Journal of Investigative Surgery

ISSN: 0894-1939 (Print) 1521-0553 (Online) Journal homepage: http://www.tandfonline.com/loi/iivs20

Efficacy of Myrtus communis L. to Inactivate the Hydatid Cyst Protoscoleces

Hossein Mahmoudvand, Shirzad Fallahi, Hormoz Mahmoudvand, Mojtaba Shakibaie, Majid Fasihi Harandi & Ebrahim Saedi Dezaki

To cite this article: Hossein Mahmoudvand, Shirzad Fallahi, Hormoz Mahmoudvand, Mojtaba Shakibaie, Majid Fasihi Harandi & Ebrahim Saedi Dezaki (2015): Efficacy of Myrtus communis L. to Inactivate the Hydatid Cyst Protoscoleces, Journal of Investigative Surgery, DOI: 10.3109/08941939.2015.1088601

To link to this article: http://dx.doi.org/10.3109/08941939.2015.1088601

Published online: 18 Dec 2015.

Journal of Investigative Surgery, Early Online, 1–7, 2015

ISSN: 0894-1939 print / 1521-0553 online
DOI: 10.3109/08941939.2015.1088601

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Efficacy of *Myrtus communis* L. to Inactivate the Hydatid Cyst Protoscoleces

Hossein Mahmoudvand,[1,2] Shirzad Fallahi,[2] Hormoz Mahmoudvand,[3] Mojtaba Shakibaie,[4] Majid Fasihi Harandi,[5] Ebrahim Saedi Dezaki[6]

[1] Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences, Khorramabad, Islamic Republic of Iran, [2] Department of Medical Parasitology and Mycology, Faculty of Medicine, Lorestan University of Medical Sciences, Khorramabad, Islamic Republic of Iran, [3] Department of Surgery, Lorestan University of Medical Sciences, Khorramabad, Islamic Republic of Iran, [4] Pharmaceutics Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Islamic Republic Iran, [5] Research Center for Hydatid Disease in Iran, University of Medical Sciences, Kerman, Islamic Republic of Iran, [6] Herbal and Traditional Medicines Research Center, Kerman University of Medical Sciences, Kerman, Islamic Republic of Iran

ABSTRACT

Purpose: The present study aims to investigate the scolicidal effects of *Myrtus communis* L. essential oil against protoscoleces of hydatid cysts and also its toxicity in mice model. Materials and *Methods*: Protoscoleces were aseptically aspirated from sheep livers having hydatid cysts. Various concentrations of the essential oil (12.5–100 μl/ml) were used for 5–30 min. Viability of protoscoleces was confirmed using eosin exclusion test (0.1% eosin staining). Moreover, 48 male NMRI mice were used to determine the acute and sub-acute toxicity of *M. communis* essential oil. One-way ANOVA with Tukey's post-hoc test was used to assess differences between experimental groups. *Results*: Findings of the present study demonstrated that the *M. communi*s essential oil at the concentration of 100 μl/ml after 5 min of exposure killed 100% protoscoleces. Similarly, the mean mortality rate of protoscoleces after 10 min of exposure to concentration of 50 μl/ml was 100%. However, lower concentrations (12.5 and 25 μl/ml) of *M. communi*s essential oil provoked a delayed protoscolicidal effects. The LD_{50} values of intraperitoneal injection of the *M. communis* essential oil was 2.23 mL/kg body wt. No significant difference ($p > .05$) was observed in the clinical chemistry and hematological parameters following oral administrations of *M. communi*s essential oil at the doses 0.05, 0.1, 0.2, and 0.4 mL/kg for 14 days. *Conclusion*: The results showed potent scolicidal activity of *M. communis* with no significant toxicity, which might be used as a natural scolicidal agent in hydatid cyst surgery.

