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In Vitro Anthelmintic Effects of Medicinal Plants Used in Czech Republic

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Abstract

The ethanol extracts of 16 Czech medicinal plants, namely, *Allium sativum* L. (Alliaceae), *Artemisia absinthium* L. (Asteraceae), *Artemisia vulgaris* L. (Asteraceae), *Carum carvi* L. (Apiaceae), *Consolida regalis* Gray (Ranunculaceae), *Cucurbita pepo* L. (Cucurbitaceae), *Daucus carota* L. (Apiaceae), *Dryopteris filix-mas* (L.) Schott (Dryopteridaceae), *Erigeron canadensis* L. (Asteraceae), *Hedera helix* L. (Araliaceae), *Inula helenium* L. (Asteraceae), *Juglans regia* L. (Juglandaceae), *Satureja hortensis* L. (Lamiaceae), *Tanacetum vulgare* L. (Asteraceae), *Thymus vulgaris* L. (Lamiaceae), and *Valeriana officinalis* L. (Valerianaceae), have been tested for their potential *in vitro* anthelmintic effect against eggs *Ascaris suum* and infectious larvae *Trichostrongylus colubriformis*. The extracts of *A. sativum, A. absinthium*, *C. carvi*, *D. carota*, and *J. regia* possessed the strongest anthelmintic effect on the embryonating eggs at all concentrations tested (62.5, 125, 250, 500, 1000, 2000 μg/mL). The best results, showing a higher effect against the infective third-stage larvae in comparison with synthetic anthelmintic Zentel (albendazole), have been obtained for *A. sativum, A. absinthium*, *C. carvi*, *C. regalis*, *I. helenium*, *J. regia*, *S. hortensis,* and *V. officinalis*.

Keywords: Anthelmintic, *Ascaris suum*, Czech medicinal plants, plant extracts, *Trichostrongylus colubriformis*.

Introduction

Increasing problems of resistance development in helminths (Geerts & Dorny, 1995; Coles, 1997) against anthelmintics have led to the proposal of screening medicinal plants for their anthelmintic activity. This resistance against synthetic anthelmintics for gastrointestinal trichostrongylids and ascarids is a worldwide problem of sheep, goat (Waller, 1994), and pig breeding (Serrano et al., 2001). The parasites *Ascaris suum* and *Trichostrongylus colubriformis* are considered responsible for serious production losses of pigs and sheep, respectively (Stewart & Hale, 1988; Barnes & Dobson, 1990).

A number of medicinal plants have been used to treat parasitic infections in man and animals (Said, 1969; Akhtar et al., 2000). Among various kinds of *in vitro* methods, the egg hatch assays (Coles et al., 1992; Ketzis et al., 2002; Pessoa et al., 2002; Alawa et al., 2003; Hounzangbe-Adote et al., 2005) and the larval motility tests (Rabel et al., 1994; Lorimer et al., 1996; Khunkitti et al., 2000; Hounzangbe-Adote et al., 2005) are commonly used to test medicinal plants against nematodes.

For the current area of the Czech Republic, the collection of plants for medicinal use was first reported after arrival of the Slavic peoples to the area of Central Europe in the era of the great migration in the fourth and fifth centuries (Drabek & Hanzlicek, 1975; Prunerova, 2006). In the Middle Ages, the first pharmacobotanic garden was established by druggist Henricus Schwab in Prague in 1341 (Baloun, 1985). The first Czech writing on collection and use of medicinal plants is a translation of Mathioli Herbal by Tadeas Hajek from Hajek, published in 1562 (Drabek, 1972). In contrast with the rich tradition of medicinal plants use in the area of the Czech Republic (Novacek, 1998; Drabek, 2004), modern ethnopharmacological studies are totally lacking. Because there are no reports on anthelmintic activities of Czech medicinal plants, we decided to evaluate ethanol extracts of 16 medicinal plants traditionally used in the

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Czech Republic for treatment of nematode infections for their *in vitro* effects against eggs of *A. suum* and larvae of *T. colubriformis*.

Materials and Methods

Plants

The species have been selected according to their traditional use for treatment of parasitical infections recorded in Czech (former Czechoslovak) pharmacognosy literature (Kresanek et al., 1977; Korbelar et al., 1978). Samples were collected from various areas of Pilsen (Domazlice and Tachov districts) and Prague regions in the Czech Republic from April to October 2005. The voucher specimens authenticated by Jan Urban are deposited at the Institute of Tropics and Subtropics, Czech University of Life Sciences, Prague. Table 1 summarizes the ethnobotanical data (botanical name and voucher specimen number, family, Czech names, parts used and ethnomedicinal uses) of the plants selected for the study.

