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REVIEW

Natural-Product-Based Solutions for Tropical Infectious Diseases

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SUMMARY About half of the world's population and 80% of the world's biodiversity can be found in the tropics. Many diseases are specific to the tropics, with at least 41 diseases caused by endemic bacteria, viruses, parasites, and fungi. Such diseases are of increasing concern, as the geographic range of tropical diseases is expanding due to climate change, urbanization, change in agricultural practices, deforestation, and loss of biodiversity. While traditional medicines have been used for centuries in the treatment of tropical diseases, the active natural compounds within these medicines remain largely unknown. In this review, we describe infectious diseases specific to the tropics, including their causative pathogens, modes of transmission, recent major outbreaks, and geographic locations. We further review current treatments for these tropical diseases, carefully consider the biodiscovery potential of the tropical biome, and discuss a range of technologies being used for drug development from natural resources. We provide a list of natural products

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with antimicrobial activity, detailing the source organisms and their effectiveness as treatment. We discuss how technological advancements, such as next-generation sequencing, are driving high-throughput natural product screening pipelines to identify compounds with therapeutic properties. This review demonstrates the impact natural products from the vast tropical biome have in the treatment of tropical infectious diseases and how high-throughput technical capacity will accelerate this discovery process.

KEYWORDS drug development, infectious disease, microbiology, natural products, tropics

INTRODUCTION

The tropics occupy a large area of the Earth's landmass from the Tropic of Cancer to the Tropic of Capricorn (Fig. 1). Tropical diseases are caused by a wide variety of pathogens, including bacteria, viruses, parasites, and fungi, that spread through various modes of transmission. The WHO defines 41 different tropical diseases, of which 21 are classified as neglected tropical diseases (NTDs) (Table 1). Traditional medicines have been used for centuries for the treatment of tropical diseases (1). Products of plants such as *Cinchona* and *Artemisia* are effectively used even today for the treatment of malaria (2). Plants are a promising source of traditional medicines, as many plants are safe with few side effects even when taken orally for prolonged periods. The long history of screening plant species by humans over millennia has led to deep-rooted knowledge of many plants that are beneficial when used correctly. Plants are also affordable and generally do not require cold-chain storage (3). The WHO has established its Traditional Medicine Strategy, which has guidelines for the assessment of herbal medicines (4). However, the active compounds of many such medicines have not been identified. Despite the encouraging identification of the neuropathic pain drug ω-conotoxin from the marine snail *Conus magus* in 1999 (5), the majority of plant and animal products have not yet been systematically investigated.

Natural products often possess a high degree of bioavailability in comparison to their synthetic counterparts (6). Therefore, it is surprising that not more natural product-based drug candidates have been identified. It is important to reflect upon this, given the recent technical advances used for the screening of natural products. Typically, it takes about 10 years and US$300 million to US$500 million in research and development (R&D)

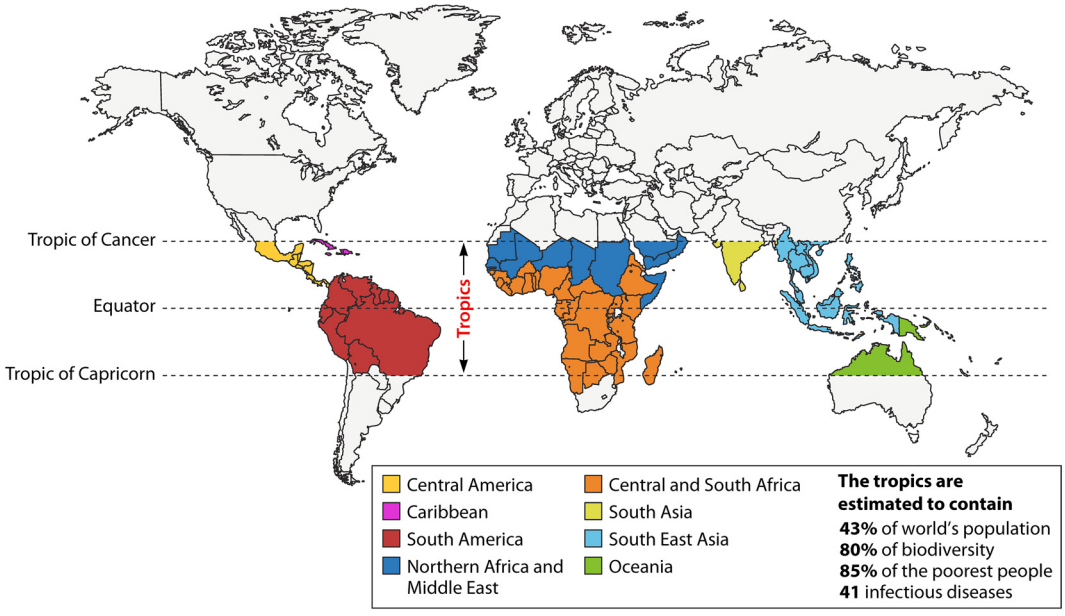

FIG 1 World map showing tropical regions. The geographical area between the Tropic of Cancer and the Tropic of Capricorn defines the tropics and occupies a large area of the Earth's landmass and oceans. The tropics span 5 continents and are home to 43% of the world's population, 80% of the biodiversity, 85% of the poorest people, and 41 infectious diseases. (Adapted from reference 243 with permission of the publisher.)

TABLE 1 Tropical infectious diseases

Organism type	Disease	NTD[a]	Pathogen(s)	Transmission	Location(s) of ongoing/recent major outbreaks[b]	Reference
Bacterium	Buruli ulcer (Bairnsdale ulcer, Daintree ulcer)	*	*Mycobacterium ulcerans*	Unclear (evidence for cutaneous contamination from infected aquatic insects, *Naucoris* spp. and *Dyplonychus* spp., and bite of infected mosquitoes)	NA	27
	Cholera		*Vibrio cholerae*	Ingestion of contaminated food/water	2018: Algeria, Niger, Zimbabwe 2017: DRC, Kenya, Mozambique, Somalia, Zambia 2016: Yemen 2015: DRC, Iraq, Tanzania	15
	Leprosy (Hansen's disease)	*	*Mycobacterium leprae*	Unclear (evidence for contamination through skin-to-skin contact with an infected individual and inhalation of contaminated droplets)	NA	26
	Melioidosis		*Burkholderia pseudomallei*	Ingestion, inhalation of contaminated dust/water Contact with contaminated soil	NA	244
	Mycetoma (actinomycetoma)	*	*Streptomyces somaliensis*, *Nocardia brasiliensis*, *Nocardia otitidiscaviarum*, *Actinomadura madurae*, *Actinomadura pelletieri*, *Pleurostomophora ochracea*	Contact of epithelia with contaminated soil or water	NA	245
	Trachoma	*	*Chlamydia trachomatis*	Direct or indirect (shared towels and clothes, flies) contact with eye or nose discharge of an infected individual	NA	246
	Tuberculosis		*Mycobacterium tuberculosis*	Inhalation of contaminated droplets	NA	12
	Yaws	*	*Treponema pallidum pertenue*	Skin-to-skin contact with an infected individual	2019: Ghana 2017: Cameroon	NA
	African trypanosomiasis (African sleeping sickness)	*	*Trypanosoma brucei gambiense*, *Trypanosoma brucei rhodesiense*	Bite of an infected tsetse fly (Glossina spp.)	NA	22
	American trypanosomiasis (Chagas disease)	*	*Trypanosoma cruzi*	Bite of infected triatomine bug (*Triatoma* spp., *Rhodnius* spp.) Ingestion of contaminated food *In utero* transmission	NA	19
	Dracunculiasis (Guinea worm disease)	*	*Dracunculus medinensis*	Ingestion of water contaminated with infected copepods	2020: Ethiopia 2019: Angola, Cameroon, Chad 2018: Angola, Chad, RSS 2017: Chad, Ethiopia 2016: Chad, Ethiopia, RSS 2015: Chad, Ethiopia, Mali, RSS	NA
	Echinococcosis	*	*Echinococcus granulosus*, *E. multilocularis*, *E. vogeli*, *E. oligarthrus*	Ingestion of contaminated food/water/soil	NA	16
Parasite	Foodborne trematodiases	*	Liver flukes (*Clonorchis sinensis*, *Fasciola gigantica*, *F. hepatica*, *Opisthorchis felineus*, *O. viverrini*) Lung flukes (*Paragonimus* spp.) Intestinal flukes (*Echinostoma* spp., *Fasciolopsis buski*)	Ingestion of contaminated food	NA	247

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TABLE 1 (Continued)

Organism type	Disease	NTD[a]	Pathogen(s)	Transmission	Location(s) of ongoing/recent major outbreaks[b]	Reference
	Leishmaniasis	*	*Leishmania* spp.	Bite of an infected sand fly (*Phlebotomus* spp., *Lutzomyia* spp.)	2019: Kenya 2018: Libya 2017: Kenya	248
	Lymphatic filariasis	*	*Wuchereria bancrofti*, *Brugia malayi*, *B. timori*	Bite of an infected female mosquito (*Anopheles* spp.)	NA	NA
	Malaria		*Plasmodium falciparum*, *P. vivax*, *P. ovale*, *P. knowlesi*, *P. malariae*	Bite of an infected mosquito (*Anopheles* spp., *Culex* spp., *Aedes* spp., *Mansonia* spp.)	2020: Vanuatu, Zimbabwe 2019: Burundi (249), Sudan 2018: Brazil 2017: Cape Verde, Costa Rica	20
	Onchocerciasis	*	*Onchocerca volvulus*	Bite of an infected black fly (*Simulium* spp.)	NA	NA
	Scabies	*	*Sarcoptes scabiei* var. hominis	Skin-to-skin contact with an infected individual	NA	13
	Schistosomiasis	*	*Schistosoma mansoni*, *S. japonicum*, *S. mekongi*, *S. guineensis*, *S. haematobium*	Contact with contaminated water	NA	250
	Soil-transmitted helminth (STH) infections	*	*Ancylostoma duodenale*, *Necator americanus*, *Ascaris lumbricoides*, *Trichuris trichiura*	Contact with contaminated soil	NA	NA
	Strongyloidiasis		*Strongyloides stercoralis*	Contact with contaminated soil	NA	251
	Taeniasis, cysticercosis	*	*Taenia solium*, *T. saginita*, *T. asiatica*	Ingestion of raw or undercooked infected beef or pork meat	NA	252
Virus	Chikungunya	*	Chikungunya virus (CHIKV)	Bite of an infected female mosquito (*Aedes* spp.)	2019: DRC 2018: Sudan 2017: Italy, Kenya, France 2016: Argentina, Kenya 2015: Senegal	21
	Crimean-Congo hemorrhagic fever		Crimean-Congo hemorrhagic fever virus (CCHFV)	Bite of an infected tick (*Hyalomma* spp.) Contact with body fluids/tissues of infected livestock Contact with body fluids of an infected individual	2020: Mali	17
	Dengue	*	Dengue virus (DENV)	Bite of an infected female mosquito (*Aedes aegypti*)	2020: Chile, Costa Rica, Indonesia, Peru, Singapore 2019: Afghanistan, Bangladesh (253), French Polynesia (254), Jamaica, Mayotte, Pakistan, Sudan 2018: Réunion Island 2017: Burkina Faso, Côte d'Ivoire, Sri Lanka, Sudan 2016: Uruguay 2015: Egypt	18

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TABLE 1 (Continued)

Organism type	Disease	NTD[a]	Pathogen(s)	Transmission	Location(s) of ongoing/recent major outbreaks[b]	Reference
	Ebola hemorrhagic fever		Ebola virus (EBOV)	Contact with an infected animal (e.g., fruit bat or nonhuman primate) Contact with body fluids of an EBOV-infected individual or dead body Contact with contaminated objects (e.g., clothes, bedding, needles, and medical equipment) Sexual transmission from semen of men who have recovered from EBOV infection	2020: DRC 2019: Uganda 2018: DRC 2017: DRC 2013–2016: West Africa[c]	14
	Japanese encephalitis		Japanese encephalitis virus (JEV)	Bites of an infected mosquito (*Aedes* spp., *Anopheles* spp., *Culex* spp., *Mansonia* spp.)	NA	255
	Lassa fever		Lassa virus	Contact with urine or feces of *Mastomys natalensis* rats (handling rats, eating contaminated food, touching contaminated household items, transepithelial contamination) Contact with body fluids of an infected individual	2020: Nigeria 2019: Nigeria 2018: Nigeria 2017: Nigeria 2016: Benin, Nigeria	NA
	Marburg hemorrhagic fever		Marburg virus (MARV)	Contact with an infected fruit bat (*Rousettus aegyptiacus*) Contact with body fluids of an infected individual	2017: Uganda	256
	Rabies	*	Rabies virus	Transcutaneous contamination with saliva of infected animals (e.g., bats, dogs)	2016: Bhutan (257)	258
	Rift Valley fever		Rift Valley fever virus (RVFV)	Bite of an infected mosquito (*Anopheles* spp., *Culex* spp., *Aedes* spp., *Mansonia* spp.) Contact with body fluids/tissues of infected livestock	2018: Kenya, Mayotte 2016: Niger 2015: Mauritania (259)	260
	Tick-borne encephalitis		Tick-borne encephalitis virus (TBEV)	Bite of an infected tick (*Ixodidae* spp.) Ingestion of raw milk from infected goats/sheep/cows	NA	261
	West Nile fever		West Nile virus (WNV)	Bites of an infected mosquito (*Culex* spp.) Contact with body fluids/tissues of infected animals *In utero* transmission	NA	262
	Yellow fever		Yellow fever virus (YFV)	Bite of an infected female mosquito (*Aedes* spp., *Haemogogus* spp.)	2020: Ethiopia, Uganda 2019: Mali 2018: Ethiopia, RSS 2017: Brazil, Nigeria 2016: Angola, Brazil, DRC, Uganda	263
	Zika		Zika virus (ZIKV)	Bite of an infected female mosquito (*Aedes* spp.) *In utero* transmission Contact with genital fluids of an infected individual	2015: Region of the Americas and others[d]	23
Fungus	Chromoblastomycosis	*	*Phialophora verrucosa*, *Fonsecaea pedrosoi*, *F. compacta*, *Cladophialophora carrionii*, *Rhinocladiella aquaspersa*	Unclear (evidence for transcutaneous contamination)	NA	264
	Lobomycosis (lacaziosis)		*Lacazia loboi*	Unclear (evidence for transcutaneous contamination)	NA	25
	Paracoccidioidomycosis		*Paracoccidioides brasiliensis*	Inhalation of spores	2015: Brazil (35)	265

(Continued on next page)

TABLE 1 (Continued)

Organism type	Disease	NTD[a]	Pathogen(s)	Transmission	Location(s) of ongoing/recent major outbreaks[b]	Reference
	Mycetoma (eumycetoma)	*	*Madurella mycetomatis*, *Curvularia lunata*, *Falciformispora senegalensis*, *Falciformispora thompkinsii*, *Trematosphaeria grisea*, *Exophiala jeanselmei*, *Medicopsis romeroi*, *Acremonium* spp., *Fusarium* spp., *Neotestudina rosatii*, *Aspergillus nidulans*, *A. flavus*, *Microsporum ferrugineum*, *M. audouinii*, *M. langeronii*, *Scedosporium apiospermum*, *S. boydii*	Contact of epithelia with contaminated soil or water	NA	245
	Rhinosporidiosis		*Rhinosporidium seeberi*	Unclear (evidence for transepithelial contamination following contact with stagnant water)	NA	24
	Sporotrichosis		*Sporothrix schenckii*	Transcutaneous inoculation from contaminated plant matter and infected cats Inhalation or ingestion of spores	NA	266
	Talaromycosis (penicilliosis marneffei)		*Talaromyces marneffei*	Transcutaneous inoculation from contaminated plant matter Inhalation or ingestion of spores	NA	267

[a]NTD, neglected tropical disease (indicated by asterisk).

[b]Recent and ongoing major outbreaks reported at https://www.cdc.gov, https://www.afro.who.int (WHO Regional Office for Africa), https://www.emro.who.int (WHO Regional Office for the Eastern Mediterranean), https://www.paho.org (WHO Regional Office for the Americas), and https://www.who.int, unless otherwise stated, over the last 5 years. The date indicates the start of the outbreak. DRC, Democratic Republic of the Congo; RSS, Republic of South Sudan; NA, not applicable.

[c]West Africa includes Guinea, Liberia, Mali, Nigeria, Senegal, and Sierra Leone.

