In vitro Protoscolicidal Effects of Cinnamomum zeylanicum Essential Oil and Its Toxicity in Mice

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ABSTRACT

Background: This study investigates the scolicidal effects of Cinnamomum zeylanicum essential oil against the protoscoleces of hydatid cysts and its toxicity in the mice model. Materials and Methods: Gas chromatography/mass spectroscopy analyses were used to identify the constituents of essential oil. Protoscoleces were treated with different concentrations of the essential oil (6.25–100 µL/mL) in each test tube for 5–30 min. The viability of protoscoleces was confirmed using eosin exclusion test (0.1% eosin staining). Forty-eight male NMRI mice were also used to determine the toxicity of C. zeylanicum essential oil (0.5–4 mL/kg). Results: The main components were found to be cinnamaldehyde (91.8%), p-metoxicinamate (1.57%), and α-pinene (1.25%). Findings indicate that C. zeylanicum essential oil with the concentrations of 100 and 50 µL/mL killed 100% of protoscoleces after 5 min of exposure. Also, the lower concentrations of C. zeylanicum essential oil motivated a late protoscolicidal effect. The LD₅₀ value of intraperitoneal injection of C. zeylanicum essential oil was 2.07 mL/kg body weight after 48 h, and the maximum nonfatal dose was 1.52 mL/kg body weight. The results also showed that there was no significant toxicity following oral administration of C. zeylanicum essential oil for 2 weeks. Conclusion: The results exhibited the favorable scolicidal activity of C. zeylanicum, which could be applied as a natural scolicidal agent in hydatid cyst surgery.

Key words: Cystic echinococcosis, gas chromatography/mass spectroscopy, hydatid cyst, protoscoleces

SUMMARY

• We evaluated the efficacy of Cinnamomum zeylanicum essential oil against hydatid cyst protoscoleces
• The viability of protoscoleces was confirmed using eosin exclusion test (0.1% eosin staining)
• Forty-eight male NMRI mice were also used to determine the toxicity of C. zeylanicum essential oil
• C. zeylanicum with potent scolicidal activity could be applied as a natural scolicidal agent in surgery.

INTRODUCTION

Cystic echinococcosis (CE) is a zoonotic parasitic disease which is caused by the larval form of Echinococcus granulosus tapeworm.[1] CE has a worldwide distribution but is mostly prevalent in the rural areas, in which it is transmitted in a cycle between dogs (definitive host), as domestic livestock, and humans, as the intermediate host.[2,3] CE may develop in humans after the accidental ingestion of tapeworm...
eggs excreted with the feces of an infected dog. The eggs hatch in the intestine of the herbivore, penetrate into the intestinal wall, and reach the livers (50%–70%), lungs (20%–30%), or any other organs through the portal system, in which they develop to a hydatid cyst. At present, surgical intervention (conventional or laparoscopic approaches) is the ideal treatment for CE. In addition, cyst puncture, aspiration, injection of chemicals, and reaspiration, chemotherapy with benzimidazole compounds, and watching and waiting for inactive, clinically silent cysts are the substitute treatments to surgery, particularly for the patients who cannot tolerate surgery. Through the surgery for dropping threat of intraoperative outflow of protoscolecites (cyst contents) and followed by the repetition of CE and secondary infection, it is necessary to apply effective protoscolicidal agents. At this time, the accessible protoscolicidal agents including hypertonic saline and silver nitrate are connected with some serious adverse effects such as biliary tract fibrosis and liver necrosis. Therefore, the expansion of new helpful scolicial agents, mainly from natural resources with little side effects and higher efficiency, is extremely important for surgeons.

