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Phytochemical and pharmacological studies on *Mikania micrantha* H.B.K. (Asteraceae)

Estudios fitoquímico y farmacológico de *Mikania micrantha* H.B.K. (Asteraceae)

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Abstract. Asteraceae is one of the largest families of Angiospermae, and contains the *Mikania* genus. Several species of this family contain polyacetylenic and thiophenic compounds which are used as taxonomic markers. Also, their phototoxic activity acts as plant defense mechanism. In this paper we report the absence of these compounds in *Mikania micrantha*.We also describe the composition of the volatile oil from the seeds and inflorescence in the species. Linalool and α-pinene were the main components of this *M. micrantha's* essential oil. This study tested the ability of extracts of *Mikania micrantha* to inhibit the mouse ear inflammation in response to topical application of 12-O-tetradecanoylphorbol-13-acetate (TPA). The antibacterial activity of extracts was also evaluated against *Bacillus subtilis* and *Escherichia coli*. Ethyl acetate extracts of this plant exhibited significant antibacterial and anti-inflammatory properties. Therefore, it could be used as a medicinal plant.

Keywords: *Mikania micrantha*; Asteraceae; Bioactivity; Essential oil; Taxonomic markers; Phototoxic compounds.

Resumen. La familia Asteraceae es una de las más grandes de las Angiospermas, y es donde se ubica al género *Mikania*. Varias especies de esta familia contienen compuestos poliacetilénicos y tiofénicos los cuales se emplean como marcadores taxonómicos, y cuya fototoxicidad puede actuar como mecanismo de defensa en la planta. En este trabajo se reporta la ausencia de estos compuestos en la especie *Mikania micrantha*. También se describe la composición de los aceites volátiles de semillas e inflorescencias en esta especie. Linalool y α-pineno son los principales componentes de este aceite esencial de *M. micrantha*. Este estudio prueba la capacidad de los extractos de *Mikania micrantha* para inhibir la inflamación inducida tópicamente por 12-O-tetradecanoylphorbol-13-acetate (TPA) en oreja de ratón y para evaluar la actividad antibacterial contra *Bacillus subtilis* y *Escherichia coli*. Los extractos de acetato de etilo obtenidos de esta planta muestran propiedades antiinflamatorias y antibacteriales significativas, por lo que esta planta bien puede ser utilizada como medicinal.

Palabras clave: *Mikania micrantha*; Asteraceae; Bioactividad; Aceite esencial; Marcadores taxonómicos; Compuestos fototóxicos.

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INTRODUCTION

Mikania micrantha H.B.K. (Asteraceae) is original from Central and South America. This genus is found in the tropics of America and Asia, and is widely known as guaco. It comprises about 300 identified species, but only 20 of them have been studied. It is used to treat fever, rheumatism, influenza and respiratory diseases. (Cabral et al., 2001; Muceneeki et al., 2009).

Terpenes are the major constituents isolated from plants of this genus and its essential oil compounds have large applications in pharmaceutic and cosmetic industries (Silva-Santos et al., 2004).

We analyzed the composition of the volatile oil from seeds and inflorescences of *Mikania micrantha.* We seeked also for the presence of phototoxic compounds, important taxonomic markers, and natural defence mechanisms against insects in species belonging to the Asteraceae family.

In this study, the pharmacological potential of *M. micrantha* was evaluated (to treat erisipela, snake beat and gastrointestinal disorders) because it is an important natural resource in the traditional medicine (Martínez et al., 2001).

Mikania micrantha has not been tested in the tetradecanoylphorbol acetate (TPA) mouse ear model of inflammation. This model may block the inflammatory response to topical TPA (Gabor, 2003). Because this plant is being used as a natural product applied to the skin, it is important to test *M. micrantha* in this model.

Antibacterial activity was tested by microplate method for determining the susceptibility of *E. coli* and *B. subtilis* to extracts of *M. micrantha* and ampicillin.

The broad activity spectrum of the extracts explains the widespread use of this plant for wound healing and other applications.

MATERIALS AND METHODS

Plant material. The plant was collected in Cuetzalan del Progreso, Puebla, Mexico, on March 13, 2006. Vouchers were deposited in the National Herbarium, Instituto de Biología, Universidad Nacional Autónoma de México (MEXU).

Preparation of extracts. The plant was divided in stem with leaves, and inflorescences with seeds, which were successively extracted at room temperature with hexane, ethyl acetate and methanol. Solvents were eliminated at reduced pressure, and the dry extracts were used for the different tests.

Essential oil. The essential oil was obtained by steam distillation of inflorescence and seeds, and was analyzed in a CG-LCR-IQUI chromatograph with an AT Aquawax column.

Pharmacological activities. The anti-inflammatory and antibacterial potential were measured using the three (hexane, ethyl acetate, methanol) extracts. The anti-inflammatory potential was tested with the mouse ear edema test, and the antibacterial potential was measured against *Bacillus subtilis* (ATCC-6051) and *Escherichia coli* (ATCC-6051).

