Anti-inflammatory and analgesic properties of Platostoma africanum (P. Beauv) leaf in mice

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Abstract

In Nigeria, traditional medicinal plants are often use to relieve pain and inflammation. Traditional healers in Nigeria folk medicine use herbs like *P. africanum (P.beauv)* to relieve pain and inflammation. The current work sought to investigate the analgesic and anti-inflammatory properties of methanol crude extract and fractions of *P. africanum* aerial part in animal models.

Extraction was accomplished through successive maceration with solvents of increasing polarity: methanol, n-hexane, and butanol. Following extraction, the crude extract and fractions were tested for central analgesic activity using the tail immersion test. Anti-inflammatory activity was tested using carrageenan induced paw edema. The extract and fractions were tested at 100, 200, and 400 mg/kg dosages. The positive control groups received pentazocine 10 mg/kg for tail immersion and diclofenac potassium 10 mg/kg for paw edema testing, whereas the negative control group received distilled water (10 mL/kg). All treatments were given orally.

P. africanum fractions demonstrated statistically significant antinociceptive efficacy in both chemical-induced peripheral and thermal-induced central pain ($p < 0.001$). The maximum dose of the hexane fraction (400 mg/kg) in 90 minutes produced the highest analgesic effects. The extract also had a statistically significant ($p < 0.001$) effect on carrageenan-induced paw edema in a dose-dependent manner. The greatest dose of butanol (400 mg/kg) in 240 minutes inhibited edema most effectively.

In general, the results of the current investigation revealed that the extract possessed strong analgesic and anti-inflammatory properties and future research is needed to identify the components responsible.

Keywords: Analgesic activity, Anti-inflammatory activity, Carrageenan, *P. africanum*, Thermal immersion test.

Introduction

Pain is a distressing perceptual and behavioral sensation caused by real or potential tissue injury. It is one of the signs / symptoms of an illness, as well as the most common reason for patients to seek medical assistance (Hofman *et al.*, 2017; Love-Jones, 2019; Raja *et al.*, 2020). Pain is also a feeling that can range from minor discomfort to excruciating agony. Pain can be localized, such as in an injury, or it can be diffuse. Analgesic medicines are commonly used to treat mild to moderate pain. Opioids and other drugs, on the other hand, may be used to treat severe pain. When pain is related to inflammatory disorders, nonsteroidal anti-inflammatory (NSAIDS) or steroidal medications are used (Jahnavi *et al.*, 2019). Inflammation is referred to as a reaction that causes redness, warmth, oedema, and soreness as a result of infectious, chemical, and physical stimuli such as bacteria, poisons, radiation, and injuries (Ghosh *et al.*, 2019; Karrat *et al.*, 2022). The inflammatory response is a defensive mechanism that seeks to limit the damaging substances. The inflammatory process begins with the production of numerous chemical mediators by macrophages and neutrophils, which are crucial for the initiation, development, control, and final resolution of the acute phase of inflammation. Monocytes play a main role in the clearing of cell debris. Monocytes are critical in the removal of cell debris. If the acute phase is not resolved, a chronic phase will develop (Oishi *et al.*, 2018). Chronic inflammatory diseases, such as ischemic heart disease, chronic kidney disease, cancer, diabetes, and neurodegenerative and autoimmune conditions, have recently been identified as the leading cause of death worldwide, accounting for more than half of all deaths (Furman *et al.*, 2019). Inflammation is the body's most prevalent adaptive response. Both pain and inflammation include a wide range of biochemical events, including enzyme activation, inflammatory release of mediators, fluid extravasation, cell migration, damage to tissues and healing (Alemu *et al.*, 2018; Dou *et al.*, 2020). The majority of anti-inflammatory medications on the market are possible inhibitors of the arachidonic acid metabolism cyclooxygenase (COX) pathway that produces prostaglandins. Prostaglandins are hyperalgesic vasodilators that cause erythema, edema, and discomfort. Nonsteroidal anti-inflammatory medicines (NSAIDs) are the most clinically significant

