Plants and their medicinal potential for controlling gastrointestinal nematodes in ruminants

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ABSTRACT: Gastrointestinal nematodes (GINs) is one of the main sanitary problems of ruminant herds, causing large economic losses to breeders. Chemical control continues to be the main measure used to combat GINs, but as resistant parasite populations spread, the efficiency of this method is becoming impaired. In light of this situation, alternative ways to control verminosis are gaining importance. Among these, phytotherapy has attracted particular attention. This review presents the current status of research on phytotherapeutic agents, their difficulties and perspectives. Plants with nematicidal activity are a potential source of chemical substances, including new molecules, that can be used to control GINs in ruminants. The principal difficulty of research into phytotherapeutic substances is to identify the bioactive compounds, because in many cases in vitro tests have shown promising results, but in vivo test results have been disappointing. Among the causes is host-drug interaction. Therefore, more careful choice of plants with anthelmintic potential by adoption of a standardized system to analyze the results of in vitro testing, combined with better understanding of the synergistic relations among metabolites and the adjustment of methods for in vivo testing, can make phytotherapy for control of GINs more effective.

Keywords: phytotherapy, tannins, flavonoids, saponins, verminosis, nematodes.

INTRODUCTION

Infections by gastrointestinal nematodes (GINs) in ruminants cause reduction in development in the birth, growth and finishing stages[1, 2, 3, 4, 5], lower milk production[6] and impaired immunocompetence[7], provoking substantial economic losses. GINs, among the various parasitoses, is one of the main factors hampering the productivity of cattle breeding[8], but it is frequently present in subclinical form, making it hard to detect, much less measure, the losses caused.

The control of parasites in ruminants mainly involves the administration of anthelmintics. The lack of knowledge of epidemiological factors, the low costs of treatment with chemical substances[9] and the...
corresponding mass use of these substances have led to the development of resistance to anthelmintics, an increasingly vexing problem that cannot be ignored by breeders\textsuperscript{[8]}.

Because of the increasing resistance of parasites, the impact of verminosis on livestock production and the growing consumer demand for animal products produced organically\textsuperscript{[9]}, many auxiliary methods to control parasites have been extensively studied, such as use of vaccines, rotated grazing\textsuperscript{[10]}, provision of nutritional supplements\textsuperscript{[11]}, selection of resistant breeding stock\textsuperscript{[12]}, use of nematophagous fungi\textsuperscript{[13, 14]} and phytotherapy\textsuperscript{[15]}. The last of these is considered particularly promising for control of GINs\textsuperscript{[16]}.

Auxiliary control measures can reduce the severity of nematodioses in ruminants. With respect to research involving plants, the possibility exists of detecting new anthelmintic molecules instead of just finding direct uses of plant extracts or feed supplements.

The purpose of this review is to present the current status of research into phytotherapeutic substances, their difficulties and perspectives, and to reiterate the need to study plants in veterinary medicine due to the growing problem of GINs infections.

**Phytotherapy**

Phytotherapy, defined as the use of plants to treat ailments, is a practice that dates to the dawn of human civilization. Nowadays plants and their extracts are still an important part of the pharmacopeia, both of so-called primitive groups and of people living in advanced societies (where phytotherapy is even making a comeback due to growing demand for natural products)\textsuperscript{[17, 18]}.

The knowledge generated by human-plant interaction, called ethnobiology, is a part of the cultural heritage of millions of people worldwide, transmitted verbally or in writing\textsuperscript{[19, 20]} (e.g., Chinese medicine).

With the increase in regional and global integration, traditional communities are exposed to the influence of modern communities, giving rise to a two-way process of cultural incorporation\textsuperscript{[17]}. As in any process of cultural fusion, losses and gains in knowledge can happen. At first, knowledge of the species used in traditional medicine can expand outside isolated communities. However, as time passes, traditional societies can be overwhelmed by outside influences, culminating in socioeconomic changes, for better or worse, and this traditional knowledge of medicinal plants fades, reducing the cultivation and/or use of native plants in favor of invasive species with cosmopolitan distribution\textsuperscript{[21]}.

