



Biochemical evaluation and molecular docking assessment of *Cymbopogon citratus* as a natural source of acetylcholine esterase (AChE)-targeting insecticides

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ABSTRACT

Acetylcholinesterase (AChE) has been an effective target for insecticide development which is a very important aspect of the global fight against insect-borne diseases. The drastic reduction in the sensitivity of insects to AChE-targeting insecticides like organophosphates and carbamates have increased the need for insecticides of natural origin. In this study, we used *Drosophila melanogaster* as a model to investigate the insecticidal and AChE inhibitory potentials of *Cymbopogon citratus* and its bioactive compounds. Flies were exposed to 100 and 200 mg/mL *C. citratus* leaf extract for a 3-h survival assay followed by 45 min exposure for negative geotaxis and biochemical assays. Molecular docking analysis of 45 bioactive compounds of the plant was conducted against *Drosophila melanogaster* AChE (DmAChE). Exposure to *C. citratus* significantly reduced the survival rate of flies throughout the exposure period and this was accompanied by a significant decrease in percentage negative geotaxis, AChE activity, catalase activity, total thiol level and a significant increase in glutathione-S-transferase (GST) activity. The bioactive compounds of *C. citratus* showed varying levels of binding affinities for the enzyme. (+)-Cymbodiactal scored highest (−9.407 kcal/mol) followed by proximadiol (−8.253 kcal/mol), geranylacetone (−8.177 kcal/mol), and rutin (−8.148 kcal/mol). The four compounds occupied the same binding pocket and interacted with important active site amino acid residues as the co-crystallized ligand (1qon). These compounds could be responsible for the insecticidal and AChE inhibitory potentials of *C. citratus* and they could be further explored in the development of AChE-targeting insecticides.

1. Introduction

Acetylcholinesterase is a cholinergic enzyme that catalyses the breakdown of acetylcholine to acetic acid and choline, thereby terminating nerve transmission at the synapses [1]. Acetylcholine is the major neurotransmitter found in the central nervous system (CNS) of both vertebrates and invertebrates alongside other transmitters, and the regulation of its activity by AChE-catalysed hydrolysis is essential for the normal functioning and/or survival of these animals. Due to its involvement in the termination of impulse transmission in insects [2], AChE has functioned as the target of many insecticides including organophosphates and carbamates [3]. Inhibition of AChE by these

insecticides causes the accumulation of acetylcholine in the synapses, making acetylcholine receptors to be permanently open and resulting in some toxicological outcomes [1,4].

The cholinergic stimulation induced by an AChE inhibitor (AChEI) may cause hyperactivity of excitable tissues, leading to fatal consequences like muscle paralysis, coma, and death. The toxicity of AChEIs is also associated with oxidative stress due to the induction of some non-cholinergic activities like the overproduction of oxygen/nitrogen-derived free radicals and alterations in the antioxidant defence system [5]. The levels and activities of some components of the antioxidant defence system are altered thereby exposing the insects to the toxic effects of the chemical [5,6].

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Several AChE-targeting insecticides have however become less effective due to the increasing resistance of insects to these chemicals. The development of resistance has altered the effective doses of insecticides and consequently increased human exposure to the toxic adverse effects of the chemicals [7]. These and other factors, such as environmental pollution, have led to the use of natural methods for insect control. Insect repellent plants are considered safe and environmentally friendly alternatives for controlling insects, which are vectors to several infectious human diseases. Some of these plants are sometimes used as ornamental plants around residential houses to repel insects and one of such plants is *Cymbopogon citratus* (lemongrass) [8,9].

Lemongrass is a perennial plant that grows in tropical and savannah regions, and it is used in traditional medicine for the treatment of some disease conditions due to their antimalarial, antimicrobial, antifungal, anti-inflammatory, sedative, antipyretic and antispasmodic effects [9]. These activities are as a result of several bioactive components of the plant, many of which have been isolated and characterized [10,11]. Some essential oil components of lemongrass are reported to be lethal to insects, probably through their ability to penetrate the system of insects via the respiratory tract or through direct contact with the body of the insects [12] to target specific enzymes of the insect's nervous system [13]. Lemongrass has been shown to have the insecticidal and insect-repellent ability towards several insects, including mosquitoes [14], houseflies [15], sand flies [16] and fruit flies (*Drosophila melanogaster*) [17], hence, it might be a source of effective and non-toxic natural insecticides. Therefore, using *Drosophila melanogaster* as a model insect, we investigated the insecticidal and AChE inhibitory potentials of *Cymbopogon citratus* and its bioactive compounds through *in vivo* and *in silico* approaches. The effects of *Cymbopogon citratus* exposure on the survival rate, negative geotactic ability, DmAChE activity and some biochemical parameters of oxidative stress (total thiol, catalase and glutathione-S-transferase) in the fruit fly were determined. In the *in silico* study, previously characterized bioactive compounds of *Cymbopogon citratus* were screened for inhibitory activity against DmAChE through molecular docking analysis to identify possible AChEIs among the compounds.

2. Materials and methods

2.1. Plant material-collection and identification

Lemongrass (*Cymbopogon citratus*) plant was obtained from Abuja, Nigeria and was authenticated at the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, a voucher specimen with the number NIPRD/H/7041 was deposited at the institute. The samples were thoroughly washed under running water after which they were air-dried for 3 weeks at room temperature. The leaves were powdered using an electric blender and the powdered sample was used for the extraction process.

2.2. Extraction

Fifty grammes (50 g) of the powdered sample of lemongrass was placed in a 500 mL conical flask and 320 mL of 70% Ethanol was poured into the flask. The flask was allowed to shake for 69 h with an orbital shaker after which the extract was filtered out into a 50 mL beaker using a muslin cloth followed by Whatman filter paper. The filtrate was placed in a water bath at 78 °C to remove the ethanol, leaving only the extract.

2.3. *Drosophila melanogaster* stock culture

Drosophila melanogaster (Canton-S strain) was obtained from the *Drosophila* Laboratory of the Department of Biochemistry, University of Ibadan, Ibadan, Nigeria. The flies were maintained and reared on a normal diet made up of cornmeal medium containing, agar-agar, 1% w/v brewer's yeast and 0.08% v/w nipargin at 37 °C under 12 h dark/light

cycle conditions.

