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Original Research Article

Anti-Inflammatory Investigations of the Ethanol Extract of *Phaulopsis falcisepala* C.B. Clarke (Acanthaceae) Whole Plant in Rodents

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ABSTRACT

Phaulopsis falcisepala C.B. Clarke (Acanthaceae) is used for treatment of fever and rheumatic pains. The study evaluated the anti-inflammatory activity of the ethanol extract of *P. falcisepala* using carrageenan, serotonin, histamine, formalin-induced oedema, cotton pellet-induced granuloma models in rats and xylene-induced oedema in mice at doses of 50, 100, 150, and 200 mg/kg orally. Acute oral toxicity and quantitative phytochemical study were also carried out. No toxic effect was observed at 5 g/kg after oral administration. The extract produced a significant ($p < 0.001$, $p < 0.0001$) dose-dependent inhibition of oedema with the maximum inhibition observed at 60 minutes (68.20%) at 150 mg/kg in the carrageenan model and at 150 mg/kg (59.74%) in xylene model. It produced significant ($p < 0.0001$) inhibition of oedema with peak effect of 64.89% inhibition at 60 minutes and 73.97% at 90 minutes in the serotonin and histamine oedema models, respectively. It markedly decreased the paw oedema induced by formalin at 100 mg/kg on days 5, 6 and 7 with percentage inhibition of oedema of 87.04%, 86.35% and 99.05%, respectively, and gave a peak inhibition of granuloma tissue development (19.94%) at 200 mg/kg in the cotton pellet granuloma model. Total phenolic, proanthocyanidin and flavonoid contents of the extract were 42.74±0.037 mg/g (gallic acid equivalent), 41.94±0.042 mg/g (catechin equivalent) and 44.62±0.010 mg/g (quercetin equivalent), respectively. The results justify the use of the plant in the preparation of ethno-medicines used in the treatment of inflammation and provide evidence for further research on its mechanism of action and use.

Keywords: *Phaulopsis falcisepala*, Carrageenan, Serotonin, Histamine, Xylene, Formalin.

Introduction

Medicinal plants are commonly used in healing and preventing ailments and diseases. They are generally considered to play beneficial roles in health care and have always been a good source of drugs. The use of medicinal plants in traditional medicine of many cultures is well documented.[1,2]

The beneficial values of medicinal plants lie in the phytochemicals present in them. Phytochemicals are biologically active, naturally occurring chemical compounds found in medicinal plants. They promote human health, prevent diseases and protect plant cells from environmental hazards.[3,4]

The World Health Organisation (WHO) recognises the increasing dependence on medicinal plants in primary health care in developing nations and is playing a major role in the recognition of traditional medicine in order to support its integration into national health systems depending on the circumstances of its use in countries and provide technical guidance and information for the safe and effective use of such medicines.[5]

Phaulopsis falcisepala C.B Clarke (Acanthaceae) is a herb with dense ovoid or short cylindrical spikes of small red or white flowers with purple marking. It is found throughout the forest zone from Senegal to South Nigeria.[6] Traditionally, the powdered plant material is applied to staunch wounds in Southern Nigeria. The plant decoction is taken as a laxative. In Ivory Coast, the plant is used to treat sores, the sap is given as a draught to stop nausea, stomach ache and it is put into bath water to treat fever, arthritis and rheumatic pains.[6] Anti-oxidant properties, alpha-amylase and alpha-glucosidase inhibitory activities of *P. falcisepala* leaves and flower extracts have been reported.[7,8]

The aim of this study is to investigate the anti-inflammatory properties of the ethanol extract of the whole plant of *P. falcisepala* in order to validate its folkloric use.

Materials and Methods

Collection and authentication of plant material

The whole plant of *P. falcisepala* was collected at Ibadan, Oyo state, Nigeria in June 2017. The plant was identified and authenticated by Mr Odewo of the Forestry Research Institute of Nigeria (FRIN). A voucher specimen (FHI 110446) was deposited in the Department of Pharmacognosy Herbarium, University of Lagos, Nigeria.

Preparation and extraction of plant material

The collected whole plant was carefully cleaned to remove dust and sand particles and cut into pieces. The plant material was air-dried, further dried in a hot air oven at 45°C and then ground to powder using a mechanical grinder and thereafter stored in a tightly covered amber coloured glass jar. The powdered material (800 g) was macerated with 96% ethanol for 72 hours at room temperature, with intermittent swirling. The resultant solution was filtered and concentrated in vacuo at 40°C using a rotary evaporator (Buchi, Switzerland) to obtain the ethanol extract which was greenish in

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colour. The yield was 10% (w/w). The extract was stored in amber coloured bottles at 4°C.

Experimental Animals

Swiss albino mice (17-30 g) and Wistar rats (110-200 g) of both sexes, used in the study were obtained from the National Agency for Food and Drugs Administration and Control (NAFDAC), Yaba, Lagos and kept in the Animal House of the College of Medicine, University of Lagos, Nigeria. The animals were housed in perforated plastic cages with wire top feeders (6 animals per cage) in a well-ventilated room at a controlled temperature and light/dark cycle (25±2°C, 12 h light/dark cycle), fed with standard rodent pellets (Livestock Feed Plc., Lagos, Nigeria) and water *ad libitum*. The animals were acclimatized to laboratory conditions for 7 days before the start of experiment. They were fasted for 12 hours prior to treatment. All animals used in the study were treated in accordance with the guideline on the use of animals as approved by Experimentation Ethics Committee of the College of Medicine, University of Lagos (CM/HREC/010/16/067).

