

## *In vitro* efficacy of *Elephantorrhiza elephantina* root extracts against adult *Paramphistomum cervi* in goats

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*Elephantorrhiza elephantina* is a medicinally important plant whose roots are used to control gastrointestinal parasites in goats. The use of plant-based anthelmintics as potential alternatives to synthetic anthelmintics in controlling gastrointestinal worms in ruminants is a promising area of research. The study was carried out to ascertain the efficacy of *E. elephantina* in controlling goat trematodes. Standard procedures were used to quantify the phytochemicals in the water, ethanol and methanol root extracts of *E. elephantina*. The quantitative phytochemical analysis revealed that alkaloids ( $93.24 \pm 1.68\%$ ), condensed tannins ( $450.52 \pm 0.15$  mg CE/g), flavonoids ( $803.93 \pm 0.13$  mg QE/g), phenols ( $476.11 \pm 0.37$  mg GAE/g) and saponins ( $83.28 \pm 1.72\%$ ) were present in *E. elephantina* root extracts. *Paramphistomum cervi* adult worms recovered from the rumen of freshly slaughtered goats were used. *In vitro* screening of ethanol, methanol and water extracts of *E. elephantina* for potential anthelmintic activity against adult *P. cervi* worm motility showed time and dose-dependent significant effects. Ethanol extracts showed a highly significant ( $P < 0.05$ ) inhibition of motility (86.67 and 96.67%) at concentrations 7.5 and 15 mg/mL respectively, after 12 h of treatment, while methanol and water extracts exhibited a significant ( $P < 0.05$ ) inhibition of motility (96.67 and 66.67% respectively) at concentration of 15 mg/mL 16 h post-exposure. Our findings confirm the efficacy of *E. elephantina* in controlling goat trematodes.

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### 1. Introduction

Gastrointestinal parasites account for huge losses experienced by the livestock industry worldwide (Verissimo et al., 2012). While presently the major way of control and treatment of these gastrointestinal parasites depends profoundly on the utilization of conventional anthelmintics, there are challenges associated with the use of these synthetic anthelmintics (Behnke et al., 2008; Keiser and Utzinger, 2008). The challenges include unavailability and inconsistent supply; high cost to resource-limited farmers, environmental pollution and the build-up of residues in animal products (Kamaraj and Abdul Rahuman, 2010). The rapidly in-

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creasing resistance to anthelmintics may perhaps be as a result of the increased rate of dosing, under-dosing, prophylactic mass treatments and repeated chronic use of the same anthelmintic drugs (Shalaby, 2013). Regular use of these anthelmintics has also led to poor development of natural immunity against gastrointestinal parasites (Ketzi et al., 2006).

One practical way of developing cheaper and effective anthelmintics is to explore potential indigenous herbal remedies used as anthelmintics (Soetan et al., 2011). Plant-derived anthelmintics is a promising area of research in trying to mitigate the challenges around the use of synthetic anthelmintic drugs in controlling gastrointestinal parasites. Alternative ethnoveterinary medicines, for example, plant extracts with anthelmintic properties are considered to be of immense potential in overcoming anthelmintic resistance (Lans et al., 2007). There have been many reports, mainly from Africa and Asia have indicated the effectiveness of medicinal plants against helminth infections in livestock (Alawa et al., 2003; Carvalho et al., 2012). Hence, there is a need to investigate the potential active compounds from these plants.

Worldwide research has shown that some plants can be utilized to diminish the level of parasitism in livestock; thus are considered alternative options to the conventional chemical anthelmintics (Githiori et al., 2006). *Elephantorrhiza elephantina* is a medicinal plant commonly utilized in ethnoveterinary medicine. Root decoctions infusions of *E. elephantina* are used to control gastrointestinal worms (Van der Merwe et al., 2001; Barros et al., 2006; McGaw and Eloff, 2008). The anthelmintic effects of this plant are due to the activity of secondary metabolites, such as tannins, alkaloids, saponins and glycosides (Salem et al., 2011; Carvalho et al., 2012; Hernandez et al., 2014). Phytochemicals are beneficial in the treatment of diseases and control of parasites that nourish on the walls of the gastrointestinal tract (Nkohla et al., 2015). Plants produce them as a defense mechanism. Research demonstrates the ability of these phytochemicals to protect humans and animals against infections (Kumar et al., 2009).

However, reports on the phytochemical analysis of crude extracts from *E. elephantina* are limited. This study sought to quantify the phytochemical constituents of *E. elephantina* in ethanol, methanol and water extracts of the root. Furthermore, the study evaluated the *in vitro* anthelmintic potential of *E. elephantina* root extracts against adult *Paramphistomum cervi* in goats.

## 2. Materials and methodology

### 2.1. Plant collection

Roots of *E. elephantina* were collected from a characteristic populace in Mt. Frere, Alfred Nzo District Municipality, Eastern Cape, South Africa. Mt. Frere lies along latitude 30°55'0" S and longitude 28°58'60" E. Immediately after harvesting, the roots were rinsed in distilled water and air-dried in the shade for 10 weeks and after that were ground into powder using a grinder with 1 mm pore size sieve (IKA- Universal Mill M20, Laboratory and Scientific Equipment Co. Pty. Ltd). The root powder was kept in airtight containers at 4 °C until further analyses.

### 2.2. Extraction procedure

Three samples of 20 g of *E. elephantina* root powder were soaked in 200 mL each of ethanol, methanol, and distilled water. The mixtures were left on an orbital shaker for 24 h and then filtered under pressure using a Buchner funnel and Whatman filter paper (12.5 cm; 111<sup>v</sup>). Then the ethanol and methanol extracts were condensed in a rotary evaporator (Laborator 4000-efficient, Heidolph, Germany) while the water extract was freeze-dried (Vir Tis benchtop K, Vir Tis Co, Gardiner, NY). The dried extracts were used for the quantitative analysis of phytochemicals. The percentage yield was calculated for each extract using the formula:

$$\text{Yield (\%)} = \frac{\text{Final weight}}{\text{Initial weight}} \times 100$$

### 2.3. Quantitative determination of phytochemicals

#### 2.3.1. Alkaloids

The gravimetric analysis for total alkaloid content was used to determine alkaloids with a few modifications (Obadoni and Ochuko, 2001). A 0.5 mL of plant extract (1 mg/mL) was combined with 100 mL of 10% acetic acid in ethanol. The mixture was covered and left standing for 4 h. Afterwards, the mixture was filtered, and the filtrate was concentrated in a water bath at 60 °C to a quarter of its initial volume. Concentrated ammonium hydroxide (25%) was added to the mixture drop-wise until the formation of a precipitate was completed. The entire mixture was left to stand, then the accumulated precipitates were rinsed with 20 mL of 0.1M dilute ammonium hydroxide and then filtered. The collected residue was oven-dried at 80 °C and weighed. The alkaloid content was calculated using the formula:

$$\text{Alkaloid(\%)} = \frac{\text{Weight of residue}}{\text{Weight of sample taken}} \times 100$$

