Antioxidant Phytochemicals of *Opuntia ficus-indica* (L.) Mill. Cladodes with Potential Anti-spasmodic Activity

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Submitted: 10-11-2016  Revised: 11-01-2017  Published: 11-10-2017

**ABSTRACT**

Background: *Opuntia ficus-indica* (OFI) (L.) Mill. (Cactaceae), a plant widespread in dry regions of the world, shows interesting biological activities (cicatrizant, antiulcer, anti-inflammatory, and hypolipidemic) and is widely used in traditional medicine. **Objectives:** Phytochemical analysis and antispasmodic effect of wild OFI cladodes were carried out. **Material and Methods:** Polyphenols and Vitamin E occurrence, in antioxidant pool of OFI cladodes, were quantified by high-performance liquid chromatography. The antispasmodic effect of OFI cladodes was assessed in isolated rabbit smooth muscle tissues. The experiments were carried out with preparations of rabbit jejunum and uterus with the spontaneous contractile activity, to evaluate the effect of cumulative concentrations of the extract on basal tone, amplitude, and frequency of contractions. **Results:** Catechin, quercetin, kaempferol, isorhamnetin and chlorogenic, ferulic, and p-coumaric acid were identified. α-, β-, and γ-tocopherols have been highlighted and α-tocopherol is the major component. OFI cladodes contain significant amount of polyphenols and tocopherols that are effective radical scavengers and inhibited ethanol 1,1-diphenyl-2-picrylhydrazyl formation by 50%. OFI cladodes caused a light inhibition of amplitude with the spontaneous contractile activity, to evaluate the effect of cumulative concentrations of cladode extract caused uterine muscle relaxation. Conclusion: The contraction of smooth muscle preparations depends on an increase in cytoplasmic free calcium ion concentration, which activates the contractile elements. The flavonoids may suppress the contractility of smooth myocytes, by an inhibition of availability of Ca2+ for muscle contraction. **Key words:** Antispasmodic activity, cladodes, high-performance liquid chromatography, *Opuntia ficus-indica* (L.) Mill, polyphenols, tocopherols

**SUMMARY**

• *Opuntia ficus-indica* (OFI) cladodes contain significant amount of polyphenols and tocopherols that are effective radical scavengers and inhibited ethanol 1,1-diphenyl-2-picrylhydrazyl formation by 50%.

• Polyphenols and Vitamin E complex occurrence in OFI cladodes were characterized by high-performance liquid chromatography

**INTRODUCTION**

*Opuntia ficus-indica* (OFI) (L.) Mill is a plant widely cultivated in many regions of the world for its fruit with a pleasant flavor and with a high content of minerals, vitamins, dietary fiber, and phytochemicals. In Sicily, the plant has found a particularly favorable environment, becoming a regular feature of the natural landscape. Not only plants grown in Sicily produce fruit that invade the markets of Northern Europe but also wild plants provide edible fruits very much appreciated by locals. In Central and South America, OFI is appreciated also for their young cladodes, known as nopalitos, which are consumed as vegetable.[11] In Sicily, cladodes are used as animal feed too. Several literature data report that OFI cladodes are helpful in many diseases and possess anti-inflammatory, hypocholesterolemic, hypoglycemic, antiulcer, and wound-healing effects.[2-6] Moreover, OFI cladodes are used in traditional medicine in gastrointestinal disorders.[7] Activities are ascribed to the presence in cladodes of mucilage, pectin, plant sterols, vitamin, and polyphenols, well known for their antioxidant properties.

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Cite this article as: Lanuzza F, Occhiuto F, Monforte MT, Tripodo MM, D’Angelo V, Galati EM. Antioxidant phytochemicals of *Opuntia ficus-indica* (L.) Mill. cladodes with potential anti-spasmodic activity. Phcog Mag 2017;13:S424-9.
In the present study, polyphenol content and Vitamin E occurrence in antioxidant pool of wild OFI L. Mill cladodes growing in a hilly area near to Messina (Sicily, Italy), were quantified by high-performance liquid chromatography (HPLC).

