European Journal of Medicinal Plants

23(2): 1-11, 2018; Article no.EJMP.41075
ISSN: 2231-0894, NLM ID: 101583475

Antispasmodic and Myorelaxant Activity of Organic Fractions from *Origanum majorana* L. on Intestinal Smooth Muscle of Rodents

Hanane Makrane[1], Mohammed Aziz[1*], Hassane Mekhfi[1], Abderrahim Ziyyat[1], Mohamed Bnouham[1], Abdelkhaleq Legssyer[1], Bernard Gressier[2] and Bruno Eto[2,3]

[1]Laboratory of Physiology, Genetic and Ethnopharmacology, Faculty of Sciences, Mohamed the First University, P.B. 717, 60000, Oujda, Morocco.
[2]Laboratory of Pharmacology, Pharmacokinetics and Clinical Pharmacy, Faculty of Pharmaceutical and Biological Sciences, Lille, France.
[3]Laboratory TransCell-Lab, Faculty of Medicine, Xavier Bichat, University Diderot-Paris7, Paris, France.

Authors' contributions

This work was carried out in collaboration between all authors. Authors H. Makrane and MA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors H. Mekhfi, AZ, MB, and AL managed the analyses of the study. Authors BE and BG managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2018/41075
Editor(s):
(1) Paolo Zucca, Department of Biomedical Sciences, University of Cagliari, Italy.
(2) Marcello Iriti, Professor, Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) Daniela Benedec, Iuliu Hatieganu University of Medicine and Pharmacy, Romania.
(2) Bencheikh Rachid, USMBA, Morocco.
(3) Qaiser Jabeen, The Islamia University of Bahawalpur, Pakistan.
Complete Peer review History: http://www.sciencedomain.org/review-history/24388

Original Research Article

Received 15th February 2018
Accepted 24th April 2018
Published 30th April 2018

ABSTRACT

Aims: *Origanum majorana* (*Lamiaceae*) is a herbaceous and perennial plant that is used in the Moroccan traditional medicine for treating gastrointestinal disorders. The objectives of this study were to confirm the antispasmodic and the myorelaxant activity of organic fractions of *Origanum majorana* (OM) in rat and rabbit jejunum.
Place and Duration of Study: Laboratory of Physiology, Genetic and Ethnopharmacology, Faculty

**Corresponding author: E-mail: azizmo5@yahoo.fr;*

of Sciences, Mohamed the First University, between September 2013 and July 2014.
Methodology: The antispasmodic and the myorelaxant test evaluated *in vitro* on rat and rabbit intestines mounted inside an isolated organ system with a temperature of 37ºC, pH 7.4 and continuous oxygenation
Results: The screening study showed those organic fractions of OM decreased the tone of contraction induced by the Carbachol 10^{-6} M and the KCl 25 mM in the jejunum. The maximum decrease was obtained by dichloromethane fraction of *Origanum majorana* (DFOM). DFOM induced dose-dependent and reversible inhibition in intestine contraction of rabbit jejunum with IC_{50} = 0.162 ± 0.002 mg/ml without any alteration of this effect in the presence of adrenergic inhibitors. Pretreatment of the tissue with this fraction (0.01-0.3 mg/ml) induced a dose-dependent shift of the dose-response curve of Carbachol and $CaCl_2$ to the right. The pharmacological inhibitors such as Atropine, L-NAME, Hexamethonium, Nifedipine and Methylene blue did not alter the relaxing effect of DFOM.
Conclusion: The results study confirms the antispasmodic and the myorelaxant effect of OM extract. Also, the results showed that adrenergic receptors, NO, guanylate cyclase or muscarinic receptors pathways did not involve in relaxation induced by DFOM suggesting that it exerts an antispasmodic effect on intestinal smooth muscle like a non-competitive antagonist towards the voltage-dependent calcium channels.

Keywords: Antispasmodic; myorelaxant; Origanum majorana; organic fractions; rabbit; rat; jejunum.

1. INTRODUCTION

Gastrointestinal problems are among the most frequently encountered diseases in humans [1]. The intestinal functional disorders that are part of these problems correspond to chronic digestive symptoms that point to a dysfunction of the lower part of the digestive tract, particularly the small intestine and the colon, and that cannot be explained by any organic abnormality [2]. They are characterised by persistent or recurrent incomprehensible pain in the abdomen [3]. These syndromes are frequently observed worldwide and can affect between 15 and 20 % of the population [4]. In Morocco, medicinal plants occupy an important role in traditional medicine, which is widely used in various fields of health [5]. These plants are believed to be an important source of new chemical substances with potential therapeutic effects [6,7]. Several medicinal plants are used for the treatment of gastrointestinal diseases such as intestinal cramps, diarrhoea and transit disorders. Among these plants, we found *Origanum majorana* (OM) belonging to the family of *Lamiaceae*. It is a herbaceous plant, naturally perennial, stem erect, reddish, covered with black hairs. The leaves are opposite, greyish-green, oval-shaped, with small white or mauve flowers, grouped in tight groups in the axils of the leaves with two spoon-shaped bracts [8]. This plant is locally known as “Merdedûch’’, present in the forest and mountainous regions of Morocco. It is widely used in the treatment of digestive tract diseases and given in colds, fevers, stomachaches and headaches [5]. Different studies have demonstrated its pharmacological properties such as: antimicrobial [9,10,11,12], apoptotic, anti-proliferative [13] anti-mutagenic [14], antioxidant [15,16,17], inhibition of blood platelet adhesion, aggregation, [18] antithrombin, antihyperglycemic [19] and antihyperlipidemic [20]. This plant contains tannins, flavonoids, hydroquinone phenolic terpenoids, phenolic glycosides [21,22], coumarin [23], isocoumarin [24,25], vitamin C, saponin, carbohydrates, proteins, amino acids [8] and more than 20 compounds of essential oil [26].

