

## Phytochemical Screening and Anti-Inflammatory Activities of Methanolic Leaf Extract of *Bauhinia monandra* in Animal Models

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**Keywords:** *B. monandra*, anti-inflammatory, edema, phytochemical studies

**Abstract.** The leaves of *Bauhinia monandra* prepared as poultices are used in many Brazilian tribes and some Nigerian communities to relieve swelling and pains, arthritis, diabetes among other ailments. These folkloric claimed benefits inspired this study in which topical and systemic anti-inflammatory activities of a methanol extract of *B. monandra* leaves (MEBmL) was assessed in rodents. The anti-inflammatory activities of *B. monandra* were determined by egg albumin-induced (systemic) paw edema in rats, and xylene-induced (topical) ear edema in mice. Treatment of rats with MEBmL at different concentration (200 and 400 mg/kg) led to significant ( $p < 0.05$ ) reduction (31.0% and 28.0% respectively) of the egg albumin-induced paw edema. Topical application of mice ear with MEBmL (50, 100 mg/kg) also significantly ( $p < 0.05$ ) reduced (39.50% and 41.87% respectively) xylene-induced ear edema. Based on the findings of the study, *B. monandra* has a promising therapeutic potential with its anti-inflammatory activities as associated with folkloric use of the plant.

### Introduction

Plant metabolites are important source of natural bioactive molecules with a vast range of pharmacological activities which are known to exert pharmacological actions in the body, thereby enhancing curative and preventive properties of different diseases [1]. Recently, the use of medicinal plants has gained global attention as most of the world's population seeks medicinal plants as therapeutic source, including compound prototypes from laboratory synthesis.

*Bauhinia monandra* also known as cow's foot, orchid tree, Napoleon plume, Flamboyant, St Thomas tree is a species of leguminous trees that belong to the Caesalpinaceae family [2]. It is an evergreen shrub or tree with a rounded crown, which can grow up to 3-15 meters tall. *B. monandra* is often planted for its beautiful flowers and ornamental foliage. It is cultivated as a garden and street tree in the tropical regions of Australia, America, Asia and West Indies [3]. *B. monandra* seeds are useful source of vitamin A, and contain high amounts linoleic and fatty acid and low amounts of mystiric acids [3].

Inflammation is a complex biological response of vascular tissues to invasion by an infectious agent, antigen challenge, physical, chemical or traumatic damage, and is a protective response involving immune cells [4], blood vessel, and molecular mediators. It is considered a mechanism of innate immunity. Although inflammation is physiological defense mechanism, too little inflammation may lead to continuous tissue damage by harmful stimulus and compromise the survival of the organism [4]. Furthermore, chronic inflammation reactions can induce, maintain or aggravate a host of diseases such as hay fever, periodontitis, atherosclerosis, rheumatoid arthritis, and some cancer [5]. Therefore, inflammation is closely regulated by the body. The non-steroidal anti-inflammatory drugs (NSAIDs) are usually used for the treatment of inflammatory conditions including osteoarthritis, rheumatoid arthritis, and musculoskeletal disorders [6]. They are generally used for symptomatic relief. However, with the vast use of NSAIDs, adverse effects have become increasingly common including gastrointestinal disorders, kidney disease, and a range of

cardiovascular events [7-8]. Therefore, new anti-inflammatory drugs without these adverse effects are needed as alternatives to NSAIDs. The use of medicinal herbs is rapidly gaining acceptance due to their effectiveness, availability, and probably low toxicity, and the progressive inefficiency of modern medicines [9].

