



Analgesic and anti-inflammatory activities of ethanol extract of *Hymenodictyon pachyantha* K.Krause stem bark

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Abstract

The cost and worrisome toxicities associated with currently available anti-inflammatory drugs necessitate continuous search for more effective, cheap, and safe anti-inflammatory agents. This study investigated the anti-inflammatory and analgesic activities of ethanol extract of *Hymenodictyon pachyantha* stem bark (EEHP). The anti-inflammatory activity of EEHP was evaluated by determining its effect on egg albumin-induced rat paw edema, phospholipase A2 (PLA2) activity, calcium chloride (CaCl₂)-induced platelet aggregatory response, and membrane stabilization. The effect of EEHP on acetic acid-induced writhing responses was used as a model for analgesic property. The presence of flavonoids, terpenoids, steroids, saponins, alkaloids, tannins, and phenols was detected in EEHP. There was no sign of toxicity and mortality 24 h post-EEHP administration, suggesting that it is safe up to 5000 mg/kg b.w. EEHP significantly inhibited egg albumin-induced rat paw edema, in a dose-dependent manner and in both early and late stages of inflammation. Similarly, EEHP significantly inhibited PLA2 activity and platelet aggregatory response. EEHP also inhibited hypotonic-induced haemolysis by 23.99, 54.99, and 92.68% at 0.2, 0.6, and 1.0 mg/ml respectively. Similarly, EEHP produced 44.64, 58.04, and 70.54% reduction in the number of writhings induced by acetic acid at 200, 400, and 600 mg/kg p.o. respectively. These results demonstrated that EEHP has analgesic and anti-inflammatory effects. The results also showed that the mechanisms of the anti-inflammatory effect may be by inhibiting PLA2 and aggregation of platelets, and stabilization of lysosomal membrane.

Keywords Medicinal plants · *Hymenodictyon pachyantha* · Phytochemicals · Acute toxicity · Inflammation

Abbreviations

EEHP	Ethanol extract of <i>Hymenodictyon pachyantha</i> stem bark
NSAIDs	Non-steroidal anti-inflammatory drugs
SAIDs	Steroidal anti-inflammatory drugs
PLA ₂	Phospholipase A2
COX	Cyclooxygenase
HRBC	Human red blood cell
PUFAs	Polyunsaturated fatty acids
EDTA	Ethylenediamine tetraacetic acid

PRP	Platelet-rich plasma
LOX	Lipoxygenase
TXA2	Thromboxane A2
LTs	Leukotrienes

Introduction

Inflammation represents a complex biological response of vascular tissues to hazardous stimuli such as damaged cells, pathogens, or irritants. The purpose of inflammatory response is to eliminate the initial cause of cell injury, and necrotic cells, and tissues damaged from the original insult and the inflammatory process, as well as to initiate tissue repair and restoration of normal tissue functions (Malaya et al. 2003). Inflammation is critically linked with the etiology of many complex disorders such as neurodegenerative diseases, allergy, cancers, autoimmune and cardiovascular diseases, metabolic syndromes (Glass et al. 2010), heart attacks, and Alzheimer's disease (Libby et al.

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2002). Classic inflammatory response is characterized by the following clinical signs: warmth, pain, redness, swelling, and loss of function (Rigler 1997).

Two major classes of drugs known as steroidal (SAIDs) and non-steroidal anti-inflammatory drugs (NSAIDs) are employed in the alleviation of inflammation. Long-term use of NSAIDs causes side effects including gastric ulceration and renal toxicity (Ezekwesili et al. 2011), since they concurrently inhibit both isoforms of cyclooxygenase (COX), the enzymes involved in the synthesis of inflammatory mediators (prostaglandins (PGs), prostacyclins, and thromboxanes). NSAIDs which are selective COX inhibitors are also reported to increase the risk of heart attack and stroke (Salmon 2006; Nelson and Cox 2008). These necessitate a continuous search for potent anti-inflammatory drugs with fewer side effects especially from natural sources as alternatives/additions to the currently available drugs. One of such efforts is to screen plants used in folk medicine to ameliorate inflammatory conditions. An example of such plant is *Hymenodictyon pachyantha* K.Krause which is used in Nigeria folk medicine for the management of inflammatory disorders. The plant is locally known as *Udele Ose* by Igbo-speaking communities in South-Eastern Nigeria, and *Qbadan* or *Farquhar* in Benin, Western Nigeria. It is distributed mostly in tropical and subtropical parts of Asia and Africa. *H. pachyantha* K.Krause has recurved calyx lobes, which are longer than the mature corollas, and has elongated ovaries. The stipules bear large deciduous glands called colleters. The corolla tube is narrow at the base, gradually widening toward the apex. The fruit is a woody capsule (Razafimandimbison and Bremer 2006). The species belonging to this genus are having oppositely arranged serrated leaves and small, clustered flowers and many seeded capsules. Stipules are linear to lanceolate or ovate 15 mm long, apex acuminate and pubescent. Leaves are deciduous: petioles are 10–60 mm long, green-white-tinged, puberulous; secondary veins are seven to ten pairs per side. Fruit, a

capsule, is ellipsoid, 2 to 2.5 cm long, growing on recurved, thick pedicels 5 to 12 mm long. Seeds are many, flat, winged all around the margin, about 1 cm long, including the wing. Bark is mostly furrowed and rough, 10–20 cm thick, exfoliating in irregularly shaped with soft scales. The wood is soft and has limited use, mostly for boxes. The wood is used as planks in building houses and boats, making boxes, packing cases, pencils, toy model making, and matches. In India, it is used as a cheaper grade of wood for making furniture, wrapper bobbins, and wool boards. The bark obtained is useful for tanning purposes while leaves are useful for dyeing and as fodder for cattle while the remaining are only useful for timber wood (David 2008). The leaves are used in animal nutrition and disease management (Chah and Igbokwe 2011) (Fig. 1).

Materials and methods

Materials

Animals

Adult Wistar albino rats of body weight between 180 and 200 g (9 ± 1 week old) were used for edema study while adult Wistar albino mice of body weight between 30 and 40 g (8 ± 1 week old) were used for acute toxicity and analgesic activity studies. The animals were purchased from the Animal Breeding Unit of Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The animals were acclimatized under standard laboratory condition in the Animal House of the Department of Biochemistry, University of Nigeria, Nsukka, for 1 week prior to the commencement of the experiment with a 12-h light and dark cycle and maintained on a regular rodent chow (Vital Growers' Mash Nig. Ltd.) and portable water ad libitum throughout the period of the experiment.



Fig. 1 Leaves and stem of *Hymenodictyon pachyantha* K.Krause. Source: Researchers

Micro-organism

The fungal organism used as source of enzymes was strains of *Aspergillus niger*. The organism was obtained from the Department of Microbiology, University of Nigeria, Nsukka.

Blood samples

The blood samples used for the membrane stabilization assay, platelet aggregation tests and phospholipase A2 activity were collected from apparently healthy voluntary donors. The blood donors were free from drug treatments for at least 2 weeks before sample collection.

Reference drugs

Indomethacin (product of Yangzhou Pharmaceutical Co. Ltd.) was used as the reference drug for paw edema, membrane stabilization, and antiplatelet aggregation assays. Prednisolone (product of Kunimed Pharmaceutical Ltd.) was used as the reference drug for the PLA₂ activity assay. Acetylsalicylic acid (also known as aspirin and product of Juhel Pharmaceuticals Ltd.) was used as the reference drug for the analgesic activity assay.

