Antioxidant Activity and Content of Phenolic Compounds and Flavonoids from Justicia spicigera

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Abstract: Aerial parts of Justicia spicigera are used in Mexican traditional medicine. Antioxidant activity of aqueous and methanolic extracts obtained from leaf, stem and flower of J. spicigera and their contents of phenolic compounds and flavonoids were evaluated in this study. Antiradical activity was proven for the capacity to scavenge the DPPH radical of each extract. The amount of total phenolic compounds was determined using the Folin-Ciocalteu reagent. Total flavonoid content was evaluated with aluminum chloride under basic conditions. For a same plant organ, extracts prepared with methanol possess a higher antiradical activity than those obtained with water. The antiradical activity of leaf and flower extracts was superior to that found for the stem extract prepared with the same solvent. Total phenolic content ranged from 1.33-5.01 g gallic acid equivalents/100 g dry weight. Leaf and flower extracts obtained with methanol or water had higher amounts of phenolic compounds than stem extract. Total flavonoid content is between 0.18 and 1.30 g catechin equivalents/100 g dry weight and the order for methanol extracts is leaf>flower>stem, whereas for the aqueous extracts this sequence is stem>flower>leaf. This is the first study describing the antioxidant activity from J. spicigera. Phenolic compounds and flavonoids contribute to this activity. The results suggest that J. spicigera is a source of antioxidant and support its use as an anti-inflammatory for the treatment of uterine cancer and against various free radical-related disorders.

Key words: Acanthaceae, muistle, uterus cancer, diarrhea, blood stimulant

INTRODUCTION

Increase of free radicals and lipid peroxidation has been associated with development of coronary heart disease, cancer and the aging process (Aruoma, 1998; Olinska et al., 2007). In order to protect the organism against free radicals, consumption of natural source antioxidants is recommended. Several studies have been conducted to determine the antioxidant properties of plants, especially those used in traditional medicine (Jang et al., 2007; Surveswaran et al., 2007). This investigation allows the identification of medicinal plants and new compounds with strong antioxidant capacity, supporting the use of medicinal plants as a natural source of potent antioxidants used for the prevention or treatment of free radical-related diseases (Surveswaran et al., 2007). In this context, phenolic compounds, flavonoids and phenols are plant chemical compounds that act as anticancer or cardioprotective agents. One such protective mechanism is due to its antioxidant activity (Yilmaz and Toledo, 2004; Boskou, 2006). However, many vegetal species have not been well studied and may be natural sources of antioxidant compounds.

Justicia spicigera Scheuchendal is a shrub belonging to the family Acanthaceae, locally known as muistle. The aerial parts of J. spicigera are used in Mexican traditional medicine for inflammation, as a blood depurative and for treatment of diarrhea and uterine cancer (Marquez et al., 1999). Ethanol extracts of leaves have demonstrated activity against Giardia duodenalis, one of the most common intestinal parasites in Mexico associated with diarrhea (Ponce-Macotela et al., 2001). Aqueous extracts have shown cytotoxic activities against human leukemic cells (Caceres-Cortes et al., 2001). Kaempferitrin, O-sitosterol-3-β-glycoside, allantoin and cryptoxanthin

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are some compounds found in *J. spicigera* (Fauler and Alam, 1982; Dominguez et al., 1990). However, antioxidant activity studies of *J. spicigera* and other species from this genus are scarce. Therefore, the purpose of the present study was to evaluate antioxidant activity of aqueous and methanolic extracts obtained from leaf, stem and flower of *J. spicigera* and their total content of phenolic compounds and flavonoids.

**MATERIALS AND METHODS**

This study was carried out during 2007 at the Department of Plant Biotechnology, Center of Biotic Products Development, Morelos México.

**Plant material:** Leaves, stems and flowers of *J. spicigera* from adult plants in the flowering state were collected in February 2007 (dry season) from km 8.5 highway (N18° 49' 35.8" W 99° 05' 39.5", altitude 1049 m. a.s.l.), Yautepec-Joxtla, Yautepec, Morelos, México. The vegetal material was identified by Mr. Rolando Ramirez, a botanist from the herbarium Humo of Autonomous University from Morelos State, Morelos, México and a voucher specimen was deposited in the same herbarium Humo.

**Extract preparation:** Flowers, leaves, or stems were dried separately at room temperature and ground with a mortar and pestle. Pulverized tissue was passed through a 0.25 mm porous sieve. To obtain methanolic extracts, dried plant material (20 mg) was soaked in methanol (10 mL) at 25°C for 30 min. The mixture was vacuum filtered with Whatman 1 filter paper. After filtration, methanol was removed under reduced pressure at 60°C in a rotary evaporator (BUCHI Rotavapor R-250, Flawi, Switzerland). Aqueous extracts were prepared by soaking dry plant material (100 mg) with water (50 mL) at 25°C for 30 min. Extracts were filtered with Whatman 1 filter paper and the filtrated extract was lyophilized using a freeze-dry system (LABCONCO Freeze Dry System 18, Kansas City, MO, USA).

