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Phytochemical analysis, oral toxicity, and in vivo antinociceptive, anti-inflammatory, and antipyretic activities of aqueous leaf extract of *Prunus africana*

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ABSTRACT

Introduction: *Prunus africana* is traditionally used in Kakamega against pain, fever, and inflammation. This research aimed to identify the phytochemicals, the antipyretic, anti-inflammatory, and antinociceptive effects, and oral toxicity of the aqueous leaf extract of *P. africana*.

Methods: The plant extract was screened for phytochemicals and minerals. The anti-inflammatory and antinociceptive effects were assessed using formalin-induced edema and pain models using Swiss-albino mice, while the antipyretic effect was evaluated through a turpentine-induced fever model using Wistar rats. Sub-acute toxicity was assessed by administering the extract orally to Wistar rats at doses of 150, 260, and 450 mg/kg for 28 days. The animals' weekly weight and biochemical parameters were measured.

Results: The extract reduced rectal temperature, edema, as well as pain in the initial and late phases ($P < 0.05$). The leaves contained carnosic acid, flavonoids, amino acids, phenolic acids, and thirteen minerals. Serum biochemistry indicated liver injury at doses of 260 and 450 mg/kg with alterations in total protein, globulin, glucose, creatinine, uric acid, and phosphorus levels compared to the normal control ($P < 0.05$).

Conclusion: The extract of *P. africana* exhibits antipyretic, antinociceptive, and anti-inflammatory effects; however, it can also cause liver damage. These findings establish a basis for additional investigation of *P. africana* for therapeutic use.

Implication for health policy/practice/research/medical education:
The leaf extract of *Prunus africana* has the potential to alleviate pain, fever, and inflammation; however, it may also induce liver toxicity. Additional toxicity assessment methods are necessary to evaluate the extent of liver damage.

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Introduction

The inflammatory response, characterized by a series of chemical mediators and cellular reactions, is triggered when cells experience injury and stress (1). Fever represents a systemic inflammatory response that extends beyond the site of infection, leading to an increase in overall body temperature (2). Pain is the perception and emotional response to harmful stimuli, resulting from an inflammatory reaction associated with tissue damage (3). Ongoing inflammation is often indicated by symptoms such as fever or pain, which are usually the first to be alleviated through medication. Uncontrolled inflammation and fever can lead to localized tissue damage, while persistent pain can severely impact the quality of life for the affected individual; therefore, medical intervention is necessary to manage these symptoms and prevent further harm.

Therapeutic approaches for these symptoms typically involve nonsteroidal anti-inflammatory drugs (NSAIDs), which inhibit the cyclooxygenase pathways; however, administering them in high doses or frequently may result in gastrointestinal issues, cardiotoxicity, and

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nephrotoxicity (4). These drawbacks necessitate the exploration of novel pharmaceuticals derived from plants. Plants possess polypharmacological properties, allowing their compounds to adopt a multi-target strategy for disease management. It is possible to isolate a single compound that exhibits enhanced efficacy with reduced toxicity (5).

Herbal remedies have been employed for centuries to address various ailments, including fever, pain, and inflammation (6), due to their perceived affordability, accessibility, effectiveness, and minimal serious side effects attributed to their antioxidant properties. However, it is essential to conduct toxicological evaluations to assess the health risks associated with their repeated use (7).

Research conducted on the aqueous stem bark extract of *Prunus africana* has demonstrated its anti-inflammatory properties through the carrageenan-induced paw edema model, revealing effective doses of 50 and 100 mg/kg without any signs of toxicity in both acute (500, 1000, and 2000 mg/kg) and sub-acute studies (50, 100, 200, and 250 mg/kg) (8). Additionally, a separate study identified phytochemicals in *P. africana* using LC-MS, including feruloyl-quinic acid, isoliquiritin, chlorogenic acid, ursolic acid, and Quercetin 3, 3'-dimethyl (9). However, no research has yet integrated the bioactive compounds found in the aqueous leaf of *P. africana* with their potential antipyretic, anti-inflammatory, antinociceptive, and oral toxicity effects. Therefore, we aimed to investigate the bioactive compounds, as well as the antipyretic, anti-inflammatory, and antinociceptive activities, along with the safety profile of the aqueous leaf extract of *P. africana* sourced from Kakamega County, Kenya, using animal models.

Materials and methods

Plant collection and identification

In August 2024, fresh leaves were collected from Murhanda village, located in the Kakamega East sub-county of Kakamega County, Kenya, at the GPS coordinates 0°15'17.028"N and 34°47'49.65"E. These leaves were subsequently transported to the National Museum of Kenya, where they were classified as *P. africana* (Hook.f.) Kalkman. A specimen of this plant has been registered under the identifier Ihazano C. C. 002, EA at the National Museums of Kenya, East African Herbarium, Nairobi, Kenya.

Chemicals, reagents, drugs, and equipment

The drugs, chemicals, equipments and reagents utilized in this research include: Diclofenac 10 mg sourced from Nila Pharmaceuticals Ltd, 20% turpentine, 5% formalin solution, double distilled water, a 2,2-diphenyl-1-picrylhydrazyl solution, methanol, sodium dihydrogen phosphate monohydrate, disodium hydrogen phosphate dehydrate, quercetin, ascorbic acid potassium ferricyanide, trichloroacetic acid, ferric chloride, Folin-Ciocalteu's reagent, gallic acid, anhydrous sodium carbonate, sodium nitrite, aluminum chloride, sodium hydroxide, chloroform, a digital rectal thermometer, a vernier caliper, and a 0.2-micron syringe, plain vials and ethylenediaminetetraacetic acid vials all obtained from Kobian Scientific. All reagents were of analytical grade.

