Drug Resistance Updates 44 (2019) 1–14

Contents lists available at ScienceDirect

Drug Resistance Updates

journal homepage: www.elsevier.com/locate/drup

Target identification and intervention strategies against amebiasis

Shruti Nagaraja, Serge Ankri*

Department of Molecular Microbiology, Ruth and Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel

ARTICLE INFO

Keywords:
Entamoeba histolytica
Amebiasis
Drug targets
Drug repurposing
Vaccination
Probiotics

ABSTRACT

Entamoeba histolytica is the etiological agent of amebiasis, which is an endemic parasitic disease in developing countries and is the cause of approximately 70,000 deaths annually. *E. histolytica* trophozoites usually reside in the colon as a non-pathogenic commensal in most infected individuals (90% of infected individuals are asymptomatic). For unknown reasons, these trophozoites can become virulent and invasive, cause amebic dysentery, and migrate to the liver where they cause hepatocellular damage. Amebiasis is usually treated either by amebicides which are classified as (a) luminal and are active against the luminal forms of the parasite, (b) tissue and are effective against those parasites that have invaded tissues, and (c) mixed and are effective against the luminal forms of the parasite and those forms which invaded the host's tissues. Of the amebicides, the luminal amebicide, metronidazole (MTZ), is the most widely used drug to treat amebiasis. Although well tolerated, concerns about its adverse effects and the possible emergence of MTZ-resistant strains of *E. histolytica* have led to the development of new therapeutic strategies against amebiasis. These strategies include improving the potency of existing amebicides, discovering new uses for approved drugs (repurposing of existing drugs), drug rediscovery, vaccination, drug targeting of essential *E. histolytica* components, and the use of probiotics and bioactive natural products. This review examines each of these strategies in the light of the current knowledge on the gut microbiota of patients with amebiasis.

1. Introduction

Amebiasis is an infection of the gastrointestinal tract caused by the protozoan parasite *Entamoeba histolytica*. *E. histolytica* infection occurs in almost all countries with poor sanitation and high rate of fecal contamination of water and food. The parasite's life cycle has two stages: the cysts which are the infective form of the parasite and are usually found in the fresh stools, and the trophozoites which are the invasive form of the parasite and are generally found in diarrheal stools. Most *E. histolytica* infections are asymptomatic. In symptomatic infections, colitis is the most frequent sign followed by liver abscesses and more rarely brain abscesses. Amebiasis is a prominent cause of severe diarrhea worldwide and is listed among the top 15 causes of diarrhea in the first two years of life in children living in the developing world (Lozano et al., 2012; Shirley et al., 2018). Amebiasis is the third cause of gastrointestinal diseases in returning international travelers after giardiasis and campylobacteriosis (Swaminathan et al., 2009). It is also estimated that amebiasis accounted for 55,500 deaths and 2.237 million disability-adjusted life years (i.e. the sum of years of life lost and years lived with disability) in 2010 (Turkeltaub et al., 2015). The diagnosis of amebiasis still relies on stool microscopy. The main drawback of stool microscopy is the challenge for an inexperienced technician to distinguish between *E. histolytica* and other non-pathogenic *Entamoeba* species that reside inside the human gut, namely *E. dispar, E. moshkovskii, E. polecki, E. coli,* and *E. hartmanni* (Saidin et al., 2018). E. histolytica Gal/GalNAc lectin-antigen stool detection by enzyme-linked immunosorbent assay (ELISA) has been developed to overcome the limitations of the microscopic diagnosis. Of note, molecular approaches based on stool polymerase chain reaction (PCR) have been considered the method of choice for diagnosis of amebiasis from fecal material due to their high sensitivity and specificity (Guevara et al., 2018; Shirley et al., 2018). Since no vaccine against amebiasis currently exists, antiamebic drugs are thus prescribed depending on the severity of the infection (for a recent review see (Martinez-Castillo et al., 2018). These drugs exist as luminal amebicides (such as paromomycin, furoate, diloxanide, and iodoquinol), and tissue amebicides depending on their site of action. Most commonly used tissue amebicides comprise of the nitroimidazole agents, tinidazole (Gonzales et al., 2019) and metronidazole (MTZ) (for a recent review see (Shirley et al., 2018)). MTZ is a prodrug which is reduced by the parasite's thioredoxin reductase and, probably by ferredoxin to produce a nitroradical anion or, if further reduced, a reactive nitroimidazole, and these two

* Corresponding author.
E-mail address: sankri@technion.ac.il (S. Ankri).

https://doi.org/10.1016/j.drup.2019.04.003
Received 14 April 2018; Received in revised form 27 April 2019; Accepted 30 April 2019

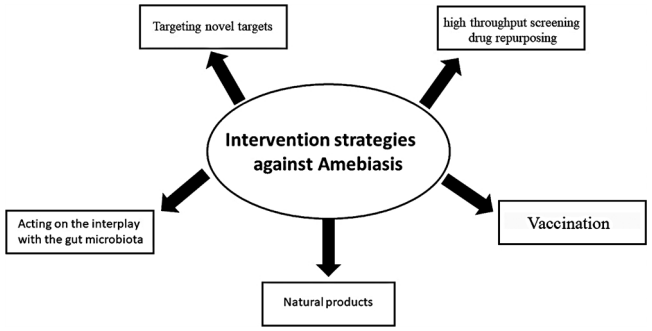

Fig. 1. Intervention strategies to combat amebiasis caused by *E. histolytica*. This figure summarizes the different strategies that are discussed in this review to combat amebiasis.

metabolites are toxic for the parasite (Leitsch et al., 2007). MTZ treatment is associated with substantial side effects such as nausea, vomiting, headaches, a metallic or bitter taste in the mouth, and more serious effects such as anorexia, ataxia, and skin rashes/itching (Andersson, 1981; Roe, 1977).

E. histolytica is capable of acquiring resistance to amebicidal concentrations of MTZ under laboratory conditions, and this drug resistance has been associated with an increased expression of iron-containing superoxide dismutase and peroxiredoxin (Wassmann et al., 1999). Moreover, partial resistance to MTZ has also been described in some clinical strains of *E. histolytica*, suggesting the emergence of MTZ-resistant strains (Bansal et al., 2004; Iyer, 2017). Thus, these observations have led to the search for new drugs with targets and modes of action distinct from those of MTZ. The aim of the current review is to summarize and to discuss these new drugs and their possible modes of action. This review will also discuss less conventional strategies to combat amebiasis (Fig. 1).

2. Finding alternative treatments to amebiasis

2.1. Finding new antiamebic organic compounds

Many potential pharmaceutical drugs contain one or several heterocyclic groups. Some of these compounds that include Schiff's bases complexed with metals, triazines, bisphosphonates, alkylphosphocholines, and diphetarsone have been tested against *E. histolytica* (Fig. 2). This section presents the results of different *in vitro* studies evaluating the effectiveness of these compounds as an anti-amebic drug and their possible mode of action.

2.1.1. Schiff bases

Schiff bases are aldehyde- or ketone-like compounds in which the carbonyl group is replaced by an imine or an azomethine group. Schiff based metal complexes have a wide variety of applications in antimicrobial and anticancer chemotherapy (Malik et al., 2018). The efficiency of different Schiff bases complexed with three different transition metals - palladium, ruthenium, and vanadium have been screened *in vitro* for antiamebic activity (Bharti et al., 2000; Maurya et al., 2006b; Neelam et al., 2000; Singh et al., 2009).

2.1.1.1. Palladium complexes of Schiff bases. Schiff bases derived from S-methyldithiocarbazate (SMDT), 2-acetylpyridine (2 Acpy), S-benzyldithiocarbazate (SBDT), thiosemicarbazones (TSC) and with the inclusion of palladium (Pd) in these complexes ([Pd(2-Acpy-SMDT)$C1_2$], [Pd(2-Acpy-SBDT)$C1_2$], and [Pd(2-Acpy-TSC)$C1_2$]), have been tested effectively against *E.histolytica*. The parent bases, SMDT, 2AcPY, and SBDT have the same amebicidal activity as MTZ (IC_{50} = 0.33 µg/ml). However, the inhibitory effect of two Pd-complexed bases, namely [Pd(2-Acpy-SBDT)$C1_2$] and [Pd(2-Acpy-SMDT)$C1_2$] was significantly higher when compared to their parent bases with an IC_{50} = 0.16 and 0.19 µg/ml, respectively (Neelam et al., 2000).

It remains unclear why palladium enhances the effectiveness of the Schiff bases but its chelation with different metals enhances the lipophilic nature of the complex and consequently increases its permeability in bacteria and fungi (Tumer et al., 1999). Therefore, it is possible that the above mentioned Pd-Schiff base complexes enter more easily in *E. histolytica* than their equivalent Schiff bases but this hypothesis still needs to be tested experimentally.

2.1.1.2. Ruthenium complexes of Schiff bases. The ability of ruthenium (Ru) to exist in two oxidation states (II and III) under physiological conditions has been exploited to develop anticancer drugs. Some similarities between the behavior of cancer cells and *E. histolytica* trophozoites may be exploited to use ruthenium-based complexes as anti-amebic drugs. First, Ru(III) is mostly inactive in a high oxygen environment, whereas in a low oxygen environment, it is reduced and consequently activated to become cytotoxic. This property has been exploited against target cancer cells as they are often living in a low oxygen environment (Gambino and Otero, 2012). *E. histolytica* also thrives in a low oxygen environment in the colon of the infected

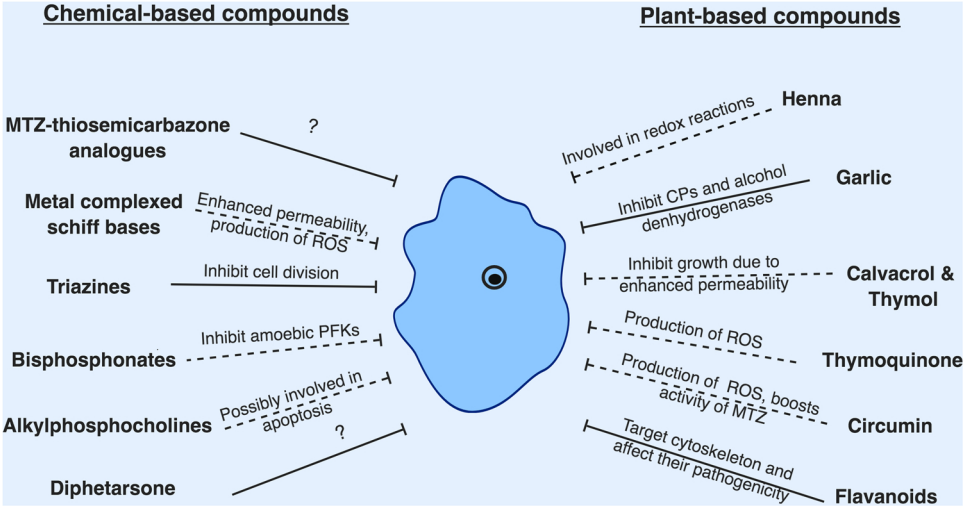

Fig. 2. Effectiveness of chemical-based and plant-based compounds against *E. histolytica*. The mode of action of different amebicidal compounds reported so far has been depicted. The solid lines represent known mechanisms, whereas the dotted lines represent a possible, yet unknown mechanism of action of these compounds.

individual (Pineda and Perdomo, 2017), and thus Ru-based compounds may be able to trigger cytotoxicity in the parasite. For example, [RuCl2(HL4)(HPTA)2]Cl_2 has a very efficient amebicidic activity compared to that of MTZ (IC_{50} = 5.2 ± 0.4μM) *vs* (IC_{50} = 6.8 ± 0.2μM). (Sarniguet et al., 2014). Since the mechanism of this amebicidal activity is not fully understood, it is possible that their interaction with nitrogen and sulfur enhances their binding to proteins and nucleic acids leading to cell death as reported for cancer cells (Thangavel et al., 2017) (Gambino and Otero, 2012).

2.1.1.3. Vanadium complexes of Schiff bases. Vanadium (V)-containing Schiff base complexes induce the production of cytotoxic reactive oxygen species (ROS) in cancer cells (Pessoa et al., 2015) (Rozzo et al., 2017) and display antibacterial activity. Their antibacterial activity is attributed to the presence of electron-donating groups attached to the bases, which activate the Schiff bases (Dikio et al., 2017). [{VO(pydx-smdt)}2μ-O] (IC_{50} = 1.25μM), an oxo-bridged binuclear vanadium Schiff base-complex, is slightly more potent as an amebicidal compound than MTZ (IC_{50} = 1.89μM) (Maurya et al., 2006a, b). Although its amebicidal mechanism is still unknown, [{VO (pydx-smdt)}2μ-O] may induce the production of ROS in the parasite (Sarniguet et al., 2014). Another property of vanadium-containing complexes is to inhibit various mammalian phosphatases (Aureliano et al., 2008; Clausen et al., 2016; McLauchlan et al., 2010; Seargeant and Stinson, 1979) and it will be interesting to test some of them on essential *E. histolytica* phosphatases (Anwar and Gourinath, 2013).

2.1.2. Quinazoline-4-(3 H)-one complexes of Schiff bases

Quinazoline-4-(3 H)-one and its derivatives possess antibacterial and antifungal properties (Grover and Kini, 2006) and antitumor activity (Al-Rashood et al., 2006). Quinazolin-4-(3 H)-one Schiff base conjugates have been developed and tested against *E. histolytica* (Tariq et al., 2018). Out of 13 compounds synthesized, only one of them (quinazolin-4(3 H)-one and 1,2,4-triazole ring) displayed potent anti-amebic activity (IC_{50} = 0.72μM) compared to MTZ (IC_{50} = 1.89μM). The mode of action of quinazolines against cancer cells involves interfering with the DNA repair pathway, transcriptional regulation, and tubulin polymerization (Orvieto et al., 2009; Raffa et al., 2004). The mode of action against *E. histolytica* remains unknown.

2.2. Triazines

Triazines are benzene-aceto-nitrile compounds bearing three nitrogen and carbon atoms, respectively. Triazines have been used by veterinarians to treat coccidiosis, equine protozoal myeloencephalitis, neosporosis, and toxoplasmosis (Stock et al., 2018). The mode of action of triazines is based on inhibition of nuclear division of protozoan organisms (Stock et al., 2018). Recently, the triazine dimer (3,3'-(((ethane-1,2-diylbis(azanediyl))bis(4-(mesityloxy)-1,3,5-triazine-6,2 diyl))bis(azanediyl))dibenzonitrile), has demonstrated potent *in vitro* and moderate *in vivo* antitrypanosomal activity (Venkatraj et al., 2018). The potential of triazines as antiamebic agents have also been tested. The authors reported that two compounds, 5c(N, N′-6-(1,3-benzodioxol-5-yl)-(1,3,5-triazine-2,4-diyl)-bis-4-chlorobenzene sulfonamide) IC_{50} = 1.05 μM, and 5d(N, N′-6-(1,3-benzodioxol-5-yl)-(1,3,5-triazine-2,4-diyl)-bis-4-nitrobenzene sulfonamide) IC_{50} = 1.02 μM are more potent *E. histolytica* inhibitors and less cytotoxic to human cells than MTZ.

5-azacytidine, one of the best known examples of synthetic triazine, is an analog of cytidine currently used against myeloid cancers (Flotho et al., 2009; Orskov and Gronbaek, 2017). 5-azacytidine at a concentration of 23 μM has no significant effect on the growth of the parasite but it impairs its cytopathic activity (ability to destroy mammalian cells) (Ali et al., 2007). Apart from this, 5-azacytidine has been used as a demethylating agent to identify genes regulated by DNA methylation in *E. histolytica*. These studies conclude that only a few genes (cysteine protease, lysozyme and heat shock protein 100) are responsive to 5-azacytidine treatment (Ali et al., 2007; Bernes et al., 2005).

