Journal of Ethnopharmacology 176 (2015) 469–474

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Gastroprotective effects and antimicrobial activity of *Lithraea molleoides* and isolated compounds against *Helicobacter pylori*

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ARTICLE INFO

Article history:
Received 25 June 2015
Received in revised form
20 October 2015
Accepted 4 November 2015
Available online 11 November 2015

Keywords:
Lithraea molleoides
Anacardiaceae
Antimicrobial
Gastric ulcer
Gastroprotection
Helicobacter pylori

Chemical compounds studied in this article:
Catechol (PubChem CID: 289)
Mannitol (PubChem CID: 6251)
Rutin (PubChem CID: 5280805)
Gallic acid (PubChem CID: 370)
Ferulic acid (PubChem CID: 445858)
Caffeic acid (PubChem CID: 689043)

ABSTRACT

Ethnopharmacological relevance: Lithraea molleoides (Vell.) Engl. (Anacardiaceae) is a medicinal plant traditionally used in South America to treat various ailments, including diseases of the digestive system.
Aim of the study: To evaluate the *in vivo* antiulcer and antimicrobial activities against *Helicobacter pylori* of *L. molleoides* and its isolated compounds.
Materials and methods: Methanolic extract 250 and 500 mg/kg, (LmE 250 and LmE 500, respectively) and infusions, 10 g and 20 g en 100 mL (LmI 10 and LmI 20, respectively) of *L. molleoides* was evaluated for antiulcer activity against 0.6N HCl, 0.2N NaOH, 200 mg/kg acetilsalicilic acid and absolute ethanol-induced gastric ulcers in rats. The degree of erosion in the glandular part of the stomach was assessed from a scoring system. Acute toxicity in mice was also evaluated. The antiulcer effect of the isolated compounds (catechol, mannitol, rutin, gallic acid, ferulic acid and caffeic acid, 100 mg/kg) was evaluated against absolute ethanol-induced gastric ulcers in rats.

The anti-*Helicobacter pylori* activity of *L. molleoides* and isolated compounds was performed using broth dilution methods.
Results: The LmE 250, LmE 500, LmI 10 and LmI 20 produced significant inhibition on the ulcer index in 0.6N HCl, 0.2N NaOH, 200 mg/kg acetilsalicilic acid and absolute ethanol- induced gastric ulcers in rats. The isolated compounds, catechol, mannitol, rutin, ferulic acid and caffeic acid were active in absolute ethanol- induced gastric ulcers in rats. *L. molleoides* and different compounds showed antimicrobial activity in all strains tested. The lowest MIC value (0. 5 μg/mL) was obtained with catechol in six of eleven strains assayed. No signs of toxicity were observed with doses up to 2 g/kg in an acute toxicity assay.
Conclusion: These findings indicate that *L. molleoides* displays potential antiulcerogenic and antimicrobial activities and the identification of active principles could support the use of this plant for the treatment of digestive affections.

1. Introduction

Lithraea molleoides (Vell.) Engl. (Anacardiaceae), known in Argentina as “molle”, “molle de beber”, “molle blanco”, “molle dulce”or “chichita”, is a tree which grows in South America, especially in Argentina, Brasil and Uruguay. Decoctions and infusion of the leaves are used by people of these countries for its medicinal properties which include stomachic, antiarthritic, hemostatic, diuretic, tonic and refreshing, sweetener and for the treatment of respiratory and digestive diseases (Ratera and Ratera, 1980; Del Vitto et al., 1997; Goleniowki et al., 2006). It is also, an ingredient of some foods (“arrope” and “aloja”) and used to improve the flavor of a traditional stimulant beverage “Mate”, widely used in

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http://dx.doi.org/10.1016/j.jep.2015.11.009
0378-8741/

South America (Soraru and Bandoni, 1978). Treatment of symptomatologies related to gastric ulcers or gastritis with medicinal plants are quite common in traditional medicine worldwide (Schmeda-Hirschmann and Yesilada, 2005). In the last decades, many efforts have been done in order to discover and/or develop new anti-ulcer drugs from natural sources (Cellini et al., 2014).

