Antinociceptive, Anti-Inflammatory and Antipyretic Properties of the Aqueous Extract of *Bauhinia purpurea* Leaves in Experimental Animals

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Key Words

Bauhinia purpurea • Aqueous extract • Antinociceptive • Anti-inflammatory • Antipyretic

Abstract

Objective: The present study was carried out to determine the antinociceptive, anti-inflammatory and antipyretic activities of the aqueous extract of *Bauhinia purpurea* leaves using animal models. **Materials and Methods:** The air-dried, powdered leaves (≈ 20 g) were soaked in distilled water (1:20; w/v) at room temperature for 72 h and the supernatant obtained was freeze-dried. The crude dried extract (≈ 2.4 g) was prepared in doses of 6.0, 30.0 and 60.0 mg/kg, and subjected to the respective antinociceptive (abdominal constriction, hot plate and formalin tests), anti-inflammatory (carrageenan-induced paw edema test) and antipyretic (brewer's yeast-induced pyrexia test) assays. **Results:** The results obtained indicate that the extract possessed significant ($p < 0.05$) antinociceptive, anti-inflammatory and antipyretic activities, which were not dependent on the doses of extract used. The highest concentration of extract was less effective as an anti-inflammatory and an antipyretic agent. **Conclusion:** This study showed that the aqueous extract of *B. purpurea* leaves possesses potential pharmacological ac-

tivities that require further investigation and, thus, confirms the folklore use of the plant in the treatment of ailments associated with pain and inflammation.

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Introduction

Bauhinia purpurea, better known as butterfly tree, is a plant that belongs to the family Leguminosae and native to India and Southern China. Although there is no documentation on its traditional use to treat diseases among the Malaysians, this plant has been used in the Indian, Sri Lankan and Pakistani folklore medicine to treat ailments like glandular swellings, skin diseases, ulcers, diarrhea, stomach tumors and wounds [1, 2]. *B. purpurea* has also been used in the treatment and control of hyperglycemia [3] and seems to possess laxative and astringent effects [1, 2]. The flowers of *B. purpurea* have also been used as a purgative in Pakistan [2], while its leaves were applied externally to the forehead to treat fever [4]. Several types of bioactive compounds have been isolated from different parts of *B. purpurea* [3, 5–7], particularly flavonoids, such as kaempferol, quercetin and isorhamnetin [3] and pacharin and four new dibenz[b,f]oxepins (2a, 3–5), named

bauhiniastatins 1–4 [7] from the plant leaves. Despite those traditional claims and successful isolation of the bioactive compounds, no in-depth scientific study has been performed on *B. purpurea* pharmacological properties. Thus, the aim of the present study was to determine the antinociceptive, anti-inflammatory and antipyretic properties of the aqueous extract of *B. purpurea* (AEBP) leaves using various animal models.

Materials and Methods

Plant Material

The leaves of *B. purpurea* were collected in June 2005 from their natural habitat in Shah Alam, Selangor, Malaysia, and were identified by Mr. Shamsul Khamis, a botanist at the Institute of Bioscience, Universiti Putra Malaysia, Selangor, Malaysia. A voucher specimen (SK 1095/05) was deposited at the Herbarium of the Laboratory of Natural Products, Institute of Bioscience, Universiti Putra Malaysia. The phytochemical screening of *B. purpurea* leaves was carried out according to the methods described by Ikhiri et al. [8].

Preparation of AEBP and Standard Drugs

The leaves of *B. purpurea* were washed and rinsed with water to remove all the dirt and unwanted particles and then air-dried for 3–4 weeks at room temperature ($27 \pm 2^\circ\text{C}$). The dried leaves were then ground into small particles, weighed (≈ 20 g) and treated with distilled water (H_2O) in the ratio of 1:20 (w/v). This mixture was then left at room temperature for 72 h and the supernatant was collected and filtered using Whatman No. 1 filter paper while the remaining plant residue was kept in an oven. The supernatant obtained, labeled as AEBP and considered as stock solution with 100% concentration/strength, was prepared in the concentrations/strengths of 10 and 50% by diluting the stock solution with H_2O for the pharmacological studies described earlier. One hundred milliliters of the obtained supernatant was also subjected to freeze-drying, yielding ≈ 0.60 g of crude dried AEBP (percentage of yield is ≈ 12.00). Based on the amount of crude dried AEBP obtained, it was estimated that the 10, 50 and 100% concentrations of AEBP were approximately equivalent to the dosages of 6.0, 30.0 and 60.0 mg/kg, respectively. 100 mg/kg acetylsalicylic acid (ASA; Bayer, Singapore), and 5 mg/kg morphine (Sigma, Germany), used for the purpose of comparison, were prepared by dissolving them in H_2O .

