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Research Article

Phytochemical Screening and Anti-Inflammatory Potential of the Organic Extracts from *Cleoserrata serrata* (Jacq.) Iltis.

María del Carmen Juárez-Vázquez[1],[2†], Alejandro Zamilpa A[3], Rosalba León-Díaz[4], Mariano Martínez-Vázquez[5], Adolfo López-Torres[6], Julieta Luna-Herrera[7], Lilian Yépez-Mulia[8], Francisco Alarcón-Aguilar[2], María Adelina Jiménez-Arellanes[1,*]

[1]*Unidad de Investigación Médica (UIM) en Farmacología, UMAE Hospital de Especialidades, Centro Médico Nacional Siglo XXI (CMN-SXXI), Instituto Mexicano del Seguro Social (IMSS). Av. Cuauhtémoc 330, Col. Doctores 06720, Ciudad de México (CdMx), MEXICO.*
[2]*Doctorado en Ciencias Biológicas y de la Salud, Universidad Autónoma Metropolitana, Av. San Rafael Atlixco 186, CdMx 09340, MEXICO.*
[3]*Centro de Investigación Biomédica del Sur (CIBIS), IMSS, Argentina 1, Col. Centro 062790, Xochitepec, Morelos, MEXICO.*
[4]*Área de Biología y Salud Integral, Instituto de Investigaciones Biológicas, Universidad Veracruzana. Luis Castelazo s/n. Col. Industrial Ánimas, Xalapa 91190, Veracruz, MEXICO.*
[5]*Instituto de Química, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Coyoacán 04510, CdMx, MEXICO.*
[6]*Instituto de Biotecnología, Universidad del Papaloapan. Circuito Central 12200, Col. Parque Industrial, Tuxtepec 68301, Oaxaca, MEXICO.*
[7]*Departamento de Inmunología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, CdMx 11340, MEXICO.*
[8]*UIM en Enfermedades Infecciosas y Parasitarias, UMAE Hospital de Pediatria, CMN-SXXI, IMSS. Av. Cuauhtémoc 330, Col. Doctores 06720, CdMx, MEXICO.*

Correspondence

M.A. Jiménez-Arellanes

UIM en Farmacología, Hospital de Especialidades, CMN Siglo XXI, IMSS, Av. Cuauhtémoc 330, Col. Doctores, 06720 Ciudad de México, MEXICO.

Phone no: (+52) (55) 5627 6900, ext. 21367;

E-mail: adelinajim08@prodigy.net.mx

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ABSTRACT

Cleoserrata serrata is used in Mexican-south traditional medicine to treat *chicleros ulcer*. The phytochemical profile and the anti-inflammatory effect from four extracts obtained by maceration proccess and of the primary fractions from two extracts are described. In addition, the antioxidant, leishmanicidal and antimycobaterial activities and LD_{50} from active extracts are reported. Anti-inflammatory activity was evaluated in TPA and carrageenan assays. Ethyl palmitate and γ-sitosterol were detected in Hexanic and CH_2Cl_2 extracts. Same compounds and stigmasta-3,5-dien-7-one, palmitic acid, phytol acetate and phytol were detected in primary fractions from CH_2Cl_2:EtOH extract. In this and in MeOH extracts a polyphenol-mixture was obtained. The MeOH extract was subjected to acid hydrolysis, and kaempferol, quercetin and scopoletin were detected in organic-phase. Polyphenol-mixture and organic-phase (IC_{50}=3730 and 2338 µg/mL) showed moderate antioxidant activity; meanwhile MeOH extract exhibited scarce activity. In carrageenan model, Hexanic extract and polyphenol-mixture showed ED_{50}=131.46 and 64.89 mg/kg, respectively. Three extracts were active but not-dose-dependent. In TPA-model, CH_2Cl_2 extract and polyphenol-mixture showed ED_{50} <0.79 mg/ear, and three extracts were active, however the effect was not-dose-dependent. CH_2Cl_2:EtOH showed antimycobacterial and leishmanicidal activities. The LD_{50} was >2 g/kg for all extracts.

Key words: Cleoserrata serrata, Anti-inflammatory effect, Organic extract, DL_{50}, Antioxidant activity, Leishmanicidal activity, Antimycobacterial activity.

INTRODUCTION

Cleoserrata serrata (Jacq.) Iltis. (Syn. *Cleome serrata* Jacq.) belongs to the family *Cleomaceae;* this specie was segregated from the *Cleome* genus and is an annual herb, mostly ruderal with ecological importance as a host plant for butterflies in Cuba.[1,2] In Mexico is distributed in the states of Guerrero, Oaxaca, Quintana Roo, San Luis Potosí, Tabasco and Veracruz, and also in Central America, northern of South America and in the Antilles.[1,3-5] *Cleoserrata* genus includes five species: *C. serrata* (Jacq.) Iltis.; *C. speciosa* (Raf.) Iltis (Syn. *Cleome speciosa* Raf.); *C. bahiana* Iltis & M.B. Costa-e-Silva ex Soares Neto & Roalson; *C. paludosa* (Willd. ex Eichler) Iltis ex Soares Neto & Roalson and *C. melanosperma* (S.Wats.) Roalson & Soares Neto.[1,5]

C. serrata is commonly known as "mastuerzo" or "zumkak", and is used in the traditional medicine from Tabasco State, Mexico.[6] In La Chontalpa and Cunduacan, Tabasco, the leaves are used as infusion or cataplasm to treat the skin lesion in patients with cutaneous leishmaniasis (caused by *Leishmania* spp) known as "*chicleros ulcer*".[7] It is also employed in southern and central regions in Mexico for skin infections.[8] In addition, Amerindian groups (Kuna, Ngöbe-Buglé and Teribe) from Panama employ the whole plant in topical warm baths and the leaves for the treatment of snake bites.[9] Recently, Alamilla-Fonseca et al.[7] described leishmanicidal and antibacterial activities for the dichloromethane:methanol (CH_2Cl_2:MeOH, 1:1) extract from *C. serrata* leaves. This extract showed mean lethal doses (LD_{50}) = 6.11 and 23.5 µg/mL against *Leishmania mexicana* (*L. mexicana*) amastigotes and promastigotes, respectively; this effect was dose-dependent. In the latter case, the author observed 60% inhibition at 100 µg/mL and 85% inhibition at 200 µg/mL. The CH_2Cl_2:MeOH extract at 10 µg/mL exhibited leishmanicidal activity on amastigotes after 4 days of culture and, at 100 µg/mL, also showed activity on promastigotes during the same time, being the most active against amastigotes. Finally, this extract was slightly active against *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) at 500 mg/disc and was positive for steroids, terpenoids, flavonoids and polyphenols test on the qualitative analysis. The essential oil obtained from *C. serrata* aerial parts contain (*Z*)-phytol (53.0%) as a main constituent and it compound showed moderate activity against *E. coli*, *Streptococcus pyogenes*, *Bacillus cereus*, *B. subtilis* and *P. aeruginosa*, and was described as anti-inflammatory agent.[10,11] At present, *C. serrata* has been scarcely investigated from chemical and biological point of view. Based in this background, in this paper we describe the phytochemistry profile of the organic extracts from *C. serrata* aerial parts, as well as their potential anti-inflammatory, antioxidant, antimycobacterial and leishmanicidal activites and their mean lethal dose (LD_{50}).

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MATERIAL AND METHODS

General experimental procedures

Analytical Thin layer chromatography-Normal phase (TLC-NP) was carried out on silica gel (Si-gel) 60 F_{254} sheets and the TLC-Reverse phase (TLC-RP) was performed on Si-gel 60 RP-18 F_{254}s pre-coated aluminum plates (0.2 mm, Merck). The TLC were sprayed with H_2SO_4 (10%) and heated by triterpenes and sterols detection, and polyphenols were detected with the Natural Product reagent (NPR, 1% MeOH diphenylboryloxyethylamine) and Polyethylene Glycol 4000 (PEG, 5% EtOH polyethylene glycol 4000) known as NPR/PEG; after that the TLC plates were observed under Ultra violet (UV) light at λ=365 nm.[12] The carbohydrates was detected by TLC-NP using n-butanol:ethyl acetate (EtOAc):acetic acid:H_2O (40:30:20:10) and by polyphenols detection was use H_2O:CH_3CN (7:3) in TLC-RP. The amino acids was detected by TLC-NP plate, using n-butanol:acetone:acetic acid:H_2O (35:35:10:20) as mobile phase and sprayed with ninhydrin reactive (30 mg of ninhydrin, 10 mL n-butanol and 0.3 mL acetic acid at 98%).

Open column chromatographic Normal phase (CC-NP) was carried out using Si-gel 60 GF_{254} (0.063-0.200 mm, Merck Millipore) and was eluted with hexane (Hex), $CHCl_3$, MeOH (Mallinckrodt or J.T. Baker), and mixture of them with increasing of polarity.

The Gas chromatograph-coupled Mass spectra (GC-MS) analysis was performed on a gas chromatograph (Agilent Technologies 6890 N) coupled to a Jeol MS-GCMATE II mass spectrometer. The GC column used was HP5 (30 m of long x 0.32 mm in diameter), and the programmed temperature (temp) was 40-310° C with a heating rate of 8° C/min. The carrier gas was He (7psi, 1mL/min), while ionization of the components of the sample was carried out in the Electronic impact mode (EI, 70 eV). The NIST mass spectrum database was employed to identify the chemical constituents in each sample by comparison. The relative percentage of each constituent from each sample was expressed as a percentage with respect to the peak area with Retention time (R*t*).

In addition, the MeOH extracts were analyzed by High Performance liquid chromatography (HPLC). This was conducted in Alliance 2695 separation module system (Waters, Milford, USA) coupled with a spectral system UV2996 PDA detector. The following analytical conditions were employed: SupelcosilTM LC-F column (5 µm, 4.6 mm x 250 mm, Sigma Aldrich, USA) with gradient system, employing 0.5% Trifluoroacetic acid (eluent A) and CH_3CN (eluent B) with the following flow: 0 to 1 min, 100-0 % B; 2 to 3 min 95-5% B/A; 4 to 20 min, 70-30% B/A; 21 to 23 min, 50-50% B/A; 24 to 25 min, 20-80% B/A, 26 to 27 min, 0-100 % B/A, and 28 to 30 min, 100-0% B. The flow rate was 0.9 mL/min and the injection volume was 10 µL. The peaks were detected at λ= 250 and 320 nm.[13] On the other hand, the *h-MeOH* ($CHCl_3$ phase obtained of the MeOH extract hydrolyzed) was analyzed by HPLC, carried out in Waters equipment (Waters, USA) comprising a 600 multisolvent delivery system with a 996-diode array detector. Equipment control, data acquisition, processing and management of the HPLC information were performed by Empower 3 software (Waters). The analytical column Phenomenex Luna® C18 (2) 100 Å, LC (250 mm x 4.6 mm, 5 µm) was utilized. The programmed temp was 40 °C, with a flow of 1 mL/min, and the injection volume was 10 µL. The elution system in gradient mode was performed. The system was 0.1% acetic acid (eluent A), CH_3CN (eluent B), and MeOH (eluent C): 0 to 25 min, 50:25:25 % A/B/C; 25 to 32 min, 90:5:5 % A/B/C; and the UV detection was made at λ= 280- 330 nm. A polyphenols mixture (*p*CSH) was submitted to Ultra-Performance Liquid Chromatography-Diode Array Detector-tandem Mass Spectrometry (UPLC-DAD-MS/MS) on chromatograph equipment (Acquity Arc, Waters). LUNA OMEGA C18 (150 mm, 2.1 mm, 1.6 µm) column was utilized, and the programmed temp was 40 °C, with a flow of 0.18 mL/min. The elution was performed in gradient mode employing the following: 0.1% formic acid (eluent A), CH_3CN (eluent B), and MeOH (eluent C), the system use was 0 to 25 min, 70:15:15 % A/B/C; 25 to 26 min, 50:25:25 % A/B/C; 27 to 40 min, 90:5:5 % A/B/C and the UV detection was carried out at λ= 330 nm. The ESI-TOF detection was performed with a spectral window of 100-1000 m/z. All chemicals reactive solvents (analytical and HPLC grade) used in this study were analytical grade and were purchased from Sigma-Aldrich or J.T. Baker.

