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Article

Seasonal and Circadian Evaluation of the *Pectis brevipedunculata* Essential Oil and Its Acaricidal Activity against *Rhipicephalus microplus* (Acari: Ixodidae)

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Pectis brevipedunculata is native species and widely available in dry and semi-arid ecosystems showing high biotechnological potential. The objective of this study was to evaluate the circadian and seasonal chemical variation of the essential oil (EO) of *P. brevipedunculata*, as well as its acaricide effect on *Rhipicephalus microplus* larvae. Aerial parts were collected and submitted to the hydrodistillation process, and the chemical composition was determined by gas chromatography mass spectrometry (GC-MS). For the assays with *R. microplus*, the larval immersion test was performed. The main constituents were citral (75% of the EO), followed by α-pinene and limonene. In the seasonality analysis, the highest yields were in the months of April (2.08%) and August (2.05%), while in the study of circadian rhythm, the percentage was 2.0% at 6 p.m. in the rainy season, and 1.2%, dry season at 6 p.m. Concerning acaricidal activity (50% lethal concentration (LC_{50})), the April (1.17 mg mL^{-1}), March (1.28 mg mL^{-1}), June (1.37 mg mL^{-1}), and October (1.27 mg mL^{-1}) oils obtained were the most active and assays performed with circadian rhythm revealed in the rain season (April) at 6 p.m. and dry season (September) at 12 a.m. LC_{50} values of 1.75 and 1.75 mg mL^{-1}, respectively. Additionally, this EO is selective to non-target organisms, i.e., ladybeetles and lacewing.

Keywords: Asteraceae, citral, acaricide, circadian, seasonal

Introduction

Natural products and their derivatives represent more than 50% of all the drugs in clinical use globally, and higher plants contribute no less than 25% of the total.[1,2] Half of the flowering plant species of the world live in tropical forests, which continue to support a vast reservoir of potential drug species. The potential for finding more therapeutic compounds is significant, and until now only 1-2% of tropical species have been studied for their pharmaceutical potential. The existence of undiscovered pharmaceuticals for modern medicine has been cited as one of the most important reasons for protecting the tropical forests.[3]

Asteraceae is the most important family among the phanerogams, representing 10% of the total angiosperm flora and comprising about 1,600 genera and 23,000 species. It is represented by about 180 genera and 1,900 species in

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different vegetation formations in Brazil. Asteraceae have a cosmopolitan distribution, being spread across all continents, but with a broader representation in temperate and semi-arid regions of the tropics and subtropics.[4,5] *Pectis* L. is the largest genus of the marigold tribe (Pectidinae: Tageteae), comprising about 90 annual and perennial species, adapted to warm regions of the New World, and occurring in savannas and openings of dry tropical forests of North America, Mexico, West Indies, Central and South America, and Pacific Islands. It is also characterized by opposite leaves with pairs of bristles at their bases, adnate phyllaries, having a floret as a single unit at maturity, and concise and densely papillose style branches.[6,7]

Pectis brevipedunculata (Gardner) Sch. Bip. (syn. *P. rubiacea* Baker) is an endemic terrestrial herb, 2-26 cm tall, pubescent stems with simple trichomes, 2.5 cm internodes; membranous opposite leaves, linear to oblong blades, acute apex, and numerous circular oil glands; dimorphic flowers with yellow ligulate corolla 3.5-4.5 mm long, 3-toothed apex, with occurrence from North to Southeast Brazil. In addition, it is an ornamental aromatic herb found in a xenophilic environment, producing valuable essential oil (EO) with a strong lemongrass odor and various therapeutic uses.[8-10] Although variation in EO composition is expected, *P. brevipedunculata* oil has citral (3,7-dimethyl-2,6-octadienal) as its primary constituent, a mixture of neral and geranial, which are two oxygenated monoterpene isomers.[8-11]

The plants produce EOs as a defense mechanism against herbivorous and pathogens, and biotic and abiotic factors usually influence their chemical compositions.[12,13] Abiotic factors, such as temperature and humidity influence the biotic organisms triggering a physiological circadian and seasonal change in plants that can be measured. The circadian cycle is correlated with the time of collecting plants throughout the day and the seasonal cycle in tropical regions with the rainy and dry seasons throughout the year.[14]

The tick *Rhipicephalus microplus* (Acari: Ixodidae) is the main ectoparasite responsible for severe economic losses to cattle breeding.[15,16] Synthetic acaricides have been most frequently used for *R. microplus* control.[17,18] On the other hand, the indiscriminate use of these synthetic acaricides has attributed resistance to ticks, as well multiple acaricide-resistance has been reported.[16,19] Thus, the plant biomolecules, such as the EOs, emerge as an alternative to synthetic chemicals in the tick control, showing significant advantages, such as the quick degradation in the environment, the synergistic effect of the constituents, selectivity to non-target organisms, and the resistance to these bioproducts which occurs in a slower form.[20-22]

Acaricidal and insecticide activity has been reported for neral and geranial, the major constituents of *P. brevipedunculata* essential oil.[23,24] However, there is no report for the acaricidal effect of essential oil obtained at the different circadian and seasonal regimes and their selectivity to a non-target organism. Thus, this study aimed to evaluate the circadian and seasonal variation of the essential oil of *P. brevipedunculata* and its acaricide effect on *R. microplus* larvae and the selectivity to non-target organisms such as *Coleomeguilla maculata* and *Eriopsis connexa* (ladybeetle) and *Chrysoperla externa* (lacewing). On the other hand, it emphasizes the need for this study as a biotechnological potential since there were no significant changes in the oil yield and chemical composition to climatic factors, making it favorable for the development of new drugs.

