Available online at www.sciencedirect.com
ScienceDirect
Phytochemistry 69 (2008) 919–927
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Chemotypic variation of essential oils in the medicinal plant, *Anemopsis californica*

Andrea L. Medina-Holguín [a], F. Omar Holguín [a], Sandra Micheletto [a], Sondra Goehle [b], Julian A. Simon [b], Mary A. O'Connell [a,*]

[a] *Department of Plant and Environmental Sciences, MSC 3Q, New Mexico State University, Las Cruces, NM 88003, USA*
[b] *Fred Hutchinson Cancer Research Center, Clinical Research Division, P.O. Box 19024, D2-100, Seattle, WA 98109-1024, USA*

Received 28 August 2007; received in revised form 15 October 2007
Available online 4 January 2008

Abstract

Anemopsis californica (Saururaceae) commonly called yerba mansa, is an important medicinal plant in many deserts in the southwestern region of North America. Populations of *A. californica,* collected throughout New Mexico, were examined for chemical variability in roots and rhizomes for select monocyclic (cymene, limonene, piperitone and thymol) and bicyclic (α-pinene, 1,8-cineole and myrtenol) monoterpenoid and phenylpropanoid (methyleugenol, isoeugenol and elemicin) derived essential oil components. Three distinct chemotypes were detected using a hierarchical clustering analysis on the concentration of 10 different analytes in three individuals from each of 17 populations. One chemotype was characterized by high elemicin concentrations, a second chemotype by high methyleugenol concentrations and the third by high piperitone and thymol concentrations. Steam distilled oil was used to screen for anticancer bioactivity. *A. californica* root oils demonstrated anti-proliferative activity against AN3CA and HeLa cells in vitro but no activity against lung, breast, prostate or colon cancer cells. The IC_{50} values for the root oil were 0.056% and 0.052% (v/v) for the AN3CA and HeLa cells, respectively.

Keywords: *Anemopsis californica*; Sauraceae; Yerba mansa; Essential oil; Chemotypes; Supercritical fluid extraction; SFE; Uterine cancer; Cervical cancer

1. Introduction

Anemopsis californica (Nutt.) Hook. and Arn. (*Houttuynia californica* Benth.et Hook.) is one of five genera belonging to the Saururaceae family. The genus contains a single species, *A. californica*, commonly known as yerba mansa. Geographically, it is found in the Southwestern region of the United States, and the northern regions of Mexico (Kelso, 1932; Caffey-Moquin, 1986). Although limited to desert biomes, yerba mansa is not a xerophyte. Populations of the plant are only found in wet, marshy and often alkaline or saline habitats surrounded by desert.

Anemopsis essential oil was studied extensively from the late 1950s to the early 1970s (Horton and Paul, 1957; Childs, 1962; Acharya and Chaubal, 1968; Sanvordeker and Chaubal, 1969; Tutupalli and Chaubal, 1971). The collective result of these works was the identification of 12 volatiles isolated from the roots and rhizomes of *Anemopsis* including methyleugenol (57%), thymol (13.8%) and piperitone (8%), and the isolation and identification of crystalline (+) asarinin. We have recently described the isolation and characterization of *Anemopsis* leaf volatiles (Medina et al., 2005). Thirty-eight compounds isolated by steam distillation or solid phase microextraction (SPME) were detected by GC/MS. Readily detectable compounds included α-pinene (1.9%), β-phellandrene (1.6%), 1,8-cineole (2.5%), piperitone (11.5%), methyleugenol (6.9%), (*E*)-caryophyllene (4.6%) and elemicin (53%).

Ethnographic information on yerba mansa is consistent. A variety of people from different cultural backgrounds and geographical areas have been interviewed and

* Corresponding author. Tel.: +1 505 646 5172; fax: +1 505 646 6041.
E-mail address: moconnel@nmsu.edu (M.A. O'Connell).

0031-9422/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2007.11.006

researchers report either similar or related uses again and again: treatment of wounds, cold and flu symptoms, pain and inflammation, as well as lung, circulatory, urinary, and digestive tract ailments (Swank, 1932; Wyman and Harris, 1947; Bean and Saubel, 1972; Bocek, 1984; Moore, 1989; Artschwager-Kay, 1996; Davidow, 1999). Both aerial and root/rhizome tissues are used medicinally; hippocratic screening of *Anemopsis* tissues demonstrates that root/rhizome, with a minimum lethal dose of 316 mg/kg, is more potent than aerial parts which showed no lethality even at the maximum dose administered 1 g/kg (Tutupalli et al., 1975). Tea made from *A. californica* leaves and roots is used to treat uterine cancer, ease menstrual cramps, induce conception, and staunch excessive bleeding after childbirth (Bocek, 1984; Artschwager-Kay, 1996); as a treatment for other gynecological conditions including yeast infection, and vaginitis (Moore, 1989; Davidow, 1999); or to treat venereal sores and ulcers (Bean and Saubel, 1972).

