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RESEARCH

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Larvicidal activity of the black pepper, *Piper nigrum* (Fam: Piperaceae) extracts on the cattle tick, *Rhipicephalus australis* (Acari: Ixodidae)

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Abstract

Background The cattle farming parasite *Rhipicephalus australis* is the main tick and one of the most important in the world from an economic point of view. Various studies have been developed in order to find plant extracts with effective acaricidal properties and environmentally friendly. Studies involving plant extracts for parasite control on commercial animal herds is a developing area in New Caledonia. Bioactive natural products play an important role as lead compounds in the development of new pesticides.

Results The ethanolic extract of *Piper nigrum* L. dried fruits as well as the ethyl acetate extract and the methanolic extract of stems exhibited 100% larvicidal activity (50 mg/mL) against *Rh. australis* larvae, the cattle tick, an hematophagous parasite. Bioguided fractionation of the ethanolic extract of dried mature fruits using the same assay led to the isolation of five compounds belonging to piperamide family. The structures of isolated compounds were elucidated using spectroscopic methods: ESI-HRMS, ^{1}H- and ^{13}C-NMR spectral data, including DEPT and 2D-NMR experiments (COSY, HSQC, HMBC, and NOESY). These include 1 compound described for the first time in *P. nigrum*, homopellitorine (2) and 4 known compounds, namely pellitorine (1), pipyaqubine (3), 2-methylpropylamide (4), and N-isobutyl-2,4-eicosadienamide (5).

Conclusion This first report on the larvicidal activity of *P. nigrum* extract and pure compounds on this tick species suggests that *P. nigrum* could be a natural biosourced alternative for the control of the larval stage of *Rh. australis*.

Keywords Piperaceae, *Piper nigrum* L., Plant extracts, *Rhipicephalus australis*, Cattle tick, Piperamides, Amide alkaloids, Botanical acaricide

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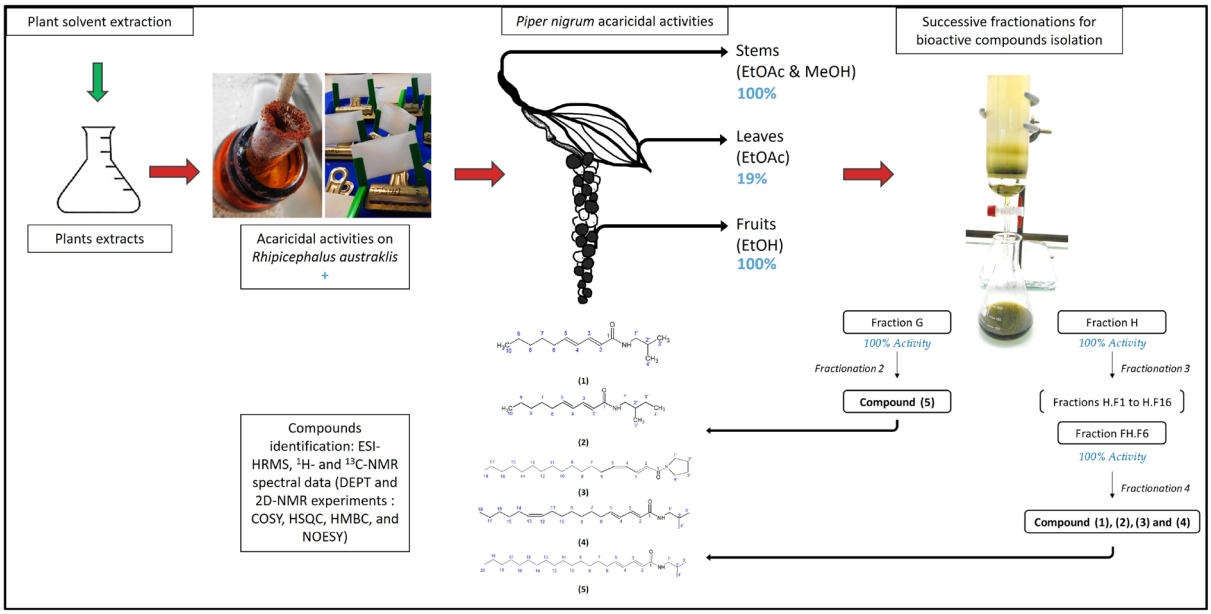
Graphical Abstract



Introduction

The cattle tick, *Rhipicephalus australis* (Canestrini 1887) was introduced in New Caledonia in 1942 and is the main health problem in cattle farming. Locally, *Rh. australis* was synonymized with the southern cattle tick, *Rhipicephalus microplus*, until *Rh. australis* was reinstated as a separate cattle tick species in 2012 [15]. This tick is the main species and one of the most important in the world from an economic point of view. It has been estimated that 80% of the world's cattle population is at risk from tick and tick-borne diseases (TBDs) causing estimated annual losses of US$ 22–30 billion [37]. The principal control method involves the use of synthetic acaricides by dip, spray, injection, or pour-on treatments [43, 48]. The continuous use of these chemical compounds has led to the selection and development of strains of *Rh. australis* resistant to organophosphates, pyrethroids and formamidine, a phenomenon that is a major concern for worldwide cattle breeders [14, 29, 43]. Moreover, such chemical control causes meat and milk contamination that can also have undesirable effects on other organisms and the environment [14, 17, 18, 27, 44].

