Original Article

Antiparasitic Efficacy of *Artemisia ludoviciana* Nutt. (Asteraceae) Essential Oil for *Acanthamoeba castellanii, Leishmania infantum* and *Trichomonas vaginalis*

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ABSTRACT

Background: *Artemisia ludoviciana* Nutt. (Asteraceae) is an aromatic, herbaceous, perennial plant and known commonly name as "White Sage", "Black Sage", "Prairie Sage" or "Cudweed Sagewort". It is traditionally used as an antispasmodic, anthelminthic, antidiarrhoeal, stomachic, hepatic colic, appetizer, and regulator of menstruation, antimalaric and antiparasitic efficiancy. **Objective:** The essential oil composition of the flowering herb of *A. ludoviciana* (AL) was investigated and for the first time, the oil was screened for antiparasitic activity. **Methods:** The chemical composition of the hydrodistilled essential oil (EO) obtained from the herb with flowered of *A. ludoviciana* was analyzed by Gas Chromatography–Flame Ionization Detector (GC-FID) and Gas Chromatography–Mass Spectrometry (GC-MS). **Results and Discussion:** The outcomes showed that the major components of oil were camphor (40.6%), 1,8-cineole (25.5%) and camphene (4.7%), among 45 identified compounds, comprising 98.5 of the total oil. In addition, the oil was tested against *Acanthamoeba castellanii*, *Leishmania infantum* and *Trichomonas vaginalis*. **Conclusion:** In this study, it is first demonstrated that *A. ludoviciana* essential oil (AL-EO) is effective against three important parasites.

Key words: *Artemisia ludoviciana*, Camphor, 1,8-Cineole, Camphene, Antiparasitic effect, GC-FID, GC/MS.

INTRODUCTION

Plants/plant extracts and their active components have been used for many centuries as treatments for diseases from headaches to parasite infections. In the last 20-30 years, researchers have seriously focused on determining whether plant-derived traditional remedies are effective and what their mode of action is. Several studies proving the effects of plants on parasite infections have been undertaken using aqueous or alcoholic extracts and essential oils (EOs).[1] The EOs generally have a broad spectrum of bioactivity, owing to the presence of several active ingredients or secondary metabolites, which work through various modes of action.[2]

The genus *Artemisia* L. comprises important medicinal plants, which have gained phytochemical attention due to their biological and chemical diversity, and essential oil production. *Artemisia* species, widespread throughout the world, are frequently used for the treatment of diseases such as malaria, hepatitis, cancer, inflammation and infections by fungi, bacteria and viruses.[2] The genus *Artemisia* includes 23 perennial aromatic herbs and shrubs that grow wild in Turkey.[3] In the literature, there are only a few papers dealing with the essential oil composition and properties of *Artemisia ludoviciana* Nutt. from among these

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species. Previous studies reported that main components of leaf oils of *A. ludoviciana* (AL) grown in USA and Mexico analyzed by gas chromatography–mass spectrometry (GC-MS) were alpha-pinene, camphene, 1,8-cineole, camphor, borneol, nonanal, linalool, carvacrol and p-alpha-dimethylbenzyl alcohol.[4,5] The dried whole plant, a leaf infusion and essential oil (EO) of *A. ludoviciana* Nutt. have traditionally been used due to antispasmodic, anthelminthic, antidiarrhoeal, stomachic, hepatic colic, appetizer, regulator of menstruation, antimalarial, and antiparasitic activity.[4,6,7] Hexane, acetone, methanol and aqueous extracts of *A. ludoviciana* were found to be active *in vitro* against the parasitic protozoa *Entamoeba histolytica* and *Giardia lamblia*.[6]

In Africa, Asia, Europe and South and North America, nearly 350 million people are at risk of leishmaniasis.[8] Between 2000 and 2014 in Turkey a total of 413 cases were reported of visceral leishmaniasis (VL) infection due to the vector *Leishmania infantum*. There were 29,845 cases of cutaneous leishmaniasis (CL) due to the vector *Leishmania tropica* from 2000-2014.[9] Treatment of the parasite uses antileishmanial medications like sodium stibogluconate, miltefosine, parmomycin, amfotericin B and pentamidine.[10] However, the effects of these medications are limited and there are serious side effects including nephrotoxic, hepatotoxic and teratogenic effects.[11,12] Globally and in Turkey, it has been reported that resistance has developed to the primary medication for treatment of CL and VL of five valuable antimony species, sodium stibogluconate (Pentostam®) and megluminantiamoniate (Glucantime®).[12-16] Due to the lack of an effective prophylactic against the disease, the toxic effects of currently-used medications and the increasing resistance to these medications, the necessity for discovery and development of new therapeutic agents has been reported.[11,17,18]