Keywords: scolicidal effects; myrtle; cystic echinococcosis; hydatid cyst; toxicity

INTRODUCTION

Human cystic echinococcosis (CE, hydatid cyst), caused by the larval stage of the dog tapeworm *Echinococcus granulosus*, remains a major public health problem on several continents and is reemerging in many countries [1, 2]. Until the 1980s, surgery was the only option for treatment of echinococcal cysts. Chemotherapy with benzimidazole compounds (albendazole and mebendazole) and, later, treatment by PAIR (cyst puncture, aspiration, injection of chemicals, and reaspiration) were subsequently introduced [3]. However, these drugs used in treatment of hydatid cysts have demonstrated different adverse effects including hepatotoxicity, severe leucopenia, thrombocytopenia, and alopecia [3, 4]. During surgery to reduce the risk of intraoperative spillage of the cyst contents (protoscoleces) and subsequently recurrence of CE and secondary infection, the use of effective scolicidal agents are obligatory [5, 6]. At present, existing scolicidal agents such as hypertonic saline and silver-nitrate which have been used for inactivation of the cyst contents, are associated with some serious adverse effects [7, 8]. Therefore, enormous efforts have been

Received 18 March 2015; accepted 26 August 2015.

Address correspondence to Dr Ebrahim Saedi Dezaki, Herbal and Traditional Medicines Research Center, Kerman University of Medical Sciences, Kerman, Islamic Republic of Iran. E-mail: saedi1358@gmail.com

Color versions of one or more figures in the article can be found online at www.tandfonline.com/iivs

made to reach new scolicidal agents especially from natural resources with low side effects and more efficacies for hydatid cyst surgery.

Myrtus communis L. (Myrtaceae) with the common name "Myrtle" is native to Southern Europe, North Africa and Asia. Different parts of this plant have been used extensively as a folk medicine for the treatment of various diseases [9]. Moreover, recent reviews have reported anti-inflammatory, antinociceptive, antioxidant, antihepatic ischemia, neuroprotective, and antimicrobial effects of *M. communi*s [10–13]. The present study was aimed to evaluate the scolicidal effects of *M. communi*s essential oil against protoscoleces *of* hydatid cysts on *in vitro* model and its toxicity in NMRI mice.

MATERIALS AND METHODS

Collection of Plant Materials

The leaves of *M. communis* were collected from rural regions of Baft district of Kerman Province, southeastern Iran, in September 2013. The identity was confirmed by a botanist at the Botany Department of Shahid Bahonar University, Kerman, Iran. A voucher specimen of the plant materials was deposited at the Herbarium of Department of Pharmacognosy, School of Pharmacy, Kerman University of Medical Science, Kerman, Iran (KF1356).

Isolation of the Essential Oil

Air-dried plant materials (200 g) were subjected to hydro-distillation for 3 hr using an all-glass Clevenger-type apparatus. The obtained essential oil was dried over anhydrous sodium sulfate and stored in darkness at 4°C in airtight glass until testing [14].

Gas Chromatography/Mass Spectrometry (GC/MS) Analysis of Essential Oil

GC Analysis

In this study, GC analysis was carried out by a Hewlett-Packard 6890 with a HP-5MS column (30 m × 0.25 mm, film thickness 0.25 mm). The column temperature was maintained at 50°C for three min and programmed to 290°C at a rate of 15°C per min, and kept constant at 290°C for five min. Injector and interface temperatures were 250 and 280°C, respectively. The flow rate of Helium as carrier gas was (1 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–C24 *n*-alkanes.

GC/MS Analysis

GC/MS analysis was performed using 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 250 and 280°C, respectively. Mass range was from 40 to 400 u. Oven temperature program was the same given above for the GC.

Identification of the essential oil components

The constituents of the essential oil 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 [15].

Drug Dilution

Dilutions of the essential oil of *M. communi*s were prepared as follow; 0.1 ml of the essential oil was dissolved in 0.87 ml of normal saline and in order to enhance the dispersal of the essential oil in normal saline, 0.03 ml of Tween 20 (Sigma-Aldrich, St Louis, MO, USA) was added to the test tube. The resulting solution was mixed adequately by a magnetic stirrer. Serial dilution was then made to obtain the essential oil at 12.5, 25, 50, and 100 μl/ml. The selection of *M. communi*s dilutions was based on the initial experiments, which also demonstrated that normal saline plus Tween 20 had no effect on the viability of protoscoleces.

Collection of Protoscoleces

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 hydatid fluid aspirated by a 50 ml syringe and aseptically transferred into a flask was left to set for 30 min for protoscoleces to settle down. The supernatant was discarded and the protoscoleces were washed two times with PBS (pH 7.2) solution. The number of protoscoleces per mL was adjusted as 2×10^3 protoscoleces in 0.9% NaCl solution with at least 90% viability rate. The viability of the protoscoleces was confirmed by their flame cell motility and impermeability to 0.1% eosin solution under a light microscope.

