

## Potential anthelmintic effect of *Capparis spinosa* (Capparidaceae) as related to its polyphenolic content and antioxidant activity

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**ABSTRACT:** *Capparis spinosa* is cultivated for several proprieties and the different parts of the plant (flower buds, fruits, leaf and seeds) have been employed in drugs, foods and cosmetics. This study aimed to explore the antioxidant potential effect and to assess the anthelmintic efficacy of an aqueous extract of *Capparis spinosa*. *In vitro* antioxidant activity of aqueous extracts from the fresh leaves and flower buds of *C. spinosa* was measured by determining free radical-scavenging activity against DPPH and ABTS radical cations. *In vitro* anthelmintic activities against eggs and adult worms of *Haemonchus contortus* from sheep were investigated in comparison to albendazole. DPPH and ABTS activities of both aqueous extracts increased in a dose dependent manner. The IC<sub>50</sub> values of aqueous extracts from fresh leaves, flowers buds and ascorbic acid in the DPPH radical scavenging assay were 101.40; 70.40 and 57.56 µg/ml, respectively. The IC<sub>50</sub> values of aqueous extracts fresh leaves, flowers buds and ascorbic acid in the ABTS radical scavenging assay were 110; 87.89 and 58.17 µg/ml, respectively. Both plant extracts showed ovicidal activity at all tested concentrations. The aqueous extract from flower buds (IC<sub>50</sub> = 2.76 mg/ml) showed higher inhibitory effects ( $P < 0.05$ ) than aqueous extract from leaves (IC<sub>50</sub> = 8.54 mg/ml) in the egg hatching assay. The aqueous extract from flower buds inhibited more worms than the aqueous extract from leaves at all tested concentrations. After 6 h of exposure, the highest tested concentrations of aqueous extracts from flower buds and leaves induced 100 and 41.66% inhibition of motility, respectively. These results show for the first time that *C. spinosa* possesses *in vitro* anthelmintic properties which may be related to its high content of phenolic compounds such as flavonoids and tannins.

**Keywords:** *Capparis spinosa*; free radical-scavenging ability; anthelmintic; *Haemonchus contortus*

### List of abbreviations

**ABTS** = 2,2'-azinobis-[3-ethylbenzthiazoline-6-sulfonic acid]; **DMSO** = dimethyl sulfoxide; **DPPH** = 2,2-diphenyl-1-picrylhydrazyl; **HPLC/MS** = chromatography/mass spectrometry; **PBS** = phosphate-buffered saline

Parasite infestations of livestock species are especially prevalent in developing countries due to poor management practices and inadequate control measures (Dhar et al. 1982). The main control measures for parasitic diseases largely rely on the repeated use of anthelmintics. However, resistance to various groups of anthelmintics is constantly increasing in worm populations, especially those found in small ruminants (Waller 1997; Van Wyk

et al. 1999). In addition, the increasing pressure from consumers to limit the use of chemical substances in livestock industry merits consideration. These two issues underpin the current search for alternatives or rather complementary solutions to chemotherapy to control gastrointestinal parasitism in sheep and goats. There is an increasing body of evidence that stockowners rely on ethno-veterinary medicine as an alternative and as a sustain-

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able control option (Deeba et al. 2009; Sindhu et al. 2010). Such use underlines a rich background of indigenous knowledge that needs to be captured (Landau et al. 2014).

*Capparis spinosa* is a flowering plant of the family capparidaceae; it is a common perennial shrub growing both wild and cultivated. The plant has a natural distribution in the coastal regions of the entire Mediterranean Sea basin. It grows in poor soils especially in dry areas, thus playing an environmental role in fixing soils and limiting erosion. Different organs of *C. spinosa* including young shoots, flower buds, fruits, leaves and seeds have been employed in drugs, foods and cosmetics (Aliyazicioglu et al. 2013). In addition, the floral buds of this plant are commonly named capers; they are harvested in spring before they blossom and are usually processed in brine.