Reviews have reported antimicrobial activity of plants and derivative essential oils, extracts, and other phytoconstituents. Essential oils are an important supply of new antimicrobial agents as a result of their two imperative properties: further protection to people and environment in addition to low risk of the appearance of microbial resistance, given that they have components which possibly will indicate a range of mechanisms of antimicrobial effects. Cinnamomum zeylanicum Blume (Lauraceae) has long been used as a spice and flavoring agent in different cultures around the world for several centuries. True cinnamon or C. zeylanicum is the inner bark of a small evergreen tree, which is considered a medication for respiratory, digestive, and gynecological disorders. A range of pharmacological functions in antitumor, anti-inflammatory, antioxidant, antimicrobial, and antidiabetic terms have been attributed to this plant. Previous investigations have found cinnamaldehyde, camphene, linalool, α-terpine, and limonene as the main constituents of C. zeylanicum essential oil. However, some factors such as location and seasonal variations could affect the chemical composition and antimicrobial activity of this essential oil. This study analyzes the chemical composition of the essential oil obtained from C. zeylanicum bark, its scolicidal effects on protoscolecites of E. granulosus, and its toxicity in mice.

MATERIALS AND METHODS

Plant material

C. zeylanicum bark was collected from rural regions of Jiroft district (Kerman, Iran) in June 2014. The plant materials were identified by Dr. Mandegari, a botanist in Herbal Medicines Research Center, Kerman University of Medical Sciences, Kerman, Iran. Voucher specimen was deposited at Herbarium of Pharmacognosy Department of Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran.

Extraction/isolation of essential oil

Air-dried bark (200 g) was subjected to hydrodistillation for 3 h by an all-glass Clevenger-type apparatus. The obtained essential oil was dried on anhydrous sodium sulfate and stored in darkness at 4°C in airtight glass vials closed under nitrogen gas until testing.

Drug dilutions

The essential oil (0.1 mL) was dissolved in 0.97 mL of normal saline. In addition, to increase the dispersal of the essential oil, 0.03 mL of Tween 20 (Sigma-Aldrich, St. Louis, MO, USA) was added to the test tube. Obtained solution was adequately mixed using a magnetic stirrer. Then, serial dilution was made to access the essential oil at concentrations of 100, 50, 25, 12.5, and 6.25 µL/mL. The selection of the essential oil dilutions was according to pilot experiments, which also indicated that normal saline plus Tween 20 had no effect on the growth of protoscolecites.

Gas chromatography/mass spectrometry analysis

Gas chromatography analysis

Gas chromatography (GC) analysis was carried out by a Shimadzu QP5050 with a HP-5MS column (30 m × 0.25 mm, film thickness 0.25 mm). The column temperature was retained at 60°C for 3 min and programmed to 180°C at a rate of 5°C/min and maintained constant at 275°C for 5 min. Injector and interface temperatures were 230 and 280°C, respectively. The flow rate of helium as carrier gas was (0.9 mL/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 coinjection of the samples with a solution containing homologous series of C8-C20 n-alkanes.

Gas chromatography/mass spectrometry analysis

GC/mass spectrometry (MS) analysis was carried out by a Thermoquest-Finnigan gas chromatograph equipped with fused silica capillary DB-5 column (30 m × 0.25 mm, film thickness 0.25 mm) coupled with a TRACE mass (Manchester, UK). Helium was used as carrier gas with ionization voltage of 70 eV. Ion source and interface temperatures were 230 and 280°C, respectively. Mass range was from 40 to 300 U. Oven temperature program was the same given above for the GC.

Identification of the essential oil components

The constituents were identified using 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 showed in the literature data.

Collection of protoscolecites

The protoscolecites of hydatid cysts were collected from the livers of naturally infected sheep and goats slaughtered at Kerman abattoir, Southeast of Iran and transferred to the Parasitology Laboratory at the Department of Parasitology and Mycology, Kerman University of Medical Sciences (Kerman, Iran). All hydatid cyst fluid aseptically aspirated by a 50 mL syringe and transferred into a glass tube was left for 30 min for protoscolecites to settle down. After throwaway the supernatant, the protoscolecites were washed two times with PBS (pH 7.2) solution. Finally, the number of protoscolecites per mL was adjusted as 2 × 10^4 protoscolecites in 0.9% NaCl solution with at least 90% viability rate.