Experimental

Collection and the plant identification

The herbaceous *P. brevipedunculata* was sampled at the campus of Universidade Federal do Maranhão (UFMA), São Luís, MA, Brazil, coordinates: 2°33'20.5"S/44°18'32.7"W. A voucher specimen was deposited in the Herbarium Rosa Mochel (SLUI), Universidade Estadual do Maranhão (UEMA), São Luís, MA, Brazil, under the No. 5287. For the seasonal study, the plant was collected from January to December 2019, at 6 a.m., and for the circadian rhythm evaluation, the collections were made in April (rainy season) and September (dry season), at 6, 9, and 12 a.m., and 3 and 6 p.m. The experiments were performed in triplicate. The plant was collected according to the Brazilian law for biodiversity protection (SisGen No. AAFB38B).

Oil extraction and composition

The entire plant of *P. brevipedunculata* (excepting root) was submitted to hydrodistillation using a Clevenger-type apparatus (100 g, 2 h).[25] For the seasonal study, the plants were air-dried for 24 h before the hydrodistillation process, while for circadian rhythm, the fresh plants were used. The oils were dried over anhydrous sodium sulfate (ISOFAR, RJ, Brazil), and their yields were calculated in percentage m/v (mL *per* 100 g).[26]

Essential oil analysis was performed on a gas chromatography mass spectrometry (GC-MS)-QP2010 (Shimadzu Corporation, Japan) using a DB-5ms capillary column (30 m × 0.25 mm × 0.25 μm film thickness, J&W Scientific, USA) and equipped with the GCMS Solution software containing libraries.[27-29] The analysis conditions were injector temperature of 250 ºC; oven temperature

programming of 35 °C for 6 min and then with a heating ramp of 10 ºC min^{-1} to 240 ºC remaining for 10 min; split mode injection for 1.0 µL of the sample (oil 6.0 µL: *n*-hexane 500 µL), split ratio 1/30; ionization by electronic impact at 70 eV; ionization source and transfer line temperatures of 250 and 200 °C, respectively. The mass spectra were obtained by automatic scanning every 0.3 s, with mass fragments in the range of 35-400 *m/z*. Quantitative data regarding the volatile constituents were obtained by peak area normalization using a gas chromatography with flame-ionization detection (GC-FID) 2010 series, operated under similar conditions of the GC-MS. Compound identification was achieved by comparison of the retention indices, determined using a homologous series of *n*-alkanes (C_8-C_{32}, Sigma-Aldrich, St. Louis, MO, USA).[30] Oil components were identified by comparing their retention indices and mass spectra (molecular mass and fragmentation pattern) with those existing in the GCMS Solution system libraries.[23]

Biological assay

Tick collection and rearing

The engorged *R. microplus* females (Santa Rita strain) were collected from artificially infected calves, washed with water, and dried with a paper towel. These engorged females were selected morphologically and kept under controlled laboratory conditions (27 ± 2 ºC and relative humidity ≥ 80%) for 15 days until the eggs were laid. After egg hatching, larvae with 14 to 21 days were used for the subsequent larval immersion test. This study was approved by the UFMA ethics committee, under No. 23.115.008186/2017-18.

Larval immersion test

The *R. microplus* larvae immersion test was performed according to Klafke *et al.*[19] Briefly, different concentrations (400-5000 µg mL^{-1}) of the EO were prepared, diluting it in 1.0% ethanol and 0.02% Triton X-100 solution, which served as a negative control of the test. Then, different concentrations (1 mL each) were transferred to 1.5 mL microtubes, and approximately 500 larvae were added to each tube, using the treatment and control solution for 10 min. The larvae were then dried, and about 100 larvae were transferred to a filter paper packet (8.5 cm × 7.5 cm), with subsequent sealing, and then were kept in an incubator at 27.0 ± 1.0 °C and 85% relative humidity for 24 h. After this time, alive and dead larvae were counted. Larvae without movement were considered dead. The experiment was performed with three replicates for each concentration.