Since ancient times, *C. spinosa* has been cultivated for medicinal purposes. It is used for arteriosclerosis, as a diuretic, kidney disinfectant and vermifuge (Soyler and Khawar 2007). In more recent investigations, aqueous extracts of *C. spinosa* exhibited several pharmacological activities, including antifungal (Ali-Shtayeh and Abu Ghdeib 1999), anti-*Leishmania* (Jacobson and Schlein 1999), antibacterial (Mahasneh 2002), anti-hyperglycaemic (Eddouks et al. 2004), chondrocyte-protective (Panico et al. 2005), hypolipidaemic (Eddouks et al. 2005), anti-allergenic, anti-histaminic (Trombetta et al. 2005), antiviral and immuno-modulatory (Arena et al. 2008) activities.

This study was aimed at investigating the antioxidant activity of crude caper and for the first time, the *in vitro* anthelmintic effect of leave and flower bud aqueous extracts against *Haemonchus contortus* from sheep compared to albendazole.

## MATERIAL AND METHODS

### Plant material and extract preparation

Fresh leaves and flower buds of *C. spinosa* were collected in Bousalem, Jendouba governorate (North of Tunisia, alt. 127 m; 36°36'N; 8°58'E), in May (spring). The plant was botanically identified according to the flora of Tunisia and a voucher specimen (Cuenod et al. 1954). Collected samples were processed for shade drying in a well ventilated room for four weeks. The dried plant parts were milled to a fine powder using an electric blender then stored in an airtight container at 4 °C until extraction.

The aqueous extracts were prepared by dissolving 100 g of powder of each plant material in 500 ml of distilled water in a glass percolator. This was allowed to macerate for 24 h at room temperature (20–25 °C) and the brew was filtered using Whatman number one filter paper. The process of percolation was repeated three times (× 500 ml). The combined filtrate of each plant material was then lyophilised. The final crude aqueous extract was transferred to a vial and kept air tight; it was stored at 4 °C until used. Obtained extracts served for the quantification of polyphenol components and the evaluation of *in vitro* anthelmintic activities.

### Aqueous plant extract analysis

Concentrations of total polyphenols, flavonoids, and tannins in aqueous extracts of fresh leaves and flowers buds of *C. spinosa* were determined using colorimetric methods. Chemical compounds were identified using chromatography/mass spectrometry (HPLC/MS).

**Total phenolic content.** Total phenolic content was assayed by a colorimetric method using the Folin-Ciocalteu reagent (FCR) according to the method of Singleton et al. (1999). An aliquot of 100 µl of each aqueous extract was added to 500 µl of FCR previously diluted in water (1/10).

A total volume of 4 ml of sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) solution was then added to the mixture and mixed thoroughly before incubation for 15 min at room temperature. The absorbance versus prepared blank was measured at 765 nm. Total phenolic content was expressed as mg gallic acid equivalents per gram of dry weight (mg of GAE/g DW) using a calibration curve with gallic acid. The calibration curve range was 50–200 mg/ml ( $R^2 = 0.95$ ). All samples were performed in triplicates.

**Total flavonoid content.** The total flavonoid content was measured by a colorimetric method using aluminium – ion  $\text{Al}^{3+}$  (Bahorun et al. 1996). To each aqueous extract sample, 1.5 ml of aluminium chloride ( $\text{AlCl}_3$ ) (2%) solution were added. The mixture was then shaken thoroughly before incubation for 10 min at room temperature. The absorbance versus prepared blank was measured at 367 nm. Total flavonoid content was expressed as milligrams of quercetin equivalents per gram of dry weight (mg of CE/g DW) with reference to a quercetin-calibration curve previously established. Concentrations ranged from 15 to

100 g/ml ( $R^2 = 0.98$ ). 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 generate anthocyanidins (Sun et al. 1998). Fifty microlitres of plant methanolic 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 per gram (mg CE/g DW) through a calibration curve with catechin. The calibration curve range was 50–600 mg/ml.

