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Urban et al. J. Anim.Plant Sci Antihelmintic Effects of Czech Plant extracts.

In vitro motility inhibition effect of Czech medicinal plant extracts on *Chabertia ovina* adults

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1 ABSTRACT
Although chabertiasis causes great economic damage to sheep farms worldwide, a limited number of studies have focused on development of antihelmintic agents effectively inhibiting *Chabertia ovina (C. ovina).* In this study, ethanol extracts of 16 Czech medicinal plants were tested for their potential *in vitro* antihelmintic activity against *C. ovina* using adult motility inhibition assay. Values of half maximal inhibitory concentration (IC_{50}) were determined for 6, 24 and 48 hour exposure at extract concentrations 0.25, 0.5, 1, and 2 mg/ml. After 6 hours, extracts of *Daucus carota* (wild carrot), *Satureja hortensis* (summer savoury), *Valeriana officinalis* (valerian), *Dryopteris filix-mas* (male fern), *Artemisia absinthium* (absinthe wormwood), *Juglans regia* (common walnut), *Hedera helix* (common ivy) and *Inula helenium* (elecampane) were more effective than positive control albendazole, with IC_{50} values 0.57, 1.15, 1.32, 1.34, 1.35, 1.60, 1.66 and 1.68 mg/mL, respectively. At 24-hour exposure IC_{50} of all extracts had significantly decreased, however, only *A. sativum*, *D. carota*, *V. officinalis* and *Tanacetum vulgare* (tansy) possessed stronger motility inhibitory effect (IC_{50} ranging from 0.30 to 0.65 mg/ml) than albendazole. All plants tested totally inhibited *C. ovina* motility at lower concentration tested (0.25 mg/mL) after 48 hours. Because of this test, the best antihelmintic activity against *C. ovina* was observed from *A. sativum*, *D. carota* and *V. officinalis*, which suggests these extracts as prospective materials for further development of novel plant-based antihelmintics against *C. ovina*. However, detailed analysis of their chemical composition and *in vivo* activity should be carried out in order to validate their antihelmintic character and verify their possible practical use.

2 INTRODUCTION
The strongylid nematode infections are known to cause great economic damage to sheep farms, especially in lambs, where they are associated with lowered live weight, reduced growth rates, occurrence of diarrhoea and increased mortality (Sweeny *et al.*, 2012). *Chabertia ovina* Fabricius (Chabertiidae) (large-mouthed bowel worm) belongs to the most

widely distributed nematodes worldwide causing severe damage to the mucosa of the colon with resulting congestion, ulceration, and small haemorrhages in sheep (Kahn and Line, 2010).Management and control of *C. ovina* in commercial sheep farms is critical because of income loss associated with reduced flock productivity. Synthetic drugs, such as benzimidazole, levamisole and albendazole are commonly used for its elimination (Wagland *et al.*, 1996; Waller *et al.* 1996; Sackett *et al.*, 2006). In the recent time, the growing problem of antihelmintic resistance of nematodes to synthetic drugs their expenses and negative impact on both animals and environment has led to development of alternative classes of antihelmintics (Waller, 1994; Besier and Love, 2003; Githiori *et al.*, 2006). Nowadays, several preparations based on plant-derived compounds or their semi-synthetic derivatives such as arecoline (*Areca catechu*), quisqualic acid (*Quisqualis indica*), santonin (*Artemisia maritima*) and artesunate (*Artemisia annua*) are used in veterinary medicine to treat nematode infections (Taylor, 2005; Dewick, 2009; Fathy, 2011).Taking into consideration relatively high incidence of *C. ovina* infections and the number of studies done in the field of antihelmintic properties of plants, only a small part is concerned with this *C. ovina*. With a few exceptions (Tariq *et al.* 2009; Silveira *et al.*, 2012), most of these available studies were using egg hatch assay (Borgsteede *et al.*, 1997; Al-Shaibani *et al.*, 2009). With the aim of presenting more accurate data on effectiveness of plant-derived agents against *C. ovina*, this study evaluated antihelmintic activity of 16 extracts using adult motility inhibition assay (AMI), which is the generally accepted as a standard method (Tritten et al, 2012).

