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Chemical composition, antioxidant activity and cytotoxicity of flower extracts from *Magnolia grandiflora* L. found in southeast Mexico

Composición química, actividad antioxidante y citotoxicidad de extractos de flores de *Magnolia grandiflora* L. encontradas en el sureste de México

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ABSTRACT

Some reports state that different structures of *M. grandiflora* contain bioactive components. Nonetheless, phytochemical studies reported that the extracted essential oils are chemically different and remarkably variable in their qualitative and quantitative compositions. Further studies on the Mexican *M. grandiflora* needs to be done. Thus, the research aimed to characterize a) the chemical composition, b) the antioxidant activity and c) cytotoxicity effect of two *M. grandiflora* flower extracts. The chemical composition was evaluated by phytochemical test followed by thin layer chromatography, UV-Vis and FTIR spectrophotometer analysis. The antioxidant activity of the flower extracts was measured by the free radical-scavenging activity (ABTS) and the stable radical of 2,2-diphenyl-1-picryl-hydrazyl (DPPH) methods, and the cytotoxicity by an *Artemia salina* bioassay. Water and ethyl flower extracts showed the presence of organic chromophores such as flavonoids. Both extracts (ethyl and water) demonstrated antioxidant activity by both ABTS (459.6 ± 8.5 and 274.2 ± 5.7 µmol TE/g, respectively) and DPPH (3210.4 ± 2.5 and 219.7 ± 0.9 µmol TE/g, respectively) methodologies, and non-cytotoxic activity (LC_{50}, µg/mL) (1285 ± 14 and 1116 ± 15, respectively). The water and ethyl extracts of *M. grandiflora* flowers found in southeast Mexico are a promissory source of chemical compounds with attributed biological activity according to the presented results.

Key words: Bioactive compounds; Biological activity; Medicinal plants.

RESUMEN

Algunos estudios afirman que diferentes estructuras de *M. grandiflora* contienen componentes bioactivos. No obstante, los estudios fitoquímicos indican que los aceites esenciales extraídos son químicamente diferentes y notablemente variables en sus composiciones cualitativas y cuantitativas. Es necesario realizar más estudios sobre *M. grandiflora* cultivada en México. Por ello, el objetivo de esta investigación fue caracterizar a) la composición química, b) la actividad antioxidante y c) el efecto citotóxico de dos extractos de flores de *M. grandiflora*. La composición química se evaluó mediante una prueba fitoquímica preliminar seguida de cromatografía en capa fina y análisis espectrofotométrico mediante UV-Vis y FTIR. La actividad antioxidante de los extractos de flores se midió por el método de la actividad reductora de radicales libres (ABTS) y el método de radicales estables de 2,2-difenil-1-picril-hidrazilo (DPPH) y la citotoxicidad por un bioensayo de *Artemia salina*. Los extractos acuoso y etílico de flores mostraron la presencia de cromóforos orgánicos como los flavonoides. Ambos extractos (etílico y acuoso) demostraron actividad antioxidante tanto por ABTS (459.6 ± 8.5 y 274.2 ± 5.7 µmol TE/g, respectivamente) y DPPH (3210.4 ± 2.5 y 219.7 ± 0.9 µmol TE/g, respectivamente); y actividad no citotóxica (CL50, µg/mL) (1285 ± 14 y 1116 ± 15, respectivamente). Los extractos acuosos y etílicos de las flores de *M. grandiflora* encontradas en el sureste de México son una fuente promisoria de compuestos químicos con actividad biológica atribuida por los resultados presentados.

Palabras clave: Actividad biológica; Compuestos bioactivos; Plantas medicinales

INTRODUCTION

The family Magnoliaceae consists of 12 genera with approximately 210 flowering plant species (Morshedlo *et al.*, 2017). *Magnolia grandiflora* is a genus distributed in tropical-subtropical regions on America and Asia as ornamental trees, with economic importance as a source of aromatic large cup-shaped flowers (Lee *et al.*, 2011; Farag and Al-Mandy, 2013). It has been used in American, Asian and Indian traditional medicine for centuries with attributed properties such as anxiety, nervous disturbance and pain controller, as well as antiseptic agent, diaphoretic, anti-inflammatory, antiseptic, and stimulant agent (Latif *et al.*, 2017; Ma *et al.*, 2020). In Mexican traditional medicine it has been used as treatment against epilepsy, spams, inflammatory and infertility diseases (Dominguez-Yescas and Vázquez-García, 2019).

