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Chemical constituents from *Zanthoxylum setulosum* (Rutaceae)

[Costituyentes químicos de *Zanthoxylum setulosum* (Rutaceae)]

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Abstract
Following our phytochemical studies of Costa Rican plants, in this work we report the isolation and identification of eight compounds from aerial parts of *Zanthoxylum setulosum* (Rutaceae). They were identified as the alkaloid skimmianine, the lignans savinin, kusunokinin, sesamin, syringaresinol and the isopentenyl ether of pluviatol, the amide aurantiamide acetate, and the triterpen lupeol. This is the first report of isolation of skimmianine from the leaves of *Z. setulosum* and its presence confirm that quinoline and benzophenanthridine alkaloids, can be considered as chemotaxonomic markers of this genus. All the isolated compounds were characterized by spectroscopic methods (including ^{1}H-NMR, ^{13}C-NMR, , HMQC, HMBC and NOESY) and comparison with the literature data.

Keywords: Rutaceae, *Zanthoxylum*, alkaloids, skimmianine, lignans.

Resumen
Continuando con el estudio fitoquímico de plantas de Costa Rica, en este trabajo informamos el aislamiento e identificación de ocho compuestos de las partes aéreas de *Zanthoxylum setulosum* (Rutaceae). Los compuestos fueron identificados como el alcaloide skimmianina, los lignanos savinina, kusunokinina, sesamina, siringaresinol y el éter isopentílico del pluviatol, la amida conocida como acetato de aurantiamida, y el triterpeno lupeol. Este es el primer informe del aislamiento de skimmianina en las hojas de *Z. setulosum*, lo cual confirma que alcaloides quinolínicos y benzofenantridinicos pueden ser considerados marcadores quimiotaxonómicos en éste género. La estructura de los compuestos aislados fue caracterizada por métodos espectroscópicos (incluyendo ^{1}HNMR, ^{13}C-NMR, HMQC, HMBC y NOESY) y comparación con datos de la literatura.

Palabras Clave: Rutaceae, *Zanthoxylum*, alcaloides, skimmianina, lignanos.

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INTRODUCTION

The *Zanthoxylum* genus that belongs to the family Rutaceae, comprises about 200 species of aromatic trees and shrubs native to the middle latitudes of North and South America, Africa, Asia, and Australia (Talapatra et al., 1973). Several members of this genus are used in traditional medicine around the world. In Costa Rica there are 12 species of this genus (Instituto Nacional de Biodiversidad INBio, http://www.inbio.ac.cr/es/default.html) and some of them are used to cure toothache, snake bites, enteritis, diarrhea, urethritis, stomatitis, rheumatism, bronchitis and hemorrhoids *(Ngane et al., 2000; Islam et al., 2001; Matu and van Staden, 2003; Ross et al*., 2004). In terms of phytochemistry, more than 90 species have been studied and among secondary metabolites that appear most frequently are alkaloids (Ahmad *et al*., 2003; de A Gonzaga *et al*., 2003; Jiang *et al*., 2007; Huang *et al*., 2008), terpens (Mathur *et al*., 1967) and lignans (Marcos *et al*., 1990; Chen *et al*., 1999; Fiorentino *et al*., 2007; Chen *et al*., 2008).

In this study, we describe the isolation and identification of eight compounds from aerial parts of *Zanthoxylum setulosum* (Rutaceae).

MATERIALS AND METHODS

General

Column chromatography (CC) was performed on silica gel 70-230 mesh (Merck). Thin layer chromatography (TLC) was conducted on silica gel 60 F_{254} (Merck). NMR spectra were obtained on Varian Mercury 400 MHz instrument, using TMS as internal standard.

Plant material

The aerial parts of *Z. setulosum* were collected in Esparza, Alajuela province, Costa Rica, in February 2005. A voucher specimen has been deposited at the herbarium Juvenal Valerio (Heredia, Costa Rica) under reference Nº 12091.

Extraction and Isolation

Air-dried and powdered aerial parts (1.35 kg of leaves) were extracted with methyl-*tert*-butyl-ether (MTBE): methanol (MeOH) 9:1 at room temperature for 24 hours. The residue (23.2 g) was obtained after removing the solvent under reduced pressure and resuspended in $CHCl_3$. The $CHCl_3$ extract was subjected to silica gel column chromatography (CC) by gradient elution with hexane: MTBE (85:15- 0:100) and finally with MTBE:MeOH (90:10-80:20) to give seven fractions (F_a–F_g).

Fraction F_b was separated by silica gel flash CC eluting with hexane: MTBE (85:15- 0:100) to obtain twenty-five fractions (F_{b1-25}). Fraction F_{b23} was repurified by preparative TLC, eluted with benzene: MTBE 9:1 to yield sesamin (**1**, 54 mg) (Bedigian *et al*., 1985). Fractions F_{b24} was repurified in a similar way to yield lupeol (**6**, 22 mg) (Wenkert *et al*., 1978). Fraction F_c was separated with silica gel flash CC and eluted with hexane: MTBE (85:15- 0:100) to obtain forty fractions (F_{c1-40}). Fraction F_{c35} was repurified by preparative TLC, eluted with benzene: CH_2Cl_2 1:1 to yield the isopentenyl ether of pluviatol (**3**, 5 mg) (Whiting, 1987). Fractions F_{c36} and F_{c38} were repurified in a similar way to give savinin (**4**, 9 mg) (Schrecker and Hartwell, 1954) and kusunokinin (**5**, 16 mg) (Lopes *et al*., 1983) respectively.

