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Original article

Phytochemical and Anti-Inflammatory Analysis of *Prunus africana* Bark Extract

Gitonga Godfrey Mutuma[1*], Ngeranwa Joseph[2], Machocho Alex King'ori[3], Kiruki Silas[4]

[1]Department of Medical Laboratory Sciences, School of Public Health, Biomedical Sciences and Technology, Masinde Muliro University of Science and Technology, Kakamega, Kenya.
[2]Department of Biochemistry, Microbiology and Biotechnology, Kenyatta University, Nairobi, Kenya.
[3] Department of Chemistry, Kenyatta University, Nairobi, Kenya.
[4]Department of Physical Sciences, Chuka University, Chuka, Kenya.

Abstract

Background and objectives: Inflammation is associated with various diseases; *Prunus africana* (Hook f.) is commonly used in Meru community of Kenya in management of inflammation. Medicinal plants contain phytochemicals associated with pharmacological activities; so, the aim of the present study was evaluating the anti-inflammatory activity of *Prunus africana* bark extract and qualitative analysis phytochemical of its phytochemicals. **Methods:** Five hundred mg of the powdered *P. africana* stem bark was extracted using 1.5 liters of dichloromethane for 24 h. The anti- inflammatory activity was evaluated against carrageenan paw induced edema in mice. The ability of the extracts to suppress the paw inflammation was expressed as a percentage inhibition of paw edema in five groups each comprising of five mice. Group I was treated with DMSO, group II with diclofenac (100 mg/Kg) and experimental groups III, IV and V with 50, 100 and 150 mg/Kg of the plant extract. The ability of the extracts to suppress the paw inflammation was expressed as a percentage inhibition of paw edema in mice. The qualitative phytochemical analysis was conducted using the standard protocols. **Results:** The percentages paw edema inhibition after the 4th h in the positive control and the experimental groups I, II and III were 13.61, 32.85, 25.15 and 5.92%, respectively. The qualitative evaluation of stem bark extract illustrated presence of tannins, saponins, flavonoids, alkaloids, quinones, cardiac glycosides, terpenoids, phenolics and coumarins. **Conclusion:** Dichloromethane stem bark extract of the *P. africana* presented anti-inflammatory activity hence a possible candidate for extraction of active anti-inflammatory compounds.

Keywords: diclofenac; inflammation; mice; phytochemistry; *Prunus africana*

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Introduction

Inflammation is a symptom associated with numerous diseases and can either be acute or chronic. Inflammation and pain are interrelated because inflammatory reactions lead to induction of genes that code for peripheral nociceptors of the dorsal ganglion resulting to increased nociceptors sensitivity [1]. Inflammation is associated with different diseases states pathogenesis and is a process associated with tumour development stages especially the initial stage, promotion, conversion of normal cell to malignancy, metastasis and invasion of other body tissues. It affects the immune system and how the body responds to various drug therapies [2]. It is common in depressive illness conditions. Acute inflammation cases are usual amongst those who are suffering from depression [3]. Administration of exogenous cytokines is associated with typical depressive behavior in health individuals [4]. Acute inflammation can be induced in laboratory animals by administering

*Corresponding author: mutumagitonga1@gmail.com

histamine [5], dilute formalin, carrageenan and lipopolysaccharide [6]. It can be quantified by checking various parameters such as paw edema [6], pro-inflammatory cells migration [7], pleurisy and lung edema [8,9], and cytokines levels [10]. Steroids and non-steroid anti-inflammatory drugs (NSAIDs) are commonly used in management of inflammation. The steroidal anti-inflammatory drugs inhibit the migration and leukocytes degranulation. The non-steroid anti-inflammatory drugs (NSAIDs) inhibit cyclo-oxygenase enzyme activity. Plant extract administration is commonly used in inflammatory condition management since they are associated with little or no adverse side effects when compared with conventional drugs [11].