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Many reports state that different plant structures of *M. grandiflora* contain active compounds such as sesquiterpenoides (Hong *et al.*, 2007), coumarins (Hussein and El-Anssary, 2019), phenylpropanoids (Cao *et al.*, 2021), lignans (Schühly *et al.*, 2009), glycosides (Wang *et al.*, 2019), alkaloids (Cho *et al.*, 2022), among others (Lim, 2014). These compounds have demonstrated biological activities such as antitumor (Chilampalli *et al.*, 2011), antimicrobial (Chang *et al.*, 1998), anti-inflammatory (Kim and Cho, 2008), anti-tyrosinase (Huang *et al.*, 2012), anti-allergic (Niitsuma *et al.*, 2001), cardioprotective (Ho and Hong, 2012) and antiviral (Lan *et al.*, 2012) activities. Moreover, in the cosmetic industry, *M. grandiflora* is used because of its anti-inflammatory and anti-acne activities exerted by its biphenols magnolol and honokiol active compounds (Mukherjee *et al.*, 2011).

Flowers of *M. grandiflora* are an important source of essential oils which are extracted with aqueous and alcoholic based techniques (Davé *et al.*, 2012). These volatile floral substances are mainly monoterpenoids, sesquiterpenoids and phenylpropanoids formed by the mevalonato-methyl erythritol phosphate (MEP) and shikimate pathways (Averesch and Krömer, 2018). The most common reported chemical constituents in *M. grandiflora* flowers are β-elemene, germacrene, bicyclogermacrene, D, β-elemene, (*E*)- *β*-ocimene, β-Caryophyllene, cyclocolorenone and geraniol (Baez *et al.*, 2012; Davé *et al.*, 2012; Lim, 2014; Morshedloo *et al.*, 2017). The antioxidant capacity exerted by many of these compounds was effective against cancerous cell proliferation without cytoxiticy (Li *et al.*, 2009; Farag and Al-Mahdy, 2013; Raut and Karuppayil, 2014).

Nonetheless, phytochemical studies reported that the extracted essential oils are chemically different and remarkably variable in their qualitative and quantitative compositions (Morshedloo *et al.*, 2017) due to methods of extraction, environmental conditions, developmental stages of flowers and genetic factors (Lim, 2014). Moreover, further studies regarding chemical compositions, antioxidant capacity and cytotoxicity of Mexican *M. grandiflora* flowers to evaluate its potential applications are necessary (Sánchez-Recillas *et al.*, 2014; Vázquez-García *et al.*, 2015). Hence, the objective of the present study was to characterize a) the chemical composition, b) the antioxidant activity and c) cytotoxicity effect of two *M. grandiflora* flower extracts.

MATERIALS AND METHODS

Plant material and chemicals

Flowers of *M. grandiflora* were collected during a rain period on June 2018 from cultivated trees in the Orizaba, Veracruz Faculty of Chemical Sciences at Universidad Veracruzana. The collected flowers were located on tomentose pedicels, erect, solitary, large, up to 20 cm in diameter. They had 6-12 white petals, narrowed at the base, and three sepals with a petaloid appearance, as well as both reproductive organs in the same flower. Guidelines on Good Agricultural and Harvesting Practices (GAP) for medicinal plants were followed (WHO, 2003). All the chemicals and solvents were obtained from Sigma-Aldrich (St. Louis, MO).

Plant extracts preparations

The *M. grandiflora* flowers were washed and then air-dried. The plant material was homogenized in a mortar and then sieved using a 3.2 mm sieve. In order to obtain 2 % (m/v) aqueous and ethyl extracts, 2 g of plant material were mixed with 100 mL of double distilled water (DDW) or 100 mL of ethyl alcohol (96 %), respectively. The aqueous extract was heated to boil for 10 min, filtered with qualitative filter paper disks (Munktell, grade 388), and then concentrated by lyiophilization for a week (lyophilizer, LABCONCO). The ethyl extract was hot continuous percolated over 72 h using Soxhlet apparatus, filtered with qualitative filter paper disks, and then concentrated under reduced pressure in a rotary evaporator (Buchi, RE111). The flower plant extracts were stored in amber flasks at 4 °C until use.

