

Methanol Extract of *Bauhinia purpurea* Leaf Possesses Anti-Ulcer Activity

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Key Words

Bauhinia purpurea · Methanol extract · Anti-ulcer activity · Flavonoids · Saponins · Polyphenols

Abstract

Objective: The aim of the present study was to determine the anti-ulcer activity of a methanol extract of *Bauhinia purpurea* leaf (MEBP). **Materials and Methods:** MEBP was administered at doses of 100, 500 and 1,000 mg/kg and its effects on acute toxicity, absolute ethanol- and indomethacin-induced gastric ulceration, and pyloric ligation tests in rats were investigated. **Results:** At a dose of 5,000 mg/kg, MEBP did not cause any signs of toxicity in rats when given orally. Oral administration of MEBP exerted anti-ulcer activity ($p < 0.05$) in all models tested. However, a dose-dependent protection was observed only in the indomethacin-induced gastric ulceration model. Histological studies supported the observed anti-ulcer activity of MEBP. In the pyloric ligation assay, MEBP significantly increased gastric wall mucus secretion ($p < 0.05$), but did not affect the acidity of the gastric contents. **Conclusion:** MEBP exhibited anti-ulcer activity, which could be due to the presence of flavonoids, saponins or other polyphenols, thereby validating the traditional use of *B. purpurea* in the treatment of ulcers.

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Introduction

Plant constituents possess a variety of pharmacological properties and these have been used in traditional medicine to cure various ailments. *Bauhinia purpurea* L. (family Fabaceae) is one of the plants that has gained interest among researchers as a potential new source of medicinal agents. *B. purpurea* has traditionally been used in the treatment of pain, rheumatism, fever, ulcers and cancerous growths in the stomach [1, 2]. Known to the Malays as *tapak kerbau* or *tapak kuda*, the plant is native to Southern Asia, Southeast Asia, Taiwan and China. Extracts of *B. purpurea* have been reported to possess various pharmacological activities [3–9]. Based on our literature search and to the best of our knowledge, no attempt has been made to scientifically demonstrate the anti-ulcer activity of *B. purpurea*. Thus, the present study aimed to evaluate the anti-ulcer activity of a methanol extract of *B. purpurea* leaf (MEBP) using various models of ulcerogenesis.

Materials and Methods

Plant Material and Extract Preparation

Leaves of *B. purpurea* were collected from their natural habitat in Shah Alam, Selangor, Malaysia, between June and September 2009, and a voucher specimen (SK 1095/05) has been deposited at

the Herbarium of the Laboratory of Natural Products, Institute of Bioscience, UPM, Serdang, Selangor, Malaysia. The detailed botanical description of the plant and the preparation of MEBP were previously described by Zakaria et al. [7]. Dried leaves of *B. purpurea* (about 875 g) were soaked in methanol for 24 h and this was repeated two more times to yield approximately 38.4 g (about 4.4%) MEBP.

Animals

Male Sprague-Dawley rats (180–220 g; 8–10 weeks old) were used in the present study and cared for, at all times, in accordance with current UPM principles and guidelines for the care of laboratory animals and the UPM ethical guidelines for investigations of experimental pain in conscious animals as described by Zakaria [2]. Animals were fasted for 48 h prior to all assays, and standard drug (30 mg/kg omeprazole) and extract were administered orally (by gavage) with distilled water (dH₂O; 10 ml/kg) as the vehicle.

Acute Toxicity Study

The acute toxicity study of MEBP was performed using a single oral dose of 5 g/kg according to the method of Mohamed et al. [10]. The effects of a single oral dose of MEBP (5,000 mg/kg) were monitored over a 14-day period, and no symptoms or signs of toxicity were observed in any of the animals treated.

Anti-Ulcerogenic Activity

Absolute Ethanol-Induced Gastric Ulceration. The absolute ethanol-induced gastric ulceration was induced according to the procedure of Noor et al. [11]. Thirty minutes after administering the test solution, gastric ulceration was induced using 1 ml/200 g body weight absolute ethanol. Fifteen minutes later, the rats were sacrificed under diethyl ether anaesthesia and the stomachs were examined for gastric erosions using a dissecting microscope (20×). The areas of ulceration were identified and measured as described by Abdullah et al. [12].

Indomethacin-Induced Gastric Ulceration. Indomethacin-induced ulcer formation was accomplished according to the method of Nwafor et al. [13]. Thirty minutes after administering the test solution, gastric ulceration was induced by oral administration of 100 mg/kg indomethacin. After 4 h, the rats were sacrificed under diethyl ether anaesthesia and the stomachs were examined for gastric erosions under a dissecting microscope (20×) as described earlier [12].

