NPC Natural Product Communications

2010
Vol. 5
No. 3
457 - 460

Seasonal Variation in the Leaf Essential Oil Composition of *Zanthoxylum clava-herculis* growing in Huntsville, Alabama

Lauren C. Eiter[a], Henry Fadamiro[b] and William N. Setzer[a,*]

[a]*Department of Chemistry, University of Alabama in Huntsville, Huntsville, AL 35899, USA*

[b]*Department of Entomology & Plant Pathology, Auburn University, AL 36849, USA*

wsetzer@chemistry.uah.edu

Received: November 23rd, 2009; Accepted: January 3rd, 2010

The seasonal variation in the chemical composition of the leaf essential oil of *Zanthoxylum clava-herculis* has been analyzed by GC-MS. Three individual trees were sampled four times during the course of the 2004 growing season. Notable differences were recorded in the essential oil yields from the three trees on the four collection dates; yields were highest in May and lowest in July The leaf essential oils were made up of 25 components, largely menthane monoterpenoids, dominated by limonene (44-73%) and 1,8-cineole (16-43%), with lesser amounts of α-thujene, linalool, γ-terpinene, and α-terpineol. The ratio of oxygenated monoterpenoids to monoterpene hydrocarbons generally increased during the season, largely reflected in the 1,8-cineole/limonene ratio.

Keywords: *Zanthoxylum clava-herculis*, Rutaceae, seasonal variation, essential oil, GC-MS, limonene, 1,8-cineole.

Zanthoxylum clava-herculis L. (Rutaceae; toothache tree, Southern prickly-ash, Hercules' club) is a small, deciduous tree growing to 10 m in height and 15 cm dbh. The flowers appear in April-May, and the fruits, which are small follicles that develop between July-September, dehisce to reveal shiny black seeds. The tree, a temperate representative of a circumtropical genus, ranges from Virginia south to Florida and west to Texas, Arkansas, and Oklahoma [1a]. The seeds are used as a condiment and a pepper substitute [1b]. The bark of *Z. clava-herculis* has been used by native Americans for chronic rheumatism, dyspepsia, dysentery, kidney trouble, heart trouble, colds, coughs, lung ailments, toothache, and nervous debility [1c,1d]. Bioactive principles include herculin and neoherculin (insecticidal), berberine and chelerythrine (antibacterial), *N*-acetylanonaine (ichthyotoxic), as well as asarinin, sesamin, *O*-prenylpiperitol, *O*-prenylxanthoxylol, and xanthoxylin [1d,2]. *Z. clava-herculis* is also a host plant of several insects, including swallowtail butterflies [3] and leaf beetles [4].

In this paper, we present the identity and seasonal variation in the chemical composition of the leaf essential oils of *Z. clava-herculis* growing in Huntsville, Alabama, during the growing season of 2004. To our knowledge, this report is the first to present the identity and seasonality of *Z. clava-herculis* leaf oil.

The yields from the collection and hydrodistillation of fresh leaves from *Z. clava-herculis* are listed in Table 1. The results show some notable differences in the essential oil yields from the three trees on the four collection dates. Yields for the three trees were highest in May and lowest in July. Among the three trees, the female one had a higher yield than the two male trees. Essential oil yields ranged from 1.1% (from the female tree in May) to 0.4% (from a male tree in July). The weather data for Huntsville, Alabama during the four collection dates in 2004 are shown in Table 2. Average temperatures were lowest in May and highest in July. Rainfall was highest in July and at least three-fold higher than in May. Average sunlight was also highest in July.

The gas chromatographic-mass spectral analyses are summarized in Table 3. A total of 25 components were detected in the leaf oils, but not all components were detected in the three plants or from the different collection dates. Monoterpene hydrocarbons and oxygenated monoterpenoids were dominant, with very small amounts of sesquiterpenes and simple oxygenated compounds (*cis*-3-hexenol and nonanal). The major components were limonene (44-73%) and 1,8-cineole (16-43%). Other components detected in relatively less amounts include α-thujene, linalool, γ-terpinene, and α-terpineol. These compounds were all detected in the three plants at the four collection dates. Limonene has also been reported as an an abundant component of *Z. limonella* [5] and *Z. piperitum* [6] fruit essential oils, while 1,8-cineole is an abundant constituent of *Z. acuminatum* leaf essential oil [7]. Linalool and α-terpineol seem to be ubiquitous components of *Zanthoxylum* leaf oils [7]. α-Thujene and γ-terpinene are common constituents of leaf oils of *Citrus* and other Rutaceae [8].

