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ORIGINAL ARTICLE

Vasorelaxant and Antioxidant Activity of Some Medicinal Plants from Campeche, Mexico

Francisco Javier Aguirre Crespo, Elias Cerino Pérez, Janice D. G. Valdovinos Estrella, Maria G. Maldonado Velazquez, Benjamin O. Ortega Morales[1], Pedro Zamora Crecencio[2], Emanuel Hernández Nuñez[3,4], Samuel E. Estrada Soto[5]

Faculty of Chemical Biological Sciences, [1]Department of Environmental Microbiology and Biotechnology, [2]Historical and Social Research Center, Autonomous University of Campeche, Campeche, [3]Department of Marine Resources, Center for Research and Advanced Studies Merida, Yucatan, [4]National Council for Science and Technology, [5]Pharmacy Faculty, Autonomous University of Morelos State, Cuernavaca, Morelos, Mexico

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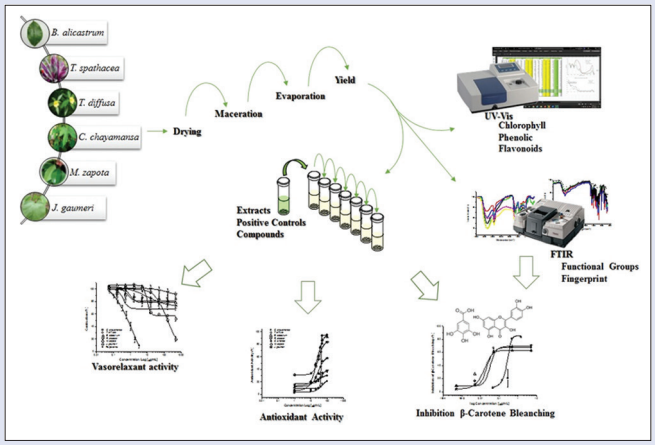
ABSTRACT

Context: *Brosimum alicastrum, Cnidoscolus chayamansa, Tradescantia spathacea, Turnera diffusa, Manilkara zapota,* and *Jatropha gaumeri* are medicinal plants recognized in Mexican Mayan Culture. **Aim:** Methanol leaves extracts of these plants were use as raw material to develop a phytochemical, spectroscopy, and pharmacological analysis. **Subjects and Methods:** Methanol maceration was carried out and were compared in terms of yield extraction, chlorophyll, simple phenolic and flavonoids content, antioxidant activity (DPPH and β-Carotene bleaching models), as well as isolated aorta rings (E+), precontracted with noradrenaline. **Results:** Best content of simple phenolic and flavonoids compounds was recorder in *B. alicastrum, J. gaumeri* and *T. diffusa. J. gaumeri* extract exert an antioxidant (β-carotene bleaching: EC_{50}: 0.8 ± 0.1 μg/mL, E_{max}: 85.7% ± 0.4%; DPPH: EC_{50}: 60.3 ± 1.8 μg/mL, E_{max}: 60.4% ± 1.8%; $P < 0.05$) and vasorelaxant (EC_{50}: 161.61 ± 7.45 μg/mL; E_{max}: 79.71% ± 3.88%; $P < 0.05$) activity in a concentration dependent-manner. Fourier transform infrared spectroscopy analysis allowed estimating a 1.26 and 2.28% of quercetin (Q) and gallic acid (GA) in *J. gaumeri*. GA exerts antioxidant activity in DPPH model (EC_{50}: 1.6 ± 0.2 μg/mL; E_{max}: 92.9% ± 3.3%) and Q/GA (1:2) mixture improves inhibition of β-carotene bleaching (EC_{50}: 0.005 ± 0.005 μg/mL; E_{max}: 69.2% ± 0.7%; $P < 0.05$). **Conclusion:** *J. gaumeri* is a medicinal plant employed in Mayan traditional medicine and GA and Q could be related to traditional uses, as well as responsible for the pharmacological effects. GA and Q interactions improve inhibition β-Carotene bleaching activity, which suggests greater solubility in lipophilic systems and potential interactions at the plasma membrane level.

Key words: Antioxidant, gallic acid, *Jatropha gaumeri,* quercetin, vasorelaxant

SUMMARY

- Methanol extracts of *Brosimum alicastrum, Cnidoscolus chayamansa, Tradescantia spathacea, Turnera diffusa, Manilkara zapota* and *Jatropha gaumeri* exerts vasorelaxant and antioxidant activity in a Concentration-Dependent Manner. Fourier transform infrared spectroscopy analysis allows the identification of Q and GA in *J. gaumeri* extract. Q/GA (1:2) mix enhances the antioxidant activity in lipophilic environment.



Abbreviations used: DPPH: 1,1diphenyl2picrylhydrazyl; E+: Aorta with endothelium; EC_{50}: Half-maximal effective concentration; E_{max}: Maximum response achievable; FTIR: Fourier transform infrared spectroscopy; Q: Quercetin; GA: Gallic acid; NA: Noradrenaline; DMSO: Dimethylsulfoxide; BHT: Dibutylhydroxytoluene; FC: Folin-ciocalteu reagent; Chl_{TOT}: Total chlorophyll content; βE: β-Carotene emulsion; PLS: Partial least-square; CRC: Concentration-response curve; ANOVA: Analysis of variance; AUC: Area under curve; ROS: Reactive oxygen species; RNS: Reactive nitrogen species.

