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Forest Ecology and Management, 16 (1986) 155–167
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

Renewable Resources from Arizona Trees and Shrubs*

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(Accepted 11 March 1986)

ABSTRACT

Carr, M.E., Mason Jr., Ch.T. and Bagby, M.O., 1986. Renewable resources from Arizona trees and shrubs. *For. Ecol. Manage.*, 16: 155–167.

Plants of 100 tree and shrub species from arid and semiarid regions of Arizona were examined botanically and chemically for their multipurpose potential as renewable sources of industrial raw materials. Analytical and botanical features are presented here for only 12 of the more promising species. Samples of the 100 species were analyzed for yields of oils, polyphenols, hydrocarbons, and crude proteins. Classes of oil constituents were identified. Contents of fatty acids and unsaponifiable matter of saponified oils were determined. Hydrocarbons were analyzed for the presence of rubber and gutta (polyisoprenes). Polyisoprenes were analyzed for average molecular weight and molecular weight distribution. Impressive yields of oil and/or polyphenol were extracted from *Asclepias linaria*, *Juglans major*, *Ligustrum japonicum*, *Mimosa biuncifera*, *Mortonia scabrella*, *Olea europaea*, and *Rhus choriophylla*. Of these, *O. europaea* and *L. Japonicum* of the Oleaceae are rapid-growing, evergreen species with low to moderate moisture requirements and very good adaptability to pollarding.

INTRODUCTION

Within the past decade, increased attention has been given to the need of developing plant species as alternative sources of fuels, chemicals, feeds, and other important materials. Worldwide supplies of natural gas and petroleum

*Presented at the Symposium on Establishment and Productivity of Tree Plantings in Semi-Arid Regions, Proceedings of the 4th International Symposium of the Caesar Kleberg Wildlife Institute, Texas A&I, Kingsville, TX, April 29—May 3, 1985.
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could be depleted by the end of this century, or shortly thereafter, and coal reserves could be exhausted within the next century, if used as a primary source of energy (Knowles et al., 1984). Presently, U.S. food crops are grown far in excess of domestic needs. New nonfood crop developments could have many benefits as well as reduce our nation's dependency upon foreign sources of strategic and essential materials (Knowles et al., 1984; Wheaton et al., 1984).

Physical, chemical, and botanical characteristics of plants are investigated at the Northern Regional Research Center in efforts to identify species with potential as new, multipurpose, energy-producing crops (Bagby et al., 1980; Buchanan et al., 1980; Carr, et al., 1985). Currently, plant species from Arizona are being studied in this program for the first time. New crop developments in underused or marginal land such as those in semiarid regions of the Southwest could stimulate industrial and economic growth without significantly competing with established crops (Bungay, 1982; Calvin, 1983; Knowles et al., 1984; Wheaton et al., 1984). In this report, botanical as well as chemical characterization data are presented for 12 of 100 Arizona species randomly collected. These 12 species yielded considerable amounts of acetone- and hexane-extractable materials and may have potential as renewable sources of raw materials in limited supply. Chemical and botanical data are discussed with respect to species previously analyzed in this program.

EXPERIMENTAL

Samples of the 12 trees and shrubs selected for discussion were collected by cutting the latest 2 to 3 years' growth during the fall of 1982 and the fall of 1983. All samples were collected closely after reaching maturity without damage. Each sample included all portions of the branches removed (stems, leaves, and fruits). Samples were collected by Robert W. Hoshaw, Professor, College of Arts and Sciences, Department of Ecology and Evolutionary Biology, University of Arizona and by Charles T. Mason, Jr., Curator of Herbarium, College of Agriculture, University of Arizona, Tucson, AZ. Voucher specimens of all species were prepared by the collectors and are filed in the USDA Northern Regional Research Center herbarium.