Prunus africana (Hook f.) species belongs to the genus *Prunus* which comprises more than 400 species. The species of great importance are about 98. Mature *P. africana* stem measures up to 1 m in diameter and the plant can grow to attain a height of approximately more than 40 m [12]. The plant bark is blackish-brown, the leaves are simple, oval shaped, alternate, shiny light green on the underside and deep green on the top side. The flowers are greenish to white in colour. The fruits are pinkish-brown, bilobed, spherical in shape measuring approximately 7 mm in length and 1.3 cm in width and when they are ripe, the thin fruit pulp turns dark red to reddish brown in colour [13]. It is mostly found in highlands with an altitude of 1500 m above sea level and is a common plant in East, Africa and West Africa, Madagascar and Comoros [14]. *Prunus africana* bark is commonly used by herbalists in management of health problems such as cancer, inflammation, viral infection [15] and benign prostate hyperplasia [16]. The pharmacological effect of the *P. africana* bark extract is associated with synergistic effect of the phytochemical compounds such as the triterpenoids [17], phytosterols [18] and ferulic acid esters [19]. Traditionally, in management of malaria, kidney disease, chest pain, bladder infections, urinary tract infections and stomach aches the bark is chewed or crushed into powder which is drunk like tea [20]. The aim of this study was to evaluate the anti-inflammatory activity of dichloromethane stem bark extracts of *P. africana* using animal model.

Materials and Methods

Ethical consideration

All animal rights and conservation in this study was in accordance to the standard ethical guidelines of the European Communities Directive 2010/63/EU (NIH, publication no. 85 – 23, revised 1985). The study protocol was approved by the Kenyatta University animal care and use committee (KU ACUC) and National Commission for Science Technology and Innovation (NACOSTI/P/16/32165/27654).

Plant material

The stem bark samples were collected from Ruriine village, North Imenti sub- county in Meru County, Kenya during August to October 2016. Local herbalists in the community were involved in selection, identification and sample collection process. The plants were authenticated by Dr. Fredrick Munyao, an acknowledged plant taxonomist at the National Museums of Kenya Herbarium. A sample voucher number NMK/BOT/PAF1/2 was deposited at the National Museum of Kenya for future reference. The *P. africana* stem bark samples were dried in a well-ventilated room and later ground using an electrical mill into fine powder.

Extraction

Five hundred mg of the powdered *P. africana* stem bark was extracted using 1.5 liters of dichloromethane for 24 h. The mixture was occasionally shaken to ensure proper mixing with the solvent. It was filtered using Whatman's filter paper No. 1 after 24 h. The filtrate was concentrated using a rotary evaporator at a temperature of 40 °C, packed in air tight containers and preserved in a freezer before the bio-screening and bioassay experimentation.

Qualitative phytochemical screening

The qualitative analysis of phytochemicals was determined following the standard protocols [21]. The following phytochemicals were tested: alkaloids, anthocyanin and betacyanin, carbohydrates, cardiac glycosides, coumarins, flavonoids, glycosides, phenolics, quinones, saponins, steroids, tannins and terpenoids.

Experimental design

Experimental animals

The anti-inflammatory testing of the plant extract was evaluated using adult healthy Swiss albino male mice weighing 25-30 g. The animals were obtained from Department of Zoological Sciences, Kenyatta University. They were given standard pellet diet and water. The experimental animals were housed in six wire mesh cages, each with five animals measuring approximately

30×30×30 cm raised about 0.75 m from the ground. The animal house ambient temperature was maintained at 25 ± 2 °C, with a normal photoperiodicity of 12 h and humidity of 35-60%. Daily replacement of wood shavings bedding was observed for hygiene purposes. The cages were well labeled from group I–VI and the experimental animals were supplied with adequate standard rodent pellets obtained from Unga limited, Kenya and water ad libitum.

