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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 phytochemical screening, fecal eggs reduction 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

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

plants. 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.

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 family of Fabaceae (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).

Figure 3. Location of Butare, the sampling site of this investigation.

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 feaces 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.

$$\mathrm{EPG} = \mathrm{Y} \times 100$$

Where, EPG: number of eggs per gram
Y: 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.

$$P = 100\left[1 - \left(\frac{\overline{X}_t}{\overline{X}_C}\right)\right]$$

Where, P: percentage of reduction of EPG
$\overline{X}_C$: Arithmetic mean of EPG of the control group of goats (group of untreated goats) between 7 days and 14 days of treatment.
$\overline{X}_t$: 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

Table 1. Phytochemical screening of *T. diversifolia.*

Group of active principles	Reagents	Initial colour	Observed colour	Colour expected	Test results
Alkaloids	Mayer	Brown- black	Brownish white	Cream formation, yellowish white	++
	Wagner	Brown- black	Reddish brown	Reddish brown(orange)	++
	Dragendorff	Brown- black	Reddish brown	Reddish brown	++
Tannins	Saline gelatin + NaCl and H_2O	Brown- black	White precipitate	White precipitate or trouble	++
	$CuSO_4$	Brown- black	White precipitate	White precipitate	++
	$FeCl_3$	Brown- black	Browinish green precipitate	Brownish green or blue- black	++
Flavonoids	HCl, CH_3OH, H_2O, Mg	Brown- black	Reddish brown for flavones	Reddish brown for flavones, red-celise for flavonols, redish violet for flavonones	++
Terpenoids-Sterols	Chloroform, H_2SO_4	Green-precipitate	Reddish brown coloration	Reddish brown coloration	++
	Chloroform, anhydride acetic,H_2SO_4	Green precipitate	Blue- brown	Blue -brown coloration	++
Saponins	-	Brown- black	Persistent Froth	persistent Frothing	++
Quinone	Ether-chloroform, HCl, ethanol, NaOH	Green precipitate	Purplish	Red or purplish	+
Leucoanthocyans	HCl_2N	Brown- black	Brownish red precipitate	Reddish violate precipitate	+ -
Anthocyans	HCl	Brown- black	Reddish brown	Red	+
	NH_3	Brown- black	Brownish blue	Blue	+
Anthraquinones	KOH	Brown- black	Reddish black	Red	+ -

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

reduction is less than 95.

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.*

Groups of active principles	Reagents	Initial colour	Colour observed	Colour expected	Test results
Alkaloids	Mayer	Yellowish brown(purplish yellow)	Yellowish white	Yellowish white	++
	Wagner	Yellowish brown	Reddish brown	Reddish brown(orange)	++
	Dragendorff	Yellowish brown	Reddish brown	Reddish brown(orange)	++
Tannins	Saline gelatin, NaCl, H_2O	Yellowish brown	White precipitate	White precipitate or trouble	++
	$CuSO_4$	Yellowish brown	White precipitate	White precipitate	++
	$FeCl_3$	Yellowish brown	Blue-black precipitate	Brownish green or blue- black	++
Flavonoids	HCl,CHOH,H2O, Mg	Yellowish brown	Reddish brown (flavones)	Red-celise for flavonol, Reddish brown for flavones, Reddish violet for flavonone	++
	Chloroform, H_2SO_4	-	Reddish brown	Reddish brown	+
	Chloroform, Anhydride acetic, H_2SO_4	-	Blue - brown	Blue-brown	++
Saponins	-	Yellowish brown	-	Persistent frothing	-
	Ether-chloroform, HCl, ethanol, NaOH	Green precipitate	Red	Red	+
Quinones	HCl_2N	Yellow	Reddish violet	Reddish violet	+
Leucoanthocians	HCl_2N	Yellow	Reddish violet	Reddish violet	+
Anthocians	HCl	Yellow	Whitish yellow	Red	+
	NH_3	Yellowish brown	Red	Blue	+
					-
Anthraquinones	KOH	Yellowish brown	Reddish yellow	Red	+
					-

