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Phytochemistry and toxicity studies of aqueous and methanol extract of naturally growing and cultivated Aloe Turkanensis

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Abstract
Despite wide usage *Aloe turkanensis* as an herbal remedy, its phytochemical profile and safety has not been properly documented. Aqueous and methanol extract of a naturally occurring and cultivated whole plant was obtained. *In vitro* toxicity using Brine Shrimp Lethality Test (BSLT) and phytochemical screening were carried out.
The extracts exhibited zero percent mortality on BSLT. Qualitative phytochemical screening of extracts from naturally growing *Aloe turkanensis* showed high concentrations of tannins. Aqueous extracts of naturally growing plant showed high concentrations of alkaloids and moderate concentration of anthraquinones, terpenoids and saponins. Methanol extract of naturally growing plant had a lower concentration of anthraquinones and terpenoids with moderate concentration of saponins and alkaloids. The cultivated species aqueous extract had a moderate concentration of anthraquinones, lower concentrations of terpenoids and steroids with negative results for saponins and alkaloids while methanol extract had moderate concentrations of terpenoids and steroids with negative results for anthraquinones, saponins and alkaloids.

Keywords: Cultivated, Naturally occurring, *Aloe turkanensis*, Brine Shrimp Lethality Test, Phytochemical screening

1. Introduction
Aloe turkanensis is a succulent monocotyledonous plant in the family *Asphodelaceae* (International Code of Botanical Nomenclature). It is a sprawling shrub with stems of up to 70 cm long. It grows in loose clumps up to 2 m diameter (Mukiama, 2005; Wabuyele, 2006).

Figures 1 and 2 are photographs *Aloe turkanensis* taken in its natural and cultivated environs

Fig 1: A photograph of cultivated *Aloe turkanensis* in Karura Forest, Kiambu County

Fig 2: A photograph of cultivated *Aloe turkanensis* in Karura Forest, Kiambu County

Turkana community uses the plant for the treatment of eye diseases, wounds, stomach ache, ringworms, burns and poultry diseases. The juice from boiled roots is added to a drink to induce vomiting, which is said to relieve persistent headaches (Schmelzer *et al*, 2008). However, the antimicrobial properties of this species have not been explored and documented.

The aim of this study was to ascertain and validate the use of the plant extract for antibacterial and antifungal activity and investigate whether there is a difference in bioactivity of a naturally occurring plant and a cultivated one.

2. Materials and Methods

2.1 Collection of the Plant, Identification and Extraction

The naturally growing *A. turkanensis* plant sample was obtained from Natira community aloe garden at the outskirts of Kakuma town, Turkana County. The County lies between latitude 3^0 37´ North and longitude 36^0 0´ East. The plant was collected during a dry season, identified in the Kenya Forestry Research Institute in Karura where the voucher specimen was deposited (At/111K). A seedling of the plant was cultivated for eight months in Karura Forest, Kiambu County.

Whole plant materials from the two plant ecotypes' were thoroughly cleaned, chopped into small pieces and aerated to dryness. In a fume chamber, dry plant materials were placed in Cunningham® grinder, ground into powder and placed in clean airtight polythene paper (Gakuya, 2001). Two hundred grams of the plant powder were extracted separately. The powdered pant materials were placed in two conical flasks; 70% v/v methanol was added into one flask while distilled water was added into the other until the powders were submerged. The flasks were corked, agitated for 96 hours at room temperature to allow proper percolation and extraction. On the fifth day, the extracts were filtered using Whatman No. 1 filter papers into other conical flasks. Each of the extract was then evaporated to dryness using a rotary evaporator and freeze-dried. The experiments were conducted at the Department of Public Health Pharmacology and Toxicology.

2.2 Phytochemical screening

Preliminary qualitative phytochemical screening was carried out on the aqueous and methanol extracts to identify the constituents using standard procedures (Harborne, 1973; Trease and Evans, 1989; Sofowora, 1993). The following compounds were tested:

2.3 Test for tannins

To test for tannins, 0.5 g of each plant extract was stirred with about 10 ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2 ml of the filtrate. Occurrence of blue-black, green or blue green precipitate was considered positive for tannins.

