Acute Toxicity, Brine Shrimp Lethality and Phytochemical Screening of *Lannea schimperi* and *Searsia longipes*

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**ABSTRACT**: *Lannea schimperi* and *Searsia longipes* are plants species under family *Anacardiaceae*. These plants have been utilized for years in traditional settings for management of an array of disease conditions; however, there is limited information about their safety (toxicity level). The aim of the current study was to evaluate the acute toxicity, cytotoxicity and phytochemical compounds of extracts from the aforementioned plants. Acute toxicity was performed *in vivo* on Swiss albino mice. Cytotoxicity was done *in vitro* on brine shrimp larvae and plants were qualitatively screened for five major groups of compounds. Both extracts exhibited good margin of safety on swiss albino mice with LD$_{50}$ (Lethal dose 50) above 2000 mg/kg body weight. *Lannea schimperi* and *Searsia longipes* expressed significant cytotoxicity on brine shrimp larvae with IC$_{50}$ (Inhibitory concentration 50) of 150.0478 mg/ml and 280.7875 mg/ml respectively. Phytochemical screenings of both extracts have revealed presence of flavonoids, saponins, tannins and glycosides. The study confirmed the previous reports on the acute toxicity of *Lannea schimperi* and *Searsia longipes*, as well as cytotoxicity of *Lannea schimperi* and for the first time reports the cytotoxicity and phytochemical compounds of *Searsia longipes*.

**INTRODUCTION**

*Lannea schimperi* and *Searsia longipes* are plants species under family *Anacardiaceae*. Genus *Lannea* comprises about 40 plant species which appears as shrubs or trees and widely distributed in Africa tropical regions. Further, the genus *Lannea* has been used for many years in different societies to manage mental disorders, gastrointestinal disorders, bacterial infections, viral infections, fungal infections, fever, as well as used as anti-bilharzia [1, 2]. *Lannea schimperi* in particular is used traditionally in some Tanzanian communities for the treatment of NIDDM (Non-insulin dependent diabetes mellitus), especially by managing some of the key symptoms manifested on diabetic patients such as polyuria, polydipsia, excessive thirst and sweating [3]. Furthermore, *Lannea schimperi* is used for the management of opportunistic diseases associated with HIV (Human Immunodeficiency virus) which includes tuberculosis, skin rashes, herpes zoster, herpes simplex and chronic diarrhea [4, 5]. Moshi and coworkers (2006) reported ethno medicinal importance of *Lannea schimperi* for the treatment of malaria and epilepsy [6]. In some Kenyan societies *Lannea schimperi* is broadly employed in management of diarrhea, stomachache and chest problems [7].
Phytochemical evaluations of the acetone and methanolic extracts from *Lannea schimperi* have revealed the presence of glycoside, triterpene, steroids, cardiac glycosides, flavonoids, phenolic glycosides, alkaloids, tannins, condensed tannins and saponins [8, 9].

Meanwhile, *Searsia* genus comprises more than 250 flowering plant species which are widely and abundantly distributed in temperate and tropical regions [11, 12]. Generally, in folk medicine extracts from various parts of the *Searsia* plants are used to prevent and cure an array of health conditions. Ethno medicinal survey has attributed the potentials of *Searsia* plants decoctions in management of toxins (Depurative), hemoptysis, inflammations, laryngitis, stomachache, traumatic fractures, spermatorrhea, snake bite, diarrhea, coughs (antitussive), dysentery, fever, jaundice, hepatitis, helminthic infections, rheumatism as well as for stimulating blood circulation [13, 14, 15, 16, 11, 17 and 18].

Regarding previously undertaken phytochemical analysis, flavonoids, (iso)flavonoids, terpenoids, anthocyanins, tannins, and organic acids were identified in some plants which belongs to *Searsia*/*Rhus* genus namely *Searsia coriaria*, *Searsia leptodictya* and *Searsia tripartitum* [19, 20, 21, 22]. However, thus far, phytochemical compounds of *Searsia longipes* have never been evaluated, hence presents a research gap for evaluation of its chemical constituents.