2.2.1. Bisphosphonates

Bisphosphonates are compounds with two phosphonate groups (PO $(OH)_2$) which are linked by esterification and a class of drugs which is currently used to treat osteoporosis (Drake et al., 2008). The competitive inhibition of *E. histolytica* phosphofructokinase (PFK) by six types of bisphosphonates was described in the eighties by Eubank and Reeves (Eubank and Reeves, 1982). In contrast to ATP-dependent PFKs of humans, PFK of *E. histolytica* uses pyrophosphate as a cofactor (Saavedra et al., 2019). PFK is a good amebicidal target as it is essential for the growth of the parasite. An extensive search for additional active bisphosphonates in *E. histolytica* led to the identification of 47 PF K inhibitors with an *in vitro* IC_{50} < 200 μM (Ghosh et al., 2004; Singh et al., 2009). Among these inhibitors, nitrogen-containing bisphosphonates were potent inhibitors of *E. histolytica* PFK (IC_{50} = 4–9 μM) (Ghosh et al., 2004). Interestingly, simple n-alkyl-1-hydroxy-1,1-bisphosphonates were very active against both *E. histolytica* and *Plasmodium falciparum* PFKs with an IC_{50} around 1μM (Ghosh et al., 2004). Apart from the *E. histolytica* PFK, bisphosphonates can inhibit other enzymes which utilize pyrophosphate as cofactor, such as pyruvate phosphate dikinase, PEP carboxytransphosphorylase, and pyrophosphate-acetate kinase (Chiba et al., 2015; Mertens, 1993; Varela-Gomez et al., 2004). Consequently, it is more accurate to consider the inhibitory action of bisphosphonates as a synergistic inhibition of different pyrophosphates-dependent *E. histolytica* enzymes rather than inhibition of PFK. The fact that bisphosphonates are poorly absorbed from the intestine (Pazianas et al., 2013) is an additional argument for the promotion of bisphosphonates as anti-amebic drugs.

2.2.2. Alkylphosphocholines

Alkylphosphocholines (*alkyl-PCs*) are a group of compounds with antineoplastic activity and consist of phosphocholine esterified to various long-chain aliphatic alcohols (Berger et al., 1987; Eibl and Unger, 1990). In contrast to most antitumor compounds, *alkyl-PCs* do not target the tumor cell's DNA but the tumor cell's membrane: they insert themselves into the lipid bilayer where they can consequently influence signaling pathways, which in turn leads to apoptotic events (Rios-Marco et al., 2017). Miltefosine, which is a hexadecyl-PC, is a drug originally prescribed as a topical treatment for patients with breast cancer skin metastases (Clive et al., 1999). This drug has been repurposed for the treatment of visceral leishmaniasis caused by *Leishmania donovani*. The drug has several modes of actions in this parasite: (a) inhibiting the synthesis of phosphatidylcholine, (b) blocking its cytochrome c oxidase activity, (c) activating the plasma membrane Ca^{2+} channel, and (d) alkalinizing rapidly the acidocalcisomes, an acidic calcium-storage organelle which is present in pathogenic, as well as non-pathogenic prokaryotes and eukaryotes (Moreno and Docampo, 2009; Pinto-Martinez et al., 2018). Miltefosine and other *alkyl-PCs* are active against *E. histolytica* and the highest antiamebic activity was found for oleyl-PC, octadecyl-PC, and nonadecenyl-PC. For these three compounds, the drug concentration at which 50% of the trophozoites were nonviable (EC_{50}) after 48 h of treatment occurred between 73 and 98 μM. The fact that alkyl-PCs can accumulate in the liver might be exploited for the treatment of amebic liver abscesses (Marschner et al., 1992).

2.2.3. Diphetarsone

Diphetarsone or Bèmarsal (Bis-(p-arsonophenylamino) 1,2-ethane are an arsenical class of compounds that has been effectively used to treat intestinal amebiasis (Keystone et al., 1983). Combination therapy of diphetarsone and spiramycin has also been used successfully against *E. histolytica* (Schneider et al., 1957). Despite its desuetude as standard treatment against amebiasis, diphetarsone may still be used as an

antiamebic compound that may represent an alternative to MTZ in case of intolerance.

2.3. Identifying new drugs against amebiasis using drug repurposing

Drug repurposing is based on the finding of new therapeutic indications for approved drugs. This is a complementary approach to traditional drug discovery that reduces the high cost and risks related with the latter. Indeed, drug repurposing is peculiarly adequate to discover new drugs for treating neglected tropical diseases, which are not a main concern of pharmaceutical firms (Berman and Radhakrishna, 2017). Until now, two high-throughput screens of drug repurposed libraries have identified auranofin, anisomycin, and prodigiosin as new antiamebic agents (Debnath et al., 2012; Ehrenkaufer et al., 2018). Auranofin targets the thioredoxin reductase in *E. histolytica* thereby making the parasite sensitive to oxidative stress (OS) (Debnath et al., 2012). Results of the phase 1 trial that involve a treatment based on 6 mg auranofin daily for seven days were encouraging, resulting in the clearance of the infection and only mild treatment-associated adverse events that were resolved without treatment (Capparelli et al., 2017).

An interesting additional example of a repurposed drug is mefloquine, a drug which is commonly used to treat malaria (Schlagenhauf, 1999) by inhibiting protein synthesis and interacting with the 80S ribosome in *Plasmodium falciparum* (Wong et al., 2017). The EC_{50} of mefloquine for *E. histolytica* trophozoites is much less than that of MTZ (1.1μM vs 5 μM, respectively). In addition, mefloquine kills *E. histolytica* cysts whereas MTZ does not (Osisanya, 1986). Despite its potential as an amebicidal compound, it is important to remember that the drug induces some adverse effects which include psychiatric side effects (Ritchie et al., 2013).

2.4. Enhancing the effectiveness of MTZ

Potentiation of well-established drugs with a metal has been used successfully against drug-resistant pathogens. For example, the combination of chloroquine with gold or platinum increases the effectiveness of chloroquine against a chloroquine-resistant strain of *Plasmodium falciparum*, one of the four etiologic agents of malaria (Navarro et al., 2014). The effectiveness of MTZ against *E. histolytica* can also be improved *in vitro* by complexation with gold, ruthenium, or copper with an IC_{50} for the copper-MTZ complex around 5-fold lower than for MTZ alone (IC_{50} = 1.81 μM) (Athar et al., 2005; Singh et al., 2009).

Potentiation of MTZ action has also been attempted by adding a thiosemicarbazone group to the drug. Eight out of eleven thiosemicarbazone analogs of MTZ that have been synthesized show a better effectiveness against *E. histolytica* than MTZ with the most efficient compound MNZ–p-TOL–TSC $C_{22}H_{22}N_6O_{3S}$ showing an IC_{50} of 0.56 μM. These thiosemicarbazone analogs of MTZ will certainly be worthwhile when MTZ-resistant *E. histolytica* strains emerge (Abid et al., 2008).

2.5. Reviving past medications against amebiasis

Anisomycin and prodigiosin were both able to kill *in vitro* MTZ-resistant parasites and prodigiosin was able to kill mature cysts (Ehrenkaufer et al., 2018). Interestingly, these drugs were used successfully more than 50 years ago to treat amebiasis on small cohorts (Balamuth and Brent, 1950; Gonzalez Constandse, 1956), emphasizing the potential of mining old data to find alternative drugs to MTZ.

2.6. Targeting essential E. histolytica components

Target identification and validation are critical steps for designing new drugs for treating *E. histolytica* infections. A non-exhaustive list of targets selected from the literature is depicted in Table 1. Some of the targets shown in Table 1 are discussed in more detail below:

2.6.1. Targeting cysteine proteases

The role of cysteine proteases (CPs) in *E. histolytica* has been recently reviewed (Siqueira-Neto et al., 2018). More than 50 genes that encode for CPs have been identified in the genome of *E. histolytica* (Irmer et al., 2009) but only four CPs, namely EhCP1, EhCP2, EhCP5, and EhCP7 are constitutively highly expressed. The expression of a fifth CP, EhCP4, is induced by mucin-producing goblet cells (He et al., 2010). The CPs in the parasite have multiple functions which include: (a) acquisition of nutrients (Que and Reed, 2000), (b) degradation of the mucosal layer of the colon (Lidell et al., 2006) and extracellular matrix (Horstmann et al., 1992; Schulte and Scholze, 1989), (c) induction of a pro-inflammatory response (Bansal et al., 2009), (d) degradation of secretory immunoglobulin (Que and Reed, 2000), (e) resistance to complement-mediated lysis (Begum et al., 2015), erythrophagocytosis (Ankri et al., 1998), and (f) involvement in encystation and excystation (Ebert et al., 2008). E-64, a potent CP inhibitor from the epoxysuccinate family of compounds, has been a key tool for studying the functions of CPs in *E. histolytica* and other parasites (Olivos-Garcia et al., 2004; Sajid and McKerrow, 2002). These studies show that *E. histolytica* trophozoites depend for their virulence on the activity of their CPs (Sajid and McKerrow, 2002). The fact that E-64d, a cell permeable CP inhibitor derivative of E-64, has been used in phase III clinical trials against muscular dystrophy (Satoyoshi, 1992) suggests that E-64d can also be tested in clinical trials against amebiasis.

In *E. histolytica*, the structural studies of EhCP1 and EhCP4 have allowed the development of specific vinyl sulfone EhCP1 inhibitors K11777 and WRR483, and the EhCP4 inhibitor WRR605. These inhibitors reduced the parasite's invasion into a SCID mouse-human colon xenograft model of amebiasis (Melendez-Lopez et al., 2007) and the level of inflammation in the mouse cecal model (He et al., 2010). However, no information about the safety and effectiveness of these inhibitors in humans is available.

2.6.2. Targeting the synthesis of L-cysteine

L-cysteine is the main antioxidant in *E. histolytica* (Fahey et al., 1984). L-cysteine can be acquired by the parasite from its environment or by *de novo* biosynthesis (Jeelani et al., 2014). Due to the paramount importance of L-cysteine for *E.histolytica* (Gillin and Diamond, 1981), the two components of the cysteine biosynthetic pathway, serine acetyl transferase (SAT) and cysteine synthase (CS), have been investigated in detail (Hussain et al., 2009; Nozaki et al., 2005). CS but not SAT was found to be essential for normal growth of the parasite in medium lacking L-cysteine (Jeelani et al., 2017).

Drug libraries, microbial secondary metabolite libraries, and microbial culture broth extracts were screened to identify inhibitors of CS1, CS2, and CS3. Among nine CS1 and CS3 inhibitors (IC_{50} values ranging from 0.31 to 490 μM vs 1.81 μM for MTZ) identified in the Mori et al., study, seven contain a naphtoquinone structure and its presence suggests that this structure contributes to CS inhibition (Mori et al., 2015). Deacetylkinamycin C and nanaomycin A (IC_{50} values of 18 and 0.8 μM, respectively) were also identified in the Mori et al., study but their cytotoxicity toward lung tissue fibroblasts precluded their utilization against *E. histolytica* (Mori et al., 2015). More recently, pencolide, a compound structurally related to the fungal metabolite, farinomalein, was identified as an inhibitor of *E. histolytica* CS1 (IC_{50} = 233 μM) and CS2 (IC_{50} = 217 μM), respectively, and consequently as an antiamebic compound (IC_{50} value in the cysteine-deprived medium = 283 μM) (Mori et al., 2018). The fungal metabolite pencolide which displays a modest mammalian cytotoxicity represents an interesting basis for the development of new inhibitors of CS with better antiamebic potency.

2.6.3. Targeting polyamine biosynthesis

Polyamines are ubiquitous naturally occurring low molecular weight aliphatic polycations which are crucial for the replication of protozoan pathogens as well (Phillips, 2018). Most efforts to block their

Table 1
Putative antiamebic targets.

Target genes	Pathway Involved	Mode of action	Techniques/ inhibitors used for the identification of target genes	References
CS	*de novo* L-cysteine biosynthesis	Cysteine deprivation-dependent antiamebic activity	Pencolide (i)/Epigenetic silencing (G3)	(Mori et al., 2018) (Jeelani et al., 2017)
CS	*de novo* L-cysteine biosynthesis	Amebicidal	Deacetylkinamycin C (i) nanaomycin A (i)	(Mori et al., 2015)
Dephospho-CoA kinase	Coenzyme A synthesis	Growth retardation	Epigenetic silencing (G3)	(Nurkanto et al., 2018a)
3′-5′ exoribonuclease EhRrp6	RNA homeostasis	Growth retardation, reduced erythrophagocytosis	Antisense RNA mediated silencing	(Singh et al., 2018)
Arginase	Depletion of L-arginine/ polyamine biosynthesis	Growth retardation	L-norvaline (i)	(Elnekave et al., 2003)
mRNA cleavage factor EhCFIm25	Messenger RNA 3'-end polyadenylation	Growth retardation	RNA aptamers	(Ospina-Villa et al., 2018)
Pantothenate kinase	CoA biosynthetic pathway	Growth retardation	Teicoplanin (i)	(Nurkanto et al., 2018b)
Src kinase	Actin cytoskeleton remodeling	Reduced erythrophagocytosis, reduced encystation	Src inhibitor-1(i)	(Lopez-Contreras et al., 2017)
CFIm25	RNA polyadenylation	Acceleration of cell proliferation, reduced mobility, reduced erythrophagocytosis	dsRNA mediated silencing	(Ospina-Villa et al., 2017)
Chitinase	Encystation	Reduction in encystation	Allosamidin (i)	(Munoz et al., 2016)
Hsp90	Stress response	Growth inhibition	Rutilantin, pararosaniline pamoate (i)	(Shahinas et al., 2015)
EhMyb1	Oxidative stress response	Sensitivity to oxidative stress	Epigenetic silencing (trigger)	(Morf et al., 2013)
EhCP112	Proteolytic activity	Reduction of virulence	siRNA	(Ocadiz-Ruiz et al., 2013)
Light subunit of the Gal/GalNAc inhi- bitable lectin (lgl1)	Adherence to target cells	Capping deficiency	Epigenetic silencing (G3)	(Bracha et al., 2006)
EhCP5	Proteolytic activity	Reduction of virulence	Epigenetic silencing (G3), Antisense RNA	(Irmer et al., 2009)
Amebapore	Cytotoxic peptide	Reduction of virulence	Epigenetic silencing (G3), Antisense RNA	(Bujanover et al., 2003) (Bracha et al., 2006) (Ankri et al., 1998)
EhMLBP	Control of LINE retrotransposon	Growth inhibition	Peptides selected by phage display, Distamycin A	(Lavi et al., 2008)
Guanine nucleotide exchange factors 2	Activation of small GTPases of the Rho family	Growth and erythrophagocytosis reduction	Dominant negative mutation	(Gonzalez De la Rosa et al., 2007)
GlcNAc-phosphatidylinositol deacetylase	Glycosylphosphatidylinositol biosynthetic pathway	Growth inhibition, endocytosis and binding to target cells reduction	Antisense RNA	(Vats et al., 2005)
Alcohol dehydrogenase 2	Fermentation of glucose	Amebicidal	Cyclopropyl (i) and cyclobutyl carbinols (i)	(Espinosa et al., 2004)
Erd2	Golgi membrane receptor	Growth inhibition / amebicidal	Antisense RNA oligomers	(Stock et al., 2001)
HP127670	Unknown	Reduction of liver abscess formation	Epigenetic silencing (trigger)	(Matthiesen et al., 2019)
Metalloprotease (EhMSP-1)	Adherence to target cells	Reduced binding on target cells, reduced phagocytosis, reduced cytopathic activity	Epigenetic silencing (G3)	(Teixeira et al., 2012)
Cysteine protease binding protein family 8	Phagosome transport of lysozymes and β-hexosaminidase	Degradation of ingested bacteria, cytopathic activity	Epigenetic silencing (G3)	(Furukawa et al., 2012)
Mitochondria carrier family	Transport of ADP/ATP across the mitosomal membrane	Growth inhibition	Epigenetic silencing (G3)	(Mi-ichi et al., 2011)
Cpn60	Mitosome-specific chaperone	Growth inhibition	Epigenetic silencing (G3)	(Mi-ichi et al., 2011)
Rhomboid protease	Cleavage of transmembrane proteins	Reduced binding on target cells, reduced phagocytosis	Epigenetic silencing (G3)	(Baxt et al., 2010)
(KERP1)	Adherence to target cells	Reduced virulence	Antisense RNA	(Santi-Rocca et al., 2008)
AIG1 (EHI_176590)	Involved in the formation of surface protrusions	Reduced virulence	Epigenetic silencing (G3)	(Nakada-Tsukui et al., 2018)
Histone deacetylase 1,2,3,6,8	Removal of acetyl groups from histone and non-histone proteins	Growth inhibition	Vorinostat(i)	(Sarita et al., 2019)

