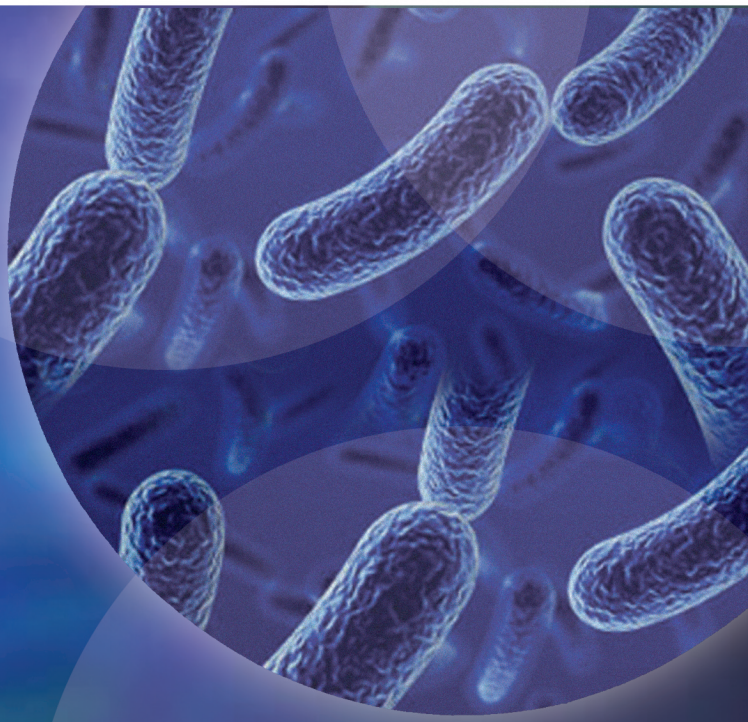
Frontiers in Clinical Drug Research

(Anti Infectives)



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Frontiers in Clinical Drug Research-Anti Infectives

(Volume 7)

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CONTENTS

PREFACE

The 7th volume of Frontiers in **Clinical Drug Research – Anti Infectives** comprises five chapters that cover a variety of topics including antivirals, treatments against some gram negative and gram negative bacteria, and an overview on a few antiprotozoal drugs that target specific pathogens.

In chapter 1, Evran *et al.*, focus on aptamers with antiviral activity, as well as the use of aptamers in viral detection platforms. They also give an overview of aptamers developed against viruses, and discuss the major hurdles in aptamer use, as well as the strategies to improve the drug potential of aptamers.

In chapter 2, Leowattana *et al.* discuss host-directed, antibiotic-adjuvant combinations and antibiotic-antibiotic combination for treating Multidrug-Resistant (MDR) gram- negative pathogens (*Acinetobacter, Enterobacteriaceae, Pseudomonas,*etc.).

In chapter 3, Barbosa and Teixeira explore the current therapeutic approaches and advances in the search for alternative solutions to inhibit the opportunistic pathogen *C. difficile.*

Rivera-Fernández *et al.* in chapter 4 of the book, review the *in vitro* and *in vivo* activities of extracts, fractions, and isolated compounds obtained from different plants against *Toxoplasma gondii,* the pathogen that causes toxoplasmosis. This chapter presents information on potential leads for novel therapeutic agents for this disease.

In the last chapter of the book by Percário *et al.,* the author describe the main Amazonian species used to treat malaria and leishmaniasis in Brazilian folk medicine, relating ethnobotanical results to chemical studies, evaluation of activities, and toxicity. Several promising compounds of plants used in traditional Amazonian medicine are described

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CHAPTER 1

Nucleic Acid and Peptide Aptamers as Potential Antiviral Drugs

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Abstract: Aptamers with target-specific binding properties have emerged as an alternative to antibodies. Nucleic acid aptamers are short single-stranded oligonucleotides that can fold into unique three-dimensional structures. Nucleic acid aptamers are selected from random libraries *in vitro* by using the SELEX (Systematic Evolution of Ligands by Exponential Enrichment) technology. Likewise, peptide aptamers are short peptides that can be selected *in vitro* by using different strategies including phage display, ribosome display, or mRNA display. Aptamers are superior to antibodies with regard to ease of production, high stability, small size, and low cost. Therefore, aptamers find broad use in different biotechnological and therapeutic applications. Among them, aptamer use in virus detection and antiviral therapy is one of the attractive applications. The present Covid-19 pandemic and life-threatening viral infections reveal the need for rapid therapeutic solutions that can efficiently target viral mechanisms. In this respect, the chapter is mainly focused on aptamers with antiviral activity, as well as the use of aptamers in viral detection platforms. First, we summarize aptamer selection technologies that can be performed *in vitro*. Among them, we briefly explain ribosome display, mRNA display and SELEX (Systematic Evolution of Ligands by Exponential Enrichment) technologies. Then, we review aptamers targeting viral proteins and viral invasion mechanisms. In addition, we give an overview of aptamers developed against viruses. We also discuss the major hurdles in aptamer use, as well as the strategies to improve the drug potential of aptamers.

