



Research Article

Spasmolytic and Antimicrobial Activities of Crude Extract of *Bongardia chrysogonum* L. Tubers

^{1,2}S. Abuhamdah, ³A. Shatarat, ⁴M. Al-Essa, ⁵H. Al-Ameer, ⁶S. Al-Olimat and ⁷P. Chazot

¹College of Pharmacy, Al-Ain University of Science and Technology, Abu Dhabi, United Arab Emirates

²Department of Biopharmaceutics and Clinical Pharmacy, Faculty of Pharmacy, The University of Jordan, Amman, Jordan

³Department of Anatomy and Histology, Faculty of Medicine, The University of Jordan, Amman, Jordan

⁴Department of Physiology and Biochemistry, Faculty of Medicine, The University of Jordan, Amman, Jordan

⁵Department of Pharmacology, Faculty of Medicine, The University of Jordan, Amman, Jordan

⁶Department of Medicinal Chemistry and Pharmacognosy, Faculty of Pharmacy, Jordan University of Science, Irbid, Jordan

⁷Department of Biosciences, Durham University, Durham, United Kingdom

Abstract

Background and Objective: Although *Bongardia chrysogonum* L. (family Berberidaceae) has been used in many countries in traditional medicine to treat gastrointestinal tract disorders, scientific evidence for this treatment is lacking. The present study was carried out to assess the antimicrobial activity and to evaluate the antispasmodic potential of *B. chrysogonum* tuber extract.

Materials and Methods: Extracts were also evaluated for antimicrobial potential against *Bacillus subtilis*, *Staphylococcus aureus*, *S. epidermidis*, *Escherichia coli* and *Pseudomonas aeruginosa* using the agar well diffusion method. Minimum inhibitory concentration and minimum bactericidal concentration were determined by using the micro dilution method. To evaluate the antispasmodic activity of *B. chrysogonum* extract on contractions induced by the spasmogens acetylcholine, BaCl₂ and KCl, contractions of rat duodenum were recorded using an isolated tissue bath chamber with an isotonic transducer and oscillographic device. Results were compared by one-way analysis of variance (ANOVA) followed by Dunnett's post test. **Results:** Preliminary phytochemical screening of the tuber extract revealed the presence of alkaloids, saponins, tannins and carbohydrates, including monosaccharides. No antimicrobial activity was observed against gram-positive or gram-negative bacteria; however, ethanolic *B. chrysogonum* tuber extract attenuated the basal contractile activity of rat duodenum smooth muscle and decreased contractions induced by acetylcholine, KCl and BaCl₂, suggesting an antimuscarinic effect and/or interference of calcium influx. Alkaloids and saponins present in the extract may account for this antispasmodic effect, suggesting multiple mechanisms that can be explored in future studies. **Conclusion:** Taken together, results demonstrate that *B. chrysogonum* tuber extract possesses antispasmodic activity but not antimicrobial activity and supports its traditional use to alleviate gastrointestinal spasms.

Key words: *Bongardia chrysogonum*, spasmolytic, antimicrobial, phytochemical screening, rat duodenum

Received:

Accepted:

Published:

Citation: S. Abuhamdah, A. Shatarat, M. Al-Essa, H. Al-Ameer, S. Al-Olimat and P. Chazot, 2017. Spasmolytic and antimicrobial activities of crude extract of *Bongardia chrysogonum* L. tubers. Int. J. Pharmacol., CC: CC-CC.

Corresponding Author: Sawsan Abuhamdah, College of Pharmacy, Al-Ain University of Science and Technology, P.O. Box 112612, Abu Dhabi, UAE
Tel: +971-2-4444696/228 Fax: 00971-2-444304

Copyright: © 2017 S. Abuhamdah *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diarrhea and irritable bowel syndrome are common functional disorders of the intestine that affect millions of people. Abdominal pain and cramps are typical symptoms¹. In Jordan and other developing countries, medicinal plants are considered effective treatment for gastrointestinal disorders such as diarrhea and irritable bowel syndrome, particularly in rural areas². Although synthetic drugs are available for these disorders, all have side effects that necessitate the search for new drugs.

Traditional medicinal plants have increasing applications and are used to develop medicine with fewer side effects. The antidiarrheal activities of plant extracts can be mediated through spasmolytic effects (intestinal smooth muscle relaxation), delayed gastrointestinal transit, slowed gut motility, increased water absorption or decreased electrolyte secretion, all of which are related to regulation of enteric nervous system³. However, scientific studies evaluating these therapeutic claims and elucidating the mechanisms of action are lacking for many of the medical plants used as antidiarrheal treatment in traditional medicine. For example, the ethnopharmacology, chemical properties and pharmacological activities of the Jordanian plant *Bongardia chrysogonum* L. (family Berberidaceae) have not yet been reported. Tubers of this plant are used in traditional medicine to treat epilepsy and gastrointestinal disorders because of their spasmolytic activity⁴. In addition, both rural communities

and researchers describe the effectiveness of these tubers for the treatment of urinary tract infections, hemorrhoids, prostatic hypertrophy, hypercholesterolemia^{5,6} and cancer⁷. The tubers are also reported to have potent hypoglycemic effects, making them useful for glucose homeostasis⁸, in addition to multiple anti-inflammatory effects⁶.

