In vitro Trypanocidal Activity of Essential Oils of Some Plants against *Trypanosoma evansi*

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ABSTRACT

In this study, the antitrypanosomal action of *Lavandula angustifolia* Mill. (Lavender), *Matricaria chamomilla* L. (chamomile) and *Ocimum basilicum* L. (Basil) essential oils was investigated against *Trypanosoma evansi*. The Gas Chromatography/Mass Spectrometry (GC/MS) was used to analyze these essential oils and identifying their chemical components. The main components present in lavender oil were 1,8-cineole (88.02%) and camphor (8.09%). In chamomile oil, bisabolol oxide (43.81%), cis- α -farnesene (17.79%) and bisabolone oxide (7.71%) were found to be the most predominant components. Basil oil is principally composed of benzene, 1-methoxy-4-(2-propenyl) (52.68%), L-linalool (29.46%) and 1, 8-cineole (9.46%). *In vitro* results revealed that all the tested oils exhibited notable trypanocidal action and inhibited the growth of *T. evansi*. The minimum antitrypanosomal activity was determined by chamomile oil and the strongest was determined by lavender oil. These findings suggest that the tested essential oils could be investigated in future studies for the treatment of *T. evansi*.

Key words: *Trypanosoma evansi*; Trypanocidal activity; Lavender; Chamomile; Basil; Plant essential oils.

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INTRODUCTION

Surra is one of the most common diseases affecting camels in North Africa, resulting in great economic loss (Giordani et al. 2016; Mahmoud et al. 2020). Surra is an animal disease that takes either the acute or a chronic form (Vanhollebeke et al. 2006). Disease caused by *T. evansi* are characterized by quick loss of weight, anemia, swollen limbs, weakness, while animals in chronic cases suffer from neural signs and paralysis of the hind limbs (Rodrigues et al. 2005).

Diminazene aceturate drug is most frequently used for treatment of trypanosomiasis in domestic animals, as it is effective besides its lower cost (Peregrine 1994). On the other hand, this drug had some disadvantages such as, it has no curative efficiency when relapses of parasitemia occur, it is nephrotoxic and hepatotoxic drug (Spinosa et al. 1999) and several cases of resistant parasites have also been reported when it used (Witola et al. 2004). Thus, it is obvious to find out the new antitrypanosomal compounds that are safer and more effective.

Many years ago, medicine has depended on essential oils in the treatment of the infectious diseases produced via various microorganisms (Nibret and Wink 2010). Plant essential oils are complex mixtures consists of different small hydrophobic components that simply cross the biological barriers and membranes. The components of plants' essential oils are able to cross the barrier of blood-brain, because of this feature of essential oils, they are being given much attention to find out new compounds which may provide basis for the development of new antitrypanosomal drugs (Costa et al. 2018).

Previous studies reported that many therapeutic plants, particularly in Africa, showed trypanocidal action due to their phytochemicals components (Razin 2017). Numerous plant essential oils have been indicated with trypanocidal action (Gevú et al. 2019). So, there is an urgent need to determine effective therapeutic agents from local medicinal plants in order to develop new trypanocides. Thus, the present work aims to assess the *in vitro* antitrypanosomal activity of *Lavandula angustifolia*, *Matricaria chamomilla* and *Ocimum basilicum* essential oils against *T. evansi*.

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MATERIALS AND METHODS

Essential Oil Isolation

Three medicinal plants were collected from North West coast, Egypt (Table 1). Formerly, they were identified by taxonomists at Medicinal Department, Desert Research Center, Egypt. The plant leaves were air dried and ground to a powder then the essential oils were isolated by hydro distillation of the powder for 4h in a steam distillation using a Clevenger-type apparatus.

Table 1: Plant species used in the present study

Plant	Common name	Family	plant part used
<i>Lavandula angustifolia</i> Mill.	Lavender	Lamiaceae	Leaves
<i>Matricaria chamomilla</i> L.	Camomile	Asteraceae	Leaves
<i>Ocimum basilicum</i> L.	Basil	Lamiaceae	Leaves

Chemical Composition of Essential Oils using Gas Chromatography/Mass Spectrometry

The Gas Chromatography/Mass Spectrometry (GC/MS) was used to analyze the three essential oils. Varian gas chromatograph equipped with auto-injector and 30-meter DB-1701 fused silica capillary column and mass spectrometer detector (MS) was used for the qualitative compound identification that is based on retention time and relative abundance of three characteristic masses. Data acquisition and processing were controlled by the chemist. High purity (99.99%) grade gases were used. Helium was used as a carrier gas and nitrogen was used as make-up gas. All samples were injected in split mode. To avoid overloading the GC column and the MS detector, the essential oils were diluted 50:1 in ethanol and then split 80:1 in the injector.