Moreover, the antispasmodic effect of OFI cladodes methanol extract was assessed in isolated rabbit smooth muscle tissues. All experiments were carried out with preparations of rabbit jejunum and uterus, with the spontaneous contractile activity, to evaluate the effect of cumulative concentrations of the extract on basal tone, amplitude, and frequency of contractions.

MATERIALS AND METHODS

Plant material

OFI (L.) Mill. cladodes were harvested from wild plants growing in hilly area near to Messina (Sicily, Italy) in April 2013. Young cladodes (20–25 cm length), without flowers and fruits, were used. The cladodes deprived of glochids were homogenized in a high-speed mixer, Ultra-Turrax for 5 min, and then lyophilized. A voucher specimen was deposited in the herbarium of our department (Department of Chemical, Biological, Pharmaceutical and Environmental Sciences) (University of Messina). Two grams of lyophilized cladodes were extracted with 200 mL of methanol for 7 days in the dark. The extract was filtered and the solvent was removed in vacuo (40°C) (Büchi Rotavapor R-205 equipped with Büchi Vacuum Controller V-800). The yield was 25.35%.

Polyphenols determination

Folin–Ciocalteu method

Aliquot of 2.5 mg of extract was dissolved in 1 mL of methanol; 100 µL of the sample was added to 200 µL of Folin–Ciocalteu reagent. After 3 min, 2 mL of water and 1 mL of sodium carbonate (15%) were added; then the test tubes were shaken and allowed to incubate in darkness for 2 h.\textsuperscript{[9]} The assay was carried out in triplicate. A blue coloration was developed, and the absorbance was read at 765 nm (Spectrophotometer Shimadzu UV-1601) at room temperature (20°C). The results were expressed as gallic acid equivalent (GAE).

High-performance liquid chromatography analysis

Chemicals

Acetonitrile and methanol both HPLC grade were purchased from Fluka (Sigma Aldrich, Milan, Italy), orthophosphoric 85% acid from Carlo Erba (Carlo Erba Reagenti SpA, Limito, Milan, Italy). High-quality water was obtained by a Milli-Q water purification system (Millipore Corporation, Bedford, MA, USA). Ferulic and chlorogenic acid, catechin, kaempferol, and isorhamnetin were purchased from Extrasynthese (Genay, France), p-coumaric acid and quercetin from Sigma Aldrich (Milan, Italy).

Equipment

Spectra System\textsuperscript{*} Gradient Pumps 4000 Menus Liquid Chromatograph (Thermo Separation Products, FL, USA), equipped with Vacuum Membrane Degasser SCM 1000 and a Rheodyne valve 8125 (loop 20 µL) (Redwood Drive Cotati, CA, USA) were used. Liquid chromatograph was coupled to a Photodiode Array Detector Spectra SYSTEM\textsuperscript{*} UV6000 LP (Thermo Separation Products, FL, USA) working in the range 220–450 nm. Chromatographic data were processed with a ChromQuest Chromatography Workstation (ThermoQuest Italy S.p.A., Rodano, Milan, Italy).

High-performance liquid chromatography analysis

About 1 g of methanol extract of lyophilized cladodes was recovered with 20 mL of methanol, filtered, and analyzed by HPLC. Analyses were carried out at room temperature with a reversed phase column Hypersil Gold 5 µm (250 mm × 4.6 mm), protected with an Hypersil Gold 5 µm (10 mm × 4 mm) drop-in guard column (Thermo Scientific, Superchrom Milan, Italy). Chromatographic separation was carried out using three eluents (A: water-85 orthophosphoric acid [99.7: 0.3 v/v]; B: methanol; C: acetonitrile) in a linear gradient program shown in Table 1. The flow rate was 0.9 mL/min. A typical chromatogram registered at 280 nm is reported in Figure 1. Qualitative analysis was carried out comparing the chromatographic behavior and the UV spectra of the components with pure standards under the same chromatographic conditions. Peak purity was established carefully by studying the DAD data of all the peaks of interest. Quantitative analysis was carried out using linear regression obtained by injecting solution of known content of standards measured at maximum adsorption wavelength of single components. The linearity response of the detector was verified in the range from 0.002 to 0.4 mg/mL for the identified compounds. The analysis was carried out in triplicate.

