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Screening Native Botanicals for Bioactivity: An Interdisciplinary Approach

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Abstract

Objective—Plant-based therapies have been used in medicine throughout recorded history. Information about the therapeutic properties of plants can often be found in local cultures as folk medicine is communicated from one generation to the next. Our objective is to identify native Louisiana plants from Creole folk medicine as a potential source of therapeutic compounds for the treatment of insulin resistance, type 2 diabetes and related disorders.

Materials and Methods—We used an interdisciplinary approach combining expertise in disciplines ranging from cultural anthropology and botany to biochemistry and endocrinology to screen native southwest Louisiana plants. Translation of accounts of Creole folk medicine yielded a list of plants with documented use in treating a variety of conditions, including inflammation. These plants were collected, vouchered and catalogued prior to extraction of the soluble components. The extracts were analyzed for bioactivity in regulating inflammatory responses in macrophages or fatty-acid induced insulin resistance in C2C12 skeletal muscle cells.

Results—Several extracts altered gene expression of inflammatory markers in macrophages. Multiplex analysis of kinase activation in insulin signaling pathways in skeletal muscle also

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Author Contributions:

AB, CR designed and conducted the experiments and evaluated data related to insulin signaling in skeletal muscle; DMC designed, processed, and evaluated anti-inflammatory data and contributed to manuscript writing; AB contributed to manuscript writing and editing; DR developed botanical extraction method and participated in data interpretation; LA acquired, catalogued and vouchered the plant material; CRB translated the Creole dialect in the 1933 Bienvenu thesis; DPT contributed to collection of plant specimens; WTC participated in overall study screening conception and determining insulin signaling targets in skeletal muscle and data interpretation; ZEF participated in data interpretation related to insulin signaling in skeletal muscle and wrote the manuscript.

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identified a subset of extracts that alter insulin-stimulated AKT phosphorylation in the presence of fatty acid-induced insulin resistance.

Conclusion—An interdisciplinary approach to screening botanical sources of therapeutic agents can be successfully applied to identify native plants used in folk medicine as potential sources of therapeutic agents in treating insulin resistance in skeletal muscle or inflammatory processes associated with obesity-related insulin resistance.

Keywords

metabolic syndrome; inflammation; obesity; ethnobotany

Introduction

The prevalence of metabolic syndrome, characterized by abdominal obesity, dyslipidemia, hypertension, insulin resistance and chronic inflammation, contributes greatly to development of Type 2 diabetes and remains as a significant public health concern [1-3]. Besides lifestyle management as the cornerstone of management, a suggested treatment for metabolic syndrome and the first suggested treatment for type 2 diabetes is metformin [4], a synthetic compound based on the hypoglycemic properties of galegine (isoamylene guanidine) found in *Galega officinalis*, a plant commonly known as French lilac or goat's rue [5]. Extracts from French lilac were used in folk medicine as early as the middle ages, and accounts of using French lilac extracts for treating symptoms typical of type 2 diabetes appeared in the 17th century [6]. Thus, the efficacy of metformin in treating hyperglycemia points to the value of botanicals as an important source of therapeutic compounds, even in an era of molecular medicine.

Plants from the genus *Artemisia* have also traditionally been used to treat diabetes, including the perennial herb *Artemisia dracunculus* or Russian tarragon [7]. Several recent studies demonstrate that an ethanolic extract of *A. dracunculus* lowers blood glucose and enhances insulin signaling in skeletal muscle in murine models of diabetes and in human skeletal muscle cells *in vitro* [8-11]. These studies support the idea that characterizing botanical sources of therapeutic compounds continues to be a viable approach to developing novel treatments for insulin resistance, type 2 diabetes and other chronic diseases. However, identifying new plant sources of botanical material requires cultural knowledge of traditional uses of medicinal plants as well as adherence to scientific nomenclature, plant classification and accepted standards of plant vouchering prior to characterization of plant preparations. We assembled a team of individuals with expertise in cultural anthropology, botany, ecology, biochemistry, and endocrinology to identify native medicinal plants from southwestern Louisiana with properties having potential therapeutic applications in treating insulin resistance and type 2 diabetes.

Our initial results indicate that our interdisciplinary approach can be successfully used to identify potential botanical sources of therapeutic agents in the treatment of metabolic syndrome and type 2 diabetes.

