Original Article

Dose-dependent Spasmolytic, Bronchodilator, and Hypotensive Activities of *Panicum miliaceum* L.

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Abstract

Panicum miliaceum L. is a medicinally effective plant used in indigenous system of medicine for a variety of ailments. However, there is no comprehensive study explaining its effectiveness in gastrointestinal tract, respiratory, and cardiovascular system ailments. This study was designed to validate the pharmacological basis for the folkloric use of *Panicum miliaceum* L. in diarrhea, asthma, and hypertension. *Panicum miliaceum* extract was analyzed to detect the presence of bioactive compounds by HPLC. The isolated rabbit jejunum, trachea, and aorta were used for in vitro experiments using tissue bath assembly coupled with Power Lab data acquisition system to explore their relative effects. *In-vivo* experiments were performed for anti-diarrheal activity. HPLC analysis revealed the presence of gallic acid, butylated hydroxytoluene, catechin, and quercetin. Concentration dependent activities were observed by relaxing K^+ (low) induced contractions having spasmolytic effect with EC50 = .358 ± .052, bronchodilator (EC50 = 2.483 ± .05793), and vasorelaxant (EC50 = .383 ± .063), probably due to the ATP dependent potassium channel activation. It was confirmed through pre-exposure of glibenclamide (specific ATP-dependent K^+ channel blocker) having similarities with cromakalim. Pm.Cr revealed its antidiarrheal via in vivo experiments on rats. This study indicates that *Panicum miliaceum* has antidiarrheal, spasmolytic, bronchodilator, and vasorelaxant activities probably due to the ATP dependent K^+ channel activation.

Keywords

Panicum miliaceum, HPLC, antidiarrheal, spasmolytic, bronchodilation, vasodilation, ATP-dependent K^+ channel opener

Introduction

Panicum miliaceum L. commonly known as proso millet, hog millet, and common millet belongs to family *Poaceae*[1] Proso millet is found in approximately all areas of Asia (especially India, Pakistan, and Bhutan) Africa, Central Russia, North America, Turkey, Australia, Greece Japan, Mongolia, and Europe. In Pakistan, it is known as Cheena or Cheeni and distributed in Sind, Punjab, Khyber Pakhtunkhwa, Gilgit, and Kashmir.[2,3]

Panicum plants are traditionally used to treat diarrhea, dysentery, dyspepsia, ulcers,[4] and constipation.[5,6] *Panicum antidotale* decoction is used in cough, throat infections,[7] respiratory tract infections,[8] and cardiovascular diseases (hyperstension).[9] *Panicum miliaceum* L. is used traditionally for treatment of coeliac diseases (abdominal discomfort and severe diarrhea). Medicinally, the grain made into a conjee is given for acidity, motion sickness, and biliousness. It is also used for the treatment of snakebite poisoning. The cooked seed is applied as a poultice for abscesses/blisters and sores/wounds. Proso millet is also useful

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against heart diseases, hypercholesterol, breast cancer, diabetes, gallstones, hematuria, inflammation, wrinkles,[10] and different viral and bacterial diseases.[11]

Proso millet is rich in phytochemicals like phytic acid, which is believed to lower cholesterol, and phytate, which is associated with reducing cancer risks, along with phenolic acids and benzoic acids.[10] It also contains chlorogenic acid, syringic acid, caffeic acid, R-coumaric acid, ferulic acid, gallic acid, hydroxycinnamic acids, carotenoids, lutein,[9] fatty acids (oleic and linolenic), starch, proteins,[12] and myoinositol hexa-phosphoric acid.[13] Moreover, gluten-free fiber and other important constituents like carbohydrates, and vitamins, zinc, calcium, phenol, magnesium, iron, potassium, phosphorous, and manganese are reported to be present.[14]

Previous studies showed that *Panicum miliaceum* L possesses anti-diabetic,[15] pro-apoptotic, anti-adipogenic,[16] and anti-cancer[9] activities. However, there is little evidence available regarding its medicinal use in gastrointestinal tract (GIT) and cardiovascular system (CVS) ailments. This study has been designed to explore the pharmacological basis for medicinal use of *Panicum miliaceum* L in diarrhea, asthma, and hypertension.

Methodology

Plant Collection, Extraction, and Fractionation

National Agricultural Research Center (NARC), Bioresource Conservation Institute, Islamabad provided seed sample of *Panicum miliaceum* L. for research purpose on 13-8-2018. Seeds were cultivated in the investigational farm area of Faculty of Agricultural Sciences and Technology Baha-Uddin Zakariya University Multan by a PhD scholar Muhammad Maqsood. The seeds were collected and identified taxonomically by Dr Zafar-Ullah Zafar, a competent taxonomist from Institute of Pure and Applied Botany of the same University and a voucher specimen (Fl.P 165) was deposited in the herbarium of the Institute of Pure and Applied Biology.

The seeds collected were cleaned, grinded into coarse powder, and macerated with solution (70% methanol+30% distilled water) in amber colored bottle with regular shaking for 4 days. Then filtration was done firstly through cloth then with Whatman filter paper. Same method was repeated for 2nd and 3rd maceration. All collected filtrates were combined; solvent was evaporated under reduced pressure on rotary evaporator (Rotavapor, BUCHI Labrotechnik AG, Model 9230, Switzerland) until a dark reddish brown paste (Pm.Cr) with approximate 16% yield was attained. The obtained extract was stored in dark colored glass bottle. At the time of experiment, Pm.Cr extract was dissolved in DMSO and distilled water.[17]

Fractionation was done by dissolving 10 g Pm.Cr in 100 mL distilled water and 100 mL dichloromethane (DCM). Two layers DCM (Pm.Dcm) and aqueous (Pm.Aq) were separated out and dried.

Chemicals and Animals

Almost 99.9% pure chemicals were used for in vivo and in vitro experimental studies. Paracetamol, aspirin, and castor oil were obtained from GSK. Glibenclamide, phenylephrine, doxazocin, losartan, cromakalim, carbachol, and loperamide were obtained from Sigma Chemical Co. (USA). Chemicals to make Krebs and Tyrode's solution were acquired from Merck (Germany). For experiments white albino rabbits (1.0-2.0 kg) and Sprague Dawley rats (150-250 g) of both genders were taken from Animal House of Faculty of Pharmacy. Animals were housed at 26 ± 1°C. All permitted Ethical rules were followed in the whole research.[18]

Phytochemical Screening

Preliminary Phytochemical Examination. The Pm.Cr and Pm.Aq were analyzed qualitatively to detect bioactive compounds.

HPLC Analysis. A simple, speedy and competent binary sloped solvent method developed by our group was used for the identification of phenolic and flavonoids compounds in Pm.Cr. HPLC containing C18 column with an internal diameter of 250*4.7 mm, flow rate of .9 mL/minutes, and ability to separate phenolic constituents was 8–9 and for flavonoids 1–4 in a period of 35–36 minutes was used. Mobile phase used were; A: acetonitrile 70% and methanol 30% and B: H2O and .5% acetic acid. The concentration, retention time, and peak areas were compared with standards.[19]

In-Vitro Assays

Preparation of Isolated Jejunum. Experiments were done with Pm.Cr) and its fractions (Pm.Aq and Pm.Dcm) on isolated tissue to observe either it has spasmodic or antispasmodic consequence on GIT.[20]

Rabbits were kept on fasting overnight before experimentation but had availability of water. After dissection, jejunum tissue (~2 cm) was prepared, and kept immediately in Tyrode's solution having proper oxygen supply. Tissue was hanged in organ bath (oxygenating Tyrode's solution @ 37°C temperature), 1 g tension was set, and experiment was performed for 45 minutes to equilibrate it. Fresh Tyrode solution was flushed with an interval of 15 minutes before exposure of extract.

