

# Investigation of in vitro amoebicidal activities of *Ornithogalum sigmoideum* on *Acanthamoeba castellanii* cysts and trophozoites

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## Abstract

**Aim:** In this study, amoebicidal activity of methanol and ringer extracts obtained from *Ornithogalum sigmoideum* against *Acanthamoeba castellanii* cyst and trophozoite forms was investigated by determining cell viability percentage and IC<sub>50</sub> values.

**Material and Methods:** The in vitro amoebicidal activities methanolic (1%) and Ringer extracts of *O. sigmoideum* prepared at varying concentrations (in the range of 40 mg/ml - 1.25 mg/ml) on *A. castellanii* trophozoites and cysts were investigated.

**Results:** IC<sub>50</sub> results observed at 1<sup>st</sup>, 3<sup>rd</sup>, 6<sup>th</sup>, 8<sup>th</sup>, 24<sup>th</sup>, 48<sup>th</sup> and 72<sup>nd</sup> hours in *A. castellanii* trophozoite form were 23.2, 15.6, 12.1, 10.1, 8.3, 6.6 and 2.9 mg/ml by using the methanolic extract of *O. sigmoideum*, and 24.2, 16.7, 13.3, 11.9, 8.9, 8.4 and 7.3, mg/ml by using the Ringer extract of *O. sigmoideum* respectively. The 40 mg/ml methanolic extract of *O. sigmoideum* killed all trophozoites at 48<sup>th</sup> and 72<sup>nd</sup> hours. The % viability rate at 72<sup>nd</sup> hour when using the Ringer extract of *O. sigmoideum* was 2.0±0.0.

**Conclusion:** The methanolic extract was found to be more effective than the Ringer extract on *Acanthamoeba* trophozoites. The sensitivity of *A. castellanii* cysts to both extracts was not very different from each other, and the sensitivity of trophozoites to both extracts was higher than that of cysts. The methanolic extracts of *O. sigmoideum* can be used as an alternative treatment option or in combination with other therapies for treating *Acanthamoeba* infections. In addition, Ringer extract of *O. sigmoideum* can also be used as an alternative to the methanolic extract.

**Keywords:** *Acanthamoeba castellanii*; Amoebicidal effect; *Ornithogalum Sigmoideum*.

## INTRODUCTION

Amoeba living free in nature has been the focus of research in the fields of medicine and veterinary medicine since 1960 since they cause lethal parasitoses in humans and animals (1). *Acanthamoeba* species are among the common protozoa found in the environment. They have a wide distribution around the world. Habitats of *Acanthamoeba* species include soil, dust, mud, air, thermal springs, sea water, swimming pools, sewage, air-cleaning units, tap (municipal) water and bottled water, hospital environment, dental treatment units, dialysis units, contact lenses, lens storage containers, lens cleansing solutions, mammalian cell cultures, bacterial and fungal growth mediums (2-7). In addition, *Acanthamoeba* species have been isolated from plants, animals including fish, amphibians, reptiles and mammals, from nasal mucosa, pharynx and thorax of apparently healthy individuals, infected brain and lung

tissues, skin lesions of immunocompromised individuals, and corneal tissues of patients with keratitis (7-10). The risk of pathogenicity is higher in immunocompromised individuals, and in case of AIDS, cancer, previous organ transplantation, immunosuppressant use, inadequate nutrition and continuous stress (7,11).

*Acanthamoeba* species have two stages in their life cycle: The first stage is the trophozoite form, which actively feeds, grows, moves and multiplies; and the second stage is the cyst form, which is more resistant to the unfavorable environmental conditions (1,12). The size of the trophozoite and cyst form varies between species. When environmental conditions are appropriate, the cyst form becomes a trophozoite (7,13,14). Major diseases caused by *Acanthamoeba* species are *Acanthamoeba* keratitis (AK), which is especially seen in contact lens users, and the highly lethal granulomatous amebic

Received: 27.07.2018 Accepted: 14.09.2018 Available online: 24.09.2018

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encephalitis (GAE) and cutaneousacanthamoebiasis. In addition, various widespread infections that are seen in immunocompromised patients, skin lesions and pneumonia are other diseases caused by *Acanthamoeba* spp. Since *Acanthamoeba* species function as vectors for transmission of many viral and bacterial diseases including those that are caused by *Legionella pneumophila*, *Pseudomonas aeruginosa*, and *Mycobacterium avium*, they are clinically important (7,15-17).

