Acaricidal activity of Piper nigrum fruit extracts on the cattle tick, Rhipicephalus (Boophilus) australis (syn. R. microplus)

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Research Article

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Abstract

Background

The cattle farming parasite *Rhipicephalus (Boophilus) 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

We screened 200 extracts obtained from 70 plant species against *R. (B.) australis* (Canestrini 1887 ; syn. *R. (B.) microplus*) (Acari, Ixodidae) larvae, the cattle tick, an haematophagous parasite The most active extracts were obtained from *Piper nigrum* L. and especially the ethanolic extract of dried fruits as well as the ethyl acetate extract and the methanolic extract of stems which all exhibited 100% larvicidal activity. Bio-guided 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 fisrt 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 *R. australis* (syn. *R. microplus*).

Introduction

The cattle tick, *Rhipicephalus australis* (Canestrini 1887) (Acari: Ixodidae), was introduced in New Caledonia in 1942 and the annual economic losses caused by this parasite are staggering. Locally, *R. australis* was synonymized with the tick formerly called *Rhipicephalus microplus*, until *R. australis* was reinstated as a separate cattle tick species in 2012 [18]. Therefore, in this study this tick is called *R. australis* (syn. *R. microplus*). This cattle farming parasite *R. australis* is the main tick and one of the most important in the world from an economic point of view. This tick causes large losses to ranchers [10, 51], and as being a disease vector, it reduces reproductive efficiency, meat and milk production and can produce ‘pinholes’ which reduce the hide value [7, 68]. The principal control method involves the use of

synthetic acaricides by dip, spray, injection or pour-on treatments [45, 50]. The continuous use of these chemical compounds has led to the selection and development of strains of *R. australis* (syn. *R. microplus*) resistant to organophosphates, pyrethroids and formamidine, a phenomenon that is a major concern for worldwide cattle breeders [17, 33, 45]. Moreover, such chemical control causes meat and milk contamination that can also have undesirable effects on other organisms and the environment [17, 20, 31, 46].

The need of new scientific investigations for alternative ways to control this tick is related to the evolution of resistance of *R. australis* (syn. *R. microplus*) to synthetic acaricides. As a matter of fact, various studies have been developed in order to find plant extracts with acaricidal properties [4, 7, 8, 12, 17, 25, 29, 32, 36, 37, 42, 46, 68] in order 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 [34].

Several studies have already been done to assess the potential acaricide activity of natural substances on *R. australis* (syn. *R. microplus*). In a first study, Borges *et al.* (2011) inventoried 55 plants belonging to 26 families tested against this parasite [7]. In a second review in 2016, Benelli *et al.* describe the results of 62 extracts on *R. australis* (syn. *R. microplus*) excluding essential oils [4]. In 2020 Quadros *et al.* listed 27 plants-derived substances with potential for tick control and prevention on *R. australis* (syn. *R. microplus*), including monoterpenes (*e. g.* α-pinene, citronellal, eucalyptol, geraniol, limonene…), spilanthol (a fatty acid amine), β-caryophyllene (a sesquiterpene), azadirachtin (a tetranotriterpenoid), allicin (an organosulfur), eugenol (a phenylpropanoid), copaifera oleoresin and digitoxin (a steroidal glycoside) [46].

In New Caledonia, several works have been devoted to the acaricidal activity of natural substances on *R. australis* (syn. *R. microplus*) larvae but mainly concerned essential oils. Lebouvier *et al.* [36, 37], showed that essential oils from endemic trees of New Caledonia could provide natural acaricides for the control of the cattle tick *R. australis* (syn. *R. microplus*). Nevertheless, the development of an alternative tick control strategy must be associated with a high safety profile as well as availability and remanence. Therefore, 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 [36, 39]. 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 5 to 13 genera and 2000 to 4000 species (depending on the taxonomical authority) and is known to have acaricidal compounds such as monoterpenes, sesquiterpenes, alkaloids and phenylpropanoids [5, 13, 14, 27, 40, 41, 44, 47, 52, 55, 69]. 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 very diverse suites of secondary bioactive metabolites [52]. *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 attention of chemists as early as 1819 when Oestred isolated piperine. Since that time, search for active constituents from different *Piper* species is

being continued and this has been intensified in recent years, particularly because of interesting biological activities of various chemicals from several *Piper* species [14, 23, 38, 52, 55, 59–63, 69]. Indeed, some *Piper* species are listed as remedies for stomach pain, asthma, bronchitis, fever, abdominal pain, haemorrhoidal afflictions, rheumatism, as anti-inflammatory and stimulant agents, but also as insect repellents, insecticidal, acaricidal, antifungal and antibiotic [19, 26, 32, 38, 49, 55, 59, 60, 61, 70]. 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 [9, 14, 26, 35, 38, 47, 53, 57, 58–63, 64, 65, 66, 69].