Each sample was allowed to dry in a sheltered area at ambient conditions in Arizona. The entire quantity of each sample collected (about 500–1000 g, moisture-free basis) was milled in a Wiley mill equipped with a screen containing 1-mm-diameter holes. A subsample of the milled material was analyzed for moisture, ash, and crude protein (% Kjeldahl nitrogen $\times$ 6.25). A subsample (about 100 g) of each milled sample was extracted in a Soxhlet apparatus, first with acetone for 48 h and then with hexane for 48 h. Acetone was allowed to evaporate from the plant residue before hexane extraction. The acetone-extracted fraction was partitioned between hexane and water/ethanol (1/7) to obtain 'oil' and 'polyphenol', respectively. Hexane extraction after acetone

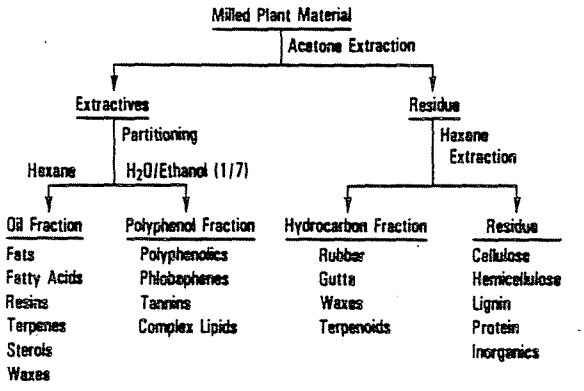

Fig. 1. Extraction and separation of plant fractions.

extraction yielded the 'hydrocarbon' fraction. Yields were determined gravimetrically after solvent removal and oven drying and were reported as percents of the moisture-free and ash-free plant sample weight.

Oils were examined for classes of constituents by thin-layer chromatography (TLC) using a standard mixture of sitosterol (a fatty acid), oleyl alcohol (a higher alcohol than sitosterol), oleic acid (a fatty acid), triolein (a triglyceride), oleyl laurate (a nonglyceride ester), and squalene (a hydrocarbon) as reference. Oils were saponified by conventional procedures (Cocks and Van Rede, 1966). Saponified oils were partitioned and analyzed gravimetrically for organic acids and unsaponifiable material. Hydrocarbons fractions were examined by infrared (IR) spectroscopy for the presence of rubber, gutta, and waxes. Average molecular weight ($\bar{M}_w$) and Mw distribution (*MWD*) were determined by gel permeation chromatography (GPC) (Swanson et al., 1979). Details of procedures used in the extractions, partitioning, and various analyses have been reported recently (Carr, 1985).

RESULTS AND DISCUSSION

Several schemes for new and/or improved utilization of plant materials for nonfood uses have been reviewed recently (Nemethy, 1984). In 1974 a program was developed at this Center and is still used for examining 'whole-plant' species as potential multipurpose oil- and hydrocarbon-producing crops. The term 'whole-plant' refers, generally, to the entire above-ground plant. However, trees and most shrubs have been necessarily sampled by collecting the latest 1 to 3 years' growth. Although such collections do not represent the 'whole-plant', they more closely represent portions of the plant that probably would be harvested in context of this program.

Figure 1 is a flow chart of the steps used to obtain plant fractions referred to as 'oil', 'polyphenol', and 'hydrocarbon' (see Experimental). Compositional

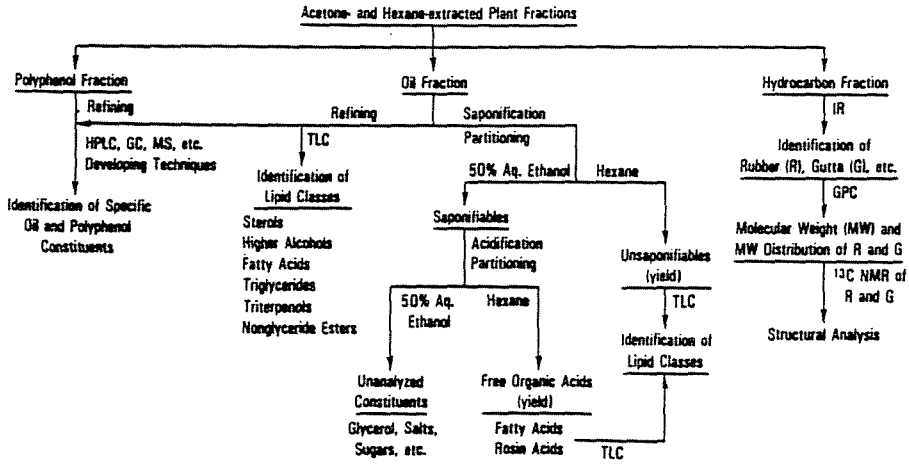

Fig. 2. Characterization of extracted plant constituents.