Methods

Ethical statement

The experimental procedures were carried out in accordance with the institutional, national, and international ethical recommendations for the use of human subjects for research (Helsinki declaration), as well as for the care and use of laboratory animals (NIH publication #85-23, revised in 1985) in experiments, clinical studies, and biodiversity rights.

Preparation of plant extract

Fresh *H. pachyantha* stem bark and leaves were collected from a habitat in Orba, Udenu Local Government Area of Enugu State, Nigeria, and was authenticated by Mr. Alfred Ozioko, a taxonomist from Bioresources Development and Conservation Programme (BDCCP) Research Centre, Nsukka, Enugu State, and voucher specimen deposited in their herbarium for reference purposes with voucher reference number #Intercedd/859. The leaves of the plants were used for identification purposes only. The stem bark was washed off of dirt using portable water, cut into small pieces and air-dried with regular turning to avoid decaying, until crispy (which took two weeks). The dried stem bark was powdered using a mechanical grinder. A known weight of the pulverized sample (1000 g) was macerated in 5 l of absolute ethanol, vigorously shaken, and allowed to stand for 72 h in a rotor. The mixture

was filtered using a mesh. Further filtration was achieved using Whatman No. 1 filter paper to remove fine particles. The filtrate was concentrated under reduced pressure using a rotary evaporator at 45 °C to obtain the crude ethanol extract of *H. pachyantha* stem bark (EEHP) which was refrigerated in an air-tight container at 4 °C until required. The percentage yield for the extract was 3.32%.

Determination of phytochemical constituents of EEHP

The qualitative and quantitative phytochemical constituents of the extract were determined using the methods earlier described by Harborne (1998) and Trease and Evans (2002).

Determination of the anti-inflammatory effect of EEHP using egg albumin-induced rat paw edema model

This was done using the method of Winter et al. (1962). The increase in the right hind paw size of the rats induced by the sub-plantar injection of freshly prepared egg albumin was used as a measure of acute inflammation. Egg albumin releases mediators of acute inflammation responsible for causing edema. The ability of the extract to inhibit this release of mediators is a measure of its anti-inflammatory effect. A total of twenty-five male rats were used for the study. They were divided into five groups of five rats each and treated as follows:

- Group 1: Received 5 ml/kg of 3% (v/v) Tween 80 in distilled water (vehicle)
- Group 2: Received 10 mg/kg body weight of indomethacin (standard drug)
- Group 3: Received 100 mg/kg body weight of EEHP
- Group 4: Received 200 mg/kg body weight of EEHP
- Group 5: Received 400 mg/kg body weight of EEHP

All the experimental rats were fasted for 18 h before the experiment to ensure uniform hydration and minimize variability in edematous response, after which, the right hind paw size of each rat at time zero (before the induction of edema) was measured using a Vernier calliper. This was followed by intraperitoneal administration of test samples (extract and standard drug) as outlined above. One hour after administration, acute inflammation was induced by injecting 0.1 ml of freshly prepared egg albumin into the sub-plantar of the right hind paw of rats. The increase in the right hind paw size of rats was subsequently measured at 0.5, 1, 2, 3, 4, and 5 h after egg albumin injection. The difference between the paw size of the injected paws at time zero and at different times after egg albumin injection was used to assess the formation of edema. These values were used in the calculation of the percentage inhibition of edema for each dose of the extract and for indomethacin at the different time intervals using the relation below:

Inhibition of paw edema (%)

$$= \frac{\text{Paw edema of control} - \text{Paw edema of test groups}}{\text{Paw edema of control}} \times 100$$

Paw edema = (Vt–Vo); where Vo = Paw edema at time zero; Vt = Paw edema at time *t* (0.5, 1, 2, 3, 4, 5 h).

Determination of the anti-inflammatory effect of EEHP using the inhibition phospholipase A2 activity model

The effect of the extract on PLA2 activity was determined using modifications of the methods of Vane (1971). Phospholipase A2 activity was assayed using its action on erythrocyte membrane; it releases free fatty acids from the membrane phospholipids thereby causing leakage, allowing haemoglobin to flow into the medium in the process. The enzyme activity is thus directly related to the amount of haemoglobin in the medium. This was measured at 418 nm since haemoglobin absorbs maximally at this wavelength.

Enzyme preparation This was done as described by Odu et al. (2018). Fungal enzyme was obtained from *Aspergillus niger* strain culture. The nutrient broth was prepared by dissolving 15 g of sabouraud dextrose agar in 1000 ml of distilled water, homogenized in a water bath for 10 min, and dispensed into 250-ml conical flasks. The conical flasks were sealed with cotton wool and foil paper. The broth was then autoclaved at 121 °C for 15 min. The broth was allowed to cool to room temperature and then, the organisms in the Petri dishes were aseptically inoculated into the broth and incubated for 72 h at room temperature. The culture was transferred into test tubes containing 3 ml phosphate buffered saline and centrifuged at 3000 rpm for 10 min. The fungal cells settled at the bottom of the test tube while the supernatant was used as the crude enzyme preparation.

Substrate preparation Fresh human blood samples were centrifuged at 3000 rpm for 10 min and the supernatant (plasma) discarded. The red cells were washed three times with equal volume of normal saline, measured and reconstituted as a 40% (v/v) suspension with phosphate buffered saline. This served as the substrate for PLA2.

Assay procedure Two millimolars CaCl₂ (0.2 ml), human red blood cell (HRBC) (0.2 ml), 0.2 ml of the crude enzyme preparation and varying concentrations of normal saline, the extract and the reference drug were incubated in test tubes for 1 h. The control contained the human red blood cell suspension, CaCl₂, and free enzyme. The blanks were treated with 0.2 ml of boiled crude enzyme separately. The incubation reaction mixtures were centrifuged at a speed of 3000 rpm for 10 min. Samples of the supernatant (1.5 ml) were diluted

with 10 ml of normal saline and the absorbance of the solutions read at 418 nm (Odu et al. 2018). Prednisolone, a known inhibitor of phospholipase A2, was used as the reference drug. The percentage maximum enzyme activity and percentage inhibition was calculated using the following relations:

$$\% \text{maximum activity} = \frac{\text{OD of test}/t}{\text{OD of control}/t} \times \frac{100}{1}$$

$$\% \text{inhibition} = 100 - \% \text{maximum activity of enzyme}$$

Determination of membrane stabilization effect of EEHP on hypotonicity-induced haemolysis of red blood cells

The effect of EEHP on haemolysis induced by distilled water was evaluated by incubating various concentrations of EEHP with HRBCs and distilled water. This assay was carried out by a modified method of Sikder et al. (2012). The assay is based on the susceptibility of RBCs' membrane to oxidative damage, made possible as a result of the preponderance of poly-unsaturated fatty acids (PUFAs) in the membranes leading to haemolysis. Haemoglobin and other internal cell components are thus released into the surrounding fluids. Absorbance readings at 418 nm are a reflection of the amount of haemoglobin in the medium. The effect of the EEHP on haemolysis induced by distilled water was evaluated by incubating 0.1 ml solution containing graded concentrations (mg/ml) of the extract with 0.1 ml of the sodium citrate treated blood, 1.8 ml of normal saline, and 0.5 ml of distilled water in a test tube at 37 °C in a water bath for 1 h. After the incubation, the test tubes were centrifuged at 3000 rpm for 10 min and the absorbances of the supernatants were read at 418 nm. The same procedure was repeated using indomethacin (a standard anti-inflammatory drug) as a positive control. The extent of haemolysis (and membrane stabilization) was assessed as changes in absorbance. The inhibition of haemolysis (%) by the extract and indomethacin were calculated using the relation below:

$$\text{Inhibition of haemolysis (\%)} = 100 \left(1 - \frac{\text{OD}_1 - \text{OD}_2}{\text{OD}_1} \right)$$

where OD₁ = absorbance of control hypotonic solution and OD₂ = absorbance of test sample.

