Veterinary Parasitology 266 (2019) 84–87

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Short communication

Towards green drugs against cestodes: Effectiveness of *Pelargonium roseum* and *Ferula gummosa* essential oils and their main component on *Echinococcus granulosus* protoscoleces

Mohaddeseh Abouhosseini Tabari[a], Mohammad Reza Youssefi[b,*], Mojtaba Nasiri[c], Mojtaba Hamidi[c], Komeil Kiani[c], Shohre Alian Samakkhah[a], Filippo Maggi[d]

[a] Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Amol, Iran
[b] Department of Veterinary Parasitology, Babol-Branch, Islamic Azad University, Babol, Iran
[c] Faculty of Veterinary Medicine, Babol-Branch, Islamic Azad University, Babol, Iran
[d] School of Pharmacy, University of Camerino, Camerino, Italy

ARTICLE INFO

Keywords:
Cystic echinococcosis
Hydatidosis
Essential oils
Protoscoleses
Surgery

ABSTRACT

Cystic echinococcosis is of great public health importance. Surgery is the efficient treatment for this infection. To minimize the risk of cyst intraoperative leakage, using scolicidals is crucial. To date, any efficacious scolicidal without side effect has not been introduced. Since essential oils of *Pelargonium roseum* and *Ferula gummosa* have shown several bioactivities, we evaluated their potential against protoscoleces of *E. granulosus* using albendazole as positive control. Furthermore, chemical composition of both essential oils was analyzed by gas chromatography-mass spectrometry (GC-MS) analyses, and their main constituents were also evaluated for scolicidal activity. Different concentrations of essential oils and their two main constituents were tested for scolicidal activity. Mortality rate was measured by eosin staining. Results of GC-MS revealed citronellol and β-pinene as the main constituents of *P. roseum* and *F. gummosa* essential oils, respectively. After 60 min of exposure to 50 µg/mL of *P. roseum* and *F. gummosa,* mean mortality rate of protoscoleces was 100%. However, β-pinene and citronellol at the same time point with only 10 µg/mL concentrations resulted in approximately higher than 80% mortality. Essential oils of *P. roseum* and *F. gummosa* showed significant toxic effect on *E. granulosus* with 50% lethal concentration (LC_{50}) values of 8.52 and 17.18 µg/mL, respectively. Based on the LC_{50} values, β-pinene (2.20 µg/mL) was the most potent scolicidal agent in the present study. The overall toxicity of β-pinene and citronellol was significantly higher than the whole essential oils of *F. gummosa* and *P. roseum*. Based on these results, β-pinene and citronellol can be considered as candidate ingredients for the development of green scolicidals.

1. Introduction

Cystic echinococcosis caused by metacestodes of *Echinococcus granulosus* is a substantial zoonosis with considerable socio-economic impact, affecting both humans and susceptible animals in many parts of the world. Adult cestodes in the small intestine of carnivorous definitive host develop eggs which are voided with the faeces and contaminate the environment. Following accidental ingestion of the eggs, metacestodes develop fluid-filled cystic structure that produces large numbers of protoscoleces within it. Cysts can occur in any organ system and clinical features of this disease vary depend on the organ involved (Thompson et al., 2017).

Surgery is the most efficient treatment of cystic echinococcosis. One of the main complications in cystic echinococcosis surgery is rupture and dissemination of infective protoscoleces during surgery (Dziri et al., 2009). An approach to manage complicated cystic echinococcosis surgery is application of scolicidal agents to prevent potential re-infection. To date, many scolicidals have been introduced, but most of them possess local or systemic side effects (Moazeni et al., 2012). Therefore, novel scolicidals with lower side effects are needed to optimize the treatment protocol of hydatidosis disease.

Nowadays, plant-derived compounds are widely considered in natural product research aimed to discover alternative sources of antiparasitic agents. However, only a limited amount of research efforts has been devoted to evaluate the scolicidal activity of selected essential oils (EO) against protoscoleces of *E. granulosus* (Maggiore et al., 2012;

* Corresponding author.
E-mail addresses: youssefi929@hotmail.com, youssefi@baboliau.ac.ir (M.R. Youssefi).

https://doi.org/10.1016/j.vetpar.2018.12.019
Received 21 September 2018; Received in revised form 27 December 2018; Accepted 29 December 2018

Moazeni et al., 2012, 2015).

