Doi: https://doi.org/10.17584/rcch.2022v16i1.13413

Volatile chemical composition of Colombian *Plectranthus amboinicus* (Lour.) Spreng. essential oil and its biocidal action against *Tribolium castaneum* (Herbst)

Composición química volátil del aceite esencial colombiano de *Plectranthus amboinicus* (Lour.) Spreng. y su acción biocida contra el *Tribolium castaneum* (Herbst)

BEATRIZ EUGENIA JARAMILLO-COLORADO[1, 3]
RAFAEL VEGA-DÍAZ[1]
CRUZ NAYIVE PINO-BENÍTEZ[2]

***Plectranthus amboinicus* (Lour.) Spreng.**

Photo: C.N. Pino-Benítez

ABSTRACT

Plectranthus amboinicus (Lour.) Spreng. is an herbaceous and aromatic plant that belongs to the Lamiaceae family. In this research work, the repellent and insecticidal activity of the essential oil (EO) of *P. amboinicus* from the Condoto township in the department of Choco (Colombia) against the flour weevil *Tribolium castaneum* (Herbst) was studied. EO was isolated by the hydrodistillation technique. The identification of the volatile metabolites of *P. amboinicus* EO was done through gas chromatography coupled to mass spectrometry (GC / MS). The contact method was used for the bioassay of the essential oil's repellent and fumigant activities. In the *Plectranthus amboinicus* EO, fifteen compounds were found. The major compound was carvacrol (75.9%) followed by α-bergamotene, *p*-cymene, α-humulene, 4-terpineol, caryophyllene oxide, β-guaiene, 1-octen-3-ol, α-muurolene, caryophyllene, 3-hexen-2-ol, γ-terpinene, isothymol, 2-carene and β-bisabolene, respectively. The highest repellent activity obtained was 83.33 and 9.67% at a concentration of 0,1% with exposure times of 2 and 4 hours, respectively. The fumigant activity was 100% at a concentration of EO 250 $\mu L\ L^{-1}$. The results indicated that the natural compounds tested may be useful alternatives to control *T. castaneum* infestation.

Additional keywords: essential oils; repellent activity; insecticidal activity, bioprospecting.

[1] Universidad de Cartagena, Faculty of Exact and Natural Sciences, Cartagena de Indias (Colombia).
ORCID Jaramillo-Colorado, B.E.: 0000-0003-1708-2367; ORCID Vega-Díaz, R.: 0000-0001-6310-5836
[2] Universidad Tecnológica del Chocó Diego Luis Córdoba, Faculty of Education Sciences, Quibdo (Colombia).
ORCID Pino-Benítez, C.N.: 0000-0001-7471-8697
[3] Corresponding author. bjaramilloc@unicartagena.edu.co

e-ISSN: 2422-3719 · ISSN-L: 2011-2173

RESUMEN

Plectranthus amboinicus (Lour.) Spreng. es una planta herbácea y aromática, pertenece a la familia de las Lamiaceae. En este trabajo de investigación se estudió la actividad repelente e insecticida del aceite esencial (AE) de *P. amboinicus* proveniente de Condoto corregimiento del departamento de Chocó (Colombia) contra el gorgojo de harina *Tribolium castaneum* Herbst. El AE se aisló mediante la técnica de hidrodestilación. La identificación de los metabolitos volátiles del AE de *P. amboinicus,* se hizo a través de cromatografía de gases acoplada a espectrometría de a masas (CG/MS). Para el bioensayo de la actividad repelente y fumigante del aceite esencial fue utilizado el método de contacto. En el AE de *Plectranthus amboinicus*, se encontraron 15 compuestos mayoritarios. El mayor componente fue carvacol (75,9%) seguido por α-bergamoteno, p-cimeno, α-humuleno, 4-terpineol, óxido cariofilleno, β-guaieno, 1-octen-3-ol, α-muuroleno, cariofileno, 3-hexen-2-ol, γ-terpineno, isotimol, 2-careno y β-bisaboleno, respectivamente. La actividad fumigante fue de 100% a la concentración del EO de 250 $\mu L\ L^{-1}$. Los resultados indicaron que los compuestos naturales probados pueden ser alternativas útiles para controlar la infestación de *T. castaneum.*

Palabras clave adicionales: aceites esenciales; actividad repelente; actividad insecticida, bioprospección.

