Phytochemicals for Liquid Fuels and Petrochemical Substitutions: Extraction Procedures and Screening Results[1]

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Various solvents were examined for their effectiveness in the extraction of nonpolar and polar compounds from plant materials for obtaining botanochemicals. Cyclohexane extraction followed by methanol gave consistently higher yields than other solvents in current use. Soxhlet extraction was found to give higher yields than shaking/decanting. Yields of nonpolar and polar extractions are reported for 80 species from the southeastern United States and southern Great Plains.

As the availability of petroleum-derived fuels and industrial feedstocks lessens due to depletion as well as economic and political developments, it is imperative that we reexamine renewable sources of organic compounds suitable as alternatives to petroleum-based substances (McLaughlin and Hoffmann, 1982; Princen, 1982; Wang and Huffman, 1981). Calvin (1979) suggested that energy plantations may one day serve this purpose. Recent demonstrations (Weisz et al., 1979; Haag et al., 1980) that plant hydrocarbons can be converted by a zeolite type catalyst (ZSM-5 type) directly to gasoline and closely-related materials underscores the technical practicality of this approach. Newly developed methods for cracking by fluid bed reactors and zeolite catalysts change the perspective of biological chemicals and fuels considerably. Haag et al. (1980) obtained very high conversions of a whole-plant methylene chloride extract of *Grindelia squarrosa* (Pursh.) Dunal. and an acetone extract of *Euphorbia lathyris* L. to liquid fuels.

Major classes of extractable hydrocarbon-like substances produced in plants that might serve as suitable substrates for catalytic conversion are terpenoids (mono-, sesqui-, di-, tri-terpene and isoprenoid polymers), phenolics (flavonoids, phenols, polyphenols), and long chain aliphatics (waxes, triglycerides, fatty acids, etc.). Many thousands of species of plants produce copious amounts of such hydrocarbon-like materials (Buchanan et al., 1978a,b; Erdman and Erdman, 1981). The conifers are particularly rich in terpenes, with pines having been utilized for many years as a source of turpentine and related (chiefly terpenoid) products.

Additional chemical feedstocks can be obtained from the extracted residue (Campos-Lopez and Roman-Alemany, 1980; Lipinsky, 1981; Princen, 1982) which can significantly increase the value of a phytochemical crop (Buchanan et al., 1980).

Much of the recent research on phytochemical crops has centered on latex-producing species (particularly *Euphorbia* species) (Calvin, 1979; William Brooks, Univ. Arizona, Office of Arid Lands Studies, pers. comm.) to be grown on semi-

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arid and arid land plantations. However, it is premature to settle on a few species for extensive developmental research without clearly demonstrating the superiority of those species as hydrocarbon sources. Further, before valid species comparisons can be made, standardized extraction procedures need to be developed.

The most detailed study on extraction procedures published to date has been that of Buchanan et al. (1978) on *Asclepias syriaca* L. They extracted with Soxhlets for 48 h, using a polar solvent (acetone, methanol, etc.), followed by 48 h of extraction with a nonpolar solvent such as hexane or cyclohexane. Total extractives yield increased with the use of the more polar solvents. However, the hydrocarbon and oil fractions (terminology of Buchanan et al., 1978) remained fairly constant.

Nemethy et al. (1978) compared 8 h Soxhlet extractions using acetone, followed by benzene with extractions using heptane followed by acetone. Using the nonpolar solvent first generally yielded a larger total extractive but the acetone extractables were then higher in oxygen content. Methylene chloride was also tried but found to give much lower yields than acetone.

The University of Arizona has also carried out research on extraction, using Soxhlets for up to 24 h with cyclohexane followed by 95% ethanol for up to 24 h (J. Hoffmann, pers. comm.). No data are available for comparisons with the work of Buchanan et al. (1978) and Nemethy et al. (1978).

Although no single procedure may be found optimal for extraction of each of the thousands of species that should be screened, some of the methods appear to be superior in extraction efficiency. If species are to be compared for hydrocarbon yields, standardization must be accepted in the initial screening.

