Inhibitory Effects of Two Varieties of Tunisian Pomegranate (*Punica granatum* L.) Extracts on Gastrointestinal Transit in Rat

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ABSTRACT The present study was undertaken to determine whether total and methanol juice extracts of two Tunisian Pomegranate (*Punica granatum* L.) varieties (Garsi and Gabsi) protect against diarrhea as well as their effects on gastrointestinal transit (GIT) in healthy rats. In this respect, male Wistar rats were used and divided into control- and pomegranate-treated groups. The antidiarrheal activity was evaluated using the castor oil-induced diarrhea method and the GIT was assessed using charcoal meal. Our results showed that total and methanol *P. granatum* juice extracts produced a significant dose-dependent protection against castor oil-induced diarrhea. Pomegranate extracts and juice also decreased the GIT significantly and dose dependently. Importantly, the Garsi variety appeared to be more effective than the Gabsi variety on these two parameters. These findings suggest that pomegranate extracts have a potent antidiarrheal property in rats confirming their efficiency in the Tunisian traditional medicine.

KEY WORDS: • diarrhea • gastrointestinal transit • minerals • pomegranate • rat

INTRODUCTION

DIARRHEA IS A MAJOR cause of morbidity and mortality, particularly among children in developing countries, including Tunisia.¹ This disease may be caused by a disturbance of many digestive functions such as bile acids² and is characterized by the discharge of semisolid or watery fecal matter from the bowel thrice, or more frequently, in 1 day.³ Diarrhea is usually accompanied by many symptoms, like abdominal discomfort, nausea, and vomiting.⁴ Generally, diarrhea might be induced by microorganism products such as enterotoxins or a combination of secretory and osmotic mechanisms that act together to induce diarrhea.⁵ The therapy of diarrhea presents a major problem due to unpredictable side effects achieved through the use of marketed drugs.⁶ For this reason, the World Health Organization (WHO) has introduced a program for diarrheal control, which involves the use of traditional herbal medicines.

Pomegranate (*Punica granatum* L.), a fruit native in the Middle East and North Africa, including Tunisia, has gained widespread popularity as a functional food and nutraceuti-

cal source.⁷ Because of its richness in phenolic compounds, sugars, and minerals,⁸ pomegranate fruit exhibits many beneficial health effects such as antioxidant,⁹ anti-inflammatory,¹⁰ chemopreventive,¹¹ antifungal,¹² and antimicrobial.¹³ However, data on its digestive effects are too minimal. *P. granatum* was demonstrated to play a role in controlling the dietary glucose absorption in the intestinal tract by decreasing the sodium-dependent glucose transporter 1 (SGLT1) expression and may contribute to blood glucose homeostasis in the diabetic patients.¹⁴ Recently, Coursodon-Boydiddle *et al.*¹⁵ suggested that pomegranate seed oil reduces intestinal damage in a rat model of necrotizing enterocolitis.

In the present study, we investigated whether various extracts of pomegranate are able to protect against castor oil-induced diarrhea and analyzed their effects on gastrointestinal transit (GIT) in healthy rats.

MATERIALS AND METHODS

Chemicals

Clonidine, yohimbine hydrochloride, gum arabic, castor oil, and charcoal meal were from Sigma Chemical Co. (Sigma-Aldrich GmbH, Steinheim, Germany). All other chemicals were of analytical grade.

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Preparation of pomegranate juice and extracts

Pomegranate fruits (Gabsi and Garsi varieties) were cultivated from the region of Gabes (south east of Tunisia) during summer 2011. The voucher specimens (No. M0811) have been deposited with the herbarium of the Higher Institute of Biotechnology of Béja. The seeds are manually separated and rapidly crushed in an electric blender. The homogenates were used as total extract or filtered through a colander (0.5 mm mesh size) to obtain the pomegranate juice. On the other hand, a part of the seeds was dried in an incubator at 50°C for 72 h and powdered in an electric blender. The powder was extracted with methanol (0.067 g/mL) at room temperature. The extract was evaporated to dryness *in vacuo* and weighed. The total and methanol pomegranate juice extracts were stored at -80°C until use.

