ACS OMEGA

Open Access

http://pubs.acs.org/journal/acsodf

Article

Larvicidal Activity of Constituents from the Main Brazilian Propolis Types: Green, Red, and Brown against *Aedes aegypti*

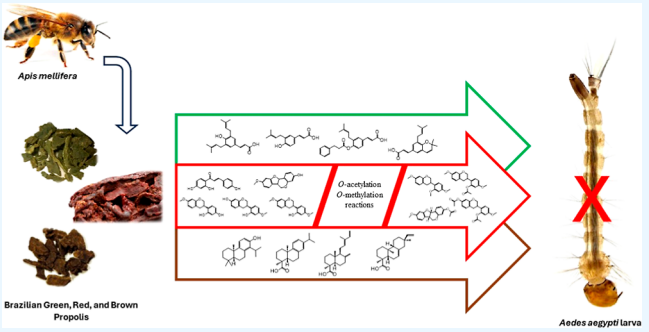
Victor P. Ribeiro, Jairo K. Bastos, Alden S. Estep, and Kumudini M. Meepagala*

Cite This: *ACS Omega* 2024, 9, 35560−35566

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: In search of environmentally benign and mammalian-friendly mosquito-mitigating compounds, we conducted an investigation into the constituents isolated from Brazilian red, brown, and green propolis. Additionally, we synthetically modified active constituents to explore the role of lipophilicity in enhancing their larvicidal activity. Honeybees collect plant resins from their habitats, mix them with saliva, and utilize them to seal their beehives. The constituents present in propolis exhibit a unique composition specific to the geographical location and the fauna of the region. As part of the plant's natural defense mechanism, propolis compounds demonstrate antibacterial, insecticidal, and phytotoxic properties. Given that several insecticides target the enzyme acetylcholinesterase, we conducted *in silico* studies to examine the interactions between propolis compounds and acetylcholinesterase through molecular docking. In this study, we present the mosquito larvicidal activities of propolis constituents.



INTRODUCTION

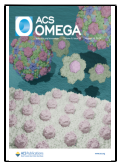
Mosquitoes serve as vectors for several viral diseases that are of importance to humans and other species. *Aedes aegypti*, in particular, is responsible for transmitting fatal diseases such as Dengue, Zika, Chikungunya, and yellow fever, with a high incidence rate primarily in tropical and subtropical regions worldwide. However, due to factors associated with climate change, incidences of these illnesses in temperate regions of the globe are increasingly common.[1] One approach to prevent these diseases is by controlling the vectors for which larvicides and insecticides are used. The life cycle of *Ae. aegypti* comprises four stages: egg, larvae, pupa, and adult, and it spends its entire immature development in aquatic environments.[2] Larvicides are used to combat larvae; however, they can lead to two main issues: the development of a population of mosquitoes resistant to larvicides and the potential toxicity of these products, which can cause harm to human and environmental health. An alternative would be natural larvicides from natural products.[3]

Propolis is a natural product produced by bees using resins collected from plants. The primary role of propolis for bees is to protect the beehive against invaders, but it also performs functions such as regulating hive temperature and in controlling parasites and microorganisms.[4] Due to its botanical origin, propolis exhibits a rich diversity of metabolites, making it widely utilized in traditional medicine primarily for immunomodulation in countries such as Brazil, Japan, and China.[5] Currently, propolis can be found in the form of extracts but is also incorporated into pharmaceutical and cosmetic formulations, showcasing not only its significant medicinal value but also its economic potential.[6]

Brazil stands out as one of the largest propolis producers. Propolis classification systems vary, considering other factors such as geographical origin, chemical composition, and even botanical sources.[7] A classification that is widely used is based on color. One of the most produced and commercialized Brazilian propolis is green propolis, characteristic of the southeastern region of Brazil, produced by *Apis mellifera* bees from resins collected from *Baccharis dracunculifolia* buds.[8] Another propolis that has gained prominence for its chemical composition and biological properties is the red type, produced in the coastal region of Northeast Brazil, characterized by the mangrove habitat, whose botanical source is a mixture of resins from *Dalbergia ecastaphyllum* and *Symphonia globulifera*.[9] Additionally, brown propolis is one of the most complex from a chemical perspective, where several botanical sources were reported in the composition of this propolis, with *Araucaria angustifolia*, *Eucaliptus* spp., and *B. dracunculifolia*, among others.[10,11]

Propolis exhibits several biological properties attributed to its various types: green propolis is renowned for its

Received: April 1, 2024
Revised: August 1, 2024
Accepted: August 5, 2024
Published: August 9, 2024



antimicrobial and antioxidant properties,[5,12] red propolis demonstrates anticancer activity,[13] and brown propolis showcases anti-inflammatory and antiparasitic properties.[14] In the present work, we evaluated the activity of the main components isolated from green, red, and brown propolis against *Ae. aegypti* mosquito larvae.

MATERIALS AND METHODS

General. Pyridine and 4-(dimethylamino)pyridine were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Acetic anhydride, hydrochloric acid (HCl), and anhydrous Na_2SO_4 were purchased from Fisher Scientific (Hampton, NH). Trimethylsilyldiazomethane was purchased from TCI America (Portland, OR).

1D and 2D NMR spectra were recorded on a Bruker Avance III-400 MHz spectrometer using $CDCl_3$ as a solvent, with TMS as an internal standard. Analytical TLC was performed on silica gel plates with a GF fluorescent indicator (250 μm, Analtech, Newark, DE). Direct analysis in real time-high-resolution mass spectrometry (DART-HRMS) of purified compounds in MeOH was acquired in an AccuTOF-DART mass spectrometer (JEOL USA, Inc. Peabody, MA, USA).

Propolis Material. Green propolis was provided by Apis Flora Ribeirão Preto, SP, Brasil. Red propolis was purchased from Cooperativa de Apicultores de Canavieiras (COAPER, Canavieiras, Bahia). Brown propolis was collected from Angatuba—São Paulo, Brazil.