Acute toxicity test in mice

The study was evaluated according to the Organisation for Economic Co-operation and Development (OECD) guideline 420 using a total of six (6) mice each which were treated with a single oral dose of the extract (5 g/kg), while the control group was given distilled water.[9] Behavioural parameters and mortality were monitored closely at 1, 4 and 24 hours after treatment for signs of toxicity. They were further observed daily for 7 days for signs of delayed toxicity and mortality.

Anti-inflammatory studies of ethanol extract of P. falcisepala

Carrageenan-induced rat paw oedema

The method of Winter *et al*. was used for this study.[10] Thirty-six (36) rats were randomly assigned to six treatment groups of six rats each (n = 6). Each group (I, II, III, IV, V and VI) was administered the plant ethanol extract (50, 100, 150, 200 mg/kg p.o), positive control (Indomethacin 10 mg/kg), and negative control (distilled water 10 mL/kg p.o.). One hour post treatment, oedema was induced by injection of carrageenan (0.1 mL, 1% w/v in saline) into the sub-plantar tissue of the right hind paw. The paw volume was measured immediately before injection of the phlogistic agent and then, at 30 minutes interval for 3 hours using the plethysmometer. Inflammation was expressed as an increase in paw volume due to carrageenan injection.

Relative anti-inflammatory activity was analysed based on reduction in paw volume and calculating percentage inhibition of oedema.

The percentage inhibition was calculated using the formula:

$$\text{Percentage inhibition} = \frac{(Ct - Co)\text{distilled water} - (Ct - Co)\text{treated}}{(Ct - Co)\text{distilled water}} \times 100$$

Where;

C_t = Paw volume at time t

C_o = Paw volume before administration of treatment and carrageenan

C_t-C_o = Paw oedema

Xylene-induced ear oedema

The xylene-induced ear oedema test was performed as previously described by Tang *et al*.[11] The study was carried out with thirty-six (36) mice randomly assigned into six groups of six mice each (n=6). Each group (I, II, III, IV, V and VI) was treated with single oral administration of ethanol extract of *P. falcisepala* (50, 100, 150, 200 mg/kg), normal saline (10 mL/kg), or dexamethasone (1 mg/kg) as positive control. Thirty (30) minutes after, oedema was induced by the application of a drop of xylene with the aid of a syringe to the inner surface of the right ear. The left ear of each animal was used as control. After 15 minutes, the mice were euthanized under ether anaesthesia and both ears were cut off and weighed. The mean of the difference between the right and left ears was determined for each group. The percentage of inflammation inhibited was calculated using the formula;

$$\text{Percentage inhibition} = \frac{(REwt - LEwt)\text{saline} - (REwt - LEwt)\text{treated}}{(REwt - LEwt)\text{saline}} \times 100$$

Where;

RE_{wt}= Right ear weight

LE_{wt}= Left ear weight

Serotonin and Histamine-induced rat paw oedema

Selected inflammatory mediators (histamine and serotonin) were used to determine the mediator of inflammation being inhibited by the plant extract. The dose with the maximum percentage inhibition in the carrageenan model was used for the screening. The assays were carried out using the method of Amann *et al*.[12]

Serotonin-induced rat paw oedema

Three groups of six rats randomly selected were treated with single oral administration of ethanol extract of *P. falcisepala* (150 mg/kg, p.o), ondasentron (10 mg/kg, p.o) or distilled water (10 mL/kg, p.o). One hour afterwards, the rats were injected with 0.1 mL serotonin (10^{-3} mg/mL) into the sub-plantar tissue of the right hind paw. Paw measurements were made before injection of serotonin and at 30 minutes interval for 3 hours after injection of the phlogistic agent using a plethysmometer. The mean of the paw volume was computed and percentage inhibition calculated.[12]

Histamine-induced rat paw oedema

Eighteen rats were randomly assigned into three groups of six rats each (n= 6). Each group was treated with single oral administration of ethanol extract of *P. falcisepala* (150 mg/kg, p.o), chlorpheniramine (10 mg/kg, p.o), distilled water (10 mL/kg, p.o). One hour afterwards, the rats were injected with 0.1 mL histamine (10^{-3} mg/mL) into the sub-plantar tissue of the right hind paw. Paw measurements were made before injection of histamine and at 30 minutes interval for 3 hours after injection of the phlogistic agent. The mean of the paw volume was computed and percentage inhibition calculated.[12]

Formalin-induced rat paw oedema

In this study, the method of Seyle *et al*. was used.[13] Thirty-six rats were randomly divided into six groups of six animals each and separately administered with distilled water (10 mL/kg p.o.), *P. falcisepala* extract (50, 100, 150 and 200 mg/kg, p.o.) and indomethacin (10 mg/kg), respectively.

