Plant Products in the Treatment and Control of Filariasis and Other Helminth Infections and Assay Systems for Antifilarial/Anthelmintic Activity

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Key words

- plant products
- filariasis
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- *in vitro* assays
- *in vivo* assays

Abstract

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Lymphatic filariasis, onchocerciasis, loiasis, and other helminth infections cause serious health problems especially in resource-limited tropical and subtropical developing countries of the world, and more than 2 billion people are infected with at least one helminth species. From times immemorial, man looked up to the plant kingdom in search of anthelmintics, antifilarials, and remedies for parasite-induced health problems. Although more than 50% of drugs in modern

medicine are derived from plants or leads from plants, a success story of plant-based anthelmintics or antifilarials is yet to be told. In the last 5 decades, more than 100 plant products were reported to be beneficial in the treatment or control of these parasitic infections but they could not be developed into viable drugs for a variety of reasons. This review focuses on the plant products reported to be useful in the control and treatment of human helminth infections with the main emphasis on filariasis and the *in vitro* and *in vivo* systems available for assaying anthelmintic activity.

Introduction

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Since the time man first discovered the plant kingdom as a rich and convenient source of food, he returned to this kingdom repeatedly and found there remedies for illness too. As a result, several knowledge bases of how to treat or prevent illness and diseases using plants were generated. Some of this knowledge was passed on through generations by “word” (“folk medicine”) and some was compiled and practiced, such as Ayurveda in India, Kampo and traditional Chinese medicine (TCM) in Japan, Taiwan, and China. Today, about 50% of the drugs used in modern medicine are of plant origin [1] and the success stories include the mito-inhibitor vinca alkaloids vincristine & vinblastin (from *Vinca rosea*) and their semisynthetic analogues vinorelbine and vindesine as anticancer agents, the topoisomerase II inhibitors etoposide and teniposide which are semisynthetic isomers of the cytotoxic podophyllotoxin from *Podophyllum* spp., the taxanes and camptothecins, also anticancer agents [2], and the antimalarial artemesinin and its derivatives from *Artemisia annua* [3]. However, there are very few success stories related to antifilarial or anthelmintic activity, with the possible exception of ivermectin which is a macrocyclic lactone derived from

Streptomyces avermitilis. This review focuses on the plant products reported to show activity against human helminth parasitic infections with the main emphasis on filarial infections and the *in vitro* and *in vivo* systems employed to assay the (antifilarial) activity.

The Parasites

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The helminth parasites represent an extreme in the spectrum of pathogens as they are probably the only multicellular pathogens infecting man and animals. The helminth parasites comprise two very distantly related taxa: (1) the round worms or nematodes belonging to Nematelminthes (Class: Nematoda); and (2) the flatworms or Platyhelminthes (Class: Cestoda and Trematoda).

Worldwide, more than 2 billion people are infected with at least one helminth species [4]. The majority of these infections occur in resource-limited tropical and subtropical developing countries of the world, where over half of the population may harbor infections [5]. Of the various helminthic infections in man, those caused by filarial parasites are particularly important because of the huge loss of man-hours they cause.

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Bibliography

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The filarial parasites (Class: Nematoda; Superfamily: Filarioidea) include approximately 500 species infecting almost all vertebrates except fish. The parasites reside in lymphatics, connective tissues, or body cavities of the vertebrate hosts, and the infection is transmitted by a blood-sucking arthropod vector.

The filarial species infecting only humans are: *Wuchereria bancrofti*, *Brugia malayi*, and *B. timori*, which are responsible for lymphatic filariasis (LF) causing debilitating disease manifestations such as “elephantiasis” and hydrocele, *Onchocerca volvulus* that causes “river blindness”, and *Loa loa* causing “loiasis” (“calabar swelling”). Other prevalent but benign human filariids are: *Acanthocheilonema perstans*, *Acanthocheilonema streptocerca*, and *Mansonella ozzardi*, as well as the less frequent minor species: *W. lewisi*, *B. beaveri*, *B. guyanensis*, *M. semiclarum*, *Dipetalonema arbuta*, *D. sprengi*, *Microfilaria bolivarensis*, and *M. rodhaini*.

LF is a major disease with ever increasing prevalence in the developing world and the second leading cause of permanent and long-term disability. Globally, about 1 billion people live in areas endemic to LF (80 countries) and thus are exposed to the risk of infection. About 120 million suffer from the infection or the chronic filarial disease manifestations such as edema of limbs, breast, external genitalia, or hydrocele [6].

It is estimated that medical treatment for acute and chronic LF manifestations costs millions of dollars each year across the endemic regions. In India alone over 10 million people per year seek treatment for LF, which accounts for a total of 30 million dollars per annum. It is thought that the measurable health care costs of treating LF are small in proportion to the individual and societal costs from lost productivity. The contribution of LF to tropical disease burden in terms of *disability adjusted life years* (DALY) – which basically indicates the amount of healthy life expectancy lost because of a disease or disability caused by it, or risk factor, including both mortality and morbidity – is around 5.94 million globally and over 2.62 million for India [7].

LF infection is spread by *Anopheles*, *Culex*, *Aedes*, and *Mansonia* species of mosquitoes. During a blood meal, the mosquito takes up the stage 1 larvae or microfilariae (mf) circulating in the blood of an infected human. In the mosquito, mf undergoes two molts to become stage 3 infective larvae (L_3) which enter the human host during a blood meal of the vector. L_3 penetrate through the local connective tissue and enter the lymphatic vessels [8] where they take 2 to 12 months to develop into adult worms through two molts. Mature male and female worms mate and produce the progeny, mf, which enter the bloodstream from where they are picked up by the mosquito during its blood meal, and the life cycle continues.

Onchocerciasis is the second major filarial disease group and affects around 18 million people, mainly in tropical Africa and Latin America [9]. The infection is presented as a spectrum of dermal and ocular lesions resulting from the presence of microfilariae in the skin and eyes. The severity of the pathology which may cause blindness has attracted a massive international effort to reduce the impact of onchocerciasis through vector control and by mass chemotherapy [10].

Among non-filarial nematode infections, the soil-transmitted helminths (STH) commonly known as intestinal worms are the most common infections worldwide and constitute an important community health problem. The causal parasites are: *Ascaris lumbricoides*, *Trichuris trichiura*, and the hookworms *Ancylostoma duodenale* and *Necator americanus*. Recent estimates suggest that *A. lumbricoides* infects over 1 billion people, *T. trichiura* 795 million, and hookworms 740 million. The greatest numbers of

STH infections occur in sub-Saharan Africa, the Americas, China, and East Asia [11]. STH affect most frequently children and produce diarrhea, abdominal pain, general malaise, and weakness that may affect working and learning capacities and impair physical growth and activity. Hookworms cause chronic intestinal blood loss leading to anemia [12–16]. A list of human helminth infections other than filariasis is given in **Table 1**.

In vitro and in vivo Systems for Screening Potential Antifilarials

Being multicellular advanced organisms displaying considerable host specificity, the helminth parasites pose several challenges in the development of convenient and reliable laboratory test systems for assaying plant and synthetic products for anthelmintic and antifilarial activity. In the case of the filarial parasite, there are two main challenges: first, there are 16 distinct human filarial parasite species and the efficacy of the products may show species specificity and parasite stage-specificity. For instance, a product may show about 80% efficacy against adult worms of *Onchocerca* spp. but only show an identical or acceptable activity against the larval microfilariae stage of *B. malayi* (unpublished observation). Consequently, to maximize the exploration, a given product, whether active or inactive against one parasite, has to be tested against life stages of multiple species. The second challenge is the availability, maintainability, and responses of some of the target parasites/parasite life stages *in vitro* and transmission of infection to nonhuman laboratory animal models. For example, the most prevalent lymphatic filariid, *W. bancrofti*, is seldom used for *in vitro* or *in vivo* screening. This is because the infection cannot be transmitted to or maintained in small laboratory animals. As a result we do not have a convenient screening model of this parasite and a source of the parasite life stages for *in vitro* use.

However, our improved understanding in the recent decades of the biology and host parasite interactions helped us developing not only useful *in vitro* systems but also successful transmission of human infections into small and larger laboratory animals for *in vivo* screening [18–31].

The different *in vitro* screen systems developed over the decades and employed for screening plant and synthetic products are given in **Table 2**. The assays employ one or more life stages (L_3 , mf, or adult worms) of the parasite depending upon the feasibility or type of activity (larvicidal, microfilaricidal, or macrofilaricidal) desired. The endpoints used in the assays include inhibition (in the parasite) of: motility, reduction of a tetrazolium salt to its formazan, parasite specific glutathione-S-transferase, enzymes involved in antioxidant generation or free radical scavenging, molting of L_3 to L_4 , embryogenesis, and mf release from female worms. The assays are employed as prescreens either singly or as a battery of two or more assays and the most frequently used battery consists of motility assay and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) reduction assay. Using this battery, a product is considered active if it causes complete inhibition in motility and/or > 50% inhibition in MTT reduction. The advantage of the assays are that a) a large number of products can be screened, b) they require a small amount of the test product for the assay, and c) the assays can be completed within 24–48 hrs [32,33]. Investigators have also used 120 hr incubation in order not to miss products that act slowly [34]. The main disadvantage of the *in vitro* pre-screens is that they detect

Table 1 Human helminth infections (other than filariasis) [17].

Disease	Parasite	Habitat	Infective agent/route	Intermediate host	Clinical manifestations
Nematode infections					
Ancylostomiasis	<i>Ancylostoma duodenale</i> , <i>A. ceylanicum</i> , <i>Necator americanus</i>	Intestine	L ₃ /per os, skin	None	Creeping eruptions, anemia, gastrointestinal (G.I.) manifestations, pot-belly, puffy face
Ascariasis	<i>Ascaris lumbricoides</i>	Intestine	Eggs/per os	None	G. I. disturbances: intestinal colic, obstruction, carbohydrate depletion, physical and mental retardation, allergy
Trichuriasis	<i>Trichuris trichiura</i>	Intestine	Eggs/per os	None	Diarrhea, dysentery, pain, rectal prolapses
Enterobiasis	<i>Enterobius vermicularis</i>	Intestine	Eggs/per os	None	Abdominal pain, dysentery, pruritus, rectal prolapses
Trichinellosis	<i>Trichinella spiralis</i>	Intestine, muscle	Encysted larvae/per os	None	G. I. disturbances, myositis, myocarditis, neurological symptoms, urticarial rash, fatal toxemia
Strongyloidiasis	<i>Strongyloides stercoralis</i>	Intestine	L ₃ /skin	None	G. I. disturbances
Trematode infections					
Schistosomiasis	<i>Schistosoma mansoni</i> , <i>S. haematobium</i> , <i>S. japonicum</i> , <i>S. mekongi</i> , <i>S. intercalatum</i>	Vasculature of G. I. or genito-urinary systems	Cercariae/skin	Snail	Acute: Dermatitis, fever, chills, nausea, abdominal pain, diarrhea, malaise, and myalgia Chronic: Bloody diarrhea (<i>S. mansoni</i>) or hematuria (<i>S. haematobium</i>)
Cestode infections					
Taeniasis and Echinococcosis (hydatid disease)	<i>Taenia solium</i> , <i>T. saginata</i> , <i>Diphyllobothrium</i> spp., <i>Hymenolepis</i> spp., <i>Echinococcus multilocularis</i>	Intestine	Eggs or cysts/per os	Pig/cow/fish	Abdominal discomfort, diarrhea, loss of appetite. Anemia in people with the fish tapeworm, neurological problems (rare)

Table 2 *In vitro* antifilarial assay systems.

