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Effect of *Eupatorium arnottianum* on Gastrointestinal Tract

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Authors' contributions

This work was carried out in collaboration between all authors. Author SG designed the study, performed the statistical analysis, wrote the protocol, managed the analyses of the study and wrote the first draft of the manuscript and final version of the manuscript. Author PC managed the analyses of the study. Authors VM and MC managed the literature searches and pharmacognostic and phytochemical research. All authors read and approved the final manuscript.

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ABSTRACT

Aims: *Eupatorium arnottianum* Griseb is used in popular medicine for gastric pains. So, the aim of this study was to investigate the possible spasmolytic effects of ethanolic and dichloromethanic extracts of the plant using preclinical methods.
Place and Duration of Study: Department of Pharmacology, School of Pharmacy and Biochemistry, Universidad de Buenos Aires, from September 2015 to May 2016.
Methodology: The effects of *Eupatorium arnottianum* extracts and its isolated compounds were evaluated on isolated rat jejunum. Intestinal transit of charcoal meal after the administration of extract was determined and compared with the control group. Also, phytochemical study was performed.
Results: The extracts inhibited non competitively the cumulative concentration–response curves induced by acetylcholine (1.10^{-9}–1.10^{-5} M), with similar inhibition at the highest concentration (2 mg/mL: 66.2±8.7% of inhibition for ethanolic extract and 61.5±10.5% of inhibition for

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dichlorometanic extract). Also, both extracts non-competitively inhibited the curve response concentration induced by $CaCl_2$ (1.10^{-4} -3. 10^{-1} M) and significantly reduced the maximal response in a concentration-dependent manner. Furthermore, *E. arnottianum* (62, 125 and 250 mg/Kg, per oral) significantly decreased the propulsion of the charcoal meal through the gastrointestinal tract (68% of inhibition for 250 mg/Kg ethanolic extract, 42% of inhibition for 125 mg/Kg dichlorometanic extract). Finally, nepetin, the major compound of the ethanolic extract and two major compounds of dichlorometanic extract, jaceosidin and nepetin, reduced the maximal response of the acethylcholine in isolated jejunum (30 µg/mL: 75% of inhibition for jaceosidin and 89% of inhibition for nepetin).

Conclusion: The results of the present study could demonstrate that *Eupatorium arnottianum* plays a spasmolytic role in gastrointestinal motility by interfering in calcium influx. Also nepetin and jaceosidin, major components of ethanolic and dichlorometanic extracts could be responsible for these properties.

Keywords: Eupatorium arnottianum; isolated jejunum; nepetin; jaceosidin; gastrointestinal transit.

1. INTRODUCTION

Functional gastrointestinal disorders affect 15–20% of the population and are related to chronic or recurrent diseases that are diagnosed on the basis of specific symptomatic criteria. Different therapies are prescribed based on the predominant symptom (s) and are often accompanied by dietary and lifestyle recommendations. However, all these symptomatic therapies have variable efficacy and often are associated with side-effects [1]. The renewed interest in the search for new drugs from natural sources has gained global attention during the last two decades. Due to rich biodiversity, with high diversity of chemicals and promising effects, plants and herbs can be used as source of drugs. Nevertheless, a small portion has been studied for its medicinal potential. Taking into account that current management of gastrointestinal motility disorders continues to prove difficult, new strategies may help to control these diseases.

Genus *Eupatorium* belongs to the Eupatorieae and it is used for different diseases. This genus comprises of nearly 1200 species distributed in tropical regions of America, Europe, Africa and Asia [2].

Particularly in Argentina, there are fifteen medicinal *Eupatorium* species described as native. They have been used by indian and rural populations as antiseptic, febrifugue, antidiarrheal, for the treatment of different kind of pains and inflammation, headaches and to cure sores and pimples [3-5].

Eupatorium arnottianum Griseb. (“clavel”, “tuji”) is an herb that grows in the North-East and Centre of Argentina and South of Bolivia. It is used by rural populations of Santa Victoria e Iruya (Salta, Argentina) for gastric pains [6] and by “kallawaya” healers from the bolivian altiplano against asthma, bronchitis, colds and topically in plasters for bone fractures and dislocations [7].

