Research, Society and Development, v. 10, n. 1, e48510111895, 2021
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v10i1.11895

Volatile oils from *Philodendron meridionale* Buturi & Sakur: chemical composition and *in vitro* effect against *Ctenocephalides felis* and leukemia cells

Óleos voláteis de *Philodendron meridionale* Buturi & Sakur: composição química e efeito *in vitro* contra *Ctenocephalides felis* e células leucêmicas

Aceites volátiles de *Philodendron meridionale* Buturi & Sakur: composición química y efecto *in vitro* contra *Ctenocephalides felis* y células leucémicas

Received: 01/12/2021 | Reviewed: 01/18/2021 | Accept: 01/21/2021 | Published: 01/25/2021

Juliane Nadal Dias Swiech
ORCID: https://orcid.org/0000-0002-5106-9767
Universidade Federal do Paraná, Brazil
E-mail: juliswiech@yahoo.com.br
Daniela Gaspardo Folquitto
ORCID: https://orcid.org/0000-0001-7572-225X
Universidade Federal do Paraná, Brazil
E-mail: danielafolquitto@gmail.com
Vanessa Barbosa Bobek
ORCID: https://orcid.org/0000-0002-2836-2076
Universidade Federal do Paraná, Brazil
E-mail: vanessabbobek@gmail.com
Amanda Migliorini Urban
ORCID: https://orcid.org/0000-0001-6805-1603
Universidade Federal do Paraná, Brazil
E-mail: amandamurban@yahoo.com.br
Luciane Mendes Monteiro
ORCID: https://orcid.org/0000-0003-2896-8147
Universidade Estadual de Ponta Grossa, Brazil
E-mail: lucianemendesmonteiro@gmail.com
Marilú Swiech
ORCID: https://orcid.org/0000-0002-5551-8750
Universidade Estadual de Ponta Grossa, Brazil
E-mail: mariluswiech@hotmail.com
Fernando Cesar Martins Betim
ORCID: https://orcid.org/0000-0002-1668-8626
Universidade Federal do Paraná, Brazil
E-mail: fernandobetim@hotmail.com
Camila Bugnoto Pereira
ORCID: https://orcid.org/0000-0001-8210-4804
Universidade Federal do Paraná, Brazil
E-mail: camilabugno@hotmail.com
Carla Cristine Kanunfre
ORCID: https://orcid.org/0000-0002-2865-3084
Universidade Estadual de Ponta Grossa, Brazil
E-mail: cckanunfre@gmail.com
Josiane de Fátima Gaspari Dias
ORCID: https://orcid.org/0000-0002-8548-8505
Universidade Federal do Paraná, Brazil
E-mail: josianefgdias@gmail.com
Rosi Zanoni da Silva
ORCID: https://orcid.org/0000-0001-6734-9520
Universidade Estadual de Ponta Grossa, Brazil
E-mail: rosizanoni@bol.com.br
Paulo Vitor Farago
ORCID: https://orcid.org/0000-0002-9934-4027
Universidade Estadual de Ponta Grossa, Brazil
E-mail: pvfarago@gmail.com
Marilis Dalarmi Miguel
ORCID: https://orcid.org/0000-0002-1126-9211
Universidade Federal do Paraná, Brazil
E-mail: marilisdmiguel@gmail.com

Obdulio Gomes Miguel
ORCID: https://orcid.org/0000-0002-2231-9130
Universidade Federal do Paraná, Brazil
E-mail: obdulio@ufpr.br

Abstract
Essential oils are an important natural source of pesticides and can be sui*table* used in ectoparasite control. The goal of this paper was to investigate the chemical composition of the volatile oils obtained from leaves and stems of *Philodendron meridionale* Buturi & Sakur by gas chromatography/mass spectrometry and to evaluate their insecticidal effect against *Ctenocephalides felis* Bouché adult insects and their cytotoxicity on human Jurkat leukemic T-cells by 3-(4,5-dimethylthiazol-2-yl)-2,5diphenyltetrazoluim bromide (MTT) reduction method. The main compound was ent-kaur-16-ene at concentrations of 20.78 and 22.46% for volatile oils from leaves and stems, respectively. These volatile oils provided insecticidal effect against *C. felis* with an average knockdown time of 9.61 and 7.35 min for fumigant test performed with the leaves and stems samples, respectively. Considering the topical application, these *P. meridionale* volatile oils extracted from leaves and stems demonstrated average knockdown values of 14.70 and 20.0 min, respectively. A very low cytotoxic effect of 9.48 and 11.32% was observed for *P. meridionale* volatile oils from leaves and stems even at the highest tested concentration of 400 $\mu g.mL^{-1}$, respectively. Therefore, it is possible to propose a biological selectivity to the volatile oils under study, which guarantees, at the same time, effect against this insect with no evident damage to human biological system.
Keywords: *Araceae*; *Philodendron;* Ent-kaur-16-ene; Cytotoxicity; Fleas; Essential oil.

Resumo
Os óleos essenciais são uma importante fonte natural de pesticidas e podem ser usados de forma adequada no controle de ectoparasitas. O objetivo deste trabalho foi investigar a composição química dos óleos voláteis obtidos de folhas e caules de *Philodendron meridionale* Buturi & Sakur por cromatografia gasosa / espectrometria de massa e avaliar seu efeito inseticida contra insetos adultos *Ctenocephalides felis* Bouché e sua citotoxicidade em linhagem celular Jurkat - células T leucêmicas humanas - pelo método de redução de brometo de 3- (4,5-dimetiltiazol-2-il) -2,5-difeniltetrazoluim (MTT). O principal composto majoritário foi o ent-kaur-16-eno em concentrações de 20,78 e 22,46% para óleos voláteis de folhas e caules, respectivamente. Esses óleos voláteis proporcionaram efeito inseticida contra *C. felis* com um tempo médio de remoção de 9,61 e 7,35 min para o teste de fumigação realizado com as amostras de folhas e caules, respectivamente. Considerando a aplicação tópica, esses óleos voláteis de *P. meridionale* extraídos de folhas e caules apresentaram valores médios de queda de 14,70 e 20,0 min, respectivamente. Um efeito citotóxico muito baixo de 9,48 e 11,32% foi observado para os óleos voláteis de *P. meridionale* de folhas e caules, mesmo na maior concentração testada de 400 $\mu g.mL^{-1}$, respectivamente. Portanto, é possível propor uma seletividade biológica aos óleos voláteis em estudo, o que garante, ao mesmo tempo, um efeito contra este inseto sem danos evidentes ao sistema biológico humano.
Palavras-chave: *Araceae*; *Philodendron*; Ent-kaur-16-eno; Citotoxicidade; Pulgas; Óleo essencial.

