Antipyretic Potential of Methanolic Stem Bark Extracts of *Harrisonia abyssinica* Oliv and *Landolphia buchananii* (Hallier F.) Stapf in Wistar Rats

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**Abstract**

Interest in herbal drugs is undergoing a renaissance at present time. Herbal agents are regarded to be more effective and comparatively safe as opposed to conventional medications which are relatively inaccessible and arguably associated with various adverse effects. *Harrisonia abyssinica* and *Landolphia buchananii* have been used by the Amuru and Embu communities to alleviate various ailments. However, despite their wide folklore use, extensive literature research reveals limited scientific evaluation of their described effects. Thus, the current study aimed to evaluate the antipyretic effects of their methanolic extracts. The plant samples were sourced from Mbeere, Embu County, Kenya. The test subjects (experimental rats) were grouped into four; normal group, a negative control group, reference group and experimental groups. The experimental groups were treated with stem bark extracts at concentration of 50 mg/kg, 100 mg/kg and 150 mg/kg. The determination of antipyretic activities was evaluated by using a 20% turpentine solution as the pyrexia inducing agent and then compared with aspirin as the reference drug. *H. abyssinica* extract reduced the rectal temperature by between 0.32% and 2.52%. Aspirin reduced the elevated rectal temperature by 1.70% and 2.32%. Qualitative pychochemical screening results showed that the extracts possessed several phytochemicals. The results of the study have confirmed the folklore use of the aforementioned plants in the suppression of pyrexia.

**Keywords:** *Harrisonia abyssinica*, *Landolphia buchananii*, Antipyretic

**Introduction**

Pyrexia is produced as an auxiliary impact of infection, malignancy or other infected states. It is the defense of the body to naturally create an environment where an infectious agent cannot withstand [1]. It is a typical medical sign which in humans is indicated by a temperature rise over the normal range of 36.5°C to 37.5°C as a result of the increase in the body temperature regulatory set-point [2]. The febrile response, whereby fever is one of the components, is physiologically intricate as it comprises of cytokine-mediated elevation in body temperature, development of acute-phase reactants, and stimulation of various immunologic systems [3].

Studies have demonstrated that the stimulation of leukocytes with bacterial products leads to the synthesis of protein mediators (cytokines), of which some possess potent endogenous pyrogen-like features [4]. These cytokines with pro-inflammatory properties are: interleukin-1 (IL-1) [5], tumor necrosis factor (TNF) and lymphotoxin, IL-6, and interferons [6]. Apparently, these pro-inflammatory cytokines reach the CNS where, through induction of central mediators such as prostaglandins, they raise the temperature set-point and cause fever [7].

IL-1 and TNF are involved in synthesis and release of PGE2 through activation of human endothelial cells [8]. IL-1 and TNF-induced effects such as the release of thromboxane from adherent neutrophils are deemed to contribute to fever, since an early increase in IL-1-mediated fever seems to be correlated with a rise in thromboxane levels in the third cerebral ventricle [9].

The prescriptions of non-steroidal anti-inflammatory drugs (NSAIDs) are among the most common worldwide as they relieve fever in many disorders [10]. All conventional NSAIDs inhibit the conversion of arachidonic acid into prostaglandin PGE2. The stage is catalyzed by cyclooxygenase (COX) within which isoenzymes COX-1 and COX-2 occur [11]. The variations in the adverse effects of NSAIDs at their anti-inflammatory doses are due to the individual NSAIDs exhibiting dissimilar potencies against COX-1 in comparison to COX-2 [12]. Most of NSAIDs exhibit more selectivity for COX-1 than for COX-2, and this forms the basis for their gastro-toxicity and their anti-thrombotic action [13]. Antipyretic medications can also cause abnormalities of the skin and the respiratory, blood, and central nervous system (CNS) [14].

Alternatively, the holistic approach to healthcare makes herbal medicine very attractive to many people and the presence of multiple active compounds in botanicals provides them with an important hypothetical edge over conventional single component drugs as they provide a potentiating response that may not be attainable by any individual compound [15]. *Harrisonia abyssinica* has been used in the treatment of fever and in other diseases [16]. The Amuru and Embu communities have used a solution made by boiling the roots of *Landolphia buchananii* and goat's soup to relieve backaches and joint pains [17]. However, despite their wide folklore use, extensive literature research reveals limited scientific evaluation on the pharmacological activities of their described effects on pyrexia. The current study was designed against this background and it specifically aimed at bio-
screening of the methanolic stem bark extracts of *H. abyssinica* and *L. buchananii* for antipyretic potential.

