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In vitro anthelmintic activity of hydroethanolic extract of *Artemisia herba aba* and *Juniperus phoenicea* on abomasal *Teladorsagia circumcincta* of sheep

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Abstract: The present study aimed to evaluate the *in vitro* anthelmintic activity of *Juniperus phoenicea* and *Artemisia herba-alba* against abomasal nematodes collected from naturally infected sheep. The anthelmintic activities of the extracts were assessed using egg hatch inhibition (EHIA) and adult motility assays (AMA) against *Teladorsagia circumcincta*. The parasites were stored in phosphate-buffered saline (PBS) until the *in vitro* assessment began. The leaf powders of *J. phoenicea* and *A. herba-alba* were extracted using the maceration procedure. Briefly, the leaf powders were mixed with a sufficient quantity of 99% ethanol and distilled water (80%/20%). The extracts were dissolved in a 3% dimethyl sulfoxide (DMSO) solution to improve water solubility. The hydroethanolic extracts were tested at concentrations of 1.562, 3.125, 6.25, 12.5, and 25 mg/ml. Adult motile worms were individually subjected to each treatment at laboratory temperature. The PBS and albendazole (25 mg/ml) were prepared and used as negative and positive controls, respectively. The test was repeated five times for each concentration. The results showed that both plant extracts exhibited anthelmintic effects. *A. herba-alba* extract at 25 mg/ml inhibited the motility of all *T. circumcincta* adult worms within 1 hour of administration, while *J. phoenicea* extract caused 100% mortality of adult worms after 4 hours at the same concentration. Neither of the extracts, at 1.562 mg/ml, had any inhibitory effect on motility. At 25 mg/ml, the *A. herba-alba* (98.67%) and *J. phoenicea* (96.24%) showed high activity with an inhibitory effect on hatchability compared to the other tested concentrations. The findings of the present study showed that hydroethanolic extracts of *A. herba-alba* and *J. phoenicea* leaves had *in vitro* anthelmintic activity on eggs and adult *T. circumcincta* of sheep.

Keywords: Phytotherapy; Botanical anthelmintics; Hydroethanolic extract; Abomasal nematode; Egg hatch test; Adult motility assay; Small ruminant

1. Introduction

Gastrointestinal nematode infestations significantly threaten livestock, particularly cattle, sheep, and goats. Recent research conducted in Algeria revealed that sheep are infested by various abomasal parasitic species (Zouyed et al., 2018). Despite the use of anthelmintics, the emergence of drug resistance in animals is becoming increasingly significant. For instance, *Teladorsagia*, *Trichostrongylus*, *Marshallagia*, and *Nematodirus* strongyles within five pilot farms in eastern Algeria were resistant to albendazole (Bentounsi et al., 2006), then found to be highly prevalent in pilot farms in the steppe zones of Eastern Algeria for benzimidazoles and ivermectin (Bentounsi et al., 2007). Another study in western Algeria focused on the link between the development of resistance to albendazole and the presence of *Marshallagia marshalli* in some agricultural establishments (Boulkaboul et al., 2010).

Therefore, combating anthelmintic resistance has become an important challenge that requires much research and reliable methods for the detection and testing of the efficacy of anthelmintic agents. *In vitro* tests are used to select plant species, their secondary metabolites, and ingredients that exhibit anthelmintic activity (Andre et al., 2017). There are several positive aspects of *in vitro* assays before *in vivo*, including less time-consuming, less expensive, needing a smaller number of animals, and permitting the evaluation of the efficacy of different anthelmintic compounds throughout the life cycle of the parasite (Demeler et al., 2013). Furthermore, The Adult Motility Assay (AMA) and Egg Hatch Inhibition Assay (EHIA) are preferred over *in vivo* approaches because they are cost-effective, simple, and provide immediate findings (De Jesús-Martínez et al., 2018).

Medicinal plants are widely used as single drugs or in combination delivery systems. The World Health Organization states that 80% of the global population depends on traditional medicine for their main healthcare need (WHO, 2003). Medicinal plant preparations containing bioactive compounds offer a promising alternative for treating and managing various health problems and infections. These preparations exhibit antimicrobial, antioxidant, anti-inflammatory, antipyretic, and immunostimulant properties (Laudato and Capasso, 2013). Algeria has a longstanding tradition of utilizing medicinal herbs, and the traditional herbal knowledge is passed from generation to generation (Boudjelal et al., 2010). In developing nations, traditional methods of controlling nematodes, used by small farmers, remain largely dependent on medicinal plants. Plants are a great source of naturally occurring substances that can be utilized as substitutes for dewormers in livestock (Ahmed et al., 2023).

Traditional veterinary knowledge remains crucial for sheep and goat farmers in the Algerian steppe. Many plant species are used in ethnoveterinary remedies (Miara et al., 2019). A recent study on the traditional use of medicinal plants for treating parasites in humans and animals in Algeria revealed plenty of ethnopharmacological knowledge. The most commonly mentioned medicinal plant is *A. herba-alba Asso* (Benlarbi et al., 2023), which is recognized for its antioxidant, anti-cancer, and anti-inflammatory properties (Khlifi et al., 2013), antifungal activity (Abu-Darwish et al., 2015), and antibacterial activities (Ouguirti et al., 2021). *J. phoenicea*, locally known as "Aràar," is a naturally widespread shrub in the Algerian steppe, become a commonly

used plant as an anthelmintic, antiseptic, and for treating wounds (Miara et al., 2019), and exhibits antioxidant and antibacterial activities (Ait-Mimoune et al., 2023) as well as antiproliferative properties (Kemal et al., 2023).

