Journal of Experimental Biology and Agricultural Sciences, February - 2023; Volume – 11(1) page 119 – 131

Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

Peruvian plant resources as potential natural controllers of adult *Aedes aegypti*

Ofelia Magdalena Córdova Paz Soldán[1], Franklin Vargas Vásquez[2*], Edmundo Venegas Casanova[3], Ricardo Diego Duarte Galhardo de Albuquerque[4]

[1]Grupo de Investigación Biomedicina Molecular y Enfermedades Metaxénicas. Departamento de Ciencias. Universidad Privada Antenor Orrego. Perú
[2]Instituto de Microbiología y Parasitología Tropical. Universidad Nacional de Trujillo. Perú
[3]Facultad de Farmacia, Universidad Nacional de Trujillo, Perú
[4]Laboratorio de Tecnologia em Produtos Naturais, Faculdade de Farmacia, Universidade Federal Fluminense, Brasil

Received – September 22, 2022; Revision – January 19, 2023; Accepted – February 23, 2023
Available Online – February 28, 2023

DOI: http://dx.doi.org/10.18006/2023.11(1).119.131

KEYWORDS

Natural pesticides

Aedes aegypti

Cymbopogum citrates

Rosmarinus officinalis

Minthostachys mollis

Biological control

ABSTRACT

Aedes aegypti is an important vector of tropical diseases like Dengue, Zika, Chikungunya, and Yellow Fever and affects mainly countries located in tropical and subtropical zones, including Peru. Synthetic insecticides are used to control this vector, but they also cause a residual effect on the environment, whereas the vector has developed resistance to these compounds, so there is a current need to search for new control alternatives, such as the use of abundant natural resources. Therefore, this work aimed to evaluate the biocidal activity of extracts and oils from *Cymbopogum citratus*, *Rosmarinus officinalis*, and *Minthostachys mollis* on adult *Aedes aegypti*, as well as to evaluate their quality parameters. Furthermore, the chemical profile of the three species was assessed by ultra-high-performance liquid chromatography coupled with mass spectrometry (LC-MS/MS). The results showed that the aqueous/ethanolic extracts and the essential oils from the three evaluated species presented a biocidal effect on adult *A. aegypti*. Regarding the analysis of the chemical profile, 15 compounds were identified in *R. officinalis*, while 29 compounds were identified from *C. citratus* and 30 compounds from *M. mollis*. Moreover, the extracts and oils presented quality parameters according to standards. In conclusion, the biocidal potential of the *C. citratus*, *R. officinalis*, and *M. mollis* on *A. aegypti* adults was reported so that they can be seen as a real natural alternative for the control of tropical diseases transmitted by this vector so that plant products are more eco-friendly and subject to lower resistance by target organisms.

* Corresponding author
E-mail: frvargasv@yahoo.es (Franklin Roger Vargas Vásquez)

Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI] (http://www.horizonpublisherindia.in/).
All rights reserved.

All the articles published by Journal of Experimental Biology and Agricultural Sciences are licensed under a Creative Commons Attribution-NonCommercial 4.0 International License Based on a work at www.jebas.org.

1 Introduction

In recent years, globalization, unplanned urbanization, and environmental problems, including climate change, are considerable factors that influence the transmission of diseases, among which vector-borne diseases are the most common (Chala and Hamde 2021). Vector-borne diseases of public health importance are those infectious diseases that are spread by organisms that carry viruses, parasites, or bacteria from one infected person (or animal) to another. These diseases account for 17% of the estimated global burden of infectious diseases and are more frequent in tropical and subtropical areas and places with problems with appropriate water access and sanitation (OPS 2019; WHO 2020).

In Peru, there is a group of diseases that share the same vector: *Aedes aegypti*, which is a hematophagous diptera distributed mainly in tropical areas, responsible for the transmission of several arboviruses that cause diseases such as dengue, Zika, yellow fever, and chikungunya (Cabezas et al. 2015; Dueñas-López 2022). The control of these diseases is linked to the eradication of the mosquito vector so that the habits of the mosquito guide its management to the elimination of breeding sites, which in turn are maintained due to poor hygiene habits of the population, which tends to discard garbage in patios, streets and vacant lots (Pereira et al. 2022).

The use of chemical insecticides is primarily adopted in Peru due to their effectiveness in reducing the populations of larvae and adults. The most widely used insecticides are organophosphates (temephos for eliminating larvae during focal treatment) and fenthion, fenitrothion and malathion for eliminating adult mosquitoes) and pyrethroids (deltamethrin, lambdacyalothrin, cypermethrin, and cyfluthrin). However, the WHO periodically monitors the emergence of resistance to the recommended pesticides and indicates the use of alternative substances that can be used, including in Peru (Lazcano et al. 2009; MINSA 2015).

Furthermore, the development of resistance in *A. aegypti* mosquitoes to synthetic insecticides is the main problem that affects control strategies caused by the intensive use of these products due to the selection of resistance genes in the populations of this species. Some authors have mentioned that the mechanism associated with resistance to organophosphate insecticides could be linked to the elevation of esterases (Lazcano et al. 2009).

Nonetheless, botanical insecticides are another accessible and low-cost control alternative for farmers and communities since several plant species have insecticidal activity. Insecticides of plant origin have the advantage of being more biodegradable than their synthetic counterparts and are related to less development of resistance, often found with immediate availability (Demirak and Canpolat 2022). The secondary metabolites produced by plants in the face of attacks by insects and predators make them natural candidates for use in controlling insect species, including agricultural pests and disease vectors (Pino et al. 2013).

In this context, plant species found mainly in Peru as *Rosmarinus officinalis*, *Cymbopogum citratus*, and *Minthostachys mollis*, demonstrated larvicidal, insecticidal, and repellent activities against different insects (Gillij et al. 2008; Duarte et al. 2015; Soonwera and Sittichok 2020; Rocha et al. 2022), so the objective of this present study was to evaluate the insecticidal potential of these species on adult *A. aegypti*.

2 Materials and Methods

2.1 Insects

Adults of *A. aegypti* were obtained from the towns of Rio Seco (Trujillo), provided by the Institute for Research in Microbiology and Tropical Parasitology (INIMYPAT) of the Program of Microbiology and Parasitology, Universidad Nacional de Trujillo (Peru).

2.2 Plant material

The collection of the plant species was carried out by the classic method of herborization. 12 kg of the *Rosmarinus officinalis* leaves were collected from Trigopampa, located at geographic coordinates with south latitude 07º53.351´, west longitude 078º34.600´, altitude 2,617 m, Otuzco province, La Libertad region, Peru, in April. On the other hand, the same amount (kg) of the *C. citratus* leaves were collected from Galindo, located at geographic coordinates with south latitude 08°4’47,08812’’, west longitude 078°5439,348´, altitude 89 m, Laredo district, Trujillo province, Peru, in February. Also, 12 kg of the *M. mollis* leaves were collected from Galindo, located at geographic coordinates with south latitude 07º53.356´, west longitude 078º34.611´, altitude 2,616 m, Otuzco province, La Libertad region, Peru, in April.

The plant material was selected from each plant species by separating the different parts to avoid mixing with each other and avoiding the inclusion of organic and inorganic residues. Then, a complete specimen of each plant was provided to the Truxillense Herbarium of the National University of Trujillo, to be deposited with the Herbarium codes N° 60304 for *R. officinalis*, N° 60305 for *C. citratus* and code N° 60306 for *M. mollis*.