Inhibitors of target genes (i); Epigenetic silencing (G3) refers to a method to silence gene expression (Mirelman et al., 2006). List of abbreviations used: CS- Cysteine synthase, CFIm- Cleavage Factor Im, Hsp90- Heat shock protein 90, EhMyb1- *E. histolytica* transcription factor upregulated during oxidative stress, EhCP- Cysteine proteinase, KERP-1: Lysine- and glutamic acid-rich protein, EhMLBP- *E. histolytica* Methylated Line Binding Protein, AIG1: Androgen- Induced Gene 1 protein.

biosynthesis have focused on identifying inhibitors that target ornithine decarboxylase (ODC), the rate-limiting step in polyamine biosynthesis. The ODC irreversible inhibitor, α-(difluoromethyl)-dl-ornithine (DFMO), and also called eflornithine, is effectively used to treat human African trypanosomiasis (sleeping sickness) caused by *Trypanosoma brucei gambiense* (Bottieau and Clerinx, 2019) and demonstrates cytotoxicity against the agent of visceral leishmaniasis, *Leishmania donovani* (Kaur et al., 1986). In contrast to *Trypanosoma* and *Leishmania* ODC, *E. histolytica* ODC is resistant to DFMO due to replacement of essential residues for substrate binding in the active site (Satya Tapas et al., 2013). L-arginase is the enzyme that functions one step upstream to ODC and catalyzes the conversion of L-arginine to L-ornithine. L-arginase was investigated as an alternative to ODC for blocking polyamine synthesis in *E. histolytica*. In addition to this role in polyamine synthesis, L-arginase is an important virulence factor that has been associated with resistance to nitrosative stress (NS) (Elnekave et al., 2003; Mortimer and Chadee, 2010) (Vincendeau et al., 2003) and to OS (Shahi et al., 2016). For these reasons, inhibitors of *E. histolytica* L-arginase were tested against the parasite trophozoites. L-norvaline is a general inhibitor of arginase (Chang et al., 1998) and inhibits the growth of *E. histolytica* at a millimolar level (Elnekave et al., 2003). L-norvaline is a food supplement, which is usually consumed by athletes suggests that it is safe to use. However, it has been recently reported that L-norvaline can impair mitochondrial functions and cause necrosis of neuroblastoma cells at concentrations as low as 125 μM (Samardzic and Rodgers, 2019). Consequently, safer *E. histolytica* arginase inhibitors that present better specificity and better effectiveness against the amebic enzyme than L-norvaline are necessary.

2.6.4. Targeting chitinase

The *E. histolytica* cyst is a biodefense threat to water and food supplies due to its resistance to chlorination and low infectious dose (10 cysts) (Ali et al., 2012). Data on the proteome of naturally occurring *E. histolytica* cysts provide important insights into the infectious cyst form and its components that can be targeted to prevent the transmission of amebiasis (Ali et al., 2012). Chitin is a long-chain polymer of N-acetylglucosamine, which is present in the exoskeleton of many arthropods and the cell wall of fungi (Nagpure et al., 2014) and *E. histolytica* cysts (Nagpure et al., 2014) but not present in mammalian cells. Chitin has been proposed as a potential target for designing new drugs for treating *E.histolytica* cysts (Mi-Ichi et al., 2016). Chitinases are hydrolytic enzymes that break down glycosidic bonds in chitin. *E. histolytica* encodes one active chitinase (EhCHT1) (Munoz et al., 2016). Experiments performed in *Entamoeba invadens* (a reptilian counterpart of *E. histolytica* that can encyst *in vitro* (Rengpien and Bailey, 1975)) showed that chitinase is inhibited by allosamidin; this drug is causing a delay in the encystation suggesting that inhibition of EhCHT1 could arrest the life cycle of *E. histolytica* and, thus, stop infection. A significant drawback of allosamidin is its high cost and limited availability in nature (Zhang et al., 2016). Due to this drawback, the development of alternate chitinase inhibitors is a real need. Some appropriate avenues of investigation can be learned from research performed on chitinase inhibitors of fungi where *in silico* methods that rely on an established chitinase structure have helped to identify and to develop new synthetic chitinase inhibitors which are certainly less expensive than natural ones (Chu et al., 2012; Hirose et al., 2010; Maccari et al., 2017). An homology-based model of EhCHT1 exists (Munoz et al., 2016) but a structural model based on crystallography data will definitively boost the development of potent EhCHT1 inhibitors.

2.7. Exploitation of natural products and their derivatives

Plants and their extracts are currently used to treat gastrointestinal diseases in many different parts of the world (Kelber et al., 2017). The plants and their active components described in this section can serve as the basis for the development of potent antiamebic drugs (Fig. 2).

2.7.1. Henna

Henna, a dye which is extracted from the plant *Lawsonia inermis*, has been used in medicine in Asia and the Middle East (Jeyaseelan et al., 2012). The active component of henna (2-hydroxy-1,4-naphthoquinone or lawsone) has antibacterial and antifungal activities (Ambrogi et al., 1970) (Yang and Lee, 2015). The biological activities of lawsone and its derivatives are mainly associated with redox reactions and the chelation of metal ions (Pradhan et al., 2012). Lawsone has been successfully used against various parasites (Afolayan et al., 2016; Motazedian et al., 2017) including *E. histolytica* (Hanke and Talaat, 1961). Hanke and Talaat reported that in patients suffering from intestinal amebiasis, the consumption of 3 g daily of *Lawsonia inermis* leaf powder for more than 15 days cured the patients in 93% of the cases (Hanke and Talaat, 1961). Interestingly, this treatment has also lead to the elimination of anaerobic bacteria present in the stool (Hanke and Talaat, 1961). Consequently, it is difficult to conclude whether this treatment was effective because of its effect on the parasite, its effect on the bacteria that the parasite feeds on (Iyer et al., 2019) or maybe both. Lawsone has also been used as basis for the synthesis of other bioactive p-quinones such as: (i) atovaquone which is currently used in combination with proguanil as an anti-malarial drug, (ii) phenyl ethers of lawsone which are potent against *Trypanosoma brucei rhodesiense* and *Leishmania donovani* (Bolognesi et al., 2008), and (iii) N-hexadecyl substituted lawsone Mannich bases which is around 10 percent more potent than MTZ in killing *E. histolytica* (Mahal et al., 2017).

2.7.2. Garlic

Garlic is widely used as a food spice and to treat and prevent many illnesses including microbial infections and cardiovascular diseases (Rivlin, 2001; Varshney and Budoff, 2016). Its active compounds include ajoene, allicin, and diallyl sulfides that contribute toward its antimicrobial properties (Ankri and Mirelman, 1999; El-Azzouny et al., 2018; Harris et al., 2001; Konaklieva and Plotkin, 2006). Allicin is produced when alliinase and its substrate alliin are put in contact in the crushed garlic clove. Allicin activity resides in its ability to interact with thiol-containing enzymes (Gruhlke et al., 2019). Allicin is a very potent antiamebic compound because only 30 μg/mL of allicin totally inhibits the parasite's growth and even a lower concentration of this compound 5 μg/mL reduces its virulence of the parasite (Ankri et al., 1997; Mirelman et al., 1987). The amebicidal effect of allicin relies on its ability to inhibit *E. histolytica*'s essential thiol-containing enzymes, namely CPs and alcohol dehydrogenases (Ankri et al., 1997).

2.7.3. Flavonoids

Flavonoids are antioxidants with variable phenolic structures and are found in many plants and vegetables. Their antioxidant activity is attributed to their ability to chelate iron (Havsteen, 1983) and the redox properties of their phenolic groups (Bors et al., 1990; Kumar and Pandey, 2013). Some flavonoids are also capable of scavenging ROS while others scavenge nitric oxide (NO) (Henneberg et al., 2013;Duarte et al., 2014). Flavonoids also have antiviral, antibacterial, and antifungal activity (Cushnie and Lamb, 2005). Martinez-Castillo et al., have recently reviewed their activity against protozoan parasites (Martinez-Castillo et al., 2018). In *E. histolytica*, flavonoids mainly target components of the parasite's cytoskeleton, such as actin and myosin II heavy chain (Bolanos et al., 2014; Calzada et al., 2017) resulting in the loss of the parasite's phagocytic, cytolytic, and migratory functions (Bolanos et al., 2014). Another flavonoid, kaempferol, targets *E. histolytica* pyruvate-ferredoxin oxidoreductase (Calzada et al., 2017), an enzyme crucial for redox regulation in the parasite (Jeelani and Nozaki, 2016). In addition to their direct effect on the parasite, the antioxidant properties of flavonoids may be associated with the regulation of the host inflammatory response against the parasite (Martinez-Castillo et al., 2018).

2.7.4. Carvacrol and thymol

Carvacrol and thymol are the main phenolic components in oregano and thyme, and are responsible for their antioxidant, antibacterial antifungal, and antiparasitic activities (Quintanilla-Licea et al., 2014; Sakkas and Papadopoulou, 2017). Exposure of *E.histolytica* trophozoites to a methanolic fraction of *Lippia graveolens* (Mexican oregano) that contains carvacrol led to 95–98% growth inhibition (IC_{50} of 44.3 μg/ml) (Quintanilla-Licea et al., 2014). Since not much is known about the amebicidal mechanism of carvacrol action, its mode of action can only be deduced from its effect on membrane permeability of bacteria (Sakkas and Papadopoulou, 2017) and another protozoan parasite *Giardia lamblia* (Machado et al., 2010) (Fig. 2). Amebicidal action of an essential oil prepared from *Thymus vulgaris* has been reported and the active components, carvacrol and thymol, are the same as whose present in L. *graveolens* (Behnia et al., 2008).

2.7.5. Thymoquinone (TQ)

TQ is the active bioingredient in the essential oil of the herb, *Nigella sativa* (black caraway) and its therapeutic potential has been recently reviewed (Goyal et al., 2017). TQ possesses anti-inflammatory and antioxidant activities (Ragheb et al., 2009), antitumor activity (Imran et al., 2018), and antimicrobial activity (Forouzanfar et al., 2014). It can also prevent the formation of bacterial biofilms (Goel and Mishra, 2018). TQ has a better amebicidal activity than MTZ (85.5% mortality at 15 mM TQ vs 70.9% mortality at 91 mM MTZ after 24 h of treatment) (Sheikh et al., 2015)). Although the mode of action of TQ in *E. histolytica* has not yet been elucidated, it exerts its antimicrobial effect by producing ROS (Goel and Mishra, 2018). The latter are generated when TQ, which belongs to a family of quinones, undergoes either enzymatic or non-enzymatic redox cycling with semiquinone radicals (Bolton et al., 2000).

2.7.6. Curcumins

Curcumin is the bioactive compound of turmeric, a flowering plant of the ginger family, *Zingiberaceae*. This ancient spice is very popular in India and many Asian countries and with garlic, it is considered as one of the pillars of traditional herbal medicine (Ak and Gulcin, 2008; Jacob et al., 2007; Kali et al., 2016; Neelofar et al., 2011). Curcumin inhibits the development of *T. cruzi* inside fibroblasts (Nagajyothi et al., 2012), kills *P. falciparum* by promoting the formation of ROS in the parasite (Cui et al., 2007), and impairs the organization of microtubules in *Giardia lamblia* (Gutierrez-Gutierrez et al., 2017). Curcumin and MTZ (100 μM of CUR + 5 μM of MTZ) have a synergistic effect in killing *E. histolytica* and this synergistic effect has also been described in other organisms (Kundur et al., 2018; Sasidharan et al., 2014).

2.7.7. Galactoglycerolipids (GGLs)

Oxalis curniculata (Oc) is a naturally occurring weed that is widely used in India to treat dysentery. Exposure of *E. histolytica* to GGLs, the active components of *O. curniculata*, showed that these compounds have good amebicidal properties (IC_{50}s of 24 ± 0.2, 35 ± 0.03, and 15 ± 0.2 μg/ml for GGL (Oc-1), GGL (Oc-2) and GGL (Oc-3), respectively, after 24 h of treatment) and low cytotoxicity against mammalian cells (Manna et al., 2010). The mechanism of action of GGLs in *E. histolytica* still requires elucidation but results from an investigation in Gram-positive and Gram-negative bacteria suggest that GGLs alter the cytoplasm membrane (Fischer et al., 2012).

3. Development of a vaccine against amebiasis

The role of the host immune response against *E. histolytica* has been recently reviewed (Begum et al., 2015). The events that trigger this immune response can be summarized as follows: inside the colon, the first defense that the parasite has to cope with is the mucus barrier. MUC2 mucin, which is a glycoprotein secreted from goblet cells, is the main component of this mucus barrier. β amylase secreted by the parasite degrades this barrier allowing the parasite to reach the layer of intestinal epithelial cells (IECs) (Thibeaux et al., 2013). Upon contact with IECs, the parasite triggers the IECs-dependent production of pro-inflammatory molecules including interleukin (IL)-1β), interleukin-8 (IL-8), and tumor necrosis factor (TNF)-α (Cornick and Chadee, 2017). These pro-inflammatory molecules, which are attracting neutrophils and macrophages, lead to the formation of ROS and reactive nitrogen species respectively (Leitsch et al., 2018; Nagaraja and Ankri, 2018). The parasite also triggers a humoral immune response which mainly targets Gal/GalNAc lectin which is present on the surface of the parasite (Nakada-Tsukui and Nozaki, 2016). Based on this information, several attempts to develop a Gal/GalNAc lectin-based vaccine (referenced in (Singh et al., 2016)) have resulted from low to complete protection against a challenge with *E. histolytica* trophozoites in different animal models including mice and gerbils. The most impressive result (100% protection) was obtained using a combination of four polylysine-linked synthetic peptide vaccine representing the Gal/GalNAc lectin heavy chain amino acid sequences. These peptides were administrated intranasally to baboons using cholera toxin as an adjuvant (Abd Alla et al., 2012). Additional *E. histolytica* antigen like the 29-kDa alkyl hydroperoxide reductase or the serine-rich *E. histolytica* protein confer significant protection against amebic liver abscesses in rodent models (Soong et al., 1995; Zhang et al., 1994).

A vaccine using a live attenuated strain of *E. histolytica* (G3) which was established by epigenetic silencing of the virulence factor amebapore A has been tested in hamsters (Bracha et al., 2006; Bujanover et al., 2003). This attenuated strain did cause IgG production in hamsters that were injected intra-peritoneally with the G3 strain (Bujanover et al., 2003). Despite this list of promising antigens, no actual vaccine is so far available against amebiasis.

4. Exploiting the interaction between *E. histolytica* and the gut microbiota

E. histolytica feeds on bacteria and cellular debris found in the large intestine (Marie and Petri, 2014). However, such feeding is very selective: only those bacteria with the appropriate recognition molecules are ingested by the parasite (Schulz et al., 1987). It has been reported that association with specific intestinal bacteria, changes the *E. histolytica* cell surface architecture (Ankri et al., 1999; Bhattacharya et al., 1992). It has also been reported that phagocytosis of pathogenic bacteria boost *E. histolytica* cytopathogenicity, increases the expression of Gal/GalNAc lectin on the cell surface, boosting cysteine proteinase activity, and promoting the resistance of the parasite to OS (Varet et al., 2018) (Galvan-Moroyoqui et al., 2008). Finally, bacteria-induced augmentation of *E. histolytica*'s virulence seems to occur only when the trophozoites phagocytose intact live cells (Bracha and Mirelman, 1984). The gut flora of patients suffering from amebiasis is different from the gut flora of patients with amebiasis. In these infected patients, the population of *Bacteroides*, *Clostridium, Lactobacillus*, *Campylobacter* is decreased, and the population of *Bifidobacterium* is increased (Verma et al., 2012). These findings suggest that the pathogenesis of amebiasis is driven by a dysregulation of the microbiome. Recent evidences support the existence of a cross-talk between the parasite and the microbiota. Oxaloacetate (OAA) for example is a ketoacid produced by bacteria that protects the parasite against OS and boosts its virulence (Shaulov et al., 2018) (Fig. 3A). Another bacterium that may contribute to *E. histolytica*'s virulence is Prevotella copri. The abundance of this anaerobic Gram-negative bacterium of the Bacteroidetes phylum has been associated with enhanced gut inflammation in both animals and humans (Larsen, 2017) and high E. histolytica burden (Gilchrist et al., 2016) (Fig. 3B).