Several studies have documented the anthelmintic application of medicinal plants and these include *Juniperus communis* (Štrbac et al., 2020), *Azadirachta indica* (Rehman et al., 2023), *Acacia nilotica* (Zabre et al., 2023), *Combretum glutinosum* (Toklo et al., 2023), *Artemisia brevifolia* (Iqbal et al., 2004), *Juniperus pinchotii* (Armstrong et al., 2013), *J. phoenicea* (Aouadi et al., 2021), and some *Artemisia L. species* (Dağ et al., 2023).

Except for some studies that revealed antiparasitic activity of some chemical components of plants such as artemisinin (Wang et al., 2015), 1,8-cineole and camphor (Zhu et al., 2013), carvone (Katiki et al., 2019), cinnamaldehyde and limonene (Katiki et al., 2017), most of the studies reported the presence of key components of the plants which do not provide any information about the effective antiparasitic compounds and their mechanism of action. The anthelmintic components cited above appear in the composition of *A. herba-alba* (1,8-cineole, carvone, camphor, and cinnamaldehyde) and *J. phoenicea* (limonene), studied in Algeria by Zouaoui et al. (2020). The same study showed the dominance of α-pinene, which revealed antiparasitic activity in the study of Rodrigues et al. (2015).

In a study by Ali et al. (2021), some mechanisms of the nematicidal activity of medicinal plants against *H. contortus* were regrouped. These mechanisms led the parasite to a state of starvation, energy deprivation, and neuromuscular disorganization, and it became unable to survive inside the host. However, no information about the action mechanisms of *A. herba-alba*, *Artemisia capillaris*, and *Artemisia maritima* was provided.

Given the importance of using *A. herba-alba* and *J. phoenicea* in Algerian traditional therapy and the emergence of antiparasitic resistance, this is the first study in Algeria to evaluate the *in vitro* anthelmintic activity of these plants against *T. circumcincta* from naturally infected sheep using egg hatch inhibition (EHIA) and adult motility assays (AMA).

2. Materials and Methods

2.1. Collection of Plant Samples

The leaves of *J. phoenicea* and *A. herba-alba* were collected in the area of Guettatecha locality, M'sila province, Northern Algeria (35°47′02″N, 5°02′05″E). After collection, identification of the plants was made by a botanist at the herbarium of the agronomic department at Batna 1 University. The plants were left to dry at room temperature for a week to facilitate leaf recovery. Subsequently, the plant materials were pounded using a coffee grinder, resulting in a fine powder, and were kept in the dark for an *in vitro* anthelmintic test.

2.2. Plant Extract Preparation

The leaf powders of *J. phoenicea* and *A. herba-alba* were extracted using the maceration procedure. Briefly, the leaf powders were mixed with a sufficient quantity of 99% ethanol and distilled water (80%/20%) for 48 hours; then, the solutions were filtered through Whatman paper. The filtrates were placed in a rotary evaporator to prepare hydro-ethanolic extracts. The extracts were dissolved in a 3% dimethyl sulfoxide (DMSO) solution to improve water solubility, producing two stock solutions at a concentration of 25 mg/ml (100 ml volume). Several dilutions were made with varying concentrations (1.562, 3.125, 6.25, 12.5, and 25 mg/ml).

2.3. Parasite Collection

Adult parasites were collected from the abomasum of a slaughtered sheep. The abomasum was collected post-slaughter and transported to the veterinary parasitology laboratory at Batna University. In the laboratory, the abomasum was cleaned using a continuous flow of water, and the worms were separated by cutting the larger curve of the abomasa. The parasites were stored in phosphate-buffered saline (PBS) until the *in vitro* assessment began. According to the technique described by Cabaret et al. (1984), with modification, a microscopic cutting of the uterus of 150 adult *T. circumcincta* using a small scalpel to release the eggs was the method used, excluding the most famous nematode model *H. contortus* frequently used for *in vitro* studies because it is not an abundant parasite in our bioclimatic stage.

2.4. Adult Motility Assay (AMA)

This test was performed using 96 micro-plate wells. Five increasing concentrations of the extracts of the plants were carried out (1.562, 3.125, 6.25, 12.5, and 25 mg/ml). A total of about 560 adults of *T. circumcincta* were used to assess the anthelmintic effect of the extracts. Identification of the parasite was based on their numerous longitudinal cuticular ridges, which were seen posteriorly, the presence of a genital cone with a single papilla that projects from the surface of the body, the presence of small prominent cervical papillae, together with other identification keys (Taylor et al., 2015; Gibbons and Khalil, 1982; Soulsby, 1982).

Eight (08) adult motile worms were individually subjected to each treatment at laboratory temperature. The PBS and albendazole (25 mg/ml) were prepared and used as negative and positive controls, respectively. The test was repeated five times for each concentration. The inhibition of the mobility of adult worms for five (05) seconds under the effect of extracts was used as the criterion for anthelmintic activity. Mobility of worms was observed at intervals of 0, 1, 2, 4, 6, and 8 hours using a binocular magnifier. With each observation, immobile worms were observed for five (05) seconds to confirm their condition. At the end of the test, the treated worms were immersed again for 20 minutes in the PBS to observe the possible resumption of the mobility of the worms.