2.4. Insecticidal assay of *Cymbopogon citratus* leaf extract in *Drosophila melanogaster*

About 20 flies of both genders, aged 3–5 days were transferred into treatment vials containing cotton wool soaked in 200 µL of 100 and 200 mg/mL *Cymbopogon citratus* leaf extract dissolved in 70% ethanol. For the control flies, the cotton wool was soaked in 200 µL of 70% ethanol only. The flies were exposed to these treatments for 3 h, and all experiments were carried out in triplicate. The number of live and dead flies in each vial were recorded every 30 min for 3 h and the survival rate was expressed as the percentage of live flies [18].

2.5. Negative geotaxis

A negative Geotaxis assay was performed as previously described by Johnson et al. [1]. The flies were treated with the extract as described above for 45 min after which they were transferred into empty treatment vials. The vials were tapped at the bottom and the number of flies that crossed the 6 cm line within 10 s was recorded.

2.6. Preparation of supernatant for biochemical assays

Flies exposed to *Cymbopogon citratus* leaf extract for 45 min, as well as the control flies, were immobilized in ice and homogenized in 0.1 M phosphate buffer, pH 7.4 at a ratio of 1 mg:10 mL. This was followed by centrifugation at 4000 rpm for 5 min and separation of supernatant into labelled Eppendorf tubes. The supernatant was used for the assay of biochemical parameters which include acetylcholine esterase, catalase and glutathione-S-transferase activities and the total thiol contents of the experimental flies.

2.7. Determination of acetylcholine esterase activity

The AChE activity was assayed using the method of Ellman et al. [19] with slight modifications as described by Abolaji et al. [20]. Briefly, 0.8 mM acetylthiocholine was added to a medium containing 0.1 M potassium phosphate buffer (pH 7.4) and 1 mM of DTNB to initiate the reaction. The absorbance was read at 412 nm every 30 s for 2 min. The activity of AChE was calculated as µmol of acetylthiocholine hydrolysed/min/mg protein.

2.8. Determination of catalase activity

Catalase activity was determined according to the method of Aebi [21]. 10 µL of the sample was added to a mixture of 50 mM potassium phosphate buffer (pH 7.0) and 300 mM H₂O₂. Absorbance was read at 240 nm every 10 s for 2 min and the activity of catalase was estimated as µmol of H₂O₂ consumed per min/mg of protein.

2.9. Determination of glutathione-S-transferase (GST) activity

The method of Habig and Jakoby [22] was used for the evaluation of GST activity. 20 µL of the sample was added to 270 µL of solution A (containing 20 µL of 0.25 M potassium phosphate buffer plus 2.5 mM EDTA, 10.5 µL of distilled water and 500 µL of 0.1 M GSH at pH 7.0 and 25 °C) followed by the addition of 10 µL of 25 mM CDNB. Absorbance was read at 340 nm 10-s interval for 5 min. GST activity was expressed in mmol/min/mg protein.

2.10. Determination of total thiol content

The total thiol content was determined according to the method of Ellman [23]. Briefly, 20 µL of the sample was added to the Ellman's reagent comprising 510 µL of 0.1 M phosphate buffer (pH 7.4), 35 µL of

1 mM DTNB and 35 μ L of distilled water. The assay medium was incubated for 30 min at room temperature after which the absorbance was read at 412 nm.

2.11. In silico analysis of the AChE inhibitory potentials of bioactive compounds of *Cymbopogon citratus*

2.11.1. Ligand preparation

Forty-five (45) bioactive compounds of *Cymbopogon citratus* were obtained from previous reports and Ethnobotanical Databases [10,11]. Using the Ligprep panel of Maestro 12.5, Schrödinger Suite 2020-3, the compounds and the standard ligand (9-(3-Iodobenzylamino)-1,2,3,4-tetrahydroacridine or PDB: 1QON) were prepared to obtain low-energy 3D structures with suitable chiralities. The ionization state for each ligand structure was generated at a physiological pH of 7.2 \pm 0.2. Stereoisomers of each ligand were computed by retaining specified chiralities while others were varied.

2.11.2. Protein preparation

The crystal structure of *Drosophila melanogaster* Acetylcholinesterase (DmAChE) with PDB ID: 1QON was obtained from the Protein Data Bank (PDB) repository. Using Glide (Schrödinger Suite 2020-3), the protein structure obtained was prepared via the protein preparation wizard panel. During the preparation processes, hydrogen atoms were added, bond orders were allocated, disulfide bonds were generated, the side chains and loops that are missing were replaced using prime. Water molecules located outside 3.0 Å of the heteroatoms were removed and the protein structure was minimized using OPLS3e and optimized using PROPKA [24,25].

2.11.3. The generation of receptor grid

The receptor grid was created to outline the position and size of the protein's active site for ligand docking. This was done using the receptor grid generation tool of Schrödinger Maestro 12.5. The position of the co-crystallized ligand (1qon) in the active site of the protein was used as the scoring grid.

2.11.4. Protein-ligand docking

The molecular docking analysis was carried out using the Glide-Ligand Docking panel of Maestro 12.5 on Schrödinger Suite 2020-3. The prepared ligands and the receptor grid file were imported into the workspace of Maestro, using standard precision (SP) docking, the ligands were docked into the binding pocket of the target protein. The vdW radius scaling factor was scaled at 0.80 with a partial charge cut-off of 0.15 for ligand atoms and the ligand sampling method was set to be flexible.

2.11.5. Molecular dynamics simulation

The molecular interaction of the best scoring compound with DmAChE was further validated through molecular dynamics simulation using the MOE 2019.0102. The NPT ensemble was performed at T = 300 K. The initialization stage was for 100ps, the length of the simulation was 500 ps and the relaxation time was 0.5 ps.

2.11.6. Statistical analyses

Data were expressed as mean + standard deviation ($n = 5$). Treated and control groups were compared using a one-way analysis of variance ANOVA followed by a post hoc test. A 95% confidence interval was used to determine statistical differences between treated and control groups, p values less than 0.05 ($p < 0.05$).