3 MATERIALS AND METHODS

3.1 Plant materials: Plant species were selected according to their traditional use for treatment of parasitical infections recorded in the literature (Korbelar *et al.*, 1978) and their promising antihelmintic activity against nematodes *Ascaris suum* and *Trichostrongylus colubriformis* confirmed in our previous studies (Urban *et al.*, 2007, 2008). Different plant parts of *Allium sativum* L. (bulb), *Artemisia absinthium* L. (areal part), *Artemisia vulgaris* L. (areal part), *Carum carvi* L. (fruit), *Consolida regalis* Gray (flower), *Cucurbita pepo* L. (seed), *Daucus carota* L. (root), *Dryopteris filix-mas* (L.)Schott (rhizome), *Erigeron canadensis* L. (areal part), *Hedera helix* L. (leaf), *Inula helenium* L. (rhizome and root), *Juglans regia* L. (pericarp), *Satureja hortensis* L. (areal part), *Tanacetum vulgare* L. (areal part), *Thymus vulgaris* L. (areal part), and *Valeriana officinalis* L. (rhizome). These were collected from various areas of Pilsen (Domazlice and Tachov districts) and Prague regions in the Czech Republic from April to October 2005. Voucher specimens were authenticated and deposited at the Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague. Detailed description of tested plants (voucher specimen number, families and ethnobotanical data) is presented in Urban *et al.* (2008).

3.2 Preparation of extracts: The plants were dried at a room temperature (20–25°C). Appropriate plant parts were grounded (15 g) and then macerated with 80% ethanol (450 mL) for 5 days. The extracts were subsequently filtered and concentrated *in vacuo* at 40°C. The residue was dissolved in 20 μL dimethyl-sulfoxide (DMSO) and in 980 μL phosphate-buffered saline (PBS; pH 7.2, 0.15 M), creating concentration 2 mg/mL. All samples were stored at -20 °C until tested.

3.3 Adult motility inhibition assay: The antihelmintic effect of 16 plant extracts against *C. ovina* adults was measured using motility inhibition assay previously described by Hounzangbe-Adote *et al.* (2005). Three specimens of adult *C. ovina* worms per well were inserted into 24-well microtitration plates. The worms were first washed in PBS buffer solution (pH 7.2, 0.15 M) and incubated at room temperature for one hour. Washing solution was discarded and 1 mL of plant extract was added to each well at concentrations 0.25, 0.5, 1 and 2 mg/mL. Positive (albendazole) and negative (2% DMSO in PBS solution) controls were included on each plate. Samples were assayed in four independent experiments each performed in duplicate. The motility of adult worms was controlled and recorded after 6, 24 and 48 hours optically using binocular lens. After each observation motility inhibition index (MII) was calculated using following the formulae: MII (%) = [(T-M)/T] x 100, where M refers to mobile (living) worms and T to total worm count. Values of MIIs were further used to calculate half-maximal inhibitory concentration (IC_{50}). Results are therefore expressed as minimal concentration of plant extract needed to inhibit motility of 50% adult *C. ovina* worm population tested.

4 RESULTS

In this study performed with 16 plants whose selection was based on data suggesting their possible antihelmintic effect, ethanol extracts of eight species possessed significant activity against *C. ovina* adult worms using motility inhibition *in vitro* assay. Complete results are shown in Table 1.

Table 1: *In vitro* motility inhibition effect of Czech medicinal plant extracts on adult *Chabertia ovina*

Plant species	IC_{50}* (mg/mL)/time exposure (h)		
	6	24	48
Allium sativum	> 2	0.30 ± 0.04	< 0.25
Artemisia absinthium	1.35 ± 0.36	1.08 ± 0.27	< 0.25
Artemisia vulgaris	> 2	1.55 ± 0.22	< 0.25
Carum carvi	> 2	1.19 ± 0.40	< 0.25
Consolida regalis	> 2	> 2	< 0.25
Cucurbita pepo	> 2	1.40 ± 0.31	< 0.25
Daucus carota	0.57 ± 0.09	0.62 ± 0.11	< 0.25
Dryopteris filix-mas	1.34 ± 0.31	1.33 ± 0.17	< 0.25
Erigeron canadensis	> 2	> 2	< 0.25
Hedera helix	1.66 ± 0.38	1.32 ± 0.30	< 0.25
Inula helenium	1.68 ± 0.39	1.05 ± 0.26	< 0.25
Juglans regia	1.60 ± 0.41	1.01 ± 0.33	< 0.25
Satureja hortensis	1.15 ± 0.26	1.26 ± 0.39	< 0.25
Tanacetum vulgare	> 2	0.65 ± 0.09	< 0.25
Thymus vulgaris	> 2	1.82 ± 0.62	< 0.25
Valeriana officinalis	1.32 ± 0.36	0.63 ± 0.11	< 0.25
Albendazole**	1.92 ± 0.38	1.00 ± 0.33	< 0.25

* half maximal inhibitory concentration expressed as mean value ± standard deviation
**positive control

