

Full Length Research Paper

## Antimicrobial activity of native and naturalized plants of Minnesota and Wisconsin

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The antimicrobial activity of aqueous ethanol extracts of stems, leaves, flowers and roots from 336 native and naturalized species (597 extracts) collected in Minnesota and Wisconsin was tested against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*. Twenty-four percent, or 142 extracts, exhibited antimicrobial activity. Extracts from *Betula papyrifera* Marshall (Betulaceae), *Centaurea maculosa* Lam. (Asteraceae), *Epilobium angustifolium* L. (Onagraceae), *Hypericum perforatum* L. (Clusiaceae), *Lythrum salicaria* L. (Lythraceae), and *Rhus glabra* L. (Anacardiaceae) inhibited all four microorganisms. Extracts from two species inhibited three microorganisms, 11 extracts (10 species) inhibited two, and 119 extracts (98 species) inhibited one microorganism with four species having inhibition zones greater than 15 mm. This is the first report describing the antimicrobial activity of *Clintonia* sp. (Liliaceae), *Comptonia peregrina* (L.) J.M. Coult. (Myricaceae), *Desmodium illinoense* A. Gray (Fabaceae), *Geum virginianum* L. (Rosaceae), leaves of *Scirpus americanus* Pers. (Cyperaceae), flower clusters of *Eupatorium maculatum* L. (Asteraceae), berries of *Smilacina racemosa* (L.) Desf (false Solomon's seal) and frozen *Hypericum perforatum* L. (Clusiaceae).

**Key words:** Antimicrobial, medicinal, native plants, antibacterial.

### INTRODUCTION

New antimicrobial agents are needed to treat diseases in humans and animals caused by drug resistant microorganisms. In addition, there is a continuing consumer demand for "natural" and/or "preservative-free" microbiologically safe foods and cosmetic products (Wijesekera, 1991; Zink, 1997). As public demand for these products increases, an opportunity exists to satisfy consumer demands while providing wholesome and safe products from plants.

Antimicrobial compounds of plant origin may occur in stems, roots, leaves, bark, flowers and fruits of plants. Plant derived phytoalexin (Beuchat et al., 1994) isothiocyanates (Delaquis and Mazza, 1995) alliacins, anthocya-

nins (Somaatmadja et al., 1964) and essential oils (Lis-Balchin and Deans, 1997) tannins and polyphenols and terpenoids (Cutter, 2000; Hao et al., 1998; Puupponen-Pimia et al., 2001) have demonstrated antibacterial and/or antifungal activities. These compounds are bactericidal and/or bacteriostatic influencing lag time, growth rate and maximum growth of microorganisms.

Herbal medicine expertise of North American Native Indian cultures has been documented for the states of Minnesota and Wisconsin in many publications in the popular literature. Indigenous herbal medicine includes knowledge regarding the appropriate plant parts, extraction, and manner of preparation as infusions, decoctions, or poultices. Often, different plant parts have had specific ethnomedical applications. For instance, the flowers, seeds and roots of *Rhus* and *Epilobium* spp. have had antiemetic, antidiarrheal, oral and respiratory aid, anti-hemorrhagic, dermatological aid, and analgesic applications

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(Moerman, 2004). Although a specific plant part might have a reported use, other parts of the plant and additional applications may remain uninvestigated or a plant may not have a recorded ethnomedical use.

Research has identified some North American species as potential medicinal crops (Small and Catling, 1999). An example is *Taxus brevifolia* Nutt., the Pacific Yew. This species has a taxane diterpenoid in the bark that is useful in treating some forms of cancer (Wani et al., 1971). In addition, *Achillea millefolium* L., *Acorus calamus* L., *Caulophyllum* species, *Echinacea pallida* (Nutt.) Nutt. var. *angustifolia* (DC.) Cronq., *Epilobium angustifolium* L., *Oenothera biennis* L., *Podophyllum peltatum* L., and *Taraxacum* species have been considered as medicinal crops (Small and Catling, 1999). Another example is *P. peltatum* L. that is being considered as an alternative crop in the southern United States (Cushman et al., 2005).

Another source of antimicrobial compounds is naturalized non-native plant introductions into North America, yet few of these species have been studied for this purpose. An example is *Lythrum salicaria* L. (purple loosestrife) that has invaded North American wetlands. *L. salicaria* has styptic and antibacterial action that can assist wound healing (Thompson et al., 1987).

A significant opportunity exists to identify new, natural plant derived antimicrobial agents for treatment of diseases or as food or cosmetic preservatives. Our objective was to evaluate the activity of aerial parts of native and naturalized species in the Upper Mississippi River Basin against organisms that cause disease and spoilage, that is, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*.

## MATERIALS AND METHODS

### Plant collection

In 2003 and 2004 aerial parts (leaves, stems and flowers) and an occasional root of native and naturalized plants were collected in Minnesota and Wisconsin. The availability of flowers on some species depended on the time of year. In 2003, 301 plant samples were collected and in 2004, 296 plant samples were collected for a total of 336 species. In 2003, all the plant samples were frozen at -10°C immediately after collection and stored from one to four months before extraction and antimicrobial activity determination. In 2004, plant samples were frozen (-10°C) immediately after collection, and within 48 h were extracted and tested.

### Preparation of extracts

Ten grams of plant material were cut into 2.5 cm pieces and combined with 10 ml of 80:20 aqueous ethanolic solvent. The mixture was pulverized with a stomacher for 2 min. The plant material varied in absorbency; if 2 ml of extract could not be pipetted, an additional 2 ml of solvent was added. The macerated mixture was left at room temperature (20°C) for 24 h. The stomacher bags were kneaded and approximately 2 ml of supernatant was transferred into a sterile microfuge tube and centrifuged at 15,000 xg for 15 min to remove remaining plant solids. Extracts

of 50 µl were placed on 6 mm sterile paper discs then the discs and solvent were placed in a biological safety cabinet where the solvent was allowed to evaporate for one hour.

### Antimicrobial screening

The extracts were screened for antimicrobial activity using the disk diffusion technique (Bauer et al., 1966). Test microorganisms included *S. aureus* (ATCC 12600), *E. coli* (ATCC 8677), *P. aeruginosa* (ATCC 9721) and *C. albicans* (ATCC 10231). *E. coli* and *P. aeruginosa* were maintained on MacConkey's agar (Becton Dickinson and Company, Microbiology Systems, Sparks, MD), *S. aureus* on blood agar and *C. albicans* on Sabouraud dextrose agar (Becton Dickinson and Company, Microbiology Systems, Sparks, MD). After 18 to 20 h of culture, each microorganism was diluted in sterile saline to an optical density of approximately 0.5 using a MacFarland standard (Becton Dickinson and Company, Microbiology Systems, Sparks, MD). A Mueller-Hinton agar plate was swabbed on three axes with a sterile cotton tipped swab, which was dipped in the freshly prepared, diluted culture. Discs containing the dried extract were placed on the freshly swabbed plates along with controls. Discs with evaporated solvent were used as a negative control and an antibiotic disc (ticarcillin, 75 mcg or chloramphenicol 30 mcg) was used as a positive control. The plates were incubated at 37°C for 18 h and zones of inhibition were measured in millimeters on three axes and the mean value reported.

