



# Effect of some plant extracts from Egyptian herbal plants against *Toxoplasma gondii* tachyzoites *in vitro*

Sara T. ELAZAB<sup>1,2)</sup>, Amal F. SOLIMAN<sup>3)</sup> and Yoshifumi NISHIKAWA<sup>2)\*</sup>

<sup>1)</sup>Department of Pharmacology, Faculty of Veterinary Medicine, Mansoura University, El-Mansoura 35516, Egypt

<sup>2)</sup>National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080-8555, Japan

<sup>3)</sup>Department of Pharmacognosy, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt

**ABSTRACT.** Nowadays, herbal extracts are considered to be a potential source for developing new drugs that will overcome resistance to conventional chemotherapeutic agents. This study was aimed to explore the efficacy of several Egyptian plant extracts against *Toxoplasma gondii* infection *in vitro* for future development of a new, safe, and effective compound for *T. gondii*. Methanol extracts from *Matricaria chamomilla* (German chamomile), *Laurus nobilis*, *Citrullus colocynthis*, *Cinnamum camphora*, *Boswellia scara*, and *Melissa officinalis* plants and oil extracts (either essential or fixed oils) of some plants such as: lemon grass (*Cymbopogon citratus*), marjoram (*Origanum majorana*), watercress (*Nasturtium officinale*), wheat germ (*Triticum aestivum*), sesame (*Sesamum indicum*), rosemary (*Salvia rosmarinus*), citronella (*Cymbopogon nardus*), clove (*Syzygium aromaticum*), jojoba (*Simmondsia chinensis*), and basil (*Ocimum basilicum*) were investigated for their anti-*Toxoplasma* activities. The methanol extracts from *C. colocynthis* and *L. nobilis* and the oil extracts from lemon grass and marjoram were active against *T. gondii* with half maximal inhibitory concentrations (IC<sub>50</sub>) of 22.86 µg/ml, 31.35 µg/ml, 4.6 µg/ml, and 26.24 µg/ml, respectively. Their selectivity index (SI) values were <10. Interestingly, the methanol extract from *M. chamomilla* and oil from citronella had the lowest IC<sub>50</sub> values for *T. gondii* (3.56 µg/ml and 2.54 µg/ml, respectively) and the highest SI values (130.33 and 15.02, respectively). In conclusion, methanol extract from *M. chamomilla* and oil from citronella might be potential sources of novel therapies for treating toxoplasmosis.

**KEY WORDS:** inhibitory concentration, medicinal plants, oil extract, selectivity index, *Toxoplasma gondii*

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Toxoplasmosis, a cosmopolitan zoonotic parasitic infection, is caused by an Apicomplexa intracellular protozoan, *Toxoplasma gondii* [38]. Members of the Felidae family are the definitive hosts of *T. gondii*. Humans can be infected by *T. gondii* through the ingestion of *T. gondii* cysts present in undercooked or raw meat of intermediate hosts such as cattle, sheep, goats, pigs, and chickens [26, 90]. It has been reported that up to one-third of the global population is affected by this disease with varying degrees of severity depending on immune status [88, 91]. Toxoplasmosis is usually asymptomatic in immune-competent people, causing chronic infection with parasite cyst formation in tissues. However, in hosts with a waned immune system as AIDS patients or chemotherapy-treated patients, it may lead to vigorous systemic disease and subsequently death [85]. Importantly, toxoplasmosis in pregnant women may lead to miscarriage or fetal abnormalities (hydrocephaly, calcification) or neurological illness in newborns [14].

Sulfadiazine and pyrimethamine are the two medications that are concomitantly used currently in the treatment of toxoplasmosis [63]. These drugs exert their action by blocking the *Toxoplasma* folate metabolism, which consequently inhibits the generation of the DNA and eventually blocks the replication of tachyzoites [19, 24]. However, there are many drawbacks that constrict the utilization of these current chemotherapeutic agents, including their restricted activity in eradicating *T. gondii* encysted bradyzoites [60, 84], which may cause serious undesirable effects such as suppression of the bone marrow, hematological reactions, embryopathies, gastrointestinal upset, and hypersensitivity [21, 31, 81]. Therefore, there is an urgent need for the development of novel, efficient, and safe compounds with less toxicity and short medicating courses.