Determination of the anti-inflammatory effect of EEHP using platelet aggregation model

This was achieved using a modification of the method of Born and Cross (1963). The aggregation of platelets leads to an increase in transmittance, therefore less absorbance of light. CaCl₂-induced platelet aggregation is thus shown by a reduction in absorbance at 520 nm. Any substance that has anti-

aggregatory effect would thus lead to increased absorption by the medium.

Preparation of platelet-rich plasma (PRP) Fresh venous blood samples (5 ml) were taken from healthy volunteers into plastic tubes containing 1% ethylene diamine tetraacetic acid (EDTA) as an anticoagulant. The tubes were centrifuged at 3000 rpm for 10 min and the supernatant was collected, diluted twice with normal saline, and used as the platelet-rich plasma (PRP) (Nwodo 1981). An aliquot of PRP (0.2 ml) was dispensed into three sets of test tubes each containing 1 ml of varying concentrations of extract (0.1, 0.2, and 0.4 mg/ml) in 3% Tween 80 (dissolved in normal saline). Also, another test tube contained an aliquot (0.2 ml) of PRP and 1 ml of 0.2 mg/ml indomethacin in 3% Tween 80. The contents of the respective tubes were made up to 2.2 ml with the vehicle. A control tube contained 2 ml of the vehicle and 0.2 ml of PRP. The tubes were allowed to incubate for 10 min after which, there was addition of 0.4 ml of 1.47% CaCl₂ solution to induce aggregation. The tests were performed in triplicate. Changes in the absorbance of the solutions were taken at intervals of 30 s for 120 s at 520 nm. The blanks contained the extract or indomethacin without PRP.

Determination of analgesic activity of EEHP using acetic acid-induced writhing test

To determine the analgesic activity of ethanol extract, the writhing test method described by Koster et al. (1959) was used. Nociception, initiated by pain receptors, is known as the neural process of encoding and processing noxious stimuli. It is the afferent activity produced in the peripheral and central nervous system by stimuli that have the potential to damage tissue (Onasanwo et al. 2012). Vehicle (5 mg/kg body weight (b.w.)), aspirin (200 mg/kg b.w.), three scalar amounts (100, 200, and 400 mg/kg b.w.) of the extract were respectively administered (p. o.) to 5 groups of 5 mice each. Thirty minutes afterwards, 10 mg/kg b.w. of 0.6% acetic acid was intraperitoneally injected into each mouse. The number of writhings and stretchings was counted over a 20-min period.

Acute toxicity test on EEHP

Investigation on the acute toxicity profile of EEHP was carried out using the modified method of Lorke (1983). This study was done in two phases and a total of eighteen mice were used. In phase 1, nine experimental mice were distributed in three groups of three mice each and were orally administered 10 mg/kg, 100 mg/kg, and 1000 mg/kg body weight of the extract respectively. The experimental mice were observed for an hour, then every 20 min for another 3 h, and finally, after 24 h post extract administration for lethality, and morphological, neurological and behavioural changes (signs of toxicity). Based on the result of phase 1, phase 2 of the acute toxicity study was conducted.

Increased doses of 1600, 2000, and 2900 mg/kg b.w. of the extract were administered respectively to nine experimental mice distributed in three groups of three mice each. The mice were also observed as described above.

Statistical analysis

The data obtained were expressed as mean \pm standard deviation (SD). Significant differences of the mean values were established by one-way and two-way analysis of variance and the acceptance level of significance was $p < 0.05$ for all the results. This was done using the Statistical Product and Service Solutions (SPSS) version 22.0.

Results

Phytochemical constituents of ethanol extract of *Hymenodictyon pachyantha* stem bark

Phytochemical analyses of the extract revealed the presence of flavonoids (1359.268 \pm 0.02 mg/100 g), terpenoids (2154.695 \pm 0.01 mg/100 g), steroids (3.782 \pm 0.05 mg/100 g), saponins (0.405 \pm 0.03 mg/100 g), alkaloids (268.856 \pm 0.12 mg/100 g), tannins (1375.930 \pm 0.08 mg/100 g), and phenols (2900.169 \pm 0.15 mg/100 g) (Table 1).

Effect of EEHP on egg albumin-induced rat paw edema

Table 2 shows the results of the effect of EEHP on the mean paw edema and percentage inhibition of egg albumin-induced edema in the rat paw recorded over a period of 5 h. A significant dose-dependent reduction in the mean paw edema was observed for all the treatment groups from 0.5 to 5 h when compared with the control. The paw size of rats treated with graded doses of EEHP

Table 1 Phytochemical contents of ethanol extract of *Hymenodictyon pachyantha* stem bark

Phytochemical	Relative abundance	Amount (mg/100 g)
Flavonoids	+++	1359.27 \pm 0.02
Terpenoids	+++	2154.70 \pm 0.01
Steroids	+	3.78 \pm 0.05
Saponins	+	0.41 \pm 0.03
Alkaloids	++	268.86 \pm 0.12
Tannins	+++	1375.93 \pm 0.08
Phenols	+++	2900.17 \pm 0.15

+Slightly present

++Moderately present

+++Highly present

Results are expressed in mean \pm standard deviation ($n = 3$)

Table 2 Effect of EEHP on egg albumin-induced rat paw edema

Treatment	Concentration (mg/kg)	Mean paw edema (cm) and percentage inhibition of edema					
		0.5 h	1.0 h	2.0 h	3.0 h	4.0 h	5.0 h
Control (3% Tween 80)	5 ml/kg	0.61 ± 0.02 ^{DE}	0.59 ± 0.01 ^{ED}	0.58 ± 0.01 ^{dCD}	0.57 ± 0.05 ^{dBC}	0.56 ± 0.01 ^{dB}	0.53 ± 0.01 ^{eA}
Indomethacin	10	0.31 ± 0.01 ^{AF} (49.18%)	0.28 ± 0.02 ^{AE} (52.54%)	0.22 ± 0.03 ^{AD} (62.07%)	0.19 ± 0.01 ^{AC} (66.67%)	0.16 ± 0.02 ^{AB} (71.43%)	0.13 ± 0.01 ^{bA} (75.47%)
Extract	100	0.35 ± 0.01 ^{BF} (12.20%)	0.31 ± 0.01 ^{BE} (27.27%)	0.28 ± 0.04 ^{BD} (36.95%)	0.25 ± 0.01 ^{BC} (44.68%)	0.22 ± 0.01 ^{CB} (50.00%)	0.19 ± 0.03 ^{dA} (57.14%)
	200	0.31 ± 0.02 ^{AF} (49.18%)	0.27 ± 0.02 ^{AE} (54.24%)	0.25 ± 0.01 ^{BD} (56.90%)	0.22 ± 0.03 ^{BC} (61.40%)	0.20 ± 0.04 ^{BB} (64.29%)	0.17 ± 0.02 ^{cA} (67.92%)
	400	0.30 ± 0.01 ^a (50.82%)	0.27 ± 0.05 ^a (54.24%)	0.21 ± 0.01 ^a (63.80%)	0.18 ± 0.03 ^a (68.42%)	0.15 ± 0.04 ^a (73.21%)	0.11 ± 0.01 ^a (79.25%)

