



## NOTE

Parasitology

# Extracts of wild Egyptian plants from the desert inhibit the growth of *Toxoplasma gondii* and *Neospora caninum* *in vitro*

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**ABSTRACT.** Wild medicinal plants have been traditionally used as antimicrobial agents. Here, we evaluated the *in vitro* activity of extracts from wild Egyptian desert plants against *Toxoplasma gondii* and *Neospora caninum*. From 12 plant extracts tested, the methanolic extracts from *Artemisia judaica*, *Cleome droserifolia*, *Trichodesma africanum*, and *Vachellia tortilis* demonstrated potent activity against the growth of *T. gondii*, with half-maximal inhibitory concentrations (IC<sub>50</sub>s) of 2.1, 12.5, 21.8, and 24.5 µg/ml, respectively. *C. droserifolia*, an ethanolic extract of *P. undulata*, *T. africanum*, *A. judaica*, and *V. tortilis* demonstrated potent efficacy against *N. caninum*, with mean IC<sub>50</sub>s of 1.0, 3.0, 3.1, 8.6, and 17.2 µg/ml, respectively. Our data suggest these extracts could provide an alternative treatment for *T. gondii* and *N. caninum* infections.

**KEYWORDS:** desert, Egypt, *Neospora caninum*, plant extract, *Toxoplasma gondii*

Toxoplasmosis, caused by the obligate intracellular protozoan parasite *Toxoplasma gondii*, is a disease of zoonotic potential and global veterinary and medical concern [19]. *T. gondii* is considered a single species in the genus *Toxoplasma*, phylum Apicomplexa [13]. Approximately 30% of the global human population has contracted the parasite as evidenced by the presence of anti-*T. gondii* antibodies. While *T. gondii* infections are usually asymptomatic, they can lead to adverse effects especially in patients who are immunocompromised or who obtain the disease congenitally [31]. In humans, *T. gondii* infection occurs through three main pathways: ingestion of undercooked meat contaminated with cysts, consumption of food or water contaminated with the infective stage (oocysts), or congenitally via vertical transmission from infected mothers to their offspring [22, 32]. Transplacental transmission occurs when women are infected during pregnancy, [12] and the timing of infection during pregnancy affects the progress of the disease [30].

*Neospora caninum*, the causative agent of neosporosis, is an obligate intracellular tissue cyst-forming coccidian parasite of the phylum Apicomplexa [11]. *N. caninum* is transmitted by two main pathways: transplacentally and horizontally [9, 10]. Previously, reported studies have shown the efficacy of chemotherapeutic agents such as anti-coccidian drugs, pyrimethamine, and trimethoprim [17]. Currently, there are no vaccines or safe chemotherapeutic agents available for food-producing livestock because of the long-term duration of treatment [10]. Therefore, identifying compounds from natural resources with anti-*Neospora* activities is still challenging.

Sulphadiazine has been found to have important inhibitory effects on *T. gondii* (half-maximal inhibitory concentration (IC<sub>50</sub>)=2.5 µg/ml) and to be associated with a reduction of the growth of these intracellular parasites and an alteration to their normal morphology [8]. In contrast, sulfonamides demonstrate little activity against *N. caninum* tachyzoites at 100 µg/ml [18]. Sulphadiazine and pyrimethamine can be used in combination for the treatment of toxoplasmosis in people, reported side effects include agranulocytosis, Stevens-Johnson syndrome, toxic epidermal necrolysis, and hepatic necrosis [3]. Therefore, developing novel chemotherapeutics from natural resources that are of low or no toxicity is beneficial for treating both toxoplasmosis and neosporosis.

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A large number of medicinal plants that produce natural products with potent anti-parasitic activity have been identified and researched [24]. Plant extracts or secondary metabolites that were considered as an alternative to commercial drugs were evaluated during the search for antiparasitic candidates. From 1981 to 2006, 1,184 new drugs were registered of which 28% were either natural products or their derivatives [24, 35]. The desert plants in this study have been reported to have wide medicinal uses (see [Supplementary Table 1](#)). Therefore, the current study aimed to evaluate the *in vitro* efficacy of plant extracts collected from Egypt against the growth of tachyzoites of both *T. gondii* and *N. caninum*.