**Chromatography/mass spectrometry (HPLC/MS) analysis and identification of phenolic compounds.** The chemical composition of both extracts was carried out by chromatography/mass spectrometry (HPLC/MS) analysis. HPLC-MS separation (series 1100, Agilent, Waldbronn, Germany) was performed using an Agilent C18 reversed-phase column (150 × 4.6 mm) maintained at 33 °C with a direct injection of 25 µl of the extract and 100 bar pressure and 0.25 ml/min flow rate. A programmable variable wavelength UV detector was used for the analytic detection. Elution was performed by gradient mode using two mobile phases (A and B). Phase A solution consisted of water and acetic acid (999/1 v/v, respectively). The phase B solution was acetonitrile. The gradient program was chosen as follows: phase B was set at 5% for the five first min, increased linearly to 100% at 65 min, remained at 100% for three min before decreasing to 5% at 69 min. Chromatographic peaks were integrated by Mass Lynx Software. The Mass spectroscopy (MS) was performed using a Micro mass Quattro Ultima PT MS model. The ion trap detector with electrospray ionization (ESI) source was used for quantification in negative ionization mode under specific operating conditions (capillary voltage (KV): 3.20; capillary temperature (C): 300; multiplier (V): 550 cone gas flow (L/Hr): 60).

The identity of the components was assigned by comparison of their retention indices HPLC-MS spectra with a homemade library or commercially available standards reported in the literature.

### Antioxidant activity evaluation

The antioxidant activity of fresh leave and flower bud aqueous extracts of *C. spinosa* was carried out by measuring elimination of DPPH (2,2-diphenyl-1-pic-

rylhydrazyl) radicals and of ABTS (2,2'-azinobis-[3-ethylbenzthiazoline-6-sulfonic acid] radical cations.

**Free radical-scavenging ability using a stable DPPH radical.** The free radical-scavenging activity of extracts was measured with 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 extract solution in methanol at different concentrations (20, 40, 60, 80, 100, 120 and 140 µg/ml), and incubated in the dark for 30 min; then, the absorbance was measured at 517 nm. Radical-scavenging activity was calculated using the following equation:

$$\text{Scavenging effect} = [(A_0 - A_1)/A_0] \times 100$$

where:

$A_0$  = the absorbance of the control

$A_1$  = the absorbance in the presence of the sample

All determinations were performed in triplicate.

**Free radical-scavenging ability using a stable ABTS radical cation.** Free radical-scavenging activity was additionally determined using the ABTS radical cation decolourization assay described by Siddhuraju (2006). ABTS was dissolved in water to a 7 mM concentration. ABTS radical cation (ABTS<sup>+</sup>) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate which was kept in the dark at room temperature for 12–16 h before use. The ABTS<sup>+</sup> was diluted to an absorbance of  $0.70 \pm 0.02$  and stored for off-line and on-line assays. A volume of 1 ml of diluted extract at different concentrations (20, 40, 60, 80, 100, 120 and 140 µg/ml) was added to 3 ml of ABTS<sup>+</sup> solution and left to stand in the dark at room temperature for 60 min. The absorbance was measured at 734 nm. The scavenging capacity was calculated as:

$$[(A_0 - A_b)/A_0] \times 100$$

where:

$A_b$  = the absorbance in the presence of the sample

$A_0$  = refers to the absorbance of ABTS<sup>+</sup> without sample

All determinations were performed in triplicate.

### In vitro anthelmintic assays

The anthelmintic efficacy of the two plant extracts against *H. contortus* was determined using two different procedures. For each assay, the eggs or adult stages were obtained from faeces and abomasum of Barbarine breed donor lambs experi-

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mentally 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 (W.A.A.V.P.) guidelines (Coles et al. 1992).

