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Original Research Article

Anti-Ulcer Effect on Indomethacin-Induced Ulcerated Mice of *Chromolaena odorata* Leaf from Vietnam and its Secondary Metabolites

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ABSTRACT

Chromolaena odorata (*C. odorata*) (L.) R. King & H. Robinson is a perennial flowering shrub with diverse habitats, including crops, grasslands, and roadsides. This plant has been widely used in Vietnamese folk medicine for gastric ulcer treatment. Hence, the present study aimed to evaluate the acute toxicity and the anti-ulcer effect of the ethanol crude extract of *C. odorata* leaves and its fractions against the indomethacin-induced gastric ulcer model in mice, and investigate the chemical constituents of the most active fraction. According to *in vivo* results, the ethyl acetate residue with the highest anti-ulcer activity significantly reduced gastric lesions in the experimental mice model with an ulcer index of 0.73 ± 0.39 and a percentage inhibition of 26.92%. Thus, this fraction was chosen for further chemical investigation. Four pure compounds (1-4) were extracted and isolated by using chromatographic methods. Based on the nuclear magnetic resonance spectroscopy, melting temperature, mass spectrometry analysis, and compared with the published literature, their structures were elucidated as 1-(4-hydroxy-3-methoxyphenyl)propane-1,2-diol (**1**), kaempferol-7-*O*-*α*-L-rhamnopyranoside (**2**), naringenin-5,7-di-*O*-*β*-D-glucopyranoside (3), and rubrosterone (4). To our best knowledge, all of these compounds were isolated for the first time from *C. odorata* leaves. These findings contribute to providing scientific evidence for the traditional use and phytochemicals of *C. odorata* leaves.

Keywords: *Chromolaena odorata*, anti-ulcer, indomethacin, phytochemicals.

Introduction

Peptic ulcer disease, including gastric and duodenal ulcers, is responsible for the increase in morbidity and mortality worldwide.[1] The main causes of almost peptic ulcer disease cases are associated with the infection of *Helicobacter pylori* and the frequent administration of nonsteroidal anti-inflammatory drugs (NSAIDs). In general, the medication therapies for peptic ulcers embraces antacids, anticholinergics, histamine-2-receptor antagonists, antibiotics, proton pump inhibitors, sucralfate, and bismuth.[2] However, these anti-ulcer agents exhibit many serious side effects such as impotence, hypersensitivity, skin rash, constipation, headache, arrhythmia, urinary retention, atrophic gastritis, blurred vision, hematopoietic alterations, gynecomastia, xerostomia.[3] Therefore, the demand for finding and developing herbal drugs with fewer adverse effects is increasing significantly.

Chromolaena odorata (*C. odorata*) (L.) R. King & H. Robinson is a medicinal plant of the *Chromolaena* genus, which was identified by King and Robinson in 1970. It also has other scientific names such as *Eupatorium conyzoides* M., *Eupatorium odoratum* L., and *Osmia odorata* L.[4] *C. odorata* is a spreading, clustering shrub that is mainly found in crops and grasslands in South Asia and West Africa.[5] This species is native to Asia, North and South America, West and South Africa, and Australia.[6,7]

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According to modern pharmacological investigations, *C. odorata* has potents of antioxidant, antidiabetic, anti-inflammatory, anti-fungal, antimicrobial, anti-dyslipidemia, anticancer, and cytoprotective activities.[8] It is proven that the chemical composition of *C. odorata* includes flavonoids (chalcone, flavone, flavonol, and aurone), alkaloids, tannins, phytates, saponins, steroids, diterpenes, anthraquinones, phenolic acids, and cyanogenic glycosides, which take responsibility for these activities.[9,10] Besides, a previous study revealed that the dried leaves of *C. odorata* contain flavonoid aglycones, terpenes, triterpenoids, saponins, tannins, and phenolic acids.[11] In addition, the leaves of *C. odorata* have traditionally been commonly used for the treatment of diarrhea, wounds, headache, skin diseases, inflammatory diseases, and stomach ulcers.[8,12] In Vietnamese folk medicine, a remedy including *Chromolaena odorata* 30 g, *Ardisia silvestris* 30 g, *Herba Hedyotidis capitellata*e 20 g, and *Stahlianthus thorelii Gagnep* 5 g is utilized to improve the stomach ulcer condition.[13] Due to the capability of good anti-ulcer activity, fast-growing, high adaptability, and wide distribution, the potential for developing raw materials and creating anti-ulcer products from the leaves of *C. odorata* is enormous. To contribute to providing the evidence for the usage and the premise for the developing anti-ulcerogenic agents derived from *C. odorata* leaves, this study aimed to evaluate the anti-ulcer effects of ethanol crude extract and its fractions of *C. odorata* leaves on the indomethacin-induced gastric ulcer mice model and investigate the phytochemical compounds of the most active fraction.

