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Research Article

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Evaluation of Naturally Occurring Phytochemicals Extracted from *Piper betel* L. against Acetylcholinesterase (AChE) Receptor: A Search for New AChE Inhibitors for Alzheimer's Disease

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Abstract Acetylcholinesterase (AChE) is essentially a feasible target for the symptomatic improvement in Alzheimer's disease (AD). Meanwhile, Alzheimer's (AD) is a neurodegenerative disease which accounts for an estimated 60%–80% of dementia cases. This study investigates bioactive constituents *Piper betel* L as possible Acetylcholinesterase (AChE) inhibitor using pharmacophore modelling and molecular docking approach. The docking score of the analogues showed that 2-[4-(diethylamino)butyl]-5-(prop-2-en-1-yl)phenol bonded to Acetylcholinesterase (AChE) with a minimum energy of -8.4 kcal/mol and was selected as the lead molecule. The lead molecule was observed to interact with 14 amino acids within the pocket of acetylcholinesterase (AChE) and they include TRP278, ASP71, TYR120, TRP83, HIS439, GLY440, GLU198, PHE329, PHE330, TYR333, GLY 334, ILE296, PHE287 and PHE289. Meanwhile, the water solubility, druggable and pharmacokinetics index of 2-[4-(diethylamino)butyl]-5-(prop-2-en-1-yl)phenol fitted the expected druglike characteristics. Our findings showed that 2-[4-(diethylamino)butyl]-5-(prop-2-en-1-yl)phenol have good affinity for acetylcholinesterase (AChE) and can serve as therapeutics for the treatment of Alzheimer's disease *via* acetylcholinesterase (AChE) inhibition.

Keywords Acetylcholinesterase (AChE), molecular docking, ADMET, *Piper betel* L

Introduction

The unpredictable pattern of novel disease outbreak globally has resulted in an ever-increasing demand for herbal medicines as a major source of health care. Owing to the high demand of herbal drugs, intense attention has been given to growing and preserving herbal plants with potent therapeutic benefit throughout the world. Meanwhile, herbal plants commonly used in primary healthcare contain a wide range of secondary metabolites that functions as therapeutic agents against chronic as well as infectious diseases [1]. It is worth mentioning that natural products sourced from herbal plants, either as pure compounds or as standardized extracts, provide boundless opportunities for novel drug leads because of the robust availability of chemical diversity. Nevertheless, a considerable impetus has been given to studies inclined to extraction, isolation, identification and biological study of plant constituents [2].

Piper betel L. otherwise known as betel leaf is commonly consumed as mouth freshener in South East Asia [3]. This plant belongs to family Piperaceae and is mostly used for wound healing. Betel leaf is also known to exhibit antimicrobial and antileshmian characteristics [4]. Meanwhile, the extraction, isolation, characterization of piper betel leaves, revealed that it contains a substantial amount of antioxidants like hydroxychavicol and eugenol [5]. Hence, repurposing the constituent of this plant may unearth its unknown therapeutic potential for other ailments.

Alzheimer's disorder (AD) is a neurodegenerative disease that has posed unmatched health challenge globally [6]. However, thirteen percent of human beings older than age 65 years and 45% of those older than age 85 years have AD, and the occurrence is increasing [7]. Meanwhile, sizable research is being conducted to understand the causes of AD and to discover high-quality treatments for the disorder. On the contrary, researchers have a shallow understanding of this disorder. Nevertheless, the production and build-up of tangles and plaques are thought to be associated to synaptic dysfunction and nervous relapse resulting in the slowly progressive and irreparable degradation of memory, which ultimately has an impact on the language, personality and reasoning [8]. Furthermore, the production of plaques and enclosures is presumably at least partly due to natural, ageing inflammations. Once they are formed, they cause more inflammation, speed up the formation of additional plaques and tangles to lead to a further cognitive decrease [9].

There is presently acetylcholinesterase (AChE) inhibitors such as donepezil, rivastigmine, and galantamine are among these first-class agents used in managing the disease[10]. These drugs enhance the level of acetylcholine an important memory and cognitive function transmitter in the brain by preventing acetylcholine from breakdown by enzyme [11]. The aforementioned therapeutic agent slows the development of the disease and may delay symptoms, they do not significantly improve or cure the disease's cognitive function. Hence, to date, no effective drug has been approved for the treatment of this disease.

The objective of this study includes the application of pharmacophore modelling, molecular docking simulation and ADME predictions as computational tools for the evaluation of phytochemical constituents of betel leaf varieties Banarasi extracted from literature [12], against acetylcholinesterase as inhibitors.

Methodology

Receptor and ligand preparation

The chemical structure of the chavibetol (CVT), eugenol (EGN) and hydroxylchavicolwith their analogues were built using ACD/ChemSketch 2018.2.5 Freeware version. The 2D conformation of analogues wasdesigned by substituting the hydroxyl functional group at position 7 and 9 for hydroxylchavicol and chavibetol respectively (see Fig 1). To impart enhance biological activity to hydroxylchavicol and chavibetol, similar functional groups were substituted for the hydroxyl groups at the aforementioned position of both ligands. The chemical structures were converted into their 3D forms and thereafter optimized using the MMF94 force field on Avogadro interface [13]. The dock-prep tools on the UCSF Chimera interface were used to prepare theoptimizedchemical structures before the molecular docking step. The crystallized structure of acetylcholinesterase (AChE) in complex E2020 (Aricept) having 2.50 Å resolution was retrieved from the Protein Data Bank (https://www.rcsb.org) with ID: 1eve. The structure consists of singledistinct chains A bounded to small chemical residues (E2020). The preparation of the biological target (1eve) was performed on the UCSF Chimera interface [14].

Molecular docking

The molecular docking simulation experiment was performed by making use of AutoDockVina software [15]. The analogue of eugenol, hydroxylchavicol and chavibetol were specifically docked to acetylcholinesterase (AChE) (ID: 1eve). To perform the docking simulation, grid box coordinate of the ligand to be substituted on the receptor (ID: 1eve) was generated. The grid box that defines the pocket of 1eve receptor was designedby making use of AutoDock Vina functionality on UCSF Chimera interface [14]. The grid box size and centre coordinates for the 1eve were x(8.34832, 3.06856), y (11.6822, 63.5594) and z (10.6474, 66.473) respectively. The ligand with the highest binding affinity for acetylcholinesterase (AChE) was selected for further *in silico* ADME assay.

