The efficacy of herbal medicines against Toxoplasma gondii during the last three decades: a systematic review

Article *in* Canadian Journal of Physiology and Pharmacology · July 2016

DOI: 10.1139/cjpp-2016-0039

CITATIONS
16

READS
4,941

9 authors, including:

Mahdi Sharif
University of Salford
20 PUBLICATIONS **301** CITATIONS
SEE PROFILE

Shahabeddin Sarvi
Mazandaran University of Medical Sciences
79 PUBLICATIONS **1,253** CITATIONS
SEE PROFILE

Abdol satar Pagheh
Birjand University of Medical Sciences
63 PUBLICATIONS **511** CITATIONS
SEE PROFILE

Shabnam Asfaram
Mazandaran University of Medical Sciences
20 PUBLICATIONS **124** CITATIONS
SEE PROFILE

NRC Research Press

REVIEW

The efficacy of herbal medicines against *Toxoplasma gondii* during the last 3 decades: a systematic review

Mahdi Sharif, Shahabeddin Sarvi, Abdol Sattar Pagheh, Shabnam Asfaram, Mohammad Taghi Rahimi, Saeed Mehrzadi, Ehsan Ahmadpour, Shirzad Gholami, and Ahmad Daryani

Abstract: The objective of the current study was to systematically review papers discussing the efficacy of medicinal herbs against *Toxoplasma gondii*. Data were systematically collected from published papers about the efficacy of herbs used against *T. gondii* globally from 1988 to 2015, from PubMed, Google Scholar, ISI Web of Science, EBSCO, Science Direct, and Scopus. Forty-nine papers were included in the current systematic review reporting the evaluation of medicinal plants against *T. gondii* globally, both in vitro and in vivo. Sixty-one plants were evaluated. Most of the studies were carried out on *Artemisia annua*. The second highest number of studies were carried out on *Glycyrrhiza glabra* extracts. RH and ME49 were the predominant parasite strains used. Additionally, Swiss-Webster and BALB/c mice were the major animal models used. Alcoholic and aqueous extracts were used more than other types of extracts. Natural compounds mentioned here may be developed as novel and more effective therapeutic agents that improve the treatment of toxoplasmosis due to their lower side effects, higher availability, and better cultural acceptance compared with those of the chemical drugs that are currently being used.

Key words: systematic review, *Toxoplasma gondii*, toxoplasmosis, medicinal herbs, plant extracts, in vitro, in vivo.

Résumé : L'objectif de la présente étude était de réaliser une synthèse des publications discutant de l'efficacité des plantes médicinales contre *Toxoplasma gondii*. À l'aide des bases de données PubMed, de Google Scholar, d'ISI Web of Science, d'EBSCO, de Science Direct et de Scopus, nous avons recueilli de manière systématique les données de l'ensemble des articles portant sur l'efficacité de plantes contre *T. gondii*, qui ont été publiés universellement de 1988 à 2015. La présente synthèse systématique comprend 49 articles rapportant l'évaluation de plantes médicinales contre *T. gondii* à l'échelle mondiale, tant in vitro qu'in vivo. Nous avons évalué 61 plantes. La plupart des études ont porté sur *Artemisia annua*. En second lieu, les études ont porté sur des extraits de *Glycyrrhiza glabra*. Les souches RH et ME49 étaient les parasites utilisés de manière prédominante. En outre, les souris Swiss-Webster et BALB/c constituaient les modèles animaux les plus utilisés. Les extraits alcooliques et aqueux étaient plus utilisés que d'autres types d'extraits. Les substances naturelles mentionnées ici pourraient être développées en tant qu'agents thérapeutiques nouveaux et plus efficaces en vue d'améliorer le traitement de la toxoplasmose : ils ont moins d'effets indésirables, ils sont plus faciles à obtenir et ils jouissent d'une meilleure acceptabilité culturelle que les médicaments synthétiques couramment utilisés. [Traduit par la Rédaction]

Mots-clés : synthèse systématique, *Toxoplasma gondii*, toxoplasmose, plantes médicinales, extraits de plantes, in vitro, in vivo.

Introduction

Toxoplasmosis is a widespread infection in all warm-blooded vertebrates and humans, caused by the ubiquitous intracellular protozoan parasite, *Toxoplasma gondii* (Dubey and Jones 2008). This parasite is one of the most common parasites; it is estimated that up to one third of the world's population have anti-*Toxoplasma* antibodies. Definitive hosts are members of the Felidae family. Generally, humans acquire the infection by the oral route via the consumption of either undercooked or raw meat contaminated with *T. gondii* cysts, food products (vegetables and fruits), or water contaminated with oocysts. Other routes of transmission include congenital transmission, organ transplantation, and blood transfusion (Dubey et al. 2006).

Although most toxoplasmosis cases in healthy individuals are asymptomatic or mild, the first exposure to *T. gondii* during pregnancy is extremely dangerous, as it can undergo transplacental transmission to the embryo, leading to serious pathological consequences, including hydrocephalus, microcephaly, blindness, abortion, and death. In addition, *T. gondii* is an opportunistic and life-threatening parasite in immunocompromised groups such as HIV patients and cancer and organ transplant patients receiving immunosuppressive drugs (Rahimi et al. 2015). Thus, treatment of human toxoplasmosis is of great importance for all the above-mentioned groups. Various pharmacological agents are available such as pyrimethamine, alone or combined with sulfadiazine. Atovaquone has been prescribed as a second-line medicine for treatment of retinochoroiditis. Azithromycin is also used as an alternative drug for treatment of cerebral and ocular toxoplasmosis in AIDS patients (Ahmadpour et al. 2014; Dubey 2008). However, it is noteworthy to mention that these drugs are not completely effective in destroying the parasite (Hill and Dubey 2002). In addition, they have serious side effects that should not be neglected.

Received 21 January 2016. Accepted 27 April 2016.

M. Sharif, S. Sarvi, A.S. Pagheh, S. Asfaram, S. Gholami, and A. Daryani. Toxoplasmosis Research Center, Mazandaran University of Medical Sciences, Sari, Iran; Department of Parasitology and Mycology, Sari Medical School, Mazandaran University of Medical Sciences, Sari, Iran.
M.T. Rahimi. Shahroud University of Medical Sciences, Shahroud, Iran.
S. Mehrzadi. Department of Pharmacology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.
E. Ahmadpour. Infectious and Tropical Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.
Corresponding author: Ahmad Daryani (email: daryanii@yahoo.com).

For example, pyrimethamine and sulfadiazine, which are the most common and available drugs for toxoplasmosis, have several side effects including neutropenia, thrombocytopenia, leucopenia, elevation in serum creatinine and serum liver enzymes, hematological abnormalities, and hypersensitivity reactions (Caumes et al. 1995; Meneceur et al. 2008; Soheilian et al. 2005).

Therefore, a newer and safer therapy for toxoplasmosis is an urgent need. Considering the side effects of anti-*Toxoplasma* drugs and serious clinical manifestations of toxoplasmosis, particularly in the fetus, contribution of the ethnopharmacological knowledge to the treatment of this parasitic infection is necessary to investigate novel anti-*Toxoplasma* compounds with high activity, high efficacy, low toxicity, and low price. Hence, the objective of the current study was to systematically review papers on the efficacy of medicinal plants used against *T. gondii* throughout the world from 1988 to 2015, to prepare comprehensive data for better and safer therapeutic choices in the future.

Materials and methods

Database search

English databases, including PubMed, Google Scholar, ISI Web of Science, EBSCO, Science Direct, and Scopus, were searched for publications related to toxoplasmosis treatment with plants worldwide, from 1988 to 2015. Keywords included were "Toxoplasmosis", "*Toxoplasma gondii*", "Plant", "Anti-*Toxoplasma*", "Drug", "Anticoccidial", "Treatment", "In vitro", "In vivo", and "Herbal extract". Moreover, the language of data collection was limited to English.

Data collection

All experimental studies that were carried out to evaluate the efficacy of either herbal extracts or derivatives against *T. gondii* worldwide both in vitro and in vivo were included, and repetitive papers were excluded. The data collected for the current study were as follows: first author, year of publication, scientific name of the plants, study areas, part of the plant from which extraction or derivation took place, positive control, parasite strain, type of cells used for cultures, animal model, route of infection, time exposure, and main results.

Results

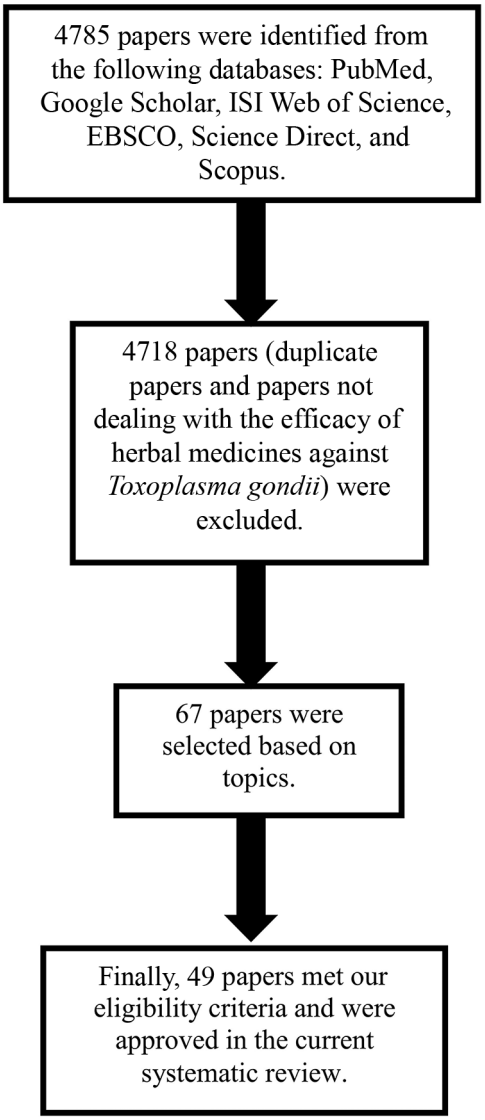
In total, 49 papers (24 in vitro, 17 in vivo, 8 in vitro and in vivo) from 1988 to 2015, were included in the current systematic review. A flowchart depicts the study design process (Fig. 1). The efficacy of 61 plant species against *T. gondii* was evaluated. Tables 1 and 2 show the characteristics of medicinal herbs tested against *T. gondii* in vitro and in vivo, respectively. Most of the studies were conducted on *Artemisia annua* (14 studies), followed by *Sophora flavescens* (4 studies), *Allium* sp., *Glycyrrhiza glabra,* and *Scutellaria baicalensis* Georgi (3 studies for each).

