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Research Article

In Vitro Assessment of Myorelaxant and Antispasmodic Effects of Stigmas, Tepals, and Leaves Hydroethanolic Extracts of *Crocus sativus*

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The valorization of *Crocus sativus* focuses mainly on the plant's stigma because it is one of the most expensive and valuable spices in the world and has great value in the food, cosmetics, and pharmacological industries. Due to this high stigma value, the other parts of the plant are considered as by-products; our study aimed to evaluate the myorelaxant and antispasmodic activities of the byproducts (tepals and leaves) to compare them with the stigma of the plant. To investigate the myorelaxant and antispasmodic activities of the *Crocus sativus* on isolated rabbit and rat jejunum, we used an *in vitro* technic with an organ bath and an isotonic transducer. Our results showed that the STG (hydroethanolic extract of stigmas) and LV (hydroethanolic extract of leaves) had a moderate myorelaxant effect with $IC_{50} = 6.61 \pm 1.5$ and 5.08 ± 0.45 mg/ml, respectively. TPL (hydroethanolic extract of tepals) had a significant ($p < 0.001$) inhibitory effect on the amplitude of the rabbit jejunum basic contractions with an $IC_{50} = 1.36 \pm 0.15$ mg/ml. TPL also caused a significant ($p < 0.001$) antispasmodic activity depending on the dose of the contraction induced by CCh (10^{-6} M) and KCl (25 mM). The antispasmodic effect of the TPL is slightly altered in the presence of nifedipine by a percentage of 26.8%. This difference is statistically significant ($p < 0.01$). Therefore, the extract could induce the inhibitory effect on L-type voltage-dependent Ca^{2+} channels, but not on the guanylate cyclase and nitric oxide pathways. That confirmed that the TPL has a comparable effect to the verapamil. The HPLC-DAD analysis of various parts of *C. sativus* shows that the three extracts contain the kaempferol flavanol compound, the STG also was revealed to be rich in carotenoids crocin, and these isomers are *trans* and *cis*-crocin, safranal, and picrocrocin. In contrast, the TPL revealed the presence of isorhamnetin and quercetin, but the LV was rich in hesperidin and mangiferin. In conclusion, this study supports the traditional use of this plant to treat digestive problems and will allow us to explore future possibilities for treating bowel spasms using natural molecules derived from saffron.

1. Introduction

Crocus sativus L. (*C. sativus*), commonly known as saffron, is a bulbous perennial male-sterile triploid medicinal plant of Iridaceae family, cultivated in Morocco and other countries. Iran is the largest producer of saffron, accounting for more than 90% of the world's saffron production. In 2019, Iran produced more than 430 tons of saffron, followed by India (22 tons),

Greece (7.2 tons), Morocco (6.5 tons), Afghanistan (6 tons), Spain (2.3 tons), Italy (1 ton), China (1 ton), and Azerbaijan (0.23 tons) [1, 2]. Many other nations produce saffron in very small amounts, including Switzerland, France, Turkey, and Australia. Saffron blooms only once a year, and their flowers are a combination of six tepals, three stamens, and three red stigmas. The dry stigmas of the flowers are used mainly as a spice for flavoring and coloring food. It is one of the world's priciest and most valuable spices and has great value in the food industry and cosmetics. The odor and flavor were attributed to three major bioactive compounds, crocin, picrocrocin, and safranal [3–5]. The main active constituents that give *C. sativus* the golden yellow-orange color are crocins [6], and picrocrocin is responsible for the bitter taste [7]. Safranal, a monoterpene aldehyde, creates odor and aroma characteristics [8].

Several pharmacological activities of C. sativus have been proven, including antioxidant [9], antidepressant [10], anti-inflammatory [11], anti-Alzheimer [12], antidiabetic [13, 14], and hepatoprotective [15] properties. It has also been found to improve the quality of life and appetite in patients with atherosclerosis [16], Additionally, the plant has been reported to possess anticancer [17] and nephroprotective activities [18, 19]. The diversity of pharmacological effects of this plant is attributed to the presence of several phenolic and carotenoid compounds.

Functional gastrointestinal dysmotility is an important part of dietary problems in the human population; among these disorders are the abdominal spasms, abdominal pain, and colic-causing problems such as diarrhea syndrome. To treat these disorders, the population has recourse to medicinal plant. Among these plants, *C. sativus* is widely used in traditional medicine to treat microbial infection and stomach pain and as a laxative [20–22].

In many studies [23–25], it is claimed that the production of saffron produces a significant amount of byproducts, many of which are discarded as unnecessary bioresidues. To obtain only 1 kg of dried stigmas, approximately 63 kg of flowers or 53 kg of tepals, 1500 kg of leaves, 100 kg of spathes, and hundreds of bulbs that are too tiny or have physical or biological defects are rejected [26]. However, this biomass represents a non-negligible source of bioactive substances that can also be employed for culinary or medicinal purposes [27]. Therefore, this study aimed to assess the myorelaxant and antispasmodic effects of the tepals and leaves of *C. sativus* and to compare them with that of the stigmas to confirm the traditional use of *C. sativus* in the therapy of gastrointestinal disorder and suggest new means of valorization.

2. Materials and Methods

2.1. Chemicals. The following chemical and standards were used: ethanol, L-NAME (L-NG-nitro arginine methyl ester), carbamylcholine chloride (carbachol, CCh), prazosin, yohimbine, propranolol, hexamethonium, methylene blue, NaCl, $MgSO_4$, $NaHCO_3$, KCl, $CaCl_2$, KH_2PO_4, glucose, dimethyl sulfoxide (DMSO), methanol, picrocrocin, crocins, kaempferol, isorhamnetin, quercetin, hesperidin, and mangiferin which were purchased from Sigma Chemical Co. (Sigma-Aldrich, USA). Atropine was provided by Research Biochemical Incorporated, USA. Verapamil was supplied by Tocris, USA. All the chemicals used were of analytical grade and solubilized in distilled water, except the nifedipine (Sigma-Aldrich, USA), which was dissolved in DMSO.

