

Research Article:

Phytochemical Screening and Antinociceptive Activity of the Hydroalcoholic Extract of *Potentilla reptans* L.Nematollah Ahangar¹ , Hossein Bakhshi Jouybari^{2,3} , Ali Davoodi² , Somayeh Shahani^{2*}

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ABSTRACT

Background: *Potentilla* species have traditionally been used as anti-inflammatory and analgesic agents in Iran and other countries.

Objectives: This study aimed to investigate the antinociceptive effect of *Potentilla reptans* L., which has a wide distribution in the north of Iran.

Methods: The biological activities of the hydroalcoholic extract of *P. reptans* aerial parts have been investigated using the acetic acid-induced writhing, hot plate, and rotarod tests in the male mice. In addition, the phytochemical profile of the extract has been evaluated.

Results: The phytochemical investigation detected secondary metabolites such as flavonoids, saponins, triterpenoids, and tannins in the extract. Moreover, the Mean±SD total phenolic and tannin contents of the extract were 251±2.08 and 111.5±1.3 mg gallic acid equivalents per gram of dried extract, respectively. Also, the Mean±SD total flavonoid content was 29.42±3.31 mg quercetin equivalents per gram of dried extract. Oral administration of the extract (100, 300, and 500 mg/kg) dose-dependently reduced the number of writhing responses induced by acetic acid and increased the reaction time in the hot-plate test. The antinociceptive effect of the extract, similar to morphine, was significantly antagonized by naloxone (4 mg/kg; IP) in the writhing test. In the rotarod test, none of the extract doses used in the experiment caused a loss of locomotor activity.

Conclusion: In this study, the hydroalcoholic extract of *P. reptans* showed a practical antinociceptive effect in hot plate and writhing tests. It seems that opioid receptors mediate the observed effect.

Introduction

T

he genus *Potentilla* (Cinquefoil), belonging to the Rosaceae family, includes about 500 species, of which 41 perennial and

herbaceous species grow in Iran. This genus is distributed widely in the northern hemisphere [1-3]. Traditionally, these plants have been used for diarrhea, wound healing, mouth ulcers, inflammation, and certain bacterial and viral infections in various parts of the world [4, 5]. *Poten-*

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tilla reptans L. is a perennial, creeping, and quinquefoliate plant with yellow flowers (popularly called creeping cinquefoil) [1]. In Iranian traditional medicine, *P. reptans* has been used for arthritis, inflammation, wound healing, diarrhea, jaundice, and epileptic disorders [6]. Based on previous studies, the main constituents of the aerial parts of *Potentilla* species such as *P. reptans* are flavonoids and tannins. The other identified constituents are triterpenoids and phenolic acids [1]. Recent pharmacological studies on the genus *Potentilla* exhibited anti-inflammatory, analgesic, antiulcer, antibacterial, anti-diarrhea, antioxidant, and cytotoxic effects [7-10].

Pain can cause physical disability and emotional distress, which affects all aspects of quality of life. Many patients with advanced and progressive diseases experience pain [11]. There are two types of pain, nociceptive and neuropathic. Nociceptive pain, as the common type, is the result of an injury or inflammation. The current drugs for the management of pain, such as nonsteroidal anti-inflammatory drugs and opiates, are often unable to alleviate pain and also cause various side effects. Accordingly, many studies have been conducted to find effective and safe alternatives, especially from natural sources. Medicinal plants have been used to relieve pain since ancient times so that they can be considered as valuable resources for the discovery of new therapeutic agents for the management of different types of pain [12-15].

Based on pharmacological potential and traditional uses of the genus *Potentilla*, we aimed to evaluate the antinociceptive activity of the hydroalcoholic extract of *Potentilla reptans* L. (HEP) in peripheral and central animal models of pain. Furthermore, the preliminary phytochemical screening and quantitative analysis of phenols, tannins, and flavonoids were performed on the extract.

Materials and Methods

Plant material and extraction

The aerial parts of *Potentilla reptans* were collected from Sari City, Mazandaran Province, Iran. The voucher specimen (E1-27-4111) was deposited at the herbarium of the Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran. The shade dried aerial parts were powdered and extracted with methanol (80%) by the maceration method. The obtained extract was concentrated by a rotary evaporator and dried by a freeze dryer. Finally, the extract was stored at 4°C until further analysis.