Validation and ADME analysis of lead molecule

The online web server, SWISS-ADME (Adsorption, Distribution, Metabolism and Excretion) was used to predict the drug-likeness, solubility uniqueness and pharmacokinetics characteristics of the lead molecule selected among the analogue (https://www.swissadme.ch).

Results and Discussion

Chavibetol (CVT), eugenol (EGN) and hydroxylchavicol are three bioactive compounds isolated from *Piper betel* L and modelled *via* pharmacophore modelling technique using similar chemical moiety for the three phytochemicals to enable comparability of their to impart primary chemical compounds (see Fig 2). Meanwhile, the target/receptor (acetylcholinesterase (AChE) (ID: 1eve)) used for this study was retrieved and processed prior to docking (see Fig 2).

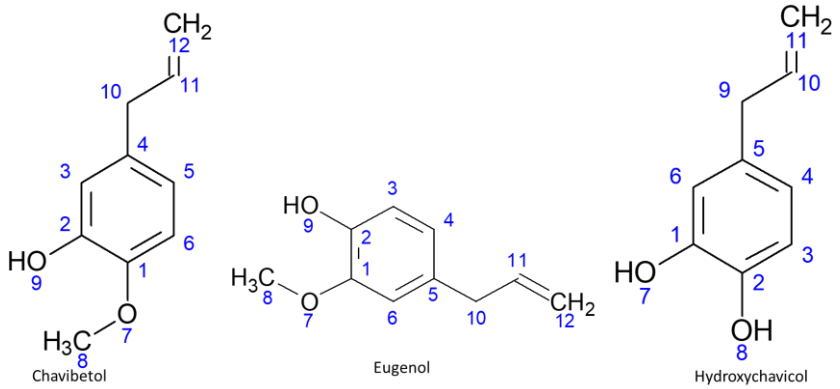


Figure 1: The numbered 2D chemical structure of chavibetol, eugenol and hydroxychavicol

Piper betel L

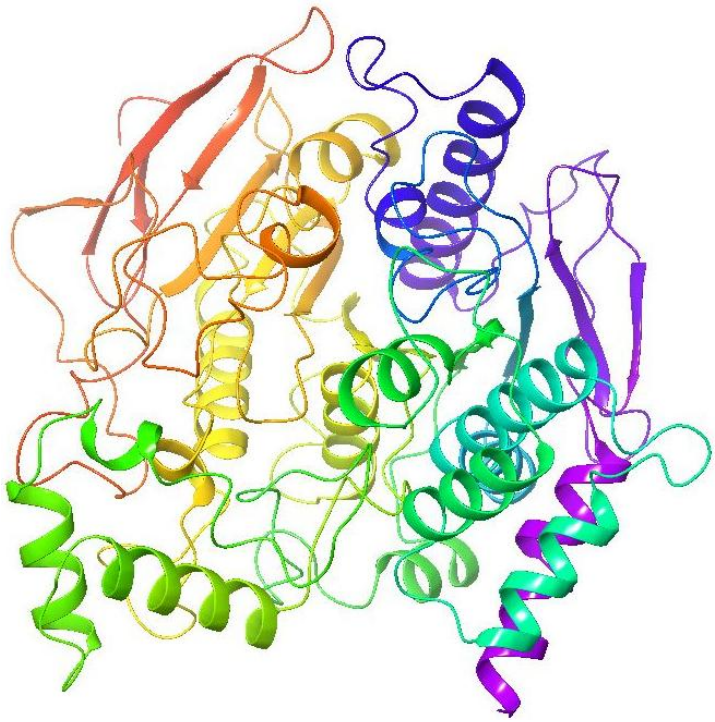
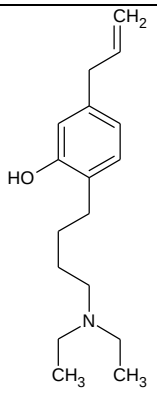
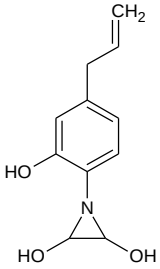
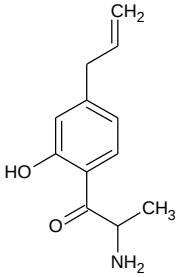
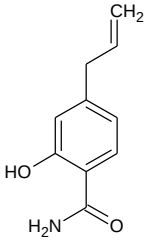
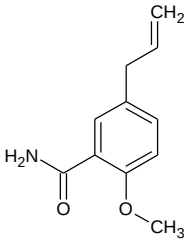
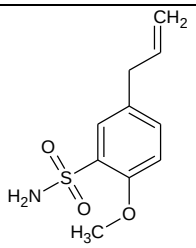
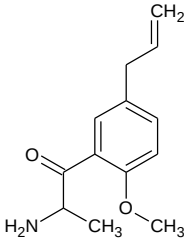
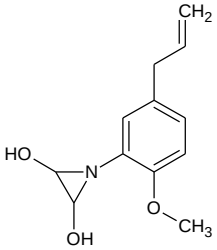
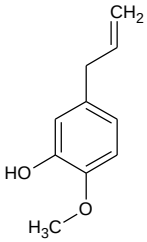
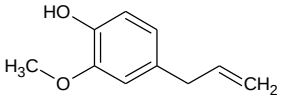
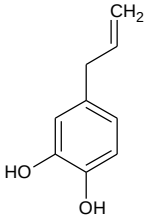


Figure 2: The 3D crystal structure of acetylcholinesterase (AChE) (ID: 1eve)

Table 1: 2D representation of ligands and their corresponding Glide score (G-Score) value calculated for the related query

Code	Score *ΔG (Kcal/mol)	Structure
CVT1	-8.4	
CVT2	-7.8	
CVT3	-7.7	
CVT4	-7.7	
HCC1	-7.6	

HCC2	-7.5	
HCC3	-7.5	
HCC4	7.3	
Chavibetol	-6.9	
Eugenol	-6.8	
Hydroxylchavicol	-6.7	