Materials and Methods

General experimental procedures

Indomethacin was purchased from Kwality Pharmaceutical, India. Misoprostol was obtained from Unimed Pharm, Korea. All other chemicals used in this research were of analytical grade. Silica gel (Merck, 0.040 – 0.063 mm) and Sephadex LH-20 (Sigma-Aldrich) were used for column chromatography. Thin layer chromatography was

performed on pre-coated silica gel plates (Merck, F_{254}), which were activated at 110ºC for 1 hour before testing. The spots were detected under UV light and by heating after spraying with cerin sulfate or acid sulfuric 10%. Nuclear magnetic resonance (NMR) spectroscopy was obtained by using a Bruker Avance 500 NMR spectrometer (Bruker Biospin, Switzerland). The mass spectrometry was recorded on an Agilent 1260 LC/MS (Agilent Technologies, USA) using atmospheric pressure chemical ionization (APCI-MS). The melting temperature was investigated with a Thermo Scientific 1401 Electrothermal MEL-TEMP 3.0 instrument (Thermo Scientific, USA).

Plant materials

The leaves of *C. odorata* were collected from Nam Dinh province, Vietnam, which is located at latitude 20º18'17"N and longitude 106º16'38"E in December 2021. The plant material was identified by Dr. Hoang Le Son at the Department of Pharmacognosy and Traditional Medicine, University of Medicine and Pharmacy, Vietnam National University (UMP-VNU), Hanoi, Vietnam. The voucher specimen (No: UMP-03) has been deposited at UMP-VNU.

Extraction and isolation

The leaves of *C. odorata* were washed, chopped, and air-dried for five days. The dried samples (1.5 kg) were macerated in ethanol (EtOH) 70% (5 L) at room temperature for 24 hours and repeated the maceration three times. The combined extracts were filtered and evaporated under a vacuum to obtain the total ethanol extract (CO.Et, 110 g). The CO.Et residue (100 g) was dispersed in distilled water and then consecutively fractioned with *n*-hexane and ethyl acetate (EtOAc) to afford the *n*-hexane extract (CO.H, 19.8 g) and the EtOAc extract (CO.EA, 39.8 g), respectively. The extracts were evaporated under low pressure and then stored at 2-4ºC for further use. The CO.EA residue (39.8 g), which showed the best activities on indomethacin-induced ulcerated mice was subjected to silica gel column chromatography (CC), using gradient systems with *n*-hexane-EtOAc (5:1 to 1:1), then EtOAc-methanol (MeOH) (5:1 to 1:1) to give five fractions (E1-E5). Fraction E1 (9.3 g) was chromatographed over silica gel, eluting with a mixture of EtOAc-MeOH (5:1) to obtain four subfractions (E1.1-E1.5). Subfraction E1.1 (1.1 g) was further separated by silica gel CC with *n*-hexane-EtOAc (4:1) to afford compound 1 (21 mg). Subfraction E1.2 (1.4 g) was purified by silica gel CC, eluting with *n*-hexane-EtOAc (8:1) to obtain compound 2 (15 mg). Chromatography of subfraction E1.3 (2.4 g) over silica gel and elution with *n*-hexane-CH_2Cl_2 (2:1) afforded compound 3 (16 mg). Subfraction E1.4 (2.2 g) was chromatographed using a reversed-phase chromatography column (Octadecyl-silica column) with 80 cm x 1.5 cm in size (stuffing length of about 70 cm) and eluted with a solvent system of MeOH-H_2O (3:1) to afford compound 4 (10 mg).

