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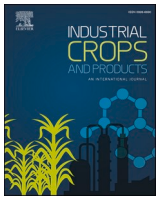

Larvicidal activity and docking study of *Ramalina complanata* and *Cladonia verticillaris* extracts and secondary metabolites against *Aedes aegypti*

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ABSTRACT

The *Aedes aegypti* mosquito is the main vector of the arboviruses that cause Zika, Yellow Fever, Dengue, and Chikungunya, incapacitating diseases that may even be fatal. There are still no vaccines for any of these viruses. Therefore, the only way to contain outbreaks is to control the vector that transmits them. In the present study, we investigated the larvicidal activity of lichen extracts (*Ramalina complanata* (Sw.) Ach., 1810 and *Cladonia verticillaris* (Raddi) Fr.), isolating components that were used against larvae of the mosquito *A. aegypti*. The ether extract from *R. complanata* exhibited an LC_{50} = 5.9 μg.ml^{-1}, while a compound isolated from this species, divaricatic acid, had an LC_{50} = 27.1 μg.ml^{-1}. The acetone extract of *C. verticilaris* exhibited an LC_{50} = 17.4 μg.ml^{-1} and the isolated compound fumarprotocetraric acid (FUM) had an LC_{50} = 13.6 μg.ml^{-1}. These results demonstrate that extracts and isolated compounds from the two lichen species have strong larvicidal activities. FUM is the most active compound in our investigation. The molecular docking studies of divaricatic acid and FUM demonstrated that they bind to the active site of *Aa*AChE1 in an energetically favorable manner. The TRP286 residue may have contributed to the more pronounced activity of fumarprotocetraric acid compared to divaricatic acid. Other aspects related to the more pronounced activity of fumarprotocetraric acid are its molecular weight and higher solubility. Our results provide evidence of the importance of studying lichen secondary metabolites as natural sources of insecticides and investigating possible interactions with *Aa*AChE1.

1. Introduction

The mosquito *Aedes aegypti* is a vector of the viruses that cause Zika, dengue, chikungunya and yellow fever, which affect thousands of people in tropical and subtropical regions throughout the world (Musso et al., 2015; World Health Organization, 2016). In Brazil, the number of cases of dengue increased by 48.1% in 2022 compared to 2021 (Brazilian Health Ministry, 2022). The most common way to prevent these diseases is to combat the transmitting mosquito by interrupting its life cycle with egg traps, larvicides and insecticides (Consoli and Oliveira, 1994). Larvicides are effective for combating larvae, which is one of the aquatic forms in the life cycle of the mosquito. However, synthetic larvicides have led to the development of resistant mosquito populations (Diniz et al., 2014). It is important to emphasize that the use of synthetic larvicides can also affect the environment and human health (Vieira Santos et al., 2017; Aiub et al., 2002).

One alternative is the use of natural larvicides derived from secondary metabolites from plants, which have been widely investigated as

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a source of promising products for combating mosquitoes and other insect pests (Budiman et al., 2021). An example is the larvicidal activity of 2-hydroxy-4-methoxy-6-propyl-methyl benzoate and (+)-usnic acid isolated from *Ramalina usnea* (lichen); these compounds exhibited significant toxicity to *A. aegypti,* with LC_{50} values of 4.85 and 4.48 $\mu g.ml^{-1}$, respectively (Moreira et al., 2016). The literature offers other examples of natural products with larvicidal activity, such as extracts and essential oils from plants and microalgae (Navarro et al., 2013). Bianco et al. (2013) reported the larvicidal activity of marine macroalgae and found that the substance responsible for the mortality of the larvae was elatol ($LC_{50} = 10.7\ \mu g.ml^{-1}$). Essential oils are an effective tool against *Aedes aegypti* mosquito larvae. There is extensive scientific literature on this subject (Budiman et al., 2021).

Lichens are a symbiotic association between fungi and algae or cyanobacteria. There are ca. 17,000 known lichen species and no less than 800 lichen-based products are used in the cosmetic, perfume, and food industries, with further uses as pollution bioindicators, dyes, and drugs. In medicine, it is possible to mention the use of lichens in folk medicine to cure some diseases, such as inflammation of the joints (arthritis), inflammation of the skin (eczema), and pulmonary disease. *Usnea longissima*, a lichen species that is highly sensitive to air pollution, is used to monitor air quality. It can also be used as a hair strengthener and hygiene product, as well as in the treatment of leg injuries, skin eruptions, and as an expectorant (Jayanthi et al., 2012).