3. Results

3.1. Survival rate and negative geotaxis of *Drosophila melanogaster* exposed to *Cymbopogon citratus* leaf extract

Exposure of flies to *Cymbopogon citratus* leaf extract caused a significant decrease in the survival rate of flies at 100 and 200 mg/mL along the 3 h of exposure (Fig. 1). The climbing rate of flies also significantly decreased at the end of the 45 min of exposure (Fig. 2).

3.2. Effects of *Cymbopogon citratus* leaf extract on AChE activity and other biochemical parameters in *D. melanogaster*

Exposure to *Cymbopogon citratus* leaf extract caused a significant decrease in the acetylcholinesterase and catalase activities of the flies at 200 mg/mL when compared with control flies (Figs. 3 and 4). GST activity significantly increased and total thiol levels significantly decreased at 100 and 200 mg/mL (Figs. 5 and 6).

3.3. Molecular docking analysis of bioactive compounds of *Cymbopogon citratus* against *Drosophila melanogaster* acetylcholine esterase (DmAChE)

Table 1 shows the docking scores of the bioactive compounds of *Cymbopogon citratus* against DmAChE. The compounds showed docking scores ranging from -9.407 to -0.484 kcal/mol against the enzyme, while the standard ligand showed a docking score of -15.402 kcal/mol. Among the *Cymbopogon citratus* compounds, (+)-Cymbodiactal scored highest followed by proximadiol, geranylacetone, and rutin while triacantanol scored lowest.

The analysis of the biological interactions showed that, in addition to other types of molecular contacts, the standard ligand interacted with the enzyme by forming pi-pi stacking interactions with TRP 83, TYR 71, TYR 370, TYR 374 and PHE 317; a halogen bond with GLY 150 and hydrophobic interactions with TYR 71, TYR 162, TYR 324, TYR 370, TYR 374, TRP 83, TRP, 321, LEU 328 and PHE 330 (Fig. 7). (+)-Cymbodiactal formed hydrogen bonds with TYR 370 and GLU 80; and hydrophobic interactions with TYR 71, TYR 162, TYR 148, TYR 370, TYR 374, TRP 472, TRP 82, LEU 159, LEU 479, ILE 484, PHE 471, and MET 476 (Fig. 8). Proximadiol formed hydrogen bonds with TYR 370 and ASP 375; and hydrophobic interactions with TRP 83, TRP 321, TYR 71, TYR 324, TYR 370, TYR 374, PHE 330, PHE 371, and LEU 328 (Fig. 9). It is important to note that the hydrogen bonds formed by (+)-Cymbodiactal and Proximadiol with TYR 370 are facilitated by water molecules. Geranyl acetone formed a hydrogen bond with TRP 83 and hydrophobic interactions with TRP 83, TRP 472, TRP 321, TYR 71, TYR 370, TYR 374, LEU 479, PHE 330, and PHE 371 (Fig. 10). Rutin formed hydrogen

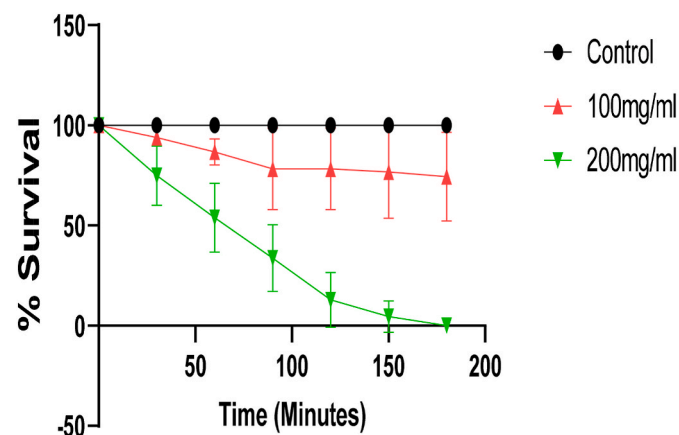


Fig. 1. The survival rate of *D. melanogaster* following exposure to *Cymbopogon citratus* leaf extract. Values are expressed as mean \pm SD ($n = 5$).

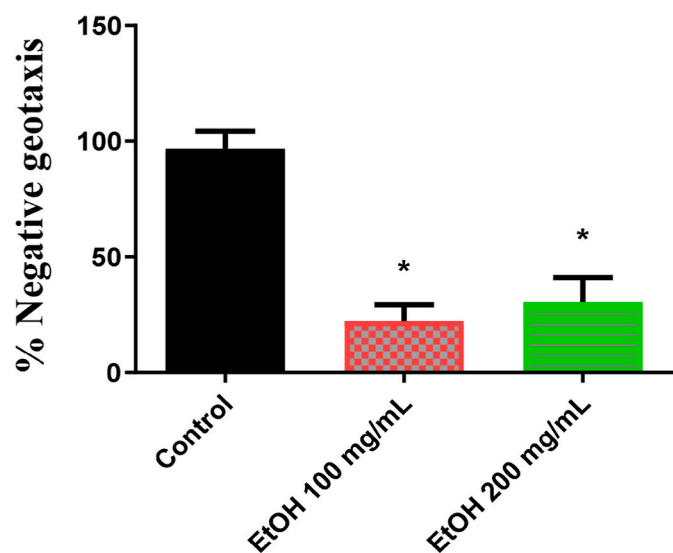


Fig. 2. % Negative geotaxis of *D. melanogaster* following exposure to *Cymbopogon citratus* leaf extract. Values are expressed as mean \pm SD (n = 5). Values with * is significant at ($p < 0.05$) versus control.

