Moringa oleifera: phytochemical detection, antioxidants, enzymes and antifungal properties

Moringa oleifera: detección fitoquímica, antioxidantes, enzimas y propiedades antifúngicas


Abstract. Moringa oleifera is a plant with a high biotechnology potential due to its high content of proteins, carbohydrates and lipids, and secondary metabolites. Some of their secondary compounds have been advocated for traditional medicinal uses. This work shows an overview of the histological organization and the composition of some biochemical components (e.g.: enzymatic inhibitors, phytochemicals, enzymatic profiles and antifungal potential) of different plant tissues. These components could be associated with the physiology and defense mechanisms of the study plant species. These activities were detected using synthetic substrates by conventional strategies based on spectrophotometrical and zymography assays. Moringa oleifera presented a typical histological array for dicotyledonous plants. According to the phytochemical assay, leaves showed the highest antioxidant activity, which could be related with their contents of phenolic compounds. Also, trypsin inhibitors were detected in different parts of the plant, indicating possible implications as defensive traits. The presence and abundance of the detected compounds could be associated with a general status of the physiology of the plant. It should be noted, however, that all components detected could be involved in plant defense mechanisms.

Keywords: Moringa oleifera; Phytochemicals; Plant defense; Trypsin inhibitors; Proteinases.

Resumen. Moringa oleifera es una planta que presenta un alto potencial biotecnológico debido a su alto contenido de proteínas, carbohidratos y lípidos. Por otra parte, algunos de sus metabolitos secundarios han sido promovidos para su uso en medicina tradicional. En el presente trabajo, se muestra una revisión de la organización histológica y la composición general de algunos de los constituyentes bioquímicos (inhibidores enzimáticos, fitoquímicos y perfiles enzimáticos) de diferentes tejidos de la planta. Dichas actividades fueron efectuadas con sustratos sintéticos por métodos convencionales basados en ensayos espectrofotométricos y zimografías. Moringa oleifera presentó un arreglo histológico típico de una dicotiledónea. De acuerdo a los ensayos fitoquímicos, las hojas mostraron la actividad antioxidante más alta, la cual podría estar asociada con sus altos contenidos de compuestos fenólicos. También se detectaron inhibidores de tripsina en diferentes partes de la planta, lo que podría tener implicancias en la formación de elementos defensivos. La presencia y abundancia de los componentes detectados podría estar asociada con un estado general de la fisiología de la planta. Los componentes detectados también podrían estar involucrados en los mecanismos de defensa de la planta.

Palabras clave: Moringa oleifera; Fitoquímicos; Defensa de plantas; Inhibidores de tripsina; Proteínas.
INTRODUCTION

*Moringa oleifera* is a widely cultivated tree considered as a multi-purpose plant. It includes its use as functional food, cleaning water material, oil extraction for biofuel production, and other applications. In a traditional way, it is used with medicinal purposes around the world due to empiric observations. These benefits have been associated with metabolites such as phenolic compounds, vitamins and proteins (Fahey, 2005; Goyal et al., 2007; Adedapo et al., 2009). Nevertheless, the complete knowledge about what kind of metabolites are present in each organ, and their ecological and biological roles, are poorly elucidated. Some studies about macromolecular characterization have been made in the last years, including a protein with the ability to agglutinate, proteinase inhibitors, lectins, carbohydrates and lipid contents (Olayemi & Alabi, 1994; Santos et al., 2005). Plant parts, including leaves, stem, roots, seeds and flowers have been reported as source of different biochemical compounds with anticarcinogenic, antiinflammmatory, antidiabetic, antioxidant, and antimicrobial effects (Fahey, 2005; Anwar et al., 2006; Goyal et al., 2007; Chumark et al., 2008; Peixoto et al., 2011). *Moringa oleifera* contains essential amino acids, carotenoids in leaves, and components with nutraceutical properties, supporting the idea of using this plant as a nutritional supplement or constituent in food preparation. Some nutritional evaluation has been carried out in leaves and stem. Studies have shown that conventional antinutritional factors like trypsin and amylase inhibitors, glucosinolates, cyanogenic glycosides, saponins and tannins were not detected, at least with the used methodology (Makkar & Becker, 1997). However, a comprehensive study including the root system is still missing. Secondary metabolites from *M. oleifera* leaves have shown antimicrobial effects against various human pathogenic bacteria, including the genera *Shigella, Pseudomonas, Salmonella* and *Bacillus*. These secondary metabolites appear to be involved in plant defense mechanisms (Clark, 1981, Chuang et al., 2007; Doughari et al., 2007; Mashiar et al., 2009). Recently, the presence of a trypsin inhibitor has been reported in leaves. This indicates an important activity against serine proteinases. This inhibitor has been stable in a wide range of pH, detergents, reducing agents and high temperatures, which coincides with the high stability reported for some proteins involved in plant defense (Bijina et al., 2011). Despite its potential applications, a comprehensive knowledge of *M. oleifera*, including its responses at the biochemical and biological levels, is still limited. Also, the biochemical implications on human health of the bioactive compounds present in this plant, their relation with the plant defense strategies against biotic and abiotic agents, as well as the physiological roles of proteins, enzymes and secondary metabolites are not well understood when ecological interactions are considered.