But in counterpoint to this pattern, recently demand in advanced societies for medicinal plants has increased markedly, prompting the search for new pharmacological substances by the drug industry, making plant genetic resources more valuable\textsuperscript{[20]}. In this sense, phytotherapy has attracted strong interest from researchers in human and veterinary medicine\textsuperscript{[22]}, in the second case with promising results regarding antiparasitic activity.

Studies of the use of plants with medicinal potential are considered urgent as a result of the rapid loss of folk knowledge\textsuperscript{[17]}. When this knowledge is associated with scientific research, the path between the choice of a target species and the demonstration of its biological effect can be shortened by the longstanding indications of medicinal activity by local people. As a disadvantage, the use of popular knowledge in scientific research can limit the chance to discover new chemical molecules, while the contrary occurs with the random choice of plants as research targets\textsuperscript{[23]}.

Therefore, more representative research about plants in a given region can be carried out as the first step in identifying plants with anthelmintic potential\textsuperscript{[24]}, or any other potential medicinal effect.

Scientific validation of phytotherapeutic substances is a necessary initial step for the correct utilization of medicinal plants or their active compounds\textsuperscript{[25]}. This step involves the conduction of \textit{in vitro} tests with plant extracts, to enable discovering the existence of anthelmintic properties. At the same time, the extracts need to be chemically characterized to identify the active compounds.

**Methods to obtain plant extracts**

The testing of plant material for biological activity starts by gathering samples and identifying the plants by species in the field, followed by processing in the laboratory. In the laboratory, except for studies of essential oils, the samples are dried and stored in plastic bags and/or cardboard boxes, protected from light. The drying interrupts the cell activity and the chemical reactions of oxidation and hydroxylation, which can produce structural changes that damage secondary metabolites\textsuperscript{[23]}.

The next steps begin with grinding or chopping the dried plant material to prepare it for extraction of metabolites, which are numerous and chosen according to the classes of metabolites desired.
The resulting extracts, targets of evaluation, are prepared in the form of liquids, pastes or powders, followed by removal of the chemicals used in the extraction process\cite{26, 27}. When the phytochemical composition of a given plant species is unknown, the usual choice is for simpler extraction methods, such as percolation, defined as static or dynamics contact with a solvent. The most common practice is to use mixtures of water and alcohol with the plant material at room temperature. Hot extraction in a Soxhlet device can also be used\cite{23}.

More advanced extraction methods aim to reduce the influence of the organic solvents often used, seeking to extract substances that are less soluble in simple water-alcohol preparations. Methanol, hexane, ethyl acetate and chloroform, among others can be used. These solvents are generally applied under high pressure and at high temperatures, for short intervals\cite{28}. In this case, the chemical bonds produced by the electrostatic interactions between the metabolites and their storage sites are ruptured by the high temperature and the compounds are carried away by the solvent (or mixture of solvents).

The last step in preparing the extracts is to evaporate the solvents, leaving the solid sample material to be tested for biological activity. Before testing, these are generally dissolved again in appropriate solutions to assure their homogeneity.

The studies of biological activity are normally preceded by chemical characterization, to learn and allow isolation of the active substances. This can be done by chromatography and spectral techniques with ultraviolet light and nuclear magnetic resonance\cite{24}.

**In vitro methods to evaluate anthelmintic plant substances**

The validation of the anthelmintic activity of plant substances starts by the submission of the extracts that contain hypothetically active substances to a variety of tests\cite{30}, which are also used to detect parasite resistance\cite{29, 30}. Among these are the egg hatching test, larval migration inhibition test, larval development inhibition test, and less frequently, the larval feeding inhibition assay and tests of inhibition of larval exsheathment and motility of larvae or adults. This last one is little utilized due to its high subjectivity.

