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Short communication

Terminalia catappa: Chemical composition, *in vitro* and *in vivo* effects on *Haemonchus contortus*

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ABSTRACT

Haemonchus contortus is the most important nematode in small ruminant systems, and has developed tolerance to all commercial anthelmintics in several countries. *In vitro* (egg hatch assay) and *in vivo* tests were performed with a multidrug strain of *Haemonchus contortus* using *Terminalia catappa* leaf, fruit pulp, and seed extracts (*in vitro*), or pulp and seed powder in lambs experimentally infected with *H. contortus*. Crude extracts from leaves, fruit pulp and seeds obtained with 70% acetone were lyophilized until used. *In vitro*, the extracts had LC_{50} = 2.48 μg/mL (seeds), LC_{50} = 4.62 μg/mL (pulp), and LC_{50} = 20 μg/mL (leaves). *In vitro*, seed and pulp extracts had LC_{50} similar to Thiabendazole (LC_{50} = 1.31 μg/mL). Condensed tannins were more concentrated in pulp extract (183.92 g of leucocyanidin/kg dry matter) than in either leaf (4.6 g) or seed (35.13 g) extracts. Phytochemical tests established that all extracts contained alkaloids, flavonoids, saponins, phenols, and terpenoids. Based on these results, *in vivo* tests were performed to evaluate the anthelmintic activity of *T. catappa* whole fruit (pulp + seed) powder. Male Santa Ines lambs were artificially infected with multidrug-resistant *H. contortus* and divided, according to similar fecal egg count (FEC) and weight, into two groups: Control (infected/untreated) and treated (infected/treated with whole fruit powder). Whole fruit powder was mixed with concentrate and provided at 2 g/kg of body weight (BW) for five days. After treatment, parasitological analysis (FEC and egg hatch assay), renal profile (urea and creatinine), liver profile (aspartate aminotransferase) and BW were determined. *In vitro* (based on LC_{50}), seed/pulp extracts had ovicidal effect similar to Thiabendazole but whole fruit powder had no anthelmintic effect on adult nematodes in the abomasum. We discuss the plausible causes of the lack of *in vivo* activity.

1. Introduction

Gastrointestinal nematodes (GIN) are the major cause of economic losses in small ruminant production. The treatment of infections caused by GIN is heavily reliant on commercial anthelmintics. However, the worldwide occurrence of multi-drug resistance GIN represents an obstacle to animal productivity. In the tropics, *Haemonchus contortus,* is a specie of great importance due to its high prevalence and pathogenicity (Almeida et al., 2010).

The lack of new commercial anthelmintic agents for small ruminants, their price, and their growing loss of efficacy have led researchers to test natural, or plant-based, products and phytochemical compounds with potential to control GIN. Research involving natural products also contributes to improve sustainable production systems, with a lower environmental impact and little to no residues passed on to either meat or milk. Effective anthelmintic phytochemicals would be of great interest to farmers, as in many countries the cost of commercial drugs is not affordable (Max, 2010).

Terminalia catappa belongs to the family Combretaceae. It is native from India, and was introduced in South America for ornamental purposes and reforestation. *T. catappa* fruit is composed of 1/3 pulp and 2/3 seed. The seeds are consumed by humans in some developing countries (Ezeokonkwo and Dodson, 2004) and are rich in crude protein (23.8%), carbohydrates (16%), K (9.3%), Ca (8.3%), and Mg (8%), and fat (51.8%) composed mostly of oleic (up to 31.5%) and linoleic (up to 29%) acids (Matos et al., 2009). Medicinal uses of *T. catappa* leaves include antioxidant (Kinoshita et al., 2007), anti-inflammatory (Fan et al., 2004), and anticancer (Chen et al., 2000), while the fruits have antidiabetic properties (Nagappa et al., 2003). Also, the leaves had anthelmintic activity against the trematode *Fischoederius cobboldi in*

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vitro (Anuracpreeda et al., 2006). *T. catappa* pulp, seed and leaves are rich source of tannins, which are plant polyphenolic compounds classified as condensed (CT) or hydrolyzable (HT). Tannins form stable complexes with protein in the rumen, increasing the proportion of undegraded rumen protein. The tannin-protein complex dissociates at the lower pH of the abomasum, resulting in a higher absorption of dietary amino acids from the duodenum (Douglas et al., 1995). Besides tannins, other phytochemical compounds such as flavonoids, alkaloids, triterpenes, coumarins may also have anthelmintic activity (Vargas-Magaña et al., 2014).

