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A literature review of plants with antiparasitic properties against horse endoparasites

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Abstract. In light of the expansion of anthelmintic resistance, this literature review focusses on plant use against common endoparasites in horses: strongyles, cyathostomins, and ascarids, all of which negatively impact the horse's quality of life. Evidence-based literature from databases such as *ScienceDirect* and *PubMed* and search engines such as *GoogleScholar* was selected, and publications that met the criteria were included in the review. The plants included in the review are native species in Europe or can be easily grown in the provided climate. The search provided a total of 5936 publications from which, after evaluation, only 12 were included in the review. The main aim of the research was to compare the efficacy of selected plants by evaluating the information of the study design, a plant component or preparation and its concentration used in the investigation, and the results obtained, while also describing the bioactive compounds that are responsible for the potential antiparasitic effect. The search covered commonly mentioned plants with anthelmintic potential for equines, and in the result, only eight of the plants had their effectiveness evaluated in the form of research, *Artemisia absinthium, Hippophae rhamnoides, Onobrychis viciifolia, Allium sativum, Inula helenium, Zingiber officinale, Cichorium intybus* and *Pimpinella* anisum. The results emphasize the necessity for further explorations to be made - the results tend to be inconsistent with each other, showing that there might be a possibility for the plants to become a part of routine anthelmintic therapy, but many of the findings have not been convincing enough so far.

Key words: horses, herbal medicine, parasites, ethnoveterinary, strongyles, cyathostmins, ascarids.

INTRODUCTION

Anthelmintic resistance has been an increasing issue for livestock owners. Since no new anthelmintics have been discovered for over 40 years, current options, benzimidazoles (fenbendazole, oxibendazole), tetrahydropyrimidines (pyrantel) and macrocyclic lactones (ivermectin, moxidectin) - have been used so widely that parasites have adapted to their mechanisms of action over time, making them less efficient. Today, most of the cyathostomin population is resistant not only to benzimidazoles and tetrahydropyrimidines but also to macrocyclic lactones (Nielsen, 2022).

The main reason for this development is the frequent use of anthelmintics as a prophylactic treatment to prevent parasite infection, as well as a treatment plan where the whole herd is treated at the same time. Another reason is the underdosing, which is mainly due to the administration of treatment through visual evaluation of the horse's weight, which prevents the drug from reaching its therapeutic dose, resulting in the survival of parasites that further develop anthelmintic resistance mechanisms. Most parasites become resistant by up-regulating cellular efflux mechanisms, increasing drug metabolism, or intervening with binding by either changing the response or decreasing the count of receptors (Fissiha & Kinde, 2021).

The most prevalent horse parasites are cyathostomins, also known as small strongyles. They can invade the mucous layer of the caecum and colon, forming a fibrous capsule - if the larva does not develop into further stages, hypobiosis can occur, causing local inflammation. In more severe cases, reactivation from the hypobiotic state can cause mass emergence of larva from the intestinal mucosa, causing larval cyathostominosis (Konstantinović, 2024). The symptoms are characterized by diarrhea, weight loss, abdominal distention, and severe typhocolitis that leads to a 50% mortality rate (Love et al., 1999; Walshe et al., 2021).

Another commonly encountered parasite is *Parascaris equorum* that affects mostly foals up to 18 months of age, because it is possible to gain acquired immunity after being infected for the first time. Parascaridosis manifests itself by causing weight loss or failure to thrive, and, in more severe cases, death (Kane, 2017).

Large strongyles are becoming less prevalent nowadays, with their most familiar representative being *Strongylus vulgaris* which infects the small intestine, causing a condition known as strongylosis (Negash et al., 2021). While migrating through the digestive tract after being ingested, they tend to damage the tissue of digestive organs, causing symptoms such as colic that can range from mild to severe. While migrating, parasites also damage the intima of blood vessels, causing vasculitis, which can progress to the development of blood clots and haemorrhagic infarctions, leading to death (Jürgenschellert et al., 2022).

Phytotherapy has the potential to become a part of the anthelmintic therapy routine- if proven effective, it could introduce new remedies, reducing the spread of anthelmintic resistance in relation to treatments that have been widely used so far. Plant secondary metabolites can be found in essential oils, aqueous and alcoholic extracts, as well as fresh pants or their parts (Štrbac et al., 2023). In contrast to primary metabolites who are responsible for maintaining a healthy cellular function, the role of secondary metabolites is the mediation of environmental response, regulation of metabolism and organismal processes. Phenolics, terpenes, and nitrogen-containing compounds are some of the widely distributed groups of secondary metabolites (Catana et al., 2017).

Phenolic compounds, flavonoids, condensed tannins (proanthocyanidins), hydrolysable tannins (gallotanins and ellagitannins), alkaloids, and phenolic acids are the secondary metabolites mentioned most in connection with anthelmintic activity (Spiegler et al., 2017). Flavonoids and tannins are believed to be capable of blocking phosphorylation reactions in parasites and inhibiting energy metabolism, leading to their death. Tannins additionally work by preventing proper nutrient ingestion from the host's cell or, when ingested, by larvae, by causing autolysis by binding to the parasite's intestinal mucosa (Symeonidou et al., 2018).

