

# Ent-labdane type diterpene with antifungal activity from *Gymnosperma glutinosum* (Spreng.) Less. (Asteraceae)

[Diterpeno tipo Ent-labdano con actividad fungicida aislado de *Gymnosperma glutinosum* (Spreng.) Less. (Asteraceae)]

Rocío SERRANO<sup>1</sup>, Tzasná HERNÁNDEZ<sup>1</sup>, Margarita CANALES<sup>1</sup>, Ana María GARCÍA-BORES<sup>1</sup>, Alfonso ROMO DE VIVAR<sup>2</sup>, Carlos L. CÉSPEDES<sup>3</sup>, and José Guillermo AVILA<sup>1\*</sup>

<sup>1</sup>Laboratorio de Fitoquímica, UBIPRO, FES Iztacala UNAM. Av. de las Barrios # 1, Los Reyes Iztacala. Tlalnepantla, Estado de México. CP 54090

<sup>2</sup>Instituto de Química, UNAM. Circuito Exterior, Ciudad Universitaria. Coyoacán, México D.F. CP 04510

<sup>3</sup>Laboratorio de Bioquímica Vegetal y Fitoquímica-Ecológica, Departamento de Ciencias Básicas, Facultad de Ciencias, Universidad del Biobío, Av. Andres Bello s/n, Casilla 447, Chillán, Chile.

## Abstract

The aim of this work was to isolate and identify the antifungal compounds present in the hexanic extract of the aerial parts of *Gymnosperma glutinosum*. The antifungal activity against six pathogenic fungi was determined in accordance with the inhibition of radial growth method. Four of those fungi *Aspergillus niger*, *Candida albicans*, *Fusarium sporotrichum* and *Trichophyton mentagrophytes* are of clinical importance and two fungi *Fusarium moniliforme* and *Rhizoctonia solani* are of agricultural importance. The bioassay-guide purification of the methanolic partition (from hexanic extract) resulted in the isolation and identification of three antifungal compounds an ent-labdane type diterpene and two methoxylated flavones, as the metabolites responsible for activity.

**Keywords:** Antifungal activity; *Gymnosperma glutinosum*; Methoxylated flavones; (+)-8S, 13S, 14R, 15-Ent-labdanetretrol.

## Resumen

El objetivo de éste trabajo fue aislar e identificar los compuestos con actividad fungicida presentes en el extracto hexánico de *Gymnosperma glutinosum*. Para evaluar la actividad antifúngica se usaron métodos de inhibición del crecimiento radial contra seis hongos patógenos; cuatro de importancia clínica: *Aspergillus niger*, *Candida albicans*, *Fusarium sporotrichum* y *Trichophyton mentagrophytes*; y dos de importancia agrícola: *Fusarium moniliforme* y *Rhizoctonia solani*. A partir de la partición metanólica (del extracto hexánico), y mediante separación biodirigida, se aislaron e identificaron 3 compuestos activos; un diterpeno con esqueleto tipo ent-labdano y 2 flavonas metoxiladas. En este reporte se muestra la actividad de los extractos y de los compuestos aislados así como la elucidación de la estructura del (+)-8S, 13S, 14R, 15-ent-labdanotretrol.

**Palabras Clave:** Actividad fungicida; Flavonas metoxiladas; *Gymnosperma glutinosum*; (+)-8S, 13S, 14R, 15-Ent-labdanotretrol.

**Recibido | Received:** June 24, 2009.

**Aceptado en Versión Corregida | Accepted in Corrected Version:** September 1, 2009.

**Publicado en Línea | Published Online:** September 30, 2009.

**Declaración de intereses | Declaration of interests:** Authors have no competing interests.

**Financiación | Funding:** This work was financially supported by a grant of DGAPA-UNAM IN- 211105-3 and Proyecto Conservación de Plantas Útiles de San Rafael Coxcatlán, a Través de Bancos de Semillas y Propagación, MGU/Useful Plants Project (UPP) - México, Royal Botanic Gardens Kew.

**This article must be cited as:** Rocío Serrano, Tzasná Hernández, Margarita Canales, Ana María García-Bores, Alfonso Romo De Vivar, Carlos L. Céspedes, and José Guillermo Avila. 2009. Ent-labdane type diterpene with antifungal activity from *Gymnosperma glutinosum* (Spreng.) Less. (Asteraceae). Bol Latinoam Caribe Plant Med Aromat 8(5):412 – 418. {EPub September 30, 2009}.

