Artemisia Species in Small Ruminant Production: their Potential Antioxidant and Anthelmintic Effects.

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Abstract

Although *Artemisia* species (spp.) are rich source of terpenes, antioxidant phenolics and flavonoids, among other biologically-active compounds, only in the past 8-10 years the genus Artemisia has been investigated for its anthelmintic and antioxidant compounds. *Artemisia annua* is a notable exception due to the production of the antimalarial compound artemisinin. These compounds can help parasitized animals to neutralize free radicals that form in their blood, boost their immune system, and help them fight gastrointestinal parasites. This manuscript will present current results on the antioxidant activity of *Artemisia* species, and focus on the genus as a potential source of antioxidant and anthelmintic compounds. Some *Artemisia* spp. had higher antioxidant capacity than conventional forages with some, e.g. *A. annua,* being a good source of minerals, protein, and antioxidants, with negligible amounts of anti-nutritional compounds. Although the anthelmintic activity of artemisinin-related drugs, dry leaves, and alcoholic extracts of *Artemisia* spp. encompass *Schistosoma, Fasciola, Trypanosoma, Eimeria* (coccidia), *Trichostrongylus*, and *Haemonchus*, not all *Artemisia* spp. are effective anthelmintic sources.

Introduction

The genus Artemisia belongs to the family Compositae (Asteraceae) and has over 300 species spread worldwide. Plants from this family are a rich source of sesquiterpene lactones, a class of natural compounds with several proved medicinal effects, including anthelmintic effect. *Artemisia annua* (annual wormwood) has been given the most attention in the past 36 years due to the pharmacological use of artemisinin (the main sesquiterpene produced by the plant) as an antimalarial recommended by the World

Health Organization to treat quinine-resistant malaria in over 50 countries in Africa, Asia, and South America. Artemisinin's second most reported use is as an anti-cancer drug (Efferth et al., 2001; Lai and Singh, 2006). In the past 8-10 years, new medicinal benefits were reported for several *Artemisia* species (spp.) due to the anti-parasitic effects of artemisinin-based compounds and the high antioxidant capacity of crude extracts of some plants of this genus. Besides artemisinin, *Artemisia* spp. are rich sources of sesquiterpene lactones and antioxidant compounds (flavonoids, phenolic acids, etc.) with potential benefits to human and animal health. The antioxidant capacities of the crude extracts of some of these plants are shown in Figure 1 and Table 1.

Current published research shows that artemisinin drugs are effective against *Leishmania, Trypanosoma* , and some viruses that affect humans. In animals, the antiparasitic activity of *Artemisia* spp. and artemisinin applies to several parasitic organisms including *Fasciola, Eimeria* (coccidia), *Trichostrongylus,* and *Haemonchus* (Table 2).

Gastrointestinal nematodes (GIN) represent a major economic hurdle in ruminant systems, and anthelmintics are estimated to account for 53% of the total costs of veterinary drugs worldwide (Diaz Lira et al., 2008). *Haemonchus contortus,* a GIN of the abomasum, leads in animal losses and is fast becoming multi-drug resistant in several regions of the world. The focus of our research group at the Agricultural Research Service (ARS), USDA, is to find alternative plants that, when fed as forage or forage supplements, have the potential to improve the health of small ruminants in grazing systems. These compounds can work as anthelmintics, directly (affecting larval establishment, larval motility, mortality, decreasing fecal egg output, impairing worm development, and decreasing egg hatchability from feces) or indirectly (balancing antioxidant blood levels, improving the nutritional status, and boosting the immune system of parasitized animals fed these plant materials). Although it is unlikely that this natural approach can totally substitute synthetic anthelmintics, it could reduce their use, make them more effective, and improve meat quality and acceptability by the growing number of consumers demanding organic, kosher, and halal products.