2.3 Preparation of plant samples

Selected plant leaves were dried at room temperature under shade, then placed in Kraft paper bags in an oven at 40 °C for 48 hours. The dried leaves were pulverized and sieved (up to a particle size of 2.0 mm) and then properly stored in amber bottles, remaining in a place without humidity and direct light.

For the essential oil extraction, 10 kg of plant material from each plant was subjected to steam distillation for 2 hours. Then, the essential oils were purified with anhydrous sodium sulfate, received in an amber glass bottle, sealed to prevent contact with light and oxygen, and stored under refrigeration at 4°C until later use. To estimate the essential oil yield, 100 g of leaves were placed in a 1 L capacity flask and conditioned in the Clevenger equipment, proceeding to distill for 2 hours. The oil obtained was measured in the graduated tube. The essential oil yield was expressed as a volume/weight percentage (ml of essential oil per 100g of plant material) (Miranda and Cuellar 2002).

To obtain the extracts, 100 g of the dried leaves were weighed, packed in a nylon bag, and then introduced into a balloon over an electric stove. Afterward, 250 mL of different solvents of increasing polarity (hexane, ethyl acetate, ethanol, distilled water) was added to begin the extraction process. Finally, each extract was filtered through a vacuum pump and stored in an amber bottle until use (Miranda and Cuellar 2002).

2.4 Determination of total solids concentration

An aliquot of 1 mL was taken from each extract, placed on a previously weighed capsule, and then put in an oven at 40°C for 24 hours. After that time, it was introduced into the desiccator (with silica gel) to cool for 30 minutes. Calculations were made by weight difference in mg/mL. This procedure was performed in triplicate (Miranda and Cuellar 2002).

2.5 Determination of the quality parameters of the plant species

2.5.1 Determination of moisture

The loss on drying method carried out the determination of water. In a previously tared cardboard box, 100 g of the fresh vegetable drug was weighed and dried in an oven at 40 °C for 48 hours until a constant weight was obtained. After, the sample was cooled in desiccators for 30 minutes (Miranda and Cuellar 2002).

2.5.2 Determination of total ashes

The determination of complete ashes was carried out by the gravimetry method. For this, 2 g of the crushed vegetable drug was weighed in a previously tared porcelain crucible, carbonized in the kitchen, and then incinerated in a muffle oven at 700°C for 2 hours. After, the sample was cooled in a desiccator for 30 minutes (Miranda and Cuellar 2002).

2.5.3 Extraction of ethanol/water-soluble substances

The extraction was carried out by the hot extraction method. Based on the solubility of soluble substances in water and ethanol 96°GL, 5g of previously dried and crushed plant leaves were weighed in a 100 mL round-bottom flask for each system. After this, 50 mL of solvent (water and ethanol 96°GL) were added, respectively, stirred, and allowed to stand for 1 hour. A reflux condenser was attached to the flask, and the sample was subjected to a constant boil for 45 min. Then, it was quickly shaken and filtered, transferred to a flask, and refluxed again. The extracts were combined and calibrated to 100 mL. Finally, 5 mL of each extract was measured in a previously tared porcelain dish, brought to dryness in a water bath, allowed to cool in a desiccator, and weighed without delay. The procedure was performed in triplicate. In addition, the calculations were obtained by gravimetry, and the content of extractable matter was expressed in mg/g of dry vegetable matter (Miranda and Cuellar 2002).

2.6 Phytochemical screening

For the phytochemical analysis, the Draggendorff test was carried out for the determination of alkaloids, as well as the foam test, for the identification of saponins, in addition to the Ballet test (for lactones), ferric chloride test (for phenolics and tannins), Borntrager test (for quinones), Nihidrine test (for free amino acids and amines), Fehling test (for reducer sugars), Gelatin test (for tannins) and Liebermann-Burchard test, for identification of terpenes, steroids, and terpenoids (Wagner and Bladt 2004).

2.7 Chemical identification of plant extracts

The chemical identification of plant extracts was carried out by ultra-high performance liquid chromatography coupled with double mass spectrometry (UHPLC-MS/MS) analysis. In a clean and dry vial, 10 mg of the samples were placed. 10 mL of ACN-H_2O solution (8:2) was added and stirred in ultrasound equipment for 20 minutes at room temperature. The obtained solutions were diluted 10 times with the same solvent to reach 0.1 mg/mL final concentration. Chromatographic separation was achieved on a Dionex Ultimate 3000 UHPLC system (Thermo Scientific) equipped with aLuna© Omega C18 100 Å, Phenomenex (150 x 2.1 mm, 1.6µm) column. Formic acid 1% (v/v) in H_2O (A) and MeCN (B) mobile phases were used. The gradient conditions were as follows: 0-1 min 90-10% B; 1-18 min 90-10% B; 18-20 min 5-95% B; 20-25 min 5-95% B. The flow of the mobile phase was 300 µL/min, and the injection volume is of 3 µL. The column temperature was kept at 40 °C. The UV chromatograms were obtained in a range between 200 and 750 nm.

The mass spectrometer Q Exactive Plus (Thermo Scientific) was equipped with an electrospray ion (ESI) source operated in positive and negative ionization modes. The "spray voltage" was kept at 3.5-2.5KV. The drying temperature was 250-300 °C, and the sheath gas flow rate was 50 AU. Nitrogen was used as the dry, fog, and collision gas, with a heat temperature of 400 °C. The collision

energy was set at 30 eV. HRESIMS and MS/MS spectra were acquired in the m/z range of 120 to 1200 amu.

2.8 Evaluation of biocidal activity

2.8.1 Stabilization of *A. aegypti* strains in the insectary

After INIMYPAT provided the papers containing *A. aegypti* from Rio Seco (Trujillo), they were transferred to plastic sources (48.5cm x 35cm x 5cm) with dechlorinated water and placed in the larval rearing boxes (polystyrene). The feeding was made with fish food (finely crushed to powder), and placed in the dechlorinated water from the first presence of larvae. When they reached the pupal stage, they were transported to the insectary, where they were placed in the rearing cages for adults and waited until the adult emerged. Once the adults emerged, they were fed with a 5% sugar solution; the females were provided with the help of a guinea pig (with blood so that it could carry out the oviposition process). For this, the guinea pig was anesthetized with Ketamine (0.1 ml) and placed in the adult rearing cage for 45 minutes so that the females could feed. Three days after the copulation between male and female, the eggs were placed in the strips of craft paper (ovitrap), and after five days (for the embryogenesis stage), the strips were removed and stored in a box at room temperature. This process was carried out until the obtaining of F2 to carry out the bioguided tests (Brogdon and Chan 1998).

2.8.2 Bioassay in *Aedes aegypti* adults

The CDC bottle biological assay method was used. Previously washed, four 250 ml wide-mouth glass bottles (CORNING) were used for each biological assay: three for the test replicates and one for the positive control (insecticide). The extracts (hexane, ethyl acetate, ethanol, and aqueous) and essential oils from the plants described in this study were evaluated. The bottles used for the experimentation were washed with hot soapy water and rinsed at least three times thoroughly. To dry the bottles, they were placed in an oven at 50°C for 15-20 minutes until completely dry. Then, each bottle was impregnated by placing 1 ml of the solution under study from each plant to be evaluated, exposing the entire internal surface of the bottle to the solution.

This procedure was carried out in a fume hood to facilitate the solvent's evaporation and guarantee the researcher's safety when working with organic solvents. The bottles were allowed to dry before starting the tests, covering them with paper or aluminum foil to protect the formulated solution from degradation due to the effect of light. Following the steps above, the positive control bottle was impregnated with 2% malathion. Soon after, using a vacuum cleaner, 15 female mosquitoes are introduced into each bottle. The timer was activated, and the bottles were examined at Time 0; the number of dead and/or alive mosquitoes was counted every 15 minutes until the total number of mosquitoes was dead or until 2 hours had passed since the beginning (Brogdon and Chan 1998).

