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Chemical analyses and evaluation of the anthelmintic effects of *Origanum majorana* essential oil*, in vitro* and *in vivo* studies

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Abstract: Because of the development of resistance in helminths against major anthelmintic drugs, the search for alternatives is necessary. Medicinal plants are being studied as an alternative source of anthelmintics against gastrointestinal nematodes. The objective of this study is to analyse the chemical composition and evaluate the anthelmintic efficacy of *Origanum majorana* essential oil. The determination of the chemical composition by gas chromatography/mass chromatography (GC/MS) revealed that the essential oil was dominated by terpenoids, particularly carvacrol (35.65%) and terpenic hydrocarbons *p*-cymene (15.82%). The *in vitro* anthelmintic effects against *Haemonchus contortus* were assessed by an egg hatch assay (EHA) and an adult worm motility assay (AWMA) compared with the reference drug albendazole. The essential oil showed ovicidal activity at all the tested concentrations (1, 2, 4 and 8 mg/ml) and more than 80% egg hatching inhibition was observed at the highest dose (8 mg/ml). Exposure to 0.5 mg/ml of the essential oil for eight hours induced a 50% inhibition in the worm motility. The *in vivo* study was performed on *H. polygyrus* by measuring the egg count reduction (ECR) and adult worm count reduction (AWCR) following the treatment of the animals with different doses (2 000, 4 000 and 5 000 mg/kg) of the plant essential oil, and 22 mg/kg of albendazole as the positive control. The results showed that 5 000 mg/kg of the essential oil inhibited the egg count and adult worm count by 76.3 and 74.0%, respectively, seven days post treatment. These findings support the possible use of *O. majorana* essential oil to control gastrointestinal nematodes.

Keywords: fresh herb; *Haemonchus contortus*; *Heligmosomoides polygyrus*; anthelmintic activity; faecal egg count; GC/MS analysis

Gastrointestinal nematode parasitism continues to represent a major problem to the health and welfare of small ruminants causing severe anaemia, weight loss, damage to gastric mucosa and villous atrophy which can be responsible for the death of the animals (Amulya et al. 2015) and may lead to important economic losses via reduction of the animal's productivity (Quijada et al. 2015;

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Furgasa et al. 2018). Compared with other parasitic nematodes, *Haemonchus contortus* is the most pathogenic helminth parasite in the world (Abdo et al. 2017).

Different studies reported the overall prevalence of *H. contortus* infection in small ruminants. In Europe, the prevalence of *H. contortus* shows high values, e.g., 77% in Switzerland, 73% in Italy (Rinaldi et al. 2015); in South Africa, the prevalence has been reported at 68% (Mushonga et al. 2017). In Tunisia, the overall prevalence of *Haemonchus* species in sheep, goats and cattle was 17, 33.6 and 7.23%, respectively (Akkari et al. 2013).

Until now, controlling animal worm infections depended almost exclusively on anthelmintic drugs (Doyle and Cotton 2019). However, the routine use of anthelmintics has led to the emergence of the resistance to all major classes in some parasites (Kaplan and Vidyashankar 2012; Milhes et al. 2017). Furthermore, the residues of some persistent chemicals in the environment have the potential to cause harm to human health and ecosystems (Waller 1997; Santos et al. 2017). Consequently, it is necessary to search for new treatment alternatives.

Nowadays, evaluation of the anthelmintic activities of medicinal plants against gastrointestinal nematodes is receiving increased attention. Products from plants could offer possible alternatives that may be sustainable and environmentally acceptable.

Origanum is a genus of a small aromatic herb belonging to the Lamiaceae family. It is widespread in Northern Africa and grows abundantly on stony slopes and in rocky mountain areas. A total of 38 species are recognised in the world (Bagci et al. 2017). In Tunisia, this plant is called "Mardgouch" and it includes three species, among them *Origanum majorana* (Bejaoui et al. 2013).

