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ORIGINAL ARTICLE

Main compounds and in vitro effectiveness of *Syzygium aromaticum* essential oil on protoscoleces of hydatid cyst

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Abstract

Surgery remains the preferred treatment for cystic echinococcosis. On the other hand, one of the side effects of hydatid cyst surgery is recurrence. Many scolicidal agents have been used for inactivation of the hydatid cyst content. However, many of these scolicidal agents may cause undesirable complications that limit their usage. The aims of this study are to investigate the chemical composition and in vitro scolicidal effect of *S. aromaticum* essential oil against protoscoleces from hydatid cysts of *Echinococcus granulosus*. The essential oil was obtained by hydrodistillation. Gas chromatography with flame ionization detection (GC-FID) and gas chromatography–mass spectrometry (GC/MS) was employed to determine the chemical composition of the essential oil. Protoscoleces were aseptically collected from infected organs (liver and lung) of animals slaughtered at the Tiaret slaughterhouse containing hydatid cysts. Protoscoleces were exposed to two concentrations of essential oil (10 and 15 μl/mL) for 5, 10, 15, and 20 min. Viability of protoscoleces was confirmed by 0.1% eosin staining. Eugenol was the major compound of the studied essential oil (78.72%) followed by β-caryophyllene (8.82%) and eugenyl acetate (8.74%). The scolicidal activity of *S. aromaticum* essential oil at the concentration of 10 μl/ml was 55.99%, 60.05%, 80.37%, and 99.76% after 5, 10, 15, and 20 min of exposure, respectively. *S. aromaticum* essential oil at the concentrations of 15 μl/ml killed 100% protoscoleces after 5 min of exposure. The present study confirmed that *S. aromaticum* essential oil has high scolicidal power in vitro. However, further studies (ex vivo and in vivo) will be needed to confirm these results.

Keywords Hydatid cyst · Scolicidal · Essential oil · *Syzygium aromaticum* · Eugenol

Introduction

Cystic echinococcosis (CE) is a chronic infection with medical and veterinary importance, which is caused by the larval stage of a cosmopolitan parasitic cestode *Echinococcus granulosus* (Eckert and Deplazes 2004; Mahmoudvand et al. 2017). CE has a worldwide distribution and is highly endemic in all North African countries (Maghreb) including Algeria, Libya, Morocco, and Tunisia (Dakkak 2010). Nevertheless, Zait et al. (2016) report that CE is an important public health problem in Algeria. This disease has been identified as a major public health and economic problem in developing countries (Mahmoudvand et al. 2014a).

Currently, there are three treatment choices for hydatidosis: surgery, PAIR (puncture, aspiration, injection, and respiration), and medicinal therapy (Larki et al. 2017; Abdel-Baki et al. 2016; Moazeni and Larki 2010). It has been suggested that surgery is still the most important treatment method with chemotherapy as the co-adjuvant treatment (Barzin et al. 2019). However, dissemination of protoscolex-rich fluid during surgery is a major cause of recurrence (Kilicoglu et al. 2008; Moazeni and Larki 2010).

Although, the preoperative destruction of the contents of the cyst and the prevention of infection of the surrounding area by the use of several chemical scolicidal agents play an important role in the success of the operation. In addition, this procedure helps prevent the return of the disease (Khuroo et al. 1993; Moazeni and Larki 2010; Kavoosi and Purfard

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2013; Lashkarizadeh et al. 2015; Mahmoudvand et al. 2017). But, most of these chemical scolicidal agents have demonstrated different side effects such as liver necrosis, sclerosing cholangitis (biliary tract fibrosis), and methemoglobinemia (Lashkarizadeh et al. 2015; Mahmoudvand et al. 2017).

The development of safe and effective new scolicidal agents is therefore of great interest (Moazeni et al. 2012a). For this, natural products and their compounds constitue the most productive source for new drug development (Rocha et al. 2005).

Syzygium aromaticum, commonly called clove, belongs to the family *Myrtaceae* (Bhuiyan et al. 2010). Clove is traditionally used as antipyretic, antiemetic, aphrodisiac, analgesic, appetizer, and disinfectant (Mahboubi and Mahboubi 2015). Likewise, it is used a remedy for asthma, disorder of digestive system, dental disorders, respiratory disorders, headaches, and sore throat (Lee et al. 2009).