The anti-inflammatory activity of the 3 extracts was carried out by the mouse ear edema test induced with TPA (12-O-tetradecanoyl-phorbol-13-acetate) (De Young et al., 1989). For each determination, three male CDI mice (25-30 g) were used; 10 mL of an ethanolic solution (0.25 mg/ml) of TPA (2.5 mg/ear) were applied to the surface of the right ear; the left ear was used as the blank; 10 min. after the application of TPA, 20 mL of the extracts (1 mg dissolved in ethanol) were applied topically After the fourth hour, the animals were sacrificed and a section (7 mm) of the ear was weighted. The increase in weight of the right ear with respect to the left one indicates the swelling produced by TPA. Percentage inhibition was calculated by comparison with the control (left ear). Indometacine (0.046, 0.085, 0.15 mg / ear) was used as drug reference.

The following experiments were carried out:

Edema A: edema induced by TPA alone,

Edema B: edema induced by TPA plus sample,

Inhibitory ratio (%) = [(Edema A - Edema B) / Edema A] x 100

Each value represents the mean of individual determinations from 3 mice. The experiment was repeated 3 times.

The bactericidal potential was determined by the microwell dilution method (Ozturk et al., 2006). The inoculum of bacteria was prepared from 18h broth cultures, and suspensions were adjusted to 0.5 McFarland standard turbidity. The Minimal Inhibitory Concentration (MIC) of *Mikania micrantha* extracts (hexane, ethyl acetate and methanol) against bacterial strains [*Escherichia coli* (ATCC-6051) and *Bacillus subtilis* (ATCC-6633)] was determined. Ninety-six well plates were prepared by dispensing 95 µl of nutrient broth and 5 µl of the inoculum into each well. A 100 µl extract from *Mikania micrantha* initially prepared at concentration of 8 µg/µl, was added into the first well. Then, 100 µl from their serial dilutions was transferred into twelve consecutive wells. Wells containing 195 µl of nutrient broth without compound and 5 µl of the inoculum were used as a test for growth. The negative control contained: 195 µl of nutrient broth, the solution solvents and 5 µl of the inoculum. This ensured that the extraction solvents had no inhibitory effects on the bacterial strains.

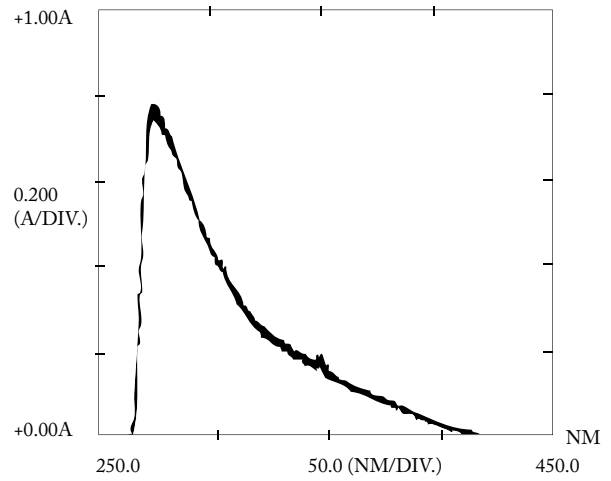
The final volume in each well was 200 µl. Ampicille anhydrous (Sigma) at the concentration range of 0.8 - 0.001 µg/µl was prepared in nutrient broth and used as standard drug for positive control. Contents of each well were mixed on a plate shaker at 300 rpm for 20s and then incubated at 37 °C for 20h. After the incubation period, microbial growth was determined by reduction of TTC (colorless compound) to formazan (red compound). With this purpose, 40 µl of 2, 3, 5-triphenyl tetrazolium chloride (TTC) (Sigma) (0.2 mg/ml) were added to each microplate well. The covered microplates were incubated at 37 °C and 100% relative humidity for 4h

and 12h. The extracts analyzed in this study were screened three times against each organism. The MIC of each extract was taken as the lowest concentration that showed no growth, and it was confirmed by plating 5µl samples from clear wells on nutrient agar medium.

RESULTS

Phototoxic compounds. We didn't find any phototoxic compounds in *Mikania micrantha* despite the fact that the Asteraceae family contains phototoxic compounds (polyacetylenes and thiophenes). The hexane extracts did not show the characteristic blue pale spots on TLC and the UV bands were not characteristic (Fig. 1). The phototoxic activity was negative.

Fig. 1. UV spectrum of hexane extracts from *Mikania micrantha*.
Fig. 1. Espectros UV de extractos de hexano en *Mikania micrantha*.



The absorption bands in the 250 and 350 region are not characteristic of polyacetylenes and thiophenes.
Las bandas de absorción en la región de 250 y 350 no son características de poliacetilenos y tiofenos.

Essential oil. Ten Components were separated by gas chromatography. The two major compounds were linalool (15.86%) and α-pinene (10.14%) (Table 1).

