

Full Length Research Paper

Antioxidant and free radical scavenging activities of plant extracts used in traditional medicine in Mexico

F. Ruiz-Terán, A. Medrano-Martínez and A. Navarro-Ocaña*

Departamento de Alimentos y Biotecnología, Facultad de Química, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, México, D.F., 04510, México.

Accepted 25 April, 2008

Twenty-two species of medicinal plants collected in the Mexican state of Morelos were selected to evaluate their free radical scavenging and antioxidant activities. The extracts from the aerial parts of the plants were obtained using hexane, acetone and methanol (66 extracts). The initial qualitative screening of antioxidants was made using two TLC methods against the stable DPPH (1,1-diphenyl-2-picrylhydrazyl-hydrate) and β -carotene-linoleic acid bleaching assay. All the extracts displayed antioxidant activity. However, the methanol extracts appeared to have the highest antioxidant activity, so they were examined further. The quantitative assays against DPPH radical, β -carotene-linoleic acid bleaching and total amount of phenols in the methanol extracts (TPME) showed nine plants as having the highest scavenging and antioxidant activities. For the nine methanolic extracts analysed, a clear relation between the total phenolic content of the extracts and their antioxidant activity was found. Plants such as *Annona squamosa* and *Sapium macrocarpum* showed two times more antioxidant activity than the commercial BHA (butylated hydroxyanisole) antioxidant. Moreover, some methanolic extracts of the plants showed activities comparable to commercial antioxidants BHA and TBHQ (*tert*-butylhydroquinone), thus making it possible to consider some of the studied plants as a potential source of antioxidants of natural origin.

Key words: Antioxidant activity, Mexican plants, total phenol content.

INTRODUCTION

Oxidation is the transfer of electrons from one atom to another and represents an essential part of both aerobic life and our metabolism, since oxygen is the ultimate electron acceptor in the electron flow system that produces energy in the form of ATP. However, problems may arise when the electron flow becomes uncoupled (transfer of unpaired single electrons), generating free radicals (Pietta, 2000).

Antioxidants are important in living organisms as well as in food because they may delay or stop formation of

free radical by giving hydrogen atoms or scavenging them. Oxidative stress is involved in the pathology of cancer, atherosclerosis, malaria and rheumatoid arthritis.

The use of traditional medicine is widespread in Mexico and plants are indeed the first source for preparing remedies in this form of alternative medicine. Among the various compounds found in plants, antioxidants are of particular importance because they might serve as leads for the development of novel drugs. Several plants used as anti-inflammatory, digestive, antinecrotic, neuroprotective, and hepatoprotective properties have recently been shown to have and antioxidant and/or antiradical scavenging mechanism as part of their activity (Perry et al., 1999; Lin and Huang, 2002). The search for natural sources of medicinal products that also have antioxidant and radical scavenging activity is on the rise (Schinella et al., 2002; VanderJagt et al., 2002).

The plants reported in this work were collected in the state of Morelos in the south of Mexico and all of them

*Corresponding author. E-mail: arturono@servidor.unam.mx.
Tel: 52-55-56225346. Fax: 52-55-56220609.

Abbreviations: DPPH, 1,1-Diphenyl-2-picrylhydrazyl-hydrate; TPME, total amount of phenol in the methanol extract; BHA, butylated hydroxyanisole; TBHQ, *tert*-butyl hydroquinone; TPC, total phenolic content introduction.

are used in traditional medicine (Argueta et al., 1994; Monroy-Ortiz and Castillo, 2000). Among the medicinal properties associated with them are the following: the fruit and bark of *Licania arborea* is used as a soap for hair infections, the latex from *Ficus obtusifolia* is employed as an anti-parasitic and also for reducing fever, *Bunchosia cannesensis* is prescribed as an anti-diarrhoeic, *Sideroxylon capiri* is used for hiccups, as an antiseptic for cleaning wounds, and women use its leaves in a water bath after giving birth. The latex of *Sapium macrocarpum* is used against scorpion stings, fever and some skin problems such as warts; its use as an anti-coagulant is also widespread. The latex of *Ficus cotinifolia* is used in the treatments of urinary infections, vomiting, malaria and against inflammatory pathologies of the spleen. The leaves of *Annona squamosa* are used in cicatrization of wounds, diarrhoea, ulcers, menstrual disorders, and also to help weight loss. The seeds of this plant are also employed as an insecticide. The leaves of *Vitex mollis* are used to treat stomach ache, digestion disorders, nervous alterations, and also scorpion stings. *Piper leucophyllum* is employed for reducing fever and its dried leaves are used for cleaning eyes and as spice in cooking. The leaves and bark of *Gliricidia sepium* are used against high fever, skin infections, urine disorders, malaria, and headache. However, its seeds are reported to be toxic. *Hamelia paten* is used to accelerate wound cicatrization. The Mexican and Central America native species of *Astianthus viminalis* is used for the curing of diabetes and malaria and to reduce hair loss. *Swietenia humilis* is used as anti-parasitic, and it is also utilized for hair care as a shampoo. It is also used with other plants in mixed herbal teas, and used as home remedies. *Stemmandenia bella* is employed for curing wounds; *Rupechta fusca* is used in some stomach disorders; *Bursera grandifolia* is used as a tooth paste and against digestive disorders; *Ziziphus amole* is prepared as infusion and it is applied for washing wounds and to treat gastric ulcers. The fruit and the latex of *Jacaratia mexicana* are used against ulcers in the mouth and digestive disorders. *Gyrocarpus jathrophifolius* leaves and bark are used as an analgesic. *Pseudobombax ellipticum* is used in respiratory disorders such as cough, and also against fever and as an anti-microbial. The stems and flowers of *Comocladia engleriana* are toxic because they produce dermatitis. The flowers and the latex of *Plumeria rubra* can be used for stopping vaginal blood shed, and tooth headache, and the latex of the plant is used against earache. Infusions are used as an eye-cleaning liquid.

