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Original Article

Antioxidant and antinociceptive effects of hydroalcoholic root extract of *Asparagus officinalis* L.

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Abstract

Introduction: *Asparagus officinalis* L. is a medicinal plant, which contains various natural bioactive phytochemicals with potential different pharmacological activities. The present study was designed to investigate the antioxidant and antinociceptive activities of the hydroalcoholic extract obtained from asparagus roots.

Methods: The plant material was extracted using ethanol 70% and preliminary phytochemical analyses were carried out. The *in vitro* antioxidant effects of the plant extract were evaluated using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radicals and total reducing ability compared to the butylated hydroxytoluene (BHT) as a standard control. The antinociceptive effects were also assessed using formalin and tail-flick test in male Wistar rats.

Results: The plant extract was relatively rich in flavonoids. The IC_{50} value for DPPH scavenging activity of the extract (1117.65±14.26 µg/ml) was significantly higher than that of BHT (64.35±4.09 µg/ml). The plant extract exerted a significantly lower total reducing ability compared to that of BHT. The extract exhibited a significant antinociceptive effect at the early stage of formalin test in the dose of 500mg/kg intraperitoneally. The results of tail-flick test also demonstrated antinociceptive effects compared to control in a dose-dependent manner. However, these antinociceptive activities were not comparable with morphine as a reference agent.

Conclusion: *A. officinalis* roots extract demonstrated considerable antioxidant and antinociceptive activities and it might be attributed to its flavonoids content.

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Keywords:
Asparagus officinalis;
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Antioxidant;
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Introduction

Asparagus officinalis L. (asparagus) from Asparagaceae family is a nutritious garden vegetable, which is native to most parts of Europe, Africa and

Asia consumed throughout the world (Poormoosavi et al., 2018). The consumable shoot of *A. officinalis* is about 20-35cm in length, which is approximately one-half to two-thirds of the stem (Zhao et al., 2011) and often used to prepare vegetable dishes, soups and salads (Hafizur et al., 2012).

Besides nutritional importance, asparagus has been demonstrated to possess many biological activities. Several studies have reported that spear and shoot extracts of *A. officinalis* showed antihypertensive (Sanae and Yasuo, 2013), hypoglycemic (Zhao et al., 2011), hypolipidemic (Zhu et al., 2011; Zhu et al., 2010), antitumor (Shao et al., 1996), hepatoprotective (Zhu et al., 2010) and antioxidant effects in different animal models. The seed extract of this plant also had anti-diabetic (Hafizur et al., 2012) and antifungal (Wang and Ng, 2001) activities. It has been reported root extracts of *A. officinalis* had beneficial impacts on oogenesis in female rats (Jashni et al., 2016), protective effects against liver and kidney damages (Poormoosavi et al., 2018) and neuroprotective activity in an animal model of neurodegenerative disease (Sakurai et al., 2014). Moreover, *A. officinalis* juice potentiated cyclophosphamide-induced antimutagenic activities in mice (Xinhui and Jing, 2001). Antioxidant activity of several *A. officinalis* cultivars have reported in many *in vitro* studies (Rodriguez et al., 2005).

The main phytochemicals responsible for *A. officinalis* bioactivity are phenolic compounds, particularly flavonoids (Makris and Rossiter, 2001; Wang et al., 2003) and hydroxycinnamic acids (Li et al., 2017; Sun et al., 2005) as well as saponins (Huang and Kong, 2006; Wang et al., 2013). However, other compounds such as carotenoids (Deli et al., 2000), glycosides (Gorianu et al., 1976) oligosaccharides and amino acids contribute to the advantageous properties of this plant (Jang et al., 2004). Furthermore, *A. officinalis* spears are rich in dietary fiber with potential therapeutic impacts (Chitrakar et al., 2019).

Bioactivities of *A. officinalis* are not related to a single compound and diversity of phytoconstituents made it a suitable candidate to assess different pharmacological effects rather than traditional nutritional usage. Previous investigations demonstrated that the antioxidant activity of *A. officinalis* extract was closely related to phenolics and flavonoids, including quercetin, kaempferol, rutin and ferulic acid (Fan et al., 2015; Lee et al., 2014). It has also shown that the presence of phenolic and flavonoid compounds were associated with anti-inflammatory and antinociceptive activity of other species of asparagus. The hydroalcoholic extract of *A. radix* (Maresca et al., 2017), *A. pubescens* (Nwafor and Okwuasaba, 2003) and *A. hamosus* (Shojaii et al., 2015), as well as the gum of *A. gummifer* (Bagheri et al., 2015) exerted a significant antinociceptive activity.