Instrumentation for HPLC Profiling of the AEBP

HPLC profiling of the AEBP was carried out in the Laboratory of Phytomedicine, Forest Research Institute of Malaysia, Kepong, Malaysia. A Waters Delta 600 with 600 Controller and Waters 2996 Photodiode Array (Milford, Mass., USA) equipped with an autosampler, online degasser and column heater was used for HPLC analysis. Data were analyzed and processed using the installed Millennium 32 Software (Waters Product). The sample was separated on a minibore Phenomenex Luna $5 \mu\text{m}$ C_{18} column (Torrance, Calif., USA) with a dimension 250×4.60 mm using a one-step linear gradient. The solvents were: (a) 0.1% orthophosphoric acid and (b) acetonitrile. The elution system was as follows:

0–12 min, 0–15% of b; 12–22 min, 15–25% of b; 22–35 min, 25–15% of b. The flow rate was 1.0 ml/min, the injection volume was 10 μl and the column oven was set at 27°C . The eluant was monitored at 200, 254 and 330 nm.

Experimental Animals

Male Balb-C mice (25–30 g; 5–7 weeks) and Sprague-Dawley rats (180–200 g; 8–10 weeks old), obtained from the Institute of Medical Research, Kuala Lumpur, Malaysia, were used in this study. All animals were handled according to the methods described elsewhere [9]. Seventy mice were equally divided into 10 groups ($n = 7$) and received (s.c.) H_2O , ASA (100 mg/kg) or MRP (5 mg/kg), and AEBP (6.0, 30.0 and 60.0 mg/kg) 30 min prior to being subjected to the abdominal constriction or hot plate tests, respectively. In the other animal group, 80 rats were equally divided into 16 groups ($n = 5$). The first batch of six groups were used in the formalin test and received (s.c.) H_2O , 100 mg/kg ASA, 5 mg/kg morphine or AEBP (6.0, 30.0 and 60.0 mg/kg), respectively, 30 min prior to being subjected to the test. The second and third batches of five groups were used in the anti-inflammatory and antipyretic studies, respectively, and received (s.c.) H_2O , 100 mg/kg ASA or AEBP (6.0, 30.0 and 60.0 mg/kg), respectively, 30 min prior to being subjected to the tests. All of the test solutions were administered at 10 ml/kg body weight.

Antinociceptive, Anti-Inflammatory and Antipyretic Assays

The antinociceptive activity of AEBP was determined using the abdominal constriction, hot plate and formalin tests. The 0.6% acetic acid-induced abdominal constriction test [10] with slight modifications was used to evaluate the chemically induced antinociceptive activity of AEBP. To assess the thermal-induced central antinociceptive activity of AEBP, the 50°C hot plate test [11] was used with slight modifications. The 5% formalin test [12] was used with slight modifications to assess the antinociceptive, non-anti-inflammatory properties of the AEBP. The 1% carrageenan-induced paw edema test [13] was used with slight modifications to assess the anti-inflammatory effect of the AEBP. The 10% brewer's yeast (BY)-induced antipyretic activity of AEBP was measured according to the method described by Reanmongkol et al. [14] but with slight modifications.

Statistical Analysis

The results are presented as mean $\pm$ standard error of mean (SEM). The one-way ANOVA test with Tukey post-hoc test was used to analyze and compare the data obtained for the abdominal constriction and formalin assays, while the two-way ANOVA test with Tukey post-hoc test was used to analyze and compare the data obtained for the hot plate, paw edema and pyrexia assays. $p < 0.05$ was used as the limit of significance.

Results

Phytochemical Screening and HPLC Profiling of the *B. purpurea* Leaves

The phytochemical screening of *B. purpurea* leaves demonstrated the presence of flavonoids, saponins, triterpenes and steroids, but no alkaloids or tannins (ta-

Fig. 1. The HPLC profiles of AEBP at 200, 254 and 330 nm.