The aim of this work is to evaluate the antioxidant activity of 66 extracts of these 22 species of plants from the Morelos State area. The antioxidants were determined using thin layer chromatography (TLC), DPPH and β -carotene. Their antioxidant activity was also quantified using spectrophotometric methods. Total phenol content was determined by the Folin-Ciocalteu method.

MATERIAL AND METHODS

Chemicals

All the chemicals used in this work were analytical grade and purchased from Sigma Aldrich Co. (St Louis, MO, USA).

Plant material

The twenty-two medicinal plant species screened in this study were collected in 2004 in Huautla, Morelos, Mexico. Their family, species and common names are shown in Table 1, together with some information concerning their popular names. They were analysed for their free radical scavenging and antioxidant activity. The aerial parts of the plants were dried at ambient temperature for 15 days. Samples were ground until they passed through a 10 μ m sieve. Specimens of these plants were deposited in the National Herbarium (MEXU), Instituto de Biología, UNAM).

Extracts preparation

Four continuous extraction processes were carried out using 27 g of the ground aerial parts of the plants. First, 100 mL of hexane were used and samples were extracted by maceration in 250 mL Erlenmeyer flasks for 24 h at room temperature. Then, one hundred milliliters of acetone were added to the ground aerial parts and lived as before during 24 h. Finally, methanol was used in the same way as the other solvents. All extracts were then filtered and the filtrates were concentrated with a vacuum rotary evaporator (Büchi) at 30°C. The concentrated extracts were stored at 4°C until they were used.

DPPH free radical scavenging activity assays

The qualitative assays were performed according to the method of Takao et al. (1994). Two milligrams of the dried extract were diluted with 1 mL of the appropriate solvent then, 20 (μ L) aliquot of each dilution of the hexane, acetone and methanol extracts was carefully loaded individually onto the baseline of the TLC plates (20 cm x 10 cm) and the sample was allowed to dry. Hexane-EtOAc (9.5:0.5), hexane-EtOAc (7:3), and EtOAc-methanol (9:1) were used as mobile phases, respectively. Once dried, the plates were sprayed with a 0.2% solution of radical-DPPH in methanol. Compounds with radical-scavenging activity showed a yellow-on-purple spot due to the discoloration of DPPH.

β -Carotene antioxidant activity assays

The assay was carried out according to the method of Cavin et al. (1998), where 2 mg of a dried extract were diluted with 1 mL of dissolvent, then an aliquot (20 μ L) of each dilution plant extract was carefully loaded individually onto the baseline of the thin layer chromatography plate 20 x 10, and the samples were allowed to dry. The mobile phase for the extracts (hexane, acetone and methanol) were the same as those mentioned above for the qualitative assays for DPPH. After drying, the plates were sprayed out with a solution of 0.05% solution of β -carotene in acetone and then the plates were exposed under a UV light (365 nm) for 1 h. A positive antioxidant reaction was considered to be present when a yellow smear appeared on a white background.

Quantitative evaluation of the free-radical scavenging

The methanolic extracts were measured in terms of their hydrogen

Table 1. Species, family and common names of the analyzed plants.

Code	Plant species	Family	Common name
1	<i>Licania arborea</i> Seem	Chysobalanaceae	Cacahuanché, cacahuaté, caña dulce, toposote
2	<i>Ficus involuta</i> Miq	Moraceae	Amate blanco
3	<i>Bunchosia cannesens</i>	Malphiaceae	Nanche de perro
4	<i>Syderoxylon capiri</i>	Sapotaceae	Capire
5	<i>Sapium macrocarpum</i> Müll Arg.	Euphorbiaceae	Palo verde, hincha huevos, venellino, lechon
6	<i>Ficus cotinifolia</i> H.B.&K	Moraceae	Texcalamate, copó, Alamo, higuierón Amate prieto
7	<i>Annona squamosa</i> L	Annonaceae	Chirimoyo, Texaltzapotl, Quantzapotl
8	<i>Vitex mollis</i>	Verbenaceae	Cuayotomate
9	<i>Piper leucophyllum</i> C.D.C	Piperaceae	Cordoncillo, Hoja santa
10	<i>Gliricidia sepium</i>	Fabaceae	Mata rata, Guerrero, Guaje, Cola de alacrán, Cacahuano Tecahuanché
11	<i>Astianthus viminalis</i> Baill	Bignoniaceae	Azochil, Axochitl, Ahuejotes, Flor de agua, Palo de agua
12	<i>Hamelia patens</i> Jacq	Rubiaceae	Coral, Trompetilla, Jicarillo
13	<i>Swietenia humilis</i> Zucc	Meliaceae	Zopilote, Cóbano, Palo de zopilote
14	<i>Stemmadenia bella</i> Miers	Apocynaceae	
15	<i>Rupechtiya fusca</i>	Polygonaceae	Guayabillo, Azulillo
16	<i>Bursera grandifolia</i> Engl	Burseraceae	Palo mulato
17	<i>Ziziphus amolle</i>	Rhamnaceae	Huixcolote, Limoncillo
18	<i>Jacaratia mexicana</i> ADC	Cariaceae	Bonete, Papayón
19	<i>Gyrocarpus jathrophifolius</i>	Hernandiaceae	Palomitas
20	<i>Pseudobombax ellipticum</i> HB&K	Bombacaceae	Rosal, clavelillo, Escibetillo
21	<i>Comocladia engleriana</i>	Anacardiaceae	Hincha huevos, Teclatia, Teclate, Teclatilla
22	<i>Plumeria rubra</i>	Apocynaceae	Cacaloxochitl, flor de mayo

