



Anti-*Toxoplasma* Activities of Some Egyptian Plant Extracts: An In Vitro Study

Sara T. Elazab¹ · Fadwa M. Arafa²

Received: 16 August 2022 / Accepted: 17 October 2022 / Published online: 30 October 2022
© The Author(s) 2022

Abstract

Purpose Toxoplasmosis is a globally widespread parasitic disease which causes major health problems in human and animals. This research was conducted to assess the effect of some Egyptian herbal extracts against *Toxoplasma gondii* (*T. gondii*) tachyzoites in vitro.

Methods The methanol extracts of *Withania somnifera*, *Cyper rotundus*, *Acacia nilotica*, *Chrysanthemum cinerariae folium*, *Anethum graveolens*, *Raphanus sativus*, *Ceratonia siliqua*, *Elettaria cardamomum* and *Cuminum cyminum* were tested against *T. gondii* tachyzoites.

Results Among the tested plants, the extracts from *Raphanus sativus*, *Cuminum cyminum*, and *Ceratonia siliqua* exhibited high anti-*Toxoplasma* activities at 50 µg/ml, relative to sulfadiazine. They showed low IC₅₀ values on *T. gondii* (7.92, 9.47 and 13.52 µg/ml, respectively) and high selectivity index values (100.79, 59.19, and 29.05, respectively). Scanning electron microscopy (SEM) findings indicated evident morphological changes in tachyzoites treated with these three herbal extracts.

Conclusion *Raphanus sativus*, *Ceratonia siliqua*, and *Cuminum cyminum* methanol extracts could be promising sources of new medicament for toxoplasmosis.

Keywords *Toxoplasma gondii* · Selectivity index · Cytotoxicity · Plant extracts · *Raphanus sativus*

Toxoplasma gondii (*T. gondii*) is an intracellular apicomplexan protozoan parasite which causes a widely distributed zoonotic disease, toxoplasmosis, in warm-blooded animals [1]. Almost 30% of the world's population is affected by toxoplasmosis [2]. The severity of toxoplasmosis differs according to the host immune status [3]. During the gestation period, *T. gondii* can cross the placental barrier and infect the fetus causing fetal malformations or abortion [4]. Nowadays, the combination of sulfonamide drugs and pyrimethamine is regarded as the first-line therapy for toxoplasmosis [5]. These drugs elicit their effects by suppressing the synthesis of DNA and or RNA of *T. gondii* via inhibiting *Toxoplasma* folic acid metabolism [6]. Nevertheless, these medicines have limitations due to numerous undesirable side effects such as hypersensitivity reactions, bone marrow

suppression, and hematological disorders, as well as their restricted efficacy in eradicating tissue cysts [7]. Thus, the development of new, efficient, and more tolerable therapies is imperative.

Medicinal herbal extracts are promising sources of novel remedies, owing to their high content of diverse bioactive compounds [8]. Previously, numerous drugs for eradicating parasitic infestations have been derived from plants, for instance, artemisinin and quinine as a therapy for malaria [9]. The goal of the present study was to investigate the potential anti-*Toxoplasma* effects of methanol extracts of some Egyptian plants. The plants utilized in this research, such as *Withania somnifera* [10, 11], *Cyper rotundus* [12], *Acacia nilotica* [13], *Chrysanthemum cinerariae folium* [14], *Anethum graveolens* [15], *Raphanus sativus* [16], *Ceratonia siliqua* [17], *Elettaria cardamomum* [18], and *Cuminum cyminum* [19], have been previously recorded to possess antiprotozoal and anthelmintic. However, to the author's knowledge, no reports are available regarding their possible effects against *T. gondii*.

Samples of *Withania somnifera* (Ashwagandha), *Cyper rotundus* (purple nutsedge), *Acacia nilotica* (gum

✉ Sara T. Elazab
sara.taha@ymail.com

¹ Department of Pharmacology, Faculty of Veterinary Medicine, Mansoura University, Mansoura 35516, Egypt

² Department of Medical Parasitology, Faculty of Medicine, Alexandria University, Alexandria, Egypt

arabic tree), *Chrysanthemum cinerariae folium* (pyrethrum), *Anethum graveolens* (dill), *Raphanus sativus* (radish), *Ceratonia siliqua* (carob), *Elettaria cardamomum* (true cardmom), and *Cuminum cyminum* (cumin) were brought from herbal drug store in Mansoura, Egypt. The conventional uses of the selected plants are listed in Table 1. Dried plants were crushed into tiny pieces and were extracted employing 70% methanol for 48 h. The resulting crude extracts were prepared at 100 mg/ml in dimethylsulfoxide (DMSO) and stored at -30°C until use.