[d]Region of the Americas and others includes Bolivia, Brazil, Cape Verde, Caribbean Islands (Aruba, Barbados, Bonaire, Cuba, Curaçao, Dominica, Dominican Republic, Grenadines, Guadeloupe, Haiti, Jamaica, Martinique Island, Puerto Rico, Saint Lucia, Saint Martin, Saint Vincent, Trinidad and Tobago, U.S. Virgin Islands), Chile, Colombia, Costa Rica, Ecuador, El Salvador, French Guiana, Guatemala, Guyana, Honduras, Maldives, Mexico, Nicaragua, Panama, Paraguay, Peru, Suriname, Venezuela, and Vietnam.

expenditure for a new product to be released into the market (7). Therefore, many pharmaceutical companies are unenthusiastic about developing drugs for tropical diseases that are primarily targeted to emerging economies of low- and middle-income countries. However, recent technological advances have made the process time efficient and cost-effective, providing an unprecedented opportunity for researchers and pharmaceutical companies to identify novel bioactive leads for commercialization (6, 8). About 50% of known plant species are thought to originate in the tropics, and one-third of those used in R&D are found in rainforests; therefore, an important opportunity also awaits developing economies of the tropics. With increasing pressure from climate change and deforestation on biodiversity, it is important for developing nations to consider both the protection of intellectual property rights of traditional knowledge holders and the overall conservation and sustainable use of medicinal plants (6). Pragmatic ways that provide access to modern health care while incorporating these considerations are urgently needed.

In this review, we describe tropical infectious diseases, the pathogens causing them, their modes of transmission, recent major outbreaks, and their geographic locations. We further detail current preventative and therapeutic treatments for tropical diseases, including any commercially licensed vaccines and promising vaccine candidates under investigation. We discuss a range of new technologies that are used for natural product discovery and drug development from natural resources focusing on high-throughput screens (HTS) and omics technologies. Finally, we discuss both approved natural products and molecules used to treat tropical diseases and additional natural products possessing antimicrobial activity with treatment potential. We hope our review will revitalize interest in natural products and drug discovery and encourage more researchers and companies to utilize recent technological advancements made.

INFECTIOUS DISEASES OF THE TROPICS

The WHO defines tropical diseases as all diseases that occur solely or principally in the tropics (Fig. 1) (9). However, this umbrella term often also includes any infectious disease that occurs in hot and humid climate. Tropical diseases are an enormous public health burden, with an estimated 1 billion people affected by at least one tropical disease, representing a significant impact on the health of people living in the tropical and subtropical regions of the world (10) (Fig. 1). Tropical diseases, including neglected tropical diseases (NTDs), are caused by a wide variety of pathogens, including bacteria, viruses, fungi, and parasites (Table 1). NTDs receive less attention from the scientific community and stakeholders of the developed countries than other tropical diseases (11). To address this shortcoming, multiple local and global nonprofit organizations such as Mission to Save the Helpless (MITOSATH, Nigeria) and the Drugs for Neglected Diseases initiative (DNDi, Switzerland) have been established to improve the health and enhance the quality of life of people affected by NTDs. The tropical diseases encompassed by these definitions are changing, with the WHO recently updating their NTD portfolio to include mycetoma, chromoblastomycosis, and scabies (69th World Health Assembly, 2016; 10th Meeting of the Strategic and Technical Advisory Group for Neglected Tropical Diseases, 2017).

Tropical diseases spread through various modes of transmission (Table 1). They can be transmitted via direct or indirect contact with infected individuals through bodily fluids or surfaces (e.g., yaws, scabies, Ebola), as well as by the inhalation of contaminated airborne droplets (e.g., tuberculosis [TB]) (12–14). Transmission may also occur by ingesting contaminated food and/or water (e.g., cholera and echinococcosis) in unsanitary environments, which persist in many tropical and subtropical countries today (15, 16). Many viral and parasitic tropical diseases are vector-borne, with transmission occurring through the bite of infected vectors, including hemipterans (Chagas disease), flies (e.g., African trypanosomiasis, leishmaniasis, onchocerciasis), mosquitoes (e.g., lymphatic filariasis, malaria, chikungunya, and dengue) and ticks (Crimean-Congo hemorrhagic fever) (17–22), among others. *In utero* transmission has also been reported for tropical diseases such as Zika virus and Chagas disease (19, 23). While the transmission mode is known for most tropical diseases,

it remains unknown for Buruli ulcer and leprosy (both listed in the WHO NTD portfolio) as well as for some fungal infections, including chromoblastomycosis, lobomycosis, and rhinosporidiosis (24–27).

Many tropical diseases have recently been or are currently responsible for major outbreaks (e.g., dracunculiasis, leishmaniasis, malaria, chikungunya, dengue, Ebola, yellow fever, and paracoccidioidomycosis) (Table 1). Although most of these outbreaks occur within the tropics, some have occurred in countries with more temperate climates. For example, France and Italy have reported outbreaks of autochthonous chikungunya in 2015 and 2016, respectively (28, 29). Similarly, locally acquired cases of Crimean-Congo hemorrhagic fever were reported in Spain in 2016 and 2018 (30). More recently, in 2019, France reported its first locally acquired case of Zika virus, which is also believed to be the first case recorded in Europe (31). While the number of total cases in each instance was relatively small (8 chikungunya cases in France, 436 chikungunya cases in Italy, 2 Crimean-Congo hemorrhagic fever cases in Spain, and 3 Zika cases in France), it illustrated the potential for such diseases in temperate climates. Such outbreaks outside the tropics highlight the potential risk for tropical diseases to spread globally; of particular concern are some of the vector-borne tropical diseases for which the competent vectors, including mosquitos, ticks, tsetse flies, and triatome bugs, are widely distributed around the world (28–32). Further, climate change, urbanization, change in agricultural practices, deforestation, and loss of biodiversity have all been implicated in increasing the potential spread of tropical diseases (33–36).

Immunization and treatment options differ widely across tropical diseases (Table 2). Currently, commercially licensed vaccines are available for only 8 of the 41 tropical diseases (cholera, TB, dengue, Ebola, Japanese encephalitis, rabies, tick-borne encephalitis, and yellow fever) (37–43), with licensing differing between countries. Many vaccine candidates are under investigation (Table 2) both for tropical diseases without any available licensed vaccine and for diseases with a current vaccine, such as TB. Indeed, the only available TB vaccine, bacillus Calmette-Guérin (*Mycobacterium bovis* BCG), provides partial protection in children but diminishes over time and is insufficient against pulmonary TB in adults (44). Although curative and/or symptomatic treatments are available for most tropical diseases, their practical efficacy remains challenged by a variety of technical, economic, and biological limitations (Table 2). With the exception of the WHO/UNICEF oral rehydration solution developed to treat cholera, the treatment of tropical diseases often relies on drugs that require strict storage conditions (45, 46). A cold chain is often unreliable or nonexistent for the tropical and subtropical regions, compromising the stability and treatment efficacy of the drugs. Additionally, the treatment of many tropical diseases may be negatively impacted by a lack of qualified health workers in the local community. For example, early intravenous injection is crucial in the treatment of many diseases (Table 2). Furthermore, access to treatment can also be impeded by the relatively high costs associated with effective drugs. For onchocerciasis and lymphatic filariasis, this economic hurdle has been overcome by the creation of the Mectizan (ivermectin) donation program (47). Finally, the global rise in antibiotic, antiparasitic, and antifungal resistance also represents a major threat to the successful treatment and management of tropical diseases (48). Unfortunately, for some tropical diseases such as dracunculiasis, lobomycosis, and rhinosporidiosis, there is currently no treatment or vaccine available, and physical extraction of the pathogens or surgical excision remains the only available option (Table 2). New treatment options are urgently needed, with discoveries from natural product platforms showing potential for the treatment and management of many tropical diseases.

NATURAL PRODUCTS AND BIODISCOVERY POTENTIAL OF THE TROPICAL BIOME

Broadly, natural products can be defined as any metabolites produced by living organisms that are largely obtained from plants, animals, and marine and microscopic organisms. Metabolites include primary and secondary metabolites. While primary metabolites such as proteins, carbohydrates, and fats are vital for the growth and

TABLE 2 Tropical infectious diseases: current treatments and vaccines[a]

Organism type	Disease	Current treatment(s)	Commercially licensed vaccine(s)[b]	Vaccine candidates under investigation [reference(s)]
Bacterium	Buruli ulcer (Bairnsdale ulcer, Daintree ulcer)	Antibiotics: rifampin, clarithromycin, streptomycin Symptomatic treatment: wound care, lymphedema management, skin grafting, physiotherapy (27) Disadvantages: Older patients may suffer from hearing loss, dizziness, and imbalance.	NA	e.g., MUL_3720 and Hsp18-based vaccines (268–271)
	Cholera	Moderate dehydration: oral administration of WHO/UNICEF oral rehydration solution Severe dehydration: intravenous administration of rehydration fluids plus antibiotic treatment Symptomatic treatment: zinc therapy for children <5 years (45, 46) Disadvantages: Antibiotics can cause nausea and vomiting and should not be given to patients with only some or no diarrhea.	Two types of licensed *Vibrio cholera* vaccines are commercially available: inactivated (Shanchol [Shantha Biotec]; Euvichol-Plus [Eubiologics]; Dukoral [SBL Vaccines]) and live attenuated (VaxChora [Emergent Biosolutions]) (37).	e.g., Dukoal, Shanechol, MORC-Vax (272)
	Leprosy (Hansen's disease)	Paucibacillary leprosy—antibiotics: rifampin, dapsone Multibacillary leprosy—antibiotics: rifampin, clofazimine, dapsone (273) Disadvantages: Antibiotics have to be taken for longer duration with follow up every 6 months for 10 years. Multidrug therapy does not provide cure in all cases of leprosy.	NA	e.g., Th1-biasing adjuvant formulation; glucopyranosyl lipid adjuvant in stable emulsion (GLA-SE, LepVax) (274–276)
	Melioidosis	Acute phase (10–14 days)—antibiotics: intravenous administration of ceftazidime or meropenem Elimination phase (3–6 months)—antibiotics: oral administration of SMX-THT or amoxicillin-clavulanic acid (277, 278) Disadvantages: Single-drug antibiotic therapy is only partially effective. Combined antibiotic therapy must be used for extended periods. SMX-THT resistance reported in Thai isolates.	NA	e.g., *purN* mutant (Δ*purN*) (279, 280)
	Mycetoma (actinomycetoma)	Antibiotics: amikacin, rifampin, SMX-THT, amoxicillin-clavulanate, imipenem, gentamicin, doxycycline (245) Disadvantages: Less effective and with many side effects, and the patients should be followed closely to assess them clinically and biochemically.	NA	e.g., epitope-based vaccine FFKEHGVPL (281, 282)
	Trachoma	Antibiotics: azithromycin, doxycycline, erythromycin, levoflaxin, ofloxacin Symptomatic treatment: surgery (246, 283) Disadvantages: It can take decades to evaluate the desired primary end point of trachoma treatment after the start of the intervention. Trials suggest merely a lowering of the risk, not a cure.	NA	e.g., subunit *Chlamydia* vaccine (*C. muridarum* recombinant MOMP [rMOMP], native trimeric conformation [nMOMP]) (284, 285)
	Tuberculosis	Antibiotic treatment of *M. tuberculosis* infection varies depending on infection form (i.e., active or latent infection), antibiotic resistance (i.e., drug-resistant or multidrug-resistant infection), infected individuals (e.g., pregnant women, children), and coinfection status (e.g., HIV infection). Isoniazid, rifampin, rifapentine, pyrazinamide, and ethambutol are some of the main antibiotics currently used (12) Disadvantages: Multidrug-resistant tuberculosis (MDR-TB) is resistant to both isoniazid (INH) and rifampin (RFP). These antibiotics have many side effects, including gastrointestinal disturbance, psychiatric disorder, arthralgia, dermatological effects, ototoxicity, nephrotoxicity, peripheral neuropathy, hypothyroidism, and epileptic seizures.	The BCG vaccine (live attenuated *Mycobacterium bovis* strain) is the only commercially licensed TB vaccine.	e.g., protein-subunit vaccine M72/AS01$_E$, live attenuated VPM1002, MTBVAC (12, 44, 286, 287)

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TABLE 2 (Continued)

Organism type	Disease	Current treatment(s)	Commercially licensed vaccine(s)[b]	Vaccine candidates under investigation [reference(s)]
	Yaws	Antibiotic: azithromycin Alternative antibiotics: benzathine penicillin, doxycycline (288) Disadvantages: Painful during deep i.m. injection of antibiotics, allergy to penicillin, structural and logistic problems related to treatment.	NA	Single-dose azithromycin for the treatment of yaws. (NIH, U.S. National Library of Medicine, ClinicalTrials.gov.)
Parasite	African trypanosomiasis (African sleeping sickness)	*T. brucei gambiense*—antiparasitics: pentamidine, eflornithine, NECT, melarsoprol, fexinidazole *T. brucei rhodesiense*—antiparasitics: suramin, melarsoprol (289) Disadvantages: Very toxic, prevalence in impoverished regions of Africa places economic constraints, small number of expensive drugs with limited efficacy and serious side effects and which are difficult to administer.	NA	e.g., invariant surface glycoproteins (ISGs), conserved variant surface glycoprotein (VSG) (290, 291)
	American trypanosomiasis (Chagas disease)	Antiparasitics: benznidazole, nifurtimox (292) Disadvantages: Significant side effects, efficacy decreases with length of the infection; treatment success difficult to measure; can take years before patients become seronegative (average, 16 years).	NA	e.g., recombinant proteins (Tc24, TSA-1 with Th1 adjuvant) (293, 294)
	Dracunculiasis (Guinea worm disease)	No commercially licensed antiparasitic drug to treat *Dracunculus medinensis* infection Physical extraction	NA	NA
	Echinococcosis	Antiparasitics: mebendazole, albendazole, praziquantel Symptomatic treatment: surgery or PAIR (percutaneous aspiration, injection of chemicals, and reaspiration) (295) Disadvantages: Gold standard methods to determine efficacy of medical treatment, biological status, effective dose not available. No standardized diagnostic and monitoring methods for long-term follow-up. Treatment outcomes improve when surgery is combined with drugs; timing of chemotherapy pre/postsurgery unclear.	NA	e.g., epitope-based vaccine (A5YTY7, A0A068WVL6) (296)
	Foodborne trematodiases	Antiparasitics: pranziquantel, triclabendazole, nitazoxanide Disadvantages: Few drugs are available, and therefore potential for emerging drug resistance is high. Reliable tests to detect parasites are not available; potential for misdiagnosis and incorrect treatment is high.	NA	e.g., recombinant Ov-TSP-2 and −3, *C. sinensis* CsTP 22.3 kDa (297, 298)
	Leishmaniasis	Antiparasitics: sodium stibogluconate, pentavalent antimonials, amphotericin B, paromomycin, miltefosine Disadvantages: 60% of patients unresponsive, drug resistance common, combination therapy required, intramuscular or intravenous injections per day for 20–28 days lead to toxicity, drug efficacy compromised due to parenteral route of administration.	NA	e.g., ChAd63-KH (299, 300)
	Lymphatic filariasis	Antiparasitics: diethylcarbamazine, ivermectin, albendazole, doxycycline Disadvantages: Temporarily clear microfilariae but not adult worms; where filariasis coexists with *Loa loa*, neurologic decline and encephalopathy are causes for concern.	NA	e.g., thioredoxin peroxidase (TPX), collagen 4 (Col4) (301–303)
	Malaria	Antiparasitic treatment of *Plasmodium* species infection varies depending on two main factors: severity status (i.e., uncomplicated, severe, cerebral) and parasite species. Atovaquone and proguanil, artemether and lumefantrine, quinine sulfate and doxycycline, mefloquine, chloroquine phosphate, primaquine phosphate, and hydroxychloroquine are some of the main antiparasitics currently used (304).	N/A	e.g., PfSPZ vaccine, chimpanzee adenovirus serotype 63 (ChAd63) (305)

(Continued on next page)

TABLE 2 (Continued)