Fig. 2. The HPLC profile of AEBP compared to the standard flavonoids at 200 nm.

ble 1).The HPLC profiles of AEBP were measured at three different wavelengths, namely 200, 254 and 330 nm (fig. 1). Comparison against standard flavonoids (i.e. catechin, rutin and fisetin) was performed at 200 nm as those standards were best detected at the said wavelength (fig. 2). Comparison made at the wavelength of 200 nm against standard flavonoids, namely catechin (retention time $R_T = 6.742$ min), rutin ($R_T = 14.402$ min) and catechin ($R_T = 21.786$ min), demonstrated that the various peaks present in the AEBP possessed R_T values that are different from the standards, except for the peak with R_T value of 21.782 min. The R_T value (21.782 min) was almost identical to that of fisetin (21.786 min) and suggests its presence in the AEBP. Although the retention time

Table 1. Phytochemical constituents of *B. purpurea*

Constituents	<i>B. purpurea</i>	Constituents	<i>B. purpurea</i>
Flavonoids	+	Alkaloids	–
Triterpenes	+	Saponins	++
Tannins	–	Steroids	+++

Flavonoids, tannins, triterpenes and steroids: + = weak color; ++ = mild color; +++ = strong color. Alkaloids: + = negligible amount of precipitate; ++ = weak precipitate; +++ = strong precipitate. Saponins: + = 1–2 cm froth; ++ = 2–3 cm froth; +++ = >3 cm froth.

Table 2. The antinociceptive activity of AEBP assessed using the hot plate test

Treatment	Hot plate latency at various intervals, s (mean $\pm$ SEM)					
	0 hours	1 hour	2 hours	3 hours	4 hours	5 hours
H ₂ O	8.00 $\pm$ 0.74	7.66 $\pm$ 0.56	8.48 $\pm$ 0.54	9.26 $\pm$ 0.92	8.08 $\pm$ 1.52	9.74 $\pm$ 0.47*
5 mg/kg morphine	7.68 $\pm$ 1.46	18.19 $\pm$ 1.61 ^a	18.97 $\pm$ 0.94 ^b	17.94 $\pm$ 0.69 ^c	15.64 $\pm$ 0.98 ^d	14.27 $\pm$ 0.78 ^e
AEBP						
10%	6.90 $\pm$ 0.47	6.60 $\pm$ 0.25	12.18 $\pm$ 1.34 ^b	15.64 $\pm$ 1.50 ^c	9.46 $\pm$ 1.61	11.68 $\pm$ 1.18
50%	8.04 $\pm$ 1.47	7.04 $\pm$ 1.02	13.02 $\pm$ 0.37 ^b	15.02 $\pm$ 1.17 ^c	10.98 $\pm$ 1.63	7.32 $\pm$ 1.76
100%	6.78 $\pm$ 1.47	7.42 $\pm$ 0.72	14.78 $\pm$ 0.73 ^b	15.06 $\pm$ 0.90 ^c	12.48 $\pm$ 1.62 ^d	11.92 $\pm$ 1.14

* $p < 0.05$ (significantly different) when compared to the control group (H₂O treated) at the 0 h time interval. Data with same superscript differed significantly ($p < 0.05$) when compared to the control group (H₂O treated) at their respective time interval. There were 7 mice in each treatment.

Table 3. The antinociceptive activity of AEBP assessed using the formalin test

Treatment	Early phase, s	Late phase, s
H ₂ O	73.00 $\pm$ 3.24	81.00 $\pm$ 2.61
5 mg/kg morphine	16.20 $\pm$ 0.98*	4.90 $\pm$ 0.97*
100 mg/kg ASA	76.40 $\pm$ 6.44	10.00 $\pm$ 3.08*
AEBP		
10%	22.92 $\pm$ 6.13*	22.88 $\pm$ 5.53
50%	34.96 $\pm$ 7.31*	29.64 $\pm$ 14.40*
100%	38.78 $\pm$ 3.97*	46.12 $\pm$ 18.98*

* $p < 0.05$ (significantly different) when compared to their respective control group (H₂O treated). There were 5 mice in each treatment.

could not give confirmation on the actual compounds that might be present in the extracts, it can be used as a comparison against standards, which have been known to possess a specific retention time.