2.5. Egg Hatch Inhibition Assay (EHIA)

The egg hatch test was carried out according to the procedure described by Coles et al. (2006) with modifications. For every concentration of plant extract, 50 eggs of *T. circumcincta* were deposited in 0.5 ml of solution inside 1.5 ml Eppendorf tubes. All tubes were incubated at 27°C for 48 hours in this assay. A drop of Lugol iodine solution was added to each tube to stop further hatching, and all the unhatched eggs and first-stage (L1) larvae were counted under a dissecting microscope (40x). Two batches were conducted: a negative reference control batch consisting of PBS, and a positive reference control batch containing albendazole at a concentration of 25 mg/ml. The experiment was conducted with five repetitions for each concentration of the extracts. Ultimately, the percentage of inhibition in the hatching of eggs was determined by employing the subsequent formula:

$$Percente\ inhibition = 100\left(1 - \frac{x_1}{x_2}\right)$$

Where x_1 represents the number of eggs hatched in the extract, and x_2 represents the number of eggs hatched in PBS.

2.6. Data analysis

All data were tabulated and analyzed using descriptive statistics, determining minimum and maximum values and average and standard deviation variables. Statistical analysis was performed using SPSS version 23. A Kruskal-Wallis test was conducted with time and concentration as independent variables and plant activities as the dependent variable.

3. Results

3.1. Adult Motility Assay (AMA)

Adult motility experiments were conducted on the adult worms; the results are presented in Table 1. Within one hour of administering a dosage of 25 mg/ml of *A. herba-alba* extract, the motility of all adult worms was reduced. In contrast, the *J. phoenicea* extract resulted in a 100% mortality rate of mature worms after 4 hours of administration at the same dose. At a concentration of 3.125 mg/ml, *A. herba-alba,* and *J. phoenicea* exhibited 30 and 15% inhibition of motility, respectively, 8 hours after treatment. Neither of the extracts, at 1.562 mg/ml, had any inhibitory effect on motility. *A. herba-alba* was more effective on worms than *J. phoenicea*. Experiments showed that as the concentration of extracts increases and as time passes, the worms' movement decreases.

3.2. Egg Hatch Inhibition Assay (EHIA)

In the egg hatch inhibition assay (EHIA), the rate of inhibition ranged from 85.10 to 100% for *A. herba-alba* and from 33.12 to 96.23 % for *J. phoenicea*. Maximal (100%) egg-hatching inhibition effect was exhibited by the extract of *A. herba-alba* at 25 mg/ml (Figure 1).

Plant	Time (h)	Extract concentrations (mg/ml) 1.562 mean± sd	3.125 mean± sd	6.25 mean± sd	12.5 mean± sd	25 mean± sd	Negative mean± sd	Positive mean± sd	P value
A. herba alba	T0	0. 0± 0. 0	0. 0± 0. 0	0. 0± 0. 0	0.0± 0. 0	0.0± 0. 0	0. 0± 0. 0	100± 0. 0	0.0001
	T1	0. 0± 0. 0	0. 0± 0. 0	22.5± 31.1	32.5± 20.9	97.5± 5.6	0. 0	100± 0. 0	0.0001
	T2	0. 0± 0. 0	0. 0± 0. 0	62.5± 15.3	77.5± 20.5	100± 0. 0	0. 0± 0. 0	100± 0. 0	0.0001
	T4	0. 0± 0. 0	0. 0± 0. 0	75.0± 38.5	100± 0. 0	100± 0. 0	0. 0± 0. 0	100± 0. 0	0.0001
	T6	0. 0± 0. 0	5. 0± 6.85	100 ± 0.0	100± 0. 0	100± 0. 0	0. 0± 0. 0	100± 0. 0	0.0001
	T8	0. 0± 0. 0	30.0± 11.2	100± 0. 0	100± 0. 0	100± 0. 0	0. 0± 0. 0	100± 0. 0	0.0001
J. phoenicea	T0	0. 0± 0. 0	0.0± 0. 0	0. 0± 0. 0	0. 0± 0. 0	0. 0± 0. 0	0. 0± 0. 0	100± 0. 0	0.0001
	T1	0. 0± 0. 0	0. 0± 0. 0	0. 0± 0. 0	0. 0± 0. 0	2.5± 5.6	0. 0± 0. 0	100± 0. 0	0.0001
	T2	0. 0± 0. 0	0. 0± 0. 0	0. 0± 0. 0	0. 0± 0. 0	50± 12.5	0. 0± 0. 0	100± 0. 0	0.0001
	T4	0. 0± 0. 0	0. 0± 0. 0	2.5± 5.6	67.5± 11.2	100± 0. 0	0. 0± 0. 0	100± 0. 0	0.0001
	T6	0. 0± 0. 0	5. 0± 6.85	12.5± 12.5	80± 6.85	100± 0. 0	0. 0± 0. 0	100± 0. 0	0.0001
	T8	0. 0± 0. 0	15± 5.6	35± 18.5	95± 6.85	100± 0. 0	0. 0± 0. 0	100± 0. 0	0.0001

Obs. Comparisons are made within the row.

Table 1 – Percentage (%) of movement inhibition effect using hydroalcoholic extracts of *Artemisia herba-alba* and *Juniperus phoenicea* against *Teladorsagia circumcincta* adult worms.

