Co-evaluation of Plant Extracts as Petrochemical Substitutes and for Biologically Active Compounds[1]

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Recent efforts to discover phytochemicals that could substitute for petroleum-derived fuels and industrial feedstocks have not given much attention to the potential of these same phytochemicals to provide sources of biologically active compounds. The suitability of extraction products made to assess specific plants as potential botanochemical sources has been evaluated for use in screening procedures for evidence of biologically active compounds. Screening procedures for antibacterial, antifungal and toxic properties are discussed. Screening results are presented for extracts of nearly 80 species of plants from the southeastern United States and southern Great Plains that had previously been evaluated as sources of botanochemicals.

The recent changes in the price of petroleum have spurred a renewed interest in plant screening to discover alternative sources of chemicals for use as fuel and chemical feedstocks (Adams, 1982; Adams and McChesney, 1983; Buchanan et al., 1978a,b; McLaughlin and Hoffmann, 1982). The availability of these extracts presents an unusual opportunity to append screenings for biologically active compounds. The extracts can be examined for possible use as sources of compounds with antibacterial, antifungal or toxic activity. In this paper, we present procedures for such screens, and report the results obtained by applying these screening procedures for 80 plant species from the southern United States and southern Great Plains (Adams and McChesney, 1983).

MATERIALS AND METHODS

Plant collections

Whole above-ground plants in the full reproductive state (flowering in angiosperms) were collected with the exception of *Juniperus monosperma, Betula nigra, Sapium sebiferum* and *Tamarix ramosissima,* in which cases, only leaves were collected. Whole-plant material (except as previously noted) from 5 plants was bulked and dried for 48 h at 70°C. The plant material was then ground in a Wiley mill to pass a 2-mm screen. All reproductive organs were discarded to facilitate comparisons among species. See Adams and McChesney (1983, Table 4) for collectors, herbarium voucher numbers, location of herbarium specimens, and authorities for Latin names.

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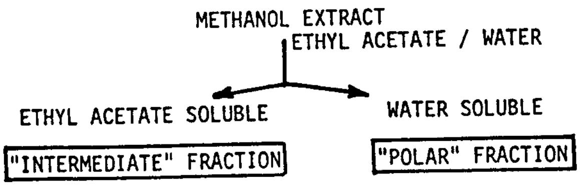
Extractions

The extracts were obtained by Soxhlet extraction for 22 h with cyclohexane ("Non-Polar") followed by methanol (22 h) as described in detail by Adams and McChesney (1983). The methanol extracts were fractionated by a rapid solvent-partitioning procedure that grossly separates the materials present based upon their relative polarities. A portion of the methanol extract was concentrated and dissolved in a mixture of ethyl acetate and water. The resultant biphasic solution was separated in a separatory funnel. Scheme 1 outlines this procedure:



Crude extract toxicity screen

Toxicities of crude extracts were determined against 2 organisms: (1) brine shrimp larvae (*Artemia salina*) using a modification of the procedure of Kinghorn et al. (1978); and (2) fruit flies (*Drosophila melanogaster*) using a modification of the procedure of Gupta and Rawlins (1966).

Cultiviation of brine shrimp

Vacuum-packed brine shrimp eggs (Longlife Fish Food Products, Harrison, NJ) were hatched in artificial seawater (20–25°C) in an open beaker. No nutrients were added. The hatching medium was constituted by diluting a synthetic sea-salt medium, "Instant Ocean" (Aquarium Systems, Inc., Eastlake, OH), with deionized water to give a product with specific gravity of about 1.025 (at 25°C). Two days after seeding, brine shrimp nauplii were separated from unhatched eggs by careful decantation.