To observe spasmodic/antispasmodic response, extract doses ware added in cumulative way. Further experiments with provoked contractions (High-K^+, Low- K^+) followed by test substance were done to discover its possible mechanisms. Spasmolytic effect of plant extract may be due to blocking of Ca^{++} channels or opening of K^+ channels.[21] The relaxation effect of plant extract on contractions triggered by Low K^+ is usually considered due to possible involvement of K^+-channel opening mechanism.[22]

To determine its K^+ channel activation, low-K^+ triggered contraction pretreated with glibenclamide was evaluated with extract doses which inhibited its relaxing effect having similar pattern as cromakalim confirmed its K^+-ATP potassium channel opening mechanism.[23]

Preparation of Isolated Trachea. Experiments were done on isolated tracheal tissue to observe response on GIT. For tissue

preparations Rabbits were kept on fasting overnight before experimentation but had availability of water. After dissection, trachea was prepared, and kept immediately in Kreb's solution having proper oxygen supply. Tissue was hanged in organ bath (oxygenating Kreb's solution @ 37°C temperature), 1 g tension was set, and experiment was performed for 45 minutes to equilibrate it. Fresh Kreb's solution was flushed with an interval of 15 minutes before exposure of extract.[20] Pm.Cr extract doses were applied against baseline and stabilized contractions triggered by CCh and Low-k to find out its cholinergic and potassium channel opening mechanisms.

Preparation of Isolated Aorta. Vasoconstrictive and/or vasodilative effect on isolated aorta preparation was tested. Aorta tissues were prepared from rabbits for which rabbits were kept on fasting overnight before experimentation but had availability of water. After dissection, trachea was removed and kept immediately in Kreb's solution having proper oxygen supply. Tissue was hanged in organ bath (oxygenating Kreb's solution @ 37°C temperature), 2 g tension was set, and experiment was performed for 45 minutes to equilibrate it. Fresh Kreb's solution was flushed with an interval of 15 minutes before exposure of extract.[20] Responses were recorded against stabilized baseline and contractions triggered by PE and low- K^+.

In-Vivo Experimentation

Anti-diarrheal Activity. Anti-diarrheal effect of Pm.Cr was evaluated by a previously reported method.[24] Thirty-five rats were taken and divided into 7 groups @ 5 rats per group (100–200 g). Control group was given normal saline (10 mL/kg) and group 2 and 3 were given cromakalim and loperamide, (positive controls). The group 4 and 5 received different doses (100 and 200 mg/kg, p.o.) of Pm.Cr and the group 6 and 7 were pretreated with glibenclamide (GB) After 1 hour, each rat was given (10 mL/kg) castor oil orally and placed in separate box lined with filter paper to observe watery fecal drops for next 1,2,3, and 4 hours.

$$\%\ \text{age protection} = \left(\frac{Fc - Ft}{Fc}\right) \times 100$$

where,
Fc = counted feces wet (control)
Ft = counted feces (wet) (test)

Statistical Analysis. The results are mean ± SEM. The median effective concentrations (EC50 value) with 95% (CI) were calculated with Graph Pad Prism (GraphPad, San Diego, California, USA: http://www.graphpad.com). The statistics was carried out using one-way (ANOVA) followed by Dunnett's test in the case of in vivo, a probability ($P < .05$) was considered statistically significant.[25]

Results

Preliminary Phytochemical Screening

Phytochemical analysis of Pm.Dcm indicated alkaloids, phenols, and terpenoids, but Pm.Aq contained flavonoids, glycosides, and tannins.

HPLC Examination

Pm.Cr retention time was compared with that of standard. Gallic acid, butylated hydroxytoluene, catechin, and quercetin were detected (Figure 1, Table 1).

Response on Jejunum. Pm.Cr and its fractions were used to assess its response on isolated tissue of jejunum. Pm.Cr exhibited its relaxing effect on naturally contracted tissue at a concentration of 3 mg/mL, stabilized contractions triggered by Low K^+, at a concentration range of .01–3 mg/ml of Pm.Cr with EC50 = .358 ± .052, 95% (C.I): .276-.464) (Figures 2 and 5A and 5B), which was inhibited in tissues pretreated with 3 μM glibenclamide (Figure 5C and 5D).

Pm.Aq exhibited its relaxing effect on naturally contracted tissue at concentration .01–5 mg/ml while contractions triggered by low K^+ relaxed with EC50 = .082 ± .111, 95% (C.I): .040-.167 mg/mL (Figures 3 and 5B).

Pm.Dcm also revealed its spasmolytic response at .01-3 dose concentration with EC50 = .334 ± .147, 95% (C.I): .120–1.063 (Figures 4 and 5C) against Low-K^+ as well as on naturally contracted tissue at concentrations .01–1 mg/ml.

Recorded responses were compared with cromakalim (standard ATP-sensitive K^+ channel opening mechanism) having its relaxing effect on contractions triggered by low K^+ with EC50 = .184 ± .165, 95% (C.I): .021–.214 mg/mL (Figure 2E). Furthermore, 3 μM glibenclamide blocked the relaxing effect of Pm.Cr on contractions triggered by low K^+ with EC50 =.17 ± .021, 95% (C.I): .041–.511 as cromakalim EC50 =.018 ± .100 95% (C.I): .02–.248 mg/mL (Figure 5E).

Response on Trachea. The crude extract and its fractions were assessed to record their response on isolated trachea. Pm.Cr exhibited its relaxing effect on contractions triggered by low K^+ and CCh (1 μM) at concentrations 1 and 3 with EC50 = 2.483 ± .05793, 95% (C.I): 1.901–3.364 and EC50 = .611 ± .1045, 95% (C.I): .334–1.154, respectively (Figures 6 and 9A). 3 μM glibenclamide blocked the relaxing effect of Pm.Cr on contractions triggered by low K^+ (Figure 9D).

Pm.Aq exhibited its relaxing effect on contractions triggered by low K^+ at .3 mg/mL with EC50 = .053 ± .1761, 95% (C.I): .012–.335. Pm.Aq exhibited its relaxing effect on contractions triggered by CCh at .03 mg/mL concentration. (Figures 7 and 9B)

Pm.Dcm exhibited its relaxing effect on contractions triggered by low K^+ and CCh (1 μM) at .3 with EC50 = .352 ± .3276. 95%