MATERIALS AND METHODS

Plant collections

Whole above-ground plants in full reproductive state (i.e., flowering in angiosperms) were collected with the exceptions 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^{R} mill to pass a 2 mm screen. All reproductive organs were discarded to facilitate comparisons among species.

Extractions

A plug of glass wool was placed in each Whatman paper thimble (33 mm × 94 mm) and both were dried for 48 h at 100°C. The thimble and glass wool plug were then placed in a desiccator for 4 h to prevent rehydration before pre-weighing.

Disposable aluminum pans were used for evaporation of the solvents from each

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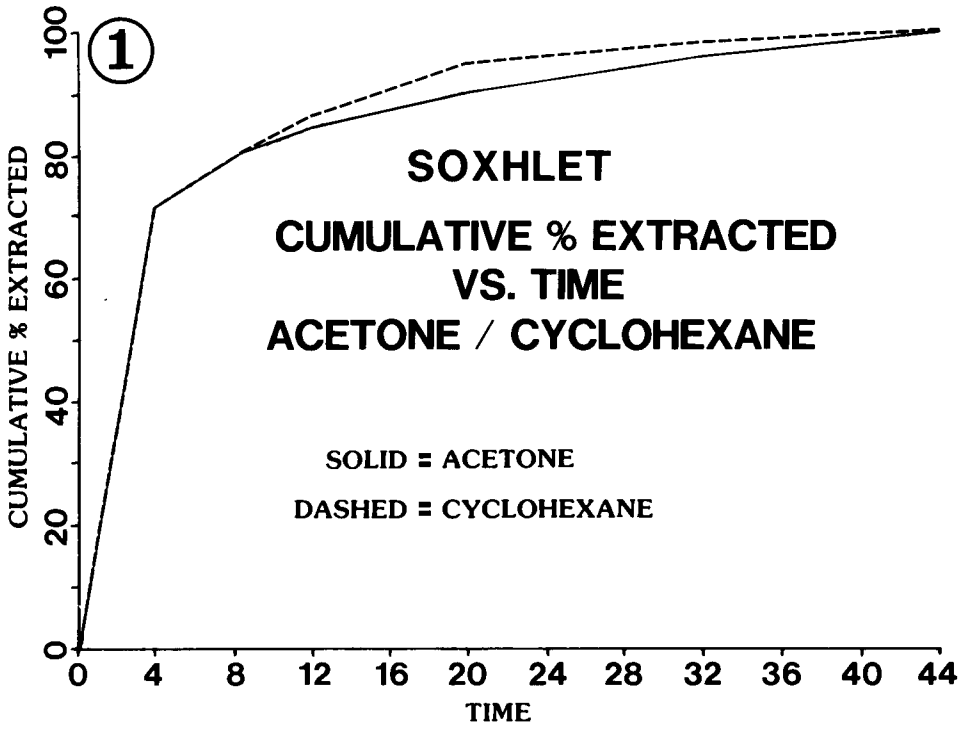
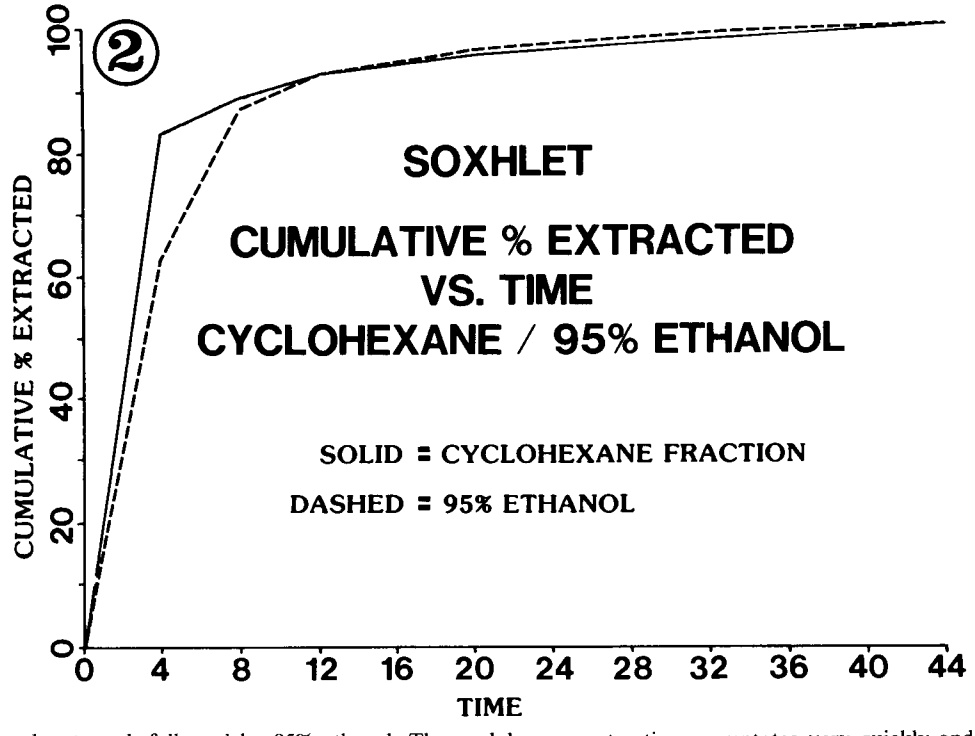
Fig. 1–2. Fig. 1. Time-course plot of the cumulative % extracted vs. time. *Asclepias latifolia* was first extracted by Soxhlet with acetone and then with cyclohexane. Note that the cyclohexane fraction appears to level off at about 32 h whereas the acetone extract still has appreciable slope. Fig. 2. Time-course plot of the cumulative % extracted vs. time for *Asclepias latifolia*. Cyclohexane was the first





