Scolicidal activity of essential oils and nanoemulsions of Satureja sahendica and Artemisia dracunculus on hydatid cysts protoscoleces

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Research Article

Keywords: Satureja sahendica, A.dracunculus, nanoemulsions, Hydatidosis

Posted Date: May 4th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1439706/v1

Abstract

Hydatidosis is a substantial zoonosis infection with a significant socio-economic burden. Surgery is the most effective treatment of hydatidosis. The utilization of scolicidal agents is a method to minimize the risk of distribution of infective protoscoleces during surgery. So far, most of the available scolicidals result in local or systemic side effects. Considering essential oils of *Artemisia dracunculus* and *Satureja sahendica* have shown several bioactivities, we decided to characterize *A.dracunculus* and *S.sahendica* nanoemulsions and evaluate the scolicidal activity of these two nanoemulsions and the EOs using albendazole as positive control. To reach this goal, 10^3 protoscoleces of *E.granulosus* in 1 ml medium M199 were exposed to four concentrations of 1, 2, 5, and 10 µg/ml of EOs and nanoemulsions at 30, 60, and 120-minute time points. For all tested plants, a significant effect of the treatments ($F_{5,60}=61.47$, $P<0.001$), the time of exposure ($F_{2,120}=170.72$, $P<0.001$) and their interaction ($F_{10,120}=4.32$, $P<0.001$) were noted. The overall mortality rate in protoscoleces after exposutre to *S. sahendica* nanoemulsion was significantly higher than other treatments and it was 83.13% (P 0.05). Among all the tested compounds of the present study, nanoemulsion of *S.sahendica* demonstrated the highest toxicity on protoscoleces and may serve as a candidate for research and development on novel scolicidas.

Introduction

Hydatidosis caused by metacestodes of *Echinococcus granulosus* is a substantial zoonosis infection with a significant socio-economic burden, affecting both humans and herbivore animals worldwide. Eggs are disposed in the environment from mature cestodes that live in the small intestine of carnivorous. Fallowing ingestion of the eggs, metacestodes generate fluid-filled cystic structures, sometimes containing great numbers of protoscoleces inside it. Cysts can affect any organ of body and make clinical feature(s) depending on the infected organ (Sachar et al. 2014).

Surgery is the most effective treatment of hydatidosis. One of the main difficulties associated with hydatidosis surgery is rupture and distribution of infective protoscoleces during surgery. A method to control complicated hydatidosis surgery is utilization of scolicidal agents to rule out possible re-infection occurrence. So far, most of the available scolicidals result in local or systemic side effects (Rouhani et al. 2013). Recent researches have focused on new resources especially safe herbals to find plant-derived compounds with scolicidal activity. Thus, we need to find novel and potent medicinal herbs with higher effectiveness and lower side effects to improve the treatment procedure of hydatidosis.

Plant biologically active compounds are used broadly in natural product investigations to spot new sources of antiparasitic agents. *Satureja sahendica* Bornm. (Labiatae family) is a native plant of western and northwest of Iran. SSB is a late-blooming species (late summer and fall), growing on rock walls and rocky hills (Alizadeh et al. 2013). Several bioactivities, including antibacterial and antifungal, have been reported for the EO of *S.sahendica* (Yousefzadi et al. 2012). Kheirandish and coworkers (2011) reported antileishmanial effect for *S.khuzestanica*, another member of the Labiatae family (Kheirandish et al. 2011). *Artemisia dracunculus* L. (tarragon) (Asteraceae family) is a perennial plant (Alasvand Zarasvand

et al. 2016) that is spread over nearly all temperate Asia, western North America, and central Europe (Zarezade et al. 2018). The EO of *A.dracunculus* L. is rich of alkaloids, flavonoids and glycosides. Mueller et al. (2004) have reported the antitumor, antifungal and insect repellent effects of *A.dracunculus* (Mueller et al. 2004). Furthermore, it has been showed that *A.dracunculus* possesses the most antileishmanial effect between all *Artemisia* species (Emami et al. 2012).

In the recent decade, nanotechnology has provided a promising potential for research and development of novel delivery systems that resulted in the better delivery of the drug to its site of action, increase in therapeutic efficacy, reduction in the needed dose, and consequently decrease in the side effects. Nanosystems have the advantages of promoting absorption, distribution and tissue uptake of the drug alongside protecting the active substance from degradation (Echeverría & Albuquerque, 2019). Several studies have reported higher efficacy of nano-based delivery systems of EOs on pathogenic organisms (Baldissera et al. 2013; de Moraes et al. 2018; Pavoni et al. 2019).

Based on the above, herein we decided to develop and characterize *A.dracunculus* and *S.sahendica* nanoemulsions and evaluate the scolicidal activity of these two nanoemulsions and the EOs against hydatid cysts protoscoleces. Also, compare the activities with albendazole as the standard treatment of hydatid cyst.