Mineral determination

The plant material was collected and dried in an oven set at 80°C. One gram of sample was placed in a stainless steel capsule and then calcined in a muffle furnace (Vecstar Furnaces; Chester field, United Kingdom) at 550°C for 4 h. After cooling, the ash was attacked by 5 mL of deionized water and 1 mL of HCl and subjected to the boil. The content of the capsule was then filtered into a volumetric flask of 100 mL and adjusted with deionized water. The potassium, magnesium, sodium, iron, and zinc concentrations in the samples were assessed with an atomic absorption spectrophotometer (AVANTA GBC Scientific Equipment Pty, Ltd, Victoria, Australia).

Condensed tannin determination

The content of condensed tannins in pomegranate extracts was determined utilizing the modified vanillin assay.¹⁶ Briefly, 250 μ L of the sample was added to 750 μ L of water, 1.5 mL of vanillin solution (4%), and 750 μ L of HCl 12 N. The reaction mixture was kept in the dark for 20 min and its optical density was measured at 500 nm. Catechin was used as a standard, and the results were expressed as mg of catechin equivalents (mg GCT/g DW).

Animal treatment

Adult male Wistar rats (weighing 200–220 g and housed five per cage) were purchased from the Pasteur Institute of Tunis and used in accordance with the local Ethic Committee of Tunis University for use and care of animals in conformity with the NIH recommendations. They were provided with food (standard pellet diet-Badr, Utique-TN) and water *ad libitum* and maintained in an animal house at controlled temperature (22°C $\pm$ 2°C) with a 12-h light–12-h dark cycle.

Castor oil-induced diarrhea

The antidiarrheal activity of pomegranate was evaluated according to the method of Awouters *et al.*¹⁷ modified by

Mukherjee *et al.*¹⁸ Thirteen groups of 10 animals each were fasted for 18 h and treated as follows:

Group 1 served as the control group and received physiological solution (NaCl, 0.9%, p.o.).

Group 2 served as the positive control group and received physiological solution (NaCl, 0.9%, p.o.).

Group 3 received diaretyl (10 mg/kg, i.p.).

Group 4 was pretreated with Gabsi variety pomegranate juice (4 mL/kg, b.w. p.o.).

Group 5 was pretreated with Garsi variety pomegranate juice (4 mL/kg, b.w. p.o.).

Group 6 was pretreated with Gabsi variety pomegranate total extract (300 mg/kg, b.w. p.o.).

Group 7 was pretreated with Garsi variety pomegranate total extract (300 mg/kg, b.w. p.o.).

Groups 8, 9, and 10 were pretreated with various doses of Gabsi variety pomegranate methanol extract (200, 400, and 800 mg/kg, b.w. p.o., respectively).

Groups 11, 12, and 13 were pretreated with various doses of Garsi variety pomegranate methanol extract (200, 400, and 800 mg/kg, b.w. p.o., respectively).

After 60 min, each animal, except group 1, received 1 mL of castor oil by gavages and placed in a separate cage. Animals were observed for defecation up to 4 h. Transparent plastic dishes were placed beneath each cage and the characteristic diarrheal droppings were noted.

Gastrointestinal propulsion

GIT was measured using the charcoal meal test.¹⁹ Thirteen groups of 10 animals each were used as follows:

Group 1 served as a control and received 1 mL of physiological solution (NaCl, 0.9%, p.o.).

Group 2 received yohimbine (1 mg/kg, b.w. i.p.).

Group 3 received clonidine (2 mg/kg, b.w. i.p.).

The groups (4–13) were treated in the same way as those indicated for diarrhea.

Two hours after treatment, different groups of rats received the standard charcoal meal (10% charcoal in 5% gum arabic). Animals were anesthetized 30 min later. Laparotomy was performed and the distance traveled by the meal compared to the total length of the small intestine was measured.

Statistical analysis

Data were analyzed by unpaired Student's *t*-test and are expressed as mean $\pm$ standard error of the mean or standard deviation. Data are representative of 10 independent experiments. All statistical tests were two-tailed, and a *P* value of .05 or less was considered significant.