\*Contactos | Contacts: Email [tuncomaclovio@correo.unam.mx](mailto:tuncomaclovio@correo.unam.mx) (J.G. Avila Acevedo). Tel: +52 5623 1136; Fax: +52 5623 1225.



BLACPMA es una publicación de la [Cooperación Latinoamericana y Caribeña de Plantas Medicinales y Aromáticas](#)

This is an open access article distributed under the terms of a Creative Commons Attribution-Non-Commercial-No Derivative Works 3.0 Unported Licence. (<http://creativecommons.org/licenses/by-nc-nd/3.0/>) which permits to copy, distribute and transmit the work, provided the original work is properly cited. You may not use this work for commercial purposes. You may not alter, transform, or build upon this work. Any of these conditions can be waived if you get permission from the copyright holder. Nothing in this license impairs or restricts the author's moral rights.

Este es un artículo de Acceso Libre bajo los términos de una licencia "Atribución Creativa Común-No Comercial-No trabajos derivados 3.0 Internacional" (<http://creativecommons.org/licenses/by-nc-nd/3.0/deed.es>) Usted es libre de copiar, distribuir y comunicar públicamente la obra bajo las condiciones siguientes: Reconocimiento. Debe reconocer los créditos de la obra de la manera especificada por el autor o el licenciadore (pero no de una manera que sugiera que tiene su apoyo o apoyan el uso que hace de su obra). No comercial. No puede utilizar esta obra para fines comerciales. Sin obras derivadas. No se puede alterar, transformar o generar una obra derivada a partir de esta obra. Al reutilizar o distribuir la obra, tiene que dejar bien claro los términos de la licencia de esta obra. Alguna de estas condiciones puede no aplicarse si se obtiene el permiso del titular de los derechos de autor. Nada en esta licencia menoscaba o restringe los derechos morales del autor.

## INTRODUCTION

One of the predominant species of the secondary vegetation of Mesoamerica is *Gymnosperma glutinosum*. This plant grows in perturbed areas in association with secondary vegetation and crops (Rzendowski and Rzendowski, 1985). In México, *G. glutinosum* is known as “Tatalencho” or “Popote” and used for the treatment of rheumatism, diarrhea, ulcerations and abscesses, as well as dermatological infections (Arias et al., 2000; Hernández et al., 2003 and Canales et al., 2005). It has been reported that *G. glutinosum* contains flavones, diterpenes, and essential oils (Yu et al., 1988, Horie et al., 1998, Maldonado et al., 1994; Canales et al., 2007). In a previous study, the hexanic extract of *G. glutinosum* was found to have antitumor activity in L5178Y-R lymphoma cells (Gomez-Flores et al., 2009). Canales et al. (2007) evaluated the antibacterial activity of extracts and compounds isolated from *G. glutinosum* grown in two ecologically different localities in México. The hexanic extract, as well as 5,7-Dihydroxy-3,6,8,2',4',5' hexamethoxyflavone, and (-)-17-Hydroxy-neo-clerod-3-en-15-oic acid, were active antibacterial substances. In addition, the hexanic extract showed antifungal activity against five species of mycelial fungi.

The aim of this work was to isolate and identify the antifungal compounds presents in the hexanic extract of the aerial parts of *G. glutinosum* collected in Zapotitlán de las Salinas, Puebla. Here, we describe the bioassay-guided purification and activity of (+)-8S, 13S, 14R, 15-ent-labdane-tetrol; 5, 7 dihydroxy 3, 6, 8-trimethoxyflavone and 5, 7 dihydroxy 3, 6, 8, 2', 4', 5'-hexamethoxyflavone.

## MATERIAL AND METHODS

### General experiment procedures

Uncorrected melting points were determined on a Fisher-Johns apparatus. IR spectra were acquired on a Nicolet Magna FT-IR 750 spectrometer. <sup>1</sup>H and <sup>13</sup>C NMR spectra were measured on a Varian-Unity Plus 500 spectrometer (at 500/125 MHz) and a Varian-Gemini 200 spectrometer (at 200/75 MHz). EI-Mass spectra were measured on a Jeol JMS-AX505HA spectrometer. Column chromatography (CC) was performed with silica gel 60 (sigma 5-2509). TLC silica gel 60 F<sub>254</sub> (Macherey-Nagel 818133) plates were used to follow the fractionation process.

