Isolation of Major Compounds and Gastroprotective Activity of *Alchemilla Caucasia* on Indomethacin Induced Gastric Ulcers in Rats

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**Abstract**

**Objective:** The Alchemilla genus, which belongs to the Rosaceae family, is known as Lady's mantle and is commonly used in traditional medicine. This study was designed to investigate the major metabolites isolation and gastroprotective effects of *Alchemilla caucasica*.

**Materials and Methods:** Phytochemical studies were carried out using column chromatography on *Alchemilla caucasica*. The gastroprotective effect of ethanol extract of this plant was tested on indomethacin-induced gastric ulcer model in rats. In addition, superoxide dismutase (SOD), malondialdehyde (MDA), and glutathione (GSH) parameters in the stomach tissue were examined.

**Results:** Quercetin-3-O-glucuronide, apigenin, and catechin were isolated from aerial parts of *Alchemilla caucasica*. When macroscopic ulcer index and histopathological results were analyzed, the extract at 200 mg/kg dose was found to be most effective. All doses of extract reduced MDA level and enhanced SOD activity and GSH level.

**Conclusion:** The results of this study showed that *Alchemilla caucasica* has significant antiulcer activity. This effect was thought to be caused by antioxidant properties of flavonoids.

**Keywords:** Antioxidants, medicinal plants, ulcer, phytochemicals

**Introduction**

Peptic ulcer is a gastrointestinal system disease characterized by mucosal damage secondary to pepsin and gastric acid secretion. *Helicobacter pylori* infection, nonsteroidal anti-inflammatory drugs (NSAIDs), critical illness, surgery, smoking, and stress are the causes of peptic ulcer disease [1]. Reactive oxygen species (ROS) cause a significant increase in the formation of gastric ulcer. Indomethacin (IND)-induced inflammation in the stomach tissue causes ROS formation [2]. Antioxidants such as superoxide dismutase (SOD) and glutathione (GSH) have protective effects against stomach damage [3].

The Alchemilla genus (family: Rosaceae) contains nearly 80 species in Turkey [4, 5]. This genus is known as Lady’s mantle and is commonly used in traditional medicine. Some of the species have been used as a diuretic, hypocholesterolemic, sedative, and memory booster [6] and have been used for diarrhea, dysmenorrhea, menopausal complaints, eczema, skin rashes, ulcers [7], liver disorder, menstruation problem, and skin diseases [8]. Species belonging to Alchemilla genus contain mainly flavonoids [4, 9]. They also include triterpenes [10, 11] and tannins [12, 13]. It is known that flavonoids have antibacterial, hepatoprotective, anti-inflammatory, anticancer, antiviral, and strong antioxidant effects [14].

*Alchemilla caucasica* is a species belonging to this genus. This study was designed 1) to isolate secondary metabolites of *A. caucasica*, 2) to evaluate the activity of its ethanol extract in IND-induced gastric ulcer model, and 3) to define its effects on antioxidant parameters in rat stomach tissue.

**Materials and Methods**

**Animals**

In the experiments, 36 male albino Wistar rats weighing 220–280 g were used. The studies were conducted in the Atatürk University’s Experimental Animal Laboratory at the Medicinal and...
Experimental Application and Research Centre in accordance with the national guidelines for animal use in experiments. An ethics permit was obtained from the local animal care committee of Atatürk University (93722986-000-E.18001/20587).

**Plant Material**

The plant samples were collected from Konaklı Mountain (Erzurum Province, Turkey). A sample of the species is stored in the Herbarium of the Faculty of Pharmacy (Atatürk University, AUEF1023).

