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Antimicrobial, anti-inflammatory, antiparasitic, and cytotoxic activities of *Galium mexicanum*

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ABSTRACT

Ethnopharmacological relevance: To study the potential benefit of the traditional Mexican medicinal plant *Galium mexicanum* Kunth (Rubiaceae). Hexane, chloroform, and methanol extracts as well as various fractions from these extracts were tested to determine antibacterial, antifungal, antiparasitic or anti-inflammatory activities in vitro.
Materials and methods: Aerial parts of the plant were extracted with various solvents and fractionated accordingly. Their antibacterial and antifungal activities were assessed on nine bacterial and four fungal strains. *Leishmania donovani* was used as a protozoan strain for antiparasitic activity. The anti-inflammatory activity of the compounds was investigated by measuring the secretion of interleukin-6 when macrophages were exposed to lipopolysaccharide.
Results: Various extracts and fractions obtained from this plant exhibit antibacterial, antifungal, antiparasitic, and anti-inflammatory activities. Of special interest was the hexane fraction HE 14b, which show antibacterial (ranging between 67 and 666 μg/ml) and antifungal (at concentrations of 333 μg/ml) activities. Also the hexane fraction HE 5 exhibited antiparasitic activity (at concentrations of 260 μg/ml), whereas the methanol fraction ME 13–15 showed a potent anti-inflammatory activity when compared to dexamethasone. Chemical analyses of the chloroform extract show the presence of triterpenes, saponins, flavonoids, sesquiterpene lactones, and glucosides, but no tannins were detected in the assayed extract.
Conclusions: The benefit of *Galium mexicanum* as a traditional medicinal plant was confirmed using antibacterial and antifungal assays in vitro. We also report for the first time, and to the best of our knowledge, antiparasitic and anti-inflammatory activities of this plant.

1. Introduction

Plants have been used medicinally all over the world for many centuries. The genus *Galium* (Rubiaceae), comprised of approximately 1300 species (Tropicos, 2010), has been used in the folk medicine of many cultures for the treatment of a variety of pathological conditions. In China, *Galium verum* is used to treat hepatitis and phlebophlogosis (Jiangsu, 1977), while in Central Serbia the same species is used to treat skin infections (wounds and acne), as a sedative, and as a diuretic (Jaric et al., 2007). Another species, *Galium tricornutum* subsp. *longipedunculatum* has been also used to treat skin infections, for treatment of kidney disorders, and as a diuretic and analgesic in North Pakistan (Shah et al., 2006). It is worth noting that although these countries are far apart, these *Galium* species have been used to treat similar disorders.

Compounds isolated from the genus *Galium* include iridoid glucosides and iridoidic acids (Iavarone et al., 1983; Uesato et al., 1984; Handjieva et al., 1996; Serrilli et al., 2008; De Rosa et al., 2000; Deliorman et al., 2001), triterpene saponins (De Rosa et al., 2000), anthraquinones (Halim et al., 1992; Koyama et al., 1993; Banthorpe and White, 1995; El-Gamal et al., 1995; Zhao et al., 2006), and flavonoids (Zhao et al., 2006).

Galium mexicanum Kunth is an endemic perennial and climbing herb from Mexico. This plant is used in Mexican folk medicine to

Abbreviations: ATCC, American type culture collection; BSA, bovine serum albumin; CEE, chloroform extract; ELISA, enzyme-linked immuno sorbent assay; FCS, fetal calf serum; HEE, hexane extract; HRP, horse radish peroxidase; IL-6, interleukin-6; LPS, lipopolysaccharide; MEE, methanol extract; PBS, phosphate buffered saline; PBS-T, phosphate buffered saline supplemented with 0.05% Tween-20; PI, propidium iodide; PMA, phorbol myristate acetate.

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treat diarrhea, stomach infections, pain and inflammation of the digestive system, stomach cleansing, chest pain, and skin diseases (Aguilar et al., 1994; INI, 1994a). Toasted and grinded leaves and stems are used for treatments, as are infusions prepared with aerial parts of the plant, which are used to wash the skin (INI, 1994b,c).

In the current study, we evaluated the presence of antimicrobial components in extracts and fractions obtained from the aerial parts of *Galium mexicanum*. In addition, we tested their anti-inflammatory and cytotoxic activities to evaluate their potential use as antibiotics. We also report antiparasitic properties in two hexane fractions obtained from this genus.