A published research showed that plants that produce both CT and HT, or mostly HT had better anthelmintic effect on *C. elegans* than plant extracts containing only CT (Katiki et al., 2013). This effect was attributed to gallic acid and/or ellagic acid present in HT. This notion was recently validated in an anthelmintic study with *Haemonchus* and *Trychostrongylus* that shows that CT containing higher percentage of galloyl groups (groups with 3 OH groups similar to gallic and ellagic acid) have significantly better anthelmintic effect than CT without galloyl groups (Quijada et al., 2015).

The objective of this work was to access the *in vitro* and *in vivo* anthelmintic activity of phytochemical components of *T. catappa* against *H. contortus*, and the role of tannins in this activity. The *in vitro* hatchability test determined the most potent extract against *Haemonchus contortus*. We performed both qualitative and quantitative phytochemical composition of the *Terminalia* extracts. *In vivo* test were conducted to determine the anthelmintic activity and safety of *T. catappa* in sheep infected with multidrug-resistant *Haemonchus*.

2. Materials and methods

2.1. T. catappa

The leaves and fruits used in this study were collected from a unique tree, located at Instituto de Zootecnia- Nova Odessa, São Paulo, Brazil (−22°77′ S, −47°29′ W, 560 m of altitude). The plant material was deposited at, and identified by the herbarium of the Instituto de Zootecnia, and received a number: BAG-IZ NO 2620. Leaves and fruits (new, green, with uniform color and no insects) were used. About 10 kg of each material was dried in a forced-air oven at 35 °C to a constant weight, then grinded to 0.5 mm size. The materials were stored in freezer −80 °C until extract preparation or animal administration. Phytochemical compounds were extracted as follow: 100 g of the grinded material was extracted with 700 mL of a 70:30 (acetone: water) containing 0.1% ascorbic acid, stirred overnight, and vacuum-filtered through Whatman #2 filter paper. Filtered samples were concentrated in a rotary evaporator at 40 °C until only water remained. The remaining aqueous phase was lyophilized, frozen, and protected from light until used.

2.2. Total phenols, total tannins and condensed tannins

Tannins were analyzed according to Makkar et al. (1993). Total phenols and total tannins, which are expressed in gram equivalent of tannic acid/kg of dry matter (DM), and condensed tannins were done according to the methodology of Porter et al. (1986) and expressed in gram equivalent of leucocyanidin/kg DM.

2.3. Phytochemicals compounds

Qualitative analysis was performed from lyophilized extracts (leaf, pulp, seed) and dried whole fruit (pulp + seed) according to Evans (2002).

Flavonoids: 0.5 g of sample was dissolved in 20 mL of 80% ethanol and filtered. 3 mL of the filtrate was mixed with 4 mL of 1% KOH. The formation of a ring on the superficial layer of the liquid indicates the presence of flavonoids. **Alkaloids:** 0.5 g of sample was mixed with 8 mL of 1% HCl, warmed and filtered. 2 mL of this solution was mixed with 6 drops of Dragendorff's reagent. If precipitation occurred, it indicates the presence of alkaloids. **Saponins:** In a test tube with screw cap, 0.5 g of sample was mixed with 6 mL of boiling distilled water. The tube was shaken for 15 s and kept still in a vertical position for 15 min. The presence of a foam column on the surface of the solution indicated the presence of saponins. **Steroids:** 2 g of sample was mixed with 2 mL of anhydride acetic and followed by 2 mL of H_2SO_4. The formation of a green solution indicates the presence of steroids. **Phenols:** 0.5 g of sample was mixed with 3 mL of distilled water. 5 drops of 10% aqueous ferric chloride were added to the solution. The formation of a dark green color indicates the presence of phenols. **Terpenoids:** 5 g of the sample were dissolved in 5 mL of acetone. 2 mL of chloroform was added to the solution, and then 3 mL of H_2SO_4 were slowly added. The formation of reddish brown color layer indicates the presence of terpenoids. **Resins:** 1 g of sample was mixed with 2 mL of acetone and added 3 drops of acetic anhydride followed by 1 mL of H_2SO_4. The presence of resin in the extract takes the appearance of a yellow-orange color. **Tannins:** 10 g of sample was mixed with 10 mL of distilled water followed by 3 mL of 1% aqueous $FeCl_3$. The formation of a dark green, dark blue, or black color in the solution indicates the presence of tannins.