**Materials and Methods**

**Collection and preparation of plant samples**

The stem barks of *H. abyssinica* and *L. buchananii* were sourced from Mbeere North, Embu County, Kenya, with the help of local herbalists. Harvesting of the stem barks was done before the onset of the dry season since humid environment facilitates the detachment of the bark. The samples were then sorted, cleaned and transported in clean burlap sacks to the Biochemistry and Biotechnology Laboratories at Kenyatta University for further processing. The plant samples were then provided to a taxonomist for botanical verification and specimen deposited at the Kenyatta University Herbarium. The stem barks of *H. abyssinica* and *L. buchananii* were air dried at room temperature being cut into small pieces till they were properly dry. The stem barks were then ground into fine homogenous powder via an electric mill and sieved to remove extraneous matters such as dirt, foreign particles and adulterants.

**Extraction**

For each plant sample, 400 g of powder was soaked separately in 1200 mL of methanol and the solution was allowed to stand for 24 h.

**Determination of antipyretic activities**

Experimental design

Wistar albino rats, Rattus norvegicus, were utilized in this study. The animals were housed in the Animal House, Department of Biochemistry and Biotechnology at Kenyatta University. They were then acclimatized for 48 h prior to the experiment. The animals were maintained under standard laboratory conditions of ambient temperature (25°C) and with 12-hour daylight. Standard rodent pellets were used to feed the experimental animals and were supplied with water ad libitum [19]. The antipyretic experiments were performed on one-month-old adult male Wistar rats.

**Data management and statistical analysis**

Experimental raw data on the rectal temperatures were tabulated on Ms Excel spread sheet program. This data were then imported to the Minitab statistical software where it was subjected to 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.*

<table>
<thead>
<tr>
<th>Group</th>
<th>Status</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>DMSO (10%)</td>
</tr>
<tr>
<td>II</td>
<td>Negative control</td>
<td>Turpentine (20%) + DMSO (10%)</td>
</tr>
<tr>
<td>III</td>
<td>Positive control</td>
<td>Turpentine (20%) + 100 mg/kg Aspirin + DMSO (10%)</td>
</tr>
<tr>
<td>IV</td>
<td>Experimental group A</td>
<td>Turpentine (20%) + 50 mg/kg extract + DMSO (10%)</td>
</tr>
<tr>
<td>V</td>
<td>Experimental group B</td>
<td>Turpentine (20%) + 100 mg/kg extract + DMSO (10%)</td>
</tr>
<tr>
<td>VI</td>
<td>Experimental group C</td>
<td>Turpentine (20%) + 150 mg/kg extract + DMSO (10%)</td>
</tr>
</tbody>
</table>

Table 1: Evaluation protocol of antipyretic activities of methanolic extracts of *Harrisonia abyssinica* and *Landolphia buchananii* in rats.

**Qualitative phytochemical screening**

Qualitative phytochemical screening was done on the extracts to screen for the presence or absence of selected phytochemical constituents using known protocols [23,24]. The secondary metabolites tested for included flavonoids, terpenoids, alkaloids, sterols, saponins, phenolics, and cardiac glycosides as these are generally associated with the antipyretic effects.

**Experimental raw data on the rectal temperatures were tabulated on Ms Excel spread sheet program. This data were then imported to the Minitab statistical software where it was subjected to 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 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.*
**Results**

**Antipyretic activity of methanolic stem bark extracts of H. abyssinica in rats**

Generally, there was a reduction in rectal temperature in turpentine-induced pyretic rats after treatment with methanolic stem bark extract of *H. abyssinica* (Table 2) (Figure 1). In the first hour after treatments, the rat groups treated with methanolic stem bark extract of *H. abyssinica* at all the dose levels (50, 100 and 150 mg/kg body weight) and with aspirin lowered the elevated temperature to 99.10%, 98.80%, 98.93% and 98.30%, respectively (Table 2) (Figure 1). The antipyretic activities exhibited by the extract were statistically insignificant (p ≥ 0.05) (Table 2) (Figure 1) when compared to the normal control group and positive control group with the reference drug, aspirin. However, treatments at the dose level of 100 and 150 mg/kg body weights recorded antipyretic activities that were statistically significant when compared to the negative control group (p ≤ 0.05) (Table 2) (Figure 1). The treatment at the dose level of 50 mg/kg body weight demonstrated antipyretic activity that was not statistically significant when compared to the negative control (p ≥ 0.05) (Table 2) (Figure 1).