medications used to treat inflammatory illnesses such as arthritis and cardiovascular disease. Because of their efficacy in a wide range of pain and inflammatory diseases, nonsteroidal anti-inflammatory medicines (NSAIDs) are among the most extensively used pharmaceuticals (Bindu *et al.*, 2020). However, due to its nonselective suppression of both constitutive (COX-1) and inducible (COX-2) isoforms of the cyclooxygenases enzymes, long-term NSAID use may cause gastro-intestinal ulcers, bleeding, cardiovascular risk, and renal issues (Richette *et al.*, 2015; Fokunang *et al.*, 2018). Because of this, novel anti-inflammatory and analgesic medications that do not have these side effects are being sought all over the world as alternatives to NSAIDs and opiates. According to the World Health Organization, over 80% of the global population still uses plant-based medications, which include the therapeutic use of plants as anti-nociceptive agents in folkloric treatment (Murugananthan *et al.*, 2013; Awad *et al.*, 2014; Murugesan *et al.*, 2014). *Platostoma africanum* P. Beauv. belongs to the Lamiaceae plant family and shares some phenotypic characteristics with Ocimum species in the same family. *Plastostoma spp*. is a tropical African genus with at least 45 species. *P. africanum* is an indigenous African herb known as "Akan-Osante" (Ghana), "Mani" (Liberia), and "Mkpri Ibok-ukpong" (Efik-Nigeria). It has a pleasantly scented mint or sage odor and is commonly found in moist locations and waste regions in Tropical African countries. The leaves are oblong, acute or abruptly acuminate, with serrated margins on the upper section, whole and wedge-shaped at the base, and 2 to 4 cm long petioles (Aikpokpodion *et al.*, 2012). *P. africanum* produces slender racemes of extremely small flowers with a white corolla. In West Africa, *P. africanum* is widely utilized in traditional medicine. In Nigeria, it is use to cure rheumatic symptoms, internal heat, and the leaves are utilized as a local haemostatic. It is also used for fever, similar to Ocimum; the root is combined with Tephrosia linearis (Leguminosae) to form a decoction used internally and externally for febrile chills and rheumatic symptoms. Based on the findings above, *P. africanum* aerial component extracts were tested for analgesic and anti-inflammatory properties in albino mice with experimentally induced pain and inflammation.

Materials and methods

Collection and processing of plant material

Fresh leaves of *Platostoma africanum* were harvested from a forest in Udenu, Enugu State, Nigeria, where they naturally flourish. A

taxonomist attached to the International Centre for Ethnomedicine and Drug Development (InterCEDD), Nsukka, Enugu State, Nigeria, identified the plant where a voucher specimen (InterCEDD 211) was deposited. The leaves were washed in tap water to remove dust and were shade-dried for two weeks. The dried leaves were then ground using a miller, weighed, packaged in an airtight container with clear labels, and kept at room temperature prior to extraction.

Experimental Animals

Healthy adult Swiss Albino mice of either sex (20–35g, and 6–8 weeks of age) were used for evaluation of the analgesic and anti-inflammatory activities of the extracts. The animals were purchased from the Animal House of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The animals were acclimatized under standard laboratory conditions in the animal house of Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology, 3 weeks prior to the commencement of the experiment. They received human care throughout the experimental period in accordance with the ethical rules and recommendations of the Enugu State University of Science and Technology Ethical Committee on the Care and Use of Laboratory Animals and the revised National Institute of Health guide for care and use of laboratory animals (Pub No.85-23, revised 1985).

Equipment, Drugs and Chemicals

Carrageenan (Sigma Chemicals Co., St Louis, USA), Methanol, n-Hexane, Butanol were purchased from Sigma Chemicals Co., St Louis, USA) and are of analytical grade. **Standard Drugs**: Diclofenac potassium by Nuel Pharmaceutical Ltd and Pentazocine by Embassy Pharmaceutical Ltd

Preparation of methanol extract

Maceration was carried out by placing 594.28 g of finely ground leaves in 4.5 L of analytical-grade methanol for 72 h based on a modified method (Ekalu *et al.*, 2020). The mixture was periodically stirred and filtered twice; first with muslin cloth and later with Whatman No 1 filter papers. The filtrate was concentrated using a rotary evaporator at 50^{o} C and then dried over a hot air oven at 35^{o} C. Prior to usage, the concentrated extract (MEcr) was weighed, labeled, and kept in a refrigerator at 4^{o} C.

Fractionation

The methanol crude extract was fractionated following the standard procedure, with different solvents according to their

increasing order of polarity; n-Hexane, followed by butanol. A 20 g of MEcr was fixed in 200 g of silica gel with methanol to form a slurry. The slurry was poured into a long glass column. The column was eluted with n-hexane (500 mL, 3 times), and butanol (500 mL, 3 times) (Ostberg-Potthoff *et al.*, 2019). The two fractions were completely evaporated using a water-bath regulated at 50 °C, to obtain a slurry form of the fractions.

