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ANTINOCICEPTIVE EFFECT OF *ELAEAGNUS ANGUSTIFOLIA* FRUIT IN MICE

HOSSEIN HOSSEINZADEH* AND MOHAMMAD REZA TAHERI

From the Faculty of Pharmacy, Mashhad University of Medical Sciences, P.O. Box 91775-1365, Mashhad, I.R. Iran.

ABSTRACT

The antinociceptive effect of *Elaeagnus angustifolia* fruits was studied in mice. The antinociceptive effect of ethanolic and boiling water extracts was studied using two thermal stimuli, the hot-plate and tail-flick tests. The intraperitoneal and oral administration of the ethanolic extract of seed (1.75-7.00 g/kg), but not pericarp and medulla had significant antinociceptive activity in the hot-plate test. Naloxone pretreatment did not inhibit the antinociceptive activity of the extract. The aqueous extract of different parts of the fruit (seed, medulla and pericarp) had antinociceptive activity in this test. The ethanolic extract of the seed had no antinociceptive effect in the tail-flick test and its effect was not abolished by naloxone. A 70% failure rate in the traction test (a muscle relaxation test) was induced by ethanolic extract of the seed (3.5 g/kg), compared with the 75% induced by the reference drug diazepam (2.5 mg/kg). It is concluded that the antinociceptive effect of *E. angustifolia* may be mediated by a supraspinal effect and muscle relaxation activity.

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Keywords: *Elaeagnus angustifolia*; Antinociceptive activity; Muscle relaxation, Hot-plate, Tail-flick, Traction test, Medicinal plants.

INTRODUCTION

The fruit of *Elaeagnus angustifolia* is used in Iranian traditional medicine as an analgesic agent for the alleviation of pain in rheumatoid arthritis patients. The Elaegnaceae have a variety of medicinal uses. The ripe fruits of *E. philipensis* have been used to treat amoebic dysentery.[8] Antibacterial agents such as epigalloctechin from *E. glabra* were identified.[7] There is a general belief that leaves and fruits of *E. angustifolia* have an antipyretic effect.[12]

This study was initiated because, to the best of our knowledge, there has been no scientific report on the antinociceptive activity of *E. angustifolia* to date.

*Corresponding author.

MATERIALS AND METHODS

Animals

Male and female albino mice 23-28 g were obtained from a random bred colony (Khorassan Javane Co, Mashhad, I.R. Iran) from the animal house of Mashhad University of Medical Sciences. Animals were housed in a colony room with a 12/12 hr light/dark cycle at 21 ±2 °C and maintained on a special diet. Animals were deprived of food for 12 h before oral administration of the extract but had free access to water.

Plant material

Fruits of *E. angustifolia* were collected from Esfarayen (a town in north of Khorassan province) and dried at room

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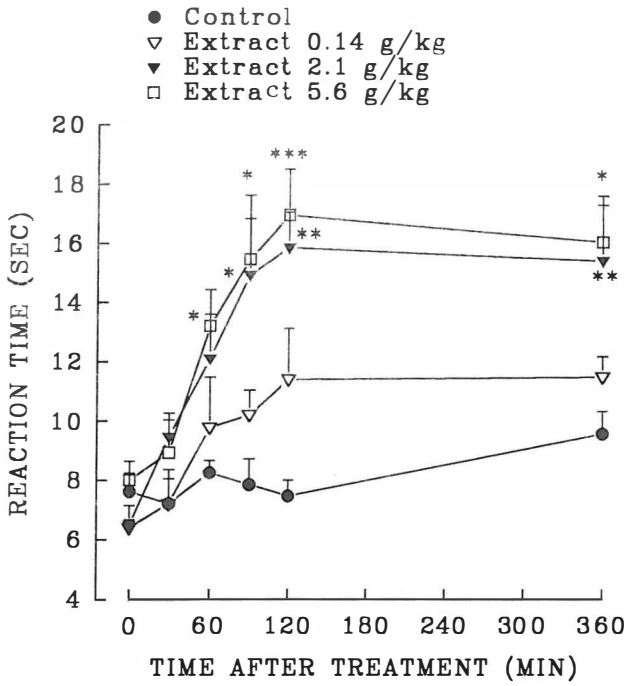

Fig. 1. Effect of different intraperitoneal doses of alcoholic extracts of *E. angustifolia* seed on pain threshold of mice in the hot-plate test. Each point represents the mean ± S.E.M. of reaction time of 7 mice. *p<0.05, **p<0.01, ***p<0.001, compared with control, Tukey-Kramer test.