For centuries, medicinal plant extracts have been utilized extensively by mankind in treating several diseases [39]. These herbal extracts possess a wide variety of bioactive compounds such as tannin, flavonoids, alkaloids, and phenolic substances, which may serve as potential alternatives to many synthetic drugs [23, 25]. Previously, several identified compounds for curing parasitic

\*Correspondence to: Nishikawa, Y.: [nisikawa@obihiro.ac.jp](mailto:nisikawa@obihiro.ac.jp)

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diseases such as malaria have been isolated from plants, for example, quinine and artemisinin [11]. In Egypt, the utilization of medicinal plants rose since Pharaonic times, and nowadays, many Egyptians still rely on medicinal plants for the treatment of several diseases [1, 55]. Considering the aforementioned drawbacks of the current anti-*Toxoplasma* remedies, it is imperative to search for novel and highly effective compounds for the treatment of toxoplasmosis with minimal side effects. Therefore, in an attempt to develop a new therapy from Egyptian herbal extracts for *T. gondii*, this study aimed to investigate the effect of methanol and oil extracts of some plants against *T. gondii*. The selected plants for this research are frequently used for treating many diseases in herbal therapy and are known to have diverse bioactive constituents. In addition, most of these plants have been previously reported to exhibit antiprotozoal and anthelmintic activity against organisms such as *Matricaria chamomilla* [37]; *Laurus nobilis* [10, 89]; *Citrullus colocynthis* [36]; *Cinnamum camphor* [76]; *Boswellia scara* [64]; *Melissa officionalis* [52]; *Cymbopogon citratus* [70]; *Origanum majorana* [46]; *Salvia rosmarinus* [7]; *Syzygium aromaticum* [50]; and *Ocimum basilicum* [20]. However, to the best of our knowledge, no literature on their potential anti-*Toxoplasma* activities is available.

## MATERIALS AND METHODS

### Parasites and cell cultures

*T. gondii* RH-GFP (a green fluorescent protein expressing- RH strain) was used in this study. African green monkey kidney (Vero) cells and human foreskin fibroblast (HFF) cells were used for parasite maintenance. Purification of the parasite was performed using a previously reported method [69].

### Plant materials, oil extracts, and chemicals

Samples of *M. chamomilla* (German chamomile), *L. nobilis* (Bay laurel), *C. colocynthis* (Colocynth), *C. camphora* (Camphor tree), *B. scara* (Frankincens), and *M. officionalis* (Lemon balm) plants were procured from an herbal drug store in Mansoura, Egypt. Table 1 illustrates the name, family, utilized parts, and the traditional uses of plants that were employed in this experiment. Plant identification was carried out by Prof. Dr. Ibrahim Mashaly from Botany Department, Faculty of Science, Mansoura University, Egypt. Plant materials were dried and ground into min pieces. Afterwards, they were extracted thrice at ambient temperature using 70% methanol (48 hr/time). Crude extracts were obtained by evaporating the filtrates under vacuum in a rotary evaporator. The obtained extracts were mixed with dimethyl sulfoxide (DMSO) at 100 mg/ml and preserved at  $-30^{\circ}\text{C}$ .

Essential oils of some plants such as lemon grass (*Cymbopogon citratus*), marjoram (*Origanum majorana*), watercress (*Nasturtium officinale*), rosemary (*Salvia rosmarinus*), citronella (*Cymbopogon nardus*), clove (*Syzygium aromaticum*), basil (*Ocimum basilicum*), and fixed oils from other plants such as sesame (*Sesamum indicum*), wheat germ (*Triticum aestivum*), and jojoba (*Simmondsia chinensis*) were supplied by the National Research Center of Medicinal and Aromatic Plants, Qalyubia, Egypt. Sulfadiazine (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 1 N NaOH (stock solution 200 mg/ml) and was used.

### In vitro Cytotoxic assay

The effect of the aforementioned plant extracts on the viability of HFF cells was investigated according to the method described by [47]. Briefly, HFF cells were grown in 96-well plates with 100  $\mu\text{l}$  Dulbecco's modified Eagle's medium (DMEM, Sigma-Aldrich) in each well (cell suspension,  $1 \times 10^5$  cells/ml in DMEM supplemented with 10% FBS). After 48 hr of incubation at  $37^{\circ}\text{C}$  in a 5%  $\text{CO}_2$  atmosphere, 100  $\mu\text{l}$  of the plant extracts dissolved in DMEM were added to the cells (final concentrations, 7.8–1,000  $\mu\text{g/ml}$ ). Wells with sulfadiazine or culture medium only served as the positive and negative controls, respectively. After 72 hr, cell

