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Original paper

In vitro scolicidal effect of *Calendula officinalis*, *Artemisia dracunculus*, *Artemisia absinthium*, and *Ferula assafoetida* extracts against hydatid cyst protoscolices

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ABSTRACT. *Echinococcus granulosus* is the etiologic agent of cystic echinococcosis. Numerous research studies have been conducted on natural scolicidal agents to inactivate protoscolices during surgery. This study was undertaken to compare the *in vitro* scolicidal effects of hydroalcoholic extracts of *Calendula officinalis*, *Artemisia dracunculus*, *Artemisia absinthium* and *Ferula assafoetida.* The scolicidal activities of the extracts were tested at different concentrations following incubation periods of 10, 30 and 60 min. The chemical composition of the hydroalcoholic extracts were analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). The major chemical components of *C. officinalis*, *A. dracunculus*, *A. absinthium* and *F. assafoetida* were identified as n-Docosane (14.17%), 2H-1-benzopyran-2-one, 7-methoxy (54.96%), n-Docosane (9.72%) and 2-methoxy-3-methyl-butyric acid, methyl ester (13.9%), respectively. The results showed that the hydroalcoholic extracts of *A. absinthium* and *F. assafoetida* at a concentration of 250 mg/ml resulted in killing 100% of the protoscolices at 60 minutes, while the concentration of 250 mg/ml of hydroalcoholic extract of *C. officinalis* and *A. dracunculus* resulted in killing 42.33% and 65.67%, respectively. The findings of the present study showed that *A. absinthium* and *F. assafoetida* have potent scolicidal effects. However, additional *in vivo* studies are required to confirm the efficacy of these plant-derived extracts against hydatid cyst for their clinical use.

Keywords: *Echinococcus granulosus*, scolicidal, *Calendula officinalis*, *Artemisia dracunculus*, *Artemisia absinthium, Ferula assafoetida*

Introduction

Echinococcosis, cystic echinococcosis, hydatidosis, or cystic hydatid disease is among the most neglected parasitic diseases. Human echinococcosis is a neglected zoonotic infection caused by larval forms (metacestodes) of taeniid tapeworms belonging to the *Echinococcus granulosus sensu lato* complex found in the small intestine of carnivores [1]. Hydatid disease is one of the most important cosmopolitan parasitic zoonotic diseases affecting humans and can cause damage to multiple organs such as the liver and lungs [2,3]. Echinococcosis is listed by the World Health Organization (WHO) among the neglected tropical diseases (NTDs) which cause devastating health, social and economic consequences to more than one billion people and their public health control is challenging [4].

Cystic echinococcosis is reported to be highly endemic in pastoral communities in different regions in South America, the Mediterranean region, Eastern Europe, Central and East Africa, the Near and the Middle East, North Africa, China, and Russia, with several millions of humans are infected [5–7].

There are two therapeutic options for treating hydatid cyst including surgery (invasive or percutaneous) and chemotherapy [5]. The current chemotherapeutic agents for the treatment of hydatid cyst are mebendazole and albendazole. Adverse side effects of such drugs may include abnormalities in liver function, abdominal pain, diarrhea, nausea, dizziness, headache, and teratogenic effects [8,9]. Scolicidal agents are used in surgery to help minimize the impact of any possible spillage and dissemination of viable protoscolices. An effective scolicidal agent is characterized by acting at low concentrations over a short exposure time and being stable after dilution with cyst fluid, non-toxic, low cost, and easily available [10,11]. Numerous research studies have been conducted on scolicidal agents to inactivate protoscolices during surgery. There is an interest to identify alternative plant-derived scolicidal agents that are readily available at low cost, have minimal side effects, and are less toxicity than the currently used chemotherpatic scolicidal agents.

Calendula officinalis (*C. officinalis*) belongs to the family Asteraceae and has been traditionally used in the treatment of organ inflammation, gastrointestinal ulcers, dysmenorrhea, and chronic infections. *C. officinalis* has antipyretic, anti-tumor, cicatrizing, antimicrobial, antifungal, and antiseptic properties, and it is used in the treatment of marks, freckles, sprain conjunctivitis, mental tension and insomnia [12].

Artemisia dracunculus (*A. dracunculus*) and *Artemisia absinthium* (*A. absinthium*) belong to the family Asteraceae. The plants have several therapeutic properties such as antispasmodic, and antiflatulence effects, and remove impurities, heal mouth ulcers and may prevent cancer, antiparasitic (leishmaniosis), insecticide, antifungal, anti-coagulant, anti-hyperlipidemia, prevent heart disease and high blood pressure, and are useful for atherosclerosis, eliminate alimentary canal disorders, nervous disorder, bad breath, and appetite suppressant properites [13].

Ferula assafoetida (*F. assafoetida*) is a herbal plant whose gum oleoresin is used in cooking and as a digestive agnet. *F. assafoetida* grows wild in the central and southern mountains of Iran. The gum oleoresin is called “anghouzeh”, “khorakoma”, or “anguzakoma” in Iran. The plant, which belongs to the family Apiaceae, is an herbaceous everlasting that grows to about two meters in height with an unpleasant odor [14]. *F. assafoetida* is also used to treat asthma, alimentary canal disorders, and intestinal parasites [15]. The oleoresin gum has antifungal, antidiabetic, antiinflammatory, anti-mutagenic, and antiviral properties [16]. The solicidal effect of such plants was not been previously explored. The present study was undertaken to determine and compare the *in vitro* scolicidal effect of the hydroalcoholic extracts of *C. officinalis*, *A. dracunculus*, *A. absinthium* and *F. assafoetida*.