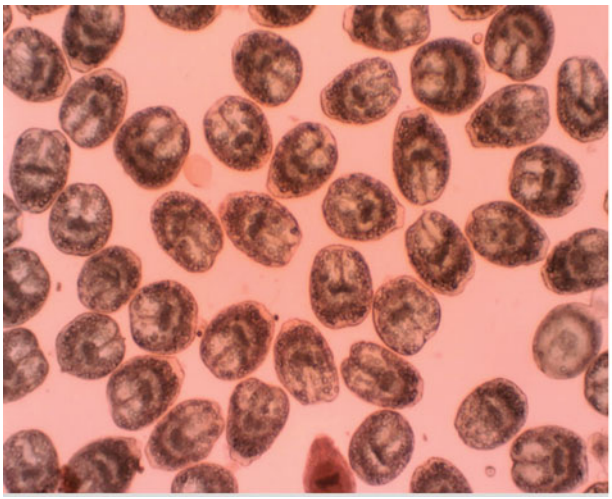

FIGURE 1 Live (colorless) protoscoleces of hydatid cysts after exposure to various concentrations of *M. communis* essential oil following various exposure times (5–30 min) with 0.1% eosin.

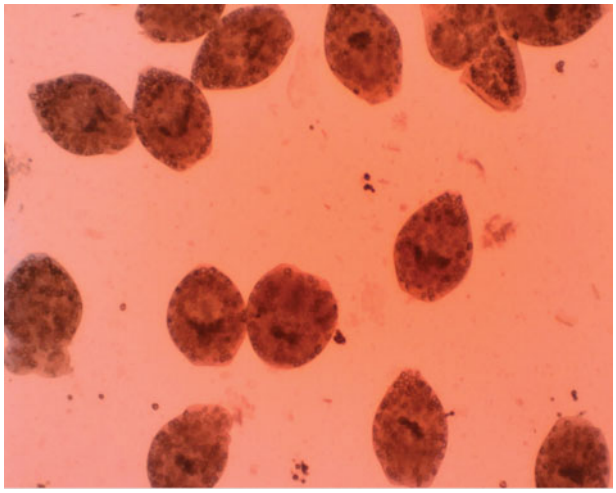

FIGURE 2 Dead (red) protoscoleces of hydatid cysts after exposure to various concentrations of *M. communis* essential oil following various exposure times (5–30 min) with 0.1% eosin.

Scolicidal Effect on Protoscoleces

To determine the scolicidal activity of essential oil of *M. communis* against protoscoleces of hydatid cysts, various concentrations of the essential oil were used for 5, 10, 20, and 30 min. Initially, 0.5 ml of the protoscoleces (2×10^3/ml) solution was placed in test tubes. Then 0.5 ml of various concentrations of the essential oil was added to each test tube. Contents of the tubes were then gently mixed and incubated at 37°C for 5, 10, 20, and 30 min. At the end of each incubation time, the upper phase was carefully removed so as not to interrupt the protoscoleces. Fifty microliters of 0.1% eosin stain (Sigma-Aldrich, St Louis, MO, USA) was then added to the remaining settled protoscoleces and mixed gently. The upper portion of the solution was discarded after 10 min of incubation. The remaining pellet of protoscoleces was then smeared on a glass slide, covered with a cover glass and examined under a light microscope. The percentage of dead protoscoleces was determined by counting 300 protoscoleces. Normal saline plus Tween 20 and hypertonic saline 20% were used as negative and positive control, respectively [16].

Viability Test

In this study, eosin exclusion test was used to determine the viability of protoscoleces of hydatid cysts [17]. 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 (Figure 1), while dead protoscoleces absorbed eosin and colored red (Figure 2).

Toxicity Test

Ethical Statement

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Kerman University of Medical Science (Permit Number: 91/27, 2013). Moreover, all efforts were made to minimize suffering.

Animals

Forty-eight male NMRI mice (three to four months old, 30–35 g) were obtained from the Animal Breeding Stock Facility of Razi Institute of Iran (Karaj, Iran). Animals were housed in a colony room with a 12:12 hr light/dark cycle at 21 ± 2°C and were handled according to standard protocols for the use of laboratory animals.

