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Original article

Molecular interaction of lavender (*Lavandula angustifolia* Mill) essential oil compounds as potential anxiolytic against α2δ subunit voltage gated calcium channel

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Abstract

The effect of brain area connectivity caused by an imbalance of the glutamate neurotransmitter in the brains patients experiencing anxiety is determined by α2δ subunit calcium channel activity. Lavender (*Lavandula angustifolia*) has been investigated to have anxiolytic effects on in vitro studies by its modulation through GABA, NMDA, and serotonin receptors, especially in the amygdala and hippocampus. However, the molecular mechanism of Lavender small molecules to reduce anxiety through calcium channels remains unclear. This study aims to evaluate lavender essential oil compounds which are potentially anxiolytic through the in-silico approach as an inhibitor of the α2δ subunit VGCC. Compound tabulations were obtained from earlier studies and collected from Pubchem database, while the three-dimensional structure of α2δ the subunit was retrieved from RCSB PDB. The physicochemical properties of the compounds were analyzed by using SwissADME and pkCSM. Binding affinity screening and molecular interaction analysis were conducted through CB-Dock web server. From 32 compounds demonstrated for docking against α2δ VGCC, four of them including geranyl formate, neryl alcohol, and phellandral have most binding site similarities compared to pregabalin by pointing at Arg217. This residue was known to exerts the pharmacological action of pregabalin to reduce anxiety and pain. The binding affinity of the geranyl formate (-6,6 kcal/mol), neryl alcohol (-6,4 kcal/mol), and phellandral (-6,2 kcal/mol) were comparable to pregabalin (-6,8 kcal/mol). Molecular dynamic was indicated to predict conformational change of α2δ subunit after superimposed. Thus, current study was indicated that geranyl formate, neryl alcohol, and phellandral as lavender essential oil small molecules has potent beneficial function as anxiolytic potential candidate.

Keywords: Anxiety, lavender essential oil, α2δ subunit, voltage gated calcium channel

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Introduction

Anxiety disorder is a mayor serious mental health problem described by stressful conditions with globally high prevalence (Khan, et al., 2020). Recently, about 3,6% million in worldwide and 4,9% Southeast Asian people are reported to face anxiety every year (Hidayati, et al., 2022). This disorder originated from high desire, trauma, and unexpected threat. As the result, anxiety risk associated with memory impairment, difficulties at solving problem, as far as its effect to somatic conditions including increased heart rate, muscle tension, nausea, and tremor (Adwas, et al., 2019; Nechita, et al., 2018). The finding of particular neurological disorder such as temporal lobe seizures, multiple sclerosis, and Huntington's disease, anxiety may accompany its presentation (Zun, et al., 2013). Along with dynamically complication which can develop the risk of anxiety disorder, adequate treatment becomes a major emergency to redeem the burden of this disorder (Tjandrarini, et al., 2020).

Previous study revealed relationship of anxiety as a symptom of stress to neural synapse dysregulation due to a homeostatic signaling imbalance of two main amino acid neurotransmitter, glutamate, and GABA (Wieronska, et al., 2011). In vitro studies have proven high glutamate release causes brain excitability in post-traumatic brain injury mice (Beitchman, et al., 2020). Additional study also shown that chronic stress-induced anxiety in mice increases presynaptic glutamate release in ventral hippocampus (Liu, et al., 2020). This phenomenon happens due to susceptibility to overexcitation that activates glutamatergic neuronal projections of anxiogenic circuits such as basolateral amygdala, ventral hippocampus, and medial prefrontal cortex (Tovote, et al., 2015). In addition to anxiety, overexcited neuron can also exacerbate other neurological disorder such as pain and epilepsy (Wieronska, et al., 2011).

