Anthelmintic activity of Tunisian chamomile (*Matricaria recutita* L.) against *Haemonchus contortus*

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Abstract

The chemical treatment of gastrointestinal parasitic diseases has been undermined by increasing resistance and high toxicity. There is an urgent need to search for alternative natural sources for the treatment of such parasites. In this respect, the present study aims to quantify phenolic compounds of chamomile (*Matricaria recutita* L.) and to study their *in vitro* anti-oxidant and anthelmintic activities in solvents with increasing polarity. *In vitro* determination of anti-oxidant capacity was carried out using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical cation methods. *In vitro* anthelmintic activity was investigated on egg-hatching inhibition and loss of motility of adult worms of *Haemonchus contortus* from sheep. The results showed that methanolic and aqueous extracts contain more total polyphenols, total flavonoids and condensed tannins than chloroformic and hexanic extracts. ABTS and DPPH assays showed that methanolic extracts had the highest anti-oxidant potency ($IC_{50} = 1.19 \, \mu g/ml$ and $1.18 \, \mu g/ml$, respectively). *In vitro* anthelmintic activity showed that both methanolic ($IC_{50} = 1.559 \, mg/ml$) and aqueous ($IC_{50} = 2.559 \, mg/ml$) extracts had the greatest effect on egg hatching and motility of worms (100% after 8 h post exposure at 8 mg/ml). A significant and positive correlation between DPPH and ABTS tests was observed for all tested extracts. Therefore, total phenolic, total flavonoid and condensed tannin values were correlated with IC_{50} from both ABTS and DPPH, and with inhibition of egg hatching. To our knowledge, this report is the first of its kind to deal with *in vitro* anthelmintic activities of chamomile extracts.

Introduction

Ovine parasitic infections by gastrointestinal helminths have an enormous economic impact in terms of morbidity, mortality, decreased production of milk, meat and wool (Chartier & Hoste, 1994; Akhtar *et al.*, 2000;

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Githiori *et al.*, 2006). Among the dominant species of nematodes of sheep, *Haemonchus contortus* causes severe damage to animal health because of its high prevalence and its pathogenicity, related to its haematophagous habits (Angulo-Cubillán *et al.*, 2010; Hoberg *et al.*, 2010; Besier *et al.*, 2016a). This blood-feeding activity is the main sign of haemonchosis, leading to anaemia, weakness and, frequently, to death (Besier *et al.*, 2016b). In Tunisia, the prevalence of *H. contortus* in sheep and goats has recently been estimated at 17.0% and 33.6%, respectively (Akkari *et al.*, 2013).

The most commonly used method to control these gastro-intestinal parasitic nematodes is based on the repeated use of chemical agents (Knox *et al.*, 2012; Leathwick, 2012). However, the repeated use of anthelmintics has several disadvantages and limitations, including high toxicity, high cost, the presence of residues in the environment and in consumer products, and the increasing resistance of parasites to drugs (Chartier & Hoste, 1994; Waller, 2006; Peña-Espinoza *et al.*, 2014; Van den Brom *et al.*, 2015). In this context, searching for promising approaches to identify new natural candidates for parasitic control in sheep is important (Alonso-Díaz *et al.*, 2010). In this regard, the present study focused on polyphenol-rich plants as a natural alternative to the classical anthelmintics.

Polyphenols have been considered to be the main active compounds responsible for anti-oxidant and biofunctional properties detected in many medicinal plants. Many *in vitro* and *in vivo* experiments have confirmed their protective activity against a multitude of human and animal conditions, such as allergy, inflammation and viral infections (Cutler *et al.*, 2008; Carocho & Ferreira, 2013). Phenolic compounds have been proposed to treat abomasal and intestinal parasitic nematodes (Hoste *et al.*, 2006). Moreover, a relationship between polyphenols, flavonoids and tannins and the anthelmintic activity against *H. contortus* has been reported in a previous study (Akkari *et al.*, 2016).

Chamomile (*Matricaria recutita* L.), an annual herbaceous plant of the Asteraceae family, is one of the most widely used and well-documented medicinal plants in the world (Gupta *et al.*, 2010) and is generally regarded as safe (GRAS) because it neither contains toxic compounds nor represents any acute toxicity for humans and animals (Bradley, 1993; Tolouee *et al.*, 2010). It has been used for centuries as a medicinal plant, mostly for a variety of illnesses related to inflammation of the skin, upper respiratory and gastrointestinal tract (Petronilho *et al.*, 2012). It has several benefits for human and animal health such as antimicrobial, antidiarrhoeal, anti-oxidant, antispasmodic, anti-inflammatory, anti-allergic and anti-depressant properties (McKay & Blumberg, 2006; Cemek *et al.*, 2010; Chandrashekhar *et al.*, 2011; Al Bahtiti, 2012; Amsterdam *et al.*, 2012; Sebai *et al.*, 2014). Chamomile is known for its richness in phenolic compounds believed to be responsible for its biological activities (Gardiner, 2007; Guimarães *et al.*, 2013). It contains phenolic compounds such as the flavonoids apigenin, quercetin, patuletin and luteolin, glucosides and coumarins (herniarin and umbelliferone) (Mann & Staba, 1986; McKay & Blumberg, 2006).