## RESULTS AND DISCUSSION

Of the 597 samples, 142 samples (24%), representing 109 different species and 53 plant families exhibited antimicrobial activity against at least one microorganism (Table 1). Extracts from six plants (*B. papyrifera* Marshall, *Centaurea maculosa* Lam, *E. angustifolium* L., *Hypericum perforatum* L., *L. salicaria* L. and *Rhus glabra* L.) inhibited growth of the four test microorganisms. Extracts from 455 samples exhibited no antimicrobial activity (Table 2).

### Plant extracts inhibiting four microorganisms

#### *Betula papyrifera*

Extracts of *B. papyrifera* Marshall (paper birch) leaves were most effective against *S. aureus* and had slightly inhibited the other three microorganisms. Previous research showed that leaf extracts (aqueous acetone) of *Betula pubescens* Ehrh, the old-world counterpart to *B. papyrifera* also had antimicrobial activity against *S. aureus*, with slight activity against *E. coli*, and no activity against *C. albicans* or *Aspergillus niger* (Rauha et al., 2000). Ethanol extracts of *B. papyrifera* bark and wood exhibited antimicrobial activity against our four bacterial species (two Gram-positive and two Gram-negative) but no antifungal activity (Omar et al., 2000). Methanolic extracts of air-dried *B. papyrifera* branches had activity against 6 of 11 bacteria (four Gram-positive, two Gram-negative) and three fungal species (McCutcheon et al., 1992; 1994). The antimicrobial activity appears to be eva-

**Table 1.** Antimicrobial activity of aqueous/ethanol plant extracts from aerial parts of plants collected in Minnesota and Wisconsin, U.S.A. in 2003 and 2004.

Botanical name	Common name	Plant part tested	Microorganisms <sup>a</sup>			
			Inhibition zones in mm			
Inhibition against four microorganisms			Sa	Ec	Pa	Ca
<i>Betula papyrifera</i> Marshall <sup>10</sup>	paper birch	leaves	9	sl	sl	sl
<i>Centaurea maculosa</i> Lam. <sup>8</sup>	spotted knapweed	leaves	11	10	7	7
<i>Epilobium angustifolium</i> L. <sup>37</sup>	Fireweed	leaves	11	7	7	15
<i>Epilobium angustifolium</i> L. <sup>37</sup>	Fireweed	flowering aerial organs	17	6	7	20
<i>Hypericum perforatum</i> L. <sup>17</sup>	St. John's Wort	flowering aerial organs	15	6	6	7
<i>Lythrum salicaria</i> L. <sup>32</sup>	purple loosestrife	flowering spikes, leaves	15	10	7	7
<i>Rhus glabra</i> L. <sup>3</sup>	sumac, smooth	green flower clusters	16	10	12	13
<i>Rhus glabra</i> L. <sup>3</sup>	sumac, smooth	leaves	13	7	10	15
Inhibition against three microorganisms						
<i>Desmodium illinoense</i> A. Gray <sup>23</sup>	prairie tick trefoil	flowering aerial organs	sl		sl	sl
<i>Scirpus americanus</i> Pers. <sup>19</sup>	bulrush	leaves	10	sl	sl	
Inhibition against two microorganisms						
<i>Clintonia</i> sp. <sup>31</sup>	Clintonia	leaves, roots	11			11
<i>Comptonia peregrina</i> (L.) J.M. Coult. <sup>35</sup>	sweet fern	aerial organs	9			15
<i>Comptonia peregrina</i> (L.) J.M. Coult. <sup>35</sup>	sweet fern	leaves	14			12
<i>Cotinus coggygria</i> Scop. <sup>3</sup>	smoke tree	leaves	13		10	
<i>Desmanthus illinoensis</i> (Michx.) MacMill. <sup>33</sup>	Illinois bundle flower	leaves, stems, flowers	15			17
<i>Epilobium angustifolium</i> L. <sup>37</sup>	fireweed	flowers	14			17
<i>Epilobium ciliatum</i> Raf. <sup>37</sup>	Amer. willow herb	leaves	11			15
<i>Eupatorium maculatum</i> L. <sup>8</sup>	Joe pye weed	flower clusters	9			sl
<i>Geum virginianum</i> L. <sup>44</sup>	rough avens	immature floral organs	10			10
<i>Juglans nigra</i> L. <sup>29</sup>	black walnut	leaves	15			12
<i>Polygonum coccineum</i> L. <sup>41</sup>	swamp smartweed	flowers	11			7
Inhibition against a single microorganism						
<i>Adiantum pedatum</i> L. <sup>1</sup>	maidenhair fern	leaves	7			
<i>Aesculus glabra</i> Willd. <sup>28</sup>	Ohio buckeye	leaves	8			
<i>Allium ramosum</i> L. <sup>2</sup>	Chinese chives	leaves	7			
<i>Ambrosia artemesifolia</i> L. <sup>8</sup>	ragweed	flowering aerial organs	10			
<i>Amorpha canescens</i> L. <sup>23</sup>	lead plant	leaves	7			
<i>Amorpha canescens</i> L. <sup>23</sup>	lead plant	flowering aerial organs	8			
<i>Anaphalis margaritacea</i> (L.) Benth. and Hook <sup>8</sup>	pearly everlasting	leaves	18			
<i>Anaphalis margaritacea</i> (L.) Benth. and Hook <sup>8</sup>	pearly everlasting	flowering aerial organs	15			
<i>Anaphalis margaritacea</i> (L.) Benth. and Hook <sup>8</sup>	pearly everlasting	flower	10			
<i>Anemone quinquefolia</i> L. <sup>43</sup>	snowdrop	leaves				12
<i>Apocynum androsaemifolium</i> L. <sup>7</sup>	spreading dogbane	leaves stem, stalk	10			
<i>Apocynum androsaemifolium</i> L. <sup>7</sup>	spreading dogbane	leaves	12			
<i>Apocynum cannabinum</i> L. <sup>7</sup>	prairie dogbane	leaves	7			
<i>Asarum canadense</i> L. <sup>6</sup>	wild ginger	leaves w/ stems	15			
<i>Asclepias tuberosa</i> L. <sup>7</sup>	butterfly weed	flowering aerial organs	7			

Table 1. contd.

