Parasitol Res (2012) 110:1985–1992
DOI 10.1007/s00436-011-2727-3

ORIGINAL PAPER

In vitro amoebicidal activity of ethanol extracts of *Arachis hypogaea* L., *Curcuma longa* L. and *Pancratium maritimum* L. on *Acanthamoeba castellanii* cysts

Nagwa Mostafa El-Sayed · Khadiga Ahmed Ismail · Sabah Abd-El-Ghany Ahmed · Mona Hafez Hetta

Received: 18 October 2011 / Accepted: 18 November 2011 / Published online: 7 December 2011

Abstract *Acanthamoeba castellanii* causes amoebic keratitis which is a painful sight-threatening disease of the eyes. Its eradication is difficult because the amoebas encyst making it highly resistant to anti-amoebic drugs, but several medicinal plants have proven to be more effective than the usual therapy. This study aimed to evaluate the in vitro amoebicidal activity of ethanol extracts of *Arachis hypogaea* L. (peanut), *Curcuma longa* L. (turmeric), and *Pancratium maritimum* L. (sea daffodil) on *A. castellanii* cysts. *Acanthamoeba* were isolated from keratitic patients, cultivated on 1.5% non-nutrient agar, and then incubated with different concentrations of plant extracts which were further evaluated for their cysticidal activity. The results showed that all extracts had significant inhibitory effect on the multiplication of *Acanthamoeba* cysts as compared to the drug control (chlorhexidine) and non-treated control, and the inhibition was time and dose dependent. The ethanol extract of *A. hypogaea* had a remarkable cysticidal effect with minimal inhibitory concentration (MIC) of 100 mg/ml in all incubation periods, while the concentrations of 10 and 1 mg/ml were able to completely inhibit growth after 48 and 72 h, respectively. The concentrations 0.1 and 0.01 mg/ml failed to completely inhibit the cyst growth, but showed growth reduction by 64.4–82.6% in all incubation periods. *C. longa* had a MIC of 1 g and 100 mg/ml after 48 and 72 h, respectively, while the concentrations 10, 1, and 0.1 mg/ml caused growth reduction by 60–90.3% in all incubation periods. *P. maritimum* had a MIC of 200 mg/ml after 72 h, while the 20-, 2-, 0.2-, and 0.02-mg/ml concentrations showed growth reduction by 34–94.3% in all incubation periods. All extracts seemed to be more effective than chlorhexidine which caused only growth reduction by 55.3–80.2% in all incubation periods and failed to completely inhibit the cyst growth. In conclusion, ethanol extracts of *A. hypogaea*, *C. longa*, and *P. maritimum* could be considered a new natural agent against the *Acanthamoeba* cyst.

N. M. El-Sayed
Parasitology Department, Research Institute of Ophthalmology, Giza, Egypt
e-mail: nagelsaka@yahoo.com

K. A. Ismail · S. A. Ahmed (✉)
Parasitology Department, Faculty of Medicine, Ain Shams University, Abbassia, Cairo, Egypt
e-mail: dr.paraped@yahoo.com

K. A. Ismail
e-mail: khadigaahmed68@yahoo.com

M. H. Hetta
Pharmacognosy Department, Faculty of Pharmacy, Beni Suef University, Beni Suef, Egypt
e-mail: monahetta@yahoo.com

Introduction

Acanthamoeba castellanii is a facultative pathogen that has a two-stage life cycle, the vegetatively growing trophozoite stage and the dormant cyst stage (Marciano-Cabral and Cabral 2003). It is the causative agent of *Acanthamoeba* keratitis, a painful sight-threatening disease of the eyes, and granulomatous amoebic encephalitis, a fatal disease of the central nervous system (Martinez and Janitschke 1985). Moreover, it has been recognized as an opportunistic human pathogen capable of causing infections in both immunocompetent and immunocompromised individuals (Torno et al. 2000). *Acanthamoeba* keratitis can result from corneal trauma or the use of improperly or poorly maintained contact lenses. The increasing number of

contact lens users enhances the frequency of the illness because the trauma and hypoxia of the corneal epithelium facilitate the invasion of the parasite into the stroma. Furthermore, inadequate asepsis leads to contamination by bacteria and fungi, producing a favorable culture medium for the growth of this protozoan (Obeid et al. 2003).

