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Antinociceptive effects of methanolic extracts of *Pistacia aethiopica* and *Warbugia ugandensis* in mice

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ABSTRACT

Pistacia aethiopica and *Warbugia ugandensis* are medicinal plants used in folk medicine among the Embu, Kenya. Despite being in use since antiquity, no empirical data is available to validate their claimed use in pain management. This study evaluated the antinociceptive effects of methanolic extracts of these plants in mice. Qualitative phytochemical profile of the methanolic extracts was also established. The antinociceptive studies used formalin pain model. Harbone and Kotaki protocols for qualitative phytochemical screening were used. Stem bark extracts of *P. aethiopica* inhibited paw licking in mice by between 47.24% - 55.13% in the early phase and by between 30.69%-52.12% in the late phase. *W. ugandensis* leaf extracts inhibited paw licking by between 38.45% - 51.85% in the early phase and by between 43.48%-65.61% in the late phase. Diclofenac sodium inhibited paw licking by between 30.33% - 30.36% in the early phase and by between 62.93% and 77.08% in the late phase. Phytochemical screening revealed presence of saponins, alkaloids, flavonoids, phenols and terpenoids. This study established existence of antinociceptive effects in the methanolic extracts of *P. aethiopica* and *W. ugandensis* in mice. The observed effects were ascribed to the presence of phytochemicals working individually or synergistically.

Keywords: Pain, Antinociceptive, *Pistacia aethiopica*, *Warbugia ugandensis*.

INTRODUCTION

Pain refers to an unpleasant sensation, or a feeling of discomfort resulting from stimulation of pain receptors in the body when tissue damage occurs or is about to occur [1]. Pain is a valuable symptom of an underlying pathology and may be vital in the diagnosis of diseases. As an essential body's defense mechanism, pain serves as a warning of a problem particularly when it is acute.

The formalin pain model in mice is considered as a chronic pain model. Formalin induces neurogenic, inflammatory and tonic pain that closely mimics clinical pain [2]. Besides, formalin model is a highly specific and reliable mode for chronic pain [3]. Formalin induces two distinct phases with quantifiable nociceptive behaviour that is characterized by lifting, licking and biting of the injected paw [4; 5]. The response to formalin injection is shown in two phases, early phase and late phase. The early phase is mainly due to direct stimulation of nociceptors particularly the C- afferent and A-delta fibres by the peripheral stimulus [6]. The late phase is as a result of inflammatory responses in the peripheral tissue and functional alterations in the dorsal horn of the spinal cord [5].

Pain aggravates distress and morbidity and if unchecked, it results in a vicious cycle of associated pathological conditions [7]. To alleviate pain medications such as NSAIDs, steroids, immunosuppressants and opioids are often used [8]. Globally, NSAIDs are among the most common prescriptions for painful conditions [9]. The mode of action of NSAIDs is mainly through inhibition of COX enzymes and eventual reduction in prostaglandins and thromboxanes synthesis [10]. The COX enzymes catalyze conversion of arachidonic acid to prostaglandins [11]. NSAIDs also inhibit the lipoxygenase and L-arginine nitric oxide pathways, thereby reducing the concentration of pro-inflammatory mediators such as nitric oxide. Reduction in prostaglandins synthesis is essential to amelioration of pain since prostaglandins are key mediators of inflammatory pain.

Despite their effectiveness in ameliorating painful and inflammatory conditions, NSAIDs possess several adverse effects [12]. Adverse effects associated with NSAIDs include delays in blood clotting, nausea, allergy, stomach ulceration and bleeding [13]. Liver damage, kidney failure and heart conditions may also occur due to extended NSAIDs use [14]. Additionally, acetaminophen has been reported to induce liver damage after prolonged use. Diclofenac sodium, a commonly used NSAID, has been reported to increase the risk to heart conditions, stroke and liver damage [15]. Nonetheless, diclofenac use may erode the stomach mucosa predisposing one to peptic ulcers. NSAIDs that particularly inhibit the COX-2 pathway

increases risk to cerebrovascular and cardiovascular conditions such as stroke and myocardial infarction [16].