Correspondence:

Dr. Francisco Javier Aguirre Crespo,
Av. Agustín Melgar S/N entre Calle 20 y Juan de la Barrera, Col. Buenavista, CP 24039, San Francisco de Campeche, Campeche, México.
E-mail: fjaguirr@uacam.mx

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INTRODUCTION

Mexican healthcare agency reports that one-third of deaths correspond to diabetes mellitus, ischemic heart disease, and cerebrovascular diseases.[1] The Mexican National Health Survey 2016 reports a prevalence of hypertension of 25.5%, of these, 40% were unaware of having hypertension. On the other hand, 79.3% of hypertensive adults diagnosed are under pharmacological treatment but only 45.6% are under control.[2]

Hypertension depends of volume ejected by the heart into the arteries, the elastance of arteries and the rate of blood flow.[3] In hypertension, endothelium relaxant or contraction soluble factors regulate vascular tone and alterations are associated with morphological and functional alterations of the endothelium.[4]

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In addition, oxidative stress increases arterial stiffness and it is associated with arterial remodeling.[5] It should be noted that lipid peroxidation is enhanced in hypertensive patients.[6]

Physiological stress are involved in the generation of oxidative lesions, metabolic disorders, and the development of chronic degenerative diseases.[7] In the vascular system, superoxide anion (O_2^-) determines the biosynthesis and bioavailability of nitric oxide (NO) and together with hydrogen peroxide (H_2O_2) regulates the functionality of the vascular system. Reactive species play an important role in the pathophysiology of arterial hypertension.[8]

One of the worldwide research lines is focusing on the research and development of synthetic and/or natural antioxidant agents,[9] which allows the generation of prophylactic and/or therapeutic options to physiological stress associated with free radicals, among other conditions. In this sense, chemical entities present in medicinal plants could have beneficial effects by reducing oxidative stress and simultaneously favoring physiological functions such as relaxation of vascular smooth muscle. In Mexico, herbal medicine is recognized as part of Traditional Medicine and is used in the maintenance and reestablishment of health as well as in the improvement of quality of life;[10] however, few documents support the safety and efficacy of traditional use. In this context, the current research was carried out to screen the antioxidant and vasorelaxant properties of *Brosimum alicastrum* Sw. (*Moraceae*), *Cnidoscolus chayamansa* McVaugh (*Euphorbiaceae*), *Tradescantia spathacea* Sw. (*Commelinaceae*), *Turnera diffusa* Willd ex Schult (*Turneraceae*), *Manilkara zapota* (L.) P. Royen (*Sapotaceae)* and *Jatropha gaumeri* Greenm (*Euphorbiaceae*) to establish bases for the systematic search of chemical entities with potential applications to physiological stress and hypertensive diseases.

SUBJECTS AND METHODS

Chemical and drugs

Noradrenaline (NA), papaverine, dimethylsulfoxide (DMSO), 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, quercetin, dibutylhydroxytoluene (BHT), β-carotene, linoleic acid, Tween 40, and Folin Ciocalteu reagent (FC) were purchased from Sigma-Aldrich Co (St. Louis, MO, USA). All other reagents were analytical grade from local sources. Every day, extract solutions were made using distilled water.

Plant material

Plant species were select using an ethnomedical criterion. Leaves of *B. alicastrum, C. chayamansa, T. spathacea, T. diffusa, M. zapota*, and *J. gaumeri* were employed. Table 1 lists voucher numbers, ethnomedical, phytochemical and pharmacological aspects of each species. All species were collected in June-2017 in San Francisco de Campeche, Campeche, México and voucher specimen were deposit in University Herbarium. Plant material dry at room temperature under shade; later it was ground and stored in hermetic plastic bags (Ziploc®).

Extraction

Crude extracts were prepared by maceration and were carried out with a constant mass/volume ratio (2:28), time (72 h periods), and temperature (T: 25°C). Methanol extracts were recovered, filter, concentrated under reduce pressure (Buchi® Rotaevaporator) and store in the refrigerator for further analysis. Three independent experiments were performed with three replicates each ($n = 9$).

Determination of chlorophyll content

Chlorophyll estimation was calculated by recording the absorbance at λ = 645 and 663 nm as well as the use of the formula Chl_{TOT} (μg/mL): 20.2 (A_{645}) +8.02 (A_{663}), previously described.[47] Each evaluated sample was subjected to three-independent experiments with three replicates each.

Determination of simple phenolic content

From GA (1.04–8.2 μg/mL) or stock extract solution (10 μg/mL) 0.4 mL were transferred 0.4 mL of FC reagent in 1 mL of distilled and incubated for 20 min in darkness. The reaction was stopped with Na_2CO_3 10% (1.6 mL) and the absorbance was recorded at 765 nm. The simple phenolic content was expressed as equivalents (mg/g) of GA present in the plant material.[48]

Determination of the flavonoid content

From a Q (1–10 μg/mL) or extract stock solution (10 mg/mL), 0.3 mL were add to 0.9 mL of MeOH, 0.15 mL $AlCl_3$10%, 0.15 mL of CH_3CO_2 K 1M and 1.8 mL of distilled water. Mixtures were incubated for 30 min at room temperature and in dark conditions and absorbance was read at 415 nm. The content of flavonoids in the aqueous extracts is expressed as equivalents (mg/g) of quercetin present in leaves of each medicinal plant.[48]