Successful treatments have been reported with the use of a combination of cationic antiseptics (polyhexamethylene biguanide, chlorhexidine) which inhibit the membrane functions, aromatic diamidines (propamidine isethionate, hexamidine, pentamidine) which inhibit DNA synthesis, aminoglycosides (neomycin, paromomycin) which inhibit protein synthesis, and imidazoles (clotrimazole, fluconazole, ketoconazole, miconazole, itraconazole) which destabilize cell walls and polyenes, such as amphotericin B (Gautom et al. 1998). However, eradication of *Acanthamoeba* from the infection site is difficult because under adverse conditions, the amoebas encyst and medical therapy is often less effective against cysts than trophozoites due to the rigid double-layered wall of the cysts which makes it highly resistant to anti-amoebic drugs. This is problematic as cysts can survive after initial successful chemotherapeutic treatment and cause relapse of the disease (Leitsch et al. 2010). In addition, the risk of drug resistance and frequent development of undesirable side effects are major limitations (Wilson 1991).

Therefore, it is important to develop more active and dynamic therapies that facilitate the continuance of the treatment by the patients. In this context, the investigation of plants used by traditional medicine is a strategy for finding alternative treatment (Brantner and Grein 1994). Antiparasitic properties of many new natural product groups have been identified with their surprising efficacy and selectivity such as plant-derived alkaloids, terpenes, and phenolics (Kayser et al. 2003). Several substances obtained from plants have been studied for the amoebicidal activity against *Acanthamoeba*, and many of these compounds have proven to be more effective than the currently used therapy (Polat et al. 2007a, 2008; Goze et al. 2009).

Curcuma longa L. (turmeric), a perennial herb, is a member of the family Zingiberaceae (ginger); it is cultivated extensively in India, China, and other countries with a tropical climate. Turmeric is used as a food additive (spice) and preservative. Curcumin which is the main bioactive component of turmeric exhibits a great variety of pharmacological activities: anti-protozoal, anti-inflammatory, antioxidant, anti-carcinogenic, anti-mutagenic, anti-coagulant, anti-fertility, anti-diabetic, anti-bacterial, anti-fungal, antiviral, anti-fibrotic, anti-venom, anti-ulcer, hypotensive, and hypocholesteremic activities (Ishita et al. 2004). Some authors have studied the antiparasitic properties of *C. longa* L. against the tropical parasites *Plasmodium*, *Leishmania*, *Trypanosoma*, *Schistosoma*, and, more generally, against other cosmopolitan parasites: nematodes, *Babesia*, *Giardia*, *Coccidia*, and *Sarcoptes* (Haddad et al. 2011).

Arachis hypogaea L. (peanut) is a member of the family Leguminosae which is distributed in the tropics and moderate regions. It is a dietary source, capable of producing stilbene-derived compounds that are considered anti-fungal. In addition, peanut stilbenoids display a diverse range of biological activities in mammalian cells including anti-inflammatory, antioxidant activities and anti-nitric oxide production (Sobolev et al. 2011). Also, flavonoids, which have been identified as a biologically active compound in this plant, were reported to have anti-cancer, anti-androgen, anti-*Leishmania*, anti-nitric oxide production, and anti-bacterial activity (Yazaki et al. 2009). Moreover, it was found that *A. hypogaea* L. exhibited anti-bacterial activity (Parekh and Chanda 2008).

Pancratium maritimum L. (sea daffodil) is a genus of flowering plants in the family Amaryllidaceae. Alkaloids are the main active constituents in this plant (Berkov et al. 2004) which exhibited anti-fungal, anti-malarial, and cytotoxic activities (Sür-Altiner et al. 1999; Sener et al. 2003; Kaya et al. 2010). Pancratistatin, which is the most important metabolite responsible for the therapeutic benefits of this plant, has been shown to have anti-viral, anti-neoplastic (Pandey et al. 2005), and antiparasitic effect against *Encephalitozoon intestinalis*, a microsporidium causing intestinal and systemic infection in immunocompromised patients (Ouarzane-Amara et al. 2001).