Assay procedure

The extracts were solubilized in 2% aqueous Tween 80 to produce a concentration of 7.0 mg/ml, and 0.2 ml of this solution in a 30-ml beaker was diluted with 2.8 ml of the brine shrimp suspension. This produced a final concentration of 400 μg/ml of extract in the test medium containing 25–50 *A. salina* larvae. The test suspensions were covered to minimize salinity changes, allowed to stand at room temperature (20–25°C) and were examined 25 h after their constitution. Control beakers were set up containing 0.2 ml of 2% aqueous Tween 80 and 2.8 ml of the brine shrimp suspension. Counts of dead shrimp were made with the aid of a stereoscopic microscope (10×) and a high intensity lamp. Animals were considered dead when all limb movements had ceased. Counts of dead brine shrimp were expressed as a percentage of the total brine shrimp per beaker.

TABLE 1. ANTIBACTERIAL ACTIVITY OF EXTRACTS.[a]

Family	Species[b]	*B. subtilis* A	B	C	*E. coli* A	B	C	*S. aureus* A	B	C	*M. smegmatis* A	B	C	*P. aeruginosa* A	B	C
Acanthaceae	*Hygrophila lacustris*	-	1	-	1	1	-	-	3	-	1	4	-	1	-	-
Anacardiaceae	*Rhus glabra*	2	4	2	-	3	-	-	3	1	2	4	3	1	2	-
Apiaceae	*Chaerophyllum tainturieri*	-	4	-	-	1	-	2	5	1	4	6	-	-	1	-
	Eryngium yuccifolium	-	2	-	-	1	-	-	3	-	5	4	-	-	1	-
	Oxypolis filiformis	3	3	5	-	-	-	6	4	7	-	7	7	1	2	-
	Torilis arvensis	-	3	-	-	-	1	-	4	-	2	5	-	-	-	-
	Trepocarpus aethusae	2	4	-	-	2	1	2	4	1	4	6	-	-	-	-
Apocynaceae	*Trachelospermum difforme*	-	1	-	-	-	-	-	-	-	3	-	-	-	-	-
Aquifoliaceae	*Ilex glabra*	-	-	-	-	-	-	-	3	2	-	3	1	-	-	-
Asclepiadaceae	*Asclepias latifolia*	-	5	-	1	-	-	1	4	-	7	9	-	1	3	-
	A. tuberosa	-	-	-	-	-	-	-	2	-	2	-	1	-	1	-
Asteraceae	*Aster praealtus*	1	-	-	-	-	-	1	4	-	3	6	-	2	1	-
	Carphephorus odoratissimus	-	5	-	-	1	-	2	7	-	-	4	1	1	3	-
	Coreopsis tinctoria	1	2	-	-	-	-	-	5	1	3	7	-	-	2	-
	Erigeron annuus	-	-	-	-	-	-	-	3	1	2	-	-	-	1	1
	E. philadelphicus	-	-	-	-	2	1	-	4	-	3	6	-	-	-	1
	Eupatorium ivifolium	-	5	-	-	1	-	2	7	-	-	4	1	1	3	-
	Grindelia squarrosa	7	1	-	1	1	-	10	6	-	15	10	-	1	1	-
	Helianthus annuus	4	5	-	-	1	-	6	5	-	9	9	-	-	1	-
	Liatris spicata	-	3	-	1	1	-	1	4	-	1	6	1	-	1	-
	L. squarrosa	-	3	-	-	1	-	1	2	-	1	5	-	-	2	1
	Rudbeckia hirta	-	-	-	-	-	-	-	2	1	3	5	-	-	1	1
	Senecio glabellus	-	-	-	-	-	-	3	1	1	-	3	-	-	-	-
	Solidago microcephala	-	1	-	-	-	-	1	4	-	2	2	-	-	-	-
	Xanthium strumarium	-	6	-	-	-	-	-	6	-	-	4	-	-	-	-
Betulaceae	*Betula nigra*	4	7	-	-	-	-	2	7	-	4	6	-	-	-	-
Caprifoliaceae	*Sambucus canadensis*	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
Caryophyllaceae	*Stellaria media*	-	-	-	-	-	-	-	3	1	5	4	1	-	2	-
Clusiaceae	*Hypericum galioides*	-	3	-	-	-	1	6	5	2	5	4	-	-	-	-
	H. gentianoides	13	40	1	-	2	1	12	27	1	4	16	1	1	1	-