At 6 hour exposure, extracts of *D. carota, S. hortensis*, *V. officinalis*, *D. filix-max*, *A. absinthium*, *J. regia*, *H. helix* and *I. helenium* were more effective than positive control albendazole, with IC_{50} values 0.57, 1.15, 1.32, 1.34, 1.35, 1.60, 1.66 and 1.68 mg/mL, respectively. The rest of the plants exhibited no inhibitory activity (IC_{50} > 2 mg/mL). The motility inhibition effect of all extracts had significantly increased after 24 hours, however, only *A. sativum*, *D. carota*, *V. officinalis* and *T. vulgare* achieved stronger activity than positive control with IC_{50} values ranging from 0.30 to 0.65 mg/ml. The rest of the plant species had considerable weaker effect ($IC_{50} \geq$ 1.01 mg/mL). All plants tested totally inhibited *C. ovina* motility at lower concentration tested (0.25 mg/mL) after 48 hours. When IC_{50} values for all time expositions were compared to those of albendazole, the stronger activity was observed for extracts of *D. carota*, and *V. officinalis*, which were more effective than positive control at both 6 and 24 hours of exposure. The significant anti-chabertial effect of both plants is also illustrated in detail by comparison of their MIIs at concentration of 1 mg/mL with those of positive and negative controls (Figure 1.).

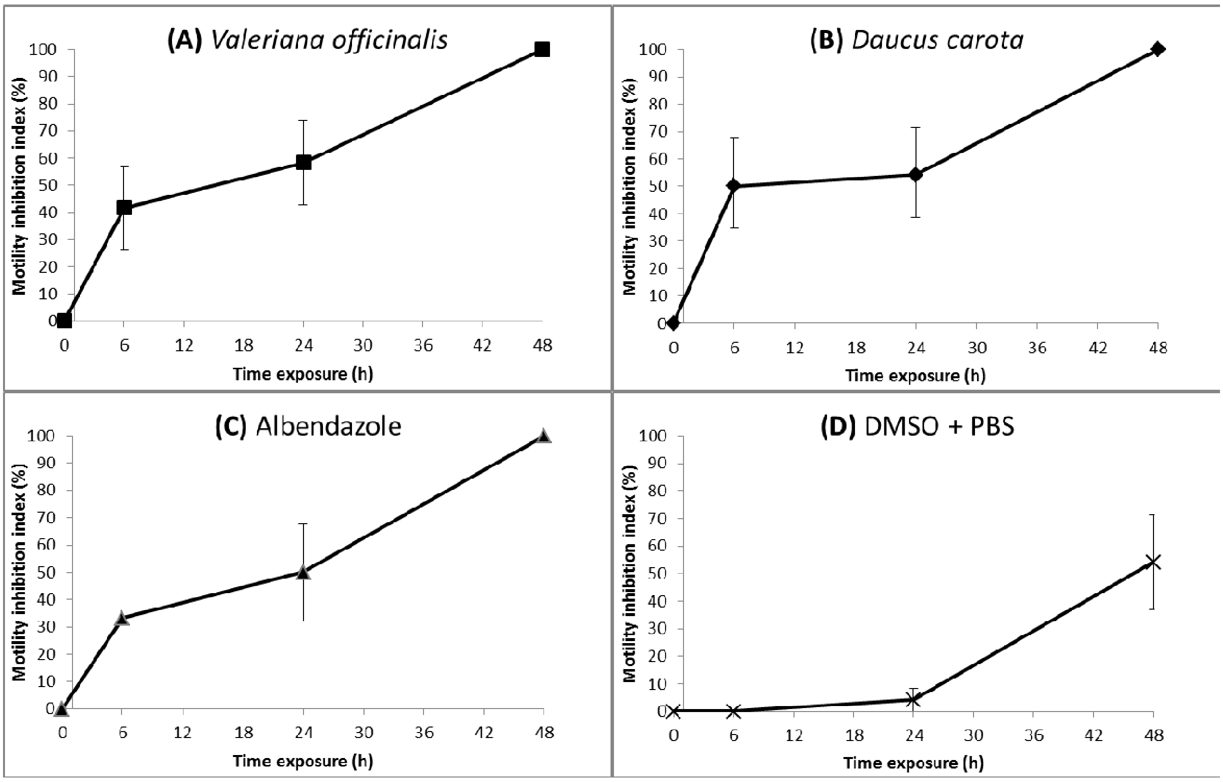

Figure 1: Motility inhibition indexes of plants (A and B) with the best anti-chabertial efficacy at concentration of 1 mg/mL in comparison to positive (C) and negative (D) controls

The stronger anti-chabertial effect than positive control has also been observed for extracts of *A. sativum*, *A. absinthium*, *C. carvi*, *D. filix-mas*, *H. helix*, *I. helenium*, *J. regia*, *S. hortensis* and *T. vulgare* for at least one of exposure times used. All plants tested totally inhibited motility of *C. ovina* at exposure of 48 hours; however, viability of tested nematodes decrease to 45.83% that significantly influenced observed antihelmintic effect of extracts tested.