Several Piperaceae have also been studied against cattle ticks [8, 12, 23, 32] and their major characteristic and active constituents could be attributed to a considerable variety of amide alkaloids [3, 54, 55, 69] and to a possible synergistic action [6, 13, 42, 43, 53, 55]. Therefore, an investigation on either major or minor compounds seems to be of particular interest.

As part of our research program towards plant-based harmless bioactive compounds for agronomic purposes in New Caledonia, 200 extracts obtained from 70 plant species (from endemic, introduced or invasive species) were tested on *R. australis* (syn. *R. microplus*). The most active extracts were obtained with *Piper nigrum*, especially with the EtOH extracts of dried fruits as well as the EtOAc and the MeOH extracts of stems which all exhibited 100% activity on the cattle tick, as determined by the FAO modified method (LPT) [67]. The acaricidal effect of *P. nigrum* extracts were compared to other chemicals and natural products described in the literature. The dried mature fruits ethanolic extract showed the best extraction yield (7%) and was selected for bio-guided fractionation. The result was the isolation and structure elucidation of 5 major compounds from the bioactive fractions, including one compound described for the first time in *P. nigrum*, previously found in the aerial part of *Piper sarmentosum* [66] and 4 compounds known for *P. nigrum*.

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). 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 and extracted (30g of dry matter in 400mL of solvent macerated for 48 hours then put in ultrasound for 20 minutes before filtration) with ethanol 95% or ethyl acetate or methanol at room temperature (solvents from Univar Solutions, Manchester, USA) and filtered under vacuum using a Buchner funnel. 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 *Rhipicephalus (Boophilus) australis* (syn. *R. (B.) microplus*) breeding was ongoing in IAC on calves. In the final stage of engorgement, female ticks were collected and used in the biological test within 24 hours. Ticks were washed with water and dried using paper towels. The female ticks were incubated at 27°C and 85% relative humidity for one week. Eggs were then collected and placed in the same conditions until larvae were two to three weeks old.

The strain was sensible to deltamethrin (RR = 0.05) and resistant to amitraz (LC99 = 8.25%) [30]: The resistance ratio (RR) corresponds to the ratio between the LC50 or LC99 of each strain studied and these same values for a sensitive strain of reference. The criteria used to define the resistance thresholds vary according to the acaricides. For deltamethrin, in accordance with the official recommendations of the FAO (1984) [21], the LC50 of each strain was compared with the one of a sensitive local reference strain, the Tiquotine strain, whose LC50 is 0.165 g/L (0.141– 0.187g/L).

If the RR50 is less than 3, the strain is considered sensible. For amitraz, there is no official recommendation on the parameters to be taken in order to assess the resistance of a tick population. In this study, the value of the LC99 was retained to measure the resistance. If the LC99 of the strain studied is less than 1 g/L, the strain is considered sensible.

Larval packet test

The FAO modified LPT method (Larval Packet Test; Stone 1962 [67]) was used to assess the acaricidal effect of samples on 14–21 days-old larvae. Nylon papers (Anowo LTD) were impregnated with 50 mg of extracts (1 mL at 50mg/mL per paper) and placed during one hour in a fume hood to allow the solvent to evaporate before being folded into packets using bulldog clips. Approximately 100 *R. australis* (syn. *R. microplus*) 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 hours. 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 LC50, six successive dilutions were tested, with the initial concentration depending on mass of extract available.

Statistical analysis

For each extract, the lethal concentration 50% (LC50) and the 95% confidence intervals (CI95%) were calculated according to Probit analysis (Finney 1971) using Poloplus® program [24].

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 aluminium plates (aluminum sheet silica gel SIL/UV254; 0.20 mm; 20x20cm) and visualized with UV light (254 and 366nm) 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, 250x10mm; particle size 5µm) 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 100µL. 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 ^1H 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 ^1H spectra and 100 MHz for ^{13}C.