The tight interaction between *E. histolytica* and the surrounding microbiota can be exploited to develop new strategies to prevent or cure amebiasis. One possible approach is to use probiotics because they have some influence on the outcome of protozoan infections (Bar et al.,

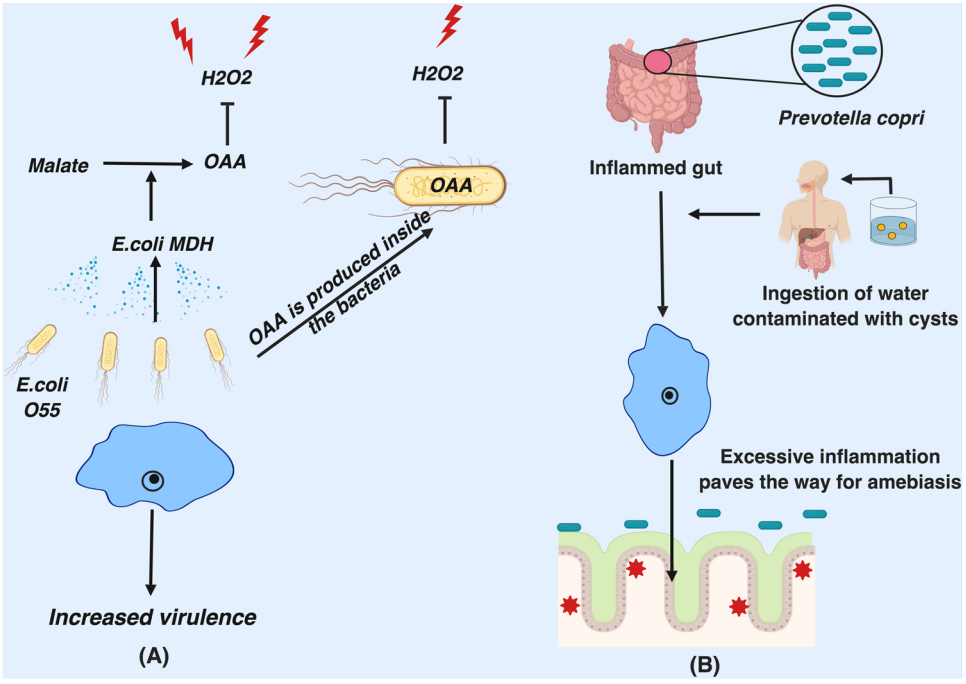

Fig. 3. Role of gut bacteria in shaping the virulence of *E. histolytica*. (A) Interaction of *Escherichia coli O55* with *E. histolytica* boosts its virulence during oxidative stress. *E. coli* secretes malate dehydrogenase (MDH), an enzyme that converts malate to oxaloacetate (OAA). OAA detoxifies hydrogen peroxide (H_2O_2) produced during inflammation. Alternatively, H_2O_2 is neutralized by OAA present inside *E. coli* before it affects *E. histolytica*. (B) The possible promotion of inflammation by *Prevotella copri* may promote the dissemination of *E. histolytica* inside the host.

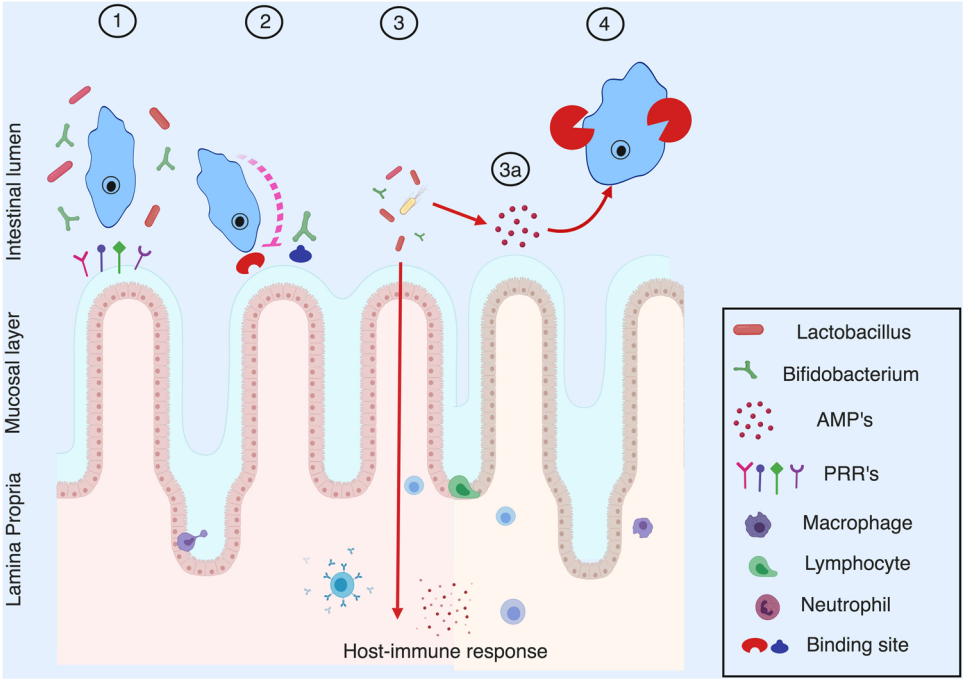

Fig. 4. Possible effect of probiotics in preventing amebiasis. (1) Probiotics compete with the parasite to bind to pathogen recognition receptors (PRRs) for penetration of the mucosal layer, (2) lead to non-specific steric hindrance which might inhibit the parasite's binding and invasion of the colonic mucosa. (3) The gut microbiota may also lead to increased host immune responses against the parasite, or (3a) produce potent antimicrobial proteins by antimicrobial products (AMPs) that may eventually destroy the parasite (4).

2015; Goyal et al., 2011; Sarjapuram et al., 2017). Probiotics are live organisms which confer a health benefit to the host when administered in adequate amounts (Hill et al., 2014). Different mechanisms are involved in the protective role of these probiotics in the gut: (1) specific competition for the binding to pathogen receptor sites on the mucosal surface, (2) formation of a barrier that prevents pathogen to access the mucosal surface, (3) production of antimicrobial products (AMP), (4) competition for nutritional substrates, and (5) enhancement of the host's innate and adaptive immune responses (Fig. 4) (Travers et al., 2011). A number of studies have been conducted to test the effectiveness of the probiotic at inhibiting adhesion of the protozoa to the intestinal mucosa surface (Mansour-Ghanaei et al., 2003; Rigothier et al., 1994). Some of the probiotics that have been tested successfully against *Entamoeba* include Saccharomyces boulardii (Mansour-Ghanaei et al., 2003), *Lactobacillus acidophilus* (Varet et al., 2018), *Lactobacillus casei* and *Enterococcus faecium* (Sarjapuram et al., 2017).

A second possible approach is to disrupt the communication between the parasite and the microbiota. The parasite uses specific ligands to bind to bacteria like the Gal/GalNAc lectin that binds *E.coli* EPEC O55 (Bracha and Mirelman, 1984), the mannose residues at its surface that binds to type 1 pili present on the surface of *Shigella flexneri* (Verdon et al., 1992) and the putative G-protein-coupled receptor 1 that binds to lipopolysaccharide (Brewer et al., 2013). Interfering with this binding has been achieved in vitro by using galactose for E.coli O55 (Bracha and Mirelman, 1984) or methyl α-D-mannopyranoside for *Shigella flexneri* (Bracha et al., 1982). At a first glance, translating these

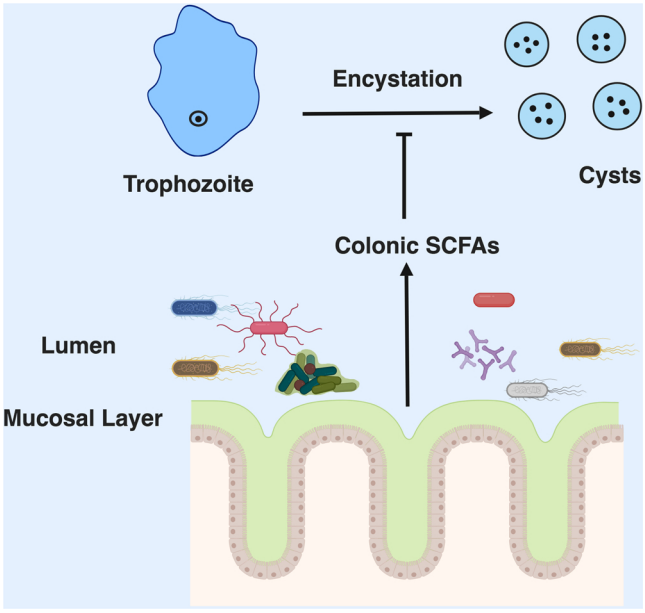

Fig. 5. Colonic Short Chain Fatty Acids (SCFAs) inhibit encystation. The colonic SCFAs are produced through anaerobic fermentation of food products by gut bacteria. These SCFAs block encystation without any effects on the trophozoites.

discoveries into therapeutics does not seem obvious. However, concrete examples support the use of simple sugars to prevent microbial infections. For example, oral administration of D-mannose can be used to prevent recurrent urinary tract infection caused by E.coli (Sihra et al., 2018) and galacto oligosaccharides which are present in human milk can reduce E. histolytica cytotoxicity (Jantscher-Krenn et al., 2012).

In addition to the abovementioned Gal/GalNAc lectin, the parasite expresses leucine-rich proteins that display structural homology with the Toll-Like-Receptor (TLR) ectodomain needed for bacterial product recognition. TLRs are microbial sensors that elicit effective cell defense mechanisms in response to bacterial infection or cell damage (Botos et al., 2011). Interestingly, the transcription of amebic Leucine-rich repeat (LRR) proteins is specifically upregulated when the parasite is incubated with Enteropathogenic E.coli (EPEC) and OS but not with EPEC or OS alone (Varet et al., 2018). This information suggests that these LRRs play a role in the cross-talk taking place between the parasite and the bacteria during OS. A number of TLR modulators has been proposed for the treatment of intestinal bowel disease (Kordjazy et al., 2018). It will be interesting to test their impact on the parasite's response to OS in the presence of bacteria.

The gut microbiota produces a plethora of metabolites resulting from the anaerobic fermentation of undigested food compounds as well as from the processing of compounds that originate from other microorganisms and from the host (Rooks and Garrett, 2016). Some of these metabolites, such as the colonic short chain fatty acid (SCFA), butyrate, or the ketoacid, OAA, have a direct influence on the parasite's biology. Colonic SCFAs inhibit its encystation (Fig. 5) (Byers et al., 2005) and OAA protects it against OS (Fig. 3A) (Shaulov et al., 2018). Influencing the level of production of these metabolites by the microbiota may represent an original strategy to control the parasite's biology and proliferation. This may be achieved by using specific probiotics, such as *Lactobacillus acidophilus* which promotes the uptake of butyrate by colonocytes (Kumar et al., 2015).

5. Conclusions and perspectives regarding therapeutic agents for eradication of amebiasis

Efforts to improve water infrastructure, to promote personal and domestic hygiene, and to encourage vaccination (Huttly et al., 1997; Saliou, 1998), have markedly contributed to the eradication of fecal-orally transmitted diseases in developed countries during the last century. Developing countries are still suffering from these diseases especially in rural regions where the incidence of infections is higher than in urban regions mostly because of the lack of water infrastructures (Gutierrez-Jimenez et al., 2019). Consequently, developing countries are still relying on chemical drugs and in rural regions where access to health services can be difficult, to folk medicine, to treat these fecal-orally transmitted diseases (Maroyi, 2016; Rawat et al., 2017). The treatment of amebiasis strongly depends on MTZ as a first-line drug because of its high efficacy and low cost (Lofmark et al., 2010). The previously mentioned side effects associated with this drug, a potential risk concerning its genotoxicity and neurotoxicity (Hernandez Ceruelos et al., 2019), and the possible emergence of MTZ-resistant *E. histolytica* strains (Bansal et al., 2004; Iyer, 2017), are genuine risks that cannot be neglected and they have been the reason for the continuous search for alternative treatments to MTZ. Such treatments may emerge from repurposing of drugs developed in the 50's like diphetarsone, anisomycin, and prodigiosin which have their effectiveness against *E. histolytica* and their safety in patients is well-established.

Among the new drugs, auranofin is probably the most promising alternative to MTZ and its effectiveness against MTZ-resistant protozoa has been established. However, the emergence of auranofin resistance in mammalian cell lines has been reported (Glennas and Rugstad, 1985; Monia et al., 1987) and the risk that such drug resistance may develop in auranofin-treated protozoa cannot be ignored.

It is also encouraging to see that many other promising active synthetic and natural compounds against *E. histolytica* exist but their characterization is still at a preliminary stage of investigation and more information on their mode of action, toxicity, and pharmacokinetics is needed. Paradoxically, allicin, whose mode of action, toxicity, and pharmacokinetics have been intensively studied, is not considered a drug by the US Food and Drug Administration (FDA) but rather a dietary supplement (Kaul and Joshi, 2001; Lawson and Hunsaker, 2018) despite strong evidences supporting its antiprotozoal activity.

Advances in the treatment of amebiasis will require investigations to identify essential and unique *E. histolytica* pathways such as those in thiol-based redox metabolism and encystation (Jeelani and Nozaki, 2016) (Herman et al., 2017). However, it will certainly benefit mostly from the development of prevention strategies including vaccination despite some problems like vaccine storage which are inherent to developing countries (Popova and Ibarra de Palacios, 2016) and probiotics as prophylactics (Heineman et al., 2012).

Funding

This study was supported by grants from the Israel Science Foundation (260/16), the Rappaport Institute, and the US-Israel Binational Science Foundation (2015211).

References

Abd Alla, M.D., Wolf, R., White, G.L., Kosanke, S.D., Cary, D., Verweij, J.J., Zhang, M.J., Ravdin, J.I., 2012. Efficacy of a Gal-lectin subunit vaccine against experimental Entamoeba histolytica infection and colitis in baboons (Papio sp.). Vaccine 30, 3068–3075.
Abid, M., Agarwal, S.M., Azam, A., 2008. Synthesis and antiamoebic activity of metronidazole thiosemicarbazone analogues. Eur. J. Med. Chem. 43, 2035–2039.
Afolayan, F.I.D., Adegbolagun, O.M., Irungu, B., Kangethe, L., Orwa, J., Anumudu, C.I., 2016. Antimalarial actions of Lawsonia inermis, Tithonia diversifolia and Chromolaena odorata in combination. J. Ethnopharmacol. 191, 188–194.
Ak, T., Gulcin, I., 2008. Antioxidant and radical scavenging properties of curcumin. Chem. Biol. Interact. 174, 27–37.
Ali, I.K., Ehrenkaufer, G.M., Hackney, J.A., Singh, U., 2007. Growth of the protozoan parasite Entamoeba histolytica in 5-azacytidine has limited effects on parasite gene expression. BMC Genomics 8, 7.
Ali, I.K., Haque, R., Siddique, A., Kabir, M., Sherman, N.E., Gray, S.A., Cangelosi, G.A., Petri Jr, W.A., 2012. Proteomic analysis of the cyst stage of Entamoeba histolytica.