A green fluorescent protein (GFP)-expressing RH strain of type I *T. gondii* (RH-GFP) and GFP-expressing Nc1 strain of *N. caninum* (Nc1-GFP) were maintained in African green monkey kidney epithelial (Vero) cells according to previously reported methods [16, 26, 27].

The plants used in this study were obtained from the field in two regions of the southern region of Egypt in the Qena Governorate (Latitude: 26° 09' 51.05" N, Longitude: 32° 43' 36.16" E). The collection was done from two sites along the Qena-Sohag and Qena-Safaga desert roads, Eastern desert, Egypt, and collection was done under the approval of the Faculty of Veterinary Medicine, South Valley University, Qena. The collection was done following the guidelines and rules of South Valley University, Qena, Egypt. A map that identifies the sites of collection is shown ([Supplementary Fig. 1](#)). Twelve plant samples were collected in May 2019 during the plant flowering season. The collected plant samples were identified by the South Valley University Herbarium, Faculty of Science, Qena, Egypt, and an official letter of identification was issued. Identification was performed according to the reported literature [4–7]. Plant taxonomy and species were further updated according to Plants of the World Online [29].

Plant samples were left to dry in the shade for 3 to 10 days. A fine powder was obtained from dried leaves, flowers, fruit, or seed parts using a kitchen blender. One hundred grams of each powdered plant material was dissolved in either 80% methanol, 70% ethanol, or distilled water for a minimum of 1 to 3 days with a ratio of 1:10 (100 g of plant powder per 1 liter of the solvent used). The plant supernatant was further collected and filtrated by a glass filtration apparatus and was then collected in a wide conical flask. It was then dissolved in a wide petri dish at room temperature for 1 to 3 days. The final crude extract was collected in centrifuge tubes and stored at –30°C until use. To test the antiprotozoal potential of the various plant extracts, they were solubilized individually in the solvent dimethyl sulfoxide (DMSO) to prepare stock solutions (100 mg/ml). The previously reported medicinal uses and the Latin binomial names of the wild plants used in this study are shown in [Supplementary Table 1](#).

To determine the cytotoxic potential of the plant extracts, cytotoxicity was evaluated against human cells using human foreskin fibroblast (HFF) cells, human embryonic kidney (293T) cells, and mouse neuroblast (N1E-115) cells. Cell suspensions ( $1 \times 10^5$  cells/ml) in Dulbecco's Modified Eagle medium (DMEM, Sigma-Aldrich, St. Louis, MI, USA) supplemented with 10% fetal bovine serum (FBS) (Nichirei Bioscience, Tokyo, Japan) were plated at 100  $\mu$ l/well in 96-well plates and incubated at 37°C in a 5% CO<sub>2</sub> atmosphere. The plant extracts were added to the cells for 72 hr at final concentrations of two-fold serial dilution starting from 1,000  $\mu$ g/ml. To evaluate cell viability, cell proliferation inhibition (%) was calculated as described previously [16, 28].

HFF cells (cell suspensions  $1 \times 10^5$  cells/ml in DMEM supplemented with 10% FBS) were plated at 100  $\mu$ l/well in 96-well plates, then *T. gondii* RH-GFP and *N. caninum* Nc1-GFP ( $5 \times 10^4$  tachyzoites/well) were added at five multiples of infection (MOI) for 4 hr. The extracellular parasites were then washed by changing the medium and the new medium with 10% FBS was added to each well. Only the plant extracts prepared in DMEM were added to the infected cells (final concentrations: 0.25–100  $\mu$ g/ml), and a medium with 0.5% dimethyl sulfoxide was used as the negative control. After being incubated for 72 hr, the fluorescence intensities of RH-GFP and Nc1-GFP were measured using a Glomax multi-detection system microplate reader (Promega Corp., Madison, WI, USA). The percentage inhibition of parasite growth was calculated according to the previous methods [16, 28]. The mean half maximal inhibitory concentration 50 (IC<sub>50</sub>) values were calculated for RH-GFP and Nc1-GFP. Data represent the mean values  $\pm$  standard deviation (SD) for three independent experiments.