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 and 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

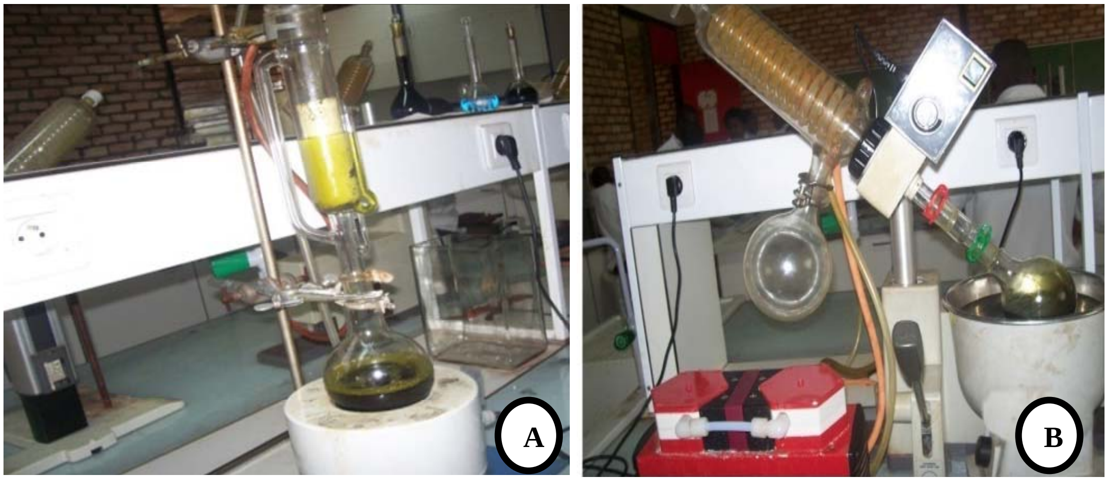

Figure 4. Soxhlet extraction assembly of (A) plant crude extracts and (B) solvents recovery.

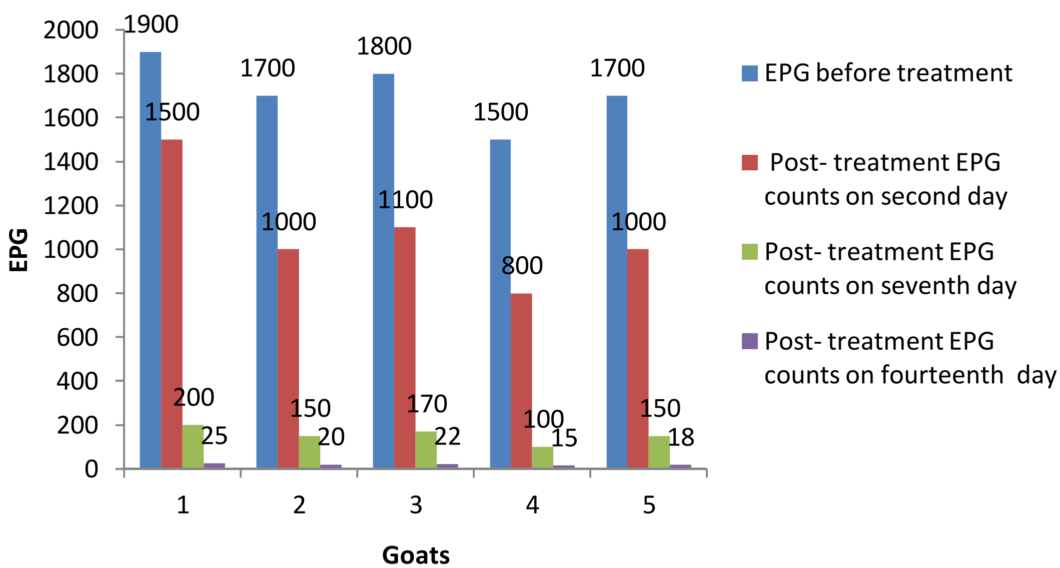

Figure 5. Number of eggs per gram after the treatment with crude extract of *T. vogelii.*

how the number of eggs per gram of feaces decreased after treatment with crud 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