Condensed tannins (CT) are currently the most studied natural class of compounds for their nutritional value (increase in protein uptake) and for reducing fecal egg counts

(FEC) in ruminants infected with GIN. However, recent publications have shown that CT from *Salix* sp. (willow) reduced FEC of *Nematodirus* and *Trychostrongylus*, but increased FEC of *Haemonchus contortus* in sheep (Diaz Lira et al., 2008) and that *Albizia anthelmintica*, with recognized anthelmintic activity and high tannin content, reduced FEC in sheep from 77-90% (Gradé & Longok 200, by Gradé et al. 2008) against mixed nematode infection, but only from 19-34% against *Haemonchus*-only infections (Gradé et al., 2008). These results strongly suggest that the research on natural anthelmintics need to expand beyond CT.

Artemisia species have negligible amounts of tannins, but are rich sources of sesquiterpene lactones and flavonoids that have reported anthelmintic activity. For instance, santonin isolated from *Artemisia* spp. was used to treat intestinal worms before the advent of synthetic anthelmintics, and has recently been used as a reference anthelmintic for in vitro studies. Artemisinin, isolated from *A. annua*, is the raw material for the most potent antimalarial after quinine and has anthelmintic activity against trematodes such as *Schistosoma* in mice, coccidia (*Eimeria*) in chicken, *Fasciola* in sheep, and *Chlonorchis* in rats (Table 2). Other artemisias (Table 2) had anthelmintic effects in vitro or in vivo against several nematodes, including *Haemonchus*, but the active compound (s) was (were) not identified. Regarding "active compound(s)", Kraft and collaborators (2003, cited by Pillay et al., 2008) reported that *Artemisia afra* had antimalarial activity against both sensitive and chloroquine-resistant *Plasmodium falciparum* in vitro, but if the crude extract was fractionated to separate flavonoids and sesquiterpene lactones, the isolated compounds never had the same remarkable activity as the whole crude extract. The authors concluded that the antimalarial activity was due to the synergistic effect of several compounds contained in the crude extract. Some of the artemisias (*annua, afra,* and *absinthium*) with antimalarial activity have also anthelmintic activity (table 2).

Anthelmintic studies with the main active compound (artemisinin or related drugs) of *A. annua*, at oral doses of 200 mg/kg were effective against *Schistossoma*, but oral doses of 10 mg/kg, once a day for a week, and 80 mg/kg were ineffective against *Haemonchus*-infected goats (Turner and Ferreira, 2005) and *Fasciola* in sheep (Keiser et al., 2008), respectively, while one single intra-muscular (i.m.) dose of 160 mg/kg reduced *Fasciola*

eggs in 65% and the worm burden in 91%, and 80 mg/kg (i.m.) reduced worm burden in 65% (Keiser et al., 2008). These results suggest that: 1) artemisinin is not as effective as its derived drugs such as artemether; 2) the dose of 10 mg/kg used in goats was too low to be effective; 3) the oral route, although more convenient, is not as effective as the i.m. route; 4) artemisinin might not be effective against *Haemonchus* but should be tested against other GIN of ruminants, such as *Eimeria* and *Fasciola*.

Our recent study showed that artemisinin is fairly stable in rumen fluid at both neutral (75% recovery) and acidic pH (95% recovery) after incubation at 39 °C for 24 hours, and that artemisinin orally fed to goats at 33 mg/kg was absorbed, transferred to the blood, and metabolized to dihydroartemisinin. Finally, a high concentration of artemisinin was found in goat feces 24 hours after oral intake (Ferreira and Gonzalez, 2008). These results are the first to indicate that artemisinin resists the rumen environment, is metabolized by goats to the active compound dihydroartemisinin, and thus could be useful to treat coccidiosis and fasciolosis in goats and sheep. However, it remains to be tested if the presence of artemisinin in feces will reduce *Haemonchus* egg hatching, thus reducing pasture infection. *Artemisia annua* and artemisinin uses for the livestock industry are currently in expansion, based on current reports of the anti-protozoal, anti-bacterial and antioxidant activities of the plant, its extracts, and its essential oil. Some animal parasites effectively controlled with *A. annua*, its essential oil, and artemisinin include *Babesia* (Kumar et al., 2003), *Eimeria* or coccidiosis (Allen et al., 1997; Arab et al., 2006; Brisibe et al., 2008), the trematodal blood fluke *Schistosoma* spp. (Lescano et al., 2004; Xiao et al., 2000), and bacteria (Juteau et al., 2002). In a recent collaborative study with African, Brazilian, and USDA-ARS colleagues, different tissues of *A. annua* were analyzed for its potential use in animal feed and scored high values for crude protein, antioxidant capacity (Table 1) and as source of amino acids, with negligible amounts of anti-nutritive components such as phytates and oxalates (Brisibe et al., 2009).