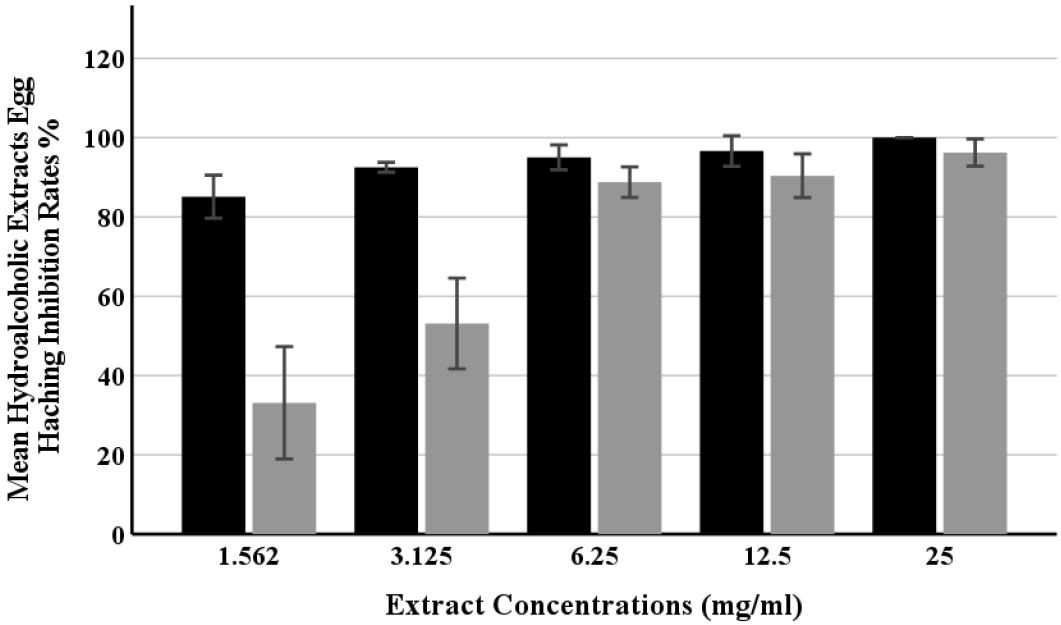

Figure 1 – Percentage (%) of egg hatching inhibition of hydro-alcoholic extracts of *Artemisia herba-alba* (black bars) and *Juniperu sphoenicea* (grey bars) against *Teladorsagia circumcincta* collected from naturally infected sheep.

4. Discussion

In the present study, an evident inhibitory *in vitro* effect of *A. herba-alba* and *J. phoenicea* leaf extracts on developing eggs and adult abomasal strongyles in naturally infected sheep was demonstrated. Our findings are consistent with those reported by Iqbal et al. (2004), who found that crude aqueous and methanolic extracts of *A. brevifolia* had anthelmintic effects on *H. contortus* adult worms, as exhibited by paralysis and/or mortality 6 hours after exposure. The research conducted by Ahmed et al. (2020) revealed that various quantities of methanolic extracts from the flowers and aerial parts of *A. herba-alba* exhibited anthelmintic activity similar to that of the conventional anthelmintic drug (albendazole at 0.25mg/ml). The anthelmintic activity of plant extracts increased with time. Accordingly, after 7 hours of exposure of adult *H. contortus* to the highest concentration (10 mg/ml), absolute mortality (100%) was produced. Furthermore, at 1 mg/ml, methanolic extracts of the flower and aerial parts of the plant showed egg-hatching inhibition rates of 98.67% and 88.3%, respectively. In the study conducted by Dağ et al. (2023) on *H. contortus*, extracts derived from various *Artemisia* species (*A. absinthium, A. abrotanum, A. annua, A. incana, and A. tournefortiana*) showed complete efficacy in the egg hatch inhibition assay across all tested concentrations (ranging from 50 to 1.5625 mg/ml) and time intervals (ranging from 1 to 48 hours). Among the studied species, it was observed that *A.annua* was the most effective and *A. tournefortiana* was the least effective species against larvae. Irum et al. (2015) showed that following the post-treatment period, the fecal egg count (FEC) significantly decreased for *Artemisia vestita* and *Artemisia maritima*. On day 28 post-treatment, the maximum reduction in fecal egg count for *A. vestita* was 87.2% at 100 mg/kg, whereas for *A. maritima* it was 84.5%. Examined extracts showed a high level of efficacy against larvae and adults.

Ouguirti et al. (2021) demonstrated the bactericidal activity of the essential oil of *A. herba-alba* growing in Algeria, which is mainly constituted by oxygenated monoterpenes (80.3%), followed by monoterpene hydrocarbons (10.8%), and a very low quantity of oxygenated sesquiterpenes (0.2 %). The major compounds are α-thujone (48.0%), β-thujone (13.4%), and camphor (13.1%). In the same country, Kadri et al. (2023) found that thujone (10.55%), borneol (5.98%), and eucalyptol (1.63%) were the major constituents of *A. herba-alba.*

On the other hand, the research conducted by Aouadi et al. (2021) on the essential oil of *J. phoenicea* demonstrated a nematicidal effect regarding worm immobility. After 8 hours of exposure, *J. phoenicea* essential oil resulted in 76.18% mortality at the highest tested concentration (1 mg/ml) and exhibited dose-dependent egg-hatching inhibition activities, which is similar to the results obtained in our study with hydroethanolic extract of the same plant. In the same study, the analysis of red juniper essential oil showed the presence of a high rate of monoterpenes (75.90%) owing to the predominance of α-pinene (74.14%), followed by linalool (2.97%), α-terpineol (0.34%) and β-myrcene (1.05%). The sesquiterpene compounds constituted 20% of the total essential oil including germacrene B (1.4%), β-caryophyllene (0.8%), and delta-cubebene (3.5%) as main components.