Results expressed as mean ± SD (*n* = 5); mean values having different lowercase letters as superscripts in a column are considered significant (*p* < 0.05). Mean values having different uppercase letters as superscripts in a row are considered significant (*p* < 0.05). Values in parenthesis () represent percentage inhibition of paw edema calculated relative to control

and indomethacin significantly decreased with time. At 5 h, the inhibition of rat paw edema for the groups treated with 100, 200, and 400 mg/kg b.w. of EEHP were 64.15%, 67.92%, and 79.25% respectively. The percentage edema inhibition for rats treated with indomethacin 10 mg/kg b.w. after 0.5 h, 1 h, 2 h, 3 h, 4 h, and 5 h was 49.18%, 52.54%, 62.07%, 66.67%, 71.43%, and 75.47% respectively. The percentage edema inhibition of the group treated with 400 mg/kg b.w. of extract was significantly higher than that of indomethacin at a 5-h period.

Effect of EEHP on phospholipase A2 activity

Table 3 shows the effect of EEHP on phospholipase A2 activity. The ethanol extract significantly inhibited the activity of PLA₂ in a dose-dependent manner when compared with the control. There was a decrease in the absorbance of the sample with increasing concentration of the extract; hence, a decrease in enzyme activity. The percentage inhibition for extracts at 0.1, 0.2, 0.3, 0.4, and 0.5 ml were 78.92%, 89.71%, 91.67%, 93.63%, and 95.59% respectively. Prednisolone, a standard anti-inflammatory drug, followed a similar trend with the enzyme activity decreasing with increasing doses of prednisolone. The absorbance of extracts 0.1, 0.2, 0.3, 0.4, and 0.5 ml was found to be significantly lower when compared with control, the same was also observed with increasing concentrations of the standard drug (prednisolone) when compared with the control.

Effect of EEHP on hypotonicity-induced haemolysis of human red blood cells

The result presented in Table 4 shows that EEHP significantly protected the human erythrocyte membrane against lyses induced by hypotonic solution compared with the control, in a dose-dependent manner. The highest percentage inhibition (92.68%) of haemolysis here was obtained at 1.0 mg/ml of the extract. This inhibitory effect of EEHP against hypotonicity-induced haemolysis of human red blood cells is comparable with that obtained with indomethacin, a standard anti-inflammatory drug.

Effect of EEHP on calcium chloride (CaCl₂)-induced platelet aggregation

Table 5 shows the effect of EEHP on CaCl₂-induced platelet aggregatory response. The extract significantly inhibited platelet aggregatory response. The inhibitory effect of EEHP against CaCl₂-induced platelet aggregation is concentration and time-dependent. The maximum inhibitory effect against CaCl₂-induced platelet aggregation was recorded at 120 s and 0.6 mg/ml EEHP concentration. This inhibitory effect by EEHP is similar to that of indomethacin.

Table 3 Effect of EEHP on phospholipase A2 activity

Treatment	Concentration (mg/ml)	Absorbance (418 nm)	% maximum enzyme activity	% inhibition
Control	–	0.21 ± 0.02 ^f	100	–
Extract	0.1	0.04 ± 0.01 ^e	21.08	78.92
	0.2	0.02 ± 0.05 ^d	10.29	89.71
	0.3	0.02 ± 0.01 ^c	8.33	91.67
	0.4	0.01 ± 0.13 ^b	6.37	93.63
	0.5	0.01 ± 0.01 ^a	4.41	95.59
Prednisolone	0.2	0.07 ± 0.01 ^a	41.67	58.33

Results expressed as mean ± SD ($n = 3$); mean values having different lowercase letters as superscripts in a column are considered significant ($p < 0.05$)

Effect of EEHP on acetic acid-induced nociceptive response in mice

The analgesic assay demonstrated that EEHP exhibited potent analgesic activity comparable to that of aspirin. The number of writhings in all doses of the extract (100, 200, 400 mg/kg body weight) and aspirin (200 mg/kg body weight) was significantly lower than the number of writhings in the control group. The analgesic effect of the extract was dose-dependent. The % analgesic activity of extracts at 100, 200, and 400 and aspirin 200 mg/kg were 44.64, 58.04, 71.43, and 70.54% respectively. This indicates that the highest dose of the extract had similar analgesic properties when compared with the standard drug, hence, may serve as the lead to development of newer anti-inflammatory drug (Table 6).

Acute toxicity profile of EEHP

There was neither death nor any sign of toxicity in the groups of mice administered 10, 100, and 1000 mg/kg body weight of EEHP for phase I as well as in the groups administered, 1900, 2600, and 5000 mg/kg body weight of EEHP for phase II (Table 7). This shows that the extract is relatively safe up to 5000 mg/kg body weight and that the LD₅₀ is above this dose.

Table 4 Effect of EEHP on hypotonicity-induced haemolysis of human red blood cells

Treatment	Concentration (mg/ml)	Hypotonic solution (ODI)	Test solution (OD2)	% inhibition of haemolysis
Control	–	1.61 ± 0.02 ^g	–	–
Extract	0.2	–	0.40 ± 0.11 ^a	23.99
	0.4	–	0.69 ± 0.05 ^c	42.96
	0.6	–	0.89 ± 0.02 ^d	54.99
	0.8	–	1.26 ± 0.01 ^e	78.18
	1.0	–	1.50 ± 0.07 ^f	92.68
Indomethacin	0.2	–	0.48 ± 0.02 ^b	29.88

Results are expressed in means ± standard deviation ($n = 3$); mean values having different letters as superscripts in a column are considered significant ($p < 0.05$)

Discussion

The present study was carried out to investigate the analgesic and anti-inflammatory effects, and mechanisms of action of ethanol extract of *Hymenodictyon pachyantha* stem bark (EEHP). The presence of flavonoids, terpenoids, phenols, steroids, saponins, alkaloids, and tannins was detected in the extract in varying proportions. Some of these phytochemicals are believed to be responsible for the anti-inflammatory activities of some plants (Garg et al. 2010; Sakat et al. 2010). For instance, flavonoids have been reported to exhibit anti-inflammatory effect in various studies (Amarlal et al. 2009; Lopez-Lazaro 2009; Shailasree et al. 2012). Similarly, terpenoids, steroids, saponins, tannins, and alkaloids have anti-inflammatory activities (Gepdireman et al. 2005; Souza et al. 2007; Das et al. 2010). In addition, the anticoagulant and anti-platelet aggregatory activity of phenolic compounds and flavonoids have been reported (Imram et al. 2012). Secondary metabolites like flavonoids and alkaloids have also been associated with analgesic properties of plants (Kumar et al. 2013; Fan et al. 2014; Afsar et al. 2015). Flavonoids are the main constituents that have capacity to interfere with eicosanoid biosynthesis pathways (Robak and Gryglewski 1996) and are suggested to suppress the release of arachidonic acid through inhibition of neutrophil degranulation (Tordera et al. 1994). Both of these