Acute Toxicity

To determine the acute toxicity, various doses of *M. communis* essential oil (0.5–4 ml/kg) were injected as intraperitoneally into four groups of six mice. The number of deaths was counted at 24 h after treatment. LD_{50} values were determined by the Probit test in SPSS software [18].

Determination of Clinical Chemistry and Hematological Parameters

Twenty-four mice were randomly divided into four groups with eight mice per group. The first group (control) was administrated normal saline orally (orogastric gavage), and the second to fourth groups were orally administrated *M. communis* essential oil at the doses of

0.05, 0.1, 0.2, and 0.4 ml/kg, respectively, for 14 consecutive days.

Following the experimental period, animals were fasted overnight and anaesthetized. According to guidelines of the Kerman University of Medical Science (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, Medetomidine) do have analgesic properties and other analgesics like opioids were not used. Moreover, due to the no signs of pain were observed in the process of the study with the anesthetic treatment utilized no specific separate analgesics were 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, surgeries were performed using instruments sterilized by autoclave and under laminar flow hood; therefore, we did not use any antibiotics. For hematological studies, total blood was collected into tubes containing ethylenediamine tetraacetic 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 amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), creatinine (Cr), blood urea nitrogen (BUN), and bilirubin (direct and total), were performed using Roche diagnostics kits (Mannheim, Germany) [19].

Statistical Analysis

Obtained results are expressed as the mean ± SEM. Data analysis was carried out by 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, $p < .05$ was considered statistically significant .

RESULTS

GC/MS Analysis of Essential Oil

Table 1 shows the identified compounds and percentage obtained by GC/MS. Twenty-five compounds were identified, representing 93.01% of the total extract. The main components were α-pinene (24.7%), 1,8-cineole (19.6%), and linalool (12.6%), respectively.

TABLE 1 Essential oil composition of *M. communi*s identified by GC/MS

No.	Compound	Percentage
1.	α-Thujene	0.88
2.	Camphene	0.58
3.	δ-3-Carene	0.73
4.	α-Pinene	24.7
5.	β –Pinene	1.28
6.	β –Myrcene	0.61
7.	α –Terpinene	0.23
8.	1,8- Cineole	19.6
9.	Methyl eugenol	1.3
10.	Linalool	12.6
11.	α-Terpinyl acetate	3.8
12.	Myrtenyl acetate	8.3
13.	α-Phellandrene	0.1
14.	β-Ocimene	0.11
15.	2,6-Octadien	0.41
16.	α-Phellandrene	0.1
17.	γ-Terpinene	0.5
18.	α-Terpinolene	0.51
19.	4-Terpineol	0.6
20.	α-Terpineol	6.1
21.	Linalyl Acetate	5.9
22.	Caryophyllene oxide	1.4
23.	α-Humulene	1.2
24.	Neryl acetate	0.14
25.	trans-Caryophyllene	1.33
	Total	93.01

TABLE 2 Scolicidal effects of *M. communis* essential oil against protoscoleces of hydatid cyst at the various concentrations following various exposure times

Concentration (μl/ml)	Exposure time (min)	Mean of mortality rate (%)
12.5	5	6.3 ± 0.3
	10	12.3 ± 1.3
	20	61.0 ± 5.3
	30	100 ± 0.0
25	5	51.6 ± 3.3
	10	83.0 ± 4.6
	20	100 ± 0.0
	30	100 ± 0.0
50	5	72.3 ± 6.0
	10	100 ± 0.0
	20	100 ± 0.0
	30	100 ± 0.0
100	5	100 ± 0.0
	10	100 ± 0.0
	20	100 ± 0.0
	30	100 ± 0.0
Normal saline + Tween 20	5	0.0 ± 0.0
	10	1.3 ± 0.1
	20	2.6 ± 1.15
	30	4.3 ± 1.15
20% Hypertonic saline	5	81.3 ± 7.6
	10	100 ± 0.0
	20	100 ± 0.0
	30	100 ± 0.0