### Plant material

*G. glutinosum* was collected in Zapotitlán Salinas, Puebla, México, at 18°24'68" north latitude and 97°25'80" west longitude. A voucher specimen was authenticated by Dra Edith López and deposited in the IZTA Herbarium, FES-Iztacala- UNAM, with the registry number IZTA 41637.

### Microbial strain

Six fungal pathogens were used: *Fusarium sporotrichum* was from ATCC NRLL 3299; *Aspergillus niger*, *Trichophyton mentagrophytes* and *Fusarium moniliforme* were donated by Dr. Cesar Flores (Laboratory of Plant Physiology, UBIPRO, FES-Iztacala) *Rhizoctonia solani* was donated by Dr. Raúl Rodríguez (INIFAP-Texcoco) and *Candida albicans* was donated by Dra Gloria Paniagua (CUSI, FES-Iztacala, UNAM). The stock cultures of the molds were maintained on Czapek Dox agar (Dibico 1106), and *C. albicans* was maintained on Dextrose Sabouraud agar (Dibico 1007).

### Antifungal activity

The assays of antifungal (mold) activity were carried out in petri dishes containing Czapek Dox agar (20 mL). A disk with a suspension of 1x10<sup>4</sup> conidia of each tested strain was placed in the center of each plate. After the mycelial colony had developed, paper disks saturated with 1 mg/disk of each extract, fractions or pure compound were placed at a distance of 0.5 cm away from the rim of the mycelial colony. The petri dishes were incubated at 23 °C for 72 h until mycelial growth had developed. Disks containing samples that had formed crescents of inhibition were considered with antifungal activity (Wang and Bun, 2002). The assay for anti-yeast activity was carried out in accordance with the disk diffusion method (Vardar-Ünlü et al., 2003).

For quantitative assays, seven doses of each extract or pure compound (0.062, 0.125, 0.250, 0.5, 1.0, 1.5 and 2.0 mg/mL) dissolved in sterile olive oil or dimethyl sulfoxide were added to rapidly mixing Czapek Dox agar (5 mL) and poured into 6 cm petri dishes. When the agar reached the room temperature, the conidia were inoculated (1x10<sup>4</sup> conidia/10 µL).

After incubation at 23 °C for 72 h, the area of the mycelial colony was measured and the inhibition of the fungal growth was determined by the following formula:

$$I (\%) = d_c - d_t / d_c \times 100$$

$d_c$ : diameter of the colony of the control culture

$d_t$ : diameter of the colony of the treated culture

The concentration-response curves were constructed with data obtained from solving the formula above. These curves were then used to calculate the IC<sub>50</sub> and IC<sub>100</sub> using the linear regression method. Ketoconazole was used as a positive control and the appropriated negative controls (solvent used for extract dilutions) were also used (Ye et al., 1999)

### Extraction and isolation

Aerial parts of *G. glutinosum* (1200 g) were shade-dried at room temperature, ground in powder and sequentially extracted with n-hexane, ethyl acetate (EtOAc) and methanol (MeOH) (100 g per 1500 mL) at room temperature. After filtration, the extracts were concentrated under low pressure at 45 °C. Finally, the yield (w/w) for each type of extract was determined.

The active extract (80 g hexane) was partitioned with methanol (M<sub>1</sub>), and hexane (H<sub>1</sub>). The active M<sub>1</sub> partition (40.27 g) was fractionized by column chromatography (CC) on silica gel (sigma 5-2509), and eluted with a hexane-EtOAc gradient (1:0 to 2:8) and EtOAc-MeOH gradient (9:1 to 1:1), yielding 31 fractions. Fractions 17 and 29 contained two active pure compounds (1 and 2, respectively). Fraction 23 (6.197 g, active) was applied to a silica gel column and eluted with a chloroform-acetone gradient (1:0 to 2:8), which provided 11 fractions. Compound 3 was isolated from fraction 7, which was eluted with chloroform-acetone 9:1.