Pelargonium roseum Willd is a species belonging to the Geraniaceae family. It is native to Southern Africa and widely grown as ornamental plant all over the world. *P. roseum* has woody stems and leaves covered with short rough hairs which give the plant a sweet odor (Carmen and Hancu, 2014). Several bioactivities including anti-trichomonal and insect repellence have been reported for the EO of *P. roseum* (Tabari et al., 2018; Tabari and Youssefi, 2018) allowing us to hypothesize its potential efficacy against cestodes of medical and veterinary importance.

Ferula gummosa Boiss. (Apiaceae) is one of the most important Iranian medicinal plants growing in the northern and western mountainous areas. This species is a resinous plant; the oleo-gum-resin of this plant species and the EO derived from it are widely used in the Iranian traditional medicine, making it a perfect candidate to be tested against *E. granulosus* cestodes (Mahboubi, 2016).

Based on earlier reports showing relevant toxicity of these EOs both on pathogens and vectors of high public health importance, we selected *P. roseum* and *F. gummosa* as new potential sources of novel natural products to be used for the development of effective and eco-friendly scolicidal drugs. Herein, the chemical composition of both EOs was analyzed by GC-MS analyses. Furthermore, we evaluated the in vitro toxicity of *P. roseum* and *F. gummosa* EOs and the main chemical constituents of both EOs for their scolicidal activity against *E. granulosus*.

2. Material and methods

2.1. Plant material and isolation of essential oils

The oleo-gum-resin of *F. gummosa* and fresh leaves of *P. roseum* were collected from Kashan, Kashan, 34.0351 °N, 51.0671 °E, Iran. *P. roseum* EO was obtained by Clevenger-type apparatus according to the method described previously (Tabari et al., 2017) and yielded 0.96%. Oleo-gum-resin of *F. gummosa* was subjected to hydro-distillation and yielded 10.2% of pale yellowish oil.

2.2. GC–MS analysis

Gas chromatography-mass spectrometry (GC–MS) analysis was performed to reveal chemical constituents of the EOs. The GC–MS analysis was carried out on an Agilent 6890 N gas chromatograph equipped with 5973 N mass selective detector and a HP5-MS capillary column (30 m × 0.25 mm i.d. × 0.1 μm film thickness). The operating conditions were as follows: the oil samples (6 μL) were diluted to 1% with *n*-hexane and the carrier gas was helium at flow rate of 1.0 mL/min; the oven temperature was programmed as follows: 60 °C held for 5 min then raised to 220 °C at 4 °C/min, then raised to 280 °C at 11 °C/min, held for 15 min; the injector and detector temperatures were set to 280 °C. The EO components were identified by correspondence of their retention indices, relative to a series of C_8-C_{30} *n*-alkanes, and the MS fragmentation with respect to those contained in commercial libraries (Adams, FFNSC2 and NIST17). The relative abundance (%) of components was calculated from the integration area of the chromatographer without using correction factor.

2.3. Collection of protoscoleces and viability assay

E. granulosus hydatid cysts were collected from livers of naturally infected sheep slaughtered at an abattoir located in Sari (Mazanadran Province, Iran). Viability of *E. granulosus* protoscoleces was assessed by 0.1% aqueous Eosin stain. Samples containing protoscoleces in the sediment with viability rate of 95% or higher were considered to be appropriate for further experiments.

2.4. Scolicidal assay

Four different concentrations (10, 20, 50, and 100 μg/mL) of *P. roseum* and *F. gummosa* EOs and their two main constituents (1, 2.5, 5 and 10 μg/mL) in normal saline solution were tested for their scolicidal activities. Dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Germany) 0.5% was used as emulsifier. In each experiment, 2 mL of the solution were poured in 24-well plates; afterwards a drop of protoscolex-rich sediment (containing at least 1×10^3 *E. granulosus* protoscoleces) was added. Thereafter, the plates were incubated at 37 °C in an incubator shaker. After each test time period (10, 30, 60 and 120 min), 100 μL of the sample were taken from each well and poured on a scaled glass slide. Mortality rates were noted by eosin staining. Control group was treated with normal saline solution containing 0.5% DMSO. Albendazole was used as positive control, at testing concentrations of 1 and 2 μg/mL in DMSO (Pensel et al., 2017). All the experiments were repeated three times for each tested concentration.

