

LEAF OIL COMPOSITIONS AND BIOACTIVITIES
OF ABACO BUSH MEDICINES

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Summary

Introduction: Four aromatic plants used in traditional “bush” medicine on Abaco Island, Bahamas were studied. *Amyris elemifera* (Rutaceae), “white torch”, is taken as a febrifuge and applied to sores and wounds, to treat influenza, and as an external bath and general tonic. *Eugenia axillaris* (Myrtaceae), “white stopper”, is used as an aphrodisiac, as well as to treat diarrhea and for bathing women after childbirth. *Lantana involucrata* (Verbenaceae), “wild sage”, is used to treat itching of the skin, measles and chicken pox. *Myrica cerifera* (Myricaceae), “bayberry”, is taken as a general tonic and diuretic. **Methods:** The leaf essential oils of the four aromatic plants were obtained by hydrodistillation and analyzed by GC-MS. The antimicrobial activity against *Bacillus cereus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Aspergillus niger*, and the *in-vitro* cytotoxicity of the oils on MDA-MB-231, MCF7, Hs 578T, Hep G2, and PC-3 human tumor cells have also been examined. **Results:** The most abundant components of *Amyris elemifera* were limonene (45.0%) and linalool (20.8%). *Eugenia axillaris* leaf oil was largely composed of α -pinene (15.5%) and dihydroagarofuran (9.2%). The leaf oil of *Lantana involucrata* was made up largely of germacrene D (21.1%), α -humulene (15.2%), and β -caryophyllene (13.7%). The most abundant essential oil components of *Myrica cerifera* were 1,8-cineole (30.7%) and α -terpineol (14.2%). *L. involucrata* leaf oil showed slight antibacterial activity against *B. cereus* and *Staph. aureus* and was weakly cytotoxic against our panel of cell lines. Neither *A. elemifera*, *E. axillaris*, nor *M. cerifera* leaf oils were appreciably antimicrobial or cytotoxic. **Conclusions:** The reported biological activities of the major constituents of *A. elemifera* leaf oil are consistent with the ethnopharmacological uses of this plant. The major components in the leaf oil and slight antimicrobial activity are consistent with the ethnobotanical use of *L. involucrata* to treat itching skin.

Key Words: *Amyris elemifera*, *Eugenia axillaris*, *Lantana involucrata*, *Myrica cerifera*, Bahamas, leaf essential oil, composition, antimicrobial activity, cytotoxic activity.

Introduction

For many generations, people living in remote locations of the Bahamas have utilized “bush medicine” to ameliorate their maladies (1). In this paper, we report on the chemical compositions, antimicrobial activities, and cytotoxic activities, of the leaf essential oils of four aromatic herbal medicines used by the people of Abaco Island, Bahamas. A leaf decoction of *Amyris elemifera* L. (Rutaceae), “white torch”, is taken as a febrifuge and applied to sores and wounds, to treat influenza, and as an external bath and general tonic (2). A decoction of the leafy branch tips of *Eugenia axillaris* (Myrtaceae), “white stopper”, is used for “building up men’s energy and body” (*i.e.*, as an aphrodisiac); it is also used to treat diarrhea and for bathing women after childbirth (1,2). *Lantana involucrata* (Verbenaceae), “wild sage”, is used in the Bahamas to treat itching of the skin, measles and chicken pox (1). In Bahamian bush medicine, a tea made from the leafy branchlets of *Myrica cerifera* (Myricaceae), “wax myrtle” or “bayberry”, is taken as a general tonic and diuretic (2).

Methods

Plant Collection. The plants were collected from Abaco Island, Bahamas, and identified by M. A. Vincent. Essential oils were obtained by hydrodistillation of freshly chopped leaves and dichloromethane extraction of the distillate (Table 1).

Table 1. Essential oil yields of Abaco bush medicines.