The criteria proposed by the World Health Organization (WHO) to assess the significance of the resistance values detected are: a) 98%–100% mortality during the recommended diagnosis period, which will indicate susceptibility in the population; b) 90%–97% mortality during the recommended diagnosis time will suggest the possibility of resistance and must be confirmed; c) <90% mortality during the recommended diagnosis time suggests resistance (WHO 2016).

2.8.3 Statistical Analysis

The data were submitted to the analysis of variance (ANOVA) and with Tukey's multiple comparison tests to determine the significant differences between the experimental and control groups with a significance level of 0.05. The InfoStat version 2018 program was used.

3 Results

3.1 Yield of essential and determination of total solids concentration

The yields of essential oils from *R. officinalis*, *C. citratus*, and *M. mollis* were 0.8, 1.0, and 0.3 % RAE (v/w), respectively. Also, the concentration of total solids in the extracts and oils was determined for each species. For *R. officinalis*, the solid concentrations in the extracts were the following: 190.03 mg/mL for the aqueous extract, 75.30 mg/mL for the ethanolic extract, and 207 µg/mL for the essential oil, while for *C. citratus*, values were: 261.23 mg/mL for the aqueous extract, 57.90 mg/mL for the ethanolic extract, and 20 µg/mL for the essential oil. For *M. mollis*, the solid concentrations were: 165.30 mg/mL for the aqueous extract, 90.30 mg/mL for the ethanolic extract, and 20 µg/mL for the essential oil (Table 1).

Table 1 Determination of the total solids concentration in the oil and extracts

Plant Species	Essential Oils	Ethanolic Extract 70° GL	Aqueous Extract
R. officinalis	20µg/mL	75.30 mg/mL	190.03 mg/mL
C. citratus	20µg/mL	57.90 mg/mL	261.23 mg/mL
M. mollis	20µg/mL	90.30 mg/mL	165.30 mg/mL

Table 2 Quality parameters of the studied plant species

Quality Parameters	Plant Species		
	R. officinalis	*C. citratus*	*M. mollis*
Water Weight Loss	52.3%	71.4%	65.3%
Total Ashes	5.32%	4.17%	7.66%
Extracted Substances	Metabolites Concentration		
H_2O	12.94 mg/ mL	21.74 mg/mL	16.86mg/mL
Etanol	11.34 mg/ mL	16.86 mg/mL	12.54mg/mL

Table 3 Phytochemical screening of the studied plant species

Assays	Metabolites	Plant Species					
		R. officinalis		*C. citratus*		*M. mollis*	
		Etanol	Water	Etanol	Water	Etanol	Water
Ferric chloride	Polyphenols	++	++	+++	+++	+++	+++
Gelatin test	Tannins	+	-	+	-	++	-
Liebermann-Burchard	Triterpenes/Steroids	-	NE	-	NE	+	NE
Borntrager	Quinones	-	NE	-	NE	NE	NE
Dragendorff	Alkaloids	-	-	-	-	++	-
Baljet	Lactones	-	NE	-	NE	-	NE
Ninhidrina	Free amines	++	++	+++	+++	+++	+++
Felhing	Reducing sugars	++	++	+++	+++	+++	+++

Here Identification: Presence (+), Absence (-); Intensity: Low (+), Moderate (++), High (+++); Not executed: (NE)

3.2 Determination of the quality parameters of the plant species

After evaluating the quality parameters of the species under study, the following results were obtained for *Rosmarinus officinalis*: water weight loss of 52.3% and total ashes equal to 5.32%. The water/ethanol soluble substances concentrations were 12.94 mg/mL (aqueous extract) and 11.34 mg/mL (ethanolic extract). For *C. citratus*, it presented a water weight loss of 71.4% and total ashes equal to 4.17 %. The concentration of water/ethanol soluble substances was 21.74 mg/mL (aqueous extract) and 16.86 mg/ml (ethanolic extract). For *M. mollis*, the water weight loss was 65.3%, and total ashes were equal to 7.66%. The concentration of water/ethanol soluble substances was 16.86 mg/mL (aqueous extract) and 12.54 mg/mL (ethanolic extract), so it can be concluded that the best extraction of secondary metabolites in both species was obtained by the aqueous extract (Table 2).

3.3 Phytochemical screening

Phytochemical screening was carried out for the three plant species. All species reported the presence of polyphenols, tannins, free amines and reducing sugars. Also, triterpenes/steroids and alkaloids were found in *M. mollis* (Table 3).

3.4 Chemical Identification by UHPLC-MS/MS

Altogether, 15 substances were identified in the ethanolic extract of *R. officinalis* (Table 4), which was shown to be composed of organic acids, flavonoids, lipid acids, and terpenoids. Among them, major reported compounds were sucrose [M-H = 341], pyroglutamic acid [M-H = 128], diethylallarate [M-H = 265], two glycosilated quercetin: quercetin-3-O-rutinoside [M-H = 609], quercetin-3-O-glucoside [M-H = 463], and three lipids: hexadecasphinganine [M+H = 274], and two isomers of heptadecasphinganine [M+H = 288] were the most common one. On the other hand, 29 substances can be identified in the extract from *C. citratus* (Table 5), in which the major compounds were sucrose, 1,3-O-di-trans-p-coumaroylglycerol [M-H = 383], 1-coumaroyl-3-feruloylglycerol [M-H = 413], as well as two lipids acids:7-Hydroxy-13,15,17-octadecatrienoic acid [M-H = 293], and 7-hydroxy-9,13-octadecadienoic acid[M-H = 295]. Moreover, 30 substances can be identified in the extract from *M. mollis* (Table 6),

Table 4 Substances identified in *R. officinalis* ethanolic extract by UHPLC-MS/MS

Substances	Rt	M-H	M+H	Main fragments (m/z)
Glutamic acid	2.12	146		128, 102, 85
Sucrose	2.16	341		179, 161, 143, 113, 101, 89, 71
Quinic acid	2.25	191		173, 127, 111
Malic acid	2.43	133		115, 72, 71
Pyroglutamic acid	2.63	128		98, 84, 82
Diethylallarate	2.73	265		179, 145
Quercetin-3-O-rutinoside	11.54	609		463, 343, 300, 271, 178
Quercetin-3-O-glucoside	11.90	463		300, 271, 178
Kaempferol-rutinoside	12.06	593		285, 255
Quercitrin	12.54	447		300, 271, 178
Hexadecasphinganine	16.15		274	256, 230, 106
Heptadecasphinganine isomer 1	16.68		288	270, 106
Heptadecasphinganine isomer 2	16.85		288	270, 106
9-Octadecenamide	17.62	282		265, 247, 149, 135, 121
Gingerol	17.70	293		236, 221