Pharmacological Studies on the AEBP

The AEBP was found to cause insignificant change in the number of abdominal constrictions at all doses used, indicating its failure to inhibit/reverse the acetic acid-induced abdominal constriction test (data not shown). The antinociceptive activity of AEBP assessed using the hot plate test in mice is shown in table 2. The extract, at all of the doses used, exhibited significant ($p < 0.05$) antinociceptive activity 2 h after subjection of the mice to the test. The activity reached its maximum effect, indicated by prolonged latency of discomfort, at the interval of 3 h

with only the activity of 60.0 mg/kg remaining to be seen until the end of the experiment. However, the activity of AEBP was less in magnitude when compared to the 5 mg/kg morphine at all time.

The antinociceptive activity of AEBP assessed using the formalin test in rats is shown in table 3. The extract, at all of the doses used, exhibited significant ($p < 0.05$) antinociceptive activity in the early and late phases of the test. In both phases of the test, the activity occurred in a dose-independent manner, with all doses of the extract exhibiting insignificant activity when compared together. The 6.0 mg/kg AEBP exhibited the highest activity, which is equieffective when compared to 5 mg/kg morphine in the early phase but to 100 mg/kg ASA in the late phase.

The anti-inflammatory activity of AEBP was assessed using the carrageenan-induced paw edema test in rats (table 4). Only at the dose of 30.0 mg/kg was the extract found to exhibit significant anti-inflammatory activity. Interestingly, the 30.0 mg/kg AEBP exhibited an equieffective activity when compared to the 100 mg/kg ASA only between the interval times of 1–4 h, after which the ASA activity was found to be greater than the extract until the end of the experiment.

The AEBP also exhibited significant ($p < 0.05$) antipyretic activity when assessed using the BY-induced pyrexia test in rats (table 5). The dose-independent antipyretic activity was observed only at the concentration 6.0 and 30.0 with the former showing remarkable activity even when compared with 100 mg/kg ASA. The 60.0 mg/kg AEBP was effective only at the interval of 1 h after the BY administration before it lost its activity until the end of the experiment. There was a remarkable decrease

Table 4. The anti-inflammatory activity of AEBP assessed using the carrageenan-induced paw edema test

Treatment	Edema volume at different time intervals, ml (mean $\pm$ SEM)								
	0 hours	0.5 hour	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours	7 hours
H ₂ O	0.16 $\pm$ 0.02	0.36 $\pm$ 0.05*	0.44 $\pm$ 0.07*	0.53 $\pm$ 0.07*	0.45 $\pm$ 0.06*	0.34 $\pm$ 0.08*	0.30 $\pm$ 0.07*	0.27 $\pm$ 0.07*	0.25 $\pm$ 0.06
100 mg/kg ASA	0.18 $\pm$ 0.02	0.24 $\pm$ 0.03 ^a	0.28 $\pm$ 0.03 ^b	0.26 $\pm$ 0.04 ^c	0.16 $\pm$ 0.05 ^d	0.11 $\pm$ 0.04 ^e	0.08 $\pm$ 0.03 ^f	0.05 $\pm$ 0.03 ^g	0.02 $\pm$ 0.02 ^h
AEBP									
10%	0.30 $\pm$ 0.08	0.46 $\pm$ 0.09	0.46 $\pm$ 0.09	0.42 $\pm$ 0.09	0.33 $\pm$ 0.10	0.30 $\pm$ 0.09	0.35 $\pm$ 0.09	0.33 $\pm$ 0.10	0.31 $\pm$ 0.10
50%	0.14 $\pm$ 0.03	0.29 $\pm$ 0.04	0.27 $\pm$ 0.02 ^b	0.23 $\pm$ 0.02 ^c	0.21 $\pm$ 0.07 ^d	0.21 $\pm$ 0.06	0.19 $\pm$ 0.03	0.18 $\pm$ 0.02	0.16 $\pm$ 0.02
100%	0.28 $\pm$ 0.07	0.41 $\pm$ 0.06	0.36 $\pm$ 0.05	0.34 $\pm$ 0.06 ^c	0.33 $\pm$ 0.06	0.35 $\pm$ 0.05	0.35 $\pm$ 0.05	0.33 $\pm$ 0.05	0.32 $\pm$ 0.05

* $p < 0.05$ (significantly different) when compared to the control group (H₂O treated) at the 0 h time interval. Data with same superscript differed significantly ($p < 0.05$) when compared to the control group (H₂O treated) at their respective time interval. There were 5 mice in each treatment.