#### 2.3.2. Condensed tannins

Determination of total condensed tannin was performed by applying the technique described by Sun et al. (1998) with a few modifications. A 0.5 mL of plant extract (1 mg/mL) was added to 3 mL of 4% vanillin-methanol. A 1.5 mL of 37% hydrochloric acid

was added to the mixture, thoroughly mixed and left standing for 15 min at room temperature. The absorbance of catechin standard (0.02 to 1 mg/mL) solution and plant extracts were recorded at 500 nm using the UV 3000 PC Spectrophotometer. Distilled water was used as the blank. Readings were taken in triplicates. The calibration curve was plotted using standard catechin. The readings were expressed as mg of catechin equivalent per g dry weight extract (mg CE/g).

### 2.3.3. Flavonoids

Flavonoid content was determined as described by Kamtekar et al. (2014) using the aluminium chloride colorimetric assay technique with some modifications. Briefly, 2 mL of distilled water and 0.15 mL of 5% sodium nitrite were put into a test-tube with 0.5 mL of the plant extract (1 mg/mL). The mixture was left standing for about 5–6 min at room temperature. After 6 min 0.15 mL of 10% aluminium chloride was added to the mixture and then was left standing for an additional 6 min. Afterwards, 1 mL of 4% sodium hydroxide was added to the mixture. A 1.2 mL of distilled water was poured to make up the volume of the mixture to 5 mL. The mixture was incubated for 15 min to develop colour. The absorbance was taken at 420 nm using a UV 3000 PC Spectrophotometer. Each assay was done in triplicates. The calibration curve was plotted using standard quercetin (0.2 to 1 mg/mL). The results were calculated as mg of quercetin equivalent for each g dry weight (mg QE/g).

### 2.3.4. Phenols

Phenol content was quantified through Folin-Ciocalteu's technique with a few modifications (Skerget et al., 2005). A 2.5 mL of Folin-Ciocalteu reagent was added to 0.5 mL of each plant extract (1 mg/mL) and vortexed for about 1 min. The mixture was held at room temperature for about 3–8 min. A 2.5 mL of 7.5% anhydrous sodium carbonate was then added and incubated in a water bath at 40 °C for 30 min to develop colour. Absorbance was then read at 765 nm using the UV 3000 PC Spectrophotometer. The blank was performed using distilled water. Each assay was done in triplicates. Standard gallic acid (0.02 to 1 mg/mL) was used to plot the calibration curve. The readings were expressed as mg of gallic acid equivalent for each g dry weight (mg GAE/g).

### 2.3.5. Saponins

The saponin content was determined following the technique of Obadoni and Ochuko (2001) with some modifications. A 20 mL of 20% ethanol was added to 0.5 mL (1 mg/mL) of the plant extract and put on a shaker for 30 min. The resulting mixture was then left in a water bath at 55 °C for 4 h. The mixture was filtered and extraction was repeated with 20 mL of 20% ethanol. The collected filtrates were concentrated to one-quarter of the original volume in a water bath at 90 °C. Afterwards, the concentrate was put into a 250 mL separating funnel and extraction was done twice with 20 mL diethyl ether. The ether layer was disposed of, while the aqueous layer was kept. A 20 mL *n*-butanol was poured to the aqueous layer, and then washing was done twice using 5 mL of 5% sodium chloride. The solution was placed in a water bath to evaporate. Then the samples were oven-dried at 40 °C. Calculation of saponin content was determined using:

$$\text{Saponin (\%)} = \frac{\text{Weight of final filtrate}}{\text{Weight of sample}} \times 100$$

## 2.4. Worm recovery

Worms were collected from freshly slaughtered goats at East London Abattoir in the Eastern Cape Province (latitude 32.9702° S and longitude 27.8872° E). Worm recovery was done following the method described by Hansen and Perry (1994). Immediately after slaughter, rumens were collected from the animals, ligated and quickly transported in a cooler box with ice to the Animal Science Laboratory at the University of Fort Hare. The contents of the rumens were washed, several times with phosphate-buffered saline (PBS) until the worms were free from debris. The species and description of the worms are as shown in Table 3. The worms were then recovered from the rumens and kept in PBS at 37 °C for 2 h.

## 2.5. Preparation of solutions

All test solutions and the standard drug solutions were freshly prepared before starting the experiments. Concentrations of 1.875, 3.75, 7.5 and 15 mg/mL were prepared for each extract by dissolving the water extract in PBS, while the ethanol and methanol extracts were dissolved in PBS mixed with 5% dimethyl sulfoxide (DMSO). A 5% DMSO/PBS mixture was used as the negative control after ascertaining that the addition of 5% DMSO did not affect the worms (Barrau et al., 2005). Valbantel® (Albendazole 1.9% m/v and Closantel sodium 3% m/v Pfizer, South Africa) was used as the positive control after it was prepared in PBS to a final concentration of 1.875 mg/mL.

## 2.6. Experimental design and treatment procedures

Adult motility inhibition assay was done following the method of Hounzangbe-Adote et al. (2005). Ethanol, methanol and water extracts of *E. elephantina* were tested at different concentrations (1.875, 3.75, 7.5 and 15 mg/mL). Ten *P. cervi* adult worms were placed in each petri dish containing 3 mL of each of the above test solutions and incubated at 37 °C. Every concentration was tested in triplicate. Worm motility was observed at 2-hour intervals for 16 h, and each observation period lasted for

**Table 1**

Percentage yield of extracts in ethanol, methanol and water solvents.

| Solvent  | Initial weight of the powder (g) | Final weight of the powder (g) | Weight of the crude extract (g) | Yield of crude extract (%) |
|----------|----------------------------------|--------------------------------|---------------------------------|----------------------------|
| Ethanol  | 20                               | 18.019                         | 1.981                           | 9.91                       |
| Methanol | 20                               | 16.401                         | 3.599                           | 18.00                      |
| Water    | 20                               | 17.41                          | 2.59                            | 12.95                      |