As far as could be ascertained, there is no published report in the literature on the anthelmintic activity of chamomile. The present study aimed to investigate the radical-scavenging activity of *M. recutita* L. flower extracts obtained using solvents of increasing polarity. Flower extracts were also tested for their anthelmintic effects against *H. contortus* from sheep. These activities were correlated with the phenolic content of the plant extracts.

Materials and methods

Plant material and preparation of extracts

Chamomile flowers were cultivated from the region of Beja (north-west of Tunisia, altitude 448 m; 36°81′N; 9°05′E) during spring 2013 and identified by Mouhiba Ben-Naceur, the Professor of Taxonomy in the Higher Institute of Biotechnology of Beja-Tunisia. Chamomile flowers were separated and thoroughly rinsed in running tap water then air dried for 14 days. The flowers were then ground to a fine powder in an electric blender (Moulinex Ovatio 2, Serris, France) and finally stored at 4°C until extraction.

Extracts were prepared by dissolving 100 g of powdered plant material in 500 ml of extraction solvent with increasing polarity (water, methanol, chloroform and hexane). After 24 h of maceration at room temperature (20–25°C, 3 × 500 ml) in the dark, to prevent light degradation, tested extracts were collected and filtered three times using Whatman No. 1 paper, then a rotary vacuum evaporator at 40°C was used in order to remove the solvent, and the aqueous filtrate was lyophilized using a lyophilizer.

Plant extract analysis

Concentrations of total polyphenols, flavonoids and tannins in flower extracts of *M. recutita* were determined by colorimetric methods.

Total phenolic content

Total phenolic content was determined by colorimetry using the Folin–Ciocalteu reagent (FCR) according to the method described by Singleton *et al.* (1999). An aliquot of 100 µl of each tested extract was added to 500 µl of FCR previously diluted in water (1/10). A total volume of 4 ml of Na_2CO_3 solution was then added to the mixture and mixed thoroughly before incubation for 15 min at room temperature. The absorbance versus a prepared blank was measured at 765 nm. Total phenolic content was expressed as mg gallic acid equivalents/g dry weight (mg of GAE/g DW) using a calibration curve with gallic acid. The calibration curve range was 50–200 mg/ml. Triplicate measurements were performed for all samples.

Total flavonoid content

Total flavonoid content was measured by the colorimetric method using aluminium ion Al^{3+} (Bahorun *et al.*, 1996). To each extracted sample, 1.5 ml of $AlCl_3$ (2%) solution were added. The mixture was then shaken thoroughly before incubation for 10 min at room temperature. The absorbance versus a prepared blank was measured at 367 nm. Total flavonoid content was expressed as mg quercetin equivalents/g dry weight (mg QE/g DW) through

a previously established quercetin calibration curve. Concentrations ranged from 15 to 100 g/ml. Triplicate measurements were performed for all samples.

Condensed tannin content

In the presence of concentrated HCl, condensed tannins were transformed by the reaction with vanillin to anthocyanins (Sun *et al.*, 1998). Fifty microlitres of each sample extract, appropriately diluted, were mixed with 3 ml of 4% methanol vanillin solution and 1.5 ml of HCl. After 15 min, the absorbance was measured at 500 nm. Condensed tannin contents were expressed as mg catechin equivalents/g dry weight (mg CE/g DW) through a calibration curve with catechin. The calibration curve range was 50–600 mg/ml.

Anti-oxidant activity evaluation

The anti-oxidant activity of each tested extract was measured using free-radical scavenging activity by DPPH (2,2-diphenyl-1-picrylhydrazyl) and by ABTS (2,2-azino-bis[3-ethylbenzthiazoline-6-sulphonic acid]) radical cation.

