PHYTOCHEMISTRY AND PHYTOTOXIC ACTIVITY OF *LAGASCEA MOLLIS* (ASTERACEAE)

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Abstract

In the course of a phytochemical study of *L. mollis* Cav. the previously reported compounds patuletin-7-O-glucoside **1**, 12-hydroxy-13-en-xanthorrhizol **2**, 11-en-13-hydroxy-xanthorrhizol **3**, xanthorrhizol **4**, 12,13-epoxy-xanthorrhizol **5**, and 1-α-angeloyloxycarotol **6**, were isolated. The EtOH extract of the aerial parts showed selective phytotoxic effects on *S. halepense*. Compounds **2** and **3** exhibited potent herbicidal activity on *S. halepense*. Additionally, at all assayed doses, compound **2** affected drastically the root growth.

Keywords: *Lagascea mollis*, Asteraceae, sesquiterpenes, flavonoids, phytotoxicity.

Resumen.

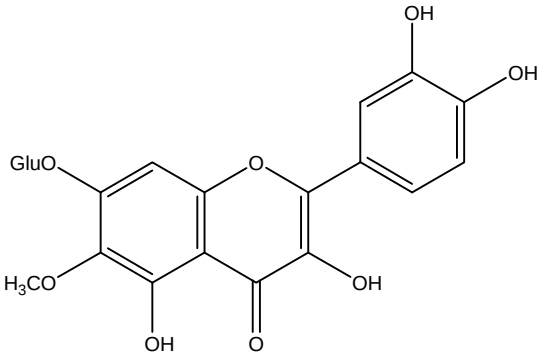
Un estudio de la química y de la actividad fitotóxica de *L. mollis* Cav. permitió el aislamiento de seis compuestos conocidos patuletina-7-O-glucósido **1,** 12-hidroxi-13-en-xanthorrhizol **2**, 11-en-13-hidroxi-xanthorrhizol **3**, xanthorrhizol **4**, 12,13-epoxi-xanthorrhizol **5** y 1-α-angeloiloxicarotol **6**. El extracto etanólico de las partes aéreas de *L. mollis* mostró actividad fitotóxica selectiva en *S. halepense*. Los compuestos **2** y **3** exhibieron potente actividad herbicida frente a *S. halepense*. El compuesto **2** afectó drásticamente el crecimiento radicular de esta especie a todas las dosis ensayadas.

Palabras clave: *Lagascea mollis*, Asteraceae, sesquiterpenos, flavonoides, fitotoxicidad

Introduction

The genus *Lagascea* (Asteraceae, tribe Heliantheae) comprises 9 species, which are distributed in Central America. *L. mollis* Cav. is a unique species that grows from Mexico to northern Argentina [1]. Previous reports about the phytochemistry of this genus include the presence of kauren-type diterpenoids, sesquiterpenoids (one of them as a sesquiterpene lactone), chromenes, acetophenones and one flavan-3-ol from *L. rigida* [2]. Flavonoid glycosides as well as dehydrofalcarinone, were obtained from *L. mollis* [3-4].

L. mollis invades cultivated fields, affecting the growth of other plants. This observation led us to suspect potential allelophatic properties of this species. In the present paper we report on the phytochemical study of *L. mollis* and the effects elicited by the whole extract on the germination and root growth of five crops namely *Allium puerrum, Avena sativa, Solanum lycopersicon, Daucus carota* and *Nicotiana tabacum* as well as the weed *Sorghum halepense*. Moreover, the bioactivity of the compounds **2**, **3** and **4** towards *S. halepense* was studied.



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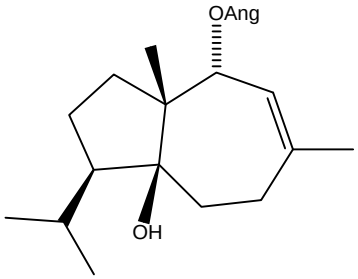

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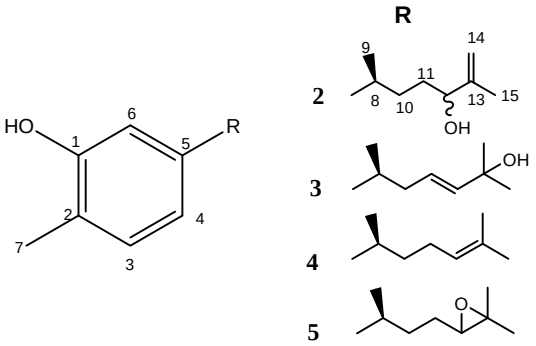