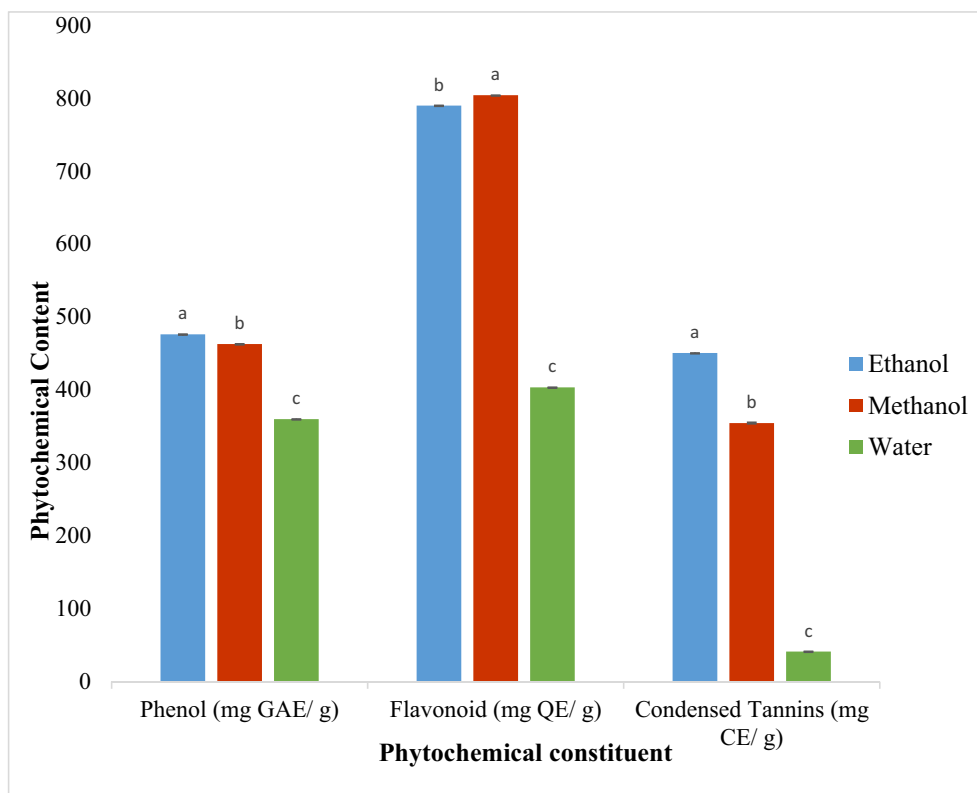
5–6 s. The number of motile worms was recorded per treatment during the observation period and expressed as a percentage. Time for motility inhibition was noted when no movement of any sort was observed except when the worms were shaken vigorously.

### 2.7. Statistical analysis

The results were presented as Mean  $\pm$  SD. Fisher's Least Significant Difference (LSD) was used to compare the difference between the ethanol, methanol and water extracts at a 5% level ( $P < 0.05$ ) using the Minitab 17 Statistical Software (2003). The mean percentages of worm motility inhibition were assessed using PROC GLM of SAS (SAS, 2003). Turkey post-hoc testing (SPSS version 22, Armonk, NY, 2013) was used to compare differences between treatment means. Two-way ANOVA test was performed to test for anthelmintic efficacy between treatment means at a 5% significance level ( $P < 0.05$ ).

### 3. Results

Methanol root extract exhibited higher yield (18.00%), followed by water (12.95%) and ethanol (9.91%) as shown in Table 1. The results of the quantitative phytochemical screening showed that *E. elephantina* root contains high amounts of alkaloids, flavonoids, phenols, saponins and condensed tannins (Fig. 1, Table 2). Higher alkaloid content was present in the water extract ( $93.24 \pm 1.68\%$ ), followed by ethanol and methanol extracts ( $52.33 \pm 1.38$  and  $34.77 \pm 0.07\%$ , respectively). There were significant differences in the saponin contents among the ethanol, methanol and water extracts at a 5% level ( $P < 0.05$ ). Saponin content was highest in ethanol extract ( $83.28 \pm 1.72\%$ ), followed by methanol extract ( $63.74 \pm 0.75\%$ ) and water extract ( $50.83 \pm 0.43\%$ ). Total condensed tannins in the roots were found to be ( $450.52 \pm 0.15$  mg CE/g) in ethanol extract, ( $354.53 \pm 1.03$  mg CE/g) in



**Fig. 1.** Phytochemical constituents in the ethanol, methanol and water extracts of *E. elephantina* roots. Results are expressed as means  $\pm$  SD of three replicates. Bar graphs with dissimilar letter superscript in the same constituent are significantly different ( $P < 0.05$ ).

**Table 2**Content of alkaloids and saponins in extracts of *E. elephantina*.

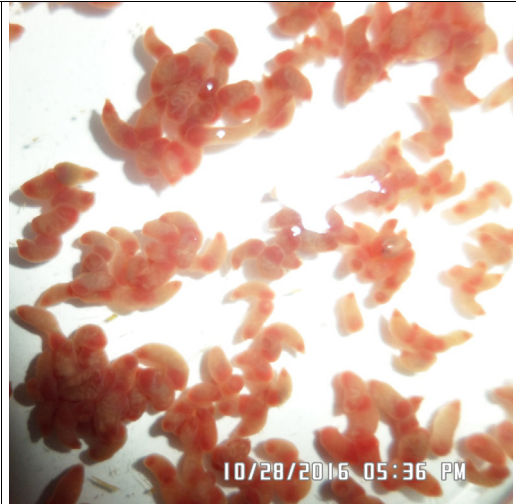
| Extract  | Alkaloids (%)             | Saponins (%)              |
|----------|---------------------------|---------------------------|
| Ethanol  | 52.33 <sup>b</sup> ± 1.38 | 83.28 <sup>a</sup> ± 1.72 |
| Methanol | 34.77 <sup>c</sup> ± 0.07 | 63.74 <sup>b</sup> ± 0.75 |
| Water    | 93.24 <sup>a</sup> ± 1.68 | 50.83 <sup>c</sup> ± 0.43 |

Results are expressed as means ± SD of three replicates. Different superscripts within a column represent significant differences at ( $P < 0.05$ ).

methanol extract and ( $41.31 \pm 0.33$  mg CE/g) in the water extract. A positive correlation was shown between standard catechin concentration and the absorbance values ( $R^2 = 0.9986$ ). Fisher's LSD analysis showed a significant difference in phytochemical quantities among the three solvents, which showed that ethanol had higher condensed tannin content than methanol and water extracts. The content of flavonoids was found to be high in ethanol ( $789.76 \pm 0.21$  mg QE/g) and methanol extracts ( $803.93 \pm 0.13$  mg QE/g) and there was no significant difference ( $P > 0.05$ ) between them. However, the flavonoid content in ethanol and methanol extracts were significantly different ( $P < 0.05$ ) from the water extract which contained lower content of flavonoids ( $403.44 \pm 0.19$  mg QE/g). A significant positive correlation was observed between standard quercetin concentration and the absorbance values ( $R^2 = 0.998$ ). The ethanol extract had the highest phenol content ( $476.11 \pm 0.37$  mg GAE/g), followed by methanol extract ( $462.86 \pm 0.31$  mg GAE/g) and lastly the water extract ( $359.91 \pm 0.24$  mg GAE/g). There was a significant difference ( $P < 0.05$ ) between phenol contents in ethanol, methanol and water extracts. A positive correlation was observed between standard gallic acid concentration and the absorbance values ( $R^2 = 0.9981$ ).