Effect on protoscolecites

To evaluate scolicidal effects, various concentrations of the essential oil were evaluated during 5, 10, 20, and 30 min. Initially, 0.5 mL of the protoscolecites (2 × 10^4/mL) solution was poured in each test tube. Then,
0.5 mL of different concentrations of the essential oil was added to test tubes. The tubes were mixed gently and then incubated at 37°C for 5, 10, 20, and 30 min. After incubation, 50 µL of 0.1% eosin stain was added to the remaining settled protoscoleces and mixed gently again. After 10 min of incubation, the upper portion of the solution was discarded. The remaining pellet of protoscoleces was then smeared on a glass slide and examined under light microscope. The mortality rate of the protoscoleces was determined by counting 300 protoscoleces.\(^2\) Moreover, normal saline plus Tween 20 and 20% hypertonic saline were used as negative and positive control, respectively.

**Viability test**

The viability was confirmed by flame cell motility and impermeability of the protoscoleces to 0.1% eosin (Sigma-Aldrich, St. Louis, MO, USA) solution under a light microscope (Smyth & Barrett, 1980). After exposure, live protoscoleces remained colorless and exhibited characteristic muscular movements and flame cell activity, whereas dead protoscoleces absorbed stain and colored red [Figure 1].\(^2\)

**Toxicity**

**Animals**

Forty-eight male NMRI mice (6–8 weeks old) were purchased from the Animal Breeding Stock Facility of Razi Institute of Iran (Karaj, Iran). Mice were kept in a colony room with a 12:12 h light/dark cycle at 21°C ± 2°C and were handled according to standard protocols for the use of laboratory animals. The experimental procedures performed in the present study were in line with the guidelines of the Kerman University of Medical Science (Kerman, Iran) for the care and use of laboratory animals (Permit no. 92/279).

**Acute toxicity effects**

To determine the acute toxicity, various doses of *C. zeylanicum* essential oil (0.5–4 mL/kg) were intraperitoneally administrated into four groups (six mice in each group). The number of deaths was counted at 48 h after treatment. LD\(_{50}\) values were determined by the Probit test in SPSS software.\(^2\)

**Determination of clinical chemistry and hematological parameters**

Twenty-four mice were randomly divided into four groups with 8 mice per group. The first group (control) was administrated normal saline orally (orogastric gavage), and the second to fourth groups were orally administrated *C. zeylanicum* essential oil at the doses of 0.05, 0.1, and 0.2 ml/kg, respectively, for 14 consecutive days. Following the experimental period, animals were fasted overnight and anesthetized. According to guidelines of the Kerman University of Medical Sciences (Kerman, Iran) for the care and use of laboratory animals, we used ketamine (100 mg/kg) and xylazine (10 mg/kg) combination for anesthesia which in it some alpha-2 adrenoreceptor agonists (i.e., xylazine and medetomidine) do have analgesic properties and other analgesics such as opioids were not used. Sodium pentobarbital (70 mg/kg, i.p.) was used as euthanasia agent and then the abdomen was opened, and blood samples were collected from the heart. In this work due to compliance with all standards of sterilization, we did not use any antibiotics. For hematological studies, total blood was collected into tubes containing ethylenediaminetetraacetic acid anticoagulant, and biochemical parameters, including hemoglobin, hematocrit, white blood cell counts, red blood cell counts, and platelet counts, were measured. To measure clinical chemistry parameters in serum, blood was collected into tubes containing no anticoagulant, allowed to clot, and serum was separated by centrifugation at 2000 g for 20 min. The assays of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatinine (Cr), blood urea nitrogen (BUN), and bilirubin (direct and total) were performed using Roche Diagnostics Kits (Mannheim, Germany).\(^30,31\)

**Statistical analysis**

Obtained results are expressed as the mean ± standard error of mean. Data analysis was carried out using SPSS statistical package version 17.0 (SPSS Inc., Chicago, IL, USA). One-way ANOVA with Tukey's post hoc test was used to assess differences between experimental groups. In addition, *P* < 0.05 was considered statistically significant.\(^32\)