2.2. Extracts Preparation. Between October and December 2021, *C. sativus* L. was obtained from "Taliouine" region of southern Morocco (30°31′54″ north, 7°55′25″ west). The plant was identified botanically by Fennane Mohammed, a professor of botany at the Scientific Institute in Rabat, Morocco. It was cataloged under the voucher code HUMPOM210 at the herbarium of the University Mohammed First in Oujda, Morocco. The proportion of each component in the plant might change depending on the *Crocus sativus* variety, the growth environment, and the cultivation technique. The stigmas typically comprise only a minor portion of the dried saffron's overall weight. Although the precise ratio of stigmas to tepals and leaves can differ, on average, the stigmas account for 0.5–1% of the weight of dried saffron. The tepals and leaves often make up the remaining weight of dried saffron.

Plant material preparation was made according to the method described by Ouahhoud et al. [14]. The different parts of *C. sativus* were manually separated and dried (using a drying oven JP SELECTA, Spain) for 4 h for the stigmas and 24 h for the tepals and leaves at 37°C. Using a ratio of 80/20 (v/v) of ethanol and water, vegetable material was macerated for 24 hours at room temperature while stirring (Witeg MS-MP8, Germany) in the dark. The extraction ratio was 2 g of plant powder to 50 ml of solvent. After the first extraction, a syringe filter (0.45 μm) was used to filter the sample, and the marc was then removed for the subsequent extraction. The whole hydroethanolic phase was then dried using a rotary evaporator (Buchi B-480, Switzerland) at 40°C after this procedure was done three times. Finally, a −20°C freezer (BOSCH, Germany) was used to preserve the dry hydroethanolic extract of the stigmas (STG), tepals (TPL), and leaves (LV). The extraction yield (measured in grams per 100 grams of dry matter) for stigmas was 60.45%, for tepals, it was 65.75%, and for leaves, it was 30.53%.

The equation used to calculate the extraction yield is

$$\text{Extraction yield}\,(\%) = \left(\frac{\text{Weight of extracted compounds}}{\text{Weight of dry plant material}}\right) \times 100. \quad (1)$$

2.3. Experimental Settings. The laboratory animals employed were males and females Wistar rats, 6–8 weeks old (200–300 g), and New Zealand rabbits, 5-6 months old (1.5–2 kg), with a proportion of 50% of each sex. All animals were kept in a house of animals of the Department of Biology, Faculty of Sciences, Mohammed First University Oujda, Morocco. In a climate-controlled room with controlled lighting (12 h: 12 h light-dark cycle) and free access to food and water, we used in each experiment six animals ($n = 6$). All animals were treated following the US National Institutes of Health's Guide for the Care and Use of Experimental Animals [28]. The Faculty of Sciences, Mohammed First University Oujda approved the study under trial registration number: 04/21-LBBEH-06 and 07/04/2021.

2.4. Tissue Preparation. We followed the previously described method used by Marghich et al. [29]. First, the animals were fasted through the night and anesthetized with light ethyl ether inhalation; then, the jejunum was rapidly removed, washed, and placed in Normal Krebs–Henseleit Buffer (KHB) solution composed of NaCl, 118; $MgSO_4$, 1.2; $NaHCO_3$, 25; KCl, 4.7; $CaCl_2$, 2.5; KH_2PO_4, 1.2; and glucose 10 mM. The KHB solution had a pH of 7.4 and was maintained at 37°C throughout the experiments with continuous oxygenation (RS Electrical AIR Pump RS-628A, China).

Segments of 2 cm were placed in isolated organ baths (10 ml). Then, the KHB solution was changed every 15 minutes to equilibrate the jejunum before adding the herbal extracts or another drug. The amplitude of the jejunum contraction is measured with a jejunum-linked isotonic transducer mounted on the isolated bath of the organ (B. Braun Melsungen AG Type 362722 #203, Germany). To calibrate the system, a weight of 1 g and 1.5 g equal to 100% of contraction was used for rat and rabbit jejunum, respectively. The concentration of the samples at a relaxant activity of 50% (IC_{50}) was calculated graphically according to the linear regression method.

2.5. Myorelaxant Effect of a Different Part of C. sativus L. in Rabbit Jejunum. The cumulative doses of hydroethanolic extracts of the different parts of the plant were added (0.3–10 mg/ml) to the organ bath after stabilization of smooth muscle basic contractions of rabbit jejunum. The most active extract (TPL) was also tested in the presence of three adrenergic receptor inhibitors (yohimbine, propranolol, and prazosin) at a concentration (5.0×10^{-5} M) for each of them. Verapamil (0.1–1 μM) was used as a positive control, respectively.

Relaxation was calculated as a percent of contraction inhibition relative to the basic rabbit jejunum contraction.

2.6. Antispasmodic Effect of Hydroethanolic Extracts of the Different Parts of Crocus sativus L. In this experiment, the jejunum was precontracted with carbachol (CCh, 10^{-6} M) or KCl (25 mM) to maintain a tone. After the tone stabilization, we added cumulated doses of different extracts (0.3–10 mg/ml). Then, the most active extract TPL was studied in more detail against pharmacological inhibitors.

The drugs inhibitors, L-NAME (10^{-4} M), atropine (10^{-6} M), blue of methylene (10^{-5} M), nifedipine (10^{-6} M), and hexamethonium (10^{-4} M), were incubated during 20 minutes. The rat jejunum precontacted with KCl (25 mM) (except for nifedipine, which was precontracted with CCh). Afterward, the TPL concentration that resulted in maximum relaxation (3 mg/ml) was added.

2.7. High-Performance Liquid Chromatography Analysis of Hydroethanolic Extracts of C. sativus. A concentration of 10 mg/ml of hydroethanolic extracts was dissolved in HPLC water and filtered using a syringe filter (0.45 μM). HPLC analysis was performed on Alliance (Waters *e*2695) coupled to a DAD (diode array detector) (Waters 2998, USA).

A reversed-phase analytical C18 column with the specifications such as micron size of 5 μm, length of 250 mm, the inner diameter of 4.6 mm, and flow rate of 1 mL/min was used to inject (20 μL) the samples from the three parts of *C. sativus*.