TABLE 1 (continued)

Family	Species	*B. subtilis* A	B	C	*E. coli* A	B	C	*S. aureus* A	B	C	*M. smegmatis* A	B	C	*P. aeruginosa* A	B	C
Cornaceae	*Cornus stricta*	-	-	-	-	-	-	-	3	-	1	3	3	-	-	-
Cupressaceae	*Juniperus monosperma*	7	7	-	2	4	-	10	10	-	10	13	-	1	2	-
Cyperaceae	*Rhynchospora corniculata*	-	3	-	1	1	-	-	3	1	-	3	-	-	2	-
	Scleria pauciflora	-	4	-	-	-	2	1	3	1	2	3	2	-	1	-
Cyrillaceae	*Cyrilla racemiflora*	2	2	1	-	6	1	-	4	-	3	6	4	-	-	1
Ericaceae	*Rhododendron serrulatum*	-	3	-	-	-	-	-	3	-	-	6	-	-	-	-
Euphorbiaceae	*Euphorbia lathyris*	4	1	-	1	1	-	5	4	-	6	10	-	1	1	-
	E. marginata	-	-	-	1	1	-	4	5	-	7	8	-	-	1	-
	Sapium sebiferum	-	-	1	1	-	-	-	5	2	1	3	1	-	1	-
	Sebastiania fruticosa	-	1	-	-	1	4	-	5	1	6	9	4	-	1	-
Fabaceae	*Melilotus alba*	-	-	-	-	-	-	-	3	1	-	2	-	-	-	-
	Psoralea psoralioides	-	-	-	-	-	-	-	4	-	-	5	-	-	-	-
	Tephrosia virginiana	2	3	-	-	-	-	1	3	-	2	6	2	-	-	-
Geraniaceae	*Geranium carolinianum*	-	2	-	-	-	1	1	2	1	3	3	4	-	-	-
Hydrophyllaceae	*Hydrolea quadrivalvis*	-	2	-	-	-	-	-	2	-	-	3	-	-	-	-
Lamiaceae	*Calamintha georgiana*	-	1	-	-	-	-	-	4	-	-	2	1	-	-	1
	Hyptis alata	-	1	-	-	-	-	-	2	1	-	1	-	-	-	-
	Pycnanthemum tenuifolium	-	-	-	-	-	-	-	2	-	2	1	-	-	-	-
Lauraceae	*Persea palustris*	4	9	-	-	-	-	3	9	-	5	6	1	-	1	-
Nymphaeaceae	*Nuphar luteum*	-	2	1	-	2	-	1	4	3	1	5	-	-	1	-
Onagraceae	*Ludwigia decurrens*	-	4	-	-	1	-	1	4	-	1	6	-	-	3	-
Plantaginaceae	*Plantago virginica*	-	-	-	-	-	-	-	2	2	3	4	-	-	-	-
Ranunculaceae	*Ranunculus bulbosus*	-	-	-	-	-	-	-	1	1	4	5	-	-	1	-
Rubiaceae	*Galium aparine*	-	-	-	-	-	-	-	3	-	-	5	-	-	-	-
Scrophylaria-ceae	*Micranthemum umbrosum*	-	3	-	-	-	-	2	5	-	-	3	-	-	2	-
Smilacaceae	*Smilax laurifolia*	1	3	-	-	-	-	-	4	1	-	2	1	-	3	1
Styracaceae	*Halesia diptera*	-	-	-	1	-	-	-	2	1	1	4	-	-	-	-

TABLE 1 (continued)