Phytochemical analysis

The phytochemical constituents of the hydroalcoholic extract of *P. reptans* were evaluated by qualitative and quantitative methods. In this study, the presence of steroids, triterpenoids, saponins, alkaloids, flavonoids, and tannin compounds were assessed. In addition, the total phenolic, tannin, and flavonoid contents were determined.

Steroids and triterpenoids

The extract was dissolved in chloroform and filtered by filter paper impregnated with anhydride sodium sulfate. Then, steroids and triterpenoids were detected by Salkowski and Liebermann-Burchard tests [16].

Saponins

Saponin compounds were detected by the foam test. A small amount of the extract was shaken with water vigorously. The formation of a stable foam for several minutes indicates the presence of saponins [17].

Alkaloids

The extract was dissolved in diluted hydrochloric acid and filtered. The filtrates were mixed with ammonia and extracted by chloroform, and dried. Moreover, the powdered residue was dissolved in hydrochloric acid and was used to test for the presence of alkaloids by Mayer, Wagner, and Dragendroff's reagents [18].

Determination of total phenolic, tannin, and flavonoid contents

Tannins were detected by precipitation method using ferric chloride and gelatin [18]. The total phenolic and tannin contents were determined by the Folin-Ciocalteu method based on a calibration curve of gallic acid at 725 nm. To estimate total tannin content, they were precipitated by polyvinylpyrrolidone (PVPP) and removed using centrifugation. Then, the phenolic content of the supernatant was determined and subtracted from the total phenolic content of the extract. The total phenolic and tannin contents were expressed as milligrams of gallic acid equivalents per gram of dried extract [19].

Flavonoids were detected by adding 5 mL ammonia and some drops of sulfuric acid. Yellow color creation shows the presence of flavonoid compounds. The total flavonoid content was determined using the aluminum chloride method based on a calibration curve of quercetin at 415 nm. The total flavonoid content was expressed as milligrams of quercetin equivalents per gram of dried extract [20].

Animal study

Male Swiss mice (20–30 g), maintained under standard environmental conditions, were used in this study. In addition, food and water were free for all animals in the process. The animals were fasted 12 hours before the study. All animal experiments were done in accordance with the Ethical Principles and Guidelines for Scientific Experiments on Animals prepared by The National Institutes of Health [21].

Acute Toxicity Test

The animals were randomly divided into six groups (5 animals/group). The control group just received distilled water (10 mL/kg), and the other groups were administered different oral doses of the HEP (500, 750, 1000, 1200, and 2000 mg/kg). Then, each animal was transferred to an individual Plexiglas cage with free access to standard food and water. Mice were observed 24 h for any symptoms of toxicity, morbidity, or mortality [21, 22].

Antinociceptive activity

Hot Plate Test

The animals (n=6) were orally received distilled water (10 mL/kg), morphine (5 mg/kg, as a reference opioid analgesic), and different doses of the HEP (100, 300, and 500 mg/kg) and then placed on a hot plate (Zharfpouyan, Iran) maintained at 55°C±3°C. The latency of nociceptive responses such as lifting/licking the hind paws or jumping was recorded at 0 (before treatment), 30, 45, 60, and 120 min after treatment. A 40-s cut-off time was used to avoid tissue damage [21, 23].

Acetic Acid-Induced Writhing Test

Nociception was induced with an intraperitoneal injection of 0.6% acetic acid (10 mL/kg) 60 min after oral administration of the above-mentioned doses of the extract, morphine, and distilled water. Ten minutes following acetic acid injection, mice were observed, and the total number of writhes (abdominal constriction and stretching of hind limbs) was recorded for 60 min. To evaluate possible participation of opioid receptors in the nociceptive effect, naloxone (4 mg/kg, IP), a competitive opioid receptor antagonist, was administered 15 min before treatment with HEP (500 mg/kg) or morphine (5 mg/kg) in a separate group of animals [24].

Evaluation of motor coordination activity

The motor coordination of mice was measured on the rotarod apparatus (Borj Sanat, Iran) at a constant speed of 6 rpm. Oral doses of 100, 300, and 500 mg/kg of the extract were administered to the animals one hour before placing them on the rotating rod. Two groups were orally received distilled water (10 mL/kg) and diazepam (2 mg/kg) as the negative and positive control, respectively. The time (in seconds) each animal could stay on the rod was recorded [25].

Statistical Analysis

Results were expressed as Mean±SEM. Statistical analysis was performed using 1-way analysis of variance (ANOVA) followed by a Tukey post hoc test in Graph-Pad Prism 6 software. P-values less than 0.05 were considered statistically significant.