Selectivity of essential oil to non-target organisms

The methodology described previously by Toledo *et al.*[31,32] was used for this bioassay. A concentration of 0.11 mg cm^2 of *P. brevipedunculata* oil was used in the bioassay, corresponding to the highest value of the LC_{50} (50% lethal concentration) applied against the *R. microplus* tick. The aliquots were dissolved in a mixture of dimethyl sulfoxide (2%), Tween 80 (1%), and distilled water, used as the solvent. Petri dishes (9 cm diameter, 1 cm depth) had their bottom-side covered with filter paper (Whatman No.1) and impregnated with 400 µL of solution. The filter paper was left to air-dry (1 h) and, subsequently, groups of five ladybeetles adults of "ladybeetle" (*Coleomeguilla maculata* or *Eriopsis connexa*) with less than 10 days age were placed in the Petri dish. Six replicates for each species and treatment were performed, and the mortality was recorded after 24 h. In the case of "green lacewing" *Chrysoperla externa* was exposed to the same concentrations that ladybeetle (0.11 mg cm^2) and with a similar procedure as described above, except that they were exposed individually in 20 smaller Petri dishes (6 cm diameter) to avoid cannibalism.[33]

Six replicates for each species and treatment were performed, and the mortality was recorded after 24 h.

Statistical analysis

Statistical significance was assessed by the multivariate analysis ($P < 0.05$), and the Pearson correlation coefficients (R) were calculated to determine the relationship between the parameters analyzed (GraphPad Prism, version 8.0, San Diego, California, USA).[34] The doses were initially transformed to log (X), and the percentage of mortality normalized; subsequently, nonlinear regression was performed to obtain the LC_{50} using the GraphPad Prism 8.0.2 software.[34]

Results and Discussion

Yield and composition of oils in the seasonal and circadian study

The yield and composition of the *P. brevipedunculata* essential oils during the seasonal study are displayed in Table 1. The yield of oils showed a higher content in January (2.1%) and May (2.1%) and the lower content in February (1.1%) and September (1.1%). Thirty-four constituents were identified and quantified by GC-MS and GC-FID, representing 99.6% of the total oils. The primary constituent of the oils was citral, a mixture of the isomers geranial (27.0 to 42.7%) and neral (22.8 to 33.2%),

followed by α-pinene (7.2 to 19.8%), and limonene (5.3 to 9.4%), comprising an average value of 83.6% of the composition of the oils. The oil from January also showed the highest geranial (42.7%) and neral (33.2%) contents and the lowest α-pinene (7.2%) and limonene (5.3%) contents when compared with the other months of the year. On the other hand, the months of March and August presented the lowest contents for geranial (27.0 and 29.6%) and neral (22.8 and 22.9%), while the α-pinene (18.4 and 19.8%) and limonene (9.4 and 8.4%) contents were highest for the seasonal period. The variation between the main constituents can also be followed in analyzing the main classes of compounds in oils: oxygenated monoterpenes (OM) and monoterpene hydrocarbons (MH). In January, OM = 82.0% and MH = 12.8%. In March, June and August, OM = 66.4, 66.1 and 64.8% and MH = 30.0, 28.4 and 30.4% (see Table 1). About 2.0% of the composition of seasonal rhythm oils was also composed of fatty acids and their derivatives.

The yield and composition of *P. brevipedunculata* essential oils during the circadian study are shown in Table 2. The average oil yields were higher in the rainy season, with 1.9% than in the dry season, with 1.1%. Low light hours (6 a.m., 2.1%; 6 p.m., 2.0%) seem to contribute to higher daily oil yields. Forty-five constituents were identified and quantified by GC-MS and GC-FID, representing 99.5% of the total oils. The lowest citral levels were observed at 9:00 a.m. (geranial, 30.3% + neral, 20.3%) and 3 p.m. (geranial, 32.2% + neral, 21.6%) in the rainy season, in contrast to the α-pinene (11.5 and 11.3%) and limonene (6.0 and 5.5%) which were the highest at the same time of day and seasonal period. The daily variation in the rainy season for oxygenated monoterpenes (OM) and monoterpene hydrocarbons (MH) was OM, 35.3.6%, and MH, 25.53%, respectively. For the dry period, the variation was OM, 38.7%, and MH, 28.3%, respectively, with more significant values. About 2.0% of the composition of seasonal rhythm oils is also composed of fatty acids and their derivatives. The rainy season showed a significant mean value for fatty acids and their derivatives, around 11.0%, compared to the dry season, only 3.1%.

Except for the common constituents identified in both seasonal and circadian cycle oils, the presence of other terpenoids and fatty acid derivatives, in small amounts, was observed in one or the other of these oils. In the seasonal study were α-thujene, germacrene B, and δ-cadinene. In contrast, the circadian study included α-campholenal, neryl and geranyl formate, ethyl nerolate, *trans-p*-menth-6-en-2,8-diol, *trans*-myrtanol acetate, citronellyl acetone and citronellyl butanoate, caryophyllene oxide, humulol, humulene epoxide II, fluorensadiol, and the linoleic and oleic acids.

In the seasonal study of *P. brevipedunculata*, the plant samples were submitted to a previous drying for 24 h to know the monthly variation in the composition of its essential oils. In the circadian study, fresh plant samples were used to know the daily variation of the constituents of essential oils. The results obtained were a slight variation in the composition of oils from the seasonal period, in contrast to a more expressive variation in the composition of oils from the circadian period. Concerning the main constituents of the oils, it is also observed that the drying process have less influence in the citral content (neral + geranial), showing a slight variation when compared to the α-pinene and limonene contents, which had significant variation between fresh and previously dried samples (see Tables 1 and 2). This influence of drying temperature on the chemical composition of *P. brevipedunculata* was evaluated by Oliveira *et al*.[8] This information seems to be very important for the economic use of the plant.