Successive isolation of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The traditional healers use primarily water as the solvent, but Parekh and Chanda (2007) found that plant extracts including *A. hypogaea* L. prepared with methanol and ethanol as solvents provided more consistent antimicrobial activity. Also, Ishita et al. (2004) reported that curcumin is soluble in ethanol, alkali, ketone, acetic acid, and chloroform and that the ethanol extract of the rhizomes was reported to have anti-amoebic activity against *Entamoeba histolytica*. In addition, Kaya et al. (2010) found that the ethanolic extract of the *P. maritimum* bulbs showed significant cytotoxic activity than n-hexane, ethyl acetate, and aqueous extracts. Phytochemical screening of ethanol extracts of *A. hypogaea* L., *C. longa* L., and *P. maritimum* resulted in the isolation of alkaloids and flavonoids which may contribute to their cytotoxic activity (Yazaki et al. 2009; Ishita et al. 2004; Berkov et al. 2004).

The aim of the present study was to evaluate the in vitro amoebicidal activity of ethanol extracts of *A. hypogaea* L., *C. longa* L., and *P. maritimum* L. on *A. castellanii* cysts.

Materials and methods

Plant materials and extraction procedure

Three medicinal plants, *A. hypogaea* L. (peanut), *C. longa* L. (turmeric), and *P. maritimum* L. (sea daffodil) were used

in this study (Figs. 1, 2, and 3). The selection of these plants was made on the basis of information gathered about their use in the traditional medicine system. The plant materials were collected from different places in Egypt and deposited in the Pharmacognosy Department, Faculty of Pharmacy, Beni Suef University. Shells (pods) that were freed from seeds of *A. hypogaea* L., rhizomes of *C. longa*, and bulbs of *P. maritimum* L. were used in the preparation of extracts. Collected plant materials were dried in the shade, ground into powdered form, and extracted in a Soxhlet apparatus with ethanol at 60°C for 6 h. Then the extracts were collected, centrifuged at 3,000 rpm for 20 min, filtrated through active charcoal, and concentrated in vacuo at 45°C, yielding a waxy material. The residues obtained were stored in a freezer until use (Lin et al. 1999).

Acanthamoeba isolation

Corneal scrapings were collected from keratitic patients attending the corneal outpatient clinic of the Research Institute of Ophthalmology (RIO), Giza, Egypt, where *Acanthamoeba* isolation and testing of the three plants' cysticidal activity were performed in the Parasitology Department of RIO, Giza, Egypt, and the Diagnostic and Research Laboratory of Parasitic Diseases, Parasitology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt. The specimens were inoculated directly onto the surface of 1.5% non-nutrient agar (NNA) plates seeded with *Escherichia coli* bacterial suspension and incubated in a humidified chamber at 30°C (Init et al. 2010). The presence of *Acanthamoeba* could be seen by the clear tracks on the *E. coli* lawn NNA produced by the feeding trophozoites of *Acanthamoeba*. Examination of the agar plate surface for the presence of amoebic growth was carried out daily for up to 7 days with light and inverted microscopes using a ×40 objective. *Acanthamoeba* was identified by the specific morphology of cyst and trophozoite. Subcultures were done after 2 weeks from positive cultures with confirmed amoebic growth by cutting a small square of agar using a sterile scalpel and placing it upside down on new NNA-*E. coli* plates. The plates were incubated in humidified chambers at 30°C and examined after 24 h. Performing sub-culturing several times facilitated the isolation of *Acanthamoeba*. *Acanthamoeba* cysts were collected from 3-week cultures. The agar surfaces were flooded with 5 ml of phosphate-buffered saline (PBS) and were gently scraped with an inoculating loop. Cysts were harvested from the suspension by centrifugation at 350×*g* for 10 min. The supernatant was aspirated, and the sediment was washed twice in PBS in order to eliminate most of the bacteria. Cysts in the resultant suspension were counted with a hemocytometer, and the suspension was standardized to be 25×10^4/ml (Perrine et al. 1995).