Antioxidant activity (1,1-diphenyl-2-picrylhydrazyl)

From extract stock solution (1 mg/mL), serial dilutions were made until reaching final concentrations (1 → 100 μg/mL). For this, 200 μL of each concentration was added to 1.8 mL of DPPH 0.1M in MeOH; they were mixed and incubated for 30 min in the dark and then, the absorbance (λ = 517 nm) was recorded. Methanol solutions of *C. sinensis* (1–50 μg/mL; positive control), GA, Q and Q/GA (0.03–32 μg/mL) were used. The percentage of remaining of DPPH was calculated using the formula:

$$\%DPPH = 1 - \frac{A_{sample\ t=30}}{A_{DPPH\ 0.1M\ t=0}} \times *100$$.[49] Finally, the use of a non-linear model was employed to determine the potency (EC_{50}, [μg/mL]) and efficacy (E_{max}, [%]) of the antioxidant activity exerted by the extracts. Each evaluated sample was subject to three independent experiments with three replicates each.

β-Carotene bleaching test

To obtain β-Carotene emulsion (βE), 1 mg of β-Carotene were dissolved in 5 mL of chloroform and 1 mL was added to 25 μL of linoleic acid with 20 μL of Tween 40; chloroform was evaporated at 40°C, 50 mL of pure water was added and shake (βE).[50] 0.3 mL of methanolic samples were added to 2.5 mL of βE to reach 0.05 → 1.5 μg/mL for BHT, 0.05 → 280 μg/mL for extracts and 0.0005 → 5.0 μg/mL for GA, Q and (Q/GA; 1:2); methanol was use as a blank sample. Absorbencies were measure at 492 nm each 15 min during 2 h. Antioxidant activity was given by the equation $([A_A - A_B]/[A_{B0} - A_{B120}]) \times 100$. A_A and A_B are the absorbencies of the test samples at each time and blank sample at 120 min, respectively, and $A_{B'0}$ and $A_{B'120}$ is the absorbance of the blank sample at $t = 0$ and $t = 120$ min, respectively. For each concentration, degradation rate was recorded and then potency and efficacy of inhibition of β-Carotene bleaching was calculated using a non-linear model. Six independent experiments were carried out on each sample evaluated.

Vasorelaxant activity

The experimental procedures were developed in concordance with recommendations of NOM-062-ZOO-1999.[51] For this, rats (*Rattus norvergicus*; male, Wistar, 275 ± 25 g) were maintained in a cycle of 12 h light/dark at 25°C; food and water consumption were *ad libitum*. The abdominal dissection allowed obtain in the thoracic aorta, it was clean of the adjacent tissue and kept in the Ringer-Krebs-Henselit solution.

Table 1: Botanical and common names of the selected plants, voucher and their ethnobotanical, phytochemical and pharmacological studies

Specie* (family) voucher	Commun and maya names	Parts employed	Traditional uses	Secondary metabolites	Pharmacological reports	Reference
Brosimum alicastrum Sw. Moraceae) 19329	Ramón, *k'an oox, xichxichcuy*	Seeds, bark, leaf	Asthma, tuberculosis, kidney diseases, hypoglicemic	Gallic acid, vanillic acid, caffeic acid, *p*-hydroxybenzoic acid, p-cumaric acid, (-)-epicatechin, starch	Hypoglysemic	[11-14]
Tradescantia spathacea Sw. (Commelinaceae) 19187	Maguey morado matlali, zopilotera	Leaf, latex	Ulcers, wounds, cancer, dysentery, headache, asthma, cough, intestinal infections, inflammation	Kaempferol, quercetin, isoquercetin, luteolin 5-glucoside, rutin, hexadecanoic acid, 1,12 octadecanoic acid, sitosterol, stigmasterol, 4- (2,4-dihydroxy-phenyl) -5-hydroxy-5H-furan-2-one), reonina	Antimutagenic, antiviral, antimicrobial	[15-20]
Turnera diffusa Willd ex Schult (Tumeraceae) 10532	Damiana *ajkits, misibkook*	Laef Branches	Anemia, bronchitis, cough, diabetes, fever, mycosis, gastrointestinal, respiratory and skin diseases	Tetrafilin B, arbutin, gonzalitosin I, damianina, tricosan-2-one, hexacosane, a-pinene, β-pinene, p-cymene, 1,8-cineole, sitosterol, apigenin-7-O-6-paracumaroil-glucoside	Anxiolytic, antibacterial, antidiabetic, antioxidant, antiobesity, antispasmodic, citotoxic, gastroprotector, hepatoprotector, enzyme inhibitor	[21,22]
Cnidoscolus chayamansa McVaugh (Euphorbiaceae) 10532	Chaya *chaykol, xchay.*	Leaf Latex	Diuretic, anti-inflammatory, energizing, pain, kidney and skin diseases, laxative, constipation, diarrhea, disentery, burns, diabetes, hypercholesterolemia, hypertension	Palmitic acid, stearic acid, oleic acid, myristic acid, arachidonic, lauric acid, 9,10, amentoflavone, kampferol-3-O-rutinoside, naringenin, catechin, protocatechuic acid, dihydromyricetin, linamarin	Antioxidant, hipoglysemic, hypocholesterolemic, hypotrygliceridemic, antitumoral	[23-31]
Manilkara zapota L. Van Royen (Sapotaceae) 4727	Chicozapote, zapote chico, *sak-ya, ya*	Fruits, seeds, bark, leaf	Astringent, diuretic, lung and kidney diseases, pain, emetic, fever, diarrhea, disentery, rheumatism, cold, leukorrhea, hypertension, insomnia, inflamation	lupeol acetate, oleanolic acid, apigenin-7-O-α-L-rhamnoside, myricetin-3-O-α-L-rhamnoside, caffeic acid, epicatechin taraxerol methyl ether, spinasterol, 6-hydroxyflavanon, (+)-dihydrokaempferol, 3,4-dihydroxybenzoic acid, taraxerol, taraxerone caffeoylquinic acid and methyl 4-O-galloylchlorogenate	Antimicrobial, citotoxic, hypoglysemic, immunostimulator, antitumoral, anti-diarrheal, anti-secretory, anti-spasmodic, anti-motility, anti-ulcer	[32-39]
Jatropha gaumerii Greenm (Euphorbiaceae) 18927	Pomolche *chulche×, xpomolche×*	Leaf	Mauthwash, ulcers, skin diseases, wounds, hemorrhoids, herpes, diarrhea, disentery, cancer	2-epi-jatrogrossidione, 15-epi-4E-jatrogrossidentadione, b-sitosterol, α-amirina, β-amirina, taraxasterol	Antibacterial, anti-inflammtory, healing, antioxidant, citotoxic	[40-46]