**Evaluation of antioxidant activity:** Free radical scavenging activity of extracts for the DPPH reagent (2, 2-diphenyl-1-picrylhydrazyl) was determined according to Miliauskas et al. (2004). Stock solution of DPPH (60 μM) in methanol was prepared daily. A sample (0.1 mL) of each extract at various concentrations (50-750 μg extract mL⁻¹) was placed in a cuvette. DPPH stock solution (3.9 mL) was added and the decrease in absorbance at 515 nm of DPPH was measured after 15 min using a UV-visible spectrophotometer (UV-160A, Shimadzu, Tokyo, Japan). Methanol was used to adjust the spectrophotometer at zero. A blank sample containing the same amount of DPPH solution was prepared and absorption was measured at 515 nm. Radical scavenging activity was calculated using the following formula:

\[
\text{Inhibition (％) = } \frac{A_b - A_e}{A_b} \times 100
\]

where, \( A_b \) is absorption of blank sample at 0 min, \( A_e \) is absorption of tested extract solution at 15 min.

The extract concentration providing 50% inhibition (IC₅₀) was calculated from the graph plotting inhibition percentage against extract concentration. All evaluations were performed in triplicate.

**Determination of total content of phenolic compounds:** Total phenolic content was measured by the methodologies reported by Shohael et al. (2002). One sample (100 μL) of extract at a concentration of 1 mg mL⁻¹ was dissolved in deionized water (2.5 mL) followed by addition of Folin-Ciocalteu reagent (0.01 mL). The mix was incubated for 6 min and 0.5 mL of sodium carbonate (20%) was added. The reaction was incubated at room temperature for 30 min until the formation of a blue complex. Absorbance was measured at 760 nm. The measurement was compared with a standard curve (2.5-25 μg mL⁻¹) of gallic acid (Sigma-Aldrich Chemical, St. Louis, MO, USA). The sample content was expressed as g GAE/100 g d.wt. of extract. A mixture of water and reagents was used as blank. Sample measurements were done in triplicate and the mean and standard deviations were calculated in each case.

**Determination of total flavonoid content:** Total flavonoid content was determined by a colorimetric method reported by Shohael et al. (2002). Extract samples (0.25 mL) at a concentration of 1 mg extract mL⁻¹ were diluted with deionized water (1.25 mL). A sodium nitrite solution at 5% (0.75 mL) was added and samples were incubated for 6 min at room temperature. Aluminum chloride at 10% (0.150 mL) was aggregated and the mixture was incubated (5 min). Finally, 0.5 mL of sodium hydroxide (1 M) was added. The mixture was brought to 2.5 mL with distilled water and incubated at 25°C for 30 min. Absorbance was measured at 510 nm. A standard curve (5-100 μg mL⁻¹) of catechin (Sigma-Aldrich Chemical) was used to calculate the flavonoid content. Results were expressed as g CE/100 g d.wt. of extract. All evaluations were performed in triplicate.

**Statistical analysis:** Significant variables were calculated and results were subjected to Kruskal-Wallis ANOVA test using SPSS Sigmastat 3.0 (SPSS Inc., Chicago IL, USA). Differences were considered significant when p<0.001.
RESULTS AND DISCUSSION

Lower IC₅₀ values are indicative of a higher antiradical activity. All extracts of *J. spicigera* are a source of radical scavenging activity, but this activity changed with the solvent used to prepare the extract (Table 1). For a same plant organ, extracts prepared with methanol possess a higher antiradical activity than those obtained with water. It was also found that organ extracts obtained with the same solvent show a differential radical scavenging capacity. Leaf or flower extracts had higher antioxidant activity than those found in stem. The order of free radical scavenging activity for methanol extracts is leaf > flower > stem, whereas for aqueous extracts this sequence is flower > leaf > stem.

Total phenolic content was from 1.33 to 5.01 g GAE/100 g d.wt. For a same solvent, the amount of phenolics is higher in flower and leaf extracts than that measured for stem extract (Table 2). Total flavonoid contents are between 0.18 and 1.30 g CE/100 g d.wt. The sequences of flavonoid contents among organs are different from those found for phenolic compounds. For methanolic extracts this sequence is leaf > flower > stem, whereas for aqueous extracts this order is stem > flower > leaf (Table 2).