complexities of these fractions are exemplified in this scheme. Utilization of such constituents could have considerable economic and strategic value (Bagby et al., 1980; Buchanan et al., 1980; Bungay, 1982; Calvin, 1983; Knowles et al., 1984; Wheaton et al., 1984).

Figure 2 outlines our scheme for analyzing the oils, polyphenols, and hydrocarbons. Up until now, oils have been characterized with respect to classes of compounds. Now, oils and polyphenols of selected species are being analyzed for specific compounds. Polyisoprenes of the hydrocarbon fractions were analyzed for weight-average molecular weight ($\bar{M}_w$) and molecular weight distribution (*MWD*). None of the hydrocarbon fractions discussed in this report was examined by ^{13}C nuclear magnetic resonance spectroscopy (NMR), which is used sometimes for conformation and structural characterization of gutta (*trans*-1,4-polyisoprene).

One scheme for additonal treatment of plant materials after removal of readily extractable fractions is presented in Fig. 3 to illustrate a concept for total biomass utilization. The plant materials can be further fractionated by an acid-catalyzed, aqueous-phenol extraction method (Johansson et al., 1982). This treatment solubilizes hemicellulose (aqueous phase) and lignin (phenolic phase). Phenol-extracted lignin can be readily separated and burned as a fuel or converted to phenol per se and/or other chemicals. Xylose, a 5-carbon sugar, is the principal hydrolyzate from hemicellulose exemplified in the scheme. This sugar can be fermented to ethanol (Slininger et al., 1982) or converted to furfural, an important industrial chemical. The concentrated, insoluble, cellulose-rich residue could be used as a high-energy animal feed or could be hydrolyzed to glucose. Cellulose of the residue is highly accessible to enzymatic hydrolysis (Johansson et al., 1982). The glucose hydrolyzate could be used,

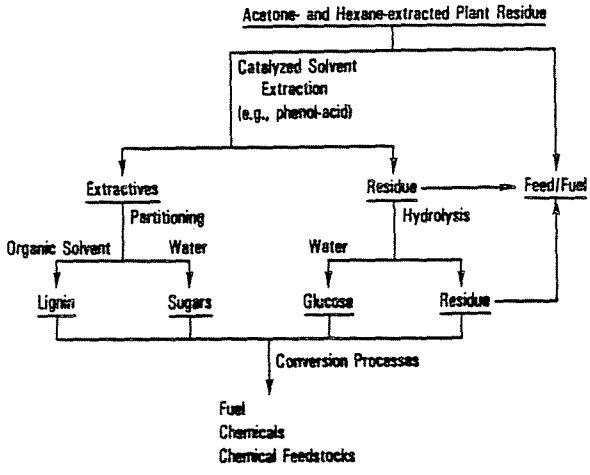

Fig. 3. Treatment of lignocellulosic residues after removal of oils, polyphenols, and hydrocarbon.

for example, to produce fuel ethanol, acetone/butanol, 2,3-butanediol, or glycerol.

Botanical and chemical features of selected species

Some botanical features of six Arizona plants with growth rates ranging from 0.5 to 1 m y^{-1} are presented in Table 1. All of these plants, except *Asclepias linaria* Cav., are culviated, and all would be expected to have considerable variations in growth rates, dependent upon a host of physical and chemical environmental factors. Yields of extracts from samples of these plants are shown in Table 2. Botanical and chemical features are discussed collectively.