Plant material, extract preparation and chemical fractionation

The *C. serrata* aerial parts were collected in Comalcalco, Tabasco, Mexico in July 2017 and herbarium specimen was deposited at the Herbarium of the Instituto Mexicano del Seguro Social from Mexico (voucher IMSSM-16307). Additionally, more plant material was collected in August 2018 and the herbarium specimen was deposited in the Herbarium of Facultad de Estudios Superiores Zaragoza, UNAM (FEZA) with voucher FEZA-17841 and was identified by Dr. Eloy Solano Camacho from FEZA, UNAM.

The aerial parts (550 g) were air-dried at room temp and ground. After that, this was extracted by the maceration process with CH_2Cl_2:EtOH (1:1) twice with occasional shaking at room temp. This extract (key CSH) was filtered and concentrated at 40°C in a vacuum system and was maintained at 25°C until its use. Another sample from plant material (429 g, dry and ground, collected in August of 2018) was extracted successively with Hex, CH_2Cl_2 and MeOH; each extract was macerated for a week at room temp, and was filtered, evaporated and was maintained at 25°C until its use. From the MeOH extract a precipitate (key: *p*MeOH) was obtained. The Hex, CH_2Cl_2 and MeOH extracts showed a similar profile to that CSH by TLC-NP and TLC-RP comparative analysis.

The CSH extract was subjected to HPLC analysis and 10 g of this extract was submitted to CC-NP on Si-gel (relation 1:20) and was eluted with Hex, EtOAc, and EtOH, and a mixture of these. This process led to obtaining 107 primary fractions of 500 mL each, these were grouped according to their chromatographic profile in TLC-NP or in TLC-RP and then were combined in 18 fraction groups as follows:

A (F7-13, 3.6 mg, Hex 100%), B (F14, 171.9 mg, Hex:EtOAc 97:3), C (F15-16, 116.3 mg, Hex:EtOAc 97:3), D (F17-24, 249.8 mg, Hex:EtOAc 97:3), E (F25-26, 15.5 mg, Hex:EtOAc 97:3), F (F27-28, 19.2 mg, Hex:EtOAc 97:3), G (F29-31, 47.7 mg, Hex:EtOAc 97:3), H (F32-40, 205.4 mg, Hex:EtOAc 97:3 and 9:1), I (F41-43, 329.7 mg, Hex:EtOAc 9:1), J (F44-46, 168.1 mg, Hex:EtOAc 9:1), K (F47-54, 492.8 mg, Hex:EtOAc 8:2), L (F55-61, 315.2 mg, Hex:EtOAc 7:3), M (F62-68, 389.3 mg, Hex:EtOAc 1:1), N (F69-84, 2214.6 mg, EtOAc and EtOAc:EtOH 75:25), Ñ (F85-90, 1878.1 mg, EtOAc:EtOH 75:25), O (F91-101, 1001.4 mg, EtOAc:EtOH 75:25 and 1:1), P (F102-103, 133.2 mg, EtOAc:EtOH 1:1) and Q (F104-107, 223.1 mg, EtOH).

The primary fractions B, C, G, K, M (low polarity fractions of CSH) and the Hex and CH_2Cl_2 extracts were submitted to GC-MS analysis. From the more polar fractions (Ñ, O, P and Q) a precipitate with key *p*CSH was obtained. It was very similar to *p*MeOH in TLC-RP, the final key of these sample was *p*CSH. In this sample, a polyphenols mixture was detected as fluorescents bands under UV light (λ =365 nm) and as yellow-coloured zone when the plate was sprayed with NP/PEG [12]. The *p*CSH (5 mg/500 µL) was passed through SPE microcolumns (500 mg, J.T. Baker Inc., Philipsburg, USA). It had been previously activated with CH_3CN 100 % (2 mL) and conditioned with CH_3CN 5%. Afterward, the sample was added, and then the microcolumn was eluted with CH_3CN 20%; this later sample was analysed by UPLC-DAD-MS/MS.

On the other hand, the MeOH extract (220.9 mg) was subjected to hydrolysis procedure using HCl 2N (15 mL) at ≈ 90°C for 2 h. Subsequently, the reaction mixture was washed with $CHCl_3$ and this organic phase with key *h-MeOH* was analyzed by HPLC and TLC-NP. In the aqueous phase, carbohydrates was detected employing some sugar standard.

Finally, the Hex extract (15 g) was submitted to CC-NP on Si-gel (relation 1:20) and was eluted with Hex, $CHCl_3$, and MeOH, and with a mixture of these; this process led to obtaining 122 primary fractions of 400 mL each one, which were grouped in 22 fraction groups according to their chromatographic profile:

I (F1-2, 59.80 mg, Hex 100%), II (F3-7, 77.1 mg, Hex100 %), III (F8-18, 10.90 mg, Hex 100%, Hex:$CHCl_3$ 95:05), IV (F19-27, 6.40 mg, Hex:$CHCl_3$ 95:05), V (F28-30, 78.5 mg, Hex:$CHCl_3$ 9:1 and 8:2), VI (F31-35, 171 mg, Hex:$CHCl_3$ 8:2), VII (F36-40, 124.30 mg, Hex:$CHCl_3$ 8:2), VIII (F41-44, 113.60 mg, Hex:$CHCl_3$ 8:2), IX (F45-53, 549.20 mg, Hex:$CHCl_3$ 7:3), X (F54-56, 192.90 mg, Hex:$CHCl_3$ 6:4), XI (F57-61, 53.50 mg, Hex:$CHCl_3$ 6:4), XII (F62-65, 52.50 mg, Hex:$CHCl_3$ 6:4), XIII (F66-68, 115.40 mg, Hex:$CHCl_3$ 1:1), XIV (F69-71, 311.40 mg, Hex:$CHCl_3$ 1:1), XV (F72-78, 1956 mg, Hex:$CHCl_3$ 1:1), XVI (F79-94, 3838.2 mg, Hex:$CHCl_3$ 4:6), XVII (F95-96, 400.50 mg, Hex:$CHCl_3$ 2:8), XVIII (F97-99, 292.20 mg, Hex:$CHCl_3$ 2:8), XIX (F100-107, 835.90 mg, $CHCl_3$ 100%), XX (F108-113, 7220.90 mg, $CHCl_3$:MeOH 8:2), XXI (F114-117, 180.50 mg, $CHCl_3$:MeOH 1:1), XXII (F118-122, 104 mg, $CHCl_3$:MeOH 3:7). Some primary fractions (III, V, VI, IX, XIII, XV, XVII, XIX, XX) were submitted to GC-MS analyses. In these primary fractions sterols and triterpenes was detected as a main constituent.

Primary fraction XV, eluted with Hex:$CHCl_3$ 1:1 (1.71 g) was submitted to CC-NP on Si-gel (1:30) and was eluted with Hex, $CHCl_3$, and MeOH, and mixture of these. This process led to obtaining 57 secondary fractions, which were grouped according to their chromatographic profile in TLC-NP and then were combined in 4 groups: XV-a (F1-31, 15 mg,), XV-b (F32-39, 317 mg), XV-c (F40-43, 559.7 mg), and XV-d (F44-57, 495.4 mg).

In vitro Antioxidant activity

Radical-scavenging activity was measured using *2*,2-Diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich D9132) as described by Brand-Williams et al. and Miliauskas et al.[14,15] The CSH, Hex, CH_2Cl_2, and MeOH extracts, *h-MeOH* (from hydrolyzed MeOH extract) and *p*CSH samples were evaluated from 500 to 5000 µg/mL, and quercetin (25-150 µg/mL) was employing as the standard control. IC_{50} was calculated from the standard calibration curve. The experiment was carried out twice.

In vitro antimycobacterial and leishmanicidal activites

The antimycobacterial assay was determined using the Microplate Alamar Blue assays (MABA) previously described,[16] employing nine strains as follows: *Mycobacterium tuberculosis* H37Rv (ATCC 27294), four strain of *M. tuberculosis* H37Rv mono-resistant [resistant to rifampicin (ATCC 35838), to isoniazid (ATCC 35822), to streptomycin (ATCC 35820) and to ethambutol (ATCC 35857)], four multidrug-resistant (MDR) clinical isolates of *M. tuberculosis* with resistance to first-line drugs (MMDO, MTY 137, SIN-4, SIN-6), and four non-tuberculosis Mycobacterium (*M. fortuitum, M. smegmatis, M. avium, M. abscessus*).

Leishmanicidal activity was determined using the assay previously described[17] on the *Leishmania mexicana* (MNYC/BZ/62/M379) promastigotes and amastigotes. Miltefosine and amphotericin B were used as reference drugs (100 to 0.8 mg/mL). All assays were carried out in triplicate. Also, the cytotoxicity of sample was determined using murine macrophage cell line J774.2 (ATCC TIB-67), and the Selectivity index (SI) was determined. The sample was dissolved in DMSO and diluted in culture medium at concentration ranging from 100 to 0.8 mg/mL in a final volume to 200 µg/mL.

Animal *in vivo* assays

Male Balb/C mice (23 ±2 g) were provided by the IMSS Bioterium and were maintained in plastic cages during a 7-day conditioning period prior to the experiments under laboratory conditions (12/12-hour light/dark cycle; temp 25 ± 2°C; humidity 55-80%) with Rodent Chow food and water *ad libitum*. The animal for each experiment were randomly selected and grouped in a group of 5 animals each one and their management was carried out according to the statutes of the International Committee for the Care and Use of Laboratory Animals (IACUC) and Mexican Official Norm (NOM-062-ZOO-1999) revised in 2016.[18] The protocol was approved by the IMSS National Committee of Scientific Research (CNIC R-2018-785-059). After each biological assay, the mice were sacrificed by cervical dislocation and incinerated in the IMSS Bioterium.

Acute toxicity (LD_{50})

This assay was performed according to that previously described.[16] The CSH and MeOH extracts were evaluated at 0.5, 1, and 2 g/kg, and the Hex and CH_2Cl_2 extracts were only evaluated at 2 g/kg. The samples were administered by intragastric (i.g.) route and were previously dissolved in vehicle [Tween 80:H_2O (1:9)], while the control groups only received vehicle (VEH).

Anti-inflammatory assays

The carrageenan induced paw oedema and TPA-induced ear oedema models were used. The assays were performed according to that previously described.[16] The animals were randomly divided and grouped (n=5) as follows: Group I: carrageenan control (vehicle), Group II-IV: carrageenan plus sample dose (extract or fraction), and Group V: carrageenan plus Ind (reference drug, 10 o 20 mg/kg). The CSH extract was tested at 50, 150, and 300 mg/kg and their primary fractions (B, C, D, I, K, M, Ñ, P, Q) were evaluated at 150 mg/kg. The Hex, CH_2Cl_2 and MeOH extracts (aerial parts) and *p*CSH were evaluated at 50, 100, and 150 mg/kg. The primary fractions VI, IX, XIII, XV, and XX (from Hex extract) were tested at 100 mg/kg. Finally, the *h-MeOH* (hydrolyzed MeOH extract) was tested at 25, 50 and 100 mg/kg. All samples were dissolved in Tween 80:H_2O (1:9) and administered by i.g. route.