Preliminary phytochemistry of plant extracts

The preliminary phytochemical properties of the flower extracts were examined for the presence of alkaloids (Dragandroff´s reagent, Wagner´s reagent and Sonneschain´s reagent) flavonoids (Shinoda test and Zinc-Hydrochloride test), glycosides (Legal test, H_2SO_4 test and Borntrager`s test), saponins (Froth forming test), tannins ($FeCl_3$ test, Vanilin-Hydrocloride test and alkaline test) and triterpenoids (Liberman test, Salkowsky test and Noller test), as described by Okeulu and Chinwe (2001). All tests were performed in triplicate.

Qualitative analysis by thin layer chromatography

Thin layer chromatography (TLC) on analytical plates over 10 cm x 20 cm silica gel 60 (Art. 1.05641) was performed in order to separate the plant extracts metabolites. Different solvent systems with different polarities were prepared and TLC studies performed to select the solvent system for a better resolution. The methodology was performed as follows: 1) Flower extracts solutions were applied on 6 mm bands, 5 mm from the bottom, 12 mm from the left edge, 4 mm apart by means of Linomat IV (Camag) on pre-coated (0.25 mm layer, Merck) TLC plates by using capillary tubes, 2) TLC was developed in a crystal chamber using hexane-Ethyl acetate (5:5 and 2:8), 3) TLC plates were dried with Camag TLC plate heater at 110 °C for 20 min, and observed under ultra violet light at 254 and 365 nm respectively, 4) Then, they were sprayed with iodine and vanillin solutions as spraying reagents, 5) Finally, the development of color in separated bands was analyzed and expressed by its retention factor (R*f*) with the formula:

$$\mathrm{Rf} = \frac{\text{Distance travel by solute}}{\text{Distance travel by solvent}} \quad (1)$$

UV-VIS and FTIR spectroscopic analysis

Flower extracts were examined under visible and UV light for proximate analysis. For UV-VIS and FTIR spectrophotometer analysis (Karpagasundari and Kulothungan, 2014), the flower plant was centrifuged at 3000 rpm for 10 min and filtered through Whatman No. 1 filter paper with a high-pressure vacuum pump. The sample was diluted 1:4 with

the aqueous and methanol solvents respectively. The flower extracts were scanned within a wavelength range of 200-800 nm using a CARY 50 VARIAN (Amsterdam, The Netherlands) and the characteristic peaks were detected. The peak values from the UV-VIS and FTIR were recorded and the analysis repeated twice for the spectrum confirmation.

***In vitro* antioxidant activity**

The flower extracts of *M. grandiflora* were tested for *in vitro* antioxidant activity by the standard methods. The total phenols content was quantified by the Folin-Ciocalteu method (Singleton and Rossi, 1965). An aliquot (0.5 mL) of each diluted polar extract was mixed with deionized water (35 mL) and 2.5 mL of Folin-Ciocalteu reagent; after 3 min of incubation, a sodium carbonate solution (20 % in water) (5 mL) was added. The solution was incubated at 70 °C for 20 min and then adjusted to 50 mL with deionized water. The UV-VIS absorbance was read at 750 nm (CARY 50 VARIAN; Amsterdam, The Netherlands). The results were expressed as mg gallic acid equivalent per gram of dry weight (mg GAE/g DW). For calibration curve, gallic acid concentrations were used between 0.05 - 0.5 mg/mL ($R^2 = 0.99$).

The method described by Medda *et al.* (2021) with some modifications was used to quantify the ABTS radical scavenging activity. The $ABTS^+$ cation radical was produced by mixing ABTS stock solution (7 mM in water) and 2.45 mm potassium persulfate at a 1:0.5 ratio respectively, and stored in the dark at room temperature for at least 16 h before use. The ABTS radical was diluted with water until an absorbance at 734 nm reached a 0.7 value. The ABTS solution was prepared fresh before each analysis. Each flower extract (7.5 µL) was mixed with 1000 µL of ABTS diluted. The reaction mix was incubated for 30 min in the dark at room temperature and the absorbance was immediately read at 734 nm using a CARY 50 Scan UV143 Vis VARIAN spectrophotometer (Amsterdam, The Netherlands). The calibration curve was prepared using a range of 0 - 4 mM ($R^2 = 0.98$) of Trolox reagent. The radical scavenging activity was estimated by the decrease of absorbance and expressed as the Trolox equivalent (TEAC) ABTS per mL of essential oil or component as follows:

