



Effect of Leaf Methanol Extract of *Boswellia Dalzielii* on the Experimental Inflammatory and Nociceptive Models in Albino Mice

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Abstract: Inflammation is a physiological protective response by the organism to remove the injurious stimuli, microbial infections, and trauma. The prolong treatment for inflammation and pain with NSAID's has been shown greater unwanted effects, so there is a rising scope for traditional medicines. *Boswellia dalzielii* leaves are used traditionally as folk remedies for the treatment of health problems caused by inflammation. The present study was an attempt to investigate the qualitative phytochemical constituents and the activity of methanol extract of *Boswellia dalzielii* leaves (MEBD) on the experimental inflammatory and nociceptive models in mice. Qualitative phytochemical constituents of the MEBD were performed using standard protocol. Anti-inflammatory activity was evaluated by studying carrageenan and egg-albumin induced paw oedema and xylene induced ear oedema animal models. Acetic acid-induced abdominal writhing, tail immersion and hot plate tests had been performed to evaluate the anti-nociceptive effect. This MEBD revealed the absence of alkaloids and saponins. The all doses (100, 200 and 400 mg) of the MEBD significantly ($p < 0.001$) inhibited the acute inflammatory process compared to the negative control. Highest percentage inhibition was $90.69 \pm 0.42\%$ in carrageenan paw oedema test 100 mg/kg, 94.01 ± 8.72 (Egg-albumin paw oedema test, 100 mg/kg) at 6 hrs, $57.43 \pm 0.03\%$ (xylene test, 400 mg/kg). In anti-nociceptive experiments, MEBD of all doses were significantly reduced ($p < 0.001$) acetic acid-induced writhing ($82.24 \pm 3.03\%$, 400 mg/kg). In addition, MEBD significantly increased the latency time ($p < 0.001$) in the tail flick (12.15 ± 0.01 , 400 mg/kg) at 90 min and in the hot plate test (13.37 ± 0.10 , 200 mg/kg) at 60 min compared to the control group. In conclusion, the study revealed that *Boswellia dalzielii* leaves, widely used in traditional medicine in North Cameroon, and affectively has antinociceptive and anti-inflammatory powers.

Keywords: *Boswellia Dalzielii*, Inflammation, Anti-inflammatory, Anti-nociceptive, Phytochemicals, Mice

1. Introduction

Inflammation (Latin, *inflammare*, to set on fire) is the complex biological responses of vascular tissues protects us from chemicals, stimuli, microbes and tissue injury and promote the regeneration of the tissue that was injured [1]. Inflammation is initiated by tissue damage which is the danger signal. This lesion activates resident inflammatory cells such as mast cells, polynuclear basophils and platelets. Once activated, these cells release chemical mediators of inflammation: prostaglandins produced by the cyclo-

oxygenase (COX) pathway, leukotrienes which result from the activation of the lipo-oxygenase (LOX) pathway, bradykinins, histamine and serotonin [2]. The inflammation involved in various pathological conditions such as rheumatoid arthritis, asthma, chronic hepatitis [3]. It was detected by the Roman Doctor Celse two thousand years ago, which resulted in four cardinal symptoms: pain, redness, heat and oedema [4]. From the site of a lesion, a so-called nociceptive nerve message is born which is conveyed along the peripheral nerves to the brain where it actually becomes pain. Nociception is the sensory process at the origin of the nervous message that

causes pain [5]. Pain signals danger and encourages people to flee or avoid it, so it has a protective function and represents an adaptive response to noxious or potentially damaging stimuli [6]. Non-steroidal anti-inflammatory drugs are commonly used for treatment of pain and inflammation. Although effective, non-steroidal anti-inflammatory drugs have many side effects, most often on the gastrointestinal and renal systems [7]. These effects appear in the form of nausea, vomiting, diarrhea, stomach pain, even ulcer and gastrointestinal bleeding or renal failure following nephrotoxicity [8, 9]. However, development of medicinal plants that have anti-inflammatory and analgesic activity without these side effects is necessary. Cameroon is a rich source of medicinal plants and natural products are believed to be an important source of new chemical substances with potential therapeutic applicability.

Boswellia dalzielii (Burseraceae) commonly is an aromatic plant locally abundant in the Sudano-Sahelian Savannah, on rocky and dry, shallow soils [10]. In Cameroon, *B. dalzielii* is also present in Mokolo, in the north-Cameroon. The tree has a characteristic pale papery bark that is peeling and ragged. The plant products (such as the gum resin) and different parts of the plant are widely employed in traditional medicine. In Northern Nigeria, the bark is boiled up in large quantity to make a wash for fever and rheumatism, gastro-intestinal troubles, wounds, asthma, pleurisy, appendicitis, dizziness, palpitations, leprosy, diarrhoea and bloating in cattle [11]. The root decoction boiled along with *Hibiscus sabdariffa* is used for the treatment of syphilis. The root decoction with *Daniellia oliveri* is used on wounds [12]. It is also used as a stomachic and has shown that the aqueous extract of the stem bark produced some anti-ulcer activity [13, 14]. A survey of the literature revealed that the anti-inflammatory activity of *Boswellia dalzielii* leaves has not been documented. In the present pharmacological study, the anti-inflammatory and anti-nociceptive effect of the methanolic extract of *Boswellia dalzielii* leaves was investigated.