The *Origanum* plant is widely used as a traditional remedy to treat various ailments such as whooping and convulsive coughs, digestive disorders, and menstrual problems (Bafana 2013). It is universally utilised in the pharmaceutical, cosmetic, food and health industries (Novak et al. 2000; Bafana 2013). The *Origanum* essential oils contain various compounds which are responsible for different biological activities. In previous studies, it has been demonstrated that *Origanum majorana* essential oils possess antimicrobial (Busatta et al. 2008; Walker et al. 2016), antioxidant (Roby et al. 2013), antiviral (Minami et al. 2003) and insecticide activity against *Anopheles labranchiae* (El-Akhal et al. 2016).

The aim of the current study was to determine the chemical composition of *Origanum majorana* essential oil and evaluate, for the first time, the *in vitro* and *in vivo* nematocidal effects against gastrointestinal nematodes.

MATERIAL AND METHODS

Plant materials: Collection and analyses

Fresh leaves and stems of *O. majorana* were collected from the region of Beja (Northwest of Tunisia, alt, 222 m; 36°30'N; 9°55'E) and analysed at the National Institute for Research and Physico-Chemical Analysis Tunisia (INRAP).

An amount of 200 g of the plant was submitted for hydro-distillation using a Clevenger type apparatus. The obtained oils were dried over anhydrous sodium sulphate and stored at 4 °C pending analysis.

The chemical composition of the essential oil was determined by gas chromatography coupled with mass spectrometry (GC/MS) methods. The following conditions were used: A capillary column (Agilent, 30 mm × 0.25 mm, film thickness 0.25 μm), the injector temperature was 250 °C, the interface line temperature was 300 °C and the mass scan ranged from 50 to 550, the electron impact was 70 eV, the carrier gas was Helium adjusted to a linear velocity of 37 cm/s, the injected volume was 1 μl.

The temperature programme was 110 °C for 2 min, raised to 180 °C at 4 °C/min, then 220 °C (2 °C/min) and finally programmed to 300 °C at a rate of 20 °C/min.

The compounds were identified by comparison to their retention time of the n-alkane standards and also by comparison of their mass spectra with those of the W8N08 and NIST08 libraries (Younsi et al. 2017).

Acute toxicity in mice

An acute oral toxicity study was conducted according to the internationally accepted standard guidelines for the use of animals (Macedo et al.

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2011). It was performed based on the methodology described by Macedo et al. (2010). A total of 48 Swiss Albino mice with an average weight of 25 g were used. They were obtained from the animal house of the Pasteur Institute of Tunis, Tunisia. The animals were housed at a standard room temperature (22 ± 1 °C, 45–75% relative humidity) with a 12 h light and dark cycle and fed standard rodent feed and water *ad libitum.* All experiments were performed according to the the National Institutes of Health recommendations (NIH publication 86-23 revised 1985), approved by the National Ethic Committee of Tunis University.

The animals were randomly divided into six groups (n = 8). The groups received the following treatment via oral administration using a gavage needle:

- Group A: (negative control): received 3% PBS Tween 80.
- Group B: treated with 1 000 mg/kg of essential oils.
- Group C: treated with 2 000 mg/kg of essential oils.
- Group D: treated with 3 000 mg/kg of essential oils.
- Group E: treated with 4 000 mg/kg of essential oils.
- Group F: treated with 5 000 mg/kg of essential oils.

After 24 h, the total number of dead animals was verified and the lethal doses were calculated (LD_{10} and LD_{50}).

In vitro anthelmintic activity

The *in vitro* anthelmintic efficacy of the plant extract on *H. contortus* was evaluated using two different tests: Egg Hatch Assay (EHA) and Adult Worm Motility Assay (AWMA). For each assay, the eggs and adult worms were obtained from Barbarine donor lambs experimentally infested with 5 000 *H. contortus* third larvae kindly provided by Professor Smaragda Sotiraki (Veterinary Research Institute, Thessaloniki, Greece). The experiments were performed following the guidelines according to the World Association of the Advancement of Veterinary Parasitology (WAAVP) (Coles et al. 1992).

Approximately five to ten grams of faeces was collected directly from the rectum of the experimentally infected sheep with *H. contortus*, crushed in water, sifted successively (300, 150, 75, and 38 μm sieves), and centrifuged for 10 min at 754.65 × *g*. The concentration of the eggs was adjusted to 200–250 eggs/ml of PBS (phosphate buffered saline). One ml of the egg solution was placed per well in 24 multi-well plates with a concentration of essential oil varying between 1, 2, 4 and 8 mg/ml and diluted in PBS Tween.

