Recent advances in identification of potential drug targets and development of novel drugs in parasitic diseases: Part V: The value of natural products in drug discovery: Helminths

Review Article

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ABSTRACT

Almost all the current drugs used to treat neglected tropical diseases have some drawbacks including cytotoxicity, limited availability, and the emergence of resistant strains. The clinical significance of a natural product was recognized as a novel target against pathogens by identifying its mechanisms of action. Such products are commonly used for treating severe parasitic diseases such as malaria, leishmaniasis, Chagas' disease, schistosomiasis, and filariasis. A significant drawback of natural products is their limited supply. However, sustainable production is possible by utilizing bioengineering technology to facilitate the biosynthesis of derivatives with similar biological activities and discover new families of natural products by cultivating bacteria, fungi, endophytes, and marine creatures. Advances in genetic engineering technology, *i.e.*, molecular farming, enabled investigators to focus on developing novel safe drugs from natural products. This review aims to highlight recent advances in discovering novel drugs extracted from natural products of herbal and marine sources for treating helminthic diseases.

Keywords: drug discovery; essential oils; helminths; marine source; natural products; propolis.

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Abbreviations: ABZ: Albendazole; **EO:** Essential oil; **IVM:** Ivermectin; **MTZ:** Metronidazole; **NO:** Nitric oxide; **NPs:** Nanoparticles; **PZQ:** Praziquantel.

Glossary: Black caraway: *Nigella sativa* (*N. sativa*); **Cinnamon:** *Cinnamomum zeylanicum* (*C. zeylanicum*); **Curcumin:** *Curcuma longa* (*C. longa*), and *Curcuma zadoaria* (*C. zadoaria*); **Garlic:** *Allium sativum* (*A. sativum*); **Ginger:** *Zingiber officinale* (*Z. officinale*); **Mugwort:** *Artemisia absinthium* (*Art. absinthium*); **Myrrh:** *Commiphora molmol* (*C. molmol*); **Pomegranate:** *Punica granatum* (*P. granatum*); **Tea tree:** *Melaleuca alternifolia* (*M. alternifolia*); **Wormwood:** *Artemisia annua* (*Art. annua*); **Zaatar:** *Zataria multiflora* (*Z. multiflora*).

INTRODUCTION

Several metabolites isolated from herbs and marine sources, as well as synthetic and semisynthetic compounds were investigated for treating parasitic diseases. In their review, Cheuka *et al.*[1] reported that the commonly studied natural products included alkaloids, quinones, chalcones, terpenes, saponins, and tannins. Zingiberaceae family members, *i.e.*, herbs with aromatic flavors; turmeric, ginger, and galangal constitute the majority of plant sources. Herbs also include flowering plants such as Chinese licorice, and different species of *Chinchona, Calophyllum Tephrosia, Cissampelos, Pera, Arnica, Lantana,* and *Psorospermum*. Besides, metabolites isolated from fruits (*e.g.*, pomegranate, pumpkin, and mangrove), vegetables (*e.g.*, onion, garlic, piper, mushroom, garawa, and eggplant), and plants (*e.g.*, wormwood, asparagus, Arabic caraway, jatropha and fern) were also included. Metabolites isolated from microbial sources include sinefungin, pan-methyltransferases, and mevinolin which is a highly competitive inhibitor of hydroxy-methylglutaryl-coenzyme A reductase, produced by *Streptomyces* spp., and *Aspergillus* spp., respectively. Notably, *Wolbachia* endosymbiosis provided a promising target for novel chemotherapy against filariasis. Finally, sponges either sea (*Plakortis* spp.), steroidal (*Pandaros* spp.), petroslid (*Neopetrosia* spp.), or encrusting turret (*Haliclonia* spp.), ascidians and colonial tunicates (*Didemnum* spp.) contain also natural products isolated from marine animals, plants, and microorganisms[1].

The present review highlighted natural products derived from herbal and marine sources investigated in the last decade (2015-2024) for development of novel drugs against parasitic infections.

Herbal medicine (Figure 1)

Extracts and essential oils (EOs) derived from wormwood or mugworts (*Artemisia* spp.), myrrh (*Commiphora* spp.), tee tree (*M. alternifolia*), and naphthoquinones attracted much attention in the literature due to their efficacy against several parasitic diseases.

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- Artemisinin, a bioactive compound derived from the EO of *Artemisia* spp. leaves, has several biological activities including anti-malarial, anti-tumor, anti-inflammatory, and anti-oxidant. All activities are attributed to *Artemisia* secondary metabolites, *e.g.*, phenolic compounds (flavonoids, terpenoids, and coumarins). In a recent report, reviewers discussed methods utilized in extraction, isolation, and characterization of their main metabolites with special emphasis on phenolics, phenolic acids, and polyphenols (flavonoids and tannins)[2].

- Myrrh (*C. molmol*), commonly grown in Arabic countries and Northeastern Africa, is traditionally used to treat wounds, ulcers, microbial infections, and inflammatory diseases. It also exerts analgesic, antioxidant, neuroprotective, anti-diabetic, and anti-cancer activities. Pharmacological properties are attributed to terpenoids (monoterpenoids and sesquiterpenoids), diterpenoids, triterpenoids, and steroids. Batiha *et al*.[3] recommended future studies to explore myrrh phytochemical components, utilizing advanced approaches in drug development, aiming to elucidate its mechanism(s) of action.

- Lam *et al*.[4] reviewed the biological activities of EO extracted from the tea tree (*M. alternifolia*) and its major monoterpene constituents. They summarized recent advances involving its inhibitory effects against malignant malaria, leishmaniasis, trypanosomiasis, *Acanthamoeba* keratitis, trichomoniasis, hydatid cyst, and anisakiasis. Its anti-parasitic effects were attributed to its competitive blockage of acetylcholinesterase activity and its ability to modulate host inflammatory responses. Moreover, it exhibited strong anti-histaminic effects, *i.e.*, beneficial in treating allergic manifestations commonly associated with helminthic infections[4].

- Quinones, organic aromatic metabolites derived from several plant families, fungi, algae, and bacteria, include benzoquinones, anthraquinones, and naphthoquinones (NQs). The latter possesses a broad spectrum of interesting biological activities for new therapeutic propositions. Peixoto and her Brazilian colleagues[5] discussed the *in vitro* and *in vivo* studies conducted to investigate NQs inhibitory activity against neglected tropical diseases with special emphasis on epoxy-α-lapachone (ELAP), a major component derived from *Tabebuia* spp., flowering plants. The following issues were concluded: 1) due to its safety and efficacy, ELAP should be processed to preclinical trials to develop novel broad-spectrum drugs against tropical diseases; 2) its proposed mechanism(s) of action included inhibition of serine proteases, immunomodulation through blocking expression of pro-inflammatory cytokines, potential inhibitory activity against different enzymes with essential role in the metabolic pathway, *e.g.*, glycolysis, and lipid metabolism; 3) its use as drug delivery system combined with available therapeutic drugs, or loaded on nanoparticles (NPs) definitely would decrease emergence of resistant strains, minimize adverse effects of current therapy, and increase stability and bioavailability as well[5].