Herbal medicines are thought to be effective, easily accessible, affordable, and arguably, have limited or no side effects [17]. *P. aethiopica* and *W. ugandensis* are medicinal plants used as remedy for pain among the Embu community in Kenya. Despite long history of use, no empirical data exists in regard to their effectiveness in management of pain. The objective of the study was to evaluate anti-nociceptive effects of methanolic extracts of *P. aethiopica* and *W. ugandensis* in mice models as a preliminary step toward development of herbal derived anti-nociceptive remedy. In this study, antinociceptive effects of the extracts were compared to the diclofenac sodium as the standard. Moreover, the study also determined the qualitative phytochemical profiles of the extracts.

MATERIALS AND METHODS

Drugs and chemicals

The drugs used in this study include: formalin (Amar formalin R H Devani Ltd), diclofenac sodium (Dynapar, Troika pharmaceuticals Ltd), methanol analytical grade (Walke Selman EA Ltd).

Instruments and materials

The instruments used in this study were; laboratory mill, electronic balance, rotary evaporator (Goel scientific glass works Ltd), plexiglass observation chamber and mirrors.

Plants under study

Warbugia ugandensis is a plant species in the family Canellaceae. It is native in Africa particularly in central, eastern and southern regions. Bark or leaves are usually used as remedy for stomach-ache, constipation, toothache, cough, fever, muscle pains, weak joints and general body pains [18].

Pistacia aethiopica is plant species in the Anacardiaceae family native to Africa and Arabian coast Peninsula. It is found in the eastern Africa countries and Yemen [18]. *P. aethiopica* is used among the Embu and Mbeere communities, Kenya, as remedy for malaria and toothache [19].

Collection and preparation of plant materials

Fresh plant samples were collected from Kevote village, Embu west sub-county in Embu County, Kenya with the assistance of traditional medical practitioners. Information on the local names of the plants, parts used, mode of preparation and the season when their curative potency is maximal was provided by the herbalists. Nonetheless, bio-conservation measures were considered during sample collection. For *W. ugandensis*, fresh leaf samples were collected whereas stem barks were collected for *P. aethiopica*. Sample collection was done during the dry season in January; the time that the herbalists believed the plant had optimal medicinal properties. The GPS coordinates for *P. aethiopica* and *W. ugandensis* were 0°26' 51.2" S 37°31' 46.5"E and 0°26'33"S 37°31'53"E respectively. The samples were then air dried under a shade after which they were transported to Kenyatta University, Biochemistry, Microbiology and Biotechnology Department laboratories for further processing. The samples were authenticated by a taxonomist at Kenyatta University, Plant Sciences Department and a sample deposited with the herbarium for future reference. The voucher numbers assigned to each plant sample were IMM001/2016 and IMM002/2016 for *P. aethiopica* and *W. ugandensis* respectively. Dried

plant materials were pulverized into a fine homogeneous powder using a laboratory mill and then put in air tight polythene bags ready for extraction.

Preparation of the methanolic extracts

For each sample, 200grams of powder was macerated separately in 1.5litres of cold methanol for 48 hours with regular agitation to extract the phytochemicals. The mixtures were first decanted then filtered using Whatman's filter paper No.1 and the filtrate concentrated under reduced pressure using rotary evaporator at a temperature of 65 degrees Celsius. The resultant concentrates were then air dried under room temperature. The final residue for *P. aethiopica* and *W. ugandensis* weighed 22 and 35grams for respectively. These were kept in airtight containers at 4 degrees Celsius awaiting bioassays.