Trichomoniosis is a common infection everywhere in the world and the infection rates are reported to show great variations from country to country and society to society. Researchers have stated that the different results obtained by different people in different regions may be due to factors such as the use of different techniques for diagnosis, and deficient and mistaken assessments.[19,20] *Trichomonas* may be treated with metronidazole, tinidazole, nimorazole, secnidazole and ornidazole. It is recommended to administer paired treatment at the same time. Metranidazole may be administered to resistant cases 4-6 weeks after first treatment. Patients developing side effects linked to metranidazole may be given agents like polyoxethylene nonylphenol, aminacrine, sodium edetate and docusate sodium for 2 weeks duration administered by the vaginal route twice per week.[21,22]

Acanthamoeba species may proliferate easily in natural environments, infected organs, and on xenic and axenic cultures. *Acanthamoeba* have been identified in natural environments like water, thermal water, sea water, soil and air. Additionally they have been reported to be isolated from human-made environments such as drinking water, bottled spring water, distilled water in laboratories, chlorinated swimming pools and contact lens storage cases.[23-26]

Granulamatous amoebic encephalitis (GAE) cases are commonly detected to involve *Acanthamoeba culbertsoni, A. castellanii* and *A. rhysoides* species. *Acanthamoeba keratitis* is a parasitosis caused by a variety of *Acanthamoeba* species. Preparatory factors are trauma, contact lens use, and contact of infected water with the cornea. It is observed in healthy individuals and causes severe ocular pain, burning, vision disruption and stromal infiltration with ring shape. Over time vision is impaired and the eye may even be lost. In *Acanthamoeba keratitis* cases, most commonly *A. castellanii* and *A. polyphaga* are identified.[27,28] For treatment a variety of medications have been tried. Among effective agents, propamidine isethionate, ketoconazole, miconazole and itraconazole may be listed. Surgical cleaning of the lesion and additionally oral and local administration of miconazole have been emphasized as effective.[29]

In this study, EO of the *A. ludoviciana* flowering herb was analyzed with GC and GC-MS and antiparasitic efficacy of the oil against *Acanthamoeba castellanii*, *Leishmania infantum* and *Trichomonas vaginalis* was examined for the first time.

MATERIALS AND METHODS

Plant material

The flowering herb of *A. ludoviciana* was obtained from Zeytinburnu Medicinal Plant Garden, Istanbul, Turkey in 2016.

Isolation of the essential oil

The plant materials were air dried at room temperature and were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus to extract essential oils. The oils were dried over anhydrous sodium sulfate to remove moisture and stored at +4°C until analyzed and tested further.

Analysis of essential oil

GC-MS analysis

The GC-MS analysis was carried out with an Agilent 5975 GC-MSD system. An Innowax FSC column (60 m x 0.25 mm, 0.25 μm film thickness) was used with helium as carrier gas (0.8 ml/min). The GC oven temperature was kept at 60°C for 10 min and programmed to 220 °C at a rate of 4°C/min, and kept constant at 220 °C for 10 min and then programmed to 240 °C at a rate of 1°C/min. Split ratio was adjusted to 40:1. The injector temperature was set at 250 °C. Mass spectra were recorded at 70 eV. Mass range was from m/z 35 to 450.

GC analysis

The GC analysis was carried out using an Agilent 6890N GC system. The FID (Flame Ionization Detector) temperature was 300°C. To obtain the same elution order with GC-MS, simultaneous auto-injection was done on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms. The analysis results are given in Table 1.

Identification of the EO components was carried out by comparison of their relative retention times with those of authentic samples or by comparison with their relative retention index (RRI) to a series of *n*-alkanes. Computer matching against commercial (Wiley GC/MS Library, MassFinder 3 Library),[30,31] in-house "Başer Library of Essential Oil Constituents" created from genuine compounds and components of known oils, as well as MS literature data,[32,33] was used for identification.

Antiparasitic Studies

Sample Supply

The study obtained *Acanthamoeba castellani* and *Trichomonas vaginalis* from Cumhuriyet University Parasitology laboratory, *Leishmania infantum* from Ege University Medical Parasitology Department and *Escherichia coli* strains from Ordu University Literature-Science Faculty Biology Department. Parasites were cultured to ensure continuity.

