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Original Article

Comparison of the scolicidal effect of *Allium sativum* and *Ferula asafoetida* extract on hydatid cyst protoscoleces *in vitro*

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Summary
Cystic echinococcosis (CE) is a zoonotic disease and with has a global distribution. Today, much research carries out to inactivate hydatid cyst protoscoleces. In particular, herbal compounds have received more attention due to their cheapness, easy access, low toxicity, and side effects. This study aimed to compare the scolicidal effect of hydroalcoholic extract of *Allium sativum* (garlic) and *Ferula asafoetida* (angozeh) on hydatid cyst protoscoleces *in vitro*. The scolicidal activity of *A. sativum* and *F. asafoetida* extracts were evaluated at concentrations of 50, 100, 150, 200, and 250 mg/ml following 10, 30, and 60 minutes of exposure. Each reaction was repeated three times. The viability of protoscoleces was examined with a 0.1% eosin stain under a light microscope. The chemical composition of two extracts was analyzed using Gas Chromatography-Mass Spectrometry (GC–MS). Statistical analyses were performed with GraphPad software version 5.0. The results of this study showed that *F. asafoetida* extract, at a concentration of 250 mg/ml after 60 minutes of exposure, killed 100% of the protoscoleces compared to the control group, but the hydroalcoholic extract of *A. sativum* at the same concentration and time, it was able to kill 98% of protoscoleces. The main chemical components of *A. sativum* and *F. asafoetida* identified as allyl methyl trisulfide (12.8%) and methyl ester (13.9%), respectively. The findings of the present study showed that *F. asafoetida* has more potent scolicidal effects than *A. sativum*. However, further studies are needed to evaluate the effectiveness of the *F. asafoetida* plant.
Keywords: Hydatid cyst, Scolicidal, *Allium sativum*, *Ferula asafoetida*, *In vitro*.

Introduction

Hydatidosis is the result of infection with the larval stage of the *Echinococcus granulosus*. This disease is one of the most important zoonotic infectious diseases in humans and animals. Hydatid cysts can affect host organs such as the liver, lungs, heart, brain, bones, spleen, and kidneys and may even lead to death (Norouzi et al., 2020). At present, surgery and chemical drugs such as benzimidazole derivatives are used to treat hydatidosis (Ahmadpour et al., 2019). Recently, the use of chemical medicines has been limited due to increased resistance of protoscoleces, liver dysfunction, abdominal pain, diarrhea, nausea, and

headache (Naseri et al., 2016; Walker et al., 2004). On the other hand, the use of Albendazole in mice has shown teratogenic effects (Maggiore et al., 2012).

The scolicidal agents used in hydatid cyst surgery should be able to kill many protoscoleces in a short time and low concentrations, non-toxic, low cost, and safe for host tissues and available (Anthony et al., 2005). So far in Iran and around the world, many chemicals and scolicidal agents have been used to inactivate protoscoleces of hydatid cysts, among them, plant compounds are essential because they are easy to access, have low side effects, and are inexpensive (Kohansal et al., 2017); Therefore, researchers have a strong tendency to evaluate and present plant extracts as an alternative.

Allium sativum (garlic) is a well-known plant and medicine with beneficial properties such as antioxidant, anti-tumor, anti-viral, anti-fungal, anti-bacterial, anti-arthritic, and anti-worm effects (Eskandarian et al., 2012). *Ferula asafoetida* is a resin obtained from plant root secretions. This plant grows wild in the central and southern mountains of Iran. Resin or gum in Iran is called "Angozeh", "Khorakoma", and "Angozakoma" and has an unpleasant odor (Davoudi Moghadam et al., 2014). This gum is not only traditionally used to treat various diseases such as asthma, gastrointestinal disorders, bloating, intestinal parasites, and neurological disorders, but also as a spice in cooking (Iranshahy and Iranshahi, 2011). This resin is known for its anti-fungal, anti-viral, anti-diabetic, and anti-inflammatory properties (Bandyopadhyay et al., 2006; Lee et al., 2009). In addition, because the people of Nepal believe that Angozeh has antispasmodic, sedative, and antiseptic properties, they consume it in their diet (Bandyopadhyay et al., 2006). The aim of this study was to compare the protoscoleces effect of hydroalcoholic extract of *A. sativum* and *F. asafoetida* on hydatid cyst protoscoleces *in vitro.*

Materials and methods

Preparation of protoscoleces

In this experimental study, 30 sheep liver and lungs were collected from slaughterhouse and transferred to the parasitology laboratory. The cyst fluid was aspirated with a sterile syringe and transferred to a test tube and left at room temperature for 10 minutes to settle the protoscoleces. After 10 minutes, the supernatant was discarded, and the protoscoleces were washed two times with PBS and stained with 0.1% eosin to evaluate the viability of the protoscoleces.

