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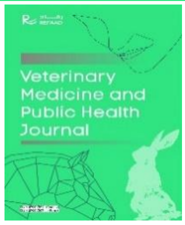

Screening Saudi Ethnomedicinal Plants for Anthelmintic and Immuno-potentiating Properties: A Proposed In *Vivo* Approach

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Review Article

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Abstract:

Objectives: This paper advocates for a practical approach to empirically determine the anthelmintic therapeutic potential and immunopotentiating properties of these plants.

Methods: Experimental animal/parasite models, namely the tapeworm *Hymenolepis diminuta* in mice, the nematode *Ascaridia* galli in chickens, as well as the tapeworm *Raillietina cesticillus* in chickens—are proposed for this purpose.

Results: Evidence of therapeutically effective and immunopotentiating medicinal plants from the Kingdom derived from this study will pave the way for the development of effective, safe, and relatively low-cost anthelmintic drugs and immunostimulants.

Conclusion: We emphasize that this is a proposed methodology paper. While our focus is on Saudi Arabia, the techniques are broadly applicable worldwide.

Keywords: *Anthelmintic; Immunopotentiation; Ethnomedicinal plants; In vivo models; Intestinal helminths; Saudi Arabia.*

1 Introduction

Helminth parasites, a diverse group of multicellular worms, are known to infect various organs and systems within the human body. Intestinal helminths are broadly categorized into two primary groups: roundworms (Nematodes) and flatworms, which include both tapeworms and flukes. Significant nematode pathogens include *Ascaris lumbricoides*, *Trichuris trichiura*, *Ancylostoma duodenale*, *Necator americanus*, and *Strongyloides stercoralis* (WHO, 2023) resulting in a range of debilitating conditions (Schlosser-Brandenburg *et al.*, 2023). Despite recent advancements in medical care, achieving complete control over human intestinal helminth infections remains a distant goal. This challenge is compounded by the absence of effective vaccines, the high cost of drug treatments, the emergence of drug resistance, and the rapid reinfection rates in environments where transmission cannot be interrupted. Given these limitations, research has increasingly focused on compounds derived from natural products to demonstrate their effectiveness as therapeutic agents against parasites. Furthermore, new approaches are exploring how boosting the host's natural immune response could serve a similar purpose. Currently, the scientific community worldwide is keenly focused on the role of various natural products, including whole medicinal plants, their extracts, and phytochemicals, in regulating the immune system (Nassar, 2022).

Saudi Arabia is known for its diverse plant flora, which includes various species of trees, herbs, and shrubs (Aati *et al*., 2019). Traditional Saudi folk medicine utilizes a significant portion of this biodiversity, with reports indicating that 96 species from 47 families are employed (Riaz Ullah *et al*., 2020). Notably, many of these plants are specifically recognized for their efficacy against tapeworms. For instance, a study by Youssef (2013) identified numerous plant species collected from different sites in Qassim with anthelmintic properties. Among these, *Capparis spinosa* has been highlighted for its dual anthelmintic and immunostimulant effects. Ethnobotanical surveys reveal that a substantial portion of the Saudi population relies on traditional medicine, either independently or in conjunction with modern treatments (Bodeker *et al*., 2005). Supporting this, an ethnopharmacological survey targeting Saudi citizens found that 80% of those interviewed had used herbal medicine for various ailments. In this context, several reports indicate that intestinal helminth infections are prevalent in humans in Saudi Arabia. These include *Ancylostoma duodenale*, *Ascaris lumbricoides*, *Enterobius vermicularis*, *Hymenolepis nana*, *Necator americanus*, *Schistosoma mansoni*, *Strongyloides stercoralis*, and *Trichuris trichiura* (Bolbol & Mahmoud ,1984; Alruzug & Khormi, 2016; Amer *et al*., 2018; Wakid ,2020 ; Al-Malki ,2021; Haouas *et al.* , 2021 ; Abdelkareem *et al*., 2022 ; Al-Refai & Wakid , 2024). Al-Malki (2021) reported a high prevalence of intestinal parasite infections in Saudi Arabia, with rates varying depending on the city and age group. The author, citing earlier studies by Al-Braiken (2008) and Zakai (2004), found that the prevalence of such infections in children ranged from 9.5% to 47.4%. These infections were noted to be either symptomatic or asymptomatic.

