Full Length Research Paper

Preliminary investigation on anthelmintic activity and phytochemical screening of leaf crude extracts of *Tithonia diversifolia* and *Tephrosia vogelii*

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This investigation lies within the framework of studying the phytochemistry of two medicinal plants *Tithonia diversifolia* and *Tephrosia vogelii* and assessing their potentials in controlling the helminthiasis in goats. The crude extracts were obtained from the leaves by Soxhlet methodology and the anthelmintic activities were tested in various laboratories in Butare. The results from this investigation showed that both *T. diversifolia* and *T. vogelii* have lots of active principles grouped in alkaloids, tannins, flavonoids, terpenoids, and sterols and exhibit anthelmintic activities in goats. The percentages of reduction of fecal eggs are 98, 97, 96 and 95% with methanol crude extract of *T. vogelii*, infusion of *T. vogelii*, methanol crude extract of *T. diversifolia* and infusion of *T. diversifolia*, respectively. This investigation showed the efficacy of both *T. vogelii* and *T. diversifolia* against gastrointestinal nematodes in goats and therefore their potentials in assuring more the animal health care in Rwanda by for treating the parasitic nematodes in goats by plant-based drugs instead of crude extracts.

Key words: Anthelmintic activity, feacal egg, gastro-intestinal nematodes, goats, phytochemical screening, traditional medicine, *Tithonia diversifolia*, *Tephrosia vogelii*, medicinal-plant, Rwanda.

INTRODUCTION

In Rwanda and elsewhere, the medicinal plants have been used by Man from the prehistoric times to present days and in all civilizations. By trial and error, he distinguished between the beneficial and poisonous...
Man has also observed that in large quantities medicinal plants may be poisonous, and learned about the usefulness of plants by observing sick animals that use some plants that they usually ignore. Today, the scientists took in this way to isolate active compounds from the medicinal plants in order to provide the way of formulating various plant-based drugs (Kabera et al., 2013). The medicinal value of plants is directly connected to the vast array of chemical compounds, known as secondary plants products or secondary metabolites, manufactured by their various biochemical pathways. Among them, some have been conformed to be active against microbes and parasites. They are usually classified in different groups namely alkaloids, flavonoids, tannins, quinones, terpenoids and saponins (Margaret and Wink, 1998; Levetin and McMahon, 1999; Njoroge and Bussmann, 2006). Researches show that the drugs from plants are better accepted by the body than synthetic substance (Bodeker et al., 2005). Although some plants may have toxic properties associated with their curative power, this does not mean necessarily to desert directly their uses. In all the ways, further knowledge on plants chemical constituents and their uses are however needed to valorize them and to see if they can be used for one or other purpose. In this way, many plants came under examination leading to extraction and characterization of their actives ingredients, where obtained results make pharmacological studies and synthesis of more potent drugs with reduced toxicity (Levetin and McMahon, 1999; Bodeker et al., 2005).

The availability of food and products of animal origin are still being the basic needs of man as they have been since the dawn of his creation. In the most developing African countries, like Rwanda and regional countries, the animal's breeding is the one of the sectors which contributes to maintain food security and provide the income for population from rural areas (Van, 2011). In spite of these important needs of animals by their nationals, the diseases have always negatively affected their production and directly or indirectly affect the nutritional as well as economical sector. Consequently, the constant challenge of nature to animal health is often also a challenge to man's health. For this reason, the maintenance of high standards of animal health is essential to the public health of any country of the third world (Vaarst et al., 2008).

In our country Rwanda, most of animals breeders have not access to modern therapy provided by modern veterinary; reason why they have recourse to the traditional medicine to treat different diseases of their animals by using two main plants namely "Kimbazi" and "Umuruku" scientifically named *Tithonia diversifolia* and *Tephrosia vogelii*, respectively. Even if the efficacy of these medicinal plants is not undisputable, more investigation should be done in many fields of science in order to know exactly their chemical composition, the diseases they treat and their posology particularly in Rwanda. It is within this framework that we conduct a survey on the phytochemical and anthelmintic activities of *T. diversifolia* and *Tephrosia vogelii*, mostly used by Rwandan traditional healers in treating their flock of small animals.

