

## Essential oil of *Rosmarinus officinalis* induces *in vitro* anthelmintic and anticoccidial effects against *Haemonchus contortus* and *Eimeria* spp. in small ruminants

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**Abstract:** This work aimed to evaluate the valorisation of the volatile oil of “*Rosmarinus officinalis* L.”, a spontaneously growing medicinal plant in Tunisia, by studying its chemical composition, anthelmintic and anticoccidial potentials against *Eimeria* spp. and *Haemonchus contortus* at different essential oil concentrations. The main compounds of the *R. officinalis* essential oil identified by GC/MS were three monoterpenes: 1,8-cineole (52.06%),  $\alpha$ -pinene (15.35%) and camphor (7.69%). The anticoccidial activity was estimated by the inhibition percentage of the oocyte sporulation in addition to the unsporulated and degenerated *Eimeria* oocysts using a haemocytometer after exposure to different essential oil concentrations. The essential oil was active against *Eimeria* spp. oocysts of sheep at IC<sub>50</sub> = 1.82  $\mu$ g/ml. Therefore, the IC<sub>50</sub> values of the anticoccidial activity of this oil examined was 1.82 mg/ml. The anthelmintic efficacy of the rosemary volatile oil against *Haemonchus contortus* was realised by two *in vitro* tests: the egg hatch assay (EHA) and the adult worm’s motility assay (AWMA), by comparing this efficacy with albendazole (anthelmintic, of reference). In the egg hatch assay, the percentage of inhibition was observed at 16 mg/ml and was 73.76% after 2 days of incubation (IC<sub>50</sub> = 11.41 mg/ml) and for the adult worm’s motility assay, it was 100% inhibition.

**Keywords:** coccidia; *Eimeria* spp.; essential oil; *Haemonchus contortus*; *Rosmarinus officinalis*; small ruminants

Coccidia and helminths are a major constraint in small ruminants’ production as they cause serious consequences in the livestock industry, such

as weight loss, stunted growth, a reduction in the milk production and poor welfare (Taylor et al. 2007). Among intestinal protozoan infections, coc-

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cidiosis, which is linked to the development of the *Eimeria* species in the intestines of young lambs, is considered one of the most economically important diseases threatening the sheep and goat industry in the world, in particular, because it remains mainly undetected due to its subclinical form (Foreyt 1990; Alzieu et al. 1999). Sheep coccidiosis is highly prevalent (Dittmar et al. 2010) and usually manifests in susceptible small ruminants following a high rate of infection (Taylor and Catchpole 1994). The severity of this infection depends on both the species of *Eimeria* and the size of the infecting dose of oocysts (Taylor et al. 2016).

Infected lambs most often show a reduction in their growth rate and an increase in the diarrhoea with a high oocyst production, however, the infection is not obligatorily followed by clinical consequences (Pout et al. 1966; Gregory et al. 1980). Despite that the mortality caused by coccidiosis is infrequent, the subclinical manifestations may have negative consequences on the flock productivity by decreasing the growth rates (Gauly et al. 2004). Previous investigations have reported the existence of a high prevalence of lambs infected by *Eimeria* in correlation with its clinical and economic consequences (Dittmar et al. 2010; Saratsis et al. 2011). Although *Eimeria* spp. could be treated by some antiparasitic compounds such as ammonia or by phenolic compounds, these products are not recommended due to their high toxicity and the occurrence of their residues in the milk and meat (Williams 1997).

In addition to coccidial infections, gastrointestinal nematodes in sheep caused by the most common species “*Haemonchus contortus*” has been reported as an important source of economic losses in the ruminant industry (Waller 2006). Perry et al. (2002) have shown that it is the most prevalent gastrointestinal parasite of small ruminants. This blood-feeding parasite may cause severe clinical consequences like anaemia, weakness and death of the animal (Besier et al. 2016). Akkari et al. (2013) showed that the inclusive prevalence of haemonchosis in goat, cattle and sheep in Tunisia was 6, 7.23 and 17.33%, respectively.

Nowadays, the use of synthetic anti-parasitic drugs has become too widespread and also overused in order to resolve parasitoses in livestock. Such uncontrolled usage has resulted in parasite-resistant populations as well as a negative impact manifested by environmental pollution.