Keywords: Antiviral aptamer, Aptasensor, Diagnostic aptamers, DNA aptamer, MRNA display, Peptide aptamer, Ribosome display, RNA aptamer, SELEX, Therapeutic aptamers.

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1. INTRODUCTION

Nucleic acid aptamers and peptide aptamers with antibody-like binding properties are promising therapeutic agents. Several aptamers are currently evaluated under clinical phase studies, but the majority of studies are focused on metabolic diseases. In this chapter, we aim to highlight the potential use of aptamers as novel antiviral agents, and their importance in diagnosis and monitoring viral infections.

The first section of the chapter gives an overview of the *in vitro* selection methods used to identify peptide and nucleic acid aptamers. Here, only some methods developed for selection from combinatorial libraries are given. This section is divided into three sub-sections as 1.1, 1.2 and 1.3 to introduce the methods of mRNA display, ribosome display and SELEX. The SELEX section is further divided into 1.4.1 and 1.4.2 to summarize two of the SELEX methods, namely cell-SELEX and bead-based SELEX. The final sub-sections 1.5 and 1.6 explore the modification strategies to improve the stability properties of aptamers for therapeutic use.

The second section of the chapter is devoted to aptamers used for the detection of viruses. The sub-sections 2.1-2.9 summarize the studies for diagnosis of Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Human Immunodeficiency Virus (HIV), Influenza, Arboviruses, SARS virus, Ebola Virus, SARS-CoV-2, and Human Papilloma Virus (HPV).

The third section of the chapter is devoted to the aptamers targeting viral proteins. Aptamers developed against several proteins of HIV, HCV, HBV, SARS, and Influenza are summarized under the sections 3.1-3.5.

1.1. The *in vitro* Selection Methods for Peptide and Nucleic Acid Aptamers

Directed evolution is a powerful tool to develop proteins with superior function and binding properties [1]. One critical step of directed evolution is the screening or selection of improved variants from a large library [2]. Advances in molecular techniques have allowed the design of highly diverse libraries of nucleic acids, peptides and antibodies [3]. To meet the demand for working with large libraries, protein display technologies have been developed for the selection, isolation and identification of proteins with the desired properties [4,5]. Display techniques are basically divided into two groups: (i) cell-free and (ii) cell-based. Cell-based approaches such as bacterial display, yeast display, mammalian cell display and phage display have some limitations regarding the efficiency of recovery and library diversity [6]. Although phage display has been widely used to select

proteins and peptides with improved binding properties [7], cell-free display methods have emerged as an alternative to overcome the limitations associated with living cells [8].

Ribosome display and mRNA display are cell-free methods, which rely on *in vitro* transcription and translation of the newly formed peptide along with its encoding mRNA. Thereby, ribosome display and mRNA display can establish a direct link between phenotype and genotype. *In vitro* display and selection approach consists of 3 steps: (i) designing the initial library, (ii) performing repetitive rounds to obtain the desired characteristics, (iii) screening and characterizing the selected variants [9]. Engineered antibodies, proteins, as well as peptides for various applications in diagnostics and therapeutics have been identified by *in vitro* selection methods [10]. Ribosome display and mRNA display enable identification of high-affinity proteins or peptide aptamers [11,12], whereas SELEX (Systematic Evolution of Ligands by Exponential enrichment) method allows *in vitro* nucleic acid aptamer selection. As shown in Table **1**, those *in vitro* methods allow working with libraries of large size. Cell-based approaches are limited by cell growth and replication [13]. In contrast, *in vitro* selection methods allow precise control of many parameters like pH, temperature, buffer conditions, and ionic strength [14]. Auxiliary components including the binding target and reaction substrates can be easily added to the selection medium, thereby eliminating the toxic effect problem that may be encountered in cell-based display methods [15].

Table 1. Library size of *in vitro* selection methods.

In vitro method	Size of library	References
SELEX	10^{14}-10^{15}	[16]
mRNA display	10^{12}-10^{13}	[14]
Ribosome display	10^{13}-10^{14}	[17]

1.2. MRNA Display

mRNA display is based on the formation of a covalent link between the target peptide/protein and the mRNA encoding it. For this aim, 3' end of mRNA is modified with puromycin, an antibiotic molecule that acts like an aminoacylated tRNA. Upon translation of the modified mRNA, puromycin enters the A region of the ribosome and forms a peptide bond with the C-terminal of the polypeptide. In this way, the puromycin-modified mRNA is covalently linked to the peptide [18]. This generates a stable link between genotype and phenotype. In addition, this stable covalent bond allows selection under stringent conditions. In mRNA