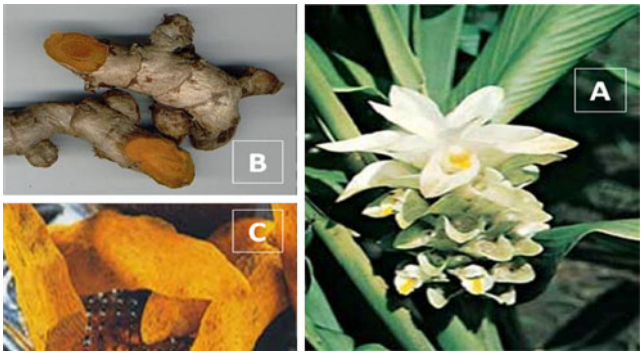

Fig. 2 *C. longa* plant (**a**) and its rhizome (**b**, **c**)

Experimental design

In order to evaluate the in vitro amoebicidal activity of ethanol extracts of *A. hypogaea* L., *C. longa* L., and *P. maritimum* L. on *A. castellanii* cysts, amoebae were incubated with different concentrations of *A. hypogaea* L.(100, 10, 1, 0.1, 0.01 mg/ml), *C. longa* L.(1,000, 100, 10, 1, 0.1 mg/ml), and *P. maritimum* L. (200, 20, 2, 0.2, 0.02 mg/ml), for different incubation periods (24, 48, and 72 h). One hundred microliters (100 μl) of the calibrated cyst suspension (25×10^4/ml) was inoculated into each well of a 96-well plate, and then the plate was left for 30 min to

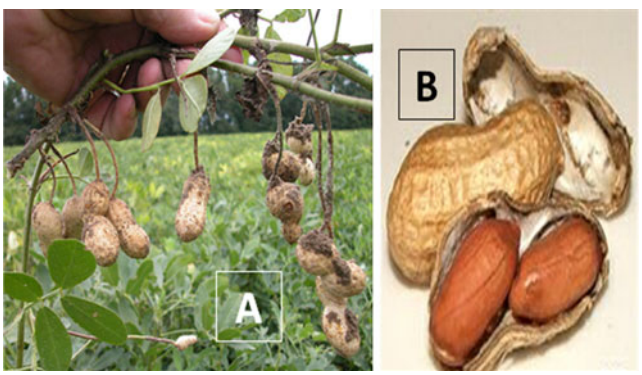

Fig. 1 *A. hypogaea* plant (**a**) and shells freed from seeds (**b**)

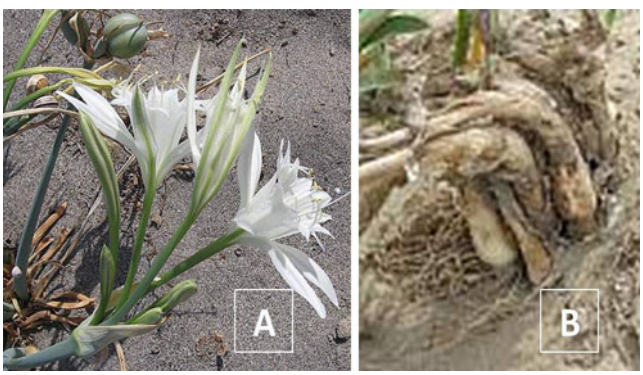

Fig. 3 *P. maritimum* plant (**a**) and its bulb (**b**)

avoid disturbance of the adherence of amoebae onto the wells' surface. Then, the PBS solution was removed, and 100 μl of each concentration of the plant extracts was added into the wells. The plate was sealed and incubated at 30°C for different incubation periods. In addition, controls containing only the parasite in PBS as a non-treated control and parasite plus 0.02% chlorhexidine gluconate (prepared from a solution 20% in H_2O CHX, C-9394; Sigma) as a reference drug control were submitted to the same procedure. Each experiment was performed in triplicate. After each incubation period, 100 μl from each test and control well was transferred into 100 μl of 0.3% basic methylene blue media. Unstained (viable) and stained (nonviable) parasites were enumerated in the hemocytometer, 10 min after stain addition. For cultures containing no viable cysts, an additional test was performed to confirm the results obtained. To evaluate their viability, it was inoculated onto NNA-*E.coli* plate, incubated at 30°C for an additional 72 h, and examined to detect any viable cysts or trophozoites (Polat et al. 2008).

