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A study of docking and dereplication of extracts from *Tradescantia pallida* and their *in vitro* cytotoxic activities to control dengue mosquitoes

Fabisleine Vieira Cabral [1], Cassia Cristina fernandes [1], Márcia Matos da Silva [2], Iara Silva Squarisi [3], Arthur Barcelos Ribeiro [3], Denise Crispim Tavares [3], Erdi Can Aytar [4] and Mayker Lazaro Dantas Miranda [5]

[1] Instituto Federal de Educação, Ciência e Tecnologia Goiano, *Campus* Rio Verde, Av. Sul Goiana, s/n, Zona Rural, 75901-970, Rio Verde, GO, Brazil

[2] Universidade Federal de Goiás, Av. Esperança, s/n, *Campus* Samambaia, 74690-900, Goiânia, GO, Brazil

[3] Universidade de Franca, Av. Dr. Armando Salles Oliveira, 201, 14.404-600, Franca, São Paulo, Brazil

[4] Usak University Faculty of Agriculture, Department of Horticulture, 64200, Uşak, Türkiye

[5] Instituto Federal de Educação, Ciência e Tecnologia do Triângulo Mineiro, *Campus* Uberlândia Centro, Rua Blanche Galassi, 150, Morada da Colina, 38411-104 Uberlândia, MG, Brazil.

Abstract: *Tradescantia pallida* is a plant known for its luxuriant purple leaves. This study aimed at extracting ethyl acetate extract (EAE) and ethanolic extract (EE) from *T. pallida* aerial parts to identify their phenolic compounds by liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS-MS) using commercial patterns and at evaluating their larvicidal and cytotoxic activities against *Aedes aegypti* larvae and tumor cell lines, respectively. To assess their larvicidal activity, different concentrations of EAE and EE (10, 100, and 1000 µg/mL) were prepared with sterile distilled water and dimethyl sulfoxide (DMSO) (1%). Then, 20 mL of every solution and 25 third-stage larvae were placed in plastic cups. Cytotoxic activity of extracts was evaluated at concentrations ranging from 31.25 to 1000 µg/mL in tumor and non-tumor human cell lines. EAE and EE were found to be toxic to *A. aegypti* larvae since LC_{50} values were 435.0 and 480.5 µg/mL, respectively. Extracts showed no cytotoxic activity after the 24-hour treatment at 1000 µg/mL concentrations. LC-ESI-MS-MS results revealed that quercetin is the compound found at the highest concentration in both ethanol and ethyl acetate extracts; it highlights its significant role in both extraction methods and its prominence in the docking study. The docking analysis of quercetin against the crystal structure of arylalkylamine *N*-acetyltransferase 2 (4FD5) from *A. aegypti* yielded a binding energy of -6.7 kcal/mol. Additionally, details about the acute toxicity profile of quercetin and its possible toxicity targets have been provided. Results showed that extracts under evaluation exhibited larvicidal effects and showed no cytotoxicity to human cells.

Keywords: *Aedes aegypti;* phenolic compounds; natural larvicide; tumor cell lines; quercetin

1. Introduction

Mosquitoes that belong to the genus *Aedes* compose a relevant class of vectors since they transmit important pathologies in the health area. Arbovirosis is a disease caused by arboviruses, which include the ones that cause dengue fever, chikungunya fever, zika fever, and yellow fever. Thus, strategies to control mosquitos (*Aedes aegypti* and *Aedes albopictus*) are essential in countries with high prevalence of the diseases, such as Brazil[1]. *A. aegypti* is the one that mainly disseminates and survives in urban areas where stagnant bodies of water are its breeding sites. As a result, the species proliferates fast due to the possibility of reproduction and availability of feeding sources [1].

Dengue fever is epidemiologically relevant in tropical and subtropical regions in Africa, Asia, and South American countries, such as Brazil. The virus that transmits it belongs to the genus *Flavivirus* and the family Flaviviridae. It has four serotypes – DEN-1, DEN-2, DEN-3, and DEN-4 –transmitted by female mosquitos mostly belonging to *A. aegypti*[2]. Classical dengue fever leads to symptoms such as high fever (from 39 to 40 °C), cephalea, tiredness, myalgia, and fatigue. Regarding dengue hemorrhagic fever, there is an alteration in vascular permeability and blood coagulation cascade. In case of

**Corresponding author: Mayker Lazaro Dantas Miranda*
Email address: maykermiranda@iftm.edu.br
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complication, the disease may lead to hypovolemic shock and death, which characterizes its most severe form [2].