Free-radical scavenging ability using a stable DPPH radical

The free-radical scavenging activity of extracts was measured by DPPH using the method described by Gorinstein *et al.* (2004). A volume of 2.8 ml solution of DPPH in methanol (0.1 mM) was added to 0.2 ml of each extract sample in methanol at different concentrations (1.56, 3.12, 6.25, 12.50, 25.00, 50.00 and 100.00 µg/ml), incubated in a dark room for 30 min, then the absorbance was measured at 517 nm. Radical scavenging activity was calculated using the following equation: Scavenging effect = $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the control and A_1 is the absorbance in the presence of the sample. The extract concentration providing 50% inhibition (IC_{50}) was calculated from the plot of inhibition percentage against extract concentration. Tests were carried out in triplicate.

Free-radical scavenging ability using a stable ABTS radical cation

The free-radical scavenging activity was determined by the ABTS radical cation decolourization assay described by Siddhuraju (2006). ABTS was dissolved in water to a 7 mM concentration. ABTS radical cation (ABTS⁺) was produced by reacting ABTS stock solution with 2.45 mM potassium persulphate and was kept in a dark room at room temperature for 12–16 h before use. The ABTS⁺ was diluted to the absorbance of 0.70 ± 0.02 and stocked for off-line and on-line assays. A volume of 1 ml of each diluted tested extract at different concentrations (1.56, 3.12, 6.25, 12.50, 25.00, 50.00 and 100.00 g/ml) was added to 3 ml of ABTS⁺ solution and kept in a dark place at room temperature for 60 min. The absorbance was measured at 734 nm. The scavenging capacity was calculated as $[(A_0 - Ab)/A_0] \times 100$, where Ab is the absorbance in the presence of the sample, and A_0 refers to the absorbance of ABTS⁺ without sample. The extract concentration providing 50% inhibition (IC_{50}) was calculated from the plot of inhibition percentage against chamomile extract concentration. All tests were performed in

triplicate. For both DPPH and ABTS assays, the same procedure was repeated with a synthetic anti-oxidant – ascorbic acid – as positive control. Anti-oxidative capacities of the extracts were compared with those of ascorbic acid.

In vitro *anthelmintic assays*

The anthelmintic efficacy tests of chamomile extracts on *H. contortus* were performed using two different procedures. For each assay, the eggs or adult stages were obtained from faeces and abomasum of Barbarine donor lambs experimentally infected by oral administration of a pure aqueous suspension of 6000 *H. contortus* third-stage larvae (L3), according to the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines (Coles *et al.*, 1992).

Egg-hatch assay

Freshly collected eggs were incubated with different extracts in quadruplet. Dried flower extracts of *M. recutita* were used as a test treatment. Untreated eggs in phosphate-buffered saline (PBS) with dimethyl sulphoxide (DMSO) (0.5%) solution were used as a negative control. Albendazole reference drug (99.8% pure standard reference, Médivet, SA, Tunisia) was dissolved in DMSO and diluted at three concentrations (0.25, 0.50 and 1.00 µg/ml); eggs treated with the different concentrations of albendazole served as positive controls. For each extract concentration, approximately 200 eggs in 1 ml of PBS were placed in each test tube. Sample extracts at different concentrations (8.0, 4.0, 2.0, 1.0, 0.5 and 0.25 mg/ml) in 1 ml PBS with DMSO (0.5%) were used. After incubation for 48 h at 27°C, egg hatching was stopped by adding Lugol's iodine solution. The numbers of L1 larvae and eggs per well were counted under a dissecting microscope at 40× magnification. The percentage of hatched eggs was determined using the ratio: number of L1 × 100/(number of eggs + number of L1).

Adult worm motility assay

This test was performed according to Hounzangbe-Adote *et al.* (2005). Adult worms were collected from a lamb, at week 6 after experimental infection. Immediately after being slaughtered, the abomasum was removed, opened and placed at 37°C in 0.9% sodium chloride solution. The collected parasites were then washed and kept in PBS solution. Eight actively moving worms were placed in Petri dishes filled with 8.0, 4.0, 2.0 and 1.0 mg/ml of *M. recutita* extracts in PBS with DMSO (0.5%), in a volume of 3 ml. Albendazole dissolved in DMSO then in PBS at a final concentration of 1 mg/ml was used as a positive control. PBS with DMSO (0.5%) was used as a negative control. All the tests were performed in triplicate. Inhibition of worm motility was the criterion of anthelmintic activity. The required delays for worm paralysis and/or complete immobility were recorded at 0, 1, 2, 4, 6 and 8 h. To test if the worms could recover their motility after incubation for 8 h, they were washed with distilled water and resuspended in PBS for 30 min. Worm death was ascertained by the absence of motility during an observation period of 5–6 s. The immobility index was calculated as follows:

Table 1. Extract yields, polyphenolic content and effect of *Matricaria recutita* extracts on free-radical scavenging capacity and egg-hatching activity.