Compounds of every oil were recognized by their mass spectra and retention indices. Retention indices of the components were compared with those of authentic samples existing in the database of laboratory. Obtained mass spectra were compared with references from the laboratory database spectra and literature data (Adams 2007).

Parasites

The strain of *T. evansi* was collected from naturally infected camel and then identified in Gene Bank (ID 8W96AJEX01R) by Barghash et al. (2016).

In vitro Trypanocidal Activity of the Tested Essential Oils

The *in vitro* antitrypanosomal action was evaluated in duplicates in 96 well micro titer plates. Ten μ L from serial dilution of the essential oil were incubated with 40 μ L of blood infected with *T. evansi* in the wells of microtiter plates. For control, the 10 μ L diluted essential oil was replaced with phosphate buffer saline in the wells of the microtiter plates. Motility of the parasites was observed under the microscope (x40) at five min intervals for one hour. The drop in motility of *T. evansi* in oil-treated blood as compared to that of *T. evansi*-loaded control blood without essential oil was taken as a measurement of antitrypanosomal activity.

Statistical analyses

The LC₅₀ and LC₉₀ were obtained from toxicity lines and performed with the Ldp-Line software.

RESULTS

Chemical Composition of Essential Oils using GC/MS

The chromatograms (Figs. 1, 2 and 3) revealed the composition of *L. angustifolia*, *M. chamomilla* and *O. basilicum* essential oils used in the present experiments. The identified components in these oils, their retention times and their relative percentages are listed in Tables 2, 3 and 4.

Twenty-five compounds were identified from lavender oil (Table 2). The main constituents were 1,8-cineole (88.02%) and camphor (8.09%) which totaled 96.11% of the chemical composition of the lavender essential oil.

A total of 35 constituents were recognized in chamomile oil corresponding to 99.99% of the total volatile constituents (Table 3). The most abundant components of this oil were bisabolol oxide (43.81%), cis- α -farnesene (17.79%), bisabolone oxide (7.71%), 2-furanmethanol, tetrahydro- α , β ,5-trimethyl-5-(4-methyl-3-cyclohexen-1-yl)-, [2S-[2 α ,5 α (R*)]]-(7.49%) and en-indicodiol (4.77%).

Basil essential oil is consisting of 25 components and it is predominantly composed of benzene, 1-methoxy-4-(2-propenyl) (52.68%), L-linalool (29.46%) and 1,8-cineole (9.46 %) which confirming 91.6% of the total volatile compounds.

In vitro Trypanocidal Activity of the Tested Essential Oils

The *in vitro* trypanocidal action of the three oils (lavender, chamomile and basil) were illustrated in Table 5. Out of three tested essential oils, lavender showed the highest trypanocidal activity (LC₅₀=0.04%, LC₉₀=1.08%) (Table 5). The oil of chamomile had the lowest trypanocidal activity (LC₅₀=0.52%, LC₉₀=4.40%). Essential oil from basil oil showed moderate activity with LC₅₀=0.23% and LC₉₀=2.86%.

DISCUSSION

For centuries, medicinal plants are used as therapeutics worldwide (El-Badry et al. 2010). Natural products of plants are significant sources of new biologically active compounds that are being chemically balanced and cause less side effects than the synthetic drugs (Ullah et al. 2016). Presently, GC/MS was used to analyze the tested essential oils and identifying their chemical components. The main components present in lavender oil were 1, 8-cineole (88.02%) and camphor (8.09%) (Table 2). The occurrence of 1,8-cineole and camphor as major components of the oil from lavender has been mentioned. The oils of the same plant are reported to mainly containing linalool, 1,8-cineole and camphor (Oroian et al. 2019). On the other hand, Mantovani et al. (2013) reported that borneol, *epi*- α -muurolol, α -bisabolol, precocene, eucalyptol, caryophyllene oxide, camphor were the major components of lavender oil. This variation in the essential oil chemical composition may result from the interaction of numerous factors such as soil composition, climatic conditions, solar radiation, collection season, condition and duration of storage and biotic factor (i.e. microorganisms, insects, etc.) (Moghaddam and Farhadi 2015).

