Antinociceptive Activity of Methanolic Stem Bark Extracts of Harrisonia Abyssinica Oliv and Landolphia Buchananii (Hallier F.) Stapf in Mice Models

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Antinociceptive Activity of Methanolic Stem Bark Extracts of *Harrisonia abyssinica* Oliv and *Landolphia buchananii* (Hallier F.) Stapf in Mice Model

Nthiga PM*, Kamau JK, Safari VZ, Ngugi MP and Mburu DN

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**Abstract**

Pain is an unpleasant sensation attributed to an interplay of sensory and cognitive mechanisms and thus a source of burden to the individual and society. Since pain acts as a source of discomfort, it requires medical suppression. There is a growing interest in the use of herbal remedies as an attractive healthcare alternative to synthetic drugs. This is due to conventional medications being expensive and arguably associated with various adverse effects, hence the necessity for herbal agents that are effective, safe and relatively cheap. *Harrisonia abyssinica* and *Landolphia buchananii* are herbs that have been utilized to manage various ailments afflicting people especially in tropical Africa. However, despite their wide folklore use, extensive literature research reveals limited evaluation on the pharmacological activities of their described effects on pain. Thus, the aim of the current study was to evaluate the antinociceptive effects of their methanolic extracts. Experimental mice were divided into a normal control group, a negative control group, a reference group and three dosage groups. The dosage groups were treated with stem bark extracts at concentration of 50 mg/kg, 100 mg/kg and 150 mg/kg. The formalin paw licking test was used to determine the antinociceptive potential. Evaluation of the antinociceptive activities was compared with diclofenac as the reference drug. The *H. abyssinica* extract reduced pain by 39.73%-81.13% (in the early phase) and 15.92%-69.84% (in the late phase) while *L. buchananii* extract reduced it by between 35.35%-47.72% (in the early phase) and 20.57%-55.17% (in the late phase). In the early phase, reduction of pain by diclofenac was by 19.97%-46.50% and 76.77-74.80% (in the late phase). Several phytochemicals were observed to be present. The traditional medicinal use of the aforementioned plants in the suppression of pain has thus been confirmed by the study results.

**Keywords:** *Harrisonia abyssinica*; *Landolphia buchananii*; Antinociceptive

**Introduction**

Pain, as a sub-modality of somatic sensation, describes a complex array of unpleasant experiences associated with actual or perceived tissue damage and exhibited by specific independent, psychological, and behavioral responses [1]. It is an intricate experience comprising of physiological response to a noxious stimulus, which in some cases is followed by an emotional response [2]. Formalin induction of pain is biphasic; an early phase, which comprises of the first to fifth minute and a late phase, which occurs fifteen to thirty minutes after pain induction. The initial acute response (early phase), indicating a neurogenic type of pain, develops immediately after formalin injection for 5 min (0-5 min), and then a sustained response (late phase), measured 15-30 min after formalin injection [3]. The early phase results from a direct effect of formalin on nociceptors [4], predominantly afferent C fibers and partly Aδ fibers [5]. The early phase can be inhibited by opioid agonists such as morphine, fentanyl, bradykinin B1 and B2 receptor antagonists [6]. The late phase is a tonic response whereby inflammatory processes are involved and is associated with activation of the neurons in the dorsal horns of the spinal cord [4]. Both phases have been proven to be inhibited by centrally acting drugs, such as opioids while those that act peripherally, like steroids and nonsteroidal anti-inflammatory drugs like aspirin, inhibit only the late phase of the test [7]. To relieve pain in many disorders, nonsteroidal anti-inflammatory drugs (NSAIDs) are prescribed routinely worldwide [8]. However, long-term administration of NSAIDs may cause severe complications such as gastric ulcers, renal damage and cardiac abnormalities because of their non-selective inhibition of cyclooxygenases (COX) enzymes [9]. Interest in herbal medicine is on the rise as they are arguably believed to be more effective and possess fewer adverse effects [10]. *Harrisonia abyssinica* and *Landolphia buchananii* have been used by the Ameru and Embu communities in Kenya to alleviate various ailments but there is no scientifically investigated report of their described effects on pain. Therefore, the study mainly aimed to evaluate the antinociceptive potential of the aforementioned plants as preliminary drug screening towards production of more efficient plant-derived analgesic agents. The study also compared antinociceptive activities between these extracts as well as with diclofenac and also aimed to determine qualitative composition of the extracts.