2 **3** **4** **5**

Experimental

General

The NMR spectra were recorded on a Bruker AC 200 spectrometer (^{1}H at 200 MHz and ^{13}C at 50 MHz) with TMS as internal reference. CC were performed on silica-gel 230-400 mesh, RPCC on C-18 silica gel, TLC was carried out on precoated Silica gel 60 F_{254} plates (Merck). Detection was achieved by UV light and spraying with vanillin reagent followed by heating.

Plant Material

The aerial parts of *L. mollis* (flowering) were collected in Salta, Argentina, on March 1998. The plant material was identified by Ing. Lázaro Novara. A voucher specimen (Nº 11028) was deposited at the Museo de la Facultad de Ciencias Naturales, Universidad Nacional de Salta.

Extraction and isolation

Air-dried aerial parts (400 g) of *L. molis* were macerated with EtOH at room temperature for 7 days. The extract was concentrated to dryness under reduced pressure at 40ºC to yield 9.20 g of a dark-green residue (crude extract), which was subjected to flash column chromatography on silica gel C-18, eluted with MeOH-H_2O 1:1 (*Fraction 1*), MeOH-H_2O 7:3 (*Fraction 2)*, MeOH-H_2O 4:1 (*Fraction 3*) and MeOH (*Fraction 4*).

Fraction 1 (0.37 g), was purified by Sephadex LH-20 with Cl_2CH_2:MeOH (9:1) as solvent followed by preparative paper chromatography using H_2O:AcOH 8.5:1.5 as solvent, to give the compound **1** (3 mg).

Fraction 2 (0.90 g), was chromatographed on a silica gel column eluting with a gradient of hexane-EtOAc to yield compounds **2** (30 mg) and **3** (35 mg).

Fraction 3 (0.55 g), was purified by repeated silica gel column chromatography eluting with hexane-Cl_2CH_2 (4:1) yielding compounds **4** (28 mg), **5** (4 mg) and **6** (15 mg).

Bioassay

Seeds. Seeds of *Allium puerrum*, *Avena sativa, Solanum lycopersicon* and *Daucus carota* were purchased from Semillería Agrosalta (Salta, Argentina). Seeds of *Nicotiana tabacum* were obtained from Cooperativa de Tabacaleros de Salta. Seeds of *Sorghum halepense* (free of pesticides) were field collected. The assay seeds were selected for uniformity of size, and all undersized and damaged seeds were discarded. Before the bioassay, seeds were washed with tap water and the surface sterilized using NaClO (10 % v/v) for 10 min, followed by several washes in sterile destilled water.

Solutions. The crude extract, as well as the assayed pure compounds were dissolved in EtOH (10, 100 and 1000 ppm).

Petri Dish Bioassays. Bioassays were carried out using plastic Petri dishes (90 mm diameter) containing a sheet of Whatman Nº 1 filter paper as support. Test solution (5 mL) was added to the filter paper in the Petri dish and dried completely in vacuo at 40ºC. The same volume of EtOH was used as control. After addition of destilled water (5 mL), ten seeds of *A. puerrum*, *A. sativa*, *S. lycopersicon*, *D. carota, N. tabacum* or *S. halepense* were placed on the filter paper, and incubated for 7 days at 25 ± 2 ºC in the dark. There were five replicates for the weed and crop species of each treatment, and parallel controls. The effects of the extract and pure compounds were determined by measuring the radicle length and counting the number of germinated seeds [5, 6].

The elongation of the roots was measured and averaged for each concentration. Inhibition of seed germination was judged by comparing the treated plant with that of the control experiment.

Statistical treatment. The germination and root length values were analysed by the Kruskal Wallis´s test; differences between the experiment and the control were significant with a value of $P \leq 0.05$.