Qualitative phytochemical screening

Standard qualitative phytochemical tests were done on the crude methanol extract and the various fractions (Harborne, 1998). The presence or absence of a specific phytochemical group was determined by visual examination of color or fronting.

Acute toxicity studies

Acute toxicity of MEcr of *P. africanum* was performed using twelve albino mice according to the method of (Lorke, 1983). Briefly, twelve albino mice weighing between 23-30 g of either sex with free access to food and clean water were used for the test. The animals were allotted to three (3) groups of three (3) animals per group. The groups received accordingly, oral doses of 10, 100 and 1000 mg/kg of MEcr of *P. africanum*, after which the animals were allowed free access to food and water and monitored for death over a period of 24 hours. The number of death was observed and recorded and the results obtained were used in phase 2 of the experiment. The result of the first phase showed no death in 1000 mg/kg MEcr of *P. africanum* group. Hence, higher doses of the extract at 1900, 2600 and 5000 mg/kg of MEcr of *P. africanum* were administered to one mouse each. The mice were allowed free access to food and water after which they were observed for death over 24hours period.

Evaluation of the analgesic activity of *P. africanum*

Thermal Response Model (Tail Immersion Test)

The tail immersion method was used to evaluate the central mechanism of analgesic activity according to the method described by (Toma *et al.*, 2003). Here the painful reactions in animals were produce by thermal stimulus, that is, by dipping the tip of the tail in hot water. Swiss albino mice were grouped and treated with 100, 200, and 400 mg/kg body weight of extract and fractions respectively. Pentazocine (10mg/kg IP) was used as a reference drug, while control group received distilled water (10mg/kg PO). The animals were fasted for 16 hours with water *ad libitum*. After administration of standard and test drugs, the basal reaction time was measured

by immersing the tail tips of the mice (last 1-2 cm) in hot water heated at temperature 55 ± 1^0C. The actual flick response of mice, that is, the time taken in seconds to withdraw it from a hot water source, was calculated and the results were compared with control group. Mice with baseline latencies of more than 10 s were eliminated from the study. A latency period of 15 s was set as the cutoff point, and the measurement was then stopped to avoid injury to mice. The latent period of the tail flick response was determined at 0, 30, 60 and 90 minutes after the administration of drugs.

Evaluation of the anti-inflammatory activity of *P. africanum*

Carrageenan-induced hind paw edema in mice

This method was carried out by inducing acute inflammation in the paws of overnight fasted mice with free access to water. The mice were injected with carrageenan (1% w/v in normal saline, 0.05mL) into the plantar side of the left hind paw. The linear paw circumference was measured using the cotton thread method for 4 hrs at 30 min intervals after the administration of the phlogistic agent (Ishola *et al.*, 2011). The effect of the extract after the induction of phlogistic agent was investigated. The mice were randomly grouped into five groups of five animals each. The first group which served as the negative control received distilled Water (10 mg/kg PO). The second group served as the positive control and received diclofenac potassium at a dose of 10 mg/kg IP. The third, fourth and the fifth groups were given methanol extract, n-hexane and butanol fractions of *P.africanum* at doses of 100, 200 and 400 mg/kg PO respectively. The reaction time (in sec) was recorded 30 min post-treatment. Paw edema inflammation was induced 1-hour post administration. The linear paw circumference was measured using the cotton thread method for 4 hours at 30 min intervals post induction.

Statistical analysis

The data obtained from the evaluation of activities were analyzed using two-way analysis of variance (ANOVA) in IBM SPSS (statistical product and service solution) version 23.0 and presented as Mean ± Std Error of Mean (SEM) value with *$p<0.05$ considered as significant.

Results

Percentage yield of *P. africanum* (P.A)

Table 1 shows the yield of the methanol extract (98 g) and the fractions of n- hexane fraction, butanol fraction, and butanol fraction with values 7.6 g, 11g, and 2.2 g respectively. The yield gotten from the leaves of *P.*

africanum after 594.28 g was soaked in 3 bottles of for 72 hours is 98 g.

So, the percentage yield is = 98 g/594.28 g) x 100

= 16.49 %.