Family	Species	*B. subtilis* A	B	C	*E. coli* A	B	C	*S. aureus* A	B	C	*M. smegmatis* A	B	C	*P. aeruginosa* A	B	C
Symplocaceae	*Symplocos tinctoria*	-	-	-	-	-	-	-	1	-	-	5	1	-	-	-
Typhaceae	*Typha latifolia*	-	-	-	-	-	-	-	4	-	2	2	2	-	-	-
Valerianaceae	*Valerianella radiata*	-	2	-	-	-	-	-	-	1	3	2	-	-	-	-
Verbenaceae	*Verbena brasiliensis*	3	4	2	3	3	-	-	5	2	2	2	1	-	2	-
Streptomycin sulfate (1 mg/ml)			9			4			6			20			6	

[a]METHANOL EXTRACTS WERE PARTITIONED (SCHEME 1) AND THE RESULTANT FRACTIONS BIOASSAYED. A=NON-POLAR (CYCLOHEXANE EXTRACT), B=INTERMEDIATE (ETHYL ACETATE FRACTION) AND C=POLAR (WATER FRACTION). ACTIVITIES AGAINST *Bacillus subtilis*, (6633); *Escherichia coli*, (10536); *Staphylococcus aureus*, ((6538); AND *Pseudomonas aeruginosa* , (15442) WERE DETERMINED AFTER 24 H. ACTIVITY AGAINST *Mycobacterium smegmatis* , (607) WAS DETERMINED AFTER 48 H. ACTIVITIES ARE REPORTED AS AVERAGE RADIUS OF THE ZONE OF INHIBITION. A (-) INDICATES NO INHIBITION. CONCENTRATIONS OF EXTRACTS APPLIED WERE 20 MG/ML.

[b]See Adams and McChesney (1983) Table 4 for collectors, herbarium voucher numbers, location of herbarium specimens, and authorities for Latin names.

Cultivation of Drosophila

Drosophila were cultured in half-pint glass bottles with 2–3 cm of growth medium (Instant *Drosophila* Medium, Formula 4-24 plain, Carolina Biological Supply, Burlington, NC) reconstituted with water covering the bottom. Two to three weeks of culture are needed before new adults emerge in sufficient quantities for use in bioassays.

Assay procedure

Instant *Drosophila* Medium (0.25 g) was weighed into 6-dr vials. Extracts were dissolved (ethanol: H_2O) and added to the medium to provide a final concentration of 20 mg extract per ml of medium. After removal of solvent (evaporation overnight in a well-ventilated hood), the medium was saturated with deionized water (0.25–0.30 ml) and allowed to stand 24 h at room temperature. Recently emerged *Drosophila* adult flies were anesthetized and added (5–10 per vial) and those dead at 48 and 72 h were counted. Counts of dead flies are expressed as a percentage of total flies per vial.

Qualitative antimicrobial screening

Qualitative antimicrobial screening was carried out using the agar-well diffusion assay against those organisms listed in Tables 1 and 2. All test organisms were obtained from the American Type Culture Collection. Crude extracts and fractions were routinely tested at a concentration of 20 mg/ml in ethanolic or aqueous ethanolic solution. Results of the qualitative screen are reported as the average radius of the zone of inhibition surrounding the well containing the test solution.

RESULTS AND DISCUSSION

A large-scale screening effort to discover potential sources of botanochemicals useful as petroleum substitutes presents an unusual opportunity to assay the same extracts for substances showing biological activity or other chemicals with specific usefulness. Toxicity to brine shrimp larvae (*Artemia salina*) and fruit flies (*Drosophila melanogaster*) may indicate toxicity for other animal species. The presence of toxicity in the extracts would indicate which plants needed more extensive evaluation of their potential for toxicity as part of the overall program objectives. These plants or their extracts might pose potential serious hazards to workers growing, gathering or processing the materials. To gain some indication of the class of compound(s) responsible for any activity present, a crude fractionation based upon solvent partitioning was performed as outlined in the materials and methods. Table 1 records the activity of the extracts as antibacterial agents. Table 2 reports the antifungal activity of the extracts, and the toxicity of the extracts is shown in Table 3.