Despite the folkloric use and the chemical composition, no scientific evaluation of this plant on gastrointestinal tract has been carried out to date. In the present work, the effects of *E. arnottianum* and two isolated compounds were evaluated.

2. MATERIALS AND METHODS

2.1 Plant Material

Aerial parts of *E. arnottianum* were collected in the sourroundings of Paraná, Entre Ríos province (Argentina), in February 2014. Specimen was identified by Ing. Juan de D. Muñoz and kept at the Herbarium of the Facultad de Ciencias Agropecuarias de Entre Ríos, leg. J. de D. Muñoz 3535.

2.1.1 Preparation of plant extracts

The air-dried and powdered aerial parts of *Eupatorium arnottianum* (700 g) were succesively extracted by maceration with dichloromethane and 50% aqueous ethanol. Extracts were filtered and concentrated in vacuum to obtain dry dichloromethanic (DCM) and 50% ethanolic extracts (EtOH). The extract was filtered and freeze-dried (yield 5.3% w/w of dried plant material for DCM and 16.2% for EtOH, respectively).

2.1.2 Fractionation of extract

The dichloromethanic extract was adsorbed on CeliteR and eluted successively with hexane, toluene, diethylether and acetone. Three main fractions F1–F3 were obtained, and were dried under vacuum. The respective yields were: for F1 49.55; F2: 16.67; F3: 1.88/100 g crude extract. F1-F3 was fractionated as described by Clavin et al. [8]. Nepetin and jaceosidin was isolated from F2 and F3.

The EtOH extract was suspended in hot distilled water and extracted in a liquid-liquid semicontinuous extractor successively with diethyl ether (FD) and ethylacetate (FE). FD was purified in a CC column of Sephadex/gradients of diclhoromethane-ethyl acethate-methanol. From one of the fractions, a compound was isolated. Other compounds were also isolated and identified from these fractions.

2.2 Drugs

Acetylcholine, atropine and quercetine were purchased from Sigma (St. Louis, MO, USA). All chemicals used to prepare Tyrode's solution were analytical grade and solubilized in distilled water. Nepetin and Jaceosidin was isolated from *E. arnottianum*. The flavonoid solutions were prepared in dimethylsulfoxide (DMSO). The final concentration of DMSO was less than 0.05% which has been proven not to induce any observable effects on muscle tone. All other drugs and solutions were prepared and used immediately.

2.3 Pharmacological Studies

2.3.1 Animals

Female Sprague Dawley rats (210±10 g) and Swiss mice (20-23 g) were used. The animals were housed in standard environmental conditions (22±1°C and a 12 h light/dark cycle), with free access to a standard commercial diet and water ad libitum. All the efforts were made to minimize animal suffering.

2.3.2 *In vitro* assay

Jejunums were prepared as previously described [9]. Rats were starved for 24 h before the experiment with free access to water. The animals were killed by decapitation without anaesthetic agent in order to avoid any influence on the relaxation responses of the tissue. Jejunums of approximately 1.00 cm in length were prepared and mounted in 10 mL organ baths containing Tyrode solution of the following composition (mM): NaCl 135, KCl 5.0, $MgCl_2$ 1.0, $NaHCO_3$ 15.0, NaH_2PO4 1.0, $CaCl_2$ 2.0, glucose 11.0. The bath solution was maintained at 36±1°C and constantly oxygenated with 95% O_2 + 5% CO_2. The preparations were allowed to equilibrate for at least 30 min under 1 g resting tension. Tissues were connected to a force displacement transducer for the measurement of isometric force. Concentration-response curves for acetylcholine (Ach) were obtained for the tissues. The Ach curves were then re-determined in the presence of different concentrations (0.5, 1.0, and 2.0 mg/mL) of *E. arnottianum* oratropine (10^{-5} M) used as positive control, which were added 20 min before. Also nepetin, jaceosidin (15 and 30 µg/mL) quercetine (15 µg/mL) were tested.

In other group of experiments and after an initial incubation, Tyrode solution was replaced by calcium-free hyperpotassic medium (K^+ 75 mM). Concentration-response curves were obtained by cumulative addition of $CaCl_2$ (10^{-4} – $3x10^{-2}$ M) in the absence and presence of different concentrations (0.5, 1.0, and 2.0 mg/mL) of *E. arnottianum* and verapamil (10^{-6} M).