Marine source (Figure 2)

Since marine creatures inhabit a challenged environment of high pressure, lack of oxygen and photosynthesis, and limited food supply, they evolved adaptation strategies. They acquired toxic compounds from others for protection from predators. Therefore, marine natural products are a rich source of biological compounds and interesting candidates for developing novel drugs. Recently, Zhang and his colleagues[6] screened the

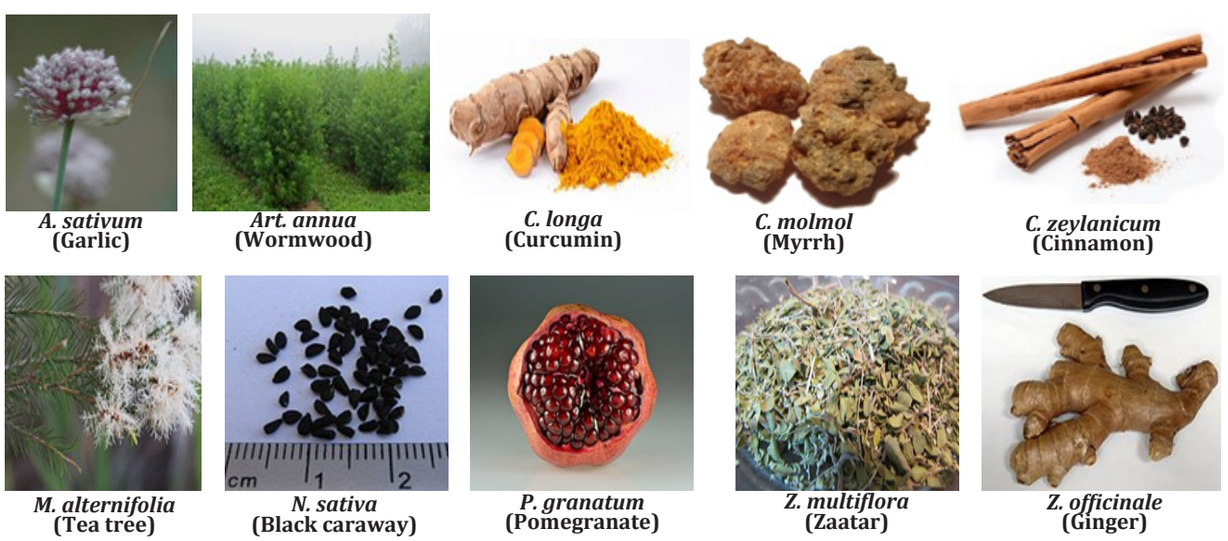
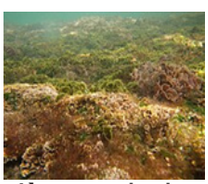


Fig. 1. Common natural herbs used in treating parasitic diseases.

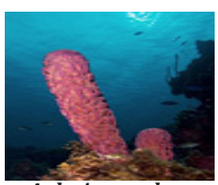
Web of Science database for marine-derived natural anti-parasite products and the following issues were concluded: 1) sponges are the major source exhibiting potent inhibitory activity against neglected tropical diseases, especially those belonging to *Monanchora, Dyside, Hyrtiso, Pseudoceratina,* and *Xestospongia*; 2) naturally derived or semisynthetic analogs were developed by structure–activity relationship analysis that exhibited higher bioactivity, and less toxicity; 3) mass production and cost-effectiveness are the major obstacles for introducing marine natural products into clinical medicine[6].

The impact of marine-derived compounds in the pharmacological industry of novel drug development was also reviewed. Tempone *et al*.[7] focused on alkaloids that comprise an extensive group of secondary metabolites produced by marine creatures. In fact, extensive chemodiversity of marine-derived alkaloids (MDAs) attracted considerable attention in the pharmacological industry. Inability to provide sustainable production encouraged the investigators towards synthesis of MDAs. The review discussed several synthetic approaches that were utilized to develop novel chemotherapeutic agents including metabolites derived from sponge (indole, guanidine, and thiazine alkaloids), algae (bromopyrrole alkaloids), and other miscellaneous alkaloids (ascididemin, and decahydroquinoline)[7].

In their report[8], it was claimed that marine epiphytic bacteria (prokaryotes) possess unique genes that express novel classes of bioactive metabolites with pharmaceutical potential inhibitory activities. Epiphytic bacteria usually inhabit marine surfaces, and as a survival strategy, they adhere to each other forming a biofilm. This continuous development of biofilm leads to a phenomenon called epibiosis. Consequently, competition among epiphytic bacteria to survive within the biofilm community leads to presence of bacterial strains with broad-spectrum inhibitory phenotypes, *i.e.*, successful epibiotic colonizers. Further studies utilizing *in silico* screening of marine surface biofilm' metabolites against developmental life cycle stages were recommended[8].

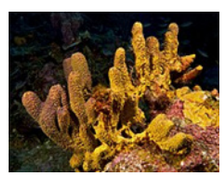


Algae growing in shallow waters



***Aplysina archer* sponge**

***Eupiectelia aspergilium* sponge**



***Pseudoceratina crassa* sponge**

***Spongia officinalis* sponge**

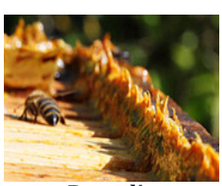
Fig. 2. Natural products of marine source.

Other sources (Figure 3)

Asfaram *et al*.[9] published a review discussing the value of propolis (bee glue) in treating several parasitic diseases. Propolis (beeswax) is an adhesive resin collected by bees from exudates produced by various plant species and is mixed with salivary and enzymatic secretions. Therefore, it is a source of several biologically active compounds such as resin, terpenoids, polyphenols, steroids, flavonoids, amino acids, phenolic acids, and essential aromatic oils. Since propolis has immunoregulatory functions and anti-oxidant capacity, several mechanisms of action were proposed such as inhibition of nitric oxide (NO), IFN-γ, TNF-α, production of interleukins and specific IgM and IgG, and induction of apoptosis[9].

A neglected source of natural products is mollusks since Heterobranchia (different-gilled snails) are rich in bioactive molecules and metabolites with diverse variability in structure and bioactivity. Avila and Angulo-Preckler[10] discussed ~450 compounds for their usefulness for ecological (snails' protection) or pharmacological (anti-inflammatory and anti-cancer) activities. Since a few mollusks nano-molecules were explored for their bioactivity, the reviewers encouraged researchers to identify, biosynthesize, and investigate mollusk molecules that would increase their use value to treat several parasitic diseases[10].

On the other hand, a growing literature suggests that primates consume plants for self-protection against pathogens. A recent review surveyed herbal plants ingested by Japanese monkeys (*Macaca fuscata yakui*). A set of plants were collected and their ethnomedicinal value was analyzed. The study investigated the methanolic extracts of 45 plant parts against *S. mansoni*, *P. falciparum, L. donovani,* and *Trypanosoma* spp. *in vitro*. Interestingly, the



Propolis (beeswax)

Macaca fuscata yakui

***Rhinella marina* (Cane toad)**

Heterobranchia

Fig. 3. Other sources of natural products.

majority exhibited broad significant activity. Since the cytotoxicity assays on mammalian cells were minimal, the investigators claimed that understanding primate diets would enhance drug development against neglected tropical diseases[11].

Anti-helminthic activity

Several phytochemical compounds derived from herbs were investigated against multiple helminthic infections. Out of ten compounds derived from *Ajania nubigena, Corydalis crispa*, and *Pleurospermum amabile*, only two compounds (luteolin, and bergapten) exhibited significant dual activity against *S. mansoni*, and *T. muris*. Since ultrastructural results showed lethal tegmental damage, the investigators suggested the use of both compounds as novel scaffolds for broad-spectrum anthelmintic drug development[12]. To improve the potency of diterpenoid 7-keto-sempervirol against adult, and larval stages of *S. mansoni*, and *F. hepatica*, Crusco *et al*.[13] screened synthesized analogues. Results showed that analogue 7d exhibited the most lethal effects on both parasites with minimum cytotoxicity to two human cell lines. In order to develop a broad-spectrum drug against neglected tropical diseases, 15 traditional medicines used in treating schistosomiasis, and onchocerciasis in Ghana were evaluated for their *in vitro* activity. Two compounds (B4-DCM, and B7-DCM) exhibited the most *in vitro* inhibitory activity against *S. mansoni*, and *O. ochengi*[14]. Later, anemonin isolated from *R. multifidus* leaves exhibited inhibitory activity against *S. mansoni* adult *in vitro*, and *L. aethiopica* promastigotes and amastigotes[15].