Anti-inflammatory activity

Anti-inflammatory activity of *P. africana* stem bark extract was set against carrageenan paw induced edema in mice and the outcome was evaluated [22]. Edema was induced in all five animal groups through subplantar injection of the freshly prepared suspension of 0.05 mL of 1% carrageenan. Group I served as the normal control and was treated with 10% DMSO intraperitoneally. Group II served as the negative control and was treated with 10% DMSO and freshly prepared suspension of 0.05 mL of 1% carrageenan. Group III served as the positive control and was treated with freshly prepared suspension of 0.05 mL of 1% (w/v) carrageenan and conventional anti-inflammatory drug (diclofenac) intraperitoneally. Group IV, V and VI were treated with 50, 100 and 150 mg/kg stem bark extract of *P. africana* intraperitoneally and freshly prepared suspension of 0.05 mL of 1% (w/v) carrageenan, respectively.

The linear circumference of the injected paw was measured in mm at 0, 1, 2, 3 and 4 after administration of carrageenan using digital vernier caliper (27 SDC041, Xuzhou Smile Trading Company Ltd., China) and recorded. The increase in paw circumference at 1, 2, 3 and 4 h after administration of carrageenan injection was adopted as the parameter for measurement of inflammation. The plant extract was administered one hour after the administration of carrageenan. The experimental animals were euthanized at the end of the study through cervical dislocation. The ability of the extracts to suppress the paw inflammation was expressed as a percentage inhibition of paw edema and was calculated according to the following equation [23]:

$$\% \text{ Paw edema inhibition} = \{(Ct - Tt)/Ct\} \times 100$$

Where Ct = Paw diameter 1 h post carrageenan administration (control); Tt = Paw diameter post treatment with plant extract at an interval of one to four h.

Statistical analysis

The values of the parameters used to ascertain the anti-inflammatory activity of the dichloromethane stem bark extract of *P. africana* was analyzed using one way analysis of variance (ANOVA) to give the descriptive statistics and the data was summarized in terms of mean ± SEM.

The quantitative data obtained on the change in paw diameter were all collected and recorded in MS–Excel. The data was subjected to descriptive statistics using version 17.0 of the Minitab statistical software package (Minitab Inc., 2017). The Analysis of Variance (ANOVA) and Tukey's post hoc test was done to compare the means separation. The data analysis was set at 95% confidence level with statistical significance of $p \leq 0.05$. All quantitative and qualitative data were presented in graphs and tables.

Results and Discussion

The qualitative evaluation of of *P. africana* extract showed presence of tannins, saponins, flavonoids, alkaloids, quinones, cardiac glycosides, terpenoids, phenols and coumarins (table 1).

Table 1. Qualitative phytochemical compositions of dichloromethane stem bark extract of *Prunus africana*

Phytochemicals	Dichloromethane stem bark extract of *Prunus africana*
Carbohydrates	++
Tannins	++
Saponins	+
Flavonoids	++
Alkaloids	++
Anthocyanin and betacyanin	-
Quinones	+
Glycosides	-
Cardiac glycosides	+
Terpenoids	++
Phenols	+
Coumarins	+
Steroids	++

Key: **+**=trace, ++=moderate, +++=intense, **-** =not present

The anti-inflammatory activity of *P. africana* stem bark extract was elucidated using carrageenan-induced paw edema in mice. The plant extract and diclofenac anti-inflammatory activities were as indicated in the table 2 and figure 1. One hour after administration of the plant extract and the conventional drug (diclofenac), the anti-inflammatory effects and percentage paw edema inhibition in the experimental mice in the normal control, negative control, positive control and the experimental groups A, B and C were 79.58, 79.58, 90.91 and 77.70% respectively (figure 1). The paw edema value in the negative control, positive control and experimental groups A, B and C were 1.06±0.07, 0.28±0.04, 0.28±0.06, 0.16±0.05 and 0.30±0.03, respectively (table 2).

