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Review Article

A Review on filaricidal activity of phytochemical extracts against filariasis and the Parasites Genomic Diversity

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Submitted: 15 October 2018
Approved: 25 October 2018
Published: 26 October 2018

Keywords: Molecular biology; Filariasis; Elephantiasis; Parasitology; Phytochemicals

Abstract

Filariasis is one of the Neglected Tropical Diseases (NTDs) known to be of serious public health importance and pose devastating socio-economic burden especially among the poor people in tropical and subtropical countries of the world. The parasite is responsible for lymphatic filariasis affecting about 1.3 billion people in 72 countries worldwide. The major parasitic agents of the infection are three closely related nematodes of clade *Onchocercidae* namely *Wuchereria bancrofti, Brugia malayi* and *B. timori* that are transmitted to human through bites by mosquitoes of genera: *Aedes, Anopheles*, *Culex* and *Mansonia*. The disease is targeted by the World Health Organization (WHO) for elimination by 2020 through the use of chemically synthesized drugs used as therapeutic agents to cure the disease but there are some setbacks. Phytochemical extracts are viewed as alternative therapy in the management of the disease. Additionally, the species have many ecological variants and are diversified in terms of their genetic fingerprint. This diversification in terms of genomic sequences as well as rapid infection rate warrant the lymphatic filarial parasites to respond differently to diagnostic and therapeutic interventions. Thus understanding the genomic diversity of the parasite will help in efficient therapeutic management of the disease, thereby eliminating it to prevent unnecessary suffering and contribute to the reduction of poverty. In this review, we have highlighted on the used for phytochemical extracts in the therapeutic management of the lymphatic and the molecular genetic diversity of the parasite was delineated.

Introduction

Lymphatic filariasis is a vector-borne infection with parasitic nematodes of family Filariodidea namely: *Wuchereria bancrofti, Brugia malayi* and *B. timori*. About 90% of the infection is reported to be caused by *Wuchereria bancrofti* and the remainder is due to infection with either *B. malayi* or *B. timori* [1]. The parasites are transmitted via mosquito bites [2,3]. Lymphatic filariasis is transmitted by different types of mosquitoe genera, for example, in urban and semi-urban areas the disease is transmitted by *Culex* mosquito, which is widespread across such habitat. In rural areas, the disease is said to be transmitted by Anopheles, while Aedes, mainly in endemic islands in the Pacific 1].

Adult worms lodge in the lymphatic vessels and disrupt the normal function of the lymphatic system. The worms can live for approximately 6 - 8 years and, during their life time, produce millions of microfilariae (immature larvae) that circulate in the blood. The death of the filarial parasite causes inflammation within the host which initiates the first step of its pathogenesis. Infections are mainly hidden and often acquired during childhood leading to a lifetime of an impaired lymphatic system, suppressing the host immunity and increased risk of debilitating episodes filarial fever known as

How to cite this article: Gumel AM, Dogara MM. A Review on filaricidal activity of phytochemical extracts against filariasis and the Parasites Genomic Diversity. Int J Clin Microbiol Biochem Technol. 2018; 1: 024-032. https://dx.doi.org/10.29328/journal.ijcmbt.1001004

adenolymphangitis (ADL). The host inflammation resulted in an altered lymphatic system and the abnormal enlargement of body parts e.g. lymphoedema, hydrocoele, and elephantiasis thus resulting in chronic disabling consequences of the damage caused by infections of the lymphatic vessels. This causes acute and chronic morbidity in humans within tropical and subtropical areas of Africa, Asia, the Western Pacific, and some parts of the Americas resulting in high public health and socio-economic burden within the affected societies [4].

According to World Health Organization (WHO) fact sheets, more than 1.3 billion people in 72 countries worldwide are threatened by lymphatic filariasis, with 856 million people in 52 countries living in areas that require preventive chemotherapy to stop the spread of infection [1]. The global baseline estimate of people affected by lymphatic filariasis was 25 million men with hydrocele and over 15 million people with lymphoedema and elephantiasis. At least 36 million people remain with these chronic disease manifestations.

Several chemotherapeutic drugs (albendazole, ivermectin, diethylcarbamazine citrate) are employed in the management of filariasis. Interestingly, the drug is said to be effective even when used as monotherapy [2]. Unfortunately, most of these synthetic drugs are characterized with adverse side effects warranting the recent surge of research on alternative therapeutic drugs.