Extraction and Isolation of Compounds. Green propolis extract was obtained by dynamic maceration of ground material (100 g) with ethanol/water (7:3) in a shaker at 120 rpm for 24 h three times. The crude extract was filtered, and the solvent was evaporated to obtain a green propolis extract (42 g). The extract (8 g) was fractionated in a flash chromatography column (5.5 cm × 13.5 cm) using vacuum on silica gel 60H (60−200 μm-Across organics) with hexane/ethyl acetate (95:5) as the mobile phase. The fractions were analyzed by TLC and combined according to the similarity of the TLC profile. The compounds were purified by preparative RP-HPLC by using a C-18 LUNA (250 × 10 mm, 5 μm, Phenomenex), using a method described by Arruda et al. (2020)[15] using a gradient of acetonitrile (55−82%) and acidic water (with 0.1% of formic acid) as the mobile phase.

The isolation process of the compounds from red propolis was previously reported by Aldana-Mejía et al. (2021).[13] Red propolis crude extract was obtained by maceration (200 g) in ethanol/water 7:3, using a shaker incubator at 30 °C and 120 rpm, for 24 h, five times. The crude extract was mixed with microcrystalline cellulose and partitioned in a solid-phase process using *n*-hexane, dichloromethane, and ethyl acetate. All of the compounds used in this study were obtained from the dichloromethane fraction. The dichloromethane fraction was fractionated using a glass column (90 × 5 cm i.d.) packed with Sephadex LH-20 (25−1000 μm; Sigma-Aldrich) as the stationary phase with ethanol (96%) as the mobile phase. The compounds were purified by semipreparative RP-HPLC with a synergy Polar-RP column (4 μm, 250 × 21.5 mm, Phenomenex) by gradient elution with acid−water (0.1% of formic acid) and acetonitrile (40−80%).

The diterpenes obtained from the brown propolis were isolated in a process described by Ribeiro et al. (2021).[16] The crude extract was obtained by maceration with ethanol/water 7:3 for 24 h three times. Then it was suspended in a solution of a minimum amount of methanol in water and partitioned with ethyl acetate and butanol. The ethyl acetate fraction was submitted to silica gel column chromatography using hexane and ethyl acetate mixture as the mobile phase furnishing five fractions. The diterpenes were purified from these fractions by using silica gel column chromatography.

Preparation of Semisynthetic Analogues. *General Method for O-Acetylation.* To the isolated compound dissolved in pyridine (1.5 mL) were added 3 equiv of acetic anhydride and catalytic amount of DMAP, and the reaction mixture was stirred for 2 h at room temperature. The reaction mixture was acidified with 1N aqueous HCl, extracted 3 times with ethyl acetate (6 mL each), and dried over anhydrous Na_2SO_4, and ethyl acetate was evaporated. The reaction product was purified using an Ultra 10g Biotage column with 0−50% ethyl acetate/hexane gradient. The identity of the product was confirmed by NMR and HR-mass spectroscopy.

General Method for O-Methylation. The compound was dissolved in methanol (3 mL) and then 4 equiv of trimethylsilyldiazomethane (ca. 10% in hexane, ca. 0.6 mol/L) was added to the solution, and the reaction mixture was stirred for 6 h at room temperature under a N_2 atmosphere. The solvent was evaporated, and the reaction product was purified using an Ultra 10 g Biotage column with 0−50% ethyl acetate/hexane gradient. The identity of the product was confirmed with NMR and HR-mass spectroscopy.

Methylation of 7-*O*-methylvestitol (**9**) (20 mg, 69.87 mmol) yields 17 mg (56.6 mmol, 81%) of compound **14** (3-(2,4-dimethoxyphenyl)-7-methoxychromane) as a white powder. High-resolution DART positive *m*/*z* 301.1479 $[M + H]^+$, calculated for $C_{18}H_{21}O_4$ 301.1439 (Figure S1, Supporting Information). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.03 (*d*, *J* = 8.3 Hz, 1H), 6.98 (*d*, *J* = 8.3 Hz, 1H), 6.50−6.47 (*m*, 2H), 6.46 (*dt*, *J* = 4.1, 2.1 Hz, 1H), 6.42 (*d*, *J* = 2.6 Hz, 1H), 4.31 (*ddd*, *J* = 10.3, 3.5, 2.0 Hz, 1H), 4.01 (*t*, *J* = 10.1 Hz, 1H), 3.82 (*s*, 3H), 3.80 (*s*, 3H), 3.77 (*s*, 3H), 3.62−3.52 (*m*, 1H), 2.98 (*dd*, *J* = 15.7, 10.6 Hz, 1H), 2.87 (*ddd*, *J* = 15.5, 5.4, 1.5 Hz, 1H) (Figure S2, Supporting Information). ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.68, 159.04, 158.28, 155.16, 130.17, 127.54, 121.89, 114.64, 107.11, 104.11, 101.37, 98.71, 70.15, 55.37, 55.35, 55.33, 31.57, 30.35 (Figure S3, Supporting Information).

Acetylation of 7-*O*-methylvestitol (**9**) (20 mg, 69.87 mmol) yields 19 mg (57.86 mmol, 82%) of compound **15** (5-methoxy-2-(7-methoxychroman-3-yl)phenyl acetate) as a white powder. High-resolution DART positive *m*/*z* 329.1387 [M + H]+, calculated for $C_{19}H_{21}O_5$ 329.1389 (Figure S4, Supporting Information). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.12 (*d*, *J* = 8.7 Hz, 1H), 6.98 (*dd*, *J* = 8.4, 0.9 Hz, 1H), 6.81 (*dd*, *J* = 8.6, 2.6 Hz, 1H), 6.64 (*d*, *J* = 2.6 Hz, 1H), 6.49 (*dd*, *J* = 8.4, 2.7 Hz, 1H), 6.44 (*d*, *J* = 2.6 Hz, 1H), 4.25 (*ddd*, *J* = 10.5, 3.5, 1.9 Hz, 1H), 3.96 (*td*, *J* = 10.5, 0.8 Hz, 1H), 3.79 (*s*, 3H), 3.78 (*s*, 3H), 3.25 (*tdd*, *J* = 10.3, 5.8, 3.5 Hz, 1H), 2.99−2.81 (*m*, 2H), 2.32 (*s*, 3H) (Figure S5, Supporting Information). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.55, 159.20, 159.11, 154.94, 149.42, 130.23, 127.93, 125.18, 114.06, 112.55, 108.45, 107.37, 101.44, 70.01, 55.48, 55.35, 31.72, 31.12, 20.99 (Figure S6, Supporting Information).