Schistosomiasis

In spite of its low efficacy against juvenile schistosomes, Praziquantel (PZQ) is the sole drug for schistosomiasis for the last four decades. Therefore, plenty of studies were conducted to develop a novel drug from natural products.

Natural extracts: Several *in vitro* and *in vivo* studies investigated extracts of *N. sativa* oil and *Chroococcus turgidus*, separately or combined[16], *P. granatum*[17], *Rauwolfia vomitoria*[18], *Callistemon citrinus*[19], *Pelargonium reinforme/sidoides*[20], *Art. annua*, *A. sativum*, and *N. sativa* extracts[21], and *Handroanthus impetiginosus*[22]. The potential efficacy was investigated against either *Schistosoma* adults *in vitro*, or experimentally in infected mice or both. Based on concentrations and incubation time, all extracts induced viability reduction, and tegmental morphological alteration. Phytochemical analysis revealed enrichment of chemical compounds that exhibited *in vitro* 100% mortality due to decreased motor activity[17,18,20-22]. Besides, it was demonstrated that *P. reinforme/sidoides* efficacy was attributed to its bioactive ingredients, tri- and tetra-oxygenated cumarine, gallic acid, and gallic acid methyl ester (polyphenols)[20]. Similar to PZQ, natural herbs significantly reduced intestinal, and hepatic burden, and hepatic granuloma count and size. Moreover, decreased viability and egg production with tegmental morphological alterations in a dose-dependent manner were recorded[16,17,19-21].

For *S. haematobium*, it was demonstrated that extracts of *Origanum majorana, Ziziphus spinachristi*, and *Salvia fruticose* exhibited 100% lethal effects on adults, and schistosomula *in vitro*. Similar inhibitory activity was obtained in experimentally infected mice, *i.e.*, *O. majorana*, and *Z. spinachristi* achieved better parasitological results than *S. fruticose*[23].

Natural or synthesized compounds: Several studies were conducted *in vitro*, or *in vivo* or both to investigate the potential efficacy of synthetic analogues of piplartine, an alkaloid in *Piper* spp.[24], hederacolchiside A1 derived from *Pulsatilla chinensis*[25], limonoids of citrus fruits[26], imizadole alkaloids derived from *Pilocarpus microphyllus*[27], several compounds derived from *Ozoroa insignis*[28], and *Albatrellus confluens*[29]. Two issues were recorded: 1) a higher concentration of limonin was required for female death to induce massive tegmental destruction exposing the sub-tegmental tissues[26]; 2) pentadecenyl phenol from *O. insignis*[28], and geranylgeranyl-2-orcinol from *A. confluens*[29] exhibited significant potency against schistosomula. Due to their low cytotoxicity against two human cell lines, the investigators recommended further *in vivo* evaluation[28,29].

Silva Torres *et al*.[30] synthesized two aurones, naturally bioactive compounds in several plants and vegetables. Both aurones exhibited lethal alteration in the tegument integrity, and motor activity *in vitro*. Oral administration to experimentally infected mice showed ~60% reduction of all parasitological parameters. Utilizing *in silico* analysis, the investigators attributed this reduction to inhibition of adenosine triphosphate (ATP) diphospho-hydrolases. In comparison to PZQ, molecular docking studies showed that both aurones interacted more actively with the ATPase nucleotide bond[30].

Azevedo *et al*.[31] tabulated different natural products and variable natural or synthesized components, and summarized their potential mechanism of actions *in vitro*, and *in vivo*. They categorized the *in vitro* anti-schistosomal effects into three mechanisms of action: 1) direct tegument alteration; 2) increased production of mitochondrial reactive oxygen species (ROS); 3) inhibition of ATPase activity. Due to their ability of schistosomicidal activity, the main outcome of *in vivo* treatment is significant reduction of egg deposition in tissues with decreased hepatic granuloma numbers and size. Some natural products exhibited several immunomodulatory activities to modulate cytokine production associated with Th1, Th2, and Th17 profiles[31].

Propolis: Compared to infected non-treated group, Brazilian green propolis significantly reduced worm, and egg burdens with increased dead eggs. Histological examination of livers showed a significant reduction in the count, and size of hepatic granulomas. The study recommended its oral administration in a combined therapy with PZQ[32].

Traditional medicine: In Ghana, two medicines were traditionally used (B4-DCM and B7-DCM) in treating schistosomiasis. While B4 was prepared from dried herbs of *Aloe vera* and *Taraxacum officinale*, B7 was prepared from *Vernonia amygdalina, Khaya senegalensis, Mangifera indica,* and *Azadirachta indica*[14]. In Egypt, *T. officinale* extract alone significantly reduced all parasitological parameters in comparison to PZQ. Its combined therapy with PZQ significantly decreased IL-6 and TNF-α levels, and inhibited hepatic DNA damage, in comparison with PZQ alone[33].

The efficacy of herbal traditional medicine consisting of menthol, and menthone was investigated for 60 d treatment regimen in *S. mansoni*-experimentally infected mice. In addition to significant reduction in all parasitological parameters, reduction of eosinophilia, and ILs 4 and 10 levels was observed[34]. Traditional Chinese medicine (*Xiaochaihu decoction*) showed significant capability to alleviate hepatic fibrosis caused by schistosomiasis *japonicum* in experimentally infected mice. Treatment for 16 w ameliorated liver pathological changes, and inhibited expression of fibro-genetic markers, *i.e.*, TGF-β1, and SMADs (2, and 4), as well as collagens (Col1A1 and Col3A1). The investigators also demonstrated that the hepatic protective effects of *X. decoction* were mediated by heat shock protein (HSP47)/TGF-β axis[35]. Exploring Kenyan medicinal plants, traditionally used for the treatment of malaria, pneumonia, and diarrhea, enabled the investigators to select seven plants; *Art. annua*, *Ajuga remota, Bredilia micranta, Cordia africana, Physalis peruviana, Prunus africana*, and *Senna didymobotrya*. Among their 22 prepared extracts, only five exhibited ~70% inhibitory activity against adults, and another two showed <20% viability against juveniles. The study recommended combined therapy with PZQ to achieve complete cure[36].

Screening studies: In 2022, three studies[37-39] were conducted to develop a novel drug against schistosomiasis. Brazilian investigators screened apyrase inhibitors from *Centella erecta*, and identified asiaticoside, one of the apyrase-binding compounds. Similar to PZQ, asiaticoside exhibited *in vivo* anti-schistosomal activity without adverse effects. Molecular docking analysis demonstrated inhibition of the active sites of *Schistosoma* ATPases[37]. *In silico* screening of several compounds derived from beetles revealed that Buprestin H exhibited significant inhibitory activity against *S. mansoni* adults and juveniles without cytotoxic activity against human cell lines. The investigators conducted a molecular docking study demonstrating its mechanism of action, targeting thioredoxin glutathione reductase[38]. Combined ligand-, and structure-based virtual screening of SistematX database of metabolites (https://sistematx.ufpb.br/) was utilized. Out of 1000 alkaloids, the investigators succeeded in selecting only five potentially active against *S. mansoni*. After *in silico* analyses for their metabolism, toxicity, and drug-like profile, only two were proposed as starting points for development of novel drugs in treating schistosomiasis[39]. More recently, a group of investigators utilized *in silico* analysis to screen 63 compounds previously characterized from *Azadirachta indica* against three *Schistosoma* drug targets; thioredoxin glutathione reductase, ATPases, and arginase. These drug targets were selected according to their roles in maintenance of redox homeostasis, *de novo* pyrimidine biosynthesis, and metabolic pathway, respectively. The investigators conducted molecular docking, ADMET properties, and molecular dynamics simulation of the hit compounds. Results revealed that Andrographolide possessed a satisfactory stability at the active site of ATPase. The study recommended future studies to explore the possibility of developing a promising anti-schistosomal drug using Andrographolide from *A. indica*[40].