Resumen
Los aceites esenciales son una importante fuente natural de pesticidas y pueden usarse de manera adecuada en el control de ectoparásitos. El objetivo de este trabajo fue investigar la composición química de los aceites volátiles obtenidos de hojas y tallos de *Philodendron meridionale* Buturi & Sakur por cromatografía de gases / espectrometría de masas y evaluar su efecto insecticida contra insectos adultos *Ctenocephalides felis* Bouché y su citotoxicidad em línea celular Jurkat - células-T leucêmicas humanos- por el método de reducción de bromuro de 3-(4,5-dimetiltiazol-2-il)-2,5difeniltetrazoluim (MTT). El compuesto principal fue ent-kaur-16-eno en concentraciones de 20,78 y 22,46% para aceites volátiles de hojas y tallos, respectivamente. Estos aceites volátiles proporcionaron un efecto insecticida contra *C. felis* con un tiempo de remoción promedio de 9,61 y 7,35 min para la prueba de fumigante realizada con las muestras de hojas y tallos, respectivamente. Considerando la aplicación tópica, estos aceites volátiles de *P. meridionale* extraídos de hojas y tallos mostraron valores promedio de caída de 14,70 y 20,0 min, respectivamente. Se observó un efecto citotóxico muy bajo de 9,48 y 11,32% para los aceites volátiles de *P. meridionale* de hojas y tallos, incluso a la concentración más alta probada de 400 $\mu g.mL^{-1}$, respectivamente. Por tanto, es posible proponer una selectividad biológica a los aceites volátiles en estudio, lo que garantiza, al mismo tiempo, efecto contra este insecto sin daño evidente al sistema biológico humano.
Palabras clave: *Araceae*; *Philodendron;* Ent-kaur-16-eno; Citotoxicidad; Pulgas; Aceite essencial.

1. Introduction

Essential oils are complex mixtures of volatile and natural compounds with strong odor. They consist of secondary metabolites produced by plants. In nature, essential oils play an important role in protecting plants and serving as antibacterial,

antiviral, antifungal, and insecticidal substances. They also act against herbivores by reducing their interest in eating them. Furthermore, they are able to attract certain insects, which disperse pollen and seeds as well as repel other undesirable insects (Bakkali *et al.*, 2008; Ellse & Wall, 2014; Barros, Garcia & Andreotti, 2019; Betim *et al.*, 2019; Rodrigues *et al*., 2020; Apolinário *et al.*, 2020). Research on essential oils has been significantly intensified in order to discovery new active molecules and formulations. In addition, researchers are interested in precursors to be used in new medicines for human and animal use with higher efficacy and safety. Many essential oils demonstrate strong pharmacological properties as anti-inflammatory, antimicrobial, anticytotoxic, antioxidant, antifungal, antinociceptive, antipyretic, healing and antileishmanial activities (Bakkali *et al*., 2008; Cardile *et al*., 2009; Ribeiro *et al*., 2010; Pereira *et al.*, 2011; Sousa, 2012; Ract *et al*., 2015; Miranda *et al*., 2016; Damasceno *et al.*, 2017; Pereira *et al*., 2017; Kauffmann *et al*., 2019).

Species of *Philodendron* present proved biological activities such as anti-hemorrhagic (Otero *et al*., 2000; Moura *et al*., 2015), antiprotozoal (Muelas-Serrano *et al*., 2000), immunosuppressive (Yang *et al*., 2014), cytotoxicity against hepatocytes (HepG2) (Hassanein *et al.*, 2011; El-Deeb *et al.*, 2012) as well as antinociceptive and anti-inflammatory (Scapinello *et al*., 2019), insecticidal (Rickli *et al.*, 2020), allelopathic (Swiech *et al.*, 2021). However, only three studies reported their biological activity with essential oils. Alliance *et al.* (2017) investigated the potential effect of essential oils from the root of *Philodendron deflexum* Poepp on larvae of *Aedes aegypti* Linnaeus and *Anopheles albirtasis* Lynch-Arribálzaga. Santiago *et al.* (2014) described the insecticidal activity of essential oils from the roots of *Philodendron bipinnatifidum* Schott on *Diabrotica speciose*. Germar and Silva *et al.* (2016) reported the antibacterial activity of the *Philodendron goeldii* essential oil G. M Barroso against *Corynebacterium glutamicum*.

Philodendron meridionale Buturi & Sakur is a plant native to Brazil (Buturi, Temponi & Sakuragui, 2014). To the best of our knowledge, this is the first study on the chemical composition of volatile oils from *P. meridionale* that investigates their *in vitro* insecticidal and cytotoxic activities. Based on this purpose, *Ctenocephalides felis* Bouché was chosen because it is the most common flea among dogs and is responsible for a variety of veterinary dermatological problems. It can also be a vector for bacteria (such as *Bartonella* sp., responsible for rickettsial diseases) and an intermediate host for Filarioidea, Cestoda, other parasites, and viruses (Lans, Turner & Khan., 2008; Batista *et al*., 2016). Considering the effect of volatile compounds on human cells (Chkhikvishvili *et al*., 2013; Pereira *et al*., 2017), tests were performed using Jurkat cells, a human *T lymphocytes* widely used to study leukemia, in order to determine the *P. meridionale* cytotoxic potential.

2. Materials and Methods

2.1 Botanical material

Plant material was collected following a previous authorization from the Biodiversity Authorization and Information System (SISBIO), an organization associated with the Brazilian Ministry of Environment (MMA), and Chico Mendes Institute for Biodiversity Conservation (ICMBi) numbered 44970-1. The botanical material was collected at the Botanical Garden Campus of Federal University of Paraná, Brazil (25°26′58″ S, 49°37′12″ W – 906 m altitude) in June, 2014. All plant material was obtained from the same sample in a sterile phase. A specimen was stored at the Municipal Botanical Museum of Curitiba, numbered 390207 and then identified by an expert botanist, Dr. M.C.S.

2.2 Obtaining the Essential oil

Extraction was carried out by hydrodistillation for 7 h in dark conditions using Clevenger apparatus (USP XXV, 2002) and dry leaves (extraction 1) or dry stems (extraction 2) from *P. meridionale*, ground in a knife mil. The oil was collected into an Eppendorf tube containing anhydrous sodium sulfate and kept at 4 ± 0.5 ºC.

2.3 Identifying the essential oil constituents

The samples were analyzed by chromatography using a GC/MS-QP 2010 Plus Shimadzu equipped with an Rtx-5MS capillary column (30 m x 0.25 mm x 0.25 µm). Ten microliters of essential oils were dissolved in 1 mL of n-hexane for each oil sample, and 1 µL of the sample solution was injected. The injector was set to splitless mode at 250°C, while the interface and the ion source were set to 300°C. Helium at a flow rate of 1 mL/min was used as the carrier gas. An injection ramp method was used, with an injector temperature of 250ºC and a column pressure of 20 psi. The starting temperature was set at 50ºC for the first 5 min. The temperature was then increased to 200 ºC at a rate of 5ºC/min. Triplicate injections were made for each sample. Mass spectra were recorded at 70 eV in a scan mode from m/z 40 to 350.

The volatile compounds from *P. meridionale* oils were identified by comparing their relative retention indices to the literature (Adams, 2007). The raw percentage from the peak area of each compound was obtained in full-scan GC/MS analyses. Further standardization was not carried out, since our aim was to identify the volatile oil compounds.

2.4 Insecticidal activity of *P. meridionale* volatile oils against *C. felis*

Adult insects were collected from well-fed infected dogs in Ponta Grossa, Paraná by combing the dog hair and transferring the specimens to plastic Falcon tubes labelled with collection data. The insects were kept in groups of six and identified by the specialist Dr. I.F.B. After confirming specimen mobility, they were transferred to Petri glass dishes of 10 cm in diameter, where they were immediately subjected to testing. They were kept in a controlled environment at 28 ± 1°C and 60 ± 5% of relative humidity.