Table 1: Leaf essential oil yields of *Zanthoxylum clava-herculis*.

Tree	Date Collected Extracted	Mass Fresh Leaves (g)	Mass Ess. Oil (g)	Yield Ess. Oil (%)
A (♂)	12-May-04	20.26	0.195	1.0
	3-Jul-04	24.57	0.104	0.4
	26-Aug-04	20.03	0.153	0.8
	23-Oct-04	25.86	0.158	0.6
B (♂)	13-May-04	18.32	0.134	0.7
	4-Jul-04	19.34	0.073	0.4
	27-Aug-04	15.76	0.132	0.8
	24-Oct-04	22.15	0.148	0.7
C (♀)	14-May-04	15.85	0.181	1.1
	5-Jul-04	18.34	0.116	0.6
	28-Aug-04	15.23	0.138	0.9
	24-Oct-04	21.27	0.151	0.7

Certain components detected in the female tree were not detected in the male trees, including γ-muurolene, valencene, γ-cadinene, and δ-cadinene. Also, α-terpineol was detected in greater amounts in the female tree than in the two male trees. Most of the remaining components were either detected in trace amounts or undetected in some trees or dates. Comparing the abundance of the major components across the collection dates, limonene was most abundant in the May collections, whereas 1,8-cineole showed a gradual increase in abundance from May to October. A similar trend was recorded for all three trees.

It is not readily apparent what factors may be responsible for the variation in essential oil chemical compositions. *Salvia libanotica* from Lebanon shows a similar increase in 1,8-cineole concentration with concomitant decrease in limonene concentration in August compared with April (57.4% cineole and 2.4% limonene in August; 47.7% cineole and 6.1% limonene in April) [9]. Similar seasonal changes have been reported for *Pinus pinea* from both southern Italy, May vs. October [10], and Spain, early June vs. early September [11a]; and for *Picea sitchensis* during the growing season [11b].

The weather data for the two-week periods prior to collection, summarized in Table 2, may explain, at least in part, the seasonal variation in *Z. clava-herculis* leaf essential oil yields recorded in this study. It may be that the relatively warm temperatures, coupled with abundant sunshine and rainfall, during the two weeks prior to the early July collection was responsible for the lower essential oil yields at that time by increasing the emission of volatiles and decreasing the amount of essential oil remaining in the leaves. Monoterpene emissions are generally maximized during the warmer part of the season. Thus, for example, monoterpene emissions for both *Pinus pinea* and *Quercus ilex* from Spain were observed in June, attributed to high temperatures and insolation [11a]. Similarly, monoterpene emissions from *Eucalyptus globulus* from Western Australia were greatest during the Australian summer (January) and lowest during the winter (July) [14].

Table 2: Weather data for Hunstville, Alabama during collection of plant materials in 2004.

	29-Apr to 12-May	20-Jun to 3-Jul	13-Aug to 26-Aug	8-Oct to 21-Oct
Av High (°C)[a]	26.7	27.9	28.6	21.2
Av Low (°C)[a]	13.1	20.4	16.6	12.9
Av Temp (°C)[a]	19.9	24.1	22.6	17.1
Rainfall (mm)[b]	40	146	23	122
Sunshine (hours)[a]	50	77	49	99
Av Daylight (hrs:min)	13:43	14:27	13:18	11:21
Tree phenology	flowering		fruiting	

[a] National Weather Service Forecast Office, Huntsville, Alabama [12].
[b] Cooperative Huntsville Area Rainfall Measurements (Alabama) [13]

Although the sample size was small and not statistically relevant, there seems to have been less chemical diversity (fewer components) in the two male trees sampled compared with the single female tree. The increased chemical diversity of the female tree was manifested in the sesquiterpenoids (γ-muurolene, valencene, γ-cadinene, and δ-cadinene) that were not detected in the male trees. In addition, with the exception of 1,8-cineole, the female tree had higher concentrations of oxygenated monoterpenoids (sabinene hydrates, linalool, α-terpineol, and terpinen-4-ol) and higher concentrations of the sesquiterpenoid β-caryophyllene. In addition to higher chemical diversity, the female tree showed slightly higher essential oil yields than either of the two male trees. Higher essential oil yield and chemical diversity in female trees has been previously observed in *Juniperus virginiana* [15]. Similarly, the floral essential oils from female *Laurus nobilis* were more chemically diverse than that from males [16a] and *Ficus carica* female trees showed greater chemical diversity in their fig volatiles than male trees [16b]. Non-volatile bark constituents of the medicinal plant *Amphiptherygium adstringens* were present in greater quantities in female than male trees [17]. Female *Cannabis sativa* plants generally produced higher levels of total cannabinoids [18a], as well as terpenoids [18b], than male plants. Since sexual reproduction is generally thought to be metabolically more costly for female plants than for males [19], increased investment in essential oils in females relative to males might represent the product of sexually biased natural selection for compounds conferring some protection against herbivores and pathogens. It is also