Extraction

The fresh leaves of *P. africana* were collected and subsequently placed in a shaded area to air dry for a duration of two weeks, after which they were ground into a fine powder. Five hundred grams of this powdered leaf material were immersed in 5 liters of double-distilled water and subjected to a thermostatic water bath at 60°C for 12 hours, with regular agitation. The resulting extract was then filtered through Whatman filter paper No. 1. The concentration of the filtrate was achieved under low pressure using a Lyovapor freeze dryer™ L-200 Buchi, Switzerland at KEMRI.

Liquid chromatography and mass spectroscopy (LC-MS)

This research involved the LC-MS spectroscopic examination of the aqueous leaf extract of *P. africana*, conducted using the Agilent 1260 Infinity HPLC system (Agilent Technologies, Palo Alto, CA) connected to an Agilent 6120 mass detector featuring a single quadrupole analyzer (Agilent Technologies, Palo Alto, CA). The analysis utilized a ZORBAX SB-C18 column measuring 4.6 by 250mm with a particle size of 3.5µm, maintained at a temperature of 40°C with certain modifications (9). The mobile phase consisted of water (A) and acetonitrile (B), each containing 0.01% formic acid. An injection volume of 3µl was employed, with a constant flow rate of 0.5 ml/min. Rutin hydrate (≥94%, Sigma-Aldrich, St Louis, MO) was diluted in a sequential manner (1-100 ng/µL) and analyzed via LC-MS to generate linearly calibrated curves (peak area versus concentration) represented by the equation: [Y = 5578.4X - 39094 (R2 = 0.9990)], which was utilized for the external quantification of the identified compounds. The identities of the compounds were confirmed through co-injections with commercially acquired samples, where available, or by consulting relevant literature.

Determination of minerals

A purified quartz carrier was used to pipette 10 µL aliquots of *P. africana* extracts into clean test tubes, which were subsequently dried on a hot plate at approximately 50°C. This sample underwent irradiation for 500 seconds using an S2 PICOFOX TXRF spectrometer operating at 50 kV and a current of 1000 µA, with a molybdenum anode. The internal standard was 25 µL of a 1000 ppm Gallium stock solution. The resulting spectra were analyzed using the S2 PICOFOX software, tailored to the selected elements. Element concentrations were calculated based on their peak net intensities, following the formula documented by (10).

Laboratory animals

Male Swiss albino mice (*Mus musculus*), aged between 3 to 4 months and weighing 17 to 31 g were utilized to assess antinociceptive and anti-inflammatory properties. For the evaluation of antipyretic effects and toxicity, male Wistar rats (*Rattus norvegicus*) of the same age range, weighing between 150 to 275 g were employed. The decision to exclude female rodents was made to prevent the hormonal fluctuations associated with the estrous cycle from introducing excessive variability into the data, which could complicate the interpretation of the findings. The rodents were sourced from the Animal Breeding Facility within the Department of Biochemistry, Microbiology, and Biotechnology at Kenyatta University. Before the commencement of the experiments, the rodents were allowed a 72-hour acclimatization period in a controlled laboratory environment, which was maintained a 12-hour light cycle and ambient temperature of 25 ˚C. They had access to food pellets and water *ad libitum*. Additionally, the animals were fasted for 12 hours before the experiments. Careful handling of the animals was ensured to prevent injury or undue stress during the experimental procedures (11), in compliance with established guidelines for laboratory animal care. The use of these animals was approved by the Kenyatta University Animal and Care Committee (PKUA/020/020).

Antipyretic evaluation

The antipyretic assessment of aqueous extracts from *P. africana* was conducted utilizing the turpentine-induced fever model as described by (12). Thirty-six rats were allocated into six groups (Table 1). A digital thermometer was prepared with lubrication and subsequently inserted approximately 3 cm into the rectum of each rat to obtain the rectal temperature. Following the initial temperature measurement, fever was induced by administering 1 mL of turpentine intraperitoneally at a dosage of 20 mL/kg body weight. Rats exhibiting a temperature increase of 0.8 °C one hour post-turpentine injection were classified as pyretic and received various oral treatments. Rectal temperatures were monitored for four hours at one-hour intervals following the administration of treatments.

Antinociceptive evaluation

The antinociceptive effects of aqueous extracts from *P. africana* were evaluated using a modified method established by (12). Thirty-six mice were randomly divided into six groups as detailed in Table 2. A 5% buffered formalin solution was prepared, and 0.05 mL was subcutaneously injected into the sub-plantar area of the right hind limb of each mouse, thirty minutes after they received oral treatments. Pain assessment was conducted by measuring the duration of paw licking in seconds. The mice's responses were monitored during the initial five minutes (neurogenic/early phase) and again at 15-30 minutes (inflammatory/late phase) following formalin administration. The percentage of pain inhibition was calculated using the following formula:

$$\% \text{ Inhibition in licking of the paw} = \frac{N-T}{N} \times 100$$

Where N is the value of the group of negative control at each phase, T is the value of the group treated at each phase.

Anti-inflammatory assessment

The identical mice injected with formalin were employed to assess paw edema. A vernier caliper was utilized to measure the paw edema. The circumferences of the hind paw were recorded as a baseline before the formalin injection. Measurements of paw circumference were taken at one, two, and three hours post-injection and documented in millimeters. Edema assessment was conducted by comparing the baseline measurements with the post-injection paw circumference readings. These values were subsequently converted into paw diameter.