Inflammation was induced in all the animals by sub-plantar injection of 0.1 mL of freshly prepared (2% $^{v}/_{v}$) formalin in the right hind paw. Paw thickness was measured 1 hr prior to and after formalin injection. The drug treatments were continued for 7 consecutive days and paw oedema was measured 1 hr after drug treatment each day.[35] The percentage inhibition of oedema was calculated using the formula:

$$\text{Percentage inhibition} = \frac{(Ct - Co)\text{distilled water} - (Ct - Co)\text{treated}}{(Ct - Co)\text{distilled water}} \times 100$$

Where;

C_t = Mean paw volume at time t

C_o = Mean paw volume before administration of treatment

C_t-C_o= Mean paw oedema

Cotton pellet-induced granuloma model

The method of Winter and Porter was used for this assay.[14] A total of thirty-six rats randomly assigned into six groups of six rats each (n= 6) were used for this study. Each group was treated with single oral administration of ethanol extract of *P. falcisepala* (50, 100, 150, 200 mg/kg), distilled water (10 mL/kg) and indomethacin (10 mg/kg) as reference standard, respectively. Thirty minutes after drug administration, an autoclaved cotton pellet of 20 ± 0.5 mg was aseptically implanted subcutaneously in the back region of the rats while anesthetized with urethane. The extract was administered once daily for 7 days. On Day 8, the animals were anesthetized again and cotton pellets were removed surgically, freed from the extraneous tissue, and dried in a hot-air oven at 60°C until the weight became constant. The dried pellets were weighed and the increment in the dry weight of the pellets was taken as a measure of granuloma formation.

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The Percentage inhibition of granuloma tissue development was calculated using the formula:

$$\text{Percentage inhibition} = \frac{\text{Weight of pellet (control)} - \text{Weight of pellet (treated)}}{\text{Weight of pellet (control)}} \times 100$$

Quantitative phytochemical analysis

Determination of Total Phenolic Content

The total phenolic content of the extract was determined by modified Folin-Ciocalteu method.[15] Gallic acid was used as a standard at concentrations of 0.01, 0.02, 0.03, 0.04 and 0.05 mg/mL prepared in methanol. A volume of 0.5 mL of the extract (1 mg/mL) and gallic acid was mixed with 1.25 mL Folin Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 mL (75 g/L) of sodium carbonate. The mixtures were vortexed for 15 seconds and allowed to incubate for 30 minutes at 40^{o}C. Absorbance was measured at 760 nm using a UV-VIS spectrophotometer. All determinations were performed in triplicates. Total phenolic content was expressed as mg gallic acid equivalent (GAE)/g dry extract using the following equation based on the calibration curve: y = 22.893x - 0.024, R^2 = 0.9944, where y is the absorbance and x is the gallic acid concentration (mg/ml).

Determination of Total Proanthocyanidin Content

Proanthocyanidin content was determined by the method of Broadhurst and Jones.[16] A volume of 0.5 mL of 1 mg/mL extract solution was mixed with 3 mL of 4% vanillin-methanol solution and 1.5 mL hydrochloric acid; the mixture was incubated for 15 min. The absorbance was measured at 500 nm. Total proanthocyanidin content was expressed as catechin equivalent (CE mg/g) using the following equation based on the calibration curve: y = 1.7367x + 0.0065, R^2 = 0.9869, where y is the absorbance and x is the catechin concentration (mg/ml).

Determination of Total Flavonoid Content

The assay was carried out according to the method of Woisky and Salatino.[17] Aluminium Chloride ($AlCl_3$) 2% in ethanol (1.5 mL) was added to 1.5 mL of the extract. Concentrations of 1 mg/mL of the extract in methanol was used, while quercetin concentrations of 0.01, 0.02, 0.03, 0.04 and 0.05 mg/ mL prepared in methanol were used to obtain the calibration curve. The absorbance was measured at 420 nm after 60 min incubation at room temperature. The estimation of total flavonoids content in the crude extract was carried out in triplicates and the average calculated. Total flavonoid content was calculated as quercetin equivalent (QE mg/g) using the following equation based on the calibration curve: y = 14.683x + 0.1482, R^2 = 0.9858, where y is the absorbance and x is the quercetin concentration (mg/mL).

Statistical analysis

The results were expressed as Mean ± Standard Error of Mean (SEM) and Percentages (%). Data were analysed using two-way analysis of variance (ANOVA) followed by Dunnett's test, with the aid of GraphPad Prism 6. Results were considered statistically significant at $p < 0.05$.

Results and Discussion

Inflammation is a pathophysiological response of living tissues to injuries, burns, microbial infections, allergens and other noxious stimulus[18,19] and it is a process in which leucocytes (white blood cells), erythrocytes and components of plasma are released into the affected tissue(s) to protect the body from infection and initiates injury repair mechanism.[20] It involves a complex array of enzyme activation, mediator release, extravasation, cell migration, tissue breakdown and repair.[21] Due to its implication in virtually all human and animal diseases, inflammation has become the focus of global scientific research, more so, since the currently used anti-inflammatory agents are prone to evoking serious adverse reactions such as gastric lesion, ulceration and renal damage.[22,23] Hence, the need for the development of newer anti-inflammatory agents from natural sources which are active and have lesser side effects to substitute for synthetic therapeutic agents.

In this study, the anti-inflammatory activity of the ethanol extract of whole plant of *P. falcisepala* was investigated after sub-plantar injection of carrageenan, serotonin, histamine and formalin in rat paws. The xylene-induced ear oedema and cotton pellet-induced granuloma models of inflammation were also used. The acute toxicity of the extract and quantitative phytochemical analysis were also evaluated.

