Acaricidal activity of flavonoids extract of *Borago officinalis* L. (Boraginaceae) against brown dog tick, *Rhipicephalus sanguineus* (Latreille, 1806)

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Abstract. *Borago officinalis* L. (Boraginaceae) is a plant of the Boraginaceae family, used in Algeria for food and medicinal purposes. This study reports the effect of flavonoids extracted from the aerial part of *Borago officinalis* L. (Boraginaceae) on the larvae and engorged adult females of the brown dog tick *Rhipicephalus sanguineus* (Latreille, 1806) using adults immersion test (AIT) and larval immersion test (LIT). For this purpose, the larvae and engorged female of *Rhipicephalus sanguineus* (Latreille, 1806) were exposed to serial dilutions of flavonoids extract (50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml) using “dipping method” in vitro. The plant extract was obtained by fractionation using appropriate solvents. The extraction yield is 22% with a flavonoids concentration equal to 129.12 µg equivalent of quercetin/ml of the extract. The chromatographic analysis by high performance thin layer chromatography (HPTLC) reveals the presence of gallic acid, vanillic acid, kaempferol, dihydroxybenzoic and quercetin. The results obtained show that the flavonoids extract of *Borago officinalis* L. (Boraginaceae) considerably reduces the oviposition and the hatching rate of the eggs of *Rhipicephalus sanguineus* (Latreille, 1806) and was shown to be toxic against newly hatched larvae of *Rhipicephalus sanguineus* (Latreille, 1806) (P < 0.05).

INTRODUCTION

Ticks are currently considered an important vector of human infectious diseases in the world, after mosquitoes (Leulmi et al., 2016). It is responsible for the maintenance and transmission of many pathogens (bacteria, helminths, protozoa and viruses) that affects domestic animals and humans (Bitam & Raoult, 2009; Remedio et al., 2015).

*R. sanguineus* is widely distributed throughout the world, and is considered a potential vector of pathogenic agents such as *Babesia canis*, *Ehrlichia canis* and *Rickettsia conorii* (Dantas-Torres, 2008). In humans, it can transmit the intracellular parasites *Rickettsia rickettsii* and *Rickettsia conorii*, the causative agents of the Mediterranean and spotted fever in Europe and North Africa (Politi et al., 2013).

In Algeria and in North Africa, Mediterranean spotted fever is a real problem in public health, and the competent vector is the dog tick *R. sanguineus* (Bitam et al., 2006). There were more than 500 cases per year with a mortality of 6%; the actual number of cases is more than double according to studies conducted by Mouffok et al. (2009) in Algeria. Many entomological investigations were carried out to describe this disease in depth (Bitam et al., 2006).

Facing this scourge, several methods of control such as physical, biological and chemical were developed. However, the...
The indiscriminate use of the acaricides has caused an emerging problem of tick resistant to acaracides in the world (Rosado-Aguilar et al., 2010).

To ensure better intervention while preserving the natural environment, new preventive methods and new products that are efficient and biodegradable are constantly searched (Saotoing et al., 2014). The use of plant products has been shown by researchers on the importance on its lower risk of side effects (Fouché et al., 2017).

Borage (B. officinalis L.) is an annual herb cultivated for medicinal and culinary uses (Asadi-Samani et al., 2014). The attention to B. officinalis L. has arisen due to their interesting potential as a source of nutrients and bioactive compounds (Benvenuti et al., 2016; Loizzo et al., 2016). The phenolic and flavonoid compounds of these plants were found to be important phytochemicals (Karimi et al., 2015).

In order to find alternatives to the synthetic pesticides and to contribute to a durable management of the environment, it seemed appropriate to evaluate for the first time in Algeria the acaricidal effect of flavonoids extracted from the aerial part of B. officinalis L. against larvae and engorged adult females of R. sanguineus.

MATERIALS AND METHODS

Plant material
The plant B. officinalis L. popularly known as “Elharcha” in local language was collected in spring (April 2017) from Boumerdes area (36° 462 003 north, 3° 282 003 east) situated at 45 km east of Algiers, Algeria. The plant was identified and authenticated by a botanist at Boumerdes University.

Tick
The ticks used in this study are species of R. sanguineus. Fully engorged females were collected from naturally infested dogs during numerous entomological surveys to carry out this study. The ticks were washed with distilled water and dried with an absorbent paper. These females were used for adult immersion test (AIT) and incubated at 30°C, 70-80% relative humidity and a 12/12 h photoperiod in an incubator equipped with a thermo-hygrometer until the eggs are laid. These eggs provided the larvae that were used just after hatching for the larval immersion test (LIT).