Experimental animals

Male Swiss albino mice (*Mus musculus*), aged between 8-12 weeks old weighing 25-35 grams were used to bio-screen for analgesic activities of the two medicinal plants. The animals were acquired from Kenya Medical Research Institute and kept in the animal research facility in the Department of Biochemistry, Microbiology and Biotechnology at Kenyatta University where experiments were carried out. A 48 hours acclimatization period was allowed prior to experimentation. The mice were kept in the standard cages and maintained under the standard laboratory conditions of ambient temperature (25 °C) and with 12-hour day light. The animals were fed on a standard rodent pellet and supplied with water *ad libitum* [20]. Ethical approval for use of animals was granted by Kenyatta university research committee.

Evaluation of antinociceptive effects

A completely randomized research design was used in evaluation of antinociceptive effects. The antinociceptive effects of the methanolic extracts of *P. aethiopica* and *W. ugandensis* were determined using formalin-induced paw licking model in mice as described by [20]. Thirty male Swiss albino mice, were randomly categorized into six groups of five animals each (n=5). The animals were first weighed and their respective weights recorded. Diclofenac sodium was used as the reference drug.

Administration of the various treatments was done through the intraperitoneal route. The volume administered in each treatment was 0.1 millimeter. Moreover, administration of the treatments was done 30 minutes prior to pain induction. Pain induction involved injection of 0.05 ml of 2.5% formalin solution into the sub-plantar region of the left hind paw.

Group I was the normal control in which the mice in which no pain was induced. Group II was the negative control in which 0.1ml of 10% DMSO was administered with subsequent pain induction. Group III was the positive control, in which, diclofenac sodium at a dose of 15 mg/kg body weight was administered. Groups IV, V and VI entailed treatment with the methanolic extracts at 50, 100 and 150 mg/kg body weight dose level. 10% DMSO solution and normal saline was used to reconstitute the methanolic extracts (Table 1).

In all groups, the mice were individually placed in a transparent plexiglass observation chamber with mirrors placed on two sides of the chamber to enhance observation of the nociceptive behaviors from all angles. The pain behavior of the mice was individually observed and recorded. The treatment protocol for analgesic assays was as follows:

Table 1: Treatment protocol for determination of antinociceptive effects of methanolic extracts of *P. aethiopica* and *W. ugandensis* in mice

Group	Status	Treatment
I	Normal control	
II	Negative control	Formalin (2.5%)
III	Positive control	Formalin+ Diclofenac (15mg/kg body weight)
IV	Experimental group I	Formalin+ Extract (50mg/kg body weight)
V	Experimental group II	Formalin+ Extract (100mg/kg body weight)
VI	Experimental group III	Formalin+ Extract (150mg/kg body weight).

The time, in seconds, that the mouse spent lifting, licking or biting the injected paw was measured using a stop watch. The nociceptive behavior was monitored and recorded according to response pattern described by [5], whereby two distinct periods of intensive licking and biting activity were identified and scored separately. The early phase, which was due to direct chemical stimulation of nociceptors, was recorded 1-5 minutes after formalin injection. The late phase, occasioned by release of inflammatory mediators, was recorded 15-30 minutes following formalin injection. The duration between the fifth and the fifteenth minute is the remission period with minimal nociceptive behaviour [21]. The percentage paw licking inhibition was computed as per the formula by [22].

$$\% \text{ Paw licking inhibition} = \frac{N-T}{N} \times 100$$

Where; N- The normal control group value for each phase

T - The treated group value for each phase

Qualitative Phytochemical Screening

The methanolic extracts of *P. aethiopica* and *W. ugandensis* were subjected to qualitative phytochemical screening for presence or absence of phytochemicals using standard methods of analysis as described by [23] and [24]. Phytochemicals screened for include: flavonoids, phenols, saponins, alkaloids, steroids, terpenoids and tannins.

Statistical analysis

One-way ANOVA followed by the Tukey’s post-test was used to analyze the data obtained from formalin pain model. P<0.05 was the critical criterion for statistical significance. Minitab statistical software version 17.1.0 was used. Unpaired student t-test was used in the comparison of the antinociceptive effects of the two plants.