To the best of our knowledge, there is no study evaluating the antinociceptive effects of *A. officinalis* roots extract (ARE). Hence, the present study aimed to screen phytoconstituents of ARE and to evaluate its possible *in vitro* antioxidant activity along with beneficial impacts on experimental pain in rats.

Materials and methods

Materials

Morphine sulfate was purchased from Temad Company (Tehran, Iran), while all chemical reagents were obtained from Sigma-Aldrich Química S.A. (Sintra, Portugal). Ethanol and methanol were prepared from Merck (Darmstadt, Germany). All solvents were pro-analysis grade and were acquired from Merck (Lisbon, Portugal). Extract was dissolved in normal saline, freshly on experimentation days.

Plant material and extraction

Fresh roots of *A. officinalis* were purchased from Safiabad Agricultural and Natural Resources Research and Education Center (Dezful, Iran) in August 2018. The shade dried and comminuted plant material (100g) was macerated with ethanol 70% in water (3x1 l) at ambient temperature for 72h. Afterward, the obtained hydroalcoholic extracts was filtered and concentrated using a rotary evaporator at 40°C to give gummy extract. The extract was kept at -20°C until other assessments.

Preliminary phytochemical analysis

The qualitative analyses of the roots phytoconstituents (alkaloids, flavonoids, saponins, tannins and terpenoids) were conducted using methods explained by Trease and Evans (Evans, 2009).

Antioxidant assay

DPPH ability

Free radical scavenging activity of the ARE was

investigated using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) method (Wong et al., 2006). Purple-colored solution of DPPH changes to stable yellow-colored solution in presence of electron-donating ability of the extract's compounds. The reaction mixture was prepared by adding 40µl of methanolic solutions of ARE or butylated hydroxytoluene (BHT, 100-1600 µg/ml) to 3ml of the freshly prepared methanolic solution of DPPH (0.1mM), followed by incubation at the ambient temperature for 30min. Thereafter, the absorbance was measured at 515nm. Each experiment was conducted three times and DPPH scavenging activity was calculated as inhibition percentage (I%) based on the following formula: $I\% = [(A_{control}-A_{sample})/A_{control}] \times 100$

The inhibition percentages were plotted against the ARE concentrations and the concentration required to scavenge 50% of DPPH radical was considered as IC_{50}.

Total reducing ability

The ferric reducing antioxidant potential of ARE was determined based on the previously described method (Yildirim et al., 2001). Different concentrations of ARE or BHT (100-800 µg/ml) were added to a mixture of 2.5ml of potassium ferricyanide (1%) and 2.5ml of phosphate buffer (0.2M, pH: 6.6), followed by incubation at 50°C for 30min. Thereafter, 2.5ml of trichloroacetic acid (10%) was added to this mixture and centrifuged at 3000rpm for 10min. At the final step, 2.5ml of distilled water and 0.5m of ferric chloride (0.1%) were added to 2.5ml of the supernatant solution and the absorbance was measured at 700nm. Each experiment was conducted three times and increased absorbance considers as increased reducing ability.

Animals and study design

Forty eight male *Wistar* rats (200-220g) were purchased from the Animal House of Hormozgan University of Medical Sciences, Bandar Abbas, Iran. All animals were kept in standard condition and had free access to food and tap water. The experimental protocols were approved by the Animal Ethics Committee of Hormozgan University of Medical Sciences (approval number; IR.HUMS.REC.1397.218).

The rats were randomly divided into four groups (six in each group); control (saline 0.9%, 1ml/kg), MS (morphine sulfate, 10mg/kg), ARE300 (*A. officinalis* roots extract, 300mg/kg) and ARE500 (*A. officinalis* roots extract, 500mg/kg). All animals were treated with a single intraperitoneal injection.