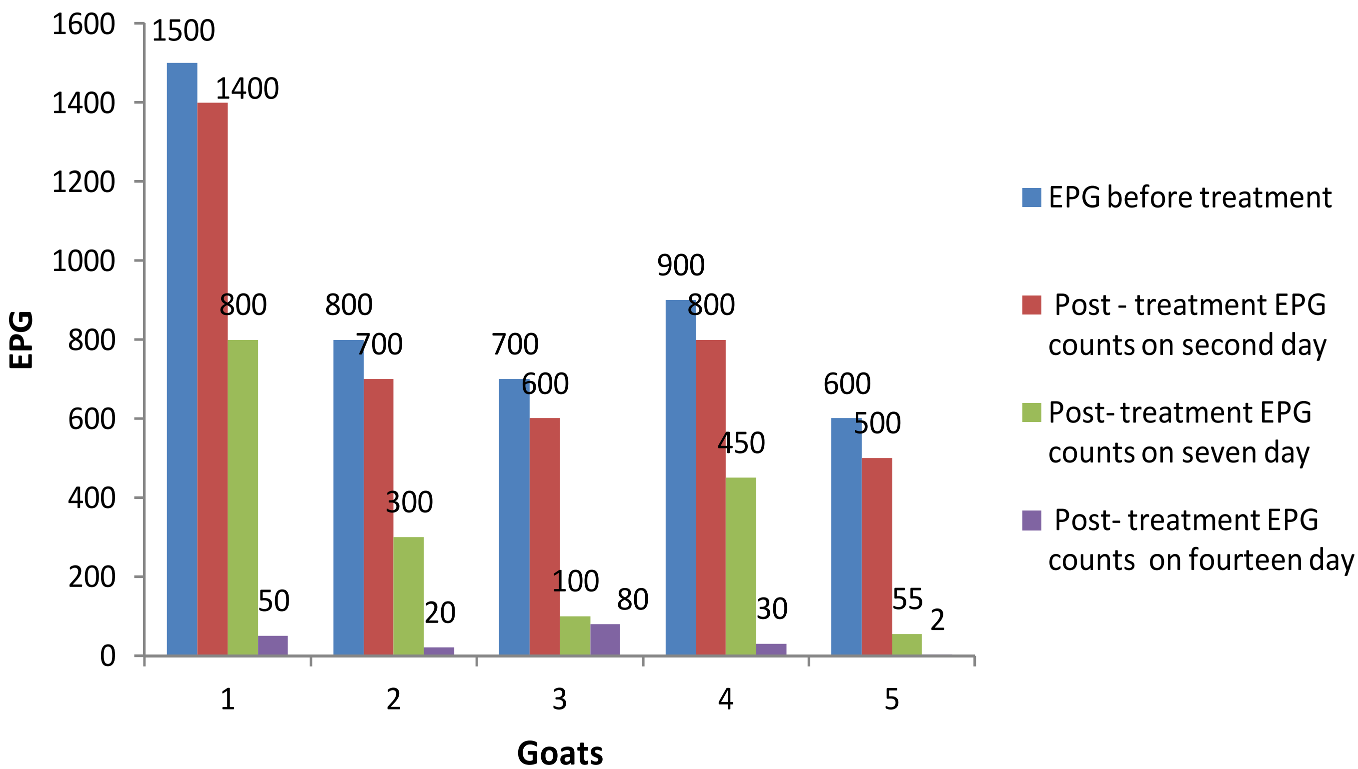

Figure 6. Number of eggs per gram after the treatment with powder infusion of *T. vogelii*