The egg hatching technique\cite{31}, initially described to evaluate the occurrence of resistance to benzimidazoles, can be adjusted for use in assays with plants to detect medicinal effects\cite{32}. These adjustments can involve small alterations such as reduction of the number of eggs tested and increase in the volume of the treatment solution, to facilitate counting the eggs, besides assuring greater contact between the eggs and extract. The testing procedures, because the compounds have unknown action, need to be designed to reduce the chances of mistaken interpretations and variations in the results. The purpose is to assess the ability of the target extract to prevent or impede the hatching of larvae from the eggs after contact with it. This can be achieved by interrupting the blastular development of the nematode embryo\cite{33}, blockage of the enzymatic pathways or interference in the action of enzymes associated with hatching\cite{34}. The larval migration inhibition test (LMIT)\cite{35} is used to evaluate the resistance to anthelmintics that act either to stimulate or inhibit the somatic musculature. It is based on evaluation of the migratory capability of third-stage larvae after a period of exposure to or incubation with the treatment solution. This technique has been subject to several modifications\cite{36}, but still suffers from low repeatability because of the difficulty of quantifying non-migrating larvae. Besides this, the methods described are restricted to using pure isolates. Demeler\cite{37} proposed a more sensitive version of the LMIT, in which the increase in accuracy can be attributed to the repeatability of the observations of migrating and non-migrating larvae. This method can also be applied to mixed isolates, since it does not involve the exsheathment of third-stage larvae, allowing identification of their genus.

The evaluation of the ability to inhibit larval development\cite{37, 38}, developed primarily for studies of resistance to benzimidazoles, levamisole and macrocyclic lactones, can also be applied to study extracts with biological activity\cite{34}, making it a more universal test because it does not depend on the molecule’s action mechanism. However, even though it is a highly accurate test, its use to study the resistance to parasiticides or even for bioprospection is subject to some limitations due to the lack of standardization to different parasite species. In this respect, larval development tests have been standardized for helminths of sheep and goats\cite{38}, pigs\cite{39} and equines\cite{40}. However, few studies have been published on bovine nematodes\cite{41}, and those that have are restricted to the genus *Cooperia*. The lack of standardization of techniques to assess phytotherapeutic substances is a factor limiting the discovery and careful assessment of new bioactive compounds. Currently plants are often evaluated and considered to be active, inferring they can be used to help control parasites, but without well-established indices of biological activity (efficacy)\cite{39}.
Standardized methods to conduct in vitro tests, as proposed by Jackson & Hoste\(^{[42]}\), are necessary to determine and compare parameters like EC50, EC90 and EC99 of different plant substances, from different regions, to indicate with greater scientific security candidates for more advanced studies in the field of veterinary parasitology.

The best way to assess a phytotherapic compound is to perform tests on the different stages of parasites, to elucidate and characterize the ways the extract of interest acts, since the inhibition action of a determined substance can be different depending on the phases of the parasite’s development\(^{[26, 42]}\).

**Compounds of plant origin with anthelmintic action**

The compounds that have been found to have nematicidal action belong to a large class of substances called secondary metabolites, which originate from the allelopathic relations between plants and their surrounding environment. These substances, as the name suggests, are not associated with the plant’s primary metabolism, so they are not indispensable to its development. They are synthesized by alternative pathways of the cell metabolism, involving shikimic acid and amino acids\(^{[43]}\), and are stored in compartments such as cellular vacuoles or trichomes (appendages of the epidermis), depending on their chemical nature, often in organs and cells that are distant from the synthesis site\(^{[44]}\).

Plants produce various substances at the same time, in response to different stimuli\(^{[45]}\). In some cases, more than one substance can be produced in response to the same stimulus or agent. Secondary metabolites can be produced in all parts of the plant (leaves, stems, roots, flowers and seeds), and the concentration is affected by the species, development stage and climatic\(^{[46]}\) and geographic conditions\(^{[47]}\).