Table 5 Effect of EEHP on calcium chloride-induced platelet aggregation

Treatment	Concentration (mg/ml)	Δ Absorbance (520 nm)			
		30 s	60 s	90 s	120 s
Control	–	0.21 ± 0.01 ^{fA}	0.21 ± 0.01 ^{fB}	0.23 ± 0.02 ^{fC}	0.21 ± 0.02 ^{fA}
Extract	0.1	0.14 ± 0.01 ^{eC}	0.14 ± 0.02 ^{eBC}	0.14 ± 0.02 ^{dB}	0.14 ± 0.05 ^{dA}
		(67.14%)	(66.82%)	(60.44%)	(66.18%)
	0.2	0.14 ± 0.02 ^{dC}	0.13 ± 0.01 ^{dBC}	0.13 ± 0.01 ^{eB}	0.13 ± 0.02 ^{eA}
		(64.76%)	(63.03%)	(58.67%)	(61.35%)
0.4	0.13 ± 0.02 ^{eB}	0.13 ± 0.01 ^{eB}	0.12 ± 0.11 ^{bA}	0.12 ± 0.01 ^{bA}	
	(60.00%)	(59.24%)	(53.78%)	(57.97%)	
0.6	0.12 ± 0.02 ^{bA}	0.12 ± 0.01 ^{bA}	0.20 ± 0.02 ^{eC}	0.20 ± 0.15 ^{eC}	
	(58.10%)	(57.35%)	(87.11%)	(95.17%)	
Indomethacin	0.2	0.13 ± 0.01 ^{bcAB}	0.13 ± 0.02 ^{eB}	0.12 ± 0.02 ^{bA}	0.12 ± 0.01 ^{bA}
		(60.48%)	(59.72%)	(53.78%)	(58.45%)

Results expressed as mean ± SD ($n = 3$); mean values having different lowercase letters as superscripts in a column are considered significant ($p < 0.05$) while mean values having different uppercase letters as superscripts in a row are considered significant ($p < 0.05$). Values in parenthesis () = % inhibition of platelet aggregation

actions result in suppression of production and activities of inflammatory mediators like PGs and lipoxygenase end-products that induce inflammation, pain, and fever.

The anti-inflammatory assays of the ethanol extract displayed significant inhibition of inflammation. This suggests that the phytoconstituents of the plant extract may have inhibited the inflammation through their suppressive action on the production of PGs. The anti-inflammatory activity of the ethanol extract of *H. pachyantha* stem bark was assessed by measuring its ability to reduce local edema induced in the rat paw by injection of a phlogistic substance (Omkar et al. 2007). Egg albumin-induced edema model has been used in anti-inflammatory studies; subcutaneous injection of egg albumin into the rat paw induces edema resulting from plasma protein-rich fluid exudation along with neutrophil extravasation. The increase in the paw volume of the rats after the injection of egg albumin correlates with the work of Ekwueme et al. (2011) that egg albumin induces paw edema in rats. The feasible mechanism of egg albumin-mediated inflammation is tri-phasic. The first phase (0–2 h) is predominantly non-phagocytic and mainly mediated by histamine and serotonin, the second phase (2–3 h) is mediated by kinin, and the last phase (3–5 h) is due to the liberation of PGs (Suba et al. 2005) which

produces edema-dependent on neutrophil mobilization. The ethanol extract at doses of 100, 200, and 400 mg/kg body weight showed good anti-inflammatory activity as it significantly inhibited the increase in paw volume from 0.5 to 5 h. This shows that the extract inhibited all the phases of the inflammatory response just as was demonstrated with *Annona vepretorum* species by Silva et al. (2015). The inhibition of the early phase of edema exhibited by the ethanol extract in this study suggests that it blocks the release of histamine and serotonin. The suppression of edema in the second and third phases of inflammation suggests that the anti-inflammatory activity of the extract may also be due to the suppression of kinin and PG formation induced by egg albumin within this period. Since these mediators cause edema by increasing vasodilatation and vascular permeability at the site of injury, the extract therefore reduces vascular permeability and fluid exudation, probably by preventing the contraction of endothelial cells and hence, suppressing edema.

Consistent with the above finding is the inhibitory effect of EEHP on phospholipase A2 activity, an acyl-hydrolase that cleaves fatty acid from membrane phospholipids in position two of the phospholipids, hydrolyzing the bond between the second fatty acid tail and the glycerol molecule and calcium

Table 6 Effect of EEHP on acetic acid-induced nociceptive response in mice

Treatment	Dosage	No. of writhings (count/20 min)	% analgesic activity
Control (3% Tween 80)	0.1 ml/10 g	28.00 ± 1.63 ^d	–
Extract	100 mg/kg	15.50 ± 2.08 ^c	44.64
	200 mg/kg	11.75 ± 0.96 ^b	58.04
	400 mg/kg	8.00 ± 0.82 ^a	71.43
Aspirin	200 mg/kg	8.25 ± 1.26 ^a	70.54

Results expressed as mean ± SD ($n = 4$); mean values having different lowercase letters as superscripts are considered significant ($p < 0.05$) down the column

Table 7 Acute toxicity profile of EEHP

Treatment	Dosage of extract (mg/kg)	Mortality rate
Phase I		
Group 1	10	0/3
Group 2	100	0/3
Group 3	1000	0/3
Phase II		
Group 1	1900	0/3
Group 2	2600	0/3
Group 3	5000	0/3

Number of mice per group (n) = 3

chloride-induced platelet aggregation. Arachidonic acid released from membrane phospholipids is acted upon by cyclooxygenase (COX) and lipoxygenase (LOX) which leads to the de novo synthesis of lipid mediators (George et al. 2014). The action of COX on arachidonic acid produces mediators such as PGE₂, PGD₂, PGI₂, and thromboxane A₂ (TXA₂), while the action of 5-LOX on arachidonic acid releases leukotrienes (LTs) such as LTB₄. The extract at concentration of 0.1 to 0.5 mg/ml exhibited a significant and concentration-dependent inhibition of PLA₂ activity. This inhibition of PLA₂ by the extract implies that it was able to suppress the release of free fatty acids from RBC membrane phospholipids and the consequent deprivation of COX and LOX substrates for the synthesis of inflammatory mediators, hence limiting their effects such as vasodilatation, vascular permeability, chemotaxis, and pain, thus preventing inflammation. This inhibition of PLA₂ also shows that the extract has potentials for preventing atherosclerosis and cancer as PLA₂ has been implicated in their aetiologies (Sato et al. 2009). The mechanism of inhibition of PLA₂ by the extract could be like that of corticosteroids which induce lipocortin synthesis (Schimmer and Parker 2001). This effect could be attributed to the presence of flavonoids in the extract as studies have shown that flavonoids inhibit PLA₂ (Wang et al. 2006; Kim et al. 2008; Messina et al. 2009). Similarly, Barbosa et al. (2012) observed that sub-fraction of *Spiranthera odoratissima* leaves which contained only tannins inhibited PLA₂ activity, suggesting that tannins might have contributed to the inhibitory effect of the ethanol extract.

It was also observed that the extract was found to significantly inhibit lysis of erythrocytes in a dose-dependent fashion. In a study on membrane stabilization effects of *Spiranthes paniculata* leaf extracts, Hossain et al. (2014) reported a similar outcome. One of the mechanisms of anti-inflammatory actions of analgesic compounds is by membrane stabilization which makes it a good model of anti-inflammatory studies (Hossain et al. 2014).