5 DISCUSSION

As far as previously described, antihelmintic activity of the most effective plants tested in this study is considered, *A. sativum*, *A. absinthium*, *I. helenium J. regia* and *T. vulgare* was found to be effective against various parasites such as *Haemonchus contortus* (Squires *et al.*, 2011; Ahmed *et al.*, 2013), *Ascaris lumbricoides* (El Garhy and Mahmoud, 2002), *Eicinia foetida* (Kale *et al.*, 2011) and *Oesophagostomum* spp. (Magi *et al.*, 2005). In addition, plant extracts of *D. carota*, *V. officinalis* and *C. carvi* showed *in vitro* antihelmintic effects against *Ascaris suum* and

Trichostrongylus colubriformis in our previous study (Urban *et al.*, 2008). Despite the existence of above-mentioned reports on antihelmintic activity of *A. sativum*, *A. absinthium*, *C. carvi*, *D. carota*, *I. helenium*, *J. regia*, *T. vulgare* and *V. officinalis*, according to our best knowledge, this is the first report on *in vitro* motility inhibition effect of these plants against *C. ovina*. This study hypothesized which bioactive chemical constituents are present in the tested plant extracts and could be responsible for antihelmintic activity. *A. sativum* contains several sulfur based aliphatic compounds, whereas allicin is considered the most important. This compound has been reported to influence growth of various nematodes including *Ascaridia galli* and *Schistosoma mansoni* both *in vitro* and *in vivo* (Lima *et al.* 2011; Velkers *et al.*, 2011). Antihelmintic activity of artesunate (water-soluble semi-synthetic drug derived from artemisinin, a compound present in *A. absinthium*) was previously described by Fathy *et al.* (2011). Its detected high *in vivo* activity together with reported safety has been recommended for clinical use in humans for treatment of some trematode infections such as *Clonorchis sinensis*, *Fasciola hepatica* and *Schistosoma japonicum*. Limonene, major component of *C. carvi* essential oil (Dewick, 2009), has previously demonstrated significant antihelmintic activity against *Ascaridia galli* both *in vitro* and *in vivo* (Abdelqader *et al.*, 2012). According to this result, it is assumable that limonene can significantly contribute to the antihelmintic effect of *C. carvi* observed in this study. Fetterer and Fleming (1991) had previously described antihelmintic activity of juglone, a quinone compound of *J. regia* pericarp, against *Haemonchus contortus* and *Ascaris suum in vitro*. Therefore, it is highly probable that juglone is accountable for antihelmintic activity of *J. regia* extract. Several studies have discussed alantolactone and isoalantolactone, terpenoids of *I. helenium* root, as compounds responsible for antihelmintic activity of the plant, however, it was discovered that their effect is relatively weak (Bruneton, 1999). Kaempferol, quercetin and their glycosylated derivatives, which have been detected in *I. helenium* roots (Spiridon *et al.*, 2013) have previously exhibited considerable antihelmintic activity against *Schistosoma mansoni* adult worms *in vitro* (Braguine *et al.*, 2012). This finding suggests that phenolic compounds together with presented lactones contribute to overall antihelmintic activity of *I. helenium* (Azaizeh *et al.*, 2013). Root of *V. officinalis* contains alkaloids, epoxy-iridoid esters (valepotriates) and various sesquiterpenoids (Letchamo *et al.*, 2004; Dewick 2009; Parveen *et al.*, 2012). Since sesquiterpene structures have previously showed to be very potent antihelmintic agents *in vitro* (Li *et al.*, 2008), sesquiterpenes can significantly contribute to antihelmintic efficacy of *V. officinalis*. Thujone, monoterpenoid naturally occurring in aerial parts of *T. vulgare* (Ramasubramaniaraja and Niranjan Babu, 2010), has previously possessed antihelmintic activity against *Ascaris lumbricoides* and *Fasciola hepatica in vitro* (Mackie *et al.*, 1955). Therefore, this compound can be responsible for detected antihelmintic activity also in this test. One of the most interesting outcomes of this study is seen in relatively high *D. carota* antihelmintic efficacy. Medicarpin and 4-hydroxymedicarpin (phytoalexins) have previously demonstrated substantial antihelmintic effect towards *Caenorhabditis elegans in vitro* (Stadler *et al.