Tocopherol determination

Chemicals

n-hexane >95% GC pure (Fluka, Sigma Aldrich S.R.L., Milan, Italy); butylhydroxytoluene (BTH) (Fluka Ag, SG Buches, Switzerland); ethyl alcohol 96%, HPLC grade hexane, isopropanol (JT Baker NL-Deventer, The Netherlands); potassium hydroxide >85% pellets (Italian Fine Chemicals, Milan, Italy); sodium phosphate dibasic dihydrate 99%, potassium dihydrogen phosphate 99.5%, sodium sulfate anhydrous 99.5%, and L-ascorbic acid 99.7% (Carlo Erba, Milan, Italy) were purchased from Supelco (Bellafonte, PA, USA, distributed by Sigma-Aldrich, Milan).

Equipment

Liquid chromatograph Perkin-Elmer LC series 4 (Norwalk Connecticut, USA), equipped with a Rheodyne valve 8125 (loop 10 µL) (Redwood Drive Cotati, CA, USA), Shimadzu RF-10AXL Fluorescence Detector (Shimadzu Corporation, Kyoto, Japan) connected to a diode array detector (SpectraSYSTEM UV6000LP–Thermo Separation Products, USA); Chrom-Card acquisition and monitoring system data processing (Fisons Instruments S.p.A., Rodano, Milan, Italy) were used.

Sample preparation

To eliminate lipid components that interfere with determination of tocopherols, it was necessary to saponify the samples.\textsuperscript{[9]} Lyophilized cladodes (0.5 g) were added of 25 mL H\textsubscript{2}O, 50 ml ethanol, and 500 mg ascorbic acid. The sample was saponified with 25 mL potassium hydroxide solution (100 g/100 mL) at a room temperature in stoppered flask de-aerated with nitrogen. The saponification was continued for 1 h in the dark, shaking occasionally. The tocopherols were not degraded by saponification at room temperature. The unsaponifiable matter was then extracted for three times, respectively, with 100, 50, and 50 mL of hexane.

| Eluent A % | Eluent B % | Eluent C % | Time (min) |
|------------|------------|------------|------------|
| 95         | 3          | 2          | 0          |
| 70         | 15         | 15         | 25         |
| 60         | 15         | 25         | 35         |
| 52         | 10         | 38         | 40         |
| 30         | 10         | 60         | 50         |
| 0          | 10         | 90         | 55         |
| 95         | 3          | 2          | 58         |

(A: water - 85 orthophosphoric acid (99.7:0.3 v/v); B: methanol; C: acetonitrile)
Extracts were combined, washed three times with, respectively, 100, 50, and 50 mL of phosphate buffer pH 7.4 and dehydrated with 30 g of anhydrous sodium sulfate. The solution was dried in a rotary evaporator at 35°C and recovered with 5 mL of hexane containing 0.3 mg/mL of BHT to protect tocopherols from oxidation. An aliquot of the extract was filtered with Millex®-GV 0.22 µm filter (Millipore Corporation, Bedford, MA 01730, USA). The standards of tocopherols were solubilized in hexane and were added with 0.3 mg/mL of BTH to make them more stable to oxidation. The solution, stored at −18°C, was stable for more than 1 month.

**Analytical method**

HPLC analysis was carried out, at room temperature, using a Chromospher 5 Si 250 mm × 2 mm column (Chrompack, The Netherlands) connected to a guard column Resolve Silica (Waters Corporation, Milford, MA, USA). The analysis was performed in isocratic elution, using a sonicated mixture of n-hexane-dioxane (98.5:1.5) (v/v), as mobile phase at a flow rate of 0.4 mL/min. Fluorimetric detection was performed at λ<sub>exc</sub> 290 nm and λ<sub>em</sub> 330 nm. A typical chromatogram is reported in Figure 2.

