Medicinal plants used as anthelmintics: Ethnomedical, pharmacological, and phytochemical studies

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ABSTRACT
Intestinal parasites delay mental and physical development in children. Infection with these parasites can result in complications during pregnancy and alter the health of newborns, which has long-term effects on educational attainment and economic productivity. The appearance of resistance against classical drug treatments generates interest in the development of new deworming alternatives. We think that research of new plants species may reveal potential antiparasitic compounds. This review is focused on the use of plants and secondary metabolites against intestinal parasites. We discuss the use of plants in traditional medicine and the use of plant secondary metabolites tried in in vitro and in vivo models when available.

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1. Introduction

According to the World Health Organization, more than 1.5 billion people, or 24% of the world's population, are infected with soil-transmitted helminths (STHs) [1]. Morbidity induced by infection with the major STHs results in an estimated 5.19 million disability-adjusted life years (DALYs) [2]. Infections are widely distributed in tropical and subtropical areas, with the greatest numbers occurring in sub-Saharan Africa, the Americas, China, and East Asia where coinfection with schistosomes and soil-transmitted helminths is common [1].
The most common and persistent parasitic nematodes that infect humans are the soil-transmitted nematodes—roundworms (Ascaris lumbricoides), whipworms (Trichuris trichiura), hookworms ( Necator americanus and Ancylostoma duodenale), and thread worms (Strongyloides stercoralis)—and the filarial nematodes that are responsible for lymphatic filariasis (LF) (Brugia sp. and Wuchereria bancrofti) and onchocerciasis (Onchocerca volvulus) [3]. The main tapeworms that affect humans are Taenia solium and Taenia saginata and medium and small tapeworms such as Hymenolepis nana, Hymenolepis disminuta, and Diphylidium caninum [4].

Detrimental health effects caused by intestinal parasites include anemia, impaired cognitive and physical development of children, complications during pregnancy, altered health of newborns, and inflammation [1]. Chronic infections can lead to bladder cancer and have long-term effects on educational attainment and economic productivity. Intestinal parasites disproportionately affect the poorest individuals, particularly in rural areas. Further, in poor and marginalized neighborhoods, infection with these parasites contributes to the cycle of poverty in vulnerable people [5]. Chronic infections are associated with considerable economic losses in the veterinary world. Studies from developing and developed countries show that the cost of deworming and the health impact of worms on livestock result in major economic losses [5].

Initial contact between host and parasite is during infection, which varies with parasite species. Strongyloides stercoralis and Schistosomes spp. penetrate the skin actively. Some STHs, including Angiostrongylus, infect the host after ingestion of undercooked food, when hands contaminated with soil are put in the mouth, or through an insect vector [6]. The mated state is a fundamental process of parasite viability inside the human host and is necessary for establishing the infection.

Only a handful of anthelmintic compounds are currently available; these are divided into several families that include the benzimidazoles, macrocyclic lactones, imidazothiazoles, and cyclic octadepsipeptides. Targets of these various treatment options are well-documented and include DNA, RNA, cytoskeletal proteins, biomembranes, and the nervous system multicellular host [4]. Host immune determinants (i.e., mechanisms that lead to the killing of the huge multicellular parasite such as filarial nematodes) are currently not well-defined and remain elusive, even though pathways involving the activation of cellular and humoral responses have been described [7]. Moreover, resistance to anthelmintics is concentrated in cities; it has been reported for almost all species of domestic animals and even in some parasites that infect human beings [8]. Studies recently show the presence of gastrointestinal nematodes that are resistant to the main commercially available anthelmintic drugs on cattle farms [9].

Therefore, we consider the research of plants species that may have antiparasitic activity, because many of these plants have been used as medicine in the past [10]. In this review, we discuss the literature examining how plants and their active compounds were used to treat conditions (in vitro and in vivo effects) consistent with STHs. Further, we review the empirical use of medicinal plants in the treatment of diseases with STH symptomatology.

2. Plant species used in ethnomedicine with anthelmintic properties

Approximately 80% of the world’s population still relies upon plants for primary health care; even today in Western medicine, and despite progress in synthetic chemistry, approximately 25% of prescription medicines are still derived either directly or indirectly from plants. A trend in phytomedicine is the use of original plant bioactive compounds with the potential for chemical modification, which will broaden phytomedical importance [10].