Formalin test

Formalin test was carried out to evaluate acute neurogenic and chronic inflammatory pain (Coderre et al., 1990). The animals were treated with saline 0.9% (1ml/kg), morphine sulfate (10mg/kg) and ARE (300 and 500mg/kg). Thirty minutes after treatment, 50µl of formalin solutions (2.5%) was injected into the dorsal surface of the animal's right hind paw. The animals were then placed in transparent camber for observation and nociceptive behavior is evaluated in both early phase (0-5) and late phase (15-20min) after formalin injection. The pain intensity rating (PIR) as a nociception index were calculated using the pain behaviors and the time of each behavior based on the following formula: $PIR = [(0\times T0)+(1\times T_1)+ (2\times T_2)+ (3\times T_3)] / 300$

The time spent in normal behavior with full contact of paw plantar surface with the floor (T0), no contact of some parts of the paw with the floor or limping (T1), elevating of the paw (T2), and vigorous shaking or licking of the paw (T3).

Tail-flick test

Tail-flick test was conducted to evaluate antinociceptive activity of ARE (D'Amour and Smith, 1941). After animal restraining in a plexiglass cylinder, radiant heat was focused on the distal end of their tail from a distance of 4-7cm. Reaction time of animals was recorded three times (with time interval of 10 seconds) and their mean was considered as baseline latency time (BL). The light intensity was then adjusted to produce mean LB between 3 and 5s, and the cut-off time (CT) of 10s was used to avoid tissue damage. Thereafter, the animals were treated with saline 0.9% (1ml/kg), morphine sulfate (10mg/kg) and ARE (300 and 500mg/kg) and the treatment latency times (TL) were measured at 15, 30, 45 and 60min after treatment. The percentage of maximal possible effect (MPE%) was calculated as index of antinociception according to the following formula: $MPE\% = [(TL-BL) / (CT-BL)] \times 100$

Evaluation of acute toxicity

Animals employed in the antinociceptive studies were

Table 1: Phytochemical screening of *Asparagus officinalis* roots extract.

	Tests	Result
1	Alkaloids	-
2	Flavonoids	++
3	Saponins	+
4	Tannins	-
5	Terpenoids	-

- Negative, + Less, ++ Moderate

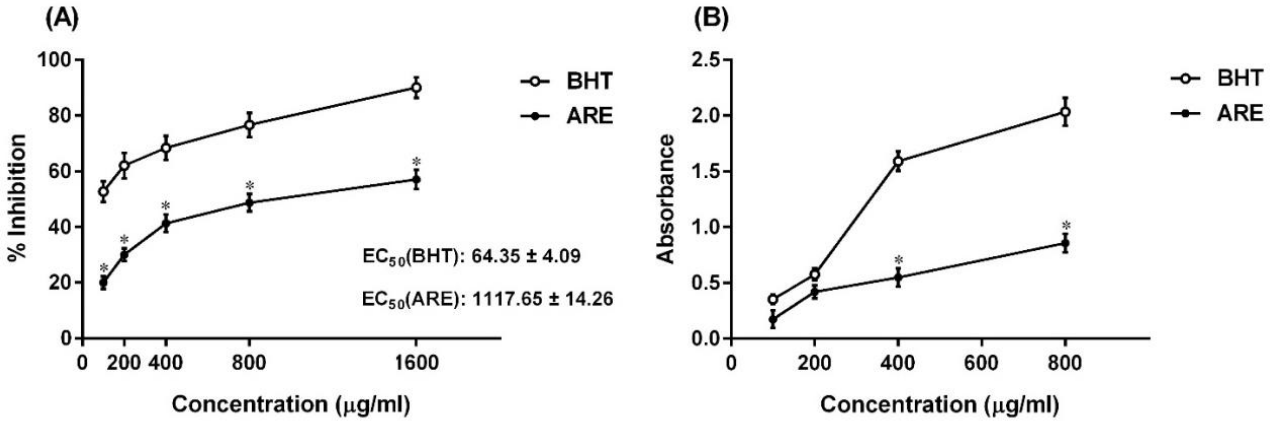

Fig.1. DPPH free radical-scavenging activity (A) and ferric reducing antioxidant power (B) of butylated hydroxytoluene (BHA) as standard and methanolic extract of *Asparagus officinalis* roots extract (ARE). Data were expressed as mean±SEM. Differences were tested by one-way ANOVA and post-hoc test of Tukey. $^{*}P<0.05$ versus control group at the same concentration points.

cared and observed for five days and morbidity or mortality was recorded.

Statistical analyses

Data were expressed as mean±SEM. Statistical analyses were performed using Statistical Package for the Social Sciences software (SPSS 16). After the assessing the normality, comparisons between the groups were conducted using One-way ANOVA analysis, followed by post hoc Tukey's test. $P < 0.05$ was considered as the significance level.