Anxiety-reducing drugs that are widely used are Selective Serotonin Re-uptake Inhibitor (SSRI) and Serotonin and Norepinephrine Reuptake Inhibitors (SNRI), which act by increasing serotonin system and decreasing norepinephrine. However, severe to mild effects have been reported from these drugs. The symptoms of headache, gastrointestinal disease, insomnia, and elevated blood pressure can't be ruled out as drug limitations (Adwas, et al., 2019; Santarsieri & Schwartz, 2015). Besides SNRI and SSRI, there is anxiolytic, anticonvulsant, and antiepileptic effects that are act by inhibits calcium influx and decreased the release of excitatory neurotransmitter glutamate in hippocampus, amygdala, and medial prefrontal cortex. This mechanism mediated by strong binding to N-type and P/Q-type potential protein α2δ subunit voltage gated

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calcium channel (VGCC) that act on the transmembrane structure of neurons (Ablinger, et al., 2020). This subunit selective ligand belongs to gabapentinoids, namely pregabalin and gabapentin. Although its recommended as adjunctive therapy, these two synthetic compounds also consider to serious side effect. High doses of gabapentinoids (>900 mg) can lead to dependency, respiratory problems, tremor, and ataxia (Lennox & Mangin, 2019). Hence, bioactive agents that can access and attenuate specific target becomes the top priority for researcher to develop the potential anxiolytic alternatives.

Aromatherapy effectivity from plant essential oil as volatile therapeutics is considered to be more tolerant and enter the limbic system such as amygdala and hippocampus easier compared to oral drugs. Lavender (*Lavandula angustifolia*) essential oil with high neuroprotective effects is widely used as an anxiolytic agent, antidepressant, and analgesic (Fung, et al., 2021). Various clinical and in vitro studies have proven this effect through neuronal receptor involved in anxiety like 5-HT receptor, NMDAr, and VGCC (Malcolm & Tallian, 2017; Garakani, et al., 2020). Although Schuwald et al. (2013) have found that Lavender compounds are able to reduce calcium influx from N-type and P/Q-type VGCC, but the exact molecular mechanism remains unclear. In silico approach simulates probable molecular mechanism through protein-ligand interaction. This study evaluates and investigates the ligands contained in Lavender in inhibiting VGCC using in silico virtual screening approach and molecular docking.

Methods

Data mining

The α2δ subunit VGCC protein (PDB ID: 7MIY) is downloaded from RCSB PDB (https://www.rcsb.org/) in PDB format. The active compound of Lavender essential oil was obtained from Jablonský et al. (2016) and Dong et al. (2020). Cannonical SMILES and three-dimensional structure of the compounds were retrieved from PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Pregabalin was used as control ligand. Protein was prepared using Discovery Studio 2019 software to eliminate water molecules and natural ligands from the protein. Ligands were then prepared to minimize the energy by using Open Babel in PyRx 0.8 software. The result then converted to PDB (.pdb) format.

Screening analysis

The physicochemical properties of Lavender essential oil compounds were predicted using SwissADME (http://www.swissadme.ch) and Graph-Based Signatures pkCSM websites (biosig.unimelb.edu.au/pkcsm/prediction). The parameters used for screening were lipophilicity, Blood Brain Barrier (BBB) permeability, Central Nervous System (CNS) permeability, hepatotoxicity, the human ether-a-go-go related gene (hERG) inhibitor, Lipinski rules of five, Total Polar Surface Area (TPSA), and cellular location. Selected compounds were then analyzed to dock screening using CB-Dock. After molecular interaction analysis for the most potential compound was selected, it subsequently analyzed for evaluation of possible toxicity based on crammer rules, skin or eye irritant properties, DNA binding adduct, mutagenicity or carcinogenicity, and Ames test properties using Toxtree (https://toxtree.sourceforge.net/).

Docking molecules & analysis for binding interaction

To simulate the capability of ligands to binding with protein, we used binding affinity screening through cavity-detection guided blind docking on CB-Dock website (http://clab.labshare.cn/cb-dock/php/blinddock.php). From all five possible binding cavity, only one was selected based on pregabalin binding pocket at lowest binding affinity score as control ligand. Selected protein-ligand complexes in pdb format were stored in rar (.zip) document. The binding types among the atoms of ligand and amino acid residue of receptor was identified using Discovery Studio Visualizer 2019.