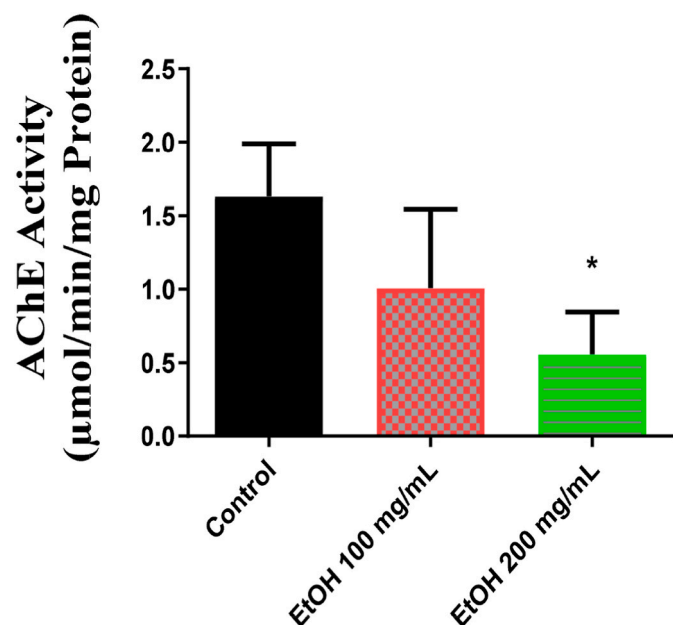


Fig. 3. Acetylcholinesterase activity of *D. melanogaster* following exposure to 100 and 200 mg/mL of *Cymbopogon citratus* leaf extract. Values are expressed as mean \pm SD (n = 5). Values with * is significant at ($p < 0.05$) versus control.

bonds with LEU 471, ASP 160, ASP 482, SER 470, and GLN 490; and hydrophobic interactions with ILE 82, ILE 161, TRP 83, TRP 472, TRP 498, LEU 471, LEU 479, and LEU 498 (Fig. 11).

3.4. Molecular modeling of biological interactions of (+)-Cymbodiactal with DmAChE

Following the molecular dynamics simulation study of the DmAChE-Cymbodiactal complex. (+)-Cymbodiactal was still occupied the same binding pocket as observed in the docking study. It retained the hydrogen bond interaction with TYR 370 and the hydrophobic interactions with TYR 71, TYR 162, TYR 148, TYR 370, TYR 374, TRP 472, TRP 82, LEU 479, ILE 484 and PHE 471 (Fig. 12).

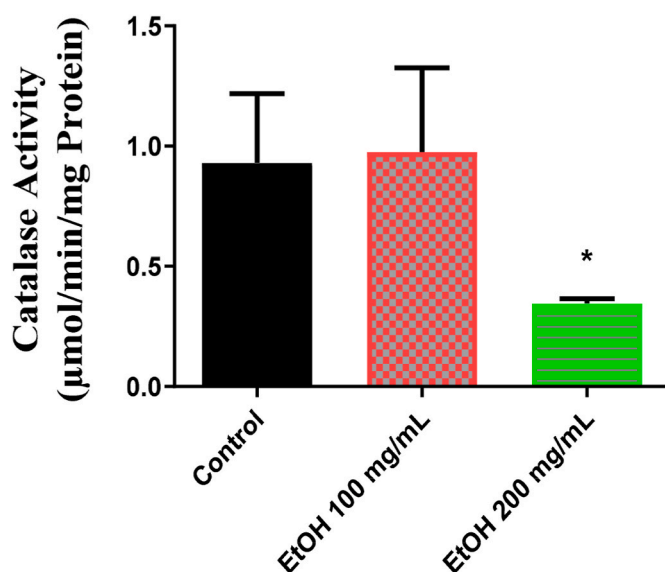


Fig. 4. Changes in catalase activity in *D. melanogaster* following exposure to 70% ethanolic extract of *Cymbopogon citratus* leaf. Values are expressed as mean \pm SD (n = 5). Values with * is significant at ($p < 0.05$) versus control.

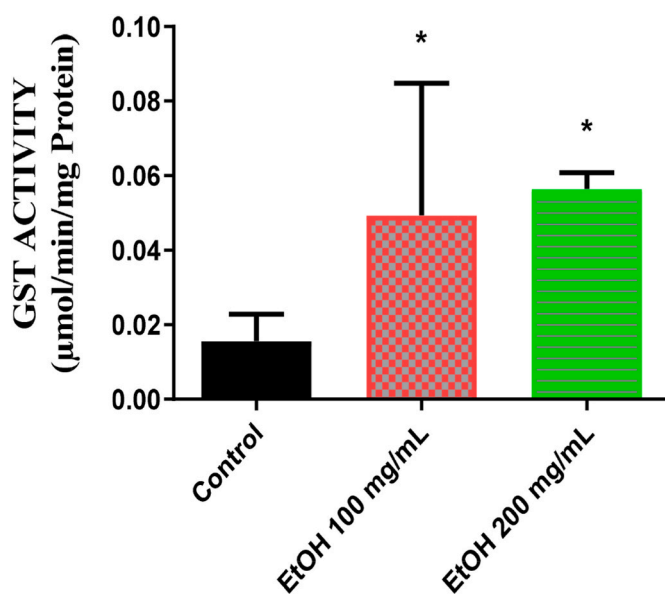


Fig. 5. Changes in glutathione-S-transferase activity in *D. melanogaster* following exposure to 70% ethanolic extract of *Cymbopogon citratus* leaf. Values are expressed as mean \pm SD (n = 5). Values with * is significant at ($p < 0.05$) versus control.

4. Discussion

Acetylcholinesterase (AChE) is an enzyme that terminates synaptic transmission at cholinergic synapses through its swift hydrolysis of acetylcholine, a neurotransmitter, and it has been an effective target for a wide array of chemical agents including the anti-Alzheimer drugs and insecticides [1,3]. Insecticide development is a very important aspect of the global fight against epidemics and pandemics. Over seventeen per cent of all infectious diseases are vector-borne and they are responsible for over 700 000 deaths yearly [26]. Insect-borne diseases like malaria and dengue hemorrhagic fever are among the deadliest infections worldwide [27] and one of the ways of controlling them is through the use of insecticides. Nevertheless, the emergence of insecticide-resistant

