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***Vernonia patens* Kunth, an Asteraceae species with phototoxic and pharmacological activity** (With 3 Tables)

***Vernonia patens* Kunth, una especie de Asterácea
con actividad fototóxica y farmacológica**
(Con 3 Tablas)

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Abstract. The presence of phototoxic compounds in stems and leaves of young plants of *Vernonia patens* Kunth was confirmed by TLC. These compounds were in smaller amounts in younger than in adult plants of this species. Only the stems presented specific activity of these compounds from the two study plant organs. It included characteristic UV bands at 200 and 300nm, and phototoxic activity against *Bacillus subtilis* (ATCC-6633). Stems and leaves of *Vernonia patens* also showed anti-inflammatory activity and bactericide potential.

Key words Asteraceae, phototoxic compounds, bactericide activity, anti-inflammatory activity.

Resumen. Se estableció la presencia de compuestos fototóxicos en tallos y hojas de plantas jóvenes de *Vernonia patens* mediante TLC. Estos compuestos se encontraron en menor cantidad en plantas jóvenes que en plantas adultas de esta especie. De las dos partes estudiadas (tallos y hojas), sólo los tallos presentaron la actividad específica de estos compuestos: ban-

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das características al UV entre 200 y 300 nm, y actividad fototóxica frente a *Bacillus subtilis* (ATCC-6633). Los tallos y hojas de *Vernonia patens* también mostraron actividad antiinflamatoria y potencial bactericida.

Palabras clave: Asteraceae, compuestos fototóxicos, actividad bactericida, actividad antiinflamatoria.

INTRODUCTION

In our systematic study of species of the Asteraceae family, searching for phototoxic compounds which could be taxonomic markers, we analyzed *Vernonia patens* plants.

Since this species have a confirmed pharmacological activity, we also determined the biological activity of the extracts: anti-inflammatory and bactericidal potentials.

MATERIALS AND METHODS

Plant material. Plants were collected in Nauzontla, Puebla, Mexico, on 13 March 2006. This is the time when this species starts flowering. Harvested plants had a very small root system. From these plants, we studied the stems and the leaves. Voucher specimens were deposited in the National Herbarium, Instituto de Biología, UNAM (MEXU).

Preparation of extracts. Plants were divided in stems and leaves. Extracts were prepared from these plant parts using hexane, ethyl acetate and methanol; the solvents were eliminated at reduced pressure. The dry extracts were used for the different tests.

Phototoxic compounds. Presence of polyacetylenes and thiophenes was evident from the hexane extracts by TLC. This was confirmed by (1) the UV spectrum of the extract, with characteristic bands, and (2) by its bactericidal potential, after being irradiated with UV-light (Daniels, 1965).

Antibacterial potential. From the three extracts of stems and leaves, the antibacterial activity was measured against *Bacillus subtilis* (ATCC-6633) and *Escherichia coli* (ATCC-6051).

The bactericidal test was performed by the paper disc diffusion method (Cavaliere, 2005). Petri dishes with agar containing a bacterial concentration of 10^6 UFC were used with this purpose. Hexane, ethyl acetate

and methanol were assayed each at concentrations of 0.25, 0.5, 1, 2, or 4 mg/ml. Petri dishes were incubated at 37 °C during 24h. Anhydrous ampicilline (0.05 mg) (Sigma) was used as a positive control. Each assay was repeated three times.

Anti-inflammatory test. The bioassay was conducted 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 µL of an ethanolic TPA solution (Table 3) were applied on the surface of the right ear. The same amount of ethanol (control) was applied on the left ear. Ten minutes after application of the TPA, 20 µL of the extracts were applied topically (1 mg dissolved in ethanol). After 4h, mice were sacrificed and an ear section (7 mm) was cut off and weighed. Increases in weight of the right compared to the left ears indicated swelling. Percentage swelling inhibition was calculated by comparison with the control (left ear). Indometacine (0.046, 0.085, 0.15 mg/ear) was used as a drug reference.

Statistical analysis. When corresponding, results were analyzed using the Student's *t* test (Steel & Torrie, 1985). Differences were considered either significant or highly significant with respect to the control when $p \leq 0.05$ or $p \leq 0.01$, respectively.

RESULTS

Phototoxic compounds. The hexane extracts contained the phototoxic compounds, which were confirmed by TLC and developed with ceric sulphate. The UV spectrum gave the characteristic bands of these compounds at 200 and 300 nm (Table 1), and the phototoxic activity showed an inhibition halo at different concentrations. Only the stem extract was active, giving an inhibition halo of (1) 7.9 mm (39.5%) at a concentration of 0.25 mg/ml, and (2) 8.9 mm (44.5%) at a concentration of 1 mg/ml, compared to ampicilline (control). It gave an halo of 20 mm (100%) at a concentration of 0.05 mg/ml.

Antibacterial potential. It was performed with hexane, ethyl acetate and methanol extracts against *Bacillus subtilis* (ATCC-6633) and *Escherichia coli* (ATCC-6051) (inoculation 10^6 UFC). Ampicilline was used as a control at a concentration of 0.05 mg/ml.

