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Full Length Research Paper

Antioxidant and antimicrobial activity of seed from plants of the Mississippi river basin

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Seeds of native and naturalized plants currently found in the Mississippi River Basin of the United States were evaluated as potential new sources of antimicrobial and antioxidant activity. Methanol extracts of seeds were tested for antioxidant levels using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant assay and for antimicrobial activity using a disk diffusion assay against *Staphylococcus aureus, Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans*. A wide range of antioxidant and antimicrobial activities were observed in the 158 species tested. Antioxidant levels ranged from 2,400 µM Trolox/100 gm (TE) to 261,384 TE. Lythrum salicaria L. (261,384 TE), Lythrum alatum Pursh (206,154 TE), *Spiraea tomentosa L*. (141,430 TE), *Rumex verticillatus L*. (123,423 TE) and *Oenothera biennis L*. (98,563 TE) had the highest levels of antioxidant activity. Extracts of seeds from 35 species had antimicrobial activity. L. salicaria L., Rumex crispus L., Rumex verticillatus L. and Spirea tomentosa L. had high levels of antioxidant activity and correspondingly high levels of antimicrobial activity against all four microorganisms. The correlation between seeds with high antioxidant levels and those with antimicrobial activity was quite low. In this study, we identified native and naturalized plants from the Mississippi River Basin as potential sources of antioxidant and antimicrobial compounds.

Key words: Antibacterial, antimicrobial, antioxidant, medicinal plants, native plants.

INTRODUCTION

A renewed interest has occurred in the last decade to search for phytochemicals of native and naturalized plants for pharmaceutical and nutritional purposes (Ho et al., 1992; Oktay et al., 2003; Wangensteen et al., 2004) with the recognition that plant-derived products have great potential as sources of pharmaceuticals (Cragg et al., 1996). Although leaves, roots, flowers, whole plants, and stems were examined for useful phytochemicals in many research projects, few reports refer to seeds as sources for pharmaceuticals. Yet, a large number of chemical compounds are present in seeds or seed coats, including alkaloids, lectins, and phenolic compounds such as lactones, tannins and flavonoids. These com-pounds probably function in the protection of seeds from microbial degradation until conditions are favorable for germination (Cai et al., 2004; Komutarin et al., 2004). Given the reactivity of these compounds, it is surprising that seeds and their extracts are not frequently utilized in medicinal applications.

Antioxidants are a group of compounds that facilitate survival in plants and may promote the health of humans that consume a variety of plant foods (Connor et al., 2002; Lampart-Szczapa et al., 2003; Mojzisova and Kuchta, 2001; Shahidi, 2000). In plants, the term antioxidant often refers to a wide range of phenolic compounds

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that vary from simple phenolic acids to highly polymerized compounds such as tannins. Phenolic compounds, or polyphenols, are categorized into 15 main classes with over 8,000 identified compounds. The largest category is the flavonoid group, comprising 13 classes with over 5,000 compounds (Fine and Candidate, 2000; Harborne, 1998; Kris-Etherton et al., 2002). In plants, polyphenols are important for structural support, as antiherbivorous substances, for attracting pollinators, for protection from ultraviolet radiation and for wound repair (Harborne, 1998).

The human body also synthesizes endogenous antioxidants such as superoxide dismutases, glutathione peroxidases, alpha-tocopherol and melatonin to counteract cellular damage by active oxygen and free radicals (Manchester et al., 2000; Mojzisova and Kuchta, 2001; Oktay et al., 2003). Many studies suggest that endogenous antioxidants, or exogenous antioxidants supplied by diet, can function as free radical scavengers and improve human health. In this latter regard, consumption of a variety of plant foods may provide additional health benefits (Connor et al., 2002; Mojzisova and Kuchta, 2001; Oktay et al., 2003; Parr and Bolwell, 2000). Antioxidants that retard the oxidation process may additionally exhibit antimicrobial activity (Cutter, 2000; Hao et al., 1998; Puupponen-Pimia et al., 2001).

The antimicrobial compounds found in plants are of interest because antibiotic resistance is becoming a worldwide public health concern especially in terms of food-borne illness and nosocomial infections (Anderson et al., 2001; Hsueh et al., 2005; Lin et al., 2005; Mora et al., 2005; Navon-Venezia et al., 2005; Vattem et al., 2004). Naturally occurring antimicrobials are being sought as replacements for synthetic preservatives such as parabens (ethyl, methyl, butyl and propyl parabens), butylated hydroxytoluene (BHT) and butylated hydroxanisole (BHA) that are under scrutiny as suspected cancer-causing agents (Bergfeld et al., 2005; Byford et al., 2002; Sun et al., 2003; Wangensteen et al., 2004).

Plants produce a multitude of organic compounds that have antimicrobial activity. The compounds are found in various plant parts such as stems, roots, leaves, bark, flowers or fruits and seeds and include alliin/allicins, isothiocyanates, plant pigments (Cutter, 2000), hydrolytic enzymes, proteins, essential oils (Smid and Gorris, 1999) , and phytoalexins or phenolic compounds (Cutter, 2000; Smid and Gorris, 1999).

Our objective was to evaluate the antioxidant and antimi-crobial activity of native and naturalized plant seeds from the Mississippi River Basin.

MATERIALS AND METHODS

Seed sources

One hundred and fifty eight seeds of native and naturalized species were evaluated in antioxidant and antimicrobial assays although not all species were evaluated in both assays. Seed samples tested in the antioxidant assay included 150 species representing 45 families of plants occurring in the Mississippi River Basin (Table 1). A majority of the seed specimens were obtained from the Department of Agronomy and Plant Genetics, University of Minnesota; and from companies specializing in native and naturally occurring plants including Prairie Moon Nursery, 31837 Bur Oak Lane, Winona, MN; Richters Seeds, 357 Highway 47, Goodwood, Ontario, LOC 1A0 Canada; Wind River Seeds 3075 Lane 51, Manderson, WY 82432; Outsidepride.com, Inc.; and Johnny's Selected Seeds, 955 Benton Avenue, Winslow, Maine 04901. Seeds were also collected from plants located in counties bordering the St. Croix River Valley. Seeds of *Comptonia peregrina* (L.) Coult (Myricaceae) were harvested in Sawyer County, WI. Collected seeds were dried at room temperature and cleaned. All seed samples were stored at 10°C until analyzed. Seeds of *Zanthoxylum americanum* P. Mill (Rutaceae) were separated from the capsule with both components tested separately. Due to the diminutive nature. Extracts of the seeds of *Spirea* sp., because of their extremely small size, were obtained from the dried flower clusters.

