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Sarah L. Miller
Department of Chemistry, University of Alabama in Huntsville Huntsville, AL 35899, USA

William A. Haber
Missouri Botanical Garden St. Louis, MO 63166, USA Apdo. 50-5655, Monteverde Puntarenas, Costa Rica, Central America

William N. Setzer
Department of Chemistry, University of Alabama in Huntsville Huntsville, AL 35899, USA

Correspondence:
William N. Setzer
Department of Chemistry, University of Alabama in Huntsville Huntsville, AL 35899, USA
Email: wsetzer@chemistry.uah.edu

Chemical composition of the leaf essential oil of an undescribed species of *Zanthoxylum* from Monteverde, Costa Rica

Sarah L. Miller, William A. Haber, William N. Setzer

Abstract

The leaf essential oil of *Zanthoxylum* sp. A "Peñas Blancas" (Rutaceae), from Monteverde, Costa Rica, was isolated by hydrodistillation and analyzed by GC-MS. A total of 12 compounds were identified in the leaf oil accounting for 100% of the total composition. The most abundant components of the essential oil of *Zanthoxylum* sp. A "Peñas Blancas" were citronellal (23.3%), limonene (16.7%), *trans*-pinocamphone (11.6%), and β-pinene (10.8%). A cluster analysis comparison with previously published leaf oil compositions of *Zanthoxylum* species revealed a high degree of interspecific as well as intraspecific variation in the genus.

Keywords: *Zanthoxylum*; composition; citronellal; limonene; *trans*-pinocamphone; β-pinene; cluster analysis.

1. Introduction

The Rutaceae is a diverse and widely studied family found throughout the world, and its largest genus is *Zanthoxylum*. Many species of *Zanthoxylum* have been extensively studied, due to their large role in the traditional medicine of various cultures[1, 2]. Upon study, these species have demonstrated antifungal, antimicrobial, anti-inflammatory, gastrostimulant, and cytotoxic properties[3-7]. Here, we report the essential oil composition of an undescribed species, *Zanthoxylum* sp. A "Peñas Blancas".

Zanthoxylum sp. A "Peñas Blancas" is a small tree, with glabrous, alternate, parapinnate leaves with 7-8 leaflets. The leaflets, 17×7 cm, are round near the base, grooved dorsally near the apex with petioles around 7-10 cm long; the leaflet apex is abruptly acuminate, the base gradually narrowing and acute, the margins crenate with translucent glands, but the lamina interior without glands. The midvein and lateral veins are impressed above, expressed below, with 8-11 lateral veins per side. The rachis is 10-26 cm long with sparse prickles. The leaves are dark green above and pale olive-green below.

2. Materials and Methods

2.1 Plant Material

Leaves of *Zanthoxylum* sp. A "Peñas Blancas" were collected from a non-reproductive individual tree growing in the Peñas Blancas River Valley of the Monteverde Cloud Forest Preserve (10.3091 N, 84.7162 W, 800 m elevation) on May 14, 2008. The plant was characterized by W. A. Haber, and a voucher specimen was deposited in the herbarium of the National Museum of Costa Rica (voucher number Haber ex Cruz 13276). The fresh leaves (45.9 g) were chopped and hydrodistilled for 4 h using a Likens-Nickerson hydrodistillation apparatus with continuous extraction with $CHCl_3$ (50 mL). The chloroform extract was then evaporated to give the essential oil (618.1 mg).

2.2 Gas Chromatography – Mass Spectrometry

The leaf essential oil of *Zanthoxylum* sp. A "Peñas Blancas" was subjected to gas chromatographic-mass spectral analysis using an Agilent 6890 GC with Agilent 5973

mass selective detector, fused silica capillary column (HP-5ms, 30 m × 0.25 mm), helium carrier gas, 1 mL/min flow rate; injection temperature 200 °C, oven temperature program: 40 °C initial temperature, hold for 10 min; increased at 3 °C/min to 200 °C; increased 2 °/min to 220 °C, and interface temp 280 °C; EIMS, electron energy, 70 eV. The sample was dissolved in $CHCl_3$ to give a 1% w/v solution; 1-µL injections using a splitless injection technique were 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 with those reported in the literature[8] and stored on the MS library [NIST database (G1036A, revision D.01.00)/ChemStation data system (G1701CA, version C.00.01.08)].

2.3 Numerical Cluster Analysis

Forty-four *Zanthoxylum* samples were treated as operational taxonomic units (OTUs). The percentage composition of the main essential oil components was used to determine the chemical relationship between the different samples of *Zanthoxylum* by cluster analysis using the NTSYSpc software, version 2.2[9]. Correlation was selected as a measure of similarity, and the unweighted pair-group method with arithmetic average (UPGMA) was used for cluster definition.