Extract samples	Extract yield (%)	Polyphenolic content			Radical scavenging activity		Anthelmintic activity
		TP (mg GAE/g DW)	TF (mg QE/g DW)	CT (mg CE/g DW)	DDPH IC$_{50}$ (µg/ml)	ABTS IC$_{50}$ (µg/ml)	Egg hatching IC$_{50}$ (mg/ml)
Aqueous extract	11.18	24.93 ± 0.41	18.37 ± 1.58	7.42 ± 0.08	1.23	1.38	2.559
Methanolic extract	10.54	42.12 ± 2.30	28.45 ± 0.19	7.42 ± 0.33	1.18	1.19	1.523
Chloroformic extract	9.12	5.34 ± 0.73	12.54 ± 0.19	0.70 ± 0.04	45.89	44.86	5.347
Hexanic extract	8.22	1.67 ± 0.20	4.64 ± 0.29	0.37 ± 0.04	69.72	64.36	–

TP, Total phenolic content expressed as mg gallic acid equivalents/g dry weight (mg GAE/g DW); TF, total flavonoid content expressed as mg quercetin equivalents/g dry weight (mg QE/g DW); TC, condensed tannin contents expressed as mg catechin equivalents/g dry weight (mg CE/g DW); DDPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid); IC$_{50}$, value of concentration causing 50% inhibition of the activity.

Immobility index (%) = 100 × (number of dead worms per Petri dish/total number of worms per Petri dish).

Statistical analysis

The statistical analysis was performed using the SPSS 10.0 software package for Windows (SPSS Inc., Chicago, Illinois, USA). SigmaPlot 12.0 (Systat Software Inc., San José, California, USA) was used to calculate the IC$_{50}$ for ABTS, DPPH and egg-hatch inhibition.

Regression was used for evaluation of the dose–response relationship using Minitab® Release 14 (Minitab SARL (France) www.minitab.com). The result of the worm motility inhibition was expressed as mean ± standard error of mean (SEM). The results of IC$_{50}$ for egg-hatching inhibition were assessed statistically using analysis of variance (ANOVA) and complemented by multiple comparisons of means by the SNK test (Student–Newman–Keuls). Spearman's correlation coefficients were calculated between anti-oxidant capacity and anthelmintic effects versus polyphenolic content, between DDPH and ABTS assays, DDPH and egg-hatching assays and, finally, between ABTS and egg-hatching assays of tested extracts (SPPS 10.0 software). Means of DDPH, ABTS scavenging activities and anthelmintic efficacy were compared by Wilcoxon test (SPSS 10.0 software). A probability of 0.05 was used as the threshold for statistical significance.

Results

Extract yields

The highest percentage yield of extraction of *M. recutita* was in the aqueous extract (11.18%) while yields of the methanolic, chloroformic and hexanic extracts were 10.54, 9.12 and 8.22%, respectively (table 1).

Total phenolic, flavonoid and condensed tannin contents

Phytochemical screening revealed the presence of polyphenols, flavonoids and condensed tannins in all the tested extracts. Their amounts varied according to the nature of the extract. The highest concentration was found in the methanolic extract (42.12 ± 2.30, 28.45 ± 0.19 and 7.42 ± 0.33 mg/g DW, respectively), while the chamomile hexanic extract gave the lowest amount of these compounds (1.67 ± 0.20, 4.64 ± 0.29 and 0.37 ± 0.04 mg/g DW, respectively) (table 1).

Anti-oxidant activity

The radical scavenging activity of chamomile extracts and ascorbic acid increased significantly in a dose-dependent manner (fig. 1). Inhibitory concentrations for methanol and aqueous extract (IC$_{50}$) were 1.19 and 1.38-µg/ml, respectively, for ABTS and 1.18 and 1.23 µg/ml, respectively, for DPPH scavenging activities. These two extracts showed important anti-oxidant activity, being close to ascorbic acid (IC$_{50}$ = 1.16 and 1.17 µg/ml for DPPH and ABTS scavenging activities, respectively) (table 1, fig. 1).

The comparison between DPPH and ABTS scavenging activities showed that there was a statistically significant correlation with aqueous, methanolic, chloroformic and hexanic extracts (r = 0.915, 0.758, 0.948 and 0.915, respectively; $P < 0.01$) (table 2). Also, a high positive correlation was observed between total polyphenols, flavonoids and condensed tannins with scavenging of DPPH (0.764, 0.722 and 0.731, respectively; $P < 0.01$) and ABTS radicals (0.722, 0.752 ($P < 0.01$) and 0.604 ($P < 0.05$), respectively) (table 3).

