Tropical Biomedicine 35(2): 383–391 (2018)

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

El haddad, D.[1*], Bitam, I.[2], Bouchenak, O.[1], Toubal, S.[1], Yahiaoui, K.[1], Arab, K.[1] and Boumaza, S.[1]
[1]Laboratory of Valorization and Conservation of Biological Resources, Faculty of Sciences, University of M'Hamed Bougara Boumerdes, 35 000 Boumerdes, Algeria
[2]Laboratory Biodiversity and Environment: Interactions, Genomes, Faculty of Biological Sciences, University of Sciences and Technology Houari Boumediene, 16 000 Algiers, Algeria
[2]Higher School of Food Sciences and Agroalimentary Industries 16 000 Algiers, Algeria
[*]Corresponding author e-mail: elhaddad-dj@hotmail.com
Received 6 September 2017; received in revised form 12 January 2018; accepted 14 January 2018

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 officunalis* 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

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 $AlCl_3$ 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):

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

$$Egg\ laying\ inhibition\ (\%) = \frac{[IE\ (control) - IE\ (treated\ group)]\ x\ 100}{IE\ (control)}$$

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).

$$RE = \frac{[weight\ of\ eggs\ (g)\ x\ percentage\ of\ haching\ x\ 20\ 000]}{weight\ of\ female\ (g)}$$

$$PE = \frac{[RE\ (control\ group) - RE\ (treated\ group)]\ x\ 100}{RE\ (control\ group)}$$

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.

$$\%\ Mortality\ (corrected) = \frac{[\%\ Mortality\ (test\ group) - \%\ Mortality\ (control\ group)]}{[100 - \%\ Mortality\ (control\ group)]\ x\ 100}$$

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).

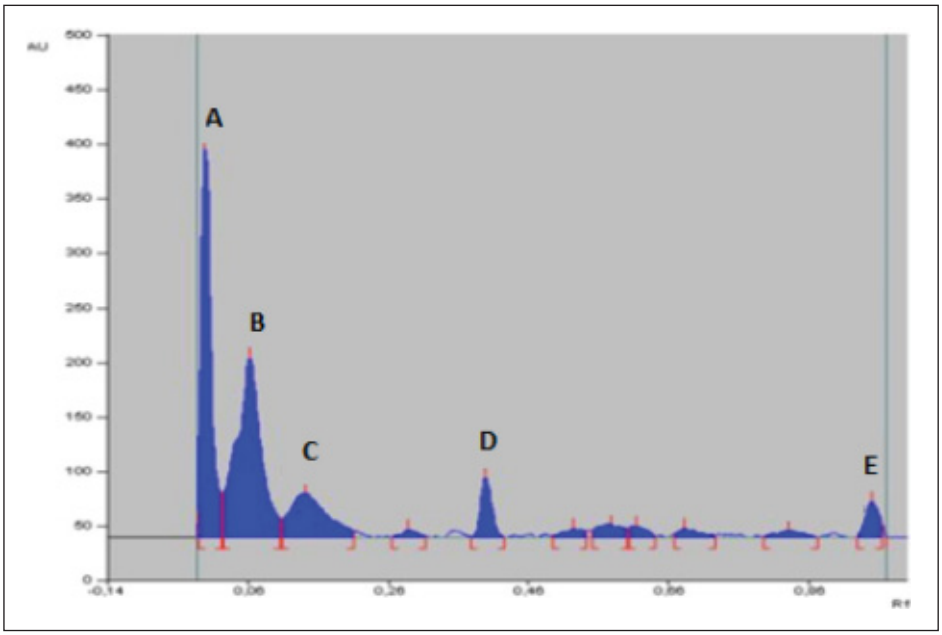

Figure 1. Chromatogram analysis by HPTLC of flavonoids extract of the areal parts from *B. officinalis* L. at wavelength 200 nm with acetone, toluene, ethanol, NH3 (45%, 45%, 7%, 3%).
A: kaempferol; B: quercetin; C: gallic acid; D: dihydroxybenzoic; E: vanillic acid

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

Samples (mg/ml)	Weight of the Ticks (g)	Weight of the Eggs (g)	IE[1]	Egg Laying Inhibition (%)
6.25	0.1858[a] (±0.0073)	0.1120[a] (±0.0072)	0.6028[a] (±0.0151)	1.5193[b] (±0.4729)
12.5	0.1948[a] (±0.0176)	0.1082[a] (±0.0062)	0.5554[a] (±0.0183)	9.2631[b] (±3.9265)
25	0.1705[a] (±0.0138)	0.0896[a] (±0.0044)	0.5255[a] (±0.0166)	14.1480[c] (±1.8295)
50	0.1818[a] (±0.0146)	0.0901[a] (±0.0017)	0.4956[a] (±0.0497)	19.0328[c] (±7.2766)
Distilled water	0.1892[a] (±0.0057)	0.1158[a] (±0.0047)	0.6121[a] (±0.0062)	0.0000[a] (±0.0000)

IE[1]: 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.

