A Review of the Current Research Trends in the Application of Medicinal Plants as a Source for Novel Therapeutic Agents Against Acanthamoeba Infections

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Abstract

Acanthamoeba keratitis (AK) is a sight-threatening infection of the cornea that mostly affects contact lens wearers. Until now, AK treatment remains very difficult due to the existence of a highly resistant cyst stage in the life cycle of Acanthamoeba which is extremely resistant to most of the available anti-amoebic compounds. Moreover, current treatment of AK is usually based in the combination of various therapeutic agents such as polyhexamethylene biguanide or chlorhexidine and propamidine isethionate. However, all the mentioned compounds have also showed toxic side effects on human keratocytes and presented poor cysticidal effect at the concentrations currently used in the established AK treatments.

Nowadays, the elucidation of novel compounds with antimicrobial and anticancer properties from plant and herbs with medicinal properties have encouraged researchers to evaluate plants as a source of new molecules with anti-trophozoite and cysticidal effects.

Thus, in recent years, many natural products have been reported to present potent anti-Acanthamoeba properties with good selectivity and minimal toxic effects. Therefore, the chemical drugs currently used for AK treatment, their drawbacks as well as the current research in medicinal plants as a source of potent anti-Acanthamoeba compounds are described in this review.

Keywords: Medicinal plant; Acanthamoeba infections; Chemical therapy.

Introduction

Acanthamoeba spp. are free-living amoebae with the potential of being opportunistic pathogens for humans and animals. There are two stages in their life cycle: an active trophozoite form and the double-walled highly resistant cyst. Trophozoites inhabit a variety of bacteria-contained niches such as fresh water bodies, hot springs, soil, drinking water, bottled water, dental treatment units, dialysis units, fluids of contact lenses and infected tissue cultures among others (Table 1.) (1). As mentioned before, the Cyst form of Acanthamoeba is highly resistant to a vast range of temperature, pH, and anti-microbial agents (2). Furthermore, this amoebic genus is the causative agent of two severe diseases in humans: Acanthamoeba keratitis which is serious corneal infection that can develop into blindness and usually

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Table 1. Characteristics of *Acanthamoeba* spp. as agents of amoebic encephalitis and amoebic keratitis (Visvesvara et al, 2007).

| Life cycle | Two forms: trophozoite and cyst |
|------------|--------------------------------|
| Morphological Features | Trophozoite: Vesicular nucleus; spine-like pseudopodia projecting from surface; cyst: wall with two layers |
| In vitro cultivation | Axenic, bacterized, and defined media; tissue culture cells; growth at 37 °C (CNS isolates) or 30 °C (keratitis isolates) |
| The most important diseases | **Granulomatous Amoebic Encephalitis (GAE)** | **Amoebic keratitis (AK)** |
| Incubation period | Weeks to months (GAE) | Days (AK) |
| High risk people | Typically Immune-compromised Individuals such as AIDS patients (GAE) | Mainly contact-lens wearers; Low secretory IgA may contribute (AK) |
| Clinical Characteristics | Headache, fever, nausea, vomiting, behavioral changes, stiff neck, lethargy, loss of consciousness, seizures, coma, and death (GAE) | Painful, sight- redness, photophobia, edema (AK) |
| Clinical course | Sub-acute course; acute stage fatal in weeks (GAE) | Penetration of amoebae into cornea; stromal ring due to PMN infiltrate (AK) |
| Laboratory diagnostic methods | Amoeba seen in CSF; Molecular method (GAE) | Not relevant (AK) |
| Neuroimaging (CT and/or MRI) | Presence of space occupying or ring enhancing lesion (GAE) | Corneal scrapings or biopsy; confocal microscopy; PCR (AK) |
| Prevention | Monitoring of environmental sources such as waters, ventilators, air conditioning units (GAE) | Use of anti-acanthamoeba lens solutions; avoiding swimming or bathing with contact lenses (AK) |
| Chemical therapy | Combination of drugs such as ketoconazole, fluconazole, itraconazole, azithromycin, sulfadiazine, amphotericin B, rifampin, voriconazole, and miltefosine (GAE) | Combination chemotherapeutic agents such as polyhexamethylene biguanide, chlorhexidine (AK) |
| Prognosis | Poor; diagnosis is often Post-mortem, only a few patients have survived (GAE) | Good with early diagnosis and proper treatment (AK) |

reported in contact lens wearers, and the fatal Granulomatous Amoebic Encephalitis (GAE) which mostly affects immunocompromised individuals (3, 4). *Acanthamoeba* also may cause other diseases such as cutaneous ulcers, abscesses, arthritis, and/or rhinosinusitis (5).