PLoS Negl. Trop. Dis. 6, e1643.
Al-Rashood, S.T., Aboldahab, I.A., Nagi, M.N., Abouzeid, L.A., Abdel-Aziz, A.A., Abdel-Hamide, S.G., Youssef, K.M., Al-Obaid, A.M., El-Subbagh, H.I., 2006. Synthesis, dihydrofolate reductase inhibition, antitumor testing, and molecular modeling study of some new 4(3H)-quinazolinone analogs. Bioorg. Med. Chem. 14, 8608–8621.
Ambrogi, V., Artini, D., De Carneri, I., Castellino, S., Dradi, E., Logemann, W., Meinardi, G., Di Somma, M., Tosolini, G., Vecchi, E., 1970. Studies on the antibacterial and antifungal properties of 1, 4-naphthoquinones. Br. J. Pharmacol. 40, 871–880.
Andersson, K.E., 1981. Pharmacokinetics of nitroimidazoles - Spectrum of adverse reactions. Scand. J. Infect. Dis. 60–67.
Ankri, S., Mirelman, D., 1999. Antimicrobial properties of allicin from garlic. Microbes Infect. 1, 125–129.
Ankri, S., Miron, T., Rabinkov, A., Wilchek, M., Mirelman, D., 1997. Allicin from garlic strongly inhibits cysteine proteinases and cytopathic effects of Entamoeba histolytica. Antimicrob. Agents Chemother. 41, 2286–2288.
Ankri, S., Stolarsky, T., Mirelman, D., 1998. Antisense inhibition of expression of cysteine proteinases does not affect Entamoeba histolytica cytopathic or haemolytic activity but inhibits phagocytosis. Mol. Microbiol. 28, 777–785.
Ankri, S., Bracha, R., Padilla-Vaca, F., Mirelman, D., 1999. Applying antisense technology to the study of entamoeba histolytica pathogenesis: response. Trends Microbiol. 7, 473–474.
Anwar, T., Gourinath, S., 2013. Analysis of the Protein phosphotome of Entamoeba histolytica reveals an intricate phosphorylation network. PLoS One 8, e78714.
Athar, F., Husain, K., Abid, M., Agarwal, S.M., Coles, S.J., Hursthouse, M.B., Maurya, M.R., Azam, A., 2005. Synthesis and anti-amoebic activity of gold(I), ruthenium(II), and copper(II) complexes of metronidazole. Chem. Biodivers. 2, 1320–1330.
Aureliano, M., Henao, F., Tiago, T., Duarte, R.O., Moura, J.J., Baruah, B., Crans, D.C., 2008. Sarcoplasmic reticulum calcium ATPase is inhibited by organic vanadium coordination compounds: pyridine-2,6-dicarboxylatodioxovanadium(V), BMOV, and an amavadine analogue. Inorg. Chem. 47, 5677–5684.
Balamuth, W., Brent, M.M., 1950. Biological studies on Entamoeba histolytica. IV. Direct action of the antibiotic, prodigiosin. Proc. Soc. Exp. Biol. Med. 75, 374–378.
Bansal, D., Sehgal, R., Chawla, Y., Mahajan, R.C., Malla, N., 2004. In vitro activity of antiamoebic drugs against clinical isolates of Entamoeba histolytica and Entamoeba dispar. Ann. Clin. Microbiol. Antimicrob. 3, 27.
Bansal, D., Ave, P., Kerneis, S., Frileux, P., Boche, O., Baglin, A.C., Dubost, G., Leguern, A.S., Prevost, M.C., Bracha, R., Mirelman, D., Guillen, N., Labruyere, E., 2009. An ex-vivo human intestinal model to study Entamoeba histolytica pathogenesis. PLoS Negl. Trop. Dis. 3, e551.
Bar, A.K., Phukan, N., Pinheiro, J., Simoes-Barbosa, A., 2015. The interplay of host microbiota and parasitic protozoans at mucosal interfaces: implications for the outcomes of infections and diseases. Plos Negl. Trop. Dis. 9.
Baxt, L.A., Rastew, E., Bracha, R., Mirelman, D., Singh, U., 2010. Downregulation of an Entamoeba histolytica rhomboid protease reveals roles in regulating parasite adhesion and phagocytosis. Eukaryot. Cell 9, 1283–1293.
Begum, S., Quach, J., Chadee, K., 2015. Immune evasion mechanisms of Entamoeba histolytica: progression to disease. Front. Microbiol. 6, 1394.
Behnia, M., Haghighi, A., Komeylizadeh, H., Tabaei, S.J., Abadi, A., 2008. Inhibitory effects of Iranian Thymus vulgaris extracts on in vitro growth of Entamoeba histolytica. Korean J. Parasitol. 46, 153–156.
Berger, M.R., Muschiol, C., Schmahl, D., Eibl, H.J., 1987. New cytostatics with experimentally different toxic profiles. Cancer Treat. Rev. 14, 307–317.
Berman, J., Radhakrishna, T., 2017. The tropical disease priority review voucher: a game-changer for tropical disease products. Am. J. Trop. Med. Hyg. 96, 11–13.
Bernes, S., Siman-Tov, R., Ankri, S., 2005. Epigenetic and classical activation of Entamoeba histolytica heat shock protein 100 (EHsp100) expression. FEBS Lett. 579, 6395–6402.
Bharti, N., Maurya, M.R., Naqvi, F., Azam, A., 2000. Synthesis and antiamoebic activity of new cyclooctadiene ruthenium(II) complexes with 2-acetylpyridine and benzimidazole derivatives. Bioorg. Med. Chem. Lett. 10, 2243–2245.
Bhattacharya, A., Ghildyal, R., Prasad, J., Bhattacharya, S., Diamond, L.S., 1992. Modulation of a surface antigen of Entamoeba histolytica in response to bacteria. Infect. Immun. 60, 1711–1713.
Bolanos, V., Diaz-Martinez, A., Soto, J., Rodriguez, M.A., Lopez-Camarillo, C., Marchat, L.A., Ramirez-Moreno, E., 2014. The flavonoid (-)-epicatechin affects cytoskeleton proteins and functions in Entamoeba histolytica. J. Proteomics 111, 74–85.
Bolognesi, M.L., Lizzi, F., Perozzo, R., Brun, R., Cavalli, A., 2008. Synthesis of a small library of 2-phenoxy-1,4-naphthoquinone and 2-phenoxy-1,4-anthraquinone derivatives bearing anti-trypanosomal and anti-leishmanial activity. Bioorg. Med. Chem. Lett. 18, 2272–2276.
Bolton, J.L., Trush, M.A., Penning, T.M., Dryhurst, G., Monks, T.J., 2000. Role of quinones in toxicology. Chem. Res. Toxicol. 13, 135–160.
Bors, W., Heller, W., Michel, C., Saran, M., 1990. Flavonoids as antioxidants: determination of radical-scavenging efficiencies. Meth. Enzymol. 186, 343–355.
Botos, I., Segal, D.M., Davies, D.R., 2011. The structural biology of Toll-like receptors. Structure 19, 447–459.
Bottieau, E., Clerinx, J., 2019. Human african trypanosomiasis: progress and stagnation. Infect. Dis. Clin. North Am. 33, 61–77.
Bracha, R., Mirelman, D., 1984. Virulence of Entamoeba histolytica trophozoites. Effects of bacteria, microaerobic conditions, and metronidazole. J. Exp. Med. 160, 353–368.
Bracha, R., Kobiler, D., Mirelman, D., 1982. Attachment and ingestion of bacteria by trophozoites of Entamoeba histolytica. Infect. Immun. 36, 396–406.
Bracha, R., Nuchamowitz, Y., Anbar, M., Mirelman, D., 2006. Transcriptional silencing of multiple genes in trophozoites of Entamoeba histolytica. PLoS Pathog. 2, e48.
Brewer, M.T., Agbedanu, P.N., Zamanian, M., Day, T.A., Carlson, S.A., 2013. Evidence for a bacterial lipopolysaccharide-recognizing G-protein-coupled receptor in the bacterial engulfment by Entamoeba histolytica. Eukaryot. Cell 12, 1433–1438.
Bujanover, S., Katz, U., Bracha, R., Mirelman, D., 2003. A virulence attenuated amoebapore-less mutant of Entamoeba histolytica and its interaction with host cells. Int. J. Parasitol. 33, 1655–1663.
Byers, J., Faigle, W., Eichinger, D., 2005. Colonic short-chain fatty acids inhibit encystation of Entamoeba invadens. Cell. Microbiol. 7, 269–279.
Calzada, F., Basurto, J.C., Barbosa, E., Velazquez, C., Hernandez, N.G., 2017. R.M. Ordonez Razo, D.M. Luna and L.Y. mulia, antiprotozoal activities of tiliroside and other compounds from Sphaeralcea angustifolia (Cav.) G. Don. Pharmacogn. Res. 9, 133–137.
Capparelli, E.V., Bricker-Ford, R., Rogers, M.J., McKerrow, J.H., Reed, S.L., 2017. Phase I clinical trial results of auranofin, a novel antiparasitic agent. Antimicrob. Agents Chemother. 61.
Chang, C.I., Liao, J.C., Kuo, L., 1998. Arginase modulates nitric oxide production in activated macrophages. Am. J. Physiol. 274, H342–348.
Chiba, Y., Kamikawa, R., Nakada-Tsukui, K., Saito-Nakano, Y., Nozaki, T., 2015. Discovery of PPi-type phosphoenolpyruvate carboxykinase genes in eukaryotes and Bacteria. J. Biol. Chem. 290, 23960–23970.
Chu, H., Wang, J., Shen, H., Yang, Y., Zhu, W., Li, G., 2012. Investigation of family 18 chitinases and inhibitors by computer-aided approaches. Curr. Drug Targets 13, 502–511.
Clausen, J.D., Bublitz, M., Arnou, B., Olesen, C., Andersen, J.P., Moller, J.V., Nissen, P., 2016. Crystal Structure of the Vanadate-Inhibited Ca(2+)-ATPase. Structure 24, 617–623.
Clive, S., Gardiner, J., Leonard, R.C., 1999. Miltefosine as a topical treatment for cutaneous metastases in breast carcinoma. Cancer Chemother. Pharmacol. 44 (Suppl), S29–30.
Cornick, S., Chadee, K., 2017. Entamoeba histolytica: host parasite interactions at the colonic epithelium. Tissue Barriers 5, e1283386.
Cui, L., Miao, J., Cui, L., 2007. Cytotoxic effect of curcumin on malaria parasite Plasmodium falciparum: inhibition of histone acetylation and generation of reactive oxygen species. Antimicrob. Agents Chemother. 51, 488–494.
Cushnie, T.P., Lamb, A.J., 2005. Antimicrobial activity of flavonoids. Int. J. Antimicrob. Agents 26, 343–356.
Debnath, A., Parsonage, D., Andrade, R.M., He, C., Cobo, E.R., Hirata, K., Chen, S., Garcia-Rivera, G., Orozco, E., Martinez, M.B., Gunatilleke, S.S., Barrios, A.M., Arkin, M.R., Poole, L.B., McKerrow, J.H., Reed, S.L., 2012. A high-throughput drug screen for Entamoeba histolytica identifies a new lead and target. Nat. Med. 18, 956–960.
Dikio, C.W., Okoli, B.J., Mtunzi, F.M., 2017. Synthesis of new anti-bacterial agents: hydrazide Schiff bases of vanadium acetylacetonate complexes. Cogent Chem. 3.
Drake, M.T., Clarke, B.L., Khosla, S., 2008. Bisphosphonates: mechanism of action and role in clinical practice. Mayo Clin. Proc. 83, 1032–1045.
Duarte, J., Francisco, V., Perez-Vizcaino, F., 2014. Modulation of nitric oxide by flavonoids. Food Funct. 5, 1653–1668.
Ebert, F., Bachmann, A., Nakada-Tsukui, K., Hennings, I., Drescher, B., Nozaki, T., Tannich, E., Bruchhaus, I., 2008. An Entamoeba cysteine peptidase specifically expressed during encystation. Parasitol. Int. 57, 521–524.
Ehrenkaufer, G.M., Suresh, S., Solow-Cordero, D., Singh, U., 2018. High-throughput screening of Entamoeba Identifies compounds which target both life cycle stages and which are effective against metronidazole resistant parasites. Front. Cell. Infect. Microbiol. 8, 276.
Eibl, H., Unger, C., 1990. Hexadecylphosphocholine: a new and selective antitumor drug. Cancer Treat. Rev. 17, 233–242.
El-Azzouny, M.M., El-Demerdash, A.S., Seadawy, H.G., Abou-Khadra, S.H., 2018. Antimicrobial effect of garlic (Allium sativum) and thyme (Zataria multiflora boiss) extracts on some food borne pathogens and their effect on virulence gene expression. Cell. Mol. Biol. 64, 79–86.
Elnekave, K., Siman-Tov, R., Ankri, S., 2003. Consumption of L-arginine mediated by Entamoeba histolytica L-arginase (EhArg) inhibits amoebicidal activity and nitric oxide production by activated macrophages. Parasite Immunol. 25, 597–608.
Espinosa, A., Clark, D., Stanley Jr, S.L., 2004. Entamoeba histolytica alcohol dehydrogenase 2 (EhADH2) as a target for anti-amoebic agents. J. Antimicrob. Chemother. 54, 56–59.
Eubank, W.B., Reeves, R.E., 1982. Analog inhibitors for the pyrophosphate-dependent phosphofructokinase of Entamoeba histolytica and their effect on culture growth. J. Parasitol. 68, 599–602.
Fahey, R.C., Newton, G.L., Arrick, B., Overdank-Bogart, T., Aley, S.B., 1984. Entamoeba histolytica: a eukaryote without glutathione metabolism. Science 224, 70–72.
Fischer, C.L., Drake, D.R., Dawson, D.V., Blanchette, D.R., Brogden, K.A., Wertz, P.W., 2012. Antibacterial activity of sphingoid bases and fatty acids against Gram-positive and Gram-negative bacteria. Antimicrob. Agents Chemother. 56, 1157–1161.
Flotho, C., Claus, R., Batz, C., Schneider, M., Sandrock, I., Ihde, S., Plass, C., Niemeyer, C.M., Lubbert, M., 2009. The DNA methyltransferase inhibitors azacitidine, decitabine and zebularine exert differential effects on cancer gene expression in acute myeloid leukemia cells. Leukemia 23, 1019–1028.
Forouzanfar, F., Bazzaz, B.S., Hosseinzadeh, H., 2014. Black cumin (Nigella sativa) and its constituent (thymoquinone): a review on antimicrobial effects. Iran. J. Basic Med. Sci. 17, 929–938.
Furukawa, A., Nakada-Tsukui, K., Nozaki, T., 2012. Novel transmembrane receptor involved in phagosome transport of lysozymes and beta-hexosaminidase in the enteric protozoan Entamoeba histolytica. PLoS Pathog. 8, e1002539.
Galvan-Moroyoqui, J.M., Del Carmen Dominguez-Robles, M., Franco, E., Meza, I., 2008. The interplay between Entamoeba and enteropathogenic bacteria modulates epithelial cell damage. PLoS Negl. Trop. Dis. 2, e266.
Gambino, D., Otero, L., 2012. Perspectives on what ruthenium-based compounds could offer in the development of potential antiparasitic drugs. Inorg. Chim. Acta Rev. 393,