Lichens are also rich in secondary metabolites with proven biological properties, such as fumarprotocetraric and lecanoric acid. Fumarprotocetraric acid has demonstrated expectorant and antioxidant activity in rats and lecanoric acid has antioxidant activity as well as antimicrobial activity, with action against Gram-positive bacteria (Gaikwad et al., 2014; Nóbrega et al., 2012; De Barros Alves et al., 2014). Extracts from the lichens *Lecanora muralis, Letharia vulpina* and *Peltigera rufescens* exhibited insecticidal activity against adult forms of the grain weevil, *Sitophilus granarius* (Emsen et al., 2015). There are studies on molecular docking of acetylcholinesterase, butyrylcholinesterase, and tyrosinase enzymes to molecules isolated from seaweeds (isoamijiol, 14-deoxyamijiol, amijidictyol, dictyodial, and 4α-acetoxydictyodial). They show that these molecules can interact with enzymes and inhibit them, evidencing the mode of action of larvicides in mosquitoes (Kilic et al., 2021). Secondary metabolites confer the protection of lichens from certain types of insects (Pöykkö et al., 2010) and constitute a promising source of compounds with insecticidal activity that could be a good option in the search for bioinsecticides for pest control programs.

Relatively few studies have addressed the insecticidal activity of substances derived from lichens against insects of public health importance. Therefore, the aim of the present study was to investigate the larvicidal activity of extracts from lichens (*Ramalina complanata* (Sw.) Ach., 1810 and *Cladonia verticillaris* (Raddi) Fr.) and isolated components of these extracts against larvae of the mosquito *A. aegypti*.

2. Methods

2.1. Lichen material

The lichen *R. complanata* was collected from the municipalities of Saloá (09° 01.399′ S; 36° 47.565′ W) in the state of Pernambuco in northeast Brazil in the winter period of the Southern Hemisphere (June 2008). The identification of the thallus was performed by an evaluation of morphological characteristics and a voucher (nº 54299) was deposited in the Geraldo Mariz Herbarium of the Botany Department of *Universidade Federal de Pernambuco* (UFPE), Recife, PE, Brazil.

The lichen *C. verticillaris* was collected from the municipality of Taquaritinga do Norte (09° 01.399′ S; 36° 47.565′ W) in the state of Pernambuco, Brazil in the winter period of the Southern Hemisphere (June 2017). The identification of the thallus was performed as described above and a voucher (nº 54301) was deposited in the Geraldo Mariz Herbarium of the Botany Department of UFPE, Recife, PE, Brazil.

2.2. Preparation of extracts and isolation of compounds

The isolation of all compounds was performed following the methods described by Asahina and Shibata (1954).

Preparation of ether extract of *R. complanata* and isolation of divaricatic acid: dried and crushed lichen (10 g) was extracted using 100 ml of diethyl ether in a soxhlet apparatus under reflux. The extract was transferred to a vacuum rotary evaporator and subsequently stored in glass vials and placed in a desiccator until constant weight was obtained. A mass of 0.870 g of dry extract was obtained (yield: 8.7%). Around 0.87 g of extract was used to isolate divaricatic acid. The glass vial (10 ml) containing the entire extract was placed in a water bath, followed by the addition of 5 ml of ethanol. A glass rod was used to mix the solid with ethanol in an attempt to dissolve the impurities contained in the divaricatic acid. The mixture was filtered using a filter paper, and the solid obtained was transferred back to the flask. Every time the solid was filtered, a very small sample was used in thin-layer chromatography (TLC) to check the purity of the divaricatic acid. This procedure was repeated until a single spot was obtained on TLC, i.e., highly purified divaricatic acid. TLC: the elution system used was toluene/dioxane/acetic acid (45:12.5:2 v/v/v), reveal in 10% sulfuric acid. The compounds' bands were observed under UV light (254/312 nm). We obtained 0.249 g of divaricatic acid (28% yield).

Preparation of the acetone extract of *C. verticillaris* and isolation of FUM: Lichen thalli (30 g) were submitted to extraction in a shaker at room temperature for 3 h using 100 ml of acetone. The extraction was repeated 3 times and the extracts were combined. The extract was kept in a freezer for 24 h then filtered. The solvent was removed in a vacuum rotary evaporator until a dry extract was obtained (2.4 g; 8% yield). TLC was used to monitor compound bands in extract preparation. Isolation of FUM from *C. verticillaris* acetone extract: 2.4 g of the acetone extract was dissolved in 20 ml of cold acetone and centrifuged at 5000 rpm for 5 min. This centrifugation procedure was repeated 4 times, until the formation of a precipitate could be observed. Then, the acetone solution was separated from the precipitate using a Pasteur pipette. The precipitate was moved to a G4 sintered glass funnel, and very gently washed with drops of ice-cold acetone. A mass of 0.50 g of FUM was obtained (20% yield).

2.3. Identification of compounds in extracts and confirmation of molecular structure

The identification and isolation of compounds was performed using thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) and proton nuclear magnetic resonance (^{1}H NMR).