Secondary metabolites can be associated with defensive mechanisms against infection (thus being good candidates for phytotherapic products), as well as against other plants competing for nutrients and protection against sunlight. They can also act to attract pollinizers to flowers, to disperse seeds and to promote nitrogen fixation\(^{[48]}\). In animals, these substances can interact with different molecules, such as enzymes and neurotransmitters, and cell sites like hormone receptors, so it is rare for a cell site to exist over which no secondary metabolite is active\(^{[49]}\).

Among the secondary metabolites are saponins, alkaloids, non-protein amino acids, tannins and other polyphenols, lignin and glycosides. Of these, tannins are most associated with anthelmintic activity\(^{[45, 48]}\).

In the case of polyphenol compounds, especially tannins, their ability to link to some proteins is well known. Tannins are classified according to their chemical structures into condensed and hydrolyzable tannins. The former are commonly found in tropical forage grasses and are subdivided into various other classes, with highlight on prodelfinidins and procyanidins for their anthelmintics activity\(^{[49]}\).

In ruminants, binding to proteins from the diet, especially those rich in prolines\(^{[49]}\), occurs in the rumen and diminishes the protein degradation by bacteria of the local microbiota. In turn, in the abomasum, because of the low pH, the tannin/protein macromolecular complex is broken down and the proteins are degraded and absorbed in the intestinal tract. Therefore, a protein-rich diet enhances the immune response to harmful agents, among them nematodes, representing a form of indirect action of these metabolites on helminth infections\(^{[50]}\).

The direct effect of tannins comes from their affinity to bind to proteins of the parasite, causing changes in its cuticle architecture as well as degeneration of the musculature and intestinal cells\(^{[50]}\). These injuries can reduce the motility of the nematode, due to the metabolic alterations arising from structural breakage of the cuticle. The parasite’s nutrition can be affected as a result of changes in the front end, and the release of eggs by females can also be impaired by destructuring of their reproductive appendage. Another effect caused by tannins occurs due to the interaction of these metabolites and the sheath of L3 larvae, preventing their exsheathment and hence impairing penetration in the host’s gastrointestinal tract. In parallel, tannins can interfere in the nutrition of the sheath of L3 larvae, preventing their exsheathment and hence impairing penetration in the host’s gastrointestinal tract. In parallel, tannins can interfere in the nutrition of the sheath of L3 larvae, preventing their exsheathment and hence impairing penetration in the host’s gastrointestinal tract. In parallel, tannins can interfere in the nutrition of the sheath of L3 larvae, preventing their exsheathment and hence impairing penetration in the host’s gastrointestinal tract. In parallel, tannins can interfere in the nutrition of the sheath of L3 larvae, preventing their exsheathment and hence impairing penetration in the host’s gastrointestinal tract. In parallel, tannins can interfere in the nutrition of the sheath of L3 larvae, preventing their exsheathment and hence impairing penetration in the host’s gastrointestinal tract. In parallel, tannins can interfere in the nutrition of the sheath of L3 larvae, preventing their exsheathment and hence impairing penetration in the host’s gastrointestinal tract. In parallel, tannins can interfere in the nutrition of the sheath of L3 larvae, preventing their exsheathment and hence impairing penetration in the host’s gastrointestinal tract. In parallel, tannins can interfere in the nutrition of the sheath of L3 larvae, preventing their exsheathment and hence impairing penetration in the host’s gastrointestinal tract. In parallel, tannins can interfere in the nutrition of the sheath of L3 larvae, preventing their exsheathment and hence impairing penetration in the host’s gastrointestinal tract. In parallel, tannins can interfere in the nutrition of the sheath of L3 larvae, preventing their exsheathment and hence impairing penetration in the host’s gastrointestinal tract. In parallel, tannins can interfere in the nutrition of the sheath of L3 larvae, preventing their exsheathment and hence impairing penetration in the host's gastrointestinal tract.