Preparation of extracts

The plants were collected and subsequently dried at a room temperature (20–25°C). Various fractions of plants according to their effect were ground (15.0 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 of dimethyl sulfoxide (DMSO) and in 980 μL of phosphate-buffered saline (PBS; pH 7.2, 0.15 M), both obtained from Lach-Ner (Neratovice, CZ), at a starting concentration of 2000 μg/mL.

Biological procedures

The effects of the 16 plant extracts of two life-cycle stages on a two-parasite-nematode, that is, the eggs and the third stage (L3), were measured by using different laboratory procedures using 96-multiwell plates. Two-fold dilutions (six) of each extract were prepared starting from 2000 μg/mL, which corresponds with the concentration range tested in previous studies by Lorimer et al. (1996), Paolini et al. (2004), Houzangbe-Adote et al. (2005), and Eguale et al. (2006). Each experiment was examined in triplicate, with the exception of larvae third stage positive controls, which were tested in duplicate. Synthetic anthelmintic Zentel (Smithkline Beecham, UK) (albendazole) and solution of DMSO (2% v/v) in PBS were assayed simultaneously as positive and negative controls, respectively.

The test of inhibitory effect of the Czech medicinal plant extracts on *A. suum* eggs was performed with slight modifications of egg hatching assay described previously by Coles et al. (1992) and Houzangbe-Adote et al. (2005), a method generally used for determination of sensitivity of Trichostrongylideae parasites. Because the eggs of *A. suum* (Ascarideae family) do not hatch spontaneously, inhibitory effect of the extracts tested on the process of full embryo development during eggs embryogenesis was determined. A brief description of the method follows: parasite eggs were freshly obtained from pigs infected with *A. suum*. The extracted eggs were repeatedly washed with saline, distributed into 96-multiwell plates creating a concentration of 50–100 eggs/well, and incubated 48 h at room temperature (20–25°C). After incubation, the eggs were placed into sulfuric acid (0.1 N) for 3 weeks until egg maturity stage. Thereafter, the number of the embryonating eggs from the individual plates were counted under a microscope by 80 × zoom; and the percentage of sample activity was determined as the ratio between the number of larvae and the number of eggs deposited per well. The average value was calculated for each concentration of all tested plants.

The larval migration inhibition (LMI) bioassay was used in order to measure inhibiting activity against infective larvae as previously described by Rabel et al. (1994), Lorimer et al. (1996), and Houzangbe-Adote et al. (2005). Briefly, the L3 larvae hatched *in vitro* from *T. colubriformis* eggs previously obtained from infected sheep were applied at a concentration of 30–40 for each well and incubated for 3 h at 20°C in the presence of plants extracts. After incubation, the larvae were washed in PBS solution (pH 7.2, 0.15 M) and briefly vortexed (with 150 turns/min for 5 min) three-times, and, after a short sedimentation (10 min), washed for the last time. Finally, the content of each well was moved on the slide, and the number of larvae with migration activity was counted under microscope (by 80 × zoom). The percentage of LMI was calculated as equal to $[(T - M)/T] \times 100$, where T represents the total number of L3 deposited in individual wells and M the number of L3 having migrated through (activity move).

Results

In our study, all extracts from 16 traditional Czech medicinal plants selected according to the ethnopharmacological data possessed *in vitro* effects on the different nematode life-cycle stages, namely on eggs *A. suum* as well as on the infective third-stage larvae *T. colubriformis,* which confirms their popular use and justifies the ethnobotanical approach in the search for biologically active extracts.

Results of assay on eggs embryogenesis are shown in Table 2. The average rate of embryonating eggs in the negative control was 80.73%, and values for positive control ranged from 10.16% to 7.12%. The effect of positive controls escalates in proportion to increasing concentration. All the tested plant extracts confirmed anthelmintic effects on the egg embryogenesis (*A. suum*) compared with negative control. However, none of the tested plant extracts in any concentrations had a higher ovicidal effect than did positive controls. The extracts from plants *A. sativum* L., *A. vulgaris* L., *C. regalis* Gray, *C. pepo* L., *D. filix-mas* (L.) Schott, *H. helix* L., *I. helenium* L., *J. regia* L., *S. hortensis* L., *T. vulgare* L., *T. vulgaris* L., and

Table 1. Ethnobotanical data on medicinal plants.