Qualitative analysis was carried out comparing the chromatographic behavior and the excitation and emission spectra of the samples with those of pure standards, obtained under the same chromatographic conditions, registered with "stopped flow" technique. To confirm the result and especially to verify the peak purity, the registration of the UV spectra in the range 220–450 nm was also carried out. α-, β-, and γ-tocopherols were identified.

Quantitative analysis was carried out using linear regression obtained by injecting solution of known content of tocopherol standards. The linearity response of the fluorescence detector was verified in the range from 0.001 and 0.05 mg/mL for α- and γ-tocopherol and 0.0001–0.008 mg/mL for β and δ-tocopherol.

**Antioxidant activity**

Radical scavenging activity of methanol extract of cladodes was assayed according to Ohinishi et al.<sup>10</sup> An ethanol 1,1-diphenyl-2-picrylhydrazyl (DPPH, Sigma-Aldrich, Milano Italy) solution (0.1 mM) was mixed with different concentration of the cladode extract in a range from 10–100 µL. The optical density change at 517 nm was measured 10 min later with a spectrophotometer (Shimadzu, model UV-1601). The scavenging activity was measured as the decrease in absorbance of the samples versus DPPH standard solution. The mean value was obtained from triplicate experiments. Results were expressed as percentage activity, and mean inhibiting concentrations were calculated using the Litchfield and Wilcoxon test.<sup>11</sup>

**Antispasmodic effect**

The effects of cumulative concentrations of the OFI cladodes extract (25–250 µg/mL) on spontaneous motility were evaluated.

**Animals**

Rabbits (New Zealand) weighing 1.7–2.0 kg, of both genders, were used. Animals were housed under standard laboratory conditions (temperature 22° ± 2°C; humidity 60% ± 4%; natural lighting) with free access to food and water. Animal care was in compliance with the Italian regulations on protection of animals used for experimental and other scientific purposes (D.M: 116192), as well as with the EEC regulations (O.J. of E.C.L. 358/1 12/18/1986).

Rabbits, fasted for 24 h, were sacrificed by cervical dislocation. The abdomen was opened and segments of jejunum and uterus were collected.

**Isolated rabbit jejunum preparation**

A total of 2 cm segments of jejunum were suspended in 20 mL tissue baths containing Tyrode’s solution (mM: KCl 2.68, NaCl 136.9, MgCl<sub>2</sub> 1.05, NaHCO<sub>3</sub> 11.90, NaHPO<sub>4</sub> 0.42, CaCl<sub>2</sub> 1.8, and glucose 5.55; pH 7.94), maintained at 37°C and aerated with a mixture of oxygen (95%) and carbon dioxide (5%). A tension of 0.5 g was applied to each tissue and kept constant throughout the experiment. Most of the strips developed spontaneous contractions within 5–30 min; strips with no spontaneous activity in this period were discarded.
The intestinal responses were recorded isotonically using a transducer (HSE F30, 372 chicks-Hugo Sachs Elektronik, Harvard Apparatus GmbH-Germany). The data were digitally recorded and collected by data acquisition software HSEACAD W (Hugo Sachs Elektronik, Harvard Apparatus GmbH-Germany) and displayed on a computer screen. Each preparation was allowed to equilibrate for at least 30 min before the addition of the cladode extract added in a cumulative manner at concentration of 25–50 µg/mL.

To examine whether muscarinic receptors are involved in the jejunum response to extract, concentration-response curve was obtained in the presence of a muscarinic receptors blocker atropine (1.0 µg/mL) (Sigma). Nifedipine (Sigma) was used as reference drug control at a dose of 0.01–1.0 µg/mL.