Organism type	Disease	Current treatment(s)	Commercially licensed vaccine(s)[b]	Vaccine candidates under investigation [reference(s)]
		Disadvantages: Rampant drug resistance, questionable safety of antimalarials, side effects such as headache, dyspepsia, diarrhea, etc. Limited data are available on their efficacy in treatment of drug-resistant and non-*falciparum* strains. Difficult to achieve required drug concentration in infants.		
	Onchocerciasis (river blindness)	Antiparasitics: ivermectin, moxidectin (306) Disadvantages: Questions remain if drugs can eliminate disease in areas of very high endemicity and loiasis coendemicity, due to severe reactions in people with *Loa loa* microfilaremia. Drug-resistant parasites are emerging following many years of treatment. Safe dose in children not determined.	NA	e.g., recombinant proteins—Ov-103 and Ov-RAL-2 (307)
	Scabies	Antiparasitics: ivermectin, permethrin, crotamiton (13) Disadvantages: Neurotoxicity has been reported in children with widespread skin damage. Potential for emergence of drug resistance. Harmful effects on health and environment. Reinfection and recrudescence are common.	NA	e.g., recombinant *Sarcoptes scabiei* chitinase-like protein 5 (rSsCLP5-based) vaccine (308, 309)
	Schistosomiasis	Antiparasitic: praziquantel (250) Disadvantages: Treatment does not prevent transmission or reinfection in areas of endemicity, as it is ineffective against juvenile parasites; prevalence will decrease only if more than 70% of the community participates; growing concerns regarding resistance, chemical residues, and cost.	NA	e.g., recombinant Sh28GST/Alhydrogel (310–313)
	STH infections	Antiparasitic: albendazole or mebendazole Disadvantages: Increasing drug resistance, treatment often followed by rapid reinfection.	NA	e.g., rAc-MTP-1, rAc-16 (314, 315)
	Strongyloidiasis	Antiparasitic: ivermectin Disadvantages: Development of drug resistance as parasite remains in the body for a long time, lack of standardization of antihelmintic treatment, toxicity, no test to detect cure currently available.	NA	e.g., DNA immunization (Sseat-6 gene), Ss-IR (*S. stercoralis* immune-reactive antigen), srHSP60 (316)
	Taeniasis, cysticercosis	Taeniasis antiparasitics: praziquantel, niclosamide Cysticercosis antiparasitics: praziquantel, albendazole Symptomatic treatment: corticosteroids, antiepileptic drugs (neurocysticercosis), surgical extraction (depending on localization of the cysts) (317) Disadvantages: Death of the parasite between the 2nd and 5th day of treatment triggers neurological symptoms and, rarely, can be fatal. Side effects of praziquantel include malaise, headache, dizziness, nausea, fever, bloody diarrhea, etc. Side effects of albendazole include hepatotoxicity, alopecia, headache, nausea, urticaria.	NA	e.g., recombinant vaccines (TSOL18 and TSOL45) (318, 319)
Virus	Chikungunya	No commercially licensed antiviral drug to treat CHIKV infection Symptomatic treatment: rest, prevention of dehydration, administration of pain relief drugs (acetaminophen or paracetamol) to reduce fever and relieve some symptoms. Aspirin and other nonsteroidal anti-inflammatory drugs can be administered once DENV infection is ruled out (21). Disadvantages: Long-term pain management required for some with recurring joint pain in 20% of patients after 1 year.	NA	e.g., live attenuated vaccine (TSI-GSD-218), live recombinant vaccine (MV-CHIKV), virus-like-particle vaccine (VRC-CHKVLP059-00-VP) (320, 321)

(Continued on next page)

TABLE 2 (Continued)

Organism type	Disease	Current treatment(s)	Commercially licensed vaccine(s)[b]	Vaccine candidates under investigation [reference(s)]
	Crimean-Congo hemorrhagic fever	No commercially licensed antiviral drug to treat CCHFV infection Symptomatic treatment: intravenous fluids and electrolyte supplementation, oxygen therapy, coinfection treatment (17) Disadvantages: Requires high-level isolation facilities with proper biocontainment procedures.	NA	e.g., CCHFV Bulgarian vaccine, CCHFV DNA vaccine (322, 323)
	Dengue	No commercially licensed antiviral drug to treat DENV infection Mild infection: treatment of symptoms with pain relief drugs (acetaminophen or paracetamol) to reduce fever and relieve some symptoms Severe infection: supportive hospital therapy (18) Disadvantages: For cases progressing to dengue hemorrhagic fever, patient requires hospitalization and extensive monitoring (recommended 4-h checks) during onset of critical phase. Vaccine requires strict cold-chain storage.	A licensed live attenuated recombinant DENV vaccine is commercially available: CYD-TDV, Dengvaxia, Sanofi Pasteur (38).	e.g., tetravalent dengue vaccine (CYD-TDV), Sanofi Pasteur's Dengvaxia (38, 324)
	Ebola hemorrhagic fever	No commercially licensed antiviral drug to treat EBOV infection Symptomatic treatment: intravenous fluids and electrolyte supplementation, oxygen therapy, antiemetic drug treatment, antidiarrheal drug treatment, coinfection treatment (14) Disadvantages: Requires high-level isolation facilities with proper biocontainment procedures. Vaccine requires strict cold-chain storage.	A live attenuated recombinant licensed EBOV vaccine is commercially available: rVSV-ZEBOV, Ervebo, Merck.	e.g., inactivated EBOVΔVP3, Ad5.EBOV GP + Ad5.EBOV NP, Ad5.EBOVGPΔTM + Ad5.EBOV (325, 326)
	Japanese encephalitis	No commercially licensed antiviral drug to treat JEV infection Symptomatic infection: supportive hospital therapy, including neurological observation, intravenous fluids and electrolyte supplementation, administration of pain relief drugs to reduce fever and relieve some symptoms, rest (255) Disadvantages: Confirmation of suspected severe cases requires CT/MRI scans, spinal fluid extraction. Inactivated vaccines require 2 doses, and others require cold-chain storage.	Three types of JEV vaccines are commercially licensed: inactivated (Ixiaro, Valneva Austria GmbH; JE-VAX, Sanofi Pasteur), live attenuated (CD.JEVAX, CDIBP), and recombinant (IMOJEV, Sanofi Pasteur) (39).	NA
	Lassa fever	No commercially licensed antiviral drug to treat Lassa virus infection Symptomatic treatment: intravenous fluids and electrolyte supplementation, oxygen therapy, coinfection treatment Disadvantages: Hospitalization required in severe cases. Ribavirin used for treatment in early stages, but it is not available in many regions and is suspected to be toxic and teratogenic.	NA	e.g., ChAdOx1-Lassa-GP (327)
	Marburg hemorrhagic fever	No commercially licensed antiviral drug to treat MARV infection Symptomatic treatment: intravenous fluids and electrolyte supplementation, oxygen therapy, coinfection treatment (256) Disadvantages: Severity of disease require hospitalization in intensive care for all affected.	NA	e.g., inactivated MARV, VRO-MARV GP, VRO-MARV NP (325, 328, 329)
	Rabies	Postexposure prophylaxis (before symptom onset): extensive wound washing, immediate vaccination, and administration of rabies immunoglobulin (if classified as severe exposure) (258) Disadvantages: Vaccine is effective after exposure but not after development of symptoms. Virtually always fatal after symptoms develop.	A licensed inactivated rabies virus vaccine is commercially available: Rabipur/Rabipor/Rabavert, GSK; Imovax Rabies, Sanofi Pasteur (40, 41).	NA

(Continued on next page)

TABLE 2 (Continued)

Organism type	Disease	Current treatment(s)	Commercially licensed vaccine(s)[b]	Vaccine candidates under investigation [reference(s)]
	Rift Valley fever	No commercially licensed antiviral drug to treat RVFV infection Mild and short-duration infection: no specific treatment required, pain relief drugs can be used to reduce fever and relieve some symptoms Severe infections: supportive hospital therapy Disadvantages: Hospitalization required in severe cases, but treatment is generally limited to supportive care.	NA	e.g., TSI-GSD-200, TSI-GSD-223 (330)
	Tick-borne encephalitis	No commercially licensed antiviral drug to treat TBEV infection Symptomatic treatment to treat neurologic symptoms (261) Disadvantages: Severe cases require hospitalization, including tracheal intubation and respiratory support. Vaccines not widely available.	A licensed inactivated TBEV vaccine is commercially available: Encepur, GSK; TICOVAC/FSME-IMMUN, Pfizer; EnceVir, NPO Microgen (42).	NA
	West Nile fever	No commercially licensed antiviral drug to treat WNV infection Mild infection: no specific treatment required, pain relief drugs can be used to reduce fever and relieve some symptoms Severe infection: supportive hospital therapy (262) Disadvantages: Hospitalization required in severe cases and can require CT/MRI scans, spinal fluid extraction.	NA	e.g., Hydrovax-001, ChimaeriVax-WN02, rWN/DEN4Δ30 (331–334)
	Yellow fever	No commercially licensed antiviral drug to treat YFV infection Mild infection: rest, dehydration prevention by drinking, administration of pain relief drugs to reduce fever and relieve some symptoms Severe infection: supportive hospital therapy Disadvantages: Hospitalization required in severe cases; however, treatment is generally limited to supportive care. Vaccine requires cold-chain storage.	A licensed live attenuated YFV vaccine is commercially available: YF17D, YF-VAX/Stamaril, Sanofi Pasteur (43)	NA
	Zika	No commercially licensed antiviral drug to treat ZIKV infection Mild infection: rest, dehydration prevention by drinking, treatment of symptoms with pain relief drugs (acetaminophen or paracetamol) to reduce fever and relieve some symptoms Severe infection: supportive hospital therapy Disadvantages: Hospitalization required in severe cases. Pregnant women require monthly monitoring for fetal growth.	NA	e.g., DNA vaccines (VRC5283, VRC5288, GLS5700), mRNA vaccines (mRNA-1325, mRNA-1893) (335, 336)
Fungus	Chromoblastomycosis	Antifungals: itraconazole, thiabendazole, posaconazole, voriconazole, terbinafine, flucytosine, fluconazole, ketoconazole, amphotericin B Symptomatic treatment: heat treatment, cryotherapy, surgery (264) Disadvantages: Amphotericin B targets cholesterol-containing membranes, leading to cellular toxicity in humans. Side effects are significant, and therefore amphotericin B is used only for critically ill patients with serious fungal infections. Side effects of common antifungals include headaches, diarrhea, rash, nausea, and muscle or joint pains. Surgery is not usually recommended, as it is thought to facilitate spread of disease.	NA	DNA-hsp65 vaccine (337)

(Continued on next page)

TABLE 2 (Continued)

Organism type	Disease	Current treatment(s)	Commercially licensed vaccine(s)[b]	Vaccine candidates under investigation [reference(s)]
	Lobomycosis (lacaziosis)	No commercially licensed antifungal drug to treat *Lacazia loboi* infection No standard treatment is available to date; surgical excision and successful treatment protocols have been reported (25, 338, 339) Disadvantages: Recurrence is common after surgery due to contaminated tools or incomplete removal due to difficulty in demarcating the lesion site. Treatment with common antifungals including amphotericin B has been found to be inadequate.	NA	NA
	Mycetoma (eumycetoma)	Antifungals: ketoconazole, itraconazole, voriconazole, posaconazole, terbinafine Symptomatic treatment: surgery, amputation (245) Disadvantages: Long treatment course. Side effects of common antifungals include headaches, diarrhea, rash, nausea, and muscle or joint pains. Amputation causes significant loss of life quality.	NA	e.g., peptides KYLQ, FEYARKHAF, FFKEHGVPL (282)
	Paracoccidioidomycosis	Antifungals: itraconazole, amphotericin B, SMX-THT Supportive hospital therapy for severe infection (265) Disadvantages: Hospitalization required in severe cases. Side effects of common antifungals include headaches, diarrhea, rash, nausea, and muscle or joint pains.	NA	e.g., antigen gp43, peptide P10 (340–342)
	Rhinosporidiosis	Combination of surgical excision and supportive medical therapy (dapsone, amphotericin B) (24) Disadvantages: Recurrence is common after excision. Treatment with amphotericin B results in significant cellular toxicity (as described above). Disease can occasionally be resistant to dapsone and may require combination therapy with other drugs.	NA	NA
	Sporotrichosis	Oral administration of saturated solution of potassium iodide Antifungals: amphotericin B, itraconazole, terbinafine (266) Disadvantages: Potassium iodide treatment typically effective for skin infection only. Side effects of common antifungals include headaches, diarrhea, rash, nausea, and muscle or joint pains. Treatment with amphotericin B results in significant cellular toxicity (as described above).	NA	e.g., humanized antibody (MAbP6E7) (343)
	Talaromycosis (penicilliosis marneffei)	Antifungals: amphotericin B, itraconazole, voriconazole (267) Disadvantages: Side effects of antifungals include headaches, diarrhea, rash, nausea, and muscle or joint pains. Treatment with amphotericin B results in significant cellular toxicity (as described above).	NA	NA

[a]BCG, bacillus Calmette-Guérin; CCHFV: Crimean-Congo hemorrhagic fever virus; CHIKV, chikungunya virus; DENV, dengue virus; EBOV, Ebola virus; JEV, Japanese encephalitis virus; MARV, Marburg virus; NECT, nifurtimox-eflornithine combination therapy; RVFV, Rift valley fever virus; SMX-THT, sulfamethoxazole-trimethoprim (cotrimoxazole); STH, soil-transmitted helminth; TBEV, tick-borne encephalitis virus; WNV, West Nile virus; YFV, yellow fever virus; ZIKV, Zika virus; i.m., intramuscular; CT/MRI, computed tomography/magnetic resonance imaging; Th1, T helper 1; NA, not applicable.
[b]Licensing may vary between countries.

development of a living organism, secondary metabolites such as alkaloids, terpenoids, and flavonoids are responsible for its survival and defense against competitors and intruders (49). These natural products are being exploited and manipulated by humans for developing novel drugs (see Drug Development from Natural Resources and Therapeutic Solutions for Infectious Diseases of the Tropics, below). With an estimated 300,000 to 500,000 plant species and ca. 2 million lower classes of organisms, these resources are considered the chemotherapeutic pool, which can be exploited for developing drugs (50).

It is estimated that 50% of known plant species originate in the tropics (Fig. 1), with 14,000 species identified from the Amazon region alone (51). Similarly, the tropical Far North Queensland region of Australia is rich in rainforest (covering 3.6 million ha) and reef biomes, and its Wet Tropics World Heritage Area alone is home to over 2,800 plant species, including 700 endemic species that occur nowhere else on Earth (52). Approximately one-third of the medicinal plants used in the research and development of pharmaceutical drugs are found in rainforests (47). However, only a limited number of tropical plants and animals have been considered for medical uses and therefore provide an unprecedented opportunity for researchers and pharmaceutical companies to identify novel bioactive leads for potential commercialization.

Within the plant kingdom, the focus of pharmaceutical research has been on flowering plants, whereas mangroves and nonflowering plants, such as mosses, ferns, hornworts, cycads, liverworts, and lycopods, remain barely studied for drug development to date and represent an untapped source of novel compounds. Similarly, tropical lichens, fungi, insects, snails, reptiles, spiders, scorpions, and amphibians are not well characterized and are worthy of pharmaceutical exploration.

DRUG DEVELOPMENT FROM NATURAL RESOURCES

Developing drugs from natural sources is a lengthy and tedious endeavor. The biodiscovery pathways include specimen identification and collection, extraction and isolation, identification, and bioactivity testing (53). The most common challenge faced by researchers in translating laboratory discoveries to commercial drugs is access to sufficiently large quantities of biological samples and lead compounds, which is considered a "valley of death." This bottleneck could be overcome through strategic collaboration between chemists (with expertise in natural products and organic synthetic chemistry), biologists (with expertise in biological processes and sample collection), immunologists (with expertise in cell- and animal-based assays), and bioinformaticians (to develop discovery platforms using large-scale genome sequence mining and shotgun metagenomics) (54).

Strategies for Drug Development from Natural Resources

It is important to understand the existing techniques, technologies, expertise, and financial resources within the pharmaceutical field in order to devise an efficient drug discovery strategy (55). Several natural products containing compounds with activity against tropical disease-causing pathogens have been discovered. However, due to the high failure rate and the significant investment required to take a promising raw natural product forward, very few compounds have overcome the bottleneck toward becoming a new standard-of-care treatment for a tropical pathogen. Currently, the most common strategies used for discovering novel drugs from natural resources are (i) the random approach based on a "find and isolate" method, (ii) the biorational approach based on ecological and ethnobotanical methods, and (iii) the chemorational approach based on chemotaxonomical considerations (56) (Fig. 2). The last strategy uses information on plant-specific chemotypes, structural similarity, and reported bioactivities (57) to guide drug screening processes. Of these three strategies, the biorational approach, especially ethnobotany-guided screening, is the most efficient one. For example, 80% of 122 plant-derived drugs were discovered based on an ethnodirected biorational approach (58). This high hit rate of novel drugs or drug leads is mainly attributable to their extended clinical uses in traditional medicines.

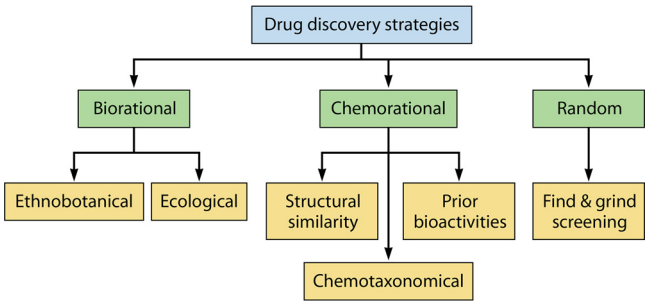

FIG 2 Strategies for searching for novel drugs from natural products. Common strategies for discovering novel drugs from natural resources include random, chemorational, and biorational approaches. The biorational approach relies on ethnobotanically focused screening and ecologically directed screening. The chemorational approach is directed by chemotaxonomical considerations. The random approach relies on high-throughput screening with no prior ethnopharmacological uses or chemotypical rationality.

