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A Molecular Docking Study of *Aloysia citrodora* Palau. Leaf Essential Oil Constituents towards Human Acetylcholinesterase: Implications for Alzheimer's disease

Sawsan Abuhamdah[1,2,*], Rushdie Abuhamdah[3,5], Melanie-Jayne Howes[4], Georgina Uttley[5] and Paul L Chazot[5]

[1]College of Pharmacy, Al Ain University, P.O. Box 112612, Abu Dhabi, UAE; [2]Department of Biopharmaceutics and Clinical Pharmacy, Faculty of Pharmacy, The University of Jordan, Amman, Jordan; [3]Department of Bioengineering, Imperial College, London; [4]Natural Capital and Plant Health Department, Jodrell Laboratory, Royal Botanic Gardens Kew, Richmond; [5]Department of Biosciences, Durham University, Durham, UK

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Abstract

Aloysia citrodora essential oil has been described as a potential new candidate for treating Alzheimer's disease (AD). It displays a range of pharmacological properties, including acetylcholinesterase (AChE) inhibition worthy of investigation. The essential oil of the fresh leaves of *A. citrodora* was obtained by hydrodistillation, and the composition of the essential oil was investigated using GC-MS (Gas Chromatography-Mass Spectrometry). Sixty compounds were identified, which were composed of oxygenated monoterpenes and sesquiterpenes. In the evaluation of the anticholinesterase activity of the essential oil through virtual screening, molecular docking and bioassay were used. Seven main components were identified namely caryophyllene oxide, geranyl acetate, β-sesquiphellandrene β-curcumene, γ-/ar-curcumene, β-/(Z)-α-bisabolene, *trans*-calamenene, and β-sesquiphellandrene. Three compounds (geranyl acetate, caryophyllene oxide and bisabolene) were available commercially and assessed for AChE inhibitory activity to validate the approach. The dose-response relationship showed that AChE inhibitory with IC_{50}= 5.3 µM for caryophyllene oxide and 244.5 µM for geranyl acetate. The molecular docking study revealed that *A. citrodora* may yield new selective inhibitors of AChE from plant-originated essential oils.

Keywords: *Aloysia citrodora*, Essential oil, Molecular docking, Acetylcholinesterase inhibition, Alzheimer's disease.

1. Introduction

Alzheimer's disease (AD) is known as the most prevalent age-associated neurodegenerative disorder and the major cause of dementia worldwide. The estimated number of individuals with dementia is 46.8 million and it is expected to reach 74.7 million by 2030 according to the 2015 World Alzheimer Report (Zhu *et al.*, 2018). It is a progressive disease characterized by a loss of basal forebrain neurons and reduced cortical and hippocampal level of acetylcholine (ACh).

AChE, is a key modulator for cholinergic neurotransmission, where it terminates impulse transmission at cholinergic synapses, by hydrolyzing the neurotransmitter acetylcholine. AD is accompanied by a decline in cholinergic functioning and, thus, to raise the level of ACh, a key enzyme in the breakdown of the ACh is a way of compensation for the lowered concentrations of ACh (Anand *et al*., 2017). The relation between the observed cholinergic dysfunction and AD severity provides a rationale for the therapeutic use of acetylcholinesterase inhibitors (AChEIs).

Up till now, no effective pharmacotherapeutic options for disease prevention exist. Only limited symptomatic treatment of AD (Habash *et al*., 2017), is available, with three AChE inhibitors, *e.g*. rivastigmine, donepezil, and galantamine, in clinical use (Jiang *et al.*, 2017). Plant secondary metabolites have been the center of attention in the search for new bioactive leads due to their chemical diversity and wide range of therapeutic effects (Korábečný et al., 2018). An increasing amount of evidence in the literature shows that essential oils (EOs) are a good source of several bioactive compounds targeting AD. They are composed of enormously complex chemical composition with volatile-derived compounds, including mostly monoterpenes and sesquiterpenes (Abuhamdah *et al*., 2015).

Lemon verbena - *Aloysia citrodora* Palau (Verbenaceae) is an aromatic plant native to Argentina, Paraguay, Brazil and various parts of the Middle East (Bahramsoltani *et al.*, 2018). The plant extracts are valuable for medicinal preparations. The plant has a gentle sedative action, a mild tonic effect upon the nervous system, and helps to counter anxiety and depression (Gil *et al.*, 2007). *A. citrodora* L. EO has been previously shown

* Corresponding author e-mail: smaabuhamdah@gmail.com.