Materials And Methods

Preparation of nanoemulsions

A.dracunculus and *S.sahendica* EO were prepared by hydrodistilation and stored at dark and cold place (4°C). The maximum ability of EOs to solubilized in Milli-Q water (Merck Millipore, France) in nanoscale was investigated by Shahavi et al., 2019, and 2016 (Shahavi et al. 2016; Shahavi et al. 2019). This method mixed oil and water phases by creating ultrasonic waves and causes the scattered phase particles to be dispersed more evenly in a nano size in the continuous phase. For this purpose, the synthesis grade of Sorbitan monolaurate (Span 80™, Merck, Germany), and Poly sorbitan monooleate (Tween 80™, Merck, Germany) at the weight ratios 56:44 was employed as the carrier of the *A.dracunculus* and *S.sahendica* oils to water nanoemulsions. EOs at concentrations of 1000 to 2000 µl/ml, with Milli-Q water and mixture of 5 wt% surfactants were blended. After that, the nanoemulsions were transferred to an ultrasonic processor (UP400S, Dr Haschler, Germany) with a maximum power of 400 W and frequency of 20 kHz with the ultrasonic time of 300 seconds, and the ultrasonic device prepared the stable nanoemulsions with the ultrasonic cycle of 0.75% and ultrasonic intensity of 208 W/cm2. Finally, the nanoemulsions droplet size distribution and mean size were determined by dynamic light scattering (Zetasizer Nano Series, ZEN 3600, Malvern, UK). Measurements were made at 25°C, and each measurement was performed three times. The Zetasizer Software (version 7.13) was used to collect and analyze the data.

Collection of hydatid cyst protoscoleces

Infected sheep livers were provided from slaughterhouse in Amol, Mazandaran province, Iran, and transferred to the parasitology laboratory of veterinary medicine faculty, Islamic Azad University, Babol Branch, Iran. The livers surfaces were washed and disinfected with 70% alcohol-soaked cotton, and the contents of cysts, including *E. granulosus* protoscoleces and fluids, were drained into sterile Erlenmeyer flasks. Afterwards, the protoscoleces were allowed to settle, then the supernatant was discarded, and the remained protoscoleces sand washed three times with normal saline. On average, for each wash step, 20 minutes were spent for settling and carefully removing liquids and holding the protoscoleces.

Viability test of the protoscoleces

To determine the viability of protoscoleces, 0.1% eosin staining method was used. In this method, after exposure to the eosin, dead protoscoleces were colored red, while, alive ones did not absorb the color. Viability was measured by counting the alive and dead protoscoleces in at least five microscopic slides.

In vitro scolicidal activity

Herein, we compared the scolicidal effect of *S.sahendica* and *A.dracunculus* EOs and their nanoemulsions relative to albendazole, the standard drug. To reach this goal, 10^3 protoscoleces of *E.granulosus* in 1 ml medium M199 were exposed to four concentrations of 1, 2, 5, and 10 µg/ml of EOs and nanoemulsions at 30, 60, and 120-minute time points. Albendazole at the concentration of 2 µg/ml was used as positive control. The negative control group did not receive any treatment except a one percent concentration of DMSO that was used as co-solvent in all of the treated groups. All of the treated wells were incubated at 37°C. At time points of 30, 60, and 120 minutes, after exposure, mortality rates were recorded for all of the tested compounds. Scolicidal assay for each concentration of the tested compounds at each time point was done in triplicate.

Statistical analysis

Repeated measures ANOVA followed Bonferroni post hoc test was used to analyze differences in different exposure times for each concentration of the tested compounds. Differences between the means of mortality rate for a different tested compound at each time point (30, 60, and 120 min) were analyzed by one-way analysis of variance (ANOVA) followed by Tukey- HSD post hoc test. Fifty and ninety percent lethal concentrations (LC_{50} and LC_{90}) were calculated by Probit regression analysis. Data analysis was performed using SPSS (version 25.0) (SPSS Inc., USA). For all analyses, ($P < 0.05$) was considered statistically significant.

Results

Particle size analysis

Particle size analysis includes mean diameter and polydispersity index (PDI). PDI is uses for estimation of the average uniformity of a particle solution. Figure 1 (A) shows the mean diameter of nanoemulsion of *Satureja sahendica* EO and (B) *Artemisia dracunculus* EO. Mean diameter of nanoemulsion of *S.*

sahendica EO and *A. dracunculus* EO were 81.3 and 88.2 nm, respectively. Nanoemulsion of *S. sahendica* and *A. dracunculus* s showed PDI values of 0.22 and 0.39, respectively. These results indicated that these formulated nanoemulsions were in nanometric scales.

Zeta potential

Particle surface charges of nanoemulsions of *S. sahendica* EO (A) and *A. dracunculus* EO (B) were showed in Fig. 3. Mean zeta potential of nanoemulsions of *S. sahendica* and *A. dracunculus* were − 43.0 and − 44.6 mV, respectively.

Scolicidal effects

Table 1 shows the mortality rates of *E. granulosus* protoscoleces over different times of exposure to *S. sahendica* and *A. dracunculus*, and their nanoemulsions in comparison to albendazole and the negative control. For all tested plants, a significant effect of the treatments ($F_{5,60} = 61.47$, $P < 0.001$), the time of exposure ($F_{2,120} = 170.72$, $P < 0.001$) and their interaction ($F_{10,120} = 4.32$, $P < 0.001$) were noted. The overall mortality rate in protoscoleces after exposutre to *S. sahendica* nanoemulsion was significantly higher than other treatments and it was 83.13% (P 0.05), while overall scolicidal activity of *A. dracunculus* nanoemulsion was equal to 71.13% and did not showed significant difference relative to *S. sahendica* EO (68.62%) ($P > 0.05$). *A. dracunculus* EO (46.44%) caused lower mortality rates in protoscoleces in comparison to *S. sahendica*. The mean mortality rate of protoscoleces in each treatment shows a significant difference between the three time points of the study. However, in the control group and albendazole, there was just a significant difference between 30 and 120 min time points (P 0.05).