Bongardia chrysogonum, commonly known as (-Uruf-el-Deek-), is a small perennial plant that is wide spread in the eastern Mediterranean region⁹, Iran, Turkey, Afghanistan and North Africa¹⁰. The plants have 20-80 cm erect scapes and tuberous rhizomes that are 2-5 cm in diameter. The 1-3 radical leaves are imparipinnate or deeply pinnatisect and petiolate, 10-25 cm long with a petiole about 1/4 as long. The leaves are horizontally spreading, with lateral pinnae in 3-8 opposite pairs and a slightly larger terminal pinna closely subtended by a pair of lateral pinnae (Fig. 1).

Previous studies have reported the presence and isolation of secondary plant metabolites such as alkaloids and triterpenoids¹¹⁻¹⁵. However, despite the wide spread use of these tubers, detailed studies have not been conducted to determine their phytochemical composition and biological properties. Therefore, the aim of this research was to determine the phytochemical constituents, antimicrobial activities and possible antispasmodic activity of *B. chrysogonum* (BC) extract. The results may provide support for the traditional use of this plant to treat intestinal disorders. This is the first study to describe antispasmodic activity of this plant.



Fig. 1: *Bongardia chrysogonum* L. plant

MATERIALS AND METHODS

Reagents: Drugs and analytical grade chemicals were purchased from Sigma (St. Louis, MO, USA). All drugs were dissolved in distilled water and dilutions were prepared fresh on the day of the experiment.

Animals: Male Wistar rats (250 ± 8.11 g) were obtained from the Faculty of Medicine, University of Jordan, subjected to a 24 h fast and given access to water *ad libitum* prior to experimentation. All procedures involving animals were carried out in accordance with Jordanian regulations for animal experimentation and care. The protocol was reviewed and approved by the laboratory animal ethics committee of the Scientific Research Council at the Deanship of Academic Research (protocol and ethics approval number UJ 218/2013-2014). The study commenced on December 1, 2014 and lasted for 24 months. All experimentation was carried out at the pharmacology research lab in the Faculty of Medicine, The University of Jordan, Amman, Jordan. All efforts were made to minimize animal suffering and reduce the numbers of animal used.

Duodenum preparation: Duodenum preparation was carried out according to the method described by Shatarat *et al.*¹⁶. After pretreating the tissue with BC extract or control, the cumulative concentration response curve obtained using acetylcholine (ACh) chloride, potassium chloride (KCl) and barium chloride (BaCl_2) was recorded isotonicity and the effect was allowed to reach a steady state at each concentration.

Plant materials and extraction: *Bongardia chrysogonum* tubers were collected in Jordan (15 km of Northwest Amman). The collected material was identified by Professor Suleiman Al-Olimat, Department of Pharmaceutical Sciences, Faculty of Pharmacy, Jordan University of Science and Technology, Irbid, Jordan. A voucher for each plant material has been deposited in the herbarium of the University for Future Reference (BC No. 5450).

Tubers were selected, dried completely under shade and then ground to a fine powder. Crude extracts were prepared by refluxing 500 g of the powdered plant material with 80% ethanol or chloroform for 3 h and maintaining the extract for 5 days at room temperature with continuous shaking (Labtech, Korea). The crude extracts were then passed through 125 mm filter paper (Albet, EEC). The volume of each filtered

solution was increased to 100 mL with solvent and then the solvent was evaporated under reduced pressure using a rotary evaporator (Heidolph Laborota, Germany) to obtain 10% crude extracts (100 mg mL^{-1}). The extracts were further dried by incubating at room temperature for 8 days. The crude extracts were either used directly or stored in an air-tight container for further use.

Assessment of antispasmodic activity: After stabilization for 30 min, the antispasmodic effect of the BC tuber ethanol extract was evaluated by using the following procedures.

Relaxant effect of BC tuber extract on ACh-induced contractions: After immersion and equilibration of the duodenum in the organ bath, the dose-response curve was obtained for ACh. An increase in ACh concentration resulted in increased smooth muscle contraction and the submaximal contractile response was obtained at 3×10^{-5} M. This concentration was selected for the cumulative concentration response curves. Increasing concentrations of BC ethanol extract were added to the organ bath 2 min before ACh administration and the effect of the BC extract was expressed as a percentage of the ACh-induced contraction.

Relaxant effect of BC tuber extract on BaCl_2 -induced contractions: Freshly isolated duodenum was also exposed to 5 mM BaCl_2 to induce contractions. Increasing concentrations of BC ethanol extract were added to the organ bath 2 min before the addition of BaCl_2 and the effect of the BC extract was expressed as a percentage of the BaCl_2 -induced contraction.