2. Materials and methods

2.1. Plant material

Galium mexicanum Kunth was collected at 2550 m above sea level in San Jose del Pacífico, Oaxaca, México (16°10′22″N 96°30′W) in September 2008. A voucher specimen numbered 14467 was deposited in the Herbarium of the Benemérita Universidad Autónoma de Puebla, México.

2.2. Preparation of plant extracts

A total of 133 g air-dried aerial parts of the plant were sequentially extracted with *n*-hexane (hexane), chloroform, and methanol after macerating the material in each solvent in three rounds for 48 h. Following filtering and solvent volatilization, the hexane, chloroform, and methanol extracts (HEE, CEE, and MEE, respectively) yielded 0.67 g (0.5%), 0.63 g (0.47%) and 20.77 g (15.62%) of residue, respectively. The extracts were chromatographed on silica gel (70–230 mesh) using 190 ml of pure or combined solvents and according to Fig. 1. A total of 84 fractions corresponding to all of the three extracts were collected and combined according to their TLC profile. Four hexanic fractions [HE (0.05 g), HE 5 (0.06 g), HE 13–14a (0.05 g), HE 14b (0.19 g)], four chloroformic fractions [CE (0.09 g), CE 5–9 (0.10 g), CE 10 (0.42 g), CE 11 (0.02 g)], and six methanolic fractions [ME (0.2 g), ME 13–15 (0.05 g), ME 17–21 (0.125 g), ME 27–28 (0.21 g), ME 29–34 (1.23 g), ME 40–41 (0.1 g)] were further analyzed for their bioactivity properties. Fractions were dried in a vacuum using a rotary evaporator and stock solutions were prepared by dissolving 20 mg of each fraction in 100 μl DMSO, following sonication (Branson 3210) for 60 min at 30 °C until the material was dissolved.

2.3. Chemical analysis of the extracts

2.3.1. Triterpenes

The presence of these compounds was determined by dissolving 10 mg of the CEE in 1 ml chloroform. The solution was supplemented with 1 ml of acetic anhydride and 2 drops of H_2SO_4. The appearance of a red, pink, green, purple or blue coloration in the interface indicates the presence of triterpenes (Domínguez, 1973).

2.3.2. Saponins

Two different methods were used to determine the presence of saponins. In the first one, 10 mg of CEE was mixed with hot water and the mixture was shaken for 30 s. The formation of stable foam indicates the presence of saponins. In the second method, a drop of Rosenthaler reagent (1 g of potassium arsenate dissolved in 100 g H_2SO_4) was added to 10 mg CEE. The appearance of a violet coloration indicates the presence of saponins (Domínguez, 1973).

2.3.3. Flavonoids

The presence of these compounds was evaluated using two methods. The first method was performed by adding 100 mg of zinc or magnesium powder to 10 mg of CEE and three drops of HCl. The appearance of a red coloration indicates the presence of flavonoids (Domínguez, 1973). In the second method, three drops of NaOH were added to 10 mg of CEE, the appearance of a yellow or orange colour indicates de presence of flavonoids (Domínguez, 1973).

2.3.4. Tannins

10 mg of CEE was dissolved in 1 ml chloroform. The mixture was filtrated and separated in two portions. The presence of tannins was evaluated by the formation of a precipitate either by adding 100 mg $FeCl_3$ or gelatine reagent [(1% gelatine (w/v)] (Domínguez, 1973).

2.3.5. Cardiotonic glucoside and sesquiterpene lactones

Two methods were used to determine the presence of these compounds. In the first method, 10 mg of chloroform extract was dissolved in 1 ml chloroform. Three drops of Baljet reagent (1% ethanolic solution of picric acid and 10% aqueous solution of NaOH) was added (Domínguez, 1973). Formation of a red or orange coloration indicates the presence of these compounds. In the second method, 10 mg of chloroform extract was dissolved in 1 ml chloroform. Three drops of pyridine, one drop of 5% sodium nitroprusside, and three drops of 10% aqueous solution of NaOH were added sequentially. A red coloration indicates the presence of cardiotonic glucosides and sesquiterpene lactones