Molecular docking

To select a lead candidates among the analogsof chavibetol (CVT), eugenol (EGN) and hydroxylchavicol, molecular interaction study was performed through ligand docking into the active site of acetylcholinesterase (AChE). The minimum energy (fullfitness score) of CVT1 (2-[4-(diethylamino)butyl]-5-(prop-2-en-1-yl)phenol), CVT2 (1-[2-hydroxy-4-(prop-2-en-1-yl)phenyl]aziridine-2,3-diol), CVT3 (2-amino-1-[2-hydroxy-4-(prop-2-en-1-yl)phenyl]propan-1-one), CVT4 (2-hydroxy-4-(prop-2-en-1-yl)benzamide), HCC1 (2-methoxy-5-(prop-2-en-1-yl)benzamide), HCC2 (2-methoxy-5-(prop-2-en-1-yl)benzene-1-sulfonamide), HCC3 (2-amino-1-[2-methoxy-5-(prop-2-en-1-yl)phenyl]propan-1-one), HCC4 (1-[2-methoxy-5-(prop-2-en-1-yl)phenyl]aziridine-2,3-diol), Chavibetol (2-methoxy-5-(prop-2-en-1-yl)phenol), Eugenol (2-methoxy-4-(prop-2-en-1-yl)phenol) and Hydroxylchavicol (4-(prop-2-en-1-yl)benzene-1,2-diol)were estimated as-8.4 kcal/mol, -7.8 kcal/mol, -7.7 kcal/mol,

-7.7 kcal/mol, -7.6 kcal/mol, -7.5 kcal/mol, -7.5 kcal/mol, 7.3 kcal/mol, -6.9 kcal/mol, -6.8 kcal/mol and -6.7 kcal/mol respectively. A comparison of the docking score showed that 2-[4-(diethylamino)butyl]-5-(prop-2-en-1-yl)phenol had the highest affinity for acetylcholinesterase (AChE), hence, was selected as the lead molecule. The lead molecule was observed to interact with 14 amino acids within the pocket of acetylcholinesterase (AChE) and they include: TRP278, ASP71, TYR120, TRP83, HIS439, GLY440, GLU198, PHE329, PHE330, TYR333, GLY 334, ILE296, PHE287 and PHE289. Meanwhile chavibetol (CVT), eugenol (EGN) and hydroxylchavicol was observed to interact with 8, 12 and 7 amino acid residues respectively within the pocket of acetylcholinesterase (AChE). The lead molecule was better exposed within the sphere of the active site of acetylcholinesterase (AChE) than chavibetol (CVT), eugenol (EGN) and hydroxylchavicol. Hence, the high the affinity of 2-[4-(diethylamino)butyl]-5-(prop-2-en-1-yl)phenol for acetylcholinesterase (AChE). Meanwhile, the three-dimensional representation of intermolecular ligand- receptor interaction of all the analogs are displayed in Figs 3 to 13.

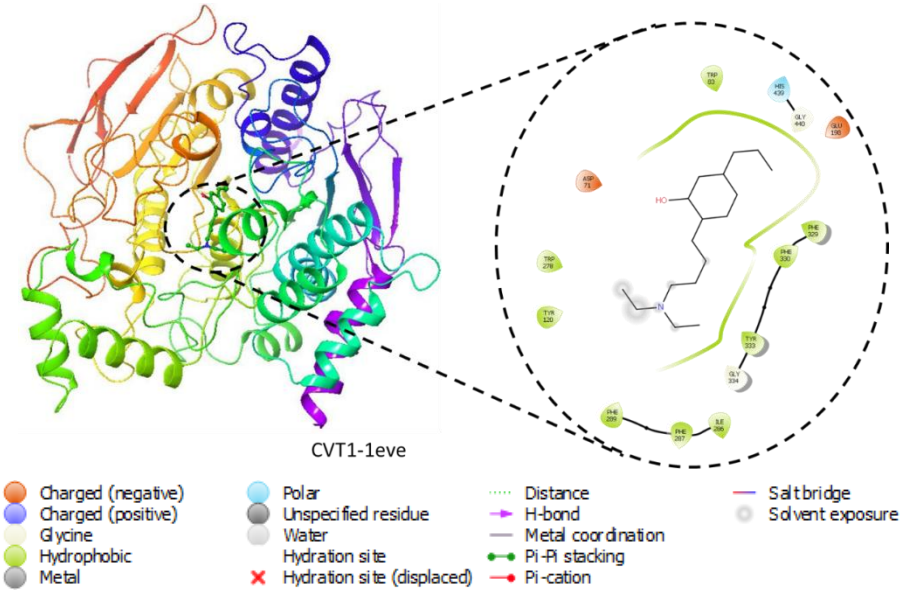


Figure 3: The3D X-ray crystal structure of 1eve complex with CVT1showing also the binding site region and the residues that constitute this binding site region

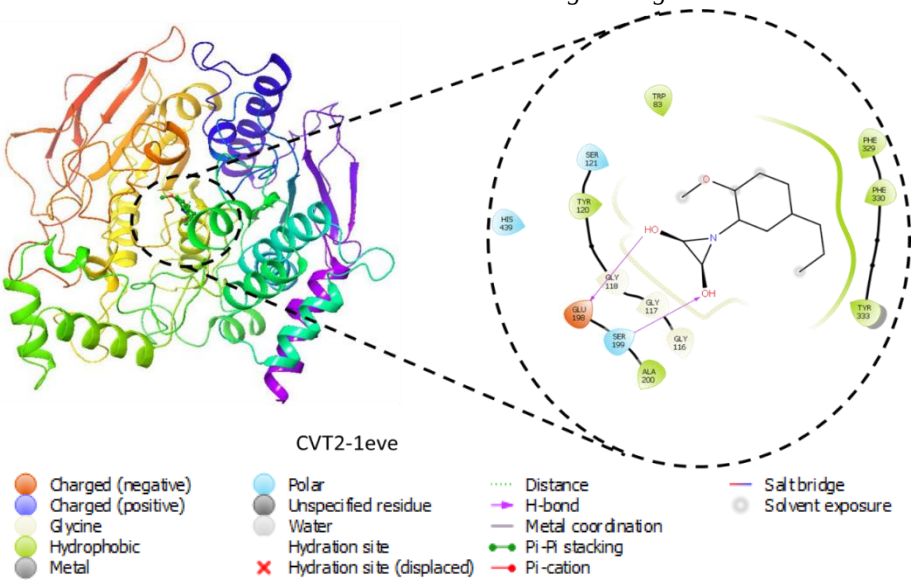


Figure 4: The 3D X-ray crystal structure of 1eve complex with CVT2showing also the binding site region and the residues that constitute this binding site region