Table 1
Total killing effect of *Satureja sahendica* and *Artemisia dracunculus*, and their nanoemulsions at different time points (30, 60, and 120 min) on protoscoleces of *Echinococcus granulosus*

Treatment	Time			Total
	30	60	120	
Satureja sahendica EO	^{a,A}59.33 ± 10.30	^{b,A}67.80 ± 12.35	^{c,A}78.73 ± 10.59	^{A}68.62 ± 10.78
Satureja sahendica nanoemulsion	^{a,B}76.26 ± 10.51	^{b,B}83.00 ± 11.28	^{c,B}90.13 ± 8.95	^{B}83.13 ± 9.93
Artemisia dracunculus EO	^{a,C}40.66 ± 8.95	^{b,C}45.86 ± 10.37	^{c,C}52.80 ± 12.11	^{C}46.44 ± 10.06
Artemisia dracunculus nanoemulsion	^{a,A}63.00 ± 9.21	^{b,A}71.33 ± 9.40	^{c,A}79.06 ± 10.02	^{A}71.13 ± 9.28
Control	^{a,D}0.66 ± 0.57	^{ab,D}2.33 ± 0.57	^{b,D}5.00 ± 1.00	^{D}2.66 ± 0.57
Albendazole	^{a,D}11.33 ± 1.15	^{ab,D}15.66 ± 0.57	^{b,D}24.00 ± 1.00	^{D}17.00 ± 0.66
P-value	0.001	0.001	0.001	0.001

*Values are means ± SD of three replicates. **The different superscripts a,b,c in the same row indicate significant differences ($P < 0.05$). ***The different superscripts A,B,C,D in the same column indicate significant differences ($P < 0.05$).

As can be observed in Fig. 3, S. *sahendica* EO showed a potent scolicidal activity against the protoscoleces of *E. granulosus*. Indeed, after 30 min of treatment, this plant EO at 25 µg/ml concentration, killed 48.66% of the parasites. On the other hand, its nanoemulsion at the same dose and time point resulted in more toxicity on protoscoleces, with 57.33% mortality rate. Also, after 30 min of treatment at 25 µg /ml, the *A. dracunculus* in the form of nanoemulsion was more effective than the EO (52.66 vs 33% mortality rate). *S. sahendica* and *A. dracunculus*, and their nanoemulsions at the concentration of 400 µg /ml after 120 min treatment, achieved significant scolicidal activity with mortality rates of 92, 100, 71.33, and 92.66%, respectively. Results showed that in both forms of EO and nanoemulsion *S. sahendica* was more effective than *A. dracunculus* against *E. granulosus* protoscolices.

Table 2 shows the 60 minute LC_{50} and LC_{90} values of the studied compounds on *E. granulosus* protoscoleces. *S. sahendica* nanoemulsion and *A. dracunculus* nanoemulsion were the most effective treatment against protoscoleces with LC_{50} values of 10.60 and 11.48 µg /ml, respectively. Moreover, based on the obtained LC_{50} values, EO of *S. sahendica* was more effective than *A. dracunculus* (LC_{50}: 25.37 vs 213.35 µg /ml).

Table 2
Lethal activity of *Satureja sahendica* and *Artemisia dracunculus*, and their nanoemulsions at 60 minute time point on protoscoleces of *Echinococcus granulosus*

Components	Concentration (µg /ml)	60 min mortality (%) ± SD[a]	LC_{50} (µg/ml) (LCL-UCL)	LC_{90} (µg/ml) (LCL-UCL)	χ^2 (df)[b]
Satureja sahendica (µg /ml)	25	53.33 ± 1.52	25.37 (11.56–39.36)	1004.81 (503.59-3919.67)	2.14 (3) n.s.
	50	55.33 ± 3.05			
	100	70.00 ± 2.00			
	200	77.00 ± 1.00			
	400	83.33 ± 3.05			
Satureja sahendica nanoemulsion (µg /ml)	25	67.33 ± 1.15	10.60 (4.07–17.95)	161.97 (116.42-271.36)	0.69 (3) n.s.
	50	74.00 ± 4.00			
	100	85.66 ± 2.08			
	200	92.66 ± 1.15			
	400	95.33 ± 0.57			
Artemisia dracunculus (µg /ml)	25	34.00 ± 2.00	213.35 (151.29-306.53)	927.90 (679.59-1586.96)	1.33 (3) n.s.
	50	42.00 ± 2.00			
	100	43.33 ± 1.15			
	200	46.00 ± 2.00			
	400	64.00 ± 2.00			
Artemisia dracunculus nanoemulsion (µg /ml)	25	57.33 ± 1.15	11.48 (1.88–24.08)	1340.32 (533.78-13677.14)	0.65 (3) n.s.
	50	66.66 ± 2.03			
	100	73.33 ± 1.15			
	200	75.33 ± 3.05			
	400	84.00 ± 2.00			

[a]values are mean ± SD of three replicates.

[b]Chi-square, df: degrees of freedom.