Table 3: Seasonal variation in leaf essential oil composition of *Zanthoxylum clava-herculis*.

RI[a]	Compound	Area (%) Tree A (♂) 5/12[b]	7/3	8/26	10/22	Tree B (♂) 5/13	7/4	8/27	10/24	Tree C (♀) 5/14	7/5	8/28	10/24
862	*cis*-3-Hexenol	0.3	tr[c]	tr	tr	tr	0.9	tr	tr	tr	0.5	0.5	tr
938	α-Thujene	0.5	3.4	5.6	0.2	5.2	4.0	tr	1.3	3.3	3.3	3.1	3.2
944	α-Pinene	0.7	0.9	tr	0.2	1.4	3.8	---	1.1	tr	tr	tr	tr
980	Sabinene	tr	tr	tr	tr	tr	0.9	tr	tr	tr	0.3	0.9	tr
981	β-Pinene	---	---	---	---	---	---	---	---	---	0.3	---	---
996	Myrcene	0.7	0.9	0.7	0.8	tr	0.7	tr	tr	0.5	0.7	0.7	tr
1026	*p*-Cymene	0.1	---	---	---	---	---	---	---	---	---	---	---
1031	Limonene	67.4	52.4	56.3	55.8	73.0	59.3	59.9	47.1	63.8	54.2	51.8	43.6
1035	1,8-Cineole	28.4	40.5	34.9	41.0	15.8	26.8	31.9	43.3	12.9	25.4	27.6	35.2
1060	γ-Terpinene	0.6	0.7	0.9	0.9	2.2	1.1	4.2	1.7	2.5	1.7	1.3	2.1
1068	*cis*-Sabinene hydrate	tr	tr	tr	tr	tr	tr	---	tr	tr	0.3	0.2	tr
1099	*trans*-Sabinene hydrate	tr	tr	tr	---	---	---	---	---	---	0.2	0.1	---
1103	Linalool	tr	tr	tr	---	2.5	1.5	2.0	2.2	11.0	6.4	7.8	11.3
1106	Nonanal	---	---	---	---	---	---	---	---	---	0.4	0.5	---
1134	*cis*-Limonene oxide	0.1	tr	---	tr	---	---	---	---	---	---	tr	---
1138	*trans*-Limonene oxide	0.1	tr	tr	tr	tr	tr	---	tr	0.3	0.3	0.3	tr
1177	Terpinen-4-ol	---	---	---	---	---	tr	---	tr	tr	0.2	0.2	tr
1192	α-Terpineol	0.7	1.1	1.6	0.9	tr	0.5	tr	0.7	2.7	3.2	3.5	3.5
1216	*cis*-Sabinene hydrate acetate	0.3	0.2	tr	0.3	tr	tr	0.8	0.6	1.5	0.9	0.7	1.1
1350	α-Terpinyl acetate	---	---	---	---	tr	0.3	1.2	2.0	tr	tr	tr	tr
1417	β-Caryophyllene	tr	tr	tr	tr	tr	0.2	tr	tr	0.6	0.5	0.4	tr
1475	γ-Muurolene	---	---	---	---	---	---	---	---	0.4	0.4	0.2	tr
1493	Valencene	---	---	---	---	---	---	---	---	tr	0.2	tr	tr
1512	γ-Cadinene	---	---	---	---	---	---	---	---	tr	0.2	tr	tr
1522	δ-Cadinene	---	---	---	---	---	---	---	---	0.4	0.4	0.2	tr

[a]RI = "Retention Index" determined with reference to a homologous series of *n*-alkanes on an HP-5ms column. [b]Date collected/extracted (day/month). [c]tr = "trace".

generally believed that production of secondary metabolites is energetically costly [19e,20].