Histopathological Analysis

Gastric tissue samples from each group with ulceration induced using absolute ethanol or indomethacin were fixed in 10% formalin and embedded in paraffin. Specimens were then sectioned (3–5 μm) and further stained with haematoxylin and eosin prior to evaluation by light microscopy [12].

Pylorus Ligation

Pylorus ligation was performed according to the method described by Shay et al. [14]. Thirty minutes after administering the test solution, pylorus ligation was performed. Four hours later, the animals were sacrificed by exposure to an overdose of diethyl ether; the stomachs were removed and the contents collected before draining into a centrifuge tube followed by centrifugation at 3,000 rpm for 10 min. The pH, juice volume and total acidity of

Table 1. Effect of various doses of MEBP and omeprazole on ethanol- and indomethacin-induced gastric lesions in rats

Model	Pre-treatment	Dose, mg/kg	Ulcer area, mm ²	Protection, %
Absolute ethanol	saline	–	107.2 ± 23.0	–
	omeprazole MEBP	30	41.3 ± 10.3*	58.3
		100	91.5 ± 35.3	14.6
		500	31.8 ± 12.8*	70.3
		1,000	18.0 ± 5.9*	83.2
Indomethacin	saline	–	9.5 ± 1.4	–
	omeprazole MEBP	30	0.0 ± 0.0*	100.0
		100	6.5 ± 2.2	31.6
		500	4.7 ± 0.8*	50.8
		1,000	5.0 ± 0.9*	47.4

Values are means ± SEM (n = 6/group). * p ≤ 0.05: data differed significantly from those of the saline-pre-treated group.

the gastric secretion were determined while gastric lesions were assessed as described earlier [12]. Although the pH is related to total acidity, the concepts are not identical, i.e. pH measures acid strength while total acidity measures the amount of acids present in the collected gastric juice.

Gastric wall mucus content was determined according to the method previously described by Corne et al. [15] while the phytochemical screening of MEBP was carried out according to the methods described by Ikhiri et al. [16].

HPLC Analysis

Ten milligrams of crude dried MEBP was dissolved in 1 ml methanol and filtered through a membrane filter with a pore size of 0.45 μm prior to analysis. The HPLC profile of MEBP was analysed by means of an HPLC system (Waters Delta 600 with 600 Controller) with a photodiode array detector (Waters 996, Milford, Mass., USA). A Phenomenex Luna (5 μm) (Torrance, Calif., USA) column was used (4.6 mm i.d. × 250 mm) and for elution of the constituents, two solvents denoted as A and B were employed. A was 0.1% aqueous formic acid, B was 0.1% formic acid in acetonitrile. Initial conditions were 85% A and 15% B with a linear gradient reaching 25% B at t = 12 min. This was maintained for 10 min after which the program returned to the initial solvent composition at t = 25 min and continued for 10 min. The flow rate used was 0.1 ml/min and the injection volume was 10 μl. The column oven was set at 27°C and the eluant was monitored at wavelengths of 254, 300 and 366 nm. The retention times and UV spectra of major peaks were analysed. The HPLC analyses were carried out in the Laboratory of Phytomedicine, Medicinal Plants Division, Forest Research Institute of Malaysia (FRIM), Kepong, Malaysia.

Statistical Analysis

The results were expressed as means ± SEM and analysed using one-way analysis of variance, followed by Dunnett's multiple-comparison tests. Results were considered significant when p ≤ 0.05.

Table 2. Histopathological evaluation of the effect of various doses of MEBP and omeprazole on ethanol-induced gastric lesions in rats

Pre-treatment	Architecture of the mucosa	Glands	Haemorrhage	Oedema	Inflammatory exudates	Leucocyte infiltration	Cellular debris
Saline	++	-	+++	++	+	+	+
30 mg/kg omeprazole	++	-	++	++	-	-	-
100 mg/kg MEBP	+	-	++	+	-	+	+
500 mg/kg MEBP	+	-	+	+	-	+	-
1,000 mg/kg MEBP	+	-	+	+	-	-	-

The severity of various features of the ethanol-induced gastric lesions were evaluated according to the following scoring scheme: - = normal; + = mild effect; ++ = moderate effect; +++ = severe effect.