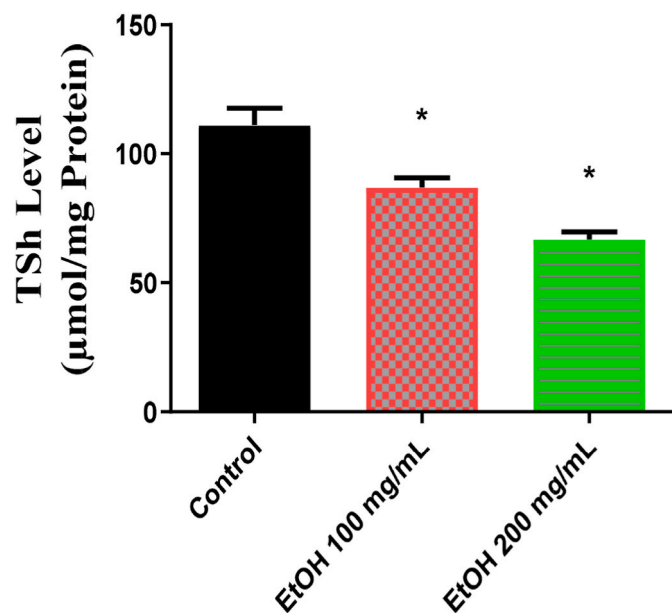


Fig. 6. Changes in total thiol level in *D. melanogaster* following exposure to 70% ethanolic extract of *Cymbopogon citratus* leaf. Values are expressed as mean \pm SD (n = 5). Values with * is significant at ($p < 0.05$) versus control.

strains of insects has rendered insecticide development a persistent struggle [3]. The drastic reduction in the sensitivity of insects to organophosphate and carbamate insecticides has elevated the effective doses of these chemicals, leading to increased human exposure to their toxic adverse effects [7]. Hence, the use of insecticides of natural origin is a good alternative. This study has not only provided a scientific basis for the alternative use of *Cymbopogon citratus* as an insecticidal/insect-repellent plant, but it has further revealed its potential as a source of AChE-targeting agents. Exposure of flies to *Cymbopogon citratus* leaf extract significantly reduced the survival rate of flies throughout the exposure period (Fig. 1). This was accompanied by a corresponding decline in the percentage negative geotaxis (Fig. 2) and the activities of AChE and catalase of the flies (Figs. 3 and 4). The GST activities of the flies were significantly elevated (Fig. 5) and the total thiol levels were significantly reduced (Fig. 6).

Percentage negative geotaxis is a measure of the locomotor performance of flies based on the number of flies that climbed beyond a defined distance within a specific time or the amount of time it takes to climb a specified height [1]. Reduction in negative geotaxis which is usually accompanied by a decreased AChE activity is regarded as an indicator of the neurotoxicity of chemicals [12]. Several studies have demonstrated a reduction in the negative geotactic capacity of flies following exposure to toxic chemicals, and as observed in this present study, the reductions were connected with AChE inhibition [1,20,28]. In our previous study, it was observed that exposure to benzo[a]pyrene-7, 8-dihydrodiol-9-10-epoxide (BPDE) caused a significant decline in the rate of survival and negative geotaxis of flies and the involvement of AChE inhibition in this activity was demonstrated both *in vivo* and *in silico* [1]. Abolaji et al. reported a significant decrease in the negative geotaxis of flies exposed to 4-vinylcyclohexene1,2-monoepoxide (VCM) and 4-Vinylcyclohexenediepoide (VCD) and it was accompanied by an inhibition of AChE activity [20]. Sharma et al. showed a positive correlation between AChE inhibition and the locomotor performance of flies [29]. The ability of *Cymbopogon citratus* to reduce the locomotor performance of flies with a corresponding reduction in DmAChE activity therefore makes it a promising source of natural neurotoxic agents against insects.

The significant reduction of the catalase activity of flies exposed to *Cymbopogon citratus* leaf extract has further authenticated the