2.4.1 Fumigant activity

The fumigant analysis was performed in triplicate using a total of 216 non-sexed adult insects. For each test and sample (n = 4), the insects were divided into three groups of six insects each. The assay was carried out as suggested by Toloza *et al.* (2006). A closed chamber system consisting of a Petri dish and its cover that allowed for the formation of vapors was used. A drop (50 µL) of each volatile oil was placed on a glass cover inside the dish. Three groups of six adult insects were monitored at 10-min intervals for 1 h. The results were expressed as average knockdown time (KT_{50}). *Melaleuca* essential oil (Via Farma, São Paulo, Brazil) was used as a positive control, while water was used as a negative control.

2.4.2 Topical action

The topical analysis (Yang *et al.*, 2004) was also performed by dividing 216 non-sexed adult insects as reported in 2.3.1. Each test was carried out in a closed chamber consisting of a Petri dish and its corresponding cover, using 30 µL of each volatile oil or positive control diluted to a 10% concentration in a mixture of propylene glycol and ethyl alcohol (1:4). This volume of solution was then placed on each insect. Three groups of six adult insects were monitored at 10-min intervals for 1 h. Results were expressed as KT_{50}. *Melaleuca* essential oil (Via Farma, São Paulo, Brazil) was used as a positive control, while a mixture of propylene glycol and ethyl alcohol (1:4) was used as a negative control.

2.5 Cytotoxic activity of *P. meridionale* volatile oils against human Jurkat leukemic T-cell line

The Jurkat cell line was purchased from the Brazilian Cell Bank (Rio de Janeiro, Brazil). The cells were grown in RPMI 1640 medium at pH 7.4 and kept in a humidified stove set to 37 ± 0.5°C, with a 5% CO_2 atmosphere. This medium consists of a mixture of salts enriched with amino acids, vitamins, and other components necessary for cellular growth, namely 10% fetal bovine serum (FBS) supplemented with sodium bicarbonate (24 $mmol.L^{-1}$), penicillin (10,000 U), and streptomycin (10 $mg.L^{-1}$).

2.5.1 Cell viability by reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT)

A stock solution (10 mg/mL) was prepared by dissolving each volatile oil in a mixture of propylene glycol and ethyl alcohol (1:4) (Virador *et al*., 1998). The stock solution was diluted in RPMI 1640 with 10% sodium dodecyl sulfate to achieve concentrations of 0, 50, 100, 200, and 400 $\mu g.mL^{-1}$ shortly before beginning the experiments (Sylvestre *et al*., 2006; Cardile *et al*., 2009). The Jurkat cells were then seeded in plates containing 96 wells, at concentrations of 1.5 x 10^4 cells per well. They were then treated with each volatile oil at different concentrations for 72 h to investigate cell viability by MTT reduction. After this time interval, the cells were transferred to 2 mL tubes and centrifuged at 1500 rpm for 5 min. The supernatant was discarded and 200 μL of a 1:10 mixture of MTT in RPMI with 10% FBS was added to each tube. The cells were kept in an incubator for 30 min and centrifuged again at 2500 rpm for 5 min. The supernatant was discarded and 200 μL of DMSO was added in order to solubilize the purple formazan dyes resulting from the MTT reduction. The color of the supernatant was measured using a UV/Vis Shimadzu-1601 spectrophotometer at 550 nm. Absorbance values were used to calculate the percentage of cell viability as per the following equation: V% = (Abs A/Abs C) x 100, where V% is the cell viability percentage, Abs A is the sample absorbance, and Abs C is the control absorbance.

2.6 Statistical analysis

The analyses were completely randomized. Insecticidal activity was tested using four treatments and nine repetitions, while cytotoxic activity was tested using five treatments for each sample and three repetitions containing 4 wells each. Results were submitted to analysis of variance (ANOVA) and compared using the Tukey test at 5% probability ($P < 0.05$). Origin 9.0 was used for statistical analysis.

3. Results

Both volatile oils from extraction 1 and extraction 2 presented lower density than water, yellow color, and similar, strong, and characteristic odors. Total yield, with respect to the mass of dry material, was calculated to be 0.070 ± 0,036% (v/w) for leaves and 0.081 ± 0,013% (v/w) for stems from *P. meridionale*.

The leaf volatile oil GC/MS data showed low concentrations of monoterpenes and triterpenes and 55.22% sesquiterpene and 41.34% diterpene concentrations. In both cases, sesquiterpene and diterpene hydrocarbons are more usual. However, the stem volatile oil demonstrated concentrations of 53.77% and 39.60% for sesquiterpenes and diterpenes, respectively. Again, hydrocarbons were the typical compounds found. Monoterpenes and triterpenes were not observed in extraction 2. The identified volatile classes and their percentages are summarized in Table 1.

Table 1. Volatile composition determined by gas chromatography/mass spectrometry in volatile oils of *Philodendron meridionale* Buturi & Sakur from leaves and stems.

		VOLATILE OIL FROM LEAVES		VOLATILE OIL FROM STEMS	
VOLATILE COMPOUND	IRt	IRc	(%)	IRc	(%)
[1]*D*-Limonene	1029	1019	0.16	—	—
[2]Linalool	1086	1082	0.77	—	—
Linalyl formate	1214	—	—	1203	1.50
[2]Pulegone	1233	1212	0.13	—	—
2-Undecanone	1293	1283	0.10	—	—
[3]*α*-Elemene	1335	1338	0.19	—	—
[3]Cyclosativene	1369	1360	0.70	—	—
[3]Copaene	1374	1368	0.82	—	—
[3]*β*-Elemene	1389	1382	1.29	—	—
[3]*α*-Gurjunene	1409	1401	14.47	1402	6.73
[3] (*Z*)-Caryophyllene	1408	1408	2.49	1408	0.74
[3]iso-Caryophyllene	1409	1409	1.18	1409	0.61
[3]*β*-Cubebene	1390	1410	0.54	—	—
[3]*β*-Gurjunene	1431	1422	0.20	—	—
[3]*α*-Bergamotene	1411	1423	0.92	1424	0.50
[3]*α*-Caryophyllene	1415	1425	2.80	1426	1.29
[4]*δ* -Cadinol	1420	—	—	1427	0.64
[3]-Copaene	1430	1437	8.45	—	—
[3]*β*-Sesquisabinene	1457	1440	0.21	—	—
[3]Aromandendrene	1441	1443	0.23	—	—
[3]*α*-Guaiene	1439	1446	2.04	—	—
[3]*β*-Farnesene	1454	1452	2.86	1454	17.12
[3]*γ*-Gurjunene	1477	—	—	1477	1.38
[3]*γ*-Muurolene	1478	1478	0.75	1479	0.71
[3]*α*-Cubebene	1478	1479	0.13	—	—
[3]Eudesma-1,4(15),11-triene	1475	—	—	1480	2.59
[3]*D*-Germacrene	1485	—	—	1481	5.59
2-Tridecanone	1495	—	—	1487	5.11
[3]*β*-Muurolene	1493	1491	0.46	1488	0.41
[3]Bicyclogermacrene	1500	1492	6.34	1490	4.51
[3]Viridiflorene	1492	1495	0.18	—	—
[3]*α*-Muurolene	1500	1497	0.42	—	—
[3]*α*-Amorphene	1482	1501	0.15	—	—
[3]*β*-Sesquiphellandrene	1521	1526	0.12	—	—
[3]*δ*-Cadinene	1522	1528	2.13	1528	2.32
[4]Cubebol	1514	1531	0.23	1529	1.45
[3]Cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-	1541	—	—	1542	0.86
[4]Palustrol	1568	1556	2.68	—	—
[4]Spathulenol	1578	1559	0.72	—	—
[4]*trans*-Sesquisabinene hydrate	1577	1567	0.08	—	—
[4] (-)-Globulol	1578	1581	0.82	—	—
tau-Cadinol acetate	—	1597	0.82	—	—
[4]Ledol	1602	—	—	1594	5.76
[4]tau-Cadinol	1640	1629	0.62	—	—
[4]2-Pentadecanone	1697	—	—	1689	0.56
[5]Atiserene	1789	1790	0.31	—	—
Hexahydro farnesyl acetone	1845	1837	0.11	—	—
[5]Neophytadiene	1830	—	—	1835	1.69
[5]Rimuene	1896	1902	6.34	1904	5.08

