NPC Natural Product Communications

2006
Vol. 1
No. 1
43 - 45

1,3,5-Trimethoxybenzene and 2,4,6-Trimethoxystyrene are the Major Components in the Leaf Oil of *Eugenia confusa* from Abaco Island, Bahamas

William N. Setzer*[,a], Joseph A. Noletto[a], and Michael A. Vincent[b]

[a]*Department of Chemistry, University of Alabama in Huntsville, Huntsville, Alabama, 35899, U.S.A*

[b]*W.S. Turrell Herbarium, Department of Botany, Miami University, Oxford, Ohio, 45056, U.S.A.*

wsetzer@chemistry.uah.edu

Received: November 17th, 2005; Accepted: January 9th, 2006

The leaf oil of *Eugenia confusa* was obtained by hydrodistillation and analyzed by GC-MS. The most abundant components were 1,3,5-trimethoxybenzene (74.0%) and 2,4,6-trimethoxystyrene (20.7%).

Keywords: *Eugenia confusa*, Myrtaceae, essential oil composition, 1,3,5-trimethoxybenzene, 2,4,6-trimethoxystyrene.

Eugenia confusa DC. (Myrtaceae), "red-berry stopper", is a tree native to south Florida, the Bahamas and the West Indies, up to 6 m tall, with light gray, scaly bark [1]. Leaves are opposite, elliptic or ovate, 4-5 cm long. The flowers are white, 2-6 mm, in leaf axils or basal scales. The fruits are solitary, one-seeded, long-stalked, red berries, about 6-7 mm in diameter.

To our knowledge, there have been neither ethnobotanical reports nor any reported phytochemical analyses of *E. confusa*. The *Eugenia* genus, however, is important in the traditional herbal medicines of many cultures. For example, leaves of *E. uniflora* are used in southern Brazil to treat bacterial infections and diarrhea [2], used as compresses for bruises in northwestern Argentina [3], and as an antihypertensive, in addition to many other uses [4]. *E. axillaris* bark is used in eastern Cuba for diabetes [5], fruits of *E. jambolana* are used to treat diabetes in Brazil [6], and leaves of *E. dysenterica* are used in southern Brazil to treat diarrhea and dysentery [7]. Clove oil, the essential oil obtained from the buds of *E. caryophyllata*, has been used as a toothache remedy; and the plant also has antiseptic, counterirritant and carminative properties [8]. Because of the ethnopharmacological importance of the genus, in addition to our interest in essential oils of the Myrtaceae [9,10], we collected and analyzed the leaf essential oil of *E. confusa* from Abaco Island, Bahamas.

The essential oil components of *E. confusa* leaf oil are listed in Table 1. The most abundant, as

Table 1: Chemical composition of *Eugenia confusa* leaf essential oil.

RI[1]	Compound	Area (%)
867	*cis*-3-Hexenol	trace
1412[2]	1,3,5-Trimethoxybenzene[3]	74.0
1550	Elemol	trace
1559	Elemicin	trace
1582	Caryophyllene oxide	trace
1621	2,4,6-Trimethoxystyrene[4]	20.7
1628	Unknown[5]	5.3

[1]RI = "Retention Index" on a HP-5ms column based on comparison with a series of homologous alkanes.
[2]Reference RI for 1,3,5-trimethoxybenzene (1405) from Noguiera *et al*. [15].
[3]GC/MS (EI), 70 eV, 280°C m/z (rel. int.): 168(M+, 100%), 139(73%), 125(17%), 109(14%), 95(8%), 79(8%), 78(8%), 69(10%), 63(5%), 52(6%). Ref (NIST) MS: 168(M+, 100%), 139(89%), 125(21%), 109(21%), 95(18%), 79(14%), 78(12%), 69(21%), 63(8%), 52(11%).
[4]GC/MS (EI), 70 eV, 280°C m/z(rel. int.): 194(M+, 83%), 179(100%), 151(30%), 121(46%), 91(13%), 77(11%), 69(8%), 51(9%). Ref (NIST) MS: 194(M+, 89%), 179(100%), 151(26%), 121(60%), 91(22%), 77(23%), 69(23%), 51(19%).
[5]GC/MS (EI), 70 eV, 280°C m/z(rel. int.): 220(M+, 8%), 205(17%), 202(16%), 187(21%), 177(22%), 162(58%), 159(58%), 147(39%), 134(22%), 131(22%), 119(100%), 117(28%), 107(35%), 105(42%), 93(41%), 91(61%), 79(29%), 77(22%).