**Extraction and Isolation Studies**

The aerial parts of the plant were dried and powdered. The powdered extract (430 g) was extracted with methanol at 40 °C (3×2 L). The methanol extract (72.77 g) was evaporated in the rotary evaporator. The dried extract was dissolved in water and subjected to liquid-liquid extraction with dichloromethane and ethyl acetate. Later, the solvents were evaporated in a rotary evaporator. Ethyl acetate, dichloromethane, and water extracts were obtained. Compound 1 was isolated from water extract using silica gel (Merck, Darmstadt, Germany) column (solvent systems: CHCl₃:MeOH:H₂O [90:10:1→50:50:50]), Sephadex LH-20 (Sigma, St. Louis, MO, USA) column (solvent system: MeOH), and then silica gel column (solvent systems: CH₃Cl:MeOH:H₂O [70:35:3, 35:35:35]). Compound 2 was isolated from ethyl acetate extract using silica gel (solvent systems: CH₃Cl:MeOH:H₂O [90:10:1→50:50:50]), reverse phase silica gel column (solvent systems: H₂O:MeOH [90:10→100:100], and Sephadex LH-20 column (solvent system: MeOH). Compound 3 was isolated from EtOAc extract using silica gel column (solvent systems: CH₃Cl:MeOH:H₂O [90:10:1→50:50:50]), Sephadex LH-20 column (solvent system: MeOH), and then silica gel column (solvent systems: CH₃Cl:MeOH:H₂O [80:20:2, 70:30:30, 60:40:40]). The structures of the compounds were determined by 1H nuclear magnetic resonance (NMR), 13C NMR, 2D NMR, and quadrupole time-of-flight (Q-TOF) mass spectrometer.

**Main Points**

- Quercetin-3-O-glucuronide, apigenin and catechin were isolated from aerial parts of Alchemilla caucasia.
- All doses of extract increased SOD activity and GHS level and decreased MDA level.
- According to the histopathological examination, IND+ALC200 group had similar characteristics with the healthy group.

**Ulcer Model**

The IND-induced ulcer model procedure was used in this experiment [15, 16]. NSAIDs are frequently referred to as the ulcerative formation model [17]. In the experiment, rats were not fed for 24 hours with free access to water. A total of 1 ml of saline was administered to healthy and IND control groups by oral gavage as vehicle. Then, 40 mg/kg dose of famotidine (FAM) (Famodin 40 mg, Sandoz Drug Company, Istanbul, Turkey) was administered to all groups except for healthy group by oral gavage. After 6 hours, thiopeptol (Thiopeptol sodium, IE Ulagay A.S., Istanbul, Turkey) at a dose of 50 mg/kg was applied intraperitoneally to all groups, and euthanasia was performed. The ulcerated areas on the stomach surfaces of rats were evaluated macroscopically and were measured by mm² paper.

**Experimental Groups of Indomethacin-Induced Ulcer Model**

Each experiment group consisted of 6 rats, which were classified as follows: 1) Healthy; 2) 25 mg/kg IND; 3) 25 mg/kg IND+40 mg/kg FAM (IND+FAM); 4) 25 mg/kg IND+50 mg/kg ALC (IND+ALC50); 5) 25 mg/kg IND+100 mg/kg ALC (IND+ALC100); and 6) 25 mg/kg IND+200 mg/kg ALC (IND+ALC200).

**Histopathological Evaluation**

The tissues were fixed in a 10% buffered formalin solution for 24 hours for histopathological examination. After fixation, tissue samples were immersed in paraffin. Sections that were 5-μm thick were cut from the paraffin-immersed tissue samples and placed on positively charged slides. Then, the samples underwent deparaffinization and rehydration and were spotted with Mayer’s hematoxylin and eosin. The sections were investigated under the microscope for histopathological changes using a light photomicroscope (Nikon Eclipse E600; USA).

**Biochemical Studies**

The tissues were stored at ~80 °C for biochemical experiments. The samples were ground with liquid nitrogen by a TissueLyser II grinding jar set. The milled tissues (50 mg) were centrifuged after mixing with 1 ml PBS buffer. SOD activity [18] and malondialdehyde (MDA) [19] and GSH levels [20] were measured at room temperature using an enzyme-linked immunosorbent assay reader [21]. A standard curve was formed by calculating the medial absorbance. Linear SOD activity and GSH and MDA concentrations were calculated according to the equation obtained from the standard absorbance.

**Statistical Analysis**

For the statistical analysis, IBM Statistics 20 version of Statistical Package for the Social Sciences (IBM SPSS Corp; Armonk, NY, USA) software was used. One-way analysis of variance and Duncan’s Multiple Comparison Test were performed. The mean values in the same column by the same letter are not significantly different from the test of Duncan. The level with the p-value <0.05 was considered significant. Results are expressed as mean ± standard deviation.

