SHORT COMMUNICATION

Phyto-Pharmacological Investigation of Ethanolic Extract of Flowers of *Bauhinia acuminata*

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Abstract

Bauhinia acuminata is a type of angiosperm in the Fabaceae family, native to South and Southeast Asia, South China, Burma, India, Nepal, Pakistan, and Sri Lanka. The common names include *B. acuminata*, mountain ebony, camel's foot tree, kachnar, and butterfly ash. Angiosperm tree could be widely used as a medicinal plant common in tropical regions. Flowers, buds, stems, roots, bark, seeds, and leaves have been used to treat many ailments since ancient times. NSAIDs are one of the most important classes of drugs used today, and several clinical problems require long-term use. As a result of long-term use, side effects, especially stomach ulcers, can worsen the patient's clinical symptoms. So there is a requirement to go for painkillers, but it is not related to problems even with chronic use. The literature says that a lot of analytical work has been done on this plant, but none of it has been evaluated for its analgesic and anti-inflammatory effects. An aqueous ethanolic extract was prepared and its analgesic, anti-inflammatory, and antipyretic effects were evaluated in animal models. The results showed that the flower extract at 200 mg/kg dose had significant analgesic, anti-inflammatory, and antipyretic effects compared to the medicinal active drugs.

Keywords: *Bauhinia acuminata*, Phytochemistry, Kanchnar, Analgesic, Anti-inflammatory, Antipyretic activity.

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Introduction

Plants are a valuable source of many secondary metabolites that are used as pharmaceuticals, agrochemicals, flavors, fragrances, dyes, biopesticides, and food additives. People have been using plants as drugs for thousands of years. All plant parts (leaves, flower buds, flower, stem, stem bark, seeds, and roots) were used in ancient medicines. A selection of anti-inflammatory drugs is one of each widely used drug class, a medicinal plant with analgesic, antimicrobial, anti-inflammatory, and antipyretic activity selected for pharmacological analysis (Bakhru *et al*., 1998; Balajirao *et al*., 1995; Rajkapoor *et al*., 2006; Parekh *et al*., 2006; Rajanna *et al*., 2011; Gupta *et al*., 2005; Gupta *et al*., 2004; Anjani *et al*., 1992; Kumar *et al*., 2005). *Bauhinia acuminata* contains sterols, flavonoids, saponins, and tannins in various elements of the plant, such as roots, flowers, and leaves, and is believed to have multiple pharmacological properties, ranging from healing, analgesic, and antipyretic properties. Nonsteroidal anti-inflammatory drugs (NSAIDs), currently available for various effects such as stomach irritation, cause excretory blood flow in the injured urinary tract and tend to prolong bleeding by suppressing platelet counts (Hakim *et al*., 2010). Therefore, we created an experiment to pharmacologically evaluate these properties of the stem flowers of the orchid tree.

Taxonomical Status

Taxonomically, *B. acuminata* is classified into the kingdom Plantae, the division Tracheophyta, the class Magnoliposida, the order Fabales, the family Fabaceae, the genus Bauhinia, and the species Acuminata. It is a deciduous plant that produces showy pink or white flowers during spring and summer. The leaves are alternate and pinnate, with two to four pairs of leaflets. The flowers are bisexual and are pollinated by insects. The fruit is a pod containing several black seeds.

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Botanical Description

B. acuminata will be a little to medium-sized evanescent tree with a short caddy and spreading crown, attaining a peak over 15 m and a diameter of 50 cm. In dry forests, the dimensions are ways lower (Akhter *et al*., 2012; Murashige *et al*., 1962). The bark is light darkish brown-grey, glossy to slightly fissured and scaled. The inner bark is crimson, fibrous and sour. The twigs are slender, zigzag; once young, mild weight green, barely bushy and angled, turning into darkish-brown gray. Leaves have minute stipules 1 to 2 metric linear unit, early caduceus, stalk puberulous to hairless, 3 to 4 cm; plate broadly ovate to circular, typically broader than long, 6 to 16 cm diameter; nerved; pointers of lobes broadly rounded, base cordate; facet hairless, decrease opaque however hairless once mature.