Plant collection

In this experimental study, *F. asafoetida* gum was collected from the Tabas county (South Khorasan province, Iran) and was identified, approved, and registered with the herbarium number 2365 in the botany section of Yazd Agricultural Research Center. *A. sativum* was purchased from the fruit and vegetable market of East Azerbaijan province. After peeling, small slices were prepared and dried in the shade. Both plants were milled using an electric grinder.

Preparation of plant extracts

The maceration method was used to prepare the hydroalcoholic extract. 100 g of plant powder was mixed with 400 ml of 70% ethanol and placed on a shaker at room temperature. After three days, the material was passed through three cleaning layers and incubated at 37 ° C until the water and alcohol evaporated completely. After complete evaporation of water and alcohol, the dry material was shaved off the bottom, and stored at 4 ° C for later use.

Evaluation of the scolicidal activity of the plant extracts

To evaluate the scolicidal effect of the extracts of the two plants, concentrations of 50, 100, 150, 200 and 250 mg/ml of plants in distilled water were prepared separately. Half a milliliter of the extracts was added to the microtubes, and a drop of protoscoleces-rich sediment (containing 2000 protoscoleces) was added. The contents of the tubes were gently mixed, and the tubes were incubated for 10, 30, and 60 minutes at 37°C. After the incubation period, the upper phase was carefully removed. Then a drop of 0.1% eosin was added to the remaining precipitate. A drop of

protoscoleces was placed on a slide, and a slide was placed on it and the dead and living protoscoleces were counted using a light microscope. The effect of scolicidal of two *A. sativum* and *F. asafoetida* plants with Albendazole as positive control and distilled water as negative control in similar doses were evaluated and compared. These experiments were repeated three times for each concentration.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

Chromatographic analysis was performed using the GC-MS instrument (Agilent19091S-433) (Agilent Technologies, CA, USA). The hydroethanolic extract was mixed with hexane (1:1). Then the mixture was put in a separator, kept for 15 minutes to form a double phase. The hexane phase was isolated and injected into the GC/MS instrument for analysis.

Statistical Analysis

Statistical analyses were performed with GraphPad software version 5 and expressed as a mean ± SD. Data were analyzed by a two-way ANOVA.

Results

The results of this experimental study showed that the hydroalcoholic extract of *A. sativum* and *F. asafoetida* has scolicidal activity in all concentrations, but *F. asafoetida* extract has a high scolicidal effect compared to *A. sativum* extract in all concentrations and times. On the other hand, the results showed that *F. asafoetida* extract, at a concentration of 250 mg/ml after 60 minutes of exposure, killed 100% of the protoscoleces, the hydroalcoholic extract of garlic at the same concentration and time was able to killed 98% of the protoscoleces. In both extracts, scolicidal activity increased with increasing concentration. The scolicidal effects of two extracts at various concentrations and exposure times against protoscoleces are shown in Table 1 and Figure 1. The lowest scolicidal effect of each extracts, at a concentration of 50 mg/ml after 10 minutes of exposure to protoscoleces, was 5.33% and 8.33%, respectively.