The secondary metabolites represent a chemical interface between plants and the surrounding environment, and their biosynthesis is frequently affected by environmental conditions, with variation in their contents and relative proportions. Seasonality, circadian rhythm, developmental stage and age, temperature, water availability, UV radiation, soil nutrients, altitude, atmospheric composition, and tissue damage influence secondary metabolism.[13]

In previous works,[9,10,35] the essential oils of *P. brevipedunculata* from Rio de Janeiro and Ceará, Brazil, were also characterized by a high percentage of citral (neral + geranial), followed by α-pinene and limonene. Nerol and geraniol, considered their precursor alcoholic enantiomers, were also identified with a much lower percentage.[36]

Essential oils from other *Pectis* species were previously reported. The oil of *Pectis texana* Cory (syn. *P. angustifolia* var. *fastigiata* (A. Gray) D.J. Keil) from Texas, USA, showed thymol (48%) as the main constituent. The oil of *Pectis papposa* Harv. & A. Gray from California, USA, presented cuminaldehyde (47%), β-pinene (27%), and carvone (12%).[37] The oil of *Pectis prostrata* Cav. from Camagüey, Cuba, exhibited perillaldehyde (70%) and limonene (16%).[38] The oils of two samples of *Pectis apodocephala* Baker from Ceará, Brazil, were characterized by significant percentages of citral (78 and 53%), followed by α-pinene (11 and 4%) and limonene (7 and 6%).[35,39] The oil of *Pectis oligocephala* (Gardner) Sch. Bip., from Ceará, Brazil, showed *p*-cymene (71%) and thymol (24%) as its primary constituents.[39] The oil of *Pectis odorata* Griseb. from Córdoba, Argentina, showed citral (50%) and limonene (50%) as their primary constituents.[40]