RESULTS

Effects of pomegranate on castor oil-induced diarrhea

We demonstrated in the present study that all rats in the castor oil group produced copious diarrhea 4 h after castor oil administration. Pretreatment with pomegranate juice and

total extract significantly reduced the number of defecations (Fig. 1A). Administration of various doses of the pomegranate methanol extract (200, 400, and 800 mg/kg, b.w.) significantly and dose dependently decreased defecation rates. The standard antidiarrheal drug, diaretyl (10 mg/kg, p.o.), produced a more marked antidiarrheal effect, but less than the methanol extract for the Gabsi variety at 800 mg/kg (Fig. 1B). Generally, the Garsi variety appears to be more effective than the Gabsi variety.

Effects of pomegranate on gastrointestinal propulsion

We further looked at the effects of pomegranate as well as pharmacological agents on GIT (Fig. 2). In the control group treated with saline solution (0.9%), the charcoal meal travelled 82% of the total length of the small intestine. In rats treated with pomegranate juice and total extract, intestinal transit was significantly decreased. Indeed, the methanol extract of *P. granatum* significantly reduced the GIT in a

dose-related manner. Importantly, the Garsi variety appears to be even more effective than the Gabsi variety. Both the pharmacological agents used for testing our methodology showed that atropine (an α_2 adrenergic agonist) and yohimbine (an α_2 adrenergic antagonist) respectively increased (12%) and decreased (34%) the gastrointestinal propulsion.

Mineral content in pomegranate

Mineral content in the seeds of two varieties of Tunisian pomegranates (Gabsi and Garsi) is given in Table 1 and showed that sodium is the most abundant element in the two varieties (52–121 mg/100 g of dry matter). Potassium comes in second place (35–89 mg/100 g of dry matter) followed by calcium (15–36 mg/100 g of dry matter). The pomegranate is also rich in iron and magnesium, while zinc is present in very small quantities. However, the Garsi variety appears richer in minerals than the Gabsi variety.

FIG. 1. Effect of pomegranate juice and total extract (TE) (A) as well as methanol extract (ME) (B) on castor oil-induced diarrhea. Animals were pretreated with pomegranate juice, total extract, various doses of methanol extract (200, 400, and 800 mg/kg, respectively, D1, D2, and D3), reference molecule (diaretyl), or vehicle (NaCl 0.9%). One hour after, animals received castor oil by gavage and observed for defecation up to 4 h ($n = 10$). **a3**, $P < .001$ compared to the castor oil group; **b2**, $P < .01$ and **b3**, $P < .001$ compared to the Gabsi variety; **c1**, $P < .05$ compared to the diaretyl group.

TABLE 1. MINERAL CONTENT IN POMEGRANATE SEED

Minerals (mg/kg)	Gabsi variety	Garsi variety
Sodium	52.37 ± 11.48	121.52 ± 11.75 a3
Potassium	35.85 ± 8.35	89.71 ± 1.47 a3
Calcium	15.62 ± 1.89	36.41 ± 2.84 a3
Iron	7.16 ± 2.10	14.33 ± 2.48 a3
Magnesium	5.12 ± 1.69	11.99 ± 3.63 a2
Zinc	0.068 ± 0.018	0.044 ± 0.027

Data are expressed as means ± standard deviation (SD) (n = 10).

a2: $p < 0.01$ and **a3**: $p < 0.001$ compared Gabsi variety.

Condensed tannin content in pomegranate

We further looked at the total and condensed tannin contents (Table 2). As expected, the pomegranate methanol extract is richer in total and condensed tannins than juice and total extract.

DISCUSSION

In the present study, we evaluated the antidiarrheal effects of two varieties of Tunisian pomegranate (Gabsi and Garsi) as well as their effects on GIT in adult healthy rats.