Acanthamoeba culture

Non-nutrient Agar

Eosin methylene blue (EMB) culture prepared according to the procedure was used to produce *E. coli*. The study used Page's amoeba saline solution. The prepared solution was placed in a 100 ml flask and left in an autoclave at 121°C for 15 min and stored at 4°C until use.

Preparation of Medium

Agar of 1.5 g was heated and dissolved in 100 mL Page solution, autoclaved for 15 min at 121°C for 15 min and distributed to petri dishes. The cultures were stored at 4°C until use.

Culture

The prepared media were watered with 0.5 mL Page solution and 24-h *E.coli* strains were spread on the agar. Samples taken from *A. castalleni* strains were seeded on the medium. Seeded parasites were left for 72 h at 26°C and trophozoites were collected from the petri dishes without harm and washed using Page solution at 1500 g for 5 min in a centrifuge. To test the viability of trophozoites, 0.4% trypan blue was used and they were counted on a hemocytometer slide.

Trichomonas vaginalis culture

Cysteine-Peptone-Liver-Maltose (CPLM) Culture Method

The CPLM medium liver extract mixture comprised 20 g Bacto liver powder mixed with 330 ml distilled water. The mixture was left for 1 h at 50ºC and then at 80ºC for protein coagulation. Later it was strained through filter paper. Ringer solution was prepared by melting 2 Ringer tablets in 1000 mL distilled water. The liver extract and Ringer solution were mixed well together. This mixture has 32 g peptone, 16 g maltose, 2.4 g L-cysteine HCl and 1.6 g Bacto agar added. The mixture was left in a water bath until the agar melted and was strained after the agar had fully melted. The strained mixture had 0.7 mL 0.5 % methylene blue added. The prepared medium had pH set to 5.8-6, was had 5 ml each distributed to 125 x 16 mm tubes and was sterilized for 20 min at 121ºC. For sterilization control a sample tube was left at 37 ºC for 24 h incubation. The tubes were stored at + 4 ºC until seeding.

Culture

Under sterile conditions 1 mL inactivated human serum (left for half an h at 56ºC, inactivated human serum was stored in a deep freeze) was added to the medium. Diluted penicillin, streptomycin and triflucan of 1 ml each was added to 1 mL physiologic serum and each tube had 0.2 mL of medication added under sterile conditions and then was left in an incubator at 37ºC. Two days later they were investigated for proliferation. Samples taken from the medium washed with sterile Ringer solution at 1500 g for 5 min in a centrifuge. To test the viability of trophozoites, 0.4 % trypan blue was used and counted on a hemocytometer slide.

Table 1: Composition of the Essential Oil of *Artemisia ludoviciana*.			
No	**RRI** [a]	**Compound**	**%**
1	1014	Tricyclene	0.2±0*b*
2	1032	α-Pinene	0.8±0
3	1035	α-Thujene	0.3±0
4	1076	Camphene	4.73±0.05
5	1118	β-Pinene	0.5±0
6	1132	Sabinene	0.3±0
7	1188	α-Terpinene	0.3±0
8	1203	2-Methyl butyl isobutyrate	0.2±0
9	1213	1,8-Cineole	25.53±0.21
10	1255	γ-Terpinene	0.7±0
11	1280	*p*-Cymene	2.73±0.05
12	1286	2-Methyl butyl 2-methyl butyrate	0.5±0
13	1450	*trans*-Linalool oxide (*Furanoid*)	0.2±0
14	1474	*trans*-Sabinene hydrate	1.07±0.87
15	1532	Camphor	40.57±0.77
16	1553	Linalool	0.33±0.05
17	1556	*cis*-Sabinene hydrate	0.3±0
18	1571	*trans*-*p*-Menth-2-en-1-ol	0.6±0
19	1586	Pinocarvone	0.8±0
20	1591	Bornyl acetate	1.27±0.05
21	1611	Terpinen-4-ol	3.5±0.08
22	1638	*cis*-*p*-Menth-2-en-1-ol	0.47±0.05
23	1648	Myrtenal	0.37±0.05
24	1663	*cis*-Verbenol	0.53±0.19
25	1670	*trans*-Pinocarveol	0.73±0.05
26	1682	δ-Terpineol	0.37±0.05
27	1683	*trans*-Verbenol	1.07±0.05
28	1706	α-Terpineol	0.2±0
29	1709	α-Terpinyl acetate	0.87±0.05
30	1719	Borneol	3.87±0.12
31	1725	Verbenone	0.77±0.05
32	1751	Carvone	0.3±0
33	1758	*cis*-Piperitol	0.23±0.05
34	1786	*ar*-Curcumene	0.1±0
35	1793	α-Campholene alcohol	0.17±0.05
36	1802	Cumin aldehyde	0.3±0
37	1804	Myrtenol	0.1±0
38	1845	*trans*-Carveol	0.37±0.05
39	1864	*p*-Cymen-8-ol	0.3±0
40	1896	*cis*-*p*-Mentha-1(7),8-diene-2-ol	0.1±0
41	2008	Caryophyllene oxide	0.3±0.08
42	2144	Spathulenol	0.33±0.05
43	2257	β-Eudesmol	0.2±0
44	2316	Caryophylla-2(12),6(13)-dien-5α-ol (=*Caryophylladienol I*)	0.1±0
45	2324	Caryophylla-2(12),6(13)-dien-5α-ol (=*Caryophylladienol II*)	0.2±0
		Total	98.47±0.41