Assay(s) (Measure/endpoint of the antifilarial activity)	Parasite(s)	Parasite life stage employed	References
Single assays			
Motility (irreversible inhibition of motility of parasite/viability)	<i>Litomosoides carinii</i> , <i>Brugia malayi</i> , <i>Acanthocheilonema viteae</i>	Mf	[35]
	<i>B. malayi</i>	Adults	[36]
	<i>B. malayi</i>	L ₃	[37]
	<i>B. malayi</i>	Adults, mf	[38]
Mf release inhibition	<i>B. malayi</i>	Adults	[38]
MTT reduction (inhibition of MTT reduction/viability)	<i>Onchocerca volvulus</i> , <i>O. gutturosa</i>	Female adults	[39]
GST Inhibition (Inhibition in parasite GST activity/viability)	<i>B. pahangi</i> , <i>B. malayi</i>	Mf, L ₃ , adults	[40]
Molting inhibition (Inhibition of L ₃ to L ₄ molting)/anti- <i>Wolbachia</i>	<i>B. malayi</i>	L ₃	[41]
Octapamine stimulation (tonic paralysis by altered membrane potentials/viability)	<i>A. viteae</i>	Adults	[42]
Two-assay battery			
Motility; reduction in lactate excretion (viability)	<i>B. patei</i> and <i>B. malayi</i>	L ₃ , adults	[43]
Motility; inhibition of respiration (viability)	<i>L. carinii</i>		[44]
Motility; MTT reduction (viability)	<i>Setaria cervi</i>	Adult, mf	[45]
	<i>O. volvulus</i>	Adults	[46–49]
	<i>O. gutturosa</i>	Adults	[47]
	<i>O. ochengi</i>	Adults, mf	[50]
	<i>A. viteae</i>	L ₃ , adults, mf	[51, 52]
	<i>B. malayi</i>	Adults, mf	[28, 53]
	<i>S. digitata</i>	Adults	[54]
Motility; GST inhibition (viability)	<i>S. cervi</i>	Adult (female)	[55]
Multiple assay battery			
Motility; MTT reduction; inhibition of microfilaria release (embryostatic effect; viability)	<i>B. malayi</i>	Adults, mf	[56]
	<i>B. pahangi</i>	Adults	[57, 58]
Antioxidant enzyme inhibition (inhibition of xanthine-oxidase, superoxide dismutase, catalase, glutathione peroxidase/viability)	<i>B. pahangi</i>	Adults	[57, 58]
	<i>L. carinii</i> , <i>S. cervi</i>	Adults	[59]

antifilarial activity of only those products which do not require metabolic activation to active pharmacophore. As a result several products that are negative *in vitro* but which may show activity after bitransformation in the host are missed. It is therefore necessary to include in the *in vitro* incubations a metabolic activation system such as the liver microsomal fraction (S9 mix) rich in most of the cytochrome P450 isoforms. An (expensive) alternative is testing all products, whether active or inactive *in vitro*, in suitable animal models of the infection.

Although tests employing animal models are labor intensive, expensive, lengthy, and often difficult to scale-up, they are sufficiently reliable, and the conclusions drawn from them are frequently transferable to human infection, provided sufficient care is taken in the selection of the host-parasite system.

Our understanding of the host-parasite interactions and immune responses of the host in human filariasis and in a variety of animal models of the human infection has greatly improved in recent years [20, 60–62] and as a result we now have well-characterized animal models for assaying antifilarial activity (● Table 3).

Historically, attempts to transmit the human filarial infections to laboratory animals were unsuccessful. This necessitated the use of alternative animal models for antifilarial drug discovery programs. One of the earliest models is *Litomosoides carinii* infection in the cotton rat. Introduced in 1944 [63], this was used as a primary screen and was instrumental in the discovery of the microfilaricide diethylcarbamazine (DEC). However a major drawback with this model was that the host can only be infected through the vector but not by manual injection of infective larvae. So, there is no way of knowing how many, if at all, infective larvae had been introduced into the host by the vector. This is important for reproducible 1) determination of the percent yield of adult worms (= % larvae surviving and developing in to adult worms), and 2) quantifying the macrofilaricidal and worm sterilizing effect of the test drug. Another model which was established in '70s is *Acanthocheilonema viteae* in the jird (*Meriones unguiculatus*) and in *Mastomys coucha*. These models overcame the deficiencies of the *L. carinii*/cotton rat model and were considered acceptable [64] as a surrogate primary screen for products against human *O. volvulus* infection. However, for a long time there was still no rodent model for human lymphatic filarial infections with *W. bancrofti* and *B. malayi*. The real breakthrough came with the successful transmission of *B. malayi* to the rodents jird [65, 66] and *Mastomys coucha* [18, 67, 68] and recently, to nonhuman primates [25, 29, 31, 69]. In *M. coucha* the infection is introduced by subcutaneous injection of a known number of L₃. In jird, in addition to the s.c. route, the infection can also be initiated by intraperitoneal instillation of a known number of adult worms or L₃. The latter method not only makes the animal microfilaremic in a short period but is especially useful for assaying macrofilaricidal efficacy of products by following the fate of instilled worms. Both the rodent models show high and sustained microfilaraemia for prolonged periods which is advantageous for assaying microfilaricides. An additional feature of the *M. coucha* is its susceptibility to develop filarial disease manifestations (unpublished observation).

Among nonhuman primates, the leaf monkeys (*Presbytis* spp.) were found to be especially susceptible to *B. malayi* infection [25, 29] and among them the Indian leaf monkey displays responses similar to those shown by human subjects harboring the infection, including the recurrent febrile and limb edema episodes, hydrocoele, and eosinophilia [29].

With the availability of adequate animal models of human filarial infection, the new product screening protocol has been revised by the WHO. At the authors' Institute the protocol conducted is largely based on WHO recommendations and is as follows:

Prescreen: *In vitro* motility and MTT assays using adult worms and mf of *B. malayi* for short-listing. This is followed by IC₅₀ (the concentration at which the parasite motility is inhibited by 50%) determination using the same assays and CC₅₀ (cytotoxic concentration at which 50% of cells are killed) determination using VERO Cell line C1008 (African green monkey kidney cells) [33, 89–91].

Primary screening: Jird bearing i.p. instilled adult worms of *B. malayi*.

Confirmation of efficacy: L₃-initiated infection of *B. malayi* in *M. coucha* or jird.

Dose optimization studies: L₃-initiated infection of *B. malayi* in *M. coucha*.

Efficacy in a non-rodent or nonhuman primate model (previously called as tertiary screen): L₃-initiated infection of *B. malayi* in non-rodent/nonhuman primate model.

Assay Systems for Screening Plants against Helminth Parasites Other Than Filariae

In vitro (primary) screens

For screening potential synthetic or plant derived anthelmintics (other than antifilarials) several nematode, cestode, and trematode parasites have been used in *in vitro* systems (● Table 4). The selection of parasites for the *in vitro* systems is apparently based on considerations such as easy availability, adaptability to laboratory conditions, ease in handling, and, when the human parasite cannot be used, the similarities between human and surrogate parasite responses to known drugs and/or taxonomical proximity of the species chosen. This approach would appear justified by several instances of intergeneric chemotherapeutic responses within the same family. Among nematodes, such a relationship is known to exist between murine oxyurid and *Enterobius vermicularis* with piperazine; between *Nippostrongylis muris* and trichostrongyles of sheep and cattle or hookworms of man and dog with bphenium and between *Strongyloides ratti* and *S. stercoralis* with dithiazanine [92].

In vivo

● Tables 5 and 6 show a list of animal models for primary and secondary screening, respectively. The *in vivo* primary and secondary screening would strengthen the results obtained in the *in vitro* prescreening. The *in vivo* screens will also demonstrate whether the spectrum of activities can be extended to the related parasites in different hosts and to efficacy in human subjects. Parasites having a short and direct life cycle needing no vector would obviously need less time and labor and would be economical to generate results. *Hymenolepis nana*, a human tapeworm, in natural condition, is cycled through intermediate host (*Tribolium confusum*) but also attains maturity in one and the same host within 15 days of incubation of eggs. This system will also facilitate the assessment of drugs against cysticercoides and adult worms in the same infected animal. Small hosts with minimum genetic variations and easily available in adequate numbers are usually preferred for reproducibility.

In spite of all the care exercised in selecting the best host-parasite system, the results obtained in experimental hosts can not totally

Table 3 Animal models of nonhuman and human filarial infections.

Parasite	Host	Vector	Initiation/introduction of infection	Site of adult worms/microfilariae	Reference
1. Rodent and non-rodent models of nonhuman filarial infections					
<i>Litomosoides carinii</i> [#]	<i>Sigmodontis hispidus</i> [#]	Mite	L ₃ (s. c.)	Pleural cavity/blood	[63, 70–74]
	<i>Mastomys coucha</i>				
	<i>Meriones unguiculatus</i>				
<i>Acanthocheilonema viteae</i>	<i>M. unguiculatus</i>	Tick	L ₃ (s. c.)	Subcutaneous and internal connective tissues/blood	[75, 76]
	<i>M. lybicus</i>				
	<i>M. persicus</i>				
	<i>M. coucha</i>				
	<i>Mesocricetus auratus</i>				
<i>Brugia pahangi</i>	<i>M. unguiculatus</i>	Mosquito	L ₃ (s. c.), adult worms (i. p.)	Testes, heart, lungs/blood	[69, 75, 77–79]
	<i>M. coucha</i>				
	CDI and Balb/c mice				
	Dog	Mosquito	L ₃ (s. c.)	Lymphatics/blood	
	Cat				[80]
<i>Dirofilaria immitis</i>	Dog	Mosquito	L ₃ (s. c.)	Heart, pulmonary arteries, venae cavae/blood	[41, 80]
2. Rodent and non-rodent models of human filarial infections					
<i>B. malayi</i> [*]	<i>M. unguiculatus</i> [*]	Mosquito	L ₃ (s. c.), adult worms (i. p.)	Lymphatics, testes, heart, lungs/blood	[21, 53, 66, 68, 69, 81–83]
	<i>M. coucha</i> [*]		L ₃ (s. c.)		
	Balb/c mice		L ₃ /adult worms (i. p.)		
<i>B. malayi</i>	Dog	Mosquito	L ₃ (s. c.)	Lymphatics/blood	[69, 80]
	Cat				
3. Nonhuman primate models of human filarial infections					
<i>B. malayi</i> ^{**}	<i>Erythrocebus patas</i>	Mosquito	L ₃ (s. c.)	Lymphatics/blood	[25, 29–31, 69, 84–86]
	<i>Nycticebus coucang</i>				
	<i>Galago crassicaudatus panganiensis</i>				
	<i>Papio cynocephalus</i>				
	<i>Cercopithecus aethiops</i>				
	<i>Macaca mulatta</i>				
	<i>Presbytis entellus</i> ^{**}				
	<i>P. melalophos</i>				
	<i>P. cristata</i>				
<i>Wuchereria bancrofti</i>	<i>P. entellus</i>	Mosquito	L ₃ (s. c.)	Lymphatics/blood	[87]
<i>Breinlia sergenti</i>	<i>Nycticebus coucang</i> , <i>Papio anubis</i> , <i>Erythrocebus patas</i>	Mosquito	L ₃ (s. c.)	Peritoneal cavity	[80, 88]
<i>Loa loa</i>	<i>Mandrillus leucophaeus</i>	Chrysops	L ₃ (s. c.)	Subcutaneous and connective tissues/blood	[80, 88]
<i>Onchocerca volvulus</i>	<i>Pan troglodytes</i>	Simulium	L ₃ (s. c.)	Subcutaneous tissues/blood	[80, 88]

[#] Earlier used as a primary screen. ^{*} Models currently employed as a primary screen for assaying potential antifilarial products at CDRI. ^{**} Models employed as final preclinical confirmation (previously called as tertiary screen) assays at CDRI

be translated to the target parasite in its natural host because the different compounds behave differently in different hosts (absorption, kinetics, resorption and distribution, etc.) [104]. This might be crucial for decision making regarding whether or not it should enter successive steps of drug development program. The different *in vitro* and *in vivo* systems, their utility and drawbacks have been recently reviewed by Keiser [101].

Plants for Filariasis

In modern medicine the drugs used for lymphatic filariasis are diethylcarbamazine (DEC) [70] and ivermectin [105], and a single-dose treatment with DEC or ivermectin, or combination of DEC or ivermectin with albendazole is currently employed in an attempt to control the infection. DEC and ivermectin are microfilaricides and therefore only useful in reducing transmission and pathology. New drugs are required to improve treatment by killing the adult worms (macrofilariae), which are long-lived, and to replace the currently used drugs before drug resistance starts appearing [106].

Table 4 *In vitro* parasite systems (other than filaria) for screening anthelmintic activity.