Materials and Methods

Identification and Cataloguing of Medicinal Plants from Southwest Louisiana

Medicinal native plants were identified from a translation [12-14] of the original Creole language accounts of plants used in Creole folk medicine recorded by Charles Bienvenu in 1933 [15]. Many of those plants currently present in southwest Louisiana were identified and collected by the National Wetlands Research Center, and a voucher was made for each specimen and deposited at the National Wetlands Research Center herbarium in Lafayette, Louisiana.

Preparation of Plant Extracts

Plants were lyophilized and extracted in 80% ethanol 10:1 by sonication in a 50 °C water bath for 1 hour. Extracts were filtered through Miracloth (Calbiochem, Billerica, MA) and centrifuged to obtain a clear solution. The clear extracts were then dried by rotary evaporation followed by lyophilization and stored at -20 °C. The extracts were resuspended in DMSO or ethanol and diluted to the indicated concentrations in cell culture media for use in the screening assays.

Cell culture

Macrophage—RAW 264.7 macrophages (American Type Culture Collection; Manassas, VA; #TIB-71) were maintained in Dulbecco's modified Eagle's medium (DMEM) with high glucose, L-glutamine, and sodium pyruvate (DML 10, Caisson, North Logan, UT) supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin, and 10% fetal bovine serum (FBS). RAW macrophage cells were incubated in a humidified chamber at 37 °C with 5% CO₂ and subcultured by cell scraping. For experiments, RAW cells were plated at a density of 4×10^5 cells/ml in 24-well plates. Cells were incubated overnight (18 hours) and washed with phosphate-buffered saline (PBS) and replaced with fresh media. Cells were treated with 10 or 100 µg/ml plant extract or vehicle (50% ethanol or DMSO) for a total of 8 hours. To elicit inflammatory responses, 1 µg/ml lipopolysaccharide (LPS, Sigma-Aldrich, St. Louis, MO) was added to macrophages after 2 hours and incubated for an additional 6 hours. To demonstrate induction of inflammation and to evaluate potential proinflammatory effects, extract treatments (100 µg/ml) without LPS were included. Cells were treated in duplicate or triplicate and experiments included vehicle controls. Media were collected and the cells were washed with PBS prior to collection with Trizol Reagent. Samples were stored at -80°C.

Macrophage cell viability with each treatment was measured using MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide] (TCI, Portland, OR) as described [16]. Cell viability of each treatment was normalized to media only control (100%).

Skeletal Muscle—Murine C2C12 myoblasts (ATCC; #CRL-1771) were cultured in DMEM, high glucose (25 mM) with 10% FBS, 2 mM glutamine, and antibiotics (100 units/ml penicillin G and 100 µg/ml streptomycin) in a humidified chamber at 37 °C and 5% CO₂. To obtain fully differentiated myotubes, the media was exchanged for DMEM, high glucose with 2% horse serum, glutamine, and antibiotics when the myoblasts reached

confluence. The media was replaced every 48 hours and the cells were maintained in this medium until fully differentiated, when the media was exchanged for DMEM, low glucose (5 mM) with 2% horse serum. The myotubes were fully formed by the fourth day post-induction. For experiments, myotubes were incubated overnight (16 hours) with fatty acid-free bovine serum albumin (BSA)-conjugated sodium palmitate (200 μ M) alone or BSA-conjugated sodium palmitate and increasing concentrations of each plant extract (1, 10 or 50 μ g/ml). Thereafter, the cells were serum deprived by exchanging the media for DMEM containing 0.3% fatty acid-free BSA for 4 hours prior to insulin stimulation (100 nM). Palmitate alone or in combination with the plant extracts remained in the media during the 4 hour serum deprivation incubation. Ten minutes after adding insulin, the cells were rinsed with cold wash buffer and harvested in cell lysis buffer (Bio-Rad, Hercules, CA) according to the manufacturer's instructions. The lysates were stored at -80°C . Control lysates were harvested from myotubes that were incubated overnight in the presence or absence of palmitate and the presence or absence of the ethanolic extract from *A. dracuncululus* termed PMI5011, as a positive control for plant extracts that enhance phosphorylation of Akt in the presence of palmitate-induced insulin resistance [10, 11].

Gene expression analysis

Total RNA was extracted from RAW macrophage cells according to manufacturer's specifications (Life Technologies) and as previously described (23). RNA integrity was evaluated by agarose gel electrophoresis. Following treatment with Deoxyribonuclease I Amplification grade (Invitrogen, Carlsbad, CA), RNA quality was checked on the NanoDrop 1000 system (Thermo Fisher Scientific, Wilmington, DE). A ratio of OD 260/280 2.0 and OD 260/230 1.8 was acceptable. First strand cDNA synthesis was performed using ABI High-Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA) with RNase I inhibitor, according to the manufacturer's instructions using 1 μ g of RNA.