Received: 31-08-2021 Accepted: 22-10-2021 Published: 06-06-2022

INTRODUCTION

Plectranthus amboinicus (Lour.) Spreng. (synonyms: Colus amboinicus [Lour.]) is an herbaceous, succulent, aromatic plant with fleshy leaves, generally less than 1 m tall. Native to tropical Asia and Africa, it is cultivated in tropical areas of the world (Tramil, 2017), and it belongs to the Lamiaceae family (The Plant List, 2013). In Colombia, it is found in the biogeographic regions of the Caribbean, Pacific, Valle del Cauca, and Valle del Magdalena (0-1,700 m altitude) (Bernal *et al*., 2019).

P. amboinicus is a medicinal species, whose extracts and/or essential oils have various ethnobotanical uses, among which are the treatment of respiratory tract disorders as a bronchodilator and antitussive (Lopes *et al*., 2017); anti-diarrheal (Shubha and Bhatt, 2015); antiparasitic (Lima *et al*., 2014; Ramli *et al*., 2014); antihyperglycemic and antihyperlipidemic (Viswanathaswamy *et al*., 2011); anti-inflammatory (Chen *et al*, 2014); larvicide (Huang *et al*., 2019); antimicrobial and antioxidant (Ajitha *et al*., 2014; Gupta and Negi, 2016; Santos *et al*., 2016; Swamy *et al*., 2017); anticancer (Yulianto *et al*., 2016); among others.

The essential oils produced by plants contain a wide range of secondary metabolites, such as derivatives of monoterpenes, sesquiterpenes, diterpenes, aromatics, hydrocarbons, and fatty acids (Dehsheikh *et al*., 2020).

Plants produce essential oils for various purposes; these have been considered a species of botanical pesticides, and some have been evaluated as insecticides against mosquitoes, flies, etc. (Cossetin *et al*., 2018; Luz *et al*., 2020). The biocidal effect of essential oils and pure compounds on insects can be manifested in various ways including toxicity, mortality, antifeedant activity, growth inhibitor, suppression of reproductive behavior, reduction of fecundity, and fertility (Jankowska *et al*., 2018). Given the diverse biological activities of *P. ambicoides,* the objective of this work was to evaluate its repellent and fumigant activity by using *the Tribolium castaneum* (Herbst) weevil as a biological model and to relate the results to the volatile chemical composition of the essential oil.

MATERIALS AND METHODS

Vegetal material

Plectranthus amboinicus (Lour.) Spreng. plants were collected in Condoto, Choco, Colombia, in 2019. Taxonomic identification was made at the National Herbarium of Colombia. The control leaves of each plant are archived as a permanent sample at the Herbarium (COL No-538449).

Extraction of the essential oil (EO)

EO was obtained through the hydrodistillation method by using Clevenger type distillation equipment (Jaramillo-Colorado *et al*., 2012). 500 g of leaves and stems from *P. amboinicus* were used, finely chopped, and submerged in boiling water by using conventional heating for 2 h. The EO was separated by decantation and then anhydrous Na_2SO_4 (Merck, Darmstadt, Germany) was added to the oil. One EO aliquot (30 μL) was diluted in 1 mL of dichloromethane (Panreac AppliChem, Darmstadt, Germany) for gas chromatography analysis (Jaramillo-Colorado *et al*., 2020).

Chromatography analysis

The EO was analyzed in an Agilent Technologies GC-MS system model 7890A Network GC coupled to a mass selective detector model 5975 (Palo Alto, CA) equipped with a split/split-less injection port (230°C, split ratio 20:1). The mass spectra were obtained by electron-impact ionization at 70 eV energy. GC conditions were as follows: A HP-5MS capillary column (30 m × 0.25 mm id × 0.25 μm df) with 5% phenyl-poly (methyl siloxane) stationary phase was used for the separation of mixtures. The initial oven temperature was 50°C for 2 min and then resumed at a rate of 5°C min· up to 250°C (5 min). The carrier gas was helium, with an inlet pressure at the head of the column of 12.667 psi at a rate of 1.172 mL min^{-1}, at 50°C. The mass spectra and Kovàts retention indexes obtained were compared to those reported in the literature (Adams, 2007).