**Materials and Methods**

**Collection and preparation of plant samples**

The fresh stem bark samples of *Harrisonia abyssinica* and *Landolphia buchananii* were collected with the help of local herbalists from their natural habitat in Mbeere, Embu County, Kenya. An acknowledged taxonomist botanically authenticated the plant samples and sample voucher deposited at the Kenyatta University herbarium. The plant samples were transported in clean burlap sacks to Kenyatta University for further processing. They were sorted, cleaned and rinsed with distilled water. They were then air dried at room temperature after being chopped separately into small pieces. The dried samples were then ground into fine powder by an electric mill.

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Extraction

A volume of 400 grams of powder for each sample was soaked separately in 1500 ml of methanol and left to stand for 24 hours. The filtration of the extracts was done using Whatmann No.1 filter papers. A rotary evaporator set at 65°C concentrated the filtrate to dryness under reduced pressure and vacuum. The resultant concentrates were packaged separately in airtight containers and stored at 4°C awaiting bioassay studies.

Experimental design

Laboratory animals: Healthy strains of Swiss albino mice (Mus musculus) of either sex weighing 20 to 30 g were employed for this study. The animal breeding colony was bred in the Animal House, Department of Biochemistry and Biotechnology at Kenyatta University. The mice were acclimatized for 48 hours before use in the experiment. Standard ethical procedures were adhered to when handling the mice.

Determination of antinociceptive activities

The antinociceptive activities of the plant extracts were determined through the formalin paw licking test. The mice were divided into six groups (n=5) and then treated as shown on (Table 1). Half an hour after administering the treatments, the formalin test was performed using a known protocol [6]. The plant extracts were prepared on the day of the experiment and were administered by intraperitoneal injection. The induction of nociceptive response (licking and biting) was performed by subcutaneous injection of 0.1 ml of 2.50% formalin in the subplantar region of the right hind paw [4,11].

The mice were then individually placed in a transparent glass chamber for observation. The time that the mice spent licking or biting the injected paw was scored [4]. The pain scores were identified for two periods of nociceptive behavior (licking/biting) and recorded separately. The first period was recorded 1-5 minutes after injecting formalin and the second period recorded 15-30 minutes after formalin injection. The percentage licking inhibition was then calculated using the formula;

\[ C - T \]
\[ C \times 100 \]

Where;

C: The vehicle-treated control group value for each phase
T: The treated group value for each phase

Qualitative phytochemical screening

Qualitative phytochemical screening was done on the extracts to identify the presence or absence of selected phytochemical constituents using known protocols [12,13]. The secondary metabolites tested for included flavonoids, terpenoids, alkaloids, sterols, saponins, phenolics, and cardiac glycosides as these are generally associated with the antinociceptive effects.

Data management and statistical analysis

Experimental raw data on licking time/latency of pain response was tabulated on Ms Excel spreadsheet program. This data was then entered to the Minitab statistical software for descriptive statistics. The results were expressed as mean ± standard error of mean (SEM) for analysis. Then one-way analysis of variance (ANOVA) was performed to compare the means of the groups, subsequently followed by Tukey’s post hoc test for pair-wise mean separations and comparisons to obtain the specific significant differences among the different groups. The comparison of the mean activities of H. abyssinica and L. buchananii extracts against pain was performed using unpaired student t-test. The values of P ≤ 0.05 were considered statistically significant. The data on licking time/latency was presented using tables and graphs.

Results

Antinociceptive activity of methanolic stem bark extracts of Harrisonia abyssinica in mice

In general, the administration of methanolic stem bark extract of Harrisonia abyssinica appreciably reduced the formalin-induced pain in both phases which was indicated by reduction in paw licking time (Table 2).

In the early phase, the percent inhibition of paw licking time upon administration of stem bark extracts of H. abyssinica at the three dose levels (50,100 and 150 mg/kg body weight) was 39.73%, 69.73% and 81.13% respectively and exhibited a dose dependent trend (Table 2). The treatment of mice with methanolic stem bark extract of H. abyssinica at the dose levels of 50 and 100 mg/kg body weight was not significantly different from that of positive control with the reference drug, diclofenac (p ≥ 0.05; Table 2). However, treatment of mice with the extract at dose level of 150 mg/kg produced a stronger antinociceptive effect compared to the positive control group (p ≤ 0.05; Table 2). All the dose levels (50,100 and 150 mg/kg body weights) of methanolic stem bark extract of H. abyssinica significantly reduced paw licking time compared to negative control (p ≤ 0.05; Table 2).

