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In vitro amoebicidal activity of *Origanum syriacum* and *Origanum laevigatum* on *Acanthamoeba castellanii* cysts and trophozoites

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ABSTRACT

In some patients, complete treatment of amoebic keratitis is difficult because of the resistance of cysts to therapeutic agents. The aim of this study was to evaluate the *in vitro* amoebicidal activity of methanolic extracts of *Origanum syriacum* and *Origanum laevigatum*. In the presence of methanolic extracts (ranging from 1.0 to 32.0 mg/ml), numbers of the viable *Acanthamoeba castellanii* trophozoites and cysts were decreased. Both extracts showed a time and dose dependent amoebicidal action on the trophozoites and cysts. Of the extracts tested, *O. syriacum* showed the stronger amoebicidal effect on the trophozoites and cysts. In the presence of 32 mg/ml extract, no viable trophozoites were observed within third hour. The extract was also found effective against the cysts within 24th hour. In the case of *O. laevigatum*, no viable trophozoites were observed within 72nd hour at the concentrations of 16 and 32 mg/ml. As expected, cysts were found more resistant to the extracts than the trophozoites.

1. Introduction

Free-living amoebae of the genus *Acanthamoeba* are commonly occurring organisms, and may be found in the soil, air, in both salt or fresh water reservoirs and on every continent, as well as in air-conditioning units, in water mains, showers, sanitary and dental equipment, dialysers, fluids for contact lenses and infected tissue cultures (Visvesvara and Stehr-Green, 1990; De Jonckheere, 1991; Mergeryan, 1991; Szenasi et al., 1998). The first suggestions that these amoebae may cause diseases afflicting humans were made in 1958 (Marciano-Cabral and Cabral, 2003). Currently, cases of granulomatous amoebic encephalitis (GAE), *Acanthamoeba* keratitis, amoebic pneumonitis and skin inflammation are observed around the world (Fowler and Carter, 1965; Callicott, 1968; Jager and Stamm, 1972; Willaert et al., 1976; Martinez et al., 1977; Martinez and Visvesvara, 1997; Marciano-Cabral et al., 2000).

Clinical symptoms of human GAE include headaches, fever, neurological disorders, such as hallucinations, disorientation and vision disorders, personality changes and coma (Martinez and Visvesvara, 1997). *Acanthamoeba* keratitis, in turn, is characterized by marked ophthalmalgia, photophobia, blue–red vision and blood extravasations. In the lungs, amoebae cause numerous inflammatory foci (AP), which are accompanied by the exudation of serous fluid containing trophozoites and cysts. Skin changes are in the form of numerous ulcerations. All of the infections are typically chronic.

In all cases of *Acanthamoeba* spp. invasions, chemotherapy poses a serious problem. A majority ends in patient death. Only a few instances of successful chemotherapy have been noted, performed using highly toxic drugs usually used for disinfection, e.g. chlorhexidine derivatives (Kitagawa et al., 2003; Seal, 2003). Effective treatment of infections of the central nervous system or eyes in immunocompetent persons has been recorded with combined therapy, commenced at an early stage of the disease (Derda et al., 2009). However, in the later stages of the disease, the majority of therapeutic agents are ineffective (Ficker et al., 1990; Dougherty et al., 1994; Horne et al., 1994; Murdoch et al., 1998).

Higher plants and microbial organisms are used as natural sources for the discovery of new drug leads. Artemisinin, quinine and licochalcone A are examples for plant-derived products, and amphotericin B and ivermectin are important antiparasitics isolated from microorganisms. Many other natural products of diverse molecular structure have demonstrated antiparasitic activity in the laboratory and represent interesting lead structures for the development of new and urgently needed antiparasitics.

Members of the genus *Origanum* comprise the most important aromatic plants throughout the world including sweet marjoram (*Origanum majorana* L.), the dittany of Crete (*Origanum dictamnus* L.), Italian oregano or pot marjoram (*Origanum onites* L.), Greek oregano or winter marjoram (*Origanum heracleoticum* L.) and Turkish wild oregano (*Origanum vulgare*) and bible hyssop or Syrian

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Table 1
Effect of *O. syriacum* methanol extract on the survival and growth of *A. castellanii* trophozoites and cysts.[a]