Antioxidant activity

Seed preparation

Seeds were pulverized using a Thompson Mill and further homogenized in a Wig-L-Bug ball mill (Crescent Dental Mfg. Co.) set at 5000 rpm for 30 s using two stainless steel balls to obtain a fine powder. Ground seeds were stored in Whirl-pak bags at 0°C until analysis. Bulk dill seeds *(A. graveolens* L.) were purchased locally as a plant reference material (PRM) or control. It was processed in the same manner as other seed samples and stored at 0°C. Ground material was weighed in sets of three that ranged from 3 to 100 mg depending on estimated antioxidant capacity. Sample size was determined as the amount that could lower the absorbance of DPPH solution by 50% and the second sample in each set was targeted to be at the 50% reduction point. When high radical scavenging activity was observed in; a) samples of 3 mg or less, or b) when the homogenized seed was oily and would clump upon grinding, the plant material was diluted with 20 micron cellulose powder. Samples having an antioxidant activity of 10,000 TE or greater were diluted. As the antioxidant activity increased dilution factors increased concomintantly resulting in dilutions of 1:5, 1:10, 1:20 and 1:40. Approximately 2/3 of the samples required dilution.

Fruits or organs associated with seeds

Some of the seed samples included investings such as wings, valves and fruits and some had attachments such as awns, bristles and coma. For purchased seeds, all investings and attachments were ground with the seeds. Many of the seeds collected from wild populations were cleaned to remove investings, attachments and fruits. Due to the minute size of the seed in the genus *Spiraea*, (1 mm) the dried flower clusters were used in extract preparation. For *Z. americanum* P. Mill. the follicular capsule and the seed were tested separately.

DPPH radical scavenging assay

The DPPH assay was performed as described (Miller et al., 2000) with some modifications. DPPH (2, 2-diphenyl-1-picrylhydrazyl) was dissolved in methanol and brought to full volume with an equal measure of distilled water. Twenty-five ml of 100 μM DPPH solution

Table 1. Antioxidant values reported in µM Trolox /100 g (TE) from DPPH (2,2-diphenyl- 1-picyrlhydrazyl) radical scavenging activity of crude seed extracts from plants found in the Mississippi River Basin. Table organization is in order of greatest to least radical scavenging activity.

Botanical Name	Family	Common Name	TE
Lythrum salicaria L.[a]	Lythraceae	purple loosestrife	261,384
Lythrum alatum Pursh	Lythraceae	winged loosestrife	206,154
Spiraea tomentosa L.[ad]	Rosaceae	steeplebush	141,430
Rumex verticillatus L. [ac]	Polygonaceae	swamp dock	123,423
Oenothera biennis L.	Onagraceae	common evening primose	98,563
Glyceria grandis S.Wats.	Poaceae	reed manna grass	70,940
Rumex crispus L.[a]	Polygonaceae	curly dock	69,029
Glyceria striata (Lam.) A.S. Hitchc.	Poaceae	fowl manna grass	67,707
Spirea alba Du Roi [a,d]	Rosaceae	meadowsweet	63,985
Impatiens capensis Meerb.	Balsaminaceae	spotted touch me not	62,381
Potentilla arguta Pursh	Rosaceae	prairie cinquefoil	59,635
Amorpha canescens Pursh	Fabaceae	lead plant	58,448
Gnaphalium obtusifolium L.	Asteraceae	sweet everlasting	57,331
Geum triflorum Pursh [b]	Rosaceae	prairie smoke	55,723
Dicentra eximia (Ker-Gawl.) Torr. [a]	Fumariaceae	wild bleeding heart	55,145
Zanthoxylum americanum P. Mill [ae]	Rutaceae	n. prickly ash	47,367
Penstemon pallidus Small	Scrophulariaceae	pale beardtongue	47,320
Dioscorea villosa L.[b]	Dioscoreaceae	wild yam	45,196
Epilobium angustifolium L.	Onagraceae	fireweed	44,606
Gaura biennis L.	Onagraceae	biennial gaura	44,583
Lespedeza capitata Michx	Fabaceae	round headed bush clover	43,945
Psoralea esculenta Pursh	Fabaceae	breadroot	43,182
Hypericum perforatum L.[a]	Clusiaceae	St. John's wort, common	38,592
Verbena hastata rosea	Verbenaceae	pink vervain	38,035
Cephalanthus occidentalis L.[b]	Rubiaceae	button bush	36,620
Tilia americana L.[a]	Tiliaceae	basswood	35,542
Liatris pycnostachya Michx.	Asteraceae	prairie blazing star	34,536
Antennaria plantaginifolia (L.) Richards	Asteraceae	pussytoes	34,450
Tephrosia virginiana (L.) Pers.	Fabaceae	goat rue	31,147
Amorpha fruticosa L.[b]	Fabaceae	false indigo	30,833
Diarrhena americana Beauv.	Poaceae	beak grass	30,830
Physocarpus opulifolius (L.) Maxim[a]	Rosaceae	ninebark	30,311
Rhamnus cathartica L.[a]	Rhamnaceae	common buckthorn	29,999
Glycyrrhiza lepidota Pursh	Fabaceae	wild licorice	29,662
Petalostemum purpureum (Vent.) Rydb.	Fabaceae	purple prairie clover	29,335
Lathyrus ochroleucus Hook.	Fabaceae	pale pea	29,237
Helenium autumnale L.[a]	Asteraceae	sneezeweed	29,087
Aster laevis L.	Asteraceae	smooth blue aster	28,716
Aster drummondii Lindl.	Asteraceae	Drummond's aster	28,259
Iris virginica shreve L.[b]	Iridaceae	southern blue flag	28,082
Desmodium canadense (L.) DC.	Fabaceae	showy tick trefoil	27,924
Taraxacum officinale G.H. Weber ex Wiggers	Asteraceae	dandelion	27,855
Hieracium canadense Michx.[c,a]	Asteraceae	Canada hawkweed	27,854
Artemisia ludoviciana Nutt.	Asteraceae	prairie sage	27,807
Scirpus fluviatilis (Torr.) Gray	Cyperaceae	river bulrush	27,506
Artemisia caudate Michx.	Asteraceae	beach wormwood	27,068
Zanthoxylum americanum P. Mill. [a]	Rutaceae	northern prickly ash	26,751
Hypericum pyramidatum L.	Clusiaceae	great St. John's wort	26,300

Table 1. Contd.