The need of new scientific investigations for alternative ways to control this tick is related to the evolution of resistance of *Rh. australis* to synthetic acaricides. As a matter of fact, various studies have been performed in order to find plant extracts with acaricidal properties [3, 6, 7, 10, 14, 22, 26, 28, 32, 33, 40, 44, 65] to discover natural compounds at least as effective as classic treatments but also environmentally friendly and susceptible to be produced on a large, commercial scale [30].

In a first study, Borges et al. [6] inventoried 55 plants belonging to 26 families tested against *Rh. australis* [6]. In a second review in 2016, Benelli et al. describe the results of 62 extracts on this parasite excluding essential oils [3]. In 2020, Quadros et al. listed 27 plants-derived substances with potential for tick control and prevention on *Rh. australis* [44].

In New Caledonia, several works have been devoted to the acaricidal activity of natural substances on *Rh. australis* larvae, but mainly concerned essential oils. Lebouvier et al. [32, 33], showed that essential oils from endemic trees of New Caledonia could provide natural acaricides for the control of the cattle tick *Rh. australis*. Nevertheless, the development of an alternative tick control strategy must be associated with a high safety profile as well as availability and remanence. Indeed, the potential toxicity of essential oils, their low extraction yield and their volatile nature reduce their valorization and application potential despite the many biological activities they may present [32, 35]. Therefore, it seems that organic solvent extracts from plants have many positive aspects for valorization in the control of cattle ticks and the Piperaceae family is a very interesting example.

Piperaceae family is represented by at least 3000 species and is known to have acaricidal compounds, such as

monoterpenes, sesquiterpenes, alkaloids, and phenylpropanoids [4, 11, 12, 24, 38, 39, 42, 45, 49, 52, 66]. The genus *Piper* is very large, and several species of *Piper* have been used as spice and in traditional medicine and bear an immense commercial, economical, and medicinal importance. Some *Piper* species have simple chemical profiles, while others, such as *Piper nigrum* contain diverse suites of secondary bioactive metabolites [49]. *Piper nigrum* L., commonly known as black pepper, is a climber originally native to India. The acrid and pungent taste of *P. nigrum* fruits attracted the attention of chemists as early as 1819 when Oestred isolated piperine. Since that time, the search for active constituents from different *Piper* species is being continued and this has been intensified in the recent years, particularly because of interesting biological activities of various chemicals from several *Piper* species [12, 20, 34, 49, 52, 56–60, 66]. Indeed, some *Piper* species are listed as remedies for stomach pain, asthma, bronchitis, fever, abdominal pain, hemorrhoidal afflictions, rheumatism, as anti-inflammatory and stimulant agents, but also as insect repellents, insecticidal, acaricidal, antifungal, and antibiotic [16, 23, 28, 34, 47, 52, 56–58, 67]. The chemistry of *Piper* species has been widely investigated, and phytochemical investigations from all parts of the world have led to the isolation of several physiologically active compounds, including alkaloids, amides, propenylphenols, lignans, neolignans, terpenes, steroids, pyrones, piperolides, chalcones, flavones, and flavanones [8, 12, 23, 31, 34, 45, 50, 54–60, 61–63, 66].

Several Piperaceae have also been studied against cattle ticks [7, 10, 20, 28] and their major characteristic and active constituents could be attributed to a considerable variety of amide alkaloids [2, 51, 52, 66] and to a possible synergistic action [5, 11, 40, 41, 50, 52]. Therefore, an investigation on either major or minor compounds seems to be of particular interest.

In this context, we evaluated the larvicidal activity of the black pepper, *Piper nigrum* (Fam: Piperaceae) extracts on the cattle tick, *Rhipicephalus australis* (Acari: Ixodidae).

Materials and methods

Plant material and extraction procedure

Fruits and aerial parts (leaves and stems) of *Piper nigrum* were collected at the IAC SRA (Agronomic Research Station of Institut Agronomique néo-Calédonien) of Pocquereux (La Foa, New Caledonia, 21°44′10,338" S 165°53′44,296" E). The IAC SRA extends over 90 hectares and hosts numerous experimental orchards devoted to tropical fruit crops as well as many plants of food interest and this is where the pepper plants are grown.

Dried fruits, dried leaves, and dried stems were grounded into powder (Grinder Moulin IKA® M20 250 mL) and extracted (30 g of dry matter in 400 mL of solvent macerated at room temperature for 48 h then put in ultrasound for 20 min before filtration). Three different solvents were used for the extraction viz. ethanol 95%, ethyl acetate, and methanol (solvents from Univar Solutions, Manchester, USA) and filtered under vacuum using a Buchner funnel (Buchner funnel JIPO). The filtrates were concentrated under reduced pressure at 40 °C. The dried crude extracts were stored at 4 °C in the IAC extracts collection.

Biological test

Preparation of tick

A *Rh. australis* breeding was ongoing in IAC on calves. The strain was sensible to deltamethrin and resistant to amitraz. In the final stage of engorgement, female ticks were collected and used in the biological test within 24 h. Ticks were rinsed by water and dried using filter papers. The female ticks were incubated at 27 °C and 85% relative humidity for 1 week. Eggs were then collected and placed in the same conditions until larvae were 2 weeks old.