Relaxant effect of BC tuber extract on KCl-induced contractions: To determine Ca^{2+} antagonist activity, 60 mM KCl was added to the organ bath to produce sustained contractions. Increasing concentrations of BC ethanol extract were then added to evaluate its inhibitory effects, which were expressed as a percentage of the KCl-induced contractions.

Solvent effect on rat duodenum contractions: The effect of the solvent (80% ethanol) on smooth muscle contractions in the isolated rat duodenum was evaluated by adding up to 100 μL ethanol to the organ bath.

Antimicrobial activity: The agar well diffusion method was used to evaluate antimicrobial activity of the BC extracts¹⁷ against *Bacillus subtilis* (ATCC 11562), *Staphylococcus aureus*

(ATCC 6538), *Staphylococcus epidermidis* (ATCC 12228), *Escherichia coli* (ATCC 29425) and *Pseudomonas aeruginosa* (ATCC 11921). Petri plates were filled with 75 mL seeded agar, which was allowed to solidify. Freshly prepared bacterial inoculum was evenly spread using a sterile cotton swab over the entire agar surface. A 6 mm hole was then punched with a sterile cork borer and 100 μ L each of ethanolic and aqueous crude BC tuber extract was poured into the well. The Petri plates were allowed to stand at room temperature for 1 h and then incubated at 37°C overnight. The solvent dimethyl sulfoxide (DMSO) was used to fill the well as a negative control and 1 mg mL⁻¹ norfloxacin was used as a positive control. The zone of inhibition (average diameter) after 24 h was determined for each treatment and compared with the controls.

Determination of minimum inhibitory concentration and minimum bactericidal concentration: To confirm the results of the agar diffusion assay, the Minimum Inhibitory Concentration (MIC) of both BC extracts was determined by the microdilution method¹⁸ using nutrient broth. The MIC is defined as the lowest concentration of an antimicrobial that inhibits visible growth of a microorganism after overnight incubation. Plant extracts were dissolved in 10% DMSO and two fold dilutions were prepared with nutrient broth. The nutrient broth containing plant extract was inoculated with 10 μ L bacterial suspension (5×10^6 CFU mL⁻¹), with nutrient broth containing DMSO serving as a negative control. To evaluate bacterial growth, 270 mg resazurin was dissolved in 40 mL sterile water and 10 μ L of the resazurin solution was added to each sample and incubated for 24 h at 37°C. Bacterial growth was detected by reading absorbance at 500 nm. Bacterial growth was indicated by a color change from purple to pink or colorless (assessed visually). The MIC was defined as the lowest plant extract concentration at which the color changed or the highest dilution that completely inhibited bacterial growth.

The samples were then spread on fresh solid media and incubated for 18 h to determine the Minimum Bactericidal Concentration (MBC) values (i.e., lowest concentration that prevents the growth of an organism). The lowest plant extract concentration that prevented all bacterial growth was reported as the MBC. The MIC and MBC were determined in triplicate experiments.

Preliminary phytochemical analysis: Qualitative phytochemical analysis of the ethanolic and chloroform BC tuber extracts was performed according to the standard

procedures described by Harborne¹², Trease and Evans¹⁸ and Wagner *et al.*¹⁹. The analysis consisted of the foam test for saponins^{12,19}, Liebermann-Burchard test for terpenoids and triterpenoids¹⁸, FeCl₃ and lead subacetate tests for tannins^{12,19}, Shinoda's test for flavonoids¹⁹, Borntrager's test for anthraquinones^{12,19}, Keller-Killiani test for cardiac glycosides¹⁸, Molisch's test for carbohydrates¹², Fehling's test for reducing sugars¹², Barfoed's test for monosaccharides¹², Millon's test for proteins¹², ninhydrin test for amino acids¹² and Mayer's test, Dragendorff's test, Wagner's test and 1% picric acid for alkaloids^{12,19}. The results are expressed as (+) for the presence and (-) for the absence of the phytochemical.

Statistical analysis: Results are expressed as Mean \pm Standard Error of the Mean (SEM) of 4-6 replicates, as indicated in the figure legends. Results were compared by one-way analysis of variance (ANOVA), followed by the post hoc Dunnett's multiple comparison test¹⁹, $p < 0.05$ was considered significant. Statistical analysis was performed using GraphPad Prism version 5.02 for Windows (GraphPad Software, San Diego, CA, USA).

RESULTS

Effect of BC ethanolic extract on spontaneous contraction in isolated rat duodenum: In all experiments, the addition of ACh, BaCl₂ (5 mM) or KCl (60 mM) caused a rapid contraction in the duodenum, which returned to the plateau state immediately. This contraction of duodenum (without BC tuber extract) was considered 100%.