donating or radical scavenging ability using the stable radical DPPH. One mL of a methanolic solution (200 mg L⁻¹) of antioxidant was placed in a tube to which 1 mL of a DPPH 3 x 10⁻⁵ M in DMSO was added. For controls, tubes with 1 mL of the extract plus 1 mL of DMSO were also prepared. Other controls have one mL of the DPPH solution plus 1 mL of DMSO, and also 2 mL of DMSO was also placed in a tube. The solutions were bubbled with nitrogen and they were left for 60 min at room temperature. Samples were read after the reaction time at 517 nm (Genesis Spectrophotometer Milton, Roy). Butylated hydroxyanisole (BHA), *tert*-butyl hydroxyquinone (TBHQ), Ferulic acid and Quercetin at 200 mg L⁻¹ were used as a reference for the radical scavenging method. The percentage of scavenging activity of the radical-DPPH was calculated using the formula described by Xiaojun et al. (1998):

$$\text{Scavenging activity (\%)} = [(A_i - A_j) / A_c] \times 100$$

where A_i = absorbance of the mixed extract with the DPPH solution, A_j = absorbance of the same extract mixed with DMSO, and A_c = absorbance of the DPPH solution with DMSO. All determinations were carried out in triplicates.

Determination of antioxidant activity with the β -carotene bleaching method

This experiment was conducted following the method described by Emmons (1999). The antioxidant capacity was measured by the ability of extracts to minimize the coupled oxidation of β -carotene and linoleic acid in an emulsified solution, which loses its orange color when reacting with the radicals. 30 mL of deionised water

were saturated with air for 30 min and then one ml of a β -carotene solution (20 mg/10 mL of chloroform) was added. One ml of this solution was placed in a tube, to which 20 mg of linoleic acid and 200 mg of Tween 80 were previously added. Chloroform was removed at 40°C under vacuum, and 50 mL of previously air saturated distilled water were added to form a stable emulsion. 5 mL of samples (200 mg L⁻¹) of the antioxidant were added to the tubes and were read at 470 nm ($T = 0$). Samples were incubated at 50°C in a water bath for 120 min and samples were read as before in a spectrophotometer (Genesis Spectrophotometer Milton, Roy). The control was prepared adding 0.3 mL of methanol in the β -carotene distilled water solution and volume was adjusted with 0.2 mL of methanol in 5 mL of distilled water. BHA, TBHQ, ferulic acid and quercetin at 200 mg L⁻¹ were used as a reference. Three repetitions were conducted for all samples. The antioxidant activity coefficient (AAC) in the extracts was calculated as earlier described by Miller et al. (1971) and Moure et al. (2000).

Total phenolic determination in the plant extracts (TPME)

The total phenolic content in plant methanolic extracts (TPME) was determined by the Folin-Ciocalteu colorimetric method. The concentration of total polyphenols from methanolic extracts was adjusted to 200 ppm in all the assays. The methanolic extract solution (1 mL) was mixed with 1 mL of the Folin-Ciocalteu reagent. After 3 min, 1 mL of saturated 35% (w/v) Na₂CO₃ was added to the mixture and distilled water was added until it reached 10 mL. The absorbance was measured at 760 nm (Genesis Spectrophotometer Milton, Roy) after 1 h of incubation at room temperature, and data were calculated using a pre-prepared gallic acid calibration curve. Values are