We first demonstrated in the present work that pomegranate juice and total extract, as well as methanol fraction, protect against castor oil-induced diarrhea by inhibiting the

number of defecations when compared with the untreated group. Castor oil-induced diarrhea has been widely studied.²⁰ However, castor oil produced permeability changes in the intestinal mucosal membrane to water and electrolytes, resulting in watery luminal content that flows rapidly through the small and large intestines.^{21,22} The active component of castor oil is the ricinoleic acid,²¹ which stimulates the production of several mediator substances that include prostaglandins, nitric oxide, platelet-activating factor, cAMP, and tachykinins.²³ The induction of diarrhea by castor oil can also be attributed to the liberation of prostaglandins by colonic cells.¹⁷ Castor oil-induced diarrhea has been shown to be attenuated by many plants extracts such as *Strychnos potatorum*,²⁴ *Amaranthus spinosus*,²⁵ *Ixora coccinea*,²⁶ *Pyrenacantha staudtii*,²⁷ *Ficus benghalensis*,²⁸ and *Matricaria recutita*²⁹ or isolated molecules such as ternatin³⁰ and piperine.³¹ Diarrhea can be also due to the direct or indirect effect of many digestive system products such as bile acids.² However, the latter has a variety of physiologic functions and was actively reabsorbed (up to 95%) in the terminal ileum.³² Disruption of the enterohepatic circulation of bile acids due to ileal disease (e.g., Crohn's or radiation ileitis) or idiopathic bile acid malabsorption causes chronic diarrhea.³³

Moreover, we also showed in the present study that the propulsion of the charcoal meal through the gastrointestinal

FIG. 2. Effect of pomegranate juice and TE (A) as well as ME (B) on gastrointestinal transit (GIT). Animals were pretreated with pomegranate juice, total extract, various doses of methanol extract (200, 400, and 800 mg/kg, respectively, D1, D2, and D3), reference molecules (atropine [Atr] and yohimbine [Yoh]), or vehicle (NaCl 0.9%). Two hours after, rats received the standard charcoal meal (10% charcoal in 5% gum arabic) and the distance traveled by the meal compared to the total length of the small intestine was measured (n = 10). **a2**, $P < .01$ and **a3**, $P < .001$ compared to the castor oil group.

TABLE 2. CONDENSED TANNIN CONTENT IN POMEGRANATE SEED

Total tannins	Gabsi variety	Garsi variety
Juice (mg GCt/mL)	1.78±0.12	3.99±0.79 a3
Total extract (mg GCt/g DW)	3.49±0.22	9.29±0.94 a3
Methanol extract (mg GCt/g DW)	17.05±2.83	48.44±11.70 a3

Data are expressed as mean±standard deviation (n=10).

a3, $P < .001$ compared to the Gabsi variety.

tract was significantly decreased by the pomegranate juice and extracts. According to the literature, our results are in line with many reports demonstrating that GIT can be decreased by several medicinal plant extracts such as *Vitis vinifera*³⁴ and *Psidium guajava*.³⁵ In contrast, we recently showed that date pulp extract and palm sap (*Phoenix dactylifera* L.) significantly and dose dependently reduce GIT activity in healthy rats.³⁶ To our knowledge, this the first study to deal with Tunisian pomegranate juice, total, and methanol extracts on GIT in the rat model. However, Tunisian pomegranate was recently shown for its richness in phenolic compounds and to have antioxidant capacity⁹ as well as a protective effect on lipopolysaccharide-induced lung inflammation.³⁷ Indeed, these molecules are the major source of antioxidant ability of pomegranate fruit, by scavenging free radicals as hydroxyl radical (OH·), which leads to digestive function disorders.³⁸

Particularly we demonstrated in the present work that pomegranate seeds contain large amounts of minerals. The sodium concentration was the highest for both varieties followed in the descending order by the potassium, calcium, iron, magnesium, and zinc. The mineral contents were generally lower than those reported in the literature.^{39,40} This variability may be due to the agro-climatic conditions as well as the variety of pomegranate trees as recently described by El-Kar *et al.*⁴¹ for nine Tunisian ecotypes. Castor oil produces a diarrheal effect due to, in part, its inhibition of intestinal Na⁺, K⁺-ATPase activity to reduce normal fluid absorption.^{42,43} Mineral content in the digestive system can affect the processes of secretion and gastrointestinal absorption of water and electrolytes. Furthermore, both mineral deficiency and mineral excess can lead to digestive disorders associated with alterations in transport gastrointestinal water and ions.⁴⁴ Consequently, pomegranate extracts could be used to correct the mineral content deregulation during diarrhea.