solvent used, followed by 95% ethanol. The cyclohexane extraction asymptotes very quickly and both have begun to level off after 20 h.

TABLE 1. COMPARISON OF EXTRACTION EFFICIENCY OF VARIOUS SOLVENTS ON *ASCLEPIAS LATIFOLIA*.

Method	Polar solv. yield %	Nonpolar solv. yield %	Total yield %
Acetone/cyclohexane	7.34	1.07	8.41
Acetone/benzene	7.33	1.47	8.80
Heptane/acetone	1.99	6.18	8.16
Cyclohexane/95% ethanol	10.13	6.37	16.50
Cyclohexane/methanol	15.56	6.25	21.81

extraction but these were found to contain a volatile coating that would contribute a source of error. Therefore, the aluminum pans were baked at 100°C for 24 h, placed in a desiccator for 4 h and then pre-weighed.

In each experiment, 20 g of oven dried (70°C, 48 h) plant material was used.

In the experiments involving sequential extractions, the marc was dried for 4 h at 100°C between each extraction to remove the solvent. The extract was placed in a pre-weighed aluminum pan and evaporated in an externally vented oven. Cyclohexane, benzene and heptane extracts were evaporated at 100°C for 24 h and then placed in a desiccator for 4 h before weighing.

Acetone, 95% ethanol, methanol and water extracts were evaporated at 100°C for 48 h and then placed in a desiccator for 4 h before weighing. The extraction thimble, glass wool and marc were dried for 48 h at 100°C and then placed in a desiccator for 4 h before weighing. During extractions with methanol or ethanol, super heating occurred due to high concentrations of solutes in solvents. Therefore, these solvents were removed after 4 h of extraction in order that fresh solvent and new boiling chips could be added. Glas-Col^{R} heating mantles (6 × 250 ml) were used in the Soxhlet extractions with 105 VAC power input. Although some volatiles are lost in the extract drying procedure, these would also likely be lost in a commercial harvesting and field drying process (Adams, 1982).

Extractions with the rotary shaker were performed in 125 ml Erlenmeyer flasks with 20 g of plant material, filled to 100 ml with solvent, and capped with aluminum foil. At the end of each shaking trial, the marc was separated from the liquid by decanting and washed twice with 100 ml of the extracting solvent and the liquids were then combined. In all extraction tests, the marc was dried for 4 h at 100°C between using the first and second solvents to prevent the first solvent from influencing the second extraction. The extracts were collected at specified times and the extraction continued using fresh solvent in the time-course studies.