Techniques for Drug Development from Natural Resources

A range of technologies spanning low to medium throughput has been available for decades, allowing the screening of viable pathogens responsible for tropical diseases. The bioassay screening protocols include *in vitro*, *ex vivo*, and *in vivo* models. For intracellular pathogens (e.g., *Trypanosoma cruzi* and *Plasmodium* spp.), cell-based screening methods adapted from conventional mammalian cell monitoring have been developed, such as the WST-1 assay (water-soluble tetrazolium) (59–61) or cell death monitoring with an array of fluorescent probes (62–65). While useful, these assays require careful consideration of the cell types used, as this choice can heavily influence the screening outcomes (66). For larger extracellular pathogens, particularly helminths, techniques are more challenging to develop. Nonetheless, a range of screening techniques have emerged over the past decade with various levels of scalability. These techniques include manual or automated video assessment (67), impedance motility monitoring (68, 69), enzymatic alamarBlue reduction (70), colorimetric (71), fluorescence (72, 73), and lactate or luminescent assays (74, 75).

Screening natural product libraries or raw products for their potential bioactive effect on pathogens can be a daunting task unless a high-throughput screen (HTS) can be developed for the target disease organism. Workflows that incorporate multiwell plates (e.g., 96 or 384 wells) can ideally be handled by robotics to allow for optimal HTS. Challenges arise when developing HTS for larger organisms, such as helminths. Often, the parasite life cycle stage that is key for treating clinical manifestation in humans is challenging to produce in sufficient quantities in the lab for adequate testing. Additionally, the physical size of the worm (millimeters to tens of centimeters long) can make large-scale handling (manual) of the parasite extremely difficult. Therefore, in many studies, the only feasible option for drug screening is to use analogues akin to the target macroscopic organism, such as easily available microscopic larval stages, or related microscopic model organisms, such as *Caenorhabditis elegans* (76, 77). While this allows simple HTS, the applicability to the desired target needs to be assessed appropriately. The limited applicability of these methods was recently highlighted in a study screening 1,280 compounds, in which neither the hookworm larva or *C. elegans* models demonstrated high fidelity as analogue models for detecting toxicity against the adult hookworm, the desired target that infects humans (78).

While the gold standard for evaluating antihelminth activity when screening drugs is visual phenotypic assessment of the parasite, the past decade has seen rapid advancement in adapting a range of HTS technologies (79). Some impedance-based methods based on either commercial cell monitoring products such as the xCELLigence system (ACEA Biosciences, Inc.) or custom "in-house" systems designed from the ground up for targeted purposes have been adapted and improved to allow antihelminth activity to be evaluated based on helminth mobility measurement (68, 80). While ultimately applicable to HTS, these methods so far have not been used for natural product screening beyond small research laboratory-based proof-of-principle studies, exploring 10 to 50 products at

a time (81–84). Additionally, HTS of drugs against macroscopic disease-causing agents has been taking advantage of the development of advanced automated microscopy (85, 86). The automated imaging of a 12- to 384-well plate(s) allows, for a reasonably low cost, assessment of pathogen viability, and therefore the drug screening can be performed by a simple visualization of the pathogen mobility.

While many of these HTS techniques are commonly used in research laboratories worldwide, pathogens that require biocontainment higher than biosafety level 2 (BSL-2) (e.g., *Mycobacterium tuberculosis*, rabies, Rift Valley fever virus [RVFV], and West Nile virus [WNV] require BSL-3; Ebola virus [EBOV], Lassa virus, and Marburg virus [MARV] require BSL-4) can be uniquely challenging, especially in the tropics, where the proportion of low-income countries remains relatively high. Advances in modern robotics have made possible the incorporation of such technologies up to BSL-4 biocontainment capacities, allowing HTS of drugs for a range of deadly pathogens, including EBOV (87). However, capability will always be limited, and extensive safety restrictions limit full incorporation of HTS methods. One alternative to phenotypic screening that bypasses the parasite supply or safety limitations is virtual drug design based on protein sequences (88–91); while still a technically challenging and expensive method, it is slowly becoming more readily available with increasing computing power coupled with a decrease in the cost of sequencing technologies.

Omics Technologies for Drug Development from Natural Resources

As critical as the search strategies are, the success of drug discovery and development also relies heavily on the successful adaptation of advanced technologies to the discovery platforms. In many countries, increasingly affordable technological innovation in the areas of genomics, metagenomics, proteomics, and metabolomics have revolutionized the drug discovery programs (53). While genomics-, transcriptomics- and proteomics-based approaches have been extensively used to better understand the biology of parasitic helminths and facilitate development of diagnostics and therapeutics, metabolomics-based approaches have been largely overlooked. High-throughput technologies and software need to be integrated to enable big data generation, mining, and interpretation of the results.

Genomics and metagenomics. Increasingly affordable sequencing technologies are changing how potential pharmaceutical drugs are being identified from natural products. While industrial investment in research programs aimed at discovering natural products suitable for pharmaceuticals has decreased in recent years (92), the use of next-generation sequencing technologies offers new screening pathways for targeted natural product discovery. Two sequencing applications in particular have the potential to revolutionize natural product discovery: large-scale genome sequence mining (93) and shotgun metagenomics (94). Large-scale genomic mining is a targeted approach, where the entire genome sequence from organisms of interest is interrogated in order to identify previously uncharacterized natural products. In contrast, shotgun metagenomics is an untargeted approach, where all sequences present in a community/environment are interrogated for novel natural products; however, with this approach, the organism of origin may not be known.

Many natural products have been discovered using genomics technologies. For example, genome mining of individual species led to the discovery of the novel polyphenolic polyketide antibiotic clostrubin from *Clostridium beijerinckii*, a strictly anaerobic bacterium (95), in addition to novel aminocoumarins from the uncommon actinomycete *Catenulispora acidiphila* DSM 44928 (96). To date, most sequence-based natural product discoveries have relied on individual genome sequences; however, the growth of high-quality, publicly available sequence data is enabling the simultaneous genome mining of thousands of species. For example, a recent study mined 10,000 actinomycetes in a search for novel phosphonic acids, an important class of natural products with known antimicrobial, antiviral, antimalarial, and herbicidal activities. This study identified a new archetypical pathway for phosphonate biosynthesis in addition to 11 previously undescribed phosphonic acid natural products (97). The authors propose

their methodology as a generalizable framework suitable for the rapid discovery of other natural product classes in order to discover lead compounds suitable for the pharmaceutical industry (97).

Functional metagenomics are also being used as screening tools for natural product discovery at both the species level (e.g., *Strepomyces* [98]) and in complex environment samples (e.g., marine [99]). These methods are becoming increasingly popular for accessing bacterially encoded secondary metabolites, as it gives access to products from the majority of bacteria that are not readily culturable. Shotgun metagenomic sequencing has several advantages in that it is unbiased and requires no species-specific lab-based preparation, but most critically, it allows access to all the organisms' collective genomes and thus provides a snapshot of the bioactive potential of entire bacterial populations in a single experiment. Additionally, the genetic information encoding the relevant biological activities are typically clustered on bacterial genomes, meaning that with limited starting material, it is possible to capture sequence describing the biological pathway of interest.

The last decade has seen an acceleration in the sequencing of microbial, fungal, and plant genomes, with tens of thousands of genomes now available in public archives, including GenBank and Ensembl. Despite the generation of this large volume of data, there exists a bottleneck in our ability to process and analyze these data in a meaningful way. In natural drug discovery, genome mining techniques have emerged as an approach to identify potential products of interest (100, 101), where secondary metabolites from biosynthetic gene clusters that encode novel bioactive metabolites are identified. In recent years, software to support genome mining have significantly matured for microbes and fungi. For example, AntiSMASH (antibiotics and secondary metabolite analysis shell) (102) uses computational methods to rapidly identify, annotate, and analyze secondary metabolite biosynthesis gene clusters identified in bacterial and fungal genomes. Many other software tools that exist are typically specific to either an organism group(s) or pathways. These include SMURF for fungal metabolites (103), BAGEL3 for prokaryotes (104), PRISM (Prediction Informatics for Secondary Metabolome) for microbial organisms (105), IMG/ABC for storing experimentally validated BCGs (106), and ASMPKS for predicting modular polyketide synthases (107). While progress has been made in regard to microbes and fungal genomes, tools available for plant-based drug discoveries are significantly lagging.

Proteomics. Over the last few decades, numerous proteomic approaches have been developed and applied to facilitate the process of identifying protein and small molecule drug candidates. Typically, bioactivity or phenotype-based drug discovery involves the development and execution of bioassay screens to guide the isolation of the active fraction leading to the eventual identification of the active compounds (108, 109). Mass spectrometry, with its ability to identify small molecules and proteins through their fragment peptides, is an integral step in both proteomics and metabolomics (see "Metabolomics," below) and has been used to characterize small molecules and natural products since the 1960s (110).

Recent improvements have been made in both the utility and sensitivity of mass spectrometers (111). An example of this progress is the recently released high-performance mass spectrometer Orbitrap Fusion Lumos Tribrid. With a resolution of up to 1,000,000 full width at half maximum (FWHM) values at *m/z* 200, this mass spectrometer combines Orbitrap, quadrupole, and linear ion trap technologies in one acquisition path, which allows it to acquire a more complex spectra at a higher rate (112, 113).

Other forms of chemistry or affinity-based fraction selection techniques range from simple solvent extractions (114) to molecular affinity, as mentioned above, as well as more complex techniques such as photoaffinity labeling, which allows potential drug compounds to be labeled with a photo cross-linker and a purification tag (109). Modern techniques taking advantage of newly discovered biochemical interactions between proteins and their ligands, such as the cellular thermal shift assay, have shown promise as drug discovery techniques. The cellular thermal shift assay is based on the rationale that protein stability can be altered by ligand binding (115), and it was recently demonstrated that studying the shift in the heat denaturation curves of the

cellular proteomes after exposure to lead compounds can identify effective binding partners (116).

Novel extraction technologies have also been developed to address chemical and biological constraints and to improve overall extraction and downstream detection efficiency. These include high-intensity pulsed electric fields combined with semibionic extraction (117), highly sensitive supercritical fluid extraction (118), high-speed countercurrent chromatography (119), and sequential extractions combining multiple techniques to extract compounds with different properties from a single source (120).

Metabolomics. Metabolomics uses multiple technologies, including high-performance liquid chromatography (HPLC), infrared spectroscopy (IR), gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), and nuclear magnetic resonance (NMR) (53). Metabolomics platforms are increasingly being used for a variety of applications, including diagnosis of diseases, infections, host-parasite interaction, biomarker and drug lead discoveries, drug target identification, drug interaction, and personalized treatments (121). Metabolomics techniques are also emerging for the identification of the secreted metabolites by tropical canine parasites, such as hookworm, tapeworm, and roundworm (122, 123). There is a need to apply metabolomics to identify biomarker compounds for many other tropical parasites in order to understand the mechanisms responsible for their parasitism and host immune evasion.

THERAPEUTIC SOLUTIONS FOR INFECTIOUS DISEASES OF THE TROPICS

Approved Therapeutic Molecules Derived from Natural Products

Historically, natural products have been employed in the treatment of many diseases affecting humans. The *Dictionary of Natural Products* (https://www.routledge.com/go/the_dictionary_of_natural_products), an authoritative and comprehensive database on natural products, lists 270,000 chemical entities, some of which are considered vital components of many modern drugs (124). Newman and Cragg recently reported that 1,881 naturally derived drugs were discovered and approved as drug entities between 1981 and 2019 (125). It is also estimated that approximately half of all medications validated between 1981 and 2010 have been sourced from natural products (126). As an example, out of 15 antiparasitic compounds used between 1981 and 2014, 60% of these have their origins in natural products (127). However, the majority of them are either semisynthetic or mimics of the natural bioactive compounds, resulting in a significantly smaller percentage (5%) of natural products sourced directly from nature and used as therapeutic molecules (126). This section highlights the most commonly used approved pharmaceutical drugs sourced from natural products.

Tetracyclines. Tetracyclines are a family of broad-spectrum antimicrobial agents that were discovered in the 1940s. They are still used for their antimicrobial activity against a range of microbes implicated in many diseases, including some tropical diseases like malaria, where it is used as primary treatment for mefloquine-resistant *Plasmodium falciparum* (128), as well as trachoma and yaws (129, 130). Chlortetracycline (initially called aureomycin due to its yellow color) and oxytetracycline (initially called terramycin, in reference to *terra*, Latin for earth) were the first of the tetracyclines isolated from *Streptomyces aureofaciens* and *Streptomyces rimosus*, respectively (131–133). Similar molecules of the same class were subsequently extracted from *S. aureofaciens*, *S. rimosus*, and *Streptomyces viridofaciens* (tetracycline and demeclocycline) or synthesized through modification of natural products (e.g., doxycycline, lymecycline, methacycline, minocycline, rolitetracycline) (131, 134). Tetracyclines are known to bind to the bacterial 30S ribosomal subunit to reversibly inhibit bacterial protein synthesis and blocking them from growing or replicating further, a mode of action called "bacteriostatic" (129). However, since the isolation of the first tetracycline-resistant bacterium, *Shigella dysenteriae* (135), multiple microbial species have been reported to have acquired resistance to the natural (first-generation and some second-generation) tetracyclines, leading to the introduction of newer (third-generation) synthetic tetracyclines (136–138).

Quinine. Quinine is a basic alkaloid prepared from the bark of the *Cinchona* plant. The WHO recommends its use in combination with clindamycin in the management of uncomplicated *P. falciparum* malaria in pregnant women who are within their first

trimester. In the first trimester of pregnancy, quinine is also recommended for treatment of chloroquine-resistant *Plasmodium vivax* malaria (139). Additionally, quinine administration has been recommended for severe malaria in adults and children if artesunate and artemether (see "Artemisin" below) are not available (139). The mechanism behind quinine's antimalaria action is not fully understood. It was demonstrated that its antimalaria activity could be from the ability of its quinoline group to cap hemozoin, which is crystallized from heme, as the parasite digests hemoglobin in red blood cells (140). Heme is chemically destructive and causes cellular damage through various means, such as oxidative stress and cytoskeletal protein disruption (141). Quinoline capping of hemozoin crystals prevents the parasite from detoxifying heme into insoluble and inert heme, thereby allowing free heme to build up, poisoning the parasite (140). Lastly, it must be noted that quinine has considerable adverse effects, which can range from impairment of hearing, tinnitus, headaches, and nausea to vertigo, vomiting, and loss of vision (142). Despite this, quinine remains a viable alternative to many approved pharmaceutical drugs due to its low cost and the emergence of resistance to other common antimalarials.

Artemisinin. The use of the *Artemisia annua* plant, also known as sweet wormwood, to treat intermittent fevers among other indications has been documented in the Chinese *materia medica* in the late 1960s (143). In the early 1970s, artemisinin, a sesquiterpene lactone, was identified as responsible for the antimalarial activity of *A. annua* (144). Although the use of nonpharmaceutical forms of *A. annua* is not recommended by the WHO, artemether, artesunate, and dihydroartemisinin, its more stable semisynthetic derivatives, are included in the artemisinin-based combination therapy (ACT) recommended for treating malaria (139, 144). Both artemether and artesunate are metabolized by the body into dihydroartemisinin, which has various toxic effects against the parasite, including alkylation and misfolding of proteins initiated by free radicals created from the cleavage of the endoperoxide bridge found within the dihydroartemisinin molecule (144–146). ACTs constitute first-line therapies for most indications of malaria, including severe malaria. Depending on the indication, these compounds are frequently used in combination with other long-acting synthetic antimalarials, such as lumefantrine or amodiaquine (139). This is because artemisinin-based compounds have a short half-life and the longer-lasting synthetic compounds can continue to provide antimalarial activity to prevent the rise of drug resistance after artemisinin reaches subtherapeutic concentrations in the body (147).

NATURAL PRODUCT DISCOVERIES FOR THE TREATMENT OF TROPICAL DISEASES

Increasingly, natural products are being examined for their suitability in the treatment of tropical diseases caused by bacteria, virus, parasites, and fungi. There are many studies highlighting the effectiveness of natural products in treating tropical diseases.