CHAPTER 2

Host-Directed, Antibiotic-Adjuvant Combination, and Antibiotic-Antibiotic Combination for Treating Multidrug-Resistant (MDR) Gram-Negative Pathogens

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Abstract: Antibiotics were firstly used for the treatment of critical infections in the 1940s. They could save patients' lives and increased life spans by improving the outcome of serious infections. Antibiotics are the most commonly used drugs in a healthcare environment. However, antibiotics are not correctly prescribed, due to improper antibiotic selection, not suitable dosing, inappropriate treatment duration, and wrong treatment in nonbacterial conditions. Consequently, the rapid emergence of resistant bacteria occurs worldwide because they could adapt and compete with environmental stress. There are 4 primary mechanisms of resistance to counter the antibiotics: (i) modification of the target, (ii) enzymatic inhibition of the antibiotics, (iii) active efflux of the antibiotics, and (iv) changing membrane permeability. Carbapenem-resistant *Pseudomonas aeruginosa* (CR-PA), CR *Acinetobacter baumannii* (CR-AB), and CR *Enterobacteriaceae* were declared by World Health Organization (WHO) as the three most important pathogens that pose the greatest threat to human health. Moreover, they are also multidrug-resistant (MDR) and usually resist almost all of the most effective antibiotics, including carbapenem and fourth-generation cephalosporin. There is an urgent need to develop new strategies to combat antibiotic resistance and preserve the existing antibiotics. Numerous approaches, including host-directed, antibiotic-adjuvant combination, and antibiotic-antibiotic combination therapy, have been put forward to bring about antibiotic efficacy against MDR pathogens.

Keywords: Antibiotic-Adjuvant Combination, Antibiotic-Antibiotic Combination, Gram-Negative Pathogens, Host-Directed, Multidrug-Resistant.

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INTRODUCTION

Bacterial infections remain a significant global health problem. Antibiotics have bring about a disease in prevalence from bacterial pathogens; however, we increasingly confront antimicrobial resistance (AMR) to these antibiotics, and the development of new antibiotics lags beyond the emergence of the bacterial resistance. The problem is not confined to human-linked habitats since different works have shown that other ecosystems, including animals, soil, and water bodies, contribute to the origin, spread, and maintenance of AMR. Moreover, multidrug-resistant (MDR) microorganisms are among the most prominent healthcare problems of the 21st century and are responsible for 60,000 - 70,000 deaths per year in the United States and Europe [1, 2]. These situations are prevalent in the low- and middle-income countries where the resistant strains are hard to detect. Recently, World Health Organization (WHO) has reported that approximately 51% and 65% of infections are resistant to penicillin and ciprofloxacin, respectively, in many countries [3]. The antibiotics are widely used in medical therapeutic facilities and selection pressure is developed that increases MDR pathogens; thus there is a further need for new-generation antibiotics with novel antibacterial properties [4, 5]. To mitigate the MDR pathogen, combination treatment is an urgent option. Combining two antibiotics comprised of a drug targeting the antibiotic resistance activity and an adjuvant are promising new therapeutic strategies [6, 7]. Furthermore, the clinical manifestation of bacterial pathogens reflects a complex interplay between the host, pathogen, and antibiotics. Owing to the innate immune response playing a crucial role in combating bacterial infection, a host-directed therapy combined with an appropriate antimicrobial agent may reduce the antibacterial resistance. This approach may led to a successful clinical outcome and resolve antimicrobial-resistant infections, however resulting in some of the hindrances to antibiotic treatments [8, 9]. This review focused on host-directed therapies by using the immunomodulatory agents that target critical host signaling enzymes exploited by bacteria for their intracellular invasion, replication, and dissemination. We also describe the treatment of MDR Gram-negative bacterial infection with antibiotic-adjuvant combination and antibiotic-antibiotic combination.

MDR GRAM-NEGATIVE PATHOGENS (GNPS)

The number of Gram-negative bacterial infections is more than Gram-positive bacterial infections worldwide. AMR among Gram-negative bacteria is an emerging global problem because of the rapid spread of resistance mechanisms and restricted treatment regimens. Moreover, the incidence of serious infections from MDR-GNPs has increased dramatically and constituted a serious threat to