Figure 1: Flower of *B. acuminata*

Flower clusters (racemes) are branchless at the ends of twigs. The little vegetation has brief, stout stalks and a stalk-like, green, narrow basal tube (hypanthium) (Figure 1). The sunshine green, pretty furry curlicue bureaucracy a pointed five-angled bud and split open on one aspect, last attached; petals five, barely unequal, wavy margined and narrowed to the bottom; five snaky stamens; extraordinarily narrow, stalked, snaky reproductive structure, with slim, green, one-celled ovary, fashion and dot-like stigma. Pods are dehiscent, strap-shaped, obliquely striate, lengthy, tough, and flat with 10 to 15 seeds in every; seeds are brown, flat, and almost circular with coriaceous seed fleece. The ideal call refers back to the variegation of the plants.

Phytochemical Properties

The *Bauhinia acuminate* contains several phytoconstituents like vit-C, Quercetin, and its derivatives. The presence of kaempferol and apigenin indicates the presence of flavonoids (Sravika *et al*., 2021). It also contains palmitic, phthalic, gallic, etc (Sebastian *et al*., 2020). Other different constituents like saponin, tannin, carbohydrates, alkaloids, and glycosides are also present in extracts. It contains a major amount of carbohydrates, followed by protein, lipids, and crude fiber. Due to the presence of phenolic compounds, it has antioxidant properties. The plant also contains cardiac glycosides. Steroids, terpenoids, resins, and amino acids are also available with the extract (Dongray *et al*., 2015 and Neelima *et al*., 2021).

Materials and Methods

Collection and Extraction

The source of flower of *B. acuminata* was collected from Mancheswar village of Bhubaneswar. The flower of *B. acuminata* was dried under shade and powdered by a mechanical grinder and passed through sieve no 40. The coarse powder of the flower was extracted through warm percolation and soxhalation at 60°C for twelve hours. The extract is washed with petroleum ether and dried in desiccators.

Animals

Wister rats (150-200g) of both sex (for anti-inflammatory & antipyretic activity) and male albino mice (for analgesic activity) weighing 20 to 25 g were randomized into four teams of six each for each experiment. Animals were acclimatized to the animal house conditions for a week. Water and diet were given *ad libitum*. Polypropylene cages were used to house all animals (3 in one cage) at a specific temperature (25 ± 2°C), and relative humidity (55–65%), and animals were kept under a 12 hours light/dark cycle in the animal house. The food was withheld ten hours before experimentation; however free access to water was allowed.

Drugs and Chemicals

Carrageenan was arranged from Sigma Chemical Co. (St Louis, MO, USA), Diclofenac sodium (cataflam) from Novartis India Ltd., Mumbai, paracetamol from Odisha Drugs and Chemicals Ltd., Bhubaneswar, India, and formalin from (Rankem). Vernier caliper was purchased from Percision India Ltd.

Drug Administration

The flower extract of *B. acuminata* was administered by suspending it in a gum arabic solution. In every model, ethanolic extract of flower of *B. acuminata* at doses of 100 and 200 mg/kg for anti-inflammatory, analgesic, and antipyretic activities, while diclofenac sodium at a dose of 10 mg/kg (for anti-inflammatory & analgesic activity) and paracetamol 100 mg/kg (for antipyretic activity) orally.

Evaluation of *in-vivo* anti-inflammatory activity (Parmar *et al*., 2006)

Paw edema was induced by injecting 0.1 mL of 1% w/v carrageenan suspended in 1% CMC into sub-plantar tissues of the left hind paw of each rat after 30 minutes of drug administration. Rats were divided into four groups, each group consisting of six animals.

Group I- Carrageenan control (C.C)
Group II- 100 mg/kg of ethanolic extract (Test 1)
Group III- 200 mg/kg of ethanolic extract (Test 2)
Group IV- 10 mg/kg of Diclofenac sodium as reference (Std.)

The intensity of paw edema was measured using a Vernier caliper at 60, 120, 180, 240, and 300 min after administration of carrageenan to each group. The following formula calculated the inhibitory activity:

Inhibition of paw edema (%) = [(EC-ET)]/EC×100

Where EC is the edema thickness of the toxicant control group and ET is the edema thickness of the treated groups.