The Plant List (2013). Version 1.1. Published on the Internet; http://www.theplantlist.org/(accessed 1st January)

For evaluation, 0.3 mm segments were stabilized (3 g; 30 min). The vasorelaxant activity induced by the extracts (0.03 → 560 μg/mL) was developed according to the methodology described.[52] Test samples and positive control (Papaverine: 0.1 → 3 μg/mL) were compared with respect to the maximum contraction induced by NA (1×10^{-7} M) by using Acqknowledge software (BIOPAC®, CA, USA). Six independent experiments were carried out on each sample.

Infrared Fourier transform-infrared spectroscopy

FTIR experiments were made from crude extracts and use the powder diffuse reflectance technique; the samples pellets were prepared with 1 mg of GA or Q, 1 mg of the extract, 199 mg of KBr, and then were mix in an agate mortar. Subsequently, pellets were loaded in Thermo Nicolet Nexus 670, under the following conditions: 400–4000 cm^{-1} for scan ranging and 4 cm^{-1} of resolution.[53] FTIR spectrum of GA, Q and Q/GA mixture (25/75, 50/50, and 75/25) has been employed for estimation of GA and Q content in the extracts derived from the species under study. The weighting of the area under the curve (AUC) at 721 y 762 cm^{-1} presents in the FTIR spectrum of Q, GA and mixtures were used to estimate Q and GA content in crude extracts from medicinal plants under study. For this, the partial least square model was used to quantify Q and GA in complex mixtures such as medicinal plants extracts.

Statistical analysis

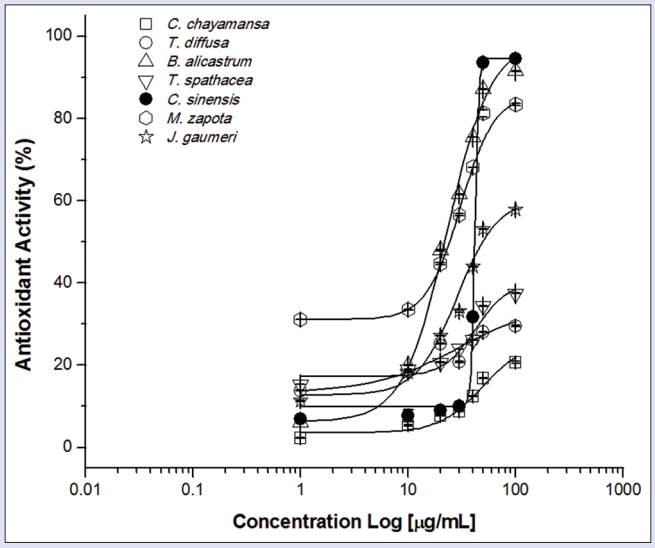
The experimental results are shown as the average ± the standard error of the mean. Experimental data of concentration-response curves (CRCs) were plot and adjusted by the non-linear employing

curve-fitting program Microcal™ Origin 8.0 (Microcal Software Inc., USA). Significance was evaluated using an analysis of variance; values of $P < 0.05$ were considered statistically significant.