TABLE 3 Clinical chemistry parameters in mice sera

Parameters	*M. communis* essential (ml/kg)				
	0.05	0.1	0.2	0.4	Control
AST (U/l)	127.2 ± 16.5	132 ± 14.3	139 ± 15.5	151 ± 16.3	124 ± 19.5
ALT (U/l)	81 ± 5.3	77 ± 6.5	89 ± 7.6	102 ± 11.6	80 ± 5.3
ALP (U/l)	259 ± 23.2	273 ± 19.5	269 ± 23.4	301 ± 23.5	280 ± 16.5
Cr (mg/dl)	0.35 ± 0.05	0.46 ± 0.1	0.51 ± 0.1	0.59 ± 0.1	0.4 ± 0.05
BUN (mg/dl)	34.3 ± 5.2	39.6 ± 4.3	42.1 ± 6.1	53.45 ± 4.6	40 ± 2.4
TB (mg/dl)	0.7 ± 0.11	0.88 ± 0.15	0.84 ± 0.2	0.65 ± 0.15	0.8 ± 0.1
DB (mg/dl)	0.23 ± 0.07	0.26 ± 0.05	0.31 ± 0.01	0.18 ± 0.02	0.2 ± 0.015

AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; Cr, creatinine; BUN, Blood urea nitrogen; TB, Total bilirubin; DB, Direct bilirubin.

Effect on Protoscoleces

Scolicidal effects of essential oil of *M. communis* at various concentrations following different exposure times are shown in Table 2. The obtained results indicated that essential oil of *M. communis* at the concentration of 100 μl/ml after 5 min of exposure killed 100% protoscoleces. Likewise, the mean of mortality rate of protoscoleces after 10 min of exposure to the concentration of 50 μl/ml was 100%. However, lower concentrations of *M. communis* essential oil provoked a delayed protoscolicidal effects; so that, at the concentration of 25 μl/ml killed 51.6, 83, 100 and 100% of the protoscoleces and at the concentration of 12.5 μl/ml killed 6.3%, 12.3%, 61%, and 100% of the protoscoleces after 5, 10, 20, and 30 min of incubation, respectively. In contrast, the mortality rate of protoscolices in the negative and positive controls was 4.3% after 30 min and 100% after 5 min of exposure, respectively. These findings also revealed that the essential oil of *M. communis* at all of concentrations had significant ($p < .05$) scolicidal effects compared with the control group.

Acute Toxicity

Acute toxicity effects of *M. communis* essential oil were evaluated on male NMRI mice. The LD_{50} value of intraperitoneal injection of the *M. communis* essential oil was 2.23 ml/kg body wt. and the maximum nonfatal doses were 1.84 ml/kg body wt.

Clinical Chemistry and Hematological Parameters

According to the results of LD_{50}, the doses of 0.05, 0.1, 0.2, and 0.4 ml/kg of *M. communis* essential oil were chosen. No death was observed in doses of 0.05, 0.1, and 0.2 ml/kg, while 16.6% death was observed in the group receiving 0.4 ml/kg of *M. communis* essential oil. Tables 3 and 4 showed the findings of the clinical chemistry and hematological parameters following oral administration of *M. communis* essential oil for two weeks. There was no significant difference ($p > .05$) between oral administrations of *M. communis* essential oil at the employed doses 0.05, 0.1, 0.2, and 0.4 ml/kg and control.

DISCUSSION

Natural products, such as plants extract, either as pure compounds or as standardized extracts, due to having low toxicity, low cost, high efficacy, and high availability provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity [20]. According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their pri-

TABLE 4 Hematology parameters in whole blood

Parameters	*M. communis* essential (ml/kg)				
	0.05	0.1	0.2	0.4	Control
RBC ($\times 10^6/\mu$l)	5.71 ± 0.23	6.1 ± 0.25	5.82 ± 0.24	6.3 ± 0.29	5.4 ± 0.23
HGB (g/dl)	11.4 ± 0.75	11.1 ± 0.71	12.2 ± 0.81	11.7 ± 0.77	10.9 ± 0.64
Hct (%)	31.5 ± 1.1	30.5 ± 0.95	35.3 ± 1.3	33.2 ± 1.25	34.6 ± 1.38
WBC ($\times 10^3/\mu$l)	3.0 ± 0.25	2.9 ± 0.18	3.1 ± 0.27	3.7 ± 0.35	2.6 ± 0.2
PLT ($\times 10^3/\mu$l)	369 ± 35	389 ± 41	373 ± 37	528 ± 0.56	411 ± 47

