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***Satureja khuzestanica* Extract Elicits Antinociceptive Activity in Several Model of Pain in Rats**

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Abstract: *Satureja khuzistanica* is used as anti-inflammatory, antinociceptive and antiseptic in folk medicine; however, its analgesic effects have not yet been clarified in different scientific models of pain. The Objective of this work was to determine the antinociceptive activity of *Satureja khuzistanica* and possible involvement of opioid system in several model of pain in rats. All experiments were carried out on male Wistar rats. The tail-flick, hot-plate and acid acetic tests were used to assess the antinociceptive effect of *Satureja khuzistanica* Extract (SKE). The extract was given at doses of 12, 25, 50 and 100 mg kg⁻¹ intraperitoneally (i.p.). Naloxone (3 mg kg⁻¹) was used to evaluate the opioid receptor involvement in SKE antinociceptive effects. The data showed that SKE caused a dose-dependent analgesic effect on tail-flick, hot-plate and acid acetic tests. The Maximum antinociceptive activity of *Satureja* extract was observed 30 min after the injection of 100 mg kg⁻¹ and significantly persisted up to 45 min. Blockage of opioid receptors by naloxone (3 mg kg⁻¹) could not prevent SKE-induced analgesic effect. In addition, SKE could potentiate the antinociceptive effect of 5 mg kg⁻¹ morphine on tail-flick test. Co-administration of 12 mg kg⁻¹ SKE with 3 mg kg⁻¹ morphine produced potent antinociceptive effects which were greater than those in morphine-treated or SKE group (p<0.001). Our results indicate that SKE has analgesic property in several models of pain and it can be used for the treatment and/or management of painful conditions.

Key words: *Satureja khuzistanica*, antinociception, tail-flick, hot-plate, acid acetic test, naloxone, rats

INTRODUCTION

The management of pain is considered to be a major clinical problem. Opioids have been used for treating moderate to severe pain but treatment with these drugs leads to the induction of side effects such as analgesic tolerance, physical dependence, emesis, constipation and drowsiness (Katzung *et al.*, 2012). In addition, Non-steroidal Anti-inflammatory Drugs (NSAIDs) as pain killers are also associated with several side effects. The most common side effects are gastric upset (intolerance),

gastric and duodenal ulcers and renal failure, while hepatotoxicity, asthma and rashes occur less frequently (Katzung *et al.*, 2012). Therefore, the finding of herbs that have analgesic property without hazardous side effects is essential skills for pain management.

Others reported the beneficial effects of different species of *Yamasaki et al.* (1998) reported that *Satureja* posses anti-HIV-1 activity (Yamasaki *et al.*, 1998). *Satureja hortensis* seed essential oil showed analgesic and anti-inflammatory effects in animal models (Hajhashemi *et al.*, 2012). Others reported antibacterial

and antifungal (Bezbradica *et al.*, 2005), antispasmodic and antidiarrhea (Hajhashemi *et al.*, 2000) and vasodilatory effects (De Rojas *et al.*, 1999) for different species of *Satureja* in different part of the world. *Satureja khuzistanica* Jamzad (Marzeh Khuzestani in Persian) is a folk medicine that is widely used in most part of Iran as an analgesic and antiseptic (Zargari, 1993). Previous studies showed the anti-inflammatory activity of *Satureja khuzistanica* (Amanlou *et al.*, 2005).

Previous studies reported that *Satureja* species posses antioxidant, anti-diabetic, antihyperlipidemic and reproduction stimulatory properties (Abdollahi *et al.*, 2003; Momtaz and Abdollahi, 2010).

Furthermore, this plant has anti-hyperalgesic effect in a rat model of diabetic neuropathy (Kaeidi *et al.*, 2013). In Persian traditional medicine *Satureja* is used for treating muscle and neuropathic pain as well as withdrawal-induced pain and related side effects (Zargari, 1993).