The hypotonic solution-induced human erythrocyte lysis test is a good model to examine membrane stabilization. In the presence of hypotonic solution such as water, the membrane of red

blood cells lyse due to osmotic uptake of water leading to extracellular release of haemoglobin. This results in increase in optical density at 418 nm. Hence, a reduction in optical density at 418 nm when RBCs are in hypotonic solution and in the presence of extract indicates that the extract has RBC's membrane stabilization effect. Similarly, lysosomal membranes are lysed during inflammation, resulting in the extracellular release of lysosomal enzymes that cause damages to nearby tissues leading to a variety of inflammatory disorders. Because human RBC membranes are similar to lysosomal membrane (Mohammedi and Atik 2014), the stabilization of membranes of RBCs by the extract thus implies that it may as well stabilize lysosomal membranes, hence, the choice of the model for the evaluation of mechanism of action of anti-inflammatory agents. The two major mechanisms by which NSAIDs act are either by inhibition of lysosomal enzymes or stabilization of lysosomal membranes. The ability of the extract to retain the integrity of RBC's membrane by suppression of hypotonic-mediated membrane lysis is suggested as a possible mechanism of anti-inflammatory activity of the extract.

Among the crucial mediators of inflammation identified in a variety of animal models, including sepsis (Wang et al. 2005), acute kidney injury, dermal inflammation (Zarbock et al. 2006), vascular injury (Katoh 2009), and myocardial infarction, are platelets. Mechanism of platelet aggregation involves platelet-to-platelet adhesion and is necessary for effective hemostasis following the initial adhesion of platelets to the site of injury. Following adhesion, platelets are activated by a number of agonists such as adenosine diphosphate (ADP) and collagen present at the sites of vascular injury. These agonists activate platelets by binding to specific receptors on the platelet surface and the eventual formation of blood clot through the deposition of fibrin from the coagulation cascade (Paez-Espinosa et al. 2012). This study demonstrated that the extract produced a concentration and time-dependent inhibition of CaCl₂-induced platelet aggregatory response, comparable with a standard anti-inflammatory drug (indomethacin). The inhibition of CaCl₂-induced platelet aggregatory response could probably be due to the extract's ability to inhibit PLA₂ and COX which are required for the synthesis of TXA₂. The inhibition of TXA₂ is imperative because it induces platelet aggregation by increasing intracellular Ca²⁺ concentration which promotes fusion of platelet granules with the membrane, thus, releasing its contents such as ADP that further promotes platelet aggregation (Sangkuhl et al. 2011).

The inhibition of platelet aggregation by the extract could also imply decreased vascular permeability and leukocyte extravasation which is mediated respectively by histamine and P-selectin normally released from the platelet granules (Zarbock et al. 2006). Since blood platelets participate in pathological thrombosis, leading to conditions like myocardial infarction, stroke, embolism and peripheral vascular thrombosis (Golino et al. 2005; Fabre and Gurney 2010), inhibition of platelet aggregation by the extract is thus indicative of its possible role as an antithrombotic

agent and could be useful in the management of the above disorders. This demonstrated antiplatelet aggregatory response by the extract could be due to the presence of phenolic compounds and flavonoids in the extract. This is because the anticoagulation and antiplatelet aggregatory activities of these phytochemicals have been reported (Hommam et al. 2000; Imram et al. 2012). In addition, phenolic compounds and flavonoids have antioxidant effects (Messina et al. 2009), and the roles of antioxidants on the inhibition of platelet activation and aggregation is established (Sobotková et al. 2009). In addition, the antiplatelet aggregatory response by the extract could also be attributed to the presence of tannins, as the anticoagulant or antiplatelet aggregation activity of tannins has also been demonstrated in many studies (Mekhfi et al. 2006; Kee et al. 2008; Kim et al. 2008).

The result of the effect of the ethanol extract against acetic acid-induced visceral pain showed appreciable analgesic activity. Pain is associated with the pathophysiology of various clinical consequences such as arthritis, muscular pain, cancer, and vascular diseases (Akhtar et al. 2013). The characteristic of pain activity generated by intraperitoneal injection of acetic acid is presented with contraction of abdominal muscle followed by extension of hind limbs and elongation of body part and such constriction is thought to be mediated by local peritoneal receptor (Bentley et al. 1983). Acetic acid-induced writhing response is a suitable method for assessing peripheral analgesic effects as it is sensitive for various classes of analgesic drugs. Pain perception in acetic acid-induced writhing assay is revealed by producing localized inflammatory response due to release of free arachidonic acid from tissue phospholipids by the help of COX, which in turn produces PGs, specifically PGE₂ and PGF₂α. The level of lipoxygenase produced during this pain induction may also increase peritoneal fluids and cause inflammation and pain by increasing capillary permeability. Thus, the substance inhibiting the writhing will have an analgesic effect preferably by inhibition of PG synthesis (Khan et al. 2010). NSAIDs relieve the pain response peripherally by inhibiting production of PGs, thromboxane, and other inflammatory mediators through their inhibitory effects on COX. The ethanol extract in this study at 100, 200, and 400 mg/kg b.w. significantly reduced the number of writhes induced by a 0.6% acetic acid solution, in a dose-dependent manner, similar to that of aspirin. Central nervous system depressants have been shown to reduce the number of writhing in acetic acid pain models (Garcia et al. 2007; Nahar et al. 2012). The inhibition of acetic acid-induced writhing by the ethanol extracts also suggests that it has a depressant effect on the central nervous system.

Acute toxicity describes the unwanted effect that occurs either immediately or at a short time interval after a single or multiple administration of such substance within 24 h. The unwanted (or adverse) effect is any effect that produces functional impairments in organs and biochemical lesions, which could alter the functioning of the organism in general or

individual organs (Akhila et al. 2007). It has been observed that an overdose of *H. pachyantha* extract is usually non-fatal; the victims tend to suffer self-limiting gastrointestinal disturbances (Anokbonggo et al. 1990). The administration of the extract by oral route was chosen to mimic the route used by the traditional healers in treating their patients. This would make any findings in the experimental animals easily translatable to what would be expected in the human subjects. On the basis of this fact, the acute toxicity study of EEHP was carried out. On administration of the extract, no immediate behavioural change was observed. The mice moved about and fed normally. After 20 min, piloerection was noticed and the animals became restless, some trying to escape through the holes in the cages. The animals did not vomit, neither was there ptosis. All of the above signs were not observed after 1 h post extract administration. There was also no mortality after 24 h post extract administration. In general, the acute toxicity test result revealed that the extract has a high safety profile, as it was tolerated by the animals up to a dose of 5000 mg/kg body weight. This entails that the no observed adverse effect level (NOAEL) estimate of extract is beyond 5000 mg/kg.

Conclusion

The results of this study infer that ethanol extract of *Hymenodictyon pachyantha* stem bark (EEHP) inhibited acetic acid-induced nociceptive response through peritoneal receptors mediated cyclooxygenase inhibition, indicating analgesic effects. This effect was found to be comparable with the standard analgesic drug, aspirin used in this study. The results also showed that the extract has promising anti-inflammatory activity that is similar to standard anti-inflammatory drugs (indomethacin and prednisolone) used. The findings suggest that the mechanisms of this anti-inflammatory effect may be by inhibiting phospholipase A₂ activity and platelet aggregation which are responsible for inflammation. The analgesic and anti-inflammatory activities of EEHP observed in this study might be linked with the phytoconstituents of the extracts as reported. This investigation provides empirical evidence for the use of *H. pachyantha* stem bark extract in folkloric treatment of inflammatory disorder.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Informed consent Not applicable.