Dose (mg/ml)	Effect on	Experimental periods						
		1 hour	3 hour	6 hour	8 hour	24 hour	48 hour	72 hour
32.0	Trophozoites	45.3 ± 2.5	0	0	0	0	0	0
	Cysts	58.0 ± 3.6	34.3 ± 11.0	31.3 ± 9.9	21.0 ± 5.6	0	0	0
16.0	Trophozoites	66.3 ± 9.1	37.3 ± 13.3	0	0	0	0	0
	Cysts	72.0 ± 8.0	71.7 ± 5.9	67.0 ± 2.6	64.0 ± 4.6	52.7 ± 4.2	43.0 ± 8.5	32.3 ± 8.7
8.0	Trophozoites	96.3 ± 1.2	78.3 ± 7.8	68.0 ± 5.2	63.7 ± 7.2	53.7 ± 7.8	36.7 ± 7.6	26.3 ± 1.5
	Cysts	97.7 ± 2.1	93.0 ± 3.6	86.3 ± 4.2	82.3 ± 4.5	77.7 ± 3.8	77.3 ± 1.2	65.0 ± 5.0
4.0	Trophozoites	97.7 ± 0.6	93.3 ± 1.5	82.7 ± 6.4	80.7 ± 4.5	75.7 ± 6.0	62.0 ± 5.3	57.0 ± 2.6
	Cysts	98.3 ± 1.5	94.7 ± 1.5	91.0 ± 1.0	84.3 ± 3.8	82.0 ± 4.0	78.7 ± 4.2	76.3 ± 6.7
2.0	Trophozoites	97.7 ± 0.6	97.3 ± 1.2	95.7 ± 0.6	95.7 ± 1.2	93.7 ± 4.9	92.0 ± 5.2	90.3 ± 4.7
	Cysts	99.3 ± 1.2	95.0 ± 3.0	94.0 ± 3.6	92.0 ± 3.0	91.3 ± 3.2	88.3 ± 1.5	87.3 ± 3.8
1.0	Trophozoites	99.0 ± 1.7	98.7 ± 1.2	96.7 ± 1.5	94.3 ± 0.6	93.7 ± 3.2	92.7 ± 2.5	89.0 ± 3.6
	Cysts	100.0 ± 0.0	97.3 ± 2.5	96.0 ± 2.0	96.3 ± 1.5	95.0 ± 1.0	94.0 ± 1.7	90.3 ± 4.7
Control	Trophozoites	100 ± 0	99.3 ± 1.2	100.0 ± 0.0	98.3 ± 0.6	98.3 ± 0.6	98.3 ± 0.6	97.7 ± 0.6
	Cysts	100 ± 0	100.0 ± 0.0	99.3 ± 1.2	98.7 ± 1.2	97.7 ± 1.2	97.0 ± 1.7	97.0 ± 1.7

[a] Date were expressed as mean ± SD.

oregano (*Origanum syriacum*); all are commercially available and exportable plants with appreciable market values (Baser et al., 1993; Kintzios, 2002). *Origanum* plant species are widely used in the flavoring of food products and alcoholic beverages as well as in perfumery for their spicy fragrance (Aligiannis et al., 2001; Sivropoulou et al., 1996; Vera and Chane-Ming, 1999). Apart from their commercial importance, these plants have long been used as spices and condiments for foods (Novak et al., 2000), and have also been used in the treating of several ailments (Ryman, 1992). Because of their potential use in different purposes, studies of the composition and biological properties of the essential oils from many *Origanum* species have been the focus of several studies (Novak et al., 2000; Milos et al., 2000; Mockute et al., 2001; Gotsiou et al., 2002).

Wide application of therapeutic agents to treat *Acanthamoeba* keratitis, sometimes, may cause undesirable reactions for humans. For this reason, research is being conducted into alternative methods of treating AK. Our research is mainly focused on plant extracts for the treatment of parasitic infections effectively as an alternative way to the classical chemical and/or synthetic methods. In this context, amoebicidal activities of some plant species have been reported by our research team (Tepe et al., 2011; Goze et al., 2009; Akin Polat et al., 2007a,b, 2008). Drugs of natural origin have already been used to treat other parasitic diseases (Arrieta et al., 2001; Kayser et al., 2003; Said Fernández et al., 2005). The aim of this study was to evaluate the *in vitro* amoebicidal activity of the methanolic extracts of *O. syriacum* and *Origanum laevigatum*. As far as our literature survey could ascertain, no report is available for the amoebicidal activities of these plant species in the literature. Therefore, this study could be assumed as the first report on this topic.