Insects rearing

Adults of *T. castaneum* were reared in oat (*Avena sativa*). Bioassays were carried out in the dark in incubators at 28-30°C and 70-80% relative humidity at the Agrochemical Research Laboratory of the Universidad de Cartagena.

Repellent activity

The repellent property of *P. amboinicus* EO was analyzed on adult specimens of *T. castaneum*. It was evaluated by using the area preference method. The oil was dissolved in acetone (Panreac AppliChem, Darmstadt, Germany) after preparing 5 solutions (1, 0.1, 0.01, 0.001, and 0.0001%). A 9-cm in diameter filter paper sheet was cut in half and 500 μL of each concentration was applied separately to one of the halves of the filter paper as evenly as possible with a micropipette. The other half (control) was treated with 500 μL of acetone. DEET (N, N-diethyl-toluamide) (Dr. Ehrenstorfer, Germany), which refers to a commercial repellent, was a positive control.

The treated and control media disks were dried at room temperature for 10 min to allow for the evaporation of the solvent. The treated and untreated halves were attached using adhesive tape and placed on Petri dishes. Twenty adult specimens (5 to 7 d old) of *T. castaneum* were placed, one by one, in the center of each filter paper disc with the help of tweezers. The dishes were then covered and, after approximately 5 min, transferred to an incubator at room temperature (Jaramillo-Colorado *et al*., 2020). Four replicates were used for each concentration. Weevil preference was measured for each Petri dish at 2 and 4 h of exposure.

To determine the percentage of repellency (PR), check the following equation following the parameters identified by Jaramillo-Colorado *et al*. (2012) (Eq. 1):

$$PR = [(Nc - Nt) / (Nc + Nt)] \times 100 \quad (1)$$

where, *Nc* number of insects in the control area (acetone) and *Nt* number of insects in the treated area (EO + acetone).

Fumigant activity

Fumigant activity was performed according to Jaramillo-Colorado *et al*. (2020). The toxic effect from *P. amboinicus* EO and terpenes were assayed on *T. castaneum*. Filter paper discs (Whatman No. 1, 2-cm in diameter), laid down at the bottom of Petri dish covers (90 × 15 mm) were used. These were impregnated with oil at doses calculated as to provide equivalent fumigant concentrations of 500, 350, 250, 150, and 50 μg mL^{-1} air of oil, respectively. Twenty adult insects (1 to 10 d old) were introduced and tightly capped (replicated four times for each concentration). Pirilan, a commercial pesticide containing methyl pirimiphos, (Syngenta, Colombia) (organophosphorus pesticide, 300 μg L^{-1} air) as an active ingredient, was used as a positive control. The mortality percentage was determined after 24 and 48 h from the start of exposure.

The percentage of mortality (% mortality) was calculated using the following Equation 2:

% mortality = [(*MT* - *MC*) / (100- *MC*)] * 100 (2)

where, *MT* and *MC* are the number of dead insects in the treated and control areas, respectively.

Statistical analysis

The results were converted into repellent and fumigant percentages and analyzed by ANOVA (Kruskal-Wallis test). Mortality rates were calculated by using the statistical formulas of Abbott and *Probit* to determine the LC_{50}, chi-square values, and related parameters. Biostat, a statistical software (Analyst Soft Robust Business Solutions, BioStat v 2009) was used, with a confidence level of 5%. Four replicates for each analysis were performed.

RESULTS AND DISCUSSION

The essential oil of *P. amboinicus* obtained by hydrodistillation presented a yield of 0.2% (w/w). Table 1 shows the major compounds found in the EO of *P. amboinicus,* extracted by hydrodistillation. Fifteen compounds with a relative area greater than 0.5% were found, where the main analytes were 3-hexen-2-ol- (z) - (0.59%), 1-octen-3-ol (1.97%), 2-carene (0.50%), *p*-cymene (3.48%), γ-terpinene (0.54%), 4-terpineol (2.53%), carvacrol (75.88%), isothymol (0.57%), β-Guaiene (2.1%), α-bergamotene (4.4%), humulene (2.7%), α-murolene (1.3%), β-bisabolene (0.50%), caryophyllene oxide (2.39%).