Wolstenholme et al. (2004) have shown that the frequent use of conventional anthelmintic drugs has led to the emergence of resistant parasitic worms in infected animals. In addition, toltrazuril resistance was recently demonstrated against ovine *Eimeria* spp. for the first time (Odden et al. 2018). Thus, new non-toxic alternatives, with a proven antiparasitic activity, are necessary.

Waller and Thamsborg (2004) propose that the exploitation of aromatic and medicinal plants have a great probability in the remediation of the gastrointestinal parasite diseases.

Recently, and from this point of view, there are many alternative approaches using bioactive compounds isolated from aromatic and medicinal plants, such as polyphenols and essential oils, which have been extensively explored as natural pathogen controls of parasites. Volatile oils or essential oils are aromatic liquid substances usually obtained by traditional distillation (hydrodistillation) techniques from many kinds of organs of aromatic and medicinal plants, and many of these organs are rich in bioactive substances like terpenoids, phenolics, acids (Negi 2012). The latter compounds could have potential effects against several pathogens (parasites, fungi, bacteria, viruses) (Brenes and Roura 2010).

Rosemary (*Rosmarinus officinalis* L.) is an evergreen undershrub with a typical aromatic odour. It belongs to the Labiatae family (Lamiaceae), which includes up to 200 genus and about 3 500 species (do Amaral Franco et al. 1972). The oil of this plant is used in perfume flavour manufacturing or aromatherapy (Mizrahi et al. 1991) and it is known to possess antioxidant properties (Tena et al. 1997; Bicchi et al. 2000; Wada et al. 2004), antitumour and antimicrobial activities (Baratta et al. 1998; Pintore et al. 2002; Almela et al. 2006).

To the best of our knowledge, there are no published *in vitro* studies on both the anthelmintic and anticoccidial capacities of rosemary essential oil against *Haemonchus contortus* and *Eimeria* spp., respectively.

The present work focused on investigating the essential oil composition of *R. officinalis* and evaluated its *in vitro* anthelmintic and anticoccidial capacities against *Haemonchus contortus* and ovine *Eimeria* spp., respectively.

Therefore, the present study provides further insight into the use of aromatic plants against gastrointestinal parasites.

## MATERIAL AND METHODS

### Preparation of plant material

Rosemary leaves were randomly collected by hand on June 2016 from the region of Djebel Chaanbi (in the centre-west of Tunisia, 1 544 m above sea level, latitude 35°10'N, longitude 8°50'E) during the vegetative and flowering stage. The leaves were rinsed and then dried in the dark at an ambient temperature for 2 weeks. The dried plant was then powdered using an electric mixer (MoulinexOvatio 2, Serris, France) and finally stored in a refrigerator at 4 °C until use.

### Extraction and analysis of volatile oil

The dried rosemary leaves were chopped and subjected to hydrodistillation. The essential oil was extracted by a hydrodistillation technique (Riahi et al. 2013) by using a Clevenger apparatus. The resulting essential oil was evaporated over anhydrous Na<sub>2</sub>SO<sub>4</sub> for one day, and then filtered and properly stored at –4 °C in tightly fixed in dark bottles.

### Gas chromatography/mass spectrometry (GC/MS) analysis

The analysis of the volatile compounds by GC was carried out on a Hewlett-Packard 6890 gas chromatograph (Palo Alto, CA, USA) equipped with a flame ionisation detector (FID) and an electronic pressure control (EPC) injector. A polar polyethylene glycol (PEG) HP Innowax capillary column (30 m × 0.25 mm, 0.25 mm film thickness; Hewlett-Packard, Palo Alto, CA, USA) was used. The flow of the carrier gas (N<sub>2</sub>) was 1.6 ml/minute. The split ratio was 60 : 1. The analysis was performed using the following temperature programme: oven temperature kept isothermally at 35 °C for 10 min, increased from 35 °C to 205 °C at the rate of 3 °C/min and kept isothermally at 205 °C for 10 minutes. The injector and detector temperatures were held at 250 °C and 300 °C, respectively. The individual peaks were identified by comparing their relative retention indices to *n*-alkanes (C<sub>6</sub>–C<sub>22</sub>) with those in the literature (Adams 2007) and/or with those authentic compounds available in our laboratory. The percentage compositions of the samples were calcu-

lated according to the area of the chromatographic peaks using the total ion current.