This report presents a brief histological and biochemical description of leaves, stem and roots of *M. oleifera*, and the distribution of metabolites often associated to plant defense.

MATERIALS AND METHODS

Histological analysis. *Moringa oleifera* plants were obtained from greenhouses of the Agronomy Faculty of Universidad Autonoma de Nuevo Leon (UANL) in Nuevo Leon, Mexico. Each plant organ was separated and fixed in a solution of formaldehyde, ethanol and acetic acid in distilled water (5:25:10:60 v/v) during 24 h. Then, samples were rinsed with distilled water for 2 h and sections of fixed organs were handmade using razor blades. Adequate sections were selected and exposed to sequential dehydration in ethanol (90%) and stained with 0.1% Safranin O solution during 4 h. These samples were thereafter washed to eliminate excess of stain with ethanol (70%) during 15 min twice. Sections were exposed to dehydration up to absolute ethanol for 10 min, and stained by immersion in 0.05% Fast Green solution for 1 min. The excess of colorant was washed away with ethanol (95%) during 5 min, and dehydrated with absolute ethanol during 15 min. This was followed by sequential immersion in ethanol-xylene solutions by gradually increasing xylene concentration up to reach 100%. Finally, preparations were mounted using Canada balsam.

Extract preparation. Four plants (= 9 month-old) were obtained, and parts were separated and cleaned (Fig. 1 A). The cortex was cut off with knives removing the central wood-hard tissue. Samples of 10 g were prepared and rinsed with distilled water for 5 min and dried. Each separated part was ground using water for the aqueous extract, and ethanol at 70% for the ethanolic extract. Macerated samples were immersed in each solvent (1:2 ratio) during 20 min and clarified by centrifugation at 10000 g for 5 min to 4 °C. The liquid fraction was obtained and kept at -70 °C for subsequent analysis.

Phytochemical assay. Both extracts were exposed to phytochemical assay using color reactions according to several protocols as follows:

**Polyphenol detection with KMnO**$_3$. To 100 μL of each sample, 100 μL of distilled water were added. Reactions were carried out by the addition of 20 μL of KMnO$_3$. The reaction was considered positive when color change was in the yellowish or greenish tones. Distilled water was used as control.

**Polyphenol detection with FeCl**$_3$. To 100 μL of each sample, 100 μL of distilled water were added and the reactions were started with addition of 20 μL of FeCl$_3$. The reaction was positive when dark-greenish, blue to dark-blue or even blackish tones were presented in the mixture. Distilled water was used as control.

**Flavonoid detection.** To 100 μL of extract some fragments of metallic magnesium and three drops of 25% HCl solution were added. A positive reaction was considered when color of the solution changed to pink, yellow or orange.