Extract preparation
The plants collected was cleaned by washing in running water and dried at room temperature for two weeks. The dried aerial parts (stem, leaf and flowers) were powdered in a plant sample grinder (Philips, France). Powder obtained (100 g) was used for extraction of flavonoids according to the protocol of Bruneton (1999). It is based on the degree of solubility of flavonoids in organic solvents comprising two major steps: The first is a maceration in methanol; the second is a series of liquid-liquid extractions with increasingly polar solvents (petroleum ether, diethyl ether, ethyl acetate and n-butanol). The extract obtained was evaporated to dryness using a rotavapor (Stuart RE300DB, UK).

Dosage of flavonoids
The aluminum trichloride method was used for the determination of flavonoids (Yi et al., 2008). For that, 1 ml of the sample was added to 1 ml of the solution of AlCl₃ at 2%. After 10 minutes of reaction, the absorbance was read at 430 nm by a spectrophotometer (Optizen 2120 UV/Vis, Korea). The concentration of flavonoids was deduced from the calibration curve established using quercetin (0-350 µg/ml). The result was expressed in micrograms equivalent of quercetin/ml of the extract.

High performance thin layer chromatography
Thin layer chromatography (TLC) was performed on TLC sheets coated with layers of silica gel 60 F254 (10 x 20 cm with 0.2 mm of thickness, Germany). After injection of 20 µl of extract and standard solutions in the form of bands using automatic syringe (CAMAG, Switzerland), CAMAG glass twin trough chamber previously saturated with the solvent for 30 minutes were used for
the plate development (Wankhade & Mulani, 2015). The mobile phase consists of acetone, toluene, ethanol and NH3 (45%, 45%, 7% and 3%).

The chromatograms were evaluated in UV light at 200nm on a CAMAG TLC scanner operated by winCATS software version 4.03.

**Adult immersion test**

Groups of 10 *R. sanguineus* engorged females were weighed and immersed for 5 minutes in Petri dishes (5.5 cm diameter, 1.5 cm high) containing 10 ml of the respective dilutions of flavonoids extract from aerial parts of *B. officinalis* L. (6.25, 12.5, 25 and 50 mg/mL). Distilled water was used as the negative control. Ticks were recovered from the solutions, dried on filter paper and incubated at 27–28°C, 70–80% relative humidity. After two weeks, the number of females laying eggs was determined. The eggs were collected, weighed, and then placed in glass tubes, which were incubated under the same conditions before described (Ribeiro et al., 2008). This test was performed in two replicates.

The index of egg laying and of the percentage of egg laying inhibition were calculated as follows (Sabatini et al., 2001):

\[
IE \text{ (index of egg laying)} = \frac{\text{weight of eggs laid (g)}}{\text{weight of females (g)}}
\]

\[
\text{Egg laying inhibition (%) = } \frac{\left[ IE \text{ (control)} - IE \text{ (treated group)} \right] \times 100}{IE \text{ (control)}}
\]

The hatching of eggs was estimated by counting using a stereoscope. The efficiency of the extract was calculated according to the following equations (Drummond et al., 1973).

\[
RE = \frac{\left[ \text{weight of eggs (g)} \times \text{percentage of hatching} \times 20\,000 \right]}{\text{weight of female (g)}}
\]

\[
PE = \frac{\left[ RE \text{ (control group)} - RE \text{ (treated group)} \right] \times 100}{RE \text{ (control group)}}
\]

Where:

RE: Reproductive Efficiency; PE: Product Efficiency

**Larval immersion test**

Larval immersion test was performed in three replicates according to the protocol proposed by Shaw (1966), with modifications. Approximately 0.01 g of eggs (equivalent of 200 embryonated eggs) were placed in bags of TNT fabric (6.0 cm x 6.0 cm). The bags were incubated in a BOD incubator at 27–28°C and 70-80% RH (12/12 h photoperiod) until the eggs started to hatch. After this period of hatching, 200 viable larvae were transferred to new bags and immersed (bags containing the larvae) for 5 minutes in Petri dishes containing 20 ml of the respective dilutions of the flavonoids extract (6.25, 12.5, 25 and 50 mg/mL). Distilled water was used as negative control. The bags were dried using filter paper and incubated at same conditions above for 48 hours. The mortality rate was calculated and corrected according to Abbott’s formula (Abbott, 1925) by counting the number of live and dead larvae.