Moreover, phenolic profile analysis of *J. phoenicea* growing in Algeria revealed that quercetin was the major compound and the antimicrobial activity against all the bacterial strains was more bacteriostatic than bactericidal (Ghouti et al., 2018). In the study of Štrbac et al. (2020), the main components of *Juniperus communis* essential oil were α-pinene (40.46%), sabinene (14.04%), myrcene (8.87%) and limonene (4.95%). The essential oil exhibited significant efficacy against sheep gastrointestinal

nematode eggs. At the highest concentration (50 mg/ml), the essential oil displayed a hatchability inhibitory effect of 96.75%, which is comparable to our result of 96.238% at 25 mg/ml. Furthermore, Armstrong et al. (2013) showed that *Juniperus pinchotii* forage material decreased the mobility of *H. contortus* larvae.

Using medicinal plants and their products as feed supplements or additives instead of chemical drugs may be an effective strategy for controlling sheep's gastrointestinal nematodes. The present study highlighted the anthelmintic properties of *A. herba-alba* and *J. phoenicea* extracts against gastrointestinal nematodes in sheep. The demonstrated effectiveness is likely attributed to the synergistic effect of the various compounds in the hydroalcoholic extracts and their diverse composition, which was identified above by Zouaoui et al. (2020). Some compounds are already recognized as medically pertinent due to their anthelmintic properties, such as quercetin, and phenolic acids (Rehman et al., 2023), monoterpene alcohols and monoterpene hydrocarbons in essential oils (Štrbac et al., 2021). Condensedtannins also may exert anthelmintic activity by reducing hatching, and decreasing larval motility (Molan, 2014). Besides, tannins have been shown to interfere with coupled oxidative phosphorylation and block ATP synthesis in *H. contortus* (Martin, 1997). In the study conducted by Maestrini et al. (2020), saponins and prosapogenins showed inhibiting effects on gastrointestinal strongyle eggs in a concentration-dependent manner. Furthermore, it was found that the activity of *Artemisia lancea* essential oil against *H. contortus* is attributed to the presence of 1,8-cineole and camphor (Ali et al., 2021), which represents a high rate in the composition of Algerian *A. herba alba.*

Based on the above, it appears that the presence of effective chemical compounds against worms leads to two types of changes in the parasite: first, structural alterations (tegmental damage, intrauterine egg destruction, lipid accumulation, glycogen depletion, and finally, worm paralysis and death), and second metabolic changes (Irreversible inhibition of the enzymes of the metabolic cascade, disrupt mitochondrial membrane potential; inhibit oxidative phosphorylation of mitochondria, activation of *caspase-3-mediated apoptosis*) (Cavalcante et al., 2016; Araujo et al., 2017; Ali et al., 2021; Resendiz-Gonzalez et al., 2024).

5. Conclusion

A. herba-alba and *J. phoenicea* exhibited *in vitro* anthelmintic activity. However, while the initial results are encouraging, further research are needed to enhance the purification process, identify bioactive components, and understand their mechanisms of action against sheep nematodes. This knowledge could aid in optimizing dosage and delivery methods, ensuring efficacy while minimizing potential side effects.

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Briefing notes: Abomasum collections were conducted in slaughterhouses that respect animal welfare according to internationally and nationally accepted standards (European Directive 86/609/EEC of 24 November 1986, and national executive decree No. 95-363 of November 11, 1995, Algeria).

6. References

Abu-Darwish MS, Cabral C, Gonçalves MJ, Cavaleiro C, Cruz MT, Efferth T, Salgueiro L. *Artemisia herba-alba* essential oil from Buseirah (South Jordan). Chemical characterization and assessment of safe antifungal and anti-inflammatory doses. Journal of Ethnopharmacology., 174:153–160, 2015. https://doi.org/10.1016/j.jep.2015.08.005

Ahmed AH, Ejo M, Feyera T, Regassa D, Mummed B, Huluka SA. *In vitro* anthelmintic activity of crude extracts of *Artemisia herba-alba* and *Punica granatum* against *Haemonchus contortus*. J Parasitol Res., 2020:4950196, 2020. https://onlinelibrary.wiley.com/doi/epdf/10.1155/2020/4950196

Ahmed H, Kilinc S G, Celik F, Kesik HK, Simsek S, Ahmad K S, Afzal MS, Farrakh S, Safdar W, Pervaiz F, Liaqat S, Zhang J, Cao J. An inventory of anthelmintic plants across the globe. Pathogens 12 (1): 131, 2023. https://doi.org/10.3390/pathogens12010131

Ait-mimoune N, Kada, Drider H. Antimicrobial and antioxidant activities of Algerian *Juniperus phoenicea* and *Salvia officinalis* essential oils. J. Mex. Chem. Soc. 67 (2), 2023. https://doi.org/10.29356/jmcs.v67i2.1921

Ali R, Rooman M, Mussarat S, Norin S, Ali S, Adnan M, Khan SN. A systematic review on comparative analysis, toxicology, and pharmacology of medicinal plants against *Haemonchus contortus*. Front. Pharmacol., 12: 644027, 2021. https://doi.org/10.3389/fphar.2021.644027

André WPP, Cavalcante GS, Ribeiro WLC, Dos Santos JML, Macedo LTF, De Paula HCB, De Morais SM, De Melo JV, Bevilaqua CML. Anthelmintic effect of thymol and thymol acetate on sheep gastrointestinal nematodes and their toxicity in mice. Rev Bras Parasitol Vet., 26 (3): 323-330, 2017. https://doi.org/10.1590/S1984-29612017056

Araújo SA, Soares AMDS, Silva CR, Almeida Júnior EB, Rocha C Q, Ferreira ATDS, Perales J, Costa-Júnior LM. In vitro anthelmintic effects of *Spigelia anthelmia* protein fractions against *Haemonchus contortus*. PloS one, 12(12), e0189803, 2017. https://doi.org/10.1371/journal.pone.0189803

Armstrong SA, Klein DR, Whitney TR, Scott CB, Muir JP, Lambert BD, Craig TM. Effect of using redberry juniper (*Juniperus pinchotii*) to reduce *Haemonchus contortus in-vitro* motility and increase ivermectin efficacy. Vet.