Fig. 1: GC/MS chromatogram of *Lavandula angustifolia*. Fig. 2: GC/MS chromatogram of *Matricaria chamomilla*. Fig. 3: GC/MS chromatogram of *Ocimum basilicum*.**Table 2:** Chemical composition of essential oils from leaves of *Lavandula angustifolia*

Retention time	Area	Compound
4.43	0.04	Tricyclene
4.66	0.58	α -Pinene
5.10	0.28	Camphene
5.68	0.10	Sabinene
5.84	0.46	2 α -Pinene
6.19	0.07	2,3-Dehydro-1,8-cineole
7.01	0.05	α -Humulene (CAS)
7.34	0.05	α -Cymene
7.42	0.09	D-Limonene
7.58	88.02	1,8-Cineole
8.41	0.08	γ -Terpinene
8.92	0.05	Trans-Sabinene hydrate
9.56	0.08	Bicyclo [2.2.1] heptan-2-one, 3,3-dimethyl-
11.17	0.07	α -Campholenal
11.67	0.18	trans-Pinocarveol
11.77	0.07	(-)-Trans Pinene
11.89	0.11	cis-Verbenol
12.04	8.09	Camphor
12.61	0.18	Pinocarvone
12.99	0.42	Isoborneol
13.30	0.23	Terpinen-4-ol
14.02	0.38	(1R)-(-)-Myrtenal
14.55	0.08	Bicyclo [3.1.1] hept-3-en-2-one, 4,6,6-trimethyl-, (1S)
29.61	0.19	(-)-Caryophyllene oxide
32.03	0.05	ϵ -Cadinol

Table 3: Chemical composition of essential oils from leaves of *Matricaria chamomilla*

Retention time	Area	Compound
7.97	0.40	1-(1-Methylcyclopentyl)-2-propyn-1-one
21.18	0.19	α -Elemene
22.36	0.14	Caryophyllene
24.01	17.79	Cis α -Farnesene
24.45	0.24	trans-p-Mentha-2,8-dienol
24.97	1.18	Germacrene D
25.10	0.17	4-(2,6,6-Trimethylcyclohexa-1,3-dienyl) but-3-en-2-one
25.31	0.28	α -Selinene (CAS)
25.53	0.88	Bicyclogermacrene
26.03	2.32	β -Farnesene
26.39	0.30	Benzene, (2-ethyl-4-methyl-1,3-pentadienyl)-, (E)-
26.46	0.29	ϵ -Cadinene (CAS)
28.32	0.43	Nerolidol
28.57	0.33	Dendrolasin (CAS)
28.87	1.16	(-)-Spathulenol
29.16	0.24	Megastigmatrienone
30.43	0.30	Lanceol, cis
30.97	0.53	3,3-(Ethylenedioxy) octan-1-ol
31.44	0.83	β -Cadinol
31.86	7.49	2-Furanmethanol, tetrahydro- β , β ,5-trimethyl-5-(4-methyl-3-cyclohexen-1-yl)-, [2S-[2 α ,5 α (R*)]]-
32.11	0.39	8-Methoxyoct-7-en-1-ol
32.19	0.20	cis-Z- β -Bisabolene epoxide
32.95	7.71	Bisabolone oxide
33.10	2.28	β -Bisabolol
33.35	0.22	Caryophyllene oxide
34.75	2.00	Chamazulene
35.64	43.81	Bisabolol oxide
36.20	0.13	2H-Pyran-3-ol, tetrahydro-2,2,6-trimethyl-6-(4-methyl-3-cyclohexen-1-yl)-, [3S-[3 α ,6 α (R*)]]-
38.47	0.55	2-Pentadecanone, 6,10,14-trimethyl-
40.37	4.77	En-in-dicycloether
41.06	0.50	1,6-Dioxaspiro [4.4] non-3-ene, 2-(2,4-hexadiynylidene)-
47.83	0.32	9,12-Octadecadienoic acid (Z, Z)-
52.50	0.24	Hexacosane
54.60	0.57	9-Octadecenamide (CAS)
57.90	0.81	Pentacosane (CAS)

Our results showed that the bisabolol oxide (43.81%), cis- α -farnesene (17.79%), and bisabolone oxide (7.71%) were found to be the most predominant components in chamomile essential oil. Other authors also identified the constituents of this oil such as Pirzad et al. (2006) identified azulene-7-ethyl-1,4-dimethyl, limonene, bisabolol oxides, bisabolone oxide and trans- β -farnesene as the major constituents of chamomile oil. On the other hand, the main constituents identified in chamomile oil were α -bisabolol, α -farnesene, β -farnesene, guaiazulene, α -bisabolol oxide A (Ayoughi et al. 2011).

In the present study, basil oil is principally composed of benzene, 1-methoxy-4-(2-propenyl) (52.68%), L-linalool (29.46%) and 1, 8-cineole (9.46%). Similarly, Santoro et al. (2007) identified linalool and 1,8-cineole as the main constituents of basil oil. Other authors identified linalool as the main component of essential oil from *O. basilicum* (El-Badry et al. 2010). Sajjadi (2006) detected methyl eugenol, methyl cinnamate and methyl chavicol, respectively as the main constituents of the basil oils. The observed variances in the essential oil content may be as a

Table 4: Chemical composition of essential oils from leaves of *Ocimum basilicum*