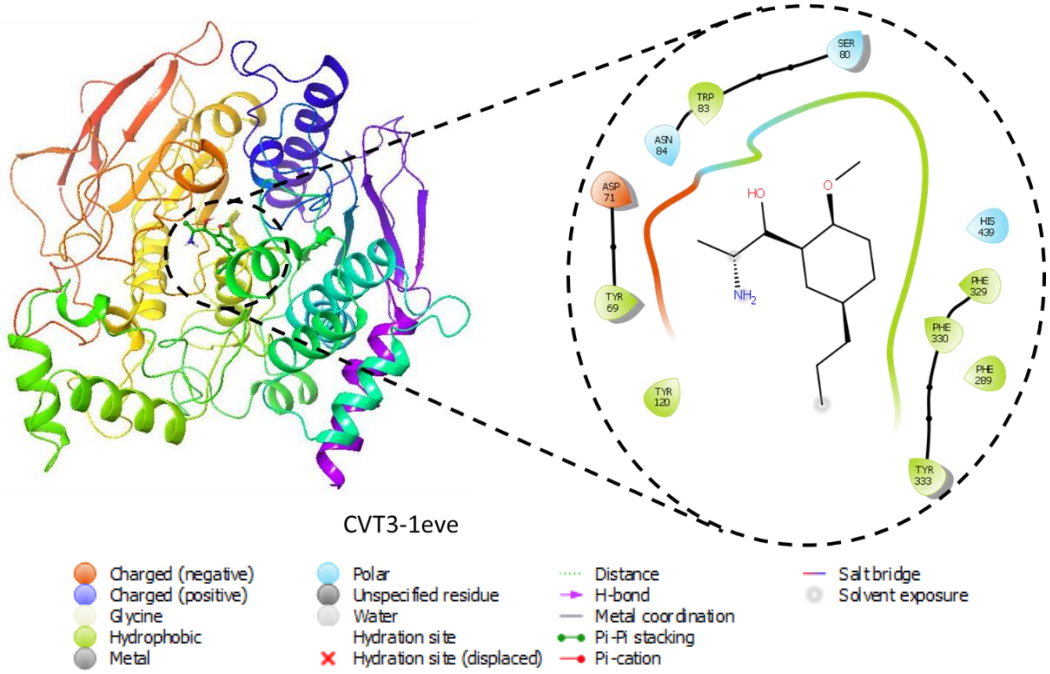


Figure 5: The 3D X-ray crystal structure of 1eve complex with CVT3 showing also the binding site region and the residues that constitute this binding site region

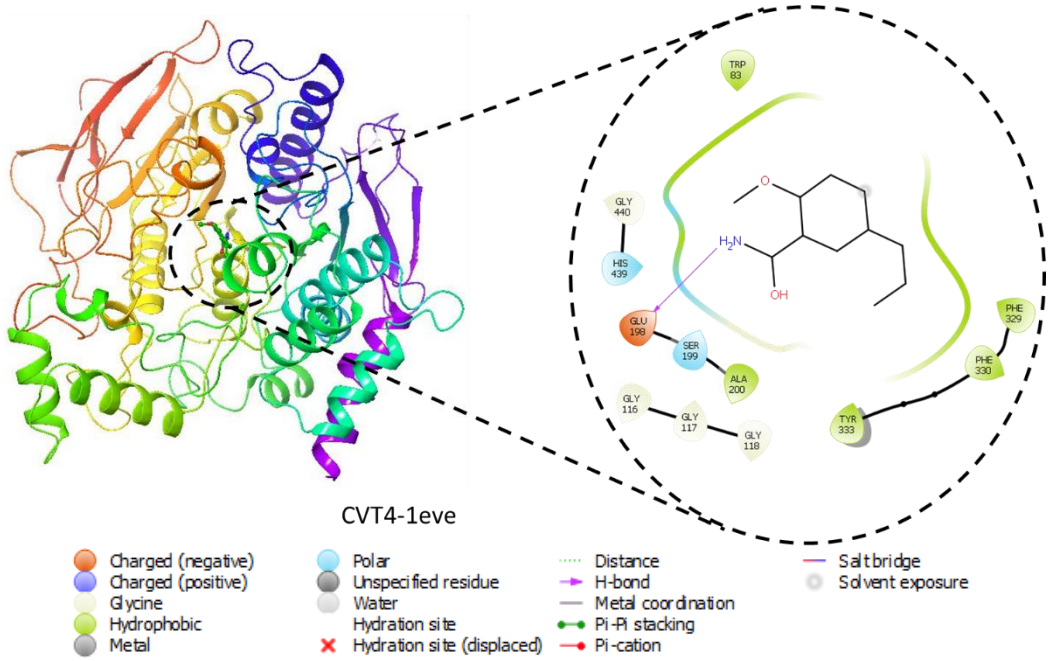


Figure 6: The 3D X-ray crystal structure of 1eve complex with CVT4 showing also the binding site region and the residues that constitute this binding site region