RESULTS

Table 2: Antinociceptive effects of methanolic stem bark extracts of *Pistacia aethiopica* in Swiss albino mice

Group	Treatment	Early phase	Late phase
Normal control		00.00 ± 0.00 (100.00±0.00) ^a	00.00 ± 0.00 (100.00±0.00) ^a
Negative control	Formalin	126.00±3.9 (0.00±0.00) ^e	103.60± 1.75 (0.00±0.00) ^e
Positive control	15mg/kg bw Diclofenac+ Formalin	88.20± 3.76 (30.33±1.44) ^d	38.40± 1.94 (62.93±1.87) ^b
Experimental groups	50 mg/kg bw <i>P. aethiopica</i> + Formalin	66.80 ± 2.08 (47.24±1.65) ^c	71.80 ± 1.53 (30.69±1.48) ^d

Antinociceptive activities of methanolic extracts of *P. aethiopica* in Swiss albino mice

Generally, the methanolic stem bark extracts of *P. aethiopica* reduced paw licking time significantly in a dose dependent manner compared to the negative control group in both early and late phases of formalin-induced pain in mice (p<0.05; Table 2). The antinociceptive effects were indicated by increased percent paw licking inhibition or reduced paw licking time on formalin-induced nociception in Swiss albino mice.

In the early phase, the methanolic stem bark extracts of *P. aethiopica* had significant pain inhibition activity indicated by percent paw licking inhibition of between 47.24% and 55.13% by the three extract doses (50, 100 and 150 mg/kg body weight). Diclofenac sodium, which was the positive control, elicited a percent paw licking inhibition of 30.33% in the early phase. The percent paw licking inhibitions by the three methanolic stem barks extracts doses were 47.24%, 52.29% and 55.13% respectively (Table 2). In this phase, the antinociceptive effects of the three dose levels of 50, 100 and 150 mg/kg body weight were statistically similar (p>0.05; Table 2). The pain inhibitory effects of the three extract dose levels were significantly higher than the effect of the reference drug in the early phase (p<0.05; Table 2).

In the late phase, the methanolic stem bark extracts of *P. aethiopica* reduced pain significantly compared to the negative control (p<0.05). The percent paw licking inhibitions by the three doses of *P. aethiopica* extract ranged between 30.69% and 52.12% while that of the diclofenac (positive control) was 62.93% (Table 2).

The antinociceptive activities of the three extract dose levels (50, 100 and 150mg/kg body weight) of *P. aethiopica* were significantly lower than that exhibited by the positive control in the late phase (p<0.05; Table 2). The analgesic effects of the methanolic stem bark extracts, at dose levels of 50 and 100 mg/kg body weight, were statistically similar (p>0.05). However, the dose level of 150mg/kg body weight caused significantly larger pain reduction than the other two extract dose levels (p<0.05; Table 2)

100 mg/kg bw+ <i>P. aethiopica</i> +Formalin	60.40 ± 2.20 (52.29±1.74) ^{bc}	67.60± 1.54 (34.75±1.48) ^d
150 mg/kg bw + <i>P. aethiopica</i> + Formalin	56.80± 2.29 (55.13±1.81) ^b	49.60± 6.30 (52.12±1.57) ^c

Values are expressed as Mean ± SEM for five animals per group. Statistical comparison were made within a column and values with the same superscript are not significantly different (One-way ANOVA followed by Tukey’s post hoc test, $p > 0.05$). Values in parenthesis indicate percent mean paw licking inhibition, bw is an abbreviation for body weight

Antinociceptive effects of methanolic leaf extracts of *Warbugia ugandensis* in Swiss albino mice

The methanolic leaf extracts of *W. ugandensis* also remarkably reduced paw licking time in the early and late phases of nociception at all the three dose levels (Table 3). In the early phase, intraperitoneal administration of the methanolic leaf extracts of *W. ugandensis*, at dose levels of 50, 100 and 150mg/kg body weight, reduced pain appreciably compared to negative and normal controls. The percent inhibitions of paw licking during the early phase by the three experimental doses (50, 100 and 150 mg/kg body weight) were 38.45%, 51.82% and 43.07% respectively. The reference drug (Diclofenac sodium) caused a percent paw licking inhibition of 30.36%. The antinociceptive effect of the extract dose level of 100mg/kg body weight was significantly higher than those of dose levels 50 and 150 mg/kg body weight ($p < 0.05$; Table

3). However, the antinociceptive activities of the three tested dose levels were significantly higher than that of diclofenac (positive control) in the early phase ($p < 0.05$).