* List of abbreviations : AD: Alzheimer's disease, ACh: Acetylcholine, AChE: Acetylcholinesterase, hAChE: human acetyl cholinesterase, ChEIs: Cholinesterase inhibitors, GC-MS: Gas chromatography–mass spectrometry, FID: Flame ionization detector, IC50: is the concentration of an inhibitor where the response (or binding) is reduced by half.

to possess a range of useful neuropharmacological properties, including potent AChE inhibitory activity and *in vitro* neuroprotective potential (Abuhamdah *et al*., 2015).

Molecular docking and binding prediction of two molecules in 3D space is a newly established model system available in the discovery of novel chemical binding hits to a specific protein target. Adopting this approach, the study of the interaction between a chemical and a protein of interest at the atomic level, permits the characterization of the binding site location in target proteins. Identifying the "best-fit" orientation of a ligand and a particular protein, by minimizing the free energy of the system, can predict both the affinity and efficacy of the molecule.

The aim of the present study is to identify the chemical composition of *A. citrodora* leaf essential oil, evaluation of the AChE inhibitory activity and molecular docking of main components of the oil inside human AChE in order to find novel inhibitors towards AD.

2. Material and Methods

2.1. Chemicals

All chemicals, reagents and solvents were purchased from Sigma-Aldrich Chemical Co. (USA). AChE from electric eel, Type V-S, Ellman's reagent, acetylthiocholine iodide, eserine, caryophyllene oxide, geranyl acetate, and bisabolene were purchased from Santa Cruz Biotechnology (Dallas, USA).

2.2. Collection of plant material, essential oil extraction and GC-MS Analysis

The leaf parts of *A. citrodora* were collected from Jordan in April, 2015. The botanical identity of the plant specimen was confirmed by Prof. of Pharmacognosy and Phytochemistry, Suleiman Olimat at Department of Pharmaceutical Sciences, Faculty of Pharmacy, Jordan University of Science & Technology, Jordan. It was authenticated to be *Aloysia citrodora* Palau belonging to family Verbenaceae. A voucher specimen of the plant is stored in Department of Pharmaceutical Sciences, The University of Jordan. 500 g of the leaves was subjected to hydrodistillation for 6 h, according to the standard procedure described in the European Pharmacopoeia and stored at 4 °C in the dark until further study. Analytical gas chromatography was carried out on a Perkin-Elmer USA, equipped with a flame ionization detector (FID) and mass spectrometry GC-MS analyses. Chemical constituents were identified by comparison of their mass spectra retention indices with those of the literature data as previously described (Abuhamdah *et al*., 2015).

2.3. Molecular Docking

The co-crystal structure of human AChE with donepezil (PDB code: 4EY7) was retrieved from Protein Data Bank. Molecular docking of compounds into the active site of hAChE was performed through Genetic Optimization for Ligand Docking (GOLD v3.0.1) software (CCDC, Cambridge, U.K), which helps to predict the ligand conformational flexibility by genetic algorithm. Empirical scoring function was optimized using ChemPLP for pose prediction and calculations of the binding affinity between two molecules, after they have been docked.

2.4. Enzymatic Assay for AChE Inhibition

AChE inhibitory activities of the test compounds was determined by the Ellman's colorimetric method (Ellman *et al.,* 1961). Briefly, reactions involved adding 140 µl Tris buffer, 20 µl DTNB, 20 µl test solution, and 20 µl AChE solution in a 96-well microplate, and incubation for 15 min at 25 °C then 10 µl ACh added and the formation of the yellow color was monitored at 412 nm utilizing a 96-well microplate reader (BioTek ELx800). The percent of inhibition was determined by using the formula: $(1\text{-}A_I/A_C) \times 100$, where A_I and A_C are the respective enzyme inhibitory activity and control. The experiments were carried out in triplicate. Eserine (physostigmine) was used as the reference drug.

2.5. Statistical analysis

IC_{50} values were determined graphically from inhibition curves using the GraphPad Prism Software version 4.00 (GraphPad Software, Inc., San Diego, CA, USA) with the log (inhibitor) *vs.* response (variable slope) function.

3. Results

3.1. Phytochemical profile of A. citrodora essential oil

We have already reported the chemical profile of the EO from *A. citrodora* (Abuhamdah *et al*., 2015). In total, 63 compounds were identified representing 93% of the total oil. The oil was characterized by a high content of monoterpenes and moderate levels of sesquiterpenes; the main compounds included geranyl acetate, curcumene, spathulenol, caryophyllene oxide, limonene, geranial, neral, and 1,8-cineole, where this chemical profile was the basis of the *in silico* study in hand (Abuhamdah *et al*., 2015).