**RESULTS**

**Gas chromatography/mass spectrometry analysis of essential oil**

Yellow-colored essential oil (yield 1.6% v/w) was obtained by

| Peak number | Compound     | RT   | Peak area (%) |
|-------------|--------------|------|---------------|
| 1           | a-pinene     | 4.81 | 1.25          |
| 2           | Cis-ciamene   | 6.73 | 0.9           |
| 3           | Camphene     | 7.54 | 0.62          |
| 4           | Ocimene      | 8.69 | 0.6           |
| 5           | Linalool     | 9.12 | 0.21          |
| 6           | Tricyclene    | 10.26| 0.4           |
| 7           | Cinnamaldehyde| 17.06| 91.8         |
| 8           | p-metricinamate| 21.05| 1.57        |
| 9           | Trans-carophyllene| 22.32| 0.31       |
| 10          | Humulene     | 24.18| 0.12          |
| 11          | Benzenethanamine| 26.47| 0.42         |
| 12          | Trans-cinnamyl acetate| 28.91| 0.61       |
| Total       |              |      | 98.81         |

RT: Retention time

**Table 2:** Scolicidal effects of *Cinnamomum zeylanicum* essential oil against protoscoleces of hydatid cyst at various concentrations following various exposure times

| Concentration (µL/mL) | Exposure time (min) | Mean of mortality rate (%) |
|-----------------------|---------------------|-----------------------------|
| 100                   | 5                   | 100±0.0                     |
| 100                   | 10                  | 100±0.0                     |
| 100                   | 20                  | 100±0.0                     |
| 100                   | 30                  | 100±0.0                     |
| 50                    | 5                   | 100±0.0                     |
| 100                   | 10                  | 100±0.0                     |
| 100                   | 20                  | 100±0.0                     |
| 100                   | 30                  | 100±0.0                     |
| 25                    | 5                   | 46.3±3.15                   |
| 100                   | 10                  | 100±0.0                     |
| 100                   | 20                  | 100±0.0                     |
| 100                   | 30                  | 100±0.0                     |
| 12.5                  | 5                   | 36.6±3.51                   |
| 100                   | 10                  | 74.3±7.0                    |
| 100                   | 20                  | 100±0.0                     |
| 100                   | 30                  | 100±0.0                     |
| 6.25                  | 5                   | 16.6±2.5                    |
| 100                   | 10                  | 43.0±2.5                    |
| 100                   | 20                  | 82.3±2.5                    |
| 100                   | 30                  | 100±2.5                     |
| Normal saline + Tween 20 | 5              | 1.6±1.15                    |
| 100                   | 10                  | 2.6±1.15                    |
| 100                   | 20                  | 3.0±0.5                     |
| 100                   | 30                  | 4.6±1.5                     |
| 20% hypertonic saline | 5                   | 76.3±7.0                    |
| 100                   | 10                  | 100±0.0                     |
| 100                   | 20                  | 100±0.0                     |
| 100                   | 30                  | 100±0.0                     |

Data are expressed as the mean±SD (n=3). SD: Standard deviation
Effect on protoscoleces
Table 2 shows the scolicidal effects of *C. zeylanicum* essential oil with different concentrations following various exposure times. *C. zeylanicum* essential oil with the concentrations of 100 and 50 μL/mL killed 100% of protoscoleces after 5 min of exposure. Likewise, the mean mortality rate of protoscoleces with the concentration of 25 μL/mL was 100% after 10 min of incubation. However, lower concentrations showed moderate protoscolicidal effects so that, at the concentration of 12.5 μL/mL, 36.6, 74.3, 100, and 100% of protoscoleces and, at the concentration of 6.25 μL/mL, 16.6, 43, 82.3, and 100% of protoscoleces were killed after 5, 10, 20, and 30 min of incubation, respectively. However, the mortality rate of protoscoleces in the negative and positive controls was 4.3% after 30 min and 100% after 5 min of exposure, respectively. Therefore, the obtained findings demonstrated that the essential oil of *C. zeylanicum* at all of these concentrations had more significant (*P < 0.05*) scolicidal effects than the control group.

