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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) isothiocynates (Delaquis and Mazza, 1995) allicins, anthocyanins (Somaatmadja et al., 1964) and essentials 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, antihemorrhagic, 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., *Caulophyllus* 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 American, 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 includeed *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 Saubouraud 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 microrganism (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[a]			
			Inhibition zones in mm			
Inhibition against four microorganisms			Sa	Ec	Pa	Ca
Betula papyrifera Marshall[10]	paper birch	leaves	9	sl	sl	sl
Centaurea maculosa Lam.[8]	spotted knapweed	leaves	11	10	7	7
Epilobium angustifolium L.[37]	fireweed	leaves	11	7	7	15
Epilobium angustifolium L.[37]	fireweed	flowering aerial organs	17	6	7	20
Hypericum perforatum L.[17]	St. John's Wort	flowering aerial organs	15	6	6	7
Lythrum salicaria L.[32]	purple loosestrife	flowering spikes, leaves	15	10	7	7
Rhus glabra L.[3]	sumac, smooth	green flower clusters	16	10	12	13
Rhus glabra L.[3]	sumac, smooth	leaves	13	7	10	15
Inhibition against three microorganisms						
Desmodium illinoense A. Gray[23]	prairie tick trefoil	flowering aerial organs	sl		sl	sl
Scirpus americanus Pers.[19]	bulrush	leaves	10	sl	sl	
Inhibition against two microorganisms						
Clintonia sp.[31]	Clintonia	leaves, roots	11			11
Comptonia peregrina (L.) J.M. Coult.[35]	sweet fern	aerial organs	9			15
Comptonia peregrina (L.) J.M. Coult.[35]	sweet fern	leaves	14			12
Cotinus coggygria Scop.[3]	smoke tree	leaves	13		10	
Desmanthus illinoensis (Michx.) MacMill.[33]	Illinois bundle flower	leaves, stems, flowers	15			17
Epilobium angustifolium L.[37]	fireweed	flowers	14			17
Epilobium ciliatum Raf.[37]	Amer. willow herb	leaves	11			15
Eupatorium maculatum L.[8]	Joe pye weed	flower clusters	9			sl
Geum virginianum L.[44]	rough avens	immature floral organs	10			10
Juglans nigra L.[29]	black walnut	leaves	15			12
Polygonum coccineum L.[41]	swamp smartweed	flowers	11			7
Inhibition against a single microorganism						
Adiantum pedatum L.[1]	maidenhair fern	leaves	7			
Aesculus glabra Willd.[28]	Ohio buckeye	leaves	8			
Allium ramosum L.[2]	Chinese chives	leaves	7			
Ambrosia artmesifolia L.[8]	ragweed	flowering aerial organs	10			
Amorpha canescens L.[23]	lead plant	leaves	7			
Amorpha canescens L.[23]	lead plant	flowering aerial organs	8			
Anaphalis margaritacea (L.) Benth. and Hook[8]	pearly everlasting	leaves	18			
Anaphalis margaritacea (L.) Benth. and Hook[8]	pearly everlasting	flowering aerial organs	15			
Anaphalis margaritacea (L.) Benth. and Hook[8]	pearly everlasting	flower	10			
Anemone quinquefolia L.[43]	snowdrop	leaves				12
Apocynum andromaesifolium L.[7]	spreading dogbane	leaves stem, stalk	10			
Apocynum androsaemifolium L.[7]	spreading dogbane	leaves	12			
Apocynum cannabium L.[7]	prairie dogbane	leaves	7			
Asarum canadense L.[6]	wild ginger	leaves w/ stems	15			
Asclepias tuberosa L.[7]	butterfly weed	flowering aerial organs	7			

Table 1. contd.

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

Table 1. contd.

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

Table 1. contd.

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

Sa=Staphylococcus aureus; Ec= Escherichia coli; Pa= Sa=Staphylococcus aureus; Ec= Escherichia coli; Pa=Pseudomonas aeruginosa; Ca=Candida albicans; sl=slight Numerical superscripts refer to familial names for each species:
[1]Adiantaceae, [2]Alliaceae, [3]Anacardiaceae, [4]Apiaceae, [5]Aquifoliaceae, [6]Aristolochiaceae, [7]Asclepiadaceae, [8]Asteraceae, [9]Balsaminaceae, [10]Betulaceae, [11]Boraginaceae, [12]Brassicaceae, [13]Caesalpiniaceae, [14]Cannabaceae, [15]Caprifoliaceae,[16]Caryophyllaceae, [17]Clusiaceae, [18]Cornaceae, [19]Cyperaceae, [20]Equisetaceae, [21]Ericaceae, [22]Euphorbiaceae, [23]Fabaceae, [24]Fagaceae,[25]Fumariaceae, [26]Geraniaceae, [27]Grossulariaceae, [28]Hippocastanaceae, [29]Juglandaceae, [30]Lamiaceae, [31]Liliaceae, [32]Lythraceae,[33]Mimosaceae, [34]Moraceae, [35]Myricaceae, [36]Myrtaceae, [37]Onagraceae, [38]Papaveraceae, [39]Pinaceae, [40]Poaceae, [41]Polygonaceae, [42]Portulacaceae, [43]Ranunculaceae, [44]Rosaceae, [45]Rutaceae, [46]Salicaceae, [47]Saxifragaceae, [48]Scrophulariaceae, [49]Solanaceae,[50]Taxaceae, [51]Tiliaceae, [52]Vitaceae

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

Centaurea maculosa

This study showed *C. maculosa* leaf extracts had inhibittion zones of 7 to10 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.

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

Table 2. contd.

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

Table 2. contd.

Stylophorum diphyllum (Michx.) Nutt.	*Verbena urticifolia* L.
Symphyotrichum ciliolatum (Lindl.) Love	*Vicia cracca* L.
Syringa vulgaris L.	*Viola canadensis* L.
Tagetes minuta L.	*Zanthoxylum americanum* Mill.
Taraxacum officinale F.H. Wigg.	*Zizia aptera* (A. Gray) Fernald
Teucrium canadense L.	
Thalictrum pubescens Pursh	
Thaspium trifoliatum (L.) A. Gray	
Thermopsis villosa (Walter) F. and B.G. Schub	
Thlaspi arvense L.	
Tradescantia virginiana L.	
Tragopogon dubius Scop.	
Trifolium ambiguum Bieb.	
Trifolium arvense L.	
Trifolium pratense L.	
Trifolium repens L.	

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 inhibitted *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 phlorglucinol 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 Gonus (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 antimicrobial 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 coggygria* 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 coggygria

Cotinus coggygria 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. coggryia* 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 antiseptics, antimicrobials, antihemorragics and as an aid in wound healing (Demirci et al., 2003; Tzakou et al., 2005). Essential oils of *C. coggygria* 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-negative 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; Grujić-vasić et al., 1990; Alkhawajah, 1997; Cha et al., 1998; Lopez et al., 2001; Qa'dan et al., 2005).

As in our study Grujić-vasić et al. (1990) reported antibacterial activity by *J. nigra* against seven microrganismss 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 microrganism 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 damselae* 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 Δ-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 effecttiveness 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 micro-organism 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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