2.3.3 *In vivo* assay

Gastrointestinal transit test was performed in mice [10]. The first group was referred to as the control and orally received the vehicle. The other groups received oral administration of *E. arnottianum* ethanolic and dichlorometanic extract at doses of 62, 125 and 250 mg/Kg, respectively. One group received the reference drug atropine by intraperitoneal route (0.1 mg/Kg). Half an hour after the treatment, individual animals were administered 1 mL of charcoal (3% charcoal in water). Thirty min later, the animals were sacrificed and the intestinal distance, moved by charcoal from pylorus to cecum, was measured.

2.4 Statistical Analysis

The pharmacological results were expressed as mean±SEM. Concentrations–response curves were plotted and experimental data were adjusted by a non-linear curve fitting program. Statistical analysis was performed by one-way analysis of variance (ANOVA), followed by Bonferroni or Dunnet´ tests. $P < .05$ and $P < .01$ were considered to be statistically significant (GraphPad Prism 5, version 5.03 for windows).

3. RESULTS AND DISCUSSION

3.1 Phytochemical Study

Purity of precipitated compound was tested by HPLC-DAD and identified by spectroscopic (UV, MS, HRMN) and chromatographic data, compared with literature. The compound identified in ethanolic extract was determined as 5,7,3',4'-tetrahydroxy-6,-methoxyflavone (nepetin) [11]. Nepetin showed to be the major compound of the EtOH, representing 0.3% w/w in the extract. Two major compounds, jaceosidin and nepetinwas isolated from DCM [8].

3.2 Pharmacological Studies

Gastrointestinal motility is regulated by a lot of substances such as nitric oxide, autacoids, serotonin, prostaglandis, gastrin. Nevertheless, acetylcholine (Ach) is considered as one of important neurotransmitter that regulates gut activity. It is a neurotransmitter released by the parasympathetic nervous system and plays an important physiological role in regulating the peristaltism of the gastrointestinal tract. External ACh stimulates the muscarinic receptors on the smooth muscle cells, eliciting contraction [12]. *E. arnottianum* antagonised the jejunum contractions induced by Ach (1.10^{-9}–1.10^{-5}M), with similar inhibition at the highest concentration (2 mg/mL: 66.2±8.7% of inhibition for EtOH extract and 61.5±10.5% of inhibition for DCM) (Fig.1 A and B). The maximal response of the agonist was reduced by both extracts in a concentration dependent manner, suggesting that they posses a spasmolytic effect. Atropine, a classical muscarinic receptor antagonist, was used as reference drug. It induced a profound inhibitory effect on the response of Ach at concentration of 1.10^{-5} M.

It is known that the muscarinic receptor M_3 elicits a direct contractile response through calcium mobilization, whereas the M_2 receptor is capable of mediating effects that potentiate the contractile response of the M_3 receptor or other Ca^{2+} mobilizing receptors [12]. The M_3 receptor, via Gq, stimulate phospholipase C, release intracellular Ca^{2+} stores, resulting in a rise in cytosolic Ca^{2+} concentration. Also the contraction induced by M_3 depends largely on Ca^{2+} entry via Ca^{2+} channels opened by membrane depolarization [13]. Taking into account this information, it could be possible that *E. arnottianum* acts on calcium channel. So, in order to investigate this hypothesis, the effect of extracts on the muscle contractions induced by $CaCl_2$ (1.10^{-4} -3.10^{-1} M) was analysed. Both extracts non-competitively inhibited the curve response concentration induced by $CaCl_2$ and significantly reduced the maximal response in a concentration-dependent manner (Fig. 2A and B). Verapamil (1.10^{-6} M), a calcium channel blocker, was used as positive control. Therefore, the observation based on the performed study could indicate that *E. arnottianum* posses a spasmolytic effect and behaves as calcium-antagonist, reducing the utilization of external calcium.