Results

Phytochemical analysis

The ARE was found to be relatively rich in flavonoids (Table 1). Fewer amounts of saponins were also present in ARE. Preliminary phytochemical screaming showed that the ARE was free of alkaloids, tannins and terpenoids.

DPPH scavenging assay

The free radical scavenging activity of the ARE is shown in Figure 1A. This antioxidant activity was increased with the increasing of concentrations. The antioxidant activity of ARE was significantly lower than that of BHT at all concentrations ($P<0.05$). The IC_{50} value of ARE (1117.65±14.26 µg/ml) was superior over BHA (64.35±4.09 µg/ml).

Ferric reducing ability Assay

The reducing powers of ferric ion as an indicator of electron-donating ability shows in Figure 1B. Increasing ARE concentration enhanced total reducing ability in a concentration-dependent manner. Reducing power of ARE was significantly lower than that of BHT at high concentrations.

Formalin test

The results of the formalin test in rats (Fig. 2) showed that ARE (500mg/kg) had a significant antinociceptive effect in the early phase ($P<0.05$), however, this effect was slighter than that of morphine sulfate. The antinociceptive effects of ARE at dose of 300mg/kg in the early phase was not significant compared to the

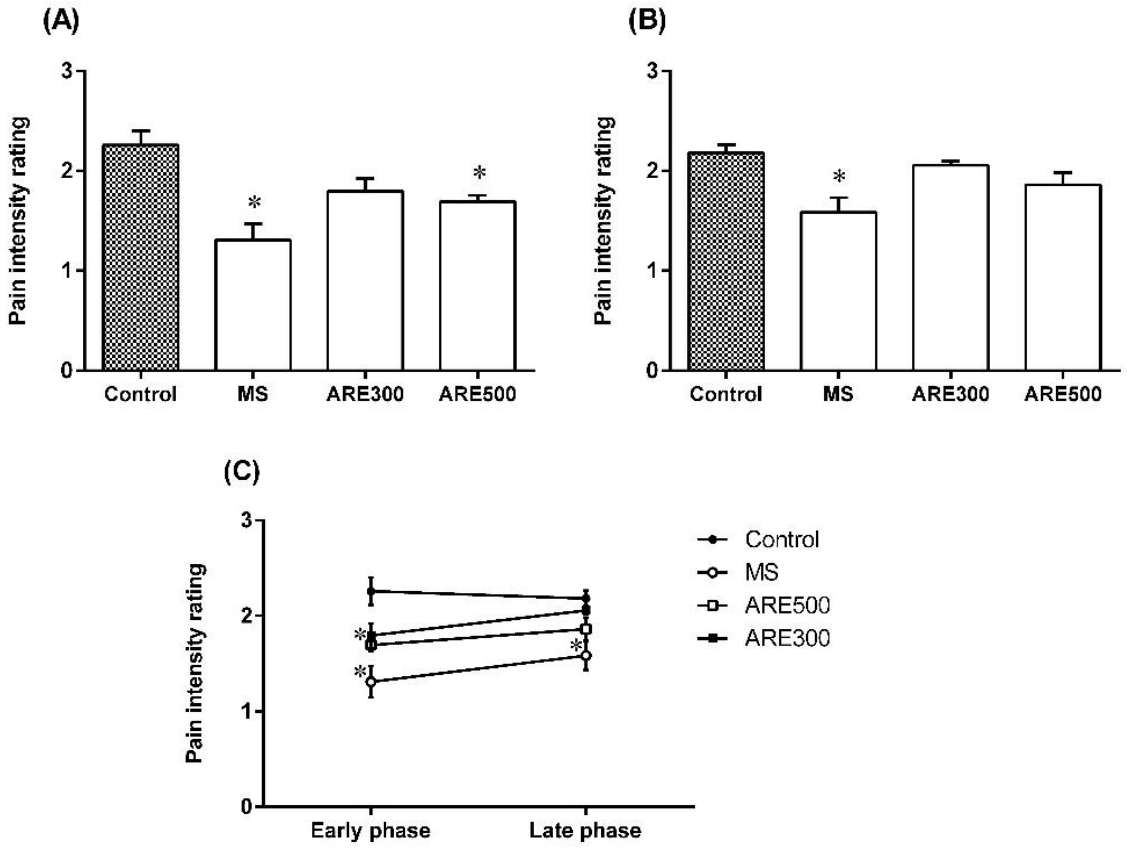

Fig.2. Antinociceptive activity in early phase (A), late phase (B) and time point (C) of the formalin test in rats. Animals received vehicle (control), morphine sulfate (MS, 10mg/kg) and *Asparagus officinalis* roots extract, ARE (300 and 500mg/kg) by intraperitoneal injection. Data were represented as mean±SEM (n=6/group). Differences were tested by one-way ANOVA and post-hoc test of Tukey. $^{*}P<0.05$ versus control group.

