SUPPLEMENTARY MATERIALS

Chemical composition, protoscolicidal effects and acute toxicity of *Pistacia atlantica* Desf fruit extract

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Abstract

The present study was designed to evaluate chemical composition and scolicidal effects of *Pistacia atlantica* Desf. extract against protoscoleces of hydatid cysts and also its acute toxicity in mice model. Various concentrations of the methanolic extract (5-50 mg/mL) were used for 10 to 60 min. Viability of protoscoleces was confirmed using eosin exclusion test (0.1%). Acute toxicity was also determined in mice model. The main components were β -myrcene (41.4%) α -pinene (32.48%), and limonene (4.66%). Findings demonstrated that *P. atlantica* extract at the concentrations of 25 and 50 mg/mL after 20 and 10 min of exposure killed 100% protoscoleces. The LD₅₀ of the intraperitoneal injection of the *P. atlantica* methanolic extract was 2.43 g/kg and the maximum non-fatal dose was 1.66 g/kg. Obtained results showed potential of *P. atlantica* extract as a natural source with no significant toxicity for the producing of new scolicidal agent for use in hydatid cyst surgery.

Keywords: hydatid cyst; scolicidal; toxicity; *P. atlantica*

3. Experimental

3.1. Plant materials and extraction

The fruits of *P. atlantica* were collected from rural regions of Lorestan province, west of Iran, from May to September 2013. They were identified by a botanist of the Botany Department of Razi Herbal Medicines Research Center, Khorramabad, Iran (LF 2522). Air dried plant materials (100 g) were separately extracted by percolation method with 80% methanol successively for 72 h. in room temperature. The extracts were passed through filter paper (Whatman No.3, Sigma, Germany) to remove plant debris. The extracts were finally concentrated in vacuum at 50°C using a rotary evaporator (Heidolph, Germany) and stored at -20°C, until using (Mahmoudvand et al., 2014b).

3.2. Gas chromatography/mass spectrometry (GC/MS) analysis of extract

In this study, chemical compositions of *P. atlantica* extract were identified by extracting them using a solid phase micro extraction (SPME) technique according to the method described by Ghiasvand et al. (2011). GC/MS was performed using by an Agilent 6890N coupled with a HP-5MS column (30 m × 0.25 mm, film thickness 0.25 mm). The column temperature was maintained at 40°C for 12 min and programmed to 180°C at a rate of 10°C per min, and kept constant at 180°C for 4 min. Injector and interface temperatures were 250 and 280°C, respectively. The flow rate of Helium as carrier gas was (1mL/min C.F). The percentages were calculated by electronic integration of FID peak areas without the use of response factors correction. Linear retention indices for all components were determined by co-injection of the samples with a solution containing homologous series of C8–C24 *n*-alkanes. The constituents of the extracts were identified by comparison of their relative retention time and mass spectra with those of standards Wiley 2001 library data of the GC/MS system or with those of reported in the literature data (Adams, 2004).

3.3. Protoscolicidal effect

The protoscoleces of hydatid cysts were obtained from the livers of naturally infected sheep and goats slaughtered at Kerman abattoir, southeastern Iran and carried to the Parasitology Laboratory at the, Kerman University of Medical Sciences, Iran. The number of protoscoleces per mL was adjusted as 2×10^3 protoscoleces in 0.9% NaCl solution with at least 90% viability rate. To evaluate the protoscolicidal activity of *P. atlantica* extract against protoscoleces of hydatid cysts, various concentrations of the extract (5-50 mg/mL) were used for 10, 20, 30 and 60 min according to the method described by Mahmoudvand et al (2014c).

Normal saline and hypertonic saline 20% were used as negative and positive control, respectively. Eosin exclusion test was used to determine the viability of protoscoleces of hydatid cysts. Eosin solution with a concentration of 0.1% (1 g of eosin powder in 1000 mL distilled water) was used for discrimination process. The live protoscoleces remained colorless and displayed characteristic muscular movements and flame cell activity after exposure to the stain, while dead protoscoleces absorbed eosin and colored red (Fig. 1).

3.4 Acute toxicity

Twenty four male NMRI mice (6–8 weeks old) were obtained from the Animal Breeding Stock Facility of Razi Institute of Iran (Karaj, Iran). Different doses of *P. atlantica* extract (0.5-4 g/kg) were injected as intraperitoneally into groups of six mice. The number of deaths was counted at 48 h after treatment. LD₅₀ values were determined by the probit test SPSS software (Hosseinzadeh et al., 2013).

3.5. Statistical analysis

All the tests were performed in triplicate. Data analysis was carried out by using SPSS statistical package (version 17.0) (SPSS Inc., Chicago, IL, USA). Differences between test and control groups were analyzed by *t*-test. In addition, $P < 0.05$ was considered statistically significant

References

- Adams, R.P, 2004. Identification of essential oil components by gas chromatography/mass spectroscopy. Illinois, USA: Carol Stream, IL: Allured Publishing Corporation.
- Brunetti, E., Kern, P., Vuitton, D.A, 2010. Writing Panel for the WHO-IWGE Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. Acta. Trop. 114(1), 1-16.
- Bozorgi, M., Memariani, Z., Mobli, M., Salehi Surmaghi, M.H., Shams-Ardekani, MR., Rahimi, R, 2013. Five *Pistacia* species (*P. vera*, *P. atlantica*, *P. terebinthus*, *P. khinjuk*, and *P. lentiscus*): a review of their traditional uses, phytochemistry, and pharmacology. Sci. World. J. doi: 10.1155/2013/219815.
- Cowan, M.M, 1999. Plant products as antimicrobial agents. Clin. Microb. Rev. 12, 564-582.