Others reported that carvacrol (78.3%) is the main components of the *Satureja* extract (Kaeidi *et al.*, 2013). Recently, the antinociceptive effects of carvacrol have been reported in some rodent pain assessing tests (Cavalcante Melo *et al.*, 2012; Guimaraes *et al.*, 2010; Guimaraes *et al.*, 2012a, b).

The composition of essential oils is widely variable for various species of *Satureja* in different parts of the world (Slavkovska *et al.*, 2001).

Based on the above facts, the present study was designed to test the hypothesis that *Satureja* extract could exert antinociceptive effects on chemical and thermal models of pain and opioid system could be involved in its possible analgesic properties in rats.

MATERIALS AND METHODS

Animals: All experiments were carried out on male Wistar rats, weighing 200-250 g, that were housed four per cage under a 12 h light/dark cycle in a room with controlled temperature (22±1°C). Food and water were available *ad libitum*. Animals were handled daily (between 9:00 and 10:00 a.m.) for 3 days, before the experiment day in order to adapt them to manipulation and minimize nonspecific stress responses. Rats were divided randomly into several experimental groups, each comprising 6-8 animals. All experiments followed the guidelines on ethical standards for investigation of experimental pain in animals (Zimmermann, 1983).

Preparation of SKE: An ethanolic *Satureja khuzistanica* Extract (SKE) was prepared in Razi Herbal Medicines Research Center (Lorestan, Iran). The healthy leaves were

dried in shade condition and to avoid decomposition of chemical constituents dried leaves were powdered and stored in clean and dry airtight containers for further studies. A sample was deposited at the herbarium of Razi Herbal Medicines Research Center. Two hundred grams of the air-dried leaves were grinded into fine powder. The powder was extracted twice, on each occasion with one liter of 80% ethyl alcohol. The collective ethanol extract was filtered and the filtrate was concentrated to dryness under reduced pressure in a rotary evaporator and the resulting ethanol extract was freeze-dried. Gas chromatography-mass spectroscopy (GC-MS) analysis of the extract showed that carvacrol (78.3%), 9-Octadecenoic acid (13.5%), hexadecanoic acid (6.7%), bis (2-ethylhexyl) phthalate (1.0%) and beta-bisabolene (0.5%) were the main compositions of the *Satureja* extract (Kaeidi *et al.*, 2013). The same extract was also used in this study.

Drugs: Aliquot portions of the crude *Satureja* extract and naloxone (Temad Co., Iran) were dissolved in warm physiological saline for use on each day of our experiments. These drugs were injected intraperitoneally (i.p.) in the volume of 1 mL kg⁻¹. Control animals received saline in the equal volume (1 mL kg⁻¹).

Tail-flick test: Antinociception was assessed by Tail-Flick test (D'Amour and Smith, 1941). The Tail-Flick latency for each rat was determined three times and the mean was designated as baseline latency before drug injection. The intensity of the beam was adjusted to produce mean control reaction time between 2 and 4 sec. The cut-off time was fixed at 10 sec in order to avoid any damage to the tail. After determination of baseline latencies, rats received intraperitoneal injection of drugs and the reaction latency was determined in different times after injection. The Tail-Flick latencies were converted to the percentage of antinociception according to the following formula:

$$\text{Antinociception (MPE) (\%)} = \frac{\text{Reaction time of test-basal reaction time}}{\text{Cut off time-basal reaction time}}$$

Hot-plate test: Rats were individually placed on a hot-plate maintained at 55±0.2°C and the time of licking of the hind paws or attempt to jump out of the beaker was recorded as the latency period. The cut-off time was 60 sec to avoid tissue damage. Before drug administration, baseline latency was examined. The paw withdrawal latency was tested after drug administration. The Maximum Possible Effect (MPE) was calculated as:

$$\text{MPE (\%)} = \frac{\text{Latency after drug administration-baseline latency}}{60\text{-baseline latency}} \times 100$$

Writhing test: Abdominal constriction induced by intraperitoneal injection of acid acetic was carried out. In this test, the animals were treated with the vehicle or different doses of SKE (50, 100 and 150 mg kg⁻¹) 30 min before the administration of acetic acid (0.6%, i.p.). The number of writhings was counted every 5 min after acetic acid injection over a period of 30 min. Writhing was indicated by abdominal constriction and full extension of hind limb.