Albendazole (99.8% pure standard reference; Medivet, S.A., Tunis, Tunisia) at the concentration of 1 μg/ml served as a positive control. PBS with Tween 80 (3%) was used as a negative control. Four replicates were used for each concentration of the extract and the control group. The plate was incubated at 27 °C. After 48 h of incubation, the egg hatching was stopped by adding 5% Lugol's iodine solution. The hatched larvae and unhatched eggs were counted under a dissecting microscope at × 40 magnification. The percentage of the hatched eggs was calculated using the ratio: The number of L1 × 100/(the number of eggs + the number of L1).

The adult live male and female worms of *H. contortus* were collected from the abomasum of the infected sheep immediately after slaughtering. The abomasum was removed, opened and placed in a 37 °C saline solution. The motile worms were placed in separate Petri dishes (n = 8), treated with different concentrations (0.5, 0.25, 0.125 mg/ml) of the *O. majorana* essential oil extract in PBS Tween 80 (3%) in a total volume of 3 ml.

Albendazole, at a concentration of 1 mg/ml, was used as a positive control and PBS Tween 80 (3%) was used as a negative control. For each treatment, three replicates were performed. The inhibition of the worm motility is the criteria for anthelmintic activity. The motility of the worms was examined after a 0, 2, 4, 6 and 8 h interval post treatment. To evaluate the worm motility after 8 h, the worms were washed, resuspended in PBS Tween for 30 minutes.

Paralysis of the worms was confirmed by the absence of motility during a 5–6 s observation period under a dissecting microscope at × 40 magnification. The immobility index (AWM) was calculated by using the following formula:

$$\text{AWM (\%)} = \text{100} \times \text{the number of dead worms/the total number of worms per Petri dish} \quad (1)$$

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In vivo anthelmintic activity

Swiss albino mice (n = 36) of both sexes weighing 20–25 g and aged 5 to 6 weeks were used in the study.

Before any experiments were undertaken, all the animals were treated with 7.5 mg/kg BW (body weight) of albendazole to eliminate any intestinal helminth infections.

The infective larvae (L_3) of *H. polygyrus* were generously provided by Dr. Rick Maizels, the University of Edinburgh, the UK. The larvae were cultured from eggs appearing in the faeces of infected mice to the L3 stage in the Petri dishes containing wet filter paper. Concisely, the egg-containing faecal materials were macerated in the wet filter paper and incubated till they hatched into the first larval (L1) stage which underwent several stages of moulting before emerging as the third stage of infective larvae as described by Adiele et al. (2013).

After acclimatisation, in all the studies, a dose of ≈100 *H. polygyrus* infective larvae (L3) contained in 0.6 ml of distilled water were given orally to each mouse. After 9 to 11 days (pre-patent period) (Smyth 1996), the infected animals were randomly divided into five groups of six individuals each. Group I served as negative and group II as a positive control: Albendazole (22 mg/kg BW). Group III, IV and V were treated with *O. majorana* essential oil as follows:

- Group III: 2 000 mg/kg of *O. majorana* essential oils.
- Group IV: 4 000 mg/kg of *O. majorana* essential oils.
- Group V: 5 000 mg/kg of *O. majorana* essential oils.

All the groups were treated for six consecutive days.

A sample of the faecal material was obtained from each mouse in the morning before the administration of the treatment on days 10, 11, 12, 13, 14 and 16 (treatment period). The faecal egg count was calculated as egg per gram (EPG) of the faecal material using a McMaster counting slide. Briefly, 2 g of the mixed faeces was macerated, washed with 60 ml of a saturated salt solution and homogenised in a porcelain mortar (Thienpont et al. 1979). The faecal eggs were calculated according to the McMaster technique (Yondo et al. 2013). The faecal egg count reduction (FECR) was calculated according the equation (Coles et al. 1992):

$$\text{FECR (\%)} = 100 \times (1 - \text{T/C}) \tag{2}$$

where:

T – the means of the FEC (faecal egg count) in the treated groups;

C – the means of the FEC in the control groups.