Parasitol., 197; 271-276, 2013. http://dx.doi.org/10.1016/j.vetpar.2013.04.021

Benlarbi F, Mimoune N, Chaachouay N, Souttou K, Saidi R, Mokhtar MR, Kaidi R, Benaissa MH. Ethnobotanical survey of the traditional antiparasitic use of medicinal plants in humans and animals in Laghouat (Southern Algeria). Veterinary World., 16 (2): 357–368, 2023. http://dx.doi.org/10.14202/vetworld.2023.357-368

Bentounsi B, Trad R, Gaous N, Kohil K, Cabaret J. Gastrointestinal nematode resistance to benzimidazoles on a sheep farm in Algeria. Veterinary Record., 158 (18): 634-55, 2006. https://doi.org/10.1136/vr.158.18.634

Bentounsi B., Attir B., Meradi S., Cabaret J. Repeated treatment faecal egg counts to identify gastrointestinal nematode resistance in a context of low-level infection of sheep on farms in eastern Algeria. Vet. Parasitol. 144 (1-2):104-10, 2007. https://doi.org/10.1016/j.vetpar.2006.09.013

Boulkaboul A, Boucif A , Senouci K. Recherche de la résistance des strongles aux anthelminthiques chez le mouton en Algérie. Revue d'élevage et de médecine vétérinaire des pays tropicaux., 63 (3-4) : 71-75, 2010. https://doi.org/10.19182/remvt.10100

Boudjelal A, Henchiri C, Sari M, Sarri D, Hendel N, Benkhaled A, Ruberto G. Herbalists and wild medicinal plants in M'Sila (North Algeria): An ethnopharmacology survey. J. Ethnopharmacol., 148(2): 395–402, 2013. https://doi.org/10.1016/j.jep.2013.03.082

Cabaret J, Morales G, Gruner I. Caracterisation de *Teladorsagia circimcincta* et *T. trifurcata*. Ann. Parasito. Hum. Comp., 59 (6); 607-617, 1984. https://doi.org/10.1051/parasite/198661155

Cavalcante GS, De Morais SM, Andre WP, Ribeiro WL, Rodrigues AL, De Lira FC, Viana JM, Bevilaqua CM. Chemical composition and *in vitro* activity of *Calotropis procera* (Ait.) latex on *Haemonchus contortus*. Veterinary parasitology, 226: 22–25, 2016. https://doi.org/10.1016/j.vetpar.2016.06.012

Coles GC, Jackson F, Pomroy WE, Prichard RK, von Samson-Himmelstjerna G, Silvestre A, Taylor MA, Vercruysse J. The detection of anthelmintic resistance in nematodes of veterinary importance. Vet Parasitol., 136 (3–4):167–185,2006. https://doi.org/10.1016/j.vetpar.2005.11.019

Costa CT, Bevilaqua CM, Morais SM, Camurça-Vasconcelos AL, Maciel MV, Braga RR, Oliveira LM. Anthelmintic activity of *Cocos nucifera L.* on intestinal nematodes of mice. Res Vet Sci. 88(1):101-3, 2010. https://doi.org/10.1016/j.rvsc.2009.05.008

Dağ SRO, Erez MS, Kozan E, Özkan AMG, Çankaya İİT. *In-vitro* Anthelmintic activity of five different *Artemisia L.* species growing in Türkiye. Pak Vet J., 43 (4): 771-777, 2023. http://dx.doi.org/10.29261/pakvetj/2023.087

De Jesús-Martínez X, Olmedo-Juárez A, Olivares-Pérez J, Zamilpa A, Mendoza de Gives P, López-Arellano M E, Rojas-Hernández S, Villa-Mancera A, Camacho-Díaz L M, Cipriano-Salazar M. *In-vitro* anthelmintic activity of methanolic extract from *Caesalpinia coriaria* J. Willd fruits against *Haemonchus contortus* eggs and infective larvae. BioMed Research International., 2018, Article ID 7375693, 6 pages https://doi.org/10.1155/2018/7375693

Demeler J, Gill JH, von Samson-Himmelstjerna G, Sangster, NC. The *in vitro* assay profile of macrocyclic lactone resistance in three species of sheep *trichostrongyloids*. Int. J. Parasitol. Drugs Drug Resist., 3: 109–118, 2013. https://doi.org/10.1016/j.ijpddr.2013.04.002

Ghouti D, Rached W, Moussaoui A, Pires TCSP., Calhelha RC., Alves MJ, Lazzouni HA, Barros L, Ferreira ICFR. Phenolic profile and *in vitro* bioactive potential of saharan *Juniperus phoenicea* L. and *Cotula cinerea* (Del) growing in Algeria. Food Funct., 9: 4664–4672, 2018. DOI https://doi.org/10.1039/C8FO01392F

Gibbons LM, Khalil LF. A key for the identification of genera of the nematode family *Trichostrongylidae* Leiper, 1912. Journal of Helminthology., 56: 185- 233, 1982. https://doi.org/10.1017/S0022149X00034581