2.5. Statistical analysis

Differences between the means of mortality rate in different exposure times in each concentration of tested compounds were analyzed by Repeated measures ANOVA followed Bonferroni *post hoc* test. Differences between the means of mortality rate in different tested compound in each time of exposure (10, 30, 60, and 120 min) were analyzed by one-way analysis of variance (ANOVA) followed by Tukey-HSD *post hoc* test. For calculation of 50 and 90% lethal concentrations (LC_{50} and LC_{90}), Probit regression analysis was used. Data analysis was done using SPSS statistical package (version 23.0) (SPSS Inc., Chicago, IL, USA). For all analyses, ($P < 0.05$) was considered statistically significant.

3. Results and discussion

3.1. Chemical composition of the essential oils

The chemical composition of *P. roseum* and *F. gummosa* EOs was shown in Tables 1 and 2. A total of 36 volatile components were identified in the EO from *P. roseum*, accounting for 99.0% of the total composition. The oxygenated monoterpenes (90.9%) dominated the oil chemical profile, with citronellol (37.7%), geraniol (17.6%) and citronellyl formate (11.0%) as the most abundant compounds. A total of 26 compounds were identified in the EO from *F. gummosa*, accounting for 89.0% of the total composition. Notably, the oil composition was dominated by monoterpene hydrocarbons (67.1%), with β-pinene (57.0%) as the predominant component.

The chemical profile of *P. roseum* in the present study was quite consistent with that previously reported by us, except for the presence of citronellyl formate (Tabari et al., 2018). On the other hand, the study of Dabiri et al., (2011) reported citronellol (27.3%), citronellyl formate (17.2%), (*E*)-caryophyllene (10.3%) as the major compounds but not geraniol as in our case. Concerning *F. gummosa*, previous studies conducted on several populations growing in Iran highlighted α-pinene (17.00–56.55%) and β-pinene (7.95–37.04%) as the two major volatile constituents of the oleo-gum-resin (Malekzadeh et al., 2018). Thus, the hallmark of the accession examined was the little amount of α-pinene (4.1%) compared with β-pinene (57.0%).

3.2. Scolicidal activity

Fig. 1 shows the mortality rates of *E. granulosus* protoscoleces over different times of exposure to *P. roseum*, *F. gummosa*, β-pinene, and citronellol in comparison to albendazole and the negative control. For all tested compounds, a significant effect of the tested concentration ($F_{3,176} = 130.582$, $P < 0.001$), the time of exposure ($F_{3,132} = 264.517$, $P < 0.001$) and their interaction ($F_{9,176} = 3.253$, $P = 0.001$) were noted.

As can be seen in Fig. 1, the EO from *P. roseum* showed a potent scolicidal activity. Indeed, after 10 min of treatment, this EO at 100 μg/

Table 1
Chemical composition of the essential oil from Pelargonium roseum.