A. aegypti is an arthropod that belongs to the family Diptera, Culicidae. The genus *Aedes* was first described in Egypt in 1762. It is believed to have been introduced into Brazil between the 16th and the 19th centuries. Destruction of several natural habitats and the adaptation of mosquitos to stagnant water in wild, rural, and urban environments with large populations have intensified the dissemination of the species in many Brazilian states since the 20th century [3].

B. Chemical insecticides, such as organochlorines, organophosphates, and carbamates, used to be widely applied to several situations. However, their use had a negative and undefinable impact on fauna, flora, and the environment, which increased interest in natural insecticides – also known as botanical insecticides – and research into them resumed [4].

Botanical insecticides, which are compounds that result from the secondary metabolism of plants, compose chemical defense processes against herbivorous insects and interfere with their basic biochemical processes, thus leading to changes in their physiology and behavior [5]. This insecticide has several advantages, such as rapid activity and degradation, low/moderate toxicity to mammals, high selectivity, and low phytotoxicity [5].

Tradescantia pallida, whose common name is purple secretia ("*taboquinha roxa"* and "*tetrapoeraba roxa*" in Brazilian Portuguese), is well-known in Brazil due to its sumptuous purple leaves [6,7]. Two studies have reported that this species exhibits biological activities, such as antimicrobial, antioxidant, antifungal, and antitumor [8,9]. It should be mentioned that this is the first report of larvicidal and cytotoxic activities of extracts from *T. pallida*.

To continue studies of *T. pallida* (Figure 1) carried out by our research group [9], this study aimed at determining phenolic compounds of ethyl acetate extract (EAE) and ethanolic extract (EE) from *T. pallida* aerial parts by liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS-MS) and their *in vitro* larvicidal and cytotoxic activities. A docking study was also carried out.

Figure 1. Aerial part of *T. pallida* (Commelinaceae)

2.Material and methods

2.1 Plant material

T. pallida (Commelinaceae) aerial parts were collected on January 21st, 2021, at 1 pm in the *Cerrado* region (17°47'25' 'S and 50°57'54"W), Rio Verde, Goiás (GO) state, Brazil. They were stored in paper bags, identified, and preserved. Plant material was identified by the botanist Erika Amaral and deposited in the herbarium that belongs to the Instituto Federal Goiano, Campus Rio Verde, GO, exsiccate no. 231-TP.

2.2. Ethyl acetate extract (EAE) and ethanolic extract (EE)

Extracts resulted from a sequential process of maceration using two types of organic solvents – ethyl acetate and ethanol – in agreement with Abubakar and Haque (2020) [10], with modifications. Three hundred mL ethyl acetate was added to a 100.0 g sample and kept at constant magnetic agitation for 2 hours. After that, the raw material was kept in contact with ethyl acetate for 4 days at room temperature, protected from light, and manually agitated once a day. The mixture that resulted from the extraction was separated by filtration, followed by evaporation of the solvent by a rotary evaporator. The residue of EAE was again extracted using a sequential process of ethanol. Extracts were performed in triplicate for both types of solvents. The results were 3.5 g EAE and 7 g EE.

2.3. Identification of phenolic compounds by LC-ESI-MS-MS

Analyses of EAE and EE were carried out at the Centro Regional para o Desenvolvimento Tecnológico e Inovação (CRTI) that belongs to the Universidade Federal de Goiás (UFG) in Goiânia, GO, Brazil. An Ultimate 3000 liquid chromatographer, Thermo Scientific, with Agilent-C18 column (4.6mm x 100mm; 3µm), coupled with a Thermo Scientific Q-Exactive high-resolution mass spectrometer, with a H-ESI source, operating in both positive and negative modes, spray voltage 3.5 kV, sheath gas 30, auxiliary gas 10, capillary temperature