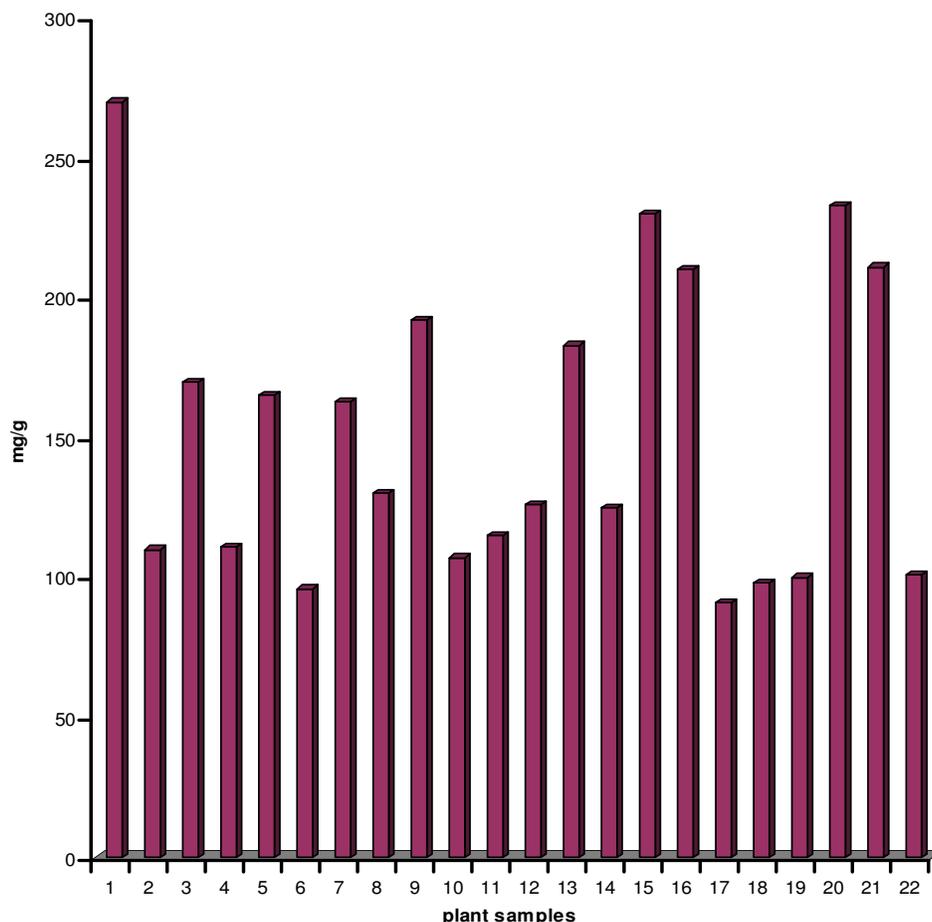


Figure 1. Total phenol content (mg/g) of methanolic plants extracts. 1: *L. arborea*, 2: *F. obtusifolia*, 3: *B. canesens*, 4: *S. capiri*, 5: *S. macrocarpum*, 6: *F. continifolia*, 7: *A. squamosa*, 8: *V. molli*, 9: *P. leucophyllum*, 10: *G. sepium*, 11: *A. humilis*, 12: *H. patens*, 13: *S. humilis*, 14: *S. bella*, 15: *R. fusca*, 16: *B. grandifolia*, 17: *Z. amolle*, 18: *J. mexicana*, 19: *G. jathrophifolius*, 20: *P. ellipticum*, 21: *C. egleriana*, and 22: *P. rubra*.

presented as the mean of triplicate analysis. Results are expressed as mg of gallic acid/g of extract (Singleton and Rossi, 1965).

RESULTS

Total phenol compounds in the plant extracts

Figure 1 shows that the highest total phenolic contents were found in the methanolic extracts of *L. arborea*, *B. canesens*, *S. capiri*, *A. squamosa*, *P. leucophyllum*, *S. humidis*, *R. fusca*, *B. grandifolia*, *P. ellipticum* and *C. egleriana*. The methanolic extract of *L. arborea* exhibited the highest total phenolic content (TPC) value.

Antioxidant activity

The lowest antioxidant activity was observed in the hexane plant extracts, but the extracts from plants of *L. arborea*, *B. grandifolia*, *Z. amolle*, *J. mexicana*, *G.*

jathrophifolius, *P. ellipticum* and *C. egleriana* had the highest radical scavenging results. The extracts obtained with acetone showed higher antioxidant activity than the hexane ones but this activity is still low if compared with that of the methanolic extracts. The extracts that had the highest antioxidant activity using acetone as a solvent were *L. arborea*, *S. macrocarpum*, *P. leucophyllum*, *B. grandifolia*, *Z. amolle*, *J. mexicana*, *G. jathrophifolius*, *P. ellipticum* and *C. egleriana*. The methanol extracts showed higher amount of phenolic compounds than those extracted with acetone and hexane. The extracts with the highest antioxidant activity were obtained from *L. arborea*, *S. macrocarpum*, *V. mollis*, *A. humilis*, *H. patens*, *S. humidis*, *S. bella*, *R. fusca*, *B. grandifolia*, *P. ellipticum* and *C. egleriana*.

Hexane extracts also showed the lowest antioxidant activity using the bleaching method with β -carotene on TLC plates and, among the hexane plant extracts, *L. arborea*, *B. grandifolia*, *Z. amolle*, *J. mexicana*, *G. jathro-*

Table 2. DPPH scavenging and bleaching with β -carotene from plants by TLC test.