This study showed a significant difference between the scolicidal effects of *F. asafoetida* hydroalcoholic extract at all concentrations compared to the negative control group (P <0.05). Also, the scolicidal effects of *A. sativum* extract at all concentrations except 50 mg/ml for 10 minutes in all receiving groups were significant compared to the negative control (P <0.05). For both extracts, the increase in lethal effects was dose-dependent and significant (P <0.05). While in the groups receiving *A. sativum* extract, all groups showed a significant difference from the positive control group (receiving albendazole) (P <0.05). Table 1 and Figure 1 compare the scolicidal activity of hydroalcoholic extracts of *A. sativum* and *F. asafoetida* on protoscoleces at different concentrations and exposure times. Figures 2 and 3 show the scolicidal activity of the hydroalcoholic extract of *A. sativum* and *F. asafoetida*. GC-MS analysis revealed that the main chemical components of *A. sativum* and *F. asafoetida* were identified as allyl methyl trisulfide (12.8%) and methyl ester (13.9%), respectively. The results of GC-MS analysis of the plant extracts are shown in Figures 4 and 5.

Discussion

To date, many scolicidal agents have been used to inactivate hydatid cyst protoscoleces, but many of these agents cause adverse effects; Therefore, their use is limited (Sharafi et al., 2017). Some studies have described the inhibitory effects of plants, their various extracts, and their components on a variety of protoscoleces. Mahdavi and Masood (2002) studied the scolicidal effect of aqueous and alcoholic extract of *Peganum harmala. L*. and their study showed that the aqueous extract of *P. harmala*, in comparison with its alcoholic extract, had a weak and insignificant effect on protoscoleces. In contrast, alcoholic extract at the same concentration and time, caused 100% mortality of protoscoleces (Mahdavi and Masood, 2002). Jafari et al. (2017) investigated the effect of aqueous extract of sour pomegranate on protoscoleces. They concluded that the concentration of 80 mg had the most significant effect after 15 minutes and caused the elimination

of 100% of protoscoleces (Jafari et al., 2017). Salehi et al. (2014) investigated the effects of aqueous and hydroalcoholic extract of barberry fruit on protoscoleces. Their study showed that aqueous extract in 5 minutes and hydroalcoholic extract in 2 minutes killed all protoscoleces. In both extracts, scolicidal activity increased with increasing dilution (Salehi et al., 2002). In another research, the lethal effect of methanolic extract of pomegranate root on protoscoleces was examined *in vitro*. Among the studied extracts, the concentration of 0.1% had strong scolicidal effects in 6 hours (Zibaei et al., 2014).

Table 1. The scolicidal effects of *A. sativum* and *F. asafoetida* extract at various concentrations and exposure times against hydatid cysts of *E. granulosus*

Concentration of the extract	Time of exposure	*A. sativum*	*F. asafoetida*	Positive control	Negative control
50 mg/ml	10 min	5.33 ± 1.15	8.33 ± 1.15	100 ± 0.00	4.66 ± 0.57
	30 min	11 ± 1.00	15 ± 1.00	100 ± 0.00	4.66 ± 1.00
	60 min	40.67 ± 2.08	60.67 ± 2.08	100 ± 0.00	4.33 ± 0.57
100 mg/ml	10 min	42.2 ± 2.00	56 ± 2.00	100 ± 0.00	4.66 ± 0.57
	30 min	45 ± 1.52	83 ± 2.00	100 ± 0.00	4 ± 1.00
	60 min	47 ± 2.08	87 ± 2.51	100 ± 0.00	4.33 ± 0.57
150 mg/ml	10 min	62 ± 2.08	68 ± 1.08	100 ± 0.00	4.66 ± 0.57
	30 min	73 ± 1.52	85.67 ± 3.05	100 ± 0.00	4 ± 1.00
	60 min	75 ± 1.5	94.67 ± 0.57	100 ± 0.00	4.33 ± 0.57
200 mg/ml	10 min	77 ± 1.00	90.33 ± 1.15	100 ± 0.00	4.66 ± 0.57
	30 min	80 ± 1.50	91.67 ± 1.50	100 ± 0.00	4 ± 1.00
	60 min	85 ± 0.75	95.67 ± 0.57	100 ± 0.00	4.33 ± 0.57
250 mg/ml	10 min	90 ± 0.57	97 ± 1.00	100 ± 0.00	4.66 ± 0.57
	30 min	93 ± 1.15	97.67 ± 0.57	100 ± 0.00	4 ± 1.00
	60 min	98 ± 0.00	100 ± 0.00	100 ± 0.00	4.33 ± 0.57

