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Anthelmintic effect of plant extracts containing condensed and hydrolyzable tannins on *Caenorhabditis elegans*, and their antioxidant capacity

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A B S T R A C T

Although tannin-rich forages are known to increase protein uptake and to reduce gastrointestinal nematode infections in grazing ruminants, most published research involves forages with condensed tannins (CT), while published literature lacks information on the anthelmintic capacity, nutritional benefits, and antioxidant capacity of alternative forages containing hydrolyzable tannins (HT). We evaluated the anthelmintic activity and the antioxidant capacity of plant extracts containing either mostly CT, mostly HT, or both CT and HT. Extracts were prepared with 70% acetone, lyophilized, redissolved to doses ranging from 1.0 mg/mL to 25 mg/mL, and tested against adult *Caenorhabditis elegans* as a test model. The extract concentrations that killed 50% (LC_{50}) or 90% (LC_{90}) of the nematodes in 24 h were determined and compared to the veterinary anthelmintic levamisole (8 mg/mL). Extracts were quantified for CT by the acid butanol assay, for HT (based on gallic acid and ellagic acid) by high-performance liquid chromatography (HPLC) and total phenolics, and for their antioxidant activity by the oxygen radical absorbance capacity (ORAC) assay. Extracts with mostly CT were *Lespedeza cuneata*, *Salix X sepulcralis*, and *Robinia pseudoacacia*. Extracts rich in HT were *Acer rubrum*, *Rosa multiflora*, and *Quercus alba*, while *Rhus typhina* had both HT and CT. The extracts with the lowest LC_{50} and LC_{90} concentrations, respectively, in the *C. elegans* assay were *Q. alba* (0.75 and 1.06 mg/mL), *R. typhina* collected in 2007 (0.65 and 2.74 mg/mL), *A. rubrum* (1.03 and 5.54 mg/mL), and *R. multiflora* (2.14 and 8.70 mg/mL). At the doses of 20 and 25 mg/mL, HT-rich, or both CT- and HT-rich, extracts were significantly more lethal to adult *C. elegans* than extracts containing only CT. All extracts were high in antioxidant capacity, with ORAC values ranging from 1800 μmoles to 4651 μmoles of trolox equivalents/g, but ORAC did not correlate with anthelmintic activity. The total phenolics test had a positive and highly significant ($r = 0.826$, $p \leq 0.01$) correlation with total hydrolyzable tannins. Plants used in this research are naturalized to the Appalachian edaphoclimatic

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conditions, but occur in temperate climate areas worldwide. They represent a rich, renewable, and unexplored source of tannins and antioxidants for grazing ruminants, whereas conventional CT-rich forages, such as *L. cuneata*, may be hard to establish and adapt to areas with temperate climate. Due to their high *in vitro* anthelmintic activity, antioxidant capacity, and their adaptability to non-arable lands, *Q. alba*, *R. typhina*, *A. rubrum*, *and R. multiflora* have a high potential to improve the health of grazing animals and must have their anthelmintic effects confirmed *in vivo* in both sheep and goats.

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1. Introduction

Gastrointestinal nematodes (GIN) lead to reductions in growth, weight gain, and carcass value, resulting in economic losses and death in small ruminants (Stear et al., 2007). Although commercial anthelmintics allowed the development of intensive ruminant grazing systems, major strongylid nematodes (including *Haemonchus contortus*) developed resistance to most commercial anthelmintics in all main goat- and sheep-producing regions of the world (Bartley et al., 2004; Besier, 2007). *In vitro* screening of potential new anthelmintic leads is the first line of action to find alternatives to failing anthelmintics. Although different nematodes have been used, the free-living soil nematode *Caenorhabditis elegans* is a model that has been used worldwide to screen plant extracts (McGaw et al., 2007; Waterman et al., 2010), purified fractions, and plant compounds (Smith et al., 2009). *C. elegans* has also been used to establish the mode of action of new anthelmintics, with results replicated in sheep infected with drug-resistant *H. contortus* (Bull et al., 2007, Kaminsky et al., 2008).