Plant species	Spots Intensity ^a					
	DPPH ^b	β -Carotene ^b	DPPH ^c	β -Carotene ^c	DPPH ^d	β -Carotene ^d
<i>Licania arborea</i> Seem	+3 ^e	+3	+2	+1	+++4	+++3
<i>Ficus involuta</i> Miq	N ^f	N	N	N	N	N
<i>Bunchosia cannesens</i>	N	N	N	N	N	N
<i>Syderoxylon capiri</i>	N	N	N	N	+3	N
<i>Sapium macrocarpum</i> Müll Arg.	N	N	+2	+2	++2	++4
<i>Ficus cotinifolia</i> H.B.&K	N	N	N	N	N	N
<i>Annona squamosa</i> L	N	N	N	N	+2	N
<i>Vitex molli</i>	N	N	N	N	+++6	+++6
<i>Piper leucophyllum</i> C.D.C	+3	+1	+++3	++2	+1	+++2
<i>Gliricidia sepium</i>	N	N	N	N	N	N
<i>Astianthus viminalis</i> Baill	N	N	N	N	N	N
<i>Hamelia patens</i> Jacq	N	N	N	N	++5	+3
<i>Swietenia humilis</i> Zucc	N	N	N	N	+++6	+++5
<i>Stemmadenia bella</i> Miers	N	N	N	N	+++4	+++4
<i>Rupechtia fusca</i>	N	N	N	+2	+++5	+++4
<i>Bursera grandifolia</i> Engl	+4	+3	+2	+2	+++4	+++4
<i>Ziziphus amolle</i>	+3	+3	+2	+2	N	N
<i>Jacaratia mexicana</i> ADC	+4	+3	+1	+1	N	N
<i>Gyrocarpus jathrophifolius</i>	+4	+3	+1	+1	N	N
<i>Pseudobombax ellipticum</i> HB&K	+4	+3	+3	+4	+++6	+++3
<i>Comocladia engleriana</i>	+4	+3	++7	++4	+++6	+++4
<i>Plumeria rubra</i>	N	+1	+	N	N	N

^a+, Weak; ++, Intermediate; +++, strong.

^bSpots of the hexane extracts of the plants on TLC plates, developed by Hex-Ethyl acetate (9.5:0.5, v/v).

^cSpots of the acetone extracts of the plants on TLC plates, developed by Hex-Ethyl acetate(7:3, v/v).

^dSpots of the methanol extracts of the plants on TLC plates, developed by Ethyl acetate-Methanol(9:1, v/v), spray reagents DPPH and β -carotene.

^eNumber of spots.

^fNegative results.

phifolius, *P. ellipticum* and *C. engleriana* showed the highest activity. These results are in good agreement with the activities shown by some hexane extracts, using the DPPH assay. In the case of the acetone extracts, high values were observed in the plant extracts of *L. arborea*, *S. macrocarpum*, *P. leucophyllum*, *B. grandifolia*, *Z. amolle*, *J. mexicana*, *G. jathrophifolius*, *P. ellipticum* and *C. engleriana*; these results also coincided with those obtained with the DPPH scavenging activities (Table 2). The highest activity values were shown in the methanol extracts of *L. arborea*, *S. macrocarpum*, *V. mollis*, *A. humilis*, *H. patens*, *S. humidis*, *S. bella*, *R. fusca*, *B. grandifolia*, *P. ellipticum* and *C. engleriana*. The antioxidant activity of methanol extracts was higher than that of acetone and hexane extracts.

Figure 2 shows that the scavenging activities in the methanolic extracts were low in samples of *L. arborea*, *F. obtusifolia* and *A. squamosa* (47.5, 53.4, 49.7%, respectively), using the DPPH assay. The extracts obtained from *F. obtusifolia*, *B. canesens*, *F. continifolia*, *V. molli*, *A. viminalis*, *H. patens*, *S. bella*, *P. ellipticum* and *C.*

engleriana have an even higher activity than that showed by BHA. However, these activities are lower than those showed by ferulic acid and TBHQ.

Heat-induced oxidation of an aqueous emulsion system of β -carotene and linoleic acid was employed as an antioxidant test reaction. In this particular model, β -carotene undergoes rapid discoloration in the absence of an antioxidant. Methanolic extracts samples were evaluated at the final concentration of 200 ppm for the essays, and BHA, BHQ, ferulic acid and quercetin, synthetic and natural antioxidants, respectively, were compared under the same conditions. Figure 3 shows that the samples with higher activity than the reference compound (BHA) were *L. arborea*, *S. bella*, *B. grandifolia*, *P. ellipticum* and *C. engleriana*. It is also shown that the samples of *B. canesens*, *A. humilis*, *S. humidis*, *R. fusca* and *G. jathrophifolius* have low activity in relation to TBHQ, and higher if compared with quercetin, ferulic acid and BHA. *H. patens* had a similar activity to that showed by BHA, and higher than the commercial natural antioxidants. It is important to point out that 14% of the