In vitro *anthelmintic assays*

Egg-hatch assay

Methanolic and aqueous extracts from chamomile showed higher inhibitory effects on egg hatching (IC$_{50}$ = 1.523, 2.559 mg/ml, respectively) (table 1). The inhibitory effect of the hexanic extract was the lowest, as it induced 42.06% egg-hatching inhibition at 8 mg/ml (fig. 2). Albendazole inhibited 91.75% of egg hatching at 1 µg/ml (IC$_{50}$ = 0.1255 µg/ml). The maximum concentration required to induce total (100%) egg-hatch inhibition for the methanolic extract was 4 mg/ml (table 1).

On the one hand, the results showed a high correlation between the inhibition of egg hatching and total polyphenols, flavonoids and tannins (r = 0.747, 0.749 and 0.818,
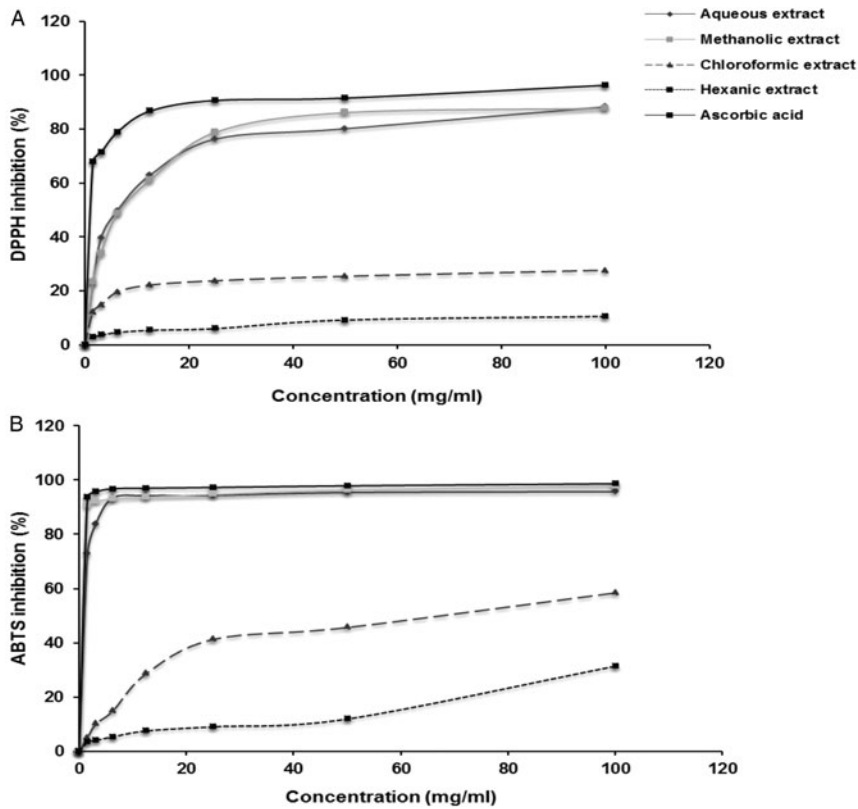
Fig. 1. Free-radical scavenging activities of aqueous, methanolic, chloroformic and hexanic extracts of *Matricaria recutita* and ascorbic acid measured by (A) DPPH and (B) ABTS methods.
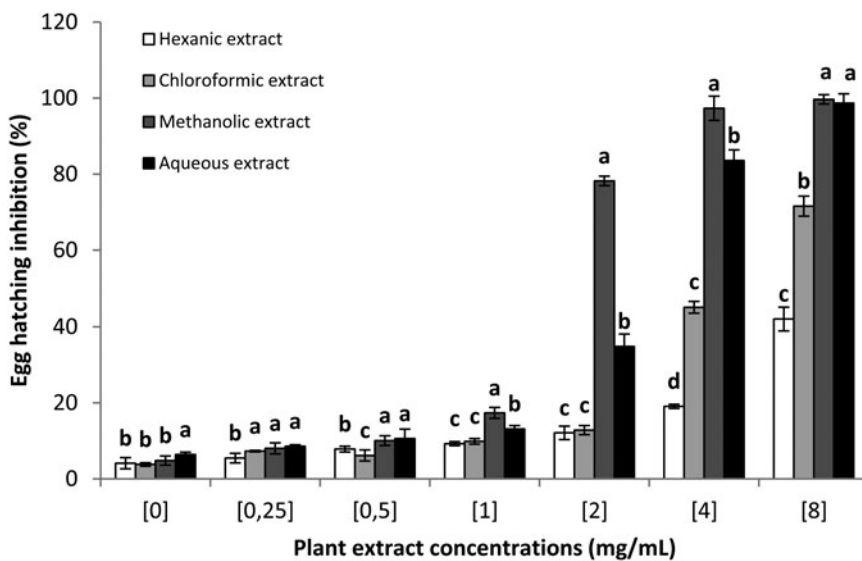