Lepidium virginicum L.[a]	Brassicaceae	peppergrass	25,948
Boltonia asteroides (L.) L'Her.	Asteraceae	false aster	25,789
Lycopus americanus Muhl.ex W. Bart	Lamiaceae	water horehound	25,398
Baptisia tinctoria (L.) R.Br ex Ait f.	Fabaceae	small yellow wild indigo	24,900
Lysimachia quadriflora L.	Primulaceae	prairie loosestrife	24,561
Linum perenne L.	Linaceae	perennial flax seed	24,370
Barbarea vulgaris Ait f.[a]	Brassicaceae	wintercress	23,698
Eupatorium purpureum L.	Asteraceae	sweet joe pyeweed	23,575
Valeriana officinalis L.	Valerianaceae	valerian	22,455
Convolvulus arvensis L.[a]	Convolvulaceae	field bindweed	22,298
Solidago speciosa Nutt.	Asteraceae	showy golden rod	21,658
Verbena hastata L.	Verbenaceae	blue vervain	21,652
Matricaria matricariodes auct. Non (Less.) Porter[a]	Asteraceae	pineapple weed	21,594
Rosa arkansana Porter	Rosaceae	prairie wild rose	21,573
Parthenium integrifolium L.	Asteraceae	wild quinine	21,148
Saliva azurea Michx ex Lam.	Lamiaceae	blue sage	20,613
Baptisia bracteata Muhl ex Ell.	Fabaceae	prairie indigo	20,081
Carex grayi Carey[b]	Cyperaceae	common bur sedge	19,700
Dodecatheon meadia L.	Primulaceae	midland shooting star	18,213
Euphorbia corollata L.	Euphorbiaceae	flowering spurge	18,082
Thalictrum pubescens Pursh [b]	Ranunculaceae	tall meadow rue	17,926
Thalictrum dasycarpum Fisch. & Ave-Lall.	Ranunculaceae	purple meadow rue	17,765
Heuchera richardsonii R. Br.	Saxifragaceae	prairie alum root	17,711
Centaurea maculosa Lam. [a]	Asteraceae	spotted knapweed	17,647
Astragalus crassicarpus Nutt.	Fabaceae	ground plum	17,206
Pedicularis lanceolata Michx.	Scrophulariaceae	marsh betony	17,187
Monarda fistulosa L.	Lamiaceae	wild bergamot	16,506
Rudbeckia hirta L.	Asteraceae	black eyed Susan	15,874
Agastache foeniculum (Pursh) Kuntze	Lamiaceae	anise hyssop	15,627
Hypericum punctatum Lam.	Clusiaceae	dotted St. John's Wort	15,317
Capsella bursa-pastoris (L.) Medik	Brassicaceae	shepherds purse	14,713
Verbascum thapsus L.	Scrophulariaceae	mullein	14,562
Aquilegia canadensis L.[a]	Ranunculaceae	columbine	14,461
Andropogon gerardii Vitman[g]	Poaceae	big blue stem	14,054
Mirabilis nyctaginea (Michx.) MacM.[a]	Nyctaginaceae	four o'clock	13,860
Ratibida columnifera (Pursh) Dunal	Asteraceae	long headed coneflower	13,500
Andropogon scoparius Michx.[g]	Poaceae	little blue stem	13,447
Monarda punctata L.	Lamiaceae	spotted bee balm	13,300
Sporobolus heterolepis (Gray) Gray[b]	Poaceae	northern dropseed	13,300
Baptisia australis (L.) R. Br ex Ait f.	Fabaceae	blue wild indigo	12,997
Rhus typhina L. [a]	Anacardiaceae	sumac, staghorn	12,989
Oxytropis lambertii Pursh	Fabaceae	purple locoweed - ns	12,961
Pinus strobus L.[h]	Pinaceae	white pine pollen (eastern)	12,951
Zizia aurea (L.) W.D.J.Koch[b]	Apiaceae	golden Alexander	12,838
Ceanothus americanus L.	Rhamnaceae	new jersey tea	12,689
Tragopogon dubius (Scop.)[a]	Asteraceae	yellow salsify	12,673
Eryngium yuccifolium Michx.[b]	Apiaceae	rattlesnake master	12,531
Veronicastrum virginicum (L.) Farw.	Scrophulariaceae	Culver's root	12,316
Delphinium virescens Nutt.[b]	Ranunculaceae	prairie larkspur	12,203
Symphoricarpos occidentalis Hook.[g]	Caprifoliaceae	western snowberry	11,929
Urtica dioica L.	Urticaceae	stinging nettle	11,753

Table 1. Contd.