**Isolated rabbit uterus preparation**

Isolated uterus preparations from virgin female rabbits were used. Myometrial segments, 1.5 cm in length, were isolated and mounted in 20 mL tissue baths containing Tyrode's solution sufficiently oxygenated and thermostated at 37°C. The tone and the contractile activity of the organ were recorded by means of an isotonic transducer (HSE F30, 372 chicks-Hugo Sachs Elektronik, Harvard Apparatus GmbH-Germany). All experiments were conducted with preparations of the uterus in spontaneous contractile activity, to evaluate the effects of cumulative concentrations of the OFI cladodes extract (5–250 µg/mL) on the basal tone, amplitude, and frequency of contractions. In some experiments, the spontaneous contractile activity of the preparation was inhibited by reducing the temperature of the bath of the organ to 30°C–32°C and changing the nutrient solution of Tyrode as follows: NaCl 9 g, KCl 0.42 g, CaCl₂·H₂O 0.08 g, NaHCO₃ 0.5 g, H₂O to 1 L, pure O₂, and pH 7. This was to assess whether the pretreatment of the preparation with the cladode extract influences the maximal contractile response induced by certain agonists, epinephrine 1.0 µg/mL, acetylcholine 1.0 µg/mL, and histamine 1.0 µg/mL (Sigma).

**Statistical analysis**

For chromatographic analysis, the results were given as mean ± standard deviation (SD) of three determinations. For biological assay, statistical comparison was carried out by one-way analysis of variance and Dunnett's test. The data represent mean ± standard error of mean (SEM), and values of P < 0.05 were considered statistically significant. This number of replicates allowed to generate a mean and SEM for each experiment. Each experiment was assessed in triplicate.

Some of biological data were also expressed as mean ± standard error of 10 determinations. The results were statistically analyzed by Student's t-test; P < 0.05 versus control was taken as statistically significant.

**RESULTS**

**Polyphenol determination**

**Folin–Ciocalteu method**

The total phenolic content of OFI cladodes methanol extract was 3.2 mg/g (SD ± 1.3). The results were expressed as GAE.

**High-performance liquid chromatography analysis**

Catechin, chlorogenic, ferulic and p-coumaric acid, quercetin, kaempferol, and isorhamnetin were identified [Figure 1]. The results are summarized in Table 2.

**Antioxidant activity**

OFI cladodes contain polyphenol compounds and Vitamin E complex that are effective radical scavengers. Actually, they inhibited DPPH formation by 50% at a concentration of 56.7 µg/mL (SD ± 1.11).

**Antispasmodic effect**

**Effects on isolated rabbit jejunum**

OFI cladodes methanol extract caused a light inhibition of amplitude and frequency of spontaneous contractions and a marked decrease in muscle basal tone of rabbit jejunum preparations. The inhibitory effect was not concentration-dependent, for doses ranging between 25–250 µg/mL [Table 4]. These inhibitory effects on spontaneous motility are also manifested in the presence of atropine, in conditions in which the motility is essentially myogenic.

**Effects on isolated rabbit uterus**

On spontaneously contracting uterus preparations, the addition of increasing concentrations of cladode extract (25–250 µg/mL) caused uterine muscle relaxation. Reduced amplitude and frequency of contractions and a reduction of muscle tone characterize the releasing effect. The strongest effect occurred at the concentration of 250 µg/mL.

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**Table 2: Polyphenols content in OFI cladodes (mean of three replications)**

| Phenols           | mg/kg±S.D |
|-------------------|-----------|
| Catechin          | 180.0±4.1 |
| Chlorogenic acid  | 25.24±2.7 |
| Ferulic acid      | 97.62±3.3 |
| p-coumaric acid   | 20.91±1.8 |
| Quercetin         | 75.13±2.8 |
| Kaempferol        | 72.97±2.0 |
| Isorhamnetin      | 917.80±4.8 |

**Table 3: Tocopherols content in OFI cladodes (mean of three replications)**

| Tocopherols | mg/kg±S.D |
|-------------|-----------|
| α-tocopherol| 98.4±3.5  |
| β-tocopherol| 18.3±1.7  |
| γ-tocopherol| 2.3±0.9   |
In quiescent uterine preparations, the extract of cladodes (250 mg/mL), when added before the reference agonist drugs (adrenaline 1.0 µg/mL, acetylcholine 1.0 µg/mL, and histamine 1.0 µg/mL), partially antagonizes their maximal contractile response [Figure 3], suggesting a nonspecific effect.

