

Research Article

Antinociceptive effect of *Pentas lanceolata* and *Ximenia americana* medicinal plants used to treat malaria traditionally in Kenya

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Background: Pain being one of the many symptoms experienced in the course of an infection, this study was designed to investigate antinociceptive activity of two medicinal plants used to treat malaria traditionally. In traditional health practice there is usually a misunderstanding on whether the plants clear the disease causing organism or they cure the pain as one of the most common symptoms. The plants were selected based on their ethnomedical literature as a means of establishing a distinction between their antinociceptive and bio-activities.

Objective: To determine antinociceptive effect of *Pentas lanceolata* and *Ximenia americana*.

Materials and Methods: The aerial parts of *P. lanceolata* and the stem bark of *X. americana* were collected from Kiangombe forest and Kerio Valley in Embu and Elgeyo Marakwet Counties respectively. The collection was done with the help of an experienced taxonomist. The plant parts were dried, and extracted with methanol and aqueous solvents. Analgesic activity was determined by the tailflick and formalin test techniques in male albino mice. The positive control used in the experiment was acetylsalicylic acid (ASA) at 100 mg/kg.

Results: Antinociceptive activity of the plant extracts in the tail flick assay was time and dose dependent, except in the case of *P. lanceolata* methanol extract. In the formalin test, the extracts significantly ($p < 0.05$) reduced the time spent in pain behavior in both the early and late phases

Conclusion: The results of this study support the use of these plants to manage pain and imply other pharmacological benefits to the host other than parasitocidal effect in malaria treatment.

Keywords: Medicinal plants, tail-flick, formalin test, antinociceptive

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1. Introduction

Pain is a vital function of the nervous system in providing the body with a warning of potential or actual injury. The nervous system detects and interprets a wide range of thermal and mechanical stimuli, as well as environmental and endogenous chemical irritants. When intense, these stimuli generate acute pain, and in the setting of persistent injury, both peripheral and central nervous system components of the pain transmission pathway exhibit tremendous plasticity,

enhancing pain signals and producing hypersensitivity. Nociceptors are the specialised sensory receptors responsible for the detection of noxious (unpleasant) stimuli, transforming the stimuli into electrical signals, which are then conducted to the central nervous system. Pain experienced due to infections is known as neuropathic pain which is caused by damage to nerves in the central or peripheral nervous system (Basbaum and Jessell, 2000; Basbaum et al, 2009). Pain being one of the many symptoms experienced in the course of an infection, this study was designed to investigate

antinociceptive activity of some selected medicinal plants with the aim of trying to demystify the ability of the plants, to cure the pain as one of the most common symptoms of many infections, rather than their ability to clear the disease causing organism; a misunderstanding that occurs in traditional health practice. The plants were selected based on their ethnomedical literature described below, as a means of establishing a distinction between their antinociceptive and bio-activities.

Pentas lanceolata is an upright tropical evergreen shrub, 3 - 4 feet tall. The juice from the roots of *P. lanceolata* locally known as *Olkilaki-olkerr* (Maasai) is drunk for malaria and depression (Kokwaro, 1993). The plant has been shown to exhibit antimalarial activity (Koch et al, 2005; Rotich et al, 2015). It also has antibacterial activity (Matu et al, 2012).

Ximeina americana L. is a small tree up to 8 m high. It belongs to the family olacaceae and is widely used to treat various ailments like malaria in Nigeria (Ogunleye and Ibitoye, 2003), skin infections, leprosy and *Trypanosoma Congolese* infection (Adieza and Minda, 2011). In Sudan, the leaves and twigs are traditionally used to treat colds, fevers, headaches, sleeping sickness, oedema, used as laxatives, and as a poison antidote. The bark is used for kidney and heart complaints as well as headaches and skin ulcers (Abdalla et al, 2013) In Kenya its locally known as *Ol-amai* (Maasai). The seed oil is used to treat cracking feet. The root decoction syphilis, diarrhea and hookworm. The leaf measles and stomachache and the fruit for tonsillitis and mouthsores (Dharani 2011; Kokwaro, 2009).

The use of *X. americana* in traditional medicine has been verified for certain disorders, anticancer (Voss et al, 2006b), antitrypanosomal (Maikai et al, 2008), antimicrobial (Maikai et al, 2009), anti-inflammatory (Soro et al, 2009a), antimalarial Rotich et al, 2015). Analgesic activity of extracts of *X. americana* leaves has also been elucidated (Siddaiah et al, 2009).

In 2013, the World Health Organization (WHO) recommended the integration of traditional medicines proved to be useful into national health care programmes (WHO Traditional Medicine Strategy 2014–2023). Plant-derived natural products are rich resources in drug development, considering, 68% of all pharmaceuticals are from plants or are plant inspired (Zhu et al, 2012). Taking this into account, this study sought to investigate the analgesic properties of *P. lanceolata* and *X. americana*.