TLC analyses followed the methods described by Culberson (1969). Chromatography was performed in the ascending direction using Merck® silica gel F254 nm chromatoplates. The solvent system was toluene-dioxane-acetic acid (80:19.5:0.5, v-v:v). After the run, bands corresponding to the compounds were viewed under a UV lamp (254 and 366 nm) and subsequently developed by spraying a 10% sulfuric acid solution and heating to 100ºC/10 min. Compounds were identified by comparison with standards of lichen phenols available in the lab.

HPLC analyses were performed in a Hitachi liquid chromatograph coupled to a UV/Vis detector, following the method described by Legaz et al. (1986). An RP18 reverse phase column was used, with pressure of 88 atm and a flow of 1 ml/min. Compounds were identified based on retention time in the column and comparison with standards available in the lab.

^{1}H NMR analysis was performed in a Varian spectrometer (Unity model) at 300 MHz.

2.4. Maintenance of Aedes aegypti colony

Larvae were obtained from the *Aedes aegypti* Linneaus (Rockefeller strain) colony of the insectarium of the UFPE Chemical Ecology Lab. The

insectarium was kept at a temperature of 27 ± 1ºC, average relative humidity of 80% and a photoperiod with 14 h of light and 10 h of darkness. The Rockefeller strain is cultivated and used in entomology as a reference of susceptibility, which enables its use in bioassays for the comparison of resistance with local populations.

2.5. Rearing of Aedes aegypti in laboratory

For the hatching of the eggs of *Aedes aegypti,* paper platforms containing eggs were placed in a cup with water from the larval rearing containers in the lab. This procedure is normally performed within 24 h. The newly hatched eggs were distributed and placed in round plastic containers with a 20-cm diameter containing approximately 450 ml of distilled water. Feeding throughout the larval stage was performed with ground Purina® dog ration.

The water in the containers was exchanged every two days until the larvae reached the pupal stage. The pupae were removed from the containers and placed in glasses, which were placed in square cages (35 cm per side) for the emergence of the adults. The adults were fed daily with a 10% sucrose solution. Chicken blood meal was offered to the females, supplied in an artificial glass apparatus at 35–36º C.

2.6. Larvicidal bioassay

The extracts and pure compounds were diluted in distilled water for the preparation of different concentrations. Tween 80 (three drops) was used as co-solvent for better homogenization in 100 ml of distilled water. Twenty *A. aegypti* larvae (L4 instar) were exposed to the treatment in 50-ml beakers containing 20 ml of the test solution. Each test was conducted in triplicate. The negative control was distilled water with Tween 80 (same concentration used to dissolve the extracts and isolated compounds – three drops per 100 ml of water) and 20 larvae (L4 instar). The positive control was Temephos (LC_{50} = 0.0033 μg.ml^{-1}), used to kill 20 larvae (L4 instar) (to confirm non-resistant colony). Mortality of the larvae exposed to the treatments was determined after 24 and 48 h. The quantity of dead larvae was recorded on a chart and lethal concentrations (LC_{50} and LC_{90}) were calculated. Larvae with no motor activity 48 h after the onset of the bioassay were considered dead. The calculation of the LC_{50} and LC_{90} for the extract and isolated compounds was performed using Probit – Package Ecotox: Analysis of Ecotoxicity (Wheeler et al., 2006; Hlina et al., 2021; De Carvalho et al., 2017).

2.7. Molecular docking of compounds

Preparation of ligands: For the molecular docking analysis, two standards (Temephos and AL200 co-crystalized ligand) and two lichen compounds were used in the preparation of the ligands. All structures of the compounds were designed, and energies were minimized using the BIOVIA Discovery Studio Visualizer (https://discover.3ds.com/discovery-studio-visualizer-download).

Preparation of macromolecule: The enzyme acetylcholinesterase 1 from *Aedes aegypti* (*Aa*AChE1) (PDB: 5FUM) was retrieved from the Protein Data Bank (PDB). All ligands, ions and molecules of water were manually removed from the original structure and hydrogen atoms were added using the BIOVIA Discovery Studio.

Docking: The docking process was performed using the Molegro Virtual Docker (MVD) program and the binding site was defined by molecular interactions between amino acid residues and the co-crystallized ligand (AL200). The grid box was set at a radius of 15 Å. The results for each calculation were given by the bond energy (Kj. mol^{-1}) for each pose of the ligand coupled to the target enzyme.

Consensus docking: The consensus analysis was based on the average auto-scaling scores (Oda et al., 2006). All scores obtained by five methods (GPU Score, Moldock Score, Rerank Score, Docking Score and Similarity Score) for each molecule were divided by the lowest value among them. The average of the normalized values (consensus) was calculated for each compound and the compounds were subsequently ranked as a function of this average.

Docking validation: Docking validation was performed by the re-dock of the co-crystallized ligand (AL200) extracted from the original PDB file (PDB ID: 5FUM) and interpreted in terms of the root mean square deviation < 2 for overlap of the re-docked ligand and crystallographic pose (Silva-Júnior et al., 2016).