Discussion

The aerial parts of *L. mollis* were extracted with EtOH. The concentrated EtOH extract, after repeated column chromatography on silica gel and on Sephadex LH-20,

yielded patuletin 7-O-glucoside **1** [7], 12-hydroxy-13-en-xanthorrhizol **2** [8], 11-en-13-hydroxy-xanthorrhizol **3** [8] , xanthorrhizol **4** [9,10], 12,13-epoxy-xanthorrhizol **5** [11, 12] and 1-α-angeloyloxycarotol **6** [13]**.** The structures of the isolated compounds were identified by a combination of spectroscopic methods (IR, MS, ^{1}H and ^{13} C NMR, and 2D NMR experiments) as well as by comparison with the literature data. The ^{13}C NMR spectra of both **2** and **5** (Table 1), showed that these compounds were recovered as a C-12 epimeric mixture.

Table 1. Data ^{13}C RMN of bisabolenes **2** and **5** (200 MHz, $CDCl_3$, TMS)

C	2[a]	5[a]
1	153.8	153.8
2	121.1	121.1
3	130.8	130.8
4	119.3	119.3
5	146.7	146.5
6	113.5	113.6, 113.4
7	15.3	15.3
8	39.5, 39.4	39.5, 39.2
9	22.4	18.7
10	34.0, 33.9	35.1, 34.7
11	32.9	27.2, 26.9
12	76.0, 76.1	64.7, 64.4
13	147.3	58.3
14	111.2, 111.1	22.5, 22.3
15	17.5, 17.4	29.8

[a] Epimeric mixture at C-12

In order to evaluate the inhibitory or stimulatory effects on germination and root growth, the EtOH extract of *L. mollis* was tested on the dycotyledons *S. lycopersicon*, *N. tabacum* and *D. carota,* and against the monocotyledons *A. puerrum*, *A. sativa* and *S. halepense.*

In general, the extract had no significant effect on germination or radicle length of the crop species (Tables 2 and 3). In *S. halepense*, the inhibitory effect was more on radicle length than on germination (Tables 2 and 3). Radicle length of *S. halepense* was drastically reduced in response to aerial parts extract of *L. mollis* at all the treatment doses (Table 2, Figure 1). At 10 ppm, radicle length declined by nearly 71%.

Table 2. Growth Inhibition Effect[a] of *L. mollis* Extract on *Nicotiana tabacum*, *Daucus carota, Solanum lycopersicon, Allium puerrum, Avena sativa* and *Sorghum halepense*.

	Dose			Control
	10 ppm	**100 ppm**	**1000 ppm**	
Nicotiana tabacum	3.80 ± 0.24 (-6.4)	3.77 ± 0.41 (-7.1)	2.98 ± 0.82 (-26.6)	4.06 ± 0.61
Daucus carota	21.93 ± 1.78 (-13.3)	24.38 ± 1.75 (-3.6)	22.79 ± 1.59 (-9.9)	25.29 ± 4.10
Solanum lycopersicon	34.49 ± 5.46 (+15.2)	35.12 ± 9.62 (+17.3)	37.12 ± 11.51 (+23.9)	29.95 ± 7.23
Avena sativa	63.68 ± 7.57 (+13.2)	57.82 ± 17.16 (+2.8)	54.45 ± 18.72 (-3.2)	56.26 ± 9.37
Allium puerrum	18.20 ± 2.83 (-11.3)	16.29 ± 3.23 (-20.6)	14.90 ± 6.01 (-27.4)	20.52 ± 2.94
Sorghum halepense	9.44 ± 4.25* (-71.3)	6.32 ± 2.10* (-80.8)	5.81 ± 1.26* (-82.4)	32.91 ± 6.97

[a]Values are the root length (mm) ± SD.
*Significant differences, compared to the control for $P < 0.05$ according to Kruskal Wallis`s test.
Figures in parenthesis indicate percent change control (+ values represent stimulation, -values represent inhibition).

Table 3. Effects of Extract from *L. mollis* on germination of test plants.