<i>Baptisia australis</i> (L.) R.Br. <sup>23</sup>	false indigo	leaves w/ few flowers	sl			
<i>Cannabis sativa</i> L. <sup>14</sup>	hemp	leaves, stems	25			
<i>Chamaecrista fasciculata</i> (L.) Moench <sup>24</sup>	partridge pea	leaves	11			
<i>Chrysanthemum leucanthemum</i> L. <sup>8</sup>	oxeye daisy	flowers	7			
<i>Cicuta maculata</i> L. <sup>4</sup>	water hemlock	flowering aerial organs	sl			
<i>Cicuta maculata</i> L. <sup>4</sup>	water hemlock	flower clusters	sl			
<i>Claytonia virginica</i> L. <sup>42</sup>	spring beauty	leaves	sl			
<i>Coreopsis palmata</i> Nutt. <sup>8</sup>	prairie coreopsis	leaves				8
<i>Cornus amomum</i> L. <sup>18</sup>	silky dogwood	leaves	10			
<i>Cornus stolonifera</i> Mill. <sup>18</sup>	red-osier dogwood	flower clusters, leaves	10			
<i>Corylus</i> sp. <sup>10</sup>	hazelnut	seed clusters, leaves	10			
<i>Cotinas coggygria</i> Scop. <sup>3</sup>	smoketree	flowers, leaves, stem	11			
<i>Desmodium canadense</i> (L.) DC <sup>23</sup>	showy tick trefoil	leaves	12			
<i>Dicentra eximia</i> (Ker Gawl.) Torr. <sup>25</sup>	bleeding heart	leaves, stem	10			
<i>Diervilla lonicera</i> Mill. <sup>15</sup>	bush honeysuckle	leaves, stem, flowers	9			
<i>Echinacea purpurea</i> (L.) Moench <sup>8</sup>	purple coneflower	leaves	10			
<i>Equisetum sylvaticum</i> L. <sup>20</sup>	woodland horsetail	leaves	10			
<i>Eucalyptus</i> sp. <sup>36</sup>	eucalyptus	leaves	15			
<i>Eupatorium maculatum</i> L. <sup>8</sup>	Joe pye weed	leaves, stems	8			
<i>Eupatorium perfoliatum</i> L. <sup>8</sup>	boneset	leaves	11			
<i>Eupatorium perfoliatum</i> L. <sup>8</sup>	boneset	flower clusters	7			
<i>Euphorbia corollata</i> L. <sup>22</sup>	flowering spurge	leaves	10			
<i>Euphorbia corollata</i> L. <sup>22</sup>	flowering spurge	aerial parts	7			
<i>Euphorbia esula</i> L. <sup>22</sup>	leafy spurge	flowers				9
<i>Euphorbia maculata</i> L. <sup>22</sup>	milk purslane	leaves				14
<i>Filipendula rubra</i> (Hill) B.L.Rob. <sup>44</sup>	Queen of the prairie	leaves	12			
<i>Geranium maculatum</i> L. <sup>26</sup>	wild geranium	flowers	7			
<i>Geranium maculatum</i> L. <sup>26</sup>	wild geranium	leaves, stems	9			
<i>Geum triflorum</i> Pursh <sup>44</sup>	prairie smoke	leaves, stems	7			
<i>Glycyrrhiza lepidota</i> Pursh <sup>23</sup>	wild licorice	leaves	11			
<i>Helenium autumnale</i> L. <sup>8</sup>	sneezeweed	leaves	7			
<i>Helianthus giganteus</i> L. <sup>8</sup>	giant sunflowers	flowers	sl			
<i>Heracleum lanatum</i> L. <sup>4</sup>	cow parsnip	seed heads, stems	10			
<i>Heracleum lanatum</i> L. <sup>4</sup>	cow parsnip	leaves	7			
<i>Hesperis matronalis</i> L. <sup>12</sup>	dame's rocket	leaves	14			
<i>Hesperis matronalis</i> L. <sup>12</sup>	dame's rocket	flower	12			
<i>Heuchera richardsonii</i> R.Br. <sup>47</sup>	prairie alum root	leaves	13			
<i>Hypericum perforatum</i> L. <sup>17</sup>	St. John's Wort	aerial parts, immature fruits	11			
<i>Ilex verticillata</i> L. <sup>5</sup>	winterberry	branch w/ leaves, berries	9			
<i>Impatiens capensis</i> Meerb <sup>9</sup>	jewelweed	leaves	sl			
<i>Juglans nigra</i> L. <sup>29</sup>	black walnut	leaves	11			
<i>Larix laricina</i> (Du Roi) K. Koch <sup>39</sup>	tamarack	leaves	7			
<i>Ledum groenlandicum</i> Oeder <sup>21</sup>	Labrador tea	leaves	9			
<i>Liatris pycnostachya</i> Michx. <sup>8</sup>	prairie blazing star	leaves	10			
<i>Lythrum salicaria</i> L. <sup>32</sup>	purple loosestrife	leaves	9			
<i>Miscanthus giganteus</i> (hybrid) <sup>40</sup>	Chinese silver grass	leaves, stalk	12			

Table 1. contd.

<i>Monarda fistulosa</i> L. <sup>30</sup>	wild bergamot	leaves	6		
<i>Monarda fistulosa</i> L. <sup>30</sup>	bergamot	flowering aerial organs	7		
<i>Morus rubra</i> L. <sup>34</sup>	red mulberry	leaves	11		
<i>Oenothera biennis</i> L. <sup>37</sup>	evening primrose	leaves	sl		
<i>Picea mariana</i> (Mill.) Britton, Sterns and Poggenb. <sup>39</sup>	black spruce	leaves	9		
<i>Pinus strobes</i> L. <sup>39</sup>	white pine	flowers	10		
<i>Polygonum coccineum</i> L. <sup>41</sup>	swamp smartweed	leaves	14		
<i>Polygonum coccineum</i> L. <sup>41</sup>	swamp smartweed	flowering aerial organs	11		
<i>Polygonum cuspidatum</i> L. <sup>41</sup>	Japanese knotweed	leaves	9		
<i>Polygonum persicaria</i> L. <sup>41</sup>	lady's thumb	leaves, stems	7		
<i>Potentilla arguta</i> Pursh <sup>44</sup>	tall potentilla	leaves			16
<i>Potentilla simplex</i> Michx. <sup>44</sup>	common cinquefoil	flowering aerial organs	11		
<i>Prunella vulgaris</i> L. <sup>30</sup>	heal-all	flowering aerial organs	7		
<i>Prunus americana</i> Marshall <sup>44</sup>	plum	leaves	7		
<i>Pycnanthemum virginianum</i> (L.) Durand B.D. Jacks. <sup>30</sup>	mountain mint	leaves	9		
<i>Quercus rubra</i> L. <sup>24</sup>	red oak	leaves			9
<i>Rhus typhina</i> L. <sup>3</sup>	staghorn sumac	berries	sl		
<i>Ribes</i> sp. <sup>27</sup>	gooseberry	leaves, twigs	10		
<i>Rosa palustris</i> Marshall <sup>44</sup>	swamp rose	aerial parts w/ rose hips	13		
<i>Rubus</i> sp. <sup>44</sup>	wild raspberry	leaves	9		
<i>Rumex acetosella</i> L. <sup>41</sup>	sheep sorrel	immature flowers	11		
<i>Rumex crispus</i> L. <sup>41</sup>	curly dock	immature flowers	12		
<i>Rumex crispus</i> L. <sup>41</sup>	curly dock	stems of immature			
<i>Rumex crispus</i> L. <sup>41</sup>	curly dock	flowers	11		
<i>Ruta graveolens</i> L. <sup>45</sup>	rue	leaves	sl		
<i>Salix petolaris</i> Sm. <sup>46</sup>	meadow willow	leaves	9		
<i>Sanguinaria Canadensis</i> L. <sup>38</sup>	bloodroot	leaves w/ stems	sl		
<i>Scirpus americanus</i> Pers. <sup>19</sup>	bulrush	flower clusters	8		
<i>Scirpus validus</i> Vahl <sup>19</sup>	great bulrush	leaves	7		
<i>Senna marilandica</i> (L.) Link <sup>13</sup>	wild senna	leaves	10		
<i>Silene latifolia</i> Poir. <sup>16</sup>	white campion	stems, leaves	9		
<i>Smilacina racemosa</i> (L.) Desf. <sup>31</sup>	false Solomon's seal	leaves stem, seeds			14
<i>Smilacina racemosa</i> (L.) Desf. <sup>31</sup>	false Solomon's seal	berries			17
<i>Solanum dulcamara</i> L. <sup>49</sup>	nightshade	berries, mixed maturity			11
<i>Solanum dulcamara</i> L. <sup>49</sup>	nightshade	flower, leaves, stems,			
<i>Solanum dulcamara</i> L. <sup>49</sup>	nightshade	fruits			11
<i>Solidago rigida</i> L. <sup>8</sup>	stiff goldenrod	leaves	sl		
<i>Solidago</i> sp. <sup>8</sup>	golden rod	leaves	7		
<i>Spiraea alba</i> Du Roi <sup>44</sup>	meadowsweet	flowers, leaves, stems	15		
<i>Spiraea tomentosa</i> L. <sup>44</sup>	steepleshub	leaves	15		
<i>Spiraea tomentosa</i> L. <sup>44</sup>	steepleshub	flowers, leaves, stems	10		
<i>Spiraea tomentosa</i> L. <sup>44</sup>	steepleshub	flowering aerial organs	13		
<i>Symphytum officinale</i> L. <sup>11</sup>	comfrey	leaves	9		
<i>Tanacetum vulgare</i> L. <sup>8</sup>	tansy	leaves, stems	sl		
<i>Tanacetum vulgare</i> L. <sup>8</sup>	tansy	flower clusters	7		
<i>Taxus</i> sp. <sup>50</sup>	yew	new spring growth	sl		
<i>Tephrosia virginiana</i> (L.) Pers. <sup>23</sup>	goats rue	leaves	11		