Several of the species examined gave extracts that showed potential for antibacterial or antifungal activity. We consider those with zones of inhibition of 8 mm or greater in the antibacterial screen as promising, and those of 6 mm or more as having promise as antifungals, except against *Aspergillus niger* where 3 mm or more is considered potential activity. Thus *Grindelia squarrosa, Hypericum gentianoides, Juniperus monosperma,* and *Persea palustris* all show antibacterial activities of interest. Several additional species may be promising with 6–

TABLE 2. ANTIFUNGAL ACTIVITY OF EXTRACTS.[a]

Family	Species	*C. albicans*[b] A	B	C	*S. cerevisiae*[b] A	B	C	*A. niger*[b] A	B	C	*T. mentagraphytes*[b] A	B	C
Acanthaceae	*Hygrophila lacustris*	1	1	1	2	1	-	-	-	-	1	3	-
Anacardiaceae	*Rhus glabra*	-	3	1	-	4	2	-	-	-	1	5	2
Apiaceae	*Chaerophyllum tainturieri*	-	-	-	1	3	-	-	-	-	1	3	-
	Eryngium yuccifolium	-	-	-	-	-	6	-	-	-	5	4	4
	Oxypolis filiformis	-	1	1	1	3	3	-	2	1	3	7	2
	Torilis arvensis	-	-	-	2	2	2	-	-	-	-	10	-
	Trepocarpus aethusae	1	1	-	2	4	-	-	3	-	4	8	-
Apocynaceae	*Trachelospermum difforme*	-	-	-	-	-	-	-	-	-	-	-	-
Aquifoliaceae	*Ilex glabra*	-	-	1	2	2	1	-	-	-	-	3	1
Asclepiadaceae	*Asclepias latifolia*	1	3	-	2	7	-	-	-	-	6	15	-
	A. tuberosa	-	-	-	-	2	-	-	-	-	-	2	-
Asteraceae	*Aster praealtus*	1	-	-	8	7	2	-	-	-	5	12	2
	Carphephorus odoratissimus	1	1	1	2	4	3	-	-	-	-	10	-
	Coreopsis tinctoria	-	2	-	4	5	-	-	-	-	-	5	1
	Erigeron annuus	-	-	-	-	-	-	-	-	-	-	-	-
	E. philadelphicus	-	2	-	-	2	-	-	-	-	5	9	-
	Eupatorium ivifolium	1	2	2	1	3	2	-	-	-	-	6	1
	Grindelia squarrosa	2	3	-	5	5	-	1	-	-	10	14	1
	Helianthus annuus	-	2	-	2	4	-	-	-	-	7	8	-
	Liatris spicata	-	1	1	1	3	1	-	-	-	-	2	1
	L. squarrosa	-	1	1	1	4	1	-	-	-	1	10	-
	Rudbeckia hirta	-	-	-	10	-	-	-	-	-	-	1	-
	Senecio glabellus	-	-	-	2	-	1	-	-	-	-	-	-
	Solidago microcephala	-	1	1	2	1	1	-	-	-	-	1	1
	Xanthium strumarium	2	6	-	-	7	-	1	7	-	7	-	-
Betulaceae	*Betula nigra*	-	-	-	-	-	-	-	-	-	-	-	-
Caprifoliaceae	*Sambucus canadensis*	-	-	-	-	-	-	-	-	-	-	-	4
Caryophyllaceae	*Stellaria media*	-	-	-	-	2	-	-	-	-	-	-	-
Clusiaceae	*Hypericum galioides*	1	1	1	2	1	1	-	-	-	4	4	1
	H. gentianoides	-	2	1	1	1	-	-	-	-	-	8	1