RH-GFP and Nc1-GFP tachyzoites ( $2 \times 10^5$ ) were pre-treated with either one of the four different extracts (*A. judaica*, *C. droserifolia*, *T. africanum*, and *V. tortilis*) (100  $\mu$ g/ml), Sulfadiazine (1 mg/ml) or Eagle's Minimum Essential Medium (EMEM) alone for 1 hr at 37°C. Then, the pre-treated parasites were added to Vero cells at 1 ml/well in a 12-well plate (MOI=2). At 2 to 3 hr post-infection, the extracellular parasites were washed away and EMEM supplemented with 8% FBS was added. After 24 hr, the infected cells were analyzed by indirect fluorescent antibody test (IFAT) using anti-*T. gondii* GRA7 rabbit IgG antibody [34] and anti-*N. caninum* GRA7 rabbit IgG antibody [25] to measure the infection rates because treatment with crude plant extracts may cause destruction of the parasite-cell membranes, resulting in leakage of GFP signal from the parasite cytosol. The infection rate for RH-GFP and Nc1-GFP was measured by counting the number of *T. gondii* GRA7-positive and *N. caninum* GRA7-positive Vero cells per 100–140 Vero cells in ten randomly different fields for each plant candidate against the untreated parasites, respectively. Estimation of the infection rate values was performed blindly for all slides. The infection rate values were calculated by IFAT as follows: [(number of parasite-positive Vero cells) / (100 randomly selected Vero cells)]  $\times$  100 [16].

Graph Pad Prism 6.0 and its updated version 8.3.4 software (Graph Pad Software Inc. La Jolla, CA, USA) were used for the analysis of the IC<sub>50</sub> values for the percentage inhibition of the parasites and host cells. The final mean IC<sub>50</sub>s of anti-RH-GFP and anti-Nc1-GFP, and against HFF cells were calculated based on three independent experiments and the data represent the mean  $\pm$  SD. For mean infection rate calculation (mean infection rate %), statistical analyses were performed using a One-way analysis of variance (ANOVA) and a Tukey-Kramer post hoc analysis ( $P < 0.05$ ).

The cytotoxic potential of all plant extracts was evaluated by two-fold serial dilutions from 1,000  $\mu$ g/ml and the mean IC<sub>50</sub> values were calculated ([Table 1](#)). The methanolic and aqueous extracts from *C. procera*, the ethanolic extract from *P. undulata*, both the methanolic and ethanolic extracts from *C. colocynthis*, the methanolic extract from *P. undulata*, and the ethanolic extract from *O. basilicum* showed the highest cytotoxicity against HFF cells with mean IC<sub>50</sub>s of 2.9  $\mu$ g/ml, 41.5  $\mu$ g/ml, 55.5  $\mu$ g/ml, 65.6

**Table 1.** Mean half-maximal inhibitory concentrations (IC<sub>50</sub>s) of extracts against *Toxoplasma gondii* (RH-GFP), *Neospora caninum* (NcI-GFP), and different host cells *in vitro*