\[
\% \text{ Mortality (corrected)} = \frac{\left[\% \text{ Mortality (test group)} - \% \text{ Mortality (control group)}\right]}{\left[100 - \% \text{ Mortality (control group)}\right]} \times 100
\]

**Statistical analysis**

Results of acaricidal activity were expressed as mean ± SD. The statistical comparison between the groups was performed with an ANOVA using the statistical presentation system, Statistica version 6. A value of P<0.05 was considered significant. The probit analysis was used to calculate lethal concentrations LC50 and LC99 (concentrations to kill 50% and 99% of larvae respectively) and their respective 95% confidence intervals (CI).

**RESULTS**

The flavonoids extract obtained has a gelatinous appearance with a dark brown coloration. The extraction yield is 22%. The
content of flavonoids obtained is 129.12µg equivalent of quercetin/ml of the extract.

HPTLC results confirmed the presence of gallic acid, vanillic acid, kaempferol, dihydroxybenzoic and the quercetin in the flavonoids extract of aerial parts *B. officinalis* L. (Figure 1).

The percentage of egg laying inhibition, referred to the average of two assays, in flavonoid dilutions from aerial parts of *B. officinalis* L. at concentrations ranging from 6.25 to 50 mg/ml which varied significantly from the negative control (F=8.14377, ddl=3, P=0.035).

Table 1 shows that the percentage inhibition of egg laying increases with increasing concentration. The best result was obtained with the concentration 50 mg/ml. The lowest concentration (6.25 mg/ml) has not an effect on fecundity inhibition when compared with control (Table 1).

**Table 1. Index of egg laying and fecundity inhibition in treated females of *Rhipicephalus sanguineus***

<table>
<thead>
<tr>
<th>Samples (mg/ml)</th>
<th>Weight of the Ticks (g)</th>
<th>Weight of the Eggs (g)</th>
<th>IE²</th>
<th>Egg Laying Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.25</td>
<td>0.1858± (±0.0073)</td>
<td>0.1120± (±0.0072)</td>
<td>0.6028± (±0.0151)</td>
<td>1.5193b (±0.4729)</td>
</tr>
<tr>
<td>12.5</td>
<td>0.1948± (±0.0176)</td>
<td>0.1082± (±0.0062)</td>
<td>0.5554± (±0.0183)</td>
<td>9.2631b (±3.9265)</td>
</tr>
<tr>
<td>25</td>
<td>0.1705± (±0.0138)</td>
<td>0.0896± (±0.0044)</td>
<td>0.5255± (±0.0166)</td>
<td>14.1480c (±1.8295)</td>
</tr>
<tr>
<td>50</td>
<td>0.1818± (±0.0146)</td>
<td>0.0901± (±0.0017)</td>
<td>0.4956± (±0.0497)</td>
<td>19.0328c (±7.2766)</td>
</tr>
<tr>
<td>Distilled water</td>
<td>0.1892± (±0.0057)</td>
<td>0.1158± (±0.0047)</td>
<td>0.6121± (±0.0062)</td>
<td>0.0000± (±0.0000)</td>
</tr>
</tbody>
</table>

IE² index of egg laying, values with the same superscript letters within a column do not show statistically significant differences by Sheffé, Tukey-Kramer and Neuman-Keuls tests (P< 0.05). The results represent the mean of two assays.

Figure 1. Chromatogram analysis by HPTLC of flavonoids extract of the aerial parts from *B. officinalis* L. at wavelength 200 nm with acetone, toluene, ethanol, NH₃ (45%, 45%, 7%, 3%).
A: kaempferol; B: quercetin; C: gallic acid; D: dihydroxybenzoic; E: vanillic acid
The percentage of hatching showed values below the control ($F=66.78$, $ddl=4$, $P<0.001$) from the concentration 12.5 mg/ml to 50 mg/ml, but the concentration 6.25 mg/ml did not show a statistically significant difference from the control (Table 2).

Table 2 showed the results for the mean of two tests for the calculation of reproductive efficiency (RE) and product efficacy (PE) for the dilutions of the flavonoids extract of the aerial parts of *B. officinalis* L. The greater effectiveness of the extract was obtained at a concentration of 50 mg/ml (PE=43.12%) and lowest effectiveness (PE=2.74%) at concentration of 6.25 mg/ml ($F=21.5871$, $ddl=3$, $P=0.006$).