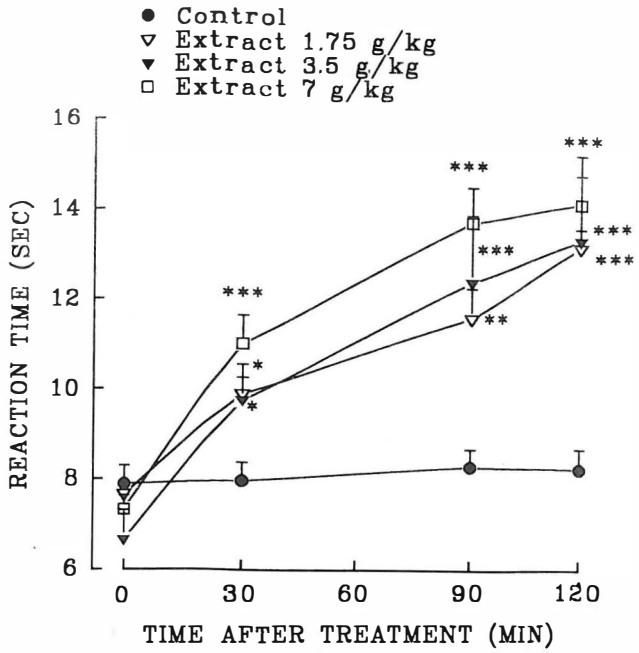

Fig. 2. Effect of different oral doses of alcoholic extracts of *E. angustifolia* seed on pain threshold of mice in the hot-plate test. Each point represents the mean ± S.E.M. of reaction time of 7 mice. *p<0.05, **p<0.01, ***p<0.001, compared with control, Tukey-Kramer test.

temperature. Three parts of fruits were separated and grinded. *E.angustifolia* was properly identified by Ferdowsi University and voucher samples were preserved for reference in the herbarium of the Department of Pharmacognosy, School of Pharmacy, Mashhad (Taheri, 97-0501-1).

Preparation of extracts

The dried and grinded products obtained from the different parts of fruits were extracted by decoction in boiling water for 15 minutes. This extract was then filtered and used for testing. Alcoholic extract was prepared using continuous extraction by ethanol in a Soxhelt apparatus. Known volumes of the extracts were evaporated to dryness under vacuum using a rotary evaporator.

Antinociceptive study

A. Hot-plate test

Hot-plate test was assessed on male and female mice. The temperature of the metal surface was maintained at 55±0.2 °C. Latency to a discomfort reaction (licking paws or jumping) was determined before and after drug administration. The cut-off time was 40 sec.

B. Tail-flick test

Tail-flick test was assessed on male and female mice.

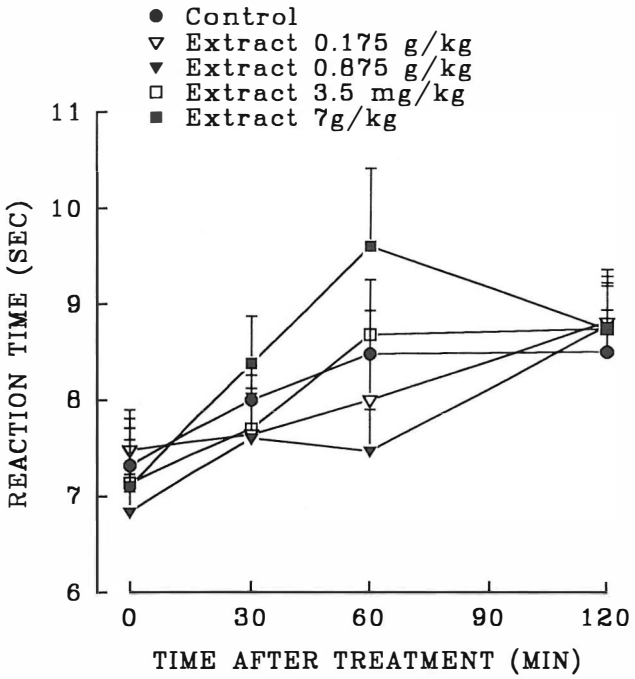

Fig. 3. Effect of different intraperitoneal doses of alcoholic extracts of *E. angustifolia* medulla on pain threshold of mice in the hot-plate test. Each point represents the mean ± S.E.M. of reaction time of 7 mice. Compared with control, Tukey-Kramer test.