Plant extract	Plant family	Plant part	Mean IC <sub>50</sub> (µg/ml)						Mean selectivity index (SI)						
			RH-GFP <sup>a</sup>		NcI-GFP <sup>b</sup>		293T cells <sup>d</sup>		NIE-115 cells <sup>e</sup>		RH-GFP		NcI-GFP		
			(±SD)	>100	(±SD)	N.T	(±SD)	HFF cells <sup>c</sup>	(±SD)	N.T	(±SD)	HFF	NIE-115	HFF	293T
<i>Aerva javanica</i> (Burm.f.) Juss. ex Schult.	Amaranthaceae	Leaves	>100	N.T	378.1 (134.0)	N.T	N.T	N.T	N.T	>3.7	N.D	N.D	N.D	N.D	N.D
<i>Anabasis setifera</i> Moq.	Amaranthaceae	Leaves	>100	N.T	1,263.6 (194.9)	N.T	N.T	N.T	>12.6	N.D	N.D	N.D	N.D	N.D	N.D
<i>Artemisia judaica</i> L.	Asteraceae	Leaves	2.1 (0.6)	8.6 (3.2)	316.8 (88.8)	346.2 (64.1)	382.7 (133.4)	150.8	164.8	182.2	36.8	40.2	44.5		
<i>Calotropis procera</i> (Aiton) Dryand.	Apocynaceae	Flowers	7.2 (3.3)	1.8 (1.6)	41.5 (22.6)	N.T	N.T	N.T	5.7	N.D	N.D	23.0	N.D	N.D	
	M80%	Flowers	1.9 (0.1)	0.9 (0.7)	2.9 (1.5)	N.T	N.T	N.T	1.5	N.D	N.D	3.0	N.D	N.D	
<i>Citrullus colocynthis</i> (L.) Schrad.	Cucurbitaceae	Seeds	>100	N.T	88.0 (14.3)	N.T	N.T	N.T	>0.8	N.D	N.D	N.D	N.D	N.D	
	M80%	Seeds	>100	N.T	65.6 (7.2)	N.T	N.T	N.T	>0.6	N.D	N.D	N.D	N.D	N.D	
<i>Cleome droserifolia</i> (Forssk.) Delile	Cleomaceae	Leaves	12.5 (9.4)	1.0 (0.3)	370.9 (95.3)	263.4 (13.8)	247.3 (127.8)	29.6	21.0	19.8	370.9	263.4	247.3		
<i>Forsskaolea tenacissima</i> L.	Urticaceae	Leaves	64.5 (12.8)	N.T	519.0 (141.9)	N.T	N.T	N.T	8.0	N.D	N.D	N.D	N.D	N.D	
<i>Ochradenus baccatus</i> Delile	Resedaceae	Fruit	>100	N.T	1,179.0 (245.4)	N.T	N.T	N.T	>11.7	N.D	N.D	N.D	N.D	N.D	
<i>Ocimum basilicum</i> L.	Lamiaceae	Leaves	46.6 (28.3)	N.T	252.6 (13.9)	N.T	N.T	N.T	5.4	N.D	N.D	N.D	N.D	N.D	
<i>Pulicaria undulata</i> (L.) C.A.Mey.	Asteraceae	Flowers	15.4 (14.5)	3.0 (3.0)	55.5 (10.7)	N.T	N.T	N.T	3.6	N.D	N.D	18.5	N.D	N.D	
	M80%	Flowers	46.9 (7.9)	N.T	197.5 (61.3)	N.T	N.T	N.T	4.2	N.D	N.D	N.D	N.D	N.D	
<i>Trichodesma africanum</i> (L.) Sm.	Boraginaceae	Leaves	21.8 (14.0)	3.1 (1.6)	413.0 (96.9)	287.9 (47.4)	145.9 (47.9)	18.9	13.2	6.7	133.2	92.8	47.0		
<i>Yachellia tortilis</i> subsp. raddiana (Savi) Kyal. & Boatwr.	Fabaceae	Seeds	24.5 (0.5)	17.2 (9.2)	554.5 (110.5)	531.5 (79.8)	262.4 (118.0)	22.6	21.6	10.7	32.2	30.9	15.2		
Sulfadiazine			99.4 µg/ml*	>1,000*				N.D							

<sup>a</sup> The mean IC<sub>50</sub> and standard deviation values against *T. gondii* (RH-GFP) were calculated from the averages of three independent experiments after a 72-hr culture of the parasites with a plant extract. <sup>b</sup> The mean IC<sub>50</sub> and standard deviation values against *N. caninum* (NcI-GFP) were calculated from the averages of three independent experiments after a 72-hr culture of the parasites with a plant extract. <sup>c</sup> The mean IC<sub>50</sub> against HFF cells was calculated from the averages of three independent experiments after a 72-hr treatment. <sup>d</sup> The mean IC<sub>50</sub> against human embryonic kidney (293T) cells was calculated from the average of three independent experiments after a 72-hr treatment. <sup>e</sup> The mean IC<sub>50</sub> against mouse neuroblast (NIE-115) cells was calculated from the average of three independent experiments after a 72-hr treatment. Experiments with extracts that had no effect with IC<sub>50</sub> >100 µg/ml were not replicated against *T. gondii* and not tested against *N. caninum*. Except for those indicated to have an ethanolic or aqueous extract, all the plant extracts were methanolic. Average IC<sub>50</sub>s from all plant extracts were calculated from three independent experiments with near values. IC<sub>50</sub>: half maximal inhibitory concentration; 50; SD: standard deviation; SI: selectivity index; RH-GFP, a green fluorescent protein expressing-RH strain of *T. gondii*; HFF: human foreskin fibroblast; N.T: not tested; N.D: not determined; M80%: 80% methanol; E70%: 70% ethanol; aq.: aqueous, \*data from [15].