This manuscript highlights the antioxidant and anthelmintic potential of several species of *Artemisia* published previously, and presents results from our ongoing research.

Antioxidant activity

Artemisia spp. have high content of flavonoid and phenolic compounds that are associated with their high antioxidant capacity. *Artemisia* spp. can score 50% or more of the total antioxidant capacity (TAC) measured by the oxygen radical absorbance capacity (ORAC) test and found in oregano (Table 1), the herb with the highest known ORAC value. There is indication that the flavonoids and phenolic acids found in leaves and crude alcoholic extracts have medicinal effects on their own, such as antioxidant and anti-cancer, or have synergistic effects with other medicinal compounds present in the plant, increasing their absorption, pharmacological activity, and permanence in the human or animal subject consuming the plant or extract (Blanke et al., 2008). Recently, plant materials with high antioxidant capacity such as *Artemisia afra* tops and grape seeds were found to attenuate coccidian infection in chickens and even increased the body weight of infected birds, similar to chickens in a control diet (Naidoo et al., 2008). *A. annua* leaves (Zheng and Wang, 2001) and crude extracts have been reported to be a good source of antioxidants (Cai et al., 2004), being among the four medicinal plants with the highest ORAC level among other Chinese medicinal plants (Zheng and Wang, 2001). This high antioxidant capacity was attributed to the high content and diversity of its leaf flavonoids, including C-glycosyl flavonoids as a possible component of the antioxidant and antiviral activity (Han et al., 2008). Flavonoids have been reported to be responsible for more of the antioxidant activity of leafy vegetables and herbs than vitamin E, C, or glutathione (Cao et al., 1996). Although the antimalarial activity of *A. annua* is mostly attributed to artemisinin, flavonoids from whole plant extracts also had antimalarial activity (Liu et al., 1992). Although it has been known that *Artemisia* spp. are rich in flavonoids, our literature search has resulted in less than 15 manuscripts focusing on the antioxidant capacity of *Artemisia* spp., and published from 2001 to 2009. One of those was the result of an international collaborative work on the nutritional and antioxidant characteristics of *A. annua* (Brisibe et al., 2009), with partial results presented in Table 1.

On Figure 1, we present results of antioxidant activity (ORAC) of some *Artemisia* spp., compared to other plants, and performed in our laboratory and elsewhere. These artemisia species, cultivated in West Virginia, showed that *A. afra* and *A. annua* had the highest ORAC values of the tested artemisias with total antioxidant capacities of 2,095

and 1,125 μmol TE/g, respectively. The ORAC values for oregano reported here (2,838 μmol TE/g) are about 1,000 ORAC units higher than the ones previously reported in the literature (ranging from 1,500-1,800 μmol TE/g). This oregano was cultivated in our greenhouses, harvested monthly, oven dried at 45 °C, and stored at room temperature for over two years before analysis.

