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Protoscolicidal Effects of *Bunium Persicum* (Boiss) Against Hydatid Cyst Protoscoleces

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Abstract. Medicinal plants have been used for treatment of various diseases. The potent scolicidal activity of medicinal plants has been also known. Thus, the current study was conducted to investigate the protoscolicidal effects of *Bunium Persicum* against hydatid cyst protoscoleces. Protoscoleces were collected from sheep's kidney having hydatid cysts. Five concentrations of *B. Persicum* essential oil (1, 3, 5, 10 and 15 mg/mL) were investigated for 10, 20, 30 and 60 min. Viability of protoscoleces was confirmed using eosin exclusive test. Regardless of the time, the rate of dead protoscoleces was increased with increasing the essential oil concentrations. The most rates of dead protoscoleces (100 %) were observed in 15 mg/mL from 10 to 60 minutes. On the basis findings this *in vitro* study, it can be advised *B. Persicum* essential oil as an effective natural scolicidal agent.
Keyword: *Bunium Persicum*, Hydatid cyst, Protoscoleces, Scolicidal activity.

Introduction

Hydatid disease is caused by a tapeworm of the genus *Echinococcus*.

This disease is known as one of the most important neglected parasitic diseases at all over the world [MAHMOUDVAND *et al.,* 2016a] and it can influence humans and domestic livestock such as cattle, sheep, camels, pigs, horses and others.

This disease is broadly found at each region of world, especially where dogs consume organs of animals infected with hydatid cyst and help to developmental cycle [AMMANN and ECKERT, 1995].

Different treatments have been used for treatment of hydatid disease including surgery, ultrasound–guided aspiration, and chemotherapy [WHO, 1996].

Chemotherapy has also been applied as an adjunct to surgery the prophylaxis against spillages having cyst [MOAZENI and MOHSENI, 2012].

The benzimidazole and albendazole are known as suitable drugs for treatment of hydatid cyst.

Most studies have been indicated the efficacy of albendazole by the disappearance the cysts less than 30 % under ideal circumstances [MOAZENI and MOHSENI, 2012].

This topic well shows that other treatments will be needed for hydatid cyst.

Much effort has been done to finding new antimicrobial compounds from various kinds and sources such as plants, animals and microorganisms. Herbal plants have been recently used to treat many diseases such as infections [MOAZENI and MOHSENI, 2012].

Bunium persicum, so called "Zireh Kohi" in Persian, is belonging to Apiaceae family, and it broadly grows in the southeast of Iran [RECHINGER, 1989].

It is known to have many benefits including carminative, anti–spasmodic, increasing breast milk and antiepileptic treatment [ZARGARI, 1996].

Its seeds are capable to treating diarrhea and dyspepsia [BASER *et al.,* 1997, CERNEA, *et al.,* 2015, SAMFIRA, *et al.,* 2015].

Studies have been also reported other benefits for its essential oil such as antinociceptive, antioxidant, anti–inflammatory and antimicrobial effects [HAJHASHEMI *et al.,* 2011, TALEI and MOSAVI 2009, SEKINE *et al.,* 2007].

Thus, the present study was conducted to investigate the protoscolicidal effects of *B. Persicum* essential oil against protoscoleces of hydatid cysts.

Material and methods

Plant material and essential oil isolation

The seeds of *B. Persicum* were purchased from Kerman, Iran at October 2015. The samples were then identified by botanist and dried at room temperature.

The dried samples (by 150 g) were subjected to hydrodistillation for 3 h by an all–glass Clevenger–type apparatus.

This work was repeated several times to obtain the enough essential oil. The essential oil achieved was dried over anhydrous sodium sulfate.

Gas chromatography (GC)–mass spectrometry (MS) analysis

GC analysis of the essential oil of *B. Persicum* was done using a Shimadzu QP 5000 (FID) chromatograph HP–5 MS capillary column (30m×250 µm, film thickness 0.25 µm).