2. Materials and Methods

2.1. Plant Material

The fresh aerial parts identified *Boswellia dalzielii* were collected from Mandaka area, Far North Region of Cameroon on December 2019. The collected plant was identified and authenticated by Prof. Mapongmetsem Pierre-Marie, a botanist of the Department of Biological Science, Faculty of Science, University of Ngaoundéré, Cameroon and confirmed by comparison with a sample preserved at Cameroon National Herbarium (CNH), Yaoundé (Cameroon). Voucher specimen has been deposited under the reference number of CNH N°20532/SRF-CAM. The collected leaves was cleaned, washed thoroughly under distilled water, air-dried at laboratory room temperature and grounded to powder.

2.2. Preparation of Plant Extract

For preparing extract, the grinded powder was

successively extracted with absolute methanol by maceration at room temperature (25°C) over period of six days with mixing at regular interval. In brief, one hundred grams of powder of the plant material were macerated in 500 ml of methanol (1: 5, w/v). After the extraction, the macerated plant material was filtered through No. 1 Whatman paper filter where repeated until the sample became clear. After the filtration, the filtrate was evaporated to dryness under reduced pressure in rotary evaporator (UV-2100 Spectrophotometer), to obtain the thick semi-solid paste stored in polyethylene bags at room temperature to serve biological tests.

2.3. Experimental Animals

The assays were carried out on albino mice weighing 25-30 grs, 90 days of aged were obtained from the Laboratoire National Vétérinaire (LANAVET), Garoua (Cameroon). The animals of both sex were grouped and housed in the laboratory animal house (six per group) at room temperature (25±2°C), 60% - 70% humidity on a 12 hours light-dark cycle. They were housed in polypropylene cages and containing paddy husk as bedding. They were acclimatized at least one week before the commencement of the experiments. The animals submitted to oral administration of the extract or drug or vehicle. Before each experiment, in order to avoid a possible interaction with food, the animals received only water for 12 h. The experimental protocol strictly approved by laboratory animal care and guidelines of the Animal Ethical Committee of the Ngaoundéré Regional Delegation of livestock; Fishes and Animal Industries Authority, Cameroon under the approval number 075/16/L/RA/DREPIA. All experiments were performed in the morning.

2.4. Phytochemical Screening

The methanolic extract of the plant extract was qualitatively screened for the presence of various phytoconstituents, which may be responsible for analgesic and anti-inflammatory effect, such as alkaloids, phenolic compounds, tannins, flavonoids, saponins, anthocyanins, terpenoids, coumarins, and reducing compounds using standard procedures [15, 16].

2.4.1. Test for Alkaloid

Place 0.1 ml of the dry vegetable powder in an Erlenmeyer flask to which 2.5 ml of 1% HCl is added. This mixture is incubated in a water bath at 50°C for 2 hours. The extract solution is divided into two equal volumes, one volume of which is treated with 1 ml of Mayer's reagent while the second volume is treated with 1 ml of Wagner's reagent. The appearance of a white (tube 1) or brown (tube 2) precipitate indicates the presence of alkaloids.

2.4.2. Test for Phenolic Compounds

To 1 ml of the methanolic extract solution contained in a test tube are introduced one or two drops of 10% alcoholic iron (III) chloride solution. The appearance of a more or less dark white or green color was a sign of the presence of

phenolic compounds.

2.4.3. Test for Tannin

To 2 ml of the methanolic extract solution contained in a test tube are introduced one or two drops of 2% alcoholic iron (III) chloride solution. The appearance of a blue-blackish or green color indicates the presence of tannins.

2.4.4. Test for Flavonoids

Macerate 2 g of the pulverized vegetable powder in 40 ml of 1% HCl diluted for 24 hours. In a test tube containing 10 ml of the filtrate is added a solution of NH₄OH. The appearance of a light yellow color in the upper part of the tube indicates the presence of flavonoids.

2.4.5. Test for Saponosides

In a test tube containing 5 ml of the methanolic extract solution, 10 ml of the distilled water are added. The tube was stirred for 2 min then left to stand. A height of persistent foam for at least 15 min, greater than 1 cm indicates the presence of saponosides.

2.4.6. Test for Anthocyanins

Put 1 g of vegetable powder in 10 ml of hot water for 5 min. To 1 ml of 10%, infused a few drops of pure HCl are added; a pink-red color appears. A few drops of NH₄OH are added, the change of the blue-purplish color a second time concludes the presence of anthocyanins.