Discussion

Presently, there are three treatment options for hydatidosis: surgery, percutaneous aspiration and medicinal treatment (Adas et al. 2009). Even though huge improvements have happened in medical and interventional radiological techniques, surgery is still the gold standard for hydatid cyst treatment (Fattahi et al. 2019). Surgery is an invasive and mostly considered a risky therapeutic option, but the aim in hydatidosis is inactivation and evacuation of cysts and preventing the recurrence of the disease (Fattahi et al. 2019). Embryonic cells existing on the hydatid cyst germinal layer can generate new protoscoleces or brood capsules. To prevent the disease recurrence, this layer should be destroyed (Fattahi et al. 2019; Moazeni et al. 2019). A suitable scolicidal agent can be described by its potency at low doses, high efficacy in a short time, high accessibility, low toxicity, and longer retention time in the cystic contents. Necessarily, novel, more appropriate, and more efficient scolicidal agents are required to improve hydatidosis treatment (Mahmoudvand et al. 2019). Historically, herbal medicines have been a popular form of complementary and alternative medicine worldwide. They are currently in demand and their popularity is increasing day by day (Mahmoudvand et al. 2019; Verma & Singh, 2008). *A.dracunculus* and *S.sahendica* were selected for the present study due to their several special features including accessibility and low price. It was reported that *A.dracunculus* showed the highest antileishmanial effect between 11 *Artemisia* genus species (Emami et al. 2012). Also, *A.dracunculus* showed a significant antileishmanial effect in an *in vivo* study model (Babaee Khou et al. 2007). However, in the present study, *A.dracunculus* EO was not as potent as the other tested EO against protoscoleces. On the other hand, by nanoformulasion, scolicidal activity of *A.dracunculus* significantly increased and resulted in 60 min LC_{50} values of 11.48 for the nanoemulsion versus 213.35 µg/ml for the EO.

In the present study, EO of *S.sahendica* at the highest tested concentration after 120 min resulted in higher than 90% mortality rates. Studies have reported several biological activities including antimicrobial and antiparasitic effects for the species of the Satureja genus. Volatile compounds in Satureja species EOs are characterized mainly with oxygenated monoterpenes including thymol and carvacrol (Tepe & Cilkiz, 2016). Most probably, some parts of scolicidal activity of *S.sahendica* EO is due to presence of carvacrol and thymol. Moreover, there are several reports on antiparasitic and scolicidal activity of carvacrol and thymol and plant containing these two terpenoids as their main constituents (Mahmoudvand et al. 2017; Moazeni et al. 2012; Tabari et al. 2017; Youssefi et al. 2019a; Youssefi et al. 2019b). In line with these, the present study showed high efficacy of *S.sahendica* EO and its nanoemulsion on protoscoleces with LC_{50} values of 25.37 and 10.60 µg/ml, respectively, which were significantly higher than *A.dracunculus* EO and its nanoemulsion.

In this research, all the tested EOs and their formulated nanoforms demonstrated higher scolicidal activity relative to the standard conventional drug, albendazole. After oral administration of albendazole, it will be metabolized to a sulfoxide metabolite. It has been showed that albendazole sulfoxide is the primary active metabolite of albendazole and responsible of its *in vivo* therapeutic effects (Adas et al. 2009). Thus, therapeutic effect of albendazole under *in vitro* conditions, as a result of lack of metabolizing system, is limited (Hardin et al. 1997). For a more reliable *in vitro* comparison between tested EOs and the

standard albendazole treatment, using albendazole sulfoxide instead of albendazole would have been a better choice.

To the best of our knowledge, the present study is the first study to evaluate the efficacy of *S.sahendica* and *A.dracunculus* EO and their nanoemulsions against hydatid cysts protoscoleces. The developed nanoemulsions in the present study were successfully in nanometric scale and showed higher scolicidal activity in comparison to the EOs. Nanoemulsions can reach the target organs more easily because of their smaller size, and higher ability to pass through the biological membranes. Among all the tested compounds of the present study, nanoemulsion of *S.sahendica* demonstrated the highest toxicity on protoscoleces and may serve as a candidate for research and development on novel scolicidas. Further *in vivo* studies are needed to warrant its safety and efficacy in hydatidosis infection model.

Declarations

Acknowledgements The authors would like to thank all worker of slaughterhouse in Amol, Mazandaran province who helped in collecting hydatid cysts.

Funding This study has been extracted from two theses of medical student at the Mashhad University of Medical Sciences. It was funded by Mashhad University of Medical Sciences, Iran as Project No. 990111. and 981830.

Competing interests The authors declare no competing interests.

Ethics approval The studies protocols were submitted, reviewed, and approved by the Ethics Committee of the Mashhad University of Medical Sciences (ethical codes: IR.MUMS.MEDICAL.REC.1399.229 and IR.MUMS.MEDICAL.REC.1399.243).

Consent to participate Not applicable.

Consent for publication All authors read and approved the manuscript.

Availability of data and material All authors ensure that all data and materials support the findings and comply with field standards.

Code availability Not applicable.

Authors' contributions MAT and MRY designed and directed the project; NN and MHS performed the experiments; MMB and SAS analyzed the data; EM and MHSH developed the theoretical framework; RH, MAT and EM wrote the article.