Table 3. Effect of various doses of MEBP and omeprazole on gastric juice parameters in the rat model of pylorus ligation

Pre-treatment	Dose, mg/kg	Ulcer area, mm ²	Protection, %	Gastric juice volume, ml	pH	Total acidity [H ⁺]
Saline	-	11.0 ± 1.0 ^a	-	7.7 ± 0.5 ^a	1.58 ± 0.66 ^a	3,426.3 ± 204.5 ^a
Omeprazole	30	2.8 ± 0.5 ^b	74.3	3.7 ± 0.8 ^b	3.27 ± 0.07 ^b	2,430.0 ± 153.7 ^b
MEBP	100	6.5 ± 0.6 ^c	40.9	10.3 ± 0.4 ^c	1.15 ± 0.04 ^a	4,313.3 ± 197.8 ^c
	500	1.8 ± 0.7 ^b	83.4	10.0 ± 0.6 ^c	1.13 ± 0.01 ^a	4,799.3 ± 484.8 ^c
	1,000	6.2 ± 0.6 ^c	44.0	9.8 ± 0.3 ^c	1.12 ± 0.02 ^a	4,191.8 ± 151.5 ^c

Data with different superscripts differed significantly ($p < 0.05$).

Results

Percentage Yield of MEBP and Acute Toxicity Study

Ethanol-Induced Gastric Ulceration. Gross pathological studies revealed that only the 500 and 1,000 mg/kg doses of MEBP exhibited significant ($p \leq 0.05$) reduction of gastric lesion development, with the extent of protection being approximately 70 and 80%, respectively, as compared with the control group (table 1). Overall, MEBP exhibited significant ($p \leq 0.05$) anti-ulcer activity in a dose-dependent manner. The results of the histopathological evaluation studies are summarized in table 2.

Indomethacin-Induced Gastric Ulceration. All doses of MEBP tested demonstrated significant ($p \leq 0.05$) anti-ulcer activity as indicated by reductions in the percentage of total ulcer area of approximately 31.6, 50.8 and 47.4%, respectively, compared to the control group (table 1).

Pylorus Ligation. All doses of MEBP demonstrated anti-ulcer activity as shown by significant ($p \leq 0.05$) reductions in total ulcer area (in the range of approximately 40–83%) compared to the control group (table 3). By

comparison, 30 mg/kg omeprazole produced 74.3% reduction in ulcer area. All doses of MEBP also caused significant ($p \leq 0.05$) increases in the volume of gastric juice released, a slight reduction in the pH of gastric contents and a concomitant increase in the total acidity of the gastric contents compared to the control group. Furthermore, both the 500 and 1,000 mg/kg doses of MEBP significantly ($p \leq 0.05$) increased the gastric wall mucus content by two-fold compared with the control group (table 4).

Histopathological Studies of Gastric Ulceration

Effect of MEBP on Ethanol-Induced Gastric Ulceration in Rats. Overall, tissues of MEBP-treated rats showed significant protection of the gastric mucosa as indicated by a reduction in ulcer areas (fig. 1a–e), haemorrhage and oedema formation, the preservation of a normal mucosal architecture and the absence of leucocyte infiltration at the highest dose used (fig. 1a₁–e₁).

Effect of MEBP on Indomethacin-Induced Gastric Ulceration in Rats. Overall, pre-treatment with MEBP showed anti-ulcer activity as demonstrated by the reduc-