Plant	Collection Date and Location	Mass of Leaves	Mass of Oil (% yield)
<i>Amyris elemifera</i>	June 10, 2002 26° 34.5' N, 77° 7.4' W, 1-2 m a.s.l.	209.9 g	7.309 g (3.48%)
<i>Eugenia axillaris</i>	June 10, 2002 26° 34' N, 77° 8' W, 1-2 m a.s.l.	228.9 g	2.642 g (1.15%)
<i>Lantana involucrata</i>	December 18, 2000 26° 31.09' N, 77° 4.26' W, 13 m a.s.l.	190.0 g	125 mg (0.0658%)
<i>Myrica cerifera</i>	June 9, 2002 26° 34.55' N, 77° 8.35' W, 1-2 m a.s.l.	141.8 g	1.772 g (1.25%)

Gas chromatographic-mass spectral analysis. The leaf essential oils were subjected to GC-MS analysis on an Agilent system consisting of a model 6890 gas chromatograph, an HP-5ms GC column, a model 5973 mass selective detector (MSD), and an Agilent ChemStation data system as described previously (3). Identification of oil components was achieved based on their retention indices (determined with reference to a homologous series of normal alkanes), and by comparison of their mass spectral fragmentation patterns (NIST database/ChemStation data system) (4).

Antimicrobial assays. The essential oils and the purified major components were screened for antimicrobial activity against Gram-positive bacteria, *Bacillus cereus* (ATCC No. 14579) and *Staphylococcus aureus* (ATCC No. 29213); Gram-negative bacteria, *Pseudomonas aeruginosa* (ATCC No. 27853) and *Escherichia coli* (ATCC No. 25922); and the fungi *Candida albicans* (ATCC No.10231) and *Aspergillus niger* (ATCC No. 16401). Minimum inhibitory concentrations (MIC) were determined using the microbroth dilution technique (5,6). The antimicrobial activities of the essential oils and components are summarized in Table 2.

Cytotoxicity assays. The essential oils and purified major components were screened for cytotoxic activity against PC-3 (human prostatic adenocarcinoma), MDA-MB-231 (human mammary adenocarcinoma), MCF7 (human mammary adenocarcinoma), Hs 578T (human ductal carcinoma), and Hep G2 (human hepatocellular carcinoma), using the MTS assay as described previously (5,6). Cytotoxic activities are summarized in Table 3.

Results

The essential oil of *A. elemifera* as revealed by GC-MS is made up of 71.3% monoterpenoids, 21.0% sesquiterpenoids, and 7.7% other compounds. The most abundant compounds are limonene (45.0%), linalool (20.8%), β -caryophyllene (5.6%), 3-hexadecanone (5.3%), caryophyllene oxide (3.9%), and β -sesquiphellandrene (3.6%). In this study, we find neither the leaf oil nor its major components to be notably antimicrobial. However, linalool is slightly active against *S. aureus*, while caryophyllene and caryophyllene oxide show slight activity against *B. cereus*. Although *A. elemifera* leaf oil shows no cytotoxic activity against our panel of tumor cell lines, β -caryophyllene and caryophyllene oxide are active. The other major components, limonene and linalool, are not cytotoxic, according to our study. GC-MS analysis of the leaf oil of *E. axillaris* shows the oil to be composed of oxygenated sesquiterpenoids (31.6%), sesquiterpene hydrocarbons (24.4%), monoterpene hydrocarbons (21.6%), and oxygenated monoterpenoids (19.4%). The most abundant constituents of *E. axillaris* leaf oil are α -pinene (15.5%), β -dihydroagarofuran (9.2%), β -caryophyllene (8.8%), α -humulene (6.9%), 1,8-cineole (6.6%), and germacrene D (6.2%). *E. axillaris* leaf oil is neither appreciably antimicrobial nor cytotoxic. In addition, none of the major components from the essential oil shows notable activity in our antimicrobial or cytotoxicity assays. The oil of *L. involucrata* is dominated by sesquiterpenoids with 67.6% sesquiterpene hydrocarbons and 24.0% oxygenated sesquiterpenoids. The most abundant components are germacrene D (21.1%), α -humulene (15.2%), β -caryophyllene (13.7%), and caryophyllene oxide (5.3%). *M. cerifera* leaf oil is made up of 57.4% oxygenated monoterpenoids, dominated by 1,8-cineole (30.7%), α -terpineol (14.2%), and 4-terpineol (9.0%); 24.4% sesquiterpene hydrocarbons, mostly β -caryophyllene (6.4%) and δ -cadinene (5.0%); and 16.8%