The human gastric pathogen *Helicobacter pylori* is able to establish persistent infections in the human stomach that can lead to severe inflammatory gastroduodenal disease including peptic ulcer disease and gastric cancer. Several authors have documented that the different gastric mucosa-associated diseases can be cured after eradication of microorganisms (Fischer et al., 2009). The antibiotics commonly used for eradication of *H. pylori* infection are amoxicillin (AMX), clarithromycin (CLA), metronidazole (MTZ), tetracycline or quinolone. *H. pylori* standard triple therapy consists in the combination of two antibiotics and a proton pump inhibitor, but the antimicrobial resistance increased drastically and endangers the successful eradication due to emerging *H. pylori* multiresistant strains (Malfertheiner, 2014). In our region the prevalence of strains resistant to CLA and MTZ is high compared to other region of the world (Vega et al., 2010).

Therefore, the increase of resistance and undesirable side effects that may occur during treatment has led to the search and testing of new options for *H. pylori* eradication. The use of natural antibacterial agents including phytochemicals and bioactive nutraceuticals is currently considered as a procedure with more effective and safer outcomes (Keenan et al., 2010). In this sense, *L. molleoides* has antimicrobial activity against several microorganisms, such as *Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus aureus* and *Mucor* sp. (Penna et al., 2001). Moreover, the hydroalcoholic extract of the aerial parts of L. molleoides, given orally at a dose of 1000 mg/kg, showed significant anti-ulcerogenic activity on ulcer induced by indomethacin and absolute alcohol in rats (Araujo et al., 2006).

The present study was designed to determine the antimicrobial activity of *L. molleoides* and the isolated compounds (catechol, mannitol, rutin, gallic acid, ferulic acid and caffeic acid) against one reference strain and ten clinical isolated *H. pylori* samples and the effects of *L. molleoides* on the gastric cytoprotective activity in four different models of experimentally induced gastric ulcer in rats.

2. Methods

2.1. Plant material

L. molleoides (Vell.) Engl. (Anacardiaceae) was collected in December 2008 in San Luis, Argentina, by M. F. Garro. The plant was identified by Dr. Luis A. Del Vitto and a voucher specimen has been deposited at the Herbarium of the Universidad Nacional de San Luis, voucher N°515.

2.2. Phytochemical analysis

The air-dried plant material (0.360 kg) was extracted with hot MeOH (3l × 3). A white solid that precipitated spontaneously from the methanolic extract purified upon repeated recrystallization from MeOH to yield compound **1** (mannitol) (18 mg). After the separation of the compound (**1**), the remaining methanolic extract was concentrated to 1.5 l, then H_2O was added (10, 20 and 30%) and partitioned between n-hexane, CCl_4, $CHCl_3$, EtOAc and n-BuOH saturated in H_2O, respectively. AcOEt extract was subjected to Sephadex LH-20 column chromatography and eluted with MeOH to afford compounds **2** (caffeic acid) (14 mg), (gallic acid) **3** (12 mg), (ferulic acid) **4** (11 mg) and **5** (catechol) (8 mg). n-BuOH saturated in H_2O extract was purified by Sephadex LH-20 (MeOH:H_2O 9:1) to yield **6** (rutin) (15 mg). The yield of the MeOH extract was 26% (w/w).

Known compounds were identified by comparing the ^{1}H NMR, ^{13}C NMR spectra and physical properties with those of authentic material.

The LmI 10 and LmI 20 of aerial parts from *L. molleoides* were prepared by following the methodology outlined in the Farmacopea Nacional Argentina (1978). The yield of the infusions were 32% and 36% (w/w), respectively.

