Preliminary Qualitative Analysis of Phytochemical Constituents of the Endemic *Aloe tororoana* Raynolds in Tororo, Eastern Uganda.

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Accepted 03 March, 2014

*Aloe tororoana* Reynolds is recorded to grow exceptionally on the rock cliffs of Tororo rock and the adjacent Osukuru hills in Tororo district, Eastern Uganda. Due to its unique distribution pattern relative to other aloe species in the same area we postulated that it could be having unique phytochemicals. This study focused on initial qualitative phytochemical analysis of leaf gel extract from *A. tororoana*. Preliminary qualitative phytochemical analysis revealed the presence of alkaloids, tannins, saponins, anthraquinones, flavonoids and cardiac glycosides to be present in the leaf gel extract of *A. tororoana*. The results indicate that *A. tororoana* can be a potential source of useful antimicrobial drugs.

**Keywords:** Medicinal plants, Aloe tororoana, phytochemicals screening.

**INTRODUCTION**

Plants belonging to the genus aloe have been exploited due to their therapeutic value for a number of decades (Crosswhite. & Crosswhite., 1984; Morton., 1961). In the recent years, there has been an increasing interest in the inner colorless leaf gel due to its curative properties. Aloe gel is reported to be one of the remarkable healing substances known because it contains a wide range of phytochemicals (Vogler & Ernest, 1999). Good scientific evidence exists on medicinal use of topical *Aloe vera* in treatment of genital herpes, psoriasis vulgaris, and seborrhea dermatitis (Ulbricht et al., 2007). Monographs from Health Canada, the German Commission E, and the World Health Organization recognizes the use of oral *Aloe vera* as a laxative (German Commission; Health Canada.; Ulbricht et al., 2007). In Uganda, two commercially important species of aloe, *A. tweediae* and *A. vera* are already been exported for foreign exchange under the BIO-trade initiative(Andama, 2012).

A wide variety of secondary metabolites are produced by plants belonging to the genus aloe. Many different classes of compounds are represented in these plants including alkaloids, anthraquinones, anthrones, bianthraquinoids, chromones, coumarins and pyrones (Arunkumar & Muthuselvam, 2009; Nwaoguikpe et al., 2010). The most important constituents of *Aloe* bitters are the anthrones, aloin A and B, and the chromones; aloesin and aloeresin A (Raynolds, 2004).

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Different studies have reported biological activities such as laxative action in animals, apoptosis (programmed cell death) in Jurkat cells (Buenz, 2008), cytotoxicity against certain breast and ovarian cancer cell lines (Metenawy et al., 1996), antimicrobial activity, dose dependent inhibitory effect on matrix metalloproteinase (MMP) enzyme (Barrantes & Guinea, 2003), anti-inflammatory and immunomodulatory effects of different classes of phytochemicals isolated from different aloe species.

*A. tororoana* Reynolds is recorded to grow exceptionally on the rock cliffs of Tororo rock and the adjacent Osukuru hills in Tororo district, Eastern Uganda (Pomeroy et al., 2002). Here, the plant grows on bare and open rock surfaces with minimum presence of other vegetation cover (Andama, 2012). On the contrary, other species of aloes in the same area grow on flat land and under ordinary soil conditions. Over the years, the local communities have used the different species of aloes to treat abdominal problems, fever, chicken and other animal diseases with undisputed success stories.

Different aloe species are believed to have variations in their phytochemical composition due to inter-species variation, varying climate and soil conditions (Botes et al., 2008). Considering the unique distribution pattern of aloes in eastern Uganda, it is reasonable to believe that *A. tororoana* could have unique phytochemicals. Nevertheless, there is no information known about the phytochemical composition of *A. tororoana*. In this study, we decided to carry out preliminary investigation of the phytochemical composition of the endemic *A. tororoana*.

**MATERIALS AND METHODS**

**Plant material collection and Identification**

The whole fresh plant, *Aloe tororoana* was collected from the rock cliffs of Tororo rock in Tororo town, Eastern Uganda. The identification of the plant was authenticated in the herbarium, Department of Botany, Makerere University.

**Sample preparation**

The leaf skin was removed by hand and the leaf gel was homogenized. This formed the leaf gel extract. The homogenate was extracted with three different solvents, water, ethanol and acetone. The different extracts were used for preliminary phytochemical analysis.

**Phytochemical analysis**

Standard procedures described by Sofowora (1993), Trease and Evans (1989) and Harborne (1973) were used for chemical identification of the phytochemicals in the three different extracts of *A. tororoana*.

**Test for Alkaloids**

3 ml of aqueous extract was stirred with 3 ml of 1% HCl on a steam bath. Mayer’s and Wagner’s reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

**Test for tannins**

About 2 ml of the aqueous extract was stirred with 2 ml of distilled water and few drops of FeCl₃ solution were added. The formation of a green precipitate was an indication for the presence of tannins.