oxygenated sesquiterpenoids. Neither the volatile leaf oil of *M. cerifera* nor its major components exhibited either antimicrobial activity or antineoplastic activity in our assays.

Table 2. Antimicrobial activity (MIC, $\mu\text{g/mL}$) of leaf essential oils and their major components of Abaco bush medicinal plants.

Material	<i>Bacillus cereus</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Candida albicans</i>	<i>Aspergillus niger</i>
<i>A. elemifera</i>	625	1250	625	625	1250	625
<i>E. axillaris</i>	625	625	625	625	625	625
<i>L. involucrata</i>	312	312	625	625	625	625
<i>M. cerifera</i>	625	625	312	625	625	625
α -Pinene	625	312	312	625	156	625
Limonene	625	312	625	1250	1250	2500
1,8-Cineole	156	625	625	625	312	312
Linalool	625	156	625	1250	625	625
4-Terpineol	1250	1250	1250	625	312	1250
α -Terpineol	625	1250	1250	625	1250	625
β -Caryophyllene	156	312	312	1250	1250	625
α -Humulene	312	312	625	1250	625	78
Germacrene D	625	156	625	1250	625	39
Caryophyllene oxide	156	1250	1250	1250	625	625
Positive Control	1.22 ¹	0.61 ¹	2.44 ¹	1.22 ¹	0.61 ²	0.61 ²

¹Gentamicin sulfate. ²Amphotericin B

Discussion

A. elemifera. Linalool and β -caryophyllene are also moderately abundant constituents of *A. diatrypa* (7). Both *A. diatrypa* leaf oil (7) and *A. balsamifera* wood essential oil (8) contain moderate amounts of β -sesquiphellandrene. Limonene is an abundant constituent of many essential oils of the Rutaceae, especially *Citrus* (9), and has exhibited cancer chemopreventive activity (10,11), possibly due to induction of glutathione-S-transferase and glutathione peroxidase (12). In addition, limonene has been shown to have muscle relaxant and sedative activities (13). Linalool, abundant in rosewood (*Aniba roseadora*) (14) and sweet basil (*Ocimum basilicum*) (15) essential oils, has been found to exhibit anti-inflammatory (16), antinociceptive (17), and sedative (18) effects. In addition, linalool inhibited the growth of bacteria and fungi (19-21) and showed antileukemic activity (22). β -Caryophyllene has shown anti-inflammatory (23) and anesthetic (24) effects in addition to anticarcinogenic (25) and antineoplastic (26) activities. Caryophyllene oxide exhibited anti-dermatophytic activity (27) and smooth-muscle-relaxant activity (28). β -Sesquiphellandrene has shown antirhinoviral (29) and antiulcer (30) activities.

The reported biological activities of the major constituents of *A. elemifera* leaf oil are consistent with the ethnopharmacological uses of the plant to reduce fever, treat symptoms of flu, treat sores and wounds, and its use as a general tonic and bath.

Table 3. Cytotoxic activity of leaf oils and their major components of Abaco medicinal plants.