Research evidence has shown that *B. monandra* possesses antidiabetic properties against experimentally induced diabetes in rats [10]. Its antidiabetic activity has been linked to the presence of antioxidant compounds [9] which had also been studied [9]. Native Bauhinia species (Leguminosae) have been widely employed in folk medicine for the treatment of diabetes. In Brazil, leaves of the species *B. purpurea* Linn., *B. forficata* Link., and *B. monandra* Kurz. are commonly used as teas for treatment of diabetes [11]. Due to the elevated costs of commercial diabetes medication, Brazilian folks often prefer the use of infusions of Bauhinia sp as alternative for treating diabetes, and other ailments [11-13]. Report have also shown a wide range of pharmacological activities from different species of this genus [11-12], but very few have reported the anti-inflammatory activity of *B. monandra*. Some bioactive compounds that have been isolated from this genus include flavonoids, tannins, lactones, steroids, terpenoids, and glycolipids [11-13]. Since there are scarce experimental studies about its anti-inflammatory activity, the scope of this study was to evaluate whether the methanolic extracts obtained from dried *B. monandra* leaves are able to promote anti-inflammation in animal models, and contributing phytoconstituents of this common medicinal plant in folk medicine.

## Materials and Methods

### Chemicals

All chemicals used in this study were of analytical grade. They were products of Sigma Aldrich, Germany. All laboratory reagents and distilled water were freshly prepared.

### Collection and extraction of plant material

Fresh aerial leaves of *B. monandra* were collected in February 2018 from the Botanical garden of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka (6°51'24"N, 7°23'45"E) where it was appropriately identified and authenticated by Dr. Nweze, a taxonomist. A voucher specimen was deposited in the herbarium unit of Department of Plant Science and Biotechnology, University of Nigeria, Nsukka with specimen voucher number, UNH 1559. The leaves obtained were washed properly to remove dust and dirt, air-dried for 3 weeks and milled with a laboratory-scale mechanical grinder (Elapaq GX160, China). The pulverized plant leaves (500 g) was cold macerated in 2L of methanol for 72 h with agitation at intervals. The extract solution was then filtered through Whatman No. 1 filter paper. The filtrate obtained was concentrated in a rotary evaporator (IKA RV3, Germany) under reduced pressure at 45°C for 3 h, and the resultant methanolic extract of *B. monandra* (MEBmL) leaves was stored at 4°C in a refrigerator for subsequent use.

### Experimental animals

Swiss albino mice (25-32g) and Wistar rats (125-200 g) of both sexes were obtained from the animal house of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka. They were housed in metal steel cages in a well-ventilated animal house under 12:12 hour light dark cycle. The animals were provided with normal pellet diet and water *ad libitum*, and allowed to acclimatize to environmental conditions for seven days prior to commencement of the experiments. The research protocols were in accordance with the ethical rules and recommendation of the University of Nigeria committee on the use and handling of laboratory animals. These principles are also in accordance to the National Research Council Guide for Care and Use of Laboratory Animals [14].

### Preliminary phytochemical screening

Phytochemical analysis was carried out on the methanolic leaf extract of *B. monandra* using standard procedures [15-16].

### Acute toxicity study

The acute toxicity study of leaf methanolic extract of *B. monandra* was evaluated according to the procedures outlined by the Organization for Economic Co-operation and Development (OECD) [17]. Eighteen Swiss albino mice of both sex grouped into three groups (six mice per group) were used for the study. Following the fasting period, a single dose of methanolic extract of *B. monandra* (100, 500, 1000 and 2000 mg/kg) were administered orally to in the treatment groups. Another six mice were administered distilled water as control group. Food was allotted to the mice exactly one hour after treatments. The mice were closely and carefully observed within the first 6 h for any indication of toxicity effect, and daily for a duration of 14 days. Animals were visually observed daily for mortality, behavioral pattern, changes in physical appearance, injury, pain and signs of illness during the duration of study.

### Evaluation of anti-inflammatory activity

Two models were used for assay of anti-inflammatory potential of methanolic extract of *B. monandra*. Thirty adult Wistar rats or adult Swiss albino mice of either sex were used. They animals were randomly divided into five groups of six animals per group. They were fasted for 18 h prior to the experiment.