**Tannin detection.** Detection was based on gelatin precipitation in saline solution. Ten tubes were prepared with 2 mL of that solution; 3 tubes were filled with a 10% solution of...
Data were analyzed using ANOVA. Growth was evaluated on each Physiological and biological properties of Moringa oleifera. Absorbance of clarified extracts of Moringa oleifera was measured at 517 nm. Controls contained 100 μL of distilled water. The radical-scavenging capacity of the extracts was calculated with the following equation and expressed as DPPH percent of inhibition:

\[
\text{Inhibition (\%)} = \left( \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \right) \times 100
\]

**Electrophoresis and zymography.** For enzyme detection, the extracts were separated by electrophoresis following Martinez-Avila et al. (2011). Briefly, 2.9 mL of 60 μM DPPH radical were added to 100 μL of each extract. Then, samples were located in a dark place, and after 30 min the absorbance (Abs) was measured at 517 nm. Controls contained 100 μL of distilled water. The radical-scavenging capacity of the extracts was calculated with the following equation and expressed as DPPH percent of inhibition:

**DDP**

H scavenging capacity. DPPH assay was carried out following Martinez-Avila et al. (2011). Briefly, 2.9 mL of 60 μM DPPH radical were added to 100 μL of each extract. Then, samples were located in a dark place, and after 30 min the absorbance (Abs) was measured at 517 nm. Controls contained 100 μL of distilled water. The radical-scavenging capacity of the extracts was calculated with the following equation and expressed as DPPH percent of inhibition:

\[
\text{Inhibition (\%)} = \left( \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \right) \times 100
\]

**Electrophoresis and zymography.** For enzyme detection, the extracts were separated by electrophoresis following an adapted protocol of Laemmli (1970). Run conditions included 20 min at 80 V and 40 min at 120 V in 10% polyacrylamide gels. Protein profiles were stained with Commassie blue. Zymography was carried out with the same conditions described above. For amylase activity, starch with low amylase activity was used to detect differences among the treatments. Means were compared using the Tukey test (p=0.05). Analyses were done using the statistical software SAS version 9.0.

**Alkaloid detection.** Samples of 600 μL of each extract were heated at 95 °C, and 100 μL of HCl (10%) were added. Tubes were left at room temperature for cooling them and then 20 μL of Wagner reagent were added and mixed. The reaction was considered positive when maroon precipitation was formed.

**Saponin detection.** Three hundred μL of each extract and 300 μL of boiling water were mixed, then cooled, and thereafter tubes were vigorously agitated. Foam formation during more than 10 min was considered a positive reaction for saponin presence.

**DDP**

H scavenging capacity. DPPH assay was carried out following Martinez-Avila et al. (2011). Briefly, 2.9 mL of 60 μM DPPH radical were added to 100 μL of each extract. Then, samples were located in a dark place, and after 30 min the absorbance (Abs) was measured at 517 nm. Controls contained 100 μL of distilled water. The radical-scavenging capacity of the extracts was calculated with the following equation and expressed as DPPH percent of inhibition:

\[
\text{Inhibition (\%)} = \left( \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \right) \times 100
\]

**Electrophoresis and zymography.** For enzyme detection, the extracts were separated by electrophoresis following an adapted protocol of Laemmli (1970). Run conditions included 20 min at 80 V and 40 min at 120 V in 10% polyacrylamide gels. Protein profiles were stained with Commassie blue. Zymography was carried out with the same conditions described above. For amylase activity, starch with low amylase activity was used to detect differences among the treatments. Means were compared using the Tukey test (p=0.05). Analyses were done using the statistical software SAS version 9.0.
RESULTS

Anatomical description. *Moringa oleifera* had a typical histological array of dicotyledonous plants. Leaflet blades were always dorsiventrally compressed. Transverse sections showed a unistratified epidermis with stomata on both sides; they were more abundant in the abaxial side. Epidermal cells were more or less isodiametric, polyhedral and uniformly arranged. Mesophyll consisted of 6 to 8 layers of chlorenchymatous tissue. Two cellular types were observed according to the shape and position of each cell. The first type was a palisade parenchyma on the adaxial face with a compact arrangement of tabular cells. The second type was a spongy parenchyma on the abaxial face, with more or less compactly-arranged cells and scarce intercellular spaces; the cells were irregularly shaped. The central bundle was collateral and usually semi-triangular shaped, with the apex pointing out towards the adaxial face, and subtended by some collenchymatous cells towards the abaxial face (Fig. 1 B and C).