3.2. Structure-based virtual screening of A. citrodora EO constituents for AChE inhibition

To perform the study described herein, the crystallographic structure of human AChE (hAChE) (PDB: 4EY7) was downloaded from protein data bank (Figure 1). The structure of hAChE in complex with donepezil is depicted as a ribbon diagram (Cheung *et al.,* 2012) (Figure 2). A centre was established around the determined binding site. 15Å radius was chosen to form the search space. Default settings for small molecule-protein docking were used throughout the simulations. Molecular docking GOLD version 3.0.1 was employed to investigate the binding mode, and the default parameters of genetic algorithms (GA) were applied to search for a reasonable binding conformation of the flexible ligands. The maximum number of GA runs was set to 10 for each compound. ChemPLP scoring function was used to evaluate the docking conformations. Docked conformations were saved in MOL2 format, and imported into Hermes for further analysis. The donepezil pose derived from GOLD was adopted as the reference compound for alignment. In order to consider the influence of crystallized water molecules on docking, donepezil was docked into the binding pocket with and without water molecules, separately docking conformations of donepezil superimposed with the X-ray crystal structure very well, which reflected that the co crystallized water had little impact on docking (Abuhamdah, 2014).

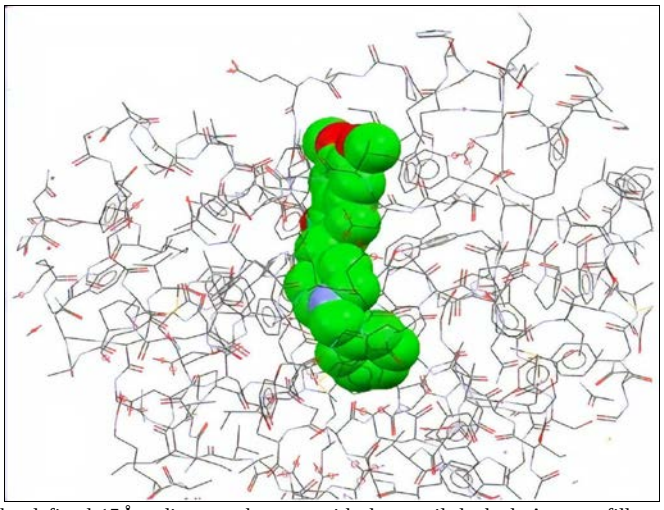

Figure 1. A magnification of the defined 15Å radius search space with donepezil docked: A space fill model. GOLD Molecular docking version 3.0.1.

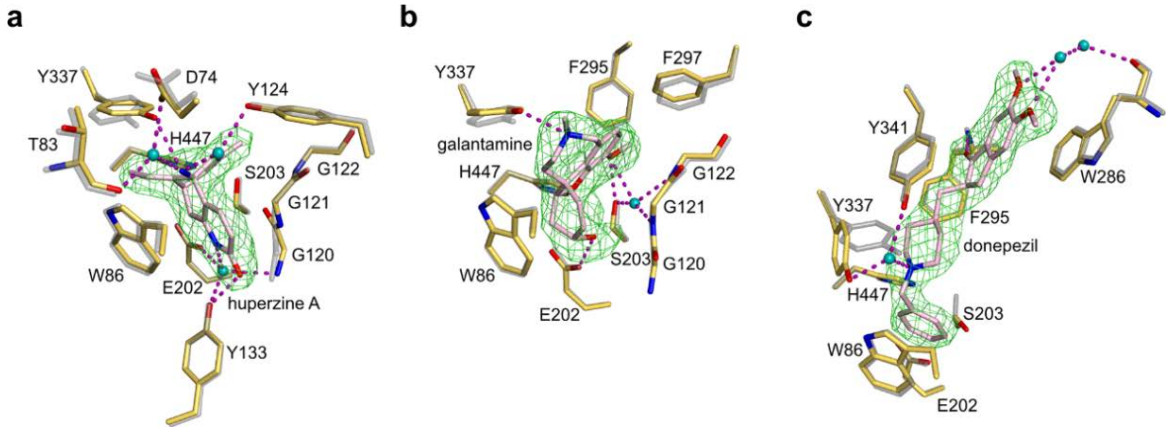

Figure 2. Close up views of the AChE active site with bound AChEI standards a) huperzine A, b) galantamine and c) donepezil. Carbons are in pink in the ligands and light yellow in residues of the complex [10]