Larval packet test

The FAO modified LPT method (Larval Packet Test; Stone 1962 [64]) was used to assess the acaricidal effect of samples on 14 days old larvae. Nylon papers (Anowo LTD) were impregnated with 50 mg of extracts (1 mL at 50 mg/mL per paper) and placed during one hour in a fume hood to allow the solvent (EtOH 95%) to evaporate before being folded into packets using bulldog clips. Approximately 100 *Rh. australis* larvae were placed into each treated nylon paper packet, which was then sealed with additional bulldog clips and placed in an incubator (27 °C; 85% RH) for 24 h. Two replicates and a control (nylon paper with solvent) for each sample were used. After exposure to the sample, the numbers of live and dead larvae were counted to calculate the percentage of larval mortality. To determine the LC_{50}, six successive dilutions were tested, with the initial concentration depending on mass of extract available.

Statistical analysis

For each extract, the lethal concentration 50% (LC_{50}), 90% (LC_{90}), 99% (LC_{99}), slope, chi-square, heterogeneity and the 95% confidence intervals (CI95%) were calculated according to Probit analysis [21] using Poloplus® program, Le Ora Software [36].

Compounds isolation

Chromatographic and spectroscopic methods for compounds isolation

The chromatography columns were performed on silica gel (Sigma-Aldrich, 70–230 mesh). Thin-layer chromatography were carried out on aluminum plates (aluminum sheet silica gel SIL/UV254; 0.20 mm; 20 × 20 cm) and visualized with UV light (254 and 366 nm) then sprayed with vanillin−H_2SO_4.

The semi preparative chromatography isolations were performed with Waters Deltaprep instrument. An HPLC column (Thermo Scientific™ Hypersil™ ODS C18 HPLC Preparative column, 250 × 10 mm; particle size 5 µm) was used for the analysis. The mobile phase consisted of milliQ water (solvent A) and acetonitrile (HPLC supra gradient grade) purchased from Unichrom (Ajax Finechem Pty. Ltd., New Zealand) (solvent B), and the flow rate was set to 2 mL.min^{-1}. The column oven was set at 35 °C. The injection volume was 100 µL. UV–Visible spectra were recorded between 200 and 400 nm.

The ESI-HRMS spectra were recorded on a QToF instrument (Agilent 6530, Les Ulis, France) in infusion mode. Ionization source conditions were drying gas temperature 325 °C, drying gas flow rate 11 L/min, nebulizer 35 psig, fragmentor 175 V, skimmer 65 V. Range of *m/z* was 200–1700. Purine ion $C_5H_4N_4$ $[M+H]^+$ (*m/z* 121.050873) and the hexakis (1H,1H,3H-tetrafluoropropoxy)-phosphazene ion $C_{18}H_{18}F_{24}N_3O_6P_3$ $[M+H]^+$ (*m/z* 922.009798) were used as internal lock masses. Full scans were acquired at a resolution of ca 11 000 (at *m/z* 922).

The ^{1}H and ^{13}C NMR 1D spectra as well as 2D spectra (COSY, HSQC, HMBC and NOESY), were recorded in $CDCl_3$ on a Bruker Avance 400 spectrometer (Sarrebourg, France) operating at 400 MHz for ^{1}H spectra and 100 MHz for ^{13}C.

Successive fractionations for compounds isolation

Fractionation 1 The ethanol crude extract (45 g; 7% yield), active at 100% against *Riphicephalus australis*, was chromatographed on normal phase silica gel column (particle size 0.060–0.200 mm). The mobile phase consisted of a stepwise gradient of acetone in CH_2Cl_2: 0% (4L), 0.25% (1.5L), 1% (2L), 2.5% (1L), 3% (2L), 4% (2L), 5% (2L), 8% (2L), 10% (4L) to end with 100% MeOH. The fractions were combined based on the thin-layer chromatography (TLC) profiles to give 17 different fractions A-Q. Among the 17 fractions obtained, two of them (Fractions G and H) showed 100% activity, and were selected for further purifications.

Fractionation 2 Further purification of fraction G (818 mg) by semi preparative chromatography on reverse phase silica gel—60 C8 rp perfluorinated (27 × 1.5 cm; particle size 0.040–0.063 mm) eluted with MeOH/H_2O (90/10—100/0 in 15 min and 100/0 for 15 min) provided 5 subfractions and compound 5.

Fractionation 3 Further purification of fraction H (5 g) by normal phase silica gel column (particle size 0.063–0.100 mm) was carried out. The mobile phase consisted of a gradient of petroleum ether and ethyl acetate from 90/10 (2L), 80/20 (4L) and 70/30 (2L) to provide 16 subfractions FH.F1 to FH.F16.

Fractionation 4 Fraction FH.F6 was subjected to further chromatography.FH.F6 (510 mg) was chromatographed by semi preparative chromatography on reverse phase silica gel – 60 C8 rp perfluorinated (27 × 1.5 cm; particle size 0.040–0.063 mm) eluted with ACN/H_2O (70/30 during 15 min; to 100/0 in 30 min and 100/0 for 15 min) to provide compounds 1, 2, 3, and 4.