Some pharmacological effects of clove oil such as anticarcinogenic, anti-asthma, anti-allergic, anti-inflammatory, acaricidal, insecticidal, anti-mutagenic, cytotoxic, anesthetic, antibacterial, antifungal, antiviral, and antioxidant properties (Mahboubi and Mahboubi 2015; Gaylor et al. 2014; Lee et al. 2009; Guan et al. 2007) were demonstrated.

The present study was undertaken to evaluate chemical composition and in vitro scolicidal activity of *Syzygium aromaticum* essential oil.

Materials and methods

Extraction of essential oil

Clove flower buds (*S. aromaticum*) were purchased from a local market in Tiaret (Algeria). Clove flower buds was crushed using a mortar and pestle. The essential oil of clove flower buds was extracted by hydrodistillation. The obtained oil was collected and dried over anhydrous sodium sulfate and stored in amber vials with screw cap in a refrigerator at 4 °C prior to analysis.

Analysis of essential oil

The chemical composition of the essential oil was analyzed using gas chromatography with flame ionization detection (GC-FID) and gas chromatography–mass spectrometry (GC/MS) at the Sarl Pyrenessences Analyses (France) according to the method previously described by Selles et al. (2018).

Collection of protoscoleces

Protoscoleces of *E. granulosus* were obtained from the infected organs (liver and lung) of sheep slaughtered at Tiaret slaughterhouse (western Algeria) and carried to the Parasitology Laboratory at the Veterinary Sciences Institute, Ibn Khaldoun University, Tiaret, Algeria.

The hydatid fluid of cysts was aseptically transferred into the glass cylinders and left to set for 30 min (Moazeni and Larki 2010; Moazeni et al. 2012b; Kavoosi and Purfard 2013). The protoscoleces were settled down at the bottom of cylinders. The supernatant was then removed, and the yielded protoscoleces were washed three times using normal saline (Moazeni et al. 2012b; Mahmoudvand et al. 2014b). A fertility test and viability was assessed by muscular movements and 0.1% eosin staining test (Daryani et al. 2009; Moazeni et al. 2012b; Mahmoudvand et al. 2014b). The live protoscoleces were finally transferred into a dark container containing normal saline and stored at 4 °C for further use.

Scolicidal assay

In this study, two concentrations of *Syzygium aromaticum* essential oil (15 μl/ml and 10 μl/ml) were used for 5, 10, 15, and 20 min. To enhance the dispersion of the essential oil in normal saline, it was dissolved in physiological saline solution 0.9% (*w*/*v*) supplemented with Tween 20 (Sigma) at a final concentration of 10% (*v*/v). The resulting solution was mixed properly using a magnetic stirrer. Half a milliliter of each concentration was placed in a test tube; 0.5 ml of protoscoleces-rich sediment was added to the tube and mixed gently. The tube was then incubated at 37 °C for 5, 10, 15, and 20 min. At the end of each incubation times, the upper phase was carefully removed so as not to interrupt the protoscoleces. Half a milliliter of 0.1% eosin stain was then added 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 glass slide, covered with a cover glass, and examined under a light microscope. The percentages of dead protoscoleces were determined by counting an average of 1100 protoscoleces. In the controls, protoscoleces were treated only with normal saline and protoscoleces were treated with a mixture of normal saline and Tween 20 diluted were used. All tests were carried out in triplicate.

Viability test

In order to evaluate the viability of protoscoleces, eosin solution with a concentration of 0.1% was used. After exposure to the stain, a live protoscoleces remained colorless and showed characteristic muscular mouvements and flame cell activity (Fig. 2), but dead protoscoleces absorbed eosin and colored red (Fig. 2). The mortality rate of protoscoleces was

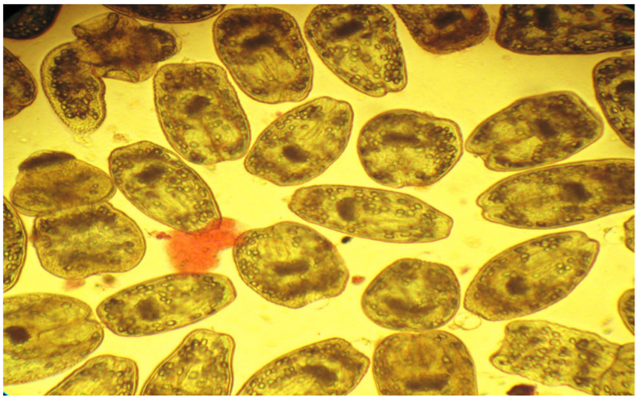

Fig 1 Live protoscoleces of hydatid cysts after exposure to 0.1% eosin GX10

determined, as the percent of dead protoscoleces to the total protoscoleces.