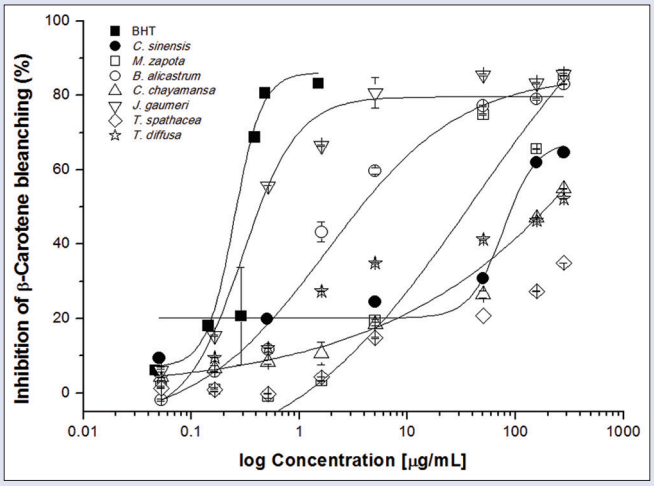
RESULTS

Effect of methanol maceration on leaves extraction

The methanol maceration procedure was suitable to obtain raw material from leaves of medicinal plants. The yield was significant ($P < 0.05$) lower in arboreal species. Methanol maceration in arboreal species (*B. alicastrum, M. zapota* and *J. gaumeri*) also showed the significant ($P < 0.05$) reduction in chlorophyll extraction. Whereas the maceration with methanol ameliorates the yield and chlorophyll extraction in *T. spathacea, C. chayamansa* and *T. diffusa*. The chemical and spectroscopic analysis allows to the estimation of simple phenolic and flavonoid compounds content in all experimental methanolic extracts. Herbal species showed low content of simple phenolic and flavonoids compounds with respect to arboreal species as well as methanol extract of *C. sinensis*, internal control employed. In this sense, *B. alicastrum* and *J. gaumeri* extracts registered the best content of simple phenols and flavonoids compounds [Table 2].



Graph 1: Antioxidant activity induced by crude extracts derived from aerial parts of medicinal plants (1 → 100 µg/mL) in DPPH model test. Results are expressed as the mean ± standard error of the mean of six experiments ($P < 0.05$ *vs. C. sinensis*)



Graph 2: β-Carotene bleaching activity induced by crude extracts derived from aerial parts of medicinal plants (0.05 → 280 µg/mL). Results are expressed as the mean ± standard error of the mean of six experiments ($P < 0.05$ *vs*. dibutylhydroxytoluene)

Table 2: Quantitative analysis of methanolic extracts derived of medicinal plants from Campeche, Campeche, Mexico

Specie	Yield (%)	Chlorophylls (mg/mL)	Simple phenols Eq. GA (µg/g)	Flavonoids Eq. Quercetin (µg/g)
Brosimum alicastrum	24.41±0.01	8.20±1.76	1.71±0.02	10.77±0.17
Tradescantia spathacea	37.39±0.07	28.94±3.31	0.47±0.14	3.52±0.04
Turnera diffusa	30.35±0.01	28.76±3.04	0.39±0.01	6.43±0.19
Cnidoscolus chayamansa	35.16±0.04	13.74±1.37	0.42±0.01	4.45±0.04
Manilkara zapota	15.39±3.39	3.56±0.63	0.59±0.01	3.3±0.03
Jatropha gaumeri	6.66±0.31	9.14±3.45	0.79±0.002	4.67±0.07
Camellia sinensis	38.57±4.09	NA	3.30±0.005	11.18±0.01

*$P<0.05$ versus *C. sinensis*. NA: No applied

Table 3: Antioxidant (2,2-diphenyl-1-picrylhydrazyl, β-carotene bleaching) and vasorelaxant activity induced by methanolic extracts derived of the selected plants

Species	Antioxidant DPPH		Inhibition β-Carotene bleaching		Vasorrelaxant	
	Potency EC_{50} (µg/mL)	Efficacy E_{max} (%)	Potency EC_{50} (µg/mL)	Efficacy E_{max} (%)	Potency EC_{50} (µg/mL)	Efficacy E_{max} (%)
Brosimum alicastrum	22.9±2.4*	98.7±6.4	6.3±0.1	83.0±0.1	>500	21.4±1.7*
Cnidoscolus chayamansa	49.6±2.4	26.1±10.7	>500	54.0±0.1	>500	16.8±3.3*
Tradescantia spathacea	42.4±9.9	40.3±6.5	>500	34.9±0.01	>500	8.5±2.7*
Turnera diffusa	28.9±3.6	34.9±14.2	233.1±0.2	52.2±0.1	>500	31.4±2.7*
Manilkara zapota	30.6±0.9*	85.8±1.7	55.6±1.6	85.4±0.1	>500	47.8±3.4*
Jatropha gaumeri	60.3±1.8	60.4±1.8	0.8±0.01	85.7±0.4	161.6±7.5	79.7±3.9
Camellia sinensis	41.8±0.3	94.6±0.8	113.4±0.04	64.6±1.2	NA	NA
BHT	NA	NA	0.36±0.01	80.6±0.2	NA	NA
Papaverine	NA	NA	NA	NA	0.68±0.35	80.9±3.0

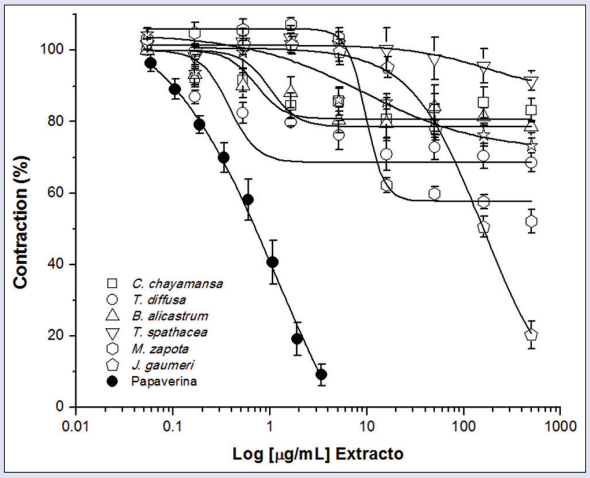
*$P<0.05$ *versus C. sinensis*. NA: No applied; BHT: Dibutylhydroxytoluene