The present study aimed to evaluate the antispasmodic and the myorelaxant activity of organic fractions from *Origanum majorana* on rodents.

2. MATERIALS AND METHODS

2.1 Chemicals and Solutions

2.1.1 Chemicals

Carbamylcholine chloride (Carbachol, CCh), propranolol, yohimbine, prazosin, nifedipine purchased from Sigma chemical co, atropine from Fluka, hexamethonium from Across organics, L-NAME from Calbiochem, dimethyl sulfoxide (DMSO) and methylene blue from Sigma-Aldrich. All chemicals used were of the analytical grade available and solubilised in distilled water except nifedipine that has dissolved in 10% DMSO. The vehicle used for

solubilization of drugs did not affect tissue contractility in the control experiments.

2.1.2 Solutions

The solutions used in this study were: Normal Krebs-Henseleit buffer (KHB) solution (in mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2 and glucose 10. Calcium free high K^+ KHB ($[K^+]$ = 76.2 mM); NaCl 48, KCl 75, $CaCl_2$ 0, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4, 1.2 and glucose 10. Calcium-free KHB (in mM); NaCl 121.7, KCl 4.7, $CaCl_2$ 0, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2 and glucose 10. All the components of these solutions were purchased from Sigma-Aldrich, made up in distilled water, and their pH was adjusted to 7.4.

2.2 Plant Material

The aerial parts of *Origanum majorana* were brought back from the Errachidia region. It was identified by Professor Fennane Mohammed, from Scientific Institute in Rabat, Morocco and registered under voucher number HUMPOM83 at the plant section of Herbarium Mohamed the First University Oujda Morocco.

2.3 Preparation of the Organic Fractions

Extraction has obtained the organic fractions from *Origanum majorana* (200 g) according to the method described by Cechinel-Filho and Yunes [27] using the Soxhlet system with solvents of increasing polarity. All fractions were filtered and dried using vacuum rotary evaporator. Yield fractions are shown in the following Table:

Table 1. Yield fractions from *Origanum majorana*

Fractions	Yields
Petroleum Ether	5,15 %
Dichloromethane	3,25 %
Ethyl Acetate	2,05 %
Methanol	20,60 %
Depleted Aqueous Extract	7,10 %

2.4 Animals

The experiments were performed on male and female Wistar rats (200-300 g) and New Zealand rabbits (1.5-2 kg). All animals were kept in an air-conditioned room, controlled lighting (12 h: 12 h light-darkness cycle) with free access to food and water, in the animal house of the department of biology, Mohamed the First University Oujda Morocco. Food was withdrawn 24 h before the experiment. All procedures concerning animals were carried out in an ethically proper way by following guidelines as set by the World Health Organization and conform to the European Community guiding principles in the care and use of animals (86/609/CEE, CE off J No.L358, 18 December 1986).

2.5 Antispasmodic and Myorelaxant Effect

Animals were sacrificed, and segments of the jejunum (2 cm) were removed and stored during the tests in the normal Krebs-Henseleit buffer (KHB) oxygenated saline. Each piece was mounted in a jejunum isolated organ bath vessel (10 ml). The fragment was subjected to a tension of 1 g and was connected to an isotonic transducer (B. Brawn Messenger AG Type 362722 # 203). Spontaneous contractions were recorded on a data logger (B. Brawn Messenger AG 861 062 Type, No. 1696). The preparation was incubated in the standard physiological KHB solution maintained at a temperature of 37 °C and pH 7.4 with continuous oxygenation for 30 min to have the same physiological conditions of the animal. The physiological fluid is changed every 15 min to balance the jejunum before adding plant extracts or other drugs. For all experiments, the effects of each dose are recorded for at least 7-8 min [28].

A secondary concentration responses curves were obtained by addition of increasing levels of the extract. The antispasmodic effect of these fractions against Carbachol (CCh) at 10^{-6} M and KCl at 25 mM has been studied on the rat jejunum. The most active fraction, dichloromethane, was studied in more detail. The myorelaxant effect of dichloromethane fraction of *Origanum majorana* (DFOM) (0.01-0.3 mg/ml) was tested on the spontaneous contractions of rabbit jejunum and in the presence of adrenergic inhibitors (yohimbine, prazosin, and propranolol at 5×10^{-5} M). Dose-response curves of Carbachol (CCh) were done for the rat's jejunum. CCh doses (3.10^{-8} M to 3.10^{-5} M) were cumulatively added to the bath in the absence and in the presence of DFOM after 10 min of contact with 0.01, 0.1 and 0.3 mg/ml. For the $CaCl_2$ test, the jejunum was allowed to stabilize in normal KHB solution and was then replaced with a Calcium-free KHB and EDTA (0.1 mM) to

remove calcium from the tissues. This solution was replaced with Calcium free high K^+ KHB. Following an incubation period of 10 min and after the confirmation of no spontaneous contractions of jejunum, $CaCl_2$ was added cumulatively (0.3 to 10 mM), every 5 min, to obtain control concentration-response curves of $CaCl_2$. The concentration-response curves were repeated following 10 min incubation with the DFOM (0.01-0.3 mg/ml).

The pharmacological inhibitors; L-NAME (10^{-4} M), methylene blue (10^{-5} M), atropine (10^{-6} M) and hexamethonium (10^{-4} M) were incubated for 20 min, after that, the rat jejunum was pre-contracted by KCl 25 mM and challenged by DFOM (0,3 mg/ml). The same protocol was applied for the nifedipine (10^{-6} M), except the smooth muscle was pre-contracted by the CCh (10^{-6} M).