Current treatment options for *Acanthamoeba* infections require a long time and cause some side effects, and also cyst forms that are seen in chronic infections can be resistant to drugs. In order to eliminate such problems alternative treatment methods are needed. There is an increasing tendency in modern medicine and pharmaceutical industry to make use of plant-based chemicals for this purpose (18,19).

The number of plants which are used for medicinal purposes in Turkey is estimated to be around 500-1,000. In addition, around 200 medicinal and aromatic plants have been reported to have potential for export (20,21).

As in all other countries, plants of medicinal importance in Turkey have long been used to treat diseases in the forms of tea or spices. These plants are also commonly used in various dyes, ornaments, fragrance and flavor industries, perfumes, food additives, cleaning products and cosmetics, and new benefits are discovered by each day (22-27).

In this study, we investigated the anti-amoebic activities of methanolic and Ringer extracts of *Ornithogalum sigmoideum* plant on *Acanthamoeba castellanii* cyst and trophozoite forms by determining cell viability percentage and  $IC_{50}$  values.

## MATERIAL and METHODS

### Preparation of plant extract

*O. sigmoideum* plant, which is known as 'sakarca' by local people, was obtained from a district bazaar in the city of Ordu. The leaves, flowers and stem parts of the plant was washed with sterile deionized water in laboratory, and then dried with blotting paper. Sixty grams of samples from the stem, leaf and flower parts of the plant were weighed in an analytical scale, and grinded in 250 ml of methanol. The resulting mixture was mixed in a shaker microclimate device for 72 hours. At the end of this time, the mixture was filtered through a filter paper for coarse filtration. Afterwards, the mixture was passed through a vacuum filter containing a bacterial filter (pore size: 0.22 µm) for elimination of bacteria. 200 ml of the filtrate was taken into a sterile volumetric flask, and the methanol was evaporated via evaporator. The final concentration of the *O. sigmoideum* extract was calculated as 40 mg/ml. (28)

### Trophozoites

*Escherichia coli* (ATCC 25922) suspension was inoculated to ringer agar growth mediums. Then, *A. castellanii* (ATCC 30010) trophozoite strain was inoculated to the same

mediums. The growth mediums were incubated at  $26 \pm 1$  °C for 3 days. At the end of this time, the petri dishes were washed with sterile Ringer solution three times in a biosafety cabinet while paying attention not to harm the trophozoites. The surface was then gently swept with a sterile loop, and collected in 15 ml sterile falcon tube, and the suspension was centrifuged at 10°C and 3000 rpm for 5 minutes. Following centrifugation, the supernatant portion was discarded, and the sediment was taken into Eppendorf tubes to be used in the experiments. The sediment portion was then taken to Thoma counting chamber, and the trophozoites were counted under a light microscope. The number of trophozoites per mL was calculated. The final concentration was adjusted to  $2 \times 10^6$  trophozoites/ml before starting the experiment. In order to test the vitality of the trophozoite, 0.4% trypan blue dye was added to the sediment, the mixture was vortexed and allowed to stand at room temperature for 3 minutes. The mixture was put onto a slide and covered with a coverglass, and examined under 40x magnification of light microscope. The trophozoites that engulfed the dye and appear as blue were evaluated as non-viable as described previously by Malatyali et al. (29)

### Cysts

After inoculating the trophozoites into the ringer agar medium, a portion of the petri dishes were incubated at  $30 \pm 1$  °C for 2 weeks to allow transformation to cyst forms. At the end of this period, the same procedures that were applied to the trophozoite forms were also applied to the cyst forms. The final concentration was adjusted to  $2 \times 10^6$  cysts/ml prior to the experiment. The experiment was initiated with 100% viability.

### Experimental Design

1 ml of the plant extract (*O. sigmoideum* 40 mg/ml) was taken and put into sterile Eppendorf tubes. Six serial dilutions were prepared to obtain different concentrations of the plant extract. The dilutions were made using 1% methanol and ringer solution. The resulting concentrations were within the range of 1.25, 2.5, 5, 10, 20, 40 mg/ml.