In vitro and ex vivo pharmacological evaluations

B. alicastrum, J. gaumeri and *M. zapota* methanolic extracts exert an antioxidant activity in a concentration-dependent manner on the DPPH antioxidant model [Graph 1 and Table 3]. CRC analysis revealed that methanolic extract of *B. alicastrum* and *M. zapota* exerts a significant antioxidant activity with better potency and efficacy than *C. sinensis* (EC_{50}: 22.9 ± 2.4 µg/mL; E_{max}: 98.7% ± 6.4%; EC_{50}: 30.6 ± 0.9 µg/mL; E_{max}: 85.8% ± 1.7% *vs.* EC_{50}: 41.8 ± 0.3 µg/mL; E_{max}: 94.6 ± 0.8; $P < 0.05\%$) extract. On the other hand, among herbaceous species only *T. diffusa* extract exerts a great potency (EC_{50}: 28.9 ± 3.6 µg/mL) of antioxidant activity but with low efficacy (E_{max}: 34.9% ± 14.2%) as free radical scavenger.

Extracts derived from arboreal species showed a significant ($P < 0.05$) inhibition of β-carotene bleaching activity. *J. gaumeri* (EC_{50}: 0.8 ± 0.01 µg/mL; E_{max}: 85.7 ± 0.4%), *B. alicastrum* (EC_{50}: 6.3 ± 0.1 µg/mL; E_{max}: 83.0 ± 0.1%) and *M. zapota* (EC_{50}: 55.6 ± 1.6 µg/mL; E_{max}: 85.4 ± 0.1%) extracts exhibited an inhibition of β-Carotene bleaching activity in a concentration-dependent manner with better potency and efficacy than *C. sinensis* (EC_{50}: 113.4 ± 0.04 µg/mL; E_{max}: 64.6 ± 1.2%) extract. Finally, methanol extracts of *C. chaymansa, T. spathacea* and *T. diffusa* do not exert a significant bleaching activity [Graph 2 and Table 3].

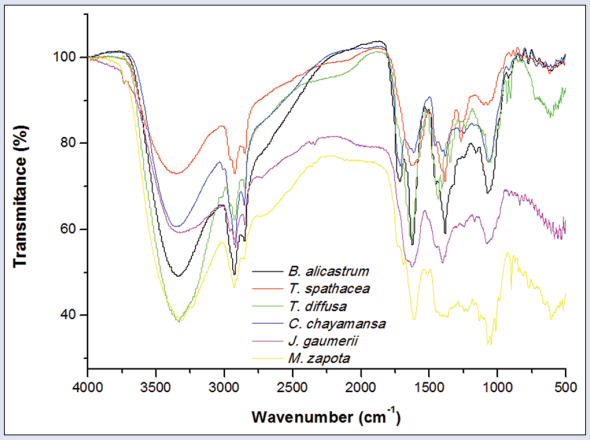
Evaluation in aortic rings (E+) pre-contracted with noradrenaline (1 µM) allows to identify that the extracts of *B. alicastrum, C. chayamansa, T. spathacea*, *M. zapota* and *T. diffusa* do not exert a significant vasorelaxant activity. However, CRC analysis revealed that *J. gaumeri* extract exerts a similar smooth muscle relaxant effect than papaverine but less potency [Graph 3 and Table 3].



Graph 3: The vasorelaxant effect induced by crude extracts derived from aerial parts of medicinal plants (0.03 → 560 µg/mL) in aortic rings isolated from rats. Results are expressed as the mean ± standard error of the mean of six experiments ($P < 0.05$ *vs.* Papaverine)

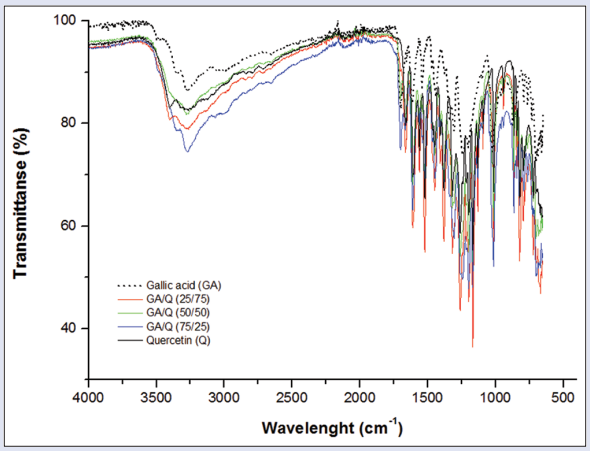
Fourier transform-infrared spectroscopy analysis and estimation of gallic acid and quercetin content