In the acute toxicity test, no death was recorded within 24 hours of oral administration of the extract and even after seven days of administration, there were no visible signs of delayed toxicity. Hence, the results indicate that extract of *P. falcisepala* orally administered could be considered as non-toxic at the dose tested.[24]

In the carrageenan-induced rat paw oedema, the extract of *P. falcisepala* (50, 100 and 150 mg/kg) produced a significant ($p < 0.001$, $p < 0.0001$) inhibition of oedema relative to the control (distilled water) in a dose-dependent manner. A peak effect of 68.20% inhibition with 150 mg/kg dose of the extract at 60 minutes was observed (Table 1). The extract compared favourably with indomethacin (10 mg/kg), which produced its peak inhibition of oedema (59.92%) at 120 minutes.

Carrageenan-induced hind paw oedema is a standard experimental model of acute inflammation. It is a suitable *in vivo* model to study anti-inflammatory effects of natural products since it involves several mediators.[25] It is also known to be sensitive to cyclo-oxygenase inhibitors and has been used to study the effect of non-steroidal anti-inflammatory drugs.[26] More so, the experimental model exhibits a high degree of reproducibility. The local injection of carrageenan induces inflammatory reaction in three different phases.[10] The first phase begins immediately after injection of carrageenan and diminishes in 1.5 hours and is mediated through the release of cytoplasmic enzymes, histamine, and serotonin from the mast cells. The second phase (1.5-2.5 hours) is maintained by kinin-like substances and the last phase (2.5-6 hours) is related to the release of prostaglandin, protease, lysosome and slow reacting substances.[27]

The results from this study suggest that the extract is a possible inhibitor of serotonin and histamine since it showed peak inhibition at 60 minutes post induction of carrageenan.

The extract significantly ($p < 0.0001$) reduced the ear oedema induced by xylene and showed peak inhibition at 150 mg/kg (59.74%). The effect produced was dose-dependent up to 150 mg/kg and compared effectively with the standard drug dexamethasone (1 mg/kg) which produced its peak inhibition of oedema as 51.72% (Table 2).

The xylene model has certain advantages in the evaluation of anti-inflammatory steroids.[28] The application of xylene induces neurogenous oedema through the release of substance P from sensory neurons which is known to cause severe vasodilatation, plasma extravasations and oedematous changes of skin which leads to an acute inflammatory response.[29] The increased thickness of ear tissues is caused by these histopathological changes. It is characterized by fluid accumulation and oedema. Suppression of this response is taken as an indication of antiphlogistic effect.[30] The observed results in this study suggest that the extract acts by inhibiting the release of substance P, thereby preventing vasodilatation and plasma extravasations of neurogenic inflammation, which are crucial in controlling the early stage of acute inflammation.[31]

To ascertain the effect of the extract on the mediators of inflammation, the extract was tested on two mediators (serotonin and histamine), using the most active dose (150 mg/kg).

P. falcisepala extract produced a significant ($p < 0.0001$) inhibition of oedema with peak effect of 64.89% inhibition at 60 minutes (Table 3) and 73.97% at 90 minutes (Table 4) in the serotonin and histamine models, respectively.

These results show that the plant extract was able to effectively inhibit the oedema induced by serotonin and histamine. Hence it can be suggested that the anti-inflammatory activity of the extract might be associated with the inhibition of the synthesis and/or action of serotonin and histamine. However, the difference in time of peak inhibition observed in the carrageenan and histamine models i.e. 60 and 90 minutes, respectively maybe due to interference of other

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mediators such as bradykinin and prostaglandins which are present when carrageenan is used in oedema induction.[28]

In the formalin-induced oedema model, *P. falcisepala* produced a significant ($p < 0.05$, $p < 0.01$, $p < 0.001$, $p < 0.0001$) inhibition of oedema relative to the control (distilled water). The extract markedly decreased the paw oedema compared to indomethacin on days 5, 6 and 7. A peak inhibitory effect of 99.05% was observed with 100 mg/kg of the extract and 85.67% with the standard drug, indomethacin on day 7 (Table 5).

Formalin induced paw oedema model is a simple model used to investigate sub-acute inflammation. It is a suitable model for screening of agents for anti-arthritic, anti-inflammatory and anti-proliferative activity,[32] since it involves infiltration of neutrophils, macrophages and proliferation of fibroblasts. The nociceptive effect of formalin is known to be biphasic, consisting of an early neurogenic component (phase 1, 0-7 minutes) followed by a later tissue-mediated response (phase 2, 15-90 minutes).[33] In the first phase, there is a release of histamine, serotonin and kinin, while the second phase is related to the release of prostaglandins.[29,33] The result from this study indicates that the extract showed significant inhibitory effect on rat paw oedema development in the latter phase (days 5, 6 and 7). This suggests that *P. falcisepala* has inhibitory effect on the release of prostaglandins and can be useful in the management of sub-acute inflammation.

The extract decreased the dry weight of granuloma significantly ($p < 0.05$, $p < 0.001$, $p < 0.0001$) when compared to the control group.

However, the peak effect produced was mild (19.94%) at 200 mg/kg. The standard drug, indomethacin (10 mg/kg) produced a peak inhibition of granuloma tissue development of 53.68% (Table 6).