The potential cytotoxic effect of these herbal extracts was assessed on Vero cells (African green monkey cells supplied from the National Cancer Institute, Cairo, Egypt) based on the technique of Montazeri et al. [29]. In brief, Vero cells were seeded in 96-well plate with 180 μl Roswell Park Memorial Institute medium (RPMI 1640 medium, Sigma, St. Louis, MO, USA) in every well (density, 2×10^4 cells/ well in RPMI 1640 medium containing 10% FBS, and 100 U/ml penicillin, 100 $\mu\text{g}/\text{ml}$ streptomycin) and incubated at 37°C under 5% CO_2 for 24 h. After that, the cells were subjected to the tested extracts at concentrations of 15.625, 31.25, 62.5, 125, 250, 500 and 1000 $\mu\text{g}/\text{ml}$. Following 24 h incubation, MTT solution (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium

bromide) was added to the cultures to measure cell viability. The optical density was determined at 490 nm with a microplate reader (Benchmark, Bio-Rad, USA). The growth inhibition (%) was calculated. The half maximal inhibitory concentration (IC_{50}) value of every plant extract on Vero cells was evaluated using GraphPad prism 5 software (GraphPad Software Inc., La Jolla, CA, USA).

To test the effect of herbal extracts on intracellular *T. gondii* invitro, Vero cells were plated in 96-well plate (density 2×10^4 cells/well/180 μl RPMI 1640 medium supplemented with 10% FBS) and kept to grow at 37°C in 5% CO_2 . After 24 h of incubation, *T. gondii* RH tachyzoites (obtained from Medical Parasitology Department, Faculty of Medicine, Alexandria University, Egypt) were added to the wells (parasite: cell ratio = 5:1). 24 h later, the culture medium was changed and the infected cells were exposed to the plant extracts dissolved in RPMI 1640 medium at 10 and 50 $\mu\text{g}/\text{ml}$. Wells with RPMI 1640 medium or sulfadiazine only were considered as negative and positive controls, respectively. Thereafter, MTT solution was added and incubated for 4 h. The optical absorbance was detected at 490 nm. The percentage of *T. gondii* tachyzoites growth inhibition (GI) was estimated based on the following equation:

Table 1 Activities of methanolic extracts from some plants on *Toxoplasma gondii* at concentrations 50 $\mu\text{g}/\text{ml}$ and 10 $\mu\text{g}/\text{ml}$

Plants	Plant part	Traditional uses	References	% Inhibition of <i>T. gondii</i> RH at concentrations ($\mu\text{g}/\text{ml}$)	
				50	10
<i>Withania somnifera</i> (Ashwagandha)	Leaf	Used as aphrodisiac, for treatment of impotency, nervous exhaustion, insomnia, chronic fatigue, skin problems and coughing	[20]	40.99 ± 6.54	24.52 ± 7.48
<i>Cyper rotundus</i> (Purple nutsedge)	Leaf	For treating gastrointestinal disorders, menstrual disturbance, bronchitis, leprosy, epilepsy and urolithiasis	[21]	17.24 ± 4.59	3.83 ± 1.76
<i>Acacia nilotica</i> (Gum arabic tree)	Leaf	As a therapy for diabetes mellitus, bronchitis, tuberculosis and pharyngitis	[22]	43.29 ± 10.68	27.20 ± 5.78
<i>Chrysanthemum cinerariae folium</i> (Pyrethrum)	Flower	To cure chest pain, hypertension, allergy, sore throat and headache	[23]	9.96 ± 2.89	4.98 ± 4.03
<i>Anethum graveolens</i> (Dill)	Seed	As a diuretic, stomachic and to cure gastrointestinal upset	[24]	31.03 ± 12.00	11.49 ± 5.26
<i>Raphanus sativus</i> (Radish)	Seed	For treating dysentery, constipation, hypertension and chronic tracheitis	[25]	73.56 ± 4.14	46.74 ± 8.62
<i>Ceratonia siliqua</i> (Carob)	Fruit	Used as antidiabetic, antidiarrheal, diuretic and antiatherosclerotic	[26]	69.35 ± 9.29	35.63 ± 4.59
<i>Elettaria cardamomum</i> (True cardmom)	Seed	As a treatment for cardiac disorders, constipation, urinary problems and tuberculosis	[27]	59.38 ± 12.66	28.73 ± 8.67
<i>Cuminum cyminum</i> (Cumin)	Seed	Used as anthelmintic agent, stomachic, carminative, antipyretic, diuretic, tonic and antidiabetic	[28]	77.78 ± 6.73	40.23 ± 13.55

Reference control (1 mg/ml sulfadiazine) exhibited anti-*Toxoplasma* activities with % of RH growth suppression of 61.30 ± 14.37 . Data are presented as mean $\pm$ SD ($n = 3$)

Table 2 Half maximal inhibitory concentration (IC₅₀) of methanol herbal extracts displaying anti-*Toxoplasma* activity and their associated selectivity indexes

Herbal extract	IC ₅₀ (μg/ml)		Selectivity index (SI)
	Vero cells	<i>T. gondii</i> RH	
<i>Raphanus sativus</i> (radish)	798.3 ± 48.10	7.92 ± 1.14	100.79
<i>Ceratonía siliqua</i> (carob)	392.8 ± 66.44	13.52 ± 4.85	29.05
<i>Cuminum cyminum</i> (cumin)	560.6 ± 51.91	9.47 ± 3.98	59.19
Sulfadiazine	> 1,000	94.41 ± 4.44	ND

IC₅₀ values were estimated depending on three independent experiments. Data are presented as mean ± SD
 ND not detected

$$GI = \frac{At - Ac}{Ac} \times 100,$$

in which At and Ac reflect the absorbance of the treated cells and control, respectively.