Table 5. The antipyretic activity of AEBP assessed using the BY-induced pyrexia test

Treatment	Body temperature at different time intervals, °C (mean $\pm$ SEM)									
	BF	0 hours	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours	7 hours	8 hours
H ₂ O	37.82 $\pm$ 0.29	37.88 $\pm$ 0.18	38.84 $\pm$ 0.16*	39.12 $\pm$ 0.11*	39.08 $\pm$ 0.30*	38.70 $\pm$ 0.11*	38.86 $\pm$ 0.27*	38.96 $\pm$ 0.22*	38.06 $\pm$ 0.53	38.41 $\pm$ 0.28
100 mg/kg ASA	37.46 $\pm$ 0.24	38.18 $\pm$ 0.22	37.48 $\pm$ 0.30 ^a	37.38 $\pm$ 0.36 ^b	37.90 $\pm$ 0.37 ^c	38.06 $\pm$ 0.23 ^d	38.32 $\pm$ 0.07	38.58 $\pm$ 0.14	38.60 $\pm$ 0.09	38.68 $\pm$ 0.15
AEBP										
10%	37.50 $\pm$ 0.37	37.02 $\pm$ 0.30	35.60 $\pm$ 0.27 ^a	35.24 $\pm$ 0.25 ^b	36.08 $\pm$ 0.25 ^c	36.32 $\pm$ 0.28 ^d	36.96 $\pm$ 0.20 ^e	37.26 $\pm$ 0.19 ^f	37.70 $\pm$ 0.07	37.86 $\pm$ 0.08
50%	38.22 $\pm$ 0.23	37.76 $\pm$ 0.18	36.34 $\pm$ 0.55 ^a	36.68 $\pm$ 0.50 ^b	37.38 $\pm$ 0.40 ^c	37.82 $\pm$ 0.29 ^d	38.38 $\pm$ 0.20	38.38 $\pm$ 0.09	38.30 $\pm$ 0.09	38.08 $\pm$ 0.04
100%	38.40 $\pm$ 0.16	37.72 $\pm$ 0.20	37.52 $\pm$ 0.18 ^a	38.76 $\pm$ 0.25	39.24 $\pm$ 0.10	39.18 $\pm$ 0.20	39.10 $\pm$ 0.06	39.42 $\pm$ 0.16	38.81 $\pm$ 0.09	38.67 $\pm$ 0.09

* $p < 0.05$ (significantly different) when compared to the control group (H₂O treated) at the 0 h time interval. Data with same superscript differed significantly ($p < 0.05$) when compared to the control group (H₂O treated) at their respective time interval. There were 5 mice in each treatment.

in the rat's rectal temperature in the first 2 h after the BY administration before the temperature was seen to increase gradually until it reached the normal rectal temperature at the end of the experiment.

Discussion

The present study has demonstrated the pharmacological potential of *B. purpurea* as an antinociceptive, anti-inflammatory and antipyretic agent when tested on various animal models. The ability of AEBP to prolong the latency to discomfort in the respective formalin and hot plate tests plausibly suggested the extract's potential to inhibit chemically and thermally induced noxious stimuli. Other than that, the ability to inhibit/reverse the former and latter tests could also be associated with the extract's potential to inhibit the inflammation-induced [15] and non-inflammation-related [16] nociception, respectively. Interestingly, according to Hunskaar et al.

[17], the ability to inhibit both types of stimuli also indicates that the extract possesses a characteristic of a strong analgesic with centrally mediated activity [18], which is also supported by the observation that the extract inhibited both phases of the formalin test. Furthermore, the antinociceptive activity seen in the early phase of the formalin test reflects the extract's potential to produce an antinociceptive, non-anti-inflammatory effect [19] when given systemically. The nonconcentration-dependent activity seen in all assays were unexpected but could be related to the terms 'therapeutic window' [20] and 'receptor downregulation' [21].