Acetylation of vestitol (**8**) (20 mg, 73.45 mmol) yields 19 mg (53.31 mmol, 72%) of compound **16** (3-(2-acetoxy-4-methoxyphenyl)chroman-7-yl acetate) as a white powder. High-resolution DART positive *m*/*z* 357.1347 $[M + H]^+$, calculated for $C_{20}H_{21}O_6$ 357.1338 (Figure S7, Supporting Information). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.11 (*d*, *J* = 8.6

https://doi.org/10.1021/acsomega.4c03132
ACS Omega 2024, 9, 35560−35566

Hz, 1H), 7.06 (*d*, *J* = 8.3 Hz, 1H), 6.82 (*dd*, *J* = 8.7, 2.6 Hz, 1H), 6.64 (*d*, *J* = 2.5 Hz, 1H), 6.62 (*d*, *J* = 2.4 Hz, 1H), 6.60 (*s*, 1H), 4.26 (*ddd*, *J* = 10.5, 3.6, 1.9 Hz, 1H), 3.96 (t, *J* = 10.6 Hz, 1H), 3.79 (*s*, 3H), 3.25 (*tdd*, *J* = 10.3, 5.7, 3.6 Hz, 1H), 3.02−2.85 (*m*, 2H), 2.32 (*s*, 3H), 2.29 (*s*, 3H) (Figure S8, Supporting Information). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.64, 169.55, 159.20, 154.76, 149.76, 149.44, 130.21, 127.85, 124.84, 119.69, 113.74, 112.60, 110.00, 108.49, 69.98, 55.49, 31.46, 31.38, 21.12, 21.00 (Figure S9, Supporting Information).

Acetylation of medicarpin (**6**) (10 mg, 36.99 mmol) yields 8 mg (25.61 mmol, 69%) of compound **17** (6aR,11aR-9-methoxy-6a,11a-dihydro-6*H*-benzofuro[3,2-*c*]chromen-3-yl acetate) as white crystals. High-resolution DART positive *m*/*z* 313.1093 $[M + H]^+$, calculated for $C_{18}H_{17}O_5$ 313.1076 (Figure S10, Supporting Information). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (*d*, *J* = 8.4 Hz, 1H), 7.14 (*dd*, *J* = 8.5, 0.9 Hz, 1H), 6.80 (*dd*, *J* = 8.4, 2.3 Hz, 1H), 6.71 (*d*, *J* = 2.3 Hz, 1H), 6.47 (*d*, *J* = 2.3 Hz, 1H), 6.45 (*d*, *J* = 1.4 Hz, 1H), 5.52 (*d*, *J* = 6.7 Hz, 1H), 4.33−4.22 (*m*, 1H), 3.77 (*s*, 3H), 3.67−3.60 (*m*, 1H), 3.57 (*ddd*, *J* = 11.0, 6.7, 4.5 Hz, 1H), 2.29 (*s*, 3H) (Figure S11, Supporting Information). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.66, 161.66, 161.03, 156.70, 152.07, 132.27, 125.24, 119.23, 118.29, 115.77, 111.20, 107.03, 97.38, 78.51, 67.07, 55.98, 39.99, 30.17, 21.58 (Figure S12, Supporting Information).

Larval Bioassay of Compounds. All compounds were solubilized in DMSO with gentle heating and agitation and then used to create stocks of 10,000 and 2000 ppm. The larval bioassay was conducted as described previously using the pesticide-susceptible ORL1952 strain of first instar *Ae. aegypti*.[17,18] Semisynthetic analogues were additionally tested with the pyrethroid-resistant Puerto Rico strain.[19] Replicate assays were conducted on three consecutive days with independently hatched cohorts of larvae. Assays were conducted in 200 μL volumes that included 5 first instar larvae, 10 μL of a 2% food slurry (2:1 pig chow/alfalfa powder), and 2 μL of each compound stock. Negative controls of DMSO and permethrin positive controls were included in each assay. Technical duplicate wells were prepared for each compound and control. Mortality was scored at 24 h, and assays were prepared and maintained at 22 °C throughout the course of the assay. Average mortality and standard deviation were calculated by summing the two technical duplicates from each assay and converting them to a percentage for each of the three replicates. PPM values shown in Tables 1 and 2 are the final concentration in the well.

Molecular Docking. The molecular docking analysis was conducted using *Ae. aegypti* acetylcholinesterase 1 (AaAChE1) as the target enzyme. The three-dimensional structure of AaAChE1 (PDB code: 5FUM) was obtained from the RCSB Protein Data Bank. Autodock Tools version 1.5.7 was used to prepare the macromolecule for the docking analysis. During the preparation, ligands, ions, and water molecules were removed from the original structure. As ligands in the docking study, the cocrystallized ligand (AL200) extracted from the original PDB file (PDB: 5FUM) was used as the standard. The isolated compounds medicarpine (**6**) and 7-*O*-methylvestitol (**9**) and the semisynthetic analogues 3-(2,4-dimethoxyphenyl)-7-methoxychromane (**14**), 5-methoxy-2-(7-methoxychroman-3-yl)phenyl acetate (**15**), and 3-(2-acetoxy-4-methoxyphenyl)-chroman-7-yl acetate (**17**) were assessed as ligands to the target enzyme. Permethrin was also included in the analysis as a standard. The structures of these compounds were drawn using the ChemDraw 15.0 program, and their respective energies were minimized using the Avogadro 1.2.0 software. Docking validation was performed by redocking the cocrystallized ligand AL200. The redocked complex was then superimposed onto the reference cocrystallized complex, and the root-mean-square deviation (RMSD) was calculated (RMSD < 2 Å). The docking process was carried out using the AutoDock Vina program.[20] The molecular interactions between the amino acid residues and the AL200 ligand were used to determine the binding site. The results of each calculation were given by the binding energy (kJ mol^{-1}) for each pose of the ligand coupled to the target enzyme binding site. The best ligand−receptor complex, observed by the affinity energy values (kcal/mol) between the ligands and the amino acid residues of AaAChE1, was used to generate 2D interaction maps with the BIOVIA Discovery Studio Visualizer.