#### 5, 7-dihydroxy-3, 6, 8-trimethoxyflavone (1):

Obtained as a yellow powder (201 mg); Identified by comparison with reported physical and spectroscopic data (Horie et al., 1998; Yu et al., 1988).

#### 5, 7-dihydroxy-3, 6, 8, 2', 4', 5'-hexamethoxyflavone (2):

Obtained as a yellow powder (55 mg); Identified by comparison with reported physical and

spectroscopic data (Horie et al., 1998; Yu et al., 1988).

#### (+)-8S, 13S, 14R, 15-ent-labdanetetrol (3):

Obtained as a white powder (166 mg); mp. 95-96°C (hexane-EtOAc);  $[\alpha]_D^{25} +5.5^\circ$  (CH<sub>3</sub>OH;  $c = 0.1$ ); IR bands  $\nu_{max}$  (CHCl<sub>3</sub>) : 3331 (OH), 1028 (C-O), 2921 (C-H) 2899, 966 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, CH<sub>3</sub>OD): see Table 2; <sup>13</sup>C NMR (125.7 MHz, CH<sub>3</sub>OD) see Table 2; EIMS  $m/z$  (rel. int.): 342 [M<sup>+</sup>, 2.65], 245 (100), 177 (31), 137 (34), 109 (36), 95 (38), 69 (40), 43 (58).

### Statistical analysis

The results of the assays for antifungal activity are expressed as the mean ± S.D. The statistical analysis was carried out using one and two way analysis of variance (ANOVA).

## RESULTS AND DISCUSSION

### Biological activity of extracts and partitions

Six species of pathogenic fungal microorganisms were used (five molds and one yeast) to evaluate the antimicrobial effect of the extracts. Inhibition of radial growth 12 mm from the surrounding disk was considered an indicator of a good inhibitory effect, while 6 mm of inhibition diameter was considered good for the yeast bioassay. Hexanic extract and the M<sub>1</sub> partition showed an inhibitory effect on the growth of test molds, whereas no effect was observed on the growth of yeast (Table 1). Quantitative results of the bioassays showed that the hexane extract and M<sub>1</sub> partition exhibited antimicrobial activity against all molds (Table 1). M<sub>1</sub> showed the lowest IC<sub>50</sub>. In this instance, the M<sub>1</sub> partition was selected for fractionation and isolation of active compounds.

Canales et al. (2007) reported the antifungal activity of the hexanic extract of *G. glutinosum* collected in two different localities in México (San Rafael and Tepeji del Río). In this report, we showed the results of the hexanic extract activity of *G. glutinosum* collected in Zapotitlán de las Salinas. Zapotitlán is an arid zone that is drier than San Rafael. This fact clearly showed that there are differences in the concentration or chemical structure of the antimicrobial metabolites synthesized by *G. glutinosum* under different environmental conditions (Lamberts et al., 1998). In general, the IC<sub>50</sub> or MIC's obtained for the hexanic extract of *G. glutinosum*

collected in Zapotitlán are smaller than those reported by Canales et al. (2007).

### Chemistry

5, 7-dihydroxy-3, 6, 8-trimethoxyflavone (**1**) and 5, 7-dihydroxy-3, 6, 8, 2', 4', 5'-hexamethoxyflavone (**2**):

Those compounds were isolated as a yellow powder, with melting points of 178-179 and 177-179°C respectively. Their IR, <sup>1</sup>H and <sup>13</sup>C NMR spectra data were identical to those reported in literature (Horie et al., 1998; Yu et al., 1988).

(+)-8*S*, 13*S*, 14*R*, 15-ent-labdanetetrol (**3**):

After fractionation of M<sub>1</sub>, it was isolated an amorphous powder (166 mg) with a melting point of 95-96 °C. The compound has a molecular formula of C<sub>20</sub>H<sub>38</sub>O<sub>4</sub> (EIMS). It possesses four hydroxyl groups, which are identified by strong absorption at 3331 cm<sup>-1</sup> in the IR spectrum. The <sup>1</sup>H NMR data showed signals for five tertiary methyl groups at δ 1.14, 1.11, 0.87, 0.84 and 0.81. In addition, an ABX system in <sup>1</sup>H NMR for protons geminal to hydroxyl functions: one methylene (δ 3.77 *dd*, 1H and δ 3.50 *dd*, 1H) and one methine (δ 3.49 *dd*, 1H) corresponding to a primary and secondary alcohol respectively (Table 2). This was confirmed by the <sup>13</sup>C NMR signals at δ 63.9 (*t*) and 78.1 (*d*). The other signal at δ 75.45 (*s*) is due to the presence of a tertiary hydroxyl geminal to a methyl group (<sup>13</sup>C δ 22.4 *q*; <sup>1</sup>H δ 1.11 *s*). An additional signal at δ 75.36 (*s*) established the presence of other tertiary hydroxyl geminal to a