TABLE 2 (continued)

Family	Species	*C. albicans*[b] A	B	C	*S. cerevisiae*[b] A	B	C	*A. niger*[b] A	B	C	*T. mentagraphytes*[b] A	B	C
Cornaceae	*Cornus stricta*	-	3	3	1	2	1	-	-	-	-	3	-
Cupressaceae	*Juniperus monosperma*	2	5	-	5	7	-	1	2	-	16	25	-
Cyperaceae	*Rhynchospora corniculata*	-	-	1	1	-	1	-	-	-	-	1	1
	Scleria pauciflora	-	1	2	1	1	1	-	-	-	-	2	1
Cyrillaceae	*Cyrilla racemiflora*	-	1	1	1	6	3	-	-	1	-	4	-
Ericaceae	*Rhododendron serrulatum*	-	-	1	-	-	1	-	-	-	-	1	-
Euphorbiaceae	*Euphorbia lathyris*	2	3	-	3	5	-	-	-	-	10	20	-
	E. marginata	1	2	-	1	5	-	-	-	-	15	9	-
	Sapium sebiferum	2	1	1	2	2	1	-	-	-	-	5	-
	Sebastiania fruticosa	-	1	-	1	1	1	-	-	-	-	5	-
Fabaceae	*Melilotus alba*	-	-	-	-	-	-	-	-	-	7	-	-
	Psoralea psoralioides	-	-	-	-	4	-	-	-	-	-	-	-
	Tephrosia virginiana	-	-	-	2	-	-	-	-	-	-	-	2
Garaniaceae	*Geranium carolinianum*	1	-	2	-	2	2	-	-	-	-	5	-
Hydrophyllaceae	*Hydrolea quadrivalvis*	-	1	1	1	1	1	-	-	-	-	-	-
Lamiaceae	*Calamintha georgiana*	1	-	-	1	1	1	-	-	-	3	6	2
	Hyptis alata	-	1	-	1	-	-	-	2	1	-	1	-
	Pycnanthemum tenuifolium	-	-	-	-	4	-	-	-	-	-	2	-
Lauraceae	*Persea palustris*	-	1	-	1	1	-	-	-	-	-	4	-
Nymphaeaceae	*Nuphar luteum*	3	1	1	4	1	1	-	-	-	3	3	-
Onagraceae	*Ludwigia decurrens*	-	1	1	-	2	1	-	-	1	-	2	1
Plantaginaceae	*Plantago virginica*	-	-	-	2	-	-	-	-	-	2	-	2
Ranunculaceae	*Ranunculus bulbosus*	-	-	-	-	-	-	-	-	-	-	3	-
Rubiaceae	*Galium aparine*	2	-	-	-	2	1	-	-	-	-	2	-
Scrophylariaceae	*Micranthemum umbrosum*	1	-	1	1	1	1	-	-	1	3	6	1
Smilacaceae	*Smilax laurifolia*	1	1	1	-	-	-	-	-	-	-	-	-
Styracaceae	*Halesia diptera*	-	-	1	-	2	5	-	-	-	1	3	5

TABLE 2 (continued)

Family	Species	*C. albicans* A	B	C	*S. cerevisiae* A	B	C	*A. niger* A	B	C	*T. mentagrophytes* A	B	C
Symplocaceae	*Symplocos tinctoria*	-	-	1	1	1	-	-	-	-	1	1	1
Typhaceae	*Typha latifolia*	-	-	-	-	-	-	-	-	-	-	-	2
Valerianaceae	*Valerianella radiata*	-	1	1	2	1	2	-	-	-	6	1	-
Verbenaceae	*Verbena brasiliensis*	-	2	-	-	3	-	-	-	-	7	5	-
Amphotericin B (1 mg/ml)			10			11			8			14	

[a] METHANOL EXTRACTS WERE PARTITIONED (SCHEME 1) AND THE RESULTANT FRACTIONS BIOASSAYED. A=NON-POLAR (CYCLOHEXANE EXTRACT), B=INTERMEDIATE (ETHYL ACETATE FRACTION) AND C=POLAR (WATER FRACTION). ACTIVITIES AGAINST ALL ORGANISMS WERE DETERMINED AFTER 48 H AND ARE REPORTED AS THE AVERAGE RADIUS OF THE ZONE OF INHIBITION. A (-) INDICATES NO INHIBITION. CONCENTRATIONS OF EXTRACTS APPLIED WERE 20 MG/ML.