Botanical name and voucher specimen number	Family	Czech names	Parts used	Ethnomedicinal uses[a]
Allium sativum L. (Urb 004)	Alliaceae	Česnek setý	Bulbs	Anthelmintic effect, arteriosclerosis, hypertensive diseases, cardiac complaint, bowel diseases
Artemisia absinthium L. (Urb 009)	Asteraceae	Pelyněk pravý	Aerial part	Anthelmintic effect, dysorexia, enteralgia, biliousness
Artemisia vulgaris L. (Urb 003)	Asteraceae	Pelyněk Černobýl	Aerial part	Antiparasitic effect, dysorexia, stomachache, dysmenorhea
Carum carvi L. (Urb 015)	Apiaceae	Kmín kořenný	Fruit	Antiparasitic and bacteriostatic effect, dysorexia, stomachache
Consolida regalis Gray (Urb 002)	Ranunculaceae	Ostrožka stračka	Flowers	Anthelmintic effect, cough
Cucurbita pepo L. (Urb 011)	Cucurbitaceae	Tykev turek	Seeds	Anthelmintic effect, ureteritis
Daucus carota L. (Urb 007)	Apiaceae	Mrkev obecná	Roots	Antiparasitic effect, liver complaint, enteritis, jaundice, and ureteritis
Dryopteris filix-mas (L.) Schott (Urb 014)	Dryopteridaceae	Kaprad' samec	Rhizomes	Antiparasitic effect, fungal infections
Erigeron canadensis L. (Urb 005)	Asteraceae	Turan kanadský	Flowering aerial part	Anthelmintic and antiparasitic effect, hemorrhage, bloody diarrhea, ureteritis
Hedera helix L. (Urb 010)	Araliaceae	Břečt'an popínavý	Leaves	Antiparasitic effect, cough, bronchitis, wounds
Inula helenium L. (Urb 016)	Asteraceae	Oman pravý	Rhizomes and roots	Anthelmintic effect, bronchitis, wounds
Juglans regia L. (Urb 001)	Juglandaceae	Ořešák královský	Pericarp	Anthelmintic effect, exanthesis, fungal infections
Satureja hortensis L. (Urb 013)	Lamiaceae	Saturejka zahradní	Aerial part	Anthelmintic effect, gastritis, enteritis
Tanacetum vulgare L. (Urb 008)	Asteraceae	Kopretina vratič	Aerial part	Anthelmintic effect , articular diseases
Thymus vulgaris L. (Urb 012)	Lamiaceae	Mateřídouška tymián	Aerial part	Antiparasitic effect, bronchitis, gastritis, dyspepsia, flatulence, diarrhea, wounds
Valeriana officinalis L. (Urb 006)	Valerianaceae	Kozlík lékařský	Rhizomes	Anthelmintic effect, bowel neurosis, insomnia, heart distress

[a]The ethnomedical information has been taken from Korbelar et al. (1978).

V. officinalis L. possessed positive dependence between their ovocidal effects and concentration levels, whereas the extracts from *A. absinthium* L., *C. carvi* L., *D. carota* L., and *E. canadensis* L. were not effective in a concentration-dependent manner. The significant anthelmintic effect on the egg embryogenesis was demonstrated by extracts from *A. sativum, A. absinthium*, *C. carvi*, *D. carota*, and *J. regia*, at all concentrations tested. Among the other plants tested, the extract of *V. officinalis* was effective in concentrations ranging between 125 and 2000 μg/mL, followed by *A. vulgaris* and *I. helenium* extracts, possessing activity at concentrations from 250 to 2000 μg/mL, *H. helix* and *T. vulgare*, showing an effect at concentrations ranging between 500 and 2000 μg/mL, and *T. vulgaris*, being active at the concentration of 2000 μg/mL.