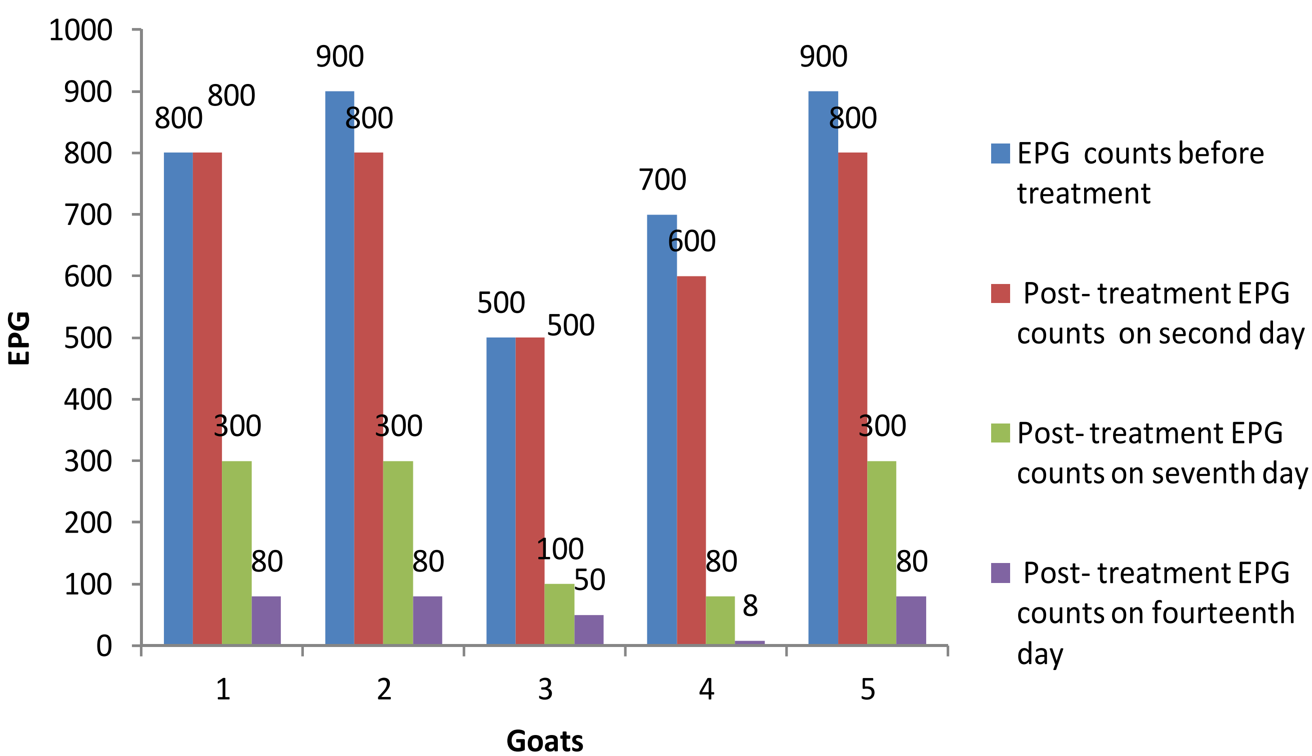

Figure 7. Number of eggs per gram after treatment by with crude extract from *T. diversifolia.*