Molecular dynamic simulation

Molecular dynamic (MD) was carried out using Webgro GROMACS simulation. Ligand topology generated by running PRODGR through ligand HETATM code submission on GlycoBioChem (http://davapc1.bioch.dundee.ac.uk/cgi-bin/prodrg/submit.html) The ligand-protein complex and ligand topology were then uploaded to Webgro protein with ligand simulation (https://simlab.uams.edu/ProteinWithLigand/index.html). The temperature simulation was set to 310 Kelvin and change the simulation time to 20 ns before submission. RMSD backbone and ligand fit to protein were then anayzed.

Results

Screening analysis of *Lavandula angustifolia* essential oil

For evaluating physicochemical properties of *Lavandula angustifolia* essential oil compounds, we conducted a virtual screening using pkCSM-pharmacokinetics and SwissADME (Table 1). Lipophilicity was predicted using consesus $LogP_{o/w}$ descriptor of SwissADME and its refers to capability of substance to transport through permeability of membrane and allocation to different tissue and organs. Consesus $LogP_{o/w}$ in the range 2.25 to 4.75 indicating better brain penetration (Alminderej, et al., 2020). The highest lipophilicity value of these compounds was 4.59. Blood Brain Barrier (BBB) is a complex structure in CNS that controls the diffusion or clearance of various material or nutrients from the blood to the brain and vice versa that are crucial to neural function. Molecules with log BBB > 0.3 are considered to rapidly crossed the BBB. The BBB permeability values of these compounds were predicted in the range 0.438 to 0.901. Similar to BBB, CNS permeability was also measured to predict ability of compounds to penetrate the CNS if the value of logPS> -2. Hepatotoxicity predicts whether the given compounds may be associated with impaired liver function. The hERG inhibitors are likely associated with development

of acquired long QT syndrome leading to fatal ventricular arrhythmia (Pires, et al., 2015). Lipinski rule of five states that good absorption occurs when the molecular weight < 500 Da, hydrogen bond donors < 5, hydrogen bond acceptors < 10, rotatable bonds < 10, and polar surface area < 140 Å. TPSA values less than 30 Å suggesting good entrance to brain (Alminderej, et al., 2020; Domínguez-Villa, et al., 2021). The result of ADME selection given 32 compounds that subsequently analyzed for the binding affinity screening. Molecular docking was used to measure the capability of ligands to bind protein at binding affinities below -6 kcal/mol. All ligands was successfully bound to α2δ subunit, with five possible binding sites including at the surface and internal locations determined by CB-Dock web server. From all binding sites, only one pocket was choosen due to its similarity with control ligand, pregabalin, at most negative binding affinity score. The screening results showed three compounds (Figure 1) which have similar ligand-protein interactions as pregabalin, namely geranyl formate, neryl alcohol, and phellandral. Toxtree toxic hazard estimation presented that from all the compounds, only neryl alcohol was available to identify, while other compounds could not be identified by Toxtree. Cramer rules of neryl alcohol showed as Class I (low) indicating low toxicity. This compound can cause serious lesions to the eye and irritating to skin. There were no DNA binding alerts identified of this compound. There was also nothing alerts for S. typhimurium mutagenicity identified from neryl alcohol

Molecular interaction of geranyl formate, neryl alcohol, and phellandral with α2δ subunit VGCC

Pregabalin as control ligand has the lowest binding affinity (-6.8 kcal/mol) following by geranyl formate (-6.6 kcal/mol), neryl alcohol (-6.4 kcal/mol), and phellandral (-6.2 kcal/mol) as can be seen in Table 2. The smaller binding affinity indicates the less energy needed to bound between the ligand and protein so they can interact easily (Agustin, et al., 2020). Pregabalin interacts with α2δ subunit at 21 residue and consist of three binding types including four electrostatic, ten hydrogen bond, and eight hydrophobic (Figure 1). It can be seen on Table 2, geranyl formate formed four hydrogen bonds like pregabalin, at residue Tyr236, Arg241, and Trp243. Neryl alcohol has hydrogen bonds at the same two residues as pregabalin, at residue Arg241 and Asp452. Phellandral formed 2 two hydrogen bonds to one residue at Arg241. It can be seen from Figure 1, the ligands always bound to Arg241 residue. It happens because the existence of hydrogen bonds between oxygen atoms and nitrogen atoms in the arginine's guanidium group. The distances of hydrogen bonds formed from those interaction varies from 2.79-2.99 Å, indicating as strong residue binding. Since the three compounds have a similar binding site compared with pregabalin, this suggest that these compounds might exhibit inhibitory activity against α2δ subunit