On day 17 (seven days after the first day of treatment), the mice were anaesthetised using chloroform. The small intestine was excised completely and the total worm counts for *H. polygyrus* were recovered and counted under a dissecting microscope.

The total worm count reduction (TWCR) was calculated by the method described by Enriquez (1993):

$$\text{TWCR (\%)} = 100 \times [(\text{Total worm count in the control group}) - (\text{Total worm count in the treated group})]/\text{Total worm count in the control group} \tag{3}$$

Statistical analysis

The *in vitro* data were analysed using Student's *t*-test. The lethal concentration values (LC_{50}) were calculated using a Graph Pad Prism. The result of the worm motility inhibition was expressed as the mean ± standard error of the mean (SEM). The means were compared using Duncan's multiple range test, the significance levels were within confidence limits of 0.05 or less.

The *F*-values and significance levels of the data were analysed using the General Linear Model (GLM) in SPSS (SPSS for Windows manual, v16.0.0.0.).

RESULTS

Chemical analysis of the *Origanum majorana* essential oils

The essential oil composition of *O. majorana* is presented in Table 1. Twenty-six compounds, representing 100% of the total oil, were identified. Carvacrol constituted the highest proportion of the essential oils (35.65%) followed by *p*-cymene (15.82%) and Thymol (2.79%). Other substances were present at low concentrations.

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Table 1. The relative percentage of the main constituents of the *Origanum majorana* essential oils detected by the GC/MS analysis

Compound	%	Rt	Compound	%	Rt
Carvacrol	35.65	14.201	Dodecane	0.64	35.723
p-cymene	15.82	6.946	Nonadecane	0.59	32.646
Heneicosane	9.37	31.508	Pentadecane	0.52	33.271
Docosane	6.85	33.391	Endo-borneol	0.49	10.568
Tricosane	5	32.378	Dodecane-8-carboxylat	0.48	23.029
Hexadecane	3.32	30.579	2-methoxy-8-chlorodi-benzofuran	0.44	24.561
Thymol	2.79	13.978	4-methoxyphenyl	0.4	26.042
Tetracosane	2.28	29.819	1S, *cis*-calamenene	0.38	19.746
Heptadecane	2.6	31.881	Tritriacontane	0.36	25.932
Hexadecane	1.57	30.579	2-methyl-5-propylnonane	0.34	23.843
Trans-caryophyllene	1.39	17.229	1-methyl-3,3-bicyclohexane	0.34	24.175
Pentacosane	1.13	29.962	α-thujene	0.31	4.98
Octadecane	1.12	30.848	Heptadecane, 7-methyl	0.29	22.51
Octadecane, 5-methyl	1.09	28.649	Eicosane	0.29	33.435
Hexane, 3,3-dimethyl	0.94	30.529	Caryophyllene oxide	0.27	21.156
2-hydroxymethyl	0.79	24.654	Docosane, 6-methyl	0.21	28.749
Tridecane, 6-propyl	0.73	25.932	Dodecane, 4-methyl	0.17	28.749
Butyl-hydroxy-toluene	0.71	19.459	O-Acetylthymol	0.12	14.794

Rt = retention time

Acute oral toxicity assay

Oral administration of *O. majorana* essential oils at single limit doses of 1 000, 2 000, 3 000, 4 000 and 5 000 mg/kg caused no signs of toxicity or mortality in all the treated mice during the observation period of 24 hours.

In vitro anthelmintic studies

The tested essential oil showed ovicidal activity at all the tested concentrations. This activity was clearly dose-dependent (Figure 1). The inhibition of the egg hatching was higher than 80% at the concentration of 8 mg/ml (IC_{50} = 3.206 mg/ml). The positive control showed 100% egg hatching inhibition at 1 μg/ml. Regarding the negative control, less than 5% failure to hatch *H. contortus* eggs was observed.