2.4. Strains and culture media

The bacterial strains used in this study included the Gram-negative strains *Acinetobacter baumannii* (ATCC BAA-747), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 14210), and *Salmonella typhimurium* (ATCC 13311). *Bacillus subtilis* (ATCC 6633), *Mycobacterium smegmatis* mc^{2}155 (ATCC 700084), *Staphylococcus aureus* (ATCC 25923), Methicillin-Resistant *Staphylococcus aureus* (MRSA) (ATCC 700698), and *Streptococcus pyogenes* (ATCC 51878) were used as representatives of Gram-positive bacteria. *Aspergillus fumigatus* (ATCC 1022), *Candida albicans* (provided by Vancouver General Hospital, British Columbia, Canada), *Cryptococcus neoformans* var. *grubii* (kindly provided by Dr. Karen Bartlet, University of British Columbia, BC, Canada), and *Trichophyton rubrum* (ATCC 18758) were tested as representatives of pathogenic fungi. The parasite *Leishmania donovani* Sudan strain 2S was assessed for antiparasitic activity of the extracts and was generously provided by Dr. Neil Reiner (University of British Columbia, Vancouver, British Columbia, Canada).

Bacterial strains were cultured in Mueller–Hinton broth (B&D) except for *Mycobacterium smegmatis*, which was cultured in Trypticase Soy broth (B&D). Bacterial stocks were maintained on the same broth supplemented with 1.5% agar (B&D) at 4 °C. All the bacterial strains were cultured at 37 °C. Fungi were cultured in Sabouraud broth (B&D), and the antifungal activity against filamentous fungi was assessed from spores. Spores of *Aspergillus fumigatus* and *Trichophyton rubrum* were carefully harvested in 2 ml Sabouraud broth containing 10% glycerol by rubbing carefully the top of sporulated colonies. Spores were aliquoted and kept at −20 °C. Filamentous fungi were incubated at 28 °C. For *Candida albicans* and *Cryptococcus neoformans*, the same protocol used for bacterial strains was followed, but using Sabouraud broth. *Leishmania donovani* promastigotes were cultured in medium M199 supplemented with 10% fetal bovine serum (Gibco), 1% penicillin and streptomycin, 20 mmol/l Hepes (Stem Cell Technologies), 6 μg/ml hemin, 2 mmol/l L-glutamine, 10 μg/ml folic acid, and 100 μmol/l adenosine at 26 °C in a EchoTherm Chilling Incubator (Torrey Pines Scientific, San Marcos, CA, USA). Every third day the organisms were split 1:10 into fresh medium.