Retention time	Area	Compound
2.78	0.16	2-Hexanone (CAS)
5.4	0.29	α -pinene
6.53	0.29	Sabinene
6.72	0.66	Geraniol formate
6.99	0.66	α -Myrcene
8.44	0.16	dl-Limonene
8.61	9.46	1,8-Cineole
9.02	0.98	1,3,6 Octatriene,3,7dimethyl
11.28	29.46	L-Linalool
13.50	0.10	Camphor (CAS)
14.46	0.09	Myrcenol
14.85	0.24	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)
15.59	0.14	Linalyl propionate
15.79	52.68	Benzene, 1-methoxy-4-(2-propenyl)
22.70	0.57	Eugenol
23.91	0.19	1,11-Dodecadiyne
24.89	0.76	Benzene, 1,2-dimethoxy-4-(2-propenyl)
25.24	0.26	trans-Caryophyllene
25.78	1.03	trans- α -Bergamotene
26.84	0.24	1,3,7-Octatriene, 3,7-dimethyl
27.96	0.58	1,9 Decadiyne
28.57	0.25	Santolina triene
29.35	0.23	1,11 Dodecadiyne
30.48	0.10	Trans-Ocimene
34.78	0.42	α -Humulene (CAS)

Table 5: *In vitro* antitrypanosomal activity of plant essential oils against *Trypanosoma evansi*

Plant essential oils	LC ₅₀ (%) (95% CI) a	LC ₉₀ (%) (95% CI)	Slope $\pm$ S.E.
Lavender	0.04 (0.02-0.07)	1.08 (0.68-2.53)	0.90 $\pm$ 0.16
Chamomile	0.52 (0.39-0.65)	4.40 (3.19-7.04)	1.39 $\pm$ 0.16
Basil	0.23 (0.13-0.34)	2.86 (2.09-4.60)	1.17 $\pm$ 0.16

a: 95% confidence intervals.

result of variations in climate, soil, plant age, season and other factors that can affect the oil composition (da Silva et al. 2018).

Previous studies have reported anti-inflammatory, anticancer, antioxidant and antimicrobial activities of plant essential oils either in cell or animal models (Sharifi-Rad et al. 2017). The present *in vitro* results revealed that all the tested essential oils exhibited notable trypanocidal action. Similarly, *O. basilicum* oil displayed *in vitro* antitrypanosomal action against *T. cruzi* (Santoro et al. 2007). In the same context, previous studies documented antiparasitic actions of the oils from lavender, chamomile and basil (Luize et al. 2005; El-Badry et al. 2010; Mantovani et al. 2013).

The current results showed that lavender, chamomile and basil exhibited different antitrypanosomal activity against *T. evansi* which was in agreement with the results of Costa et al. (2018) who found that *Lavandula luisieri* exhibited antitrypanosomal action against *T. brucei brucei*. Some other plant essential oils exhibited antitrypanosomal properties against *T. evansi* such as essential oils from *Carapa guaianensis*, *Schinus molle* (Baldișsera et al. 2013), and *Melaleuca alternifolia* (Baldișsera et al. 2014), that are in coordinated with the present results. Similarly, many essential oils have been tested for their antitrypanosomal activity against another

species of *Trypanosoma*. In this context, essential oils from *Juniperus oxycedrus* (Costa et al. 2018) exhibited antitrypanosomal action against *T. brucei brucei*. Likewise, essential oils of *Rosmarinus officinalis* (Pereira et al. 2017) and *Renealmia chrysotricha* (Gevú et al. 2019) displayed effective antiparasitic action against *T. cruzi*.

Presently, lavender, chamomile and basil essential oils inhibited the growth of *T. evansi* that was detected either by disappearing, dissection or non-motility within 60minutes of incubation. Bakkali et al. (2008) stated that the essential oils could interact with the parasite cell membrane, causing loss of membrane permeability and subsequent cell death. Taking into consideration the number of the oil components and the interactions among them, it is probably that the essential oils have further cell targets such as proteins and lipids (Borges et al. 2012). Therefore, the present study suggest that the tested oils could be investigated in future studies for the treatment of *T. evansi*.

Conclusion

The antitrypanosomal activity of *L. angustifolia* (Lavender), *M. chamomilla* (Chammomile) and *O. basilicum* (Basil) against *T. evansi* (ID 8W96AJEX01R) has been reported in the present study for the first time. Our results revealed that these plants could be a potential source of antitrypanosomal agents. In the current study, GC/MS was used to identify the chemical composition of these essential oils. However, investigations to identify the active antitrypanosomal compounds from these plants are in progress. It is hoped that these active compounds could be developed as antitrypanosomal agent.

Authors' contributions

EAR conducted the experiments, carried out the statistical analysis, constructed tables and figures and wrote/revised the manuscript. HS and TRA supervisor the work and revised the manuscript. TSB save the strain of *T. evansi* in liquid nitrogen. All authors read and approved the final manuscript.

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