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

Plants	Plant part	Traditional uses	References	% inhibition of RH-GFP at 50 $\mu\text{g/ml}$	% inhibition of RH-GFP at 10 $\mu\text{g/ml}$
<i>Boswellia scara</i> (Frankincens)	Resins	To cure bronchial and urinary infections	[42]	$18.28 \pm 8.11$	0.00
<i>Cinnamum camphora</i> (Camphor tree)	Leaf	Used for treating for inflammatory diseases such as bronchitis, rheumatism, and sprains	[80]	$54.95 \pm 18.97$	$34.73 \pm 10.01$
<i>Citrullus colocynthis</i> (Colocynth)	Fruit	Used for treating respiratory diseases, diabetes, obesity, insecticide, purgative, anthelmintic, and molluscicide	[3, 28, 78]	$97.07 \pm 8.7$	$65.73 \pm 4.45$
<i>Laurus nobilis</i> (Bay laurel)	Leaf	Used for treating gastrointestinal diseases	[22]	$74.78 \pm 12.01$	0.00
<i>Matricaria chamomilla</i> (German chamomile)	Flower	Used for skin irritations, wounds eczema, ulcers, bruises, burns, gout, neuralgia, sciatica, mastitis rheumatic pain, and hemorrhoids	[8, 79]	$83.36 \pm 7.69$	$67.37 \pm 14.52$
<i>Melissa officionalis</i> (Lemon balm)	Leaf	Used for treating CNS problems such as nervous agitation, sleep disorders, depression, and gastrointestinal diseases	[30, 34]	$17.96 \pm 12.03$	$3.14 \pm 4.29$

% inhibition of a green fluorescent protein expressing-RH strain of *T. gondii* (RH-GFP) of 1 mg/ml Sulfadiazine:  $67.93 \pm 10.56$ . Values are the mean  $\pm$  SD of triplicate samples, and data are a representative of three independent experiments.

counting kit-8 (CCK-8, Dojindo, Kumamoto, Japan) was added to determine cell viability. Their optical densities were detected at 450 nm using an MTP-120 microplate reader (Corona Electric, Hitachinaka, Japan). HFF cell growth suppression (%) was estimated using the following equation:

$$\frac{\text{the absorbance of cells treated with extracts}}{\text{the absorbance of cells cultured with medium alone}} \times 100$$

GraphPad Prism 5 software was used to determine the half maximal inhibitory concentration (IC<sub>50</sub>) values on HFF cells (GraphPad Software Inc., La Jolla, CA, USA).

### Anti-Toxoplasma activity

HFF cells were grown at 100 µl/well in 96-well microtiter plates (cell suspension 1 × 10<sup>5</sup> cells/ml in DMEM supplemented with 10% FBS) and incubated at 37°C in a 5% CO<sub>2</sub> atmosphere for 48 hr. Then, the cells were infected with *T. gondii* RH-GFP (5 × 10<sup>4</sup> tachyzoites/ well) for 4 hr, and the extracellular parasites were removed. Afterward, the infected cells were subjected to the herbal extracts prepared in DMEM. All plant extracts (methanol and oil extracts) were tested for their anti-*Toxoplasma* activity at 50 and 10 µg/ml. Then, the plant extracts showing percentage inhibition of RH-GFP growth at 50 µg/ml higher than that produced by sulfadiazine were investigated at concentrations (0.5–100 µg/ml) to determine their IC<sub>50</sub> values against *T. gondii*. Sulfadiazine and media were added as positive and negative controls, respectively. After 3 days of incubation, a microplate reader (GloMax-Multi Detection System, Promega, Madison, WI, USA) was used to measure the fluorescence intensity of RH-GFP. The growth inhibition (%) of RH-GFP was determined as reported previously [47]. IC<sub>50</sub> values of the herbal extract on *T. gondii* were determined with the aid of GraphPad Prism 5 software.