Nonetheless, regarding the exploitation of this plants in management of diseases as afore discussed, it is therefore of high imperative to evaluate their margin of safety. Hence, the present study aimed to evaluate the toxicity of these plants by using multi-faceted approaches.

**MATERIALS AND METHODS**

**Sample collection**

Sample collection was done at Endasaki ward found in Manyara region. Prior to sample collection, on site identification of the plants were performed by a botanist from National herbarium of Tanzania. Following identification, specimens were taken and stored in National herbarium of Tanzania with accession number NM 01 and NM 02 for *Lannea schimperi* and *Searsia longipes* respectively.

**Sample processing and extraction**

After collection, samples were dried in absence of direct sunlight. Thereafter, stem barks sizes were reduced by using grinder and extraction was done by using methanol. Briefly, stem bark samples were socked in methanol for two days, and afterward the extracts were filtered and solvents were recovered by using rotary evaporator. Following solvent recovery, 35g and 45g concentrates (crude extracts) of *Searsia longipes* and *Lannea schimperi* were obtained respectively.

**Acute toxicity test of the extracts on mice**

Acute toxicological study was performed to evaluate immediate toxic effects of the methanolic extracts of *Searsia longipes* and *Lannea schimperi* to experimental animals. Study was conducted in abidance to the stapes stipulated in OECD (Organization for Economic Cooperation and Development) guideline number 427. Briefly, two groups of mice each containing 3 female mice with the body weight of 28 ± 2 g and an age of 10 to 12 weeks were selected and kept for 5 days before dosing for acclimatization to the new laboratory environment. One group was used for *Lannea schimperi* extract and other group for *Searsia longipes* extract. On the fifth day, mice were fastened overnight with only supply of water *ad libitum*. Thereafter, mice in each group were administered their respective extract dose of 2000 mg/kg body weight. Distilled water was used as the diluent solution to prepare the aforementioned dose and each animal received 0.5 ml. Following drug administration, water and food were further withheld for 1 hour and afterward animal were observed for 24 hour with much attention given on first four hours. Death and any significant behavioral and physiological changes such as coma, convulsion, tremors, salivation and respiratory problems were carefully examined and recorded [23]. Additionally, observation was prolonged for 14 days to observe any delayed response and this test was done twice as illustrated in Figure 1.
Figure 1. Three (3) female mice were used and it was expected that if 2-3 mice would have died following the administration of 2000 mg/kg dose, the next lower limiting concentration would be used as stipulated in OECD guideline number 423. And if 0-1 mouse died, the procedure in stage one would be repeated and if the result remained the same, it would therefore concluded that the lethal dose 50 of the extract is > 2000 mg/kg bwt.

Cytotoxicity of the extracts on Brine shrimp larvae

Media Preparation

Media were used to mimic the natural environment suitable for the survival of the Brine shrimp larvae. Media were prepared on the basis of the protocol prescribed by Meyer and coworkers (1967). Briefly, Sea salt was obtained after evaporation of sea water collected from Indian Ocean at Dar es Salaam coast. Artificial seawater was thereafter prepared at the concentration of 3.8 g/L by diluting the obtained sea salt with distilled water. Following preparation, artificial sea water was filtered and afterward transferred into a sterilized tank that has been divided into two compartments by perforated polythene wall. One compartment of the tank was large and covered meanwhile the other compartment was small and not covered. Shrimp eggs (500 mg) were sprinkled into the large and covered compartment of the tank and a lamp was illuminated on the uncovered part in order to attract the hatched shrimps. The mature nauplii were collected between 24 and 36 hours of hatching. Hence, the obtained brine shrimp larvae were used as indicator organisms for general cytotoxicity assessment [24].