2% acetic acid in water (solvent A) and methanol (solvent B) were employed in a gradient of binary solvents as follows for elution: initial 80% A and 20% B, 20 minutes of 100% B, 25 minutes of 100% B, 30 minutes of 50% A and 50% B, and 35 minutes of 80% A and 20% B. The UV detection was done between 220 and 575 nm. By contrasting retention times and maximum wavelengths with reference standards (picrocrocin, crocin, kaempferol, isorhamnetin, quercetin, kaempferol, hesperidin, mangiferin, and others that are not detected), specific compounds were determined.

2.8. Statistical Analysis. Statistical analysis was performed in all pharmacological tests and the results were expressed as an average ± SEM. In addition, the difference between the groups was calculated using one-way variance analysis (ANOVA) and then by a post hoc Tukey test, using GraphPad Prism (version 5, San Diego, CA, USA). When p is less than 5% (5% is α), it was considered significant in all tests performed. The linear regression method was used to obtain an inhibitory concentration of 50% (IC_{50}). We operated in each experiment six animals ($n = 6$). Figure 1 shows the experimental design.

3. Results

3.1. Myorelaxant Effect on Jejunum Isolated Rabbit. STG had a moderate myorelaxant effect up to 10 mg/ml (Figure 2(a)) with an $IC_{50} = 6.61 \pm 1.5$ mg/ml (Table 1). The LV has a myorelaxant effect better than the first extract with a product up to 5 mg/ml (Figure 2(b)) with an $IC_{50} = 5.08 \pm 0.45$ mg/ml (Table 1). Regarding TPL, a significant inhibitory effect on the amplitude of basic rabbit jejunum contractions was shown. They reduced these contractions depending on the dose with an inhibitory concentration 50 (IC_{50}) of spontaneous contraction equal to 1.36 ± 0.15 mg/ml (Table 1) and a maximum inhibitory effect at a dose of 3 mg/ml; this inhibition is reversible and comparable to that found by verapamil (Figure 2(d)). Therefore, TPL has the best myorelaxant effect compared to the other extracts.

In the presence of three adrenergic inhibitors, rabbit basal jejunum contraction is inhibited by 3 mg/ml of TPL and is identical to the effect of the extract alone (Figure 3).

3.2. Antispasmodic Activity on Isolated Rat Jejunum Precontracted by CCh or KCl. Figure 4 demonstrates the inhibitory effect of different extracts on the contraction of the rat jejunum precontracted with CCh (10^{-6} M). STG and LV reduced the maximum tone induced by CCh depending on the dose ranging from 0.3 to 5 mg/ml (Figures 4(a) and 4(b)) with an $IC_{50} = 2.11 \pm 0.35$ and 1.89 ± 0.17 mg/ml, respectively (Table 1). TPL caused a significant dose-dependent antispasmodic effect on the contraction induced by CCh with a product up to 3 mg/ml (Figure 4(c)) with an $IC_{50} = 1.05 \pm 0.07$ mg/ml (Table 1).

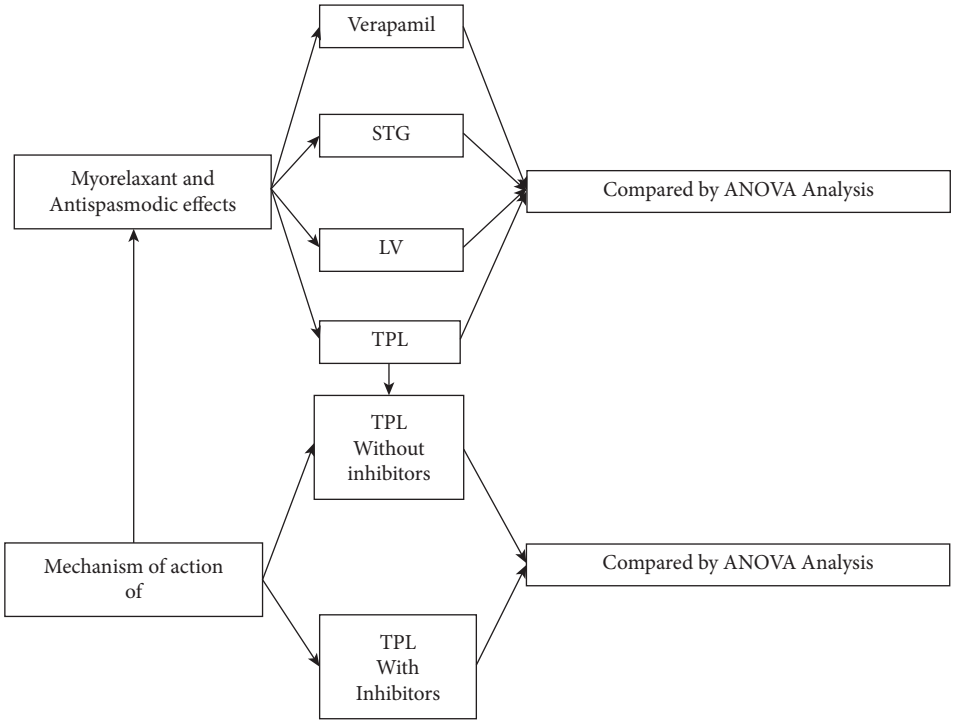

Figure 1: Experimental design of the study. STG, hydroethanolic extract of stigmas; TPL, hydroethanolic extract of tepals; LV, hydroethanolic extract of leaves.

On the other hand, Figures 5(a)–5(c) show the same result of the three extracts with a significant inhibitory effect on KCl-induced contraction with a better effect of TPL, which caused a significant effect at 1 mg/ml. In contrast, the maximum response was reached at 3 mg/ml. The IC_{50} of antispasmodic impacts are summarized in Table 1.

TPL decreased the percentage contraction of rat jejunum preincubated with methylene blue, L-NAME, hexamethonium, and atropine. The difference between the effects obtained by TPL without and with incubation of one of these inhibitors is statistically insignificant (Figure 6(a)). The antispasmodic effect of the TPL was slightly altered in the presence of nifedipine by a percentage of 26.8%. This difference is statistically significant (p‹0.01) (Figure 6(b)).

3.3. High-Performance Liquid Chromatography Analysis of Hydroethanolic Extracts of C. sativus. Figures 7(a)–7(c) and Table 2 (A, B, and C) illustrate the HPLC-DAD chromatograms and the chemical constitution of various parts of *C. sativus*, respectively.