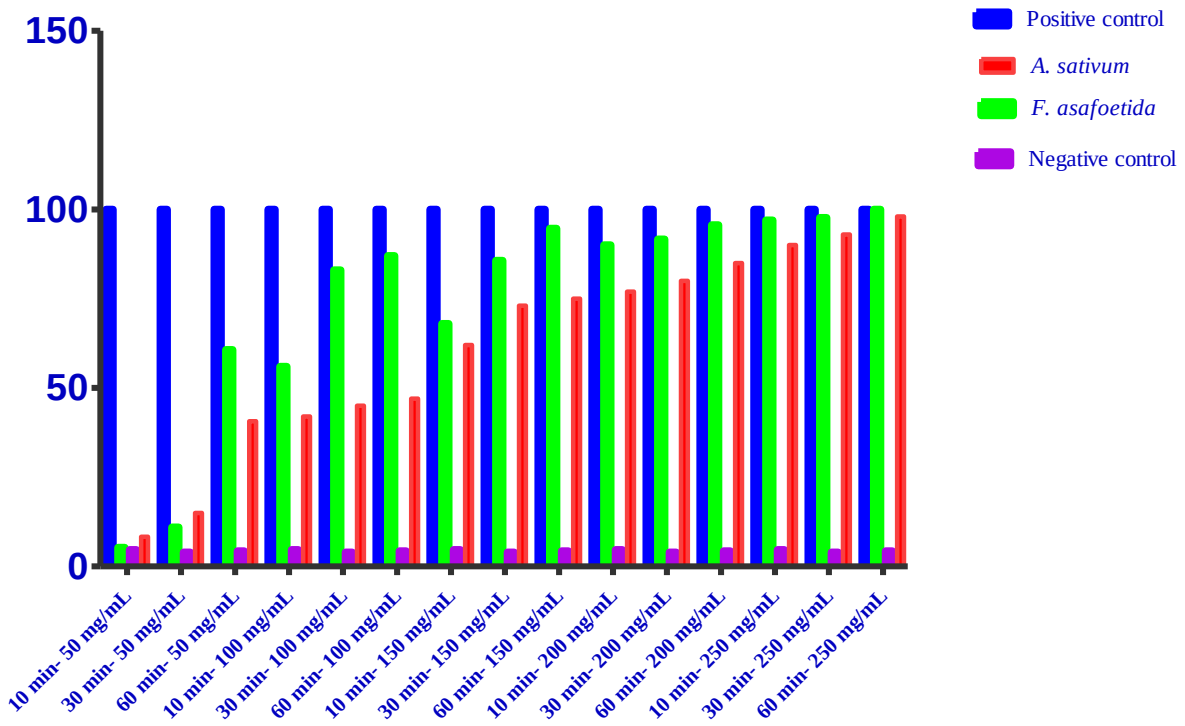

Fig. 1. The scolicidal effects of *A. sativum* and *F. asafoetida* extract at various concentrations and exposure times against hydatid cysts of *E. granulosus*