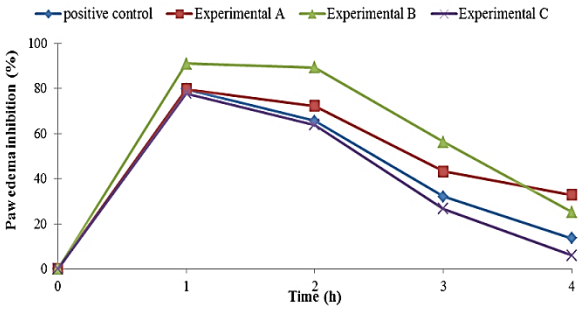

Figure 1. The inhibition percentage of paw edema by dichloromethane stem bark extracts of *Prunus africana* in mice model; the animals in experimental group A, B and C were treated with dichloromethane stem bark extract of *P. Africana* at a dose level of 50, 100 and 150 mg/Kg, respectively.

There was a significant difference in the paw diameter size reduction between these groups with an F value equal to 51.03 at $p < 0.05$ (table 2). The anti-inflammatory activity of the 50, 100 and 150 mg/Kg doses were not significantly different with that of diclofenac $p < 0.05$ (table 2). Values are expressed as Mean ± SEM for five animals per group. Statistical comparison was made within a column and values with different superscript are significantly different by one-way ANOVA followed by Tukey's post hoc test ($p<0.05$).

Two hours after administration of the plant extract the anti-inflammatory effects and percentage paw edema inhibition in the experimental mice in the normal control, negative control, positive control and the experimental groups A, B and C were 65.46, 72.24, 89.19 and 63.76% (figure 1). The paw edema value in the negative control, positive control and experimental groups A, B and C were 1.18±0.07, 0.62±0.05, 0.54±0.04, 0.34±0.05 and 0.64±0.02 respectively (table 2). There was a significant difference in the paw diameter size reduction between these groups with F value = 43.43 at $p < 0.05$ (table 2). The extract at 150 mg/Kg showed anti-inflammatory activity which was not statistically different regarding the conventional drug (diclofenac) (table 2). The anti-inflammatory activity of the 50 and 100 mg/Kg doses was significantly different with that of diclofenac at $p < 0.05$ (table 2).

Three hours after administration of the plant extract the antiinflammatory effects and percentage paw edema inhibition in the experimental mice in the normal control, negative control, positive control and the experimental groups A, B and C were 32.07, 43.19, 56.15 and 26.52% respectively (figure 1). The paw edema value in the negative control, positive control and experimental groups A, B and C were 1.08±0.07, 0.82±0.04, 0.70±0.02, 0.56±0.04 and 0.88±0.04 respectively (table 2). There was a significant difference in the paw diameter size reduction between these groups with a F = 20.28 at $p < 0.05$ (table 2). The extract of *P. africana* at the dose of 150 mg/Kg presented anti-inflammatory activity which showed no significant different regarding the conventional drug (table 1). The effects of 50 and 100 mg/Kg doses were statistically significant different with that of at $p < 0.05$ (table 2). Four h after administration, the anti-inflammatory effects and percentage paw edema inhibition in the experimental mice in the positive control and the experimental groups A, B and C was 13.62, 32.85, 25.15 and 5.92% respectively (figure 1). The paw edema value in the negative control, positive control and experimental groups A, B and C were 1.04±0.06, 0.94±0.03, 0.74±0.04, 0.82±0.04 and 1.02±0.06 respectively (table 2). There was a significant difference in the paw diameter size reduction between the groups with F value = 9.72 at $p < 0.05$ (table 2). 50, 100 and 150 mg/Kg showed anti-inflammatory activity which was significantly different compared to the conventional drug (diclofenac) (table 2).

Most of the drugs used in the management of inflammation are associated with various side effects [24]. Herbal medicines are mostly accepted because of their potency, less side effects, affordability, accessibility and cultural acceptability [25]. In the present study, the anti-inflammatory activity of *P. africana* stem bark extract of was evaluated in Swiss albino mice using carrageenan. Carrageenan is commonly used for screening anti-inflammatory activity of new agents because it involves routine and simple use of animal model [26,27].