The results show that the three extracts contain the kaempferol flavanol compound, the STG also was revealed to be rich in carotenoids crocin, and the isomers are *trans* and *cis*-crocin, safranal, and picrocrocin, while the TPL revealed the presence of isorhamnetin and quercetin, but the LV was rich in hesperidin and mangiferin.

4. Discussion

The gastrointestinal tract (GI) involves multiple ingestion tasks, treating and absorbing nutrients, and disposing of wastes on time. These tasks are easier by several stereotyped motor models that rely on the intrinsic rhythmicity of smooth muscles that produce spontaneous phasic contractions in many areas of the bowel. The source of this spontaneous phasic contraction is a slow electrical that spreads through the interstitial cells of Cajal (ICC). This mechanism leads to a depolarization/repolarization cycle [30]. The spontaneous contraction of rabbit jejunum is the best model to study the myorelaxant activity of the different extracts of the plant because their amplitude was clear, easier, and larger to evaluate than the rat jejunum. This contraction is reduced moderately by the STG and the LV. However, the TPL significantly inhibited and reduced the amplitude of rabbit jejunum spontaneous contractions depending on the dose with an $IC_{50} = 1.36 \pm 0.15$ mg/ml. In addition, the inhibitory effect of the TPL was restored after a double rinse with a physiological solution; therefore, the TPL has a myorelaxant impact reversibly. This inhibition can be explained by the fact that the TPL directly affects the source of this spontaneous phasic contraction by acting directly on ICC or on smooth muscle cells. The effect obtained by the TPL is comparable to that of other plants like *Artemisia campestris* [29] and *Warionia saharae* [31]. To understand the mechanism of action of the plant, we used three adrenergic inhibitors: propranolol, yohimbine, and, prazosin; antagonist of β, $\alpha 1$, and $\alpha 2$, respectively, because the adrenergic pathway is the main way to inhibit the intestinal smooth muscle contraction [32]. In the presence of these inhibitors, rabbit basal jejunum contraction was inhibited by 3 mg/ml of TPL and identical to the effect of the extract alone. This suggests that the impact of the TPL did not act via the adrenergic receptor. Another study on tepals aqueous extract demonstrates an antagonistic effect on rat-isolated vas deferens muscle via an adrenergic pathway [33]. Most tested plant extracts traditionally used for diseases of the

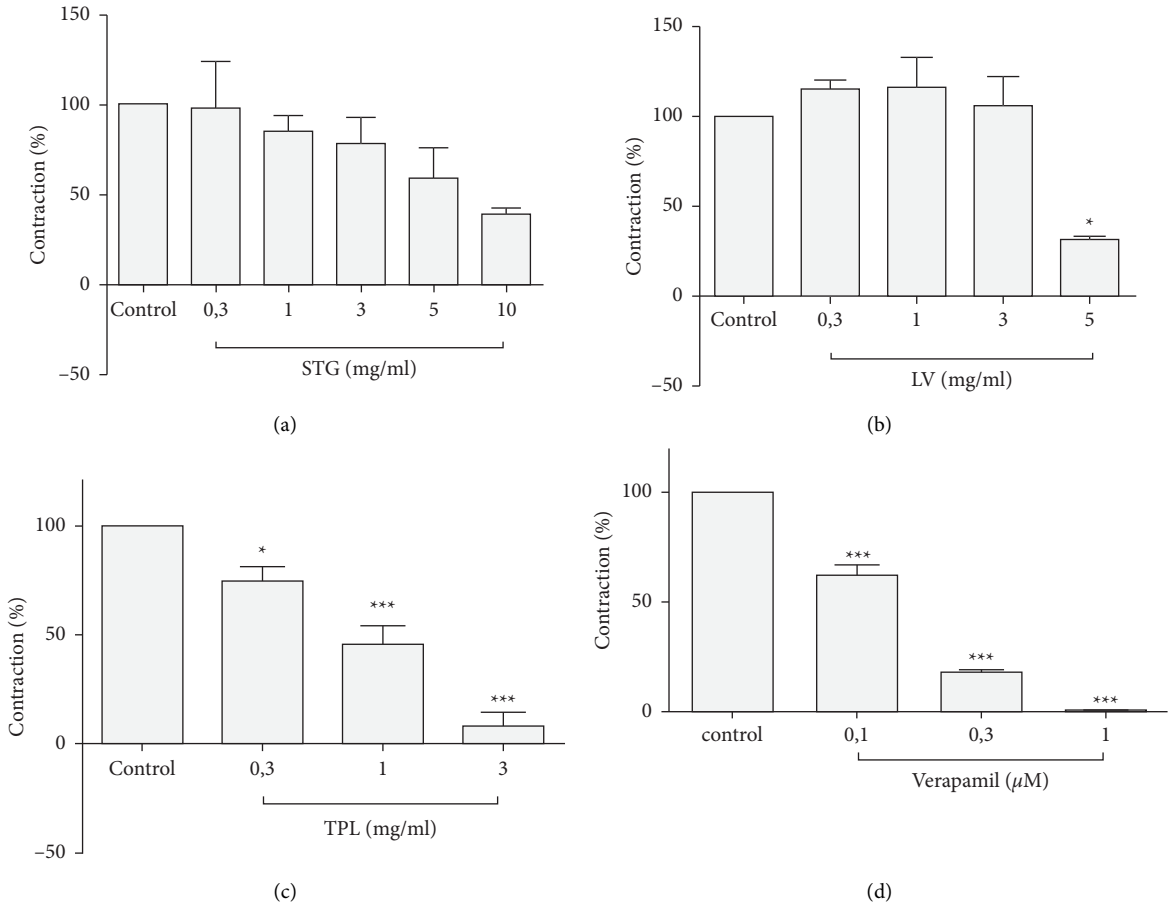

Figure 2: Myorelaxant effect of STG (a), LV (b), TPL (c), and verapamil (d) on spontaneous contractions of rabbit jejunum. STG, hydroethanolic extract of stigmas; TPL, hydroethanolic extract of tepals; LV, hydroethanolic extract of leaves. $^{*}p < 0.05$; $^{***}p < 0.001$. The difference was statistically significant compared to the control (mean ± SEM; $n = 6$).

Table 1: The IC_{50} value of different hydroethanolic extracts of *C. sativus* in antispasmodic and myorelaxant effects.