350 °C, auxiliary gas temperature 250 °C, tube lens 55 and mass range *m/z* 150-700, was used. HPLC analysis was carried out with deionized water which was acidified with 0.1% formic acid (mobile phase A, v/v) and methanol acidified with 0.1% formic acid (mobile phase B, v/v). Gradient programming started at 93:07 (A:B %), 70:30 (A:B %) for 10 minutes, 50:50 (A:B %) for 5 minutes, 30:70 (A:B %) for 3 minutes, 20:80 (A:B %) for 2 minutes, 100 (B %) for 3 minutes, kept for 3 minutes and 93:07 (A:B %) for 2 minutes, kept for 2 minutes. Runtime was 33 minutes at a flow rate of 0.3 mL/min, injection volume was 10 µL, and column temperature was 20 °C. In the fragmentation study, Parallel Reaction Monitoring (PRM) was conducted with collision energies (NCE) of 15 and 30. A stock solution with methanol standards at 1 mg/mL concentration was used to identify phenolic compounds. Stock solutions were used for preparing the solution of the mixture of standards at the concentration of 50 µg/mL. The analysis of the standard mix was carried out in the conditions used for the samples. Standards of phenolic compounds were gallic acid, ellagic acid, protocatechuic acid, gentisic acid, vanillic acid, caffeic acid, ferulic acid, *p*-coumaric, catechin, epicatechin, luteolin, kaempferol, quercetin, rutin and naringenin (Sigma-Aldrich®). The Xcalibur™ software program processed data.

2.4. Larvicidal activity of EAE and EE

The methodology used for evaluating larvicidal activity quantitatively was adapted from Betim et al. (2019) [11]. To evaluate extract activities, *A. aegypti* eggs (Rockefeller strain from the Fundação Oswaldo Cruz) were hatched in the laboratory by a BOD oven at 25 ± 3 °C, at 80% relative humidity, and fed with fish food (Alcon basic, MEP 200 complex) up to the third stage of larval development. EAE and EE were diluted in 0.5% dimethyl sulfoxide (DMSO) at the following concentrations: 1000, 100, and 10 µg/mL. After hatching, ten larvae were treated with water, DMSO, EAE, and EE for 24 h. Afterward, alive and dead larvae were counted in every treatment. The trial was conducted in triplicate (30 larvae per sample concentration).

The probit analysis was used to determine the values of lethal concentration (LC50 and LC90), which correspond to 95% confidence intervals. The chi-square test was also carried out in the trial. All statistical analyses were conducted using IBM SPSS Statistics, version 20.0 (IBM Corp., Armonk, NY, EUA).

2.5. Cytotoxic activity of EAE and EE

In this study, different human cell lines were used for evaluating the cytotoxic activity of extracts: non-tumoral fibroblasts (GM07492A), cervical adenocarcinoma (HeLa), breast adenocarcinoma (MCF-7) and glioblastoma (U-251MG). Cells were cultured in Ham's Nutrient Mixture F10 (HAM-F10) and Dulbecco's Modified Eagle's medium (1:1; Sigma-Aldrich) supplemented with 10% fetal bovine serum (Cultilab), antibiotics (0.01-mg/mL streptomycin and 0.005-mg/mL penicillin; Sigma-Aldrich) and 2.38-mg/mL Hepes (Sigma-Aldrich) at 37°C with 5% CO_2. All in vitro assays were performed on three different days to ensure reproducibility.

Cytotoxicity was evaluated using the colorimetric toxicology assay in vitro - Kit XTT (Roche Diagnostics), which agreed with the manufacturer's recommendations. Cell cultures were treated for 24 h with extract concentrations that ranged from 31.25 to 1000 µg/mL. The choice of tested concentrations was based on the solubility limit. Negative (without any treatment), solvent (DMSO 1%), and positive (cisplatin; Sigma-Aldrich) control cultures were included. The treatment and analysis procedures were conducted as described by Carnizello et al. (2016) [12]. The GraphPad Prism program performed A non-linear regression analysis to calculate the sample concentration that inhibits 50% of cell viability (IC_{50}, half maximal inhibitory concentration).

2.6. Molecular docking studies

Molecules were drawn in the Chem-Draw Ultra 18.0 program, while their minimal energy forms were obtained in the Chem 3D 18.0 program and saved in Mol2 format. The Protein Data Bank was used to record enzymes (PDB). Crystal structures of arylalkylamine *N*-Acetyltransferase 2 from *A. aegypti* (4FD5) (1.64 Å) were chosen and preserved in PDB format. Molecule-enzyme interactions using AutoDock Vina 1.5.7 software and binding energies (kcal/mol) were determined [13]. 2D and 3D visuals are demonstrated by the BIOVIA Discovery Studio Visualizer software [14].