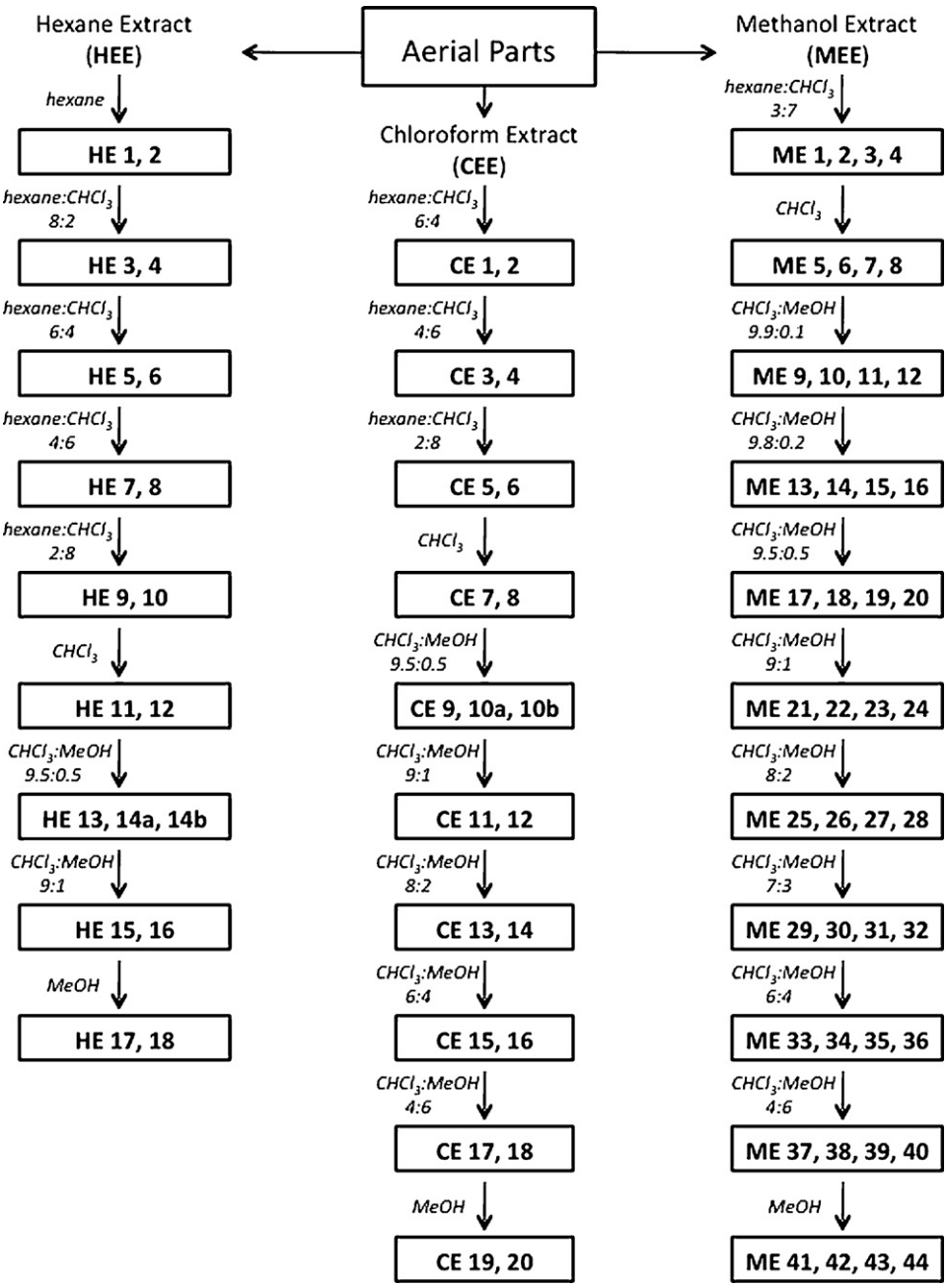

Fig. 1. Diagram showing the fractionation of the hexane, chloroform and methanol extracts extracted from the aerial parts of *Galium mexicanum*. Ratios of the combined solvents are expressed in volume/volume.

2.5. Antimicrobial and antiparasitic assays

A microdilution assay was used to assess the antimicrobial activities using a 96-well plate and according to published protocols (NCCLS, 2006). Bacterial strains were grown overnight by shaking at 37 °C. The inoculum density for the bacterial strains was adjusted to be equivalent to 0.5 in the McFarland scale (McFarland, 1907) by using an optical density of 0.080–0.100 at 625 nm. Extracts and fractions at concentrations of 1, 5, 10, 50, 67, 166, 333, 500, 999, 1500 and 2000 μg/ml were evaluated in a final volume of 150 μl/well. Minimal inhibitory concentrations (MICs) were determined by incubating the organisms in 96-well microplates for 24 h at 37 °C with the exception of fungi, which were cultured at 28 °C. The endpoints were determined when no turbidity in the well was observed.

Plates were sealed and incubated by shaking at 37 °C for 24 h. DMSO and untreated inoculum were used as negative controls, while gentamicin was used as a positive control. Antifungal activities were performed in RPMI 1640 medium supplemented with L-glutamine and without $NaHCO_3$ (Gibco). Fungal strains were grown at 28 °C for 24 h using the same format of 96-well plates, and in the case of *Trichophyton rubrum* and *Aspergillus fumigatus* plates were incubated at the same temperature but for a period of 72 h. DMSO and untreated inoculum were used as negative controls, while fluconazole and amphotericin B were used as positive controls.

The evaluation of antiparasitic activity was performed in 24-well flat bottom plates containing 1×10^6 promastigotes/well. Compounds were evaluated at a final concentration of 67, 166, 333, 500, 999 and 1500 μg/ml. Untreated parasites and DMSO were used

as negative controls. Motility and number of parasites were registered at 24, 48 and 72 h, after staining the sample with 0.4% Trypan blue solution.

2.6. Cytotoxicity assays

Monocytic cell line THP-1 (ATCC 202) was cultured in RPMI 1640 (Hyclone) supplemented with 5% fetal calf serum (FCS) (Hyclone), and 2 mM L-glutamine (StemCell Technologies). THP-1 cells were dispensed at a concentration of 3×10^5 well^{-1} in a 96-well plate, and incubated with compound concentrations ranging between 26 and 2600 μg/ml. Plates were placed at 37 °C in a humidified atmosphere supplemented with 5% CO_2 for 24 h. Non treated THP-1 cells or supplemented with DMSO were used as negative controls. THP-1 cells exposed to 5% hydrogen peroxide were used as a positive control. Propidium iodide (PI) was used to evaluate cell damage and according to published procedures (Pick et al., 2004). The half maximal inhibitory concentration (IC_{50}) was calculated by plotting the compound concentrations against the percentage of damaged cells.