Analyses and illustrations: The analyses of the interactions between the ligands and amino acid residues of *Aa*AChE1 were performed using the BIOVIA Discovery Studio. The same program was used for the illustrations.

3. Results and discussion

3.1. Physicochemical analysis of lichen metabolites

The isolation of the compounds was accompanied by TLC. For FUM, the retention factor (RF) = 0.18 corroborates the data reported in the literature (Martins et al., 2018). The RF for divaricatic acid was 0.56. The purity of the compounds was evaluated using HPLC. The divaricatic acid had 99% purity (Fig. 1) and a retention time of 16.47 min (Figs. 1 and 2).

FUM had a retention time of 2.79 min and 93.79% purity (Fig. 2).

3.1.1. C. verticillaris

The spectroscopic analysis was performed using H^1 NMR and the results confirmed data reported by Huneck and Yoshimura (1996):

Divaricatic acid: (400 MHz, $CdCl_3$)δ 0.98 (6 H, T, 5.4 Hz, Me, C18, 27); 1.68 (4 H, *m,* CH2 – C-16 and 26); 2.97 (4 H, *m,* CH2, C-17, 25); 2.93 (3 H, *s,*OCH3, C-20); 6.38 (2 H, *d,* 2.4 Hz, C4, 11); 6.64 (1 H, *d,* 2.4 Hz, C15); 6.76 (1 H, *d,* 2.4 Hz, C-6); 10.65 (2 H, *s*, OH, C7, 22).

Fumarprotocetraric acid (FUM): ^{1}H NMR (300 MHz, DMSO-d_6) δ_H (H; *mult.*; int.): 2.38 (3 H; *s*; CH_3-9), 2.41 (3 H; *s*; CH_3-9′). 5.26 (2 H; *s*; CH_2-8′), 6.60 (2 H; *s*; CH-2′; CH-3′), 6.80 (1 H; *s*; CH-5), 10.53 (1 H; *s*; CH-8), 11.93 (1 H; *s*; C-4-OH or C-2′-OH).

3.2. Larvicidal activity of compounds

The LC_{50} and LC_{90} of the lichen compounds against the *A. aegypti* larvae are displayed in Table 1. The ether extract from *R. complanata* had 50% lethality at a concentration of 5.9 μg.ml^{-1}, whereas the compound isolated from this lichen (divaricatic acid) had an LC_{50} of 27.1 μg.ml^{-1}, demonstrating that the extract had greater larvicidal action compared to the isolated compound. This finding may be due to the synergism between divaricatic acid and other chemical components of the extract that were also active in the larvicidal tests. Further investigation is necessary to identify these active compounds in the extract. The synergistic effect was well discussed by Pavela (2008) in a study in which the authors showed that a mixture of compounds improved the mortality of the insects compared with compounds tested separately.

The acetone extract of *C. verticilaris* had an LC_{50} of 17.4 μg.ml^{-1} and its isolated compound (FUM) had an LC_{50} of 13.6 μg.ml^{-1} (Table 1), demonstrating that the isolated compound had greater larvicidal activity than that of the extract. Thus, FUM is the active compound of this extract in terms of larvicidal activity for *Aedes aegypti*.

The insecticidal potential of FUM has been reported in the literature. Martins et al. (2018) demonstrated the insecticidal action of FUM for the development of *Nasutitermes corniger*. Lectin isolated from the lichen *C. verticillaris* also presented insecticidal action against *Nasutitermes corniger* (Silva et al., 2009).

Other compounds isolated from lichens have been described as bioinsecticides. Usnic acid isolated from the lichen *Ramalina usnea* caused 50% mortality of *A. aegypti* larvae exposed to the compound at a concentration of 4.48 μg.ml^{-1} (Moreira et al., 2016). Extracts from the lichens *Lecanora muralis*, *Letharia vulpina* and *Peltigera rufescens* caused the