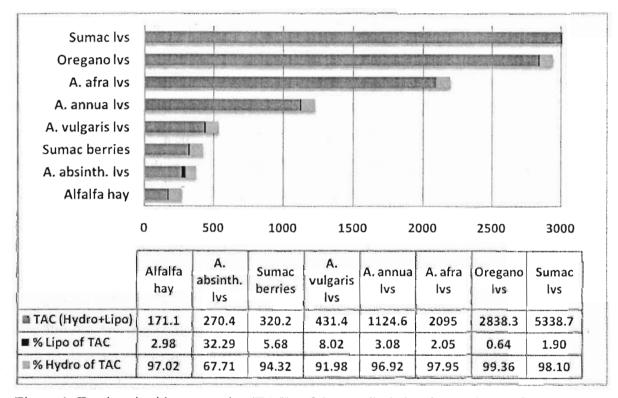|	Alfalfa hay	A. absinth. lvs	Sumac berries	A. vulgaris lvs	A. annua lvs	A. afra lvs	Oregano lvs	Sumac lvs
TAC (Hydro+Lipo)	171.1	270.4	320.2	431.4	1124.6	2095	2838.3	5338.7
% Lipo of TAC	2.98	32.29	5.68	8.02	3.08	2.05	0.64	1.90
% Hydro of TAC	97.02	67.71	94.32	91.98	96.92	97.95	99.36	98.10

Figure 1. Total antioxidant capacity (TAC) of leaves (lvs), berries, and hay of some atemisias determined by the oxygen radical absorbance capacity (ORAC) and compared to other plants and forages. The TAC (dark gray) was separated into percentages of its hydrophilic (light gray) and lipophilic (black) fractions. The TAC of sumac leaves is off the chart.

Artemisia vulgaris presented an ORAC value (TAC) of 431, equivalent to sericea lespedeza and forage grasses, such as switchgrass (Table 2), but this was a variegated, ornamental, cultivar and the wild type needs to be tested. It is interesting to notice that staghorn sumac leaves scored over 5,000 and could be a valuable antioxidant source to use as a feed additive because it has over 10 times the ORAC value of lespedeza, forage

grasses, and some artemisias. It is also worth noticing that the percent of the TAC pertaining to the lipophilic fraction was less than 10%, except for *A. absinthium*, confirming that vitamins A, C, D, and E (all lipophilic) are only minor components of the plant antioxidant activity. The hydrophilic fraction represented most of the TAC. This fraction contains the flavonoids, phenolics acids, and tannins produced by the plants. This is also indicated by the fact that both aqueous and ethanolic extracts of a Brazilian and of a Chinese *A. annua* had hydrophilic ORAC values of 2,123, 2,535 and 1,960 μmol TE/g, respectively (Table 1). These ORAC values, added to the total phenolics data (Table 1) indicate that artemisia leaves and crude extracts are a valuable source of antioxidants to animals, compared to lespedeza and trefoil (Table 1). The fact that the ORAC values for the Brazilian *A. annua* were higher than for the Chinese indicate that the Brazilian cultivar is a higher source of antioxidants than the Chinese, which agrees with a recent study (Bilia et al., 2006) that states that the Brazilian cultivar had a higher content of flavonoids than the Chinese cultivar. Interestingly, *A. annua* leaf ORAC of a cloned Swiss cultivar that was freeze-dried and compared to the oven-dried (40 °C) leaves indicated that there was no statistical difference in total antioxidant activity (TAC), although the lipophilic ORAC values were lower for the oven-dried samples (Table 1). These results indicate that lipophilic vitamins (A, C, D, and E) might have degraded during oven drying, again confirming reports that flavonoids have more antioxidant power than vitamins (Cao et al., 1996). Thus, our results agree with those of others in that the bulk of the antioxidant activity represented in the ORAC-TAC (ORAC-H + ORAC-L) is mostly present in the hydrophilic (ORAC-L) fraction with the lipophilic fraction (ORAC-L) representing less than 10% of the TAC. Besides the high antioxidant capacity of *A. annua*, recent work with our African collaborators has established the plant as a good source of amino acids (18%), crude protein (27%), minerals (mainly potassium and calcium in the leaves), while low in anti-nutritive components such as phytates and oxalates (Brisibe et al., 2009). High protein content translates into important essential amino acids needed for the development of healthy bones, muscles, skin and blood, while potassium maintains water balance in the body as an electrolyte, participates in the transmission of nerve impulses to muscles, and in the maintenance of normal blood