### Testing Amoebicidal Activity of Plant Extracts on *A. castellanii* Trophozoites and Cysts

100 µl of *A. castellanii* trophozoite/cyst suspension was added to each sterile Eppendorf tube. Then, 100 µl from each of the previously prepared plant extract methanol (1%)/ringer solution dilutions was added. The tubes were vortexed, and then incubated at  $26 \pm 1$  °C. Eppendorf tubes were taken out from the incubator at 1<sup>st</sup>, 3<sup>rd</sup>, 6<sup>th</sup>, 8<sup>th</sup>, 24<sup>th</sup>, 48<sup>th</sup> and 72<sup>nd</sup> hours, respectively for performing the counts. Prior to counting, cell viability was assessed by treating with 0.4% trypan blue. 20 µl of 0.4% trypan blue dye solution was added to each of the seven sterile Eppendorf tubes. Then, 20 µl of each of the 40 mg/ml, 20 mg/ml, 10 mg/ml, 5 mg/ml, 2.5 mg/ml, and 1.25 mg/ml dilutions series, and control solution (contains only methanol (1%)/ringer solution and *A. castellanii* (trophozoite/cyst) suspension but no plant extract) was added to the tubes, and vortexed. After allowing it to stand for 3 minutes at room temperature, the number of viable cells in each tube

was examined using a Thoma counting chamber, starting from the tube with highest concentration. Trophozoite and cyst counts were assessed separately for both ringer and methanol dilution series. All experiments were performed with three repeats, and statistical analyses were applied to determine the cell viability percentage.

### Statistical Analysis

The data regarding the changes observed at 1<sup>st</sup>, 3<sup>rd</sup>, 6<sup>th</sup>, 8<sup>th</sup>, 24<sup>th</sup>, 48<sup>th</sup> and 72<sup>nd</sup> hours in control and plant extracts were entered to Microsoft Excel and SPSS programs. SPSS 18 package software was used to perform descriptive data analysis, and to generate the graphics. Data are expressed as mean  $\pm$  standard deviation (SD). Comparative analyses were performed within 95% confidence interval.  $p < 0.05$  was accepted as statistically significant.

Cell viability % results indicating the amoebicidal activity of the extracts were analyzed with multiple comparison test (Post-hoc) in SPSS 18 software. Binary comparison of the groups was performed with Tukey's test. For calculating IC<sub>50</sub> (plant extract concentration showing lethal effect on half of the trophozoites; 50% inhibitory concentration), logarithmic regression analysis was employed. The graph plotted in the logarithmic regression analysis was used to determine the IC<sub>50</sub> value. The amoebicidal actions observed with different concentrations of plant extracts

at different intervals were compared against the control cells, and expressed as percent inhibition (% cell death). IC<sub>50</sub> values of plant extracts in different concentrations were determined after experiments performed in three replicates.

### RESULTS

In this study, the amoebicidal actions of ringer and methanolic (1%) extracts obtained from *O. sigmoideum* on *A. castellanii* cysts and trophozoites were investigated by determining cell viability percentage (%) and IC<sub>50</sub> values. The data are expressed as mean  $\pm$  standard error and a, b, c, d, e, f were different statistical values from 72. hours,  $p (0.05)$  as shown in Table 1, Table 3.

The methanolic (1%) extract of *O. sigmoideum* in 40 mg/ml concentration showed very strong amoebicidal effect, and no viable parasites were detected at 48th hour (Table 1, Figures 2,3). IC<sub>50</sub> value of methanolic extract of the plant observed at 48<sup>th</sup> hour was 6.6 mg/ml (Table 2). The Ringer extract of *O. sigmoideum* in 40 mg/ml concentration also showed a strong amoebicidal effect with 2.0 $\pm$ 0.0 % viability rate at 72<sup>nd</sup> hour (Table 3, Figures 5, 6). IC<sub>50</sub> value of Ringer extract of the plant at 72<sup>nd</sup> hour was calculated as 7.3 mg/ml (Table 4).