Most of the surveys used alcoholic (16 studies) and aqueous extracts (13 studies) of the plants. In vitro, human foreskin fibroblast (HFF) (8 studies) and Vero cells (6 studies) were used. The high virulent strain (RH) (37 studies) was predominantly used both in vitro and in vivo, followed by the low virulent strain (ME49) (4 studies). Notably, Swiss-Webster (4 studies) and BALB/c mice (5 studies) were the most commonly used animal models in the reviewed papers. Two studies were conducted on humans to evaluate the effect of artemisinin and artemether on *T. gondii* in schizophrenia patients.

Discussion

The aim of this study was to systematically review the efficacy of medicinal herbs against different strains of *T. gondii*, using documented data from the literature review. Toxoplasmosis is one of the most significant issues that continue to be a serious threat to public health. Laboratory studies have shown that strains of

Fig. 1. Flowchart describing the study design process.



T. gondii have significant resistance to a wide range of inhibitors, including those most frequently used by the patients (McFadden et al. 2001; Pfefferkorn et al. 1992). Furthermore, long-term drug treatment causes clinical failure due to the emergence of drug-resistant parasite variants (Pfefferkorn and Borotz 1994; Reynolds et al. 2001). On the other hand, high cost and low availability of some anti-*Toxoplasma* drugs, especially in poor countries, leads to an increase in the prescription of traditional medicines. In the past 30 years, scientists have seriously begun to study the efficacy of herbal drugs and their modes of action using in vivo and in vitro assays. In some studies, both conditions have been used (Benoit-Vical et al. 2000; Jones 1996).

Swiss-Webster and BALB/c mice are considered as the best model for toxoplasmosis investigation. Mice have the characteristics of easy breeding, small size, and short generation times, and are inexpensive and very sensitive to *T. gondii*. Parasite virulence in vivo is dependent on several factors, such as the animal model, route and dose of administration, the parasite stage, and the parasite strain (Ferreira et al. 2001; Asgari et al. 2013). Although strains of *T. gondii* are genetically similar, they show strong phenotypic differences in laboratory mice; type I (RH strain) is virulent (lethal) for naive mice while types II and III are significantly less virulent, because they may or may not cause death of naive

mice (Behnke et al. 2011). Our findings indicated that type I (RH strain) tachyzoites were the most commonly used strain to evaluate the effects of medicinal herbs in the reviewed studies. This may be due to their severe virulence and similar invasion conditions in the body (Araujo and Slifer 2003). However, some studies used low virulence strains, such as ME49, for determination of brain cyst burden and evaluation of anti-*T. gondii* activities. de Oliveira et al. (2009) evaluated the effects of *A. annua* L. on susceptibility to infection with *T. gondii* in mice and HFF using RH and ME49 strains. Cysts of the moderately virulent ME49 strain were obtained from the brain tissues of large vesper mice (*Calomys callosus*) that were infected 30–45 days earlier by the oral route. In vivo assays indicated that the infusion of *A. annua* effectively controls the infection with ME49 strain (100% survival rate, 30 days after infection) but could not control the infection caused by the virulent RH strain (20%–50% survival rate).

Various studies have shown that a number of antimalarial plants were evaluated for the treatment of *Toxoplasma* (Sepulveda-Arias et al. 2014). The anti-parasitic effect of these plants and their derivatives was not restricted to *Plasmodium* because they were also found to be inhibitors of *T. gondii*. These 2 parasites belong to the phylum Apicomplexa; they have common metabolic pathways and potential drug targets. Several drugs have already been known to be effective against both parasites, such as *Qinghaosu* (artemisinin), *Cinnamomum comphora, Lippia multiflora,* and *Vernonia colorata* (Benoit-Vical et al. 2000; Holfels et al. 1994). Experiments showed that compounds such as artemether, artesunate, and artemisinin produced by *A. annua* were used as antimalarial drugs and have shown some effect in inhibiting the growth of *T. gondii* (Sarciron et al. 2000).

Cells used for *T. gondii* in vitro often included HFF and Vero cells. Studies have shown that the propagation of *T. gondii* parasites in high quality cells produces tachyzoites with high yield and viability, but with minimal host cell contamination. Therefore, appropriate selection of host cells for reproduction of the parasites is important for the examination of anti-*Toxoplasma* activities (Saadatnia et al. 2010). Our findings indicate that alcoholic and aqueous extracts were used more than other types of extracts. The extraction method plays a major role in determining the concentration of constituents. For example, alkamides are the main hydrophobic component of plant root extracts and ethanol extraction results in greater concentrations of alkamides, whereas the more hydrophilic polysaccharides are retained in aqueous extracts (Hall 2003).

In this survey, *Artemisia* was the most commonly used extract against *T. gondii*. The genus *Artemisia* includes over 400 species, considered as common types of wormwood that are native in temperate Asia, but naturalized all over the world. Artemisinin, as an effective compound, is generally present in the leaves and flowers of the plant. Ridley and Hudson (1998) indicated that artemisinin destroys parasitic cells by producing highly reactive oxygen-based free radicals or electrophilic intermediates, alkylating and oxidizing proteins and lipids of parasite membranes and inactivating channel proteins. In addition, the effect of artemisinin is mediated through interfering with the electron transport chain in the mitochondrial membrane through free radicals and consequently leading to mitochondrial dysfunction. Ferreira et al. (2010) noted that the flavonoids of *A. annua* leaves have been involved in the destruction of CYP450 enzyme, which is responsible for altering the absorption and metabolism of artemisinin in the body. *Artemisia* efficacy against other species of parasites was studied. Lans et al. (2007) used *A. annua* for treatment of roundworms, pinworms, and *Giardia* in pigs. Studies have shown that extracts of *A. annua* were 81.6%–83.2% effective against the development of *Cryptosporidium parvum* in animal models. Two studies were conducted on humans to evaluate of the effect of artemisinin and artemether on *T. gondii* in schizophrenia patients, but it did not show clinical benefit of these derivatives for schizophrenia symptoms. However, they did find that adjunctive artemisinin reduced the levels of antibodies to gliadin, a food antigen (Dickerson et al. 2011; Wang et al. 2014). Generally, anti-*Toxoplasma* effects of medical plants such as survival rate, decreased cell injury, parasite load, and brain cyst burdens were evaluated in various studies. Hence, some medical plants that showed significant anti-*Toxoplasma* effects are introduced.

Glycyrrhiza glabra L. is native of southeastern Europe and southwestern Asia. *Glycyrrhiza glabra* has a variety of biological effects such as antimicrobial, anticancer, and antiviral activities. The main components are triterpenes, saponins, glycyrrhizin, and glycyrrhetic acid (Sharma and Agrawal 2013). Choi et al. (2008) evaluated the effects of *G. glabra* extract on the cell proliferation and cell viability of *T. gondii* using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Results indicated that the extract effectively had an anti-proliferative effect against *T. gondii* through direct inhibition of the parasite in infected host cells.

Eurycoma longifolia Jack is a flowering plant belonging to family Simaroubaceae, native to Indonesia and Malaysia. Extracts derived from the roots of this plant were found to have antimalarial and cytotoxic activities that were linked to the quassinoids, eurycomanone, 13α (21)-epoxy and 13, 21-dihydro analogues (Chan et al. 1986). Kavitha et al. (2012) evaluated the anti-*T. gondii* activity of 1 fraction of methanol extract and 4 fractions of the roots of *E. longifolia* in Vero cells. The assay of anti-*Toxoplasma* activity showed the greatest effect with TAF 355 and TAF 401 fractions.

Vernonia colorata is a known species of the *Vernonia* genus. The plant is extensively used throughout Africa in traditional medicine. Chukwujekwu et al. (2009) have reported excellent antimalarial activity for a pure molecule (vernodalin) isolated from this plant. Benoit-Vical et. al (2000) showed that *V. colorata* was significantly effective for toxoplasmosis treatment as it strongly inhibited *Toxoplasma* growth at concentrations > 10 mg/L, with a half maximal inhibitory concentration (IC_{50}) of 16.3 mg/L.

Ginkgo biloba (Ginkgoaceae) is a traditional herbal medicine in China with ingredients such as ginkgolic acids, chromocor, and polysaccharides. Ginkgolic acids significantly inhibited *T. gondii* DNA and protein synthesis, even at low concentrations (3.13, 6.25, or 12.5 μg/mL). Moreover, the toxicity of ginkgolic acids to HFF cells was lower than that of azithromycin (Chen et al. 2008).