The tested extracts exhibiting percentage of inhibition of tachyzoites growth at 50 μg/ml more than that obtained by sulfadiazine were further analyzed at concentrations (0.78–100 μg /ml) to evaluate their IC₅₀ values against *T. gondii*. GraphPad prism 5 software was employed to estimate the IC₅₀ values of the extracts on *T. gondii*. Selectivity index (SI) of the samples was calculated using the following equation as described by Montazeri et al. [29]:

$$SI = \frac{\text{IC}_{50} \text{ of the herbal extracts on Vero cells}}{\text{IC}_{50} \text{ of the herbal extracts on } T. gondii}$$

To assess the anti-*Toxoplasma* activities of the methanol extracts of *Raphanus sativus*, *Ceratonía siliqua*, and *Cuminum cyminum* by scanning electron microscopy (SEM), following incubation of 10⁵ *Toxoplasma* tachyzoites with each plant extract (at 10 μg/ml) or sulfadiazine (at 100 μg/ml) for 2 h, tachyzoites were loaded on a slide, fixed with 2% paraformaldehyde and 2.5% glutaraldehyde in sodium cacodylate buffer (0.1 M, pH 7.4) and rinsed with cacodylate buffer. Thereafter, the slide was exposed to 1% osmium tetroxide buffer for 2–4 h. Then, they were dehydrated in serial ascending concentrations of ethanol. Finally, the tachyzoites were embedded in Epon Resin, and 20 nm gold particles were utilized for coating prior to investigation using a JOEL-JSM-IT 200, Japan, scanning microscope [30].

The effects of the herbal extracts on *T. gondii* at concentrations 50 and 10 μg/ml are elucidated in Table 1. They showed varying levels of anti-*Toxoplasma* effects at 50 μg/ml, starting from 9.96 up to 77.78% RH growth inhibition. From the nine examined plants, the extracts derived from *Raphanus sativus*, *Cuminum cyminum*, and *Ceratonía siliqua* displayed higher % of RH growth inhibition relative to sulfadiazine at 1 mg/ml (61.30%). Moreover, as presented in Table 2, these three herbal extracts revealed lower IC₅₀ values (7.92, 9.47, and 13.52 μg/ml, respectively) than sulfadiazine (94.41 μg/ml). In addition, based on the “hit” criteria (SI ≥ 10) previously reported by Banzragchgarav

et al. [31], the three plants exhibited promising SI values of 100.79, 59.19 and 29.05, respectively. The SI value was regarded as a reliable marker for the selective activity of the remedy [32].

Furthermore, morphological analysis of untreated *T. gondii* tachyzoites using SEM exhibited crescent shape with smooth surface (Fig. 1A). In contrast, tachyzoites treated with *Raphanus sativus* methanol extract showed numerous protrusions (arrow), ridges and depressions (Fig. 1C, D). Moreover, those exposed to *Ceratonía siliqua* extract revealed loss of crescentic morphology and ballooning with multiple crystalline deposits on the surface (Fig. 1E, F). Meanwhile, tachyzoites treated with *Cuminum cyminum* extract displayed irregular rough surface with multiple furrows and deep depressions (Fig. 1G, H).

Raphanus sativus, commonly known as radish, is one of the cruciferous vegetables widely consumed all over the world [33]. Earlier literature has recorded several pharmacological effects of *Raphanus sativus*, including antioxidant [34], anti-inflammatory [35], antimicrobial [36], antileishmanial [16], antitumor [37], antiulcer [38] and antiurolithiatic [39] activities. These biological values of radish are attributed to its phytoconstituents of flavonoids, alkaloids, and phenols [40]. The anti-*Toxoplasma* activity of *Raphanus sativus* extract observed in this study may be due to one or more of its bioactive constituents. Phenolic compounds have been demonstrated to exert potent inhibitory effect on *Leishmania donovani* promastigotes and amastigotes by chelating iron, causing morphological alterations and cell cycle disruption [41]. Moreover, prior studies have proved the antagonistic effect of flavonoids against several protozoa such as *Plasmodium falciparum*, *Entamoeba histolytica*, *Cryptosporidium parvum*, and *Leishmania donovani* [42–45]. Additionally, it has been announced that alkaloids have a noticeable activity against *Trypanosoma brucei rhodesiense*, *Leishmania donovani*, and *Plasmodium falciparum* [46–48]. The anti-*toxoplasma* activity of *Raphanus sativus* in this study was ascertained by the ultrastructural alterations noticed by SEM represented as membrane irregularities (protrusions, ridges and depression). These morphological changes may be attributed to its flavonoids content. It has been declared that flavonoids disrupt cytoplasmic and