Rt: Retention time

Table 5 Substances identified in *C. citratus* ethanolic extract by UHPLC-MS/MS

Substances	Rt	M-H	Main fragments (m/z)
Sucrose	2.16	341	179, 161, 143, 113, 101, 89, 71
Shikimicacid	2.46	173	155, 137, 129, 111
Pyroglutamic acid	2.63	128	98, 84, 82
Aesculetin	11.41	177	135, 105, 89
Caffeic acid	11.53	179	135, 107
2”-O-Rhamnosyl- 6-C-Glucosyl luteolin	11.90	593	473, 447, 429, 327, 285
Apigenin-pentosyl-glucose	11.91	563	503, 425, 399, 298
6-C-Glucosyl luteolin	12.08	447	327, 285
Apigenin-rhamnosyl-glucose	12.52	577	415, 323, 293, 269
Luteolin 7-neohesperidoside	12.81	593	447, 285
Apigenin-8-C-glucoside-2'-rhamnoside	12.86	577	415, 311, 298, 269
6-C-Pentosyl-8-C-hexosyl apigenin	12.90	563	473, 417, 399, 298
7-C-Glucosyl luteolin	13.06	447	327, 285
Trans-coumaric acid	13.20	163	119, 93
Ferulic acid	13.61	193	178, 149, 134
3',6'-diferuloylsucrose	14.29	693	517, 337, 193
Acetyl-3',6'-diferuloylsucrose	14.97	735	559, 337, 193
Acetyl-3',6'-diferuloylsucrose isomer	15.18	735	559, 337, 193
8-C-rhamnoside apigenin	15.24	415	311, 253
6-C-rhamnoside apigenin	15.73	415	397, 353, 311. 253
Luteolin	15.87	285	175, 151
9,12,13-Trihydroxy-10-octadecenoic acid	16.72	329	311, 139
1,3-O-di-trans-p-Coumaroylglycerol	16,99	383	219, 163, 119
1-Coumaroyl-3-feruloylglycerol	17.15	413	398, 249, 219, 163, 119
Diferuloylglycerol	17.27	443	428, 249, 193, 175, 149, 134
16-Hydroxy-9-oxo-10,12,14-octadecatrienoic acid	18.46	307	289, 235, 211, 199, 185
12,13-Dihydroxy-9-octadecenoic acid	20.18	313	295, 277, 201, 171, 155, 127
7-Hydroxy-13,15,17-octadecatrienoic acid	21.53	293	275, 256, 223, 195, 177, 111
7-hydroxy-9,13-octadecadienoic acid	22.28	295	277, 259, 193, 183, 171, 113

Rt: Retention time

Table 6 Substances identified in *M. mollis* ethanolic extract by UHPLC-MS/MS

Substances	Rt	M-H	M+H	Main fragments (m/z)
Mannitol	2.18	181		163, 149, 131, 119, 101, 89, 71, 59
Quinic acid	2.30	191		173, 127, 111
Shikimic acid	2.46	173		155, 137, 129, 111
Malic acid	2.54	133		115, 72, 71
Caffeoylquinic acid	6.75	353		191, 179, 173, 161, 135, 111, 93
Caffeic acid	11.79	179		135, 107
Quercetin-3-O-rutinoside	12.27	609		300, 271, 178
Kaempferol-3-O-rutinoside	12.83	593		447, 285, 255
Naringenin-7-O-rutinoside	12.92	579		271, 227, 175, 151
Isoquercitrin	13.22	463		300, 271, 178
Naringenin-4´-O-rutinoside	13.44	579		271, 227, 175, 151
Kaempferol-3-O-glucoside	13.57	447		284, 255
Naringenin-7-O-glucoside	13.91	433		271, 227, 175, 151
Rosmarinic acid	14.33	359		197, 179
Isosakuranetin-7-O-rutinoside	14.90	639 [M-H+FA]		285, 226
Isosakuranin	15.75	447		285, 270, 241, 196
Chalconosakuranetin	15.83	493 [M-H+FA]		285, 179, 161, 135
Tricoumaroylspermidine	16.09	582		436, 342, 316, 145
9,12,13-Trihydroxy-10,15-octadecadienoic acid	16.25	327		309, 291, 137
9,12,13-Trihydroxy-10-octadecenoic acid	16.78	329		311, 139
Naringenin	16.94	271		227, 151, 107
Hesperetin	17.24	301		286, 257, 242
Irigenin	17.83	359		344, 329, 314, 193
2-Amino-1,3-heptadecanediol	18.50		288	270, 106, 88, 70
Eupatilin	19.14	343		328, 313, 298, 270
Isosakuranetin	19.26	285		270, 243, 151
Citronellicacid	20.01		153	135, 109, 107, 93, 81
Gardenin B	21.66		359	344, 329, 298, 135
3-Hydroxy-11-ursen-28,13-olide	22.44		455	437, 409, 391, 219
Enoxolone	22.73	471		425, 407, 271, 217

Rt: Retention time

in which the major compounds were isosakuranetin-7-O-rutinoside [M-H+FA = 639], naringenin [M-H = 271], isosakuranetin [M-H = 285], and citronellic acid [M+H = 153].

3.5 Evaluation of biocidal activity

The analysis of variance (ANOVA) reported that the analyzed data are statistically significant $p<0.05$, which establishes that there are differences between the means of the percent mortality (biocidal effect) using each plant extract, essential oil, or positive control against adults of *A. aegypti*. The biocidal activity evaluation on *A. aegypti* showed that all the essential oils caused 100% mortality of *A. aegypti* adults within 105 minutes and above 90% mortality in 30 minutes, being more efficient than all the extracts evaluated.

The statistically significant difference was determined between groups; the Tukey statistical test was used, where it was reported a $p=0.9999$ between the essential oils and the positive control (2% malathion). On the other hand, the comparison between aqueous and

ethanolic extracts reported a *p*-value above 0.9732. In all cases, the significance level was more significant than p=0.05 of standard significance, which in turn means that these extracts have comparable efficacy since there is no statistically significant difference in their biocidal effect against adults of *A. aegypti*. All ethanolic extracts caused 100% mortality in 105 min. However, the aqueous extracts of *R. officinalis* and *C. citratus* caused observed mortality in shorter periods than ethanolic extracts (30 min versus 45 min for *R. officinalis* and 15 min versus 45 min for *C. citratus*, respectively) (Figures 1, 2, and 3).

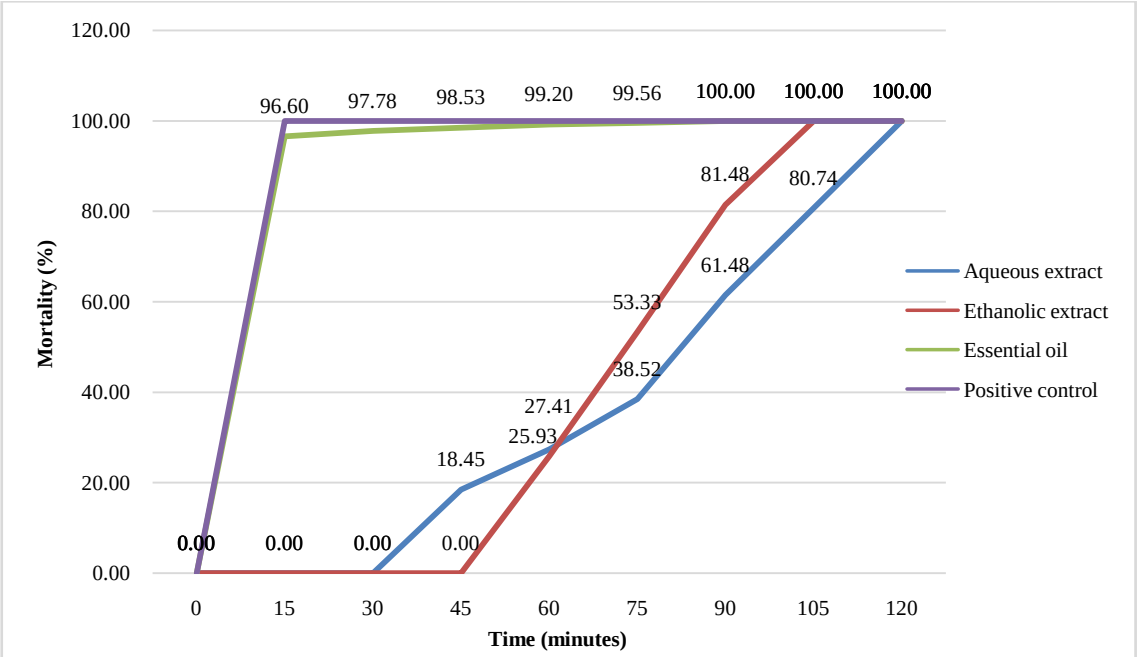

Figure 1 Percentage of mortality of adults of *A. aegypti* exposed to different extracts and oils from *R. officinalis* and 2% malathion (positive control)

