TRADITIONAL AND INTEGRATIVE MEDICINE

Trad Intrgr Med, Volume 1 , Issue 4, Autumn 2016

Original Research

The Effect of Methanolic and Ethanolic Extracts of *Ruta graveolens* L. Leaves on Formalin-Induced Inflammation in Rats

Sayid Mahdi Mirghazanfari[1, 2], Alireza Abbassian[3*], Mohammad Kamalinejad ,[4] Seyyed Morteza Karimian[5], Ahmad Massoud[6]

[1]*Students' Scientific Research Center, Tehran University of Medical Sciences, Tehran, Iran*
[2]*Department of Traditional Medicine, School of Medicine, AJA University of Medical Sciences; Tehran, Iran.*
[3]*Department of Iranian Traditional Medicine, School of Traditional Medicine, Tehran University of Medical Sciences, Tehran, Iran.*
[4]*Department of Pharmacognosy, School of Pharmacy, Shahid Beheshti University, Tehran, Iran.*
[5]*Department of Physiology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.*
[6]*Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.*

Received: 28 March 2016 **Revised:** 14 May 2016 **Accepted:** 21 May 2016

Abstract

The phenomenon of inflammation is the base of many acute and chronic diseases. New effective anti-inflammatory drugs are needed to battle side effects of acute and especially chronic inflammation and incomplete treatment of patients suffering from these side effects. For this purpose, in the present study, we evaluated Ruta *graveolens* L. *(R. graveolens)* as a folk herb for treatment of inflammation. In an experimental-interventional study, anti - inflammatory effects of *R. graveolens*, a Persian Medicine (PM) plant, were examined with formalin-induced hind paw edema model in rats. Sodium Salicylate (300mg/kg, ip, SS) was injected as a positive control group and compared with three doses of methanolic extract of R. *graveolens* (Meth) (50, 100 and 200 mg/kg; i.p.), two doses of ethanolic extract of *R. graveolens* (Eth) (50 and 100 mg/kg; i.p.) and a group of distilled water (6 mL/kg; i.p.). The results showed that *R. graveolens* has acute and chronic anti-inflammatory properties and prevents inflammation. These effects were found to be effective like SS.

Keywords: *Ruta graveolens L.*, Inflammation, Formalin test, Traditional Iranian Medicine, Methanolic extract, Ethanolic extract

Citation: Mirghazanfari SM, Abbassian A, Kamalinejad M, Karimian SM, Massoud A. **The Effect of Methanolic and Ethanolic Extracts of *Ruta graveolens* L. Leaves on Formalin-Induced Inflammation in Rats.** Trad Integr Med 2016; 1(4): 136 - 141

1. INTRODUCTION

Anti-inflammatory drugs such as NonSteroidal Anti-Inflammation Drugs (NSAID's) and glucocorticoids are the most frequently used drugs in prescriptions; but it is believed that current drugs are not useful in all cases and have many side effects [1- 4]. Therefore, searching for alternatives seems to be essential.

Traditional Persian (Iranian) Medicine (PM) is one of the old medical schools with historical manuscripts and books which contain valuable herbal experiences. Therefore, searching through traditional books can be beneficial for finding possible candidates (i.e. suitable herbs) for anti-inflammatory research. This approach has been emphasized by World Health Organization (WHO) [5].

Ruta graveolens L. or *Sodab* in Persian, is one of medicinal herbs which is listed in anti-inflammatory herbs in PM manuscripts. It is

*Corresponding Author: Alireza Abbassian
Email: Abbasian@sina.tums.ac.ir

commonly used in joint and nerve inflammations such as sciatica. Also, traditional PM physicians have used it in lung and liver inflammations, soft tissue inflammations (which named it Khanazar) as well as colorectal inflammations like hemorrhoid (Bavasar)[6].

There are some old studies about anti-inflammatory effects of *R. graveolens* [7, 8, 9]. Also, there are several research about anti-microbial [10, 11, 12], anti-platelet aggregation [13], anti-fertility [14, 15, 16], anti-leishmanial and anti-fungal [17, 18], anti-parasitic [19, 20], anti-cancer [21, 22], cardiovascular [23], anti-spasm [24], anti-hypertension [25] effects of the herb and some other studies are about *R. graveolens* analysis and toxicity [26, 27]

In this study, we reconsidered anti-inflammatory effects of *R. graveolens.*

2. METHODS

Plant material

R. graveolens leaves were obtained from the National Botanical Garden of Iran (Tehran, Iran), identified by G. Amin and a voucher specimen (No. *Ruta graveolens L.* 6638-THE) was deposited in the herbarium of the School of Pharmacy, Tehran University of Medical Sciences.