Asclepias linaria Cav. is a low-growing subshrub (sometimes herbaceous), which requires little moisture and is commonly found on dry, rocky slopes and mesas of southern Arizona (and Mexico). Little is known about its adaptability to pollarding. However, stems die back to the ground and typically regenerate. Specimens of this species gave the highest yield of oil (8.7% on a moisture-free, ash-free sample weight basis) of the 100 Arizona tree and shrub species analyzed and, also, gave a very substantial amount of hydrocarbon (1.9%). Eight other *Asclepias* species previously analyzed in this program have yielded significant amounts of hydrocarbon, but none has given this much oil. Hydrocarbon of *Asclepias linaria* was primarily rubber with a weight-average molecular weight ($\bar{M}_w$) of 101,000 and a molecular weight distribution (*MWD*) of 5.5. *MWD* is $\bar{M}_w/\bar{M}_n$, in which $\bar{M}_n$ is the number-average molecular weight (Swanson et al., 1979). Such low-molecular-weight rubbers, compared to guayule rubber (1,310,000, Swanson et al., 1979) could be used, for example, in processing rubber and adhesive products or as hydrocarbon feedstocks (Buch-

TABLE 1

Botanical characteristics of selected Arizona trees and shrubs

Family Species Common name	Voucher number and collection site[a]	Life cycle[b] and habit	Growth rate, m/y[c] (height, m)	Moisture need	Adaptability to pollarding[d]	Horticultural zone and primary geographic distribution in U.S.	
						Zone	Distribution
Anacardiaceae							
Schinus molle L. California peppertree	80852 adobe soil, cultivated	Evergreen tree	1 (7-15)	Moderate	Excellent	9	SW states
Rhus lancea L. f. African sumac	80636 adobe soil, cultivated	Evergreen tree	1 (5-8)	Low to moderate	Good	8	SW states
Asclepiadaceae							
Asclepias linaria Cav. None	80176 rocky soil, near stream, wild	Subshrub	0.5 (0.5-1)	Low	Good	7,8	S states
Caprifoliaceae							
Viburnum suspensum Lindl. Sandankwa viburnum	80628 loamy soil, cultivated	Evergreen shrub	0.5 (2-2.5)	Moderate	Excellent	8	S and SW states
Oleaceae							
Ligustrum japonicum Thunb. Japanese privet	80733 adobe soil, cultivated	Evergreen shrub	1 (3-4)	Moderate	Very good	8-10	S states
Olea europaea L. Common olive	80745 adobe soil, cultivated	Evergreen tree	1 (5-15)	Moderate to low	Very good	8-10	S CA, S AZ

[a]All species were from Pima County, Arizona. Voucer specimens are kept at the Northern Regional Research Center herbarium, Peoria, IL.
[b]Perennials.
[c]Estimated average growth rate for young trees and shrubs. Specific environmental conditions are available from the authors.
[d]Arbitrary qualitative ratings of poor, fair, good, very good, and excellent were used, based on collectors observations. Quantitative information was not available.

TABLE 2

Yields of extracts and protein for selected Arizona trees and shrubs[a,b]

Species	Growth rate	Extracts			Total extracts (%)	Crude protein[c] (%)	Total extracts plus protein (%)
		Oil (%)	Poly-phenol (%)	Hydro-carbon (%)			
Asclepias linaria	Moderate	8.7	11.7	1.9	22.3	8.5	30.8
Ligustrum japonicum	Rapid	5.3	27.4	0.2	32.9	13.6	46.5
Olea europaea	Rapid	2.6	29.4	0.1	32.1	7.3	39.4
Rhus lancea	Rapid	3.3	18.1	0.1	21.5	9.6	31.1
Schinus molle	Rapid	4.7	9.5	0.1	14.3	11.6	25.9
Viburnum suspensum	Moderate	4.8	22.6	0.2	27.6	9.0	36.6

[a]See Table 1.
[b]Yields are % of the plant sample weight (moisture-free and ash-free basis).
[c]% Kjeldahl nitrogen × 6.25.

anan et al., 1980). Yields of polyphenol (11.7%) and crude protein (8.5%) for *Asclepias linaria* were moderate.