2.7. Anti-inflammatory assay

This assay was performed only in those compounds showing low cytotoxicity. THP-1 cells at a concentration of 5×10^4 cells/well were dispensed in a 96-well plate, and activated after addition of 100 ng/ml phorbol myristate acetate (PMA) (Sigma). Plates were incubated at 37 °C in a humidified atmosphere of 5% CO_2 for 24 h. Then, differentiated cells were gently washed with fresh medium (3×), and incubated in presence of the compounds for 6 h. Next, cells were gently washed with fresh medium (3×), and an inflammatory response was initiated upon addition of 100 ng/ml of lipopolysaccharide (LPS) from *Escherichia coli* (Sigma). After 3 h, plates were centrifuged at 1000 rpm for 5 min, and the supernatant was transferred to a new 96-well plate and kept at −20 °C for later analysis. Interleukin-6 (IL-6) (R&D) was used to evaluate the anti-inflammatory activity using a sandwich ELISA. Briefly, 96-well plates were coated with 20 μg hIL-6 overnight at 4 °C. Next day, plates were washed with phosphate buffered saline (PBS) supplemented with 0.05% Tween-20 (PBS-T), and blocked with 3% bovine serum albumin (BSA) dissolved in PBS overnight at 4 °C. The blocking agent was washed away with PBS-T (3×) and 50 μl of the supernatant was added to hIL-6. After 2 h incubation, wells were washed again with PBS-T (3×), and exposed to biotinylated anti-hIL-6 (R&D) and according to the instruction of the manufacturer. After 1 h incubation, wells were washed again with PBS-T (3×), and avidin-HRP (BD Opt EIA) diluted 1:250 in 3% BSA was added. After 1 h, wells were washed with PBS-T (3×) and the presence of IL-6 was evaluated by addition of 3,3′,5,5′-tetramethylbenzidine until a blue colour developed. Reactions were stopped by addition of 25 μl of 1 M sulphuric acid solution. Absorbance was read in an ELISA reader (Bio-Rad) at 450 nm. THP-1 cells exposed to DMSO alone or DMSO combined to LPS was used as negative controls, while dexametasone was used as a positive control. Values are expressed in percentage of inflammation after normalization to LPS.

2.8. Statistical analysis

Student *t*-test was used for statistical analysis. A p value <0.05 was considered significant.

3. Results

3.1. Chemical constituents of the extracts

Chemical analyses of the CEE show the presence of triterpenes, saponins, flavonoids, sesquiterpene lactones, and glucosides (data not shown). No tannins were detected in the assayed extract. These results are in agreement with previous works, which reported the isolation of similar components from other *Galium* species (Iavarone et al., 1983; Uesato et al., 1984; Handjieva et al., 1996; De Rosa et al., 2000; Deliorman et al., 2001; Zhao et al., 2006; Serrilli et al., 2008).

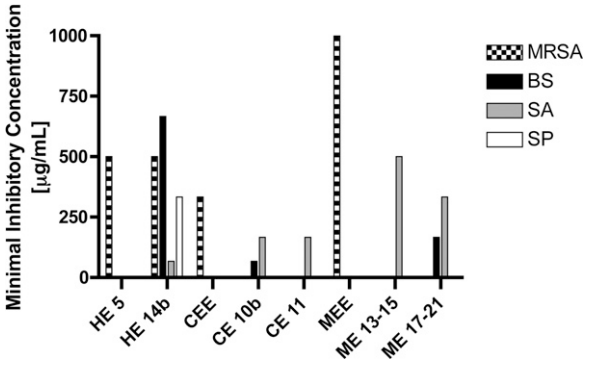

Fig. 2. Antibacterial activity exhibited by extracts and fractions of *Galium mexicanum*. Shown is the bacterial growth inhibition when bacterial strains were exposed to different concentrations of the tested compounds. BS, *Bacillus subtilis*; MRSA, methicillin-resistant *Staphylococcus aureus*; SA, *Staphylococcus aureus*; SP, *Streptococcus pyogenes*. HE, CE, and ME are hexane, chloroform, and methanol fractions and CEE and MEE are chloroform and methanol extracts, respectively.