Besides tannins, lectins, which are non-immunological proteins, have direct action on the fecundity of Teladorsagia circuncincta (Stademann) and Trichostrongylus colubriformis (Giles) and indirect effect by activation of the local immune response of the host, with increase of eosinophils and mucus-producing cells and a tendency to increase the number of the T-helper cells in the mucosa of the gastrointestinal tract of infected individuals\(^{[51]}\). The direct action of concanavalin-A, a lectin, was demonstrated in Strongyloides ratti (Sandground) from their connection to the sugar terminals present in the parasite’s amphid receptors, affecting its chemokinetic capacity\(^{[52]}\).
Two species of helminths – T. circuncincta and Haemonchus contortus (Rudolphi) – can be distinguished from each other in their different development phases by using lectins[54]. This is due to the ability of lectins to bind to the sugars located on the parasite’s surface. In this sense, the chemical composition of the cuticle in relation to surface carbohydrates can vary among species, and these lectins can show a degree of binding selectivity. This fact should be considered when studying these substances, in particular their ability to control parasites. Besides this, some lectins can act to inhibit ribosomes[44], impeding protein synthesis.

Saponins have an amphipathic characteristic, acting as detergent solutions. When ingested, they are absorbed promptly or as micelles by the intestinal cells[45]. When in contact with biological membranes, some saponins can destabilize them, thus influencing the ionic exchange[46], culminating with an increase in the concentration of calcium ions in the intracytoplasmic environment, and as a consequence raising the concentration of pro-apoptotic calcium-dependent proteins, as observed for dammaranes, a class of saponins[53]. In the gut of mammals exposed to contact with saponins, the absorption of molecules and transport of sugars are impaired[55], probably weakening the helminths in the same way[46], by contact via the blood in the case of hematopagous helminths, or directly from ingesta rich in saponins. Saponins contained in Medicago sativa (Linnaeus) have demonstrated an inhibiting effect on the development of nematodes that parasitize plants[57].

Representatives of other classes of secondary metabolites have also shown anthelmintic activity, such as anethole and thymol (terpenes), extracted from Croton zehtneri (Pax et Hoffm.) and Lippia sidoides (Cham.), based on inhibition of egg hatching and larval development of H. contortus[58]. The sesquiterpene longifolene, a secondary metabolite, was shown to have ovicidal and larvicidal action on H. contortus, through the ability to mimic the juvenile hormone[59]. Lorimer et al.[60] also identified the action of polyphenol compounds to inhibit the migration of third-stage nematode larvae.

Plant extracts are complex mixtures containing a wide diversity of secondary metabolites in many concentration ranges. When these extracts are tested, in many cases two or more chemical substance acting synergistically are responsible for the antiparasitic effect, none of which would be effective alone[43].

The effect of condensed tannins, prodelfphinidins and procyanidins to inhibit the exsheathment H. contortus larvae can be enhanced by association with polyphenols such as quercetin and luteolin, with the synergistic effect depending on the tannin’s structure. Procyanidins were found to be less active[61], indicating the different activity levels among tannins[62]. A similar effect was observed with caffeic acid, whose ovicidal action was enhanced by quercetin. A basic condition for the synergistic effect in this case was the maintenance of a fixed quercetin: caffeic acid ratio in the extract. When this ratio was altered in the extracts as a consequence of the methods and solvents used for extraction, the ovicidal effect was reduced[63].

On the other hand, negative (antagonistic) interactions can also occur. Polyphenol compounds of Lysiloma latissilquum (L.) Benth and Theobroma cacao (Linnaeus) were implicated in reducing the anthelmintic activity of acetone: water extracts of two plants on H. contortus eggs, as shown by the increase of the nematocidal effect after incubation of the extracts with polyvinyl polypyrrolidone, an inhibitor of polyphenols[59].

In vitro tests of the anthelmintic efficacy of phytotherapic substances

The first step in the process of validating phytotherapic substances is to conduct in vitro tests of the plant extracts, considering only the candidate plant and target parasite, free of external conditions, such as environment and host[25]. In this respect, many plant species have been evaluated regarding anthelmintic action (Table 1).

The large majority of investigations of the effect of phytotherapic substances on parasites have focused on sheep, with many fewer studies aimed at other livestock species. Two factors can be mentioned as responsible for this research bias: the larger number or reports of resistance and the greater pathogenicity of the parasites.