Experimental animals

The Healthy male Wistar albino mice (weighing 180 – 200 g) were purchased from the National Institute of Hygiene and Epidemiology. Animals were maintained individually in polycarbonate cages in a colony room under standard environmental conditions including a 12h light/dark cycle, temperature-controlled (25 ± 3ºC), and were offered pelleted food and water *ad libitum.*[14] All procedures were conducted following protocols (permission number of UMP/082022) approved by the Ethics Committee of UMP-VNU and complied with the international instructions for animal research.

Acute toxicity assessment

The safety doses of the CO.Et, CO.H, and CO.EA extracts were determined through an acute oral toxicity test following the method of Litchfield – Wilcoxon.[15] Wistar male albino mice were assigned randomly into six groups (ten in each group) and fasted overnight. Each group was orally administered with gradually increasing doses of test samples (up to 2000 mg/kg). The general condition, behavior changes, signs of toxicity (vomiting, convulsions, agitation, etc), and mortality of mice groups were observed strictly for three days to evaluate the median lethal dose (LD_{50}) values. The observation was continued for seven days for any signs of delayed toxicity.

In vivo anti-ulcer assay

The *in vivo* anti-ulcer effect of the total ethanol extract, along with *n*-hexane and ethyl acetate fractions of *C. odorata* leaves was evaluated on an experimental mice model of indomethacin-induced ulcers following the previously described methods with some modifications.[16] According to ethnomedicinal practitioners, the dose levels of *C. odorata* extracts equivalent to 30 g of fresh medicinal leaves per day for humans were chosen. Briefly, Wistar male albino mice were randomly divided into six groups (n = 10/group) and treated orally with the following protocols:

Group I (negative control group): Distilled water (10 mL/kg).
Group II (ulcerated control group): Indomethacin (40 mg/kg) + distilled water (10 mL/kg).
Group III (positive control group): Indomethacin (40 mg/kg) + misoprostol (50 µg/kg).
Group IV (treated group): Indomethacin (40 mg/kg) + CO.Et (250 mg/kg).
Group V (treated group): Indomethacin (40 mg/kg) + CO.H (49.5 mg/kg).
Group VI (treated group): Indomethacin (40 mg/kg) + CO.EA (99.5 mg/kg).

The animals were treated with reference drug (misoprostol) or *C. odorata* extracts once daily for seven days. On the 7th day, after one hour of treatment, mice from groups II, III, IV, V, and VI were orally administered a single dose of indomethacin (40 mg/kg). Before administering indomethacin, mice fasted for 18 hours. After 6 hours of ulcer induction, all mice were anesthetized with thiopental and sacrificed by cervical dislocation. The part of the alimentary canal from the esophagus (proximal to the cardia) to the small intestine (3 cm from the pylorus) was cut separately. The stomachs were removed and opened with scissors following a great curvature. They were then rinsed with saline to remove the blood clots and gastric contents, absorbed the ulcer surface with formaldehyde 5%, and fixed on a sponge with pins for the determination of ulcer index.

Evaluation index

The ulcers were observed under a 10-fold binocular microscope to assess lesions. The severity of the ulcers was measured according to the point scale as scored as 0 (Normal colored stomach), 0.5 (Red colored stomach), 1 (Spot ulcers), 1.5 (Hemorrhagic streak), 2 (Deep ulcers), and 3 (Perforation ulcers).[17]

The ulcer index (UI) was assessed by the average ulcer scores and calculated by the following formula:

$UI = U_N + U_S + U_A \times 0.1$

where UI = Ulcer index, U_N = Ulcer number, U_S = Ulcer score, U_A = Ulcer surface area.

The percentage of inhibition (% I) was measured by the following equation:

$$\% I = \frac{UI_{control\ group} - UI_{treated\ group}}{UI_{control\ group}} x\ 100$$

Statistical analysis

The results were presented as mean ± standard deviation (SD). Microsoft Excel 2016 and SPSS 22.0 software using one-way ANOVA and independent sample *t*-test were utilized for analyzing the experimental data. The difference between variables was statistically significant when $p < 0.05$.