[5]*cis*-Biformene	1931	1940	4.01	—	—
[6] (*E*)-3-Methyl-5-((1R,4aR,8aR)-5,5,8a-trimethyl-2-methylenedecahydronaphthale	—	—	—	1942	2.96
L-Ascorbic acid 2,6-dihexadecanoate	1968	1950	0.15	—	—
[5]Hibaene	1931	1952	0.45	—	—
[5]Cembrene	1937	1954	0.56	1952	5.22
[5]*α*-Springene	1969	1967	0.13	1968	1.49
[5]Eicosane	2000	1993	0.51	—	—
[5]Phyllocladene	2016	—	—	2005	1.08
[5]*trans*-Biformene	2026	2022	6.85	—	—
[5]Ent-kaur-16-ene	2043	2040	20.78	2040	21.46
[6]Verticiol	2273	2267	1.40	—	—
Octacosane, 1-iodo	—	2295	0.93	—	—
[6] (*E*)-Labda-8(17),12-diene-15,16-dial	2383	—	—	2386	0.62
[7]Squalene	2831	2828	0.28	—	—
Compounds identified (Total)			97.90		93.37
[1]Hydrocarbon monoterpenes			0.16		0.00
[2]Oxygenated monoterpenes			0.90		0.00
[3]Hydrocarbon sesquiterpenes			50.07		45.36
[4]Oxygenated sesquiterpenes			5.15		8.41
[5]Hydrocarbon diterpenes			39.94		36.02
[6]Oxygenated diterpenes			1.40		3.58
[7]Hydrocarbon triterpenes			0.28		0.00

IRt = retention index (Adams, 2007), IRc = retention index calculated, % = percentage of componente, (—) means non-detected. Source: The authors.

Table 1 presents the volatile compounds extracted from leaf (extraction 1) and stem samples (extraction 2) of *P. meridionale*. Considering the resulting chromatograms, these samples revealed 50 and 28 identified peaks, respectively.

The major volatile compounds found in the leaves were the diterpenes ent-kaur-16-ene (20.78%), *trans*-biformene (6.85%), and rimuene (6.34%), as well as the sesquiterpenes *α*-gurjunene (14.47%), *β*-copaene (8.45%), and bicyclogermacrene (6.34%). The main volatile compounds found in the stem extraction were the diterpene ent-kaur-16-ene (21.46%) and cembrene (5.22%). Sesquiterpenes were also observed, namely *β*-farnesene (17.12%), *α*-gurjunene (6.73%), ledol (5.76%), and D-germacrene (5.59%) (Table 1).

In general, the volatile oils from leaves and stems of *P. meridionale* presented insecticidal activity against *C. felis* in both fumigant and topical trials.

The fumigant assay resulted in statistically significant KT_{50} values of 9.61 ± 0.03 min, 7.35 ± 0.02 min, and 17.24 ± 0.03 min for leaf extraction, stem extraction and positive control in all-pairwise comparisons, respectively. No specimens were affected by the negative control, even after 360 min exposure time. Regarding the topical application, statistically significant KT_{50} values of 14.70 ± 0.02 min, 20.0 ± 0.02 min, and 36.76 ± 0.04 min were verified for leaf extraction, stem extraction, and positive control in all-pairwise comparisons, respectively. No KT_{50} value was determined for the negative control after 360 min of topical application. The percentage of affected specimens in different time intervals is seen in Table 2.

Table 2. Affected specimens of *Ctenocephalides felis* Bouche (%) in different time intervals after exposure to volatile *Philodendron meridionale* Buturi & Sakur from leaves and stems.

TIME INTERVAL (MIN)	FUMIGANT USE (%)				TOPICAL APPLICATION (%)			
	Negative control	Positive control	Volatile oil from leaves	Volatile oil from stems	Negative control	Positive control	Volatile oil from leaves	Volatile oil from stems
10 – 20	0[1]	52	16	68	0	0	34	16
20 – 30	0	16	42	16	0	16	34	34
30 – 40	0	16	0	16	0	16	16	34
40 – 50	0	16	42	—	0	16	16	0
50 – 60	0	—[2]	—	—	0	16	—	16

[1] 0 means absence of activity; [2] — means end of the activity in a previous time interval. Source: The authors.

In general, *P. meridionale* volatile oils seem to be non-cytotoxic to human Jurkat leukemic T-cell line. The most substantial reductions in cell viability were 9.47 ± 0.06% and 11.32 ± 0.07% for leaf extraction and stem extraction, respectively. These values were observed for the highest concentration tested (400 $\mu g.mL^{-1}$), while the other concentrations revealed lower values as illustrated in Table 3. Considering the low effect of *P. meridionale* volatile oils against Jurkat cell line, it was not possible to calculate the half maximal inhibitory concentration (IC_{50}) since no cell death greater than 50% was achieved.

Table 3. Cell viability od human Jurkat Leukemic T-Cell line after exposure to volatile *Philodendron meridionale* Buturi & Sakur from leaves and stems at different concentrations.

VOLATILE OIL	VOLATILE OIL FROM LEAVES		VOLATILE OIL FROM STEMS	
CONCENTRATION ($\mu g.mL^{-1}$)	Cell viability (%)[1]	Cell death (%)[2]	Cell viability (%)	Cell death (%)
50	101.85 ± 0.04	0	95.01 ± 0.04	4.99
100	98.94 ± 0.04	1.06	91.69 ± 0.07	8.31
200	94.15 ± 0.02	5.85	90.32 ± 0.02	9.68
400	90.52 ± 0.06	9.48	88.68 ± 0.07	11.32

The values of cell viability did not differ significantly within 0.05 (ANOVA and Tukey Test). [1] mean ± standard deviation; [2] mean value obtained from the cell growth control set at 100%. Source: The authors.

4. Discussion

Despite differences in some particular volatile compounds, this study confirmed the lack of remarkable changes in the percentage of sesquiterpenes and diterpenes obtained from *P. meridionale* leaves and stems. This information is supported by the fact that these two volatile samples share 13 of their major components (Table 1). Based on the chemical composition of *P. meridionale* volatile oils, it was initially expected that both samples would provide similar biological activities.

Ottobelli *et al.* (2011) found concentrations of 9.63 and 82.11% for monoterpenes and sesquiterpenes, respectively, when investigating an essential oil from *Philodendron scabrum* k. Krause stems. Castellar *et al.* (2013) observed concentrations of 24.3 and 72.6% for monoterpenes and sesquiterpenes in an essential oil from *Philodendron fragrantissimum* Kunth roots. Santiago *et al.* (2014) obtained 3.7 and 91.6% monoterpenes and sesquiterpenes in an essential oil from *P. bipinnatifidum* roots. Therefore, the *P. meridionale* volatile oils followed the well-known profile reported for the genus, since they demonstrated low content of monoterpenes and higher amount of sesquiterpenes. However, the volatile composition of both volatile oils from *P. meridionale* presented a higher composition of diterpenes which was not observed in other species from *Philodendron*. This result suggests that the volatile composition of our species can be better classified as resin oil than essential oil.