The main compound found in this oil was carvacrol, which is a phenolic monoterpenoid that has a wide range of bioactivities, such as clinical applications (antioxidant, antimicrobial, and anticancer properties), (Sharifi-Rad *et al*., 2018), repellent, acaricide (Tabari *et al*., 2017, 2015), and insecticidal (Youssefi *et al*., 2019), among others.

The chemical composition in this study differs slightly from the results obtained through other essential oils of *P. amboinicus* from other countries, *i.e.,* in the EO from Paraiba, Brazil, where the principal compounds found were carvacrol (33.50), *p*-cymene (28.20%) and γ-terpinene (14.77%). While a study in India reported the caryophyllene, caryophyllene oxide, aromadendrene oxide, and selinene as the majority components (Vishnu *et al*., 2021), in Malaysia the main constituents of the *P. amboinicus* EOs were carvacrol (43-45%), γ-terpinene (11-16%), and *p*-cymene (12-16%) (Arumugam *et al*., 2020), and in Taiwan, these were was carvacrol (61,5%), β-Caryophyllene (12.79%), γ-terpinene (8.51%) and *p*-cymene (9.42%) (Huang *et al*., 2019). The variation in the proportion

Table 1. Major compounds found in the essential oil of *P. amboinicus*, obtained by GC-MS.

Peak, No.	Compound	T_R (min)	Molecular ion	I_k (HP-5)	Relative area (%)
1	3-Hexen-2-ol	4.24	100.16	857	0.6±0.08
2	1-Octen-3-ol	6.54	128.21	979	2.0±0.50
3	2-Carene	7.36	136.23	1010	0.5±0.05
4	*p*-Cymene	7.57	134.22	1048	3.5±0.50
5	γ-Terpinene	8.38	136.23	1063	0.6±0.22
6	4-Terpineol	11.66	154.25	1089	2.5±0.50
7	Carvacrol	16.03	150.22	1298	75.9±1.20
8	Isothymol	16.99	150.22	1299	0.6±0.09
9	Caryophyllene	18.42	204.35	1420	1.0±0.22
10	α-Bergamotene	18.54	204.35	1431	4.4±0.20
11	α-Humulene	18.79	204.35	1440	2.7±0.50
12	β-Guaiene	19.28	204.35	1455	2.1±050
13	α-Muurolene	20.13	204.35	1499	1.3±0.60
14	β-Bisabolene	20.23	204.35	1509	0.5±0.05
15	Caryophyllene oxide	21.69	220.35	1582	2.4±0.60

T_R: Retention time. I_k: Kováts index performed in apolar column HP-5 (5% phenyl -95% polymethyl siloxane) (30 m × 0.25 mm di × 0.25 um df).

Table 2. Repellent activity of the essential oil of *Plectranthus amboinicus*, and *Stay off Amazonic*, a commercial repellent (DEET) against *Tribolium castaneum* at different exposure times.

Essential oil (EO)	Concentrations (%)	Repellent activity (%)[a]	
		2 h	4 h
P. amboinicus	1	78.67 ± 5.51	84.67 ± 5.42
	0.1	83.33 ± 2.70	92.67± 2.06
	0.01	64.67 ± 6.75	70.00± 5.25
	0.001	48.67± 5.68	58.00 ± 5.36
	0.0001	18.67 ± 2.74	34.33 ± 4.75
DEET (N,N-diethyl-toluamide - *Stay off*)	1	76.25 ± 5.25	78.00 ± 8.44
	0.1	50.05 ± 6.54	60.85 ± 6.61
	0.01	40.62 ± 7.24	54.75 ± 2.74
	0.001	16.44 ± 3.36	18.20 ± 6.75
	0.0001	10.98 ± 5.42	16.25 ± 2.06

Repellent activity value = mean ± standard deviation.

and yield percentage of essential oils and their chemical composition can be due to the influence of agroecology and environmental conditions (Aguiar *et al*., 2015).

The results of the repellent activity of the essential oil of *P. amboinicus* and a commercial repellent against *T. castaneum*, are shown in table 2.

The essential oil of *P. amboinicus* presented the highest percentage of repellency at a concentration of 0.1% at 2 and 4 h of exposure (83.33 and 92.67%, respectively). EO results were compared to those of the commercial repellent based on DEET (N, N-diethyltoluamide). At the concentration of 0.1% DEET, the repellency percentages obtained at 2 and 4 h of exposure against *T. castaneun* were 50.05 and 60.85%, respectively. Significant differences (Kruskal-Wallis test $P<0.05$) were found between the concentrations for the repellent percentage.