a RRI Relative retention indices calculated against *n*-alkanes on the HP Innowax column;
b mean % calculated from Flame Ionization Detector (FID) data ± SD (n=3).

Leishmania culture

To ready-made RPMI-1640 medium, 10 % fetal bovine serum added. The medium had 10,000 IU penicillin, 10 µg streptomycin and 40 µg triflucan added as antibiotics. *Leishmania* culture had samples taken with a sterile glass Pasteur pipette and these were seeded on medium and incubated at 25ºC. Four days later the medium was checked, if proliferation had occurred the study began. To test the viability of promastigotes, 0.4 % trypan blue was used and they were counted on a hemocytometer slide.

Preparation of essential oil concentrations

Essential oil prepared with physiologic serum at concentrations of 32, 16, 8, 4, 2 and 1 mg/mL and 200 µL was distributed into sterile eppendorf tubes.

Experimental stage

Final concentrations were set to $20x10^6$ trophozoite/mL for *A. castellani,* $51x10^6$ trophozoite/ml for *T. vaginalis* and $45x10^6$ trophozoite/ml for *L. infantum* and these were added to the 200 µL tubes and incubated at room temperature. The viability of the parasites was checked at certain times and noted. Tubes with no live cells identified had control seeding performed again and no proliferation was observed in any. Parasites without added essential oil were left in the same environment as controls. EO and control group were diluted with saline.

Statistical Assessment

Data firstly were subjected to logarithmic transformation to ensure the assumption of homogeneity of variance. To determine whether there was a significant difference between viability of the parasite species and dose administered, the transformed data had the repeated measures variance analysis and the Tukey multiple comparison test applied. The analysis results returned the data to their original form and present the research findings as n, mean and standard deviation. The results were accepted as having significance level of $p \leq 0.05$. All statistical calculations were performed with the SPSS 22.0 V statistical packet program.

RESULTS AND DISCUSSIONS

In the study, the antiparasitic effect of *A. ludoviciana*-EO on *T. vaginalis*, *A. castellani* and *L. infantum* was evaluated and the percentage yield of EO of *A. ludoviciana* flowering herb was calculated on a dry weight basis (0.092 %). The compounds of AL-EO were showed in Table 1.

Each measurement in the study was taken twice and the means were calculated. The repeated measures variance analysis Table for the viability levels of the parasite species according to dose and time is given in Table 2, with descriptive statistics (mean and standard deviation) given in Table 3.

When Table 2 is investigated, there was a significant difference determined in the viability of the parasites depending on time, parasite species and doses. Additionally, the effect of dose*time interaction was determined to have a significant effect on parasite viability. In this situation as the dose increased, it may be interpreted that the rate of reduction in parasite viability fell. Again, there was a reduction in parasite viability linked to time observed which is shown in Figures 1, 2 and 3. Additionally, the cause of the difference in parasite viability levels depending on parasite species and doses may be explained by the different rates of antiparasitic effect according to plant species.

When Table 3 is examined, it is observed that *T. vaginalis* was completely dead in the 48th h.

As seen in Table 3, as the concentration of EO increased, the viability rates of the parasites reduced. At the start of the experiment the concentration of $20x10^6$ trophozoite/mL of *A. castellani* had final concentration in the control tube of $17x10^6$ trophozoite/mL, the inital $51x10^6$ trophozoite/mL for *T. vaginalis* had final concentration in the control tube of $27x10^6$ trophozoite/mL and the inital $45x10^6$ trophozoite/mL for *L. infantum* had final concentration in the control tube of $45x10^6$ trophozoite/mL.