Since protein kinases (PKs) are essential molecules involved in several cellular processes such as signaling, growth, differentiation, and metabolism, they are potential drug targets. In fact, *Schistosoma* kinome revealed polo-like kinases, mitogen-activated protein kinases, and tyrosine kinases. An updated online database for PK inhibitors (https://www.icoa.fr/pkidb/index.html) was utilized by German researchers. In their review, they discussed several approaches and selected PKs inhibitors against schistosomes such as artemisinin, genistein derived from *Felmingia vestita*, and several anti-cancer therapies for drug repurposing[41]. In addition, Azevedo *et al*.[31] summarized recent advances in molecular and computational technology that allowed optimization of time and resources for efficient screening of alternative new compounds. In addition to *in silico* screening analysis to select and design new compounds against a characterized potential molecular target, online proteomic and genomic databases (*e.g.*, https://parasite.wormbase.org/, https://www.rcsb.org) were constructed demonstrating the 3D structure of several *Schistosoma* validated and putative drug targets. For future studies, the reviewers recommended four drug targets; thioredoxin glutathione reductase, glutathione-S-transferase, histone deacetylase, and 20S proteasome. Furthermore, modern evolutionary technology in genome sequencing adds deep understanding of drug resistance, *Schistosoma* gene expression, and establishment of drug-parasite target relationship. Artificial intelligence and machine learning are currently utilized in identification

of drug targets and development of novel therapy using molecular docking and protein structure online database (https://www.alphafold.ebi.ac.uk/)[31].

Nanotechnology: To enhance curcumin solubility and bioavailability, nanoemulsion of the ethanolic extract of *C. longa* crude powder was evaluated for its pharmacokinetic properties on *S. mansoni* life cycle stages *in vitro*. It showed anti-schistosomal effect on cercariae, schistosomulae, males and females at 30 min, 24 h, 48 h, and 72 h, respectively. Due to its potent inhibitory activity against juveniles, the investigators recommended curcumin nanoemulsion in combination with PZQ in treating schistosomiasis *mansoni*[42]. Later, the prophylactic and therapeutic effects of ginger-derived NPs were evaluated in *S. mansoni*-experimentally infected mice either alone or combined with PZQ or Mefloquine. Results revealed a slight prophylactic effect, while the highest therapeutic effect, *i.e.*, 100% reduction of all parasitological parameters, was achieved in combined therapy with Mefloquine[43].

Natural products with other therapeutic roles in schistosomiasis

Antioxidant: Several herbal aqueous or methanolic extracts exhibited amelioration of liver functions, and significantly improved all oxidative stress biomarkers (NO reduction, and conserved expressions of hepatic glutathione, glutathione-S-transferase, and superoxide dismutase). They included *Clerodendrum umbellatum*[44], *Ceratonia siliqua*[45], *Sida pilosa*[46], *Ziziphus spina-christi*[47,48], combined *A. sativum* and *C. longa*[49], and Fenugreek oil derived from *Trigonella foenum*[50].

Anti-fibrotic activity: In a Saudi-Egyptian collaborative work, two studies were conducted to investigate the ameliorative effects of *C. siliqua* extract[45], and *Z. spina-christi leaf* extract[47] on liver fibrosis of mice infected with *S. mansoni*. Compared to PZQ, both extracts exhibited significant anti-fibrotic activity observed by decreased collagen deposition. It was attributed to downregulation of tissue inhibitors of matrix metalloproteinases, and α-smooth muscle actin expression[45,47].

Anti-apoptotic: Two studies lessened hepatic apoptosis observed by reduction of caspase-3, and *bax* gene expression, and increased Bcl-2 level[45,47]. Notably, *bax* gene is the core regulator of the intrinsic pathway of apoptosis, and Bcl-2 is a neuroprotective, anti-apoptotic protein. More recently, Fenugreek oil derived from *Trigonella foenum* exhibited significant anti-apoptotic activity. When administered with PZQ, it restored caspase-7 to its normal value with significant reduction of annexin-V level[50].

Anti-angiogenic, and anti-proliferative activities: Two studies investigated the anti-angiogenic effect of combined Kalobin-PZQ[20], and *Z. spina-christi* leaf methanol extract[48]. In both studies, immunohistochemical staining demonstrated reduced vascular endothelial growth factor (VEGF) expression in both hepatocytes and sinusoids. It is worth mentioning that VEGF plays an important role in chronic schistosomiasis since it significantly increases during liver fibrosis. It regulates several pro-fibrotic and immune cytokine genes in hepatic stellate cells, including integrin, fibronectin, INF-γ, IL-6 and IL-10[20,48]. In the second study, *Z. spina-christi* anti-proliferative activity was demonstrated. Compared to PZQ, it showed significant reduction of both hepatic granuloma, and Ki-67 protein, a protein detected only in rapidly dividing cells[48].

Immunomodulatory activity: Fucoidan, a sulfated polysaccharide derived from brown seaweed *Fucus vesiculosus* showed immunomodulatory activity *in vitro*, and *in vivo*. *In vitro*, stimulated splenocytes with fucoidan demonstrated increased Treg cells, and expression of chemokine receptors. Decreased hepatic phosphorylated p65, and pro-inflammatory cytokines (IL-6, IL-12 and TNF-α), with increased anti-inflammatory cytokines (ILs 4 and 13) were recorded in experimentally infected mice. The study concluded that fucoidan reduced hepatic pathology through its immunomodulatory activity[51]. In a recent study, investigators evaluated hydro-alcoholic extract (HAE) derived from *Chenopodium ambrosioides* in amelioration of schistosomiasis-related hepatic granulomas. Results revealed that independent of the phase of treatment, HAE reduced eosinophils, IgE, IFN-γ, TNF-α, and IL-4, as well as hepatic granulomas. In contrast, dependent on the phase of treatment, HCE increased IL-10 levels and the number of T and B lymphocytes, and macrophages, *i.e.*, increased formation of hepatic granulomas. The study attributed this result to secondary compounds in HAE, *e.g.*, kaempferol, quercetin and their derivatives[52[.

Clinical trials: Compared to PZQ, *Art. annua*, and *Art. afra*, were used in treating 800 patients in a double-blind randomized clinical trial. While all *Artemisia*-treated patients showed negative stool microscopic examination within two weeks of treatment, PZQ-treated patients showed similar results after three weeks. Further implementation on a wide global scale was recommended[53].

Hydatid cyst

During the last decade, several studies were conducted utilizing different herbal medicines for treating hydatid cysts. Although the only clinically curative approach is surgery, however, an efficient compound with scolicidal activity is essentially required during surgery to prevent recurrence of secondaries. All *in vitro* studies used 0.1% eosin to evaluate the viability of protoscoleces, collected from fertile hydatid cysts, after exposure to different concentrations of the herbal extract. The majority of studies performed additional *ex vivo* assessment and evaluated the cytotoxic effects in experimentally

infected mice. An ideal scolicidal extract should show 100% inhibitory activity at a low concentration, in a short time of exposure, and without cytotoxic effects.