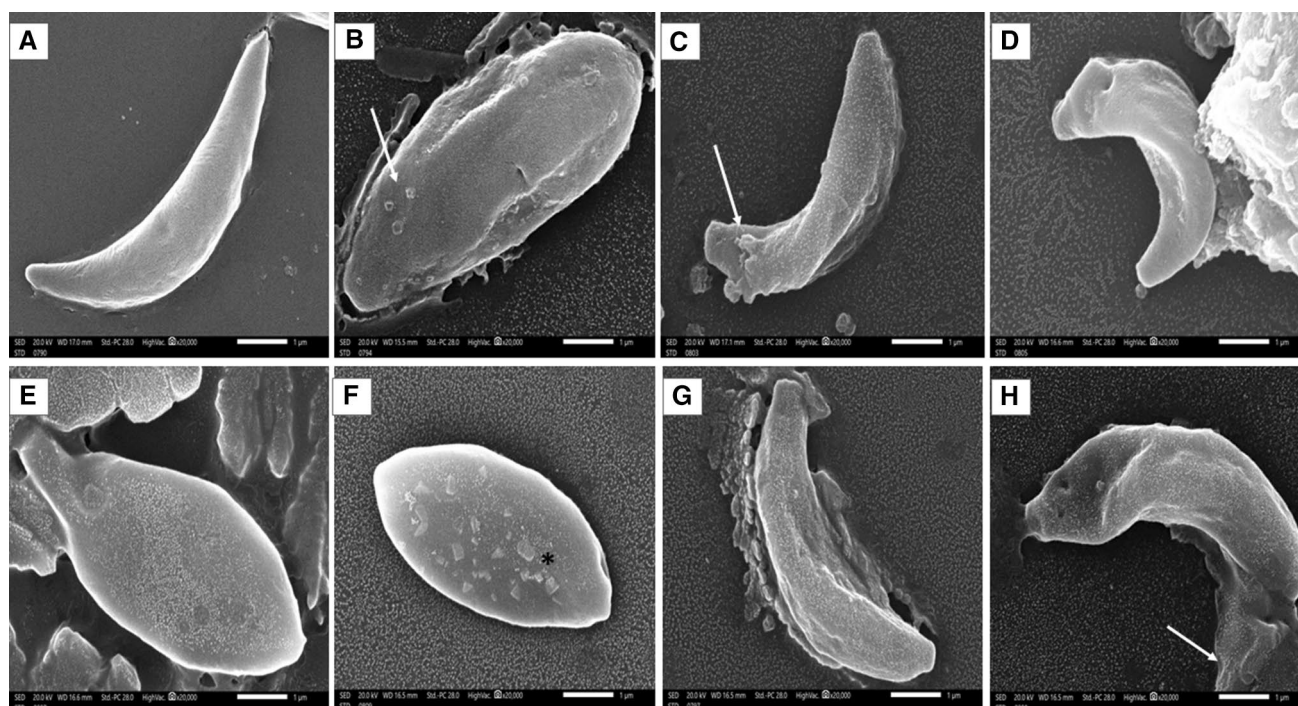


Fig. 1 Scanning electron microscopy (SEM) images of *Toxoplasma gondii* tachyzoites showing: **A** non-treated crescent-shaped tachyzoite with smooth regular surface ($\times 20,000$). **B** Tachyzoite treated with sulfadiazine (at $100 \mu\text{g/ml}$) showing loss of characteristic crescentic morphology with irregular rough surface, multiple deep ridges and surface blebs (arrow) ($\times 20,000$). **C**, **D** Tachyzoites treated with *Raphanus sativus* (radish) methanol extract ($10 \mu\text{g/ml}$) showing numerous protrusions (arrow), ridges and depressions ($\times 20,000$). **E**,

F Tachyzoites treated with *Ceratonia siliqua* (carob) methanol extract ($10 \mu\text{g/ml}$) exhibited loss of crescentic morphology and ballooning. Multiple crystalline deposits were noticed on the surface (asterisk) ($\times 20,000$). **G**, **H** Tachyzoites treated with *Cuminum cyminum* (cumin) methanol extract ($10 \mu\text{g/ml}$) revealed irregular rough surface with multiple furrows and deep depressions. Loss of tachyzoite surface integrity with leakage of cytoplasmic contents was also noticed (arrow) ($\times 20,000$)

plasma membrane integrity [49, 50]. They also inhibit tyrosine, protein kinases, topoisomerase activity, mitochondrion function, and fatty acid type II synthesis [51, 52]. Thereby, they can compromise the mitochondria of tachyzoites and suppress the signaling of cell survival or death factors [53].

Cuminum cyminum (Cumin) is an annual aromatic plant belonging to Apiaceae family and possesses diverse nutraceutical and pharmaceutical features [54]. It has been declared to exhibit antioxidant, antibacterial, antifungal, antidiabetic, antistress, hypolipidemic, immunostimulant and memory-improving effects [55, 56]. The main ingredients identified in cumin seed extract were flavonoids, isoflavonoids, flavonoid glycosides, alkaloids, lignins, monoterpenoid glycosides and phenolic compounds [57, 58]. The distinct constituents of cumin may contribute to its anti-*Toxoplasma* action reported in this study. Nevertheless, further exploration into the mode of action of *Cuminum cyminum* methanol extract against *T. gondii* is needed.