Synthesized cDNAs were diluted 25-fold and 5 μ L of dilution was used for qPCR with 12.5 μ L of Power SYBR Green PCR master mix (Applied Biosystems, Warrington, UK), 0.5 μ L primers (6 μ M) and BPC (Biotechnology Performance Certified) grade water (Sigma) to a final reaction volume of 25 μ L. Exon-spanning primer sequences [17] were as follows: *β -actin* forward 5'- AAC CGT GAA AAG ATG ACC CAG AT - 3', reverse: 5'- CAC AGC CTG GAT GGC TAC GT-3', *IL-1 β* forward 5'- CAA CCA ACA AGT GAT ATT CTC CAT - 3', reverse 5'- GAT CCA CAC TCT CCA GCT GCA - 3', *iNOS* forward 5'- CCC TCC TGA TCT TGT GTT GGA - 3', reverse 5'- TCA ACC CGA GCT CCT GGA A-3', *COX-2* forward 5' – TGG TGC CTG GTC TGA TGA TG -3', reverse 5'- GTG GTA ACC GCT CAG GTG TTG-3', *TNF α* forward 5' – TGG GAG TAG ACA AGG TAC AAC CC – 3', reverse 5'- CAT CTT CTC AAA ATT CGA GTG ACA A - 3'.

All experimental samples were run in triplicate and each reaction plate included controls without template added. Quantitative PCR amplifications were performed on an ABI 7300 Real-Time PCR System (Applied Biosystems) with the following thermal cycler profile: 2 min, 50°C ; 10 min, 95°C ; 15 s, 95°C for 40 cycles; 1 min, 60°C for the dissociation stage; 15 s, 95°C ; 1 min, 60°C ; 15 s, 95°C .

Samples were analyzed by the comparative Ct method and normalized with respect to the average Ct value of *β-actin*. Vehicle with LPS served as the calibrator for Ct analysis and was assigned a relative quantitation (RQ) value of 1.0. Lower RQ values indicated inhibition of gene expression and therefore greater anti-inflammatory activity. Percent inhibition was determined by $[(1-RQ)*100]$ and anti-inflammatory potential was denoted by percent inhibition (>90%, >60% or >30% of selected targets).

Protein Expression Analysis

Whole cell lysates were sonicated while on ice to ensure complete disruption of the cellular membranes. Cellular debris was removed via centrifugation at $4,500 \times g$ for 20 minutes at 4 °C. Protein concentration of the supernatants was determined using a BCA assay (Sigma-Aldrich) according to the manufacturer's directions. Multiplex analysis (BioPlex, Bio-Rad) of phosphoproteins involved in insulin signaling in skeletal muscle was carried out using 2 µg protein per replicate. Western blot analysis of Akt phosphorylation (anti-phospho-AKT^{ser473} rabbit polyclonal antibody #9271, Cell Signaling, Danvers, MA) as an indicator of insulin signaling was carried out using 60 µg total protein. The C2C12 lysates were resolved by SDS-PAGE and subjected to immunoblotting using infrared detection (Odyssey Imaging System, LI-COR, Lincoln, NE). Total c-JUN or total Akt expression was used as a calibrator (anti-c-JUN rabbit polyclonal antibody #A302-958A, Bethyl Laboratories, Montgomery, TX; anti-Akt mouse monoclonal antibody #2920, Cell Signaling) in the western blot analyses.

Statistical Analysis

Statistical significance was determined using unpaired two-tailed *t*-test or Dunnett's multiple comparison test on GraphPad Prism 5 software (GraphPad Software, La Jolla, CA) or SAS 9.3 (Cary, NC). Variability is expressed as the mean $\pm$ standard deviation.

Results

Selected extracts regulate expression of anti-inflammatory markers

Of the plants identified as having medicinal properties, thirty different preparations of plants have been identified, catalogued and vouchered, prior to extraction of the soluble components for bioactivity screening (**Figure 1**). Twenty plant extracts (**Table 1**) were evaluated for anti-inflammatory activity using gene expression of markers of inflammatory responses in macrophages, *TNFα*, *IL-1β*, *COX-2* and *iNOS* [18]. Of the twenty plant extracts evaluated, ten showed greater than 30% inhibition in the gene expression of at least one of the inflammatory markers without cytotoxic effects when treated at 100 µg/ml (**Table 1**). None of the extracts elicited inflammatory gene expression without LPS. Anti-inflammatory activity was most effective against *IL-1β* mRNA expression, which was potently inhibited (> 90%) by five extracts. Interestingly, the three extracts that demonstrated greater than 50% inhibition of *TNFα* expression did not inhibit gene expression of the other inflammatory markers. Extracts of *Saururus cernuus* (Lizard's Tail) and *Persea borbonia* (Red Bay) have records of use as treatments for inflammation in Creole folk medicine [15] and were both found to inhibit *IL-1β* and *COX-2* expression.