**Figure 1.** Flowering plant of *T. diversifolia*.

*T. diversifolia* (locally known as Kimbazi or Icyicamahirwe) is an impressive flowering plant belonging to the Eudicots class, *Asterale* order and in the *Asteraceae* family (Schilling and Panero, 1996). *T. diversifolia* is used for different purposes such as ornamental purpose because of its characteristic bitter taste. It has been also used to induce a fever, to help fight poisoning, although not used for direct medicinal purposes (Schilling and Panero, 1996; Jama et al., 2000). In traditional medicine, it has been used to treat some ailments such as throat and liver ailments, stomach upset, and diarrhea in livestock. It is also used as an anti-diabetic, anti-malaria, anti-inflammatory, antibacterial, antimicrobial and potential cancer chemopreventive (Touqeer et al., 2013; Hoffmann and Fnimh, 2003; Adebayo et al., 2009) (Figure 1).

*Tephrosia vogelii* (locally named Umuruku) is a soft woody branching herb with dense foliage reaching up to 0.5-4 m tall and belongs into the class of Magnoliopsida, order of Fabales and in the *Fabaceae* family (Gadzirayi et al., 2009). It is used in various applications in human daily life. It is used by the farmers to improve the soil fertility because of the nitrogen found in their leaves and seeds. It is also used as firewood, as an insecticide against storage pests and mites on plants, as piscicidal although this last use is now illegal in many countries.
Figure 2. Blooming plant of *T. vogelii* grown in Cyarwa cy’Imana (Butare, Rwanda).

Because of the rotenone obtained in the leaves and seeds. In traditional medicine, *T. vogelii* is used to treat many animal ailments such as skin diseases and intestinal worms. It has antibacterial activities against *Staphylococcus aureus* and *Bacillus subtilis*. It is also used to treat ectoparasites and endoparasites in cattle (Blommaert, 1950; Hoffman, 2003; Hammond et al., 1997; Colin, 2011) (Figure 2).

**MATERIALS AND METHODS**

**Plants materials and animals fecal collection**

After the dew was removed by the morning sun, the fresh and mature leaves of *T. diversifolia* were harvested from the arboretum of the University of Rwanda (UR) the leaves of *Tephrosia vogelii* leaves were collected from “Cyarwa cy’Imana” (near Agateme centre) during wet season. The collected leaves were then dried under weak sunlight and ground into powder that was stored in cool place for further oil extraction. The feces samples were collected from a flock of 30 goats of the School of Agri-Veterinary of Kabutare (EAVK) and stored for further tests. These sampling sites are located in Butare, Eastern Province with geographic coordinates 2°36′S 29°45′E (Figure 3).

**Extraction and phytochemical screening**

The plant crude extracts were obtained by using the Soxhlet extractor and n-hexane and methanol as solvents. The obtained crude extracts were used in evaluation of anthelmintic activity of both plants and the phytochemical screening method was carried out in the standard procedures (Table 1 and Figure 3).

**Reduction of eggs per gram of feaces (EPG) after treatment with crude extract and powder infusion**

The fecal samples collected from the goats of EAV Kabutare; were examined under microscopy by using fecal flotation methods based on principle that parasites are less dense than the fluid flotation medium; this method helped to know the goats infected by parasites. McMaster method using McMaster eggs-counting slide and microscope was employed to quantify the eggs per gram of feaces (Margaret et al, 1994).

**Assessment of anthelmintic activity of extracts**

To evaluate the anthelmintic activity, McMaster Method was used before and after the treatment of the goats by using methanol crude extracts and powder infusion of these two plants studied here in order to know the reduction of eggs after treatment. The number of eggs per gram was calculated by using the formula below.

\[
\text{EPG} = Y \times 100
\]

Where, EPG: number of eggs per gram

\[Y: \text{Number of egg counted in both chambers of McMaster egg-counting slide.}\]

To qualify the efficacy of solutions from both plants (*T. diversifolia* and *T. vogelii*) against goats’ helminthes, the test of reduction of EPG was used to calculate the percentage of reduction of eggs in feaces is calculated by using the formula below.

\[
P = 100 \left[1 - \left(\frac{X_t}{X_C}\right)\right]
\]

Where, P: percentage of reduction of EPG

\[X_C: \text{Arithmetic mean of EPG of the control group of goats (group of untreated goats) between 7 days and 14 days of treatment.}\]

\[X_t: \text{Arithmetic mean of EPG of group of treated goats between 7 days and 14 days of treatment.}\]

If the percentage reduction is greater or equal to 95%, the solution is effective and the solution is ineffective if the percentage

Figure 3. Location of Butare, the sampling site of this investigation.
Table 1. Phytochemical screening of T. diversifolia.