103–114.
Ghosh, S., Chan, J.M., Lea, C.R., Meints, G.A., Lewis, J.C., Tovian, Z.S., Flessner, R.M., Loftus, T.C., Bruchhaus, I., Kendrick, H., Croft, S.L., Kemp, R.G., Kobayashi, S., Nozaki, T., Oldfield, E., 2004. Effects of bisphosphonates on the growth of Entamoeba histolytica and Plasmodium species in vitro and in vivo. J. Med. Chem. 47, 175–187.
Gilchrist, C.A., Petri, S.E., Schneider, B.N., Reichman, D.J., Jiang, N.N., Begum, S., Watanabe, K., Jansen, C.S., Elliott, K.P., Burgess, S.L., Ma, J.Z., Alam, M., Kabir, M., Haque, R., Petri, W.A., 2016. Role of the gut microbiota of children in diarrhea due to the protozoan parasite Entamoeba histolytica. J. Infect. Dis. 213, 1579–1585.
Gillin, F.D., Diamond, L.S., 1981. Entamoeba histolytica and Giardia lamblia: growth responses to reducing agents. Exp. Parasitol. 51, 382–391.
Glennas, A., Rugstad, H.E., 1985. Acquired resistance to auranofin in cultured human cells. Scand. J. Rheumatol. 14, 230–238.
Goel, S., Mishra, P., 2018. Thymoquinone inhibits biofilm formation and has selective antibacterial activity due to ROS generation. Appl. Microbiol. Biotechnol. 102, 1955–1967.
Gonzales, M.L.M., Dans, L.F., Sio-Aguilar, J., 2019. Antiamoebic drugs for treating amoebic colitis. Cochrane Database Syst. Rev. 1, CD006085.
Gonzalez Constandse, R., 1956. [Anisomycin in intestinal amebiasis; study of 30 clinical cases]. Prensa Med. Mex. 21, 114–115.
Gonzalez De la Rosa, C.H., Arias-Romero, L.E., de Jesus Almaraz-Barrera, M., Hernandez-Rivas, R., Sosa-Peinado, A., Rojo-Dominguez, A., Robles-Flores, M., Vargas, M., 2007. EhGEF2, a Dbl-RhoGEF from Entamoeba histolytica has atypical biochemical properties and participates in essential cellular processes. Mol. Biochem. Parasitol. 151, 70–80.
Goyal, N., Tiwari, R.P., Shukla, G., 2011. Lactobacillus rhamnosus GG as an effective probiotic for murine giardiasis. Interdiscip. Perspect. Infect. Dis.(2011), 795219.
Goyal, S.N., Prajapati, C.P., Gore, P.R., Patil, C.R., Mahajan, U.B., Sharma, C., Talla, S.P., Ojha, S.K., 2017. Therapeutic potential and pharmaceutical development of thymoquinone: a multitargeted molecule of natural origin. Front. Pharmacol. 8, 656.
Grover, G., Kini, S.G., 2006. Synthesis and evaluation of new quinazolone derivatives of nalidixic acid as potential antibacterial and antifungal agents. Eur. J. Med. Chem. 41, 256–262.
Gruhlke, M.C.H., Antelmann, H., Bernhardt, J., Kloubert, V., Rink, L., Slusarenko, A.J., 2019. The human allicin-proteome: S-thioallylation of proteins by the garlic defence substance allicin and its biological effects. Free Radic. Biol. Med. 131, 144–153.
Guevara, A., Vicuna, Y., Costales, D., Vivero, S., Anselmi, M., Bisoffi, Z., Formenti, F., 2018. Use of real-time polymerase chain reaction to differentiate between pathogenic Entamoeba histolytica and the nonpathogenic Entamoeba dispar in Ecuador. Am. J. Trop. Med. Hyg.
Gutierrez-Gutierrez, F., Palomo-Ligas, L., Hernandez-Hernandez, J.M., Perez-Rangel, A., Aguayo-Ortiz, R., Hernandez-Campos, A., Castillo, R., Gonzalez-Pozos, S., Cortes-Zarate, R., Ramirez-Herrera, M.A., Mendoza-Magana, M.L., Castillo-Romero, A., 2017. Curcumin alters the cytoskeleton and microtubule organization on trophozoites of Giardia lamblia. Acta Trop. 172, 113–121.
Gutierrez-Jimenez, J., Luna-Cazares, L.M., Cruz, L.M., De Aquino-Lopez, J.A., Sandoval-Gomez, D., Leon-Ortiz, A.T., Hernandez-Shilon, J.A., Constantino-Jonapa, L.A., Matamoros, W.A., Vidal, J.E., 2019. Children from a rural region in the Chiapas Highlands, Mexico, show an increased risk of stunting and intestinal parasitoses when compared with urban children. Bol. Med. Hosp. Infant. Mex. 76, 18–26.
Hanke, M., Talaat, S.M., 1961. The biochemistry and phsiology of henna (Lawsonia alba): its use as a remedy for intestinal amoebiasis. Trans. R. Soc. Trop. Med. Hyg. 55, 56–62.
Harris, J.C., Cottrell, S.L., Plummer, S., Lloyd, D., 2001. Antimicrobial properties of Allium sativum (garlic). Appl. Microbiol. Biotechnol. 57, 282–286.
Havsteen, B., 1983. Flavonoids, a class of natural products of high pharmacological potency. Biochem. Pharmacol. 32, 1141–1148.
He, C., Nora, G.P., Schneider, E.L., Kerr, I.D., Hansell, E., Hirata, K., Gonzalez, D., Sajid, M., Boyd, S.E., Hruz, P., Cobo, E.R., Le, C., Liu, W.T., Eckmann, L., Dorrestein, P.C., Houpt, E.R., Brinen, L.S., Craik, C.S., Roush, W.R., McKerrow, J., Reed, S.L., 2010. A novel Entamoeba histolytica cysteine proteinase, EhCP4, is key for invasive amebiasis and a therapeutic target. J. Biol. Chem. 285, 18516–18527.
Heineman, J., Bubenik, S., McClave, S., Martindale, R., 2012. Fighting fire with fire: is it time to use probiotics to manage pathogenic bacterial diseases? Curr. Gastroenterol. Rep. 14, 343–348.
Henneberg, R., Otuki, M.F., Furman, A.E., Hermann, P., do Nascimento, A.J., Leonart, M.S., 2013. Protective effect of flavonoids against reactive oxygen species production in sickle cell anemia patients treated with hydroxyurea. Rev. Bras. Hematol. Hemoter. 35, 52–55.
Herman, E., Siegesmund, M.A., Bottery, M.J., van Aerle, R., Shather, M.M., Caler, E., Dacks, J.B., van der Giezen, M., 2017. Membrane trafficking modulation during Entamoeba encystation. Sci. Rep. 7, 12854.
Hernandez Ceruelos, A., Romero-Quezada, L.C., Ruvalcaba Ledezma, J.C., Lopez Contreras, L., 2019. Therapeutic uses of metronidazole and its side effects: an update. Eur. Rev. Med. Pharmacol. Sci. 23, 397–401.
Hill, C., Guarner, F., Reid, G., Gibson, G.R., Merenstein, D.J., Pot, B., Morelli, L., Canani, R.B., Flint, H.J., Salminen, S., Calder, P.C., Sanders, M.E., 2014. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat. Rev. Gastroenterol. Hepatol. 11, 506–514.
Hirose, T., Sunazuka, T., Omura, S., 2010. Recent development of two chitinase inhibitors, Argifin and Argadin, produced by soil microorganisms. Proc. Jpn. Acad., Ser. B, Phys. Biol. Sci. 86, 85–102.
Horstmann, R.D., Leippe, M., Tannich, E., 1992. Host tissue destruction by Entamoeba histolytica: molecules mediating adhesion, cytolysis, and proteolysis. Mem. Inst. Oswaldo Cruz 87 (Suppl. 5), 57–60.
Hussain, S., Ali, V., Jeelani, G., Nozaki, T., 2009. Isoform-dependent feedback regulation of serine O-acetyltransferase isoenzymes involved in L-cysteine biosynthesis of Entamoeba histolytica. Mol. Biochem. Parasitol. 163, 39–47.
Huttly, S.R., Morris, S.S., Pisani, V., 1997. Prevention of diarrhoea in young children in developing countries. Bull. World Health Organ. 75, 163–174.
Imran, M., Rauf, A., Khan, I.A., Shahbaz, M., Qaisrani, T.B., Fatmawati, S., Abu-Izneid, T., Imran, A., Rahman, K.U., Gondal, T.A., 2018. Thymoquinone: a novel strategy to combat cancer: a review. Biomed. Pharmacother. 106, 390–402.
Irmer, H., Tillack, M., Biller, L., Handal, G., Leippe, M., Roeder, T., Tannich, E., Bruchhaus, I., 2009. Major cysteine peptidases of Entamoeba histolytica are required for aggregation and digestion of erythrocytes but are dispensable for phagocytosis and cytopathogenicity. Mol. Microbiol. 72, 658–667.
Iyer, L.R., Naik, S., Paul, J., 2017. Antioxidant enzyme profile of two clinical isolates of *Entamoeba histolytica* varying in sensitivity to antiamoebic drugs. World J. Clin. Infect. Dis. 7, 21–23.
Iyer, L.R., Verma, A.K., Paul, J., Bhattacharya, A., 2019. Phagocytosis of gut Bacteria by Entamoeba histolytica. Front. Cell. Infect. Microbiol. 9, 34.
Jacob, A., Wu, R., Zhou, M., Wang, P., 2007. Mechanism of the anti-inflammatory effect of curcumin: PPAR-gamma activation. PPAR Res. (2007), 89369.
Jantscher-Krenn, E., Lauwaet, T., Bliss, L.A., Reed, S.L., Gillin, F.D., Bode, L., 2012. Human milk oligosaccharides reduce Entamoeba histolytica attachment and cytotoxicity in vitro. Br. J. Nutr. 108, 1839–1846.
Jeelani, G., Nozaki, T., 2016. Entamoeba thiol-based redox metabolism: a potential target for drug development. Mol. Biochem. Parasitol. 206, 39–45.
Jeelani, G., Sato, D., Soga, T., Watanabe, H., Nozaki, T., 2014. Mass spectrometric analysis of L-cysteine metabolism: physiological role and fate of L-cysteine in the enteric protozoan parasite Entamoeba histolytica. mBio 5, e01995.
Jeelani, G., Sato, D., Soga, T., Nozaki, T., 2017. Genetic, metabolomic and transcriptomic analyses of the de novo L-cysteine biosynthetic pathway in the enteric protozoan parasite Entamoeba histolytica. Sci. Rep. 7, 15649.
Jeyaseelan, E.C., Jenothiny, S., Pathmanathan, M.K., Jeyadevan, J.P., 2012. Antibacterial activity of sequentially extracted organic solvent extracts of fruits, flowers and leaves of Lawsonia inermis L. from Jaffna. Asian Pac. J. Trop. Biomed. 2, 798–802.
Kali, A., Bhuvaneshwar, D., Charles, P.M., Seetha, K.S., 2016. Antibacterial synergy of curcumin with antibiotics against biofilm producing clinical bacterial isolates. J. Basic Clin. Pharm. 7, 93–96.
Kaul, P.N., Joshi, B.S., 2001. Alternative medicine: herbal drugs and their critical appraisal–part II, Progress in drug research. Fortschritte der Arzneimittelforschung. Progres des recherches pharmaceutiques 57, 1–75.
Kaur, K., Emmett, K., McCann, P.P., Sjoerdsma, A., Ullman, B., 1986. Effects of DL-alpha-difluoromethylornithine on Leishmania donovani promastigotes. J. Protozool. 33, 518–521.
Kelber, O., Bauer, R., Kubelka, W., 2017. Phytotherapy in Functional Gastrointestinal Disorders. Dig. Dis. 35 (Suppl. 1), 36–42.
Keystone, J.S., Proctor, E., Glenn, C., McIntyre, L., 1983. Safety and efficacy of diphetarsone in the treatment of amoebiasis, non-pathogenic amoebiasis and trichuriasis. Trans. R. Soc. Trop. Med. Hyg. 77, 84–86.
Konaklieva, M.I., Plotkin, B.J., 2006. Antimicrobial properties of organosulfur anti-infectives: a review of patent literature 1999-2005. Recent Pat. Antiinfect. Drug Discov. 1, 177–180.
Kordjazy, N., Haj-Mirzaian, A., Haj-Mirzaian, A., Rohani, M.M., Gelfand, E.W., Rezaei, N., Abdolghaffari, A.H., 2018. Role of toll-like receptors in inflammatory bowel disease. Pharmacol. Res. 129, 204–215.
Kumar, S., Pandey, A.K., 2013. Chemistry and biological activities of flavonoids: an overview. TheScientificWorldJournal(2013), 162750.
Kumar, A., Alrefai, W.A., Borthakur, A., Dudeja, P.K., 2015. Lactobacillus acidophilus counteracts enteropathogenic E. Coli-induced inhibition of butyrate uptake in intestinal epithelial cells. Am. J. Physiol. Gastrointest. Liver Physiol. 309, G602–607.
Kundur, S., Prayag, A., Selvakumar, P., Nguyen, H., McKee, L., Cruz, C., Srinivasan, A., Shoyele, S., Lakshmikuttyamma, A., 2018. Synergistic anticancer action of quercetin and curcumin against triple-negative breast cancer cell lines. J. Cell. Physiol.
Larsen, J.M., 2017. The immune response to Prevotella bacteria in chronic inflammatory disease. Immunology 151, 363–374.
Lavi, T., Siman-Tov, R., Ankri, S., 2008. EhMLBP is an essential constituent of the Entamoeba histolytica epigenetic machinery and a potential drug target. Mol. Microbiol. 69, 55–66.
Lawson, L.D., Hunsaker, S.M., 2018. Allicin bioavailability and bioequivalence from garlic supplements and garlic foods. Nutrients 10.
Leitsch, D., Kolarich, D., Wilson, I.B., Altmann, F., Duchene, M., 2007. Nitroimidazole action in Entamoeba histolytica: a central role for thioredoxin reductase. PLoS Biol. 5, e211.
Leitsch, D., Williams, C.F., Hrdy, I., 2018. Redox pathways as drug targets in microaerophilic parasites. Trends Parasitol. 34, 576–589.
Lidell, M.E., Moncada, D.M., Chadee, K., Hansson, G.C., 2006. Entamoeba histolytica cysteine proteases cleave the MUC2 mucin in its C-terminal domain and dissolve the protective colonic mucus gel. Proc. Natl. Acad. Sci. U. S. A. 103, 9298–9303.
Lofmark, S., Edlund, C., Nord, C.E., 2010. Metronidazole is still the drug of choice for treatment of anaerobic infections. Clin. Infect. Dis. 50 (Suppl. 1), S16–23.
Lopez-Contreras, L., Hernandez-Ramirez, V.I., Herrera-Martinez, M., Montano, S., Constantino-Jonapa, L.A., Chavez-Munguia, B., Talamas-Rohana, P., 2017. Structural and functional characterization of the divergent Entamoeba src using Src inhibitor-1. Parasit. Vectors 10, 500.
Lozano, R., Naghavi, M., Foreman, K., Lim, S., Shibuya, K., Aboyans, V., Abraham, J., Adair, T., Aggarwal, R., Ahn, S.Y., Alvarado, M., Anderson, H.R., Anderson, L.M., Andrews, K.G., Atkinson, C., Baddour, L.M., Barker-Collo, S., Bartels, D.H., Bell, M.L., Benjamin, E.J., Bennett, D., Bhalla, K., Bikbov, B., Bin Abdulhak, A., Birbeck, G.,

Blyth, F., Bolliger, I., Boufous, S., Bucello, C., Burch, M., Burney, P., Carapetis, J., Chen, H., Chou, D., Chugh, S.S., Coffeng, L.E., Colan, S.D., Colquhoun, S., Colson, K.E., Condon, J., Connor, M.D., Cooper, L.T., Corriere, M., Cortinovis, M., de Vaccaro, K.C., Couser, W., Cowie, B.C., Criqui, M.H., Cross, M., Dabhadkar, K.C., Dahodwala, N., De Leo, D., Degenhardt, L., Delossantos, A., Denenberg, J., Des Jarlais, D.C., Dharmaratne, S.D., Dorsey, E.R., Driscoll, T., Duber, H., Ebel, B., Erwin, P.J., Espindola, P., Ezzati, M., Feigin, V., Flaxman, A.D., Forouzanfar, M.H., Fowkes, F.G., Franklin, R., Fransen, M., Freeman, M.K., Gabriel, S.E., Gakidou, E., Gaspari, F., Gillum, R.F., Gonzalez-Medina, D., Halasa, Y.A., Haring, D., Harrison, J.E., Havmoeller, R., Hay, R.J., Hoen, B., Hotez, P.J., Hoy, D., Jacobsen, K.H., James, S.L., Jasrasaria, R., Jayaraman, S., Johns, N., Karthikeyan, G., Kassebaum, N., Keren, A., Khoo, J.P., Knowlton, L.M., Kobusingye, O., Koranteng, A., Krishnamurthi, R., Lipnick, M., Lipshultz, S.E., Ohno, S.L., Mabweijano, J., MacIntyre, M.F., Mallinger, L., March, L., Marks, G.B., Marks, R., Matsumori, A., Matzopoulos, R., Mayosi, B.M., McAnulty, J.H., McDermott, M.M., McGrath, J., Mensah, G.A., Merriman, T.R., Michaud, C., Miller, M., Miller, T.R., Mock, C., Mocumbi, A.O., Mokdad, A.A., Moran, A., Mulholland, K., Nair, M.N., Naldi, L., Narayan, K.M., Nasseri, K., Norman, P., O'Donnell, M., Omer, S.B., Ortblad, K., Osborne, R., Ozgediz, D., Pahari, B., Pandian, J.D., Rivero, A.P., Padilla, R.P., Perez-Ruiz, F., Perico, N., Phillips, D., Pierce, K., Pope 3rd, C.A., Porrini, E., Pourmalek, F., Raju, M., Ranganathan, D., Rehm, J.T., Rein, D.B., Remuzzi, G., Rivara, F.P., Roberts, T., De Leon, F.R., Rosenfeld, L.C., Rushton, L., Sacco, R.L., Salomon, J.A., Sampson, U., Sanman, E., Schwebel, D.C., Segui-Gomez, M., Shepard, D.S., Singh, D., Singleton, J., Sliwa, K., Smith, E., Steer, A., Taylor, J.A., Thomas, B., Tleyjeh, I.M., Towbin, J.A., Truelsen, T., Undurraga, E.A., Venketasubramanian, N., Vijayakumar, L., Vos, T., Wagner, G.R., Wang, M., Wang, W., Watt, K., Weinstock, M.A., Weintraub, R., Wilkinson, J.D., Woolf, A.D., Wulf, S., Yeh, P.H., Yip, P., Zabetian, A., Zheng, Z.J., Lopez, A.D., Murray, C.J., AlMazroa, M.A., Memish, Z.A., 2012. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 380, 2095–2128.