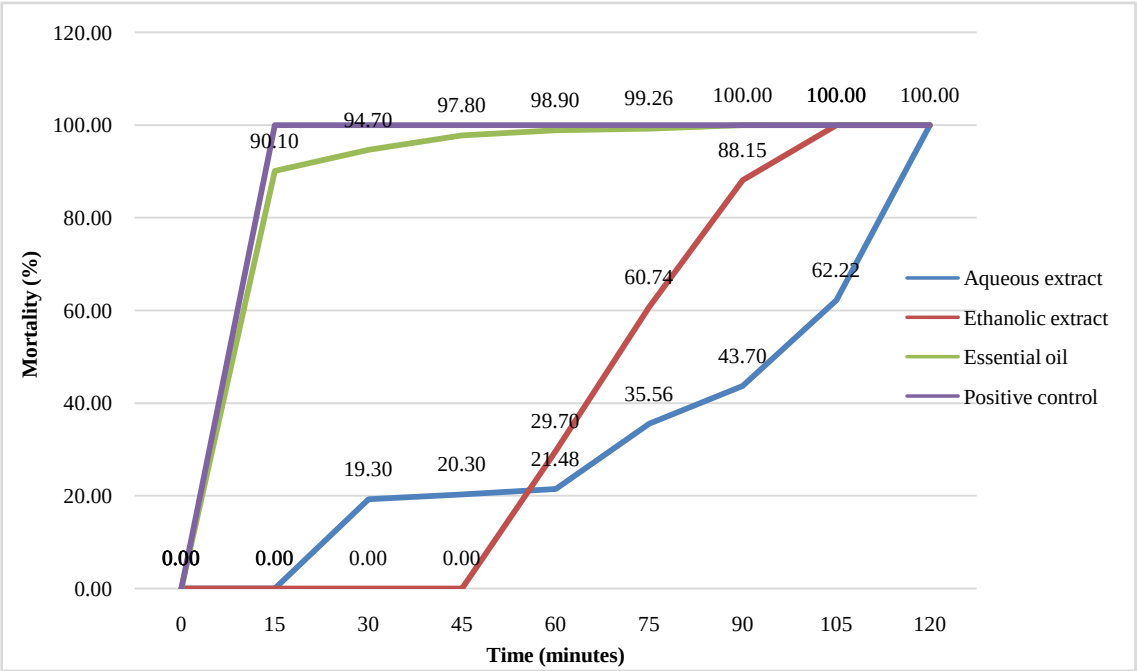

Figure 2 Percentage of mortality of adults of *A. aegypti* exposed to different extracts and oils from *C. citratus* and 2% malathion (positive control)

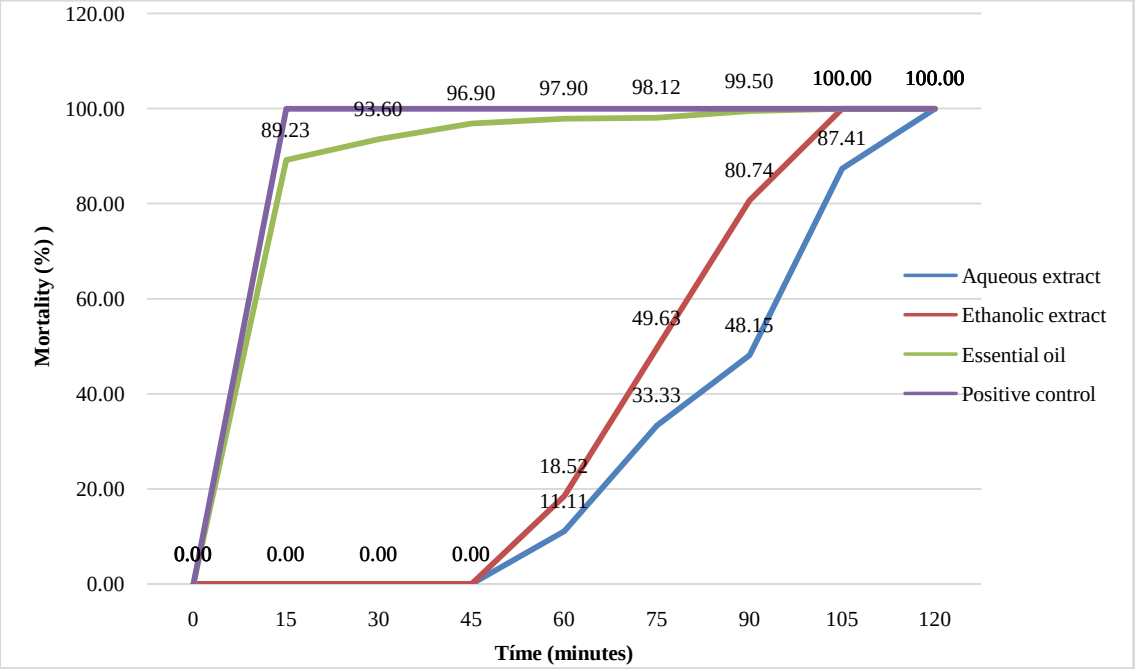

Figure 3 Percentage of mortality of adults of *A. aegypti* exposed to different extracts and oils from *M. mollis* and 2% malathion (positive control)

4 Discussion

The plant species *R. officinalis* yielded 0.8% of the fresh leaves' essential oil. The literature indicates that the yield of essential leaf oil is 0.61% for the tissue in the fresh state and 0.98% for the dry tissue, so compared to the result obtained, the value found in this work is within the expected range. It has been shown that the main components of the essential oil of *R. officinalis* are camphor (5.0-21%), 1,8-cineole (15-55%), α-pinene (9.0-26%), borneol (1.5-5.0%), camphene (2.5–12%), β-pinene (2.0–9.0%) and limonene (1.5–5.0%) in proportions that vary according to the vegetative stage and bioclimatic conditions (López 2008; Andrade et al. 2018). In turn, *C. citratus* presented a yield of 1.0% for the essential oil from its fresh leaves. The literature reports that the fresh plant of *C. citratus* provides essential oil between 0.5 – 0.7%, and the obtained oil is of a transparent yellow liquid with a citrus scent. These results agree with the findings of Rodriguez et al. (2006), those who received 0.7% of essential oil from this plant species collected from the town of Trujillo, included in the same region mentioned in this study (Rodriguez et al. 2006). Compared with the yield obtained, the plant species provides a high percentage of essential oil. This could be due to different agroclimatic factors such as the salinity of the soil, altitude, and water content, which in turn could also influence the quality and quantity of essential oils (Ekpenyong et al. 2014). According to different studies with *C. citratus*, essential oils, geranial, neral, and myrcene were the main components of the species (Soto-Ortiz et al. 2002; Rodriguez et al. 2006; Gbenou et al. 2013). On the other hand, the yield of the essential oil obtained from the fresh leaves of *M. mollis* was 0.3 %, which is in agreement with the essential oil ranges (0.25 - 4.93%) reported in the literature. The predominant components of essential oil are pulgeone and menthone. This includes a study by Orbegozo and Rodriguez (2018), which obtained 0.6% v/w in a species collected in the same region (Santiago de Chuco, La Libertad). All the essential oil values obtained in this study are within the acceptable ranges, and the place of origin was found to have a slight effect on this parameter (Van Baren et al. 2014; Orbegozo and Rodriguez 2018). In addition, *M. mollis* is very heterogeneous in its genetics and morphology, and other abiotic factors previously mentioned can influence the composition of the essential oil (Linares 2020).