Sub-acute oral toxicity study

A sub-acute oral toxicity study was performed by the test guidelines set forth by the Organization for Economic Cooperation and Development (OECD) 407 (13). The rats were divided into four groups, each consisting of five rats. Group 1 served as the control and received 0.1 mL of normal saline orally, while groups II, III, and IV were administered doses of 150, 260, and 450 mg/kg orally, respectively, daily for 28 days. Throughout the study, the rats were provided with rodent pellets and water. Their weights were recorded before the commencement of dosing, weekly during the treatment phase, and on the day of sacrifice. On the 29th day, the rats were weighed again,

Table 1. the protocol employed to evaluate the antipyretic effects of aqueous leaf extracts of *Prunus africana* in rats

Group	Treatment
I (Normal control)	Normal saline (0.1 mL)
II (Negative control)	Turpentine (1 mL of 20 mL/kg bw) + 0.1 mL normal saline
III (Positive control)	Turpentine (1 mL of 20 mL/kg bw) + 15 mg/kg bw diclofenac in normal saline (0.1 mL)
IV (Experimental A)	Turpentine (1 mL of 20 mL/kg bw) + 50 mg/kg bw extract in normal saline (0.1 mL)
V (Experimental B)	Turpentine (1 mL of 20 mL/kg bw) + 100 mg/kg bw extract in normal saline (0.1 mL)
VI (Experimental C)	Turpentine (1 mL of 20 mL/kg bw) + 150 mg/kg bw extract in normal saline (0.1 mL)

Table 2. The protocol employed to evaluate the antinociceptive and anti-inflammatory properties of *Prunus africana* aqueous leaf extracts in mice

Group	Treatment
I (Normal control)	0.1 mL of Normal saline
II (Negative control)	5% formalin (0.05 mL) + 0.1mL of normal saline
III (Positive control)	5% formalin (0.05 mL) + 15 mg/kg diclofenac in normal saline (0.1 mL)
IV (Experimental A)	5% formalin (0.05 mL) + 50 mg/kg extract in normal saline (0.1 mL)
V (Experimental B)	5% formalin (0.05 mL) + 100 mg/kg extract in normal saline (0.1 mL)
VI (Experimental C)	5% formalin (0.05 mL) + 150 mg/kg extract in normal saline (0.1 mL)

and following an overnight fast, they were euthanized using chloroform. This sacrifice was essential for the sub-acute toxicity assessment to facilitate the collection of blood samples for serum analysis and to inspect the organs for any indications of toxicity. The relative organ weights of the liver, kidney, and heart for each rat were calculated as documented in the literature (14).
Relative organ weight = [Absolute organ weight (g) / Body weight (g) of rats on sacrificial day] × 100.

Biochemical parameters screening

On the 29th day, all rats were sacrificed, and blood was extracted from each rat's heart. One milliliter of blood was divided into two portions; one portion was placed in standard vials, while the other was placed in vials containing ethylenediaminetetraacetic acid (EDTA). The blood samples in both the EDTA-free and standard vials were subsequently subjected to centrifugation at 3000 rpm for 10 minutes in a Bench Centrifuge to separate plasma and serum, respectively, which were then promptly analyzed using the Biochemistry Mindra (Bs-230 model). The serum biomarkers assessed included globulin, albumin, alanine aminotransferase, total protein, aspartate aminotransferase, creatinine, uric acid, phosphorus and glucose.

Statistical analysis

Statistical analysis was conducted using One-way repeated measures ANOVA, followed by Bonferroni correction across the time intervals for each experiment, while One-way ANOVA with Tukey's post hoc test was employed for multiple comparisons. The significance threshold was set at $P<0.05$.

Results

Percentage yield and LC-MS analysis of *Prunus africana* aqueous leaf extract

The aqueous leaves of *P. africana* yielded 46.27 g of crude extract, corresponding to a yield of 9.25% (w/w). The identification of the compounds was conducted using linear calibration curves represented by the equation: [Y = 5578.4X – 39094 (R2 = 0.9990)], which facilitated the external quantification of the identified compounds. In total, 25 compounds were identified and quantified in the aqueous leaves of *P. africana*, comprising 9 amino acids, 10 flavonoids, 1 phenolic diterpene, and 5 phenolic acids, utilizing an LC-MS quadrupole. Figure 1 illustrates the chromatograms obtained in positive ion mode, while Table 3 details the abundance of each compound.

Mineral composition

Various concentrations of macro and microelements were detected and measured in the aqueous leaf extract of *P. africana* (Table 4). The x-axis represents the energy of the emitted X-rays, while the y-axis denotes the intensity of these X-rays (Figure 2). The peaks observed in the spectrum correspond to specific elements, with the peak energy indicating the element and the peak intensity (height) reflecting its concentration.

In vivo antipyretic effects

All administered doses of the extract and diclofenac significantly reduced rectal temperature from the initial measurement to the second hour ($P \leq 0.05$), achieving levels comparable to the normal control. In the third and fourth hours, rectal temperature remained consistent with that of the second hour, as illustrated in Table 5. Furthermore, all doses of the extract and diclofenac exhibited a lower temperature after the fourth hour when compared to the negative control.

In vivo anti-inflammatory effects

The reduction of paw edema was consistently observed from the first to the third hour for both the 50 and 100 mg/kg doses of the extract and diclofenac. Additionally, these doses produced a paw size smaller than that of the negative control by the end of the third hour. In contrast, the 150 mg/kg dose showed no inhibition of paw edema (see Table 6), although the edema was still reduced than that seen in the negative control.