methyl group (<sup>13</sup>C δ 24.14 *q*; <sup>1</sup>H δ 1.15 *s*). The <sup>13</sup>C NMR and DEPT spectra revealed 20 carbon signals, which were shown by DEPT experiment to be 5 methyls, 8 methylene, 3 methines and 4 quaternary carbons. The hydrogen and carbon connectivities in **3** were deduced from the results of two-dimensional <sup>1</sup>H-<sup>1</sup>H COSY, HSQC and HMBC experiments. The exact position of the methyl functions, were provided by a two-dimensional HMBC spectrum (Table 2). The attachment of methyl groups 18 and 19 at C-4 was clearly illustrated by the cross-peak of methyl groups at δ 33.8 and 21.9 with H-5 represented by a *dd* centered at δ 0.94. Likewise, the attachment of methyl 20 at C-10 was determined by the cross-peak of the signal at δ 16.8 with H-9 at δ 1.07. These signals show excellent correlation with a diterpene with *ent-labdane* skeleton (**3**) whose structure is similar to the (+)-ent-Labd-7-en-13*S*, 14*R*, 15-triol isolated by Maldonado (1994). In compound **3**, the double bond 7-8 does not exist, probably because that unsaturated bond has been hydrated and given rise to a hydroxyl group that is geminal with respect to methyl 17. This methyl shows a signal in <sup>1</sup>H NMR spectrum at δ 1.15 and this displacement is due to the paramagnetic effect of the OH. A two dimensional NOESY experiment indicated the α position of the methyl 17, which exhibited interactions with methyls 20, 19 and 16 as well as H-7α (δ 1.80, *ddd* *J*= 12.5, 6, 3). All the above mentioned features demonstrate that compound **3** is (+)-8*S*, 13*S*, 14*R*, 15-ent-labdanetetrol (Fig. 1).

**Table 1.** Antifungal activity (IC<sub>50</sub> µg/mL) of crude extracts and partitions from *Gymnosperma glutinosum*

| Strain    | Extract and/or Partition |                |                |           |           | CONTROL      |
|-----------|--------------------------|----------------|----------------|-----------|-----------|--------------|
|           | Hexane                   | H <sub>1</sub> | M <sub>1</sub> | EtOAc     | MeOH      | Ketoconazole |
| <i>An</i> | 125                      | 250            | 125            | <i>na</i> | <i>na</i> | 7            |
| <i>Fm</i> | 250                      | 250            | 125            | >1000     | <i>na</i> | 14           |
| <i>Fs</i> | 500                      | >500           | 125            | >1000     | <i>na</i> | 7            |
| <i>Rs</i> | 60                       | 125            | 60             | >1000     | <i>na</i> | 7            |
| <i>Tm</i> | 60                       | 60             | 60             | <i>na</i> | <i>na</i> | 3.5          |
| <i>Ca</i> | <i>na</i>                | <i>na</i>      | <i>na</i>      | <i>na</i> | <i>na</i> | 94           |

*na*= no activity; Key: *An*= *A. niger*; *Fm*= *F. moniliforme*; *Fs*= *F. sporotrichum*; *Rs*= *R. solani*; *Tm*= *T. mentagrophytes*; EtOAc= Ethyl Acetate, MeOH= Methanol. ANOVA: *F*= 3.72; *P*< 0.05.