Table 1. Larvicidal Activity of Compounds Isolated from Green, Red, and Brown Propolis

sample	mortality (% ± SD)	
	100 PPM	20 PPM
(**1**) artepillin C	6.7 ± 5.8	0 ± 0
(**2**) baccharin	0 ± 0	0 ± 0
(**3**) drupanin	3.3 ± 5.8	0 ± 0
(**4**) 2,2-dimethyl-6-carboxyethenyl-2*H*-1-benzopyran	0 ± 0	0 ± 0
(**5**) isoliquiritigenin	93.3 ± 11.5	13.3 ± 11.5
(**6**) medicarpin	96.7 ± 5.8	3.3 ± 5.8
(**7**) neovestitol	93.3 ± 5.8	3.3 ± 5.8
(**8**) vestitol	13.3 ± 11.5	0 ± 0
(**9**) methylvestitol	93.3 ± 11.5	33.3 ± 5.8
(**10**) totarol	0 ± 0	0 ± 0
(**11**) dehydroabietic acid	83.3 ± 20.8	3.3 ± 5.8
(**12**) communic acid	26.7 ± 5.8	3.3 ± 5.8
(**13**) isopimaric acid	0 ± 0	0 ± 0
permethrin (0.008 ppm)	81.7 ± 16.1	
DMSO	0 ± 0	

RESULTS

From green propolis, 4 prenylated compounds derived from 4-hydroxycinnamic acid were isolated: artepillin C (**1**), baccharin (**2**), drupanin (**3**), and 2,2-dimethyl-6-carboxyethenyl-2*H*-1-benzopyran (**4**). Red propolis provided 5 compounds: a chalcone, isoliquiritigenin (**5**), the pterocarpan medicarpin (**6**), and three isoflavonoids, neovestitol (**7**), vestitol (**8**), and 7-*O*-methylvestitol (**9**). Four diterpenes were isolated from brown propolis: totarol (**10**), dehydroabietic acid (**11**), communic acid (**12**), and isopimaric acid (**13**) (Figure 1). All the ^{1}H and ^{13}C NMR data from the isolated compounds are presented in Supporting Information section.

Regarding larvicidal activity, the compounds derived from green propolis exhibited a weak activity against *Ae. aegypti* larvae, while baccharin and 2-dimethyl-6-carboxyethenyl-2*H*-1-benzopyran were inactive, artepillin C and drupanin induced a mortality of 6.7 and 3.3% of larvae, respectively, at 100 PPM (Table 1). However, a similar pattern was observed for the brown propolis compounds with higher activity. Totarol and isopimaric acid were inactive, while communic acid and dehydroabietic acid induced a mortality of 6.7 and 3.3%, respectively, at 100 PPM. In contrast, the red propolis compounds demonstrated significantly higher activity, with all of them being active. Isoliquiritigenin, neovestitol, and

https://doi.org/10.1021/acsomega.4c03132
ACS Omega 2024, 9, 35560−35566

Table 2. Larvicidal Activity of Semisynthetic Analogues

sample	mortality (% ± SD)			
	ORL1952		PR	
	100 PPM	20 PPM	100 PPM	20 PPM
(14)	93.3 ± 11.5	50 ± 43.6	73.3 ± 25.2	13.3 ± 15.3
(15)	56.7 ± 37.9	3.3 ± 5.8	26.7 ± 25.2	0 ± 0
(16)	13.3 ± 11.5	0 ± 0	23.3 ± 5.8	0 ± 0
(17)	13.3 ± 23.1	0 ± 0	3.3 ± 5.8	0 ± 0
permethrin (0.008 ppm)	90 ± 8.7		0 ± 0	
DMSO	0 ± 0		0 ± 0	

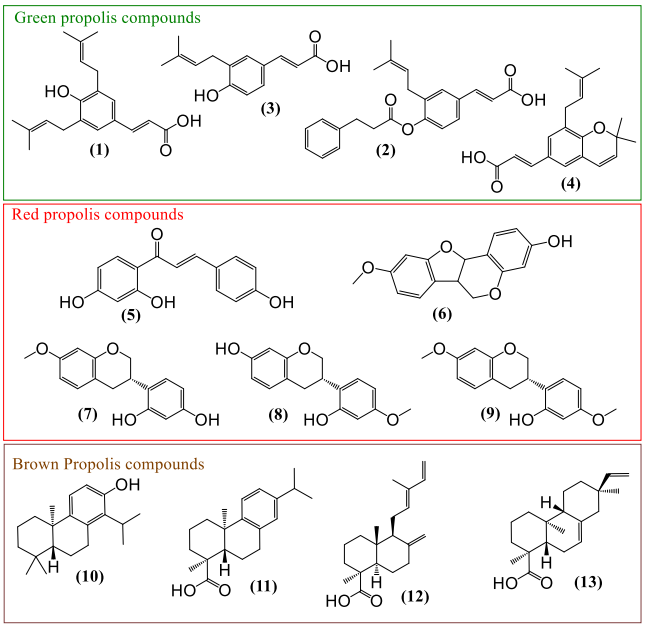


Figure 1. Chemical structures of compounds isolated from green, red, and brown propolis.

methylvestitol caused a mortality of 93.3% of the larvae at a concentration of 100 PPM, while medicarpin induced a mortality of 96.7% of the larvae at the same concentration.

The semisynthetic analogues had their larvicidal activities evaluated against *Ae. aegypti* (Table 2). Among the analogues evaluated, two compounds maintained the same activity observed as their precursors, 3-(2,4-dimethoxyphenyl)-7-methoxychromane (**14**) and 3-(2-acetoxy-4-methoxyphenyl)-chroman-7-yl acetate (**16**), while the analogues 5-methoxy-2-(7-methoxychroman-3-yl)phenyl acetate (**15**) and 3-(2-acetoxy-4-methoxyphenyl)chroman-7-yl acetate (**17**) presented a lower larval mortality rate compared to that of the precursors.