**Table 1. Percent (%) viability effect of *O. sigmoideum* methanol extract on *A. castellanii* trophozoites and cysts at different concentrations**

Concentration	Morphological form	Experimental period						
		1. hour	3. hour	6. hour	8. hour	24. hour	48. hour	72. hour
40 mg/ml	Trophozoits	6.3 $\pm$ 0.3	3.6 $\pm$ 0.3	2.0 $\pm$ 0.0	1.3 $\pm$ 0.6	0.6 $\pm$ 0.6	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
	Cyst	94.3 $\pm$ 0.3 <sup>a</sup>	92.3 $\pm$ 0.3 <sup>b</sup>	90.0 $\pm$ 0.0 <sup>c</sup>	89.0 $\pm$ 0.5 <sup>d</sup>	88.3 $\pm$ 0.3 <sup>e</sup>	87.0 $\pm$ 0.5 <sup>f</sup>	85.0 $\pm$ 0.0
20 mg/ml	Trophozoits	63.6 $\pm$ 0.8 <sup>a</sup>	41.3 $\pm$ 0.8 <sup>b</sup>	16.3 $\pm$ 1.2 <sup>c</sup>	9.0 $\pm$ 0.5	6.6 $\pm$ 0.3	5.3 $\pm$ 0.3	3.0 $\pm$ 0.0
	Cyst	95.3 $\pm$ 0.3 <sup>a</sup>	93.6 $\pm$ 0.3 <sup>b</sup>	92.0 $\pm$ 0.0 <sup>c</sup>	91.0 $\pm$ 0.0 <sup>d</sup>	89.3 $\pm$ 0.3 <sup>e</sup>	87.6 $\pm$ 0.3	87.0 $\pm$ 0.0
20 mg/ml	Trophozoits	82.0 $\pm$ 2.5 <sup>a</sup>	74.3 $\pm$ 3.1 <sup>b</sup>	66.3 $\pm$ 1.7 <sup>c</sup>	53.3 $\pm$ 3.1 <sup>d</sup>	42.0 $\pm$ 1.1 <sup>e</sup>	28.6 $\pm$ 1.4 <sup>f</sup>	16.0 $\pm$ 3.2
	Cyst	96.3 $\pm$ 0.3 <sup>a</sup>	94.6 $\pm$ 0.3 <sup>b</sup>	93.0 $\pm$ 0.0 <sup>c</sup>	91.3 $\pm$ 0.3 <sup>d</sup>	90.3 $\pm$ 0.3 <sup>e</sup>	88.6 $\pm$ 0.3	87.6 $\pm$ 0.3
5 mg/ml	Trophozoits	94.6 $\pm$ 0.6 <sup>a</sup>	88.6 $\pm$ 0.3 <sup>b</sup>	84.0 $\pm$ 0.5 <sup>c</sup>	83.0 $\pm$ 0.0 <sup>d</sup>	76.3 $\pm$ 1.7 <sup>e</sup>	68.6 $\pm$ 1.2 <sup>f</sup>	41.6 $\pm$ 1.2
	Cyst	97.0 $\pm$ 0.0 <sup>a</sup>	96.0 $\pm$ 0.0 <sup>b</sup>	93.3 $\pm$ 0.3 <sup>c</sup>	92.3 $\pm$ 0.3 <sup>d</sup>	92.3 $\pm$ 0.3 <sup>e</sup>	90.6 $\pm$ 0.3 <sup>f</sup>	89.0 $\pm$ 0.0
2.5 mg/ml	Trophozoits	98.0 $\pm$ 0.0 <sup>a</sup>	93.3 $\pm$ 0.3 <sup>b</sup>	90.6 $\pm$ 0.3 <sup>c</sup>	87.0 $\pm$ 0.0 <sup>d</sup>	83.0 $\pm$ 0.5 <sup>e</sup>	72.0 $\pm$ 2.5 <sup>f</sup>	52.6 $\pm$ 1.7
	Cyst	98.0 $\pm$ 0.0 <sup>a</sup>	96.6 $\pm$ 0.3 <sup>b</sup>	95.3 $\pm$ 0.3 <sup>c</sup>	93.3 $\pm$ 0.3 <sup>d</sup>	92.6 $\pm$ 0.3 <sup>e</sup>	91.6 $\pm$ 0.3 <sup>f</sup>	90.0 $\pm$ 0.0
1.25 mg/ml	Trophozoits	98.6 $\pm$ 0.6 <sup>a</sup>	95.0 $\pm$ 0.0 <sup>b</sup>	93.3 $\pm$ 0.3 <sup>c</sup>	89.6 $\pm$ 0.6 <sup>d</sup>	85.3 $\pm$ 0.8 <sup>e</sup>	78.0 $\pm$ 1.5 <sup>f</sup>	63.6 $\pm$ 0.3
	Cyst	99.0 $\pm$ 0.0 <sup>a</sup>	97.6 $\pm$ 0.3 <sup>b</sup>	95.6 $\pm$ 0.3 <sup>c</sup>	94.6 $\pm$ 0.3 <sup>d</sup>	93.6 $\pm$ 0.3 <sup>e</sup>	91.6 $\pm$ 0.3	90.6 $\pm$ 0.3
Control	Trophozoits	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	99.0 $\pm$ 0.0	98.0 $\pm$ 0.8	97.0 $\pm$ 0.0
	Cyst	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	99.3 $\pm$ 0.3	99.3 $\pm$ 0.3	99.0 $\pm$ 0.0