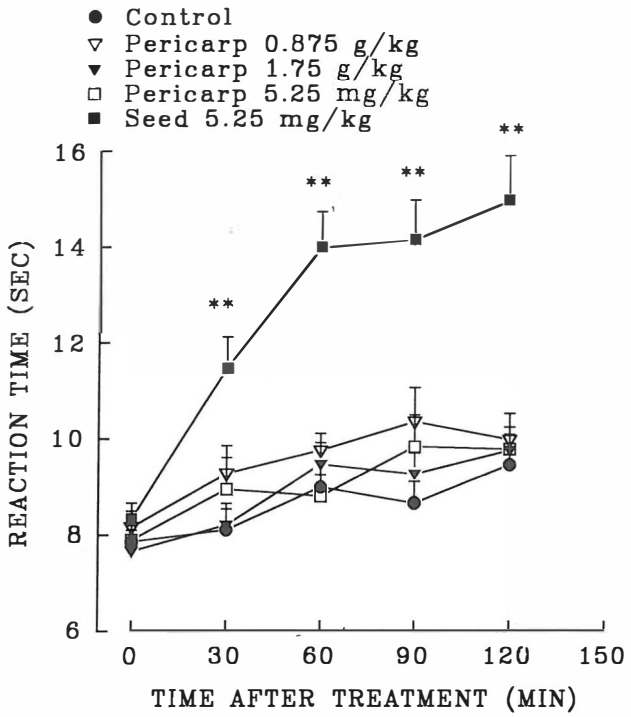

Fig. 4. Effect of different intraperitoneal doses of alcoholic extracts of *E. angustifolia* seed and pericarp on pain threshold of mice in the hot-plate test. Each point represents the mean ± S.E.M. of reaction time of 7 mice. **$p<0.01$, compared with control, Tukey-Kramer test.

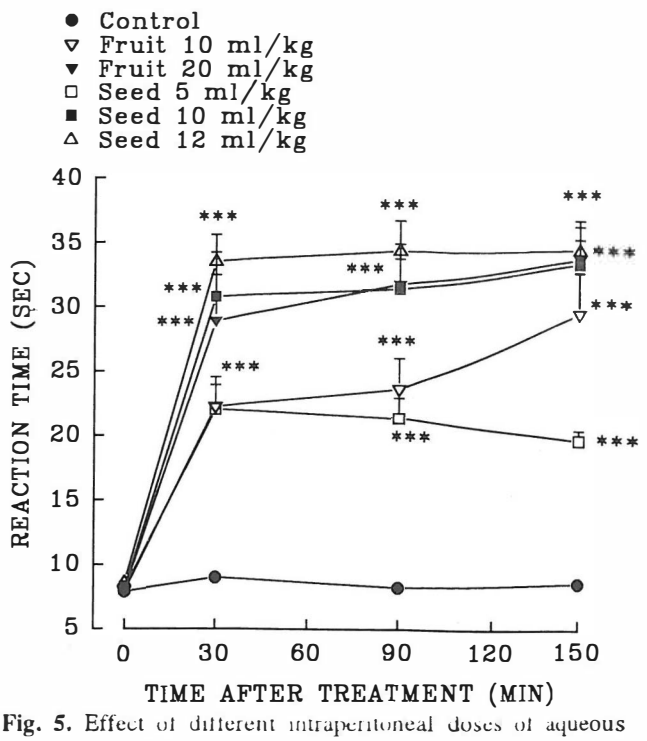

Fig. 5. Effect of different intraperitoneal doses of aqueous extracts of *E. angustifolia* seed and fruits on pain threshold of mice in the hot-plate test. Each point represents the mean ± S.E.M. of reaction time of 9 mice. ***$p<0.001$, compared with control, Tukey-Kramer test.

The base line and cut-off time were 2-3 sec and 10 sec, respectively.