3.2. Antibacterial activity

The antibacterial activities of HEE, CEE, and MEE extracts of aerial parts of *Galium mexicanum* were assayed on four Gram-negative and five Gram-positive bacteria. The growth of four Gram-positive strains was inhibited by CEE and MEE, and also by six fractions. All Gram-negative strains assayed were resistant to all of the tested samples. Most of the growth inhibition of Gram-positive bacteria was observed in the concentration range of 166–666 μg/ml except for fractions HE 14b, which inhibited specifically the growth of *Staphylococcus aureus* at a final concentration of 67 μg/ml (Fig. 2). Surprisingly, fraction HE 14b inhibited the growth of the four Gram-positive bacteria suggesting the presence of a single or a mixture of potent antibacterial compounds. Interesting, the CEE was able to inhibit the growth of MRSA, but not *Staphylococcus aureus* and *Bacillus subtilis*, which were inhibited by other two fractions obtained from the same extract, indicating that the CEE possesses an additive or synergistic antibacterial effect against MRSA and is concentration dependent for *Staphylococcus aureus* and *Bacillus subtilis*. The same tendency was observed with the MEE and its two derived fractions against the same strains.

3.3. Antifungal activity

Fractions HE 5, ME 13–15, and ME 40–41 show activity against *Trichophyton rubrum* at concentrations ranging between 333 and 500 μg/ml (Fig. 3). The growth of the yeast *Cryptococcus neoformans* was inhibited by fractions HE 14b and ME 13–15 at concentrations of 333 and 999 μg/ml, respectively; while the growth of *Candida albicans* was inhibited by CE 10b at a concentration of 666 μg/ml (Fig. 3). None of the extracts or fractions shows antifungal activity against *Aspergillus fumigatus*.

3.4. Antiparasitic activity

From all the extracts and fractions analyzed, only two fractions HE 5 and HE 14b, show antiparasitic activities against *Leishmania donovani* promastigotes. Of special interest is fraction HE 5, which inhibited the growth of the parasite at concentrations of 333 μg/ml

Table 1
Minimum inhibitory concentration (μg/ml) and half maximal inhibitory concentrations (IC_{50}) of *Galium mexicanum* extracts and fractions.

Compound	Bacteria				Fungi			IC_{50} (±SD)
	BS	MRSA	SA	SP	CA	CN	TR	
HE 5	R	500	R	R	R	R	333	1398.5 ± 72.3
HE 14b	666	500	67	333	R	333	R	228.5 ± 19.1
CEE	R	333	R	R	R	R	R	415.0 ± 49.5
CE 10b	67	R	166	R	666	R	R	382.5 ± 53.0
CE 11	R	R	166	R	R	R	R	193.5 ± 6.4
MEE	R	999	R	R	R	R	R	206 ± 5.6
ME 13–15	R	R	666	R	R	999	500	951.5 ± 12.0
ME 17–21	166	R	333	R	R	R	R	260 ± 42.4
ME 40–41	R	R	R	R	R	R	333	1000 ± 70.7

BS = *Bacillus subtilis*, MRSA = methicillin-resistant *Staphylococcus aureus*, SA = *Staphylococcus aureus*, SP = *Streptococcus pyogenes*, CA = *Candida albicans*, CN = *Cryptococcus neoformans*, TR = *Trichophyton rubrum*, R = resistant, CEE = chloroform extract, MEE = methanol extract, HE = hexane fraction, CE = chloroform fraction, ME = methanol fraction.

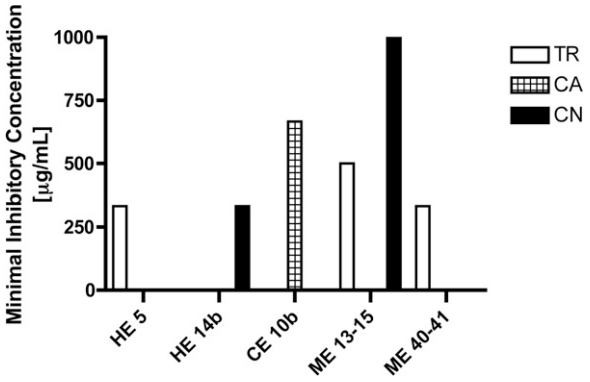

Fig. 3. Antifungal activity exhibited by fractions of *Galium mexicanum*. Shown is the fungal growth inhibition when fungal strains were exposed to different concentrations of the tested compounds. CA, *Candida albicans*; CN, *Cryptococcus neoformans*; TR, *Trichophyton rubrum*. HE, CE, and ME are hexane, chloroform and methanol fractions, respectively.

for the evaluated period of 72 h. Fraction HE 14b show also antiparasitic activity but at a concentration of 999 μg/ml (Fig. 4).