This paper advocates for a practical approach to empirically determine the anthelmintic therapeutic potential and immunopotentiating properties of these plants using experimental animal/parasite models. Specifically, it proposes using the tapeworm *Hymenolepis diminuta* in mice, the nematode *Ascaridia galli* in chickens, and the tapeworm *Raillietina cesticillus* in chickens. The choice of these specific models is based on the fact that these parasites are present in their natural respective definitive hosts in various localities in Saudi Arabia (Ramadan & Abu-Znada ,1989; Zahrani *et al.*, 2012; Banaja *et al.*, 2013; Allam ,2019; Ramadan & Abu-Znada ,2019), and the intermediate hosts they require are also prevalent in the Kingdom (Gharsan *et al.* ,2018).

2 Materials and Methods

2.1 Establishment of parasite lifecycles

Adult *H. diminuta* can be recovered from wild-caught rodents while *R. cesticillus* and A. galli can be recovered from local backyard and free-range chickens at post-mortem. The lifecycles of *H. diminuta* and *R. cesticillus* can be propagated in laboratory-reared insect intermediate host (e.g. the common flour beetles *Tribolium confusum* and *Tribolium castaneum*, and the mealworm beetle Tenebrio molitor) by feeding parasite eggs (*H. diminuta*) or shredded gravid segments (*R. cesticillus*) (Elowni ,1980) allowing for infective cysticercoids (larvae) to develop in the insects. A. galli, on the other hand, has a direct lifecycle and does not require an intermediate host.

2.2 Experimental infection with parasites

Mice can be infected with *H. diminuta* using infective cysticercoids recovered by dissection of infected beetles. The required number of larvae, not more than 30 days old in the insect, is administered to mice by gavage needles. Cysticercoid of *R. cesticillus* will be administered to chickens by feeding them in gelatin capsules, a technique superior to the commonly used dropper method (Elowni ,1984). Infection with *A. galli*, on the other hand, will be transmitted to chickens by feeding infective larvae from embryonated parasite eggs cultured *in vitro* (Permin *et al.*,1997).

2.3 Treatment and parasite recovery

Infected animals will undergo oral treatment with plant extracts, guided by protocols established by the implementing institution derived from literature and local ethnomedicinal practices. Subsequently, their intestines will be opened to assess for the presence of worms using a technique described by Hopkins *et al*. (1972). This method effectively reveals "destrobilated worms" —those that consist solely of a scolex and the neck region, having either lost their chain of segments ("strobila") or failed to develop a strobila initially.

2.4 Assessment of the therapeutic efficacy of extracts

In both tapeworm and nematode infections, assessments will be conducted at necropsy by counting residual intestinal parasites and measuring their biomass (mg dry weight). These results will be compared against untreated controls that received a similar number of infective larvae. Differences will be analyzed statistically.

2.5 Assessment of the immunopotentiating effect

H. diminuta is rejected (expelled) by the mouse host within only 7-10 days after an infection initiated with cysticercoids. Mice develop a robust Th2-mediated immune response that peaks around 8 days post-infection leading to the expulsion of worms (McKay, 2010; Persaud *et al.*, 2007; Li *et al.*, 2021; Rajeev *et al.* ,2024). Accordingly, to assess the immunopotentiator properties of plant extracts, the experimental design should involve the use of three distinct mice groups: a group that had been receiving plant extract over a defined period of time prior to subsequent "challenge" infection (see below) (experimental group), a group given a 6-cysticercoids infection but no previous extract treatment (immune control group), and a group given neither cysticercoid infection nor plant extract (naïve control group). All three groups will undergo a challenge with *H. diminuta* through the surgical transplantation of single strobilate worms from cortisone-immunosuppressed mouse donors into the duodenum (Hopkins & Zajac ,1976) or by the oral administration of 6 cysticercoids. After being challenged, mice from all three groups will be euthanized and their intestines examined for parasites. Any parasites found will be counted, and their dry weight *(biomass)* will be measured. When mice are surgically implanted with strobilate worms, they will be euthanized 5 days later. At this point, naïve control mice are not expected to have rejected the worms, since a strobilate *H. diminuta* transplant is not rejected in less than 7 days by a naïve mouse, compared to about 4 days for an immune recipient (Hopkins & Zajac ,1976). Therefore, for a plant material to be considered an effective immuno-potentiator, the survival and growth of transplanted worms in plant-treated mice should be significantly different from those in naïve mice, ideally resembling or even surpassing the results seen in the immune control group. Based on the author's experience, day eight post-challenge with cysticercoids reveals the greatest difference in infection rejection between naïve and immune mice.