In vivo antinociceptive effects

During the initial phase, all extract doses (50, 100, and 150 mg/kg) along with diclofenac reduced pain levels by 9.38%, 11.51%, 13.07% and 7.58%, respectively, when compared to the negative control group. In the second phase, the extract doses of 50, 100 and 150 mg/kg as well as diclofenac reduced pain by 86.40%, 85.05%, 45.07% and 68.46%, respectively, compared to the negative control group. In the second phase, the 50 and 100 mg/kg doses

Table 3. Phytochemical profile of the *Prunus africana* aqueous leaf extract analysed by liquid chromatography-mass spectroscopy in positive ion mode

Retention time (min)	Abundance (%)	Chemical formula	Compound
5.12	2.3	$C_6H_{14}N_2O_2$	Lysine
5.95	13.5	$C_5H_9NO_2$	Proline
6.57	7.6	$C_5H_{11}NO_2$	Valine
8.32	0.4	$C_5H_{11}NO_2S$	Methionine
11.21	12.4	$C_6H_{13}NO_2$	Isoleucine
12.03	13.6	$C_6H_{13}NO_3$	Leucine
12.85	2.6	$C_9H_{11}NO_3$	Tyrosine
14.29	0.6	$C_{15}H_{14}O_6$	Catechin
17.10	0.3	$C_4H_9NO_3$	Threonine
17.30	3.1	$C_{20}H_{28}O_4$	Carsonic acid
18.60	3.5	$C_{16}H_{12}O_7$	Isorhamnetin
18.60	3.6	$C_{15}H_{14}O_6$	Protocathechuic acid hexoside
18.72	15.0	$C_9H_{11}NO_2$	Phenylalanine
19.76	5.7	$C_{18}H_{16}O_8$	Rosmarinic acid
20.14	2.7	$C_{16}H_{14}O_{16}$	Hesperetin
23.75	1.2	$C_{16}H_{18}O_9$	3-O-caffeoylquinic acid
24.71	0.2	$C_{27}H_{30}O_{16}$	Rutin
25.13	1.9	$C_{16}H_{18}O_9$	5-O-caffeoylquinic acid
25.13	0.4	$C_{15}H_{10}O_6$	Kaempferol
25.34	0.4	$C_{15}H_{10}O_{16}$	Luteolin
25.86	1.3	$C_{16}H_{18}O_8$	Coumaroyl quinic acid
26.22	0.7	$C_{15}H_{10}O_6$	Scutellarein
26.72	0.4	$C_{15}H_{10}O_6$	Kaempferol 3-O-glucoside
28.00	0.5	$C_{23}H_{22}O_{12}$	Quercetin acetyl hexoside
28.00	0.3	$C_{16}H_{14}O_5$	Sakuranetin

of the extract demonstrated a greater pain inhibition than diclofenac. However, at the 150 mg/kg dose, the leaf extract exhibited a lesser pain inhibition compared to diclofenac and the lower doses (refer to Table 7).

Sub-acute oral toxicity effects

In the investigation of sub-acute toxicity, rats administered doses of 260 and 450 mg/kg of the extract exhibited no significant weight gain during the third and fourth weeks. Conversely, at a dose of 150 mg/kg, the rats experienced weight gain until the third week, followed by a lack of significant weight gain in the fourth week, mirroring the control group (refer to Table 8). The weight change (the difference in body weight recorded at the end of the study compared to the initial weight) was consistent across all groups; however, the liver size was reduced at the doses of 260 and 450 mg/kg (Table 8).

Serum biochemistry

The oral administration of the aqueous extract of *P. africana* for 28 days resulted in a significant reduction in total protein, globulins, and phosphorus levels at doses of 260 and 450 mg/kg when compared to the normal control group ($P < 0.05$). Additionally, the glucose concentration at the 450 mg/kg dosage was lower than that of the normal control ($P < 0.05$). Furthermore, there was a decrease in both creatinine and uric acid levels across all administered doses relative to the normal control rats ($P < 0.05$) (Table 9).

Discussion

The bioactive components of *P. africana* exhibit significant diversity, influenced by geographical, genetic, and environmental factors (15). This research uncovered 25 compounds in the aqueous leaf extract of *P. africana* sourced from Kakamega County, Kenya, including nine amino acids previously unreported. The LC-MS analysis of the methanol bark extract from Uganda identified compounds such as chlorogenic acid, astragalin, luteoloside, hyperin, naringenin, and mesoporphyrin IX (16), with only chlorogenic acid being common with the findings of this study. Another investigation identified various compounds, including ursolic acid, isoliquiritin, prunetin, procyanidin B5, and tocopherolcinnamtannin

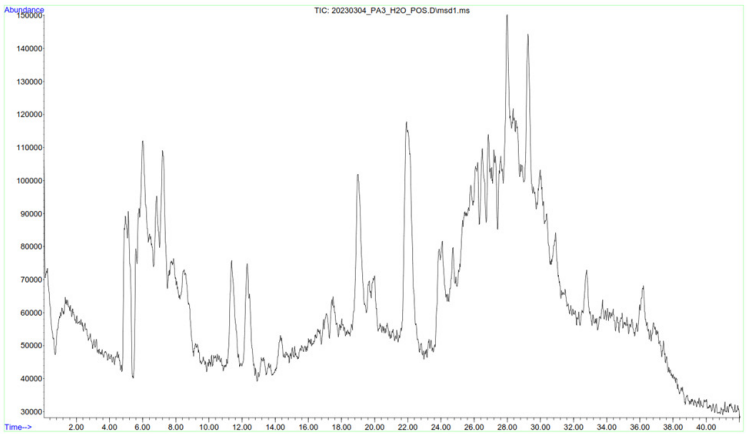


Figure 1. Chromatogram of the aqueous leaf extract *of Prunus africana* acquired through liquid chromatography-mass spectrometry using a single quadrupole. The chromatogram indicates the peak shape of an analyte, with the height of the peak proportional to the abundance (concentration) of that analyte at a specific retention time.