In the study, the 64 mg/mL concentration of AL-EO inhibited the proliferation of *A. castellani* trophozoites in the first three h and had rapid effect with amoebicidal effect in the first 24 h. Again, the 64 mg/mL concentration inhibited proliferation of the parasitic trophozoites of *T. vaginalis* in the first three h and had rapid effect with anti-tricomonal effect in the first 8 h. Additionally, the 64 mg/mL concentration of EO inhibited the proliferation of trophozoites of *L. infantum* in the first three h and had rapid effect with antileishmanial effect in the first 14 h.

When the Figures 1,2,3 are investigated, it is observed that the viability rates for the parasites effectively fell depending on dose.

To assess the viability rates of the parasites according to dose, the Tukey test was performed and results are given in Table 4.

When the Table 3 is examined, 64 mg/ml dose significantly reduced viability. The reductions in viability rates of the parasites with doses of 32 mg/ml, 16 mg/ml and

Table 2: Variance analysis table of parasite species according to time and dose.

Source of variance	Type III Sum of Squares	df	Mean Square	F	Sig.
Time	8.353	1	8.353	6.333	0.020
Time * parasite_species	7.182	2	3.591	2.722	0.089
Time * doses	120.154	6	20.026	15.182	<0.001
Time * parasite_species* doses	49.678	12	4.140	3.138	0.011
Error (time)	27.700	21	1.319		
Parasite_species	71.870	2	35.935	14.096	<0.001
Doses	505.789	6	84.298	33.068	<0.001
Parasite_species * doses	40.192	12	3.349	1.314	0.282
Error	53.534	21	2.549		

Table 3: Effects of *A. ludoviciana* essential oil on the proliferation of *A. castellani* and *T. vaginalis* and *L. infantum*.

Parasites	Dose (mg/ml)	Experimental periods									
A. castellani	AL-EO	3 h		24 h		54 h		76 h		120 h	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
	1.0	$19.5x10^6$	1.44	$9.5x10^6$	1.08	$8x10^6$	1.00	$1x10^6$	1.00	$1x10^6$	1.00
	2.0	$17x10^6$	1.54	$8x10^6$	1.17	$5.5x10^6$	1.14	$1x10^6$	1.00	$1x10^6$	1.00
	4.0	$5.5x10^6$	1.14	$5x10^6$	1.00	$4x10^6$	3.55	$0.5x10^6$	17484.00	$0.5x10^6$	17484.00
	8.0	$5x10^6$	1.00	$4x10^6$	1.00	$4x10^6$	1.00	$2.5x10^6$	17484.00	$0.5x10^6$	17484.00
	16.0	$4.5x10^6$	1.17	$3x10^6$	1.63	$2x10^6$	1.00	$0.5x10^6$	17484.00	$0.5x10^6$	17484.00
	32.0	$4x10^6$	1.00	$2x10^6$	1.00	$2x10^6$	2.17	$0.5x10^6$	17484.00	$0.5x10^6$	17484.00
	64.0	$1.0x10^6$	1.00	0	1.00	0	1.00	0	1.00	0	1.00
T. vaginalis	AL-EO	3 h		8 h		24 h		35 h		48h	
	1.0	$25x10^6$	2.17	$16x10^6$	2.07	$7.5x10^6$	1.10	$2.4x10^6$	3.55	$1x10^6$	17484.00
	2.0	$23x10^6$	2.67	$14x10^6$	1.75	$4.5x10^6$	1.33	$2.2x10^6$	3.12	$1x10^6$	17484.00
	4.0	$14x10^6$	4.65	$8.5x10^6$	2.17	$2x10^6$	2.67	$1.5x10^6$	1.63	0	1.00
	8.0	$14x10^6$	4.50	$6.3x10^6$	1.91	$2x10^6$	2.67	0	1.00	0	1.00
	16.0	$11x10^6$	4.54	$4.4x10^6$	1.17	$1.5x10^6$	1.63	0	1.00	0	1.00
	32.0	$10x10^6$	4.16	$2x10^6$	1.00	$1x10^6$	2.17	0	1.00	0	1.00
	64.0	$1.5 x10^6$	1.63	0	1.00	0	1.00	0	1.00	0	1.00
L. infantum	AL-EO	3 h		14 h		24 h		54 h		78 h	
	1.0	$10x10^6$	1.33	$8x10^6$	1.38	$6x10^6$	1.00	$4x10^6$	1.00	0	1.00
	2.0	$10x10^6$	1.00	$7x10^6$	1.00	$3x10^6$	1.63	$3x10^6$	1.00	0	1.00
	4.0	$10x10^6$	1.00	$4x10^6$	1.00	$2x10^6$	1.00	$0.5x10^6$	17484.00	0	1.00
	8.0	$5x10^6$	1.33	$4x10^6$	1.44	$1x10^6$	1.00	0	1.00	0	1.00
	16.0	$4x10^6$	1.44	$1x10^6$	1.00	0	1.00	0	1.00	0	1.00
	32.0	$2x10^6$	1.00	$0.5x10^6$	17484.00	0	1.00	0	1.00	0	1.00
	64.0	$1.0 x10^6$	1.00	0	1.00	0	1.00	0	1.00	0	1.00