Allium cepa (onion), a plant that is widely cultivated in most countries in the world, has a long history of medicinal use. It contains various chemicals that have unique biological activities such as tianshic acid, *N-trans*-feruloyltyramine, beta-sitosterol-3betaglucopyranoside-6-palmitate, sitosterol, daucosterol, tryptophan, and adenine. Onion juice is commonly used to prevent bacterial, fungal, and parasitic infections (Helen et al. 2000). Abu El Ezz et al. (2011) reported that *A. cepa* had effective anti-parasitic activity in a mouse model of *Cryptosporidium* infection. Efficacy of this plant against *T. gondii* has been shown in various studies. Garedaghi and Bahavarnia (2014) evaluated the effects of *A. cepa* on testis degeneration in male rats infected by *T. gondii* (RH strain). Results showed that using freshly prepared onion juice was effective in improving sperm health parameters and increasing the sperm number, viability, and motility in rats.

Zingiber officinale (ginger) has been widely used as an herbal medicine. Constituents of ginger include volatile oil, phenolic derivatives (zingerone), and oleoresin (gingerols and shogaols), which are the main antioxidant compounds in ginger (Rashidian et al. 2014). Ginger is used in the treatment of filaria and protoscolices (Baqer et al. 2014; Mostafa et al. 2011). Choi et al. (2013) evaluated the antiparasitic effect of ginger root extract (GE) and GE/F1 (fraction 1 obtained from GE) against *T. gondii* in vitro and in vivo. They showed that GE/F1 anti-*Toxoplasma* effects are due to inactivation of apoptotic proteins in infected host cells.

Myristica fragrans Houttuyn (nutmeg) belongs to family Myristicaceae and is native to Indonesia, Caribbean Islands, and India.

Table 1. In vitro studies on the anti-*Toxoplasma* effects of herbal medicine.

No.	Plants	Used part/derivative	Extract	Positive control	Strain	Parasite inoculation	Cells	Concentration	Result	Effectivity	Reference
1	*Artemisia annua*	Arteether	Ethyl ether	Roxithromycin	RH	—	Macrophage monolayer	0.01–400 μg/mL	Arteether showed inhibitory effect on the infected cells and *Toxoplasma* as low as 0.1 μg/mL	No	Chang and Pechère 1988
2	*A. annua*	Artemisinin Dihydroartemisinin Artemether Arteether 1-Propyl-ether-QHS 1-Butyl-ether-QHS Sec-butyl-ether-QHS		Pyrimethamine	RH	2×10^2	Fibroblast	0.01, 0.1, 1, and 10 μg/mL	Artemether was the most effective, with a potency of 10-fold greater than that of artemisinin	Yes	Ke et al. 1990
3	*A. annua*	Arteether	—	—	RH	—	Enterocyte or mouse peritoneal macrophages, M199-FCS	0.02, 0.2, 0.25, 0.5, 1.0, and 2.0 μg/mL	Concentration of 0.5 μg/mL showed inhibitory effect	Yes	Holfels et al. 1994
4	*A. annua*	Artesunate Dihydroartemisinin	Aqueous	Pyrimethamine	DUR	3×10^4	THP-1	0.1–0.5 μg/mL of dihydroartemisinin	Inhibition of parasite growth of 70% with dihydroartemisinin was seen. Artesunate and dihydroartemisinin decreased the number of tachyzoites to 40%–50%	Yes	Sarciron et al. 2000
5	*Azadirachta indica, Cinnamomum camphora, Lippia multiflora, Vernonia colorata, Guiera senegalensis, Combretum micranthum, Ximenia americana, Cochlospermum planchonii, Sida acuta*	Different parts	Aqueous	Pyrimethamine	RH	2×10^3	MRC5	0.1–50 mg/L	*V. colorata* showed inhibition of parasite growth. No inhibitory effect was observed with *A. indica, C. camphora, S. acuta, C. planchoni*, and *X. americana*	Yes	Benoit-Vical et al. 2000
6	*Gentiana scabra*	—	Methanol Aqueous	—	RH	—	Macrophage	Maximal nitrite concentration in macrophages: 23.22 and 24.07 μmol/L after water and methanolic extracts	*T. gondii* was inhibited	Yes	Kang et al. 2003
7	*Sophora flavescens, Sinomenium acutum, Pulsatilla koreana, Ulmus macrocarpa, Torilis japonica*	Different parts	Alcohol	Pyrimethamine Sulfadiazine	RH	1.0×10^5 (single concentraion) or 1.0×10^3 to 1.0×10^5 (dilution series)	ED	19.5–625 ng/mL	The reduction rates of *T. gondii* in minimal ranges: *T. japonica* (53.1%), *P. koreana* (38.4%), *S. flavescens* (27.2%)	Yes	Youn et al. 2003
8	*S. flavescens, T. japonica*	FractionsTJ1, TJ2/ SF1–SF5	Ethanol	Sulfadiazine	—	—	ED	0.356–2.850 ng/mL	TJ2 fraction of *T. japonica* and 4 fractions (SF1–SF4) of *S. flavescens* showed anti-protozoal activity, TJ1 and SF5 exhibited lower inhibitory rates	Yes	Youn et al. 2004
9	*A. annua*	Artemisinin and synthetic derivatives: Artemether, 2a, 2b, 2c, 2d, and Deoxy-2a	Nonacetal Hydrolytically	Trimethoprim	2 F	—	HFF	—	Artemether, 2b, 2c, and 2d inhibited *T. gondii* at less than 1 μg/mL. 2a inhibited at concentrations between 2 and 3 μg/mL	Yes	Jones-Brando et al. 2006

Table 1 (*continued*).

No.	Plants	Used part/derivative	Extract	Positive control	Strain	Parasite inoculation	Cells	Concentration	Result	Effectivity	Reference
10	Changqing capsule	—	—	Spiramycin Pyrimethamine Azithromycin	—	2.5×10^4	—	Different doses	Changqing capsule eliminated tachyzoites better than the current clinical drugs	Yes	Zhang et al. 2006
11	Huangqin, liquorice, purslane, *Inonotus obliquus*	—	—	—	RH	—	Vero		Huangqin and liquorice can inhibit the growth and reproduction of *T. gondii* but the effects of the inhibition in vitro of purslane, and *I. obliquus* are feeblish	Yes	Wang et al. 2007
12	*A. annua*	—	—	Thapsigargin	RH 2 F	—	HFF	—	Artemisinin treatment altered intracellular calcium in parasites by increasing the periodicity of calcium oscillations	Yes	Nagamune et al. 2007*a*
13	*A. annua*	Dihydroartemisinin Artemether Artesunate 9-epi-10 deoxoartemisinin 2-deoxyartemisinin artemisone	—	Pyrimethamine 5-fluoro-2-deoxyuridine	RH 2 F	—	HFF	10 nmol/L to 100 μmol/L	Artemisinin mutants were resistant to the induction of protein secretion from micronemes	Yes	Nagamune et al. 2007*b*
14	*Arecae pericarpium, S. flavescens, Meliae cortex, Rheum undulatum, Pulsatilla, Acorus gramineus* Soland, *Dryopteris crassirhizoma, Quisqualis indica, Fraxinus rhynchophylla* Hance, *G. glabra, Portulaca oleracea, Schizandra chinensis* Baillon, *Zingiber officinale, Angelica gigas, Artemisia scoparia*	—	Methanol	Pyrimethamine Sulfadiazine Spiramycin	RH	3×10^5	HeLa	0.11–2.28 mg/mL	Anti-*T. gondii* activity was observed with *Z. officinale* (EC_{50} = 0.18 mg/mL) and *S. flavescens* (EC_{50} = 0.20 mg/mL)	Yes	Choi et al. 2008
15	*Ginkgo biloba*	Sarcotesta	Petroleum ether	Azithromycin	RH	5×10^4	HFF	3.13–100 μg/mL	After 48 h, *T. gondii* DNA and protein synthesis were minimal, no visible parasites in HFF	Yes	Chen et al. 2008
16	*Fraxinus rhychophylla*	Oleuropein	—	Sulfadiazine Pyrimethamine	RH	—	Madin Darby bovine kidney cell	—	In the cells, the selectivity of oleuropein was 8.9	Yes	Jiang et al. 2008
17	*A. annua*	Artemiside Artemisone	—	Benzylpiperazine Pyrimidylpiperazine	RH 2 F	10^6	HFF	0.01–1.0 μmol/L	The compounds inhibited parasite growth, with EC_{50}s ranging from 0.11 to 0.21 μmol/L and with EC_{90}s ranging from 0.17 to 0.37 μmol/L	Yes	Dunay et al. 2009
18	*A. annua*	3a, 3b, 3c, 3d, 3e, 3f, and Deoxy-3a	—	Trimethoprim	RH	1.6×10^5	HFF	10 mg/L	5 of the 7 new derivatives, 3a–3c, 3e, and 3f, effectively inhibited *T. gondii* growth (IC_{50} = 1.0–4.4 mmol/L)	Yes	D'Angelo et al. 2009

Table 1 (*continued*).