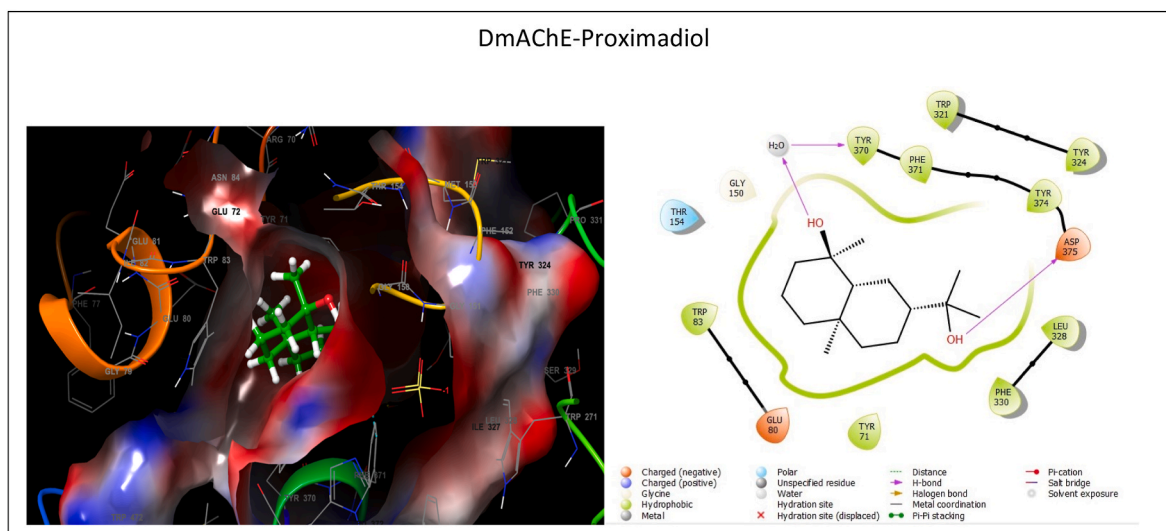
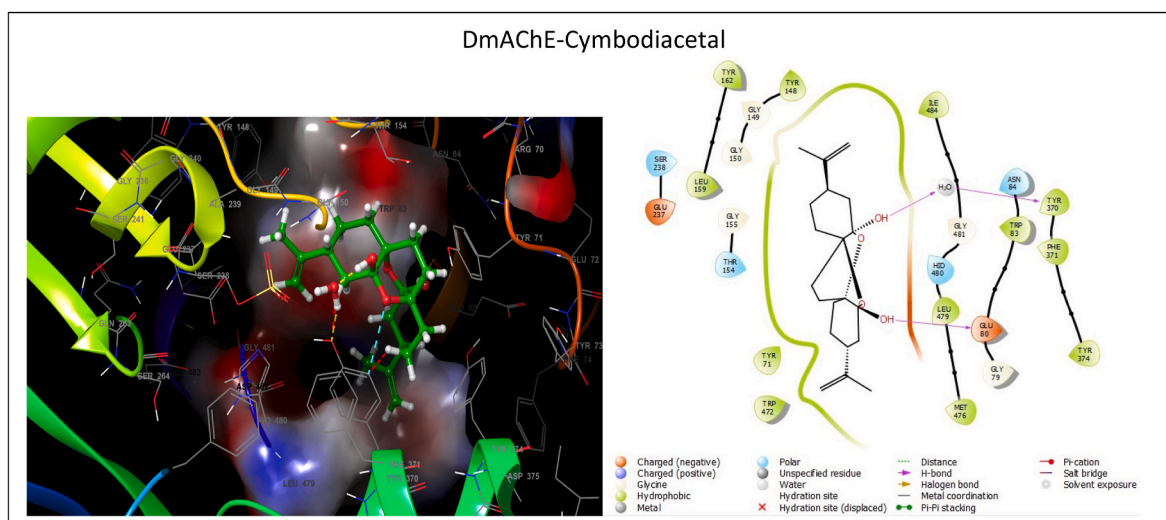
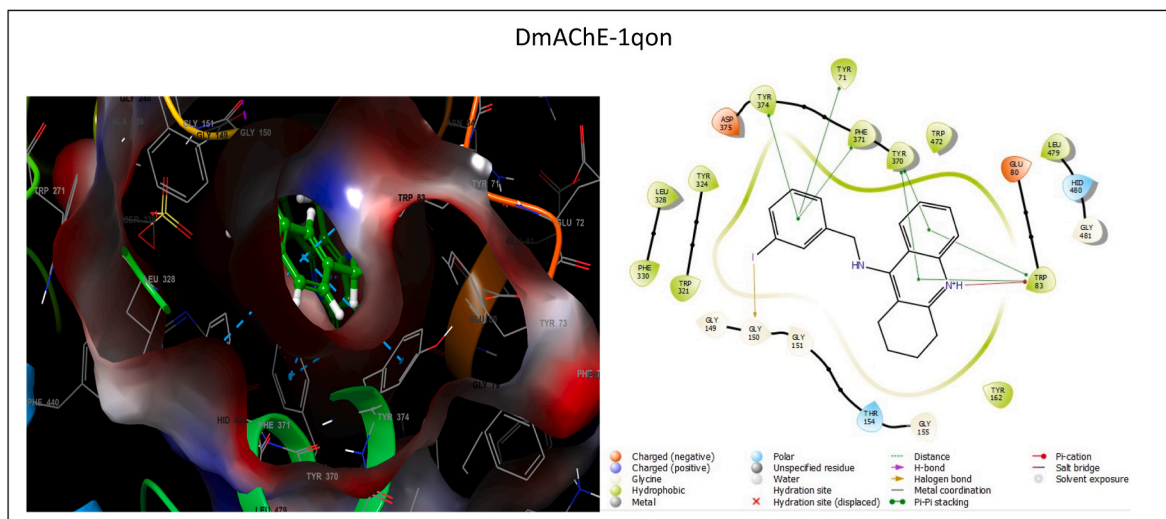
Table 1

Docking scores of the bioactive compounds of *Cymbopogon citratus* against DmAChE.

Compound	PubChem CID	Docking score (kcal/mol)
1QON (standard ligand)	448167	-15.402
(+)-Cymbodiacetal	11336586	-9.407
Proximadiol	165258	-8.253
Geranylacetone	1549778	-8.177
Rutin	5280805	-8.148
Alpha-Camphorene	101750	-8.085
Farnesol	445070	-7.97
Hexacosanol	68171	-7.096
Piperitone	6987	-7.025
Eucalyptol	2758	-7.014
Beta-Caryophyllene	5281515	-6.989
Farnesal	5280598	-6.975
(+)-Alpha-Terpineol	442501	-6.901
Geranyl acetate	1549026	-6.884
Isopulegol	170833	-6.88
(-)-Pinene	15837102	-6.833
Borneol	64685	-6.83
Alpha-terpineol	17100	-6.783
Alpha-pinene	6654	-6.747
(+)-2-Carene	600647	-6.696
Quercetin	5280343	-6.677
Citronellal	7794	-6.517
Geraniol	637566	-6.482
Methyl Eugenol	7127	-6.478
Luteolin	5280445	-6.418
Nerol	643820	-6.37
Citronellol	8842	-6.145
Citral	638011	-6.14
10-(Ethylaminocarbonyl)capric acid	454075	-6.136
Neral	643779	-6.121
Linalyl acetate	8294	-6.063
Limonene	22311	-6.004
Geranic acid	5275520	-5.929
Estragole	8815	-5.891
Citronellyl Acetate	9017	-5.84
Citronellic Acid	10402	-5.82
Linalool	6549	-5.452
Myrcene	31253	-5.328
Methyl-heptenone	54531913	-5.068
Methyl-heptenol	20745	-4.8
Caprylic acid	379	-4.732
Isovaleraldehyde	11552	-4.683
(-)-Linalool	443158	-4.373
Isovaleric acid	10430	-4.01
Furfural	7362	-3.785
Triacantanol	68972	-0.484

insecticidal activity of the plant. The enzyme catalase is an essential component of the antioxidant defence system of organisms and it catalyses the metabolism of hydrogen peroxide (H_2O_2) to water (H_2O) and molecular (O_2) thereby maintaining redox balance [1]. Inhibition of catalase activity is one of the toxicological consequences of exposure to toxic compounds and it is one of the mechanisms underlying the insecticidal action of many insecticides. Paluzar and Sagiroglu evaluated the inhibitory effect of some pesticides on the activity of catalase in an *in vitro* study [30]. All the compounds tested (Deltamethrin, Dichlorvos, Malathion and Lambda-cyhalothrin) inhibited the enzyme either competitively or non-competitively. Inhibition of catalase which could be a result of chemical-induced oxidative stress might limit the protective role of the enzyme thereby exposing the insects to the detrimental effects of the chemical [1].