2.4.7. Test for Sterols and Triterpenes

Sterols and triterpenes have been investigated by the Liebermann reaction. Five (5) ml of each of the extract were evaporated on a sand bath. The residue is dissolved hot in 1 ml of acetic anhydride; we added 0.5 ml of concentrated sulfuric acid to the triturate. The appearance of a purple or violet ring at the interphase, turning blue and then green, indicated a positive reaction.

2.4.8. Test for Coumarins

In a capsule, 5 ml of ethereal extract is evaporated, and then 2 ml of hot water is added to the residue. The solution is shared between 2 test tubes. To the contents of one of the tubes, 0.5 ml of 25% NH₄OH is added. The fluorescence is observed under U.V at 366 nm. Intense fluorescence in the tube where ammonia was added indicates the presence of coumarins.

2.4.9. Test for Reducing Compounds

Their detection consists in introducing 1 ml of the extract solution into a test tube, then 2 ml of Fehling's solution (1 ml of Fehling A and 1 ml of Fehling B liquor) are introduced, then heated the tube in a water bath at 40°C for 8 min. A positive test is characterized by the appearance of a brick red precipitate.

2.5. Evaluation of Anti-Inflammatory Activity of the Extract

2.5.1. Topical Oedema Induced by Carrageenan

This assay was done according to first previously reported technique of Winter et al. [17]. Five groups of six animals of

each group were allotted to different treatment groups. Group I served as the control mice and was pre-treated with normal saline, 10 mL/kg body weight. Groups II, the standard reference group was given p.o, an aqueous solution of Diclofenac sodium (10 mg/kg of body weight), and Group III, IV and V received p.o 100, 200, 400 mg/kg body weight of MEBD, respectively. One hour after the treatment, paw oedema was induced by a single 0.1 ml plantar aponeurosis injection of 1% carrageenan (Sigma-Aldrich, USA) in 0.9% normal saline into the right hind paw of each conscious mouse (25-30 grs). Mice paw volume was measured before the injection of the "irritant" substance and after at regular selected time intervals (0, 1, 2, 3, 4, 5 and 6 hrs) using a vernier caliper. Results were expressed as Oedema inhibitory activity (Pi) of the extract and were calculated according to the following ratio:

$$Pi = \frac{[(Dt-D0)control - (Dt-D0)treated]}{(Dt-D0)control} \times 100 \quad (1)$$

Where Dt is the average diameter for each group at different time points after carrageenan injection and D0 the average diameter for each group before carrageenan injection

2.5.2. Egg-Albumin Induced Hind Paw Oedema

The test was carried out as described by Okoye and Osadede [18]. The experimental animals (n=6 per group) were fasted for 24 hours and deprived of water only during the experiment. The animals were divided into five groups each comprising of six mice. Acute inflammation was induced by injecting 0.1 ml of fresh undiluted egg albumin into the planter region of the right hind paw of mice subcutaneously. The mice of group I, as a vehicle-treated control group were given 10 ml/kg BW of NaCl 0, 9%, orally. Group II, as positive control group, was given Diclofenac sodium 10 mg /kg BW, orally. Groups III – V received MEBD with 3 levels of doses, 100; 200; and 400 mg/kg BW. The solutions of MEBD (100, 200, 400 mg/kg, orally) and standard drug, diclofenac sodium (10 mg/kg) was administered one hour prior to induction of oedema. The paw thickness was measured at hourly interval for 6 h using a digital vernier caliper.

2.5.3. Xylene-Induced Ear Oedema

The test was done according to previously reported technique of Igbe et al. [19]. Concisely; after an overnight fasting, mice were divided into five groups of six animals each. Normal saline (10 mL/kg p.o.) was administered to 1st group served as control, diclofenac sodium (10 mg/kg p.o.) to 2nd group and MEBD (100, 200 and 400 mg/kg p.o.) given to 3rd, 4th and 5th group. One hour after the treatment, topical inflammation was induced on the posterior and anterior surface of the right ear of each mouse by application of 0.05 mL of xylene using a dropper pipette. The left ear of the same mouse served as control. One hour later, the thickness of the ear is measured by a digital caliper for each mouse [20]. The ear weight changes produced by irritant was calculated as the difference of ear weight of the right and left ear (treated vs. untreated) and% inhibition was calculated as

follows:

$$\% \text{ Inhibition} = \frac{A-B}{A} \times 100 \quad (2)$$

Where A and B denote ear swelling of the negative group and ear swelling of the drug groups, respectively.