No	Component[a]	RI exp.[b]	RI lit.[c]	%[d]	ID[e]
1	α-pinene	927	932	0.5 ± 0.1	a,b,c
2	myrcene	990	988	Tr[f]	a,b,c
3	*p*-cymene	1022	1020	0.1 ± 0.0	a,b,c
4	limonene	1025	1024	0.3 ± 0.0	a,b,c
5	*cis*-linalool oxide	1071	1067	0.1 ± 0.0	a,b
6	*trans*-linalool oxide	1087	1084	Tr	a,b
7	linalool	1100	1095	6.4 ± 1.1	a,b,c
8	*cis*-rose oxide	1110	1106	1.2 ± 0.3	a,b
9	*trans*-rose oxide	1126	1122	0.4 ± 0.1	a,b
10	menthone	1150	1148	3.0 ± 0.6	a,b
11	*iso*-menthone	1160	1158	3.8 ± 0.8	a,b
12	α-terpineol	1187	1186	0.5 ± 0.1	a,b,c
13	γ-terpineol	1195	1199	Tr	a,b
14	citronellol	1232	1223	37.7 ± 3.6	a,b,c
15	neral	1241	1235	0.2 ± 0.0	a,b,c
16	geraniol	1257	1249	17.6 ± 2.8	a,b,c
17	geranial	1271	1264	0.4 ± 0.1	a,b,c
18	citronellyl formate	1276	1271	11.0 ± 1.9	a,b
19	geranyl formate	1303	1298	4.3 ± 0.9	a,b
20	α-cubebene	1344	1345	0.1 ± 0.0	a,b
21	citronellyl acetate	1355	1350	0.6 ± 0.2	a,b,c
22	α-copaene	1368	1374	0.3 ± 0.1	a,b
23	β-bourbonene	1376	1387	0.8 ± 0.2	a,b
24	geranyl acetate	1385	1379	0.6 ± 0.1	a,b
25	(*E*)-caryophyllene	1409	1417	1.2 ± 0.3	a,b,c
26	6,9-guaiadiene	1435	1442	0.1 ± 0.0	a,b
27	citronellyl propanoate	1444	1444	1.2 ± 0.3	a,b
28	geranyl propanoate	1476	1476	0.5 ± 0.1	a,b
29	α-muurolene	1493	1500	Tr	a,b
30	γ-cadinene	1505	1513	0.1 ± 0.0	a,b
31	*trans*-calamenene	1516	1521	0.5 ± 0.1	a,b
32	δ-cadinene	1517	1522	0.4 ± 0.1	a,b
33	citronellyl butanoate	1529	1530	0.3 ± 0.0	a,b
34	α-calacorene	1534	1542	Tr	a,b
35	geranyl butanoate	1562	1562	1.0 ± 0.2	a,b
36	10-*epi*-γ-eudesmol	1607	1622	3.6 ± 0.6	a,b
	Total identified (%)			99.0 ± 0.4	
	Grouped compounds (%)				
	Monoterpene hydrocarbons			1.0	
	Oxygenated monoterpenes			90.9	
	Sesquiterpene hydrocarbons			3.5	
	Oxygenated sesquiterpenes			3.6	

[a] The order of components is according to the elution from a HP-5MS (30 m × 0.25 mm i.d. × 0.1 μm f.t.) capillary column.
[b] Linear retention index calculated using the Van den Dool and Kratz (1963) formula.
[c] Linear retention index value taken from Adams (2007).
[d] Relative percentage values are mean of three determinations ± SD.
[e] Identification method: a, comparision of the calculated RI with that of Adams (2007); b, MS matching with ADAMS, FFNSC2 and NIST 17 libraries; c, comparison with analytical standard (Sigma-Aldrich).
[f] Tr, traces, % < 0.1.

Table 2
Chemical composition of the essential oil from Ferula gummosa.

No	Component[a]	RI[b]	RI Lit.[c]	%[d]	ID[e]
1	α-thujene	921	924	1.0 ± 0.2	a,b
2	α-pinene	927	932	4.1 ± 0.8	a,b,c
3	camphene	940	946	0.1 ± 0.0	a,b,c
4	sabinene	966	969	0.4 ± 0.1	a,b,c
5	β-pinene	969	974	57.0 ± 3.1	a,b,c
6	myrcene	990	988	1.3 ± 0.3	a,b,c
7	α-phellandrene	1003	1003	Tr[f]	a,b,c
8	δ-3-carene	1008	1008	1.2 ± 0.3	a,b,c
9	*p*-cymene	1022	1020	0.3 ± 0.1	a,b,c
10	β-phellandrene	1025	1024	0.9 ± 0.2	a,b
11	(*Z*)-β-ocimene	1037	1034	0.7 ± 0.2	a,b,c
12	(*E*)-β-ocimene	1047	1044	0.1 ± 0.0	a,b,c
13	*trans*-pinocarveol	1134	1135	Tr	a,b,c
14	carvacrol, methyl ether	1243	1241	0.4 ± 0.1	a,b
15	bornyl acetate	1282	1287	0.2 ± 0.0	a,b,c
16	α-terpinyl acetate	1347	1346	0.1 ± 0.0	a,b
17	α-copaene	1368	1374	0.1 ± 0.0	a,b
18	2-*epi*-β-funebrene	1401	1411	2.5 ± 0.5	a,b
19	β-cedrene	1407	1419	5.9 ± 1.0	a,b
20	α-humulene	1443	1452	Tr	a,b,c
21	unknown sesquiterpene $M^+ = 204$	1460		7.4 ± 1.5	b
22	β-acoradiene	1465	1469	0.3 ± 0.0	a,b
23	*ar*-curcumene	1479	1479	0.3 ± 0.0	a,b
24	δ-cadinene	1517	1522	0.4 ± 0.1	a,b
25	β-sesquiphellandrene	1519	1521	0.4 ± 0.1	a,b
26	β-acorenone	1679	1697	11.4 ± 2.1	a,b
	Total identified (%)			96.3 ± 1.3	
	Grouped compounds (%)				
	Monoterpene hydrocarbons			67.1	
	Oxygenated monoterpenes			0.7	
	Sesquiterpene hydrocarbons			17.1	
	Oxygenated sesquiterpenes			11.4	