The volatile compounds analysis by GC/MS was performed on an HP 5890 (II) gas chromatograph interfaced with an HP 5972 mass spectrometer (Palo Alto, CA, USA) with electron impact ionisation (70 eV ionisation energy). An HP-5 MS capillary column (30 m × 0.25 mm, coated with 5% phenyl methyl silicone, 95% dimethylpolysiloxane, 0.25 mm film thickness; Hewlett-Packard, Palo Alto, CA, USA) was used. The column temperature was programmed to rise from 50 °C to 240 °C at a rate of 5 °C/minute. The ion source temperature was 230 °C and the quadrupole temperature was 150 °C. The front inlet temperature was 250 °C. The carrier gas was helium with a flow rate of 1.2 ml/min; the split ratio was 60 : 1. The scan time and mass range were 1 s and 40–300 *m/z* at 3.62 amu/scan, respectively. Identification of the volatile compounds was undertaken by matching their recorded mass spectra with those stored in the Wiley/NBS mass spectral library of the GC/MS data system and other published mass spectra (Adams 2007).

### Anthelmintic activity of rosemary essential oil against *Haemonchus contortus*

Following the instructions of the World Association for the Advancement of Veterinary Parasitology, an anthelmintic *in vitro* assay was conducted by Coles et al. (1992). Firstly, a Barbarine parasite-donor lamb weighing approximately 30 kg was drenched with albendazole (10 mg/kg) to obtain a parasite free animal.

After anthelmintic treatment during fourteen days, a coproscopic test was performed to confirm the absence of gastrointestinal nematode (GIN) eggs. Then, the animal was orally infected with 5 000 infective *H. contortus* L3.

### Egg hatching assay

The parasite eggs were collected from the faeces of infected donor sheep, crushed in water, sifted successively (300, 150, 70, and 38 µm sieves). The final suspension was then centrifuged for 10 min at 754.65 g. A solution of NaCl (sodium chloride) with a density of 1.2 was added to the pellet and then centrifuged again for 10 min at 754.65 g. The

obtained suspension was washed thoroughly with sterile water through a 38 µm mesh. The *in vitro* test was assessed in 24-multiwell plates by adding 1 ml of the egg solution containing approximately 200 eggs to 1 ml of *Rosmarinus officinalis* essential oil at different concentrations (1, 2, 4, 8 and 16 mg/ml) in each well with four replicates (at each concentration). Albendazole (positive control) and phosphate buffered saline (negative control) were used as the authentic standard.

In addition, Lugol's solution (50 µl) was added to the individual wells to stop the egg hatching process after 48 h of incubation at 27 °C. Finally, according to the following formula, the percentage of unhatched eggs used for plant extracts and control were calculated microscopically at × 40 magnification:

$$\text{Egg hatch assay (EHI)} = \frac{[(\text{number of eggs})/(\text{larvae number} + \text{number of eggs})] \times 100}{(1)}$$

### Motility test of adult worm

The helminth motility assay was performed six weeks post-experimental infection in accordance with the described method of Hounzangbe-Adote et al. (2005). After the slaughter of the experimentally infected lamb, the abomasum was removed, opened and the content was placed in a 0.9% saline solution at 37 °C and incubated per group of eight in Petri dishes with the *R. officinalis* oil at the concentrations of 1, 2, 4, 8 and 16 mg/ml.

For the positive control, the adult worms were incubated with 1 µg/ml of albendazole and with phosphate buffered saline (PBS) as a negative control. Each treatment was conducted with three replicates and the parasite mortality was evaluated per intervals of 0, 1, 2, 4 and 8 h following this formula:

$$\text{Immobility index (\%)} = \frac{100 \times (\text{number of dead worms})}{\text{total number of worms}} \quad (2)$$

### *In vitro* anticoccidial property of *Rosmarinus officinalis*

#### OOCYST ISOLATION

Naturally infected lambs from a farm with a known history of coccidiosis served as the oocyst

donors. After identification of the positive samples containing high numbers of oocysts by a modified McMaster method (Henriksen and Christensen 1992; Meyer et al. 1999), the oocysts were isolated by a simple flotation technique (Roepstorff and Nansen 1998). In this method, a flotation solution of saturated sodium chloride (500 g of glucose/l) was used.