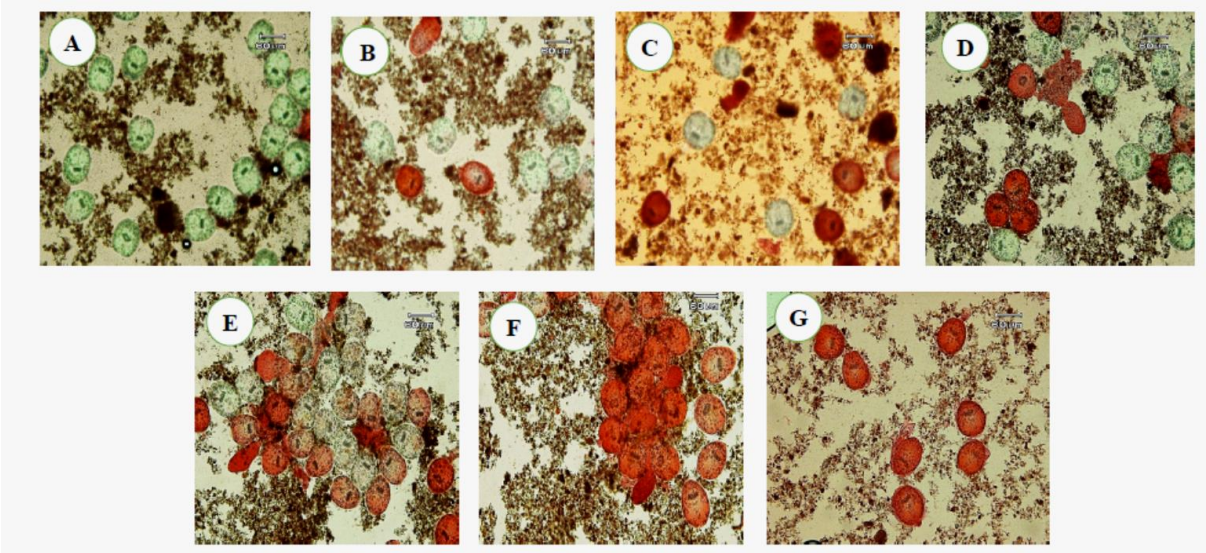

Fig. 2. Images of scolicidal activity of hydroalcoholic extract of *A. sativum*; **A:** Negative control, **B:** Concentration of 50 mg/ml, **C:** Concentration of 100 mg/ml, **D:** Concentration of 150 mg/ml, **E:** Concentration of 200 mg/ml, **F:** Concentration 250 mg/ml, **G:** Positive control.

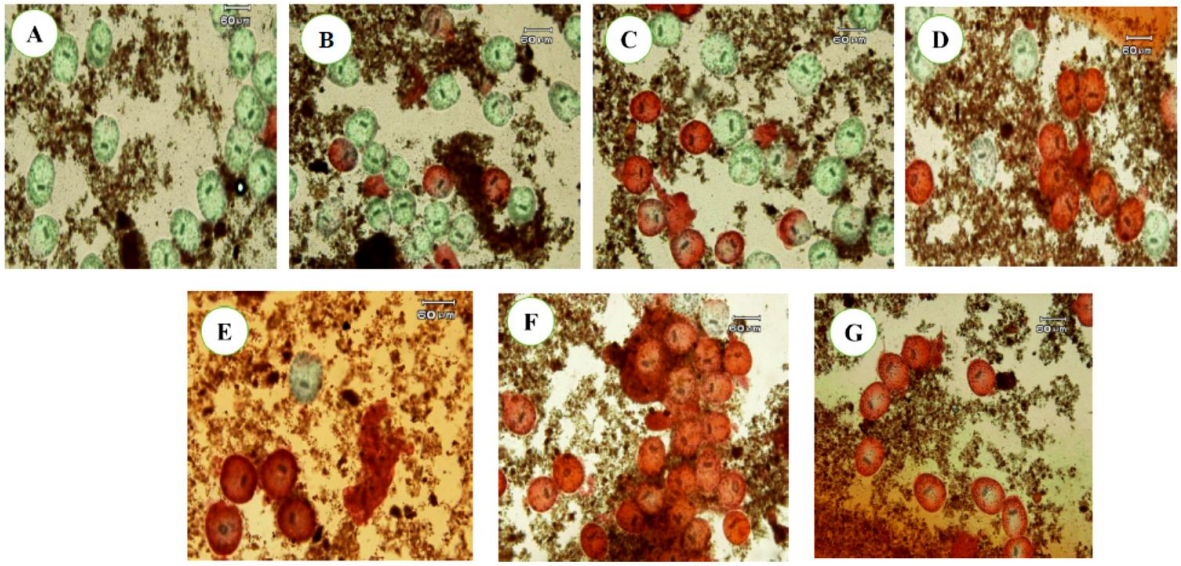

Fig. 3. Images of scolicidal activity of hydroalcoholic extract of *F. asafoetida*; **A:** Negative control, **B:** Concentration of 50 mg/ml, **C:** Concentration of 100 mg/ml, **D:** Concentration of 150 mg/ml, **E:** Concentration of 200 mg/ml, **F:** Concentration 250 mg/ml, **G:** Positive control.