It is a sulphated polysaccharide obtained from carrageen moss, a sea weed [28]. Carrageenan is associated with severe inflammation reaction after subcutaneous administration to the hind paw of either mice or rat [29]. Carrageenan is also used when evaluating natural products anti-inflammatory activity [30]. Edema associated with carrageenan administration occurs in two different phases: early and late. The early phase is associated with release of histamine, cytokinins and serotonin while late phase is associated with production and release of oxygen derived free radicals, prostaglandins and lysosome and proteases enzymes [30]. The extract of *P. africana* in this study showed strong anti-inflammatory activity in carrageenan induced inflammation in Swiss albino mice through paw diameter reduction in both early and late phases.

Table 2. Effects of dichloromethane stem bark extract of *Prunus africana* on carrageenan induced paw edema in mice (n=5); values are expressed as Mean ± SEM for five animals per group. Statistical comparison were made within a column and values with different superscript are significantly different by one-way ANOVA followed by Turkey's post hoc test ($p < 0.05$).

Groups	Treatment	Edema value in mm (Mean ± SEM)			
		1st h	2nd h	3rd h	4th h
Negative control	DMSO + Carrageenan	1.06±0.07^{a}	1.18±0.07^{a}	1.08±0.07^{a}	1.04±0.06^{a}
Positive control	Carrageenan+ Diclofenac (100 mg/Kg)	0.28±0.04^{b}	0.62±0.05^{b}	0.82±0.04^{b}	0.94±0.03^{ab}
Experimental A	Carrageenan+ *P. africana* extract 50 mg/Kg	0.28±0.06^{b}	0.54±0.04^{bc}	0.70±0.02^{bc}	0.74±0.04^{c}
Experimental B	Carrageenan+ *P. africana* extract 100 mg/Kg	0.16±0.05^{b}	0.34±0.05^{c}	0.56±0.04^{c}	0.82±0.04^{bc}
Experimental C	Carrageenan+ *P. africana* extract 150 mg/Kg	0.30±0.03^{b}	0.64±0.02^{b}	0.88±0.04^{b}	1.02±0.06^{a}
	F Value	51.03	43.43	20.28	9.72
	p Value	0.00	0.00	0.00	0.00

The anti-inflammatory activities of the plant extract could be attributed to inhibition of the inflammatory mediators in both early and late phases there by suppressing edema. The results are correlated with other studies. In vivo and in vitro anti-inflammatory and toxicity investigation of the *Rumex vesicarius* Linn. bark methanol extract indicated some anti-inflammatory activities [31]. Additionally, anti-inflammatory activities in *Myenus obscura* and *Caesalpinia volkensi* extract in animal models demonstrated positive results [32]. Therefore, there is a possibility that the extract of *P. africana* stem would be capable of inhibiting prostaglandin production which initiates the inflammation process. The plant extract may have inhibited the cyclo-oxygenase enzyme action a vital catalyzing enzyme in the prostaglandins production from arachidonic acid.

The extract of *P. africana* demonstrated a dose dependent anti-inflammatory response which was observed from the 1st to the 4th h. The paw edema inhibition was higher in the first two hours. The low percentage change in the 4th hour could be attributed with slow rate of absorption of the active phytochemical compounds. In the 4th hour, there might be high plasma concentration levels of the anti-inflammatory associated phytochemical compounds sufficient to reduce inflammation. In the first three hours, lower paw edema inhibition may be attributed to the fact that the phytochemical must be bio-transformed to give active anti-inflammatory agents [33]. The dose levels used in this study were 50, 100, and 150 mg/kg body weight. This was similar to dose levels used in previous studies [32,34]. The plant extracts at the dose of 150 mg/kg demonstrated more considerable anti-inflammatory activity compared to 50 and 100 mg/kg doses. This could be attributed to low active phytochemicals concentration which might have been metabolized and excreted quickly. The anti-inflammatory activity observed correlates well with results obtained in previous studies regarding *Mytenus obscura* and *Caesalpinia volkensi* anti-inflammatory activities [32]. Similarly, the methanol extract of *T. brownii* stem bark demonstrated dose dependent anti-inflammatory response [35].