Phytochemical extracts are reported to have potential bioactivity against lymphatic filariasis [5-10]. Among them are those obtained from *Andrographis paniculata* [11], *Azadirachta indica* [12], *Haliclona oculata* [13] *Polyalthiasu aveolens* [14]. This article highlighted a review on recent literatures that reported the efficacy of some phytochmical extracts against filarial parasites. It also explores the genetic diversity that exists within different strains of the filarial parasites. It is believed that understanding the genomic diversity of the parasite will help in efficient therapeutic management of the disease, thereby eliminating it to prevent unnecessary suffering and contribute to the reduction of poverty.

Phyto-chemicals extracts used as filaricidal agents

Ricinus communis (Family: Euphorbiaceae): *The plant is commonly known as castor oil plant* found in both the tropical and temperate climates of the world. Literature has documented that *R. communis* seeds extracts posses bioactivity against warts, cold tumours, been widely used as a human laxative-cathartic agent [15]. The filaricidal effect of organic solvent extract of *R. communis* seeds against filarial parasite *B. malayi* at varying dosage was reported [15]. The research indicates dose dependent filaricidal activity (40-90%). In contrast, Nisha et al. [16] reported that treatment with ethanol fraction (1 mg/ml) of *R. communis* seed extract caused a complete suppression of *S. digitata* microfilarial growth within 1hr, 40 min. In fact, Nisha et al. [16], established that using the extract, incur 72.39% microfilarial growth inhibition [16].

Haliclona oculata (Family: Chalinidae): *The demospongie (Haliclona oculata)* a marine of family *Chalinidae* is known to possess a variety of bioactivity against several diseases such as cancer [17], neurodegeneration [18], type-2 diabetes [19], fungal and microbial infections [20,21]. The bioactivity of these sponges is thought to be due to possession of novel sterols, metabolites including steroids, terpenoids, alkaloids, cyclic peptides and unsaturated fatty acids [13]. Gupta *et al.* (2012) reported that administering methanolic extract, of *H. oculata* at 100 mg/kg for five consecutive days by sub-cutaneous route demonstrated significant macrofilaricidal efficacy of 51.3%, 64% and 70.7%, respectively. Chemical analyses of the extract revealed that it contained a mixture of four alkaloids (Figure 1) namely mimosamycin, xestospongin-C, xestospongin-D and araguspongin-C together with few minor compounds [13]. This observation was found to be in resonance with Lakshmi et al. [22], on anti-filarial activity of another species, *H. exigua* against lymphatic *B. malayi*. *In vitro* and *in vivo*

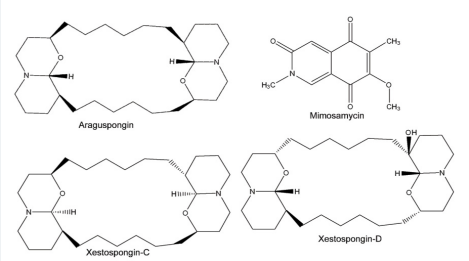

Figure 1: *Alkaloids extracts of Haliclona spp.*

study showed that 31.25 µg/ml concentrations of the crude alcoholic extract soluble fractions were able to kill the adult worms. Whereas the chloroform extract was found to be effective at lower concentration (15.6 µg/ml), and the observed bioactivity was attributed due to the presence of araguspongin-C.

Azadirachta indica (Family: Meliaceae): Popularly called as 'Neem tree', the plant is a large evergreen tree with its height reaching up to 50 ft. [23]. It is widely distributed within the hot tropical regions especially in India and West Africa [24]. The leaves and bark of this tree has a bitter taste of triterpenoid azadirachtin [25]. Almost every part of this tree is reported to be used in complementary medicine for the cure of different ailments such as anti-microbial [26], anti-inflammatory [27], anti-cancer [28], anti-malarial [29], anti-ulcerogenic [30] and anti-filarial [23] activities. Al-Rofaai et al. [12] reported the effect of *A. indica* leaf extract against helminthes *Teladorsagia circumcincta*. Employing organic solvents extraction and aqueous fractionation methods, they found that the first stage larvae (L_1) were shown to be more sensitive having the lowest LC_{50} at 7.15 mg/ml of the extract as compared to 24.91 mg/ml on infective stage larvae (L_3). Other workers employing distilled alcoholic and aqueous extracts of *A. indica* flowers showed that it has potential anti-filarial activity against microfilariae of *Setariacervi* [23]. The study also showed that the inhibition was concentration dependent, and both extracts were found to have almost similar lethal effect on the microfilariae of *S. cervi*, with LC_{50} being 15 and 18 ng/ml, respectively [23].