Material	Cytotoxicity				
	MDA-MB-231	MCF7	Hs 578T	PC-3	Hep-G2
<i>A. elemifera</i> ¹	0	23.37(3.34)	16.95(11.49)	6.91(28.68)	19.41(6.22)
<i>E. axillaris</i>	0	11.99(3.30)	8.77(3.00)	0	16.45(5.63)
<i>L. involucrata</i>	42.66(19.80)	30.21(1.76)	95.79(1.43)	67.47(6.38)	92.21(2.42)
<i>M. cerifera</i>	0	0	2.74(9.31)	3.23(4.89)	14.76(18.01)
α -Pinene ²	> 735	> 735	> 1840	> 735	970(260)
Limonene	> 735	> 735	> 1840	> 735	1150(110)
1,8-Cineole	> 650	> 650	> 1625	> 650	> 1625
Linalool	> 650	> 650	> 1625	> 650	> 1625
4-Terpineol	> 650	> 650	> 1625	> 650	> 1625
α -Terpineol	> 650	> 650	> 1625	> 650	> 1625
β -Caryophyllene	155(1)	190(26)	383(41)	153(6)	347(74)
α -Humulene	108(1)	131(18)	251(26)	> 490	260(23)
Germacrene D	331(12)	341(12)	270(26)	127(10)	272(19)
Caryophyllene oxide	324(33)	178(33)	350(1)	150(3)	116(8)
Doxorubicin hydrochloride ³	44.6(4.1)	49.4(3.3)	17.9(2.1)	63.7(8.8)	2.28(0.40)

¹Cytotoxicity for essential oils is expressed as % kill at 250 μ g/mL for Hs 578T and Hep-G2 cells; % kill at 100 μ g/mL for MDA-MB-231, MCF7, and PC-3 cells; standard deviations are shown in parentheses. ²Cytotoxicity for pure compounds is expressed as LC_{50} values in μ M; standard deviations are shown in parentheses. ³Positive control.

***E. axillaris*.** α -Pinene, β -caryophyllene, α -humulene, 1,8-cineole, and germacrene D are relatively common in *Eugenia* spp. Thus, α -pinene is abundant in the leaf oil of *E. jambolana* (31), 1,8-cineole is the major component of *E. haitiensis* essential oil (32), β -caryophyllene and α -humulene are abundant constituents of *E. dysenterica* leaf essential oil (33), and germacrene D is abundant in *E. stipitata* fruits (34). β -Dihydroagarofuran has been found in the many essential oils, but has apparently not been previously detected in *Eugenia* essential oils.

***L. involucrata*.** The presence of germacrene D, α -humulene, and β -caryophyllene as major components is consistent with the ethnopharmacological use of *L. involucrata* to treat skin problems. Thus, commercially marketed herbal treatments for skin problems include goldenrod (*Solidago canadensis*) oil, rich in germacrene D (35); salves from purple coneflower (*Echinacea purpurea*) root extract, rich in germacrene D and β -caryophyllene (36); and lotions from extracts of hops (*Humulus lupulus*) "flowers", rich in α -humulene and β -caryophyllene (37).

Both *Lantana camara* (38) and *L. aculeata* (39) are used in traditional medicine to treat itching and other skin problems. *L. camara* essential oil has abundant quantities of α -humulene and β -caryophyllene (40), and *L. aculeata* essential oil is high in β -caryophyllene (39). *L. xenica* is also rich in β -caryophyllene and germacrene D (41). The essential oils of *L. camara* (38,40), *L. aculeata* (39), *L. xenica* (41), and *L. achyranthifolia* (42) have exhibited weak antimicrobial activity, consistent with the results of *L. involucrata* leaf oil.

***M. cerifera*.** The large percentage of 1,8-cineole may account for its use in traditional herbal medicine. *Eucalyptus* oil and tea tree (*Melaleuca alternifolia*) oil are commercially important medicines, and these essential oils are also rich in 1,8-cineole (43). 1,8-Cineole has been reported to exhibit insecticidal (32), antimicrobial (19), and antioxidant (44) activity, and may synergistically enhance the biological activities of other essential oil components (45).

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