Parasite (host)	Life stages	Assay(s)/measure of the anthelmintic activity	References
Nematodes			
<i>Trichostrongylus colubriformis</i> (human infection)	Eggs, L ₃ (infective larvae), adult worms	Egg hatch inhibition assay (EH), larval development assay (LD), larval migration inhibition (LMI), adult worm viability	[93–96]
<i>Haemonchus contortus</i> (ruminants)	Eggs, infective larvae, adult worms	Egg hatch inhibition assay (EH), larval migration inhibition (LMI)	[93, 97, 98]
<i>Ancylostoma caninum</i> (human) <i>Necator americanus</i> (human)	Larvae, adults	Motility/irreversible inhibition of motility	[93]
<i>Ascaris suum</i> (pig & human) <i>A. lumbricoides</i> (human) <i>Ascaridia galli</i> (chicken/turkey) <i>Heterakis gallinarum</i> (chicken/turkey) <i>Toxocara canis</i>	Adult worms, 2nd stage larva	Motility/irreversible inhibition of motility	[99, 100]
Cestodes			
<i>Railletina echinobothrida</i> (fowl)	Adult worms	Motility/irreversible inhibition of motility	[99]
Trematodes			
<i>Paramphistomum</i> sp. (cattle, sheep, goat)	Adult worms	Motility/irreversible inhibition of motility	[99]
<i>Schistosoma mansoni</i> <i>Fasciola hepatica</i> <i>Echinostoma caproni</i> (all human infections)	Adult worms	Motility/irreversible inhibition of motility	[101]

Table 5 Helminth parasites used for *in vivo* primary screening* [17, 100, 102, 103].

Parasite	Host/intermediate host	Infective agent/route	Model for	Clinical correlation [®]
Nematodes				
<i>Ascaris suis</i> (<i>suum</i>)	Mouse/none	Eggs/per os	Ascariasis	+++
<i>Necator americanus</i>	Hamster/none	L ₃ /skin	Ancylostomiasis	+++
<i>Ancylostoma ceylanicum</i>	Hamster/none	L ₃ /per os	-do-	+++
<i>Nippostrongylus brasiliensis</i>	Hamster/none	L ₃ /per os	-do-	+++
Cestodes				
<i>Hymenolepis nana</i>	Mouse/beetle [#]	Eggs/per os	Taeniasis	+++
<i>H. diminuta</i>	Rat/beetle	Eggs/per os	-do-	++
Trematodes				
<i>Fasciola hepatica</i>	Rat, rabbit/snail	Metacercariae/per os	Fascioliasis	++
<i>F. gigantica</i>	Rat/rabbit/snail	-do-	-do-	++
<i>Schistosoma mansoni</i>	Mouse, hamster/snail	Cercariae/skin	Schistosomiasis	+++
<i>S. japonicum</i>	Mouse/snail	-do-	-do-	+++
	Hamster/snail	-do-	-do-	++
	Rabbit/snail	-do-	-do-	++
	Guinea pig/snail	-do-	-do-	++

* Intermediate host not obligatory; [®] scale: ++ = moderate; +++ = high

Table 6 Test models for *in vivo* secondary screening [17].

Parasite	Host/intermediate host	Model for	Clinical correlation
Nematodes			
<i>Ascaridia galli</i>	Chicken/none	Ascariasis	+++
<i>Toxocara canis</i>	Dog/none	-do-	++
<i>Toxascaris leonina</i>	Cat/none	-do-	++
<i>Ancylostoma caninum</i>	Dog/none	Ancylostomiasis	+++
<i>A. brasiliense</i>	Dog, cat/none	-do-	+++
<i>A. tubaeforme</i>	Cat/none	-do-	++
Cestodes			
<i>Taenia hydatigena</i>	Dog/ruminants	-do-	+++
<i>T. taeniaeformis</i>	Cat/rodents	-do-	+++
<i>Dipylidium caninum</i>	Dog/fleas	Taeniasis	+++
<i>T. pisiformis</i>	Dog/rabbit	-do-	+++
<i>Cysticercus pisiformis</i> (larva of <i>T. taeniaeformis</i>)	Rabbit	Cysticercosis	++
<i>Echinococcus cyst</i> (intermediate stage of <i>E. granulosus</i>)	Rabbit	Hydatid disease	++

In the last few decades a lot of emphasis has been laid on the development of antifilarial agents from plant or natural products by many investigators [32, 48, 82, 107, 108] and to develop traditional plant-based medical preparations complementary or alternative medicines (CAM) supported by scientific validation of efficacy and safety and quality control of the preparations. Although there are few specific reports on the antifilarial properties of plant extracts or products, it is not unusual to find these indications cited amongst a general list of medicinal plants [109, 110]. Some plants are reported to be active against tissue dwelling nematodes and various filarial species and are used in traditional systems of medicine [111]. **Table 7** shows a list of plants studied for activity against filarial parasites [112]. Some of these are tested for their antifilarial activity mostly *in vitro* and some *in vivo*. Among these, *Streblus asper*, a plant used in traditional medicine for lymphedema, is the only plant that has been studied extensively and systematically *in vitro* as well as *in vivo* and whose active constituents were chemically characterized. A preparation of plant decoction named “filacid” made from the stem bark of *S. asper* has been administered to over 5000 filarial patients at a filaria clinic in Varanasi, India, during the period 1970–1987 [113, 114] and was found to be effective in the treatment of filarial lymphedema, filarial chyluria, and other conditions of the disease. In comparative trials, other plants used in traditional medicine were found to be less effective (viz. *Crataeva nurvala* [7%], *Argyreia nervosa* [48%], *Butea monosperma* [12%]) than “filacid” in the treatment of filarial lymphedema [112, 113]. Later, the stem bark extract was found to be active against several filarial species including *B. malayi* *in vitro* and *in vivo*. The active principles were identified as two cardiac glycosides, asperoside and strebloside [107]. For onchocerciasis, there are relatively fewer reports of plant-based traditional medicine in the literature. *Aloe barteri* was cited for the treatment of *O. volvulus*-induced skin conditions [109]. Another plant, *Cassia aubrevellei*, which is believed in Liberia to be useful in skin conditions associated with onchocerciasis, was found to be inactive against female parasites recovered from nodules of patients [115]. On the contrary, the plant extract increased the density of skin microfilariae [116]. Extracts of some Cameroonian plants like *Carapa procera*, *Pachypodanthium staudth*, and *Polyalthia suaveolens* were also found to be effective against filarial parasites. The active principles of these plants were identified as carapolide A and oliverine and were tested against *O. volvulus* *in vitro* by Titanji et al. [117]. Cardol, a phenolic compound isolated from *Anacardium occidentale* is reported to be active against bovine filariid *S. cervi* *in vitro* [118]. Other *in vitro/in vivo* investigations of plant extracts have also been reported against various filarial species [116, 119–123]. Both aqueous and alcoholic extracts of the leaves of *Mallotus philippensis* and *Sencio nudicaulis* were effective in inhibiting the movements of the nerve-muscle (n.m.) preparation of *S. cervi*. The stimulatory response of acetylcholine was blocked by the aqueous extract on whole worm movements [124]. The effect of *S. nudicaulis* extracts was different from that produced by the calcium channel blocker nifedipine on the whole worm and n.m. preparation. While nifedipine blocks the stimulant effect of Ach, the extract of *S. nudicaulis* fails to do so. This response bears similarity with DEC, which also does not block AchE response. However, interpretation of these activities in terms of target filarial infections *in vivo* is difficult.

The majority of the filaricidal applications of plant products reported in the early literature are for the treatment of guinea worm (*Dracunculus* sp.) which was earlier considered as a filarial

parasite but is now included in a separate group. Plant extracts are in many cases applied externally to the sore caused by guinea worm indicating that most of the observed effects may be due to direct topical effect of the agent on the parasite or wound. Several plant products were also reported active when given orally. Root decoction of *Combretum micranathum* was reported to help in expelling guinea worms in infested patients; inflammation around the lesions was also reduced [125]. The leaves of *Elaeophorbia drupifera* and *Hillieria latifolia* taken in combination with a palm soup preparation were found to be guinea wormicidal [125].

In laboratory investigations several plant products were identified with antifilarial activity. *In vitro*, macrofilaricidal activity was shown by ethanolic and aqueous extracts of the medicinal plant *Cardiospermum halicacabum* against *B. pahangi* in terms of reduced motility of both male and female adult worms and reduced microfilarial release and motility (ethanolic extract) [126]. Methanolic extracts of the root of *Vitex negundo* L. (containing alkaloids, saponin, and flavonoids) and leaves of *Aegle marmelos* Corr. (containing coumarins) produced complete loss of motility of microfilariae of *B. malayi* [127]. Extracts of *Butea monosperma* leaves and roots showed significant inhibition of motility in a dose-dependent manner of *B. malayi* microfilariae [32]. In animal models, crude extract and hexane fraction of marine red alga *Botryocladia leptopoda* killed adult filarial parasites of *L. sigmodontis* and *A. viteae* and caused sterilization of *B. malayi* female worms [128]. Crude extract and chloroform fraction of the stem portion of the plant *Lantana camara* showed adulticidal and female worm sterilizing activity against *B. malayi* in *M. coucha* and in jirds with i.p. instilled *B. malayi* adult worms. Oleanonic and oleanolic acids isolated from the hexane and chloroform fractions showed considerable antifilarial activity on *B. malayi* *in vitro*. Inhibition of motility and subsequent mortality of adult worms of *S. digitata* was produced *in vitro* by extracts of *Cedrus deodara*, *Ricinus communis*, *Sphaeranthus indicus*, and *Centratherum anthelminticum* in decreasing order [54]. Crude extract (and an active fraction of it) of *Trachyspermum ammi* fruit inhibited motility and killed *S. digitata* worms *in vitro* and showed macrofilaricidal and female sterilizing efficacy *in vivo* against *B. malayi* in *M. coucha*. The active compound was isolated and found to be a phenolic monoterpene [129]. Potential micro- and macrofilaricidal efficacy against *B. pahangi* was shown by ethanol extract of leaves of *Neurolaena lobata*, a Guatemalan medicinal plant [58]. Aqueous, butanol, and hexane extracts of *Caesalpinia bonducella*-seed kernel demonstrated microfilaricidal, macrofilaricidal, and female-sterilizing efficacy against *L. sigmodontis* in cotton rats and microfilaricidal activity against *B. malayi* in *M. coucha* [130].

Plants for Helminthiasis (Other Than Filariasis)

The literature concerning the use of plants as anthelmintics (**Table 8**) is more extensive [158, 159] and preparations from many of these plants are in current use. With few exceptions, e.g. the investigation by Kiuchi et al. [111] on tissue dwelling nematodes, the majority of them are effective against intestinal helminths. The most widely known and investigated anthelmintics of plant origin are ascaridole, derived from *Chenopodium ambrosioides* [158] and the phloroglucinols aspidin and deaspidin, from the male fern *Dryopteris filix mas*. They are used effectively to treat tapeworm infections [160]. Some of the plant

Table 7 Plant products with activity in filariasis or against the parasites.