Column chromatographic was performed on silica gel 60 G (Merck), 0.063–0.200 mm. TLC was carried out on Kieselgel mit Fluoreszent-Indicator UV 254 (Macherey–Nagel), 0.25 mm thick plates and LH Sephadex. ^{1}H NMR and ^{13}C NMR spectra were obtained at 200.13 MHz on a Bruker AC-200 spectrometer (chemical shift δ in ppm, coupling constant J in Hz), TMS was used as internal standard (Saad et al., 1987; Foo et al., 2000). **C1** ^{1}H NMR (200 MHz, D_2O): 3.88 (s), 3.90 (s), 3.91 (s), 3.97 (s); **C2** ^{1}H NMR (200 MHz, DMSO-d6): 7.07 (1H, s, H-2), 6.8 (1H, d, J= 8Hz, H-5), 7.1 (1H , d, J= 8 Hz, H-6), 7.6 (1H, d, J= 15 Hz, H-7), 6.3 (1H,d, J=15 Hz, H-8); **C3** ^{1}H NMR (200 MHz, DMSO-d6): 7.15 (2H, s, H-3 and H-7); **C4** ^{1}H NMR (200 MHz, DMSO-d6): 3.80 (3H, s, -OCH3), 7.09 (1H, s, H-2), 6.9 (1H, d, J= 8Hz, H-5), 7.1 (1H , d, J= 8 Hz, H-6), 7.6 (1H, d, J= 15 Hz, H-7), 6.3 (1H,d, J=15 Hz, H-8); **C5** ^{1}H NMR (200 MHz, DMSO-d6): 6.96 (2H, s, H-3, H-6), 6.88 (2H, s, H-4, H-5); **C6** ^{1}H NMR (200 MHz, DMSO-d6): 6.21(1H, d, J=2, H-6), 6.40 (1H, d, J=2, H-8),7.55 (1H, d, J=2.1, H-2′), 6.86 (1H, d J=9, H-5′),7.56 (1H, dd, J=9, 2.1, H-6′), 9.71 (1H, s, C4′-OH), 9.21 (1H, s, C3′-OH), 12.62 (1H, s, C5-OH), 10.86 (1H, s, C7-OH), 5.35 (1H, d, J=7.4, H1-G),5.12 (1H, d, J=1.9, H1-R), 1.00 (3H, d, J=6.1,CH3R . [R and G represent signals from rhamnose and glucose moieties, respectively].

2.3. Antimicrobial activity of L. molleoides against Helicobacter pylori

2.3.1. Strains and culture conditions

H. pylori NCTC 11638 (reference strain), a kind gift from Dr. Manuel López-Brea, Microbiology Service of Hospital Universitario de la Princesa, Madrid, Spain and ten clinical isolates obtained from gastric antral biopsy specimens were used for this study. *H. pylori* strains were grown in Mueller-Hinton agar (MHA) supplemented with 7% horse blood (MHA-B) and identified by microscopy, urease, catalase and oxidase tests.

2.3.2. Antibacterial activity of L. molleoides

The antibacterial activity of *L. molleoides* extract against *H. pylori* strains was assayed by broth microdilution method using Mueller Hinton Broth (MHB) according to CLSI guidelines (2007). Serial dilutions of AMX (Sigma-Aldrich Co., St Louis, MO) were used as a control in the susceptibility test. Twofold dilutions of extract were performed to obtain the following final concentrations: from 500 to 4 μg/mL for *L. molleoides* extracts and from 125 to 0.08 μg/mL for AMX. Broth microdilution methods were carried out in 96-well microtitre plates. In each well were dispensed aliquots (100 μl) of each dilution of extract and aliquots (100 μl) of each bacterial suspension adjusted to a scale of 0.5 on the Mac Farland standard (1×10^8 colony forming units (CFUs)/mL). Two hundred microlitres of extract, bacterial suspensions, MHB and 0.9% saline were also included as controls. Plates were incubated in microaerobic condition at 37 °C for 3 days. The evaluation of the results were done by colorimetric evaluation using 2,3,5-Triphenyl tetrazolium chloride (TTC) as an indicator. Minimal inhibitory concentration (MIC) was measured by determining the smallest amount of extract or antibiotic needed to inhibit the visible growth of the microorganism. All tests were performed in duplicate.

2.3.3. Minimal inhibitory concentration (MIC) of isolated compounds of L. molleoides

MIC of isolated compounds was determined by conventional broth dilution method as previously described. Serial dilutions of different compounds (250–0.008 μg/mL) were performed with 0.9% saline and MIC was determined following incubation at 37 °C for 3 days under microaerophilic conditions. MICs of CLA (Abbott Laboratories, Argentina) and MTZ (Sigma Chemical Co., St. Louis, MO) were also determined using serial dilutions ranging from 128 to 0.008 μg/mL. Resistance was defined as the CLA MIC being ≥ 1 μg/mL l and MTZ MIC being ≥ 8 μg/mL.

2.4. Animals

The experiments were performed on male Wistar rats (200–220 g) and albine mice of either sex (25–30 g) with free access to standard food and water, in a 12-h day–night cycle (lights on from 07:00 to 19:00 h), at a constant temperature of 22 ± 3 °C (with periodic cycles of air changes) and a relative humidity of about 50–60%. Acclimatization of animals was done for two days before the commencement of the experiment. The animals were randomly assigned to different groups. All the animals were obtained from the Bioterium of the Facultad de Química, Bioquímica y Farmacia of Universidad Nacional de San Luis (Argentina) and the experiment were in compliance with the A.N.M.A.T. (Administración Nacional de Medicamentos, Alimentos y Tecnología Médica, 1996) No 6344/96 for animal care guide-lines and were also authorized by Institutional Committee for the Care and Use of Laboratory Animals (Acronym: C.I.C.U.A.) of our institution (protocol No. F-129/13, F-133/13 and F-143/13 in Resolution 010–14).