Studies on the composition of volatile oils from *Philodendron* plants are still scarce. Limonene and copaene have been found in some other species of this genus, while caryophyllene has been observed in all of those studied taxa with some different stereochemical orientations and concentrations (Viana, Andrade-Neto & Pouliquen, 2002; Bezerra *et al*., 2002; Ottobelli *et al*., 2011; Castellar *et al*., 2013; Santiago *et al*., 2014; Bacchus *et al.*, 2015; Joffard *et al*., 2017). Germacrene has also been investigated by Silva *et al.* (2016) for *Philodendron maximum* K. Krause, as well as by Joffard *et al.* (2017) for *Philodendron melinonii* Brongn and *P. fragrantissimum*. No other similarities were observed between the composition of *P. meridionale* essential oils and those of other species from *Philodendron*.

Certain terpenes, mainly elemene, copaene, gurjunene, caryophyllene, cubebene, sesquisabinene, muurolene, germacrene, cadinol, and biformene, were detected in more than one stereochemical orientation (Table 1). These data demonstrate that there are compounds being produced through the same metabolic pathway. In spite of this chemical relationship, these stereoisomers can provoke different effects on the biological systems and can result in dissimilar properties from a pharmacological point of view.

This study verified a high concentration of ent-kaur-16-ene. This is the first report of this diterpene in *Philodendron*. This compound is a secondary metabolite that is biosynthetically derived from the cyclization and subsequent modification of the bicyclic carbon backbone of geranylgeranyl pyrophosphate via oxidation, reduction, acetylation, methylation, and glycosylation (Toyomasu & Sassa, 2010; Riehl *et al*., 2015). This compound is also a precursor of steviol glycosides.

Many compounds obtained through metabolically processing of ent-kaurene present anticancer activity – for example, in SK-HEP1 human hepatocellular carcinoma cells activated by adenosine monophosphate-activated protein kinase and cytochrome p53 and inhibited by nuclear factor kB and the telomerase enzyme (Riehl *et al*., 2015). Ding *et al.* (2010) correlated the structures of eight ent-kaurene diterpenes and their structural substituents with activity in Hep G2 tumor cells, whereas Zu-Yin *et al.* (2007) demonstrated the use of ent-kaurene in apoptosis of Jurkat and myeloid leukemia HL-60, U937, and K562 cells. However, although this compound was a major component of the volatile oils in this study, it did not demonstrate mitochondrial/non-mitochondrial cytotoxic action as demonstrated by MTT testing with the Jurkat cell line. There was minimal decrease in cell viability in the concentrations used (Table 3), allowing for cellular development. According to Sylvestre *et al.* (2006), IC_{50} values of 200-300 μg/mL are indicative of very weak cytotoxic activity. Taking all these into account, one might suggest that a substance, though predominant, does not present the same effect when it is isolated. Therefore, it can be hypothesized that other volatile compounds present in the *P. meridionale* essential oils provided a synergic protective effect against Jurkat cell death which supported the high results of cell viability experimentally observed (Table 3).

The KT_{50} results demonstrate remarkable insecticidal potential (Table 2) by the fumigant activity and the topical application, even when compared to other studies (Yang *et al*., 2004; Toloza *et al.*, 2006; Toloza *et al*., 2008). Yang *et al.* (2004) studied essential oils from *Eucalyptus cinerea* F. Muell and tested their fumigant potential against *Pediculus humanus capitis* Linnaeus. The KT_{50} value of 6.2 min that was observed is somewhat similar to what was found in this study.

Meanwhile, Toloza *et al.* (2008) obtained values of 25.57, 35.01, and 31.31 min for *Eucalyptus grandis* W. Hill, *Eucalyptus camaldulensis* Dehnh, and *Eucalyptus tereticornis* Smith, respectively. These values are higher than the ones observed here. In that sense, *P. meridionale* volatile oils can be further used in the development of novel products to combat fleas in animals and humans.

Secondary metabolites play an important role in the resistance of plants to insects. According to Ibrahim *et al.* (2001), they can yield contact toxicity by penetrating the cuticle of the insect, fumigant activity by entering through the respiratory system, and ingestant effects by moving through the digestive system. The chemical features of essential oils contribute to their insecticidal activity, particularly in the toxokinetic and toxicodynamic stages of penetration, distribution, metabolism, and interaction with the site of action. This is due to the lipophilicity of the molecules present, which allows easy permeability of the insect integument with respect to topical action. In addition, these compounds are characterized by their high vapor tension, enabling fumigant activity (Sfara, Zerba & Alzogaray, 2009). Ellse and Wall (2014) attested that the fumigant action is connected to a neurotoxic pathway, rather than simply a mechanical one. This was confirmed by Genovese, Mclean and Khan (2012), who pointed out the possible interference of essential oils as neuromodulators of octopamine and gamma amino butyric acid receptors. In addition, the natural hydrophobicity of the oils could have mechanical effects on the parasites by preventing them from excreting water by perspiration, thereby resulting in osmotic stress or suffocation (Ellse & Wall, 2014; Burgess, 2009). This effect could also competitively inhibit the enzyme acetylcholinesterase by occupying the hydrophobic area in its active center (Ryan & Byrne, 1988). Finally, insect feeding behavior depends on integrating the central nervous system with chemoreceptors located in the legs, parts of the mouth, and oral cavity. Insecticides might therefore act on these chemoreceptors, thereby inhibiting feeding; this is characterized as an antifeedant activity (Santiago *et al*., 2014).

Schmelz *et al.* (2014) stated that the sequential activity of the enzyme ent-kaurene synthase produces diterpene phytoalexins that present antifeedant activity in insects. This information highlights both its nature as a defense mechanism of the plant as well as its mechanism of action as a bio-pesticide. In this case, in addition to its own insecticidal effect, ent-kaur-16-ene could serve as a precursor to substances that act as insecticides. Ultimately, the concentrations and synergetic or complementary actions resulting from the mixture of terpenes that are present in *P. meridionale* volatile oils play an extremely important role in their insecticidal activity (Ibrahim *et al*., 2001; Yang *et al*., 2004; Izumi *et al*., 2013; Ellse & Wall, 2014).

In that sense, the great finding of this paper is related to the confirmation of an effective insecticidal activity against the parasite *C. felis* with no pronounced effects on cell viability of human Jurkat leukemic T-cells. Thus, it is possible to propose a biological selectivity to the volatile oils under study, which guarantees, at the same time, effect against this insect with no evident damage to the human biological system.

In addition, this study shows that *P. meridionale* volatile oils work against ectoparasites, *e.g. C. felis*, and that they can be used as an alternative to more traditional insecticides like organochloride, organophosphate, and pyrethroid compounds whose use has been restricted due to their harmful environmental and human health effects. Moreover, they are also feasible compounds to be used in organic farming (Ellse & Wall, 2014). However, not all plants have the ability to accumulate volatile oils. On the other hand, *P. meridionale* has particular anatomical structures, such as secretory cells (idioblasts), cavities and ducts focused on producing natural products (Swiech *et al*., 2016) that can be successfully used to obtain volatile oils. In that sense, this work paves the way for using *P. meridionale* volatile oils as raw materials to obtain novel insecticidal products to treat animal and human ectoparasites.