## RESULTS

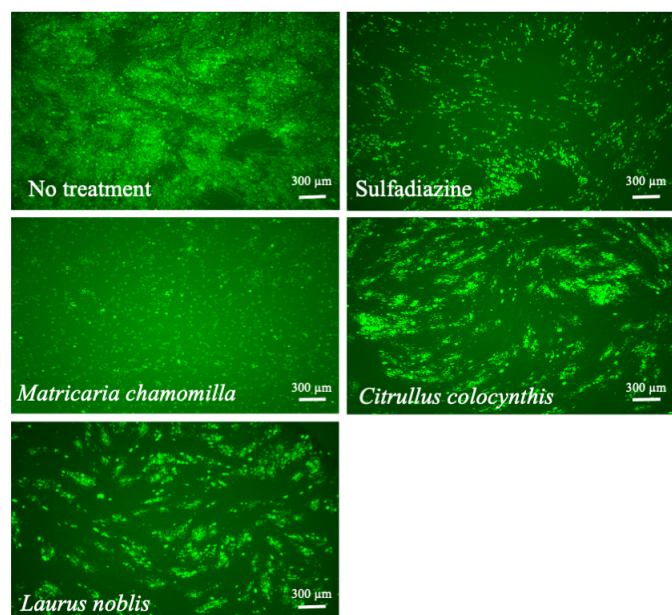
To investigate the anti-*Toxoplasma* effects of herbal extracts, we examined the fluorescence intensity of RH-GFP. Tables 1 and 2 show the activities of the aforementioned plant extracts against *T. gondii* at concentrations of 50 and 10 µg/ml. The methanol extracts obtained from *M. chamomilla* (German chamomile), *C. colocynthis*, and *L. nobilis* had high anti-*Toxoplasma* activities compared with 1 mg/ml sulfadiazine (67.93%). They inhibited RH-GFP growth at 50 µg/ml showing 83.36 ± 7.69, 97.07 ± 8.7, and 7478 ± 12.01% inhibition, respectively (Table 1). Moreover, among the oil extracts at 50 µg/ml, lemon grass, citronella, and marjoram showed high percentage inhibition of 93.20 ± 8.16, 87.69 ± 3.33, and 63.36 ± 6.66, respectively (Table 2). Figures 1 and 2 show the anti-*Toxoplasma* activities of sulfadiazine (1 mg/ml), *M. chamomilla* (50 µg/ml), *C. colocynthis* (10 µg/ml), *L. nobilis* (50 µg/ml), lemon grass oil (10 µg/ml), citronella oil (10 µg/ml), and marjoram oil (50 µg/ml).

Table 3 illustrates, according to *in vitro* screening, that the IC<sub>50</sub> values of plant extracts showed high anti-*Toxoplasma* activities. All examined plant extracts showed lower IC<sub>50</sub> values than sulfadiazine (IC<sub>50</sub>=99.4 µg/ml). The methanol extracts of *C. colocynthis*

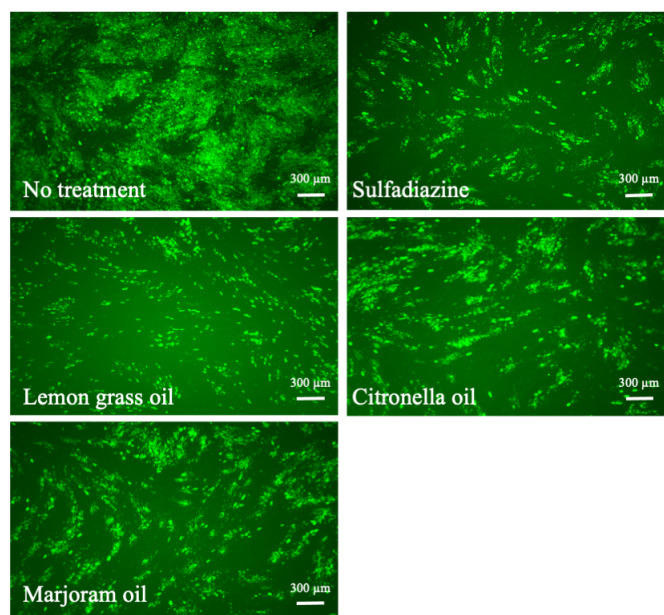
**Table 2.** Activities of oil extracts from some plants on *Toxoplasma gondii* at concentrations 50 µg/ml and 10 µg/ml