Helium was used as carrier gas at a flow rate 0.9 mL/min (split ratio 1:43) with an injection volume of 0.1 µL.

Injector temperature was set at 280 °C. Oven primary temperature was kept at 60 ° C and gradually increased to 275 °C at 10 °C /min, held for 10 min.

MS analysis was done by a Shimadzu QP 5050 operating at 70 eV ionization energy supplied with an HP–5 capillary column (30m×250 µm, film thickness 0.25 µm) with helium as the carrier gas. Retention indices were determined by retention times of n–alkanes (C8–C28) and by computer programming. The components were also identified by their relative retention time and mass spectra with standards, NIST version8 library data of the GC/MS system, and previous data [ADAMS 2004, BUTNARIU, and BOSTAN, 2011, BUTNARIU, 2012].

Collection of protoscoleces

Protoscoleces were collected from kidneys the sheep's infected at Ahvaz industrial slaughter, Iran.

The cyst fluids were aspirated and transferred to Falcon tubes. Fluids were then centrifuged at 2000 rpm for 3 minutes and upper fluids were removed.

Protoscoleces present in lower sediments were washed for 3 times and viability of protoscoleces was confirmed using eosin exclusive test.

In the current study, five concentrations of *B. Persicum* essential oil (1, 3, 5, 10 and 15 mg/mL) were investigated for 10, 20, 30 and 60 min. Then 2.5 mL of each solution was placed in test tubes and then 15 mL of protoscoleces–rich sediment was added. The contents of the tubes were gently mixed. The tubes were then incubated at 37°C for 10, 20, 30 and 60 min. At the end of each incubation time the upper phase was carefully removed so as not to disturb the protoscoleces.

One milliliter of 0.1 % eosin stain was then transferred to the remaining settled protoscoleces and mixed gently.

The upper portion of the solution was discarded after 15 min of incubation.

The remaining pellet of protoscoleces was then smeared on a manually scaled glass slide which had been covered with a cover glass (24×50 mm), and examined under a light microscope.

The dead protoscoleces (%) were investigated by counting a minimum of 500 protoscoleces. Non-treated protoscoleces were calculated as a control group in each experiment. The experiments were done in triplicate.

Statistical analysis

The data were analyzed using SPSS software (version 16) and Kruskal–Wallis test. Also, significant differences were considered at $P<0.05$.

Results and discussion

The data for GC/MS analysis of *B. Persicum* essential oil are presented in Table 1.

Analysis found 23 components in *B. Persicum* essential oil that constitute by 96 % of *B. Persicum* essential oil.

The main components were including β–terpinene–7–al (28 %) γ–

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terpinene (25.7 %) and cuminaldehyde (26.1 %).

Table 1.

GC/MS analysis of *B. Persicum* essential oil

	Components	Retention indices	% Composition
1	p–cymen–8–ol	1183	0.1
2	p–menth–1–en–7–al	1283	0.2
3	Thujene	927	0.2
4	Trans–sabiene–hydrate	1104	0.3
5	Myrcene	998	0.6
6	Limonene	1032	2.8
7	Terpinene	1016	0.1
8	Trans 2–p–menthen–7–ol	1272	0.2
9	Myrteyl acetate	1316	0.4
10	γ–terpinene	1060	25.7
11	β–pinene	982	1.2
12	pinene	935	0.7
13	Cis sabinene hydrate	1073	0.2
14	Isobomyl acetate	1290	0.1
15	Cuminaldehyde	1261	26.1
16	Sabinene	960	0.4
17	Trans–chrysanthenol	1199	1.5
18	β–terpinene–7–al	1291	28
19	p–cymene	1027	6.3
20	β–phellandrene	1011	0.1
21	Terpinolene	1087	0.3
22	E–Myromide	1123	0.2
23	1,8–cineole	1035	0.2
24	Linalool	1099	0.1

Scolicidal effects of *B. Persicum* essential oil against hydatid cysts at different concentration and time are presented in Table 2. Results indicated that higher concentrations higher concentrations of *B. Persicum* essential oil (10 and 15 mg/mL) could kill 100 % hydatid cysts after 20 min exposure.