Hounzangbe-Adote MS, Paolini V, Fouraste I, Moutairou K, Hoste H. *In vitro* effects of four tropical plants on three life-cycle stages of the parasitic nematode, *Haemonchus contortus*. Res. Vet. Sci. 78 (2): 155–160, 2005. https://doi.org/10.1016/j.rvsc.2004.05.009

Iqbal Z, Lateef M, Ashraf M, Jabbar A. Anthelmintic activity of *Artemisia brevifolia* in sheep. Journal of Ethnopharmacology., 93: 265–268, 2004. https://doi.org/10.1016/j.jep.2004.03.046

Irum S, Ahmed H, Mukhtar M, Mushtaq M, Mirza B, Donskow-Łysoniewska K, Qayyum M, Simsek S. Anthelmintic activity of *Artemisia vestita* Wall ex DC. and *Artemisia maritima L.* against *Haemonchus contortus* from sheep. Vet Parasitol., 212 (3-4): 451-5, 2015. https://doi.org/10.1016/j.vetpar.2015.06.028

Jia J, Qin Y, Zhang L, Guo C, Wang Y, Yue X, Qian J. Artemisinin inhibits gallbladder cancer cell lines through triggering cell cycle arrest and apoptosis. Mol Med Rep., 13(5): 4461-8, 2016. https://doi.org/10.3892/mmr.2016.5073

Kadri M, Mouane A, Goubi S, Ramdan F, Yahia A. Composition, antimicrobial and antioxidant activity of *Artemisia Herba-Alba* essential oil from northeast Algeria (Khenchela region). Acta Periodica Technologica., 54: 1-335,2023. https://doi.org/10.2298/APT2354151K

Katiki LM, Araujo RC, Ziegelmeyer L, Gomes ACP, Gutmanis G, Rodrigues L, Bueno MS, Veríssimo CJ, Louvandini H, Ferreira JFS, Amarante AFT. Evaluation of encapsulated anethole and carvone in lambs artificially- and naturally-infected with *Haemonchus contortus*. Exp Parasitol., 197: 36-42, 2019. https://doi.org/10.1016/j.exppara.2019.01.002

Katiki LM, Barbieri AME, Araujo RC, Veríssimo CJ, Louvandini H, Ferreira JFS. Synergistic interaction of ten essential oils against *Haemonchus contortus in vitro*. Vet Parasitol., 243:47-51, 2017. https://doi.org/10.1016/j.vetpar.2017.06.008

Kemal M Er, Bakchiche B, Kemal M, Cheraif K, Kara Y, Bardaweel SK, Miguel MG, Yildiz O, Ghareeb MA. Six

Algerian plants: phenolic profile, antioxidant, antimicrobial activities associated with different simulated gastrointestinal digestion phases and antiproliferative properties. Journal of Herbal Medicine., 38: 100636, 2023. https://doi.org/10.1016/j.hermed.2023.100636

Khlifi D, Sghaier RM, Amouri S, Laouini D, Hamdi M, Bouajila J. Composition and anti-oxidant, anti-cancer and anti-inflammatory activities of *Artemisia herba-alba*, *Ruta chalpensis L.* and *Peganum harmala L.* Food and Chemical Toxicology., 55: 202–208, 2013. https://doi.org/10.1016/j.fct.2013.01.004

Laudato M, Capasso R. Useful plants for animal therapy. Alternative Medicine., 1(1):1, 2013. https://doi.org/10.13172/2052-7845-1-1-327

Li W, Mo W, Shen D, Sun L, Wang J, Lu S, Gitschier JM, Zhou B. Yeast model uncovers dual roles of mitochondria in action of artemisinin. PLoS Genet., 1(3):e36, 2005. https://doi.org/10.1371/journal.pgen.0010036

Martin RJ. Modes of action of anthelmintic drugs. Veterinary Journal., 154(1):11-34, 1997. https://doi.org/10.1016/s1090-0233(05)80005-x

Meriem A, Msaada K, Sebai E, Aidi Wannes W, Salah Abbassi M, Akkari H. Antioxidant, anthelmintic and antibacterial activities of red juniper (*Juniperus phoenicea L.*) essential oil. Journal of Essential Oil Research., 34 (2): 163–172, 2021. https://doi.org/10.1080/10412905.2021.1941338

Maestrini M, Tava A, Mancini S, Tedesco D, Perrucci S. *In Vitro* Anthelmintic activity of saponins from *medicago spp*. against sheep gastrointestinal nematodes. Molecules., 25 (2): 242, 2020. https://doi.org/10.3390/molecules25020242

Miara Mohamed Djamel, Bendif Hamdi, Ouabeda Asmahane, Rebbas Khellaf, Ait Hammoua Mohammed, Amirat Mokhtar, Greenee Alex, Teixidor-Toneuf Irene. Ethnoveterinary remedies used in the Algerian steppe: exploring the relationship with traditional human herbal medicine. Journal of Ethnopharmacology., 244; 112164, 2019. https://doi.org/10.1016/j.jep.2019.112164

Molan AL. Effect of purified condensed tannins from pine bark on larval motility, egg hatching and larval development of *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* (Nematoda: *Trichostrongylidae*). Folia Parasitol (Praha)., 61(4):371-376, 2014. https://folia.paru.cas.cz/pdfs/fol/2014/04/10.pdf