Evaluation of the drug efficacy was done by:

- Counting the number of trophozoites using the hemocytometer after each period of incubation
- Calculation of the percent of growth reduction according to the equation (Palmas et al. 1984)

$$\text{Percent of growth reduction} = a - b/a \times 100$$

where,

a is the mean number of trophozoites/cysts in control cultures

b is the mean number of trophozoites/cysts in drug-treated cultures

- Determination of the minimal inhibitory concentration (MIC) as the lowest concentration of the tested plant extracts and chlorhexidine 0.02% in which no viable organism was observed (Meingasser and Thurner 1979)

Statistical analysis

Data were presented as the mean ± SD of triplicate determinations and percent of growth inhibition. The means were analyzed by one-way ANOVA followed by Student's *t* test, and the difference was considered significant when the *p* value was <0.05 and highly significant when the *p* value was <0.001.

Ethical consideration

An informed consent was taken from all the patients after explaining the aim of the study to them. The study was approved by the Research Ethics Committee, Faculty of Medicine, Ain Shams University.

Results

The results of the present study are shown in Tables 1, 2, and 3 and Figs. 4, 5, and 6.

Discussion

Acanthamoeba keratitis is a severe, potentially sight-threatening ocular infection characterized by progressive corneal inflammation and ulceration, and if it is not

Table 1 Effect of *A. hypogaea* ethanol extract on the in vitro growth of *A. castellanii* cysts for different incubation periods

Dosage of treatment	Duration of treatment (hours)					
	24 h		48 h		72 h	
	Mean±SD	% of growth inhibition	Mean±SD	% of growth inhibition	Mean±SD	% of growth inhibition
Non-treated control	25.3±4.2	0	25±3	0	23±6.2	0
Chlorhexidine 0.02% (drug control)	11.3±3.2	55.3	8.7±2.1	65.6	4.7±1.5	79.6
A. hypogaea, 100 mg/ml	0**,***	100	0**,***	100	0*,***	100
A. hypogaea, 10 mg/ml	1.3±1.5*,***	94.9	0**,***	100	0*,***	100
A. hypogaea, 1 mg/ml	3.7±1.5*,***	85.8	1.7±1.5**,***	93.2	0*,***	100
A. hypogaea, 0.1 mg/ml	6.3±1.5*	75.1	5±2.6*	80	4±1*	82.6
A. hypogaea, 0.01 mg/ml	9±1*	64.4	6.3±2.1*	74.8	4.3±2.1*	81.3

*p<0.05, statistically significant difference in comparison to non-treated control in the same time interval; **p<0.001, statistically highly significant difference in comparison to non-treated control in the same time interval; ***p<0.05, statistically significant difference in comparison to drug control in the same time interval

Table 2 Effect of *C. longa* ethanol extract on the in vitro growth of *A. castellanii* cysts for different incubation periods

Dosage of treatment	Duration of treatment (hours)					
	24 h		48 h		72 h	
	Mean±SD	% of growth inhibition	Mean±SD	% of growth inhibition	Mean±SD	% of growth inhibition
Non-treated culture control	25±5	0	24±1	0	23.7±3.5	0
Chlorhexidine 0.02% (drug control)	11±3.6	56	8.7±1.5	64.2	4.7±2.1	80.2
C. longa, 1 g/ml	1.3±1.5*,***	94.8	0**,***	100	0**,***	100
C. longa, 100 mg/ml	3±2*,***	88	1.7±1.5**,***	93	0**,***	100
C. longa, 10 mg/ml	5.3±3*	78.8	3.7±2.5**,***	84.8	2.3±2.1*	90.3
C. longa, 1 mg/ml	8.3±3.5*	66.8	6.3±3*	74.1	4±2*	83.1
C. longa, 0.1 mg	10±4*	60	9±4*	63	7.7±3.5*	67.5