5. Final Considerations

Volatile oils from *P. meridionale* are an important source of terpenes, mainly sesquiterpenes and diterpenes, which demonstrates a remarkable potential for the development of veterinary and human medicines against fleas. It is possible that

the major volatile compound found in *P. meridionale* leaves and stems, ent-kaur-16-ene, is partially responsible for the insecticidal effects obtained in this study; however, the synergistic effect of all other volatile constituents appears to be important.

These volatile oils could be further used as bio-pesticides, given that they showed high efficacy in combating *C. felis* both topically and as a fumigant agent. Furthermore, they did not change the enzymatic activity of the mitochondrial/non-mitochondrial succinate dehydrogenase of human Jurkat leukemic T-cells, a primary target that could lead to cell death. As such, they also demonstrated safety to be applied on animals and humans with no risk of cell damage.

Future studies may address other biological and toxicological actions, as well as the insecticidal action on other adult insects ora t stages of development.

Acknowledgments

We are grateful to Dr. Mônica Cássia Sakuragui for her assistance in identifying the botanical material and to Dr. Ivana de Freitas Bárbola for the identification of the investigated insect.

Funding

This work was supported by the Coordination for the improvement of higher education personnel (CAPES) [grant number 40001016042P8].

References

Adams, R. P. (2007). *Identification of Essential Oil Components by Gas Chromatography/mass Spectrometry*. (4th ed.). Allured Publishing Corporation.

Alliance, D., Santos, C. B. R., Barbosa, L. M. C., Gomes, J. S., Lobato, C. C., Viana, J. C., Ramos, R. S., Ferreira, R. M. A., & Souto, R. N. P. (2017). Chemical Study, Predictions "*In Silico*" and Larvicide Activity of the Essential Oil of Root *Philodendron deflexum* Poepp. *JCTN*. 14(7), 3330-3337. https://doi.org/10.1166/jctn.2017.6630.

Apolinário, R., Nogueira, J., Costa, M. G. S., Santos-Mallet, J., Santos, M. G., Azambuja, P., Mello, C. B., Gonzalez, M. S., Rocha, L. & Feder, M. D. (2020). Insecticidal activity of Pilocarpus spicatus Saint-Hilaire (Rutaceae) essential oil against the crop pest Dysdercus peruvianus (Guérin-Méneville, 1831) and Oncopeltus fasciatus (Dallas, 1852). *Research, Society and Development*, *9*(11), e90091110489. https://doi.org/10.33448/rsd-v9i11.10489

Bacchus, N. E., Oliveira, W. N., Marangon, C., & Moraes, C. M. (2015). Caracterização Química do Óleo Essencial de *Philodendron sp*. no Município de São João da Baliza, Roraima. *Orbital: Electron J. Chem*. 7(4), 376-381. http://dx.doi.org/10.17807/orbital.v7i4.774.

Bakkali, F., Averbeck, S., Averbeck, D. & Idaomar, M. (2008). Biological effects of essential oils: a review. *Food Chem Toxicol*. 46, 446-475. https://doi.org/10.1016/j.fct.2007.09.106.

Barros, J. C., Garcia, M. V., & Andreotti, R. In: Andreotti, R., Garcia, M. V., & Koller, W. W. (2019). *Carrapatos na cadeia produtiva de bovinos.* Brasília, DF: Embrapa, 2019. 240 p.

Batista, L. C. S. O., Cid, Y. P., Almeida, A. P., Prudêncio, E. R., Riger, C. J., Souza, M. A. A., Coumendouros, K., & Chaves, D. S. A. (2016). *In vitro* efficacy of essential oils and extracts of *Schinus molle* L. against *Ctenocephalides felis felis*. *Parasitology*. 143, 627-638. https://doi.org/10.1017/S0031182016000081.

Betim, F. C. M., Oliveira, C. F., Souza, A. M., Szabo, E. M., Zanin, S. M. W., Miguel, O. G., Miguel, M. D. & Dias, J. F. G. (2019). *Ocotea nutans* (Nees) Mez (Lauraceae): chemical composition, antioxidant capacity and biological properties of essential oil. *Braz. J. Pharm. Sci*. 55, 1-10. http://dx.doi.org/10.1590/s2175-97902019000118284

Bezerra, M. Z. B., Andrade-Neto, M., Freitas, R. M., & Feitosa, C. M. (2002). The root oil of *Philodendron imbe* Schott (Araceae). *J Essent Oil Res*. 14, 56-57. https://doi.org/10.1080/10412905.2002.9699763.

Burgess, I. F. (2009). The mode of action of dimeticone 4% lotion against head lice, *Pediculus capitis*. *BMC Pharmacology*. 9, 1-8. https://doi.org/10.1186/1471-2210-9-3.

Buturi, C. V., Temponi, L. G. & Sakuragui, C. M. (2014). A new species of *Philodendron* Schott (Araceae) in Paraná, Brazil. *Phytotaxa*. 174, 144-148. http://dx.doi.org/10.11646/phytotaxa.174.3.2.

Cardile, V., Russo, A., Formisano, C., Rigano, D., Senatore, F., Arnold, N. A. & Piozzi, F. (2009). Essential oils of *Salvia bracteata* and *Salvia rubifolia* from lebanon: chemical composition, antimicrobial activity and inhibitory effect on human melanoma cells. *J Ethnopharmacol*. 126(2), 265-272. https://doi.org/10.1016/j.jep.2009.08.034.

Castellar, A., Oliveira, D. R., Leitão, S. G., Bizzo, H. R., Soares, M. L. C., Kinupp, V. F. & Veiga-Junior, V. F. (2013). Essential oil from *Philodendron fragrantissimum*, an aromatic Araceae from Amazonia, Brazil. *J Essent Oil Res*. 25(3), 194-197. http://dx.doi.org/10.1080/10412905.2012.751557.

Chkhikvishvili, I., Sanikidze, T., Gogia, N., Mchedlishvili, T., Enukidze, M., Machavariani, M., Vinokur, Y. & Rodov, V. (2013). Rosmarinic Acid-Rich Extracts of Summer Savory (*Satureja hortensis* L.) Protect Jurkat T Cells against Oxidative Stress. *Oxid Med Cell Longev*. 9 pages. Article ID 456253. http://dx.doi.org/10.1155/2013/456253.

Damasceno, C. S. B., Oliveira, L. F. O., Szabo, E. M., Souza, A. M., Dias, J. F. G., Miguel, M. D. & Miguel, O. G. (2017). Chemical composition, antioxidant and biological activity of *Ocotea bicolor* Vattimo-Gil (LAURACEAE) essential oil. *Braz. J. Pharm. Sci*. 53(4), e17298. http://dx.doi.org/10.1590/s2175-97902017000417298

Ding, L., Hou, Q., Zhou, Q., Zhang, Q., Hou, T. & Liu, G. (2010). Structure–activity relationships of eight ent-kaurene diterpenoids from three *Isodon* plants. *Res Chem Intermed.* 36, 443-452. https://doi.org/10.1007/s11164-010-0144-3

El-Deeb, A. S., Abdel-Aleem, I. M., Elamin, S. M., Refahy, L. A. & El-Shazly, M. A. (2012). Antiproliferative effect of phenolic glucosides isolated from *Philodendron bipinnatifidum* on HepG2 cells. *Phytopharmacol***.** 3(2), 351-358.