The main aim of the study was to analyse the available evidence-based publications to evaluate whether phytotherapy has a potential of becoming a part of anthelmintic therapy or prophylaxis routine. It is theorised that plant- based therapy could be a solution to the increasing concerns of the anthelmintic resistance. Incorporating phytotherapy in daily management of endoparasites could also provide economical benefits to farmers since some of the plants can be grown locally.

Of the 12 publications reviewed, information was obtained about 17 different plant preparations used for research. Pellets were the most widely used plant preparation (23%), followed by hydroalcoholic extracts prepared with ethanol or an unspecified solvent (17%). Fresh or dried plants (12%), methanolic extracts (12%), aqueous extracts (12%) and decoctions (12%) were used for an equal number of tests. For the *in vitro* research on the efficacy of sainfoin pellets, a solution was obtained with tap water (6%) or dimethylsulfoxide (6%) as a solvent.

There is significant gap between folk knowledge and science-based conclusions on plant use as anthelmintic remedies. This study tries to compile available information and evaluate plant extract use and level of evidence. Thus, showing directions for future studies.

Reduction of helminth resistance and use of natural remedies is one of the one health for all initiatives. This study focusses on herbal remedies that have shown potential anthelmintic properties and describes the role of their biologically active compounds and preparation methods to reach a therapeutic effect.

MATERIALS AND METHODS

Before searching for eligible publications, inclusion and exclusion criteria were defined for the results to be suitable for the main objectives of the review. The publications included had horses and their characteristic endoparasites as the main study object, the research was carried out *in vivo* or *in vitro* and described plants that are representative of Europe and its climate. Exclusion criteria stated that literature that was not available in English or focused on other species was not used in the creation of the final review. Publications that described the anthelmintic properties of plants that cannot be found in Europe were also excluded, as well as those that did not conduct research using *in vivo* or *in vitro* methods, did not describe the plant parts or preparation that were used or the parameters that described the effectiveness of treatment. Due to the limited number of sources, the publication date was not considered when testing for eligibility. Each record was screened by one reviewer. To ensure the accuracy of the results found, double verification was performed by checking the available records twice within one day of the previous search.

The search was carried out in the *ScienceDirect* and PubMed databases as well as the *Google Scholar* search engine, using keywords that consisted of the botanical name of the plant in English or Latin and the combination 'AND horse AND anthelmintic'. During the screening, a protocol was filled, providing the information of the total number of entries obtained for each keyword, separating the non-eligible entries and duplicates from the included publications. In total, the search provided 5,936 results, of which

80 duplicates and 214 entries that were not available in English were excluded during the identification phase (Page et al., 2021).

Of the eleven plants that were commonly mentioned in ethnoveterinary records as a potentially effective remedy against endoparasite invasion, the authors were able to find evidence-based research for eight of them *(Artemisia absinthium, Hippophae rhamnoides, Onobrychis viciifolia, Allium sativum, Inula helenium, Zingiber officinale, Cichorium intybus, Pimpinella anisum),* excluding *Gentiana lutea, Gentiana asclepidaea* and *Thymus vulgaris* from the further study. (Lynn, 2006, Peachey et al., 2015, Grimm et al., 2022).