In the larval immersion test, the flavonoids extract of *B. officinalis* L proved toxic killing almost 100% of *R. sanguineus* larvae at a concentration of 50 mg/ml after 48 h of exposure ($F=71.168$, $ddl=4$, $P<0.001$). At the lowest concentration (6.25 mg/ml), the extract showed efficiency above 45%. The larval LC50 and LC99 values calculated from the probit analysis are respectively 7.58 mg/ml (IC: 7.48 – 8.08) and 48.60 mg/ml (IC: 45.46 – 48.84).

**DISCUSSION**

Plants are a rich source of bioactive compounds such as phenolic compounds, terpenoids, coumarins and alkaloids (Lee *et al.*, 2010). The use of these natural products in the control of brown dog tick *R. Sanguineus* has been the focus of research in many countries, especially with the increased resistance that these organisms present to commercial acaricides (Miller *et al.*, 2001). This resistance is due to the indiscriminate use of chemical substances that can provoke intoxication in animals and their handlers, and possible contamination of the environment with chemical wastes (Delmonte *et al.*, 2017).

In this study, the yield of extraction is 22% with a concentration of 129.12 µg equivalent of quercetin/ml of the extract. This result is comparable to that reported by Affi Chaouache *et al.* (2014), who obtained a yield of 37.6% working on the same plant harvested in Tiziouzou (Algeria). This variation may be the result of the influence of abiotic factors on the synthesis of these secondary metabolites. Indeed, environmental factors (temperature, humidity, light intensity, supply of water, minerals and CO2) influence the growth of a plant and secondary metabolite production (Akula & Ravishankar, 2011).

The chromatographic characterization by HPTLC reveals the presence of several flavonoic compounds such as gallic acid, vanillic acid, kaempferol, dihydroxybenzoic and quercetin. Affi Chaouache *et al.* (2014), using the HPLC technique indicate that this plant share the presence of gallic acid and quercetin. The flower's methanolic extract of the herbal plant *B. officinalis* L. harvested in spring (April) from Iran were analysed for its bioactive compounds using RP-HPLC analyses and confirmed the presence of gallic acid, pyrogallol, salicylic acid, caffeic acid, myricetin, rutin and daidzein (Karimi *et al.*, 2017). Additionally, the extract obtained from the fresh flowers of *B. officinalis* L. collected...
in Iran were examined by GC and GC-MS. Forty three compounds were identified. The main compound determined in flower extract was acetic acid, heptanoic acid, propanoic acid with a predominance of the carboxylic acid class (Saadatian et al., 2017). Some of the constituents of the flavonoid extract described in this work have a range of biological activities already described in the literature; for example, the gallic acid and quercetin identified in flavonoid extract from *B. officinalis* L., which show antibacterial and antioxidant activities (Affi Chaouache et al., 2014).

Recently, only a few works evaluated the *in vitro* efficacy of plant extracts against *R. sanguineus* but several recent works evaluated the efficacy against other species of *Rhipicephalus* (da Silva et al., 2016). In this study, adult immersion test for *R. sanguineus* showed very promising results (P<0.05). The most effective concentration tested was 50 mg/ml, showing 19.03% of egg laying inhibition and 63.42% of hatchability. The concentrations 25, 12.5 and 6.25 mg/ml reduced the percentage of egg laying to 14.14, 9.26, 1.51%, and the percentage of hatchability at 74.72, 83.64, and 91.50% respectively. There was a direct relationship between the concentration of the samples tested and the result of egg laying inhibition and the percentage of hatchability.

This result is consistent with those found by Politi et al. (2012), by working on the acaricidal activity of the ethanolic extract of *Tagetes patula* L. (Asteraceae) against *R. sanguineus* revealing a percentage of egg laying inhibition of 21.5% at the concentration 50 mg/ml. These authors (Politi et al., 2015), found that the egg hatchability of *R. sanguineus* using the 70% ethanolic extract of the aerial parts of *T. patula* was 31.5% at a concentration of 12.5 mg/ml and 3.02% at 100 mg/ml. da Silva et al. (2016), obtained 100% efficacy of *Tagetes minuta* (Asteraceae) essential oil at 20% against larvae, nymphs and adults of the tick *R. sanguineus*.