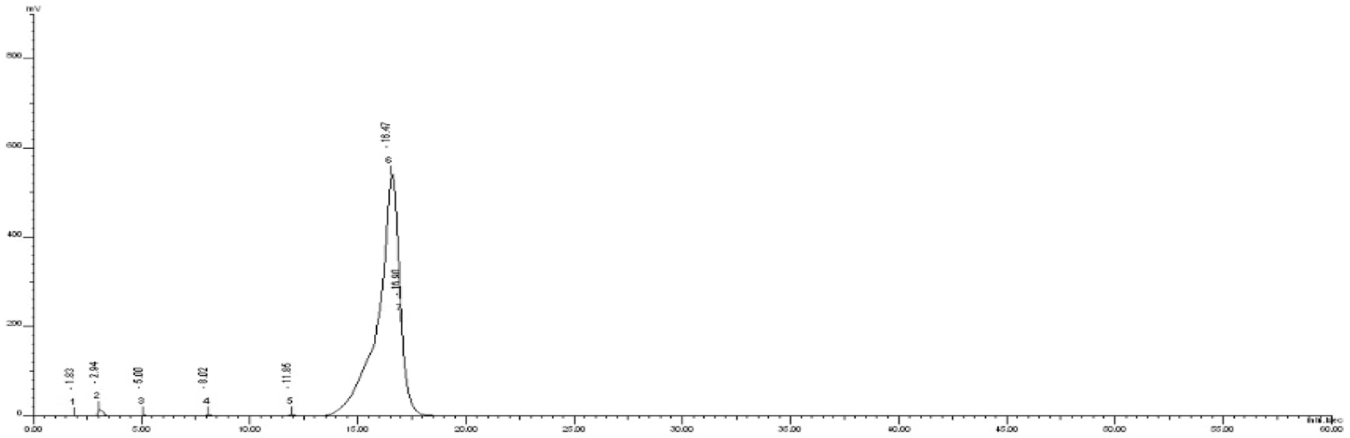

Fig. 1. HPLC chromatogram. Divaricatic acid isolated from *R. complanata*.

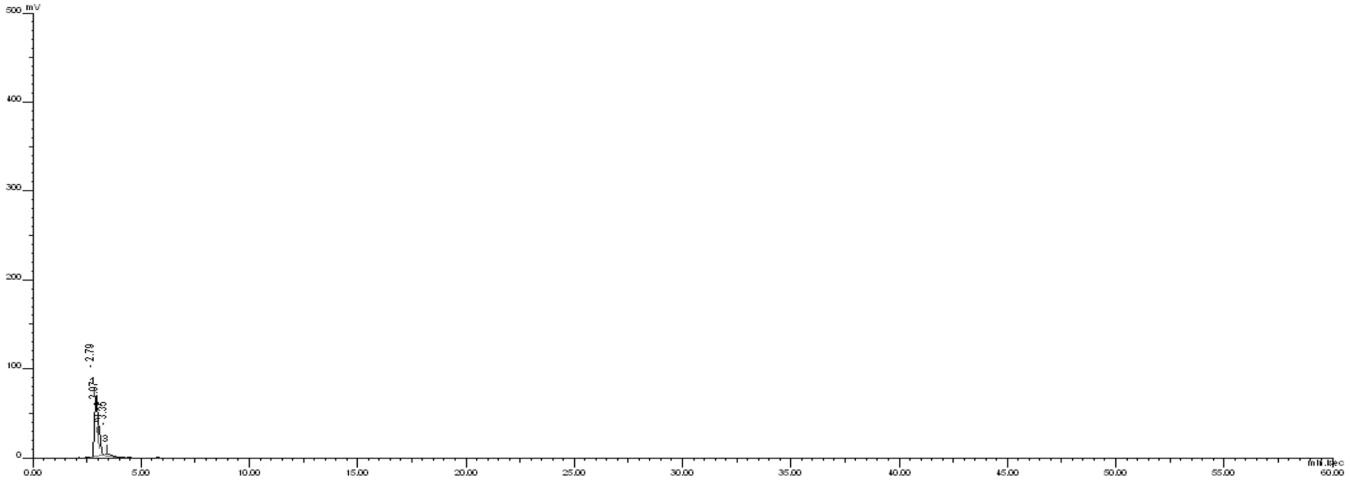

Fig. 2. HPLC chromatogram. Fumarprotocetraric acid (FUM) isolated from.

Table 1
Larvicidal data (LC_{50} and LC_{90}) for *A. aegypti* exposed to lichen extracts, isolated compounds and Temephos.

Material	N[1]	X[2]	Slop (SE)	DF[2]	Larvicidal activity*	
					LC_{50}^{4} (95% CI)[3*]	LC_{90}^{4} (95% CI)[3*]
Temephos	520	0.5687	0.348	4	0.0033 (0.00299–0.00370)	0.0102 (0.00777–0,0158)
Ether extract of *R. Complanata*	400	0,2091	0.705	3	5.9 (2.92–8.03)	15.0 (12.6–18.3)
Divaricatic acid	350	0,7179	0.403	3	27.1 (19.3–36.1)	60.4 (42.9–162)
Acetone extract of *C. verticilaris*	300	0.624	0.419	3	17.4 (15.5–19.3)	34.9 (31.0–40.7)
Fumarprotocetraric acid (FUM)	300	0,0814	0.590	3	13.6 (3.36–27.1)	22.1 (19.5–23.5)

1Numder of insects used in test, [2]degrees of freedom, [3]lethal concentration and confidence interval; [4] $\mu g.ml^{-1}$. *LC values are considered significantly different when 95% CI fail to overlap ($P < 0.05$).

death of adult forms of the grain weevil, *Sitophilus granarius* (Emsen et al., 2015).

The results in Table 1 of the larvicidal tests (LC_{50} and LC_{90}) with *Aedes aegypti* exposed to the lichen extracts and compounds isolated from these extracts are similar to those reported for synthetic compounds derived from thiosemicarbazones by Da Silva et al. (2015). The most active compounds in the study had an LC_{50} ranging from 5.8 to 28.8 $\mu g.ml^{-1}$.