Adrographis paniculata Linn (Family: Acanthaceae): *Andrographispaniculata* (Green Chiretta) is an annual herbaceous plant belonging to the family *Acanthaceae,* native to Southeast Asian. It has been traditionally used for centuries in Ayurvedic medicine. The herb has been revered for treating infectious diseases and highly regarded as having a preventative effect for many diseases, due to its powerful immune strengthening benefits [31]. Extensive research literatures has revealed that *A. paniculata* has a broad range of pharmacological activities in different ailments such as anti-analgesic [32], anti-oxidant [33], anti-biofilm [34], gastro-protective [35], wound healing [36], hepato-protective [37], anti-filarial [11], anti-cancer [38], anti-malarial [39] and anti-termites [40]. It has been reported that the prophylactic effect of *Andrographis* was able to stop the catastrophic effect of the deadly flu virus of 1919 global epidemic from reaching India [41]. In fact, its bioactive diterpenoid andrographolide and its analogs were reported to block the MCF-7 breast cancer cells cycle at the G0-G1 phase [42].

Kumarappan et al. [43] studied the anti-filarial activity of alcoholic extract of *A. paniculata,* the study reported that aqueous extract of the leaves showed microfilaricidal activity on *Dipetalonema reconditum* within 40 min, both *in vitro* and *in vivo*. Administration of the extract (0.06 ml/Kg body weight) reduced the number of the microfilariae in infected dogs by more than 85% (Dutta & Sukul, 1982). Earlier, Zaridah et al. [44] reported the filaricidal activity of *A. paniculata* aqueous leaf extract against *B.malayi*. The authors analyzed the anti-filarial activity of the extract using relative movability (RM) value of the adult worm over a period of 24 hrs resulting in the total death of the parasite when treated with 5 or 10 mg/ml of the extract.

Hibiscus species (Family: Malvaceae): *H. sabdariffa* (roselle) is a native of tropics, used for the production of fiber and infusions that are normally used as beverages. It is a woody shrub of annual to perennial seasoning. It is reported to have several medicinal efficacies especially on hypertensive patients [45]. The plants is said to be rich in anthocyanins, as well as dihydroxybenzoic acid. Daphniphylline forms the major pigment, while the dried calyces contain the flavonoids gossypetin, hibiscetine and sabdaretine. In addition, a small amount of delphinidin-3-monoglucoside, cyaniding-3-monoglucoside, and delphinidin were also present [45]. Moreover, the seeds were reported to be a good source of lipid-soluble antioxidants, particularly gamma-tocopherol [46]. Recently, ethanolic extract of *H. sabdariffa* leafs were reported to exhibit filaricidal activity against *B. malayi* (Saxena *et al.*, 2011). The efficacy of the plant extract filaricidal activity was assessed using both the *in vivo* and *in vitro* motility and MTT reduction assays on the microfilariae (mf) and adult worms. The authors found that administering the leaf-extract at 500 mg/ml for 5 days, incurred about 30% macrofilaricidal efficacy and 42% sterilization of female worms in *Meriones unguiculatus*. On the other hand, feeding *M. coucha* with the extract (1g/kg, for 5 days) exerted 57% macrofilaricidal with 64% sterilizing effect on female worms (Saxena *et al.*, 2011). In a similar studies, the crude methanolic extract of *H. mutabilis* (confederate rose) and the isolated bioactive molecule 'ferulic acid' were tested against bovine *S. cervi* [47]. The authors reported that both the extract and the bioactive molecule 'ferulic acid' exerted significant filaricidal activities against *S. cervi* [47].

Cardiospermum halicacabum (Family: Sapindaceae): *Cardiospermum halicacabum* (balloon vine), is a climbing plant (Figure 2) widely distributed in tropical and subtropical regions of Africa and Asia [48]. This plant has been reported to have bioactivity such as homoeopathic [48] and anti-diarrheal efficacy [49], anti-microbial [50,51]. The filaricidal activity of ethanolic and aqueous extracts of *C. halicacabum* against *B. pahangi* was previously reported is [52]. The researchers found activity on adult worms and the amount of microfilarial released by female worms was concentration and time dependent. For example, using 500 µg/ml, the authors observed the aqueous extract to significantly reduce motility of adult females after 24 h of exposure, the release of microfilariae from female worms on day 2 and the motility of the adult males after 3 days. However, the aqueous extract at this concentration (500 µg/ml) did not affect the motility of microfilariae with the exception of those in higher concentration extracts. In contrast, 500 µg/ml of the ethanol extract was found to rapidly reduce the motility of microfilariae on day 2. Furthermore, higher concentrations of ethanol extracts (2 mg/ml) inhibit both the motility of adult worms and the release of microfilariae from females [52].