In the late phase, the methanolic leaf extract of *W. ugandensis*, at dose levels 50, 100 and 150 mg/kg body weight, significantly reduced paw licking compared to the negative control ($p < 0.05$; Table 3). The percent inhibitions in paw licking time by the three extract dose levels (50, 100 and 150 mg/kg body weight) were 43.48%, 63.80% and 60.67% respectively. The percent paw licking inhibition by the 150 and 100mg/kg body weight extract dose levels were statistically similar to that of the reference drug ($p > 0.05$; Table 3). However, the antinociceptive activity of 50mg/kg body weight extract dose level was significantly lower than those of the 100 and 150 mg/kg body weight extract dose levels ($p < 0.05$).

Table 3: Antinociceptive effects of methanol leaf extracts of *Warbugia ugandensis* in Swiss albino mice

Group	Treatment	Early phase	Late phase
Normal control		00.00 ± 0.00 (100.00±0.00) ^e	00.00 ± 0.00 (100.00±0.00) ^a
Negative control	Formalin	121.20±3.06 (0.00±0.00) ^a	101.20± 2.92 (00.00±0.00) ^e
Positive control	15mg/kg bw Diclofenac+ Formalin	55.40 ± 4.72 (30.36±2.10) ^d	23.20± 3.68 (77.08±3.64) ^d
	50 mg/kg bw <i>W. ugandensis</i> + Formalin	74.60 ± 2.27 (38.45±1.87) ^b	57.20 ± 4.78 (43.48±4.72) ^b
Experimental groups	100 mg/kg bw <i>W. ugandensis</i> + Formalin	58.40 ± 3.08 (51.82±2.54) ^c	34.80± 2.75 (63.80±2.71) ^{cd}
	150 mg/kg bw <i>W. ugandensis</i> + Formalin	69.00 ± 2.00 (43.07±1.65) ^b	39.80± 2.11 (60.67±2.07) ^c

Values are expressed as Mean ± SEM for five animals per group. Statistical comparison were made within a column and values with the same superscript are not significantly different (One-way ANOVA followed by Tukey’s post hoc test; $p > 0.05$). Values in parenthesis indicate percent mean paw licking inhibition, bw is an abbreviation for body weight

Comparison of antinociceptive effects of *P. aethiopica* and *W. ugandensis*

The antinociceptive effects of the two studied medicinal plants on formalin-induced pain in mice models were compared. In the early phase, the percentage paw licking time inhibitions of *P. aethiopica*, at the dose levels of 50 and 150mg/kg body weight, were significantly higher than those of *W. ugandensis* at the two dose levels ($p < 0.05$; Figure 1). However, at the dose level 100mg/kg body weight, the two medicinal plants were equally effective ($p > 0.05$; Figure 1).

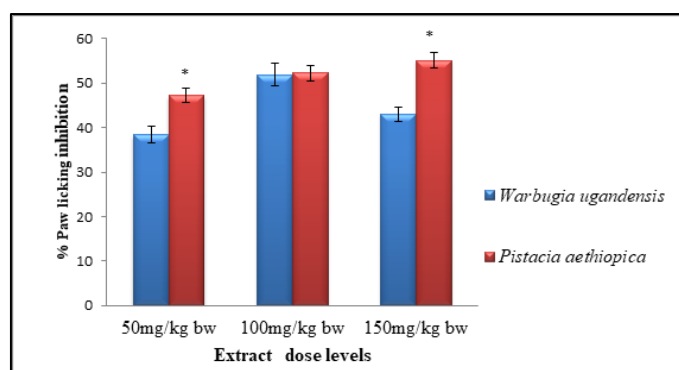


Figure 1: Comparison of paw licking inhibition of methanol extracts of *P. aethiopica* and *W. ugandensis* in the early phase of formalin-induced nociception. * indicates variation in the analgesic effect between the two plants under study (Unpaired t-test; $p < 0.05$)

On the other hand, in the late phase, the methanolic leaf extracts of *W. ugandensis*, at the three dose levels (50,100 and 150 mg/kg body

weight), was significantly more effective than the methanolic stem bark extracts of *P. aethiopica* ($p < 0.05$; Figure 2).