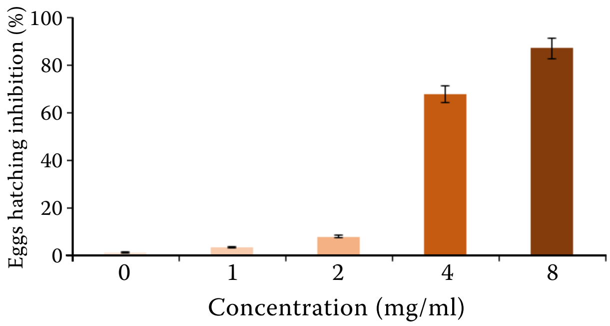

Figure 1. The dose-dependent profile of the *Haemonchus contortus* egg hatching inhibition submitted to the increasing concentrations of the *Origanum majorana* essential oil (1; 2; 4; and 8 mg/ml)

Origanum majorana essential oil exhibited the effective inhibition of worm motility against *H. contortus* (Table 2). At the highest tested concentration (0.5 mg/ml), the essential oil induced 50% mortality, after 8h exposure. The mortality of the worms in albendazole (1 mg/ml) was 91.6 % within 8 h post exposure. The negative control showed no effect on the worm motility.

In vivo anthelmintic assay

The mean eggs and the percentage reduction in the faecal egg counts of the mice treated with the different doses of *O. majorana* essential oil and albendazole are presented in Table 3. The results revealed a significant decrease in the faecal egg

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Table 2. The *in vitro* anthelmintic treatments of the *Origanum majorana* essential oil on the *Haemonchus contortus* adult worm

Treatments	Concentrations (mg/ml)	% of *Haemonchus contortus* worms showing mortality post-exposure to various treatments (mean ± SEM)						
		0 h	1 h	2 h	4 h	6 h	8 h	PBS
Origanum majorana essential oil	0.125	0% ± 0.0	0% ± 0.0	0% ± 0.0	0% ± 0.0	0% ± 0.0	0% ± 0.0	0% ± 0.0
	0.25	0% ± 0.0	0% ± 0.0	0% ± 0.0	0% ± 0.0	12.5% ± 0.57	25% ± 0.57	0% ± 0.0
	0.5	0% ± 0.0	0% ± 0.0	0% ± 0.0	0% ± 0.0	25% ± 0.57	50% ± 0.57	0% ± 0.0
Albendazole with 1 mg/ml (positive control)		0% ± 0.0	0% ± 0.0	0% ± 0.0	25 % ± 0.17	58.33% ± 0.14	91.6% ± 0.15	0% ± 0.0
PBS (negative control)		0% ± 0.0	0% ± 0.0	0% ± 0.0	0% ± 0.0	0% ± 0.0	0% ± 0.0	0% ± 0.0

PBS = phosphate buffered saline; SEM = standard error of the mean

Table 3. The mean variation ± the standard deviation and the percentage of the faecal egg count reduction (FECR) in the experimentally infected mice with *Heligmosomoides polygyrus* (at day 3 and day 7) after treatment with phosphate buffered saline (PBS) Tween 80 (3%), albendazole and different doses of the *Origanum majorana* essential oil

	Doses (mg/kg)	Period: D0	D1	D2	D3	D4	D5	D6	D7
Essential oil	2 000	29 300 ± 4 572	18 700 ± 367	26 000 ± 4 532	27 200 ± 3 797[c] (19)	19 400 ± 5 634	30 000 ± 245	27 300 ± 1 143	30 100 ± 3 715[ade] (21.2)
	4 000	19 600 ± 1 143	21 500 ± 6 859	18 000 ± 2 409	18 200 ± 4 736[d] (43.9)	13 300 ± 1 134	16 000 ± 4 654	14 700 ± 2 368	14 000 ± 2 082[abc] (63.3)
	5 000	17 000 ± 8 410	13 400 ± 490	20 500 ± 2 000	19 000 ± 1 184[e] (36.1)	10 200 ± 163	15 000 ± 2 123	14 066 ± 3 266	9 900 ± 2 164[abc] (74)
Albendazole (22 mg/kg) (positive control)		21 100 ± 4 654	16 100 ± 1 960	12 800 ± 5 960	10 950 ± 2 817[a] (60.1)	8 600 ± 1 796	725 ± 216	5 750 ± 2 327	3 200 ± 82[bcde] (91.6)
PBS Tween 80 (3%) (negative control)		23 000 ± 2 654	17 700 ± 9 104	32 100 ± 41	28 000 ± 9 675[b] (0.0)	37 300 ± 6 532	35 200 ± 4 534	35 000 ± 3 797	38 200 ± 4 409[ade] (0.0)