2.4. Multi-drug resistant Haemonchus contortus

This strain was previously determined to be multi-drug resistant by FECRT (Almeida et al., 2010). This strain is resistant to the main anthelmintics found in the market: Benzimidazoles (including Albendazole and Thiabendazole), tetrahydropirimidines (Levamisole), organophosphates (Trichlorfon), macrocyclic lactones (Ivermectin, Moxidectin) and salicylanilides (Closantel). Susceptibility or resistance to Thiabendazole was evaluated by the *in vitro* egg hatch assay (Von Samson-Himmelstjerna et al., 2009). These authors established that a resistant strain should have LC_{50} > 0.1 μg Thiabendazole/mL. In our tests, Thiabendazole LC_{50} was 1.31 μg/mL.

2.5. In vitro egg hatch assay (EHA)

Three lambs artificially infected with the multi-drug resistant *H. contortus* strain were used as egg donors. About 5 g of feces were collected directly from the rectum, dissolved in water at 37 °C and then filtered with 1000; 105; 55 and 25 μm sieves. Eggs retained by the latter sieve (Jackson and Hoste, 2010) were counted and used for tests. Quantification of eggs were done under a microscope and from five drops (10 μL each) of the egg solution. The average number of eggs was calculated for 10 μL and the volume that contains hundred eggs was adjusted per well. Tests were performed with 6 repetitions per concentration and incubated at 27 °C/24 h with extracts at doses ranging from 25 mg/mL–0.0006 mg/mL. Each dilution had half of the concentration of the previous extract (25–12.5–6.25–3.12–1.56–0.78–0.39–0.19–0.09–0.04–0.02–0.01–0.005–0.002–0.00012–0.0006 mg/mL). All extracts, positive and negative control had DMSO at 2% in order to facilitate dissolution in distilled water. (Thiabendazole – Sigma-Aldrich was tested at doses ranging from 1 to 0.1 μg/mL) according to Von Samson-Himmelstjerna et al. (2009). The tannin chelator poly vinyl poly pyrrolidone (PVPP – Sigma–Aldrich) at 60 μg/mL was mixed to pulp, seed and leaf extracts at 20 μg/mL (3:1) were also evaluated in EHA.

2.6. In vivo tests

All procedures were approved by the Ethics Committee of Animal Use from CENA-USP and received a number: 2013-1. Fourteen five-month-old male 'Santa Ines' lambs, mean body weight of 22 kg, free of parasites (cleaned with Monepantel – Zolvix®) were artificially infected with 4000 *H. contortus* L3 larvae of a multidrug-resistant strain

Table 1
Dry matter (DM) at 100 °C, organic matter (OM), crude protein (CP), lignin and mineral elements (ME) of lambs diet: hay, concentrate, total diet and dried and powdered fruit (pulp + seed) of *T. catappa*.

SAMPLE	DM	OM[a]	CP[a]	Lignin[a]	ME[a]
Hay	873.81	945.93	117.26	69.08	54.07
Concentrate[b]	946.46	969.21	154.25	14.56	30.79
Total diet	932.76	954.87	116.57	54.00	45.13
Pulp + seed powder	942.21	946.30	59.94	268.94	53.70

DM expressed in g/kg of green matter.
[a] Values expressed in g/kg of dried matter.
[b] 85,5% corn meal, 11% soybean meal, 1,5% ovine mineral salt, 1% NaCl and 1% limestone.