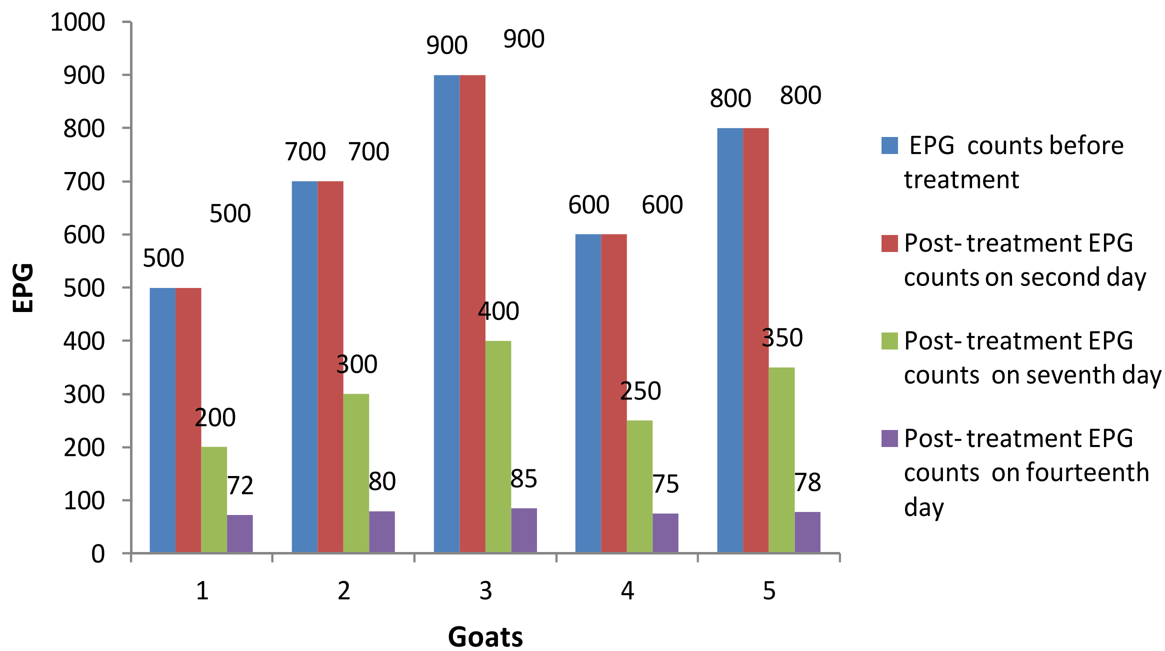

Figure 8. Number of eggs per gram after the treatment of goats with powder infusion of *T. diversifolia.*

Table 3. Results of the calculation of the percentage of EPG reduction.

Type of treatment	% of EPG reduction
Methanolic crude extract from *T. vogelii* leaves	98
Infusion of powder from *T. vogelii* leaves	97
Methanolic crude extract from *T. diversifolia* leaves	96
Infusion of powder from *T. diversifolia* leaves	95

against animals's parasites should be the positive alternative to the use of synthetic anthelmintics such as Thiabendazole, Phenothiazine and Levamizole which have often raised objections.

REFERENCES

Adebayo JO, Balogun EA, Oyeleke SA (2009). Toxicity study of the aqueous extract of *Tithonia diversifolia* leaves using selected biochemical parameters in rats. Pharmacogn. Res. 2(3):143-147.

Blommaert KLJ (1950). The plant *Tephrosia vogelii (*Hook. F, as a Fresh water fish poison. Roy. Soc. So. Afr. Trans. 32:247-263.

Bodeker G, Ong CK, Grundy C, Burford, SK (2005). WHO Global Atlas of Traditional, Complementary and Alternative Medicine. Vol. 1 Centre for Health development, WHO Press, Geneva, Switzerland, pp. 344-347

Colin S (2011). Fundemental of animal science*. 1st edition,* Cengage Learning, United State, pp. 389-391.

Dzenda T, Ayo JA, Adelaiye AB, Adaudi AO (2007). Ethno-medical and veterinary uses of *Tephrosia vogelii* hook. F: A review Niger. Vet. J. 28(3):24-39.

Edeki AL (1997). Toxicity, anthelmintic and electromyographic studies of methanolic extracts of *Tephrosia vogelii.* D.V.M. Project, Dept. Vet. Physiol. Pharmacol., A.B.U., Zaria Nig. p. 28.

Ezeonwumelu JOC, Omolo RG, Ajayi AM, Agwu E, Tanayen JK, Adiukwu CP, Oyewale AA, Adzu B, Okoruwa AG, Ogbonnia SO (2012). Studies of phytochemical screening, acute toxicity and anti-diarrhoeal effect of aqueous extract of Kenyan *Tithonia diversifolia* leaves in rats. Br. J. Pharm. Toxicol. 3(3):127-134.