Bacteria

In a very comprehensive review of recipes used by traditional healers in Burundi, Ngezahayo and colleagues recently identified a list of 155 different plant species belonging to 51 families and 139 genera used to prepare treatments for microbial tropical diseases of bacterial origin (148). Similarly, based on local folklore, Gupta et al. have collected 35 different plant species from India with anecdotal evidence of antituberculosis activity (149). Upon further examination, the ethanol extracts of 11 of those plants showed clear antimycobacterial activity (Table 3). There is also evidence that many plants from the Ivory Coast, Ghana, and Benin used to treat Buruli ulcer contain active ingredients with *in vitro* and *in vivo* activity against *Mycobacterium ulcerans* (150–156) (Table 3). Additionally, a plant-based treatment using *Capparis zeylanica* has been associated with a reduction of the diarrhea in patients suffering from cholera (157) (Table 3). Several other studies have described plant-based treatments of cholera (148), leprosy (148, 158, 159), and yaws (148); however, these studies do not provide clear evidence of the implication of the natural products in the improvement of the symptoms. Traditionally, most of these plant-based treatments are applied either as a maceration, powder, or decoction, indicating that the active ingredients within some of these plants may have topical and/or oral antibacterial activity.

TABLE 3 Active compounds from natural products with activity against tropical disease-causing bacteria[a]

Disease	Description and pathogenesis	Geographical distribution	Product family/class	Extracts/ natural product	Product source or origin	Extraction method	Efficacy/assessment model	Biological activity	Reference(s)
Buruli ulcer (BU)	BU is caused by *Mycobacterium ulcerans*. Pathogenesis of BU relies on mycolactone, a polyketide-derived macrolide. Its mode of transmission remains poorly understood, but the current hypothesis is that the disease is transmitted from stagnant bodies of water or mosquitoes.	BU was first described in Australia but has been reported from 33 countries worldwide, including West Africa, Central and South America, and the Western Pacific. About 73% of the total global cases have been reported from Côte d'Ivoire, Ghana, and Benin.	Naphthofurans	Rifampin	*Amycolatopsis rifamycinica*	Extracted from fermentation culture of the bacterium	Clinically used for treating BU	Oral administration of rifampin (10 mg/kg orally once daily)	27, 344
			Benzene and substituted derivatives	Streptomycin	*Streptomyces griseus*	Extracted from fermentation culture of the bacterium	Clinically used for treating BU	Intramuscular injection, 15 mg/kg of body weight for 8 weeks	345
			Alkaloids	Holadysamine	*Holarrhena floribunda*	50 g of powder was macerated and extracted using 70% ethanol.	*In vitro*: well diffusion assays	Compound inhibited the growth of *M. ulcerans* at MIC of 50 μg/ml	155
				Holophyllinol Holamine Holaphyllamine				Compound inhibited the growth of *M. ulcerans* at MIC of 125 μg/ml	
				Crude extract	*Moringa oleifera*	Extracted with water	Children with skin lesions clinically suggestive of BU (2–15 years old) were given normal diet spiked with 330 ml of *M. oleifera*/child at each meal.	Children's ulcers decreased from 72 mm to 48 mm on day 56 after administration of water extract of (330 ml) *M. oleifera*.	154
					Aglaonema commutatum	Leaves boiled in water for 5 min	200 μg/ml of extract was prepared and diluted with medium (1st to 8th dilution); MIC was determined at final concentrations (25% [vol/vol] to 0.20% [vol/vol]) corresponding to 50 μg/ml to 0.4 μg/ml.	*In vitro* activity with MIC of 40 μg/ml	346
					Aloe vera *Alstonia boonei*	Leaves macerated in water			
					Capsicum annum	Fruit macerated in water			
					Gratiola officinalis	Bark boiled in water for 20 min		*In vitro* activity with MIC of 1.56–25 μg/ml	
					Jatropha curcas	Leaves macerated in 70% ethanol		*In vitro* activity with MIC of 250 μg/ml	
					Spigelia anthelmia	Leaves and grains boiled in water for 5 min		*In vitro* activity with MIC of 6.25–25 μg/ml	
					Syzygium aromaticum	Seeds boiled in water for 20 min		*In vitro* activity with MIC of 25 μg/ml	
					Zea mays and *Spigelia anthelmia*	Grains and leaves boiled in water for 5 min		*In vitro* activity with MIC of 6.25–25 μg/ml	
					Zanthoxylum zanthoxyloides	Roots boiled in water for 20 min		*In vitro* activity with MIC of 12.5–25 μg/ml	
Trachoma	Trachoma is a bacterial infection of the eyes and genitals, which is spread through flies or direct contact. The eye infection can lead to blindness.	Trachoma is widespread across Africa, Asia, and Central and South America, with the highest prevalence in Ethiopia and South Sudan.	Flavonoids	Baicalin	*Scutellariae baicalensis*	Purchased commercially, dissolved in DMSO	Female mice were infected with *Chlamydia trachomatis*, followed by 1 mM intravaginal rinse treatment.	Reduced bacterial counts by 78% after 5 days and 99.9% after 11 days	178
			Flavonoids	Luteolin	Wide range of plants such as trees, herbs, and vegetables. Sourced from Extrasynthese, Genay, France	Purchased commercially, dissolved in DMSO	*Chlamydia trachomatis* bacterial challenge, followed by 2 mg/kg intraperitoneal injection treatment	Showed 25% and 37% fewer pathogen-positive mice at days 6 and 13, respectively	
			Flavonoids	Catechin	Various vascular plants, sourced from tea leaves	Boiling in water	*In vitro* HL cells (human airway epithelium line) cultured with	A 0.4-mg/ml concentration applied topically	

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TABLE 3 (Continued)

Disease	Description and pathogenesis	Geographical distribution	Product family/class	Extracts/ natural product	Product source or origin	Extraction method	Efficacy/assessment model	Biological activity	Reference(s)
							bacteria and treatments applied in culture medium	was completely inhibitory	
			Polyphenols	Flavones, flavonols, coumarins, gallates	Various vascular plants	Purchased commercially, dissolved in DMSO	*In vitro* HL cells (human airway epithelium line) cultured with bacteria and treatments applied in culture medium	A 50 μM concentration was highly active (85%–100% inhibition)	
Tubercu-losis	TB is a pulmonary disease that is initiated by the deposition of *Mycobacterium tuberculosis*, contained in aerosol droplets, onto lung alveolar surfaces. The progression of the disease can have several outcomes, determined largely by the response of the host immune system.	Most new cases of TB are in Asia and Africa.	Quinonoids	Plumbagin and crotonate plumbagin	Root of *Plumbago indica* Linn collected from Orissa, India	Multiple extraction methods	Broth microdilution assay Resazurin microplate assay (REMA)	Inhibition of thymidylate synthase MIC of 0.25–16 μg/ml	160–162
			Flavonoid	Kaempferol and its benzyl derivative	Leaf extract of *Rhoeo spathacea*, *Pluchea indica* from Indonesia	*In silico* modeling of molecular structures	AutoDock Vina, followed by 50-ns molecular dynamics simulation using YASARA	*In silico* inhibitor of the CYP121 *M. tuberculosis* enzyme	163
			Naphthoquinones	Maritinone	Stem bark extract of *Diospyros anisandra*	Maceration and liquid-liquid fractionation	Cytotoxicity assay using Vero cells and peripheral blood mononuclear cells	Bacteriostatic activity MIC of 1.56–3.33 μg/ml	164
				3,3′-Biplumbagin				Bacteriostatic activity MIC of 1.56–3.33 μg/ml	
			Iridoids-plumeride	Plumericin/ isoplumericin	Stem bark of *Plumeria bicolor*	Extracted by methanol	Tetrazolium bromide assay	Bacteriostatic activity MIC of 1.5–2.1 μg/ml	165
			Piperidines	Dipiperidine derivatives	*Piper nigrum*	Not applicable	Luciferase growth inhibition assay, *in vivo M. tuberculosis* induced weight loss in mouse and human trials	Disease reduction and bacterial growth inhibition	170
								Bacteriostatic activity MIC in the range of 4.0–32.0 μg/ml	171–174
			Gallic acid-derivatives	3-*O*-methyl-alkylgallates	*Loranthus micranthus*	Maceration	Bactericidal assay	Bacteriostatic activity MIC of 6.25 μM	166
			Coumarin-type compound	Collinin	*Zanthoxylum schinifolium* found in Korea, China, and Japan	Extracted by methanol and isolated using HPLC	Microbial cell viability assay	Bacteriostatic activity MIC of 3.13–6.25 μg/ml in culture broth and 6.25–12.5 μg/ml inside cells	167
			Acridone alkaloid	(i) Hydroxy-1, 3-dimethoxy-10-methyl-9-acridone, (ii) 1-hydroxy-3-methoxy-10-methyl-9-acridone, (iii) 3-hydroxy-1, 5, 6-trimethoxy-9-acridone	Stem bark of *Zanthoxylum leprieurii* from Mpigi District, Uganda	Crude extract extracted with methanol column chromatography	Microplate alamarBlue assay	Bacteriostatic activity MIC of 5.1 μg/ml	168
			Cucurbitacins	Ursolic acid	Ripe deseeded fruit of *Citrullus colocynthis* collected from Rajasthan, India	Extracted with petroleum ether, chloroform, methanol, and water	Maceration chromatography, bacterial viability assay	Bacteriostatic activity MIC of 50 μg/ml	169
			Cucurbitacins	Cucurbitacin	Ripe deseeded fruit of *Citrullus colocynthis* collected from Rajasthan, India	Extracted with petroleum ether, chloroform, methanol, and water	Maceration chromatography, bacterial viability assay	Bacteriostatic activity MIC of 25 μg/ml	169

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TABLE 3 (Continued)

Disease	Description and pathogenesis	Geographical distribution	Product family/class	Extracts/ natural product	Product source or origin	Extraction method	Efficacy/assessment model	Biological activity	Reference(s)
			Sponge-derived bengamide	Bengamide B	*Tedania* sp. collected from East Diamond Islet, Queensland, Australia	Marine sponge extract, isolated using HPLC	Intracellular mycobacterial activity assay	Interference with methionine aminopep-tidase activity MIC of 0.39–1.56 μg/ml	175
			Alkaloid	Halicyclamine A	*Haliclona* sp.	Marine sponge extract, extracted with methanol and isolated using HPLC	MTT assay	Bacteriostatic activity MIC of 1–5 μg/ml under aerobic and hypoxic conditions	176
			Steroidal alkaloid	Plakinamide P	*Plakina* sp. collected from Crooked Island, Bahamas	Marine sponge extract, extracted with heptane, ethyl acetate-ethanol mixture, ethanol, and methanol HPLC	Autoluminescence bacterial viability assay	Bactericidal activity MIC of 1.8 μg/ml	347
			Not specifically identified in this paper, but some active products were discussed		Bark of *Alstonia scholaris* Roots of *Glycyrrhiza glabra* Seeds of *Holarrhena antidysenterica* Fruits of *Mallotus philippensis* Tubers of *Eulophia nuda* Leaves of *Cocculus hirsutus* Tubers of *Pueraria tuberosa* Roots of *Cyperus rotundus* Rhizome of *Curcuma caesia* Floral head of *Sphaeranthus indicus* Roots of *Plumbago zeylanica*	Plant material collected from four districts in Madhya Pradesh were dried, ground, and then extracted with 95% ethanol to obtain crude extract.	*In vitro* measurement of plant extract bacteriostatic activity against 7 strains of *M. tuberculosis* via resazurin microtiter plate assay (REMA) compared to cytotoxicity against THP-1 macrophage cells measured via flow cytometry to derive a selectivity index (IC_{50} cells/MIC)	Selectivity index of 4 to >8 Selectivity index of 8 to >32 Selectivity index of 8 to >32 Selectivity index of 8 to >32 Selectivity index of 8 to 32 Selectivity index of 4 to >8 Selectivity index of 1 to 8 Selectivity index of 16 to >64 Selectivity index of 4 to 16 Selectivity index of 8 to 16 Selectivity index of 32 to 64	149

[a]DMSO, dimethyl sulfoxide; HPLC, high-performance liquid chromatography; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide; IC_{50}, 50% inhibitory concentration.

In contrast to the vast knowledge about the source organisms (mainly plants), relatively little is known about the identity of the active compounds within these organisms exerting antimicrobial activity (Table 3). Over the last few years, some groups around the world have isolated a number of natural compounds with proven *in vitro* activity against *M. tuberculosis* (Table 3). Plumbagin and other quinonoid compounds have shown a strong bacteriostatic effect in a number of independent studies (160–162). In addition, various other plant-derived compounds, such as kaempferol (163), maritinone (164), 3,3′-biplumbagin (164), plumericin and isoplumericin (165), 3-*O*-methyl-alkylgallates (166), collinin (167), acridone alkaloids (168), ursolic acid (169), and cucurbitacin (169), have been reported to have bacteriostatic effects on *M. tuberculosis in vitro* (Table 3). Importantly, piperidine-based active compounds isolated from *Piper nigrum* have already progressed to preclinical and clinical trials in animals and humans (170–174) (Table 3). Another promising source for antituberculosis drugs appears to be marine sponges (175–177) (Table 3). Quan and colleagues recently reported on the antituberculosis activity of bengamide B derived from the marine sponge *Tedania* sp. (175). Other studies have demonstrated strong bacteriostatic effects of halicyclamine A (176) and plakinamide P (177), derived from the sponges *Haliclona* sp. and *Plakina* sp., respectively. In a study from Benin, four steroidal alkaloids (holadysamine, holophyllinol, holamine, and holaphyllamine) from the plant species *Holarrhena floribunda* showed bacteriostatic activity against *Mycobacterium ulcerans* (155). Several active natural products, including baicalin, luteolin, catechins, flavonoids, and polyphenols, from a variety of tropical plants have also been shown to improve the eyesight of patients infected with *Chlamydia trachomatis* (178). Preclinical studies in mouse models of cholera have also shown a reduction in diarrhea when mice were treated with farnesol isolated from the *Acacia farnesiana* tree (179) or with sulfated polysaccharides isolated from the red seaweed *Gracilaria cervicornis* (180).

Parasites

Multiple natural products have been shown to have antiparasitic activity against pathogens responsible for lymphatic filariasis (181–186), leishmaniasis (187), and Chagas disease (187) (Table 4). Similarly, a large range of natural products from the tropics have been commonly used for treating malaria (188–190) (Table 4). More recently, by combining nanoparticle technologies and natural extracts from the leaves of the plant *Indigofera oblongifolia*, Dkhil et al. have demonstrated the promising antiplasmodial and hepatoprotective activity of silver nanoparticles in a *Plasmodium chabaudi*-infected mouse model (191). Nanocarrier-based drug delivery systems have received enormous attention in the past few years (192). Antimicrobial drugs become more efficacious when adsorbed, entrapped, or linked to polysaccharides such as dextran, besides increasing the surface area for the drug and achieving targeted drug delivery (193). A snapshot of intravenously administered tetramethylrhodamine-isothiocyanate (TRITC)-conjugated dextran in the blood vessels of mice with malaria is shown (Fig. 3). Dextran-based carriers are being tested for many diseases, including for codelivery of buparvaquone (BPQ) and polymyxin B (PB) against leishmaniasis and to enhance cross presentation of subunit vaccines against melioidosis (194, 195). Two active compounds against *Plasmodium* spp., cryptolepine and dichroine, extracted from *Cryptolepis sanguinolenta* and *Dichroa febrifuga* (Ch'ang Shan) roots, respectively, have been identified (196–198) (Table 4). Recently, bisaprasin, a bromotyrosine alkaloid isolated from *Aplysinella rhax*, a Fijian marine sponge, has exhibited moderate antiparasitic activity against *P. falciparum* (199) (Table 4).