All the detected oocysts in the flotation solution were weighed and counted in order to determine their number per gram of faeces (opg). The unsporulated oocysts were stored at +4 °C in Cr<sub>2</sub>K<sub>2</sub>O<sub>7</sub> 2% (w/v) and then washed three times with 1% Tween 80 in PBS before utilisation. For the better viability of the oocytes utilised in this work, they were less than two weeks old and stored at +4 °C.

#### IN VITRO SPORULATION INHIBITION ASSAY

Approximately 5 × 10<sup>4</sup> oocysts per vial were subjected to various tests using four rosemary essential oil concentrations (0.125, 0.250, 0.5 and 1 mg/ml in 1% Tween 80 dissolved in phosphate buffer saline). In addition, six repetitions were used for each essential oil concentration. Moreover, the same batch of oocysts was utilised throughout the experiments in order to ensure a constant *Eimeria* spp. composition.

However, the unsporulated oocytes were oxygenated using an air pump at 26 °C. Upon 48 h exposure to the respective essential oil concentrations, the oocysts were first cleaned with PBS, and then, one hundred oocysts were placed in a droplet and immediately counted in order to determine the sporulation percent under a light microscope (× 200 magnification).

#### Statistical analysis

The *in vitro* data were expressed as means values ± standard deviation. The statistical analysis was performed by using statistical program package Statistica v5.1 software (Statsoft 1998).

A one-way analysis of variance (ANOVA) and Duncan's multiple range test were used in order to perform pairwise comparisons between the individual groups. The differences between the individual means were deemed to be significant at *P* < 0.05. The IC<sub>50</sub> concentrations were calculated by means of regression analysis.



## RESULTS

### *Rosmarinus officinalis* essential oil composition

The *R. officinalis* essential oil yield was  $1.5 \pm 0.12\%$  (w/w). The volatile oil was analysed by using a GC/MS, each compound was identified according to its retention index (RI) and by matching

Table 1. Essential oil composition of the *R. officinalis* leaves

Component*	RI	(%)	Identification
$\alpha$ -Thujene	932	0.15	GC/MS, RI
Tricyclene	927	0.17	GC/MS, RI
$\alpha$ -Pinene	936	15.35	GC/MS
$\beta$ -Pinene	936	5.74	GC/MS, RI
Camphene	950	5.34	GC/MS, RI
$\beta$ -Myrcene	987	1.48	GC/MS
$\alpha$ -Phellandrene	1 002	0.19	GC/MS, RI
$\Delta$ -3-Carene	1 010	0.18	GC/MS, RI
$\alpha$ -Terpinene	1 013	0.45	GC/MS, RI
<i>p</i> -Cymene	1 015	1.27	GC/MS
$\gamma$ -Terpinene	1 051	0.65	GC/MS, RI
$\alpha$ -Terpinolene	1 082	0.26	GC/MS, RI
Camphor	1 123	7.69	GC/MS, RI
Borneol	1 150	2.28	GC/MS, RI
$\Delta$ -Terpineol	1 155	0.40	GC/MS
Terpinen-4-ol	1 164	0.59	GC/MS, RI
$\alpha$ -Terpineol	1 176	1.92	GC/MS, RI
1,8-Cineole	1 213	52.06	GC/MS, RI
Bornyl acetate	1 270	0.25	GC/MS, RI
$\alpha$ -Copaene	1 379	0.17	GC/MS, RI
$\beta$ -Caryophyllene	1 421	2.21	GC/MS, RI
$\alpha$ -Humulene	1 455	0.22	GC/MS, RI
$\gamma$ -Muurolene	1 474	0.13	GC/MS
$\Delta$ -Cadinene	1 520	0.19	GC/MS, RI
Linalool	1 553	0.64	GC/MS, RI
<b>Chemical classes</b>			
Monoterpene hydrocarbons		31.23	
Oxygenated monoterpenes		65.83	
Sesquiterpene hydrocarbons		2.75	
Oxygenated sesquiterpenes		0.17	
Total identified		99.98	

GC/MS = gas chromatography coupled to mass spectrometry; RI = retention index on HP-5 MS

\*Compounds are listed in order of their elution in HP-5-MS (non-polar column)

its mass spectra compared with those published in the literature.