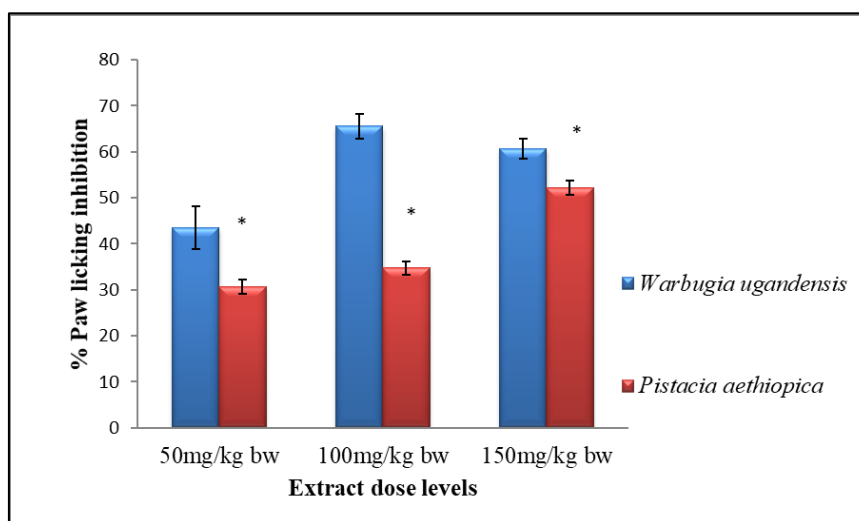


Figure 2: Comparison of paw licking inhibition by the methanol extracts of *P. aethiopica* and *W. ugandensis* during the late phase of formalin induced nociception. * denotes statistical variation in the effects of the methanolic extracts of the two plants (Unpaired t-test; $p < 0.05$).

Qualitative phytochemical profiles of the methanolic extracts of *P. aethiopica* stem bark and *W. ugandensis* leaves

Qualitative phytochemical analysis of the methanolic leaf extracts of *Warbugia ugandensis* and stem bark extracts of *Pistacia aethiopica*. Saponins, terpenoids, flavonoids, steroid and phenols were present in both plant extracts. Additionally, *P. aethiopica* had alkaloids and tannins that were absent in *W. ugandensis*.

Table 4: Phytochemical composition of methanolic extracts of *Warbugia ugandensis* leaves and *Pistacia aethiopica* stem bark

Phytochemical	<i>Warbugia ugandensis</i>	<i>Pistacia aethiopica</i>
Saponins	+	+
Alkaloids	-	+
Terpenoids	+	+
Flavonoids	+	+
Steroids	+	+
Phenols	+	+
Tannins	-	+

The positive sign (+) indicates presence while the negative sign (-) indicates absence.

DISCUSSION

The methanolic extracts of *P. aethiopica* and *W. ugandensis* significantly suppressed formalin-induced pain in mice in both phases of formalin-induced nociception (Tables 2 and 3). The antinociceptive activities were indicated by reduction in paw licking time, which was expressed as percent paw licking inhibition. The inhibition of formalin-induced pain in both phases, by the methanolic extracts of *P. aethiopica* and *W. ugandensis*, suggests that the antinociceptive effects elicited by the phyto-constituents, present in the methanolic extracts of the two plants, involved both central and peripheral mechanisms of antinociceptive in mice. Nevertheless, the methanolic extracts of *P. aethiopica* and *W. ugandensis* did not equally inhibit pain in both of phases (Tables 2 and 3). In addition, the inhibition of both phases of formalin-induced pain implied that the methanolic extracts of both

plants contained not only antinociceptive but also anti-inflammatory active principles given the inhibition of the neurogenic and inflammatory pain of the formalin model [25].