Effect	IC50 (mg/ml)		
	Antispasmodic effect		Myorelaxant effect
	CCh (10^{-6} M)	KCl (25 mM)	Spontaneous contraction
TPL	$1.05 \pm 0.07^{*}$	$1.28 \pm 0.13^{**}$	$1.36 \pm 0.15^{**}$
LV	1.89 ± 0.17^{NS}	2.63 ± 0.09^{NS}	5.08 ± 0.45^{NS}
STG	2.11 ± 0.35	2.33 ± 0.22	6.61 ± 1.5

STG, hydroethanolic extract of stigmas; TPL, hydroethanolic extract of tepals; LV, hydroethanolic extract of leaves; NS, not significant, $^{*}p < 0.05$; $^{**}p < 0.01$. The comparison was made between the STG and the byproducts of saffron (TPL and LV) (mean ± SEM; $n = 6$). IC_{50} = the concentration of samples that give 50% relaxant activity.

digestive tract had a myorelaxant impact that does not pass through the adrenergic receptor pathway; we quote, for example, *Origanum majorana* and *Rubia tinctorum* [34, 35].

The CCh, an analog of acetylcholine (Ach), or KCl (25 mM) produced a maximum jejunum contraction taken as 100%; these experimental models are frequently used to evaluate the antispasmodic effect and their mechanism of action of drugs and herbal extracts [36–38]. In these two experimental models, we found that TPL has a much greater antispasmodic effect on rat jejunums than the other two saffron extracts (STG, LV).

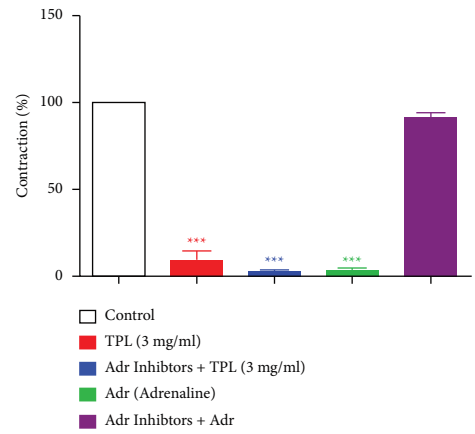

Figure 3: Myorelaxant effect of the TPL (3 mg/ml) in the absence and presence of adrenergic inhibitors (prazosin + yohimbine + propranolol). TPL, hydroethanolic extract of tepals. $^{***}p < 0.001$, the difference was statistically significant compared to the control (mean ± SEM; $n = 5$).

The depolarization of the smooth muscle cells and the increase in the frequency of the action potentials lead to a rise in the intracellular calcium and the activation of the myosin light chain, causing smooth muscle contraction

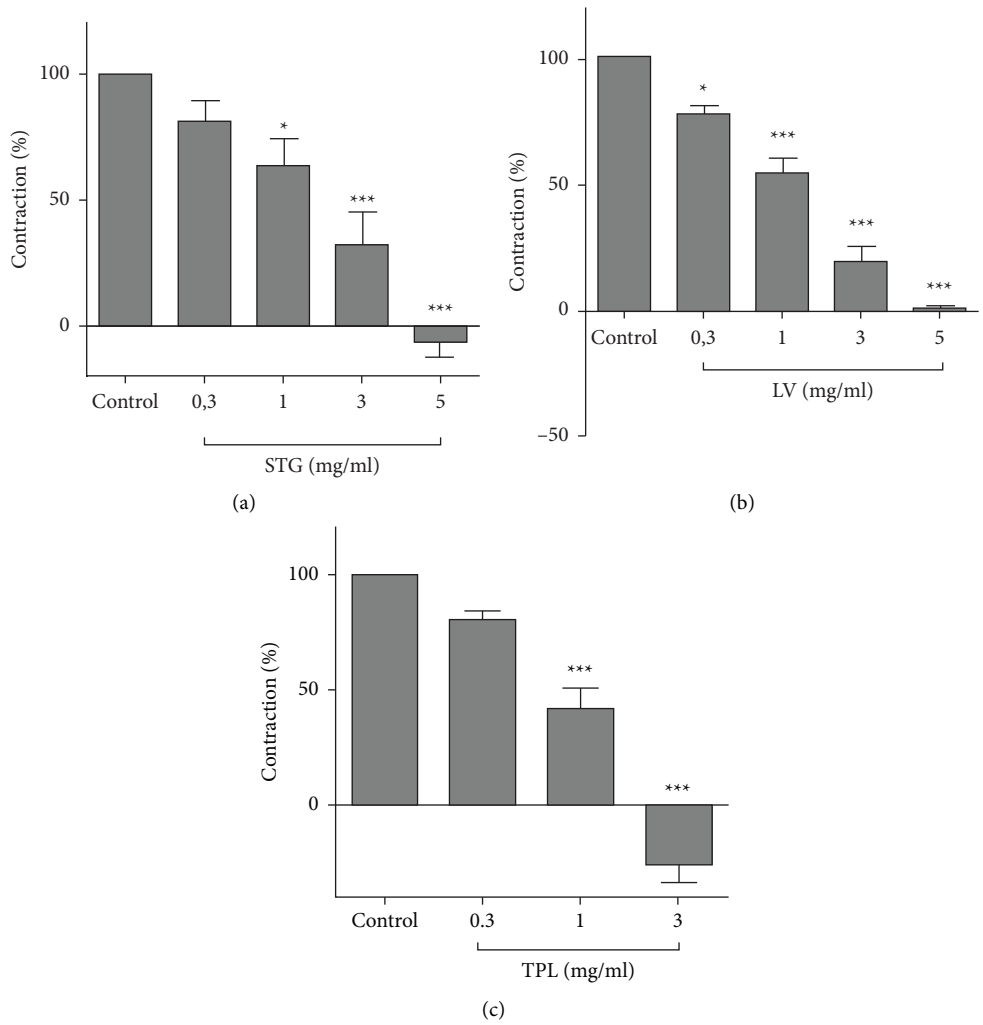

Figure 4: Antispasmodic effect of STG (a), LV (b), and TPL (c) on rat jejunum precontracted by CCh (10^{-6} M). STG, hydroethanolic extract of stigmas; TPL, hydroethanolic extract of tepals; LV, hydroethanolic extract of leaves. $^{*}p < 0.05$; $^{***}p < 0.001$. The difference was statistically significant compared to the control (mean ± SEM; $n = 6$).

induced by CCh [39]. Also, CCh is released from functional synapses after the depolarization of the cells [40], and it binds to M_3 postsynaptic receptors on the smooth muscle cells to build contraction through G-proteins [41]. The contraction induced by KCl is based on the depolarization of muscle fibers, resulting in an increase in potassium, which leads to the activation of voltage-dependent L-type calcium channels. Therefore, the intracellular calcium concentration increases and contraction occurred. Therefore, the jejunal smooth muscle contraction was mainly due to the rise in the concentration of cytoplasmic calcium ions [42].