Fasuyi AO, Dairo FAS, Ibitayo FJ (2010). Ensiling wild sunflower (Tithonia diversifolia) leaves with sugar cane molasses. Livest. Res. Rural Dev. 22(3):42-49.

Fluck H (1955). The influences of climate on the active principles in medicinal plants. J. Pharm. Pharmacol. 7:361-383.

Gadzirayi CT, Mutandwa E, Marizvikuru M, Tembinkosi C (2009). Utilization of *Tephrosia vogelii* in controlling ticks in dairy cows by small-scale commercial farmers in Zimbabwe. Afr. J. Biotechnol. 8(17):4134-4136.

Hammond JA, Fielding D, Bishop SC (1997). Prospects for plant anthelmintics in tropical veternary medicine. Vet. Res. Commun. 21(3):213-228.

Hoffmann D, Fnimh AHG (2003). Medical Herbalism: The Science and Practice of Herbal Medicine. Healing Arts Press, Inner Traditions, Bear & Company. pp. 90-111.

Jama B, Palm CA, Buresh RJ, Niang A, Gachengo C, Nziguheba G, Amadalo B (2000). *Tithonia diversifolia* as green manure for soil fertility improvement in Western Kenya. Agrofor. Syst. 49:201-221.

Kabera J, Ugirinshuti V, Nyirabageni A (2013). Potential aromatic plants for herbal tea in Rwanda. Frankfurt, Germany, pp. 3-10.

Kalume MK, Losson B, Angenot L, Tits M, Wauters JN, Frédérich M, Saegerman C (2012). Rotenoids content and *in vitro* acaricidal activity of *Tephrosia vogelii* leaf extract on the tick *Rhipicephalus appendiculatus.* Vet. Parasitol. 190(1-2): 204-209.

Langat KB, Siele KD, Nanyingi OM, Mutai KC, Rukunga MG, Mwandawiro C, Ireri L, Tonui KW (2012). Larvicidal Action of Extracts from Tithonia diversifolia Against the Dengue Mosquito Aedes aegypti (Diptera: Culicidae). J. Biol. Active Prod. Nat. 2(1): 46-49.

Levetin E, McMahon K (1999). Plant and society, 2nd ed, Mc Graw-Hill, Dubuque, IA, pp. 315-330.

Margaret FR, Wink M (1998). Alkaloids: Biochemical, Ecology and Medicinal application. Plenum Press, New York, pp. 87-324.

Margaret W, Russell L, Anne M (1994). Veterinary clinical parasitology. 6th Edition, Lawa State University Press, pp. 3-9.

Njoroge GN, Bussmann RW (2006). Herbal usage and informant concensus in ethnoveterinary management of cattle diseases among Kikuyus (Central Kenya). J. Ethnopharmacol. 108:332-339.

Schilling E, Panero JL (1996) - Phylogenetic reticulation in subtribe *Helianthinae*. Am. J. Bot. 83(7):939-948.

Siamba DN, Okitoi LO, Watai MK, Wachira AM, Lukibisi FB, Mukisira EA (2007). Efficacy of *Tephrosia vogelli* and *Vernonia amygdalina* as anthelmintics against *Ascaridia galli* in indigenous chicken. Livest. Res. Rural Dev. 19(12):176-182.

Touqeer S, Saeed MA, Ajaib A (2013). A Review on the Phytochemistry and Pharmacology of Genus *Tephrosia*. Phytopharmacology 4(3): 598-637

Vaarst M, Padel S, Younie D, Hovi M, Sundrum A, Rymer C (2008). Animal health challenges and veterinary aspects of organic livestock farming identified through a 3 year EU network project. Open Vet. Sci. J. 2:111-116.

Van AJ (2011). The role of reproductive technologies in breeding schemes for livestock populations in developing countries. Livest. Sci. 136:29-37.