3. Results and Discussion

The essential oil of the fresh leaves of *Zanthoxylum* "Peñas Blancas" was recovered in 1.35% yield, and a total of 12 components were identified in the oil (Table 1). The oil was composed of 35% monoterpenoid hydrocarbons, 52% oxygenated monoterpenoids, and 13% sesquiterpenoid hydrocarbons. The major components of the oil were citronellal (23.3%), limonene (16.7%), *trans*-pinocamphone (11.6%), and β-pinene (10.8%). Phytochemicals such as citronellal and limonene are found in substantial amounts in many *Zanthoxylum* species[10-12], and many of the lesser abundant components of the oil like α-pinene, linalool, and germacrene D are common throughout the Rutaceae[13-15]. The presence of several of the oxygenated monoterpenoids, *trans*-pinocamphone, pinocampheol, myrtenal, and *trans*-dihydrocarvone, however, was unexpected and these components have not been found in any other studied *Zanthoxylum* species.

Table 1: Chemical Composition of the leaf oil of *Zanthoxylum* sp. A "Peñas Blancas".

RI	Compound	% Composition
943	α-Pinene	7.5
977	β-Pinene	10.8
1025	Limonene	16.7
1098	Linalool	7.3
1150	Citronellal	23.3
1154	*trans*-Pinocamphone	11.6
1171	Pinocampheol	4.9
1193	Myrtenal	3.7
1202	*trans*-Dihydrocarvone	1.3
1418	(*E*)-Caryophyllene	4.3
1480	Germacrene D	6.9
1494	Bicyclogermacrene	1.7

In order to place *Zanthoxylum* sp. A "Peñas Blancas" in chemical context with other members of the genus, a cluster analysis of the major essential oil components has been carried out and is shown in Figure 1. The analysis reveals a great degree of variability and plasticity in *Zanthoxylum* essential oil composition. There is a high degree of intraspecific variation as well as interspecific variation. Thus, for example, the sample of *Z. rhoifolium* from Costa Rica[13] is chemically very different from those from Brazil [16-18] and *Z. fagara* oils from Cuba[19] and Costa Rica[10] have very different chemical profiles.

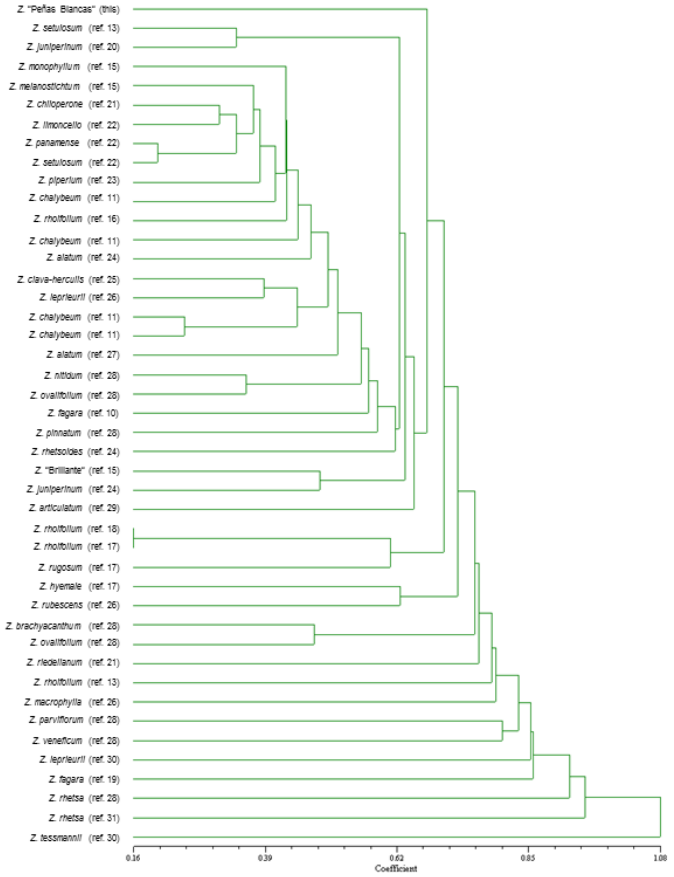

Fig 1: Dendrogram obtained by cluster analysis of the percentage composition of essential oils from *Zanthoxylum* samples, based on correlation and using the unweighted pair-group method with arithmetic average (UPGMA).

4. Conclusions

The leaf oil composition of *Zanthoxylum* sp. A "Peñas Blancas" as revealed in this study is chemically distinct from other *Zanthoxylum* oils (see above), and this is reflected in the cluster analysis.

5. Acknowledgments

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