GAE is a relatively rare disease. Clinical characteristics include headache, fever, nausea, vomiting, behavioral changes, stiff neck, lethargy and increased intracranial pressure. In later stages of the infection also symptoms such as loss of consciousness, seizures, coma, and death have been reported. Approximately more than 150 cases have been reported worldwide (6, 7).

*Acanthamoeba* keratitis (AK) usually manifests in the early stages of infection with inflammation, eye redness, epithelial defects and photophobia, edema and intense pain. Moreover, if it is not diagnosed and treated on time, it may even end in blindness (8). Previous studies in the early to mid-1980 reported an exponential increase in the number of individuals infected with this amoeba (9). This is mainly due to an increased number of soft contact lens wearers and improper use and maintenance of the lenses.
Furthermore, it is worthy to mention that 85% of AK cases are detected in soft contact lens wearers (10, 11). In a more recent study in 2007, AK reported case were estimated to be higher than 3000 (6). Therefore, it is clear that the number of AK reported cases continues to rise worldwide.

Methodology based on search strategy

A systematic review based on database sources such as Medline, PubMed, Scopus and Google scholar was conducted in this study. No restrictions were placed on study date, design or language of publication including all valuable and relevant information containing the keywords Acanthamoeba and therapy. We also referred to the databases of Medline, PubMed, Scopus and Google scholar and the keywords Acanthamoeba and Amoebic Keratitis, and words including treatment, medicinal plants and herbal medicine. Furthermore, information in books associated to Acanthamoeba and treatment strategy was also included as well as abstracts and full articles that were written in English and showed to be relevant to the topic described above. Only reports and studies with minimal relevance were excluded from this study.

Current therapy of Acanthamoeba infection

Chemical treatment and their drawback

Effective treatment of CNS-related infections due to Acanthamoeba has been recorded as a combined treatment, normally started at an early stage of the infection. However, in the later stages of the infection, the majority of therapeutic agents were reported not to be effective (12). Overall, combination chemotherapies were found more successful than single-drug therapies. Therefore, usual therapeutic agents reported so far include a combination of drugs such as ketoconazole, fluconazole, itraconazole, pentamidine isethionate, azithromycin, sulfadiazine, amphotericin B, rifampicin, voriconazole and miltefosine (12). Because of ineffective therapy, GAE is often deadly, thus less than 10 GAE patients have recovered with the application of the drugs mentioned above (13).

Regarding, Acanthamoeba keratitis (AK) treatments reported so far, the combination of chemotherapeutic agents such as polyhexamethylene biguanide, which destroys cell membranes, and propamidine isethionate, which inhibits DNA synthesis (14, 15) is often used. Moreover, chlorhexidine, alone or in combination with other drugs, has also been applied for AK treatment (16, 17). Unfortunately, propamidine is poorly cysticidal and even resistance to this compound has been reported in Acanthamoeba strains (18, 19).

In the case of a persistent infection with inflammation, corticosteroids may be used. However, their use is controversial because they cause suppression of the immunological response of the patient. Moreover, corticosteroids produce inhibition of the processes of encystation and excystation of Acanthamoeba, which could be a cause for the appearance of resistance problems (1). Recent studies have highlighted an association of topical corticosteroids and a diagnostic delay of AK (1, 15, 20).

It is also important to mention that the described combination treatment are normally only active against the trophozoite stage and therefore, Acanthamoeba cysts could remain viable and lead to serious and frequent recurrences of keratitis. Moreover, resistance of the double walled cysts is mainly due to cellulose molecules presented in the inner layer of the cysts. In addition, the majority of drugs mentioned above are highly toxic to human keratocytes. Furthermore, the required treatment duration for the listed drugs is very long and may last up to six months (21, 22).