2.6 Statistical Analysis

Pharmacological responses are shown as mean values ± standard error of the mean (SEM) for separate experiments using tissues from 6 animals. Student's t-test performed the difference between extracts and controls (mean comparisons) and the determination of significance level. Differences are considered statistically significant when the probability value $P < 0.05$. The 50% inhibitory concentration (IC_{50}) was determined using linear regression.

3. RESULTS

The organic fractions from *Origanum majorana* have reduced the maximum tone induced by CCh 10^{-6} M (Fig.1) and KCl 25 mM (Fig.2) on the rat jejunum with the IC_{50} represented in Table 2.

According to these results, we note that the dichloromethane fraction gave the most significant effect compared to the other fractions, then for the study of the mechanism of action we focused on this fraction.

DFOM inhibited spontaneous contractions of rabbit jejunum by reversible and dose-dependent manner with an IC_{50} = 0,162 ± 0,002 mg/ml (Fig. 3). Decreases in amplitude were accompanied by a reduced tone of contractions. Even in the presence of adrenergic blockers, Propranolol (5.10^{-5} M), Prazosin (5.10^{-5} M) and Yohimbin (5.10^{-5} M), the DFOM still induced a complete inhibition of the contraction of smooth muscle fibres of the rabbit jejunum. This inhibition was comparable to that obtained with the fraction alone without inhibitors.

The effect of CCh and $CaCl_2$ dose-response in the presence and absence of DFOM was also studied on rat jejunum. The extract showed a significant inhibitory effect from 0.01 mg/ml on the maximum induced by CCh or $CaCl_2$ while shifting the dose-response curves of contraction to the right. At a dose 0.3 mg/ml, the fraction showed a total inhibition of the contraction of the jejunum (Fig. 4).

The DFOM has decreased the percentage of contraction of rat intestinal smooth muscle pre-incubated with L-NAME, methylene blue, atropine and hexamethonium by 97.4%, 96.49%, 95.45% and 116.6% respectively. The difference between the control and each test is hugely significant, but the difference between the relaxation induced by DFOM without incubation with one of these inhibitors and that obtained after incubation is statistically not significant (Fig. 5A).

The DFOM has also decreased the percentage of contraction of the rat jejunum preincubated with nifedipine and pre-contracted with CCh by 94.29%. The difference between the control and the test is hugely significant, but the difference between the relaxation induced by DFOM without incubation with nifedipine and that obtained after incubation is statistically no significant (Fig. 5B).

Table 2. Comparison of relaxation induced by the five organic fractions using their IC_{50}

Fractions	IC_{50} (mg/ml)	
	CCh 10^{-6} M	KCl 25 mM
Dichloromethane	0,107 ± 0,006	0,112 ± 0,006
Petroleum ether	0,149 ± 0,005	0,122 ± 0,007
Ethyl acetate	0,670 ± 0,091	0,833 ± 0,153
Methanol	1,679 ± 0,202	7,808 ± 3,743
Depleted aqueous extract	1,874 ± 0,117	17,343 ± 2,176