Pharmacological activities

Anti-inflammatory potential: The hexane and ethyl acetate extracts showed a significant anti-inflammatory activity; the methanol extracts were inactive. The hexane extract from stems and leaves showed 49.69% inhibition of the mouse ear edema; the indometacine control had 71.02%, and the hexane extract had 21.33% (Table 2). Seed and inflorescence extracts had 51.75% inhibition. Inhibition from the ethyl acetate extract from stem and leaves was 88.30%. Seeds and inflorescence extracts showed 54.62% of ear edema inhibition, corresponding to 16.4% (Table 2).

Table 1. Identification of essential oil components by gas chromatography.
Tabla 1. Identificación de componentes de aceites esenciales por cromatografía gaseosa.

Peak	Name compound	Retention time (min)	Peak Area (%)	Concentration (%)
1	α-pinene	3.231	19.17	10.143
2	camphene	4.354	0.35	0.187
3	β-pinene	5.911	16.49	8.724
4	α -felandrene	6.662	0.75	0.395
5	β -ocimene	7.131	13.45	7.118
6	linalool	20.616	29.97	15.860
7	geranyl acetate	21.282	1.57	0.829
8	terpineol	24.391	11.92	6.310
9	geraniol	27.622	5.46	2.892
10	thymol	32.051	0.87	0.458

Table 2. Anti-inflammatory activity of *Mikania micrantha*.
Tabla 2. Actividad antiinflamatoria de *Mikania micrantha.*

Topically administered (TPA 2.5 mg /ear)			
Extract doses: 1 mg / ear		% Inhibition of edema	
Extracts		**Hex**	**AcOEt**
Leaves and Stems		**49.69** ± 2.62*	**88.30** ± 0.38*
Seeds and inflorescences		**51.75** ± 1.67*	**54.62** ± 1.65*
Indometacine	**Drug doses** (mg / ear)	**Drug reference**	
	0.046	**27** ± 4.70*	
	0.085	**50** ± 3.40*	
	0.150	**71** ± 0.62**	

Effect on TPA-induced mouse ear edema. Values are the mean of n=3 ± 1 standard deviation. Results were analyzed by a student's t test, and values of * p≤0.05, ** p≤0.01 are considered *significantly or **highly significantly different, respectively, from controls. Methanolic extracts did not have anti-inflammatory activity.
Efectos en la oreja del ratón inducidos por TPA. Los valores son promedio de n=3 ± 1 desviación estándar. Los resultados fueron analizados por la prueba de student, y los valores de * p≤0.05, ** p≤0.01 son considerados *significativamente o ** altamente significativamente diferentes, respectivamente, respecto a los controles. Los extractos metanólicos no tuvieron actividad anti-inflamatoria.

Antibacterial activity: The MIC tests revealed that both bacterial strains are sensitive to the three crude extracts from leaves and stems, and seeds and inflorescences of *Mikania micrantha* between 0.5 - 4 µg/µl.

Escherichia coli had a MIC of 1 µg/µl in presence of the ethyl acetate extract from each part of the plant. The methanol extract from seeds and inflorescences was less active, while the

hexane extracts, and methanolic extract from leaves and stems have the same inhibitory effect at 2 µg/µl.

Bacillus subtilis was more sensitive to the ethyl acetate extract from each part of the plant, and had a small MIC 0.5 µg/µl. Leaf and stem extracts were twice as active as seed and inflorescence extracts against this bacteria.

DISCUSSION

Apparently this Asterace, collected in March 2006 in Cuetzalan del Progreso, Puebla, Mexico, did not present any phototoxic compounds.

Linalool and α-pinene are the main volatile oil components of seeds and inflorescences in *Mikania micrantha*. Such composition can explain the antibacterial and antiinflamatory properties of this species.

The activity of leaves plus stem, and seeds and inflorescences, was bigger in the ethyl acetate than in the hexane extract.

Many authors attributed the pharmacological effect of guaco to coumarin (1,2-benzopirone) (Dos Santos et al., 2006). However, our results made clear that this is not the only bioactive component present in the extracts we tested. Ethyl acetate is a solvent capable of extract coumarin and several terpenes and phenolic compounds, which have a wide range of biological activities. These include plant growth-regulators, secondary compounds against insects, and anti-bacterial properties (Huang et al., 2009). Some flavonoids and dicaffeoylquinic acid butyl esters have been recently described as bioactive for *M. micrantha* (Wei et al., 2004).

We showed by the TPA model of mouse ear inflammation that the secondary metabolites tested, which were contained in the ethyl acetate extract of *M. micrantha,* have the best biological activity. However, the methanol extracts were inactive.

The highest growth inhibitory concentration against *E. coli* was in the methanol extract, followed by the hexane and ethyl acetate extracts. For *Bacillus subtilis* the smallest concentration for both plant parts was in the ethyl acetate extract, followed by the concentration in the methanol and hexane extracts. Therefore, the ethyl acetate extract has the bioactive compounds that can produce a desirable pharmacological effect.

Our results demonstrated that *M. micrantha* can be used as a medicinal plant.

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