The present larvicidal data of the lichens against *Aedes aegypti* are similar to data reported in studies using the plant *Piper corcovadensis*. Da Silva et al. (2016) demonstrated that the essential oil from the leaves of this plant had an LC_{50} of 30.52 $\mu g.ml^{-1}$ and the major constituent of the oil (1-butyl-3,4-methylenedioxybenzene) had an LC50 = 22.1 $\mu g.ml^{-1}$. In a larvicidal study involving *Aedes aegypti*, Albuquerque et al. (2022) demonstrated that the hexane extract of the leaves of the same plant had an LC50 = 18.23 $\mu g.ml^{-1}$ and the compound beta-germacrene-D-4-ol

isolated from the extract had an LC50 = 6.71 μg.ml^{-1}.

The present findings suggest that the lichen extracts and isolated compounds show promising insecticidal potential against *Aedes aegypti* when compared to the results obtained with essential oils compiled in the review by Budiman et al. (2021) and with seaweed extracts compiled by Ali et al. (2013).

The LC_{50} and LC_{90} of *R. complanata* ether extract are comparable to those of several plant extracts and isolated compounds described in the literature (Pavela, and Pavela et al., 2019, 2021). The leaf ethanolic extract of *Andrographis paniculata* (Burm.f.) Nees showed an LC_{50} = 12 ppm and the petroleum ether leaf extract of *Argemone mexicana* L. an LC_{50} = 49 ppm. But there are plant extracts showing even better results, such as the extract of *Artemisia annua* L. with an LC50 = 0.7 ppm and the root essential oil from *Carlina acaulis* L. with an LC_{50} = 2 ppm. The LC_{50} values of compounds isolated from lichens in this study ranged from 13 to 27 ppm. Studies show that compounds isolated from plants have data comparable to ours or even better: beta-sitosterol (LC_{50} = 11.5 ppm), emodin (LC_{50} = 1.9 ppm), and alpha-mangostin (LC_{50} = 2.2 ppm), among others.

In silico molecular docking studies and absorption, distribution, metabolism, excretion and toxicity (ADMET) prediction studies were then performed with the isolated compounds (divaricatic acid and fumarprotocetraric acid) to obtain information on the possible mechanism of the insecticidal action.

3.3. Molecular docking

Molecular docking is an approach that enables the determination of parameters that lead to the best results by enabling a better sampling of conformation at the binding site (Silva-Júnior et al., 2016). Thus, molecular docking was performed with divaricatic acid and fumarprotocetraric acid and the active site of the enzyme acetylcholinesterase 1 from *Aedes aegypti* (*Aa*AChE1). *Aa*AChE1 is an important molecular target for the control of the vector and is responsible for AChE-mediated resistance to insecticides (Engdahl et al., 2016).

All ligands investigated (divaricatic acid, fumarprotocetraric acid and the standard compound temephos) were able to interact with amino acid residues at the active site of *Aa*AChE1 from *Aedes aegypti*, docking in an energetically favorable manner (Table 2).

The consensus averages resulting from the scores for the best pose of each compound enabled establishing a ranking among the molecules studied and the compounds used as standards (Table 2). AL200 (co-crystallized ligand) had the best consensus and, consequently, the most favorable interaction. Divaricatic acid had the second-best average consensus (0.7116), followed by temephos (0.6455). Among the compounds studied, fumarprotocetraric acid (FUM) had the lowest average consensus (0.6455), proving to be the least energetically favorable among the compounds tested.

Although the *in silico* analysis resulted in a different order for the compounds when compared to the experimental LC_{50} results, which demonstrated that fumarprotocetraric acid was more active than divaricatic acid, other factors, such as the type of interactions and ADME parameters are fundamental to the understanding of biological activity. Thus, the aim of this step was to ascertain how many and what types of interaction may be determinant of greater inhibitory activity. The results are displayed in Table 3.

Table 2
Ranking of compounds with best average consensus at active site of *Aa*AChE1.

Compound	Consensus average
AL200*	0.9610
Divaricatic acid	0.7116
Temephos	0.6455
Fumarprotocetraric acid	0.6455

* Co-crystallized ligand at active site of AChE1 (PDB: 5FUM).

Few *in silico* studies have investigated interactions between lichen phenols and biological targets. Igoli et al. (2014) evaluated interactions of lichesterinic, protolichesterinic and fumarprotocetraric acids isolated from *Cetraria islandica* with riboflavin kinase, sterol-14α-demethylase and glutathione synthetase. The authors found that hydrophobic factors were intensely related to the high affinity with the biological targets analyzed. Indeed, a greater number of amino acid residues were found in hydrophobic reactions with the lichen compounds evaluated in the present study. Divaricatic acid presented seven hydrophobic reactions with the amino acid residues ASP74, TRP86, PHE297, TYR337, PHE338, HIS447 and GLY448, whereas fumarprotocetraric acid presented six hydrophobic reactions with the residues TYR72, TRP286, SER293 and PHE295.