Materials and Methods

Preparation of protoscolices

Thirty hydatid cysts were collected from the liver and lungs of naturally infected sheep from Tabriz industrial slaughterhouse and transferred to the parasitology laboratory of the Faculty of Veterinary Medicine in University of Tabriz. The cyst fluid was aspirated using a sterile syringe. The cyst fluid was transferred to a glass container and immobilized at room temperature for 30 minutes. The supernatant was discarded and the protoscolices were washed three times with PBS. Protoscolices were stained using 0.1% eosin to assess their viability. Batches of protoscolices that were over 90% viable were selected for further testing.

Plant collection

F. assafoetida was collected from the Tabas region (Yazd Province) in Iran. The plant species was confirmed by botanists from Yazd Agricultural Research, Yazd, Iran (with herbarium number 1360). In the present study, *C. officinalis*, *A. dracunculus* and *A. absinthium* were purchased from herb shop in East Azerbaijan province, and the species was identified and confirmed by the East Azerbaijan Agricultural Research Center, Tabriz, Iran (with herbarium numbers 350, 720 and 722, respectively). The gum of *F. assafoetida* and arial parts of *C. officinalis*, *A. dracunculus* and *A. absinthium* were ground into a powder form using an electrical blender. The powder obtained from each dried plant was dissolved in 100 ml of 70% ethanol and were kept overnight at room temperature.

Preparation of plant extracts

The hydroethanolic extracts of the plants were prepared by maceration of 100 g of the dried powdered plants in 70% aqueous ethanol for three days at room temperature, then filtrated through filter paper (Whatman Ltd., Buckinghamshire, UK).

Table 1. The scolicidal effects of *C. officinalis*, *A. dracunculus*, *A. absinthium* and *F. asafoetida* extracts at various concentrations and exposure times against hydatid cysts of *E. granulosus*

Concentration of the extract	Time of exposure	*C. officinalis*	*A. dracunculus*	*A. absinthium*	*F. assafoetida*	Positive control	Negative control
50 mg/ml	10 min	8 ± 1.00	5.33 ± 1.52	70 ± 2.00	8.33 ± 1.15	100 ± 0.00	4.66 ± 0.57
	30 min	11 ± 1.00	29.67 ± 1.53	75 ± 2.00	20 ± 1.00	100 ± 0.00	4 ± 1.00
	60 min	10.33 ± 1.52	53.33 ± 2.08	82.67 ± 2.51	60.67 ± 2.08	100 ± 0.00	4.33 ± 0.57
100 mg/ml	10 min	11.67 ± 1.52	22.67 ± 2.30	89 ± 1.00	56 ± 2.00	100 ± 0.00	4.66 ± 0.57
	30 min	12.67 ± 1.52	35 ± 2.64	83.67 ± 1.52	83 ± 2.00	100 ± 0.00	4 ± 1.00
	60 min	13.33 ± 2.08	42.67 ± 2.51	89 ± 1.00	87 ± 2.51	100 ± 0.00	4.33 ± 0.57
150 mg/ml	10 min	12.33 ± 1.52	48.67 ± 1.52	91.67 ± 1.52	68 ± 6.08	100 ± 0.00	4.66 ± 0.57
	30 min	13.67 ± 1.52	54 ± 1.00	93.67 ± 1.15	85.67 ± 3.05	100 ± 0.00	4 ± 1.00
	60 min	15 ± 2.64	60.67 ± 2.08	95.33 ± 1.52	94.67 ± 0.57	100 ± 0.00	4.33 ± 0.5
200 mg/ml	10 min	21.67 ± 2.08	60 ± 2.00	95.67 ± 0.57	90.33 ± 1.15	100 ± 0.00	4.66 ± 0.57
	30 min	24 ± 2.00	61.33 ± 2.51	96 ± 1.00	91.67 ± 1.50	100 ± 0.00	4 ± 1.00
	60 min	28.33 ± 1.52	62 ± 2.00	97 ± 1.00	95.67 ± 0.57	100 ± 0.00	4.33 ± 0.57
250 mg/ml	10 min	34.33 ± 4.04	61 ± 2.00	97.33 ± 0.57	97 ± 1.00	100 ± 0.00	4.66 ± 0.57
	30 min	38 ± 2.00	64.33 ± 1.15	98.67 ± 0.57	97.67 ± 0.57	100 ± 0.00	4 ± 1.00
	60 min	42.33 ± 2.51	65.67 ± 2.08	100 ± 0.00	100 ± 0.00	100 ± 0.00	4.33 ± 0.57

The alcohol in the sample was removed by a vacuum rotary evaporator (Model VV 2000, Heidolph, Germany). The extracts were concentrated using an incubator at a temperature of 37°C to obtain a dry powder extract.

Evaluation of the in vitro scolicidal activity of the plant extracts

In order to determine the scolicidal effect of the four plant extracts, the hydroalcoholic extracts were added to distilled sterile water to obtain 50, 100, 150, 200 and 250 mg/ml dilutions. One ml of the extracts was poured into a microtube and a drop of protoscolices (2000 protoscolices) was added. The contents of the tubes were quickly mixed and incubated at 37°C for 10, 30, and 60 minutes, then the supernatant was discarded. To assess the viability of the protoscolices, a 0.1% solution of eosin stain was added to the bottom tube protoscolices and mixed gently, which were later spread on a glass slide, covered with a cover slip (24×50 mm), and examined using a light microscope. The number of dead protoscolices was determined by counting at least 500 of them. The experiments were performed in triplicate. Normal saline and mebendazole (5g/100 ml) solutions were used as negative and positive controls, respectively.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

Chromatographic analysis was performed using Gas Chromatography-Mass Spectrometry instrument (Agilent19091S-433) (Agilent Technologies, CA, USA). The hydroethanolic extract was mixed with hexane (Merck KGaA, Darmstadt, Germany) (1:1), and the solution was placed on the shaker for 1 hour until it was homogeneously mixed. Then the mixture was put in a separator, kept for 15 minutes to form a