RBC, red blood cell; HGB, hemoglobin; Hct, hematocrit; WBC, white blood cell; PLT, platelet.

mary health care needs. *M. communi*s broadly grows in the central and southern parts of Iran. Different parts of this plant are used in traditional medicine to treat a wide range of diseases, such as infectious ones[9]. This work was designed to evaluate the scolicidal effects of *M. communi*s essential oil on an *in vitro* model. At present, an ideal scolicidal agent to reduce the risk of protoscoleces spillage during hydatid cyst surgery is described by its potency at lower concentrations, high efficacy in a shorter time of exposure, stability in the presence of cystic fluid, scolicidal ability inside a cyst, lower toxicity, higher availability, and ability for rapid preparation [1]. So far, the scolicidal effects of various chemical and natural products have been proven [21–30]. However, as previously mentioned existing scolicidal agents are associated with adverse effects and controversial efficacy. Our results revealed that *M. communi*s essential oil at the concentrations of 100 and 50 μl/ml killed 100% protoscoleces after 5 and 10 min of incubation, respectively. These results indicated that scolicidal activity of this plant is comparable with the existing scolicidal agents such as 20% hypertonic saline (15 min), 20% silver nitrate (20 min), 0.5–1% cetrimide (10 min), H_2O_2 3% (15 min), and 95% ethyl alcohol (15 min). Thus, findings of the present investigation supported the idea that *M. communi*s might be a natural source for producing of a new scolicidal agent for use in hydatid cyst surgery. The phytochemical screening of *M. communi*s leaves exhibited the presence of terpenoids, flavonoids, and tannins in this plant [9]. Individual activities of these compounds have previously been shown [31]. Additionally, several investigations have reported the potent antimicrobial activities of these compounds and their derivatives such as α-pinene, limonen, terpinene, thymol, and carvacrole [32–35]. In this study, we found that the main components of *M. communi*s essential oil are the monoterpene hydrocarbons such as α-pinene (24.7%), 1,8-cineole (19.6%), and linalool (12.6%). Therefore, phytoconstituents in this plant could be responsible for their scolicidal effects though their exact mode of action is poorly understood. However, in the case of antimicrobial mechanism of some terpenoids compounds such as monoterpens, as the main components of *M. communi*s, Sikkema et al. (1995) revealed that they diffuse into pathogen and damage cell membrane structures [36]. On the other hand, other reports suggested that the antimicrobial activity is related to ability of terpenes to affect not only the permeability but also other functions of the cell membranes, these compounds might cross the cell membranes, thus penetrating into the interior of the cell and interacting with critical intracellular sites [37, 38].

Now, current scolicidal agents such as hypertonic saline and silver-nitrate are associated with some adverse effects including sclerosing cholangitis (biliary tract fibrosis), liver necrosis, and methemoglobinemia [7, 8]. In regard to the toxicity effects of *M. communi*s essential oil, we found that the LD_{50} values of intraperitoneal injection of the *M. communi*s essential oil was 2.23 ml/kg body wt. Liver and renal enzyme activities such as ALT, AST, ALP, Bilirubin (total, direct), Cr, and BUN are the major characteristics of liver and renal function. In the present study, no significant difference ($p > .05$) was observed in the clinical chemistry and hematological parameters following oral administrations of *M. communi*s essential oil for 14 days. However, in rare cases, systemic administration of myrtle oil as a drug may lead to nausea, vomiting and diarrhea [9]. Uehleke and Brinkschulte also demonstrated that (1979) in people receiving therapeutic doses of this essential oil (1–2 ml/day), the appearance of adverse effects on liver is unlikely to happen [39]. In addition, Habibzadeh Khameneh et al (2014) reported that *M. communi*s at low concentrations (<500 μg/ml) had no significant cytotoxic effects; while at higher concentrations (>625 μg/ml) represented significant cytotoxic effects against Hela and MCF7 cell lines [40]. Thus, these findings suggest that *M. communi*s essential oil at the concentrations used in the present study had no significant toxicity and could be consider safe for host. However, more experiments such as histopathological tests are needed for confirmation of that essential oil is unlikely to induce some serious side effects such as sclerosing cholangitis.

In conclusion, the results showed promising scolicidal activity of *M. communis* which might be used as a natural scolicidal agent to reduce the risk of protoscoleces spillage during hydatid cyst surgery. However, further studies will be needed to confirm these results by checking the essential oil in a clinical setting as a new scolicidal agent.