**Table 2.**  $^{13}\text{C}$  NMR (125.7 MHz),  $^1\text{H}$  NMR (500 MHz), HMBC and NOESY spectroscopic data<sup>a, b</sup> for compound **3**.

| C <sup>b</sup> | $\delta_{\text{C}}$ | $\delta_{\text{H}}$ (J Hz)                               | HMBC                           | NOESY                   |
|----------------|---------------------|--|--------------------------------|-------------------------|
| 1              | 41.0 <i>t</i>       | 1.71 <i>m</i><br>1.69 <i>m</i>                           | 5, 9<br>5, 9                   |                         |
| 2              | 19.5 <i>t</i>       | 1.41 <i>m</i><br>1.36 <i>m</i>                           | 10<br>10                       |                         |
| 3              | 43.2 <i>t</i>       | 1.37 <i>m</i><br>1.17 <i>m</i>                           | 4, 5, 18, 19<br>4, 5, 18, 19   |                         |
| 4              | 34.2 <i>s</i>       |  |                                |                         |
| 5              | 57.6 <i>d</i>       | 0.94 <i>dd</i> (2.5, 2.5)                                | 2, 3, 4, 6, 7, 18, 19, 20      | 9, 18                   |
| 6              | 19.5 <i>t</i>       | 1.66 <i>m</i><br>1.55 <i>ddd</i> (12.5, 7.5, 3.5)        | 3, 5, 7, 17<br>3, 5, 7, 17     |                         |
| 7              | 44.9 <i>t</i>       | 1.80 <i>ddd</i> (12.5, 6, 3)<br>1.43 <i>m</i>            | 5, 6, 8, 9, 11, 17<br>5, 9, 17 |                         |
| 8              | 75.36 <i>s</i>      |  |                                |                         |
| 9              | 63.1 <i>d</i>       | 1.07 <i>dd</i> (3.0, 7.0)                                | 1, 5, 6, 7, 8, 20              | 5, 18                   |
| 10             | 40.5 <i>s</i>       |  |                                |                         |
| 11             | 21.5 <i>t</i>       | 1.63 <i>m</i><br>1.33 <i>m</i>                           | 7<br>7                         |                         |
| 12             | 43.2 <i>t</i>       | 1.72 <i>br d</i> (8.5)<br>1.47 <i>m</i>                  | 8, 9, 13, 16<br>8, 9, 13, 16   |                         |
| 13             | 75.45 <i>s</i>      |  |                                |                         |
| 14             | 78.2 <i>d</i>       | 3.49 <i>dd</i> (16.0, 8.0)                               | 12, 13, 15                     |                         |
| 15             | 63.9 <i>t</i>       | 3.77 <i>dd</i> (10.5, 2.5)<br>3.50 <i>dd</i> (16.5, 8.0) | 13, 14<br>13, 14               |                         |
| 16             | 22.4 <i>q</i>       | 1.11 <i>s</i>  | 13                             | 17                      |
| 17             | 24.1 <i>q</i>       | 1.14 <i>s</i>  | 7, 8, 9                        | 7 $\alpha$ , 16, 19, 20 |
| 18             | 33.9 <i>q</i>       | 0.87 <i>s</i>  | 3, 4, 5, 19                    | 5, 9                    |
| 19             | 21.9 <i>q</i>       | 0.81 <i>s</i>  | 3, 4, 5, 18, 20                | 16, 17, 20              |
| 20             | 16.1 <i>q</i>       | 0.83 <i>s</i>  | 1, 2, 4, 5, 9, 17, 19          | 16, 17, 20              |

