Antioxidant Activity Assessment of Plants Used in Huastec Traditional Medicine, Mexico

R.C. Díaz T.¹, G. Espinosa R.², C.A. Ilizaliturri H.², D. González M.², V.G Cilia L.²*

¹ Programa Multidisciplinario de Posgrado en Ciencias Ambientales, Universidad Autónoma de San Luis Potosí, México.
² Facultad de Medicina-Coordinación para la Innovación y Aplicación de la Ciencia y la Tecnología (CIACYT). Universidad Autónoma de San Luis Potosí, México.

*Corresponding Author: V.G Cilia L., CIACYT. Avenida Sierra Leona No. 550, Colonia Lomas Segunda Sección ZIP 78210. San Luis Potosí, SLP, México.

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ABSTRACT

The antioxidant capacity of Ocotea tampicensis (Meissner) Hemsley, Bursera simaruba (L.) Sarg, Pseudobombax ellipticum (Kunth) Dugand, Hamelia patens Jacq and Croton reflexifolius Kunth was evaluated. Ethanolic and aqueous extractions were performed at concentrations of 15, 30, 60, 120 and 240 mg / 100ml. Colorimetric tests ABTS, DPPH and FRAP were used. The results were expressed as equivalent antioxidant capacity ascorbic acid (AEAC) and percentage capture of free radicals. The aqueous extracts of O. tampicensis and B. simaruba showed the higher percentage capture of free radicals at a concentration of 240mg / 100ml. We hypothesize that the ability to capture free radicals of the species mentioned, are partly responsible for the analgesic and anti-inflammatory activity of these plants used in Huastec traditional medicine.

Keyword: Antioxidants; free radicals; inflammation; traditional medicine

INTRODUCTION

It is commonly accepted that in a situation of oxidative stress, reactive oxygen species (ROS) and free radicals (FR) are generated and have an important role in the pathogenesis of various serious diseases, such as neurodegenerative disorders, cancer, cardiovascular diseases, atherosclerosis, cataracts, and inflammation [1, 2]. It is reported that some medicinal plants contain a wide variety of compounds with antioxidant activity which can play an important role in adsorbing and neutralizing FR and ROS [2, 3, 4]. An antioxidant is a substance that prevents the oxidation, neutralizes the oxidizing action of free radicals by releasing electrons, maintaining cellular stability [5, 6].

The use of traditional medicine is wide-spread and plants still represent a large source of natural antioxidants that might serve as leads.
for the development of novel drugs. Studies of medicinal plants as potential antioxidants and modeling of the possible mechanisms of their inhibitory actions are of significant interest [7]. Many of these antioxidant capacities are associated with lower occurrence and lower mortality rates of several human diseases [8]. Plants exhibiting antioxidant substances, often turn have anti-inflammatory effect [9]. Factors involved in the inflammatory process include physical and chemical stimulants that are released during the immune response and by tissue damage. ROS play a significant role in the inflammatory response; the resulting oxidative stress may lead Inflammation and other chronic diseases. Several anti-inflammatory, neuroprotective, and hepatoprotective drugs have been shown to have an antiradical scavenging mechanism as part of their activity [2,5].

The Huastec area is located in the northeast of Mexico, in which two indigenous groups live Teenek and Nahuatl, these groups maintain the use of traditional medicine, it can be observed in its traditional markets and primary health care practices of its inhabitants. The study was aimed at evaluating the antioxidant activity of five traditional medicinal plants used for pain related ailments in Huastec traditional medicine. The antioxidant properties of the medicinal plant extracts were investigated with ABTS, DPPH and FRAP methods, which are the most widely used methods for assessing antioxidant activity associated with radical scavenging [9, 10, 11].

**MATERIALS AND METHODS**

**Chemicals**

Ammonium salt 2,2’-azinobis-(3- etilbenzotiazolin-6-sulfónico) (ABTS) (Fluka Chemicals), potassium persulfate (Panreac), monosodium phosphate (Panreac), 2,2-difenil-1-picrilhidrazil (DPPH) (Sigma), methanol (Merck), hydrochloric acid (Panreac), 2,4,6-tripirydyl-s-triazine (TPTZ) (Fluka Chemicals), ferric chloride (Panreac), sodium acetate (Panreac), ascorbic acid (CTR scientific). All chemicals were analytic grade.