Chronic inflammation occurs by means of the development of proliferate cells and are marked chiefly by new connective tissue formation which can be in granuloma form. Granulomas form in response to immune mediation when macrophages and lymphocytes

accumulate around inert foreign particles that have not been eliminated, together with epitheloid and giant cells derived from macrophages to form a ball of cell.[34] In chronic inflammatory states, the efficacy of anti-inflammatory agents can be indicated by the inhibition of fibroblasts and infiltration of neutrophils and exudation.[36]

The cotton pellet-induced granuloma method is a known model for screening anti-inflammatory activity in the chronic phase of inflammation,[37] which is characterized by monocyte infiltration, fibroblast proliferation, angiogenesis, and exudation.[38] The model is widely used to evaluate the transudative and proliferative components of chronic inflammation and can serve as a sub-chronic and chronic inflammatory test model for the study of anti-arthritic substances. The moist weight of the pellets correlates with transude and the dry weight of the pellet correlates with the amount of granulomatous tissue formed.[39] The poor inhibition of cotton pellet-induced granuloma by *P. falcisepala* suggests that the plant may not be effective in inhibiting granulocyte infiltration, synthesis of collagen and mucopolysaccharide, preventing angiogenesis and increasing the number of fibroblast during granuloma tissue formation and thus, may not be used in the treatment of chronic inflammatory conditions.[40]

Quantitative analysis of the plant extract gave total phenolic, proanthocyanidin and flavonoid contents of 42.74 ± 0.037 mg GAE/g dry weight, 41.94 ± 0.042 mg CE/g dry weight and 44.62 ± 0.010 mg QE/g dry weight, respectively.

Phytochemical studies of medicinal plants are important in other to ensure reproducible quality of herbal medicines which contributes to their efficacy and safety.[41] Previous studies have shown that anti-inflammatory and analgesic effects can be as a result of the high polyphenol content of plants especially phenolics and flavonoids.[42] Flavonoids have been shown to inhibit the cyclooxygenase enzyme and act by regulating inflammatory mediators.[41] They prevent the synthesis of prostaglandins by inhibiting the enzyme prostaglandin synthase, specifically the endoperoxide.[43] Anthocyanins have been reported to lower prostaglandin levels by inhibiting COX-2, thus acting as anti-inflammatory agents in inflamed connective tissue and joints.[44] The extraction and quantification of these phenolic compounds in medicinal plants are therefore necessary to allow for assessment and eventual value added utilization. The polyphenolic constituents present in the extract may be responsible for the observed anti-inflammatory activity in this study.

Table 1: Effect of ethanol extract *P. falcisepala* on Carrageenan induced rat paw oedema

		Increase in paw volume						
Treatment	**Dose (mg/kg)**	**T_0**	**T_{30}**	**T_{60}**	**T_{90}**	**T_{120}**	**T_{150}**	**T_{180}**
P. falcisepala	50	2.85 ± 0.03	4.07 ± 0.09**** (44.79)	4.11 ± 0.13*** (41.94)	4.53 ± 0.14** (39.57)	4.39 ± 0.13** (41.22)	4.79 ± 0.06** (42.60)	4.80 ± 0.22* (38.09)
P. falcisepala	100	3.26 ± 0.17	4.25 ± 0.11*** (55.20)	3.95 ± 0.08*** (53.24)	4.66 ± 0.23* (49.64)	4.72 ± 0.15* (44.27)	5.25 ± 0.17 (41.12)	5.56 ± 0.14 (26.98)
P. falcisepala	150	3.35 ± 0.13	4.13 ± 0.19**** (64.71)	4.04 ± 0.27*** (68.20)	4.31 ± 0.26*** (64.75)	4.63 ± 0.21* (51.15)	4.99 ± 0.29* (51.48)	5.19 ± 0.30 (41.59)
P. falcisepala	200	2.81 ± 0.11	4.20 ± 0.34*** (36.65)	4.19 ± 0.43** (35.94)	4.41 ± 0.45** (42.08)	4.92 ± 0.25 (20.22)	5.43 ± 0.34 (22.19)	5.52 ± 0.18 (13.65)
Indomethacin	10	3.05 ± 0.18	4.00 ± 0.24**** (57.46)	4.02 ± 0.24*** (55.76)	4.36 ± 0.31** (53.24)	4.11 ± 0.25*** (59.92)	5.28 ± 0.60 (34.32)	5.11 ± 0.61 (34.92)
Control	10 mL/kg	3.08 ± 0.07	5.29 ± 0.12	5.25 ± 0.14	5.86 ± 0.34	5.70 ± 0.45	6.46 ± 0.39	6.23 ± 0.46

Values are expressed as mean ± SEM. (n=6). Figure in parenthesis indicate percentage inhibition of oedema development
****$p < 0.0001$, ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ vs. Control (Two way ANOVA, Bonferroni's Multiple Comparison)

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Table 2: Effect of *P. falcisepala* on xylene induced ear oedema in mice

Treatment	Dose (mg/kg)	Increase in ear weight (mg)	% Inhibition
P. falcisepala	50	2.90 ± 0.30	25.64
P. falcisepala	100	1.87 ± 0.22***	52.05
P. falcisepala	150	1.57 ± 0.31****	59.74
P. falcisepala	200	2.13 ± 0.24***	45.38
Dexamethasone	1	1.90 ± 0.23***	51.72
Distilled water	10 (ml/kg)	3.90 ± 0.37	-

Values are expressed as Mean ± SEM. (n = 6). ****p < 0.0001, ***p < 0.001 compared to the Control (One way ANOVA, Dunnet's Multiple Comparison)

Conclusion

This study shows the efficacy of *P. falcisepala* as an efficient anti-inflammatory agent in acute and sub-acute inflammatory conditions. It thus justifies the use of *P. falcisepala* in preparations used in the treatment of ailments associated with inflammation. Isolation of active principles responsible for the observed anti-inflammatory activity of the plant and investigation of its mechanism of action are on-going.