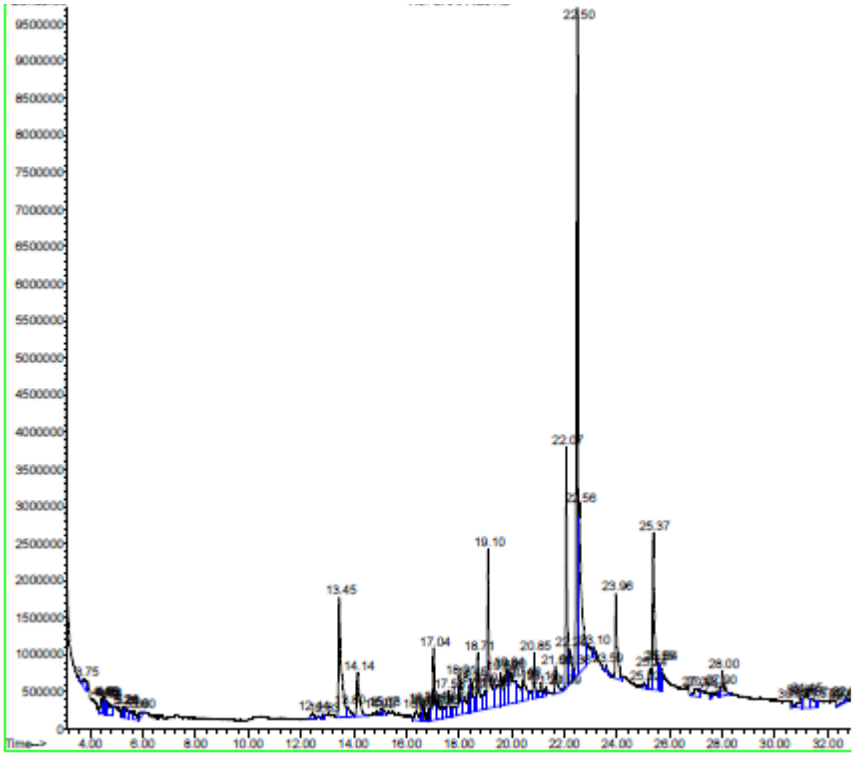

Fig. 4. GC-MS analysis of *A. sativum* extract

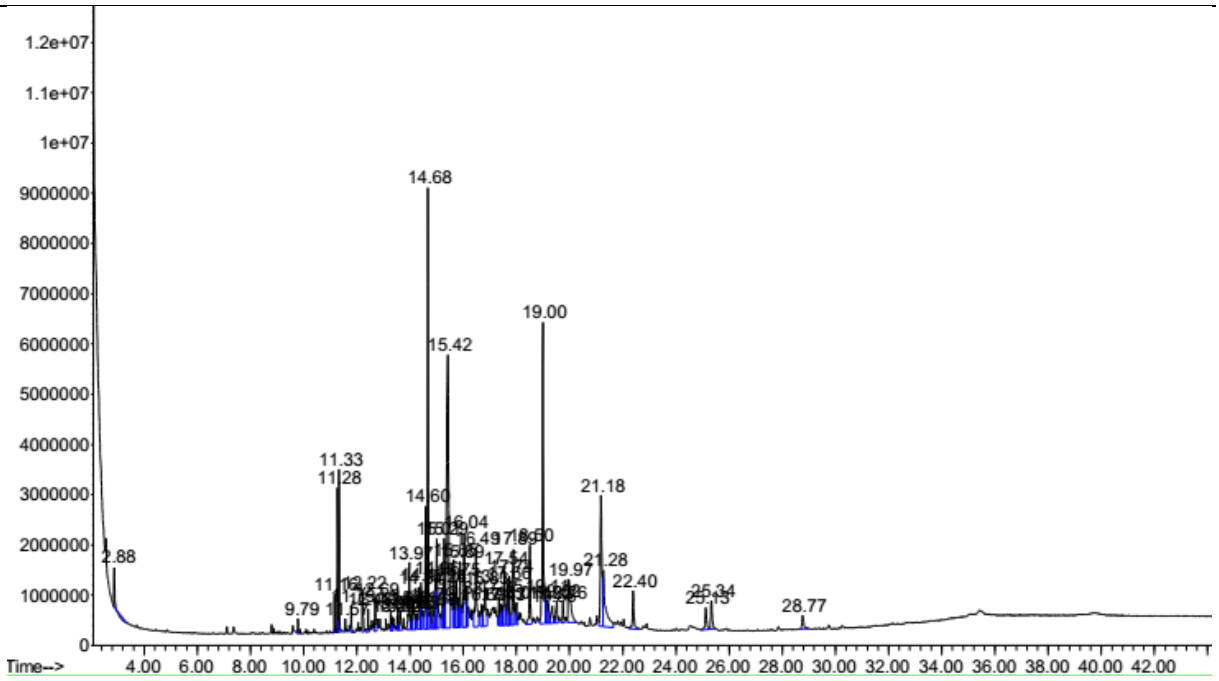

Fig. 5. GC-MS analysis of *F. asafoetida* extract

Bahrami et al. (2016) investigated the effect of *Lepidium sativum* on protoscoleces and concluded that a concentration of 15 mg had the most significant effect after 60 minutes (Bahrami et al., 2016). Feyzi et al. (2015) examined the effect of *Artemisia aucheri* and *Zingiber officinale* and concluded that methanolic extract of *Z. officinale* at a concentration of 100 mg/ml killed all protoscoleces in 40 min, while *A. aucheri* extract at all concentrations studied had little effect on protoscoleces (Feyzi et al., 2015). In an examination, studied the lethal effect of *Ceratonia silique* on hydatid cyst protoscoleces in vitro. According to the results, *C. silique* extract at a concentration of 50 mg/ml after 30 minutes caused the killing of all protoscoleces (Malekifard and Keramati, 2018). Moazeni and Nazer (2010) survived the effect of methanolic extract of *A. sativum* (garlic) on protoscoleces. They showed that a concentration of 25 mg/ml in 60 minutes eliminated 100% of protoscoleces (Moazeni and Nazer, 2010). The difference between this study and the present study is that in this study, the greatest effect of scolicidal is garlic 100%, but in the present study, 98% was obtained, which is probably due to the difference in the type of extract (methanolic and ethanolic), and unfortunately, in the Moazeni and Nazer study GC-MS has not been used to compare the differences in the composition of the extracts. Sadjjadi et al. (2008) used chloroformic extract of *A. sativum*, and the results revealed that concentration of 200 mg/ml had the highest scolicidal activity (Sadjjadi et al., 2008). In this study, the mortality rate (99.58 ± 1.63) was obtained, while in our study, 98% was obtained, which are almost close to each other. In a study, Moazani et