[b] *Candida albicans*, (10231); *Saccharomyces cerevisiae*, (9763); *Aspergillus niger*, (16888); *Trichophyton mentogrophytes*, (9972).

TABLE 3. TOXICITY OF EXTRACTS.[a]

Family	Species	% Dead *D. melanogaster* A	B	C	% Dead *A. salina* A	B	C
Acanthaceae	*Hygrophila lacustris*	33	71	0	0	0	7
Anacardiaceae	*Rhus glabra*	0	0	0	0	0	0
Apiaceae	*Chaerophyllum tainturieri*	60	80	86	85	50	5
	Eryngium yuccifolium	60	40	0	0	85	0
	Oxypolis filiformis	7	17	0	100	100	78
	Torilis arvensis	50	50	14	100	100	2
	Trepocarpus aethusae	100	20	0	100	97	0
Apocynaceae	*Trachelospermum difforme*	0	0	0	0	0	0
Aquifoliaceae	*Ilex glabra*	0	0	0	6	0	0
Asclepiadaceae	*Asclepias tuberosa*	40	75	100	0	90	54
Asteraceae	*Aster praealtus*	100	0	0	73	90	81
	Carphephorus odoratissimus	0	33	0	75	100	50
	Coreopsis tinctoria	55	100	71	85	90	0
	Erigeron annuus	0	0	0	0	94	0
	E. philadelphicus	83	75	25	100	100	0
	Eupatorium ivifolium	17	14	0	0	67	10
	Liatris spicata	18	8	0	8	95	0
	L. squarrosa	9	0	0	100	100	3
	Rudbeckia hirta	0	100	0	0	63	0
	Senecio glabellus	20	60	83	80	80	0
	Solidago microcephala	43	25	0	0	0	26
	Xanthium strumarium	14	0	0	100	100	0
Betulaceae	*Betula nigra*	86	50	33	0	0	0
Caprifoliaceae	*Sambucus canadensis*	20	25	50	0	0	0
Caryophyllaceae	*Stellaria media*	80	100	67	50	50	0

TABLE 3 (continued)

Family	Species	% Dead *D. melanogaster* A	B	C	% Dead *A. salina* A	B	C
Clusiaceae	*Hypericum galioides*	20	17	0	55	61	23
	H. gentianoides	10	0	0	49	100	0
Cornaceae	*Cornus stricta*	0	0	0	0	0	0
Cyperaceae	*Rhynchospora corniculata*	6	0	8	0	16	0
	Scleria pauciflora	45	0	0	0	0	28
Cyrillaceae	*Cyrilla racemiflora*	65	0	8	27	0	6
Ericaceae	*Rhododendron serrulatum*	9	0	0	0	0	0
Euphorbiaceae	*Sapium sebiferum*	83	83	0	0	40	0
	Sebastiania fruticosa	7	0	8	31	100	5
Fabaceae	*Melilotus alba*	17	63	0	0	0	5
	Psoralea psoralioides	67	0	33	20	92	0
	Tephrosia virginiana	100	0	0	100	98	75
Geraniaceae	*Geranium carolinianum*	0	100	20	4	25	100
Hydrophyllaceae	*Hydrolea quadrivalvis*	33	0	0	0	22	0
Lamiaceae	*Calamintha georgiana*	0	0	0	0	0	19
	Hyptis alata	57	33	0	0	0	0
	Pycnanthemum tenuifolium	100	37	50	0	0	0
Lauraceae	*Persea palustris*	45	0	38	92	98	0
Nymphaeaceae	*Nuphar luteum*	80	0	0	4	97	65
Onagraceae	*Ludwigia decurrens*	0	8	0	55	50	86
Plantaginaceae	*Plantago virginica*	50	60	0	50	17	0
Ranunculaceae	*Ranunculus bulbosus*	0	60	20	0	0	0
Rubiaceae	*Galium aparine*	17	100	0	50	91	0
Scrophulariaceae	*Micranthemum umbrosum*	29	45	9	60	100	95