**Test for saponins**

5 ml of aqueous extract was shaken vigorously with 5 ml of distilled water in a test tube and warmed. The formation of stable foam was taken as an indication for the presence of saponins.

**Test for phlobatannins**

About 2 ml of aqueous extract was added to 2 ml of 1% HCl and the mixture was boiled. Deposition of a red precipitate was taken as an evidence for the presence of phlobatannins.

**Test for flavonoids**

To 1 ml of aqueous extract was added 1 ml of 10% lead acetate solution. The formation of a yellow precipitate was taken as a positive test for flavonoids.

**Tests for anthraquinones**

a)  *Borntrager’s test*: 3 ml of aqueous extract was shaken with 3 ml of benzene, filtered and 5 ml of 10% ammonia solution was added to the filtrate. The mixture was shaken and the presence of a pink, red or violet colour in the ammonical (lower) phase indicates the presence of free anthraquinones.

b)  3 ml of the aqueous extract was boiled with 3 ml of aqueous sulphuric acid and filtered while hot. 3 ml of benzene was added to the filtrate and shaken. The benzene layer was separated and 3 ml of 10% NH₃ added. A pink, red or violet colouration in the ammonical (lower) phase indicates the presence of anthraquinone derivatives.
Table 1. Qualitative phytochemical analysis of A. tororoana.

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Aqueous Extract</th>
<th>Ethanol Extract</th>
<th>Acetone Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Barbaloins</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Cardiac glycosides</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phlobatannins</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ Phytochemical present - phytochemical absent

**Test for terpenoids**

2 ml of the organic extract was dissolved in 2 ml of chloroform and evaporated to dryness. 2 ml of concentrated sulphuric acid was then added and heated for about 2 min. A greyish colour indicates the presence of terpenoids.

**Tests for steroids**

(i) 2 ml of ethanol extract was dissolved in 2 ml of chloroform and 2 ml concentrated sulphuric acid were added. Formation of a red colour in the lower chloroform layer was used as an indicator for the presence of steroids.

(ii) 2 ml of the ethanol extract was dissolved in 2 ml of chloroform and treated with sulphuric and acetic acids. The development of a greenish colour was taken as an indicator for the presence of steroids.

**Tests for carbohydrates**

a) **Molisch’s test:** 3 ml of the aqueous extract was added to 2 ml of Molisch’s reagent and the resulting mixture shaken properly. 2 ml of concentrated H₂SO₄ was then poured carefully down the side of the test tube. A violet ring at the interphase indicates the presence of carbohydrate.

b) To 3 ml of the aqueous extract was added about 1 ml of iodine solution. A purple colouration at the interphase indicates the presence of carbohydrates.

c) **Keller-Kiliani** test: 2 ml of each extract was dissolved in 2 ml of glacial acetic acid containing one drop of FeCl₃ solution. The mixture was then poured into a test tube containing 1 ml of concentrated H₂SO₄. A brown ring at the interphase indicates the presence of a deoxy sugar, characteristic of cardenolides.

**Tests for glycosides**

a) **Liebermann’s test:** 2 ml of the acetone extract was dissolved in 2 ml of chloroform and 2 ml of acetic acid was added and the solution cooled well in ice. Sulphuric acid was then added carefully. A colour change from violet to blue to green indicates the presence of a steroidal nucleus (that is, a glycone portion of glycoside).

b) **Salkowski’s test:** 2 ml of each extract was dissolved in 2 ml of chloroform. 2 ml of sulphuric acid was added carefully and shaken gently. A reddish brown colour indicates the presence of a steroidal ring (that is, a glycone portion of glycoside).

(c) **Keller-Kiliani** test: 2 ml of each extract was dissolved in 2 ml of glacial acetic acid containing one drop of FeCl₃ solution. The mixture was then poured into a test tube containing 1 ml of concentrated H₂SO₄. A brown ring at the interphase indicates the presence of a deoxy sugar, characteristic of cardenolides.

**RESULTS AND DISCUSSIONS**

The present study focused mainly on qualitative chemical evaluation of the phytochemical constituents of fresh leave gel extract from the endemic A. tororoana. The study revealed the presence of alkaloids, tannins, anthraquinones, Barbaloins, cardiac glycosides, and flavonoids, (Table 1). The phytochemicals present have been shown to possess medicinal activity as well as physiological activity in previous studies. These results
show that *A. tororoana* can be a potential source of useful compounds that can be used as leads to synthesize new antimicrobial drugs. The presence of these phytochemicals justifies the traditional medicinal uses of this plant by the local communities.

Further studies are currently being conducted to isolate, identify, quantify, characterize and elucidate the structures of the bioactive compounds. Another study is currently focused on investigating the antimicrobial activities of the leaf gel extract from this plant.

**ACKNOWLEDGEMENT**

The authors are grateful to Busitema University for financial facilitation to undertake this preliminary study.

**REFERENCES**