[a–e]The numbers with the same letter are significantly different

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Table 4. The percentage of the total worm count reduction of *Heligmosomoides polygyrus* after treatment with phosphate buffered saline (PBS), albendazole and the doses of the *Origanum majorana* essential oil

Drugs	Doses (mg/kg)	Mean worm intensity after treatment ± standard deviation	% of reduction of total worm count
Essential oils	2 000	77.17 ± 1.22[d]	11.63
	4 000	32.67 ± 1.63[c]	62.59
	5 000	20.67 ± 4.90[bc]	76.33
Albendazole (positive control)	22	17.83 ± 2.04[b]	79.58
PBS Tween 80 (negative control)		87.33 ± 6.53[a]	0

[a–d]The numbers with the same letter in the same column are not significantly different

TWC = total worm count; TWCR = total worm count reduction

counts of the animals during the treatment period. The oral administration of the 5 000 mg/kg doses of the essential oil (day 7) showed a significant egg reduction (74%) when compared to the negative control group and the animals treated with the 2 000 mg/kg doses ($P < 0.05$). This reduction was slightly lower when compared with albendazole (91.6%). From day 3, the comparison of the mean egg output was not significant between the different doses and negative controls.

The essential oil showed nematocidal activity at all the doses used in this experiment (Table 4). A strong effect (76.3%) was observed in the mice treated with the highest concentration of 5 000 mg/kg, which is similar to albendazole (79.58%). The reduction percentage of the parasitic burden at the lowest dose (2 000 mg/kg) was 11.63% which was statistically indistinguishable from the negative control ($P > 0.05$).

DISCUSSION

This study was performed to validate the *in vitro* and *in vivo* anthelmintic effects of *O. majorana* essential oil against gastrointestinal nematodes.

The *Origanum* plant was selected based on its medicinal virtues. The essential oil was known to possess antiparasitic (Pensel et al. 2014), antioxidant (Nejla and Moncef 2006) and antimicrobial activity (Vagi et al. 2005). To the best of our knowledge, the current study is the first report of the nematocidal activity of *O. majorana*.

Oils are complex mixtures that may contain a greater number of compounds in different quantities (Sell 2006). In our study, 36 constituents of the *O. majorana* oil were identified representing 100% of the oil compositions. The main compounds found were: carvacrol (35.65%), *p*-cymene (15.82) and thymol (2.79%). Regarding our results, the chemical composition was not in agreement with the previous studies performed on samples collected from the North-East region of Tunisia which reported that the main compound was terpinen-4-ol (29.13–32.57%) (Sellami et al. 2009). In fact, terpinen-4-ol was the major compound from the *O. majorana* essential oil collected from India and Argentina (Vera and Chane-Ming 1999; Banchio et al. 2008). Many factors may be responsible for the chemical difference between our results and those previously reported, such as geographical location (Paolini et al. 2010) and genetic factors (Sangwan et al. 2001). Our results are in agreement with those of Kokkini (1997), who found that thymol and the carvacrol characterise all "*oregano*" type essential oils.

A good nematocidal activity was achieved with the *O. majorana* essential oil against *H. contortus* by reducing 86.97% of the egg hatching at a dose of 8 mg/ml. Also, a mortality of 50% of the adult worms was obtained after 8 h exposure. Our results depict, for the first time, the effect against *H. contortus*. However, the *Origanum* genus is showing higher effects against other parasites. Indeed, Santoro et al. (2007) showed that the essential oils of *Oregano* were active against the protozoan *Trypanosoma cruzi*, measured by growth (IC 50/24 h = 175 µg/ml) and ultrastructure (IC 50/24 h = 115 µg/ml). Furthermore, Gaur et al. (2018) evaluated the ability of the *Origanum vulgare* essential oil to inhibit *Cryptosporidium parvum* infectivity of HCT-8 cells. The loss of cell viability started at 125 µg/ml. The efficacy of the *Origanum* essential oil was also demonstrated *in vitro* on *Echinococcus granulosus* at the concentration of 10 µg/ml (Pensel et al. 2014). The anthelmintic activity of essential oils from various Tunisian plants against *H. contortus* has previously been reported. The maximum inhibition effectiveness rates of the *Artemisia campestris* essential oil on the egg hatching and adult motility were 100% and 66.6% at 2 mg/ml and 0.5 mg/ml,