Results

Phytochemical screening

The yield of the hydroalcoholic extract was 19.2% (w/w). The phytochemical analysis of the extract was revealed the presence of saponins, triterpenoids, flavonoids, and high amounts of phenolic compounds and tannins.

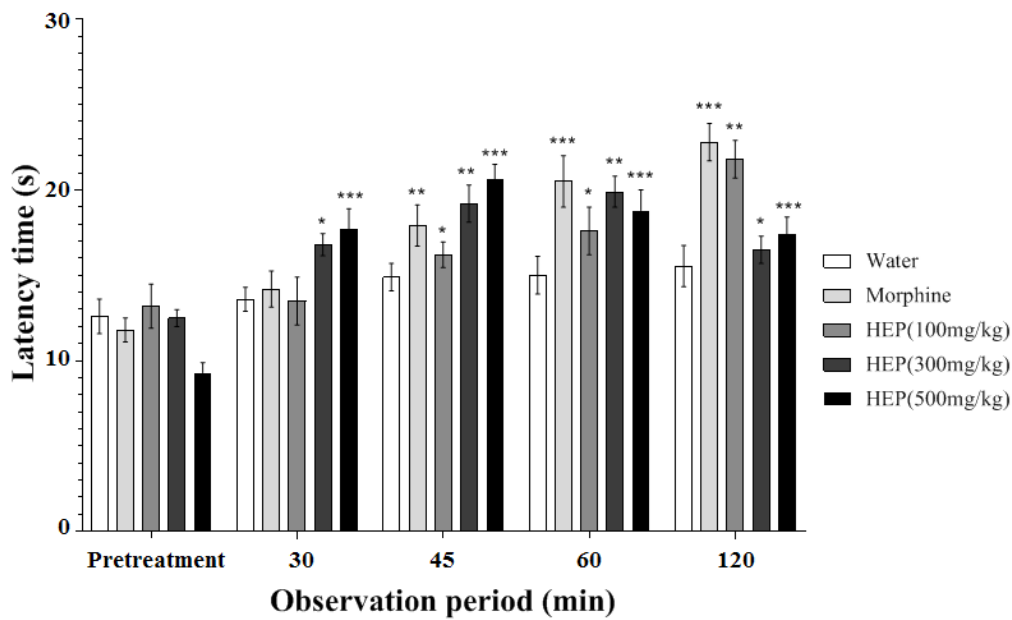
The Mean±SD total phenolic and tannin contents of HEP, determined by the Folin-Ciocalteu method, were respectively 251±2.08 and 111.5±1.3 mg gallic acid equivalents per gram of dried extract. The Mean±SD total content of flavonoids was 29.42±3.31 mg quercetin equivalents per gram of dried extract.

Acute Toxicity Test

The oral administration of a single dose up to 2000 mg/kg of HEP showed neither toxicity nor mortality during 24 h observation of subjects.

Hot Plate Test

Antinociceptive effects of different oral doses (100, 300, 500 mg/kg) of HEP in the hot plate test have been shown in Figure 1. Also, 100 mg/kg dose of HEP significantly increased latency time in 45, 60, and 120 min compared to baseline. Besides, 300 and 500 mg/kg doses of HEP could significantly increase latency in all recorded times. The highest latency time was caused by 500 mg/kg 45 min following administration. It should be noted that latency time decreased after 45 min despite a significant difference with baseline. Moreover, morphine (5 mg/kg) sig-



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Figure 1. Effect of the Hydroalcoholic Extract of *Potentilla reptans* L (HEP) (100, 300, 500 mg/kg) and morphine (5 mg/kg) on hot plate test in mice

Each column represents Mean±SEM. *P<0.05, **P<0.01, ***P<0.001 compared to the reaction time in pretreatment groups.

nificantly raised latency time in 45, 60, and 120 min compared to baseline. Antinociceptive activity of 300 and 500 mg/kg of HEP in 45 min was comparable to morphine.