In the hexane extract, leaves showed no activity against *Bacillus subtilis* at concentrations of 0.25, 0.5, 1.0, 2.0 and 4.0 mg/ml. Stems had

activity at 0.25 and 1 mg/ml, with an inhibition halo of 10.8 mm (54%) and 15 mm (75%), respectively, while the inhibition halo was 20 mm in the control. Stems and leaves did not present activity against *Escherichia coli* (Table 2).

Table 1. Characteristic U.V. absorption of polyacetylenes.

Tabla 1. Absorción de U.V. característica de poliacetilenos.

| <i>Vermonia patens</i> | Peaks (mm) | Abs (AU) |
|----------------------------|------------|----------|
| Hexane extract from Leaves | 247 | 0.226 |
| | 266 | 0.370 |
| | 280 | 0.110 |
| Hexane extract from Stems | 247 | 0.536 |
| | 266 | 0.489 |
| | 296 | 0.295 |

Absorption bands in the 200 and 300 nm regions are characteristic of polyacetylenes.
Bandas de absorción en el rango de 200 a 300 nm son características de poliacetilenos.

Table 2. Antibacterial activity of leaf and stem extracts of *V. patens*.

Tabla 2. Actividad antibacteriana de extractos de hoja y tallo de *V. patens*.

| Hexane Extract | | | |
|-----------------------|--|---|--------------------------|
| Sample | <i>Bacillus subtilis</i> Halo of inhibition (mm) | <i>Escherichia coli</i> Halo of inhibition (mm) | Concentration (mg/ml) |
| Control (ampicilline) | 20 ± 0.01 | 17 ± 0.06 | 0.05 |
| Leaves | - | - | 0.25 |
| | - | - | 0.50 |
| | - | - | 1.00 |
| | - | - | 2.00 |
| | - | - | 4.00 |
| Stem | 10.8 ± 0.29 | - | 0.25 |
| | - | - | 0.50 |
| | 15 ± 0.15 | - | 1.00 |
| | - | - | 2.00 |
| | - | - | 4.00 |

| Ethyl acetate Extract | | | |
|-----------------------|-------------|-------------|------|
| Leaves | - | - | 0.25 |
| | - | - | 0.50 |
| | - | 6.0 ± 0.02* | 1.00 |
| | - | 7.0 ± 0.02 | 2.00 |
| | 7.0 ± 0.01* | 8.0 ± 0.03 | 4.00 |
| Stem | - | 6.3 ± 0.71* | 0.25 |
| | - | 6.5 ± 0.71 | 0.50 |
| | - | 7.6 ± 0.61 | 1.00 |
| | - | 7.9 ± 0.42 | 2.00 |
| | 7.2 ± 0.27* | 9.6 ± 0.51 | 4.00 |
| Methanol Extract | | | |
| Leaves | - | - | 0.25 |
| | - | - | 0.50 |
| | - | - | 1.00 |
| | 9.3 ± 0.15* | 8.9 ± 0.20* | 2.00 |
| | 10 ± 0.20 | 9.2 ± 0.10 | 4.00 |
| Stem | - | 6.3 ± 0.70* | 0.25 |
| | - | 6.6 ± 0.51 | 0.50 |
| | 7.0 ± 0.01 | 7.6 ± 0.61 | 1.00 |
| | 8.0 ± 0.05 | 8.9 ± 0.20 | 2.00 |
| | 10.3 ± 0.30 | 9.9 ± 0.10 | 4.00 |

-: Without activity

*: Response corresponding to a Minimum Inhibitory Concentration (MIC)

Microorganism concentration in plate: 10⁶ UFC.

Positive control: Anhydrous ampicilline [D(-)- α -Aminobenzylpenicillin](Sigma).

Zone of inhibition, including the diameter of the filter paper disc (5 mm); values are the mean of three replicates.

-: Sin actividad.

*: Respuesta correspondiente a una concentración inhibitoria mínima (CIM). Concentración de microorganismos en la placa: 10⁶ UFC.

Control: Ampicilina anhidra [D(-)- α -Aminobencilpenicilina](Sigma).

Zona de inhibición, incluyendo el diámetro del disco de papel de filtro (5 mm); los valores son el promedio de 3 repeticiones.

The AcOEt extract from leaves presented activity against *B. subtilis* at a concentration of 4 mg/ml (halo of 7 mm, 35% inhibition), and against *E. coli* at concentrations of 1, 2 and 4 mg/ml (halo of 6, 7 and 8 mm, respectively, which determined 30, 35 and 40% inhibition of bacterial growth, respectively). Stems presented an inhibition halo of 7.2 mm (36%) at a concentration of 4 mg/ml against *B. subtilis*. The inhibition halo was of 6.3, 6.5, 7.6, 7.9 or 9.6 mm at concentrations of 0.25, 0.50, 1.0, 2.0 or 4 mg/ml, respectively, against *E. coli*. This determined 31.5, 32.5, 38, 39.5 and 48% growth inhibition, respectively (Table 2).