2.7. Prediction of toxicity of chemicals

The chemical toxicity of selected phytocompounds has been predicted. The 3D structures of quercetin were saved by PROTOX-II (https://tox-new.charite.de/protox_II/) web servers (Charite University of Medicine, Institute of Physiology, Structural Bioinformatics Group, Berlin, Germany) [15-18].

3. Results and Discussion

The main phenolic compounds identified in EAE and EE are shown in Table 1. The following 15 phenolic compounds were investigated: gallic acid, ellagic acid, protocatechuic acid, gentisic acid, vanillic acid, caffeic acid, ferulic acid, *p*-coumaric acid, catechin, epicatechin, luteolin, kaempferol, quercetin, rutin, and naringenin. Eight were found in both extracts under study: gallic acid, protocatechuic acid, gentisic acid, vanillic acid, caffeic acid, *p*-coumaric acid, ferulic acid, and quercetin (Table 1). Other non-identified compounds were also found in the extracts.

Table 1. Data on phenolic compounds found in EAE and EE identified by commercial patterns.

Retention Time (T_R) (min)	T_R *Standard* (min)	Name	Molecular formula	Molecular Mass	Detected Mass $[M-H]^-$	Calculated Mass $[M-H]^-$	Error (ppm)	Fragments *m/z*	EAE	EE
10.40	10.48	**Gallic acid**	$C_7H_6O_5$	170.02153	169.01520	169.01425	-6.70	125.02300	**yes**	**yes**
14.91	14.88	**Protocatechuic acid**	$C_7H_6O_4$	154.02661	153.01812	153.01933	-7.90	109.02814	**yes**	**yes**
18.40	18.41	**Gentisic acid**	$C_7H_6O_4$	154.02661	153.01932	153.01933	-5.40	109.02812	**yes**	**yes**
19.79	19.83	**Caffeic acid**	$C_9H_8O_4$	180.04226	179.03392	179.03498	-5.92	135.04390	**yes**	**yes**
19.81	19.90	**Vanillic acid**	$C_8H_8O_4$	168.04226	167.03406	167.03498	-5.50	152.01029	**yes**	**yes**
22.07	22.13	***p*-coumaric acid**	$C_9H_8O_3$	164.04734	163.03889	163.04007	-7.23	119.04889	**yes**	**yes**
22.39	22.41	**Ferulic acid**	$C_{10}H_{10}O_4$	194.05791	193.05002	193.05063	-7.07	178.02612; 134.03608	**yes**	**yes**
25.30	25.32	**Quercetin**	$C_{15}H_{10}O_7$	302.04266	302.23600	302.03538	-7.02	178.99752; 151.00242; 121.02815	**yes**	**yes**

In various ethnic communities, *Tradescantia* species have been used as ethnopharmacological moieties against diabetes [19]. The extract from extraction with chloroform as a solvent showed the presence of polyphenols, flavonoids, polysaccharides, and glycosaponins, whose remarkable therapeutic activities against diabetes were reported by previous studies [19]. Butnariu et al.[20] indicated the presence of phenolic compounds and flavonoids, such as rutin, luteolin, and apigenin, in addition to anthocyanins and tannins. Lima et al. (2019) [21] showed promising fungicide results of the aqueous natural dye extract from *T. pallida* against *Fusarium solani*, *Sclerotinia sclerotiorum*, and *Colletotrichum gloeosporioides*. Kamiya et al. (2019) [14] reported the effects of aqueous extract from *T. pallida* on *Pseudomonas aeruginosa* growth and biofilm formation. They also stated that aqueous extracts from *T. pallida* inhibited bacterial growth and biofilm formation significantly, whereas methanolic extracts inhibited neither [22]. Extract from *T. pallida* leaves has already been used to prepare zinc oxide nanoparticles (ZnO NPs), and studies have shown good luminescence properties and cytotoxicity against cervical cancer line [23]. Likewise, hydroalcoholic extract from *T. pallida* leaves has already determined its antimicrobial potential, and the results were relevant against different Gram-positive and Gram-negative bacteria[6]. Extracts from *T. pallida* leaves grown in Korea showed high antioxidant activity and α-glucosidase inhibition[24]. Two recent Brazilian studies described the potential of extracts from *T. pallida*. One showed that aqueous extract from *T. pallida* affects the oviposition activity of *Plutella xylostella* by decreasing the mean number of daily eggs and fertility [25]. The other addressed the chemical composition of hexane extract and showed its high activity against three phytopathogenic fungi [9]. The oldest study of this species of Commelinaceae found in the literature was carried out by Shi et al. (1992) [26,] who only investigated its chemical composition and highlighted two major anthocyanins in extracts under evaluation.