Statistical analysis

In the present study, all the tests were performed in triplicate. Statistical analysis was performed by R software (version 3.3.0/2016-05-03). Differences between test and control groups were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post hoc test. In addition, $P < 0.05$ was considered statistically significant.

Results

Chemical composition of *S. aromaticum* essential oil

The composition of *S. aromaticum* essential oils is presented in Table 1. The main constituents were Eugenol (78.72%), followed β-caryophyllene (8.82%) and eugenyl acetate (8.74%).

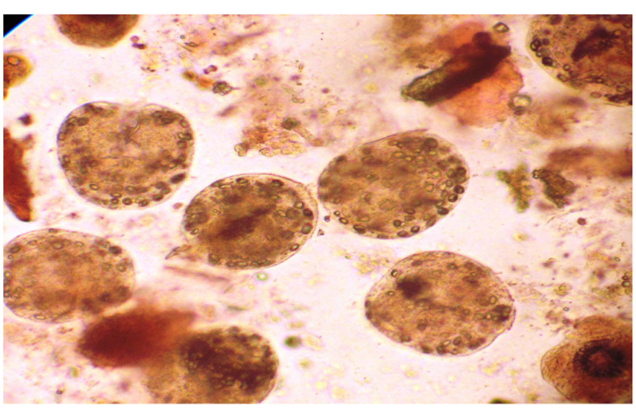

Fig. 2 Dead protoscoleces of hydatid cysts after exposure to 15 μl/ml of *S. aromaticum* essential oil with 0.1% eosin GX10

Table 1 Major components of *S. aromaticum* essential oil determined by GC-FID and GC-MS

Compounds	Percentage
Eugenol	78.72
β-Caryophyllene	8.82
Eugenyl acetate	8.74

Scolicidal activity

Results of the effectiveness of different concentrations of *S. aromaticum* essential oils as a scolicidal agent are shown in Tables 2 and 3. The scolicidal activity of *S. aromaticum* essential oils at the concentration of 10 μl/ml was 55.99%, 60.05%, 80.37%, and 99.76% after 5, 10, 15, and 20 min of application, respectively. Significant differences comparing the control groups after 20 and 15 min of exposure were shown at this concentration ($P < 0.01$).

Findings showed that essential oil at the concentrations of 15 μl/ml killed 100% protoscoleces after 5 min of exposure. These findings demonstrated that *S. aromaticum* essential oils at the concentration of 15 μl/ml were extremely significant comparing to the control groups at all exposure times ($P < 0.0001$).

Discussion

The surgical operation is considered as the most efficient method for the treatment of hydatid disease. On the other hand, one of the side effects of hydatid cyst surgery is recurrence (Shahnazi et al. 2016). To date, many scolicidal agents including some plant extracts, mannitol, albendazole, chlorhexidine gluconate, honey, hypertonic saline, silver nitrate, cetrimide, ethyl alcohol, H_2O_2, and povidone-iodine have been used for inactivation of the hydatid cyst content (Sharafi et al. 2017). However, many of these scolicidal agents may cause undesirable complications that limit their usage (Kavoosi and Purfard 2013). Therefore, the development of new scolicidal agents with low side effects and more efficacies is an urgent need for surgeons (Adas et al. 2009; Mahmoudvand et al. 2014b).

In the present study, *S. aromaticum* essential oil presented high scolicidal activity. The scolicidal power of this essential oil at the dose of 15 μl/ml was 100% after 5 min of exposure. However, the scolicidal activity was decrease at 10 μl/ml. The rates of dead were 99.76%, 80.37%, 60.05%, and 55.99% after 20, 15, 10, and 5 min of exposure, respectively.