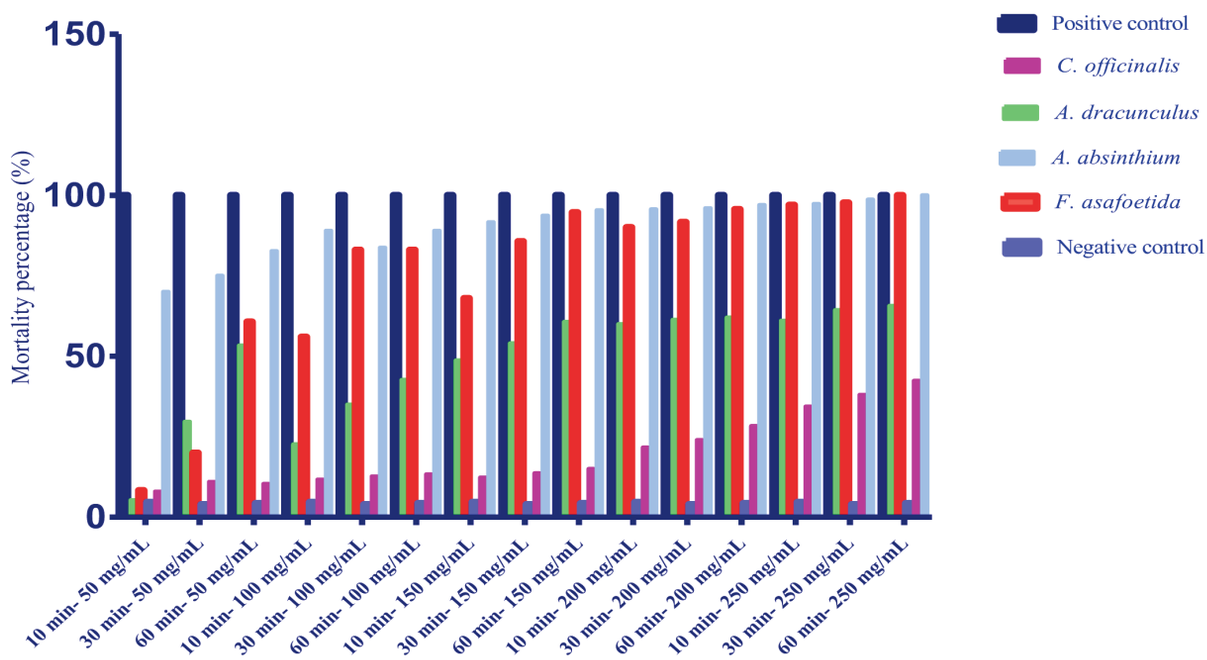

Figure 1. The scolicidal effects of *C. officinalis*, *A. dracunculus*, *A. absinthium* and *F. assafoetida* extracts at various concentrations and exposure times against hydatid cysts of *E. granulosus*

double phase and the hexane phase was isolated and injected into the GC/MS instrument for analysis.

Statistical analysis

Data were analyzed using GraphPad Prism software program version 5 and expressed as a mean ± SD. Data were analyzed by a two-way ANOVA and Student's two-tailed t-test for the comparison between the test and control.

Ethical statement

The study project was approved by the ethics committee of University of Tabriz approval number IR.TU.REC.1398.10 (Code Project: 39114). A permission was granted by Tabriz industrial slaughterhouse to collect liver tissue samples from the animals for this study.

Results

Findings of the present study showed that *A. absinthium* and *F. assafoetida* extracts at a concentration of 250 mg/ml killed 100% of protoscolices after 60 minutes of exposure, while the concentration of 250 mg/ml of *C. officinalis* and *A. dracunculus* resulted in killing 42.33% and 65.67%, respectively at the same exposure time. The scolicidal effect of *A. dracunculus* extract at the concentration of 50 mg/ml at 10 minutes exposure time was lower (5.33%). Among the four plant extracts, *A. absinthium* has the highest scolicidal activity (70%) at the lowest concentration and lowest time (50 mg/ml at 10 minutes). The mortality rate of hydatid cyst protoscolices after exposure times to different concentrations of the hydroalcoholic extracts of the four plants are shown in table 1 and figure 1.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis showed that the major constituent of *C. officinalis* is n-Docosane (14.17%), *A. dracunculus* is 2H-1-benzopyran-2-one, 7-methoxy (54.96%), *A. absinthium* is n-Docosane (9.72%) and that of *F. assafoetida* gum is 2-methoxy-3-methyl-butyric acid, methyl ester (13.9%). The results of GC/MS analysis of the plant extracts were shown in figures 2–5. The results showed that *A. absinthium* and *F. assafoetida* significantly killed the protoscolices and these plant derived extracts are recommended to be used as a potential scolicidal agent of plant origin.