2.6. Evaluation of Anti-nociceptive Activity of the Extract

2.6.1. Acetic Acid-Induced Abdominal Writhing Test

The antinociceptive effect of the MEBD was investigated in mice using the method described by Koster *et al.* [21]. Concisely; after an overnight fasting, the Swiss albino mice were divided into five groups of six animals each. The control animal was treated by vehicle (10 mL/kg, group I). The reference analgesic drug, Diclofenac sodium (100 mg/kg, group II) and Animals were pretreated with MEBD (100, 200 and 400 mg/kg, group III, IV and V, respectively). The different solutions were orally administrated to mice 30 min prior to acetic acid injection. Each mouse was injected intraperitoneally with 0.2 mL of 1% acetic acid (v/v) at a dose of 10 mL/kg body weight. After a latency period of 5 minutes, this injection produces abdominal writhing responses by activating the chemo-sensitive nociceptors which manifests itself in the mouse by a movement of stretching of the hind legs and twisting of the dorso-abdominal musculature, called spasms. The analgesic effect is assessed by counting these cramps for 20 minutes after the injection of the algogenic agent [22]. The percentage of analgesic activity was expressed as percentage reduction of the number of stretching and calculated using the following formula [23]:

$$\text{Percent reduction} = 100 - \frac{X_t}{X_c} \times 100 \quad (3)$$

where, X_t =Average number of writhes in treated group;
 X_c =Average number of writhes in control group.

The ability of the MEBD was significantly reduced and the number of acetic acid-induced writhes was taken as an analgesic activity.

2.6.2. Tail Immersion Method

Analgesic activity was assessed by tail immersion method as described by Mohemad *et al.* [24]. Concisely, the albino mice of either sex weighing 25-30 g were fasted overnight with ad libitum access to water. Thirty mice were divided into five groups of six animals each as follows: Group I: negative control group received normal saline (10 mL/kg body weight), Group II: standard group (diclofenac sodium, 100 mg/kg body weight), and Group III, IV and V, test groups (100, 200 and 400 mg/kg body weight, respectively). The animals are allowed to adapt to the cages for 30 min before testing. The distal part of the tail of each animal was marked (5 cm). This marked part of the tail was immersed in a beaker of freshly filled water of exactly $50 \pm 1.0^\circ\text{C}$. Within a few seconds the mouse reacted by withdrawing the tail. The time taken to withdraw the tail was noted as reaction time. A cut off time of 10 seconds was maintained at $50 \pm 1.0^\circ\text{C}$ to prevent tissue damage. After respective drug treatment, tail of

each animal was immersed in a beaker of freshly filled water of exactly $50 \pm 1.0^\circ\text{C}$ and reaction time was measured at 0, 30, 60, 90, and 180 min, respectively.

2.6.3. Hot Plate Method

The hot Plate test, a nociceptive model first proposed by Koster *et al.* [21] was used. Mice of either sex were divided into five groups each comprising six animals. All animals were fasted overnight. Three groups were given different doses of the plant extract, while one group I was given a vehicle (control, 10 mL/kg, p.o.), the group II was given standard drug, Diclofenac sodium (reference group, 10 mg/kg, p.o.), and the group III, IV, and V, the test groups (100, 200 and 400 mg/kg, p.o., respectively). The animals were placed on a hot plate maintained at a temperature of $54 \pm 1^\circ\text{C}$. The variable measured in this test is the latency of the 1st jump made by the animal, it can react by shaking the paws, licking the paws or avoidance jumps. The reaction time is an indication of the animal's resistance to pain and is used to assess the effectiveness of analgesic substances. The reaction time for the animal to lick the paw or jump off the hot plate was taken as the latency. This was repeated after 30 min, 60 min, 120 and 240 min of the exact time given. Only mice that showed initial nociceptive response within 20 seconds were selected and used for the study. The cut off of measuring the animals' reactions was limited to 20 seconds because of the risk of tissue damage.

2.7. Statistics

Data was processed on Graph Pad Prism software (version 5.3). The comparison of the different percentages of inhibition was made by analysis of variances (ANOVA) followed by Dunnett's test at risk α equal to 0.05.

3. Results

3.1. Phytochemical Screening

According to the results mentioned in Table 1 concerning the Preliminary phytochemical screening of MEBD, we note the richness of the plant studied in phenolic compounds, flavonoids, anthocyanins, steroids/triterpens and reducing compounds, and absence of alkaloids saponosides, and tannins. The result of phytochemical test has been summarized in the table 1.

Table 1. Phytochemical constituents of leaf methanol extract of *Boswellia dalzielii*.

Phytochemicals	Results
Alkaloids	P-
Phenolic compound	P+
Tannins	P-
Flavonoids	P+
Saponins	P-
Anthocyanins	P+
Terpenoids & Steroids	P+
Reducing compound	P+

“P+” stands for the presence and “P-” indicates the absence of Phytochemicals

3.2. Anti-Inflammatory Test

3.2.1. Topical Oedema Induced by Carrageenan

The anti-inflammatory effects of the MEBD on carrageenan induced hind paw oedema and percentages of inhibition in mice are shown in Table 2. The results obtained indicates that the diameter of the leg measured by the caliper showing the increase as a function of time i.e. 5.84±0.02 mm, 6.94±0.02 mm, 6.23±0.01 mm at 1, 3 and 6 hrs., respectively in the control group. The preventive oral administration of diclofenac solution at a dose of 10 mg / kg BW which is a non-steroidal anti-inflammatory drug results in a significant reduction in edema with a maximum percentage inhibition of 69.89±0.39% at 3 hours of treatment. MEBD administered at the doses of 100 and 400 mg / kg manifest its inhibitory effect from the sixth and first hours after the injection of carrageenan with a percentage inhibition of 90.69±0.42% and 79.93±8.97%, respectively. The dose of the MEBD of 200 mg / kg prevented formation of oedema with a maximum percentage inhibition of 53.87±4.72% at 6 hours.