Name of the plant	Family	Part used/product	Activity against	Reference
<i>Adenia gummifera</i>	Passifloraceae	Root	Filariasis, hydrocoele	[131]
<i>Aegle marmelos</i> Corr.	Euphorbiaceae	Leaf	Mf of <i>B. malayi</i> (<i>in vitro</i>)	[32, 127]
<i>Afstonia boonei</i>	Apocynaceae	Bark, fresh latex, fresh stem bark	Loaiasis, filarial swellings	[132–134]
<i>Agrimonia eupatoria</i>	Rosaceae	Agrimophol	<i>Schistosoma</i> sp <i>Taenia</i> sp	[135]
<i>Alstonia congensis</i>	Apocynaceae	Latex	Loaiasis, filarial swellings (bandaged along with crushed bark of <i>Erythrophleum guineense</i>)	[109]
<i>Alstonia scholaris</i>	Apocynaceae	Latex, bark	Filariasis, elephantiasis	[136]
<i>Aloe barteri</i>	Liliaceae	Leaf	Guinea worm, disease causing white skin patches (onchocerciasis?)	[109]
<i>Ammannia multiflora</i>	Lythraceae	Leaf	Sight problems, including those caused by filaria	[131]
<i>Andrographis paniculata</i>	Acanthaceae	Dried leaf	Filariasis, mf of <i>D. reconditum</i> in dogs (<i>in vivo</i> and <i>in vitro</i>) and adults of <i>B. malayi</i> in rodents	[120]
<i>Argyrea speciosa</i>	Convolvulaceae	Whole plant	Filariasis, parasitic skin diseases, active <i>in vitro</i> against <i>S. cervi</i>	[137, 138]
<i>Azadirachta indica</i>	Meliaceae	Leaf, flower	<i>S. digitata</i> (<i>in vitro</i>)	[139, 140]
<i>Boerhavia repens</i>	Nyctaginaceae	Immature shoots	Elephantiasis	[109]
<i>Botryocladia leptopoda</i>	Rhodymeniaceae	Red algae	<i>L. sigmodontis</i> , <i>A. viteae</i> , <i>B. malayi</i> (<i>in vivo</i>)	[128]
<i>Butea monosperma</i>	Leguminosae-Papilionaceae	Root and leaf	Mf of <i>B. malayi</i> (<i>in vitro</i>)	[127]
<i>Caesalpinia bonducella</i>	Caesalpinaceae	Seed kernel	<i>S. digitata</i> (<i>in vitro</i>), <i>L. sigmodontis</i> , <i>B. malayi</i> (<i>in vivo</i>)	[130]
<i>Calotropis gigantea</i>	Asclepiadaceae	Leaf, latex	Filariasis, elephantiasis, skin changes, <i>S. digitata</i> (<i>in vitro</i>)	[109, 140, 141]
<i>Calotropis procera</i>	Asclepiadaceae	Whole plant/milky juice, dried aerial parts, root, bark, latex	Guinea worm; filariasis, elephantiasis	[109, 136, 142–144]
<i>Carapa procera</i>	Meliaceae	Dried fruit, seed	Filariasis, <i>O. volvulus</i> (<i>in vitro</i>), parasitic skin disease	[117, 145]
<i>Cardiospermum halicacabum</i>	Sapindaceae	Plant	<i>B. pahangi</i> adult and Mf (<i>in vitro</i>)	[126]
<i>Cassia alata</i>	Leguminosae	Fresh leaf juice	Parasitic skin disease	[117, 122]
<i>Cassia aubrevillei</i>	Leguminosae	Root, bark	Onchocerciasis, skin microfilaricidal, active <i>in vitro</i> against <i>O. volvulus</i> mf	[138]
<i>Cassia occidentalis</i>	Leguminosae	Leaf, seed	Guinea worm, parasitic skin diseases acute lymphedema, skin changes	[109]
<i>Cassia tora</i>	Leguminosae	Dried leaf	Parasitic skin diseases	[109, 141]
<i>Cayaponia martiana</i>	Cucurbitaceae	Root	Elephantiasis	[109]
<i>Cedrus deodara</i>	Pinaceae	Plant extract	<i>S. digitata</i> adults (<i>in vitro</i>)	[54]
<i>Centratherum anthelminticum</i>	Asteraceae	Plant extract	<i>S. cervi</i> , <i>S. digitata</i> adults (<i>in vitro</i>)	[54]
<i>Cinnamomum culilawan</i>	Lauraceae	Bark	Rubeifacient for filarial lymphangitis	[109]
<i>Cleistopholis glauca</i>	Annonaceae	Dried bark	Filariasis, inactive <i>in vitro</i> against <i>O. volvulus</i>	[109]
<i>Clerodendrum capitum</i>	Verbenaceae	Root	Elephantiasis	[109]
<i>Crossopteryx febrifuga</i>	Rubiaceae	Fresh fruit juice	Eye filaria	[125]
<i>Cyrotomium fortunei</i>	Polypodiaceae	Dried rhizome	Filariasis	[133]
<i>Daniella thurifera</i>	Leguminosae	Gum	Parasitic skin diseases	[146]
<i>Delonix elata</i>	Leguminosae	Whole plant	Filariasis, elephantiasis	[109]
<i>Dichrostachys cinerea</i> D. glomerata	Leguminosae	Dried stem bark, inner bark	Elephantiasis	[142, 147]
<i>Dombeya amaniensis</i>	Steruliaceae	Root	Filariasis/lymphatic disorders	[148]
<i>Eclipta alba</i>	Compositae	Dried whole plant	Elephantiasis	[109]
<i>Elaeophorbia drupifera</i>	Euphorbiaceae	Leaf	Guinea worm, filariasis	[131, 145]
		Leaf	Guinea worm, used with <i>Hillieria latifolia</i>	[109]
<i>Elephantopus scaber</i>	Compositae	Dried root	Filariasis	[141]
<i>Emicostema littorale</i>	Gentianaceae	Whole plant	Filariasis, microfilaricidal <i>in vitro</i> against <i>Conspicuum guindiensis</i>	[125]
<i>Erythrophleum guineense</i>	Leguminosae	Crushed bark	Loaiasis (filarial swellings), used with <i>Alstonia congensis</i>	[149]
<i>Erythrophleum ivorense</i>	Leguminosae	Dried stem bark	Loaiasis (filarial swellings) used in <i>O. volvulus</i>	[119]
<i>Eucalyptus robusta</i>	Myrtaceae	Leaf	Microfilariasis	[109]
<i>Guiera senegalensis</i>	Combretaceae	Leaf	Parasitic skin diseases, guinea worm inflammatory swellings	[109]
<i>Hillieria latifolia</i>	Phytolaccaceae	Whole plant, leaf	Guinea worm, used with <i>Elaeophorbia drupifera</i> ; filariasis, <i>O. volvulus</i> (<i>in vitro</i>)	[109, 150]

continued next page

Table 7 Plant products with activity in filariasis or against the parasites. (continued)

Name of the plant	Family	Part used/product	Activity against	Reference
<i>Jatropha curcas</i>	Euphorbiaceae	Seed oil, leaf, whole plant	Guinea worm, rubefacient for parasitic skin diseases	[122, 125, 141]
<i>Kigelia africana</i>	Bignoniaceae	Whole plant	Elephantiasis of scrotum	[109]
<i>Lantana camara</i>	Verbenaceae	Stem	<i>A. viteae</i> , <i>B. malayi</i>	[108]
<i>Limeum pterocarpum</i>	Molluginaceae	Aerial parts	Filariasis	[141]
<i>Lawsonia inermis</i>	Lythraceae	Leaf	<i>S. digitata</i> (in vitro)	[140]
<i>Lycopodium rubrum</i>	Lycopodiaceae	Whole plant	Elephantiasis	[151]
<i>Mallotus philippensis</i>	Euphorbiaceae	Leaf	<i>S. cervi</i> (in vitro)	[124]
<i>Melia azadirachta</i>	Meliaceae	Bark	Filariasis, component (15%) of FILARIN	[152]
<i>Microglossa afzelii</i>	Compositae	Dried leaf	Filariasis, <i>O. volvulus</i> (inactive in vitro)	[124]
<i>Mussaenda elegans</i>	Rubiaceae	Leaf	Elephantiasis	[109, 125]
<i>Myrianthus arboreus</i>	Moraceae	Dried stem bark	Filariasis, <i>O. volvulus</i> (inactive in vitro)	[109]
<i>Newbouldia laevis</i>	Bignoniaceae	Root and leaf	Elephantiasis, scrotal elephantiasis, orchitis	[122, 153]
<i>Neurolaena lobata</i>	Asteraceae	Leaf	<i>B. pahangi</i> adults (in vitro)	[58]
<i>Ocimum sanctum</i>	Lamiaceae	Leaf	<i>S. digitata</i> (in vitro)	[140]
<i>Ochrocarpus africanus</i>	Guttiferae	Root/resinous sap	Parasitic skin diseases	[109]
<i>Odyndea gabunensis</i>	Simaroubaceae	Dried stem bark	Filariasis, <i>O. volvulus</i> (inactive in vitro)	[154]
<i>Pachyelasma tessmanii</i>	Leguminosae	Dried fruit	Filariasis, <i>O. volvulus</i> (in vitro)	[122]
<i>Pachylobus edulis</i>	Buseraceae	Bark	Parasitic skin diseases	[141]
<i>Pachypodanthium staudtii</i>	Annonaceae	Dried stem bark	Filariasis, <i>O. volvulus</i> (in vitro)	[117, 122]
<i>Physoedra longipes</i>	Cucurbitaceae	Whole plant	Elephantiasis of scrotum	[117, 122]
<i>Phytotria tanganyikensis</i>	Rubiaceae	Leaf	Elephantiasis	[109]
<i>Raphia farinifera</i>	Palmae	Dried fruit	Filariasis, <i>O. volvulus</i> (in vitro)	[155]
		Dried leaf	Filariasis, <i>O. volvulus</i> (in vitro)	[122]
<i>Ricinus communis</i>	Euphorbiaceae	Plant extract, leaf	<i>S. digitata</i> adults (in vitro); Mf of <i>B. malayi</i> (in vitro)	[32, 54, 127]
<i>Richiea caparoides</i>	Capparidaceae	Leaf, root	Filariasis; guinea worm	[122, 141]
<i>Rynchosia hirta</i>	Leguminosae	Whole plant	Filariasis, elephantiasis	[156]
<i>Sargentodoxa cuneata</i>	Sargentodoxaceae	Dried stem	Filariasis	[147]
<i>Sencio nudicaulis</i>	Asteraceae	Leaf	<i>S. cervi</i> mf (in vitro)	[109]
<i>Sphaeranthus indicus</i>	Asteraceae	Plant extract	<i>S. digitata</i> adults (in vitro)	[54]
<i>Streblus asper</i>	Urtaceae	Stem bark	Filarial lymphedema, micro- and macro-filaricidal, <i>S. cervi</i> , <i>L. carinii</i> , <i>B. malayi</i> , <i>A. viteae</i> , <i>B. malayi</i>	[107, 113, 114, 123, 157]
<i>Trachyspermum ammi</i>	Apiaceae	Fruit	<i>S. digitata</i> adults (in vitro), <i>B. malayi</i> (in vivo)	[129]
<i>Terminalia chebula</i>	Combretaceae	Not known	Filariasis	[109]
<i>Tinospora cordifolia</i>	Menispermaceae	Not known	Filariasis (acute lymphedema, skin changes)	[112]
<i>Xerodermis stuhlmannii</i>	Leguminosae	Root	Elephantiasis	[138]
<i>Vitex negundo</i> L.	Euphorbiaceae	Root, leaf	Mf of <i>B. malayi</i> (in vitro)	[32, 127]
<i>Zingiber officinale</i>	Zingiberaceae	Fresh rhizome	Filariasis, <i>D. immitis</i> (microfilaricidal) (acute lymphedema)	[121, 152, 154]

products are vermicides while others are vermifuges. Oil of che-nopodium (ascaridole) is effective against *Ascaris* and hook-worms but is highly toxic. Aspidum is one of the oldest used anthelmintics obtained from the rhizomes of the fern *Dryopteris filix mass.* Polyhydric phenol is its active principle (filicic acid and filicin). The product has specific action against intestinal cestodes: *Diphyllobothrium*, *Taenias*, *Hymenolepis* spp and others, and acts probably by paralyzing the muscles of parasites. However, the drug is toxic and causes polyneuritis and paralysis of the iris. Santonin is the oil obtained from the seeds of *Artemisia maritima*. Flowering tops of this plant were used by physicians of Greece as early as 60 AD. The decoction of the stem bark of *Zanthoxylum liebmannianum* decreased the count of intestinal nematode eggs in naturally infected sheep while the chloroform extract was found to be toxic to *Ascaris suum*. Alpha-sanshool from *Z. liebmannianum* was found to be the active compound. However, alpha-sanshool induced tonic-clonic seizures in mice and thus has some toxicity [161]. Thymol obtained from *Thymus vulgaris* is a monohydric phenol (methyl isopropyl phenol) and is used as vermicide in eliminating hookworms and *Trichinella spi-*

ralis. Thymol is not active against *Ascaris*, *Trichuris*, and *Enterobius*. It is neurotoxic and affects kidneys. Pelletierin is an alkaloid obtained from the pomegranate tree, *Punica granatum*, and is active against *Taenia* sp. but causes headache, dizziness, nausea, vomiting, and diarrhea with colic pain; it is also known to be neurotoxic. Arecanut, the seed of *Areca catechu* is also known for its anthelmintic action; its active principle is arecoline, a colorless liquid. Dried flowers of *Hagenia abyssinica*, commonly called as “Kousso” or Cusso, are used against tapeworm (*Taenia* sp.) infections. Their active principle is identified as kosatxin. Palasonin derived from *Butea frondosa* and its piperavine salts were found to be active against *A. lumbricoides* [162]. Although these are not advantageous over existing synthetic anthelmintics (benzimidazole derivatives and ivermectin), they are effective in expelling the worms if used as a purgative. Interestingly, some plant anthelmintics directly inhibit the worms’ motility due to cholinergic agonist/antagonist action as in the case of arecolin. However, the action of all these agents depends on several host factors too.

Table 8 Plants with activity against helminths other than filariidae.