**Egg hatch assay.** Freshly collected eggs were washed repeatedly with distilled water and incubated with different extracts in quadruplets. Aqueous extracts of *C. spinosa* leaves and flower buds were used as test treatment. Albendazole (99.8% pure standard reference, Médivét, S.A.) was used as reference drug (positive control). Untreated eggs in (PBS) phosphate-buffered saline (pH 7.2) solution were used as negative control. For each extract concentration, approximately 200 eggs were distributed into 24-multiwell plates (1 ml per well). Aqueous extracts at concentrations of 1.0; 2.0; 4.0; 8.0 and 16.0 mg/ml were diluted in PBS and were added to the wells (1 ml per well). Albendazole dissolved in DMSO and diluted at three concentrations (0.25; 0.5 and 1 µg/ml); the highest concentration served as positive control.

After 48 h incubation 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 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 an experimentally infected lamb six weeks after infection. Immediately after being slaughtered, the abomasum was removed, opened and placed in 0.9% sodium chloride solution at 37 °C. The collected parasites were then washed and kept in PBS solution. Eight actively moving worms were placed in Petri dishes containing 50.0; 25.0 and 12.5 mg/ml of *C. spinosa* aqueous extracts in PBS in a total volume of 3 ml. Albendazole dissolved in DMSO and diluted in PBS at a final concentration of 0.5 mg/ml was used as positive control. PBS was used as negative control. Three replicates were performed for each treatment. Inhibition of worm motility was the rationale for anthelmintic drug activity estimation. The required time for paralysis or complete inactivity and immobility was recorded at 0; 1; 2; 4; 6 and 8 h. To estimate the revival of the worm motility, the extracts and albendazole were washed away after 8 h incubation, and parasites were resuspended in PBS for 30 min. Death of worms was ascertained by absence of any motility during an observation period

of 5–6 s for each concentration under a dissecting microscope at × 40 magnification. The immobility index was calculated as follows:

$$\text{Immobility index (\%)} = 100 \times \frac{\text{number of dead worms}}{\text{total number of worms per Petri dish}}$$

### Statistical analysis

The statistical analysis was performed using the SPSS-10.0 software package for Windows. IC<sub>50</sub> for egg hatch inhibition and free radical-scavenging activities were calculated by probit analysis. The results of the worm motility inhibition, DDPH, ABTS scavenging activity and anthelmintic efficacy assays were expressed as mean ± standard error of mean (S.E.M). Results were statistically evaluated using analysis of variance (ANOVA) and complemented by multiple comparisons of means using the SNK test (Student-Newman-Keuls).

## RESULTS

### Chemical analysis of *Capparis spinosa* aqueous extract

**Total phenols, flavonoid and tannin contents.** The amounts of total phenols, flavonoids and condensed tannins were determined from calibration

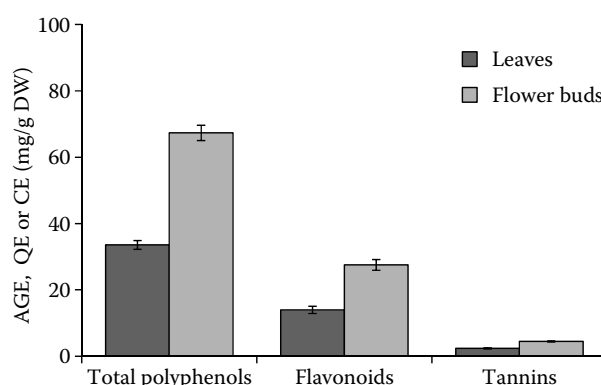


Figure 1. Total phenolic, flavonoid and condensed tannin contents of caper leaves and flower bud aqueous extracts. Total phenolic content expressed as mg gallic acid equivalents per gram of dry weight (mg of GAE/g DW); total flavonoid content expressed as milligrams of quercetin equivalents per gram of dry weight (mg of QE/g DW); condensed tannin contents expressed as mg catechin equivalents per gram (mg CE/g DW); data are given as mean ± SD; *n* = 3