2.5. Drugs used

Clarithromycin and metronidazole were purchased from Abbott Laboratories, Argentina, and Sigma Chemical Co., St. Louis, MO, respectively. All others chemicals used were of reagent grade and obtained from the local market.

2.6. Induction of gastric lesions

Gastric lesions were produced according to the method of Robert et al. (1979). Male Wistar rats, randomly assigned into groups ($n=6$–8), were deprived of food for 24 h prior to starting the experiments and had free access to water. All rats were housed in wire mesh-bottomed cages throughout the study to prevent coprophagy. The necrotizing agents (0.6N HCl, 0.2N NaOH, 200 mg/kg acetilsalicilic acid or absolute ethanol) were administered orally (1 mL), and 1 h later, the animals were sacrificed (euthanization with CO_2). The stomachs were removed, opened along the greater curvature and washed gently with ice-cold saline solution. The degree of erosion in the glandular part of the stomach was assessed from a scoring system designed by Marazzi-Uberti and Turba (1984) from 0 (no erosions) to 5 (maximal damage). The results were expressed in terms of an Ulcer Index which is the average severity of erosions per rat for each group. All these values were divided by the number of animals. The LmE 250, LmE 500 or LmI 10 and LmI 20 or compounds catechol, mannitol, rutin, gallic acid, ferulic acid and caffeic acid (100 mg/kg), omeprazole (60 mg/kg) or vehicle were administered 60 min prior to the necrotizing agent (*p.o.*).

2.7. Acute toxicity testing

Acute toxicity of the infusion of *L. molleoides* was investigated on albine mice (OECD Guidelines, 2001). *L. molleoides* lyophilized aqueous extract was re-dissolved in distilled water and administered intragastrically at the doses of 5, 50, 300 and 2000 mg/kg. The animals were divided into one control group and four treated groups of six animals each, including both sex and weighing 25–30 g. The control group received saline and each treated group received orally simple doses of the infusion of *L. molleoides* (5, 50, 300 and 2000 mg/kg body weight). The mice were allowed access to standard laboratory feed and water *ad libitum*. The animals were under observation during 14 consecutive days, to record body weight (first, seventh and fourteenth days), toxic symptoms (restlessness respiratory, distress, diarrhea or convulsions) and mortality. At the end of the experiment, the animals were killed by cervical dislocation and kidney, spleen and liver were observed macroscopically, the relative weight (organ/body) was determined.

2.8. Statistical analysis

Statistical analysis was performed using GraphPad Prism version 5.00 for Windows and GraphPad InStat version 3.00 for Windows (GraphPad Software, San Diego, California, USA, www.graphpad.com). All data are expressed as the mean $\pm$ S.E.M. (Standard Error of Mean). A probability of $p < 0.05$ was considered significant.

3. Results

Results of the antimicrobial activity of *L. molleoides* extract and the isolated compounds are summarized in Table 1. The *L. molleoides* extract showed significant inhibitory activity against strains tested with a MIC ranging from 125 to 8 μg/mL. The CLA and MTZ resistant strains showed higher MIC values for any *L. molleoides* extract or isolated compounds. The lowest MIC value (0.5 μg/mL) was obtained with catechol in six of eleven strains assayed.

The necrotizing agents 0.6N HCl, 0.2N NaOH, 200 mg/kg acetilsalicilic acid and absolute ethanol produced gastric ulcers in all

Table 1
MICs of *Lithraea molleoides* extract and isolated compounds for eleven strains of *Helicobacter pylori*.[a]

L. molleoides extract and pure compound	MIC (μg/mL) of *H. pylori* strains										
	NCTC 11638	HP796	HP857	HP160	HP162	HP172	HP173	HP198	HP206	HP211	HP213
L.molleoides extract	16	125	64	16	64	32	125	64	32	8	8
catechol	16	64	16	0.5	32	0.5	64	0.5	0.5	0.5	0.5
mannitol	32	125	32	32	32	64	64	32	32	32	32
rutin	8	125	64	64	64	64	64	64	64	8	8
gallic acid	64	125	64	1	125	32	64	32	32	1	1
ferulic acid	125	125	125	125	125	125	125	125	125	125	125
caffeic acid	32	125	125	125	64	16	16	64	32	16	16
CLA	0.5	4	0.25	0.5	2	2	4	0.016	0.008	0.5	0.25
MTZ	0.25	16	1	8	8	8	16	0.032	0.5	2	1

[a] Values are average derived from two determinations.