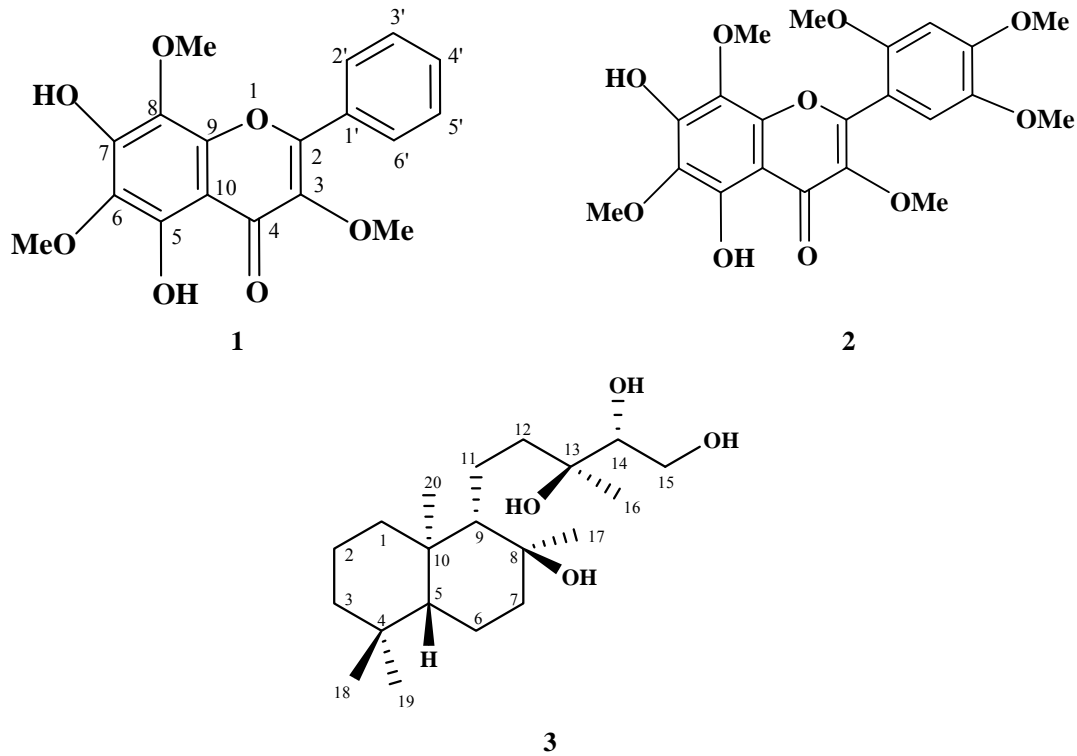
<sup>a</sup> Measured in CD<sub>3</sub>OD, <sup>b</sup> Assignments were made with the aid of the  $^1\text{H}$ - $^1\text{H}$  COSY and HSQC

### Antifungal activity of the isolated compounds

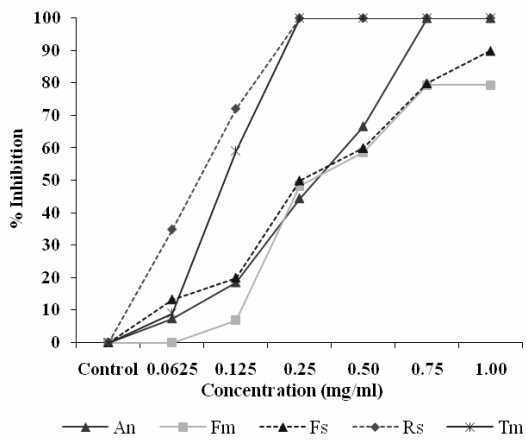
In previous work (Canales et al., 2007) compound **2** was isolated, however, it wasn't evaluated against fungi. Moreover, it did not have a bioassay-guide purification to study the isolated antifungal compounds present in hexanic extract of *G. glutinosum*. Compound **3** was active against the five molds tested. The concentration-response curve (Fig. 2) showed that the most sensitive strains were *R. solani* and *T. mentagrophytes* with an IC<sub>100</sub> of 0.230 and 0.240 mg/mL (in both cases R<sup>2</sup>= 9.4) respectively, and an IC<sub>50</sub> of 0.100 and 0.130 mg/mL, respectively. *F. moniliforme*, *F. sporotrichum*, and *A. niger* showed an IC<sub>50</sub> of 0.5, 0.4 and 0.36 mg/mL, (R<sup>2</sup>= 0.9, 0.92

and 0.98), respectively. The flavones (compounds **1** and **2**) were active only against *R. solani* (Fig. 3); in this case, the IC<sub>100</sub> of compounds **1** and **2** was of 0.420 and 0.230 mg/mL (in both cases, R<sup>2</sup>= 0.96), respectively. *T. mentagrophytes* is a pathogenic fungus in skin, and *R. solani* is a pest in commercial crops. It is important to find antimicrobial compounds specific for pathogenic strains which have little toxic effect on most microorganisms this allows for the target to be attacked without altering the beneficial, normal microbial flora of humans and crops.