Table 1. Major compounds in *Capparis spinosa* aqueous extracts identified using liquid chromatography/mass spectrophotometry

| Fresh leaves M/Z (M-H)-RT (min)                                     | Flowers buds M/Z (M-H)-RT (min)                           |
|---|---|
| Quercetin (301) 25.5  | Quercetin (301) 25.5                                      |
| Quercetin 3- <i>O</i> -glucoside (463) 31.5                         |   |
| Quercetin 3- <i>O</i> -glucoside-7- <i>O</i> -rhamnoside (608) 32.5 | Rutin or Quercetin 3- <i>O</i> -rutinoside (609) 29.5     |
| Quercetin-7- <i>O</i> -D-glucopyranoside (608) 30.5                 | Quercetin-3-glucoside-7-rhamnoside (607) 34.5             |
| Ferulic acid (193) 24   | Kaempferol-3- <i>O</i> -glucoside (593) 32                |
| <i>P</i> -coumaric acid (163) 19.5                                  | Kaempferol-3- <i>O</i> -(2-rhamnosyl)-rutinoside (591) 41 |
| Caffeic acid (179) 14.5   | Caffeic acid (179) 14.5                                   |

RT = retention time

curves previously established using gallic acid, quercetin and catechin as standards, respectively. Figure 1 depicts tannin and flavonoid contents of plant aqueous extracts and their respective total phenol contents.

The total polyphenol content of *C. spinosa* aqueous extracts was higher in plants at the flowering stage than at the vegetative stage, ranging from 67.29 in flowers buds to 33.55 mg GAE/g DW in fresh leaves. A similar tendency was observed for flavonoid and condensed tannin contents. Thus, higher amounts of flavonoids and condensed tannins were found in flowers (27.54 mg QE/g DW and 4.46 mg CE/g DW) compared to fresh leaves (13.97 mg QE/g DW and 2.38 mg CE/g DW), respectively.

**Identification and quantification of phenolic compounds using HPLC-MS.** Based on literature references (Incocencio et al. 2000) and HPLC-MS, analysis of both fresh leaves and aqueous extracts from flower buds of capers revealed the presence of a large number of flavonoids and phenolic compounds. Quercetin derivatives (Rutin or Quercetin 3-*O*-rutinoside, Quercetin-3-glu-

coside-7-rhamnoside) and kaempferol derivatives (Kaempferol-3-*O*-glucoside, Kaempferol-3-*O*-(2-rhamnosyl)-rutinoside) were the most abundant flavonoids present in flowers buds. Quercetin and its glycoside derivatives such as Quercetin 3-*O*-glucoside, Quercetin-3-*O*-glucoside-7-*O*-rhamnoside and Quercetin-7-*O*-D-glucopyranoside were present in fresh leaves. In addition, caffeic acid, ferulic acid and *p*-coumaric acid were the most abundant phenolic acids in aqueous caper extracts (Table 1).

### Antioxidant activity

The difference in DPPH activities between fresh leaves and flower bud aqueous extracts was highly significant ( $P < 0.001$ ). Antioxidant activities increased in a dose-dependent manner; and were found to range from 10.4–63.2% and 25–77.5%, respectively, as compared to 34.4–87.5% for ascorbic acid. The  $IC_{50}$  values of fresh leaves, flower bud aqueous extracts and ascorbic acid in the DPPH

Table 2. The half maximal inhibitory concentration ( $IC_{50}$ ) of *Capparis spinosa* aqueous extracts with respect to radical elimination and *Haemonchus contortus* egg hatching

| Treatment                             | $IC_{50}$                   |
|---------------------------------------|-----------------------------|
| DDPH free radical-scavenging activity | flower bud aqueous extract  |
|                                       | fresh leave aqueous extract |
|                                       | ascorbic acid               |
| ABTS free radical-scavenging activity | flower bud aqueous extract  |
|                                       | fresh leave aqueous extract |
|                                       | ascorbic acid               |
| Egg hatch inhibition                  | flower bud aqueous extract  |
|                                       | fresh leave aqueous extract |
|                                       | albendazole                 |