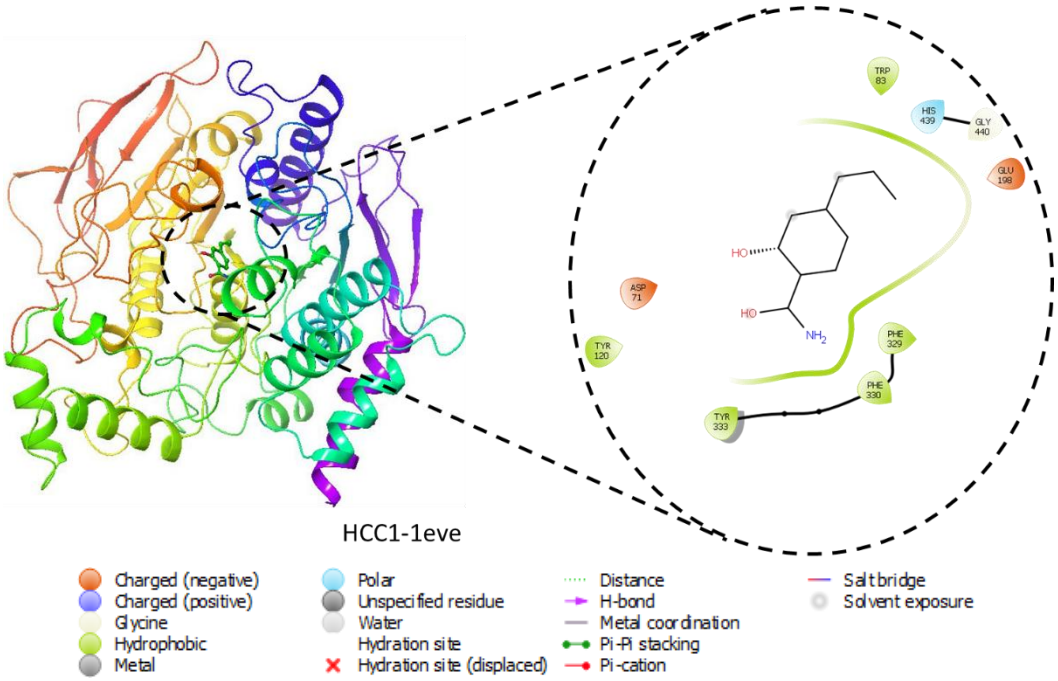


Figure 7: The 3D X-ray crystal structure of 1eve complex with HCC1 showing also the binding site region and the residues that constitute this binding site region

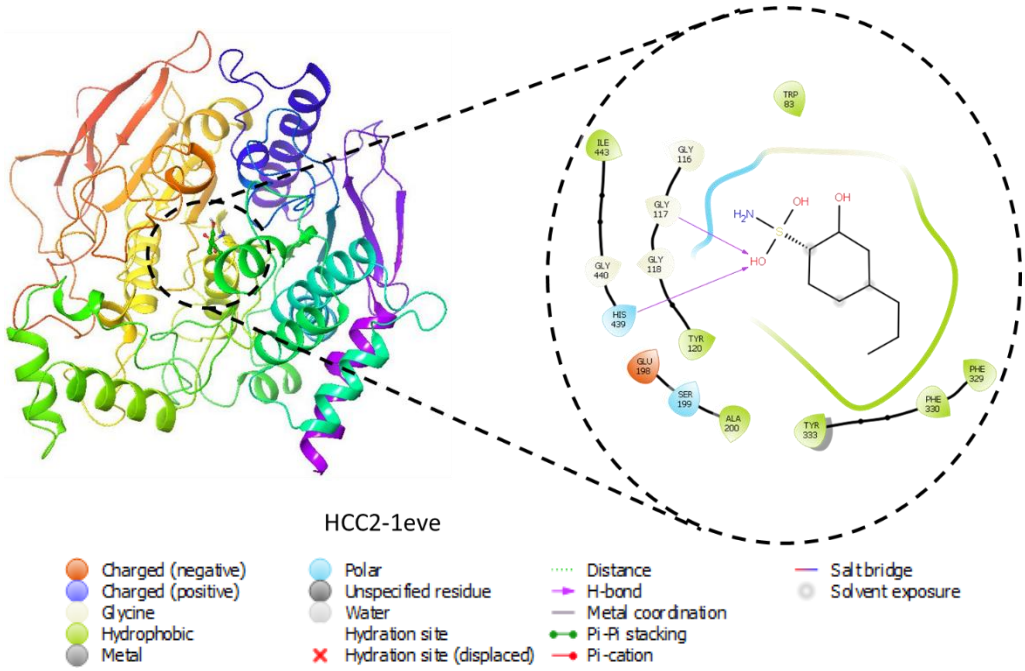


Figure 8: The 3D X-ray crystal structure of 1eve complex with HCC2 showing also the binding site region and the residues that constitute this binding site region

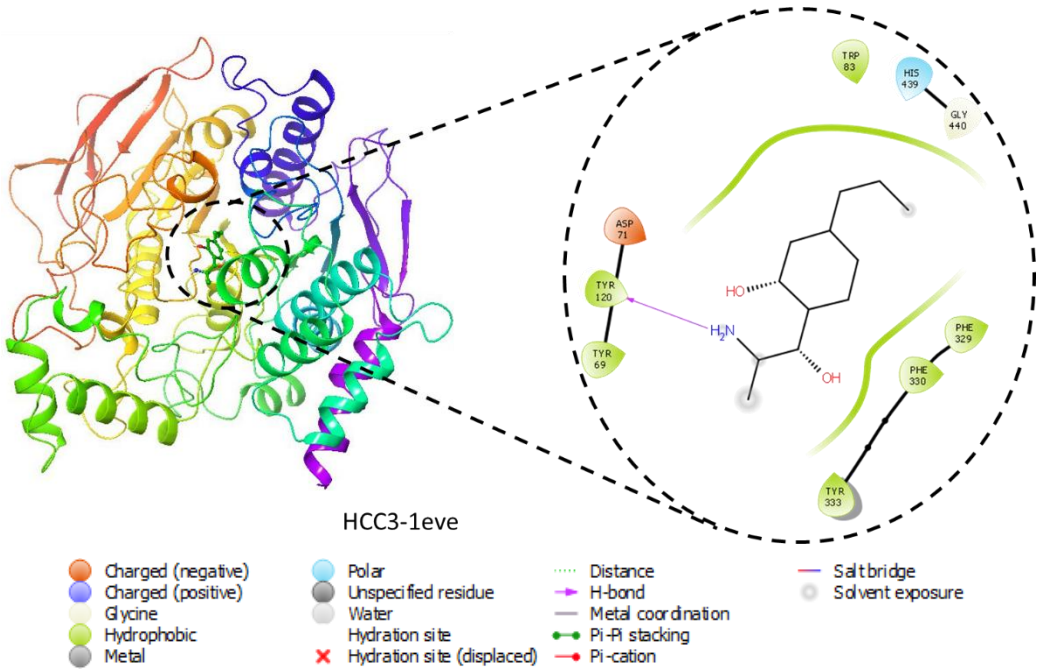


Figure 9: The 3D X-ray crystal structure of 1eve complex with HCC3 showing also the binding site region and the residues that constitute this binding site region