Fig. 2. Dose-dependent profile of the egg-hatching inhibition of *Haemonchus contortus* submitted to increasing concentrations of plant extract (0, 0.25, 0.5, 1, 2, 4, 8 mg/ml). Standard errors of the mean are shown on each bar. Values labelled with superscript letters (a–d) are significantly different ($P < 0.001$), $n = 4$.

Table 2. Pearson's correlation coefficient between DDPH and ABTS assays, DDPH and egg-hatching assays, and ABTS and egg-hatching assays of tested extracts of *Matricaria recutita*.

	Correlation coefficient		
Extract samples	DDPH vs. ABTS	DDPH vs. egg hatching	ABTS vs. egg hatching
Aqueous extract	0.915**	0.701**	0.874**
Methanolic extract	0.758**	0.640**	0.922**
Chloroformic extract	0.948**	0.706**	0.767**
Hexanic extract	0.915**	0.813**	0.747**

**, $P < 0.01$.

respectively, $P < 0.01$) (table 3). On the other hand, the inhibition of egg hatching was significantly correlated with scavenging of both DPPH ($r > 0.640$; $P < 0.01$) and ABTS radicals ($r > 0.747$; $P < 0.01$) in all tested extracts (table 2).

Adult worm motility assay

All extracts produced *in vitro* showed dose-dependent activity on adult parasites. The methanolic extract, however, killed more worms than the other extracts at all concentrations tested ($P < 0.05$). After 8 h, it induced 91.77% mortality at the highest concentration tested (8 mg/ml), while the aqueous extract induced only 75.05% mortality at the same concentration (table 4). There was 82.26% mortality of worms in albendazole within 8 h post-exposure. However, the worms in the DMSO negative control solution showed neither paralysis nor mortality. Finally, no worm recovered motility in the PBS revival test.

Discussion

In this study, *M. recutita* flower extracts recovered using solvents of increasing polarity were examined for their anti-oxidant and anthelmintic activities.

First, the results of the phytochemical study showed that *M. recutita* was rich in polyphenols, flavonoids and condensed tannins. Their quantities were higher in methanolic and aqueous extracts compared to extracts in other organic solvents (chloroform and hexane). The phenolic contents of the tested extracts were higher than that of the decoction extract from Tunisian *M. recutita* (Sebai et al., 2015) and they were higher than those of the extracts of Brazilian chamomile tea and infusions (Guimarães

Table 3. Correlation coefficients of total phenolic, total flavonoid and condensed tannin contents versus DPPH, ABTS radical scavenging activity and egg-hatching inhibition of *M. recutita* extracts.

	DDPH	ABTS	Egg hatching
Total phenolic	−0.764**	−0.722**	−0.747**
Total flavonoid	−0.722**	−0.752**	−0.749**
Condensed tannin	−0.731**	−0.604*	−0.818**

**, $P < 0.01$; *, $P < 0.05$.

et al., 2013; Zielinski et al., 2014). However, many other studies showed that ethanolic and aqueous extracts contain more phenolic compounds than the extracts tested in this study (Vinha et al., 2012; Alibabaei et al., 2014). This could be attributed to the geographic origin and the variety of chamomile (Sharafzadeh & Alizadeh, 2011) as well as the climatic conditions and the extraction process (Papagiannopoulos et al., 2004; Sebai et al., 2015). Moreover, the extracting solvent has a prominent impact on the amount and nature of the compounds recovered, so the difference in phytochemical profile can modify the efficacy and the biological activity of the extract (Makris & Kefalas, 2004; Naczk & Shahidi, 2006; Sifaoui et al., 2014).

The results of this study are consistent with previous results from other studies, which confirmed the high concentration of phenolic compounds in chamomile (Guimarães et al., 2013; Mekinić et al., 2014). Many authors showed that polar solvents, especially methanol and ethanol, are more efficient in extracting these phenolic compounds because they have high polarity and good solubility (Roby et al., 2013; Formisano et al., 2015).