Table 2. Reproductive efficiency and product efficiency of flavonoids extract of *Borago officinalis* L.

Samples (mg/ml)	W^1_{eggs} (g)	H^2_{eggs} (%)	RE[3]	PE[4] (%)
6.25	0.1120^{a} (±0.0072)	91.50^{a} (±0.73)	$1\ 101\ 105.37^{a}$ (±18 889.64)	2.74^{a} (±2.81)
12.5	0.1082^{a} (±0.0062)	83.64^{ab} (±0.42)	$932\ 876.97^{ab}$ (±26 008.19)	17.58^{ab} (±3.27)
25	0.0896^{a} (±0.0044)	74.72^{b} (±2.03)	$786\ 938.90^{bc}$ (±3 369.46)	30.52^{bc} (±0.52)
50	0.0901^{a} (±0.0017)	63.42^{c} (±1.61)	$645\ 098.36^{c}$ (±55 868.79)	43.12^{c} (±4.26)
Distilled water	0.1158^{a} (±0.0047)	92.73^{a} (±0.29)	$1\ 132\ 712.6^{a}$ (±13 361.05)	–

[1] W_{eggs} : total weight of eggs laid; [2] H_{eggs}: percentage of hatching of eggs laid; [3] RE : reproductive efficiency; [4] PE : efficiency of the product (percentage). 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.

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 Afif Chaouach *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 CO_2) 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 flavonoic 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 flavonoic extract from *B. officinalis* L., which show antibacterial and antioxydant 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

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

Samples (mg/ml)	Number of living larvae	Number of death larvae	(%) Mortality[1]
6.25	109.67^{b} (±20.45)	90.33^{b} (±20.45)	45.04^{b} (±10.24)
12.5	54.33^{c} (±8.95)	145.67^{c} (±8.85)	72.71^{c} (±4.47)
25	24.00^{cd} (±5.61)	176.00^{cd} (±5.61)	87.88^{cd} (±2.80)
50	0.67^{d} (±0.41)	199.33^{d} (±0.41)	99.54^{d} (±0.22)
Distilled water	178.33^{a} (±2.48)	21.67^{a} (±2.48)	10.83^{a} (±1.24)

[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 *R. sanguineus* larvae 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, dihydrohybenzoic 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.

Acknowledgment. We thank Mr Toubal Kader and Mr Djemaa Mohamed who helped us in collecting the plant and the ticks.

REFERENCES

Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology* **18**(2): 265-267.

Afif Chaouach, T., Arab, K. & Bendahou, M. (2014). Phytochemical screening of Algerian *Borago officinalis* L. and evaluation of its antioxidant and antimicrobial activities against respiratory pathogens. *International Journal of Phytomedicine* **6**(3): 369-376.

Asadi-Samani, M., Bahmani, M. & Rafieian-Kopaei, M. (2014). The chemical composition, botanical characteristic and biological activities of *Borago officinalis*: A review. *Asian Pacific Journal of Tropical Medicine* **7**: S22-S28.

Benvenuti, S., Bortolotti, E. & Maggini, R. (2016). Antioxidant power, anthocyanin content and organoleptic performance of edible flowers. *Scientia Horticulturae* **199**: 170-177.

Bitam, I., Parola, P., Matsumoto, K., Rolain, J.M., Baziz, B., Boubidi, S.C., Harrat, Z., Belkaid, M. & Raoult, D. (2006). First molecular detection of *R. conorii*, *R. aeschlimannii*, and *R. massiliae* in ticks from Algeria. *Annals of the New York Academy of Sciences* **1078**(1): 368-372.

Bitam, I. & Raoult, D. (2009). Other tick-borne diseases in Europe. In : *Lyme Borreliosis*. *Karger Publishers* **37**: 130-154.

Bruneton, J. (1999). Pharmacognosie et phytochimie des plantes médicinales. 3rd Edition. Paris : Tec & Doc, p575.

Dantas-Torres, F. (2008). The brown dog tick, *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae): from taxonomy to control. *Veterinary Parasitology* **152**(3): 173-185.

da Silva, E.M.G., da Silva Rodrigues, V., de Oliveira Jorge, J., Osava, C.F., Szabó, M.P.J., Garcia, M.V. & Andreotti, R. (2016). Efficacy of *Tagetes minuta* (Asteraceae) essential oil against *Rhipicephalus sanguineus* (Acari: Ixodidae) on infested dogs and *in vitro*. *Experimental and Applied Acarology* **70**(4): 483-489.