Successive fractionations for compounds isolation

Fractionation 1. The ethanol crude extract (45g; 7% yield), active at 100% against *Riphicephalus australis* (syn. *R. microplus*), 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 (27x1.5 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 sub-fractions and **compound 5**.

Fractionation 3. Further purification of fraction H (5g) 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 sub-fractions 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 (27x1.5 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

The Table 1 shows the acaricidal activities of *Piper nigrum* crude extracts on *R. australis* (syn. *R. microplus*). The leaves EtOAc extract showed only 19% of larvae mortality and so no LC50 was calculated. The stems extracts (EtOAc and MeOH) and the fruits EtOH extracts (mature and green/unripe) showed 100% activity against 21days-old larvae of *R. australis* (syn. *R. microplus*) and respectively LC50 of 0.034, 0.4, 0.25 (Fig. 1) and 0.15%.

Table 1
Acaricidal activities of *Piper nigrum* crude extracts at 50 mg/mL and LC50.

Plant parts extracts	Solvant	Mortality (%)	LC50 (%)
Stems	EtOAc	**100**	**0,034** (0,34 mg/mL)
	MeOH	**100**	**0,4** (4 mg/mL)
Fruits (Mature)	EtOH	**100**	**0,2499** (2,499 mg/mL)
Fruits (Green)	EtOH	**100**	**0,1533** (1,533 mg/mL)
Leaves	EtOAc	19	-

The dried mature fruits ethanolic extract showed the best extraction yield (7%) and was selected for bio-guided 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. 2).

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. 2) identified by spectroscopic analysis (HRMS and NMR, Table 2) and by comparison to published data. Structures of **compounds (1)-(5)** are presented in Fig. 3.

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 $[2M + Na]^+$ (100), 692.5723 $[3M + Na]^+$ (10); (corresponding to a formula $C_{14}H_{25}NO$, calculated 223.1936). The spectroscopic data matched to those found in literature [40, 38, 66].

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 $[3M + Na]^+$ (2); (corresponding to a formula $C_{15}H_{27}NO$, calculated 237.2092). The spectroscopic data matched to those found in literature [6, 57, 66].

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 $[2M + H + Na]^+$ (2); (corresponding to a formula $C_{22}H_{39}NO$, calculated 333.3031). The spectroscopic data matched to those found in literature [26].

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 $[2M + Na]^+$ (15); (corresponding to a formula $C_{22}H_{39}NO$, calculated 333.5512). The spectroscopic data matched to those found in literature [35].

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 $[2M + H]^+$ (2), 749.6904 $[2M + Na]^+$ (5); (corresponding to a formula $C_{24}H_{45}NO$, calculated 363.6202). The spectroscopic data matched to those found in 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.), tobacco army worm, *Aedes aegypti* and for several more lepidopteran and hymeopteran herbivorous insects [19, 26, 28, 38, 43, 49, 53, 54, 55, 59, 60, 61]. Godara *et al.* (2018) observed that methanolic extract of dried fruits of *P. nigrum* significantly affected mortality rates of adults engorged females of *Rhipicephalus (Boophilus) australis* (syn. *R. (B.) microplus*) in a dose-dependent manner with an additional effect on the reproductive physiology of ticks by inhibiting oviposition and the LC50 value of methanolic extract was calculated as 0.48% (0.46–0.49) [25]. Our results are thus the first to report the larvicidal activity of *P. nigrum* fruit extracts on this tick species.

In this work, the dried mature and unripe (green) fruits ethanolic extracts showed LC50 of 2,499 mg/mL and 1,533 mg/mL (0.25 and 0.15%), respectively, on *R. australis* (syn. *R. microplus*) larvae. Furthermore,

the ethyl acetate extract and the methanol extract from *P. nigrum* stems showed a LC50 of 0.34 mg/mL and 4 mg/mL (0.034% and 0.4%), respectively, on the larval stage of *R. australis* (syn. *R. microplus*).