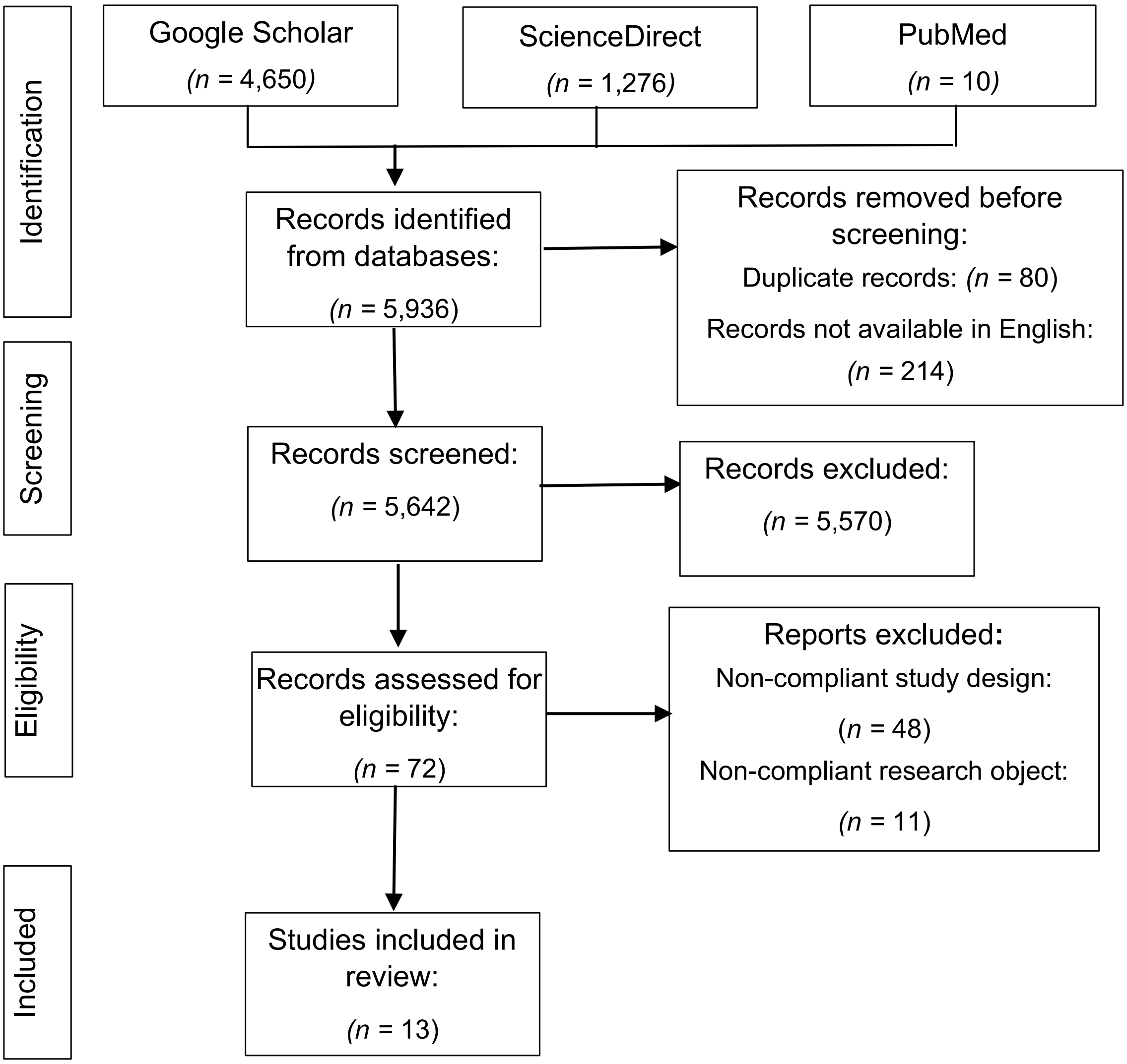

Figure 1. PRISMA flow diagram.

During the screening phase, the compliance of the publications with keywords was evaluated, marking 5,570 publications as non-eligible for further use due to either not suitable study design or providing information on the wrong study object - for example, other farm animals. Lastly, after careful evaluation of the study design and the research object, of 72 publications, only 12 were included in the final review (Fig. 1).

RESULTS AND DISCUSSION

One of the most researched plants with antiparasitic potential is sainfoin (*Onobychis viciifolia*). The legume is rich in polyphenols, making it not only a worthy food supplement but also provides it with the ability to affect the motility of larvae (Grimm et al., 2022). Proanthocyanidins, which are a type of condensed tannins, are among the primary polyphenolic compounds known for their anthelmintic activity. Due to the protein- binding ability, tannins do not degrade in the stomach, making their way to the small intestine, where they promote the absorption of amino acids. The increased number of amino acids helps to strengthen the host's immune system, boosting its ability to fight parasitic infection (Mukherjee et al., 2016).

Of five selected publications, four used *in vivo* methods, exploring the anthelmintic efficacy of commercial dehydrated sainfoin pellets. An overview of the study methods and plant preparations is provided below (Table 2).

Grimm et al. (2022) performed a study in which horses were separated into three groups with a control group receiving a standard diet consisting of hay and wheat bran and two groups that received 8.5% or 17.0% of their daily dry matter intake (% DM intake) of commercially purchased dehydrated sainfoin pellets in addition to the standard diet, substituting the exact percentage of dry matter intake for wheat. The study lasted 84 days and samples were collected weekly. On the 28th day, the horses were treated with fenbendazole. During the preanthelmintic treatment period, FEC (faecal egg count) was the lowest in the group fed with the highest sainfoin content, followed by the other group of horses fed sainfoin, while in the control group the FEC value was the highest. To evaluate the results of the post-anthelmintic treatment period with fenbendazole, FECRT (faecal egg reduction count test) was performed, and the results indicated its reduced efficacy, with the egg reduction count reaching only 67%, which was not affected by diet choices, as the result was similar in all three groups. Regarding FEC values after fenbendazole treatment, no dietary impact on the results was observed. While a decrease in larval motility was observed in all groups during the 84-day trial period, larvae obtained from horses fed sainfoin reached a significantly lower motility score than those in the control group. Reduced motility could help reduce pasture contamination in the future (Grimm et al., 2022).

Malsa et al. (2022) evaluated the effect of sainfoin on cyathostomins in a 78-day *in vivo* trial. The treatment group received dehydrated sainfoin pellets and the control group was fed alfalfa pellets for a 21-day period with weekly collection of faecal samples. For the FEC assay, despite the 35% decrease during the 3-week trial period, the result did not differ significantly from the control group. In the case of LDA (larval development assay), the decrease in development was similar in both groups. However, sainfoin-fed horses had a lower increase in larval development between day 14 and day 21. At the end of the experimental diet, all horses were treated with ivermectin, and ERP (egg reappearance period) was evaluated according to FECR calculations. In both groups, the cyathostomin eggs reappeared 42 days after receiving anthelmintic therapy. On day 78 the sainfoin group had a lower FECR value (89.6%) than the control group (98.9%) and excreted more parasite eggs. This observation could be explained by the fact that sainfoin appears to have a negative impact on the concentration of ivermectin in plasma by reducing its maximum concentration (Malsa et al., 2022).