Research involving human participants and/or animals The study was approved by the Ethics Committee of the Mashhad University of Medical Sciences (ethical codes: IR.MUMS.MEDICAL.REC.1399.229 and IR.MUMS.MEDICAL.REC.1399.243).

References

1. Adas, G., Arikan, S., Kemik, O., Oner, A., Sahip, N. and Karatepe, O., 2009. Use of albendazole sulfoxide, albendazole sulfone, and combined solutions as scolicidal agents on hydatid cysts (in vitro study). World Journal of Gastroenterology, 15, 112. https://doi.org/10.3748/WJG.15.112.
2. Alasvand Zarasvand, M., Madani, M. and Modaresi. M., 2016. The Effect of Hydroalcoholic Extract of *Artemisia dracunculus* L. (Tarragon) on *Candida albicans* Infection in Mice. Jundishapur Journal of Natural Pharmaceutical Products, 11, 29911. https://doi.org/10.17795/jjnpp-29911.
3. Alizadeh, M.A., Arab, H.A., Tabaie, R. and Nasiri, M., 2013. Evaluation of seed and seedling emergence enhancement of some population of Sahandy savory (*Satureja sahendica*) by gibberlic acid, potasium nitrate, pre-cooling, physical and chemical scarification treatment. Pakistan Journal of Biological Sciences, 16, 1208–1211. https://doi.org/10.3923/pjbs.2013.1208.1211.
4. Babaee Khou, L., Mohebali, M., Lahiji, N. and Mehrabi Tavana, A., 2007. The therapeutic effects of *Eucalyptus*, *Myrtus*, *Ferula*, *Aretmisia*, *Allium* and *Urtica* extracts against cutaneous leishmaniasis caused by *Leishmanaia major* in small white mice (out-bred). Hakim Research Journal, 10, 21–27.
5. Baldissera, M.D., D.a Silva, A.S., Oliveira, C.B., Zimmermann, C.E., Vaucher, R.A., Santos, R.C., Rech, V.C., Tonin, A.A., Giongo, J.L. and Mattos, C.B., 2013. Trypanocidal activity of the essential oils in their conventional and nanoemulsion forms: in vitro tests. Experimental Parasitology, 134, 356–361. https://doi.org/10.1016/j.exppara.2013.03.035.
6. de Moraes, A.R.D.P., Tavares, G.D., Rocha, F.J.S., de Paula, E. and Giorgio, S., 2018. Effects of nanoemulsions prepared with essential oils of copaiba-and andiroba against *Leishmania infantum* and *Leishmania amazonensis* infections. Experimental Parasitology, 187, 12–21. https://doi.org/10.1016/j.exppara.2018.03.005.
7. Echeverría, J. and Albuquerque, R.D.D.G.d., 2019. Nanoemulsions of essential oils: new tool for control of vector-borne diseases and in vitro effects on some parasitic agents. Medicines, 6, 42. https://doi.org/10.3390/medicines6020042.
8. Emami, S.A., Rabe, S.Z.T., Ahi, A. and Mahmoudi, M., 2012. Inhibitory activity of eleven Artemisia species from Iran against *Leishmania major* parasites. Iranian Journal of Basic Medical Sciences, 15, 807. https://doi.org/10.22038/IJBMS.2012.4854.
9. Fattahi, A.S., Fattahi Masoom, S.H., Lorestani, F., Fakhlai, M., Abtahi Mehrjerdi, F.S., Gazanchian, M. and Nouri Hosseini, G., 2019. Clinical Outcomes of Modified versus Traditional Technique for the Surgery of Hydatid Cyst of the Liver: A Case Control Study. Middle East Journal of Digestive Diseases, 11, 152–157. https://doi:10.15171/mejdd.2019.142.
10. Hardin, T.C., Najvar, L.K., Rizzo, J., Fothergill, A.W., Rinaldi, M.G. and Graybill, J.R., 1997. Discrepancy between in vitro and in vivo antifungal activity of albendazole. Journal of Medical and Veterinary Mycology, 35, 153–158.
11. Kheirandish, F., Delfan, B., Farhadi, S., Ezatpour, B., Khamesipour, A., Kazemi, B., Ebrahimzade, F. and Rashidipour, M., 2011. The effect of *Satureja khuzestanica* essential oil on the lesions induced by

Leishmania major in BALB/c mice. African Journal of Pharmacy and Pharmacology, 5, 648–653. https://doi.org/10.5897/AJPP11.130.