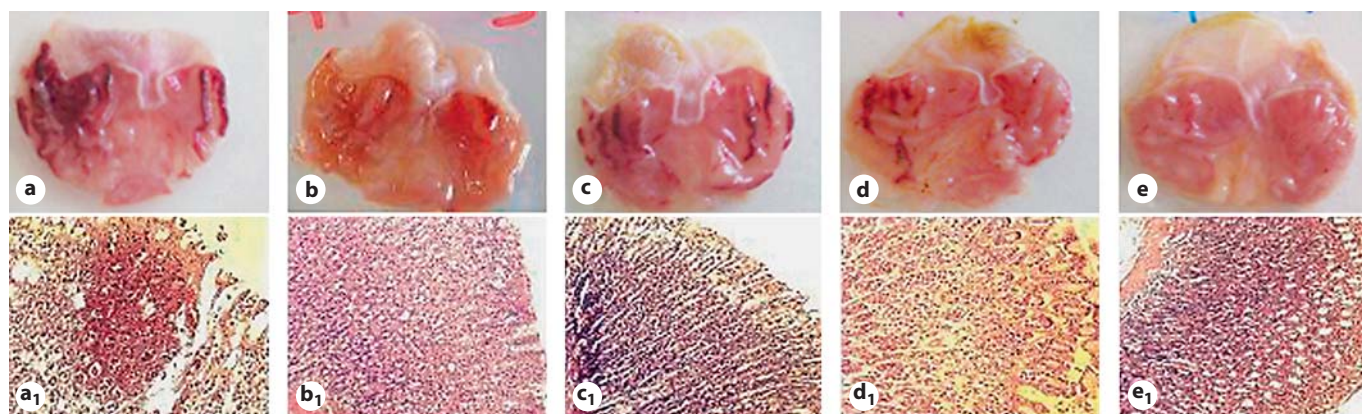


Fig. 1. Anti-ulcer activity of MEBP against absolute ethanol-induced ulcer. **a** Stomach of an ulcer control rat. **b** Stomach of a rat pre-treated with 30 mg/kg omeprazole. **c** Stomach of a rat treated with 100 mg/kg MEBP. **d** Stomach of a rat treated with 500 mg/kg MEBP. **e** Stomach of a rat pre-treated with 1,000 mg/kg MEBP. The corresponding histopathological sections are shown below. **a₁** Stomach of the ulcer control animal showing a severe effect on the mucosa with haemorrhagic erosion, oedema, moderate leucocyte infiltration and cellular debris. **b₁** Stomach of an omeprazole-

treated animal showing a moderate effect on the mucosa with moderate haemorrhage and oedema. **c₁** Stomach of a rat treated with 100 mg/kg MEBP showing a mild effect on the mucosa with moderate haemorrhage and mild oedema, leucocyte infiltration and cellular debris. **d₁** Stomach of a rat treated with 500 mg/kg MEBP showing an almost normal mucosa with mild haemorrhage, oedema and leucocyte infiltration. **e₁** Stomach of a rat treated with 1,000 mg/kg MEBP showing an almost normal mucosa with mild haemorrhage and oedema.

Table 4. Effect of various doses of MEBP and omeprazole on gastric wall mucus content

Pre-treatment	Dose, mg/kg	Gastric wall mucus (Alcian blue $\mu\text{g/g}$ wet tissue)
Saline	–	37.7 ± 3.6^a
Omeprazole	30	36.1 ± 0.5^a
MEBP	100	45.3 ± 3.9^a
	500	97.7 ± 3.2^c
	1,000	79.8 ± 12.9^b

Data with different superscripts differed significantly ($p < 0.05$).

tion in ulcer area and the presence of a normal mucosal architecture, less haemorrhage development and oedema formation as compared to the untreated (control) group (results not shown).

Phytochemical Constituents and HPLC Profile of MEBP. The phytochemical screening of MEBP demonstrated the presence of flavonoids, saponins, condensed tannins and steroids, but no triterpenes or alkaloids. The HPLC profile of MEBP is shown in figure 2a. Two major peaks appeared in the chromatogram at all wavelengths tested at retention times of 12.0 and 14.1 min. Further

analysis demonstrated that the two peaks showed λ_{max} values in the region of 253 nm and 296–354 nm, respectively (fig. 2b).

Discussion

The present study demonstrated that MEBP exerts an anti-ulcer activity against absolute ethanol- and indomethacin-induced gastric ulceration, and in pylorus ligation models in rats and Bighetti et al. [17] previously reported that the ethanol-induced ulcer model can be used to screen drugs for cytoprotective activity. The cytoprotective activity can result from an increase in the synthesis of prostaglandins that, in turn, stimulates mucus and bicarbonate production. Ethanol can either rapidly penetrate the gastric mucosa to cause lipid peroxidation or be metabolized to form superoxide anion and hydroxyl radicals in the gastric mucosa [18]. These reactive metabolites can react with most of the cell components causing changes in their structures and loss of their functions, or be involved in other processes that ultimately result in oxidative damage [19], which in turn leads to gastric mucosal injury. The ability of MEBP to scavenge superoxide anion and other reactive oxygen species has been reported elsewhere [7], and may contribute to the observed