Transverse sections obtained from the middle part of the third apical growing internode presented an epidermal cell layer. It was subtended by two or three rows of collenchymatous cells (identified because of their irregularly thickened walls), followed by cortical parenchyma cells with around 20 layers up to the vascular region. Immersed in the cortical parenchyma, there were fibers arrayed in separate bundles with a cylindrical disposition. The vascular system was an ectophloic siphonostele, with well-developed piths; such array had around six-layers of phloem with a cylindrical distribution surrounding an eight-layer vascular cambium, represented by rectangular, thin-walled cells. Xylem was represented by vascular rows, which increased in diameter from the center to the periphery of the section. They were also distributed in a cylindrical way forming a pith of parenchyma with wide, thin-walled cells (Fig. 1 D and E).

The root showed a multistratified epidermis in transversal section, with wide tabular, thin-walled cells with up to 9 layers in general. There were also extra rows of 5 to 6 cells forming elevations which gave the transversal section a “daisy-flower” shape. There was a cortical region constituted of parenchyma cells, with sclerenchyma cells separated in a cylindrical array reaching the limit with the endodermal region. The casparian band was formed by cells with lignified walls enclosing the vascular region. Xylem was distributed in five poles when the root was young, but no poles could be clearly distinguished in mature roots. Phloem was inserted between xylem poles. No pith was observed (Fig. D and E).

Phytochemical detection. The phytochemical constituents present in the aqueous and ethanolic leaf, stem, root and stem-cortex extracts of *M. oleifera* were mainly detected by basic colored-reactions. The phytochemical assay showed differences among plant parts as indicated in Table 1, confirming the presence of phenolic compounds, alkaloids, flavonoids and the absence of saponins and tannins. The assay showed a more diverse phytochemical composition on leaves, with the presence of alkaloids, FeCl₃-reacting-polyphenolic compounds and flavonoids. On the other
hand, a similar phytochemical pattern was observed for the stem and roots, where the predominant group was the KMNO$_3$-reacting polyphenolic compound. No presence of saponins and tannins were detected for both extracts in all plant parts with the methods used. Both types of polyphenolic compounds were assayed under the same conditions. However, the velocity and intensity of reactions, used as indicative of relative abundance, allowed detecting gradual differences among the leaves, stem and roots, where the amount of KMNO$_3$-reacting polyphenolic compounds seemed to be higher. For FeCl$_3$-reacting-polyphenolic compound and flavonoid detection, the reaction time was extended for stem and root and no changes were observed. This indicated that very low amounts (or total absence) of these compounds were present in such organs. Phytochemicals like phenolic compounds are usually related to several biological activities, including antioxidant potential and radical scavenging, which is relevant for therapeutics and nutraceutical applications. In this sense, DPPH radical scavenging was carried out in all *M. oleifera* parts trying to determine the richer organ in antioxidant potential and the best system for extraction. Our results indicated that activity was present in both extracts, and that higher or minor activities were on leaves or stem and roots, respectively (Table 2).

**Enzymatic profiles.** An enzymatic profile was established as an indicator for the metabolic state of each organ using zymology techniques. This is because of information about enzymes at each organ could be associated to endogenous roles such as reserve mobilization, protein degradation or plant defense. The proteolytic and amylolytic assay of both extracts included general substrates. For proteinases, the assay was through a wide pH range. Most of the detected enzymes were obtained in aqueous extracts. In general, proteinases showed a greater activity at pH 5 or 8, while a minor proteolytic activity was recovered in the ethanolic extract (Fig. 2). The leaf aqueous extracts showed the highest intensity for proteinases at pH 5; nevertheless, the leaf proteolytic profile was similar to that on the stem, stem cortex and roots. The pattern of proteolytic activities showed a population of two main bands. The first band showed a molecular weight of 40 kDa, and the second one a weight close to 27 kDa (Fig. 2 A and B). In the case of ethanolic extracts, only a band of activity at pH 5 was observed in the stem. The signals corresponding to active enzymes at pH 5 were less visible when incubation was at pH 6. At pH 7 and 8 a different proteolytic pattern was visualized. In aqueous extracts, a band of 80 and another one of 42 kDa were detected at pH 7; bands were more intense at pH 8 as indicated by the arrows (Fig. 2 C and D). In addition, a pattern of three bands of 30, 33 and 39 kDa were detected at pH 8 as indicated by the arrows (Fig. 2 C and D). In addition, a pattern of three bands of 30, 33 and 39 kDa were detected in the cortex after incubation at pH 7, and the same, but more intense, pattern was observed at pH 8 in all the extracts. The intensity, however, was highest in the ethanolic extracts. Amylase activity was detected in both extracts (Fig. 3); in aqueous extract, amylase activity in the leaf was present as two bands. The more intense band showed a molecular weight around 90 kDa, while it was only 50 kDa in the less intense band. In the case of stem and cortex, the amylolytic activity was intense in the 90 kDa position, showing a continuous hydrolysis throughout the wall. In the root aqueous extract, that activity was less intense in the 90 kDa position; also, a band of 30 kDa was observed in the stem, stem cortex and roots. No other signals were detected in the aqueous extracts. Amylase activity in the leaves, stem and roots was detected with low intensity in ethanolic extracts. This was even increasing the incubation time. In the case of the ethanolic extract for the cortex, a main