In the late phase, the methanolic stem bark extract of H. abyssinica at the three dose levels (50,100 and 150 mg/kg body weight) reduced

<table>
<thead>
<tr>
<th>Group</th>
<th>Status</th>
<th>Treatment</th>
<th>Phase I (seconds)</th>
<th>Phase II (seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>DMSO only</td>
<td>00.00 ± 0.00c</td>
<td>00.00 ± 0.00c</td>
</tr>
<tr>
<td>II</td>
<td>Negative control</td>
<td>Formalin+10% DMSO</td>
<td>11.30 ± 13.9a</td>
<td>143.80 ± 8.14a</td>
</tr>
<tr>
<td>III</td>
<td>Positive control</td>
<td>Formalin+15 mg/kg Diclofenac+10% DMSO</td>
<td>55.40 ± 4.72c (46.50)</td>
<td>33.20 ± 5.89(76.77)</td>
</tr>
<tr>
<td>IV</td>
<td>Experimental group A</td>
<td>Formalin+50 mg/kg extract+10% DMSO</td>
<td>103.70 ± 4.34c (93.73)</td>
<td>119.6 ± 4.01(15.92)</td>
</tr>
<tr>
<td>V</td>
<td>Experimental group B</td>
<td>Formalin+100 mg/kg extract+10% DMSO</td>
<td>66.80 ± 7.14c (39.73)</td>
<td>50.40 ± 7.32(63.80)</td>
</tr>
<tr>
<td>VI</td>
<td>Experimental group C</td>
<td>Formalin+150 mg/kg extract+10% DMSO</td>
<td>50.40 ± 7.83 (26.83)</td>
<td>41.60 ± 10.5(69.64)</td>
</tr>
</tbody>
</table>

Table 1: Evaluation protocol of antinociceptive activities of methanolic extracts of Harrisonia abyssinica and Landolphia buchananii in mice

Values are expressed as Mean ± SEM for five animals per group. Statistical comparison were made within a column and values without the same superscript are significantly different by one-way ANOVA followed by Tukey’s post hoc test (p ≤ 0.05). Figures in parenthesis indicate percent paw licking inhibition. Formalin=2.5%; DMSO=10%; Diclofenac=15 mg/kg.

Antinociceptive effects of methanolic stem bark extract of Harrisonia abyssinica Oliv. on formalin-induced pain in mice
formalin induced pain in mice in a dose dependent manner by 15.92%, 63.80% and 69.84% respectively. The dose levels (100 and 150 mg/kg body weight) of methanolic stem bark extract of H. abyssinica significantly reduced paw licking time compared to negative control (p ≤ 0.05; Table 2). However, the treatment of mice with the extract at the dose level of 50 mg/kg body weight was statistically insignificant when compared with the negative control group (p ≥ 0.05; Table 2). The treatment of mice with methanolic stem bark extract of H. abyssinica at the dose levels of 100 and 150 mg/kg body weight was comparable with that of positive control with the reference drug, diclofenac (p ≥ 0.05; Table 2).

**Antinociceptive activity of methanolic stem bark extracts of Landolphia buchananii in mice**

On the other hand, the administration of methanolic stem bark extract of L. buchananii also generally reduced the formalin-induced pain in both phases. In the early phase, methanolic stem bark extract of L. buchananii, at the three dose levels (50,100 and 150 mg/kg body weight), reduced formalin-induced pain by 35.35%, 40.80 and 47.72% respectively in a dose dependent fashion (Table 3).

Treatments with the extract at all the dose levels (50,100 and 150 mg/kg body weights) significantly inhibited the paw licking response time in mice compared to the negative control group (p ≤ 0.05; Table 3). The antinociceptive effectiveness of the doses at the three dose levels (50, 100 and 150 mg/kg body weights) was statistically insignificant from each other (p ≥ 0.05; Table 3). In this phase, the methanolic stem bark extract of L. buchananii at the dose levels of 50 mg/kg and 100 mg/kg body weight exhibited a decrease in pain that was comparable to diclofenac (reference drug) (p ≥ 0.05; Table 3). However, the antinociceptive effect of the extract at the dose level of 150 mg/kg body weight was stronger with a 47.72% licking inhibition when compared to the positive control group which had a 19.97% licking inhibition (p ≤ 0.05; Table 3).