$$\%\ of\ ABTS\ inhibition = \frac{(Cabs - Sabs)}{Cabs}\ x\ 100 \qquad (2)$$

Where C_{abs} is the control absorbance at t = 0 (containing all reagents except the test compound), and S_{abs} is the sample absorbance of the test compound after 30 min. The results were expressed in µmol TE/g extract. All determinations were performed in triplicate. Antioxidant activity was expressed as IC_{50}, defined as the concentration of the test material required to cause a 50 % decrease in initial ABTS concentration.

The antioxidant activity of the flower extracts was also measured by the stable radical of 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (Brand-Williams *et al.*, 1995). DPPH radical solution (100 µM) in 80 % (v/v) aqueous methanol was prepared. Test samples were prepared by mixing each flower extract (10 µL) and DPPH solution (190 µL), mixed, and then incubating in the dark at 37 °C for 20 min. All tests were performed in triplicate, with vitamin E as a positive control. The absorbance values were measured at 517 nm against a methanol blank (CARY 50 Scan Uv143 Vis VARIAN; Amsterdam, The Netherlands). Trolox was used as a standard for calibration curve (range between 6 - 21 µM; $R^2 = 0.97$). The % of inhibition was calculated with the formula:

$$\%\ of\ DPPH\ inhibition = \frac{(C_{\mathrm{abs}} - S_{\mathrm{abs}})}{C_{\mathrm{abs}}}\ \mathrm{x}\ 100 \qquad (3)$$

Where C_{abs} is the control absorbance at t = 0 (containing all reagents except the test compound), and S_{abs} is the sample absorbance of the test compound after 20 min. The results were expressed in µmol Trolox equivalent for g extract (µmol TE/g extract). Antioxidant activity was expressed as IC_{50}, defined as the concentration of the test material required to cause a 50 % decrease in initial DPPH concentration.

Cytotoxicity bioassay

For determining the cytotoxicity of flower extracts, a brine shrimp (*Artemia salina)* lethality bioassay was carried out (Krishnaraju *et al.*, 2005). Brine shrimp were hatched using brine shrimp eggs in a conical flask (1 L) with sterile seawater (38 g/L, pH 8.5 adjusted with Na_2CO_3) with aeration. After 24 h, 15 mL of yeast solution 0.06 % was added to the conical flask as larvae feeding; 48 h after the egg's incubation, active nauplii free from eggshells were collected, counted and placed in each vial containing 4.5 mL of brine solution. After 24 h of exposure to different concentrations (1 - 5,000 µg/mL) of the flower plant extracts (in triplicate per dose), surviving larvae were counted. The lethality (%) was determined by comparing the mean surviving nauplii and control tubes. The LC_{50} values were obtained from the best-fit line plotted concentration vs percentage lethality. Potassium dichromate ($K_2Cr_2O_7$) was used as positive control in the bioassay. Sterile seawater was used as negative control.

Data treatment and statistical analysis

Data are expressed as the means of three biological replicates. Results of phenolic compounds, $ABTS^+$ and cytotoxicity determinations are expressed as the means and standard deviations of three replicates. When needed, results were compared using an analysis of variance (ANOVA) with STATISTICA software (StatSoft 10.0; Tulsa, Ok, USA). Significant difference Fisher test ($p \leq 0.05$) was used to compare means.

RESULTS AND DISCUSSION

Preliminary phytochemistry of flower extracts

In the preliminary phytochemical analysis of the flower extracts, the presence of alkaloids, flavonoids, tannins, glycosides, coumarins, quinones, sesquiterpene lactones, saponins, and triterpenoids were examined (Table 1). The re-

Table 1. Phytochemical tests to determine metabolites from water and ethyl extracts.
Tabla 1. Ensayos fitoquímicos para determinar metabolitos a partir extractos acuosos y etílicos.