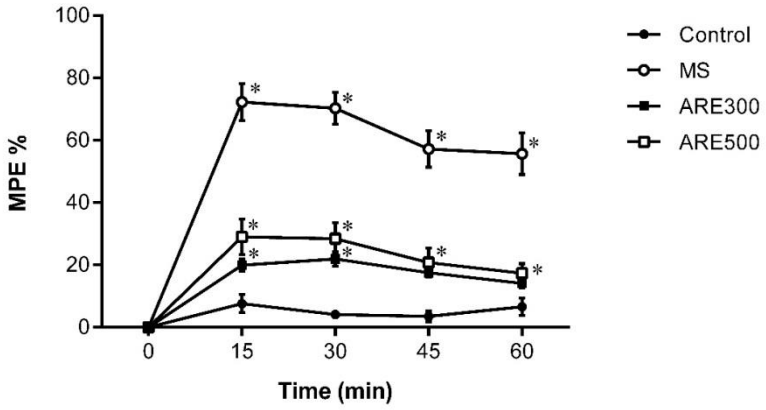

Fig.3. Time-dependent antinociceptive activity on the tail-flick assay in rats. Animals received vehicle (control), morphine sulfate (MS, 10 mg/kg), *Asparagus officinalis* roots extract and ARE (300 and 500mg/kg) by intraperitoneal injection. Data were represented as mean±SEM (n=6/group). Differences were tested by one-way ANOVA and post-hoc test of Tukey. $^{*}P<0.05$ versus control group at the same time points.

control group. Although ARE (300 and 500mg/kg) decreased formalin-induced pain in the late phase of the test, these effects were not statically significant.

Tail-flick

The results of tail-flick thermal analgesia test in rats (Fig. 3) demonstrated that ARE at both doses of 300 and 500mg/kg showed a significant anti-nociceptive activity ($P<0.05$) in a dose-dependent manner compared to that of control group. The analgesic

activity of ARE was less potent than that of morphine sulfate (10mg/kg) at each time point after administration (P<0.05).

Acute toxicity

When ARE was studied by intraperitoneal administration in rats, no mortality and signs of toxicity were observed in doses of 300 and 500mg/kg.

Discussion

Asparagus, which is used for nutritive purposes in the making of vegetable dishes, soups and salads worldwide, has many pharmacological activities. To the best of our knowledge, there is no study investigating the antinociceptive effects of Iranian native *A. officinalis* roots extract. Therefore, the present study aimed to screen phytochemical compounds and to assess the *in vitro* antioxidant activity and the possible antinociceptive effects of ARE in rodent. The obtained results revealed the presence of flavonoids and fewer amounts of saponins in the plant extracts. The extract also showed antioxidant effects and relative analgesic activity compared to the standard controls.

The qualitative phytochemical analysis of ARE showed the presence of relative amounts flavonoids and saponins. In consist with these results, previous studies have shown that the ethanolic and methanolic extracts of *A. officinalis* roots form other geographical regions are rich in flavonoids and polyphenolic compounds (Symes et al., 2018) and saponins (Huang and Kong, 2006). Furthermore, other parts of these plant have high content of flavonoids (Makris and Rossiter, 2001; Wang et al., 2003) and saponins (Wang et al., 2013). Considering these information, a large body of evidence have demonstrated that flavonoids and saponins as well as other secondary metabolites have a crucial role against oxidative stress and nociceptive processes (Ielciu et al., 2019; Puente-Garza et al., 2018; Soares-Bezerra et al., 2013; Wei et al., 2019). These compounds were identified to have inhibitory activities against five proteases, which potentially involved in the radical scavenging activity as well as anti-inflammatory process (Martínez et al., 2012).