Other studies have shown the potential of essential oils from *P. amboinicus* and their blend as mosquito repellents against *A. aegypti*, the vector of dengue, chikungunya, and yellow fever (Lalthazuali and Mathew, 2017), as well as against bites of *Lutzomyia migonei*, the Leishmania vector (Nieves *et al*., 2010), and *L.* (*Viannia*) *braziliensis* (Lima *et al*., 2014).

The results obtained for the fumigant activity of the essential oil of *P. amboinicus* against the flour weevil (*T. castaneum*) are recorded in table 3. Therein, it can be observed that at a concentration of 250 μg mL^{-1} of AE air it reached a mortality rate of 100%.

Table 3. Fumigant activity of essential oil from *P. amboinicus* against *T. castaneum*.

Concentrations (μg mL^{-1})	Mortality (%)	
	24 h	48 h
50	17.50 ± 2.5	21.61 ± 3.0
150	73.33 ± 1.9	85.00 ± 1.8
250	100 ± 0	100 ± 0
350	100 ± 0	100 ± 0
500	100 ± 0	100 ± 0

The values reflect the average of the four replicates ± the standard mean deviation.

Significant differences (Kruskal-Wallis test, ($P<0.05$) were found between the concentrations and the mortality percentage.

Table 4 exhibits the mean lethal concentrations (LC_{50}) obtained for the essential oil of *P. amboinicus* in two exposure periods. The fumigant toxicity of the EO was evaluated on adult *T. castaneum* weevils.

The interpretation for 95% FL is that, with 95% confidence, the required lethal concentration to achieve 50% mortality in the study population species will be within the lower limit and upper limit.

The results of the *probit* analysis showed that the pyrimiphos methyl (positive control), at 24 h of exposure obtained an LC_{50} = 188.673 [114.824; 246.333] μg mL^{-1} air, at 48 h at LC_{50} = 84.2145 [77.023; 147.414] μg mL^{-1} air, pirimiphos-methyl. The *P. amboinicus* EO's had a toxicity level in the first 24 h like that of the pirimiphos-methyl, but 2.16 times more lethal in the first 48 h times than the essential oil under study,

Table 4. Toxicity of the essential oil from *P. amboinicus* and pirimiphos-methyl against *T. castaneum.*

Treatments	Period (h)	LC_{50} (95% FL)	χ^2 (df)	Slope ± SE
P. amboinicus	24	182.070 [158.040; 209.753]	0,998 (39)	1.977± 0.031
	48	136.937 [116.743; 160.623]	1.000 (36)	1.751 ± 0.035
Commercial insecticide (pirimiphos-methyl)	24	188.673 [114.824; 246.333]	1.449 (3)	0.0198 ± 0.0015
	48	84.2145 [77.023; 147.414]	1.758 (3)	0.0159 ± 0.0015

FL: Fiducial limits, χ^2: Chi-square, df: Degrees of freedom, SE: standard error, *n=5*.

because *P. amboinicus* EO yielded an LC_{50} = 182.070 μg mL^{-1} air at 24 h, and LC_{50} = 136.937 μg mL^{-1} air at 48 h of exposure.

The EO from *P. amboinicus* evaluated in this study showed significant fumigant and repellent activity. According to the literature, the main compound found in the oil, carvacrol, had insecticidal activity and neurophysiological effects against *Cimex lectularius* L (Gaire *et al*., 2019); *Culex pipiens pallens* (Youssefi *et al*., 2019; Ma *et al*., 2014); and *R. dominica* and *L. serricone* (Ramadan *et al*., 2020)*, Ixodes ricinus* (Tabari *et al*., 2017); *Dermanyssus gallinae* (Tabari *et al*., 2015); among others.

Carvacrol has delocalized electron and hydroxyl groups. Ultee *et al*. (2002) suggested that the hydroxyl group and delocalized electron of carvacrol is essential for biological activities.

CONCLUSIONS

The results obtained from the essential oil from *P. amboinicus* display great potential to develop natural biocides to control *Tribolium castaneun* Herbst due to its terpene-rich chemical composition.