Metabolites	Test	Water extract	Ethyl extract
Alkaloids	Dragandroff	-*	-
	Wagner	+	+
	Sonneschain	-	-
Flavonoids	Shinoda	-	-
	Zinc-Hydrochloride	+	++
Tannins	$FeCl_3$	++	+++
	Vanilin-Hydrocloride	-	-
	Alkaline	-	-
Glycosides	Borntrager	-	-
	Legal	-	-
	Baljet	-	-
Coumarins	Erlich	-	-
	NH_4OH	-	-
	NaOH	-	-
Quinones	NaOH	-	-
	H_2SO_4	-	-
Sesquiterpene lactones	Lactones	-	-
Saponins	Saponins	+	+
Triterpenoids	Liberman	-	-
	Salkowsky	-	+++

* The preliminary results of secondary metabolites recognition are expressed with the symbols (+) and (-), where (+ + +) indicates a fairly marked presence of the reaction and (-) indicates the absence of the metabolite.

sults of the preliminary phytochemical tests were compared in both extracts, differing only in two metabolites. Flavonoids and triterpenoids test showed marked presence on the ethyl extract. Positive results were obtained in the alkaloid reaction using Wagner's reagent for both samples. Serna-González and Guzman-Vazquez (2010) stated that alkaloids from Magnoliaceae family have relaxing properties. Meanwhile, the reaction for flavonoids and tannins indicates the presence of phenolic compounds which confirms a great variety of biological properties, such as antitoxic, antitumor, antiviral, antimicrobial, anti-inflammatory, antibacterial, antiallergic, fungicidal and insecticidal, among others (Tungmunnithum *et al*., 2018).

Positive results were obtained for saponins, presenting a moderate content of this metabolite, which has not been reported in studies previously carried out with *M. grandiflora*. As it is known, in the literature saponins may be associated with numerous biological activities that include anti-inflammatory, antibacterial, antifungal, and antiviral (Troisi *et al*., 2014). Although the presence in *M. grandiflora* is moderate, it could have an influence on its anti-inflammatory and antibacterial effects.

Triterpenoids test with the Salkowski reagent fairly marked positive (+++) with the ethyl extract. Triterpenes are from the family of terpenes, and there are previously reported sesquiterpenoids and triterpenes in *M. grandiflora* (Del Valle *et al*., 2004). This metabolite was only found in the alcoholic extract, which may indicate that has a higher affinity for alcohol. The previous situation also occurred when performing the glycosides test, giving only positive for the aqueous extract with the Baljet reaction, but negative results were obtained with the alcoholic extract. Glycosides have been used to treat congestive heart failure (Ávalos-García and Pérez-Urria, 2009). This result is related with the study carried out by Del Valle *et al.* (2004) entitled "Studies of *M. grandiflora* extracts on guinea pig heart muscle", which mentions that the crude extracts of leaves and petals of *M. grandiflora* have a positive inotropic effect for the heart, as well as a coronary vasodilation. According to this, in several regions of Mexico, *M. grandiflora* is known for its effect on diseases of the heart, which is one of his greatest recognitions in herbal medicine.

Qualitative analysis by thin layer chromatography

Thin-layer chromatography was performed in order to obtain a separation of metabolites present in the flower extracts of *M. grandiflora*. Hexane: Ethyl acetate was used as the mobile phase, at different concentrations (5:5 and 2:8). Each separate analyte was marked and the distance traveled by each one was measured to calculate its Rf. The values obtained from both extracts are shown in Table 2. The preliminary results of the RF at the 5:5 dilution indicate the possible presence of one isolated analyte, and at the 2:8 dilution the presence of two isolated analytes in both extracts is shown, corroborated with the three repetitions performed, since there is not much difference between their Rf. These 3 different metabolites are due to the fact that the dilutions are of different polarity. The 2:8 dilutions are more polar than the 5:5 dilutions. Thus, we can observe the separation of more analytes. The more retained analytes near the origin tend to be of higher polarity since they are fixedly adsorbed to the active centers of the stationary phase, in this case silica gel, whereas the nonpolar ones will elute more easily (Sherma and Fried, 2003). The advantage of thin-layer chromatography as a preliminary analysis technique for extracts should be highlighted, since it gives us an idea of their composition (Qu *et al*., 2018).

UV-VIS and FTIR spectroscopic analysis

The UV-VIS analysis was performed to identify phytoconstituents present in ethyl and water extracts of *M. grandiflora*. The mentioned analysis was performed to identify the chemical compounds that contains σ-bonds, π-bonds and lone pair of electrons, chromophores and aromatic rings. The qualitative UV-VIS profile of both *M. grandiflora* extracts were determined between the wavelengths of 200 nm and 800 nm, in order to obtain a proper baseline and due to the peaks and sharpness. The performed analysis of ethyl extract showed peaks at 260 nm and 360 nm respectively (Figure 1a).