The retention index, percent composition and the identity of the rosemary essential oil compounds are listed in Table 1 showing that the studied oil was resolved into 25 components representing 99.98% of the total identified compounds. The major constituents were 1,8-cineole (52.06%),  $\alpha$ -pinene (15.35%), camphor (7.69%),  $\beta$ -pinene (5.74%) camphene (5.34%), borneol (2.28%) and  $\beta$ -caryophyllene (2.21%) (Table 1). Other compounds were present at amount of about 1%, such as  $\alpha$ -terpineol (1.92%),  $\beta$ -myrcene (1.48%), and *p*-cymene (1.27%). The remaining compounds were detected at a percentage less than 1%. The oxygenated monoterpenes (65.83%) and monoterpene hydrocarbons (31.23%) were the predominant class.

### Egg hatch assay

As mentioned in Figure 1, all the tested doses of *R. officinalis* essential oil manifested a very promising ovicidal activity ( $IC_{50} = 11.41$  mg/ml). The egg hatch inhibition was dose dependent ( $P < 0.05$ ). We reported a 73.76% egg hatching inhibition at 16 mg/ml.

### Adult worm motility assay

The rosemary essential oil inhibition on the worm motility in each treatment varied significantly ( $P < 0.05$ ) from 0% to 100%. The obtained results showed that no mortality was observed in the negative control.

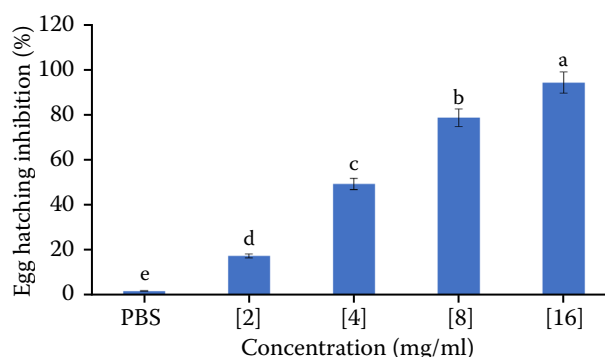


Figure 1. Effect of the *R. officinalis* volatile oil concentration on the hatching eggs of *Haemonchus contortus*. The means with different letters (a–e) are significantly different at  $P < 0.05$

Table 2. Anthelmintic activity of volatile oil of *R. officinalis* on adult *Haemonchus contortus*

Treatments	Concentrations (mg/ml)	Mortality (%) of worms of <i>H. contortus</i> after exposure to the different treatments (means $\pm$ SD)						
		0 h	1 h	2 h	4 h	6 h	8 h	PBS
<i>Rosmarinus officinalis</i> volatile oil	2	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	12.50 $\pm$ 0.00	29.16 $\pm$ 0.44	91.66 $\pm$ 0.44	100.00 $\pm$ 0.00	0.00 $\pm$ 0.00
	4	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	16.66 $\pm$ 0.44	41.66 $\pm$ 0.44	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	0.00 $\pm$ 0.00
	8	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	25.00 $\pm$ 0.00	50.00 $\pm$ 0.66	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	0.00 $\pm$ 0.00
	16	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	25.66 $\pm$ 0.66	62.50 $\pm$ 0.44	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	0.00 $\pm$ 0.00
Albendazole* (1 mg/ml)		0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	29.16 $\pm$ 0.44	37.50 $\pm$ 0.66	66.66 $\pm$ 1.33	100.00 $\pm$ 0.00	0.00 $\pm$ 0.00
PBS		0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00

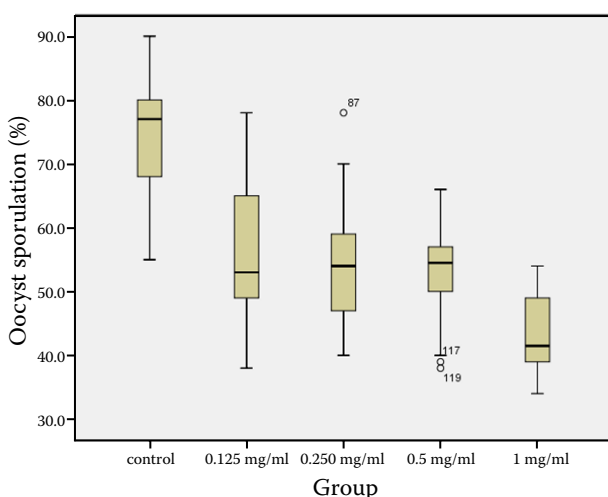
PBS = phosphate buffered saline; SD = standard deviation