The extracts of *E. elephantina* showed time and dose-dependent anthelmintic activity on the adult *P. cervi*. Generally, all *E. elephantina* extracts showed anthelmintic effects against the adult worms (Table 4; Figs. 2, 3, 4 and 5, respectively). The extracts of *E. elephantina* roots significantly inhibited adult worm motility over the 16 h treatment period ( $P < 0.05$ ). The positive control (Valbantel) showed 100% motility inhibition within 10 h while the negative control (PBS) showed 100% motility. Ethanol extracts showed a highly significant ( $P < 0.05$ ) inhibition of motility (86.67 and 96.67%) at concentrations 7.5 and 15 mg/mL respectively, after 12 h of treatment. However, there was no significant difference in the inhibition of motility between ethanol (15 mg/mL) and the positive control; while ethanol (7.5 mg/mL) was significantly different from the positive control at 12 h post-exposure. Methanol and water extracts exhibited significantly different ( $P < 0.05$ ) motility inhibition (96.67 and 66.67%, respectively) at concentration of 15 mg/mL 16 h post-exposure. There was no significant difference in the inhibition of motility by methanol (15 mg/mL) and positive control at 16 h post-exposure ( $P > 0.05$ ).

**Table 3**Picture showing adult *P. cervi* recovered from the rumen of goats.

|   |  |
|---|--|
|  | <p><b>Description</b></p> <p><i>Paramphistomum cervi</i> are parasitic flat worms (Platyhelminth), in the class Trematoda and belong to the Paramphistomatidae family. <i>P. cervi</i> adults are small, conical/ pear-shaped (the anterior end is tapered while the posterior is broad) and maggot-like flukes about a centimetre long and light red in colour when fresh. The adult worms live in the rumen, attach by suckers and feed from the host rumen. The adult worms cause little or no damage at all whilst the larvae in the intestines cause Paramphistomosis whose symptoms include excessive diarrhoea, anaemia, lethargy which may lead to death when not treated (Taylor <i>et al.</i>, 2007)</p> |
| <p>Adult <i>P. cervi</i> in the negative control (PBS)</p>                          |  |

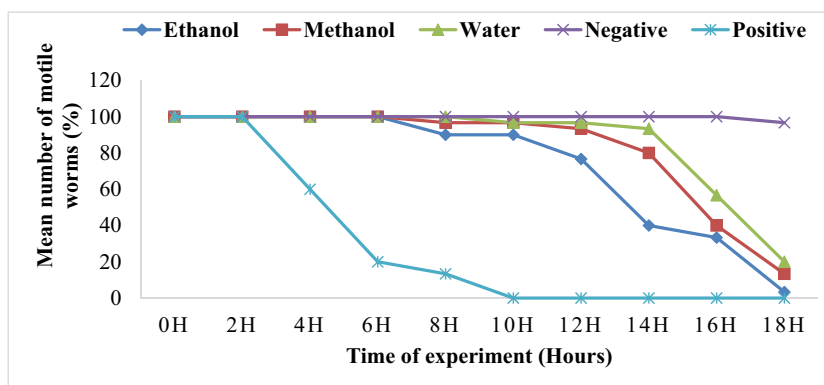
**Table 4***In vitro* effect of *E. elephantina* extracts on adult *P. cervi* in comparison with Valbanel.