Overall, the reported and worrying lack of effective chemotherapeutic agents, have urged researchers in this field to search for novel compounds as a high priority for the treatment of Acanthamoeba infections. Thus, there is a raising trend to shift resources from chemical drugs to natural origin compounds (mainly isolated from plants and herbs) (23).

Animal-based natural products

Magainins, are defense peptides with antimicrobial activity that have been described to be secreted by the African clawed frog (Xenopus laevis). These compounds cover the skin of the animal and have been reported to create an exclusive membrane-targeted mechanism of action against pathogenic agents. The reported
mechanism of action involves a change in the ion conductance of membrane barriers. Magainins have been reported to be active against gram-positive and gram-negative bacteria and present anti-viral, anti-fungal and anti-parasitic effects. In the case of *Acanthamoeba*, two of the known magainins so far, MSI-103 and MSI-94 have been reported to induce amoebistatic and amoebicidal effects at concentrations from 20 to 40 µg/mL (24). Further evaluation of these compounds as anti-*Acanthamoeba* agents should be carried out against the cyst stage and also by developing *in-vivo* studies.

**Plant-based treatments**

In recent years, many researchers working on novel therapeutic options against *Acanthamoeba* infections have focused their studies on the application of medicinal plants as a source of novel molecules with higher anti-amoebic activity and lower toxicity representing an alternative method to currently used synthetic molecules. Many plant extracts have been reported in the literature as powerful inhibitors of microbial agents including bacteria, parasites and fungi. In the case of *Acanthamoeba*, various medicinal plants and herbal extracts have been evaluated as sources of amoebicidal agents and even some of the evaluated plants have been proven to be useful for therapeutic options even *in-vivo*. Some of the test plants and herbs until now include:

- Thymus (25), *Satureja cuneifolia* and *Melissa officinalis* (26), *Ipomoea* sp., *Kaempferia galanga*, *Cananga odorata* (27), *Trigonella Foenum Graecum* (28), *Origanum syriacum* and *Origanum laevigatum* (29), *Pouzolzia indica* (30), *Garlic* (33), *Arachis hypogaea L.*, *Curcuma longa L.* and *Pancratium maritimum L.* (34), *Peucedanum*

### Table 2. Several medicinal plants with reported activity against *Acanthamoeba* spp.

| Plant                        | Extract       | Effective Concentration (trophozoite) | Effect time (trophozoite) | Percentages of viable trophozoites | Effective Concentration (cyst) | Effective time (cyst) | Percentages of viable cysts |
|------------------------------|---------------|---------------------------------------|---------------------------|-----------------------------------|-------------------------------|------------------------|---------------------------|
| Thymus siptyleus subsp.      | Methanol      | 32 mg/mL                              | 3 h                       | 0                                 | 32 mg/mL                      | 12 h                   | 0                         |
| *Sipyleus var. Sipyleus*     |               |                                       |                           |                                   |                               |                        |                           |
| *Sipyleus*                   |               |                                       |                           |                                   |                               |                        |                           |
| Satureja cuneifolia          | Methanol      | 32 mg/mL                              | 24 h                      | 0                                 | 32 mg/mL                      | 72 h                   | 53/07                     |
| Melissa officinalis          | Methanol      | 32 mg/mL                              | 72 h                      | 55/07                             | 32 mg/ mL                     | 72 h                   | 70/0                      |
| Trigonella foenum graecum    | Chloroformic  | 10 mg/mL                              | 48 h                      | 0                                 | 10 mg/mL                      | 72 h                   | 0                         |
| *Origanum syriacum*          | Methanol      | 32 mg/mL                              | 3 h                       | 0                                 | 32 mg/mL                      | 24 h                   | 0                         |
| *Helianthemum lippii*        | Ethyl acetate | N/A*                                  | N/A*                      | N/A*                              | 500 mg/mL                     | 72 h                   | 25                        |
| *Arachis hypogaea L.*        | Ethanol       | N/A*                                  | N/A*                      | N/A*                              | 100 mg/mL                     | 24 h                   | 0                         |
| *Curcuma longa L.*           | Ethanol       | N/A*                                  | N/A*                      | N/A*                              | 1 g/ml (MIC)                  | 48 h                   | 0                         |
| *Pancratium maritimum L.*    | Ethanol       | N/A*                                  | N/A*                      | N/A*                              | 200 mg/mL (MIC)               | 72 h                   | 0                         |
| *Inula oculus-christi (L)*   | Aqueous       | 32 mg/mL                              | 24 h                      | 0                                 | 32 mg/mL                      | 72 h                   | 74/7                      |