2. Materials and Methods

2.1 Collection of plant material

Plants specimen were collected with the help of an experienced taxonomist, authenticated and voucher specimens deposited at the East African Herbarium National Museums, of Kenya.

Aerial parts of *Pentas lanceolata* were collected from Kiangombe forest, Siakago, Embu County while *Ximeina americana* stem bark was collected from Kerio Valley, Elgeyo Marakwet County in Kenya (Table 1).

Table 1: Plants parts collected, location and voucher numbers

Plant Name	Family	Location collected	Part collected	Voucher No.
<i>P. lanceolata</i> (Forssk.)	Rubiaceae	Kiangombe forest, Siakago,	Aerial parts	739
<i>X. americana</i> L.	Olacaceae	Kerio Valley	Stem bark	711

2.2 Extraction of plant material

The plant parts were air dried at room temperature under shade and pulverized using a laboratory mill. The resulting powders were packed in air tight polythene bags, labeled and stored in the dark until used.

Each plant sample was extracted with water and methanol. For the methanol extracts 50 g of the powdered plant material was macerated with 500 ml of methanol at room temperature for 24 h and filtered through Whatman filter paper No. 1. The plant material was re-extracted with 300 ml methanol for the same period and the filtrates pooled and concentrated under vacuum at 40 °C until dry. The concentrate was weighed and transferred to an air tight sample bottle and stored at 4 °C until used. Another 50 g of the same sample was extracted once with 500 ml of distilled water in a water bath at 60 °C for 1 h, filtered and lyophilized in a freeze dryer. The dry extracts for all the samples similarly treated were weighed into airtight containers and stored at 4 °C until used.

2.3 Drugs and test agents

Acetylsalicylic acid (ASA) (CSPC OUYI Pharmaceutical Co. Ltd, China, Batch No. 130339) was used as a reference drug. Formalin used was of analytical grade. Sterile normal saline was used as a negative control (0.2ml per animal). ASA (100mg/kg) and extracts (100mg/kg, 200mg/kg) were dissolved in normal saline on the day of the experiment prior to use.

2.4 Experimental Animals

Male Swiss albino mice weighing 25-30 grams were used for both the tail flick and formalin test. Food and water were given ad libitum. Animals were allowed 7 days for acclimatization. They were kept at temperature of 22° C to 25° C and relative humidity of 50%. The experiments were conducted as per the guidelines issued by the International Association for the Study of Pain in experimental animals (Zimmermann, 1983).

2.4 Tail -Flick test

The tail flick test measures the response threshold of high intensity heat stimuli when applied to the animal's tail. The latency between onset of the stimulus and a rapid withdrawal of the tail is noted and recorded in seconds. The mice were brought to the test room, their weight recorded and then they were allowed to acclimatize to the test environment. Baseline latencies of the animals were recorded and the intensity of the

heat adjusted to produce latencies of 3 to 4 seconds. The light beam was focused approximately 15mm from the tip. The stimulus cutoff was set at 10 seconds to avoid tissue damage in the absence of a withdrawal reflex. The mice were treated with the plant extracts (100 and 200 mg/kg, i.p.) or acetylsalicylic acid (ASA) 100mg/kg, i.p. as a reference drug 30 min before the experiment and observed after 30 min and 1 hr. The time from onset of stimulation to a rapid flick of the tail from the light beam was recorded.

2.5 Formalin test

The method used, was similar to that described previously (Hunskar and Hole, 1987) with slight modifications. Mice were removed from their initial cages, weight taken and then allowed to acclimate for 30 min in the test environment. They were then randomly grouped into groups of six and pretreated 30 min prior to the formalin injection with either the plant extracts (200 mg/kg, i.p), normal saline or ASA (100 mg/kg, i.p). The normal saline and ASA served as the negative and positive controls respectively. The mice were then injected with 20 μ l of 5% formalin on the dorsal surface of the right hind paw and immediately returned to the observation chamber. The time (in seconds) spent in licking and biting of the injected paw by the animals, was taken as an indicator of nociception response. This was measured for 0-10 min (first phase) and 15-30min second phase after formalin injection. The data was presented as mean \pm s.e.m of time(s) spent in pain behavior. The mean of time (s) spent in pain behavior for the extract was compared with that of the control. The formalin test model is biphasic, it measures both neurogenic pain (first phase) and inflammatory pain (second phase) (Adzu et al, 2014).

2.6 Data analysis

Results were expressed as Mean \pm SEM. The data was analyzed using one-way analysis of Variance (ANOVA)

test and subsequently subjected to turkey post hoc test for multiple comparisons $p < 0.05$ were considered statistically significant.