Statistical analysis: The results are expressed as Mean±SEM. The difference in MPE% (antinociception) and also mean number of abdominal writhing in and between groups over the time course of study was determined by one or two-way analysis of variance (ANOVA), respectively followed by the Newman-keuls test with 5% level of significance (p<0.05).

RESULTS

Analgesic effect of *Satureja khuzestanica* extract (SKE) on tail-flick test: There were no significant differences in baseline tail-flick latency in all experimental groups. In addition, administration of vehicle had no significant effect on nociceptive threshold. SKE at dose of 25 mg kg⁻¹ i.p. showed analgesia at 15 min after injection (p<0.001, respectively). Intraperitoneal administration of 50 mg kg⁻¹ SKE also exerted significant antinociceptive activity at 15 and 30 min (p<0.001, p<0.05, respectively). *Satureja* extract in dose 100 mg kg⁻¹ exerted an antinociceptive activity which appeared 15 min after injection and peaked at 30 min and significantly persisted up to 45 min (Fig. 1). The analgesic effect of 100 mg kg⁻¹ SKE was greater than 25 and 50 mg kg⁻¹ at 30, 45 min after injection.

Comparison of analgesic effects of 100 mg kg⁻¹ (i.p.) SKE 15, 30, 45, 60 and 120 min after injection on tail-flick showed that SKE in presence or absence of opioid antagonist naloxone had equal effect (Fig. 2).

Analgesic effect of *Satureja khuzestanica* extract (SKE) on hot-plate test: Hot-plate data showed that SKE (100 mg kg⁻¹ i.p.) induced an antinociceptive effect which appeared 15 min after the injection, reaching a peak at 30 min and persisting significant up to 45 min. In addition, 50 mg kg⁻¹ SKE had a significant analgesic property 15 and 30 min after the injection (p<0.01 and p<0.001, respectively). Administration of vehicle and 25 mg kg⁻¹ SKE did not show any nociceptive response (Fig. 3).

Naloxone pretreatment had no influence on the effects of 100 mg kg⁻¹ SKE on nociceptive threshold (Fig. 4).

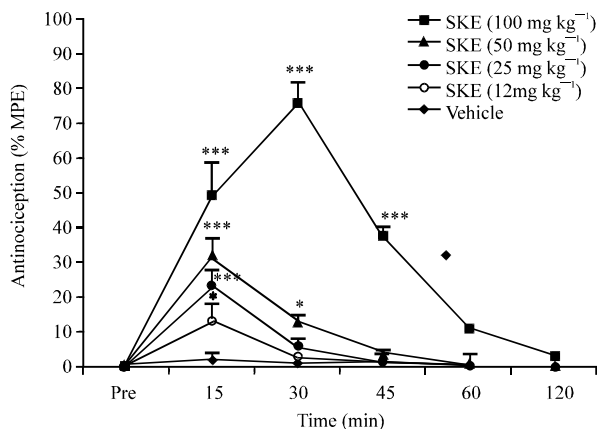


Fig. 1: Antinociceptive effects of intraperitoneal (i.p.) *Satureja khuzestanica* extract (SKE) on tail-flick test in rats. Values represent Mean±SEM (n = 6-8). *p<0.05, **p<0.01 and ***p<0.001 significantly different vs. vehicle-treated group at the same time