*N/A* = Not Applicable  
**MIC** = Minimum Inhibitory Concentration.
caucasicum, P. palimbioides, P. chryseum, P. longibraeoleatum (35), Salvia staminea, L. oculus-christi (L) (36), Pterocaulon polystachyum (37), Pastinaca armenea (Fisch. &C.A.Mey.), L. oculus-christi (L) (38), Tunisian olive (39), Propolis (40) and Buddleia cordata (41).

In Table 2, a list of several medicinal plants and herbs with reported amoebicidal and cysticidal effect is included and are described next:

**Thymus sipyleus** subsp. *Sipyleus var. sipyleus*

*In-vitro* effect of methanolic extracts of *Thymus sipyleus* subsp. *Sipyleus var. sipyleus* was tested against *Acanthamoeba* trophozoites (1.0 to 32 mg/mL). The effective activity was observed at 32 mg/mL. However, it is important to mention that this medicinal plant presented no toxicity to human keratocytes even at the highest concentration tested (32 mg/mL). A bio-guided fractionation analysis of *Thymus sipyleus* could help to find the active compounds within this plant against *Acanthamoeba* in the near future (25).

**Allium sativum** (garlic)

The anti-*Acanthamoeba* effects of the methanol extracts of *Allium sativum* (garlic) have been tested against *Acanthamoeba* trophozoites and cysts *in-vitro*. Interestingly, an amoebicidal and cysticidal activity was described for this plant species being dose and time dependent. Moreover, the tested extract was not toxic even at 3.9 mg/mL. Therefore, *Allium sativum* should be further studied in order to elucidate the novel anti-amoebic compounds presented in this plant (33).

**Ziziphus vulgaris** and **Trigonella foenum graecum** (Fenugreek)

Recent research carried out in our laboratories have shown that the aqueous extracts of *Ziziphus vulgaris* and *Trigonella foenum graecum* are active against both the trophozoite and cyst stages of *Acanthamoeba*. In the case of *Trigonella foenum graecum* concentration of 400 mg/mL was able to eliminate trophozoites and cysts when incubated at a concentration of 750 mg/mL, after 24 h in both cases. In comparison, *Ziziphus vulgaris* aqueous extracts were able to eliminate *Acanthamoeba* trophozoites at a concentration of 200 mg/mL and cysts at 500 mg/mL, after 24 h of incubation (unpublished data). It should be mentioned that both plants did not show toxicity when tested on cell culture at the highest evaluated concentrations.

**Arachis hypogaea L., Curcuma longa L. and Pancratium maritimum L.**

The cysticidal activity of *Arachis hypogaea L.*, *Curcuma longa L.* and *Pancratium maritimum L.* was evaluated against *Acanthamoeba castellani* cysts *in vitro*. The obtained results revealed that the ethanol extract of *A. hypogaea L* had a cysticidal effect with a minimal inhibitory concentration (MIC) of 100 mg/mL in all the tested hours (24, 48, 72 h). *Curcuma longa* extracts showed MIC of 1 g/mL at 48 h and 100 mg/mL after 72 h. *Pancratium maritimum L.* also showed a MIC of 200 mg/mL after 72 h (34).

**Origanum syriacum** and **Origanum laevigatum**

*In vitro* evaluation of the amoebicidal activity of methanolic extracts of *Origanum syriacum* and *Origanum laevigatum* against *Acanthamoeba castellanii*, have shown that concentrations of 32 mg/mL of *Origanum syriacum* extracts, were able to eliminate trophozoites after 3 h. Moreover, incubation of cysts with extracts at the same concentration, revealed a cysticidal activity after 24 h. In the case of *O. laevigatum*, anti-trophozoite activity was observed after 72 h of incubation with extracts at a concentration of 16 mg/mL (29).