Cytotoxicity assay

_Lannea schimperi_ and _Searsia longipes_ extracts were dissolved in dimethyl sulphoxide (DMSO) to make a stock solution of 40 mg/ml each. From the stock solution, 7 (Seven) different concentrations of 4 µg/ml, 8 µg/ml, 24 µg/ml, 40 µg/ml, 80 µg/ml, 120 µg/ml, and 240 µg/ml were tested in duplicate. Cyclophosphamide drug and DMSO were used as positive and negative control respectively [24]. In every tested concentration including positive and negative control, 10 brine shrimp larvae were used. Following 24 hours of larvae subjection to different concentrations of the extracts, number of viable nauplii which were motile and didn’t sediment to the bottom of the test container were counted under the illumination condition. Regarding the Meyers et al. (1982) report, cytotoxicity (LC50) of the extracts below 1000 µg/ml was considered to be potent [24].

Qualitative phytochemical screening

Phytochemical screening was performed to identify groups of compound present in the _Searsia longipes_ and _Lannea schimperi_ methanolic extracts. In this regard, a total of five
groups of compounds namely terpenoids, glycosides, flavonoids, tannins and saponins were analyzed by using qualitative analytical methods described by Gul et al. (2017) [25].

**Terpenoids:** 1g of the crude extracts were dissolved in 10 ml of distilled water each and thereafter warmed until were completely dissolved. Solutions were filtered by using whatman filter paper number one. Afterward, two (2) ml of the extracts aqueous solutions were placed on the test tubes and then 2 ml of the acetic acid and H$_2$SO$_4$ were added. Hence, formation of reddish brown coloration indicated the presence of the terpenoids.

**Glycosides:** 2ml of the extracts aqueous solution was measured and dispensed in test tubes. 1 ml of the acetic acid was added, followed by addition of 1 to 2 drops of FeCl$_3$. Afterward, 1 ml of the concentrated sulfuric acid was also added. Thus, formation of violet ring indicated the presence of the glycosides.

**Tannins:** 2ml of the filtered aqueous solution of the extracts were measured and poured into the test tubes. Thereafter 2 ml of the 5% FeCl$_3$ were added and solutions were observed for the formation of yellow precipitate which portrayed the presence of the tannins.

**Saponins:** 2g of the crude extracts were dissolved in 10 ml of the distilled water and then warmed until dissolved completely. Thereafter, the solutions were filtered and 5 ml of the extracts were placed in test tubes. Extracts in the tubes were shaken vigorously until formation of the stable persistent froth. Afterward, 2 to 3 drops of olive oil were added and again the solutions were shaken vigorously and then observed for the formation of the emulsion. Hence, emulsion formation portrayed the presence of the saponins.

**Flavonoids:** 2 g of the extracts were dissolved in 10 ml of the distilled water and then heated until they dissolve completely. The solutions were filtered and 2 ml of the filtrates were dispensed in the test tubes. 2 to 3 drops of HCL (Hydrochloric Acid) and magnesium turning were added and thereafter solutions were observed for the formation of the pink precipitate which indicates the presence of flavonoids.

**Data analysis**

Data were analyzed by using Microsoft excel software, where by IC$_{50}$ values were calculated at 95% confidence interval.

**RESULTS**

**Acute toxicity of the extracts**

Following administration of 2000 mg/kg bw of *Lannea schimperi* and *Searsia longipes* extracts, none of the treated mice have died and due to that the lethal dose 50 of the extracts were regarded to be greater than 2000 mg/kg bw (Table 1). Further, mice were observed individually for the behavioral changes, whereby *Lannea schimperi* seemed to induce tremor, sleepy and respiratory changes (Table 2). Meanwhile, *Searsia longipes* caused reduction of somatomotor activity and respiratory changes (Table 2).