The data are expressed as mean  $\pm$  standard error

a, b, c, d, e, f: different statistical values from 72. hours,  $p (0.05)$

a: 1. - 72.hours; b: 3. - 72.hours; c: 6.-72.hours; d: 8.-72.h; e: 24 - 72.hours; f: 48th - 72nd hour

**Table 2. IC<sub>50</sub> value of *O. sigmoideum* methanol extract on *A. castellanii* trophozoites at different concentrations**

<i>A. castellanii</i>	Experimental period	50% inhibitor concentration (IC <sub>50</sub> )
Trophozoite	72	2.9 mg/ml
	48	6.6 mg/ml
	2	8.3 mg/ml
	8	10.1 mg/ml
	6	12.1 mg/ml
	3	15.6 mg/ml
	1	23.2 mg/ml

**Table 3. Percent (%) viability effect of *O. sigmoideum* Ringer extract at different concentrations on *A. castellanii* trophozoites and cysts**

Concentration	Morphological form	Experimental period						
		1. hour	3. hour	6. hour	8. hour	24. hour	48. hour	72. hour
40 mg/ml	Trophozoites	13.6±0.6 <sup>a</sup>	10.0±0.5 <sup>b</sup>	7.6±0.3 <sup>c</sup>	4.0±0.0	2.3±0.3	2.0±0.0	2.0±0.0
	Cyst	95.0±0.0 <sup>a</sup>	92.0±0.0 <sup>b</sup>	91.0±0.0 <sup>c</sup>	90.0±0.0 <sup>d</sup>	89.6±0.3 <sup>e</sup>	89.0±0.0 <sup>f</sup>	87.0±0.0
20 mg/ml	Trophozoites	65.3±1.2 <sup>a</sup>	43.3±0.3 <sup>b</sup>	21.0±0.5 <sup>c</sup>	9.6±0.6 <sup>d</sup>	8.6±0.8 <sup>e</sup>	6.0±0.5	4.0±0.0
	Cyst	96.0±0.0 <sup>a</sup>	94.6±0.3 <sup>b</sup>	93.0±0.0 <sup>c</sup>	92.0±0.5 <sup>d</sup>	91.6±0.3 <sup>e</sup>	90.0±0.0	89.0±0.0
10 mg/ml	Trophozoites	86.0±0.5 <sup>a</sup>	77.6±0.3 <sup>b</sup>	72.0±0.5 <sup>c</sup>	64.6±0.3 <sup>d</sup>	44.3±0.3 <sup>e</sup>	43.3±0.8	40.6±1.2
	Cyst	97.3±0.3 <sup>a</sup>	95.6±0.3 <sup>b</sup>	94.6±0.3 <sup>c</sup>	92.6±0.3 <sup>d</sup>	92.0±0.0 <sup>e</sup>	91.6±0.3	90.3±0.3
5 mg/ml	Trophozoites	97.0±0.5 <sup>a</sup>	89.3±0.3 <sup>b</sup>	86.3±1.2 <sup>c</sup>	83.0±0.5 <sup>d</sup>	79.6±0.3 <sup>e</sup>	76.0±0.5 <sup>f</sup>	64.3±0.3
	Cyst	98.0±0.0 <sup>a</sup>	96.3±0.3 <sup>b</sup>	95.3±0.3 <sup>c</sup>	94.0±0.5 <sup>d</sup>	93.3±0.0 <sup>e</sup>	92.0±0.0	91.0±0.0
2.5 mg/ml	Trophozoites	98.0±0.0 <sup>a</sup>	93.0±0.0 <sup>b</sup>	91.0±0.0 <sup>c</sup>	86.6±0.3 <sup>d</sup>	83.3±0.3 <sup>e</sup>	79.0±0.5	75.6±0.8
	Cyst	98.3±0.3 <sup>a</sup>	96.6±0.3 <sup>b</sup>	96.0±0.0 <sup>c</sup>	94.6±0.3 <sup>d</sup>	93.6±0.3 <sup>e</sup>	92.6±0.3	92.0±0.0
1.25 mg/ml	Trophozoites	100.0±0.0 <sup>a</sup>	96.3±0.8 <sup>b</sup>	93.0±0.0 <sup>c</sup>	88.6±0.8 <sup>d</sup>	87.3±1.2 <sup>e</sup>	83.6±0.3	80.6±0.3
	Cyst	99.0±0.0 <sup>a</sup>	98.0±0.0 <sup>b</sup>	97.0±0.0 <sup>c</sup>	95.6±0.3 <sup>d</sup>	94.6±0.3 <sup>e</sup>	94.0±0.0	93.0±0.0
Control	Trophozoites	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	99.0±0.6
	Cyst	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0