Z. clava-herculis is known to be a host plant for the giant swallowtail butterfly, *Papilio cresphontes* [3]. Other host plants for *P. cresphontes* include *Amyris elemifera*, *Ptelea trifoliata*, *Casimiroa edulis*, *Zanthoxylum fagara*, and *Citrus* spp. [3,21]. To our knowledge, it is not currently known what phytochemicals may serve as attractants for *P. cresphontes* to *Z. clava-herculis* or other members of the Rutaceae. *A. elemifera* leaf essential oil is rich in limonene and linalool [22] and these two monoterpenoids are abundant in *Citrus* leaf oils as well [8,23]. Although linalool has been found in many *Zanthoxylum* spp. [7], limonene was not detected in *Z. fagara* [24a]. Similarly, *P. trifoliata* leaf oil showed the presence of linalool, but no limonene [24b]. An analysis of *C. edulis* leaf oil has shown it to contain neither linalool nor limonene [25a]. Studies are ongoing in our laboratories to determine if leaf essential oils play a role in the attraction of swallowtail butterflies to *Z. clava-herculis* and other Rutaceae plants.

Experimental

Plant material: A sampling of mature leaves from different locations on three different trees growing in second growth forest at 187 m elevation on the western foot of Green Mountain, Huntsville, Alabama (34° 38' 46" N, 86° 33' 27" W) were collected at four different times of the year (mid-May, early July, late August, and late October) during 2004. The leaves were collected in the morning (approximately 8:00 am), immediately chopped, and hydrodistilled for 4 h using a Likens-Nickerson hydrodistillation-extraction apparatus. Essential oil yields are summarized in Table 1.

Gas chromatographic/mass spectral analysis: The leaf oils of the *Zanthoxylum clava-herculis* samples were subjected to GC-MS analysis as described previously [25a]. The components [25b] from the GC-MS analyses are listed in Table 3.

Acknowledgments – WNS is grateful to an anonymous private donor for the generous gift of the GC-MS instrumentation and to Professor R. O. Lawton for helpful discussions.

References

[1] (a) Radford AE, Ahles HE, Bell CR. (**1968**) *Manual of the Vascular Flora of the Carolinas*, University of North Carolina Press, Chapel Hill, North Carolina; (b) Coon N. (**1975**) *The Dictionary of Useful Plants*. Rodale Press, Emmaus, Pennsylvania; (c) Foster S, Duke JA. (**1990**) *Medicinal Plants*. Houghton Mifflin, Boston; (d) Lewis WH, Elvin-Lewis, MPF. (**1977**) *Medical Botany*. Wiley, New York.

[2] (a) Jacobson M. (**1948**) Herculin, a pungent insecticidal constituents of southern prickly ash bark. *Journal of the American Chemical Society*, ***70***, 4234-4237; (b) Grieve M. (**1971**) *A Modern Herbal*, Vol ***I***. Dover, New York; (c) Rao KV, Davies R. (**1986**) The ichthyotoxic principles of *Zanthoxylum clava-herculis*. *Journal of Natural Products*, ***49***, 340-342; (d) Gibbons S, Leimkugel J, Oluwatuyi M, Heinrich M. (**2003**) Activity of *Zanthoxylum clava-herculis* extracts against multi-drug resistant methicillin-resistant *Staphylococcus aureus* (mdr-MRSA). *Phytotherapy Research*, ***17***, 274-275.

[3] Scott JA. (**1992**) *The Butterflies of North America*. Stanford University Press, Stanford, California.

[4] (a) Jolivet P, Hawkenswood TJ. (**1995**) *Host-Plants of Chrysomelidae of the World*. Backhuys Publishers, Leiden; (b) Keralis JD, Riley EG. (**2007**) Observations on the life history of *Derospidea brevicollis* (LeConte) (Coleoptera: Chrysomelidae) when feeding upon *Zanthoxylum clava-herculis* L. in southeast Texas. 2007 Annual Meeting of the Entomological Society of America.

[5] Itthipanichpong C, Ruangrungsi N, Pattanaautsahakit C. (**2002**) Chemical compositions and pharmacological effects of essential oil from the fruit of *Zanthoxylum limonella*. *Journal of the Medical Association of Thailand*, ***85*** **Suppl 1**, S344-S354.