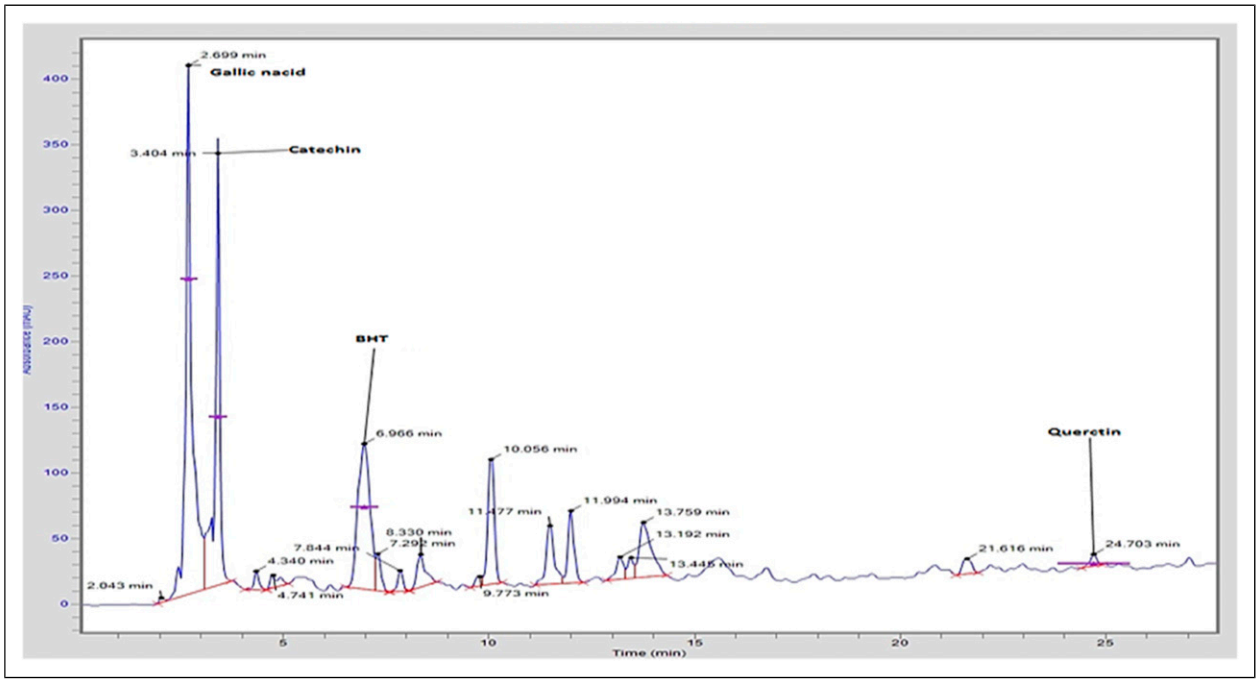

Figure 1. *HPLC- chromatogram of Panicum miliaceum L.*

Table 1. Comparison of standards and *panicum miliaceum* L.

Standards	Retention Time (minutes)	Compounds detected	Retention Time (minutes)
Gallic acid	2.806	Gallic acid	2.700
Catechin	3.387	Catechin	3.405
Butylated hydroxytoluene	7.041	Butylated hydroxytoluene	6.967
Quercetin	24.894	Quercetin	24.704

(C.I): .050–.976 and, 95% (C.I): .010–.786, respectively (Figures 8 and 9C).

Recorded responses were compared with cromakalim having its relaxing effect on contractions triggered by low K^+ with EC50 = .013 ± .001, 95% (C.I): .01–0. Furthermore, 3 μM glibenclamide blocked the relaxing effect of cromakalim having EC50 = .14, 95% (C.I): .031–.401 mg/mL (Figure 9E).

Response on Aorta. Pm.Cr tested on aorta did not produce any effect on baseline. Contractions triggered by low K^+ and PE (1 μM) relaxed with Pm.Cr at a concentration of .01–3 and .01–5, respectively, with EC50 = .383 ± .063, (95% (C.I): .271–.548 mg/mL and EC50 = 05.29 ± .315, (95% (C.I): 3.993–11.56 (Figures 10 and 12A). Glibenclamide @ 3 μM blocked the relaxing effect of Pm.Cr on contractions triggered by low K^+ (Figure 12B).

Pm.Aq completely relaxed contractions triggered by PE (1 μM) at the concentration of .01–3 with EC50 = .092 ± .010, (95% (C.I): .044–.1915 mg/mL but had not against low-K^+. Pm.Dcm fraction revealed its vasorelaxant effect on contractions triggered by low K^+ at concentration of .01–3 with EC50 = .864 ± .0011, 95% (C.I): .658–2.433 mg/mL. It also relaxed stabilized contraction of PE (1 μM) at .01–5 with EC50 = .02931 ± .213, 95% (C.I): .004–.280 mg/mL (Figure 11).

Recorded responses were compared with cromakalim which showed relaxing effect on contractions triggered by low K^+ with EC50 = .01, 95% (C.I): .02–.277 mg/mL. Furthermore, 3 μM glibenclamide blocked the relaxing effect of cromakalim with EC50 = .15 (95% (C.I): .032–.437 mg/mL (Figure 12).

In-vivo Experimentation

Anti-diarrheal Effect of Pm.Cr. Pm.Cr exhibited potent anti-diarrheal effect, with comparison to control group, the tests groups had percentage protection of 64.65% ($P < .01$) and 88.89% ($P < .005$), respectively. The cromakalim, loperamide, and saline group showed 65.81, 77.9, and 20.4% protection, respectively. When the effect of Pm.Cr pretreated with GB decreased to 34.1 and 22.5%, with cromakalim, it was reduced to 25.6% (Figure 13).

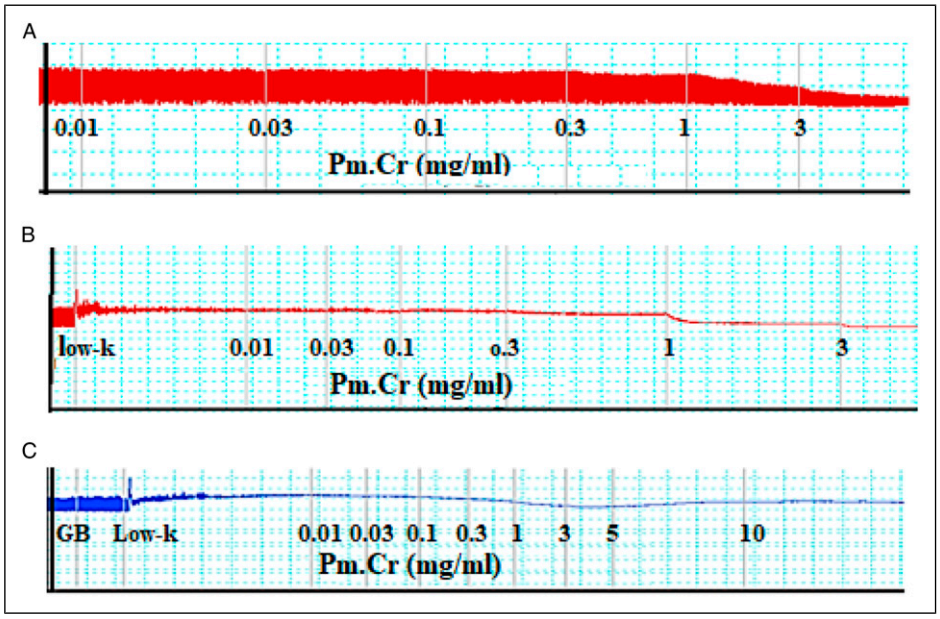

Figure 2. *Pm.Cr extract effect on (A)* natural(spontaneous) contractions *(B)* Low-K induced contractions *(C)* Low-K induced contractions pre-treated with 3 μML^{-1} glibenclamide.

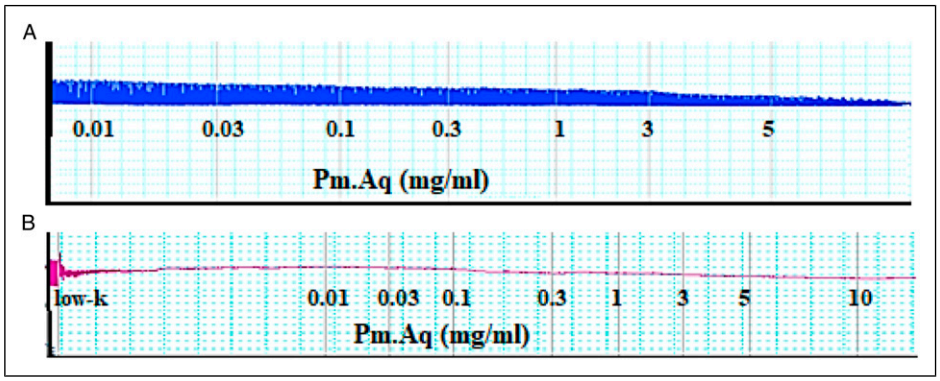

Figure 3. *Pm.Aq extract effect on (A)* natural (spontaneous) contractions *(B)* Low-K^+ induced contractions.