In general, several mechanisms of action could be used to explain the observed antinociceptive activity of AEBP. The ability to inhibit/reverse the centrally synthesized prostaglandins or COX [22] could be one of the possible mechanisms that contribute to the central antinociceptive, as well as antipyretic activities of the AEBP seen in the present study. The involvement of the opioid system in the AEBP antinociceptive activity could also be sug-

gested based on the claim by Chan et al. [18] and Hosseinzadeh and Younesi [23] that centrally acting drugs like opioids affect both phases of the formalin and hot plate tests, respectively. Although the AEBP did not cause any significant change in the abdominal constriction test, the effect of AEBP on peripheral prostaglandins or COX should not be ruled out based on the significant anti-inflammatory activity as discussed in the next paragraph. Further, the failure of AEBP to cause changes in the number of abdominal constrictions does not mean that the extract is inactive against the chemically noxious stimuli, but rather could possibly be due to the types of stimuli or assays used as the extract also produced positive results in the formalin test, another type of chemically induced nociceptive assay. Interestingly, the compound of the triterpene glycoside type has been reported to give positive result in the hot plate but not the abdominal constriction tests [24]. Although the present study did not aim at isolation and identification of bioactive compounds, the presence of triterpenes in the AEBP could possibly lead to the observed activities. The anti-inflammatory and antipyretic activities of AEBP could also be linked to the ability of the extract to influence the peripheral and central COX activity or prostaglandin synthesis [18]. This fact is supported by claims that the carrageenan-induced inflammation is a COX-dependent response and is more effectively controlled with arachidonate cyclo-oxygenase but not arachidonate lipo-oxygenase inhibitors [25]. In addition, the pyrexia mechanism involved the activation of central COX or production of prostaglandins within the central nervous system [22]. The ability of AEBP to inhibit/reverse the effect of prostaglandins or COX has been explained in the above paragraph.

The phytochemical screening of AEBP demonstrated the presence of flavonoids, saponins, steroids and triterpenes, which are suggested to act synergistically to exert the observed pharmacological activity [26]. Interestingly, compounds like flavonoids [27] and triterpenes [28], in part, have been shown to possess anti-inflammatory activity, and the claim made by Attaway and Zaborsky [29] that compounds with anti-inflammatory activity also possess antinociceptive activity seems to support our findings on the AEBP pharmacological activities. Based on the classes of compounds detected in AEBP, several mechanisms of action could be used to explain the observed activities of the extract. Flavonoids are potent inhibitors of nitric oxide synthase type 2 that are involved in the synthesis of NO [30] via indirect blockade of the cyclo-oxygenase and/or lipoxygenase pathways [31], and

of the protein kinase C and L-arginine/NO pathways [32] that have been known to take part in a series of molecular events that lead to antinociceptive [33] and anti-inflammatory [27] activities. The links between release of NO from the endothelial cells during vasorelaxation, the ability of flavonoids to induce vasodilation and the importance of vasodilation in the antinociceptive and anti-inflammatory mechanisms have also been reported and are worth mentioning [34, 35]. Further, Middleton et al. [36] have reported on the flavonoids' potential to inhibit phospholipase A2 and phospholipase C, which are important enzymes in a cascade of inflammatory process. The anti-inflammatory activity of AEBP, on the other hand, could be associated with the inhibitory effect of flavonoids and triterpenes on the nuclear factor- κ B [37]. Saponin has also been reported to exert antinociceptive activity via modulation of the GABA_A, NMDA and non-NMDA receptors at the supraspinal level [38]. Suh et al. [38] have also reported that the nonopioid-mediated saponin antinociception is modulated via activation of the descending serotonin and α_2 -adrenergic pathways.

Conclusion

The present study demonstrated that the AEBP possessed various potential pharmacological properties, namely antinociceptive, anti-inflammatory and antipyretic activities. Phytochemical screening revealed the presence of compounds like flavonoids, triterpenes, saponins and steroids, which were expected to act synergistically to produce the observed activities. Thus, the present study confirmed the folklore use of *B. purpurea* for the treatment of various ailments, and the plant's potential pharmacological activities merit further investigation.

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