\*Albendazole is a positive control; phosphate buffered saline is a negative control

However, the high concentration (16 mg/ml) resulted in 100% mortality. The worms exposed to the positive control (albendazole) for 8 h exhibited the highest anthelmintic activity, with a mortality rate of 100% at 1 mg/ml (Table 2). In addition, in the PBS (negative control), there is no worm recovered motility.

### Effects of *R. officinalis* essential oil on coccidian sporulation

In Figure 2, the median sporulation values with the respective interquartile ranges (IQR) are provided.



Essential oil concentration	Control	0.125 mg/ml	0.250 mg/ml	0.5 mg/ml	1 mg/ml
Median	77.00	53.00	54.00	54.50	41.50
Interquartile range (IQR)	12.25	17.25	12.00	7.50	10.00

Figure 2. Median sporulation values with the respective interquartile ranges (IQR) are provided

Treatment with the *R. officinalis* essential oil significantly ( $P < 0.001$ ) decreased the oocyst median sporulation rates when compared with the control (median: 77.0, IQR: 12.2) irrespective of the essential oil concentration; however, with a considerable effect at the highest concentration of 1 mg/ml (median: 41.5, IQR: 10.0). The  $IC_{50}$  value of this essential oil was 1.82.

### DISCUSSION

In this study, the extracted essential oil from the rosemary leaves was used for the first time to assess its *in vitro* anthelmintic and anticoccidial activities in sheep by referencing to its chemical composition.

The obtained results showed that the rosemary leaf essential oil yield was  $1.50 \pm 0.12\%$  on a dry weight basis (w/w). Compared to previous reports, the yield of our plant was found to be higher than the values from samples collected from Antalya (0.45–0.50%) (Uysal and Tugrul 2009) and from Cukurova (0.27–0.65%) (Kirici and Inan 2001) and lower than some others collected from Tunisia (1.01–2.10%) (Furnier et al. 1983) and (1.09–2.10%) essential oils from Cukurova (Kirpik 2005).

In Tunisia, some environmental conditions, such as the summer season collection areas (Mkaddem et al. 2007), the geographic locations, the genetic variations, the harvest period of the year and the growth conditions, which could modify the volatile oil composition (Benlarbi et al. 2014; Aboukhalid et al. 2016), could explain the obtained higher essential oil yield.

From a literature point of view, the essential oil of *R. officinalis* was found to be rich in both monoterpenes and monoterpene derivatives (95–98%) and

sesquiterpenes (2–5%) (Angioni et al. 2004; Diaz-Maroto et al. 2007).

The rosemary essential oil composition shows a predominance of a monoterpene ketone (1,8-cineole, 52.06%), followed by a monoterpene hydrocarbon ( $\alpha$ -pinene, 15.35%), camphor (7.69%),  $\beta$ -pinene (5.74%), borneol (2.28%) and camphene (5.34%). From the essential oil composition's point of view, previous studies from Iran, India and Tunisia show that there are similarities with our results (Laiq ur Rahman et al. 2007; Kiarostami et al. 2009; Akrouf et al. 2010). Szumny et al. (2010) reported that the Polish fresh *Rosmarinus officinalis* essential oil analysed and identified by GC/MS showed that  $\alpha$ -pinene (33.3%), bornyl acetate (14.8%), camphene (13.8%), and 1,8-cineole (12.3%) were the main compounds.

A previous study reported by Celik et al. (2007) demonstrate that there are many significant variations of rosemary extract compositions with regards to the extraction solvent, technique of extraction, geographic position and harvesting time of the plant material. In addition, Assis et al. (2003) reported that natural compounds from the plant materials were considered as promising ways of applications in the pharmaceutical field to treat some anthelmintic diseases.