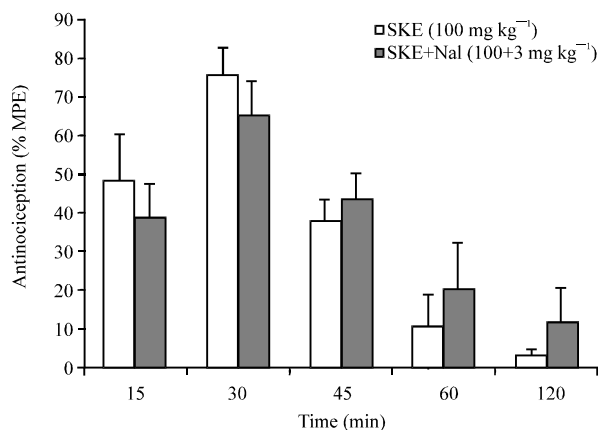


Fig. 2: Antinociceptive effects of 100 mg kg⁻¹ *Satureja khuzestanica* extract (SKE) on tail-flick test in rats in presence or absence of naloxone (3 mg kg⁻¹ i.p.). Values represent Mean±SEM (n = 6-8). There are no significant differences between analgesic scores in experimental groups

Analgesic effect of *Satureja khuzestanica* extract (SKE) on acid acetic test: A significant reduction of writhes in tested animals compared to those in the control group was considered as an antinociceptive response. Administration of vehicle had no significant effect on nociceptive responses in this test (Fig. 5). Intraperitoneal injection of SKE at doses of 50 mg kg⁻¹ did not reduce acid acetic-induced nociceptive responses during both phases. There was a significant reduction in pain response with 100 and 150 mg kg⁻¹ SKE.

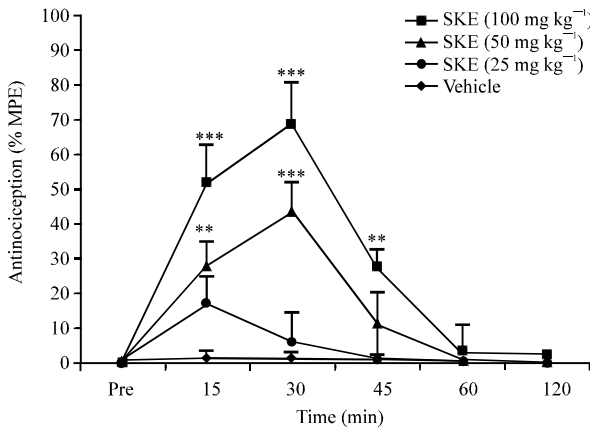


Fig. 3: Antinociceptive effects of intraperitoneal (i.p.) *Satureja khuzestanica* extract (SKE) on hot-plate test in rats. Values represent Mean±SEM (n = 6-8). **p<0.01 and ***p<0.001 significantly different vs. vehicle-treated group at the same time

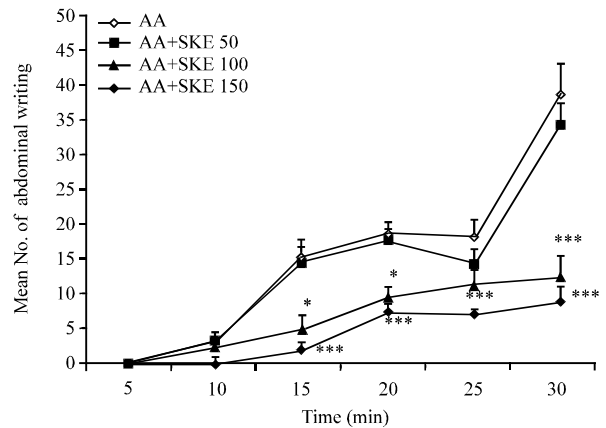


Fig. 5: Antinociceptive effects of intraperitoneal (i.p.) *Satureja khuzestanica* extract (SKE) on acetic acid-induced visceral pain in rats. Values represent Mean±SEM (n = 6-8). *p<0.05 and ***p<0.001 significantly different vs. acetic acid-injected animals at the same time