Collas et al. (2018) conducted combined research, including both *in vivo* and *in vitro* methods, and exploring the impact of sainfoin on horse strongyles. For the in vivo assay, the horses were divided into three groups and fed commercial sainfoin pellets, a high protein diet or a control diet for 18 days. The egg count decreased significantly in all three groups with no significant differences between the diet groups, and the number of expelled nematodes also did not vary much between the groups. For LDA, 700 g of sainfoin pellets were added to 422 mL of tap water, obtaining four concentrations. Although lower concentrations of sainfoin did not show considerable efficacy, 29% of the added sainfoin decreased larval development by 82%. In the case of EHA (egg hatching assay), samples were prepared from 42 g of sainfoin pellets and 420 mL of 0.5% dimethyl sulfoxide. Reduced hatching (by 37%) was observed at a concentration of 7.5 mg mL^{-1} and above (Collas et al., 2018).

Laroche et al. (2024) performed the most recent *in vitro* study. Four diet plans were used: high-fibre or high-starch diet alone as control groups or combined with manufactured sainfoin pellets. The results of the larval motility assay indicated that the larvae obtained from horses fed sainfoin were less mobile. FEC was consistent in the group of horses that received a high-fibre diet, but an increase in FEC was observed with time in the group fed a high starch content. Adding sainfoin pellets to the high-starch diet for a group receiving both seemed to slow down the increase in egg excretion. However, the additional sainfoin did not affect egg excretion in the high-fibre group (Laroche et al., 2024).

Obtained information is quite inconsistent: while one study demonstrates a decrease in FEC for horses fed with sainfoin (Grimm et al., 2022), other three did not find any significant impact on this parameter (Collas et al., 2018; Malsa et al., 2022; Laroche et al., 2024). The effect on ERP and FECRT after treatment with standard anthelmintics was also not sufficient and an interaction between ivermectin and sainfoin was observed, raising concerns about the possibility of cotreatment in the future. However, in all publications that observed the impact on larval motility, fewer mobile larvae were detected in groups fed sainfoin.

Regarding *Parascaris equorum*, *in vitro* research was conducted, comparing the efficacy between the aqueous extract of commercial sainfoin stems and leaves and thiabendazole. Despite the inhibition of embryonation and larval motility that increased along with the increase in sainfoin concentration, a significant count of eggs was still able to reach the 4th stage of development. For example, at the highest concentration of 1,000 μg mL^{-1}, the mortality rate reached approximately 26%, but 20% of the eggs were able to reach full development. However, the anthelmintic effect is still considered sustainable (George, 2022). Thiabendazole is believed to show a lower mortality rate, because its mechanism of action involves binding with β-tubulin, preventing microtubule polymerisation, therefore preventing eggs from entering the development stage, but not affecting them sufficiently strongly to cause death (Tydén et al., 2016). The larval mortality rate of sainfoin could be explained by the potential mechanism of action of condensed tannins: by binding to the egg surface and damaging it, the attempt to repair damage causes depletion of the energy reserves of the egg, decreasing the development and chances of survival (Vargas-Magaña et al., 2014).

Garlic (*Allium sativum*) is often mentioned in folk medicine because of its antibacterial properties and its activity as a vermifuge (Lynn, 2006). The main bioactive compound is allicin, an organic sulphur compound that only exists in freshly cut or crushed garlic bulbs due to its need to be activated by an enzymatic reaction which is triggered by the injury of the bulb. Allicin is chemically unstable and can survive only for a few hours at room temperature before further metabolising (Londhe & Nipate, 2011).

The garlic extract was assessed against ascariasis *in vitro*. The results of the larval bioassay showed the lack of impact on larval motility at each concentration used (Rakhshandehroo et al., 2017).

The authors were able to find four publications on the ability of garlic to interact with horse strongyles. Cernea et al. (2009) conducted an *in vitro* study. In the case of the EHA test, complete inhibition of egg hatching was observed at the 6.25% concentration followed by 85.5% inhibition at a dilution of 3.15%. No correlation was observed between concentration and efficacy. The results of LDA indicated that the highest treatment efficacy was achieved at concentrations ranging from 6.25% to 25%, preventing the complete development of stage 3 larvae (Cernea et al., 2009). Tavassoli et al. (2018) performed *in vitro* research using a hydroalcoholic extract as well. After evaluating the larval count under the microscope, it was concluded that all concentrations of *Allium sativum* were effective against the larval stage of *Strongylus* spp., eliminating at least 82.75% of larvae during the 48-hour period. The concentration of 100 mg mL^{-1} showed the greatest effect by reaching a 100% larval death rate at the end of the 48-hour period (Tavassoli et al., 2018). The results of both studies are consistent with each other, showing the potential to completely inhibit larval development (Cernea et al., 2009, Tavassoli et al., 2018).