**Equipment**

The equipment used for the quantification of ABTS, DPPH and FRAP was a UV-visible spectrophotometer (Thermo Scientific BioMate 3S).

**Plant material**

The selection of medicinal plants was based on ethnopharmacological interviews. The various data (local name, medicinal uses, used parts of plant, method of preparation and administration) were collected from local inhabitants having knowledge of the curative properties of these plants. Plant materials were collected from Tocoy and Tanjacnej in the municipality of San Antonio, San Luis Potosí, México (Table 1). The plants were authenticated by Dr. Javier Fortanelli Martinez from Universidad Autónoma de San Luis Potosí. Voucher specimens were prepared, identified and were deposited in the Isidro Palacios herbarium from Universidad Autónoma de San Luis Potosí for record purposes.
Table 1: Medicinal plants used in the treatment of pain in Tanjajnec and Tocoy, San Luis Potosí, México

<table>
<thead>
<tr>
<th>Family</th>
<th>Specie</th>
<th>Local name</th>
<th>Used part</th>
<th>Use</th>
<th>Voucher number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lauraceae</td>
<td><em>Ocotea tampisensis</em></td>
<td>Tok’té</td>
<td>leaf</td>
<td>Headache</td>
<td>34071</td>
</tr>
<tr>
<td>Euphorbiaceae</td>
<td><em>Croton reflexifolius</em></td>
<td>Oli</td>
<td>sap</td>
<td>Toothache</td>
<td>33390</td>
</tr>
<tr>
<td>Burseraceae</td>
<td><em>Bursera simaruba</em></td>
<td>Chaca</td>
<td>cortex</td>
<td>Headache</td>
<td>43905</td>
</tr>
<tr>
<td>Rubiaceae</td>
<td><em>Hamelia patens</em></td>
<td>Chacloc</td>
<td>leaf</td>
<td>Antinflammatory</td>
<td>30845</td>
</tr>
<tr>
<td>Bombacaceae</td>
<td><em>Pseudobombax ellipticum</em></td>
<td>Mococ</td>
<td>cortex</td>
<td>Stomach pain</td>
<td>31345</td>
</tr>
</tbody>
</table>

Plant extracts
Each plant recollected was dried for 72 hours at room temperature covered with paper bags, later each part separately (leaves, bark and roots) was pulverized employing a blender. The pulverized material was stored in paper bags until further maceration.

Preparation of aqueous extracts
In a beaker glass 500 ml of distilled water were placed, then was placed on a hot plate (Thermolyne), before the water reached the boiling point was added the pulverized plant. After was removed and allowed to macerate for one hour in a closed container. Later was filtered twice with filter paper Whatman No. 2. Absolute ethyl alcohol and deionized water (70:30) was added and allowed to macerate in an amber bottle and sheltered in the dark for a week. The concentrated aqueous extracts were lyophilized and stored at −20 °C in amber glass bottles until used. Ethanolic extracts of *Hamelia patens* Jaqc. (Rubiaceae), *O. tampicensis* (Meissner) Hemsley (Lauraceae), *Croton reflexifolius* Kunth (Euphorbiaceae) were performed. For each of the extracts concentrations of 15, 30, 60, 120 and 240 mg/100 ml were done.

Antioxidant assessment

**ABTS method**
The antioxidant capacity of the samples was measured by using the method described by Re et al. [13] with some variations. This method is based on the ability of a substance to scavenge the ABTS•+ radical compared with a standard antioxidant (ascorbic acid) in a dose response curve. The ABTS+ radical was generated by reacting a 7 mM ABTS aqueous solution with 2.45 mM K3S2O8 in the dark for 24 h, at room temperature. This solution was then diluted in 5 mM phosphate buffer saline (PBS) at pH 7.4 to
an absorbance of 0.70 at 734 nm and equilibrated at 30°C for 15 min prior to use. 2 ml of the ABTS** solution was taken and 100 µl of the solution of the plant extract to different concentrations of 15, 30, 60, 120 and 240 mg/100 ml were added. Subsequently, were mixed for 30 seconds and the absorbance at 734 nm at 1, 2, 3, 4, 5, 6, and 30 minutes were measured. All measurements were made in triplicate. The percentage of inhibition at 734 nm (Thermo Scientific BioMate 3S UV-Visible) is calculated and plotted as a function of concentration of antioxidants and of ascorbic acid for the standard reference data.