Beside the phytochemical composition, free-radical scavenging activity of different extracts was investigated by two methods: DPPH and ABTS. Results showed that all chamomile extracts were characterized by important anti-oxidant activities for both tests. Methanolic and aqueous extracts showed the best scavenging activity, with lower values compared to chloroformic and hexanic extracts as well as other plant extracts well known for their anti-oxidant properties (Kelebek et al., 2009; Zhao et al., 2011).

For all extracts, a significant and positive correlation between DPPH and ABTS tests was observed. Therefore, the current results highlight that total phenolic, total flavonoid and condensed tannin contents were positively correlated with both ABTS and DPPH radical assays. This means that the good anti-oxidant capacity of chamomile is mainly related to the high levels of polyphenols and flavonoids. This correlation was supported by many authors and is common among the majority of plants (Pyo et al., 2004; Hamad et al., 2010; Guimarães et al., 2013; Akkari et al., 2016). The findings of this study corroborate previous reports suggesting that phenols and flavonoids contained in chamomile methanolic extract strongly contribute to the anti-oxidant activity (Formisano et al., 2015). Recent findings suggest that especially quercetin (flavonol), chlorogenic acid, caffeic acid, p-coumaric acid and ferulic acid (phenolic acids) are the primary sources of anti-oxidant ability of chamomile methanolic extract, by scavenging free radicals such as the hydroxyl radical (OH), which is the major cause of lipid peroxidation (Nováková et al., 2010; Roby et al., 2013).

Our results confirm the anthelmintic effect of chamomile extracts on *H. contortus*. Methanolic extract totally inhibited egg hatching at low concentration (4 mg/ml) compared to aqueous, chloroformic and hexanic extracts. These results confirm those of a previous study, in which the methanolic extract from *Rubus ulmifolius* inhibited 95.66% of egg hatching at 4 mg/ml (Akkari et al., 2016). However, ethanolic extracts of *Artemisia campestris* and *Thymys capitatus* had good activity at a lower concentration (2 mg/ml) (Boubaker Elandolsi et al., 2013; Akkari et al., 2014).

Table 4. *In vitro* anthelmintic efficacy of extracts of *Matricaria recutita* on *Haemonchus contortus*.

Treatment	C (mg/ml)	Mortality rate (%) of *Haemonchus contortus* worms post-exposure to various treatments (mean ± SEM)						
		0 h	1 h	2 h	4 h	6 h	8 h	PBS
Aqueous extract	1	0 ± 0.0	0 ± 0.0	0 ± 0.0	8.25 ± 0.05	10.87 ± 0.15	16.33 ± 0.05	0 ± 0.0
	2	0 ± 0.0	0 ± 0.0	0 ± 0.0	19.37 ± 0.15	21.08 ± 0.08	34.15 ± 0.03	0 ± 0.0
	4	0 ± 0.0	0 ± 0.0	0 ± 0.0	31.50 ± 0.11	35.26 ± 0.09	48.27 ± 0.08	0 ± 0.0
	8	0 ± 0.0	0 ± 0.0	0 ± 0.0	35.29 ± 0.11	59.33 ± 0.05	75.05 ± 0.02	0 ± 0.0
Methanolic extract	1	0 ± 0.0	0 ± 0.0	0 ± 0.0	12.71 ± 0.01	20.03 ± 0.11	33.45 ± 0.07	0 ± 0.0
	2	0 ± 0.0	0 ± 0.0	0 ± 0.0	33.45 ± 0.08	39.63 ± 0.07	58.51 ± 0.13	0 ± 0.0
	4	0 ± 0.0	0 ± 0.0	0 ± 0.0	41.71 ± 0.05	58.91 ± 0.02	79.25 ± 0.05	0 ± 0.0
	8	0 ± 0.0	0 ± 0.0	0 ± 0.0	50.12 ± 0.07	74.36 ± 0.06	91.77 ± 0.06	0 ± 0.0
Chloroformic extract	1	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	2.37 ± 0.11	12.64 ± 0.10	0 ± 0.0
	2	0 ± 0.0	0 ± 0.0	0 ± 0.0	7.46 ± 0.06	12.33 ± 0.08	19.25 ± 0.05	0 ± 0.0
	4	0 ± 0.0	0 ± 0.0	0 ± 0.0	12.51 ± 0.11	27.33 ± 0.03	33.46 ± 0.08	0 ± 0.0
	8	0 ± 0.0	0 ± 0.0	0 ± 0.0	16.52 ± 0.07	34.12 ± 0.06	48.07 ± 0.07	0 ± 0.0
Hexanic extract	1	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	2.82 ± 0.01	8,48 ± 0.09	0 ± 0.0
	2	0 ± 0.0	0 ± 0.0	0 ± 0.0	4.27 ± 0.06	6.39 ± 0.05	10.39 ± 0.13	0 ± 0.0
	4	0 ± 0.0	0 ± 0.0	0 ± 0.0	8.52 ± 0.09	9.33 ± 0.11	12.56 ± 0.01	0 ± 0.0
	8	0 ± 0.0	0 ± 0.0	0 ± 0.0	12.68 ± 0.09	18.36 ± 0.07	25.25 ± 0.05	0 ± 0.0
Albendazole[a]	1	0 ± 0.0	0 ± 0.0	0 ± 0.0	21.55 ± 1.22	71.96 ± 1.22	82.26 ± 0.01	0 ± 0.0
PBS with DMSO 0.5%[a]		0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0