The molecular docking analysis of the compounds revealed favorable binding energy, ranging from −9.3 to −10.6 kcal mol^{-1} against the target model AaAChE1 of *Ae. aegypti* (Table 3). In the *in silico* molecular docking study, different types of intermolecular interactions were observed between the amino acids located in the active site of AaAChE1 and the compounds under investigation (Figure 2).

DISCUSSION

The different types of propolis used in this study were produced by bee species of *A. mellifera*. Several factors can exert influence on the chemical composition of propolis, including the geographic location of the hives. As bees use

Table 3. Affinity Energy and Amino Acid Residues Involved in Interaction between Compounds and the Active Site of *Aa*AChE1

compound	affinity energy (kcal mol^{-1})	interactions
(6)	−10.2	ASP74, TRP86, TYR124, TRP286, ARG296, PHE297, TYR337, TYR341, HIS447
(9)	−9.7	TRP286, LEU289, TYR338, PHE338, TYR341
(14)	−9.3	TYR72, TRP86, TRP286, PHE295, TYR337, PHE338, TYR341
(15)	−10.1	TYR72, ASP74, TRP86, TYR124, TRP286, TYR337, PHE338, TYR341, HIS447
(17)	−10.6	TRP86, TRP286, SER293, ILE294, TYR337, PHE338, TYR341, GLY448
AL200	−11.1	TRP86, TRP286, TYR337, PHE338, TYR341, HIS447

plant resins to produce propolis, the region's biodiversity directly impacts the compounds present in each type of propolis.[21]

The compounds isolated from the three distinct types of propolis employed in this study underscore the great chemical diversity of propolis. This chemical diversity is directly correlated with the wide range of pharmacological applications attributed to propolis. Propolis is generally associated with potent antioxidant activity due to the high concentration of phenolic compounds; however, we can observe that brown propolis is predominantly composed of diterpene compounds. Despite the extensive scientific literature supporting the various biological applications of propolis, studies assessing the larvicidal potential of its constituents still need to be explored. Therefore, the effect of these isolated compounds against *Ae. aegypti* larvae was evaluated.

The primary botanical source of red propolis is *D. ecastaphyllum*, which is responsible for the presence of isoflavonoids, flavanones, chalcones, and pterocarpans. Pluempanupat and coauthors (2013) evaluated the larvicidal potential of *Dalbergia oliveri* and isolated isoflavonoids against *Ae. aegypti* larvae.[22] The highest larvicidal effect was observed with dichloromethane and hexane fractions of *D. oliveri*. Medicarpin, isolated from the dichloromethane fraction, was the most active compound against *Ae. aegypti*.[22] Similarly, Bezerra-Silva et al. (2015) evaluated the larvicidal potential of *Bowdichia virgilioides* against *Ae. aegypti* larvae and found that medicarpin present in the extract exhibited strong larvicidal properties.[23] These findings corroborate our results since medicarpin had the greatest larvicidal effect and highlight the effectiveness of medicarpin as a potent larvicidal agent against *Ae. aegypti* larvae.

A notable aspect of the results obtained in the larvicidal bioassay is the divergent activities observed among compounds