Table 2
Effects of *Lithraea molleoides* on gastric ulcers induced by different necrotizing agents in Wistar rats.

Treatment	Ulcer index	Percentage inhibition of ulceration
HCl (control)	4.78 ± 0.19	–
HCl+LmI 10	2.15 ± 0.20***	55.03
HCl+LmI 20	1.7 ± 0.29***	64.44
HCl+LmE 250	2.57 ± 0.37***	46.24
HCl+LmE 500	1.16 ± 0.6***	74.75
HCl+omeprazole 60 mg/kg	2.00 ± 0.52**	54.75
NaOH (control)	4.79 ± 0.20	–
NaOH+LmI 10	3.95 ± 0.25*	17.54
NaOH+LmI 20	1.52 ± 0.51***	68.23
NaOH+LmE 250	1.25 ± 0.47***	73.79
NaOH+LmE 500	0.59 ± 0.15***	87.62
NaOH+omeprazole 60 mg/kg	2.90 ± 0.50*	38.42
ASS(control)	4.75 ± 0.12	–
ASA+LmI 10	3.79 ± 0.33*	20.22
ASA+LmI 20	2.25 ± 0.25***	66.26
ASA+LmE 250	1.19 ± 0.18***	74.95
ASA+LmE 500	0.67 ± 0.15***	85.09
ASA+omeprazole 60 mg/kg	1.77 ± 0.39***	37.26
EtOH (control)	4.75 ± 0.17	–
EtOH+LmI 10	3.62 ± 1.4*	23.29
EtOH+LmI 20	2.45 ± 0.21***	48.43
EtOH+LmE 250	1.58 ± 0.17***	66.74
EtOH+LmE 500	0.80 ± 0.10***	83.16
EtOH+omeprazole 60 mg/kg	3.00 ± 0.28***	36.84

Effects of 10% and 20% infusions (LmI 10 and LmI 20) and methanolic extract 250 and 500 mg/kg, (LmE 250, LmE 500) of *L. molleoides* on gastric ulcers induced by different necrotizing agents (0.6N HCl, 0.2N NaOH, 200 mg/kg acetylsalicylic acid, ASA, and absolute ethanol, EtOH) in Wistar rats (n=6–8). All values were expressed as mean ± SEM. Asterisks denote significant differences from the control: $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$ *vs.* control (Student´s *t*-test).

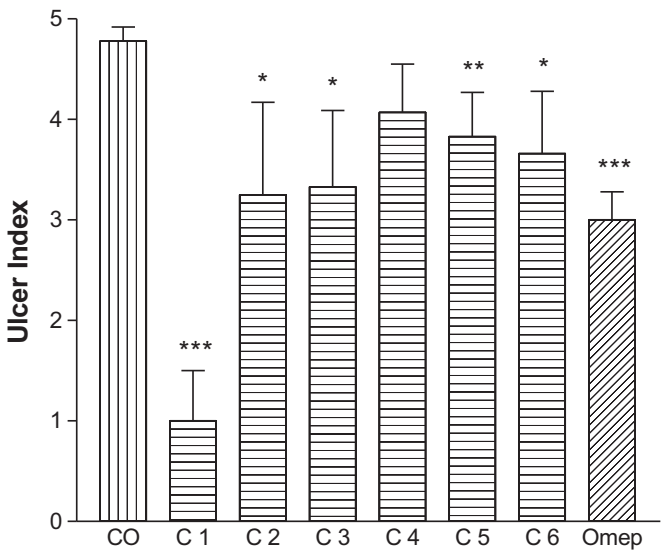

Fig. 1. Effects of compounds isolated from *Lithraea molleoides* on ethanol-induced gastric lesions in rats. Effects of compounds isolated from *Lithraea molleoides* on ethanol-induced gastric lesions in rats (C 1: catechol; C 2: mannitol; C 3: rutin; C 4: gallic acid; C 5: ferulic acid; C 6: caffeic acid) (100 mg/kg, *p.o.*). The damage was expressed as Ulcer Index (score 0–5, normal mucosa to maximal damage). Vehicle+EtOH served as the control (CO). Omep=omeprazole (60 mg/kg). Asterisks denote significant differences from the control ($^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$, Student´s *t*-test). All values were expressed as mean ± S.E.M.