Ellse, L. & Wall, R. (2014). The use of essential oils in veterinary ectoparasite control: a review. *Med Vet Entomol*. 28, 233–243. https://doi.org/10.1111/mve.12033.

Genovese, A. G., Mclean, M. K. & Khan, S. A. (2012). Adverse reactions from essential oil-containing natural flea products exempted from Environmental Protection Agency regulations in dogs and cats. *J Vet Emerg Crit Care*. 22, 470–475. https://doi.org/10.1111/j.1476-4431.2012.00780.x.

Hassanein, H. I., El-Ahwany, E. G., Salah, F. M., Hammam, O. A., Refai, L. & Hamed, M. (2011). Extracts of five medicinal herbs induced cytotoxicity in both hepatoma and myeloma cell lines. *J Cancer Sci Ther.* 3(10), 239-243. https://doi.org/10.4172/1948-5956.1000097

Ibrahim, M., Kainulainen, P., Aflatuni, A., Tiilikkala, K. & Holopainen, J. K. (2001). Insecticidal, repellent, antimicrobial activity and phytotoxicity of essential oils: with special reference to limonene and its suitability for control of insect pests. *Agri Food Sci Finl*. 10, 243-259. https://doi.org/10.23986/afsci.5697.

Izumi, E., Ueda-Nakamura, T., Veiga-Júnior, V. F. & Nakamura, C. V. (2013). Toxicity of Oleoresins from the Genus *Copaifera* in *Trypanosoma cruzi*: A Comparative Study. *Plant Med*. 79, 952–958. http://dx.doi.org/ 10.1055/s-0032-1328712

Joffard, N., Legendre, L., Gibernau, M. & Pascal, L. (2017). Differential accumulation of Volatile Organic Compounds (VOCs) by leaves and roots of two Guianese *Philodendron* species, *P. fragrantissimum* Kunth and *P. melinonii* Brongn. *Chem Biodivers*. 14(4). https://doi.org/10.1002/cbdv.201600415.

Kauffmann, C., Giacomin, A. C., Arossi, K., Pacheco, L. A., Hoehne, L., Freitas, E. M., Machado, G. M. C., Cavalheiro, M. M. C., Gnoatto, S. C. B. & Ethu, E. M. (2019). Antileishmanial *in vitro* activity of essential oil from *Myrciaria plinioides*, a native species from Southern Brazil. *Braz. J. Pharm. Sci.*, 55, 1-8. http://dx.doi.org/10.1590/s2175-97902019000217584

Lans, C., Turner, N. & Khan, T. (2008). Medicinal plant treatments for fleas and ear problems of cats and dogs in British Columbia, Canada. *Parasitol Res*. 103, 889–898. https://doi.org/10.1007/s00436-008-1073-6.

Miranda, C. A. S. F., Cardoso, M. G., Batista, L. R., Rodrigues, L. M. A. & Figueiredo, A. C. S. (2016). Óleos essenciais de folhas de diversas espécies: propriedades antioxidantes e antibacterianas no crescimento espécies patogênicas. *Rev. Ciênc. Agron*. 47(1), 213-220. http://dx.doi.org/10.5935/1806-6690.20160025.

Moura, V. M., Sousa, L. F., Santos, M. C., Raposo, J. D. A., Lima, A. E., Oliveira, R. B., Silva, M. N., & Mourão, R. H. V. (2015). Plants used to treat snakebites in Santarém, western Pará, Brazil: An assessment of their effectiveness in inhibiting hemorrhagic activity induced by *Bothrops jararaca* venom. *J Ethnopharmacol.* 161, 224-232. http://dx.doi.org/10.1016/j.jep.2014.12.020.

Muelas-Serrano, S., Nogal, J. J., Martinez-Diaz, R. A., Escario, J. A., Martinez-Fernandez, A.R. & Gomez-Barrio, A. (2000). *In vitro* screening of american plant extracts on *Trypanosoma cruzi* and *Trichomonas vaginalis*. *J Ethnopharmacol*. 71(1), 101-107. https://*doi*.org/10.1016/S0378-8741(99)00185-3

Otero, R., Fonnegra, R., Jimenez, S. L., Nuneza, V., Evans, N., Alzate, S. P., Garcia, M. E., Saldarriaga, M., Del Valle, G., Osorio, R. G., Diaz, A., Valderrama, R., Duque, A. & Velez, H.N. (2000). Snakebites and ethnobotany in the northwest region of Colombia Part I: Traditional use of plants. *J Ethnopharmacol.* 71, 493–504.

Ottobelli, I., Facundo, V. A., Zuliani, J., Luz, C. C., Brasil, H. O. B., Militão, J. S. L. T. & Braz-Filho, R. (2011). Estudo químico de duas plantas medicinais da amazônia: *Philodendron scabrum* k. Krause (ARACEAE) e *Vatairea guianensis* Aubl. (FABACEAE). *Acta Amazôn*. 41(3), 393-400. http://dx.doi.org/10.1590/S0044-59672011000300009.

Pereira, C. B., Kanunfre, C. C., Farago, P. V., Borsato, D. M., Budel, J. M., Maia, B. H. L. N. S., Campesatto, E. A., Sartoratto, A., Miguel, M. D. & Miguel, O. G. (2017). Cytotoxic mechanism of *Baccharis milleflora* (Less.) DC. essential oil. *Toxicol in Vitro*. 42, 214–221. http://dx.doi.org/10.1016/j.tiv.2017.04.031

Pereira, F. O., Wanderley, P. A., Viana, F. A. C., Lima, R. B., Sousa, F. B., Santos, S. G. & Lima, E. O. (2011). Effects of *Cymbopogon winterianus* Jowitt ex Bor essential oil on the growth and morphogenesis of *Trichophyton mentagrophytes*. *Braz. J. Pharm. Sci.*, 47(1), 145-153. http://dx.doi.org/10.1590/S1984-82502011000100018

Ract, J. N. R., Soares, F. A. S. M., Rodrigues, H. G., Bortolon, J. R., Murata, G. M., Gonçalves, M. I. A., Hatanaka, E., Curi, R. & Gioielli, L. A. (2015). Production of vegetable oil blends and structured lipids and their effect on wound healing. *Braz. J. Pharm. Sci.*, 51(2), 415-427. http://dx.doi.org/10.1590/S1984-82502015000200019

Ribeiro, R. V., Silva, R. M., Lima, J. C. S. & Martins, D. T. O. (2010). Antiinflammatory, antinociceptive and antipyretic effects of hydroethanolic extract from *Macrosiphonia velame* (A. St.-Hil.) M. Arg. in animal models. *Braz. J. Pharm. Sci.*, 46(3), 515-523. http://dx.doi.org/10.1590/S1984-82502010000300015

Rickli, M. E., Pradella, H. Q, Gomes, M. G., Belini, B. de P., Bortolucci, W. de C., Fernandez, C. M. M., Colauto, N. B., Linde, G. A. & Gazim, Z. C. (2020). Bioinsecticidal activity of *Philodendron bipinnatifidum*. *Research, Society and Development*, *9*(11), e85791110464. https://doi.org/10.33448/rsd-v9i11.10464

Riehl, P.S., Deporre, Y.C., Armaly, A.M., Groso, E.J. & Schindler, C.S. (2015). New avenues for the synthesis of ent-kaurene diterpenoids. *Tetrahedron*. 71, 6629-6650. https://doi.org/10.1002/chin.201542238.