Cristani, M., D'Arrigo, M., Mandalari, G., Castelli, F., Sarpietro, M.G., Micieli, D., Venuti, V., Bisignano, G., Saija, A., Trombetta, D, 2007. Interaction of four monoterpenes contained in essential oils with model membranes: implications for their antibacterial activity. J. Agric. Food. Chem. 55(15), 6300-6308.

Ghiasvand, A. R., M. Nasser, S. Farsizaeh, M. H. Meshkatsadat, R. Sadeghi-Sarabi, S. Shadabi and M. Borzoei , 2011. Chemical characterization of cultivated *Tagetes minuta* L. by use of ultrasound-assisted head space SPME and GC–MS. Chromatographia 73(9-10), 1031-1035.

Hosseinzadeh, H., Sadeghi Shakib, S., Khadem Sameni, A., Taghiabadi, E, 2013. Acute and subacute toxicity of safranal, a constituent of saffron, in mice and rats. Iran J Pharm Res 12(1):93-99.

Loomis, T.A, 1968. Essential of toxicology. Philadelphia: Lea and Febige.

Mahmoudvand, H., Saedi Dezaki, E., Kheirandish, F., Ezatpour, B., Jahanbakhsh, S., Fasihi Harandi, M, 2014a. Scolicidal effects of black cumin seed (*Nigella sativa*) essential oil on hydatid cysts. Korean. J. Parasitol. 52(6), 653-659.

Mahmoudvand, H., Tavakoli, R., Sharififar, F., Minaie, K., Ezatpour, B., Jahanbakhsh, S., Sharifi, I., 2014b. Leishmanicidal and cytotoxic activities of *Nigella sativa* and its active principle, thymoquinone. Pharm. Biol. 4:1-6.

Mahmoudvand, H., Sharififar, F., Saedi Dezaki, E., Ezatpour, B., Jahanbakhsh, S., Fasihi Harandi, M, 2014c. Protoscolicidal effect of *Berberis vulgaris* root extract and its main compound, berberine in cystic echinococcosis. Iranian. J. Parasitol. 9(4): 26-34.

Mahmoudvand, H., Fasihi Harandi, M., Shakibaie, M., Aflatoonian, M.R., Makki, M.S., Jahanbakhsh, S, 2014d. Scolicidal effects of biogenic selenium nanoparticles against protoscolices of hydatid cysts. Int. J. Surg. 12(5): 399-403.

Rodrigues, K.A., Amorim, L.V., Dias, C.N., Moraes. D.F., Carneiro, S.M., Carvalho, F.A, 2015. *Syzygium cumini* (L.) Skeels essential oil and its major constituent α -pinene exhibit anti-*Leishmania* activity through immunomodulation *in vitro*. J Ethnopharmacol. 3, 160: 32-40.

Sokovic, M., van Griensven, L.G, 2006. Antimicrobial activity of essential oils and their components against the three major pathogens of the cultivated button mushroom, *Agaricus bisorus*. *Eur. J. Plant. Pathol.* 116, 211-224.

Yesil Celiktaş, O., Hames Kocabas, E.E. Bedir E., Vardar Sukan F, 2007. Antimicrobial activities of methanol extracts and essential oils of *Rosmarinus officinalis*, depending on location and seasonal variations. *Food Chem*, 100, 553–559.

World Health Organization (WHO) informal working group on echinococcosis, 1996. *Bull WHO* 74, 231–242.

Table S1. Fruit extract composition of *P. atlantica* identified by GC/MS.

No.	Compound	Retention time	% of Components
1.	α -Pinene	6.68	32.48
2.	Sabinene	7.85	3.066
3.	β -Myrcene	8.33	41.04
4.	α -Terpinene	8.72	0.377
5.	delta.3-Carene	8.89	1.337
6.	Limonene	9.51	4.66
7.	cis-Ocimene	9.78	1.621
8.	trans-Ocimene	10.13	1.100
9.	γ -Terpinene	10.49	0.478
10.	α -Terpinolene	11.47	0.807
11.	Linalool	12.01	1.019
	(E)-4,8-Dimethyl-1,3,7-		
12.	nonatriene	12.40	2.390
13.	Alloocimene	12.88	1.363
14.	Verbenol	13.56	0.582
15.	Piperitone	17.25	0.392
16.	α -Terpinenyl acetat	20.30	0.422
17.	trans-Caryophyllene	22.47	1.368
18.	α -Caryophyllene	23.47	0.388
19.	β -Santalene	24.15	0.403
20.	isoseychellene	24.64	0.258
21.	delta.-Cadinene	25.37	0.228
22.	Nerolidol	26.46	0.683
23.	Verbenyl ethyl ether	26.99	0.292
24.	Mayuron	28.46	0.172
25.	11-n-Decyldocosane	30.74	1.339
26.	1-Hexacosene	32.54	0.234
Total			97.14

Figure S1. Live (left) and dead (right) protoscoleces of hydatid cysts after exposure to *Pistacia atlantica* Desf fruit extract.