Eighty-eight AChE inhibitors in this study (including donepezil, galantamine, huperzine, rivastigmine and tacrine) were taken from Cheung *et al.* (2012) all of which were reported by the same laboratory with similar experimental conditions and procedures to obtain bioactivity data for the compounds. The 3D chemical structures of these acetylcholinesterase inhibitors were sketched using ChemBioDraw Ultra (Ver12.0.2.1076 Cambridge Soft Corp., Cambridge, MA) then saved in mol file formats; these were considered as the training set. All chemical constituents detected in *A. citrodora* EO (Abuhamdah et al., 2015) were sketched using ChemBioDraw Ultra and also saved in mol file format. Each compound was tested for ten genetic algorithm runs, generating ten docking poses for each compound, with the top five conformations saved for analysis. Each docking run was repeated twice. The optimal docking protocol and scoring function was chosen, and the output options created sub-directories for each ligand conformation docked in order of best fit. The scores of these top hits had values comparable to those possessed by optimal conformations of huperzine A, rivastigmine, donepezil, and galantamine (the well-known alkaloid type of drug as AChE inhibitor) in the active site (Figure 2). The top scoring hits from *A. citrodora* EO constituents were imported to Hermes software to view crystal structures with docked ligands (Figures 3A, B, C, D, E, F and G). The top seven scoring hits showed high similarities in the protein residues with which they interact, both with themselves and known AChE inhibitors, such as huperzine A and donepezil, as shown in Table 1. The most common interactions were with TYR 341 + 337, PHE 338 and HIS 447, respectively. The majority of these interactions involved hydrogen bonding, hydrophobic and π-π interactions (Abuhamdah, 2014).

Seven hit compounds exhibit a good docking score when compared to the standard donepezil. *Trans*-calamenene has six stacking interaction with TYR337, TYR341, TRP286, HIS447 and ASP74. On the other hand, (Z)-α-bisabolene has five stacking interactions with TYR337, TYR341, TRP286, PHE 338 and VAL 294. Geranyl acetate and caryophyllene oxide both have four stacking interactions; these were TYR 341 PHE 338 PHE 295 HIS 447 and TYR337, TYR341, TYR124 and HIS447 respectively. β-curcumene, ar-curcumene, and β-sesquiphellandrene were active hits with three stacking interactions, with all sharing the interaction with TYR341. (β-curcumene TYR337, TYR341, HIS447; ar-curcumene TYR337, TYR341, VAL 294; β-sesquiphellandrene TYR341, PHE 338, VAL 294).

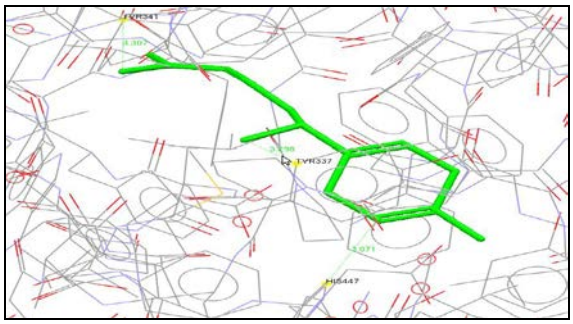

Figure 3A. 1st Ranked Hit: Close up views of the hAChE active site with bound **β-curcumene**, (carbon atoms in green) showing Ligand-Protein interactions points (yellow) at TYR 341, TYR337, HIS 447. β-curcumene had the highest hit score of PLP 75.64.

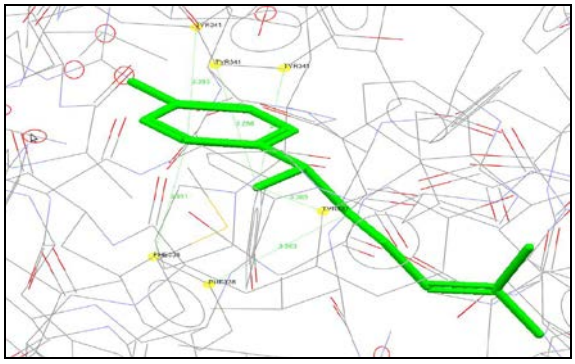

Figure 3B. **2nd Ranked Hit**: Close up views of the hAChE active site with bound **ar-curcumene** (carbon atoms in green), showing Ligand-Protein interactions points (yellow) at TYR 341, TYR 337, PHE 338.