Molecular diversity of the parasite

Currently, WHO has launched a massive campaign on the global program to eliminate lymphatic filariasis (GPELF), through elimination of its causative agents: *W. bancrofti*, *B. malayi*, and *B. timori* (Figure 3), through a combination of mass drug administration

Figure 2: *Cardiospermum halicacabum.*

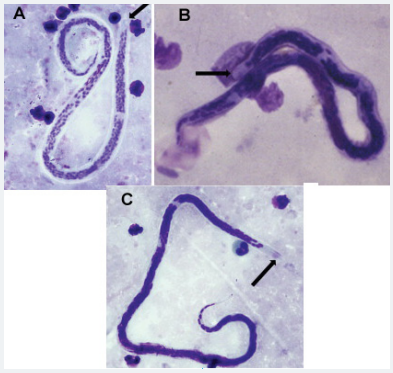

Figure 3: Filarial parasites (a) *W. bancrofti*, (b) *B. malayi*, (C) *B. tumori*. Note: arrow indicates the cephalic space [59]..

(MDA) and integrated vector management (IVM) [53]. The current goal of GPELF is to eliminate lymphatic filariasis by the year 2020. Despite constituting a major public health problem posed by lymphatic filariasis in many tropical and subtropical regions, the parasites' genetics and genomics remains poorly understood [54].

Current tools available to monitor LF are limited to diagnostic tests targeting DNA repeats, filarial antigens, and anti-filarial antibodies. While these tools are useful for detection and surveillance, unfortunately, elimination programs have yet to take full advantage of molecular typing for inferring infection history, strain fingerprinting, and evolution.

To this end, molecular genetics have proved to be a valuable tool in deciphering the evolutionary history of parasites as well as improving the knowledge of infectious disease epidemiology [53]. Genetic analysis provides a glimpse into the epidemic history of a parasite that can complement epidemiological analysis or even in some cases replacing missing surveillance data. With the advent of polymorphic molecular markers for the filarial parasites especially bacnroftian filaria, it is now possible to apply population genetic analysis to understand lymphatic filariasis.

Molecular typing approaches have included whole mitochondrial genomes, genotyping, targeted sequencing, and random amplified polymorphic DNA (RAPDs) in order to systematically delineate different species variants of the parasites. For example, mitochondrial (mt) genome sequences have enabled comparison of population genetics and evolution for numerous free-living and parasitic nematodes. Ramesh et al. [54] have compared the complete mt genome of *W. bancrofti* through analysis of isolates from Papua New Guinea, India and West Africa. Using mt genome sequence for *B. malayi* as a reference, the researchers were able to assemble the sequences for each isolate and annotate it. The study described that the length of the *W. bancrofti* mt genome is approximately 13,637 nucleotides, it also contains 2 ribosomal RNAs (Figure 4), 22 transfer RNAs, 12 protein-coding genes, and is characterized by a 74.6% AT content [54]. It further asserts that the *W. bancrofti* mt gene order is identical to that reported for *Onchocerca volvulus*, *Dirofilaria immitis*, *Setaria digitata* and *B. malayi* [54].

Papua New Guinea researchers identified 5 major strains that were widespread and many minor strains some of which exhibit geographic stratification. Genome data, while rare, has been utilized to reconstruct evolutionary relationships among taxa of the filarial Onchocercidae and identify gene synteny. Their phylogeny reveals that speciation from the common ancestor of both *B. malayi* and *W. bancrofti* occurred around 5–6 million years ago with shared ancestry to other filarial nematodes as recent as 15 million years ago.

Previous study had employed the use of comparison of random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP)

profiles to identify robust molecular markers for bancroftian parasite of Indian variant [55]. Using RAPD marker (OP8) primer after digestion with λDNA/*Hin*dIII and ΦX174/*Hae*III, the researchers were able to reveal variations between the parasite populations (Figure 5) collected from different regions [55].

In contrast to the above studies, Ghedin et al. [56] have sequenced ~90 megabase pairs genome of *B. malayi* and inferred that the parasite has between 14,500 and 17,800 protein-coding genes [57], have reviewed the comparative genomics of nematodes. The review highlighted that analysis of conserved synteny between *B. malayi* and *C. elegans* revealed a preponderance of within-chromosome linkage, but little local synteny [57,58]. *B. malayi* has five chromosome pairs, and comparisons of genes linked in *B. malayi* against *C. elegans* suggest that the changed chromosome number is the result of a fusion between genomic segments corresponding to *C. elegans* chromosomes I and III. However, since the related filarioid of *Setaria* is reported as having n=6 [57], the fusion could have occurred in the onchocercine lineage.