Furthermore, the values found for the quality parameters of three species studied in this work proved to be within the range recommended by the reference documents in Peru (Villar 1999; Miranda 2002; British Pharmacopoeia 2022;). In addition, the study of Orbegozo and Rodriguez (2018) demonstrated the value of 7.58 % of total ashes in a sample of *M. mollis* from the La Libertad region, corroborating the result described in this work.

The study determining the concentration of solids in extracts and oils demonstrated greater water efficiency in concentrating the metabolites in all the species evaluated. The quantitative and qualitative yield of the extraction depends on the nature of the extracted compounds and the solvent used, as well as on factors such as the nature of the sample, concentration of the solvent,

temperature, contact time, particle size, and mass-solvent ratio among others (Soto and Rosales 2016).

In the chemical identification by UHPLC-MS/MS, 15 substances were identified for *R. officinalis*, whereas 29 compounds were for *C. citratus* and 30 for *M. mollis*. Flavonoids, mainly querecetin compounds, besides organic acids, sugars, lipids, and gingerol, a phenolic constituent, were the main substances identified in *R. officinalis*; these results agree with the previous literature of this species (Achour et al. 2017; Mena et al. 2020). In the *C. citratus* analysis, phenolic acids like caffeic, ferulic, and coumaric acid conjugates, as well as the flavonoids apigenin and luteolin and lipid acids were the principal bioactive constituents and are also found in other works as species markers (Sousa et al. 2021; Bhaskar et al. 2021). Moreover, the chemical analysis of *M. mollis* extracts showed the presence of flavonoids, including isosakuranetin and naringenin, as major compounds, besides phenolic acids and terpenoids, which in turn are characteristics of the genus *Minthostachys* (Faraone et al. 2021).

The lethality of essential oils can vary greatly, depending on factors such as chemical composition, plant species, part of the plant extracted, maturity, and extraction method (Isman 2020). Essential oils are a mixture of various chemical components that produce different toxic effects on the insect's organism, so it is likely that *A. aegypti* cannot easily develop an adaptation that leads to resistance (Wahyuni 2012; Dias and Moraes 2014). Many studies have shown the neurotoxic actions of essential oils, causing paralysis followed by death, in insects. This characteristic allows considering the components of essential oils as potential bioinsecticides. This mechanism might occur through the inhibition of acetylcholinesterase (AChE), or triggering effects similar to those produced by conventional insecticides such as temephos or organophosphate cholinesterase inhibitors used in vector-borne disease control programs (Kostyukowsky et al. 2002; WHO 2009). Another mechanism is through the octopaminergic system. Available data showed that essential oils could increase the level of cAMP and calcium in nerve cells. Therefore, essential oils are interesting bioinsecticide candidates (Jankowska et al. 2018). Furthermore, the literature revealed that essential oils rich in phenylpropanoids, oxygenated sesquiterpenes, and monoterpene hydrocarbons have significant larvicidal activity against *A. aegypti* (Dias and Moraes 2014). According to Rodriguez et al. (2006), the main compounds responsible for insecticidal activity are of low polarity (Rodriguez et al. 2006).

Regarding this point, Isman (2000) indicates that monoterpenes and phenylpropanoids were the most identified compounds in essential oils, extracts, and purified fractions, which stand out in larvicidal activity (Isman 2000). In addition, to postulate that the toxicity of these phytometabolites may be associated with their non-polar property, which increases the ability of the compound to penetrate the hydrophobic cuticle of the larvae and enhances its harmful effect on the insect compared to polar compounds (Vincent and Wegst 2004). Scientific evidence showed that the essential oils of *R. officinalis*, *C. citratus*, and *M. mollis* have insect repellent, insecticide, larvicide, adulticide, ovicide, and oviposition dissuasive activities, being applied in the control of pathogens and insects (Prajapati et al. 2005; Gillij et al. 2008; Waliwitya et al. 2009; Vera et al. 2014; Soonwera and Phasomkusolsil 2016; Alegre 2016; Azeem et al. 2019; Oladeji et al. 2019; Soonwera and Sittichok, 2020;). Moreover, the components found in these essential oils, such as pulegone, menthone, thymol, eugenol, trans-anetol, and citronellal have shown high larvicidal activity (Waliwitya et al. 2009).

The insecticidal activity of the extracts from *R. officinalis* is probably related to the presence of quercetin, kaempferol conjugates, and gingerol. Quercetin possesses an antifeedant effect against *A. aegypti*. The proposed mechanism suggests that quercetin oxidation by larvae generates reactive oxygen species, which in turn can degrade the nutritional quality of food present in the gut lumen of the insects. In addition, quercetin also inhibits transhydrogenase activity, negatively impacting their development and therefore leading them to death (Pessoa et al. 2018). Also, kaempferol inhibits the ecdysone 20-monooxygenase enzyme in females, suppressing their oviposition (Mitchell et al. 1993), and gingerol is a growth insect regulator and has antifeedant activity (Agarwal et al. 2001). In turn, the activity of *C. citratus* extracts can be explained by the presence of apigenin conjugates that share the exact mechanism of action with kaempferol and by the high content of phenolic acids that can inhibit insect acetylcholinesterase (Maazoun et al. 2017). Also, the presence of caffeic acid and rosmarinic acid in *M. mollis* extracts acts on the reduction of insect herbivory in several ways, such as reducing fertility, discouraging feeding, oviposition, and shortening the insect life span (Isman 2006; Dawkar et al. 2013). This effect is added to the mechanisms related to flavonoids and kaempferol conjugates and acid triterpenes (enoxolone and 3-Hydroxy-11-ursen-28,13-olide), which in turn provide antifeedant action in insects (González-Coloma et al. 2011).

In sum, the essential oils and extracts from the three species, found mainly in Peru, provide a promising source for insecticidal applications due to their significant biocidal activity and possible toxicological safety for mammals and the environment since they are more easily degraded by natural ecosystem mechanisms (Bhatt et al. 2013).

Conclusions

The research demonstrated the insecticidal activity of essential oils and extracts of the plant Peruvian resources *R. officinalis*, *C. citratus*, and *M. mollis* against *A. aegypti* adults. The study also

determined the chemical profile of ethanolic extracts from three species, including 15 substances for R. officinalis, 29 for C. citratus, and 30 for *M. mollis*. Furthermore, the study of quality parameters for the essential oil and extracts indicated that they agree with recommended quality standards so that these phytoproducts can be seen as more eco-friendly biological controllers of *A. aegypti* when compared with synthetic insecticides.

Acknowledgments

The authors thank FONCEDYT-ProCiencia for project 164-2018 financing support.

Conflict of Interest

The authors have no conflict of interests.

References

Achour, M., Mateos, R., Ben Fredj, M., Mitraoui, A., Bravo, L., & Saguem, S. (2017). A Comprehensive Characterisation of Rosemary tea Obtained from *Rosmarinus officinalis* L. Collected in a sub-Humid Area of Tunisia. *Phytochemical Analysis*, *29*, 87-100.