Regarding their larvicidal potential, EAE and EE exhibited reasonable activity due to their mortality rates and low LC_{50} values against *A. aegypti* larvae (Table 2). The most satisfactory activity was exhibited by EAE, whose LC_{50} was 435.0 µg/mL and whose mortality rate was excellent even at the lowest concentration under evaluation (10 µg/mL). On the other hand, EE also exhibited a promising value of LC_{50}, i. e., 480.5 µg/mL (Table 2).

Table 2. Mortality and lethal concentrations of extracts from *T. pallida* against *A. aegypti* larvae

SAMPLE	CONCENTRATION (µg/mL)	MORTALITY (%) ± SD	LC_{50} (µg/mL) (LCMIN – LCMAX)	LC_{90} (µg/mL) (LCMIN – LCMAX)	X^2	(df)
EAE	10 100 1000	20.57 ± 0.60 35.98 ± 0.60 98.60 ± 0.60	435.00 (210.20 – 1320.50)	> 1000	1.75	1
EE	10 100 1000	15.30 ± 0.60 20.33 ± 0.60 65.70 ± 0.60	480.50 (230.60 – 1380.40)	> 1000	1.90	1

Ethyl acetate extract from *T. pallida* aerial parts (**EAE**); Ethanolic extract from *T. pallida* aerial parts (**EE**); Level of confidence between minimum and maximum concentrations of extract for larvicidal activity (**LCMin - LCMax**). $\mathbf{x^2}$ = chi-square test. (**df**) = degree of freedom. **Note:** there was no mortality in the negative control; larva mortality was 100% in the positive control. The confidence interval was equal to or above 95%.

Silva et al.[27] have reinforced that the larvicidal activity of some samples may be considered efficient when LC_{50} values range between 100 and 750 µg/mL[27]. Other family Commelinaceae species have already proven active against *A. aegypti* larvae. An example is the *Commelina benghalensis*, which caused the death of 80% of larvae in 24h [28]. It may be explained by its high concentration of phenolic compounds [28]. Ethanolic extract from *Commelina erecta* also exhibited larvicidal activity since its LC_{50} was above 500 µg/mL[29].

In general, mortality rates determined at 1000 µg/mL were 98.60% and 65.70% in the cases of EAE and EE, respectively (Table 2). Both rates were found to be satisfactory due to the phenolic compound quercetin, which was recently described as an active compound that can cause the death of *A. aegypti* larvae [30]. Quercetin nanosuspension has already proven to be an excellent alternative to control *A. aegypti*, since it led to larva death and prevented larvae that had survived from emerging from the water [31].

It is known that there is fast cell proliferation from the larval stage of *A. aegypti* up to its adulthood. However, since it is known that quercetins recover cuticles of larva epithelium, activities of active compounds of EAE and EE have likely occurred in intestine mesentery cells. As a response to the toxic effect of any compound, mesentery cells produce toxins which, depending on the level of aggression, destroy their cells. Thus,

The leading cause of death would be malnutrition, resulting from intestine inefficiency in absorbing nutrients needed for larval growth and development [32]. In general, the toxicity mechanism of phenolic compounds may be due to their antioxidant properties, which go along with damage to the epithelial membrane of mosquito larval guts [33]. In addition, the authors suggest that the difference in LC_{50} for EAE and EE may be due to the high concentration of phenolic compounds in EAE.

In sum, neither EAE nor EE showed cytotoxicity on tumor and non-tumor cell lines in the experimental conditions under investigation (Table 3). It should be emphasized that both extracts showed larvicidal activity at non-cytotoxic concentrations.

Table 3. IC_{50} values (µg/mL) of EAE and EE against different human cell lines after 24 h of treatment at various concentrations (31.25 to 1000 µg/mL).