world public health in the last decade [10 - 12]. The development of MDR strains (non-susceptible to >1 agent in >3 antimicrobial categories), extensively drug-resistant (XDR) strains (non-susceptible to >1 agent in all but <2 antimicrobial categories), and pan-drug-resistant (PDR) strains (non-susceptible to all antimicrobial agents), has increased abruptly. These infections have caused increasingly worsening morbidity and mortality and escalating treatment problems. Furthermore, they also have a great economic impact on healthcare environments due to the rising costs from prolonged hospitalizations. Moreover, the alarming aspect of these is that relatively few new antibiotics have been approved in recent years [13]. GNPs are the common causes of urinary tract infections (UTIs), intra-abdominal infections (IAIs), ventilator-associated pneumonia (VAP), and septicemia. Mainly, *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa* are significant bacteria in the hospital infection, which account for 27% of all pathogens and 70% of all GNPs, causing healthcare-related infections [14]. The most common GNP causing VAP, and the second most common bacteria causing catheter-associated UTIs, is *P. aeruginosa*. The most common GNP causing central line-associated circulatory infections is *K. pneumoniae*. The most common GNPs causing UTIs and the second most common bacteria causing all of the healthcare-associated infections are *E. coli*. A majority of the mortalities are related to MDR-GNPs infections caused by carbapenem-resistant *Enterobacteriaceae* (CRE), extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*, MDR *P. aeruginosa*, and MDR *Acinetobacter baumannii* [15 - 18].

MECHANISM OF RESISTANT DRUGS

The expression of antibiotic inactivating enzymes and non-enzymatic pathways formulates the mechanism of AMR in GNP. The mechanisms take place by increasing the intrinsic resistance due to mutations in chromosomal genes or acquired mobile genetic elements carrying resistance genes. These include aminoglycosides modifying enzymes, plasmid encoding-lactamases, or non-enzymatic mechanisms like Qnr (plasmid-borne quinolone resistance gene) for fluoroquinolone (FQ) [19 - 21]. Notably, more than 10% of the *Enterobacteriaceae* resisted 3rd generation cephalosporin, and approximately 2-7% of it resisted carbapenem. This resistance is caused by the fast distribution of extended-spectrum beta-lactamase (ESBL) producing strains Fig. (**1**). Additionally, the rates of carbapenem resistance for *K. pneumonia* are more than 25%. While *P. aeruginosa* and *A. baumannii* having resistance to carbapenem account for 20 to 40% and 40 to 70%, respectively [22].

CHAPTER 3

Bioactive Substances as Anti-infective Strategies Against *Clostridioides Difficile*

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Abstract: The incidence and severity of diarrhea associated with *Clostridioides difficile* increased exponentially worldwide until 2004. But during the last few years, a downward trend has been observed globally, except in some European countries and Asia.

Until recently, *C. difficile* was the primary cause of nosocomial infection following antibiotic exposure, presenting a high rate of mortality and morbidity. However, the emergence and spread of a hypervirulent strain (BI/NAP1/027) and an increase in the incidence of community-acquired *C. difficile* infection (CDI), especially in populations not previously considered at high risk, have contributed to alterations in the infection epidemiology. After initial treatment with broad-spectrum antibiotics, CDI recurrence is the cause of substantial morbidity, indicating that alternative strategies to the usual therapeutics are urgently needed.

Several studies have investigated probiotics to assess their preventative and/or prophylactic effects on CDI, but their use is still controversial. Other anti-infective alternatives, such as bacteriocins and phage therapy, appear as promising answers for CDI treatment.

This review explores the current therapy approaches and the advances in searching for alternative solutions to inhibit the opportunistic pathogen *C. difficile*.

Keywords: Antibiotics, Antimicrobials, Bacteriocins, Bacteriophages, Clostridioides difficile infection (CDI), Fecal microbiota transplantation, Fidaxomicin, Monoclonal antibodies, Metronidazole, Non-toxigenic spores, Probiotics, Recurrent CDI, Synthetic polymers, Vaccines, Vancomycin.

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INTRODUCTION

Clostridioides difficile (previously *Clostridium difficile*) [1] is a Gram-positive, endospore-forming, and toxin-producing anaerobic species. It was originally isolated from neonates' stools in 1935 [2] as an inoffensive inhabitant of commensal intestinal microbiota. However, in 1978 *C. difficile* was recognized as an important cause of antimicrobial-associated diarrhea in hospitalized individuals [3].

Spores of *C. difficile* can be found ubiquitously in the environment. Once ingested, these highly resistant spores can survive to the gastrointestinal tract barriers and remain inactive, resisting the host's immune system mechanisms. *Clostridioides difficile* produces major toxins responsible for mild-to-severe forms of gastrointestinal infections, ranging from asymptomatic intestinal colonization, self-limiting mild diarrhea to severe or life-threatening pseudomembranous colitis, toxic megacolon, sepsis, and death [4, 5]. The main predisposing factor for developing *C. difficile* infection (CDI) is antibiotic therapy, particularly with broad-spectrum antibiotics [6]. The normal gastrointestinal microbiome acts as a colonization barrier, preventing the spore's germination and their return to a toxin-producing vegetative state [5]. Disturbance of the gastrointestinal microbiome due to antibiotic exposure allows spore germination and vegetative growth of *C. difficile* [6]. Vegetative cells enter the mucus layer and adhere to intestinal epithelial cells, proliferating and colonizing the large intestine, where they produce and release toxins [7].