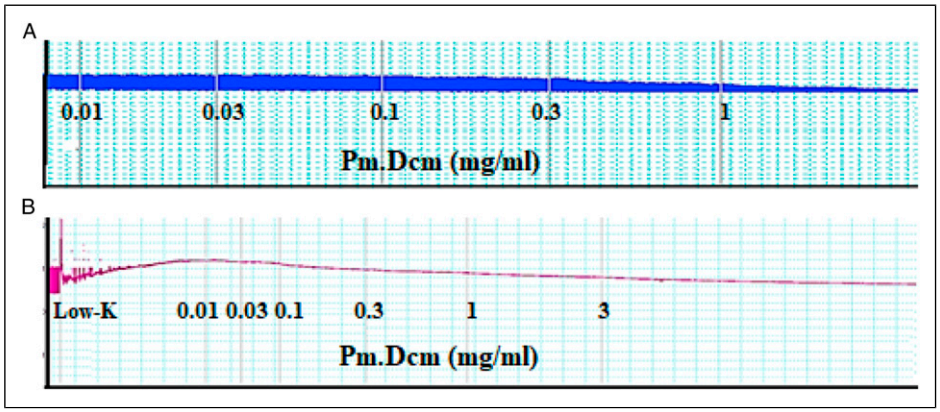

Figure 4. *Impact of Pm.Dcm on (A)* natural contractions in jejunum *(B)* on contractions triggered by Low-K^+.

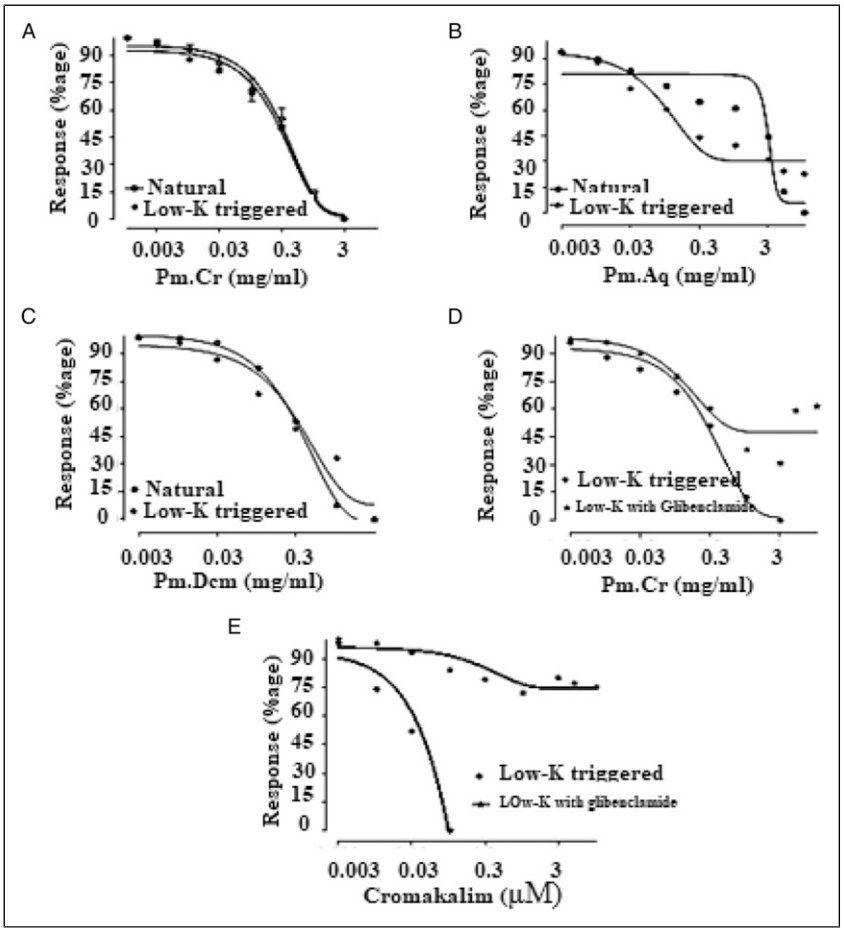

Figure 5. *Graphical representation of sigmoidal dose response curves. (A)* Pm.Cr *(B)* Pm.Aq *(C)* Pm.Dcm on natural (spontaneous) and Low-K^+ induced contractions *(D)* Pm.Cr in presence of 3 μML^{-1} glibenclamide and *(E)* cromakalim effect low-K^+ triggered contractions in presence and absence of glibenclamide in isolated rabbit jejunum.

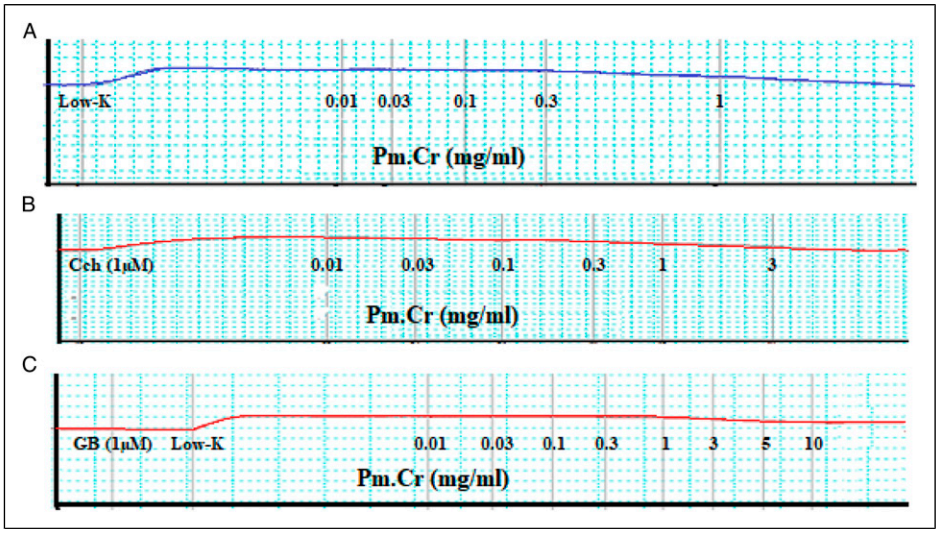

Figure 6. *Effect of Pm.Cr on contractions triggered by (A)* Low-K *(B)* CCh(1 μM) *(C)* Low-K with Glibenclamide in trachea.