Both the nematode *A. galli* and the tapeworm *R. cesticillus* evoke a protective immune response in their avian chicken host (Gray , 1972; 1973; 1976; Shohana *et al.* 2023). However, although both are intestinal parasites, they differ significantly in their developmental patterns. *A. galli* undergoes an obligatory histotrophic larval phase in the intestinal mucosa, lasting approximately 17-18 days, before the larvae return to the intestinal lumen to mature into adults around 28-30 days post-ingestion (Hauck ,2025). In contrast, *R. cesticillus* is restricted to the intestinal lumen, with only the worm's scolices attaching to the mucosa. Consequently, for *A. galli*-infected chickens, precisely timing necropsies after product treatment is crucial. This allows researchers to evaluate treatment effects on both the difficult-to-reach mucosal larval stages and the later-stage luminal adult parasites. To test for therapeutic efficacy, *R. cesticillus* infections can be initiated with a low dose of cysticercoids in young chicks. Conversely, to assess the immune response, a high dose of up to 100 cysticercoids is necessary and sufficient to evoke a protective immune response (Elowni & Hopkins, 1981). This dose should be administered to chickens aged 6-8 weeks, as they will have reached full immunological competence by this stage (Lebedev *et al.*, 2024).

3 Discussion

Our study utilizes models with a significant advantage: the parasites employed do not multiply within the host after the administration of infective larvae. This means a single tapeworm cysticercoid larva or nematode larva develops into only one adult parasite. Consequently, this design eliminates the unpredictable errors that would arise with parasites that reproduce in their hosts, where replication can occur to an unpredictable or unmeasurable degree. This precision enhances the reliability of results, allowing for more accurate assessment of the efficacy of treatments.

Beyond their inherent reliability, our models provide valuable insights into targeting difficult parasitic stages. Intestinal helminth infections are a significant treatment challenge because larval stages can become embedded within host tissues. Some key examples in humans include infections with the dwarf tapeworm, *Hymenolepis nana*, where autoinfection leads to the development and build-up of infective cysticercoids within the intestinal villi and presence of larvae and parthenogenetic females of *Strongyloides stercoralis* embedded within the crypts of Lieberkühn in the small intestine epithelium. The phenomenon is also encountered in infections with hypobiotic (arrested) trichostrongylid larvae in ruminants, nodular worms (*Oesophagostomum* species) in various animal species, and the spiruriid nematode *Draschia megastoma* in the glandular mucosa of the equine stomach. In this regard, *A. galli*, the nematode model used in the present study, undergoes a histotrophic phase of development in the intestinal mucosa (Hauck ,2025). Demonstrating a natural product's efficacy against the embedded larvae of this parasite would strongly indicate that the product possesses valuable physicochemical and pharmacological properties. This ability to target hidden, tissue-dwelling parasites is crucial, as it paves the way for the development of novel therapeutic agents against hidden, tissue-dwelling parasites in both humans and animals.

Furthermore, the characteristics of these models can offer unique insights into potential mechanisms of action. In Cyclophyllidean tapeworms, strobilation (the formation of strobila) is attributed to budding from the neck region, an area highly active in DNA synthesis and cell division. In this regard, observing destrobilated worms following treatment, as opposed to the complete elimination of the parasite, provides crucial insight into an investigational product's possible mode of action. Such an observation would suggest a selective targeting of this active neck region, highlighting a specific pathway of anti-parasitic effect.

While this study provides a description of three effective models, the principles demonstrated here can be extended to other parasitic systems. For example, researchers may consider utilizing *Raillietina tetragona*, a davaineid intestinal tapeworm found in poultry. Its intermediate host, the ant *Pachycondyla sennaarensis* (Mohammed *et al.*, 1988) (now reclassified as *Brachyponera sennaarensis* (Mayr, 1862), is prevalent in Saudi Arabia (Al-Khalifa *et al.* 2010; Sharaf *et al.*, 2023), as is the parasite in its definitive host (Zahrani *et al.*, 2012). This parasite model has been successfully employed in our laboratory to evaluate the anthelmintic activity of *Jatropha curcas* (Osman *et al.*, 2007), a perennial shrub historically used in traditional medicine to treat a range of ailments, as well as evaluating the cestocidal activity of medications such as benzimidazoles (Nurelhuda *et al.*, 1989 a), praziquantel (Nurelhuda *et al.*, 1989 b), and niclosamide (Elowni *et al.*,1989) using cysticercoid larvae from naturally infected ants. Similarly, experimentation with *Choanotaenia infundibulum*, a tapeworm of significant prevalence in both domestic chickens and rock doves in the Taif region of Saudi Arabia (Zahrani *et al.*, 2012), could also prove valuable, building upon the methodological framework established in this study.

4 Conclusions

Study-derived evidence of therapeutically effective and immunopotentiating of native plants in the Kingdom, supported by clinical trials, will pave the way for ethnomedical applications against intestinal helminths, ultimately leading to the development of effective, safe, and relatively low-cost anthelmintic drugs.

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