<table>
<thead>
<tr>
<th>Group of active principles</th>
<th>Reagents</th>
<th>Initial colour</th>
<th>Observed colour</th>
<th>Colour expected</th>
<th>Test results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Mayer</td>
<td>Brown-black</td>
<td>Brownish white</td>
<td>Cream formation, yellowish white</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Wagner</td>
<td>Brown-black</td>
<td>Reddish brown</td>
<td>Reddish brown(orange)</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Dragendorff</td>
<td>Brown-black</td>
<td>Reddish brown</td>
<td>Reddish brown</td>
<td>++</td>
</tr>
<tr>
<td>Tannins</td>
<td>Saline gelatin NaCl and H2O</td>
<td>Brown-black</td>
<td>White precipitate</td>
<td>White precipitate or trouble</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>CuSO4</td>
<td>Brown-black</td>
<td>White precipitate</td>
<td>Brownish green precipitate</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>FeCl3</td>
<td>Brown-black</td>
<td>Brownish green precipitate</td>
<td>Brownish green or blue-black</td>
<td>++</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>HCl, CH3OH, H2O, Mg</td>
<td>Brown-black</td>
<td>Reddish brown for flavones</td>
<td>Reddish brown for flavones, red-celise for flavonols, redish violet for flavonones</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Chloroform, H2SO4</td>
<td>Green-precipitate</td>
<td>Reddish coloration brown</td>
<td>Reddish brown coloration</td>
<td>++</td>
</tr>
<tr>
<td>Terpenoids-Sterols</td>
<td>Chloroform, anhydride acetic, H2SO4</td>
<td>Green precipitate</td>
<td>Blue-brown</td>
<td>Blue-brown coloration</td>
<td>++</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>Brown-black</td>
<td>Persistent</td>
<td>persistent</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Froth</td>
<td>Frothing</td>
<td></td>
</tr>
<tr>
<td>Quinone</td>
<td>Ether-chloroform, HCl, ethanol, NaOH</td>
<td>Green precipitate</td>
<td>Purplish</td>
<td>Red or purplish</td>
<td>+</td>
</tr>
<tr>
<td>Leucoanthocyans</td>
<td>HClN</td>
<td>Brown-black</td>
<td>Brownish precipitate</td>
<td>Reddish violate precipitate</td>
<td>+ -</td>
</tr>
<tr>
<td>Anthocyanins</td>
<td>HCl</td>
<td>Brown-black</td>
<td>Reddish brown</td>
<td>Red</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>NH3</td>
<td>Brown-black</td>
<td>Brownish blue</td>
<td>Blue</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>KOH</td>
<td>Brown-black</td>
<td>Reddish black</td>
<td>Red</td>
<td>+ -</td>
</tr>
</tbody>
</table>

Caption: ++: abundant, +: present, ±: no decision, -: absent

RESULTS AND DISCUSSION

Phytochemical screening of T. diversifolia showed that alkaloids, tannins, flavonoids including their derivatives such as the rotenoids, saponins and terpenoids- steroids are in abundance in this plant. There is no decision for Anthraquinones and leucoanthocyanins because the observed colour is not very similar to expected colour. These results led us to the same observations that were made by previous research on this plant (Fasuyi et al., 2010; Kalume et al., 2012; Ezeonwumelu, 2012; Siamba et al., 2007). The Table 2 shows that alkaloids, tannins, flavonoids and terpenoids-steroids are abundant in the leaves of Tephrosia vogelii while the compounds of saponins group are absent. The same situation was also observed in previous phytochemical screening of this plant which was carried out in the past by Dzenda and his collaborators (2012). There is no decision for anthraquinones and anthocyanins because the colour observed for anthraquinone is not very similar to the expected one while for anthocyanins the colour expected in acidic medium is observed in basic medium and the colour to be observed in acidic medium was absent.
Table 2. Phytochemical screening of *T. Vogelii*.