GST is another component of the antioxidant defence system of organisms. This superfamily of enzymes catalyses the conjugation of toxic (electrophilic) intermediates with glutathione, thus shielding the cells from the toxic effects of foreign chemicals [1]. Therefore, the increased GST activity observed in the flies exposed to lemongrass in this study is part of the animal's response to the active components of the plant. But this response could have been counteracted by other toxicological effects of the extract on the flies. These include the reduction of total thiol



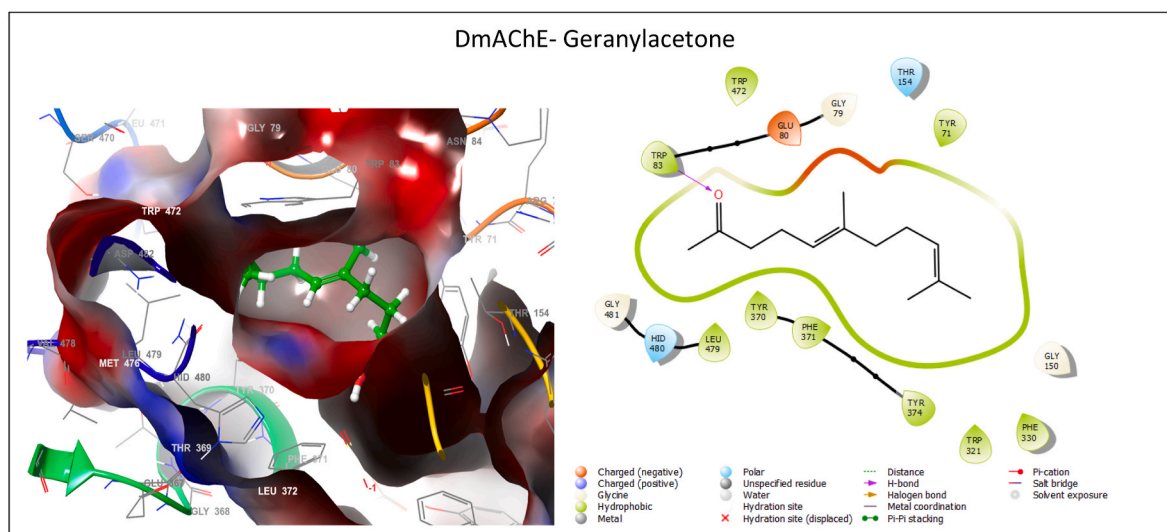


Fig. 10. 3D and 2D representations of the molecular interactions of Geranylacetone with DmAChE.

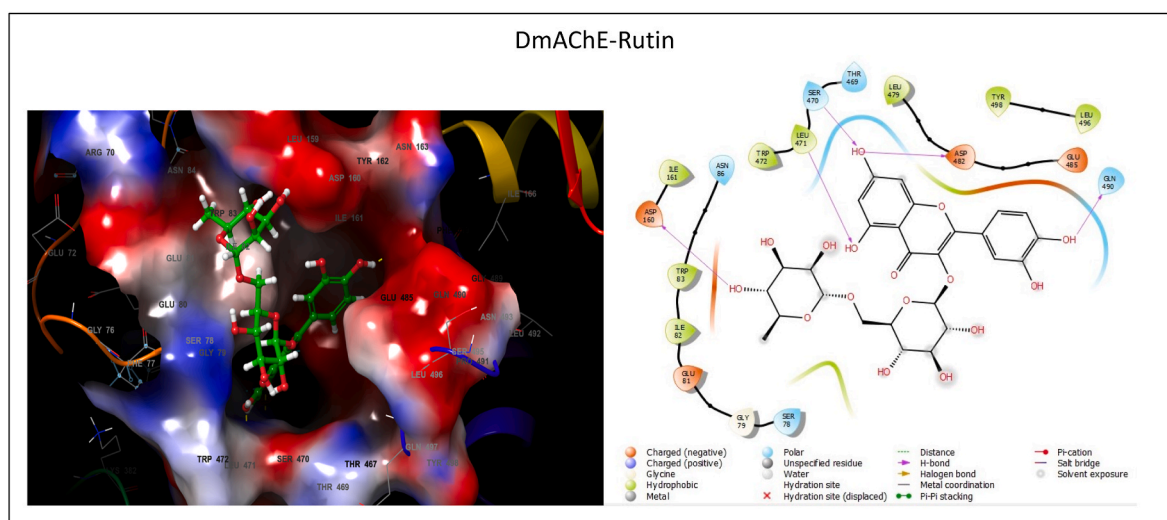


Fig. 11. 3D and 2D representations of the molecular interactions of Rutin with DmAChE.

levels in addition to the AChE and catalase inhibition. Thiols (glutathione, homocysteine, cysteinylglycine and cysteine) are a class of naturally occurring protective biomolecules that contain a sulfhydryl group (-SH). Due to their nucleophilic structure, they are able to offer protection by reacting with electrophilic intermediates [1]. A significant decrease in total thiol level as observed in the lemon grass-exposed flies in this study, therefore, predisposes the animals to the toxic effects of the extract.

To identify potential AChE inhibitors in *Cymbopogon citratus*, molecular docking analysis of some previously characterized bioactive compounds of the plant was conducted against DmAChE. The 45 compounds showed varying levels of binding affinities with docking scores ranging from -9.407 to -0.484 kcal/mol against the enzyme. The four top-scoring *Cymbopogon citratus* compounds, are (+)-Cymbodiactal (-9.407 kcal/mol), Proximadiol (-8.253 kcal/mol), Geranylacetone (-8.177 kcal/mol) and Rutin (-8.148 kcal/mol). The 2D and 3D models of the protein-ligand interactions showed that the four compounds occupied the same binding pocket as the co-crystallized ligand (1qon). The interaction of 1qon with DmAChE in this study (Fig. 7) is similar to that described by Harel et al. [3] As observed in this study, the tacrine moiety of the inhibitor was found to make planar pi-pi interactions with

TRP 83 and TYR 370 in addition to other types of contacts at the active-site gorge of the enzyme. Aromatic amino-acid residues like TYR 71, TRP 83, TYR 324, PHE 330, TYR 370, PHE 371, TYR 374, TRP 472 and HIS 480 at the active-site gorge are reported to play significant roles in binding the enzyme to the inhibitor [3]. This is in line with the results obtained in this study and it shows the reliability of the docking programme used.