3. Results

3.1 Evaluation of pain in the tail flick assay

The results of the tail flick test revealed significant ($p < 0.05$) antinociceptive activity of the plant extracts when compared to the control. However the group given *X. americana* extracts at a dose of 100 mg/kg i.p had insignificant reaction time at 30 min of the observation period. The reaction time for the mice given the plant extracts increased at one hour of the observation period, with the exception of *P. lanceolata* methanol extract. This was also true for the dosage whereby the reaction time increased with increase in dosage except for *P. lanceolata* water extract. The standard drug acetylsalicylic acid (aspirin) at a dose of 100 mg/kg had little analgesic activity (Table 2). Three out of the six mice, given *X. americana* extracts at a dose of 200 mg/kg i.p. died within 24 hours.

3.2 Evaluation of the formalin induced pain

In general, the plant extracts significantly ($p < 0.05$) reduced the time spent in pain behavior in both the early(0-10 min) and late (15-40 min) phases compared to normal saline which acted as the negative control (Figure 1). The antinociceptive effect of the water and methanol extract of *P. lanceolata* was not significantly different ($p = 0.367$) from that of acetyl salicylic acid in the first phase. The same pattern was witnessed again in the second phase with the exception of *P. lanceolata* water extract. The water and methanol extracts of *X. americana* had better activity than acetyl salicylic acid which was the reference drug in the two phases. The activity of *X. americana* methanol extract was superior to ASA and all the other plant extracts (Table 3).

Table 2 Antinociceptive activity of *P. lanceolata* and *X. americana* in the tail flick assay

Extracts	Plant parts	Dose (mg/kg, i.p)	Response time (in seconds)	
			After 30 min	After 1 hour
Water extracts				
<i>P. lanceolata</i>	Aerial parts	100	7.27 \pm 1.10	7.77 \pm 1.09
<i>P. lanceolata</i>	Aerial parts	200	4.95 \pm 0.83	6.27 \pm 1.07
<i>X. americana</i>	Stem bark	100	3.52 \pm 0.36	5.72 \pm 1.06
<i>X. americana</i>	Stem bark	200	6.83 \pm 1.11	7.47 \pm 0.88
Methanol extracts				
<i>P. lanceolata</i>	Aerial parts	100	5.65 \pm 0.99	4.21 \pm 0.39
<i>P. lanceolata</i>	Aerial parts	200	6.86 \pm 1.22	4.37 \pm 0.82
<i>X. americana</i>	Stem bark	100	3.92 \pm 0.51	5.37 \pm 0.95
<i>X. americana</i>	Stem bark	200	4.42 \pm 0.55	7.80 \pm 1.15
Control			3.63 \pm 0.43	3.2 \pm 0.47
ASA			4.76 \pm 1.04	4.67 \pm 0.86

Values expressed as mean \pm S.E.M of 6 animals.

Table 3: Effect of *X. americana* and *P. lanceolata* on the formalin test

Treatment	Dose (mg/kg i.p)	Phase one	Phase two
		Time spent in pain behavior. (Sec.)	Time spent in pain behavior. (Sec.)
<i>X. americana</i> methanol extract	200	10.83±1.14	14.00±1.92
<i>X. americana</i> water extract	200	15.17±1.49	29.33±6.01
<i>P. lanceolata</i> methanol extract	200	25.67±1.65	51.33±8.39
<i>P. lanceolata</i> water extract	200	29.00±3.30	31.00±3.80
Normal Saline	-	45.67±2.91	85.50±1.88
ASA	100	31.67±1.23	53.33±2.03

Values are mean± SE of 6 animals.