Acute toxicity
Acute toxicity effects of *C. zeylanicum* essential oil on male NMRI mice were determined. The LD$_{50}$ value of intraperitoneal injection of *C. zeylanicum* essential oil was 2.07 mL/kg body weight after 24 h, and the maximum nonfatal dose was 1.52 mL/kg body weight.

Clinical chemistry and hematological parameters
In the present study and according to the results of LD$_{50}$, the doses of 0.05, 0.1, and 0.4 mL/kg of *C. zeylanicum* essential oil were selected. The obtained findings indicated that no death was observed in doses of 0.05, 0.1, and 0.2 mL/kg after 2 weeks. Tables 3 and 4 are shown the results of the clinical chemistry and hematological parameters following oral administration of *C. zeylanicum* essential oil for 2 weeks. There was no significant difference (*P > 0.05*) between oral administrations of *C. zeylanicum* essential oil at the doses 0.05, 0.1, and 0.4 mL/kg and control.

DISCUSSION
Consistent with the World Health Organization, a perfect scolicidal agent for dropping the risk of protoscoleces spillage through hydatid cyst surgery is explained by elevated effectiveness in a shorter time of contact, high influence at lower doses, higher accessibility, constancy in the attendance of cystic fluid, and lower toxicity.[10] Historically, herbs and spices as pure compounds, because of having low toxicity, low cost, high efficacy, and high availability, give boundless opportunities for new drug improvement.[13,31] Therefore, this investigation was designed to analyze the chemical composition of the essential oil obtained from *C. zeylanicum* barks, its scolicidal effects on the hydrated cyst protoscoleces, and its acute toxicity in the mice model.

The obtained findings demonstrated that *C. zeylanicum* at concentrations of 100 and 50 μL/mL completely killed hydatid cyst protoscoleces after 5 min of incubation, whereas lower concentrations of *C. zeylanicum* essential oil motivated deferred protoscolicidal effects. It was also found that the scolicidal activity of *C. zeylanicum* was analogous with the current scolicidal agents such as 20% hypertonic saline, 20% silver nitrate, 0.5%–1% cetrizime, H$_2$O$_2$ 3% (15 min), and 95% ethyl alcohol with potent scolicidal effects between 10 and 20 min.

The common scolicidal agents had serious side effects such as sclerosing cholangitis (biliary tract fibrosis), liver necrosis, and methemoglobinemia.[10,31] For this reason, the present results supported the idea that *C. zeylanicum* could be a natural origin for producing a new protoscolicidal agent which can be applied in CE surgery. Previously, Sharifiar et al.[34] reported the presence of high amounts of tannin and alkaloid as well as lack of sapinons in the phytochemical screening of *C. zeylanicum* barks. Individual activities of these compounds were proven by Cowan.[35] It was found that the main components were cinnamaldehyde (91.8%), α-metoxicinamate (1.57%), and α-pinene (1.25%).

In line with the present results, Saleem et al. reported cinnamaldehyde (49.15%), limonene (15.10%), and α-pinene (1.25%) as the main components of *C. zeylanicum* bark essential oil extracted by hydrodistillation method.[16] In several studies, antimicrobial activities of cinnamaldehyde as the main component of *C. zeylanicum* against some pathogenic strains have been shown.[36] Therefore, phytoconstituents in this plant could be answerable for their scolicidal effects although their accurate manner of action is inadequately understood. However, some researchers have shown its effects on the energy creation of microorganisms.[37] The possible inhibition mechanisms of energy generation are the inhibition of glucose uptake or utilization of glucose and effects on membrane permeability.[37] Moreover, in the case of the antimicrobial mechanism of some monoterpene hydrocarbons such as limonene and α-pine, Sikkema et al. indicated that they were dim into pathogen and hurt cell membrane structures.[38] In addition, other studies demonstrated that the antimicrobial activity of these compounds is related to their capability to affect not only permeability but also other functions of cell wall; these compounds might cross the cell membranes and thus penetrate into the interior of the cell and interact with the critical intracellular sites.[39,40] Previous investigations on laboratory animals have demonstrated that liver and kidney is the main object limb of drug toxicity.[41,42] Injury to the structural integrity of the liver is evaluated by increased serum levels of enzymes such as ALT, AST, ALP, and bilirubin. On the other hand, the Cr blood test and BUN are
used to evaluate kidney function. Acute and subacute toxicity is the first steps of evaluation toxicity effects all drugs. In these methods, tested drugs used in both routes of oral and intraperitoneal administration. In this study, we have used manifold concentrations in comparison with concentrations applied for its protoscolicidal scolicidal effects.