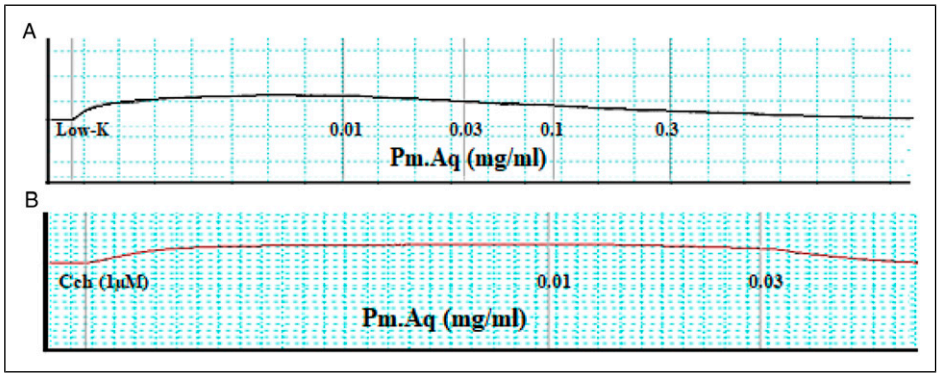

Figure 7. *Effect of Pm.Aq on contractions triggered by (A)* Low-K *(B)* CCh(1 μM) in trachea.

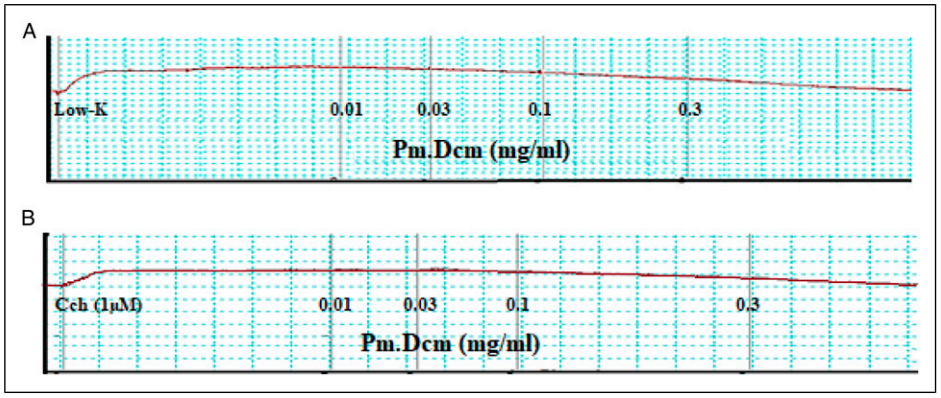

Figure 8. *Effect of Pm.Dcm on contractions triggered by (A) Low-K (B)* CCh(1 μM) in trachea.

Discussion

Medicinal plants are gaining importance worldwide because of their potential therapeutic use and lack of side effects. Based on the folk medicinal use of *Panicum miliaceum* in hyperactive GIT, respiratory and CV disorders,[4,8,9] this study was designed to evaluate the antidiarrheal, anti-spasmodic, anti-asthmatic, and vasorelaxant activity of plant extract by in vitro method. To get an insight into the mechanisms, the jejunum, trachea, and aorta of isolated rabbit were used.

It has been perceived that plants exhibit inhibitory effects via activation of K^+ channel.[20]The use of low-K^+ induced depolarization causes opening of K^+ channels. Due to presence of K^+ channels in intestinal and epithelial cells, K^+ channel openers produce relaxation of smooth muscle by decreasing intracellular calcium via hyperpolarization of membrane.[26]

To evaluate whether the inhibitory effect of Pm.Cr is due to involvement of K^+-channels, it was tested on stabilized contractions of jejunum triggered by low-K^+, where it caused complete relaxation. The observed spasmolytic response may be due to the opening of potassium channels.[22] Moreover, this antispasmodic activity may be attributed to presence of flavonoid (catechin, quercetin),[27,28] alkaloids,[29] tannins,[30] and quercetin[31] which are detected by preliminary phytochemical screening and HPLC studies. This K^+ channel opening activity was found to be more dominant in organic fraction as compared to aqueous fraction. Potassium channel opener drugs have widespread therapeutic use in gastrointestinal disorders like diarrhea,[32] in CVS as antihypertensive,[33] and as bronchodilator in hyperactive airways diseases like asthma and cough.[34]

Cromakalim (ATP dependent K^+ channel opener) inhibited low K^+ induced contractions. To confirm the K^+ channel activation mechanism, low-K^+ triggered contractions pretreated with glibenclamide was tested with extract doses which inhibited its relaxing effect having similar pattern as cromakalim confirmed opening of K^+-ATP channel.[35]

To check it bronchodilator effect, Pm.Cr was applied on contractions triggered by low K^+ and CCh. Pm.Cr started a reduction of low- K^+ and CCh-triggered contractions, being more potent against low K^+. The results proposed that

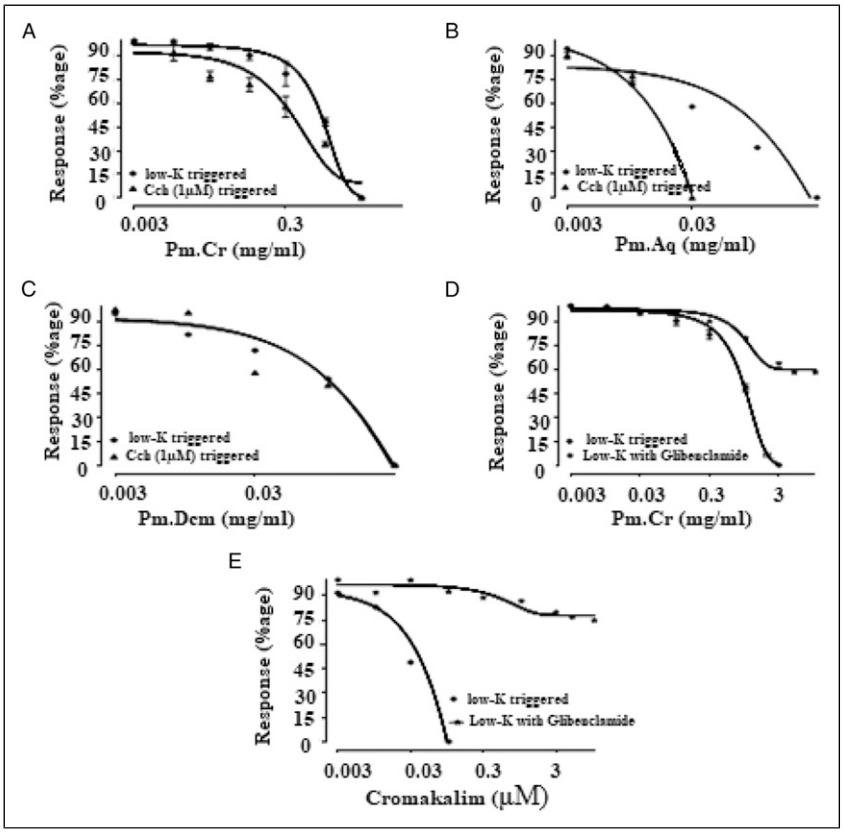

Figure 9. *Concentration dependent relaxing effect of Panicum miliaceum response (A)* Pm.Cr *(B)* Pm.Aq *(C)* Pm.Dcm effect on low-K^+ and CCh triggered contractions & *(D)* Pm.Cr *(E)* cromakalim effect on low-K^+ triggered contractions in presence and absence of glibenclamide on tracheal tissue.