Table 1: Yield of extract and fractions of P.A

Extract and fractions	**Initial weight (Mx) (g)**	**Crude extract (My) (g)**	**Percentage yield%= My/Mx × 100**
Methanol crude extract	594.28	98	16.49
n-Hexane fraction	40	7.6	19
Butanol fraction	40	11	27.5
Ethyl acetate fraction	40	2.2	5.5

Preliminary phytochemical screening

Qualitative phytochemical composition of aerial part of *Platostoma africanum*

The result of qualitative phytochemical screening of methanol extract, n-hexane and butanol fractions of aerial part of *P. africanum* showed that it contained, Terpenoids, Flavonoids, Tannins, Saponins and Steroids as shown in **Table 2** below.

Table 2: Qualitative phytochemical constituent of aerial part of *P. africanum*

S/No	**Phytochemical constituents**	**Qualitative Remarks**		
		Methanol	**n-hexane**	**Butanol**
1	Terpenoids	+	+	+
2	Flavonoids	+	+	+
3	Tannins	+	+	+
4	Alkaloids	+	+	+
5	Saponins	+	+	+

6	Steroids	+	+	–
7	Glycosides	–	–	–
8	Reducing Sugar	–	–	–

Key: + Present, - Absent

Acute toxicity study of methanol extract of aerial part of P.A

The result showed that the methanol crude extract of aerial part of *P. africanum* was not toxic and there was no sign of behavioral changes and physiological alterations even up to the dose of 5000mg/kg body weight. This suggests that the extract is safe up to 5000 mg/Kg.

Analgesic activity

Tail immersion method

Table 4 shows the result of the analgesic activity of methanol extract (ME), and fractions of *P. africanum* measured by tail immersion method. At a dose of 200 mg/kg, the ME of *P. africanum* showed a significant ($p < 0.001$) decrease in analgesic effect when compared with the positive control (PC). However, there is a significant increase in the reaction time at a dose of 400mg/kg when compared with the negative control (NC) at $p < 0.001$, with the maximum reaction time at 90 minutes and beyond. The butanol fraction (BF) showed a significant $p < 0.001$ increase in analgesic activity at a dose of 200mg/kg as compared with the NC and the analgesic effect increased with the passage of time and achieved peak level after 60mins. The hexane fraction showed a significant $p < 0.001$ increase in analgesic activity at a dose of 200 mg/kg when compared with the NC and maximal analgesic activity was achieve after 60 minutes.

Table 4: Effect of the crude extract and fractions of P.A on the reaction time of tail immersion method in mice.

Tail flick latency time (sec)					
Groups (Treatment)	**Dose (mg/kg)**	**Baseline**	**30 mins**	**60 mins**	**90 mins**

NC	10	3.04 ± 0.21	2.33±0.43***	2.20±0.21	1.91±0.32***
PC	10	2.81 ± 0.34	3.68±0.29	3.55±0.17###	4.23±0.43
Methanol Crude	100	1.96 ± 0.52***#	2.23±0.46***###	2.13±0.31###	2.40±0.71###
	200	2.08±0.51**	2.00±0.27###	2.37±0.51###	2.72±0.36###
	400	3.24 ± 0.41	3.51±0.43	3.52±0.21***	4.24±0.46***
Butanol Fraction	100	1.90±0.11***#	2.44±0.41###	2.23±0.31###	2.32±0.52***#
	200	2.53 ± 0.23	3.86±0.11***	4.06±0.50***	4.17±0.58***
	400	2.33 ± 0.33	2.00±0.31###	2.44±0.43	2.91±0.31**###
N – Hexane Fraction	100	1.86±0.45***##	2.64±0.41##	2.13±0.55###	2.22±0.20###
	200	2.33±0.32	3.56±0.61***	4.16±0.32	4.07±0.11***
	400	2.43±0.34	2.40±0.31###	2.54±0.08##	2.81±0.37*###

Results are presented as means ± standard deviation, n=5, *, ** and ***: significantly different from the NC at $p < 0.05$, 0.01 and 0.001 respectively. #, ## and ###: significantly different from the PC at $p < 0.05$, 0.01 and 0.001 respectively. Two-way, ANOVA followed by Turkey HSD.

Anti-inflammatory activity

Carrageenan-induced hind paw edema in mice.