No.	Plants	Used part/derivative	Extract	Positive control	Strain	Parasite inoculation	Cells	Concentration	Result	Effectivity	Reference
19	*A. annua*	Aerial parts	Aqueous	Sulfadiazine	RH	2×10^5	HFF	80–2500 μg/mL	Viability of HFF cells in the presence of different concentrations of *A. annua* and sulfadiazine was above 72%	Yes	de Oliveira et al. 2009
20	*Olea europaea*	Fruits of olive	Two-phase extraction	—	RH	—	Vero	—	Maslinic acid blocked entry of the parasite to the cell in an ID50 of 46 μmol/L and 8 μmol/L at 24 and 48 h of treatment	Yes	De Pablos et al. 2010
21	*Radix glycyrrhizae, Radix scutellariae, Radix stemonae, Rhizoma Cyrtomii, Radix sophora flavescentis*	—	—	Sulfadiazine sodium	RH	—	Ana-1	—	*R. glycyrrhizae, R. scutellariae*, and *R. stemonae* could inhibit the proliferation of *T. gondii*	Yes	Jing et al. 2011
22	*Melia azedarach* (cinnamon) *Azadirachta indica* (neem)	Leaves	Aqueous	—	RH	5:1 parasite–host cell ratio	Vero	0.15, 0.3, 0.5, 1, 2, 3, and 5 mg/mL	At higher doses, a reduction of about 90% was observed	Yes	Melo et al. 2011
23	*Eurycoma longifolia*	Root fractions (TAF 273, TAF 355, TAF 191, and TAF 401)	Methanol	Clindamycin	RH	3×10^5	Vero	1.56–100 μg/mL	Significant anti-*T. gondii* activity was observed with TAF 355 (EC_{50} = 0.369 μg/mL) and TAF 401 (EC_{50} = 0.882 μg/mL)	Yes	Kavitha et al. 2012
24	*Coptis chinenis* (goldenthread)	Berberine and its analogs 7a, 7b, 7c, 8a, 8b, and 8c	Alkaloids	Trimethoprim	RH	50	HFF	0.32–320 μg/mL	ID50 of less than 50 nmol/L and therapeutic indices (TI) up to 4000 were observed	Yes	Krivogorsky et al. 2012
25	*Myristica fragrans* Houtt. (nutmeg)	Fruits	Essential oil	Clindamycin	—	6×10^5	Vero	100 μg/mL to the lowest 3.125 μg/mL	Significant inhibiting activity with EC_{50} of 24.45 μg/mL	Yes	Pillai et al. 2012
26	*Scutellaria baicalensis*	—	Ethanol, Baicalin, Baicalein	—	RH	—	—	—	The effect of the ethanol extraction was better than of baicalin. The baicalien was the best role in *Scutellaria* on the inhibition of *Toxoplasma*	Yes	Wang et al. 2012
27	*Astragalus membranaceus, S. baicalensis*	Root	Aqueous	Cotrimoxazole	RH	5×10^4	HeLa	1, 10, and 100 mg/mL	Reduction of tachyzoites in SbE were higher than those in AmE	Yes	Yang et al. 2012
28	*Psidium guajava*, *Tinospora crispa*	Leaves Stem	Aqueous Methanol	Clindamycin	RH	6×10^5	Vero	200, 100, 50, 25, 12.5, 6.25, and 3.125 μg/mL	*T. crispa* stem extract (EC_{50} = 7.71 ± 1.56 μg/mL) and *P. guajava* leaves extract (EC_{50} = 121.18 ± 6.56 μg/mL)	Yes	Lee et al. 2012
29	*Z. officinale*, *Ginkgo biloba*, *Glycyrrhiza glabra*	Roots of ginger and *G. glabra*, leaves of *G. biloba*, GE/F1 fraction	Methanol	Sulfadiazine	RH	5×10^5	C6	30–240 μg/mL	GE (ginger extract) and GE/F1 inhibited viability of *T. gondii* compared with other extracts	Yes	Choi et al. 2013
30	*Vanilla*	Resvan	—	—	ME49	1×10^4	RAW 264.7 macrophage	645 μg/mL	Vanillin presents anti-*Toxoplasma* activity with IC_{50}: 645 μg/mL, but resvan does not show any	Yes	Oliveira et al. 2014

Table 1 (*concluded*).

No.	Plants	Used part/derivative	Extract	Positive control	Strain	Parasite inoculation	Cells	Concentration	Result	Effectivity	Reference
31	*Dictamnus dasycarpus*	—	Ethanol	Sulfadiazine	RH	—	—	—	The selectivity of *D. dasycarpus* was 7.52 compared with sulfadiazine 2.08	Yes	Hong et al. 2014
32	*Sambucos nigra*	Fruits and leaves	Methanol	Pyrimethamine	RH	4×10^6	—	5, 10, 25, and 50 mg/mL	Fruit extract was better than leaf. 5 mg/mL after 120 min killed 100% of tachyzoites	Yes	Daryani et al. 2015

Note: RH, High virulent strain; DUR, low virulent strain with chronic infection in mice; 2 F, causing acute infection in mice; ME49, low virulent strain; EC_{50}, half maximal effective concentration; EC_{90}, 90% maximal effective concentration; IC_{50}, half maximal inhibitory concentration; ID50, 50% inhibitory doses.

Anthelmintic and insecticide activities are the most important properties of this plant (Dwivedi et al. 2011). Anti-*Toxoplasma* activity depends on major compounds such as myristicin, limonene, eugenol, and terpinen-4-ol in the structure of the plant (Bakkali et al. 2008). Pillai et al. (2012) showed a potential anti-parasitic activity of this plant against *T. gondii* on Vero cell lines. This plant strongly inhibited *T. gondii* growth, with an IC_{50} value of 24.5 g/mL and low cytotoxic effects.

Astragalus membranaceus and *Scutellaria baicalensis* were other effective medicinal plants against *T. gondii*. These plants are broadly used as traditional herbal medicines in China, Japan, and Korea to treat bacterial and viral infections, as anti-inflammatory agents and immune stimulants. The main constituents of *A. membranaceus* are saponins, flavonoids, polysaccharides, amino acids, and trace elements (Yeh et al. 2014). The main chemical compounds of *S. baicalensis* include baicalein, baicalin, wogonin, and sitosterol. Baicalin and baicalein are the major antibacterial components of this plant (Blach-Olszewska and Lamer-Zarawska 2008). Yang et al. (2010) reported that these traditional herbs significantly inhibited the intracellular replication of *T. gondii* in treated cells (compared with untreated cells). Thus, the protective immunity against *T. gondii* could be notably enhanced by these extracts.

Myrrh is an aromatic resin obtained from the stem of the herbal tree *Commiphora molmol*. It contains a resin (myrrh), which is a combination of a volatile oil, gum, and a bitter principle (al-Awadi et al. 1991). Many studies have shown that myrrh is effective in the treatment of human schistosomiasis, fascioliasis, heterophyidiasis, and dicrocoeliasis (Abo-Madyan et al. 2004; Al-Mathal and Fouad 2004). In addition, it was shown to be very effective in the treatment of infections with *Trichomonas vaginalis* (Al-Zanbagi 2007). Al-Zanbagi and Zelai (2008) evaluated the anti-*Toxoplasma* activity of myrrh in murine models infected with the RH strain. Myrrh extract reduced the mean number of tachyzoites in the peritoneal fluid of infected mice 4 days after infection; for the treatment with 100 and 200 mg/kg per day, the growth inhibitions were 96.6% and 100%, respectively.

Nigella sativa is a plant native to the Middle East, southern Asia, and Eastern Europe and is known commonly as black cumin (Mohtashami et al. 2015). This plant has potential effect against *Plasmodium falciparum, Leishmania*, *Trypanosoma* spp., *Entamoeba histolytica,* and *C. parvum* (Abdulelah and Zainal-Abidin 2007; Fattahi Bafghi et al. 2011; Kayser et al. 2003; Tempone et al. 2005). Rayan et al. (2011) proved the anti-*Toxoplasma* activity of black seed oil from *N. sativa*, as this oil was able to significantly protect mice infected with the ME49 strain against death and brain cyst burdens compared with the infected untreated control. The antiprotozoal activity of this plant may be attributed to the presence of different classes of alkaloids and phenolics.

Sambucus nigra is a flowering plant belonging to family Adoxaceae. The main components in this plant include cyanidin-3-glucoside, cyanidin-3-sambubioside, and small amounts of anthocyanins, flavonols, and flavonol esters (Sidor and Gramza-Michałowska 2015). It has substantial effects such as anti-*Helicobacter pylori*, antiprotozoal, and antibacterial activities. Daryani et al. (2015) evaluated the anti-*Toxoplasma* activity of a methanolic extract of *S. nigra* fruits and leaves in infected macrophages. Results of this study confirmed that *S. nigra* has satisfactory efficacy in vitro and that the parasiticidal effect of the fruit extract was significantly better than that of the leaf extract.

As shown by these studies, many new natural products have revealed considerable efficacy and high success against *Toxoplasma,* both in vitro and in vivo. However, some of these studies suffer from drawbacks that could influence the accuracy of the obtained results. These limitations include the wide range of herbal extract doses used and different extract preparation methods, as well as that some studies were performed only in vitro and were not investigated in vivo.

Table 2. In vivo studies on anti-*Toxoplasma* effects of herbal medicine.