Regarding leishmaniasis, cubebin isolated from *Piper cubeba* has been described as a potent parasiticidal (200) (Table 4). A recent study conducted by de Souza and colleagues has found that the *Psidium brownianum*-derived natural compounds quercetin, myricetin, and gallic acid as well as the *Psidium guajava*-derived ethanolic extract and the flavonoid and tannic fractions show antiparasitic activity against both *Leishmania brasiliensis* and *Leishmania infantum* parasites (201) (Table 4). Similarly, a sesquiterpene lactone, parthenolide, derived from *Tanacetum parthenium*, shows antileishmania activity against *Leishmania amazonensis* in both *in vitro* and *in vivo* studies (202–204). In the context of lymphatic filariasis, galactolipids isolated from the leaves of the tropical tree *Bauhinia racemosa* have been characterized as promising antifilarial agents in both *in vitro* and *in vivo* models (205) (Table 4). Several *in vitro* studies

TABLE 4 Active compounds from natural products with activity against tropical disease-causing parasites

Disease	Description and pathogenesis	Geographical distribution	Product family/class	Extracts/natural product	Product source or origin	Extraction method	Efficacy/assessment model	Biological activity	Reference(s)
American trypanosomiasis (Chagas disease)	A vector-borne parasitic disease caused by blood-sucking bugs infected with *Trypanosoma cruzi* and spread through bites from insect vectors	Americas	Solenopsins alkaloids	Solenopsin	*Solenosis invicta, S. saevissima*	Fire ants' mounds isolated; solenopsin alkaloids extracted with hexane and further purified with hexane–acetone silica columns; alkaloids compared with known mass spectra.	*In vitro:* Tested against proliferation of *T. cruzi* epimastigote forms of Dm-28c and CL-Brener.	Solenopsin extracts used at 0.25–0.5 × IC_{50} values for up to 8 days; growth capacity recovered after solenopsins were removed (inhibition reversible).	208
			Bromotyrosine alkaloids	Bisaprasin	*Aplysinella rhax*	Marine sponge extract partitioned using modified Kupchan partitioning technique; reversed-phase solid-phase extraction used for further fractionation	*In vitro: T. cruzi* Tulahuen C2C4 strain, expressing LacZ; L6 rat skeletal muscle cells with 5 μl of compound	Moderate parasiticidal activity; IC_{50}, 19 μM	348
			Unspecified		*Handroanthus impetiginosa* *Ageratum conyzoides* *Ruta graveolens*	Crude extracts prepared from powdered aerial parts; macerated in hydroalcoholic solution followed by evaporation at 35 degrees for 2 h	*In vitro:* Cytotoxicity measured in MTT assay (concentrations from 1.0 to 0.03125 kg body/ml) against *Trypanosoma cruzi* trypomastigotes (CL strain; Brener) and murine J774.G8 macrophages.	Lethal to *T. cruzi* trypomas-tigotes at 0.5, 0.25, and 0.125 kg body/ml of *H. impetiginosus* extract *A. conyzoides* extract toxic at most of the concentrations (exception, 0.0625 kg body/ml) *R. graveolens* extract showed increased mortality of trypomastigotes compared to *H. impetiginosa* extract. Cell invasion inhibition: Pretreatment with *A. conyzoides*, *H. impetiginosus*, and *R. graveolens* reduced no. of *Trypanosoma cruzi* trypomastigote-infected cells	187
			Sesquiterpene lactones	Psilostachyin (Psi)	*Ambrosia tenuifolia*	Unspecified	*In vitro:* Growth inhibition of *T. cruzi* epimastigotes, (percent inhibition and IC_{50}) estimated in the presence of hemin concentrations of 0–20 kg body/liter. *In vivo:* Swiss-Webster mice (one female and one male) treated intraperitoneally or orally at 25–400 mg/kg; MTD values determined on animal survival rates and behavior alteration after 48 h	Maximum inhibitory capacity without hemin (IC_{50}, 4.74 μM). Reduction of IC_{50} value in the presence of 20 kg body/liter of hemin. Psilostachyin A: EC_{50} at 24 h of 33 ± 1 μg/ml against bloodstream trypomastigotes. *In vivo*: Reductions in parasitemia from 40 to 70% at 0.5–50 mg/kg; Psilostachyin A did not protect against animal mortality	209, 210
				Psilostachyin C (PsiC)	*Ambrosia scabra*	Extraction using the aerial parts of *A. scabra* by macer-ation with dichlorom-ethane and methanol at room temp.	*In vitro:* Bloodstream trypomastigotes cultured with 0.1–100 μg/ml PsiC; peritoneal macrophages infected with transfected trypo-mastigotes expressing β-galactosidase cultured with 0.01–10 μg/ml to 10 μg/ml. *In vivo:* CF1 and C3H/HeN mice infected i.p. with bloodstream *T. cruzi* trypomastigotes; treated daily with 1 mg/kg body/day of PsiC. Parasitemia and mice mortality assessed.	Increased no. of epimastigotes observed after treatment with GSH and PsiC compared with PsiC alone. *In vivo*: PsiC-treated animals showed a twofold reduction in parasites, but succumbed to infection from day 20 (30-day survival rate of 20% vs 0% in untreated mice).	349

(Continued on next page)

TABLE 4 (Continued)

Disease	Description and patho-genesis	Geographical distribution	Product family/class	Extracts/natural product	Product source or origin	Extraction method	Efficacy/assessment model	Biological activity	Reference(s)
Human African trypanosomiasis	A vector-borne parasitic disease caused by insect vectors tsetse flies infected with *Trypanosoma brucei* and spread through bites from the insect.	Sub-Saharan Africa	Phenolic compounds	Bisdemethoxycurcumin, demethoxy-curcumin, oregonin, broussochalcone A, 3-deoxysappan-chalcone, xanthoan-gelol, 7-(4″-hydroxy-3″-methoxy-phenyl)-1-phenylhept-4-en-3-one, 4-hydroxy-3-methox-ycinnamal-dehyde, obovatal, honokiol, 1′S-1′-acetoxychavicol acetate, saucerneol D, manassantin A, manassantin B, kushenol F, apigenin, eupatilin, morusin, 3-deoxysappanone B, 6,8-diprenylorobol, genistin, sophoricoside	*Curcuma longa, Alnus japonica, Broussonetia papyrifera, Caesalpinia sappan, Angelica keiskei, Cinnamomum cassia, Machilus thunbergii, Alpinia galanga, Saururus chinensis, Sophora flavescens, Agrimonia pilosa, Artemisia vulgaris, Morus alba*	Identified from the Chungnam National University (Korea) proprietary library of 440 natural products from medicinal plants.	*In vitro:* growth inhibition assay of bloodstream form *T. brucei brucei* strain 427 treated with test compounds compared to cytotoxicity assays of HEK239T and HepG2 cell lines treated with test compounds to derive a selectivity index (CC_{50} cells/EC_{50} *T. brucei brucei*)	Selectivity index ranging from 2.29 to >46.34	211
			Guajanolide sesquiterpene lactone	Cynaropicrin	*Centaurea salmantica* L. *(Asteraceae)*	Dried aerial parts of *C. salmantica* were defatted with n-hexane (Scharlau), dried and then extracted exhaustively with ethyl acetate (Scharlau) yielding crude extract.	*In vitro:* Cardiac cells were infected with Y and Colombiana strains of *T. cruzi* with increasing nontoxic concentrations of the compounds. Death rates and EC_{50} were calculated *In vivo:* Swiss-Webster mice (one female and one male) treated intraperitoneally or orally at 25–400 mg/kg; MTD values determined on animal survival rates and behavior alteration after 48 hours	Cynaropicrin moderately effective on intracellular proliferative forms (EC_{50} at 48 h of >0.75 μg/ml). *In vivo*: Cynaropicrin (25 and 50 mg/kg/day i.p.) showed no reduction in parasitemia; 100% mortality in all groups.	210, 212
			Sesquiterpene lactone	Deoxyelephantopin	*Elephantopus scaber* Linn.	Dried powder of *E. scaber* soaked with methanol for 3 days. Extracts concentrated with rotary vacuum evaporator.	*In vitro: T. brucei rhodesience* (strain STIB 900) parasite and mouse skeletal (L-6) cell were incubated with compound at 90–0.123 μg/ml over 72 h	Ethyl acetate fraction showed highest antitrypanosomal activity (0.21 ± 0.04 μg/ml) with a lower cytotoxicity (6.25 ± 0.77 μg/ml).	350
Leishmaniasis	A parasitic disease caused by *Leishmania* parasites and spread through bites from sand flies	Tropics and subtro-pics of Africa and Asia, and southern Europe	Lignans Amides	Cubebin Piplartine	*Piper cueba*	Materials extracted with n-hexane, ethyl acetate, and methanol and concentrated under vacuum on rotary evaporator	*In vitro: L. donovani* promastigote MTT cell cytotoxicity assay. *In vivo: L. donovani* amastigotes (intracardiac injection) in golden hamsters were administered ± compound for 10 days.	*P. cubeba* showed > 90% inhibition of promastigotes of *L. donovani* at 100 μg/ml; extracts from *P. retro-fractum* >75% inhibition at 20 μg/ml. *In vivo*: Treatment with piplartine (30 mg/kg/10 ml i.p.) reduced parasitic burden and spleen weight.	200
			Multiple families	Quinic acid, catequin/ epicatequin, ellagic acid, gallocatechol as well as myricetin, quercetin, cafeoil, and their associated derivatives, were identified from analyzed extracts	*Psidium brownianum* *Psidium guajava*	Crushed leaves were extracted with 100% ethanol for 96 h, filtered, then dried in a rotary evaporator. Flavonoid fractions were extracted from the ethanol extract with hexane, chloroform, then ethyl acetate. Tannic fractions were extracted from the ethanol extract with 7:3 acetone/water,	*In vitro* Leishmanicide assay against epimastigotes compared to cytotoxicity against NCTC 929 fibroblasts exposed to 1,000 and 500 μg/ml of the respective extract	84.02–94.25% of epimastigote forms killed compared to 38.86–40.97% fibroblasts at 1,000 μg/ml 46.6–95.55% of epimastigote forms killed compared to 36.66–37.9% of fibroblasts at 1,000 μg/ml	201

(Continued on next page)

TABLE 4 (Continued)

Disease	Description and pathogenesis	Geographical distribution	Product family/class	Extracts/natural product	Product source or origin	Extraction method	Efficacy/assessment model	Biological activity	Reference(s)
						filtered and concentrated, then further extracted with petroleum ether.			
			Unspecified		*Handroanthus impetiginosa* *Ageratum conyzoides* *Ruta graveolens*	Crude extracts prepared from powdered aerial parts of the plant; macerated in hydroalcoholic solution, followed by evaporation	*In vitro*: Cytotoxicity measured using MTT assay with extract concentrations of 1.0 to 0.03125 mg/ml	*A. conyzoides* extract caused increased mortality in *L. amazonensis* promastigote; treatment with *H. impetiginosus* showed lower parasite mortality; treatment with *R. graveolens* showed significant increase in mortality rate. Invasion assay: *Leishmania amazonensis* pretreated with *A. conyzoides* and *R. graveolens* exhibited decreased entry into macrophages; *H. impetiginosus* had no impact on invasion and infection.	187
			Sesquiterpene lactone	Parthenolide	*Tanacetum parthenium*	Maceration in ethanol-water and extraction at room temp in the dark.	*In vitro:* Antiamastigote activity was assessed using *L. amazonensis*-infected (MHOM/BR/75/Josefa strain) J774G8 macrophages treated with compound.	Extract inhibited promastigote growth (IC_{50}, 29 μg/ml after 48 h). Parthenolide (IC_{50}, 0.37 μg/ml) induced partial lysis of the promastigotes at 5 μg/ml. At 1 μg/ml, it resulted in 90% growth inhibition. Cytotoxic assay: Macrophages treated with parthenolide showed CC_{50} of 14 μg/ml.	203
						Purchased from Sigma-Aldrich (Germany)	*In vitro:* Parasite growth assay on *L. amazonesis* treated with 70% parthenolide	IC_{50} of 1.3 μM and IC_{90} of 3.3 μM	204
				Sesquiterpene lactone-rich fraction		Extraction of aerial parts of plant with ethanol and distilled water followed by sequential chromatography with hexane, ethyl acetate, dichloromethane, and methanol.	*In vivo*: Mice infected with *L. amazonensis* metacyclic promastigotes by injection into the footpad. Mice were treated intramuscularly with test fraction after lesion development. Mice were assessed for lesion growth and parasite load	Sesquiterpine lactone-rich-fragment-treated mice had reduced lesion development. Parasite load was significantly reduced	202
Lymphatic filariasis	A parasitic disease caused by micropic, thread-like worms transmitted via mosquito bites	Africa, the Americas, Pacific and Asia	Galactolipids	1, 2-di-O-linolenoyl-3-O-α-galactopyranosyl-(1→6)-O-β-galacto-pyranosyl glycerol	*Bauhinia racemosa*	Successive column chromatographic separations on normal and reverse phase silica gel was used to extract subfraction F4	*In vivo*: Active compounds identified in n-butanol fraction F4 were evaluated intraperitoneally (i.p.) for five consecutive days using 50 mg/kg	Galactolipid 1, 2, and 3 had 58.3%, 45.8%, and 54.15% adult worm mortality, respectively, with long chain fatty acid alcohol 8 showing 49.95% adulticidal activity over control.	205
			Unknown	Hexane fraction	*Botryocladia leptopoda*	95% ethanol was used to extract powdered, dried material with the combined extract concentrated at below 50°C in a rotavapour and dried under high vacuum. Ethanol extract fractionation was done into four parts: hexane, chloroform, n-butanol soluble, and n-butanol insoluble.	*In vitro:* Crude extracts/fractions tested against adult female parasites of *L. sigmodontis*, *A. viteae*, and *B. malayi*. The crude extract was tested at 250 μg/ml while the fractions were tested at various serial twofold dilutions from 500 down to 15.6 μg/ml. Worms were incubated in triplicate at 37°C overnight in the RPMI 1640 medium containing antibiotics.	Slight decrease in microfilarial levels was observed on day 8 post-treatment in both *L. sigmodontis*- and *A. viteae-infected* animals. Microfilarial levels continuously decreased until the end of the observation period, finishing with decreases in microfilarial density of 82% and 97%, respectively.	181
			Triterpenoids	Oleanonic acid/oleanolic acid	*Lantana camara*	Combined extract concentrated under reduced pressure at below 40°C, then dried under high	*In vivo:* Mastomys rat showing rises in microfilaremia that had 2–3-mo-old *A. viteae* infections or 5–6-mo-old *B. malayi*	95.04% reduction in microfilaremia on day 8 when treated with 1 g/kg of crude extract for 5 consecutive days. The crude extract caused high mortality in the adult *B. malayi*	182

(Continued on next page)

TABLE 4 (Continued)

Disease	Description and pathogenesis	Geographical distribution	Product family/class	Extracts/natural product	Product source or origin	Extraction method	Efficacy/assessment model	Biological activity	Reference(s)
						vacuum. Fractionation done with different solvents to obtain fractions including n-hexane, chloroform, n-butanol, and aqueous fractions.	infections were selected. Extracts suspended in 0.1% Tween 80 and administered orally at varying doses for 5 days. Two pure compounds were administered both orally and intraperitoneally.	transplanted gerbil model on day 42 (72%) or day 60 (80%) of subcutaneous or oral treatment.	
			Unknown	Hexane fraction	*Butea monosperma*	Powdered *Butea monosperma* L. plant leaves were extracted with petroleum ether, hexane ethanol, and methanol. Extract was dissolved in dimethylsulfoxide to obtain different concentrations for further study.	*In vitro:* Crude methanol, hexane-ethanol, and antibiotic ciprofloxacin extracts were used for antifilarial screening on adult *Setaria cervi*.	Methanol concn (0.25 to 5.0 mg/ml), hexane-ethanol concentrations (0.50 to 10.0 mg/ml) and ciproflo--xacin concn (1.0 to 20.0 mg/ml) caused complete immobilization of the worms at 5 to 24 h exposure.	351
			Flavonoids	4,5-diethyl-3′-ethoxy-pyro-flavone	*Vitex negundo*	Powdered *Vitex negundo* L. leaves were extracted with petroleum ether (60°C–80°C), chloroform, ethyl acetate, and methanol using the percolation method.	*In vitro:* 0.01% streptopenicillin and 10% heat-inactivated fetal calf serum were mixed in DMEM and the worms added. 100 μl diluted extract of *Vitex negundo* was added and worms incubated at 37°C for 24 h in 5% CO_2 incubator and motility observed.	*Vitex negundo* fraction 3 concentrations from 0.005 to 0.02 mg/ml caused complete immobilization of the worms at incubation at 2 h.	352
			Phenylpropanoids	Coumarins	*Aegle marmelos* Corr.	Leaves from *Aegle marmelos* Corr. were extracted by 70% ethanolic extraction process.	*In vitro:* 100 microfilariae mixed in 100 μl of RPMI of were added to each well. Plates were incubated at 37°C for 48 hours in 5% CO_2 incubator. Microfilaria motility assessed after incubation by microscopy.	Dose-dependent loss of microfilaria motility observed for herbal extracts from *Aegle marmelos* Corr. leaves.	353
			Unknown	Hexane fraction	*Caesalpinia bonducella*	Kernel seeds air dried and extracted with 95% ethanol with the extract concentrated under reduced pressure $<$45°C using a Rotavapor	*In vitro:* Microfilaricidal activity was assessed in animals killed on day 42 or 91 post-treatment. Cottom rat pleural cavities and various tissues from *Mastomys coucha* were examined in saline for motility and cell adhesion on the worm surface.	With *L. sigmodontis* on cotton rats, the extract of seed kernel showed 60.7, 72.4, and 98.4% microfilaricidal action respectively on days 8, 21, and 42 post-treatment with 96.0% overall adulticidal activity.	186
			Limonoid	Gedunin	*Xylocarpus granatum*	1 kg of air-dried, powdered fruits were extracted with 50% ethanol and combined extracts filtered, concentrated under reduced pressure below 50°C. The powder was fractionated into chloroform-soluble and chloroform-insoluble fractions by maceration.	*In vitro:* Actively motile female worms exposed to various concentrations in 48-well culture plate containing 1,000 μl media. RPMI 1640 medium containing antibiotics and 10% fetal bovine serum was used. Following drug exposure, worm motility was recorded microscopically.	The crude aqueous ethanolic extract of fruit of *Xylocarpus* killed adult *B. malayi* and microfilariae at 125 and 62.5 μg/ml. This compares to the standard drug ivermectin, which killed adult worms at 7.8 μg/ml and microfilariae at 125 μg/ml concn.	185