by this study, are 1,3,5-trimethoxybenzene (74.0%) and 2,4,6-trimethoxystyrene (20.7%). The abundant presence of these aromatic compounds in *E. confusa* leaf oil was completely unexpected. Thus, there is no evidence of either 1,3,5-trimethoxybenzene or 2,4,6-trimethoxystyrene in other *Eugenia* essential oils, including *E. jambolana* [11], *E. uniflora* [10,12-14], *E. dysenterica* [7], *E. caryophyllata*, and *E. axillaris* (unpublished results from this laboratory). 1,3,5-Trimethoxybenzene has been found, however, in the floral essential oils of *Clusia* [15] and *Rosa* spp. [16,17]. Both 1,3,5-trimethoxybenzene and 2,4,6-trimethoxystyrene have been found in the essential oils of *Zieria* spp. [18,19]. Additionally, 2,4,6-trimethoxytoluene is an abundant component of *Stockwellia quadrifida* leaf oil [20].

Experimental

Plant material: Leaves of *E. confusa* were collected from a single plant from Abaco Island, Bahamas (26° 34.55' N, 77° 8.35' W, 1-2 m a.s.l) on 7 June 2002. The plant was identified by M. A. Vincent by comparison with specimens at the W. S. Turrell Herbarium. A voucher specimen has been deposited in the University of Alabama in Huntsville herbarium. The essential oil was obtained by hydrodistillation of the freshly chopped leaves (247.1 g) and dichloromethane extraction of the distillate to give 1.577 g (0.638% yield) of leaf oil.

Gas chromatography-mass spectrometry: The leaf essential oil was subjected to GC-MS analysis on an Agilent system consisting of a model 6890 gas chromatograph, a model 5973 mass selective detector, and an Agilent ChemStation data system. The GC column was an HP-5ms fused silica capillary with a (5% phenyl)-methylpolysiloxane stationary phase, film thickness of 0.25 μm, a length of 30 m, and an internal diameter of 0.25 mm. The carrier gas was helium with a column head pressure of 7.07 psi and flow rate of 1.0 mL/min. Inlet temperature was 200°C and MSD detector temperature was 280°C. The GC oven temperature program was used as follows: 40°C initial temperature, hold for 10 min; increased at 3°C/min to 200°C; increased 2 °/min to 220°C. The sample was dissolved in CH_2Cl_2 and a split injection technique was used. 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) [21].

Acknowledgments - Support for this work was provided by a generous grant from an anonymous private donor. We are grateful to Forest Heights Academy, Marsh Harbor, for allowing us the use of their laboratory facilities on Abaco.

References

[1] Scurlock JP. (**1990**) *Native Trees and Shrubs of the Florida Keys*. Lauren & Herbert, Inc., Sugarloaf Shores, Florida.

[2] Coelho de Souza G, Haas APS, von Poser GL, Schapoval EES, Elisabetsky E. (**2004**) Ethnopharmacological studies of antimicrobial remedies in the south of Brazil. *Journal of Ethnopharmacology*, ***90***, 135-143.

[3] Hilgert NI. (**2001**) Plants used in home medicine in the Zenta River basin, Northwest Argentina. *Journal of Ethnopharmacology*, ***76***, 11-34.

[4] Consolini AE, Sarubbio MG. (**2002**) Pharmacological effects of *Eugenia uniflora* (Myrtaceae) aqueous crude extract on rat's heart. *Journal of Ethnopharmacology*, ***81***, 57-63.

[5] Canoa JH, Volpato G. (**2004**) Herbal mixtures in the traditional medicine of Eastern Cuba. *Journal of Ethnopharmacology*, ***90***, 293-316.