Table 3 includes results from the LMI test. LMI observed for the larvae of the negative control was 15.86%. Positive controls had LMI measured of Zentel from 31.25% to 78.26%. The effect of the positive control escalates in proportion to increasing concentration. With the exception of extracts from *C. carvi* and *H. helix*, all plant extracts tested showed a concentration-dependent effect on migration inhibition of the infective third-stage larvae of *T. colubriformis*. A significant anthelmintic effect against the infective third-stage larvae was demonstrated by extracts from *A. sativum, A. absinthium*, *C. carvi*, *D. filix-mas*, *I. helenium*, *J. regia*, *S. hortensis*, and *V. officinalis*. The best results, showing a higher effect against the infective third-stage larvae in comparison with Zentel, were obtained for *A. absinthium*, *I. helenium*, *V. officinalis*, at a concentration of 62.5 μg/mL, followed by *I. helenium* and *J. regia* (125 μg/mL), *A. absinthium*, *J. regia*, *C. regalis* (ranging from 250 to 500 μg/mL), and *V. officinalis* (2000 μg/mL). The same results were achieved for extracts from *S. hortensis* and *C. carvi*, both inhibiting infective third-stage larvae in all concentrations ranging from 62.5 to 500 μg/mL, and *A. sativum* within the range of concentrations from 62.5 to 1000 μg/mL.

Discussion and Conclusions

In previous studies, extracts from medicinal plants possessed *in vitro* anthelmintic (antiparasitic) activities in various kinds of *in vitro* tests, including species tested in our

Table 2. Ovicidal effect (o) of ethanol extracts from some species of Czech medicinal plants.

Species and controls	Yield[a] (%)	Concentration of extracts (μg/mL)					
		62.5	125	250	500	1000	2000
		O ± SD (%)					
A. sativum	12.67	64.94 ± 9.09	54.53 ± 0.22	57.86 ± 4.46	59.37 ± 13.27	53.46 ± 5.61	38.44 ± 5.15
A. absinthium	20.07	65.95 ± 8.38	63.79 ± 9.39	60.44 ± 2.41	65.55 ± 1.65	64.67 ± 1.28	67.25 ± 7.32
A. vulgaris	13.33	76.93 ± 2.62	70.98 ± 3.42	68.24 ± 3.44	65.03 ± 4.40	61.40 ± 3.51	63.68 ± 7.10
C. carvi	7.87	60.16 ± 3.67	64.02 ± 2.59	58.10 ± 5.24	63.21 ± 5.07	59.15 ± 1.18	57.95 ± 0.69
C. regalis	25.20	91.90 ± 2.13	89.00 ± 2.22	86.67 ± 3.24	82.05 ± 1.15	81.54 ± 3.25	76.56 ± 2.02
C. pepo	17.60	85.03 ± 9.02	82.52 ± 7.54	76.08 ± 11.06	72.64 ± 2,31	70.83 ± 8.91	69.43 ± 5.43
D. carota	12.27	70.50 ± 3.14	69.01 ± 1.43	70.50 ± 4.11	64.79 ± 2.11	59.77 ± 1.28	59.80 ± 4.43
D. filix-mas	28.40	97.39 ± 0.59	94.79 ± 2.09	88.48 ± 0.78	83.09 ± 0.39	78.83 ± 0.33	69.51 ± 0.40
E. canadensis	18.58	81.61 ± 2.95	84.29 ± 1.16	73.74 ± 7.18	76.59 ± 3.88	76.33 ± 2.46	70.65 ± 2.12
H. helix	27.55	91.13 ± 3.40	71.40 ± 2.32	74.35 ± 3.13	68.97 ± 8.72	63.70 ± 5.71	60.65 ± 3.50
I. helenium	18.40	84.10 ± 2.55	77.03 ± 2.93	69.48 ± 3.18	69.64 ± 3.68	66.37 ± 1.21	63.12 ± 3.36
J. regia	29.27	66.76 ± 0.67	64.33 ± 2.73	64.23 ± 5.69	62.73 ± 1.18	61.05 ± 2.24	52.97 ± 2.89
S. hortensis	16.47	90.69 ± 4.22	84.86 ± 1.87	86.91 ± 3.73	73.64 ± 1.45	69.00 ± 9.68	68.74 ± 1.35
T. vulgare	23.07	76.57 ± 4.58	74.77 ± 7.22	70.55 ± 6.50	66.79 ± 4.18	58.39 ± 3.86	54.02 ± 3.61
T. vulgaris	12.60	93.11 ± 2.40	90.54 ± 6.29	76.41 ± 9.58	76.29 ± 17.96	68.53 ± 13.47	58.14 ± 6.06
V. officinalis	18.80	83.24 ± 2.59	64.66 ± 9.81	61.86 ± 2.08	62.39 ± 4.86	62.38 ± 2.69	60.68 ± 5.23
Zentel[b]		10.16 ± 1.99	10.13 ± 1.28	7.97 ± 1.17	9.83 ± 4.41	7.90 ± 1.65	7.12 ± 3.88
PBS with DMSO [c]		80.73 ± 5.81	80.73 ± 5.81	80.73 ± 5.81	80.73 ± 5.81	80.73 ± 5.81	80.73 ± 5.81