In the TPA-induced ear oedema model, the animals were randomly divided and grouped (n=5) as follows: Group I: TPA control (vehicle), Group II-IV: TPA plus sample dose (extract or fraction), and Group V: TPA plus Ind (reference drug, 2 mg/ear). All samples (CSH, Hex, CH_2Cl_2 and MeOH extracts, *p*CSH and *h-MeOH*) were evaluated at 0.5, 1, and 2 mg/ear and applied topically, and were previously dissolved in acetone. In addition, primary fractions from the CSH and Hex extracts were tested at 2 mg/ear and were solubilized in acetone. Only fraction V (insoluble in acetone) was solubilized in $CHCl_3$.

Statistical analysis

Sigma Plot 14.0 statistical software was utilized for the analysis of the results and graphic elaboration. Data is presented as standard error of the mean (SEM). BW gain values in acute toxicity test were analyzed with bifactorial Analysis of variance (ANOVA) and with a post hoc Student Newman Keuls (SNK) test. Results of $p<0.05$ were considered statistically significant. For ear oedema weight in TPA and the development of paw oedema in the carrageenan model one-way ANOVA followed by the Dunnett post-hoc test $p<0.05$ was considered significant. Finally, for relative weight of each organ in acute toxicity a Kruskal-Wallis test (ANOVA by ranks) was carried out, in addition

to a post hoc SNK test, in which relevant out comes were those with a value of $p<0.05$.

RESULTS

Phytochemical analysis

By maceration process at room temp, 198.93 g of the CSH extract was obtained from 550 g of dry leaves, which represents a yield of 36.16 %. In addition, 7.18 g (1.67%), 2.78 g (0.64%) and 33.83 g (7.88%) of the Hex, CH_2Cl_2, and MeOH extracts, respectively, were obtained from 429 g of dry material after the successive extraction process.

The main compounds detected in the CSH extract were fatty acids, triterpenes, and sterols (by TLC-NP analyses) and polyphenols mixture (by TLC-RP analyses). The CSH (A) and MeOH (B) extracts were analyzed by HPLC for polyphenols detection (Figure 1). In both chromatograms, between 8.2 and 10 min several peaks were observed that correspond to polyphenols. In the HPLC chromatogram of the MeOH extract (Figure 1B) a main peak with R*t* = 8.44 min with typical UV bands (at 266 and 348) for glycosylated flavonoids was observed. In addition, both extracts (CSH and MeOH) showed the presence of some amino acids and some carbohydrates (detected by TLC-NP analysis), and these were compared with commercial standards. In the MeOH extract, phenylalanine with an Rf = 0.63, alanine (Rf = 0.4), glycine (Rf = 0.30) and glutamine (Rf = 0.34) and in the CSH extract only alanine (Rf = 0.4) were observed. In both extract, sucrose (Rf = 0.33) was detected as main constituents.

By GC-MS analysis of the Hex extract, 9,12-octadecadienoic acid ethyl ester (20.52%, as a main compound), γ-sitosterol (20.62%), vitamin E (6.30%) and ethyl palmitate (7.85%) were detected. In the CH_2Cl_2 extract, γ-sitosterol (30.19%, as a main compound), ethyl palmitate (3.05%) and palmitic acid (9.96%) as well as vitamin E and stigmasterol were detected (Table 1). In addition, in primary fractions B, C, G, K and M (with low polarity) obtained from the CSH extract, ethyl palmitate (5.83 %); γ-sitosterol (45.65%); stigmasta-3,5-dien-7-one (5.09%); palmitic acid (23.76%); phytol (31.14 %) and phytol acetate (51.20 %) as the main constituents (with >5 % in the chromatogram) were detected, and the other minor constituents are described in Table 1.

The *p*CSH obtained from the most polar primary fractions Ñ, O, P and Q of the CSH extract and from MeOH extract contain mainly a polyphenols mixture. This sample was subjected to UPLC-DAD-MS/MS, and in the chromatogram (Figure 2) four peaks with R*t* = 19.4 (main), 23.1, 26.9, and 27.6 min were observed; in this MS spectra, a peak with R*t* =19.4 min showed a *m/z* = 755.20, that corresponds to a glucoflavonoid; which most likely corresponds to a kaempferol-glycoside derivate, because it shows a typical UV wave (λ = 264 and 348 nm) for this type of compounds. In this sample, other constituents such as scopoletin, sugar (such as sucrose) and some amino acids were also detected.

In addition, the MeOH extract (that contain *p*CSH) was hydrolysed under acid condition; after that, the *h-MeOH* was obtained. In this *h-MeOH* sample kaempferol, quercetin and scopoletin were identified, these compounds showed a Rf = 0.37, 0.20 and 0.68 on TLC-NP using $CHCl_3$:MeOH (9:1), the Rf values was similar to commercial standard. In the HPLC chromatogram from the *h-MeOH*, kaempferol showed an R*t* = 23.00 min with λ = 265.1 and 363.6 nm (Figure 3B), similar R*t* with commercial standard (Figure 3A). Another two main compounds were observed in this chromatogram with an R*t* = 10.28 min (λ = 234.8 and 322.7 nm) and 10.52 min (λ = 272.7 and 345.4 nm); these latter two compounds are under isolation for chemical identification. It must be mentioned that the main peak, with R*t* = 8.44 min observed in the HPLC chromatogram from the MeOH extract (Figure 1B) disappeared when the hydrolysis process was performed (Figure 3B). In addition, the scopoletin was quantified on *h-MeOH* by HPLC employing the condition described by Pérez-González et al.,[19] this sample contain 51.24 mg of scopoletin/g of dry sample (Figure 4A and 4B).

On the other hand, the active Hex extract was fractionated by CC-NP and this process allowed to obtain 22 groups of fractions. The main compounds detected by TLC-NP in this extract were fatty acids, triterpenes, and sterols. The majority fractions XV to XX show the presence of *β*-sitosterol as a main compound. Fraction XV (1.71 g, primary fraction with good yield) was subjected to next CC-NP and four secondary fractions groups were obtained. In the secondary fraction XV-a, β-amyrin, lupeol acetate, β-amyrin acetate and stigmastan-3,5-diene were identified. Stigmastan-3,5-diene and β-sitosterol were also identified in secondary fractions XV-b and XV-c (as a main constituents) and some fatty acids such as ethyl palmitate and palmitic acid was detected in fraction XV-d.

In vitro Antioxidant activity

The CSH and MeOH extracts showed a poor antioxidant activity with IC_{50} = 10,598 and 5,843 µg/mL, respectively, respect to quercetin (IC_{50} = 92.13 µg/mL). Conversely, *p*CSH and the *h-MeOH* exhibited a better activity with IC_{50}= 3,736 and 2,338 µg/mL, respectively; this last value was better than the CSH and MeOH extracts. The Hex and CH_2Cl_2 extracts were inactive (IC_{50} > 5 mg/mL).

Antimycobacterial and Leishmanicidal activities

The CSH extract was more active against *M. tuberculosis* MTY-137 and *M. tuberculosis* H37Rv showed a Minimum Inhibitory Concentration (MIC) = 12.5 and 25 µg/mL, respectively, and had moderate activity against *M. tuberculosis* H37Rv resistant to ethambutol and MDR *M. tuberculosis* SIN-4 with MIC = 50 µg/mL. All extracts were inactive against the rest of *M. tuberculosis* and non-tuberculosis mycobacterium strains with MIC ≥ 100 µg/mL.

With regard to the results of leishmanicidal activity, the CSH extract showed a one-half inhibitory concentration (IC_{50}) = 4.91 and 13.02 µg/mL against promastigotes and amastigotes of *L. mexicana*, respectively. In addition, the one-half cytotoxic concentration (CC_{50}) = 129.04 and 13.02 µg/mL and the Selectivity index (SI) = 27.97 and 9.9 were determined against the promastigotes and amastigotes of *L. mexicana*, respectively.

Acute oral toxicity study

The LD_{50} value for the Hex, CH_2Cl_2, MeOH and CSH extracts was >2 g/kg in Balb/C male mice, administered by i.g. route. The Body Weight (BW) gain of the animals treated with the CSH extract (2 g/kg) was increased. On day 9, the BW gain was 1.14 g and for day 14, it was 1.42 g with respect to VEH group (0.16 and 0.66 g, respectively). This BW gain was higher in the group that received the CSH extract at 2 g/kg; on the other hand, the groups treated with this extract at doses low (0.5 and 1 g/kg) showed a similar BW gain with respect to the VEH group (Figure 5). No changes were detected in the relative weight of the organs (spleen, liver kidney), nor was any alteration of these organs observed at the macroscopic level (data not shown).

The MeOH extract at 0.5 mg/kg did not affect the BW gain when was administered by i.g. route (Figure 6), this BW gain value was similar to VEH group. However, this extract at 2 g/kg decreased the BW gain, on days 7 and 14, it showed values of 0.44 and 0.89 g with regard to VEH group (1.21 and 1.50 g, respectively). The BW loss may be related to an increase in mucus in the feces of the mice that received the extract. Besides, the Hex and CH_2Cl_2 extracts at 2 g/kg favored the BW gain (1.46 and 1.79 g/kg, respectively), this increase was slightly better than that observed in the VEH group, 1.1 g/kg (Figure 6).

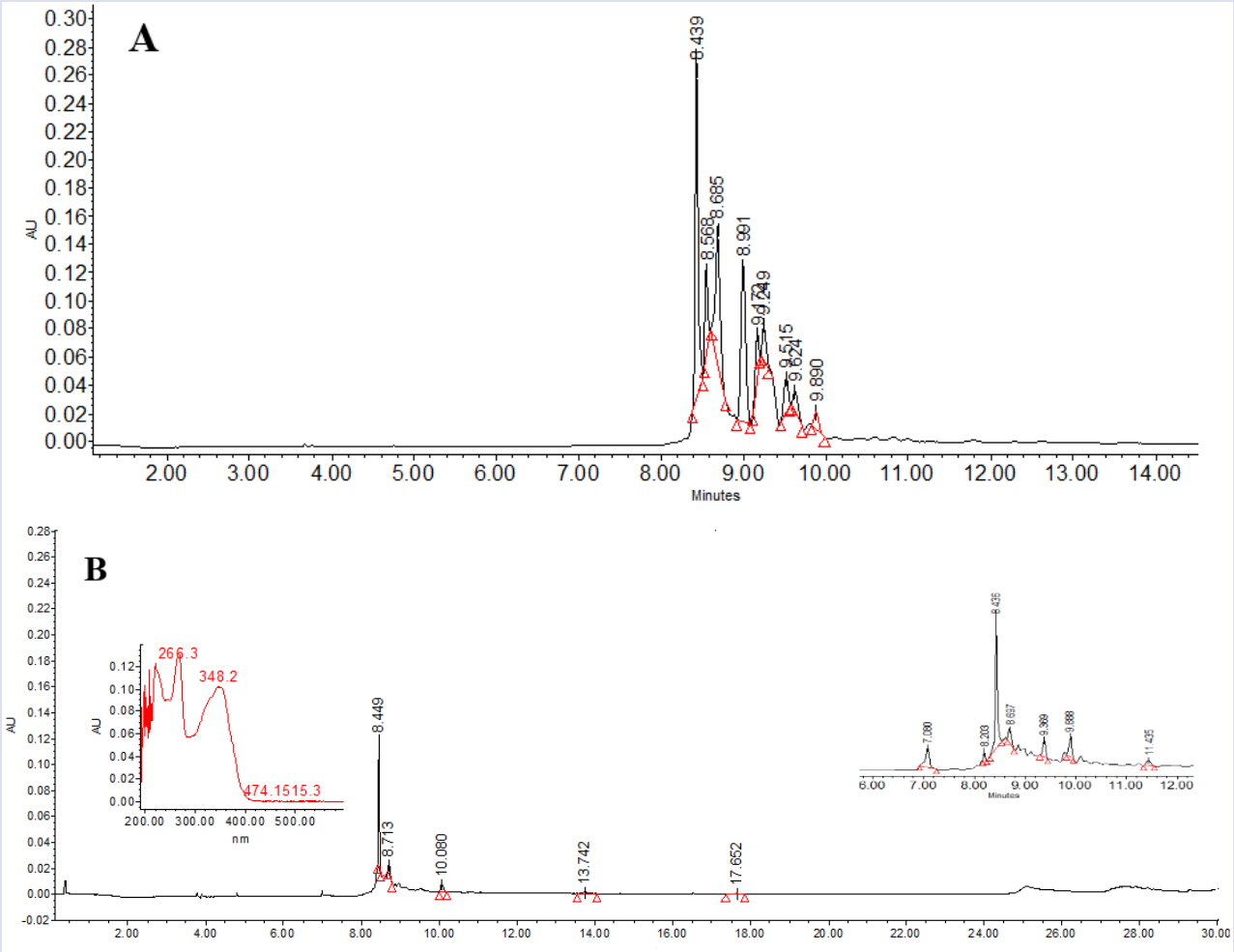

Figure 1: HPLC chromatogram of the CSH (A) and MeOH (B) extracts from *Cleoserrata serrata*.