In the late phase, the methanolic stem bark extract of L. buchananii at the dose levels (50, and 100 mg/kg body weights) also reduced formalin-induced pain in mice (Table 3). However, the paw licking reduction activity of the extract at the dose level of 150 mg/kg body weight was statistically insignificant compared to the negative control group (p ≥ 0.05; Table 3). The percent paw licking inhibitions of the stem bark extract at the three dose levels were 55.17%, 39.14% and 20.57% respectively (Table 3). The treatment at the dose level of 50 mg/kg bodyweight was comparable to that of positive control with the reference drug, diclofenac (p ≥ 0.05; Table 3). However, treatments at the dose levels of 50 and 100 mg/kg body weights were not statistically significant from one another (p ≥ 0.05; Table 3).

**Comparison between the antinociceptive activity of Harrisonia abyssinica and Landolphia buchananii**

In comparison, the methanolic stem bark extract of H. abyssinica exhibited more effective antinociceptive effect at the dose levels of 100 and 150 mg/kg body weight than methanolic stem bark extract of L. buchananii during the early phase at p values of 0.01 and 0.02 respectively (Figure 1). However, in this phase, the comparison of the antinociceptive effectiveness at the dose level of 50 mg/kg body weight of the two extracts was not significantly different from each other (p = 0.60; Figure 1).

In the late phase, the antinociceptive effect of methanolic stem bark extract of H. abyssinica was more efficacious at the dose levels of 100 and 150 mg/kg body weights than that of L. buchananii at p values of 0.034 and 0.002 respectively (Figure 2). However, L. buchananii exhibited more antinociceptive activity than H. abyssinica at the dose level of 50 mg/kg body weight (p=0.00; Figure 2).

**Qualitative Phytochemical Screening**

Qualitative phytochemical screening revealed the presence of flavonoids, terpenoids, alkaloids, sterols, saponins, phenolics and cardiac glycosides (Table 4).

**Discussion**

Clinically, several broad processes are associated with pain:

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Phase I (seconds)</th>
<th>Phase II (seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>DMSO only</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt; (100.00)</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt; (100.00)</td>
</tr>
<tr>
<td>Negative control</td>
<td>Formalin+DMSO</td>
<td>154.10 ± 13.90&lt;sup&gt;a&lt;/sup&gt; (00.00)</td>
<td>156.40 ± 7.76&lt;sup&gt;a&lt;/sup&gt; (00.00)</td>
</tr>
<tr>
<td>Positive control</td>
<td>Formalin+DMSO+Diclofenac</td>
<td>119.80 ± 6.91&lt;sup&gt;a&lt;/sup&gt; (19.97)</td>
<td>40.40 ± 16.30&lt;sup&gt;a&lt;/sup&gt; (74.80)</td>
</tr>
<tr>
<td>Methanolic Stem bark extracts</td>
<td>50 mg/kg+Formalin+DMSO</td>
<td>96.40 ± 4.88&lt;sup&gt;a&lt;/sup&gt; (35.35)</td>
<td>69.60 ± 5.58&lt;sup&gt;a&lt;/sup&gt; (55.17)</td>
</tr>
<tr>
<td></td>
<td>100 mg/kg+Formalin+DMSO</td>
<td>93.20 ± 14.10&lt;sup&gt;a&lt;/sup&gt; (40.80)</td>
<td>95.11 ± 11.70&lt;sup&gt;a&lt;/sup&gt; (38.14)</td>
</tr>
<tr>
<td></td>
<td>150 mg/kg+Formalin+DMSO</td>
<td>77.40 ± 11.30&lt;sup&gt;a&lt;/sup&gt; (47.72)</td>
<td>125.00 ± 12.50&lt;sup&gt;a&lt;/sup&gt; (20.57)</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM for five animals per group. Statistical comparison were made within a column and values without the same superscript are significantly different by one-way ANOVA followed by Tukey’s post hoc test (p ≤ 0.05). Figures in parenthesis indicate percent paw licking inhibition. Formalin=2.5%, DMSO=10%, Diclofenac=15 mg/kg.

Table 3: Antinociceptive effects of methanolic stem bark extracts of L. buchananii in the early phase of the formalin test.