Results

Acaricidal effect of crude extracts

Table 1 shows the acaricidal activities of *Piper nigrum* crude extracts against *Rh. australis*. The leaves EtOAc extract showed only 19% of larval mortality and so no lethal concentrations were calculated. The stems extracts (EtOAc and MeOH) and the fruits EtOH extracts (mature and green/unripe) showed 100% activity against

Table 1 Acaricidal activities of *Piper nigrum* crude extracts at 50 mg/mL

Plant parts extracts	Solvent	Mortality (%)	LC_{50} (%)	LC_{90} (%)	LC_{99} (%)	Slope	Chi-square	Heterogeneity
Stems	EtOAc	100	0.034 [0.033–0.036]	0.048 [0.045–0.052]	0.064 [0.058–0.071]	8.65 ± 0.39	20.48	1.71
	MeOH	100	0.411 [0.400–0.422]	0.545 [0.527–0.567]	0.686 [0.655–0.724]	10.45 ± 0.41	4.94	0.55
Fruits (Mature)	EtOH	100	0.250 [0.240–0.260]	0.325 [0.310–0.343]	0.402 [0.377–0.435]	11.26 ± 0.66	0.77	0.08
Fruits (Green)	EtOH	100	0.153 [0.147–0.160]	0.212 [0.201–0.228]	0.277 [0.255–0.307]	9.07 ± 0.62	3.22	0.32
Leaves	EtOAc	19	–	–	–	–	–	–

Values in square brackets correspond to the confidence interval

14 day-old larval *Rh. australis* so their LC_{50}, $LC_{90,}$ and LC_{99} were calculated and as compared to the literature.

The dried mature fruits ethanolic extract showed the best extraction yield (7%) and was selected for bioguided fractionation. Among the 17 fractions obtained, two of them (Fraction G, fraction H, and fraction H.F6) showed 100% activity and were selected for further purification of their major compounds (Fig. 1).

Isolation and identification of pure compounds (1)−(5)

Bioassay-guided fractionation of the *Piper nigrum* dry mature fruits EtOH extract afforded five pure compounds (Fig. 1) identified by spectroscopic analysis (HRMS and NMR, Table 2) and by comparison to the published data. Structures of compounds (1)−(5) are presented in Fig. 2.

Compound (1)—Pellitorine ((2E,4E)-N-isobutyldecadienamide)

For ^{1}H and ^{13}C NMR spectra, see Table 2; HRMS *m/z* (%): 224.2024 $[M+H]^+$ (60), 246.1848 $[M+Na]^+$ (100), 287.2124 (25), 469.3787 $[2\ M+Na]^+$ (100), 692.5723 $[3\ M+Na]^+$ (10); (corresponding to a formula $C_{14}H_{25}NO$, calculated 223.1936). The spectroscopic data matched to those found in literature [34, 38, 63].

Compound (2)—Homopellitorine (N-2′-methylbutyl-2E,4E-decadienamide)

For ^{1}H and ^{13}C NMR spectra, see Table 2; HRMS *m/z* (%): 260.2004 $[M+Na]^+$ (60), 274.2165 (50), 344.1552 (40), 413.2689 $[M+Na+C_9H_{15}ON]^+$ (100), 734.4350 $[3\ M+Na]^+$ (2); (corresponding to a formula $C_{15}H_{27}NO$, calculated 237.2092). The spectroscopic data matched to those found in literature [5, 54, 63].

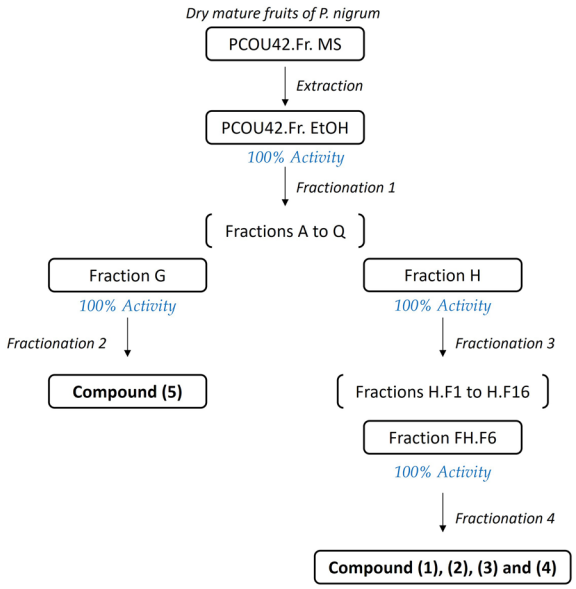


Fig. 1 Fractionation steps of *P. nigrum* dried mature fruits ethanolic extract

Compound (3)—Pipyaqubine or pirrollidide (N-pyrrolidyl-2,4-octadecadienamide)

For ^{1}H and ^{13}C NMR spectra, see Table 2; HRMS *m/z* (%): 290.2706 $[M\text{-}C_3H_7]^+$ (70), 356.1535 $[M+Na]^+$ (100), 476.3711 $[MH+2(C_5H_{11})]^+$ (5), 691.3224 $[2\ M+H+Na]^+$ (2); (corresponding to a formula $C_{22}H_{39}NO$, calculated 333.3031). The spectroscopic data matched to those found in the literature [23].