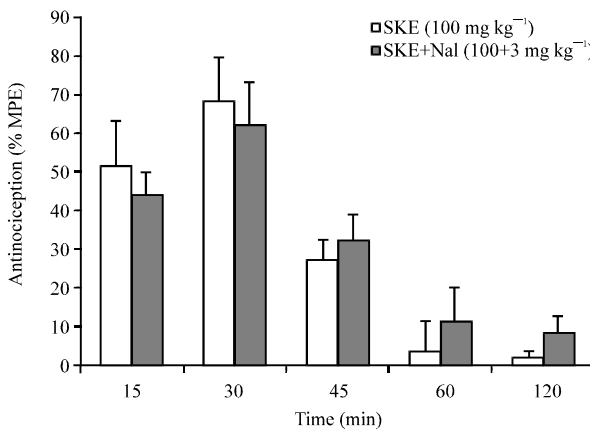


Fig. 4: Antinociceptive effects of 100 mg kg⁻¹ *Satureja khuzestanica* extract (SKE) on hot-plate test in rats in presence or absence of naloxone (3 mg kg⁻¹ i.p.). Values represent Mean±SEM (n = 6-8). There are no significant differences between analgesic scores in experimental groups

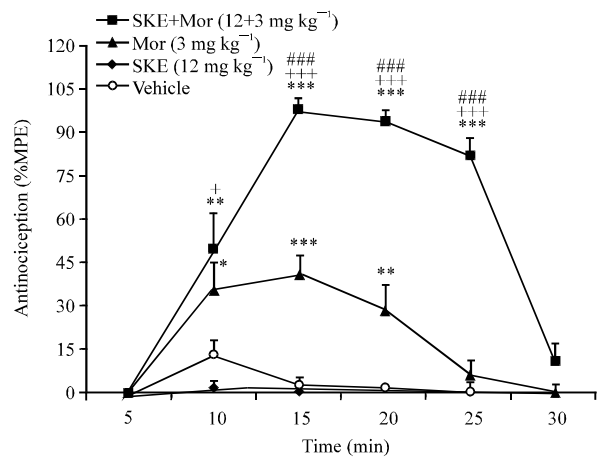


Fig. 6: Effect of 12 mg kg⁻¹ *Satureja khuzestanica* extract (SKE), 3 mg kg⁻¹ morphine and SKE plus morphine on nociceptive threshold in rats. The tail-flick test was used to assess the effect of drugs. Values represent Mean±SEM (n = 6-8). *p<0.05, **p<0.01 and ***p<0.001 significantly different vs. vehicle-treated group at the same time. +p<0.05 +++p<0.001 as compared with SKE-treated group at the same time. ####p<0.001 as compared with morphine-treated group at the same time

Effect of *Satureja khuzestanica* extract (SKE) on analgesic effects of morphine: The tail-flick test was used to assess the effect of drugs. As it is shown in Fig. 6, SKE (12 mg kg⁻¹ i.p.) had no significant effects on nociceptive threshold. Morphine (3 mg kg⁻¹) produced moderate analgesic responses 15, 30 and 45 min after injection. Co-administration of 12 mg kg⁻¹ SKE with 3 mg kg⁻¹ morphine produced potent antinociceptive effects which were greater than those in morphine-treated

or SKE group (p<0.001). This analgesic activity reached a peak 30 min after injection and lasted for about 120 min.

DISCUSSION

Present results showed that SKE posses analgesic activity in tail flick, hot plate and acetic acid-induced abdominal writhings in a dose dependent manner. Also our results showed that *Satureja khuzestanica* analgesic activity was not reversed by naloxone pretreatment. Despite the number of papers published on *Satureja khuzestanica*, none has focused on its influence on nociceptive threshold and its analgesic activity.