Table 1. Antioxidant capacity of *Artemisia annua* and a few conventional forages expressed in oxygen radical absorbance capacity (ORAC) for the hydrophilic (Hydro) and lipophilic (Lipo) fractions and as total antioxidant capacity (TAC) in μmol of Trolox equivalents (TE) per gram of dry weight (DW). Total phenolics of *Artemisia annua* are expressed in mg of gallic acid equivalent per gram of dry weight, adapted from (Brisibe et al., 2009). Oregano ORAC is given for comparison, as the highest ORAC value reported for an herb.[z]

PLANT SOURCE	ORAC-HYDRO	ORAC-LIPO	(LIPO X 100)) TAC	ORAC-TAC	TOTAL PHENOLICS
MEDICINALS	(μmol TE/g DW)	(μmol TE/g DW)	(%)	(μmol TE/g DW)	(mg GAE/g DW)
A. annua					
Flowers[y]	1,196	38.0	3.0	1,234	51.0
Leaves[y]	1,090	34.6	3.2	1,125	40.5
Stems[y]	288	4.0	1.4	292	18.0
Roots[y]	284	2.6	0.9	287	13.6
Water extract (Br.)	2,123	ND	-	ND	72.0[x]
EtOH extract (Br.)	2,535	ND	-	ND	77.5[x]
EtOH extract (Ch.)	1,960	ND	-	ND	86.0[x]
FD leaves (Sw.)	1,029.0	58.6	5.4	1,087	ND
OD leaves (Sw.)	1,042.0	39.6	3.7	1,082	ND
Artemisia afra	2,052	43	2.1	2,095	ND
A.vulgaris	370.5	34.6	8.0	405.1	ND
A. absinthium	183.1	87.3	32.3	270.4	ND
Oreganum [w]	2,820	18.3	0.65	2,839	66.8
FORAGES					
Lespedeza tops	530.2	9.3	1.7	539.5	ND
Lotus sp. tops (trefoil)	323.4	5.8	1.8	329.2	ND
Switchgrass	316.5	14.58	4.4	331.12	51.7[x]
Alfalfa hay	166	6.0	3.6	172	12.3

[z] ND – Not determined, Br – Brazilian cultivar, Ch. – Chinese cultivar, Sw. – Swiss cultivar, EtOH – Ethanol, FD – Freeze dried, OD – Oven dried.

[y]Data from (Brisibe et al., 2009).

[x]Determined by the Prussian blue Method.

[w]Greenhouse-cultivated oregano at the AFSRC.

pressure. Potassium was also the main mineral found in *A. annua* leaves cultivated in an Appalachian soil (Ferreira, 2007).

Anti-parasitic activity

Artemisia species with reported anthelmintic and anti-parasitic activity are presented in Table 2. Although results vary according to the parasite model used, life stage of the parasite, and host animal tested, it is clear that polar extracts obtained with water or ethanol have a greater anthelmintic activity than non-polar extracts obtained with hexane, which usually have low or no anthelmintic activity. These results indicate that antioxidant flavonoids (hydrophilic fraction) might play a role in anthelmintic activity.

Although some artemisias had promising anthelmintic effects, their possible neurotoxic effects should be kept in mind such as those of *A. absinthium* in rats, if provided in doses higher than 2% of a commercial extract in drinking water (Mukinda and Syce, 2007) and those of *A. afra* aqueous extract which, although non-lethal at 1g/kg of body weight, induced under-activity, diarrhea, and salivation in rats (Mukinda and Syce, 2007). This indicates that oral doses lower than 1g/kg of body weight must be used when testing *A. afra* anthelmintic effects in animals to prevent undesirable effects. However, oral doses of 1.0 and 2.0g/kg of body weight of *A. absinthium* aqueous and ethanolic extract significantly reduced FEC in sheep infected with a mixture of gastrointestinal nematodes, without apparent toxicity to the animals. In vitro, both extracts reduced the motility of *Haemonchus contortus* at 25 mg/mL (Tariq et al., 2009), and both aqueous and ethanolic extracts were effective in vitro and in vivo, but ethanolic outperformed the aqueous extracts. These results agree with others (Urban et al., 2008) who found that ethanolic extracts of *A. absinthium* significantly reduced egg development and juvenile (L3) larval motility of *Ascaris suum* in vitro.