In fact, literature provided a huge number of Iranian publications investigating herbal plants. Among them were EOs of *C. zeylanicum*[54], *C. longa*[55], *C. zadoaria*[56], *Smyrnium olusatrum*[57], *Ferula macrecolea*[58], *Z. multiflora*[59]. They included also extracts of *Myrtus communis*[60], *Rheum ribes* L.[61], *Capparis spinosa* L.[62], and both *Urtica dioica* and *Cassia fistula* fruits[63]. Methanolic extracts of *Pistacia khinjuk*[64], *A. sativum*[65], *Pipper longum*[66], and *Rhus coriaria* (Sumac)[67]; ethanolic extract of *Ziziphora tenuior*[68], and hydroalcoholic extract of *Taxus baccata* L.[69], and *Art. absinthium* and *Ferula assafoetida*[70] were also included. Regarding marine products, spines and shells extractions from *Echinometra mathaei*, obtained from the Persian Gulf showed scolicidal activity *in vitro*[71]. Natural products other than herbal medicine were also investigated, and exhibited potent scolicidal activity, *e.g.*, ozone gas[72].

Similarly, several Egyptian studies were also conducted investigating the scolicidal activity of *Salvadora persica* root extracts[73], and hydroalcoholic extract of *P. granatum* peel, and *N. sativa*[74]. Although extract of *N. sativa* showed more scolicidal potency than that of *P. granatum* peel, their efficacies were of moderate significant correlation to exposure time, and concentrations[74]. To increase its efficacy, another Egyptian study investigated *N. sativa* EO either alone, or combined with ABZ, and utilized chitosan NPs as a drug delivery system. Promising results were obtained using *N. sativa* EO loaded on chitosan NPs. A significant decrease of TNF-α with increased NO levels occurred indicating increased host immune response[75]. Moreover, histopathological studies revealed complete eradication of hydatid cyst with significant improvement of inflammation, necrosis, and hepatic congestion in mice treated with *N. sativa* EO loaded on NPs[76].

In a study conducted in Algeria, the investigators demonstrated that the aqueous extract of *Atriplex halimus* leaves exhibited significant *in vitro* scolicidal activity in a time- and dose-dependent manner, without cytotoxic effects against murine peritoneal macrophages[77]. In Jordan, a group of investigators showed that methanolic extracts of *Citrullus colocynthis, Ruta graveolens*, and *Peganum harmala* exhibited higher therapeutic effects than ABZ. In addition, *R. graveolens* showed the best histological results with complete germinal layer detachment[78].

Water-soluble alkaloids from traditional Chinese medicine *Sophora moorcroftiana* (Fabaceae) showed scolicidal activity, with a significant increase in cytokines expression[79]. Recently, two Chinese reports were published, where the first study demonstrated that crocin, a carotenoid chemical compound found in the flowers of crocus and gardenia, exhibited a moderate inhibitory activity against *E. multilocularis* metacestodes, and protoscoleces *in vitro*. The *in vivo* study showed a significant reduction in hydatid cyst size after crocin administration. Ultrastructural results revealed that crocin caused structural damage to both germinal and laminated layers with increased collagen deposition due to the inhibition of matrix metalloproteinases expression[80]. Results of the second study showed that *Capparis spinosa* fruit extract had *in vitro* scolicidal effects on the larvae of *E. granulosus* (*Sensu stricto*) as demonstrated by ultrastructural disruption of metacestodes, and protoscoleces[81].

Noal *et al*.[82] showed that two acetate fractions (N-butanol and ethyl) derived from *Blepharocalyx salicifolius* Berg also exhibited scolicidal activity against *E. ortleppi* protoscoleces. A group of Argentine investigators conducted two studies. *Stevia multiaristata* extract quickly decreased protoscoleces viability, and loss of the tegmental turgidity, with subsequent germinal layer collapse[83]. Since *Humulus lupulus*, a bittering follower plant, exhibited sedative, digestive, anti-inflammatory, and antimicrobial effects, the second study investigated the *in vitro* efficacy of its variable methanolic extracts against *E. granulosus* (*S. stricto*) protoscoleces. Due to their highest content of flavonoids, total polyphenols, and saponins, Mapuche and Victoria varieties exhibited significant protoscolicidal effects compared to others, *i.e.*, Bullion, Cascade, and Traful. Both varieties caused ~50% decrease of protoscoleces viability, with complete ultrastructural altered contraction after 18 d. Since the beer-making industry leaves *H. lupulus* are an agricultural by-product, the study proposed their use as a source of secondary metabolites with protoscolicidal activity[84].

Phytochemical analysis revealed that the main components were cinnamaldehyde in *C. zeylanicum*[54], isofuranodiene, a C15 organic sesquiterpene in *Smyrnium olusatrum*[57], terpinolene in *F. macrecolea* EO[58], carvacrol in *Z. multiflora*[59], flavonoids, tannins, and terpenoids in *C. spinose*[62], and gallic acid in both *Atriplex halimus*[77], and *B. salicifolius*[82]. The mechanism of action was attributed to the initiation of protoscolices programmed cell death as demonstrated by significant increase of caspases activity[58,60,71].

In their report[85], reviewers claimed that effective compounds derived from natural products that were investigated in treating hydatid cysts were scolicidal and/or cysticidal. Scolicidal compounds are administered intra-cystically before surgical intervention to prevent recurrence during surgical or percutaneous intervention. The main obstacle in this approach is the highly variable procedures involved in their preparation, making it difficult to

assess different reported studies. Besides, scolicidal compounds should be investigated *in vivo* for intra-cyst solubility and bioavailability, and *in vitro* for their potent efficacy against metacestodes. It is worth mentioning that efficient solubility and bioavailability are solved using NPs technology. On the other hand, several factors control the efficacy of cysticidal drugs such as the number, size, location, developmental stage, and cyst fertility[85]. Later, two recent reviews[86,87] summarized usefulness of herbal medicine in treating hydatid cyst and both reviews recommended further research to: 1) explore the *in vivo* mechanisms of action, hence increasing pharmacokinetics properties, and improving their therapeutic efficacy; and 2) investigate the other *E. granulosus* genotypes since the majority of studies were conducted only on genotype G1. Both reviews claimed that clinical trials are essentially required.

Nematocidal activity

Increased temperatures and global climate changes were associated with increased prevalence of diseases caused by parasitic nematodes, with subsequent emergence of anti-nematode drug resistance. No doubt, the impact of plants, fish, and livestock nematodes accelerates transmission of zoonotic diseases. Using *C. elegans* as a nematode model, a bioassay-guided isolation of compounds from *Tetradenia riparia* leaves with potent nematocidal efficacy, was conducted. The investigators identified only one compound (sandaracopimaradiene) with LC50 value (5.4 µg/ml), and recommended further studies to investigate its anti-nematode activity *in vitro* and *in vivo*[88]. Utilizing the same approach, another study identified three compounds in *Warburgia ugandensis*. They were polygodial, waburganal, and alpha-linolenic acid with low LC50 value. Structure-activity relationship demonstrated that polygodial possessed a selective structural motif responsible for its potent activity. The proposed mechanism of action was inhibition of mitochondrial ATP synthesis[89].