During these tests, various metabolites have been detected as having nematocidal action. The anthelmintic action has been identified by the direct effect of the metabolites on the free-living parasite stages and can be confirmed when the metabolites are incubated with inhibitory substance, such as polyvinyl polypyrrolidone (PVPP).

The ethanol fraction of Jatropha curcas (Linnaeus) (a plant rich in tannins) was evaluated by tests of inhibition of egg hatching and larval exsheathment, with respective inhibition rates of 99.8% and 81.1%. To assess the influence of the presence of tannins on the efficacy of the ethanol extract, PVPP...
Table 1. Plant with anthelmintic potential and their respective spectrums of activity and mechanisms of action based on in vitro ests.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Parasites evaluated</th>
<th>Susceptible parasite phase</th>
<th>Mechanism of action</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flemingia vestita</td>
<td>Trematoda and Cestoda</td>
<td>Adults</td>
<td>Destructuring of the cuticular epithelium</td>
<td>(64)</td>
</tr>
<tr>
<td>Peltophorum africanum</td>
<td>Trichostrongylus colubriformis</td>
<td>Eggs and L1, L2 and L3 larvae</td>
<td>Inhibition of hatching and larval development</td>
<td>(65)</td>
</tr>
<tr>
<td>Allocasuarina torulosa, Neolitrea dealbata, Acacia holaricea, Acacia salicina, Callitrisendlicheri e Casuarina cunninghamiana</td>
<td>Haemonchus placei and Cooperia sp.</td>
<td>L3 larvae</td>
<td>Inhibition of motility</td>
<td>(66)</td>
</tr>
<tr>
<td>Allocasuarina torulosa, Neolitrea dealbata, Acacia holaricea, Acacia salicina, Callitrisendlicheri e Casuarina cunninghamiana</td>
<td>Caenorhabditis elegans</td>
<td>Juveniles</td>
<td>Inhibition of motility</td>
<td>(67)</td>
</tr>
<tr>
<td>Tabernaemontana citrifolia</td>
<td>H. contortus</td>
<td>Eggs, larvae and adults</td>
<td>Inhibition of hatching, development and motility of larvae and adults</td>
<td>(68)</td>
</tr>
<tr>
<td>Melia azedarach</td>
<td>H. contortus</td>
<td>Eggs and larvae</td>
<td>Inhibition of hatching and larval development</td>
<td>(69)</td>
</tr>
<tr>
<td>Markhamia obtusifolia</td>
<td>T. colubriformis</td>
<td>Eggs</td>
<td>Inhibition of hatching</td>
<td>(70)</td>
</tr>
<tr>
<td>Jatropha curcas</td>
<td>H. contortus</td>
<td>Eggs and larvae</td>
<td>Inhibition of hatching and exsheathment</td>
<td>(71)</td>
</tr>
<tr>
<td>Acacia baileyana, Acacia melanoxylon, A. podalyrrifolia, Alectryon oleifolius, Duboisia hopwoodii, Eucalyptus gomphocephala e Santalum spicatum</td>
<td>Cyathostominae</td>
<td>Larvae</td>
<td>Inhibition of larval development</td>
<td>(72)</td>
</tr>
<tr>
<td>Pistacia lentiscus e Phillyrea latifolia</td>
<td>Teladorsagia circuncincta, T. colubriformis and Chabertia ovina</td>
<td>Larve</td>
<td>Inibição do desenvolvimento larval</td>
<td>(73)</td>
</tr>
<tr>
<td>Acacia mearnsii</td>
<td>H. contortus, Trichostrongylus sp., Oesophagostomum sp., and Cooperia sp.</td>
<td>Eggs and larvae</td>
<td>Inhibition of hatching and motility</td>
<td>(74)</td>
</tr>
<tr>
<td>Citrus sinensis e Melaleuca quinquenervia</td>
<td>H. contortus</td>
<td>Eggs and larvae</td>
<td>Inhibition of hatching and larval development</td>
<td>(75)</td>
</tr>
<tr>
<td>Artemisia annua</td>
<td>Bunostomum sp., Cooperia sp. e Trichostrongylus sp.</td>
<td>Eggs and larvae</td>
<td>Inhibition of hatching and motility</td>
<td>(76)</td>
</tr>
</tbody>
</table>
was added. After this addition, the hatching inhibition declined to 91.9%, indicating a weak influence of tannins on the blastular development, while in the exsheathment inhibition test, the inhibition was reverted, demonstrating that tannins are more effective against the larval stages[71].