### Table 1. Phytochemical assay results.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Aqueous extract</th>
<th>Ethanolic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>+ + + + - - + + + +</td>
<td>- - - - - - - - - -</td>
</tr>
<tr>
<td>Stem</td>
<td>- + + + - - - - + + +</td>
<td>- - - - - - - - - -</td>
</tr>
<tr>
<td>Cortex</td>
<td>- + + + + + + + + + +</td>
<td>- - - - - - - - - -</td>
</tr>
<tr>
<td>Roots</td>
<td>- - - - - - - - - - -</td>
<td>- - - - - - - - - -</td>
</tr>
</tbody>
</table>

These results were obtained by color reactions and qualitative comparison. P1, FeCl$_3$-reacting-polyphenolic compounds; P2, KMNO$_3$-reacting polyphenolic compounds; F, flavonoids; A, alkaloids; T, tannins; S, saponins. Negative detection is indicated by -, positive reaction is indicated by +, ++ and ++++. All reactions were done by triplicate on at least three plants.

### Table 2. DPPH' scavenging capacity.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Aqueous extracts</th>
<th>Ethanolic extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem</td>
<td>20.47 ± 0.455</td>
<td>30.91 ± 1.77</td>
</tr>
<tr>
<td>Leaves</td>
<td>85.20 ± 0.001</td>
<td>89.67 ± 0.75</td>
</tr>
<tr>
<td>Roots</td>
<td>14.27 ± 0.344</td>
<td>29.48 ± 1.55</td>
</tr>
</tbody>
</table>

The capacity was expressed as the percentage of DPPH inhibition for each analyzed part and extract. Values are the mean ± 1 standard deviation of n=3.

La capacidad secuestradora fue expresada como el porcentaje de inhibición de DPPH para cada parte vegetal y extracto analizado. Los valores son el promedio ± 1 desviación estándar de n=3.
band of amylolytic activity was observed at 90 kDa position, and no other signal was detected.

**Trypsin inhibitor detection.** Trypsin inhibitor content as the main proteinaceous antinutritional factor was determined in aqueous and ethanolic extracts as indicated in Table 3. The inhibitory activity was spread throughout all the plant, but higher inhibitory unit content was detected in ethanolic extract of roots, and lower contents were presented by stem and cortex in aqueous extract. The specific activity was related to the amount of protein in each extract.

**Antifungal effect.** Micelial growth of *A. solani* did not present significant differences in both aqueous and etha-

![Fig. 2](image-url). Proteolytic profiles of extracts from *M. oleifera* vegetative plant parts. Clear bands indicate the proteolytic activity. (A) Profile of enzymes active at pH 5, black arrow indicates a 40 kDa band, and white arrow points out a band of 27 kDa; (B) Profile at pH 6, black and white arrows indicate positions for 40 and 27 kDa bands, respectively; (C) Profile at pH 7, broad black arrow indicates 80 kDa band, and broad white arrow points out at 42 kDa band, both notoriously active at the cortex water extract (CW), thin black and white arrows showed the pattern of 30, 33, 39 kDa bands of activity from the cortex in CW and CE; (D) Profile at pH 8, broad arrows indicates the same pattern as in (C), but more intense at this pH, the pattern of 30, 33 and 39 kDa bands was present in all at pH 8. L: leaf, S: stem, R: root and C: cortex. The solvents are indicated by W (water) and E (ethanolic). M, molecular weight standards.
Physiological and biological properties of *Moringa oleifera*