Table 2. Qualitative analysis by thin layer chromatography results of the Rf obtained both solutions.
Tabla 2. Análisis cualitativo por cromatografía en capa fina de los resultados de la Rf obtenida de ambas soluciones.

2:8 Dilution		Distance (cm)	Rf*
Ethyl extract	A1	1.5	0.254
	A2	1.7	0.309
	A3	1.7	0.309
	B1	4	0.678
	B2	4.7	0.855
	B3	4.8	0.873
Water extract	A1	1.4	0.237
	A2	1.7	0.309
	A3	1.9	0.345
	B1	4.1	0.695
	B2	4.8	0.873
	B3	4.8	0.873

5:5 Dilution		Distance (cm)	Rf
Ethyl extract	A1	2.4	0.421
	A2	4.1	0.745
	A3	4.1	0.745
Water extract	A1	3.2	0.582
	A2	3.9	0.709
	A3	3.9	0.709

* The retention factor (RF) is the result of (a) the distance of flower extract divided by (b) the distance of the eluent over the thin layer chromatography.

* El factor de retención (RF) es el resultado de (a) la distancia del extracto floral dividida por (b) la distancia del eluyente sobre la cromatografía en capa fina.

While the performed analysis of water extract showed peaks at 240 nm, 260 nm and 360 nm respectively (Figure 1b). The absorption spectrum of the *M. grandiflora* flower extract from the 400 nm to 800 nm region was not detectable.

In this analysis the peaks of absorbance at 200 to 400 nm are correlated to the presence of unsaturated groups and heteroatoms such as O, S, N (Njoku *et al.*, 2013). In both extracts, the spectrum shows many peaks from 300 nm to 400 nm which confirms the presence of organic chromophores within the *M. grandiflora* flower extracts. Nonetheless, the UV-VIS analysis has some limitations by the inherent difficulties to record the peaks to any particular constituents in the system. The UV-VIS results should be complemented with any other analytical technique such as FTIR and GC/MS techniques to a proper extract characterization and constituent identification (Karpagasundari and Kulothungan, 2014).

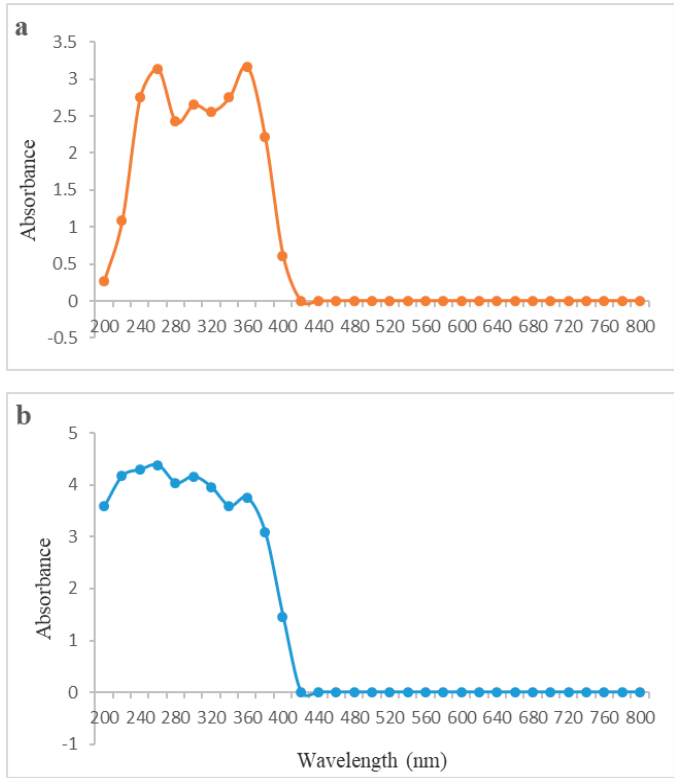

Figure 1. UV-VIS wavelength absorbance analysis of *M. grandiflora* flower extracts. a) ethyl extract; b) water extract.
Figura 1. Análisis de absorbancia de longitud de onda UV-VIS de extractos de flores de *M. grandiflora*. a) extracto de etílico; b) extracto acuoso.