TABLE 3 (continued)

Family	Species	% Dead *D. melanogaster* A	B	C	% Dead *A. salina* A	B	C
Smilacaceae	*Smilax laurifolia*	0	40	0	0	0	0
Styracaceae	*Halesia diptera*	8	20	8	3	30	70
Symplocaceae	*Symplocos tinctoria*	7	20	7	21	50	17
Typhaceae	*Typha latifolia*	100	83	83	61	0	0
Valerianaceae	*Valerianella radiata*	0	0	0	6	16	3
Verbenaceae	*Verbena brasiliensis*	0	50	0	20	75	8
Water only			11			2	
Ethanol only			5			NT	
2% Tween 80 only			NT			8	

[a]METHANOL EXTRACTS WERE PARTITIONED (SCHEME 1) AND THE RESULTANT FRACTIONS BIOASSAYED. A=NON-POLAR (CYCLOHEXANE EXTRACT), B=INTERMEDIATE (ETHYL ACETATE FRACTION) AND C=POLAR (WATER FRACTION). TOXICITY IS REPORTED AS PERCENT OF ANIMALS DEAD OR IMMOBILE AFTER 24 H FOR *Artemia salina* LARVAE AND AFTER 72 H FOR *Drosophila melanogaster* ADULTS.

8 mm zones of inhibition. As antifungals, *Asclepias latifolia, Aster praealtus, Carphephorus odoratissimus, Euphorbia lathyris, Euphorbia marginata, Grindelia squarrosa, Helianthus annuus, Juniperus monosperma, Trepocarpus aethusae,* and *Xanthium strumarium* all show potential. Only *Trepocarpus aethusae* and *Xanthium strumarium* show significant activity against *Aspergillus niger.*

Ten species extracts showed greater than 80% lethality in one or more of the fractions (Scheme 1) against both *Artemia salina* and *Drosophila melanogaster.* This would suggest that a relatively high percentage of the plants may prove toxic. A detailed examination of the toxic effects of the residues remaining from the extraction procedure will be needed to determine the feed value of this material for domestic animals. The use of these residues as animal feed or feed extenders has been suggested to improve the economics of the overall system. Extracts of several other species showed significant toxicity to one or the other of the organisms (Table 3). Admittedly these organisms may be relatively sensitive but in our opinion this is desirable in an indicator organism. Those species that show potential as a souce of botanochemicals (high percentage of total extractables) but also show the potential for toxicity in the fruit fly and brine shrimp assays may be further evaluated for toxicity in more expensive laboratory animal assays.

CONCLUSION

We have demonstrated that screening for biologically active compunds in the extracts can be carried out in conjunction with hydrocarbon screening. This aspect represents an unusual opportunity for secondary (or tertiary) screening of hydrocarbon-producing species for multiuse. Several of the species tested in this study show promise as potential sources of antibacterial or antifungal substances. Nickell (1959) has reported that extracts prepared solely for evaluation as antimicrobials gave evidence that many plants contained substances of potential as antimicrobial agents. The use of the rapid and inexpensive fruit fly and brine shrimp toxicity screens provides useful information upon which to base decisions for more extensive toxicity evaluation as well as the potential of the extracts to yield insecticidal substances.

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