The results of this study are consistent with previous studies on antinociceptive activities of medicinal plants that observed antinociceptive activities in both phases of formalin-induced nociception [26], demonstrated that DCM: Methanolic leaf and root bark extracts of *Carissa edulis* inhibited pain in both phases of formalin-induced pain. Similarly, the methanolic stem bark extracts of *Harrisonia abyssinica* Oliv. and *Landolphia buchanani* Hallier F. reduced paw licking in both phases of formalin induced nociception in mice [27]. Studies by [28] revealed that ethanolic extracts of *Curcuma zedoaria* rhizome ameliorated pain in both early and late phases of formalin-induced nociception. Ethylacetate fraction of *Cassia fistula* L. exhibited antinociceptive activities in early and late phases of formalin-induced nociception in rats [29]. Moreover, *Allium sativum* powder suppressed formalin-induced nociception in mice in both phases of formalin-induced nociception [30].

The mean percent paw licking inhibitions by the three *P. aethiopica* extract dose levels indicate that the methanolic extracts had higher antinociceptive effect in the early phase than in the late phase (Table 2). This may be ascribed to fast diffusion of the active principles across the cell membranes into peritoneal cavity. Therefore, there was fast onset of antinociceptive effects [31]. Additionally, the phytoconstituents present in the methanolic extracts of *P. aethiopica* might have exerted antinociceptive effects directly without undergoing any biotransformation. However, there is a possibility that the methanolic extracts of *P. aethiopica* may have had high concentrations of opioid like analgesic phyto-constituents that exerted a central antinociceptive effect that was observed as heightened anti-nociceptive effect in the early phase.

On the other hand, the antinociceptive effects of the methanolic extracts of *W. ugandensis* were higher in the late phase than in the early phase (Table 3). This could have been due to slow or delayed diffusion and absorption of the active principles into the peritoneal cavity which in turn caused delayed antinociceptive effects. The slow diffusion of

bioactive compounds may also explain why antinociceptive effect increased with time leading to a higher effect in the late phase. Furthermore, the higher antinociceptive effect in the late phase may imply that the phytoconstituents in the methanolic extracts of *W. ugandensis* needed biotransformation into more active antinociceptive agents [32]. Nonetheless, the possibility of higher concentration of peripherally acting active principles cannot be ignored.

This study used three extract dose levels, 50, 100 and 150mg/kg body weight that were administered intraperitoneally. The rationale behind the use of these three doses levels was to evaluate the dose-response effect and therefore, determine whether or not the extracts exhibited a dose related pharmacological effect. The minimum dose was determined after a pilot study that revealed 50mg/kg body weight to be a suitable dose level for an observable pharmacological effect.

The antinociceptive effects elicited by 100 and 150 mg/kg body weight extract dose levels of *P. aethiopica* were higher than that of the 50mg/kg body weight (Figure 1 and Table 2). This indicates the antinociceptive activities of *P. aethiopica* were dose dependent in both early and late phases of the formalin-induced nociception. The more pronounced antinociceptive activities at higher dose levels may have been due to higher concentration of bio-active compounds that exerted greater antinociceptive effects. Such a dose dependent effect was reported earlier by [27] while working on antinociceptive activity of *Harrisonia abyssinica* and *Landolphia buchani* in mice models, in which the extracts exhibited dose dependent antinociceptive activities in the early phase.