Fraction F_d was subjected to silica gel flash CC and eluted with hexane: MTBE (85:15- 0:100) to give fifty fractions (F_{d1-50}). Fraction F_{d43} was repurified by preparative TLC, eluted with benzene: MTBE 9:1 to yield aurantiamide acetate (**7**, 34 mg) (Talapatra et al., 1980).

Finally, fraction F_e was separated by silica gel flash CC eluting with hexane: MTBE (85:15- 0:100) to produce sixty fractions (F_{d1-60}). Fraction F_{e55} was repurified by preparative TLC, eluted with benzene: CH_2Cl_2: MTBE 4:4:2 to yield skimmianine (**8**, 5 mg) (Dreyer and Brenner, 1980) and syringaresinol (**2**, 14 mg) (Sharp *et al*., 2001).

The structures of the eight compounds (figure 1), including one alkaloid, five lignans, one triterpen, and one amide were identified by spectroscopic methods (^1H and ^{13}C NMR, including COSY, HMQC and HMBC) and comparison with the literature data.

RESULTS AND DISCUSSION

The plants of genus *Zanthoxylum* are well know to contain several types of compounds as amides, coumarins, flavonoids, and mainly lignans and alkaloids which show a wide range of pharmacological activities. Previous phytochemical investigations on the aerial parts of *Z. setulosum* have yielded the lignans sesamin, syringaresinol and methylarctigenin, the alkaloid kokusaginin, the sterol 22-oxo-24-methylcholest-5-en-3β-ol, the triterpen lupeol, and other components as cerotic acid, pungenin and several flavanones (Angulo Ortiz and Cuca Suarez, 2002).

In this work, we report for the first time that *Z. setulosum* is a source of skimmianine. Previous studies have shown that this alkaloid presents several biological activities e.g *in vitro* antiviral effect against hepatitis B virus (Yang and Chen, 2008), citotoxic activity (Chen *et al*., 2005) and antimicrobial activity (Hanawa *et al*., 2004). Moreover, the isolation of this compounds confirm that quinoline and benzophenanthridine alkaloids, can be considered as chemotaxonomy markers of this genus (Sun and Duan, 1996).

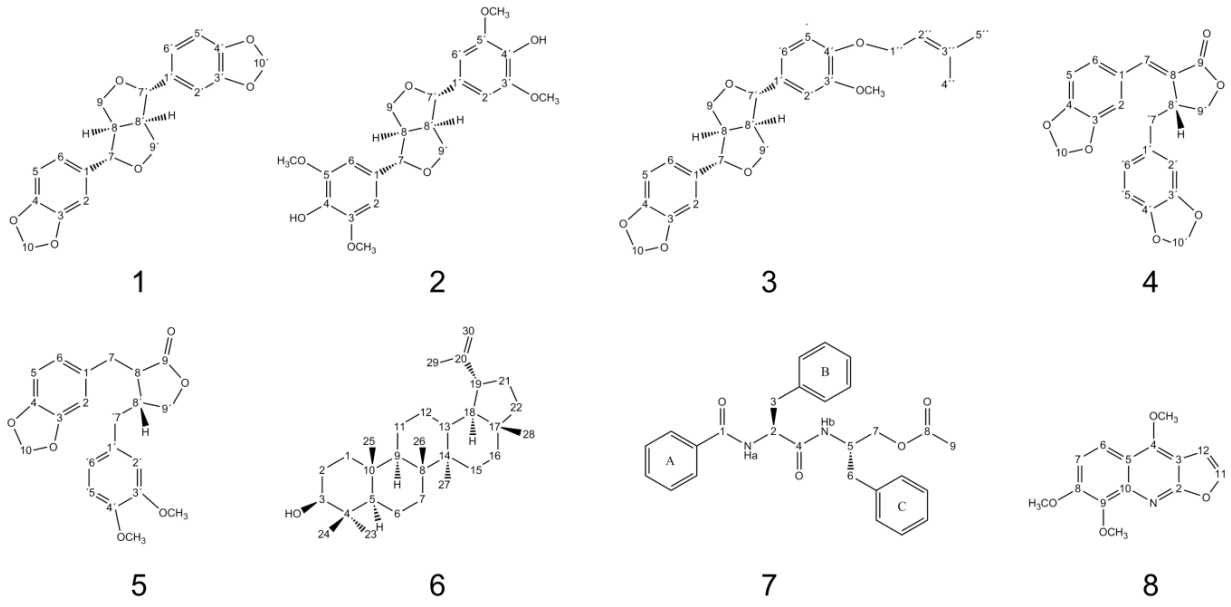

Figure 1. Structures of compounds **1**–**8** isolated from *Z. setulosum*: skimmianine (**8**), the lignans savinin (**4**), kusunokinin (**5**), sesamin (**1**), syringaresinol (**2**) and the isopentenyl ether of pluviatol (**3**), the amide aurantiamide acetate (**7**), and the triterpen lupeol (**6**).

In addition, the lignans savinin, kusunokinin, the isopentenyl ether of pluviatol, and the amide aurantiamide acetate are also report for the first time in the aerial parts of *Z. setulosum*. Finally, in agreement with the previous report by Angulo Ortiz and Cuca Suarez (2002) the lignans sesamin and syringaresinol and the triterpen lupeol were also isolated from *Z. setulosum*.

CONCLUSIONS

In this new phytochemical study of the aerial parts of *Z. setulosum*, we report the isolation and identification of eight known compounds. They were identified as the alkaloid skimmianine, the lignans savinin, kusunokinin, sesamin, syringaresinol and the isopentenyl ether of pluviatol, the amide aurantiamide acetate, and the triterpen lupeol.

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