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Antioxidant and anti-inflammatory activity of *Moussonia deppeana*

[Actividad antioxidante y anti-inflamatoria de *Moussonia deppeana*]

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Abstract

*Moussonia deppeana* (Schdl. & Cham) Hanst is a species of Mexican Medicinal Flora used in Veracruz state, to treat sufferings related to stomach pain, renal diseases, cough, tumors and inflammation. Obtained results showed that EtOAc extract was the most active in free radical scavenging test DPPH (C16 18.3±3.4 μg/mL) with 41% of reducing power respect to ascorbic acid and total content of polyphenols was smaller (328.9±7.6 mg GAE/g) than the found in the ethanol extract (388.6±6.2 mg GAE/g). Anti-inflammatory activity was evaluated by topical application of the extracts (doses 2 mg/ear) giving a greater inhibition in hexane and EtOAc extracts (39 and 28%, respectively). The model of paw edema was evaluated in EtOAc extract, observing a similar inhibition to indomethacin (43% with 100 mg of dose) at the first hour. These results support the biological effect attributed in their traditional use.

Keywords: Anti-inflammatory, Antioxidant, *Moussiana deppeana*, Ethnopharmacology, Medicinal Plants.

Resumen

*Moussonia deppeana* (Schdl. & Cham) Hanst, es una especie de la Flora Medicinal Mexicana usada en el estado de Veracruz, para tratar padecimientos relacionados con dolor estomacal, enfermedades renales, tos, tumores e inflamación. Los resultados obtenidos mostraron que el extracto de EtOAc fue el más activo en la prueba de DPPH (C16 18.3±3.4 μg/mL), con un poder reductor de 41% respecto al ácido ascórbico y el contenido total de polifenoles fue menor (328.9±7.6 mg GAE/g) al encontrado en el extracto etánolico (388.6±6.2 mg GAE/g). La actividad anti-inflamatoria evaluada mediante aplicación tópica de los extractos (dosis de 2 mg/oruga) dio mayor inhibición con el extracto hexánico, seguido del EtOAc (39 y 28%, respectivamente). El modelo del edema plantar fue evaluado únicamente en el extracto de EtOAc observándose una inhibición similar a indometacina (43% a dosis de 100 mg de extracto) en la primera hora. Los resultados apoyan el efecto biológico atribuido en su uso tradicional.


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INTRODUCTION

*Moussonia deppeana* (Schdl. & Cham) Hanst, belongs to Gesneriaceae family (synonyms: *Kolheria deppeana*, *Gesneria deppeana*, *Moussonia elongate*), this species is broad distributed since Mexico to Panama. In folklore medicinal is commonly known as clachichinole, tlachichinole, tochomitillo or valletina (EscalaIanté, 1988). This plant is frequently used by Mexican people in traditional medicine because of their curative properties (stomach inflammation, diarrhea, ulcer, kidney disease, vaginal infection and some tumors). In this sense, several extracts of these plants have been studied as antiprotozoal (Calzada et al., 1998) and some reports revealed the presence of β-sitosterol, β-D-glucosyl-sitosterol, ursolic acid, oleaonic acid, 2β,3β-dihydroxy-olean-12-en-28-oic acid, 2α,3α-dihydroxy-olean-12-en-28-oic acid (Noguera et al., 1994); 2-methyl-anthaquinone, chromanone and stigmasterol (Reyes-Blas, 1995).

Gesneriaceae family is very extensive and includes tropical herbs and shrubs; many of them are growth as ornamentals (Martínez, 1969; Alcántara and Luna, 2001). In some members of this family have been isolated anthocyanins, flavonoids and flavones (Díaz, 1976; Gould and Lister, 2006). Of particular interest was the isolation of some glycosylated rutine derivatives by Robinson and Tood (Robinson et al., 1934), which have a well-defined anti-inflammatory activity.

By the other hand, is well known that oxygen and nitrogen reactive species play important roles in normal physiological processes, protection from pathogens, cellular signaling pathways, and regulation of vascular tone (Valko et al., 2007); also, they are related to development of tissue damage in various human diseases such as cancer, aging, neurodegenerative disease, malaria and pathological events in living organism (Gutteridge, 1994).