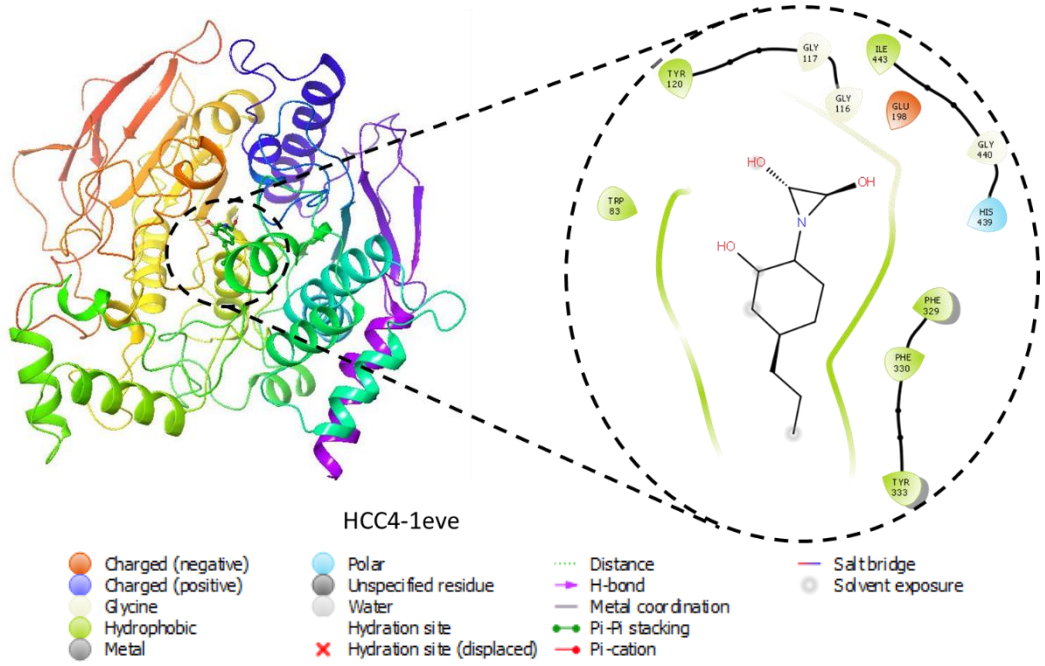


Figure 10: The 3D X-ray crystal structure of 1eve complex with HCC4 showing also the binding site region and the residues that constitute this binding site region

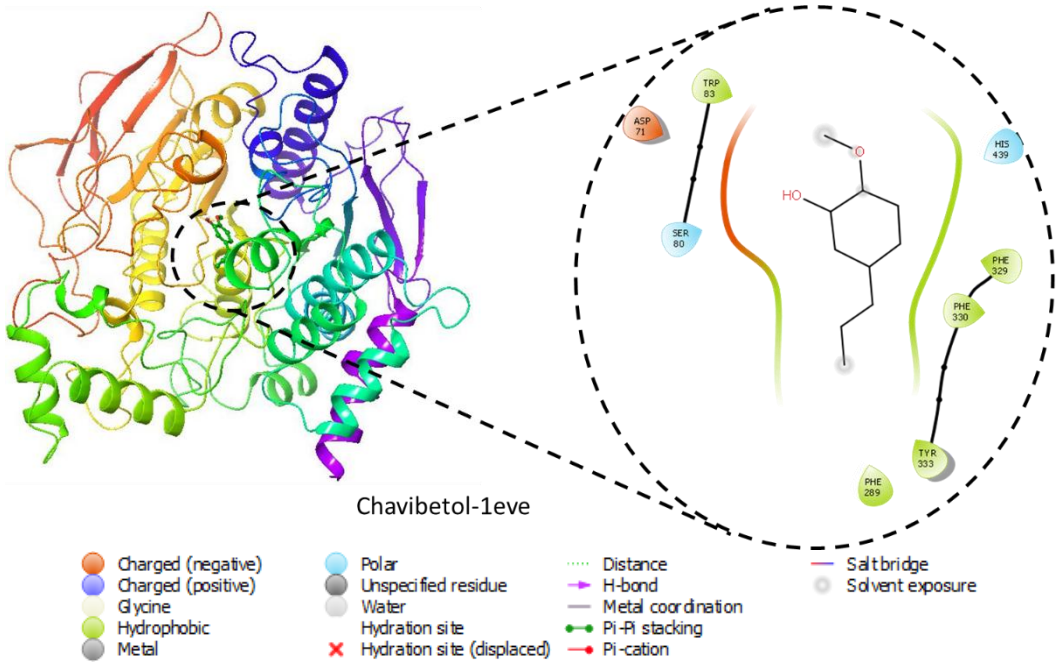


Figure 11: The 3D X-ray crystal structure of 1eve complex with chavibetol showing also the binding site region and the residues that constitute this binding site region

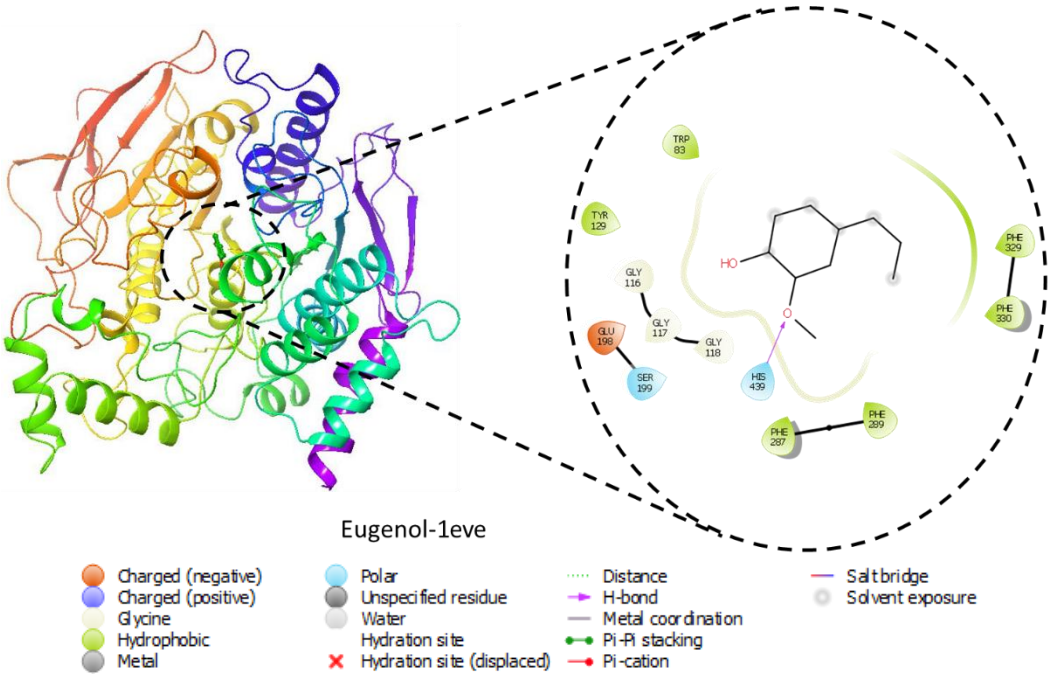


Figure 12: The 3D X-ray crystal structure of 1eve complex with eugenol showing also the binding site region and the residues that constitute this binding site region