With regard to the toxicity of *C. zeylanicum* essential oil, it was found that the essential oil showed no mortality up to the dose of 2 mL/kg. However, 25% of mortality occurred at the dose of 4 mL/kg. No significant differences (*P > 0.05*) in the clinical chemistry and hematological parameters following oral administrations of *C. zeylanicum* essential oil for 14 days were detected. According to the toxicity classification, *C. zeylanicum* essential oil induced no significant toxicity among male NMRI mice.\(^{[43]}\)

**CONCLUSION**

The present study demonstrated the scolicidal activity of *C. zeylanicum* which could be used as a natural scolicidal agent to reduce the risk of spillage protoscolecies during CE surgery. However, further investigation is required to confirm these findings through studying the essential oil in a clinical setting as a new scolicidal agent.

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**Conflicts of interest**

There are no conflicts of interest.

**REFERENCES**

1. Guidelines for treatment of cystic and alveolar echinococcosis in humans. WHO Informal Working Group on Echinococcosis. Bull World Health Organ 1996;74:231-42.
2. Fashi Harandi M, Budke CM, Rostami S. The monetary burden of cystic echinococcosis in Iran. PLoS Negl Trop Dis 2012;6:e1915.
3. Mahmouvdand H, Mirbadie SR, Sadooghan S, Fashi Harandi M, Jahankish S, Saedi Dezaki E. Chemical composition and scolicidal activity of *Zatania multiflora* Boiss essential oil. J Essent Oil Res 2017;29:42-7.
4. Brasnet E, Kern P, Vutton DA, Writing Panel for the WHO-WGE. Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. Acta Trop 2010;114-1:16.
5. Mahmouvdand H, Kheirandish F, Ghaseemi Kia M, Tavakoli Kareshk A, Yarahmadi M. Chemical composition, protoscolidal effects and acute toxicity of *Platia* atlantica Desf. fruit extract. Nat Prod Res 2015;7:1-4.
6. Armbranzas C, Gutiérrez-Cuadra M, Faríñas MC. Hydatidosis: Epidemiological, clinical, diagnostic and therapeutic aspects. Rev Esp Quimioter 2015;28:116-24.
7. Mahmouvdand H, Tavakoli Oliaei R, Mirbadie SR, Kheirandish F, Tavakoli Kareshk A, Eztapour B, et al. Efficacy and safety of *Bunium persicum* (Boiss) to inactivate protoscolecies during hydatid cyst operations. Surg Infect (Larchmt) 2016;17:713-9.
8. Georgiou GK, Lianos GD, Lazaros A, Harissis A, Manganos A, Donigi G, et al. Surgical management of hydatid liver disease. Int J Surg 2015;20:118-22.
9. Teimoni H, Sabzi F, Hassani V, Nadii S, Mahmouvdand H. Base deficit in immediate postoperative period of open heart surgery and outcome of patients. Acta Med Iran 2007;47:227-32.
10. Rajabi MA. Fatal reactions and methaemoglobinemia after silver nitrate irrigation of hydatid cyst. Surg Pract 2009;13:1-7.
11. Mahmouvdand H, Fashi Harandi M, Shabekia M, Asadi A, Marjani M, Akhlaghi M, et al. Scolicidal effects of biogenic selenium nanoparticles against protoscolecies of hydatid cysts. Int J Nanomedicine 2014;9:1299-403.