Importantly, we showed that pomegranate methanol extract is richer in condensed tannins than juice and total extract. However, Tunisian pomegranate was previously known for its richness in tannins.⁴¹ Indeed, besides their antioxidant activity, these molecules are implicated in the regulation of GIT.²⁴ Tannins are present in many plants and can denature protein to form the protein tannate complex, which makes the intestinal mucosa more resistant and reduces secretion.⁴⁵ Tannins may be responsible for the inhibition of GIT and thereafter the antidiarrheal activity.

In conclusion, our data indicate that pomegranate extracts and juice have inhibiting effects on GIT and possesses antidiarrheal activity in rats. These findings confirm the basis

for the use of *P. granatum* seeds in traditional medicine for the treatment and/or management of diarrhea in some rural communities in Tunisia.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

REFERENCES

1. Bryce J, Boschi-Pinto C, Shibuya K, Black RE; WHO: Child Health Epidemiology Reference Group. WHO estimates of the causes of death in children. *Lancet* 2005;365:1147–1152.
2. Odunsi-Shiyanbade ST, Camilleri M, McKinzie S, Burton D, Carlson P, Busciglio IA, Lamsam J, Singh R, Zinsmeister AR: Effects of chenodeoxycholate and a bile acid sequestrant, colestevlam, on intestinal transit and bowel function. *Clin Gastroenterol Hepatol* 2010;8:159–165.
3. Suleiman MM, Dzenda T, Sani CA: Antidiarrhoeal activity of the methanol stem-bark extract of *Annona senegalensis* Pers. (Annonaceae). *J Ethnopharmacol* 2008;116:125–130.
4. Scarpignato C, Rampal P: Prevention and treatment of traveler's diarrhea: a clinical pharmacological approach. *Chemotherapy* 1995;4:48–81.
5. Guarino A, Canani RB, Casola A, Pozio E, Russo R, Bruzzese E, Fontana M, Rubino A: Human intestinal cryptosporidiosis: secretory diarrhea and enterotoxic activity in Caco-2 cells. *J Infect Dis* 1995;171:976–983.
6. Ammar RB, Sghaier MB, Boubaker J, Bhouri W, Naffeti A, Skandrani I, Bouhlel I, Kilani S, Ghedira K, Chekir-Ghedira L: Antioxidant activity and inhibition of aflatoxin B1-, nifuroxazide-, and sodium azide-induced mutagenicity by extracts from *Rhamnus alaternus* L. *Chem Biol Interact* 2008;174:1–10.
7. Johanningsmeier SD, Harris GK: Pomegranate as a functional food and nutraceutical source. *Annu Rev Food Sci Technol* 2011;2:181–201.
8. Elfalleh W, Nasri N, Marzougui N, Thabti I, M'rabet A, Yahya Y, Lachiheb B, Guasmi F, Ferchichi A: Physico-chemical properties and DPPH-ABTS scavenging activity of some local pomegranate (*Punica granatum*) ecotypes. *Int J Food Sci Nutr* 2009;60:197–210.
9. Elfalleh W, Tlili N, Nasri N, Yahia Y, Hannachi H, Chaira N, Ying M, Ferchichi A: Antioxidant capacities of phenolic compounds and tocopherols from Tunisian pomegranate (*Punica granatum*) fruits. *J Food Sci* 2011;76:707–713.
10. Winand J, Schneider YJ: The anti-inflammatory effect of a pomegranate husk extract on inflamed adipocytes and macrophages cultivated independently, but not on the inflammatory vicious cycle between adipocytes and macrophages. *Food Funct* 2014;5:310–318.
11. Bishayee A, Bhatia D, Thoppil RJ, Darvesh AS, Nevo E, Lansky EP: Pomegranate-mediated chemoprevention of experimental hepatocarcinogenesis involves Nrf2-regulated antioxidant mechanisms. *Carcinogenesis* 2011;32:888–896.
12. Pai MB, Prashant GM, Murlikrishna KS, Shivakumar KM, Chandu GN: Antifungal efficacy of *Punica granatum*, *Acacia*