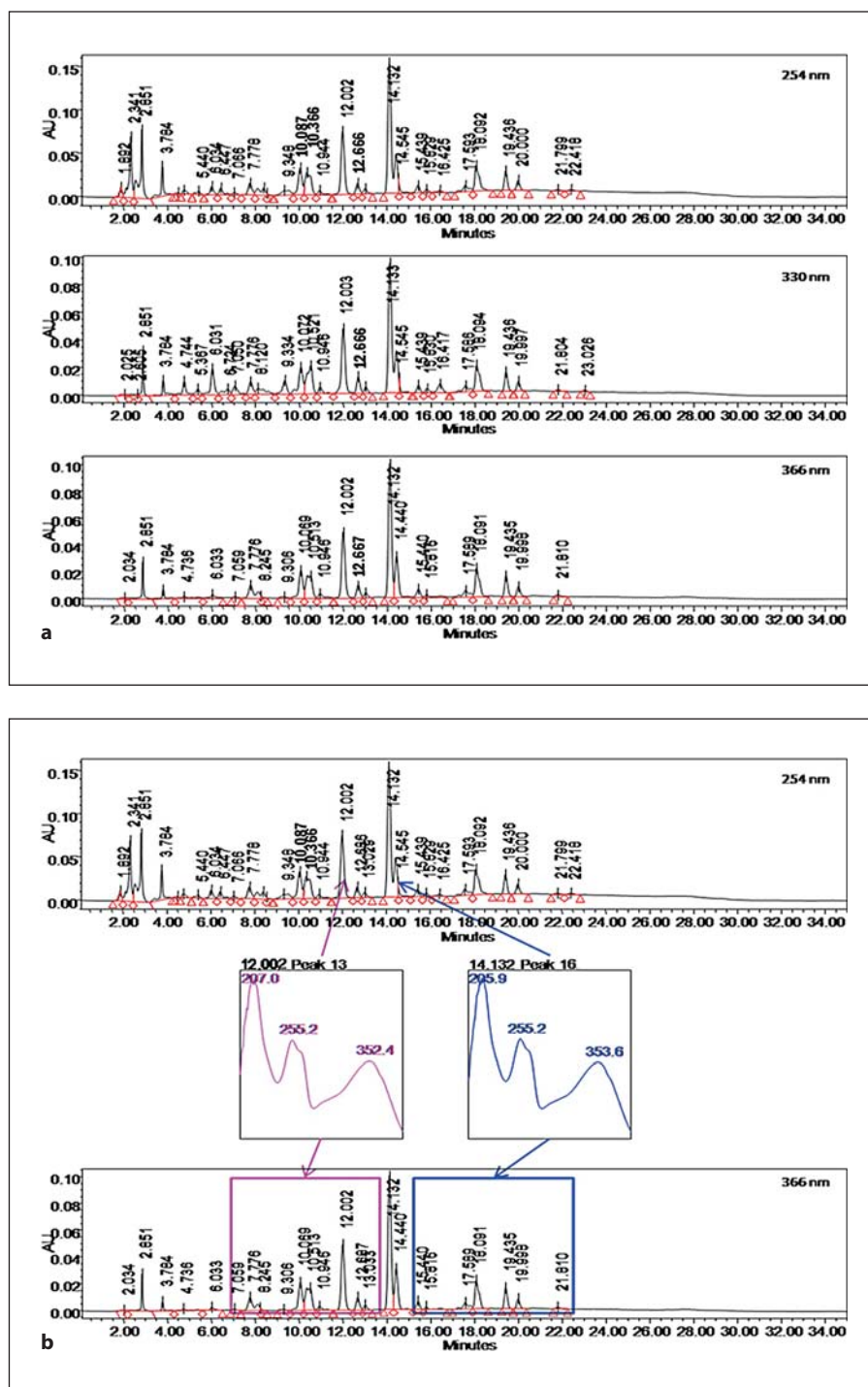


Fig. 2. **a** HPLC profile of MECP at the wavelengths of 254, 330 and 366 nm. **b** UV spectra analysis of peak 13 (retention time = 12.0 min) and peak 16 (retention time = 14.1 min) of MECP at 366 nm exhibiting λ_{\max} at 240–280 and 300–380 nm, suggesting, in part, the presence of flavonoid-based compounds.

anti-ulcer activity [12]. Moreover, the anti-inflammatory effect of MECP reported earlier [2] also supported the observed anti-ulcer activity based on a report indicating that suppression of neutrophil infiltration during inflammation enhances gastric ulcer healing [20]. Al-

though there is no report on the ability of MECP to induce prostaglandin synthesis, one could postulate that it might induce the synthesis of certain type of prostaglandins that play a protective, instead of an injurious, role in the gastric mucosa [22].