Discussion

So far, many scolicidal chemotherapeutic agents have been used to inactivate protoscolices, however their therapeutic use is limited due to their reproted harmful side effects. Adverse side effects have been reported for hypertonic saline (20%), cetrimide (0.5–1%), silver nitrate (20%), ethyl alcohol (95%)

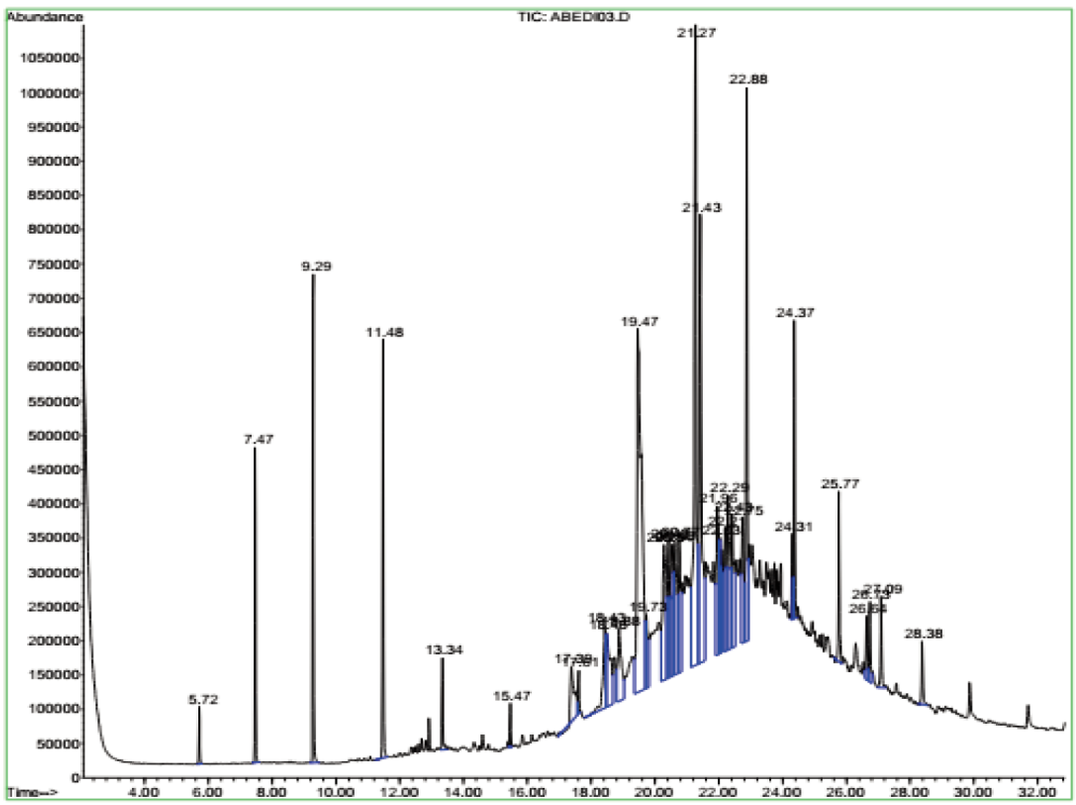

Figure 2**.** Gas-Chromatography/Mass Spectrometry (GC-MS) analysis of *C. officinalis* extract

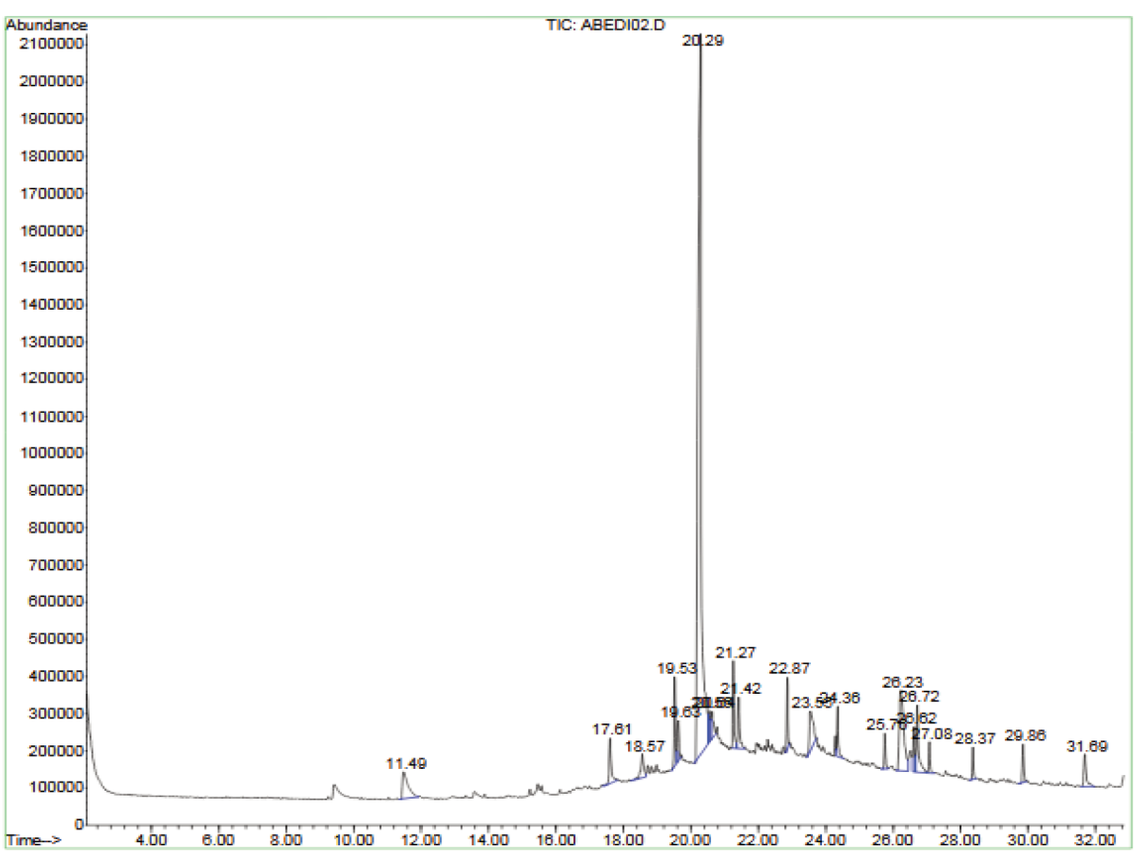

Figure 3. Gas-Chromatography/Mass Spectrometry (GC-MS) analysis of *A. dracunculus* extract