### Egg albumin-induced paw edema

The rat paw method as described by Akah et al. [18] was used for the test. The rats were divided into five groups of six per group. Group I, II, III, and IV were administered methanolic extract of *B. monandra* (100, 200, 400 mg/kg, i.p.) and indomethacin (10 mg/kg, i.p.) suspended in distilled water respectively. Group V received distilled water only (1 mL/kg, i.p.) as control group. Approximately one hour after treatment, inflammation of the hind paw was induced by injecting 0.1 mL of undiluted fresh egg albumin into the subplanter surface of the right hind paw of rats. The right hind paw volumes of the rats were determined on the principle of volume displacement using the Digital Plethysmometer. Paw volumes were measured at 0.5, 1, 2, 3, 4 and 5 hours after the injection of egg albumin. The mean edema at every time interval was determined in terms of difference in volume displacement at time t, and zero time ( $V_t - V_0$ ). Percent inhibition of edema was calculated using the formula:

$$\% \text{ inhibition} = [(V_c - V_t) \div V_c] \times 100 ,$$

where  $V_c$  = the volume of edema of the control (distilled water treated) group at time t,  $V_t$  = the volume of edema at corresponding time of the treated rats.

### Xylene-induced ear edema

The acute topical inflammation in mice ear was evaluated by a modification of the method described by Tjolsen et al. was used [19]. The mice were divided into five groups of six per group. Group I, II, III and IV received 2 drops of methanolic extract of *B. monandra* (25, 50, 100 mg/kg) and indomethacin (10 mg/kg) applied on the anterior surface of the right ear. Group V received distilled water (2 drops) as control group. Topical inflammation was immediately induced on the posterior surface of the same right ear by application of 0.05 mL of xylene. Fifteen minutes after induction, mice were killed by overdose chloroform anesthesia. Circular sections of both right (treated) and left (untreated) ears were punched out using a 6 mm cork borer and weighed appropriately. The mean ear edema was determined in terms of weight difference between the earplugs. Percent inhibition of edema was calculated using the formula:

$$\% \text{ inhibition} = 100 \times [1 - (R_t - L_t) \div (R_c - L_c)] ,$$

where  $R_t$  = mean weight of right earplug of treated animals,  $L_t$  = mean weight of left earplug of treated animals,  $R_c$  = mean weight of right earplug of control animals,  $L_c$  = mean weight of left earplug of control animals.

### Statistical analysis

This was done using SPSS version 16.0 (SPSS Inc. Chicago, IL. USA). All values are expressed as mean  $\pm$  SEM. Data were analyzed student t-test and one-way ANOVA. Difference between means were considered statistically significant at  $P < 0.05$ .

## Results

### Preliminary phytochemical screening

The phytochemical constituents of methanolic extract of *B. monandra* are shown in Table 1. The extract contains abundance of important phytochemicals of active medicinal constituents. Phytochemicals found present include reducing sugar, proteins, carbohydrates, flavonoids, saponins, resins, steroids, oils, terpenoids, alkaloids, tannins, and cardiac glycosides.

**Table 1.** Preliminary phytochemical screening of methanolic extract of *B. monandra* leaves

Phytoconstituents	Inference
Reducing sugar	+
Proteins	+++
Carbohydrate	+
Flavonoid	++
Saponin	++
Acidic compounds	+
Resin	+
Steroids	++
Oils	++
Terpenoid	++
Alkaloid	++
Tannins	+++
Cardiac glycosides	+

+: present; ++: moderately present; +++: highly present

### Acute Toxicity study ( $LD_{50}$ )

Result of the acute toxicity study showed that no early or late mortality or any significant change in behavioral and physical activities was observed in mice administered up to 1 g/kg of methanolic extract of *B. monandra* leaves. No animal showed any significant alteration in behavioral, physiological and physical activities. The indication is that the oral administration of *B. monandra* extract did not produce any visible toxic effect.