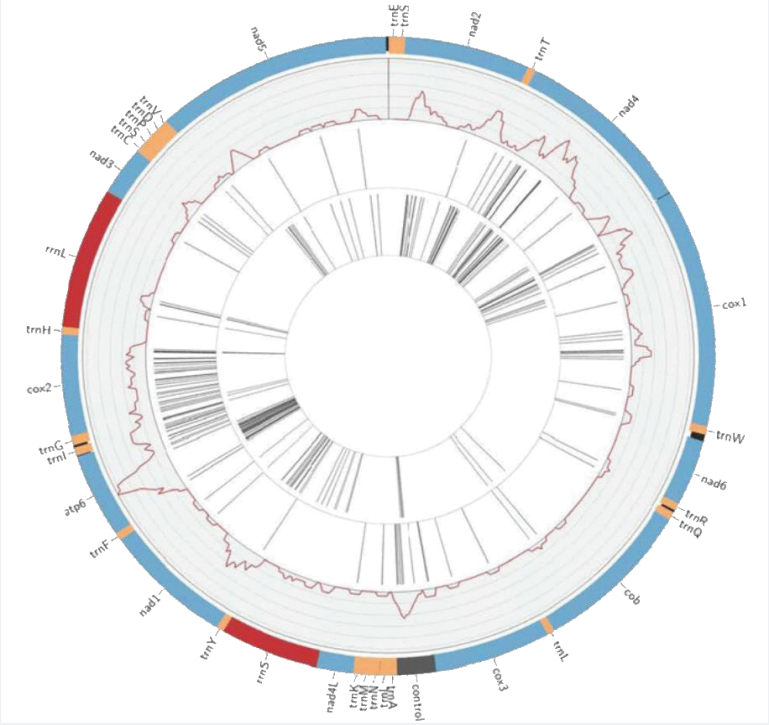

Figure 4: Circos constructed *W. bancrofti* mt genome showing protein coding sequences regions (blue), tRNAs (orange), rRNAs (red), AT control region represented in grey, and intergenic regions are black adopted from Ramesh, et al. [54].

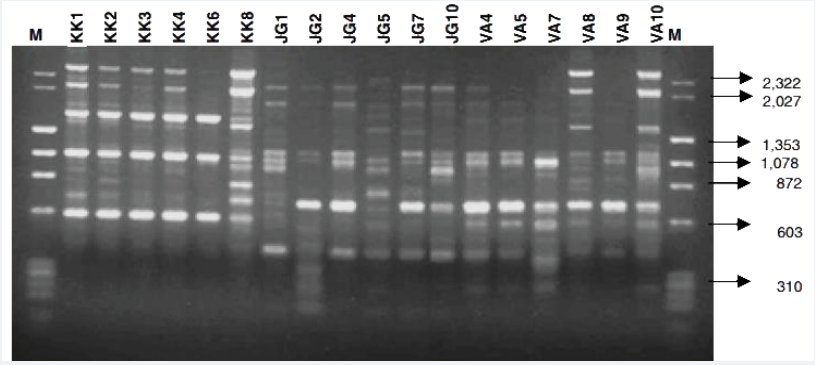

Figure 5: RAPD Profile of isolates of *W. bancrofti* from different Indian regions using Operan-08 RAPD primer (λDNA/*Hin*dIII and ΦX174/*Hae*III digest) adopted from Patra, et al. [55].

Conclusion

Lymphatic filariasis affects about 1.3 billion people in 72 countries worldwide. The major parasitic agents of the infection are three closely related nematodes of clade *Onchocercidae* namely *Wuchereria bancrofti, Brugia malayi* and *B. timori*. Chemically synthesized drugs are known to be used as therapeutic agents to manage the disease but with some setbacks such as carcinogenic impurities and adverse side effects. Thus prompting the current observed challenges encountered in its therapeutic management. Phytochemical extracts on the other hand, have proved to be effective against the filarial parasites. Again, the parasites species have many ecological variants that are almost similar to one another and to related taxa. We believe that understanding the genomic diversity of the parasite will help in efficient therapeutic management of the disease, thereby eliminating it to prevent unnecessary suffering and contribute to the reduction of poverty.

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