The results obtained in this study show that the methanolic extracts of *W. ugandensis* caused a non-dose dependent antinociceptive activity whereby the highest antinociceptive effect was observed at 100mg/kg body weight dose level in both phases (Table 3). This suggests that the 100mg/kg body weight extract dose level was a better antinociceptive agent compared to the 150mg/kg body weight. Therefore, a higher dose level (150mg/kg body weight) produced less antinociceptive effect than the 100mg/kg body weight dose level (Table 3). This scenario may be attributed to the saturation of the active principles at the binding sites of the enzymes or proteins involved in antinociceptive mechanisms. It implies that any dose beyond 100mg/kg body weight could not yield any significant increase in antinociceptive effect. On the contrary, a dose beyond 100mg/kg body weight led to a decline in the antinociceptive activity. [33], observed a similar scenario whereby a higher extract dose level produced a diminished effect compared to a lower dose. The decline in pharmacological effect, beyond optimal dose, can also be ascribed to a natural pharmacokinetic mechanism within the animal that initiates excretion and clearance of the drug in order to avoid toxic effects by limiting their biological action [34].

The antinociceptive activities of the methanolic extracts of *P. aethiopica* and *W. ugandensis* at 50mg/kg body weight dose level were less potent than higher doses in both phases (Tables 2 and 3). Probably, at lower dose levels the active principles in the extracts might have been metabolized, cleared or inactivated at a faster rate. Besides, the concentration of the active principles, at 50mg/kg body weight dose level, may have had limited the pharmacological effects [31].

The results suggest that the methanolic extracts of *P. aethiopica* and *W. ugandensis* exerted considerable antinociceptive effects in formalin-induced pain in mice (Table 2 and 3). The methanolic extracts might have acted through metabolic inhibition of the COX pathway in a similar version to that of NSAIDs. The antinociceptive activities observed in this study may be attributed to phytochemicals present in

the methanolic extracts of *P. aethiopica* and *W. ugandensis* (Table 4). Flavonoids have been found to inhibit lipoxygenase and cyclooxygenase pathways that are responsible for peripheral nociception [35]. Moreover, flavonoids inhibit prostaglandin biosynthesis by inhibiting the function of prostaglandin synthase [36]. Flavonoids have been shown to target synthesis of prostaglandins which are involved in pain perception through opioidergic mechanism [37; 36]. Additionally, flavonoids are one of the nitric oxide synthase inhibitors that derail production of nitric oxide, which is a key agent of inflammatory nociception [38]. Flavonoids prevent activation of N-methyl-D-aspartate (NMDA) receptors and lower the levels of intracellular calcium. These cause a decrease in the activity of nitric oxide synthase enzyme and phospholipase A₂ with eventual decline in NO and prostaglandin production [39].

Alkaloids and tannins may also have been responsible for the observed antinociceptive activities. Previous studies have demonstrated that antinociceptive activities may be linked to tannins [40; 41], alkaloids [42] and steroidal compounds [44]. Terpenoids have also been associated with antinociceptive activity through inhibition of thrombocyte aggregation and interference with signal transduction of pain mechanisms [45]. Saponins have also been associated with antinociceptive activities by modulation of the GABA_A, NMDA and non-NMDA receptors for central nociception [46]. A study by [47], linked antinociceptive activities of saponins to non-opioid mediated activity through activation of the descending serotonin and α_2 -adrenergic pathways.

Therefore, this study postulates that synergistic activities of flavonoids, tannins, terpenoids, saponins and steroids were responsible for the observed antinociceptive activities. However, the precise mechanism of these active principles is still obscure.

CONCLUSION

This study concludes that the methanolic extracts of *P. aethiopica* and *W. ugandensis* have effects antinociceptive in mice. The observed antinociceptive activities of the methanolic extracts of *P. aethiopica* and *W. ugandensis* were due to phytochemicals present in the extracts such as flavonoids, terpenoids, tannins, phenols and saponins that have been previously associated with analgesic effects. The antinociceptive potencies of *P. aethiopica* and *W. ugandensis* demonstrated in this study supports, at least in part, the folkloric uses of these plants in management of painful conditions. Moreover, this study provided empirical data on the antinociceptive potencies of these plants which is an important initial step towards development of a standardized herbal pain remedy.

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Conflict of interest

The authors declare no conflict of interests

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