*Justicia spicigera* belongs to the Acanthaceae family and their antioxidant activity and contents of phenolic compounds and flavonoids are in agreement with those values reported for other members of this same plant family. However, there are differences depending on the plant species. The highest antioxidant activity found in the leaf methanolic extract of *J. spicigera* (48.86 µg mL⁻¹) is higher than that evaluated for whole plants of *Didiplanta verticillata* (785.67 µg mL⁻¹) but lower than those reported for *Hygrophila auriculata* (20.33 µg mL⁻¹), *Blepharis linifolia* (44 µg mL⁻¹) and *Lepidagathis anobrya* (16.33 µg mL⁻¹) (Sawadogo et al., 2006). Similarly, the highest phenolic compound content was found in the leaf methanolic extract of *J. spicigera* (5.01 g GAE/100 g d.wt.). This value is higher than that reported for whole plants of *Didiplanta verticillata* (2.82 g GAE/100 g), whereas it is lower than that reported for *H. auriculata* (17.75 g GAE/100 g) and *B. linearifolia* (15.38 g GAE/100 g) (Sawadogo et al., 2006).

Extracts of flower and leaf of *J. spicigera* obtained with the same solvent showed antioxidant activity higher than that of stem. Likewise, the amount of phenolics was higher in leaf and flower extracts than that measured for stem extracts. Thus, results of this study indicate that phenolic compounds may be contributing to the antioxidant activity found in leaf, stem and flower of *J. spicigera*. Positive correlations have been reported between total phenolic content and antioxidant activity of the extracts of other plant species of Acanthaceae (Sawadogo et al., 2006; Surveswaran et al., 2007). Similarly, other correlations between total phenolic content and antioxidant activity have been reported for 70 plant species used in folk medicine (Katalinic et al., 2006) and for 45 plants used in Chinese traditional medicine (Jang et al., 2007).

Flavonoids identified as kaempferitrin, O-sitosterol-3-β-glycoside, allantoin and cryptoxanthin have been reported in *J. spicigera* and may also supply antiradical activity according to their chemical characteristics (Euler and Alam, 1982; Domínguez et al., 1990). However, there are no specific studies about their contribution to the antioxidant activity in this plant. In this study, flavonoids were evaluated in the three plant organs and for a same organ the content changed with the solvent used for the extraction. Different mixers of flavonoids may be obtained from a same vegetal organ extracted with methanol or water and with different antiradical activity, making the suggestion of a relationship of flavonoid content with the antioxidant activity difficult. For example, leaf aqueous extract with the highest flavonoid level showed the lowest antiradical activity. Detailed studies of the extraction, composition and molecular structure of phenolic compounds and particularly of flavonoids from *J. spicigera* are needed for understanding their contribution to the antiradical activity as present in their natural form.

Table 1: Antioxidant activity of organ extracts from *J. spicigera* obtained with methanol and water

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Flower</th>
<th>Leaf</th>
<th>Stem</th>
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</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>54.03±1.77a</td>
<td>48.86±1.25c</td>
<td>391.41±6.15c</td>
</tr>
<tr>
<td>Water</td>
<td>60.08±1.71b</td>
<td>174.72±4.48</td>
<td>944.67±8.35</td>
</tr>
</tbody>
</table>

Results are Mean±SD (n = 3). Values with different lower case letter(s) are significantly different (p<0.001) as measured by Tukey’s test.

Table 2: Total content of phenolic compounds (g GAE per 100 g d.wt.) and flavonoids (g CE per 100 g d.wt.) of extracts from *J. spicigera*

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Flower</th>
<th>Leaf</th>
<th>Stem</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenolic</td>
<td>4.11±0.04a</td>
<td>5.01±0.02b</td>
<td>3.91±0.02c</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>0.70±0.09a</td>
<td>1.16±0.04b</td>
<td>0.58±0.13c</td>
</tr>
</tbody>
</table>

Results are Mean±SD (n = 3). Values with different lower case letter(s) are significantly different (p<0.001) as measured by Tukey’s test.
The differential distribution of phenolics content and radical scavenging activity found in organs of *J. spicigera* was also reported for *Beta vulgaris*, where leaf methanolic extracts have higher antioxidant activity than stem and there is a positive correlation with phenolic content (Pyo et al., 2004). This differential distribution of antioxidant activity between organs is probably due to the presence of differential mixtures of the chemical compounds with antioxidant activity. Synergism among chemical compounds generating antioxidant activity is not dependent only on the concentration and structure of each one. Interaction with other elements of the mixture is another factor that may also contribute to free radical scavenger activity (Castro et al., 2006).

Aerial parts of *J. spicigera*, principally the leaves, are used as antipyretics, anti-inflammatory, anticancer agents, blood depurate and for treatment of diarrhea (Márquez et al., 1999). Results of this study show for the first time, the antioxidant activity of leaf, flower and stem of *J. spicigera*. Phenolic compounds and flavonoids contribute to this activity. Likewise, it suggests a more accurate use of *J. spicigera* in traditional medicine against diverse free radical-related disorders.

**ACKNOWLEDGMENT**

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**REFERENCES**