RESULTS AND DISCUSSION

Since extraction times of from 8 h (Nemethy et al., 1978) to 48 h (Buchanan et al., 1978) for each solvent have been used, the first priority was to examine a time-course extraction experiment. The initial system investigated was the acetone/cyclohexane method used by the USDA Laboratory in Peoria, IL (Buchanan et al., 1978). Fig. 1 shows the results of collecting fractions at the end of 4, 8, 12, 20, 32, and 44 h for both the acetone and cyclohexane extractions (in that order) of *Asclepias latifolia*. Whereas the cyclohexane extraction appears to as-

TABLE 2. COMPARISON OF SHAKING/DECANTING (18 H) VS. SOXHLET EXTRACTION (20 H).

	Cyclohexane yield	95% ethanol yield	Total yield	C/E[a] ratio	Rel. eff.[b]
Asclepias latifolia					
Shaking	4.58	4.34	8.92	1.06	
Soxhlet	6.37	10.13	16.50	0.63	0.54
Euphorbia marginata					
Shaking	6.89	2.82	9.71	2.44	
Soxhlet	7.53	5.99	13.52	1.26	0.72
Grindelia squarrosa					
Shaking	6.95	2.53	9.48	2.74	
Soxhlet	8.92	4.42	13.34	2.02	0.71
Helianthus annuus					
Shaking	3.41	3.18	6.59	1.07	
Soxhlet	4.98	5.77	10.75	0.86	0.61

[a] Cyclohexane extract yield divided by ethanol extract yield.
[b] Total shaking/decanting extract yield divided by total Soxhlet extract yield.

ymptote between hours 32 and 44, the acetone extraction has appreciable slope in the interval. A time-course extraction curve for a second solvent system (cyclohexane/95% ethanol) is shown in Fig. 2. After 20 h of extraction, both cyclohexane and 95% ethanol had removed more than 95% of the total extracted (44 h).

Comparisons of the extraction efficiency of different solvent systems on *Asclepias latifolia* (Table 1) indicated that cyclohexane/95% ethanol and cyclohexane/methanol were much more efficient than acetone/cyclohexane, acetone/benzene, or heptane/acetone. When acetone/benzene was used, the first solvent (acetone-polar) yielded 7.33% and the second (benzene-nonpolar) yielded only 1.47%. However, when heptane (nonpolar) was used as the first solvent, it resulted in a yield of 6.18% and with acetone (polar) yielding only 1.99%. Nemethy et al. (1978) postulates that using a nonpolar compound as the first solvent removes waxes and allows a more efficient extraction by the polar solvent. This does not appear to be the case with *Asclepias latifolia,* but this species has very little surface wax and may not represent a fair test of the hypothesis.

Although Soxhlet extraction has been used in previous hydrocarbon screen-studies, the shaking/decanting is commonly used in natural product research, particularly for the extraction of flavonoids (Mabry et al., 1970). Due to the large number of extractions that can be carried out simultaneously by shaking/decanting, it was important to investigate this procedure and compare it with Soxhlet extractions. Total yields by shaking have a relative efficiency (compared to the Soxhlet method) of between 0.54 and 0.62 (Table 2). Another interesting aspect is that the ratio of cyclohexane to ethanol fractions (C/E) is not consistent between the shaking and the Soxhlet method. For example, *Asclepias latifolia* yielded approximately equal amounts of the cyclohexane and ethanol fractions by the shaking/decanting method whereas it yielded nearly twice as much ethanol fraction as cyclohexane fraction with the Soxhlet extraction method. The trend among the 4 species (Table 2) is to have greater total yields and relatively greater amounts

TABLE 3. COMPARISONS AMONG SOXHLET EXTRACTIONS USING CYCLOHEXANE FOLLOWED BY ACETONE, 95% AQUEOUS ETHANOL OR METHANOL.[a]

Plant species	Average % yield			
	C	A	E	M
Asclepias latifolia	6.31	1.83	10.13	15.56
Asclepias speciosa	4.56	3.63	16.73	17.84
Euphorbia lathyris	6.61	1.46	7.38	19.13
Euphorbia marginata	9.10	2.27	6.36	12.41
Grindelia squarrosa	12.00	3.77	4.58	10.52
Helianthus annuus	5.23	2.01	5.77	15.18
Juniperus monosperma	14.38	6.13	17.72	24.10

[a] C = cyclohexane, A = acetone, E = 95% aqueous ethanol, M = methanol.

of the ethanol fraction by the Soxhlet method. In addition, the shaking/decanting method presents a problem when decanting in that some of the very fine material (from grinding) must be filtered out after decanting. This fine material presents a problem in handling samples and quantifying yields accurately.