Silphium laciniatum L.[b]	Asteraceae	compass plant	11,450
Bromus kalmii Gray	Poaceae	prairie brome	11,100
Pedicularis canadensis L.	Scrophulariaceae	wood betony	10,964
Scirpus cyperinus (L.) Kunth	Cyperaceae	wool grass	10,922
Eleocharis acicularis (L.) Roemer & J.A. Schultes	Cyperaceae	spikerush	10,852
Sphaeralcea coccinea (Nutt.) Rydb.	Malvaceae	scarlet globemallow	10,498
Astragalus canadensis L.	Fabaceae	Canadian milkvetch	10,347
Sorghastrum nutans (L.) Nash[g]	Poaceae	Indian grass	10,259
Galium boreale L.	Rubiaceae	northern bedstraw	9,458
Plantago purshii Roemer & JA Schultes	Plantaginaceae	woolly plantain	9,352
Comptonia peregrina (L.) Coult.	Myricaceae	sweet fern	9,120
Grindelia squarrosa (Pursh) Dunal	Asteraceae	gumweed	9,059
Onosmodium molle Michx.	Boraginaceae	marbleseed	9,050
Scrophularia lanceolata Pursh	Scrophulariaceae	figwort	9,019
Phalaris arundinaceae L.[g]	Poaceae	reed canary grass	8,885
Desmanthus illinoensis (Michx.) MacM ex B.L. Robins. & Fern.	Fabaceae	Illinois Bundleflower	8,812
Melanthium virginicum L.[b]	Liliaceae	bunch flower	8,715
Elymus virginicus L.[cn]	Poaceae	virginia wildrye	8,660
*Leonurus cardiaca*L.[c]	Lamiaceae	Motherwort	8,591
Cassia fasciculata Michx.	Fabaceae	partridge pea	8,465
Hystrix patula Moenchh	Poaceae	bottlebrush grass	8,249
Scirpus atrovirens Willd.	Cyperaceae	dark green bulrush	8,034
Spartina pectinata Bosc ex Link[g]	Poaceae	cord grass	7,908
Anethum graveolens L.	Apiaceae	dill	7,828
Saponaria officinalis L.	Caryophyllaceae	soapwort	7,495
Bouteloua curtipendula (Michx.) Torr.	Poaceae	sideoats grama	7,441
Phlox maculata L.	Polemoniaceae	wild sweet william	7,237
Panicum lanuginosum Ell., non Bosc ex Spreng.	Poaceae	panic grass	7,045
Agropyron trachycaulum (Link) Malte ex H.F. Lewis [g]	Poaceae	slender wheat grass	6,858
Silene cserei Baumg.[a]	Caryophyllaceae	lesser campion	6,821
Asclepias tuberosa L.[b]	Asclepiadaceae	butterfly weed	6,605
Heracleum maximum Bartr.[b]	Apiaceae	cow parsnip	6,591
Sporobolus asper (Michx.) Kunth [n]	Poaceae	rough drop seed	6,258
Aralia racemosa L.	Araliaceae	spikenard	6,192
Asclepias syriaca L.[b]	Asclepiadaceae	common milkweed	6,115
Thlaspi arvense L.	Brassicaceae	pennycress	6,086
Lychnis alba P. Mill.[a]	Caryophyllaceae	white campion	6,051
Calamagrostis canadensis (Michx.) Beauv.	Poaceae	blue joint grass	5,251
Stellaria media (L.) Vill.	Caryophyllaceae	chickweed	4,915
Allium stelatum Fraser	Liliaceae	prairie onion	4,897
Veronica officinalis L.	Scrophulariaceae	speedwell	4,506
Panicum virgatum L.[g]	Poaceae	switchgrass	4,313
Sporobolus cryptandrus (Torr.) Gray	Poaceae	sand dropseed	3,817
Tripsacum dactyloides (L.) L.[b]	Poaceae	eastern gamma grass	3,786
Buchloe dactyloides (Nutt.) Engelm.[f]	Poaceae	buffalo grass	3,694
Smilacina racemosa (L.) Desf.	Liliaceae	Solomon's plume	3,421
Lupinus perennis L.	Fabaceae	wild lupine	3,280
Stipa spartea Trin.[b]	Poaceae	porcupine grass	2,961
Tradescantia bracteata Small ex Britt.	Commelinaceae	prairie spiderwort	2,767
Juncus effusus L.	Juncaceae	common rush	2,380
Polygonatum canaliculatum (Muhl.) Pursh	Liliaceae	Solomon's seal	2,345

[a]wild collection, [b]seed with fruit,[c] two seed sources averaged,[d] flower spikes w/ seeds,[e] fruit only,[f] treated seed

[g]seed purity ranges from 68.82%-99.9%: Respective seed purity %: 90.47, 83.88, 80.19, 89.04, 90.92, 87.57, 76.74, 93.76, 99.9, 87.69, 68.82, 98.09, [h] pollen.

was added to the ground seed samples in 40 ml glass tubes with Teflon-lined screw cap tubes. Samples were incubated at 35°C on a shaker bed at 60 strokes/min. for four hours. The samples were filtered into glass culture tubes, decanted into cuvettes and the absorption read at 517 nm in a Milton Roy Spectronic 21D spectrophotometer calibrated with a 50/50 methanol/distilled water blank. Absorption values were taken simultaneously on DPPH blank solution at zero time and after four hours. The readings for

each sample were plotted on a standard curve from the reaction of Trolox with DPPH and the data then converted to antioxidant activity reported as Trolox Equivalents (moles Trolox/100 gm) or TE. All samples were run in duplicate, 29 samples were run in triplicate, 6 samples had four replications, 5 samples had five replications and 1 sample had seven replications. The difference in replications from one sample to another was due to the need to estimate the appropriate sample weight to achieve the 50% reduction point. Samples of the seeds of dill, the plant reference material, were run with each batch. Values were reported as the means of total runs.

Antioxidants in plant material may be water soluble, fat soluble, insoluble or bound to cell walls, and react at different rates. Therefore, the radical scavenging end-point of 50% reduction (SC_{50}) permitted a measurable point within a reasonable amount of time. This DPPH method has the advantage that the entire sample is able to react with the free radical and the four-hour mixing period allows time for reaction with weak antioxidants (Prakash et al., 2001). Methanol was chosen as the organic solvent for its wide solubility properties for low molecular weight and moderately polar substances, including phenolic compounds (Kuo et al., 2002; Miller et al., 2000; Prakash et al., 2001).

Antimicrobial activity

Extracts from seeds were tested for antimicrobial activity against four microorganisms, Gram-positive *Staphyloccocus aureus* (ATCC 12600), Gram-negative *Escherichia coli* (ATCC 8677) and *Pseudomonas aeruginosa* (ATCC 9721), and the yeast *Candida albicans* (ATCC 10231), using the disk diffusion assay technique (Bauer et al., 1966).