### Effect of methanolic extract of *B. monandra* leaves (MEBmL) on egg albumin-induced paw edema in rats

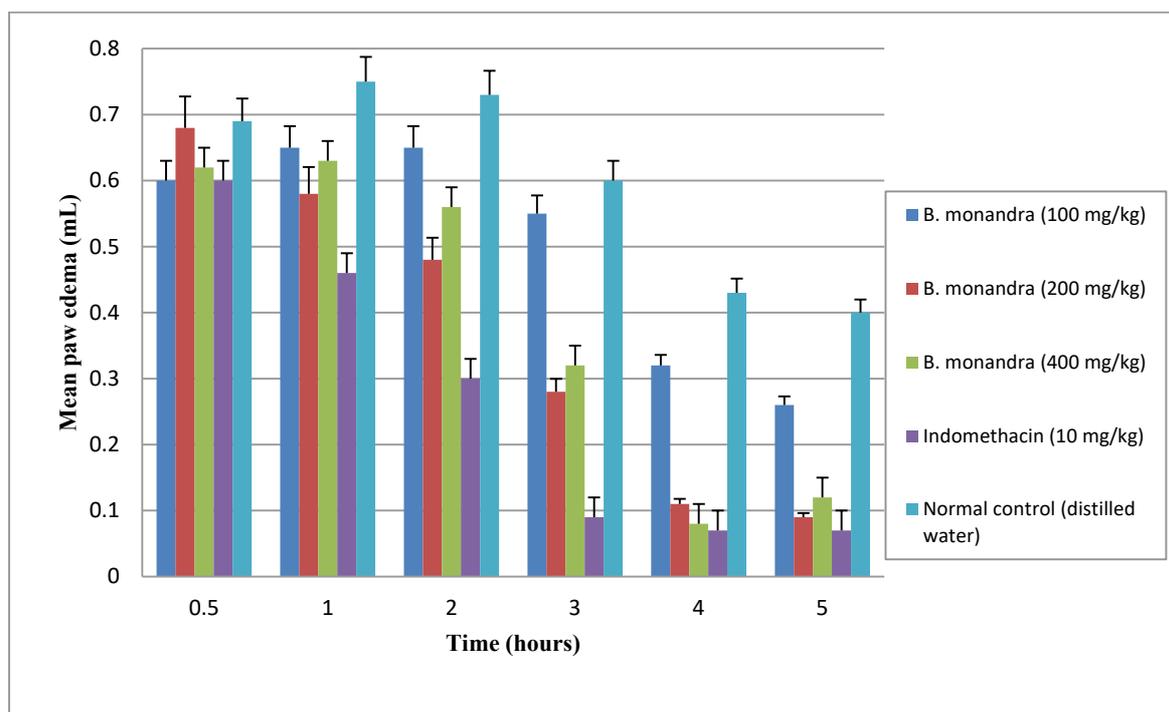
Pre-treatment of rats with MEBmL (200 and 400 mg/kg) caused significant ( $p < 0.05$ ) reduction of egg albumin-induced inflammation and a rapid resolution of paw edema over a 5-h period, except at a dose of 100 mg/kg (Fig. 1). Administration of MEBmL showed a significant

( $p < 0.05$ ) anti-inflammatory activity (MEBmL 200 and 400 mg/kg) reducing the egg paw edema in a non dose-dependent manner (Table 2). Two hours after egg albumin injection, a significant ( $p < 0.05$ ) reduction (25% and 17%) in paw edema was observed in rats treated with MEBmL 200 and 400 mg/kg respectively. After 5 h, a significant ( $p < 0.05$ ) paw edema reduction was observed with MEBmL at these concentrations. The rats treated with Indomethacin (10 mg/kg) showed the best anti-inflammatory activity (33%) after 5 h (Figure 1). The edema inhibition demonstrated by MEBM was similar to that obtained in positive control (Indomethacin, 10 mg/kg).