Since the preliminary experiments indicated that methanol extracted more material than ethanol (Table 1) for *Asclepias latifolia,* several species were chosen for additional comparisons. The general trend (Table 3) shows that methanol removes from about 50% more (*A. latifolia*) to over 250% more (*Helianthus annuus*) polar material than ethanol. Although the fuel and chemical feedstock value of the polar fraction is not known at present (Lipinsky, 1981; Buchanan et al., 1980), it would appear that if plants are transported to an extraction facility, one might want to extract as much material as possible for use as special products. Additional extraction should also reduce the toxicity of the marc. This is an important consideration in evaluating the marc for livestock feed.

A time-course extraction of *Asclepias latifolia* with cyclohexane/methanol (Fig. 3) shows that methanol did not reach an asymptote after 44 h of extraction. This would lead one to expect that methanol would not be as efficient as ethanol (Fig. 2) since ethanol nears the asymptote at about 22 h. This is not the case however (Table 3). In every taxon, methanol extracted greater amounts than ethanol.

In view of the differences among the various extraction method efficiencies, Soxhlet extraction with cyclohexane followed by methanol for 20 h each was selected. Recent experiments (Adams, 1982) have shown that hexane can be substituted for cyclohexane.

Eighty species of plants were examined by the cyclohexane/methanol Soxhlet procedure. Percent yields for each solvent and total extractives are reported in Table 4. From these results, it is clear that there is considerable variation in quantity of total extractables, from a low of 8.30% to a high of 52.55%. Furthermore, most species gave roughly 15–25% of total extractables with the major portion composed of the more polar materials (methanol fraction). Five of the 80 species examined gave yields in excess of 30%: *Baccharis neglecta,* 32.55%; *Ilex glabra,* 32.20%; *Juniperus monosperma* (leaves), 38.48%; *Sapium sebiferum* (leaves), 32.70%; and *Rhus glabra,* 52.55%. Only 4 species gave cyclohexane extractable yields in excess of 10%: *Grindelia squarrosa,* 12.0%; *Juniperus mon-*

TABLE 4. YIELDS OF CYCLOHEXANE AND METHANOL EXTRACTIONS.