The four top-scoring lemon grass compounds were also found to interact with these important active site amino acid residues. Apart from their hydrophobic contacts with the amino acids, they also formed hydrogen bonds with two or more active site residues. (+)-Cymbodiactal formed hydrogen bonds with TYR 370 and GLU 80, Proximadiol with TYR 370 and ASP 375, Geranyl acetone with TRP 83 and Rutin with LEU 471, ASP 160, ASP 482, SER 470 and GLN 490. These interactions, therefore, make them potential inhibitors of DmAChE and possible contributors to the insecticidal activities of lemongrass. It is also of interest to note that the hydrogen bonds formed by (+)-Cymbodiactal and Proximadiol with TYR 370 in the docking study are facilitated by water molecules. Active site water molecules play very crucial roles in protein-ligand binding and this has attracted a lot of attention. AChEs have been found to contain quite a number of gorge water molecules

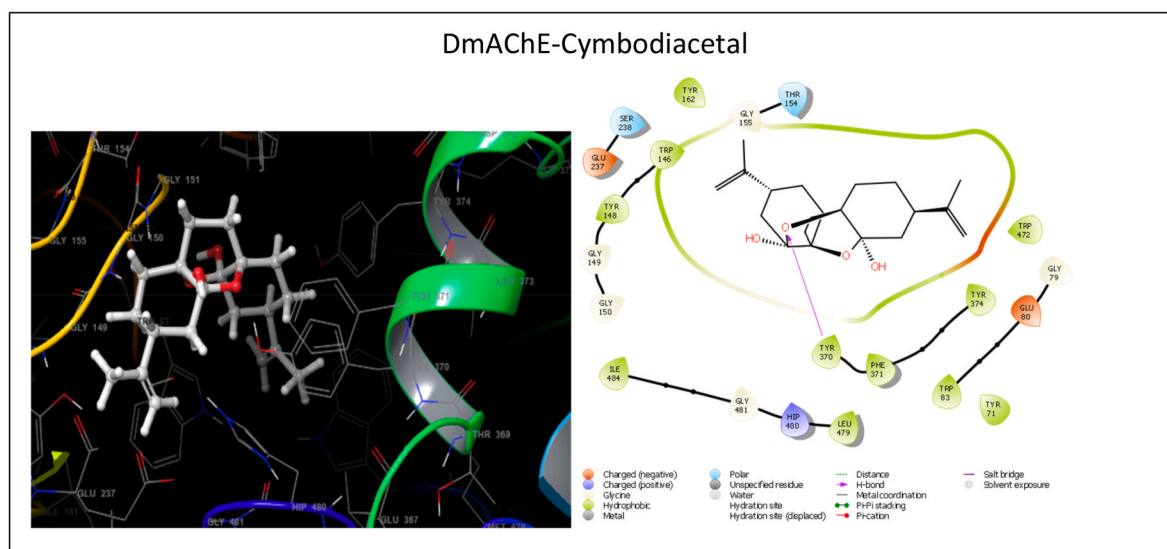


Fig. 12. 3D and 2D representations of the molecular interactions of (+)-Cymbodiactal with DmAChE after Molecular Dynamics Simulation.

which exhibit properties that aid catalysis and molecular interactions with ligands [31]. These properties could have contributed to the high docking scores exhibited by (+)-Cymbodiactal and Proximadiol for DmAChE compared with other *Cymbopogon citratus* compounds.

In the molecular dynamics simulation study of the DmAChE-Cymbodiactal complex, which was carried out to further validate the docking results and investigate the effects of ligand and protein flexibility on the observed molecular interactions. (+)-Cymbodiactal was still observed to occupy the same binding pocket as observed in the docking study. It retained the hydrogen bond interaction with TYR 370 and the hydrophobic interactions with most of the amino acid residues observed in the docking study. This further authenticates the possible inhibitory activity of this compound against *Drosophila* AChE.

Cymbodiactal is a bis-monoterpenoid first isolated from *Cymbopogon martini* by Bottini et al. [32]. Monoterpenoids, which are mostly found in plant essential oils, are among the most effective class of natural pesticides. These compounds are recognized as insecticides, insect repellents and acaricides [33]. Monoterpenoids are also explored as potent inhibitors of AChE and as promising alternatives for the treatment of neurological disorders like Alzheimer's Disease (AD) [34, 35]. This present study, therefore, identifies (+)-Cymbodiactal, a bis-monoterpenoid from *Cymbopogon* species as one of the probable AChE-targeting agents and potential insecticides in lemongrass. Proximadiol, the second top-scoring *Cymbopogon citratus* compound identified in this study [36], belongs to a category of terpenoids called sesquiterpenoids which are known to exhibit a wide range of biological activities [37]. Previous studies have reported the ability of some sesquiterpenoids to regulate cholinergic transmission through AChE inhibition [37]. The third promising compound is Geranyl acetone which is a monoterpene ketone. This volatile oil component of plants is responsible for the flavouring and fragrance properties of many plants [38,39] and as suggested by the molecular docking analysis conducted in this study, it is likely to contribute to the insecticidal property of lemongrass. Rutin is a flavonol also known as rutoside, quercetin-3-rutinoside, and sophorin and it is an essential nutritional constituent of foodstuff. It has been shown to exhibit several pharmacological activities and its insecticidal property has been reported [40]. Its interaction with DmAChE in this study has projected it as one of the possible AChE-targeting agents in *Cymbopogon citratus*.

5. Conclusion

Exposure of *Drosophila melanogaster* to 100 and 200 mg/mL

Cymbopogon citratus leaf extract induced a neurotoxic effect in the flies as revealed by the increased mortality, decreased negative geotaxis and inhibition of AChE activity in the exposed flies. The toxic effect was further supported by a reduction in catalase activity and total thiol contents of the flies. The bioactive compounds of *Cymbopogon citratus* showed possible binding affinities for *Drosophila melanogaster* AChE and the four top-scoring compounds are (+)-Cymbodiactal, proximadiol, geranylacetone, and rutin. The compounds occupied the same binding pocket and interacted with similar active site amino acid residues as the co-crystallized ligand (1qon). These compounds are suggested to be responsible for the insecticidal and AChE inhibitory potentials of *Cymbopogon citratus* and they could be further explored in the development of AChE-targeting insecticides.

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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

Data will be made available on request.

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