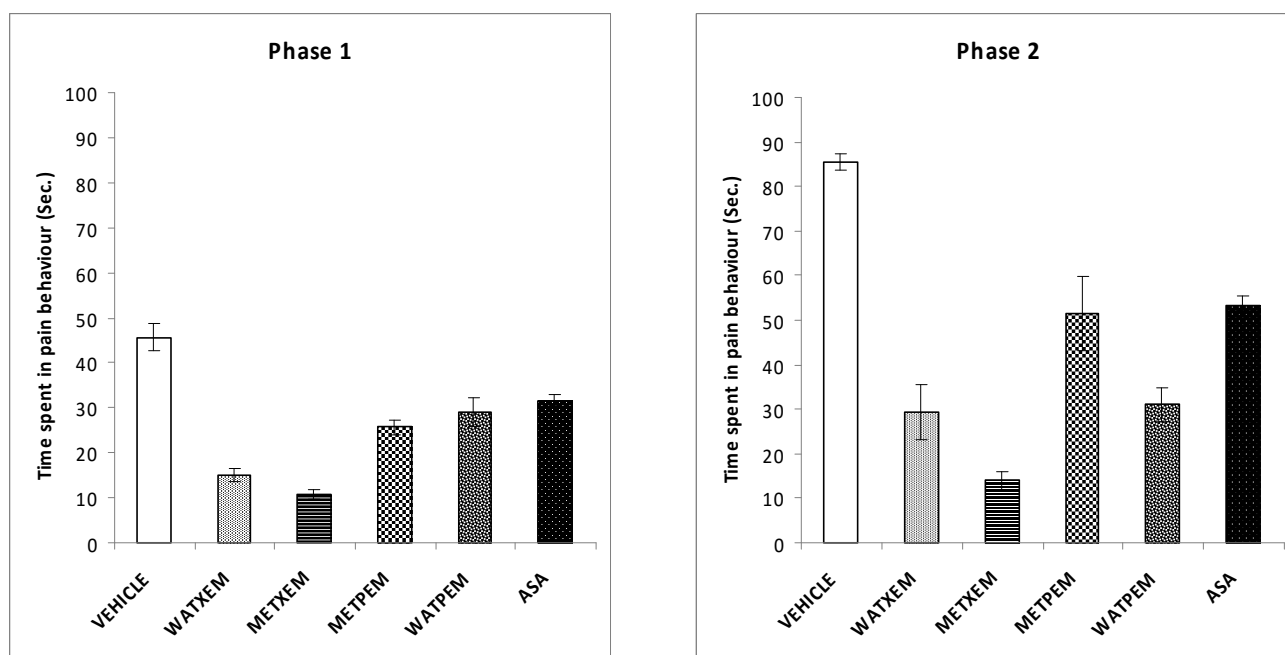


Figure 1: Effects of methanol extract of *X. americana* (METXEM), water extract of *X. americana* (WATXEM), methanol extract of *P. lanceolata* (METPEM) and water extract of *P. lanceolata* (WATPEM) on the formalin test

3.2 Evaluation of the formalin induced pain

In general, the plant extracts significantly ($p < 0.05$) reduced the time spent in pain behavior in both the early (0-10 min) and late (15-40 min) phases compared to normal saline which acted as the negative control (Figure 1). The antinociceptive effect of the water and methanol extract of *P. lanceolata* was not significantly different ($p = 0.367$) from that of acetyl salicylic acid in the first phase.

The same pattern was witnessed again in the second phase with the exception of *P. lanceolata* water extract. The water and methanol extracts of *X. americana* had better activity than acetyl salicylic acid which was the reference drug in the two phases. The activity of *X. americana* methanol extract was superior to ASA and all the other plant extracts (Table 3).

4. Discussion

In this study, the two plants *P. lanceolata* and *X. americana* exhibited potent analgesic activity in both phases of the formalin test, with methanol extract of *X. americana* showing superior antinociceptive activity. The formalin test model is biphasic, and measures both neurogenic pain (first phase), and inflammatory pain (second phase) (Adzu et al, 2014).

The first phase is a short response which is deemed to reflect the activity of C-fiber afferent nociceptors. The second phase follows a short inactive period. It is continuous and prolonged and is thought to be due to central sensitization of the spinal dorsal horn neurons as a result of the initial onslaught from C-fiber nociceptive afferents during the first phase (Gong et al, 2014).

The results for this study justify the use of the two plants to manage pain by herbalists. The results also indicate that other than the direct parasiticidal effect supported by various papers (Koch et al., 2005; Rotich et al, 2015) in the treatment of malaria, the two plants also possess other pharmacological benefits to the host for instance acting as analgesics. In Kenya the two plants are used to treat malaria locally. Malaria presents with fever, headache, muscle pains, chills, vomiting and general malaise (CDC, 2014).

Water and methanol extracts of the aerial parts of *P. lanceolata* at a dose of 200mg/kg i.p. significantly ($p < 0.05$) reduced pain in the formalin test and was comparable to ASA the reference drug. Other parts of the two plants have been shown to have antinociceptive activity in other studies too. Ethanol and ethyl acetate of the leaf extracts of *P. lanceolata* was shown to have analgesic activity in the acetic acid induced writhing method (Suman et al, 2014). Hemamalini et al, 2011 reported analgesic potential of the methanol extract of *X. americana* leaf in the tail flick and hot plate tests as well.

Even though methanol extract of *X. americana* stem bark, was seen to have better activity, at 200mg/kg i.p. In the tail flick assay, 3 out of 6 mice, died within 24 hours indicating that this extract was toxic at that dose. This implies that care should be taken in the administration of the stem bark extracts of *X. americana*. Rotich et al (2015) reported toxicity of the stem bark extracts of *X. americana* in the acute toxicity assay too. Toxicity of other parts of this plant has been reported as well in other studies, for instance, toxicity of the roots (Voss et al, 2006a; Soro et al, 2009b; Olabissi et al, 2011) and leaves by (Kamita et al, 2014) has been reported.

Conflict of Interest Declaration

The authors declare no conflict of interest.

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