(Continued on next page)

TABLE 4 (Continued)

Disease	Description and pathogenesis	Geographical distribution	Product family/class	Extracts/natural product	Product source or origin	Extraction method	Efficacy/assessment model	Biological activity	Reference(s)
Malaria	A mosquito-borne parasitic disease transmitted via mosquito bites	Sub-Saharan Africa and South Asia	Indoloquinolone alkaloid	Cryptolepine	*Cryptolepis sanguinolenta*	650 g of powdered roots boiled for 30 min in 5 liters of distilled water which was decanted and filtered. Filtrate freeze-dried to obtain crude extract referred to as cryptolepis (CPS).	*In vitro:* Cryptolepine mixed with the aqueous root extract of *C. sanguinolenta* were used to assess gametocyte survival after drug exposure using a resazurin-based assay.	*Cryptolepis sanguinolenta* and its major alkaloid, cryptolepine exhibited high inhibitory activity against late-stage *P. falciparum* gametocytes (NF54).	354
			Quinazolinone alkaloid	Febrifugine	*Dichroa febrifuga*	Dried roots were ground and macerated in 14 liters of methanol at room temp for a wk. After filtration, solvent was evaporated to obtain crude methanol extract, which was then suspended with the alkaloidal portion separated by chromatography on a silica gel 60 column	*In vitro:* Mice were treated orally or subcutaneously daily from day 3-10 to day 10 with either candidate antimalarial drugs or vehicle alone (negative control). Five doses for each group were tested. Films were Giemsa stained and examined microscopically to determine parasitemia level.	R237645 (halofuginone) was found to be the most active febrifugine analog against the parasites.	355
			Bromopyrrole alkaloids	Bisaprasin	*Aplysinella rhax*	The sponge sample was extracted with methanol followed by DCM and dried and partitioned following the modified Kupchan liquid-liquid partitioning technique.	*In vitro:* Compounds 1–6 and 9–10 were tested in duplicate with a 16-point dose-response curve (starting concentrations of 63–115 μM). Following incubation, LDH activity was measured.	Bisaprasin, a biphenylic dimer derived from psammaplin A, showed moderate activity at 19 and 29 μM against *T. cruzi* Tulahuen C4 while psammaplin A showed activity at 30 and 60 μM against *P. falciparum* 3D7 strain.	348
			Not determined		*Cochlospermum planchonii*	N'Dribala extract was prepared daily by decoction of 50 g of dried *Cochlospermum planchonii* tuberous root powder mixed with 1,500 ml of boiling water.	*In vivo*: Parasite density was estimated by two readers. Full clinical examination and blood sampling of patients was conducted on days 0, 2, and 5.	At D2, 17 N'Dribala-treated patients had Pd $>$ 500 parasites/mm^3 compared to all CQ-treated patients, with Pd $<$ 500 parasites/mm^3. At D5, seven N'Dribala-treated patients had Pd $>$ 500 parasites/mm^3, with 57% of CQ-treated and 52% N'Dribala-treated patients having Pd = 0	356
Schistosomiasis	A parasitic disease caused by worms transmitted through contact with contaminated freshwater	Africa, Asia and the Americas	Lactones	Vernodalin	*Vernonia amygdalina*	1 kg of air-dried plant leaves was ground coarsely and added to a stoppered container with water. Mixture was strained, the damp solid material pressed, and the combined liquid clarified by filtration.	*In vitro:* 1.0 ml from each of the homogenates added to test tubes and left undisturbed for 10 min at 25°C. Mixture incubated for 5 min at 55°C, then removed and cooled with running water. Absorbance read at 650 nm against the blank.	Treatment of the intermediary host *Bulinus truncatus* with various concentrations of *Vernonia amygdalina* leaf extracts caused reductions in the activity of acetylcholinesterase in snail haemolymph, muscle, hepatopancreas, and intestine.	357
			Phenolics Phloroglucinols Phloroglucinol Alkyl-phenylketones	Aspidin Flavaspidic acid Methylene-bis-aspidinol Desaspidin	*Dryopteris* spp. *Dryopteris* spp. *Dryopteris* spp. *Dryopteris* spp.	Dihydroartemisinin was ground in a ball miller with dimethyl sulfoxide, Tween 80, and 1% carboxymethylcellulose sodium, yielding suspension solutions containing 8, 12, 16, or 24 g dihydroartemisin.	*In vitro:* Adult worm pairs were incubated for 24 h with phloroglucinol derivatives (range 10-100 μM). Viability assays were performed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.	All worm pairs died after 24 h of incubation with aspidin, flavaspidic acid, methylene-bis-aspidinol, and desaspidin. Worms showed decreased motor activity with tegumental alterations that were incubated with aspidin and flavaspidic acid, while worms showed decreased motor activity without tegumental alterations when incubated with methylene-bis-aspidinol and desaspidin.	207

[a]MTD, maximum tolerated dose; GSH, glutathione; i.p., intraperitoneally; CC_{50}, 50% cytotoxic concentration; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide; DMEM, Dulbecco modified Eagle medium; DCM, dichloromethane; LDH, lactate dehydrogenase; Pd, parasite density; CQ, chloroquine.

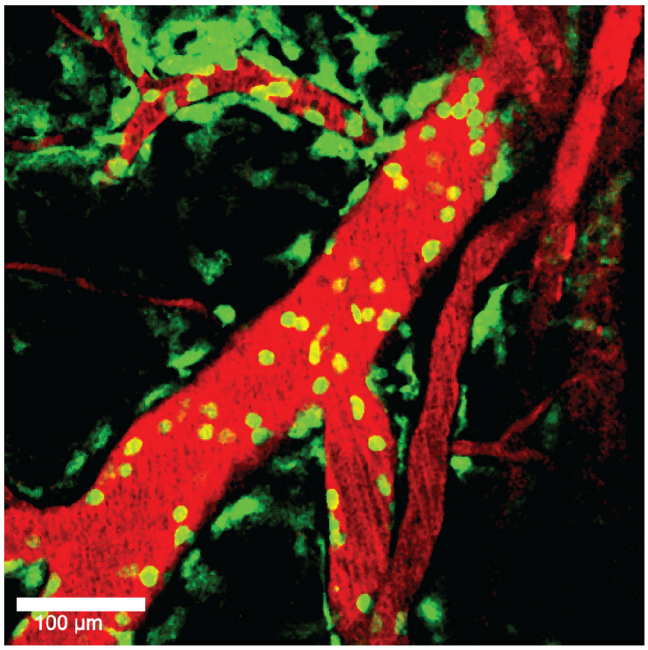

FIG 3 Visualizing drug delivery in the microvasculature. MacGreen mice were infected with *Plasmodium berghei* ANKA for subsequent intravital imaging of the brain microvasculature when the mice were showing clinical signs of malaria. Blood vessels were infused with tetramethylrhodamine-isothiocyanate (TRITC)-conjugated dextran (red). Moderate levels of leukocyte accumulation are seen both within and outside the blood vessels (green).

have identified promising natural compounds for treating schistosomiasis; among them are vernodalin, aspidin, flavaspidic acid, methylene-bis-aspidinol, and desaspidin (206, 207) (Table 4). The current antihelminthic drug, praziquantel, is not effective against the *Schistosoma mansoni* schistosomulum stage. Recently, four lead natural chemotherapeutic agents, including isomyristicin, bergapten, luteolin, and linalool oxide acetate, which were discovered from medicinal plants, showed efficacy against the schistosomulum stage and against multiple phylogenetically distinct parasites (82). Finally, some studies focusing on Chagas diseases have identified solenopsin alkaloids extracted from fire ants (*Solenosis invicta* and *Solenosis saevissima*), bisaprasin, and psilostachyin and psilostachyin C (sesquiterpene lactones isolated from *Ambrosia tenuifolia* and *Ambrosia scabra*) as promising antitrypanosomal agents (199, 208–210) (Table 4). Of note, the antiparasitic activity of the natural compounds described by de Souza and colleagues against *L. brasiliensis* and *L. infantum* was also observed against *Trypanosoma cruzi* (201). In the context of human African trypanosomiasis (HAT), phenolic compounds extracted from medicinal plants *in vitro* (211) and a sesquiterpene lactone, cynaropicrin (from *Asteraceae* plants *in vivo*), have exhibited potent activity against *T. brucei* (210, 212). Further reviews of active natural products against tropical parasites can be found in studies by Cockram and Smith (213) and Herrera Acevedo et al. (190).

Viruses

Natural products as potential novel therapeutics have been explored, especially in the context of dengue fever (214, 215). Oliveira and colleagues provide a detailed review of natural products that showed antiviral activities against dengue virus (DENV) and chikungunya virus (216). A Malaysian open-label randomized controlled trial (RCT) that included patients suffering from dengue fever and dengue hemorrhagic fever has demonstrated that papaya extract was effective in improving symptoms by increasing the platelet count, as well as the expression of some genes implicated in the *de novo* regeneration of platelets, less than 2 days after receiving the first dose of fresh *Carica papaya* leaf juice (217) (Table 5). Similar results have been obtained in an Indonesian RCT in which *C. papaya* leaf extract was encapsulated and administered orally (218) (Table 5). Additionally, antiviral activity against the four serotypes of DENV (DENV-1, -2,

TABLE 5 Active compounds from natural products with activity against tropical disease-causing viruses[a]

Disease	Description and pathogenesis	Geographical distribution	Product family/class	Extracts/ natural product	Product source or origin	Extraction method	Efficacy/assessment model	Biological activity	Reference
Chikungunya	Chikungunya virus is transmitted through the bite of an infected mosquito	Mostly Africa, Asia, and the Americas	Diterpenoid esters and potentially other unidentified families	Several identified and unidentified compounds	11 *Euphorbia* species plants collected from Corsica island, France	Solid-liquid or liquid-liquid extraction with ethyl acetate, methanol, or water from powdered plant material, such as whole plant, leaves, stems, roots, or aerial parts. Forty-five extracts from parts of 11 Euphorbiaceae plants were tested.	*In vitro* cell quantification of Vero cell cultures treated with plant extracts only or chikungunya virus inoculum and plant extract to derive a selectivity index (CC_{50} Vero cells/EC_{50} virus).	Selectivity index ranging from 1 to >47 (e.g., 4.1 μg/ml EC_{50} virus vs >100 μg/ml CC_{50} Vero cells).	229
			Flavonoids	Luteolin and apigenin (major constituents of the fraction with antiviral activity)	*Cynodon dactylon* plants collected in the Western Ghats region around the Madurai District of Tamil Nadu, India	Powdered plant material defatted with petroleum ether, extracted with ethanol, and fractionated through silica gel. Five fractions were screened in a pilot, with 1 displaying sufficient antiviral activity for further testing.	*In vitro* cell quantification of Vero cells treated with titrated active fraction compared to Vero cells incubated with chikungunya virus inoculum, followed by treatment with titrated active fraction. Validated by reverse transcription-PCR of chikungunya virus E2 gene.	Expressed as a percentage of the untreated control, 25 μg/μl of active fraction resulted in 88% antiviral activity while displaying minimal cytotoxicity. Band displaying E2 gene product was significantly reduced.	228
			Macrocyclic lactone	Abamectin	*Streptomyces avermitilis*	Identified from 2,933 compounds from three compound libraries: Spectrum (Micro Source Discovery Systems), NIH Clinical Collection I, and an FDA-approved drug library (ENZO Life Sciences).	*In vitro* cell quantification of BHK-21 and Huh-7.5 cells treated with test compounds and quantification of viral replication in chikungunya virus-infected BHK-21 and Huh-7.5 cells treated with test compounds to derive a selectivity index (CC_{50} cells/EC_{50} virus). Validated by reverse transcription-PCR and Western blotting of chikungunya virus RNA and proteins.	Selectivity index of 19.2 in BHK-21 cells and 10.9 in Huh-7.5 cells. Bands for chikungunya virus RNA and proteins are significantly reduced.	230
				Ivermectin				Selectivity index of 62.4 in BHK-21 cells and 4.1 in Huh-7.5 cells. Bands for chikungunya virus RNA and proteins are significantly reduced.	
			Benzylisoquinoline alkaloids	Berberine	*Berberis vulgaris*			Selectivity indexes of >55.64 in BHK-21 cells and >52.6 in Huh-7.5 cells. Bands for chikungunya virus RNA and proteins are significantly reduced.	
Dengue	Dengue viruses (DENV-1, -2, -3, and -4) are spread through the bite of an infected *Aedes* species mosquito	Mostly Africa, Asia, and South America	Polysaccharides	Galactan	*Cryptonemia crenulata* collected from Cupe's beach, Pernambuco State, Brazil	Powdered whole-plant material extracted with water, followed by KCl fractionation and DEAE-Sephacel chromatography.	*In vitro* plaque reduction and virus yield inhibition assay on cell lines exposed to different dengue virus strains.	IC_{50} of 5.2–13.9 μg/ml against DENV-3 plaque formation or virus yield in three cell lines. IC_{50} of 3.6–14.7 μg/ml against DENV-2 plaque formation or virus yield in three cell lines.	221
				Kappa carrageenan	*Gymnogongrus griffithsiae* collected in Caioba, Parana State, Brazil	Powdered whole-plant material extracted with water, followed by KCl fractionation.		IC_{50} of 5.2–13.9 μg/ml against DENV-3 plaque formation or virus yield in three cell lines. IC_{50} of 0.31–1.8 μg/ml against DENV-2 plaque formation or virus yield in three cell lines.	
			Flavonoid	Baicalein	*Scutellaria baicalensis*	Purchased from the Indofine Chemical Company (USA).	*In vitro* cytotoxicity compared to focus formation unit reduction and DENV-2 RNA quantitative reverse transcription-PCR of Vero cells infected with DENV-2 after various conditions of baicalein treatment to derive a selectivity index (CC_{50} cells/IC_{50} virus).	Selectivity index of 16.1 for antiviral adsorption activity. Selectivity index of 17.8 for postviral adsorption activity. Selectivity index of 21.3 for antiviral activity during continuous treatment.	222
			Protoberberine alkaloid	Palmatine	*Coptis chinensis*	Purchased from Chengdu Mansite Pharmaceutical Company (China).	*In vitro* cytotoxicity compared to virus titer measurements from Vero cells infected with DENV-2 to derive a selectivity index (CC_{50} Vero cells/EC_{50} virus).	Selectivity index of 39.	223
			Triterpenoid	Glycyrrhizin	*Glycyrrhiza uralensis*, *G. glabra*	Purchased from Sigma-Aldrich Chimie SARL (France).	*In vitro* cytotoxicity in confluent or proliferating Vero cells compared to virus-induced pathogenicity measurements from Vero cells infected with DENV-1, -2, and -4 to derive a selectivity index (CC_{50} Vero cells/EC_{50} virus).	Selectivity index of 6 for DENV-1 and DENV-2. Selectivity index of 4 for DENV-4.	224

(Continued on next page)

TABLE 5 (Continued)