Acknowledgments

Authors thank funding from *Ministerio de Ciencia, Tecnología e Innovación, Ministerio de Educación Nacional, Ministerio de Industria, Comercio y Turismo*, and *ICETEX*, Programa *Ecosistema Científico-Colombia Científica* from *Fondo Francisco José de Caldas*; Grant RC-FP44842-212-2018. *Universidad Tecnológica del Chocó*, Natural Products Group, and Dr. Yuber Palacios; Research Groups Support Program Research Vicepresidency at *Universidad de Cartagena* (2016-2020).

Conflict of Interest: The manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

BIBLIOGRAPHY REFERENCES

Adams, R.P. 2007. Identification of essential oil components by gas chromatography/mass spectroscopy. 4th ed. Allured Publishing, Carol Stream, IL.

Aguiar, J.J.S., C.P.B. Sousa, M.K.A. Araruna, M.K.N. Silva, A.C. Portelo, J.C. Lopes, V.R.A. Carvalho, F.G. Figueredo, V.C.N. Bitu, H.D.M. Coutinho, T.A.S. Miranda, and E.F.F. Matias. 2015. Antibacterial and modifying-antibiotic activities of the essential oils of *Ocimum gratissimum* L. and *Plectranthus amboinicus* L. Eur. J. Integr. Med. 7(2), 151-156. Doi: 10.1016/j.eujim.2014.10.005

Ajitha, B., Y.A.K. Reddy, and P.S. Reddy. 2014. Biosynthesis of silver nanoparticles using *Plectranthus amboinicus* leaf extract and its antimicrobial activity. Spectrochim. Acta A Mol. Biomol. Spectrosc. 128, 257-262. Doi: 10.1016/j.saa.2014.02.105

Arumugam, G., U.R. Sinniah, M.K. Swamy, and P.T. Lynch. 2020. Micropropagation and essential oil characterization of *Plectranthus amboinicus* (Lour.) Sprengel, an aromatic medicinal plant. In Vitro Cell. Dev. Biol.-Plant. 56, 491-503. Doi: 10.1007/s11627-020-10056-1

Bernal, R., S.R. Gradstein, and M. Celis (eds.). 2019. Catálogo de plantas y líquenes de Colombia. In: *database* Universidad Nacional de Colombia, Instituto de Ciencias Naturales, http://catalogoplantasdecolombia.unal.edu.co; consulted: 10-06-2021.

Chen, Y.-S., H.-M. Yu, J.-J. Shie, T.-J. Cheng, C.-Y. Wu, J.-M. Fang, and C.-H. Wong. 2014. Chemical constituents of *Plectranthus amboinicus* and the synthetic analogs possess anti-inflammatory activity. Bioorg. Med. Chem. 22(5), 1766-1772. Doi: 10.1016/j.bmc.2014.01.009

Cossetin, L.F., E.M.T. Santi, J.F. Cossetin, J.B. Dillmann, M.D. Baldissera, O.I. Garlet, T.P. Souza, L. Loebens, B.M. Heinzmann, M.M. Machado, and S.G. Monteiro. 2018. *In vitro* safety and efficacy of lavender essential oil (Lamiales: Lamiaceae) as an insecticide against houseflies (Diptera: Muscidae) and blowflies (Diptera: Calliphoridae). J. Econ. Entomol. 111(4), 1974-1982. Doi: 10.1093/jee/toy145

Dehsheikh, A.B., M.M. Sourestani, P.B. Dehsheikh, J. Mottaghipisheh, S. Vitalini, and M. Iriti. 2020. Monoterpenes: Essential oil components with valuable features. Mini. Rev. Med. Chem. 20(11), 958-974. Doi: 10.2174/1389557520666200122144703

Gaire, S., M.E. Scharf, and Gondhalekar. 2019. Toxicity and neurophysiological impacts of plant essential oil components on bed bugs (Cimicidae: Hemiptera). Sci. Rep. 9(1), 3961. Doi: 10.1038/s41598-019-40275-5

Gupta, S.K. and P.S. Negi. 2016. Antibacterial activity of indian borage (*Plectranthus amboinicus* Benth) leaf extracts in food systems and against natural microflora in chicken. meat. Food Technol. Biotechnol. 54(1), 90-96. Doi: 10.17113/ftb.54.01.16.3973