Gut-targeted therapies for CDI have a relatively direct effect on a mild disease. Nevertheless, the same is not often verified in patients with severe disease [8], and recurrence of CDI occurs in many patients after initial treatment with broad-spectrum antibiotics [9]. Since the main clinical challenges are the recurrence of CDI and the resistance to antibiotics currently used in the therapy, alternative therapeutic strategies are urgently needed.

This chapter intends to review the therapy approaches recommended for CDI treatment and explore the advances in searching for new therapies, emphasizing natural alternatives to currently used antibiotics to inhibit the opportunistic pathogen *C. difficile*.

PATHOGENESIS AND EPIDEMIOLOGY OF *C. DIFFICILE*

The main virulence factor mediating the pathogenesis of *C. difficile* disease is the production of two large toxins, toxin A and toxin B [10, 11], encoded by *tcdA* and *tcdB* genes, respectively. These are located within a region of the chromosome identified as pathogenicity locus or PaLoc [11]. Some strains can also produce a

binary toxin, *C. difficile* transferase (CDT) [12]. This toxin is encoded by *cdtA* and *cdtB* genes that are located outside the PaLoc region at the part of the binary toxin locus in the genome (CdtLoc) [13]. Although there is no evidence that it causes disease *per se*, this binary toxin has been associated with more severe disease [14].

Epidemiology of *C. difficile* has been changing since the mid-2000s; community-associated infections have become more frequent among younger and relatively healthy individuals due to unknown predisposing factors, antibiotic therapy, and previous hospitalization status [15, 16]. The epidemiology of *C. difficile* in children is characterized by the transient colonization of different toxigenic and non-toxigenic strains at different times [17] and, despite the severe forms of CDI are still less common in children [18, 19], rates of recurrent CDI are similar to those in adults.

The emergence and spread of a new hypervirulent *C. difficile* BI/NAP1/027 strain (characterized as BI group by restriction endonuclease analysis, North American pulsed-field type NAP1 by pulse-field gel electrophoresis, and ribotype 027 by polymerase chain reaction ribotyping) [20] may be correlated with the new epidemiology of CDI, due to its increased sporulation [21] or hyperexpression of toxins [22]. The mechanisms by which transmission occurs in the community are not yet known.

CURRENT APPROACHES IN THE TREATMENT OF CDI

Antibiotic therapy is the treatment of choice for CDI. The severity of the disease dictates the specific treatment to be used, based on guideline recommendations [17, 23]. Until recently, vancomycin (bacterial cell wall synthesis inhibition) and metronidazole (inhibition of deoxyribonucleic acid synthesis and DNA degradation) were the first-choice antibiotics. However, recurrent CDI with significant morbidity and mortality upon the cessation of their administration triggered the development of a new and specific RNA synthesis inhibitor antibiotic, fidaxomicin (previously designated as OPT-80) [24]. The use of fidaxomicin was approved in adults (May 2011) and in children (January 2020) by the US Food and Drug Administration (FDA). It does not cause significant changes to the intestinal microbiome of infected patients during [25] and after treatment [26] compared to vancomycin, resulting in lower rates of relapse. Additionally, treatment with fidaxomicin showed reduced acquisition and overgrowth of vancomycin-resistant enterococci and *Candida* spp [27], unlike the treatment with vancomycin. Frequent use of vancomycin should be avoided since it leads to increased colonization of vancomycin-resistant enterococci, increasing the CDI recurrence risk [28]. In addition to the lower cure rates in severe CDI

CHAPTER 4

Anti-*Toxoplasma* Drug Discovery and Natural Products: a Brief Overview

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Abstract: *Toxoplasma gondii*, an apicomplexan protozoan that is considered an opportunistic parasite of medical and veterinary interest, causes toxoplasmosis, which may be asymptomatic in the immunocompetent host, while fatal in the immunocompromised patient. A combination of pyrimethamine-sulfadiazine is the treatment of choice; nevertheless, these two drugs produce severe side effects and are only effective against acute toxoplasmosis, hence, less toxic novel compounds with anti-toxoplasma activity are greatly needed. Natural compounds seem to be a promising source to identify lead compounds against *T. gondii*. In this review, the *in vitro* and *in vivo* activities of extracts, fractions, and isolated compounds obtained from different plants are described. In addition, some biological and pathological generalities of the parasite are reviewed as well. Data were obtained from a bibliographic search throughout digital faculty libraries, Google Scholar search engine, Science Direct, SciELO databases, and the National Center for Biotechnology Information (NCBI). Founded records include the evaluation of 58 extracts, 14 fractions, and 7 compounds belonging to 53 species of 33 plant families. Predominant studies were made on *in vivo* RH *T. gondii* tachyzoites strain and very few in *in vivo* models of both acute and chronic toxoplasmosis. Research of natural compounds against *T. gondii* deserves more attention in order to identify novel drugs useful in the control of toxoplasmosis.