In a previous experiment in collaboration with Drs. Hart and Dr. Wang (American Institute of Goat Research, Langston, OK), goats infected with GIN and fed *A. ludoviciana* flowering tops and *A. annua* leaves for four or six days, respectively, showed a 34% and 4% decrease in FEC, respectively, 14 days after treatments started, while control animals showed a 32% increase in FEC (Hart et al., 2008). This study indicates that *A. ludoviciana* might have a better anthelmintic activity than *A. annua*, but the study

should be repeated with both species at the same developmental stage (vegetative or flowering) and also using ethanolic crude extracts instead of only crushed leaves. *Artemisia annua* leaves were found to have similar in vitro organic matter (IVOM) disappearance as alfalfa (63% vs. 67%) indicating that artemisinin (determined as 1.4% on a dry matter basis) did not impair ruminal microflora or overall digestibility in goats (Turner and Ferreira, 2005). The same authors tested oral artemisinin at 10 mg/kg body weight for six days in Boer goats artificially infected with *Haemonchus contortus*, but found no significant reduction in FEC between infected and control, untreated, goats. Although the dose was later determined to be too low to be effective, the data showed a slight decrease in FEC of artemisinin-treated animals compared to a slight increase in FEC in infected, untreated goats. One should also keep in mind that *Haemonchus contortus* is a very resilient nematode that sets high standards for anthelmintic screening with plant-derived compounds.

Tests done in collaboration with Dr. Anne Zajac (Virginia Tech), and using gerbils (a small rodent) artificially infected with *Haemonchus contortus* and treated with artemisinin alone have not reproduced the results obtained for artemisinin drugs in mice or sheep infected with *Schistosoma* and *Fasciola*, respectively, while results with crude *Artemisia* spp. extracts and its essential oil are still to be evaluated. In vitro tests with some individual compounds that occur in *A. annua* and crude ethanolic extracts of *A. annua* have shown some potential to inhibit the motility of *Haemonchus* larvae (Dr. Joyce Foster, personal communication).

Currently, in vitro anthelmintic results, obtained with artemisinin elsewhere, could not be reproduced with *A. annua* in the gerbil model system. Although this might indicate that *A. annua* has no effect on *Haemonchus* and that other species should be tested, it could also mean that: 1) doses tested so far are too low to be effective, 2) the gerbil system does not host *Haemonchus* long enough (only 9 days) for the treatment to produce an effect (animals treated for 5 days only), and 3) the relationship between metabolized plant secondary metabolites and *Haemonchus* might be different in goats and sheep (polygastric ruminants) than in gerbils (a monogastric rodent) infected with the parasite.

Table 2.   Anthelmintic activity of artemisia species, artemisinin, and *A. annua* essential oil on internal parasites of animals and humans studied in vitro (parasite outside of host) or in vivo in different hosts, such as mice and sheep.