[6] Jiang, L, Kubota K. (**2004**) Differences in the volatile components and their odor characteristics of green and ripe fruits and dried pericarp of Japanese pepper (*Xanthoxylum piperitum* DC.). *Journal of Agricultural and Food Chemistry*, ***52***, 4197-4203.

[7] Setzer WN, Noletto JA, Lawton RO, Haber WA. (**2005**) Leaf essential oil composition of five *Zanthoxylum* species from Monteverde, Costa Rica. *Molecular Diversity*, ***9***, 3-13.

[8] (a) Lota ML, de Rocca Serra D, Tomi F, Casanova J. (**2000**) Chemical variability of peel and leaf essential oils of mandarins from *Citrus reticulata* Blanco. *Biochemical Systematics and Ecology*, ***28***, 61-78; (b) Lota ML, de Rocca Serra D, Tomi F, Casanova J. (**2001**) Chemical variability of peel and leaf essential oils of 15 species of mandarins. *Biochemical Systematics and Ecology*, ***29***, 77-104; (c) Lota ML, de Rocca Serra D, Tomi F, Jacquemond C, Casanova J. (**2002**) Volatile components of peel and leaf oils of lemon and lime species. *Journal of Agricultural and Food Chemistry*, ***50***, 796-805; (d) Gancel AL, Olle D, Ollitrault P, Luro F, Brillouet JM. (**2002**) Leaf and peel volatile compounds of an interspecific citrus somatic hybrid [*Citrus aurantifolia* (Christm.) Swing. + *Citrus paradisi* Macfayden]. *Flavour and Fragrance Journal*, ***17***, 416–424; (e) Gancel AL, Ollitrault P, Froelicher Y, Tomi F, Jacquemond C, Luro F, Brillouet JM. (**2003**) Leaf volatile compounds of seven citrus somatic tetraploid hybrids sharing willow leaf mandarin (*Citrus deliciosa* Ten.) as their common parent. *Journal of Agricultural and Food Chemistry*, ***51***, 6006-6013.

[9] Farhat GN, Affara NI, Gali-Muhtasib HU. (**2001**) Seasonal changes in the composition of the essential oil extract of East Mediterranean sage (*Salvia libanotica*) and its toxicity in mice. *Toxicon*, ***39***, 1601-1605.

[10] Staudt M, Bertin N, Hansen U, Seufert G, Ciccioli P, Foster P, Frenzel B, Fugit JL. (**1997**) Seasonal and diurnal patters of monoterpene emissions from *Pinus pinea* (L.) under field conditions. *Atmospheric Environment*, ***31***, 145-156.

[11] (a) Sabillón D, Cremades LV. (**2001**) Diurnal and seasonal variation of monoterpene emission rates for two typical Mediterranean species (*Pinus pinea* and *Quercus ilex*) from field measurements – relationship with temperature and PAR. *Atmospheric Environment*, ***35***, 4419-4431; (b) Hrutfiord BF, Hopley SM, Gara RI. (**1974**) Monoterpenes in Sitka spruce: Within tree and seasonal variation. *Phytochemistry*, ***13***, 2167-2170.

[12] National Weather Service Forecast Office, Huntsville, Alabama, http://www.srh.noaa.gov/hun/climate/data/f6/f6.php?site=HSV.

[13] Cooperative Huntsville Area Rainfall Measurements (Alabama) http://weather.msfc.nasa.gov/charm/.

[14] He C, Murray F, Lyons T. (**2000**) Seasonal variations in monoterpene emissions from *Eucalyptus* species. *Chemosphere*, ***2***, 65-76.

[15] Setzer WN, Whitaker KW, Lawton RO. (**1992**) A chemical ecological study of the components of the essential oil of Eastern red cedar (*Juniperus virginiana*) from three habitats in Huntsville, Alabama. *Castanea*, ***57***, 209-212.

[16] (a) Flamini G, Cioni PL, Morelli I. (**2002**) Differences in the fragrances of pollen and different floral parts of male and female flowers of *Laurus nobilis*. *Journal of Agricultural and Food Chemistry*, ***50***, 4647-4652; (b) Gibernau M, Buser HR, Frey JE, Hossaert-McKey M. (**1997**) Volatile compounds from extracts of figs of *Ficus carica*. *Phytochemistry*, ***46***, 241-244.

[17] Olivera Ortega AG, Soto Hernandez M, Martınez Vazquez M, Terrazas Salgado T, Solares Arenas F. (**1999**) Phytochemical study of cuachalalate (*Amphiptherygium adstringens*, Schiede ex Schlecht). *Journal of Ethnopharmacology*, ***68***, 109-113.