Ligustrum japonicum Thunb. is a rather large, evergreen shrub, which is cultivated throughout the southern states. Shrubs grow well after pruning, particularly if grown in light garden soil with moderate amounts of water. This species yielded a very high combined amount of oil (5.3%) plus polyphenol (27.4%), a substantial amount of apparent protein (13.6%), and the greatest total yield of extracts plus protein (46.5%). The hydrocarbon yield was low (0.2%). In our screening program, it has been rare for any one species to yield a high amount of oil as well as a high amount of polyphenol. Such species previously analyzed here include *Populus tremuloides* Michx. (22% polyphenol + 5.0% oil) and *Rhus glabra* L. (20.2% polyphenol + 5.9% oil).

Olea europaea L. is a broad-leaved, evergreen tree, native to the Mediterranean region, which is well known commercially in many countries for its fruit (common olive) and as an ornamental. This species is very resistant to heat and drought, needs good drainage and grows well in hot interior valleys of southern California and southern Arizona. It occasionally establishes itself as in Napa, California. The trees appear to be very adaptable to pollarding, especially when young. Maximum tree heights are quite variable (5–15 m). *Olea europaea* gave the highest polyphenol yield (29.4%) of the 100 Arizona tree and shrub species examined. However, yields of oil, hydrocarbon, and protein were very moderate. We have previously reported a polyphenol yield of 33.2% for *Parkinsonia aculeata* L., which has been studied extensively by others for various reasons (Everitt, 1983; Felker et al., 1983; Thorn et al., 1983).

Viburnum suspensum Lindl. is an evergreen shrub with moderate moisture needs and, probably, excellent adaptability to pollarding. This species yielded

substantial amounts of oil (4.8%) and polyphenol (22.6%). Extracts from this species yielded known flavonol glycosides and an acetylated coumarin glucoside (Iwagawa and Hase, 1984).

Rhus lancea L.f. is a fairly large evergreen tree with a broad, spreading top, which has become naturalized in the Southwest. It tolerates a wide range of soil and moisture conditions but needs good drainage. Growth rate is fast when young, and the species appears to be very adaptable to pollarding. Some *Rhus* species (e.g., *R. typhina* L. and *R. glabra* L.) contain high concentrations of tannins, useful as tanning and adhesive agents (Buchanan et al., 1980). *R. lancea* had a substantial amount of polyphenol (18.1%), a moderate amount of oil (3.3%), an average amount of protein (9.6%), and very little hydrocarbon (0.1%). Of nine *Rhus* species previously analyzed by us, most have given significant amounts of polyphenol. Recently, new flavonoids from the leaves of *R. lancea* have been characterized (Ramachandran et al., 1983).

Schinus molle L. is a large, evergreen tree with an excellent growth rate, especially after pruning. This species originated from Peru and has been grown as an ornamental tree for at least 100 years in southern California. It establishes in grassland oil fields, coastal sage scrub, and disturbed coastal chaparral of southern California. Ability of this species to naturalize in comparison to *Schinus terebinthifolius* Raddi (a related taxon) has been studied recently (Nilsen and Muller, 1980). Fruit of this species yields a volatile oil that has been used in pharmaceutical and flavoring products and as a substitute for black pepper. Fatty acids, enzymes, and volatile constituents of the fruit oil produced by *Schinus molle* have been studied recently (Diamantoglou and Kull, 1981; Bar-Nun et al., 1981; Bernhard et al., 1983). In our work, this species yielded a noteworthy amount of oil but had the lowest total yield of extractives of species listed in Table 2.

Tables 3 and 4 show botanical and chemical information for six species that are relatively slow-growing compared to those listed in Table 1. Of these species, only *Pittosporum tobira* (Thunb.) Ait. is cultivated. All six species yielded substantial quantities of extractives. *Rhus choriophylla* is an evergreen tree (or shrub), which can be found on rocky slopes, often on limestone. Its adaptability to pollarding may not be good. However, chemically this species was outstanding with respect to high yields of both oil (7.0%) and polyphenol (20.0%) from a single species (Table 4). The hydrocarbon of this species was a mixture of rubber and waxes with molecular weights of $<10,000$.