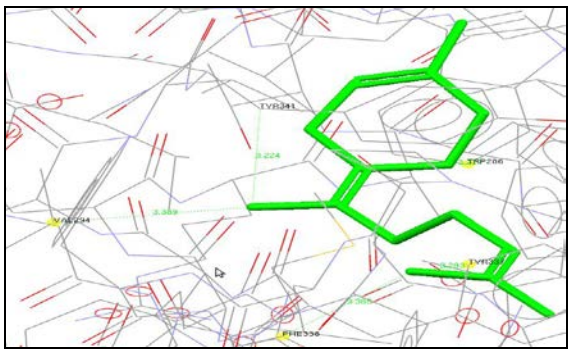

Figure 3C. 3rd Ranked Hit: Close up views of the HssAChE active site with bound **(Z)-α-bisobolene** (carbon atoms in green) showing Ligand-Protein interactions points (yellow) at TYR 341, TYR 337, TRP 286, PHE 338 and VAL 294**.**

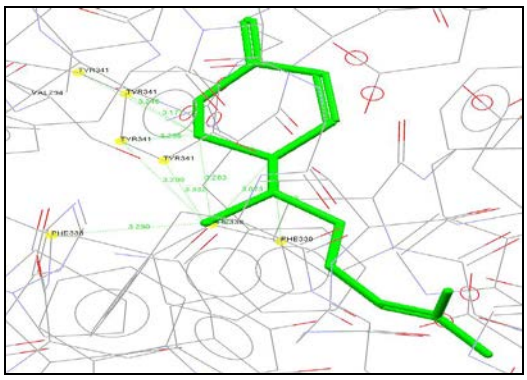

Figure 3D. 4th Ranked Hit: Close up views of the hAChE active site with bound **β-sesquiphellandrene** (carbon atoms in green), showing Ligand-Protein interactions points (yellow) at TYR 341, PHE 338, VAL 294.

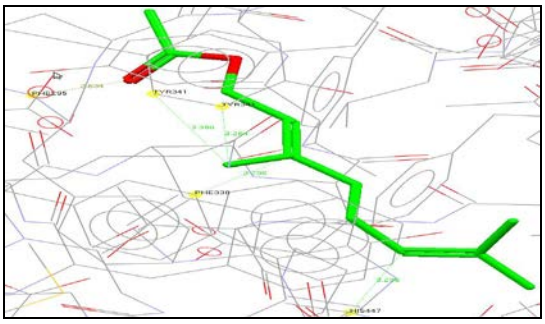

Figure 3E. 5th Ranked Hit: Close up views of the hAChE active site with bound **geranyl acetate** (carbon atoms shown in green, Oxygen atoms in red) showing Ligand-Protein interactions points (yellow) at TYR 341, PHE 338, PHE 295, HIS 447.

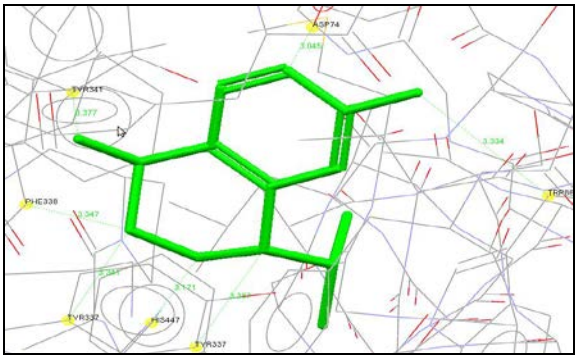

Figure 3F. 6th Ranked Hit: Close up views of the HssAChE active site with bound **trans-calamenene,** (carbon atoms in green) showing Ligand-Protein interactions points (yellow) at TYR 341, TYR337, PHE338, HIS 447, TRP86, and ASP74.

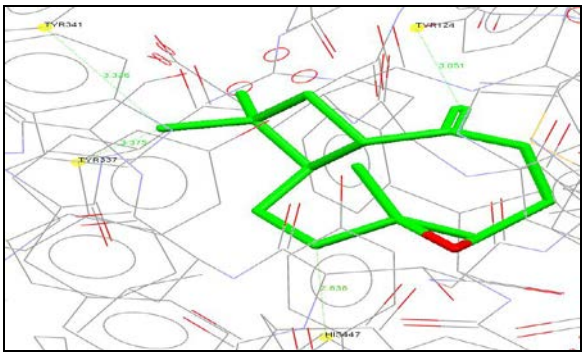

Figure 3G. 7nd Ranked Hit: Close up views of the hAChE active site with bound **caryophyllene oxide** (carbon atoms shown in green, oxygen atoms in red) showing Ligand-Protein interactions points (yellow) at TYR 341, TYR337, TYR124 HIS 447.

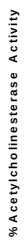

Figure 4. Acetylcholinesterase inhibitory activities of caryophyllene oxide and geranyl acetate. Values are mean +/- SD for n=3 replicate determinations. Eserine (IC_{50} = 0.67 nM) used as positive control.