The indomethacin-induced ulcer model was employed as it can be used to assess cytoprotective and gastric acid secretion activities [17]. According to Wallace [21], indomethacin suppresses prostaglandin synthesis, and the ability of the extract to attenuate indomethacin-induced gastric ulceration would seem to be inconsistent with the previously reported anti-inflammatory activity of the extract [2, 7]. The observed anti-ulcer activity could be due to the ability of the extract to induce the synthesis of protective prostaglandins (e.g. prostaglandins of the E and I series) as described by Wallace [21] while the anti-inflammatory activity reported by Zakaria et al. [2] is believed to be attributed to the ability of MEBP to attenuate the effect of injurious prostaglandins [21]. In this regard, it is interesting to consider the anti-inflammatory and anti-ulcer activities of liquorice [22]. The anti-inflammatory activity of liquorice assessed using the carrageenan-induced paw oedema test may be due to its effect on the adrenal gland, which is responsible for producing cortisol. In addition, liquorice-derived compounds (i.e. glycyrrhizin) have been reported to increase the concentration of prostaglandins in the digestive system, which induces mucus secretion from the stomach, leading to the healing of ulcers [22]. Several possible pathways have been suggested and some were applied to justify the presence of anti-inflammatory and anti-ulcer activities in MEBP as given below. Firstly, MEBP may act to increase the concentration of prostaglandins in the digestive system as seen with the anti-ulcer study while inhibiting the production and/or release of locally produced prostaglandins in the anti-inflammatory study. Secondly, MEBP might act on the adrenal gland, which is responsible for producing the body's own anti-inflammatory adrenal steroid hormone, cortisol, instead of acting at the level of prostaglandin synthesis [22]. Thirdly, the anti-ulcer and anti-inflammatory activities may both be related to the antioxidant activity of the extract [7, 23]. Furthermore, MEBP also might produce multiple non-specific anti-ulcer effects as seen with boswellic acid, a pure compound isolated from *Boswellia serrata*, instead of suppressing the prostaglandin synthesis [24].

The pyloric ligation model has sometimes been used to determine the mode of action of anti-ulcer agents. This assay is believed to result in the increased release of pepsin, as well as to the accumulation of gastric acid, which can cause damage to gastric mucosal cells, resulting in gastric ulceration [25]. The ability of the mucosal defence barrier to protect against the actions of acid and pepsin depends on the quality and quantity of gastric mucus secretion, both of which are dependent on prostaglandins

[18, 21]. Thus, MEBP may exhibit anti-ulcer activity by reducing gastric acid secretion and/or enhancing mucus secretion in this experimental model.

Previous phytochemical studies have revealed that the leaf of *B. purpurea* contains – in order of decreasing quantities – steroids, saponins, triterpenes and flavonoids [2] while recent studies have demonstrated that MEBP contains only flavonoids, saponins, condensed tannins and steroids. Furthermore, MEBP was found to contain the second highest amount of total phenolic content after the aqueous extract, followed by the plant's chloroform extract [7]. Detailed phytochemical studies on the leaf of *B. purpurea* revealed the presence of dimeric flavonoids [26] and β -sitosterol [27]. The presence of flavonoids as reported by Yadav and Bhadoria [26], in particular, was consistent with our preliminary HPLC analysis of MEBP wherein two of the major peaks detected in the UV spectra (i.e. 207–253 and 205–354 nm) may represent flavonoid-type compounds (e.g. five major subgroups of flavonoids are flavonols, flavones, dihydroflavonols, flavanonols and flavanones) [28]. According to Tsimogiannis et al. [28], the UV-Vis spectra of flavonoids include two absorbance bands, termed band A, that lie in the range of 310–350 nm for flavones and 350–385 nm for flavonols, and band B that is found in the range of 250–290 nm and this is similar in all the aforementioned flavonoid subgroups. In the case of flavanones and dihydroflavonols, the wavelength of band A is often in the range of 300–330 nm while band B falls in the range of 277–295 nm. Furthermore, flavonols, as well as many polyphenols, have been reported to show maximal absorbance at wavelengths between 270 and 290 nm [28]. We have earlier reported the presence of flavonoids in MEBP [7], which is consistent with the UV spectra of MEBP obtained in the present study. Flavonoids [29], in particular, have been reported to possess anti-ulcer properties and could be responsible for the activity of MEBP we have observed. The presence of phenolic compounds in MEBP might also suggest that antioxidant effects could contribute to the anti-ulcer activity of the extract [12].

Conclusion

MEBP exhibited anti-ulcer activity, which could be ascribed to its flavonoid content and/or its antioxidant activity. These findings validate the traditional use of *B. purpurea* in the treatment of gastric ulceration.

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