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Short communication

# Anthelmintic and cytotoxic activities of extracts of *Markhamia obtusifolia* Sprague (Bignoniaceae)

F. Nchu[a,b,c,*], J.B. Githiori[c], L.J. McGaw[c], J.N. Eloff[c]

[a] Department of Horticultural Sciences, Faculty of Applied Sciences, Cape Peninsula University of Technology, P.O. Box 652, Cape Town 8000, South Africa
[b] Percy FitzPatrick Institute of African Ornithology, DST/NRF Centre of Excellence, University of Cape Town, Private bag X03, Rondebosch 7701, Cape Town, South Africa
[c] Phytomedicine Programme, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa

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ABSTRACT

The anthelmintic activity of *Markhamia obtusifolia* Sprague (Bignoniaceae) leaf extracts was evaluated against the ruminant gastrointestinal nematode parasite *Trichostrongylus colubriformis* (Nematoda: Strongylida) using the *in vitro* egg hatch test. Also, the cytotoxic activity of aqueous extracts of *M. obtusifolia* was evaluated in cell line cytotoxicity assays. The results indicated that the effective concentration ($EC_{50}$) for the water extract of *M. obtusifolia* leaves (0.46 mg/mL; Confidence Interval [CI] 0.3–0.5 mg/mL) was significantly lower than the $EC_{50}$ for the acetone extract of *M. obtusifolia* (0.8 mg/mL; CI 0.7–1 mg/mL). Aqueous extracts were twice as potent as the acetone extracts. The $EC_{90}$ (0.2 mg/mL; CI 0.1–0.02) for thiabendazole (positive control) was significantly lower than the $EC_{90}$ for the water extract of *M. obtusifolia* (10.7 mg/mL; CI 8.3–13.7 mg/mL). In the cytotoxicity bioassay, the lethal concentration ($LC_{50}$) for the aqueous extract of *M. obtusifolia* was 0.476 mg/mL, which was relatively high (low toxicity) in comparison to the highly toxic berberine ($LC_{50}$ = 9.80 μg/mL). The current study showed that *M. obtusifolia* plant extracts possess anthelmintic activity and are relatively non-cytotoxic, thus providing support for their use in traditional veterinary practices.



## 1. Introduction

In most developing countries, gastrointestinal nematodes impact negatively on livestock production (Satrija et al., 2001). Gastrointestinal parasites cause clinical and subclinical infections that reduce animal survival and depress growth rates, wool and milk production, and reproductive performance (Alawa et al., 2010). Parasitism has been ranked high among factors that threaten livestock production worldwide (Perry et al., 2001).

Moreover drugs needed for the treatment of endoparasites of humans or livestock are usually expensive and often out of reach for the populations of developing countries (Amin et al., 2009). Furthermore, there is rapid development of anthelmintic resistance (Melo et al., 2003). According to Alawa et al. (2003), declining funding and rising costs of veterinary services cause difficulties in access to these services by resource-poor communities. Alternative methods of control are thus required that are both practical and economical for farm production systems. Anthelmintics derived from plant parts that are used traditionally for treatment of parasitic infections in human and animals may offer an alternative to minimize some of these problems (Akhtar et al., 2000). In addition, the growth

* Corresponding author at: Department of Horticultural Sciences, Faculty of Applied Sciences, Cape Peninsula University of Technology, P.O. Box 652, Cape Town 8000, South Africa. Tel.: +27 214603196; fax: +27 214603193.
*E-mail address:* felixnchu@gmail.com (F. Nchu).

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of organic livestock farming globally, which is claimed to be less toxic to man and the environment might favor the continuous use of traditional medicinal plants in Africa for treatment of endoparasitic infections caused by intestinal worms.

Suitable anthelmintic bioassays are being used to evaluate extracts from different plant species in order to validate some of the traditional claims about their efficacy. For example, the species *Vernonia amygdalina* Delile (Asteraceae) and *Annona senegalensis* Pers (Anonnaceae) are used by local livestock farmers in Nigeria as anthelmintics and their aqueous extracts showed *in vitro* anthelmintic activities (Alawa et al., 2003). *Spilgelia anthelmia* L. (Loganiaceae), which is used for the treatment of gastrointestinal helminthiasis in folk medicine of Brazil, Panama and Costa Rica also showed *in vitro* ovicidal and larvicidal activity against *Haemonchus contortus* Cobb (Nematoda: Strongylida) (Assis et al., 2003).