Table 4. The concentration of mineral elements in the aqueous leaf extracts of *Prunus africana*

Mineral element	Concentration (µg/g)	Quantities of mineral given orally to each rat for the three doses (µg/g) Extract doses (mg/kg weight of the body)			RDA (µg/day)
		150	260	450	
Cl	165 ± 14	3.58875	6.2205	10.76625	1673.076923
K	3810 ± 258	82.8675	143.637	248.6025	4461.538462
Ca	763 ± 91	16.59525	28.7651	49.78575	2230.769231
Ti	4.55 ± 0.55	0.0989625	0.171535	0.2968875	1.963076923
Mn	11.20 ± 0.20	0.2436	0.42224	0.7308	5.130769231
Fe	6.36 ± 0.86	0.13833	0.239772	0.41499	17.8461385
Ni	1.04 ± 0.02	0.02262	0.039208	0.06786	0.58
Cu	0.56 ± 0.04	0.01218	0.021112	0.03654	3.346153846
Zn	5.84 ± 0.01	0.12702	0.220168	0.38106	24.53846154
Br	1.35 ± 0.15	0.029145	0.050895	0.0880875	17.84615385
Rb	9.72 ± 0.11	0.21141	0.3664444	0.63423	11.15384615
Sr	9.77 ± 0.58	0.2124975	0.368329	0.6374925	4.305384615
Pb	< 0.05	< 0.0010875	< 0.001885	< 0.0032625	0.2230769231

Results are shown as mean ± SD (n = 3). The Mean falls below the detection limit of the TXRF system.

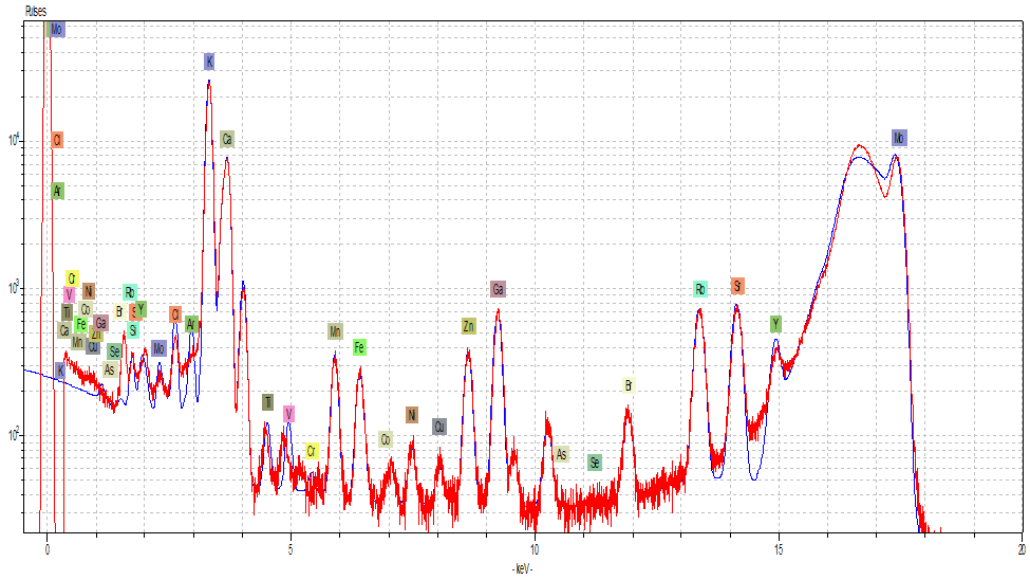


Figure 2. The spectrum obtained from the total X-ray fluorescence analysis of the aqueous leaf extract of *Prunus africana*. The peaks observed in the spectrum correlate to specific elements, with the peak energy specifying the element and the peak height representing its concentration.

A2, in the aqueous bark extracts from Muguga, Nairobi County, Kenya, none of which were found in this study (9). Conversely, the methanol leaf and stem bark extracts from Kereita forest in Kiambu County, Kenya, revealed the presence of catechin, chlorogenic acid, oleic acid, kaempferol, rutin, apigenin, quercetin, zeatin, campesterol, and ursolic acid, with six compounds overlapping with those identified in this research (17).