Moreover, two Cameroonian studies demonstrated the usefulness of natural products such as *Lophira lanceolata*[90], and *Acacia nilotica* fruits[91] as potential compounds with broad nematocidal activity. Both studies investigated the *in vitro* anthelmintic activities against *C. elegans* drug resistant strains, and *O. ochengi*. While ethanolic, and methanol-methylene chloride extracts of *L. lanceolata* killed 50% of *C. elegans* with low IC50 value, *L. lanceolata* leave extracts were lethal to ABZ- and IVM-*C. elegans* resistant strains after 72 h. Results of *in vitro* cytotoxicity, and *in vivo* acute toxicity revealed that both extracts were relatively safe[90]. In a hydro-alcoholic extract (CE), a constituent in its most active fraction (CG), and four related proanthocyanidins from *Acacia nilotica* fruits exhibited potent inhibition against *C. elegans in vitro*. Both *in vitro* cytotoxicity on *Caco-2* cell line, and *in vivo* acute oral toxicity on Wistar rats revealed that crude extract and pure constituents were safe to use[91].

Liu *et al*.[92] reviewed the studies conducted to investigate the efficacy of compounds derived from natural products against parasitic nematodes. They claimed that 1) most of the investigated compounds were isolated using bioassay-guided purification approach that guarantees identification of the major bioactive compounds in the assayed herb; 2) parameters used for evaluation included death, motility, and egg production bioassays; 3) isolated purified compounds included fatty acids, saponins, and phenolic compounds; 4) synergy was commonly reported between bioactive compounds even isolated from the same plant; 5) all investigated compounds proved *in vitro* potent efficacy, but few were *in vivo* investigated; 6) the major mechanism of action was mitochondrial inhibition.

Ascariasis

A wide variety of *in vitro* anti-*Ascaris* activity was observed in investigating ethanolic extracts of 29 medicinal plants used in Ghana, and the Caribbean. The extracts of *Clausena anisata, Zanthoxylum zanthoxyloides* and *P. granatum* were recognized to have high potent anti-*Ascaris* activity at low LC50 value; hence, it was recommended that further investigations are warranted to validate their use as a complementary daily dosage with mass drug administration program[93]. Later, the results of a primary schools-based clinical trial revealed that papaya (*Carica papaya*) seeds, supplemented to routine school meals, exhibited reduction of *A. lumbricoides* burden[94].

Hookworm infections

Crude hydroalcoholic extract of Ethiopian traditional medicine (*Embelia schimperi*) exhibited nematocidal activity against *N. americanus in vitro*. Additionally, the, *E. schimperi* major component of diammonium salt of embelin, showed 85.3% parasite clearance in experimentally infected mice[95]. Phytochemical analysis of *Dichapetalum filicaule* revealed dichapetalins, triterpenoids, and glycerol monostearate. All exhibited potent inhibitory activity against *N. americanus in vitro* as recorded from egg hatching inhibition assay, however, dichapetalin X showed the lowest LC50 value[96].

Extracts of three nematophagous fungi, *Paecilomyces lilacinus*, and two *Trichoderma* spp. (*T. harzianum*, and *T. virens*) showed significant ovicidal activity against *Ancylostoma* eggs[97]. Ethanolic, and methanolic extracts of *Andrographis paniculate* leaves showed the highest *in vitro* ovicidal activity against *A. duodenale*, whereas its ethyl acetate extract had the highest larvicidal activity. The phytochemical analysis revealed that *A. paniculate* nematocidal

activity was attributed to andrographolide, the major diterpene component. Additionally, its pure fraction showed significant ovicidal, and larvicidal activities at low concentration[98]. The ovicidal activity against *Ancylostoma* eggs was also demonstrated using hydroalcoholic extract of *Schinus terebinthifolia*; which was mainly attributed to ethyl gallate, myricitrin, and gallic acid compounds[99].

In spite of utilizing mass drug administration program for 15 y to control soil-transmitted helminth infections, hookworm infection remained common in Mayuge district, Uganda. In a randomized controlled trial, a study investigated the efficacy of dual- versus single-dose ABZ, either with or without avocado (*Persea americana*) against hookworm infections in 222 schoolchildren. Three weeks after treatment, both cure and egg reduction rates were significantly reduced in children receiving dual ABZ dose, and avocado exhibited higher reduction rates without significance. The investigators recommended future studies exploring avocado' pharmacokinetic properties to improve ABZ therapeutic efficacy[100].

Strongyloidiasis

Utilizing egg hatching, and larval motility tests, a Brazilian group of investigators conducted three studies investigating the usefulness of natural products against *S. venezuelensis* eggs, and larvae *in vitro*. Compared with two control drugs (ABZ, and IVM), latex from *Carica papaya*, and purified papain, the major bioactive component, were effective ovicidal, and larvicidal compounds. Purified papain' ovicidal activity showed a dose-dependent response[101]. In the second study, the investigators proposed benzyl isothiocyanate, carpaine, and carpasemine, isolated from *C. papaya* seed hexane extract, as promising compounds for treatment of strongyloidiasis[102]. Similar ovicidal, and larvicidal activities were obtained on investigating extracts, and isolated compounds from *Siparuna guianensis*. Ethanol extract, and aqueous fraction were as effective as ABZ, and IVM. Their phytochemical analysis revealed phenolic compounds, tannins and flavonoids[103].

Additionally, another three natural products, *Argemone mexicana*[104], *Manilkara zapota*[105], and *Spondias mombin* L[106] were investigated against *S. venezuelensis* eggs, and larvae *in vitro*. Berberine, an *A. mexicana* main component, was the most effective, followed by the methanolic, and crude extracts with the least LC50 value. It also showed an efficient anti-oxidant activity without hemolytic activity against human erythrocytes[104]. Ethanolic extract, phenolic-rich compounds, and chlorogenic acid from *M. zapota* leaves were proposed potential phytomedicine for treatment of drug-resistant infections[105]. Comparable to IVM, ethanolic extract of *S. mombin*, and its fractions showed better *in vitro* results, and fraction 4 exhibited 100% mortality in 12 h, and 4 h at concentrations of 50, and 400 µg/ml, respectively. Ultrastructural results revealed cuticle wrinkling, and peeling, while IVM caused only wrinkling. The investigators attributed anti-*Strongyloides* effects to the high enrichment of monoaromatic phenolic lipids in fraction 4[106].

Anisakiasis

Utilizing online electronic search, Spanish investigators evaluated the *in vitro* and *in vivo* efficacy of several natural products (extracts, EOs, and major components) against L3 larvae of *A. simplex*. *In silico* analysis of the results showed that *M. alternifolia* EO, and turmerone isolated from *C. longa* exhibited the highest efficacy *in vitro*. *In vivo*, perillaldehyde, a *Perilla frutescens* OE' major component was the most active compound[107].

Trichinosis

The ethanolic extract of *Art. annua* was efficient in treating intestinal trichinosis since it significantly reduced intestinal adult count. Compared with infected untreated mice, it restored the normal intestinal architecture, reduced edema, alleviated inflammation as demonstrated by reduced inflammatory infiltrate, and intestinal TGF-β expression[108]. Treatment with extracts of *Z. officinale*, and *C. zeylanicum* exhibited similar results in comparison to ABZ-Prednisolone combined treatment. Both extracts significantly reduced intestinal adults, and muscular larvae counts. Moreover, *Z. officinale* treated mice showed the highest reduction of TNF-α level, and the ultrastructural results demonstrated degenerative effects on both adults and larvae[109]. Compared to ABZ., the anti-*T. spiralis* activity of pumpkin decoction, and its combination with honey was investigated in the intestinal phase of murine trichinosis. Combined treatment exhibited the highest reduction rate (83.2%) with significant reduced intestinal cyclo-oxygenase-2 expression, NO and TNF-α, and increased TGF-1β, and IL-17 as well as antioxidant enzymes' activities[110].

A recent Egyotian study concluded that ethanolic propolis extract has a potential *in vitro* and *in vivo* efficacy against both adult worms and muscle larvae. Since it is safe, and commercially available product, the investigators recommended its use as a prophylactic supplement or in combination with ALB to increase its effect as it showed anti-fibrotic, anti-inflammatory, and antiapoptotic effect[111] .