Name of the plant	Family	Part/product used	Target parasite	Reference
<i>Albizzia anthelmintica</i> <i>A. lebbek</i>	Mimosaceae	Bark	<i>Hymenolepis diminuta</i>	[167]
<i>Allium sativum</i>	Liliaceae	Bulbs	Nematodes*, helminths	[173]
<i>Areca catechu</i>	Arecaceae	Seed	<i>Ascaris</i> sp.	[135]
<i>Azadirachta indica</i>	Meliaceae	Leaf	Helminthes*	[139]
<i>Bauhinia purpurea</i>	Caesalpiniaceae	Leaf	Helminthes*	[174]
<i>Butea frondosa</i>	Papilionaceae	Seed	<i>A. lumbricoides</i> in man, <i>T. canis</i> in dogs	[162, 175]
<i>Camellia sinensis</i>	Theaceae	Green tea	Infective larvae of <i>Teladorsagia circumcincta</i> and <i>Trichostrongylus</i> (in vitro)	[176]
<i>Carica papaya</i>	Caricaceae	Seed, latex	Rat tapeworm, intestinal nematode	[168, 177, 178]
<i>Centratherum anthelminticum</i>	Asteraceae	Seed	Tapeworm	[179]
<i>Chenopodium ambrosioides</i>	Chenopodiaceae	Leaf, seed (oil of chenopodium)	<i>Ascaris</i> sp, hookworm	[173]
<i>Coriandrum sativum</i>	Apiaceae	Crude extract of seed	<i>Haemonchus contortus</i> (in vitro and in vivo)	[172]
<i>Cucurbita maxima</i>	Cucurbitaceae	Seed	Helminths*	[175, 177, 179]
<i>Cucurbita pepo</i>	Cucurbitaceae	Seed	<i>Hymenolepis nana</i> , <i>Dicrocoelium dendriticum</i>	[175, 177, 179]
<i>Cyathocline purpurea</i>	Asteraceae	Essential oil of aerial part	Tapeworm and hookworm	[177]
<i>Datura metel</i>	Solonaceae	Fruit or flower	<i>A. galli</i>	[180]
<i>Delonix regia</i>	Caesalpiniaceae	Flower	<i>H. contortus</i>	[179]
<i>Digenea simplex</i>	Rhodophyceae	Kainic acid	<i>Ascaris</i> sp.	[135]
<i>Embelia schimperi</i>	Myrsinaceae	Dried fruit	<i>Hymenolepis diminuta</i> (in vitro & in vivo)	[165]
<i>Evolvulus alsinoides</i>	Convolvulaceae	Crude extract	Helminths*	[180]
<i>Flemingia vestita</i>	Fabaceae	Root, tuber-peel	Trematode, cestode, <i>A. suum</i> , <i>A. lumbricoides</i>	[99]
<i>Ficus glabrata</i> , <i>F. spp.</i>	Moraceae	Latex	<i>A. suum</i> , <i>Strongiloides</i> , <i>Trichuris</i> , <i>S. obvelata</i>	[164, 181]
<i>Limnophila repens</i>	Scrophulariaceae	Oil	Helminths*	[175]
<i>Leucas caphalotes</i>	Lamiaceae	Leaf	Helminths*	[174]
<i>Luffa echinata</i>	Cucurbitaceae	Seed	Helminths*	[175]
<i>Mallotus philippinensis</i>	Euphorbiaceae	Fruit (kamalin)	<i>Diphylobothrium latum</i>	[175]
<i>Matteuccia orientalis</i>	Onocleaceae	Root	<i>Fasciola hepatica</i>	[182]
<i>Milletia thonningii</i>	Papilionaceae	Seed	<i>Schistosoma mansoni</i>	[183]
<i>Onobrychis viciifolia</i> Scop.	Fabaceae	Plant (as forage)	<i>H. contortus</i>	[169]
<i>Piliostigma thonningii</i>	Caesalpiniaceae	Bark	<i>A. galli</i>	[184]
<i>Polygonum glabrum</i>	Polygonaceae	Leaf	Helminths*	[185]
<i>Psorelea corylifolia</i>	Papilionaceae	Seed	Helminths*	[175]
<i>Quisqualis indica</i>	Combretaceae	Quisqualic acid	Nematodes*	[177]
<i>Taverniera abyssinica</i>	Leguminosae	Dried root	Nematodes*	[186]
<i>Urginea indica</i>	Liliaceae	Bulb	<i>A. suum</i>	[187]
<i>Struthiola argentea</i>	Thymelaeaceae	Plant	Helminth (in vitro)	[171]
<i>Teloxys graveolens</i> (Willd.)	Chenopodiaceae	Plant	<i>F. hepatica</i> , <i>A. galli</i>	[170]
<i>Zanthoxylum liebmannianum</i>	Rutaceae	Stem bark	Intestinal nematode of sheep, <i>A. suum</i>	[161]

* Parasite(s) not specified

The oil fractions from *Limnophila conferta* syn. *L. repens* Benth and *L. heterophylla* (Roxb) Benth syn. var. *reflexa* (Benth) Hook. f. belonging to Scrophulariaceae, and from *Buddleja asiatica* Lour (Buddleia), *B. neemda* Ham. ex Roxb. syn. *B. asiatica* Lour. and *B. globosa* Hope belonging to the family Buddlejaceae were found to show good anthelmintic activity [163].

The latex of some species of *Ficus* (Moraceae) has been traditionally used as vermifuge in Central and South America. However, due to high acute toxicity (hemorrhagic enteritis), latexes are not recommended for use in traditional medicine [164]. Extract of the dried fruits/crushed seeds of *Embelia schimperi* Vatke, belonging to the family Myrsinaceae, is used by the Masai people of Tanzania and Kenya who believe that it eliminates adult *Taenia saginata*, the beef tapeworm. It was effective against tapeworms *Hymenolepis diminuta* in a rat model. No significant *in vivo* effect was observed against *H. microstoma*, the trematode *Echinostoma caproni*, and the nematode *Heligmosomoides polygyrus* in mice, although the worms could be killed *in vitro*. These results indicate that the crushed seeds of *E. schimperi* taken orally by the Masai people, indeed have an anthelmintic activity against human

intestinal tapeworms [165]. The West African legume *Milletia thonningii* is used in Ghana as an anthelmintic and as a purgative [166]. A chloroform extract of the seeds of *Milletia thonningii* which is known to be molluscicidal and cercaricidal was topically applied to mouse skin 2 and 24 hours prior to exposure to *S. mansoni* cercariae. The presence of *M. thonningii* extract components on the surface of the skin appeared to be effective in preventing subsequent establishment of infection. The compound responsible for the activity is thought to be the isoflavonoid alpinumiso-flavone. The aqueous extract of *Albizzia anthelmintica* bark showed high anthelmintic activity (68–100%) against experimental *H. diminuta* infection in albino rats; it was not toxic. The water extract from *A. lebbek* bark was less effective against the cestode and was toxic to rats at a high dose [167]. Papaya latex (*Carica papaya*) showed an antiparasitic efficacy against *Heligmosomoides polygyrus* in a mice model [168].

Sainfoin (*Onobrychis viciifolia*) extracts containing condensed tannins inhibited the migration of 3rd-stage larvae of *Haemonchus contortus* [169]. 5,7-Dihydroxyflavanone (pinocembrine) from the acetone extract of *Teloxys graveolens* (Willd.) Weber

(Chenopodiaceae) exhibited fasciolicide, ovicide, and larvicide activities on newly excysted *Fasciola hepatica*, on infective eggs of *A. galli*, and on stage 3 larvae of *Stomoxys calcitrans*, respectively [170]. One of the flavones from *Struthiola argentea* (Thymelaeaceae) identified as 5,6,2',5',6'-pentamethoxy-3',4'-methylenedioxyflavone, demonstrated the most potent anthelmintic activity with 90% inhibition of larval motility *in vitro* [171]. The crude aqueous and hydro-alcoholic extracts of the seeds of *Coriandrum sativum* (Apiaceae) inhibited hatching of eggs of *H. contortus* completely in a dose-dependent manner. The hydro-alcoholic extract showed better *in vitro* activity against adult parasites than the aqueous one [172].

Conclusions and Prospects

From times immemorial, the plant kingdom has been a veritable source of medicinal agents, and there are several success stories of plant derived drugs even in modern medicine. However, our efforts to discover plant products or develop new drugs on plant-based leads for the control and treatment of filariasis and other helminth infections have not been met with much success. In the last five decades, more than 100 plant products were reported to be beneficial in the treatment or control of these parasitic infections but they could not be developed into viable drugs for a variety of reasons. New animal models of human infection developed in recent years improved our understanding of host parasite interactions and provided us better models to identify anthelmintic/antifilarial activity of plant products. It is expected that the newer assay batteries coupled with quality controlled systematic plant identification, collection, storage, processing, etc., and the state-of-the-art technology of chemical "fingerprinting" of the products would help us build some success stories in the area of filariasis/helminth infections.

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References

- Harborne JB. Phytochemical methods. A guide to modern techniques of plant analysis, 3rd edition. London: Chapman & Hall; 1998
- Butler MS, Newman DJ. Mother Nature's gifts to diseases of man: the impact of natural products on anti-infective, anticholesteremics and anticancer drug discovery. *Prog Drug Res* 2008; 65: 13–44
- Kuhn T, Wang Y. Artemisinin – an innovative cornerstone for anti-malaria therapy. *Prog Drug Res* 2008; 66: 385–422
- de Silva NR, Brooker S, Hotez PJ, Montresor A, Engels D, Savioli L. Soil-transmitted helminth infections: updating the global picture. *Trends Parasitol* 2003; 19: 547–551
- Fincham JE, Markus MB, Adams VJ. Could control of soil-transmitted helminth infection influence the HIV/AIDS pandemic. *Acta Trop* 2003; 86: 315–333
- Molyneux D. Lymphatic filariasis (elephantiasis) elimination: a public health success and development opportunity. *Filaria J* 2003; 2: 13
- WHO. The global burden of disease: 2004. Available at http://www.who.int/healthinfo/global_burden_disease/estimates_regional/en/index.html. Accessed October 10, 2010
- Maizels RM, Gomez-Escobar N, Gregory WF, Murray J, Zang X. Immune evasion genes from filarial nematodes. *Int J Parasitol* 2001; 31: 889–898
- WHO. Onchocerciasis and its control. Report of a WHO Expert Committee on onchocerciasis control. *World Health Organ Tech Rep Ser* 1995; 852: 1–104
- Molyneux DH. Onchocerciasis control in West Africa: Current status and future of the onchocerciasis control programme. *Parasitol Today* 1995; 11: 399–402
- WHO. Soil-transmitted helminthiasis. Number of children treated 2007–2008: update on the 2010 global target. *Wkly Epidemiol Rec* 2010; 85: 141–147
- Stephenson LS, Latham MC, Kinoti SN, Kurz KM, Brigham H. Improvements in physical fitness of Kenyan schoolboys infected with hookworm, *Trichuris trichiura* and *Ascaris lumbricoides* following a single dose of albendazole. *Trans R Soc Trop Med Hyg* 1990; 84: 277–282
- Nokes C, Grantham-McGregor SM, Sawyer AW, Cooper ES, Bundy DA. Parasitic helminth infection and cognitive function in school children. *Proc Biol Sci* 1992; 247: 77–81
- Adams EJ, Stephenson LS, Latham MC, Kinoti SN. Physical activity and growth of Kenyan school children with hookworm, *Trichuris trichiura* and *Ascaris lumbricoides* infections are improved after treatment with albendazole. *J Nutr* 1994; 124: 1199–1206
- Koroma MM, Williams RA, de la Haye RR, Hodges M. Effects of albendazole on growth of primary school children and the prevalence and intensity of soil-transmitted helminths in Sierra Leone. *J Trop Pediatr* 1996; 42: 371–372
- Stoltzfus RJ, Albonico M, Chwaya HM, Savioli L, Tielsch J, Schulze K, Yip R. Hemoglobin determination of hookworm-related blood loss and its role in iron deficiency in African children. *Am J Trop Med Hyg* 1996; 55: 399–404
- Katiyar JC, Gupta S, Sharma S. Experimental models in drug development for helminthic diseases. *Rev Infect Dis* 1989; 11: 638–654
- Murthy PK, Tyagi K, Roy Chowdhury TK, Sen AB. Susceptibility of *Mastomys natalensis* (GRA strain) to a subperiodic strain of human *Brugia malayi*. *Indian J Med Res* 1983; 77: 623–630
- Murthy PK, Chowdhury TK, Sen AB. Indian cat (*Felis indica*) as a host to subperiodic Malaysian strain of human *Brugia malayi*. *J Commun Dis* 1983; 15: 100–105
- Murthy PK, Srivastava AK, Joshi A, Sen AB, Murthy PSR, Ghatak S. Physiopathophysiology changes during infection in multimammate rats. I: Histological studies. *IRCS Med Sci* 1986; 14: 1106–1107
- Tyagi K, Murthy PK, Chatterjee RK, Sen AB. Chemotherapeutic response of *Brugia malayi* to antifilarials in *Mastomys natalensis*. *Indian J Parasitol* 1986; 10: 195–207
- Katiyar JC, Misra A, Gupta S, Visen PKS, Murthy PK, Sen AB. Efficacy of a substituted methyl benzimidazole carbamate against developing and adult helminth parasites. *Vet Parasitol* 1987; 23: 193–204
- Murthy PK, Tyagi K, Sen AB. Attempt to infect *Tatera indica* with sub-periodic strain of *Brugia malayi*. *Indian J Med Res* 1987; 85: 471–472
- Katiyar JC, Gupta S, Visen PK, Murthy PK, Misra A, Kumar S, Sarin JP. Methyl 5-[4-(2-pyridinyl)-1-piperazinylcarbonyl]-1H-benzimidazol-2-yl carbamate: efficacy against developing and adult helminths by topical application. *Indian J Exp Biol* 1988; 26: 715–719
- Murthy PK, Tyagi K, Chatterjee RK. Indian langur (*Presbytis entellus*) as experimental host for *Brugia malayi* infection. *Curr Sci* 1990; 59: 1236–1239
- Murthy PK, Fatma N, Chatterjee RK. Comparative susceptibility of ivermectin against different filarial parasites. *CSIR Golden Jubilee Symposium*, Lucknow, India; 1992
- Tyagi K, Murthy PK, Chatterjee RK. *Brugia malayi* in Indian leaf monkey (*Presbytis entellus*). Response to repeated exposures of infective larvae. *Curr Sci* 1996; 70: 164–167
- Murthy PK, Chatterjee RK. Evaluation of two *in vitro* test systems employing *Brugia malayi* parasite for screening of potential antifilarials. *Curr Sci* 1999; 77: 1084–1089
- Murthy PK, Khan MA, Rajani HB, Srivastava VML. Preadult stage of parasite is involved in the development of filarial limb edema in *Brugia malayi*-infected Indian leaf monkey (*Presbytis entellus*). *Clin Diagn Lab Immunol* 2002; 9: 913–918
- Dube A, Murthy PK, Puri SK, Misra-Bhattacharya S. *Presbytis entellus*: a primate model for parasitic disease research. *Trends Parasitol* 2004; 20: 358–360
- Murthy PK, Srivastava K, Murthy PSR. Responses of *Brugia malayi*-*Presbytis entellus*, a non-human primate model of filariasis, to DEC, ivermectin and CDRI compound 82–437. *Curr Sci* 2004; 86: 101–108