For this, we studied the effect of the TPL by contracting the smooth muscles with a medium rich in potassium. The latter causes the opening of voltage-dependent Ca^{2+} channels and, consequently, calcium entry from the extracellular medium to the intracellular medium. The myorelaxant activity of the TPL is comparable to that obtained by the verapamil, a standard voltage-dependent calcium channel inhibitor used as a positive control [43]. Therefore, this extract could act in the same way as verapamil. These findings are supported by the fact that the relaxing effect of the TPL is reduced significantly in the presence of nifedipine. Thus, the extract can act as a calcium antagonist and may suppress the contractility of smooth myocytes. Razavi et al. [44] demonstrated that the saffron inhibits L-type voltage-dependent calcium channels to induce relaxation in isolated rat aorta.

Based on this data, we can confirm that the three extracts have an antispasmodic effect on the intestinal smooth muscle cells by inhibiting the calcium channels or repolarizing their plasma membrane. A previous research has shown that the antispasmodic effect of herbal medicine is mediated through anticholinergic and NO/cGMP pathways [29, 45, 46]. For that reason, we would like to assess the impact of the TPL on the cholinergic receptor, by using hexamethonium (nicotinic cholinergic inhibitor) and atropine (muscarinic cholinergic inhibitor). These inhibitors did not influence the relaxing effect found by the TPL. Therefore, the extract did not act on the cholinergic receptors pathway. At the other hand, a previous research

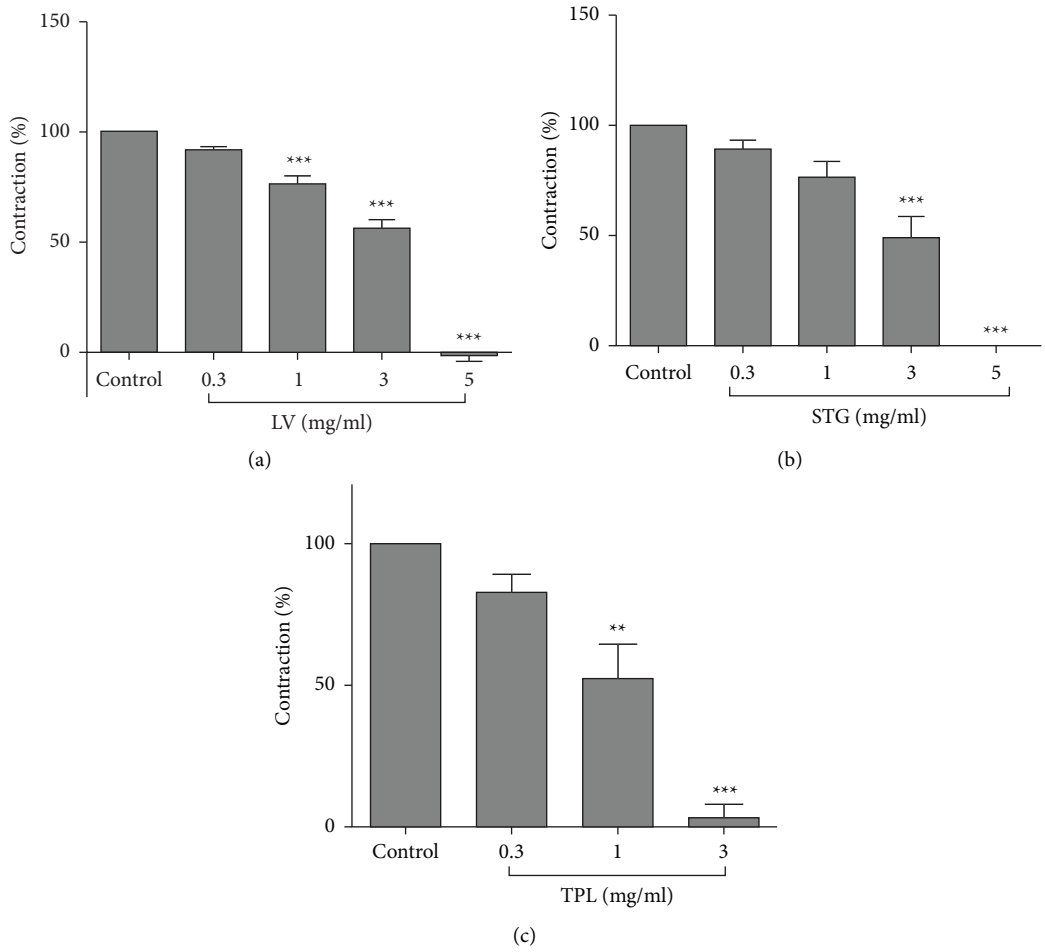

Figure 5: Antispasmodic effect of hydroethanolic extracts of LV (a), STG (b), and TPL (c) on rat jejunum precontracted with KCl 25 mM. STG, hydroethanolic extract of stigmas; TPL, hydroethanolic extract of tepals; LV, hydroethanolic extract of leaves. $^{*}p < 0.05$; $^{***}p < 0.001$. The difference was statistically significant compared to the control (mean ± SEM; $n = 6$).

shows that the relaxing effect of the aqueous extracts of *C. sativus* on the trachea is passed through specifically by the muscarinic receptors [47]. The results of Fatehi et al. [33] suggested that the inhibitory effect of *Crocus sativus* tepals' extract on the contraction of both isolated guinea-pig ileum and rat vas deferens induced by electrical field stimulation was a postsynaptic effect. TPL had a comparable antispasmodic impact with and without the presence of the L-NAME (inhibitor of nitrogen monoxide (NO)) [48] or methylene blue (inhibitor of guanylate cyclase pathway). That is to say, the TPL did not act on NO/cGMP pathway. Nemati et al. showed an inhibitory effect of the *C. sativus* on histamine (H_1) receptors [49]. Several studies on *C. sativus* demonstrated a relaxant effect on several smooth muscle-like vascular smooth muscle [44], tracheal smooth muscle [50], urogenital, and gastrointestinal smooth muscle. The hydroethanolic extract of *Crocus sativus* had an important relaxant effect on electrical field stimulation-induced contraction of guinea pig ileum [33].