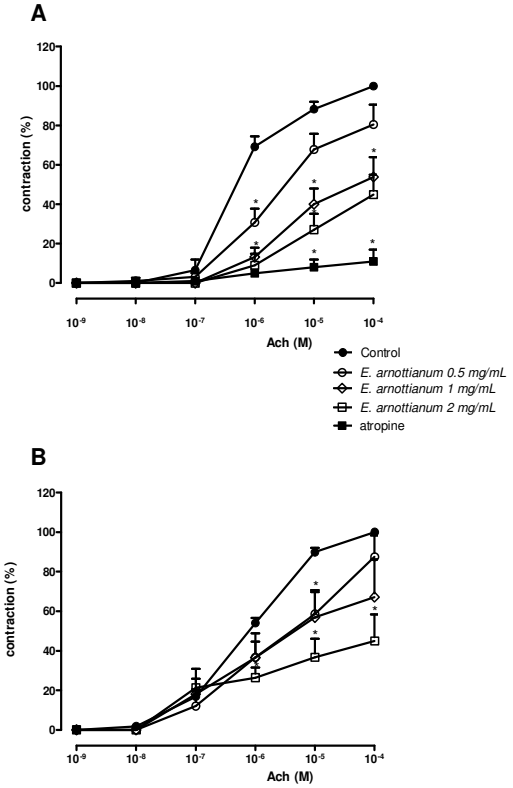

Fig. 1. Effect of different concentrations *Eupatorium arnottianum* ethanolic extract (A) and dichloromethanic extract (B) on cumulative log concentration–response curves for acethyl choline (Ach). Symbols represent mean±SEM, n=5 experiments

**P=.05 (E. arnottianum vs. control group), One way ANOVA, followed by Bonferroni test*

Gastrointestinal motility disorders are defined as ailments associated with inadequate, incoordinated, or excessive gastrointestinal muscular activity, resulting in abnormal propulsion of gastric or intestinal content. Normal

peristalsis along the gastrointestinal tract results from serie of control mechanisms involving extrinsic parasympathetic and sympathetic pathways, intrinsic nervous system and electrical and contractile properties of smooth muscle cells. Since, physiological responses from ion fluxes on gut smooth muscle organize contractions to propel content in the stomach and colon [14], the *in vivo* evaluation of the extract try to support antispasmodic action observed. Interestingly, *E. arnottianum* (62, 125 and 250 mg/Kg, per oral) significantly decreased the propulsion of the charcoal meal through the gastrointestinal tract as compared with the control group (Fig. 3), so strongly supported the *in vitro* spasmolytic effect observed with EtOH and DCM extracts. Also, the effect induced by atropine (0.1 mg/Kg), reference group, is shown in the same figure.

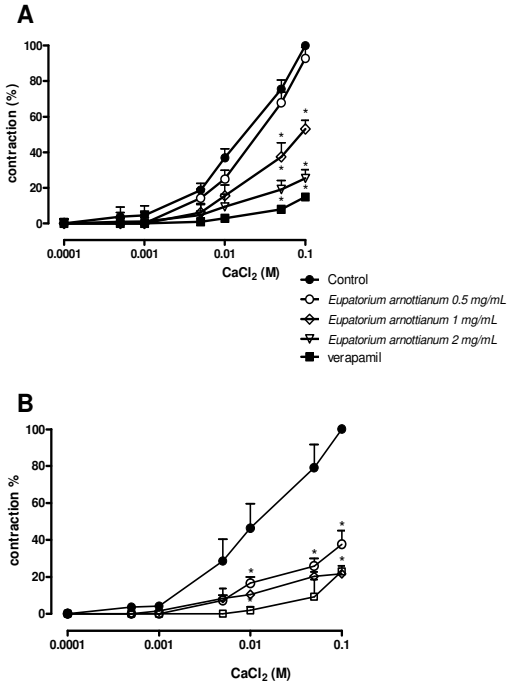

Fig. 2. Effect of different concentrations *Eupatorium arnottianum*ethanolic extract (A) and dichloromethanic extract (B) on cumulative log concentration–response curves for calcium chloride. Symbols represent mean±SEM, n=5 experiments.
**P=.05 (E. arnottianum vs. control group), One way ANOVA, followed by Bonferroni test*

Aiming to identify substances that could be responsible for the activity showed in this study, two compounds identified from *E. arnottianum*, nepetin and jaceosidin were studied. Both compounds reduced the maximal response of the Ach at 15 and 30 µg/mL. Quercetin was used as reference drug in the system (15 µg/mL). Previous studies showed that nepetin exerts anti-inflammatory effects in *in vitro* and *in vivo* systems, inhibiting cyclooxigenase-2 and reducing reactive oxygen species [8,15]. Also, jaceosidin stimulates angiogenesis by activating the VEGFR2/FAK/PI3K/AKT/NF-kB signaling pathway [16] and exerts topical anti-inflammatory effects [17]. But, this is the first time that jacesosidin and nepetin showed to possess antispasmodic activity *in vitro*.