Extract preparation

Seeds were pulverized with a Thompson Mill 24 h before the assay was begun and stored overnight at 10ºC. On the day of testing, 5 ml of 50% aqueous methanol prepared with double-distilled sterile water was added to 2 gm of plant material. Seed extracts displayed a wide range of absorption characteristics thus the ground seeds were allowed to undergo a swelling period for one hour at room temperature. After the swelling period, the ground material was subjected to a vortex mixer for a visual determination of flowability. To maximize the concentration of the extract, the amount of the 50% aqueous methanol was minimized to the minimal amount necessary to allow the solution to be flowable in the tube. The vials containing the ground seed material/solvent mixture were placed on the shaker bed and mixed at 60 strokes/min for 18 h at room temperature. The samples were centrifuged to obtain 2 ml of supernatant. The supernatant was transferred into sterile microfuge tubes, re-centrifuged and 50 l of extract applied to 6 mm sterile disks. Negative control discs contained 50 l extraction fluid only. The discs were allowed to stand at room temperature for a minimum of one hour or until control discs were dry.

Kirby-Bauer disk diffusion susceptibility testing

The test organisms *S. aureus, P. aeruginosa, E. coli and C. albicans* were grown at 37ºC on blood agar, MacConkey agar or Sabouraud dextrose agar, respectively. After 18 to 20 h incubation, each microorganism was diluted in sterile double distilled water to an approximate optical density of 0.5 using a MacFarland standard (Becton Dickinson Microbiology Systems, 7 Loveton Circle, Sparks, MD 21152). Mueller-Hinton agar plates were swabbed on three axes with a sterile swab dipped in the freshly prepared diluted culture. When negative control disks were dry, all disks with extracts were transferred to Mueller Hinton plates. The disks were placed on the freshly swabbed plates along with two positive controls consisting of the plant reference material (PRM) that consisted of an extract of the berries of *Rhus typhina* L. (staghorn sumac)] and an antimicrobial compound. The antibiotic Ticarcillin 75 mcg was used as the positive control for the bacteria. The antifungal essential oil blend, “RC”, from YoungLiving was used as the positive control for the yeast. The anti-yeast activity of the essential oil blend “RC” was evaluated with three other blends, “Melrose”, “Purification” and “Thieves”. “RC” was chosen as the control due to its smaller inhibition zones and thus avoided interference with other inhibition zones. Each extract was run in triplicate. The plates were inverted and incubated at 37° C for 18 h. The diameters of inhibition zones were measured with a ruler on three axes and averaged. Those extracts exhibiting partial zones of inhibition were also measured and recorded. Partial inhibition was defined as zones of clearing with the presence of one or more colonies within the zone of inhibition. Zone of inhibition measurements were reported as an average of the replicates showing antimicrobial activity.

Inhibition zones of 8 to 10 mm (using 6 mm disks) are considered to be significant when testing plant extracts for antimicrobial activity (McCutcheon et al., 1992; Omar et al., 2000; Tepe et al., 2005).

Data analysis

Excel statistical software was used to compare the level of antioxidant activity (TE) to antimicrobial activity (inhibition zones in mm). A regression line was generated and the R^2 calculated (Figure 1).

RESULTS AND DISCUSSION

Antioxidant activity of seed extracts

The antioxidant activity measured in μM Trolox Equivalents /100 g (TE) ranged from 2,345 TE for *Polygonatum canaliculatum* (Muhl) Pursh (Solomon’s seal) to 261,500 TE in *Lythrum salicaria* L. (purple loosestrife); a more than a 100-fold difference. It is difficult to compare antioxidant values between different methods of analysis, thus antioxidant levels were categorized into seven ranges based on this data.

Eighty-six percent of the samples had antioxidant activity from 2,300 to 40,000 TE (Table 1). Antioxidant levels above 40,000 TE are considered high levels of radical scavenging activity. Ranges of activity were divided into 7 categories:

1) 43 samples (29%) from 2,300 to 10,000 TE,
2) 42 samples (28%) from 10,001 to 20,000 TE
3) 43 samples (29%) from 20,000 to 40,000 TE
4) 12 samples (8%) from 40,001 to 60,000 TE
5) 5 samples (3%) from 60,001 to 80,000 TE
6) 2 samples (1%) from 80,001 to 130,000 TE
7) 3 samples (2%) from 130,001 to 270,000 TE

A wide range of antioxidant activity in plant material is not uncommon (Cai, 2004). Direct comparison of antioxidant values among studies, however, is difficult due to the variation in analytical methods. In a study evaluating the

antioxidant activity of 112 Chinese medicinal plants using the ABTS method, the samples had a 400-fold range in total antioxidant activity from 44 to 17,674 μM Trolox/100 g DW. A relatively large number of species (44%) were also in the lower range of 100.1 to 500.0 μM /100 g/DW (Cai, 2004).

Antioxidant activity of seeds vs. common fruits and vegetables

Miller et al. (2000) using the DPPH method, found that the antioxidant activity of 20 common fresh vegetables and fruits ranged from 50 to1400 TE/100 g. In this study, common vegetables tested included red cabbage at 1400, garlic at 1,300, broccoli at 600, spinach at 500, carrots and tomatoes at 200 TE/100g. Twenty fresh fruits were also tested and ranged from 100 to 5,500 TE/100 g and included blackberries at 5,500, blueberries at 3,300, red grape at 1,700, orange at 600, and watermelon at 100 TE/100g. The antioxidant activity of native plant seeds far exceeded the greatest antioxidant value of fresh fruits and vegetables reported by Miller et al. (2000), but these noted differences are related to the percent of dry matter present in the plant material. Since seeds typically have a lower moisture content then fresh produce, higher antioxidant levels might be anticipated.

Wang, et al. (1996) and Cao, et al. (1996) also observed that the antioxidant activity of samples containing larger amounts of dry biomass were higher than fresh samples with the degree of increased activity apparently directly related to the percentage of dry biomass. As a concentrated source of antioxidants, dry seeds from genera such as *Lythrum*, *Rumex*, *Spiraea* and *Oenothera*, may provide an excellent source of antioxidants in constrast to fresh vegetables or fruits. In addition, certain plant families such as the Lythraceae, Polygonaceae, Rosaceae and Onagraceae appear to contain species with higher antioxidant levels warranting further investigation of species within specific plant families (Table 1).