The activity of several Piperaceae extracts on cattle ticks have also been studied. Ferraz *et al.* (2010) observed that the essential oil of aerial parts of *Piper mikanianum* had a LC50 = 0.233% on tick larvae and that the essential oil of aerial parts of *P. xylosteoides* had a LC50 = 0.615% while the essential oil of aerial parts of *P. amalago* was inactive [23]. Da Silva Lima *et al.* (2014) showed that fruits hexane extract of *P. tuberculanum* showed the greatest efficacy (LC50 = 0.004%) followed by the ethyl ether, ethanol and methanol extracts with LC50 of 0.008%, 0.273% and 0.449%, respectively [12]. The results presented by Fernandez *et al.* (2018), indicated a LC50 of 0.00517% for piperovatine isolated from the roots of *Piper corcovadensis* and that piperovatine tested *ex situ* in an open environment at its *in vitro* LC99 of 25.41µg/mL had an efficiency of 96.63%, indicating that piperovatine kept its larvicidal action in an open environment [22]. Braga *et al.* (2018) showed that the LC50 of *Piper tuberculatum* extracts after 24 hours 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. On the engorged females, the highest efficacy rates were obtained at the concentration of 50 mg/mL, corresponding to 71.57%, 68.38% and 37.03% of the fruit, leaf and stem extracts, respectively. The main effect of the ethanol extracts was on the egg hatching rate of ticks, with a reduction of 55.63% for the fruit and leaf extracts, and 20.82% for the stem extract [8]. Jyoti *et al.* (2022) reports the acaricidal activity of *P. longum* fruit extracts, at different concentrations (0.625–10%) of alcoholic and aqueous extracts, against both larval and adult stages of amitraz resistant population of cattle tick. Indeed, a dose-dependent mortality response on larval stages was recorded for both extracts and higher acaricidal property was exhibited by the alcoholic extract with LC50 and LC95 (95% CL) values of 0.488% (0.48–0.49) and 1.39% (1.35–1.44), respectively. Similarly, against adult engorged females, ethanolic extract showed higher acaricidal property with LC50 and LC95 (95% CL) values of 4.67% (4.61–4.74) and 12.38% (12.05–12.73), respectively [32]. Moreover, Barrios *et al.* (2022) reports that the use of *Piper tuberculatum* extracts (100 mg/ml) was highly effective as an acaricide, reaching a high mortality rate in adult *R. australis* (syn. *R. microplus*) ticks showing a mortality of 78% after 24 hours in adult ticks, reaching 100% at 9 days and the LC50 for the *P. tuberculatum* EtOH extract (leaves, stems, fruits, and seeds) was 20.30 mg/mL (2.3%) after 24 hours. This study also 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 [3].

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. Our isolated piperamides are the major compounds of the bioactive sub-fractions (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 *R. australis* (syn. *R. microplus*) larvae [11]. According to Yu *et al.* (2022), the major characteristic and active constituents of *P. nigrum* fruits are amide alkaloids [69]. 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 [53]. Indeed, Rodrigues *et al.* (2020) showed that pellitorine, pipyaqubine and piperine had LC50 of 20, 31 and 10 µg/mL, respectively, on *Aedes aegypti* larvae [26, 49]. Ee *et al.* (2010) showed that pellitorine could be a potential anti-cancer hit compound [15] and we can find in literature that pellitorine and piperine exhibited also antibacterial [48] and insecticidal [56] activities. Furthermore, Miyakado *et al.* (1979, 1980) 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 [42, 43]. 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 [38]. One important fact is that the efficacity of *Piper* extracts as botanical insecticides has been correlated with the concentration of piperamides present [54, 55]. 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. [47].

Piperamides can thus be considered as important bioactive compounds having a synergistic action [6, 13, 53, 55]. 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 [52]. 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, the leaves and the 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 [69, 70]. 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 [69].

Conclusions

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 [38].

Phytochemical studies on *Piper* spp. have been conducted to find potential pharmaceuticals or pesticides but the most interesting investigations pertain on the synergy effects. This is particularly important when considering *Piper* genus phytochemistry, because these species have plethora of defensive compounds that could potentially interact with each other. Appropriate tests of pure compounds, whole plant extracts and pertinent mixtures should be performed to ascertain their potential in controlling small-scale insect outbreaks and reducing the likelihood of resistance development [13, 55].

To conclude, plant-based formulations enable expansion of organic agriculture and may even be used as an auxiliary in conventional production systems [7, 2].