### Table 2: Effects of intra peritoneal administration of methanolic stem bark extract of *Harrisonia abyssinica* Oliv on turpentine-induced pyrexia in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Percent change in rectal temperature (°C) after drug administration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 h</td>
</tr>
<tr>
<td>Normal</td>
<td>DMSO only</td>
<td>100 ± 0.00</td>
</tr>
<tr>
<td>Negative control</td>
<td>Turpentine+DMSO</td>
<td>100 ± 0.00</td>
</tr>
<tr>
<td>Positive control</td>
<td>Turpentine+DMSO+Aspirin</td>
<td>100 ± 0.00</td>
</tr>
<tr>
<td>Methanolic</td>
<td>Turpentine+50 mg/kg</td>
<td>100 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>Turpentine+100 mg/kg</td>
<td>100 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>Turpentine+150 mg/kg</td>
<td>100 ± 0.00</td>
</tr>
</tbody>
</table>

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 by one-way ANOVA followed by Tukey’s post hoc test (p ≥ 0.05). Turpentine = 20%; DMSO = 10%; Aspirin = 100 mg/kg.

In the second hour, all the rat groups treated with methanolic stem bark extract of *H. abyssinica* at the three dose levels (50,100 and 150 mg/kg body weight) lowered rectal temperature by 1.09%, 1.33% and 1.68%, respectively (Table 2). The treatments of the extract recorded antipyretic activity that was statistically significant when compared to the negative control (p ≤ 0.05) (Table 2). The antipyretic effectiveness of the extract at the dose level of 100 mg/kg body weight was comparable to the reference drug, aspirin (p ≥ 0.05) (Table 2). However, the antipyretic activity of the extracts at the dose levels of 50 and 150 mg/kg body weight was statistically insignificant compared with the normal group (p ≥ 0.05) (Table 2).

In the third hour, pyrexia was reduced in a dose dependent manner and the trend was maintained up to the fourth hours of the test period (Table 2) (Figure 1). All the rat groups treated with methanolic stem bark extract of *H. abyssinica* at the three dose levels (50,100 and 150 mg/kg body weights) lowered rectal temperature by 1.09%, 1.33% and 1.68%, respectively (Table 2). At this hour, the antipyretic activity at the dose levels 100 and 150 mg/kg body weights was comparable to that of the positive control (p ≥ 0.05) (Table 2). The treatments of the extract recorded antipyretic effectiveness that was statistically significant compared with the normal and negative control groups (p ≤ 0.05) (Table 2).

Four hours after treatments, the stem bark extracts of *H. abyssinica* at the dose levels of 50,100 and 150 mg/kg body weight lowered turpentine-induced fever to 98.77%, 98.69% and 98.26%, respectively (Table 2) (Figure 1). The treatments of the extracts at the dose levels of 100 and 150 mg/kg body weight exhibited antipyretic effectiveness that was comparable to the positive control (p ≥ 0.05) (Table 2) but...
comparable to the normal and negative control groups (p ≤ 0.05) (Table 2).

**Antipyretic activity of methanolic stem bark extracts of *L. buchananii* in rats**

Similarly, there was a reduction in rectal temperature in turpentine-induced pyretic rats upon administration of methanolic stem bark extracts of *L. buchananii* (Table 3) (Figure 2). This demonstrates the plant possesses antipyretic potential. In the first hour after treatment, only the rat groups treated with methanolic stem bark extract of *L. buchananii* at the dose levels of 150 mg/kg body weight reduced the elevated rectal temperature to normal (Table 3). The three dose levels (50, 100 and 150 mg/kg body weight) lowered the elevated rectal temperature by 1.47%, 1.02% and 0.32% respectively while aspirin reduced rectal temperature by 1.72% (Table 3) (Figure 2). The treatments of the extract at the dose level of 50 and 100 mg/kg body weight exhibited antipyretic effectiveness that was comparable to the positive control with the reference drug, aspirin (p ≥ 0.05) (Table 3).

**Table 3:** Effects of intraperitoneal administration of methanolic stem bark extract of *Landolphia buchananii* (Hallier f.) Stapf on turpentine-induced pyrexia in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Percent change in rectal temperature (°C) after drug administration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 h</td>
</tr>
<tr>
<td>Normal control</td>
<td>DMSO</td>
<td>100 ± 0.00</td>
</tr>
<tr>
<td>Negative control</td>
<td>Turpentine+DMSO</td>
<td>100 ± 0.00</td>
</tr>
<tr>
<td>Positive control</td>
<td>Turpentine+DMSO+Aspirin</td>
<td>100 ± 0.00</td>
</tr>
<tr>
<td>Methanolic</td>
<td>Turpentine+50 mg/kg</td>
<td>100 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>Turpentine+100 mg/kg</td>
<td>100 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>Turpentine+150 mg/kg</td>
<td>100 ± 0.00</td>
</tr>
</tbody>
</table>

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 by one-way ANOVA followed by Tukey's post hoc test (p ≥ 0.05). Turpentine =20%; DMSO = 10%; Aspirin = 100 mg/kg.