Effect of BC tuber extract on ACh-induced contractions in isolated rat duodenum: A single application of 3×10^{-5} M ACh evoked a contraction of 100% (Fig. 2), which was inhibited in a concentration-dependent manner by 0.15- 2.5 mg mL⁻¹ BC tuber extract. This relaxant effect was reversible and the spontaneous activity returned to normal after washing the preparation.

Effect of BC tuber extract on BaCl₂-induced contractions of isolated rat duodenum: The contractile response produced by a single application of BaCl₂ (5 mM) was considered 100% ($n = 6$). The results showed that BC tuber extracts (0.15-2.5 mg mL⁻¹) decreased the contractile response evoked by BaCl₂. No significant decrease was observed with 0.15 mg mL⁻¹ BC extract; however, contractions were 84.4% of the control ($p < 0.01$) with 0.5 mg mL⁻¹ extract (Fig. 3).

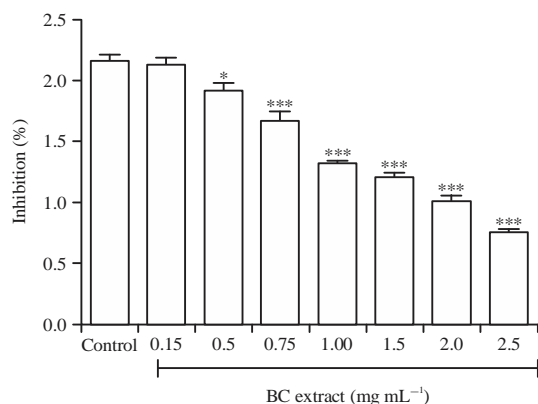


Fig. 2: Concentration-dependent inhibitory effects of crude BC tuber extract on spontaneous contractions and contractions induced by 3×10^{-5} M ACh in isolated rat duodenal smooth muscle

Results are expressed as Mean \pm SEM (n = 4), *p<0.01, ***p<0.001 (ANOVA followed by Dunnett's test, IC₅₀= 1.25 mg mL⁻¹)

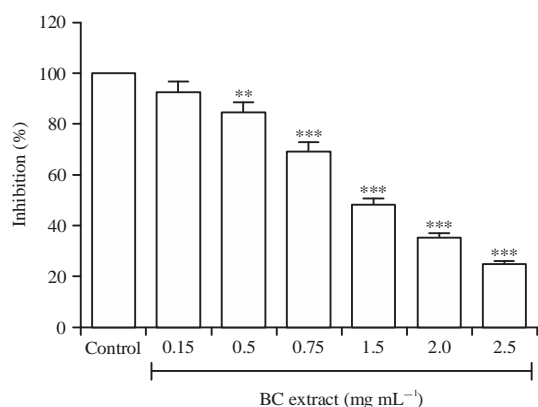


Fig. 3: Concentration-dependent inhibitory effects of crude BC tuber extract on spontaneous contractions and contractions induced by 5 mM BaCl₂ in isolated rat duodenal smooth muscle

Results are expressed as Mean \pm SEM (n = 6), **p<0.01, ***p<0.001 (ANOVA followed by Dunnett's test, IC₅₀ = 1.5 mg mL⁻¹)

Effect of BC tuber extract on KCl-induced contractions in isolated rat duodenum:

The addition of KCl (60 mM) caused sustained contractions (n = 4) in isolated rat duodenum (Fig. 4). The KCl-induced contractions were not significantly affected by 0.15-2 mg mL⁻¹ BC extract; however, 250 mg mL⁻¹ BC tuber extract decreased KCl-induced contractile responses to 53 \pm 6% that of the control.

Solvent effect on rat duodenum contraction: To determine whether the solvent used to prepare the BC tuber extract (70% v/v ethanol) could induce smooth muscle contraction,

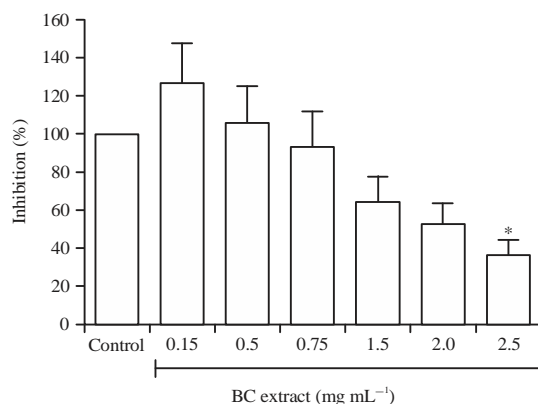


Fig. 4: Concentration-dependent inhibitory effects of crude BC tuber extract on spontaneous contractions and contractions induced by 60 mM KCl in isolated rat duodenal smooth muscle

Results are expressed as Mean \pm SEM (n = 4), *p<0.01 (ANOVA followed by Dunnett's test)