Declaration of Interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

ACKNOWLEDGMENTS

The authors would like to thank Mr. Mirbadie and Mr. Sadooghian for preparation of *M. communi*s essential oil and Dr. Ghasemi Kia for collection hydatid cysts.

REFERENCES

[1] World Health Organization (WHO) informal working group on echinococcosis. *Bull WHO* 1996;74:231–242.

[2] Eckert J, Deplazes P. Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. *Clin Microbiol Rev* 2004;17:107–135.

[3] Brunetti E, Kern P, Vuitton DA. Writing Panel for the WHO-IWGE Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. *Acta Trop* 2010;114(1):1–16.

[4] Junghanss T, da Silva AM, Horton J, et al. Clinical management of cystic echinococcosis: state of the art, problems, and perspectives. *Am J Trop Med Hyg* 2008;79(3):301–311.
[5] McManus DP, Zhang W, Li J. Echinococcosis. *Lancet* 2003;362:1295–1304.
[6] Besim H, Karayalcin K, Hamamci O. Scolicidal agents in hydatid cyst surgery. *HPB Surg* 1998;10:347–351.
[7] Sahin M, Eryilmaz R, Bulbuloglu E. The effect of scolicidal agents on liver and biliary tree (experimental study). *J Invest Surg* 2004;17(6):323–6.
[8] Rajabi MA. Fatal reactions and methaemoglobinaemia after silver nitrate irrigation of hydatid cyst. *Surg Pract* 2009;13:2–7.
[9] Alipour G, Dashti S, Hosseinzadeh H. Review of pharmacological effects of *Myrtus communis L.* and its active constituents. *Phytother Res* 2014;28:1125–1136.
[10] 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;4:242–247.
[11] Mahmoudvand H, Ezzatkhah F, Sharififar F, et al. Antileishmanial and cytotoxic effects of essential oil and methanolic extract of *Myrtus communis* L. *Korean J Parasitol* 2015;53(1):21–27.
[12] Ferchichi H, Salouage I, Bacha S, et al. Effect of *Myrtus Communis* L. extracts on attenuation of liver normothermic ischemia-reperfusion injury. *J Transplant Technol Res S* 2011;3:2161–2191.
[13] Tumen I, Senol FS, Orhan IE. Inhibitory potential of the leaves and berries of *Myrtus communis* L. (myrtle) against enzymes linked to neurodegenerative diseases and their antioxidant actions. *Int J Food Sci Nutr* 2012;63:387–392.
[14] Mahmoudvand H, Sepahvand A, Jahanbakhsh S, et al. Evaluation of antifungal activities of the essential oil and various extracts of *Nigella sativa* and its main component, thymoquinone against pathogenic dermatophyte strains. *J Mycol Med* 2014;24(4):155–161.
[15] Adams RP. *Identification of essential oil components by gas chromatography/mass spectroscopy*; 2004 Illinois, USA: Carol Stream, IL: Allured Publishing Corporation.
[16] Moazeni M, Saharkhiz MJ, Hosseini AA. *In vitro* lethal effect of ajowan (*Trachyspermum ammi* L.) essential oil on hydatid cyst protoscoleces. *Vet Parasitol* 2012;187:203–208.
[17] Smyth JD, Barrett NJ. Procedures for testing the viability of human hydatid cysts following surgical removal, especially after chemotherapy. *Trans R Soc Trop Med Hyg* 1980;74:649–652.
[18] Hosseinzadeh H, Sadeghi Shakib S, Khadem Sameni A, et al. Acute and subacute toxicity of safranal, a constituent of saffron, in mice and rats. *Iran J Pharm Res* 2013;12(1):93–99.
[19] Shakibaie M, Shahverdi AR, Faramarzi MA, et al. Acute and subacute toxicity of novel biogenic selenium nanoparticles in mice. *Pharm Biol* 2013;51(1):58–63.
[20] Cosa P, Vlietinck AJ, Berghe DV, et al. Anti-infective potential of natural products: how to develop a stronger *in vitro* 'proof-of-concept'. *J Ethnopharmacol* 2003;106:290–302.