[a] The order of components is according to the elution from a HP-5MS (30 m × 0.25 mm i.d. × 0.1μm f.t.) capillary column.
[b] Linear retention index calculated using the Van Den Dool and Kratz (1963) formula.
[c] Linear retention index value taken from Adams (2007).
[d] Relative percentage values are mean of three determinations ± SD.
[e] Identification method: a, comparision of the calculated RI with that of Adams (2007); b, MS matching with ADAMS, FFNSC2 and NIST 17 libraries; c, comparison with analytical standard (Sigma-Aldrich).
[f] Tr, traces, % < 0.1.

mL, killed 62.15% of the protoscoleces. On the other hand, the EO from *F. gummosa* at the same dose and time point was less toxic to protoscoleces, with 32.64% mortality. *P.* roseum and *F. gummosa* EOs at the concentration of 50 μg/mL after 60 min of treatment killed 100% protoscoleces. The mean mortality rate of protoscoleces at 50 and 100 μg/mL concentrations of *P. roseum*, and *F. gummosa* EOs in comparison between 60 and 120 min time of exposure didn't show any significant difference ($P < 0.05$), while at 10 and 20 μg/mL between 60 and 120 min time of exposure a significant difference was noted ($P < 0.05$). EO of *P. roseum* and *F. gummosa* showed promising scolicidal effects with LC_{50} values of 8.52 and 17.18 μg/mL, respectively. After 60 min of exposure, 2.5 μg/ml beta-pinene resulted in the higher than 50%, mortality rate in protoscoleces, whilst the same result was noted in citronellol at the concentration of 5 μg/ml. The mean mortality rate of protoscoleces after 60 min exposure to β-pinene and citronellol at the concentrations of 10 μg/mL was higher than 80%. The mean mortality rate of protoscoleces at 1 μg/ml β-pinene and citronellol between 10 and 30 min of exposure didn't show any significant difference ($P < 0.05$) but at 2.5, 5, 10 μg/ml concentrations between all exposure times significant difference was observed ($P < 0.05$) (Fig. 1). Besides, based on the obtained LC_{50} values, β-pinene (LC_{50} of 2.20 μg/mL) was a more active scolicidal agent than citronellol (LC_{50} of 4.88 μg/mL).

A number of researches have reported that the whole EO is generally more potent in comparison with its individual major components, demonstrating that the minor constituents might have a synergistic effect and be important for the bioactivity of the EO (Burt, 2004). In the present study, EO of *P. roseum* at the concentration of 10 μg/mL after 120 min caused 79.90% mortality in protoscoleces of hydatid cyst; however, its main constituent, citronellol, at the same concentration and time point attained 99.33% of mortality rate. According to the result, by comparing LC_{50} values of *P. roseum* EO and its major component citronellol (8.52 vs 4.88 μg/mL), we can assume that antagonistic effects between citronellol and other minor components of the EO occur. EO of *F. gummosa* at the concentration of 10 μg/mL after 120 min caused 57.66% mortality in protoscoleces. However, its main constituent, β-pinene, at the same concentration and time point attained 97.66% of mortality rate. These results led to LC_{50} values of 17.18 μg/mL for *F. gummosa* EO and 2.20 μg/mL for β-pinene; thus also in this case a significant higher scolicidal activity for the major constituent in comparison to the whole EO was observed. It can be