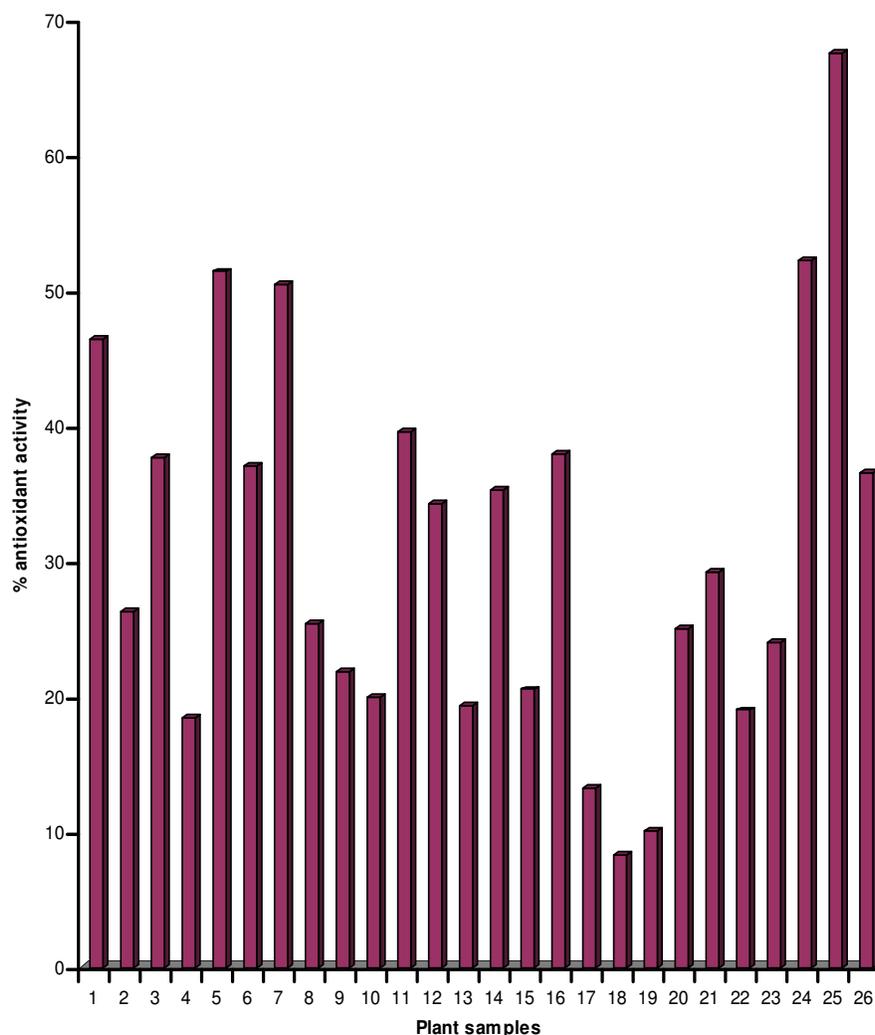


Figure 2. Percentage of the DPPH activity of methanolic plants extracts. 1: *L. arborea*, 2: *F. obtusifolia*, 3: *B. canesens*, 4: *S. capiri*, 5: *S. macrocarpum*, 6: *F. continifolia*, 7: *A. squamosa*, 8: *V. molli*, 9: *P. leucophyllum*, 10: *G. sepium*, 11: *A. humilis*, 12: *H. patens*, 13: *S. humilis*, 14: *S. bella*, 15: *R. fusca*, 16: *B. grandifolia*, 17: *Z. amolle*, 18: *J. mexicana*, 19: *G. jathrophifolius*, 20: *P. ellipticum*, 21: *C. egleriana*, and 22: *P. rubra*, 23: BHA, 24: TBHQ, 25: ferulic acid and 26: quercetin.

analysed plants had high antioxidant activities and 23% of all samples showed a significant antioxidant activity.

DISCUSSION

Among all the analyzed extracts, a significant total phenolic content, radical scavenging and antioxidant activity were found mainly in the plant extracts of *L. arborea*, *B. grandifolia*, *P. ellipticum* and *C. egleriana* obtained with hexane, acetone and methanol. In general, extracts with the highest radical scavenging and antioxidant activity showed the highest phenolic content, and an important relation was found among these parameters.

The qualitative antioxidant, the scavenging activities

and the total phenol obtained in the acetone and MeOH plant extracts may be related due to the presence of phenolic compounds such as flavonoides, tannins, coumarins, xanthones, procyanidins and benzoic and hydroxycinnamic acids, because these compounds contain an aromatic hydroxy moiety. Direct evidence of the mechanism of these extracts was not obtained in this study.

Most of the plant extracts showed relatively high total phenol values and the plant extracts of *L. arborea*, *B. grandifolia*, *P. ellipticum* and *C. egleriana* were among those which values were high. In general, extracts with high radical scavenging and antioxidant activities showed the highest phenolic content, and an important relation was found among these parameters. The plant extracts