The four extracts (BAHA 4-1, CRCA 1-1, SACE 3-1, SACE 3-2) that showed the greatest inhibitory activity were prioritized for evaluation and tested at 10 µg/ml to confirm inhibitory effects on IL-1 β . All four extracts showed significant inhibition of IL-1 β gene expression at 10 and 100 µg/ml (**Figure 2**).

The majority of plant extracts evaluated had greater than 90% cell viability. Extracts of *Solanum americanum* (Black Nightshade, berries and roots) and *S. cernuus* (roots) showed clear cytotoxicity with less than 50% macrophage cell viability and were not further evaluated. Extracts from new leaves of *P. borbonica* also demonstrated cytotoxic activity with 76% cell viability. Thus, decreased gene expression with *P. borbonica* (PEBO-4-1B), *S. cernuus* (SACE 3-4) and *S. americanum* (SOAM 2-3 and 2-4) treatment may be due in part to cytotoxicity.

Insulin signaling in skeletal muscle is regulated by a subset of extracts

Experimental control conditions were established using sodium palmitate, a fatty acid shown to inhibit insulin signaling in C2C12 myotubes [19]. The ethanol extract from *A. dracuncululus*, termed PMI5011, that enhances insulin signaling in skeletal muscle [8, 10, 11] was used as a positive control. To establish the assay conditions, phosphorylation of Akt kinase was used as a surrogate for insulin signaling due to the central role of Akt activation in growth factor and inflammatory signaling in skeletal muscle [20]. As shown in **Figure 3A, B** overnight treatment with 200 µM sodium palmitate decreases insulin-mediated Akt phosphorylation 2.5 fold, an effect that was reversed by the presence of PMI5011 (10 µg/ml) during the overnight incubation. Western blot analysis of Akt phosphorylation using a subset of the extracts indicates a range of responses can be detected using the palmitate-induced insulin resistance model in C2C12 myotubes (**Figure 3C, D**).

To date, multiplex analysis has been carried out with twenty-one extracts from the native plants (**Table 1**). Representative examples of the screen demonstrate a range of effects related to insulin signaling (**Figure 4A**). Extracts from *Celtis laevigata* (Hackberry) bark did not enhance phosphorylation of Akt in the presence of insulin resistance while extracts from the leaves and stems of *Melia azedarach* (Chinaberry) inhibited phosphorylation of Akt in a dose-dependent manner. Extracts from *Sambucus canadensis* inflorescences (Elderberry flowers), however, enhanced phosphorylation of Akt at 1, 10 and 50 µg/ml and were as effective as PMI5011 at enhancing insulin signaling when present at 50 µg/ml. Although *C. laevigata* bark had no effect on Akt phosphorylation, IRS-1 phosphorylation was stimulated by the *C. laevigata* extract at 1 µg/ml, suggesting the effect of this extract is mechanistically different from PMI5011 (**Figure 4B**). Similarly, the *C. laevigata* bark extract increased phosphorylation of the mitogen activated kinase ERK 1/2, an effect not observed with PMI5011 (**Figure 4C**).

Discussion

The aim of this collaborative project was to identify therapeutic natural products related to the treatment of metabolic syndrome and type 2 diabetes. We focused on plants native to southwest Louisiana that were identified by Creole traditional healers as having medicinal use, as recorded in Creole language by Charles Bienvenu in 1933 [15] and translated from

the original Creole dialect in 2010 [12-14]. Our initial screens identified a subset of plant extracts that alter inflammatory marker gene expression in macrophages and a separate subset of extracts with bioactivity toward regulating insulin signaling in skeletal muscle.