Huang, H.-T., C.-C. Lin, T.-C. Kuo, S.-J. Chen, and R.-N. Huang. 2019. Phytochemical composition and larvicidal activity of essential oils from herbal plants. Planta 250, 59-68. Doi: 10.1007/s00425-019-03147-w

Jankowska, M., J. Rogalska, J. Wyszkowska, and M. Stankiewicz. 2018. Molecular targets for components of essential oils in the insect nervous system. A review. Molecules 23(1), 34. Doi: 10.3390/molecules23010034

Jaramillo-Colorado, B.E., I.P. Martelo, and E. Duarte. 2012. Antioxidant and repellent activities of the essential oil from Colombian *Triphasia trifolia* (Burm. f.) P. Wilson. J. Agric. Food Chem. 60(25), 6364-6368. Doi: 10.1021/jf300461k

Jaramillo-Colorado, B.E., F.M. Palacio-Herrera, and C.N. Pino-Benitez. 2020. Volatile chemical composition of Colombian *Piper gorgonillense* Trel. & Yunck. essential oil and its repellent and fumigant activity against *Tribolium castaneum* Herbst. Rev. Colomb. Cienc. Hortic. 14(3), 424-433. Doi: 10.17584/rcch.2020v14i3.11061

Lalthazuali and N. Mathew. 2017. Mosquito repellent activity of volatile oils from selected aromatic plants. Parasitol. Res. 116(2), 821-825. Doi: 10.1007/s00436-016-5351-4

Lima, S.C.G., M.J. Teixeira, J.E.G. Lopes Júnior, S.M. Morais, A.F. Torres, M.A. Braga, R.O. Rodrigues, G.M.P. Santiago, A.C. Martins, and A.T. Nagao-Dias. 2014. *In vitro* and *in vivo* leishmanicidal activity of *Astronium fraxinifolium* (Schott) and *Plectranthus amboinicus* (Lour.) Spreng against *Leishmania (Viannia) braziliensis*. BioMed. Res. Int. 2014, 848293. Doi: 10.1155/2014/848293

Lopes, P.Q., F.B. Carneiro, A.L.B. Souza, S.G. Santos, E.E. Oliveira, and L.A.L. Soarez. 2017. Technological evaluation of emulsions containing the volatile oil from leaves of *Plectranthus amboinicus* Lour. Pharmacogn. Mag. 13(49), 159-167.

Luz, T.R.S.A., L.S.S. Mesquita, F.M.M. Amaral, and D.F. Coutinho. 2020. Essential oils and their chemical constituents against *Aedes aegypti* L. (Diptera: Culicidae) larvae. Acta Trop. 212, 105705. Doi: 10.1016/j.actatropica.2020.105705

Ma, W.-B., J.-T. Feng, Z.-L. Jiang, H. Wu, Z.-Q. Ma, and X. Zhang. 2014. Fumigant activity of eleven essential oil compounds and their selected binary mixtures against *Culex pipiens pallens* (Diptera: Culicidae). Parasitol. Res. 113(10), 3631-3637. Doi: 10.1007/s00436-014-4028-0

Nieves, E., J. Fernández Méndez, J. Lias, M. Rondón, and B. Briceño. 2010. Actividad repelente de aceites esenciales contra las picaduras de *Lutzomyia migonei* (Diptera: Psychodidae). Rev. Biol. Trop. 58(4),1549-1560.

Ramadan, G.R.M., S.A.S. Abdelgaleil, M.S. Shawir, A.S. El-Bakary, K.Y. Kun, and T.W. Phillips. 2020. Terpenoids, DEET and short-chain fatty acids as toxicants and repellents for *Rhyzopertha dominica* (Coleoptera: Bostrichidae) and *Lasioderma serricorne* (Coleoptera: Ptinidae). J. Stored. Prod. Res. 87, 101610. 10.1016/j.jspr.2020.101610

Ramli, N., P.O.S. Ahamed, H.M. Elhady, and M. Taher. 2014. Antimalarial activity of Malaysian *Plectranthus amboinicus* against *Plasmodium berghei*. Pharmacogn. Res. 6(4), 280-284. Doi: 10.4103/0974-8490.138248