12. Mahmoudvand, H., Mirbadie, S.R., Sadooghian, S., Harandi, M.F., Jahanbakhsh, S. and Saedi Dezaki, E., 2017. Chemical composition and scolicidal activity of *Zataria multiflora* Boiss essential oil. Journal of Essential Oil Research, 29, 42–47. https://doi.org/10.1080/10412905.2016.1201546.
13. Mahmoudvand, H., Pakravanan, M., Aflatoonian, M.R., Khalaf, A.K., Niazi, M., Mirbadie, S.R., Tavakoli Kareshk, A. and Khatami, M., 2019. Efficacy and safety of Curcuma longa essential oil to inactivate hydatid cyst protoscoleces. BMC Complementary and Alternative Medicine, 19, 187. https://doi:10.1186/s12906-019-2527-3.
14. Moazeni, M., Saharkhiz, M.J. and Alavi, A.M., 2019. The lethal effect of a nano emulsion of Satureja hortensis essential oil on protoscoleces and germinal layer of hydatid cysts. Iranian Journal of Parasitology, 14, 214–222. https://doi.org/10.18502/ijpa.v14i2.1133.
15. Moazeni, M., Saharkhiz, M.J., Hoseini, A.A. and Alavi, A.M., 2012. In vitro scolicidal effect of *Satureja khuzistanica* (Jamzad) essential oil. Asian Pacific Journal of Tropical Biomedicine, 2, 616–620. https://doi.org/10.1016/S2221-1691(12)60107-5.
16. Mueller, M.S., Runyambo, N., Wagner, I., Borrmann, S., Dietz, K. and Heide, L., 2004. Randomized controlled trial of a traditional preparation of *Artemisia annua* L.(Annual Wormwood) in the treatment of malaria. Transactions of the Royal Society of Tropical Medicine and Hygiene, 98, 318–321. https://doi.org/10.1016/j.trstmh.2003.09.001.
17. Pavoni, L., Pavela, R., Cespi, M., Bonacucina, G., Maggi, F., Zeni, V., Canale, A., Lucchi, A., Bruschi, F. and Benelli, G., 2019. Green micro-and nanoemulsions for managing parasites, vectors and pests. Nanomaterials, 9, 1285. https://doi.org/10.3390/nano9091285.
18. Rouhani, S., Salehi, N., Kamalinejad, M. and Zayeri, F., 2013. Efficacy of *Berberis vulgaris aqueous* extract on viability of *Echinococcus granulosus* protoscolices. Journal of Investigative Surgery, 26, 347–351. https://doi.org/10.3109/08941939.2013.818746.
19. Sachar, S., Goyal, S. and Sangwan, S., 2014. Uncommon locations and presentations of hydatid cyst. Annals of Medical and Health Sciences Research, 4, 447–452. https://doi.org/10.4103/2141-9248.133476.
20. Shahavi, M.H., Hosseini, M., Jahanshahi, M., Meyer, R.L. and Darzi, G.N., 2016. Clove oil nanoemulsion as an effective antibacterial agent: Taguchi optimization method. Desalination and Water Treatment, 57, 18379–18390. https://doi.org/10.1080/19443994.2015.1092893.
21. Shahavi, M.H., Hosseini, M., Jahanshahi, M., Meyer, R.L. and Najafpour Darzi, G., 2019. Evaluation of critical parameters for preparation of stable clove oil nanoemulsion. Arabian Journal of Chemistry, 12, 3225–3230. https://doi.org/10.1016/j.arabjc.2015.08.024.
22. Tabari, M.A., Youssefı, M.R., Maggi, F. and Benelli, G., 2017. Toxic and repellent activity of selected monoterpenoids (thymol, carvacrol and linalool) against the castor bean tick, *Ixodes ricinus* (Acari: Ixodidae). Veterinary Parasitology, 245, 86–91. https://doi.org/10.1016/j.vetpar.2017.08.012.

23. Tepe, B. and Cilkiz, M., 2016. A pharmacological and phytochemical overview on *Satureja*. Pharmaceutical Biology, 54, 375–412. https://doi.org/10.3109/13880209.2015.1043560.
24. Verma, S. and Singh, S., 2008. Current and future status of herbal medicines. Veterinary world, 1, 347. https://doi.org/10.5455/VETWORLD.2008.347-350.
25. Yousefzadi, M., Riahi-Madvar, A., Hadian, J., Rezaee, F. and Rafiee, R., 2012. In vitro cytotoxic and antimicrobial activity of essential oil from *Satureja sahendica*. Toxicological & Environmental Chemistry, 94, 1735–1745. https://doi.org/10.1080/02772248.2012.728606.
26. Youssefi, M.R., Moghaddas, E., Tabari, M.A., Moghadamnia, A.A., Hosseini, S.M., Farash, B.R.H., Ebrahimi, M.A., Mousavi, N.N., Fata, A. and Maggi, F., 2019a. In vitro and in vivo effectiveness of carvacrol, thymol and linalool against *Leishmania infantum*. Molecules, 24, 2072. https://doi.org/10.3390/molecules24112072
27. Youssefi, M.R., Tabari, M.A., Esfandiari, A., Kazemi, S., Moghadamnia, A.A., Sut, S., Dall'Acqua, S., Benelli, G. and Maggi, F., 2019b. Efficacy of two monoterpenoids, carvacrol and thymol, and their combinations against eggs and larvae of the west nile vector *Culex pipiens*. Molecules, 24, 1867. https://doi.org/10.3390/molecules24101867.
28. Zarezade, V., Moludi, J., Mostafazadeh, M., Mohammadi, M. and Veisi, A., 2018. Antioxidant and hepatoprotective effects of *Artemisia dracunculus* against CCl(4)-induced hepatotoxicity in rats. Avicenna Journal of Phytomedicine, 8, 51–62. https://doi.org/10.22038/AJP.2017.19137.1738.