(Almeida et al., 2010), also used for the *in vitro* test. Animals were allocated in individual cages of 1 m × 2 m, and supplied with hay, concentrate, water and mineral salt. At 40 days post infection, animals were divided in 2 groups with similar fecal egg count (FEC), hematocrit and body weight (BW). One group remained infected, but untreated, while the other was infected and treated with *T. catappa* pulp + seed powder.

2.6.1. Control group (n = 6)

Animals received 1.5% of BW of *Cynodon* hay and 1.5% of BW of concentrate (corn and soymeal plus mineral salt and vitamins) with approximately 12% of protein in the total diet (Table 1), and water *ad libitum*.

2.6.2. T. catappa group (n = 8)

Animals were fed concentrate (1.5% of BW) and hay (1.5% of BW) similar to control group. Treated animals consumed 2 g/kg of BW of dried and grinded whole fruit *T. catappa*-concentrate mix in the morning once a day for 5 days, and then offered *Cynodon* hay after treatment.

2.7. Evaluations

2.7.1. Feces

Feces were collected directly from the rectum to perform FEC before, 7, 14, 21 and 28 days after treatment and at 7, 14, 21 and 28 days after treatment. The EHA was used to evaluate the residual effect of *T. catappa* in feces.

2.7.2. Blood

Blood was collected before, 7, 14, 21 and 28 days after treatment with *T. catappa* from jugular vein in tubes containing EDTA as anticoagulant to determine microhematocrit and without EDTA to recover serum. Levels of urea, creatinine, and aspartate aminotransferase (AST) were established in the serum. The commercial reagent Labtest® was used for the biochemical analysis and adjusted to 37 °C. Reactions were read with the semiautomatic biochemical analyzer spectrophotometer (Bio 200®, Bioplus Laboratory Products Ltda, Barueri, SP, Brazil). Reference values: urea (17.12–42.8 mg/dL), creatinine (1.2–1.9 mg/dL) and AST (60–280 UI/L) (Kaneko et al., 1997; Meyer and Harvey, 2004).

2.7.3. Weight

Weight was recorded before and 28 days after *Terminalia* treatments.

2.8. Statistics

For *in vitro* tests, data were analyzed to establish the lethal concentration that killed 50% of eggs (LC_{50}) with estimated 95% fiducial limits by a regression curve generated by the SAS® probit program (SAS v. 9.2® Cary, NC). The LC_{50} was used to classify the anthelmintic activity of the extracts.

For *in vivo* tests, FEC was log (x + 10) transformed to stabilize variance. SAS® was used to analyze FEC and blood variables through proc mixed using repeated measures. Pairwise comparisons of significant main effect were evaluated with Tukey's test. Differences were considered significant when $p \leq 0.05$.

Table 2
Total phenols, total tannins, and condensed tannins from leaf, pulp and seed extracts and powdered fruit (pulp + seed powder) of *T. catappa*.

SAMPLE	Total phenols[a]	Total tannins[a]	Condensed Tannins[b]
Leaf extract	612.26	586.19	4.61
Pulp extract	361.18	298.93	183.92
Seed extract	311.39	282.84	35.13
Pulp + seed powder	72.97	57.16	33.24

[a] values expressed in grams of tannic acid/kg of dry matter.
[b] values expressed in grams of leucocyanidin/kg of dry matter.