the animals treated. A pretreatment with LmE 250, LmE 500, LmI 10 and LmI 20 induced inhibition of gastric ulcers, compared with the control group ($p<0.05$ or $p<0.001$, Table 2). Omeprazole (60 mg/kg) significantly also reduced all the gastric ulcer induced by ulcerogenic agents studied ($p<0.05$, $p<0.01$ or $p<0.001$). Compounds catechol, mannitol, rutin, ferulic acid and caffeic acid isolated from *L. molleoides* protect against experimental ulcer induced by absolute ethanol at a dose of 100 mg/kg ($p<0.05$, $p<0.01$ or $p<0.001$, Fig. 1). Gallic acid did not show gastroprotective effect in this model of experimental ulcer.

Moreover, *L. molleoides* was studied for acute oral toxicity as per revised OECD guidelines No 423. *L. molleoides* was devoid of any toxicity up to 2000 mg/kg in mice by oral route. No toxic symptoms or death occurred during the experiment. The body weight of the animals treated with the infusion of *L. molleoides* has not showed significant differences compared to control group. Relative wet weight of organs (kidney, spleen and liver) did not show any significant change when was compared with the control group.

4. Discussion

The development of effective acid suppressant drugs and identification of *H. pylori* as a major ulcerogen agents, were two important events that contributed to decreasing rates of peptic ulcer disease. However, this disease remains an important health problem, especially for chronic users of non-steroidal anti-inflammatory drugs and the patients infected with *H. pylori* resistant strains (Lemos et al., 2012). The eradication of *H. pylori* can therefore, contribute to the treatment and prevention of these gastroduodenal diseases.

Several natural products used for the treatment of gastric disorders, including stomach ache and ulcers have shown antimicrobial activity against *H. pylori* (Palacios-Espinosa et al., 2014). Phenolic acids such as ferulic, gallic and caffeic acid, possessed potential ulcer preventive activity in vitro, that include inhibition of H+, K+-ATPase and *H. pylori* growth. Also, is has been shown that gallic acid contributes significantly to anti-oxidant activity (Siddaraju et al. 2009).

In this study, ferulic acid showed moderate activity MICs 125 μg/mL by all strains; while gallic and caffeic acid showed range MICs 32–125 μg/mL for most of the strains. However three strains were inhibited with MIC values of 1 ug/mL for gallic acid and four strains were inhibited with MIC values of 16 ug/mL for caffeic acid.

Rutin is a flavonoid that possesses antimicrobial activity due to damage in bacterial cell against some Gram-positive and mostly for fastidious Gram-negative pathogenic bacteria (Maddox et al., 2010). Our results showed MIC lower values than the obtained by other authors with *Rubus ulmifolius* and *Rhus verniciflua* Stokes (Martini et al., 2009; Suk et al., 2011).

H. pylori produces oxygen radicals and induces cyclooxygenase (COX) 2 expression in gastric epithelial cells during gastric ulceration and carcinogenesis. Also, *H. pylori* significantly stimulates the production of lipid peroxide, an indicator of oxidative damage, during the infection. Mannitol is a known hydroxyl radical scavenger and may attenuate *H. pylori*-induced gastric inflammation by both an inhibitory effect on *H. pylori*-induced COX-2 expression and by inhibiting lipid peroxidation (Kim et al., 2002). In this work mannitol showed antimicrobial activity against *H. pylori* with MICs values of 32 μg/mL for all strains except CLA and MTZ resistant strains which showed MIC values 64 μg/mL. The present study demonstrates that *L. molleoides* extract and their isolated compounds have inhibitory activity against *H. pylori* strains; however, discrepancies between strains were observed. These differences can be attributed to the susceptibility patterns of strains used in this study. *L. molleoides* extract showed MIC values ranged 32–125 μg/mL against all *H. pylori* MTZ resistant strains, except for HP 160. Regarding antibacterial activity of each compounds assayed, CLA and MTZ resistant strains were inhibited at

concentrations greater that 32 μg/mL. In this study, CLA and MTZ *H. pylori* resistant strains harboring cagA+ (data not show). In this sense, the observation that resistant strain cagA+ show a different behavior towards compounds i.e. was less susceptible, could be explained on the genomic diversity of *H. pylori* strains under selection by antibiotic or presence of virulence gene such as pathogenicity island cagA. Further studies on mode of action of *L. molleoides* and their compounds may contribute to the characterization as new anti-*H. pylori* agent of natural origin.