Considering the economic and agricultural importance of *Piper nigrum* as well as the extraction yields, plant parts of *P. nigrum* are very interesting compared to essential oils from other plants which could also present neurotoxic effects. However, more studies are necessary to isolate and test specific compounds in bioassays with ticks in the larval and adult stages and to determine their safety to humans and other animals. Regulations requiring ecotoxicity studies and the evaluation of possible collateral effects should be applied and education about how to use these compounds and the risks that they impose may minimize the possible negative impacts [46]. As adult ticks are the main problem for livestock in terms of damages, research studies on tick's larvae emphasis a more strategic and preventive control. Finally, as Quadros *et al.* (2020) 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 [46]. Finally, as Salehi *et al.* (2019) 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 [52].

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.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

The datasets, concerning the 200 plant extracts screened against *Rhipicephalus australis* (syn. *R. microplus*), generated and/or analyzed during the current study are not publicly available because these data concern a confidential database, collection of natural extracts, belonging to the IAC but are available from the corresponding author on reasonable request.

Availability of data and material

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].

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Marine Toussirot 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 & editing.

Paul Coulerie performed the plants collection, extractions and chromatographic analyses and bio-guided fractionations for compounds isolation.

Thomas Hue 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.

Alexandre Maciuk performed the HRMS, 1H and 13C NMR analysis for compounds identification and reviewed the draft.

Valérie Kagy 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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Tables

Table 2 is available in the Supplementary Files section.