Combined therapy with ABZ: During 2021-2024, several Egyptian studies investigated the efficacy of combined treatment in intestinal and muscular murine trichinosis. Extract of *P. granatum* administered alone or combined with ABZ, exhibited higher efficacy than ABZ monotherapy in intestinal trichinosis. In the muscular phase, combined treatment exhibited significant immunomodulatory effects that reduced muscular $CD4^+$ expression, and extensively alleviated associated myositis[112]. Combined treatment (*C. molmol*-ABZ) proved more efficacious than if ABZ was

combined with *Lipidium sativum*[113]. Investigating the efficacy of *Cyperus rotundus* methanolic extract and its derivate fractions (ethyl acetate, petroleum ether, and butanol) revealed that butanol exhibited the highest anti-*Trichinella* activity *in vitro*, with the lowest LC50 value. When butanol was administered either alone or combined with ABZ to experimentally infected mice, significant decrease of all parasitological and histopathological parameters with maximal therapeutic effects were recorded in combined therapy. Phytochemical analysis of the methanolic extract revealed several active compounds including phenolic acids, flavonoids, sterols, and triterpenoids[114]. To increase curcumin bioavailability, a fourth Egyptian study explored the potential efficacy of curcumin NPs alone and in combination with ABZ on murine trichinosis. Combined treatment showed the highest reduction rates in intestinal adult (99.3%), and muscular larva (95.9%) counts. There was a significant improvement in the inflammatory response recorded by decreased expression of VEGF, and matrix metalloproteinase 9, due to curcumin anti-angiogenetic activity. Creatinine kinase, and malonaldehyde level were also significantly decreased with improvement of total antioxidant capacity[115].

Immunoprotective activity: Although *Lonicera caerulea* (honeyberry) extract did not show anti-*Trichinella* efficacy, it immunoprotected mice from trichinosis. The study suggested that it affected the dynamics of *Trichinella* expulsion from the intestines through stimulation of splenic lymphocyte proliferation, and altered B ($CD19^+$), and T ($CD3^+$, $CD8^+$) cell counts[116].

Diseases caused by filarial nematodes

Since endosymbiont *Wolbachia* presents a potential promising drug target in filarial nematodes, several studies were conducted investigating natural products, and antibiotics for their anti-*Wolbachia* activity. Methanolic extracts of *Melaleuca cajuputi* flowers significantly suppressed *Wolbachia* endosymbionts in *Aedes albopictus* Aa23 cells[117]. The screening approach demonstrated that kirromycins, isolated from *Streptomyces* spp., significantly depleted *Wolbachia* in *Drosophila* cells *in vitro* and at a lower LC50 value compared with Doxycycline, an anti-*Wolbachia* drug. *Ex vivo* results revealed that kirromycins eliminated endosymbiosis in *B. pahangi* ovaries with higher efficacy compared with Doxycycline. Reassuringly, no cytotoxic effects were recorded against two mammalian cell lines[118]. In *Litomosoides sigmodontis* rodent model, corallopyronin A, a preclinical anti-*Wolbachia* drug, administered alone for two weeks, cleared >90% of endosymbiosis from microfilariae with subsequent blockage of adult stage development. When administered combined with ABZ for a week, a sustained reduction of >99% of endosymbiosis from adults was achieved, with subsequent complete inhibition of embryogenesis[119]. More recently, utilizing structure-based drug design approach, the investigators identified six novel inhibitors targeting *Wolbachia* surface protein (WSP). Using homology modelling techniques, out of 15 generated WSP 3D structures, only one was selected due to its reasonable quality. High throughput virtual screening utilizing three online libraries (42,883 natural products from African and Chinese databases) identified 23 compounds with efficient selective inhibitory activity on *Wolbachia*. Due to satisfactory pharmacological profiles and insignificant toxicity, only six inhibitors were proposed as potential novel anti-*Wolbachia* agents for future studies[120].

Lymphatic filariasis

Methanolic extracts of *M. cajuputi* flowers significantly reduced viability, inhibited motility of *B. pahangi* adults, and decreased microfilarial release. The inhibition of *Wolbachia* endosymbionts was proposed as its mechanism of action[117]. To identify the bioactive constituent, the crude ethanolic extract of *Taxodium distichum* was solvent fractionated (F 1-4). Both the crude extract and F3 exhibited *in vitro* 80-100% reduction of *B. malayi* adults, and microfilaria. *In vivo* studies utilizing two animal models (*Mastomys coucha*, and *Meriones unguiculatus*) showed that the crude extract killed all adults in >80% of infected animals. While F3 showed macrofilaricidal activity in *M. coucha*, it exhibited embryostatic effects in *M. unguiculatus*. The study concluded that *T. distichum* ethanolic extract possessed potent anti-filarial activity, and its bioactive constituent was labdane diterpenoid[121]. Later, the same group of investigators observed that flowers of *Calotropis procera*, used traditionally to alleviate symptoms of elephantiasis, had not yet been investigated for its anti-filarial activity. Ethanolic extract, and its hexane fraction exhibited *in vitro* microfilaricidal, and macrofilaricidal activity against *B. malayi*. Their oral administration to the previously mentioned rodent models, recorded the following results: 1) ethanolic extract killed ~50% *B. malayi* adults in both models; 2) in a dose-dependent manner, hexane fraction killed 12-60% of *B. malayi* adults only in *M. coucha* model; 3) both extract and fraction suppressed microfilaraemia up to 91-, and 35- days post treatment, respectively. Phytochemical analysis revealed that lupeol, β-sitosterol, and triacontanol were the major constituents in hexane fraction. The investigators recommended further studies investigating each component separately or combined to elucidate their anti-filarial activity[122]. Additionally, extracts of *Daniellia oliveri*, and *Psorospermum febrifugum*, traditional Cameroonian medicines, showed selective inhibitory action against *B. pahangi in vitro* without cytotoxic effects[122].

Onchocerciasis

Cameroonian researchers reviewed the 13 studies conducted to investigate anti-*Onchocerca* natural products from 1990 to 2017. *Anacardium occidentale,*

Euphorbia hirta and *Acacia nilotica* were the most commonly investigated, and polycarpol, voacamine, voacangine, as well as ellagic, gallic, and gentisic acids were among the isolated compounds with anti-*Onchocerca* activity. Although the majority of these compounds showed satisfactory *in vivo* efficacy, and safety, none of them reached clinical trial stage. This is mainly due to the limited accessibility through the *Onchocerca* nodular wall, and bioavailability inside the cutaneous nodules[124]. Later, extracts of *D. oliveri*, and *P. febrifugum* showed *in vitro* filaricidal activity against *O. ochengi* adults, and microfilaria, suggesting their potential therapeutic use in treating onchocerciasis, especially when associated with lymphatic filariasis[123].