Agarwal, M., Walia, S., Dhingra, S., & Khambay, B.P. (2001). Activity of compounds isolated/derived from *Zingiber officinale* Roscoe (ginger) rhizomes. *Pest Management Science*, *57*, 289-300.

Alegre, A. (2016). Efecto tóxico del extracto acuoso, etanólico y hexánico de *Minthostachys mollis*, *Annona muricata*, *Lupinus mutabilis* y *Chenopodium quinoa*s obre *Tetranychus urticae* (Trombidiformes: Tetranychidae) y *Chrysoperla* externa (Neuroptera: Chrysopidae). Graduation Thesis, Universidad Ricardo Palma, Peru.

Andrade, J.M, Faustino, C., Garcia, C., Ladeiras,D., Reis, C.P., & Rijo, P. (2018). *Rosmarinus officinalis* L.: an update review of its phytochemistry and biological activity. *Future Science*, *4*, FSO283.

Azeem, M., Zaman, T., Tahir, M., Haris, A., Iqbal, Z., & Binyameen, M. (2019). Chemical composition and repellent activity of native plants essential oils against dengue mosquito, *Aedes aegypti*. *Industrial Crops and Products*, *140*, 111609.

Bhaskar, K., Sassykova, L.R., Prabhahar, M., ShebhaPercis, E., Nalini, A., Jenish, T., Jayarajan, J., & Sendilvelan, S. (2021). Analysis of *Cymbopogon citratus*, *Pinus sylvestris* and *Syzygium cumini* biodiesel feed stocks for its fatty acid composition. *Materials Today: Proceedings*, *45*, 5970-5977.

Bhatt, S., Gething, P., Brady, O., Messina, J., Farlow, A., & Moyes, C. (2013). The global distribution and burden of dengue. *Nature*, *496*, 504-507.

British Pharmacopoeia. (2022). London: British Medicinal Herbal Association.

Brogdon, W., & Chan A. (1998). Instrucciones para la Evaluación de la Resistencia a Insecticida en Vectores mediante del Ensayo Biológico de la Botella de los CDC. Atlanta: CDC. Avaliable in: https://www.cdc.gov/malaria/resources/pdf/fsp/ir_manual/ir_cdc_bioassay_es.pdf

Cabezas, C., Fiestas, V., García, M., Palomino, M., Mamani, E., & Donaires, F. (2015). Dengue en el Perú: A un cuarto de Siglo de su reemergencia. *Revista Peruana de Medicina Experimental y Salud Pública*, *32*, 146-156.

Chala, B., & Hamde, F. (2021). Emerging and Re-emerging Vector-Borne Infectious Diseases and the Challenges for Control: A Review. *Frontiers in Public Health*, 9, 715759.

Dawkar, V., Chikate, Y., Lomate, P., Dholakia, B., Gupta, V., & Giri, A. (2013). Molecular insights into resistance mechanisms of Lepidopteran insect pests against toxicants. *Journal of Proteome Research*, *12*, 4727–4737.

Demirak, M.S.S., & Canpolat, E. (2022). Plant-Based Bioinsecticides for Mosquito Control: Impact on Insecticide Resistance and Disease Transmission. *Insects*, *13*, 162.

Dias, C.N., & Moraes, D.F.C. (2014). Essential oils and their compounds as *Aedes aegypti* L. (Diptera: Culicidae) larvicides: review. *Parasitology Research*, *113*, 565-592.

Duarte, J.L., Amado, J.R.R., Oliveira, A.E.M.F.M., Cruz,R.A.S, Ferreira, A.M., Souto, R.N.P., Falcão, D.Q., Carvalho, J.C.T., & Fernandes, C.P. (2015). Evaluation of larvicidal activity of a nanoemulsion of *Rosmarinus officinalis* essential oil. *Brazilian Journal of Pharmacognosy*, *25*, 189-192.

Dueñas-López, M.A. (2022). *Aedes aegypti* (yellow fever mosquito). *CABI Compendium*. Avaliable in: https://www.cabidigitallibrary.org/doi/pdf/10.1079/cabicompendium.94883

Ekpenyong, C.E., Akpan, E.E., & Daniel, N.E. (2014). Phytochemical Constituents, Therapeutic Applications and Toxicological Profile of *Cymbopogon citratus* Stapf (DC) Leaf Extract. *Journal of Pharmacognosy and Phytochemistry*, *3*,133-141.

Faraone, I., Russo, D., Genovese, S., Milella, L., Monne, M., Epifano, F., & Fiorito, S. (2021). Screening of in vitro and in silico α-amylase, α-glucosidase, and lipase inhibitory activity of oxyprenylated natural compounds and semisynthetic derivatives. *Phytochemistry*, *187*, 112781.

Gbenou, J.D., Ahounou, J.F., Akakpo, H.B., Laleye, A., Yayi, E., Gbaguidi, F. (2013). Phytochemical composition of *Cymbopogon citratus* and *Eucalyptus citriodora* essential oils and their anti-inflammatory and analgesic properties on Wistar rats. *Molecular Biology Reports*, *40*, 1127-1134.

Gillij, Y.G., Gleiser, R.M., & Zygadlo, J.A. (2008). Mosquito repellent activity of essential oils of aromatic plants growing in Argentina. *Bioresource Technology*, *99*, 2507-2515.

González-Coloma, A., López-Balboa, G., Santana, O., Reina, M., & Fraga, B.M. (2011). Triterpene-based plant defenses. *Phytochemistry Reviews*, *10*, 245–260.

Isman, M.B. (2000). Plant essential oils for pest and disease management. *Crop Protection*, *19*, 603-608.

Isman, M.B. (2006). Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annual Reviews in Entomology*, *51*, 45–66.

Isman, M.B. (2020). Bioinsecticides based on plant essential oils. *Zeitschrift für Naturforschung C*, *75*, 7-8.

Jankowska, M., Rogalska, J., Wyszkowska, J., & Stankiewicz, M. (2018). Molecular Targets for Components of Essential Oils in the Insect Nervous System—A Review. *Molecules*, *23*, 34.

Kostyukovsky, M., Rafaeli, A., Gileadi, C., Demchenko, & N., Shaaya, E. (2002). Activation of octopaminergic receptors by essential oil constituents isolated from aromatic plants: possible mode of action against insect pests. *Pest Management Science*, *58*, 1101-1106.

Lazcano, J.A., Rodríguez, M.M., San Martín, J.L., Romero, J.E., & Montoya, R. (2009). Evaluación de la resistencia a insecticidas de una cepa de *Aedes aegypti* de El Salvador. *Revista Panamericana de Salud Pública*, *26*, 229–234.

Linares V. (2020). Consideraciones para el uso y estudio de la "muña" peruana *Minthostachys mollis* (Benth.) Griseb y *Minthostachys setosa*(Briq.) Epling. *Ethnobotany Research and Applications*, *19*, 1-9.

López L. (2008). El romero. Planta aromática con efectosantioxidantes. *Revista Offarm*, *27*, 60-63.

Maazoun, A.M., Ben Hiel, T., Hamdi, S.H., Belhadj, F., Ben Jemâa, J.M., & Marzouki, M.N. (2017). Screening for insecticidal potential and acetylcholinesterase activity inhibition of *Urginea maritima* bulbs extract for the control of *Sitophilus oryzae* (L.). *Journal of Asia-Pacific Entomology*, *20*, 752-760.