Only fumarprotocetraric acid had an interaction in common with the co-crystallized ligand and temephos (TRP286). This interaction may be related to its greater activity in comparison to divaricatic acid. Moreover, an unfavorable interaction was found between divaricatic acid and the residue SER125. The 2D images of the interactions between the compounds and amino acid residues of the active site of *Aa*AChE1 are presented in Fig. 3.

The ADMET parameters of the compounds were predicted *in silico*. The results are presented in Table 4.

The *in silico* ADMET analysis furnishes data that can assist in the understanding of the greater activity of fumarprotocetraric acid compared to divaricatic acid despite the lower interaction capacity with *Aa*AChE1.

The first variable that stands out is molar mass. The standard compounds and fumarprotocetraric acid have a similar molecular weight and greater than 400 g/mol, whereas divaricatic acid has a molecular weight 388.41 g/mol. Studies in the literature report different responses for biological activity as a function of the molecular weight of the ligand (Barbucci et al., 1998; Chen et al., 2015; Jiménez-Rosés et al., 2021). The second point of interest is the cLogP = 1.86, which gives fumarprotocetraric acid greater solubility in an aqueous medium. Lastly, divaricatic acid did not exhibit toxicity for any of the parameters tested, whereas fumarprotocetraric acid demonstrated a low probability of being mutagenic and a low risk to reproduction. However, FUM exhibited a high probability of being an irritant.

4. Conclusions

The extracts from the lichens *R. complanata* and *C. verticilaris* and the compounds isolated from these extracts (divaricatic and fumarprotocetraric acid, respectively) demonstrated larvicidal activity against *A. aegypti*. Fumarprotocetraric acid was the more active compound and was able to eliminate 50% of the larvae in any instar at a lower concentration of 13.6 μg.ml^{-1}. The molecular docking studies demonstrated that the lichen compounds bind to the active site of *Aa*AChE1 in an energetically favorable manner. The greater influence of hydrophobic forces in the interaction with the active site of the receptor is compatible with data in studies found in the literature that relate the hydrophobic

Table 3
Ranking of compounds in terms of larvicidal activity (LC_{50}) and amino acid residues involved in interaction between compounds and active site of *Aa*AChE1.

Compound	INTERACTIONS
AL200	TRP86, TRP286, TYR337, PHE338, TYR341, HIS447 and GLY448
Divaricatic acid	ASP74, TRP86, TYR124, SER125, PHE297, TYR337, PHE338, HIS447 and GLY448
Temephos	TYR72, ASP74, TRP86, GLY120, GLY121, GLU202, TRP286, TYR337, TYR341 and HIS447
Fumarprotocetraric acid	TYR72, TYR124, TRP286, SER293, PHE295 and ARG296