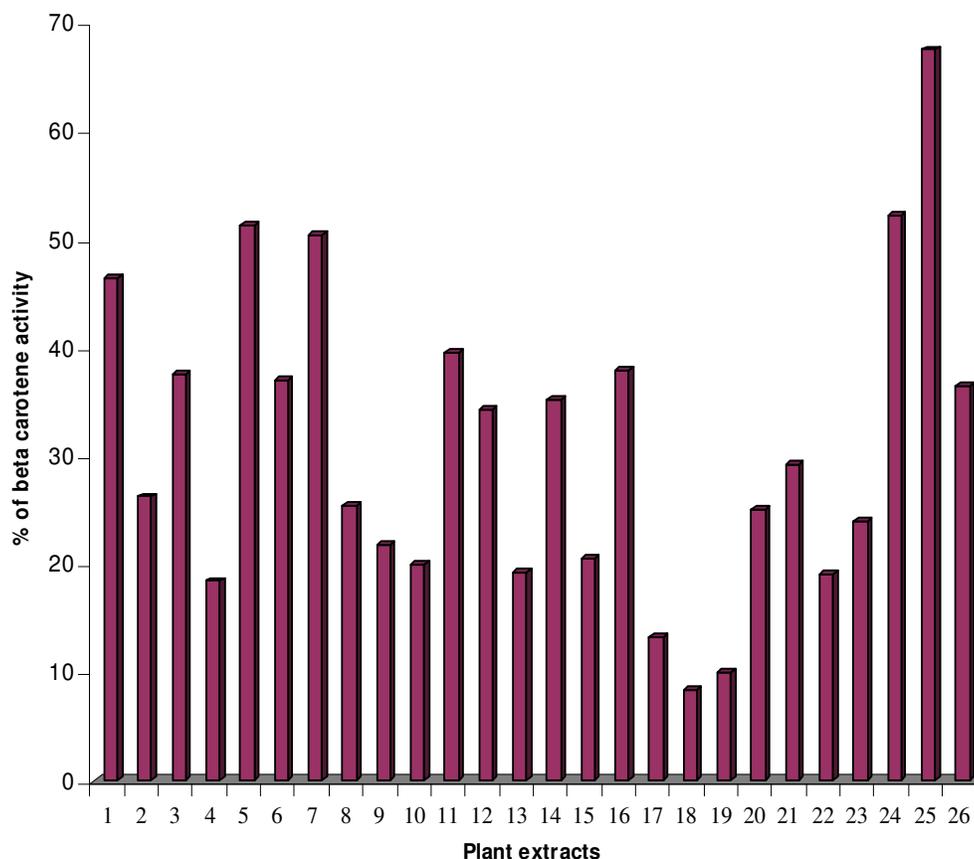


Figure 3. Percentage of the β -carotene activity of methanolic plants extracts. 1: *L. arborea*, 2: *F. obtusifolia*, 3: *B. canesens*, 4: *S. capiri*, 5: *S. macrocarpum*, 6: *F. continifolia*, 7: *A. squamosa*, 8: *V. molli*, 9: *P. leucophyllum*, 10: *G. sepium*, 11: *A. humilis*, 12: *H. patens*, 13: *S. humilis*, 14: *S. bella*, 15: *R. fusca*, 16: *B. grandifolia*, 17: *Z. amolle*, 18: *J. mexicana*, 19: *G. jathrophifolius*, 20: *P. ellipticum*, 21: *C. eglariana*, and 22: *P. rubra*, 23: BHA, 24: TBHQ, 25: ferulic acid and 26: quercetin.

that showed a relation between total phenolic content and high antioxidant activity were the methanolic plant extracts of *L. arborea*, *B. canesens*, *S. macrocarpum*, *A. squamosa*, *A. humilis*, *S. bella*, *B. grandifolia*, *P. ellipticum*, and *C. eglariana*. Only *L. arborea*, *B. grandifolia*, *P. ellipticum*, and *C. eglariana* exhibited the best results in the antioxidant and radical scavenging activities and the highest total phenol values. The plant extracts of *L. arborea*, *S. macrocarpum*, *A. squamosa* and *B. canesens* exhibited the highest antioxidant activity and the total phenol values, and the plant extracts of *L. arborea*, *B. canesens*, *S. macrocarpum*, *A. squamosa*, *A. humilis*, *B. grandifolia* and *C. eglariana* showed high scavenging activities and total phenol values.

Some of the methanolic extracts showed quite a strong antioxidant and radical scavenging activities; in some cases, even higher than the synthetic and natural antioxidant reference compounds. Methanolic extracts of the *L. arborea*, *S. macrocarpum* and *A. squamosa* showed similar values to those of TBHQ and ferulic acid and

higher than those of BHA and quercetin in the scavenging and antioxidant activity measured with β -carotene essays. Plants of the genera *Licania*, *Sapium* and *Annona* exhibited higher or similar values to the antioxidant and scavenging activities in relation to synthetic and natural antioxidants. The presence of the polyphenolic compounds in those genera has already been reported (De Oliveira et al., 1987; Ahmad et al., 1991; Braca et al., 2002).

The methanolic extracts had more polar components and these contributed towards the scavenging and antioxidant activities and total phenolic content. The antioxidant activities shown by MeOH extracts could be related to the presence of the polyphenolic compounds.

The plants that showed high antioxidant values have been used in some Mexican areas for different purposes as remedies, or even as food additives. Limonoids from the seeds of *Swietenia humilis* have been characterized (Jiménez et al., 1998). The leaves of *Piper leucophyllum* are used as a condiment in some traditional food in

México, and there are reports of antioxidant activity in plants of species of the same genera used in traditional medicine elsewhere (Heras et al., 1998; Velázquez et al., 2003). Some species of genera *Bursera* have been reported to have antimicrobial properties too (Camporese et al., 2003). Antibacterial, molluscicidal and cytotoxic activity was previously reported from *Plumeria rubra* in traditional medicinal plants from Thailand (Hamburger et al., 1991). Neuropharmacologic effects in rats have been shown by Morales et al. (2001) using extracts of *Gliricidia sepium*.

The results exhibited by *L. arborea* are related to the antioxidant activity shown by other species of this genus, such as *L. licaniaeflora*. Flavonoids, both glycosides and aglycones, have been isolated from many species of the genus *Licania* (Braca et al., 2002a,b). This study suggests that more-polar components present in these medicinal plants contributed towards their increased antioxidant activity. This study reveals that the Morelos flora can be a potential source of new antioxidants that could be used in a variety of fields. Although the active principles responsible for the antioxidant activity of the tested extracts have not been isolated in this work, its results are useful for further analysis.