**Figure 2:** The percent change of rectal temperature by methanolic stem bark extract of *Landolphia buchananii* on turpentine-induced pyretic rats.

In the second hour, all the groups treated with methanolic stem bark extract of *L. buchananii* at the three dose levels (50, 100 and 150 mg/kg body weight) lowered rectal temperature to 98.53%, 98.22% and 98.49%, respectively (Figure 2). The treatments of the extract at the dose level of 50 and 100 mg/kg body weight recorded antipyrretic activity that was comparable to the positive control with the reference drug, aspirin (p ≥ 0.05) (Table 3). However, the antipyretic activity of the extract at the dose level of 150 mg/kg body weight was statistically insignificant when compared with the normal and negative control groups (p ≥ 0.05) (Table 3).

In the third hour, the methanolic stem bark extract of *L. buchananii* at all dose levels lowered elevated rectal temperature by 1.49%, 2.32% and 0.56% respectively (Table 3). The methanolic stem bark extracts of *L. buchananii* at the dose levels of 50 and 100 mg/kg body weight exhibited antipyretic effect that was comparable to positive control with the reference drug, aspirin (p ≥ 0.05) (Table 3).
Four hours after treatment, the stem bark extract of *L. buchananii* at dose levels of 50, 100 and 150 mg/kg body weight appreciably lowered turpentine-induced fever to 98.40%, 97.48% and 99.23%, respectively (Table 3) (Figure 2). The methanolic stem bark extract of *L. buchananii* at the dose level of 100 mg/kg body weight exhibited strong fever lowering activity by 2.52%. The antipyretic activity at this dose level as well as at the dose level of 50 mg/kg body weight was comparable to that of positive control with the reference drug (p ≥ 0.05) (Table 3).

**Comparison between the antipyretic activities of *H. abyssinica* and *L. buchananii***

In comparison, the antipyretic activity of the methanolic stem bark extracts of *H. abyssinica* and *L. buchananii* did not significantly differ from each other at the dose levels of 50 mg/kg body weight during the 4 h of the test period at p values of 0.075, 0.057, 0.054 and 0.397 respectively. At the dose level of 100 mg/kg, the antipyretic activity of *H. abyssinica* and *L. buchananii* did not differ significantly during the first 3 h (p values at 0.705, 0.313 and 0.081, respectively) but during the fourth hours, *L. buchananii* exhibited stronger antipyretic effect compared to *H. abyssinica* at p value of 0.039. *H. abyssinica* exhibited more effective antipyretic activity than *L. buchananii* at the dose level of 150 mg/kg body weight during all the 4 h of the test period at p values of 0.042, 0.050, 0.005 and 0.009, respectively.

**Qualitative phytochemical screening**

Qualitative phytochemical screening of the methanolic stem bark extracts of *H. abyssinica* and *L. buchananii* revealed the presence of alkaloids, flavonoids, saponins, phenolics, terpenoids and steroids. Cardiac glycosides were, however, absent in *H. abyssinica*. Steroids were in trace amounts in the methanolic stem bark extract of *L. buchananii* which was also the case of saponins in the methanolic stem bark extracts of *H. abyssinica* (Table 4).

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th><em>H. abyssinica</em> stem bark extract</th>
<th><em>L. buchananii</em> stem bark extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>+(trace)</td>
</tr>
<tr>
<td>Saponins</td>
<td>+(trace)</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Phenolics</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Present phytochemical are denoted by (+) sign, absent phytochemical are denoted by (−) sign while +(trace) denotes slightly present phytochemical.

**Discussion**

The current study was designed to evaluate the antipyretic properties of the methanolic stem bark extracts of *H. abyssinica* and *L. buchananii*. The evaluation of antipyretic properties of the extracts was performed on turpentine-induced pyretic rats. Exogenous pyrogenic stimuli such as turpentine and lipopolysaccharides are inducers of fever by their ability to produce pro-inflammatory cytokines [25].

Turpentine is refined from Pinus palustris mill and produces local inflammation with an acute phase fever upon administration into experimental animals [26]. Turpentine exerts a direct influence on various tissues, including the brain and only small doses are need to produce an increase in body temperature. Moreover, the experimental animals acquire tolerance to turpentine in a less easy manner than to other pyrogens [27]. Therefore, these findings guided the selection of turpentine as the pyrogen for the present study.