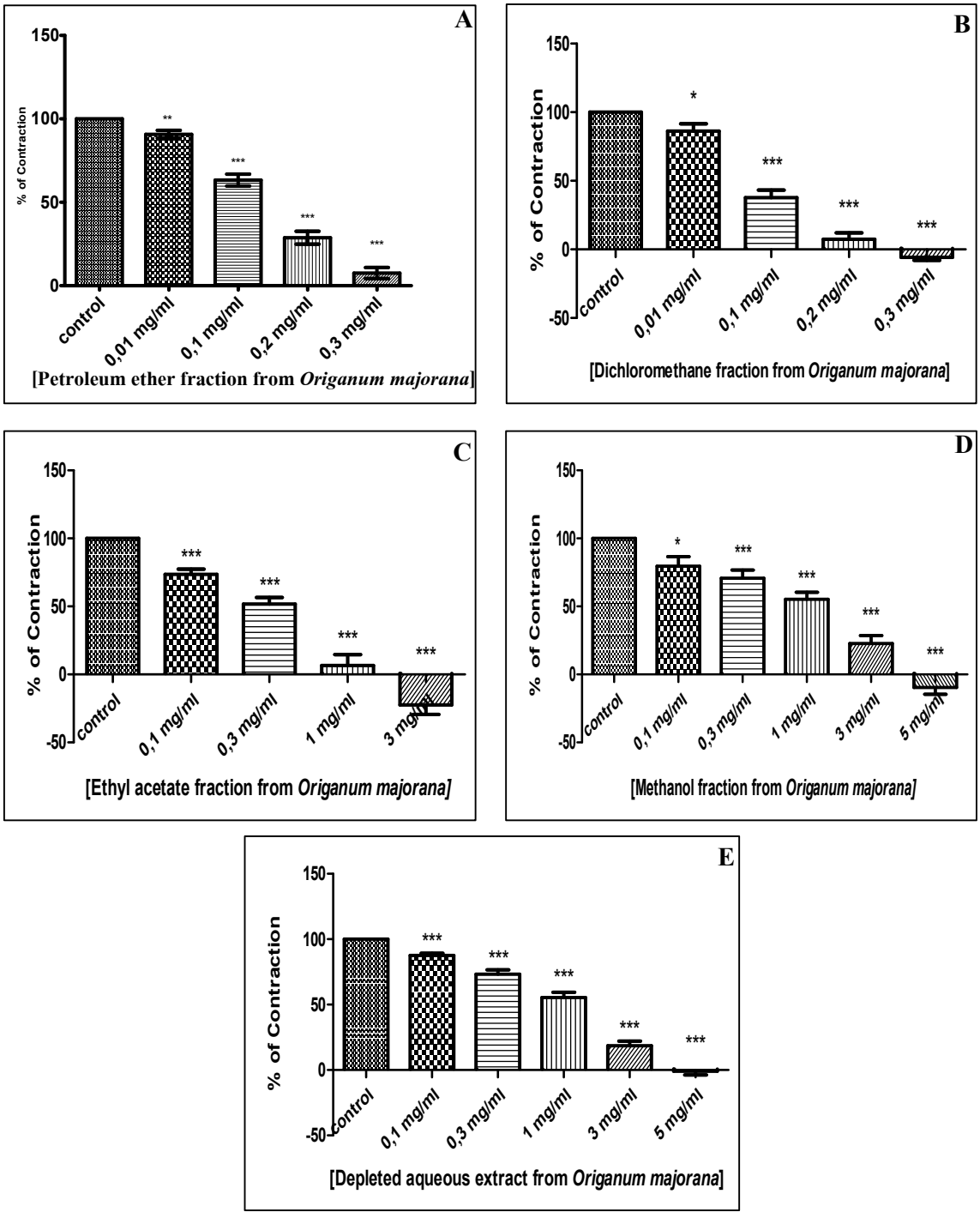

Fig. 1. Effect of the petroleum ether (A), dichloromethane (B), ethyl acetate (C), methanol (D) and depleted aqueous extract (E) fractions of *Origanum majorana* on rat jejunum pre-contracted by CCh 10^{-6} M

Test drugs: Significant from normal control, P < 0.05; ** P < 0.01;*
**** P < 0.001 Mean ± S.E.M = Mean values ± Standard error utilizing six experiments*

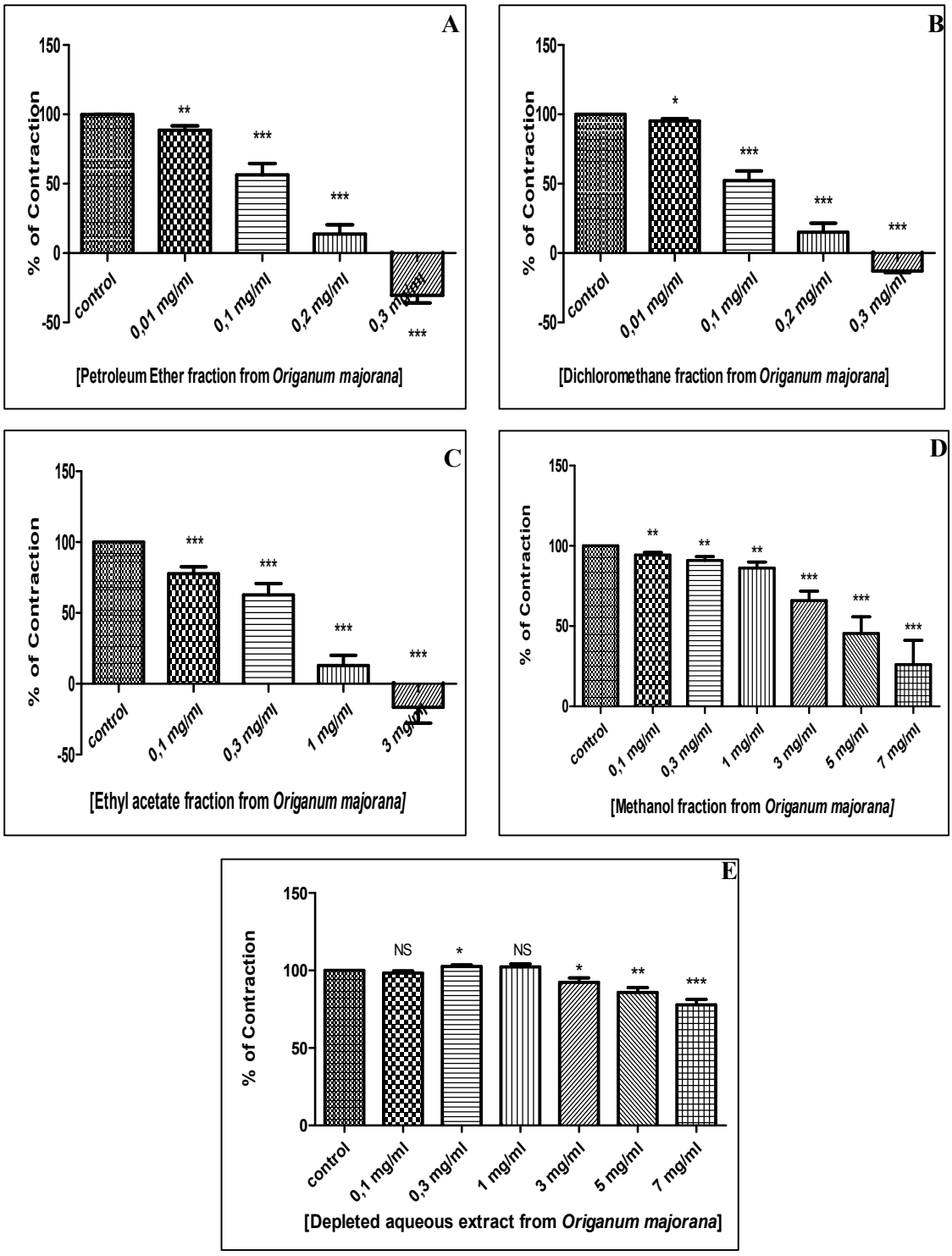

Fig. 2. Effect of the petroleum ether (A), dichloromethane (B), ethyl acetate (C), methanol (D) and depleted aqueous extract (E) fractions of *Origanum majorana* on rat jejunum pre-contracted by KCl 25 mM