**Table 2.** Effect of methanolic extract of *B. monandra* leaves on egg albumin induced paw edema in rats

Treatment Groups	Mean paw edema (mL) and inhibition of edema (%)					
	0.5 h	1.0 h	2.0 h	3.0 h	4.0 h	5.0 h
Group I (100 mg/kg of MEBmL)	0.60±0.01** (9.00)	0.65±0.03*** (10.00)	0.65±0.03 (8.00)	0.55±0.07 (5.00)	0.32±0.04 (11.00)	0.26±0.03 (14.00)
Group II (200 mg/kg of MEBmL)	0.68±0.06 (5.00)	0.58±0.00*** (17.00)	0.48±0.00* (25.00)	0.28±0.01** (32.00)	0.11±0.03* (32.00)	0.09±0.01** (31.00)
Group III (400 mg/kg of MEBmL)	0.62±0.01** (7.00)	0.63±0.03** (12.00)	0.56±0.07 (17.00)	0.32±0.04* (28.00)	0.08±0.01** (35.00)	0.12±0.04* (28.00)
Group IV (10 mg/kg of Indomethacin)	0.60±0.01** (9.00)	0.46±0.01*** (32.00)	0.30±0.00** (43.00)	0.09±0.00*** (51.00)	0.07±0.03** (36.00)	0.07±0.03** (33.00)
Group V (Normal Control)	0.69±0.01	0.75±0.01	0.73±0.09	0.60±0.06	0.43±0.07	0.40±0.06

\*\*\* $p < 0.001$ ; \*\* $p < 0.01$ ; \* $p < 0.05$ , when all groups compared to control group. All values are represented as mean  $\pm$  SEM (n=5). MEBM=Methanolic extract of *B. monandra*. All values in parenthesis ( ) are percentage inhibition of edema calculated relative to control.



**Figure 1.** Effect of methanolic extract of *B. monandra* leaves on egg albumin-induced paw edema in rats

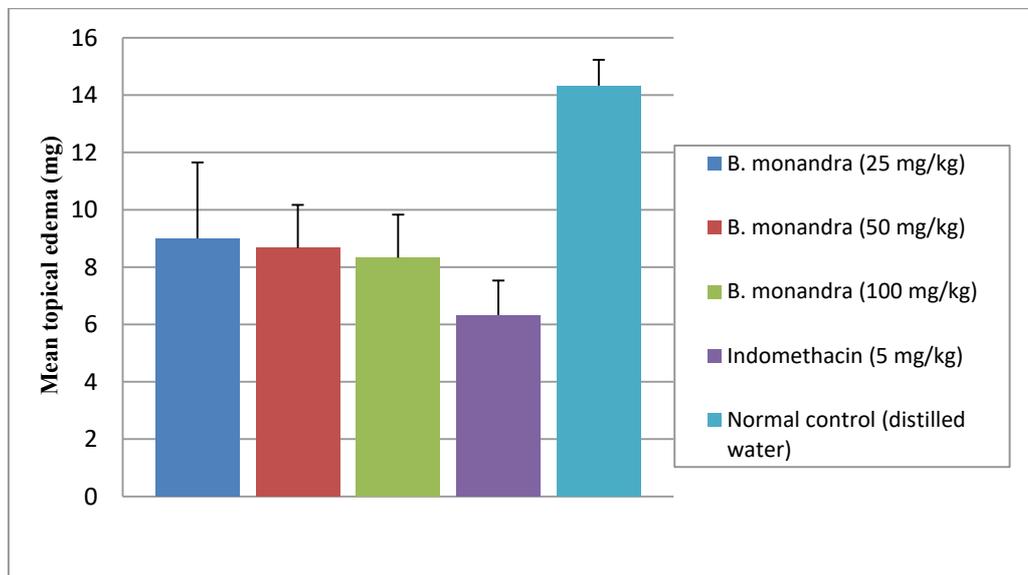
### Effect of methanolic extract of *B. monandra* leaves (MEBmL) on xylene-induced topical ear edema in mice

The administration of MEBmL in different concentrations (25, 50 and 100 mg/kg) showed a significant reduction (37.19, 39.50 and 40.87 respectively) in the topical ear edema induced by xylene application, compared with the normal control group (Table 3). The group treated with indomethacin (5 mg/kg) showed significant reduction (55.83%) of ear edema (Fig. 2). The anti-inflammatory activity produced by MEBmL (100 mg/kg) is comparable to that produced by the positive control (Indomethacin, 5 mg/kg).