12. Tavakoli Kareshk A, Keyhani A, Mahmouvdand H, Tavakoli Oliaei R, Asadi A, Andishmand M, et al. Efficacy of the *Bunium persicum* (Boiss) essential oil against acute toxoplasmosis in mice model. Iran J Parasitol 2015;10:625-31.
13. Cos P, Vlehnk A, Bengehe DV, Maes L, Anti-infective potential of natural products: How to develop a stronger in vitro ‘proof-of-concept’. J Ethnopharmacol 2008;106:290-302.
14. Rezaeeifar M, Befhanezhad M, Moradi M, Mehrabani M, Mahmouvdand H. Antibacterial effects of various extracts of *Amygdalus eburnea* on some most common bacteria in burning. Pharm Lett 2016;8:110-2.
15. Rezaeeifar R, Ayatollahi Moustavi SA, Mehrabani M, Sepahvand A. Evaluation of the antifungal effects of various extracts of *Amygdalus eburnea* on some fungal pathogens. Pharm Chem 2016;8:140-2.
16. Ranasinghe P, Perera S, Gunatilake M, Abeywardene E, Gunapala N, Premakumara S, et al. Effects of *Cinnamomum zeylanicum* (Ceylon cinnamon) on blood glucose and lipids in a diabetic and healthy rat model. Pharmacognosy Res 2012;4:73-9.
17. Mahmouvdand H, Ezzakhal F, Sharififar F, Sharii I, Dezaki ES. Antileishmanial and cytotoxic effects of essential oil and methanolic extract of *Myrtus communis*. J Parasitol Res 2010;5:21-7.
18. Sahoo HB, Sahoo SK, Sarangi SP, Sagar R, Kori ML. Anti-diarrhoeal investigation from aqueous extract of *Cuminum cyminum* Linn. Seed in Albino rats. Pharmacog Res 2014;6:204-9.
19. Saleem M, Bhatti HH, Jilliani MI, Hanif MA. Bioanalytical evaluation of *Cinnamomum zeylanicum* essential oil. Nat Prod Res 2015;21:1-3.
20. Kawatra P, Rajagopal R, Cinnamon: Mystic powers of a minute ingredient. Pharmacognosy Res 2015;7 Suppl 1:S1-6.
21. Ranasinghe P, Pigeas S, Premakumara GA, Galappaththy Z, Constantine GR, Katulanda P. Medicinal properties of ‘true’ cinnamon (*Cinnamomum zeylanicum*): A systematic review. BMC Complement Altern Med 2013;13:275.
22. Rao PV, Gan SH. Cinnamon: A multifaceted medicinal plant. Evid Based Complement Alternat Med 2014;2014:643942.
23. Celiktas O, Kocabas EH, Bedir E, Sukan FV, Oezk T, Basar KH. Antimicrobial activities of methanol extracts and essential oils of *Rosmarinus officinalis*, depending on location and seasonal variations. Food Chem 2007;100:553-9.
24. Saedi Dezaki E, Mahmouvdand H, Sharififar F, Fallahi S, Monzooti L, Eztakhal F. Chemical composition along with anti-leishmanial and cytotoxic activity of *Zatania multilora*. Pharm Biol 2015;8:1-7.
25. Adams RP. Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy. Illinois, USA: Allured Publishing Corporation; 2004.
26. Mahmouvdand H, Dezaki ES, Kheirandish F, Eztapour B, Jahankish S, Harandi MF. Scolidal effects of black cumin seed (*Nigella sativa*) essential oil on hydatid cysts. Korean J Parasitol 2014;52:453-9.
27. Mahmouvdand H, Saedi Dezaki E, Sharififar F, Eztapour B, Jahankish S, Fashi Harandi M. Protoscoedic effect of *Berberis vulgaris* root extract and its main compound, berberine in cystic echinococcosis. Iran J Parasitol 2014;9:503-10.