Between 50,000 and 70,000 plants species are used in traditional and modern medicinal methods [10]. Traditional medicine holds great promise as a source of effective treatments, including anthelmintic agents. Traditional medicine is readily available to people, especially in tropical and developing countries [11]. Therefore, plants remain an important aspect of phytochemical studies. The anthelmintic properties of plant species used in traditional medicine is provided in Table S1 of the supporting information.

3. Plant extracts characterized in in vitro and in vivo studies

One potential reason for the limited number of available anthelmintics is the difficulty in identifying lead compounds using high throughput assays. Parasitic nematodes have a complex life cycle with several biological stages (egg, larvae, and adult worm) and adequate experimental systems are not available for every relevant parasite or stage of life. Most reported screens are in vitro studies using diverse biological models like Ascaris lumbricoids, Schistosomes mansoni, Taenia solium, alternative parasites commonly used for animal relevant helminths (Haemonchus contortus, Ascaris suum, and Taenia crassiceps), and free-living species such as Pheretima posthuma and Caenorhabditis elegans. The use of C. elegans has been instrumental in improving our mechanistic understanding of several anthelmintic compounds [12].

In vitro assays are quick to perform and economical compared to in vivo tests. Either a single or battery of in vitro assays may be employed to prescreen compounds prior to in vivo testing. The most frequently used battery consists of a motility assay, an egg hatch inhibition (EHI) assay, a larval development (LD) assay, a larval migration inhibition (LMI) assay, and an assay to measure adult worm viability such as a 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) reduction assay. A compound is considered active if it causes complete inhibition of motility and/or >50% inhibition in the MTT reduction assay [13].

A detailed list of plant extracts with anthelmintic activity in vitro is provided in Table S2 of the supporting information. Although many researchers report the effectiveness of the extracts differently, we collected and presented 2 measurements: the inhibitory concentration 50 (IC50) and the minimum lethal concentration (MLC). We used a cut off effectiveness value of 2000 μg/ml. The most potent extract in Table S2 of the supporting information is from the dichloromethane fruit of Piper chaba with an IC50 of 0.77 μg/ml against S. mansoni. Piper chaba is widely distributed in Southeast Asia. The fruit of this plant is commonly called ‘Dee Plee’ in Thailand and has been used as an anti-flatulent, expectorant, antitussive, antifungal, uterus-contracting agent, sedative-hypnotic, appetite enhancer, anthelmintic, and counterirritant in the traditional medicine of Thailand [14,15]. Moreover, the aqueous acetone extract from the fruit of Piper chaba was found to have hepatoprotective effects [16]. Some amides including piperchabamides A-F, piperoleine B, piperamine, pipernonaline, piperlongumine, retrofractamides A-C, guineensine, piperchabamides B, E, D, N-isobutyl-(2E,4E)-deca-dienamide, N-isobutyl-(2E,4E)-dodecadienamide, N-isobutyl-(2E,4E,14Z)-eicosatrienamide, and piperchabasoides A and B have been isolated from the methanol extract of the P. chava fruit [17,18]. Bornyl piperate, piperlongumine, and piperine were isolated from the chloroform extract of the Piper chaba root. Bornyl piperate and piperlongumine have been found to possess potent antifungal and cytotoxic activities. Bornyl piperate and piperlongumine demonstrated weak activity against Leishmania donovani promastigotes when compared against the
standard drug, pentamidine [15].

Other potent extracts include those from hidroalcoholic leaves of Musa paradisiaca (banana), which inhibit *H. contortus* (IC₅₀ = 2.13).

Other activities have been described for the peel or the fruit of Musa paradisiaca including anti-leishmaniosis activity [19,20], antioxidative properties [21–23], and anti-microbial properties.
In vitro, the anti-leishmanial activities of triterpenes and sterols isolated from *Musa paradisiaca* fruit peel have been studied. Three compounds showed similar activity against promastigote and were identified as cycloeucalenone, 31-norcyclolaudenone, [24-26].
and 24-methylene-cicloartanol [19].