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radical scavenging assay averaged 101.40; 70.40 and 57.56  $\mu\text{g/ml}$ , respectively. The difference between the ABTS activity in fresh leaves and flower bud aqueous extracts was highly significant ( $P < 0.001$ ). Likewise, it increased in a dose-dependent manner, and was found to range between 9.8–58 and 14.6–75%, respectively, as compared to 25.6–90% for ascorbic acid. The  $\text{IC}_{50}$  values of fresh leaves, flower bud aqueous extracts and ascorbic acid in the ABTS radical scavenging assay were 110; 87.89 and 58.17  $\mu\text{g/ml}$ , respectively (Table 2).

### Egg hatch assay

Albendazole inhibited egg hatch already at low concentrations ( $\text{IC}_{50} = 0.314 \mu\text{g/ml}$ ), indicating susceptibility of the strain of *H. contortus* used in the current study. There was a difference in the dose-dependent ovicidal activity of both plant extracts ( $P < 0.05$ ). Flower bud aqueous extracts ( $\text{IC}_{50} = 2.76 \text{ mg/ml}$ ) showed higher inhibitory effects than leave aqueous extracts ( $\text{IC}_{50} = 8.54 \text{ mg/ml}$ ) on egg hatching ( $P < 0.05$ ) (Table 2). For the highest tested concentrations of both flower bud and leave extracts (16  $\text{mg/ml}$ ), there were 3% and 9% larvae (L1) in the incubated tubes, respectively (Figure 2).

### Adult worm motility

The mortality of worms in albendazole within 8 h post-exposure (PE) reached 75%. However, the

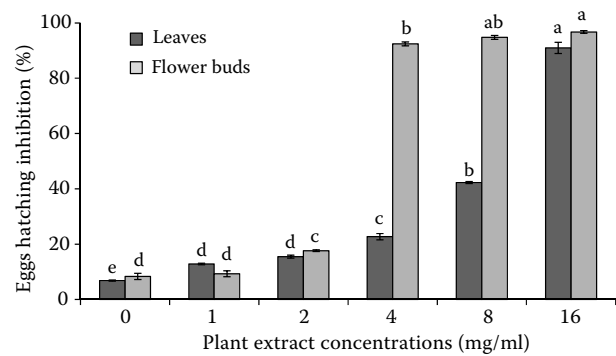


Figure 2. Dose-dependent profile of *Haemonchus contortus* egg hatching in the presence of increasing concentrations of *Capparis spinosa* aqueous extracts (1; 2; 4; 8 and 16  $\text{mg/ml}$ ); mean  $\pm$  SEM;  $n = 4$

Values labelled with superscript letters (a–e) are significantly different ( $P < 0.001$ )

worms in PBS negative control solution showed neither paralysis nor mortality. Finally, no worm recovered motility in the PBS revival test. Aqueous extract of *C. spinosa* flower buds inhibited more worms than the leave aqueous extract at all tested concentrations (Table 3); this effect was higher ( $P < 0.001$ ) at 8 h PE. Highest inhibition motility (100%) of worms was observed 6 h PE in flower bud aqueous extracts at 50  $\text{mg/ml}$  (Table 3).