Oxidative stress is an imbalance between intracellular production of reactive oxygen and nitrogen species and the capability of cells to neutralize their harmful impacts by antioxidants. The oxidation of macromolecules including nucleic acids, protein, lipids and carbohydrate by these toxic free radicals causes cellular senescence and death, and involves in the pathophysiology of many diseases (Halliwell, 2006). Potential antioxidant effects of natural compounds from various plant sources have investigated in recent years (Pohl and Thoo Lin, 2018; Soccio et al., 2018). In the present study, ARE revealed significant and promising DPPH radical scavenging activity and ferric ion reducing ability in a concentration-dependent manner. The antioxidant effects of ARE agree with previous studies investigating the antioxidant properties of roots extract of different *A. officinalis* cultivars (Symes et al., 2018; Zhang et al., 2019). Moreover, the extracts of other species of asparagus have also demonstrated antioxidant activity in different *in vitro* and *in vivo* models (Karuna et al., 2018; Kongkaneramit et al., 2011; Lei et al., 2017).

These antioxidant activities of ARE are essentially due to the presence of polyphenolic compounds, which were confirmed by the results of primarily phytochemical screening. Previous studies have mentioned that polyphenols among plant secondary metabolites play a main role in attenuation of lipid peroxidation (Stavrou et al., 2018; Sureda et al., 2014). Flavonoids especially have been reported to be bioactive compounds for antioxidant properties of *A. officinalis* extract (Fan et al., 2015; Lee et al., 2014).

Nociception is the response of sensory nervous system to damaging or potentially damaging noxious stimuli (Sneddon, 2018). Chemical, thermal and mechanical stimulation of nociceptors cause a signal, which passes to central nervous system and results in feeling of pain (Hosseini et al., 2011; Sneddon, 2018). Analgesic compound are mostly used in mono- or combination therapy to relive this sensation (Raffa, 2001). Considering the fact that administration of opioids and other non-narcotic analgesic medicines possess a wide range of adverse effects (Slater et al., 2010), natural products are currently investigating to develop novel therapeutic agents for nociception (Tsuchiya, 2017).

The formalin test is considered as a standard model for determination of potential analgesic activities. Formalin exerts a biphasic pain procedure, including early phase with a short-lasting pain response

followed by a short period of quiet interphase and late phase with inflammatory pain (Tjolsen et al., 1992). Moreover, centrally acting antinociceptive agents alleviate both early and late phases, and analgesic agent with peripheral mechanism only inhibit pain in the late phase. The present study revealed that ARE (300 and 500mg/kg) had antinociceptive activity on the acute and inflammatory pains. However, the high dose of ARE on the acute pain reached significant level. It can be claimed that the analgesic mechanism of ARE is both central and peripheral.

The tail-flick test is another standard method for assessment of analgesic activity. In this test, the thermal noxious stimulation activates peripheral nociceptors and leads to reflex of tail withdrawal at the fastest possible time (Hole and Tjolsen, 1993). Modulation of descending pain pathway and activation of spinal reflex arc might be involved in the mechanism of tail flick (Nakamura et al., 1986). In the present tail-flick test, ARE at doses of 300 and 500mg/kg demonstrated a significant anti-nociceptive activity in a dose-dependent manner. The obtained results confirmed that ARE had central analgesic activity.

Several studies have shown compounds with antioxidant activity can possess antinociception through attenuation of lipid peroxidation and overproduction of prostaglandin from phospholipids (Osukoya et al., 2016). Therefore, the observed analgesic effects of ARE could be due to the presence of antioxidant compounds. On the other hand, the antinociceptive activity might be the result of the secondary plant metabolites. It has been reported that flavonoids, which are present in ARE, inhibits the Ca^{2+} inward current and release of analgesia and proinflammatory mediators (Kempuraj et al., 2005).

Previous study conducted on the other species of asparagus has shown the analgesic activity of plant extract associated with plant secondary metabolites (Hassan et al., 2008). However, more investigations are necessary to determine the phytoconstituents responsible for the antioxidant and analgesic effects and to identify the exact mechanism of antinociceptive activities.

Conclusion

Asparagus officinalis roots extract demonstrated antioxidant and antinociceptive activities and it might be attributed to the presence of flavonoid constituents. More future studies are necessary to assess the phytochemical compounds responsible for the antioxidant and antinociceptive activities and to distinguish the exact mechanism of the analgesic effects. Moreover, additional studies should be carried out to ensure the safety of extract.

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Conflict of interest

The authors declare that they have no conflict of interest.

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