Ceratonia siliqua (carob), a member of Leguminosae family, is broadly cultivated in Mediterranean countries, and in some parts of the USA and Australia [59]. Previous reports have revealed antimicrobial, antioxidant, and

anticancer effects of carob [60]. Furthermore, *Ceratonia siliqua* has been shown to have potent anthelmintic action against gastrointestinal nematodes (*Haemonchus contortus* and *Trichostrongylus colubriformis*) [61]. The major phenolic components detected in *Ceratonia siliqua* are kaempferol, tannic acid, catechin hydrate and polydatin [62]. The findings of the current research suggested *Ceratonia siliqua* as a promising candidate in combating toxoplasmosis.

Conclusively, the present study elucidated that the methanol extract of *Raphanus sativus*, *Cuminum cyminum* and *Ceratonia siliqua* have significant inhibitory effect on the growth of *T. gondii* RH tachyzoites in vitro. However, future studies are warranted to investigate their efficacy in vivo and to explore their underlying mechanism of action.

Acknowledgements The authors thank the staff members of the Tissue Culture Unit, Medical Technology Center, Medical Research Institute, Alexandria University, for their help.

Author Contribution SE and FA designed and conducted the experiment. SE prepared the methanol extracts of the used medicinal plants.

SE and FA analyzed the data of this work. SE wrote the manuscript. All authors read and approved the final manuscript.

Funding Open access funding provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB).

Declarations

Conflict of Interest The authors declare no conflict of interests.

Ethical Statement All procedures conducted in the current work were reviewed and accepted by the Ethics Committee, Faculty of Medicine, Alexandria University, Alexandria, Egypt (IRB No. 00012098; approval No. 0305458).