### DISCUSSION

For preliminary screening of anthelmintic activity, investigators prefer *in vitro* tests including adult

Table 3. *In vitro* anthelmintic efficacy of *Capparis spinosa* crude aqueous extracts on *Haemonchus contortus*

| Treatment   | Concentration (mg/ml) | Mortality rate (%) of <i>Haemonchus contortus</i> worms post-exposure to various treatments (mean $\pm$ SE) |              |                 |                               |                               |                               |              |
|---|-----------------------|---|--------------|-----------------|-------------------------------|-------------------------------|-------------------------------|--------------|
|   |                       | 0   | 1            | 2               | 4                             | 6                             | 8                             | PBS          |
|   |                       | (hours)   |              |                 |                               |                               |                               |              |
| Flower bud aqueous extract                          | 12.5                  | 0 $\pm$ 0.00  | 0 $\pm$ 0.00 | 0 $\pm$ 0.00    | 0 $\pm$ 0.00 <sup>c</sup>     | 0 $\pm$ 0.00 <sup>c</sup>     | 0 $\pm$ 0.00 <sup>c</sup>     | 0 $\pm$ 0.00 |
|   | 25                    | 0 $\pm$ 0.00  | 0 $\pm$ 0.00 | 0 $\pm$ 0.00    | 16.66 $\pm$ 0.07 <sup>b</sup> | 45.83 $\pm$ 0.07 <sup>b</sup> | 75 $\pm$ 0.00 <sup>b</sup>    | 0 $\pm$ 0.00 |
|   | 50                    | 0 $\pm$ 0.00  | 0 $\pm$ 0.00 | 12.5 $\pm$ 0.00 | 45.83 $\pm$ 0.07 <sup>a</sup> | 100 $\pm$ 0.00 <sup>a</sup>   | 100 $\pm$ 0.00 <sup>a</sup>   | 0 $\pm$ 0.00 |
| Fresh leave aqueous extract                         | 12.5                  | 0 $\pm$ 0.00  | 0 $\pm$ 0.00 | 0 $\pm$ 0.00    | 0 $\pm$ 0.00                  | 0 $\pm$ 0.00 <sup>b</sup>     | 0 $\pm$ 0.00 <sup>c</sup>     | 0 $\pm$ 0.00 |
|   | 25                    | 0 $\pm$ 0.00  | 0 $\pm$ 0.00 | 0 $\pm$ 0.00    | 0 $\pm$ 0.00                  | 4.16 $\pm$ 0.07 <sup>b</sup>  | 20.83 $\pm$ 0.07 <sup>b</sup> | 0 $\pm$ 0.00 |
|   | 50                    | 0 $\pm$ 0.00  | 0 $\pm$ 0.00 | 0 $\pm$ 0.00    | 8.33 $\pm$ 0.14               | 41.66 $\pm$ 0.19 <sup>a</sup> | 70.83 $\pm$ 0.07 <sup>a</sup> | 0 $\pm$ 0.00 |
| Positive control (albendazole at 1 $\text{mg/ml}$ ) |                       | 0 $\pm$ 0.00  | 0 $\pm$ 0.00 | 0 $\pm$ 0.00    | 29.16 $\pm$ 0.14              | 58.33 $\pm$ 0.26              | 75 $\pm$ 0.21                 | 0 $\pm$ 0.00 |
| Negative control (PBS)                              |                       | 0 $\pm$ 0.00  | 0 $\pm$ 0.00 | 0 $\pm$ 0.00    | 0 $\pm$ 0.00                  | 0 $\pm$ 0.00                  | 0 $\pm$ 0.00                  | 0 $\pm$ 0.00 |

Values labelled with superscript letters (a–c) are significantly different ( $P < 0.05$ ),  $n = 3$

paralysis tests (Parveen 1991), and egg hatch assays (Alawa et al. 2003). Fresh leave and flower bud aqueous extracts of *C. spinosa* inhibited more than 90% of *H. contortus* egg hatching at the concentration of 16 mg/ml ( $IC_{50}$  = 8.54 and 2.76 mg/ml respectively). This is lower than our previously reported figures in *Thymus capitatus* and *Artemisia campestris* samples (100% inhibition at 2 mg/ml concentration) (Elandolsi et al. 2013; Akkari et al. 2014).