No.	Plant	Used part/Derivative	Extract	Positive control	Animal/Strain	Treatment/Route	Result	Effectivity	Reference
1	*Artemisia annua*	Arteether	Ethyl ether	Roxithromycin	Swiss-Webster RH	1, 10, 25, 50, 100, 200, 400, and 600 mg/kg for 5 days i.p.	With 200 mg/kg, there was an increase in survival, 20% of mice living until the 15th day after challenge, but all died of toxoplasmosis on the 16th day	No	Chang and Pechère 1988
2	*A. annua*	Artesunate Dihydroartemisinin	Aqueous	Sulfadiazine Pyrimethamine	OF1 mice DUR	100 mg (3 times a day) for 5 days orally	Decrease in cerebral cysts of 40%	Yes	Sarciron et al. 2000
3	*Allium sativum* (garlic)	Different parts	Aqueous	—	BALB/c RH	100, 200, 400, and 500 mg/kg per day orally	Survival rate of 100% until the fifth day	Yes	Khoushzaban et al. 2007
4	*Commiphora molmol* (myrrh)	Resin	Aqueous	Spiramycin	CBA/J RH	100 and 200 mg/kg per day i.p.	With 100 and 200 mg/kg per day of myrrh, the percentage of growth inhibition were 96.6% and 100%, respectively	Yes	Al-Zanbagi 2007
5	*Juglans mandshurica*	—	—	Acetylspiramycin	Mice RH	0.068, 0.135, and 0.278 g/kg per day	*J. mandshurica* enhanced macrophage phagocytic function on *T. gondii*	Yes	Wen et al. 2008
6	*Fraxinus rhynchophylla*	Oleuropein	—	Sulfadiazine Pyrimethamine	Mice RH	24, 125, and 300 mg/kg once a day for 4 days orally	The inhibition ratio was 55.4% after treatment with 300 mg/kg oleuropein	Yes	Jiang et al. 2008
7	*Curcuma longa*	—	Ethanol	—	BALB/c RH	—	No tachyzoites were found in the peritoneal fluid of infected mice	Yes	Al-Zanbagi and Zelai 2008
8	*Peganum harmala*	—	Aqueous	Pyrimethamine	BALB/c RH	100, 200, 300, 400, and 500 μg/kg for 7 days orally	100% of mice living until the 5th day after challenge (Effective dose 300 μg/kg)	Yes	Khoshzaban et al. 2008
9	*A. annua*	Artemisone Artemiside	—	Sulfadiazine	C57BL/6 PRU	10 mg/kg every day for 8 days	60% of the artemiside-treated mice and more than 50% of the artemisone-treated mice survived from infection	Effective in acute form	Dunay et al. 2009
10	*Piper nigrum*, *Capsicum frutescens*, *Cinnamomum cassia*, *Curcuma longa*	Oil	Aqueous Ethanol	Spiramycin	Swiss albino RH	100 and 200 mg/kg per day for 7 days i.p.	The most effective extract was *Curcuma longa* ethanol extract with 98.6% and 99.2% inhibition of the growth *T. gondii* in 100 and 200 doses	Yes	Al-Zanbagi 2009
11	*A. annua*	Aerial parts	Aqueous	Sulfadiazine	Calomys callous C57BL/6 and BALB/c RH, Me49	10 mg/kg per day during 5 days s.c.	Survival rates ranged from 20% to 50%	Yes	de Oliveira et al. 2009
12	*Thymus broussonetii*	Aerial parts	Oil	—	OF1 mice PRU	20 μg/animal several days orally	Essential oils of thyme had a blocking effect on the appearance of the cysts	Yes	Dahbi et al. 2010
13	*Astragalus membranaceus* (AmE), *Scutellaria baicalensis E* (SbE)	Root	Aqueous	—	Mice RH	AmE 75 mg/kg per day SbE 50 mg/kg per day 21 days orally	Treatment with AmE or SbE, had prolonged survival time and Th1-type cellular immune response	Yes	Yang et al. 2010
14	*Nigella sativa* (black seed or black cumin)	Oil	—	—	Swiss albino Me49 cyst	5 mL/kg body mass per day for 14 days orally	Reduced brain cyst burdens and enhanced expression of iNOS	Yes	Rayan et al. 2011
15	*Panax ginseng*	Root	—	—	ICR mice RH	50 μg s.c.	Mice immunized showed an increased survival time compared with control mice	Yes	Qu et al. 2011

Table 2 (*concluded*).

No.	Plant	Used part/Derivative	Extract	Positive control	Animal/Strain	Treatment/Route	Result	Effectivity	Reference
16	*Juniperus procera*	Different parts	Aqueous	Spiramycin	Mice RH	Orally	The stem, leaves, and fruits of *J. procera* caused 48%, 50%, and 53.5% inhibition of *T. gondii*	Yes	Al-Zanbagi 2011
17	*A. annua*	—	Aqueous	Placebo	66 participants with schizophrenia	100 mg of artemisinin orally twice a day	In this human study, neither groups had significant changes in antibodies to *T. gondii*	No	Dickerson et al. 2011
18	*Allium. cepa* (onion)	Fruit	Aqueous	—	Wistar albino RH	1 mL of onion juice for 13 days orally	Onion juice had strong antioxidant potential and decreasing cell injury	Yes	Gharadaghi et al. 2012
19	*Zingiber officinale* (ginger), *Ginkgo biloba*, *Glycyrrhiza glabra*	Roots of ginger and *G. glabra*, leaves of *G. biloba*, GE/F1 fraction	Methanol	Sulfadiazine	BALB/c RH	50, 500 μg/mL	GE/F1 causing the inactivation of apoptotic proteins in infected host cells through the direct inhibition of *T. gondii*	Yes	Choi et al. 2013
20	*P. ginseng*	Root (ginsenoside Re)	—	—	ICR mice RH	50 μg s.c.	Vaccinated mice displayed significantly increased survival time	Yes	Qu et al. 2013
21	*A. cepa* (onion)	Fruit	—	—	Wistar albino rats RH	0.5 g/rat, 1 g/rat of onion juice for 30 days	Serum proteins and testis mass were not decreased in the control and onion groups. Total antioxidant capacity increased in the groups that received onion juice	Yes	Garedaghi and Bahavarnia 2014
22	*A. membranaceus*	Root	—	Atovaquone	BALB/c RH	0.075 mg/g for 7 days orally	*Astragalus* increased the levels of IL-2	Yes	Sönmez et al. 2014
23	*A. annua*	LEW3–27 CPH4–136	—	—	CBA/J or Swiss-Webster RHMe49 cyst	3–50 mg/kg twice daily for 1–7 days i.p.	In mouse model of acute toxoplasmosis, both derivatives showed modest efficacy but in mouse model of chronic, significantly decreased brain cyst burden	Yes	Schultz et al. 2014
24	*Vanilla*	Resvan	—	Sulfadiazine	Swiss-Webster Me49 cyst	Resvan (500 mg/kg) or vanillin (500 mg/kg) for 6 days orally	Resvan showed antioxidative activity (84.9%) comparing with vanillin (19.4%) at 1000 μg/mL. Vanillin presents anti-*Toxoplasma* activity, while resvan did not show any	Yes	Oliveira et al. 2014
25	*A. annua*	Artemether	Ethyl ether	Trihexyphenidyl and clonazepam	100 participants	80 mg artemether orally	In this human study, artemether as an adjunct to risperidone could not to reduce cognitive deficits of schizophrenia	No	Wang et al. 2014

Note: RH, High virulent strain; DUR, low virulent strain with chronic infection in mice; ME49, low virulent strain.

Conclusion

This study clarifies the status of the examined herbal products as anti-*Toxoplasma*. Natural compounds in this review would contribute to the search for novel and more effective therapeutic applications in toxoplasmosis due to lower side effects, higher availability, and better cultural acceptance compared with the chemical drugs that are currently being used. The authors highly recommend further preclinical and clinical investigations on the medicinal plants that have shown promising and desired outcomes.

Conflict of interest

The authors declare that there is no conflict of interest associated with this work.

Acknowledgements

We thank Vice Chancellors for Research of Mazandaran University of Medical Sciences for financial support (grant no. 1662).

References

Abdulelah, H.A.A., and Zainal-Abidin, B.A.H. 2007. In vivo anti-malarial tests of *Nigella sativa* (Black Seed) different extracts. Am. J. Pharm. Toxicol. **2**(2): 46–50. doi:10.3844/ajptsp.2007.46.50.

Abo-Madyan, A.A., Morsy, T.A., Motawea, S.M., and Morsy, A.T. 2004. Clinical trial of Mirazid in treatment of human fascioliasis, Ezbet El-Bakly (Tamyia Center) Al-Fayoum Governorate. J. Egypt Soc. Parasitol. **34**(3): 807–818. PMID: 15587309.

Abu El Ezz, N.M.T., Khalil, F.A.M., and Shaapan, R.M. 2011. Therapeutic effect of onion (*Allium cepa*) and cinnamon (*Cinnamomum zeylanicum*) oils on cryptosporidiosis in experimentally infected mice. Global Vet. **13**: 179–183.

Ahmadpour, E., Daryani, A., Sharif, M., Sarvi, S., Aarabi, M., Mizani, A., et al. 2014. Toxoplasmosis in immunocompromised patients in Iran: a systematic review and meta-analysis. J. Infect. Dev. Ctries. **8**(12): 1503–1510. PMID: 25500647.

al-Awadi, F., Fatania, H., and Shamte, U. 1991. The effect of a plants mixture extract on liver gluconeogenesis in streptozotocin induced diabetic rats. Diabetes Res. **18**(4): 163–168. PMID:1842751.

Al-Mathal, E.M., and Fouad, M.A. 2004. Myrrh (*Commiphora molmol*) in treatment of human and sheep *Dicrocoeliasis dendriticum* in Saudi Arabia. J. Egypt Soc. Parasitol. **34**(2): 713–720. PMID:15287191.

Al-Zanbagi, N. 2007. Comparative study of myrrh and metronidazole effect on *Trichomonas vaginalis* in vitro. J. Union Arab Biolog. **8**: 47–68.