Muscle relaxation study-Traction test

The animals were suspended by their hind legs from a taut metal wire and the time taken to get hold of the wire with their front legs was recorded; they were considered to have passed or failed the test according to whether this did or did not occur within 5 sec. Failure was considered to be synonymous with muscle relaxation. Experiments were performed 30, 60, 90 and 120 min after administration of the extract.

Statistical analysis

The data of the antinociceptive activity was expressed as mean values ± S.E.M. and tested with analysis of variance followed by the multiple comparison test of Tukey-Kramer. In the traction test, the data were evaluated by Fischer test. Discrepancies with $p<0.05$ were considered statistically significant.

RESULTS

The intraperitoneal and oral administration of alcoholic seed extracts of *E. angustifolia* showed antinociceptive

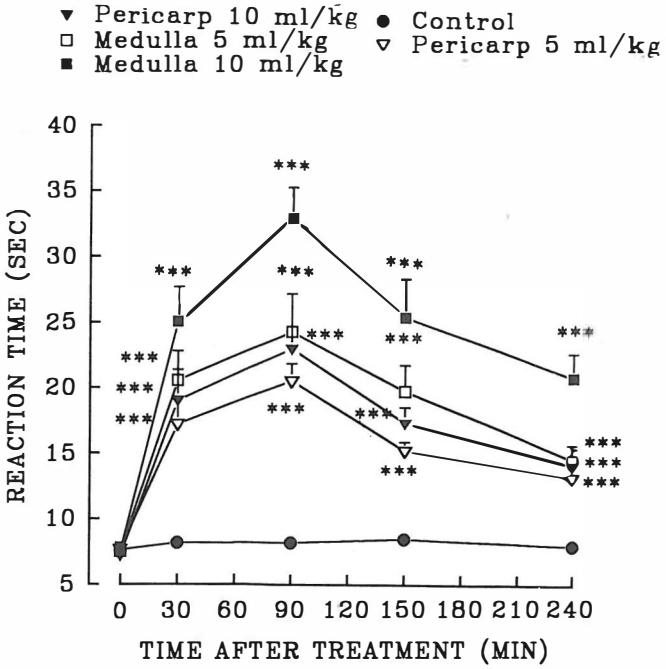

Fig. 6. Effect of different intraperitoneal doses of aqueous extracts of *E. angustifolia* pericarp and medulla on pain threshold of mice in the hot-plate test. Each point represents the mean ± S.E.M. of reaction time of 8 mice. ***$p<0.001$, compared with control, Tukey-Kramer test.

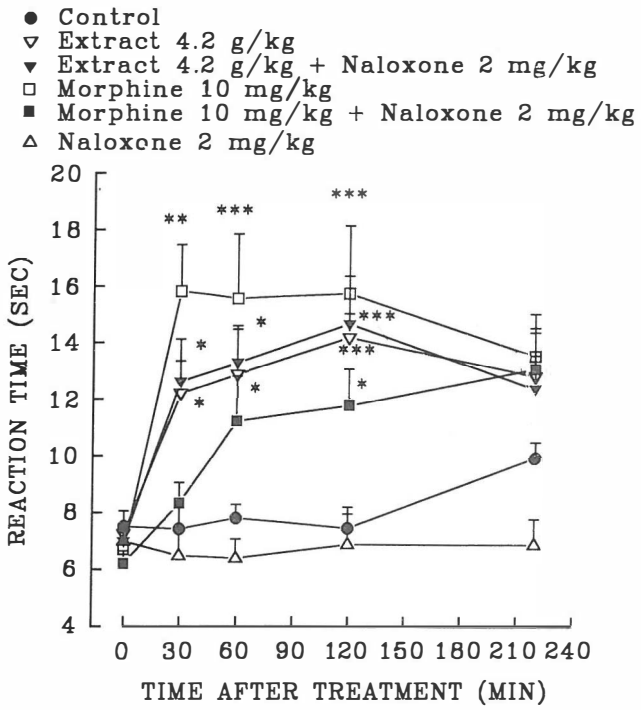

Fig. 7. Effect of naloxone on the alcoholic extract of *E. angustifolia* seed and morphine on pain threshold of mice in the hot-plate test. Each point represents the mean ± S.E.M. of reaction time of 7 mice. *$p<0.05$, **$p<0.01$, ***$p<0.001$, compared with control, Tukey-Kramer test.