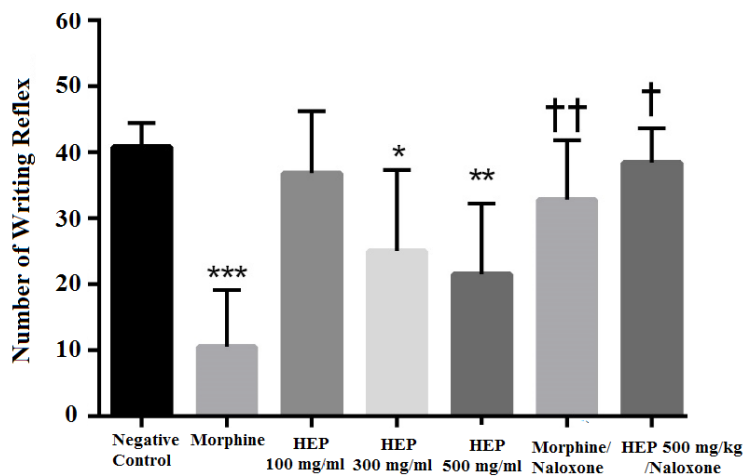
the animals with naloxone (4 mg/kg, IP) decreased the antinociceptive effects in both extract (P<0.05) and morphine (P<0.01) groups (Figure 2).

Acetic Acid-Induced Writhing Test

Oral administration of the extract at doses of 300 and 500 mg/kg exhibited a significant reduction in writhing response compared to the control group. Pretreatment of

Evaluation of motor coordination activity

The administration of HEP (100, 300, 500 mg/kg) had no significant effect on the motor coordination of mice compared with the control group. In contrast, pretreat-

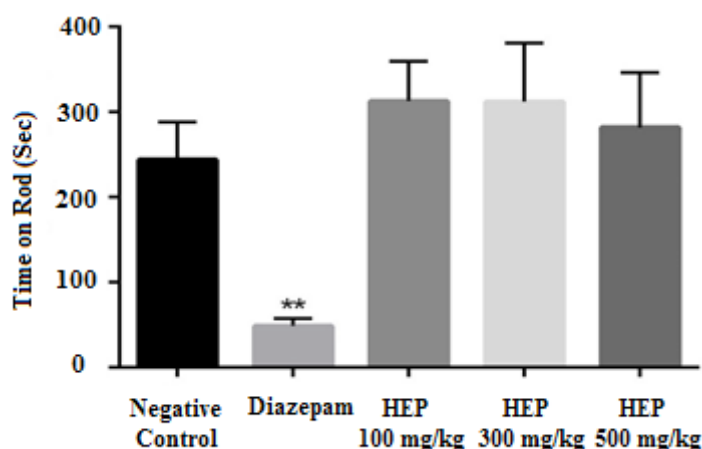


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Figure 2. Effect of the Hydroalcoholic Extract of *Potentilla reptans* L (HEP) (100, 300, 500 mg/kg) and Morphine (5 mg/kg) on acetic acid-induced writhing test in mice

Each column represents Mean±SEM; *P<0.05, **P<0.01, ***P<0.001 compared to the control group; †P<0.05 compared to HEP 500 mg/ml group, ††P<0.01 compared to morphine group.

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Figure 3. Effect of the Hydroalcoholic Extract of *Potentilla reptans* L. (HEP) (100, 300, 500 mg/kg) and Diazepam (2 mg/kg) on motor coordination activity by rotarod test in mice

Each column represents Mean±SEM; **P<0.01 compared to the control group.

ment with diazepam (2 mg/kg, IP) significantly reduced the time spent on rotating rod (P<0.01) (Figure 3).

Discussion

In different parts of the world, *Potentilla* species have been used in traditional medicine for treating inflammation and painful disorders. Based on our results, the hydroalcoholic extract of *P. reptans*, as one of the native species in Iran, exhibited antinociceptive activity in the animal models. In the hot plate test, 300 and 500 mg/kg doses of the extract had antinociceptive activity in all desired times. Hot plate is a good and reliable method for screening the antinociceptive activity of compounds acting in the central nervous system. So, we could say that parts of the antinociceptive effect of *P. reptans* are mediated via central pain pathways modulation.

In the writhing method, the hydroalcoholic extract of *P. reptans* dose-dependently reduced the abdominal constrictions and twitches induced by acetic acid. Acetic acid injection leads to the activation of chemosensitive nociceptors by endogenous mediators, such as bradykinin, serotonin, histamine, and prostaglandins (PG E₂, PGF₂α). This method can be used to evaluate the analgesic activity of compounds resembling opioids and nonsteroidal anti-inflammatory agents [26].