Al-Zanbagi, N.A. 2009. In vivo effect of some home spices extracts on the *Toxoplasma gondii* Tachyzoites. J. Family Community Med. **16**(2): 59–65. PMID: 23012192.

Al-Zanbagi, N.A. 2011. Noticeable effect of Juniperus procera as *Toxoplasma gondii* tachyzoites inhibitor in vivo. Int. J. Health Welln. Soc. **1**(2): 197–204. doi:10.18848/2156-8960/CGP/v01i02/41159.

Al-Zanbagi, N.A., and Zelai, N.T. 2008. Two methods for attenuating *Toxoplasma gondii* tachyzoites RH strain by using ethanol extract of Curcuma longa. J. Egypt Soc. Parasitol. **38**(3): 965–976. PMID:19209778.

Araujo, F.G., and Slifer, T. 2003. Different strains of *Toxoplasma gondii* induce different cytokine responses in CBA/Ca mice. Infect. Immun. **71**(7): 4171–4174. doi:10.1128/IAI.71.7.4171-4174.2003. PMID:12819111.

Asgari, Q., Keshavarz, H., Shojaee, S., Motazedian, M.H., Mohebali, M., Miri, R., et al. 2013. In vitro and in vivo potential of RH strain of *Toxoplasma gondii* (Type I) in tissue cyst forming. Iran. J. Parasitol. **8**(3): 367–375. PMID:24454428.

Bakkali, F., Averbeck, S., Averbeck, D., and Idaomar, M. 2008. Biological effects of essential oils – a review. Food Chem. Toxicol. **46**(2): 446–475. doi:10.1016/j.fct.2007.09.106. PMID:17996351.

Baqer, N.N., Khuder, M.H., and Amer, N. 2014. Antiprotoscolices effects of ethanolic extract of *Zingiber officinale* against *Echinococcus granulosus* invitro and invivo. Int. J. Adv. Res. **2**(10): 59–68.

Behnke, M.S., Khan, A., Wootton, J.C., Dubey, J.P., Tang, K., and Sibley, L.D. 2011. Virulence differences in *Toxoplasma* mediated by amplification of a family of polymorphic pseudokinases. Proc. Natl. Acad. Sci. U.S.A. **108**(23): 9631–9636. doi:10.1073/pnas.1015338108. PMID:21586633.

Benoit-Vical, F., Santillana-Hayat, M., Kone-Bamba, D., Mallie, M., and Derouin, F. 2000. Anti-*Toxoplasma* activity of vegetal extracts used in West African traditional medicine. Parasite, **7**(1): 3–7. doi:10.1051/parasite/2000071003. PMID:10743641.

Blach-Olszewska, Z., and Lamer-Zarawska, E. 2008. Come back to root-Therapeutic activities of *Scutellaria baicalensis* root in aspect of innate immunity regulation-Part I. Adv. Clin. Exp. Med. **17**(3): 337–345.

Caumes, E., Bocquet, H., Guermonprez, G., Rogeaux, O., Bricaire, F., Katlama, C., and Gentilini, M. 1995. Adverse cutaneous reactions to pyrimethamine/sulfadiazine and pyrimethamine/clindamycin in patients with AIDS and toxoplasmic encephalitis. Clin. Infect. Dis. **21**(3): 656–658. doi:10.1093/clinids/21.3.656. PMID:8527561.

Chan, K.L., O'Neill, M.J., Phillipson, J.D., and Warhurst, D.C. 1986. Plants as sources of antimalarial drugs. Part 3 *Eurycoma longifolia*. Planta Medica, **52**(2): 105–107. doi:10.1055/s-2007-969091. PMID:3725929.

Chang, H.R., and Pechère, J.C. 1988. Arteether, a qinghaosu derivative, in toxoplasmosis. Trans. R. Soc. Trop. Med. Hyg. **82**: 867. doi:10.1016/0035-9203(88)90021-1. PMID:3256990.

Chen, S.X., Wu, L., Jiang, X.G., Feng, Y.Y., and Cao, J.P. 2008. Anti-*Toxoplasma gondii* activity of GAS in vitro. J. Ethnopharmacol. **118**(3): 503–507. doi:10.1016/j.jep.2008.05.023. PMID:18602775.

Choi, K.M., Gang, J., and Yun, J. 2008. Anti-*Toxoplasma gondii* RH strain activity of herbal extracts used in traditional medicine. Int. J. Antimicrob. Agents. **32**(4): 360–362. doi:10.1016/j.ijantimicag.2008.04.012. PMID:18619816.

Choi, W., Jiang, M., and Chu, J. 2013. Antiparasitic effects of *Zingiber officinale* (Ginger) extract against *Toxoplasma gondii*. J. Appl. Biomed. **11**(1): 15–26. doi:10.2478/v10136-012-0014-y.

Chukwujekwu, J.C., Lategan, C.A., Smith, P.J., Van Heerden, F.R., and Van Staden, J. 2009. Antiplasmodial and cytotoxic activity of isolated sesquiterpene lactones from the acetone leaf extract of *Vernonia colorata*. S. Afr. J. Bot. **75**(1): 176–179. doi:10.1016/j.sajb.2008.10.001.

Dahbi, A., Bellete, B., Flori, P., Hssaine, A., Elhachimi, Y., Raberin, H., et al. 2010. The effect of essential oils from *Thymus broussonetii* Boiss on transmission of *Toxoplasma gondii* cysts in mice. Parasitol. Res. **107**(1): 55–58. doi:10.1007/s00436-010-1832-z. PMID:20336317.

D'Angelo, J.G., Bordón, C., Posner, G.H., Yolken, R., and Jones-Brando, L. 2009. Artemisinin derivatives inhibit *Toxoplasma gondii* in vitro at multiple steps in the lytic cycle. J. Antimicrob. Chemother. **63**(1): 146–150. doi:10.1093/jac/dkn451. PMID:18988681.

Daryani, A., Ebrahimzadeh, M.A., Sharif, M., Ahmadpour, E., Edalatian, S., Rahimi Esboei, B., and Sarvi, S. 2015. Anti-*Toxoplasma* activities of methanolic extract of *Sambucus nigra* (Caprifoliaceae) fruits and leaves. Rev. Biol. Trop. **63**(1): 7–12. doi:10.15517/rbt.v63i1.14545. PMID:26299111.

de Oliveira, T.C., Silva, D.A., Rostkowska, C., Béla, S.R., Ferro, E.A., Magalhães, P.M., and Mineo, J.R. 2009. *Toxoplasma gondii*: effects of *Artemisia annua* L. on susceptibility to infection in experimental models in vitro and in vivo. Exp. Parasitol. **122**(3): 233–241. doi:10.1016/j.exppara.2009.04.010. PMID:19389400.

De Pablos, L.M., González, G., Rodrigues, R., García Granados, A., Parra, A., and Osuna, A. 2010. Action of a pentacyclic triterpenoid, maslinic acid, against *Toxoplasma gondii*. J. Nat. Prod. **73**(5): 831–834. doi:10.1021/np900749b. PMID: 20441162.

Dickerson, F., Stallings, C., Vaughan, C., Origoni, A., Goga, J., Khushalani, S., and Yolken, R. 2011. Artemisinin reduces the level of antibodies to gliadin in schizophrenia. Schizophr. Res. **129**(2–3): 196–200. doi:10.1016/j.schres.2011.04.010. PMID:21546216.

Dubey, J.P. 2008. The history of *Toxoplasma gondii* — the first 100 years. J. Eukaryot. Microbiol. **55**(6): 467–475. doi:10.1111/j.1550-7408.2008.00345.x. PMID: 19120791.

Dubey, J.P., and Jones, J.L. 2008. *Toxoplasma gondii* infection in humans and animals in the United States. Int. J. Parasitol. **38**(11): 1257–1278. doi:10.1016/j.ijpara.2008.03.007. PMID:18508057.

Dubey, J.P., Su, C., Cortés, J.A., Sundar, N., Gomez-Marin, J.E., Polo, L.J., et al. 2006. Prevalence of *Toxoplasma gondii* in cats from Colombia, South America and genetic characterization of *T. gondii* isolates. Vet. Parasitol. **141**(1–2): 42–47. doi:10.1016/j.vetpar.2006.04.037. PMID:16797845.

Dunay, I.R., Chan, W.C., Haynes, R.K., and Sibley, L.D. 2009. Artemisone and artemiside control acute and reactivated toxoplasmosis in a murine model. Antimicrob. Agents Chemother. **53**(10): 4450–4456. doi:10.1128/AAC.00502-09. PMID:19635951.

Dwivedi, G., Bairagi, M., Rawal, D., and Rawal, S. 2011. Anthelmintic activity of *Myristica fragrans* (Nutmeg) extract. Res. J. Pharm. Biol. Chem. Sci. **2**(3): 315.

Fattahi Bafghi, A., Vahidi, A.R., Anvari, M.H., Barzegar, K., and Ghafourzadeh, M. 2011. The in vivo antileishmanial activity of alcoholic extract from *Nigella sativa* seeds. Afr. J. Microbiol. Res. **5**(12): 1504–1510. doi:10.5897/AJMR11.009.

Ferreira, A.M., Martins, M.S., and Vitor, R.W.A. 2001. Virulence for BALB/c mice and antigenic diversity of eight *Toxoplasma gondii* strains isolated from animals and humans in Brazil. Parasite, **8**: 99–105. doi:10.1051/parasite/2001082099. PMID:11474987.