The FTIR spectrum was performed to identify the functional group of the active components based on the peak absorbance detected at the infrared radiation. The FTIR spectrum of the *M. grandiflora* flower extracts in the form of wave numbers (cm^{-1}) are shown in table 3. The wave absorption numbers at 3,348.22 and 3,428.32 are due to the stretching hydroxyl groups (Pramila *et al.*, 2012). The band at 2,930.02 is due to symmetric stretching of saturated (sp^3) carbon (Karpagasundari and Kulothungan, 2014). The bands at 1,579.98 and 1,629.78 are assigned to the bending mode of absorbed water, since plant extracts even ethyl extracts are known to have a strong affinity for water (Oliveira *et al.*, 2016). The bands at 1,550.02 and 1,540.02 are due to C=C stretching related to the aromatic structure of both extracts (Al-Shareefi *et al.*, 2019). The vibrational absorption band at 1,402.22 was related to rocking of methyl group (Ashokkumar *et al.*, 2014). A band at 1,253.97 was related to C-O stretching (Oliveira *et al.*, 2016). The bands at 577.97 and 587.97 were due to the aromatic ring out of plane bending (Carballo *et al.*, 2008).

Table 3. Structural features of *M. grandiflora* flower extracts by FTIR.
Tabla 3. Características de la estructura de extractos de flores de *M. grandiflora* por FTIR.

Ethyl extract	Wave numbers (cm^{-1})	Assignments
	3,348.22	-OH stretch
	1,579.98	Alkene C=C
	1,550.02	C=C stretching
	577.97	Aromatic ring
Water extract		
	3,428.32	-OH stretch
	2,930.02	C-H stretch
	1,629.78	Alkene C=C
	1,540.02	C=C stretching
	1,402.22	C-H bending
	1,253.97	C-O stretch
	587.97	Aromatic ring

In vitro antioxidant activity

Extracts of aromatic plants are known to have antioxidant properties, thus this offers the possibility of being used as natural preservatives for food and cosmetics (Bendif *et al.*, 2017). In the present study, the antioxidant activity of *M. grandiflora* flower extracts was investigated (Table 4). In general, the antioxidant activity of plant extracts is primarily due to phenolic compounds and, in *M. grandiflora* flower extracts, the presence of different groups of phenolic compounds such as phenolic acids, flavonoids, and diterpenoids, are responsible for the observed antioxidant properties (Elansary *et al.*, 2019). The total phenolic content (TPC) (expressed as gallic acid equivalents) is often used as an approximately measurement of the antioxidant power of a plant extract (Wu *et al.*, 2018). The TPC values reported for both *M. grandiflora* flower extracts, which were determined by the Folin-Ciocalteu assay, are also reported in table 4. From the obtained data, slight differences in TPC values can be observed between water and ethyl extracts, with the former exhibiting the highest TPC. These results are consistent with the concentrations of the major chemical compounds showed in table 1.

The flower extracts showed moderate antioxidant activity as reported in all the assays where Trolox was used as reference. The greater antioxidant activity observed in ethyl *M. grandiflora* flower extracts could be attributed to their phytochemical properties (Tables 1-3) and/or to their synergistic effects (Garza *et al.*, 2019). Elansary *et al.* (2019), also concluded that *Magnolia acuminata* had the highest antioxidant activity in comparison with other plant extracts; which was attributed to phytochemicals such as catechin and catechin derivatives.

The antioxidant activity of the flower extracts was measured by the ABTS and DPPH methods. The relatively stable nitrogen-centered free radical DPPH is ubiquitously used to measure the scavenging ability of different phytochemicals, extracts or essential oils and their antioxidant

Table 4. Antioxidant activity of *M. grandiflora* ethyl and water flower extracts.
Tabla 4. Actividad antioxidante de extractos etílicos y acuosos de flores de *M. grandiflora*.