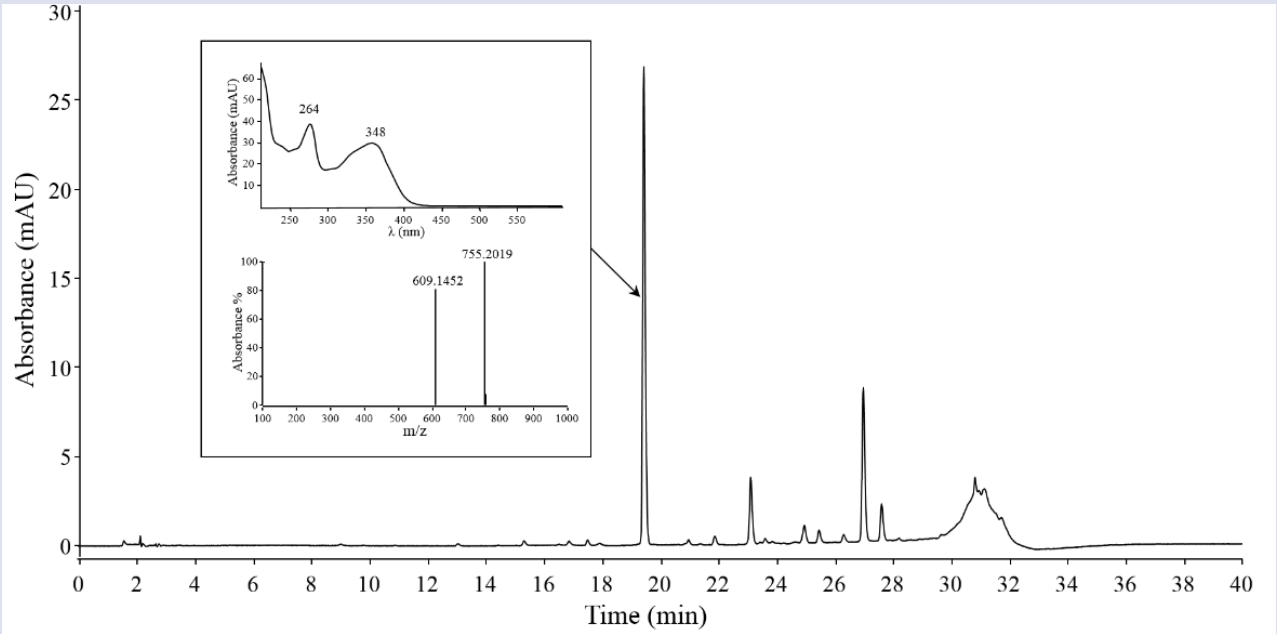

Figure 2: UPLC-DAD-MS/MS chromatogram of the *p*CSH, the main compound (Rt=19.4 min) showed a λ at 264 and 348 nm and MS/MS spectrum ([M-H] showed a m/z = 755.2019 with ([M-H]-= $C_{33}H_{39}O_{20}$.

Table 1: GC-MS analysis of phytocomponents identified in Hex and CH_2Cl_2 extracts and in primary fractions obtained from CSH extract of the *Cleoserrata serrata*.

Sample	RT	Compound name	Molecular formula	MW	% area
Hex extract	22.42	Hexadecanoic acid, ethyl ester (Ethyl palmitate)	$C_{18}H_{36}O_2$	284	7.85
	24.39	9,12-Octadecadienoic acid ethyl ester	$C_{20}H_{36}O_2$	308	20.52
	32.97	γ-Tocopherol	$C_{28}H_{48}O_2$	416	1.78
	33.61	Vitamin E	$C_{29}H_{50}O_2$	430	6.30
	34.45	Campesterol	$C_{28}H_{48}O$	400	3.81
	35.22	γ-Sitosterol	$C_{29}H_{50}O$	414	20.62
CH_2Cl_2 extract	22.25	n-Hexadecanoic acid (Palmitic acid)	$C_{16}H_{32}O_2$	256	9.96
	22.42	Hexadecanoic acid, ethyl ester (Ethyl palmitate)	$C_{18}H_{36}O_2$	284	3.05
	33.64	Vitamin E	$C_{29}H_{50}O_2$	430	6.33
	34.68	Stigmasterol	C29H48O	412	6.15
	35.22	γ-Sitosterol	$C_{29}H_{50}O$	414	30.19
Primary Fractions from CSH extract					
B	22.38	Hexadecanoic acid, ethyl ester (Ethyl palmitate)	$C_{18}H_{36}O_2$	284	5.83
	23.97	Octadecanoic acid, methyl ester (Methyl stearate)	$C_{19}H_{38}O_2$	298	1.95
	37.1	3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Phytol)	-	278	31.14
C	20.46	2-Pentadecanone, 6,10,14-trimethyl	-	250	2.79
	20.95	Phytol	-	278	1.77
	35.28	γ-Sitosterol	$C_{29}H_{50}O$	414	45.65
G	35.96	Stigmasta-3,5-dien-7-one	$C_{29}H_{46}O$	410	5.09
	36.31	Stigmast-4-en-3-one	$C_{29}H_{48}O$	412	3.08
K	20.44	Phytol, acetate	-	123	51.20
	22.16	n-Hexadecanoic acid (Palmitic acid)	$C_{16}H_{32}O_2$	256	23.76
M	24.5	Octadecanoic acid (stearic acid)	$C_{18}H_{36}O_2$	284	2.90
	33.58	(+)-ç-Tocopherol, O-methyl	$C_{29}H_{50}O_2$	430	3.36
	35.16	γ-Sitosterol	$C_{29}H_{50}O$	414	13.87
	37.99	Stigmastane-3,6-dione, (5à)-	-	428	4.79

RT: retention time; MW: molecular weight.

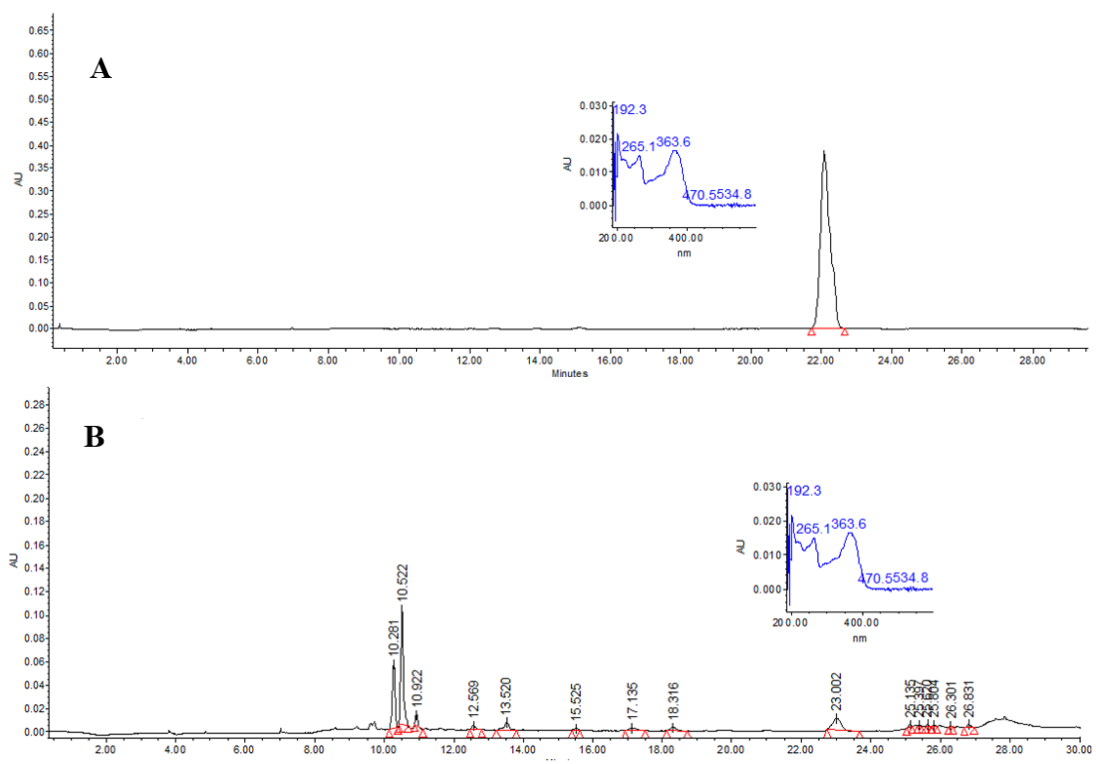

Figure 3: HPLC chromatogram of kaempferol standard (A) and MeOH extract hydrolyzed (B).

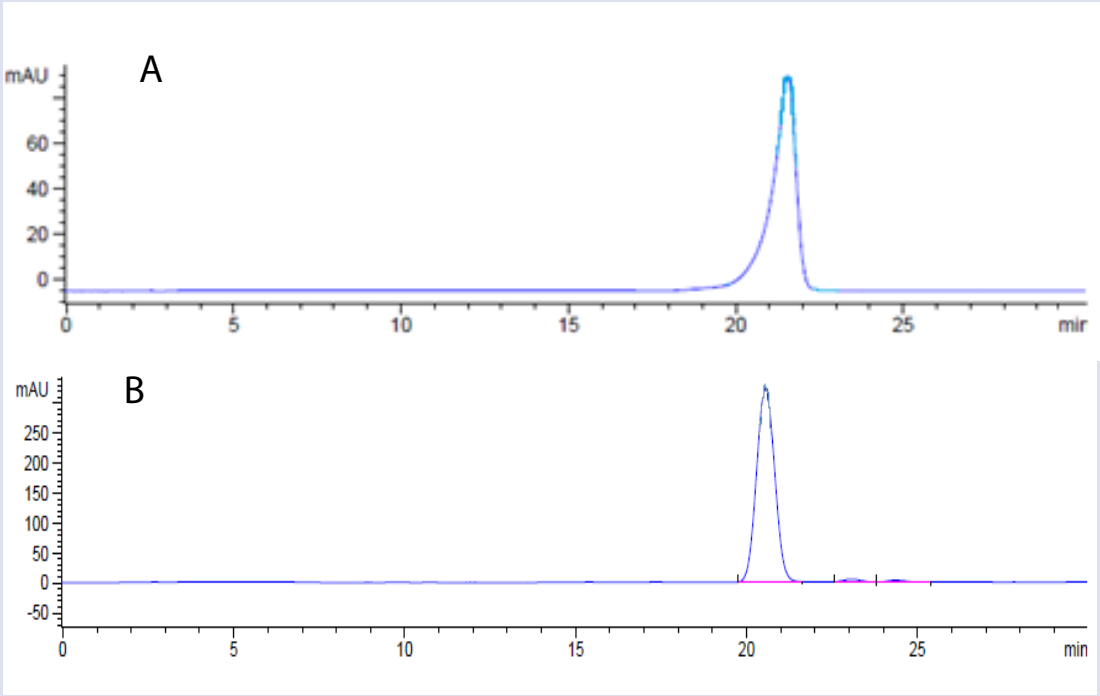

Figure 4: HPLC chromatogram of scopoletin standard (A) and MeOH extract hydrolyzed (B).