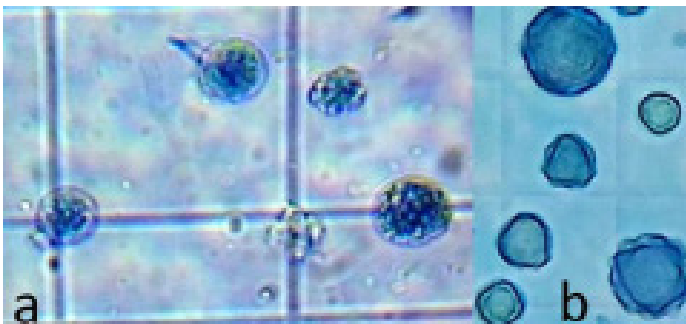
The data are expressed as mean ± standard error

a, b, c, d, e, f: different statistical values from 72. hours, p (0.05)

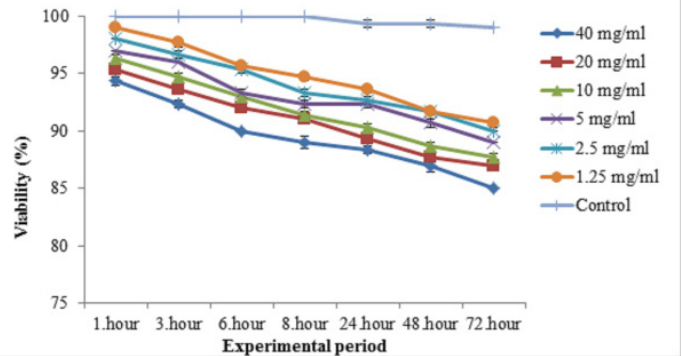
a: 1. - 72.hours; b: 3. - 72.hours; c: 6.-72. hours; d: 8.-72.; e: 24.- 72.hours; f: 48th - 72nd hour

**Table 4. IC50 value of *O. sigmoideum* Ringer extract on *A. castellanii* trophozoites at different concentrations**

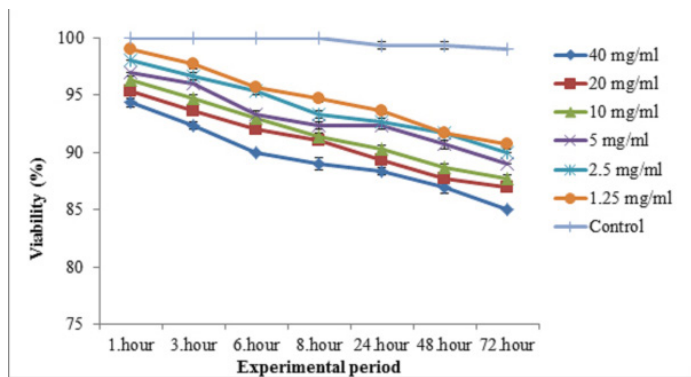
<i>A. castellanii</i>	Experimental period	50% inhibitor concentration (IC <sub>50</sub> )	
		Trophozoite	
	72		7.3 mg/ml
	48		8.4 mg/ml
	24		8.9 mg/ml
	8		11.9 mg/ml
	6		13.3 mg/ml
	3		16.7 mg/ml
	1		24.2 mg/ml