The result of anti-inflammatory effect of methanol crude extract (MEcr), butanol fraction (BF), and n- hexane fraction (HF) of *P. africanum*, Carrageenan-induced hind paw edema in mice model is shown in table 5. Methanol extract exhibited significant ($p < 0.001$) decrease in inhibition of edema at 100 and 200 mg/kg dose when compared to positive control. The BF showed significant ($p < 0.001$) increase in anti-inflammatory activity at a dose of 100, 200, and 400 g/kg when compared to NC. The BF exhibited maximum anti-inflammatory effect at a dose of 200 and 400 mg/kg after 240 minutes when compared to the NC. The anti- inflammatory effect increases dose dependently with time

and peak at 240mins. The HF showed a significant ($p < 0.001$) increase in anti-inflammatory activity at a dose of 200 mg/kg as compared with negative control. HF exhibited maximum anti-inflammatory effect at a dose of 200 mg/kg after 210 minutes.

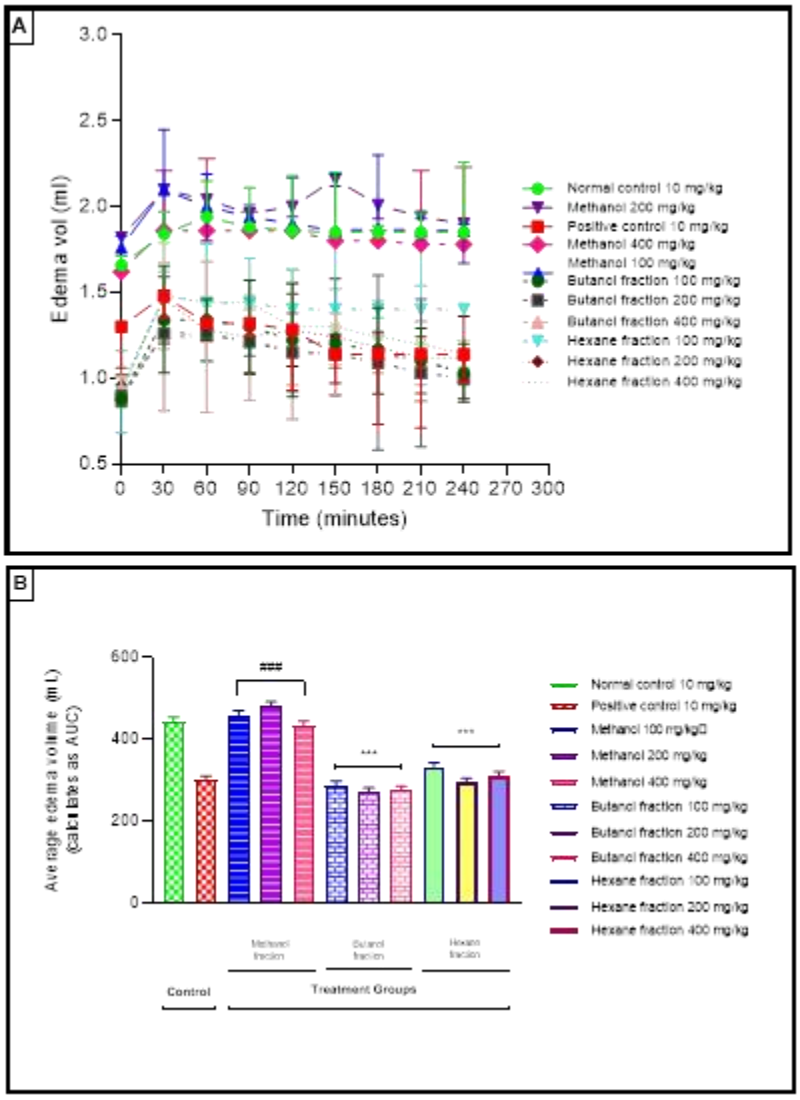

Figure 1: Carrageenan-induced hind paw edema in mice.

Results are presented as means ± standard deviation, n=5, ***: significantly different from the NC at $p < 0.001$. ###: significantly different from the PC at $p < 0.001$ respectively. Two-way, ANOVA followed by Turkey HSD.