*Test drugs: Significant from normal control, * P < 0.05; ** P < 0.01; *** P < 0.001 and NS: No Significant. Mean ± S.E.M = Mean values ± Standard error by means of six experiments*

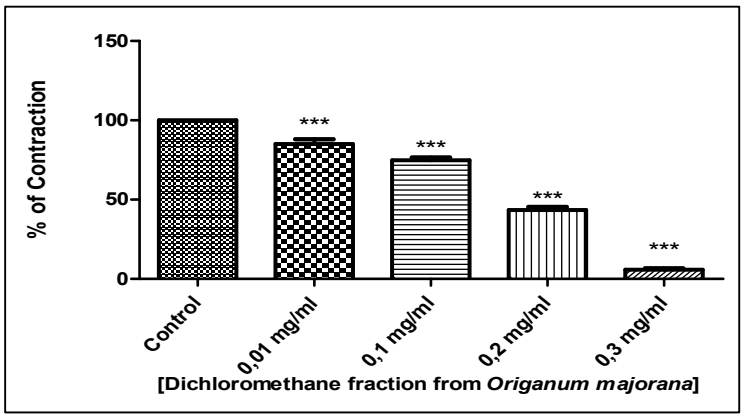

Fig. 3. Effect of the dichloromethane fraction of *Origanum majorana* on the amplitude of contractions of the rabbit jejunum.

*Test drugs: Significant from normal control, *** P < 0.001. Mean ± S.E.M = Mean values ± Standard error utilizing six experiments*

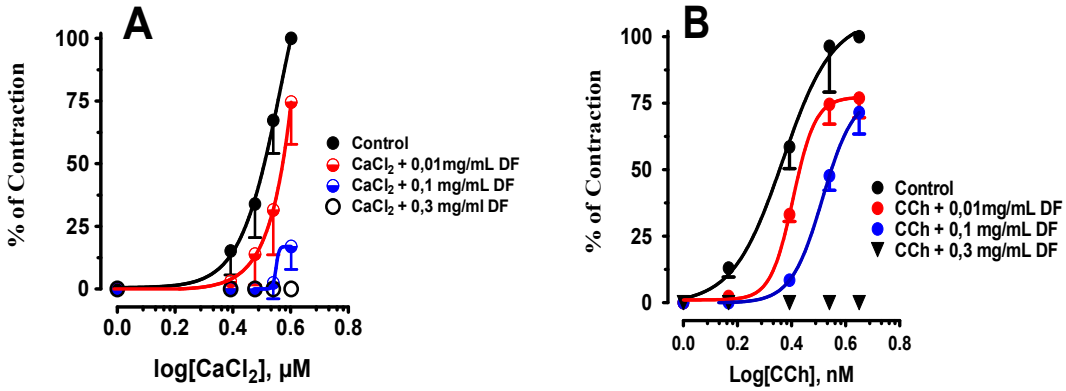

Fig. 4. Dose-response curve of $CaCl_2$ (A) and Carbachol (B) in the presence and absence of the dichloromethane fraction (DF) of *Origanum majorana on the rat jejunum*

Mean ± S.E.M = Mean values ± Standard error by means of six experiments.

4. DISCUSSION

In this study, we investigated the antispasmodic activity of organic fractions (petroleum ether, dichloromethane, ethyl acetate, methanol and depleted aqueous extract) from *Origanum majorana*. All these fractions showed marked antispasmodic business, but the most important is the dichloromethane fraction. It is for this reason that we have continued the research on the mechanism of action on this fraction.

The jejunum of rabbit was used to study the spontaneous basal contractions because their amplitude was easier to evaluate than the rat jejunum. The present data showed that the DFOM exercised a concentration-dependent reversible inhibitory effect on the smooth muscle. As envisaged we fosxund that adrenaline inhibits the rabbit intestinal spontaneous contractions by beta receptor effect (actin α and β adrenergic receptors) [29]. The blocking of adrenergic receptors, α_1 by the prazosin, α_2 by the yohimbine and β by the propranolol, showed that adrenaline did not have any effect on the contractions (data not shown); while the dichloromethane fraction at the concentration of 0.3 mg/ml previously shown to produce a clear myorelaxant effect caused the inhibition of these contractions.he results suggest that these effects were not mediated through the adrenergic receptors. Similar results have been found with other plants such as *Cravetta crassipes* [30] and *Cistus ladaniferus* [31].