The stem and root extracts of *Trianthema portulacastrum* have an IC$_{50}$ of 2.41 µg/ml against the eggs of *H. contortus*; the dicloromethane bark of *Michelia champaca* (IC$_{50}$ = 7.29) is effective against *S. masoni*; and the diclorometane root extract of *Plumango indica* as an IC$_{50}$ = 9.71 when tested with *C. elegans*.
Even though there is often a significant correlation between *in vitro* tests and *in vivo* studies of anthelmintics and parasite drug resistance, discrepancies between *in vivo* and *in vitro* studies have been reported [27]. *In vivo* tests are sufficiently reliable and conclusions drawn from them are frequently transferable to human infection. Although tests employing animal models are labor intensive, expensive, time-consuming, and often difficult to scale-up, they provide valuable insights provided that sufficient care is taken when selecting the host:parasite system [11]. Because some of the HTS also affect farm animals, there are a variety of *in vivo* models and Table S3 shows the *in vivo* effects of some plant extracts. Direct comparisons of the effects are complicated because of the differences in dosing (timing, amount, etc.) and the biological differences between the models examined.

4. *In vitro* studies of secondary metabolites

In phytochemical studies, isolation of the active compound is a crucial step in the discovery of new anthelmintic drugs. The aim is not only to find new chemical compounds effective against helminths, but also the replacement of commonly used synthetic chemicals, which would provide an organic method to treat parasites using plants with anthelmintic properties [10]. For example, an alternative strategy for controlling gastrointestinal parasites is the use of plant secondary metabolites [28]. Table S3 of the supporting information shows secondary metabolites with anthelmintic activities. We collected 2 reported measurements from previous studies: Inhibitory Concentration 50 (IC50) and Concentration Effective (CE). Additionally, structures of these secondary metabolites are shown in Fig. 1. The lowest IC50 in Table S4 of the supporting information is for pinostrobin (4) against *T. crassiceps* (0.001 mM), followed by garginielliptone FC (13) on *S. mansoni* (0.005 mM), and lupeol (16) against *C. elegans* (0.006 mM).

5. Secondary plant metabolites with *in vivo* activity

Table S5 of the supporting information highlights several secondary metabolites that have gone from *in vitro* to *in vivo* testing; several biological models generally used were fish, sheep, or mouse. Additionally, structures of these secondary metabolites are shown in Fig. 2. However, in early studies looking for effective treatments against intestinal parasites in humans, various plant species were used based on ethnomedical information that today are responsible for many of the known pharmacological secondary metabolites. For example, santonin (74), a sesquiterpene lactone isolated from *Artemisia santonica*, was used in the 1950s against ascariasis and expulse *A. lubricoides*. However, due to its toxic effects, it was replaced by benzimidazole derivatives that are still currently used. Today, santonin is studied as an anti-tumor treatment in several types of cells [29]. The weed *Chenopodium ambrosioides* (L.), known as Epazote in Mesoamerica and as Paico in the Andes, has been used for many centuries as an anthelmintic [30]. The isolated secondary metabolite responsible for the anti-ascariasis action was called ascaridole (1). Fatalities arising from the consumption of *Chenopodium* oil were probably due, in most cases, to overdoses [31]; however, they led to the decline in the use of commercial oil and promoted the use of more modern medications. Ascaridole has demonstrated activity against other parasites such as *Plasmodium falciparum* and *Leishmania sp.* [32]. Further, it has antifungal [33] and anti-*H. pylori* activities. Another molecule that exists even for humans is Toosendanin (96) is a triterpenoid derivative that exists in the fruits of *Fructus toosendan* and the leaves and cortexes of *Melia toosendan*. It has antifeedant effects and is poisonous. Modern pharmacological studies show that the
main component in fructus toosendan and cortex meliae with an antiascarasis effect is toosendanin [34]. In addition to its role as an anti-roundworm drug, toosendanin is also effective against botulism. Toosendanin antagonizes the effect of nerve repression induced by botulinum at the neuromuscular junction [35]. It is important to have more information on molecular mechanisms involved in both the effect on the parasite and the adverse effects to develop better drugs.

6. Conclusions

According to the literature review, there is a great diversity of species whose extracts have shown potential anthelmintic activity. In contrast, the number of isolated secondary plant metabolites is
lower, which demonstrates the need for further assessment studies and preclinical trials in order to obtain new anthelmintics. New anthelmintics are needed for the pharmacological arsenal because the drugs currently available are not entirely satisfactory.

Conflicts of interest

Authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2017.02.005.

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