Results and Discussion

Acute toxicity test

The acute toxicity of the CO.Et, CO.H, and CO.EA extracts at ascending doses from 100 mg/kg to 2000 mg/kg was assessed. After the testing period, mice from all groups were still healthy with normal physical activities. At the highest dose of 2000 mg/kg, all extracts of *C. odorata* leaves did not show any sign of toxicity or mortality in mice. Hence, the median lethal dose (LD_{50}) of the leaf extracts of *C. odorata* is higher than 2000 mg/kg. These results were consistent with those of

previous acute toxicity reports, which indicated that adult mice could well-tolerate the *C. odorata* leaf extracts at doses between 10 – 5000 mg/kg.[18]

Anti-ulcer effect of C. odorata leaf extracts

The anti-ulcer activity of the total ethanol extract and solvent fractions of *C. odorata* leaves was evaluated using a gastric ulcerated mice model induced by indomethacin. This agent is widely used to produce a gastric ulcer experimental model due to its higher ulcerogenic ability than other NSAIDs.[19] It is proved that indomethacin promotes the reduction of prostaglandin synthesis by inhibiting both cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) enzymes, which is a necessary condition for gastric tissue damage occurring.[20,21] In this study, the anti-ulcer capacity of *C. odorata* leaf extracts was compared to misoprostol, a synthetic analog of prostaglandin E1. Various reports showed that misoprostol exposes a dose-related protective effect on NSAID-induced ulcers. The protection mechanisms of misoprostol include the inhibition of gastric acid secretion and the enhancement of gastric mucosal resistance to injury.[22]

Based on the results represented in Table 1, the administration of indomethacin created gastric lesions with 100% of mice in the vehicle control group having ulcers. Misoprostol significantly reduced the rate of ulcers caused by indomethacin compared with the normal control group ($p < 0.05$). When compared to the ulcerated control group, the leaf extracts of *C. odorata* at the tested doses tended to decrease the rate of indomethacin-induced ulcers with the ulceration proportion of 91.1%, 80.6%, 71.8% for the CO.Et, CO.H, and CO.EA, respectively. However, these differences were not statistically significant ($p > 0.05$). Regarding the ulcer index and the percentage of ulcer inhibition, the misoprostol-treated group had significantly decreased the gastric lesions induced by indomethacin with a percentage inhibition of 24.04% when compared to the ulcerated control group (Table 2 and Figure 1). Among fractions, at the dose levels equivalent to clinical doses, the CO.EA performed the greatest protective effects on indomethacin-induced ulcers with the lowest ulcer index (0.73 ± 0.39) and the highest percentage inhibition (26.92%). A significant reduction was seen in the ulcer index and percentage inhibition of both fractions as compared to the vehicle group ($p < 0.05$). By contrast, the total ethanol extract CO.Et failed to inhibit gastric lesions with higher ulcer index and percentage inhibition than those of the vehicle group.

Gastric ulcer is known to cause by inflammation occurring in the gastric mucosal. Hence, anti-inflammatory agents that can inhibit the generation of inflammatory mediators or the action of inflammatory cells are considered general treatment therapies for this disease.[23] In addition, antioxidants are supposed to contribute the gastric ulcer healing due to their abilities of oxidative damage reduction, cell and tissue protection, and creation of favorable conditions for tissue healing.[24] Previous studies have determined the anti-inflammatory and antioxidant activities of the *C. odorata* leaf extracts which were exerted by the presence of substance groups including alkaloids, flavonoids, polyphenols, tannins, saponins, and triterpenes.[4,25] This could be the reason for the anti-ulcer effect of *C. odorata* leaves on the indomethacin-induced ulcerated mice model. The difference in the chemical composition and the content of biomolecules might lead to the difference in the anti-ulcer potential of their extracts and fractions.

Isolated compounds and structural elucidation

The chromatographic methods led to the separation of four compounds (1–4, Figure 2) from the bioactive ethyl acetate fraction of *C. odorata* leaves. The structures of these compounds were determined by melting temperature, mass spectrometry (MS), nuclear magnetic resonance (NMR) spectroscopy, and comparison with the published reference data. Their relative concentrations in the CO.EA were 0.053%, 0.038%, 0.040%, and 0.025% for compounds 1–4, respectively. All compounds were isolated for the first time from *C. odorata*.