- nilotica*, *Cuminum cyminum* and *Foeniculum vulgare* on *Candida albicans*: an *in vitro* study. *Indian J Dent Res* 2010;21:334–336.
13. Türkylmaz M, Tağı Ş, Dereli U, Özkan M: Effects of various pressing programs and yields on the antioxidant activity, antimicrobial activity, phenolic content and colour of pomegranate juices. *Food Chem* 2013;138:1810–1818.
 14. Kim HK, Baek SS, Cho HY: Inhibitory effect of pomegranate on intestinal sodium dependent glucose uptake. *Am J Chin Med* 2011;39:1015–1027.
 15. Coursodon-Boydiddle CF, Snarrenberg CL, Adkins-Rieck CK, Bassaganya-Riera J, Hontecillas R, Lawrence P, Brenna JT, Jouni ZE, Dvorak B: Pomegranate seed oil reduces intestinal damage in a rat model of necrotizing enterocolitis. *Am J Physiol Gastrointest Liver Physiol* 2012;303:744–751.
 16. Price ML, Van-Scovo S, Buttler LG: A critical evaluation of the Vanillin reaction as an assay for tannin in sorghum grain. *J Agric Food Chem* 1978;26:1214–1218.
 17. Awouters F, Niemegeers CJE, Lenaerts FM, Janssen PAJ: Delay of castor oil diarrhoea in rats: a new way to evaluate inhibitors of prostaglandin biosynthesis. *J Pharm Pharmacol* 1978;30:41–45.
 18. Mukherjee PK, Saha K, Murugesan T, Mandal SC, Pal M, Saha BP: Screening of antidiarrheal profile of some plant extracts of specific regions of West Bengal, India. *J Ethnopharmacol* 1998; 60:85–89.
 19. Ali BH, Bashir AA: The effect of alpha 2-receptor agonists and antagonists on gastrointestinal transit time. *Clin Exp Pharmacol Physiol* 1993;20:1–6.
 20. Gaginella TS, Stewart JJ, Olsen WA, Bass P: Actions of ricinoleic acid and structurally related fatty acids on the gastrointestinal tract. II. Effects on water and electrolyte absorption *in vitro*. *J Pharmacol Exp Ther* 1975;195:355–361.
 21. Karim A, Mekhfi H, Ziyat A, Legssyer A, Bnouham M, Amrani S, Atmani F, Melhaoui A, Aziz M: Anti-diarrhoeal activity of crude aqueous extract of *Rubia tinctorum* L. roots in rodents. *J Smooth Muscle Res* 2010;46:119–123.
 22. Izzo AA, Mascolo N, Capasso R, Germanò MP, De Pasquale R, Capasso F: Inhibitory effect of cannabinoid agonists on gastric emptying in the rat. *Naunyn Schmiedeberg's Arch Pharmacol* 1999;360:221–223.
 23. Izzo AA: Castor oil: an update on mechanism of action. *Phytother Res* 1996;10:109–111.
 24. Biswas S, Murugesan T, Sinha S, Maiti K, Gayen JR, Pal M, Saha BP: Antidiarrhoeal activity of *Strychnos potatorum* seed extract in rats. *Fitoterapia* 2002;73:43–47.
 25. Hussain Z, Amresh G, Singh S, Rao CV: Antidiarrheal and anti-ulcer activity of *Amaranthus spinosus* in experimental animals. *Pharm Biol* 2009;47:932–939.
 26. Maniyar Y, Bhixavatimath P, Agashikar NV: Antidiarrheal activity of flowers of *Ixora coccinea* Linn. in rats. *J Ayurveda Integr Med* 2010;1:287–291.
 27. Awe EO, Kolawole SO, Wakeel KO, Abiodun OO: Antidiarrheal activity of *Pyrenacantha staudtii* Engl. (Icacinaeae) aqueous leaf extract in rodents. *J Ethnopharmacol* 2011;137:148–153.
 28. Patil VV, Bhangale SC, Chaudhari KP, Kakade RT, Thakare VM, Bonde CG, Patil VR: Evaluation of the antidiarrheal activity of the plant extracts of *Ficus* species. *Zhong Xi Yi Jie He Xue Bao* 2012;10:3473–3452.