Juglans major (Torr.) A. Heller, the well-known Arizona walnut tree, contained high oil content (7.0%) but moderate contents of polyphenol, hydrocarbon, and protein.

Mortonia scabrella Gray is a native evergreen shrub with low moisture requirements and is found on dry plains, mesas, and slopes. Its adaptability to pollarding is unknown. This species is noteworthy in this study not only for its high yield of oil (6.6%) but perhaps also for its multipurpose potential

TABLE 3

Botanical characteristics of some relatively slow-growing Arizona trees and shrubs[a]

Family Species Common Name	Voucher number and collection site[b]	Habit	Growth rate, m/y (height, m)	Moisture need	Adaptability to pollarding	Horticultural zone and primary geographic distribution in U.S.	
						Zone	Distribution
Anacardiaceae							
Rhus choriophylla Woot. and Standl. Evergreen sumac	80366 gravel road hillside open area	Evergreen tree or shrub	0.2 (2-3)	Low to moderate	Poor	6-8	W Texas, S New Mexico, SE Arizona
Celastraceae							
Mortonia scabrella Gray Utah mortonia	80883 rocky limestone hillside	Evergreen shrub	0.15 (1-2)	Low	Unknown	7,8	W Texas to S Arizona
Juglandaceae							
Juglans major (Torr.) A. Heller Arizona walnut	80221 sandy river bottom, dry surface	Deciduous tree	0.3 (12-15)	Moderate	Probably not good	6-8	W Texas, New Mexico, Arizona
Leguminosae							
Cercidium microphyllum (Torr.) Rose & Johnson Littleleaf palo verde	80742 rocky foothills, open	Deciduous tree	0.3-0.4 (to 8)	Moderate	Poor to fair	8-10	S and W Arizona, S California
Mimosa biuncifera Benth. Wait-a-minute bush	80744 rocky hillside, slight drainage	Deciduous shrub	0.2 (1-2)	Low	Good	7	W Texas to SE Arizona
Pittosporaceae							
Pittosporum tobira (Thunb.) Ait. Japanese mock orange	80383 adobe soil, cultivated	Evergreen shrub	0.15 (2-5)	Moderate	Good	8-10	Throughout S. states

[a]Growth rates are less than those shown in Table 1. See footnote c, Table 1.
[b]Arizona counties were Santa Cruz for 80366; Cochise for 80883; Ima for 80221, 80742, and 80744; Maricopa for 80383.

TABLE 4

Yields of extracts and protein for some relatively slow-growing Arizona trees and shrubs[a]

Species	Extracts			Total extracts (%)	Crude protein (%)	Total fractions (%)
	Oil (%)	Poly-phenol (%)	Hydro-carbon (%)			
Cercidium microphyllum	4.9	11.8	0.2	16.9	10.4	27.3
Juglans major	7.0	9.4	0.2	16.6	10.4	27.0
Mimosa biuncifera	1.5	28.7	0.1	30.3	11.7	42.0
Mortonia scabrella	6.6	16.0	0.7	23.3	7.5	30.8
Pittosporum tobira	4.1	5.1	2.3	11.5	8.8	20.3
Rhus choriophylla	7.0	20.0	0.4	27.5	7.0	34.3

[a]See Table 3, footnote a. See Table 2, footnotes a and b for yield basis.

(16.0% polyphenol and 0.7% hydrocarbon). Its hydrocarbon was rubber with an $\bar{M}_w$ of 168,000 ($MWD = 3.3$).

Mimosa biuncifera Benth. is a deciduous shrub, which requires little moisture and thrives well in dry mesas, rocky slopes, and chaparral. This species is an efficient soil binder and forms dense thickets that serve as forage for livestock. Its adaptability to pollarding is expected to be good. *Mimosa biuncifera* was the second highest polyphenol-yielding (28.7%) species of Arizona trees and shrubs analyzed. Crude protein content was the highest of the six species listed.

Pittosporum tobira (Thunb.) Ait. is an ornamental, evergreen shrub that is cultivated throughout the southern states and used for landscaping (Chase, 1981; Ingram et al., 1981). This species was noteworthy for yielding a substantial amount of hydrocarbon (2.3%) plus a moderate amount of oil (4.1%). Very few species yield more than 1% hydrocarbon. Rubber of this hydrocarbon had a very low molecular weight ($\bar{M}_w = 27{,}400$).