**Peucedanum caucasicum, P. palimbioides, P. chryseum and P. longibraeoleatum**

Amoebicidal activity of the methanolic extracts of *Peucedanum caucasicum, P. palimbioides, P. chryseum* and *P. longibraeoleatum* has been examined *in-vitro*. The obtained results in this study determined that *P. longibraeoleatum* extracts presented the strongest amoebicidal effect against *Acanthamoeba*. Thus, elimination of *Acanthamoeba* trophozoites and cysts was observed between 24 and 72 h of incubation with
extracts at a concentration of 32 mg/mL (35).

Salvia staminea and Salvia caespitosa

Amoebicidal activity of Salvia species has been evaluated against Acanthamoeba castellani in-vitro. The reported results revealed that S. staminea presented anti-Acanthamoeba effect. Moreover, the methanolic extracts of S. staminea were shown not to be toxic to human cells even at concentrations of 16 mg/mL (36).

Satureja cuneifolia and Melissa officinalis, Ipomoea sp., Kaempferia galanga, Cananga odorata, Solidago virgaurea, Solidago graminifolia, Pouzolzia indica, Ptero caulon polystachyum, Pastinaca armenea (Fisch. &C.A.Mey.), Inula oculus-christi (L.), Tunisian olive tree (Olea Europaea), Propolis and Buddleia cordata.

M. officinalis has been reported to present moderate amoebicidal and cysticidal effects but S. cuneifolia presented the highest effect against trophozoites and cysts of Acanthamoeba (26). Moreover, in another study the effect of the polar and nonpolar extracts of various plants from Southeast Asia was evaluated for their in-vitro amoebicidal activity against different species of Acanthamoeba including A. culbertsoni, A. castellani, and A. polyphaga. The obtained results revealed that of the 200 tested plants, three species/genera (Ipomoea sp., Kaempferia galanga, and Cananga odorata) were active against Acanthamoeba. Furthermore, Gastrochilus panduratum extract had a lytic effect when evaluated against A. polyphaga and amoebistatic effects against A. castellani and A. culbertsoni species (27).

An in-vitro assay developed to evaluate the amoebicidal activity of the chloroformic fraction of Trigonella foenum graecum has also reported this fraction to present anti-Acanthamoebae effects (28). In another study, four fractions of the methanolic extract of Pouzolzia indica were reported to present cysticidal effects (30). The amoebicidal activity of different parts of plants such as flowers, roots and leaves of Rubus chamaemorus, Pueraria lobata, Solidago virgaurea and Solidago graminifolia extracts were examined in-vitro. The tested extracts presented in-vitro and in-vivo against Acanthamoeba. Moreover, these tested extracts presented not toxic effect for the animals used in the in-vivo assay (31).

The ethyl acetate and methanol extracts of Helianthemum lippii (L.) have been reported to present activity against Acanthamoeba castellani cysts being the ethyl acetate extract, the most active extract against Acanthamoeba (32). Ptero caulon polystachyum (hexane fraction) extracts have been reported to eliminate 66%-70% of Acanthamoeba trophozoites after 48-72 h of incubation (37). In the same study, I. oculus showed the strongest amoebicidal effect when compared to Pastinaca armenea (38).

Olive trees have also been reported to be able to inhibit the trophozoite stage of Acanthamoeba castellanii Neff. In this study, the activity of Olive Leaf Extracts (OLE) showed Inhibitory Concentrations of the 50% of the population (IC$_{50}$) ranging from 8.234 μg/mL in the case of the alcoholic mixture of the Dhokkar variety, to 33.661 ± 1.398 μg/mL for the methanolic extract of the toffehi variety (39).

Propolis extracts have also been tested and reported to be cysticidal after incubation of Acanthamoeba cysts with concentrations higher than 15.62 mg/mL at 48 h or longer. Moreover, ethanolic extracts of Propolis have been reported to be active against Acanthamoeba trophozoites and cysts (40).

An in-vitro assay to evaluate the amoebicidal activity of the aqueous and methanolic extracts of Buddleia cordata against 29 strains of free-living amoebae, reported that the aqueous extract was active against 14 amoebic strains whereas the methanolic one was active against 16 strains. Nevertheless, the observed effects induced only amoebistatic effects against the tested strains. Moreover, no cysticidal activity was observed in any extract after 24 h of incubation and at concentrations up to 32 mg/ml(41).