Conflict of interest

The authors declare no conflict of interest.

Table 3: Effect of extract of *P. falcisepala* on serotonin induced right paw oedema in rats

		Increase in paw volume						
Treatment	**Dose (mg/Kg)**	**T_0**	**T_{30}**	**T_{60}**	**T_{90}**	**T_{120}**	**T_{150}**	**T_{180}**
P. falcisepala	150	4.24 ± 0.13	5.48 ± 0.22* (45.27)	5.17 ± 0.18**** (64.89)	5.27 ± 0.18** (54.82)	5.25 ± 0.14** (53.35)	5.33 ± 0.16 (45.37)	5.29 ± 0.17 (43.20)
Ondasentron	10	3.98 ± 0.11	4.99 ± 0.29**** (55.61)	4.99 ± 0.27**** (61.94)	4.74 ± 0.16**** (66.64)	4.74 ± 0.09**** (65.20)	4.80 ± 0.07*** (59.22)	4.70 ± 0.16*** (61.48)
Distilled water	10 mL/kg	3.88 ± 0.03	6.15 ± 0.11	6.54 ± 0.21	6.16 ± 0.18	6.04 ± 0.29	5.88 ± 0.33	5.73 ± 0.13

Values are expressed as mean ± SEM. (n=6). Figure in parenthesis indicate percentage inhibition of oedema development
****p < 0.0001, ***p < 0.001, **p < 0.01, *p < 0.05 vs. Control (Two way ANOVA, Tukey's Multiple Comparisons)

Table 4: Effect of extract of *P. falcisepala* on histamine induced right paw oedema in rats

		Increase in paw volume						
Treatment	**Dose (mg/Kg)**	**T_0**	**T_{30}**	**T_{60}**	**T_{90}**	**T_{120}**	**T_{150}**	**T_{180}**
P. falcisepala	150	4.06 ± 0.15	4.78 ± 0.23 (39.64)	4.65 ± 0.17**** (66.76)	4.57 ± 0.11**** (73.97)	4.85 ± 0.06 (35.46)	4.94 ± 0.05 (34.04)	5.04 ± 0.04* (38.54)
Chlorpheniramine	10	4.03 ± 0.07	4.86 ± 0.8 (30.25)	4.80 ± 0.2**** (56.92)	4.91 ± 0.22**** (55.10)	4.68 ± 0.19* (46.87)	4.89 ± 0.21 (35.66)	5.06 ± 0.17* (35.10)
Distilled water	10 mL/kg	4.14 ± 0.19	5.33 ± 0.2	5.94 ± 0.31	6.12 ± 0.19	5.37 ± 0.22	5.48 ± 0.20	5.74 ± 0.22

Values are expressed as mean ± SEM. (n=6). Figure in parenthesis indicate percentage inhibition of oedema development
****p < 0.0001, ***p < 0.001, **p < 0.01, *p < 0.05 vs. Control (Two way ANOVA, Tukey's Multiple Comparisons)

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Table 5: Effect of ethanol extract *P. falcisepala* on formalin induced rat paw oedema

		Increase in paw volume							
Treatment	**Dose (mg/kg)**	**T_0**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
P. falcisepala	50	5.92 ± 0.23	7.67 ± 0.04 (33.77)	7.10 ± 0.12 (31.10)	6.46 ± 0.16 (52.86)	6.46 ± 0.12 (56.59)	6.40 ± 0.13 (61.24)	5.99 ± 0.03**** (95.42)	5.97 ± 0.07*** (96.63)
P. falcisepala	100	5.73 ± 0.02	7.73 ± 0.23 (24.45)	6.94 ± 0.19 (29.07)	6.60 ± 0.14 (25.36)	6.39 ± 0.14 (47.23)	5.89 ± 0.13* (87.04)	5.95 ± 0.05**** (86.35)	5.74 ± 0.09**** (99.05)
P. falcisepala	150	5.66 ± 0.10	7.48 ± 0.35 (31.13)	6.89 ± 0.20 (27.91)	6.53 ± 0.06 (24.79)	6.37 ± 0.06 (43.67)	5.8 ± 0.11** (88.76)	5.91 ± 0.05**** (84.76)	5.72 ± 0.09**** (95.99)
P. falcisepala	200	5.43 ± 0.10	7.67 ± 0.09 (15.25)	6.82 ± 0.06 (19.28)	6.30 ± 0.11 (25.21)	6.09 ± 0.09 (47.49)	5.89 ± 0.10* (62.69)	5.84 ± 0.06**** (74.90)	5.61 ± 0.10**** (88.41)
Indomethacin	10	6.01 ± 0.17	7.79 ± 0.11 (32.89)	7.28 ± 0.10 (30.91)	6.86 ± 0.17 (27.36)	6.82 ± 0.09 (36.28)	6.84 ± 0.08 (35.58)	6.70 ± 0.12 (59.66)	6.24 ± 0.16* (85.67)
Control	10 mL/kg	5.31 ± 0.23	7.95 ± 0.39	7.03 ± 0.18	6.47 ± 0.05	6.57 ± 0.06	6.57 ± 0.09	6.98 ± 0.15	6.89 ± 0.15