In vitro screening of 14 extracts from *Tragia benthami*, and *Piper umbellatum* revealed that only nine extracts exhibited 100% activity on all investigated stages. However, methylene chloride extract of *P. umbellatum* leaves showed the highest activity. Interestingly, out of the most active extracts, only three exhibited anti-*L. loa* microfilarial activity. All extracts showed selective toxicity against *O. ochengi* with neither cytotoxic effects, nor acute toxicity to BALB/c infected mice[125]. The anti-*Onchocerca* activity of *L. lanceolata*[90], and *A. nilotica*[91] extracts showed promising results against *O. ochengi*. Phytochemical analysis of the investigated herbs revealed enrichment of terpenoids, hydrocarbons, tannins, and fatty acids derivatives. Screening extracts of 15 Ghanian traditional medicines, 11 exhibited high to moderate inhibition of *O. ochengi* microfilariae and males, while only 6 exhibited >60% inhibitory activity against females. The most active extract (B2-DCM) was prepared from *Phyllanthus niruri, Syzygium aromaticum, Xylopia aethiopica,* and *Tapinanthus bangwensis*. Three other extracts, prepared from two anti-schistosomal (B4-DCM, and B7-DCM), and one anti-*Onchocerca* (O1) traditional medicines recorded satisfactory LC50. Notably, O1 was prepared from *Mangifera indica, Momordica charantia*, *Z. officinale*, and *Xylopia aethiopica*. The *in vitro* study showed that O1 was the most effective against females[14]. Later, six novel *Wolbachia* inhibitors, selected *via* high throughput virtual screening of natural products from African and Chinese databases, were proposed potential novel drugs for treatment of onchocerciasis, particularly for *O. ochengi*. and *O. gutturosa*[120].

Twelve extracts derived from two Cameroonian medicinal plants traditionally used in treating onchocerciasis; *Lantana camara* and *Tamarindus indica*, were investigated against *O. ochengi*, and *L. loa* adult viability, and microfilaria motility *in vitro*. Cytotoxic effects, and anti-*Onchocerca* activity were evaluated against monkey kidney epithelial cells, and experimentally infected BALB/c mice, respectively. Results revealed that all extracts exhibited 100% inhibitory activity against *O. ochengi* adults and microfilaria, however, the highest activity was recorded with hexane extract of *L. camara* leaves. *In vivo* studies showed that lantadene A, isolated from *L. camara* methylene chloride extract, exhibited potent activity against *O. ochengi*, and *L. loa*, however, its activity against *L. loa* was lower than its anti-*Onchocerca* activity. Because IVM is contraindicated in treating onchocerciasis in endemic areas of loaiasis, the investigators proposed lantadene A as a potential therapeutic for both parasites, which warrants further investigation[126]. Recently, hydroethanolic extracts of *Aframomum melegueta, Xylopia aethiopica*, and *Khaya senegalensis* exhibited immunomodulatory effects among patients with hyperactive onchocerciasis (*i.e.*, severe cutaneous inflammation, or generalized onchocerciasis), through downregulating both Th17, and Th2 responses[127].

CONCLUDING REMARKS

1. Several EOs, and bioactive metabolites isolated from herbal and marine sources, as well as derived from microorganisms, and insects open new avenues in the progress of developing novel drugs for treatment of parasitic diseases. Alkaloids, quinones, chalcones, terpenes, saponins, tannins, tunicates, ascidians, sinefungin and mevinolin constitute the widely investigated bioactive compounds, and metabolites. Other investigated natural sources included synthetic compounds naturally occurring in vegetables and fruits (aurones), and sponges (synthetic alkaloids). Propolis, mollusca metabolites derived from Heterobranchia, Buprestin H isolated from beetles, and ecofriendly green synthesis were also investigated for their potential therapeutic efficacy against several parasitic diseases.
2. Due to their efficacy against a wide range of parasitic diseases, several herbs gained much attraction such as extracts and EOs of wormwood, and mugwort (*Artemisia* spp.), myrrh (*C. molmol*), garlic (*A. sativum*), pomegranate (*P. granatum*), black caraway (*N. sativa*), ginger (*C. zadoaria*, and *Z. officinale*), curcumin (*C. longa*), and tee tree (*M. alternifolia*), as well as derived compounds such as naphthoquinones, chalcones, carvacrols, and steroidal lactones.
3. Several studies evaluated extracts of natural products with potential broad-spectrum efficacy against multiple helminths, *e.g.*, B4-DCM and B7-DCM in treatment of schistosomiasis and onchocerciasis, marine epiphytic bacteria, and hydroalcoholic extracts of *A. nilotica* and *L. lanceolata* with nematocidal activity, kirromycins isolated from *Streptomyces* spp. and other inhibitors of *Wolbachia* endosymbiosis with filaricidal activity.
4. Drug discovery for development of novel drugs has several advantages including unexpansive drugs for low-income patients, capability for long-term treatment without adverse side effects, targeting multiple parasite' targets, and no possibility for emergence of resistant strains. However, lower concentration of bioactive compounds, low

bioavailability and solubility, absence of standard methods for preparation, and uniform optimization regarding quality difference among investigated compounds were the main reported challenges facing novel drugs development. Besides, few proposed compounds reached clinical trials due to lack of studies on structure-activity relation to design selective inhibitors with high binding affinity toward the parasite targets.

5. For developing novel drugs from natural sources, three approaches were utilized, however, phenotypic screening using online libraries is the most widely used strategy. NatureBank, PhytoQuest Phytopure, comprehensive marine natural products database, and African flora database are the most commonly reported online libraries. Other studies utilized *in silico* or biochemical approach, where the former is conducted on a proposed drug target, and the latter on repurposing or modifying approved drugs using potential specific scaffolds.
6. Evaluation of traditional medicines gained much attention in the literature. They included Chinese *X. decoction* (schistosomiasis *japonicum*), Jamaican *E. foetidum* (strongyloidiasis), Indian *C. procera*, and Cameroonian *D. oliveri* and *P. febrifugum* (lymphatic filariasis), six Cameroonian plants; *A. nilotica, C. articulates, T. benthami, P. umbellatum, L. camara*, and *T. indica* (onchocerciasis).
7. Since the introduction of nanotechnology, NPs, nanoemulsions, and nanofibers, as well as liposomes were efficient safe drug delivery tools to overcome low bioavailability. Examples included curcumin nanoemulsion, and ginger-derived NPs either alone or combined with Mefloquine (schistosomiasis), and *N. sativa* EO loaded on chitosan NPs (hydatid cyst).
8. In spite of the huge literature investigating usefulness of natural products in treatment of parasitic diseases, only few reached clinical trials, *e.g.*, *Artemisia* spp. in treatment of schistosomiasis, papaya seeds in ascariasis, and avocado in hookworm infections.
9. Use of natural products to modulate host immune response towards an efficient cell-mediated immunity for parasite elimination was recommended. Therefore, natural products were used as adjuvant therapy combined with commonly used drugs, *e.g.*, *P. granatum*, *C. molmol*, *C. rotundus* and propolis in trichinosis.
10. In schistosomiasis, herbs with immunomodulatory, anti-oxidant, and anti-fibrotic activities were recommended. Hydroalcoholic extract of *C. ambrosioides*, and Fucoidan of *F. vesiculosus* reduced hepatic pathology through reduction of eosinophils, and IgE, and immunomodulation of pro-inflammatory and anti-inflammatory cytokines. Allicin, and curcumin were also suggested to improve the anti-oxidant hepatic capacity. On the other hand, herbs with anti-fibrotic activity proved effective in treatment of chronic schistosomiasis with liver fibrosis, *e.g.*, *C. siliqua* extract, *Z. spinachristi* leaf extract, Fenugreek oil derived from *T. foenum*.
11. In hydatid cyst, development of novel scolicidal compounds from natural products is urgently required for intracystal application before surgical intervention to prevent recurrence. However, this process is challenged by three issues: 1) intra-cyst solubility and bioavailability; 2) efficacy against metacestodes *in vitro* and *in vivo*; 3) variable *E. granulosus* genotypes.
12. In diseases caused by trematodes, cestodes, and intestinal nematodes, several drug targets were proposed including several proteases, protein kinases, ATPases, and calcium channel transporters.
13. Although several natural products and traditional herbal medicine were investigated against diseases caused by filaria, *Wolbachia* endosymbiosis remains the main drug target.

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