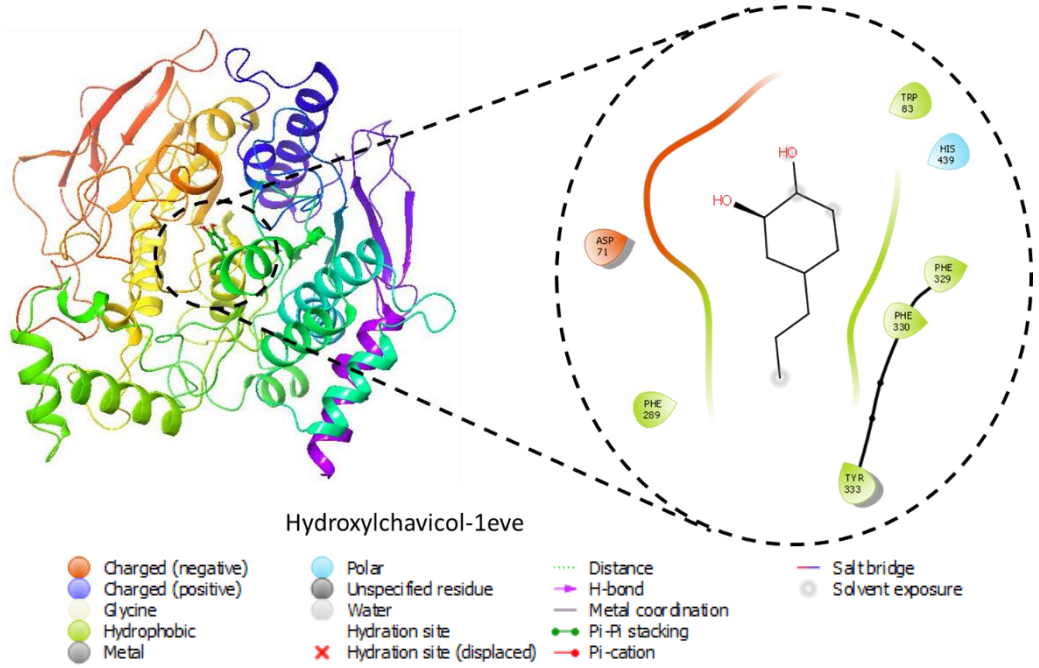


Figure 13: The 3D X-ray crystal structure of 1eve complex with hydroxylchavicol showing also the binding site region and the residues that constitute this binding site region

ADME assessment of acetylcholinesterase (AChE) inhibitors

The bioavailability radar of 2-[4-(diethylamino)butyl]-5-(prop-2-en-1-yl)phenol was used to observe the physicochemical space of the lead molecule employed for the pharmacokinetics and drug-likeness predictions (see Fig 14).

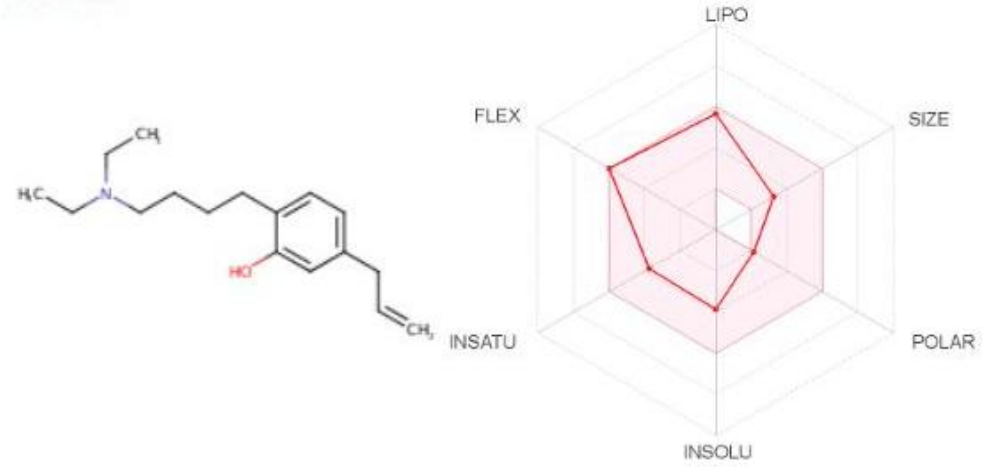


Figure 14: The bioavailability radar of 2-[4-(diethylamino)butyl]-5-(prop-2-en-1-yl)phenol using Swiss ADME predictor

The *in silico* absorption, distribution, metabolism and excretion (ADME) of the lead molecule (CVT1) was obtained using SWISSADME online webserver. This server is a modelling tool for rational drug design. The interaction between therapeutic agents and the human body is a two-way process. The therapeutic agent may alter a biochemical process in the human body which in turn can result in receptor inhibition, activation, and signal pathway blocking. Thereafter, the human body will dispose therapeutic agents *via* absorption, distribution, metabolism, and excretion. These two biochemical processes are interactional and simultaneous and lead to desired pharmacological function or undesirable side effects to man. Hence, for a compound to be druggable, the chemical

must satisfy ADME properties[16]. The physiochemical properties of the 2-[4-(diethylamino)butyl]-5-(prop-2-en-1-yl)phenol include: 19 heavy atoms, 2 hydrogen bond acceptors, 1 hydrogen bond donors, molar refractivity of 84.08and topological polar surface area (TPSA) of the molecule is found to be 23.47 $Å^2$. Meanwhile, the lipophilicity of the molecule includes: iLOGP is 3.86, XLOGP3 is 4.38, WLOGP is 3.79, MLOGP is 3.60, SILICOS-IT is 4.54 and Consensus P0/W is 4.01. Pharmacokinetic data were acquired and displayed using the molecule falling in egg's yolk model. As shown in Fig 15, 2-[4-(diethylamino)butyl]-5-(prop-2-en-1-yl)phenol was observed to penetrate blood-brain barrier and this could be by passive diffusion or via a variety of catalyzed transport systems that carries the therapeutic agent into the brain (carrier-mediated transport, receptor-mediated transcytosis) or out of the brain (active efflux) [16]. This indicates that 2-[4-(diethylamino)butyl]-5-(prop-2-en-1-yl)phenol will be suitable for the target disease.