In this sense, antioxidant capacity of medicinal plants and herbs has been linked to in vivo protection from oxidative stress in numerous studies (Prior et al., 2005) but rarely has been associated with anti-inflammatory capacity (Jensen et al., 2008). For this reason, the present study is aimed to the evaluation of antioxidant and acute anti-inflammatory effect of several extracts of *M. deppeana* growing in Mexico by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay (Brand-Williams et al., 1995; Miliauskas et al., 2004); reducing power with FeCl₃ test (Oyaizu, 1986); total polyphenols by Folin-Ciocalteau assay (Spanos and Wrosltad, 1990) as well as 12-O-tetradecanoylphorbol 13-acetate (TPA) induced mouse ear edema model (Young and De Young, 1989) and carrageenan induced mouse paw edema model (Levy, 1969) for determination of anti-inflammatory properties.

MATERIALS AND METHODS

All chemical used were analytical grade. 1, 1-Diphenyl-2-picryl hydrazyl (DPPH), Folin-Ciocalteau’s reagent, potassium ferricyanide (K₃Fe[CN]₆), ferric chloride (FeCl₃), gallic acid monohydrate, ascorbic acid, carrageenan lambda, 12-O-tetradecanoylphorbol 13-acetate and solvents were obtained from Sigma-Aldrich (México).

Absorbance in colorimetric determinations was measured in a UV-Vis Varian spectrophotometer Cary100 model.

Vegetal material

*M. deppeana* was collected in Rancho Viejo-Cinco Palos Municipality of Coatepec, Veracruz State, Mexico in October 2007. The taxonomic identification of plants was confirmed by Luis Hermann Bojorquez Galván, a taxonomist. A voucher specimen (CIB8987) has been deposited in the Instituto de Investigaciones Biológicas Herbarium of Universidad Veracruzana.

Preparation of crude plant extracts

About 800 g of aerial part of plant material were cut into small pieces, dried at room temperature and extracted by exhaustive maceration in darkness with different solvents. Three extracts were obtained successively and solvent was removed using rotary evaporator (Hexane, 1.68 g; EtOAc, 2.01 g and EtOH, 7.3 g). Crude extracts were kept in amber colored glass vials at room temperature for further use.

Phytochemical analysis

Phytochemical analysis of the plant extracts was undertaken using standard qualitative methods (color test and/or Thin Layer Chromatography, TLC). Two milligrams of each extract were dissolved in chloroform (5 mL) before application to TLC plates (2×6 cm). The elution systems were benzene/acetone (9:1) for hexane and EtOAc extracts meanwhile for the ethanol extract was used a mixture butanol/water/
acetic acid (6:3:1). The revealing agents were: Dragendorff solution (for alkaloids), AlCl<sub>3</sub> 1% in ethanol (for flavonoids), ZnCl<sub>2</sub> (for sapogenins), KOH 10% in ethanol (for coumarins), perchloric acid (for sterols) and NaOH (for quinones) (Domínguez, 1973; Kaufman et al., 2006).

**Animals**

All animals employed in the experiments were CD1 male mice (20 -25 g) obtained from Facultad de Medicina of the Universidad Veracruzana, Xalapa. The animals were acclimated for one week in photoperiods adjusted to 12 hours of light and 12 hours darkness daily and 50-55% relative humidity with standard pellet diet (Rodent chow) and drink *ad libitum*. This work was performed according to the Guide for the Care and Use of Laboratory Animals (National Academy of Sciences, 1996) and the Official Mexican Norm (NOM-062-ZOO-1999).

**Determination of Antioxidant Capacity**

**Free radical scavenging by the use of DPPH radical**

The DPPH radical scavenging capacity of each extract was determined according to Brand-Williams method modified by Miliauskas, 2004. DPPH radicals have and absorption maximum at 517 nm, which disappears with reduction by an antioxidant compound. The DPPH radical solution in methanol (9 x 10<sup>-5</sup> M) was freshly prepared, and 2.9 mL of this solution was mixed with 100 µL of methanolic solutions of plant extracts at several concentrations (33, 16.5 and 8.25 µg/mL). The samples were incubated for 30 min at 37 °C in a water bath, and decrease in absorbance at 517 nm was measured (A<sub>E</sub>). A blank sample containing 100 µL of methanol in the DPPH radical solution was prepared daily, and its absorbance was measured (A<sub>B</sub>). Radical scavenging activity was calculated using the following formula:

\[
\% \text{ Inhibition} = \left( \frac{A_B - A_E}{A_B} \right) \times 100
\]