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Keywords: Anti-*Toxoplasma*, Bradyzoite, Medicinal plants, Natural products, Plant extracts, *Toxoplasma gondii*, Toxoplasmicide, Tissue cysts, Tachyzoite, IC^{50}.

INTRODUCTION

Based on their multiple active metabolites with therapeutic properties, natural products have been used for thousands of years to treat human malaises. Nowadays, natural products are still used by approximately four out of five people worldwide as teas, tinctures, extracts or alimentary supplements. Throughout the modern history of mankind, natural compounds have been used to design novel drugs with different uses, such as antimicrobials, antipyretics, and anti-inflammatories. The main antimalarials commonly used around the world were synthesized from natural compounds. Chloroquine was developed from quinine, an alkaloid obtained from the Cinchona stem bark; artesunate and artemether were derived from an artemisinin compound found in *Artemisia annua* leaves [1 - 4]. Based on the fact that plants have been a successful source of antimalarials, the search for novel anti-*Toxoplasma* compounds from natural products has recently increased [5 - 9]. In this review, the *in vitro* and *in vivo* activities of extracts, fractions, and isolated compounds obtained from different plants are described. To better understand the need to develop new treatments, some general aspects regarding *Toxoplasma gondii* biology and toxoplasmosis control, need to be understood. These aspects are herein briefly described.

T. gondii is an apicomplexan parasite of medical and veterinary importance and perhaps the most successful and ubiquitous protozoan. During its life cycle, the parasite develops different infectious phases, as tachyzoites, tissue cysts containing bradyzoites and oocysts [10, 11]. Crescent shape intracellular tachyzoites are responsible for the acute phase of the infection and can disseminate through blood or lymph to invade all nucleated cells [11] (Fig. **1**). Proinflammatory cytokines (mostly interferon-gamma) participate in a not well characterized molecular pathway that allows tachyzoites transformation into bradyzoites and tissue cysts, that are most frequently observed in the immunocompetent host brain [12, 13] (Fig. **2**). The slow proliferation of bradyzoites contained in tissue cysts causes chronic infections and can eventually interconvert into tachyzoites [10]. Oocysts, which are the resistance phase of the parasite, are found in cat feces and become infectious after approximately five days in the environment withstanding extreme conditions for several months [10]. *T. gondii* is considered a food-borne parasite as it can be transmitted by the consumption of raw or poorly cooked meat infected with tissue cysts or by the ingesta of food and water contaminated with infectious oocysts [11]. Tachyzoite infections are rarely reported to occur after drinking unpasteurized milk or by

laboratory accidents. Infection can also occur by transplacental route, transplantation of infected organs or by blood transfusion. *T. gondii* life cycle can be observed in Fig. (**3**). Cats´ hygiene habits, as well as parasites´ life cycle, make direct transmission difficult. *T. gondii* infection goes clinically unnoticed in the immunocompetent host whereas it can be lethal in immunocompromised patients [14, 15]. Unspecific clinical manifestations, such as lymphadenitis, fever, odynophagia, cephalea, myalgias, and in rare cases hepatosplenomegaly, pulmonary or cardiac symptoms are reported in the acute phase. Toxoplasmic encephalitis can lead to death in AIDS patients, while the parasite can cause abortion or malformations in the newborn [13, 16 - 18].