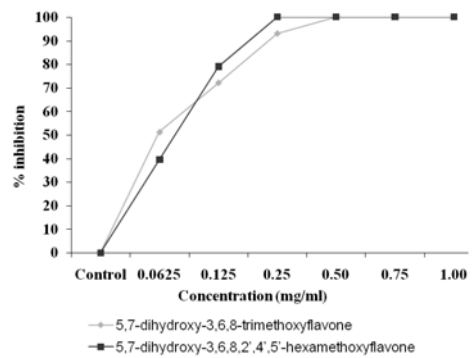
**Figure 1.** Chemical structures of isolate compounds from *G. glutinosum*.



**Figure 2.** Effect of compound 3 on the growth of mycelial fungus.



**Figure 3.** Effect of compounds 1 and 2 on the growth of *R. solani*.



Key: An= *A. niger*; Fm= *F. moniliforme*;  
 Fs= *F. sporotrichum*; Rs= *R. solani*;  
 Tm= *T. mentagrophytes*

## CONCLUSION

This is the first report of an ent-labdane derivative with antifungal activity isolated from *Gymnosperma glutinosum*.

## REFERENCES

- Arias TAA, Valverde VMT, Reyes SJ. 2000. Las plantas de la región de Zapotitlán Salinas, Puebla. Instituto Nacional de Ecología, México, pp. 8-9, 22.
- Canales M, Hernández T, Caballero J, Romo VA, Avila G, Duran A, Lira R. 2005. Informant consensus factor and antibacterial activity of the medicinal plants used by the people of San Rafael Coxcatlán, Puebla, México. *J Ethnopharmacol* 97(3):429-439.
- Canales M, Hernández T, Serrano R, Hernández LB, Duran A, Ríos V, Sigrist S, Hernández HL, Garcia AM, Angeles-López O, Fernández-Araiza MA, Avila G. 2007. Antimicrobial and general toxicity activities of *Gymnosperma glutinosum*: a comparative study. *J Ethnopharmacol* 110(2):343-347.
- Gómez-Flores R, Verástegui-Rodríguez L, Quintanilla-Licea R, Tamez-Guerra P, Monreal-Cuevas E, Tamez-Guerra R, Rodríguez-Padilla C. 2009. Antitumor properties of *Gymnosperma glutinosum* leaf extracts. *Cancer Invest.* 27(2):149-155.
- Hernández T, Canales M, Ávila JG, Durán A, Caballero J, Romo VA, Lira R. 2003. Ethnobotany and antibacterial activity of some plants used in traditional medicine of Zapotitlán de las Salinas, Puebla (México). *J Ethnopharmacol* 88(2-3):181-188.
- Horie T, Ohtsuru Y, Shibata K, Yamashiata K, Tsukayama M, Kawamura Y. 1998. <sup>13</sup>C NMR spectral assignment of the A-ring of polyoxygenated flavones. *Phytochemistry* 47(5):865-874.
- Lambers H, Chopin FS, Pons TL. 1998. *Plant Physiological Ecology*. Springer, New York, p. 427.
- Maldonado E, Segura CR, Ortega A, Calderón JS, Fronczek FR. 1994. Ent-labdane and neo-clerodane diterpenes from *Gymnosperma glutinosum*. *Phytochemistry* 35(3):721-724.
- Rzendowski J, Rzendowski GC. 1985. *Flora Fanerogámica del Valle de México*. Vol II. Escuela Nacional de Ciencias Biológicas IPN e Instituto de Ecología, México, pp. 501, 581.
- Vardar-Ünlü G, Candan F, Sökmen A, Daferera D, Polissiou M, Sökmen M, Dönmez E Tepe B. 2003. Antimicrobial and antioxidant activity of the essential oil and methanol extracts of *Thymus pectinatus* Fisch. et Mey. Var. *pectinatus* (Lamiaceae). *J Agric Food Chem* 51(1):63-67.
- Wang H, Bun BN. 2002. Isolation of an antifungal thaumatin-like protein from kiwi fruits. *Phytochemistry* 61(1):1-6.
- Ye XY, Wang HX, Ng TB. 1999. First chromatographic isolation of an antifungal thaumatin-like protein from French bean legumes and demonstration of its antifungal activity. *Biochem Biophys Res Commun* 263:130-134.
- Yu S, Fang N, Mabry TJ. 1988. Flavonoids from *Gymnosperma glutinosum*. *Phytochemistry* 27(1):171-177.