Conclusion

To date, the beneficial effect of herbal medicine in many conditions such as primary dysmenorrhea, patients with diabetes and many more are studied (42).
Acanthamoeba keratitis is a medical challenge for most ophthalmologists. This severe corneal disease is usually treated with combination drugs such as polyhexamethylene biguanide or chlorhexidine and propamidine isethionate (15). Current therapeutic options present toxic side effects to human keratocytes and present null or low cysticidal effect (18).

In summary, many natural products have been reported to present high anti-Acanthamoeba activities in the recent years. Therefore, plants extracts should be considered as a highly important and powerful source for the search of novel anti-Acanthamoeba compounds in the near future.

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References

(1) Lorenzo-Morales J, Martín-Navarro CM, López-Arencibia A, Arnalich-Montiel F, Piñero JE and Valladares B. Acanthamoeba keratitis: an emerging disease gathering importance worldwide? Trends Parasitol. (2013) 29: 181-7.

(2) Arnalich-Montiel F, Jaumandreu L, Leal M, Valladares B and Lorenzo-Morales J. Scleral and intraocular amoebic dissemination in Acanthamoeba keratitis. Cornea. (2013) 32: 1625-7.

(3) Rezaeian M, Farnia Sh, Niyyati M and Rahimi F. Amoebic keratitis in Iran (1997-2007). Iranian J. Parasitol. (2007) 2: 1-6.

(4) Selby D, Chandra RS, Rakusan TA, Loechelt B, Markle B and Visvesvara GS. Amoebic osteomyelitis in a child with acquired immunodeficiency syndrome; a case report. Pediatr. Pathol. Lab. Med. (1998) 18: 89–95.

(5) Lorenzo-Morales J, Marciano-Cabral F, Lindo JF, Visvesvara GS and Maciver SK. Pathogenicity of amoebae. Exp. Parasitol. (2010) 126: 2-3.

(6) Govinda S, Visvesvara GS, Moura H and Schuster FL. Pathogenic and opportunistic free-living amoebae: Acanthamoebas pp, Balamuthia mandrillaris, Naegleriafowleri, and Sappinia didiploidea. Immunol. Med. Microbiol. (2007) 50: 1-26.

(7) Trabelsi H, Dendana F, Sellami A, Sellami H, Cheikhrouhou F, Neji S, Makni F and Ayadi A. Pathogenic free-living amoebae: epidemiology and clinical review. Pathol. Biol. (2012) 60: 399-405.

(8) Niyyati M, Lasjerdi Z, Haghighi A and Nazemalhosseini Mojarad E. Contamination of clinical settings to highly pathogenic Acanthamoeba in Iran. Occupational Health (2012) 2: 8.

(9) Kristin M and Hammersmith KM. Diagnosis and management of Acanthamoeba keratitis. Curr. Opin. Ophthalmol. (2006) 17: 327–31.

(10) Wynter-Allison Z, Lorenzo Morales J, Calder D, Radlein K, Ortega-Rivas A and Lindo JF. Acanthamoeba infection as a cause of severe keratitis in a soft contact lens wearer in Jamaica. Am. J. Trop. Med. Hyg. (2005) 73: 92-4.

(11) Niyyati M, Rahimi F, Lasjerdi Z and Rezaeian M. Potentially pathogenic free-living Amoebae in contact lenses of the asymptomatic contact lens wearers. Iran. J. Parasitol. (2014) 9: 14-9.

(12) Webster D, Umar I, Kolyvas G, Bilbao J, Guiot MC, Duplisea K, Varnstrom Y and Visvesvara GS. Treatment of granulomatous amoebic encephalitis with voriconazole and miltefosine in an immunocompetent soldier. Am. J. Trop. Med. Hyg. (2012) 87: 715–8.

(13) Walochnik J, Aichelburg A, Assadian O, Steuer A, Visvesvara G, Vetter N and Aspöck H. Granulomatous amoebic encephalitis caused by Acanthamoeba amoebae of genotype T4, in a human immunodeficiency virus-negative patient. J. Clin. Microbiol. (2008) 46: 38–40.