**Determination of total phenolic content**

The total phenolic concentration was determined using the Folin-Ciocalteu’s reagent according to the Spanos and Wrosltad, 1990. To 50 µL of each sample (1 mg/mL, three replicates), 2.5 mL 1/10 dilution of Folin-Ciocalteu’s reagent and 2 mL of Na<sub>2</sub>CO<sub>3</sub> (7.5 %, w/v) were added and incubated at 45 °C for 15 min. The absorbance of all samples was measured at 765 nm using a UV-Vis spectrophotometer. Results were expressed as gallic acid equivalent (µg/mL) by using the following equation, which was obtained from standard gallic acid graph (range 20 to1000 µg/mL).

\[
\text{Absorbance} = 0.001 \times [\text{GAE(µg/mL)}] + 0.075
\]

**Determination of reducing power**

The reducing power was determined according to the method described by Oyaizu, 1986. A 0.125 mL aliquot of extract (1 mg/mL) was mixed with 1.25 mL of 200 mM sodium phosphate buffer (pH 6.6) and 1.25 mL of 1% of potassium ferricyanide. The mixture was then incubated at 50 °C for 20 min. After 1.25 mL of 10% trichloroacetic acid (w/v) were added, the mixture was centrifugated at 650 g for 10 min. A 2.5 mL aliquot of the upper layer was mixed with 5 mL of destilled water and 1 mL of 0.1% ferric chloride, and the absorbance at 700 nm was measured. The obtained value was compared with the ascorbic acid value as standard.

**Anti-inflammatory evaluation**

12-O-tetradecanoylphorbol 13-acetate (TPA)-induced mouse ear edema.

Irritant dermatitis was induced on the right ear by topical application of 2.5 µg TPA in 25 µL of acetone according to the methodology reported by Young and De Young, 1989. TPA was applied on both the inner and outer surfaces of the ears. The extracts (doses 2 mg/ear) and the indomethacin (doses 2 mg/ear) were applied topically 30 minutes after TPA to the right ear (E<sub>T</sub>), the left ear received vehicle (E<sub>0</sub>). In the control group the right ear received only TPA (E<sub>T</sub>) and the other ear acetone (E<sub>0</sub>). In all groups the edema was allowed to develop for 6 hours; afterwards the animals were sacrificed by dislocation cervical and plugs (diameter of 6 mm) of the central portion were taken from both ears and weighted. The inhibition of auricular edema was calculated with the difference between weight ears of the animal treatment with the extract or indomethacin and the control group.
% Inhibition = \left( \frac{(E_t - E_o)_{\text{control}} - (E'_t - E'_o)_{\text{treated}}}{(E_t - E_o)_{\text{control}}} \right) \times 100

Carrageenan-induced mouse paw edema

20 µL/paw of a 1% carrageenan solution was injected into the sub-planter region of the left hind paw of the mouse. The increment in the paw thick was determined with a digital micrometer at 1, 3, 5 and 7 h after carrageenan administration. A reference group was administered intraperitoneally with indomethacin (doses 5 mg/kg). The extract was administered at doses of 100 and 300 mg/kg by the same via before carrageenan administration. The control group received vehicle only. The percent edema inhibition was calculated for each animal group in comparison to group treated with vehicle (10% Tween 80) according to the Olajide et al, 2000.

Statistical analysis of data

Data are presented as means ± S. D. of at least triplicate experiments. For in vivo experiments, data is reported as the means ± S.E.M. Significant differences between groups were determined by analysis of variance (ANOVA) complemented with Dunnett’s test using the software Statistica version 7 from StatSoft, Inc. (2004), p<0.05 was considered significant.

RESULTS AND DISCUSSION

Reactive oxygen species (ROS) may be involved in the etiologies of several human diseases as atherosclerosis, ischemic injury, cancer and neurodegenerative diseases, as well as in processes like inflammation and ageing (Edmonds, 2000). Also, there is evidence that antioxidants may be useful in preventing the deleterious consequences of oxidative stress and there is increasing interest in the protective biochemical functions of natural antioxidants containing in medicinal plants. In this sense, our attention has been focused in the antioxidant and anti-inflammatory properties of M. deppeana, a medicinal plant for the treatment of diabetes, stomach inflammation, kidney diseases, cough, and tumoral disease (Cano-Asseleih, 1997).

The phytochemical screening (Table 1) revealed the presence of phenolic compounds (flavonoids and coumarins) in EtOAc and EtOH extracts. In the hexane and EtOAc extracts were possible to observe a positive result for sterols and quinones, which supports the presence of some metabolites previously reported (Noguera et al., 1994 and Reyes-Blas, 1995).