For possible evaluation of opioid receptors involvement, naloxone was injected 15 min before 500 mg/kg administration. By increasing the number of writhes close to the negative control, for both extract and morphine, we could conclude that the opioidergic system is among the targets of HPE active ingredients.

Since sedation could affect the reaction time to noxious stimuli, motor coordination was assessed with the rotarod test [23]. None of the effective antinociceptive doses of the extract changed the locomotor activity and neuromuscular coordination in mice.

Based on recent studies, some species of *Potentilla* showed anti-inflammatory and antinociceptive activities. Antinociceptive effect of acacetin and chrysin, two flavones isolated from *P. eveshita*, was evaluated by formalin and writhing tests. Both compounds showed strong antinociceptive activity. Molecular docking of chrysin displayed remarkable interaction between chrysin and cyclooxygenase-2 (COX-2) enzyme [27, 28].

In another study, umbelliferone, the coumarin-based derivative isolated from *P. eveshita*, was reported as a potent analgesic and anti-inflammatory agent. The IP administration of 5 and 10 mg/kg of umbelliferone reduced pain behavior dose-dependently in hot plate and formalin tests [29].

Potentilla species are rich in phenolic compounds such as flavonoids, phenolic acids, and tannins. Based on our results, the amounts of phenolic compounds and tannins in the hydroalcoholic extract of *P. reptans* were considerable. The presence of hydrolyzable tannins has been reported in *Potentilla* species. In one study, agrimoniin, the main ellagitannin of *P. recta*, demonstrated free radical scavenging effect and in vitro anti-inflammatory activity due to lipoxidase (LOX) inhibition. The inhibitory effect of the isolated compound on LOX can reduce the inflammatory mediators related to pain and inflammatory diseases [30, 31].

Several cinnamic acid derivatives such as p-coumaric acid, ferulic acid, and caffeic acid and also flavonoids such as kaempferol, quercetin, and isorhamnetin have been isolated from *P. reptans*. Based on previous studies, caffeic acid exhibits peripheral analgesic activity against inflammatory pain by several mechanisms involving up-regulation of nuclear factor-kB and thus overexpression of pain and inflammatory mediators. In addition, kaempferol and kaempferol-3-O-glucoside showed anti-inflammatory and analgesic effects in various in vivo experimental models. Possible mechanisms for the analgesic action of these compounds involved interaction with COX and LOX [1, 32].

In a recent study, intraperitoneal administration of the hydroalcoholic extract of *P. reptans* leaves, collected from the west part of Iran, at doses of 50 and 100 mg/kg exhibited significant antinociceptive effects in writhing, tail-flick, and formalin tests [33]. Similar to our study, naloxone significantly reduced the antinociceptive activity of the extract. In our previous work, the methanol extract of *Geum iranicum* roots (the Rosaceae family) showed anti-inflammatory and antinociceptive activity via modulation of opioid receptors. It seems that various bioactive compounds, including phenol compounds, might be responsible for the observed effects [23].

Ellagic acid is a polyphenolic compound found in the aerial part of *P. reptans* and more than 20 other *Potentilla* species. This compound is also generated by the hydrolysis of ellagitannin [1]. The antinociceptive potential of ellagic acid and its derivatives has been evaluated in some studies. Mansouri et al. reported that the systemic and peripheral antinociceptive effects of ellagic acid interact with opioid receptors. As a result, ellagic acid could be one of the main compounds responsible for the antinociceptive activity of *P. reptans* [34].

Conclusion

Based on the present study, the oral administration of the hydroalcoholic extract of *P. reptans* had considerable antinociceptive effects in an animal model. It was shown that the observed effects might be mediated mainly through opioid receptors. The phytochemical analysis indicated the presence of high levels of phenolic compounds that may be responsible for the antinociceptive activities of the extract. Further studies are needed to identify the effective constituents in *P. reptans* extract and to determine the molecular mechanisms of action.

Ethical Considerations

Compliance with ethical guidelines

All experimental protocols were in accordance with the National Institutes of Health (NIH) guidelines and approved by the Ethics Committee of Mazandaran University of Medical Sciences, Sari (Code: IR.MAZUMS.REC. 95.1277).

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Authors' contributions

Conceptualization, methodology, validation, formal analysis, writing – review & editing: Nematollah Ahangar and Somayeh Shahani; Investigation, formal analysis, writing - original draft: Hossein Bakhshi Jouybari and Ali Davoodi.

Conflict of interest

The authors declared no conflict of interest.

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