| Treatment                   | Conc (mg/mL) | Motility inhibition (%) of adult <i>P. cervi</i> worms at different hours (Mean $\pm$ SD) |                                  |                                  |                                    |                                   |                                    |                                   |                                    |                                    |                                  |  |
|-----------------------------|--------------|---|----------------------------------|----------------------------------|------------------------------------|-----------------------------------|------------------------------------|-----------------------------------|------------------------------------|------------------------------------|----------------------------------|--|
|                             |              | 0   | 2                                | 4                                | 6                                  | 8                                 | 10                                 | 12                                | 14                                 | 16                                 | 18                               |  |
| Ethanol                     | 1.875        | 100.0 <sup>a</sup><br>$\pm$ 0.00  | 100.0 <sup>a</sup><br>$\pm$ 0.00 | 100.0 <sup>a</sup><br>$\pm$ 0.00 | 100.0 <sup>a</sup><br>$\pm$ 0.00   | 90.0 <sup>be</sup><br>$\pm$ 0.00  | 90.0 <sup>bg</sup><br>$\pm$ 0.00   | 76.7 <sup>ae</sup><br>$\pm$ 15.28 | 40.0 <sup>efg</sup><br>$\pm$ 10.00 | 33.3 <sup>bcd</sup><br>$\pm$ 15.28 | 3.3 <sup>c</sup><br>$\pm$ 5.77   |  |
|                             | 3.75         | 100.0 <sup>a</sup><br>$\pm$ 0.00  | 100.0 <sup>a</sup><br>$\pm$ 0.00 | 100.0 <sup>a</sup><br>$\pm$ 0.00 | 90.0 <sup>a</sup><br>$\pm$ 10.00   | 86.7 <sup>ae</sup><br>$\pm$ 11.55 | 83.3 <sup>agh</sup><br>$\pm$ 15.28 | 66.7 <sup>gh</sup><br>$\pm$ 5.77  | 26.7 <sup>fgh</sup><br>$\pm$ 20.82 | 26.7 <sup>bcd</sup><br>$\pm$ 15.28 | 0.0 <sup>c</sup><br>$\pm$ 0.00   |  |
|                             | 7.5          | 100.0 <sup>a</sup><br>$\pm$ 0.00  | 100.0 <sup>a</sup><br>$\pm$ 0.00 | 93.3 <sup>ac</sup><br>$\pm$ 5.77 | 93.3 <sup>ad</sup><br>$\pm$ 5.77   | 80.0 <sup>be</sup><br>$\pm$ 10.00 | 70.0 <sup>ch</sup><br>$\pm$ 10.00  | 13.3 <sup>c</sup><br>$\pm$ 5.77   | 6.7 <sup>h</sup><br>$\pm$ 5.77     | 3.3 <sup>d</sup><br>$\pm$ 5.77     | 0.0 <sup>c</sup><br>$\pm$ 0.00   |  |
|                             | 15           | 100.0 <sup>a</sup><br>$\pm$ 0.00  | 100.0 <sup>a</sup><br>$\pm$ 0.00 | 86.7 <sup>ac</sup><br>$\pm$ 5.77 | 70.0 <sup>be</sup><br>$\pm$ 10.00  | 50.0 <sup>c</sup><br>$\pm$ 5.77   | 30.0 <sup>e</sup><br>$\pm$ 10.00   | 3.3 <sup>cg</sup><br>$\pm$ 5.77   | 0.0 <sup>i</sup><br>$\pm$ 0.00     | 0.0 <sup>d</sup><br>$\pm$ 0.00     | 0.0 <sup>c</sup><br>$\pm$ 0.00   |  |
| Methanol                    | 1.875        | 100.0 <sup>a</sup><br>$\pm$ 0.00  | 100.0 <sup>a</sup><br>$\pm$ 0.00 | 100.0 <sup>a</sup><br>$\pm$ 0.00 | 100.0 <sup>a</sup><br>$\pm$ 0.00   | 96.7 <sup>a</sup><br>$\pm$ 5.77   | 96.7 <sup>a</sup><br>$\pm$ 5.77    | 96.7 <sup>a</sup><br>$\pm$ 5.77   | 80.0 <sup>aj</sup><br>$\pm$ 10.00  | 40.0 <sup>bc</sup><br>$\pm$ 10.00  | 13.3 <sup>bc</sup><br>$\pm$ 5.77 |  |
|                             | 3.75         | 100.0 <sup>a</sup><br>$\pm$ 0.00  | 100.0 <sup>a</sup><br>$\pm$ 0.00 | 100.0 <sup>a</sup><br>$\pm$ 0.00 | 96.7 <sup>a</sup><br>$\pm$ 5.77    | 96.7 <sup>a</sup><br>$\pm$ 5.77   | 93.3 <sup>ag</sup><br>$\pm$ 0.577  | 86.7 <sup>ae</sup><br>$\pm$ 5.77  | 70.0 <sup>bj</sup><br>$\pm$ 10.00  | 33.3 <sup>bcd</sup><br>$\pm$ 15.28 | 6.7 <sup>bc</sup><br>$\pm$ 5.77  |  |
|                             | 7.5          | 100.0 <sup>a</sup><br>$\pm$ 0.00  | 100.0 <sup>a</sup><br>$\pm$ 0.00 | 100.0 <sup>a</sup><br>$\pm$ 0.00 | 90.0 <sup>a</sup><br>$\pm$ 10.00   | 86.7 <sup>ae</sup><br>$\pm$ 5.77  | 76.7 <sup>agh</sup><br>$\pm$ 20.82 | 70.0 <sup>bh</sup><br>$\pm$ 10.00 | 40.0 <sup>efg</sup><br>$\pm$ 10.00 | 10.0 <sup>d</sup><br>$\pm$ 10.00   | 3.3 <sup>c</sup><br>$\pm$ 5.77   |  |
|                             | 15           | 100.0 <sup>a</sup><br>$\pm$ 0.00  | 100.0 <sup>a</sup><br>$\pm$ 0.00 | 100.0 <sup>a</sup><br>$\pm$ 0.00 | 83.3 <sup>ade</sup><br>$\pm$ 15.28 | 80.0 <sup>be</sup><br>$\pm$ 10.00 | 56.7 <sup>dh</sup><br>$\pm$ 5.77   | 56.7 <sup>de</sup><br>$\pm$ 11.55 | 33.3 <sup>g</sup><br>$\pm$ 5.77    | 3.3 <sup>d</sup><br>$\pm$ 5.77     | 0.0 <sup>c</sup><br>$\pm$ 0.00   |  |
| Water                       | 1.875        | 100.0 <sup>a</sup><br>$\pm$ 0.00  | 100.0 <sup>a</sup><br>$\pm$ 0.00 | 100.0 <sup>a</sup><br>$\pm$ 0.00 | 100.0 <sup>a</sup><br>$\pm$ 0.00   | 100.0 <sup>a</sup><br>$\pm$ 0.00  | 96.7 <sup>a</sup><br>$\pm$ 5.77    | 96.7 <sup>a</sup><br>$\pm$ 5.77   | 93.3 <sup>a</sup><br>$\pm$ 5.77    | 56.7 <sup>b</sup><br>$\pm$ 15.28   | 20.0 <sup>b</sup><br>$\pm$ 10.00 |  |
|                             | 3.75         | 100.0 <sup>a</sup><br>$\pm$ 0.00  | 100.0 <sup>a</sup><br>$\pm$ 0.00 | 100.0 <sup>a</sup><br>$\pm$ 0.00 | 100.0 <sup>a</sup><br>$\pm$ 0.00   | 96.7 <sup>a</sup><br>$\pm$ 5.77   | 96.7 <sup>a</sup><br>$\pm$ 5.77    | 96.7 <sup>a</sup><br>$\pm$ 5.77   | 86.7 <sup>a</sup><br>$\pm$ 5.77    | 43.3 <sup>bc</sup><br>$\pm$ 5.77   | 16.7 <sup>b</sup><br>$\pm$ 5.77  |  |
|                             | 7.5          | 100.0 <sup>a</sup><br>$\pm$ 0.00  | 100.0 <sup>a</sup><br>$\pm$ 0.00 | 100.0 <sup>a</sup><br>$\pm$ 0.00 | 96.7 <sup>a</sup><br>$\pm$ 5.77    | 93.3 <sup>ae</sup><br>$\pm$ 5.77  | 83.3 <sup>cg</sup><br>$\pm$ 5.77   | 70.0 <sup>bh</sup><br>$\pm$ 10.00 | 56.7 <sup>bef</sup><br>$\pm$ 15.28 | 40.0 <sup>bc</sup><br>$\pm$ 0.00   | 6.7 <sup>bc</sup><br>$\pm$ 5.77  |  |
|                             | 15           | 100.0 <sup>a</sup><br>$\pm$ 0.00  | 100.0 <sup>a</sup><br>$\pm$ 0.00 | 96.7 <sup>a</sup><br>$\pm$ 5.77  | 93.3 <sup>ad</sup><br>$\pm$ 5.77   | 86.7 <sup>ae</sup><br>$\pm$ 5.77  | 70.0 <sup>ch</sup><br>$\pm$ 10.00  | 60.0 <sup>be</sup><br>$\pm$ 10.00 | 36.7 <sup>cef</sup><br>$\pm$ 15.28 | 33.3 <sup>c</sup><br>$\pm$ 5.77    | 0.0 <sup>c</sup><br>$\pm$ 0.00   |  |
| Positive control (Valbanel) | 1.875        | 100.0 <sup>a</sup><br>$\pm$ 0.00  | 100.0 <sup>a</sup><br>$\pm$ 0.00 | 60.0 <sup>b</sup><br>$\pm$ 10.00 | 20.0 <sup>c</sup><br>$\pm$ 10.00   | 13.3 <sup>d</sup><br>$\pm$ 5.77   | 0.0 <sup>f</sup><br>$\pm$ 5.77     | 0.0 <sup>g</sup><br>$\pm$ 0.00    | 0.0 <sup>i</sup><br>$\pm$ 0.00     | 0.0 <sup>d</sup><br>$\pm$ 0.00     | 0.0 <sup>c</sup><br>$\pm$ 0.00   |  |
| Negative control (PBS)      |              | 100.0 <sup>a</sup><br>$\pm$ 0.00  | 100.0 <sup>a</sup><br>$\pm$ 0.00 | 100.0 <sup>a</sup><br>$\pm$ 0.00 | 100.0 <sup>a</sup><br>$\pm$ 0.00   | 100.0 <sup>a</sup><br>$\pm$ 0.00  | 100.0 <sup>a</sup><br>$\pm$ 0.00   | 100.0 <sup>a</sup><br>$\pm$ 0.00  | 100.0 <sup>a</sup><br>$\pm$ 0.00   | 100.0 <sup>a</sup><br>$\pm$ 0.00   | 96.7 <sup>a</sup><br>$\pm$ 5.77  |  |