Disease	Description and pathogenesis	Geographical distribution	Product family/class	Extracts/ natural product	Product source or origin	Extraction method	Efficacy/assessment model	Biological activity	Reference
			Not specifically identified		*Carica papaya*	Fresh juice extracted using a juice extractor from Sekaki variant *C. papaya* leaves sourced from a private plantation in Selangor, Malaysia.	Open-label randomized controlled human trial involving recruited patients from the dengue ward in a hospital at Selangor, Malaysia. Patients received 50 g of fresh juice, 15 min after breakfast, for 3 days.	Increased platelet count and increased ALOX12 and PTAFR expression in patients from the intervention group.	217
						Capsules containing material extracted from *C. papaya* leaves with ethanol registered for sale in Indonesia.	Human trial involving dengue patients in Indonesia who received 24 capsules of *C. papaya* leaf extract taken twice daily.	Increased platelet counts, shorter hospitalization, and increased stability of hematocrit in patients from the intervention group.	218
			Multiple chemical classes	Carvone/limonene/bicyclose-squiphellandrene/ piperitenone/piperitone/ β-bourboneno	*Lippia alba*	Experimental plant material was grown from propagation cuttings collected from the Cundinamarca and Antioquia regions in Colombia. Essential oil mixtures were extracted from plant material via microwave-assisted hydrodistillation in a Clevenger-type apparatus and identified through chromatography and spectroscopy.	*In vitro* cytotoxicity in Vero cells compared to virus titer measure-ments from Vero cells infected with treated DENV-1, -2, -3, and -4 to derive a selectivity index (CC_{50} Vero cells/EC_{50} virus).	Selectivity index of 4–349.	219
				Geranial/neral/limonene/ 1,8-cineole/spathulenol/ geraniol/*trans*-β-cariofilene/ nerol/geranyl acetate	*Lippia citriodora*			Selectivity index of 2–30.	
Ebola	A rare and deadly viral disease caused by genus *Ebolavirus*. The disease is transmitted through direct contact with an infected animal or sick or dead Ebola virus-infected human	Sub-Saharan Africa	Bisbenzylisoquinoline alkaloid	Tetrandrine[b]	*Stephaniae tetrandra* and other *Menispermacae* plant species	Tetrandrine was purchased from an unspecified commercial vendor.	*In vivo* mouse model of EBOV infection via the i.p. route. Mice were treated i.p. with tetrandrine periodically. Viral load in blood was measured via plaque-forming assays.	Significant protection against EBOV-induced weight loss, mortality, and clinical score. 1,000-fold decrease in viral load at day 3 of infection.	232
			Not specifically identified		*Prunella vulgaris*	Dried fruit spikes were purchased from Tong Ren Tang Health Pharmaceutical Co., Ltd. (China), and were soaked and then boiled in deionized water.	*In vitro* viral infection inhibition assay on pretreated Vero E6 cells infected with recombinant eGFP-Zaire EBOV virus.	>50% reduction in infection in the presence of 6.25 μg/ml of aqueous *Prunella vulgaris* extract.	220
Japanese encephalitis	Japanese encephalitis is a flavivirus transmitted via bite from an infected *Culex* species mosquito	Asia and the western Pacific	Triterpenoid	Glycyrrhizin	*Glycyrrhiza uralensis, G. glabra*	Purchased from Sigma-Aldrich Chimie SARL (France).	*In vitro* cytotoxicity in confluent or proliferating Vero cells compared to virus-induced pathogenicity measurements of Vero cells infected with JEV to derive a selectivity index (CC_{50} Vero cells/EC_{50} virus).	Selectivity index of 7.	224
			Dihydroxyanthraquinone	Aloe-emodin	*Rheum palmatum*	Purchased from Sigma Chemical Co. (USA).	*In vitro* cytotoxicity in HL-CZ and TE-671 cells compared to a plaque reduction assay on HL-CZ and TE-671 cells pretreated with aloe-emodin and then infected with T1P1 strain of JEV to derive a therapeutic index (CC_{50} cells/IC_{50} virus).	Therapeutic index of >1,500.	225
West Nile fever	A mosquito-borne disease caused by vectors infected with West Nile virus spread through bite of an infected mosquito	Africa, parts of Europe, the Americas, Middle East, West Asia, and Australia	Triterpenoid	Glycyrrhizin	*Glycyrrhiza uralensis, G. glabra*	Purchased from Sigma-Aldrich Chimie SARL (France).	*In vitro* cytotoxicity in confluent or proliferating Vero cells compared to virus-induced pathogenicity measurements from Vero cells infected with WNV to derive a selectivity index (CC_{50} Vero cells/EC_{50} virus).	Selectivity index of 11 for proliferating healthy cells and >13 for confluent healthy cells.	224
			Protoberberine alkaloid	Palmatine	*Coptis chinensis*	Purchased from Chengdu Mansite Pharmaceutical Company (China).	*In vitro* cytotoxicity compared to virus titer measurements from Vero cells infected with WNV to derive a selectivity index (CC_{50} Vero cells/EC_{50} virus).	Selectivity index of 286.	223
Yellow fever	A rare mosquito-borne viral infection spread through bite of an infected *Aedes* or *Haemagogus* species mosquito	Tropical and subtropical areas of Africa and South America	Terpenes	Carvone/limonene/ bicyclosesquiphellandrene	*Lippia alba* collected from Jordan Sube, Colombia	Essential oil mixtures were extracted by microwave-assisted hydrodistillation in a Clevenger-type apparatus, followed by chromatographic and spectroscopic identification.	*In vitro* cytotoxicity in Vero cells compared to a plaque reduction assay of Vero cells infected with YFV treated with extracted essential oils to derive a CC_{50}/MIC ratio.	CC_{50}/MIC ratio of 22.9.	226
				Carvacrol/thymol/γ-terpinene	*Lippia origanoides* collected from Jordan Sube, Colombia			CC_{50}/MIC ratio of 26.4.	
				trans-Sabinene hydrate/thymol/ carvacryl methyl ether/γ-terpineno/*p*-cimene	*Oreganum vulgare* collected from Marinilla, Colombia			CC_{50}/MIC ratio of 26.5.	
				α-Thujone/β-thujone/1,8-cineole/*trans*-carveol/ *trans*-carveol/sabinene	*Artemisia vulgaris* collected from Armenia, Colombia			CC_{50}/MIC ratio of 8.8.	

(Continued on next page)

TABLE 5 (Continued)

Disease	Description and pathogenesis	Geographical distribution	Product family/class	Extracts/ natural product	Product source or origin	Extraction method	Efficacy/assessment model	Biological activity	Reference
			Protoberberine alkaloid	Palmatine	*Coptis chinensis*	Purchased from Chengdu Mansite Pharmaceutical Company (China).	*In vitro* cytotoxicity compared to virus titer measurements from Vero cells infected with YFV to derive a selectivity index (CC_{50} Vero cells/EC_{50} virus).	Selectivity index of 141.	223
			Triterpenoid	Glycyrrhizin	*Glycyrrhiza uralensis, G. glabra*	Purchased from Sigma-Aldrich Chimie SARL (France).	*In vitro* cytotoxicity in confluent or proliferating Vero cells compared to virus-induced pathogenicity measurements from Vero cells infected with two strains of YFV to derive a selectivity index (CC_{50} Vero cells/EC_{50} virus).	Selectivity index of 5 to >6 against both strains of YFV.	224
Zika	A mosquito-borne viral infection spread through bite of an infected *Aedes* species mosquito, by sex, and by pregnant mother to fetus	Africa, Asia, and the Americas	Anthraquinone	Emodin	*Rheum palmatum, Cassia obtusifolia, Polygonum multiflorum, Aloe barbadensis*	Not mentioned.	*In vitro* cytotoxicity in Vero E6 cells compared to plaque-forming measurements from Vero E6 cells incubated with ZIKV to derive a selectivity index (CC_{50} Vero cells/IC_{50} virus).	Selectivity index of 21.44.	231
			Benzylisoquinoline alkaloids	Berberine	*Berberis vulgaris*			Selectivity index of 5.65.	
			Triterpenoid	Glycyrrhizin	*Glycyrrhiza uralensis, G. glabra*	Purchased from Sigma-Aldrich Chimie SARL (France).	*In vitro* cytotoxicity in confluent or proliferating Vero cells compared to virus-induced pathogenicity measurements from Vero cells infected with ZKV to derive a selectivity index (CC_{50} Vero/EC_{50} virus).	Selectivity index of >7.	224

[a]i.p., intraperitoneal; eGFP, enhanced green fluorescent protein; ALOX12, arachidonate 12-lipoxygenase; PTAFR, platelet-activating factor receptor.
[b]Tetrandrine, originally isolated from Chinese and Japanese herbs, is now produced synthetically.

-3, and -4) has also been reported for *Lippia* species plants (219) (Table 5). Inhibitory activities of natural products against Ebola have also been explored, and aqueous extract of *Prunella vulgaris* has been shown to be a potent inhibitor of EBOV entry *in vitro* (220) (Table 5).

Natural compounds such as galactan (221), kappa carrageenan (221), baicalein (222), and palmatine (223) have been shown to have antiviral activity against DENV-2, whereas glycyrrhizin has displayed antiviral effects against both DENV-1 and DENV-2 (224) (Table 5). Furthermore, glycyrrhizin has also shown antiviral activity against Japanese encephalitis virus (JEV), West Nile virus (WNV). and yellow fever virus (YFV) (224) (Table 5). Antiviral activity against WNV and YFV has also been described for palmatine (223), whereas anti-JEV activity has been reported for aloe emodin, a natural anthraquinone derived from *Rheum palmatum* (225) (Table 5). Additionally, a Colombian study revealed that essential oils extracted from *Lippia alba*, *Lippia origanoides*, *Oreganum vulgare*, and *Artemisia vulgaris* show inhibitory effects against YFV (226) (Table 5). The natural compounds from these plants include, among others, carvone, limonene, bicyclosesquiphellandrene, carvacrol, thymol, gamma-terpinene, *trans*-sabinene hydrate, and sabinene (226) (Table 5). Antiviral activity against other mosquito-transmitted viruses, including chikungunya virus (CHIKV) and Zika virus (ZIKV), has also been demonstrated for jatrophane esters (227), luteolin (228), apigenin (228), ethanolic extracts from several *Euphorbia* species (229), abamectin and ivermectin (230), berberine (230, 231), and emodin (231) (Table 5). Finally, tetrandine isolated from radix stephaniae tetrandrine, the dried root of *Stephania tetrandra*, was described as a potent EBOV entry inhibitor (232) (Table 5).

Fungi

Several studies have described plant-based treatments for various tropical fungal diseases (233–237) (Table 6). Interestingly, propolis (bee glue) from the honey bee *Apis mellifera* also demonstrates strong fungicidal effects against *Paracoccidioides brasiliensis* and *Sporothrix schenckii* (238, 239). Although the active antifungal product within propolis remains elusive, it was shown that *p*-coumaric acid appears to be the major compound (239).

Some studies have also investigated the antifungal activity of compounds isolated from natural products (Table 6). Medina-Alarcon and colleagues have reported that chalconoids derived from *Cinnamomum verum* display fungicidal activity against *Paracoccidiodes brasiliensis* and *Paracoccidioides lutzii* (240). Fungicidal effects against paracoccidioidomycosis have also been demonstrated with methyl linolenate, caryophyllene oxide, and *trans*-nerolidol derived from the Brazilian medicinal plant *Baccharis dracunculifolia* (241).

CONCLUSIONS

Here, we examine how natural products are being harnessed to develop solutions to the diseases of the tropics. Our literature search revealed that while there is vast knowledge of medicinal use from many tropical plants and animals, overall, the underlying active compounds remain largely unknown or else restricted to crude extracts. Further, while many emerging natural products have been tested for their efficacy against various tropical diseases, much of the work currently is done using *in vitro* assays. Given that the Amazon rainforest alone harbors at least 15,000 plant species (242) and the Great Barrier Reef is home to 1,400 species of coral, 3,000 species of mollusks, and 630 species of echinoderms (Australian Government Great Barrier Reef Marine Park Authority; http://www.gbrmpa.gov.au/, accessed 20 April 2020), the potential reservoir of natural products for treating tropical diseases is enormous. While we describe many natural products containing compounds with activity against tropical disease-causing pathogens, we find that there is currently a discovery bottleneck due to the high failure rate and the significant investment required to take a promising raw natural product forward in order to become a new standard-of-care treatment for a tropical pathogen. This limitation is being overcome with improved high-throughput technical capacity for natural product isolation and identification in many tropical areas of the world, ensuring that more of these natural reservoirs will likely reveal their pharmaceutical secrets in the near future.

TABLE 6 Active compounds from natural products with activity against tropical disease-causing fungi[a]

Disease	Description and pathogenesis	Geographical distribution	Product family/class	Extracts/natural product	Product source	Extraction method	Efficacy/assessment model	Biological activity	Reference(s)
Paracoccidioidomycosis	Fungal infection caused by the fungus *Paracoccidioides*	Central and South America	Flavonoids	Chalconoids	*Cinnamomum verum*	Nanoemulsion	*In vitro*: Antifungal activities of the compounds 2′ chalc and NLS + 2′ chalc were tested at different concentrations, 0.24–250 μg/ml, in a 96-well plate. MICs were observed via color change. MFCs were assessed for growth.	The MIC is the lowest compound concentration at which colonies were observed. NLS + 2′ chalc showed significant antifungal activity.	240
			Terpenes	Methyl linolenate Caryophyllene oxide *Trans*-nerolidol	*Baccharis dracunculifolia* *Baccharis dracunculifolia* *Baccharis dracunculifolia*	Dried plant part was extracted with ethanol and fractionated through silica gel. 105 fractions were collected; ursolic acid, methyl linolenate, caryophyllene oxide, and *trans*-nerolidol were isolated.	*In vitro*: Fungal strains inoculated with the fractions were visually compared with drug-free growth control. The MIC for which the well was optically clear was observed.	The MIC is the lowest compound concentration at which colonies were observed.	241
			Terpenes	Caryophyllene, kaurenoic acid, copalic acid	*Copaifera langsdorffii*	Copaiba resin oil was extracted from the trunk of a *C. langsdorffii* tree. A nanoemulsion of copaiba resin oil (CopaPlu) was formed by dissolving the extract and Pluronic F-127 in ethanol, followed by evaporation at 60°C.	*In vitro*: Copaiba resin oil and CopaPlu were tested at different concentrations against isolates of *P. lutzi*, *P. brasiliensis*, *P. americana*, and *P. restrepiensis*. The MIC was noted, and the MFC of 20 ml of each well with no visible fungal growth was examined.	Copaíba resin oil and CopaPlu inhibited the growth of all isolates.	235
			Terpenes	Ethyl hydrocinnamate, spathulenol	*Baccharis dracunculifolia*	Aerial parts of *B. dracunculifolia* and *P. regnellii* were extracted by maceration with 80% ethanol and concentrated with hydroalcoholic extract, distilled in water, and extracted with hexane dichloromethane and ethyl acetate.	*In vitro*: Fungal strains were inoculated with the extracts and visually compared with drug-free growth control. The MIC for which the well was optically clear was observed.	The hexane fractions from both *B. dracunculifolia* and *P. regnellii* showed the best MIC value (7.8–30 μg/ml) against *P. brasiliensis*.	236, 241
			Phenols	1-Methoxy-4-(1-propenyl) benzene, apiol	*Piper regnellii*				
				Crude propolis	*Apis mellifera*	Frozen propolis was ground and extracted with ethanol. The extracts were filtered and diluted in distilled water.	*In vitro*: The number of CFU of *P. brasiliensis* was assessed.	Fungicidal activities of macrophages of *P. brasiliensis*.	238
Chromoblastomycosis	Chronic fungal disease caused by a variety of genera of the order Chaetothyriales	Africa and South America	Not specifically identified		*Pterocaulon alopecuroides*	Dried, powdered aerial parts of *P. alopecuroides* were macerated in methanol for 3 days at a ratio of 1:10 (wt/vol).	*In vitro*: The absence of organism growth indicates fungicidal activity.	Crude methanolic extract of *P. alopecuroides* inhibited organism growth of all chromoblastomycosis agents tested.	233
Mycetomas	Chronic fungal infection of the skin and subcutaneous tissue caused by eumycetoma found in soil and water.	Sub-Saharan Africa, Central and South America, Asia	Phytosterols	Stigmatriene	*Boswellia papyrifera*	Dried, powdered samples were macerated in methanol for 7 days at room temperature. Crude methanol extract, hexane fraction, and a defatted methanol fraction were obtained from the vacuum-dried methanolic extract through liquid-liquid separation using hexane.	*In vitro*: Isolates were cultured for 10 days at 37°C. The MIC was defined as the first well in which no growth was visible.	Although all crude methanolic extracts were able to inhibit *M. mycetomatis*, lower concentrations of stigmatriene inhibited *M. mycetomatis* the most.	234
				Crude extract Crude extract	*Acacia nubica* *Nigella sativa*				
Sporotrichosis	Fungal infection caused by the fungus *Sporothrix*.	South America, China, South Africa		*O*-glucosyl-flavonoids, 16 prenylated benzophenone	*Vismia guianensis*	A mixture of stem bark or leaves of *V. guianensis* powder was soaked in ethanol and solvent solution at 10% (wt/vol), filtered, and reextracted with solvents, and extract was evaporated and lyophilized.	*In vitro*: *V. guianensis* extracts were tested in *S. schenckii* isolates from humans. The MIC is the lowest antifungal agent concentration that inhibits fungal growth. Mice: Oral administration at a dose of 10 mg/kg body weight.	*In vitro*: Treatment of *S. schenckii* with *V. guianensis* extracts inhibits the growth of the organism significantly. Mice: Clinically used for treating *S. schenckii*.	237
				Crude propolis	*Apis mellifera*	Hydroalcoholic extract of frozen brown propolis was evaporated, and dry matter was dissolved in phosphate buffer, emulsified, filtered, and diluted in sterile distilled water.	*In vitro*: Hydroalcoholic extract of brown propolis was tested *in vitro* against *Sporothrix brasiliensis* isolates. The MIC was noted, and the MFC of 10 ml of each well with no visible fungal growth was examined.	Treatment of *Sporothrix brasiliensis* with brown Brazilian propolis inhibited growth of 100% of the isolates.	239

[a]Drugs are currently in (pre)clinical trials. 2′ chalc, 2′-hydroxychalcone; NLS, nanoemulsion; MFC, minimum fungicidal concentration.

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