References

- Afsar T, Khan MR, Razak S, Ullah S, Mirza B (2015) Antipyretic, anti-inflammatory and analgesic activity of *Acacia hydaspica* R. Parker and its phytochemical analysis. *BMC Complement Altern Med* 15: 136–137
- Akhila JS, Deepa S, Alwar MC (2007) Acute toxicity studies and determination of median lethal dose. *Curr Sci* 93:917–920
- Akhtar MS, Malik A, Saleem MS, Murtaza G (2013) Comparative analgesic and anti-inflammatory activities of two polyherbal tablet formulations (Aujaie and Surangeen) in rats. *Trop J Pharm Res* 12: 603–607
- Amarlal S, Mira L, Nogueira JM, da Silva AP, Helena FM (2009) Plant extracts with anti-inflammatory properties- a new approach for characterization of their bioactive compounds and establishment of structure-antioxidant activity relationships. *Bioorg Med Chem* 17(5):1876–1883
- Anokbonggo WW, Odoi-Adome R, Oluju PM (1990) Traditional methods of diarrhoeal management in Uganda. *Bull World Health Organ* 68:359–363
- Barbosa DBM, Nascimento MVM, Lino RC, Magalhães MR, Florentino IF, Honório TCD, Galdino PM, Bara MTF, de Paula JR, Costa EA (2012) Mechanism involved in the anti-inflammatory effect of *Spiranthera odoratissima* (Manacá). *Braz J Pharmacogn* 22(1): 137–143
- Bentley GA, Newton SH, Starr J (1983) Studies on the antinociceptive action of α -agonist drugs and their interactions with opioid mechanisms. *Br J Pharmacol* 79(1):125–134
- Born GVR, Cross MJ (1963) Inhibition of aggregation of blood platelets by substances related to adenosine diphosphate. *J Physiol* 166:29–30
- Chah JM, Igboke EM (2011) Plants used for small ruminant nutrition in the Eastern Guinea Savanna region of Nigeria. *Livest Res Rural Dev* 23(8):1–11
- Das S, Haldar PK, Pramanik G, Suresh RB (2010) Evaluation of anti-inflammatory activity of *Clerodendron infortunatum* Linn extract in rats. *Glob J Pharmacol* 4(1):48–50
- David JM (2008) *Mabberley's plant-book*, 3rd edn. Cambridge University Press, Cambridge
- Ekwueme FN, Oje OA, Nwodo OFC, Ozoemena NF (2011) Anti-inflammatory capacity of the aqueous leaf extract of *Senna mimosoides* on inhibition of rat oedema, platelet aggregatory activity and prostaglandin synthase activity. *J Med Plant Res* 5(14):3028–3036
- Ezekwesili CN, Obidoa O, Nwodo OFC, Ezekwesili-Ofili JO (2011) Toxicity of *Acalypha torta* (Muell) leaves ethanolic extract in mice and rat. *Anim Res Int* 8(1):1315–1322
- Fabre J, Gurney ME (2010) Limitations of current therapies to prevent thrombosis: a need for novel strategies. *Mol Biosyst* 6:305–315
- Fan S, Ali C, Basri DF (2014) Evaluation of analgesic activity of the methanol extract from the galls of *Quercus infectoria* (Olivier) in rats. *Evid Based Complement Alternat Med* 7:742–758
- Garcia A, Shankar H, Murugappan S, Kim S, Kunapuli SP (2007) Regulation and functional consequences of ADP receptor-mediated ERK2 activation in platelets. *Biochem J* 404:299–308
- Garg VKR, Jain M, Sharma PKR, Garg G (2010) Anti-inflammatory activity of *Spinacia oleracea*. *Int J Pharm Pharm Res* 1(1):1–4
- George A, Chinnappan S, Chintamaneni M, Kotak CV, Choudhary Y, Kueper T, Radhakrishnan AK (2014) Anti-inflammatory effects of *Polygonum minus* (Huds) extract (Lineminus™) in *in-vitro* enzyme assays and carrageenan induced paw oedema. *BMC Complement Altern Med* 14:1–7
- Gepdireman A, Mshvildadze V, Suleyman H, Elias R (2005) Acute anti-inflammatory activity of four saponins isolated from ivy: alpha-hederin, hederasaponin-C, hederacolchiside-E and hederacolchiside-F in carrageenan-induced rat paw oedema. *Phytomedicine* 12(6-7):440–444
- Glass CK, Saijo K, Winner B, Marchetto MC, Gage FH (2010) Mechanisms underlying inflammation in neurodegeneration. *Cell* 140:918–934
- Golino P, Loffredo F, Riegler L (2005) Novel antithrombotic strategies in cardiovascular diseases. *Curr Opin Investig Drugs* 6:298–306
- Harborne JB (1998) *Phytochemical methods: a guide to modern techniques of plant analysis*, 2nd edn. Chapman and Hall Limited, London
- Hommam MM, Taniguchi O, Okak MN, Tsuma T (2000) Inhibitory effects of lignans and flavonoids in saibokuto, a herbal medicine for bronchial asthma, on the release of leukotrienes from human polymorphonuclear leukocytes. *Planta Med* 66:88–91
- Hossain MM, Ahamed SK, Dewan SMR, Hassan MM, Istiaq A, Islam MS, Moghal MMR (2014) *In vivo* antipyretic, antiemetic, *in vitro* membrane stabilization, antimicrobial and cytotoxic activities of different extracts from *Spilanthes paniculata* leaves. *Biol Res* 47: 1–5
- Imram I, Liagat H, Sagheer A, Nasir R, Shahid R, Ghulam A, Muhammad Y (2012) Antiplatelet activity of methanolic extract of *Acacia leucophloea* bark. *J Med Plant Res* 6(25):4185–4188
- Katoh N (2009) Platelets as versatile regulators of cutaneous inflammation. *J Dermatol Sci* 53:89–95
- Kee NL, Mnonopi N, Davids H, Naudé RJ, Frost CL (2008) Antithrombotic, anticoagulant and anticancer activities of selected medicinal plants of South Africa. *Afr J Biotechnol* 7:217–223
- Khan H, Saeed M, Gilani AH, Khan MA, Dar A (2010) The antinociceptive activity of *Polygonatum verticillatum* rhizomes in pain models. *J Ethnopharmacol* 127:521–527
- Kim HP, Park H, Son KH, Chang HW, Kang SS (2008) Biochemical pharmacology of biflavonoids: implications for anti-inflammatory action. *Arch Pharm Res* 31(3):265–273
- Koster R, Anderson M, de Beer ER (1959) Acetic acid for analgesic screening. *Fed Proc* 18:412–416
- Kumar S, Bajwa BS, Kuldeep S, Kalia AN (2013) Anti-inflammatory activity of herbal plants: a review. *IJAPBC* 2(2):272–281
- Libby P, Ridker PM, Maseri A (2002) Inflammation and atherosclerosis. *Circulation* 105:1135–1143
- Lopez-Lazaro M (2009) Distribution and biological activities of the flavonoid luteolin. *Mini-Rev Med Chem* 9:31–59
- Lorke D (1983) A new approach to practical acute toxicity testing. *Arch Toxicol* 54:275–287
- Malaya G, Upal KM, Ramanathan SK, Thangavel SK (2003) Studies on anti-inflammatory, analgesic and antipyretic properties of methanolic extract of *Caesalpinia bonducella* leaves in experiment animal models. *Iran J Pharmacol Ther* 2:30–34
- Mekhfii M, ElHaouari M, Bnouham M, Aziz M, Ziyat A, Legssyer A (2006) Effects of extracts and tannins from *Arbutus unedo* leaves on rat platelet aggregation. *Phytother Res* 20:135–139
- Messina S, Bitto A, Aguenouz M, Mazzeo A, Miqllorato A, Polito F, Irrera N, Altavilla D, Vita GL, Russo M, Naro A, De Pasquale MG, Rizzuto E, Musaro A, Squadrito F, Vita G (2009) Flavocoxid