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Concentration (mg/kg bwt)</th>
<th>No of live mice</th>
<th>LD$_{50}$(mg/kg)</th>
<th>GHS</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSM</td>
<td>2000</td>
<td>3</td>
<td>&gt; 2000</td>
<td>Category 5</td>
</tr>
<tr>
<td>SLM</td>
<td>2000</td>
<td>3</td>
<td>&gt; 2000</td>
<td>Category 5</td>
</tr>
</tbody>
</table>

*Lethal dose 50, *Global harmonized classification system, *Body weight
Table 2. Mortality, physiological and behavioral assessment of mice following administration of 2000 mg/kg bwt of the extracts.

<table>
<thead>
<tr>
<th>Observation</th>
<th>LSM</th>
<th>SLM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M1</td>
<td>M2</td>
</tr>
<tr>
<td>Comma</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Convulsion</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Tremors</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Salivation</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Skin and fur</td>
<td>Normal Normal Normal Normal Normal Normal Normal</td>
<td></td>
</tr>
<tr>
<td>Eye and mucosal membrane</td>
<td>Normal Normal Normal Normal Normal Normal Normal</td>
<td></td>
</tr>
<tr>
<td>Somatomotor activity</td>
<td>Normal Normal Normal Reduced Reduced Reduced Reduced</td>
<td></td>
</tr>
<tr>
<td>Lethargy</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Sleepy</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Respiratory changes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Death</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

M1 = Mouse 1, M2 = Mouse 2, M3 = Mouse 3, LSM – Lannea schimperi Methanolic extract, SLM – Searsia longipes Methanolic extract

Cytotoxicity Test on Brine Shrimp larvae

Both Lannea schimperi and Searsia longipes extracts demonstrated concentration dependent mortality of the brine shrimp larvae. Whereby, the mortality seemed to increase as the concentrations of the extracts increased (Figure 2). Nonetheless, both plants were significant toxic to brine shrimp larvae and divulged lethal concentrations 50 (LD_{50}) of 150 and 280 µg/ml for Lannea schimperi and Searsia longipes respectively as depicted in Table 3.

Figure 2. Mortality of brine shrimp larvae at different concentrations of Lannea schimperi (LSM) and Searsia longipes (SLM).

Table 3. Cytotoxicity of the extracts.

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>*L_{50} (µg/ml)</th>
<th>95% CI; (µg/ml)</th>
<th>Regression equation</th>
<th>Retention factor (R^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSM</td>
<td>150.0478</td>
<td>118.0559 - 199.1434</td>
<td>Y = 87.647logx - 140.74</td>
<td>0.9542</td>
</tr>
<tr>
<td>RLM</td>
<td>280.7875</td>
<td>210 - 375.1040</td>
<td>Y = 96.664logx - 186.67</td>
<td>0.9745</td>
</tr>
<tr>
<td>*Cyclophosphamide</td>
<td>16.365</td>
<td>12.006 - 22.305</td>
<td>Y = 69.968logx - 34.9360</td>
<td>0.994929</td>
</tr>
</tbody>
</table>

*Lethal Concentration 50, *CI = Confidence Interval, *Positive control
**Qualitative phytochemical screening of the extracts**

Methanolic extracts of *Lannea schimperi* and *Searsia longipes* were screened for five major groups of compounds namely terpenoids, flavonoids, glycosides, tannins and saponins. Hence, following phytochemical screening both extracts were found to contain flavonoids, glycosides, tannins and saponins as displayed in Table 4.