3.2.2. Egg-Albumin Induced Hind Paw Oedema

The anti-inflammatory effects of the MEBD on egg-albumin induced hind paw oedema and percentages of inhibition in mice are shown in Table 3. One hour, after administration of normal saline to the negative control group, egg-albumin caused a significant increase in the diameter of the mouse paw with a maximum diameter of 5.99±0.01 mm at 3 hrs. The

preventive oral administration of diclofenac at a dose of 10 mg / kg orally very significantly prevents the increase in the volume of the mouse paw compared to the negative control. It is 5.03±0.02 at 4 hours after the injection of egg-albumin, i.e. a maximum percentage inhibition of 63.85±1.85%. The pretreatment by MEBD exhibits a highly significant inhibition at all the doses (100, 200 and 400 mg / kg) i.e. 4.78±0.01 mm, 4.72±0.03 mm and 4.82±0.01 mm of the diameter of the paw with percentages of inhibition of 94.01±8.72% at 6 hrs, 91.32±5.78% at 6 hrs and 92.16±0.40% at 2 hrs, respectively.

3.2.3. Xylene-Induced Ear Oedema

The anti-inflammatory effects of the MEBD on xylene-induced ear oedema and percentages of inhibition in mice are shown in Table 4. Compared to the control group, MEBD at doses tested (100 and 200 mg/kg) produced most significantly inhibited ($P < 0.001$) ear oedema i.e. percentage inhibition of 45.71% and 41.22%, respectively. Oral administration of this extract at a dose of 400 mg / kg (56.33%), one hour before the induction of inflammation, exerts a highly significant anti-inflammatory effect ($p < 0.001$) in comparison with the group control, considered to be 100% inflammation. Diclofenac (10 mg/kg), a standard anti-inflammatory drug, had a highly significant ($P < 0.001$) inhibitory effect on the auricle swelling of mice induced by xylene (56.73%).

Table 2. Effect of methanolic extract of the *Boswellia dalzielii* leaves on carrageenan-induced paw oedema in mice.

Groups	Paw oedema thickness (% Inhibition)						
	0 hr	1 hr	2 hr	3 hr	4 hrs	5 hrs	6 hrs
Control	4.68±0.01	5.84±0.01	5.89±0.01	6.94±0.02	6.36±0.01	6.19±0.01	6.23±0.01
Diclo 10 mg/kg	4.13±0.01	5.02±0.01 ^c	4.91±0.02 ^c	4.73±0.01 ^c	5.26±0.01 ^c	4.58±0.01 ^c	4.65±0.01 ^c
	-	(12.47±1.33)	(25.50±3.00)	(69.86±0.39)	(23.63±0.90)	(65.97±1.13)	(61.30±0.77)
MEBD 100 mg/kg	4.22±0.01	5.73±0.01 ^a	5.14±0.01 ^c	5.68±0.01 ^b	5.35±0.01 ^c	4.64±0.01 ^c	4.35±0.01 ^c
	-	(07.38±0.71)	(15.11±1.74)	(12.90±0.66)	(25.40±1.23)	(68.44±0.59)	(90.69±0.42)
MEBD 200 mg/kg	4.01±0.01	4.49±0.01 ^c	4.76±0.01 ^c	4.89±0.01 ^c	5.01±0.01 ^c	4.74±0.01 ^c	4.23±0.01 ^c
	-	(27.69±1.23)	(7.78±0.85)	(29.99±1.46)	(9.86±1.35)	(20.87±7.31)	(53.87±4.72)
MEBD 400 mg/kg	4.16±0.01	4.43±0.01 ^c	4.84±0.01 ^c	5.78±0.01 ^a	5.06±0.01 ^c	4.57±0.01 ^c	4.25±0.06 ^c
	-	(79.93±8.97)	(15.45±10.28)	(9.14±10.54)	(19.02±10.54)	(78.37±5.54)	(61.19±7.31)

MEBD=leaf methanol extract of *Boswellia dalzielii*, SEM=standard error of mean, Diclo=Diclofenac sodium, n=6 where, n is the number of mice. Values in the table are expressed as mean±SEM, ^ap < 0.05, ^bp < 0.01, ^cp < 0.001 significantly different in comparison with control. The data was analyzed by ANOVA followed by Dunnett's tests.

Table 3. Effect of leaf methanolic extract of *Boswellia dalzielii* on egg-albumin induced paw oedema in mice.