Santos, F.A.V., C.G. Serra, R.J.A.C. Bezerra, F.G. Figueredo, Edinardo, F.F. Matias, I.R.A. Menezes, J.G.M. Costa, and H.D.M. Coutinho. 2016. Antibacterial activity of *Plectranthus amboinicus* Lour (Lamiaceae) essential oil against *Streptococcus mutans*. Eur. J. Integr. Med. 8(3), 293-297. Doi: 10.1016/j.eujim.2015.11.021

Sharifi-Rad, M., E.M. Varoni, M. Iriti, M. Martorell, W.N. Setzer, M. Del Mar Contreras, B. Salehi, A. Soltani-Nejad, S. Rajabi, M. Tajbakhsh, and J. Sharifi-Rad. 2018. Carvacrol and human health: A comprehensive review. Phytother. Res. 32(9), 1675-1687. Doi: 10.1002/ptr.6103

Shubha, J.R. and P. Bhatt. 2015. *Plectranthus amboinicus* leaves stimulate growth of probiotic *L. plantarum*: Evidence for ethnobotanical use in diarrhea. J. Ethnopharmacol. 166, 220-227. Doi: 10.1016/j.jep.2015.02.055

Swamy, M.K., G. Arumugam, R. Kaur, A. Ghasemzadeh, M.M. Yusoff, and U.R. Sinniah. 2017. GC-MS based metabolite profiling, antioxidant and antimicrobial properties of different solvent extracts of Malaysian *Plectranthus amboinicus* leaves. Evid. Based Complement. Altern. Med. 2017, 1517683. Doi: 10.1155/2017/1517683

Tabari, M.A., M.R. Youssefi, A. Barimani, and A. Araghi. 2015. Carvacrol as a potent natural acaricide against *Dermanyssus gallinae*. Parasitol. Res. 114(10), 3801-3806. Doi: 10.1007/s00436-015-4610-0

Tabari, M.A., M.R. Youssefi, F. Maggi, and G. Benelli. 2017. Toxic and repellent activity of selected monoterpenoids (thymol, carvacrol and linalool) against the castor bean tick, *Ixodes ricinus* (Acari: Ixodidae). Vet. Parasitol. 245, 86-91. Doi: 10.1016/j.vetpar.2017.08.012

The Plant List. 2013. Version 1.1. In: *database*, http://www.theplantlist.org/; consulted: May, 2021.

Tramil. 2017. *Plectranthus amboinicus*. In: Framacopedia, http://www.tramil.net/es/plant/plectranthus-amboinicus; consulted: July, 2021.

Ultee, A., M.H.J. Bennik, and R. Moezelaar. 2002. The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen *Bacillus cereus*. Appl. Environ. Microbiol. 68(4), 1561-1568. Doi: 10.1128/AEM.68.4.1561-1568.2002)

Vishnu Priya, N., U.G. Vinitha, and MM. Sundaram. 2021. Preparation of chitosan-based antimicrobial active food packaging film incorporated with *Plectranthus amboinicus* essential oil. Biocatal. Agric. Biotechnol. 34, 102021. Doi: 10.1016/j.bcab.2021.102021

Viswanathaswamy, A.H.M., B.C. Koti, A. Gore, A.H.M. Thippeswamy, and R.V. Kulkarni. 2011. Antihyperglycemic and antihyperlipidemic activity of *Plectranthus amboinicus* on normal and alloxan-induced diabetic rats. Indian J. Pharm. Sci. 73(2), 139-145. Doi: 10.4103/0250-474x.91572

Youssefi, M.R., M.A. Tabari, A. Esfandiari, S. Kazemi, A.A. Moghadamnia, S. Sut, S. Dall'Acqua, G. Benelli, and F. Maggi. 2019. Efficacy of two monoterpenoids, carvacrol and thymol, and their combinations against eggs and larvae of the West Nile Vector *Culex pipiens*. Molecules 24(10), 1867. Doi: 10.3390/molecules24101867

Yulianto, W., N. Andarwulan, P.E. Giriwono, and J. Pamungkas. 2016. HPLC-based metabolomics to identify cytotoxic compounds from *Plectranthus amboinicus* (Lour.) Spreng against human breast cancer MCF-7Cells. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 1039, 28-34. Doi: 10.1016/j.jchromb.2016.10.024