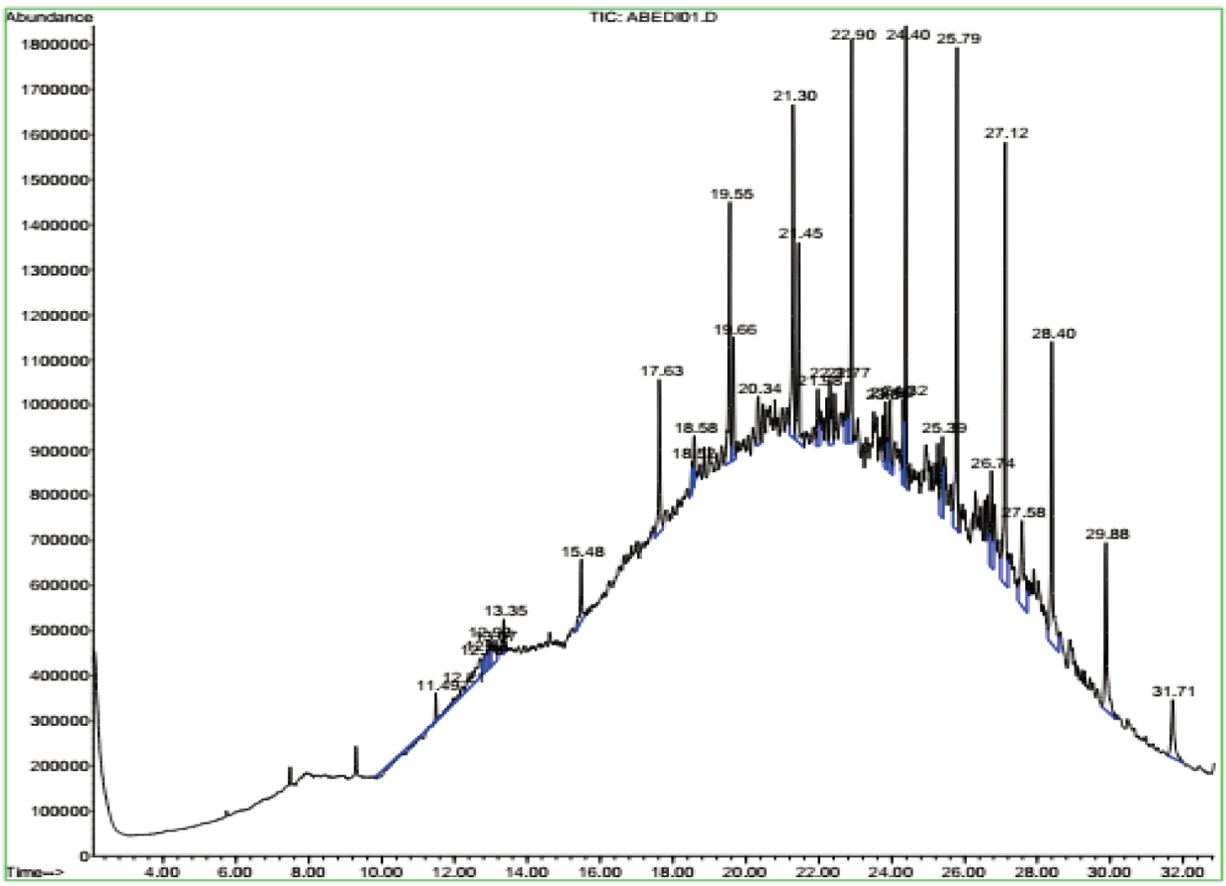

Figure 4. Gas-Chromatography/Mass Spectrometry (GC-MS) analysis of *A. absinthium* extract

and albendazole sulfoxide (20 mg/ml) [17]. A number of studies reported the antimicrobial inhibitory effects of *C. officinalis*, *A. dracunculus*, *A. absinthium* and *F. assafoetida* [12,13,16]. In the present study, the solicidal effects of hydroalcoholic extracts of four plants on the protoscolices of hydatid cyst were determined for the first time. The findings of the present study showed that *A. absinthium* and *F. assafoetida* extracts at a concentration of 250 mg/ml killed 100% of protoscolices after 60 minutes of exposure, while the concentration of 250 mg/ml of *C. officinalis* and *A. dracunculus* resulted in killing 42.33% and 65.67%, respectively at the same exposure time.

The antiparasitic effects of the four plants tested in the present study were previously reported, including the anti-leishmaniosis activity of *C. officinalis* flower on *Leishmania major* promastigotes [18], anti-leishmanicidal activity against *L. donovani* amastigote, anti-trypanosomal activity against *Trypanosoma brucei* [19], anti-*Trichomonas* activity against *Trichomonas vaginalis* [20], anti-leishmaniosis activity of *A. dracunculus* on *Leishmania major* promastigotes [21,22], schistosomicidal activity of *A. absinthium* against *Schistosoma mansoni* worm [23], antiparasitic activity of *A. absinthium* against *Hymenolepis nana* worm [24], antiparasitic activity of *A. absinthium* on *Nosema ceranae* (Microsporidia) [25], anti-leishmaniosis activity of *A. absinthium* on *Leishmania amazonensis* and *L. major* promastigotes [26,27], antiparasitic activity of *F. assafoetida* on *Strongylus* spp., *Haemonchus contortus,* protoscolices of *E. granulosus* [28–30], anti-*Trichomonas* activity of *F. assafoetida* against *T. vaginalis* [31], and anti-giardiosis activity of *F. assafoetida* [32].