Table 1. Seasonal variation of the *Pectis brevipedunculata* essential oil

Yield / %			2.1	1.1	1.3	1.4	2.1	1.3	1.8	1.5	1.1	1.9	1.3	1.2
Constituent / %	RI_C	RI_L	January	February	March	April	May	June	July	August	September	October	November	December
α-Thujene	928	924[a]			0.1					0.1	0.1			
α-Pinene[b]	935	932[a]	7.2	15.4	18.4	17.7	16.5	17.9	16.2	19.8	18.1	18.4	18.1	18.6
Sabinene	975	969[a]	0.3	0.6	0.7	0.6	0.7	0.8	0.6	0.8	0.8	0.7	0.8	0.8
β-Pinene	979	974[a]		0.3	0.4	0.3	0.3	0.4	0.3	0.4	0.4	0.3	0.4	0.4
6-Methyl-5-hepten-2-one	988	986[c]	0.6	1.5	1.0	0.9	1.2	1.5	1.0	0.7	1.1	0.6	1.1	0.6
Myrcene	992	988[a]		0.3	0.4	0.3	0.3		0.3	0.3	0.3	0.2	0.4	0.2
Limonene[b]	1032	1024[a]	5.3	8.3	9.4	8.6	9.4	9.0	8.1	8.4	7.5	8.1	7.5	8.3
(*E*)-β-Ocimene	1050	1046[a]		0.4	0.6	0.4	0.6	0.3	0.4	0.6	0.5	0.2	0.5	0.2
3-Methyl-1,2-cyclohexanedione	1094	1089[a]	0.3	0.2	0.3	0.3	0.4	0.4	0.3	0.5	0.2	0.3	0.2	0.3
Linalool	1102	1095[a]	0.7	0.9	1.3	1.3	1.3	1.5	1.2	0.9	0.8	1.1	0.9	1.3
exo-Isocitral	1147	1140[c]		0.2	0.2	0.2		0.3	0.2	0.2	0.2	0.1	0.4	0.1
(*Z*)-Isocitral	1165	1160[a]	0.4	1.3	0.9	0.8	0.7	0.8	1.0	0.9	1.3	0.6	1.5	0.6
(*E*)-Isocitral	1183	1177[a]	0.9	2.3	1.5	1.5	1.1	1.3	1.7	1.5	2.0	1.2	2.1	1.2
Terpinen-4-ol	1186	1180[a]	0.3	0.3	0.3	0.3	0.2	0.3	0.2	0.2	0.2	0.1	0.3	0.2
α-Terpineol	1200	1195[c]	0.3	0.3	0.4	0.4	0.4	0.4	0.3	0.3	0.2	0.2	0.3	0.2
Nerol	1228	1227[a]	0.3	0.7	3.0	1.2	1.7	1.7	1.1	1.7	0.6	0.8	0.5	0.9
Neral[b]	1244	1235[a]	33.2	27.5	22.8	26.3	24.8	24.0	26.7	22.9	26.3	25.6	26.0	25.1
Geraniol	1253	1249[a]	1.9	2.5	8.5	3.9	5.0	4.4	3.4	6.2	2.3	3.5	2.0	3.7
Carvenone	1261	1255[a]	0.5	0.1	0.1	0.1	0.1	0.3	0.1		0.2	0.1	0.3	
Geranial[b]	1273	1264[a]	42.7	33.9	27.0	32.0	31.4	29.9	33.9	29.6	34.5	34.4	33.0	33.8
1-Tridecene	1295	1290[a]	0.3	0.2		0.1	0.3	0.3	0.2	0.7	0.2	0.1	0.2	0.2
2,4-Octanediol	1339	1339[a]	0.3			0.2		0.5	0.2			0.3	0.4	0.3
trans-*p*-Menth-6-en-2,8-diol	1369	1371[a]	0.4		0.2	0.3	0.2	0.8	0.1			0.1	0.5	0.1
2-Undecen-1-ol	1374	1370[c]	0.5		0.1	0.2	0.1	0.8	0.3			0.5	0.7	0.6
Geranyl acetate	1379	1379[a]	0.4	0.2	0.2	0.2	0.3	0.4	0.2	0.4	0.2	0.3	0.3	0.4
β-Elemene	1396	1389[a]	0.6	0.3	0.3	0.3	0.4	0.5	0.3	0.3	0.2	0.2	0.2	0.3
(*E*)-Caryophyllene	1430	1424[c]	0.6	0.5	0.4	0.4	0.6	0.4	0.4	0.7	0.4	0.4	0.4	0.4
trans-Prenyl limonene	1467	1357[a]	0.7	0.5	0.5	0.5	0.6	0.4	0.5	0.7	0.3	0.4	0.3	0.4
Germacrene D	1492	1484		0.3	0.3	0.2	0.3		0.1	0.3	0.1		0.1	
α-Alaskene	1520	1515[c]	0.3	0.3	0.2		0.3		0.1	0.4	0.2	0.1		0.1
δ-Cadinene	1526	1522		0.1			0.1			0.1				
α-Muurolol (Torreyol)	1649	1644[a]	0.3	0.2	0.2	0.1	0.1		0.1	0.1	0.1	0.2	0.1	0.2
Valerianol	1663	1657[c]	0.2	0.1	0.1					0.1	0.1	0.2		0.1
Linoleic acid	2133	2132[a]						0.3				0.1	0.1	0.1
Monoterpene hydrocarbons / %			12.8	25.3	30.0	27.9	27.8	28.4	25.9	30.4	27.7	27.9	27.7	28.5
Oxygenated monoterpenes / %			82.0	70.2	66.4	68.5	67.2	66.1	70.1	64.8	68.8	68.1	68.1	67.6
Sesquiterpene hydrocarbons / %			2.2	2.0	1.7	1.4	2.3	1.3	1.4	2.5	1.2	1.1	1.0	1.2
Oxygenated sesquiterpenes / %			0.5	0.3	0.3	0.1	0.1		0.1	0.2	0.2	0.4	0.1	0.3
Fatty acids and derivatives / %			2.0	1.9	1.4	1.7	2.0	3.8	2.0	1.9	1.5	1.9	2.7	2.1
Total / %			99.5	99.7	99.8	99.6	99.4	99.6	99.5	99.7	99.4	99.4	99.6	99.7

[a]Reference 26; [b]main constituents; [c]reference 27. RI_C: retention index calculated (Durabond-5ms column); RI_L: retention index from literature.

Samples of *Pectis elongata* from Martinique, West Indies, have produced essential oils rich in citral (39 to 67%).[41] The oil of *Pectis floribunda* A. Rich. (syn. *Pectis elongata* Kunth), existing in Cuba, showed perillaldehyde (44%), limonene (10%), and *cis*- and *trans*-limonene oxide (8%) as their primary constituents.[42] Also, the oils of two *Pectis elongata* chemotypes from the Amazon presented citral (neral, 39% + geranial, 48%) plus perillaldehyde

Table 2. Circadian rhythm of the *Pectis brevipedunculata* essential oil chemical composition during the rainy and dry seasons