**DISCUSSION AND CONCLUSION**

From the results, it is evident that OFI cladodes contain significant amount of polyphenols and Vitamin E complex (tocopherols). The content of these substances is related at the stadium in plant development, as well to the pedoclimatic conditions in which such wild plants develop. The polyphenol and tocopherol content of the cladodes fully justify both the antioxidant activity and the biological effects reported in literature. Certainly, tocopherols provide to the biological effect of the cladodes. Even OFI cladodes, as well as the fruits, could be a good source of natural antioxidant.

The jejunum of rabbit was used to study the spontaneous basal contractions because their amplitude was easier to evaluate than the rat jejunum.[12]

Our results demonstrate that cladode extract shows antispasmodic activity. This effect could depend on the presence of catechin, a natural compound that relaxes both spontaneous and high K+ (80 mM)-induced contraction in rabbit jejunum. The contraction of smooth muscle preparations depends on an increase in cytoplasmic free calcium ion concentration, which activates the contractile elements. Catechin, by acting as calcium antagonists,[13] may suppress the contractility of smooth myocytes.

Moreover, cladodes contain some flavonoids that can be responsible for the antispasmodic effect. Literature data report that quercetin may have effects on intestinal motility both in vivo and in vitro[14,15] and kaempferol inhibits guinea pig ileum-induced contraction.[16] Furthermore, isorhamnetin, the predominant flavonoid in fruit, is present in significant amount in cladode. Seddik Ameur et al.[17] report that an isorhamnetin glycoside possesses antispasmodic activity.

Other authors suggest that the antispasmodic effect displayed by flavonoids is related to interference with calcium influx and/or calcium release from intracellular stores. They found that pretreatment with a calcium blocker, as verapamil, increased the inhibitory actions of flavonoids.[18,19] The cladode extract shows a relaxant effect on rabbit uterus and, in our experimental conditions, partially reversed the maximal contractile response of uterine tissue induced by acetylcholine, histamine, and adrenaline.

Smooth muscles contraction, in response to agonists, is initiated by the increase of the cytoplasmic free Ca2+ concentration.[20] These agonists, by activating phospholipase C, produce inositol triphosphate which stimulates sarcoplasmic reticulum to release the internal Ca2+ stores.[21] This facilitates the inflow of extracellular calcium through the receptor-operated calcium channel.[22] Other mechanisms may still be involved in the described effects.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