Buono et al. (2019) study observed the effects of garlic using an *in vivo* approach. Three study groups were involved: one received 40 g of fresh crushed garlic daily; another was fed 40 g of commercial dry garlic flakes along with 160 g of oat and an untreated control group. Despite the success previously mentioned in *in vitro* studies conducted by Cernea et al. (2009) and Tavassoli et al. (2018), *in vivo* results showed that no significant differences were found between treatment and control groups, with the presence of *Cyathostominae* and *S.vulgaris* in all samples. On day twenty-eight, the FECR values were 28.3% for the fresh garlic group and 13.7% for the dried garlic group, indicating a significant anthelmintic resistance, since FECRT values lower than 85% are considered resistant (Nielsen et al., 2013, Buono et al., 2019).

Sea buckthorn (*Hippophae rhamnoides*) is known to be rich in bioactive compounds. The juice from its fruit contains polyphenol derivatives, flavonoids gallocatechin, epicatechin, quercetin, kaempferol. Although the mechanism of their action has not been fully researched, it is theorised that flavonoids, especially quercetin, are more potent against endoparasites than tannins that are also present in buckthorn juice. Glycosilated quercetin has improved bioavailability and can bind to the eggshell of the nematode, causing a delay in osmosis that causes changes in protein metabolism that negatively affect egg hatching (Maestrini et al., 2022). Raileanu et al. (2015) using an *in vitro* approach observed that at the concentration of 50% of sea buckthorn decoction, the hatching percentage reached its peak of 98.59%, surpassing even the control group by 10%. The hatching percentage decreased along with the decrease in concentration, but the changes were not linear. The greatest inhibition was recorded at a concentration of 1.56%, where hatching was observed in only 2.44% of the eggs. During the LDA, a strong effect on larval development was observed, reaching even 1.59%

development with the 12.50% decoction. Like EHA, higher concentrations of decoction were less effective (Raileanu et al., 2015). Catana et al. (2017) performed another *in vitro* assay. For the EHA an average hatching percentage of 77.78% was observed. In the case of LDA, around 5.56% of the eggs reached the hatching stage, showing a MIC value of 0.1425%, which indicates that the efficacy of lower concentrations should be explored (Catana et al., 2017). When comparing the results of EHA, it is notable that a strong ability to prevent egg hatching was observed in both studies. However, the decoction showed a lower hatching percentage at almost all concentrations. When comparing the results of LDA, the average percentage of larval development for sea buckthorn decoction is 13,55%, but the aqueous extract showed a development of 5.56%, demonstrating a higher potency (Raileanu et al., 2015, Catana et al., 2017).

Wormwood (*Artemisia absinthium*) is attributed to vermifuge properties provided by essential oil containing monoterpenes thujone and thujole, sesquiterpene lactones (artemisinin), and flavonoids (Lynn, 2006). Caution is required when using wormwood or its extracts, since thujone is a neurotoxic compound. Due to the risk of neurotoxicity, it is not recommended for pregnant or lactating mares and should not be used long-term. It is also crucial not to exceed daily doses of products containing wormwood recommended by manufacturers (Poppenga, 2001). The oral LD50 (median lethal dose) value of thujone in mice has been determined as 192 mg kg^{-1} (Pelkonen et al., 2013). Compared to other plants, wormwood is one of the most researched natural anthelmintics with three *in vitro* studies available on horse strongyles (Cernea et al., 2009, Raileanu et al., 2015, Catana et al., 2017). Raileanu et al. (2015) conducted *in vitro* research, measuring the impact of the hydroalcoholic extract of wormwood on horse strongyles. The results of EHA indicated that higher concentrations of wormwood are less effective in the prevention of egg hatching, with the hatching percentage reaching its maximum (82.35%) at the 25% concentration, while inhibiting hatching completely at the 12.50% dilution. However, even the highest hatching percentages did not surpass the 88.57% hatching observed in the control group. For LDA, no linear correlation was observed with the minimal percentage of development (5.00%) reached at the 50% dilution, then increased significantly at concentrations ranging from 25% to 12.50% and then showed a decrease again. However, the development percentage was less than 40% in all cases, which was notably lower than observed in the control sample, where 91.94% of the larvae developed (Raileanu et al., 2015). Catana et al. (2017) explored the efficacy of aqueous extract. The EHA assay showed a limited ability to inhibit hatching, with the average hatching percentage being 79.12%, reaching a minimum at the 0.04% dilution. The large value of LC50 (lethal concentration 50) (685.3521%) indicated that for the tested strongyles population, extracts at a concentration greater than 25% should be used to achieve a sufficient effect. Since the MIC value from LDA was negative (-1.8535%), lower concentrations have to be explored to assess the efficacy of wormwood (Catana et al., 2017). Cernea et al. (2009) also used hydroalcoholic extract and observed that the minimum hatching percentage during EHA was at the maximum concentration of 25%, but even then, it reached 64.29%. The decrease in concentrations caused a notable increase in egg hatching, reaching 100% at the lowest concentrations (0.78% and 1.56%), which exceeded even the percentage in the control group (93.81%). With the LC50 value calculated as 35.9413%, the authors described the efficacy in eggs as average. The LDA results showed that at 25% extract concentration, 0% of the larvae had developed. The efficacy decreased along with the decrease in concentration, reaching 100% larval development at concentrations of 1.56% and lower. The authors concluded that with an LC50 value of 22.7616%, wormwood demonstrates weak larvicidal activity (Cernea et al., 2009). It is notable that hydroalcoholic extracts showed better results than aqueous extract by exhibiting lower LC50 values (Table 1).