- 32 Sahare KN, Anandharaman V, Meshram VG, Meshram SU, Gajalakshmi D, Goswami K, Reddy MV. *In vitro* effect of four herbal plants on the motility of *Brugia malayi* microfilariae. *Indian J Med Res* 2008; 127: 467–471
- 33 Lakshmi V, Joseph SK, Srivastava S, Verma SK, Sahoo MK, Dube V, Mishra SK, Murthy PK. Antifilarial activity *in vitro* and *in vivo* of some flavonoids tested against *Brugia malayi*. *Acta Trop*, advance online publication 6 July 2010; DOI: 10.1016/j.actatropica.2010.06.006
- 34 Townson S, Tagboto S, McGarry HF, Egerton GL, Taylor MJ. Onchocerca parasites and Wolbachia endosymbionts: evaluation of a spectrum of antibiotic types for activity against *Onchocerca gutturosa* *in vitro*. *Filaria J* 2006; 5: 4
- 35 Zahner H, Johri GN, Kohler P, Striebel HP, Franz M. *In vitro* effects of 2-tert-butyl-benzothiazole derivatives on microfilariae of *Litomosoides carinii*, *Brugia malayi* and *Acanthocheilonema viteae*. *Arzneimittelforschung* 1991; 41: 764–768
- 36 Zaridah MZ, Idid SZ, Omar AW, Khozirah S. *In vitro* antifilarial effects of three plant species against adult worms of subperiodic *Brugia malayi*. *J Ethnopharmacol* 2001; 78: 79–84
- 37 Tippawangkosol P, Choochote W, Na-Bangchang K, Jitpakdi A, Pitasawat B, Riyong D. Comparative assessment of the *in vitro* sensitivity of *Brugia malayi* infective larvae to albendazole, diethylcarbamazine and ivermectin alone and in combination. *Southeast Asian J Trop Med Public Health* 2004; 35 (Suppl. 2): 15–21
- 38 Rao RU, Huang Y, Fischer K, Fischer PU, Weil GJ. *Brugia malayi*: Effects of nitazoxanide and tizoxanide on adult worms and microfilariae of filarial nematodes. *Exp Parasitol* 2009; 121: 38–45
- 39 Comley JC, Townson S, Rees MJ, Dobinson A. The further application of MTT-formazan colorimetry to studies on filarial worm viability. *Trop Med Parasitol* 1989; 40: 311–316
- 40 Rao UR, Salinas G, Mehta K, Klei TR. Identification and localization of glutathione S-transferase as a potential target enzyme in *Brugia* species. *Parasitol Res* 2000; 86: 908–915
- 41 Smith HL, Rajan TV. Tetracycline inhibits development of the infective-stage larvae of filarial nematodes *in vitro*. *Exp Parasitol* 2000; 95: 265–270
- 42 Tiwari P, Tripathi LM, Raghu KG, Srivastava VM. *Acanthocheilonema viteae*: octopamine and its physiological role. *Exp Parasitol* 2004; 108: 53–58
- 43 Walter RD, Wittich RM, Kuhlow F. Filaricidal effect of mefloquine on adults and microfilariae of *Brugia pateri* and *Brugia malayi*. *Trop Med Parasitol* 1987; 38: 55–56
- 44 Davies KP, Zahner H, Kohler P. *Litomosoides carinii*: mode of action *in vitro* of benzothiazole and amoscanate derivatives with antifilarial activity. *Exp Parasitol* 1989; 68: 382–391
- 45 Singhal KC, Saxena PN, Johri MB. Studies on the use of *Setaria cervi* for *in vitro* antifilarial screening. *Jpn J Pharmacol* 1973; 23: 793–797
- 46 Comley JC, Szopa TM, Strote K, Buttner M, Darge K, Buttner DW. A preliminary assessment of the feasibility of evaluating promising antifilarials *in vitro* against adult *Onchocerca volvulus*. *Parasitology* 1989; 99 (Pt. 3): 417–425
- 47 Townson S, Shay KE, Dobinson AR, Connelly C, Comley JC, Zea-Flores G. *Onchocerca gutturosa* and *O. volvulus*: studies on the viability and drug responses of cryopreserved adult worms *in vitro*. *Trans R Soc Trop Med Hyg* 1989; 83: 664–669
- 48 Comley JC. New macrofilaricidal leads from plants? *Trop Med Parasitol* 1990; 41: 1–9
- 49 Strote G, Darge K, Bonow I. Morphological alterations of male *Onchocerca volvulus* after *in vitro* exposure to mel w and milbemycin a confirming the results of viability tests. *Trop Med Parasitol* 1990; 41: 429–436
- 50 Cho-Ngwa F, Daggfeldt A, Titanji VP, Gronvik KO. Preparation and characterization of specific monoclonal antibodies for the detection of adult worm infections in onchocerciasis. *Hybridoma (Larchmt)* 2005; 24: 283–290
- 51 Mukherjee M, Misra S, Chatterjee RK. Development of *in vitro* screening system for assessment of antifilarial activity of compounds. *Acta Trop* 1998; 70: 251–255
- 52 Singh DP, Misra S, Chatterjee RK. A new technique of *in vitro* assay of antifilarials using different life-forms of *Acanthocheilonema viteae*. *Jpn J Exp Med* 1990; 60: 303–309
- 53 Singh BK, Mishra M, Saxena N, Yadav GP, Maulik PR, Sahoo MK, Gaur RL, Murthy PK, Tripathi RP. Synthesis of 2-sulfanyl-6-methyl-1,4-dihydropyrimidines as a new class of antifilarial agents. *Eur J Med Chem* 2008; 43: 2717–2723
- 54 Nisha M, Kalyanasundaram M, Paily KP, Abidha, Vanamail P, Balaraman K. *In vitro* screening of medicinal plant extracts for macrofilaricidal activity. *Parasitol Res* 2007; 100: 575–579
- 55 Awasthi SK, Mishra N, Dixit SK, Singh A, Yadav M, Yadav SS, Rathaur S. Antifilarial activity of 1,3-diarylpropen-1-one: effect on glutathione-S-transferase, a phase II detoxification enzyme. *Am J Trop Med Hyg* 2009; 80: 764–768
- 56 Rao R, Well GJ. *In vitro* effects of antibiotics on *Brugia malayi* worm survival and reproduction. *J Parasitol* 2002; 88: 605–611
- 57 Gunawardena NK, Fujimaki Y, Aoki Y, Mishima N, Ezaki T, Uni S, Kimura E. Differential effects of diethylcarbamazine, tetracycline and the combination on *Brugia pahangi* adult females *in vitro*. *Parasitol Int* 2005; 54: 253–259
- 58 Fujimaki Y, Kamachi T, Yanagi T, Caceres A, Maki J, Aoki Y. Macrofilaricidal and microfilaricidal effects of *Neurolepta lobata*, a Guatemalan medicinal plant, on *Brugia pahangi*. *J Helminthol* 2005; 79: 23–28
- 59 Batra S, Chatterjee RK, Srivastava VM. Antioxidant system of *Litomosoides carinii* and *Setaria cervi*: effect of a macrofilaricidal agent. *Vet Parasitol* 1992; 43: 93–103
- 60 Tyagi K, Murthy PK, Sen AB. Sequential changes in the antibody response of *Mastomys natalensis* consequent to *Brugia malayi* infection. *Indian J Med Res* 1985; 81: 269–274
- 61 Tyagi K, Murthy PK, Sen AB. Effect of some known antifilarials on the immune responses of *Mastomys natalensis* infected with *Brugia malayi*. *Indian J Med Res* 1986; 83: 155–161
- 62 Srivastava AK, Murthy PK, Joshi A, Sen AB, Murthy PSR, Ghatak S. Physiological changes during infection in multimammate rats. II. Enzyme studies. *IRCS Med Sci* 1986; 14: 1108–1109
- 63 Culbertson JT, Rose HM. Chemotherapy of filariasis in the cotton rat by administration of neostam. *Science* 1944; 99: 245
- 64 Tropical Disease Research-TDR. Seventh Programme Report. Chapter 4 Filariasis. Geneva: WHO; 1985: 1–20
- 65 Ash LR, Riley JM. Development of subperiodic *Brugia malayi* in the jird, *Meriones unguiculatus*, with notes on infections in other rodents. *J Parasitol* 1970; 56: 969–973
- 66 Murthy PK, Murthy PS, Tyagi K, Chatterjee RK. Fate of infective larvae of *Brugia malayi* in the peritoneal cavity of *Mastomys natalensis* and *Meriones unguiculatus*. *Folia Parasitol (Praha)* 1997; 44: 302–304
- 67 Petranyi G, Mieth H, Leitner I. *Mastomys natalensis* as an experimental host for *Brugia malayi* subperiodic. *Southeast Asian J Trop Med Public Health* 1975; 6: 328–337
- 68 Zahner H, Schares G. Experimental chemotherapy of filariasis: comparative evaluation of the efficacy of filaricidal compounds in *Mastomys coucha* infected with *Litomosoides carinii*, *Acanthocheilonema viteae*, *Brugia malayi* and *B. pahangi*. *Acta Trop* 1993; 52: 221–266
- 69 Mak JW, Navaratnam V, Ramachandran CP. Experimental chemotherapy of lymphatic filariasis. A review. *Ann Trop Med Parasitol* 1991; 85: 131–137
- 70 Hewitt RI, Wallace WS, White E, Subbarow Y. Experimental chemotherapy of filariasis; experimental methods for testing drugs against naturally acquired filarial infections in cotton rats and dogs. *J Lab Clin Med* 1947; 32: 1293–1303
- 71 Sewell P, Hawking F. Chemotherapy of experimental filariasis. *Br J Pharmacol Chemother* 1950; 5: 239–260
- 72 Chatterjee RK, Fatma N, Agarwal VK, Sharma S, Anand N. Comparative antifilarial efficacy of the N-oxides of diethylcarbamazine and two of its analogues. *Trop Med Parasitol* 1989; 40: 474–475
- 73 Fatma N, Sharma S, Chatterjee RK. 2,2'-Dicarbomethoxyamino-5,5'-dibenzimidazolyl ketone – a new antifilarial agent. *Acta Trop* 1989; 46: 311–321
- 74 Srivastava SK, Chauhan PM, Bhaduri AP, Murthy PK, Chatterjee RK. Secondary amines as new pharmacophores for macrofilaricidal drug design. *Bioorg Med Chem Lett* 2000; 10: 313–314
- 75 Stables JN, Lees GM, Rankin R. The potential of mice as animal models for antifilarial screening. *Trop Med Parasitol* 1988; 39: 25–28
- 76 Singh DP, Rathore S, Misra S, Chatterjee RK, Ghatak S, Sen AB. Studies on the causation of adverse reactions in microfilaraemic host following diethylcarbamazine therapy (*Dipetalonema viteae* in *Mastomys natalensis*). *Trop Med Parasitol* 1985; 36: 21–24
- 77 Kinnamon KE, Klayman DL, Poon BT, McCall JW, Dzimiński MT, Rowan SJ. Filariasis testing in a jird model: new drug leads from some old standbys. *Am J Trop Med Hyg* 1994; 51: 791–796