**REFERENCES**

1. Lahansi S, Kouhila M, Mahrouz M, Fliou M. Moisture adsorption-desorption isotherms of prickly pear cladode (*Opuntia ficus-indica*) at different temperatures. Energy Convers Manag 2003;44:923-36.
2. Trovato A, Mondello MR, Monforte MT, Rossitto A, Galati EM. Biological activities of *Opuntia ficus indica* (L.) Mill. (Cactaceae) cladodes. Acta Horticult 2002;581:291-4.
3. Galati EM, Mondello MR, Monforte M, Galluzzo M, Miceli N, Tripodo MM. Effect of *Opuntia ficus indica* (L.) Mill. cladodes in the wound-healing process. J Prof Assoc Cactus Dev 2003;5:1-16.
4. Halmi S, Benlakssira B, Bechtarzi K, Djemour Z, Deabalah H, Riachi F, et al. Antihyperglycemic activity of prickly pear (*Opuntia ficus-indica*) aqueous extract. Int J Med Aromat Plants 2012;2:540-3.
5. Galati EM, Monforte MT, Miceli N, Mondello MR, Taviano MF, Galluzzo M, et al. *Opuntia ficus indica* (L.) Mill. mucilages show cytoprotective effect on gastric mucosa in rat. Phytother Res 2007;21:344-6.
6. Galati EM, Tripodo MM, Trovato A, d'Agostino A, Monforte MT. Biological activity of *Opuntia ficus-indica* cladodes II: Effect on experimental hypercholesterolemia in rats. Pharm Biol 2003;41:175-9.
7. Feuagang JM, Konarski P, Zou D, Stintzing FC, Zou C. Nutritional and medicinal use of Cactus pear (*Opuntia spp.*) cladodes and fruits. Front Biosci 2006;11:2574-89.
8. Gao X, Ohlander M, Jeppsson N, Bjork L, Trajkovski V. Changes in Antioxidant Effect and their Relationships to Phytonutrients in Fruits of Sea Buckthorn (*Hippophae rhamnoides*) during Maturation. J Agric Food Chem 2000;48:1485-90.
9. Currò P, Miceli G, Lanuzza F. Determinazione mediante HPLC di tocoferoli in campioni commerciali di latte. Riv Ital Sostanze Grasse 1989;66:527-30.
10. Ohnishi M, Morishita H, Iwahashi H, Shizu T, Yoshiki S, Kimura M, et al. Inhibitory effects of chlorogenic acid on in vivo and in vitro. Phytochemistry 1994;36:579-83.
11. Litchfield JT, Wilこon, F. A simplified method of evaluating dose-effect experiments. J Pharmacol Exp Ther 1949;96:99-113.
12. Aziz M, Tab N, Karim A, Mekhfi H, Bnouham M, Ziyyat A, et al. Relaxant effect of aqueous extract of *Citrus ladanifera* on rat intestinal smooth muscles. Fitoterapia 2006;77:425-8.
13. Ghayur MN, Khan H, Gilani AH. Antispasmodic, bronchodilator and vasodilator activities of (+)-catechin, a naturally occurring flavonoid. Arch Pharm Res 2007;30:970-5.
14. Di Carlo G, Mascolo N, Izzo AA, Capasso F. Effect of quercetin on gastrointestinal tract in rats and mice. Phytoter Res 1994;8:42-46.
15. Amira S, Rotondo A, Mula F. Relaxant effect of flavonoids on the mouse isolated stomach: structure-activity relationships. Eur J Pharmacol 2008;599:128-30.
16. Desire O, Rivère C, Razafindrazaka R, Gossens L, Moreau S, Guillon J, et al. Antispasmodic and antioxidant activities of fractions and bioactive constituent davidigin isolated from Mascarenhasia arborescens. J Ethnopharmacol 2010;130:320-8.
17. Seddik Anour A, Negab I, Zouzou F, Legseir B. Anti-inflammatory and antispasmodic activities of isorhamnetin glycosides isolated from Opuntia ficus-indica (L.) Mill. flowers. RJPBCS 2016;7:432-7.
18. Capasso A, Pinto A, Mascolo N, Autore G, Capasso F. Reduction of agonist-induced contractions of guinea-pig isolated ileum by flavonoids. Phytoter Res 1991;5:85-7.
19. Capasso A, Pinto A, Capasso F. 1991. Inhibitory effects of quercetin and other flavonoids on electrically-induced contraction of guinea-pig isolated ileum. J Ethnopharmacol 1991;34:279-81.
20. Karaki H, Weiss G. Mini-review: calcium release in smooth muscles. Life Sci 1988;42:111-22.
21. Hashimoto R, Hirata M, Ito Y. A role of inositol 1,4,5-triphosphate in the initiation of agonist-induced contractions of dog tracheal muscle. Br J Pharmacol 1985;86:191-9.
22. Zhang WW, Li Y, Wang XQ, Tian F, Cao H, Wang MW, et al. Effects of magnolol and honokiol derived from traditional chinese herbal remedies on gastrointestinal movement. World J Gastroenterol 2005;11:4414-18.