Table 1. Comparison of LC50 values between different preparations of Artemisia absinthium plants

Plant preparation	LC50 (%)		Reference
	EHA	LDA	
Hydroalcoholic extract	-5.3128	-3.2751	(Raileanu et al., 2015)
Aqueous extract	685.3521	-	(Catana et al., 2017)
Hydroalcoholic extract	35.9413	22.7616	(Cernea et al., 2009)

George (2022) focused on the interactions between the aqueous extract of wormwood and *Parascaris equorum*. The extract, obtained from commercial leaf and stem mix, was compared with two control groups, water and thiabendazole. An egg development assay concluded that along with an increase in wormwood concentration, the number of dead or unembryonated eggs increased. The percentage of dead larvae exceeded the percentage of dead larvae in the thiabendazole group in all concentrations of wormwood extract. However, the number of unembryonated larvae was larger in the thiabendazole group (George, 2022).

The leaves, roots and rhizomes of chicory (*Cichorium intybus*) contain sesquiterpene lactones (lactucin, lactucopicrin, 8-deoxylactucin) which not only provide the bitter taste of this plant but are also the main bioactive compounds regarding anthelmintic activity (Peña-Espinoza et al., 2018). Although not explored completely, it is theorised that the mechanism of action of sesquiterpene lactones is the inhibition of enzyme acetylcholinesterase which leads to neuromuscular paralysis and parasites death (Valente et al., 2021). Chicory also contains condensed tannins, although their concentration is comparatively low, flavonoids (anthocyanins, kaempferol and quercetin) (Rodríguez-Hernández et al., 2023). The effect of chicory on horse cyathostomins has been evaluated in a study, published by Malsa et al. (2024) During the 45-day period, two groups of naturally infected horses were grazing either in a pasture sown with chicory or a mesophile grassland. For the *in vivo* part of this research, FEC was performed and the results indicate an immense potential for chicory as the future anthelmintic treatment, since its FEC value at the end of the trial was only 13.8% lower than for horses treated with pyrantel. Furthermore, the reduction in FEC began around the 16th day of the study, leading to the idea that shorter treatments would also be effective. The LDA results did not differ significantly between day 0 and day 16th, but further on showed a lower percentage of developed larvae in chicory group, with the final count of developed larvae being 2.9 times lower than in control group. For the *in vitro* experiment, LDA was performed on the cyathostomin isolate, evaluating its sensitivity to methanolic sesquiterpene extract, obtained from fresh chicory leaves. The isolate containing pyrantel-sensitive parasites responded to treatment differently than the isolate containing pyrantel-resistant parasites (Malsa et al., 2024).

Ginger (*Zingiber officinale*) has a capacity to increase immunity in conjunction with its potential parasiticidal activity (Lynn, 2006). Ginger root contains polyphenol derivatives (gingerenone, dehydrogingerdione, shogaol, gingerol, and hexahydrocurcumin) that have been proven to suppress tapeworm (*Hymenolepis nana*) invasion by causing paralysis of these nematodes that are primarily affecting humans (Lin et al., 2014). An *in vitro* study, carried out by Catana et al. (2017), has been published, and observed the aqueous extract of ginger. The results of the EHA test showed that the average strongyles egg hatching percentage was 73.1%, which was less than in the control sample without extract added. With the LC50 value calculated in negative values, a strong inhibitory effect is determined, implying a need to assess lower extract concentrations. In the case of LDA, the ability of ginger to inhibit the development of L3 larvae was determined with the minimal inhibitory concentration (MIC) reaching negative values; indicates that ginger can affect larval development even in incredibly low concentrations (Catana et al., 2017).

While the anthelmintic properties of anise (*Pimpinella anisum*) have not yet been thoroughly described, it is theorised that the bioactive compounds found in the essential oil of anise seeds play a role in the eradication of parasites. One of the most important bioactive compounds is anethole, which not only gives the seeds their signature scent but also provides an effect as a vermifuge (Sun et al., 2019). Its estragole isomer, in combination with linalool, showed a strong ovicidal effect against sheep nematodes (Štrbac et al., 2021). Catana et al. (2017) evaluated the potency of anise against equine strongyles. A control group was used with no added decoction. In the case of the EHA test, no significant activity was observed, showing a limited ability to inhibit egg hatching, with the average hatching percentage being 80.56%. However, the anise decoction showed maximum efficacy in LDA compared to other plants tested, with the MIC value -0.2623% (Catana et al., 2017).