Compound (4)—N-isobutyl-2E,4E,12Z-octadecatrienamide (2-Methylpropylamide)

For ^{1}H and ^{13}C NMR spectra, see Table 2; HRMS *m/z* (%): 334.3131 $[M+H]^+$ (30), 356.2960 $[M+Na]^+$ (100), 689.5981 $[2\ M+Na]^+$ (15); (corresponding to a formula $C_{22}H_{39}NO$, calculated 333.5512). The spectroscopic data matched to those found in literature [31].

Compound (5)—N-isobutyl-2E,4E-eicosadienamide (N-isobutyleicosa-trans-2,trans-4-dienamide)

For ^{1}H and ^{13}C NMR spectra, see Table 2; HRMS *m/z* (%): 364.3589 $[M+H]^+$ (50), 386.3404 $[M+Na]^+$ (100), 727.7101 $[2\ M+H]^+$ (2), 749.6904 $[2\ M+Na]^+$ (5); (corresponding to a formula $C_{24}H_{45}NO$, calculated 363.6202). The spectroscopic data matched to those found in the literature [1].

Discussion

Piper nigrum is already known for its insecticidal and acaricidal activities. Extracts from different parts were shown to be toxic for houseflies (*Musca domestica* L.), rice weevils (*Sitophilus oryzae* L.), cowpea weevils (*Callosobruchus maculatus* F.), *Aedes aegypti* and for several more lepidopteran and hymeopteran herbivorous insects [16, 23, 25, 34, 41, 47, 50–52, 54, 56–58, 61].

Godara et al. [22] observed that methanolic extract of dried fruits of *P. nigrum* significantly affected mortality rates of adults engorged females of *Rh. australis* in a dose-dependent manner with an additional effect on the reproductive physiology of ticks by inhibiting oviposition and the LC_{50} value of methanolic extract was calculated as 0.48% (0.46–0.49) [22]. Nonetheless, no researches have been done on the larvicidal activity of *Piper nigrum* against *Rh. australis* although [23] demonstrated that *P. nigrum* could induce mortality on *Culex pipiens pallens* and *Aedes egypti* larvae. The research studies on larvae stage could emphase a more strategic and preventive control. This is why in this study we focused on the

Table 2 ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) for compounds 1–5 ($CDCl_3$)

(1) PELLITORINE (m/z = 223,1936—$C_{14}H_{25}NO$)			**(2) HOMOPELLITORINE (m/z = 237,2092—$C_{15}H_{27}NO$)**			**(3) PIPYAQUBINE (m/z: 333,3031—$C_{22}H_{39}NO$)**		
Position	δ^{1}H / I / m / *J* (Hz)	δ^{13}C	Position	δ^{1}H / I / m / *J* (Hz)	δ^{13}C	Position	δ^{1}H / I / m / *J* (Hz)	δ^{13}C
1 (C = O)	–	166.33	1 (C = O)	–	166.42	1 (C = O)	–	165.9
2	5.74 / 1H / *d* / 15.2	121.51	2	5.7 / 1H / *d* / 14.9	121.67	2	6.22 / 1H / *d* / 14.8	118.7
3	7.17 / 1H / *dd* / 15; 10	141.26	3	7.15 / 1H / *dd* / 15.1; 10	141.35	3	7.19 / 1H / *dd* / 10.5; 4.3	142
4	6.07 / 2H / *m*	128.06	4	6.1 / 1H / *dd* / 15.6; 9.5	128.17	4	6.15 / 1H / *dd* / 15.1; 10.8	129.01
5		143.15	5	6.03 / 1H / *dd* / 15.3; 6.2	143.26	5	6.03 / 1H / *m*	143.1
6	2.12 / 2H / *q* / 6.9	32.78	6	2.1 / 2H / *dd* / 13.2; 6.6	32.97	6	2.1 / 2H / *q* / 7.2	33.1
7	1.39 / 2H / *m*	31.23	7	1.25 / 6H / *m*	29.08	7—16	1.2—1.31 / 20H / *m*	29—32
8	1.28 / 4H / *m*	28.34	8		28.6	17	1.4 / 2H / *m*	22.8
9		22.33	9		22.5	18	0.9 / 3H / *t*	14.31
10	0.86 / 3H / *t* / 6.8	13.86	10	0.85 / 3H / *m*	14.08			
						1′-4′	3.47—3.58 / 4H / *m*	43–47
NH	5.59 / 1H / *brs*	–	NH	5.4 / 1H / *brs*	–	2′-3′	1.55—1.62 / 4H / *m*	24.8—26.8
1′	3.14 / 2H / *t* / 6	46.84	1′	3.14 / 2H / *t* / 6.5	46.9			
2′	1.78 / 1H / *m*	28.49	2′	1.38 / 1 H / *t* / 6.7	31.75			
3′	0.9 / 6H / *d* / 6.8	19.99	3′	1.77 / 2H / *sept.* / 6.7	28.81			
4′			4′	0.9 / 6H / *d* / 6.6	20.13			
			5′		20.13			