Given the well-established link between obesity-related metabolic inflammation and insulin resistance (reviewed in [21]), we employed a macrophage-based anti-inflammatory screen to identify therapeutic natural products. With this approach, we identified several extracts that have anti-inflammatory properties, an important consideration in identifying treatment strategies for metabolic syndrome and type 2 diabetes. Pro-inflammatory changes related to obesity are mediated by macrophage recruitment in response to cytokine signaling in adipose tissue [22], ultimately affecting insulin responsiveness in the liver, pancreas and skeletal muscle [21]. As the primary site of glucose disposal, skeletal muscle insulin resistance is a major contributor to the systemic insulin resistance that occurs in obesity-induced metabolic inflammation. With this in mind, the Creole medicinal plants identified as having anti-inflammatory properties may be new sources of compounds for alleviating the inflammatory responses underlying metabolic syndrome.

Ten plant extracts demonstrated anti-inflammatory activity against at least one of the selected markers. The most potent extracts in the macrophage screen were of *Baccharis halimifolia* (Groundsel Bush), *Croton capitatus* (Goat Weed), and *S. cernuus* (Lizard's Tail), demonstrating dose dependent effects with greater than 90% inhibition at 100 µg/ml and greater than 50% inhibition at 10 µg/ml. Notably, extracts from parts of the *S. cernuus* and *P. borbonia* (Red Bay) plants, identified as anti-inflammatory in Creole traditional medicine, inhibited expression of *TNFα*, *IL-1β*, *COX-2* and *iNOS*, suggesting the extracts contain compounds with potential use as therapeutic agents in treating metabolic inflammation originating in obese visceral adipose tissue.

Our parallel screen in C2C12 myotubes identified extracts from *S. canadensis* (Elderberry) that significantly enhance Akt phosphorylation under insulin resistant conditions although the extract did not influence cytokine expression in macrophages. Thus, the *S. canadensis* extract has a direct positive effect on insulin signaling in skeletal muscle *in vitro* via a mechanism that may be independent of cytokine signaling. Finally, our screen also identified extracts that have potentially negative effects on insulin signaling in skeletal muscle. An extract from *C. laevigata* (Hackberry) significantly increases phosphorylation of the insulin receptor substrate-1 (IRS-1) at serine^{636/639} as well as phosphorylation of ERK1/2^{Thr202/Tyr204;Thr185/Tyr187}, which targets IRS-1^{Ser636/639} phosphorylation [28], but had no bioactivity toward Akt phosphorylation in skeletal muscle. Unlike tyrosine phosphorylation of IRS-1, excessive serine phosphorylation of IRS-1 impairs insulin signaling and is promoted by inflammatory cytokines such as TNFα [29]. Consistent with our findings in the skeletal muscle screen, extracts from *C. laevigata* did not inhibit inflammatory cytokine expression in macrophage.

Taken together, the current results support the validity of our interdisciplinary approach to identifying and screening native plants for bioactivity related to inflammation and insulin signaling in skeletal muscle. Our initial screen has identified several extracts with anti-inflammatory properties and at least one extract that enhanced insulin signaling in skeletal

muscle. As our screen of native plants continues, extracts that are active *in vitro* will be prioritized for phytochemical characterization using bioactivity-guided fractionations. Follow-up evaluation will include dose response tests *in vitro* in macrophage and skeletal muscle cells, followed by *in vivo* experiments. Additionally, phytochemical characterization will be undertaken to identify bioactive constituents and large scale extraction procedures will be developed with consideration to the traditional preparations. The results of the screening program can then be used to relate Creole folk medicine to modern scientific research.

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Abbreviations

AKT	protein kinase B
COX-2	cyclooxygenase-2 enzyme
ERK	extracellular signal-regulated kinase
iNOS	inducible nitric oxide synthase
IL-1β	interleukin-1 β
IRS-1	insulin receptor substrate-1

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Figure 1. Botanical Screening Workflow

Each medicinal plant was identified at the University of Louisiana at Lafayette (ULL) and collected, catalogued and vouchered at the National Wetlands Research Center (NWRC). The extracts were prepared at Rutgers University and bioactivity was analyzed at Rutgers University and the Pennington Biomedical Research Center (PBRC) Botanical Research Center.

Figure 2. Inhibition of proinflammatory IL-1 β mRNA expression by a prioritized subset of botanical extracts

Lipopolysaccharide (LPS)-induced IL-1 β gene expression in RAW macrophages was assayed using realtime qRT-PCR and reported as relative IL-1 β gene expression when normalized to β -actin gene expression and calibrated to vehicle (VEH) with LPS using the

Ct method. Each extract was added at 10 μ g/ml or 100 μ g/ml in presence of LPS. Data are presented as the mean $\pm$ standard error of the mean of three independent experiments. Significant differences were determined by Dunnett's multiple comparison tests (* p <0.05, ** p <0.01, *** p <0.001)