Phytochemical screening of *P. africana* extract demonstrated the presence of alkaloids, cardiac glycosides, flavonoids, saponins, steroids and terpenoids (table 2). Alkaloids, cardiac glycosides, flavonoids, saponins, steroids and terpenoids are reported to inhibit prostaglandins synthesis pathway [36]. Flavonoids are also associated with cyclo-oxygenase, lipo-oxygenase, phospholipase and TNF-α enzymes involved in the metabolism of arachidonic acid [37,38]. Saponins have been reported to inhibit inflammation process hence they can be used as anti-inflammatory agents [39]. Flavonoids and saponins synergistically act by inhibiting enzymes linked to inflammations like the nitric oxide synthase, lipoxygenase and cyclooxygenase which are very important in the processes of inflammatory mediators production and arachidonic acid metabolism [39]. Inflammation process is also associated with free radical production commonly in the carrageenan test late phase [40]. Flavonoids, tannins, terpenoids and phenolic compounds scavenge free radicals because of their antioxidants activities and are also involved in wound healing process [41]. The presence of alkaloids, cardiac glycosides, flavonoids, saponins, steroids and terpenoids present in *P. africana* dichloromethane extract may either act singly, additively and or synergistically as anti-inflammatory agents. Traditional medicine therapeutic benefits are associated with combination of different active principles [42].

In this study, *P. africana* extract was effective in management of inflammation. This can be associated with presence of active anti-inflammatory phytochemical compounds. The anti-inflammatory activity in the first and second hour of extract was less than that observed in the third and fourth hour which indicates that the pharmacologically active phytocompounds might

undergo biotransformation to gain the anti-inflammatory activity or it took time to pass across the peritoneum cavity.

The extract activity was compared with the anti-inflammatory activity of the standard drug diclofenac. Regarding the qualitative analysis, several secondary metabolites including alkaloids and flavonoids were present in the extract. Alkaloids and flavonoids from various plant sources are associated with significant anti-inflammatory activity according to previous studies. Flavonoids exhibit anti-inflammatory activity by inhibiting the metabolism of the arachidonic acid [43]. Alkaloids are the largest class of plant secondary metabolites and are associated with various pharmacological activities including anti-inflammatory activity [44,45]. The anti-inflammatory activity associated with *P. africana* extract is probably due to the action of these phytochemicals individually or in synergistic actions. The extract demonstrated a higher anti-inflammatory activity compared to the conventional drug (diclofenac).

This study clearly demonstrated that stem bark extracts of *P. africana* presented significant anti-inflammatory activity in carrageenan edema induced in Swiss albino mice. Thus, *P. africana* is a possible candidate for extraction of active anti-inflammatory compounds which could be used in pain management. *Prunus africana* extract showed anti-inflammatory activities in animal model, thereby establishing a pharmacological basis for its use in inflammation management in folk medicine. In all the three doses the extract significantly inhibited edema through both peripheral and central mechanisms. The classes of phytochemicals identified in dichloromethane *P. africana* stem bark extract are known to contribute to anti-inflammatory activities. Therefore, this study scientifically supports the traditional use of *P. africana* stem bark extract for management of inflammation.

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Author contributions

Gitonga Godfrey Mutuma designed the experiments and wrote the first draft of the paper; Ngeranwa Joseph contributed in animal handling and experimentation; Machocho Alex King'ori contributed in the phytochemical experiments; Kiruki Silas contributed in biochemical experiments and analysis.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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Abbreviations

ANOVA: Analysis of Variance; DMSO: Dimethyl sulfoxide; NIH: National Institute of Health; NSAIDs: Non steroid anti-inflammatory drugs; TNF-α: Tumor necrosis factor alpha