1-(4-hydroxy-3-methoxyphenyl)propane-1,2-diol (1): Colorless oil; APCI-MS *m/z* 197.1 $[M-H]^-$: 197.1, 165.8, 123.2, 73.9; ^{1}H-NMR (500 MHz, CD_3OD, δ_H ppm): 4.27 (1H, d, J = 7.6 Hz, H-1), 3.82 (1H, dq, J = 6.4, 7.6 Hz, H-2), 6.80 (1H, d, J = 2.4 Hz, H-2'), 6.95 (1H, d, J = 8.0 Hz, H-5'), 6.78 (1H, dd, J = 2.0, 8.4 Hz, H-6'), 3.87 (3H, s, 3'-OCH_3); ^{13}C-NMR (125 MHz, CD_3OD, δ_C ppm): 72.9 (C-1), 80.2 (C-2), 19.2 (C-3), 134.7 (C-1'), 111.5 (C-2'), 148.8 (C-3'), 147.1 (C-4'), 115.8 (C-5'), 120.9 (C-6'), 56.3 (3'-OCH_3).

Compound 1 was obtained as a colorless oil with molecular formula $C_{10}H_{14}O_4$ based on the pseudo-molecular negative ion peak signal at *m/z* 197.1 $[M-H]^-$ on the APCI-MS. In the ^{1}H-NMR spectrum of compound 1, the presence of the 4-hydroxy-3-methoxyphenyl group was confirmed by three aromatic proton signals at δ_H 6.95 (1H, d, J = 8.0 Hz, H-5'), 6.80 (1H, d, J = 2.4 Hz, H-2'), 6.78 (1H, dd, J = 2.0, 8.4 Hz, H-6'), a methoxy proton signal at δ_H 3.87 (3H, s, 3'-OMe), and an NOE between the signals of δ_H 6.95 and 3.87. The ^{13}C-NMR and DEPT spectra revealed that compound 1 has ten carbons in total, including two methyl carbons at δ_C 19.2 (C-3), 56.3 (3'-OMe), five methine carbons at δ_C 72.9 (C-1), 80.2 (C-2), 111.5 (C-2'), 115.8 (C-5'), 120.9 (C-6'), and three quaternary carbons at δ_C 134.7 (C-1'), 147.1 (C-4'), 148.8 (C-3'). The structure of compound 1 was assigned as 1-(4-hydroxy-3-methoxyphenyl)propane-1,2-diol which was consistent with the reported literature data.[26]

Kaempferol-7-*O*-*α*-L-rhamnopyranoside (2): Yellow amorphous powder; melting point 313 – 314ºC; ^{1}H-NMR (500 MHz, DMSO, δ_H ppm): 12.47 (1H, s, H-5), 6.21 (1H, d, J = 2.2 Hz, H-6), 6.41 (1H, d, J = 1.5 Hz, H-8), 8.08 (2H, d, J = 8.7 Hz, H-2'/H-6'), 6.93 (2H, d, J = 8.7 Hz, H-3'/H-5'), 5.54 (1H, s, H-1"), 1.10 (3H, d, J = 6.3 Hz, H-6"); ^{13}C-NMR (125 MHz, DMSO, δ_c ppm): 155.7 (C-2), 135.9 (C-3), 176.0 (C-4), 160.4 (C-5), 98.5 (C-6), 162.1 (C-7), 94.3 (C-8), 155.9 (C-9), 105.1 (C-10), 122.0 (C-1'), 129.7 (C-2'/C-6'), 115.5 (C-3'/C-5'), 159.3 (C-4'), 98.8 (C-1"), 70.3 (C-2"), 70.1 (C-3"), 72.0 (C-4"), 69.6 (C-5"), 18.0 (C-6").

Compound 2 was obtained as a yellow amorphous powder with a melting point range of 313 – 314ºC. In the ^{1}H-NMR spectrum of compound 2, the signals of the 4',5,7-trihydroxy flavonol skeletons appeared as one set of meta-coupled aromatic protons at δ_H 6.41 (1H, d, J = 1.5 Hz, H-8), 6.21 (1H, d, J = 2.2 Hz, H-6) and two sets of orthologous coupled aromatic protons at δ_H 8.08 (1H, d, J = 8.7 Hz, H-2'/ H-6') and 6.93 (1H, d, J = 8.7 Hz, H-3'/ H-5'). In addition, two doublet signals at δ_H 5.54 (1H, s, H-1") and 1.10 (3H, d, J = 6.3 Hz, H-6") suggested the presence of a rhamnose half ring in the structure of compound 2. Besides, the ^{13}C-NMR data showed two carbon signals at δ_C 162.1 (C-7) and 155.7 (C-2), which revealed the binding of the rhamnose moiety to the C-7 site of the aglycone. These values were consistent with other flavonol 7-O-rhamnoside values.