Discussion

Considering the socioeconomic impacts of pain and inflammation and having the knowledge of potential herbal medicines from traditionally claimed plants, the need for searching effective analgesic and anti-inflammatory drugs with minimal untoward effects from traditional medicinal plants seems reasonable (Yuan *et al.*, 2016). *P.africanum* is among the widely used traditional medicinal plants in Nigerian folk medicine for treating pain, and different inflammatory conditions (Borokini *et al.*, 2012; Oguntibeju, 2018). It may therefore be worthwhile scientifically to investigate the analgesic and anti-inflammatory activities of the leaf of *P. aficanum* in the mice model to substantiate its claimed traditional use.

Tail-Flick Test is a nociceptive assay based on the measurement of the latency of the avoidance response to thermal stimulus in rodents. A thermal stimulus is applied to the tail, when the animal feels discomfort; it reacts by a sudden tail movement. The tail flick reaction time is then measured and used as an index of animal pain sensitivity (Xie, 2011; Famitafreshi *et al.*, 2017). In this test, both extract and fractions showed analgesic effect and could increase pain tolerance in mice. The analgesic effect of both extract and fractions could be due to their contents of various phytochemicals, such as flavonoids, tannins, and phenolic compounds. Flavonoids and tannins have demonstrated anti-inflammatory and analgesic activity in several studies (Shojaii *et al.*, 2015; Komakech *et al.*, 2019). Flavonoids have been shown to reduce the production of arachidonic acid, prostaglandins, and leukotrienes, and to reduce the high levels of intracellular Ca2þ. They may interact with 5-HT_2A and 5-HT_3 receptors which might be involved in the mechanism of analgesic activity (Karrat *et al.*, 2022). On the other hand, phenolic compounds have demonstrated anti-inflammatory activity in in vivo and in vitro studies by controlling the levels of different inflammatory markers such as COX-2 (David *et al.*, 2020; Mićović *et al.*, 2022). Moreover, flavonoids and tannins also have antioxidant activity by removing free radicals that may be involved in stimulating pain (Mondal *et al.*, 2020). The current study is similar to work done by (Kaushik *et al.*, 2012; Muhammad *et al.*, 2012; Hasan *et al.*, 2014). The tail immersion test was considered to be selective to examine compounds acting through the opioid receptor; the extract/fractions increased the pain threshold which means basal latency, which indicates that it may act via centrally mediated analgesic mechanism. Narcotic

analgesics inhibit both peripheral and central mechanism of pain, while nonsteroidal anti-inflammatory drugs inhibit only peripheral pain (Edinoff *et al.*, 2021). The extract inhibits pain with both mechanisms, suggesting that the plant extract may act as a narcotic analgesic.

Flavonoids may increase the amount of endogenous serotonin or may interact with 5-HT_2A and 5-HT_3 receptors which may be involved in the mechanism of central analgesic activity (Kaushik *et al.*, 2012). The results of the present study have shown that the crude extract of the investigated plant exhibited very high anti-inflammatory and analgesic activities. These activities may be linked to the presence of polyphenolic compounds present in the extract. The ability of flavonoids to inhibit eicosanoid biosynthesis has been documented (Samardžić *et al.*, 2018). Eicosanoids, such as prostaglandins, are involved in various immunological responses and are the end products of the cyclooxygenase and lipoxygenase pathways (Dennis *et al.*, 2015). Further, flavonoids are able to inhibit neutrophils degranulation and thereby decrease the release of arachidonic acid (Gupta *et al.*, 2016). Thus, the presence of flavonoids in the extract/fractions of *P. africanum* might be responsible for the anti-inflammatory and analgesic activity in Swiss albino mice.

Carrageenan-induced hind paw edema is a prototype model that is employed to evaluate the anti-inflammatory potentials of various natural and synthetic products as well as to determine the possible mechanisms involved in inflammation (Hisamuddin *et al.*, 2019; Yimer *et al.*, 2020). Carrageenan is a phlogistic, non-antigenic agent and is devoid of apparent systemic effect. It is also believed that the experimental model exhibited a high degree of reproducibility in acute phase inflammation. In the present study, acute inflammation was induced by sub planar injection of carrageenan (1%v/v in normal saline) in the left hind paws of the mice. Following the induction of carrageenan, an acute localized inflammation was induced through the sequential release of various endogenous inflammatory mediators (Ahmad *et al.*, 2015; Yimer *et al.*, 2020). The release of these endogenous mediators was biphasic. The early phase (0 and 2.5 h) after carrageenan induction is mainly mediated by the release of histamine, serotonin, and bradykinin. These mediators are attributed to inflammation by increasing vascular permeability in the damaged tissue surroundings. The late phase which is sustained by the overproduction of COX-2