ARTEMISIA SPECIES	ORGANISM (ANIMAL)	REFERENCE
A. annua (Oil)	*Enterococcus* sp. (in vitro)	(Juteau et al., 2002)
A. annua/ (artemisinin)	*Schistossoma* (mice); coccidia (chicken); *Fasciola* (sheep); *Chlonorchis* sp (rat); *Fasciola gigantica* (in vitro)	(Allen et al., 1998; Keiser et al., 2008; Keiser et al., 2006; Shalaby et al., 2009; Utzinger et al., 2002)
A. afra	Coccidia (chicken); *C. elegans*(in vitro)	(McGaw et al., 2000; Naidoo et al., 2008)
A. absinthium	Nematodes (soil), *Haemonchus* (sheep), *Trichinella* (rat)	(Caner et al., 2008; Korayem et al., 1993; Tariq et al., 2009; Urban et al., 2008)
A. brevifolia	sheep	(Iqbal et al., 2004)
A. capillaris	*Angiostrongylus (mice)*	(Lai, 2006)
A. herba-alba	*Haemonchus* (goats)	(Idris et al., 1982)
A. maritima	*Haemonchus* (cattle)	(Jangde et al., 2001)
A. santonica	*Ascaris lumbricoids* (in vitro)	(El Garhy and Mahmoud, 2002)
A. sativum	*A. suum, Tricghostrongylus(*in vitro*)*	(Urban et al., 2008)
A. sieberi	Coccidia (chicken)	(Allen et al., 1998; Arab et al., 2006)
A. siversiana	Tapeworm (mice)	(Singhal, 1983)
A. vulgaris	*Trichinella spiralis* (rat)	(Caner et al., 2008)

Conclusions

Although plants are being investigated and referred to as alternative therapies for failing synthetic anthelmintic, antimalarial, and anti-bacterial drugs, it unlikely that these plants will ever replace pharmaceutical drugs currently in use. However, they have the potential to decrease the use these drugs or make them more effective, if used in combination.

Several articles dealing with the anthelmintic effects of plants exist, but only few articles approach the use of these plants in combined therapy with synthetic drugs to treat multi-drug resistant nematodes. Recently, the extract of *Artemisia capillaris* was shown to have synergistic effect when used with albendazole against *Angiostrongylus cantonensis* at 100mg/kg/day, for 7 days. The concept of synergism is not new and focuses on using crude plant extracts (several compounds together) instead of one active component (natural or synthetic). Synergism has been better studied in parasitic diseases that kill millions of people every year, such as malaria. For instance, artemisinin present in traditional teas has been shown to have similar antimalarial effects than pure artemisinin as a drug, although the tea contains one third of the concentration used in artemisinin antimalarial drugs. This effect is attributed to the tea flavonoids, which potentiate artemisinin effect. The *A. annua* bioactive flavonoids chrysosplenol-D and chrysoplenetin showed week activity against a drug-resistant strain of the *Staphylococcus aureus* bacteria, but were remarkably bactericidal when combined with berberin (a plant alkaloid) and the drug norfloxacin, indicating that these natural compounds rendered the drug-resistant bacteria susceptible to norfloxacin again (Stermitz et al., 2002). Currently, artemisinin (as artemether) is recommended by the World Health Organization as a combination therapy, with a synthetic antimalarial drug (lumefantrine), as the first-line antimalarial treatment in over 50 countries afflicted by chloroquine-resistant malaria. The use of artemisinin alone is not recommended. Both the combinations of artemisinin with quinine and artemisinin with curcumin (all plant compounds) showed synergistic effect against malaria in vitro (Gupta et al., 2002; Nandakumar et al., 2006) and in mice (Nandakumar et al., 2006). Polyphenolic compounds from tea such as epigallocatechin gallate and epicatechin gallate had antimalarial effects on their own, and improved the antimalarial effects of artemisinin (Sannella et al., 2007). Schistosomiasis, caused by a blood trematode, is controlled successfully in South Africa by crude plant extracts where

21 plant species showed in vitro activity at 50mg/ml of the aqueous extract, killing 66-100% of the juvenile worms (schistosomula), with 9/21 plants being lethal at 25mg/ml, and 6/21 plants being lethal at 6.25mg/ml (Sparg et al., 2000). Synergism have been gaining momentum in the past 15 years and have been documented for several herbs through tests in vitro and in vivo and have been reviewed elsewhere (Williamson, 2001).

The accumulating and compelling evidence in the literature, and the lack of promising results obtained so far with artemisinin alone against *Haemonchus*, indicate that crude herbal extracts, herbal combinations, and the combination of herbal extracts and anthelmintic drugs need to be tested for their potential combined effects against gastrointestinal nematodes.

Acknowledgements

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