The conduction of confirmatory tests after incubation of extracts with antagonists of the bioactive substances is crucial, but is not always done due to the absence of known antagonists for all the different classes of metabolites.

During in vitro evaluations of phytotherapeutic substances, some factors should be observed to avoid false positive or false negative results regarding biological activity. Among these factors are temperature, choice of solvents, hygiene of lab equipment (pipettes, incubation plates, glassware and ovens), packaging of extracts, solubilization of extracts, quality of water used in the tests, precision of the operator and pH of the solutions, the last often being neglected despite its fundamental importance. For example Haemonchus placei (Place) eggs are rendered unviable when incubated for 24 hours at pH ≤ 3.0. This is a problem when using some commercial formulations containing benzimidazoles, which are often diluted in strongly acid vehicles. The same applies to phytotherapeutic substances: the pH can interfere in the viability of the parasite at different development stages, and above all can interfere in the stability of the metabolites in solution. Therefore, it is important to keep the pH of the medium as near as possible to 7.0, which can be done using a phosphate-buffered saline (PBS) solution.

**Reports of anthelmintic efficacy of phytotherapeutic substances: in vivo tests**

Leaves and fruits of Myrsine africana (Linnaeus) and Rapanea melanophloeos (Linnaeus), used as anthelmintics in rural communities in Kenya, were evaluated for activity by the egg count reduction test in the feces of male lambs artificially infected with H. contortus. The extracts were obtained from concoction of leaves (125 g/500 mL of water/animal) and fruits (50 g/250 mL of water/animal) of M. africana and R. melanophloeos and the resulting extracts were given orally to the animals in a single dose. No anthelmintic effect was observed for either of the plants evaluated[74]. Despite these results, the authors mentioned that this does not mean the leaves and fruits of the species evaluated do not have anthelmintic activity, because intrinsic factors of the plants such as vegetative phase and age, besides environmental factors, can influence the composition and concentration of chemical compounds from the exemplars tested; Thus when testing the same species grown under other climate conditions, in different geographic locations or in other vegetative phases, the results can be different. Besides this, some plants can be active only against certain parasite species and not against others. Finally, helminths can be naturally resistant to determined substances in the plants tested, making it advantageous to test mixed isolates in investigative studies of medicinal plants.

Another complicating factor is the possible differences in susceptibility to plant metabolites among strains of the same species of parasite from different geographic regions, as in the case of H. contortus. Strains of that species from different regions showed different sensitivity levels to tannins from Accacia pennatula (Schltdl. & Cham.) Benth. and Onobrychis viciifolia (Scop.), although this difference in sensitivity was not related to geographic region[77], since strains from nearby regions behaved differently when exposed to these polyphenols. Therefore, it is not possible to relate the collection area of a plant rich in tannins with its activity on parasite strains from distant areas, demystifying the idea that a sample of tannin or other metabolite from a tropical area should be more active against parasites from temperate areas, for example. The differences in anthelmintic activity can also be related to the development stage of the parasite[74].

Besides factors related to the environment, parasites and plants, consideration should also go to those related to the animals, especially in the case of ruminants. The ruminal flora can have a strong influence on the activity of substances administered orally, so that chemical substances indicated as bioactive through in vitro assays can undergo structural changes, losing their activity in the rumen[78]. This can explain the failures of some in vivo tests of substances known to be bioactive. Therefore, other administration routes should be evaluated, aiming to eliminate the influence of the ruminal flora. Also, great care should be taken in storing extracts to prevent their degradation.