*$p<0.05$, statistically significant difference in comparison to non-treated control in the same time interval; **$p<0.001$, statistically highly significant difference in comparison to non-treated control in the same time interval; ***$p<0.05$, statistically significant difference in comparison to drug control in the same time interval

diagnosed early and treated aggressively, the corneal epithelium becomes ulcerated with stromal infiltration, leading to perforation, ring infiltrate, and finally, loss of vision (Marciano-Cabral and Cabral 2003). The high failure rate of medication may be partially due to poor absorption of topical anti-amoebic drugs by the thickened sclera (Hirano and Sai 1999) or the ineffectiveness of these drugs in killing the highly resistant cysts and recurrence in premature stopping of treatment (Kumar and Lioyd 2002). Chlorhexidine at 0.02% concentration is a good drug of choice in the initial therapy of *Acanthamoeba* keratitis. It acts by the binding of its highly charged positive molecules to the mucopolysaccharide plug of the ostiole, resulting in penetration through it to the internalized amoeba, where they bind to the phospholipid bilayer of the cell membrane of the internalized amoeba. This results in membrane damage with irreversible loss of calcium and cell electrolytes from the cytoplasm causing cell lysis and death (Seal et al. 2003). However, it is difficult to believe that a chemical destroying the membrane of the amoeba should not at the same time affect the plasma membranes of the ocular cells, where the epithelium ulcerates, the keratocytes disappear, and the endothelium does not function properly. With continued medical treatment, the iris cells die, the lens cells die, and cataract develops (Ehlers and Hjortdal 2004).

Medicinal plants as *Thymus* (Polat et al. 2007a), *Salvia staminea* (Goze et al. 2009), *Ipomoea* sp., *Kaempferia galanga*, *Cananga odorata* (Chu et al. 1998), *Teucrium polium*, *Teucrium chamaedrys* (Tepe et al. 2011a, b), *Pastinaca armenea*, *Inula oculus-christi* (Degerli et al. 2011),

Table 3 Effect of *P. maritimum* ethanol extract on the in vitro growth of *A. castellanii* cysts for different incubation periods

Dosage of treatment	Duration of treatment (hours)					
	24 h		48 h		72 h	
	Mean±SD	% of growth inhibition	Mean±SD	% of growth inhibition	Mean±SD	% of growth inhibition
Non-treated culture control	24.7±3	0	24±3	0	22.7±3	0
Chlorhexidine 0.02% (drug control)	11±4	55.5	8.7±2.1	63.8	4.7±2.1	80
P. maritimum, 200 mg/ml	4±2.6*	83.8	1.3±0.6**,***	94.6	0**,***	100
P. maritimum, 20 mg/ml	6.7±3*	72.9	3±1**,***	87.5	1.3±1.5**	94.3
P. maritimum, 2 mg/ml	10±4.6*	59.5	6±1*	75	3.3±1.5*	85.5
P. maritimum, 0.2 mg/ml	12.7±5*	49	8.7±1.1*	64.2	5.7±2.1*	74.8
P. maritimum, 0.02 mg/ml	16.3±6	34	12±2.6*	50	9±2.6*	60.4

*$p<0.05$, statistically significant difference in comparison to non-treated control in the same time interval; **$p<0.001$, statistically highly significant difference in comparison to non-treated control in the same time interval; ***$p<0.05$, statistically significant difference in comparison to drug control in the same time interval

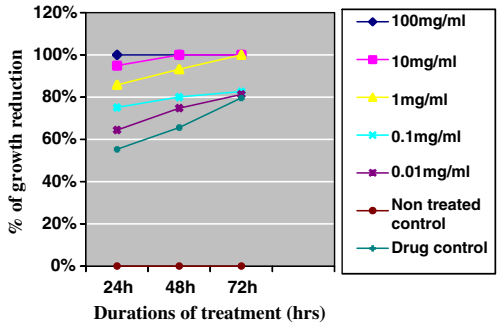

Fig. 4 Percentage of growth reduction of *Acanthamoeba* cysts in culture medium after exposure to different concentrations of *A. hypogaea* for different incubation periods