Table 1: The proportion of ulcerated mice treated with the leaf extracts of *C. odorata*.

	Percentage of mice with ulcers (%)
Group I	0
Group II	100
Group III	63.9[a]
Group IV	91.1
Group V	80.6
Group VI	71.8

Note: [a]$p < 0.05$ compared with the normal control group (Group I)

Table 2: Effect of *C. odorata* leaf extracts on indomethacin-induced ulcers in mice

	Ulcer index	**% inhibition**
Group II	1.04 ± 0.14	–
Group III	0.78 ± 0.21[a]	24.04[a]
Group IV	1.05 ± 0.24	–
Group V	0.86 ± 0.19[a]	17.31[a]
Group VI	0.73 ± 0.39[a]	26.92[a]

Note: Data are presented as mean ± SD (n = 10 for each group). [a]$p < 0.05$ compared with the ulcerated control group (Group II)

According to the analysis of spectral data and spectral comparison with previously published literature, it can be confirmed that compound 2 was kaempferol-7-*O*-*α*-L-rhamnopyranoside.[27]

Naringenin-5,7-di-*O*-*β*-D-glucopyranoside (3) – White powder; melting point 203 – 205°C; ^{1}H-NMR (500 MHz, DMSO, δ_H ppm): 5.43 (1H, dd, *J* = 2.7, 13.3 Hz, H-2α), 2.84 (1H, dd, *J* = 2.7, 16.4 Hz, H-2β), 3.19 (1H , dd, J = 13.7, 16.4 Hz, H-3), 6.51 (1H, d, *J* = 2.5 Hz, H-6), 6.32 (1H, d, *J* = 2.5 Hz, H-8), 7.31 (2H, d, *J* = 8.6 Hz, H-2'/H-6'), 6.78 (2H, d, *J* = 8.6 Hz, H-3'/H-5'), 9.61 (1H, s, H-4'), 5.08 (1H, d, *J* = 7.1 Hz, H-1"), 4.97 (1H, d, *J* = 8.4 Hz, H-1'"); ^{13}C-NMR (125 MHz, DMSO, δ_c ppm): 78.6 (C-2), 44.6 (C-3), 191.0 (C-4), 163.3 (C-5), 99.3 (C-6), 163.9 (C-7), 98.1 (C-8), 159.9 (C-9), 106.8 (C-10), 128.9 (C-1'), 128.3 (C-2'/C-6'), 115.2 (C-3'/C-5'), 157.7 (C-4'), 102.4 (C-1"), 73.3 (C-2"), 77.4 (C-3"), 69.8 (C-4"), 75.8 (C-5"), 60.9 (C-6"), 99.3 (C-1'"), 73.0 (C-2'"), 77.0 (C-3'"), 69.6 (C-4'"), 76.4 (C-5'"), 60.9 (C-6'").

Compound 3 was isolated as a white powder with a melting point range of 203 – 205°C. The ^{1}H-NMR spectroscopic data showed assignable signals to a dihydropyrone radical in the flavanone structure by a characteristic ABX spin system at δ_H 5.43 (1H, dd, *J* = 2.71, 13.3 Hz, H-2), 3.19 (1H, dd, *J* = 13.7, 16.4 Hz, unclear), 2.84 (1H, dd, *J* = 2.7, 16.4 Hz, H-3*cis*) and a pair of meta-bonded aromatic protons at δ_H 6.51 (1H, d, *J* = 2.5 Hz, H-6), 6.32 (1H, d, *J* = 2.5 Hz, H-8). Besides, there were signals of the AA´BB´ type aromatic protons at δ_H 7.31 (2H, d, *J* = 8.6 Hz, H-2'/H-6'), 6.78 (2H, d, *J* = 8.6 Hz, H-3'/H-5'). Furthermore, the presence of two sugar moieties was confirmed by two anomeric protons signal at δ_H 5.08 (1H, d, *J* = 7.1 Hz, H-1"), 4.97 (1H, d, *J* = 8.4 Hz, H-1'"). Compound 3 was identified as naringenin-5,7-di-*O*-*β*-D-glucopyranoside based on the analysis of spectral data and spectra comparison with the reported literature.[28]