[a]Albendazole is a positive control; PBS with DMSO 0.5% is a negative control.
C, concentration.

The findings of the current study also provide evidence that chamomile extracts have significant impact on adult *H. contortus*, observed in terms of worm paralysis and/or death at different post-treatment intervals at even low concentrations. Previous studies revealed that *R. ulmifolius* exhibited significant activity at the same concentrations (Akkari *et al.*, 2016); meanwhile, *A. campestris* and *T. capitatus* were active at lower concentrations (100% inhibition at 2 mg/ml) (Boubaker Elandolsi *et al.*, 2013; Akkari *et al.*, 2014).

Based on these results, a significant linear positive correlation between total polyphenols, flavonoids and tannin contents and anthelmintic activity was observed. Akkari *et al.* (2016) reported the same result with *Rubus* extracts. Many hypotheses have been proposed to explain the antiparasitic activity of these compounds, which are known to interfere with energy generation in parasites by uncoupling oxidative phosphorylation (Athnasiadou *et al.*, 2001).

Tannins are known for their protein-binding ability, which can protect proteins from degradation in the rumen, and increase protein flow to the small intestine and amino acid absorption (Min *et al.*, 2003; Waghorn & McNabb, 2003; Hoste *et al.*, 2006). Their antiparasitic activity on adults can be explained by their contact with the nematode's cuticle, buccal cavity, oesophagus and reproductive tract (Hoste *et al.*, 2012; Martínez-Ortíz-de-Montellano *et al.*, 2013). Moreover, polyphenolic compounds might interfere with enzymes secreted or excreted by the worms in the local environment, or with enzymes involved in metabolic pathways that are essential for nematode functions (Athanasiadou *et al.*, 2001).

The study of structural changes in adult *H. contortus* after *in vitro* exposure to medicinal plants has been the focus of several authors. Martínez-Ortíz-de-Montellano *et al.* (2013) studied two plants rich in tannins – sainfoin (*Onobrychis viciifolia*) and tzalam (*Lysiloma latisiliquum*) – and showed that their effects were observed mainly in the cuticle, the cephalic region, the vulva and the anus of female worms. Microscopic observations showed longitudinal and transverse folds and thick crests in the cuticle. These lesions were observed either on the whole body or on patches along the whole body of the nematode, including the cephalic parts and the rest of the body, as well as the distal part of the worms. Thus, aggregates around the oral capsule, female vulva or anus have been observed and are similar under both *in vivo* and *in vitro* conditions.

The strong correlation between anti-oxidant and anti-parasitic activity of chamomile can be explained by its rich flavonoid content. Interestingly, it has been suggested that anti-oxidant flavonoids might have anthelmintic activity (Ferreira, 2011) and anti-oxidant levels are also an indicator of flavonoids that can potentiate commercial anthelmintics. In fact, the anti-oxidant flavonoid quercetin is widely found in plants, and has been reported to increase the anthelmintic activity of moxidectin in lambs (Dupuy *et al.*, 2003). Nevertheless, chamomile can be used to reduce parasitic burdens and to improve both animal production and health (Wangchuk, 2008).

Based on the results of the present study, chamomile extracts showed significant dose-dependent free-radical scavenging and *in vitro* anthelmintic activity against *H. contortus*, as ascertained by worm motility and egg-hatching inhibition. The presence of phenolic compounds, such as flavonoids and tannins, is particularly important for expressing various bioactivities. Chamomile may thus be exploited for the development of new natural anti-oxidant and anthelmintic agents. However, bio-guided purification will be necessary to isolate and purify bioactive compounds from chamomile flower extracts, to evaluate their *in vivo* activities and to study the structural changes in the worms by electron microscopy. This will be of great value to determine the mode of action of chamomile on the worms.

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Conflict of interest

None.

Ethical standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national guides on the care and use of laboratory animals and have been approved by the institutional committee of the Institut Pasteur, Tunis.

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