3.5. Cytotoxic and anti-inflammatory activities

Samples that showed antibacterial, antifungal, or antiparasitic activities were assayed on the human-derived monocyte cell line THP-1 to determine their cytotoxic effects. Macrophages were exposed to the highest concentration of the compound showing bioactivity. The samples were exposed to the cells for 24 h and analyzed by flow cytometry. Most of the samples were toxic to

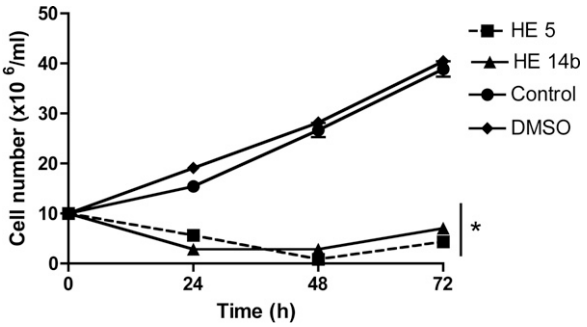

Fig. 4. Antiparasitic activity exhibited by fractions of *Galium mexicanum*. *Leishmania donovani* promastigote growth inhibition was evaluated after incubation of the parasites with hexane fractions. Untreated promastigotes and DMSO were used as negative controls. Shown is the mean ± SD of two independent experiments. *p value < 0.05.

macrophages with cell damage greater than 75% (Fig. 5). However, fractions HE 5, ME 13–15, and ME 40–41 show no cytotoxic effects. The half maximal inhibitory concentrations (IC_{50}) was calculated and listed in Table 1.

The secretion of IL-6 from macrophages exposed to the samples prior to the induction of an inflammation by LPS was used to assess the anti-inflammatory activities of the samples. Results show that fractions HE 5 and ME 13–15 were effective to reduce the secretion of IL-6 by 50% and 90%, respectively (Fig. 6), indicating that both fractions contain anti-inflammatory substances.

4. Discussion

In Mexico, people living in rural areas do not have an easy access to conventional allopathic treatments because of limited health services and low socioeconomic status. Therefore, in these places, plants can provide an important and needed source of therapeutic medicinal compounds. In Mexican folk medicine, the aerial parts of *Galium mexicanum* are used to treat infections, specifically, skin diseases and other digestive illnesses (INI, 1994a,b,c).

In this work, various extracts and fractions of *Galium mexicanum* were found to possess antimicrobial activity confirming its traditional use for dermatological diseases. Antibacterial activities

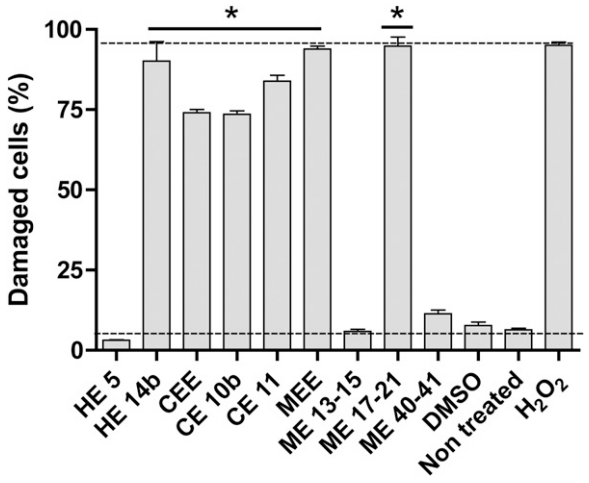

Fig. 5. Cytotoxic effects of extracts and fractions of *Galium mexicanum*. THP-1 cells were used to asses the cytotoxic effects of bioactive compounds using propidium iodide staining. Dashed lines represent treatment with 5% H_2O_2 (upper line) (positive control), and non treated cells (lower line) (negative control). Shown is the mean ± SD of three independent experiments. HE, CE, and ME are hexane, chloroform, and methanol fractions and CEE and MEE are chloroform and methanol extracts, respectively.