The phytochemicals identified in this research exhibited antipyretic, anti-inflammatory, and analgesic properties. Quercetin's analgesic effects are mediated through opioidergic and GABAergic pathways, and it mitigates inflammation by inhibiting the upregulation of mitogen-activated protein kinases as well as the production of TNF-α and IL-1β (18). Rutin alleviates inflammatory pain by inhibiting P2X7 receptors in mast cells (19). Both rutin and quercetin also decrease the expression of inducible nitric oxide synthase (iNOS), cyclooxygenase 2 (COX-2), and nuclear factor κappa B (NF-κB) during inflammatory responses (20). Luteolin obstructs critical inflammatory signaling pathways, such as NF-κB and Janus Kinase-Signal Transducer and Activator of Transcription (JAK-STAT), leading to a reduction in the expression of inflammatory mediators like TNF-α, COX-2, IL-6, and iNOS, while alleviating pain through the GABAergic system (21). Protocatechuic acid exerts its anti-inflammatory effects by inhibiting c-Jun N-terminal Kinase (JNK) (22) and its analgesic effects are achieved

Table 5. Effects of the aqueous leaf extracts of *Prunus africana* on turpentine-induced fever in rats

Treatment	Temperature (°C)				
	0 h	1 h	2 h	3 h	4 h
Normal control	36.08±0.07^{Aa}	36.05±0.04^{Aa}	36.03±0.02^{Aa}	36.07±0.05^{Aa}	36.15±0.10^{Aa}
Negative control	38.22±0.26^{Ba}	38.15±0.26^{Ca}	38.27±0.21^{Ba}	38.12±0.15^{Ba}	38.23±0.07^{Ba}
Diclofenac 15 mg/kg	38.20±0.08^{Bc}	37.35±0.10^{Bb}	36.40±0.09^{Aa}	36.27±0.10^{Aa}	36.25±0.16^{Aa}
50 mg/kg *Prunus africana*	38.18±0.10^{Bc}	37.33±0.14^{Bb}	36.40±0.09^{Aa}	36.38±0.08^{Aa}	36.37±0.06^{Aa}
100 mg/kg *Prunus africana*	38.08±0.11^{Bd}	37.58±0.08^{BCc}	36.48±0.07^{Ab}	36.07±0.08^{Aa}	36.00±0.07^{Aa}
150 mg/kg *Prunus africana*	38.07±0.07^{Bc}	37.48±0.14^{Bb}	36.40±0.08^{Aa}	35.98±0.06^{Aa}	36.17±0.06^{Aa}

Results are presented as mean ± SEM (n = 6). Values sharing different upper-case letters down the column indicate significant difference at $P < 0.05$, as determined by independent One-way ANOVA and Tukey's post hoc analysis for comparisons among the six groups at each time point. Similarly, values with different lower-case letters along the column show significant difference at $P < 0.05$, analyzed using Repeated Measures ANOVA followed by Bonferroni correction for comparisons across the five time points for each group.

Table 6. Anti-inflammatory effects of the aqueous leaf extracts of *Prunus africana* on formalin-induced edema in mice

Treatment	Paw diameter (mm)			
	0 h	1 h	2 h	3 h
Normal control	4.35±0.07^{Aa}	4.35±0.07^{Aa}	4.35±0.07^{Aa}	4.35±0.07^{Aa}
Negative control	4.75±0.03^{Bb}	5.05±0.05^{Ca}	5.07±0.03^{Ca}	5.07±0.06^{Ca}
Diclofenac 15 mg/kg	4.67±0.07^{Bc}	4.46±0.06^{ABb}	4.38±0.05^{Aab}	4.33±0.06^{Aa}
50 mg/kg *P. africana*	4.70±0.05^{Bb}	4.49±0.05^{ABa}	4.41±0.03^{Aa}	4.38±0.05^{Aa}
100 mg/kg *P. africana*	4.65±0.06^{Bb}	4.43±0.05^{ABa}	4.33±0.06^{Aa}	4.30±0.07^{Aa}
150 mg/kg *P. africana*	4.67±0.07^{Ba}	4.67±0.07^{Ba}	4.67±0.07^{Ba}	4.67±0.07^{Ba}

Results are displayed as Mean ± SEM (n = 6). Values sharing the same upper-case letters down the column are not significantly different at $P < 0.05$, as determined by independent one-way ANOVA and Tukey's post hoc test for comparisons among the six groups at each time point. Values sharing the same lower-case letters along the column are not significantly different at $P < 0.05$, as assessed by Repeated Measures ANOVA followed by Bonferroni correction for comparisons across the five time points for each group.

Table 7. Antinociceptive effects of the aqueous leaf extract of *Prunus africana* on formalin-induced pain in mice

Treatment	Paw licking in seconds	
	Early phase (1-5 min)	Late phase (15-30 min)
Normal control	0.00±0.00 (100.00±0.00)^{c}	0.00±0.00 (100.00±0.00)^{e}
Negative control	206.17±2.39 (0.00+0.00)^{a}	279.33±13.33 (0.00±0.00)^{a}
Diclofenac 15 mg/kg (Positive control)	190.33±15.87 (7.58±1.87)^{b}	88.17±15.99 (68.46±5.81)^{c}
50 mg/kg *P. africana*	184.50±6.14 (10.51±2.73)^{b}	41.00±7.33 (86.40±3.11)^{de}
100 mg/kg *P. africana*	179.17±3.77 (13.07±1.76)^{b}	43.00±9.76 (85.05 ±3.06)^{d}
150 mg/kg *P. africana*	186.83±3.82 (9.38±1.45)^{b}	153.17±7.46 (45.07±1.55)^{b}

Results are shown as mean ± SEM (n = 6). Parenthesis values reveal the percentage of inhibition in paw licking relative to the negative control. Values with different letters differ significantly ($P < 0.05$). Statistical analysis was performed using independent One-way ANOVA, followed by Tukey's test for comparisons down the column.