and its pro- inflammatory PGs product, with infiltration of polymorphonuclear leucocytes (neutrophils), took place 2.5–6hrs post carrageenan induction (Yimer *et al.*, 2020). There are also other chemicals mediators released during the late phase of inflammation such as, oxygen-derived free radicals like superoxide anion (O2-) and hydroxyl radicals (OH−), nitric oxide (NO) which play an important role in the development and progression of acute inflammation (Bhattacharya, 2015; Valko *et al.*, 2016). The butanol and hexane fractions at all test doses employed (100, 200 and 400 mg/kg) dose dependently decreased the formation of edema starting from 1 hr post carrageenan induction and the effects persisted ($p < 0.001$) till the 4th hr of observation. The effect of the extract started from the 1st phase (1 hr) and continued till the 4th hr (second phase) of inflammation. This observation suggested that bioactive constituents in the extract and fractions may suppress both phases of acute inflammation by interfering with the release and/or activity of the chemical mediators and by suppressing free radical induced oxidation.

The maximum anti-inflammatory effect of butanol fractions at a dose of 200 and 400 mg/kg were observed at the 4th time of observation with the respective values of 1.00±0.05, 1.04±0.05 respectively. This suggests that the fractions anti- inflammatory effect was dose dependent manner. The edema inhibition potential shown by the higher dose of the extract (400 mg/kg) was comparable with that of the standard drug (*diclofenac potassium 10 mg/kg*) with a value of 1.14±0.05 at the 4th time of observation. Furthermore, the hexane fraction at a dose of 200 mg/kg exhibited the maximum anti-inflammatory effect as shown in Table 5. The significant anti-inflammatory effects shown by the fractions and the standard drug (diclofenac potassium mg/kg) evidenced that their effects started in the early phase of inflammation by inhibiting endogenous inflammatory mediators such as serotonin and histamine as they are involve in the early phase of inflammation. While the effects of edema inhibition reached maximum at the 4th hour suggests that both the extract/ fractions and the standard drug have profound anti-inflammatory effects against various endogenous inflammatory mediators that are involve in the late phase of inflammation such as, COX, different PG analogues, BK and/or leukotriene or they could have, free radical scavenging activities. The anti-inflammatory action of *P. africanum* fractions in the present study can be supported by previous reports from scientific journals that stated that plants which contain mainly alkaloids, flavonoids,

saponins, and tannins phenolic compounds, glycosides, coumarins and triterpenoid chemical constituents showed strong anti-inflammatory effects (Ayeni *et al.*, 2022). Therefore, it can be deduced that the anti-inflammatory effect of *P. africanum* extract / fractions in the present study may be due to the presence of alkaloids, flavonoids, saponins, tannins, and triterpenoids.

Alkaloids exert its anti- inflammatory activity through interfering with indubitable COX expression and production of PGE2, inhibiting pro-inflammatory cytokines production like IL-1β, IL- 6, TNF-α,. Terpenoids exert its anti-inflammatory effect through inhibition of PLA_2 activity, inhibition of TNF-α production, inhibition of iNOS expression, inhibition of COX-2 expression, and inhibition of NF-κB activation. While saponins are believed to interfere with iNOS expression, inhibits COX-2 expression and subsequent production of PGE2, and exert their sequential anti-inflammatory effects (Sanna *et al.*, 2020). Furthermore, polyphenols exert their anti-inflammatory properties through inhibition of the production of inflammatory cytokines and chemokines and suppressing the activity of (COX and iNOS and thereby decreasing the production of reactive oxygen and nitrogen species) (Yang *et al.*, 2014; Yahfoufi *et al.*, 2018). The results obtained from the present study were in line with the findings of others (Kaushik *et al.*, 2012; Alemu *et al.*, 2018; Yimer *et al.*, 2020), that demonstrated the analgesic and anti-inflammatory activities of medicinal plants in a dose dependent manner.

Conclusion

In general, the anti-inflammatory and analgesic activity of *P. africanum* extract/fractions can be attributed to the combined effects of the several active phytoconstituents stated above. Further research into fractionation to find the most active fraction, constituent extraction, binding tests, and electrophysiological methods may be beneficial in fully elucidating the analgesic and anti-inflammatory properties of *P. africanum* leaf.

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