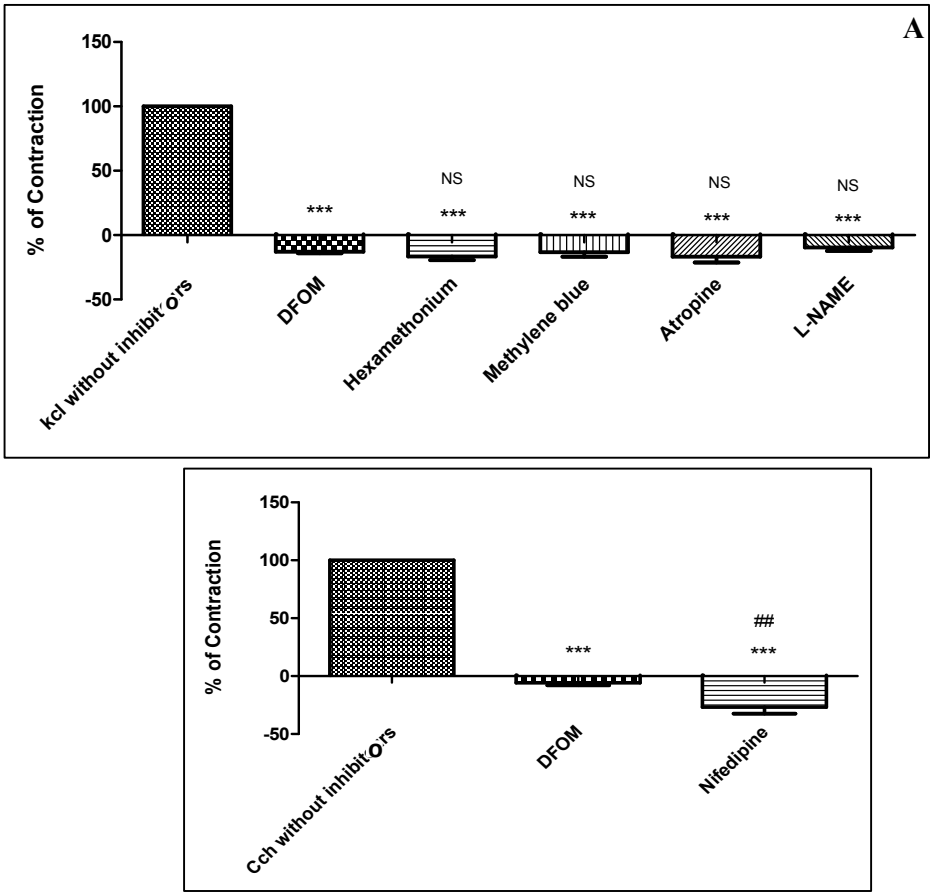

Fig. 5. Effect of the dichloromethane fraction of *Origanum majorana* (0.3 mg/ml) on contractions of the rat jejunum pre-incubated with hexamethonium10^{-4} M, methylene blue 10^{-5} M, atropine 10^{-6} M and L-NAME 10^{-4} M for 20 min, and then pre-contracted by KCl 25 mM (A) or pre-incubated with nifedipine10^{-6} M, and then pre-contracted by CCh 10^{-6} M (B)

*Test drugs: significant from normal control, ## P < 0.01; *** P < 0.001 and NS: No Significant (*** compared to the control and ##, NS compared to the fraction without inhibitors). Mean ± S.E.M = Mean values ± Standard error using six experiments*

The DFOM also caused a dose-dependent relaxation of rat jejunum tone pre-contracted by KCl 25 mM and Carbachol 10^{-6} M; which is a structural analogue of acetylcholine; it has the advantage to be not degraded in the physiological environment [32,33]. In the presence of different doses of this fraction, the maximum response to $CaCl_2$ and CCh was significantly reduced, with shifting the dose-response curves to the right. So this inhibitory effect is similar to that of a non-competitive antagonist towards the voltage-dependent calcium channels and the muscarinic receptors, respectively [34,35].

Among the substances that contract intestinal smooth muscle is acetylcholine. This latter acts either on the muscarinic receptors M_2 or M_3 [36,37] by decreasing the level of intracellular cAMP (inhibition of the adenylate cyclase enzyme) and by activation of PLC (increase in production of inositol triphosphate (IP_3) and diacylglycerol (DAG)) respectively [38,39,40]. To evaluate if the DFOM acts via the cholinergic receptor pathway, we used the hexamethonium; nicotinic cholinergic antagonist [41], and the atropine; an antagonist of the muscarinic cholinergic receptor [42]. These results showed us that DFOM does not act on cholinergic receptors.

Nifedipine selectively inhibits, at very low concentrations, the entry of calcium ions into the voltage-gated L-type channels [43,44]. The use

of the DFOM in the presence of this substance showed that it does not affect the voltage-gated L-type channels.

We wanted to evaluate if the relaxation effect of our extract passes through the NO and guanylate cyclase pathways. The use of pharmacological inhibitors such as L-NAME, an inhibitor of nitrogen monoxide (NO) [45] and methylene blue, an inhibitor of guanylate cyclase pathway [46] did not show any alteration in the effect of DFOM which gave an effect comparable to that obtained without the presence of these inhibitors. So DFOM did not act on these metabolic pathways.

The antispasmodic effect is concentrated in the organic fractions, and more predominantly in the nonpolar fractions of this plant. They are rich in lipids and essential oils which contains various compounds of the families of monoterpenols (terpinene-4-ol (22.85 %), (E)-sabinene hydride (15.94 %), etc.), monoterpenes (gamma-terpinene (12.60 %), sabinene (7.65 %), etc.), sesquiterpenes (beta-caryophyllene (2.49 %), bicyclogermacrene (1.22 %)) and terpene esters (linalyl acetate (1.70 %)) [47,48]. Among these compounds, we find the terpinenes [49], terpineols [50], limonene and linalool [51] known by their antispasmodic activity.

5. CONCLUSION

The antispasmodic effect of DFOM is similar to that of a non-competitive antagonist towards the voltage dependent calcium channels. This relaxation effect did not act on adrenergic receptors, NO, guanylate cyclase and the muscarinic receptors pathways. These results confirm favorably the use of marjoram in Moroccan traditional medicine as an antispasmodic intestinal agent.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, ethical approval has been collected and preserved by the authors.