Antimicrobial activity in seeds

Of the 146 seed extracts tested for antimicrobial activity, 35 showed some degree of activity against one or more of the four microorganisms tested (Table 2). All antimicrobial activity was recorded including those plants exhibiting partial activity. The number of seed extracts that inhibited the growth of a specific microbe is as follows: *S. aureus* was inhibited by 29 seed extracts; *C. albicans* by 12 seed extracts; *P. aeruginosa* by 7 seed extracts and *E. coli* by 5 seed extracts. *L. salicaria* L., *Rumex crispus* L., *Spirea tomentosa* L. and *Rumex verticillatus* L. in addition to the plant reference material (*Rhus typina* L.) inhibited the growth of the four tested microorganisms. *L. alatum* Pursh inhibited three microorganism, namely *S. aureus, P. aeruginosa, and C. albicans. P. maculate* L. inhibited only *E. coli* and *P. aeruginosa. Scirpus fluviatilis* (Torr.) *Gray, Verbena hastata* L.*, Zanthoxylum americanum* P. Mill*., and Lepe-dium virginicum* L. inhibited *S. aureus* and *C. albicans*. Twenty-three seed extracts inhibited a single microbe with 20 inhibiting *S. aureus*, three extracts inhibiting C*. albicans* and one inhibiting *P. aeruginosa* (Table 2).

Plant families

Species from 21 of the 45 plant families in this study exhibited some antimicrobial activity (Table 2). Plant families with the largest number of species represented were Asteraceae (21), Fabaceae (23) and Poaceae (20). The percent of species within these families that exhibited antimicrobial activity was 5, 21 and 10%, respectively. Families such as Lythraceae, Onagraceae, Polygonaceae, Primulaceae and Verbenaceae were represented by two or three species with all species having antimicrobial activity. Three of the six species from Rosaceae had antimicrobial activity.

Seeds of species exhibiting high antimicrobial activity

***Lythrum salicaria* L**.

Seed extracts of *Lythrum salicaria* had inhibition zones of 8, 11, 17 and 22 mm against *E. coli*, *P. aeruginosa*, *S. aureus* and *C. albicans* , respectively. Previously reported antimicrobial activity of *L. salicaria* had zones of inhibition (clear, 4 - 10 mm) against *E. coli*, (moderate, 3 - 4 mm) against *C. albicans* and (slight, 1 - 3 mm) against *S. aureus* (Rauha et al., 2000). Dulger and Gonus (2004) reported that leaves of *L. salicaria* produced inhibition zones of 12, 10, 10, and 8 mm against *S. aureus*, *Bacillus cereus* , *Mycobacterium smegmatis* and *Micro-coccus luteus*, respectively. Although *E. coli* and *C. albicans* were included in the study of Dulger and Gonus (2004), antimicrobial activity was not observed. Two studies have characterized the antimicrobial constituents of aerial organs of *L. salicaria* (Becker, 2005; Rauha et al., 2001). Rauha et al. (2001) summarized previously identified polyphenols including 15 tannins, 6 flavonoids, 8 phenolics, 7 phthalates, the sterol beta-sitosterol and the terpene loliolide. Becker et al. (2005) collected flowering aerial parts of L. salicaria and identified two antifungal triterpenoids, oleanolic and ursolic acid, and the antibacterial compound hexahydroxydiphenoyl ester (vescalagin). Flavonoids (flavon-c -glucosides) identified included vitexin, isovitexin, orientin and isoorientin but did not exhibit antibacterial activity (Becker et al., 2005). Flavonoids, the largest group of phenolic compounds thus far identified, are now widely considered to promote human health. However, this promotion may not be antimicrobial in nature.

Table 2. Antimicrobial and antioxidant activity of crude seed extracts from plants found in the Mississippi River Basin.

				Microorganism inhibition zones (mm) [abc]				Anti-oxidant level[d]
Plant Family	**Botanical name**	**Common name**	**Notes[e]**	***S. aureus***	***E. coli***	***P. aeruginosa***	***C. albicans***	**TE/100 gm**
Active against four microorganism								
Anacardiaceae	*Rhus typhina* L.[f]	staghorn sumac	WC/SI	17	10	14	17***	12,989
Lythraceae	*Lythrum salicaria* L.	purple loosestrife	WC	17	8	11	22	261,384
Polygonaceae	*Rumex crispus* L.	curly dock	WC	13	7	10	19	69,029
	Rumex verticillatus L.	swamp dock	WC	14	9*	10	16	123,423
Rosaceae	*Spiraea tomentosa* L.	steeplebush	Spikes WC	13	8***	7***	9***	141,430
Active against three microorganisms								
Lythraceae	*Lythrum alatum* Pursh	winged loosestrife		14		7	22	206,154
Active against two microorganisms								
Brassicaceae	*Lepedium virginicum* L.	peppergrass	WC	10***			12	25,948
Cyperaceae	*Scirpus fluviatilis* (Torr.) Gray	river bulrush	SI	7			14	8,034
Polemoniaceae	*Phlox maculata* L.	wild sweet william			14**	19**		7,237
Rutaceae	*Zanthoxylum americanum* P. Mill.	northern prickly ash	capsule WC	9***			11***	47,367
Verbenaceae	*Verbena hastata* L.	blue vervain		9			14^	21,652
Active against one microorganism								
Asteraceae	*Helenium autumnale* L.	Sneezeweed	WC	20				29,087
Convolvulaceae	*Convolvulus arvensis* L.	field bindweed	WC	8				22,298
Cyperaceae	*Carex grayi* Carey	common bur sedge	SI	10				19,700
Fabaceae	*Amorpha fruticosa* L.	false indigo	SI	17				30,833
Fabaceae	*Baptisia australis* (L.) R. Br ex Ait f.	blue wild indigo		9***				12,997
Fabaceae	*Baptisia bracteata* Muhl ex Ell.	prairie indigo		10***				20,081
Fabaceae	*Cassia fasciculata* Michx.	partridge pea		7				8,465
Fabaceae	*Psoralea esculenta* Pursh	breadroot				7***		43,182
Fumariaceae	*Dicentra exemia* (Ker-Gawl.)Torr.	wild bleeding heart	WC	9				55,145

Table 1. Contd.