Ferreira, J.F., Luthria, D.L., Sasaki, T., and Heyerick, A. 2010. Flavonoids from *Artemisia annua* L. as antioxidants and their potential synergism with artemisinin against malaria and cancer. Molecules, **15**(5): 3135–3170. doi:10.3390/molecules15053135. PMID:20657468.

Garedaghi, Y., and Bahavarnia, S.R. 2014. Repairing effect of *Allium cepa* on testis degeneration caused by *Toxoplasma gondii* in the rat. Int. J. Women's Health Reproduction Sci. **2**(2): 80–89. doi:10.15296/ijwhr.2014.12.

Gharadaghi, Y., Shojaee, S., Khaki, A., Hatef, A., Ahmadi Ashtiani, H.R., Rastegar, H., and Fathiazad, F. 2012. Modulating effect of *Allium cepa* on kidney apoptosis caused by *Toxoplasma gondii*. Adv. Pharm. Bull. **2**(1): 1–6. doi:10.5681/apb.2012.001. PMID:24312764.

Hall, C., III. 2003. *Echinacea* as a functional food ingredient. Adv. Food Nutr. Res. **47**: 113–173. doi:10.1016/S1043-4526(03)47003-4. PMID:14639783.

Helen, A., Krishnakumar, K., Vijayammal, P.L., and Augusti, K.T. 2000. Antioxi-

dant effect of onion oil (*Allium cepa*. Linn) on the damages induced by nicotine in rats as compared to alpha-tocopherol. Toxicol. Lett. **116**(1): 61–68. PMID: 10906423.

Hill, D., and Dubey, J.P. 2002. *Toxoplasma gondii*: transmission, diagnosis and prevention. Clin. Microbiol. Infect. **8**(10): 634–640. doi:10.1046/j.1469-0691.2002.00485.x. PMID:12390281.

Holfels, E., McAuley, J., Mack, D., Milhous, W.K., and McLeod, R. 1994. In vitro effects of artemisinin ether, cycloguanil hydrochloride (alone and in combination with sulfadiazine), quinine sulfate, mefloquine, primaquine phosphate, trifluoperazine hydrochloride, and verapamil on *Toxoplasma gondii*. Antimicrob. Agents Chemother. **38**(6): 1392–1396. doi:10.1128/AAC.38.6.1392. PMID:8092843.

Hong, S., Lee, H.-A., Lee, Y.-S., Chung, Y.-H., and Kim, O. 2014. Anti-toxoplasmosis effect of *Dictamnus dasycarpus* extract against *Toxoplasma gondii*. J. Biomed. Res. **15**(1): 7–11. doi:10.12729/jbr.2014.15.1.007.

Jiang, J.H., Jin, C.M., Kim, Y.C., Kim, H.S., Park, W.C., and Park, H. 2008. Anti-toxoplasmosis effects of oleuropein isolated from *Fraxinus rhychophylla*. Biol. Pharm. Bull. **31**(12): 2273–2276. doi:10.1248/bpb.31.2273. PMID:19043212.

Jing, Z.Y., Jiang, W., Wang, Q., Chen, Y., Yang, K., Song, N., et al. 2011. Inhibition effects of five Chinese herbal medicines on *Toxoplasma gondii* proliferation in vitro. Chin. Vet. Sci. **2**(22): 853–857.

Jones, F.A. 1996. Herbs — useful plants. Their role in history and today. Eur. J. Gastroenterol. Hepatol. **8**(12): 1227–1231. doi:10.1097/00042737-199612000-00018. PMID:8980946.

Jones-Brando, L., D'Angelo, J., Posner, G.H., and Yolken, R. 2006. In vitro inhibition of *Toxoplasma gondii* by four new derivatives of artemisinin. Antimicrob. Agents Chemother. **50**(12): 4206–4208. doi:10.1128/AAC.00793-06. PMID: 17060514.

Kang, S.G., Ryang, Y.S., and Kim, I. 2003. Effects of *Gentiana scabra* var. *buergeri* extract on Toxoplasmastic activity of macrophages. J. Exp. Biol. Sci. **9**(2): 85–91.

Kavitha, N., Noordin, R., Chan, K.-L., and Sasidharan, S. 2012. In vitro anti-*Toxoplasma gondii* activity of root extract/fractions of *Eurycoma longifolia* Jack. BMC. Complement. Altern. Med. **12**: 91. doi:10.1186/1472-6882-12-91. PMID: 22781137.

Kayser, O., Kiderlen, A.F., and Croft, S.L. 2003. Natural products as antiparasitic drugs. Parasitol. Res. **90**: S55–S62. doi:10.1007/s00436-002-0768-3. PMID: 12937967.

Ke, O.Y., Krug, E.C., Marr, J.J., and Berens, R.L. 1990. Inhibition of growth of *Toxoplasma gondii* by qinghaosu and derivatives. Antimicrob. Agents Chemother. **34**(10): 1961–1965. doi:10.1128/AAC.34.10.1961. PMID:2291661.

Khoshzaban, F., Ghaffarifar, F., Sharafi, M., and Ghasemi Nikou, S. 2008. Effect of *Peganum harmala* on acute toxoplasmosis in mice. Daneshvar Med. **15**(75): 27–36.

Khoushzaban, F., Ghazanfari, T., Ghaffarif, F., Sharifi, M., and Ghasemi, N.S. 2007. The effect of garlic extract on acute toxoplasmosis in mice. Iranian J. Med. Arom. Plant. **3**(37): 295–306.

Krivogorsky, B., Pernat, J.A., Douglas, K.A., Czerniecki, N.J., and Grundt, P. 2012. Structure–activity studies of some berberine analogs as inhibitors of *Toxoplasma gondii*. Bioorg. Med. Chem. Lett. **22**(8): 2980–2982. doi:10.1016/j.bmcl.2012.02.038. PMID:22425568.

Lans, C., Turner, N., Khan, T., and Brauer, G. 2007. Ethnoveterinary medicines used to treat endoparasites and stomach problems in pigs and pets in British Columbia, Canada. Vet. Parasitol. **148**(3–4): 325–340. doi:10.1016/j.vetpar.2007.06.014. PMID:17628343.

Lee, W.C., Mahmud, R., Noordin, R., Piaru, S.P., Perumal, S., and Ismail, S. 2012. Alkaloids content, cytotoxicity and anti-*Toxoplasma gondii* activity of *Psidium guajava* L. and *Tinospora crispa*. Bangl. J. Pharmacol. **7**(4): 272–276. doi:10.3329/bjp.v7i4.12499.

McFadden, D.C., Camps, M., and Boothroyd, J.C. 2001. Resistance as a tool in the study of old and new drug targets in *Toxoplasma*. Drug Resist. Updat. **4**(2): 79–84. doi:10.1054/drup.2001.0184. PMID:11512524.

Melo, E.J.T., Vilela, K.J., and Carvalho, C.S. 2011. Effects of aqueous leaf extracts of Azadirachta indica A. Juss. (neem) and Melia azedarach L. (Santa Barbara or cinnamon) on the intracellular development of *Toxoplasma gondii*. Rev. Bras. Plantas Med. Botucatu. **13**(2): 215–222. doi:10.1590/S1516-05722011000200014.

Meneceur, P., Bouldouyre, M.-A., Aubert, D., Villena, I., Menotti, J., Sauvage, V., et al. 2008. In vitro susceptibility of various genotypic strains of *Toxoplasma gondii* to pyrimethamine, sulfadiazine, and atovaquone. Antimicrob. Agents Chemother. **52**(4): 1269–1277. doi:10.1128/AAC.01203-07. PMID:18212105.

Mohtashami, R., Huseini, H.F., Heydari, M., Amini, M., Sadeqhi, Z., Ghaznavi, H., and Mehrzadi, S. 2015. Efficacy and safety of honey based formulation of *Nigella sativa* seed oil in functional dyspepsia: A double blind randomized controlled clinical trial. J. Ethnopharmacol. **175**: 147–152. doi:10.1016/j.jep.2015.09.022. PMID:26386381.

Mostafa, O.M., Eid, R.A., and Adly, M.A. 2011. Antischistosomal activity of ginger (*Zingiber officinale*) against *Schistosoma mansoni* harbored in C57 mice. Parasitol. Res. **109**(2): 395–403. doi:10.1007/s00436-011-2267-x. PMID:21327992.

Nagamune, K., Beatty, W.L., and Sibley, L.D. 2007*a*. Artemisinin induces calcium-dependent protein secretion in the protozoan parasite *Toxoplasma gondii*. Eukaryot. Cell, **6**(11): 2147–2156. doi:10.1128/EC.00262-07. PMID:17766463.

Nagamune, K., Moreno, S.N., and Sibley, L.D. 2007*b*. Artemisinin-resistant mutants of *Toxoplasma gondii* have altered calcium homeostasis. Antimicrob. Agents Chemother. **51**(11): 3816–3823. doi:10.1128/AAC.00582-07. PMID: 17698618.

Oliveira, C.B., Meurer, Y.S., Oliveira, M.G., Medeiros, W.M., Silva, F.O., Brito, A.C., et al. 2014. Comparative study on the antioxidant and anti-*Toxoplasma* activities of vanillin and its resorcinarene derivative. Molecules, **19**(5): 5898–5912. doi:10.3390/molecules19055898. PMID:24810805.

Pfefferkorn, E.R., and Borotz, S.E. 1994. Comparison of mutants of *Toxoplasma gondii* selected for resistance to azithromycin, spiramycin, or clindamycin. Antimicrob. Agents Chemother. **38**(1): 31–37. doi:10.1128/AAC.38.1.31. PMID: 8141576.