Cercidium microphyllum (Torr.) Rose and Johnson is a spiny, bushy, deciduous tree that is found in well-drained terrain such as gravelly and rocky slopes. This species yielded significant quantities of oil (4.9%), polyphenol (11.8%), and crude protein (10.4%).

Oil constituents

Classes of oil constituents of species with 4.7% or more oil are shown in Table 5. All of the listed species contained sterols, free fatty alcohols less polar than sterols, free fatty acids, and one or more types of esters. Oils of *Cercidium microphyllum* and *Juglans major* had nonglyceride (NG) and triglyceride (TG) esters, respectively. Oils of *Ligustrum japonicum* and *Pittosporum tobira* both

TABLE 5

Analyses of whole-plant oils before and after saponification

Species	Oil yield (%)[a]	Classes of oil lipids[b]			Saponified oil fractions	
		Fatty acids alcohols, and sterols	Esters	Hydrocarbon	Unsaponifiable matter yield (%)[c]	Fatty acid yield, (%)[c]
Asclepias linaria	8.7	yes	TT, NG	yes	61	no data
Cercidium microphyllum	4.9	yes	NG	trace	53	35
Ligustrum japonicum	5.3	yes	TG, TT, NG	trace	38	43
Schinus molle	4.7	yes	TG, NG	yes	53	39
Viburnum suspensum	4.8	yes	TG, NG	yes	72	22
Juglans major	7.0	yes	TG	yes	35	57
Pittosporum tobira	6.6	yes	TG, TT, NG	yes	33	38
Rhus choriophylla	7.0	yes	TG, NG	yes	63	28

[a]Yields in percent are on a moisture-free and ash-free plant sample basis.
[b]Classes were indicated by thin layer chromatography. TT = Triterpenol, NG = nonglyceride, and TG = triglyceride esters, respectively.
[c]Percent of oil sample weight (see Experimental).

had three TLC spots for esters, which were NG, TG, and probably triterpenol (TT) esters. *Asclepias linaria* oil exhibited TLC spots for TT and NG esters. The remaining species listed had spots for TG and NG esters. Also, Table 5 shows amounts of fatty acids (FA) and unsaponifiable matter (UM) from oils that were saponified. Five of the 8 oils gave a range of 53–72% UM (% of refined oil sample weight). Most 'whole-plant' oils analyzed here had about 40–60% UM. Few had as much UM (72%) as *Viburnum suspensum*. In general, the plant oils have a higher UM:FA ratio than common vegetable seed oils.

CONCLUSIONS

The development of new-crop biomass for production of fuels, chemicals, and other industrial materials will require a concentrated and steady level of multidisciplinary efforts. The process of selecting wild or cultivated species for new-crop evaluation would involve several basic steps including collections and evaluations of germplasm, agronomic and horticultural evaluations, breeding, chemical and utilization studies, scale-ups, and commercializations. For this report, we randomly collected plant samples of 100 tree and shrub species from Arizona and attempted to identify the relatively promising ones for semiarid regions, primarily, using chemical and botanical criteria estab-

lished at this Center in 1974. *Asclepias linaria, Juglans major, Ligustrum japonicum, Mimosa biuncifera, Mortonia scabrella, Olea europaea,* and *Rhus choriophylla* yielded very substantial amounts of oils, polyphenols, and/or hydrocarbons when compared to some 1000 species examined in this program. These and six other species discussed are possibly worthy of further chemical and botanical study. However, many other species need to be screened, and the high-yielding species need to be analyzed using a considerably broader base of criteria before selecting any species for crop studies. Currently, a program at this Center is being developed to identify, characterize, and utilize specific constituents of selected species.

ACKNOWLEDGEMENTS

We wish to thank Dr. Robert W. Hoshaw for plant sample collections, Charles L. Swanson for GPC information, and D.W. Ehmke for analytical assistance.

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