Figures

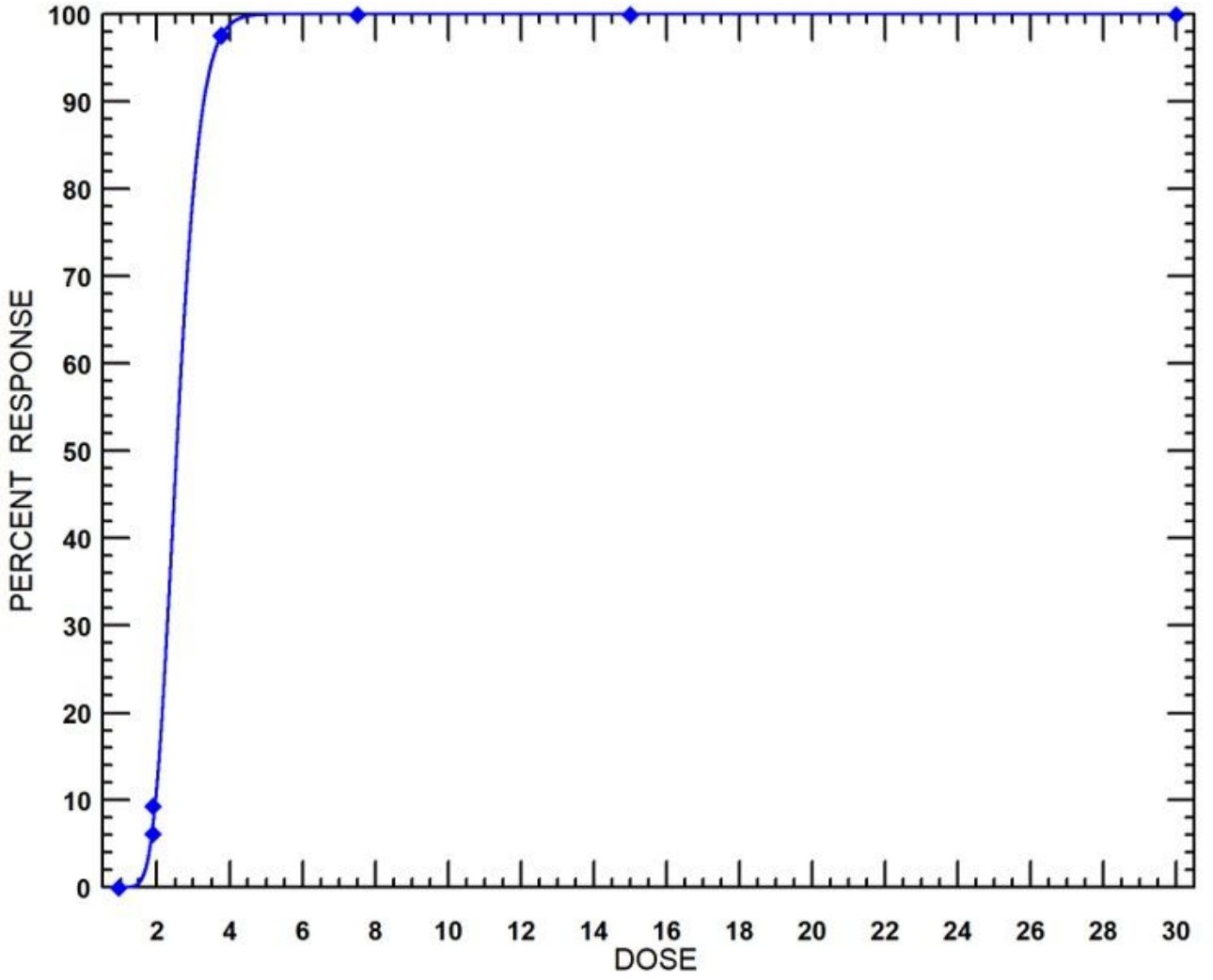

Figure 1

LC50 of the EtOH extract of *Piper nigrum* mature fruits *Calculated according to Probit analysis (Finney 1971) using Poloplus® program.*