abcde fghij Means with the same lowercase letters within the same column are not significantly different ( $P < 0.05$ ).

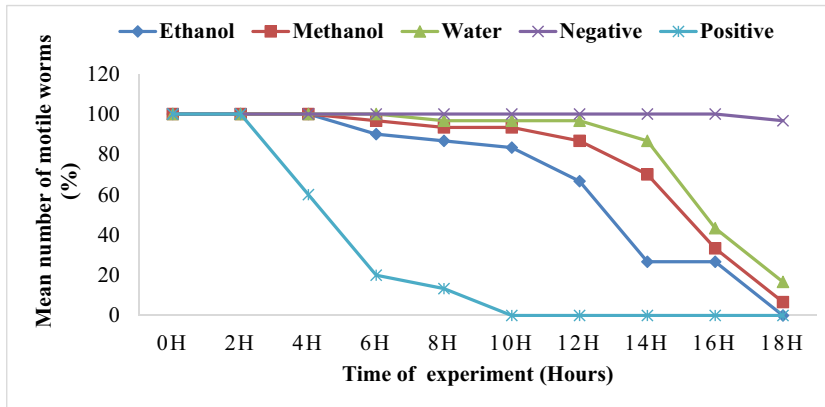
#### 4. Discussion

The findings of this work have shown that the root extracts of *E. elephantina* exhibited anthelmintic activity against the adult *P. cervi* motility that was significantly different ( $P < 0.05$ ) from the positive control (Valbanel). These findings are similar to studies by Maphosa et al. (2010) and Sanhokwe et al. (2016) who reported anthelmintic activity of root fractions of *E. elephantina* against nematodes. The results of this study also exhibited time and dose-dependent anthelmintic activity which resulted in the inhibition of motility. Thus increasing the concentration of the extracts caused early motility inhibition. The time and dose-dependent anthelmintic effects could be due to more compounds with anthelmintic activity being present at higher concentrations of the extract (Maphosa and Masika, 2012). The ethanol extract was the most effective at the test concentration of 7.5 and 15 mg/mL compared to methanol and aqueous extracts. This could be attributed to other molecules present or the different mechanisms of action against the parasites. Methanolic and aqueous extracts exhibited significantly different ( $P < 0.05$ ) motility inhibition (96.67 and 66.67%, respectively) at a concentration of 15 mg/mL, 16 h post-exposure. The effect of most worm expellers such as albendazole is to cause worm paralysis leading to expulsion of the worm *via* faecal matter of the host (Islam et al., 2019).

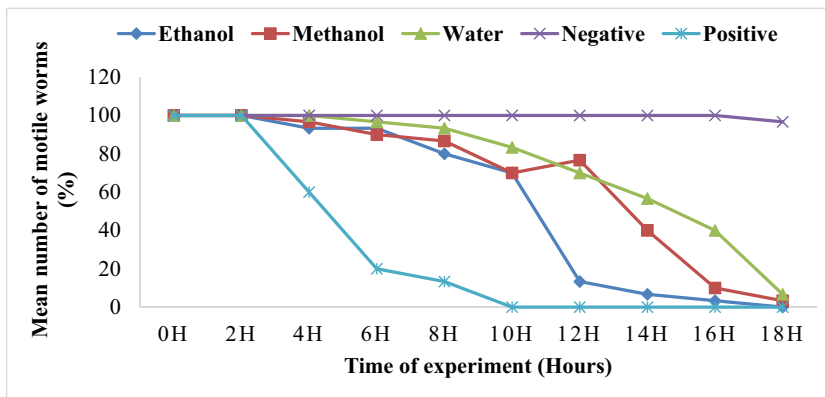


**Fig. 2.** Time- and dose-dependent *in vitro* anthelmintic activity of ethanol, methanol and water extracts of *E. elephantina* (at a concentration of 1.875 mg/mL) in comparison with positive (Valbanel 1.875 mg/mL) and negative (PBS) controls on adult *P. cervi*.





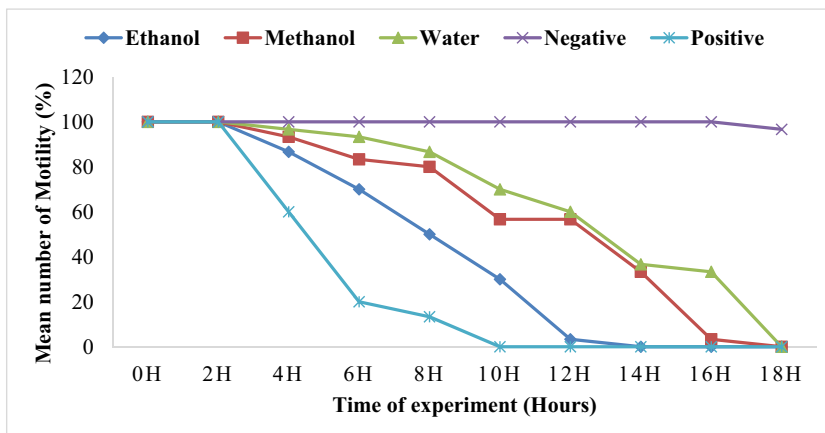
**Fig. 3.** Time- and dose-dependent *in vitro* anthelmintic activity of ethanol, methanol and water extracts of *E. elephantina* (at a concentration of 3.75 mg/mL) in comparison with positive (Valbanel 1.875 mg/mL) and negative (PBS) controls on adult *P. cervi*.