 29. Sebai H, Jabri MA, Souli A, Rtibi K, Selmi S, Tebourbi O, El-Benna J, Sakly M: Antidiarrheal and antioxidant activities of chamomile (*Matricaria recutita* L.) decoction extract in rats. *J Ethnopharmacol* 2014;152:327–332.
 30. Rao VS, Santos FA, Sobreira TT, Souza MF, Melo CL, Silveira ER: Investigations on the gastroprotective and antidiarrhoeal properties of ternatin, a tetramethoxyflavone from *Egletes viscosa*. *Planta Med* 1997;63:146–149.
 31. Bajad S, Bedi KL, Singla AK, Johri RK: Antidiarrhoeal activity of piperine in mice. *Planta Med* 2001;67:284–287.
 32. Hofmann AF: The continuing importance of bile acids in liver and intestinal disease. *Arch Intern Med* 1999;159:2647–2658.
 33. Wedlake L, A'herm R, Russell D, Thomas K, Walters J, Andreyev H: Systematic review: the prevalence of idiopathic bile acid malabsorption (I-BAM) as diagnosed by SeHCAT scanning in patients with diarrhoea-predominant irritable bowel syndrome (IBS). *Aliment Pharmacol Ther* 2009;30:707–717.
 34. Vitali F, Bonina FP, Saija A, Tomaino A, Fonte G, Pennisi C, Tita B: Studies on antidiarrhoeal activity of an extract of wine from Jacques grapes in mice. *Phytother Res* 2005;19:924–927.
 35. Ojewole JA, Awe EO, Chiwororo WD: Antidiarrhoeal activity of *Psidium guajava* Linn. (Myrtaceae) leaf aqueous extract in rodents. *J Smooth Muscle Res* 2008;44:195–207.
 36. Souli A, Sebai H, Rtibi K, Chehimi L, Sakly M, Amri M, El-Benna J: Effects of dates pulp extract and palm sap (*Phoenix dactylifera* L.) on gastrointestinal transit activity in healthy rats. *J Med Food* 2014;17:782–786.
 37. Bachoual R, Talmoudi W, Boussetta T, Braut F, El-Benna J: An aqueous pomegranate peel extract inhibits neutrophil myeloperoxidase *in vitro* and attenuates lung inflammation in mice. *Food Chem Toxicol* 2011;49:1224–1228.
 38. Tembhurne SV, Sakarkar DM: Effects of *Murraya koenigii* leaf extract on impaired gastrointestinal motility in streptozotocin-induced diabetic rats. *Zhong Xi Yi Jie He Xue Bao* 2011;9:913–919.
 39. Al-Maiman SA, Ahmad D: Changes in physical and chemical properties during pomegranate (*Punica granatum* L.) fruit maturation. *Food Chem* 2002;76:437–441.
 40. Fadavi A, Barzegar M, Azizl MH, Bayat M: Note physicochemical composition of ten pomegranate cultivars (*Punica granatum* L.) grown in Iran. *Food Sci Technol Int* 2005;11:113–119.
 41. El-Kar C, Ferchichi A, Attia F, Bouajila J: Pomegranate (*Punica granatum*) juices: chemical composition, micronutrient cations, and antioxidant capacity. *J Food Sci* 2011;76:795–800.
 42. Phillips RA, Love AH, Mitchell TG, Neptune EM Jr.: Cathartics and the sodium pump. *Nature* 1965;206:1367–1368.
 43. Nell G, Rummel W: Action mechanism of secretagogue drugs. In: *Pharmacology of Intestinal Permeation, second ed.* (Csaky TZ, ed.). Springer-Verlag, Berlin, 1984, pp. 461–508.
 44. Franceschi S, Bidoli E, Negri E, Zamboni P, Talamini R, Ruol A, Parpinel M, Levi F, Simonato L, La Vecchia C: Role of macronutrients, vitamins and minerals in the aetiology of squamous-cell carcinoma of the oesophagus. *Int J Cancer* 2000;86:626–631.
 45. Kouitcheu MLB, Penlap BV, Kouam J, Ngadjui BT, Fomum ZT, Etoa FX: Evaluation of antidiarrhoeal activity of the stem bark of *Cylicodiscus gabunensis* (mimosaceae). *Afr J Biotechnol* 2006;5: 1062–1066.