28. Mahmouvdand H, Asadi A, Harandi MF, Sharififar F, Jahankish S, Dezaki ES. In vitro lethal effects of various extracts of *Nigella sativa* seed on hydatid cyst protoscolecies. Iran J Basic Med Sci 2014;17:1001-6.
29. Hosseinzadeh H, Khoshdel M, Ghorbani M. Antinociceptive, anti-inflammatory effects and acute toxicity of aqueous and ethanolic extracts of *Myrtus communis* L. aerial parts in mice. J Acupunct Meridian Stud 2011;4:242-7.
30. Khoo ZY, Teh CC, Rao NK, Chin JH. Evaluation of the toxic effect of star fruit on serum biochemical parameters in rats. Pharmacogn Mag 2010;6:120-4.
31. Mahmouvdand H, Fallahi S, Mahmouvdand H, Shakhbae M, Harandi MF, Dezaki ES. Efficacy of *Myrtus communis* L. to inactivate the hydatid cyst protoscolecies. J Invest Surg 2015;18:1-7.
32. Jahankish S, Azadpour M, Tavakoli Kareshk A, Keyhani A, Mahmouvdand H. *Zatania multilora* Bios: Lethal effects of methanolic extract against protoscolecies of *Echinococcus granulosus*. J Parasitol Res 2016;8:1289-92.
33. Kheirandish F, Delfan B, Mahmouvdand H, Moradi N, Eztapour B, Ebrahimzedeh F, et al. Antileishmanial, antioxidant, and cytotoxic activities of *Quercus inferotina* Olivier extract. Biomed Pharmacother 2016;82:208-15.
34. Sharififar F, Mosahi MH, Dehghan-Nudehe G, Ameiri A, Alisahi F, Pourevanat A. Bioassay screening of the essential oil and various extracts from 4 spices medicinal plants. Pak J Pharm Sci 2009;22:317-22.
35. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev 1999;12:564-82.
36. Ali SM, Khan AA, Ibrahim M, Musaddiq A, Ahmed KS, Polasa H, et al. Antimicrobial activities of eugenol and cinnamaldehyde against the human gastric pathogen *Helicobacter pylori*. Ann Clin Microbiol Antimicrob 2005;4:20.
37. Gill AG, Hooley RA. Mechanisms of bactericidal action of cinnamaldehyde against *Listeria monocytogenes* and of eugenol against *L. monocytogenes* and *Lactobacillus sakei*. Appl Environ Microbiol 2004;70:5760-5.
38. Sikkema J, de Bont JA, Poolman B. Mechanisms of membrane toxicity of hydrocarbons. Microbiol Rev 1995;59:201-22.

39. Ismail A, Lamia H, Mohsen H, Sarnia G, Bassem J. Chemical composition and antifungal activity of three Anacardiaceae species grown in Tunisia. Sci Int 2013;1:148-64.

40. Cristani M, D’Arrigo M, Mandalani G, Castelli F, Sarpietro MG, Miceli D, et al. Interaction of four monoterpenes contained in essential oils with model membranes: Implications for their antibacterial activity. J Agric Food Chem 2007;55:6300-8.

41. Diskin CJ, Tomasso CL, Alper JC, Glaser ML, Fliegel SE. Long-term selenium exposure. Arch Intern Med 1979;139:824-6.

42. Mahmoudvand H, Kheirandish F, Dezaki ES, Shamsaddini S, Harandi MF. Chemical composition, efficacy and safety of Pistacia vera (var. Fandoghi) to inactivate protoscoleces during hydatid cyst surgery. Biomed Pharmacother 2016;82:393-8.

43. Loomis TA, Hayes AW. Toxicologic testing methods. In: Loomis TA, Hayes AW, editors. Loomis’s Essentials of Toxicology. San Diego, CA: Academic Press, Inc.; 1996. p. 205-48.