Rubrosterone (4) – White crystals; melting point 245 – 247°C; ^{1}H-NMR (500 MHz, CD_3OD, δ_H ppm): 3.84 (1H, brs, H-2), 3.95 (1H, m, H-3), 5.93 (1H, brs, H-7), 0.89 (3H, s, H-18), 1.01 (3H, s, H-19); ^{13}C-NMR (125 MHz, CD_3OD, δ_c ppm): 37.3 (C-1), 68.6 (C-2), 68.4 (C-3), 32.8 (C-4), 51.9 (C-5), 205.9 (C-6), 122.4 (C-7), 164.6 (C-8), 35.8 (C-9), 39.3 (C-10), 20.6 (C-11), 29.1 (C-12), 54.1 (C-13), 79.6 (C-14), 34.0 (C-15), 24.5 (C-16), 220.2 (C-17), 17.6 (C-18), 24.9 (C-19).

Compound 4 was obtained as white crystals with a melting point range of 245 – 247°C. The ^{1}H-NMR data presented two singlet signals at δ_H 1.01 (3H, s, H-19), 0.89 (3H, s, H-18) corresponding to two tertiary methyl groups, along with a signal of an olefinic proton at δ_H 5.93 (1H, brs), and two signals of oxymethine groups at δ_H 3.95 (1H, m, H-3), 3.84 (1H, brs, H-2). Furthermore, the linear position and separation pattern of carbonyl hydrogen signals indicated that two oxymethine groups have beta orientation. On the ^{13}C-NMR and DEPT spectra, there were nineteen carbon signals in total, including two carbons of carbonyl groups at δ_C 205.9 (C-6), 220.2 (C-17), two olefinic carbons at δ_C 122.4 (C-7), 164.6 (C-8), three quaternary carbons at δ_C 39.3 (C-10), 54.1 (C-13), 79.6 (C-14), four methine carbons at δ_C 68.6 (C-2), 68.4 (C-3), 51.9 (C-5), 35.8 (C-9), six methylene carbons at δ_C 37.3 (C-1), 32.8 (C-4), 20.6 (C-11), 29.1 (C-12), 34.0 (C-15), 24.5 (C-16), and two methyl carbons at δ_C 17.6 (C-18), 24.9 (C-19). The detailed analysis of spectra data and comparison with those of the reference data allowed the determination of compound 4 as rubrosterone.[29]

According to previous reports, compound 2 had high abilities of antioxidant and anti-inflammatory effects. It exposed strong antioxidant activities with the IC_{50} values of 12.3 ± 0.5 µg/mL and 7.4 ± 1.2 µg/mL for the DPPH and ABTS assays, compared to the positive control quercetin (IC_{50} 10.2 ± 0.3 µg/mL and 12.2 ± 0.5 µg/mL, accordingly).[30] Meanwhile, its anti-inflammatory potent was exerted through the inhibition of the lipopolysaccharide-induced nitric oxide production in RAW 264.7 cells and the reduction of the prostaglandin E2 accumulation.[31]Although still limited in the pharmacological studies, compounds 1, 3, and 4 were proved to be major phenolic components of some medicinal plants which performed good antioxidant, anti-inflammatory, and anti-ulcer activities.[26,32-36] Therefore, all four isolated substances might take responsibility for the anti-ulcer effect of *C. odorata* leaves.