Figures

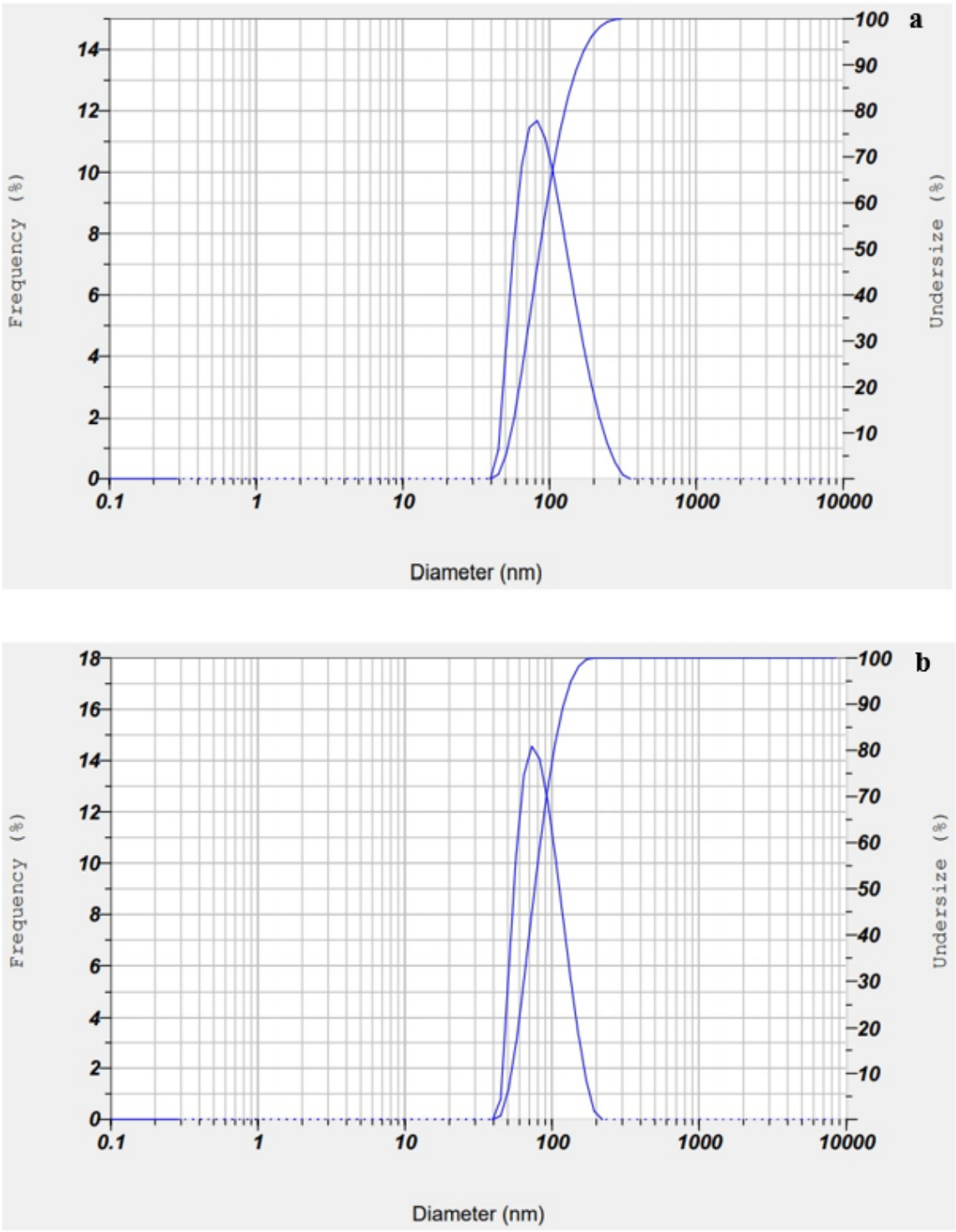

Figure 1

Particle size and polydispersity index (PDI) for nanoemulsions of *Satureja sahendica* (a) and *Artemisia dracunculus* (b)