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respectively (Abidi et al. 2018). The essential oil of *Ruta chalepensis* inhibited 100% of the *H. contortus* egg hatching at 1 mg/ml and 87.5 % of the adult worm motility at 1 mg/ml (Akkari et al. 2015). *Citrus aurantifolia*, *Anthemis nobilis* and *Lavandula officinalis* essential oils prevented more than 95% of the *H. contortus* eggs from hatching at 6.25 mg/ml, and all the essential oils induced completely inhibited the motility within the first 8–12 h of observation (Ferreira et al. 2018).

The acute toxicity study in mice revealed that the essential oil did not cause any alteration in the animal's behaviour and food and water consumption at the single limit doses of 1 000, 2 000, 3 000, 4 000 and 5 000 mg/kg during the whole period of study. According to the Organization for Economic Cooperation and Development (OECD 2008) guidelines, this result indicates that the *O. majorana* essential oil was non-toxic.

To achieve more authenticated scientific data about the anthelmintic properties, *in vivo* assays were performed using Albino Swiss mice infected with *H. polygyrus*. In the present study, the *O. majorana* essential oil showed effects on the FEC and the TWC of *H. polygyrus.* The effects of the anthelmintic assays were dose dependent and the best activity was more visible at the highest dose of 5 000 mg/kg by day 7 post-treatment, and resulted in a 76.33% worm burden reduction and a 74% reduction in the faecal egg count. Similar findings of the dose-dependent anthelmintic effects of the *A. campestris* essential oil against *H. polygyrus* have also been reported in our laboratory (Abidi et al. 2018). Satrija et al. (1995) tested the anthelmintic activity of *Papaya latex* in mice infected with *H. polygyrus* and found a high reduction in the worm counts at the highest dose (8 g/kg). Furthermore, Grzybek et al. (2016) showed that *Pumpkin* seed extracts used to treat mice infected with *H. polygyrus* was effective in reducing both the FEC and adult stages at the highest tested dose (8 g/kg). While Githiori et al. (2003) have revealed that *Albizia anthelmintica* had no *in vivo* significant anthelmintic effect in the *H. polygyrus* egg count reduction.

Origanum majorana essential oil possesses significant *in vitro* and *in vivo* anthelmintic effects. This anthelmintic activity can be attributed to the abundance of carvacrol, *p*-cymene and thymol. According to Zhu et al. (2013), *Arisaema franchetianum* essential oil, whose major compound is carvacrol, exhibited the effective inhibition of larval development against *H. contortus*. Additionally, carvacrol is the main compound from the essential oil of *Oregano*, which displays *in vitro* activity against *Echinococcus granulosus* (Fabbri et al. 2016). Commonly, the mains components are responsible for the biological properties of the essential oil (El-Akhal et al. 2016). It is apparent from literature that thymol, which is the major constituent of *Lippia sidoides*, inhibited more than 99% of the *H. contortus* egg hatching and larval development (Camurca-Vasconcelos et al. 2007). Similar results were found by Ferreira et al. (2016) who attributed the *in vitro* anthelmintic activity of the *Thymus vulgaris L*. essential oil to its main component thymol against *H. contortus*. According to Jain et al. (2018), *p*-cymene was found to be the second most common and abundant component of the essential oil of *Trachyspermum ammi*, which possesses a strong antipathogen activity. However, it is possible to suggest that the major components and the other oil compounds act synergistically to establish the final effect (Marie-Magdeleine et al. 2009).

Despite the difference in its chemical composition, essential oils (EOs) have been considered potentially promising compounds for the treatment of many parasitic diseases such as gastrointestinal nematodes (Olounlade et al. 2012).

Based on the results of the current study, it can be considered that the *O. majorana* essential oil possesses nematocidal activity against gastrointestinal nematodes. These findings suggest that *O. majorana* can be an alternative to synthetic anthelmintics. However, further research is necessary to understand the mechanism(s) of these activities and to identify the bioactive compounds in order to make the use of this plant both easy and possible.

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Conflict of interest

The authors declare no conflict of interest.

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