*, 1994). Because of presence of structurally related compound (6-methoxymellein) in root of *D. carota* (Dewick, 2009) it is presumable that this compound could be responsible for relatively high antihelmintic activity of this plant. However, further analysis will be needed for identification of the main anti-chabertial agent of *D. carota* extract. The practical usability of plants as antihelmintic agents primarily depends on their toxicity and this fact should be considered in potential application of their extracts as veterinary pharmaceutics. There have been some indices that contented sulfuric compounds in *A. sativum* may be potentially toxic to sheep individuals, causing allergic reactions, flatulence, nausea, and abdominal discomfort (Balasinska and Kulasek, 2004; Barceloux, 2008). However, studies performed with *A. sativum* extracts showed that it can be safely fed to sheep in large quantities (Fredrickson *et al.*, 1995; Nowroozi-Asl *et al.*, 2010). Due to lack of relevant data dealing with potential toxicity of *A. absinthium*, *C. carvi*, *D. carota*, *I. helenium*, *J. regia* and *V. officinalis* to sheep, further toxicological information for these species is presented for organisms other than small ruminants. *A. absinthium* essential oil is sold as a dietary supplement in some countries over the world to treat various human diseases including digestive disorders (Wojcikowski *et al.*, 2004). Several cases of acute liver and kidney failure (together with nausea, vomiting, stomach pain, headache, dizziness, seizures, numbness of the legs and arms, delirium, and paralysis) have been reported after intake of *A. absinthium* essential oil (Weisbord *et al.*, 1997; Luyckx and Naicker, 2008). Symptoms of acute toxicity were also observed after ingestion of *T. vulgare* (Foster *et al.*, 1999; Barceloux, 2008) in humans, even though it has commonly been used as herbal medicine in several countries (Lahlou, *et al.*, 2008; Alvarez *et al.*, 2011). Thujone is considered as main toxic component of both *A. absinthium* and *T. vulgare* (Pelkonen *et al.*, 2013). Alqasoumi *et al.* (2012) determined a maximum tolerance dose and genotoxicity of *C. carvi* water suspension on mice and suggest that oral administration is safe. It was discovered that extract of *D. carota* root contains carotatoxin. Upon injection to mice, compound was found to possess neurotoxic symptoms and its LD_{50} was settled to 100 mg/kg (Crosby and Aharonson, 1967). There have been some rare reports of *D. carota* causing mild intoxications to horses and cattle upon feeding. This phenomenon was predicated to carotatoxin content, however, its concentration in fresh *D. carota* material was found to be very low (10-20 ppm). A fatal toxicological effect is seen only if large quantities are eaten. *D. carota* is therefore considered being safe to its potential consumer (Tavares *et al.*, 2008). Lactones presented in *I. helenium* can cause allergic reactions and in higher doses can induce vomiting, diarrhoea and other problems in humans (Warshaw and Zug, 1996). Crude extract exhibited cytotoxic effect *in vitro* (Dorn *et al.*, 2006). Several authors discourage prolonged use; however, extracts of rhizomes and roots of *I. helenium* are accepted as preparations of choice to treat various human illnesses (Bruneton, 1999). The chief constituent of *J. regia* leaves and pericarp is juglone, which had previously demonstrated *in vitro* cytotoxicity (Inbaraj and Chignell, 2004; Spiridonov *et al.*, 2005). The registry of toxic effects of chemical substances describes juglone as potential mutagenic and carcinogenic agent (Thakur, 2011). Furthermore, Van den Berg and Labadie (1990) described dermal allergy localized at various parts of body after application of juglone to skin. However, *J. regia* is an ingredient of plant-based medications and acute toxicity in humans has not been reported after oral application of the drug (Bruneton, 1999). Constituents presented in *V. officinalis* root (baldrinals, valepotriates) are believed to possess cytotoxic, mutagenic and teratogenic properties (Bos *et al.*, 1998). Nevertheless, until now, these effects have only been observed *in vitro*. *V. officinalis* is widely accepted as dietary supplement and tranquilizer both in human and veterinary medicine (Dewick, 2009). Health risk after prolonged use is therefore negligible (Bruneton, 1999).