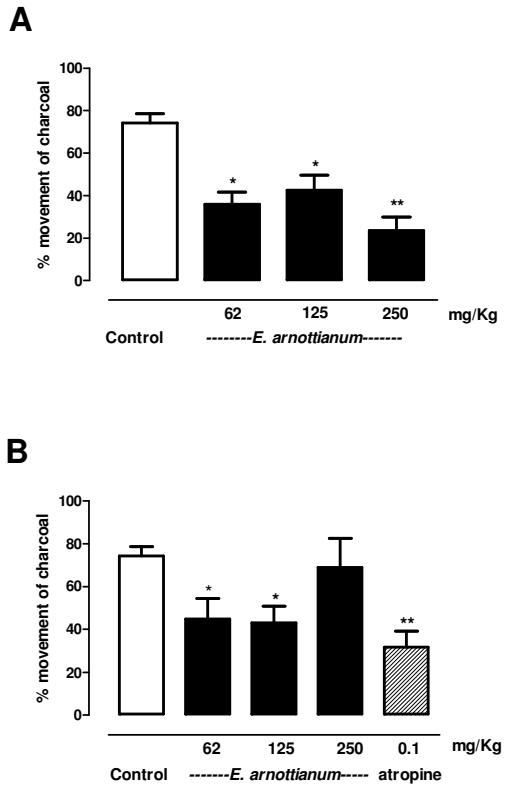

Fig. 3. Effect of different concentrations *Eupatorium arnottianum*ethanolic extract (A) and dichloromethanic extract (B) on gastrointestinal transit test. Each bar represents mean±SEM, n=5 experiments
**P=.05, **P=.01 (E. arnottianum vs. control group), One way ANOVA, followed by Dunnet test*

Direct smooth muscle relaxants, anticholinergic agents or calcium channel blockers have been used as antispasmodic drugs for decades in therapy for different gastrointestinal ailments such as inflammatory bowel disesase [18]. Considering the activity observed in this study

and that *E. arnottianum* has shown to posses anti-inflammatory and antinociceptive actions previously [8,19], this plant or its isolated compounds could emerge as alternative for treatment of gastrointestinal inflammatory disorders.

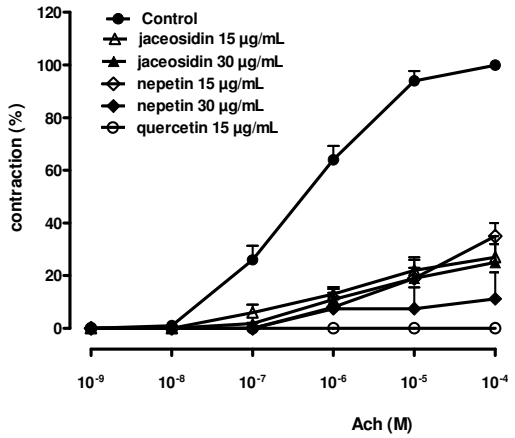

Fig. 4. Effect of jaceosidin (15-30 µg/mL), nepetin (15-30 µg/mL) and quercetin (15 µg/mL) on cumulative log concentration–response curves for Ach. Symbols represent Mean±SEM, n=5 experiments

**P=.05 (phytochemical vs. control group), One way ANOVA, followed by Bonferroni test*

4. CONCLUSION

The results of the present study could demonstrate that *Eupatorium arnottianum* plays a spasmolytic role in gastrointestinal motility by interfering in calcium influx. Also nepetin and jaceosidin, major components of ethanolic and dichlorometanic extracts could be responsible for these properties.