- 78 Kinnamon KE, Engle RR, Poon BT, Ellis WY, McCall JW, Dzimiński MT. Anticancer agents suppressive for adult parasites of filariasis in Mongolian jirds. *Proc Soc Exp Biol Med* 2000; 224: 45–49
- 79 Fujimaki Y, Sithithaworn P, Mitsui Y, Aoki Y. Delayed macrofilaricidal activity of diethylcarbamazine against *Brugia pahangi* in Mongolian jirds. *J Helminthol* 2004; 78: 293–295
- 80 Lammler G, Herzog H, Gruner D. Development of chemotherapeutic agents of parasitic diseases. Amsterdam: North Holland Publishing Company; 1975: 157
- 81 Sadanaga A, Hayashi Y, Tanaka H, Nogami S, Shirasaka A. Evaluation of the transplantation method of adult *Brugia malayi* into the jird, *Meriones unguiculatus*, for testing macrofilaricides. *Jpn J Exp Med* 1984; 54: 275–277
- 82 Gaur RL, Dixit S, Sahoo MK, Khanna M, Singh S, Murthy PK. Anti-filarial activity of novel formulations of albendazole against experimental brugian filariasis. *Parasitology* 2007; 134: 537–544
- 83 Tiwari VK, Tewari N, Katiyar D, Tripathi RP, Arora K, Gupta S, Ahmad R, Srivastava AK, Khan MA, Murthy PK, Walter RD. Synthesis and antifilarial evaluation of N1,Nn-xylofuranosylated diaminoalkanes. *Bio-org Med Chem* 2003; 11: 1789–1800
- 84 Mak JW, Lam PL, Rain AN, Suresh K. Effect of ivermectin against subperiodic *Brugia malayi* infection in the leaf monkey, *Presbytis cristata*. *Parasitol Res* 1988; 74: 383–385
- 85 Mak JW, Lam PL, Choong MF, Suresh K. Antifilarial activity of intravenous suramin and oral diethylcarbamazine citrate on subperiodic *Brugia malayi* in the leaf-monkey, *Presbytis cristata*. *J Helminthol* 1990; 64: 96–99
- 86 Murthy PK, Tyagi K, Sen AB. Susceptibility of *Macaca mulatta* to a subperiodic strain of *Brugia malayi*. *Indian J Parasitol* 1986; 10: 189–192
- 87 Misra S, Tyagi K, Chatterjee RK. Experimental transmission of nocturnally periodic *Wuchereria bancrofti* to Indian leaf monkey (*Presbytis entellus*). *Exp Parasitol* 1997; 86: 155–157
- 88 Schacher JF. Laboratory models in filariasis: a review of filarial life-cycle patterns. *Southeast Asian J Trop Med Public Health* 1973; 4: 336–349
- 89 Huber W, Koella JC. A comparison of three methods of estimating EC50 in studies of drug resistance of malaria parasites. *Acta Trop* 1993; 55: 257–261
- 90 Page C, Page M, Noel C. A new fluorimetric assay for cytotoxicity measurements *in vitro*. *Int J Oncol* 1993; 3: 473–476
- 91 Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983; 65: 55–63
- 92 Duwel D. Laboratory methods in the screening of anthelmintics. *Prog Drug Res* 1975; 19: 48–63
- 93 Colgrave ML, Kotze AC, Kopp S, McCarthy JS, Coleman GT, Craik DJ. Anthelmintic activity of cyclotides: *In vitro* studies with canine and human hookworms. *Acta Trop* 2009; 109: 163–166
- 94 Hounzangbe-Adote S, Fouraste I, Moutairou K, Hoste H. *In vitro* effects of four tropical plants on the activity and development of the parasitic nematode, *Trichostrongylus colubriformis*. *J Helminthol* 2005; 79: 29–33
- 95 Bizimenyera ES, Githiori JB, Eloff JN, Swan GE. *In vitro* activity of *Peltophorum africanum* Sond. (Fabaceae) extracts on the egg hatching and larval development of the parasitic nematode *Trichostrongylus colubriformis*. *Vet Parasitol* 2006; 142: 336–343
- 96 Alonso-Diaz MA, Torres-Acosta JF, Sandoval-Castro CA, Capetillo-Leal C, Brunet S, Hoste H. Effects of four tropical tanniniferous plant extracts on the inhibition of larval migration and the exsheathment process of *Trichostrongylus colubriformis* infective stage. *Vet Parasitol* 2008; 153: 187–192
- 97 Alonso-Diaz MA, Torres-Acosta JF, Sandoval-Castro CA, Aguilar-Caballero AJ, Hoste H. *In vitro* larval migration and kinetics of exsheathment of *Haemonchus contortus* larvae exposed to four tropical tanniniferous plant extracts. *Vet Parasitol* 2008; 153: 313–319
- 98 Maphosa V, Masika PJ, Bizimenyera ES, Eloff JN. *In-vitro* anthelmintic activity of crude aqueous extracts of *Aloe ferox*, *Leonotis leonurus* and *Elephantorrhiza elephantina* against *Haemonchus contortus*. *Trop Anim Health Prod* 2010; 42: 301–307
- 99 Tandon V, Pal P, Roy B, Rao HS, Reddy KS. *In vitro* anthelmintic activity of root-tuber extract of *Flemingia vestita*, an indigenous plant in Shillong, India. *Parasitol Res* 1997; 83: 492–498
- 100 Marquez-Navarro A, Noguera-Torres B, Hernandez-Campos A, Soria-Arteche O, Castillo R, Rodriguez-Morales S, Yopez-Mulia L, Hernandez-Luis F. Anthelmintic activity of benzimidazole derivatives against *Toxocara canis* second-stage larvae and *Hymenolepis nana* adults. *Acta Trop* 2009; 109: 232–235
- 101 Keiser J. *In vitro* and *in vivo* trematode models for chemotherapeutic studies. *Parasitology* 2010; 137: 589–603
- 102 Xue J, Xiao SH, Xu LL, Qiang HQ. The effect of tribendimidine and its metabolites against *Necator americanus* in golden hamsters and *Nippostrongylus braziliensis* in rats. *Parasitol Res* 2010; 106: 775–781
- 103 Albonico M, Allen H, Chitsulo L, Engels D, Gabrielli AF, Savioli L. Controlling soil-transmitted helminthiasis in pre-school-age children through preventive chemotherapy. *PLoS Negl Trop Dis* 2008; 2: e126
- 104 Boray JC, Happich FA, Andrews JC. Studies on the suitability of the albino rat for testing anthelmintic activity against *Fasciola hepatica*. *Ann Trop Med Parasitol* 1967; 61: 104–111
- 105 Campbell WC, Fisher MH, Stapley EO, Albers-Schonberg G, Jacob TA. Ivermectin: a potent new antiparasitic agent. *Science* 1983; 221: 823–828
- 106 Behm CA, Bendig MM, McCarter JP, Sluder AE. RNAi-based discovery and validation of new drug targets in filarial nematodes. *Trends Parasitol* 2005; 21: 97–100
- 107 Chatterjee RK, Fatma N, Murthy PK, Sinha P, Kulshreshtha DK, Dhawan BN. Macrofilaricidal activity of the stem bark of *Streblus asper* and its major active constituents. *Drug Dev Res* 1992; 26: 67–78
- 108 Misra N, Sharma M, Raj K, Dangi A, Srivastava S, Misra-Bhattacharya S. Chemical constituents and antifilarial activity of *Lantana camara* against human lymphatic filariid *Brugia malayi* and rodent filariid *Acanthocheilonema viteae* maintained in rodent hosts. *Parasitol Res* 2007; 100: 439–448
- 109 Dalziel JM. The useful plants of west tropical Africa. London: The Crown Agents for the Colonies; 1937: 612
- 110 Burkill HM. The useful plants of west tropical Africa, 2nd edition. Kew: Royal Botanic Gardens; 1985: 960
- 111 Kiuchi F, Miyashita N, Tsuda Y, Kondo K, Yoshimura H. Studies on crude drugs effective on visceral larva migrans. I. Identification of larvicidal principles in betel nuts. *Chem Pharm Bull (Tokyo)* 1987; 35: 2880–2886
- 112 Murthy PK. Anthelmintics from medicinal plants. In: Sood ML, editor. *Helminthology in India*. India: International Book Distributors; 2003: 377–392
- 113 Singh NP, Singh VK. *Streblus asper* Lour. – An ancient Indian drug for the cure of filariasis. *Acta Bot Indica* 1987; 15: 108–109
- 114 Singh NP, Ram ER. Filaria and its herbal cure. *New Botanist* 1988; 15: 201–205
- 115 Kilian HD. Preliminary data on the effects of an extract from *Cassia aubrevillei* in onchocerciasis. Hamburg: Liberia Research Unit of the Tropical Institute; 1987: 33–34
- 116 Kilian HD, John K, Buttner DW, Kraus L. *In vivo* and *in vitro* effects of extracts of *Cassia aubrevillei* in onchocerciasis. O-NOW Symposium on Onchocerciasis, Leiden, Netherlands; 1989
- 117 Titanji VPK, Evehe MS, Ayafor JF, Kimbu SF. Novel *Onchocerca volvulus* filaricides from *Carapa procera*, *Polyalthia suaveolens* and *Pachypodanthium staudii*. O-NOW Symposium on Onchocerciasis, Leiden, Netherlands; 1989
- 118 Suresh M, Kaleysa Raj R. Cardol: The antifilarial principle from *Anacardium occidentale*. *Curr Sci* 1990; 59: 477–479
- 119 Kulangara AC, Subramanian R. Preliminary studies on the effects of certain compounds on the filarial worm of the lizard, including an estimate of the toxicity of sodium flouride. *Indian J Med Res* 1960; 48: 698–704
- 120 Dutta A, Sukul NC. Filaricidal properties of a wild herb, *Andrographis paniculata*. *J Helminthol* 1982; 56: 81–84
- 121 Datta A, Sukul NC. Antifilarial effect of *Zingiber officinale* on *Dirofilaria immitis*. *J Helminthol* 1987; 61: 268–270
- 122 Titanji VPK, Ayafor JF, Mulufi JP, Mbacham WF. *In vitro* killing of *Onchocerca volvulus* (Filaroidea) adults and microfilariae by selected Cameroonian medicinal plant extracts. *Fitoterapia* 1987; 58: 338–339
- 123 Parveen N, Singhal KC, Khan NU. Screening of some plant extracts for their potential antifilarial activity using *Setaria cervi* as test organism. International Conference on Recent Advances in Medicinal, Aromatic and Spice Crops, New Delhi, India; 1989