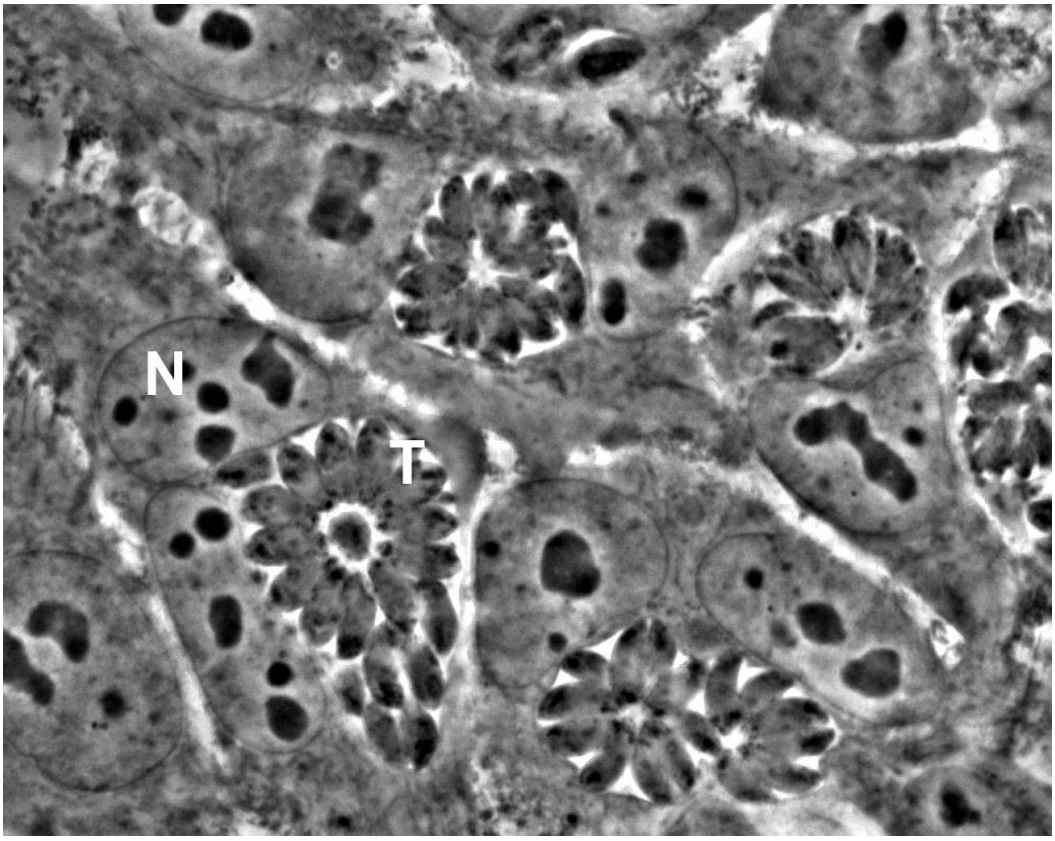

Fig. (1). Phase contrast image of *T. gondii* RH strain tachyzoites in Hep-2 cell culture forming rosettes inside the host cell. N Hep-2 cell nucleus, T tachyzoites. Image obtained at School of Medicine UNAM by Rivera-Fernández N.

CHAPTER 5

Development of Antimalarial and Antileishmanial Drugs from Amazonian Biodiversity

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Abstract: The search for therapeutic alternatives for the treatment of malaria and leishmaniasis is particularly important, given the increase in parasitic resistance to available drugs, as well as the high toxicity of those drugs. In this context, the Amazon region can make an important contribution through its high biodiversity of plants, many of which are informally used by local populations for the treatment of malaria, and leishmaniasis. This chapter aims to describe the main Amazonian species used to treat malaria and leishmaniasis in Brazilian folk medicine, relating ethnobotanical results to chemical studies, evaluation of activities, and toxicity. Different studies report the treatment of malaria with plants, with the most cited species being *Aspidosperma nitidum* Benth. (Apocynaceae); *Geissospermum sericeum* (Sagot.) Benth & Hook (Apocynaceae); *Euterpe precatoria* Mart. (Arecaceae); *Persea americana* Mill (Lauraceae); *Bertholletia excelsa* Bonpl (Lecythidaceae); *Portulaca pilosa* L. (Portulaceae); *Ampelozizyphus amazonicus* Ducke (Rhamnaceae). Additionally, traditional Amazonian populations use plants for the treatment of wounds, a clinical aspect associated with leishmaniasis, with the most cited genus being *Copaiba* and *Jatropha*. The antileishmanial activity of copaiba oil has been demonstrated, and it seems that this activity is related to terpenes. Another genus that deserves attention is *Musa*, used for the treatment of severe wounds. The leishmanicidal activity of triterpenes isolated from *Musa paradisiaca* and its anacardic acid and synthetic derivatives, which have been used against *Leishmania infantum chagasi*, was also tested. In summary, several isolated compounds of plants used in traditional Amazonian medicine are promising as antimalarial and antileishmanial drugs.

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INTRODUCTION

The recognition of the environmental limits of the modern development model has imposed the need for new forms of global governance upon the planetary environment, requiring proposals for sustainable development that oppose the worsening of environmental degradation and biodiversity loss [1].

In Brazil, the Amazon region and its people have been threatened by short-sighted, profit-driven economic interests, driving the increase in deforestation in an increasingly chaotic way. According to the Real-Time Legal Amazon Deforestation Detection System (DETER), deforestation alerts were recorded in an area of 4,219.3 square kilometers in 2018, and in 2019, 9,165.6 square kilometers of forest were deforested – more than double the area recorded in the previous year.

This accelerated deforestation will probably result in the extinction of many plant species, which will have negative impacts on the culture of the use of medicinal plants by the peoples of the Amazon. In 2005, it was estimated that about 180 indigenous peoples (approximately 208,000 individuals) lived in the Amazon, in addition to 357 remaining *quilombola* (maroon) communities and thousands of rubber tapper, riverside or babassu communities [2]. In fact, in addition to its biodiversity in terms of plant and animal species, due to the different ethnicities of its peoples, the Amazon also displays a wide spectrum of cultural diversity.