Table 1. contd.

<i>Thalictrum dasycarpum</i> Fisch. and Ave'-Lall. <sup>43</sup>	purple meadow rue	leaves, stem	7			
<i>Thalictrum dioicum</i> L. <sup>43</sup>	early meadow rue	leaves, stem	7			
<i>Tilia americana</i> L. <sup>51</sup>	basswood	developing seeds	10			
<i>Tilia americana</i> L. <sup>51</sup>	basswood	flowers clusters, bracts	7			
<i>Tragopogon pratensis</i> L. <sup>8</sup>	oyster plant	seed heads	9			
<i>Trillium grandiflorum</i> (Michx.) Salisb <sup>31</sup>	big white trillium	leaves				sl
<i>Vaccinium angustifolium</i> Aiton <sup>21</sup>	blueberry	leaves, stems	7			
<i>Verbascum thapsus</i> L. <sup>48</sup>	mullein	flowering spike	sl			
<i>Vernonia fasciculata</i> Michx. <sup>8</sup>	common ironweed	leaves	sl			
<i>Veronicastrum virginicum</i> (L.) Farw. <sup>48</sup>	Culver's root	leaves	7			
<i>Vitis aestivalis</i> Michx. <sup>52</sup>	wild grape	leaves	7			
<i>Zizia aurea</i> L. <sup>4</sup>	golden alexanders	seed heads, stems, leaves	15			
<b>Controls</b>						
Aqueous ethanol						
Ticarcillin				27	20	
Chloramphenicol			27			

Sa=Staphylococcus aureus; Ec= Escherichia coli; Pa= Pseudomonas aeruginosa; Ca=Candida albicans; sl=slight Numerical superscripts refer to familial names for each species:

<sup>1</sup>Adiantaceae, <sup>2</sup>Alliaceae, <sup>3</sup>Anacardiaceae, <sup>4</sup>Apiaceae, <sup>5</sup>Aquifoliaceae, <sup>6</sup>Aristolochiaceae, <sup>7</sup>Asclepiadaceae, <sup>8</sup>Asteraceae, <sup>9</sup>Balsaminaceae, <sup>10</sup>Betulaceae, <sup>11</sup>Boraginaceae, <sup>12</sup>Brassicaceae, <sup>13</sup>Capsalpinaceae, <sup>14</sup>Cannabaceae, <sup>15</sup>Caprifoliaceae, <sup>16</sup>Caryophyllaceae, <sup>17</sup>Clusiaceae, <sup>18</sup>Cornaceae, <sup>19</sup>Cyperaceae, <sup>20</sup>Equisetaceae, <sup>21</sup>Ericaceae, <sup>22</sup>Euphorbiaceae, <sup>23</sup>Fabaceae, <sup>24</sup>Fagaceae, <sup>25</sup>Fumariaceae, <sup>26</sup>Geraniaceae, <sup>27</sup>Grossulariaceae, <sup>28</sup>Hippocastanaceae, <sup>29</sup>Juglandaceae, <sup>30</sup>Lamiaceae, <sup>31</sup>Liliaceae, <sup>32</sup>Lythraceae, <sup>33</sup>Mimosaceae, <sup>34</sup>Moraceae, <sup>35</sup>Myricaceae, <sup>36</sup>Myrtaceae, <sup>37</sup>Onagraceae, <sup>38</sup>Papaveraceae, <sup>39</sup>Pinaceae, <sup>40</sup>Poaceae, <sup>41</sup>Polygonaceae, <sup>42</sup>Portulacaceae, <sup>43</sup>Ranunculaceae, <sup>44</sup>Rosaceae, <sup>45</sup>Rutaceae, <sup>46</sup>Salicaceae, <sup>47</sup>Saxifragaceae, <sup>48</sup>Scrophulariaceae, <sup>49</sup>Solanaceae, <sup>50</sup>Taxaceae, <sup>51</sup>Tiliaceae, <sup>52</sup>Vitaceae

associated with butenolols that were found in the buds (Demirci et al., 2000).

### ***Centaurea maculosa***

This study showed *C. maculosa* leaf extracts had inhibition zones of 7 to 10 mm (Table 1). A (+)-catechin enantiomer synthesized by this plant has been shown to possess antibacterial and antifungal activities while a (-)-catechin was phytotoxic (Veluri et al., 2004). Antimicrobial activity has been reported from methanol extracts of *Centaurea aintensis* and *Centaurea erengoides* flowers (Barbour et al., 2004), the essential oils of *Centaurea sessilis* and *Centaurea armena* (Yayli et al., 2005) and the dry heads of *Centaurea diffusa* (Skliar et al., 2005). Secondary metabolites including sesquiterpene lactones were identified in extracts of the aerial parts of *Centaurea deusta* and had antibacterial and antifungal activity (Karioti et al., 2002).