Preparation of methanolic extract (METH)

R. graveolens leaves were cleaned and powdered. 40 g of powdered leaves was mixed with 200 mL methanol (Merck, extra pure) in a magnet mixer for 5 min, soaked for 3 days, filtered and dried at room temperature. Finally, 1.54 g dry extract was obtained. The dry extract was then suspended in distilled water and administered intraperitoneally (i.p.).

Preparation of ethanolic extract (ETH)

After Cleansing and powdering of *R. graveolens* leaves, 7.5 g of the powder was boiled in 1500 mL of distilled water for 5 min and the obtained solution was then subjected to filtration. The filtrate was subsequently freezdried (Eyela Rikakikai Co. LTD Freezdryer FD-1), giving 4gr dry extract which was stored at -18°C. The powdered extract was then used to make solutions of different concentrations in distilled water.

Animals

In this study we employed male NMRI rats weighing 240 to 360 g (Pasteur Institute, Iran). They were housed in Plexiglas cages in groups of 7, with free access to food and water on a 12 h light 12 h dark cycle, ambient temperature (250 ± 10C) and relative humidity (55±5%).

Anti-inflammatory test

For anti-inflammatory activity, the formalin-induced edema model was used [28]. Rats were injected with 0.05 mL of 2.5 % formaldehyde solution into the sub-plantar region of the right hind paw. The negative control group received distilled water (DW) and the positive control group was treated with 300 mg/kg intraperitoneal (i.p.) dose of Sodium Salicylate (SS, Merck). Experimental groups were treated with 50 and 100 mg/kg i.p. doses of ETH and 50, 100 and 200 mg/kg i.p. dose of METH. The volume of injection was 4 mL/kg of body weight for DW, SS, ETH and METH treatments. The paw volume was measured before and after formalin injection and the difference in these two volumes was used as indication of the degree of inflammation. Drugs were injected 35 min before formalin injection and the paw volume was measured just before injection of drug and 1 h after formalin injection (95 min after drug injection) in acute administration studies, and daily for 7 days, in chronic administration studies. In chronic studies, animals received drugs in days 1-7 just after paw volume measurement. The paw volume was measured using a mercury-balance Plethysmometer technique [29].

Statistical analysis

The results of the experiments are expressed as mean±SEM. The differences were estimated by means of one-way ANOVA followed by LSD's test for the acute studies, and by means of Student's unpaired *t*-test for the chronic studies.

When the P value was <0.05, the difference was considered significant.

3. RESULTS

Compared with DW, ethanol extract (water-alcohol) at a dose of 100 mg/kg (ETH100) and SS at a dose of 300 mg/kg had significant acute and chronic anti-inflammatory properties (from second to seventh days); however, no significant difference was observed between ethanol extract and SS in this respect. None of the other extract-doses had anti-inflammatory properties (Figures 1 and 2).

Inflammation percentages of the ETH100 group on days 2 to 7 and the SS group on days 2 and 5 were significantly different with the DW group (Figure 3).