As a result of this fact, another important issue arises, which is the understanding of the process of occupation of the Amazon and the impact on the health of indigenous peoples and people who settled in the region. In this sense, this process stimulated the occurrence of several epidemics and created an asymmetry in the access to health services. For example, in metropolises, such as Belém and Manaus, health services are structured, while in remote locations within the forest, due to the great difficulty of access, the only therapeutic alternative available to treat diseases has often been the use of medicinal plants [3].

Among these diseases, malaria has been affecting the Amazonian people for centuries. A study conducted in 1885 showed that the Amazon was already plagued by the disease, and the possible explanation for this fact results from the intense migration that occurred to this region in the nineteenth century, resulting from rubber extraction activities and the construction of the Madeira-Mamoré railroad. In this scenario, many immigrants ended up dying from malaria – which

is considered the second major epidemic witnessed by Osvaldo Cruz and Carlos Chagas [4]. At the same time, as opposed to the high mortality experienced by immigrants, the riverside populations survived in this hostile environment due to their ancient knowledge of many native and exotic plant species to treat malaria and its symptoms, and this medicinal information was orally transmitted from one generation to another [5].

Deforestation in the Amazon is still a serious medical and public health problem, as it creates conditions for the development of several tropical endemic diseases, such as American Tegumentary Leishmaniasis (ATL). During deforestation, the rodent population migrates to other areas in search of natural shelters, and phlebotomic fauna, which previously engaged in hematophagy using these small mammals, begin to seek out humans for this purpose [6], thus transmitting the etiological agents of various diseases, such as malaria and leishmaniasis, among others. In this context, plant species that have historically been used in Amazonian folk medicine as healing agents and for wound treatment have shown promise for the treatment of tegumentary leishmaniasis.

This chapter aims to describe the main Amazonian species that are used in folk medicine for the treatment of malaria and leishmaniasis, relating ethnobotanical results to chemical studies, evaluation of activities, and toxicity. Initially, a search was performed for ethnobotanical studies available in different databases, and species with claims of use for malaria and leishmaniasis, for wound treatment, or as healing agents were selected.

Medicinal Plants used for the Treatment of Malaria and Leishmaniasis

Ethnobotanical studies have already been conducted in some regions of the Amazon, but in other regions, there is a lack of scientific studies aimed at describing the uses of plants for medicinal purposes. A range of factors contributes to create difficulties in conducting such studies, such as the large dimension of the territory of the Brazilian Amazon, and difficulties in moving across certain regions due to the lack of connecting roads, implying the need for air transport associated with river transport, which greatly increases the cost of a study. Another important factor is the reduced number of researchers in the ethnobotany field that reside in this region, in addition to scarce funding for these studies because of public policies related to research funding in Brazil.

Notwithstanding, among the studies already published, there are reports of popular use of plants for the treatment of malaria for more than 100 plant species of the region. The most cited families were Apocynaceae, Araceae, Arecaceae, Euphobiaceae, Fabaceae, Menispermaceae, Rhamnaceae and Simaroubacea. Table **1** summarizes the species that were mentioned in the available literature,

SUBJECT INDEX

A

B

C

D

E

F

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H

I

L

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N

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Q

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S

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V

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X

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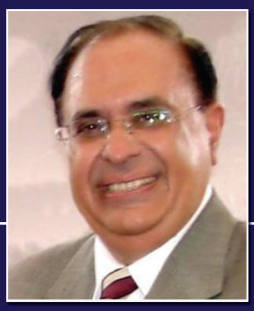

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Prof. Atta-ur-Rahman, Ph.D. in Organic Chemistry from Cambridge University (1968) has 1,232 international publications (45 international patents and 341 books). He received the following awards: Fellow Royal Society (FRS) London (2006), UNESCO Science Prize (1999), Honorary Life Fellow Kings College, Cambridge University (2007), Academician (Foreign Member) Chinese Academy of Sciences (2015), Highest Civil Award for Foreigners of China (Friendship Award, 2014), High Civil Award Austria ("Grosse Goldene Ehrenzeischen am Bande") (2007), Foreign Fellow Chinese Chemical Society (2013), Sc.D. Cambridge University (UK) (1987), TWAS (Italy) Prize (2009). He was the President of Network of Academies of Sciences of Islamic Countries (NASIC), Vice President TWAS (Italy), Foreign Fellow Korean Academy of Science & Technology, President Pakistan Academy of Sciences (2003-2006) and (2011 – 2014). He was the Federal Minister for Science and Technology of Pakistan (2000 – 2002), Federal Minister of Education (2002) and Chairman Higher Education Commission/Federal Minister (2002-2008), Coordinator General of COMSTECH (OIC Ministerial Committee) (1996-2012), and the Editor-in-Chief of Current Medicinal Chemistry.