https://doi.org/10.1021/acsomega.4c03132
ACS Omega 2024, 9, 35560−35566

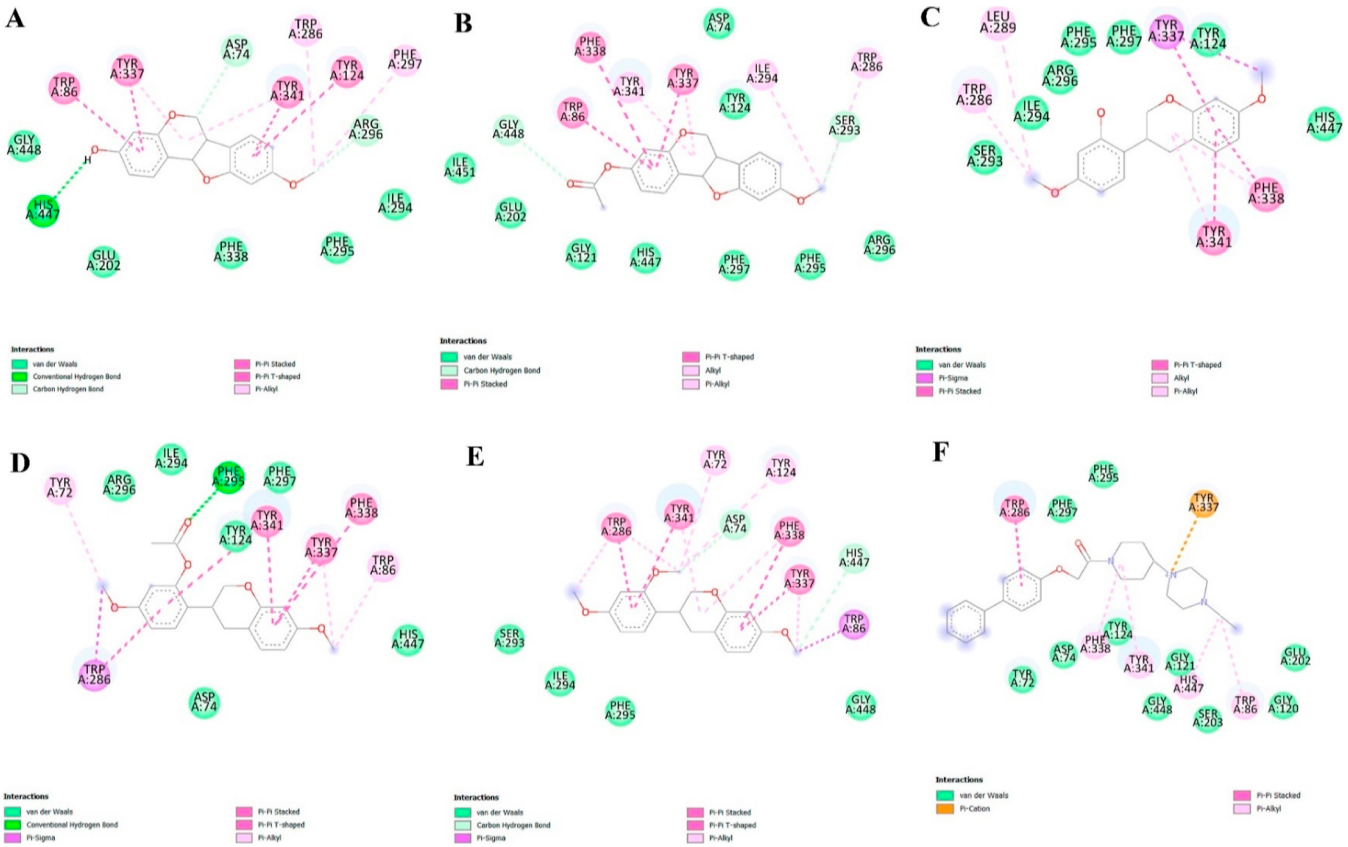


Figure 2. Compounds' interaction with the active site of AaAChE1. (A) Medicarpin (**6**); (B) 3-(2-acetoxy-4-methoxyphenyl)chroman-7-yl acetate (**17**); (C) 7-*O*-methylvestitol (**9**); (D) 5-methoxy-2-(7-methoxychroman-3-yl)phenyl acetate (**15**); (E) 3-(2,4-dimethoxyphenyl)-7-methoxychromane (**14**); (F) co-crystallized ligand AL200.

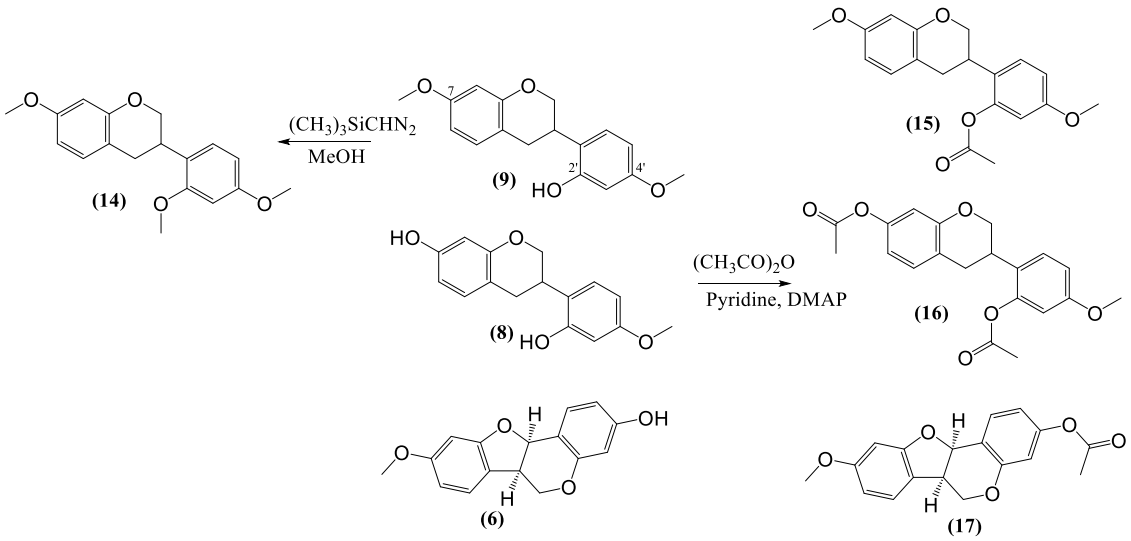


Figure 3. Chemical structures of semisynthetic analogues obtained from 7-*O*-methylvestitol, vestitol, and medicarpin.

neovestitol (**7**), 7-*O*-methylvestitol (**9**), and vestitol (**8**). Upon closer analysis, it is possible to observe that the structural differences are related to the presence of methoxy and hydroxyl groups in positions 2′, 4′, and 7. Therefore, it is possible to infer that the functional groups in these positions play a role in the observed activity. Therefore, we carried out structural modifications in vestitol (**8**), 7-*O*-methylvestitol (**9**), and medicarpin (**6**) through acetylation and methylation of hydroxyl groups (Figure 3).

Various insecticides, including organophosphates and carbamates, have acetylcholinesterase (AChE) as a target enzyme. AChE plays a vital role in the termination of impulse transmission by rapid hydrolysis of the neurotransmitter acetylcholine in multiple cholinergic pathways within both the central and peripheral nervous systems.[24] Inhibition of AChE causes an accumulation of acetylcholine, leading to overstimulation of the nervous system, leading to the death of mosquitoes. Mosquitoes express two forms of AChE, AChE1

https://doi.org/10.1021/acsomega.4c03132
ACS Omega 2024, 9, 35560−35566

and AChE2 enzymes. AChE1 is the isoform responsible for AChE-mediated insecticide resistance and exhibits greater catalytic activity compared to AChE2.[25] In this way, a molecular docking analysis was conducted on AaAChE1 from *Ae. aegypti*. The results revealed that all of the ligands analyzed could interact with amino acid residues within the active site of AaAChE1 in *Ae. aegypti*. Our in silico findings suggest that the compounds investigated in this research have the potential to effectively bind to the active site of AaAChE1. These interactions can help to understand how the compounds bind to and interact with the AaAChE1 active site.

Many natural compounds interact with the AChE enzyme. The study conducted by Silva et al. (2023) explored the molecular interactions between the compound fumarprotocetraric acid isolated from lichens and the AaAChE1 enzyme. The findings emphasize the significance of hydrophobic interactions, which are strongly correlated with a high affinity for the target enzyme. In our *in silico* studies, several hydrophobic interactions were identified between the compounds and the active site of AaAChE1. A common interaction was observed at the TRP286 amino acid residue shared by the cocrystallized ligand (AL200) and the insecticide temephos, and this common interaction was associated with the enhanced activity.[3] All compounds analyzed showed interaction with TRP286. Medicarpin (**6**) presented a Pi-alkyl-type interaction and the analogue 3-(2,4-dimethoxyphenyl)-7-methoxychromane (**14**), three interactions with TRP286.