3. Results

3.1. Phytochemicals present in the extracts and whole fruit

Plant samples were dried, grinded and extracted with 70% acetone in water. The solvents were evaporated to dryness and samples were redissolved to desired concentration with 2% DMSO in distilled water. The acetone extracts of pulp and seed contained higher concentrations of phenols, total tannins and condensed tannins than the dried (unextracted) whole fruit (pulp + seed) powder. Tannins (g/tannic acid/Kg. DM) were analyzed according to Makkar et al. (1993) and condensed tannins (g/leucocyanidin/Kg. DM) were done according to the methodology of Porter et al. (1986). Leaf extracts had at least twice the amount of total tannins and total phenols than pulp and seed extract, although leaf extract was poor in condensed tannins (Table 2). In qualitative analysis, extracts presented alkaloids, flavonoids, saponins, phenols, tannins and terpenoids (Table 4). Analysis of whole fruit did not detect alkaloids or steroids and condensed tannins was at 33.24 g/leucocyanidin/Kg. DM.

3.2. In vitro effects of extracts

Seed extract had the lowest LC_{50} with values similar to

Table 3
Lethal concentration (LC_{50}) and fiducial limits of seed, pulp, leaf extracts of *T. catappa* and Thiabendazole. Control, PVPP (60 µg/mL), extracts (20 µg/mL) + PVPP (60 µg/mL) (1:3 ratio) evaluated in egg hatch assay with *H. contortus* eggs.

SAMPLE	LC_{50} (fiducial limits)
Seed extract	2.48 µg/mL (2.37 µg/mL–2.60 µg/mL)
Pulp extract	4.62 µg/mL (4.40 µg/mL–4.48 µg/mL)
Leaf extract	20 µg/mL (18 µg/mL–21 µg/mL)
Thiabendazole	1.31 µg/mL (1.18 µg/mL–1.45 µg/mL)

SAMPLE	Percentage of hatching (st.dev)
PVPP	98.03 (1.27)
Seed extract + PVPP	0
Pulp extract + PVPP	80.29 (4.98)
Leaf extract + PVPP	68.97 (4.63)

Table 4
Qualitative phytochemical screening of leaf, pulp and seed extracts and dried and grinded fruit (pulp + seed powder) of *T. catappa*.

	Leaf extract	Pulp extract	Seed extract	Pulp + seed powder
Alkaloids	+	+	+	−
Flavonoids	+	+	+	+
Saponins	+	+	+	+
Steroids	−	−	−	−
Phenols	+	+	+	+
Terpenoids	+	+	+	+
Tannins	+	+	+	+

+ present; − absent.

Thiabendazole. Pulp and leaf extracts also had low LC_{50}. All the extracts at 20 μg/mL were added of a tannin chelating agent poly vinyl poly pirrolidone (PVPP) at 60 μg/mL (1:3 ratio). Although seed extract had 0% egg hatching, pulp extract presented 80.29% and leaf extract 68.97% hatchability (Table 3). Negative control with PVPP at 60 μg/mL presented 98.03% egg hatching.

3.3. Effect of Terminalia on parasitological parameters (FEC, hematocrit, body weight, in vitro hatchability post treatments)

The administration of 2 g *Terminalia*/kg/BW once a day for five consecutive days had no effect on either the fecal egg count nor on the hatchability of eggs post treatments. Similar hematocrit and body weight were found between animals treated with *T. catappa* pulp + seed powder and animals in the control group (Table 5).

3.4. Effect of Terminalia fruit in toxicity tests

Urea, creatinine, and aspartate aminotransferase were evaluated after treatments with *T. catappa* and no differences were found between groups of treated and control animals (Table 6).

4. Discussion

Many authors reported plant tannins to be effective against GIN (Min et al., 2004; Alonso-Díaz et al., 2008; Oliveira et al., 2011; Hoste et al., 2006; Paolini et al., 2003; Brunet et al., 2008; Athanasiadou et al., 2001). *Terminalia* (Indian almond) is a very common tree in tropical countries, easily recognized by its wide leaves and typical almond-shaped fruits. The validation of its use for parasites control could make the plant an adjuvant in the control of worms that are counterproductive in small ruminant production.