Maccari, G., Deodato, D., Fiorucci, D., Orofino, F., Truglio, G.I., Pasero, C., Martini, R., De Luca, F., Docquier, J.D., Botta, M., 2017. Design and synthesis of a novel inhibitor of T. Viride chitinase through an in silico target fishing protocol. Bioorg. Med. Chem. Lett. 27, 3332–3336.

Machado, M., Dinis, A.M., Salgueiro, L., Cavaleiro, C., Custodio, J.B., Sousa Mdo, C., 2010. Anti-Giardia activity of phenolic-rich essential oils: effects of Thymbra capitata, Origanum virens, Thymus zygis subsp. sylvestris, and Lippia graveolens on trophozoites growth, viability, adherence, and ultrastructure. Parasitol. Res. 106, 1205–1215.

Mahal, K., Ahmad, A., Schmitt, F., Lockhauserbaumer, J., Starz, K., Pradhan, R., Padhye, S., Sarkar, F.H., Koko, W.S., Schobert, R., Ersfeld, K., Biersack, B., 2017. Improved anticancer and antiparasitic activity of new lawsone Mannich bases. Eur. J. Med. Chem. 126, 421–431.

Malik, M.A., Dar, O.A., Gull, P., Wani, M.Y., Hashmi, A.A., 2018. Heterocyclic Schiff base transition metal complexes in antimicrobial and anticancer chemotherapy. MedChemComm 9, 409–436.

Manna, D., Dutta, P.K., Achari, B., Lohia, A., 2010. A novel galacto-glycerolipid from Oxalis corniculata kills Entamoeba histolytica and Giardia lamblia. Antimicrob. Agents Chemother. 54, 4825–4832.

Mansour-Ghanaei, F., Dehbashi, N., Yazdanparast, K., Shafaghi, A., 2003. Efficacy of saccharomyces boulardii with antibiotics in acute amoebiasis. World J. Gastroenterol. 9, 1832–1833.

Marie, C., Petri Jr, W.A., 2014. Regulation of virulence of Entamoeba histolytica. Annu. Rev. Microbiol.

Maroyi, A., 2016. Treatment of diarrhoea using traditional medicines: contemporary research in South Africa and Zimbabwe. AJTCAM 13, 5–10.

Marschner, N., Kotting, J., Eibl, H., Unger, C., 1992. Distribution of hexadecylphosphocholine and octadecyl-methyl-glycero-3-phosphocholine in rat tissues during steady-state treatment. Cancer Chemother. Pharmacol. 31, 18–22.

Martinez-Castillo, M., Pacheco-Yepez, J., Flores-Huerta, N., Guzman-Tellez, P., Jarillo-Luna, R.A., Cardenas-Jaramillo, L.M., Campos-Rodriguez, R., Shibayama, M., 2018. Flavonoids as a natural treatment against Entamoeba histolytica. Front. Cell. Infect. Microbiol. 8, 209.

Matthiesen, J., Lender, C., Haferkorn, A., Fehling, H., Meyer, M., Matthies, T., Tannich, E., Roeder, T., Lotter, H., Bruchhaus, I., 2019. Trigger-induced RNAi gene silencing to identify pathogenicity factors of Entamoeba histolytica. FASEB J. 33, 1658–1668.

Maurya, M.R., Kumar, A., Abid, M., Azam, A., 2006a. Dioxovanadium(V) and mu-oxo bis [oxovanadium(V)] complexes containing thiosemicarbazone based ONS donor set and their antiamoebic activity. Inorg. Chim. Acta Rev. 359, 2439–2447.

Maurya, M.R., Kumar, A., Bhat, A.R., Azam, A., Bader, C., Rehder, D., 2006b. Dioxo- and oxovanadium(V) complexes of thiohydrazone ONS donor ligands: synthesis, characterization, reactivity, and antiamoebic activity. Inorg. Chem. 45, 1260–1269.

McLauchlan, C.C., Hooker, J.D., Jones, M.A., Dymon, Z., Backhus, E.A., Greiner, B.A., Dorner, N.A., Youkhana, M.A., Manus, L.M., 2010. Inhibition of acid, alkaline, and tyrosine (PTP1B) phosphatases by novel vanadium complexes. J. Inorg. Biochem. 104, 274–281.

Melendez-Lopez, S.G., Herdman, S., Hirata, K., Choi, M.H., Choe, Y., Craik, C., Caffrey, C.R., Hansell, E., Chavez-Munguia, B., Chen, Y.T., Roush, W.R., McKerrow, J., Eckmann, L., Guo, J., Stanley Jr, S.L., Reed, S.L., 2007. Use of recombinant Entamoeba histolytica cysteine proteinase 1 to identify a potent inhibitor of amebic invasion in a human colonic model. Eukaryot. Cell 6, 1130–1136.

Mertens, E., 1993. ATP versus pyrophosphate: glycolysis revisited in parasitic protists. Parasitol. Today 9, 122–126.

Mi-ichi, F., Makiuchi, T., Furukawa, A., Sato, D., Nozaki, T., 2011. Sulfate activation in mitosomes plays an important role in the proliferation of Entamoeba histolytica. PLoS Negl. Trop. Dis. 5, e1263.

Mi-Ichi, F., Yoshida, H., Hamano, S., 2016. Entamoeba encystation: new targets to prevent the transmission of Amebiasis. PLoS Pathog. 12, e1005845.

Mirelman, D., Monheit, D., Varon, S., 1987. Inhibition of growth of Entamoeba histolytica by allicin, the active principle of garlic extract (Allium sativum). J. Infect. Dis. 156, 243–244.

Mirelman, D., Anbar, M., Nuchamowitz, Y., Bracha, R., 2006. Epigenetic silencing of gene expression in Entamoeba histolytica. Arch. Med. Res. 37, 226–233.

Monia, B.P., Butt, T.R., Mirabelli, C.K., Ecker, D.J., Sternberg, E., Crooke, S.T., 1987. Induction of metallothionein is correlated with resistance to auranofin, a gold compound, in Chinese hamster ovary cells. Mol. Pharmacol. 31, 21–26.

Moreno, S.N., Docampo, R., 2009. The role of acidocalcisomes in parasitic protists. J. Eukaryot. Microbiol. 56, 208–213.

Morf, L., Pearson, R.J., Wang, A.S., Singh, U., 2013. Robust gene silencing mediated by antisense small RNAs in the pathogenic protist Entamoeba histolytica. Nucleic Acids Res. 41, 9424–9437.

Mori, M., Jeelani, G., Masuda, Y., Sakai, K., Tsukui, K., Waluyo, D., Tarwadi, Y. Watanabe, Nonaka, K., Matsumoto, A., Omura, S., Nozaki, T., Shiomi, K., 2015. Identification of natural inhibitors of Entamoeba histolytica cysteine synthase from microbial secondary metabolites. Front. Microbiol. 6, 962.

Mori, M., Tsuge, S., Fukasawa, W., Jeelani, G., Nakada-Tsukui, K., Nonaka, K., Matsumoto, A., Omura, S., Nozaki, T., Shiomi, K., 2018. Discovery of antiamebic compounds that inhibit cysteine synthase from the enteric parasitic protist Entamoeba histolytica by screening of microbial secondary metabolites. Front. Cell. Infect. Microbiol. 8, 409.

Mortimer, L., Chadee, K., 2010. The immunopathogenesis of Entamoeba histolytica. Exp. Parasitol. 126, 366–380.

Motazedian, M.H., Mikaeili, F., Mohebali, M., Miri, R., Habibi, P., Kamarloie, S., 2017. The antileishmanial effects of Lowsonia inermis and Cedrus libani on Leishmania major promastigotes: an in vitro study. J. Parasit. Dis. 41, 375–379.

Munoz, P.L.A., Minchaca, A.Z., Mares, R.E., Ramos, M.A., 2016. Activity, stability and folding analysis of the chitinase from Entamoeba histolytica. Parasitol. Int. 65, 70–77.

Nagajyothi, F., Zhao, D., Weiss, L.M., Tanowitz, H.B., 2012. Curcumin treatment provides protection against Trypanosoma cruzi infection. Parasitol. Res. 110, 2491–2499.

Nagaraja, S., Ankri, S., 2018. Utilization of different omic approaches to unravel stress response mechanisms in the parasite Entamoeba histolytica. Front. Cell. Infect. Microbiol. 8, 19.

Nagpure, A., Choudhary, B., Gupta, R.K., 2014. Chitinases: in agriculture and human healthcare. Crit. Rev. Biotechnol. 34, 215–232.

Nakada-Tsukui, K., Nozaki, T., 2016. Immune response of Amebiasis and immune evasion by Entamoeba histolytica. Front. Immunol. 7, 175.

Nakada-Tsukui, K., Sekizuka, T., Sato-Ebine, E., Escueta-de Cadiz, A., Ji, D.D., Tomii, K., Kuroda, M., Nozaki, T., 2018. AIG1 affects in vitro and in vivo virulence in clinical isolates of Entamoeba histolytica. PLoS Pathog. 14, e1006882.

Navarro, M., Castro, W., Madamet, M., Amalvict, R., Benoit, N., Pradines, B., 2014. Metal-chloroquine derivatives as possible anti-malarial drugs: evaluation of anti-malarial activity and mode of action. Malar. J. 13, 471.

Neelam, B., Mannar, M., Fehmida, N., Alok, B., Sudha, B., Amir, A., 2000. Palladium(II) complexes of NS donor ligandsderived from S-methyl-dithiocarbazate, S-benzyl-dithiocarbazateand thiosemicarbazide as antiamoebic agents. Eur. J. Med. Chem. 35, 481–486.

Neelofar, K., Shreaz, S., Rimple, B., Muralidhar, S., Nikhat, M., Khan, L.A., 2011. Curcumin as a promising anticandidal of clinical interest. Can. J. Microbiol. 57, 204–210.

Nozaki, T., Ali, V., Tokoro, M., 2005. Sulfur-containing amino acid metabolism in parasitic protozoa. Adv. Parasitol. 60, 1–99.

Nurkanto, A., Jeelani, G., Yamamoto, T., Hishiki, T., Naito, Y., Suematsu, M., Hashimoto, T., Nozaki, T., 2018a. Biochemical, Metabolomic, and Genetic Analyses of Dephospho Coenzyme A Kinase Involved in Coenzyme A Biosynthesis in the Human Enteric Parasite Entamoeba histolytica. Front. Microbiol. 9, 2902.

Nurkanto, A., Jeelani, G., Yamamoto, T., Naito, Y., Hishiki, T., Mori, M., Suematsu, M., Shiomi, K., Hashimoto, T., Nozaki, T., 2018b. Characterization and validation of Entamoeba histolytica pantothenate kinase as a novel anti-amebic drug target. Int. J. Parasitol.-Drug 8, 125–136.

Ocadiz-Ruiz, R., Fonseca, W., Martinez, M.B., Ocadiz-Quintanar, R., Orozco, E., Rodriguez, M.A., 2013. Effect of the silencing of the Ehcp112 gene on the in vitro virulence of Entamoeba histolytica. Parasit. Vectors 6, 248.

Olivos-Garcia, A., Tello, E., Nequiz-Avendano, M., Gonzalez-Canto, A., Lopez-Vancell, R., Garcia de Leon, M.C., Montfort, I., Perez-Tamayo, R., 2004. Cysteine proteinase activity is required for survival of the parasite in experimental acute amoebic liver abscesses in hamsters. Parasitology 129, 19–25.

Orskov, A.D., Gronbaek, K., 2017. DNA Methyltransferase Inhibitors in Myeloid Cancer: Clonal Eradication or Clonal Differentiation? Cancer J. 23, 277–285.

Orvieto, F., Branca, D., Giomini, C., Jones, P., Koch, U., Ontoria, J.M., Palumbi, M.C., Rowley, M., Toniatti, C., Muraglia, E., 2009. Identification of substituted pyrazolo [1,5-a]quinazolin-5(4H)-one as potent poly(ADP-ribose)polymerase-1 (PARP-1) inhibitors. Bioorg. Med. Chem. Lett. 19, 4196–4200.

Osisanya, J.O., 1986. Comparative in vitro activity of mefloquine, diloxanide furoate and other conventionally used amoebicides against entamoeba histolytica. East Afr. Med. J. 63, 263–268.

Ospina-Villa, J.D., Guillen, N., Lopez-Camarillo, C., Soto-Sanchez, J., Ramirez-Moreno, E., Garcia-Vazquez, R., Castanon-Sanchez, C.A., Betanzos, A., Marchat, L.A., 2017. Silencing the cleavage factor CFIm25 as a new strategy to control Entamoeba histolytica parasite. J. Microbiol. 55, 783–791.

Ospina-Villa, J.D., Dufour, A., Weber, C., Ramirez-Moreno, E., Zamorano-Carrillo, A., Guillen, N., Lopez-Camarillo, C., Marchat, L.A., 2018. Targeting the polyadenylation factor EhCFIm25 with RNA aptamers controls survival in Entamoeba histolytica. Sci.