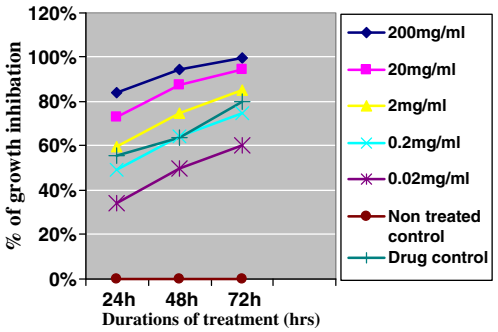

Fig. 6 Percentage of growth reduction of *A. castellanii* cysts in culture medium after exposure to different concentrations of *P. maritimum* for different incubation periods

Peucedanum species (Malatyali et al. 2011), *Rubus chamaemorus*, *Pueraria lobata*, *Solidago virgaurea*, *Solidago graminifolia* (Derda et al. 2009), *Pterocaulon polystachyum* (Ródio et al. 2008), and *Allium sativum* (Polat et al. 2007b, 2008) extracts have proven to be effective growth inhibitors to *Acanthamoeba* than the currently used therapy. From this point of view, our study was carried out to evaluate the in vitro amoebicidal activity of ethanol extracts of *A. hypogaea* L., *C. longa* L., and *P. maritimum* L. on *A. castellanii* cysts in comparison to chlorhexidine gluconate as a commonly used drug control. To our knowledge, this is the first time for these medicinal plants to be investigated for their amoebicidal effect *on A. castellanii* cysts.

Results of the present study demonstrated that the ethanol extract of *A. hypogaea* L. had a remarkable inhibitory effect on growth of *A. castellanii* cysts with MIC of 100, 10, and 1 mg/ml after 24, 48, and 72 h, respectively. Meanwhile, the concentrations 0.1 and 0.01 mg/ml showed growth reduction by 64.4–82.6% in all incubation periods. In comparison, chlorhexidine 0.02% (drug control) showed inhibitory effect on the growth of *A. castellanii* cysts by 55.3–80.2% in all incubation periods. The obtained cysticidal effect of *A. hypogaea* L. in this study may be attributed to quercetin, plant-derived flavonoids which represent a major component responsible for its biological actions (Wang et al. 2008). It has been reported that quercetin had inhibited DNA synthesis and arrested cell cycle progression in *Leishmania donovani* promastigotes, leading to apoptosis (Koide et al. 2002).

Some authors have studied the antiparasitic properties of *C. longa* L. where it exhibited anti-*E. histolytica* (Ishita et al. 2004) and anti-*Toxocara canis* activities (Kiuchi et al. 1993). Also, Haddad et al. (2011) reported that *Curcuma* and its associated bioactive compounds exhibited parasiticidal activity against the tropical parasites *Plasmodium*, *Leishmania*, *Trypanosoma*, *Schistosoma*, and more generally against other cosmopolitan parasites (nematodes, *Babesia*, *Giardia*, *Coccidia*, and *Sarcoptes*). Safety evaluation studies indicated that both *C. longa* L. (turmeric) and curcumin are well tolerated at a very high dose without any toxic effects. Thus, both turmeric and curcumin have the potential for the development of modern medicine for the treatment of various diseases (Ishita et al. 2004).

In the present study, the ethanol extract of *C. longa* L. produced an inhibitory effect on *A. castellanii* cyst growth with MIC of 1 g and 100 mg/ml after 48 and 72 h, respectively. When used in a dose of 10 mg/ml, it failed to cause complete inhibition of *A. castellanii* cyst growth, but showed maximal inhibition by 90.2% after 72 h, while 1- and 0.1-mg/ml concentrations showed growth reduction by 60–83.1% in all incubation periods. In agreement with these results, curcumin had in vitro anti-cryptosporidial activity (>95% inhibition of parasite growth) at 50 μM after 24 h (Shahiduzzaman et al. 2009). Also, curcumin inhibited chloroquine-resistant *Plasmodium falciparum* growth in culture in a dose-dependent manner with 50% inhibitory concentration (IC_{50}) of ~5 μM (Reddy et al. 2005). The activity of curcumin against promastigotes (extracellular) and amastigotes (intracellular) forms of *Leishmania amazonensis* had an excellent inhibitory activity with IC_{50} of 24 μM or 9 mg/ml (Araújo et al. 1999). In addition, Peret-Almeida et al. (2008) found that the essential oil of *C. longa* L. inhibited the growth of several bacteria, and its effectiveness was comparable to that of the traditional antibiotics chloramphenicol and amphotericin.