Analgesic activity

The hot plate method and the writhing test were used to measure the analgesic activity.

Hot plate method (Hendershot *et al*.,1959)

Albino mice were placed one by one on the plate and maintained a specific temperature of 55 ± 1°C for 30 seconds. Time of reaction for animals to lick their hind paws or to leap out after placing it on the hot plate was noted. Standard group animals were maintained by administering 10mg/ kg Diclofenac sodium, 60 minutes before placing the animals on the hot plate. The mice were administered with standard drug and claimed extract. The time between placing the heated surface and shaking or licking the paw or jumping was recorded. To prevent tissue damage, an automatic 30msec cut-off was used. The reaction time for mice was recorded at 0, ½, 1, 2, and 3 hours.

Writhing test (Gerhard *et al*., 2002)

The animals fasted overnight before the test. Mice had been injected with intra-peritoneal 0.1 mL of 0.6% acetic acid after 30 minutes of the treatment. The standard group is treated with diclofenac sodium (10 mg/kg). Similarly, the test groups were treated with 100 and 200 mg/kg of extract.

Table 1: Anti-inflammatory Activity of *B. acuminata* flower extract

Treatment dose (mg/kg)	*The difference in paw circumference in cm at time points*				
	1 hour	*2 hours*	*3 hours*	*4 hours*	*5 hours*
Carrageenan control (C.C)	0.91 ± 0.26	1.21 ± 0.14	1.44 ± 0.13	0.99 ± 0.19	0.71 ± 0.13
Diclofenac sodium (10 mg/kg)	0.6 ± 0.10^{***a}	0.12 ± 0.07^{***a}	0 ± 0015^{***a}	0 ± 0.08^{***a}	0.023 ± 0.08^{**a}
Test 1 (100 mg/kg)	0.36 ± 0.08^{***a}	0.27 ± 0.05^{***a}	0.19 ± 0.05^{***a*b}	0.21 ± 0.05^{***a}	0.18 ± 0.05^{***a}
Test 2 (200 mg/kg)	0.10 ± 0.05^{***ab}	0.08 ± 0.04^{***a}	0.03 ± 0.10^{***a}	0.04 ± 0.08^{***a}	0.06 ± 0.04^{***a}

n = 6, Values are expressed as mean ± SEM,
a: when compared with control, b: when compared with reference standard. (**p<0.01, ***p<0.001)

Table 2: Analgesic Effect of *B. acuminata* flower extract

Group	*Dose mg/kg*	*Reaction time (see) (Meant SEM)*
Control (vehicle)	1-mL/100 g	3.08 ± 0.16
Diclofenac sodium (10 mg/kg)	10	14.52 ± 0.30^{***a}
Test 1 (100 mg/kg)	100	4.56 ± 0.37^{**b}
Test 2 (200 mg/kg)	200	9.61 ± 0.17^{***ab}

n = 6, Values are expressed as mean ± SEM,
a: when compared with control, b: when compared with the reference standard. (**p<0.01, ***p<0.001)

Table 3: Effect on acetic acid-induced writhing in mice

Treatment dose (mg/kg)	*The average number of writhes*			
	30 minutes	*60 minutes*	*120 minutes*	*180 minutes*
Control (vehicle)	12 ± 1.32	14 ± 1.18	14.5 ± 1.08	12.05 ± 1.11
Diclofenac sodium (10 mg/kg)	1.56 ± 1.76^{***a}	4.42 ± 1.62^{***a}	6.0 ± 1.38^{***a}	11.05 ± 1.02^{***a}
Test 1 (100 mg/kg)	2.80 ± 2.11^{***a}	7.75 ± 2.02^{***a}	9.24 ± 1.10^{***a}	12.36 ± 2.06^{***a}
Test 2 (200 mg/kg)	2.05 ± 2.01^{***a}	6.60 ± 2.22^{***a}	8.12 ± 2.01^{***a}	11.68 ± 2.21^{***a}

n = 6, Values are expressed as mean ± SEM., a: when compared with control (***p<0.001)

Table 4: Percent protection of writhing effect

Group	*Percentage Protection*			
	30 minutes	*60 minutes*	*120 minutes*	*180 minutes*
Diclofenac sodium (10 mg/kg)	87	72	59	23
Test 1 (100 mg/kg)	78	41	34	9
Test 2 (200 mg/kg)	82	52	45	13

The mice were then located personally into glass beakers and observed for 10 minutes. The number of writhes was recorded for every animal in the group.