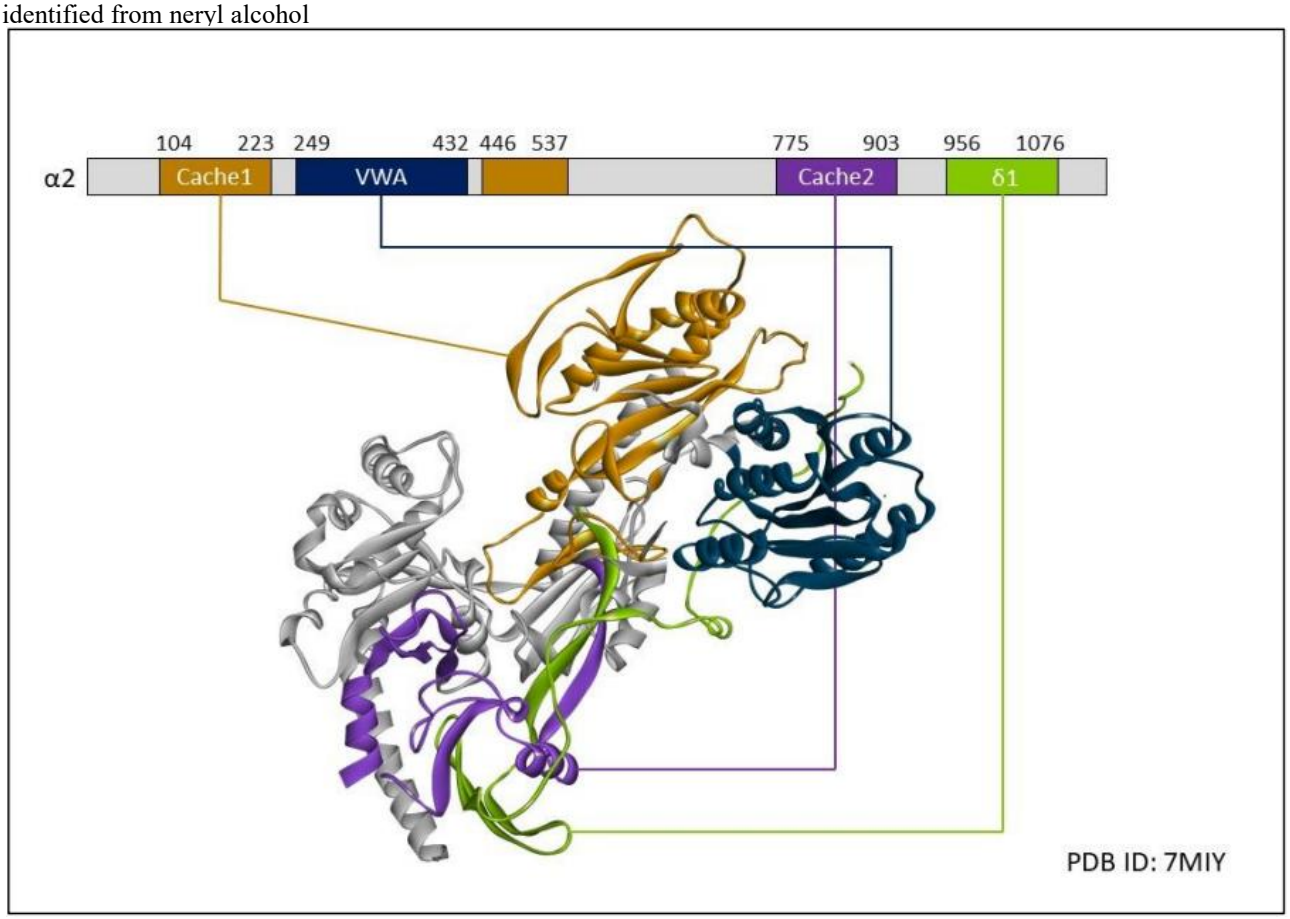

Figure 1. Voltage Gated Calcium Channel α2δ-1 subunit (7MIY) domains. Yellow boxes show cache1 domain, while purple shows cache2. Blue region indicates von-willebrand factor A (VWA). The green region indicates δ1. Gray region shows structural domain. Referred to Eroglu, et al. (2009)

Table 1. Screening result of Lavender essential oil compounds by using pkCSM and SwissADME

Compounds	SMILES	CID	Lipophilicity (Log Po/w)	BBB permeability	CNS permeability	Hepatotoxicity	hERG inhibitor	Lipinski	TPSA (Å)	Cellular Location
Camphene	CC1(C2CCC(C2)C1=C)C	6616	3.43	0.787	-1.71	No	No	Yes	0	Membrane
β-Myrcene	CC(=CCCC(=C)C=C)C	31253	3.43	0.781	-1.902	No	No	Yes	0	Membrane
Limonene	CC1=CCC(CC1)C(=C)	22311	2.79	0.743	-2.324	No	No	Yes	0	Extracellular, membrane
cis-β-Ocimene	CC(=CCC=C(C)C=C)C	5320250	3.4	0.761	-1.848	No	No	Yes	0	Extracellular, membrane
Linalool	CC(=CCCC(C)(C=C)O)C	6549	2.66	0.598	-2.339	No	No	Yes	20.23	Extracellular, membrane
Camphor	CC1(C2CCC1(C(=O)C2)C)C	2537	2.37	0.612	-2.158	No	No	Yes	17.07	Membrane