The leaf methanolic extract produced an halo of inhibition of 9.3 and 10 mm (46.5 and 50%) at 2 and 4 mg/ml concentrations, respectively, against *B. subtilis*. At the same concentrations, the inhibition halo was of 8.9 (2 mg/ml) and 9.2 (4 mg/ml) mm with 44.5 and 46% of bacterial growth inhibition, respectively, against *E. coli*. The stem extract showed an halo of 7, 8 and 10.3 mm (35, 40 and 51% growth inhibition, respectively), at concentrations of 1, 2 and 4 mg/ml, respectively, against *B. subtilis*. Against *E. coli*, the halo was from 6.3 to 9.9 mm at concentrations from 0.25 to 4.0 mg/ml. Growth inhibition ranged from 31.5 to 49.5% (Table 2).

Table 3. Anti-inflammatory activity of *V. patens*.

Tabla 3. Actividad antiinflamatoria de *V. patens*.

| Topically administrated (TPA 2.5 mg/ear) | | | | |
|--|---------------------|-----------------------|------------------|------------------|
| Extract doses: 1 mg /ear | | % Inhibition of edema | | |
| Extracts | | Hex | AcOet | MeOH |
| Stems | | 74.78 +/- 0.32** | 21.86 +/- 0.87* | 80.25 +/- 0.92** |
| Leaves | | 31.09 +/- 1.55** | 57.55 +/- 1.36** | 50.13 +/- 1.01** |
| 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 3 replicates ± 1 standard deviation. Results were analyzed by the student's t - test, and values at * p ≤ 0.05 or ** p ≤ 0.01 are considered *significant or **highly significant, respectively, compared with the control. | | | | |
| <i>Efecto sobre el edema de la oreja de ratón inducido por TPA. Los valores son el promedio de 3 repeticiones ± 1 desviación estándar. Los resultados se analizaron usando la prueba t de student, y los valores a * p ≤ 0,05 o ** p ≤ 0,01 se consideran *significativos o **altamente significativos, respectivamente, comparado con el control.</i> | | | | |

Anti-inflammatory test. It was performed with the stem and leaf extracts by the mouse ear edema test with TPA. Inhibition of the edema using plant stems were 74.8, 21.9 and 80.2% with the hexane, ethyl acetate and methanolic extracts, respectively (Table 3). In leaves, edema inhibition was 31.1% using the hexane extract; 57.5% using the ethyl acetate extract, and 50.1% utilizing the methanolic extract. These results highlight the anti-inflammatory activity of the extracts.

DISCUSSION

Phototoxic compounds. It is interesting the amount of phototoxic compounds that leaves and stems have developed at the flowering phenological stage.

Phototoxic compounds were found in stems and leaves, and their presence was made evident by TLC. The characteristic pale blue color of these compounds appeared when the plate was developed with ceric sulphate. It is more intense in stems than leaves. Their presence was also confirmed by the UV spectrum of the hexane extracts, and by the phototoxic activity against *Bacillus subtilis*, using the Petri dish agar method (Daniels, 1965).

The amount of phototoxic compounds at the initiation of the flowering stage was smaller than that in other Asteraceae at flowering time. This suggests that small changes at the study phenological stage may greatly influence the obtained results.

The only active parts were the stems; presence of the compounds was confirmed by their UV spectrum and phototoxic activity.

Antibacterial activity. Plant leaf and stem extracts (hexane, ethyl acetate and methanol) have different activity against *Escherichia coli* (ATCC-6051) or *Bacillus subtilis* (ATCC-6633).

The hexane extract from leaves did not have activity against both bacteria species. Although plant stems had a good activity against *B. subtilis*, they did not have any activity against *E. coli*.

Ethyl acetate extracts from leaves and stems inhibited growth of both bacteria species. Stems, however, showed more activity against *E. coli* than against *B. subtilis*. Leaf and stem methanolic extracts were active against *E. coli* and *B. subtilis*, although stems were more active than leaves.

These results indicate that leaf and stem methanolic extracts show the highest activity against the study bacteria. Ethyl acetate extracts from leaves and stems had also good activity against *E. coli*. While stem hexane extracts have little activity against bacteria, there was no effect when using leaf hexane extracts.

Anti-inflammatory activity. The three study extracts (hexane, ethyl acetate and methanol) were active. Extracts from the stem were the most active, showing a higher ear edema inhibition than indometacine (0.15 mg/ear) (drug reference). Leaf ethyl acetate and methanol extracts were more active than indometacine (0.085 mg/ear) (Table 3).

CONCLUSION

The high inhibitory values of the hexane, ethyl acetate and methanol extracts from the stems of *Vernonia patens* clearly demonstrate that this plant has a good pharmacological activity. In the near future, it could be used as a medicinal plant for the treatment of inflammatory processes.

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