Biological material: helminths, eggs from *Ascaris suum*. Parts used are provided in Table 1.
O: ovicidal effect (O) = $M/T \times 100$. SD (%), standard deviation. T, total number of eggs deposited in individual wells; M, the ratio between the number of larvae.
[a] Residue of the extract in terms of starting material.
[b] Albendazole, positive control.
[c] Negative control, 2% DMSO in PBS solution (pH 7.2, 0.15 M).

Table 3. Larval migration inhibition (LMI) of ethanol extracts from some species of Czech medicinal plants.

Species and controls	Concentration of extracts (μg/mL)					
	62.5	125	250	500	1000	2000
	LMI ± SD (%)					
A. sativum	48.72 ± 11.59	55.00 ± 2.89	58.33 ± 8.56	61.29 ± 3.89	63.41 ± 3.89	66.67 ± 12.94
A. absinthium	35.00 ± 12.80	37.78 ± 7.07	48.49 ± 8.37	60.00 ± 4.44	61.76 ± 4.17	76.92 ± 2.64
A. vulgaris	24.20 ± 8.70	27.94 ± 8.38	32.91 ± 17.48	33.33 ± 4.11	38.84 ± 8.71	42.65 ± 7.17
C. carvi	29.63 ± 4.20	23.81 ± 18.78	36.67 ± 25.17	40.91 ± 11.30	40.00 ± 16.25	38.18 ± 3.05
C. regalis	30.71 ± 4.60	30.34 ± 13.01	46.88 ± 19.87	42.86 ± 9.82	53.49 ± 1.89	51.67 ± 3.21
C. pepo	39.13 ± 13.30	30.00 ± 4.28	37.50 ± 17.89	41.67 ± 19.76	45.00 ± 14.46	48.28 ± 2.52
D. carota	41.94 ± 18.00	48.78 ± 22.22	52.17 ± 17.77	57.14 ± 20.20	57.89 ± 12.13	58.33 ± 16.00
D. filix-mas	25.45 ± 6.16	30.77 ± 15.98	40.68 ± 4.33	37.35 ± 6.28	48.72 ± 8.35	66.67 ± 16.92
E. canadensis	23.81 ± 20.09	26.32 ± 18.54	35.29 ± 21.47	38.46 ± 16.07	35.71 ± 21.73	39.13 ± 24.17
H. helix	21.95 ± 6.83	25.00 ± 25.26	25.93 ± 11.52	29.41 ± 4.21	27.03 ± 2.64	28.57 ± 7.74
I. helenium	48.10 ± 4.52	48.33 ± 3.65	41.18 ± 6.94	55.56 ± 18.32	48.48 ± 13.96	68.75 ± 2.81
J. regia	27.94 ± 0.75	40.00 ± 8.22	43.55 ± 2.83	47.76 ± 23.46	53.66 ± 2.98	58.97 ± 20.36
S. hortensis	47.02 ± 9.32	48.19 ± 21.04	50.00 ± 14.04	53.33 ± 12.32	52.38 ± 7.14	60.00 ± 26.90
T. vulgaris	25.14 ± 4.59	34.78 ± 8.82	35.19 ± 14.81	35.21 ± 23.12	35.71 ± 26.81	40.40 ± 16.12
T. vulgare	17.95 ± 13.41	27.44 ± 10.50	31.97 ± 16.91	37.50 ± 22.16	45.45 ± 11.98	49.15 ± 11.57
V. officinalis	50.00 ± 0.00	50.00 ± 22.36	48.61 ± 15.12	61.22 ± 2.45	62.50 ± 14.66	87.50 ± 29.32
Zentel[a]	31.25 ± 0.58	38.78 ± 6.28	41.30 ± 3.08	50.00 ± 3.65	62.75 ± 6.85	78.26 ± 4.82
PBS with DMSO[b]	15.86 ± 5.25	15.86 ± 5.25	15.86 ± 5.25	15.86 ± 5.25	15.86 ± 5.25	15.86 ± 5.25