$\mu\text{g/ml}$ , 88.0  $\mu\text{g/ml}$ , 197.5  $\mu\text{g/ml}$ , and 252.6  $\mu\text{g/ml}$ , respectively. Extracts from plants *A. judaica*, *C. droserifolia*, *A. javanica*, *T. africanum*, *F. tenacissima*, and *V. tortilis* exhibited moderate-to-weak toxicity against HFF cells with mean  $\text{IC}_{50}$ s of 316.8  $\mu\text{g/ml}$ , 370.9  $\mu\text{g/ml}$ , 378.1  $\mu\text{g/ml}$ , 413.0  $\mu\text{g/ml}$ , 519.0  $\mu\text{g/ml}$ , and 554.5  $\mu\text{g/ml}$ , respectively. Extracts from *A. setifera* and *O. baccatus* were nontoxic or safe for HFF cells as cytotoxicity was not observed and their mean  $\text{IC}_{50}$ s were  $>1,000$   $\mu\text{g/ml}$  of 1,263.6  $\mu\text{g/ml}$ , and 1,179.0  $\mu\text{g/ml}$ , respectively (Table 1).

The cytotoxic potential of the selected potent candidates *A. judaica*, *C. droserifolia*, *T. africanum*, and *V. tortilis* were further assessed against Human embryonic kidney (293T) cells and mouse neuroblast (N1E-115) cells. The mean  $\text{IC}_{50}$ s of *A. judaica*, *C. droserifolia*, *T. africanum*, and *V. tortilis* against 293T cells were 346.2  $\mu\text{g/ml}$ , 263.4  $\mu\text{g/ml}$ , 287.9  $\mu\text{g/ml}$ , 531.5  $\mu\text{g/ml}$ , respectively. While their mean  $\text{IC}_{50}$ s against N1E-115 cells were 382.7  $\mu\text{g/ml}$ , 247.3  $\mu\text{g/ml}$ , 145.9  $\mu\text{g/ml}$ , 262.4  $\mu\text{g/ml}$ , respectively (Table 1).

The activities of different types of Egyptian plant extracts were evaluated against the growth of *T. gondii* and *N. caninum* *in vitro*. Among the twelve plant extracts tested, four extracts, from *A. judaica*, *C. droserifolia*, *T. africanum*, and *V. tortilis*, showed potent activity against RH-GFP growth *in vitro* with mean  $\text{IC}_{50}$ s of 2.1, 12.5, 21.8, and 24.5  $\mu\text{g/ml}$ , respectively. Moreover, their mean selectivity index (SI) values against HFF cells were 150.8, 29.6, 18.9, and 22.6, respectively (Table 1). The mean SI values of the previously mentioned extracts against 293T cells were 164.8, 21.0, 13.2, and 21.6, respectively (Table 1). Furthermore, Their SI values against N1E-115 cells were 182.2, 19.8, 6.7, and 10.7, respectively (Table 1).

Plant extracts showing partial efficacy against *T. gondii* tachyzoites were an aqueous extract from *Calotropis procera*, an ethanolic extract from *P. undulata*, an ethanolic extract from *O. basilicum*, a methanolic extract from *P. undulata*, and a methanolic extract from *F. tenacissima*, their mean  $\text{IC}_{50}$ s were 7.2  $\mu\text{g/ml}$ , 15.4  $\mu\text{g/ml}$ , 46.6  $\mu\text{g/ml}$ , 46.9  $\mu\text{g/ml}$ , and 64.5  $\mu\text{g/ml}$ , respectively. Moreover, their mean SI values were 5.7, 3.6, 5.4, 4.2, and 8.0, respectively (Table 1). The methanolic extract from *C. procera* exhibited weak efficacy against *T. gondii* *in vitro* with a mean  $\text{IC}_{50}$  of 1.9  $\mu\text{g/ml}$  and a mean SI of 1.5, as it exhibited high cytotoxicity against HFF cells with a mean  $\text{IC}_{50}$  of 2.9  $\mu\text{g/ml}$ . We considered the extracts from *A. javanica*, *A. setifera*, and *O. baccatus*, along with both methanolic and ethanolic extracts of *Citrullus colocynthis*, to have no efficacy against the *in vitro* growth of *T. gondii* as the  $\text{IC}_{50}$ s were  $>100$   $\mu\text{g/ml}$  (Table 1).