The underlying mechanism (s) involved in SKE analgesic activity is not determined yet, however, the analgesic effect on acute and visceral pain could be mediated both by central and peripheral mechanisms. So the main constituents of SKE may be involved in its analgesic property.

It is well known that carvacrol is the main constituents of *Satureja khuzestanica*, which is thought to be responsible for their pharmacological effects. HPLC analysis of SKE in our laboratory showed that the major constituent of our extract is also carvacrol, comprising 78.3% of the extract (Kaeidi *et al.*, 2013).

Recently the antinociceptive activities of carvacrol have been demonstrated in animal models. Chiu *et al.* (2012) reported that *Plectranthus amboinicus* extract which contain carvacrol shows analgesic and anti-inflammatory effect through inhibition of proinflammatory (TNF- α and NF- κ B) mediators.

Also it has been reported that carvacrol as the main constituents of the essential oil from the dried flower buds of clove, *Eugenia caryophyllata* L. Merr. and Perry (Myrtaceae) is used as a topical application to relieve pain (Chaieb *et al.*, 2007).

In mice, it has been reported that carvacrol produces anti-pronociceptive effect against acetic acid-induced abdominal writhing without opioid participation. In addition it significantly inhibits both the early (neurogenic pain) and the late (inflammatory pain) phases of formalin-induced licking, capsaicin-and glutamate-induced pain behavior (Guimaraes *et al.*, 2010). Systemic pretreatment with carvacrol inhibited the development of mechanical hypernociception and edema induced by carrageenan and TNF- α (Guimaraes *et al.*, 2012a). Furthermore it can significantly increase in the latency response on the hot-plate test and also is effective in reducing the nociceptive behaviour in orofacial pain (Guimaraes *et al.*, 2010, 2012a).

Cavalcante Melo *et al.* (2012) reported that the antinociceptive effect of carvacrol in the acetic acid-induced abdominal constriction and formalin tests in mice were not reversed by naloxone or L-arginine which is in complete agreements with our results

(Cavalcante Melo *et al.*, 2012). It means its antinociceptive activity may not act through the opioid system or through inhibition of the nitric oxide pathway (Cavalcante Melo *et al.*, 2012). Based on our results, it is suggested that SKE possesses antinociceptive activity that may not act through the opioid system.

Antinociceptive and anti-inflammatory activities of other species of *Satureja* (*hortensis*) seed essential oil and extracts have been reported in formalin and acid acetic tests in mice (Hajhashemi *et al.*, 2012).

Ghazanfari *et al.* (2006) reported the beneficial effects of *Satureja khuzestanica* Jamzad essential oil on the mouse model of inflammatory bowel disease.

In this study three experimental models of pain were used to assess the analgesic property of the ethanolic extract of SKE. The methods were selected such that both centrally and peripherally mediated effects were investigated. The tail-flick and hot-plate tests revealed central activity. Our result suggests the antinociceptive effect of SKE at the spinal and supraspinal levels using tail-flick and hot-plate tests. However, the pattern of antinociceptive effects on hot-plate and tail-flick tests was not different.

Also SKE showed significant analgesic activity in acetic acid induced visceral pain which is in complete agreements with previous reports (Cavalcante Melo *et al.*, 2012). The acetic acid writhing test as a standard pain assessment induces not only abdominal contraction but also gastro-intestinal ileus and is simply measurable by direct observation of the number of contractions. Visceral pain induced by acetic acid irritation is possibly mediated via., local inflammation and release of substance p (Friese *et al.*, 1997).

Nociceptive information is processed and integrated peripherally as well as at spinal and supraspinal levels within the central nervous system (Jessell and Iversen, 1977; Pasternak, 1993). Tail-flick test is a spinally integrated nociceptive reflex, while hot plate test is a complex response which is supraspinally integrated. Thus, the equal observed effects indicate that the spinal and supraspinal mechanisms are involved in the antinociceptive effects of SKE (Jessell and Iversen, 1977; Pasternak, 1993).