Family	Genus-species	Voucher	Cyclohexane %	Methanol %	Total %
Acanthaceae	*Hygrophila lacustris* (Schlecht. & Cham.) Nees	*McDaniel 24689, MISS*	2.10	27.15	29.25
Agavaceae	*Yucca glauca* Nutt. ex Fraser	*Adams 2903, SRCG*	1.45	15.98	17.43
Amaranthaceae	*Amaranthus arenicola* I.M. Johnston	*Adams 2893, SRCG*	1.15	15.14	16.29
Anacardiaceae	*Rhus glabra* L.	*Stewart 1092, MISS*	6.35	46.20	52.55
Apiaceae	*Chaerophyllum tainturieri* Hook.	*Searcy 1272, MISS*	3.25	12.90	16.15
	Eryngium yuccifolium Michx.	*Stewart 1081, MISS*	1.80	14.95	16.75
	Oxypolis filiformis (Walt.) Britt.	*McDaniel 24402, MISS*	5.20	9.75	14.95
	Torilis arvensis (Huds.) Link	*Stewart 1031, MISS*	4.00	16.15	20.15
	Trepocarpus aethusae Nutt. ex D.C.	*Stewart 1032, MISS*	7.45	20.08	27.53
Apocynaceae	*Trachelospermum difforme* (Walt.) Gray	*Stewart 1094, MISS*	3.65	16.40	20.05
Aquifoliaceae	*Ilex glabra* (L.) Gray	*McDaniel 24693, MISS*	4.50	27.70	32.20
Asclepiadaceae	*Asclepias latifolia* Raf.	*Adams 2664, SRCG*	6.31	15.56	21.87
	A. pumila (Gray) Vail	*Adams 2897, SRCG*	4.78	18.47	23.25
	A. speciosa Torr.	*Adams 2909, SRCG*	4.72	18.67	23.39
	A. syriaca L.	*Adams 3095, SRCG*	4.23	18.87	23.11
	A. tuberosa L.	*Cole 2091, SRCG*	4.10	20.70	24.80
	Calotropis procera (Ait.) R. Br.	*Adams 3116, SRCG*	4.66	16.78	21.44
Asteraceae	*Artemisia filifolia* Torr.	*Adams 2973, SRCG*	4.49	20.01	24.50
	Aster praealtus Poir.	*McDaniel 24691, MISS*	3.45	10.70	14.15
	Baccharis neglecta Britt.	*Adams 3121, SRCG*	5.20	27.35	32.55
	Carphephorus odoratissimus (J.F. Gmel.) Herbert	*McDaniel 24512, MISS*	7.05	11.95	19.00
	Conyza canadensis (L.) Cronq.	*Adams 2900, SRCG*	3.01	10.94	13.95
	Coreopsis tinctoria Nutt.	*Stewart 1029, MISS*	3.40	21.65	25.05
	Erigeron annuus (L.) Pers.	*Stewart 1079, MISS*	4.40	16.75	21.15
	E. philadelphicus L.	*Stewart 1270, MISS*	3.50	19.05	22.55
	Eupatorium ivifolium L.	*McDaniel 24586, MISS*	2.75	9.45	12.20
	Grindelia squarrosa (Pursh) Dunal	*Adams 2894, SRCG*	12.00	10.52	22.52
	Helianthus annuus L.	*Adams 2895, SRCG*	5.23	15.18	20.41
	Liatris spicata (L.) Willd.	*McDaniel 24353, MISS*	6.85	7.20	14.05
	L. squarrosa (L.) Michx.	*McDaniel 24356, MISS*	3.35	10.70	14.05
	Machaeranthera tanacetifolia (H.B.K.) Nees	*Adams 2904, SRCG*	5.87	14.21	20.10
	Rudbeckia hirta L.	*Stewart 1084, MISS*	2.55	12.00	14.55
	Senecio glabellus Poir.	*Searcy 1268, MISS*	1.20	12.65	13.85
	Solidago microcephala (Greene) Bush	*McDaniel 24694, MISS*	7.45	16.70	24.15
	Xanthium strumarium L.	*McChesney 57, MISS*	3.50	15.90	19.40
Betulaceae	*Betula nigra* L.	*Stewart 1093, MISS*	3.50	16.45	19.95
Cactaceae	*Mammillaria heyderi var. meiacantha* (Engelm.) L. Benson	*Weedin 1262, SRCG*	12.82	4.59	17.41
Caprifoliaceae	*Sambucus canadensis* L.	*Stewart 1087, MISS*	8.15	13.05	21.40
Caryophyllaceae	*Stellaria media* (L.) Vill.	*Searcy 1267, MISS*	4.50	13.55	18.05
Chenopodiaceae	*Kochia scoparia* (L.) Schrad.	*Adams 2896, SRCG*	1.04	11.85	12.89
	Salsola kali L.	*Adams 2898, SRCG*	0.96	18.05	19.01
Clusiaceae	*Hypericum galioides* Lam.	*McDaniel 24686, MISS*	4.15	17.20	21.35
	H. gentianoides (L.) B.S.P.	*McDaniel 24354, MISS*	5.10	23.45	28.55
Cornaceae	*Cornus stricta* Lam.	*McDaniel 24393, MISS*	4.40	13.95	18.35
Cucurbitaceae	*Cucurbita foetidissima* H.B.K.	*Adams 2906, SRCG*	2.33	16.41	18.74
Cupressaceae	*Juniperus monosperma* (Engelm.) Sarg.	*Adams 2905, SRCG*	14.38	24.10	38.48
Cyperaceae	*Rhynchospora corniculata* (Lam.) Gray	*McDaniel 24398, MISS*	1.55	17.15	18.70
	Scleria pauciflora Muhl. ex Willd.	*McDaniel 24343, MISS*	2.80	16.80	19.60
Cyrillaceae	*Cyrilla racemiflora* L.	*McDaniel 24405, MISS*	5.60	20.25	25.85
Ericaceae	*Rhododendron serrulatum* (Small) Millais	*McDaniel 24411, MISS*	3.55	11.60	15.15
Euphorbiaceae	*Euphorbia lathyris* L.	*Adams 2911, SRCG*	6.61	19.13	25.74
	E. marginata Pursh	*Adams 2907, SRCG*	9.10	12.41	21.51
	Sapium sebiferum (L.) Roxb.	*McDaniel 24688, MISS*	10.30	22.40	32.70
	Sebastiania fruticosa (Bartr.) Fern.	*McDaniel 24396, MISS*	3.95	24.30	28.25
Fabaceae	*Melilotus alba* Medic.	*Stewart 1096, MISS*	1.65	22.75	24.40
	Psoralea psoralioides (Walt.) Cory	*Stewart 1073, MISS*	4.90	16.51	21.41
	Tephrosia virginiana (L.) Pers.	*Stewart 1080, MISS*	3.85	10.95	14.80
Geraniaceae	*Geranium carolinianum* L.	*Searcy 1273, MISS*	2.05	9.70	11.75
Hydrophyllaceae	*Hydrolea quadrivalvis* Walt.	*McDaniel 24413, MISS*	3.45	19.10	22.55