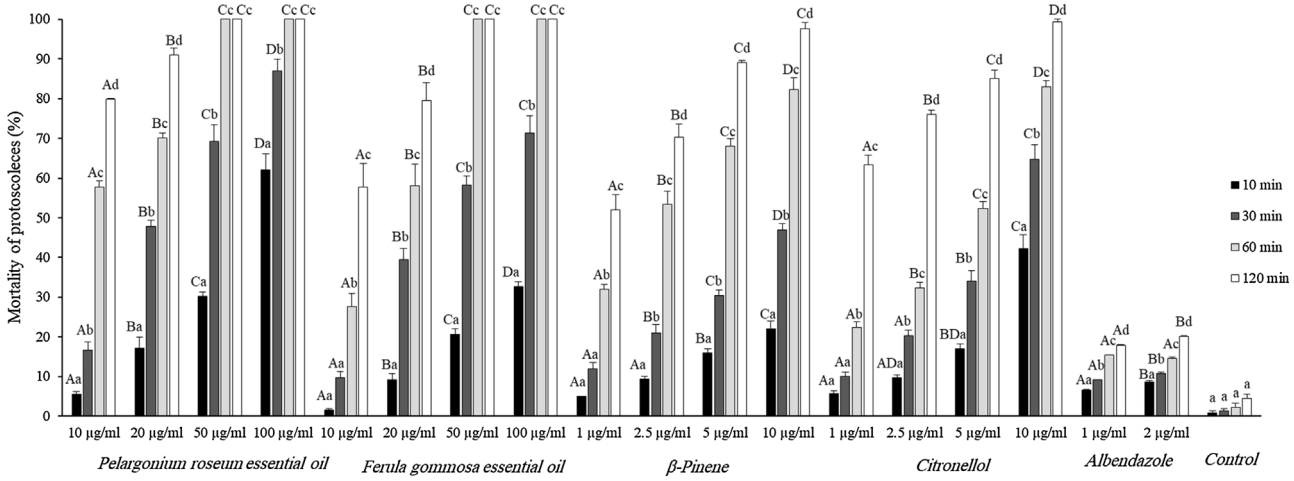

Fig. 1. Mortality (%) of *Echinococcus granulosus* protoscoleces over different times of exposure to *Pelargonium roseum and Ferula gummosa* essential oils, and their pure major constituents β-pinene and citronellol, in comparison to albendazole and the negative control.
T-bars represent SE. Within each concentration of tested compounds, columns marked with different letters (lowercase) are significantly different between times of exposure (Repeated measures ANOVA, Bonferroni test, $P < 0.05$).
Within each tested compounds, columns marked with different letters (uppercase) in each time of exposure (10, 30, 60, and 120 min separately) are significantly different between concentrations (ANOVA, Tukey's HSD test, $P < 0.05$).

speculated that also in the EO of *F. gummosa*, other minor components may have had an antagonistic effect on the scolicidal activity of β-pinene. Possible antagonistic activity between components of EOs has been previously reported (Botelho et al., 2007; Cox et al., 2001). For instance, it has been shown that mixture of terpinen-4-ol and *p*-cymene, two monoterpene compounds from *Melaleuca alternifolia* (tea tree) EO, in 50:50 w/w ratio resulted in reduced bioactivity, and terpinen-4-ol on its own was significantly more active as an antimicrobial agent against *Staphylococcus aureus* (Cox et al., 2001). Botelho et al. (2007) have demonstrated higher efficacy of thymol and carvacrol, the major components of *Lippia sidoides* EO, in comparison with the whole EO, suggesting that the minor components may contribute to an antagonistic effect on the activity of the whole EO (Botelho et al., 2007). Albendazole, tested as positive control, led to significant mortality of protoscoleces, if compared with the control group. After 120 min of treatment at the doses of 1 and 2 µg/mL, mortality rates of protoscoleces were 17.95% and 20.17%, respectively.

From a physiological point of view, the excellent scolicidal activity we observed for *P. roseum* and *F. gummosa* EOs can be due to their major constituents, citronellol and β-pinene, which are able to alter permeability of cell membranes causing accumulation and damages in excretory and intestinal cells of some parasites such as *Anisakis simplex* (Hierro et al., 2006).

Results of the present study shed light on the importance of minor constituents of the EOs which may exert antagonistic effect on the bioactivity of the whole EO. Further studies for evaluating scolicidal activity of other components of these EOs and their possible interactions in combinations are needed to find mixture containing different substances with potential synergistic effects which is significantly important because complex mixtures can hinder the ability of parasite to intoxicate substances and the emergence of resistance.

Conflict of interest

The Authors declare no competing interest.

Acknowledgement

The authors would like to express their gratitude to the Prof. Dr. Giovanni Benelli, University of Pisa, for his insightful comments and review.