Elecampane (*Inula helenium*) rhizomes and roots are filled with sesquiterpene lactones (alantolactone, isoalantolactone) and polysaccharide inulin (Stojanović -Radić et al., 2012, Zhao et al., 2024). Using a hydroalcoholic solution, a strong inhibitory effect on hatching was observed during the EHA test - at the dilution of 25%, only 8.33% of the eggs hatched. The effectiveness decreased along with the decrease in concentration, but the hatching percentage remained relatively low until the concentration went below 3.12% - and even then, it was lower than in the control group. The LDA also showed promising results: the lowest hatching percentage was observed at the 12.50% concentration, with only 9.52% of larvae having developed further than the L1 stage (Cernea et al., 2009).

The evidence base for the anthelmintic potential of eleven plants was inspected and authors were able to find publications for eight of them to create a summary (Table 2).

Table 2. Plants used for horse endoparasites

Scientific name	Bioactive compounds with anthelmintic activity	Plant preparation	Dosage	Study design*	Parasites family	Reference
Allium sativum	Volatile organic sulfur derivative (Allicin)	Hydroalcoholic extract	1.25, 2.5, 5, 10, 50, 100 mg mL^{-1}	*In vitro* (LDA)	*Strongylidae*	(Tavassoli et al., 2018)
		Hydroalcoholic extract	0.78% 1.56%, 3.12%, 6.25%. 12.5%, 25%	*In vitro* (LDA, EHA)	*Strongylidae*	(Cernea et al., 2009)
		Methanolic extract from the bulbous part	50, 70, 100, 125 mg mL^{-1}	*In vitro* (LB)	*Ascarididae*	(Rakhshandehroo et al., 2017)
	Monoterpenes (thujone), sesquiterpene lactones (artemisin), flavonoids, condensed tannins	Hydroalcoholic extract	1.56%, 3.12%, 6.25%, 12.50%, 25%, 50%	*In vitro* (EHA, LDA)	*Strongylidae*	(Raileanu et al., 2015
		Aqueous extract from stems and leaves	100–1,000 µg mL^{-1}	*In vitro* (egg development test)	*Ascarididae*	(George, 2022)
		Hydroalcoholic extract	0.78% 1.56%, 3.12%, 6.25%. 12.5%, 25%	*In vitro* (EHA, LDA)	*Strongylidae*	(Cernea et al., 2009)
Cichorium intybus	Sesquiterpene lactones (lactucin, lactucopricin), flavonoids (anthocyanin, kaempferol, quercetin)	Pasture sown with chicory	89% of the bites	*In vivo* (FEC, FECRT, LDA)	*Strongylidae*	(Malsa et al., 2024)
		Methanolic extract of sesquiterpene lactones derived from fresh chicory leaves	1–5,000 µg mL^{-1}	*In vitro* (LDA)		
Hippophae rhamnoides	Flavonoids (gallocatechin, epicatechin, quercetin, kaempferol), condensed tannins, hydrolyzable tannins	Decoction	1.56%, 3.12%, 6.25%, 12.50%, 25%, 50%	*In vitro* (EHA, LDA)	*Strongylidae*	(Raileanu et al., 2015)
		Aqueous extract	From 0.04% to 50%	*In vitro* (FEC, LDA)	*Strongylidae*	(Catana et al., 2017)

Table 2 (continued)

Inula helenium	Sesquiterpene lactones (alantolactone, isoalantolactone), monoterpenes, inulin	Hydroalcoholic extract	0.78% 1.56%, 3.12%, 6.25%. 12.5%, 25%,	*In vitro* (EHA, LDA)	*Strongylidae*	(Cernea et al., 2009)
Onobrychis viciifolia	Condensed tannins	Aqueous extract from stems and leaves	100–1,000 µg mL^{-1}	*In vitro* (egg development test)	*Ascarididae*	(George, 2022)
		Dehydrated sainfoin pellets, commercial	8.5 or 17.0 (% DM intake)	*In vivo* (FEC, FECRT, larval motility assay)	*Strongylidae*	(Grimm et al., 2022)
		Dehydrated sainfoin pellets, commercial	70.0 (% DM intake)	*In vivo* (FEC, LDA, ERP)	*Strongylidae*	(Malsa et al., 2022)
		Dehydrated sainfoin pellets, commercial	14.2 (% DM intake or 17.6 (% DM intake)	*In vivo* (FEC, larval motility assay)	*Strongylidae*	(Laroche et al., 2024)
		Dehydrated sainfoin pellets, commercial	70.0 (% DM intake)	*In vivo* (FEC, nematode expulsion)	*Strongylidae*	(Collas et al., 2018)
		Water or DMSO solution from sainfoin pellets	LDA: 0%, 6%, 12%, 19%; EHA: 3.6, 7.7, 15, 30 mg mL^{-1}	*In vitro* (LDA, EHA)		
Pimpinella anisum	Phenylpropanoids from essential oil (anethole, estragole)	Decoction	From 0.04% to 50%	*In vitro* (FEC, LDA)	*Strongylidae*	(Catana et al., 2017)
Zingiber officinale	Polyphenol derivatives (gingerenone, dehydrogingerdione, shogaol, gingerol, hexahydrocurcumin)	Aqueous extract	From 0.04% to 50%	*In vitro* (FEC, LDA)	*Strongylidae*	(Catana et al., 2017)

*Study methods: FEC = faecal egg count; LDA = larval development assay; FECRT = faecal egg reduction test; EHA – egg hatching assay; LB – larval bioassay; ERP – egg reappearance period.