TABLE 4. (CONTINUED)

Family	Genus-species	Voucher	Cyclohexane %	Methanol %	Total %
Lamiaceae	*Calamintha georgiana* (Harper) Shinners	*McDaniel 24692, MISS*	1.65	6.65	8.30
	Hyptis alata (Raf.) Shinners	*McDaniel 24695, MISS*	1.95	8.00	9.95
	Pycnanthemum tenuifolium Schrad.	*Stewart 1089, MISS*	3.90	16.90	20.80
Lauraceae	*Persea palustris* (Raf.) Sarg.	*McDaniel 24395, MISS*	5.55	15.55	21.10
Loasaceae	*Mentzelia decapetala* (Pursh) Urban & Gilg	*Adams 2902, SRCG*	1.65	21.94	23.59
Martyniaceae	*Martynia louisianica* P. Mill.	*Adams 2972, SRCG*	4.44	19.08	23.52
Nymphaeaceae	*Nuphar luteum* (L.) Sibthorp & Sm.	*McDaniel 24690, MISS*	2.10	13.90	16.00
Onagraceae	*Ludwigia decurrens* Walt.	*McDaniel 24399, MISS*	1.75	10.80	12.55
Plantaginaceae	*Plantago virginica* L.	*Searcy 1277, MISS*	0.40	13.29	14.69
Poaceae	*Sorghum vulgare* Pers.	*Adams 2992, SRCG*	1.02	18.89	19.91
Ranunculaceae	*Ranunculus bulbosus* L.	*Searcy 1276, MISS*	1.00	21.75	22.75
Rubiaceae	*Galium aparine* L.	*Searcy 1274, MISS*	0.85	26.85	27.70
Scrophulariaceae	*Micranthemum umbrosum* (Walt.) Blake	*McDaniel 24400, MISS*	5.05	14.35	19.40
Smilacaceae	*Smilax laurifolia* L.	*McDaniel 24412, MISS*	3.75	14.95	18.70
Solanaceae	*Solanum elaeagnifolium* Cav.	*Adams 2899, SRCG*	2.00	10.88	12.88
Styracaceae	*Halesia diptera* Ellis	*McDaniel 24397, MISS*	5.50	16.50	22.00
Symplocaceae	*Symplocos tinctoria*(L.) L'Her.	*McDaniel 24394, MISS*	9.50	7.70	17.20
Tamaricaceae	*Tamarix ramosissima* Ledeb.	*Adams 2901, SRCG*	1.47	18.00	19.47
Typhaceae	*Typha latifolia* L.	*Stewart 1030, MISS*	2.50	13.70	16.20
Valerianaceae	*Valerianella radiata* (L.) Dufr.	*Searcy 1269, MISS*	3.35	16.30	19.65
Verbenaceae	*Verbena brasiliensis* Vell.	*Stewart 1100, MISS*	3.95	13.65	17.60