6 CONCLUSION

In summary, the current study proved *in vitro* antihelmintic activity of ethanol extracts of eight plant species, namely *A. sativum*, *A. absinthium*, *C. carvi*, *D. carota, I. helenium*, *J. regia*, *T. vulgare* and *V. officinalis*. Even though, *A. absinthium*, *I. helenium*, *J. regia* and *T. vulgare* has been shown to significantly inhibit motility of *C. ovina*, their possible toxicity should be considered when employing these in veterinary medicine. Therefore, extracts of *A. sativum*, *D.*

carota and *V. officinalis* seems to be the prospective materials for further development of novel plant-based antihelmintics against *Chabertia ovina*. Especially extracts of *D. carota* and *V. officinalis*, which produced stronger or equal effect than albendazole at all times of experimental exposure, deserve more research attention. However, detailed analysis of their chemical composition and *in vivo* antihelmintic activity should be carried out in order to verify their possible practical use.

7 CONFLICT OF INTEREST

All authors disclose that they have no financial and personal relationships with other people or organization that could inappropriately influence (bias) their work, including employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registration, and grants or other funding.

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9 REFERENCES

Abdelqader, A., Qarallah, B., Al-Ramamneh, D. and Das, G., (2012). Antihelmintic effects of citrus peels ethanolic extracts against *Ascaridia galli*. Vet. Parasitol. 188: 78-84.

Ahmed, M., Laing, M.D. and Nsahlai, I.V. (2013). *In vitro* antihelmintic activity of crude extracts of selected medicinal plants against *Haemonchus contortus* from sheep. J. Helminthol. 87: 174-179.

Al-Shaibani, I.R.M., Phulan, M.S. and Shiekh, M. (2009). Antihelmintic activity of *Fumaria parviflora* (Fumariaceae) against gastrointestinal nematodes of sheep. Int. J. Agric. Biol. 11: 431-436.

Alvarez, A.L., Habtemariam, S., Juan-Badaturuge, M., Jackson, C. and Parra, F. (2011). *In vitro* anti HSV-1 and HSV-2 activity of *Tanacetum vulgare* extracts and isolated compounds: An approach to their mechanisms of action. Phytother. Res. 25: 296-301.

Azaizeh, H., Halahleh, F., Abbas, N., Markovics, A., Muklada, H., Ungar, E.D. and Landau, S.Y. (2013). Polyphenols from *Pistacia lentiscus* and *Phillyrea latifolia* impair the exsheathment of gastro-intestinal nematode larvae. Vet. Parasitol. 191: 44-50.

Balasinska, B. and Kulasek, G. (2004). Garlic and its impact on animal and human health. Med. Weter. 60: 1151-1155.

Barceloux, D.G. (2008). Medical toxicology of natural substances: Foods, fungi, medicinal herbs, plants and venomous animals. Wiley & Sons, Chichester, 1200 pp.

Besier, R.B. and Love, S.C.J. (2003). Antihelmintic resistance in sheep nematodes in Australia: the need for new approaches. Aust. J. Exp. Agric. 43: 1383–1391.

Borgsteede, F.H.M., Pekelder, J.J., Dercksen, D.P., Sol, J., Vellema, P., Gaasenbeek, C.P.H. and Van Der Linden, J.N. (1997). A survey of antihelmintic resistance in nematodes of sheep in the Netherlands. Vet. Quart. 19: 167-172.

Bos, R., Hendriks, H., Scheffer, J.J.C. and Woerdenbag, H.J. (1998). Cytotoxic potential of valerian constituents and valerian tinctures. Phytomedicine 5: 219-225.

Braguine, C.G., Bertanha, C.S., Gonçalves, U.O., Magalhaes, L.G., Rodrigues, V., Melleiro Gimenez, V.M., Groppo, M., Silva, M.L.A.E., Cunha, W.R., Januário, A.H. and Pauletti, P.M. (2012): Schistosomicidal evaluation of flavonoids from two species of *Styrax* against *Schistosoma mansoni* adult worms. Pharm. Biol. 50: 925-929.

Bruneton, J., (1999). Pharmacognosy, phytochemistry, medicinal plants (2nd ed.). Lavoisier, New York, 1117 pp.

Crosby, D.G. and Aharonson, N. (1967). The structure of carotatoxin, a natural toxicant from carrot. Tetrahedron 23: 465-472.

Dewick, P.M. (2009). Medicinal natural products: A biosynthetic approach, 3rd ed. Wiley & Sons, Chichester, 550 pp.

Dorn, D.C., Alexenizer, M., Hengstler, J.G. and Dorn, A. (2006). Tumor cell specific toxicity of *Inula helenium* extracts. Phytother. Res. 20: 970-980.

El Garhy, M.F. and Mahmoud L.H. (2002). Anthelminthic efficacy of traditional herbs on *Ascaris lumbricoide*s. J. Egypt. Soc. Parasitol. 32: 893–900.

Fathy, F.M. (2011). Antihelmintic effect of artesunate in experimental heterophyid infection. J. Egyp. Soc. Parasitol. 41: 469-483.

Fetterer, R.H. and Fleming, M.W. (1991). Effects of plumbagin on development of the parasitic nematodes *Haemonchus contortus* and *Ascaris suum*. Comp. Biochem. Phys. C. 100: 539-542.

Fredrickson, E.L., Estell, R.E., Havstad, K.M., Shupe, W.L. and Murray, L.W. (1995). Potential toxicity and feed value of onions for sheep. Lives. Prod. Sci. 42: 45-54.