Yield / %			2.1	1.9	1.8	1.8	2.0	1.3	1.1	1.1	0.9	1.2
Constituent / %	RI_C	RI_L	April (rainy season)					September (dry season)				
			6 a.m.	9 a.m.	12 a.m.	3 p.m.	6 p.m.	6 a.m.	9 a.m.	12 a.m.	3 p.m.	6 p.m.
α-Pinene[a]	935	932[b]	4.7	11.5	8.2	11.3	8.2	8.4	12.6	14.0	12.6	10.1
Sabinene	975	969[b]			0.2			0.3	0.4	0.5	0.4	0.4
β-Pinene	979	974[b]							0.2	0.2	0.2	0.1
6-Methyl-5-hepten-2-one	988	986[c]	0.4	0.4	0.7	0.4	0.7	0.4	0.8	0.5	0.8	0.5
Myrcene	992	988[b]								0.1	0.1	
Limonene[a]	1032	1024[b]	3.4	6.0	6.1	5.5	5.7	4.8	8.0	6.8	7.8	5.6
(*E*)-β-Ocimene	1050	1046[b]								0.3	0.1	
3-Methyl-1,2-cyclohexanedione	1094	1089[b]		0.4	0.2			0.2	0.3	0.3	0.2	0.3
Linalool	1102	1095[b]	0.6	0.4	0.7	0.3	0.9	0.7	1.1	0.5	0.8	0.5
α-Campholenal	1131	1126[c]		1.5		1.2	0.4		0.3			
exo-Isocitral	1147	1140[c]						0.1		0.1	0.1	0.2
(*Z*)-Isocitral	1165	1160[b]			0.4		0.4	0.5	0.4	0.6	0.5	0.7
(*E*)-Isocitral	1183	1177[b]		0.4	0.8		0.7	1.1	0.9	1.2	1.0	1.1
Terpinen-4-ol	1186	1180[b]								0.1	0.1	
α-Terpineol	1200	1195[c]						0.2	0.3	0.1	0.2	
Nerol	1228	1227[b]	1.1	0.4	0.9	0.4	0.7	1.3	0.5	0.9	0.6	1.0
Neral[a]	1243	1235[b]	28.4	20.3	29.7	21.6	27.5	28.9	28.1	27.0	29.2	28.2
Geraniol	1253	1249[b]	4.5	2.5	3.4	2.0	2.9	5.6	2.4	5.0	2.8	4.7
Carvenone	1261	1255[b]	0.7	0.4	0.5	0.4	0.6	0.4	0.5	0.2	0.3	0.3
Geranial[a]	1273	1264[b]	39.9	30.3	38.8	32.2	34.7	41.1	36.7	38.1	38.1	39.7
Neryl formate	1287	1280[b]	0.3	0.4		0.7	0.4					
1-Tridecene	1294	1290[b]	2.2	1.8	0.6	1.3	0.6	0.9	0.5	0.6	0.3	1.1
Geranyl formate	1300	1298[b]	0.5	0.4		0.3						
Undecanal	1306	1305[b]	0.4			0.5	0.4					
2,4-Octanediol	1338	1339[b]	1.4	4.2	1.1	4.1	2.0	0.3	0.8	0.1	0.4	0.6
Ethyl nerolate	1348	1351[b]	0.4	2.7	0.5	2.3	1.5	0.3			0.2	0.3
trans-p-Menth-6-en-2,8-diol	1369	1371[b]	1.1	1.0	1.1	0.8	2.1	0.6	0.8	0.3	0.4	0.6
2-Undecen-1-ol	1374	1370[c]	2.5	5.9	1.7	6.1	2.9	0.7	1.2	0.3	0.7	1.1
Geranyl acetate	1379	1379[b]	0.7	0.7	0.4	0.5	0.5	0.5	0.4	0.4	0.3	0.4
trans-Myrtanol acetate	1387	1385[b]		1.2	0.3	1.1	0.5					
β-Elemene	1396	1389[b]	0.7	0.4	0.4	0.3	0.4	0.6	0.5	0.2	0.3	0.2
(*E*)-Caryophyllene	1430	1424[c]			0.2			0.5	0.2	0.5	0.2	0.5
Citronellylacetone	1438	1435[c]	0.7	0.6	0.3	0.7	0.4		0.2			
trans-Prenyl limonene	1467	1357[b]			0.3			0.5	0.3	0.4	0.3	0.3
α-Alaskene	1520	1515[c]	0.3					0.2	0.2	0.1	0.1	0.2
Citronellyl butanoate	1531	1530[b]	0.2		0.3		0.2				0.1	
Caryophyllene oxide	1595	1587[c]	0.5	0.6		0.4	0.3	0.2				0.2
Humulol	1605	1604[c]		0.4		0.4						
Humulene epoxide II	1623	1613[b]	0.3	0.4	0.2	0.3	0.4	0.1	0.2			
α-Muurolol (torreyol)	1649	1644[b]	0.3		0.2			0.2	0.2	0.1	0.1	0.2
Valerianol	1663	1657[c]						0.2				0.1
Fluorensadiol	1872	1869[b]	0.4	0.5		0.4						
Linoleic acid	2133	2132[b]	1.1	1.9	0.8	1.8	1.4	0.1	0.4		0.3	0.3
Oleic acid	2141	2141[b]	0.7	1.2	0.4	1.1	0.8				0.1	0.2
Monoterpene hydrocarbons / %			8.1	17.5	14.5	16.8	13.9	13.5	21.2	21.9	21.2	16.2
Oxygenated monoteterpenes / %			79.8	64.0	78.5	65.3	75.4	81.3	72.9	74.5	74.7	77.7
Sesquiterpene hydrocarbons / %			1.0	0.4	0.9	0.3	0.4	1.8	1.2	1.2	0.9	1.2
Oxygenated sesquiterpenes / %			1.5	1.9	0.4	1.5	0.7	0.7	0.4	0.1	0.1	0.5
Fatty acids and derivatives / %			8.7	15.8	5.5	15.3	8.8	2.6	4.0	1.8	2.8	4.1
Total / %			99.1	99.2	99.8	99.2	99.2	99.9	99.7	99.5	99.7	99.7

[a]Main constituents; [b]reference 26; [c]reference 27. RI_C: retention index calculated (Durabond-5ms column); RI_L: retention index from literature.