**DPPH method**
The method described by Brand-Williams [14] with some variations was used. 400 µl of the extract or standard was added to 3 ml of DPPH in methanol solution (69 mg/ml) in a test tube. This solution was shacked and left in the dark at room temperature, proceeding to the measurement of absorbance at 20, 30, 60 and 120 minutes at 520 nm (Thermo Scientific BioMate 3S UV-Visible). All measurements were made in triplicate. Ascorbic acid was used as the standard.

**FRAP method**
The ability to reduce ferric ions was measured using a modified version of the method described by Benzie and Strain [15]. To 900 µl of FRAP reagent (2.5 ml of 10 mM TPTZ solution, 2.5 ml of 20 mM FeCl₃ and 25 ml of 0.3 mM sodium acetate buffer at pH 3.6) were added 120 µl of extract to evaluate and the reaction mixture was incubated in a water bath at 37°C. The increase in absorbance at 593 nm (Thermo Scientific BioMate 3S UV-Visible) was measured at 30 minutes. The blank was prepared with 900 µl of FRAP and 120 µl of double-distilled deionized water. Acid ascorbic was used as standard.

**Data analysis**
The absorbances were interpolated with its respective calibration curve for each method, using the following formula:

\[ y = mx + b \]

Where:

- \( y \) = A° of problem solution
- \( x \) = problem solution concentration
- \( b \) = intercept
- \( m \) = slope of the line

Clearing concentrations was as follows: \( x = (y-b)/m \) obtaining the ascorbic acid equivalent antioxidant capacity (VCEAC). Also was calculated the rate of capture of free radicals or radical inhibition in the presence of the extract is using the formula:

\[ \% \text{ inhibition} = \frac{(A^i - A^f)/A^i \times 100}{1} \]

Where:

- \( A^i \) = Radical adjusted initial absorbance of the desired wavelength
- \( A^f \) = Final absorbance of the radical with extract

**Statistical analysis**
All values were expressed as means ± S.E.M. Data were analyzed statistically by one-way ANOVA followed by Tukey’s multiple comparison using Statistic software 5 version. Differences were considered significant at \( p < 0.05 \).

**RESULTS**
**Antioxidant activity with ABTS method**
The aqueous extract of *O. tampicensis* (240mg/100ml) and the ethanolic extract of *C. reflexifolius* (240mg/100ml), showed the highest AEAC index (inhibition percentage) with 79.9% y 76.4% respectively (\( p<0.05^* \)), and the aqueous extract of *B. simaruba* (240mg/100ml) was the lowest with 23.8% (Table 2). The highest antioxidant activity was observed to the 30 minutes at 240mg/100ml for all extracts (\( p=0.0000^* \)). There was a statistically significant
difference between extracts and concentrations, being the highest dose (240 mg/100 ml) which had higher antioxidant activity in all extracts. The aqueous extract of *O. tampicensis* and the ethanolic extract of *C. reflexifolius* were the extracts with the best antioxidant activity at all times of observation (Figure 1).

### Table 2: Percentage of inhibition by ABTS method

<table>
<thead>
<tr>
<th>Specie</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>(<em>Ocotea tampicensis</em>) (aqu)</td>
<td>79.9 ± 4.74*</td>
</tr>
<tr>
<td>(<em>Croton reflexifolius</em>) (eth)</td>
<td>76.4 ± 4.49*</td>
</tr>
<tr>
<td>(<em>Hamelia patens</em>) (eth)</td>
<td>70.1 ± 5.93</td>
</tr>
<tr>
<td>(<em>Pseudobombax ellipticum</em>) (aqu)</td>
<td>58.7 ± 4.66</td>
</tr>
<tr>
<td>(<em>Ocotea tampicensis</em>) (eth)</td>
<td>42.9 ± 5.41</td>
</tr>
<tr>
<td>(<em>Bursera simaruba</em>) (aqu)</td>
<td>23.8 ± 6.29</td>
</tr>
</tbody>
</table>

aqua (aqueous) eth (ethanolic), * p<0.05

**Fig 1.** Antioxidant activity of aqueous and ethanol extracts of Huastec medicinal plants with the ABTS method at 240 mg/100 ml (p=0.0000*).