In addition, the effects showed in the present study could support the ethnomedical use of this plant and contributes to the scientific knowledge of the properties of our medicinal flora.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the Ethical Committee for the Care and Use of Laboratory Animals of School of Pharmacy and Biochemistry, Universidad de Buenos Aires (Ethics approval: Exp-FFyB 738657/11, Res 3438).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Chovet M. Gastrointestinal functional bowel disorders: New therapies. Curr Opin Chem Biol. 2000;4(4):428-32.
2. Zhang M, Wu M, Zhang J, Irwin D, Gu Y, Shi Q. Chemical constituents of plants from the genus eupatorium. Chem Biodivers. 2008;5(1):40-55.
3. Toursarkissian M. Plantas medicinales Argentinas. Buenos Aires: Hemisferio Sur; 1980. (Spanish).
4. Martínez Crovetto R. Las plantas utilizadas en medicina popular del no de corrientes. Tucumán: Fundación Miguel Lillo; 1981. (Spanish).
5. Zardini E. Etnobotánica de compuestas Argentinas con especial referencia a su uso farmacológico. Acta Farm Bonaerense. 1984;3:77-99. (Spanish).
6. Iharlegui L, Hurrell J. Asteraceae de interés etnobotánico de los departamentos de Santa Victoria e Iruya. Ecognicion. 1992;3:3-18. (Spanish).
7. Giraoult L. Kallawaya. Curanderos itinerantes de los Andes: Investigación sobre prácticas medicinales y mágicas. La Paz: UNICEF; 1987. (Spanish).
8. Clavin M, Gorzalczany S, Macho A, Muñoz E, Ferraro G, Acevedo C, et al. Anti-inflammatory activity of flavonoids from *Eupatorium arnottianum*. J Ethnopharmacol. 2007;112(3):585–9.
9. Gorzalczany S, Moscatelli V, Ferraro G. Artemisia copa aqueous extract as vasorelaxant and hypotensive agent. J Ethnopharmacol. 2013;148:56–61.
10. Sadraeia H, Asghari G, Shams M. Antidiarrheal action of hydroalcoholic extract of *Pycnocycla spinosa* in comparison with loperamide and

dicyclomine. Iran J Pharm Res. 2011;10(4):835-41.
11. Lee S, Chung H, Maier C, Wood A, Dixon R, Mabry T. Estrogenic flavonoids from *Artemisia vulgaris* L. J Agr Food Chem. 1998;46:3325-29.
12. Ehlert F. Contractile role of M2 and M3 muscarinic receptors in gastrointestinal, airway and urinary bladder smooth muscle. Life Sci. 2003;74:355–66.
13. Unno T, Kwon S, Okamoto H, Irie Y, Kato Y, Matsuyama H, et al. Receptor signaling mechanisms underlying muscarinic agonist evoked contraction in guinea-pig ileal longitudinal smooth muscle. Br J Pharmacol. 2003;139:337-50.
14. Valentin N, Acosta A, Camilleri M. Early investigational therapeutics for gastrointestinal motility disorders: From animal studies to phase II trials. Expert Opin Investig Drugs. 2015;24(6):769-79.
15. Lee C, Lin A, Hsu L, Fang J, Chiang Y, Tsai M, et al. Eupafolin ameliorates COX-2 e expression and PGE2 production in particulate pollutants- exposed human keratinocytes through ROS/MAPKs pathways. J Ethnopharmacol. 2016;189: 300-9.
16. Lee T, Jung H, Park K, Bang M, Baek N, Kim J. Jaceosidin. A natural flavone, promotes angiogenesis via activation of VEGFR2/FAK/PI3K/AKT/NF-iB signaling pathways in endothelial cells. ExpBiol Med. 2014;239:1325-34.
17. Youngpyo N, Mijung C, Heehong H, Maan-Gee L, Byoung-Mog K, Won-Ha L, et al. Natural flavone jaceosidin is a neuroinflammation inhibitor. Phytother Res. 2013;27(3):404-11.
18. Annaházi A, Róka R, Rosztóczy A, Wittmann T. Role of antispasmodics in the treatment of irritable bowel syndrome. World J Gastroenterol. 2014;20(20):6031-43.
19. Clavin M, Gorzalczany S, Miño J, Karadian C, Martino V, Ferraro G, et al. Antinociceptive effect of some Argentine medicinal species of Eupatorium. Phytother Res. 2000;14(4):275-7.

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