Extracts with high SI values ( $> 10$ ) were further analyzed for their anti-*Toxoplasma* effects by examining the intensity of fluorescence of RH-GFP after 72 hr of treatment. *A. judaica*, *C. droserifolia*, *T. africanum*, and *V. tortilis* inhibited RH-GFP signal growth at a concentration of 100  $\mu\text{g/ml}$  (Fig. 1A) when compared with the untreated parasites, and more than sulfadiazine did when tested at a concentration of 1 mg/ml.

To examine the effect of each plant extract on extracellular *T. gondii*, purified parasites were pre-treated extracellularly with 100  $\mu\text{g/ml}$  of each extract (Fig. 1B). The mean parasite infection rate values after 24 hr of pre-treatment with *A. judaica*, *C. droserifolia*, *T. africanum*, and *V. tortilis* were 6.7%, 6.8%, 9.6%, and 10.6%, respectively, which were significantly lower than the mean parasite infection rate of the untreated parasites (29.1%) (Fig. 1B).

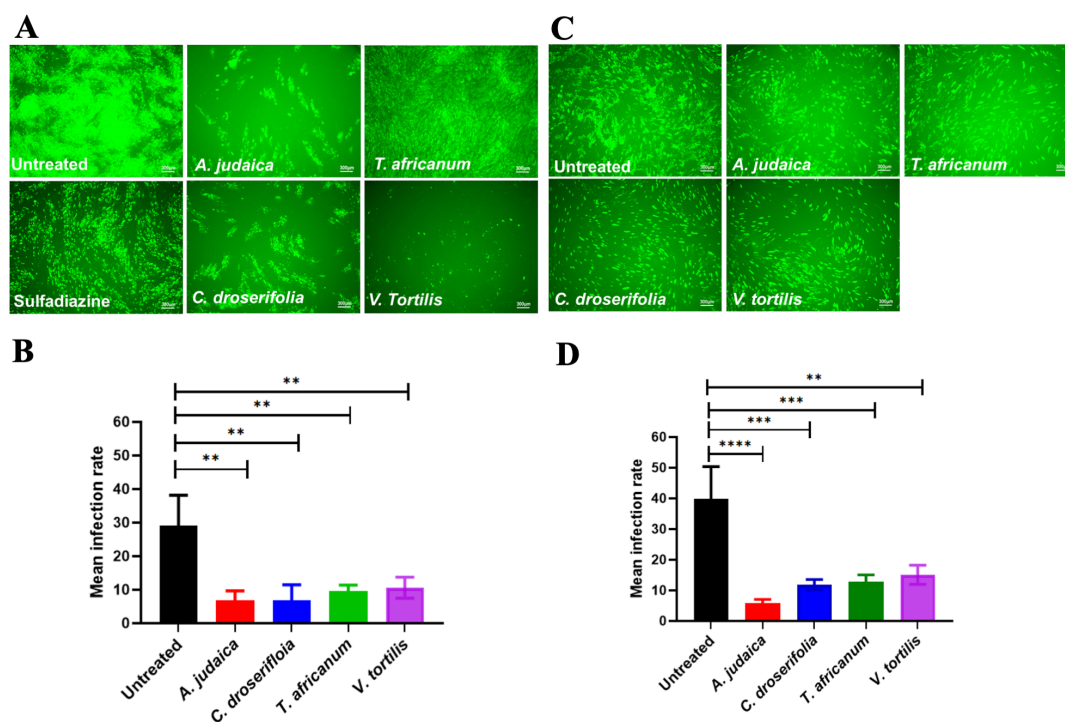
Plant extracts with potent and partial activities against RH-GFP growth were evaluated against the growth of *N. caninum* (Nc1-GFP) tachyzoites *in vitro* since this parasite is closely related to *T. gondii*. The results showed that *C. droserifolia*, *T. africanum*, *A. judaica*, and *V. tortilis* exhibited potent efficacy against the growth of Nc1-GFP with mean  $\text{IC}_{50}$ s of 1.0  $\mu\text{g/ml}$ , 3.1  $\mu\text{g/ml}$ , 8.6  $\mu\text{g/ml}$ , and 17.2  $\mu\text{g/ml}$ , respectively. In addition, their mean SI values were 370.9, 133.2, 36.8, and 32.2, respectively (Table 1). The growth inhibition of extracts from *A. judaica*, *C. droserifolia*, *T. africanum*, and *V. tortilis* plants against the growth of Nc1-GFP at a concentration of 100  $\mu\text{g/ml}$  after 72 hr or treatment was shown (Fig. 1C). Moreover, methanolic and aqueous extracts of *C. procera* and an ethanolic extract of *P. undulata* exhibited partial efficacy against Nc1-GFP with mean  $\text{IC}_{50}$ s of 0.9  $\mu\text{g/ml}$ , 1.8  $\mu\text{g/ml}$ , and 3.0  $\mu\text{g/ml}$ , respectively. In addition, their mean SI against HFF cells values were 3.0, 23.0, and 18.5, respectively (Table 1). The mean SI values of the *A. judaica*, *C. droserifolia*, *T. africanum*, and *V. tortilis* extracts against 293T cells were 40.2, 263.4, 92.8, and 30.9, respectively (Table 1). Furthermore, their mean SI values against N1E-115 cells were 44.5, 247.3, 47.0, and 15.2, respectively (Table 1).