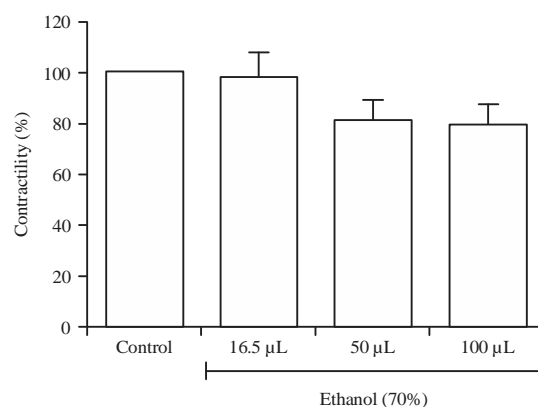


Fig. 5: Effect of 70% ethanol on isolated rat duodenum compared with control (no treatment)

Results are expressed as Mean \pm SEM (n = 4)

up to 100 μ L ethanol was added to the organ bath. Spontaneous rhythmic contractions were not significantly affected by the addition of ethanol (Fig. 5).

Antimicrobial activity: The antimicrobial potential of BC was qualitatively evaluated by the presence or absence of inhibition zones and the inhibition zone diameter. The MIC and MBC were determined by using the agar well diffusion method, with norfloxacin serving as a positive control. Results summarized in Table 1 and 2 show that ethanolic BC tuber extract did not inhibit the growth of either Gram-positive or Gram-negative bacteria. Using ethanolic and aqueous tuber extracts, no antimicrobial activity was detected against *B. subtilis*, *S. aureus*, *S. epidermidis*, *E. coli* or *P. aeruginosa*,

Table 1: Antimicrobial activity recorded as zone of inhibition of *Bongardia chrysogonum* extracts against selected bacterial strains, using norfloxacin as a positive control

Microorganisms	Zone of inhibition (mm)		
	Ethanollic extract	Aqueous extract	Norfloxacin
<i>Bacillus subtilis</i>	1.00±0.10	1.0±0.02	25.11±0.40
<i>Staphylococcus aureus</i>	3.00±0.30	2.5±0.03	22.05±0.35
<i>Staphylococcus epidermidis</i>	4.08±0.02	2.0±0.01	26.02±0.20
<i>Escherichia coli</i>	2.04±0.03	0.5±0.01	19.40±0.05
<i>Pseudomonas aeruginosa</i>	1.00±0.00	0.3±0.05	17.11±0.03

Results are expressed as Mean ± SEM

Table 2: Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC) of *Bongardia chrysogonum* extracts against selected bacterial strains, using norfloxacin as a positive control

Microorganisms	Ethanollic extract (µg mL ⁻¹)		Aqueous extract (µg mL ⁻¹)		Norfloxacin (µg mL ⁻¹)	
	MIC	MBC	MIC	MBC	MIC	MBC
<i>Bacillus subtilis</i>	620±7.0	800±11	600±7.0	790±10	3±0.4	10±0.05
<i>Staphylococcus aureus</i>	750±2.1	850±19	780±5.2	900±14	1±0.11	3±0.03
<i>Staphylococcus epidermidis</i>	900±7.8	950±4.0	800±7.8	890±4.0	2±0.4	9±0.4
<i>Escherichia coli</i>	820±2.5	850±7.0	855±4.2	820±7.0	5±0.9	15±14
<i>Pseudomonas aeruginosa</i>	950±4.2	980±5.6	900±4.2	890±7.4	20±12	90±18

Results are expressed as Mean ± SEM

Table 3: Qualitative phytochemical screening of *Bongardia chrysogonum* tubers

No	Phytoconstituents	Tests/reagents used	Ethanollic extract	Chloroform
1	Carbohydrates	Molisch's test	0	0
2	Reducing sugars	Fehling test	-	-
3	Monosaccharides	Barfoed's test	0	0
4	Proteins	Millon's test	-	-
5	Amino acids	Ninhydrin test	-	-
6	Flavonoids	Shinoda's test	-	-
7	Terpenes/steroids	Liebermann-Burchard's test	-	-
8	Alkaloids	Mayer's test	+++	0
		Dragendorff's test	+++	0
		Wagner's test	+++	0
		Picric acid (1%) test	0	0
9	Cardiac glucoside	Keller-Killani test	-	-
10	Anthraquinones	Borntrager's test	-	-
11	Tannins	Ferric chloride test	0	0
		Lead subacetate test	0	0
12	Saponins	Foam test	+++	-

+++ : Highly present, ++ : Moderately present, + : Slightly present, - : Absence of bioactive compound

suggesting that the plant tuber lacks antimicrobial activity. This is the first study reporting antimicrobial activity of this plant species against selected bacteria strains under these assay conditions.