References

Adams, R.P., 2007. Identification of Essential Oil Components by Gas Chromatograph/ Mass Spectrometry. Allured Publishing Corporation, Carol Stream, Illinois.
Botelho, M., Nogueira, N., Bastos, G., Fonseca, S., Lemos, T., Matos, F., Montenegro, D., Heukelbach, J., Rao, V., Brito, G., 2007. Antimicrobial activity of the essential oil from *Lippia sidoides*, carvacrol and thymol against oral pathogens. Braz. J. Med. Biol. Res. 40, 349–356.
Burt, S., 2004. Essential oils: their antibacterial properties and potential applications in foods—a review. Int. J. Food Microbiol. 94, 223–253.
Carmen, G., Hancu, G., 2014. Antimicrobial and antifungal activity of *Pelargonium roseum* essential oils. Adv. Pharm. Bull. 4, 511.
Cox, S., Mann, C., Markham, J., 2001. Interactions between components of the essential oil of *Melaleuca alternifolia*. J. Appl. Microbiol. 91, 492–497.
Dabiri, M., Sefidkon, F., Yousefi, M., Bashiribod, S., 2011. Volatile components of Pelargonium roseum R. Br. J. Essent. Oil Bear. Pl. 14, 114–117.
Dziri, C., Haouet, K., Fingerhut, A., Zaouche, A., 2009. Management of cystic echinococcosis complications and dissemination: where is the evidence? World J. Surg. 33, 1266–1273.
Hierro, I., Valero, A., Navarro, M.C., 2006. In vivo larvicidal activity of monoterpenic derivatives from aromatic plants against L3 larvae of *Anisakis simplex* sl. Phytomedicine 13, 527–531.
Maggiore, M.A., Albanese, A.A., Gende, L.B., Eguaras, M.J., Denegri, G.M., Elissondo, M.C., 2012. Anthelmintic effect of Mentha spp. essential oils on Echinococcus granulosus protoscoleces and metacestodes. Parasitol. Res. 110, 1103–1112.
Mahboubi, M., 2016. *Ferula gummosa*, a traditional medicine with novel applications. J. Diet. Suppl. 13, 700–718.
Malekzadeh, M., Angourani, H.R., Yazdinezhad, A., Hassan, M., Abiodun, F., Hazrati, S., 2018. Evaluation of volatile oil in indigenous populations of *Ferula gummosa* boiss. J. Essent. Oil Bear. Plants 21, 206–213.
Moazeni, M., Saharkhiz, M.J., Hosseini, A.A., 2012. In vitro lethal effect of ajowan (*Trachyspermum ammi* L.) essential oil on hydatid cyst protoscoleces. Vet. Parasitol. 187, 203–208.
Moazeni, M., Larki, S., Pirmoradi, G., Rahdar, M., 2015. Scolicidal effect of the aromatic water of *Zataria multiflora*: an in vitro study. Comp. Clin. Path. 24, 1057–1062.
Pensel, P.E., Elissondo, N., Gambino, G., Gamboa, G.U., Benoit, J., Elissondo, M.C., 2017. Experimental cystic echinococcosis therapy: in vitro and in vivo combined 5-fluorouracil/albendazole treatment. Vet. Parasitol. 245, 62–70.
Tabari, M.A., Youssefi, M.R., 2018. *In vitro* and *in vivo* evaluations of *Pelargonium roseum* essential oil activity against *Trichomonas gallinae*. Avicenna J. Phytomed. 8, 136.
Tabari, M.A., Youssefi, M.R., Esfandiari, A., Benelli, G., 2017. Toxicity of β-citronellol, geraniol and linalool from *Pelargonium roseum* essential oil against the West Nile and filariasis vector *Culex pipiens* (Diptera: culicidae). Res. Vet. Sci. 114, 36–40.
Tabari, M.A., Moghaddam, H.A., Maggi, F., Benelli, G., 2018. Anxiolytic and antidepressant activities of *Pelargonium roseum* essential oil on Swiss albino mice: possible involvement of serotonergic transmission. Phytother. Res. 32, 1014–1022.
Thompson, A., Lymbery, A.J., Deplazes, P., 2017. Echinococcus and Echinococcosis 95 Academic Press.
Van Den Dool, H., Kratz, P.D.J.A., 1963. Generalization of the retention index system including linear temperature programmed gas–liquid partition chromatography. J. Chromatogr. 11, 463–471.