(4) *N*-isobutyl-2,4,12(*E,E,Z*)-octadecatrieamide (m/z = 333,5512—$C_{22}H_{39}NO$)			**5) *N*-isobutyl-2,4-eicosadienamide (m/z: 363,6202—$C_{24}H_{45}NO$)**		
Position	δ^{1}H / I / m / *J* (Hz)	δ^{13}C	Position	δ^{1}H / I / m / *J* (Hz)	δ^{13}C
1 (C = O)	–	166.6	1 (C = O)	–	167 (HMBC)
2	5.75 / 1H / *d* / 15.2	121.9	2	5.72 / 1H / *d* / 15	121
3	7.18 / 1H / *dd* / 15.1; 10	141.4	3	7.17 / 1H / *dd* / 15.1; 9.9	141
4	6.1 / 1H / *dd* / 14.9; 9.8	128.4	4	6.1 / 1H / *dd* / 15; 9.6	128
5	6.02 / 1H / *dd* / 15.5; 6.4	143.3	5	6.03 / 1H / *dd* / 15.2; 6.4	143
6	2.11 / 2H / *q*	33.15	6	2.12 / 1H / *q*	32.18
7	1.38 / 2H / *t* / 6	29; 29.92; 29.52; 29.35	7	1.54 / 2H / *t* / 7.2	29
8	1.22–1.31 / 12H / *m*		8–19	1.23 / 24H / *s*	29.3–29.9
9			20	0.9 / 3H / *d* / 6.6	20.37
10					
11	1.9 / 4H / *m*	27.11	NH	5.43 / 1H / *brs*	–
12	5.32 / 2H / *t* / 5.6	130	1′	3.14 / 1H / *t* / 6.4	47.19
13		130.1	2′	1.78 / 2 H / *sept* / 6.8	28.9
14	1.9 / 4H / *m*	27.36	3′	0.86 / 6H / *t* / 6.8	14.37
15	1.22–1.31 / 12H / *m*	28.87	4′		
16		32.17			
17		22.54			
18	0.86 / 3H / *m*	14.2			
NH	5.55 / 1H / *brs*	–			
1′	3.13 / 2H / *t* / 6.4	47.14			
2′	1.77 / 1 H / *sept* / 6.8	28.83			
3′	0.9 / 6H / *d* / 6.6	20.35			
4′					

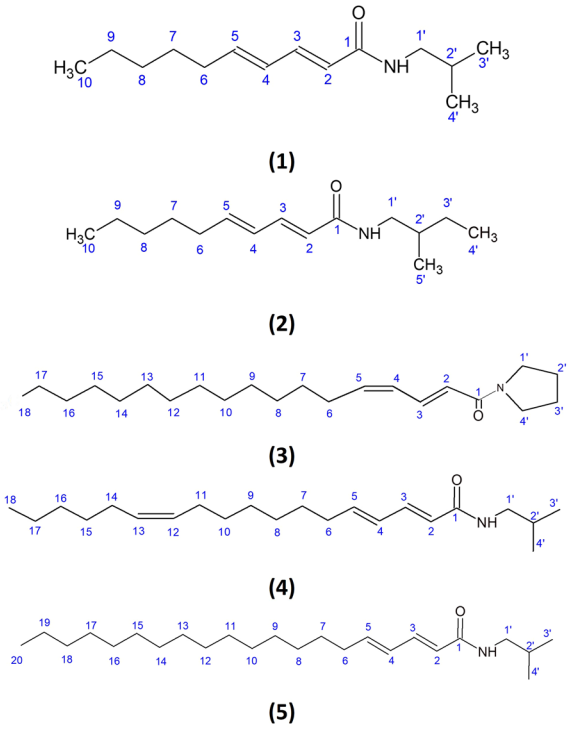


Fig. 2 Structures of compounds (1)—(5) Pellitorine (**1**), homopellitorine (**2**), pipyaqubine (**3**), 2-methylpropylamide (**4**) and N-isobutyl-2,4-eicosadienamide (**5**)

larvicidal activity against *Rh. australis* and our results are thus the first to report the larvicidal activity of *P. nigrum* fruit extracts on this tick species.

The activity of several Piperaceae extracts on cattle ticks larval stage have also been studied (Table 3).

Da Silva Lima et al. [10] showed that fruits hexane extract of *P. tuberculatum* showed the greatest efficacy (LC_{50} = 0.004%) against tick larvae followed by the ethyl ether, ethanol and methanol extracts with LC_{50} of 0.008%, 0.273%, and 0.449%, respectively [10]. However, hexane and ethyl ether being apolar solvents, we were more interested in alcoholic extracts in comparison with our results and in a development context for more adaptable and reproducible application tests. Therefore, in this work, we found ethanolic extracts (50 mg/mL) of *Piper nigrum* dried mature and unripe (green) fruits showing LC_{50} of 2.499 mg/mL and 1.533 mg/mL (0.25 and 0.15%), respectively, on *Rh. australis* larvae. We can therefore, cite that Jyoti et al. [28] reported a dose-dependent mortality response on larval stages for both *Piper longum* extracts and higher acaricidal property was exhibited by the alcoholic extract with LC_{50} and LC_{95} values of 0.488% (0.48–0.49) and 1.39% (1.35–1.44), respectively [28]. Braga et al. [7] showed that the LC_{50} of *Piper tuberculatum* extracts after 24 h of exposure were 3.62, 3.99 and 5.30 mg/mL (0.36, 0.40 and 0.530%) for fruit, stem and leaf extracts, respectively.