Values are expressed as mean ± SEM. (n=6). Figure in parenthesis indicate percentage inhibition of oedema development.
****p< 0.0001, ***p< 0.001, **p< 0.01, *p< 0.05 vs. Control (Two way ANOVA, Tukey's Multiple Comparison)

Table 6: Effect of *P. falcisepala* on cotton pellet-induced granuloma in rats

Treatment	**Dose (mg/kg)**	**Dry weight of cotton pellet (mg)**	**% Inhibition**
P. falcisepala	50	47.67 ± 0.01	7.30
P. falcisepala	100	44.75 ± 0.01	13.00
P. falcisepala	150	44.33 ± 0.01*	13.78
P. falcisepala	200	41.17 ± 0.01***	19.94
Indomethacin	10	23.83 ± 0.01****	53.68
Distilled water	10 (ml/kg)	51.42 ± 0.01	-

Values are expressed as mean ± SEM. (n=6).
****p< 0.0001, ***p< 0.001, *p< 0.05 vs. Control (One way ANOVA, Tukey's Multiple Comparison)

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Goldman P. Herbal Medicine Today and the Roots of Modern Pharmacology. USA. Ann Int Med. 2001; 135(8):594-600.
2. Gaikwad BS, Mohan GK, Rani MS. Phytochemicals for Diabetes Management. Pharm Crop. 2014; 5 (1):11-28.
3. Ojiako EN and Okoye IE. Phytochemical studies and antimicrobial activity of *Anthocleista djalonensis*. J Chem Pharm Res. 2015; 7(5):70-72.
4. Nyamai DW, Arika W, Ogola PE, Njagi ENM, Ngugi MP. Medicinally Important Phytochemicals: An Untapped Research Avenue. Research and Review, J Pharmacog Phytochem. 2016; 4(1):35-49.
5. WHO. WHO Traditional Medicine Strategy: 2014-2023; 2013. 78 p.
6. Burkill HM. The useful plants of West Tropical Africa. 2nd Edition. Volume1, Families A-D. Royal Botanical Gardens, Kew, Richmond, United Kingdom, 1985. 23 p.