ACKNOWLEDGEMENTS

This work was supported by grant from CNRST, Morocco (project URAC-40). Authors are also thankful to Badraoui Mustapha and Ramdaoui Karim for their technical support and animal breeding.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Choung RS, Talley NJ. Novel mechanisms in functional dyspepsia. World J Gastroenterol. 2006;12(5):673–677.
   Ducrotté P. Physiopathologie et traitement des troubles fonctionnels intestinaux. Gastro-entérologie. [9-058-A-10]; French. 2005;400-412.
   DOI: 10.1016/S1155-1968(05)39221-2
2. Lee KJ, Kindt S, Tack J. Pathophysiology of functional dyspepsia. Best Pract Res Clin Gastroenterol. 2004;18(4):707-16.
3. Mimidis K, Tack J. Pathogenesis of dyspepsia. Dig Dis. 2008;26:194-202.
4. Bellakhdar J. La pharmacopée marocaine traditionnelle. Médecine arabe ancienne et savoirs populaires. Ibis Press. French. 1997;349.
5. Farnsworth NR. Screening plants for new medicines. In: Wilson EO, editor. Biodiversity, Part II. Washington: National Academy Press. 1989;83-97.
6. Blumenthal M. Herbal medicines. Austin: Integrative Medicine Communications. 2000;419-23.
7. Pimple BP, Patel AN, Kadam PV, Patil MJ. Microscopic evaluation and physic-chemical analysis of *Origanum majorana* Linn leaves. Asian Pac J Trop Dis. 2012a; 897-903.
8. Busatta C, Vidal RS, Popiolski AS, Mossi AJ, Dariva C, Rodrigues MRA, et al. Application of *Origanum majorana* L. essential oil as an antimicrobial agent in sausage. Food Microbiol. 2008;25(1):207-211.
9. Mohammed N, Yasmen SH, Nohir G. Antimicrobial activity of water and ethanol marjoram (*Origanum marjorana* L.) extract. The 6th Arab and 3rd International Annual Scientific Conference on: Development of Higher Specific Education Programs in Egypt and the Arab World in the Light of Knowledge Era Requirements Faculty of Specific Education Mansoura University Egypt. 2011;2350-2366.

10. Leeja L, Thoppil JE. Antimicrobial activity of methanol extract of *Origanum majorana* L. (Sweet marjoram). J. Environ. Biol. 2007;28(1):145-146.
11. Vàgi E, Rapavi E, Hadolin M, Vásárhelyiné Perédi K, Balázs A, Blázovics A. Phenolic and triterpenoid antioxidants from *Origanum majorana* L. herb and extracts obtained with different solvents. J Agric Food Chem. 2005a;53(1):17-21.
12. Abdel-Massih RM, Fares R, Bazzi S, El-Chami N, Baydoun E. The apoptotic and anti-proliferative activity of *Origanum majorana* extracts on human leukemic cell line. Leuk Res. 2010;34(8):1052-1056.
13. Sameer H. Qari. *In vitro* evaluation of the anti-mutagenic effect of *Origanum majorana* extract on the meristemetic root cells of *Vicia faba*. J Taibah Univ Sci. 2008;1:6-11.
14. Suhaj M. Spice antioxidants isolation and their antiradical activity: A review. J Food Compost Anal. 2006;19(6–7):531-537.
15. Vagi E, Simandi B, Suhajda A, Hethelyi E. Essential oil composition and antimicrobial activity of *Origanum majorana* L. extracts obtained with ethyl alcohol and supercritical carbon dioxide. Food Res. Int. 2005b;38(1):51-57.
16. Jun WJ, Han BK, Yu KW, Kim MS, Chang IS, Kim HY. Antioxidant effects of *Origanum majorana* L. on superoxide anion radicals. Food Chem. 2001;75:439-44.
17. Yazdanparast R, Shahriyary L. Comparative effects of *Artemisia dracunculus*, *Satureja hortensis* and *Origanum majorana* on inhibition of blood platelet adhesion, aggregation and secretion. Vasc Pharmacol. 2008;48:32-37.
18. Pimple BP, Kadam PV, Patil MJ. Ulcer healing properties of different extracts of *Origanum majorana* in streptozotocin-nicotinamide induced diabetic rats. Asian Pac J Trop Dis. 2012b;312-318.
19. Pimple BP, Kadam PV, Patil MJ. Comparative antihyperglycaemic and antihyperlipidemic effect of *Origanum majorana* extracts in NIDDM Rats. Orient Pharm Exp Med. 2012c;12(1):41-50.
20. El-Ashmawy IM, El-Nahas AF, Salama OM. Protective effect of volatile oil, alcoholic and aqueous extracts of *Origanum majorana* on lead acetate toxicity in mice. Basic Clin Pharmacol Toxicol. 2005;97(4):238-43.
21. Cipak L, Grausova L, Miadokova E, Novotny L, Rauko P. Dual activity of triterpenoids: Apoptotic versus anti-differentiation effects. Arch Toxicol. 2006; 80:429-35.
22. Saadali B, Boriky D, Blaghen M, Vanhaelen M, Talbi M. Alkamides from *Artemisia dracunculus*. Phytochemistry. 2001;58(7):1083-6.
23. Engelmeier D, Hadacek F, Hofer O, Lutz-kutschera G, Nagl M, Wurz G, Greger H. Antifungal 3-butylisocoumarins from *Asteraceae–Anthemideae*. J Nat Prod. 2004;67(1):19–25.
24. Lutz-Kutschera G, Engelmeier D, Hadacek F, Werner A, Greger H, Hofer O. Synthesis of side chain substituted 3-butyli-socoumarins and absolute confisxgura-tions of natural isocoumarins from *Artemisia dracunculus*. Monatsch Chem. 2003;134:1195-1206.
25. Hajlaoui H, Mighri H, Aouni M, Gharsallah N, Kadri A. Chemical composition and in vitro evaluation of antioxidant, antimicrobial, cytotoxicity and anti-acetylcholinesterase properties of Tunisian *Origanum majorana* L. essential oil. Microb Pathog. 2016;95:86-94.
26. Cechinel-Filho V, Yunes RA. Estratégias paraaobtenção de compostos farmacologicamente ativos a partir de plantas medicinais: Conceitos sobre modificação estrutural para otimização da atividade. Quím. Nova [online]. Portugal. 1998;21(1):99-105.
27. Karim A, Berrabah M, Mekhfi H, Ziyyat A, Legssyer A, Bouali B, et al. Effect of essential oil of *Anthemis mauritiana* Maire & Sennen flowers on intestinal smooth muscle contractility. J Smooth Muscle Res. 2010;46(1):65-75.
28. Martin C, Martin A, Varnet B. Pharmacologie comparée de l'adrénaline et de la noradrénaline. Ann Anesthesiol Fr. French. 1981;22:85-95.
29. Amos S, Okwuasaba FK, Gamaniel K, Akah P, Wambebe C. Inhibitory effect of the aqueous extract of *Cravetta crassipes* leaves on gastrointestinal anduterine smooth muscle preparations isolated from rabbit, guinea pigs and rats. J Ethnopharmacol. 1998;61(3):209-213.
30. Aziz M, Tab N, Karim A, Mekhfi H, Bnouham M, Ziyyat A, et al. Relaxant effect of aqueous extract of *Cistus ladaniferus* on rodent intestinal