It can thus be considered that the ethanolic extracts of the fruits of *Piper nigrum* show the best LC_{50} of 0.2% on average for the fruits (ripe and green) as compared to the ethanolic extract of *Piper tuberculatum* fruits having an LC_{50} of 0.3% on average (0.3% [7] and 0.27% [10]) and the ethanolic extract of *Piper longum fruits* having an LC_{50} of 0.5% [28] on tick larvae (Table 3).

Furthermore, in our work, the ethyl acetate extract and the methanol extract from *P. nigrum* stems showed a LC_{50} of 0.34 mg/mL and 4 mg/mL (0.034% and 0.4%), respectively, on the larval stage of *Rh. australis*. Braga

Table 3 Larvicidal activity of different Piperaceae extracts against *Rhipicephalus microplus**

Piperaceae	Part of plant	Extract	Target *Rhipicephalus microplus**	LC_{50} (%)	LC_{95} (%)	References
Piper amalago	Aerial parts	Essential oil	Larvae	0	–	[20]
Piper corcovadensis	Roots	Isolated piperovatine	Larvae	0.00517	–	[19]
Piper longum	Fruit	Ethanol	Larval of amitraz resistant strain	0.488	1.39	[28]
Piper mikanianum	Aerial parts	Essential oil	Larvae	0.233	–	[20]
Piper tuberculatum	Fruits	Hexane	Larvae	0.004	–	[10]
Piper tuberculatum	Fruits	Ethyl ether	Larvae	0.008	–	[10]
Piper tuberculatum	Fruits	Ethanol	Larvae	0.273	0,25–0,29	[10]
Piper tuberculatum	Fruits	Methanol	Larvae	0.449	–	[10]
Piper tuberculatum	Fruits	Ethanol	Larvae	0.36	–	[7]
Piper tuberculatum	Stems	Ethanol	Larvae	0.40	–	[7]
Piper tuberculatum	Leaves	Ethanol	Larvae	0.53	–	[7]
Piper xylosteoides	Aerial parts	Essential oil	Larvae	0.615	–	[20]

* In New Caledonia, *Rh. australis* was synonymized with the southern cattle tick, *Rhipicephalus microplus*, until *Rh. australis* was reinstated as a separate cattle tick species in 2012

et al. [7] showed that the LC_{50} of *Piper tuberculatum* extracts on *Rh. microplus* larvae after 24 h of exposure were 3.62, 3.99 and 5.30 mg/mL (0.36, 0.40, and 0.530%) for fruit, stem and leaf extracts, respectively [7]. Ferraz et al. [20] observed that the essential oil of aerial parts of *Piper mikanianum* had a LC_{50} = 0.233% on tick larvae and that the essential oil of aerial parts of *P. xylosteoides* had a LC_{50} = 0.615% while the essential oil of aerial parts of *P. amalago* was inactive [20].

Moreover, Barrios et al. [2] highlights that the acaricidal property of *P. tuberculatum* can be attributed to the fact that its leaves and stems contain a considerable variety of amides and other compounds active against ectoparasites [2]. Indeed, according to Yu et al. [66], the major characteristic and active constituents of *P. nigrum* fruits are amide alkaloids [66].

In our study, the structures of isolated compounds were elucidated using spectroscopic methods: ESI-HRMS, ^{1}H- and ^{13}C-NMR spectral data, including DEPT and 2D-NMR experiments (COSY, HSQC, HMBC, and NOESY). These include one compound described for the first time in *P. nigrum*, *homopellitorine (2)* and 4 compounds previously described in *P. nigrum*, namely *pellitorine (1)*, *pipyaqubine (3)*, *2-methylpropylamide (4),* and *N-isobutyl-2,4-eicosadienamide (5)*. Moreover, the chromatographic profiles showed that piperine was the major compound of the fruit and stem extracts. The final quantities of the isolated compounds did not allow to determine their LC_{50} and LC_{90}. However, in an application context, we would like to highlight the use of crude extracts obtained with waste from pepper cultivation. Our isolated piperamides are the major compounds of the bioactive subfractions (100% activity on larvae) from which they are derived, and we can therefore hypothesize that they are partly responsible for the acaricidal activity observed with probably a synergistic action with other compounds. Da Silva et al. suggested that berberine and piperine alkaloids have an in vitro acaricidal action on *Rh. australis* larvae [9]. In 2002, Scott et al. already concluded that the biological activity of *P. tuberculatum* may be due to compounds present in smaller proportion with a synergic effect of several piperamides [50]. Indeed, Rodrigues et al. [47] showed that pellitorine, pipyaqubine, and piperine had LC_{50} of 20, 31 and 10 µg/mL, respectively, on *Aedes aegypti* larvae [23, 47]. Ee et al. [13] showed that pellitorine could be a potential anticancer hit compound [13] and we can find in literature that pellitorine and piperine exhibited also antibacterial [46] and insecticidal [53] activities. Furthermore, Miyakado et al. [40, 41] highlighted the insecticidal effect of different piperamides: pellitorine, pipercide, dihydropipercide, and guineensine. They attributed the high toxicity of the crude extracts of *P. nigrum* to a synergistic action carried by the different Piperaceae amides [40, 46]. In 2005, Lee et al. pointed out bioactive constituents (fungicidal, insecticidal, and mosquito larvicidal activities) derived from Piperaceae fruits to be pipernolanine, piperoctadecalidine, pellitorine, guineensine, pipercide, and retrofractamide A [34]. One important fact is that the efficacity of *Piper* extracts as botanical insecticides has been correlated with the concentration of piperamides present [51, 52]. Moreover in 2015, Ramesh et al. showed that sesamin, piperine, guineensine, pellitorine, trichostachine, and 4,5-dihydropiperlonguminine were considered to be the six marker compounds in *Piper nigrum* L. [45].