7. Adesegun SA, Fajana A, Orabueze CI, Coker HAB. Evaluation of Antioxidant Properties of *Phaulopsis falcisepala* C.B.Cl. (Acanthaceae). Evid Based Comp Alt Med. 2009; 6(2):227-231.
8. Abiodun OO, Tijani R, Ogbole O, Ajaiyeoba E. Antioxidant, alpha-amylase and alpha-glucosidase inhibitory activities of leaf and flower extracts and fractions of *Phaulopsis falcisepala* C. B. Clarke. Acta Pharm Sci. 2018; 56(4):23-33.
9. Organization for Economic Cooperation and Development (OECD) Test Guideline 420: Acute Oral Toxicity- Fixed dose procedure. Paris, France. 2001. 1-14 p.
10. Winter CA, Risley EA, Nuss W. Carrageenan-induced oedema in hind paw of rats as an assay for antiinflammatory drugs. Proc Soc Exp Biol Med. 1962; 111:544-547.
11. Tang X, Lin Z, Cai W, Chen N, Shen L. Antiinflammatory effect of 3-acethylaconitine. Acta Pharmacol Sin. 1984; 5:85-89.
12. Amann R, Schuligo R, Lanz I, Donnerer J. Histamine-induced edema in the rat paw — effect of capsaicin denervation and a CGRP receptor antagonist. Eur J Pharmacol. 1995; 279(2-3):27-231.
13. Seyle H. Further studies concerning the participation of the adrenal cortex in the pathogenesis of arthritis. Br Med J. 1949; 2:1129-1135.
14. Winter CA and Porter CC. Effect of alteration in side chain upon anti-inflammatory and liver glycogen activity of hydrocortisone esters. J Am Pharm Ass Sci. 1957; 46:515-519.
15. Wolfe K, Wu X, Liu RH. Antioxidant Activity of Apple Peels. J Agric Food Chem. 2003; 51(3):609-614.
16. Broadhurst RB and Jones WT. Analysis of condensed tannins using acidified vanillin. J Sci Food Agric. 1978; 48(3):788–794.
17. Woisky RG and Salatino A. Analysis of propolis: some parameters and procedures for chemical quality control. J Apic Res. 1998; 37:99-105.
18. Yoon J and Baek SJ. Molecular targets of dietary polyphenols with anti- inflammatory Properties. Yonsei Med J. 2005; 46:585–596.
19. Mansour S, Djebli N, Ozkan EE, Mat A. *In vivo* anti-inflammatory activity and chemical composition of *Hypericum scabroides*. Asian Pac J Trop Biomed. 2014; 7 (Suppl 1):S514-S520.
20. Kotas ME and Medzhitov R. "Homeostasis, inflammation, and disease susceptibility." Cell 2015; 160 (5):816–827.
21. Sowjanya R, Shankar M, Sireesha B, Naik A, Yudharaj P. An overview of inflammation and plant having anti-inflammatory activity. Int J Phytopharm Res. 2017; 7(1):25-32.
22. Sowemimo A, Onakoya M, Fageyinbo MS, Fadoju T. Studies of the anti-inflammatory and anti-nociceptive properties of *Blepharis maderaspatensis* leaves. Rev Bras Farmacogn. 2013; 23(5):830-835.
23. Tapiero H, Ba GN, Couvreur P, Tew KD. Polyunsaturated fatty acids (PUFA) and eicosanoids in human health and pathologies. Biomed Pharmacother. 2002; 56:215-222.
24. Igbe I, Ching FP, Eromon A. Anti-inflammatory activity of aqueous fruit pulp extract of Hunteria umbellate K. Schum in acute and chronic inflammation. Acta Pol. Pharm. 2010; 67(1):81-85.
25. Park JH, Son KH, Kim SW, Chang HW, Bae K, Kang SS, Kim HP. Anti-inflammatory activity of *Synurus deltoids*. Phytother Res. 2004; 18:930-933.
26. Masresha B, Makonnen E, Debella A. *In vivo* anti-inflammatory activity of *Ocimum suave* in mice. J Ethnopharmacol. 2012; 142:201-205.
27. Vinegar R, Truax JF, Selph JL, Johnston PR, Venable AL, McKenzie KK. Pathway to carrageenan-induced inflammation in the hind limb of the rat. Federation Proceedings. 1987; 46(1):118-126.
28. Choudhary M, Kumar V, Gupta P, Singh S. Investigation of antiarthritic potential of *Plumeria alba* L. leaves in acute and chronic models of arthritis. BioMed Res Int. 2014; 1-12.
29. Junping K, Yun N, Wang N, Liang L, Zhi-Hong H. Analgesic and anti-inflammatory activities of total extract and individual fractions of Chinese medicinal plants *Polyrhachis lamellidens*. Biol Pharm Bull. 2005; 28:176-180.
30. Atta AH and Alkofahi A. Anti-nociceptive and anti-inflammatory effects of some Jordanian medicinal plant extracts. J Ethnopharmacol. 1998; 60:117-124.
31. Medzhitov R. Origin and physiological roles of inflammation. Nature 2008; 454(7203):428-435.
32. Tadiwos Y, Nedi T, Ephrem E. Analgesic and anti-inflammatory activities of 80% methanol root extract of *Jasminum abbyssicum* Hochst. Ex. Dc. (Oleaceae) in mice. J Ethnopharmacol. 2017; 202:281-289.
33. Dubuisson D and Dennis SG. The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. Pain 1978; 4(2):161-174.
34. Serhan CN and Savil J. Resolution of inflammation: the beginning programs, the end. Nat Immunol. 2005; 6(12):1191-1197.
35. Perez RM, Perez S, Zavala MA, Salazar M. Antiinflammatory activity of the bark of *Hippocratea exsela*. J Ethnopharmacol. 1995; 47:85-90.
36. Anosike CA, Obidoa O, Ezeanyika LUS. The anti-inflammatory activity of garden egg *(Solanum aethiopicum*) on egg albumin-induced oedema and granuloma tissue formation in rats. Asian Pac J Trop Biomed. 2012; 5(1):62-66.
37. Meshram GG, Kumar A, Rizvi W, Tripathi CD, Khan RA. Evaluation of the anti-inflammatory activity of the aqueous and ethanolic extracts of the leaves of *Albizzia lebbeck* in rats. J Trad Comp Med. 2016; 6:172-175.
38. Majno G. Chronic inflammation: links with angiogenesis and wound healing. Am J Pathol. 1998; 153:1035.
39. Paschapur MS, Patil MB, Kumar R, Sachin RP. Evaluation of anti-inflammatory activity of ethanolic extract of *Borassus flabellifer* L. male flowers (inflorescences) in experimental animals. J Med Plants Res. 2009; 3(2):49-54.
40. Babu PN, Pandikumar P, Ignacimuthu S. Anti-inflammatory activity of *Albizia lebbeck* Benth., an ethnomedicinal plant, in acute and chronic animal models of inflammation. J Ethnopharmacol. 2009; 125:356-360.
41. Ohemu TL, Agunu A, Dafam DG, Olotu PN. Pharmacognostic studies of the stem bark of *Enantia chlorantha* Oliver (Annonaceae). Niger J Nat Prod Med. 2015; 19:122-125.
42. Orhan DD, Hartevioglu A, Kupeli E, Yesilada E. *In vivo* anti-inflammatory and anti-nociceptive activity of the crude extract and fractions from *Rosa canina* L. fruits. J Ethnopharmacol. 2007; 112:394-400.
43. Alcaatraz MJ and Jimenez MJ. Flavonoids as anti-inflammatory agents. Fitoterapia 1998; 59:25-38.
44. Howell AB. Cranberry proanthocyanidins and the maintenance of urinary tract health. Crit Rev Food Sci Nutr. 2002; 42:273-278.