Biological material: helminths, exsheathed L3 larvae from *Trichostrongylus colubriformis*. Parts used and yields are provided in Tables 1 and 2, respectively.
LMI, larval migration inhibition (%) = $[(T - M)/T] \times 100$. SD (%), standard deviation. T, total number of L3 deposited in individual wells; M, number of L3 having migrated through (activity move).
[a] Albendazole, positive control.
[b] Negative control, 2% DMSO in PBS solution (pH 7.2, 0.15 M).

study such as *A. sativum* (Raj, 1975; Harris et al., 2000), *D. carota* (Momin & Nair, 2002), *H. helix* (Julien et al., 1985; Majester-Savornin et al., 1991), *I. helenium* (El Garhy et al., 2002), and *T. vulgaris* (Mikus et al., 2000).

In our study, all the tested plant extracts showed inhibitory effects on egg embryogenesis. The observed reduction remained, however, relatively limited, as maximal reductions in embryonating eggs was around 53%, except for *A. sativum* whose inhibitory effect reached 39%. Similarly, the influence of plant extracts on the third-stage larvae measured by the LMI test was observed in the case of all the plant extracts tested. However, the results significantly differ in their effective range. The total effect was observed for all concentrations on the level of 22–87% and for a concentration of 1000 μg/mL, ranging from 27% to 63%. We can compare the result with the LMI test performed with seven plant extracts against the larvae of *T. colubriformis*, where at the same concentration (1000 μg/mL), the range was from 37% to 63% (Molan et al., 2000).

In conclusion, all ethanol extracts from 16 Czech medicinal plants tested showed anthelmintic effects *in vitro* against eggs of *A. suum* and larvae of *T. colubriformis*. The extracts of *A. sativum, A. absinthium*, *C. regalis*, *C. carvi*, *I. helenium*, *J. regia*, *S. hortensis*, and *V. officinalis* showed the best activity against the infective third-stage larvae of *T. colubriformis* in the LMI test, and a significant anthelmintic effect on egg embryogenesis (*A. suum*) was demonstrated by extracts from *A. sativum, A. absinthium*, *C. carvi*, *D. carota*, and *J. regia*.

In summary, based on the results of the above-described experiments and literature data indicating low levels of toxicity for extracts from *A. sativum* (Saxena et al., 2005; Velasco-Velázquez et al., 2006), *A. absinthium* (Chiasson et al., 2001; Muto et al., 2003), *C. carvi* (Kumar & Singh, 2006), *I. helenium* (Spiridonov et al., 2005; Dorn et al., 2006), *J. regia* (Inbaraj & Chignell, 2004; Bhatia et al., 2006), *S. hortensis* (Zani et al., 1991), and *V. officinalis* (Romero-Jimenez et al., 2005; Yao et al., 2007), we suggest that some of these plant materials could be prospective sources for development of new antiparasitical herbal remedies. However, further phytochemical studies are required to determine the types of compounds responsible for the anthelmintic properties of these species. In addition, to the best of our knowledge, this is the first ethnopharmacological report on anthelmintic activity of medicinal plants traditionally used in the Czech Republic for treatment of nematode infections.

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References

Akhtar MS, Kabal Z, Khan MN, Lateef M (2000): Anthelmintic activity of medicinal plants with particular reference to their use in animals in Indo-Pakistan subcontinent. *Small Rumin Res 38*: 99–107.

Alawa CBI, Adamu AM, Gefu JO, Ajanusi OJ, Abdu PA, Chiezey NP, Alawa JN, Bowman DD (2003): *In vitro* screening of two Nigerian medicinal plants (*Vernonia amygdalina* and *Annona senegalensis*) for anthelmintic activity. *Vet Parasitol 113*: 73–81.

Baloun J (1985): Medicinal plant gardens in the history of Czechoslovak pharmacy (the 14th to the 18th century). *Cesk Farm 34*: 449–452.

Barnes EH, Dobson RJ (1990): Population dynamics of *Trichostrongylus colubriformis* in sheep: Computer model to simulate grazing systems and the evolution of anthelmintic resistance. *Int J Parasitol 20*: 823–831.

Bhatia K, Rahman S, Ali M, Raisuddin S (2006): *In vitro* antioxidant activity of *Juglans regia* L. bark extract and its protective effect on cyclophosphamide-induced urotoxicity in mice. *Redox Rep 11*: 273–279.