Conversely, to these studies, Estrela et al. (2017), showed that the leaves ethanolic extract of *Hyptis suaveolens* did not present acaricidal activity for females of *R. sanguineus* at concentrations of 0.1, 1 and 10%.

Ravindran et al. (2011), reported the blocking effect of the hatching of ethanol extract of the *Leucas aspera* against the ticks eggs of *R. (Boophilus) annulatus*. Divya et al. (2014), revealed a statistically significant acaricidal effect (P<0.05) of *Leucas indica* alkaloids at concentrations of 25 mg/ml and 50 mg/ml against *R. (Boophilus) annulatus*. In another study, Ribeiro et al. (2008), using the hexane extract of *Calea serrata*, found values of percentage of egg laying inhibition of *R. (Bophilus) microplus* between 11.7% and 14.6% (Table 3).

The larval immersion tests showed statistically significant results (P<0.001). The flavonoids extract of *B. officinalis* L. at 50 mg/ml had mortality rates greater than 99% (99.54%). At the lowest concentration (6.25 mg/ml), the extract showed mortality rates of 45.04%. The larval LC50 and LC99 values obtained in this study were respectively 7.58 mg/ml and 48.60 mg/ml, thus showing a powerful acaricidal effect of the flavonoid extract of *B. officinalis* L.

### Table 3. Larval mortality rate of *Rhipicephalus sanguineus* front dilutions of flavonoid extract of *Borago officinalis* L.

<table>
<thead>
<tr>
<th>Samples (mg/ml)</th>
<th>Number of living larvae</th>
<th>Number of death larvae (%)</th>
<th>Mortality¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.25</td>
<td>109.67ᵇ (±20.45)</td>
<td>90.33ᵇ (±20.45)</td>
<td>45.04ᵇ (±10.24)</td>
</tr>
<tr>
<td>12.5</td>
<td>54.33ᶜ (±8.95)</td>
<td>145.67ᶜ (±8.85)</td>
<td>72.71ᶜ (±4.47)</td>
</tr>
<tr>
<td>25</td>
<td>24.00⁰ᵈ (±5.61)</td>
<td>176.00⁰ᵈ (±5.61)</td>
<td>87.88⁰ᵈ (±2.80)</td>
</tr>
<tr>
<td>50</td>
<td>0.67ᵈ (±0.41)</td>
<td>199.33ᵈ (±0.41)</td>
<td>99.54ᵈ (±0.22)</td>
</tr>
<tr>
<td>Distilled water</td>
<td>178.33ᵃ (±2.48)</td>
<td>21.67ᵃ (±2.48)</td>
<td>10.83ᵃ (±1.24)</td>
</tr>
</tbody>
</table>

¹ The mortality rate was calculated using Abbott’s formula, due to the mortality rate in the control group was close to 10.0%. Values with the same superscript letters within a column do not show statistically significant differences by Sheffé, Tukey-Kramer and Neuman-Keuls tests (P<0.05). The values are the mean of three assays.
extract of aerial parts of *B. officinalis* L. against larvae of *R. sanguineus*. The acaricidal activity of the essential oil of *Lipia sidoides* against unengorged larvae of *R. sanguineus* at concentrations of 2.35-18.80 mg/ml, resulting in 20.6-99% larval mortality. The LC90 value was 11.56 mg/ml (Gomes et al., 2014). Politti et al. (2012), obtained a mortality rate of 99.78% at the concentration of 50 mg/ml using the 70% ethanoic extract of aerial parts of *T. patula* with 7.43 mg/ml of LC50 and 108.25 mg/ml of LC99. Similar results was reported by Ribeiro et al. (2007), however, using a methanolic extract of *Hypericum polyanthemum* at 50, 25, 12.5 and 6.25 mg/ml, mortality rates of 100%, 96.7%, 84.7% and 52.7% were obtained respectively.

The flavonoic extract of *B. officinalis* L. showed toxicity against the tick *R. sanguineus*. At a concentration of 50 mg/ml, the extract significantly reduced the egg laying (18.12%) and eliminated more than 99% of the larvae. This toxicity is related to flavonoic compounds, including gallic acid, vanillic acid, kaempferol, dihydroxybenzoic acid and quercetin. This extract may present an alternative to control *R. sanguineus* by reducing the development of the immature stages of this tick’s life cycle.

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REFERENCES