Rep. 8, 5720.
Pazianas, M., Abrahamsen, B., Ferrari, S., Russell, R.G., 2013. Eliminating the need for fasting with oral administration of bisphosphonates. Ther. Clin. Risk Manag. 9, 395–402.
Pessoa, J.C., Etcheverry, S., Gambino, D., 2015. Vanadium compounds in medicine. Coord. Chem. Rev. 301, 24–48.
Phillips, M.A., 2018. Polyamines in protozoan pathogens. J. Biol. Chem. 293, 18746–18756.
Pineda, E., Perdomo, D., 2017. Entamoeba histolytica under Oxidative Stress: What Countermeasure Mechanisms Are in Place? Cells 6.
Pinto-Martinez, A.K., Rodriguez-Duran, J., Serrano-Martin, X., Hernandez-Rodriguez, V., Benaim, G., 2018. Mechanism of action of miltefosine on leishmania donovani involves the impairment of acidocalcisome function and the activation of the sphingosine-dependent plasma membrane Ca(2+) channel. Antimicrob. Agents Chemother. 62.
Popova, O., Ibarra de Palacios, P., 2016. Reaching more children with vaccines in developing countries: key challenges of innovation and delivery. Curr. Med. Res. Opin. 32, 177–181.
Pradhan, R., Dandawate, P., Vyas, A., Padhye, S., Biersack, B., Schobert, R., Ahmad, A., Sarkar, F.H., 2012. From body art to anticancer activities: perspectives on medicinal properties of henna. Curr. Drug Targets 13, 1777–1798.
Satya Tapas, P., Kumar, P., Madhubala, R., Tomar, S., 2013. Structural insight into DFMO resistant ornithine decarboxylase from Entamoeba histolytica: an inkling to adaptive evolution. PLoS One 8, e53397.
Que, X., Reed, S.L., 2000. Cysteine proteinases and the pathogenesis of amebiasis. Clin. Microbiol. Rev. 13, 196–206.
Quintanilla-Licea, R., Mata-Cardenas, B.D., Vargas-Villarreal, J., Bazaldua-Rodriguez, A.F., Kavimngeles-Hernandez, I., Garza-Gonzalez, J.N., Hernandez-Garcia, M.E., 2014. Antiprotozoal activity against Entamoeba histolytica of plants used in northeast Mexican traditional medicine. Bioactive compounds from Lippia graveolens and Ruta chalepensis. Molecules 19, 21044–21065.
Raffa, D., Edler, M.C., Daidone, G., Maggio, B., Merikech, M., Plescia, S., Schillaci, D., Bai, R., Hamel, E., 2004. Synthesis, cytotoxicity, and inhibitory effects on tubulin polymerization of a new 3-heterocyclo substituted 2-styrylquinazolinones. Eur. J. Med. Chem. 39, 299–304.
Ragheb, A., Attia, A., Eldin, W.S., Elbarbry, F., Gazarin, S., Shoker, A., 2009. The protective effect of thymoquinone, an anti-oxidant and anti-inflammatory agent, against renal injury: a review. Saudi J. Kidney Dis. Transplant. 20, 741–752.
Rawat, P., Singh, P.K., Kumar, V., 2017. Evidence based traditional anti-diarrheal medicinal plants and their phytocompounds. Biomed. Pharmacother. 96, 1453–1464.
Rengpien, S., Bailey, G.B., 1975. Differentiation of Entamoeba: a new medium and optimal conditions for axenic encystation of E. invadens. J. Parasitol. 61, 24–30.
Rigothier, M.C., Maccario, J., Gayral, P., 1994. Inhibitory activity of saccharomyces yeasts on the adhesion of Entamoeba histolytica trophozoites to human erythrocytes in vitro. Parasitol. Res. 80, 10–15.
Rios-Marco, P., Marco, C., Galvez, X., Jimenez-Lopez, J.M., Carrasco, M.P., 2017. Alkylphospholipids: an update on molecular mechanisms and clinical relevance, Biochimica et biophysica acta. Biomembranes 1859 1657–1667.
Ritchie, E.C., Block, J., Nevin, R.L., 2013. Psychiatric side effects of mefloquine: applications to forensic psychiatry. J. Am. Acad. Psychiatry Law 41, 224–235.
Rivlin, R.S., 2001. Historical perspective on the use of garlic. J. Nutr. 131, 951S–954S.
Roe, F.J., 1977. Metronidazole: review of uses and toxicity. J. Antimicrob. Chemother. 3, 205–212.
Rooks, M.G., Garrett, W.S., 2016. Gut microbiota, metabolites and host immunity, Nature reviews. Immunology 16, 341–352.
Rozzo, C., Sanna, D., Garribba, E., Serra, M., Cantara, A., Palmieri, G., Pisano, M., 2017. Antitumoral effect of vanadium compounds in malignant melanoma cell lines. J. Inorg. Biochem. 174, 14–24.
Saavedra, E., Encalada, R., Vazquez, C., Olivos-Garcia, A., Michels, P.A.M., Moreno-Sanchez, R., 2019. Control and regulation of the pyrophosphate-dependent glucose metabolism in Entamoeba histolytica. Mol. Biochem. Parasitol.
Saidin, S., Othman, N., Noordin, R., 2018. Update on laboratory diagnosis of amoebiasis. Eur. J. Clin. Microbiol. Infect. Dis.
Sajid, M., McKerrow, J.H., 2002. Cysteine proteases of parasitic organisms. Mol. Biochem. Parasitol. 120, 1–21.
Sakkas, H., Papadopoulou, C., 2017. Antimicrobial activity of basil, oregano, and thyme essential oils. J. Microbiol. Biotechnol. 27, 429–438.
Saliou, P., 1998. [Vaccines inf the prevention of fecal hazards]. Bull. Soc. Pathol. Exot. 91, 445–449.
Samardzic, K., Rodgers, K.J., 2019. Cytotoxicity and mitochondrial dysfunction caused by the dietary supplement l-norvaline. Toxicol. Vitr. 56, 163–171.
Santi-Rocca, J., Weber, C., Guigon, G., Sismeiro, O., Coppee, J.Y., Guillen, N., 2008. The lysine- and glutamic acid-rich protein KERP1 plays a role in Entamoeba histolytica liver abscess pathogenesis. Cell. Microbiol. 10, 202–217.
Sarita, M., Luis Alejandro, C.J., Yudibeth, S.L., Veronica Ivonne, H.R., Ceruelos Alejandra, H., Luis Carlos, R.Q., Ledezma Jesus Carlos, R., Patricia, T.R., Contreras Luilli, L., 2019. Vorinostat, a possible alternative to Metronidazole for the treatment of amebiasis caused by Entamoeba histolytica. J. Biomol. Struct. Dyn. 1–9.
Sarjapuram, N., Mekala, N., Singh, M., Tatu, U., 2017. The potential of Lactobacillus casei and entercoccus faecium combination as a preventive probiotic against Entamoeba. Probiotics Antimicrob. Proteins 9, 142–149.
Sarniguet, C., Toloza, J., Cipriani, M., Lapier, M., Vieites, M., Toledano-Magana, Y., Garcia-Ramos, J.C., Ruiz-Azuara, L., Moreno, V., Maya, J.D., Azar, C.O., Gambino, D., Otero, L., 2014. Water-soluble ruthenium complexes bearing activity against protozoan parasites. Biol. Trace Elem. Res. 159, 379–392.
Sasidharan, N.K., Sreekala, S.R., Jacob, J., Nambisan, B., 2014. In vitro synergistic effect of curcumin in combination with third generation cephalosporins against bacteria associated with infectious diarrhea. Biomed Res. Int.(2014), 561456.
Satoyoshi, E., 1992. Therapeutic trials on progressive muscular dystrophy. Intern. Med. 31, 841–846.
Schlagenhauf, P., 1999. Mefloquine for malaria chemoprophylaxis 1992-1998: a review. J. Travel Med. 6, 122–133.
Schneider, J., Dupoux, R., Montezin, G., 1957. [Treatment of intestinal amebiasis by diphetarsone-spiramycin. (6. 753 RP.)]. Bull. Soc. Pathol. Exot. Filiales 50, 600–606.
Schulte, W., Scholze, H., 1989. Action of the major protease from Entamoeba histolytica on proteins of the extracellular matrix. J. Protozool. 36, 538–543.
Schulz, T.F., Kollaritsch, H., Hengster, P., Stemberger, H., Scheiner, O., Wiedemann, G., Dierich, M.P., 1987. Molecular weight analysis of Entamoeba histolytica antigens recognized by IgG and IgM antibodies in the sera of patients with amoebiasis. Trop. Med. Parasitol. 38, 149–152.
Seargeant, L.E., Stinson, R.A., 1979. Inhibition of human alkaline-phosphatases by vanadate. Biochem. J. 181, 247–250.
Shahi, P., Trebicz-Geffen, M., Nagaraja, S., Alterzon-Baumel, S., Hertz, R., Methling, K., Lalk, M., Ankri, S., 2016. Proteomic identification of oxidized proteins in Entamoeba histolytica by resin-assisted capture: insights into the role of arginase in resistance to oxidative stress. PLoS Negl. Trop. Dis. 10, e0004340.
Shahinas, D., Debnath, A., Benedict, C., McKerrow, J.H., Pillai, D.R., 2015. Heat shock protein 90 inhibitors repurposed against Entamoeba histolytica. Front. Microbiol. 6, 368.
Shaulov, Y., Shimokawa, C., Trebicz-Geffen, M., Nagaraja, S., Methling, K., Lalk, M., Weiss-Cerem, L., Lamm, A.T., Hisaeda, H., Ankri, S., 2018. Escherichia coli mediated resistance of Entamoeba histolytica to oxidative stress is triggered by oxaloacetate. PLoS Pathog. 14, e1007295.
Sheikh, B.Y., Taha, M.M.E., Koko, W.S., Abdelwahab, S.I., 2015. Antimicrobial effects of Thymoquinone on Entamoeba histolytica and Giardia lamblia. Pharmacogn. Prelim. Phytochem. Investig. Jatrophae Curcatis Semen 8, 168–170.
Shirley, D.T., Farr, L., Watanabe, K., Moonah, S., 2018. A review of the global burden, new diagnostics, and current therapeutics for amebiasis. Open Forum Infect. Dis. 5, ofy161.
Sihra, N., Goodman, A., Zakri, R., Sahai, A., Malde, S., 2018. Nonantibiotic prevention and management of recurrent urinary tract infection, Nature reviews. Urology 15, 750–776.
Singh, S., Bharti, N., Mohapatra, P.P., 2009. Chemistry and biology of synthetic and naturally occurring antiamoebic agents. Chem. Rev. 109, 1900–1947.
Singh, R.S., Walia, A.K., Kanwar, J.R., Kennedy, J.F., 2016. Amoebiasis vaccine development: a snapshot on E. Histolytica with emphasis on perspectives of Gal/GalNAc lectin. Int. J. Biol. Macromol. 91, 258–268.
Singh, S.S., Naiyer, S., Bharadwaj, R., Kumar, A., Singh, Y.P., Ray, A.K., Subbarao, N., Bhattacharya, A., Bhattacharya, S., 2018. Stress-induced nuclear depletion of Entamoeba histolytica 3'-5' exoribonuclease EhRrp6 and its role in growth and erythrophagocytosis. J. Biol. Chem. 293, 16242–16260.
Siqueira-Neto, J.L., Debnath, A., McCall, L.I., Bernatchez, J.A., Ndao, M., Reed, S.L., Rosenthal, P.J., 2018. Cysteine proteases in protozoan parasites. PLoS Negl. Trop. Dis. 12, e0006512.
Soong, C.J., Torian, B.E., Abd-Alla, M.D., Jackson, T.F., Gatharim, V., Ravdin, J.I., 1995. Protection of gerbils from amebic liver abscess by immunization with recombinant Entamoeba histolytica 29-kilodalton antigen. Infect. Immun. 63, 472–477.
Stock, R.P., Olvera, A., Sanchez, R., Saralegui, A., Scarfi, S., Sanchez-Lopez, R., Ramos, M.A., Boffa, L.C., Benatti, U., Alagon, A., 2001. Inhibition of gene expression in Entamoeba histolytica with antisense peptide nucleic acid oligomers. Nat. Biotechnol. 19, 231–234.
Stock, M.L., Elazab, S.T., Hsu, W.H., 2018. Review of triazine antiprotozoal drugs used in veterinary medicine. J. Vet. Pharmacol. Ther. 41, 184–194.
Swaminathan, A., Torresi, J., Schlagenhauf, P., Thursky, K., Wilder-Smith, A., Connor, B.A., Schwartz, E., Vonsonnenberg, F., Keystone, J., O'Brien, D.P., GeoSentinel, N., 2009. A global study of pathogens and host risk factors associated with infectious gastrointestinal disease in returned international travellers. J. Infect. 59, 19–27.
Tariq, S., Avecilla, F., Sharma, G.P., Mondal, N., Azam, A., 2018. Design, synthesis and biological evaluation of quinazolin-4(3H)-one Schiff base conjugates as potential antiamoebic agents. J. Saudi Chem. Soc. 22, 306–315.
Teixeira, J.E., Sateriale, A., Bessoff, K.E., Huston, C.D., 2012. Control of Entamoeba histolytica adherence involves metallosurface protease 1, an M8 family surface metalloprotease with homology to leishmanolysin. Infect. Immun. 80, 2165–2176.
Thangavel, P., Viswanath, B., Kim, S., 2017. Recent developments in the nanostructured materials functionalized with ruthenium complexes for targeted drug delivery to tumors. Int. J. Nanomedicine 12, 2749–2758.
Thibeaux, R., Weber, C., Hon, C.C., Dillies, M.A., Ave, P., Coppee, J.Y., Labruyere, E., Guillen, N., 2013. Identification of the virulence landscape essential for Entamoeba histolytica invasion of the human Colon. PLoS Pathog. 9, e1003824.
Travers, M.A., Florent, I., Kohl, L., Grellier, P., 2011. Probiotics for the control of parasites: an overview. J. Parasitol. Res.(2011), 610769.
Tumer, M., Koksal, H., Sener, M.K., Serin, S., 1999. Antimicrobial activity studies of the binuclear metal complexes derived from tridentate Schiff base ligands. Trans. Metal Chem. 24, 414–420.
Turkeltaub, J.A., McCarty 3rd, T.R., Hotez, P.J., 2015. The intestinal protozoa: emerging impact on global health and development. Curr. Opin. Gastroenterol. 31, 38–44.
Varela-Gomez, M., Moreno-Sanchez, R., Pardo, J.P., Perez-Montfort, R., 2004. Kinetic mechanism and metabolic role of pyruvate phosphate dikinase from Entamoeba histolytica. J. Biol. Chem. 279, 54124–54130.
Varet, H., Shaulov, Y., Sismeiro, O., Trebicz-Geffen, M., Legendre, R., Coppee, J.Y., Ankri, S., Guillen, N., 2018. Enteric bacteria boost defences against oxidative stress in Entamoeba histolytica. Sci. Rep. 8, 9042.

Varshney, R., Budoff, M.J., 2016. Garlic and heart disease. J. Nutr. 146, 416S–421S.
Vats, D., Vishwakarma, R.A., Bhattacharya, S., Bhattacharya, A., 2005. Reduction of cell surface glycosylphosphatidylinositol conjugates in Entamoeba histolytica by antisense blocking of E. histolytica GlcNAc-phosphatidylinositol deacetylase expression: effect on cell proliferation, endocytosis, and adhesion to target cells. Infect. Immun. 73, 8381–8392.
Venkatraj, M., Salado, I.G., Heeres, J., Joossens, J., Lewi, P.J., Caljon, G., Maes, L., Van der Veken, P., Augustyns, K., 2018. Novel triazine dimers with potent antitrypanosomal activity. Eur. J. Med. Chem. 143, 306–319.
Verdon, R., Mirelman, D., Sansonetti, P.J., 1992. A model of interaction between Entamoeba histolytica and Shigella flexneri. Res. Microbiol. 143, 67–74.
Verma, A.K., Verma, R., Ahuja, V., Paul, J., 2012. Real-time analysis of gut flora in Entamoeba histolytica infected patients of Northern India. BMC Microbiol. 12, 183.
Vincendeau, P., Gobert, A.P., Daulouede, S., Moynet, D., Mossalayi, M.D., 2003. Arginases in parasitic diseases. Trends Parasitol. 19, 9–12.
Wassmann, C., Hellberg, A., Tannich, E., Bruchhaus, I., 1999. Metronidazole resistance in the protozoan parasite Entamoeba histolytica is associated with increased expression of iron-containing superoxide dismutase and peroxiredoxin and decreased expression of ferredoxin 1 and flavin reductase. J. Biol. Chem. 274, 26051–26056.
Wong, W., Bai, X.C., Sleebs, B.E., Triglia, T., Brown, A., Thompson, J.K., Jackson, K.E., Hanssen, E., Marapana, D.S., Fernandez, I.S., Ralph, S.A., Cowman, A.F., Scheres, S.H.W., Baum, J., 2017. Mefloquine targets the Plasmodium falciparum 80S ribosome to inhibit protein synthesis. Nat. Microbiol. 2, 17031.
Yang, J.Y., Lee, H.S., 2015. Antimicrobial activities of active component isolated from Lawsonia inermis leaves and structure-activity relationships of its analogues against food-borne bacteria. J. Food Sci. Technol. 52, 2446–2451.
Zhang, T., Cieslak, P.R., Stanley Jr, S.L., 1994. Protection of gerbils from amebic liver abscess by immunization with a recombinant Entamoeba histolytica antigen. Infect. Immun. 62, 1166–1170.
Zhang, J., Liu, J., Ma, Y., Ren, D., Cheng, P., Zhao, J., Zhang, F., Yao, Y., 2016. One-pot synthesis and antifungal activity against plant pathogens of quinazolinone derivatives containing an amide moiety. Bioorg. Med. Chem. Lett. 26, 2273–2277.