Ouguirti Nl, Bahri F, Bouyahyaoui A, Wanner J. Chemical characterization and bioactivities assessment of *Artemisia herba-alba Asso* essential oil from south-western Algeria. Nat. Volatiles & Essent. Oils., 8(2): 27-36, 2021. https://doi.org/10.37929/nveo.844309

Rehman TU, El-Mansi AA, Alhag SK, Al-Shuraym LA, Saeed Z, Arif M, Rashid M, Bhutta ZA, Zaman MA. Antiparasitic activity of methanolic and ethyl acetate extracts of *Azadirachta indica* against *Haemonchus contortus*. Pakistan Veterinary Journal., 43 (1): 199-203, 2023. http://dx.doi.org/10.29261/pakvetj/2023.014

Reséndiz-González G, Olmedo-Juárez A, González-Garduño R, Cortes-Morales JA, González-Cortazar M, Sánchez-Mendoza AE, López-Arellano ME, Mercado-Márquez C, Lara-Bueno A, Higuera-Piedrahita RI. Anthelmintic efficacy of an organic fraction from *Guazuma ulmifolia* leaves and evaluation of reactive oxidative stress on *Haemonchus contortus*. Experimental parasitology, 261, 108768, 2024. https://doi.org/10.1016/j.exppara.2024.108768

Rodrigues KA, Amorim LV, Dias CN, Moraes DF, Carneiro SM, Carvalho FA. *Syzygium cumini (L.)* Skeels essential oil and its major constituent α-pinene exhibit anti-Leishmania activity through immunomodulation *in vitro*. J Ethnopharmacol., 160:32-40, 2015. https://doi.org/10.1016/j.jep.2014.11.024

Soulsby EJL. Helminths, arthropods and protozoa of domesticated animals. London: Baillière Tindall, 1982, 809pp. https://doi.org/10.1016/0035-9203(84)90110-X

Štrbaca F, Boscob A, Amadesib A, Rinaldic L, Stojanovića D, Simind N, Orčićd D, Pušiće I, Krnjajićf S, Ratajace. *In vitro* ovicidal effect of common juniper (*Juniperus communis l.*) essential oil on sheep gastrointestinal nematodes. Veterinarski Pregled., 1 (1), 2020. ISSN: 2683-5932; 2683-5924. https://rimsi.imsi.bg.ac.rs/bitstream/handle/123456789/1885/bitstream_4385.pdf?sequence=1&isAllowed=y

Štrbac F, Bosco A, Amadesi A, Rinaldi L, Stojanović D, Simin N, Orčić D, Pušić I, Krnjajić S, Ratajac R, 2021. Ovicidal potential of five different essential oils to control gastrointestinal nematodes of sheep. Pak Vet J., 41(3): 353-358, 2021. http://dx.doi.org/10.29261/pakvetj/2021.026

Taylor MA, Coop RL, Wall RL. Veterinary Parasitology 4th Edition., Wiley-Blackwell Online ISBN:9781119073680, 2015, 1032 Pages. https://doi.org/10.1002/9781119073680.fmatter

Toklo PM, Challaton KP, Assogba MF, Akakpo GA, Kifouly AH, Alowanou GG, Hounzangbe-Adote S, Yayi E C, Gbenou J D. Phytochemical screening, *in-vitro* and *in vivo* effects of an aqueous extract of the bark of *Combretum glutinosum* Perr ex DC. (*Combretaceae*) on gastrointestinal strongyles. Phytomedicine Plus., 3 (4): 100491, 2023. doi: https://doi.org/10.1016/j.phyplu.2023.100491

Vaarst M, Padel S, Younie D, Hovi M, Sundrum A, Rymer C. Animal health challenges and veterinary aspects of organic livestock farming identified through a 3 year EU network project. The Open Veterinary Science Journal., 2 (1): 111-116, 2008. https://doi.org/10.2174/1874318808002010111

Wang J, Zhang CJ, Chia WN, Loh CC, Li Z, Lee YM, He Y, Yuan LX, Lim TK, Liu M, Liew CX, Lee YQ, Zhang J, Lu N, Lim CT, Hua ZC, Liu B, Shen HM, Tan KS, Lin Q. Haem-activated promiscuous targeting of artemisinin in *Plasmodium falciparum*. Nat Commun., 6:10111, 2015. https://doi.org/10.1038/ncomms10111

Zabre G, Tindano B, Dicko A, Bayala B, Kabore A, Belem AMG, Tamboura HH. *In-vitro* Anthelmintic Efficacy of *Acacia nilotica* Pods on Eggs and Adult Worms of *Haemonchus contortus*. The Indian Journal of Veterinary Sciences and Biotechnology., 19 (6): 90, 2023. https://doi.org/10.48165/ijvsbt.19.6.18

Zouaoui N, Chenchouni H, Bouguerra A, Massouras T, Barkat M. Characterization of volatile organic compounds from six aromatic and medicinal plant species growing wild in north African drylands. NFS Journal., 18; 19–28, 2020. https://doi.org/10.1016/j.nfs.2019.12.001

Zouyed I, Cabaret J, Bentounsi B. Climate influences assemblages of abomasal nematodes of sheep on steppe pastures in the east of Algeria. Journal of helminthology., 92 (1): 34-41, 2018. https://doi.org/10.1017/S0022149X16000845

Zhu L, Dai J, Yang L, Qiu J. *In vitro* ovicidal and larvicidal activity of the essential oil of *Artemisia lancea* against *Haemonchus contortus* (*Strongylida*). Vet. Parasitol., 195 (1-2), 112–117, 2013. https://doi.org/10.1016/j.vetpar.2012.12.050