(14) Hargrave SL, McCulley JP and Husseini Z. Results of a trial of combined propamidine isethionate and neomycin therapy for Acanthamoeba keratitis. Brolene Study Group. Ophthalmol. (1999) 106: 952–7.

(15) Khojasteh H, Niyyati M, Rezaei S, Mohebali M, Farnia S, Kazemi-Rad E, Roozafooz R, Sianati H, Rezaeian M and Heidari M. Identifying differentially expressed genes in trophozoites and cysts of Acanthamoeba T4 genotype: Implications for developing new treatments for Acanthamoeba keratitis. Eur. J. Protistol. (2014) 51: 34-41.

(16) Kosrirukvongs P, Wanaichiwanawin D and Visvesvara GS. Treatment of Acanthamoeba keratitis with chlorhexidine. Ophthalmol. (1999) 106: 798–802.

(17) Arnalich-Montiel F, Almendral A, Arnalich F,
Valladares B and Lorenzo-Morales J. Mixed *Acanthamoeba* and multidrug-resistant *Achromobacter* xylooxidans in late-onset keratitis after laser in situ keratomileusis. *J. Cataract Refract Surg.* (2012) 38: 1853-6.

(18) Ficker L, Seal D, Warhurst D and Wright P. *Acanthamoeba* keratitis—resistanceto medical therapy. *Eye.* (1990) 4: 835–8.

(19) Lorenzo-Morales J, Martín-Navarro CM, López-Arencibia A, Santana-Morales MA, Afonso-Lehmann RN, Maciver SK, Valladares B and Martínez-Carrero E. Therapeutic potential of a combination of two gene-specific small interfering RNAs against clinical strains of *Acanthamoeba*. *Antimicrob. Agents Chemother.* (2010) 54: 5151-5.

(20) Logar J and Kraut A. *Acanthamoeba* corneal infection in a contact lens wearer. *J. Infect.* (1997) 35: 237-40.

(21) Reinhard T and Sundmacher R. Clinical aspects and therapy of *Acanthamoeba* keratitis. *Ophthalmologe.* (2000) 97: 446–59.

(22) Niyyati M, Lorenzo-Morales J, Rezaie S, Rahimi F, Martín-Navarro CM, Mohembali M, Maghsoud AH, Farnia S, Valladares B and Rezaeian M. First report of a mixed infection due to *Acanthamoeba* genotype T3 and *Fahidiakmpha* in a cosmetic soft contact lens wearer in Iran. *Exp. Parasitol.* (2010) 126: 89-90.

(23) Shinwari ZK. Medicinal plants research in Pakistan. *J. Med. Plan. Res.* (2010) 4: 161-76.

(24) Schuster FL and Levandovsky M. Chemosensory responses of *Acanthamoeba castellanii*: visual analysis of random movement and responses to chemical signals. *J. Eukayot. Microbiol.* (1996) 43: 150-8.

(25) Polat ZA, Tepe B and Vural A. In-vitro effectiveness of *Thymus sibyleus* subsp. *sibyleus* var. *sibyleus* on *Acanthamoeba castellanii* and its cytotoxic potential on corneal cells. *Parasitol. Res.* (2007) 101: 1551-5.

(26) Malatyali E, Tepe B, Degerli S and Berk S. In-vitro amoebicidal activities of *Satureja cuneifolia* and *Melissa officinalis* on *Acanthamoeba castellanii* cysts and trophozoites. *Parasitol. Res.* (2012) 110: 2175–80.

(27) Chu DM, Miles H, Toney D, Ngyuen C and Marciano-Cabral F. Amebicidal activity of plant extracts from **Trigonella Foenum Graecum** and **Pastinaca armenea** (Fisch. & C.A.Mey.) and **Inula oculus-christi** (L.) on *Acanthamoeba castellanii* cysts and trophozoites. *Parasitol. Res.* (2012) 110: 2175–80.

(28) Dodangeh S, Niyyati M, Kamalinejad M. Anti-*Acanthamoeba* Activities of Chloroformic Fractions of *Trigonella Foenum Gracum* (Seed) and Their Cytotoxicity on Mice Macrophage Cell. Noveel Biomed. (2011) 82: 237-46.