acute inflammation

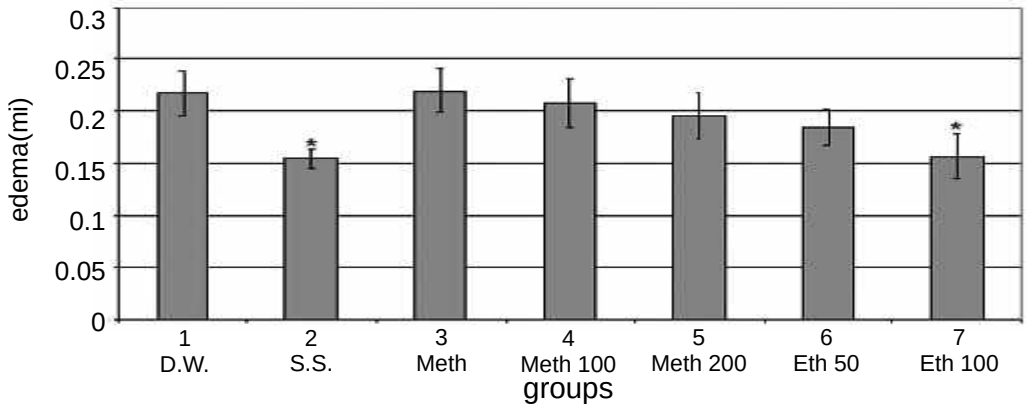

Figure 1: The amount of acute edema (inflammation) in different groups of rats.
*=Groups that have a significant acute anti-inflammatory effect (P-value<0.05) compared with D.W. group.
D.W. = Distilled Water, S.S. = Sodium Salicylate, Meth=Methanolic extract of Rue, Eth=Ethanolic extract of Rue, ml=mil-litter, 50 and 100 and 200 are extracts doses in mil-gram per each kilo-gram of body weight (mg/kg).

chronic inflammation

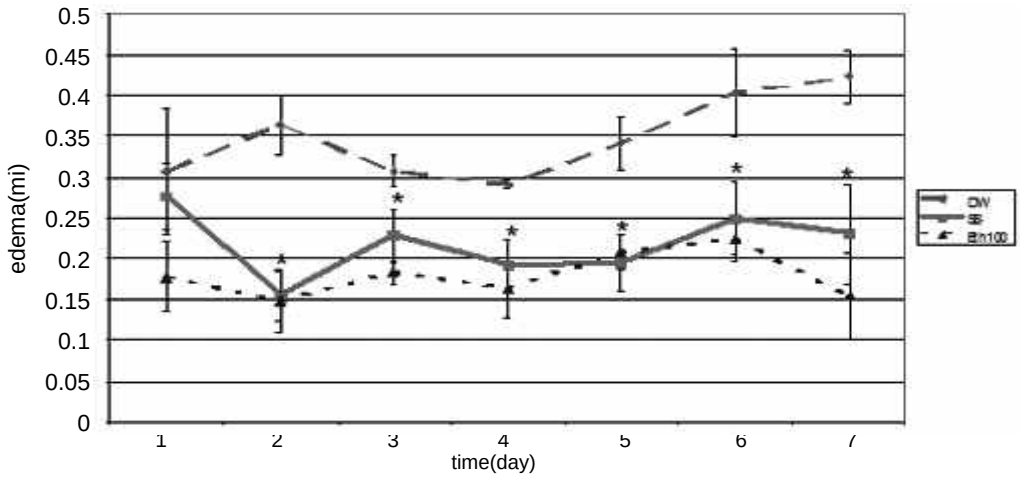

Figure 2: The amount of chronic edema (inflammation) in different groups of rats.
*=The day in which a significant anti-inflammatory effect (P-value<0.05) for both S.S. and Eth100 were seen compared with D.W. group.
D.W. = Distilled Water, S.S. = Sodium Salicylate, Eth=Ethanolic extract of Rue, ml=mil-litter, 100 is extract dose in mil-gram per each kilo-gram of body weight (mg/kg).

Percent of
chronic inflammation

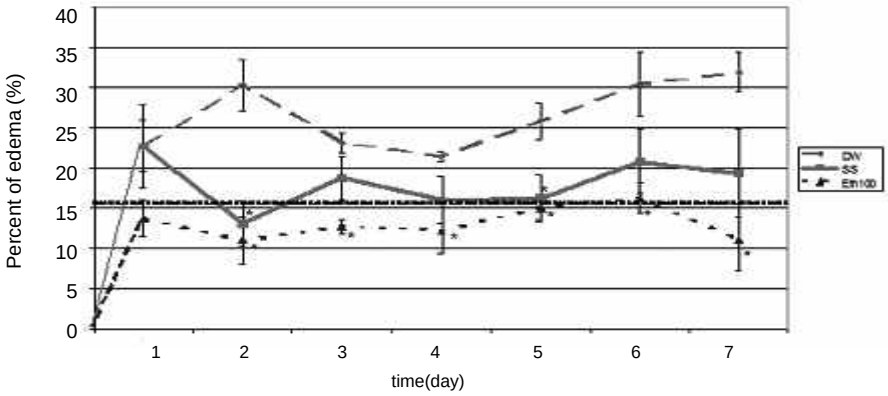

Figure 3: Percentage of chronic edema (inflammation) in three groups: control (D.W.), control (S.S.) and treatment (Eth.100). *=Days in which, in comparison with the control group, significant chronic anti-inflammatory effects were observed in the corresponding group (p-value?0.05). Dotted line indicates the maximum inflammation of 50%. D.W.: distilled water, S.S.: sodium salicylate, Eth.100: ethanol extract at the dose of 100 mg per kg of body weight (mg/kg).
Day 1 refers to the first day of chronic inflammation (the second day of the experiment). Sample size in each group was 7.