Chiasson H, Belanger A, Bostanian N, Vincent C, Poliquin A (2001): Acaricidal properties of *Artemisia absinthium* and *Tanacetum vulgare* (*Asteraceae*) essential oils obtained by three methods of extraction. *J Econ Entomol 94*: 167–171.

Coles GC (1997): Nematode control practices and anthelmintic resistance on Britisch sheep farms. *Vet Rec 141*: 91–93.

Coles GC, Bauer C, Borgsteede F, Geerts S, Klei TR, Tailor MA, Waller PJ (1992): World association for advancement in veterinary parasitology (WAAVP) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. *Vet Parasitol 44*: 35–43.

Dorn DC, Alexenizer M, Hengstler JG, Dorn A (2006): Tumor cell specific toxicity of *Inula helenium* extracts. *Phytother Res 20*: 970–980.

Drabek P (1972): The family pharmacy in the year 1595. *Cesk Farm 21*: 197–198.

Drabek P (2004): S.K. Zeidler (1620–1689) on drugs. *Ceska Slov Farm 53*: 47–50.

Drabek P, Hanzlicek Z (1975): 700 years of Czech pharmacy. *Cas Lek Ces 114*: 1285–1289.

El Garhy MF, Mahmoud LH (2002): Anthelminthic efficacy of traditional herbs on *Ascaris lumbricoides*. *J Egypt Soc Parasitol 32*: 893–900.

Eguale T, Tilahun G, Gidey M, Mekonnen Y (2006): *In vitro* anthelmintic activities of four Ethiopian medicinal plants against *Haemonchus contortus*. *Pharmacologyonline 3*: 153–165.

Geerts S, Dorny P (1995): Anthelmintic resistance in helminths of animals and man in the tropics. *Bull Seances Acad R Sci Outre-Mer 41*: 401–424.

Harris JC, Plummer S, Turner MP, Lloyd D (2000): The microaerophilic flagellate *Giardia intestinalis*: *Allium sativum* (garlic) is an effective antigiardial. *Microbiology 146*: 3119–3127.

Hounzangbe-Adote MS, Zinsou FE, Affognon KJ, Koutinhouin B, Adamou-N'Diaye M, Moutairou K (2001): Efficacité antiparasitaire de la poudre des graines de papaye (*Carica papaya*) sur les strongles gastro-intestinaux des moutons Djallonké au sud du Benin. *Rev élev Méd Vét Pays Trop 54*: 225–229.

Hounzangbe-Adote MS, 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.

Inbaraj JJ, Chignell CF (2004): Cytotoxic action of juglone and plumbagin: A mechanistic study using HaCaT keratinocytes. *Chem Res Toxicol 17*: 55–62.

Julien J, Gasquet M, Maillard C, Balansard G, Timon-David P (1985): Extracts of the ivy plant, *Hedera helix*, and their anthelmintic activity on liver flukes. *Planta Med 3*: 205–208.

Ketzis JK, Tailor A, Bowman DD, Brown DL, Warnick LD, Erb HN (2002): *Chenopodium ambrosioides* and its essential oil as treatments for *Haemonchus contortus* and mixed adult-nematode infections in goats. *Small Rumin Res 44*: 193–200.

Khunkitti W, Fujimaki Y, Aoki Y (2000): *In vitro* antifilarial activity of extracts of the medicinal plant *Cardiospermum halicacabum* against *Brugia pahangi*. *J Helminthol 74*: 241–246.

Korbelar J, Endris Z, Krejca J (1978): Nase rostliny v lekarstvi. *Avicenum, Zdravotnicke nakladatelstvi, Praha*.

Kresanek J, Krejca J (1977): Atlas liecivych rostlin a lesnych plodov. *Osveta, Bratislava, CSSR*.

Kumar P, Singh DK (2006): Molluscicidal activity of *Ferula asafoetida*, *Syzygium aromaticum* and *Carum carvi* and their active components against the snail *Lymnaea acuminata*. *Chemosphere 63*: 1568–1574.

Lorimer SD, Perry NB, Foster LM, Burgess EJ (1996): A nematode larval motility inhibition assay screening plant extracts a natural products. *J Agric Food Chem 44*: 2842–2845.