2. Materials and methods

2.1. Preparation of the methanol extracts

The air-dried and finely ground samples were extracted by using a method described elsewhere (Sokmen et al., 1999). Briefly, the sample, weighing about 100 g, was extracted in a Soxhlet apparatus with methanol (MeOH) at 60 °C for 6 hour. The extract was then filtered and concentrated under vacuum at 45 °C, yielding a waxy material (4.63%, 2.94%, 2.56%, and 3.25% w/w, respectively). Finally, the extracts were then lyophilized and kept in the dark at +4 °C until tested.

2.2. Trophozoites

A clinical isolate of *Acanthamoeba castellanii* was kindly supplied by Dr. Beattie Tara (University of Strathclyde, Glasgow). *A. castellanii* strain was cultured on non-nutrient agar plates (NNA) coated with *Escherichia coli* at 26 °C. Trophozoites in the stage of exponential growth (72–96 hour) were gently removed from the base of NNA culture plates with a sterile cell scraper. The trophozoites in the

Table 2
Effect of *O. laevigatum* methanol extract on the survival and growth of *A. castellanii* trophozoites and cysts.[a]

Dose (mg/ml)	Effect on	Experimental periods						
		1 hour	3 hour	6 hour	8 hour	24 hour	48 hour	72 hour
32.0	Trophozoites	82.3 ± 2.5	79.7 ± 3.5	76.0 ± 4.0	54.0 ± 7.2	36.7 ± 1.5	21.3 ± 3.2	0
	Cysts	94.7 ± 4.2	90.7 ± 2.1	87.0 ± 2.6	72.7 ± 10.0	70.0 ± 3.5	60.7 ± 4.2	52.3 ± 11.6
16.0	Trophozoites	86.0 ± 1.0	80.0 ± 2.0	78.7 ± 8.1	59.7 ± 8.1	45.3 ± 1.5	36.0 ± 5.3	0
	Cysts	96.0 ± 2.6	92.7 ± 4.9	88.3 ± 7.6	77.0 ± 4.6	74.7 ± 6.1	72.3 ± 2.5	67.7 ± 2.5
8.0	Trophozoites	94.7 ± 1.5	88.0 ± 2.6	82.3 ± 4.5	79.3 ± 1.5	63.3 ± 4.7	50.3 ± 5.5	48.3 ± 6.0
	Cysts	97.0 ± 1.0	94.3 ± 3.8	89.3 ± 3.1	82.7 ± 1.5	79.3 ± 3.1	74.3 ± 2.1	71.0 ± 1.7
4.0	Trophozoites	96.3 ± 1.5	93.7 ± 3.5	86.0 ± 4.0	82.3 ± 2.5	76.3 ± 1.5	66.7 ± 6.1	61.7 ± 3.8
	Cysts	97.3 ± 0.6	96.3 ± 1.5	91.0 ± 1.0	90.3 ± 4.5	83.0 ± 1.0	81.3 ± 1.5	79.3 ± 3.1
2.0	Trophozoites	96.7 ± 2.3	94.0 ± 3.6	91.3 ± 1.5	86.3 ± 6.0	80.3 ± 1.5	80.0 ± 4.0	71.0 ± 2.6
	Cysts	99.3 ± 1.2	96.3 ± 1.5	94.7 ± 2.5	92.7 ± 2.1	91.7 ± 3.8	88.3 ± 1.5	87.3 ± 3.8
1.0	Trophozoites	97.0 ± 2.6	98.7 ± 1.2	96.7 ± 1.5	94.3 ± 0.6	93.7 ± 3.2	92.7 ± 2.5	89.0 ± 3.6
	Cysts	100.0 ± 0.0	98.3 ± 2.9	96.0 ± 2.0	94.7 ± 4.2	93.7 ± 3.2	92.7 ± 3.2	89.3 ± 1.2
Control	Trophozoites	99.7 ± 0.6	99.3 ± 1.2	99.3 ± 1.2	98.3 ± 0.6	98.3 ± 0.6	98.3 ± 0.6	97.7 ± 0.6
	Cysts	100.0 ± 0.0	99.3 ± 1.2	99.3 ± 1.2	98.7 ± 1.2	97.7 ± 1.2	97.0 ± 1.0	97.0 ± 1.7

[a] Date were expressed as mean ± SD.