- counteracts muscle necrosis and improves properties in mdx mice: a comparison study with methylprednisolone. *Neurology* 220(2): 349–358
- Mohammedi Z, Atik F (2014) Haemolytic activity of different herbal extracts used in Algeria. *IJPSR* 5(8):495–500
- Nahar L, Zahan R, Morshed MTI, Haque A, Alam Z, Mosaddik A (2012) Antioxidant, analgesic and CNS depressant effects of *Synedrella nodiflora*. *Pharm J* 4(31):29–36
- Nelson DL, Cox MM (2008) Principles of biochemistry, 5th edn. W. H Freeman and Company, New York
- Nwodo OFC (1981). Elucidation of the nature of some pharmacologically active substances extractible from the seeds of *Abrus precatorius*. A Ph.D thesis submitted to the Department of Biochemistry, University of London
- Odu NM, Tchimine KM, Ogugua VN, Obonga W, Sunday PGA, Nwanguma BC (2018) Anti-inflammatory property of the methanol leaf extract of *Parinari kerstingii* (ENGL) in rats. *J Pharmacogn Phytother* 10(9):158–166
- Omkar A, Jeeja T, Chhaya G (2007) Evaluation of anti-inflammatory activity of *Nyctanthes arbour-tristis* and *Onosma echioides*. *Pharmacogn Mag* 2(8):258–260
- Onasanwo SA, Fabyi TD, Oluwole FS, Olaleye SB (2012) Analgesic and anti-inflammatory properties of the leaf extracts of *Anacardium occidentale* in the laboratory rodents. *Niger J Physiol Sci* 27:65–71
- Paez-Espinosa E, Murad JP, Khasawneh FT (2012) Aspirin: pharmacology and clinical applications. *Thrombosis* 2012:1–15
- Razafimandimbison SG, Bremer B (2006) Taxonomic revision of the tribe Hymenodictyeae (Rubiaceae, Cinchonoideae). *Bot J Linn Soc* 152:331–386
- Rigler DJ (1997) Veterinary pathology. Williams and Wilkins Publishers, Philadelphia, pp 113–157
- Robak J, Gryglewski RJ (1996) Bioactivity of flavonoids. *Pol J Pharmacol* 48(6):555–564
- Sakat S, Juvekar AR, Gambhire MN (2010) *In vitro* antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. *IJPPS* 2(1):146–155
- Salmon SD (2006) Cyclooxygenase-2 inhibitors and cardiovascular risk. *Curr Opin Cardiol* 21(6):613–617
- Sangkuhl K, Shuldiner AR, Klein TE, Altman RB (2011) Platelet aggregation pathway. *Pharmacogenet Genomics* 21(8):516–521
- Sato H, Taketomi Y, Isogai Y, Masuda S, Kobayashi T, Yamamoto K, Murakami M (2009) Group III secreted phospholipase A₂ transgenic mice spontaneously develop inflammation. *Biochem J* 421:17–27
- Schimmer BP, Parker KL (2001) Adrenocorticotrophic hormone; adrenocortical steroids and their synthetic analogs; inhibitors of the synthesis and actions of adrenocortical hormones. In: Gilman AG, Hardman JG, Limbird LE (eds) Goodman and Gilman's the pharmacological basis of therapeutics, 10th edn. McGraw Hill Company, New York, pp 1649–1677
- Shailasree S, Ruma K, Kini KR, Niranjana SR, Prakash HS (2012) Potential anti-inflammatory bioactives from medicinal plants of Western Ghats India. *Phcog Commn* 2:2–12
- Sikder MAA, Millat MS, Sultana A, Kaiser MA, Rashid MA (2012) *In vitro* membrane stabilizing activity, total phenolic content, cytotoxic, thrombolytic and antimicrobial activities of *Calliandra surinamensis* (wall.). *J Pharmacogn Phytochem* 1:40–44
- Silva JC, Araújo C, de Lima-Saraiva SRG, de Oliveira-Junior RG, Diniz TC, Wanderley C, Palheta-Junior RC, Mendes RL, Guimarães AG, Quintans-Junior LJ, Almeida JR (2015) Antinociceptive and anti-inflammatory activities of the ethanolic extract of *Annona vepretorum* Mart. (Annonaceae) in rodents. *BMC Complement Altern Med* 15(197):1–10
- Sobotková A, Mášová-Chrastinová L, Suttar J, Štikarová J, Májek P, Reicheltová Z, Kotlín R, Weisel JW, Malý M, Jan E, Dyr JE (2009) Antioxidant change platelet responses to various stimulating events. *Free Radic Biol Med* 47:1707–1714
- Souza SM, Aquino LC, Milach ACJ, Bandeira MA, Nobre ME, Viana GS (2007) Anti-inflammatory and anti-ulcer properties of tannins from *Myracrodruon urundeuva* Allemão (Anacardiaceae) in rodents. *Phytother Res* 21(3):220–225
- Suba V, Murugesan T, Kumaravelrajan R, Mandal SC, Saha BP (2005) Antiinflammatory, analgesic and antiperoxidative efficacy of *Barleria lupinina* Lindl extract. *Phytother Res* 19:695–699
- Tordera M, Ferrandiz ML, Alcaraz MJ (1994) Influence of anti-inflammatory flavonoids on degranulation and arachidonic acid release in rat neutrophils. *Z Naturforsch J Biosci* 49(3–4):235–240
- Trease GE, Evans W (2002) Pharmacognosy, 15th edn. Saunders Publishers, London
- Vane JR (1971) Inhibition of prostaglandin synthesis as a mechanism of action for aspirin like drugs. *Nat New Biol* 231:232–235
- Wang Z, Ramanadham S, Ma ZA, Bao S, Mancuso DJ, Gross RW (2005) Group VIA phospholipase A₂ forms a signaling complex with the calcium/calmodulin-dependent protein kinase II beta expressed in pancreatic islet beta-cells. *J Biol Chem* 280:6840–6849
- Wang S, Voisin MB, Larbi KY, Dangerfield J, Scheiermann C, Tran M, Maxwell PH, Sorokin L, Nourshargh S (2006) Venular basement membranes contain specific matrix protein low expression regions that act as exit points for emigrating neutrophils. *J Exp Med* 203: 1519–1532
- Winter CA, Risley EA, Nuss GW (1962) Carrageenin-induced oedema in hind paw of the rats as an assay for anti-inflammatory drugs. *PSEBM* 111:544–547
- Zarbock A, Singbartl K, Ley K (2006) Complete reversal of acid-induced acute lung injury by blocking of platelet-neutrophil aggregation. *J Clin Invest* 116:3211–3219

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