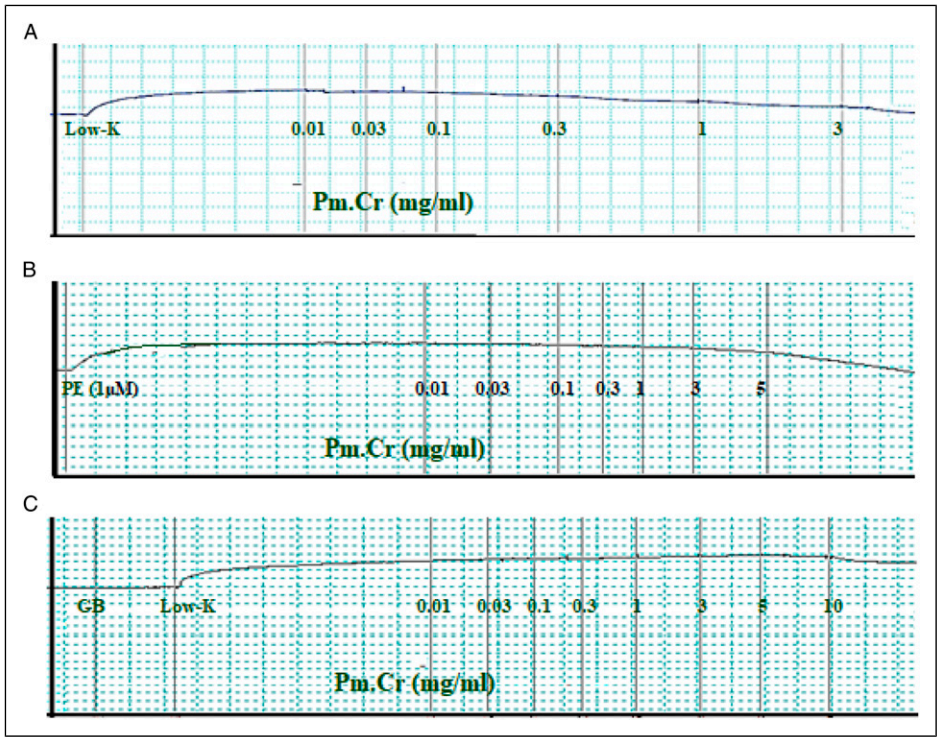

Figure 10. *Effect of Pm.Cr on contractions triggered by (A)* Low-K *(B)* PE(1 μM) *(C)* Low-K with Glibenclamide in aorta.

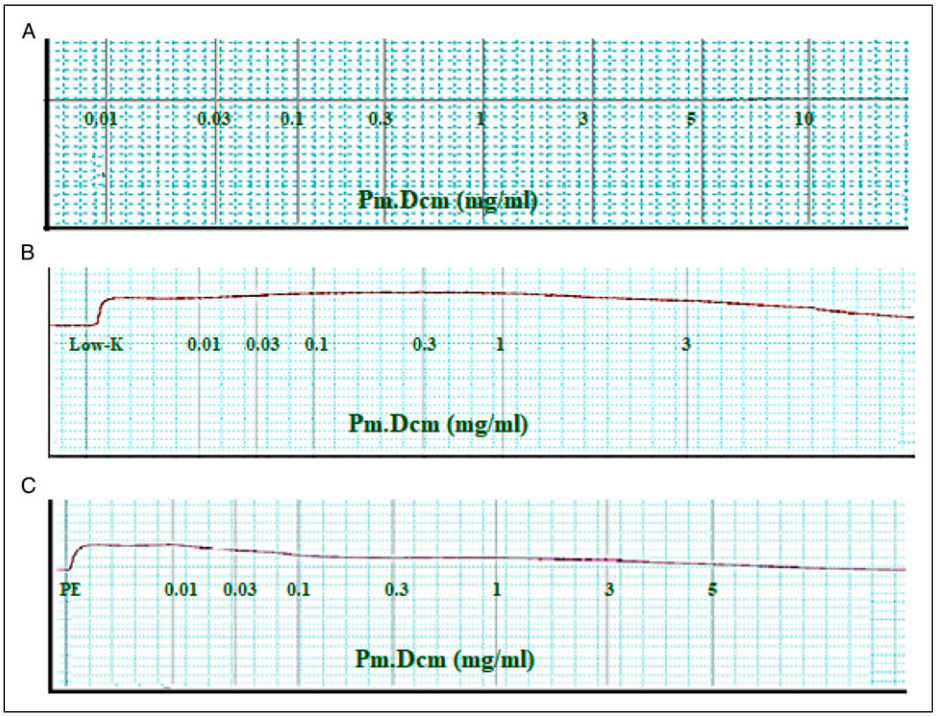

Figure 11. *Effect of Pm.Dcm on provoked contractions of (A)* Pm.Dcm *(B)* Low-K^+ *(C)* PE(1 μM) in aorta.

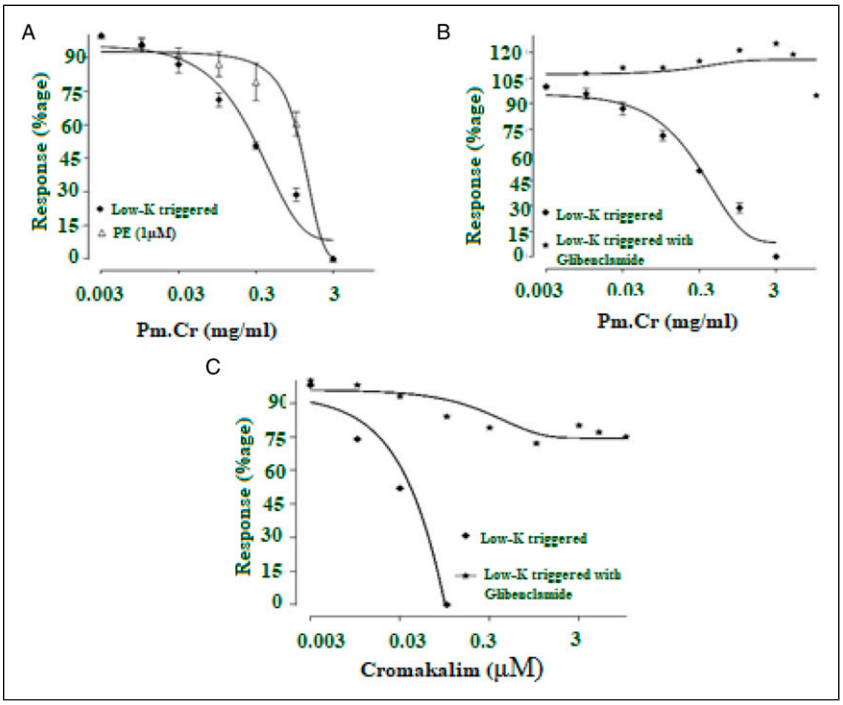

Figure 12. *Concentration dependent relaxing effect of Panicum miliaceum response; (A)* Pm.Cr on low-K^+ and PE triggered & *(B)* Pm.Cr *(C)* Cromakalim effect on low-K^+ triggered contractions with and without glibenclamide on aortic tissue.