**Antioxidant method with DPPH method**

The aqueous extract of *O. tampicensis* of 240mg/ml at 120 minutes showed the highest AEAC index with 82.57 % (p<0.05*), but its ethanolic extract (240 mg/100ml) was the lowest with 34.2% (Table 3). There was a statistically significant difference between extracts and concentrations, being the
highest dose (240 mg / 100 ml) which had higher antioxidant activity in all extracts (p<0.05*). The highest antioxidant activity was observed to the 120 minutes at 240mg/100ml aqueous extract of *O. tampicensis* (240 mg / 100 ml) was the extract with the best for all extracts. There was significant difference between extracts and concentrations, being the highest dose (240 mg / 100 ml) which had higher antioxidant activity in all extracts. The antioxidant activity at all times of observation (Figure 2).

### Table 3: Percentage of inhibition by DPPH method

<table>
<thead>
<tr>
<th>Specie</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>(240mg/ml)</em></td>
<td></td>
</tr>
<tr>
<td><em>Ocotea tampicensis</em> (aqu)</td>
<td>82.5 ± 5.28*</td>
</tr>
<tr>
<td><em>Croton reflexifolius</em> (eth)</td>
<td>69.9 ± 6.78</td>
</tr>
<tr>
<td><em>Pseudobombax ellipticum</em> (aqu)</td>
<td>64.2 ± 3.62</td>
</tr>
<tr>
<td><em>Hamelia patens</em> (eth)</td>
<td>55.8 ± 4.21</td>
</tr>
<tr>
<td><em>Bursera simaruba</em> (aqu)</td>
<td>46.1 ± 4.75</td>
</tr>
<tr>
<td><em>Ocotea tampicensis</em> (eth)</td>
<td>34.2 ± 3.29</td>
</tr>
</tbody>
</table>

aqu (aqueous) eth (ethanolic), * = p<0.05

Fig 2. Antioxidant activity of aqueous and ethanol extracts of Huastec medicinal Plants with the DPPH method at 240 mg/100ml (p=0.05*).
et= ethanolic; ac= aqueous.

**Antioxidant activity with FRAP method**
The aqueous extract of *B. simaruba* (240mg/ml) and the ethanolic extract of *O. tampicensis* (240mg/ml), showed the highest AEAC index with 78.4% and 77.1%; (inhibition percentage) at 240 minutes. The aqueous extract of *O. tampicensis* (240mg/ml) was the lowest with a percentage of inhibition 34.6% (Table 4). The
differences between extracts was statistically significant (p<0.05*). To all extracts the concentration of 240mg/ml showed the best antioxidant activity (p=0.0000*) at 30 minutes (Figure 3).

Table 4: Percentage of inhibition by FRAP method

<table>
<thead>
<tr>
<th>Specie</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bursera simaruba</em> (aqu)</td>
<td>78.4 ± 4.27*</td>
</tr>
<tr>
<td><em>Ocotea tampicensis</em> (eth)</td>
<td>77.1 ± 5.70*</td>
</tr>
<tr>
<td><em>Hamelia patens</em> (eth)</td>
<td>65.5 ± 3.45</td>
</tr>
<tr>
<td><em>Pseudobombax ellipticum</em> (aqu)</td>
<td>49.3 ± 6.26</td>
</tr>
<tr>
<td><em>Croton reflexifolius</em> (eth)</td>
<td>40.0 ± 4.87</td>
</tr>
<tr>
<td><em>Ocotea tampicensis</em> (aqu)</td>
<td>34.6 ± 6.42</td>
</tr>
</tbody>
</table>

aqu (aqueous) eth (ethanolic); *= p<0.05

Fig 3. Antioxidant activity of aqueous and ethanol extracts of Huastec medicinal plants with the FRAP method. p=0.0000*

et= ethanolic; ac= aqueous.