Preliminary phytochemical analysis: Preliminary phytochemical screening of ethanollic and chloroform BC tuber extracts revealed the presence of the following secondary metabolites: Alkaloids, tannins, monosaccharides and other carbohydrates (Table 3). Saponins were detected only in the ethanollic extract. Analysis of the chloroform BC tuber extract showed relatively small levels of alkaloids and

tannins, whereas the ethanollic extract showed relatively high levels of alkaloids, saponins and tannins.

DISCUSSION

Our results (Fig. 2-5) showed that BC tuber extract was able to not only relax the tone of spontaneous contractions in the isolated rat duodenum but also attenuated the spasmogenic effects of ACh, BaCl₂ and KCL at high concentrations. These spasmogens cause contractions by different mechanisms. The spasmolytic effect of BC tuber extract was reversible and the spontaneous activity returned

to normal after washing the tissue three times. Furthermore, the effect of the ethanolic extract was concentration-dependent.

ACh, KCl and BaCl₂-induced contractions are commonly used to analyze the spasmolytic activity and/or mechanism of action of drugs and plant extracts *in vitro*. The effects of ACh are receptor-mediated, whereas the effects of KCl are Ca²⁺ channel-mediated and BaCl₂ is a nonspecific smooth muscle spasmogen²⁰⁻²³. The rhythmic contractions of smooth muscle cells depend on an endogenous pacemaker driven by the cytosolic Ca²⁺ oscillator, which is responsible for the periodic release of Ca²⁺ from the endoplasmic reticulum. These pulses of Ca²⁺ often cause membrane depolarization²⁴. The study showed that BC extract inhibits ACh-induced contractions in a dose-dependent manner, suggesting an anticholinergic action. ACh-induced contractions in the rat ileum involve two different mechanisms involving muscarinic receptors²⁵. The ACh binds to M₂ and M₃ receptors in the gut tissue, causing smooth muscle contraction by promoting calcium influx via receptor-operated calcium channels²⁶. In addition, ACh promotes inositol triphosphate synthesis via phospholipase c activation, which in turn increases calcium release from the sarcoplasmic reticulum²⁴. The results showed that ACh alone causes contractions in isolated rat duodenum, and ethanolic BC tuber extract markedly decreased ACh-induced contractions. This result suggests that ethanolic BC tuber extract exerts strong spasmolytic activity by blocking cholinergic receptors, which may therefore be a potential target for BC.

High barium ion concentrations in the extracellular fluid depolarize the smooth muscle membrane and open voltage-dependent calcium channels, resulting in calcium influx²³. The results of this study showed that BC tuber extract significantly p<0.05 inhibited BaCl₂-induced contractions. This inhibitory effect did not involve a decrease in smooth muscle myofilament sensitivity to Ca²⁺, because the effect was not reversed by washing. It is possible that BC tuber extract contains compounds that interfere with Ca²⁺ channel activity or with Ca²⁺ ion release from intracellular stores.

L-type Ca²⁺ channels have been identified in the rat intestine. High K⁺ stimulation provokes membrane depolarization and is the most common method used to introduce Ca²⁺ into the cell without receptor stimulation^{22,23}. It has been reported that high K⁺ concentrations (>30 mM) open voltage-dependent calcium channels, causing Ca²⁺ influx and smooth muscle contraction²⁰. In this study, 60 mM K⁺ was used to cause depolarization of the tissue. The addition of BC tuber extract inhibited the K⁺-induced contractions only at the highest concentration tested (2.5 mg mL⁻¹).

BaCl₂ depolarizes the smooth muscle membrane and opens voltage-dependent Ca⁺ channels, resulting in Ca²⁺ influx. The BC tuber extract significantly p<0.05 inhibited BaCl₂-induced contractions. This relaxant effect of BC tuber extract on BaCl₂-induced contractions was concentration-dependent and greater than its effect on KCl-induced contractions. These results suggest that the antispasmodic activity of BC tuber extract may be at least partially mediated by targeting voltage-dependent Ca⁺ channels.

Phytochemical studies of the BC tuber demonstrated the presence of alkaloids¹¹⁻¹³ and saponins¹⁴ as the main secondary metabolites, with potential biological activities. Therefore, the spasmolytic activity of BC tuber extract observed in this study may be attributed in part to these metabolites. However, additional studies are needed to test this hypothesis. Antispasmodic activity could be attributed individual bioactive constituents or may be the result of a general inhibitory effect of multiple constituents that inhibit Ca²⁺ influx.

Preliminary phytochemical screening is useful to detect bioactive compounds and may lead to drug discovery and development. Results of these tests may also provide evidence for the traditional medical uses of *B. Chrysogonum* and claims about its antispasmodic effects. Future studies are needed to isolate, identify, characterize and elucidate the structure of bioactive compounds of BC.