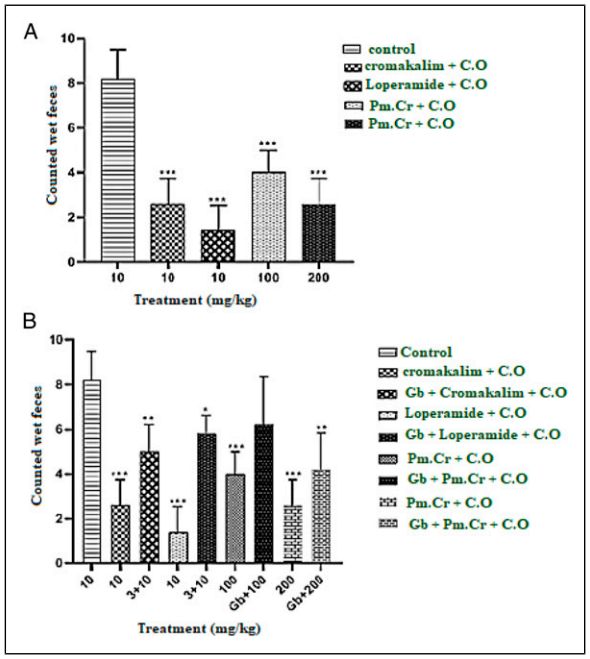

Figure 13. *Pm.Cr effect on diarrhea stimulated by castor oil in rats, (A)* dose dependent antidiarrheal effect of Pm.Cr, cromakalim, and Loperamide *(B)* dose dependent antidiarrheal effect of Pm.Cr, cromakalim, and Loperamide pretreated with glibenclamide. Applied Dunnett's test and 1-way ANOVA. $^{**}P < .01$ and $^{***}P < .005$ comparison was done with control.

bronchodilator activity is due to K^+ channel opening mechanism. Besides, flavonoids have potent bronchodilator effects and presence of flavonoids is confirmed by preliminary phytochemical and HPLC screening.[36,37]

Potassium channel opener drugs have widespread therapeutic usage in hypertension.[32] Pm.Cr was further investigated for possible effects on cardiovascular systems. Pm.Cr was applied on low K^+, and PE-triggered contractions in the isolated aorta. Pm.Cr resulted in relaxation of low K^+ and PE-triggered contractions. The relaxation of low K^+ contractions showed momentous inhibition in GB pretreated tissues, which confirms the influence of K^+-ATP channels as a mode of vasodilation that is used for hypertension treatment.[38] Catechin is used in the treatment of hypertension[28] while gallic acid acts as a cardio protective in cardiovascular problems.[39]

In castor oil induced diarrhea, Pm.Cr showed antidiarrheal effect like cromakalim,[40] which is spasmolytic and antidiarrheal activity. Castor oil increases intestinal liquid and promotes diarrhea because of its active constituent, ricinoleic acid (hydrolysis of oil),[41,42] and produces contractions in colon.[43] The detected antidiarrheal properties of the plant extract may be due to involvement of potassium channels which decreased by pretreatment with glibenclamide (GB; an ATP-dependent K+ channel blocker).[44] The observed antidiarrheal activity which may be due to the presence of flavonoids (catechin and quercetin), tannins, and terpenoids present in extract.[45]

Conclusion

The in vitro and in vivo studies of *Panicum miliaceum* L. revealed the antispasmodic, bronchodilator, vasorelaxant, and anti-diarrheal activities. The potent antispasmodic, anti-diarrheal, bronchodilator, and vasorelaxant activities are probably mediated due to the opening of ATP dependent K^+ channel activation. It provides momentous basis of its folkoric use for complaints of GIT, respiratory, and CVS.

Nomanclature

Aq	Aquous
CCh	Carbamylcholine
CI	Confidence interval
CTZ	Chemo receptor trigger zone
(EC50)	Efefctive concentration that gives half the maximum response
GB	Glibenclamide
KATP	ATP-dependent potassium channel
Pm.Cr	Methanolic crude extract of *Panicum miliaceum*
Pm.Dcm	Dichloromethane fraction of *Panicum miliaceum*
Pm.Aq	Aqueous fraction of *Panicum miliaceum*
MLC	Myosin light chain
NS	Normal saline
PE	Phenylephrine
Pm.Cr	Methanolic crude extract of *Panicum miliaceum*
Pm.Dcm	Dichloromethane fraction of *Panicum miliaceum*
Pm.Aq	Aqueous fraction of *Panicum miliaceum*
SEM	Standard error mean

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Authors' Contributions

FS and MA planned the project. MA and AAA performed the experiments as well as worked on the statistical analysis of data and results interpretation. AAA, MH, LC, MI, CG, and FS drafted the manuscript. All authors have read and agreed to the submission of the manuscript.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval

Ethical approval was attained from Ethical Committee of Baha-Uddin Zakariya University, Multan (EC /12PhL-S/2018) dated 26 March 2018. Researchers agreed using the approved informed consent documented before their enrollment into study. All experiments on animals were performed in accordance with the relevant guidelines and regulations of Commission of Laboratory Animal Resources of Life Sciences (NRC, 2011), and was permitted by the Ethical Committee of BZU, Multan.

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References

1. Habiyaremye C, Matanguihan JB, D'Alpoim Guedes J, Ganjyal GM, Whiteman MR, Kidwell KK, et al. Proso millet (*Panicum miliaceum* L.) and its potential for cultivation in the Pacific Northwest, US: a review. *Front Plant Sci*. 2017:19-67.
2. Chandrasekara A, Shahidi F. Bioaccessibility and antioxidant potential of millet grain phenolics as affected by simulated in vitro digestion and microbial fermentation. *J Funct Foods*. 2012;4(1):226-237.
3. Lu H, Zhang J, Liu K-b, et al. Earliest domestication of common millet (*Panicum miliaceum*) in East Asia extended to 10,000 years ago. *Proc Natl Acad Sci Unit States Am*. 2009;106(18): 7367-7372.
4. Sivasankari B, Anandharaj M, Gunasekaran P. An ethnobotanical study of indigenous knowledge on medicinal plants used by the village peoples of Thoppampatti, Dindigul district, Tamilnadu, India. *J Ethnopharmacol*. 2014;153(2):408-423.
5. Abouri M, El Mousadik A, Msanda F, Boubaker H, Saadi B, Cherifi K. An ethnobotanical survey of medicinal plants used in the Tata Province, Morocco. *Int J Med Plant Res*. 2012;1(7): 99-123.
6. Zubair M, Khan S, Khan S, Bilal Hussain S, Haq AU, Jamil A. Ethnobotanical study of pakistan's Southern Punjab Tehsil of Dunyapur. *Int J Multidisplin Res Stud*. 2019;02(09):40-52.
7. Ahmad S, Alam K, Wariss HM, Anjum S, Mukhtar M. Ethnobotanical studies of plant resources of Cholistan desert, Pakistan. *Int J Sci Res*. 2014;3(6):1782-1788.
8. Malik S, Ahmad S, Sadiq A, et al. A comparative ethnobotanical study of Cholistan (an arid area) and Pothwar (a semi-arid area) of Pakistan for traditional medicines. *J Ethnobiol Ethnomed*. 2015;11(1):31.
9. Zhang L, Liu R, Niu W. Phytochemical and antiproliferative activity of proso millet. *PLoS One*. 2014;9(8):e104058. DOI: 10.1371/journal.pone.0104058.
10. Fongnzossie Fedoung E, Zra T, Nyangono Biyegue C, Nouga Bissoue A, Baraye S, Tsabang N. Herbal cosmetics knowledge of Arab-Choa and Kotoko ethnic groups in the semi-arid areas of far North Cameroon: ethnobotanical assessment and phytochemical review. *Cosmetics*. 2018;5(2):31.
11. Sala A, Recio M, Giner RM, Máñez S, Tournier H, Schinella G, Ríos JL. Anti-inflammatory and antioxidant properties of Helichrysum italicum. *J Pharm Pharmacol*. 2002;54(3): 365-371.
12. Shen R, Ma Y, Jiang L, Dong J, Zhu Y, Ren G. Chemical composition, antioxidant, and antiproliferative activities of nine Chinese proso millet varieties. *Food Agric Immunol*. 2018; 29(1):625-637.
13. Bora P, Das P, Bhattacharyya R, Saikia A. Effect of processing on the phytochemical content and antioxidant capacity of proso millet (*Panicum miliaceum* L.) milled fractions. *Int J Chem Stud*. 2018;6(4):18-22.
14. Lestienne I, Buisson M, Lullien-Pellerin V, Picq C, Trèche S. Losses of nutrients and anti-nutritional factors during abrasive decortication of two pearl millet cultivars (*Pennisetum glaucum*). *Food Chem*. 2007;100(4):1316-1323.
15. Park K-O, Ito Y, Nagasawa T, Choi M-R, Nishizawa N. Effects of dietary Korean proso-millet protein on plasma adiponectin, HDL cholesterol, insulin levels, and gene expression in obese type 2 diabetic mice. *Biosci Biotechnol Biochem*. 2008;72(11): 2918-2925.
16. Bobescu E, Covaciu A, Rus H, et al. Correlation of cardiovascular risk factors and biomarkers with platelet reactivity in coronary artery disease. *Am J Therapeut*. 2019;26(5):563-569.
17. Gohar UF, Iqbal I, Shah Z, et al. COVID-19: recent developments in therapeutic approaches. In: Alothamn SI, Henidi HA, eds *Alternative Medicine Interventions for COVID-19*. Cham, Switzerland: Springer; 2021:249-274.
18. NRC (National Research Council). *Guide for the Care and Use of Laboratory Animals*. 8th ed.. Washington, DC: NAP; 2011: 1-7.
19. Mradu G, Saumyakanti S, Sohini M, Arup M. HPLC profiles of standard phenolic compounds present in medicinal plants. *Int J Pharmacogn Phytochem Res*. 2012;4(3):162-167.
20. Saqib F, Janbaz KH. Rationalizing ethnopharmacological uses of *Alternanthera sessilis*: a folk medicinal plant of Pakistan to manage diarrhea, asthma and hypertension. *J Ethnopharmacol*. 2016;182:110-121. DOI: 10.1016/j.jep.2016.02.017.
21. Janbaz KH, Akram S, Saqib F, Khalid M. Antispasmodic activity of *Symplocos paniculata* is mediated through opening of ATP-dependent K+ channel. *Bangladesh J Pharmacol*. 2016;11: 495-500.
22. Masuzawa K, Matsuda T, Asano M. Evidence that pinacidil may promote the opening of ATP-sensitive K+ channels yet inhibit the opening of Ca2+-activated K+ channels in K+ -contracted canine mesenteric artery. *Br J Pharmacol*. 1990;100(1): 143-149.
23. Yanagisawa T, Teshigawara T, Taira N. Cytoplasmic calcium and the relaxation of canine coronary arterial smooth muscle