Oil extracts	Plant part	Traditional uses	References	% inhibition of RH-GFP at 50 µg/ml	% inhibition of RH-GFP at 10 µg/ml
Basil	Leaf	For treatment of abdominal cramps, gastroenteritis, acne, wounds, and vitiligo	[33, 82]	50.03 ± 4.46	22.53 ± 1.53
Citronella	Aerial part	Treatment of intestinal parasites and digestive and menstrual disorders and also used as antipyretic	[44, 56]	87.69 ± 3.33	48.60 ± 8.22
Clove	Flower	For treating nausea, vomiting and flatulence, tuberculosis, cholera, malaria, candida, worms, viruses, different bacterial and protozoan infections	[13]	59.51 ± 4.42	54.56 ± 4.54
Jojoba	Seed	For treating sore throat, wounds, and warts	[59]	16.29 ± 11.34	13.46 ± 6.56
Lemon grass	Aerial part	Used for reducing cholesterol level, particularly in hypercholesterolemic patients	[40]	93.20 ± 8.16	56.14 ± 2.26
Marjoram	Leaf	For treating chest diseases, sore throat, cough, rheumatic pain, nervous disorders, insomnia	[15, 29]	63.36 ± 6.66	6.10 ± 1.69
Rosemary	Leaf	For treatment of bronchial asthma, abdominal colic, peptic ulcer, and cardiac diseases	[4, 77]	24.05 ± 7.5	20.85 ± 7.57
Sesame	Seeds	For treating urinary troubles and healing wound	[65]	44.87 ± 0.82	1.74 ± 2.21
Watercress	Leaf	For treatment of abdominal pain	[9, 72]	53.62 ± 8.52	20.31 ± 9.12
Wheat germ	Germ	Used for treating hypercholesteremia and diabetes	[16]	43.51 ± 16.21	35.96 ± 1.57

Values are the mean ± SD of triplicate samples, and data are a representative of three independent experiments. RH-GFP, a green fluorescent protein expressing-RH strain of *T. gondii*.



**Fig. 1.** Anti-*Toxoplasma* activities of *Matricaria chamomilla* (German chamomile), *Citrullus colocynthis*, and *Laurus nobilis*: expressed as representative images of a green fluorescent protein expressing-RH strain of *T. gondii*-infected human foreskin fibroblast cells treated with sulfadiazine (1 mg/ml), or either *M. chamomilla* (German chamomile) (50 µg/ml), *C. colocynthis* (10 µg/ml), or *L. nobilis* (50 µg/ml). Scale bar=300 µm.



**Fig. 2.** Anti-*Toxoplasma* activities of lemon grass, citronella, and marjoram oils: expressed as representative images of a green fluorescent protein expressing-RH strain of *T. gondii*-infected human foreskin fibroblast cells treated with sulfadiazine (1 mg/ml), or either lemon grass oil (10 µg/ml), citronella oil (10 µg/ml), or marjoram oil (50 µg/ml). Scale bar=300 µm.

**Table 3.** Half maximal inhibitory concentration (IC<sub>50</sub>) values and selectivity index values of methanol and oil herbal extracts

Herbal extracts	IC <sub>50</sub> (µg/ml) on HFF cells	IC <sub>50</sub> (µg/ml) on RH-GFP	Selectivity index (SI)
Citronella oil	38.16 ± 8.16	2.54 ± 1.01	15.02
<i>Citrullus colocynthis</i> (Colocynth)	27.74 ± 0.74	22.86 ± 3.43	1.21
<i>Laurus nobilis</i> (Bay laurel)	173.50 ± 55.57	31.35 ± 17.01	5.53
Lemon grass oil	28.76 ± 2.67	4.57 ± 0.21	6.25
Marjoram oil	148.95 ± 61.87	26.24 ± 7.63	5.67
<i>Matricaria chamomilla</i> (German chamomile)	464 ± 9.05	3.56 ± 2.21	130.33
Sulfadiazine	>1,000*	99.40*	N.D.

IC<sub>50</sub> values were calculated based on three independent experiments. Data are expressed as mean ± SD. \*Data from [47]. N.D.=not detected. HFF, human foreskin fibroblast; RH-GFP, a green fluorescent protein expressing-RH strain of *T. gondii*.

and *L. nobilis* and the oil extracts of lemon grass and marjoram had high anti-*Toxoplasma* activities (IC<sub>50</sub>=22.86 µg/ml, 31.35 µg/ml, 4.6 µg/ml, and 26.24 µg/ml, respectively). However, their selectivity index values were relatively low, as the selectivity index (SI) values for *T. gondii* were 1.21, 5.5, 6.25, and 5.67, respectively. Interestingly, the methanol extract from *M. chamomilla* and oil extract from citronella had the lowest IC<sub>50</sub> values (3.56 µg/ml and 2.54 µg/ml, respectively) and the highest SI values (130.33 and 15.02, respectively) for *T. gondii*.