by targeting KATP type K^+ channels and adenosine A1 receptors (23). Chlorogenic acid demonstrates anti-inflammatory activity by upregulating and inhibiting COX-2 through the attenuation of NF-κB and JNK/activation protein-1 (AP-1) signaling pathways, while its analgesic effects are mediated via the GABAergic system (24). Carnosic acid influences various inflammatory signaling pathways, including MAPK, NF-κB, and STAT3, and its antipyretic effects are realized by blocking the formation of prostaglandin-E2 (PGE2) through selective inhibition of microsomal prostaglandin E2 synthase-1 (mPGES-1) (25). Lysine inhibits inflammation by modulating the phosphorylation of Extracellular Signal-Regulated Kinase (ERK) and NF-κB within the MAPK signaling pathway (26), while exerting analgesic effects by modulating the sodium channel Nav1.8 system (27). Zinc (Zn) serves as an analgesic agent by binding with high affinity to the NR2A subunit of the NMDA receptors (28). Additionally, Zn^{2+} modulates inflammation by enhancing IKBα levels and preventing the activation of the NF-κB signaling pathway (29). Leucine and isoleucine regulate the phosphorylation of NF-kB and JNK, as well as the production of IL-8, serving as anti-inflammatory agents (30). Methionine acts to suppress the activation of the NF-κB signaling pathway (31). Collectively, these compounds may have functioned in a synergistic or additive manner

Table 8. Mean body weight (grams) of rats and organ index following the daily oral administration of aqueous leaf extracts of *Prunus africana* for 28 days

Treatment (mg/kg)	Weekly weight of normal rats (g)					Mean Δ in weight	Organ index (g)		
	0 day	7th day	14th day	21th day	28th day		Heart	Liver	Kidneys
Normal control	126.00±7.50^{a}	175.20±6.97^{b}	197.20±8.90^{c}	219.00±7.99^{d}	225.80±7.53^{d}	99.8±6.41^{A}	0.43±0.02^{A}	3.84±0.156^{B}	0.76±0.02^{A}
150	141.20±4.19^{a}	172.40±7.37^{b}	196.20±6.65^{c}	230.20±4.96^{d}	239.40±5.12^{d}	98.2±5.71^{A}	0.37±0.01^{A}	3.50±0.04^{AB}	0.74±0.03^{A}
260	140.60±3.53^{a}	193.00±7.06^{b}	215.40±6.09^{c}	229.80±7.19^{c}	230.60±6.91^{c}	90.00±5.17^{A}	0.39±0.02^{A}	3.36±0.07^{A}	0.73±0.03^{A}
450	142.40±7.55^{a}	175.20±7.94^{b}	219.00±4.61^{c}	231.00±8.34^{c}	233.00±7.46^{c}	90.6±6.85^{A}	0.39±0.02^{A}	3.39±0.05^{A}	0.75±0.03^{A}

Results are presented as mean ± SEM (n = 5). Values sharing the same upper-case letters down the column are not significantly different at $P < 0.05$, as determined by independent One-way ANOVA and Tukey's post hoc test for comparison with the normal control. Values sharing the same lower-case letters along the column are not significantly different at $P < 0.05$, as assessed by repeated measures ANOVA followed by Bonferroni correction for comparison across the five time points for each group.

Table 9. Effects of administering aqueous leaf extracts of *Prunus africana* orally daily for 28 days on the serum biochemistry of rats

Biochemical parameters	Treatment doses (mg/kg body weight)			
	Control	150	260	450
TP (g/L)	76.32±0.99^{B}	72.12±1.12^{AB}	69.96±1.24^{A}	70.96±1.79^{A}
ALB (g/L)	36.48±0.45^{A}	36.76±0.67^{A}	35.76±0.96^{A}	37.32±1.69^{A}
GLB (g/L)	39.84±0.72^{B}	35.12±0.36^{AB}	34.20±0.72^{A}	33.64±1.97^{A}
AST (U/L)	226.40±12.35^{AB}	239.94±19.93^{B}	208.16±24.50^{AB}	176.60±19.75^{A}
ALT (U/L)	56.16±1.96^{AB}	64.26±3.11^{B}	55.28±5.85^{AB}	49.08±3.84^{A}
GLU (mmol/L)	4.66±0.10^{B}	4.14±0.20^{AB}	4.06±0.17^{AB}	3.86±0.50^{A}
ALP (U/L)	145.40±10.88^{A}	162.64±2.89^{A}	141.00±6.99^{A}	143.88±12.96^{A}
GGT (U/L))	1.04±0.17^{A}	1.40±0.36^{A}	1.04±0.32^{A}	1.52±0.30^{A}
CREAT (µmol/L)	75.52±3.92^{C}	50.96±1.34^{A}	51.44±3.30^{A}	62.44±0.80^{B}
UA (µmol/L)	78.68±2.43^{C}	69.20±1.16^{B}	64.92±3.16^{B}	55.38±1.41^{A}
PHOS (mmol/L)	1.50±0.02^{B}	1.43±0.03^{B}	1.19±0.07^{A}	1.15±0.04^{A}

Results are displayed as mean ± SEM (n = 5). Values sharing the same letters across the rows are not significantly different at $P < 0.05$ compared to the normal control; this was assessed using one-way ANOVA followed by Turkey's test. GLB refers to globulin, ALB to albumin, ALT to alanine aminotransferase, TP to total protein, AST to aspartate aminotransferase, UA to uric acid, CREAT to creatinine, PHOS to phosphorus, and GLU to glucose.

to reduce pain and inflammation.