The effect of a methanolic extract of *Allium sativum* on protoscolices was previously determined and it showed a high scolicidal activity (100%) at a concentration of 25 mg/ml at 60 min [33]. It was previously reported that a chloroform extrcat of *A. sativum* high protoscolicidal activity (99.58±1.63) at a concentration of 200 mg/ml. It was previously reported that 10 mg/ml and 25 mg/ml of methanolic extracts of *Zataria multiflora* killed 100% of protoscolices after 3 min and 1 min, respectively [34]. Recently, the hydroalcoholic extract of *Taxus baccata L.* at a concentration of 150 mg/ml was previously reported to kill 66.6% of the

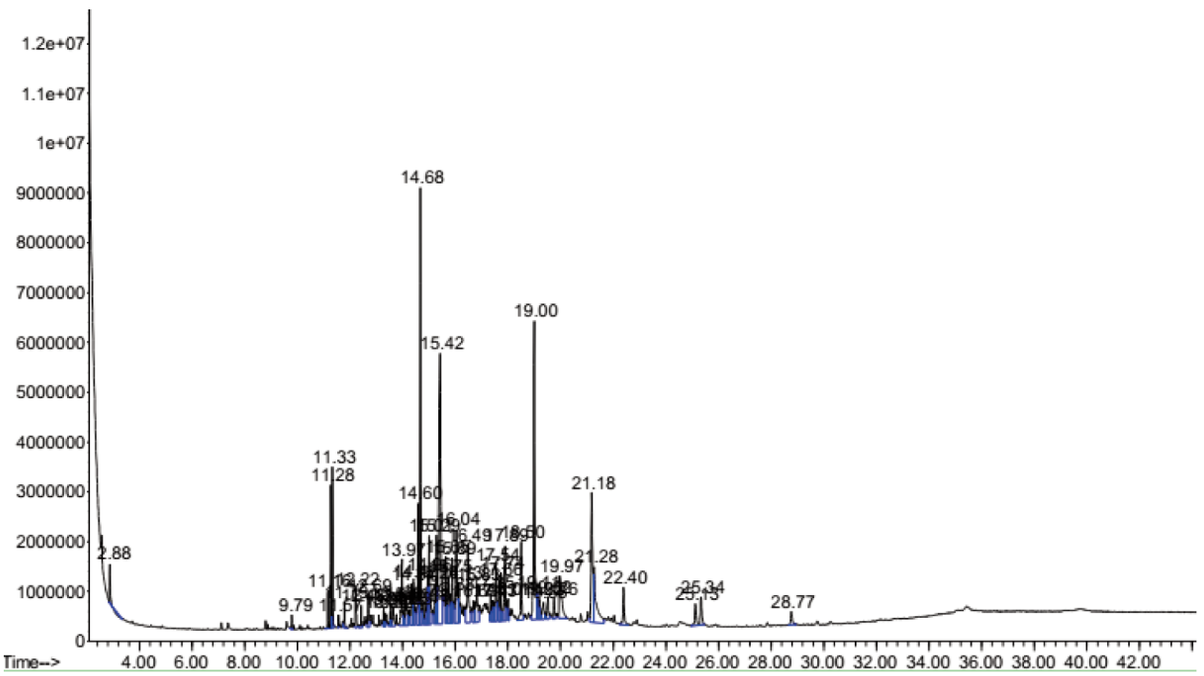

Figure 5. Gas-Chromatography/Mass Spectrometry (GC-MS) analysis of *F. assafoetida* extract

protoscolices at 60 minutes [35]. It was previously reported that protoscolices were killed following 10 min of exposure time at concentrations higher than 17 µg/ml of the essential oil of *Z. multiflora* [36]. Mahmoudvand et al. [37] reported that the essential oil of *Nigella sativa* (the blackseed) at a concentration of 10 mg/ml following 10 min exposure time eliminated 100% of protoscolices. The scolicidal effect of barberry (*Berberis vulgaris*) extract was reported to be 100% following 5 min exposure at 4 mg/ml [38]. The scolicidal effect of Ajowan (*Trachyspermum ammi*) essential oil was observed at a concentration of 10 mg/ml following 10 min exposure time [39]. *In vitro* scolicidal effect of *Satureja khuzistanica* essential oil at concentrations of 5 and 10 mg/ml, was 100% [40]. The differences in the results of different studies may be explained by the differences in plants, concentrations and time of exposure. The results of the present study showed that *A. absinthium* and *F. assafoetida* have more scolicidal properties than *C. officinalis* and *A. dracunculus*. *A. absinthium* and *F. assafoetida* have potential for use as plant derived in hydatid cyst surgery. Out of the four tested plants in the present study, *A. absinthium* and *F. assafoetida* showed the highest scolicidal effect. GC-MS analysis revealed the major chemical components of *A. absinthium* and *F. assafoetida* were identified as n-Docosane (9.72%) and 2-methoxy-3-methyl-butyric acid, methyl ester (13.9%) respectively, but the n-Docosane can not play a major role as an anti-parasitc component because it is 14.17% in the *C. officinalis* plant and probably components other than the n-Docosane may have anti-parasitic properties. In case of *F. assafoetida* plant, most of the extract components was 2-methoxy-3-methyl-butyric acid, methyl ester (13.9%), which may have anti-parasitic properties along with other components. Further *in vivo* investigations on the four plant extracts and their individual chemical constituents are required to delineate the scolicidal properties of the four plants tested in the present study before the potential clinical applications of these plants can be determined.