Treatments	**Cell lines**			
	GM07492A	**HeLa**	**MCF-7**	**U-251**
EAE	> 1000	> 1000	> 1000	> 1000
EE	> 1000	> 1000	> 1000	> 1000
Positive control	41.99 ± 1.33	69.26 ± 1.94	54.16 ± 0.42	102.03 ± 8.05

EAE - Ethyl Acetate Extract; **EE** - Ethanolic Extract. **IC_{50}** - sample concentration that inhibits 50% of cell viability; **GM07492A** - non-tumor fibroblasts; **HeLa** - cervical adenocarcinoma; **MCF-7** - breast adenocarcinoma; **U-251MG** - glioblastoma. **Positive control** - cisplatin.

LC-MS results revealed that quercetin is the compound found at the highest concentration in ethanol and ethyl acetate extracts, underscoring its significant role in both extraction methods and its prominence in the docking study. The docking analysis of quercetin against the crystal structure of arylalkylamine *N*-acetyltransferase 2 (4FD5) from *A. aegypti* yielded a binding energy of -6.7 kcal/mol (Table 4).

Table 4. Docking scores and report of predicted interactions of docked conformations of compounds against the crystal structure of arylalkylamine *N*-Acetyltransferase 2 from *A. aegypti* (4FD5).

Ligand	Protein	Binding Energy (kcal/mol)	Amino acid	Interaction	Distance
Quercetin	4FD5	-6.7	: [001:H2 - A: THR169:OG1	Conventional Hydrogen Bond	2.53
			: [001:H3 - A:ASP170:O	Conventional Hydrogen Bond	1.77
			: [001:H7 - A:ASP33:O	Conventional Hydrogen Bond	1.93
			: [001:H8 - A: ASP33:OD2	Conventional Hydrogen Bond	2.32
			A: PRO35:HD1 -: [001: O6	Carbon Hydrogen Bond	1.78
			A: SER176:HB2 -: [001: O1	Carbon Hydrogen Bond	2.57
			: IOD307:I1 -: [001	Pi-Anion	3.44
			A: SER176: HG -: [001	Pi-Donor Hydrogen Bond	2.65
			A: PHE30 -: [001	Pi-Pi T-shaped	4.92
			: [001 - A:PRO35	Pi-Alkyl	5.41

The interactions include several conventional hydrogen bonds (Figure 2), such as between [001] and A: THR169 at the distance of 2.53 Å and between [001] and A: ASP170 at 1.77 Å. Other

notable interactions are the hydrogen bond between [001] and A: ASP33 (1.93 Å) and the hydrogen bond between [001] and A: ASP33 (2.32 Å). Additionally, there are carbon-hydrogen bonds between A: PRO35 and [001] (1.78 Å) and between A: SER176 and [001] (2.57 Å). A pi-anion interaction was found between [001: IOD307] and A at 3.44 Å, while a pi-donor hydrogen bond was observed between A: SER176 and [001] at 2.65 Å. The pi-pi T-shaped interaction between A and [001] has a distance of 4.92 Å, and a pi-alkyl interaction was found between [001] and A with a distance of 5.41 Å. These interactions suggest quercetin has a strong binding affinity and highlight its potential toxic effects on *A. aegypti* larvae.