### ***Epilobium angustifolium***

Two separate extracts of *E. angustifolium* L. (fireweed) showed antimicrobial activity against all four microorganisms with inhibitions zones from 6 to 18 mm. Inhibition zones produced by the leaf extract ranged from 7 to 15

mm and extract from the flowering aerial parts were 7 to 20 mm (Table 2). These results agree with those from a previous study in which the antimicrobial activity of air-dried *E. angustifolium* was reported (Rauha et al., 2000). Extracts from fresh aerial parts of five *Epilobium* species including *E. angustifolium* had antimicrobial activity (Battinelli et al., 2001). *E. angustifolium* also showed good inhibitory action against *Klebsiella pneumoniae*, *P. aeruginosa* with an especially high level of activity against *Microsporum canis*; however, no inhibitory effect was recorded for *E. coli* (Battinelli et al., 2001). Methanolic extracts of *Epilobium minutum* inhibited three microorganisms including Gram-negative *K. pneumonia*, *P. aeruginosa*, and methicillin resistant *S. aureus* but had no activity against nine fungal species (McCutcheon et al., 1992; 1994).

### ***Hypericum perforatum***

A number of studies have reported the antimicrobial activity of *Hypericum* species throughout the world, including *H. perforatum* (McCutcheon et al., 1992, 1994; Sakar and Tamer, 1990; Rabanal et al., 2002; Avato et al., 2004; Dall'Agnol et al., 2003, 2005; Barnes et al., 2001). These latter studies prepared extracts from dried aerial plant parts. Our study is the first to test an extract prepared from frozen plant material and also confirms the

**Table 2.** Alphabetical list of species that did not show antimicrobial activity. Generally, aerial portions of the plants were sampled including leaves, flowers, stems and fruits.

<p><i>Acer negundo</i> L.  <i>Achillea millefolium</i> L.  <i>Aconitum carmichealii</i> DeBeaux  <i>Agastache foeniculum</i> (Pursh) Kuntze  <i>Alliaria petiolata</i> (M.Bieb.) Cavara and Grande  <i>Allium stellatum</i> Ker Gawl.  <i>Allium tuberosum</i> Rottl. Ex Spreng  <i>Amaranthus retroflexus</i> L.  <i>Ambrosia trifida</i> L.  <i>Amorpha fruticosa</i> L.  <i>Andromeda glaucophylla</i> Link  <i>Andropogon gerardii</i> Vitman  <i>Andropogon gerardii</i> Vitman  <i>Anemone canadensis</i> L.  <i>Anemone patens</i> L.  <i>Anemone virginiana</i> L.  <i>Angelica</i> sp.  <i>Antennaria parvifolia</i> Nutt.  <i>Apios americana</i> Medik.  <i>Aquilegia canadensis</i> L.  <i>Arctium lappa</i> L.  <i>Artemisia absinthium</i> L.  <i>Artemisia ludoviciana</i> Nutt.  <i>Castilleja coccinea</i> (L.) Spreng  <i>Catalpa speciosa</i> Warder  <i>Celtis occidentalis</i> L.  <i>Centaurea maculosa</i> Lam.  <i>Chelone glabra</i> L.  <i>Chenopodium album</i> L.  <i>Chrysanthemum leucanthemum</i> L.  <i>Cichorium intybus</i> L.  <i>Cimicifuga racemosa</i> (L.) Nutt.  <i>Cirsium arvense</i> (L.) Scop.  <i>Commelina communis</i> L.  <i>Convallaria majalis</i> L.  <i>Cornus alternifolia</i> L.f.  <i>Cornus canadensis</i> L.  <i>Coronilla varia</i> L.  <i>Dalea candida</i> Michx.  <i>Dalea purpurea</i> Vent  <i>Daucus carota</i> L.  <i>Dryopteris filix-mas</i> L.  <i>Echinacea pallida</i> Nutt.  <i>Echinacea purpurea</i> (L.) Moench  <i>Echinocystis lobata</i> (Michx.) Torr. and A. Gray  <i>Elymus canadensis</i> L.  <i>Helianthus laetiflorus</i> Pers.  <i>Helianthus maximilianii</i> Schrad.</p>	<p><i>Artemisia verlotiorum</i> LaMotte  <i>Asclepias incarnata</i> L.  <i>Asclepias syriaca</i> L.  <i>Asclepias variegata</i> L.  <i>Asclepias verticillata</i> L.  <i>Aster ericoides</i> L.  <i>Aster umbellatus</i> Mill  <i>Astragalus canadensis</i> L.  <i>Astragalus mollissimus</i> Torr.  <i>Baptisia lactea</i> (Raf.) Thieret  <i>Barbarea vulgaris</i> R. Br.  <i>Belamcanda chinensis</i> (L.) DC.  <i>Berteroa incana</i> (L.) DC.  <i>Bidens vulgata</i> Greene  <i>Bouteloua curtipendula</i> (Michx.) Torr.  <i>Brassica</i> sp.  <i>Calendula officinalis</i> L.  <i>Calla palustris</i> L.  <i>Caltha palustris</i> L.  <i>Campanula americana</i> L.  <i>Campanula rapunculoides</i> L.  <i>Campanula rotundifolia</i> L.  <i>Carex</i> sp.  <i>Elymus trachycaulus</i> (Link) Gould ex Shinners  <i>Equisetum arvense</i> L.  <i>Equisetum hyemale</i> L.  <i>Erigeron canadensis</i> L.  <i>Erigeron philadelphicus</i> L.  <i>Erigeron strigosus</i> Muhl.  <i>Eryngium yuccifolium</i> Michx.  <i>Eupatorium aromaticum</i> L.  <i>Eupatorium purpureum</i> L. <i>Eupatorium rugosum</i>  Houttuyn. <i>Euphorbia maculata</i> L. <i>Euphorbia</i>  <i>marginata</i> Pursh <i>Euthamia graminifolia</i> (L.) Nutt.  <i>Euthamia tenuifolia</i> (Pursh) Nutt.  <i>Galeopsis tetrahit</i> L.  <i>Galinsoga quadriradiata</i> Ruiz and Pav.  <i>Galium aparine</i> L.  <i>Galium boreale</i> L.  <i>Gaura biennis</i> L.  <i>Gentiana puberulenta</i> J.S. Pringle  <i>Glechoma hederaceae</i> L.  <i>Glycyrrhiza lepidota</i> Pursh  <i>Grindelia squarrosa</i>(Pursh) Dunal  <i>Lithospermum canescens</i> (Michx.) Lehm.  <i>Lobelia siphilitica</i> L.  <i>Lonicera japonica</i> Thunb.  <i>Lonicera oblongifolia</i> (Goldie) Hook</p>
<p><i>Helianthus pauciflorus</i> Nutt.  <i>Helianthus tuberosus</i> L.</p>	

Table 2. contd.