In the present study, significant anthelmintic effects of both *C. spinosa* extracts on adult *H. contortus* were observed in terms of paralysis and/or death of the worms at different hours post-treatment and the highest tested concentrations (42 and 100% 6 h PE respectively in fresh leave and flower bud aqueous extracts at 50 mg/ml). Our previous studies revealed that *T. capitatus* and *A. campestris* from the arid region of Tunisia exhibited significant *in vitro* anthelmintic activity at much lower concentrations (2 mg/ml) (Elandolsi et al. 2013; Akkari et al. 2014). Several studies have reported that the effect of plant extracts on adult worms can be observed only at very high concentrations. For example, *Euphorbia helioscopia* methanolic extract induces the highest inhibition of nematode motility (98%) at 50 mg/ml (Bashir et al. 2012); a methanol extract of *Artemisia brevifolia* at a concentration of 25 mg/ml exhibited significant *in vitro* anthelmintic activity on adult *H. contortus*, while the aqueous extract had no significant effect (Iqbal et al. 2004).

The higher anthelmintic activity of flower bud compared to fresh leave aqueous extracts on the eggs and adults of *H. contortus* could be due to the high concentrations of phenolic active compounds, such as flavonoids and tannins, in *C. spinosa* flower buds (Figure 1). In fact, flavonoids and condensed tannins are the chemical constituents likely to be responsible for anthelmintic activity (Hoste et al. 2006; Akkari et al. 2008a; Akkari et al. 2008b; Paria et al. 2012). Moreover, the dose-dependent relationship between the concentration of tannins and/or flavonoid compounds and anthelmintic activity has been repeatedly demonstrated in both *in vitro* (Molan et al. 2003; Hoste et al. 2006) and *in vivo* assays (Hoste et al. 2006; Terrill et al. 2009).

In the studied plant parts, the high content of phenolic compound in fresh leaves and flower bud aqueous extracts is an indication of their strong antioxidant activity in comparison to synthetic antioxidants. Several studies have demonstrated a correlation between antioxidant activity of ca-

per extracts and phenolic content (Aliyazicioglu et al. 2013; Abdul Ameer 2016). In fact, it has been suggested that antioxidant flavonoids might have anthelmintic activity (Ferreira 2011). According to Hasanuzzaman et al. (2013), phenolic phytochemicals are thought to promote optimum health partly via their antioxidant effects thereby protecting cellular components against free radical-induced damage.

In the present study, it was revealed that profiles of both aqueous extracts were dominated by flavonoid derivatives; quercetin, rutin and kaempferol were the most abundant polyphenolic constituents. These flavonoids have a wide range of pharmacological activities including antioxidant, anti-inflammatory, antimicrobial and anti-allergenic activities (Shaik et al. 2006; Calderon-Montana et al. 2011). In addition, Lasisi and Kareem (2011) reported that quercetin present in *Bridelia ferruginea* stem barks has anthelmintic activity. Thus, our results indicate that quercetin, rutin and kaempferol are the probable active molecules of both plant extracts. Nevertheless, the considerable activity of the extracts suggests a possible additive or synergistic effect between these major components and the other plant extract components. This is supported by the finding of Klongsiriwet et al. (2015) who revealed a possible synergistic inhibition of *H. contortus* exsheathment by flavonoid monomers and condensed tannins. Indeed, both extracts are a complex mixture of compounds that can interact with multiple molecular targets in various stages of parasite development.

Crude aqueous extracts of *C. spinosa* showed potential *in vitro* dose-dependent antioxidant and anthelmintic activity against *H. contortus*. The presence of phenolic compounds such as flavonoids and tannins are particularly important for these bioactivities. This plant species can potentially be used to formulate new and more potent natural antioxidant and anti-nematode drugs. Further trials using different doses and concentrations against different parasite species and stages are required to determine the full spectrum of anthelmintic activity of this medicinal plant species in controlling ruminant gastrointestinal nematodes.

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