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Montoya JG, Liesenfeld O (2004) The lancet. Toxoplasmosis. [https://doi.org/10.1016/S0140-6736\(04\)16412-X](https://doi.org/10.1016/S0140-6736(04)16412-X)
- Montazeri M, Mehrzadi S, Sharif M, Sarvi S, Tanzifi A, Aghayan SA, Daryani A (2018) Drug resistance in *Toxoplasma gondii*. Front Microbiol 9:2587. <https://doi.org/10.3389/fmicb.2018.02587>
- Yan C, Liang LJ, Zheng K, Zhu XQ (2016) Impact of environmental factors on the emergence, transmission and distribution of *Toxoplasma gondii*. Parasit Vectors 9:1–7. <https://doi.org/10.1186/s13071-016-1432-6>
- Dégbé M, Debierre-Grockieo F, Tété-Bénissan A, Débare H, Aklikokou K, Dimier-Poisson I, Gbeassor M (2018) Extracts of tectona grandis and Vernonia amygdalina have anti-*Toxoplasma* and pro-inflammatory properties in vitro. Parasite. <https://doi.org/10.1051/parasite/2018014>
- Schmidt DR, Høgh B, Andersen O, Hansen SH, Dalhoff K, Petersen E (2006) Treatment of infants with congenital toxoplasmosis: tolerability and plasma concentrations of sulfadiazine and pyrimethamine. Eur J Pediatr 165:19–25. <https://doi.org/10.1007/s00431-005-1665-4>
- Choi WH, Lee IA (2018) Evaluation of anti-*Toxoplasma gondii* effect of ursolic acid as a novel toxoplasmosis inhibitor. Pharmacuticals 11:1–11. <https://doi.org/10.3390/ph11020043>
- Furtado JM, Smith JR, Belfort R, Gattey D, Winthrop K (2011) Toxoplasmosis: una amenaza global. J Glob Infect Dis 3:281–284. <https://doi.org/10.4103/0974-777X.83536>
- Dias DA, Urban S, Roessner U (2012) A Historical overview of natural products in drug discovery. Metabolites. <https://doi.org/10.3390/metabo2020303>
- Batista R, Júnior DJS, A, De Oliveira AB, (2009) Plant-derived antimalarial agents: new leads and efficient phytochemicals Part II. Non-alkaloid nat prod Mol 14:3037–3072. <https://doi.org/10.3390/molecules14083037>
- Dikasso D, Makonnen E, Debella A, Abebe D, Urga K, Makonnen W, Guta M (2006) Anti-malarial activity of *withania somnifera* L. Dunal extracts in mice. Ethiop Med J 44:279–285
- Chandrasekaran S, Veronica J, Sundar S, Maurya R (2017) Alcoholic fractions F5 and F6 from *Withania somnifera* leaves show a potent antileishmanial and immunomodulatory activities to control experimental Visceral Leishmaniasis. Front Med 4:55. <https://doi.org/10.3389/fmed.2017.00055>
- Kasala S, Ramanjaneyulu K, Himabindhu J, Alluri R, Babu RR (2016) Preliminary phytochemical screening and in vitro anthelmintic activity of *Cyperus rotundus* (L.). J Pharmacogn Phytochem 5:407–409
- Zabré G, Kaboré A, Bayala B, Katiki LM, Costa-Júnior LM, Tamboura HH, Louvandini H (2017) Comparison of the in vitro anthelmintic effects of *Acacia nilotica* and *acacia raddiana*. Parasite. <https://doi.org/10.1051/parasite/2017044>
- Wachira GK, Muregi FW, Kimani FT, Tolo FM, Keter L, Irungu B (2018) Evaluation of antiplasmodial activity and safety of flower extracts of *chrysanthemum cinerariaefolium*, singly and in combination with chloroquine, lumefantrine and piperazine. Eur J Med Plants 22:1–14. <https://doi.org/10.9734/EJMP/2018/39246>
- Castro LM, Pinto NB, Moura MQ, Villela MM, Capella GA, Freitas RA, Berne MEA (2020) Anthelmintic action of the anethum graveolens essential oil on *Haemonchus contortus* eggs and larvae. Braz J Biol 81:183–188. <https://doi.org/10.1590/1519-6984.225856>
- Younus I, Siddiq A (2016) In-Vitro antileishmanial activity of *Raphanus sativus* L. var. caudatus. J Basic Appl Sci 12:242–244. <https://doi.org/10.6000/1927-5129.2016.12.37>
- Custodio L, Marques L, Mayor A, Alonso P, Albericio F, Romano A (2008) Evaluation of the antimalarial activity of extracts of carob tree (*Ceratonia siliqua* L.). Planta Med. <https://doi.org/10.1055/s-0028-1084047>
- Almohammed HI, Alkhaibari AM, Alanazi AD (2022) Antiparasitic effects of *Elettaria cardamomum* L. essential oil and its main compounds, 1–8 Cineole alone and in combination with albendazole against *Echinococcus granulosus* protoscoleces. Saudi J Biol Sci 29:2811–2818. <https://doi.org/10.1016/j.sjbs.2022.01.005>
- Bagirova M, Dinparvar S, Allahverdiyev AM, Unal K, Abamor ES, Novruzova M (2020) Investigation of antileishmanial activities of cuminum cyminum based green silver nanoparticles on *L. tropica* promastigotes and amastigotes in vitro. Acta Trop. <https://doi.org/10.1016/j.actatropica.2020.105498>
- Verma SK, Kumar A (2011) Therapeutic uses of *Withania somnifera* (Ashwagandha) with a note on withanolides and its pharmacological actions. Asian J Pharm Clin Res 4:1–4
- Peerzada AM, Ali HH, Naeem M, Latif M, Bukhari AH, Tanveer A (2015) *Cyperus rotundus* L.: traditional uses, phytochemistry, and pharmacological activities. J Ethnopharmacol 174:540–560. <https://doi.org/10.1016/j.jep.2015.08.012>
- Rather LJ, Mohammad F (2015) *Acacia nilotica* (L.): a review of its traditional uses, phytochemistry, and pharmacology. Sustain Chem Pharm 2:12–30. <https://doi.org/10.1016/j.scp.2015.08.002>
- Shahrajabian MH, Sun W, Zandi P, Cheng Q (2019) A review of *Chrysanthemum*, the eastern queen in traditional Chinese medicine with healing power in modern pharmaceutical sciences. Appl Ecol Environ Res 17:13355–13369. https://doi.org/10.15666/aecer/1706_1335513369
- Jana S, Shekhawat GS (2010) *Anethum graveolens*: an Indian traditional medicinal herb and spice. Pharmacogn Rev 4:179–184. <https://doi.org/10.4103/0973-7847.70915>
- Sham TT, Yuen ACY, Ng YF, Chan CO, Mok DKW, Chan SW (2013) A review of the phytochemistry and pharmacological activities of raphani semen. Evid based Complement Altern Med 2013:1–16. <https://doi.org/10.1155/2013/636194>