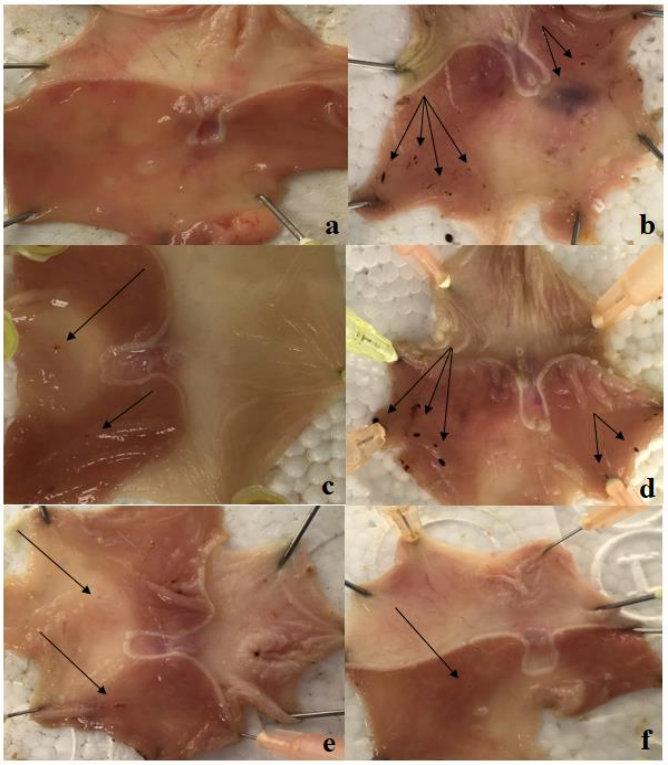

Figure 1: Ulcerative lesions in experimental mice groups: (a) normal, (b) indomethacin control, (c) treated with misoprostol, (d) treated with CO.Et, (e) treated with CO.H, and (f) treated with CO.EA

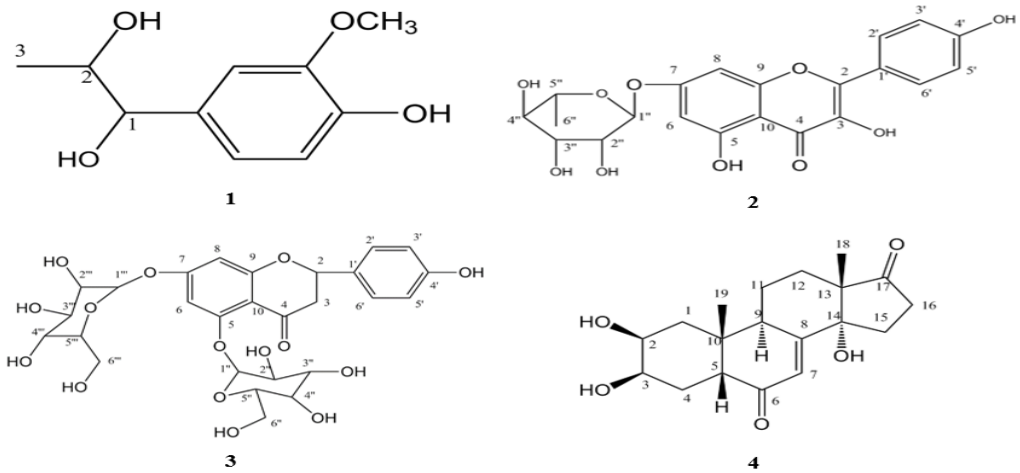

Figure 2: Structure of isolated compounds (1-4) from the ethyl acetate fraction of *C. odorata* leaves.

Conclusion

In summary, this study demonstrated the acute toxicity and the anti-ulcer ability of the total ethanol extract and its fractions of *C. odorata* leaves. Among them, at the dose levels equivalent to clinical doses, the ethyl acetate fraction exhibited the greatest protective effect on the indomethacin-induced ulcerated mice model. From this fraction, four known compounds including 1-(4-hydroxy-3-methoxyphenyl)propane-1,2-diol (1), kaempferol-7-*O*-*α*-L-rhamnopyranoside (2), naringenin-5,7-di-*O*-*β*-D-glucopyranoside (3), and rubrosterone (4) were isolated and purified by the chromatographic methods. Their structures were determined by spectroscopic data and comparison with those of previously published references. These pure compounds were isolated for the first time from the leaves of *C. odorata*. Our findings indicate that the *C. odorata* leaves may be a promising candidate for the treatment of gastric ulcers. Further studies are needed to understand the mechanisms of the therapeutic activity of the *C. odorata* leaves and identify more bioactive compounds from this medicinal plant.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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