No detectable antimicrobial activity against gram-positive and gram-negative bacteria was observed for crude BC tuber extract using the agar disk diffusion and microdilution assays. These negative results may be due to the nature of the phytochemical components of the plant and the bacteria used in the antimicrobial tests. The three major chemical constituents of pharmacological interest in the BC tuber extract were saponins, alkaloids and tannins, all of which contribute considerably to its biological activity. Previous studies report that saponins show only weak or no growth inhibition against both gram-positive and gram-negative bacteria (MIC>000 g mL⁻¹)^{27,28}. Alkaloids can have potent antimicrobial activities but this effect depends on their chemical nature, their structural class and the type of bacteria tested²⁹, which could explain the negative antimicrobial assay results. Tannins have potential as antibacterial compounds when used alone³⁰ but may be present in insufficient concentrations in the BC tuber extracts to exert antimicrobial effects. In addition, other phytochemicals present in this plant may alter this activity.

No previous studies have published results of phytochemical screening, anti microbial assays or antispasmodic tests of *B. chrysogonum*. Results of this preliminary study indicate the need for future research to

elucidate the bioactive constituents of this plant with respect to the pharmacological activities studied here.

CONCLUSION AND FUTURE RECOMMENDATION

This study showed that BC tuber extract exhibits significant antispasmodic activity on isolated rat duodenum, providing a scientific basis for the folk medicinal use of *B. chrysogonum* in the treatment of gastrointestinal disorders. Although no antimicrobial activity of the plant extract was detected, phytochemical analysis and published data demonstrate the presence of alkaloids and saponins, which may account for the antispasmodic activity. The phytochemical compounds identified in this study may be responsible for the beneficial effects of *B. chrysogonum*. Extracts from this plant may therefore serve as a good source for useful drugs; however, future research is needed to isolate, purify and characterize the bioactive constituents responsible for the antispasmodic activity of *B. chrysogonum*.

SIGNIFICANCE STATEMENTS

This study investigated the antimicrobial properties and antispasmodic potential of *Bongardia chrysogonum* L. tuber extract in an isolated rat duodenum model, demonstrating that this extract may have beneficial effects as a novel spasmolytic. This study lends pharmacological credence to the folkloric, ethnomedical use of these plant tubers as a natural remedy for the treatment of abdominal cramps and various gastrointestinal tract disorders.

ACKNOWLEDGMENTS

This project was sponsored by the Deanship of Scientific Research at the University of Jordan (grant number SRF/JU/218/2013-2014). I would like to extend my thanks to the University of Jordan for giving me (main author) a sabbatical leave for the academic year 2016-2017.

REFERENCES

1. Gurib-Fakim, A., 2006. Medicinal plants: Traditions of yesterday and drugs of tomorrow. *Mol. Aspects Med.*, 27: 1-93.
2. Abu-Irmaileh, B.E. and F.U. Afifi, 2003. Herbal medicine in Jordan with special emphasis on commonly used herbs. *J. Ethnopharmacol.*, 89: 193-197.
3. Kumar, D., K. Ganguly, H.V. Hegde, P.A. Patil and S.D. Kholkute, 2015. Spasmolytic effect of traditional herbal formulation on guinea pig ileum. *J. Ayurveda Integr. Med.*, 6: 194-197.

4. Baydoun, S., L. Chalak, H. Dalleh and N. Arnold, 2015. Ethnopharmacological survey of medicinal plants used in traditional medicine by the communities of Mount Hermon, Lebanon. *J. Ethnopharmacol.*, 173: 139-156.
5. Oran, S.A. and D.M. Al-Eisawi, 2015. Ethnobotanical survey of the medicinal plants in the central mountains (North-South) in Jordan. *J. Biodiver. Environ. Sci.*, 6: 381-400.
6. Arslan, A., E.A. Cakmak, M. Ozaslan, B. Cengiz and C. Bagci *et al.*, 2005. The effects of *Bongardia chrysogonum* (L.) spach extract on the serum parameters and liver, kidney and spleen tissues in rats. *Biotechnol. Biotechnol. Equip.*, 19: 170-179.
7. Assaf, A.M., R.N. Haddadin, N.A. Aldouri, R. Alabbassi, S. Mashallah, M. Mohammad and Y. Bustanji, 2013. Anti-cancer, anti-inflammatory and anti-microbial activities of plant extracts used against hematological tumors in traditional medicine of Jordan. *J. Ethnopharmacol.*, 145: 728-736.
8. Dokuyucu, R., K.H. Gozukara, O. Ozcan, N.K. Sefil, A. Nacar, A. Dokuyucu and M. Inci, 2016. The effect of *Bongardia chrysogonum* on prostate tissue in a rat model of STZ-induced diabetes. *SpringerPlus*, Vol. 5. 10.1186/s40064-016-2973-z.
9. Arnold, N., S. Baydoun, L. Chalak and T. Raus, 2015. A contribution to the flora and ethnobotanical knowledge of Mount Hermon, Lebanon. *Flora Mediterr.*, 25: 13-55.
10. Karl, R. and A. Strid, 2009. *Bongardia chrysogonum* (Berberidaceae) rediscovered on the East Aegean island of Chios. *Phytol. Balcanica*, 15: 337-342.
11. Dunnett, C.W., 1955. A multiple comparison procedure for comparing several treatments with a control. *J. Am. Stat. Assoc.*, 50: 1096-1121.
12. Harborne, J.B., 1984. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. 3rd Edn., Chapman and Hall, London, UK., ISBN-13: 9780412572708, pp: 84-274.
13. Alfatafta, A.A., M.H. Abu Zarga, S.S. Sabri, A.J. Freyer and M. Shamma, 1989. An investigation of *Bongardia chrysogonum*. *J. Nat. Prod.*, 52: 818-821.
14. Atta-ur-Rahman, D. Shahwar, M. Iqbal Choudhary, B. Sener, G. Toker and K.H.C. Baser, 1998. New alkaloids from *Bongardia chrysogonum*. *Nat. Prod. Lett.*, 12: 161-173.
15. Baser, K.H.C., G. Toker and B. Sener, 1993. Saponins from *Bongardia chrysogonum* (L.) Spach. growing in Turkey. *Acta Horticulturae*, 333: 175-180.
16. Shatarat, A., S. Abuhamdah, M. Al-Essa, F. Mohammad and S. Al-Olimat, 2014. Pharmacological effects of Peganum harmala L. Root Extract on Isolated Rat small Intestine. *Pharmacogn. Commun.*, 4: 56-61.
17. Atta-ur-Rahman, D. Shahwar, M.I. Choudhary, B. Sener, G. Toker and K.H.C. Baser, 2000. Triterpenoid saponins from *Bongardia chrysogonum*. *J. Nat. Prod.*, 63: 251-253.
18. Trease, G.E. and M.C. Evans, 1983. *Textbook of Pharmacognosy*. Bailliere Tindall, London.