The anthelmintic potential of essential oils from Eucalyptus citriodora (Hook.) in free form and nanoencapsulated (chitosan matrix) was evaluated for reduction of egg count in feces of naturally infected sheep, given in a single oral dose. The efficacy levels were similar, close to 50%, on the tenth day after treatment. This was the maximum efficacy, so the protective effect of nanoencapsulation was not observed. Although no increase in efficacy was found, other matrixes for formation of nanoparticles can be investigated[79], such as the use of calcium detergents and starch, among others.
The anthelmintic activity of powdered bark of *Albizia anthelmintica* (Brongn.), given orally, to naturally infected sheep was demonstrated, with fecal egg count reductions varying from 66.5 to 78.3%[80].

A dose-dependent anthelmintic effect of a mixture of different aboveground parts (except thorns) of *Cereus jamacaru* (P.D.C.) was observed on *H. contortus* and *T. colubriformis* in artificially infected sheep, with reduction between 18 and 65% in the fecal egg counts after weekly doses of 64.6g/sheep for six weeks[81].

Most in vivo investigations of plants with potential anthelmintic activity have involved the fecal egg count reduction test, which is practical and rapid, but has low sensitivity[82] and accuracy[83]. When a reduction of the egg count is not observed, various factors can be involved, so the plant should not be considered inactive. Likewise, when a reduction in the egg count is observed, this does not necessarily mean a reduction of the parasite load, because it can be related to a reduction of the fecundity of the parasites[84]. This does not diminish the importance of plants with this activity, since it helps reduce environmental contamination.

The difference between the effect of reducing fecundity and elimination of the parasite from the animal, excluding plants that act only inhibiting oviposition, is related to the concentration and nature of the secondary metabolites and the time of the parasites’ exposure to the plant compounds[84]. This time can be only a few weeks, possibly explaining failures with many plants, especially when the test substance is given in a single dose. In this case, the activity is nutraceutical rather than phytotherapeutic[29], and the evaluation methods are different for these distinct strategies. Therefore, conclusive assessments of the anthelmintic action of phytotherapeutic substances in in vivo models should include controlled anthelmintic tests.

It should be mentioned that many phytotherapeutic compounds can have toxic, effects, especially when administered in large quantities to production animals. For instance, the secondary metabolites present in *Jatropha curcas* (Linnaeus) cause alterations in the circulatory, respiratory and gastrointestinal tracts of goats[85].

Therefore, toxicity assays should be conducted before in vivo tests[25], to provide knowledge of the potential toxicity for establishment of the concentrations of the phytotherapeutic substances to be given to the animals, to prevent undesirable effects on the hosts.

**CONCLUSIONS**

Research involving plants with nematicidal activity can reveal potential sources of chemical substances that can be used in auxiliary activities to control GINs in ruminants and also to find new anthelmintic molecules.

The main difficulty of research with phytotherapeutic substances is not the identification of bioactive compounds. The main problem is that promising in vitro test results may not be borne out with the same magnitude through in vivo tests. This is due to the chemical variations of the plants and physiology of the digestive tract of ruminants, which can impede the biological activity of a given metabolite, or even because of the inadequacy of the methods used, leading to divergent results, as happens in quantification of eggs and the parasite loads of treated animals.

Most in vivo studies involve oral administration of compounds, and since ruminants have a very efficient fermentation chamber, a large portion of the metabolites can be rapidly degraded into inert compounds. Nanotechnology can be an alternative for controlled drug release, along with other ways to protect the metabolites from being degraded by the ruminal microbiota, allowing them to remain active in the abomasum and intestine. Another alternative is administration of extract preparations by routes other than oral.

The isolation of bioactive substances does not always lead to good results, because many metabolites reach their highest activity level when associated with others. Therefore, new research lines should be developed, seeking to better understand the chemical relations between metabolites and their influences on biological activity.

**REFERENCES**