Table 1. Ligand-protein interactions between top seven hit *A. citrodora* EO compounds and hAChE active site

	TYR 133	TYR 337	TYR 341	TYR 124	TRP 86	TRP 286	PHE 338	PHE 295	HIS 447	VAL 294	ASP 74
Huperzine A	X	X									
Donepezil		X	X		X	X					
β-Curcumene		X	X						X		
ar-Curcumene		X	X				X				
(Z)-α-Bisabolene		X	X			X	X			X	
***Trans*-Calamenene**		X	X		X		X		X		X
Caryophyllene oxide		X	X	X					X		
β-Sesquiphellandrene			X				X			X	
Geranyl acetate			X				X	X	X		

3.3. In vitro Experimental Studies

Three of the seven highest-ranking novel hits were commercially available to be evaluated against AChE *in vitro*. Initially, hits were screened against AChE on TLC plate. For these compounds with inhibitory potential, we performed a quantitative AChE inhibitory assay using the *in vitro* Ellman's method. The AChE inhibitor, eserine (IC_{50} 6.7 nM), was used as reference inhibitor. The resulting dose-response data were fitted using GraphPad Prism to estimate AChEI, IC_{50} values for two representative active hits, caryophyllene oxide (5.3 μM) and geranyl acetate (244.5 μM) (Figure 4). (Z)-α-Bisabolene was, apparently, not active.

4. Discussion

AChE is an attractive target for AD treatment because cholinergic deficit is a consistent symptom in the early stages of the disease [2]. Cholinesterases (ChEs) are type of α/β hydrolases that are responsible for the hydrolysis ACh into choline and acetic acid, an essential step for the restoration of the cholinergic neurons. These are divided into two sister enzymes as acetylcholinesterase (AChE; EC 3.1.1.7) and butyrylcholinesterase (BChE; EC 3.1.1.8) (Lewis *et al.*, 2002; McLaurin *et al.*, 2000. Bartolini *et al*., 2003)

Knowledge of the protein structure of AChE is essential for understanding its remarkable catalytic efficacy, the possibilities for rational drug design and developing therapeutic approaches. The structure of hAChE consists of a 12-stranded central beta-sheets surrounded by 14 α-helices. The protein is composed of five major domains: 1. catalytic site (Ser203, Glu334 and His447 residues); 2. anionic subsite (Trp86, Tyr130, Tyr337 and Phe338 residues); 3. acyl binding pocket, (Phe295 and Phe297 residues); 4. oxyanion hole (Gly121, Gly122 and Ala204 residues); 5. the peripheral anionic site (Asp74, Tyr124, Trp286, and Tyr341) (Habash *et al*., 2017; Lu *et al.*, 2011; Harel *et al*., 1996; Greenblatt *et al.*, 1999; Kryger *et al.*, 1999; Raves *et al*., 1997; Ambure *et al.*, 2014; Gupta *et al.*, 2011).

Literature review displayed promising evidence that supports the use of EOs for reversing cognitive and memory impairment of AD. Several studies regarding the cholinesterase inhibitory potential of EOs have been widely reported. The EOs of *Nigella sativa, Salvia officinalis, Acorus gramineus, Lavandula angustifolia, Melissa officinalis, Mentha piperita, Rosmarinus officinalis, Jasminum sambac, and Piper nigrum* have been proven for their putative effects *in vivo* studies together with clinical trial data (Adewusi *et al.*, 2010; Ryan and Byrne, 1988; Hansch and Deutsch, 1966; Miyazawa *et al.*, 1997; Perry *et al*., 1996 and Perry *et al.*, 2000).

A. citrodora EO also showed promising results in terms of AChE inhibition (Abuhamdah *et al*., 2015). In the present study, the essential oils of the fresh leaves of the plant, were obtained by hydrodistillation and chemically defined. The search for new AChE inhibitors, involved predicting interactions that occur between the ligand and the active site residues of the enzyme, and optimization of each single component of the oil using an algorithm scoring functions to choose the one that is most likely to be active with potential AChE inhibitory activity. Seven compounds were identified by using these molecular modeling studies. Four common interactions were detected for these active hits including: Tyr341 at peripheral anionic site, Tyr337, and Phe338 at anionic subsite domain and His447 catalytic anionic site domain respectively, and three major types of molecular interactions were exhibited: hydrogen bonds, hydrophobic interactions, and π-π interactions, visualized on the highest-scored poses.