In many cases, the presence of flavonoids, quinones and coumarins are associated with antioxidant properties due to their role in several human diseases where ROS could be involved. On the other hand, these components could be presents actively in inflammatory processes, in fact, antioxidant/anti-inflammatory activity have been related intrinsically to these chemical substances (Takahashi and Shibamoto, 2008; Jensen et al., 2008).

In order to evaluate the antioxidant efficiency of plant extracts, the radical scavenging capacity based in DPPH assay was determined and the results are shown in Table 2. In this sense, the percentage of inhibition of the DPPH radical varied from 41.1% for the hexane extract to 92.4% for the EtOAc extract, which represents a variation of approximately 2-fold respect to the hexane extract. The EtOAc extract showed the highest antioxidant activity index (AAI) (Scherer and Teixeira-Godoy, 2009) following EtOH extract and Hexane extract (1.9, 1.6 and 0.9, respectively).

Table 2. Antioxidant capacity of the extracts of M. deppeana based on DPPH test.

<table>
<thead>
<tr>
<th>Extract</th>
<th>DPPH Inhibition (µg/mL)</th>
<th>DPPH IC50 (µg/mL)</th>
<th>AAI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane</td>
<td>41.1 ± 1.2</td>
<td>40.5 ± 1.3</td>
<td>0.9</td>
</tr>
<tr>
<td>EtOAc</td>
<td>92.4 ± 2.1</td>
<td>18.3 ± 3.4</td>
<td>1.9</td>
</tr>
<tr>
<td>EtOH</td>
<td>70.1 ± 1.8</td>
<td>22.0 ± 0.4</td>
<td>1.6</td>
</tr>
<tr>
<td>Phenylbutazone</td>
<td>47.8 ± 4.4</td>
<td>34.4 ± 3.2</td>
<td>1.0</td>
</tr>
<tr>
<td>Rutin</td>
<td>93.5 ± 1.1</td>
<td>4.8 ± 0.1</td>
<td>7.4</td>
</tr>
<tr>
<td>Ascorbic acid**</td>
<td>100 ± 0.6</td>
<td>0.6 ± 0.1</td>
<td>61.2</td>
</tr>
</tbody>
</table>

Data expressed as mean ± SD (n=3). DPPH radical solution in methanol (9 x 10⁻³ M, 35.48 µg/mL) using (33µg/mL) of plant extract and ascorbic acid and rutin (5 µg/mL). * AAI = Final concentration of DPPH (µg.mL⁻¹) / IC50 (µg.mL⁻¹). ** No significance differences in IC50 were observed between this study and results previously reported (Sharma and Bhat, 2009).
The DPPH free radical scavenger capacity of the extracts was compared with well known antioxidant (ascorbic acid and rutin) and anti-inflammatory compound phenylbutazone. Despite the fact that extracts had been a significant antioxidant activity index (ca. 2, see Table 2), the obtained values were lower than rutine and ascobic acid (7.4, 61.2 respectively) but slightly higher than phenylbutazone in the case of EtOAc extract (1.0 vs 1.9).

Taking into account the results in radical-scavenging assay, it was expected that total phenol content and reducing power had presented the same behavior (Paško et al., 2009); the EtOH extract had the highest concentration of total phenol (388.6±6.2 µg GA/mL, Table 3) close to the EtOAc extract (328.9±7.6 µgGA/mL, Table 3). In the reducing power assay the EtOAc extract had shown the highest value over the EtOH extract (41.3% vs 29.0% respectively, Table 3). These observations indicated a linkage between phenolics concentration, reducing power and antioxidant activity; on the other hand, in all assays hexane extract always showed the lowest values in each test (Table 2 and 3).

Table 3. Total phenolic content and power reducing of the extracts of M. deppeana.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Total phenols</th>
<th>Reducing power (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane</td>
<td>20.6 ± 1.5</td>
<td>5.3 ± 0.4</td>
</tr>
<tr>
<td>EtOAc</td>
<td>328.9 ± 7.6</td>
<td>41.3 ± 0.5</td>
</tr>
<tr>
<td>EtOH</td>
<td>388.6 ± 6.2</td>
<td>29.0 ± 1.1</td>
</tr>
</tbody>
</table>

Data expressed as mean ± SD (n=3). Total phenols expressed in mgs. of Gallic acid equivalent per g of sample (mgGAE/g). Reducing power is expressed with respect to ascorbic acid (33µg/mL).