Conclusion

From the twenty two plants studied in this work, the methanol extracts from *L. arborea*, *B. canesens*, *S. macrocarpum*, *A. squamosa*, *S. bella*, *B. grandifolia*, *P. ellipticum* and *C. eglariana* have antioxidant activities, and they could also be used as a source of natural antioxidants. Further pharmacological studies are underway to identify the active constituents of the plant extracts responsible for the showed activities.

REFERENCES

- Ahmad M, Jain N, Kamil M, Ilyas M (1991). 6-Hydroxykaempferol 7-rutinoside from leaves of *Sapium eugniaefolium*. *Phytochemistry*. 30: 2815-2816.
- Argueta A, Cano LM, Rodarte ME (1994). Atlas de las plantas de la medicina tradicional I y II. Instituto Nacional Indigenista, México, pp. 537-538.
- Braca A, Sortino C, Politi M, Morelli I, Mendez (2002a). Antioxidant activity of flavonoids from *Licania licaniaeflora* J. *Etnopharmacol*. 79: 379-381.
- Braca A, Luna D, Mendez J, Morelli I (2002b). Flavonoids from *Licania apetala* and *Licania licaniaeflora* (Chrysobalanaceae). *Biochem. Systematics Ecol*. 30: 271-273.
- Camporese A, Balick MJ, Arvigo R, Esposito RG, Morsellino N, De Simone F, Tubaro A (2003). Screening of anti-bacterial activity of medicinal plants from Belice (Central America) J. *Ethnopharmacol*. 87: 103-107.
- Cavin A, Hostettmann W, Dyatmyko W, Potterat O (1998). Antioxidant and lipophilic constituents of *Tinospora crispa*. *Planta Med*. 64: 393-396.
- De las Heras B, Slowing K, Benedí J, Carreto E, Ortega T, Toledo C, Bermejo P, Iglesias I, Abad MJ, Gómez-Serranillos P, Liso PA, Villar A, Chiriboga X (1998). Antiinflammatory and antioxidant activity of plants used in traditional medicine in Ecuador. *J. Ethnopharmacol*. 61: 161-166.
- De Oliveira AB, De Oliveira GG, Carazza F, Maia JGS (1987). Geovanine, a new azaanthracene alkaloid from *Annona ambotany aubl*. *Phytochemistry* 26: 2650-2651.
- Hamburger MO, Cordell GA, Ruangrunsi N (1991). Traditional medicinal plants of Thailand XVII Biologically active constituents of *Plumeria rubra*. *J. Ethnopharmacol*. 33: 289-292.
- Jiménez A, Villarreal C, Toscano RA, Cook M, Arnason T, Bye R, Mata R (1998). Limonoids from *Swietenia humilis* and *Guarea grandifolia* (Meliaceae). *Phytochemistry* 49: 1981-1988.
- Lin CC, Huang PC (2002). Antioxidant and hepatoprotective effects of *Acanthopanax senticosus*. *Phytother. Res*. 14: 489-494.
- Miller HE (1971). A simplified method for the evaluation of antioxidants. *J. Am. Oil Chem. Soc*. 48: 92-97.
- Monroy-Ortiz C, Castillo EP (2000). Plantas Medicinales Utilizadas en el Estado de Morelos, UAEM, México, Chapter 2.
- Morales-Cifuentes C, Gómez-Serranillos MP, Iglesias I, Villar del Fresno AM (2001). Neuropharmacological profile of ethnomedicinal plants of Guatemala J. *Ethnopharmacol*. 76: 223-228.
- Moure A, Franco D, Sineiro J, Domínguez H, Núñez JM, Lema MJ (2000). Evaluation of extracts from *Gevuina avellana* hulls as antioxidants. *J. Agric. Food Chem*. 48: 3890-3897.
- Perry EK, Pickering AT, Wang WW, Houghton PJ, Perru NS (1999). Medicinal plants and Alzheimer's disease :from ethnobotany to phytotherapy . *J. Pharm. Pharmacol*. 51: 527-534.
- Pietta P (2000). Flavonoids as antioxidants. *J. Nat. Prod*. pp. 1035-1042.
- Schinella GR, Tournier HA, Prieto JM, Mordujovich de Buschiazzo P, Ríos JL (2002). Antioxidant activity of antiinflammatory plant extracts. *Life Sci*. 70: 1023-1033.
- Singleton VL, Rossi JA (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Viticult* 16: 55-61.
- Takao T, Kitatani F, Watanabe N, Yagi A, Sakata K (1994). A simple screening method for antioxidants and isolation of several antioxidants produced by marine bacteria from fish and shellfish. *Bioscience. Biotechnol. Biochem*. 58: 1780-1783.
- Velázquez E, Tournier HA, Mordujovich de Buschiazzo P, Saavedra G, Schinella GR (2003). Antioxidant activity of Paraguayan plant extracts. *Fitoterapia* 74: 91-97.
- VanderJagt TJ, Ghattas R, VanderJagt DJ, Crossey M, Glew RH (2002). Comparison of the total antioxidant content of 30 widely used medicinal plants of New Mexico. *Life Sci*. 70: 1035-1040.
- Xiaojun Y, Tadahiro N, Xiao F (1998). Antioxidative activities in some common seaweeds. *Plants foods for Human Nutr*. 52: 253-262.