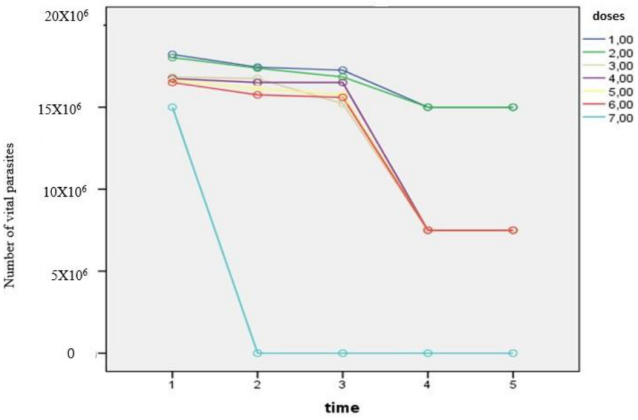

Figure 1: Viability graph of *A. castallani* according to time and dose.
Doses: 1=1mg/ml, 2= 2mg/ml, 3= 4 mg/ml, 4= 8 mg/ml, 5= 16 mg/ml, 6=32 mg/ml, 7=64mg/ml
Time: 1=3 h, 2=24 h, 3=54 h, 4=76 h, 5=120 h

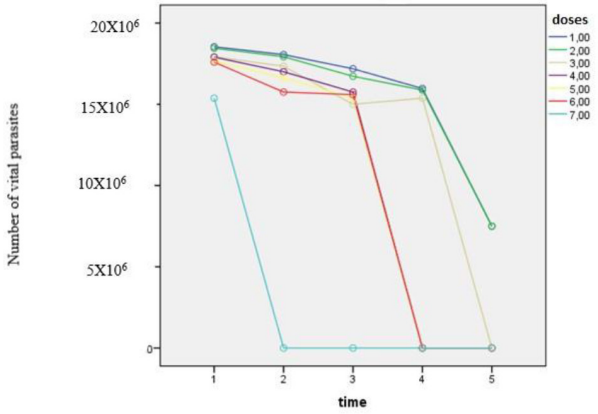

Figure 2: Viability graph of *T. vaginalis* according to time and dose.
Doses: 1=1mg/ml, 2= 2mg/ml, 3= 4 mg/ml, 4= 8 mg/ml, 5= 16 mg/ml, 6=32 mg/ml, 7=64mg/ml
Time: 1=3 h, 2=8 h, 3=24 h, 4=35 h, 5=48 h.

Table 4: Viability values for parasites according to dose.

doses	N	Subset			
		d	c	b	a
7.00	6	1.2100			
6.00	6		3.6015		
5.00	6		3.8573	3.8573	
4.00	6		4.3630	4.3630	
3.00	6			4.9792	4.9792
2.00	6				6.0177
1.00	6				6.0922
Sig.		1.000	0.533	0.141	0.147

Means for groups in homogeneous subsets are displayed. Based on observed means. The error term is Mean Square (Error) = .510.
a. Uses Harmonic Mean Sample Size = 6.000.
b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.
c. Alpha = .05.
Doses: 1=1mg/ml, 2= 2mg/ml, 3= 4 mg/ml, 4= 8 mg/ml, 5= 16 mg/ml, 6=32 mg/ml, 7=64mg/ml

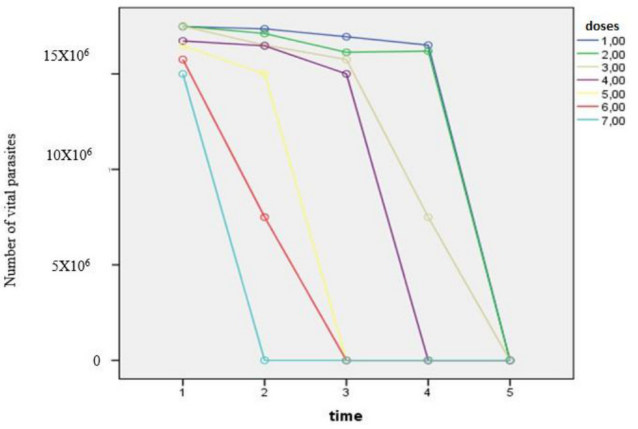

Figure 3: Infantum according to time and dose.
Doses: 1=1mg/ml, 2= 2mg/ml, 3= 4 mg/ml, 4= 8 mg/ml, 5= 16 mg/ml, 6=32 mg/ml, 7=64mg/ml
Time: 1=3 h, 2=14 h, 3=24 h, 4=54 h, 5=78 h.