Bands observed between 3600 and 3300 cm^{-1} correspond to stretching vibrations of OH groups typical of water, alcohols, phenols, flavonoids as well as amides [Graph 4]. Peaks at 2900–2800 cm^{-1} are associated with narrowing and deformation vibrations specific to $-CH_3$ and $-CH_2$ from lipids, methoxy derivatives, aldehydes, and *cis* double bonds. 1750–1600 cm^{-1} complex area corresponds to bending vibration of N-H (amino acids), C = O stretching (ketones, aldehydes, esters), free fatty acids (1710 cm^{-1}), and glycerides (1740 cm^{-1}). A 1600–1500 cm^{-1} area corresponds to aromatic domains and N-H bending vibrations. 1450–1300 corresponds to stretching vibrations C-O and C-C present in amides and phenyl groups, respectively. Stretching vibrations of carbonyl C-O or O-H bending were observed at 1300–1100 cm^{-1} area. Signals at 1030, 1050, 1105, and 1130 cm^{-1} were associated with stretching vibrations C-O of mono-, oligo- and carbohydrates. Finally, <1000 cm^{-1} area was observed C-H bending vibrations that could correspond to isoprenoids.[54-57]



Graph 4: Fourier transform infrared spectroscopy spectra of pure methanolic extracts of the aerial parts of the medicinal plant species under study (dried solid mass, KBr)

Quantitative determination of the inorganic and organic matter,[58] as well as natural products[59,60] are demonstrated by KBr-FTIR in the transmission mode. In this sense, the presence of GA and Q in methanol extracts was carried out by analysis of peaks into a fingerprint-FTIR region. The FTIR spectra of Q, GA and Q/GA mixtures (25/75, 50/50, and 75/25) are presented in Graph 5. In 1100–600 cm^{-1} region, Q and GA showed 10 and 13 bands, respectively. AUC at (725–717 cm^{-1}) and (756.5–767.8 cm^{-1}) were employed to estimates Q and GA, respectively. AUC of Q, GA and mixtures (25/75, 50/50, and 75/25) of FTIR spectrum allow us identified a high linear correlation ($r^2 = 0.87$) and ($r^2 = 0.93$), respectively. This allows estimating 1.26 and 2.28% of Q and GA in *J. gaumeri* methanol extract. These metabolites are not identified in *T. spathacea, T. diffusa, M. zapota, B. alicastrum* and *C. chayamansa,* however, their presence is not ruled out.



Graph 5: Fourier transform infrared spectroscopy spectra of pure quercetin, gallic acid, and mixtures (25/75, 50/50 and 75/25) (Dried solid mass, KBr)

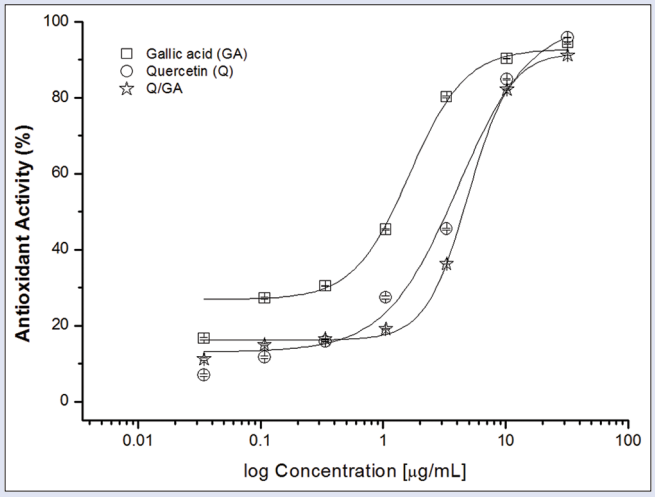
In vitro evaluations of Q, GA and Q/GA (1:2) mixture

Finally, the antioxidant activity of GA, Q, and Q/GA (1:2) mix was evaluated by using the DPPH and bleaching of β-carotene models. GA, Q, and Q/GA exerts antioxidant activities in both DPPH and β-carotene bleaching models in a concentration-dependent manner [Graphs 6, 7 and Table 4]. The CRC analysis allows identified that Q/GA (1:2) mixture was markedly shifting to the right and reduce the radical scavenging activity in the DPPH model, with respect to GA. On the other hand, the CRC of inhibition β-carotene bleaching activity exerted for Q/GA mix was significant ($P < 0.05$) shift to the left with respect to GA and Q as well as BHT (positive control).