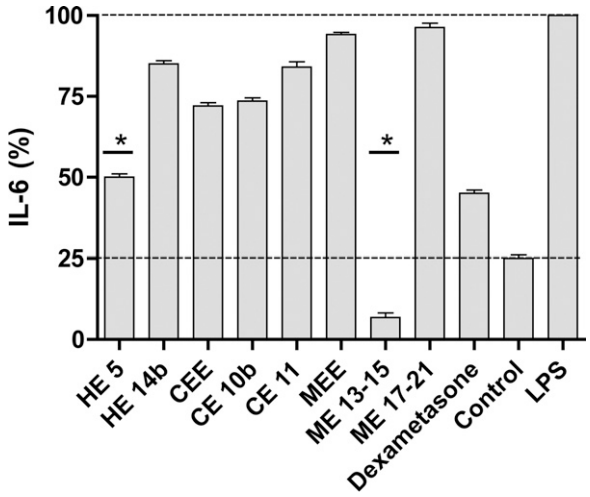

Fig. 6. Anti-inflammatory activity of extracts and fractions from *Galium mexicanum*. An inflammatory process was elicited by exposing macrophages to LPS. Extracts and fractions were exposed to macrophages prior to the addition of LPS. The anti-inflammatory process was evaluated by measuring the secretion of IL-6 into the supernatant. Shown is the mean ± SD of three independent experiments. HE, CE, and ME are hexane, chloroform, and methanol fractions and CEE and MEE are chloroform and methanol extracts, respectively. *p value < 0.05.

were observed only in Gram-positive, but not in Gram-negative strains. Of special interest is the activity of the CEE and MEE and the hexane fractions HE 5 and HE 14b (Fig. 2), which exhibited antibacterial activity against the multi-drug resistant pathogen MRSA, a prevalent worldwide strain infecting approximately 20,000 people annually in the United States, and is also associated with significant increases in the length of hospitalization (Cosgrove et al., 2005; Klevens et al., 2007). Interestingly, although we did not observe any antibacterial activities against Gram-negative strains, in a previous work using a purified anthraquinone isolated from the ethanol extract of *Galium verum*, antimicrobial activities against four Gram-negative strains were reported (Zhao et al., 2006). This discrepancy can originate from either the solvent used for the extraction (ethanol) or the absence or very low concentrations of this specific anthraquinone in *Galium mexicanum*. Another work reported significant antibacterial activity against the Gram-positive strains *Staphylococcus aureus* and *Bacillus subtilis* (Jan et al., 2009), which is in agreement with our results (Fig. 2). However, in the same work, antimicrobial activity against the Gram-negative strain *Pseudomonas aeruginosa* was reported, but no significant antimicrobial activities were measured when other four Gram-negative strains were assessed using an ethanol extract of *Pseudomonas tricornutum* (Jan et al., 2009). The present study found a strong activity of various extracts and fractions against Gram-positive bacteria.

Five fractions from *Galium mexicanum* show antifungal activities against the skin-associated fungi *Candida albicans* and *Trichophyton rubrum*, and also against *Cryptococcus neoformans*, a pathogen associated to lung infections and basal meningitis (Fig. 3). Although we observed antifungal activity against *Candida albicans* only in a chloroform fraction (CE 10b), our results agree with published work that reported no activity of the MEE of *Galium verum* (Yigit et al., 2008).

Surprisingly, two fractions of the hexane extract HE 5 and HE 14b, show strong inhibitory effects on the growth of parasite *Leishmania donovani*, while the same HE 5 and the ME 13–15 fractions showed significant anti-inflammatory effects on macrophages induced with LPS. To the best of our knowledge, there are no previous published works about the antiparasitic or anti-inflammatory activities of the genus *Galium*. In addition, most of the samples that exhibited any bioactivity were cytotoxic to macrophages except fractions HE 5, ME 13–15 and ME 29–34 (Fig. 5).

5. Conclusion

Galium mexicanum is a potential source of bioactive compounds that exhibit antimicrobial, antiparasitic, and anti-inflammatory activities. Of special interest is the potency of two fractions of the hexane extract, which show strong anti-*Leishmania* activity, and a methanol fraction that was able to significantly reduce inflammation induced by macrophages exposed to LPS. The further isolation and identification of the individual constituents present in the various fractions is currently under investigation in our laboratory.

Acknowledgement

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