Groups	Paw oedema thickness, Mean±SEM (% Inhibition)						
	0 hr	1 hr	2 hr	3 hrs	4 hrs	5 hrs	6 hrs
Control	4.75±0.01	5.59±0.01	5.89±0.01	5.99±0.01	5.54±0.01	5.13±0.01	4.74±0.21
Diclofenac sodium	4.75±0.01	5.18±0.01 ^c	5.43±0.01 ^c	5.54±0.01 ^c	5.03±0.02 ^c	4.94±0.01 ^c	4.84±0.01 ^c
	-	(49.36±3.61)	(39.07±0.52)	(35.07±1.04)	(63.85±1.85)	(49.61±3.16)	(63.64±3.44)
MEBD 100 mg/kg	4.77±0.01	4.92±0.01 ^c	5.24±0.01 ^c	5.39±0.01 ^c	5.09±0.01 ^c	4.95±0.01 ^c	4.78±0.01 ^c
	-	(81.43±1.23)	(58.42±0.80)	(49.51±0.34)	(59.58±1.15)	(50.75±1.83)	(94.01±8.72)
MEBD 200 mg/kg	4.73±0.01	4.82±0.17 ^c	5.53±0.01 ^c	5.63±0.01 ^c	5.04±0.01 ^c	4.83±0.01 ^c	4.72±0.03 ^c
	-	(88.50±20.08)	(28.44±0.38)	(25.80±0.97)	(59.87±1.66)	(71.15±2.30)	(91.32±5.78)
MEBD 400 mg/kg	4.74±0.01	5.43±0.01 ^c	4.82±0.01 ^c	4.96±0.03 ^c	5.27±0.01 ^c	5.03±0.01 ^c	4.84±0.11 ^c
	-	(16.72±1.31)	(92.16±0.40)	(82.06±2.43)	(31.98±2.01)	(20.77±3.23)	(77.95±9.09)

MEBD=leaf methanol extract of *Boswellia dalzielii*, SEM=standard error of mean, n=6 where, n is the number of mice. Values in the table are expressed as mean±SEM, ^cp < 0.001 significantly different in comparison with control. The data was analyzed by ANOVA followed by Dunnett's test.

Table 4. Effect of leaf methanolic extract of *Boswellia dalzielii* on xylene-induced ear oedema in mice.

Group	Dose	Oedema weight (mg)	% Inhibition
Control	-	4.73±0.02	-
Diclofenac sodium	10 mg/kg	2.20±0.40 ^c	53.38±865
MEBD	100 mg/kg	2.52±0.01 ^c	46.58±0.27
MEBD	200 mg/kg	2.06±0.01 ^c	56.30±0.10
MEBD	400 mg/kg	2.09±0.55 ^c	57.43±0.13

MEBD=leaf methanol extract of *Boswellia dalzielii*, SEM=standard error of mean, n=6 where, n is the number of mice. Values in the table are expressed as mean±SEM, ^c p < 0.001 significantly different in comparison with control. The data was analyzed by ANOVA followed by Dunnett's test.

3.3. Anti-Nociceptive Test

3.3.1. Acetic Acid-Induced Abdominal Writhing Test

The effect of methanol extract of *Boswellia dalzielii* leaves on acetic acid-induced writhing in mice are shown in Table 5. The negative control group displayed maximal writhing (36.67±2.06) while the oral administration of MEBD at 100, and 400 mg/kg doses had significantly decreased (p < 0.001) the number of writhing movements induced by the i. p. administration of the acetic acid with a maximal percentage inhibition of 70.35±2.90% and 81.24±3.03%, respectively. Though, 100 and 400 mg/kg of extract produced greater analgesic effect than Diclofenac (64.04±2.04%). The results revealed that the MEDP showed also very strong writhing inhibitory effect at 200 mg/kg (56.63±2.32%) which value close to the effect compared with the negative control. Diclofenac sodium used as the standard drug inhibited

46.39% abdominal writhing and statistically significantly reduced reflex writhing (p < 0.001) in comparison to the negative control.

3.3.2. Tail Immersion Test

The effect of methanol extract of *Boswellia dalzielii* leaves on tail immersion response in mice are shown in Table 6. The results of the test showed that the MEBD showed highly significant increase (P < 0.001) in heat tolerance at all the time compared to control. At all doses (100, 200 and 400 mg/kg, p.o.) within 60 min (10.51±0.08 sec) and reaching to maximum at 90 min (12.48±0.08 sec) and then decreased after 180 min (6.45±0.16 sec) observation period. In 10 mg/kg dose (10.44±0.12 sec), the standard group (Diclofenac sodium) showed significant pain reaction time which is comparable with the negative control group.

Table 5. Effect of leaf methanolic extract of *Boswellia dalzielii* on acetic acid-induced abdominal writhing in mice.

Group	Dose	Number of writhes (per 20 min)	% Inhibition (%)
Control	-	36.67±2.06	-
Diclofenac sodium	100 mg/kg	13.16±0.76 ^c	64.04±2.04
MEBD	100 mg/kg	10.83±0.76 ^c	70.35±2.90
MEBD	200 mg/kg	15.50±0.54 ^c	56.63±2.32
MEBD	400 mg/kg	6.83±0.76 ^c	81.24±3.03

MEBD=leaf methanol extract of *Boswellia dalzielii*, SEM=standard error of mean, n=6 where, n is the number of mice. Values in the table are expressed as mean±SEM, ^c p < 0.001 significantly different in comparison with control. The data was analyzed by ANOVA followed by Dunnett's test.