Comparing our results with other essential oils, *R. officinalis* was found to be more active than *Ocimum gratissimum* which, at 50 mg/ml, induced an efficiency of 100% against *H. contortus* eggs (Pessoa et al. 2002) while *Eucalyptus globulus* reached significant results on the egg hatching (99.3% at 21.75 mg/ml) (Macedo et al. 2010). The observed variation between the studies in the efficacy of the tested essential oil could be due to the variation in the essential oil constituents and the parasite genetic variation. In fact, this activity could be attributed to its major compound 1,8-cineole which was present at 52.06% to the synergy between the different oil compounds. According to previous studies, 1,8-cineole was found to exhibit an interesting anthelmintic activity against *H. contortus*. In fact, at 10 mg/ml, 1,8-cineole induced a 74.8% egg hatching inhibition, a 65.2% larval development inhibition and a 60.3% inhibition of larval migration (Zhu et al. 2013).

The anthelmintic potential detected in this work might be related to the other minor compounds found in the oil or to the interaction between several compounds. The high nematocidal activity was

assessed with *R. officinalis* essential oil by achieving the total adult worm mortality in all of the used concentrations after 8 h of incubation.

Previous studies in our laboratory have demonstrated that the *Ruta chalepensis* volatile oil provoked an 87.5% *H. contortus* worm mortality at 1 mg/ml. The antiparasitic effect of *R. officinalis* was not limited to its anthelmintic activity, a good antileishmanial activity was also observed against the promastigote of *Leishmania major*  $IC_{50} = 0.08 \mu\text{l/ml}$  (Shokri et al. 2017).

It should be noticed that in a coccidian infection, the prophylactic treatment (antiparasitic drugs) is generally administered with food. From this point of view, these drugs appear a source of 2 main perturbations.

The main problem arises from the presence of the residue of this drug in the products of poultry (Kennedy et al. 1998; McEvoy 2002). The second problem was the high resistance of the parasite to the used drugs (Stephan et al. 1997), which was also recently shown regarding the treatment of ovine coccidiosis with toltrazuril (Odden et al. 2018), apart from the widespread resistance issues in the poultry industry. In consequence, and according to the above-mentioned problems, it appears that it is essential to undertake a future search for bioactive compounds extracted from material having a strong coccidiocidal potential without any eventual residue that could be dangerous both for the consumer and for the animal.

The sporulation inhibiting effect of the *R. officinalis* oil was demonstrated in the present study by means of a previously established *in vitro* sporulation inhibition assay (Saratsis et al. 2011), which, however, did not previously produce a significant sporulation inhibition when polyphenol rich sainfoin (*Onobrychis viciifolia*) extracts were tested. A previous study by Allen et al. (1997) showed that the bioactive compounds of the *Artemisia annua* essential oil has an anticoccidial activity because of the hydrophobic properties and low molecular weight (Boyom et al. 2003) where it causes a mechanism of diffusion through the cellular membrane of the parasites causing an energy and ion leak which leads to apoptosis or the degeneration of the oocysts.

The studies by Saini et al. (2003a) argue that oregano essential oil reduces the principle of coccidiosis in broilers even if coccidiosis is expressed. This volatile oil can decrease the necrotic enteritis

(Saini et al. 2003b). The process of action of these beneficial results remains unexplained.

In this context, future studies are needed to elucidate and/or determine the mechanism of the action of the *R. officinalis* oil and its bioactive compounds against coccidian and helminth parasites in order to be incorporated in future control programmes of livestock parasitic diseases.

In conclusion, coccidiosis and helminthiasis of lambs are very important diseases and the experience of chemical treatments using chemical antiparasitic drugs have highlighted that further future research must be undertaken in order to find the best solutions. In this way, and to the best of our knowledge, this is the first report dealing with the use of essential oils of Tunisian medicinal plants (rosemary) to study their potential anticoccidial and anthelmintic actions against coccidiosis and haemonchosis of lambs.

Thus, we noted a significant reduction in the total number of sporulated oocysts, *Haemonchus* egg hatching and adult worm motility, which is considered as a promising finding that can reduce the risk of infection.

In addition, more future work must be undertaken with the goal to isolate and purify the bioactive compounds involved in the above anthelmintic and anticoccidial activities.

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## Conflict of interest

The authors declare no conflict of interest.

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