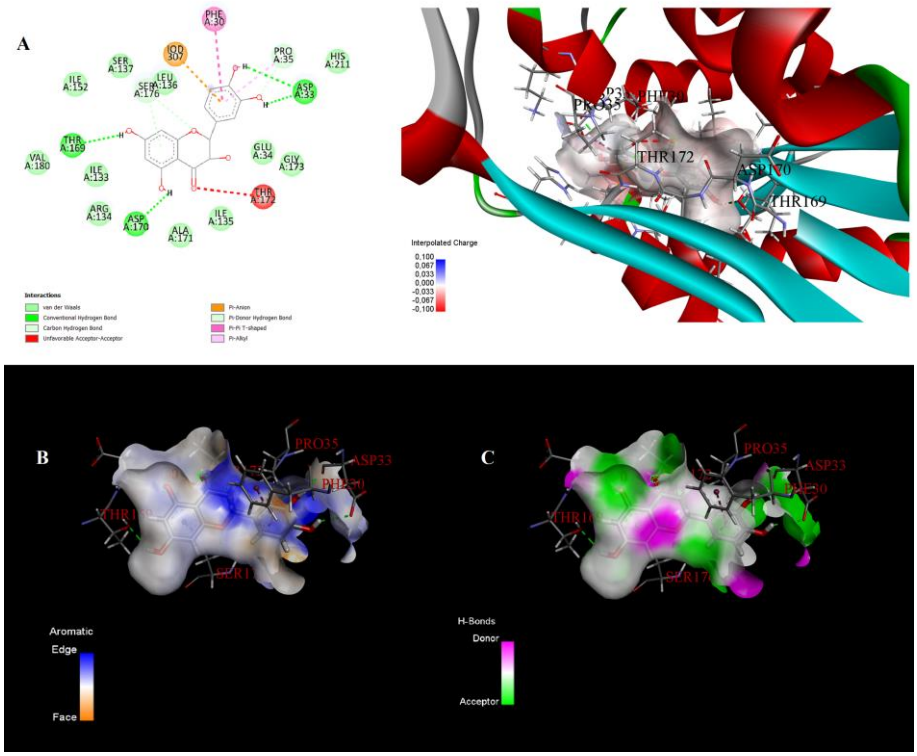


Figure 2. Interaction diagram of quercetin with 4FD5 proteins in terms of A) 2D structure; B) 3D Interpolated charge; C) H-bonds; and D) Aromatic.

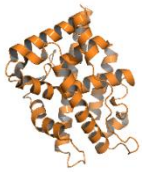
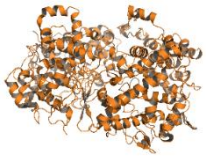
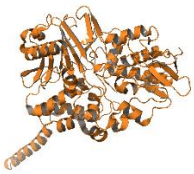
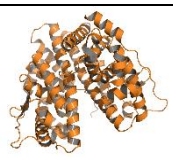
In addition to its high binding affinity shown by the docking study, the broad toxicological profile and potential targets of quercetin should be considered. Table 5 provides the acute toxicity profile of quercetin, whose LD_{50} value is 159 mg/kg. According to the predicted toxicology class, quercetin is classified as active for carcinogenicity and nutritional toxicity but inactive for immunotoxicity and clinical toxicity. It indicates that quercetin may pose potential risks for cancer and nutritional toxicity, but it neither affects the immune function significantly nor presents clinical toxicity in the conditions under investigation.

Table 5. Acute toxicity profile values of quercetin.

Toxicity Model	LD_{50} (mg/kg)	Predicted Toxicology Class	Carcinogenicity	Immunotoxicity	Immunotoxicity	Cytotoxicity	Clinical toxicity	Nutritional toxicity
	159	3	Active	Inactive	Active	Inactive	Inactive	Active

Table 6 shows possible toxicity targets for quercetin. It shows the average pharmacophore fit and similarity to known ligands for various targets. For instance, quercetin exhibits a high average similarity of 90.1% with known ligands for Amine Oxidase A and a notable average pharmacophore fit of 42.27% for Prostaglandin G/H Synthase 1. These results suggest that quercetin interacts significantly with these targets, possibly contributing to its toxicological effects.

Table 6. Details about possible toxicity targets of quercetin.

	Toxicity Target	Avg Pharmacophore Fit	Avg Similarity Known Ligands
	Androgen Receptor	1.69%	79.43%
	Prostaglandin G/H Synthase 1	42.27%	83.51%
	Amine Oxidase A	36.22%	90.1%
	Estrogen receptor 2	0.39%	72.97%

3. Conclusion

This study showed that ethyl acetate extract (EAE) and ethanolic extract (EE) from *T. pallida* aerial parts exhibited larvicidal activity against third-instar larvae of *A. aegypty*. It should be emphasized that both extracts showed larvicidal activity at non-cytotoxic concentrations. This study also highlighted that plants from the Commelinaceae family are rich sources of botanical insecticides, which may play an important role in the ongoing efforts to reduce the mosquito population and the transmission of mosquito-borne diseases to humans. Hence, the potential of *T. pallida* aerial parts may be developed to become a source of bioinsecticides.

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Disclosure statement
No potential conflict of interest was reported by the author(s).

Author contributions
FVC collected aerial parts of *T. pallida* and prepared both EAE and EE. CCF and MMS interpreted the data that resulted from LC-ESI-MS-MS. ISS, ABR, and DCT carried out cytotoxic activities on EAE and EE. MLDM participated in the design and coordination. ECA carried out the docking study. All authors contributed to the critical reading of the final manuscript and approved its submission.

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