**Table 4.** Groups of compounds present in *Lannea schimperi* and *Searsia longipes* extracts.

<table>
<thead>
<tr>
<th>Phytochemical compounds</th>
<th>LSM</th>
<th>SLM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terpenoids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponnins</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+= present, - = absent

**DISCUSSION**

**Acute toxicity**

In this study, acute toxicity tests were performed to evaluated safety of *Lannea schimperi* and *Searsia longipes* on Swiss albino mice. Following administration of 2000 mg/kg bwt dose to a group containing 3 mice, mortality and behavioral changes were assessed. For *Searsia longipes*, 30 minutes after dosing animals demonstrated respiratory problems and was manifested by rapid and existence of voice during breathing. This character disappeared after 4 hours of observation. Other changes such as tremors, sleeping and reduced food and water intake were also observed. However, all of the aforementioned changes were disappeared after four hours. Regarding mortality rate, none of the treated mice died after administration of 2000 mg/kg dose. Henceforth, the lethal dose 50 of the extracts are therefore regarded to be greater than 2000 mg/kg, thus it falls under category five (5) of the GHS as stipulated in OECD guideline number 423. This observation agrees with the Olorunnisola and coworkers (2017) report on the acute toxicity of the acetone leaf extract of *Searsia longipes*, whereby the latter was reported to have the LD 50 above 5000 mg/kg body weight [10]. Moreover, the finding is in line with Dialo and coworkers (2009, 2010) report on the acute toxicity of *Lannea kerstingii* which shares similar genus with *Lannea schimperi* [27].

**Cytotoxicity**

Both extract from *Lannea schimperi* and *Searsia longipes* demonstrated concentration dependent mortality induction and significant cytotoxicity on brine shrimp larvae with the IC 50 of 150 µg/ml and 280 µg/ml respectively. *Lannea schimperi* seemed to be more potent since it expressed lower IC 50 than that of the *Searsia longipes*. The result is not far from Haule and coworkers (2012) report, which reported the cytotoxicity of the acetone extract from *Lannea schimperi* to be 128 µg/ml [27]. The overall cytotoxicity findings are in line with Meyers and coworkers (1982) protocol on the brine shrimp lethality assay, which demonstrated the cytotoxicity of the plant extract below
1000 µg/ml to be significant active [24]. In the light of correlation between brine shrimp and cancer cell cytotoxicity, therefore toxicity expressed by these plants may be related to their ability to kill cancer cells [24, 28]. Hence, further studies are warranted to evaluate the cytotoxicity of these plants on cancer cells. Moreover, in vitro and in vivo toxicity results did not express correlation. This result is in harmony with that of the comparative study done by Sánchez and coworkers, (1993). Meanwhile it disagrees with the findings of the comparative study done by Parra et al. (2001) [28]. Hence, based on current findings brine shrimp lethality assay should not replace toxicity studies in animal models, despite the advantages of the former in economic point of view.

**Phytochemical screening**

Phytochemical screenings of the extracts from both *Lannea schimperi* and *Searsia longipes*, have exhibited the presence of flavonoids, tannins, saponins and glycosides. These findings are in harmony with the Egbe and coworkers (2016) report, which demonstrated the presence of flavonoids, tannins, saponins and glycosides in the acetone extract of the *Lannea schimperi* [8]. For the *Searsia longipes* in particular, this is the first study to report its phytochemical constituents. However, results are in harmony with the findings from phytochemical screening of other plants under *Searsia* genus, which reported to poses flavonoids, saponins and tannins [29, 20]. Nonetheless, the two plants seemed to possess similar phytochemical compounds, and this may be due close relatedness since they share a family as well as habitat from which they were collected.

**CONCLUSIONS**

Both plants have shown good margin of safety to mice and significant toxicity to brine shrimp larvae. Nonetheless, these plants possesses various phytochemical compounds namely flavonoids, tannins, saponins and glycosides. Therefore, the present study confirmed the previous report on the acute toxicity of *Lannea schimperi* and *Searsia longipes*, as well as cytotoxicity and phytochemical screening of *Lannea schimperi*. Nevertheless, for the first time it reports the cytotoxicity and phytochemical constitutes of *Searsia longipes*. However, these tests are not enough; hence more specific toxicity studies may be conducted to evaluate toxicity of the extracts on normal and cancer cell lines.

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**Conflict of interest**

The study is declared by authors to have no conflict of interest

**REFERENCES**