![Figure 1: Percent licking inhibition by methanolic stem bark extracts of H. abyssinica and L. buchananii in the early phase of the formalin test.](image1)

![Figure 2: Percent licking inhibition by methanolic stem bark extracts of H. abyssinica and L. buchananii in the late phase of the formalin test.](image2)
The antinociceptive effect of H. abyssinica at the dose of 150 mg/kg body weight (in the early phase) and L. buchananii at the dose levels of 100 and 150 mg/kg body weight (in the late phase) was greater and statistically significant compared to diclofenac (p ≤ 0.05; Table 1; Table 2) which might suggest a better inhibition of algogenic agents such as prostaglandins or a better mimicry of the diclofenac action.

The antinociceptive effects of methanolic stem bark extracts of H. abyssinica and L. buchananii might be due to one or several phytoconstituents present in the extracts. Several studies have shown the antinociceptive activity of such compounds. A number of flavonoids have been reported to produce analgesic activity [27]. Some studies have demonstrated that flavonoids inhibit prostaglandin synthetase [28]. Since prostaglandins are involved in pain perception and flavonoids have inhibitory effects against them, it might be implied that reduced presence of prostaglandins by flavonoids present in H. abyssinica and L. buchananii might be responsible for their antinociceptive effects.

Alkaloids, which were present in both extracts have been documented to possess potent analgesic activities [29] and thus might be contributory to the observed antinociceptive effects of H. abyssinica and L. buchananii. An evaluation of the several alkaloids from Ziziphus oxyphylla has demonstrated antinociception in both phases of the formalin test [30]. Terpenoids have also been linked with antinociceptive activity. For instance, a study on 1, 8-cineole terpenoid has demonstrated antinociceptive effect in the formalin test through a non-opioid mechanism [31]. Phenols, which were also detected in both extracts, have been shown to exert antinociceptive effect in mice in an opioid-mediated action according to a study on the extract of Emilia sonchifolia [32]. Saponins, which were also found in both extracts although in trace amounts in H. abyssinica extracts although in trace amounts in H. abyssinica extracts although in trace amounts in H. abyssinica extracts although in trace amounts in H. abyssinica extracts although in trace amounts in H. abyssinica extracts although in trace amounts in H. abyssinica extracts although in trace amounts in H. abyssinica extracts although in trace amounts in H. abyssinica extracts although in trace amounts in H. abyssinica extracts although in trace amounts in H. abyssinica extracts although in trace amounts in H. abyssinica extracts although in trace amounts in H. abyssinica extracts although in trace amounts in H. abyssinica extracts although in trace amounts in H. abyssinica extracts although in trace amounts in H. abyssinica extracts although in trace amounts in H. abyssinica extracts although in trace amounts in H. abyssinica extracts although in trace amounts in H. abyssinica extracts although in trace amounts in H. abyssinica extracts although in trace amounts in H. abyssinica extracts although in trace amounts in H. abyssinica extracts although in trace amounts in H. abyssinica extracts although in trace amounts in H. abyssinica extracts although in trace amounts in H. abyssinica extracts although in trace amounts in H. abyssinica.
sterol constituents possess analgesic actions. For instance, β-sitosterol which has previously been isolated from *H. abyssinica* extract [34] has been shown to exert analgesic effect according to a study using leaf extracts of *Oxalis corniculata* [35] and therefore, sterol constituents present in both extracts might also be contributory to the observed antinociceptive effects.

It is noteworthy to mention a limitation in this study regarding the use of either sex of mice in performing antinociceptive studies since pain sensitivity is gender sensitive as documented by a recent study [36].

### Conclusion

In conclusion, the present study has demonstrated the antinociceptive potential of methanolic stem bark extracts of *Harrisonia abyssinica* and *Landolphia buchananii* in mice. The methanolic stem bark extracts of *H. abyssinica* and *L. buchananii* were able to inhibit pain sensation of both phases. Thus it might be likely to find opioid analgesics as well as analgesics in *H. abyssinica* and *L. buchananii* that act by inhibition of inflammatory pathways responsible for pain. Therefore, the methanolic stem bark extracts of *H. abyssinica* and *L. buchananii* extracts might prove useful in managing painful complications and serve as a more effective alternative treatment strategy than the conventional synthetic drugs. The present study, therefore, scientifically confirms the traditional use of *Harrisonia abyssinica* and *Landolphia buchananii* for management of painful conditions.

### References


### Citation