<table>
<thead>
<tr>
<th>Groups of active principles</th>
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<th>Initial colour</th>
<th>Colour observed</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Mayer</td>
<td>Yellowish brown (purplish yellow)</td>
<td>Yellowish white</td>
<td>Yellowish white</td>
<td>++</td>
</tr>
<tr>
<td>Wagner</td>
<td>Yellowish brown</td>
<td>Reddish brown</td>
<td>Reddish brown (orange)</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Dragendorff</td>
<td>Yellowish brown</td>
<td>White precipitate</td>
<td>White precipitate or trouble</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Saline gelatin, NaCl, H₂O</td>
<td>Yellowish brown</td>
<td>White precipitate</td>
<td>White precipitate</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Tannins</td>
<td>CuSO₄</td>
<td>Yellowish brown</td>
<td>White precipitate</td>
<td>White precipitate</td>
<td>++</td>
</tr>
<tr>
<td>FeCl₃</td>
<td>Yellowish brown</td>
<td>Blue-black</td>
<td>Brownish</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Flavonoids</td>
<td>HCl, CHO₉, Mg</td>
<td>Yellowish brown (flavones)</td>
<td>Reddish brown</td>
<td>Red-celise for flavonol, reddish brown for flavones, reddish violet for flavone</td>
<td></td>
</tr>
<tr>
<td>Chloroform, H₂SO₄</td>
<td>Reddish brown</td>
<td>Reddish brown</td>
<td>++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloroform, Anhydride acetic, H₂SO₄</td>
<td>Blue-brown</td>
<td>Blue-brown</td>
<td>++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saponins</td>
<td>Ether-chloroform, HCl, ethanol, NaOH</td>
<td>Yellowish brown</td>
<td>Persistent frothing</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Quinones</td>
<td>HCl₂N</td>
<td>Yellow</td>
<td>Reddish violet</td>
<td>Reddish violet</td>
<td>+</td>
</tr>
<tr>
<td>Leucoanthocians</td>
<td>HCl₂N</td>
<td>Yellow</td>
<td>Reddish violet</td>
<td>Reddish violet</td>
<td>+</td>
</tr>
<tr>
<td>Anthocians</td>
<td>HCl</td>
<td>Yellow</td>
<td>Whitish yellow</td>
<td>Red</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>NH₃</td>
<td>Yellowish brown</td>
<td>Red</td>
<td>Blue</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>KOH</td>
<td>Yellowish brown</td>
<td>Reddish yellow</td>
<td>Red</td>
<td>+</td>
</tr>
</tbody>
</table>

Caption: ++: abundant, +: present, ±: no decision, -: absent

From what we learn from Fluck (1955), the climate has influence in one way or another to the active compounds of medicinal plants. Therefore, the abundance of some of the aforementioned active compounds should also be explained by the good quality of the Rwandan climate under which the plants have grown and leaves were harvested.

Phytochemical screening of both *T. diversifolia* and *T. vogelii* showed that these plants have nearly the same chemical compositions; therefore it will not be surprising if both plants have anthelmintic activity. The results obtained from Soxhlet extraction show that there is a lot of crude extract with methanol extraction more than with n-hexane as solvents (Figure 4). Usually, the methanol is a high polar solvent and dissolves the polar compounds present in plant tissues while n-hexane which is non polar solvent dissolves the non polar compounds present in the plant tissues. Therefore, these two statements of this investigation led to conclude that these plants contain more polar compounds than non polar compounds in their leaves.

The histograms (Figures 5, 6, 7 and 8) generated by the analysis of the results obtained from the tests of reduction of EPG were by Microsoft Excel show clearly...
how the number of eggs per gram of feaces decreased after treatment with crude extracts of these medicinal plants. The high reduction rate was observed between seventh day and fourteenth day of the treatment with methanol crude extracts and powder infusions from both plants.

Given that the percentages obtained in this study is above 95 everywhere (Table 3), the solution is effective and therefore, the extracts from the leaves of both *T. diversifolia* and *T. vogelii* are effective against gastro-helminthes in goats. In other worlds, the bioactivity of phytochemicals varied significantly, the same observations with Langat and his collaborators (2012). In additions, these results show that the methanol crude extracts are more effective than the powder infusion and so the crude extracts should contain more active principles than the powder. From these percentages of EPG reduction, it has been observed that *T. vogelii* is more effective against helminthes for goats than *T. diversifolia* though the different is not significant.

In conclusion, this present investigation revealed that both *T. vogelii* and *T. diversifolia* exhibit a remarkable anthelmintic activity in goats of Rwanda. The results of this investigation are supported by previous findings from the studies carried out on these plants (Edeki, 1997). The use of these plants in making the plant-based drugs

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**Figure 4.** Soxhlet extraction assembly of (A) plant crude extracts and (B) solvents recovery.

**Figure 5.** Number of eggs per gram after the treatment with crude extract of *T. vogelii*.
Figure 6. Number of eggs per gram after the treatment with powder infusion of \textit{T. vogelii}.

Figure 7. Number of eggs per gram after treatment by with crude extract from \textit{T. diversifolia}.
against animals’ parasites should be the positive alternative to the use of synthetic anthelmintics such as Thiabendazole, Phenothiazine and Levamizole which have often raised objections.

REFERENCES