Several studies reported the scolicidal effect of essential oil. Similarly, 10 mg/ml of *Nigella sativa* essential oil and 12.5 μl/ml and 25 μl/ml of *Zataria multiflora* essential oil killed 100% of protoscoleces at different times of exposure (Mahmoudvand et al. 2014a; Mahmoudvand et al. 2017). Likewise, these essential oils exert a

Table 2 Scolicidal effect of *S. aromaticum* essential oils at concentration of 10 μl/ml after various exposure times

		Control normal saline	Control Tween 20	*S. aromaticum* essential oils
5 min	Protoscolices (mean ± SD)	1778	1956	1674.33 ± 513.62
	Death protoscolices (mean ± SD)	372	370	786.33 ± 319.21
	Mortality rate (%)	20.92%	18.91%	55.99 ± 3.26%
10 min	Protoscolices (mean ± SD)	1331	1481	1898.67 ± 364.75
	Death protoscolices (mean ± SD)	322	371	1162.33 ± 407.26
	Mortality rate (%)	24.19%	25.05%	60.05 ± 9.43%
15 min	Protoscolices (mean ± SD)	1402	1077	1236.00 ± 166.56
	Death protoscolices (mean ± SD)	317	265	995.67 ± 220.21
	Mortality rate (%)	22.61%	24.6%	80.37 ± 13.76%
20 min	Protoscolices (mean ± SD)	1115	1245	1214.67 ± 40.46
	Death protoscolices (mean ± SD)	328	361	1211.67 ± 49.63
	Mortality rate (%)	29.42%	29%	99.76 ± 0,08%

scolicidal effect of 100% after 20 min of exposure at the dose of 1 mg/ml and 6.25 μl/ml, respectively (Mahmoudvand et al. 2014a, Mahmoudvand et al. 2017). However, lower rates of scolicidal effect were observed for essential oils of *Nigella sativa* at doses of 0.1 mg/ml and 0.01 mg/ml and *Zataria multiflora* at a dose of 3.125 μl/ml (Mahmoudvand et al. 2014a, Mahmoudvand et al. 2017).

Keyhani et al. (2017) mentioned that *Cuminum cyminum* essential oil at the concentrations of 50 and 25 μl/ml killed 100% protoscoleces after 10 and 20 min exposure, respectively. Nevertheless, this same essential oil killed 100% protoscoleces after 30 min and 60 min exposure at doses of 12.5 μl/ml and 6.5 μl/ml, respectively.

In contrast, Moazeni et al. (2012b) noticed 5 mg/ml and 10 mg/ml of *Satureja khuzistanica* essential oil killed 100% of protoscoleces after 60 and 10 min of exposure.

However, Kavoosi and Purfard (2013) observed a 100% mortality rate of protoscolex after 10 min of exposure at doses of 17.5 μg/ml of essential oil from *Zataria multiflora* and 60 μg/mL of essential oil from *Ferula asafoetida.*

The results of the present study showed that *S. aromaticum* has better and faster scolicidal activity compared with certain other plants studied in some other regions of the world. For instance, Mahmoudvand et al. (2019) studied the effect of *Curcuma longa* essential oil on protoscoleces, and reported 100% killing activity at concentrations of 200, 100, and 50 μl/ml after 5, 10, and 20 min, respectively. In the evaluation of chemical composition of *S. aromaticum* essential oil using GC-FID and GC/MS, we found that the main compounds are eugenol (78.72%), β-caryophyllene (8.82%) and eugenyl acetate (8.74%). Similar results were mentioned by several studies (Mahboubi and Mahboubi 2015; Safrudin et al. 2015; Huang et al. 2013). Nevertheless,

Table 3 Scolicidal effect of *S. aromaticum* essential oils at concentration of 15 μl/ml after various exposure times

		Control normal saline	Control Tween 20	*S. aromaticum* essential oils
5 min	Protoscolices (mean ± SD)	1382	1349	949.33 ± 265.03
	Death protoscolices (mean ± SD)	313	408	949.33 ± 265.03
	Mortality rate (%)	22.65%	30.24%	100%
10 min	Protoscolices (mean ± SD)	1230	1470	905.33 ± 55.93
	Death protoscolices (mean ± SD)	309	325	905.33 ± 55.93
	Mortality rate (%)	25.12%	22.11%	100%
15 min	Protoscolices (mean ± SD)	1131	945	1306.33 ± 47.37
	Death protoscolices (mean ± SD)	200	164	1306.33 ± 47.37
	Mortality rate (%)	17.68%	17.35%	100%
20 min	Protoscolices (mean ± SD)	1276	1627	1178.67 ± 85.17
	Death protoscolices (mean ± SD)	280	391	1178.67 ± 85.17
	Mortality rate (%)	21.94%	24.03%	100%