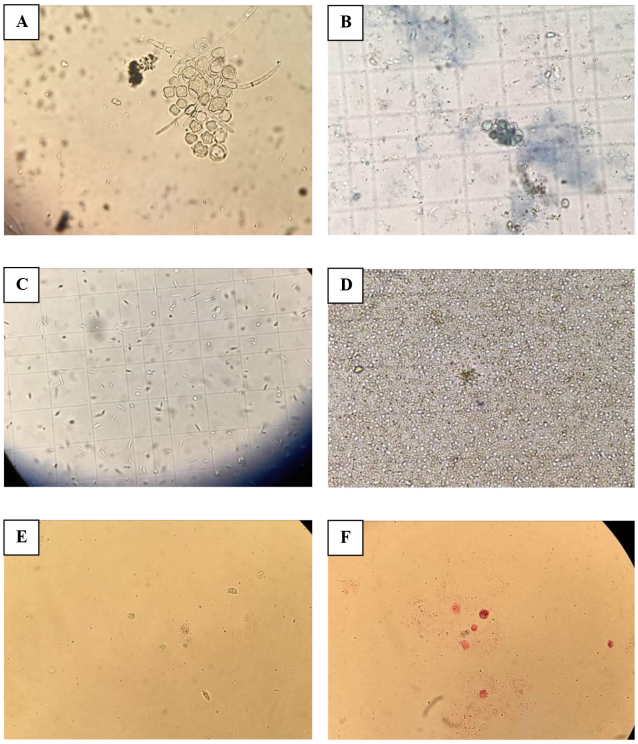

Figure 4: A. Viable *Acanthamoeba cyst*; B. Image of died *Acanthamoeba* after treatment with metilen blue; C. Viable *Leishmania* promastigot; D. Image of died *Leishmania* promastigot after treatment with metilen blue; E. Viable *Trichomonas* trophozoit; F. Image of died *Trichomonas* trophozoit after treatment with 0.1% eosin (x40).

8 mg/ml were similar. The weakest effect on viability was found for doses of 1 mg/ml and 2 mg/ml. The antiparasitic efficacy of AL-EO was showed in Figure 4. Literature information showed that for Leishmaniasis treatment in addition to different anti-leismanial agents like five valuable antimony compounds, amphotericin B deoxycholate, miltefosine, paromomycin, sitamaquine,

azole and pentamidine, thermotherapy and cryotherapy applications may be used.[34-36] The efficacy of the five valuable antimony compounds used for treatment is reported to be above 90%, though rare local and systemic side effects like myalgia, arthralgia, stomach pain, hepatitis, pancreatitis and increases in a variety of laboratory values have been reported.[36]

When resistance to antimony compounds develops, for Visseral leishmaniasis treatment intravenous or intramuscular amphotericin B is used. As amphotericin B displays broad distribution through the body, it has side effects like infusion reactions, nephrotoxicity, hypokalemia and myocarditis and patients must be admitted for 4-5 weeks.[34]

The toxic side effects of anti-leishmanial medications used for treatment and the development of resistance of the parasite to these medications have led to studies related to definition and formulation of new molecules to date.[37] Different studies have been completed;[12-18] from a study showing the anti-leishmanial activity of ethanol extracts from *Allium sativa* (garlic) and *Azadirachta indica* (Neem), locally used for treatment of Cutaneous leishmaniasis in the Sudan, had no significant difference when compared to pentostam,[16] to studies emphasizing that the anti-leishmanial efficacy on promatigotes and amastigotes of *Leishmania tropica* was higher compared to two antibiotics in the macrolid group of azithromycin and clarithromycin.[38] The AL-EO presented in this study can be considered for use as an antileishmanial.