Iridaceae	*Iris virginica shreve* L.	southern blue flag	SI	8				28,082
Liliaceae	*Melanthium virginicum* L.	bunch flower	SI				19	8,715
Onagraceae	*Epilobium angustifolium* L.	fireweed		7				44,606
Onagraceae	*Gaura biennis* L.	biennial gaura		9				44,583
Onagraceae	*Oenothera biennis* L.	common evening primose		11				98,563
Poaceae	*Diarrhena Americana* Beauv.	beak grass		9				30,830
Poaceae	*Glyceria grandis* S. Wats.	reed manna grass		11				70,940
Primulaceae	*Lysimachia quadriflora* Sims	prairie loosestrife		7				24,561
Primulaceae	*Dodecatheon meadia* L.	midland shooting star					10	18,213
Rhamnaceae	*Rhamnus cathartica* L.	common buckthorn	WC	12				29,999
Rosaceae	*Physocarpus opulifolius* (L.) Maxim	ninebark	WC	8				30,311
Rosaceae	*Potentilla arguta* Pursh	prairie cinquefoil					17	59,635
Tiliaceae	*Tilia americana* L.	basswood	WC/SI	7**				35,542
Verbenaceae	*Verbena hastata rosea*	pink vervain		9				38,035
Controls								
Ticarcillin				47	32	25		
RC							15	
Aqueous MeOH								

[a]Results reported as mean (n = 3) of inhibition zone including 6 mm disk with exception of *Verbena hastata* (n = 2). [b]*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*. [c]PI = partial inhibition: *PI on one plate, full activity on other 2; ** PI on two plates, full activity on third; *** PI on three plates. , ^ full activity on 2 plates, none on the third. [d]Notes: WC, wild collection; SI, with seed and fruit or associated organs; spikes with seed; capsule without seed. [e]TE/100g = moles Trolox per 100 gm seed material for *R. typhina (hirta)* antioxidant value represents seed without pericarp

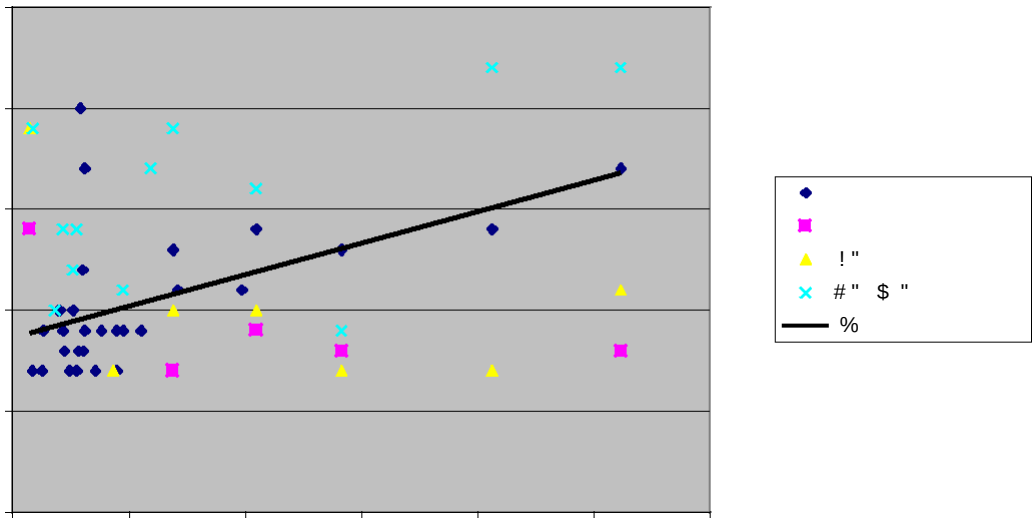

Figure 1. The correlation between antioxidant level (TE) versus antimicrobial activity (inhibition zone in mm) of the 35 active seed extracts. R^2 values: *Staphylococcus aureus*, 0.21; *Escherichia coli*, 0.34; *Pseudomonas aeruginosa*, 0.24; *Candida albicans*, 0.24.

***Rumex crispus* L. and *Rumex verticillatus* L.**

Both *R. crispus* and *R. verticillatus* showed antimicrobial activity against the four tested microorganisms with inhibition zones of 13/14, 7/9, 10/10 and 19/16, respectively, against *S. aureus*, *E. coli*, *P. aeruginosa*, and *C. albicans*, respectively (Table 2). Schnitzler et al. (1996) reported that plant material from *R. crispus* was a promising treatment against the parasitic protozoa *Leishmania mexicana*, Yildirim et al. (2001) reported that ether extracts of *R. crispus* leaves had antimicrobial activity against *S. aureus* and *Bacillus subtilis* (10 and 8 mm inhibition zones, respectively). The ether extracts of the seeds exhibited activity only against *S. aureus* (11 mm) even though cultures of *E. coli*, *P. aeruginosa* and *C. albicans* were included in the study.

R. crispus has also been shown to have antifungal activity. Kim et al. (2004) found root extracts of *R. crispus* to be 100% effective against the barley powdery mildew organism, *Erysiphe graminis sp horde,* and 90% effective against *Phytopthora infestans*, the causative organism of tomato late blight.

***Rhus typhina* L.**

Berries of *R. typhina* were used as the plant reference material (control) and showed clear inhibition zones of 17, 10, and 14 mm against *S. aureus*, *E. coli*, *P. aerugi-nosa*, respectively; and a partial inhibition zone of 17 mm against *C. albicans*. The drupes of *R. typhina* minus the pericarp were included in the antimicrobial assay but showed no activity. The extract including the pericarp showed activity against the four test organisms. This result indicates that the antimicrobial activity of some seeds is attributed to the phytochemicals contained in the fruits and hairs surrounding the seeds.