**Fig. 4.** Time- and dose-dependent *in vitro* anthelmintic activity of ethanol, methanol and water extracts of *E. elephantina* (at a concentration of 7.5 mg/mL) in comparison with positive (Valbanel 1.875 mg/mL) and negative (PBS) controls on adult *P. cervi*.

Therefore, the methanolic extract at the test concentration of 15 mg/mL showed a significant anthelmintic activity as compared to the positive control (Valbanel).

In this present study, the anthelmintic effects of *E. elephantina* could be attributed to one or more of the phytochemicals present in its roots such as alkaloids, condensed tannins, flavonoids, phenols and saponins. However, the precise mechanism of action



**Fig. 5.** Time- and dose-dependent *in vitro* anthelmintic activity of ethanol, methanol and water extracts of *E. elephantina* (at a concentration of 15 mg/mL) in comparison with positive (Valbanel 1.875 mg/mL) and negative (PBS) controls on adult *P. cervi*.

of these phytochemicals against gastrointestinal nematodes is not well understood as their effects might be additive, synergistic, or antagonistic and act at single or multiple target sites (Wynn and Fougere, 2007; Hernandez et al., 2014). Tannins are postulated to cause anthelmintic activity by either binding to the proteins found in the gastrointestinal tract of the host or to glycoprotein on the cuticle of the parasite resulting in the death of the worms (Hoste et al., 2012; Williams et al., 2014). Also, tannins are thought to interfere with energy generation by uncoupling oxidative phosphorylation (Roy et al., 2010; Weldemariam et al., 2015) which can lead to worm death. On the other hand, saponins are reported to disrupt the cell membrane of the parasites thereby changing the morphology of the cells in the cuticle (Hrckova and Velebny, 2013). Saponins may also affect feed intake and nourishment of the parasites, resulting in parasites dying (Hernandez et al., 2014). Alkaloids have been reported to act on the central nervous system and are thought to cause paralysis of worms (Roy et al., 2010; Weldemariam et al., 2015).

## 5. Conclusions and recommendations

This study showed that aqueous, ethanol and methanol extracts of *E. elephantina* root exhibited anthelmintic activity: they inhibited motility in adult *P. cervi*. Therefore, further research is needed to determine their bioactivities *in vivo* as *E. elephantina* could contribute to the development of novel anthelmintic agents. The findings thereof may be useful in developing phytotherapeutic products that are more cost-effective, safer, and accessible with reduced risk of resistance than the conventional drugs presently in use.

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## Declaration of competing interest

The authors report that there are no known conflicts of interest associated with this publication. The authors alone are responsible for the content and writing of this article.

## References

- Alawa, C.B.I., Adamu, A.M., Gefu, J.O., Ajanusi, O.J., Abdu, P.A., Chiezey, N.P., Alawa, J.N., Bowman, D.D., 2003. *In vitro* screening of two Nigerian medicinal plants (*Vernonia amygdalina* and *Annona senegalensis*) for anthelmintic activity. *Vet. Parasitol.* 113, 73–81.
- Barrau, E., Fabre, N., Fouraste, I., Hoste, H., 2005. Effect of bioactive compounds from Sainfoin (*Onobrychis vicifolia* Scop.) on the *in vitro* larval migration of *Haemonchus contortus*: role of tannins and flavonol glycosides. *Parasitology* 131, 531–538.
- Barros, I.M.C., Lopes, L.D.G., Borges, M.O.R., Borges, A.C.R., Ribeiro, M.N.S., Freire, S.M.F., 2006. Anti-inflammatory and anti-nociceptive activities of *Pluchea quitic* (D.C.) ethanolic extract. *J. Ethnopharmacol.* 106, 317–320.
- Behnke, J.M., Buttle, D.J., Stepek, G., Lowe, A., Duce, I.R., 2008. Developing novel anthelmintics from plant cysteine proteinases. *Parasite Vectors* 1, 29.
- Carvalho, C.O., Chagas, A.S., Cotinguiba, F., Furlan, M., Brito, L.G., Chaves, F.C.M., Stephan, M.P., Bizzo, H.R., Amarante, A.F.T., 2012. The anthelmintic effect of plant extracts on *Haemonchus contortus* and *Strongyloides venezuelensis*. *Vet. Parasitol.* 183, 260–268.
- Githiori, J.B., Athanasiadou, S., Thamsborg, S.M., 2006. Use of plants in novel approaches for control of gastrointestinal helminths in livestock with emphasis on small ruminants. *Vet. Parasitol.* 139, 308–320.
- Hansen, J., Perry, B., 1994. The Epidemiology, Diagnosis and Control of Helminth Parasites of Ruminants. International Laboratory for Research on Animal Diseases (ILRAD), Addis Ababa, Ethiopia, pp. 90–100.
- Hernandez, P.B., Salem, A.Z.M., Elghandour, M.M.M.Y., Cipriano-Salazar, M., Cruz-Lagunas, B., Camacho, L.M., 2014. Anthelmintic effects of *Salix babylonica* L. and *Leucaena leucocephala* lam. Extracts in growing lambs. *Trop. Anim. Health Prod.* 46, 173–178.
- Hoste, H., Martinez-Ortiz-De-Montellano, C., Manolaraki, F., Brunet, S., Ojeda-Robertos, N., 2012. Direct and indirect effects of bioactive tannin-rich tropical and temperate legumes against nematode infections. *Vet. Parasitol.* 186, 18–27.
- Hounzangbe-Adote, M.S., Paolini, V., Fouraste, I., Moutairou, K., Hoste, H., 2005. *In vitro* effects of four tropical plants on three life-cycle stages of the parasitic nematode, *Haemonchus contortus*. *Res. Vet. Sci.* 78, 155–160.
- Hrckova, G., Velebny, S., 2013. Pharmacological potential of selected natural compounds in the control of parasitic diseases. *Springer Briefs in Pharmaceutical Science and Drug Development*. Springer, New York, pp. 37–39.
- Islam, M.R., Zahra, S.F.T., Sumon, S.I., Parvin, S., Hasan, K., Ahmed, M., Siddique, M.A.T., Haque, T., 2019. Evaluation of anthelmintic activity of ethanolic extracts of *Carica papaya* leaves using *Paramphistomum cervi* and *Haemonchus contortus*. *Afr. J. Pharm. Pharmacol.* 13, 146–150.
- Kamaraj, C., Abdul Rahman, A., 2010. Efficacy of anthelmintic properties of medicinal plant extracts against *Haemonchus contortus*. *Res. Vet. Sci.* 91, 400–404.
- Kamtekar, S., Keer, V., Patil, V., 2014. Estimation of phenolic content, flavonoid content, antioxidant and alpha amylase inhibitory activity of marketed polyherbal formulation. *J. Appl. Pharm. Sci.* 4, 061–065.
- Keiser, J., Utzinger, J., 2008. Efficacy of current drugs against soil-transmitted helminth infections. *J. Am. Med. Assoc.* 299, 1937–1948.
- Ketzis, J.K., Verccruyse, J., Stromberg, B.E., Larsen, M., Athanasiadou, S., Houdijk, J.G.M., 2006. Evaluation of efficacy expectations for novel and non-chemical helminth control strategies in ruminants. 4th international conference - novel approaches to the control of Helminth parasites of livestock. 4th novel approaches conference. *Vet. Parasitol.* 31, 321–335.
- Kumar, A., Ilavarasan, R., Jayachandran, T., Decaraman, M., Aravindhan, P., Padmanabhan, N., Krishnan, M.R., 2009. Phytochemical investigation on a tropical plant, *Syzygium cumini* from Kattuppalayam, Erode District, Tamil Nadu, South India. *Pak. J. Nutr.* 8, 83–85.
- Lans, C., Turner, N., Khan, T., Brauer, G., 2007. Ethnoveterinary medicines used to treat endoparasites and stomach problems in pigs and pets in British Columbia, Canada. *Vet. Parasitol.* 148, 325–340.
- Maphosa, V., Masika, P.J., 2012. Anthelmintic screening of fractions of *Elephantorrhiza elephantina* root extract against *Haemonchus contortus*. *Trop. Anim. Health Prod.* 44, 159–163.
- Maphosa, V., Masika, P.J., Bizimenyera, E.S., Eloff, J.N., 2010. *In-vitro* anthelmintic activity of crude aqueous extracts of *Aloe ferox*, *Leonotis leonurus* and *Elephantorrhiza elephantina* against *Haemonchus contortus*. *Trop. Anim. Health Prod.* 42, 301–307.