## DISCUSSION

Over the last three decades, the high resistance of infectious organisms to chemotherapeutic agents has accounted for the failure of routine medication in the management of some infections [12]. Among the growing resistance problems, the current anti-*Toxoplasma* drugs have been reported to be less effective against parasites owing to genetic mutation [62]. Hence, there is an increasing demand for the development of new remedies for toxoplasmosis. Recently, great attention has been paid to the discovery of novel antiparasitic agents from medicinal plants. It has been reported that several herbal extracts were effective against *T. gondii* such as *Eurycoma longifolia* Jack [41, 43], *Curcuma* [5], *Artemisia annua* L. [27, 67], and *Myristica fragrans* Houtt [74]. The screening of herbal extracts to determine anti-*Toxoplasma* efficacy is considered a method for developing new drugs for

toxoplasmosis. In the present study, the methanol extracts of six plants and oil extracts of ten plants were investigated *in vitro*, for the first time, for their antitoxoplasmal effects against *T. gondii*.

All the examined plant extracts revealed varying degrees of anti-*Toxoplasma* activities at 50 µg/ml, ranging from 16.29 to 97.07%. Of all the extracts tested, the methanol extracts obtained from *M. chamomilla* (German chamomile), *C. colocythis*, and *L. nobilis* and the essential oils of lemon grass, citronella, and marjoram exhibited higher growth inhibition than sulfadiazine. Importantly, *M. chamomilla* (German chamomile) methanol extract and citronella oil, with the lowest IC<sub>50</sub> values for *T. gondii* RH-GFP (3.56 µg/ml and 2.54 µg/ml, respectively) and the highest SI values (130.33 and 15.02, respectively), were demonstrated to be the most potent. These SI values were considered as hallmarks for the selective activity of the therapy.

*M. chamomilla* (German chamomile) is a member of the Asteraceae family. In folk medicine, chamomile flowers have traditionally been used to alleviate upper respiratory and gastrointestinal tract illnesses and inflammatory conditions of the skin [73] and as a remedy for nightmares, insomnia, and hysteria. It has been reported to have anti-oxidant, antispasmodic, antidiarrheal, anti-inflammatory, anti-allergic, antimicrobial, and antidepressant features [2, 6, 17, 18, 58, 83]. Additionally, chamomile is known for its efficacy in the control and management of prostate, breast, and ovarian cancer [45]. Furthermore, Tunisian chamomile has been reported to have strong leishmanicidal activity [37]. *M. chamomilla* (German chamomile) possesses several compounds that are responsible for its biological activities [32, 35]. The major phenolic compounds found in chamomile flowers are flavonoids, quercetin, patuletin, apigenin, luteolin, glucosides, and coumarins (umbelliferone and herniarin) [54, 58]. The findings of our study indicate that the methanol extract of chamomile flowers is an effective anti-*Toxoplasma* agent. Hence, this effect on *T. gondii* growth may be attributed to any one or a combination of its bioactive ingredients.

Flavonoids have been shown to possess activity against *Cryptosporidium parvum*, *Leishmania donovani*, *Entamoeba histolytica*, and *Plasmodium falciparum* [48, 49, 57, 61]. Moreover, it has been revealed that apigenin was effective against *Leishmania tropica* amastigote [66]. In addition, previous reports have shown that coumarins exhibit activity against the promastigote form of *Leishmania major* and *Trypanosoma cruzi* [53, 87]. Therefore, future studies on the anti-*Toxoplasma* activities of these compounds that were identified in *M. chamomilla* (German chamomile) flowers are needed.

Citronella grass (*Cymbopogon nardus* Rendle) belongs to the Poaceae family. Citronella oil is commonly used as an antipyretic, diuretic, and antispasmodic agent and for the treatment of intestinal parasites [44, 56]. It is widely used as an insect repellent, especially as a repellent against fleas, mosquitoes, and biting flies [68]. In addition, citronella oil is used in the manufacture of detergents, soaps, fragrances, and perfumes [71]. The major components detected in the citronella grass essential oil were monoterpenes, which include geraniol (28.62%), citronellal (23.62%), and citronellol (17.10%) [75]. The present study showed that citronella oil has acceptable activity against *T. gondii* RH *in vitro*, with an SI value >10. The anti-*Toxoplasma* activity of citronella oil is probably owed to its chemical constituents. Monoterpenes have been reported to exert antileishmanial activity [51, 86]. However, further studies are needed to explore the mechanism of action of citronella oil against *T. gondii*.

In conclusion, our results revealed that the methanol extract from *M. chamomilla* (German chamomile) and oil extract from citronella are effective against *T. gondii* *in vitro* and may serve as potential drugs for the treatment of toxoplasmosis. However, their efficacy *in vivo* needs to be investigated in a future study.

**CONFLICT OF INTEREST.** The authors declare no conflict of interests

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