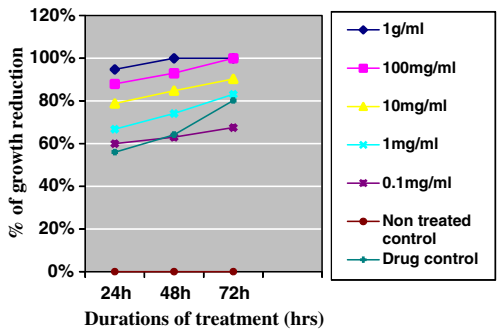

Fig. 5 Percentage of growth reduction of *Acanthamoeba* cysts in culture medium after exposure to different concentrations of *C. longa* for different incubation periods

The cysticidal effect of *C. longa* L. obtained in this study may be attributed to the curcumin which is a major component responsible for its biological actions (Ishita et al. 2004), and its possible mechanism of action as antiparasitic agent has been suggested from several studies. It has been reported that curcumin treatment at a total dose of 400 mg/kg body weight had modulated cellular and humoral immune responses of infected mice leading to a significant reduction of parasite burden and liver pathology in acute murine schistosomiasis *mansoni* (Allam 2009). Also, Cui et al. (2007) explained that cytotoxic effect of curcumin on malaria parasite (*P. falciparum*) resulted from damage of both its mitochondrial and nuclear DNA. In addition, Pérez-Arriaga et al. (2006) concluded that curcumin exhibited a cytotoxic effect in *Giardia lamblia*, inhibited the parasite growth and adherent capacity, induced morphological alterations due to protrusions formed under the cytoplasmic membrane, deformation due to swelling and cell agglutination, and provoked apoptosis-like changes. These previous activities may be involved in the amoebicidal activity of *C. longa* L. against *A. castellanii* cyst.

Regarding the inhibitory effect of *P. maritimum* L. extract on *A. castellanii cyst* growth, the results showed a MIC of 200 mg/ml after 72 h, while the 20- and 2-mg/ml concentrations showed maximal growth reduction by 94.3% and 85.5%, respectively, after 72 h. But, its lower concentrations of 0.2 and 0.02 mg/ml showed growth reduction by 34–74.8 in all incubation periods. These results go more or less with Sür-Altiner et al. (1999) who reported that the methanol extract of *P. maritimum* bulbs showed no inhibitory effect against the bacteria investigated, but it proved to be an effective anti-fungal as miconazole which is an anti-amoebic drug that destabilizes cell walls of *Acanthamoeba*. The main active constituents in this plant are alkaloids (Berkov et al. 2004), which exhibited anti-malarial (Sener et al. 2003), anti-viral (Pandey et al. 2005), and anti-tumor activities (Kaya et al. 2010). Of these alkaloids, pancratistatin and 7-deoxynarciclasine that were extracted and purified from *Pancratium littorale*, a member of the family Amaryllidaceae (the same family to which *P. maritimum* belongs), have been reported to inhibit the infection with microsporidium (*E. intestinalis*) without affecting the host cell. Because of the importance of cyclin-dependent kinase and their regulators in the multiplication and development of eukaryotes, these enzymes represent attractive potential targets for antiparasitic chemotherapy. It was found that pancratistatin and 7-deoxynarciclasine are cyclin kinase inhibitors (Ouarzane-Amara et al. 2001). The previous effect may be responsible for the obtained cysticidal activity of *P. maritimum* in this study. In addition, cytotoxic phenolic compounds (phenolic acids and flavonoids) isolated from *P. maritimum* (Youssef et al. 1998) may contribute to the observed activity of its extract.

In conclusion, ethanol extracts of *A. hypogaea* L., *C. longa* L., and *P. maritimum* L. could be considered a new promising natural agent against *Acanthamoeba* cyst. Further in vivo and in vitro studies will be needed to evaluate, standardize the doses of these natural products, and confirm the efficiency of their biological effects.

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