[18] (a) Hemphill JK, Turner JC, Mahlberg PG. (**1980**) Cannabinoid content of individual plant organs from different geographical strains of *Cannabis sativa* L. *Journal of Natural Products*, ***43***, 112-122; (b) Martin L, Smith DM, Farmilo CG. (**1961**) Essential oil from fresh *Cannabis sativa* and its use in identification. *Nature*, ***191***, 774-776.

[19] (a) Elmqvist T, Cates RG, Harper JK, Gardfjell H. (**1991**) Flowering in males and females of a Utah willow, *Salix rigida* and effects on growth, tannins, phenolic glycosides, and sugars. *Oikos*, ***61***, 65-72; (b) Rocheleau AF, Houle G. (**2001**) Different cost of reproduction for the males and females of the rare dioecious shrub *Corema conradii* (Empetraceae). *American Journal of Botany*, ***88***, 659-666. (c) Obeso JR. (**2002**) The costs of reproduction in plants. *New Phytologist*, ***155***, 321-348; (d) Espírito-Santo MM, Madeira BG, Neves FS, Faria ML, Fagundes FM, Fernandes GW. (**2003**) Sexual differences in reproductive phenology and their consequences for the demography of *Baccharis dranunculifolia* (Asteraceae), a dioecious tropical shrub. *Annals of Botany*, ***91***, 13-19; (e) Bañuelos MJ, Sierra M, Obeso JR. (**2004**) Sex, secondary compounds and asymmetry. Effects on plant–herbivore interaction in a dioecious shrub. *Acta Oecologica*, ***25***, 151-157; (f) Wheelwright NT, Logan BA. (**2004**) Previous-year reproduction reduces photosynthetic capacity and slows lifetime growth in females of a neotropical tree. *Proceedings of the National Academy of Sciences of the United States of America*, ***101***, 8051-8055.

[20] Bazzaz FA, Chiariello NR, Coley PD, Pitelka LF. (**1987**) Allocating resources to reproduction and defense. *Bioscience*, ***37***, 58-67.

[21] McAuslane HJ. (**2009**) Giant swallowtail, orangedog, *Papilio cresphontes* Cramer (Insecta: Lepidoptera: Papilionidae). University of Florida Cooperative Extension Service, document EENY-008 (IN134).

[22] Schmidt JM, Noletto JA, Vogler B, Setzer WN. (**2006**) Abaco bush medicine: chemical composition of the essential oils of four aromatic medicinal plants from Abaco Island, Bahamas. *Journal of Herbs, Spices, and Medicinal Plants*, ***12***, 43-65.

[23] (a) Attaway JA, Pieringer AP, Barabas LJ. (**1967**) A study of the percentage variation in peel and leaf oil terpenes during one season. *Phytochemistry*, ***6***, 25-32; (b) Blanco Tirado C, Stashenko EE, Combariza MY, Martinez JR. (**1995**) Comparative study of Colombian citrus oils by high-resolution gas chromatography and gas chromatography-mass spectrometry. *Journal of Chromatography, Part A*, ***697***, 501-513; (c) Vekiari SA, Protopapadakis EE, Papadopoulou P, Papanicolaou D, Panou C, Vamvakias M. (**2002**) Composition and seasonal variation of the essential oil from leaves and peel of a Cretan lemon variety. *Journal of Agricultural and Food Chemistry*, ***50***, 147-153.

[24] (a) Setzer WN, Schmidt JM, Eiter LC, Haber WA. (**2005**) The leaf oil composition of *Zanthoxylum fagara* (L.) Sarg. from Monteverde, Costa Rica, and its biological activities. *Journal of Essential Oil Research*, ***17***, 333-335; (b) Takaku S, Setzer WN. (**2007**) Chemical composition of the leaf essential oil of *Ptelea trifoliata*. *Journal of Essential Oil-Bearing Plants*, ***10***, 104-108.

[25] (a) Miller SL, Haber WA, Setzer WN. (**2009**) Chemical composition of the leaf essential oil of *Casimiroa edulis* La Llave & Lex. (Rutaceae) from Monteverde, Costa Rica. *Natural Product Communications*, ***4***, 425-426; (b) Adams RP. (**2007**) Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry, 4th Ed. Allured Publishing Corporation. Carol Stream, Illinois.