Mena, P., Cirilini, M., Tassotti, M., Herrlinger, K.A, Dall'asta, C., & Del Rio, D. (2020). Phytochemical Profiling of Flavonoids, Phenolic Acids, Terpenoids, and Volatile Fraction of a Rosemary (*Rosmarinus officinalis* L.) Extract. *Molecules*, *21*, 1576.

MINSA. (2015). Norma técnica de salud para la implementación de la vigilancia y control del *Aedes aegypti*, vector del dengue y la fiebre de chikungunya y la prevención del ingreso del *Aedes albopictus* en el territorio nacional. Avaliable in: http://www.datosabiertos.gob.pe/sites/default/files/recursos/2017/09/NTS %201162015%20%20VIGILANCIA%20Y%20CONTROL%20DEL%20AEDES%20AEGY PTI.pdf.

Miranda, M. (2002). *Métodos de análisis de drogas y extractos*. Instituto de Farmacia y Alimentos, Universidad de la Habana, Cuba.

Miranda, M., & Cuellar, A. (2002). *Manual de Prácticas de Laboratorio: Farmacognosia y Productos Naturales*. Instituto de Farmacia y Alimentos, Habana. 1-68.

Mitchell, M.J., Keogh, D.P., Crooks, J.R., & Smith, S.L. (1993). Effects of plant flavonoids and other allelochemicals on insect cytochrome P-450 dependent steroid hydroxylase activity. *Insect Biochemistry and Molecular Biology*, *23*, 65-71.

Oladeji, O.S., Adelowo, F.E., Ayodele, D.T., & Odelade, K.A. (2019). Phytochemistry and pharmacological activities of *Cymbopogon citratus*: A review. *Scientific African*, *6*, e00137.

Orbegozo, H., & Rodríguez, K. (2018). *Características farmacognósticas y rendimiento del aceite esencial de las hojas de* Minthostachys mollis *"muña"*. PhD Thesis, Universidad Nacional de Trujillo, Peru. 51 p.

Organización Panamericana de Salud (OPS). (2019). Otras enfermedades transmitidas por vectores. Avaliable in: https://www.paho.org/per/index.php?option=com_content&view=article&id=4085:otras-enfermedades-transmitidas-por-vectores&Itemid=1097

Pereira, L.E.C., Ferreira, E.M., Picinato, M.A.C., Mathias, L.A., Arcêncio, R.A., Barbosa, K.F.D., & Ferraudo, A.S. (2022). Community knowledge on dengue in territories under risk in the state of São Paulo. *Arquivos do Instituto Biológico*, *89*, 1-9.

Pessoa, L.Z.S., Duarte, J.L., Ferreira, R.M.A., Oliveira, A.E.M.F.M., Cruz, R.A.S., Faustino, S.M.M., Carvalho, J.C.T., Fernandes,C.P., Souto, R.N.P., & Araújo, R.S. (2018). Nanosuspension of quercetin: preparation, characterization and effects against *Aedes aegypti* larvae. *Brazilian Journal of Pharmacognosy*, *28*, 618-625.

Pino, O., Sánchez, Y., & Rojas, M.M. (2013). Plant secondary metabolites as alternatives in pest management. II: An overview of their potential in Cuba. *Revista de Protección Vegetal*, *28*, 95-108.

Prajapati, V., Tripathi, A.K., Aggarwal, K.K., & Khanuja, S.P.S. (2005). Insecticidal, repellent and oviposition-deterrent activity of selected essential oils against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. *Bioresources Technology*, *96*, 1749-1757.

Rocha, L.M., Rodrigues, R.D.S., Guimarães, P.H.V., & Gonsalves, J.K.M.C. (2022). Larvicide potential of essential oils from Brazilian plants against *Aedes aegypti*. *Research, Society and Development, 11*, e53211226140.

Rodríguez, W., Castro, L., Sánchez, Y., Gómez, J.E., & Correa, M. (2006). Composición química del aceite esencial de las hojas de *Cymbopogon nardus* y *Cymbopogon citratus*. *Momentos de Ciencia*, *3*, 44-50.

Soonwera, M., & Phasomkusolsil, S. (2016). Effect of *Cymbopogon citrates* (lemongrass) and *Syzygium aromaticum* (clove) oils on the morphology and mortality of *Aedes aegypti* and *Anopheles dirus* larvae. *Parasitology Research*, *115*, 1691-1703.

Soonwera, M., & Sittichok, S. (2020). Adulticidal activities of *Cymbopogon citratus* (Stapf.) and *Eucalyptus globulus* (Labill.) essential oils and of their synergistic combinations against *Aedes aegypti* (L.), *Aedes albopictus* (Skuse), and *Musca domestica* (L.). *Environmental Science and Pollution Research International*, *27*, 20201-20214.

Soto-Ortiz, R., Vega-Marrero, G., & Tamajón-Navarro, A.L. (2002). Instructivo técnico del cultivo de *Cymbopogon citratus* (D.C) Stapf (caña santa). *Revista Cubana de Plantas Medicinales*, *7*(2), 55-68.

Soto, M., & Rosales, M. (2016). Efecto del solvente y de la relación masa/solvente sobre la extracción de compuestos fenólicos y la capacidad antioxidante de extractos de corteza de *Pinus durangensis* y *Quercus sideroxyla*. *Maderas: Ciencia & Tecnología, 18*, 701-714.

Sousa, R., Figueirinha, A., Batista, M.T., & Pina, M.E. (2021). Formulation Effects in the Antioxidant Activity of Extract from the Leaves of *Cymbopogon citratus* (DC) Stapf. *Molecules*, *26*, 4518.

Van Baren, C.M., Di Leo Lira, P., Elechosa, M.A., Molina, A.M., Juárez, M.A., & Martínez, A. (2014). New insights into the chemical biodiversity of *Minthostachys mollis* in Argentina. *Biochemical Systematics and Ecology*, *57*, 374-383.

Vera, S.S., Zambrano, D.F., Méndez-Sanchez, S.C., Rodríguez-Sanabria, F., Stashenko, E.E., & Duque Luna, J.E. (2014). Essential oils with insecticidal activity against larvae of *Aedes aegypti* (Diptera: Culicidae). *Parasitology Research*, *113*, 2647-2654.

Villar, A. (1999). Farmacognosia General. Síntesis S.A. Madrid, Spain.

Vincent, J., & Wegst, U. (2004). Design and mechanical properties of insect cuticle. *Arthropod Structure & Development*, *33*, 187-199.

Wagner, H., & Bladt, S. (2004). *Plant Drug Analysis: a thin layer chromatography atlas*, 2nd edn: Berlin: Springer, Berlin, Germany.

Wahyuni, D. (2012). Larvicidal Activity of Essential Oils of Piper betle from the Indonesian Plants against *Aedes Aegypti* L. *Journal of Applied Environment Biological Science*, *2*, 6.

Waliwitiya, R., Kennedy, C.J., & Lowenberger, C.A. (2009). Larvicidal and oviposition-altering activity of monoterpenoids, trans-anithole and rosemary oil to the yellow fever mosquito *Aedes aegypti* (Diptera: Culicidae). *Pest Management Science*, *65*, 241-248.

World Health Organization (WHO). (2009). *Dengue: guidelines for diagnosis, treatment, prevention, and control*. New Edn: Geneva: World Health Organization.

World Health Organization (WHO). (2016). *Monitoring and managing insecticide resistance in Aedes mosquito populations*. New Edn: Geneva: World Health Organization.World Health Organization (WHO). (2020). *Vector-borne diseases*. New Edn: Geneva: World Health Organization.