Untill now, different researchers have reported plant extracts or essential oils with amoebicidal effects such as *Thymus sipyleus* Boiss. subsp. *sipyleus* Boiss. var. *sipyleus* L. at 32.0 mg/mL,[39] *Trigonella foenum-graecum* L. at 400 mg/mL,[40] *Peucedanum caucasicum* (Bieb.) C. Koch., *P. palimbioides* Boiss., *P. chryseum* Boiss. et Heldr. and *P. longibracteolatum* Parolly and Nordt at 32 mg/mL,[41] *Origanum syriacum* L. and *O. laevigatum* Boiss. at 32.0 mg/mL.[42] Additionally, they reported the doses used were not toxic. The AL-EO presented in this study may be used as an antiamoebicidal. Treatment of *T. vaginalis* uses 5-nitroimidazole medications. However, due to many resistant isolates, research into new therapeutic medications has gained importance. In this way, different medications have been trialed researching plants and metabolites.[43] Fernandes *et. al.* used 29 diluted extracts from plants as antiparasitics and reported four plant extracts showed tricomonidal efficacy.[44] These plants were *Securidaca longepedunculata* Fresen. (Polygalaceae, 0.10 mg/mL), *Solanum aculeastrum* Dun. (Solanaceae, 1.06 mg/mL), *Piper kapense* L.f. (Piperaceae, 11.19 mg/mL) and *Cassine transvaalensis* (Burtt. Davy) Codd (Celastraceae, 9.69 mg/mL). Ivanescu *et. al.* reported that the main metabolite of Artemisinin of dihydroartemisinin was an antiparasitic medication against Plasmodium, Schistosoma, Toxoplasma, *Trichomonas vaginalis*, *Leishmania* and *Giardia lintestinalis*.[45] Moon *et. al.* reported that two essential oils derived from *Lavandula angustifolia* Mill. and *Lavandula* × *intermedia* were antiparasitic against *Giardia intestinalis, Trichomonas vaginalis* and the fish pathogen *Hexamita* inflata.[46] The AL-EO presented in this study is considered for use as an antitrichomonal.

In the literature, studies on the EO composition of some *Artemisia* species were reported and these oils were found to have high antiparasitic activity.[47,48] According to results, *Artemisia* species were divided into two sub-groups with regard to EO composition; the first group was characterized by the presence of camphor and 1.8-cineole and the second group included mostly α-thujone. These essential oils possess antiparasitic efficiency. Also, these components are known to have antiparasitic efficiency.[47,49,50]

CONCLUSION

In this study, it was revealed for the first time that EO of *A. ludoviciana* was effective against three important parasites. It is thought that this strong efficacy is due to the camphor and 1,8-cineole identified as the main components of the oil. For AL-EO to be used as medication, there is a need for *in vitro* macrophage cultures for efficacy against *Leishmania* amastigotes and *in vivo* experimental animal model control studies. Again, it is concluded there is a need for advanced studies to research the antitrichomonal and amoebicidal efficacy. At the same time this oil, with determined antiprotozoal activity, is identified to require active molecule purification and clarification to research the cytotoxic mechanism.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ABBREVIATIONS

AL: *Artemisia ludoviciana*; **EO:** Essential oil; **RRI:** Relative retention indices; **FID:** Flame Ionization Detector; **GC-MS:** Gas chromatography-mass spectrometry; **CPLM:** Cysteine-Peptone-Liver-Maltose; **GAE:** Granulamatous amoebic encephalitis; **VL:** Visceral leishmaniasis; **CL:** Cutaneous leishmaniasis; **MAVA:** maleic anhydride-co-vinyl acetate.

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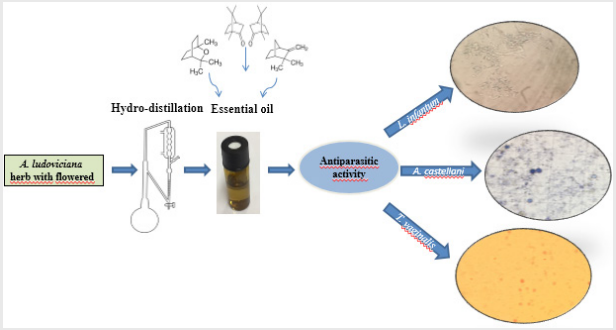
PICTORIAL ABSTRACT



SUMMARY

In this study, it was found to effect *Acanthamoeba castellani*, *Leishmania infantum* and *Trichomonas vaginalis* of *Artemisia ludoviciana* essential oil for the first time. It is assume that this potent efficacy is due to the camphor and 1,8-cineole identified as the main components of the oil. It is a need for advanced studies, such as edited some *in vitro* and *in vivo* experimental models, for research to strong antiprotozoal effects of *A. ludoviciana* oil.

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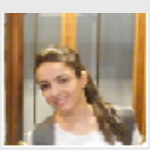

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