Piperamides can thus be considered as important bioactive compounds having a synergistic action [5, 11, 50, 52]. It also seems that piperine inhibits several metabolic enzymes and increases the oral bioavailability of many drugs and nutrients. Piperine enhances therapeutic effects and helps digestion by stimulating the intestinal and pancreatic enzymes [49]. As a matter of fact, our results are very interesting as *Piper nigrum* L. fruits are commonly cultivated, used and available worldwide.

The crops of *Piper nigrum* for the food industry generate a lot of waste (pericarp, stems and leaves usually considered as wastes during making of pepper) that can become sustainable sources in circular bioeconomy. Dried fruits, leaves and stems, as renewable parts of the plant, could be waste materials to recycle. Indeed, many studies have shown that *P. nigrum* is valued for its medicinal properties for treating pain, chills, rheumatism, flu, muscular aches and that its fruits shown antibacterial, antioxidant, anticancer, antimutagenic, antidiabetic, anti-inflammatory, analgesic, anticonvulsant, or neuroprotective effects [66, 67]. Thus, the acaricidal properties and the medicinal properties of the different parts of *P. nigrum* lead us to think that it is a plant to be valued for various applications. Indeed, as Yu et al. point out, we can consider that all this knowledge contributed to maximizing the use of different parts of *P. nigrum* as added-value resources for the food and pharmaceutical industries application [66]. Finally, as Quadros et al. [44] point out, for the development of commercial natural organic biopesticides it is important to consider the availability of the plant resource, the need for chemical standardization and quality control, the long-term stability, storage and transportation [44]. Finally, as Salehi et al. [49] highlighted, most of the studies were performed using in vitro models, so in vivo experimental approaches are needed to validate *Piper* spp extracts as acaricides [49].

Conclusion

The EtOH extracts of *Piper nigrum* dried fruits were the most active extracts (50 mg/mL) against *Rh. australis*. The dried mature fruits ethanolic extract showed the

best extraction yield (7%). and was selected for bioguided fractionation that led to the isolation and structure elucidation of 5 major compounds involved in the acaricidal activity, including one compound described for the first time in *P. nigrum.* Furthermore, as adult ticks are the main problem for livestock in terms of damages, the research studies on tick's larvae emphasis a more strategic and preventive control. Phytochemical studies on *Piper* spp. have been conducted to find potential pharmaceuticals or pesticides, but the most interesting investigations pertain on the synergy interactions.

Overall, the research findings clearly explain the feasibility of *Piper nigrum* aerial parts (fruits and stems) as potent naturals acaricides for the cattle tick control but also highlight the need for more investigations on the synergistic effects of phytochemical compounds.

Abbreviations

CH_2Cl_2	Dichloromethane
$CDCl_3$	Deuterated chloroform
CI	Confidence intervals
COSY	Correlated spectroscopy
ESI	Electrospray ionization
EtOAc	Ethyl acetate
EtOH	Ethanol
HMBC	Heteronuclear multiple bond correlation
HRMS	High-resolution mass spectrometry
HSQC	Heteronuclear single quantum coherence
IAC	Institut Agronomique néo-Calédonien
LC	Lethal concentration
LPT	Larval packet test
MeOH	Methanol
NMR	Nuclear magnetic resonance
NOESY	Nuclear Overhauser effect spectroscopy
RH	Relative humidity
RR	Resistance ratio
SRA	Agronomic research station
TBDs	Tick-borne diseases

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Author contributions
MT analyzed and interpreted all the data, carried out the bibliographical research, wrote the draft, performed the HRMS, 1H and 13C NMR interpretation and compounds identification. Writing—review and editing. PC performed the plants collection, extractions and chromatographic analyses and bioguided fractionations for compounds isolation. TH performed the acaricidal activities, as determined by the FAO modified method (LPT) and the statistical analysis using Poloplus® program and participated in the writing of the paper. AM performed the HRMS, 1H and 13C NMR analysis for compounds identification and reviewed the draft. VK supervised the work as team leader and participated in the bibliographic research as well as the writing of the paper. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
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Competing interests
The authors declare that they have no competing interests.

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