<p><i>Heliopsis helianthoides</i> (L.) Sweet  <i>Heracleum lanatum</i> Michx.  <i>Hieracium aurantiacum</i> L.  <i>Hydrangea</i> sp.  <i>Hydrophyllum virginianum</i> L.  <i>Hylotelephium spectabile</i> (Boreau) H. Ohba  <i>Impatiens pallida</i> Nutt.  <i>Inula helenium</i> L.  <i>Iris</i> sp.  <i>Iris virginica</i> L.  <i>Juniperus communis</i> L.  <i>Kuhnia eupatorioides</i> L.  <i>Lactuca</i> sp.  <i>Lathyrus ochroleucus</i> Hook.  <i>Leonurus cardiaca</i> L.  <i>Leonurus</i> sp.  <i>Lepidium virginicum</i> L.  <i>Liatris aspera</i> Michx.  <i>Lilium michiganense</i> Farw  <i>Linum rigidum</i> Pursh.  <i>Osmunda cinnamomea</i> L.  <i>Osmunda claytoniana</i> L.  <i>Oxalis stricta</i> L.  <i>Panicum</i> sp.  <i>Panicum virgatum</i> L.  <i>Parthenium integrifolium</i> L.  <i>Pastinaca sativa</i> L.  <i>Pedicularis lanceolata</i> Michx.  <i>Pediomelum argophyllum</i> (Pursh) J.W. Grimes  <i>Penstemon grandiflorus</i> Nutt.  <i>Phlox pilosa</i> L.  <i>Phragmites australis</i> (Cav.) Trin. Ex Steud.  <i>Physostegia virginiana</i> (L.) Benth.  <i>Pilea punula</i> Lindl., nom. Conserv.  <i>Plantago major</i> L.  <i>Podophyllum peltatum</i> L.  <i>Polygonatum biflorum</i> (Walter) Elliott  <i>Polygonum amphibium</i> L.  <i>Polygonum aviculare</i> L.  <i>Polygonum cilinode</i> Michx.  <i>Polygonum sagittatum</i> L.  <i>Prenanthes alba</i> L.  <i>Prenanthes racemosa</i> Michx.  <i>Solidago astate is</i> L.  <i>Solidago ptarmicoides</i> (Nees) B. Boivin  <i>Sonchus asper</i> (L.) Hill  <i>Sorghastrum nutans</i> (L.) Nash  <i>Spartina pectinata</i> Link  <i>Stachys palustris</i> L.  <i>Stellaria graminea</i> L.</p>	<p><i>Lonicera prolifera</i> (Kirchn.) Rehder  <i>Lotus corniculatus</i> L.  <i>Lupinus perennis</i> L.  <i>Lycopus americanus</i> L.  <i>Lysimachia astate</i> L.  <i>Lysimachia lanceolata</i> Walter  <i>Malva neglecta</i> Wallr.  <i>Matricaria matricarioides</i> (Less.) Porter  <i>Medicago lupulina</i> L.  <i>Medicago sativa</i> L.  <i>Melilotus alba</i> Medik.  <i>Melilotus officinalis</i> (L.) Pall.  <i>Melothria pendula</i> L.  <i>Mentha arvensis</i> L.  <i>Mentha nypacalx</i>  <i>Mentha</i> sp.  <i>Mimulus ringens</i> L.  <i>Mirabilis nyctaginea</i> (Michx.) MacMill.  <i>Monarda astate i</i> L.  <i>Nepeta cataria</i> L  <i>Pteridium aquilinum</i> (L.) Kuhn  <i>Oenothera biennis</i> L.  <i>Oenothera fruticosa</i> L.  <i>Ranunculus acris</i> L.  <i>Ratibida pinnata</i> (Vent.) Barnhart  <i>Rhamnus cathartica</i> L.  <i>Rubus flagellaris</i> Willd.  <i>Rudbeckia hirta</i> L.  <i>Salix exigua</i> Nutt.  <i>Sambucus astate is</i> L.  <i>Sambucus racemosa</i> L.  <i>Sanicula marilandica</i> L.  <i>Saponaria officinalis</i> L.  <i>Schizachyrium scoparium</i> (Michx.) Nash  <i>Scrophularia lanceolata</i> Pursh  <i>Scutellaria baicalensis</i> Georgi.  <i>Senecio</i> sp  <i>Senna hebecarpa</i> (Fernald) H.S. I and B  <i>Silene vulgaris</i> (Moench) Garcke  <i>Silphium lacinatum</i> L.  <i>Silphium perfoliatum</i> L.  <i>Silphium terebinthinaceum</i> L.  <i>Solanum carolinense</i> L.  <i>Typha latifolia</i> L.  <i>Ulmus</i> sp  <i>Urtica dioica</i> L.  <i>Valeriana officinalis</i> L.  <i>Verbascum thapus</i> L.  <i>Verbena astate</i> L.  <i>Verbena stricta</i> Vent.</p>
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**Table 2.** contd.

<p><i>Stylophorum diphyllum</i> (Michx.) Nutt.  <i>Symphotrichum ciliolatum</i> (Lindl.) Love  <i>Syringa vulgaris</i> L.  <i>Tagetes minuta</i> L.  <i>Taraxacum officinale</i> F.H. Wigg.  <i>Teucrium canadense</i> L.  <i>Thalictrum pubescens</i> Pursh  <i>Thaspium trifoliatum</i> (L.) A. Gray  <i>Thermopsis villosa</i> (Walter) F. and B.G. Schub  <i>Thlaspi arvense</i> L.  <i>Tradescantia virginiana</i> L.  <i>Tragopogon dubius</i> Scop.  <i>Trifolium ambiguum</i> Bieb.  <i>Trifolium arvense</i> L.  <i>Trifolium pratense</i> L.  <i>Trifolium repens</i> L.</p>	<p><i>Verbena urticifolia</i> L.  <i>Vicia cracca</i> L.  <i>Viola canadensis</i> L.  <i>Zanthoxylum americanum</i> Mill.  <i>Zizia aptera</i> (A. Gray) Fernald</p>
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antimicrobial activity of *H. perforatum* (Table 1). The degree of antimicrobial activity seems to be affected by the date of collection. Samples harvested in July of 2003 and 2004 did not display antimicrobial activity, whereas, samples collected in August were active. Flowering aerial parts collected earlier in 2004 inhibited our four test microorganisms. However, the aerial parts including immature seed heads collected later in 2004 only inhibited *S. aureus*. Seasonal variations of phyto-chemical production probably occur in this species. Multiple chemical constituents are reported to contribute to the bioactivity of *Hypericum* spp. Hyperforin, a phloroglucinol present in *H. perforatum* and some other species of *Hypericum*, is the primary component responsible for the antimicrobial activity (Avato et al., 2004; Dall'Agnol et al., 2003, 2005; Barnes et al., 2001). Hypericin has also been identified as the active component responsible for killing avian influenza virus H5N1 *in vitro* (Wang et al., 2006).

#### ***Lythrum salicaria***

Extracts of the flowering spikes and leaves of *L. salicaria* L., (purple loosestrife) showed antimicrobial activity against all four of the test microorganisms (Table 1). This observation is supported by additional reports on the antimicrobial activity of *L. salicaria* extracts against *S. aureus*, *E. coli*, *C. albicans*, *Bacillus cereus*, *Mycobacterium smegmatis* and *Micrococcus luteus* (Rauha et al., 2000; Dulger and Gonuz, 2004). Although *E. coli* and *C. albicans* were included in the study of Dulger and Gonuz (2004), inhibition of these microorganisms was not reported.