2.4 Borntrager's test for anthraquinones

To test for presence of anthraquinones 0.2 g of each plant extract was shaken with 10 ml of benzene and then filtered. Five milliliters of 10% ammonia solution were then added to the filtrate and thereafter shaken. Appearance of a pink, red or violet color in the ammoniacal (lower) phase was considered positive.

2.5 Liebermann-Burchard test terpenoids

To test for terpenoids, two 0.2 g of each plant extract 2 ml of acetic acid was added, the solution was cooled well in ice followed by the addition of concentrated sulfuric acid (H_2SO_4) carefully. Color development from violet to blue or bluish-green was considered positive

2.6 Test for saponins

To test for presence of saponins, 1 gram of each portion was boiled with 5 ml of distilled water and then filtered. To the filtrate, about 3 ml of distilled water was added and shaken vigorously for about 5 minutes. A persistence frothing was considered positive

2.7 Test for flavonoids

Shinoda's test was used to test the presence of flavonoids where 0.5 g of each portion was dissolved in ethanol, warmed and then filtered. Three pieces of magnesium chips were then added to the filtrate followed by a few drops of concentrated hydrochloric acid (HCL). A pinks, orange, or red to purple coloration were considered positive.

2.8 Test for alkaloids

To test for presence of alkaloids, 1 gram of the each portion of the plant extract was stirred with 5 ml of 1% aqueous HCL on a water bath and then filtered. Of the filtrate, 1 ml was taken individually into 2 test-tubes. To 1 ml, Mayer's reagent was added and appearance of buff-colored precipitate was considered positive

3. Test for bioactivity using Brine Shrimp Lethality Test

Standard Brine shrimp eggs were sourced from JBL Novo Termaad GMBH and CO in Germany.

3.1 Hatching the Brine Shrimp eggs

Thirty-three (33) grams marine salt was weighed on an electric weighing machine and transferred into 1 liter conical flask. Distilled water was added gradually concurrently stirring to dissolve marine salt. When all marine salt had dissolved distilled water was added to 1 liter mark to constitute the marine salt solution.

Brine shrimp eggs were hatched in shallow rectangular plastic double chambered box with a dividing wall which had 1-2 mm holes. The box was filled with marine salt solution (33 g of marine salt in 1 liter of distilled water). Using a spatula about 50 mg of brine shrimp eggs was sprinkled and about 5 mg of dry yeast, which served as food for the nauplii was sprinkled in the dark compartment. The other compartment was illuminated through a hole in the lid of the box and kept under a light source using a 40 watts electric bulb. After 48 hours, the phototropic nauplii were collected by use of a Pasteur pipette from the lighted compartment and subjected to brine shrimp lethality test (Gakuya, (2001).

3.2 Cytotoxicity bioassay

Three dilutions were prepared by transferring 500 µl, 50 µl and 5 µl of plant extract each for a set of five graduated tubes. Ten shrimps were transferred into each of the vial using Pasteur pipette and marine salt solution was added to the 5 ml mark to make dilutions of 1000 µg/ml, 100 µg/ml and 10 µg/ml. five graduated vials were set for each dilution and a further five for the control. The tubes were left at room temperature and the number of live larvae counted after 24 hours. The percentage mortality was determined for each dilution and controls. Where control deaths occurred within 24 hours, the data were corrected using the formulae: % death= {(test-control)/ control} ×100 (Gakuya, 2001).