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References

[1] Silva A.M. 2010. Human echinococcosis: a neglected

disease. *Gastroenterology Research and Practice* 2010: article number 583297. doi:10.1155/2010/583297

[2] Moro P., Schantz P.M. 2009. Echinococcosis: a review. *International Journal of Infectious Diseases* 13(2): 125–133. doi:10.1016/j.ijid.2008.03.037

[3] Mirbadie S.R., Zivdari M., Kalani H., Vafaei M.R., Izadi S., Jabalameli Z., Mohammadi M., Yadagiri G., Heydarian P., Mirzaei F., Mohaghegh M.A. 2020. Molecular identification of *Echinococcus granulosus sensu lato* by mitochondrial COX1 and SSU-rDNA markers in dogs in the west of Iran. *Gene Reports* 19: article number 100616. doi:10.1016/j.genrep.2020.100616

[4] WHO. Neglected tropical diseases. 2022. Geneva, Switzerland. https://www.who.int/health-topics/neglected-tropical-diseases#tab=tab_1

[5] Brunetti E., Kern P., Vuitton D.A. 2010. Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. *Acta Tropica* 114: 1–6. doi:10.1016/j.actatropica.2009.11.001

[6] Sadjjadi SM. 2006. Present situation of echinococcosis in the Middle East and Arabic North Africa. *Parasitology International* 55: S197–S202. doi:10.1016/j.parint.2005.11.030

[7] Zhang W., Zhang Z., Wu W., Shi B., Li J., Zhou X., Wen H., McManus D.P. 2015. Epidemiology and control of echinococcosis in central Asia, with particular reference to the People's Republic of China. *Acta Tropica* 141(PtB): 235–243. doi:10.1016/j.actatropica.2014.03.014

[8] Walker M., Rossignol J.F., Torgerson P., Hemphill A. 2004. *In vitro* effects of nitazoxanide on *Echinococcus granulosus* protoscoleces and metacestodes. *Journal of Antimicrobial Chemotherapy* 54(3): 609–616. doi:10.1093/jac/dkh386

[9] 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. *Parasitology Research* 110: 1103–1112. doi:10.1007/s00436-011-2595-x

[10] Anthony J., Fyfe L., Smith H. 2005. Plant active components-a resource for antiparasitic agents? *Trends in Parasitology* 21(10): 462–468. doi:10.1016/j.pt.2005.08.004

[11] Norouzi R., Ataei A., Hejazy M., Noreddin A., El Zowalaty M.E. 2020. Scolicidal effects of nanoparticles against hydatid cyst protoscolices *in vitro*. *International Journal of Nanomedicine* 15: 1095–1100. doi:10.2147/IJN.S228538

[12] Arora D., Rani A., Sharma A. 2013. A review on phytochemistry and ethnopharmacological aspects of genus *Calendula*. *Pharmacognosy Review* 7(14): 179–187. doi:10.4103/0973-7847.120520

[13] Obolskiy D., Pischel I., Feistel B., Glotov N., Heinrich M. 2011. *Artemisia dracunculus* L. (tarragon): a critical review of its traditional use, chemical composition, pharmacology, and safety. *Journal of Agricultural and Food Chemistry* 59(21): 11367–11384. doi:10.1021/jf202277w

[14] Davoudi Moghadam H., Sani A.M., Mehraban Sangatash M. 2014. Effect of oleo-gum of resin of *Ferula assafoetida* on groowth of some food and crop contaminating microbes. *International Journal of Advanced Biological and Biomedical Research* 2: 2788–2794. https://iranjournals.nlai.ir/ handle/123456789/87 114

[15] Bandyopadhyay D., Basak B., Chatterjee A., Lai T.K., Banerji A., Banerji J., Neuman A., Prangé, T. 2006. Saradaferin, a new sesquiterpenoid coumarin from *Ferula assafoetida*. *Natural Product Research* 20(10): 961–965. doi:10.1080/14786410600823431

[16] Iranshahy M., Iranshahi M. 2011. Traditional uses, phytochemistry and pharmacology of asafoetida (*Ferula assafoetida* oleo-gum-resin) – a review. *Journal of Ethnopharmacology* 134(1): 1–10. doi:10.1016/j.jep.2010.11.067

[17] Sharafi S.M., Sefiddashti R.R., Sanei B., Yousefi M., Darani H.Y. 2017. Scolicidal agents for protoscolices of *Echinococcus granulosus* hydatid cyst: review of literature. *Journal of Research in Medical Sciences* 22: article number 92 doi:10.4103/jrms.JRMS_1030_16

[18] Nikmehr B., Ghaznavi H., Rahbar A., Sadr S., Mehrzadi S. 2014. *In vitro* anti-leishmanial activity of methanolic extracts of *Calendula officinalis* flowers, *Datura stramonium* seeds, and *Salvia officinalis* leaves. *Chinese Journal of Natural Medicines* 12(6): 423–427. doi:10.1016/S1875-5364(14)60066-2

[19] Samra R.M., Maatooq G.T., Zaki A.A. 2021. A new antiprotozoal compound from *Calendula officinalis*. *Natural Product Research.* doi:10.1080/14786419.2021.2023868

[20] Samochowiec E., Urbanska L., Manka W., Stolarska E. 1979. [Assessment of the action of *Calendula officinalis* and *Echinacea angustifolia* extracts on *Trichomonas vaginalis in vitro*]. *Wiadomości Parazytologiczne* 25(1): 77–81 (in Polish with summary in English).

[21] Mirzaei F., Bafghi A.F., Mohaghegh M.A., Jaliani H.Z., Faridnia R., Kalani, H. 2016. *In vitro* anti-leishmanial activity of S*atureja hortensis* and *Artemisia dracunculus* extracts on *Leishmania major* promastigotes. *Journal of Parasitic Diseases* 40(4): 1571–1574. doi:10.1007/s12639-015-0730-9

[22] Rezaei R., Tappeh K.H., Seyyedi S., Mikaili P. 2017. The anti-leishmanial efficacy of *Artemisia dracunculus* ethanolic extract *in vitro* and its effects on IFN-γ and IL-4 response. *Iranian Journal of Parasitology* 12(3): 398–407.