The active site of AChE contains two main subsites, the esteratic subsite consisting in a histidine residue His447 and anionic subsites, of a tryptophan residue Trp84 corresponding to the catalytic machinery and the choline-binding pocket. Most non-alkaloidal AChE inhibitors, which include terpenes, flavonoids, and other phenolic compounds, seem to act as non-competitive inhibitors that bind to peripheral anionic sites (PAS) mainly represented by the residues Tyr70, Asp74, Try121, Trp279, and Tyr334. The seven hit compounds in our study exhibited a good docking score by interaction with His447, Tyr334, and Trp84, consistent with previous studies in the literature (Johnson and Moore, 2006). The information gained from these data indicated that the 3D structures of the active compounds and their molecular docking data to locate binding sites for ACh and inhibitors is a promising strategy to identify putative novel AChE inhibitors that are highly selective for their binding sites. The four new inhibitors in this case identified from *A. citrodora* will guide future experimental studies on these constituents and their potential for providing lead chemical structures for drug discovery. Chemical synthesis of the other three novel hits: β-curcumene, *trans*-calamenene, and β-sesquiphellandrene- as well as the isolated pure isomer β-/(Z)-α-bisabolene need to be available to further validate our model.

5. Conclusions

A molecular docking study predicting ligand-target interactions at a molecular level of *A. citrodora* essential oil components within human AChE, enables the identity of seven hit compounds forming favorable interactions at

the active site of the enzyme. *In vitro* evaluation of three available binding hits leads to discovery of two new active hits, namely caryophyllene oxide and geranyl acetate. Reflecting on our results, we can propose that these hits are promising candidates for future AD symptom-relieving drugs.

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Competing interests

The authors have declared no conflict of interest.

References

Abuhamdah S, Abuhamdah R, Howes MJ, Al-Olimat S, Ennaceur A and Chazot PL. 2015. Pharmacological and neuroprotective profile of an essential oil derived from leaves of *Aloysia citrodora* Palau. *J.Pharm.Pharmacol,* **67:** 1306-1315.

Abuhamdah R, 2014. Chemical, molecular pharmacology and neuroprotective properties of the essential oil derived from *Aloysia citrodora* Palau. PhD Thesis, Durham University, United Kingdom.

Adewusi EA, Moodley N and Steenkamp V. 2010. Medicinal plants with cholinesterase inhibitory activity: a review. *Afr. J. Biotechnol,* **9:** 8257-8276.

Ambure P, Kar S and Roy K.2014. Pharmacophore mapping based virtual screening followed by molecular docking studies in search of potential acetylcholinesterase inhibitors as anti-Alzheimer's agents. *BioSystems*, **116:**10-20.

Anand A, Patience A, Sharma N and Khurana N. 2017. The present and future of pharmacotherapy of Alzheimer's disease: a comprehensive review. *Eur. J. Pharmacol*,**15:** 364-375.

Rostamiasrabadi P, Shahpiri Z, Marques AM, Rahimi R and Farzaei H.2018. *Aloysia citrodora Paláu* (Lemon verbena): a review of phytochemistry and pharmacology. *J Ethnopharmacol,* **10:**34-51.

Bartolini M, Bertucci C., Cavrini, V., and Andrisano, V. 2003. β-Amyloid aggregation induced by human acetylcholinesterase: inhibition studies. *Biochem. Pharmacol,* **65:** 407-416.

Cheung J, Rudolph MJ, Burshteyn F, Cassidy MS, Gary EN, Love J, Franklin C and Height J. 2012. Structures of human acetylcholinesterase in complex with pharmacologically important ligands. *J. Med. Chem,* **55**: 10282-10286.

Ellman G, Courtney K, Andresjr V and Featherstone R. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol,* **7:** 88–95.

Fujiwara M, Yagi N and Miyazawa M. 2010. Acetylcholinesterase inhibitory activity of volatile oil from *Peltophorum dasyrachis* Kurz ex Bakar (yellow batai) and bisabolane-type sesquiterpenoids. *J. Agric. Food Chem,* **10:** 2824-2829.

Gil A, Van Baren CM, Di Leo Lira PM and Bandoni AL.2007. Identification of the genotype from the content and composition of the essential oil of lemon verbena (Aloysia citriodora Palau). *J. Agric. Food Chem,* **55**: 8664-8669.

Greenblatt H, Kryger G, Lewis T, Silman, I and Sussman, J.L 1999. Structure of acetylcholinesterase complexed with (−)-galanthamine at 2.3 Å resolution. *FEBS Lett,* **463:** 321-326.