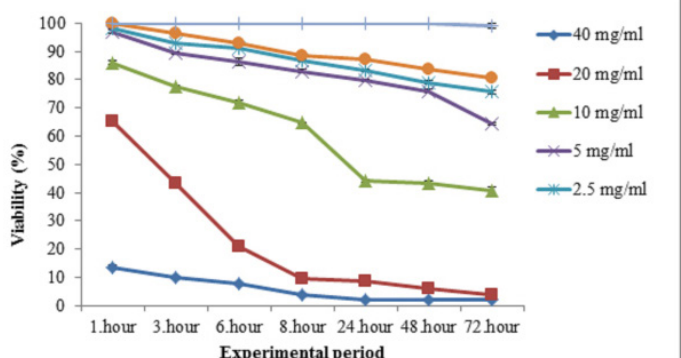
**Figure 1.** Image of *A. castellanii* trophozoites (a), cyst (b) that died in a light microscope (40X)



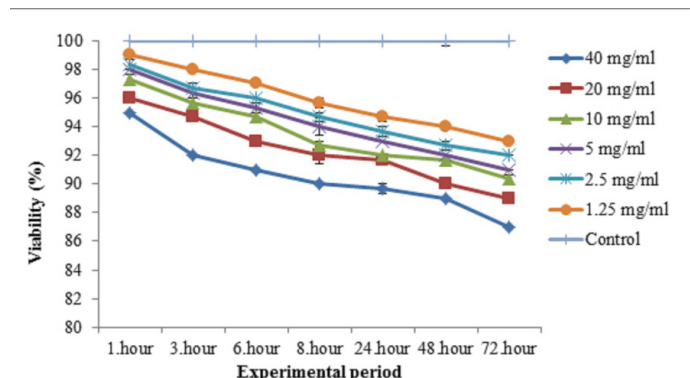
**Figure 3.** Diagram of amoebicidal effect on *A. castellanii* cysts at different times of *O. sigmoideum* prepared at different concentrations with methanol



**Figure 2.** Diagram of amoebicidal activity on *A. castellanii* trophozoites at different times of *O. sigmoideum* prepared at different concentrations with methanol



**Figure 4.** Diagram of amoebicidal activity on *A. castellanii* trophozoite at different concentrations of *O. sigmoideum* prepared at different concentrations with Ringer



**Figure 5.** Diagram of amoebicidal activity on *A. castellanii* cysts at different times of *O. sigmoideum* prepared by different concentrations of Ringer

The methanolic extract of *O. sigmoideum* plant was found to have higher amoebicidal activity on Acanthamoebatrophozoites when compared to the Ringer extract of the plant. *A. castellanii* trophozoites were found to be more susceptible than the cyst forms to both methanolic and Ringer extracts of *O. sigmoideum* plant. Both extracts showed similar action on the cysts (Tables 1, 3).

## DISCUSSION

Currently available antiparasitic drugs may fail in treating Acanthamoebainfections as they are not sufficiently effective in certain anatomic regions including the central nervous system and the eye. Current treatment of Acanthamoeba keratitis includes cationic antiseptics such as chlorhexidine and polyhexamethylenebiguanid, either alone, or two of them combined. Since granulomatous amebic encephalitis (GAE) is seen less frequently than Acanthamoeba keratitis, no standard treatment scheme for GAE has been established yet (30,31). Cationic antiseptics, antifungals and macrolide group of antibiotics have been tested in vitro and in vivo for treatment, but successful results could not be achieved consistently (31,32). Some anti-amoebic drugs only have amoebistatic activity, and molecules used in the treatment can show cytotoxic effect against the host. Prolonged use of anti-amoebic drugs may not be tolerated well by the patient, and in time, microorganisms can develop resistance to these agents as well (12,30). Because of these problems, there is an ongoing search for a drug that is effective on both trophozoite and cyst forms, well tolerated and non-cytotoxic (1,31). To avoid problems due to the lack of the desired potency and selectivity of the current treatments, long treatment durations and the related side effects, and resistance development by cyst forms that are found in chronic infections, alternative treatment methods are needed. A plant-based chemical may be the best candidate for an alternative treatment modality, as there is an increasing tendency in modern medicine and pharmaceutical industry for using plant-based chemicals in treatment (18,19).