Phytochemical analyses of leaves, pulp and seeds of *T. catappa* indicated the presence of alkaloids (leaf only), flavonoids, saponins, phenolics, terpenoids, and tannins. Regarding tannins, although our phytochemical tests indicated 18.4% condensed tannins (CT) in pulp extract, CT were present in 3.5% or less in leaves and seeds. The percent of total tannins and total phenols indicate that the majority of tannins present in *Terminalia catappa* are hydrolyzable tannins (HT). Plants produce HT as ellagitannins or gallotannins. Fruits of three *Terminalia* species were rich in mostly ellagitannins (Moilanen et al., 2013; Pfundstein et al., 2010). Although the literature is rich in reports on the anthelmintic effects of CT, it is poor on the study of HT on GIN (Spiegler et al., 2017). There is even fewer reports that discriminate CT and HT in browse plants and their anthelmintic effects. Katiki et al. (2013) reported that plant extracts that contain both CT and HT, or mainly HT were more lethal to *C. elegans* than *Lespedeza cuneata* (mostly CT). Both ellagitanins and gallotannins are rich in ellagic and gallic acid, both containing moieties with three OH groups, while procyanidins (a type of CT) have moieties with only two −OH groups. Recently, CT with monomeric subunits that hydrolyze into prodelphinidins (three −OH groups per subunit) had better anthelmintic effects than CT that give rise to proanthocyanidins (two −OH groups per subunit). This clearly shows that CT that have subunits similar to HT have more potential to control GIN, as previously indicated by Katiki et al. (2013).

Terminalia pulp extract had approximately 5 times more condensed tannins than the seed extract and about 45 times more condensed tannins than the leaf extract. *In vitro* egg hatch assay were used to screen the extracts and indicated that pulp and seed extracts had higher ovicidal activity compared to leaf extract. Interestingly, even after addition of PVPP, seed extracts at 20 μg/mL still reduced egg hatching in 100% (Table 3). Considering that PVPP chelates tannins, phenolics, and flavonoids, the seed extracts had other anthelmintic compounds not chelated by PVPP. Seed extracts also had alkaloids, saponins, and terpenoids, all of which can be extracted with 70% acetone and may have

Table 5
Mean (st. dev.) of packed cell volume (hematocrit), fecal egg count (FEC), percentage of hatching (EHA) after treatments and weight of lambs infected with multidrug-resistant *H. contortus* and treated with *T. catappa* pulp + seed powder 2 g/kg/BW/5 days, evaluated before treatment, 1, 2, 3 and 4 weeks after treatments.

	Hematocrit (%)		FEC		EHA		Weight (Kg)	
	Control	*Terminalia*	Control	*Terminalia*	Control	*Terminalia*	Control	*Terminalia*
before treat.	29.1(3.2)	30.0 (2.2)	1133 (856)	937 (718)	–	–	21.9 (3.4)	22,9 (3.6)
1 week	–	–	2767 (3605)	2100 (2045)	78.7 (4.6)	83.7 (7.7)	–	–
2 weeks	29.0 (2.7)	27.7 (2.4)	1850 (1001)	1606 (1552)	93.2 (2.2)	93.1 (3.0)	–	–
3 weeks	30.0(2.8)	30.1 (2.6)	2458 (2339)	3,318.75 (3325)	83.8 (10.8)	91.8 (3.5)	–	–
4 weeks	30.8 (1.9)	31.0 (2.2)	2467 (1696)	2606 (2510)	94.1 (3.8)	93 (3.4)	24.4 (3.0)	24.4 (4.0)
Average overall	29.71 (0.4)	29.75 (0.2)	2114 (345)	2135 (399)	87.4(5.3)	90.4(4.4)	23.6 (0.9)	23.1 (1.0)

Table 6
Mean (st. dev.) of urea, creatinine, and aspartate aminotransferase (AST) of lambs artificially infected with multidrug-resistant *H. contortus* before treatment with *T. catappa* fruit, 2 and 4 weeks after treatment.