Pfefferkorn, E.R., Nothnagel, R.F., and Borotz, S.E. 1992. Parasiticidal effect of clindamycin on *Toxoplasma gondii* grown in cultured cells and selection of a drug-resistant mutant. Antimicrob. Agents Chemother. **36**(5): 1091–1096. doi: 10.1128/AAC.36.5.1091. PMID:1510399.

Pillai, S., Mahmud, R., Lee, W.C., and Perumal, S. 2012. Anti-parasitic activity of *Myristica fragrans* Houtt. essential oil against *Toxoplasma gondii* parasite. APCBEE Procedia, **2**: 92–96. doi:10.1016/j.apcbee.2012.06.017.

Qu, D.-F., Yu, H.-J., Liu, Z., Zhang, D.-F., Zhou, Q.-J., Zhang, H.-L., and Du, A.-F. 2011. Ginsenoside Rg1 enhances immune response induced by recombinant *Toxoplasma gondii* SAG1 antigen. Vet. Parasitol. **179**(1–3): 28–34. doi:10.1016/j.vetpar.2011.02.008. PMID:21439733.

Qu, D., Han, J., and Du, A. 2013. Enhancement of protective immune response to recombinant *Toxoplasma gondii* ROP18 antigen by ginsenoside Re. Exp. Parasitol. **135**(2): 234–239. doi:10.1016/j.exppara.2013.07.013. PMID:23896123.

Rahimi, M.T., Mahdavi, S.A., Javadian, B., Rezaei, R., Moosazadeh, M., et al. 2015. High seroprevalence of *Toxoplasma gondii* antibody in HIV/AIDS individuals from North of Iran. Iran. J. Parasitol. **10**(4): 584–589. PMID:26811725.

Rashidian, A., Mehrzadi, S., Ghannadi, A.R., Mahzooni, P., Sadr, S., and Minaiyan, M. 2014. Protective effect of ginger volatile oil against acetic acid-induced colitis in rats: a light microscopic evaluation. J. Integr. Med. **12**(2): 115–120. doi:10.1016/S2095-4964(14)60011-X. PMID:24666677.

Rayan, H.Z., Wagih, H.M., and Atwa, M.M. 2011. Efficacy of black seed oil from Nigella sativa against murine infection with cysts of Me49 strain of *Toxoplasma gondii*. PUJ. **4**(2): 165–176.

Reynolds, M.G., Oh, J., and Roos, D.S. 2001. In vitro generation of novel pyrimethamine resistance mutations in the *Toxoplasma gondii* dihydrofolate reductase. Antimicrob. Agents Chemother. **45**(4): 1271–1277. doi:10.1128/AAC.45.4.1271-1277.2001. PMID:11257045.

Ridley, R., and Hudson, N. 1998. Oxidative stress and antimalarial drugs. Curr. Biol. **8**: 346–349.

Saadatnia, G., Haj Ghani, H., Khoo, B.Y., Maimunah, A., and Rahmah, N. 2010. optimization of *Toxoplasma gondii* cultivation in VERO cell line. Trop. Biomed. **27**(1): 125–130. PMID:20562822.

Sarciron, M.E., Saccharin, C., Petavy, A.F., and Peyron, F. 2000. Effects of artesunate, dihydroartemisinin, and an artesunate-dihydroartemisinin combination against *Toxoplasma gondii*. Am. J. Trop. Med. Hyg. **62**(1): 73–76. PMID: 10761727.

Schultz, T.L., Hencken, C.P., Woodard, L.E., Posner, G.H., Yolken, R.H., Jones-Brando, L., and Carruthers, V.B. 2014. A thiazole derivative of artemisinin moderately reduces *Toxoplasma gondii* cyst burden in infected mice. J. Parasitol. **100**(4): 516–521. doi:10.1645/13-451.1. PMID:24524228.

Sepulveda-Arias, J.C., Veloza, L.A., and Mantilla-Muriel, L.E. 2014. Anti-*Toxoplasma* activity of natural products: a review. Recent. Pat. Antiinfect. Drug. Discov. **9**(3): 186–194. doi:10.2174/1574891X10666150410120321. PMID: 25858302.

Sharma, V., and Agrawal, R.C. 2013. *Glycyrrhiza glabra* — a plant for the future. Mint. J. Pharm. Med. Sci. **2**(3): 15–20.

Sidor, A., and Gramza-Michałowska, A. 2015. Advanced research on the antioxidant and health benefit of elderberry (*Sambucus nigra*) in food – a review. J. Funct. Foods. **18**: 941–958. doi:10.1016/j.jff.2014.07.012.

Soheilian, M., Sadoughi, M.-M., Ghajarnia, M., Dehghan, M.H., Yazdani, S., Behboudi, H., et al. 2005. Prospective randomized trial of trimethoprim/sulfamethoxazole versus pyrimethamine and sulfadiazine in the treatment of ocular toxoplasmosis. Ophthalmology, **112**(11): 1876–1882. doi:10.1016/j.ophtha.2005.05.025. PMID:16171866.

Sönmez, N., Büyükbaba Boral, Ö., Kaşali, K., and Tekeli, F. 2014. Effects of atovaquone and astragalus combination on the treatment and IL-2, IL-12, IFN-γ levels on mouse models of acute toxoplasmosis. Mikrobiyol. Bul. **48**(4): 639–651. doi:10.5578/mb.8025. PMID:25492659.

Tempone, A.G., Borborema, S.E., de Andrade, H.F., Jr., de Amorim Gualda, N.C., Yogi, A., Carvalho, C.S., et al. 2005. Antiprotozoal activity of Brazilian plant extracts from isoquinoline alkaloid-producing families. Phytomedicine, **12**(5): 382–390. doi:10.1016/j.phymed.2003.10.007. PMID:15957374.

Wang, H.-L., Xiang, Y.-T., Li, Q.-Y., Wang, X.-P., Liu, Z.-C., Hao, S.-S., et al. 2014. The effect of artemether on psychotic symptoms and cognitive impairment in first-episode, antipsychotic drug-naive persons with schizophrenia seropositive to *Toxoplasma gondii*. J. psychiatr. Res. **53**: 119–124. doi:10.1016/j.jpsychires.2014.02.016. PMID:24656425.

Wang, M.-J., Ju, Y.-L., and Li, L. 2007. The comparison of the effects about four kinds of the Chinese traditional medicine inhibit *Toxoplasma* in vitro. J. Anim. Husb. Vet. Med. **10**: 6.

Wang, Y.-C., Ju, Y.-L., and Yao, L. 2012. Comparison of the inhibition of three

extracts of Scutellaria baicalensis to *Toxoplasma* in vitro. J. Agric. Sci. Yanbian. Uni. **1**: 19.

Wen, Q.-J., Li, S.-H., Cui, L.M., Liu, B., and Zhang, Y. 2008. Effects of *Juglans mandshurica* maxim extracts on *Toxoplasma gondii* swallowed function of peritoneal macrophage in mice. J. Pathogen. Biol. **8**: 591–592, 597.

Yang, X., Huang, S., Chen, J., Song, N., Wang, L., Zhang, Z., et al. 2010. Evaluation of the adjuvant properties of *Astragalus membranaceus* and *Scutellaria baicalensis* GEORGI in the immune protection induced by UV-attenuated *Toxoplasma gondii* in mouse models. Vaccine, **28**(3): 737–743. doi:10.1016/j.vaccine.2009.10.065. PMID:19887128.

Yang, X., Huang, B., Chen, J., Huang, S., Zheng, H., Lun, Z.-R., et al. 2012. In vitro effects of aqueous extracts of *Astragalus membranaceus* and *Scutellaria baicalensis* GEORGI on *Toxoplasma gondii*. Parasitol. Res. **110**(6): 2221–2227. doi:10.1007/s00436-011-2752-2. PMID:22179265.

Yeh, T.-S., Chuang, H.-L., Huang, W.-C., Chen, Y.-M., Huang, C.-C., and Hsu, M.-C. 2014. *Astragalus membranaceus* improves exercise performance and ameliorates exercise-induced fatigue in trained mice. Molecules, **19**(3): 2793–2807. doi:10.3390/molecules19032793. PMID:24595275.

Youn, H.J., Lakritz, J., Kim, D.Y., Rottinghaus, G.E., and Marsh, A.E. 2003. Anti-protozoal efficacy of medicinal herb extracts against *Toxoplasma gondii* and *Neospora caninum*. Vet. Parasitol. **116**(1): 7–14. doi:10.1016/S0304-4017(03)00154-7. PMID:14519322.

Youn, H.J., Lakritz, J., Rottinghaus, G.E., Seo, H.S., Kim, D.Y., Cho, M.H., and Marsh, A.E. 2004. Anti-protozoal efficacy of high performance liquid chromatography fractions of *Torilis japonica* and *Sophora flavescens* extracts on *Neospora caninum* and *Toxoplasma gondii*. Vet. Parasitol. **125**(3–4): 409–414. doi:10.1016/j.vetpar.2004.08.002. PMID:15482896.

Zhang, W., Fang, F.R., Liu, Y.J., Yang, L.D., Luo, R.Y., Gong, F., et al. 2006. In vitro effect of combined traditional Chinese medicine (Changqing capsule) on the tachyzoites of *Toxoplasma gondii*. Chin. J. Parasitol. Parasit. Disease. **24**(1): 56–58. [In Chinese.] PMID:16866146.