- McGaw, L.J., Eloff, J.N., 2008. African plants with potential for development into Ethnoveterinary products. In: Gurib-Fakim, A. (Ed.), *Novel Plant Bioresources: Applications in Food, Medicine and Cosmetics*. John Wiley & Sons, Ltd, Chichester, UK.
- Nkohla, M.B., Gxasheka, M., Lyu, Z., Qin, N., Tyasi, T.L., 2015. Effects of *Elephantorrhiza elephantina* as an anthelmintic against gastrointestinal parasites in goats. *Int. J. Agric. Res. Rev.* 3, 337–342.
- Obadoni, B.O., Ochuko, P.O., 2001. Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. *Global J. Pure Appl. Sci.* 8, 203–208.
- Roy, H., Chakraborty, A., Bhanja, S., Nayak, B.S., Mishra, S.R., Ellaiah, P., 2010. Preliminary phytochemical investigation and anthelmintic activity of *Acanthospermum hispidum* DC. *J. Pharm. Sci. Technol.* 2, 217–221.
- Salem, A.Z.M., Salem, M.Z.M., González-Ronquillo, M., Camacho, L.M., Cipriano, M., 2011. Major chemical constituents of *Leucaena leucocephala* and *Salix babylonica* leaf extracts. *J. Trop. Agric.* 49, 95–98.
- Sanhokwe, M., Mupangwa, J., Masika, P.J., Maphosa, V., Muchenje, V., 2016. Medicinal plants used to control internal and external parasites in goats. *Onderstepoort J. Vet. Res.* 83, 1016.
- Shalaby, H.A., 2013. Anthelmintics resistance; how to overcome it? *Iran J. Parasitol.* 8, 18–32.
- Skerget, M., Kotnik, P., Hadolin, M., Rizner, H.A., Simoncic, M., Knez, Z., 2005. Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. *Food Chem.* 89, 191–198.
- Soetan, K.O., Lasisi, O.T., Agboluaje, A.K., 2011. Comparative assessment of *in vitro* anthelmintic effects of the aqueous extracts of the seeds and leaves of the African locust bean (*Parkia biglobosa*) on bovine nematode eggs. *J. Cell Anim. Biol.* 5, 109–112.
- Sun, J.S., Tsuang, Y.W., Chen, J.J., Huang, W.C., Hang, Y.S., Lu, F.J., 1998. An ultra-weak chemiluminescence study on oxidative stress in rabbits following acute thermal injury. *Burns* 24, 225–231.
- Van Der Merwe, D., Swan, G.E., Botha, C.J., 2001. Use of ethnoveterinary medicinal plants in cattle by Setswanaspeaking people in the Madikwe area of the North West Province of South Africa. *J. S. Afr. Vet. Assoc.* 72, 189–196.
- Verissimo, C.J., Niciura, S.C.M., Alberti, A.L.L., Rodrigues, C.F.C., Barbosa, C.M.P., Chiebao, D.P., Cardoso, D., da Silva, G.S., Pereira, J.R., Margatho, L.F.F., da Costa, R.L.D., Nardon, R.F., Ueno, T.E.H., Curci, V.C.L.M., Molento, M.B., 2012. Multidrug and multispecies resistance in sheep flocks from São Paulo state, Brazil. *Vet. Parasitol.* 187, 209–216.
- Weldemariam, Y., Abadi, K., Gebremariam, G., Brhane, G., 2015. Phytochemical screening, anthelmintic and antioxidant activity of *Eucalyptus globules* essential oils: local cough remedy plant. *World J. Pharm. Pharm. Sci.* 4, 52–60.
- Williams, A.R., Frygasas, C., Ramsay, A., Mueller-Harvey, I., Thamsborg, S.M., 2014. Direct anthelmintic effects of condensed tannins from diverse plant sources against *Ascaris suum*. *PLoS One* 9, e97053.
- Wynn, S.G., Fougere, B.J., 2007. Introduction: Why use herbal medicine. In: Wynn, S.G., Fougere, B.J. (Eds.), *Veterinary Herbal Medicine*. Library of Congress Cataloging-in-Publication Data, p. 695.