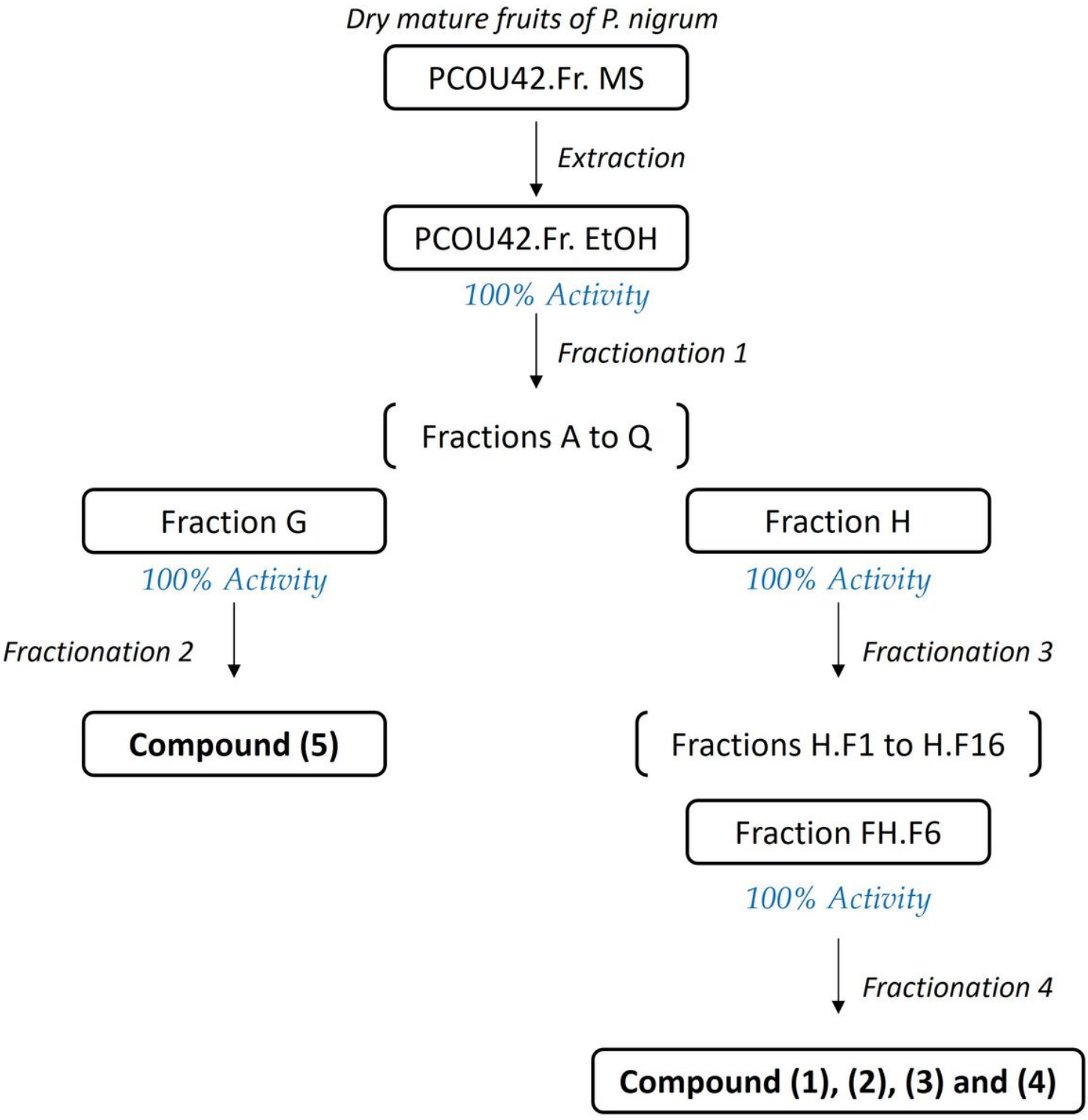

Figure 2

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

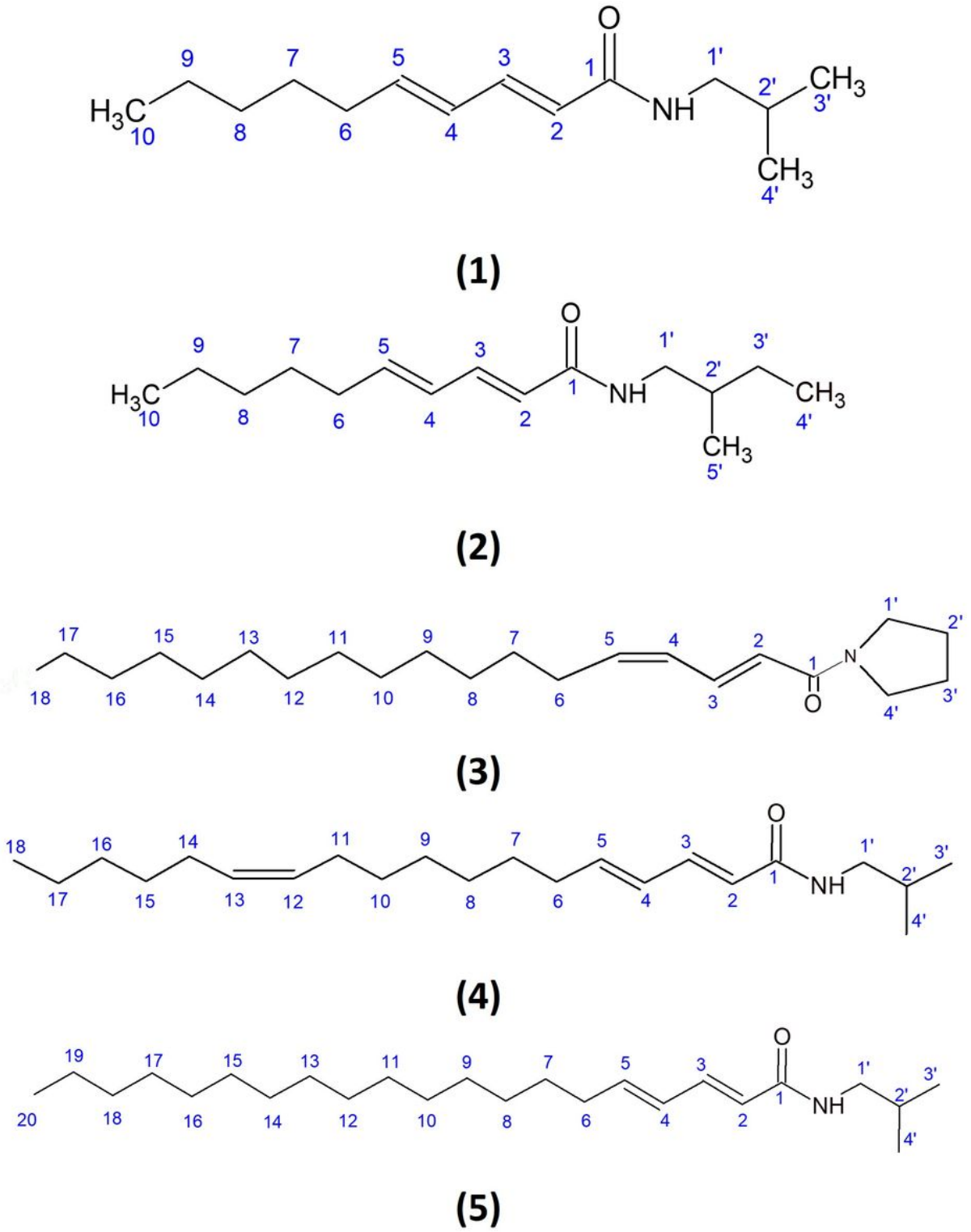

Figure 3

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

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table2Toussirotetal2022.xlsx
- GraphicalabstractToussirotetal2022.jpg