#### ***Rhus glabra* and *R. typhina***

Two separate extracts of *Rhus glabra* L. leaves and

green flower clusters exhibited antimicrobial activity (Table 1). Inhibition zones of the leaf extract ranged from 7 to 15 mm and the extracts of the green flower clusters from 10 to 16 mm. Previous studies reported antibacterial and antifungal activity of extracts prepared from air-dried branches of *R. glabra* (McCutcheon et al., 1992, 1994). *R. glabra* branches showed the broadest spectrum of antimicrobial activity inhibiting 11 microorganisms including four Gram-positive and seven Gram-negative (McCutcheon et al., 1992). Antimicrobial activity from dehydrated unripened and ripened fruits of *Rhus coriaria* L. has also been observed (Nasar-Abbas and Kadir Halkman, 2004).

#### **Plant extracts inhibiting three microorganisms**

##### ***Desmodium illinoense***

Although many *Desmodium* species are used in ethnomedicine, this study is the first report of antimicrobial activity from the genus. *Desmodium illinoense* A. Gray (prairie tick trefoil) showed antimicrobial activity against *S. aureus*, *P. aeruginosa* and *C. albicans* (Table 1). Other species in this genus have been used as an anti-inflammatory, anticatarrhal, and anti-nociceptive (Rathi et al., 2004), analgesic and anticonvulsion (N'gouemo et al., 1996), and antileishmanial (Mishra et al., 2005; Singh et al., 2005).

##### ***Scirpus americanus***

This study is the first to report antimicrobial activity of a *Scirpus* species (American bulrush). Extracts of *Scirpus americanus* Pers. inhibited the three microorganisms, *S. aureus*, *E. coli* and *P. aeruginosa* (Table 1). We have uncovered no reports on the use of *Scirpus* sp. as an an-

timicrobial in North American.

### Plant extracts inhibiting two microorganisms

Eleven extracts from 10 plant species inhibited *S. aureus* and *C. albicans* (Table 1). Those having inhibitory activity were extracts from the leaves of *Clintonia* sp., aerial organs of *Comptonia peregrina* (L.) J.M. Coult., the leaves of *C. peregrina*, flowering organs of *Desmanthus illinoensis* (Michx.) MacM. (Illinois bundle flower), leaves of *Epilobium ciliatum* Raf. (American willow herb), immature flower clusters *Eupatorium maculatum* L. (Joe pye weed), immature floral organs of *Geum virginianum* L. (rough avens), leaves of *Juglans nigra* L. (black walnut) leaves and flowers of *Polygonum coccineum* L. *Cotinus coggygia* Scop. (smoke tree) inhibited *S. aureus* and *P. aeruginosa* but had no activity against *C. albicans*.

#### **Clintonia sp.**

This study is the first report of antibacterial activity in a *Clintonia* sp. An extract of *Clintonia* sp. leaves and roots inhibited *S. aureus* and *C. albicans* (Table 1). Fresh leaves of *Clintonia borealis* (Ait.) Raf. (blue bead lily) extracted with ethanol were reported to have antifungal activity against *M. gypseum* and *Trichophyton mentagrophytes* (Jones et al., 2000). Hence, it may not be surprising that we observed inhibitory activity against *C. albicans*.

#### **Comptonia peregrina**

*Comptonia peregrina* (L.) J.M. Coult. (sweet ferns) are aromatic and secrete resin from numerous capitate-stalked glands on the leaves, especially on the lower surface. Our study is the first to report antimicrobial activity from *C. peregrina*. Two separate extracts of *C. peregrina* aerial parts and leaves inhibited *S. aureus* and *C. albicans* with the respective inhibitions zones of 9/15 and 14/12 mm (Table 1). Twenty- seven compounds have been identified in the essential oil of sweet ferns with the following probably accounting for the observed antimicrobial activity of extracts and include cineol, gamma terpinene, and caryophyllene in the highest concentrations (Halim and Collins, 1973; Lawrence and Weaver, 1974).

#### **Cotinus coggygia**

*Cotinus coggygia* Scop. (smoke tree) is a non- native species to the mid-western United States but is commonly grown as a garden shrub. It is closely related to the genus *Rhus* (sumacs). Leaf extracts of *C. coggygia* showed inhibition zones of 13 and 10 mm against *S. aureus* and *P. aeruginosa* (Table 1). In Bulgaria, the leaves are widely used in folk medicine for gastric ulcers, antidiarrhetic, anti-inflammatory, paradontosis (Ivanova et

al., 2005). In some countries extracts are used as anti-septics, antimicrobials, antihemorrhagics and as an aid in wound healing (Demirci et al., 2003; Tzakou et al., 2005). Essential oils of *C. coggygia* leaves, inflorescences and infructescences have been found to be high in monoterpenes such as limonene, myrcene, sabinene and alpha-pinene (Demirci et al., 2003; Tzakou et al., 2005). Medicinal benefits of monoterpenes, especially limonene, are being studied for cancer prevention and treatment while monoterpenols possess antibacterial and antifungal properties (Bowles, 2003).

#### **Desmanthus illinoensis**

*Desmanthus illinoensis* (Michx.) MacMill. (Illinois bundle flower) extracts from flowering aerial parts produced inhibitions zones of 15 and 17 mm against *S. aureus* and *C. albicans* respectively (Table 1). Quercetin, myricitrin and gallic acid esters of myricitrin have been isolated from bundle flower and shown to have antibacterial activity against *Bacillus sphaericus*, *Bacillus thuriengensis*, *Bacillus subtilis* and *Pseudomonas mallophilia* (Nicollier and Thompson, 1983).

#### **Eupatorium maculatum**

The flower clusters of *E. maculatum* L. (Joe pye weed) yielded an extract with slight antimicrobial against *C. albicans* and a 9 mm inhibition zone against *S. aureus* (Table 1). *Eupatorium salvia* Colla from Chile is used as an antiseptic/antimicrobial for infected wounds and insect bites (Urzua et al., 1998) and *Eupatorium glutinosum* from Ecuador and Peru is used as an astringent, antirheumatic, and antimicrobial (El-Seedi et al., 2002). The antimicrobial activity of *E. salvia* has been attributed to the presence of diterpenoids (Urzua et al., 1998). Aerial parts of *Eupatorium aschenbornianum* (Rios et al., 2003) and leaves and twigs of *E. glutinosum* (El-Seedi et al., 2002) had antimicrobial activity that was again mainly attributed to the presence of diterpenoids. Our study is the first to report antimicrobial activity of extracts from flower clusters.

#### **Geum sp.**

Extracts from *Geum virginianum* L. (rough avens) had antimicrobial activity with inhibition zones of 10 mm against *S. aureus* and *Candida albicans* (Table 1). This is the first report on the antimicrobial activity of this species. However, extracts of other *Geum* species have been reported to have antimicrobial activity. These include *Geum macrophyllum* Willd. var. *macrophyllum* (McCutcheon et al., 1994) and *Geum rivale* L. (Panizzi et al., 2000). Extracts of *Geum macrophyllum* roots showed antifungal activity against nine fungal species although five had incomplete inhibition (McCutcheon et al., 1994). Extracts of flowering aerial parts of *G. rivale* L. had anti-

antimicrobial activity against Gram- positive, Gram-nega- tive and mycetous microorganisms. The crude methanol extract had the most antimicrobial activity (Panizzi et al., 2000).