We found no references documenting the antimicrobial activity of *R. typhina*, however, some references note antimicrobial activity in a related species, *R. glabra* L. (smooth sumac). In a British Colombia ethnobotanical study, extracts of the branches of *R. glabra* restricted growth of eleven microorganisms including *E. coli* (inhibition zone > 25 mm), two strains of *P. aeruginosa* (inhibition zone >25/10.1- 15 mm), and two strains of methicillin resistant *S. aureus* (inhibition zone >25 mm/15.1-20 mm) with a inhibition zone > 8 mm considered as significant antimicrobial activity.

Isolated antibacterial compounds from *R. glabra* branches that were initially extracted with methanol and fractionated with $CHCl_3$ were identified as 3,4,5-trihydroxybenzoic acid (gallic acid), methyl gallate (methyl ester of gallic acid) and 4-methoxy-3,5-dihydroxybenzoic acid (Saxena et al., 1994) . The methylated derivatives of gallic acid were found to be 2.5 to 80-fold more active against *E. coli,* P. *aeruginosa* and *S. aureus* although the gallic acid antibacterial activity was weak (Saxena et al., 1994). Gallic acid and gallotannin were identified in the leaves of *R. typhina* (Frohlich et al., 2002; Werner et al.,

2004).

Spiraea tomentosa L.

S. tomentosa inhibited the four tested microorganisms although *S. alba* inhibited none. Antimicrobial activities of *S. tomentosa* have not been previously described.

Relationships between antioxidant and antimicrobial activity

We attempted to evaluate the relationship between seed antioxidant and antimicrobial activities because antioxidants are known to exhibit antimicrobial activity (Cutter, 2000; Hao et al., 1998; Puupponen-Pimia et al., 2001). Figure 1 compares the antioxidant level (TE) to the antimicrobial activity (inhibitions zones in mm) of the 35 active crude seed extracts against the four tested microorganisms. Antioxidant activity, with an R^2 of 0.29, explained only 29% of the variation in antimicrobial activity (Figure 1). Correlation coefficients between antioxidant and antimicrobial activity for individual microorganisms were: *S. aureus* (0.21), *E. coli* (0.34), *P. aeruginosa* (0.24) and *C. albicans* (0.24). Of the seed extracts effective against Gram-positive *S. aureus* , 62% (18 of 29) had antioxidant levels below 40,000 TE, with the remainder ranging from 44,606 to 261,384 TE. Conversely, greater antimicrobial activity against the two Gram-negative bacteria, *E. coli* at 80% (4 of 5), and *P. aeruginosa,* 86% (6 of 7) was observed in plant species with antioxidant levels above 40,000 TE. Of the seed extracts effective against *C. albicans*, 50% had antioxidant activity above 40,000 TE and 50% had less than 40,000 TE (6 of 12).

The five species that inhibited growth of all four microbes had antioxidant activity above 69,000 TE with the exception of *R. typhina*. However, the antioxidant level for *R. typhina* reflects the cleaned seed without the pericarp, which appears to be the portion of the fruit with the antimicrobial activity. Although high antioxidant activity did not guarantee antimicrobial activity against all four organisms, all species above 69,000 TE such as *Glyceria grandis* S. Wats., *Oenothera biennis* L. and *Lythrum alatum* Pursh did have some antimicrobial activity. While there appeared to be less antimicrobial activity in species with lower antioxidant levels, species such as *Amorpha fruticosa* L. (30,833 TE), *Helenium autumnale* L. (29,087 TE), *Melanthium virginicum* L. (8,715 TE) and *Phlox maculata* L. (7,237 TE) produced large inhibition zones of 17, 20, 19, and 19 mm, respectively, against one or two microorganisms.

Seeds, protective bracts, associated organs, and fruits

Nine of the 35 active seed extracts included bracts, protective hairs, or the entire pericarp. For *Zanthoxylum americanum* P. Mill*.,* the extract of the follicular capsule showed antimicrobial activity whereas the extract of the seed did not. These organs associated with seeds must not be overlooked when screeing for antimicrobials or antioxidants. Plants that are traditionally used by Thai herbalists for antimicrobial activity against enterohaemorrhagic *E. coli* O157:H7 reported that the fruits of *Punica granatum* L. (Punicaceae) and *Quercus infectoria* Oliv. (Fagaceae) were found to significantly inhibit growth of several strains of *E. coli* (Voravuthikunchai et al., 2004). Sesame seed coats (*Sesamum indicum* L.) have also been studied for their preservative qualities by inhibiting lipid peroxidation (Chang et al., 2002). Future studies of native plants in the Mississippi River Basin may want to test seed and associated organs separately to identify active plant materials.

Summary

We have quantified seed extracts of native and naturalized plants of the upper Midwestern United States that have a wide range of antioxidant and antimicrobial activity. Radical scavenging or antioxidant activity ranged from 2,400 to 261,284 TE. These values far exceed the antioxidant activity of highly touted “healthy” high antioxidant foods such as blackberries (5,500 TE) and blueberries (3,300 TE; Miller et al., 2000). Of the 146 seed extracts tested for antimicrobial activity, 34 inhibited microbial growth with five seed samples inhibiting all four microorganisms with large inhibition zones up to 22 mm. The screening of plants with high antioxidant values may not identify species with effective antimicrobial activity because the two parameters are not highly correlated. High antioxidant levels could not predict antimicrobial activity against the tested microorganisms. Examples are *O. biennis* L. and *Glyceria grandis* S. Wats. That showed antioxidant activity above 69,000 TE but showed activity against only a single microbe (Table 2). Conversely, some plant species with antioxidant levels as low as 7000 to 8000 TE, exhibited large inhibition antimicrobial inhibition zones up to 19 mm, but also against a single microorganism (Table 2).

Plant antioxidant and antimicrobial activity may vary based on time of harvest, storage temperatures and extraction methods.

Seasonal changes, environmental factors and stage of plant development effect the produc-tion and distribution of bioactive constituents in the plant. Other factors affecting capture of active phyto-chemicas are the plant organ being extracted, the types of solvents, the extraction period and extraction conditions.

Even with these challenges, screening native plants is useful for revealing antimicrobial and antioxidant activity that may lead to the development of new products for use as nutritional (antioxidants) and pharmaceutical agents.

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