Table 6. Effect of leaf methanolic extract of *Boswellia dalzielii* on tail immersion response in mice.

Groups	Dose (mg/kg)	Observation time (Hr) Mean reaction time±S. E. M (sec)				
		0 hr	30 min	60 min	90 min	180 min
Control	-	3.29±0.03	3.24±0.02	3.50±0.03	3.30±0.06	3.56±0.01
Diclo	100	3.44±0.02	8.39±0.19 ^c	7.46±0.11 ^c	9.48±0.12 ^c	10.44±0.12 ^c
MEBD	100	3.27±0.05	7.43±0.12 ^c	10.51±0.08 ^c	12.48±0.08 ^c	6.45±0.16 ^c
MEBD	200	3.45±0.06	9.40±0.19 ^c	9.43±0.13 ^c	7.43±0.14 ^c	5.39±0.19 ^c
MEBD	400	3.48±0.05	8.32±0.21 ^c	7.55±0.10 ^c	11.37±0.20 ^c	8.41±0.20 ^c

MEBD=leaf methanol extract of *Boswellia dalzielii*, S. E. M=standard error of mean, Diclo=Diclofenac sodium, n=6 where, n is the number of mice. Values in the table are expressed as mean±SEM, ^c p < 0.001 significantly different in comparison with control. The data was analyzed by ANOVA followed by Dunnett's test.

3.3.3. Hot Plate Test

The effect of methanol extract of *Boswellia dalzielii* leaves on hot plate test in mice are shown in Table 7. The control group showed no significant difference in reaction time at different time interval. The preventive oral administration of MEBD (100 mg / kg and 400 mg / kg) exhibited significant anti-nociceptive activity in mice at all the time. In the same time interval, the analgesic activity of MEBD at dose 100 mg

/ kg caused significant increase in the mice reaction time suggesting a maximum analgesic activity of 13.37±0.10 seconds when compared to the untreated control group reaction time of (2.45±0.09) at 60 minutes. The result of hot plate in this study suggests that the oral administration of diclofenac sodium (10 mg/kg) showed a significant (P < 0.001) maximal possible effect (10.43±0.14) at 240 min when compared to control.

Table 7. Effect of leaf methanolic extract of *Boswellia dalzielii* leaves on hot plate response in mice.

Group	Doses (mg/kg)	Observation time (min) Mean reaction time±S. E. M (sec)				
		0 min	30 min	60 min	120 min	240 min
Control	-	2.36±0.01	2.54±0.16	2.45±0.09	2.52±0.11	2.50±0.03
Diclofenac	100 mg/kg	2.51±0.08	7.40±0.17 ^c	5.49±0.10 ^c	6.50±0.10 ^c	10.43±0.14 ^c
MEBD	100 mg/kg	2.42±0.15	9.41±0.08 ^c	7.45±0.11 ^c	4.51±0.10 ^c	6.41±0.09 ^c
MEBD	200 mg/kg	2.55±0.08	11.44±0.11 ^c	13.37±0.10 ^c	6.47±0.10 ^c	7.51±0.06 ^c
MEBD	400 mg/kg	2.49±0.11	5.53±0.05 ^c	9.53±0.07 ^c	11.40±0.11 ^c	7.41±0.08 ^c

MEBD=leaf methanol extract of *Boswellia dalzielii*, SEM=standard error of mean, n=6 where, n is the number of mice. Values in the table are expressed as mean±SEM, ^c p < 0.001 significantly different in comparison with control. The data was analyzed by ANOVA followed by Dunnett's test.

4. Discussion

From the results obtained in table (Table 1), the phytochemical screening of MEBD revealed the presence of phenolic compounds, flavonoids, anthocyanins, steroids/triterpenes and reducing compounds, and absence of alkaloids saponosides, and tannins. In literature, the phytochemical screening of extract ethanol leaf of *Boswellia dalzielii* leaves revealed the presence of alkaloid, coumarin, flavonoid, tannin, anthocyanin, carbohydrates, anthraquinone and terpenoid [25]. This abundance of active compounds having various pharmacological properties gives the plant remarkable properties which could justify its multiple therapeutic indications and its use in traditional medicine as an anti-inflammatory and antinociceptive.