Extracts	Polyphenols (mg GAE/g dw)	ABTS		DPPH	
		TEAC* (µmol TE/g)	IC50# (µg/mL)	TEAC* (µmol TE/g)	IC50# (µg/mL)
Ethyl	46.8 ± 3.2[a‡]	459.6 ± 8.5[a]	39.4 ± 1.5[b]	3,210.4 ± 2.5[a]	54.5 ± 1.1[b]
Water	34.3 ± 1.3[b]	274.2 ± 5.7[b]	51.8 ± 0.9[a]	219.7 ± 0.9[b]	63.3 ± 0.4[a]
Control			3.8 ± 0.4[c]		3.02 ± 0.2[c]

*TEAC: Trolox equivalent (TE) antioxidant concentration.
#IC_{50}: The concentration giving a reduction of 50 %.
‡Each value is the mean of three replicates ± Standard deviation. Different letters mean significant difference (Fisher, $p \leq 0.05$).

*TEAC: Concentración antioxidante de Equivalentes Trolox (TE).
#IC_{50}: La concentración necesaria para reducir el 50 %.
‡Cada valor es la media de tres repeticiones ± Desviación estándar. Letras diferentes significan diferencia significativa (Fisher, $p \leq 0$ .05).

effects on DPPH radical depend on their hydrogen donating ability (Dastmalchi *et al.*, 2007). The water extract of *M. grandiflora* flowers reduced the concentration of DPPH by 72.09 ± 6 % with and efficacy higher than that of the ethanolic extract (63.5 ± 7 %). Vitamin E, included as positive control in the assay, showed the greatest ability to scavenge the DPPH free radical (88 ± 8 %) at the same test concentrations of 5 mg/mL. As previously mentioned, both flower *M. grandiflora* extracts have mainly components such as terpenes and phenolic compounds, and it can be assumed that these are responsible for the high percentage of ABTS and DPPH inhibition (Yoon, 2014). Moreover, flower extracts from other Magnoliaceae species have chemical antioxidant compound such as honokiol and magnolol (Yang *et al.*, 2018).

Cytotoxicity bioassay

A brine shrimp (*Artemia salina)* lethality bioassay was carried out to determine the cytotoxicity of flower extracts. Table 5 shows the LC_{50} of the flower *M. grandiflora* extracts. Both, water and ethyl extracts, did not show toxicity with LC_{50} higher than 1,000 µg/mL, unlike control, as expected, considered as cytotoxic due to a LC_{50} lower than 100 µg/mL (Yadav and Mohite, 2020). There is information about cyto-

Table 5. Cytotoxicity test by lethality brine shrimp (*Artemia salina)* bioassay.
Tabla 5. Prueba de citotoxicidad por bioensayo de letalidad en camarones en salmuera (*Artemia salina*).

Flower plant extract	LC_{50} (µg/mL)
Water extract	1,116 ± 15[b*]
Ethyl extract	1,285 ± 14[a]
Control (+)	10.3 ± 1.7[c]
Negative (-)	0

LC_{50}: Lethal concentration to kill 50 % of brine shrimp. $LC_{50} < 100$ µg/mL means toxicity level. *Each value is the mean of three replicates ± Standard deviation. Different letters mean significant difference (Fisher, $p \leq 0.05$).

DL_{50}: Concentración letal para matar el 50 % de las artemias. $DL_{50} < 100$ µg mL^{-1} significa un nivel de toxicidad. *Cada valor es la media de tres repeticiones ± Desviación estándar. Letras diferentes significan diferencia significativa (Fisher, $p \leq 0.05$).\s\s

toxicity test of *M. grandiflora* flower extracts carried out by *Artemia salina* bioassays. Nonetheless, Martínez-Báez *et al.* (2016) found a moderate cytotoxicity with a LC_{50} of 400 µg/mL, in a methanolic *M. grandiflora* plant extract. In our results, the non-cytotoxicity of both flower extracts could be related to some phenolic compounds such as magnolol which has cytoprotective activity (Zhang *et al.*, 2019).

CONCLUSIONS

The preliminary phytochemical test demonstrated the presence mainly of flavonoids, terpenes, tannins and alkaloids in both extracts. The thin layer chromatography, the UV-Vis and FTIR spectroscopic analyses showed the presence of organic chromophores such as flavonoids. The water and ethyl flower extracts showed antioxidant and non-cytotoxic activity. The water and ethyl extracts of *M. grandiflora* flowers found in southeast Mexico are a promissory source of chemical compounds with the attributed biological activities by the presented results. Nonetheless, ethyl extracts exerted more antioxidant activity. *M. grandiflora* flower extracts could be used in medicinal and cosmetic industries by their exerted chemical antioxidant properties and by their non-cytotoxic effects.

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