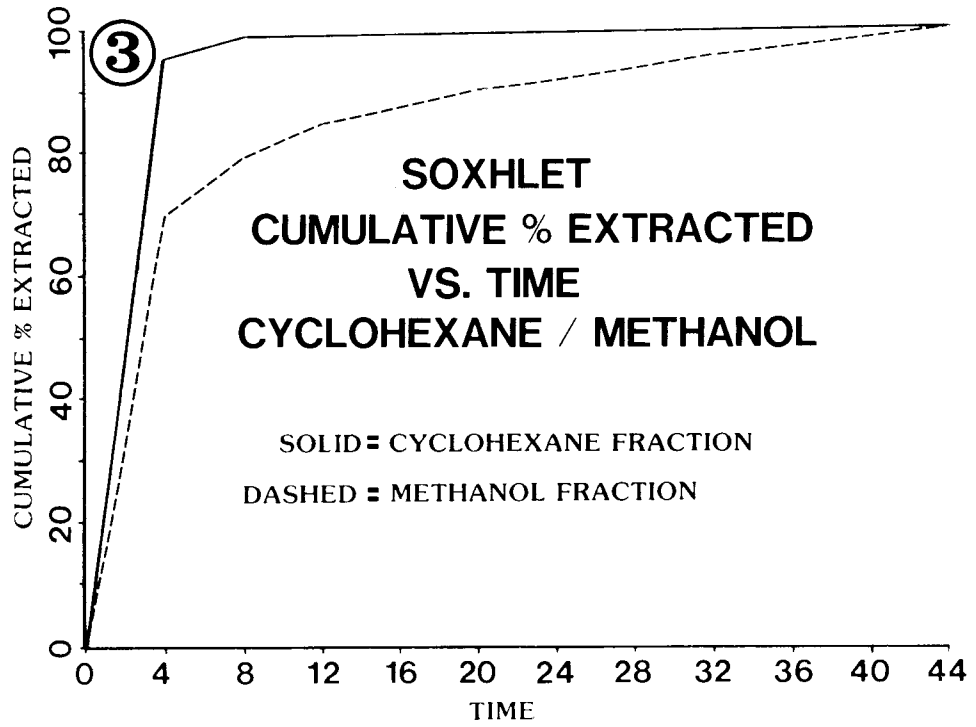


Fig. 3. Time-course experiment of the cumulative % extracted vs. time for *Asclepias latifolia*. Cyclohexane was the first solvent used, followed by methanol. Although methanol removes more material than ethanol after 20 h (Table 1), the extraction curve is much flatter suggesting that an additional class of semisoluble components is being extracted.

osperma (leaves), 14.38%; *Mammillaria heydei* var. *meiacantha,* 12.82%; and *Sapium sebiferum* (leaves), 10.30%. The nonpolar fraction appears to be the most readily hydrocracked by the zeolite catalyst procedure (Haag et al., 1980). These or closely-related species may prove viable sources of fuels. The methanol fraction generally contains fermentable compounds which could be used in alcohol production (Adams, 1982; Calvin, 1979) as well as sources of compounds for chemical feedstocks.

CONCLUSION

The Soxhlet extraction methods appear to be superior to shaking/decanting. Soxhlet extraction with cyclohexane/methanol achieved the highest yields followed by cyclohexane/95% ethanol. It is estimated that more than 95% of the extractables are removed in 44 h Soxhlet extraction with cyclohexane and then with methanol, and about 90% of the available extractives are removed in 20 h of cyclohexane followed by 20 h of methanol. For initial plant screening, it appears that a 20 h cyclohexane Soxhlet extraction followed by a 20 h methanol Soxhlet extraction represents a good compromise between yield and time constraints.

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