Rodrigues, F. C., Almeida-Bezerra, J. W., Fidelis, K. R., Costa, A. R., Souza, M. A. de, Costa, M. H. N. da, Santos, L. T. dos, Gonçalo, M. A. B. F., Menezes, S. A. de, Sousa, S. M. de, Araujo, E. K. R. de, Braga, A. V. P., Ribeiro, T. G., Costa, A. R. de S., Santos, M. A. F. dos, Nunes, R. G. dos S. & Rocha, M. I. (2020). Chemical characterization and insecticidal potential of the essential oil of *Ocimum gratissimum* L. (Lamiaceae) against *Nauphoeta cinerea* (Blaberidae). *Research, Society and Development*, *9*(9), e72996412. https://doi.org/10.33448/rsd-v9i9.6412

Ryan, M. F. & Byrne, O. (1988). Plant-insect coevolution and inhibition of acetylcholinesterase. *J Chem Ecol*. 14(10), 1965-1975. https://doi.org/10.1007/BF01013489.

Santiago, J. A., Cardoso, M. G., Figueiredo, A. C. S., Moraes, J. C., Assis, F. A., Teixeira, M. L., Santiago, W. D., Sales, T. A., Camargo, K. C. & Nelson, D. L. (2014). Chemical Characterization and Application of the Essential Oils from *Chenopodium ambrosioides* and *Philodendron bipinnatifidum* in the Control of *Diabrotica speciosa* (Coleoptera: Chrysomelidae). *Am J Plant Sci*. 5(26), 3994-4002. https://doi.org/10.4236/ajps.2014.526417

Scapinello, J., Müller, L. G., Schindler, M. C. Z., Anzolin, G. S., Siebel, A. M., Boligon, A. A., Niero, R., Saraiva, T. E. S., Maus, N. P., Betti, A. H., Oliveira, J. V., Magro, J. D. & Oliveira, D. (2019). Antinociceptive and anti-inflammatory activities of *Philodendron bipinnatifidum* Schott ex Endl (Araceae). *J Ethnopharmacol.* 23(236), 21-30. https://doi.org/10.1016/j.jep.2019.02.037

Schmelz, E. A., Huffaker, A., Sims, J. W., Christensen, S. A., Lu, X., Okada, K. & Peters, R. J. (2014). Biosynthesis, elicitation and roles of monocot terpenoid Phytoalexin. The *Plant J*. 79, 659-678. https://doi.org/10.1111/tpj.12436.

Sfara, V., Zerba, E. N. & Alzogaray, R. A. (2009). Fumigant Insecticidal Activity and Repellent Effect of Five Essential Oils and Seven Monoterpenes on First-Instar Nymphs of *Rhodnius prolixus. J Med Entomol*. 46(3), 511-515. https://doi.org/10.1603/033.046.0315.

Silva, J. I. M., Souza, M. C. S., Soares, M. L. C., Costa, R. C. & Nunez, C. V. (2016). Óleos essenciais das raízes das espécies de *Philodendron maximum, P. solimoesense e P. goeldii* (ARACEAE). *Rev Fitos*. 10(1), 77-86. http://dx.doi.org/10.5935/2446-4775.20160008

Sousa, D. P. (2012). Medicinal Essential Oils: Chemical, Pharmacological and Therapeutic Aspects. Nova Science Publishers.

Swiech, J. N. D., Bobek, V. B., Folquitto, D. G., Silva, R. Z., Budel, J. M., Farago, P. V., Miguel, M. D. & Miguel, O. G. (2016). Morpho-anatomy of the vegetative organs of *Philodendron meridionale* Buturi & Sakur. *Lat Am J Pharm*. 10, 2142-2148.

Swiech, J. N. D., Folquitto, D. G., Bobek, V. B., Urban, A. M., Betim, F. C. M., Oliveira, L. F., Pereira, C. B., Merino, F. J. Z., Dias, J. de F. G., Silva, R. Z. da Farago, P. V., Miguel, M. D. & Miguel, O. G. (2021). Phytotoxic and Enzymatic Study of *Philodendron meridionale* on seeds of *Lactuca sativa* L. *Research, Society and Development*, *10*(1), e5610111336. https://doi.org/10.33448/rsd-v10i1.11336

Sylvestre, M., Pichette, A., Longtin, A., Nagau, F. & Legault, J. (2006). Essential oil analysis and anticancer activity of leaf essential oil of *Croton flavens* L. from Guadeloupe. *J Ethnopharmacol*. 103(1), 99-102. https://doi.org/10.1016/j.jep.2005.07.011.

Toloza, A. C., Zygadlo, J., Cueto, G. M., Biurrun, F., Zerba, E. & Picollo, M. I. (2006). Fumigant and repellent properties of essential oils and component compounds against permethrin-resistant *Pediculus humanus capitis* (Anoplura: Pediculidae) from Argentina. *J Med Entomol*. 43(5), 889-895. https://doi.org/10.1603/0022-2585.

Toloza, A. C., Zerba, E., Masuh, H. & Picollo, M. I. (2008). Interspecific hybridization of *Eucalyptus* as a potential tool to improve the bioactivity of essential oils against permethrin-resistant head lice from Argentina. *Bioresour Technol*. 99, 7341-7347. https://doi.org/10.1016/j.biortech.2007.12.067.

Toyomasu, T. & Sassa, T. (2010). In *Diterpenes in Comprehensive Natural Products* II. Chemistry and Biology; Mander, L., Liu H. W., Eds.; Newnes: 1.

Viana, F. A., Andrade-Neto, M. & Pouliquen, Y. B. M. (2002). Chemical composition of the essential oil from roots of *Philodendron acutatum* Schott. *J Essent Oil Res*. 14, 172-174. https://doi.org/10.1080/10412905.2002.9699814.

Virador, V. M., Kobayashi, N., Matsunaga, J. & Hearing, V. J. (1998). A standardized protocol for assessing regulators of pigmentation. *Anal Biochem*. 270, 207-219. https://doi.org/10.1006/abio.1999.4090.

Yang, N., Wang, J., Liu, C., Song, Y., Zhang, S., Zi, J., Zhan, J., Masilamani, M., Cox, A, Nowak-Wegrzyn, A., Sampson, H. & Li, X. (2014). Berberine and limonin suppress IgE production by human B cells and peripheral blood mononuclear cells from food-allergic patients. *Ann Allergy Asthma Immunol.* 113(5), 556-564. https://doi.org/10.1016/j.anai.2014.07.021

Yang, Y., Lee, H., Clark, J. M. & Ahn, Y. (2004). Insecticidal activity of plant essential oils against *Pediculus humanus capitis* (Anoplura: Pediculidae). *J Med Entomol***.** 41, 699-704. https://doi.org/10.1603/0022-2585-41.4.699.

Zu-Yin, Y., Yu-Guang, L., He, X., Ya-Jun, S., Bo, D., Rui, H., Ya-Li, F., Zhen-Hu, Z., Ze-Yuan, L., Qin-Shi, Z., Sheng-Qi, W., Jia-Pei, C., Bing-Zhi, M. & Yu-Wen, C. (2007). Melissoidesin G, a diterpenoid purified from *Isodon melissoides*, induces leukemic-cell apoptosis through induction of redox imbalance and exhibits synergy with other anticancer agents. *Int. J. Cancer*. 121, 2084-2094. https://doi.org/10.1002/ijc.22945.