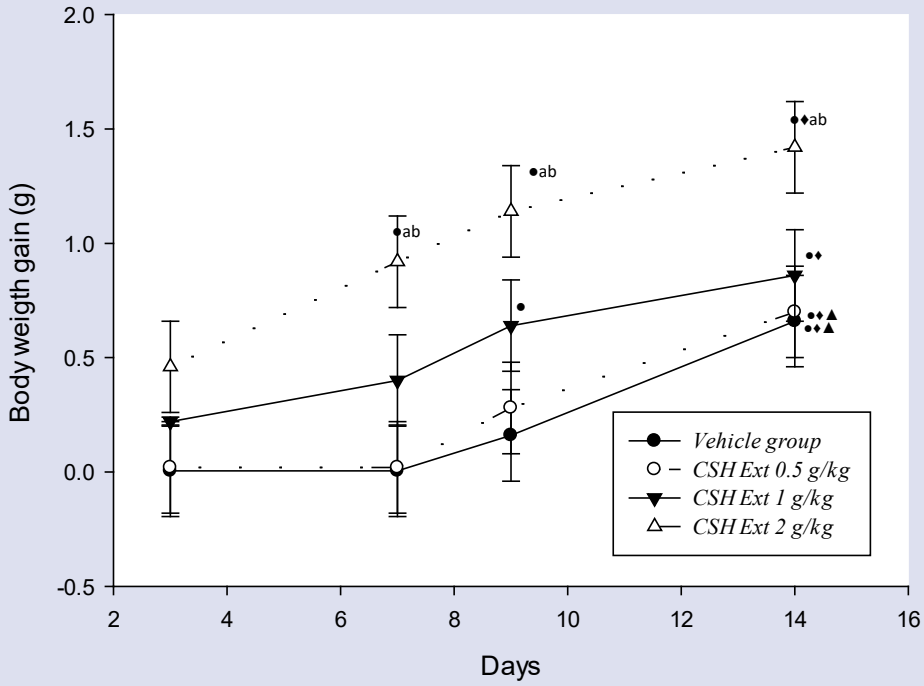

Figure 5: Effect of CSH extract from *Cleoserrata serrata* on the BW gain in a single dose intragastric administration on Balb/C male mice. Data presented as mean (±) standard error (s.e.). Statistical analysis two-way ANOVA, post hoc SNK test (p≤0.05); avs Vehicle group; bvs 0.5 g/kg; cvs 1 g/kg; ●vs Day 3; ♦ vs Day 7; ▲vs Day 9; n=5 per group.

Carrageenan model

In Table 2, the results of anti-inflammatory activity of the CSH, Hex, CH_2Cl_2, and MeOH extracts are described, determined at 5 h. Only, the Hex extract and *p*CSH exhibited a dose-dependent effect with ED_{50}= 131.46 and 64.89 mg/kg, respectively. On the other hand, the CSH extract inhibited the plantar oedema with 60.83, 34.49 and 45.66 % inhibition at 300, 150 and 50 mg/kg, respectively. The CH_2Cl_2 and MeOH extracts from *C. serrata* (aerial parts) were more active at lower doses (50 to 150 mg/kg) with 41 to 52 % inhibition, respectively. However, this effect was not dose dependent for the three extracts (Table 2).

Some primary fractions (B, C, D, I, K, M, Ñ, P and Q) obtained from the CSH extract were tested at 150 mg/kg. The percentage of inhibition was ≥23 % for D, I, K, M, Ñ, Q and P (Table 3) and the primary fractions B, and C exhibited less anti-inflammatory activity (≤16 %) with respect to Ind. This reference drug at 10 and 20 mg/kg showed 65.85 and 53.82 % of inhibition. In this biological test, different doses

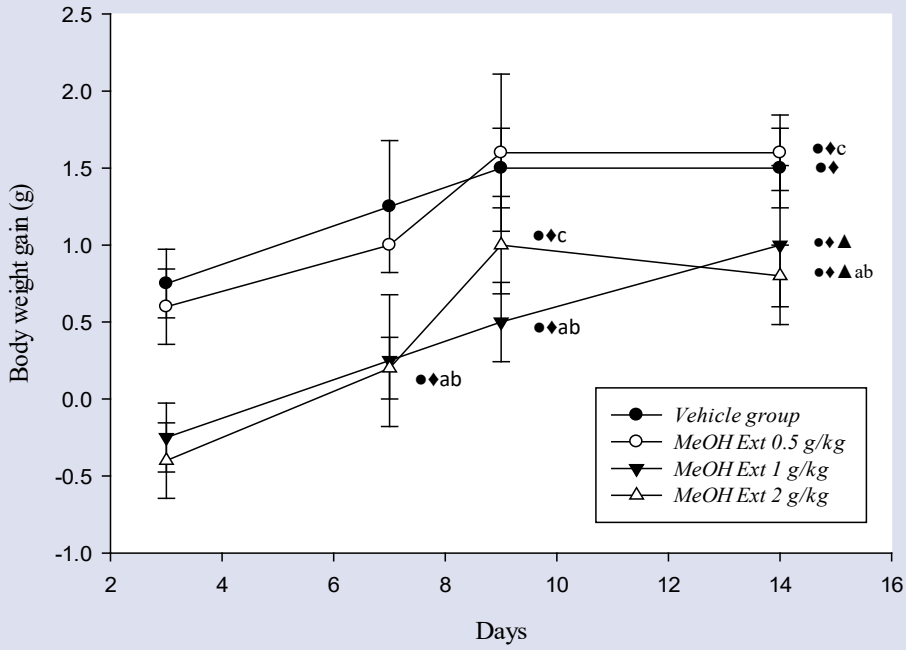

Figure 6 : Effect of MeOH extracts from *Cleoserrata serrata* on the BW gain in a single dose intragastric administration on Balb/C male mice.

Data presented as mean (±) standard error (s.e.). Statistical analysis two-way ANOVA, post hoc SNK test (p≤0.05); avs Vehicle group; bvs 0.5 g/kg; cvs 1 g/kg; ●vs Day 3; ♦ vs Day 7; ▲vs Day 9; n=5 per group.

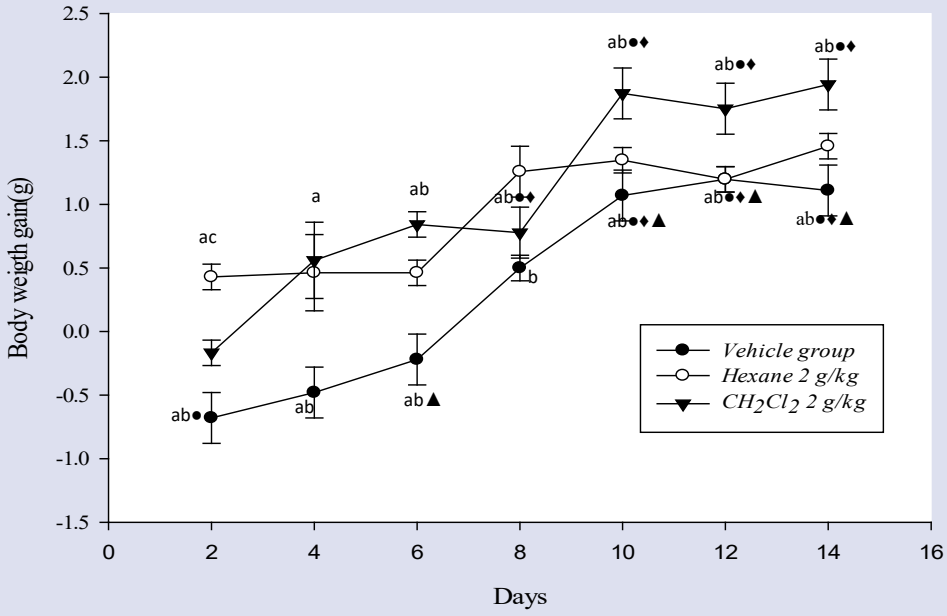

Figure 7: Effect of extracts CH_2Cl_2 and Hex from *Cleoserrata serrata* on the BW gain in a single dose intragastric administration on Balb/C male mice.

Data presented as mean (±) standard error (s.e.). Statistical analysis two-way ANOVA, post hoc SNK test (p≤0.05); avs Vehicle group; bvs Hex 2 g/kg; cvs CH2Cl2 2 g/kg; ●vs Day 4; ♦vs Day 6; ▲vs Day 8; n=5 per group.

of Ind were employed because variation in the % inhibition was found, due to the instability of this drug when it was stored at room temp.

On the other hand, some primary fractions obtained from active Hex extract (VI, IX, XIII, XV, and XXI) were tested at 100 mg/kg. Only, fraction VI showed a good anti-inflammatory activity with 55.23% inhibition, this effect that was better than Ind (42.92%) and the fractions IX, XIII, XV and XXI exhibited a poor activity with values < 27.69 % (Table 4).

Finally, the *h-MeOH* (from the hydrolysed MeOH extract) was tested at 25, 50 and 100 mg/kg, and the results are described in Table 5. This sample inhibited the inflammatory process since the third h, exhibiting 42.17, 26.05 and 23.49 % of inhibition, respectively. However, the anti-inflammatory effect observed in the fifth hour was less than that observed in the third hour, showing 30.44, 5.53 and 5.31 % of inhibition. This effect improved at the seventh hour (38.44, 25.63 and 15.02 % inhibition at 25, 50 and 100 mg/kg, respectively) and its effect was better than that of IND (13.04 %).

Table 2: Anti-inflammatory activity of *Cleoserrata serrata* organic extracts, and *p*CSH on the acute inflammation induced with carrageenan.

Treatment	Dose (mg/kg)	Paw edema formation (mm)*	Inhibition (%)	ED_{50} (mg/kg)
Carrageenan	-	0.90±0.06	-	
Ind	20	0.35±0.01[a]	60.95	
CSH extract	50	0.49±0.01[a]	45.66	
	150	0.59±0.07[a]	34.49	
	300	0.35±0.04[a]	60.83	
CH_2Cl_2 extract	50	0.49±0.11[a]	45.61	
	100	0.52±0.00[a]	41.44	
	150	0.42±0.05[a]	52.48	
Carrageenan	-	0.78±0.05	-	
Ind	18	0.40±0.06	48.07	
Hex extract	50	0.63±0.08	18.59	131.46
	100	0.51±0.07[a]	34.76	R^2=0.93
	150	0.32±0.04[a]	58.20	
MeOH extract	50	0.42±0.02[a]	42.07	
	100	0.46±0.03[a]	41.38	
	150	0.40±0.00[a]	48.26	
pCSH	50	0.41±0.03[a]	47.59	64.89
	100	0.36±0.03[a]	53.80	R^2=0.99
	150	0.32±0.05[a]	58.63	

Each group represents the mean (±) and standard error (s.e.). Statistical analysis one-way ANOVA, followed by the Dunnett post-hoc test ($p \leq 0.05$) [a]vs carrageenan; n=5. *Time: 5 hour.

Table 3: Anti-inflammatory activity of the primary fractions from CSH extract (*Cleoserrata serrata*) on the acute inflammation induced with carrageenan.