4. DISCUSSION

A number of plants have been investigated for their anti-inflammatory effects worldwide. Although many studies have been inspired by traditional and complementary medicine, unfortunately, in most cases, findings have not been reported in these studies. Anti-inflammatory effects of *R. graveolens* have been emphasized in Iranian complementary medicine. In this study, we tried to test this as a hypothesis with common methodologies. Low number of studies on this specific plant and its anti-inflammatory effects worldwide [9, 10, 11] was another motivation to conduct this study.

In this study, both alcohol (methanol) and water-alcohol (ethanol 80%) solvents were used, as some of the effective substances of plants are water-soluble and some are fat-soluble. On the one hand, methanol is generally known as the best solvent and separator of plant effective substances [33], while the solubilizing power of ethanol and solubility of ethanol extract in water (to prepare different doses) were small in this study, and there was a volume restriction for IP injection; therefore, methanol extract was used more often.

The doses used in this study were selected based on the results of the pre-test (the test carried out in low sample size before the main experiment) and doses used in similar studies on other plants [33, 35, 36] (to compare the results with those results). The following can be discussed about the findings:

1. At similar doses, ETH extract has greater anti-inflammatory effect compared to the METH extract. Therefore, it can be inferred that the anti-inflammatory agent in *R. graveolens* is more water-soluble than fat-soluble (alcohol). A previous study confirmed this as well [32]. This study proved that "rutine" is better extracted by boiling water compared to alcohol (ethanol) or water-alcohol. Therefore, water extract of *R. graveolens* probably has stronger anti-inflammatory effects. Iranian complementary medicine experts have also recommended the use of water plant extracts for treatment [37]. The present findings are in agreement with traditional medicine.

2. *R. graveolens* has acute and chronic anti-inflammatory properties, which is at least 3 times more powerful than that of SS because at one-third of the dose of SS, they had similar anti-inflammatory effects. In addition, firstly, *R. graveolens's* dose is based on concentrated extract that contains some water and impurities and is not dried and purified (such as powders SS). Secondly, as mentioned in the previous section, only a small amount of anti-inflammatory substance exists in this type of extract. Thus, if the water, pure and dry *R.*

graveolens extract is investigated, we would almost certainly observe effects much stronger than aspirin.

3. *R. graveolens* was more effective in controlling (preventing) the inflammation because, as shown in Figure 3, the percentage of inflammation corresponding to the extract group was less than the maximum limit of 50% on all days of the experiment, and almost on all days, it had a significant difference with the DW group. However, the percentage of inflammation corresponding to the SS groups were almost always higher than the 50% maximum inflammation limit and had a significant difference with the DW group in only two days.

4. A research conducted on the effective substance of *R. graveolens*, "rutine", reported that its amount is at a maximum (5%) when the plant flowers, while its amount is much lower (2.7%) in mid-October [33]. The amount of effective substance was found to be higher in the leaves than in the whole plant (leaves and stem) and higher in new wet plant than in the dry one. Since dried *R. graveolens* leaves (flowered and prepared in mid-December) were used in our study and considering the discussion number 1, it can be concluded that the maximum anti-inflammatory effect of *R. graveolens* was not achieved here. To achieve this goal, another similar study should be conducted on fresh water flowered *R. graveolens* leaf extract.

5. Similarities between our findings and those of other studies in terms of the effects of DW and SS on inflammation show that the findings of present study are reliable and valid.

Final results obtained from this research include:

1. *R. graveolens* has acute and chronic anti-inflammatory properties and prevents inflammation. These effects were found to be effective like SS.

2. The highest anti-inflammatory effects of *R. graveolens* can be obtained from its water extracts, not its alcohol extracts.

3. Despite the prevailing belief in the society and scholars, herbs, natural remedies and alternative medicine can also be used in acute cases and emergencies in addition to disease prevention and treatment of chronic diseases.

4. If we do not want to view the Iranian traditional medicine treatment orders with full confidence, at least we can be neutral and express these recipe and solutions as researchable hypotheses with a scientific spirit. In most cases, their claims would be proven, and we can therefore achieve more effective and less complicated prevention and treatment methods.