4. Results

4.1 Phytochemicals present in *Aloe turkanensis*

On qualitative phytochemical screening, methanol and aqueous extracts of naturally growing *Aloe turkanensis* exhibited high concentration of tannins but gave negative results for flavonoids. Aqueous extracts from naturally

growing plants were shown to have high concentrations of alkaloids with moderate concentration of anthraquinones, terpenoids, steroids and saponins. Methanol extract of naturally growing plant had a lower concentration of anthraquinones, steroids and terpenoids with moderate concentration of saponins and alkaloids. For the cultivated species, aqueous extract had a moderate concentration of anthraquinones, lower concentrations of terpenoids and steroids with negative results for saponins and alkaloids while methanol extract had moderate concentrations of terpenoids and steroids with negative results for anthraquinones, saponins and alkaloids. The high bioactivity of naturally growing *Aloe turkanensis* as compared to cultivated *Aloe turkanensis* plant species can be attributed to the difference in the level of phytochemicals/secondary metabolites with naturally growing *Aloe turkanensis* having higher concentration of alkaloids and anthraquinones as opposed to cultivated *Aloe turkanensis* species. These metabolites have been documented to be responsible for the antibacterial activity (Kazmi *et al.,* 1994; Ghoshal *et al*., 1996; Rattmann *et al*., 2005).

Table 1 shows the phytochemicals concentration in aqueous and methanol extracts of naturally growing and cultivated *Aloe turkanensis*

Table 1: Qualitative phytochemical screening of methanol and water extracts of different ecotypes of Aloe turkanensis

Samples	Tannins	Anthraquinones	Terpenoids/ steroids	Saponins	Flavonoids	Alkaloids
Aq-Eco 1	+++	++	++	++	-	+++
Met-Eco 1	+++	+	+	++	-	++
Aq-Eco 2	+++	++	+	-	-	-
Met-Eco 2	+++	-	++	-	-	-

Key

Aq-Eco-1 Aqueous extract of naturally growing *Aloe turkanensis*

Met-Eco 1 Methanol extract of naturally growing *Aloe turkanensis*

Aq-Eco-2 Aqueous extract of cultivated *Aloe turkanensis* plant

Met-Eco 2 Methanol extract of cultivated *Aloe turkanensis* plant

+++ Higher Concentration

++ Moderately higher concentration

\+ Lower Concentration

\- Negative results

4.2 Effects of Aloe turkanensis on Brine shrimp larvae

At a dosage of 1000 µg/ml, methanol and aqueous extract of the naturally growing *A. turkanensis* and the cultivated one exhibited a zero percent mortality when tested against *Artemia*

5. Discussion

This study shows a variation of phytochemicals in *A. turkanensis* acquired from its natural environment and a cultivated species. In table 1 there is a high concentration of phytochemicals in the naturally growing *Aloe turkanensis* as compared to the cultivated species of the plant. It is also worth noting that some phytochemicals metabolites found in the naturally growing plant are absent in the cultivated species. This phytochemical variation is an attribute of differences in soil, age, seasons, climate and type of vegetation among the ecological zones (Daniel *et al.,* 2011). For this reason, most work by conservationists on medicinal plants should be with those people who own, manage or make use of these species, or else own or manage the land on which they grow. The conservationists need to identify the conditions at field sites that are most favourable for releasing the potential offered by medicinal plants to achieve maximum value of the plant in terms of phytochemicals, conservation and sustainable development. It is in working with such stakeholders that the special meanings of medicinal plants to people can best be 'exploited' (Alan, 2004).

This study showed that aqueous and methanol extract of *Aloe turkanensis* had no lethal implication on Brine shrimp larvae even at high extract concentrations of 1000 µg/ml yet the plant extracts has been shown to have antibacterial activity against *Staphylococcus aureus* and *Bacillus cereus* (Rukenya, 2014). This indicates that lack of lethality to Brine shrimp does not mean absence of biological activity (Mwangi *et al*., 1999). Therefore the safety of *Aloe turkanensis* when used as a herbal remedy by the Turkana community is justifiable.

6. Conclusion

1. Both aqueous and methanol extracts of naturally growing and cultivated *Aloe turkanensis* showed moderate to high concentrations of phytochemicals
2. Both aqueous and methanol extracts of naturally growing and cultivated plant were found non-toxic at a concentration of 1000 µg/ml with a 100% survival of Brine Shrimp larva

7. Acknowledgement

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