The extracts with potent activity were also tested against extracellular *N. caninum*. The mean parasite infection rate values after 24 hr of pre-treatment with *A. judaica*, *C. droserifolia*, *T. africanum*, and *V. tortilis* extracts were 5.9%, 11.8%, 12.9%, and 15.1%, respectively, which were significantly lower than the values from the untreated parasites (39.8%) (Fig. 1D).

Among all the tested plants, *A. judaica* showed the highest efficacy against the growth of *T. gondii* with a mean  $\text{IC}_{50}$  of 2.1  $\mu\text{g/ml}$  (Table 1) and potent efficacy against Nc1-GFP with a mean  $\text{IC}_{50}$  of 8.6  $\mu\text{g/ml}$  (Table 1). The *Artemisia* genus belongs to the plant family Asteraceae. The genus includes about 500 species, many of which are known for their medicinal properties [14], and some plants in this genus have been reported to have medicinal value against protozoan parasites. *A. judaica* has been traditionally used to treat different diseases, recently, its ethanolic extract inhibits the protozoan parasite *Blastocystis* at a concentration range from 250 to 4,000  $\mu\text{g/ml}$  [21]. These doses of *A. judaica* were higher than those used in our present study (100  $\mu\text{g/ml}$ ) against the Apicomplexan parasites. Artemisinin and its derivatives from *A. annua* have been reported to treat many different species of protozoan parasites such as *Leishmania* sp., *Trypanosoma* sp., *T. gondii*, *N. caninum*, *Eimeria tenella*, *Acanthamoeba castellanii*, *Naegleria fowleri*, *Cryptosporidium parvum*, *Giardia lamblia*, and *Babesia* sp. [20]. In our study, *A. judaica* demonstrated potent effects against both *T. gondii* and *N. caninum*-despite a lack of information about the presence of artemisinin in *A. judaica* plant.

*C. droserifolia* is another species with medicinal value widely used traditionally in Egypt and Jordan. It has been reported that an ethanol extract can treat hyperglycemia in diabetic male albino rats [33]. Phytochemical screening studies on *Cleome* species have shown that it contains diverse, beneficial secondary products such as terpenoids, flavonoids, phenolics, and alkaloids, and suggest the use of plants from this genus in pharmacological investigations [2].