**Table 3.** Effect of methanolic extract of *B. monandra* (MEBM) on xylene-induced topical ear edema in mice

Treatment Groups	Mean ear edema (mg)	Inhibition of edema (%)
Group I (25 mg/kg of MEBmL)	9.00±2.65	37.19
Group II (50 mg/kg of MEBmL)	8.67±1.45*	39.50
Group III (100 mg/kg of MEBmL)	8.33±1.45*	41.87
Group IV (5 mg/kg of Indomethacin)	6.33±1.20**	55.83
Group V (Normal control)	14.33±0.88	-

\*\* $p < 0.01$ ; \* $p < 0.05$ , when all groups compared to control group. All values are represented as mean  $\pm$  SEM (n=5). MEBM=Methanolic extract of *B. monandra*. All values of percentage inhibition of edema calculated relative to control



**Figure 2.** Effect of methanolic extract of *B. monandra* leaves (MEBmL) fifteen minutes after xylene-induced topical ear edema in mice

### Discussion

Inflammation is basically a body protective mechanism which is aimed at ridding the body of both the initial cause of cell injury (e.g. microbes, toxins) and the sequelae of such injury (e.g. necrotic cells and tissues) [20]. Counter-regulatory mechanisms which are also known as innate anti-inflammatory mechanisms serve to control inflammatory responses and prevent them from causing unwanted damage to the host. These may include termination, break down and dissipation of secreted mediators when the offending agent is eliminated. Failure of these innate anti-

inflammatory mechanisms results to inflammatory diseases such as rheumatism and arthritis [20]. The discovery of acetylsalicylic acid (aspirin) from the bark of the Willow plant (*Salix alba*) more than a century ago, have given lead to other non-steroidal and steroidal anti-inflammatory drugs. However, continuous use and administration of these drugs is associated with some serious side effects including renal problems, gastrointestinal irritation and ulceration [21]. Therefore, research on inflammation is gaining focus on global scientific study because of the unmet need of discovering potent anti-inflammatory molecules that are devoid of these side effects as alternatives. This has prompted scientific investigation of medicinal plants used in traditional and folk medicine for treating fever, pains, and rheumatoid arthritis [22].

The present study investigated the anti-inflammatory activity of the methanolic extract of *Bauhinia monandra* (MEBmL) on experimentally induced edema in rodents. The leaves of *B. monandra* are used by the Amofu people in Nigeria to relieve swellings and treat arthritic joints. The flowers and buds of plants of this genus are commonly used to treat dysentery, sore throat, skin diseases, or remedy for dysentery [23-25]. These folkloric claims prompted the present study in which anti-inflammatory activities of *B. monandra* extract were evaluated in rodents and was found effective in reducing inflammation in both topical and systemic inflammation.

The result shows that MEBmL significantly inhibited acute inflammation on egg albumin-induced edema in rats pre-treated with MEBmL, both at the early and later phase of edema. The edema inhibition demonstrated by MEBmL was comparable to that demonstrated by the standard drug, indomethacin. This result supports a previous study which evaluated the anti-inflammatory and antinociceptive activities of *B. monandra* leaf lecithin [26]. The study revealed that lecithin isolated from the leaf of *B. monandra* (given at 15, 30 and 60 mg/kg) yielded a significant dose-dependent inflammatory inhibition on carrageenan-induced paw edema. Preliminary phytochemical screening of MEBmL revealed the presence of alkaloids, tannins, flavonoids, terpenoids, saponin, glycosides, steroids and acidic compounds (Table 1). Although based on the study we were unable to relate these constituents with the anti-inflammatory activities observed, bioactive constituents in plants are responsible for their therapeutic activities [1]. For instance, a study identified lecithin from leaves of *B. monandra* as the possessing anti-inflammatory and antinociceptive activities [26]. Condensed plant tannins from the seed extract of Marwa (*Origanum majorana*) were reported by Dhull and coworkers [27] to possess antioxidant properties. Kaur et al. [28] reported that bioactive compounds from Tulsi (*Ocimum tenuiflorum*) seeds possess antioxidant activities and DNA damage protection.