Treatment	Paw edema formation (mm)	Inhibition (%)
Carrageenan	1.19±0.02	-
Ind (10 mg/kg)	0.40 ±0.02[a]	65.85
Primary fraction from CSH extract (150 mg/kg)		
B	1.06±0.01	10.85
D	0.88±0.2	25.95
I	0.90±0.01	24.46
K	0.72±0.01	39.85
M	0.58±0.01[a]	50.92
Ñ	0.71±0.05[a]	40.60
Q	0.47±0.01[a]	59.94
Carrageenan	1.10±0.07	-
Ind (20 mg/kg)	0.51±0.02[a]	53.82
C	0.93±0.01	15.75
P	0.84±0.01[a]	23.99

Each group represents the mean (±) and standard error (s.e.). Statistical analysis one-way ANOVA, followed by the Dunnett post-hoc test ($p \leq 0.05$). [a]vs carrageenan; n=5.

Table 4: Anti-inflammatory activity of the primary fractions from Hex extract (*Cleoserrata serrata*) on the acute inflammation induced with carrageenan.

Treatment	Paw edema formation (mm)	Inhibition (%)
Carrageenan	0.66±0.04	-
Ind (10 mg/kg)	0.38 ±0.01[a]	42.92
Primary fraction from Hex extract (100 mg/kg)		
VI	0.37±0.03 [a]	55.23
IX	0.48±0.05	27.69
XIII	0.64±0.05	3.66
XV	0.49±0.05	26.68
XX1	0.53±0.06	20.97

Each group represents the mean (±) and standard error (s.e.). Statistical analysis one-way ANOVA, followed by the Dunnett post-hoc test ($p \leq 0.05$). [a]vs carrageenan; n=5.

Table 5: Anti-inflammatory activity of the hydrolyzed MeOH extract of *Cleoserrata serrata* on the acute inflammation induced with carrageenan.

Treatment	Dosis	Paw edema formation (mm) and inhibition porcentage			
	(mg/kg)	3 h	5 h	7 h	24 h
Carrageenan	-	0.61±0.02	0.72±0.02	0.79±0.02	0.51±0.04
Ind	20	0.48±0.03 **(21.7%)**	0.43±0.00 **(40.10%)**	0.69±0.03 **(13.04%)**	0.34±0.03 **(40.43%)**
h-MeOH	25	0.35±0.05 **(42.17%)**	0.50±0.05 **(30.44%)**	0.49±0.06 **(38.44%)**	0.34±0.04 **(39.58%)**
	50	0.45±0.04 **(26.05%)**	0.68±0.03 **(5.53%)**	0.59±0.06 **(25.63%)**	0.39±0.04 **(34.53%)**
	100	0.47±0.04 **(23.49%)**	0.68±0.07 **(5.31%)**	0.067±0.06 **(15.02%)**	0.35±0.05 **(41.24%)**

Table 6: Anti-inflammatory activity of *Cleoserrata serrata* organic extracts, hydrolyzed MeOH extract and precipitate pCSH on the acute inflammation induced with TPA.

Treatment	Doses (mg/ear)	Auricular edema (mg)	Inhibition %	ED_{50} (mg/ear)
TPA	-	8.34±0.23	-	
Ind	2	3.45±0.59	58.59	
CSH extract	0.5	7.38±0.5	11.51	
	1	2.78±0.34[a]	66.66	
	2	6.04±0.60[a]	27.57	
TPA	-	7.39±0.52	-	
Ind	2	3.28±0.85[a]	55.66	
CH_2Cl_2 extract	0.5	4.4±1.29[a]	40.52	
	1	3.4±0.79[a]	54.04	0.79
	2	2.22±0.31	69.92	R^2=0.99
Hex extract	0.5	4.06±0.72[a]	45.12	
	1	4.36±0.79[a]	41.07	
	2	6.4± 0.37	13.50	
MeOH extract	0.5	4.54±0.53	38.64	
	1	3.42±0.43[a]	53.77	
	2	3.94±0.77[a]	46.75	
TPA	-	6.4±0.42	-	
Ind	2	0.88±0. 06[a]	86.25	
***p*CSH**	0.5	1.92±0.52[a]	69.92	
	1	1.78±0.43[a]	72.08	0.47
	2	1.670±0.17[a]	73.82	R^2=0.99
TPA	-			
Ind			61.93	
h-MeOH	0.5	0.95	55.04	0.28
	1	1.46	65.96	R^2=0.99
	2	0.99	66.61	

Data presented as mean (±) standard error (s.e.) and indicate the percent of inhibition edema with respect to TPA group. Statistical analysis one-way ANOVA, followed by the Dunnett post-hoc test ($p \leq 0.05$) [a]vs TPA; n=5.

At 24 h this percentage of inhibition was better (reaching >34.53%), and it is noteworth mentioning that the *h-MeOH* at 100 mg/kg showed a similar anti-inflammatory effect to IND (41.24 % vs 40.43 %).

TPA model

The results obtained in this assay are described in Tables 6-8. The CH_2Cl_2 extract, *p*CSH and *h-MeOH* exhibited dose-dependent effect with ED_{50}= 0.79, 0.47 and 0.28 mg/ear, respectively and possess a better anti-inflammatory activity than Ind (ED_{50}= 1.82 mg/ear), being more active *h-MeOH*. On the other hand, the CSH extract showed 66.66 % oedema inhibition at 1 mg/ear; this activity was similar to Ind (2 mg/ear with 58.59%). Nonetheless, this inhibitory effect was not dose-dependent. The Hex and MeOH extract revealed moderate anti-inflammatory activity (>38.64 % of inhibition); however, the effect was not dose-dependent because a high dose (2 mg/ear) showed poor inhibition (Table 6).

In addition, the primary fractions B, C, I, K, M, Ñ, and P (at 2 mg/ear) showed >46 % inhibition (Table 7), and two primary fractions (D and Q) exhibited scarce (< 24.56 % of inhibition) anti-inflammatory activity.

Finally, the primary fractions III, V, VI, IX, XIII, XV and XX (from Hex extract) were also active, the results are described in Table 8. These primary fractions were tested at 2 mg/kg, and five of these showed >45 % of inhibition of the inflammatory process; but, fractions XVII and XIX showed poor activity (< 29.6 % of inhibition).

DISCUSSION

Previously, Alamilla-Fonseca et al.,[7] reported the presence of sterols, terpenes and polyphenols in the CH_2Cl_2:MeOH extract from *C. serrata* leaves by qualitative tests; however, the isolation and chemical identification of this component have not yet been described. In the

Table 7: Anti-inflammatory activity of the primary fractions from CSH extract of the *Cleoserrata serrata* on the acute inflammation induced with TPA.

Treatment	Auricular edema (mg)	Inhibition %
TPA	7.26±0.39	-
Ind (2 mg/ear)	2.87±0.66[a]	60.39
Primary fraction from CSH extract (2 mg/ear)		
B	0.82±0.38[a]	88.70
D	6.15±0.23	15.28
I	3.92±0.51[a]	46
TPA	12.82±0.65	-
Ind (2 mg/ear)	7.17±0.1.02[a]	44.05
C	3.6±0.55[a]	71.92
K	6.52±1.20[a]	49.12
M	3.15±0.69[a]	75.43
Ñ	6.67±0.59[a]	47.95
P	6.3±1.06[a]	50.87
Q	9.67±0.56	24.56

Data presented as mean (±) standard error (s.e.) and indicate the percent of inhibition edema with respect to TPA group. Statistical analysis one-way ANOVA, followed by the Dunnett post-hoc test (p≤0.05). [a]vs TPA; n=5.

Table 8: Anti-inflammatory activity of the primary fractions from Hex extract of the *Cleoserrata serrata* on the acute inflammation induced with TPA.

Treatment	Auricular edema (mg)	Inhibition %
TPA	9.37±0.86	-
Ind (2 mg/ear)	2.57±0.68[a]	72.53
Primary fraction from Hex extract (2 mg/ear)		
III	5.12±0.79[a]	45.33
V	2.97±0.49[a]	68.27
VI	1.52±0.45 [a]	83.73
IX	3.76±0.15[a]	59.82
XIII	1.45±0.32 [a]	84.53
XV	4.17±0.95[a]	55.46
XVII	6.6±0.80[a]	29.60
XIX	7.36±0.54	21.42
XX	2.7±0.53[a]	71.20

Data presented as mean (±) standard error (s.e.) and indicate the percent of inhibition edema with respect to TPA group. Statistical analysis one-way ANOVA, followed by the Dunnett post-hoc test (p≤0.05). [a]vs TPA; n=5.

CSH extract fatty acids, triterpenes, and sterols were detected as main compounds, using TLC-NP analyses, and a polyphenols mixture was also detected with TLC-RP plates and HPLC analyses.

The CSH and MeOH extracts were analyzed by HPLC to search polyphenols. In both chromatograms, some peaks were observed between 8.2 and 10 min, which correspond to polyphenols, showing typical UV bands (at 266 and 348 nm) for glycosylated flavonoids. On the other hand, in both extracts, amino acids (such as alanine, glycine, glutamine, and phenylalanine) were also detected. The TLC-NP profile of the MeOH extract was similar to Red Bull® (energy beverage), it contains taurine and L-carnitine,[20] and also contains phenylalanine, alanine, glycine and glutamine, while in the CSH extract only alanine was detected. Previously, it was described that some species of the *Cleomaceae* family are consumed in certain places in Africa and Asia as edible vegetable for their nutritional value and for their content of proteins and amino acids.[5,21,22] For example, *Cleome gynandra* leaves have 0.39 and 0.37 g of protein/g of dry weight material collected in the dry and rainy season.[23] In aqueous and saline extracts from *Corynandra viscosa* (Syn. *Cleome viscosa*) seeds DL-aspartic acid, L-hydroxy proline, L-proline, DL-serine, 3-DL-alanine, DL-2-amino-N-butyric acid and L-tyrosine were reported.[24] In *Gynandropsis gynandra (L.) Briq* (Syn *Cleome gynandra L.*) oil seeds the presence of protein (29.5%), lipid (27.7%) and some amino acids, such as glutamic acid, arginine, aspartic acid, lysine, tyrosine, and histidine were described. This amino acid profile is comparable to that of the content of the oil from leguminous seeds.[25] To our knowledge, *Cleoserrata serrata* is only used as a medicinal species in south-eastern Mexico and to date the presence of amino acids in this medicinal species had not been described. In the CSH and MeOH extracts, we also reported for the first time the presence of carbohydrates such as sucrose.

Continuing with the chemical screening, in the active Hex extract (by GC-MS analyses) 9,12-octadecadienoic acid ethyl ester, γ-sitosterol, vitamin E and ethyl palmitate as the main compounds were identified. This extract was chemically fractionated and in primary fractions XV to XX, *β*-sitosterol was detected as a main compound. The primary fraction FXV was sub fractionated by CC-NP, because it primary fraction was obtained at a good amount, and four secondary fractions groups (FXVa-d) were obtained. In FXVa, β-amyrin, lupeol acetate, β-amyrin acetate and stigmastan-3,5-diene were identified. In addition, ethyl palmitate and palmitic acid were also obtained from fraction FV-d. In FV-b and FV-c, β-sitosterol was detected as main compound. β-Amyrin, lupeol acetate, β-amyrin acetate, stigmastan-3,5-diene and β-sitosterol were identified by comparison of the Rf value with their respective standards previously isolated from *Cnidoscolus tehuacanensis* and *C. chayamansa* leaves. Some of these compounds possess significant anti-inflammatory activity.[26,27] These compounds have been previously described for *Gynandropis gynandra.*[28,29] but not for *C. serrata*. In the CH_2Cl_2 extract, γ-sitosterol (main compound), ethyl palmitate, palmitic acid, vitamin E and stigmasterol (minority components) were also identified as a main constituents.