CONCLUSIONS

Red propolis compounds demonstrated higher larvicidal activity against *Ae. aegypti* compared to the green and the brown propolis. Medicarpin (**6**) was the most active compound. The integration of computational approaches such as molecular docking with experimental studies can contribute to the elucidation of the mechanism of action and improvement of natural insecticidal compounds, contributing to the development of effective and safer insecticides for mosquito control. Our results reinforce the importance of Propolis as a source of bioactive natural compounds and the diversity of its utilization.

ASSOCIATED CONTENT

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon request.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c03132.

NMR and HR-DART-MS data of the isolated compounds; *in silico* determined physical−chemical properties from compounds; and *Aedes aegypti* acetylcholinesterase enzyme with the docked ligands and intermolecular interactions (PDF)

AUTHOR INFORMATION

Corresponding Author

Kumudini M. Meepagala − *Agricultural Research Service, U.S. Department of Agriculture, Natural Products Utilization Research Unit, University, Mississippi 38677, United States;* orcid.org/0000-0001-6071-1670; Email: kumudini.meepagala@usda.gov

Authors

Victor P. Ribeiro − *Agricultural Research Service, U.S. Department of Agriculture, Natural Products Utilization Research Unit, University, Mississippi 38677, United States*

Jairo K. Bastos − *School of Pharmaceutical Sciences of Ribeirão Preto − University of São Paulo, Ribeirão Preto 14040-930, Brazil;* orcid.org/0000-0001-8641-9686

Alden S. Estep − *USDA-ARS, Mosquito and Fly Research Unit, Gainesville, Florida 32608, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.4c03132

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This research was supported in part by an appointment to the Agricultural Research Service (ARS) Research Participation Program administered by the Oak Ridge Institute for Science and Education (ORISE) through an interagency agreement between the U.S. Department of Energy (DOE) and the U.S. Department of Agriculture (USDA). ORISE is managed by ORAU under DOE contract number DE-SC0014664. All opinions expressed in this paper are the author's and do not necessarily reflect the policies and views of USDA, DOE, or ORAU/ORISE.

REFERENCES

(1) Silvério, M. R. S.; Espindola, L. S.; Lopes, N. P.; Vieira, P. C. Plant Natural Products for the Control of *Aedes aegypti*: The Main Vector of Important Arboviruses. *Molecules* **2020**, *25*, 3484.

(2) Machado, F. P.; Folly, D.; Salas Enriquez, J. J.; Mello, C. B.; Esteves, R.; Araújo, R. S.; Toledo, P. F. S.; Mantilla-Afanador, J. G.; Santos, M. G.; Oliveira, E. E.; Ricci-Junior, E.; Rocha, L. Nanoemulsion of *Ocotea indecora* (Shott) Mez essential oil: Larvicidal effects against *Aedes aegypti*. *Ind. Crops Prod.* **2023**, *192*, 116031.

(3) da Silva, A. S.; de Oliveira Farias de Aguiar, J. C. R.; da Silva Nascimento, J.; Costa, E. C. S.; dos Santos, F. H. G.; Andrade de Araújo, H. D.; da Silva, N. H.; Pereira, E. C.; Martins, M. C.; Falcão, E. P. S.; Scotti, L.; Scotti, M. T.; do Amaral Ferraz Navarro, D. M. Larvicidal activity and docking study of *Ramalina complanata* and *Cladonia verticillaris* extracts and secondary metabolites against *Aedes aegypti*. *Ind. Crops Prod.* **2023**, *195*, 116425.

(4) Arruda, C.; Ribeiro, V. P.; Mejía, J. A. A.; Almeida, M. O.; Goulart, M. O.; Candido, A. C. B. B.; dos Santos, R. A.; Magalhães, L. G.; Martins, C. H. G.; Bastos, J. K. Green Propolis: Cytotoxic and Leishmanicidal Activities of Artepillin C, p-Coumaric Acid, and Their Degradation Products. *Rev. Bras. Farmacogn.* **2020**, *30*, 169−176.

(5) Barbosa, E. V.; Assumpção, Y. M.; Teixeira, I. M.; Pereira, R. F. A.; Ribeiro, V. P.; Bastos, J. K.; Cardoso, C. V.; Liberal, M. H. T.; Penna, B. A.; Rocha, L. M. *In vitro* comparison between antimicrobial and antibiofilm effects of Green Propolis and *Baccharis dracunculifolia* against *Staphylococcus pseudintermedius* isolate. *An. Acad. Bras. Cienc.* **2022**, *94*, No. e20211103.

(6) Beserra, F. P.; Gushiken, L. F. S.; Hussni, M. F.; Ribeiro, V. P.; Bonamin, F.; Jackson, C. J.; Pellizzon, C. H.; Bastos, J. K. Artepillin C as an outstanding phenolic compound of Brazilian green propolis for disease treatment: A review on pharmacological aspects. *Phytother. Res.* **2021**, *35*, 2274−2286.

(7) Giovanini de Oliveira Sartori, A.; Papa Spada, F.; Pena Ribeiro, V.; Rosalen, P. L.; Ikegaki, M.; Kenupp Bastos, J.; de Alencar, S. M. An insight into the botanical origins of propolis from permanent preservation and reforestation areas of southern Brazil. *Sci. Rep.* **2021**, *11*, 22043.