Even though the use of herbal remedies to treat animal diseases is widespread, there is a possible threat of herbal preparations being toxic (Van der Merwe et al., 2001; Masika et al., 2000). Plant extracts may simultaneously have high anthelmintic activities and high non-selective cytotoxic activities. Results from numerous studies suggest that tannin components in plant extracts have anthelmintic and cytotoxic activities (Takechi et al., 1985; Bizimenyera et al., 2006; Jiang et al., 2008; Ramalingam et al., 2010). Tannins have been linked to a case of poisoning in avian species (Kinde, 1988). Methods such as cell line cytotoxicity assays are widely used for determining toxic effects of plant extract *in vitro* (McGaw et al., 2007).

*Markhamia obtusifolia* is a perennial shrub belonging to the family Bignoniaceae, and grows naturally in southern and eastern Africa (Germishuizen and Meyer, 2003). Ethnomedically, roots of *M. obtusifolia* are used for the treatment of hookworm in parts of Tanzania (Chhabra and Mahunnah, 1994). However, no experimental evidence of the anthelmintic activity of the species has been found in the available literature. This study was carried out to validate the anthelmintic activity of *M. obtusifolia* on *Trichostrongylus colubriformis* (Nematoda: Strongylida) by evaluating efficacy of extracts of the plant to inhibit egg hatching. The *in vitro* cytotoxic effects of extracts were also determined to investigate the possible relationship between cytotoxicity and anthelmintic efficacy.

## 2. Materials and methods

### 2.1. Plant material

Fresh leaves of *M. obtusifolia* were harvested from the Lowveld National Botanical Gardens (Nelspruit), South Africa in April 2005. The origin of the tree is recorded in the database of the garden's herbarium with number 15/94. Leaves were dried under shade at room temperature (21–27 °C) for a period of 1 month. The dried leaves were ground using a Jankel and Kunkel Model A 10 mill into fine powder.

### 2.2. Plant extraction

#### 2.2.1. Preparation of plant material and extraction

The powdered leaf material (2 g) was separately extracted with acetone and water (20 mL) in a centrifuge tube by shaking for 15 min and centrifuging at 300 × *g* for 5 min. The insoluble materials were separated from the supernatant by filtering through Whatman No. 1 filter paper. Extraction was repeated two times per extractant by replacing solvent after each extraction. The combined filtrate obtained following acetone extraction was transferred into a pre-weighed glass beaker and the solvent evaporated by a stream of air at room temperature. Dry aqueous extract was obtained by subjecting the combined aqueous suspension to freeze-drying.

### 2.3. Egg preparation

Egg recovery and preparation was based on the procedure described by Bizimenyera et al. (2006). Faeces were collected from lambs with monospecific infections of *T. colubriformis*. The *T. colubriformis* strain was highly susceptible to anthelmintic agents and was provided by Dr Jan van Wyk from the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria. The lambs were housed on a concrete floor indoors, fed hay and commercial concentrate pellets and had free access to water. This study was approved by the Animal Ethics Committee, University of Pretoria.

### 2.4. Egg hatch assay

The *in vitro* egg hatch assay was based on the method described by Coles et al. (1992) with slight modifications. It is used to test ovicidal effects of plant extracts (Ademola et al., 2004; Alawa et al., 2003; Assis et al., 2003; Molan et al., 2003). Briefly, approximately 100 eggs in 200 μL of egg suspension were pipetted into each well of a 48-well microtitre plate. In one set of test wells, 200 μL of the acetone plant extract in a mixture of acetone and water (1:3) in concentrations of 50, 10, 2, 0.5, 0.4, 0.08 mg/mL were added. One hundred percent (100%) acetone was found to be toxic to *T. colubriformis*. A similar volume (200 μL) of aqueous plant extract in distilled water at concentrations of 100, 10, 1, 0.2, 0.04 and 0.008 mg/mL was added to a second set of wells. There were three replicates per concentration. Thiabendazole (Sigma®) in sterile distilled water solution with 25% acetone at concentrations of 25.8, 5.16, 1.032, 0.2064, 0.04128 and 0.008256 μg/mL in triplicate was used as a positive control while the diluents distilled water or the acetone–water mixture (1:3) were negative controls. The plates were incubated under humidified conditions at ambient temperature (23 °C) for 48 h. A drop of Lugol's iodine solution was added to each well to stop further hatching, and all the unhatched eggs and $L_1$ larvae in each well were counted. Percent inhibition of egg hatching was calculated using the following equation (Rabel et al., 1994):

$$\%\text{inhibition} = \frac{\text{number of eggs exposed} - \text{the number of eggs hatched}}{\text{number of eggs exposed}} \times 100.$$