On the other hand, in primary fractions (B, C, G, K and M) obtained from the CSH extract, the same compounds, as well as phytol and phytol acetate were found as main constituents. It is noteworthy that phytol has been reported in the essential oil of the aerial parts from *Cleoserrata serrata* (collected in India);[5,10] it has also been described in *Cleome monophyla*.[30]. Therefore, this is the first report that describes the presence of this type of compound (except for phytol) in the organic extract from *C. serrata.*

In *p*CSH obtained from more polar primary fractions (Ñ, O, P and Q) of the CSH extract and from the MeOH extract, a polyphenols mixture was detected by TLC-RP and HPLC analyses. In the UPLC-DAD-MS/MS chromatogram four peaks were observed. The main peaks observed (R*t* = 19.4 min) showed a *m/z* = 755.20 ($C_{33}H_{39}O_{20}$) and 609 (this fragment indicates the loss of sugar in C-7), and a typical UV wave (λ = 264 and 348 nm) which corresponds to glycosylate flavonoids; this is most likely a kaempferol-glycoside derivate, these UV wave indicates the presence of a kaempferol nucleus with carbohydrates at C-3 and C-7.[31] The most likely chemical structure of the main compound present in the MeOH extract and in *p*CSH is kaempferol-3-sophoroside-7-glucoside. at present, we are in process of isolation and identification, in order to be able to establish the structure unequivocally. Because so far, we only have the ^{1}H-NMR spectrum and in this we observe signals for the kaempferol nucleus and signals for the anomeric proton of three sugars; however, with this data we can partially establish the chemical structure. In this context, Fushiya et al.[32] reported two bioactive flavonoids isolate from the MeOH extract of the *Cleome droserifolia* aerial parts. These flavonoids were identified as 5,4'-dihydroxy-6,7,8,3',5'-pentamethoxiflavone with λ at 276 and 333 nm (*m/z* 404.11, $C_{20}H_{20}O_9$) and 5,4'-dihydroxy-6,7,8,3'-tetramethoxyflavone with λ at 275 and 342 nm (*m/z* 374.10, $C_{19}H_{18}O_8$). In addition, the main metabolites described in several species of the *Cleomaceae* family are phenolic compounds: cumarinolignoids, polymethoxyflavonols, glucoflavonoids, flavanones or flavones and their glycosides.[1,33]

On the other hand, the MeOH extract (that contains a polyphenols mixture, *p*CSH) was hydrolysed and in the *h-MeOH*, kaempferol, quercetin and scopoletin were identified by TLC-NP and HPLC analyses. It should be mentioned that the main peak with Rt = 8.44 min observed in the HPLC chromatogram from the MeOH extract disappeared when this extract was hydrolyzed and when it was analyzed under the same HPLC conditions. Some glucopyranosides of kaempferol have been described in species of the *Cleomaceae* family: from *Cleome arabica* 3-*O*-glucosyl-7-*O*-rhamnosyl-, 3-7-di-O-rhamnopyranosi-des, and 3-0-glucopyranosides of kaempferol have been isolated.[34] Kaempferol-4'-methoxy-3,7-*O*-di-rhamnoside was obtained from *C. droserifolia* and cleomeside K, L, and M were obtained from *Corynandra chelidonii* (Syn. *Cleome chelidonii*).[34-36] To our knowledge, it is the first report that describes the presence of scopoletin and glucopyranosides of the kaempferol in *C. serrata.*

With respect to antioxidant activity, only *p*CSH and the *h-MeOH* showed a moderate antioxidant activity, and the CSH and MeOH extracts exhibited weak antioxidant activity with respect to standard quercetin. This antioxidant activity for *C. serrata* was low compared to that of other genus (*Cleome* and *Corynandra*), which belong to *Cleomaceae* family.[37] For example, the aqueous and EtOH extracts from *Cleome rutidosperma* (leaves) showed 53.13 and 57.13% DPPH inhibition, respectively, at 250 µg/mL.[38] In addition; the EtOH extract from the aerial parts and roots of *Corynandra chelidonii* (Syn. *Cleome chelidonii* L.f.) collected in the Materu region (India) showed an IC_{50} = 1525 and 850.50 µg/mL, respectively.[39] The MeOH extracts of *Cleome iberica* showed an IC_{50} = 120.25 µg/mL in the DPPH assay,[40] and the MeOH 70 % extract from the leaves and stem of *Corynandra viscosa* (Syn. *Cleome viscosa*) showed IC_{50} = 373.18 and 511.10 µg/mL, respectively.[41] Meanwhile, *Cleome arabica* (EtOH extract from leaves) showed an IC_{50}= 90 µg/mL and the root extract had an IC_{50}= 970 µg/mL.[42] To date, the antioxidant activity for *C. serrata* has not been described, and the results obtained in this work indicate that this species showed poor antioxidant activity because the polar extracts from this medicinal plant contain principally glycosylated flavonoids; however, the *p*CSH showed a better activity due to its high contents of polyphenol compounds. Additionally, the *h-MeOH* (hydrolyzed sample from MeOH extract) showed better antioxidant activity because it contains free flavonoids such as kaempferol, quercetin and also contains scopoletin, these two compounds have significant antioxidant activity.

Regarding the biological evaluation, the CSH extract inhibited the growth of *M. tuberculosis* MTY-137 and *M. tuberculosis* H37Rv (MIC <25 µg/mL) and was moderately active against *M. tuberculosis* H37Rv resistant to ethambutol and MDR *M. tuberculosis* SIN-4 exhibited (MIC = 50 µg/mL). This is the first work that describes the antimycobacterial activity of *C. serrata* and this species constitutes an important source of antimycobacterial compounds. With regard to the leishmanicidal activity, the CSH extract was more active against amastigotes of *L. mexicana*. Previously, has been described that CH_2Cl_2:MeOH extract showed an LD_{50} = 23.2 and 6.11 µg/mL against the promastigotes and amastigotes of *L. mexicana*.[7] With these results, we are able to confirm that the organic extract possesses good leishmanicidal activity.

To date, the LD_{50} values for organic or aqueous extracts of *Cleoserrata serrata* have not been described. The Hex, CH_2Cl_2, MeOH and CSH extracts showed a LD_{50} >2 g/kg in Balb/C male mice. Only, the Hex and CH_2Cl_2 extracts favored the BW gain, this increase was similar to that the VEH group. LD_{50} values allow these extracts to be considered within Category 5 of substances according to OECD TG 423.[43] Elufioye and Onoja[44] described an increase in BW in ICR mice (male and female) treated with the MeOH extract of the *Corynandra viscosa* (Syn. *Cleome viscosa*) whole plant, when it was administered at 10, 100, 1000, 2900 and 5000 mg/kg by i.g. route with single doses; the mice were observed during 14 days and, at the end of the experiment period the BW was registered. The BW values were 18.6, 22.0, 22.6, 30 and 28 g, respectively, with regard to the VEH group (19 g). In addition, these authors did not find any abnormalities in organ relative weight, and nor lethality registered. The authors concluded that this behavior in terms of the BW increase was due to the nutritional value of the plant. This medicinal plant is used to treat fever, inflammation, liver disease, bronchitis and diarrhea in the Ayurvedic system.

Regarding the topic and systemic anti-inflammatory activity of the *C. serrata*, this manuscript constitutes the first report. In the carrageenan model (systemic effect), the Hex extract and *p*CSH exhibited a good anti-inflammatory activity (showing ED_{50}= 131.46 and 64.89 mg/kg), and the *p*CSH showed a better activity with respect to CSH and MeOH extracts. The CSH extract inhibited the plantar oedema, showing 60.83% of inhibition a high dose (300 mg/kg). On the other hand, the CH_2Cl_2 and MeOH extracts from *C. serrata* were more active at lower doses (<150 mg/kg) showing > 40% of inhibition, but this effect was not dose-dependent. The anti-inflammatory activity of the Hex and CH_2Cl_2 extract is due to content of the lipophilic compound (such as β-amyrin, lupeol acetate, β-amyrin acetate, stigmastan-3,5-diene, β- or γ-sitosterol and other) and the activity observed by *p*CSH and MeOH extract is due to the presence of the polyphenol mixture (kaempferol and quercetin-glycoside derivate) and other compounds such as scopoletin, amino acids and carbohydrates.

The CSH extract was fractionated and some primary fractions showed a better anti-inflammatory activity that the original extract when was tested at 150 mg/kg (≥23 % of inhibition). These fractions have a lipophilic compound (such as stearic acid, palmitic acid, β-sitosterol, phytol acetate and stigmastan-3,5-diene) as a main compound, and from polar primary fractions Ñ-Q a *p*CSH (polyphenol mixture) was obtained, this sample contain some kaempferol-glycoside derivate (perhaps kaempferol-3-sophoroside-7-glucoside). Previously, has been described that phytol possess anti-inflammatory activity.[11] It is also well known that some genus from the *Cleomaceae* family, such as *Cleome, Corynandra, Gynandropsis,* and *Tarenaya* showed acute anti-inflammatory activity on this model, and some authors have described that polyphenols are responsible for this biological activity.[5] From the MeOH (85%) extract obtained from the fresh flowers of *C. viscosa*, quercetin-3-O-(2"-acetyl)-glycoside was isolated and showed a good anti-inflammatory activity in the carrageenan model at doses of 100 and 200 mg/kg administered by i.p. route, showing 51.7 and 45% inhibition (at 3h).[45] Other glucoflavonoids (5,4´-dihydroxy-6,7,8,3´,5´-pentamethoxyflavone and 5,4´-dihydroxy-6,7,8,3´-tetra-methoxyflavone) isolated from *Cleome droserifolia* and cleomiscosin A-C isolated from *C. viscosa* have been described as anti-inflammatory compounds,[32,46] but these compounds were tested in another inflammatory assay. The polyphenols mixture (*p*CSH) present in the CSH and MeOH extracts from *C. serrata* may contribute to the anti-inflammatory properties, although the anti-inflammatory activity is also due to the content of lipophilic compounds.

Finally, the *h-MeOH* (hydrolyzed MeOH extract) showed a significant anti-inflammatory activity (>30% of oedema inhibition) at low doses (25 mg/kg), in this sample kaempferol, quercetin and scopoletin were detected; the anti-inflammatory activity for these compounds is well described.[19] The effect of this sample was better that IND (21.7 % of inhibition) at the same time.

In the topic model (TPA assay), the CH_2Cl_2 extract, *p*CSH, and *h-MeOH* were the most active showing an ED_{50}= 0.79, 0.47 and 0.28 mg/ear, respectively. The latter sample was the most active and it contain kaempferol, quercetin and scopoletin as a main constituent. Previously, has been described that these compounds possess significant anti-inflammatory activity.[19] In addition, the CSH extract showed a similar percentage of inhibition that Ind (66.66 % vs 58.59% at 1 mg/ear), but

the effect was not dose-dependent. The Hex extract exhibited moderate anti-inflammatory activity (>41.07 % of inhibition at 0.5 and 1 mg/ear); however, this effect was not dose-dependent, because a high dose (2 mg/ear) showed a poor inhibition (13.50 %). The MeOH extract also showed anti-inflammatory activity (>38.64 % of oedema inhibition at three doses tested); but this effect was also not dose-dependent.

Additionally, primary fractions (B, C, I, K, M, Ñ, and P, tested at 2 mg/ear) from the CSH extract were active, showing >46 % inhibition and only two primary fractions (D and Q) exhibited poor anti-inflammatory activity. These primary fractions were active as the original extract. The good anti-inflammatory activity of these fractions is due to the lipophilic compounds (such as phytol, phytol acetate, ethyl palmitate, 9,12-octadecadienoic acid ethyl ester, β-sitosterol, palmitic acid and others) present in this extract, plus the polyphenol mixture. Previously, it has been reported that this type of compounds exhibited good topic anti-inflammatory in *in vivo* models (TPA, croton oil-induced ear oedema).[11,47,48]

Finally, the anti-inflammatory activity in the TPA assay (topic model) for the Hex extract and from its primary fraction is due to the presence of the lipophilic compounds such as: lupeol acetate, β-amyrin acetate, β-amyrin, phytol, phytol acetate, stigmastan-3,5-diene, and β-sitosterol; these were the main constituents. Previously, the anti-inflammatory activity of this type of compounds has been described.[5,10,11,16,19,26,27] To our knowledge, this is the first paper that describes a partial chemical profile of the organic extracts of the *Cleoserrata serrata*. The anti-inflammatory, and leishmanicidal activities for these extracts validates the use in traditional medicine of the *Cleoserrata serrata*. Furthermore, we are describing the antimycobaterial activity herein, to our knowledge for the first time for this species.