ACKNOWLEDGEMENT

The authors would like to thank the Research Deputy of Tehran University of Medical Sciences for funding this research under the code 132/9067 from the research projects of Center for Scientific Student Research. We also thank the authorities of the Institute of Medicinal Plants of Iranian Academic Center for Education, Culture and Research for providing the plants, as well as the staff of Department of Pharmacology of Shahed University and Department of Physiology of Tehran University of Medical Sciences for their cooperation.

REFERENCES

[1] Katzung BG. Basic and clinical pharmacology. McGraw-Hill, New York, 8th ed. (2001) 1088-1089.

[2] Chowdhury MA, Abdellatif KRA, Don Y, Das D, Suresh MR and Knaus EE. Synthesis of celecoxib analogues possessing an N-Difluoromethyl-1, 2-dihydropyrid-2-one s-Lipoxygenase pharmacophore: Biological evaluation as dual inhibitors of cyclooxygenases and 5-Lipoxygenase with anti-inflammatory activity. J. Med. Chem. (2009) 52:1525-1529.

[3] Tranvsky K?Fischer M, V?gtle-Junkert U and Schreyger F. Efficacy and safety of 5% ibuprofen cream treatment in knee osteoarthritis. Results of a randomized, double blind, placebo controlled study. J. Rheumatol. (2004) 31(3): 565-72.

[4] Van Haselen RA and Fisher PA. A randomized Controlled trial Comparing topical piroxicam gel with a homeopathic gel in osteoarthritis of the knee. Rheumatol. (Oxford). (2000) 39(7): 714-9.

[5] Choi SH. WHO traditional medicine strategy and activities. "Standardization with evidence-based approaches". J Acupunct Meridian Stud. 2008 Dec; 1(2):153-4. doi: 10.1016/S2005-2901(09)60037-6.?

[6] Momene Tonkaboni SM. Tohfe of Hakim Momen. Nashre Shahr, Tehran, (2008) 241-2.

[7] Atta AH, Alkofahi A.Anti-nociceptive and anti-inflammatory effects of some Jordanian medicinal plant extracts. J Ethnopharmacol. 1998 Mar;60(2):117-24