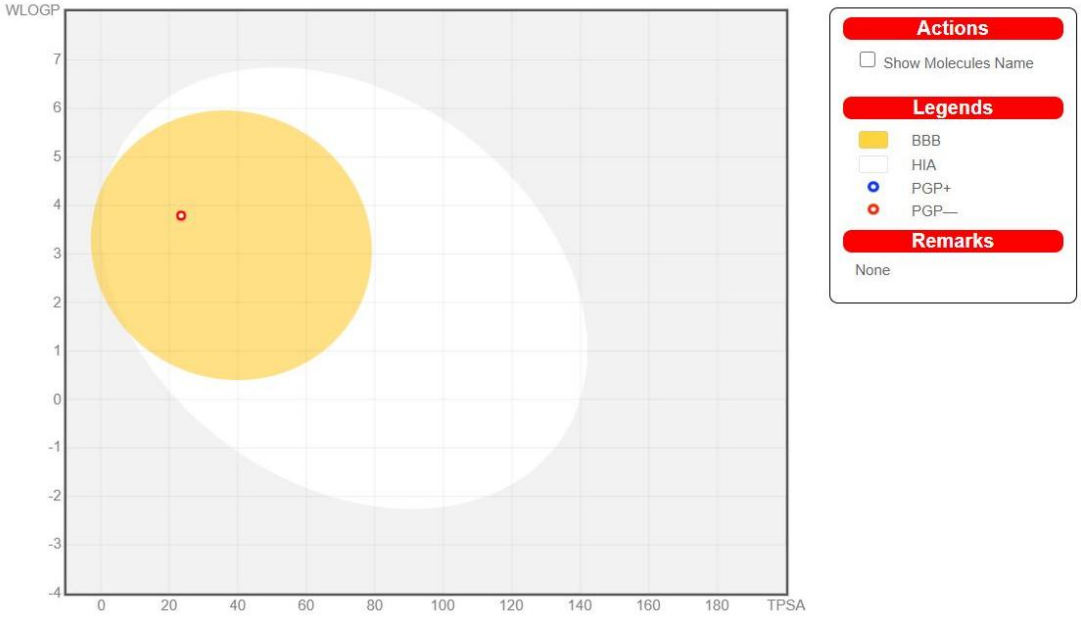


Figure 14: Molecule falling in egg's yolk prediction 2-[4-(diethylamino)butyl]-5-(prop-2-en-1-yl)phenol

Table 2: Water solubility of 2-[4-(diethylamino)butyl]-5-(prop-2-en-1-yl)phenol

Log *S* (ESOL)		-3.86
	Solubility	$3.61e^{+02}$ mg ml^{-1}; $1.38e^{-04}$ mol ml^{-1}
	Class	soluble
Log *S* (Ali)		-4.59
	Solubility	$6.73e^{+03}$ mg ml^{-1}; $2.58e^{-5}$ mol ml^{-1}
	Class	Moderately soluble
Log *S* (SILICOS-IT)		-5.22
	Solubility	$1.59e^{+03}$ $mgml^{-1}$; $6.08e^{-06}$ mol ml^{-1}
	Class	Moderately soluble

The gastrointestinal absorption (GI) was also noticed to be high suggesting good adsorption phase. Meanwhile, 2-[4-(diethylamino)butyl]-5-(prop-2-en-1-yl)phenol was also observed to be moderately soluble (see Table). Isoenzymes such as CYP1A2, CYP2C19 and CYP2D6 were inhibited by 2-[4-(diethylamino)butyl]-5-(prop-2-en-1-yl)phenol. On the contrary, CYP3A4 and CYP2C9 isoenzymes were not inhibited by 2-[4-(diethylamino)butyl]-5-(prop-2-en-1-yl)phenol. This suggests probable drug-drug interaction that may result in cytotoxicity. Hence, it is

important to thoroughly evaluate 2-[4-(diethylamino)butyl]-5-(prop-2-en-1-yl)phenol as a prospective therapeutic agent. Finally, -[4-(diethylamino)butyl]-5-(prop-2-en-1-yl)phenol was observed to obeys Lipinski's Ghose, Egan, Veber and Muegge rules with no violation (see Table 4). The lead molecule was also observed to have bioavailability 0.55.

Table 3: Pharmacokinetics of 2-[4-(diethylamino)butyl]-5-(prop-2-en-1-yl)phenol.

GI adsorption	High
BBB permeant	Yes
P-gp substrate	No
CYP 1A2	Yes
CYP2C19	Yes
CYP2C9	No
CYP2D6	Yes
CYP3A4	No
Log Kp (skin permeation)	-4.78 cm s^{-1}

Table 4: Druglikeness of 2-[4-(diethylamino)butyl]-5-(prop-2-en-1-yl)phenol.

Lipinski	Yes, 0 violation
Ghose	Yes
Veber	Yes
Egan	Yes
Muegge	Yes
Bioavailability score	0.55

Conclusion

The main objective of the present study was to perform pharmacophore modelling and the molecular docking studies of phytochemicals (Chavibetol, Eugenol and Hydroxychavicol) isolated from *Piper betel* L. The docking score of the analogues and the phytochemicals were ranked. The results revealed that all the analogues tested, showed higher dock score than chavibetol, eugenol and hydroxychavicol. Hence, 2-[4-(diethylamino)butyl]-5-(prop-2-en-1-yl)phenol was selected as the lead compound. The ADME characteristics of the lead compound showed favourable pharmacokinetics and druggable nature of the molecule. Hence, we recommend further *in vitro* and *in vivo* assay of 2-[4-(diethylamino)butyl]-5-(prop-2-en-1-yl)phenol asacetylcholinesterase (AChE) inhibitor.

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Declaration of interest

The authors declare no conflict of interest.

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