There have been a growing number of studies in the recent years on amoebicidal activity of essential oils or

extracts obtained from plants. Some of these studies use Acanthamoeba species to investigate the amoebicidal effect. However, there is limited information on the mechanisms responsible for the action of essential oils or plant extracts that are found to be effective.

The amoebicidal activities of various plant species on *A. castellanii* trophozoite and cyst forms have been investigated in in vitro experiments using extracts prepared with methanol. Göze et al. (33) studied in vitro activity of methanolic extracts of *Salvia* species (*Salvia caespitosa* and *Salvia staminea*) on *A. castellanii* trophozoites and cysts, and found the methanolic extract of *S. staminea* to have more potent amoebicidal activity. Malatyali et al. (29) evaluated the in vitro amoebicidal activities of methanolic extracts of *Peucedanum caucasicum*, *Peucedanum palimbioides*, *Peucedanum chryseum* and *Peucedanum longibracteolatum* on *A. castellanii* trophozoites and cysts, and found *P. longibracteolatum* to have the most potent amoebicidal effect. Değerli et al. (34) investigated the methanolic extracts of *Origanum syriacum* and *Origanum laevigatum* plants on *A. castellanii* trophozoites and cysts. The authors reported that *O. syriacum* showed the most potent amoebicidal activity.

Various solvents other than methanol have also been used by many researchers to prepare plant extracts. For instance, Topalkara et al. (35) compared the in vitro amoebicidal activity of the ethanolic extract of propolis on trophozoite and cysts forms of *A. castellanii*, and observed a potent amoebicidal effect on both forms. Nagwa et al. (36) evaluated the in vitro amoebicidal activities of ethanolic extracts prepared from *Arachishypogaea* L., *Curcuma longa* L. and *Pancreatium maritimum* L. plants on *A. castellanii* cysts, and they reported that the ethanolic extracts of *A. hypogaea*, *C. longa* and *P. maritimum* were more effective against Acanthamoeba cysts when compared to chlorhexidine. Badria et al. (37) examined the amoebicidal activities of ethyl-acetate and methanolic extracts of *Helianthemum lippii* (L.) on *A. castellanii* cysts isolated from a patient with amebic keratitis, and noted that ethyl-acetate extract was more effective than the methanolic extract of the plant. Mahboob et al. (38) studied the in vitro amoebicidal activities of ethyl-acetate, aquatic and buthanolic extracts of *Lonicera japonica* plant prepared using the dried flower buds. They found the ethyl-acetate extract to have the most potent amoebicidal activity.

In addition to the methanolic extract of *O. sigmoideum*, another extract was prepared from the plant using Ringer solution in the present study. In vitro amoebicidal activities of varying concentrations of methanolic and Ringer extracts of *O. sigmoideum* on trophozoite and cyst forms of *A. castellanii* were investigated. The Ringer extract was found to have lower amoebicidal activity than the methanolic extract on both trophozoite forms of *A. castellanii*.

The 40 mg/ml methanolic extract of *O. sigmoideum* killed all trophozoites at 48<sup>th</sup> and 72<sup>nd</sup> hours. While, the % viability

rate at 72nd hour at the Ringer extract of *O. sigmoideum* was 2.0±0.0.

## CONCLUSION

The results of the present study demonstrate that Ringer extracts can as well be used as an alternative to the methanolic extracts. It is concluded that extracts obtained from *O. sigmoideum* under controlled conditions can be used as an alternative treatment option or in combination with other therapies for treating *Acanthamoeba* infections. Plant extracts examined in this study showed amoebicidal activity against both trophozoite and cyst forms of *A. castellanii*. It is recommended that whether the concentrations investigated in the present study are cytotoxic for mammalian cells, or have toxic effects on experimental animals is examined with in vivo studies, and the mechanism of action for the active substances responsible for the biological action is investigated in future studies.

**Acknowledgements:** The study was supported by Ordu University, Scientific Research Projects Unit (SRP), with project code BY-1705. We would like to thank SRP for their support

**Competing interests:** The authors declare that they have no competing interest.

**Financial Disclosure:** Ordu University, Scientific Research Projects Unit (SRP), with project code BY-1705.

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