(8) Bernardes, C. T. V.; Ribeiro, V. P.; de Carvalho, T. C.; Furtado, R. A.; Jacometti Cardoso Furtado, N. A.; Bastos, J. K. Disinfectant

activities of extracts and metabolites from *Baccharis dracunculifolia* DC. *Lett. Appl. Microbiol.* **2022**, *75*, 261−270.
(9) Aldana-Mejía, J. A.; de Miranda, A. M.; Ccana-Ccapatinta, G. V.; de Araújo, L. S.; Ribeiro, V. P.; Arruda, C.; Nascimento, S.; Squarisi, I.; Esperandim, T.; de Freitas, K. S.; Ozelin, S. D.; Tavares, D. C.; Ramalho, F. S.; Bastos, J. K. Genotoxicity and toxicological evaluations of Brazilian red propolis oral ingestion in a preclinical rodent model. *J. Ethnopharmacol.* **2023**, *303*, 115920.
(10) Serafim, M. S.; Rodrigues, D. M.; Ribeiro, V. P.; Ccana-Ccapatinta, G. V.; Groppo, M.; Martins, C. H. G.; Ambrósio, S. R.; Bastos, J. K. *Eucalyptus botryoides*' resin and its new 2- *O* -galloyl-1,6- *O* -di- *trans* - *p* -coumaroyl- *β* -D-glycopyranoside compound display good antimicrobial activity. *Nat. Prod. Res.* **2023**, *37*, 618−627.
(11) Santos, M. F. C.; Oliveira, L. C.; Ribeiro, V. P.; Soares, M. G.; Morae, G. d. O. I.; Sartori, A. G. d. O.; Rosalen, P. L.; Bastos, J. K.; de Alencar, S. M.; Veneziani, R. C. S.; Ambrósio, S. R. Isolation of diterpenes from Araucaria sp Brazilian brown propolis and development of a validated high-performance liquid chromatography method for its analysis. *J. Sep. Sci.* **2021**, *44*, 3089−3097.
(12) Son, N. T.; Ribeiro, V. P.; Teixeira, M. V. S.; Furtado, N. A. J. C.; Bastos, J. K. Northeastern Caatinga Green Propolis: Volatile Oil and Antimicrobial Activity. *Chem. Nat. Compd.* **2023**, *59*, 971−973.
(13) Aldana-Mejía, J. A.; Ccana-Ccapatinta, G. V.; Ribeiro, V. P.; Arruda, C.; Veneziani, R. C. S.; Ambrósio, S. R.; Bastos, J. K. A validated HPLC-UV method for the analysis of phenolic compounds in Brazilian red propolis and *Dalbergia ecastaphyllum*. *J. Pharm. Biomed. Anal.* **2021**, *198*, 114029.
(14) Ribeiro, V. P.; Aldana-Mejia, J. A.; Arruda, C.; Candido, A. C. B. B.; Magalhães, L. G.; Oliveira, N. D.; Veneziani, R. C. S.; Bastos, J. K.; Ambrósio, S. R. A RP-HPLC-DAD method for analysis of Brazilian southeast brown propolis and its leishmanicidal properties. *Biomed. Chromatogr.* **2023**, *37*, No. e5634.
(15) Arruda, C.; Pena Ribeiro, V.; Oliveira Almeida, M.; Aldana Mejía, J. A.; Casoti, R.; Kenupp Bastos, J. Effect of light, oxygen, and temperature on the stability of artepillin C and p-coumaric acid from Brazilian green propolis. *J. Pharm. Biomed. Anal.* **2020**, *178*, 112922.
(16) Ribeiro, V. P.; Arruda, C.; Mejía, J. A. A.; Candido, A. C. B. B.; dos Santos, R. A.; Magalhães, L. G.; Bastos, J. K. Brazilian southeast brown propolis: gas chromatography method development for its volatile oil analysis, its antimicrobial and leishmanicidal activities evaluation. *Phytochem. Anal.* **2021**, *32*, 404−411.
(17) Meepagala, K. M.; Estep, A. S.; Becnel, J. J. Larvicidal and adulticidal activity of chroman and chromene analogues against susceptible and permethrin-resistant mosquito strains. *J. Agric. Food Chem.* **2016**, *64*, 4914−4920.
(18) Meepagala, K. M.; Estep, A. S.; Clausen, B. M.; Becnel, J. J. Mosquitocidal activity of a naturally occurring isochroman and synthetic analogs from the plant pathogenic fungus, Diaporthe eres against *Aedes aegypti* (Diptera: Culicidae). *J. Med. Entomol.* **2018**, *55*, 969−974.
(19) Estep, A. S.; Sanscrainte, N. D.; Waits, C. M.; Louton, J. E.; Becnel, J. J. Resistance status and resistance mechanisms in a strain of *Aedes aegypti* (Diptera: Culicidae) from Puerto Rico. *J. Med. Entomol.* **2017**, *54*, 1643−1648.
(20) Trott, O.; Olson, A. J. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.* **2010**, *31*, 455−461.
(21) Son, N. T.; Ribeiro, V. P.; Bastos, J. K. Flavonoids from green propolis of the Northeastern Brazilian Caatinga *Mimosa tenuiflora* (Willd.) Poir.: A chemotaxonomic aspect. *Biochem. Syst. Ecol.* **2022**, *104*, 104473.
(22) Pluempanupat, S.; Kumrungsee, N.; Pluempanupat, W.; Ngamkitpinyo, K.; Chavasiri, W.; Bullangpoti, V.; Koul, O. Laboratory evaluation of *Dalbergia oliveri* (Fabaceae: Fabales) extracts and isolated isoflavonoids on *Aedes aegypti* (Diptera: Culicidae) mosquitoes. *Ind. Crops Prod.* **2013**, *44*, 653−658.
(23) Bezerra-Silva, P. C.; Santos, J. C.; Santos, G. K.; Dutra, K. A.; Santana, A. L.; Maranhão, C. A.; Nascimento, M. S.; Navarro, D. M.; Bieber, L. W. Extract of *Bowdichia virgilioides* and maackiain as larvicidal agent against *Aedes aegypti* mosquito. *Exp. Parasitol.* **2015**, *153*, 160−164.
(24) Engdahl, C.; Knutsson, S.; Fredriksson, S. . Å.; Linusson, A.; Bucht, G.; Ekström, F. Acetylcholinesterases from the Disease Vectors *Aedes aegypti* and Anopheles gambiae: Functional Characterization and Comparisons with Vertebrate Orthologues. *PLoS One* **2015**, *10*, No. e0138598.
(25) Engdahl, C.; Knutsson, S.; Ekström, F.; Linusson, A. Discovery of Selective Inhibitors Targeting Acetylcholinesterase 1 from Disease-Transmitting Mosquitoes. *J. Med. Chem.* **2016**, *59*, 9409−9421.

https://doi.org/10.1021/acsomega.4c03132
ACS Omega 2024, 9, 35560−35566