	Dose			Control
	10 ppm	**100 ppm**	**1000 ppm**	
Nicotiana tabacum	92.0 ± 4.5 (+4.5)	84.0 ± 20.7 (-4.5)	72.0 ± 14.8 (-18.2)	88.0 ± 16.4
Daucus carota	94.0 ± 5.5 (+4.4)	82.0 ± 13.0 (-8.9)	96.0 ± 8.9(+6.7)	90.0 ± 10.0
Solanum lycopersicon	88.0 ± 8.4 (-4.3)	88.0 ± 8.4 (-4.3)	80.0 ± 7.1 (-13.0)	92.0 ± 4.5
Avena sativa	84.0 ± 11.4 (-6.7)	80.0 ± 18.7 (-11.1)	72.0 ± 14.8 (-20.0)	90.0 ±10.0
Allium puerrum	70.0 ± 15.8 (+8.6)	62.0 ± 14.8 (-3.1)	54.0 ± 11.4 (-15.6)	64.0 ± 21.9
Sorghum halepense	92.0 ± 8.4 (0.0)	74.0 ± 15.2 (-19.6)	52.0 ± 13.0* (-43.5)	92.0 ± 13.4

Values are presented as percentage of seed germination ± SD.
*Significant differences, compared to the control for $P < 0.05$ according to Kruskal Wallis`s test.
Figures in parenthesis indicate percent change control (+ values represent stimulation, -values represent inhibition).

The effect of the three isolated major secondary metabolites **2**, **3** and **4** on germination and root growth of *S. halepense* was examined (Table 4).

Table 4. Effects of bisabolenes **2**, **3** and **4** on radicle growth[a] and germination[b] of *S. halepense*.

Compound	Radicle length (mm)				Germination		
	10 ppm	100 ppm	1000 ppm		10 ppm	100 ppm	1000 ppm
2	14.02 ± 2.91* (-47.7)	5.90 ± 0.97* (-78.0)	3.24 ± 0.53* (-87.9)		52.0 ± 8.4 (-42.0)	34.0 ± 5.5* (-62.0)	22.0 ± 4.5* (-76.0)
3	28.64 ± 3.33 (+7.0)	13.92 ± 2.43* (-48.0)	13.04 ± 2.70* (-51.3)		68.0 ± 8.4 (-24.0)	44.0 ± 5.5* (-51.0)	40.0 ± 12.2* (-56.0)
4	32.14 ± 3.12 (20.0)	30.22 ± 3.97 (+13.0)	33.54 ± 6.20 (25.2)		50.0 ± 7.1 (-44.0)	48.0 ± 4.5 (-47.0)	26.0 ± 8.9* (-71.0)
Control		26.78 ± 4.09				90.0 ± 17.30	

[a]Values are the root length (mm) ± SD.
[b]Values are presented as percentage of seed germination ± SD.
*Significant differences, compared to the control for $P < 0.05$ according to Kruskal Wallis`s test.
Figures in parenthesis indicate percent change control (+ values represent stimulation, -values represent inhibition).

Compounds **2** and **3** showed significant inhibitory activity on germination at 100 ppm and 1000 ppm. Compound **4** was less active. This compound only inhibited germination of *S. halepense* at the highest dose tested.

Statistical analysis of our data (Kruskal-Wallis's test) indicated that **4** had no significant effects on radicle length of *S. halepense* (Table 4, Figure 1). Compound **2** showed significant inhibitory effects on root growth of *S. halepense* at all tested concentrations. This compound reduced radicle length by about 87.9 % at 1000 ppm and retained 47.7 % of inhibition at the lower concentration tested (10 ppm). Compound **3** caused significant reduction of root length at 100 (-48.0 %) and 1000 ppm (-51.3%).

Conclusions

Investigation of the aerial parts of *L. mollis* led to the isolation and characterization of four bisabolene-type sesquiterpenes **2-5** and one carotane-type sesquiterpene **6** which are now reported for the first time in the genus *Lagascea*. The isolation of **1** was consistent with a previous report on the *L. mollis* phytochemistry [3].

Results indicate that bisabolene-type sesquiterpenes **2-5**, predominate in *L. mollis*. These compounds have been previously obtained from *Iostephane heterophylla* [8, 11], which belongs to the same subtribe (Helianthineae) [14]. These findings may indicate relative relationship between *Lagascea* and *Iostephane*.

The results obtained in the present study are the first report on phytotoxic properties of *L. mollis.* The *L. mollis* extract showed selective bioactivity against weed and crop species.

Although several biological activities have been previously reported for compounds **2-4** [8, 11, 12, 15, 16], the biological activity on the plant growth has not been reported. Both bisabolenes **2** and **3**, are natural products with potent herbicidal activity under laboratory conditions. Thus, the study of structure-activity relationship of these compounds described here and their analogs would be important to investigate in the future.

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