(52 to 82%) and limonene (34 to 44%) as their primary constituents.[6]

Pectis species have pleasant scents-like as citric, cumin, and oregano, due to the presence of monoterpene constituents in their volatile compositions. The main C10-skeletal monoterpenes found in *Pectis* oils can be depicted according to their biosynthetic pathways: (**1**) nerol/neral and geraniol/geranial arranged in an acyclic-type skeleton, (**2**) α-pinene and β-pinene in a pinane-type skeleton, and (**3**) limonene, perillaldehyde, cuminaldehyde, carvone, *p*-cymene and thymol in a *p*-menthane-type skeleton (see Figure 1).[6,43]

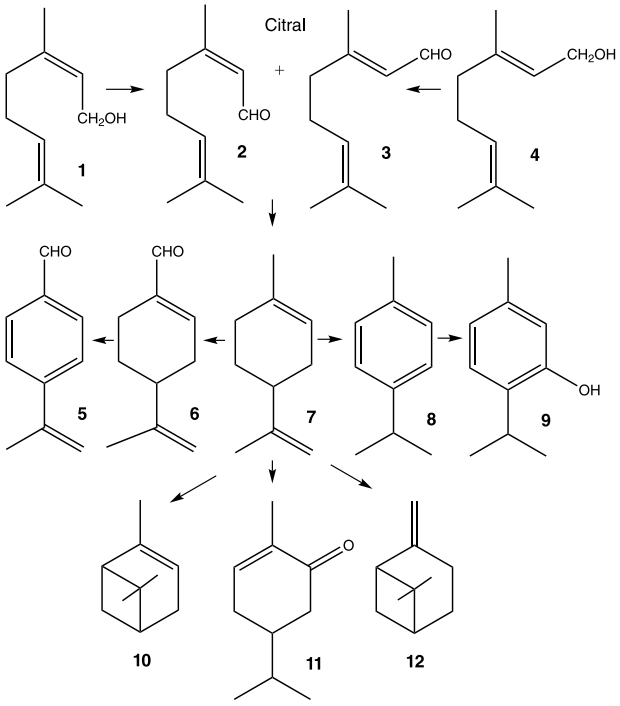

Figure 1. Monoterpenes found in *Pectis* oils and their biosynthetic relationship, involving interconversion by isomerization, cyclization, hydroxylation, and aromatization reactions. (**1**) Nerol, (**2**) neral, (**3**) geranial, (**4**) geraniol, (**5**) cuminaldehyde, (**6**) perillaldehyde, (**7**) limonene, (**8**) *p*-cymene, (**9**) thymol, (**10**) α-pinene, (**11**) carvone, and (**12**) β-pinene.

Acaricidal activity assay

The lethal concentration of the *P. brevipedunculata* EO exhibited variation according to the collection month and hours. The EO obtained in March, April and October was the most toxics to *R. microplus* with the LC_{50} = 1.280 (1.126-1.413); LC_{50} = 1.170 (1.158-1.191); LC_{50} = 1.270 (1.089-1.408) mg mL^{-1}, respectively; conversely, the lowest lethal concentration was observed for May LC_{50} = 2.840 mg mL^{-1} (2.706-2.972), (Table 3). In the circadian rhythm, the oil obtained in the rainy season at 6 p.m. was the most toxic, with LC_{50} = 1.750 mg mL^{-1} (1.709-1.798) and the essential oil extracted at 6 p.m. was the least toxic with LC_{50} = > 5 mg mL^{-1} (Figure 2). On the dry season, the essential oil extracted at 12 p.m. was the most toxic LC_{50} = 1.750 (1.669-1.823) and the extracted at 9 a.m. and 3 p.m. were the less toxics with a LC_{50} = > 5 mg mL^{-1} (Figure 2).

Selectivity to non-target organism

Essential oil of *P. brevipedunculata* was selective to non-target organisms. Neither predator insect (*E. connexa*, *C. maculate*) nor the lacewings (*C. externa*) died with the applied concentration (0.11 mg cm^2) corresponding to the highest LC_{50} (7 mg mL^{-1}) estimated to *R. microplus* (Figure 3).

The high production of neral and geranial could be related to direct defense against herbivorous due to the toxicity previously demonstrated.[36,44-48] However, the plants also need to attract pollinators and benefic organisms. Previous studies[49-53] related that emission in moderate quantities of α-pinene alone or with limonene

Table 3. Efficacy of the oils of *Pectis brevipedunculata* against the *Rhipicephalus microplus* larvae

Oil/seasonal	Month	LC_{50} / (mg mL^{-1})	$CI_{95\%}$	R^2
Pectis brevipedunculata	January	1.89af	1.744-2.054	0.94
	February	1.79a	1.716-1.868	0.98
	March	1.28beg	1.126-1.413	0.87
	April	1.17bg	1.158-1.191	0.99
	May	2.84c	2.706-2.972	0.98
	June	1.37de	1.248-1.475	0.92
	July	1.78a	1.725-1.839	0.99
	August	1.96f	1.925-2.000	0.99
	September	2.02f	1.967-2.077	0.99
	October	1.27gbe	1.089-1.408	0.85
	November	1.72ha	1.642-1.794	0.97
	December	2.38i	2.299-2.476	0.98

The same lowercase letters in the rows inside each seasonal period do not have differences by the CI 95%. LC_{50}: concentration at which 50% of the *R. microplus* larvae died; 95% CI: confidence interval at 95% probability; R^2: coefficient of determination.