Gupta S, Fallarero A, Järvinen P, Karlsson D, Johnson MS, Vuorela PM and Mohan CG. 2011. Discovery of dual binding site acetylcholinesterase inhibitors identified by pharmacophore modeling and sequential virtual screening techniques. *Bioorg. Med. Chem. Lett,* **21:** 1105-1112.

Habash M, Abuhamdah S, Younis K and Taha M. 2017. Docking-based comparative intermolecular contacts analysis and *in silico* screening reveal new potent acetylcholinesterase inhibitor. *Med. Chem. Res,* **26:** 2768–2784.

Hansch C. and Deutsch, W.1966. The use of substituent constants in the study of structure-activity relationships in cholinesterase inhibitors. *Biochim. Biophys. Acta* **126:** 117-128.

Harel, M., Quinn, D.M., Nair, H.K., Silman, I. and Sussman, J.L. 1996. The X-ray structure of a transition state analog complex reveals the molecular origins of the catalytic power and substrate specificity of acetylcholinesterase. *J. Am. Chem. Soc,* **118:** 2340–2346.

Jiang Y, Gao H and Turdu G.2017. Traditional Chinese medicinal herbs as potential AChE inhibitors for anti-Alzheimer's disease: a review. *Bioorg. Chem,* **75:** 50-61.

Johnson, G., and Moore, S. W. 2006. The peripheral anionic site of acetylcholinesterase: structure, functions and potential role in rational drug design. *Curr. Pharm. Des,* **12:** 217–225.

Korábečný J, Nepovimová E, Cikánková T, Špilovská K, Vašková L, Mezeiová E, Kuča K and Hroudová J. 2018. Newly developed drugs for Alzheimer's disease in relation to energy metabolism, cholinergic and monoaminergic neurotransmission. *Neuroscience,* **1:** 191-206.

Kryger G, Silman I and Sussman JL. 1999. Structure of acetylcholinesterase complexed with E2020 (Aricep): implications for the design of new anti-Alzheimer drugs. *Structure,* **7:** 297-307.

Lewis WG, Green LG, Grynszpan F, Radić Z, Carlier PR, Taylor P, Finn MG, Sharpless KB. 2002. Click chemistry *in situ*: acetylcholinesterase as a reaction vessel for the selective assembly of a femtomolar inhibitor from an array of building blocks. *Angew Chem. Int. Ed. Engl*,**15:** 1053-1057.

Lu SH, Wu JW, Liu HL, Zhao JH, Liu KT, Chuang CK, Lin HY, Tsai WB and Ho Y. 2011. The discovery of potential acetylcholinesterase inhibitors: a combination of pharmacophore modeling, virtual screening, and molecular docking studies. *J. Biomed. Sci*, **18:** 8-21.

McLaurin J, Yang DS, Yip CM and Fraser PE. 2000. Review: modulating factors in amyloid-6fibril formation. *J. Struct. Biol,* **130:** 259–270.

Miyazawa M, Watanabe H, Kameoka H.1997. Inhibition of acetylcholinesterase activity by monoterpenoids with a p-menthane skeleton. *J. Agric. Food Chem,* **45:** 677– 679.

Perry N, Court G, Bidet N, Court J and Perry E, 1996. European herbs with cholinergic activities: potential in dementia therapy. *Int. J. Geriatr. Psychiatry,* **11:** 1063-1069.

Perry N, Houghton J, Theobald A, Jenner P and Perry E. 2000. *In vitro* inhibition of human erythrocyte acetylcholinesterase by *Salvia lavandulaefolia* essential oil and constituent terpenes. *J. Pharm. Pharmacol,* **52:** 895-902.

Raves ML, Harel M, Pang YP, Silman I, Kozikowski AP and Sussman L.1997. Structure of acetylcholinesterase complexed with the nootropic alkaloid, (-)-huperzine A. *Nat. Struct. Biol,* **4:** 57-63.

Ryan M and Byrne O. 1988. Plant-insect coevolution and inhibition of acetylcholinesterase. *J. Chem. Ecol,* **14:** 1965-1975.

Savelev S, Okello E, Perry NS, Wilkins RM and Perry E. 2003. Synergistic and antagonistic interactions of anticholinesterase terpenoids in *Salvia lavandulaefolia* essential oil. *Pharmacol. Biochem. Behav,* **75:** 661-668.

Zhu J, Yang H, Chen Y, Lin H, Li Q, Mo J, Bian Y, Pei Y and Sun H. 2018. Synthesis, pharmacology and molecular docking on multifunctional tacrine-ferulic acid hybrids as cholinesterase inhibitors against Alzheimer's disease. *J. Enzyme Inhib. Med. Chem*, **33:** 496-506.