**Results**

**Isolation and Structure Identification**

Quercetin-3-O-glucuronide (Miquelanin) (Compound 1): C₃₀H₄₂O₁₃ m/z 479.0820 [M+H]+; 1H-NMR (400 MHz, CD₃OD) δ 3.47-3.63 (av, H-2′-H-5′), 5.32 (d, J 7.2 Hz, H-1′), 6.19 (d, J 2.1 Hz, H-6), 6.38 (d, J 2.1 Hz, H-8), 6.86 (d, J 8.5 Hz, H-5′), 7.49 (dd, J 8.5, 2.2 Hz, H-6), 7.94 (d, J 2.1 Hz, H-2′). 13C-NMR (100 MHz, CD₃OD) δ 157.1 (C-2), 134.4 (C-3), 178.0 (C-4), 161.5 (C-5), 98.5 (C-6), 164.7 (C-7), 93.3 (C-8), 157.8 (C-9), 104.3 (C-10), 121.4 (C-1′), 117.4 (C-2′), 144.5 (C-3′), 1485 (C-4′), 1166 (C-5′), 121.3 (C-6′), 103.1 (C-1″), 74.1 (C-2″), 76.7 (C-3″), 71.9 (C-4″), 762 (C-5″), 175.0 (C-6″).

Apigenin (Compound 2): C₁₅H₁₀O₅ δ 291.0853 [M+H]+; 1H-NMR (400 MHz, DMSO-d₆) δ 6.19 (d, J 2.0 Hz, H-6), 6.48 (d, J 2.0 Hz, H-6), 6.79 (s, H-3), 6.92 (d, J 8.0 Hz, H-3′, 5′), 7.93 (d, J 8.0 Hz, H-2′, 6′), 12.97 (s, 5-OH). 13C-NMR (100 MHz, DMSO-d₆) δ 164.7 (C-2), 103.3 (C-3), 182.2 (C-4), 161.9 (C-5), 99.3 (C-6), 169.9 (C-7), 94.4 (C-8), 157.8 (C-9), 104.1 (C-10), 1216.1 (C-1′), 128.9 (C-1″), 116.4 (C-2″), 161.6 (C-3″), 116.4 (C-4″), 128.9 (C-5″), 116.4 (C-6″).

Catechin (Compound 3): C₁₅H₁₀O₇ m/z 291.0853 [M+H]+; 1H-NMR (400 MHz, CD₃OD) δ 2.52 (dd, J 16.1, 8.1 Hz, H-4a), –2.87 (dd, J 16.1, 5.4 Hz, H-4b), –3.99 (m, H-3), –4.58 (d, J 7.5 Hz, H-2), 5.87 (d, J 2.3 Hz, H-8), –5.95 (d, J 2.3 Hz, H-6), 6.73 (dd, J 8.2, 1.8 Hz, H-6′), 6.78 (d, J 8.0 Hz, H-5′), 6.86 (d, J 1.8 Hz, H-2′). 13C-NMR (100 MHz, CD₃OD) δ 81.42 (C-2), 67.42 (C-3), 27.11 (C-4), 156.18 (C-5), 94.91 (C-6), 156.45 (C-7), 94.12 (C-8), 155.52 (C-9), 99.43 (C-10), 130.84 (C-1′), 113.87 (C-2′), 144.83 (C-3′), 144.85 (C-4′), 114.68 (C-5′), 118.63 (C-6′).
Macroscopic Ulcer Index
When macroscopic ulcer indexes were examined, the extract at 200 mg/kg concentration showed a strong antiulcer activity (89%, p<0.05), which is compatible to the standard drug FAM (97%, p<0.05). The ulcer index area of extract (50, 100, and 200 mg/kg doses), FAM (40 mg/kg dose), and control groups are shown in Figure 1.

Histopathological Evaluation
The tissue sections were evaluated for histopathological changes under a microscope. In the healthy group, it was observed that the gastric pits were normal, and the parietal and surface mucous cells had a healthy appearance. In the IND group, epithelial losses and irregular gastric pits were observed in the mucosa; the necrotic appearance of the surface mucous cells and the increase of lymphatic cells in the lamina propria were remarkable. There was also an increase in eosinophilic staining properties of some parietal cells. The IND+ALC200 group had a similar appearance to a healthy group. Nevertheless, some epithelial cells were cast. Histopathological ulcerated area scoring results are shown in Table 1. Macroscopic photographs and the histopathological results are shown in Figure 2.