[8] Patricia Andersen-Parrado. Alleviating arthritic inflammation with homeopathy & good nutrition. Atlanta. 1997Jul ;59(7) : 20-21
[9] Al-Okbi SY, El-Sayed EM, Ammar NM, El-Sayed NK, Abou-El Kassem LT.Effect of Ruta graveolens L. and Euphorbia peplus L. anti-inflammatory extracts on nutritional status of rats and the safety of their use. Indian J Exp Biol. 2002 Jan; 40(1):45-8.
[10] Wolters B, Eilert U.Antimicrobial substances in callus cultures of Ruta graveolens. Planta Med. 1981 Oct;43(2):166-74
[11] Ojala T, Remes S, Haansuu P, Vuorela H, Hiltunen R, Haahtela K, Vuorela P.Antimicrobial activity of some coumarin containing herbal plants growing in Finland. J Ethnopharmacol. 2000 Nov; 73(1-2):299-305.
[12] Alzoreky NS, Nakahara K.Antibacterial activity of extracts from some edible plants commonly consumed in Asia. Int J Food Microbiol. 2003 Feb 15; 80(3):223-30.
[13] Wu TS, Shi LS, Wang JJ, Iou SC, Chang HC, Chen YP, Kuo YH, Chang YL, Teng CM . Cytotoxic and antiplatelet aggregation principles of Ruta graveolens?? J Chinese Chem?Societ. 2003 FEB; 50 (1): 171-178.
[14] Kong YC, Lau CP, Wat KH, Ng KH, But PP, Cheng KF, Waterman PG. Antifertility principle of Ruta graveolens. Planta Med. 1989 Apr; 55(2):176-8.
[15] Prakash AO, Saxena V, Shukla S, Tewari RK, Mathur S, Gupta A, Sharma S, Mathur R.Anti-implantation activity of some indigenous plants in rats. Acta Eur Fertil. 1985 Nov-Dec; 16(6):441-8.
[16] Gandhi M, Lal R, Sankaranarayanan A, Sharma PL.Post-coital antifertility action of Ruta graveolens in female rats and hamsters. J Ethnopharmacol. 1991 Aug; 34(1):49-59.
[17] Ahua KM, Ioset JR? Ransijn A, Mauel J, Mavi S, Hostettmann K. Antileishmanial and antifungal acridone derivatives from the roots of Thamnosma rhodesica[Rutaceae] . Phytochemistry.2004 APR; 65 (7): 963-968.
[18] Trovato A, Monforte MT, Forestieri AM, Pizzimenti F.In vitro anti-mycotic activity of some medicinal plants containing flavonoids. Boll Chim Farm. 2000 Sep-Oct; 139(5):225-7.
[19] Guarrera PM.Traditional antihelmintic, antiparasitic and repellent uses of plants in Central Italy. J Ethnopharmacol. 1999 Dec 15; 68(1-3):183-92.
[20] Banerji P, Banerji P.Intracranial cysticercosis: an effective treatment with alternative medicines. In Vivo. 2001 Mar-Apr; 15(2):181-4.
[21] Trovato A, Monforte MT, Rossitto A, Forestieri AM.In vitro cytotoxic effect of some medicinal plants containing flavonoids. Boll Chim Farm. 1996Apr135(4):263-6.
[22] Pathak S, Multani AS, Banerji P, Banerji P.Ruta 6 selectively induces cell death in brain cancer cells but proliferation in normal peripheral blood lymphocytes: A novel treatment for human brain cancer. Int J Oncol.? 2003 Oct;23(4):975-82
[23] Chiu KW, Fung AY.The cardiovascular effects of green beans (Phaseolus aureus), common rue (Ruta graveolens), and kelp (Laminaria japonica) in rats. Gen Pharmacol. 1997 Nov; 29(5):859-62.
[24] Ovak I, Buzas G, Minker E, Koltai M, Szendrei K. [The isolation of additional spasmolytic substances from ruta graveolens l.] Naturwissenschaften. 1965 May;52:263
[25] Chiu K. W. and Fung A. Y. L. The Hypotensive Effects of Green Bean (Phaseolus aureus), Common Rue (Ruta graveolens) and Kelp (Laminaria japonica) in Rats. Phytother. Res. 1997; 11: 203-206. ?
[26] Novak I, Buzas G, Minker E, Koltai M, Szendrei K.[Studies of the hormones of the Ruta graveolens 3] Planta Med. 1966 May;14(2):151-6.
[27] Moreira MD, Picanco MC, Barbosa LCD, Guedes RNC, da Silva LM Toxicity of leaf extracts of Ageratum conyzoides to lepidoptera pests of horticultural crops . Biological Agriculture & Horticulture. 2004; 22 (3): 251-260.
[28] Kumar VL and Basu N. Anti-inflammatory activity of the latex of Calotropis procera. J. Ethnopharmacol.. (1994) 44: 123- 125.
[29] Fereidoni M, Ahmadiani A, Semnanian S and Javan M. An accurate and simple method for measurement of paw edema. J. Pharmacol. Toxicol. Methods. (2000) 43: 11-14.
[30] Vazquez B, Avila G, Seura D and Escalante B. Anti-inflammatory activity of extracts from Aloe vera gel. J. Ethnopharmacol. (1996) 55: 69-75.?
[31] Suh N, Honda T, Finaly HJ, Barchowsky A, Williams C, Benoit NE, Xie QW, Nathan C, Gribble GW and Spon MB. Novel triterpenoids suppress inducible nitric oxide synthase (iNOS) and inducible cyclooxygenase (COX-2) in mouse macrophages. Cancer. Res. (1998) 58: 717-723.
[32] Huss U, Ringbom T, Perera P, Bohlin L and Vasange M. Screening of ubiquitous plant constituents for COX-2 inhibition with a scintillation proximity based assay. J. Nat. Prod. (2002) 65: 1517-1521.
[33] Afshari J., Delazar A. Rurin from Ruta graveolens. Pharm.D. Thesis. Faculty of pharmacy, Tehran University of Medical Sciences.1994?
[34] Abbassian A. Anti-inflammatory effects of Urtica Pilulifera l. MD Thesis. Faculty of medicine, Tehran University of Medical Sciences. 2007?
[35] Fereiduni M. Anti-inflammatory effects of Sambucus ebulus??. MS Thesis. Shahid Beheshti University of Medical Sciences. 1997?
[36] Javan M. Anti-inflammatory effects of Trigonella foenum - graecum. MS Thesis. Shahid Beheshti University of Medical Sciences. 1997?
[37] Dehkhoda Dictionary Software. Tehran University Press. Version 2