(29) Degerli S, Tepe B, Celiksoz B, Berk S and Malatyali E. In-vitro amoebicidal activity of *Origanum syriacum* and *Origanum laevigatum* on *Acanthamoeba castellanii* cysts and trophozoites. *Exp. Parasitol.* (2012) 131: 20-4.

(30) Roongruangchai K, Kummalue T, Sookkua T and Roongruangchai J. Several fractions of *Pouzolzia indica* methanolic extract were lethal to the *Acanthamoeba* cyst: in-vitro study. *Siriraj Med. J.* (2009) 61: 297-300.

(31) Derda M, Hadaš E and Thiem B. Plant extracts as natural amoebicidal agents. *Parasitol. Res.* (2009) 104: 705-8.

(32) Badria FA, Hetta MH, Sarhan RM and El-Din HME. Lethal effects of *Helianthemum lippii* (L.) on *Acanthamoeba castellanicyst* in-vitro. *Korean J. Parasitol.* (2014) 3: 243-9.

(33) Polat ZA, Vural A, Ozan F, Tepe B, Ozcelik S and Cetin A. In vitro evaluation of the amoebicidal activity of garlic (Allium sativum) extract on *Acanthamoeba* castellanii and its cytoxic potential on corneal cells. HYPERLINK “https://www.ncbi.nlm.nih.gov/pubmed/18370873” J Ocul Pharmacol Ther. (2008) 24: 8-14.

(34) El-Sayed IS, Ismail K, Ahmed S and Hetta M. *In-vitro* amoebicidal activity of ethanol extracts of *Arachis hypogaea* L., *Curcuma longa* L. and *Pancuratum maritimum* L. on *Acanthamoeba castellanii* cysts. *Parasitol. Res.* (2011) 110: 1985-92.

(35) Malatyali E, Tepe B, Degerli S, Berk S and Akpulat HA. *In-vitro* amoebicidal activity of four *Puccinellum* species on *Acanthamoeba castellanii* cysts and trophozoites. *Parasitol. Res.* (2012) 110: 167-74.

(36) Goze I, Alim A, Dag S, Tepe B and Polat ZA. *In-vitro* amoebicidal activity of *Salvia staminea* and *Salvia caespitosa* on *Acanthamoeba castellanii* and their cytoxic potentials on corneal cells. *J. Ocul. Pharmacol. Ther.* (2009) 25: 293-8.

(37) Sauter IP, Santos JC, Cibulski MA, Roche PM, Poser GL and Rott MB. *In-vitro* evaluation of the amoebicidal activity of *Pterocaulon polystachyum* (Asteraceae) against trophozoites of *Acanthamoeba castellanii*. *Parasitol. Res.* (2011) 109: 1367-71.

(38) Degerli S, Berk S, Malatyali E and Tepe B. Screening of the *in-vitro* amoebicidal activities of *Pastinaca armenea* (Fisch. & C.A.Mey.) and *Inula oculus-christi* (L.) on *Acanthamoeba castellanii* cysts and trophozoites. *Parasitol. Res.* (2012) 110: 565-70.

(39) Sifaoui I, López-Arencibia A, Martín-Navarro CM, Chammen N, Mejri M, Lorenzo-Morales J, Abderrabba M and Piñero JE. Activity assessment of **Tunisian olive** leaf extracts against the trophozoite stage of *Acanthamoeba castellanii*. *Parasitol. Res.* (2013) 112: 2825-9.

(40) Topalkara A, Vural A, Polat Z, Toker MI, Ariči MK, Ozan F and Cetin A. *In-vitro* amoebicidal activity of **propolis** on *Acanthamoeba castellanii*. *J. Ocul. Pharmacol. Ther.* (2007) 23: 40-5.

(41) Rodriguez-Zaragoza S, Ordaz C, Avila G, Muñoz JL, Arciniegas A and Romo de Vivar A. *In-vitro* evaluation of the amoebicidal activity of *Buddleia cordata* (Loganiaceae, H.B.K.) on several strains of *Acanthamoeba*. *J. Ethnopharmacol.* (1999) 66: 327-34.

(42) Mirabi P, Alamolhoda SH, Esmaeilzadeh S and Mojtab F. Effect of medicinal herbs on primary dysmenorrhoea- a systematic review. *Iran. J. Pharm. Res.* (2014) 13: 757-67.

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