Foster, S., Duke, J.A. and Peterson R.T. (1999). A field guide to medicinal plants and herbs: Of eastern and central North America (Peterson Field Guides), 2nd ed. Houghton Mifflin Harcourt, New York, 432 pp.

Heath, K.B. (2001). A fatal case of apparent water hemlock poisoning. Vet. Hum. Toxicol. 43: 35-36.

Hounzangbe-Adote, M.S., Paolini, V., Fouraste, I., Moutairou, K. and 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.

Githiori J.B., Athansiadou S. and Thamsborg S.M. (2006). The use of plants novel approaches for control of gastro-intestinal helminths in livestock with emphasis on small ruminants. Vet. Parasitol. 139: 136-140.

Inbaraj, J.J. and Chignell, C.F. (2004). Cytotoxic action of juglone and plumbagin: a mechanistic study using HaCaT keratinocytes. Chem. Res. Toxicol. 17: 55-62.

Kahn, C.M. and Line S. (2010). The Merck veterinary manual, 10th ed. Merck Sharp & Dohme Corp., New Jersey, 2945 pp.

Kale, A.A., Gaikwad, S.A., Kamblea, G.S., Deshpande, N.R. and Salvekar, J.P. (2011). *In vitro* antihelmintic activity of stem bark of *Juglans regia* L. J. Chem. Pharm. Res. 3: 298-302.

Korbelar, J., Endris, Z. and Krejca, J. (1978). Nase rostliny v lekarstvi. Avicenum, Zdravotnicke nakladatelstvi, Praha, 501 pp.

Lahlou, S., Israili, Z.H. and Lyoussi, B. (2008). Acute and chronic toxicity of a lyophilised aqueous extract of *Tanacetum vulgare* leaves in rodents. J. Ethnopharmacol. 117: 221-227.

Letchamo, W., Ward, W., Heard, B. and Heard, D. (2004). Essential oil of *Valeriana officinalis* L. cultivars and their antimicrobial activity as influenced by harvesting time under commercial organic cultivation. J. Agric. Food Chem. 52: 3915-3919.

Li, G.-H., Duan, M., Yu, Z.-F., Li, L., Dong, J.-Y., Wang, X.-B., Guo, J.-W., Huang, R., Wang, M. and Zhang, K.-Q. (2008).

Stereumin A-E, sesquiterpenoids from the fungus *Stereum* sp. CCTCC AF 207024. Phytochemistry 69: 1439-1445.

Lima, C.M.B.L., de Sousa Freitas, F.I., de Morais, L.C.S.L., das Santos Cavalcanti, M.G., da Silva, L.F., Padilha, R.J.R., Barbosa, C.G.S., das Santos, F.A.B., Alves, L.C. and de Fatima Formiga Melo Diniz, M., (2011). Ultrastructural study on the morphological changes to male worms of *Schistosoma mansoni* after *in vitro* exposure to allicin. Rev. Soc. Bras. Med. Tro. 44: 327-330.

Luyckx, V.A. and Naicker, S. (2008). Acute kidney injury associated with the use of traditional medicines. Nat. Clin. Pract. Nephr. 4: 664-671.

Magi, E., Talvik, H. and Jarvis, T. (2005). *In vivo* studies of the effect of medicinal herbs on the pig nodular worm (*Oesophagostomum* spp.). Helminthologia 42: 67-69.

Mackie, A., Stewart, G.M., Cutler, A.A. and Misra, A.L. (1955). *In vitro* tests of chemical compounds on *Ascaris lumbricoides* and *Fasciola hepatica*. Brit. J. Pharm. Chemoth. 10: 7-11.

Nowroozi-Asl, A., Nazifi, S., Badiei, K., Oliaei, A. and Khazen, A. (2010). Effect of garlic extract on malignant ovine theileriosis. Indian Vet. J. 87: 975-977.

Pelkonen, O., Abass, K. and Wiesner, J. (2013). Thujone and thujone-containing herbal medicinal and botanical products: Toxicological assessment. Regul. Toxicol. Pharm. 65: 100-107.

Ramasubramaniaraja, R. and Niranjan Babu, M. (2010). Antihelminthic studies and medicinal herbs - An overview. Int. J. Pharm. Sci. Rev. Res. 5: 39-47

Sackett, D., Holmes, P., Abbot, K., Jephcott, S. and Barber, M. (2006). Assessing the economic cost of endemic disease on the profitability of Australian beef cattle and sheep producers. In: MLA Final Report. Meat and Livestock Australia, North Sydney, pp. 33–38.