[6] Pepato MT, Mori DM, Baviera AM, Harami JB, Vendramini RC, Brunetti IL. (**2005**) Fruit of the jambolan tree (*Eugenia jambolana* Lam.) and experimental diabetes. *Journal of Ethnopharmacology*, ***96***, 43-48.

[7] Costa TR, Fernandes OFL, Santos SC, Oliveira CMA, Liao LM, Ferri PH, Paula JR, Ferreira HD, Sales BHN, Silva MdRR. (**2000**) Antifungal activity of volatile constituents of *Eugenia dysenterica* leaf oil. *Journal of Ethnopharmacology*, ***72***, 111–117.

[8] Pourgholami MH, Kamalinejad M, Javadi M, Majzoob S, Sayyah M. (**1999**) Evaluation of the anticonvulsant activity of the essential oil of *Eugenia caryophyllata* in male mice. *Journal of Ethnopharmacology*, ***64***, 167-171.

[9] Setzer WN, Setzer MC, Moriarity DM, Bates RB, Haber WA. (**1999**) Biological activity of the essential oil of *Myrcianthes* sp. nov. "black fruit" from Monteverde, Costa Rica. *Planta Medica*, ***65***, 468-469.

[10] Ogunwande IA, Olawore NO, Ekundayo O, Walker TM, Schmidt JM, Setzer WN. (**2005**) Studies on the essential oils composition, antibacterial and cytotoxicity of *Eugenia uniflora* L. *International Journal of Aromatherapy*, ***15***, 147–152.

[11] Craveiro AA, Andrade CHS, Matos FJA, Alencar JW, Machaw MIL. (**1983**) Essential oil of *Eugenia jambolana*. *Journal of Natural Products*, ***46***, 591-592.

[12] Weyerstahl P, Marschall-Weyerstahl H, Christiansen C, Oguntimein BO, Adeoye AO. (**1988**) Volatile constituents of *Eugenia uniflora* leaf oil. *Planta Medica*, ***54***, 546-549.

[13] El-Shabrawy AO. (**1995**) Essential oil composition and tannin contents of the leaves of *Eugenia uniflora* L. grown in Egypt. *Bulletin of the Faculty of Pharmacy (Cairo University)*, ***33***, 17-21.

[14] de Morais SM, Craveiro AA, Machado MIL, Alencar JW, Matos FJA. (**1996**) Volatile constituents of *Eugenia uniflora* leaf oil from northeastern Brazil. *Journal of Essential Oil Research*, ***8***, 449-451.

[15] Nogueira PCdL, Bittrich V, Shepherd GJ, Lopes AV, Marsaioli AJ. (**2001**) The ecological and taxonomic importance of flower volatiles of *Clusia* species (Guttiferae). *Phytochemistry*, ***56***, 443-452.

[16] Scalliet G, Journot N, Jullien F, Baudino S, Magnard JL, Channeliere S, Vergne P, Dumas C, Bendahmane M, Cock JM, Hugueneya P. (**2002**) Biosynthesis of the major scent components 3,5-dimethoxytoluene and 1,3,5-trimethoxybenzene by novel rose *O*-methyltransferases. *FEBS Letters*, ***523***, 113-118.

[17] Wu S, Watanabe N, Mita S, Dohra H, Ueda Y, Shibuya M, Ebizuka Y. (**2004**) The key role of phloroglucinol *O*-methyltransferase in the biosynthesis of *Rosa chinensis* volatile 1,3,5-trimethoxybenzene. *Plant Physiology*, ***135***, 95-102.

[18] Southwell IA. (**1981**) Methoxystyrenes from the genus *Zieria*. *Phytochemistry*, ***20***, 1448-1450.

[19] Flynn TM, Southwell IA. (**1987**) Essential oil constituents of the genus *Zieria*. *Phytochemistry*, ***26***, 1673-1686.

[20] Brophy JJ, Fookes CJR, Houset APN. (**1991**) 2,4,6-Trimethoxytoluene from the genus '*Stockwellia*'. *Phytochemistry*, ***31***, 324-324.

[21] Adams RP. (**1995**) *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy.* Allured Publishing Corp., Carol Stream, Illinois.