contractions. Fitoterapia. 2006;77(6):425-428.

31. Hardman JG, Umbird LE, Gliman AG, Goodman G. The pharmacological basis of therapeutics. 10th ed. McGraw Hill, New York; 2001.
32. Sy Gy, Sarr A, Dièye AM, Faye B. Myorelaxant and antispasmodique effects of the aqueous extract of *Mitragyna inermis* barks on wistar rat ileum. Fitoterapia. 2004;75(5):447-450.
33. Boegge SC, Kesper S, Verspohl EJ, Nahrsted A. Reduction of Ach induced contraction of rat isolated ileum by coptisine, (+)-caffeoylmalic acid, *Chelidonium majus* and *Corydalis lutea* extracts. Planta Medica. 1996;62(2):173-174.
34. Hajhashemi V, Sadraei H, Ghannadi AR, Mohseni M. Antispasmodic and Anti-diarrhoeal effect of *Satureja hortensis* L. essential oil. J Ethnopharmacol. 2000; 71(1-2):187-192.
35. Goyal RK. Identification, localization and classification of muscarinic receptor subtypes in the gut. Life Sci. 1988; 43(26):2209-2220.
36. Levey AI. Immunological localization of M_1-M_5, muscarinic acetylcholine receptors in peripheral tissue and brain. Life Sci. 1993;52(5–6):441-448.
37. Espanol AJ, Sales ME. Participation of nitric oxide synthase and cyclo-oxygenase in the signal transduction pathway of ileal muscarinic acetylcholine receptors. Pharmacol Res. 2000;42:489-493.
38. Sales ME, Sterin-Borda L, Rodriguez M, Borda ES. Intracellular signals coupled to different rat ileal muscarinic receptor subtypes. Cell Signal. 1997;9:373-378.
39. Monteiro FS, Carvalho AFS, Marques EC, Ribeiro RM, Borges ACR, Borges MOR. Antidiarrhoeal and antispasmodic activity of leaves of *Syzygium cumini* L. (Myrtaceae) mediated through calcium channel blockage. Afr J Pharm Pharmacol. 2018;12(1):11-18.
40. Barlow RB, Franks M, Pearson JDM. A comparison of the affinities of antagonists for acetylcholine receptors in the ileum, bronchial muscle and iris of the guinea-pig. Br J Pharmacol. 1972;46(2):300-312.
41. Arunlakhshana O, Schild HO. Some quantitative uses of drug antagonists. Br J Pharmacol Chemother. 1959;14(1):48-58.
42. Daniel EE, Boddy G, Bong A, Cho WA. New model of pacing in the mouse intestine. Am J Physiol Gastrointest Liver Physiol. 2004;286:253-262.
43. Grasa L, Rebollar E, Arruebo MP, Plaza MA, Murillo MD. The role of Ca^{2+} in the contractility of rabbit small intestine *in vitro*. J Physiol Pharmacol. 2004;55(3):639-650.
44. Kopincová J, Púzserová A, Bernátová I. L-NAME in the cardiovascular system - nitric oxide synthase activator? Pharmacol Rep. 2012;64(3):511-520.
45. Gruetter CA, Greutter DY, Lyon JE, Kadowitz PJ, Ignarro LJ. Relationship between cyclic guanosine 3'-5' monophosphate formation and relaxation of coronary arterial smooth muscle by glyceryl trinitrate, nitrite and nitric oxide: effects of methylene blue and haemoglobin. J. Pharmacol. Exp. Ther. 1981;219(1):181-186.
46. Komaitis ME. Composition of the essential oil of marjoram (*Origanum majorana L.*). Food Chem. 1992;45:117-118.
47. Baj T, Baryluk A, Sieniawska E. Application of mixture design for optimum antioxidant activity of mixtures of essential oils from *Ocimum basilicum* L., *Origanum majorana* L. and *Rosmarinus officinalis* L. Ind Crops Prod. 2018;115:52-61.
48. Astudillo A, Hong E, Bye R, Navarette A. Antispasmodic activity of extracts and compounds of *Acalypha phleoides Cav.* Phytother Res. 2004;18(2):102-106.
49. Couic-Marinier F, Lobstein A. Composition chimique des huiles essentielles. Actual Pharm. French. 2013;52(525):22-25.
50. Martinetti P. Mon guide des Huiles essentielles. Editions LANORE, François-Xavier Sorlot, éditeur. French. 2013;256.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/24388