	Urea(mg/dL)		Creatinine (mg/dL)		AST (UI/L)	
	Control	*Terminalia*	Control	*Terminalia*	Control	*Terminalia*
Before	22.1 (7.6)	30.1 (7.4)	1.2 (0.1)	1.1 (0.3)	113.2 (15.1)	116.1 (18.2)
2 weeks	29.8 (7.4)	34.6 (6.8)	1.4 (0.2)	1.2 (0.2)	109.7 (13.8)	115 (22.1)
4 weeks	22.1 (4.7)	28 (9.6)	1.3 (1.8)	1.3 (0.2)	100.7 (13.1)	86.6 (35.9)

Reference values for Urea (17.12–42.8 mg/dL), Creatinine (1.2–1.9 mg/dL) and AST (60–280 UI/L). Kanekoet al. (1997); Mayes and Harvey (2004).

acted (alone or together) to inhibit egg hatching. In addition, seeds of *T. catappa* are a rich source of oleic (31.5%) and linoleic (29%) acids (Matos et al., 2009), also extractable with 70% acetone. Although we found no report of anthelmintic plant fatty acids, Stadler et al. (1994) reported that, among the fatty acids isolated from basidiomycetes and tested on *C. elegans*, the most active were coriolic acid and linoleic acid with LD_{50} values ranging from 5 to 10 mg/mL. Although less active than seed and pulp extracts, the leaf extract also had good *in vitro* anthelmintic activity (LC_{50} = 0.02 mg/ mL) compared to other extracts cited in the literature. Maciel et al. (2006) reported a LC_{50} = 0.36 mg/ mL for the ethanolic extract of *Melia azadirach*; Kamaraj et al. (2011) reported 2.9 mg/mL for metanolic extract of *Andrographis paniculata;* Kamaraj and Rahuman (2011) reported a LC_{50} = 4.25 mg/mL for the acetone extract of *Terminalia chebula* seeds in the egg hatch assay.

Terminalia catappa leaves have twice the concentration of total phenols and total tannins compared to pulp and seed extracts. Its leaves have high concentrations of the hydrolysable tannins (HT) gallic acid, ellagic acid, terflavins A, terflavins B and C, tergallagin, tercatain, teratin, punicalin, punicalagin, chebulagic acid, geraniin, granatin B, and corilagin (Fahmy et al., 2015). Among them, punicalagin is the major leaf HT (Tanaka et al., 1986). Leaves also contain the phenolic acids ferulic, vanillic, coumaric, and hydroxybenzoic and the flavonoids orientin, issorientin, vitexin, isovitexin, galloylvitexin, gallocatechin, epicatechin, apigallocatechin, galloyl-epigallocatechin (Fahmy et al., 2015). Engström et al. (2016) evaluated HT for its ovicidal activity and found that among 33 HT tested, geraniin (also present in *T. catappa* leaves) had an average of 27% activity in EHA (the highest dose evaluated was 2.0 mM).

Because other plant secondary compounds, such as rutin (Barrau et al., 2005) may exhibit anthelmintic activity, the tannin chelator PVPP was added to the extracts. PVPP chelates and inactivates not only tannins, but also phenolic compounds and flavonoids (Makkar et al., 1993; Doner et al., 1993). In fact, when PVPP was added to the flavonoid rutin *in vitro*, it presented similar results to the negative control (Barrau et al., 2005). In our study, extracts of leaves, pulp and seed at 20 μg/mL added of 60 μg/mL PVPP presented total or partial ovicidal activity. Barrau et al. (2005) and Vargas-Magaña et al. (2014) found similar results when PVPP was added to tannin-rich extracts, suggesting that the interactions between tannins and other secondary compounds affect *H. contortus* eggs.

Although *in vitro* results are not always confirmed *in vivo*, sometimes the effort is rewarded. For instance, the alkaloid nicotine, present in crude extracts of *Nicotiana tabacum* was appointed as the possible anthelmintic agent in both *in vitro* and *in vivo* studies (Iqbal et al., 2006). These authors reported significant increase in mortality of adult *Haemonchus in vitro* at 25 mg/mL of the extract. *In vivo*, the crude methanolic extract at 3 g/kg BW for five days reduced FEC of sheep naturally infected with GIN (including *Haemonchus*) in 73%, while the crude aqueous extract reduced FEC in 49%.