al. (2014a) showed that methanolic extract of *Zataria multiflora* has a high scolicidal effect on hydatid cysts at concentrations of 10 mg/ml and 25 mg/ml after 3 minutes and 1 minute, respectively, 100% of the protoscoleces were destroyed (Moazani et al., 2014a). In another study, all protoscoleces were killed after 10 minutes of exposure to concentrations of more than 17 µg/ml of *Z. multiflora* essential oil (Kavoosi and Purfard., 2013). Mahmoudvand et al. (2014a) showed that *Nigella sativa* essential oil at a concentration of 10 mg/ml killed 100% of the protoscoleces 10 minutes after exposure (Mahmoudvand et al., 2014a). In a study barberry at the concentration of 4 mg/ml after

5 minutes had a 100% scolicidal effect (Rouhani et al., 2013). Norouzi et al. (2020) obtained a scolicidal effect of hydroalcoholic extract of *Taxus baccata L.* at a concentration of 150 mg/ml, 66.6% (Norouzi et al., 2020). Probably, the difference in the results of different studies is due to the difference in the type of plant, the type of extract, the difference in the concentration measurement units, and exposure time.

Conclusion

In general, the findings of this study indicate that the scolicidal activity of hydroalcoholic extract of *F. asafoetida* plant is high, so that at a concentration of 250 mg/ml after 60 minutes of exposure to protoscoleces, 100% of them are destroyed. It eliminates and suggests the potential of this plant as a natural scolicidal agent for use in hydatid cyst surgery. This study was performed *in vitro* condition, so it is necessary to do it *in vivo* to determine the exact concentration of the effective effect of this plant extract, and its possible side effects on internal organs. Check to get the results applied.

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Conflict of interests

The authors declare that there is no conflict of interest.

Ethical approval

Not applicable.

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