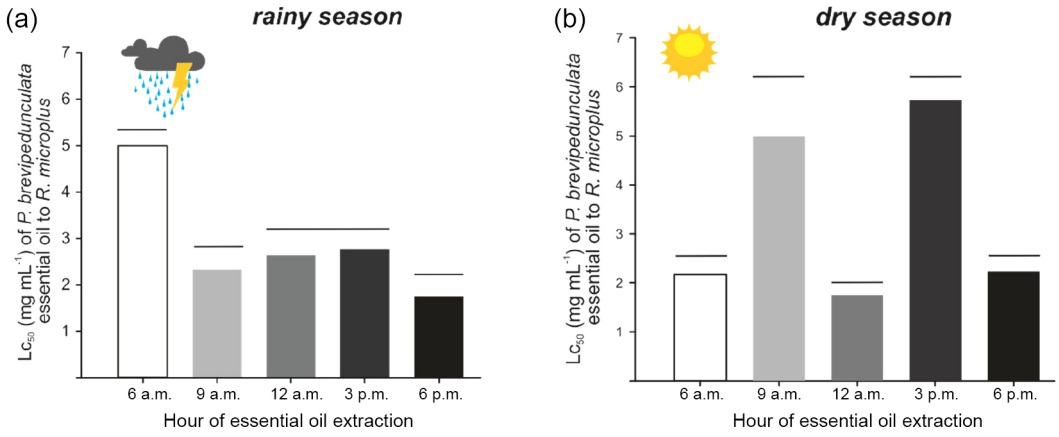

Figure 2. Variation of toxicity of the essential oil of *Pectis brevipedunculata* against the *Rhipicephalus microplus* accord to the season and hour of extraction. Horizontal lines on the same level or grouping the same bars do not significantly differ by the confidential interval 95%.

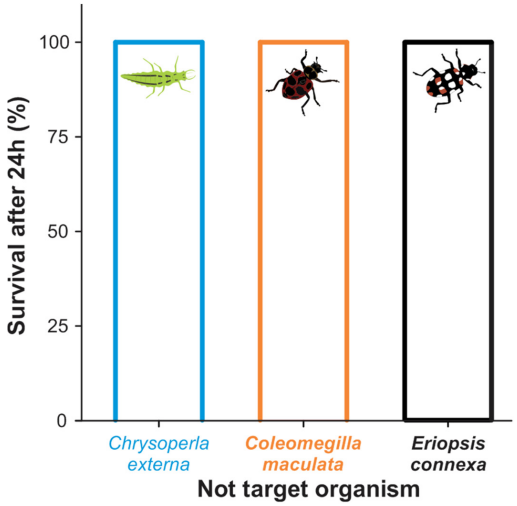

Figure 3. Survival of non-target organisms after 24 h of exposed to highest LC_{50} estimated to *R. mictoplus*.

attract pollinators, parasitoids and predator insects. This would also explain the higher production in the dry season, and the balanced production of these compounds during the highest solar radiation that is when the arthropods are most actives. As the production of biocompounds by *P. brevipedunculata* varied with the seasonal and the hours of day, this directly influenced in the toxicity of this essential oil against *R. microplus*. Here, essential oil extracted in March, April and October was most toxic against this tick; as well as the extracted at 6 p.m. and 12 a.m. for rainy and dry season, respectively. Although exist few studies on acaricidal proprieties of this plant, *R. microplus* resistant to amidines and synthetic pyrethroids or organophosphates are susceptive to EO with neral and geranial or citral isolated.[12,24] On the other hand, the toxicity of these compounds against different organisms was also demonstrated.[8,47,48] So, EO of *P. brevipedunculata* can be an alternative for the control *R. microplus*, with a major efficiency if this product is extracted in certain seasonal periods, without affecting non-target organisms.

Conclusions

The species *P. brevipedunculata* can represent an important source of bioproducts to control *R. microplus* due to the high yield of EO; and with less chemical variation in their compounds, both in the seasonal and circadian period. Additionally, this essential oil is selective to non-target organisms, i.e., ladybeetles and lacewings.

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Author Contributions

MBPC, ASL, LMCJ, LOVJ, JGSM and CQR were responsible for data curation, investigation, methodology and writing original draft; MBPC, ASL, OSM, CJSM, JSLN, JGSM and CQR for chemical analysis and writing original draft; ASL, LMCJ, SHCA, EEO and LMCJ for biological studies; CQR for project administration; LMCJ and CQR for funding acquisition; LMCJ, JGSM and CQR for supervision; MBPC, ASL, LVJ, LOVJ, JGSM and CQR for visualization.

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