Plant-derived anthelmintic compounds should have low or no toxicity to animals, low impact on the environment, and leave no undesirable residues in animal products. As such, condensed tannins (CT) have been extensively tested for their anthelmintic effect and nutritional benefits (Iqbal et al., 2002; Mueller-Harvey, 2006), but other plant products may also have anthelmintic activity. Hydrolyzable plant tannins (HT) have not received similar attention because they are widely believed to be toxic to animals. Current literature indicates that consumption of large amounts of both CT and HT by ruminants may reduce feed intake, feed digestibility and nitrogen retention, fecal output, and cause toxicity, while neither is toxic if consumed in moderation (Frutos et al., 2004; Hervás et al., 2003). Tolerance to tannins varies with ruminant species, and may increase with a high-protein diet (Makkar, 2003), while tannin-binding proteins may also influence animal tolerance.

The conventional CT-rich forages sericea lespedeza (*Lespedeza cuneata*), birdsfoot trefoil (*Lotus corniculatus*), and sulla (*Hedysarum coronarium*) have been evaluated for their anthelmintic activity. However, tree leaves may also be rich in CT. Lambs infected with mixed GIN, and fed willow (*Salix* sp.) leaves had reduced worm burdens compared to untreated lambs kept on control pasture (Diaz Lira et al., 2008), or on alfalfa (*Medicago sativa*) (Mupeyo et al., 2011). Trees and shrubs can also be rich sources of HT with antibacterial (Funatogawa et al., 2004) and antiparasitic (Kolodziej et al., 2001; Shuaibu et al., 2008) effects. For instance, ethanolic extracts of red oak (*Quercus rubra*) containing high levels of tannins had anthelmintic effect *in vitro* against both third stage larvae (L_3) and adult *H. contortus* (Hoste et al., 2006). Tannin fractions of *Quercus petraea* bark inhibited *C. elegans* reproduction and motility with IC_{50} = 125 μg/mL (CT fraction) and IC_{50} = 500 μg/mL (ellagitannins and other HT tannins), while mebendazole had IC_{50} = 10 μg/mL (König et al., 1994).

Other compounds including terpenes, saponins, alkaloids and the antioxidant flavonoids and phenolic compounds also may have anthelmintic effects. The flavonoids gallocatechin and epigallocatechin (prodelphinidins) inhibited both egg hatching and larval development of *Trichostrongylus colubriformis* (Molan et al., 2003). The greater anthelmintic effect of prodelphinidins (containing gallic acid) compared to procyanidins (no gallic acid) was confirmed using the larval exsheathment assay with *H. contortus* and *T. colubriformis* (Brunet and Hoste, 2006). Interestingly, flavonoids containing gallic acid units have high antioxidant capacity (Rice-Evans et al., 1997), which may contribute to their anthelmintic activity. The antioxidant flavonoids narigenin and hesperidin have been found to have both *in vitro* and *in vivo* activity against adults of the filarioid nematode, *Brugia malayi* (Lakshmi et al., 2010). Polyflavonol antioxidants are also tannins, which have the additional benefit of increasing protein absorption. Thus, the beneficial effects of tannin-rich forages on the immune system reported by some researchers might be a combined effect of the flavonoids, tannins and of the increased protein in their diet (Athanasiadou and Houdijk, 2010).

Some unconventional forages rich in tannins, crude protein, and antioxidants that merit testing for anthelmintic activity, are also palatable to ruminants. Goats consumed over 50% of their daily diet in *Rhus microphilla* (littleleaf sumac) (Rhee et al., 2000) and 25–75% black locust (*Robinia pseudoacacia*), although black locust caused reduced feed intake (Snyder et al., 2007). Small ruminants and cattle consume multiflora rose (*Rosa multiflora*), although goats eat it more avidly than other ruminants (Luginbuhl et al., 1998). Different oak species (*Quercus* sp.) have been fed, as leaves or fruits, to small (Manolaraki et al., 2010) and large ruminants (Sharma et al., 2008) without refusal and with anthelmintic and nutritional benefits, respectively.

Our objectives were to evaluate leaf extracts from plants naturalized to the Appalachian region for their (1) content of CT and HT, (2) antioxidant activity (expressed as total phenolics and oxygen radical absorbance capacity-ORAC), and (3) anthelmintic activity *in vitro*.

We tested extracts of staghorn sumac (*Rhus typhina*, Anacardiaceae), black locust (*R. pseudoacacia*, Fabaceae