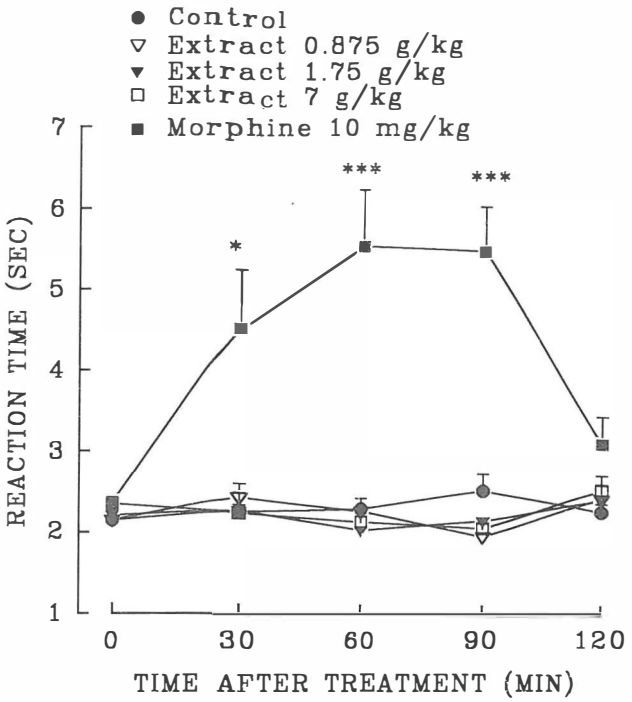

Fig. 8. Effect of intraperitoneal doses of alcoholic extracts of *E. angustifolia* seed and morphine on pain threshold of mice in the tail-flick test. Each point represents the mean ± S.E.M. of reaction time of 7 mice. *$p<0.05$, ***$p<0.001$, compared with control, Tukey-Kramer test.

activity (Figs. 1 and 2).

The intraperitoneal injection of alcoholic extracts of medulla and pericarp portions of *E. angustifolia* did not exert antinociceptive activity (Figs. 3 and 4).

Boiling water extracts of the different parts of the fruit (pericarp, cortex and seed) showed significant antinociceptive activity (Figs. 5 and 6).

Morphine sulfate significantly increased the reaction time of mice. Pretreatment by naloxone (2 mg/kg), which was not efficient by itself on heat-induced pain, did not abolish the antinociceptive effect of alcoholic extract of the seed of *E. angustifolia* (Fig. 7). The efficacy of the high dose of alcoholic seed extract of *E. angustifolia* was similar to a dose of 10 mg/kg of morphine (Fig. 7).

In the tail-flick test, the intraperitoneal injection of different doses of alcoholic extracts of the seed did not show antinociceptive activity, while morphine (10 mg/kg) effectively induced antinociceptive activity (Fig. 8).

Muscle relaxation was induced by 7 g/kg of the alcoholic extract of the seed. At the time of maximum effect, 60 min after administration, a 75% failure rate in the traction test was induced by the extract compared to 81% induced by the reference drug diazepam (Fig. 9).

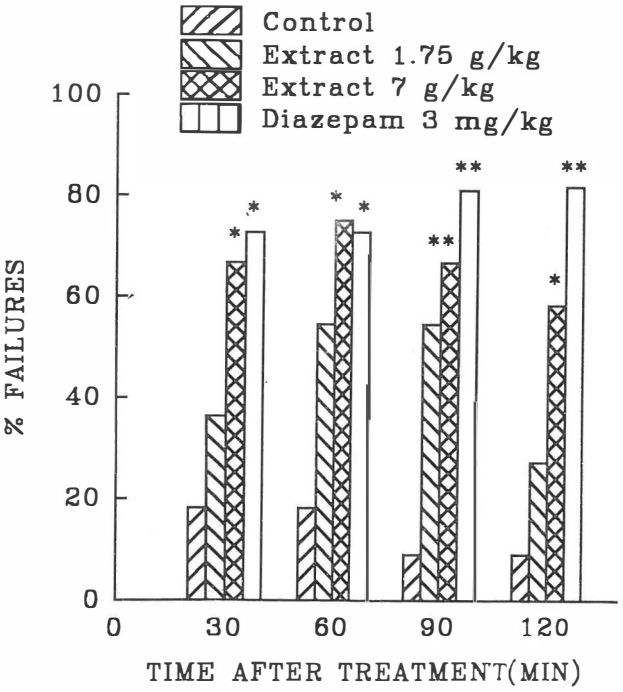

Fig. 9. Effect of intraperitoneal doses of alcoholic extracts of *E. angustifolia* seed and diazepam on traction test performance. Each point represents the mean of the percent of failures of 7 mice. *$p<0.05$, **$p<0.01$, compared with control, Fischer test.