19. Wagner, H., S. Bladt and E.M. Zgainski, 1984. Plant Drug-Analysis a Thin Layer Chromatography Atlas. Springer, New York.
20. Kishore, D.V. and R. Rahman, 2012. Spasmolytic activity of *Casuarina equisetifolia* bark extract. Int. J. Pharm. Sci. Res., 3: 1452-1456.
21. Karamenderes, C. and S. Apaydin, 2003. Antispasmodic effect of *Achillea nobilis* L. subsp. *sipylea* (O. Schwarz) Bessler on the rat isolated duodenum. J. Ethnopharmacol., 84: 175-179.
22. Brankovic, S., D. Kitic, M. Radenkovic, S. Veljkovic, T. Jankovic, K. Savikin and G. Zdunic, 2011. Spasmolytic activity of the ethanol extract of *Sideritis raeseri* spp. *raeseri* Boiss. & Heldr. on the isolated rat ileum contractions. J. Med. Food, 14: 495-498.
23. Zhang, W.W., Y. Li, X.Q. Wang, F. Tian, H. Cao, M.W. Wang and Q.S. Sun, 2005. Effects of magnolol and honokiol derived from traditional Chinese herbal remedies on gastrointestinal movement. World J. Gastroenterol., 11: 4414-4418.
24. Moradi, M.T., M. Rafieian-Koupaei, R. Imani-Rastabi, J. Nasiri, M. Shahrani, Z. Rabiei and Z. Alibabaei, 2013. Antispasmodic effects of yarrow (*Achillea millefolium* L.) extract in the isolated ileum of rat. Afr. J. Tradit Complement Altern. Med., 10: 499-503.
25. Borgi, W. and N. Chouchane, 2009. Anti-spasmodic effects of *Zizyphus lotus*(L.) Desf. extracts on isolated rat duodenum. J. Ethnopharmacol., 126: 571-573.
26. Iorizzi, M., V. Lanzotti, G. Ranalli, S. de Marino and F. Zollo, 2002. Antimicrobial furostanol saponins from the seeds of *Capsicum annum*L. Var. *acuminatum*. J. Agric. Food Chem., 50: 4310-4316.
27. Sparg, S.G., M.E. Light and J. van Staden, 2004. Biological activities and distribution of plant saponins. J. Ethnopharmacol., 94: 219-243.
28. Kuroda, T. and W. Ogawa, 2017. Search for novel antibacterial compounds and targets. Yakugaku Zasshi, 137: 383-388.
29. Cushnie, T.P.T., B. Cushnie and A.J. Lamb, 2014. Alkaloids: An overview of their antibacterial, antibiotic-enhancing and antivirulence activities. Int. J. Antim. Agents, 44: 377-386.
30. Naz, R., H. Ayub, S. Nawaz, Z.U. Islam and T. Yasmin *et al.*, 2017. Antimicrobial activity, toxicity and anti-inflammatory potential of methanolic extracts of four ethnomedicinal plant species from Punjab, Pakistan. BMC Complem. Altern. Med., Vol. 17. 10.1186/s12906-017-1815-z.