Present results showed that SKE could potentiate the antinociceptive property of sub-analgesic doses of morphine. Also, the results showed that the combination of sub-analgesic doses of *Satureja* extract and morphine, which were ineffective alone, produced a significant analgesic effect in the tail flick model of pain. The underlying mechanism (s) by which *Satureja* extract potentiates the analgesic of morphine is not determined

yet and it may be worth that further investigation and elucidation regarding the mechanism (s) of SKE extract-induced analgesia to be performed. This may be clinically important in the management of acute pain. Therefore, it can be used for the treatment and/or management of painful conditions.

CONCLUSION

In summary, this study shows that the ethanolic extract of *Satureja khuzestanica* has significant antinociceptive effects in different models of pain in rats. The analgesic property of this plant might be related with the carvacrol, as the main constituents of the SKE. The antinociceptive effect of SKE in tail flick, hot plate and the acetic acid-induced abdominal constriction were not reversed by naloxone. It means that SKE antinociceptive activity may not act through the opioid system. Also our results showed that SKE could potentiate the antinociceptive property of sub-analgesic doses of morphine.

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REFERENCES

- Abdollahi, M., A. Salehnia, S.H. Mortazavi, M. Ebrahimi and A. Shafiee *et al.*, 2003. Antioxidant, antidiabetic, antihyperlipidemic, reproduction stimulatory properties and safety of essential oil of *Satureja khuzestanica* in rat *in vivo*: A oxycopharmacological study. *Med. Sci. Monit.*, 9: 331-335.
- Amanlou, M., F. Dadkhah, A. Salehnia, H. Farsam and A.R. Dehpour, 2005. An anti-inflammatory and anti-nociceptive effects of hydrochloric extract of *Satureja khuzistanica* Jamzad extract. *J. Pharm. Pharmaceut. Sci.*, 8: 102-106.
- Bezbradica, D.I., J.M. Tomovic, M.S. Vukasinovic, S. Siler-Marinkovic and M.M. Ristic, 2005. Composition and antimicrobial activity of essential oil of *Satureja montana* L. collected in Serbia and Montenegro. *J. Essential Oil Res.*, 17: 462-465.
- Cavalcante Melo, F.H., E.R.V. Rios, N.F.M. Rocha, M.C.O. Cito and M.L. Fernandes *et al.*, 2012. Antinociceptive activity of carvacrol (5-isopropyl-2-methylphenol) in mice. *J. Pharm. Pharmacol.*, 64: 1722-1729.
- Chaieb, K., H. Hajlaoui, T. Zmantar, A.B. Kahla-Nakbi, M. Rouabhia, K. Mahdouani and A. Bakhrouf, 2007. The chemical composition and biological activity of essential oil, *Eugenia cryophyllata* (*Syzygium aromaticum* L. Myrtaceae): A short review. *Phytother. Res.*, 21: 501-506.
- Chiu, Y.J., T.H. Huang, C.S. Chiu, T.C. Lu, Y.W. Chen, W.H. Peng and C.Y. Chen, 2012. Analgesic and antiinflammatory activities of the aqueous extract from *Plectranthus amboinicus* (Lour.) Spreng. Both *in vitro* and *in vivo*. *Evid. Based Complement Alternat Med.*, Vol. 2012 10.1155/2012/508137
- D'Amour, F.E. and D.L. Smith, 1941. A method for determining loss of pain sensation. *J. Pharmacol. Exp. Therapeut.*, 72: 74-79.
- De Rojas, V.R.S., B. Somoza, T. Ortega, A.M. Villar and T. Tejerina, 1999. Vasodilatory effect in rat aorta of eriodictyol obtained from *Satureja obovata*. *Planta Med.*, 65: 234-238.
- Friese, N., E. Chevalier, F. Angel, X. Pascaud, J.L. Junien, S.G. Dahl and P.J. Riviere, 1997. Reversal by K-agonists of peritoneal irritation-induced ileus and visceral pain in rats. *Life Sci.*, 60: 625-634.
- Ghazanfari, G., B. Minaie, N. Yasa, L. Ashtaral-Nakhai and A. Mohammadirad *et al.*, 2006. Biochemical and histopathological evidences for beneficial effects of *Satureja khuzestanica jamzad* essential oil on the mouse model of inflammatory bowel diseases. *Toxicol. Mechanisms Methods*, 16: 365-372.
- Guimaraes, A.G., G.F. Oliveira, M.S. Melo, S.C. Cavalcanti and A.R. Antonioli *et al.*, 2010. Bioassay-guided evaluation of antioxidant and antinociceptive activities of carvacrol. *Basic. Clin. Pharmacol. Toxicol.*, 107: 949-957.
- Guimaraes, A.G., F.V. Silva, M.A. Xavier, M.R. Santos and R.C. Oliveira *et al.*, 2012a. Orofacial analgesic-like activity of carvacrol in rodents. *Z. Naturforsch C*, 67: 481-485.
- Guimaraes, A.G., M.A. Xavier, M.T. de Santana, E.A. Camargo and C.A. Santos *et al.*, 2012b. Carvacrol attenuates mechanical hypernociception and inflammatory response. *Naunyn Schmiedebergs Arch. Pharmacol.*, 385: 253-263.
- Hajhashemi, V., B. Zolfaghari and A. Yousefi, 2012. Antinociceptive and anti-inflammatory activities of *Satureja hortensis* seed essential oil, hydroalcoholic and polyphenolic extracts in animal models. *Med. Princ Pract.*, 21: 178-182.