26. Rtibi K, Selmi S, Grami D, Amri M, Eto B, El-Benna J, Marzouki L (2017) Chemical constituents and pharmacological actions of carob pods and leaves (*Ceratonia siliqua* L.) on the gastrointestinal tract: a review. *Biomed Pharmacother* 93:522–528. <https://doi.org/10.1016/j.biopha.2017.06.088>
27. Ashokkumar K, Murugan M, Dhanya MK, Warkentin TD (2020) Botany, traditional uses phytochemistry and biological activities of cardamom [*Elettaria cardamomum* (L.) Maton]—a critical review. *J ethnopharmacol*. <https://doi.org/10.1016/j.jep.2019.112244>
28. Mnif S, Aifa S (2015) Cumin (*Cuminum cyminum* L.) from traditional uses to potential biomedical applications. *Chem biodivers* 12:733–742. <https://doi.org/10.1002/cbdv.201400305>
29. Montazeri M, Mirzaee F, Daryani A, Naeimayi R, Karimabad SM, Arjmandi HK, Shahani S (2020) Anti-*Toxoplasma* activities of the hydroalcoholic extract of some *brassicaceae* species. *Ad Biomed Res* 9:1–5. https://doi.org/10.4103/abr.abr_206_19
30. Khosravi M, Mohammad Rahimi H, Doroud D, Mirsamadi ES, Mirjalali H, Zali MR (2020) In vitro evaluation of mannosylated paromomycin-loaded solid lipid nanoparticles on acute toxoplasmosis. *Front Cell Infect Microbiol* 10:33. <https://doi.org/10.3389/fcimb.2020.00033>
31. Banzragchgarav O, Batkhui J, Myagmarsuren P, Battsetseg B, Battur B, Nishikawa Y (2021) In vitro potentially active anti-plasmodium and anti-toxoplasma mongolian plant extracts. *Acta Parasitol* 66:1442–1447. <https://doi.org/10.1007/s11686-021-00401-8>
32. Elazab ST, Soliman AF, Nishikawa Y (2020) Effect of some plant extracts from Egyptian herbal plants against toxoplasma gondii tachyzoites in vitro. *J Vet Med Sci* 83:100–107. <https://doi.org/10.1292/jvms.20-0458>
33. Luo X, Zhang H, Duan Y, Chen G (2018) Protective effects of radish (*Raphanus sativus* L.) leaves extract against hydrogen peroxide-induced oxidative damage in human fetal lung fibroblast (MRC-5) cells. *Biomed Pharmacother* 103:406–414. <https://doi.org/10.1016/j.biopha.2018.04.049>
34. Beevi SS, Mangamoori LN, Gowda BB (2012) Polyphenolics profile and antioxidant properties of raphanus sativus L. *Nat Prod Res*. <https://doi.org/10.1080/14786419.2010.521884>
35. Kim KH, Moon E, Kim SY, Choi SU, Lee JH, Lee KR (2014) 4-Methylthio-butanyl derivatives from the seeds of raphanus sativus and their biological evaluation on anti-inflammatory and antitumor activities. *J Ethnopharmacol* 151:503–508. <https://doi.org/10.1016/j.jep.2013.11.003>
36. Aruna G, Yerragunt VG, Raju AB (2012) Photochemistry and pharmacology of raphanus sativus. *Int J Drug Formulation Res* 3:43–52
37. Hecht SS, Kenney PM, Wang M, Trushin N, Upadhyaya P (2000) Effects of phenethyl isothiocyanate and benzyl isothiocyanate, individually and in combination, on lung tumorigenesis induced in A/J mice by benzo [a] pyrene and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. *Cancer lett* 150:49–56. [https://doi.org/10.1016/S0304-3835\(99\)00373-0](https://doi.org/10.1016/S0304-3835(99)00373-0)
38. Alqasoumi S, Yahya MA, Al-Howiriny T, Rafatullah S (2008) Gastroprotective effect of radish raphanus sativus L. on experimental gastric ulcer models in rats. *Farmacia LVI* 52(2):204–214
39. Vargas R, Perez RM, Perez S, Zavala MA, Perez C (1999) Antiuro-lithiatic activity of *Raphanus sativus* aqueous extract on rats. *J Ethnopharmacol* 68:335–338. [https://doi.org/10.1016/S0378-8741\(99\)00105-1](https://doi.org/10.1016/S0378-8741(99)00105-1)
40. Shin T, Ahn M, Kim GO, Park SU (2015) Biological activity of various radish species. *Orient Pharm Exp Med* 15:105–111. <https://doi.org/10.1007/s13596-015-0183-9>
41. Antwi CA, Amisigo CM, Adjimani JP, Gwira TM (2019) In vitro activity and mode of action of phenolic compounds on leishmania donovani. *PLOS Negl Trop Dis* 13:e0007206. <https://doi.org/10.1371/journal.pntd.0007206>
42. Mead JR, McNair N (2006) Antiparasitic activity of flavonoids and isoflavones against cryptosporidium parvum and encephalitozoon-intestinalis. *FEMS Microbiol Lett* 259:153–157. <https://doi.org/10.1111/j.1574-6968.2006.00263.x>
43. Lehane AM, Saliba KJ (2008) Common dietary flavonoids inhibit the growth of the intraerythrocytic malaria parasite. *BMC Res Notes* 1:1–5. <https://doi.org/10.1186/1756-0500-1-26>
44. Lewin G, Cojean S, Gupta S, Verma A, Puri SK, Loiseau PM (2011) In vitro antileishmanial properties of new flavonoids against leishmania donovani. *Biomed Prev Nutr* 1:168–171. <https://doi.org/10.1016/j.bionut.2011.06.012>