Chemical polymorphisms or chemotypes have been reported for many medicinal plants (Mockute et al., 2001; Russell and Southwell, 2003; Curado et al., 2006). Douglas et al. (2004) conducted a study to determine whether triketone rich chemotypes of *Leptospermum scoparium* are present in New Zealand and define the boundaries of chemotypic variation. Ten chemotypes were identified, two of which contained high levels of triketones. This analysis indicated a link between the two triketone rich populations although they were spatially very distant. In a related study based on the analysis of *L. scoparium* grown from seed collected at 15 sites around the country, they identified three chemotypes, and determined that oil composition was largely genetically controlled (Perry et al., 1997).

Rapid screening protocols have been developed for the purposes of identifying natural products with anticancer properties (Simon et al., 2000; Bjornsti, 2002). Assaying plants known to have either useful or associated chemistries maximizes efficiency, as these are most likely to demonstrate activity (Balunas and Kinghorn, 2005). An herbal remedy, such as yerba mansa, is an excellent candidate for anticancer screening. The objective of this study was to determine if there were different chemotypes among the different populations of *A. californica* grown and used by herbalists in New Mexico. The bioactivity of root extracts was determined by screening for inhibition of growth of in vitro cultured cancer cell lines.

2. Results and discussion

2.1. Characterization of root oil

The essential oil composition of New Mexico *Anemopsis* root tissue was characterized. Root tissue from one population, San Pedro in Rio Arriba Co. was selected for triplicate steam distillations (Table 1). This population was chosen for steam distillation based on the large amount of tissue available. The most abundant compounds in the oil were methyleugenol, thymol, and elemicin. This is the first published report of myrtenol, anethole and elemicin in *Anemopsis* root tissue. The level of the major component methyleugenol corresponds to previously published level in root oils characterized by Acharya and Chaubal (1968). In that study using plants from California, methyleugenol comprised 55% of the root oil, with thymol at 13% and piperitone at 5%. The reduced levels of piperitone and thymol in the San Pedro sample could reflect different genetic sources of the plant material.

Table 1
Characterization of *Anemopsis* root essential oil

Compound	% Peak area (avg ± SD)
α-Pinene	0.2 ± 0.0
Cymene	0.1 ± 0.1
Limonene	0.2 ± 0.0
1,8-Cineole	0.3 ± 0.0
Myrtenol	1.5 ± 0.3
Anethole	1.0 ± 0.1
Piperitone	0.4 ± 0.0
Thymol	4.2 ± 0.4
Methyleugenol	59 ± 2
Elemicin	2.7 ± 0.2

Triplicate steam distillations of root tissue were characterized using GC/MS, and abundant compounds identified by matching mass spectral data to the mass spectra of standard compounds. Quantities are expressed as % peak area, compounds are listed in order of increasing retention time.

2.2. Chemotypic variation in Anemopsis populations

Root tissue was collected in the Fall 2001 from 17 populations of *Anemopsis*. Plants were collected in seven counties, from Northern to Southern New Mexico; populations were located at altitudes between 1198 and 1793 m (Fig. 1). This represents zones 4–8 on the United States Department of Agriculture plant hardiness map (http://www.usna.usda.gov/Hardzone/ushzmap.html).

The concentration of select monocyclic and bicyclic monoterpenoid and phenylpropanoid derived essential oil components in the 17 *Anemopsis* populations are presented in Tables 2–4. These extracts were prepared by standard SFE methods with trapping of the analytes in methanol and then the composition was determined using GC/MS. There was remarkable variability between populations across all compound classes. Of the 10 compounds characterized only three, thymol, α-pinene and methyleugenol were found in all plants collected at each of the 17 sites. The abundances of monocyclic monoterpenoid compounds were reported in Table 2; thymol was usually the most abundant compound in this class, while cymene was the least abundant. The abundances of bicyclic monoterpenoid compounds were reported in Table 3; although 1,8-cineole and myrtenol were found in only 13 or 12 of the 17 populations, respectively, in some populations these bicyclic monoterpenoids were found at higher concentrations than α-pinene. The abundances of phenylpropanoid derived