The propagation of free radical can bring many adverse reactions leading to extensive tissue damage. Lipids, proteins and DNA are very susceptible to attack by free radical (Yu et al., 1992). In this way, all antioxidant test evaluated in this work showed that M. deppeana has a great amount of antioxidant compounds that may offer resistance against oxidative stress by scavenging the free radicals and inhibiting lipid peroxidation.

Given the results in the antioxidant assays, our attention now was focused in the evaluation of the anti-inflammatory activity. In the ear edema induced with topic application of TPA, the hexane and EtOAc extracts produced the mayor inhibition of the edema, 39 and 28%, respectively (Table 4). At 2 mg/ear dose, the inhibition edema was similar between hexane extract and esculetin (39 and 38% respectively, Table 4) but lower than indomethacin (69%). In this test, ethanol extract did not show a significant inhibition.

Table 4. Anti-inflammatory activity of M. deppeana on ear edema induced with TPA.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Extract</th>
<th>Dose (mg/ear)</th>
<th>Edema inhibition (mg)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>M. deppeana</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hexane</td>
<td>1</td>
<td>8.8 ± 0.6*</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>EtOAc</td>
<td>2</td>
<td>10.4 ± 0.7</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>2</td>
<td>13.8 ± 0.5</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Indomethacin</td>
<td>2</td>
<td>4.5 ± 0.5*</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>β-sitosterol</td>
<td>1</td>
<td>9.9 ± 0.5*</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Esculetin</td>
<td>1</td>
<td>8.9 ± 0.8*</td>
<td>38</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S. E. M. The inhibition (%) was calculated respect to control group. * ANOVA-Dunnett’s, p < 0.05, n = 6.

In this regard, inflammation induced by TPA leads to protein kinase C activation, which is related to activation of phospholipase A2 with releases arachidonic acid from membrane cells that is metabolized to prostaglandins and leukotrienes (Recio et al., 2000). The inhibition observed in hexane extract could be related to this mechanism.

According to the obtained results in preliminary anti-inflammatory test (TPA), the hexane and EtOAc extract have the major inhibition; however, we chosen the EtOAc, due to the low solubility presents in hexane extract, in order to confirm the biological effect with the paw edema induced with the carrageenan model in mouse applied by systemic route.

The EtOAc extract of M. deppeana (100 and 300 mg/kg dose) applied 30 min before of carrageenan did not reduce the edema formation significantly respect to the control group. In contrast, the indomethacin to 5 mg/kg dose reduced the edema formation in all time registered.

Surprisingly, paw edema decrease 43% with lower doses of the extract (100 mg/Kg) only at the first hour; this effect was similar to indomethacin (Table 5).

In the acute inflammation induced by carrageenan, levels of substance P in inflamed paw increase within 15 minutes after induction, these levels remained elevated during the first 2 h of inflammation (Gilligan et al., 1994). Other mediators of inflammation as serotonin, histamine and bradykinin play important roles during the early
phase of carrageenan edema (Di Rosa et al., 1971). According that the observed effect in EtOAc extract could be related to some of these inflammation mediators.

**Table 5.** Anti-inflammatory activity of EtOAc extract of *M. deppeana* on paw edema produced with carrageenan test.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Paw edema (mm) and % inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>1</td>
<td>0.7±0.1</td>
</tr>
<tr>
<td>3</td>
<td>1.1±0.1</td>
</tr>
<tr>
<td>5</td>
<td>1.2±0.1</td>
</tr>
<tr>
<td>7</td>
<td>1.0±0.1</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. The inhibition (%) was calculated respect to control group. * ANOVA-Dunnett’s, p < 0.05, n = 8.

With these results, it was not possible to find a correlation between antioxidant and anti-inflammatory effects on the models used. In fact, those observations indicate that responsible compounds of both activities are not the same; because in the anti-inflammatory topical test (TPA) the hexanic extract was the best and the EtOAc extract had the higher antioxidant activity. Further investigations in the phytochemistry field and other biological activities are being developed in our group in order to get more information respect to action mechanism and chemical composition.

**CONCLUSION**

Anti-inflammatory activity was observed in the hexanic and EtOAc extracts of *M. deppeana* only by topical application. The systemic administration of low doses of EtOAc extract produced a similar inhibition percent observed with indomethacine only a short period of time. In the case of the antioxidant activity, the EtOAc was the best extract evaluated following by EtOH and Hexane extract; however, it was not possible to find a correlation between the anti-inflammatory and antioxidant activities with the used assays. Finally, the biological activity of *M. deppeana* observed in the polar extracts supports its traditional use.

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Antioxidant and Anti-inflammatory Activity of Moussonia deppeana