DISCUSSION

The present results indicate that the aqueous extracts of different parts of *E. angustifolia* fruit exhibit antinociceptive activity. The alcoholic and aqueous extract of the seed had antinociceptive activity but the alcoholic extract of other parts, i.e., the cortex and pericarp, did not show antinociceptive activity. This may indicate that different constituents are involved in antinociceptive activity.

Lack of effect of naloxone, an opioid receptor antagonist, on the antinociceptive activity of the alcoholic seed extract suggests that this extract may not have a morphine-like activity profile.

In the tail-flick test, the alcoholic extract had no antinociceptive effect. As the tail-flick test has a spinal mechanism,[4] the antinociceptive effect of the extract is therefore not mediated by a spinal mechanism.

The alcoholic extract of the seed exhibited muscle relaxant activity in a preliminary test. This effect may partly be involved in the antinociceptive activity of the extract. However, the traction test is not specific[9] and other tests–such as electrophysiology experiments–may show the exact effect of the extract.

As *E. angustifolia* has flavonoid components[1,6] and some flavonoids such as chrysin have partial agonistic effects on benzodiazepine receptors, [10-11] muscle relaxant activity may be due to these agents. Further studies, including separation of the different fractions of the fruit and radioligand binding technique, are needed.

Some flavonoid components also have antinociceptive and anti-inflammatory effects.[2-3,5] As *E. angustifolia* has flavonoid constituents, thus there is the possibility that antinociceptive activity is mediated by these agents.

It is concluded that the aqueous and alcoholic extracts of *E. angustifolia* fruit have antinociceptive effects. The antinociceptive activity of the alcoholic extract may partially be mediated by muscle relaxation. Both muscle relaxation and antinociceptive activity of the extract make it suitable for the treatment of some musculoskeletal disorders.

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REFERENCES

1. Dembinska MW, Rudnicki R, Saniewski M: Flavonoids in leaves of *Elaeagnus angustifolia*. Phytochem 12: 3015-3016, 1973.
2. Luzclarena C, Otero JA: The analgesic activity of *Hedyosmum bonplanianum*: flavonoid glycosides. Planta Med 59: 26-27, 1991.
3. Mariano M, Bye R: Analgesic and antipyretic activities of an aqueous extract and of the flavone Linarin of *Buddleia cordata*. Plant Med 62: 137-140, 1995.
4. Mohrland JS: Pain pathway: potential sites for analgesic action. In: Lednicer D, (ed), Central Analgesics. New York: Johnwill and Sons, pp. 1-30, 1982.
5. Muthiah NS, Vijayasekaran V: Anti-inflammatory activity of flavone and its methoxy derivative: structure activity study. Ind J Pharmac Sci 55: 180-183, 1993.
6. Nikolaeva AG, Krivenchuk PE, Prokopenko AP: *Elaeagnus angustifolia* flavonoids. Farm 26: 56-60, 1971.
7. Nishino C, Enoki N, Tawata S, Mori A: Antimicrobial activity of flavonoids against *Staphylococcus epidermidis*, a skin bacterium. Agric Biol Chem 51: 139-143, 1987.
8. Perry LM: Medicinal Plants of the East and Southeast Asia. London: MIT Press, p. 131, 1980.
9. Vogel HG, Vogel WH: Drug Discovery and Evaluation, Pharmacological Assays. Berlin: Springer, pp. 211-212, 1997.
10. Viola H, Paladini AC: Isolation of pharmacologically active benzodiazepine receptor ligands from *Tilia tomentosa* (Tiliaceae). J Ethnopharmacol 44: 47-53, 1994.
11. Wolfman C, Medina JH: Possible anxiolytic effects of chrysin, a central benzodiazepine receptor ligand isolated from *Passiflora coerulea*. Pharmacol Behav 47: 1-4, 1992.
12. Zargari A: Medicinal Plants. Tehran University Press, Vol. 4: pp. 275-277, 1990.