[21] Kayaalp C, Balkan M, Aydin C, et al. Hypertonic saline in hydatid disease. *World J Surg* 2001;25:975–979.
[22] Caglar R, Yuzbasioglu MF, Bulbuloglu E, et al. *In vitro* effectiveness of different chemical agents on scolices of hydatid cyst. *J Invest Surg* 2001;21:71–75.
[23] Erzurumlu K, Hokelek M, Baris S, et al. Effect of albendazolesulfoxide solution on the scolices and the hepatobiliary system. *Eur Surg Res* 1998;30:433–438.
[24] Landa Garcıáa JI, Alonso E, Gonzalez-Uriarte J. Evaluation of scolicidal agents in an experimental hydatid disease model. *Eur Surg Res* 1997;29:202–208.
[25] Mahmoudvand H, Fasihi Harandi M, Shakibaie M, et al. Scolicidal effects of biogenic selenium nanoparticles against protoscolices of hydatid cysts. *Int J Surg* 2014;12(5):399–403.
[26] Puryan K, Karadayi K, Topcu O. Chlorhexidine gluconate: an ideal scolicidal agent in the treatment of intraperitoneal hydatidosis. *World J Surg* 2005;29:227–230.
[27] Mahmoudvand H, Kheirandish F, Ghasemi Kia M, et al. Chemical composition, protoscolicidal effects and acute toxicity of *Pistacia atlantica* Desf. fruit extract. *Nat Prod Res* 2015;7:1–4.
[28] Mahmoudvand H, Dezaki ES, Kheirandish F, et al. Scolicidal effects of black cumin seed (*Nigella sativa*) essential oil on hydatid cysts. *Korean J Parasitol* 2014;52(6): 653–659.
[29] Mahmoudvand H, Sharififar F, Saedi Dezaki E, et al. Protoscolecidal effect of *Berberis vulgaris* root extract and its main compound, berberine in cystic echinococcosis. *Iranian J Parasitol* 2014;9(4):26–34.
[30] Mahmoudvand H, Asadi A, Harandi MF, et al. In vitro lethal effects of various extracts of *Nigella sativa* seed on hydatid cyst protoscoleces. *Iran J Basic Med Sci* 2014;7(12):1001–1006.
[31] Cowan MM. Plant products as antimicrobial agents. *Clin Microbi Rev* 1992;12:564–582.
[32] Sokovic M, van Griensven LG. Antimicrobial activity of essential oils and their components against the three major pathogens of the cultivated button mushroom, Agaricus bisorus. *Eur J Plant Pathol* 2006;116:211–224.
[33] Abbaszadeh S, Sharifzadeh A, Shokri H, et al. Antifungal efficacy of thymol, carvacrol, eugenol and menthol as alternative agents to control the growth of food-relevant fungi. *J Myco Med* 2014;24 (2):51–56.
[34] de Melo JO, Bitencourt TA, Fachin AL, et al. Antidermatophytic and antileishmanial activities of essential oils from *Lippia gracilis* Schauer genotypes. *Acta Trop* 2013;128(1):110–115.
[35] Vardar-Unlu G, Yağmuroğlu A, Unlu M. Evaluation of *in vitro* activity of carvacrol against *Candida albicans* strains. *Nat Pro Res* 2013;24(12):1189–93.
[36] Sikkema J, De Bont DA, Poolman B. Mechanisms of membrane toxicity of hydrocarbons. *Microbiol Mol Biol Rev* 1995;59:201–222.
[37] Cristani M, D'Arrigo M, Mandalari G, et al. Interaction of four monoterpenes contained in essential oils with model membranes: implications for their antibacterial activity. *J Agric Food Chem* 2007;55(15):6300–6308.
[38] Ezatpour B, Saedi Dezaki E, Mahmoudvand H, et al. In vitro and in vivo antileishmanial effects of *Pistacia khinjuk* against *Leishmania tropica* and *Leishmania major*. *Evid Based Complement Alternat Med* 2015; 2015:149707.
[39] Uehleke H, Brinkschulte-Freitas M. Oral toxicity of an essential oil from myrtle and adaptive liver stimulation. *Toxicology* 1979;12(3):335–342.
[40] Habibzadeh Khameneh S, Nemati F, Shiravi A. Cytotoxic effects of *Mytrtus communis* extract on Hela and MCF7 cancer cells. *J Animal Biol* 2014;6(2):21–28.