molecules.

Boldo is a common medicinal plant in folk medicine and is used primarily for the treatment of liver ailments and urinary tract infections. The present study showed that an ethanol extract of boldo leaf had a antibacterial action against all four Gram-positive bacteria tested. The essential oil of boldo leaf has been previously shown to exhibit a potent antimicrobial activity against *S. pyogenes*, *Micrococcus* sp. and *Candida* sp. (Vila et al., 1999). To maximize antibacterial activity of boldo, 50% or higher ethanol concentrations would be suitable for the preparation of the extracts.

All three levels of ethanolic extracts from the strobile of hops were active against the Gram-positive bacteria tested and the 90% ethanol extract was the most active of all the plant extracts against *S. aureus* and MRSA. The strobile of hops has been

shown to possess strong antibacterial activity in several other studies (Langezaal et al, 1992; Haas and Barsoumian 1994). Hops contains three major active components:  $\alpha$  acids (humulone),  $\beta$  acids (lupulone) and xanthohumol, all of which have been shown to possess antimicrobial activity (Koetter and Biendl, 2010). The antimicrobial activity of hops acids is mainly against Gram-positive bacteria (Haas and Barsoumian, 1994) and not active against most Gram-negative microorganisms except *Helicobacter pylori* (Ohsugi et al. 1997). Using a paper disk overlay technique, Langezaal et al. (1992) demonstrated that growth of *E. coli*, *B. subtilis*, *S. aureus*, and some fungal cultures was strongly inhibited by the extracts as opposed to the essential oil of hops. All the Gram-negative bacteria used in the present study were resistant to the hops extract.

Extracts of licorice roots had almost the same level of antibacterial activity as hops, indicating that even a very diluted extract possesses strong bactericidal action. Phenolic compounds isolated from licorice are apparently effective as antibacterial agents against *S. aureus*, including MRSA (Hatano et al., 2000), and a licorice extract has been demonstrated to have a potent bactericidal effect against *Enterococcus faecalis* (Badr et al., 2011). A study in India, suggested licorice could inhibit the growth of both Gram-positive and Gram-negative bacteria, including *Mycobacterium tuberculosis* (Gupta et al., 2008). In the present study, licorice produced, except for *K. pneumoniae*, only a mild inhibition of Gram-negative bacteria in the broth assay. The 90% ethanol extract of licorice showed activity against the *K. pneumoniae*.

Yerba mansa root extracts were effective in inhibiting the Gram-positive bacteria tested in our study. Earlier work has demonstrated the steam-distilled oil of yerba mansa leaf is active against *S. aureus*, *S. pneumoniae* and *Geotrichum candidum* (Medina et al., 2005). Use of root extract in the current study demonstrated that the root also produces antibacterial compounds against Gram-positive bacteria.

The 90% ethanol extract of usnea demonstrated a higher activity against *S. epidermidis* and MRSA than the 50% and 70% ethanol extracts. According to



Weckesser et al. (2007), usnea extract and usnic acid effectively inhibited the growth of several Gram-positive bacteria, including penicillin-resistant *Staphylococcus aureus*, methicillin resistant *S. aureus* (MRSA), and *Enterococcus faecalis*. The inhibition spectrum and MIC and MBC of usnea extract and usnic acid are similar (Engel et al., 2007), suggesting that the antimicrobial activity of usnea extract is primarily mediated by usnic acid.

The extract of *E. angustifolia* roots did not show any significant bioactivity towards any of the bacteria, only a slight inhibition of *S. pyogenes*. The MRSA strain, which was sensitive to the other tested plant extracts, was not inhibited by the *Echinacea*. Other studies (Barnes et al., 2005) suggest that *Echinacea* may possess significant immune modulatory activity and is used in the prevention and treatment of upper respiratory infection.

For buchu leaf, 50% ethanol extract seems to possess the highest activity against the MRSA strain, showing that the zone of inhibition decreased as the concentration of ethanol increased. At all ethanol concentrations, buchu leaf extract showed a considerable antibacterial activity against *S. pyogenes*. Scientific studies on the antimicrobial properties of buchu leaves have not been done, except for a few experiments with essential oil from the leaf of the plant (Lis-Balchin et al., 2001; Moolla et al., 2007).

Similar to buchu leaf, Oregon grape root extract was more active at 50% ethanol than at higher levels. While the Oregon grape extract showed no significant activity towards *S. aureus*, antibacterial activity was observed against *S. pyogenes*. Earlier *in vitro* antimicrobial tests with crude extract from Oregon grape bark and its two main protoberberine alkaloids, berberine and jatrorrhizine, showed varying degrees of antibacterial activity against twenty strains of coagulase-negative *Staphylococci* and some other bacteria that cause skin infections (Slobodníková et al., 2004). Our data support this observation, indicating ethanol extracts of Oregon grape root can inhibit the growth of *S. epidermis* and *S. pyogenes*, the organisms commonly associated with skin infections.

According to the World Health Organization, infectious diseases are a significant cause of world-

wide morbidity and mortality, accounting for approximately 50% of all deaths in tropical countries (WHO, 2003). In the US, infectious disease hospitalization rates have increased over time and are associated with substantial morbidity, mortality, and economic consequences (Yorita-Christensen et al., 2009). Additionally, antimicrobial resistance to antibiotics is emerging as a serious health issue and alternatives to treat infectious diseases in the future need to be developed (Abascal and Yarnell, 2002).

A number of studies have voiced the necessity of developing alternative antimicrobial drugs (Poole, 2002; Sibanda and Okoh, 2007). Plant antimicrobials would appear to be an excellent choice (Mahady, 2005). Our study revealed that, except for usnea and *E. angustifolia*, the other plants used in this study produced strong antimicrobials and may offer prospective new treatments for bacterial infections, including multi-drug resistant bacteria. The benefit of antimicrobial properties from these plants can only be achieved, however, by using a specific solvent and solvent concentration in extracting the plant materials. The study of chemical profiles of extracts obtained with different ethanol levels warrants future research to determine the active extract constituents at each ethanol level.

In spite of the number of published scientific articles around the globe that describe the antimicrobial activities of plant extracts, systematic studies conducted on the effects of solvent concentration on the antimicrobial activity are lacking. Due to the complex nature of the phytochemicals present in a plant extract, the extraction solvent system needs to be considered. The present study provides data on the importance of selection of an appropriate solvent concentration and indicates that ethanol extracts of plants can offer significant potential for the development of novel antibacterial therapies.

#### ACKNOWLEDGEMENTS

The authors thank Bethany Hammons, New Mexico State University for technical support throughout the study.

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