Delmonte, C., Cruz, P.B., Zeringóta, V., de Mello, V., Ferreira, F., Amaral, M.D.P.H. & Daemon, E. (2017). Evaluation of the acaricidal activity of thymol incorporated in two formulations for topical use against immature stages of *Rhipicephalus sanguineus* sensu lato (Latreille, 1806) (Acari: Ixodidae). *Parasitology Research* **116**(11): 2957-2964.

Divya, T.M., Soorya, V.C., Amithamol, K.K., Juliet, S., Ravindran, R., Nair, S.N. & Ajithkumar, K.G. (2014). Acaricidal activity of alkaloid fractions of *Leucas indica* Spreng against *Rhipicephalus (Boophilus) annulatus* tick. *Tropical Biomedicine* **31**(1): 46-53.

Drummond, R.O., Ernst, S.E., Trevino, J.L., Gladney, W.J. & Graham, O.H. (1973). *Boophilus annulatus* and *B. microplus*: Laboratory Tests of Insecticides. *Journal of Economic Entomology* **66**(1): 130-133.

Estrela, D.D.S., de Oliveira, R.P., de Matos, J.G., Cardoso, D.P., de Sousa, P.F.P., de Souza, J.S.N., da Silva, P.O., de Oliveira, J.M.G. & Farias, M. P. O. (2017). *In vitro* evaluation of the acaricidal effect of ethanolic extract of *Hyptis suaveolens* (L.) Poit (Lamiaceae) leaves on engorged females of *Rhipicephalus sanguineus* (Latraille, 1806) (Acari: Ixodidae). *Pubvet* **11**(9): 854-859.

Fouché, G., Eloff, J.N. & Wellington, K. (2017). Evaluation of South African Plants with acaricide activity against ticks. *World Academy of Science, Engineering and Technology, International Journal of Medical, Health, Biomedical, Bioengineering and Pharmaceutical Engineering* **11**(6): 381-385.

Gomes, G.A., Monteiro, C.M.O., de Santana Julião, L., Maturano, R., Senra, T.O.S., Zeringóta, V., Calmon, F., da Silva Matos, R., Daemon, E. & de Carvalho, M.G. (2014). Acaricidal activity of essential oil from *Lipia sidoides* on unengorged larvae and nymph of *Rhipicephalus sanguineus* (Acari: ixodidae) and *Amblyomma cajennense* (Acari: ixodidae). *Experimental Parasitology* **137**: 41-45.

Karimi, E., Jaafar, H., Ghasemzadeh, A. & Ebrahimi, M. (2015). Fatty acid composition, antioxidant and antibacterial properties of the microwave aqueous extract of three varieties of *Labisia pumila* Benth. *Biological Research* **48**(9): 1-6.

Karimi, E., Oskoueian, E., Karimi, A., Noura, R. & Ebrahimi, M. (2017). *Borago officinalis* L. flower: a comprehensive study on bioactive compounds and its health-promoting properties. *Journal of Food Measurement and Characterization*, 1-13.

Lee, C.H., Lee, S.G. & Lee, H.S. (2010). Acaricidal effects of *Thymus vulgaris* leaf-derived materials and monoterpene alcohols against *Dermatophagoides* spp. *Journal of the Korean Society for Applied Biological Chemistry* **53**(2): 170-174.

Leulmi, H., Aouadi, A., Bitam, I., Bessas, A., Benakhla, A., Raoult, D. & Parola, P. (2016). Detection of *Bartonella tamiae*, *Coxiella burnetii* and rickettsiae in arthropods and tissues from wild and domestic animals in northeastern Algeria. *Parasites & Vectors* **9**(1): 27.

Loizzo, M.R., Pugliese, A., Bonesi, M., Tenula, M.C., Menichini, F., Xiao, G. & Tundis, R. (2016). Edible flowers: a rich source of phytochemicals with antioxidant and hypoglycemic properties. *Journal of Agricultural and Food Chemistry* **64**(12): 2467- 2474.

Miller, R.J., George, J.E., Guerrero, F., Carpenter, L. & Welch, J.B. (2001). Characterization of acaricide resistance in *Rhipicephalus sanguineus* (Latreille) (Acari: Ixodidae) collected from the Corozal Army Veterinary Quarantine Center. Panama. *Journal of Medical Entomology* **38**(2): 298-302.

Mouffok, N., Parola, P., Lepidi, H. & Raoult, D. (2009). Mediterranean spotted fever in Algeria – new trends. *International Journal of Infectious Diseases* **13**(2): 227-235.

Politi, F.A.S., de Souza-Moreira, T.M., Rodrigues, E.R., de Queiroz, G.M., Figueira, G.M., Januário, A.H., Berenger, J.M., Socolovschi, C., Parola, P. & Pietro, R.C.L.R. (2013). Chemical characterization and acaricide potential of essential oil from aerial parts of *Tagetes patula* L. (Asteraceae) against engorged adult females of *Rhipicephalus sanguineus* (Latreille, 1806). *Parasitology Research* **112**(6): 2261-2268.