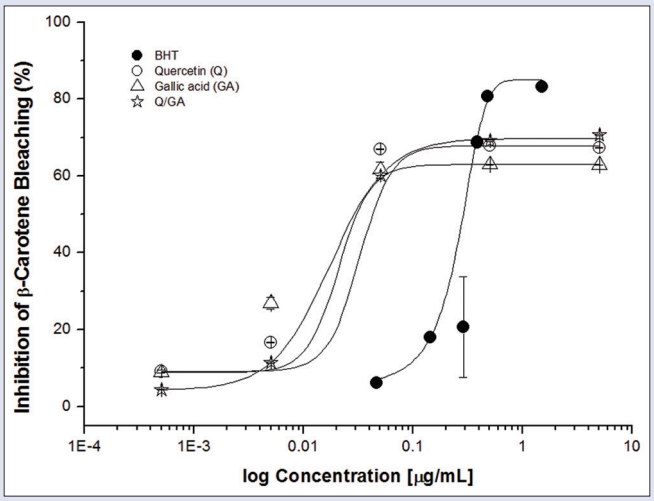
DISCUSSION

Plantae kingdom registers a huge diversity of chemical structures, which represent an important source of new drugs. For the present work, *B. alicastrum, C. chayamansa, T. spathacea, T. diffusa*, *M. zapota* and *J. gaumeri* were select for their ethnomedical and pharmacological reports as well as the presence of secondary metabolites with pharmacological reports [Table 1]. Maceration is a method included in the Mexican Pharmacopoeia[61] and it is relatively advantageous to obtain the raw material from tissues derived of medicinal plants because solvents dissolve secondary metabolites in function to the polarity, temperature, and time. The assay can be employed with other polar or nonpolar solvents to obtain both lipophilic and hydrophilic chemical entities. As can be seen in Table 2, variations in the yield as well as in the estimation of the content of chlorophyll, simple phenolic compounds, and flavonoids were registered. These results allow demonstrating the interspecies biological variability and need to use specific standard conditions for harvest and extraction of the raw material. In the study of natural products, seasonal variations, drying, and storage influence in the production of high-quality herbal products.[62] In the same way, different extraction methods are distinguished and each one presents advantages and disadvantages.[63] This context reflects the need for the employment of diverse strategies and technologies for the characterization, identification, and production of natural products.[64]

The potential emergent area is the natural antioxidants agents for their actions on ROS and RNS species generated in endothelial and smooth muscle cells from the vascular system.[65] In this sense, to explore the antioxidant and vasorelaxant effects of methanolic extracts from medicinal plants were employed DPPH and β-Carotene *in vitro* tests and noradrenaline-precontracted aorta rings as an *ex vivo* model. Methanol extracts exert antioxidant activity and the best potency and efficacy were observed in extracts derived from arboreal species (*B. alicastrum*, *M. zapota* and *J. gaumeri*), and no polar metabolites and polar compounds could be related with high radical scavenging activity [Graphs 1, 2 and Table 3]. The increased potency in the inhibition β-carotene bleaching test with respect to DPPH model [Table 3] suggests that *J. gaumeri* methanol extract could have nonpolar antioxidant metabolites. In fact, metabolites such as as α/β-amyrin,[66] sterols,[67] gallic acid,[68] and quercetin[69] act as lipid peroxidation inhibitors, however, previous studies suggest that the DPPH method is independent of the substrate polarity,[50] in this sense, metabolites as β-amyrin,[70] taraxerol,[34] β-sitosterol,[71] gallic acid[35] and quercetin[72] exert DPPH free radical scavenging activity. Quantification of natural products using FTIR has been previously reported.[73,74] Analysis of the FTIR spectrum of Q and GA allow the estimation of these metabolites in a 1:2 ratio [Graphs 4 and 5]. The mix of Q/GA exert the best parameter of potency and efficacy in the inhibition β-carotene



Graph 6: Antioxidant (1,1diphenyl2picrylhydrazyl) activity induced by GA, Q, and mix Q/GA (1:2) identified in leaves of *Jatropha gaumeri*. Results are expressed as the mean ± standard error of the mean of six experiments ($P < 0.05$)



Graph 7: Antioxidant (β carotene) activity induced by GA, Q, and mix Q/GA (1:2) identified in leaves of *Jatropha gaumeri*. Results are expressed as the mean ± standard error of the mean of six experiments ($P < 0.05$)

Table 4: Antioxidant activity induced by Quercetin, Gallic Acid and mix (1:2)

Compound	Antioxidant DPPH		Inhibition β-Carotene bleaching	
	Potency CE_{50} (μg/mL)	Efficacy E_{max} (%)	Potency CE_{50} (μg/mL)	Efficacy E_{max} (%)
Q	4.08±0.63	99.9±3.8	0.036±0.0001	69.6±0.06
GA	1.64±0.2	92.9±3.3	0.037±0.001	66.2±0.1
Q: GA (1:2)	4.89±0.2	91.8±0.7	0.017±0.0006	72.2±0.6
BHT	NA	NA	0.36±0.01	80.6±0.2

*$P<0.05$ versus BHT. NA: No applied; BHT: Dibutylhydroxytoluene; DPPH: 2,2-diphenyl-1-picrylhydrazyl; Q: Quercetin; GA: Gallic acid

bleaching test [Graph 7 and Table 4] these results suggesting that lipophilic environment favor the interaction between them, reduction of ionized species, increase of non-ionized species and better interaction with lipophilic molecules.

Simple phenolic compounds, flavonoids,[75] and triterpenes[76] have been reported to exert vasorelaxant effects on rat aortic rings. α/β-amyrin open K^+ channel.[77] β-sitosterol does not affect acetylcholine-induced relaxation.[78] GA modulate hemodynamic parameters[79] and quercetin, induce relaxation in a concentration-dependent manner.[80] Partial vasorelaxant effects induced by *J. gaumeri* [Graph 3 and Table 3] methanol extract suggest a great diversity of chemical entities and a low abundance of those with vasorelaxant activity. In this context, metabolites such as taraxerol, β-sitosterol, α/β-amyrin as well as gallic acid and quercetin identified in leaves methanolic extracts of *J. gaumeri* work together in a lipophilic environment and could be participating in the antioxidant and vasorelaxant effects.

CONCLUSION

J. gaumeri is a medicinal plant employed in Mayan traditional medicine and GA and Q could be related to traditional uses, as well as responsible for the pharmacological effects. GA and Q interactions improve inhibition β Carotene bleaching activity, which suggests greater solubility in lipophilic systems and potential interactions at the plasma membrane level.

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Conflicts of interest

There are no conflicts of interest.

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