After 4 h of the test period, the methanolic stem bark extracts of *H. abyssinica* and *L. buchananii* produced appreciable antipyretic activity against turpentine-induced fever in rats. These findings were relatable with the effects of other herbal extracts in animal models. Similar work demonstrated effective antipyretic activity of ethanolic leaf extract of *P. pinnata* (L.) Pierre on brewer's yeast-induced pyrexia in rats [28]. Another related study demonstrated antipyretic activity of methanolic stem bark extracts of Bauhinia racemosa in animal models [29].

To relieve pyrexia in many conditions, NSAIDs are prescribed regularly in routine practice [10]. NSAIDs act through the inhibition of PG biosynthesis due to the irreversible acetylation of the COX site, leaving the peroxidase activity of the enzyme unaffected [30]. Generally, the antipyretic mechanism of NSAIDs comprises inhibition of prostaglandin biosynthesis within the hypothalamus [31]. The ability of the extracts to cross the brain blood barrier is a factor that may have contributed to the observed antipyretic activity of the extracts. More so, the extracts may have reduced the temperature of the test animals by stimulating the body to produce its own antipyretic substances such as vasopressin and arginine due to the presence of the various phytochemicals present in the extracts [32].

The dose ranges employed in this study were within the dose ranges used in a study while evaluating the antipyretic effect of alcohol leaf and root bark extracts of Carissa edulis in rats [33]. Another study examining the antipyretic effect of methanolic extracts and various solvent fractions of Diospyros lotus used dose levels of 50 and 100 mg/kg body weight [34].

The methanolic stem bark extracts of *H. abyssinica* and *L. buchananii* did not lower rectal temperature during the first and second hours of the test period as effectively as in the third and fourth hours (Tables 2 and 3) (Figures 1 and 2). The highest antipyretic effect of the *H. abyssinica* stem bark extract was by 1.21% and 1.29% in the first and second hours respectively, while 1.68% and 1.74% was observed in the third and fourth hours, respectively. On the other hand, the highest antipyretic effect by *L. buchananii* stem bark extracts was by 1.47% and 1.78% in the first and second hours respectively, while 2.33% and 2.52% was observed in the third and fourth hours, respectively. The reason might be due to the fact that the active components in the extracts required to be transformed to become antipyretic.

That the stem bark extracts of *H. abyssinica* demonstrated a dose dependent response on rectal temperature (in the later hours of the test period) lowering effect in turpentine-induced pyretic rats, was in agreement with a study which noted dose dependent antipyretic effects of methanolic stem bark extract of Acacia leucophloea in yeast-induced hyperthermic rats [35]. Another related study on the antipyretic effect of methanolic extract of Hygrocybe cantharellus, a dose dependent lowering trend was noted in brewer's yeast-induced...
The dose dependent antipyretic trend of stem bark extract of *H. abyssinica* may be attributed to the diffusion of the active components in a passive manner in the peritoneal cavity.

However, the non-concentration-dependent effect seen in the antipyretic effect of *L. buchananii* may be because of a phenomenon known as the therapeutic window [37]. Some drugs possess a remarkable feature whereby the therapeutic effect is exerted only over a limited range of drug doses or plasma drug concentrations. If the doses extend beyond this narrow therapeutic range they exhibit a decline in effects [38].

The 100 mg/kg body weight dose level of the methanolic stem bark extracts of *L. buchananii* was more effective than aspirin especially at the third and fourth hour suggests a possibly better blockage of prostaglandins biosynthesis by the active principles in the extracts.

The antipyretic activity of the methanolic stem bark extracts of *H. abyssinica* and *L. buchananii* might be due to the action of one or more phytoconstituents present in the extracts. In fact, the antipyretic activity may be attributed to the synergism of the various phytochemical compounds in the extracts which may have also aided to nullify the toxic effects (if any) of the individual constituents [39]. Qualitative phytochemical screening revealed that the methanolic stem bark extracts of *H. abyssinica* and *L. buchananii* contains steroids, saponins, phenolics, alkaloids, flavonoids and terpenoids (Table 4). Some of these phytochemicals exhibit inhibitory action on COX enzyme, consequently inhibiting fever.

Conclusion

In conclusion, the present study has demonstrated the antipyretic potential of methanolic stem bark extracts of *H. abyssinica* and *L. buchananii* in animal models. The significant reduction in pyrexia in rats upon treatment with reference drugs and also with different doses of extracts, reveal that *H. abyssinica* and *L. buchananii* might be endowed with potent antipyretic properties. The present study, therefore, scientifically confirms the traditional use of *H. abyssinica* and *L. buchananii* for management of fever conditions.

References

acetate extract of Acacia catechu Willd in albino rats. Indian J Pharmacol 38: 408-413.