Regarding the *in vivo* evaluation, *T. catappa* whole fruit powder was administered for 5 days leading the animals to restrict their consumption of powdered fruit due to the high astringency of the powder and due to the dose of 2 g/kg BW (or approximately 44 g/animal/day). However, animals did consume the whole dose by mixing the fruit powder with soybean meal in a cleaned feeder. After daily dose consumption, they received *Cynodon* hay forage hay. However, *in vivo* tests did not validate our *in vitro* results when animals were fed the whole fruit (pulp + seed) powder at 2 g/kg LW for 5 days. It has been known that small ruminants can degrade HT in their rumen (McSweeney et al., 2001; Nelson et al., 1995). Recently, goats fed oak leaves had bacterial isolates that degraded HT, such as gallic acid in the rumen (Kumar et al., 2014). More interestingly, although gallic acid suffered some degradation at pH 2–3, its degradation was almost complete after 90 min under pH = 5 and pH = 7 (Benitez et al., 2005). This strongly indicates that HT can be degraded not only by rumen microorganism, but also by the rumen neutral pH (6.8). Thus, animals adapted to diets rich in HT will use tanniferous plants for their nutrition, but most HT may degrade in the rumen before they can exert significant anthelmintic affect. That problem could be circumvented by tannin microencapsulation to resist rumen degradation.

Regarding a possible post-treatment effect from *T. catappa*, egg hatch in feces was evaluated and no anthelmintic effect was observed through the egg hatch assay. Our results were contrary to Max (2010) who found that wattle tannins (1.3 g/kg. BW/day, during 3 consecutive days) reduced both worm burden and hatchability of eggs 6–9 days after oral treatments.

The hematocrit was evaluated before, 2, 3 and 4 weeks after treatment considering that *Haemonchus* infection may cause severe anemia. Some studies with natural products to control parasites demonstrated that phytochemicals sometimes do not kill GIN immediately, but may increases host resilience, prevent FEC from escalating, and maintain hematocrit of infected animals (Cala et al., 2014). These authors reported that both artemisinin-treated and levamisole groups had similar hematocrit, which were within the normal range for sheep, animal body weights were not affected, and infected-untreated sheep had FEC twice as high as artemisinin-treated sheep. Some natural products may have phytochemicals that may cause kidney and/or liver damage depending on the administered dosage. Thus, during the experiment, we evaluated urea and creatinine levels to detect changes in kidney function, and aspartate aminotransferase (AST) to detect liver changes. These parameters were all at normal levels for the species.

Pulp + seed powder had 3.3% CT, 5.7% total tannins, and 7.3% total phenols. Total phenols and total tannins fraction represent the concentration of HT and non-tannin phenolics (e.g., flavonoids). Thus, the leaf extract was the highest in total phenols (61%), total tannins (58.6%), but the lowest in CT (0.46%). The leaf extract was the next highest source of both HT and CT (Table 2). Their high content of HT of the ellagitannin type also confers a high antioxidant capacity, as previously shown (Katiki et al., 2013).Thus, leaf extract should both be tested *in vivo* for their potential anthelmintic activity.

5. Conclusion

Terminalia extracts made with 70% acetone had excellent *in vitro* activity, with seed extracts having the best anthelmintic activity. Besides tannins, phenolics and flavonoids (chelated by PVPP) other plant secondary compounds in plant tissues, such as fatty acids, saponins, alkaloids, and terpenoids may also have contributed to the anthelmintic activity *in vitro*. However, powdered whole fruit had no anthelmintic effect in sheep infected with multidrug-resistant *H. contortus*. Leaf aqueous acetone extracts had the highest concentrations of total tannins and phenols, but lowest concentrations of CT, indicating that leaves are the richest sources of HT and phenolics in *T. catappa* and should be tested *in vivo*. However, the problem of bacterial and ruminal pH degradation have to be addressed, regardless of the plant material used.

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