**DISCUSSION**

*Moringa oleifera* plants are used as a food source with valuable properties in humans (Anwar et al., 2007; Goyal et al., 2007; Adedapo et al., 2009). Therefore, knowledge about bioactive components through the whole plant, and their relative abundances their potential biological effects could help to design strategies focused on the appropriate utilization of this resource. Current descriptive studies contribute to support the ideas of alternative uses, genetic improvement, and the study of defense strategies to infer ecological interactions and adaptations. In this work, a general histological description for *M. oleifera*, a phytochemical content analysis, and some enzymatic profiles give a perspective about the accumulation and distribution of some metabolites in some parts but not in others, leading to relate their distribution with possible physiological and defensive roles.

There were plenty of non detected metabolites in this work; differences in phytochemical constitutions were observed. In the case of leaves, there was an exclusive detection of FeCl₃-reacting polyphenolic compounds together with alkaloids. Also, the presence of KMNO₄-reacting polyphenolic compounds and flavonoids was registered. These compounds could contribute to protect the foliage area from abiotic and biotic stresses; the last phenolic group was shared with the stem but

---

**Table 3. Trypsin inhibitor content.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>TIU/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>LW</td>
<td>712 ± 24</td>
</tr>
<tr>
<td>SW</td>
<td>318 ± 6</td>
</tr>
<tr>
<td>RW</td>
<td>578 ± 96</td>
</tr>
<tr>
<td>CW</td>
<td>352 ± 7</td>
</tr>
<tr>
<td>LE</td>
<td>432 ± 10</td>
</tr>
<tr>
<td>SE</td>
<td>718 ± 178</td>
</tr>
<tr>
<td>RE</td>
<td>1112 ± 248</td>
</tr>
<tr>
<td>CE</td>
<td>432 ± 10</td>
</tr>
</tbody>
</table>

Trypsin inhibitory units (TIU). Plant parts: L, leaf; S, stem; R, root; C, cortex. Solvents: W (water) and E (ethanolic) extracts. Values are the mean ± 1 standard deviation of n=3.

---

**Fig. 3.** Amylase activity assay in the extracts. Broad black arrow indicates the position of a 90 kDa band of amylase activity, broad white arrow indicates the position for a 50 kDa band present in the leaf and stem, black dotted arrow points out the position of a 30 kDa band. L: leaf, S: stem, R: root and C: cortex, and W (water) and E (ethanol). M: molecular weight standards.

**Fig. 3.** Ensayo de actividad de amilasa en los extractos. La flecha ancha y negra indica la posición de una banda de 90 kDa, la flecha ancha y blanca indica la posición de una banda de 50 kDa presente en la hoja y tallo, la flecha negra punteada señala la posición de una banda de 30 kDa. L: hoja, S: tallo, R: raíz y C: corteza, y W (agua) y E (etanólicos). M: estándares de peso molecular.
not with the root. Also, other differential accumulation was registered in the case of the relative detection for KMNO$_4$-reacting polyphenolic compounds with increasing amounts from leaves to stems and more intense signal in roots. Such distribution could obey to either the need or not of those compounds for ecological interactions. For example, the presence and accumulation of alkaloids and polyphenols in foliage is related to their deterrent and potential toxic effects to herbivores and insects (Coley, 1988; Macel et al., 2005). In the case of KMNO$_4$-reacting polyphenolic compounds, they showed a gradual accumulation according to the intensity of the signal reaction, increasing it towards the root where the presence of these compounds was the only one detected. Thus, the variation in the profile of compounds among different plant parts probably corresponds to their chemical nature, function and signals perceived from the environment (Flores et al., 1999).

The enzymatic profiles for proteinases and amylases also corresponded with variations among plant parts, with more intensity in signals in stem and roots than in leaves, which could be related with the translocation or utilization of reserves. For example, amylases seemed less abundant in leaves. This very likely indicates the leaves as a source organ, actively producing sugars which are then translocated to sink organs (i.e., stem, roots) where they are stored as starch, and breakdown by amylases, to provide energy to the sinks (Göttlicher et al., 2006).