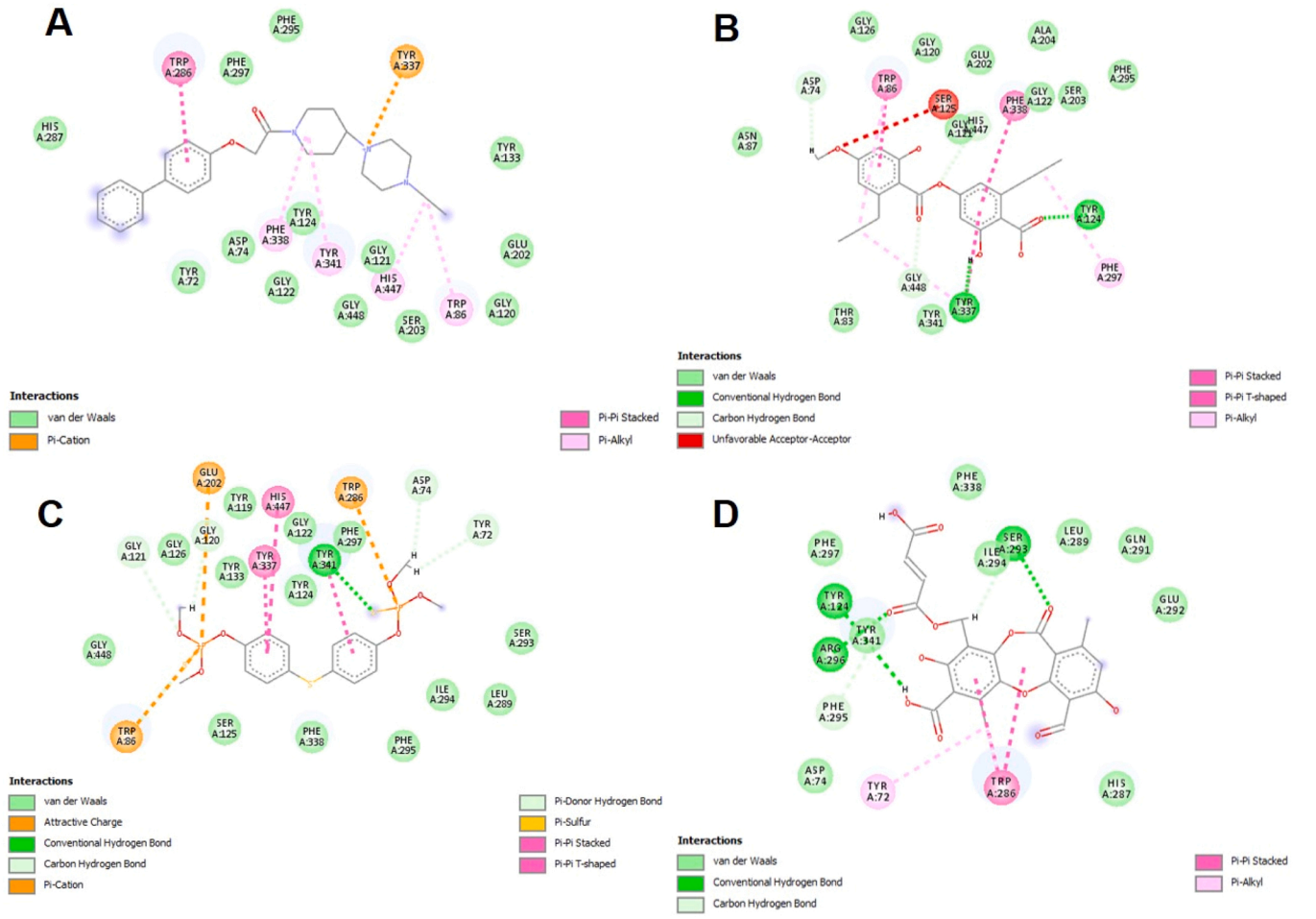

Fig. 3. Interaction with active site of *Aa*AChE1. (A) Interactions between AL200 and amino acid residues of active site of *Aa*AChE1. (B) Interactions between divaricatic acid and amino acid residues of active site of *Aa*AChE1. (C) Interactions between temephos and amino acid residues of active site of *Aa*AChE1. (D) Interactions between fumarprotocetraric acid and amino acid residues of active site of *Aa*AChE1.

Table 4
Pharmacokinetic parameters and toxicity determined *in silico*.

Parameters	Divaricatic acid	Fumarprotocetraric acid	Temephos	AL200
Mol weight	388.41	472.36	466.47	407.36
cLogP	4.24	1.86	5.62	3.58
cLogS	-4.07	-4.81	-6.49	-3.18
H-Acceptors	7	12	6	5
H-Donors	3	4	0	0
PSA	113.29	193.96	164.48	36.02
Druglikeness	-3.42	-5.53	-3.94	8.15
Mutagenic	None	Low	Low	None
Tumorigenic	None	Low	High	None
RE	None	Low	High	None
Irritant	None	High	High	None

*PSA = polar surface area; RE = reproductive effect.

nature of lichen phenols to affinity with other biological targets. Moreover, the TRP286 residue may have contributed to the greater activity of fumarprotocetraric acid compared to divaricatic acid. Other aspects related to the greater activity of fumarprotocetraric acid are its molecular weight and greater solubility. Despite being more active than divaricatic acid, fumarprotocetraric acid has possible toxic effects that need to be investigated further.

The present results demonstrate the importance of studying lichen secondary metabolites as a natural source of insecticides and investigating possible interactions with *Aa*AChE1. The results of the *in silico* molecular docking and ADMET studies can assist in understanding the mechanism of insecticidal action and the optimization of the activity of these compounds.

CRediT authorship contribution statement

André Severino da Silva: Conceptualization, Investigation, Methodology, Validation, Writing. **Júlio César Ribeiro de Oliveira Farias de Aguiar:** Methodology, Investigation, Supervision. **Jéssica da Silva Nascimento:** Methodology, Investigation. **Erick Caíque Santos Costa:** Docking studies. **Fábio Henrique Galdino dos Santos:** Writing, **Hallysson Douglas Andrade de Araújo:** Methodology. **Nicácio Henrique da Silva:** Supervision. **Eugênia Cristina Pereira:** Conceptualization and Investigation. **Mônica Cristina Martins:** Methodology and Investigation. **Emerson Peter Silva Falcão:** Funding acquisition, Investigation, Resources, Visualization, Project administration, Writing – review & editing. **Luciana Scotti:** docking study. **Marcus Tullius Scotti:** docking study. **Daniela Maria do Amaral Ferraz Navarro:** Funding acquisition, Investigation, Resources, Visualization, Project administration, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

No data was used for the research described in the article.

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