### ***Juglans nigra***

Leaf extracts from *Juglans nigra* L. had antimicrobial activity against *S. aureus* and *C. albicans* (Table 1), supporting similar findings for other *Juglans* species (Omar et al., 2000; Clark et al., 1990; Gruji -vasi et al., 1990; Alkhawajah, 1997; Cha et al., 1998; Lopez et al., 2001; Qa'dan et al., 2005).

As in our study Gruji -vasi et al. (1990) reported antibacterial activity by *J. nigra* against seven microorganismss including the four tested in this study. In contrast, our studies found no activity against either *E. coli* or *P. aeruginosa*. The lack of activity against these two organisms could be associated with the time of collection or the age of tested plants.

One of the compounds believed to contribute to the biological activities of various species of *Juglans* is juglone, a naphthoquinone, which is found in all plant organs in most members of the Juglandaceae (Clark et al., 1990). Juglone (5-hydroxy-1,4-naphthoquinone) was reported to have good activity against the test yeasts and fungi including *C. albicans*, *Saccharomyces cerevisiae*, *Cryptococcus neoformans*, *Aspergillus flavus* and *Aspergillus fumigatus* but having only moderate activity against bacteria indicating that additional phytochemicals contribute to the antimicrobial activity of *Juglans* spp.

### ***Polygonum coccineum***

Extracts from many species of *Polygonum* including knotweed and smartweed, have been found to possess antimicrobial activity (Mackeen et al., 1997; Penna et al., 2001; Kumagai et al., 2005). We found flower extract of *Polygonum coccineum* (swamp smartweed) to have antimicrobial activity against *S. aureus* and *C. albicans* (Table 1). Other studies have reported extracts of the leaves, aerial portions, leaves, rhizomes and whole plants of *Polygonum minus* Huds. (Mackeencre, 1997), *Polygonum punctatum* Elliot (Lopez et al., 2001), *P. punctatum* var. *aquatile* (Martins) [(Penna et al., 2001)] and *Polygonum sachalinense* F. Schmidt ex Maxim (Kumagai, 2005) respectively, to have antimicrobial activity. *P. minus* leaf ethanolic extracts have shown antimicrobial activity against *P. aeruginosa* and had suppressive activity on a human cervical carcinoma cell-line (Mackeen et al., 1997). *P. punctatum* methanol extracts of aerial parts exhibited antiviral and antibacterial activity (Lopez et al., 2001). Dichloromethane extracts of *P. punctatum* var. *aquatile* leaves and rhizomes inhibited five microorganisms where methanol extracts inhibited one microorganism and none were inhibited by ethanol or aqueous extracts (Penna et al., 2001). Extracts of whole

plants of *P. sachalinense* have been reported to show antifungal and antimicrobial activity with special interest in its activity against the fish pathogen *Photobacterium damsela* subsp. *piscicida* (Kumagai et al., 2005).

### **Plant extracts inhibiting a single microorganism**

There were 119 plant extracts, representing 98 species having antimicrobial activity against one microorganism. *S. aureus* was inhibited by 108 extracts and *C. albicans* by 11. Several extracts had large inhibition zones over 15 mm and are noted below.

#### ***Anaphalis margaritacea***

*Anaphalis margaritacea* L. (Benth and Hook) [pearly everlasting] leaf extract inhibited *S. aureus* with an inhibition zone of 18 mm. Chemical investigations of the flowering aerial parts of *A. margaritacea* have identified flavonoids (Wollenweber et al., 1993), and diterpenes and hydroxylactones as active constituents with two diterpenes having antibacterial activity against *B. cereus*, *P. aeruginosa* and *E. coli* (Ahmed et al., 2004).

#### ***Cannabis sativa***

*Cannabis sativa* L. extracts had very good antimicrobial activity against only *S. aureus* with an inhibition zone of 25 mm. This inhibitory zone was nearly equivalent to the controls. Wasim et al. (1995) reported antimicrobial activity from ethanol and petroleum ether extracts against multiple microorganisms. We found antimicrobial activity against only one, a Gram-positive cocci. Essential oils extracted from five fiber varieties of *C. sativa* had antimicrobial activity with the degree of antimicrobial activity varying between cultivars (Novak et al., 2001). The main components of the essential oils reported were alpha-pinene, myrcene, *trans*-beta-ocimene, alpha-terpi-nolene, *trans*-caryophyllene and alpha-humulene. Alpha-terpinolene was the component that varied the most between cultivars (Novak et al., 2001). Cannabidiol (CBD) has also been identified as a component of hemp oil effective against Gram-positive bacteria and yeast (Leizer et al., 2000). A strong correlation exists between the antimicrobial activity and the level of cannabidiolic acid (CBD) found in this species (Leizer et al., 2000). The *C. sativa* chemotypes grown in northern latitudes are reported to have a higher ratio of CBD to -9-tetrahydro-cannabinol (THC) resulting in stronger antimicrobial activity (Leizer et al., 2000).

#### ***Potentilla arguta***

*Potentilla arguta* Pursh leaf extracts had antimicrobial activity against *C. albicans* with an inhibition zone of 16 mm. Other studies have reported antibacterial activity of *P. arguta* as well as antifungal and antiviral properties

(McCutcheon et al., 1992, 1994, 1995). McCutcheon et al, (1992, 1994, 1995) reported that methanol root extracts of *P. arguta* inhibited nine bacterial species, four fungal species and the bovine respiratory syncytial virus, *Paramyxoviridae*.

### ***Smilacina racemosa***

The extract from the mature fruits of *Smilacina racemosa* (L.) Desf. (false Solomon's seal) exhibited a 17 mm inhibition zone against *C. albicans*. Methanolic extracts of the rhizomes of *S. racemosa* were reported to have no antibacterial, antifungal or antiviral activity (McCutcheon et al. 1992, 1994, 1995). Our study is the first to report antimicrobial activity of the berries.

### **Summary**

Our study has identified 142 plant extracts from 109 species that have significant antimicrobial activity. The effectiveness of antimicrobial activity could be viewed as significant based on either the number of microorganisms inhibited or the intensity of antimicrobial action based on the size of the zone of inhibition. Twenty-four percent of all the plants investigated had activity against at least one test microorganism. Various extracts from *B. papyrifera*, *C. maculosa*, *E. angustifolium*, *H. perforatum*, *L. salicaria* and *R. glabra* inhibited the growth of all microorganisms used in this study. Two plant species *D. illinoense* and *S. americanus* inhibited three microorganisms, eleven extracts (10 species) inhibited two microorganisms while 119 extracts (98 species) inhibited at least one microorganism.

Results of these studies indicate that further searches and characterizations of plants for antimicrobial compounds are warranted. In addition, research on synergistic combinations of extracts with broad spectrum or a high degree of inhibition against a particular microorganism would seem worthwhile.

As the search for new antimicrobial agents intensifies, plant extracts may provide attractive alternate sources of molecules for consideration. As drug resistance becomes an increasing problem and as consumer demand for products with natural preservative grows, perhaps it is these molecules that may form the basis of future antimicrobial research efforts.

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