The obtained literature portrays the predominance of *in vitro* assays when evaluating the anthelmintic effect (50% *in vitro*, 33% *in vivo*, 17% combined studies). While *in vitro* studies tend to be safer for living organisms, the controlled environment may fail to replicate the conditions in a living organism, providing inaccurate results (Graudejus et al., 2018). The inconsistency of the results has also been noticed in the reviewed publications. While *Allium sativum* showed a strong inhibitory effect on larval development and egg hatching *in vitro,* results from a study using live horses indicated a diminished ability to reduce faecal egg count (Cernea et al., 2009; Rakhshandehroo et al., 2017; Tavassoli et al., 2018; Buono et al., 2019). Similar inconsistency was observed in the case of *Onobrychis viciifolia*: while the *in vitro* results indicated a good effect on FEC and LDA, the vivo observations showed insufficient ability in FECRT measurements and nematode expulsion after additional pharmacotherapy. However, the results of both study designs indicated the ability of sainfoin to affect larvae and their motility (Collas et al., 2018; Grimm et al., 2022; Malsa et al., 2022; Laroche et al., 2024).

The sea buckthorn demonstrated a strong potency *in vitro* against strongyles, inhibiting larval development and egg hatching (Raileanu et al., 2015; Catana et al., 2017). *In vitro* studies of elecampane demonstrated an ability to prevent egg hatching by more than 90% and the same level of efficacy was observed in the larval development assay (Cernea et al., 2009). Decoction of anise and aqueous extract of ginger were tested within the scope of the same *in vitro* study and both showed weak impact on strongyles egg hatching but were effective in the prevention of larval development. (Catana et al., 2017) The efficacy of wormwood varied between publications: 33.3% of studies exhibited a good ability to inhibit egg hatching while other 66.7% proved the lack of efficacy in the provided concentrations. Similar situation was observed regarding larval development: two-thirds of publications proved wormwood to be efficient and able to inhibit larval development up to 100%, while in the third study larvicidal effect was labelled as weak (Cernea et al., 2009; Raileanu et al., 2015; Catana et al., 2017). Regarding obtained results about wormwood, it is important to note that all the described results were obtained from *in vitro* studies and to draw more statistically significant conclusions, an *in vivo* study is needed. The diversity of FEC results between *in vitro* and *in vivo* studies in garlic and sainfoin also emphasizes the necessity for expanded *in vivo* research in the future.

While the focus is put on the eradication of *Strongylidae* due to higher mortality rate, natural remedies of *Parascaris equorum* should also be sought. While the methanolic extract of garlic did not provide the desired larvicidal effect, two aqueous extracts obtained from sainfoin, or wormwood were sufficient (George, 2022).

When comparing the effectiveness of different preparations, it should be considered that faecal samples containing parasite eggs were collected at geographically diverse places and it is known that the anthelmintic resistance and sensitivity to therapy might vary not only by regions, but also in herds nearby each other. For most plants, the comparison of preparations was not possible due to different study designs, measured parameters or parasite species. However, a comparison between hydroalcoholic and aqueous extracts of wormwood was possible, since all three *in vitro* studies were focused on *Strongylidae* and used EHA for the evaluation. When comparing LC50 values, significantly lower concentrations of hydroalcoholic extracts were needed to reach lethal concentration 50% for the EHA test (Cernea et al., 2009; Raileanu et al., 2015; Catana et al., 2017).

Regarding secondary metabolites, it is difficult to attribute the anthelmintic properties to just one biologically active compound, since in most plants the composition is quite complex. However, when observing the biologically active substance content in plants with the most promising study outcomes, a presence of either sesquiterpene lactones, flavonoids, tannins or them combined was detected in all of them.

While the evidence bases to support the future of plant-based remedies needs further expansion, the acquired results and centuries-long use in folk medicine justify the need for further research in the form of *in vivo* studies and the exploration of more plant preparations. With the increasing anthelmintic resistance against routine anthelmintics and lack of new pharmacological treatments, herbal medicine has an immense potential as an aid in parasiticidal treatment.

CONCLUSIONS

After summarising the records obtained of bioactive compounds that are responsible for the anthelmintic activity of previously mentioned plants, the most mentioned secondary metabolites are condensed tannins (20%), flavonoids (20%) and sesquiterpene lactones (20%), followed by monoterpenes found in essential oils (15%) and phenol derivatives (10%). Less common biologically active groups with antiparasitic properties are polysaccharides (5%), sulphur derivatives (5%) and another essential oil component, phenylpropanoids (5%).

From the records available in the literature, chicory turned out to be the most efficient remedy against strongyles, exhibiting promising results both *in vivo* and *in vitro*, suggesting that enriching pastures with chicory might be helpful in the prevention and eradication of strongyles.

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