Silveira, R.X., Chagas, A.C.S., Botura, M.B., Batatinha, M.J.M., Katiki, L.M., Carvalho, C.O., Bevilaqua, C.M.L., Branco, A., Machado, E.A.A., Borges, S.L. and Almeida, M.A.O. (2012). Action of sisal (*Agave sisalana* Perrine) extract in the *in vitro* development of sheep and goat gastrointestinal nematodes. Exp. Parasitol. 131: 162-168.

Spiridon, I., Nechita, C.B., Niculaua, M., Silion, M., Armatu, A., Teaca, C.-A. and Bodirlau, R. (2013). Antioxidant and chemical properties of *Inula helenium* root extracts. Cent. Eur. J. Chem. 11: 1699-1709.

Spiridonov, N.A., Konovalov, D.A. and Arkhipov, V.V. (2005). Cytotoxicity of some Russian ethnomedicinal plants and plant compounds. Phytother. Res. 19: 428-432.

Stadler, M., Dagne, E. and Anke, H. (1994). Nematicidal activities of two phytoalexins from *Taverniera abyssinica*. Planta Med. 60: 550-552.

Squires, J.M., Ferreira, J.F.S., Lindsay, D.S. and Zajac, A.M. (2011). Effects of artemisinin and *Artemisia* extracts on *Haemonchus contortus* in gerbils (*Meriones unguiculatus*). Vet. Parasitol. 175: 103-108.

Sweeny, J.P.A, Robertson, I.D., Ryan, U.M., Jacobson, C. and Woodgate, R.G. (2012). Impacts of naturally acquired protozoa and strongylid nematode infections on growth and faecal attributes in lambs. Vet. Parasitol. 184: 298-308.

Tariq, K.A., Chishti, M.Z., Ahmad, F. and Shawl, A.S. (2009). Antihelmintic activity of extracts of *Artemisia absinthium* against ovine nematodes. Vet. Parasitol. 160: 83-88.

Tavares, A.C., Goncalves, M.J., Cavaleiro, C., Cruz, M.T., Lopes, M.C., Canhoto, J. and Salgueiro, L.R. (2008). Essential oil of *Daucus carota* subsp. *halophilus*:

Composition, antifungal activity and cytotoxicity. J. Ethnopharmacol. 119: 129-134.

Taylor, L., (2005). The healing power of rainforest herbs. Square One Publishers, New York, 528 pp.

Thakur, A., (2011): Juglone: A therapeutic phytochemical from *Juglans regia* L. J. Med. Plants Res. 5: 5324-5330.

Tritten, L., Braissant, O. and Keiser, J. (2012). Comparison of novel and existing tools for studying drug sensitivity against the hookworm *Ancylostoma ceylanicum in vitro*. Parasitology 139: 348-357.

Urban, J., Kokoska, L. and Langrova, I. (2007). Antihelmintic effects of certain Czech medicinal plants on *Ascaris suum* and *Trichostrongylus colubriformis*. Planta Med. 73: 921-921.

Urban, J., Kokoska, L., Langrova, I. and Matejkova, J. (2008). *In vitro* antihelmintic effects of medicinal plants used in Czech Republic. Pharm. Biol. 46: 808-813.

Van den Berg, A.J.J. and Labadie, R.P. (1990). Quinones. In: Methods in Plant Biochemistry vol. 1, Plant phenolics, (Harborne, J.B., Ed.), Academic Press, London, 451-491 pp.

Velkers, F.C., Dieho, K., Pecher, F.W.M., Vernooij, J.C.M., Van Eck, J.H.H. and Landman, W.J.M. (2011). Efficacy of allicin from garlic against *Ascaridia galli* infection in chickens. Poultry Sci. 90: 364-368.

Wagland, B.M., Emery, D.L., and McClure, S.J. (1996). Studies on the host-parasite relationship between *Trichostrongylus colubriformis* and susceptible and resistant sheep. Int. J. Parasitol. 26: 1279-1286.

Waller, P.J. (1994). The development of antihelmintic resistance in ruminant livestock. Acta. Trop. 56: 233-243.

Waller, P.J., Echevarria, F., Eddi, C., Maciel, S., Nari, A. and Hansen, J.W. (1996). The prevalence of antihelmintic resistance in nematode parasites of sheep in Southern Latin America: General overview. Vet. Parasitol. 62: 181-187.

Warshaw, E.M. and K.A., Zug (1996). Sesquiterpene lactone allergy. Am. J. Contact Dermat. 7: 1-23.

Weisbord, S.D., Soule, J.B. and Kimmel, P.L. (1997). Poison on line - acute renal failure caused by oil of wormwood purchased through the internet. New Engl. J. Med. 337: 825-827.

Wojcikowski, K., Johnson, D.W., and Gobé, G. (2004). Medicinal herbal extracts - renal friend or foe? Part one: The toxicities of medicinal herbs. Nephrology 9: 313-318.