Antipyretic Activity (Al-Ghamdi *et al*., 2001)

For the development of hyperthermia, induce 2, 4-dinitrophenol (DNP), 10 mL/kg i.p. Within 30 minutes, the temperature was raised. After 30 minutes, all the groups were handled as earlier with different doses. The standard group was treated with paracetamol (100 mg/kg) and the test groups were with extracts (100 and 200 mg/kg). The rectal temperatures within the animals were recorded using a digital thermometer for a length of 4 hours at hourly intervals.

Result and Discussion

Anti-inflammatory activity

After sincere observation and calculation, it was found that the anti-inflammatory effect was significant in both doses (100 and 200 mg/kg) compared with the control group. And the higher dose (200 mg/kg) was reliably significant with the diclofenac sodium. The result is shown in Table 1.

Analgesic activity

Hot plate method

It was found that compared with the control group and diclofenac sodium, i.e., the reference group, the reaction time increases significantly with an increase in dose. The reaction time with the different treatment groups is shown in Table 2.

Writhing test

Compared with the control group, the percent protection to writhing is significantly higher for extract. But it is less as compared with the reference or standard group. The result of the writhing test is shown in Tables 3 & 4.

Table 5: Effect of hydro-alcoholic extract of flower of *B. acuminata* on 2,4 DNP-induced pyrexia in rats

Treatment dose (mg/kg)	*Temperature in °C*				
	0 hour	*1 hours*	*2 hours*	*3hr*	*4hr*
Positive Control	37.6 ± 0.022	37.65 ± 0.022	38.25 ± 0.022	38.35 ± 0.022	39.25 ± 0.022
PCM (100 mg/kg)	38.17 ± 0.02^{***a}	36.8 ± 0.042^{**a}	36.59 ± 0.042^{**a}	36.55 ± 0.062^{***a}	36.4 ± 0.062^{***a}
Test 1 (100 mg/kg)	38.46 ± 0.02^{***ab}	37.80 ± 0.08^{***ab}	37.50 ± 0.12^{***ab}	37.01 ± 0.017^{***ab}	36.97 ± 0.11^{***ab*}
Test 2 (200 mg/kg)	38.4 ± 0.15^{***b}	37.1 ± 0.12^{***ab}	36.78 ± 0.052^{***ab}	36.72 ± 0.047^{***ab}	36.60 ± 0.054^{***a}

n = 6, Values are expressed as mean ± SEM,
a: when compared with control, b: when compared with reference standard. (*p<0.05, **p<0.01, ***p<0.001) PCM = Paracetamol

Antipyretic activity

The rectal temperatures of the different groups (control, test 1, 2, and std.) were measured with a digital thermometer in each hr difference. The results were depicted in Table 5. The temperature of Test 1 and 2 groups (100 & 200 mg/kg) were reduced significantly when compared with std. one (paracetamol 100 mg/kg).

Conclusion

The ethanolic flower extract of *B. acuminata* was studied at different doses (100 and 200 mg/kg) for analgesic, antipyretic, and anti-inflammatory activity. The ethanolic flower extracts possess analgesic and anti-inflammatory activity. Due to the presence of flavonoids, they may possess analgesic activity. It reduces inflammation comparably with diclofenac sodium. Similarly, the extract possesses antipyretic activity in rat models comparably with paracetamol.

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Author's Contributions

SS, ABS, and NKS designed all the experiments and performed identification of the suitability of methods. JS, AKM, and MS drafted and reviewed the manuscript. NKS and AKM performed the analysis section. All authors have read and approved the manuscript.

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Availability of data and materials

The datasets generated and analyzed during the current study are available with the corresponding author. "Data can be acquired from the first author upon request."

Conflict of interest

None

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