The carrageenan test was selected in this study, because of its sensitivity in detecting acute phase of inflammatory response [26]. It appears from Table 2 that this freshly prepared pathogen induces an increase in volume in the left paw of the mouse, considered to be a characteristic sign of inflammation and constitutes a very important parameter in the evaluation of anti- activity acute inflammatory disease of several compounds [27]. In the present study, the increase in paw diameter was greatest in the control group, with a peak percentage of 56.93±4.33% at the third hour. The results showed that the diclofenac sodium group effectively inhibited the formation of edema by the third hour (26.94%). Preventive administration of MEBD at doses of 100, 200 and 400 mg / kg had shown highly significant reduction in edema at the sixth hour 90.69±0.42 and 53.87±4.72, and 79.93±8.97 (second hour), respectively. These results may reflect the presence of active substances such as tannins and flavonoids which would inhibit the production of pro-inflammatory mediators such as histamine, serotonin, leukotrienes, and prostaglandins [28]. In addition, our results agree with those of Alamgeer et al. which indicate that the anti-inflammatory effect can be explained in part by the presence of polyphenols [29].

The egg albumin-induced paw edema is the pertinent animal models to evaluate acute inflammation [30]. In the current investigation, the egg albumin-induced oedema model revealed that MEBD showed highly significant (p < 0.001) anti-inflammatory activity. The significant anti-inflammatory effect of MEBD at all doses (100, 200 and 400 mg/kg BW) was superior to that of Diclofenac sodium (10 mg/kg BW). In addition, it has exhibited highly significant

activity at the all the both phases, which is related to the inhibition of serotonin, histamine, bradykinin, kinins and prostaglandins release, which inhibits the first and later phase of inflammation.

The anti-edema effect of MEBD was investigated using the xylene-induced ear edema model in mice. In this pattern of acute inflammation, the sign of inflammation is edema, which is characterized by vasodilation and infiltration of leukocytes, vasoactive amines (histamine and serotonin), and neutrophils [31-33]. The results obtained in the present study show that preventive administration of diclofenac (10 mg / kg) reduced edema with a percent inhibition of 71.95%. In addition, the efficacy of MEBDs in the therapy of the xylene-induced inflammation model was shown with a maximum percent inhibition of 57.43±0.03% (400 mg / kg).

The Writhing test is a chemical visceral pain model used to assess the peripheral antinociceptive activity. The intraperitoneally injection of acetic acid produced nociception in the form of contraction of the abdominal muscle accompanied by an extension of the forelimbs and body elongation [34]. The contractions induced by the injection i. p. acetic acid are a method used to induce peripheral pain by injecting irritants such as acetic acid into mice. In this abdominal writhing (or visceral pain model) model, arachidonic acid and prostaglandin biosynthesis may play a role in the nociceptive mechanism [35]. In fact, the mechanism of the onset of pain is through an increase in the intraperitoneal fluid of substances such as prostaglandins, serotonin, histamine, bradykinin which will stimulate peripheral nociceptive receptors located at the peritoneal level [36]. A potent inhibitory effect was exerted by the extract in the mouse writhing assay (a useful test for evaluating mild analgesic, non-steroidal anti-inflammatory agents) and this effect may be due to inhibition of the synthesis of the arachidonic acid metabolites. The effect of the extract on this pain models indicates that it may be centrally acting.

The tail immersion test was used to evaluate the central antinociceptive efficacy of MEBD by thermal nociceptive models. The results shown that the MEBD extract had strong antinociceptive on this test compared to negative control with the high dose showing the highest reaction time at all the time, which is even more significant than diclofenac sodium (9.48±0.12) at the same time. Results from the above tests suggest that the phytochemical present in the MEBD may be attributed its rich content of phenolic compounds, flavonoids and steroids/triterpenes, as these group known to analgesic

effect.

The hot-plate test is a specific test carried out to verify involvement of a central mechanism with compounds / drugs showing antinociceptive activity [37]. The paws of mice are very sensitive to heat at temperature which does not damage the skin [35]. The reaction time was taken as the parameter for the evaluation of analgesic activity. From the results obtained, it can be seen that MEBD significantly extended the latency period in the hotplate test. This prolonged reaction time indicates the analgesic activity of the methanol extract. MEBD was found to have antinociceptive activity in the hot plate test, which is a specific central antinociceptive test.

5. Conclusion

Medicinal plants have proved to be a rich source of therapeutic agents since ancient times to cure human diseases. This study evaluated the scientific basis for the use of *Boswellia dalzielii* leaves on pain and inflammation. In accordance to the results obtained in this study it has been observed that the MEBD has marked beneficial effects against acute anti-inflammatory and analgesic properties which are mediated via peripheral and central inhibitory mechanisms. Finally, this study provides a rationale for the use of this plant in pain and inflammatory disorders in folk medicine. Further study required to confirm the mechanism and exact phytoconstituents responsible for its anti-inflammatory and analgesic activity.

Authors' Contributions

This work was carried out by the collaboration of all authors. Author Jeweldai Vedekoi participated in experiments, statistical analysis and interpretation of all the data. Author Sokeng Dongmo Selestin helped in the plant collection procedure and contributed to the writing of the article. Author Kamtchouing Pierre prepared of the manuscript and interpreted all experiments. All the authors read and confirmed the final version of the manuscript for publication.

Conflicts of Interest

The authors declare that they have no competing interests.

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Availability of Data and Materials

All data obtained during this study are available from the first author or corresponding author upon request.

Consent for Publication

Not applicable.

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