Biochemical Evaluation
When biochemical parameters were analyzed, it was observed that the extract at 200 mg/kg concentration increased the SOD activity (p<0.05) and GSH level (p<0.05) at the highest rate compared with the standard drug FAM. In addition, the extract at 200 mg/kg concentration decreased the MDA level at highest degree (p<0.05). The results of the SOD activity and GSH and MDA levels are shown in Figure 3.

Discussion
The genus Alchemilla belongs to the Rosaceae family. This genus is rich in flavonoids and has traditionally various medicinal usage. Flavonoids are good antioxidant substances [22]. They are also thought to have antiulcer activities [23]. One of the most important hypotheses in the formation of ulcers caused by NSAIDs is increased oxidative stress and disruption of antioxidant system. When we looked at the literature, we did not encounter any phytochemical activity and antiulcer activity studies on A. caucasica. Therefore, secondary metabolites isolation was carried out by column chromatography on the aerial parts of A. caucasica in this study. The activity of the ethanol extract of A. caucasica in IND-induced gastric ulcer model was evaluated, and the effects on antioxidant parameters in rat stomach tissue were defined.

Three compounds were isolated from the aerial parts of A. caucasica using column chromatography. The results of NMR of these compounds were found to be compatible with the literature. The first component was quercetin-3-O-glucuronide (miquelianin) [24, 25], the second component was apigenin [26], and the third component was catechin [27]. The genus Alchemilla contains mainly different flavonoids [4, 9]. The result of our study supported this information.

Gastroprotective effect of A. caucasica ethanol extract on IND-induced gastric ulcers in rats was investigated. In addition, the effect of the extract on antioxidant parameters in rat stomach tissues was evaluated biochemically. It was observed that all concentrations of plant extracts significantly reduced ulcerated index areas (Figure 1). According to the histopathological examination, drop-out of epithelial cells, irregular pit observation, and necrotic appearance are directly related to ulcers. When ulcerated area index and histopathological

| Table 1. Histopathological ulcerated area scoring results |
|----------------------------------------------------------|
| Lymphatic cell increase | Hemoragy | Epithelial cell loss |
|-------------------------|----------|---------------------|
| ALC50                   | ++       | ++                  |
| ALC100                  | +        | –                   |
| ALC200                  | –        | –                   |
| IND                     | +++      | +                   |
| IND+FAM                 | –        | –                   |
| Healthy                 | –        | –                   |

Histopathological damage: – (none), + (little damage), ++ (moderate damage), +++ (severe damage) ALC: Alchemilla caucasica extract (50, 100, and 200 mg/kg concentrations). IND: Indomethacin; FAM: Famotidine
results were evaluated, it was observed that IND+ALC200 group was similar to the healthy group. However, some spilled epithelial cells have been seen. Macroscopic and histopathological results are shown in Figure 2. It is reported that some species belonging to the genus *Alchemilla* are used traditionally in the ulcer treatment [7]. The results of this study have been found compatible with this literature.

One of the most important hypotheses in the formation of ulcers caused by NSAIDs is increased oxidative stress and disruption of antioxidant system. Flavonoids have high antioxidant effects and are thought to have antiulcer effect. In this study, it was determined that *A. caucasica* contains some flavonoids. As a result of the study, it was observed that all tested doses had antiulcer effects. We think that significant effects of this plant on IND-induced ulcer is due to the single or synergistic effects of its antioxidant flavonoids or other undetectable compounds.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the Ethics Committee of Ataturk University (93722986-000-E.1800120587).

**Informed Consent:** N/A

**Author Contributions:** Concept - E.S.K.; Design - E.S.K., Y.B., A.A.; E.T; Supervision - E.S.K., Y.B., A.A.; Resources - E.S.K., Y.B., C.K.; Materials - E.S.K., Y.B., A.A., E.T; Data Collection and/or Processing - E.S.K., U.O., A.K.; Analysis and/or Interpretation - E.S.K., Y.B., A.A., E.T., C.K., A.K.; Literature Search - E.S.K., U.O.; Writing Manuscript E.S.K.; - Critical Review - E.Ç.

**Conflict of Interest:** Authors have no conflicts of interest to declare.

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**Figure 3.** SOD activity and GSH and MDA levels. SOD: superoxide dismutase; GSH: glutathione; MDA: malondialdehyde.
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