45. Machado M, Pires P, Dinis AM, Santos-Rosa M, Alves V, Salgueiro L, Sousa MC (2012) Monoterpenic aldehydes as potential anti-Leishmania agents: activity of cymbopogon citratus and citral on L. infantum, L. tropica and L. major. *Exp Parasitol* 130:223–231. <https://doi.org/10.1016/j.exppara.2011.12.012>
46. Schwikkard S, Van Heerden FR (2002) Antimalarial activity of plant metabolites. *Nat Prod Rep* 19:675–692. <https://doi.org/10.1039/b008980j>
47. dos Santos DA, Vieira PC, Silva MFGF, Fernandes JB, Rattray L, Croft SL (2009) Antiparasitic activities of acridone alkaloids from swinglea glutinosa (Bl.) Merr. *J Braz Chem Soci* 20:644–651. <https://doi.org/10.1590/S0103-50532009000400008>
48. Nnadi CO, Nwodo NJ, Kaiser M, Brun R, Schmidt TJ (2017) Steroid Alkaloids from holarrhena africana with strong activity against trypanosoma brucei rhodesiense. *Molecules* 22:1129. <https://doi.org/10.3390/molecules22071129>
49. Cushnie TT, Lamb AJ (2005) Antimicrobial activity of flavonoids. *Int J Antimicrob Agent* 26:343–356. <https://doi.org/10.1016/j.ijantimicag.2005.09.002>
50. Abugri DA, Witola WH (2020) Interaction of apigenin-7-O-glucoside with pyrimethamine against *Toxoplasma gondii* growth. *J Parasit Dis* 44:221–229. <https://doi.org/10.1007/s12639-019-01185-5>
51. Tasdemir D, Kaiser M, Brun R, Yardley V, Schmidt TJ, Tosun F, Ru'edi P (2006) Antitrypanosomal and antileishmanial activities of flavonoids and their analogues: in vitro, in vivo, structure-activity relationship, and quantitative structure-activity relationship studies. *Antimicrob Agent Chemother* 5:1352–1364. <https://doi.org/10.1128/AAC.50.4.1352-1364.2006>
52. Smiljkovic M, Stanisavljevic D, Stojkovic D, Petrovic I, Vicentic JM, Popovic J, Grdadolnik SG, Markovic D, Sankovic-Babice S, Glamoclija J, Stevanovic M (2017) Apigenin-7-O-glucoside versus apigenin: Insight into the modes of anticandidal and cytotoxic actions. *EXCLI J* 16:795–807
53. Szweczyk A, Wojtczak L (2002) Mitochondria as a pharmacological target. *Pharmacol Rev* 54:101–127. <https://doi.org/10.1124/pr.54.1.101>
54. Allaq AA, Sidik NJ, Abdul-Aziz A, Ahmed IA (2020) Cumin (*Cuminum cyminum* L.): a review of its ethnopharmacology, phytochemistry. *Biomed Res and Ther* 7:4016–4021. <https://doi.org/10.15419/bmrat.v7i9.634>
55. Satyanarayana S, Sushruta K, Sarm GS, Srinivas N, Raju GS (2004) Antioxidant activity of the aqueous extracts of spicy food additives—evaluation and comparison with ascorbic acid in vitro systems. *J Herb pharmacother* 4:1–10. https://doi.org/10.1080/J157v04n02_01
56. Al-Snafi AE (2016) The pharmacological activities of cuminum cyminum—a review. *IOSR J Pharm* 6:46–65
57. Ishikawa T, Takayanagi T, Kitajima J (2002) Water-soluble constituents of cumin: monoterpenoid glucosides. *Chem Pharm Bull* 50:1471–1478. <https://doi.org/10.1248/cpb.50.1471>
58. Takayanagi T, Ishikawa T, Kitajima J (2003) Sesquiterpene lactone glucosides and alkyl glycosides from the fruit of cumin. *Phytochemistry* 63:479–484. [https://doi.org/10.1016/S0031-9422\(03\)00103-1](https://doi.org/10.1016/S0031-9422(03)00103-1)
59. Custódio L, Fernandes E, Escapa AL, Fajardo A, Aliqué R, Alberício F, Romano A (2011) Antioxidant and cytotoxic activities of

- carob tree fruit pulps are strongly influenced by gender and cultivar. *J Agric Food Chem* 59:7005–7012. <https://doi.org/10.1021/jf200838f>
60. El Hajaji H, Lachkar N, Alaoui K, Cherrah Y, Farah A, Ennabili A, Lachkar M (2010) Antioxidant properties and total phenolic content of three varieties of carob tree leaves from morocco. *Rec Nat Prod* 4:193–204. <https://doi.org/10.1016/j.arabjc.2010.06.053>
61. Saratsi K, Hoste H, Voutzourakis N, Tzanidakis N, Stefanakis A, Thamsborg SM, Sotiraki S (2020) Feeding of carob (*Ceratonia siliqua*) to sheep infected with gastrointestinal nematodes reduces faecal egg counts and worm fecundity. *Vet Parasitol* 284:109200. <https://doi.org/10.1016/j.vetpar.2020.109200>
62. Rtibi K, Selmi S, Jabri MA, Mamadou G, Limas-Nzouzi N, Sebai H, Amri M (2016) Effects of aqueous extracts from ceratonia siliqua l. pods on small intestinal motility in rats and jejunal permeability in mice. *RSC Adv* 6:44345–44353. <https://doi.org/10.1039/C6RA03457H>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.