Based on the results obtained, we will continue with the isolation and chemical identification of glucoflavonoids; which can serve as biomarkers of the active extract. In addition, we will carry out the anti-inflammatory evaluation in the chronic inflammation model of the more active extracts and it is also necessary to determine the subacute toxicity of each extract. This paper is a contribution to the study of medicinal plants and their importance in the search for bioactive substances.

CONCLUSION

Cleoserrata serrata is used in Mexican traditional medicine to treat cutaneous leishmaniasis and, also is used in inflammatory disease. All extracts and *p*CSH exhibited a good anti-inflammatory activity, being more active the *h-MeOH*. On carrageenan assay, the Hex extract and *p*CSH showed ED_{50} = 131.46 and 64.89 mg/kg and the CSH, CH_2Cl_2 and MeOH extracts were moderately active (>34.49 % of inhibition). Primary fractions from CSH and Hex extracts also showed a good anti-inflammatory activity. On TPA assay, *h-MeOH* was more active that Ind, and CH_2Cl_2 extract, *p*CSH showed a good topic anti-inflammatory effect. The CSH, Hex and MeOH extracts were also active but their effect was no dose-dependent. Primary fractions from CSH and from Hex extracts showed a better topic anti-inflammatory effect.

The Hex and CH_2Cl_2 extracts and their primary fractions (with low- and medium-polarity from the CSH and the Hex extract) contain triterpenes, fatty acids and sterols such as: β- and γ-sitosterol, β-amyrin, lupeol acetate, β-amyrin acetate, stigmastadiene phytol, phytol acetate, β- and γ-sitosterol, 9,12-octadecadienoic acid ethyl ester, vitamin E, ethyl palmitate and palmitic acid as a main constituent. In the *p*CSH sample a kaempferol glycoside derivate, some amino acids and carbohydrates were detected. In active *h-MeOH*, kaempferol, quercetin and scopoletin were identified, and in carrageenan assay it showed a better anti-inflammatory effect at 25 mg/kg from 3 h to 24 h (a similar effect to IND), although this effect was not dose-dependent, however at 24 h this effect remained. In TPA assay, this sample showed a significant ED_{50} (0.28 mg/ear).

CSH extract showed anti-inflammatory effect and also, moderate antimycobacterial and leishmanicidal activities; therefore, it is the most recommended to continue investigating because it contains lipo- and hydrophilic compounds such as triterpenes, sterols, fatty acids, some amino acids, as well as kaempferol glycoside derivate. All extracts showed a LD_{50} > 2 g/kg in Balb/C mice and CSH extract favored the body weight gain. Respect to antioxidant activity, only *p*CSH, and *h-MeOH* were moderately active. This is the first report that describes phytochemical profile and anti-inflammatory effect as well as the DL_{50} and their antimycobacterial activity for this medicinal species.

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COMPETING INTERESTS

This study is taken in part from the Ph.D. thesis of MdC. Juárez-Vázquez (Doctorado en Ciencias Biológicas y de la Salud, UAM). All authors have read and approved the final version of the manuscript and declare that they have no conflicts of interest.

AUTHORS´CONTRIBUTION

MdelCJ and RLD were the responsible for the experimental work (technical work, interpretation of the results, collect material vegetal, statistical analyses, and elaboration/revision of the manuscript) of the research project; JLH and LYM carried out the antimycobacterial and leishmanicidal assays; AZ and ATL performed the HPLC analysis; MMV and FAA collaborated in the preparation/revision of the manuscript; MAJA is responsible of research project, elaboration of the research project and preparation/revision of the manuscript.

ETHICS APPROVAL

Balb/C mice were provided by the IMSS Bioterium. The animal experiments were performed following the statutes of the International Committee for the Care and Use of Laboratory Animals (IACUC) and Mexican Official Norm (NOM-062-ZOO-1999) revised in 2016. The protocol was approved by the National Committee of Scientific Research form IMSS (CNIC R-2018-785-059). After each biological assay, the mice were sacrificed by cervical dislocation and incinerated in the IMSS Bioterium.

CONFLICTS OF INTEREST

All authors read and approved the research protocol and agreed to participate in its development**.**

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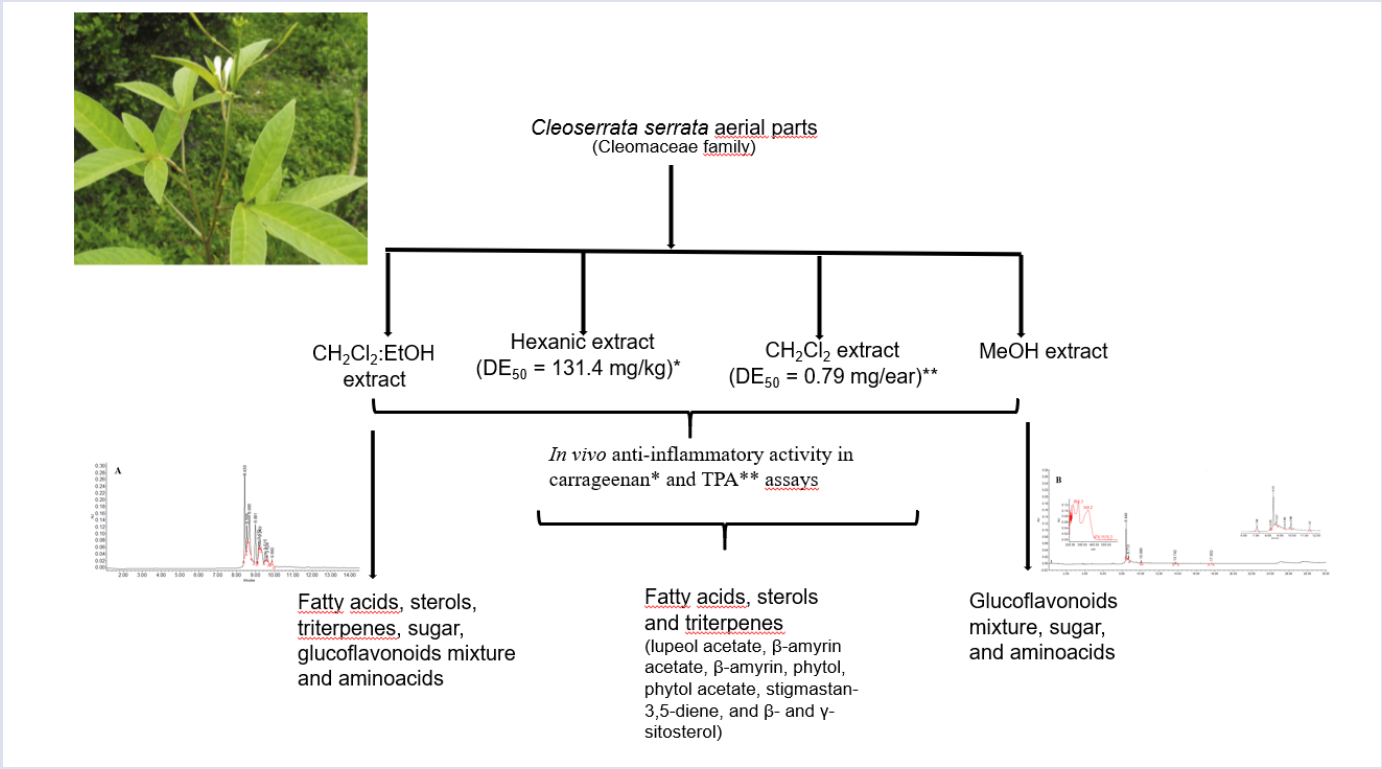
GRAPHICAL ABSTRACT



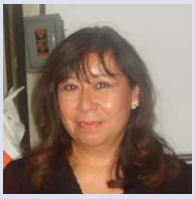
ABOUT AUTHORS

María del Carmen Juárez-Vázquez studied Biology at the Facultad de Estudios Superiores Zaragoza, Universidad Nacional Autónoma de México. She has postgraduate studies in Ciencias Biológicas, UNAM and is currently doing his PhD in Ciencias Biológicas y de la Salud, UAM. Her research focuses on the search for anti-inflammatory and immunomodulatory alternatives from medicinal plants. Their results are published in some international journals.

Adolfo López-Torres received his PhD in Chemistry from the Universidad de Guanajuato, Mexico in 2014 developing different methods to determinate global DNA and RNA methylation using HPLC-FLD and GC-MS. Adolfo is currently Lecturer in Analytical Chemistry at Universidad del Papaloapan, Mexico. His current research projects are focused on developing methodologies for metabolomic and proteomic analysis using HPLC-MS in organisms of biotechnological and biomedical interest.

Alejandro Zamilpa Researcher in the Department of Phytochemistry, CIBIS-IMSS. Specialization in the field of isolation and chemical caracterization of bioactive compounds from medicinal plants. Has published several research papers on the solution of the mexican prioritary helth problems.

Dr. Lilián Yépez-Mulia Full time Researcher, Unidad de Investigación Médica en Enfermedades Infecciosas y Parasitarias. Research Lines: Evaluation of new giardicidal, trichomonicidal, leishmanicidal and trypanocidal compounds, characterization of their mechanism of action. Evaluation and Characterization of the activity of new tubulin despolimeration agents in cancer lines.



Dr. Julieta Luna Herrera Department of Immunology, Professor at Escuela Nacional de Ciencias Biológicas of Instituto Politécnico Nacional, México City, since 1995. Graduated in Chemistry Bacteriology and Parasitology. Master and Doctor in Science in Immunology. Posdoctoral research associate at Mycobacteriology Research Laboratory at the University of Illinois at Chicago from 1991-1995. Current research on antimycobacterial and antinocardia activities of medicinal plants and new compounds. Studies on immunomodulatory activities of medicinal plants and new compounds. Studies on drug resistant mechanisms in mycobacteria and nocardia.

Rosalba León-Díaz has a Ph.D. in Sciences, in the area of toxicology and experimental pathology, from the National Autonomous University of Mexico. She is currently a researcher at the Universidad Veracruzana, in the Biological Research Institute.

Professor Mariano Martínez-Vázquez currently works at the Institute of Chemistry of the National Autonomous University of Mexico. His main lines of research are to obtain possible drugs from nature with anticancer properties and against drug-resistant bacteria. So far, he has published 143 articles in international journals. He has been Visiting Professor at the Universities of Padova in Italy and Loja in Ecuador.

Francisco Javier Alarcón Aguilar, Ph.D. Titular Professor. Pharmacology Laboratory, Health Science Department, D.C.B.S. Universidad Autónoma Metropolitana. México. Research topic: Pharmacology of medicinal plants and diseases related to metabolic syndrome. Aim: To perform pharmacological chemical research of natural products to a mechanistical level, incurring into the rational design of computer-assisted drugs and in general implementing cutting-edge techniques that allow the elucidation of its mechanisms of action at the molecular level. Achievements until July 2021: Research articles 1011; Thesis of grade: 50; International specialized scientific events 125; Upgrade courses 10; Awards 32; Citations to articles: more than 3500. Patents: 1.

Maria Adelina Jiménez Arellanes, I am a senior researcher at the Medical Research Unit in Pharmacology of the Hospital de Especialidades CMN Siglo XXI, IMSS. The main line of research is explore the hepatoprotective and antitubercular potential of medicinal plants, their acute and subacute toxicity. She focuses on obtaining and identifying bioactive compounds from medicinal plants.

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