- Hajhashemi, V., H. Sadrae, A.R. Ghannadi and M. Mohseni, 2000. Antispasmodic and anti-diarrhoeal effect of *Satureja hortensis* L. essential oil. *J. Ethnopharmacol.*, 71: 187-192.
- Jessell, T.M. and L.L. Iversen, 1977. Opiate analgesics inhibit substance P release from rat trigeminal nucleus. *Nature*, 268: 549-551.
- Kaeidi, A., S. Esmaeili-Mahani, M. Abbasnejad, V. Sheibani, B. Rasouljan, Z. Hajjalizadeh and H. Pasban-Aliabadi, 2013. *Satureja khuzestanica* attenuates apoptosis in hyperglycemic PC12 cells and spinal cord of diabetic rats. *J. Nat. Med.*, 67: 61-69.
- Katzung, B.G., S.B. Masters and A.J. Trevor, 2012. *Basic and Clinical Pharmacology*. 12th Edn., McGraw-Hill, NY., USA.
- Momtaz, S. and M. Abdollahi, 2010. An update on pharmacology of *Satureja* species: From antioxidant, antimicrobial, antidiabetes and anti-hyperlipidemic to reproductive stimulation. *Int. J. Pharmacol.*, 6: 346-353.
- Pasternak, G.W., 1993. Pharmacological mechanisms of opioid analgesics. *Clin. Neuropharmacol.*, 16: 1-18.
- Slavkovska, V., J. Jancic, S. Bojovic, S. Milosavljevic and D. Djokovic, 2001. Variability of essential oils of *Satureja montana* L. and *Satureja kitaibelii* Wierzb. ex Heuff. from the central part of the Balkan peninsula. *Phytochemistry*, 57: 71-76.
- Yamasaki, K., M. Nakamo, T. Kawahata, H. Mori and T. Otake *et al.*, 1998. Anti-HIV-1 activity of herbs in Labiatae. *Biol. Pharm. Bull.*, 21: 829-833.
- Zargari, A., 1993. *Medicinal Plants*. 4th Edn., Tehran University Publications, Tehran.
- Zimmermann, M., 1983. Ethical guidelines for investigations of experimental pain in conscious animals. *Pain*, 16: 109-110.