[23] de Almeida L.M.S., Carvalho L.S.A.D., Gazolla

M.C., Silva Pinto P.L., Silva M.P.N.D., de Moraes J., Da Silva Filho A.A. 2016. Flavonoids and sesquiterpene lactones from *Artemisia absinthium* and *Tanacetum parthenium* against *Schistosoma mansoni* worms. *Evidence-Based Complementary and Alternative Medicine* 2016: article number 9521349. doi:10.1155/2016/9521349.

[24] Beshay E. 2018. Therapeutic efficacy of A*rtemisia absinthium* against *Hymenolepis nana: in vitro* and *in vivo* studies in comparison with the anthelmintic praziquantel. *Journal of Helminthology* 92(3): 298–308. doi:10.1017/S0022149X17000529

[25] Porrini M.P., Fernández N.J., Garrido P.M. 2011. *In vivo* evaluation of antiparasitic activity of plant extracts on *Nosema ceranae* (Microsporidia). *Apidologie* 42(6)**:** 700–707. doi:10.1007/s13592-011-0076-y

[26] Tamargo B., Monzote L., Piñón A., Machín L., García M., Scull R., Setzer W.N. 2017. *In vitro* and *in vivo* evaluation of essential oil from *Artemisia absinthium* L. formulated in nanocochleates against cutaneous leishmaniasis. *Medicines* 4(2): article number 38. doi:10.3390/medicines4020038

[27] Bafghi A.F., Bagheri S.M., Hejazian S.H. 2014. Antileishmanial activity of *Ferula assafoetida* oleo gum resin against *Leishmania major*: an *in vitro* study. *Journal of Ayurveda and Integrative Medicine* 5(4): 223–226. doi:10.4103/0975-9476.146567

[28] Tavassoli M., Jalilzadeh-Amin G., Fard V.R.B., Esfandiarpour R. 2018. The *in vitro* effect of *Ferula asafoetida* and *Allium sativum* extracts on *Strongylus* spp. *Annals of Parasitology* 64(1): 59–63. doi:10.17420/ap6401.133

[29] Jabbar A., Iqbal M.Z., Ashraf, M. 2022. Effects of *Ferula assafoetida*, closantel, albendazole, oxfendazole, and ivermectin against *Haemonchus contortus* in goats and sheep. *Tropical Animal Health and Production* 54(2): article number 107. doi:10.1007/s11250-022-03111-z

[30] Alyousif M.S., Al-Abodi H.R., Almohammed H., Alanazi A.D., Mahmoudvand H., Shalamzari M.H., Salimikia I. 2021. Chemical composition, apoptotic activity, and antiparasitic effects of *Ferula macrecolea* essential oil against *Echinococcus granulosus* protoscoleces. *Molecules* 26(4): article number 888. doi:10.3390/molecules26040888

[31] Fakhrieh Kashan Z., Delavari M., Arbabi M., Hooshyar H. 2017. Therapeutic effects of Iranian herbal extracts against *Trichomonas vaginalis*. *Iranian Biomedical Journal* 21(5): 285–293. doi:10.18869/acadpub.ibj.21.5.285

[32] Nazer M.R., Abbaszadeh S., Anbari K., Shams, M. 2019. A review of the most important medicinal herbs affecting giardiasis. *Journal of Herbmed Pharmacology* 8: 78–84. doi:10.15171/jhp.2019.13

[33] Moazeni M., Nazer A. 2010. *In vitro* effectiveness of garlic (*Allium sativum*) extract on scolices of hydatid cyst. *World Journal of Surgery* 34(11): 2677–2681. doi:10.1007/s00268-010-0718-7

[34] Moazeni M., Larki S., Oryan A., Saharkhiz M.J. 2014. Preventive and therapeutic effects of *Zataria multiflora* methanolic extract on hydatid cyst: an *in vivo* study. *Veterinary Parasitology* 205(1– 2): 107–112. doi:10.1016/j.vetpar.2014.07.006

[35] Norouzi R., Hejazy M., Azizi D., Ataei A. 2020. The effect of *Taxus baccata* L. extract on hydatid cyst protoscolices *in vitro*. *Archives of Razi Institute* 75(4): 473–480. doi:10.22092/ari.2019.125573.1310

[36] Kavoosi G., Purfard A.M. 2013. Scolicidal effectiveness of essential oil from *Zataria multiflora* and *Ferula assafoetida*: disparity between phenolic monoterpenes and disulphide compounds. *Comparative Clinical Pathology* 22: 999–1005. doi:10.1007/s00580-012-1518-2

[37] Mahmoudvand H., Dezaki E.S., Kheirandish F., Ezatpour B., Jahanbakhsh S., Harandi M.F. 2014. Scolicidal effects of black cumin seed (*Nigella sativa*) essential oil on hydatid cysts. *Korean Journal of Parasitology* 52(6): 653–659. doi:10.3347/kjp.2014.52.6.653

[38] Rouhani S., Salehi N., Kamalinejad M., Zayeri F. 2013. Efficacy of *Berberis vulgaris* aqueous extract on viability of *Echinococcus granulosus* protoscolices. *Journal of Investigative Surgery* 26(6): 347–351. doi:10.3109/08941939.2013.818746

[39] Moazeni M., Mohseni M. 2012. Sumac (*Rhus coriaria* L.): scolicidal activity on hydatid cyst protoscolices. *Surgical Science* 3(9): 452–456. doi:10.4236/ss.2012.39089

[40] Moazeni M., Saharkhiz M.J., Hoseini A.A., Alavi, A.M. 2012. *In vitro* scolicidal effect of *Satureja khuzistanica* (Jamzad) essential oil. *Asian Pacific Journal of Tropical Biomedicine* 2(8): 616–620. doi:10.1016/S2221-1691(12)60107-5

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