produced by cromakalim, pinacidil, and nicorandil. *Br J Pharmacol*. 1990;101:157-165.
24. Khan A, Rehman NU, AlKharfy KM, Gilani AH. Antidiarrhoeal and antispasmodic activities of Salvia officinalis are mediated through activation of K^+ channels. *Bangladesh J Pharmacol*. 2011;6:110-116.
25. Dawson-Saunders B, Trapp RG. *Basic and Clinical Biostatistics*. East Norwalk, CT: Prentice-Hall International; 1990:100-121.
26. Mehmood MH, Siddiqi HS, Gilani AH. The antidiarrheal and spasmolytic activities of *Phyllanthus emblica* are mediated through dual blockade of muscarinic receptors and Ca2+ channels. *J Ethnopharmacol*. 2011;133(2):856-865.
27. Zhu Y, Zhang S. Antibacterial activity and mechanism of lacidophilin from *Lactobacillus pentosus* against *Staphylococcus aureus* and. *Escherichia coli Front Microbiol*. 2016;34:71-79.
28. Ghayur MN, Khan H, Gilani AH. Antispasmodic, bronchodilator and vasodilator activities of (+)-catechin, a naturally occurring flavonoid. *Arch Pharm Res (Seoul)*. 2007;30(8):970-975.
29. Gilani AH, Ghayur MN, Khalid A, Choudhary MI. Presence of antispasmodic, antidiarrheal, antisecretory, calcium antagonist and acetylcholinesterase inhibitory steroidal alkaloids in *Sarcococca saligna*. *Planta Med*. 2005;71(02):120-125.
30. Azhar I, Ahmed SW, Usmanghani K. *Tannins: Their Chemistry and Bioactivity*. Karachi, Pakistan: University of Karachi press; 1997:121-125.
31. Syarifah-Noratiqah S-B, Zulfarina MS, Ahmad SU, Fairus S, Naina-Mohamed I. The pharmacological potential of oil palm phenolics (OPP) individual components. *Int J Med Sci*. 2019; 16(5):711-719.
32. Gilani AH, Khan A-u, Jabeen Q, Subhan F, Ghafar R. Antispasmodic and blood pressure lowering effects of *Valeriana wallichii* are mediated through K+ channel activation. *J Ethnopharmacol*. 2005;100(3):347-352.
33. Shah SA, Mehmood MH, Khan M, Bukhari IA, Gilani AH. Vasodilator effect of 1-trifluoromethoxyphenyl-3-(1-propionylpiperidin-4-yl) urea is predominantly mediated through activation of voltage-dependent K+ channels. *Trop J Pharmaceut Res*. 2018;17(6):1019-1024.
34. Gilani A-H, Rehman N-u, Khan A, Alkharfy KM. Studies on bronchodilator activity of salvia officinalis (Sage): possible involvement of K+ channel activation and phosphodiesterase inhibition. *Phytother Res*. 2015;29(9):1323-1329.
35. Vogalis F. Potassium channels in gastrointestinal smooth muscle. *J Auton Pharmacol*. 2000;20:207-219.
36. Bobescu E, Covaciu A, Rus H, et al. Correlation of cardiovascular risk factors and biomarkers with platelet reactivity in coronary artery disease. *Am J Therapeut*. 2019;26(5):563-569.
37. Pietta P. Flavonoids in medicinal plants. In: Rive-Evans CV, Packer L, eds *Flavonoids in Health and Disease*. New York, NY: Marcel Dekker; 1998:61-110.
38. Edwards G, Weston AH. Pharmacology of the potassium channel openers. *Cardiovasc Drugs Ther*. 1995;9:185-193.
39. Nayeem N, Asdaq SMB, Salem H, AHEl-Alfqy S. Gallic acid: a promising lead molecule for drug development. *J Appl Pharmacol*. 2016;8(2):1-4.
40. Poggioli R, Benelli A, Arletti R, Cavazzuti E, Bertolini A. K+ channel openers delay intestinal transit and have antidiarrheal activity. *Eur J Pharmacol*. 1995;287:207-209.
41. Iwao I, Terada Y. On the mechanism of diarrhea due to castor oil. *Jpn J Pharmacol*. 1962;12:137-145.
42. Gaginella TS, Phillips SF. Ricinoleic acid: current view of an ancient oil. *Am J Dig Dis*. 1975;20:1171-1177.
43. Croci T, Landi M, Emonds-Alt X, Le Fur G, Maffrand J-P, Manara L. Role of tachykinins in castor oil diarrhoea in rats. *Br J Pharmacol*. 1997;121:375-380.
44. Franck H, Puschmann A, Schusdziarra V, Allescher H-D. Functional evidence for a glibenclamide-sensitive K+ channel in rat ileal smooth muscle. *Eur J Pharmacol*. 1994;271:379-386.
45. Kim JE, Lee MR, Park JJ, et al. Quercetin promotes gastrointestinal motility and mucin secretion in loperamide-induced constipation of SD rats through regulation of the mAChRs downstream signal. *Pharmaceut Biol*. 2018;56(1):309-317.