**DISCUSSION**

The objective of this study was assessment the antioxidant activity of some medicinal plants used in Huastec traditional medicine for pain-related ailments with three different methods (ABTS, DPPH, and FRAP). Several authors mention the need for more than one type of method for assessment the antioxidant activity of plants due to the different mechanisms of
action of antioxidants [10, 16, 17, 18]. *Ocotea tampicensis* showed the best antioxidant activity with ABTS and DPPH methods while *B. simaruba* was the best with FRAP method. There is no previous report about the compounds presents in this species but in Lauraceae family have been found neolignan, lignans, alkaloids, flavonoids and diterpenes compounds with antioxidant capacity [19]. These compounds are known to have ability to capture free radicals and have a lot of medicinal properties, especially with anti-inflammatory, analgesic, cardiovascular and anti-tumor effects [20]. Probably these compounds are responsible of the antioxidant capacity of *O. tampicensis* in DPPH and ABTS tests. The aqueous extract of *O. tampicensis* presented the highest antioxidant activity by the DPPH and ABTS methods, this indicates that was a better extraction of secondary metabolites with high capacity to capture free radicals in the aqueous medium, because the type of secondary metabolites and the solubility of the molecules, are factors that determine the antioxidant activity of the extracts in the presence of the colorimetric tests used [3]. Scavenging of DPPH radical is related to the inhibition of lipid peroxidation [21] and DPPH radical involves a hydrogen atom transfer process [22] this assay, the good antioxidant activity on DPPH radical of aqueous extract of *O. tampicensis* may be attributed to a direct role in trapping FR by donating hydrogen atom, probably this ability is in part responsible for the for use of *O. tampicensis* in treatment of pain in the Huastec traditional medicine. *Bursera simaruba* has various medicinal uses in Central America some of them against various types of pain (headache and stomach pain), also used to treat urinary tract infections, burns, nasal bleeding and fever [21, 22, 23]. In traditional medicine teenek *B. simaruba* is used for headache. The FRAP method allows to evaluate the antioxidant activity of substances capable of reducing metals involved in oxidative processes [24]. The aqueous extract of *B. simaruba*, had better antioxidant activity by the FRAP method, indicating that this extract has a very high ability to lower metals, probably this ability is in part responsible for the anti-inflammatory activity of *B. simaruba* in the Huastec traditional medicine. The Burseraceae family has diverse pharmacological properties including anti-inflammatory, antiviral action against nematodes, antifungal, antimicrobial and hepatoprotective activities, and has been tested in the treatment of leukemia and carcinomas [25]. Medicinal plants of the Burseraceae family are characterized by the presence of various compounds with analgesic, anti-inflammatory and antioxidant capacity, as terpenes, flavonoids, phenols, coumarins, sterols, resins, and gum oleoresin [20, 26, 27]. Particularly *B. simaruba* have anti-inflammation effects [26, 28], so it is used in traditional medicine in Mexico and Central America. The mechanism by which act secondary metabolites with antioxidant capacity is by inhibition or neutralization of FR in processes of inflammation in the body, so in the presence of antioxidants it is performed FR inhibition [29, 30]. For their ability to inhibit FR, antioxidants are considered as secondary metabolites with anti-inflammatory activity and thus help relieve the pain caused by inflammation associated with the release of FR [31]. It has been established that reactive oxygen species (ROS) are implicated in inflammation [32]. There exists a link of antioxidants with respect to scavenging ROS and anti-inflammatory effects and therefore play an important role in the treatment of inflammatory diseases [33]. Several studies suggest that antioxidant and anti-inflammatory agents could be beneficial in the prevention and treatment of pathologies associated to inflammatory process [2]. The demonstration of antioxidant activities by *Ocotea tampicensis* and *Bursera simaruba* may confirm this relationship. The extracts analyzed
in this study obtained from plants used in Huastec traditional medicine to mitigate various types of pain, owe their analgesic activity to secondary metabolites with antioxidant activity. Hence, these activities may justify the ethnomedicinal use of these plants in treat of pain in Huastec traditional medicine.

CONCLUSION
In the present study is supported the use of Ocotea tampicensis and Bursera simaruba in Huastec traditional medicine in the treatment of various types of pain, due to its ability to capture free radicals associated to inflammatory processes by the release of free radicals. It is important carry out anti-inflammatory and pain assessment to verify the analgesic activity of the plants analyzed in the present study. Likewise, is important for safe use in traditional medicine, conducted tests LD50 (to assess their toxicity and thus determine the safe dose for consumption. The results obtained in this study provide scientific information that could aid in the isolation of potential pharmacologically active compounds from some of these medicinal plants in future research.

ACKNOWLEDGEMENT
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CONFLICT OF INTEREST STATEMENT
The authors declare that they have no competing interests.

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