Politi, F.A.S., Figueira, G.M., Araújo, A.M., Sampieri, B.R., Mathias, M.I.C., Szabó, M.P.J., Bechara, G.H., dos Santos, L.C., Vilegas, W. & Pietro, R.C.L.R. (2012). Acaricidal activity of ethanolic extract from aerial parts of *Tagetes patula* L. (Asteraceae) against larvae and engorged adult females of *Rhipicephalus sanguineus* (Latreille, 1806). *Parasites & Vectors* **5**(1): 295.

Politi, F.A.S., Figueira, G.M., Camargo-Mathias, M.I., Berenger, J.M., Parola, P. & Pietro, R.C.L.R. (2015). Action of ethanolic extract from aerial parts of *Tagetes patula* L. (Asteraceae) on hatchability and embryogenesis of *Rhipicephalus sanguineus* eggs (Acari: Ixodidae). *Industrial Crops and Products* **67**: 55-61.

Akula, R. & Ravishankar, G.A. (2011). Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signaling & Behavior* **6**(11): 1720-1731.

Ravindran, R., Juliet, S., Sunil, A.R., Ajith Kumar, K.G., Nair, S.N., Amithamol, K.K., Shynu, M., Rawat, A.K.S. & Ghosh, S. (2011). Eclosion blocking effect of extract of *Leucas aspera* (Lamiiaceae) on *Rhipicephalus (Boophilus) annulatus*. *Veterinary Parasitology* **179**(1): 287-290.

Remedio, R.N., Nunes, P.H., Anholeto, L.A., Oliveira, P.R. & Camargo-Mathias, M.I. (2015). Morphological effects of neem (*Azadirachta indica* A. Juss) seed oil with known azadirachtin concentrations on the oocytes of semi-engorged *Rhipicephalus sanguineus* ticks (Acari: Ixodidae). *Parasitology Research* **114**(2): 431-444.

Ribeiro, V.L.S., Avancini, C., Gonçalves, K., Toigo, E. & von Poser, G. (2008). Acaricidal activity of *Calea serrata* (Asteraceae) on *Boophilus microplus* and *Rhipicephalus sanguineus*. *Veterinary Parasitology* **151**(2): 351-354.

Ribeiro, V.L.S., Toigo, E., Bordignon, S.A., Gonçalves, K. & Von Poser, G. (2007). Acaricidal properties of extracts from the aerial parts of *Hypericum polyanthemum* on the cattle tick *Boophilus microplus*. *Veterinary Parasitology* **147**(1): 199-203.

Rosado-Aguilar, J.A., Aguilar-Caballero, A., Rodriguez-Vivas, R.I., Borges- Argaez, R., Garcia-Vazquez, Z. & Mendez-Gonzalez, M. (2010). Acaricidal activity of extracts from *Petiveria alliacea* (Phytolaccaceae) against the cattle tick, *Rhipicephalus (Boophilus) microplus*. *Veterinary Parasitology* **168**(3): 299-303.

Saadatian, M., Aghaei, M., Farahpour, M. & Aghaei, M. (2017). Chemical Compositions of Flowers extract of Borage (*Borago officinalis* L.) in wild population from Urmia district, Iran. *Journal of Essential Oil Bearing Plants* **20**(1): 289-292.

Sabatini, G.A., Kemp, D.H., Hughes, S., Nari, A. & Hansen, J. (2001). Tests to determine LC 50 and discriminating doses for macrocyclic lactones against the cattle tick, *Boophilus microplus*. *Veterinary Parasitology* **95**(1): 53-62.

Saotoing, P., Tchuenguem, F.F., Dawe, A. & Ngatarang, C. (2014). Évaluation de l'effet insecticide de l'extrait acétonique des feuilles séchées de *Calotropis procera* Ait. (Asclepiadaceae) chez les adultes de *Anopheles gambiae*, Maroua (Cameroun). *International Journal of Biological and Chemical Sciences* **8**(4): 1438-1444.

Shaw, R.D. (1966). Culture of an organophosphorus-resistant strain of *Boophilus microplus* (Can.) and an assessment of its resistance spectrum. *Bulletin of Entomological Research* **56**(3): 389-405.

Wankhade, M.S. & Mulani, R.M. (2015). Preliminary phytochemical screening and chromatographic fingerprint analysis of acacia leucopholea roxb. methanolic leaf and bark extract by hptlc technique. *Indo American Journal of Pharmaceutical Research* **4**(1): 184-191.

Yi, Z., Yu, Y., Liang, Y. & Zeng, B. (2008). *In vitro* antioxidant and antimicrobial activities of the extract of *Pericarpium Citri* Reticulatae of a new Citrus cultivar and its main flavonoids. *LWT-Food Science and Technology* **41**(4): 597-603.