The administration of formalin into the hind paw triggers a biphasic pain response; the initial phase is due to the direct activation of nociceptors, whereas the subsequent phase involves a period of central sensitization and sensory input in the dorsal horn, during which inflammatory processes take place. Central analgesics work by elevating the pain threshold and modifying the body's physiological response to pain, while peripheral analgesics function by inhibiting the generation of impulses at the chemoreceptor pain site (32).

In this research, both *P. africana* and diclofenac demonstrated a significant reduction in pain during both phases compared to the negative control, with a more pronounced inhibition observed in the late phase than in the early phase. This suggests that the plant extracts were more effective in alleviating inflammatory pain. In the first phase, the pain relief was minimal, indicating that the extract contained a lower concentration of phytochemicals recognized for increasing pain thresholds, thereby obstructing the transmission of pain signals and modifying the perception of pain. The analgesic effect of aqueous extracts from *P. africana* was independent of dosage during the late phase. The doses of 50 and 100 mg/kg demonstrated a significantly greater decrease in paw licking compared to the 150 mg/kg dose and diclofenac. Various medications can interact in ways that diminish the overall effect, leading to a result that is less than expected, or conversely, can produce a more substantial effect than anticipated, depending on their individual properties. A specific concentration of the drugs is required to achieve synergistic efficacy. The inhibitory effect is due to the saturation of binding sites at higher concentrations of drugs, leading to a substantial amount of the drug remaining unbound in the plasma, which allows for distribution to other organs and subsequently results in antagonistic effects. The compound potentially involved in this process may be proline, which at high concentrations is associated with the enhancement of glutamate activity, thereby increasing excitatory synaptic transmission in the spinal region by depolarizing neurons and elevating synaptic activity (33).

Formalin-induced paw edema in rodents serves as an effective method for assessing acute inflammation, closely resembling human arthritis (34). The administration of doses of 50 and 100 mg/kg of the extract and diclofenac led to a decrease in paw size in comparison to the negative control. It is possible that the plant mitigated inflammation in a manner akin to diclofenac by inhibiting the binding of arachidonic acid to COX-2 and COX-1, thereby decreasing the synthesis of prostanoids such as PGE2, thromboxanes, and prostacyclins, which are key contributors to inflammatory responses (35). However, the administration of 150 mg/kg dose of the extract did not result in a reduction of paw size after 3 hours, although the size did not increase to match that of the negative control. This may be attributed to the phenomenon where, at higher drug concentrations, the quantity of the drug that binds approaches the maximum limit dictated by the availability of binding sites. Consequently, this leads to the saturation of active sites, leaving a significant portion of the drug unbound in the plasma, which then distributes to other organs, producing inhibitory effects. This phenomenon can be ascribed to branched-chain amino acids, specifically leucine, valine, and isoleucine, which, when present in elevated concentrations, promote inflammation, oxidative stress, and the migration of human peripheral blood mononuclear cells through the activation of mTORC1 (36). A previous research indicated a contrasting result regarding the anti-inflammatory properties of the methanol extract from the stem bark of *P. africana* sourced from Meru County, Kenya, revealing that a dosage of 150 mg/kg exhibited a more significant effect compared to the lower dosages of 50 and 100 mg/kg (37). This discrepancy may be attributed to the variations in phytochemical constituents present in these plants.

Fever is a component of the acute phase response to inflammation, tissue injury, or infection. After four hours of monitoring, the aqueous leaf extracts of *P. africana* demonstrated a notable decrease in the temperature of the pyretic rats, bringing it down to the level observed in the normal control rats. This effect was comparable to that of diclofenac, which functions by inhibiting the synthesis of prostaglandin E2 (38). All three administered doses resulted in a comparable effect in reducing fever. The optimal effective dose of the extract for fever management is 50 mg/kg, as higher doses do not yield any notable efficacy.

This research indicates that the administration of leaf extracts from *P. africana* resulted in liver damage. Symptoms of liver injury were observed at doses of 260 and 450 mg/kg. This was demonstrated by a reduction in liver size, hypoglycemia due to severe liver impairment affecting glucose metabolism, decreased globulin levels from diminished production linked to liver damage (39), hypophosphatemia from increased phosphate uptake for hepatocyte regeneration (40), hypouricemia resulting from the lack of a substance that facilitates uric acid reabsorption in the liver, and a decline in creatinine levels due to a greater than 50% reduction in hepatic creatine production (41). In contrast, the aqueous leaf extracts of *P. africana* obtained from Mount Kenya showed no indications of toxicity during sub-acute testing at doses of 50, 100, 200, and 250 mg/kg (8).

Conclusion

Based on the findings of this research, it can be concluded that the aqueous leaf extract of *P. africana* sourced from Kakamega County exhibits significant acute anti-inflammatory properties, along with antinociceptive and antipyretic effects. Additionally, the aqueous leaf extract of *P. africana* may induce toxic effects at doses of 260 and 450 mg/kg. However, since the therapeutic doses administered for alleviating fever, pain, and inflammation are below these thresholds, adverse reactions are not anticipated. Notably, the aqueous leaf extract of *P. africana* contained a variety of phytochemicals that have not been previously identified in earlier extracts of this species. Prior investigations on *P. africana* primarily utilized the stem bark, employed different extraction solvents, and sourced the plants from various locations. This variation may account for the differences in acute anti-inflammatory and antinociceptive effects, as well as sub-acute toxicity, when compared to earlier studies involving *P. africana*.

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Conflict of interests

Authors declare no conflict of interest.

Ethical considerations

The ethical clearance was approved by the Kenyatta University Animal and Care Committee (PKUA/020/020).

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