The gastroprotective effect of *L. molleoides* was investigated by several animal models of acute gastric injury induced by necrotizing agents, i.e., ethanol, a strong acid, 0.6N HCl, a strong base, 0.2N NaOH and NSAID, 200 mg/kg ASA (Robert et al., 1979).

NSAIDs induce injury/bleeding via three key pathways: inhibition of cyclooxygenase (COX)-1 activity, inhibition of COX-2 activity, and direct cytotoxic effects on the epithelium. The most important of the systemic effects of NSAIDs, in terms of inducing gastric ulceration, is their ability to suppress prostaglandin synthesis (Wallace, 2008). LmE and LmI significantly inhibited the gastric injury caused by ASA at all tested doses. This result suggests that the gastroprotective mechanism could be attributed at an increase of prostaglandin synthesis.

Non-NSAID experimental models of gastric ulcer induction include those based on ethanol, HCl and NaOH. Ethanol is known as a cause of gastric damage by altering protective factors, including decreasing mucus production and blood circulation within the mucosa. In addition, the gastric damage caused by ethanol may be due to the generation of reactive species, decreased cell proliferation, and an exacerbated inflammatory response (Choi et al., 2009). In this study, LmE 250, LmE 500, LmI 10 and LmI 20 were able to protect the gastric mucosa against the damage caused by ethanol, HCl and NaOH. This effect may be attributed to the anti-inflammatory activity previously demonstrated by Gorzalczany et al. (2011).

Our test of ethanol-induced ulcer using LmE or LmI gave similar gastroprotective results than what was obtained by each compound isolated. Barros et al. (2008) demonstrated that caffeic and ferulic acids possess gastroprotective activity, due to inhibition of the development of ulcers induced by physical and chemical agents. In this study, the treatments with caffeic and ferulic acids significantly decreased the gastric damage.

On the other hand, rutin has been reported to prevent gastric mucosal ulceration in several animal models (La Casa et al., 2000). Also, allylpyrocatechol provided ulcer healing against indomethacin-induced gastric ulceration in mice under an optimized treatment regime (Banerjee et al., 2008). The gastroprotective effect of mannitol may be attributed to luminal osmolality and delayed gastric emptying which act to lower the concentration of the necrotizing agent and secreted acid (Gharzouli et al., 2001). In this work, rutin, catechol and mannitol showed significant gastroprotective effect against ethanol-induced gastric damage, being catechol the most active. On the basis of these results, the *L. molleoides* gastroprotective activity could be due, at least in part, to the presence of catechol, mannitol, rutin, ferulic and caffeic acid. Nevertheless, further investigations are required in order to clarify the precise contributions of each compound to *L. molleoides* gastroprotection.

This work provides new information about the anti *H. pylori* potential and gastroprotective effect of *L. molleoides* and provides scientific basis for the popular use of this plant for digestive affections.

5. Conclusions

The results indicate that *L. molleoides* extracts have good antimicrobial activity against *H. pylori* strains. We demonstrated that this antimicrobial activity may be due to the presence of phenolic acid such as gallic, caffeic and ferulic, rutin, catechol or mannitol compounds described in the phytochemical profile of *L. molleoides*.

Moreover, this study demonstrated a significant antiulcer activity of *L. molleoides* using different experimental models of experimental ulcer in rats. The *L. molleoides* effect could be due, at least in part, mainly to the presence of catechol, mannitol and rutin which showed antimicrobial activity at low MIC values associated with increased protective capacity.

L. molleoides could provide an alternative therapy for *H. pylori* infection and avoid the problem of resistance associated with current antibiotic treatment. The identification of active principles could support the use of this plant for the treatment of digestive affections.

Declaration of interest statement

The authors declare that they have no competing interests.

Acknowledgments

Financial support was provided by UNSL (PROICO 2-1412, PROICO 2-3014 and PROICO 2-3614).

This paper is a part of the Garro MF doctoral thesis.

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