Majester-Savornin B, Elias R, Diaz-Lanza AM, Balansard G, Gasquet M, Delmas F (1991): Saponins of the ivy plant, *Hedera helix*, and their leishmanicidic activity. *Planta Med 57*: 260–262.

Mikus J, Harkenthal M, Steverding D, Reichling J (2000): *In vitro* effect of essential oils and isolated mono- and sesquiterpenes on *Leishmania major* and *Trypanosoma brucei*. *Planta Med 66*: 366–368.

Molan AL, Waghorn GC, Min BR, McNabb WC (2000): The effects of condensed tannins from seven herbages on *Trichostrongylus colubriformis* larval migration *in vitro*. *Folia Parasitol 47*: 39–44.

Momin RA, Nair MG (2002): Pest-managing efficacy of *trans*-asarone isolated from *Daucus carota* L. seeds. *J Agric Food Chem 50*: 4475–4478.

Muto T, Watanabe T, Okamura M, Moto M, Kashida Y, Mitsumori K (2003): Thirteen-week repeated dose toxicity study of wormwood (*Artemisia absinthium*) extract in rats. *J Toxicol Sci 28*: 471–478.

Novacek L (1998): Development of the scientific basis of Czech pharmacy 1939-1945. *Ceska Slov Farm 47*: 279–283.

Paolini V, Fouraste I, Hoste H (2004): *In vitro* effects of three woody plant and sainfoin extracts on 3rd-stage larvae and adult worms of three gastrointestinal nematodes. *Parasitology 129*: 69–77.

Pessoa LM, Morais SM, Bevilaqua CML, Luciano JHS (2002): Anthelmintic activity of essential oil of *Ocimum gratissimum* Linn. and eugenol against *Haemonchus contortus*. *Vet Parasitol 109*: 59–63.

Prunerova M (2006): Development of pharmacy in the Czech Republic. *Cas Lek Ces 145*: 815–816.

Rabel B, Mc Gregor R, Douch PGC (1994): Improved bioassay for estimation of inhibitory effects of ovine gastrointestinal mucus and anthelmintics on nematode larval migration. *Int J Parasitol 24*: 671–676.

Raj RK (1975): Screening of indigenous plants for anthelmintic action against human *Ascaris lumbricoides*: Part-II. *Indian J Physiol Pharmacol 19*: 47–49.

Romero-Jimenez M, Campos-Sanchez J, Analla M, Munoz-Serrano A, Alonso-Moraga A (2005): Genotoxicity and anti-genotoxicity of some traditional medicinal herbs. *Mutat Res 585*: 147–155.

Said M (1969): *Hamdard Pharmacopeia of Eastern Medicine*. Karachi, Pakistan, Hamdard National Foundation.

Saxena PN, Chauhan LK, Gupta SK (2005): Cytogenetic effects of commercial formulation of cypermethrin in root meristem cells of *Allium sativum*: Spectroscopic basis of chromosome damage. *Toxicology 216*: 244–252.

Serrano FJ, Reina D, Frontera E, Roepstorff A, Navarrete (2001): Resistance against migrating *Ascaris suum* larvae in pigs immunized with infective eggs or adult worm antigens. *Parasitology 122*: 699–707.

Spiridonov NA, Konovalov DA, Arkhipov VV (2005): Cytotoxicity of some Russian ethnomedicinal plants and plant compounds. *Phytother Res 19*: 428–432.

Stewart TB, Hale OM (1988): Losses to internal parasites in swine production. *J Anim Sci 66*: 1548–1554.

Waller PJ (1994): The development of anthelmintic resistance in ruminant livestock. *Acta Trop 56*: 233–243.

Yao M, Ritchie HE, Brown-Woodman PD (2007): A developmental toxocity-screening test of valerian. *J Ethnopharmacol 113*: 204–209.

Velasco-Velazquez MA, Maldonado PD, Barrera D, Torres V, Zentella-Dehesa A, Pedraza-Chaverri J (2006): Aged garlic extract induces proliferation and ameliorates gentamicin-induced toxicity in LLC-PK1 cells. *Phytother Res 20*: 76–78.

Zani F, Massimo G, Benvenuti S, Bianchi A, Albasini A, Melegari M, Vampa G, Bellotti A, Mazza P (1991): Studies on the genotoxic properties of essential oils with *Bacillus subtilis* rec-assay and Salmonella/microsome reversion assay. *Planta Med 57*: 237–241.