- 124 Singh R, Singhal KC, Khan NU. Antifilarial activity of the leaves of *Malotus philippensis* Lam. on *Setaria cervi* (Nematode Filarioidea) *in vitro*. Indian J Physiol Pharmacol 1997; 41: 397–403
- 125 Ampofo O. "Plants that heal" World Health. The Magazine of the World Health Organization 1977; 26–30
- 126 Khunkitti W, Fujimaki Y, Aoki Y. *In vitro* antifilarial activity of extracts of the medicinal plant *Cardiospermum halicacabum* against *Brugia pahangi*. J Helminthol 2000; 74: 241–246
- 127 Sahare KN, Anandhraman V, Meshram VG, Meshram SU, Reddy MV, Tuman PM, Goswami K. Anti-microfilarial activity of methanolic extract of *Vitex negundo* and *Aegle marmelos* and their phytochemical analysis. Indian J Exp Biol 2008; 46: 128–131
- 128 Lakshmi V, Saxena A, Pandey K, Bajpai P, Misra-Bhattacharya S. Antifilarial activity of *Zoanthus* species (Phylum Coelenterata, Class Anthozoa) against human lymphatic filaria, *Brugia malayi*. Parasitol Res 2004; 93: 268–273
- 129 Mathew N, Misra-Bhattacharya S, Perumal V, Muthuswamy K. Antifilarial lead molecules isolated from *Trachyspermum ammi*. Molecules 2008; 13: 2156–2168
- 130 Gaur RL, Sahoo MK, Dixit S, Fatma N, Rastogi S, Kulshreshtha DK, Chatterjee RK, Murthy PK. Antifilarial activity of *Caesalpinia bonducella* against experimental filarial infections. Indian J Med Res 2008; 128: 65–70
- 131 Haerdi F. Die Eingeborenen-Heilpflanzen des Ulanga-Distriktes Tanganyikas (Ostafrika). Acta Trop Suppl 8 1964: 1–278
- 132 Oguakwa JU, Galeffi C, Messana I, Patamia M, Nicoletti M, Marini-Betolo GB. Research on African medicinal plants. III New alkaloids from *Alstonia boonei* De Wild Gazzetta. Chim Ital 1983; 113: 533–535
- 133 Oliver-Bever BEP. Anti-infective activity of higher plants. Medicinal plants in Tropical West Africa. Cambridge: Cambridge University Press; 1986: 123–190
- 134 Ojewole JAO. Studies on the pharmacology of echitamine, an alkaloid from the stem bark of *Alstonia boonei* L. (Apocynaceae). Int J Crude Drugs Res 1984; 22: 121–143
- 135 Farnsworth NR, Akerele O, Bingel AS, Soejarto DD, Guo Z. Medicinal plants in therapy. Bull World Health Organ 1985; 63: 965–981
- 136 Guha Bakshi DN, Sensarma P, Pal DC. A lexicon of medicinal plants in India. Calcutta: Naya Prokash; 1999: 290
- 137 Watt JMM, Breyer-Brandwijk G. The medicinal and poisonous plants of Southern and Eastern Africa, 2nd edition. London: E. & S. Livingstone, Ltd.; 1962
- 138 Githens TS. Drug plants of Africa, African handbooks No. 8. Philadelphia: University of Pennsylvania Press; 1948: 125
- 139 Satyavati GV, Gupta AK, Tandon N. Medicinal plants of India. New Delhi, India: Indian Council of Medical Research; 1986
- 140 Banu MJ, Nallaiappan K, Dhandayuthapani S. Mitochondrial malate dehydrogenase and malic enzyme of a filarial worm *Setaria digitata*: some properties and effects of drugs and herbal extracts. Jpn J Med Sci Biol 1992; 45: 137–150
- 141 Ayensu ES. Medicinal plants of West Africa. Algonac, Michigan: Reference Publications Inc.; 1978: 330
- 142 Deka L, Majumdar R, Dutta AM. Some Ayurvedic important plants from district Karnrup (Assam). Ancient Sci Life 1983; 3: 108–115
- 143 Al-Yahya MA, Al-Meshal IA, Mossa JS, Tariq M. Phytochemical and pharmacological studies on *Calotropis procera*. 3rd International Conference of Traditional and Folk Medicine, Lecatecas, Mexico; 1985
- 144 Al-Yahya MA. Phytochemical studies on the plants used in traditional medicine of Saudi Arabia. Fitoterapia 1986; 47: 179–182
- 145 Sahu TR. Less known uses of weeds as medicinal plants. Ancient Sci Life 1984; 4: 245–249
- 146 Hedberg I, Hedberg O, Madati PJ, Mshigeni KE, Mshiu EN, Samuelsson G. Inventory of plants used in traditional medicine in Tanzania. II. Plants of the families Dilleniaceae – Opiliaceae. J Ethnopharmacol 1983; 9: 105–127
- 147 Iwu MM. Empirical investigation of dietary plants used in Igbo Ethnomedicine. New York: Nina Etkined Redgroove Publishing Company; 1986: 116
- 148 Hedberg I, Hedberg O, Madati PJ, Mshigeni KE, Mshiu EN, Samuelsson G. Inventory of plants used in traditional medicine in Tanzania. Part III. Plants of the families Papilionaceae–Vitaceae. J Ethnopharmacol 1983; 9: 237–260
- 149 Maheshwari JK, Singh KK, Saba S. Ethno-medicinal uses of plants by the Thurus of Kheri District, U.P. Bull Med Ethnobot Res 1980; 1: 318–337
- 150 Whistler WA. Traditional and herbal medicine in the Cook Islands. J Ethnopharmacol 1985; 13: 239–280
- 151 Baoua M, Fayn J, Bassiere J. Preliminary phytochemical testing of some medicinal plants of Niger. Planta Med 1976; 10: 251–266
- 152 Dragendorff G. Die Heilpflanzen der verschiedenen Völker und Zeiten. Stuttgart: Ferdinand Enke; 1898
- 153 Lewis WH, Manony PFE. Medical botany: plants affecting man's health. New York: John Wiley and Sons; 1977: 240
- 154 Vasileva B. Plantes mediques Conakrynales de Guinea. Republic de Guinea: Conakry; 1969
- 155 Kokward JO. Medicinal plants of East Africa. Nairobi, Kenya: East Africa Literature Bureau; 1976
- 156 Kerharo J, Adam JG. Pharmacopée sénégalaise traditionnelle. Paris: Vigot; 1974
- 157 Parveen N, Singhal KC, Khan NU, Singhal P. Potential antifilarial activity of *Streblus asper* against *Setaria cervi* (Nematoda: Filarioidea). Indian J Pharmacol 1989; 21: 16–21
- 158 Kliks MM. Studies on the traditional herb anthelmintic *Chenopodium ambrosioides* L. Ethnopharmacological evaluation and clinical field trials. Soc Sci Med 1985; 21: 879–886
- 159 Ibrahim MA, Nwude N, Ogunsui RA, Aliu YO. Screening of West African plants for anthelmintic activity. ILCA Bull 1984; 17: 19–23
- 160 Takki S. Anthelmintic effect of fern preparations and their action on some liver function tests in man. Ann Med Exp Fenn 1967; 45: 341–351
- 161 Navarrete A, Hong E. Anthelmintic properties of alpha-sanshool from *Zanthoxylum liebmannianum*. Planta Med 1996; 62: 250–251
- 162 Raj RK, Kurup PA. Anthelmintic activity, toxicity and other pharmacological properties of palasonin, the active principle of *Butea frondosa* seeds and its piperazine salt. Indian J Med Res 1968; 56: 1818–1825
- 163 Rastogi RP, Mehrotra BN. Compendium of Indian medicinal plants. New Delhi: CDRI/CSIR/PID; 1995: 125, 435
- 164 de Amorin A, Borba HR, Carauta JP, Lopes D, Kaplan MA. Anthelmintic activity of the latex of *Ficus* species. J Ethnopharmacol 1999; 64: 255–258
- 165 Bogh HO, Andreassen J, Lemmich J. Anthelmintic usage of extracts of *Embelia schimperi* from Tanzania. J Ethnopharmacol 1996; 50: 35–42
- 166 Perrett S, Whitfield PJ, Sanderson L, Bartlett A. The plant molluscicide *Millettia thonningii* (Leguminosae) as a topical antischistosomal agent. J Ethnopharmacol 1995; 47: 49–54
- 167 Galal M, Bashir AK, Salih AM, Adam SE. Activity of water extracts of *Albizia anthelmintica* and *A. lebbeck* barks against experimental *Hymenolepis diminuta* infection in rats. J Ethnopharmacol 1991; 31: 333–337
- 168 Satrija F, Nansen P, Murtini S, He S. Anthelmintic activity of papaya latex against patent *Heligmosomoides polygyrus* infections in mice. J Ethnopharmacol 1995; 48: 161–164
- 169 Barrau E, Fabre N, Fouraste I, Hoste H. Effect of bioactive compounds from Sainfoin (*Onobrychis viciifolia* Scop.) on the *in vitro* larval migration of *Haemonchus contortus*: role of tannins and flavonol glycosides. Parasitology 2005; 131: 531–538
- 170 Del Rayo Camacho M, Sanchez B, Quiroz H, Contreras JL, Mata R. Pino-cembrine: a bioactive flavanone from *Teloxys graveolens*. J Ethnopharmacol 1991; 31: 383–389
- 171 Ayers S, Zink DL, Mohn K, Powell JS, Brown CM, Murphy T, Brand R, Pretorius S, Stevenson D, Thompson D, Singh SB. Flavones from *Struthiola argentea* with anthelmintic activity *in vitro*. Phytochemistry 2008; 69: 541–545
- 172 Egale T, Tilahun G, Debella A, Feleke A, Makonnen E. *In vitro* and *in vivo* anthelmintic activity of crude extracts of *Coriandrum sativum* against *Haemonchus contortus*. J Ethnopharmacol 2007; 110: 428–433
- 173 Booth S, Johns T, Lopez-Palacios CY. Factors influencing self-diagnosis and treatment of perceived helminthic infection in a rural Guatemalan community. Soc Sci Med 1993; 37: 531–539
- 174 Jain SK, Sinha BK, Saklani A. Some interesting medicinal plants known among several tribes of India. Ethnobotany 1989; 1: 89–100
- 175 Rastogi RP, Mehrotra BN. Compendium of Indian medicinal plants. New Delhi: CDRI/CSIR/PID; 1990
- 176 Molan AL, Sivakumaran S, Spencer PA, Meagher LP. Green tea flavan-3-ols and oligomeric proanthocyanidins inhibit the motility of infective larvae of *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* *in vitro*. Res Vet Sci 2004; 77: 239–243
- 177 Rastogi RP, Mehrotra BN. Compendium of Indian medicinal plants. New Delhi: CDRI/CSIR/PID; 1993

- 178 Satrija F, Nansen P, Bjorn H, Murtini S, He S. Effect of papaya latex against *Ascaris suum* in naturally infected pigs. *J Helminthol* 1994; 68: 343–346
- 179 Rastogi RP, Mehrotra BN. Compendium of Indian medicinal plants. New Delhi: CDRI/CSIR/PID; 1991
- 180 Dhar ML, Dhar MM, Dhawan BN, Mehrotra BN, Ray C. Screening of Indian plants for biological activity. *Indian J Exp Biol* 1968; 6: 232–247
- 181 Hansson A, Veliz G, Naquira C, Amren M, Arroyo M, Arevalo G. Preclinical and clinical studies with latex from *Ficus glabrata* HBK, a traditional intestinal anthelmintic in the Amazonian area. *J Ethnopharmacol* 1986; 17: 105–138
- 182 Shiramizu K, Tuchida T, Aru M. Anthelmintic effect of cude preparation containing the root of *Matteuccia orientalis* on bovine fasciolosis. *J Jpn Vet Med Assoc* 1993; 46: 561–564
- 183 Perrett S, Whitfield PJ, Bartlett A, Sanderson L. Attenuation of *Schistosoma mansoni* cercariae with a molluscicide derived from *Millettia thonningii*. *Parasitology* 1994; 109 (Pt. 5): 559–563
- 184 Asuzu IU, Onu UO. Anthelmintic activity of the ethanolic extract of *Piliostigma thonningii* bark in *Ascaridia galli* infected chickens. *Fitoterapia* 1994; 65: 291–297
- 185 Muddathir AK, Balansard G, Timon-David P, Babadjamian A, Yogoub AK, Julien MJ. Anthelmintic properties of *Polygonum glabrum*. *J Pharm Pharmacol* 1987; 39: 296–300
- 186 Stadler M, Dagne E, Anke H. Nematicidal activities of two phytoalexins from *Taverniera abyssinica*. *Planta Med* 1994; 60: 550–552
- 187 Aye T, Mya B, Saw H, Tin M, Marlar L, Po Aung S. Screening of some medicinal plants reputed for anthelmintic activity on *in vitro* test models. *Myanmar Health Sci Res J* 1993; 5: 79–84