Results also showed that lower concentrations (3 and 5 mg/mL) could show more efficient scolicidal effects at more interval times.

Table 2.

Scolicidal effects of BP essential oil against protoscoleces of hydatid cysts at the various contents and times

	10 min	**20 min**	**30 min**	**60 min**
Control	5.6	5.9	8.1	8.9
1 mg/mL	6.1	7.5	10.2	17.0
3 mg/mL	21.8	23.1	23.9	39.2
5 mg/mL	45.6	49.3	65.8	80.0
10 mg/mL	95.6	100	100	100
15 mg/mL	100	100	100	100

The present study found β–terpinene, γ–terpinene and cuminaldehyde as main components of *B. Persicum* essential oil.

Other studies found γ–terpinene, cuminal, and p–cymene [HAJHASHEMI *et al.*, 2011, BUTU, *et al.*, 2015, PENTEA, *et al.*, 2015] and/or cuminaldehyde, γ–terpinene, p–cymene, cuminyl alcohol, and limonene as main components of *B. Persicum* essential oil [FOROUMADI *et al.*, 2002, BUTNARIU, *et al.*, 2016, BUTU, *et al.*, 2014, BUTNARIU, *et al.*, 2015].

Studies have been reported that some factors including species, climate, collection time and growth stage can influence chemical composition of essential oil [YESIL CELIKTAS *et al.*, 2007; SAEDI DEZAKI *et al.*, 2015, BUTNARIU and CORADINI, 2012].

Our findings showed that higher concentrations of *B. Persicum* essential

oil (10 and 15 mg/mL) could kill 100% hydatid cysts after 20 min exposure, while concentration of 15 mg/mL could kill 100% hydatid cysts on 10 minutes.

Results also showed that lower concentrations (3 and 5 mg/mL) could efficiently show more scolicidal effects at more interval times. A study has been shown that *B. Persicum* essential oil could show potent scolicidal activity without significant toxicity [MAHMOUDVAND *et al.*, 2016b].

Other study showed that methanolic extract of garlic could kill 100 % protoscoleces at concentration 25 mg/mL after 60 min of exposure [PESSOA *et al.*, 2002].

Very little chemotherapeutic agents are found to have medical management of hydatid disease induced–*Echinococcus granulosus* [BLANTON *et al.*, 1998, FERENCZ, *et al.*, 2012, RODINO, *et al.*, 2014]. The control parasitic diseases are usually made with synthetic anthelmintics, but this substance may have undesirable complications which restricts their use. Drug resistance promoted researchers to find the alternatives, such as medicinal plants [PESSOA et al., 2002, BUTNARIU, *et al.*, 2012].

Studies have been reported some components including γ–terpinene and cuminaldehyde as antimicrobial against some pathogenic microbial strains [CARSON *et al.*, 1995; NAVEED *et al.*, 2013, BUTNARIU and GIUCHICI, 2011]. Regarding previous studies, it can be stated that phytoconstituents present in *B. Persicum* essential oil could be responsible for scolicidal activity. The mechanism is not still known. Sikkema and collab. showed that these components diffuse into pathogens and can destroy cell membrane structures. Interestingly, lower concentrations of *B. Persicum* essential oil could remove higher rate of protoscoleces at more time [SIKKEMA *et al.*, 1995, BUTNARIU, 2014]. This means that lower concentrations will be needed more time to showing scolicidal effects.

Conclusions

This in vitro study showed scolicidal effects of *B. Persicum* essential oil especially at higher concentrations (10 and 15 mg/mL) at shorter time and lower concentration (5mg/mL) at longer time. These findings allowed us to advising *B. Persicum* essential oil as source of new compounds which could be used as an effective scolicidal agent. Further studies will be needed to find and isolate these active compounds. On the basis our findings, it can be advised *B. Persicum* essential oil for traditionally medicine.

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