The vascular and cellular events of inflammation play an important role in the activation of cyclooxygenase (COX-1 and/or COX-2) resulting in release of multiple inflammatory mediators such as histamine, serotonin, bradykinin, prostaglandins [29-30]. The synergistic action of these inflammatory mediators at the local site of inflammatory induction leads to increased vascular permeability and blood flow, hence edema formation [31]. Considering the significant reduction by *B. monandra* in paw edema induced by egg albumin at different time intervals, we can indicate that *B. monandra* may have inhibitory action on the release of inflammatory mediators. Report by Solomon et al. [32] showed that *B. tomentosa*, a species of *Bauhinia* inhibited inflammation *in vitro*. The result in the present study and supported by Solomon et al. indicted. Topical application of *B. monandra* also inhibited acute inflammation induced by xylene in mice. This particular model of xylene-induced ear edema has a good predictive value for screening of anti-inflammatory agents. The phlogistic agent, xylene, when applied topically causes an instant irritation of the mice ear that leads to fluid accumulation/edema – a characteristic of an acute inflammatory response [20]. Therefore, the suppression of this response indicates an anti-inflammatory effect. The methanolic extract of *B. monandra* inhibited inflammation of both egg albumin and xylene-induced edema in a dose related manner. We however could not give specific reason for the dose dependency.

NSAIDs reduce inflammation by inhibiting the activity of COX-1 and/or COX-2 [33]; however, prolonged use of NSAIDs confers some side effects to patients, including gastric lesions and kidney disease [708]. Studies have also reported that plants from *Bauhinia* genus has antinociceptive and anti-inflammatory [26], anti-oxidant [32], anti-ulcer [34] properties and confers

nephroprotective effect against cisplatin-induced renal damage [35]. These properties of *Bauhinia* genus suggest multiple benefits of *B. monandra*, and indicative as a possible anti-inflammatory agent devoid of serious side effects such as gastric lesions, and kidney problems.

Preliminary phytochemical studies showed that the plant is rich in flavonoids, glycosides, terpenoids. Although the present study did not investigate the association of these constituents and anti-inflammatory property, some of these bioactive constituents have been previously reported in previous studies to inhibit the production and release of pro-inflammatory mediators [20]. Plant terpenoids and alkaloids have been reported to possess anti-inflammatory activities and suppress inflammatory responses in rodents [36-38]. Prostaglandins and nitric oxide biosynthesis are involved in inflammation. Nitric oxide synthase and cyclooxygenase (COX-2) are responsible for production of many chemical mediators of inflammation [20]. Flavonoids have been reported to possess potent inhibitory effect on both enzymes [39-40]. Flavonoids modulate inflammatory responses by accelerating the destruction and antagonizing the action of the chemical mediators involved in inflammatory reactions.

## Conclusions

The results from the present study show that *B. monandra* has potent anti-inflammatory properties and this could explain the vast use and benefit of this plant in folk medicine. Furthermore, the study also provided experimental evidence for the effectiveness of *B. monandra* in folk medicine for treating inflammation-associated diseases such as rheumatism. Therefore, *B. monandra* should be further investigated as potential source of anti-inflammatory compounds for pharmacological purposes.

## Conflicts of Interest

The authors declare no conflicts of interest regarding this manuscript. The authors alone are responsible for the content and writing of the manuscript.

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