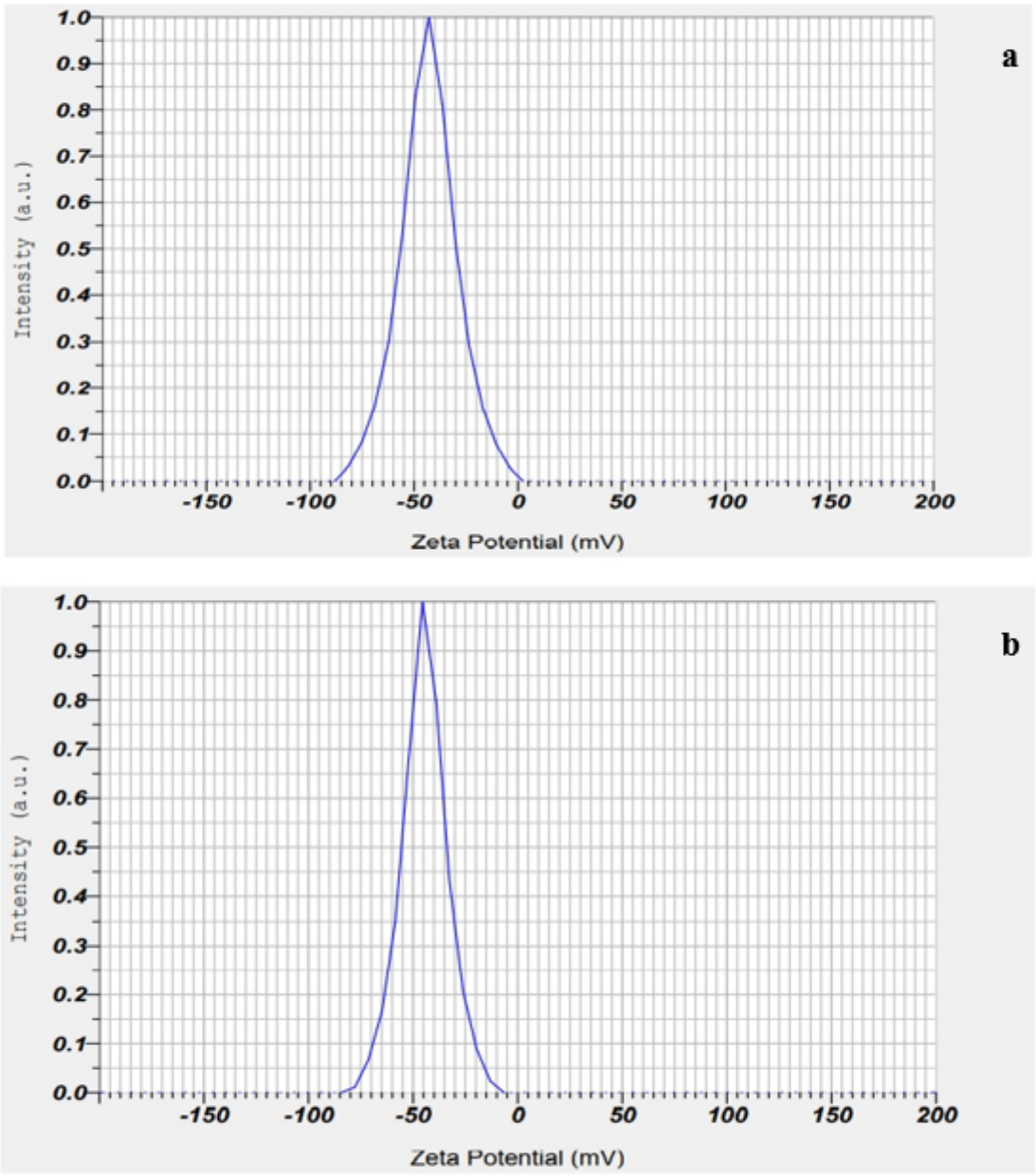

Figure 2

Zeta potential of nanoemulsions of *Satureja sahendica* (a) and *Artemisia dracunculus* (b)

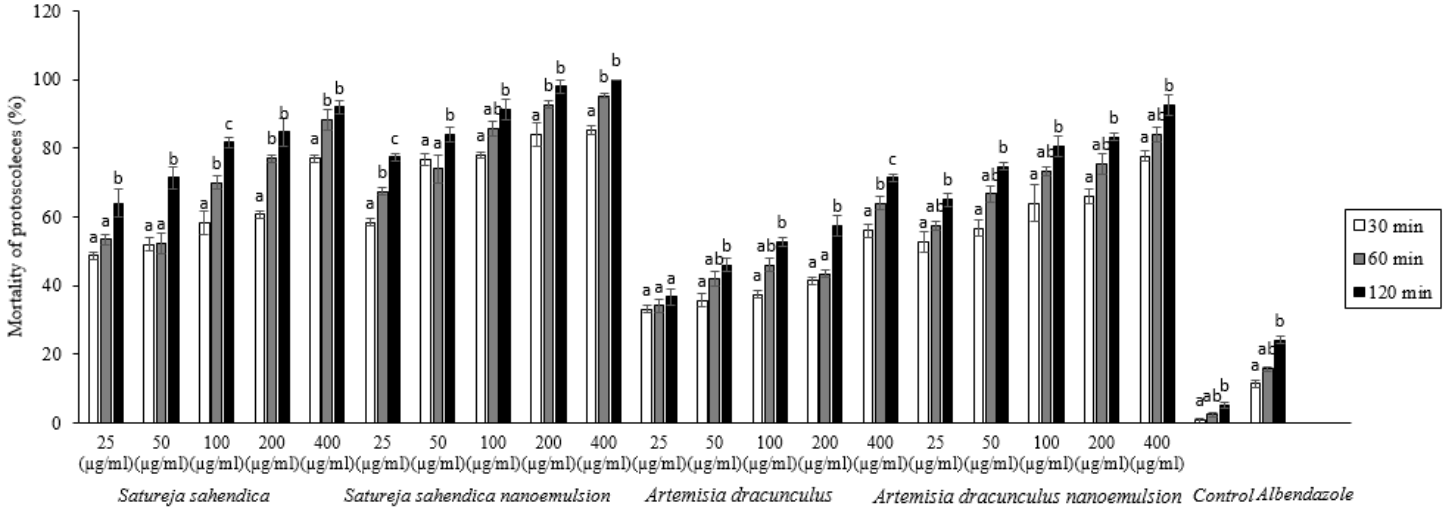

Figure 3

Mortality (%) of *Echinococcus granulosus* protoscoleces over different times of exposure to *Satureja sahendica* and *Artemisia dracunculus,* and their nanoemulsions in comparison to albendazole and the negative control

Data are means±SD. Within each tested concentration of plants and their nano emulsions, column marked with different letters (lowercase) are significantly different between times of exposure (Repeated measures ANOVA, Bonferroni test, $P<0.05$)