Figure 3. Regulation of Akt phosphorylation in skeletal muscle by botanical extracts can be assayed using C2C12 myotubes

C2C12 myotubes were incubated in the presence of sodium palmitate (200 μ M) to induce insulin resistance as assayed by inhibition of insulin-mediated Akt phosphorylation in the absence or presence of botanical extracts. (A) Western blot analysis of Akt phosphorylation using an ethanolic extract from *Artemisia dracuncululus* (PMI5011) as a positive control. (B) Quantitation of the western blot shown in (A). (C) Western blot analysis of Akt phosphorylation in the presence of a subset of botanical extracts. a=*Sambucus canadensis* inflorescence (SACA 1-1); b=*Melia azedarach* stems (MEAZ 10-2); c=*M. azedarach* leaves (MEAZ 10-1); d=*Celtis laevigata* leaves (CELA 8-1). (D) Quantitation of the western blot shown in (C). Statistical significance reported as the mean $\pm$ standard deviation compared to samples containing palmitate and insulin alone, * $p < 0.05$

Figure 4. Multiplex analysis of insulin signaling proteins in C2C12 myotubes

(A) Akt phosphorylation is stimulated by extracts from *Sambucus canadensis* (SACA 1-1) at each extract concentration, trends downward at 50 μg/ml with the *Celtis laevigata* (CELA 8-1) extract and is inhibited in a dose-dependent manner by extracts from *Melia azedarach* leaves (MEAZ 10-1) and stems (MEAZ 10-2). (B) *C. laevigata* extracts increase IRS-1 phosphorylation at serine 636/639 and (C) ERK 1/2 phosphorylation at threonine 202/tyrosine 204 (ERK1) and threonine185/tyrosine 187 (ERK2). Statistical significance reported as the mean +/- standard deviation compared to samples containing palmitate and insulin alone, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table 1

Inhibition of lipopolysaccharide-induced proinflammatory gene expression in macrophages

Plant ID	Latin Name	English Name	Plant Part	TNF α	IL-1 β	COX-2	iNOS
BAHA 4-1	<i>Baccharis halimifolia</i>	Groundsel Bush	Stems and Leaves	-	xxx	-	-
CAAM 1-1	<i>Callicarpa americana</i>	Beautyberry	Berries	xx	-	-	-
CELA 8-1	<i>Celtis laevigata</i>	Hackberry, Sugarberry	Bark	-	-	-	-
CRCA 1-1	<i>Croton capitatus</i>	Goat Weed	Leaves	-	xxx	-	x
ERHE 1-1	<i>Erythrina herbacea</i>	Coral Bean	Berries	xx	-	-	-
MEAZ 10-1	<i>Melia azedarach</i>	Chinaberry	Leaves	-	x	-	-
MEAZ 10-2	<i>Melia azedarach</i>	Chinaberry	Stems	-	-	-	-
PEBO 5-1A	<i>Persea borbonia</i>	Red Bay	Old Leaves	-	xx	x	x
PEBO 5-1B~	<i>Persea borbonia</i>	Red Bay	New Leaves	-	xxx	x	-
SACA 1-1	<i>Sambucus canadensis</i>	Elderberry	Inflorescences	-	-	-	-
SACA 1-2	<i>Sambucus canadensis</i>	Elderberry	Leaves	-	xx	x	x
SACA1-3B	<i>Sambucus canadensis</i>	Elderberry	Young Stems	-	-	-	-
SACA 1-5	<i>Sambucus canadensis</i>	Elderberry	Inflorescences	-	-	-	-
SACA 2-1	<i>Sambucus canadensis</i>	Elderberry	Stems and Inflorescences	xx	-	-	-
SACE 3-1	<i>Saururus cernuus</i>	Lizard's Tail	Leaves	-	xxx	xx	-
SACE 3-2	<i>Saururus cernuus</i>	Lizard's Tail	Inflorescences	-	xxx	-	xx
SACE 3-3	<i>Saururus cernuus</i>	Lizard's Tail	Stems	-	-	-	-
SACE 3-4~	<i>Saururus cernuus</i>	Lizard's Tail	Roots	-	xxx	xx	x
SOAM 2-3~	<i>Solanum americanum</i>	American Black Nightshade	Green Berries	-	-	-	-
SOAM 2-4~	<i>Solanum americanum</i>	American Black Nightshade	Roots	-	xx	-	-

x: >30% inhibition, xx: >50% inhibition, xxx: >90% inhibition Treated with 100 μ g/ml plant extract

~ Cytotoxicity detected