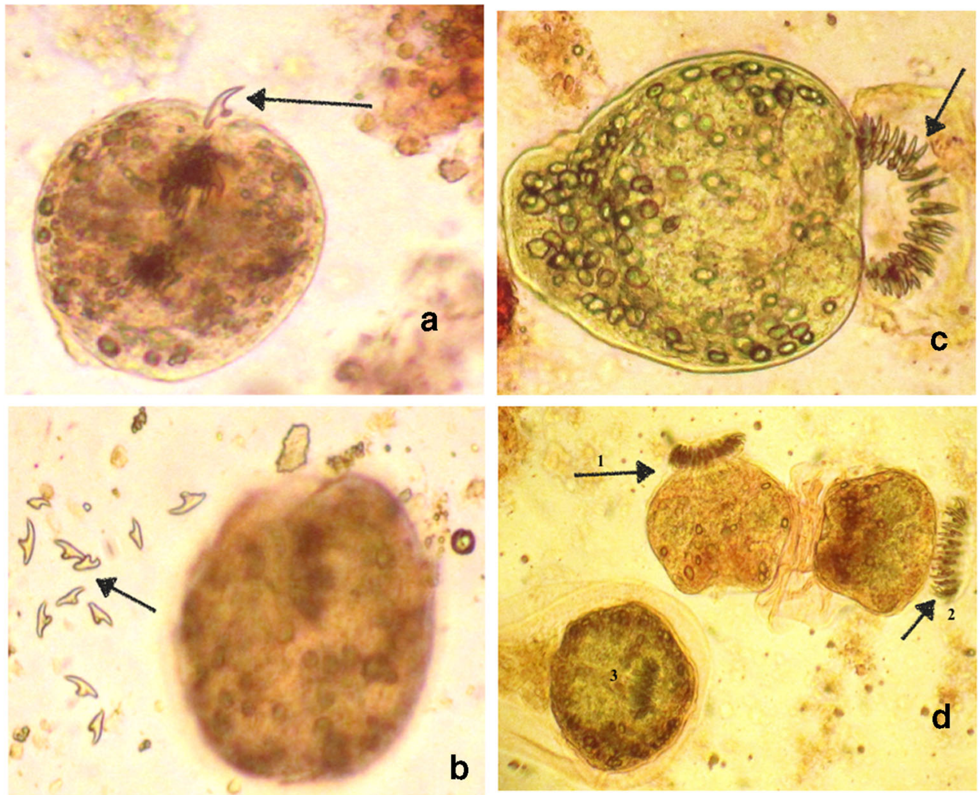

Fig. 3 Disintegration and rupture of the protoscoleces wall accompanied by a release of the hooks. **a** Hook release GrX40; **b** Hooks released next to the dead protoscolece GX40; **c** Release of the hooks in the form of a crown by a dead protoscolece GX40; **d** (1 and 2) Release of the hooks in the form of a crown by a dead protoscolece, (3) deformation of protoscolece GX10

variations in percentages are found. However, Sokamte et al. (2016) and Lee et al. (2009) reported that eugenol is the major compound associated with other chemotype such as δ-cadinene, β-elemene, 2-propanone, methylhydrazone, cyclopentane, methyl, furan, tetrahydro-3-methyl and α- caryphyllene.

In the present survey, the scolicidal effect at a dose of 15 μl/ml was associated with disintegration and rupture of the protoscoleces wall accompanied by a release of the hooks (Fig. 3). Nazzaro et al. (2013) reported that eugenol alters the cell membrane, whereas Devi et al. (2010) demonstrated that eugenol has the ability to disintegrate the membrane. Eugenol causes denaturantion of proteins and modification of the permeability of the cell membrane by reaction with these phospholipids(Guan et al. 2007). These suggestions and the richness of this essential oil in eugenol can explain the phenomenon observed in this study.

Conclusion

The present study suggests that *S. aromaticum* essential oil is a rich source of eugenol that could be used as a natural scolicidal agent to reduce the risk of spillage of protoscoleces during hydatid cyst surgery due to the promising scolicidal effects against hydatid cyst protoscoleces in vitro. Even though cloves are well known in food preparation, their possible side effects when used as a scolicidal agent require further investigation. Nevertheless, additional studies (ex vivo, in vivo) will be needed to prove these outcomes by examination of essential oil as a new scolicidal agent in a clinical setting.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval and consent to participate This paper does not contain any studies with human participants or animals performed by any of the authors.

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