Extracted oil from *C. droserifolia* diluted in methanol was reported to have antimicrobial efficacy against Gram-positive bacteria



**Fig. 1.** Effect of plant extracts against *Toxoplasma gondii* (RH-GFP) and *Neospora caninum* (Nc1-GFP) growth *in vitro* (A) Representative images of a green fluorescent protein-expressing RH strain of *T. gondii* (RH-GFP)-infected human foreskin fibroblast cells after treatment with 100 μg/ml of each of the following plant extracts: *Artemisia judaica*, *Trichodesma africanum*, *Cleome droserifolia*, or *Vachellia tortilis*, and sulfadiazine (1 mg/ml), or medium alone, Scale bar=300 μm. (B) Effects of the selected potent plant extracts at 100 μg/ml on extracellular *T. gondii* *in vitro* determined by indirect fluorescent antibody test (IFAT). RH-GFP was pretreated with either one of the four different extracts (*A. judaica*, *T. africanum*, *C. droserifolia*, or *V. tortilis*) (100 μg/ml/plant extract), or EMEM with 8% FBS (1 ml/tube) and Sulfadiazine (1 mg/ml) and the infection rate for RH-GFP was measured. Each bar represents the mean ± SD of three wells per plant sample. The results were representative of two independent experiments of similar data. Statistically significant differences were determined by a one-way ANOVA and a Tukey-Kramer post hoc analysis (\*\* $P < 0.01$ ). (C) Representative images of a green fluorescent protein-expressing Nc1 strain of *N. caninum* (Nc1-GFP)-infected human foreskin fibroblast cells after treatment of 100 μg/ml of either one of the plant extracts (*Artemisia judaica*, *Trichodesma africanum*, *Cleome droserifolia*, or *Vachellia tortilis*) or medium alone, Scale bar=300 μm. (D) Effects of the selected potent plant extracts at 100 μg/ml on extracellular *N. caninum* *in vitro* determined by IFAT. The Nc1-GFP line was pre-treated with one of the four extracts (*A. judaica*, *T. africanum*, *C. droserifolia*, *V. tortilis*), and the infection rate for Nc1-GFP was measured. Each bar represents the mean ± SD of three wells per plant sample. The results were representative of two independent experiments of similar data. Statistically significant differences were determined by a one-way ANOVA and a Tukey-Kramer post hoc analysis (\*\* $P < 0.01$ , \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$ ).

including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Bacillus cereus*, and *Micrococcus luteus* with minimum inhibitory concentrations (MICs) of 23.7, 28.7, 36.0, 14.7, and 35.3 μg/ml, respectively. These results demonstrate that *C. droserifolia* may have components that can kill pathogenic microorganisms and suggest the need for future therapeutic investigations of *Cleome* species [23]. In our study, we demonstrated the same results from *Cleome* leaves. This exhibited potent efficacy against both *T. gondii* and *N. caninum* using lower concentrations than in the previously mentioned study [1], with mean IC<sub>50</sub>s of 12.5 and 1.0 μg/ml, respectively (Table 1).

A 100 mg/ml concentration of the methanolic extract of *T. africanum* was previously reported to have an antibacterial effect against Gram-negative bacteria such as *Proteus vulgaris*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and *S. aureus* and Gram-positive bacteria and fungi such as *Bacillus cereus*, *Salmonella typhi*, and *Candida albicans* with mean inhibition zones of 13.5, 11.5, and 17.5 mm, respectively [1]. In our study, *T. africanum* was tested against two different protozoal parasites at lower concentrations starting from 100 μg/ml, and its mean IC<sub>50</sub> against *T. gondii* and *N. caninum* was 21.8 and 3.1 μg/ml, respectively (Table 1).

Our data reveal that different wild plant extracts collected from Egypt with low or no cytotoxicity demonstrate potent activity against the growth of both *T. gondii* and *N. caninum* tachyzoites *in vitro*. These plants may be useful in the development of medications used to treat toxoplasmosis and neosporosis in the future. We did not characterize the main active components responsible for their efficacy which is a limitation of our study. Because rare plants were not used in the study, we plan to characterize and identify the main active components of the potent crude extracts by spectroscopic methods, including nuclear magnetic resonance and high-resolution mass spectrometer. In the future study, we will try to isolate the active components and evaluate their efficacies *in vivo* against both parasites in subsequent studies.

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

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