Usually, antioxidant properties of plant extracts have been attributed to the presence of polyphenolic compounds, which have great potential as antimicrobial agents. Measurement of antioxidant activity is the most widely accepted analysis to attribute the several benefits of phenolic compounds (Yemis et al. 2008). Leaves showed the highest antioxidant potential, which coincided with the positive signals obtained for polyphenolic and flavonoid compounds. They are probably responsible for such antioxidant activity. Similar findings have been reported by Gardner et al. (2000) on juices from vegetables, pineapple and apple. As a result, the leaf could be considered the most relevant part of $M. oleifera$ as antioxidant source in comparison to other parts of the plant. The fact of its high antioxidant activity is according with findings in previous reports (Chumark et al., 2008; Sreelatha & Padma, 2009).

Usually, trypsin inhibitors are associated to endogenous regulation of proteinases. Their role as constitutive or inducible proteins in complex responses for plant defense has been reported; trypsin-like proteinase inhibitors seem to be a conserved and widely dispersed defense strategy in plants (Ryan, 1990; Cipollini & Bergelson, 2001). In a previous report, proteinase inhibitors with activity against serine proteinases including thrombin, chymotrypsin, elastase and some cysteine proteinases were obtained from seeds, leaves, stem, flowers and bark; this assay was focused in proteinases of therapeutic and industrial importance (Bijina et al., 2011). In the present work, bovine trypsin inhibitor activity was detected in leaves,

**Fig. 4.** Effect of $M. oleifera$ aqueous extracts on the growth of some phytopathogenic fungi. There was no significant effect on $A. solani$ (A) and $F. oxysporum$ (B) growth. Growth was affected by all the aqueous extracts in $P. parasitica$ (C); the root extract inhibited around 70% of growth. White triangles indicate the control, and black squares, black triangles and black circles indicate leaf, stem and root aqueous extracts, respectively.

**Fig. 4.** Efecto de los extractos acuosos en el crecimiento de algunos hongos fitopatógenos. No hubo efecto significativo sobre el crecimiento de $A. solani$ (A) y $F. oxysporum$ (B). El crecimiento fue afectado por todos los extractos acuosos en $P. parasitica$ (C); el extracto radical inhibió alrededor del 70% del crecimiento. Los triángulos blancos indican el control, cuadros negros, triángulos negros y círculos negros indican extractos acuosos de hoja, tallo y raíz, respectivamente.
Physiological and biological properties of *Moringa oleifera*

stem, roots and cortex in aqueous and ethanolic extracts. This would indicate that *M. oleifera* is a potential source for inhibitors with different properties in all vegetative plant parts. Nevertheless, if the inhibitory activity is involved in plant defense or in endogenous regulation is still unknown.

Evidently, more studies on the general biochemical and molecular biology for this plant species will lead to new and directed applications for this resource, and a more comprehensive study of metabolites, enzymes and other compounds in *M. oleifera*. Their variation through time, developmental stage, or as a particular metabolic state in each organ could lead to improve understanding of plant responses with physiological and ecological implications, looking for a better use of this resource.

Numerous studies have been conducted on different parts of *M. oleifera*, but there is a direct need to isolate and identify compounds associated with biotechnological applications. Nevertheless, it is worth mentioning that endogenous roles of these compounds are still poorly understood. Since this plant has been proposed as a crop, it is planted around the world, which implies it needs to grow under different environmental conditions. Thereafter, it is expected that there will be some variation in the presence or concentration of certain metabolites in different parts of the tree. Despite it is a studied plant, changes in the general physiology and biochemical composition among populations adapted to particular environmental conditions are not known. Here, the presence of some compounds considered as defensive elements has been shown, and their distribution in vegetative plant parts has been established. However, detailed studies for examining the endogenous roles of the study compounds are yet required.

**ACKNOWLEDGEMENTS**

This work was supported by PAICYT 2010, an institutional program in Universidad Autónoma de Nuevo León (UANL) for Science and Technology promotion.

**REFERENCES**