The phytochemical analysis of our plant showed that the three extracts contain the kaempferol flavanol compound, the STG also was revealed to be rich in carotenoids crocin, and the isomers are *trans* and *cis*-crocins, safranal, and picrocrocin. In contrast, the TPL revealed the presence of isorhamnetin and quercetin, but the LV was rich in hesperidin and mangiferin. Additionally, other studies confirmed the existence of these compounds in *C. sativus* extracts [51–53]. This can lead to a relation between these molecules and the potential antispasmodic effect obtained. That is confirmed by the fact that quercetin reduced intestinal transit and motility in mice [54]. Other studies funded by Lozoya et al. [55, 56] showed that quercetin had a calcium antagonist effect and was a relatively potent relaxant of ileum smooth muscle contraction and also can induce relaxation of human gastric smooth muscle directly thought the K^{+}_{ATP} channels [57]. Another study confirms the quercetin's relaxant effect on rabbit-isolated bladder smooth muscle contractions [58]. Mangiferin also exhibits an antispasmodic effect on tracheal contraction [59]. The *in silic*o study performed by Wahid et al. [60] demonstrated that the kaempferol inhibited the expression of the target genes involved in smooth muscle contraction and calcium-mediated signaling. In general, flavonols are known for their effective antispasmodics effect on smooth muscles. This

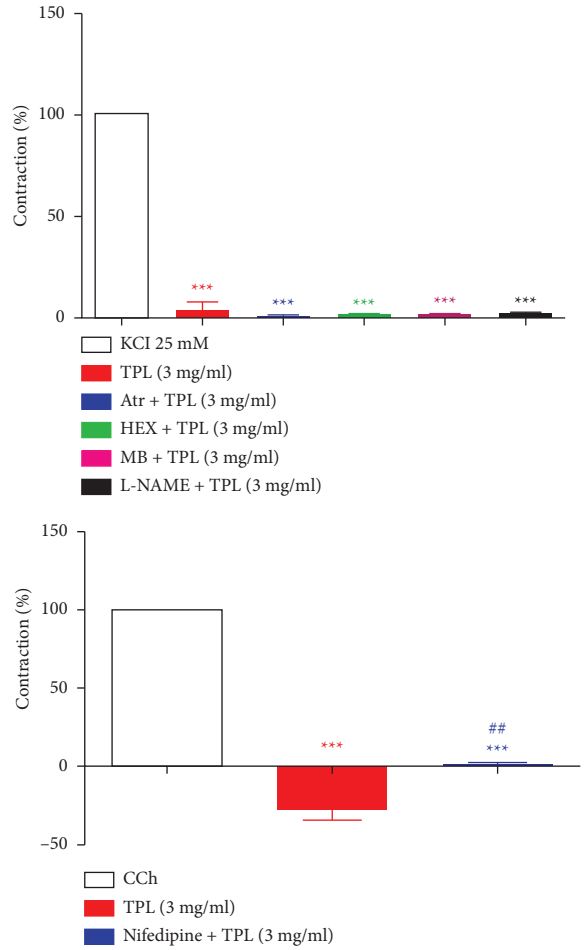

Figure 6: Antispasmodic effect of TPL (3 mg/ml) on the contraction of rat jejunum preincubated with atropine (Atr), hexamethonium (HEX), methylene blue (MB), and L-NAME and then precontracted with KCl 25 mM (a) or preincubated with nifedipine and precontracted with CCh 10^{-6} M (b). TPL, hydroethanolic extract of tepals. *** $p < 0.001$. The difference was statistically significant compared to the control (KCl 25 mM or CCh). ##<0.01): the comparison made between the effect of the extracts without and with the preincubation by the inhibitors (mean ± SEM; $n = 6$).

activity may be related to the suppression of the contractility of smooth myocites, by antagonism of Ca^{2+} uptake [61]. In addition, isorhamnetin presented an important inhibition of abdominal contraction [62].

The results of the various experiments confirmed the necessity of using these byproducts, which can be valorized or utilized for other purposes in several ways. Tepals and leaves of *C. sativus* are cheaper and produced in large quantities compared to the stigma, so they could be considered a suitable source for various uses. Moreover, we have demonstrated that the tepals extract had a great antispasmodic effect compared to the other part extract of saffron. The different tepal extracts have shown many other pharmacological effects such as antioxidant, antidepressant, antihypertensive, antitussive, immunomodulatory, hepatoprotective, antinociceptive, renoprotective, antidiabetic, and antibacterial activities [63]. Based on these properties, saffron tepals could be used as a preferred or complementary drug in various diseases. As kaempferol, quercetin, and isorhamnetin are the major flavonoids in TPL, it would be very interesting to test them individually on the digestive tract. On the other side, *C. sativus* byproducts could be applied to agriculture as biostimulants [64] and antifungals [65]. In terms of economic impact, using *C. sativus* byproducts can provide farmers with an additional source of income and create new opportunities for value-added products. Additionally, using *C. sativus* byproducts can also help to reduce waste and make the cultivation of saffron more sustainable. However, it is important to note that the market for *C. sativus* byproducts is still relatively small and may take some time before it becomes fully developed.

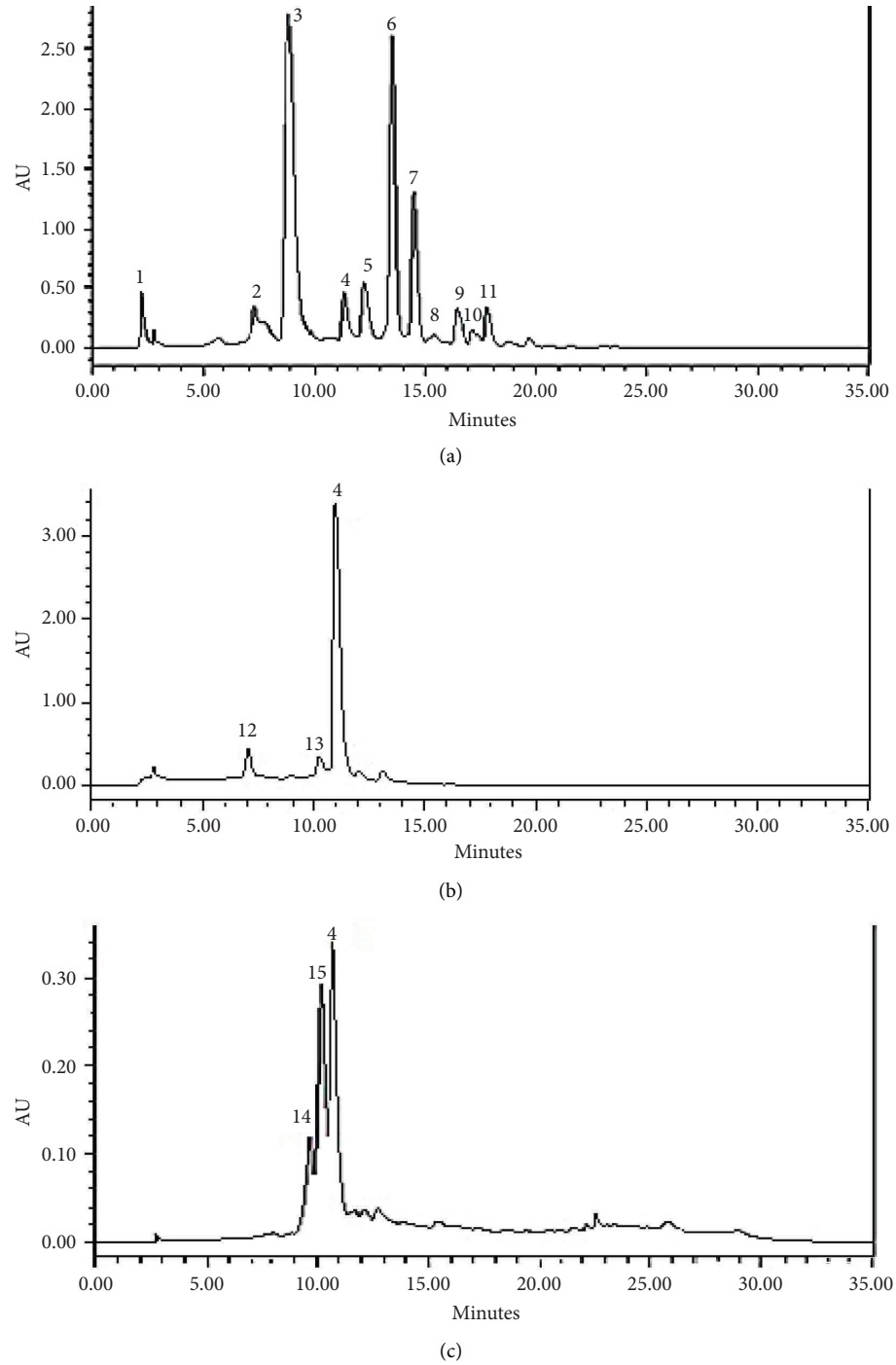

Figure 7: HPLC-DAD chromatograms of STG (a), TPL (b), and LV (c). STG, hydroethanolic extract of stigmas; TPL, hydroethanolic extract of tepals; LV, hydroethanolic extract of leaves. 1: picrocrocin; 2: kaempferol glucoside; 3: picrocrocin acid form; 4: kaempferol; 5: *trans*-crocin-5; 6: *trans*-crocin-4; 7: *trans*-crocin-3; 8: *trans*-crocin-2; 9: *cis*-crocin-4; 10: *cis*-crocin-3; 11: *trans*-crocin-1; 12: isorhamnetin; 13: quercetin; 14: hesperidin; 15: mangiferin.

Table 2: Chemical composition of STG (A), TPL (B), and LV (C).

Peak number	Compound	Retention time (min)	% of area
A			
1	Picrocrocin	2.225	2.84
2	Kaempferol glucoside	7.328	3.44
3	Picrocrocin acid form	8.871	41.39
4	Kaempferol	11.340	4.11
5	*trans*-Crocin-5	12.296	5.59
6	*trans*-Crocin-4	13.503	20.54
7	*trans*-Crocin-3	14.47	10.03
8	*trans*-Crocin-2	15.426	0.63
9	*cis*-Crocin-4	16.435	2.79
10	*cis*-Crocin-3	17.177	1.13
11	*trans*-Crocin-1	17.748	2.6
B			
12	Isorhamnetin	7.051	6.12
13	Quercetin	10.223	5.72
4	Kaempferol	11.035	76.97
C			
14	Hesperidin	9.668	13.54
15	Mangiferin	10.15	41.52
4	Kaempferol	10.721	44.94

Crocin-1, ester-monoglucoside–crocétine; Crocin-2, ester-di-(β-D-glucoside)–crocétine; Crocin-3, ester-(β-D-glucoside)-(β-gentiobioside)–crocétine; Crocin-4, ester-di-(β-D-gentiobioside)–crocétine; Crocin-5, ester-(β-D-triglucoside)-(β-D-gentiobioside)–crocétine; STG, hydroethanolic extract of stigmas; TPL, hydroethanolic extract of tepals; LV, hydroethanolic extract of leaves.

5. Conclusion

In conclusion, the data obtained have provided evidence for the antispasmodic and myorelaxant activities of the hydroethanolic extracts of *C. sativus* tepals mediated possibly via the calcium channel inhibition but not on the guanylate cyclase and nitric oxide pathways. The action of one or several substances synergistically found in *C. sativus* may explain the traditional use of *C. sativus* to treat various disorders such as colic and diarrhea by regulating the contractile response and will allow us to explore future possibilities for the treatment of bowel spasms using natural molecules derived from saffron.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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