Terpinen-4-ol	CC1=CCC(CC1)(C(C)C)O	11230	2.6	0.563	-2.473	No	No	Yes	20.23	Extracellular, membrane
Hotrienol	CC(=C)C=CCC(C)(C=C)O	5366264	2.51	0.622	-2.349	No	No	Yes	20.23	Not available
Geranyl acetate	CC(=CCCC(=CCOC(=O)C)C)C	1549026	3.21	0.566	-2.199	No	No	Yes	26.3	Extracellular, membrane
Geranyl formate	CC(=CCCC(=CCOC=O)C)C	5282109	3.05	0.597	-2.206	No	No	Yes	26.3	Extracellular, membrane
Geranyl vinyl ether	CC(=CCCC(=CCOC=C)C)C	5365842	3.67	0.714	-2.174	No	No	Yes	9.23	-
Longifolene	CC1(CCCC2(C3C1C(C2=C)CC3)C)C	289151	4.5	0.808	-1.949	No	No	Yes	0	Not available
p-Cymene	CC1=CC=C(C=C1)C(C)C	7463	3.5	0.478	-1.397	No	No	Yes	0	Extracellular, membrane
β-Cymene	CC1=CC(=CC=C1)C(C)C	10812	3.58	0.475	-1.397	No	No	Yes	0	Membrane
β-trans-Ocimene	CC(=CCC=C(C)C=C)C	5281553	3.4	0.761	-1.848	No	No	Yes	0	Extracellular, membrane
α-Terpinolen	CC1=CCC(=C(C)C)CC1	11463	3.4	0.695	-2.317	No	No	Yes	0	Extracellular, membrane
1-Pentylallyl acetate	CCCCCC(C=C)OC(=O)C	17121	2.69	0.55	-2.331	No	No	Yes	26.3	Membrane
(4E,6Z)-allo-Ocimene	CC=C(C)C=CC=C(C)C	5371125	3.37	0.746	-1.805	No	No	Yes	0	-
(-)-Borneol	CC1(C2CCC1(C(C2)O)C)C	1201518	2.38	0.646	-2.331	No	No	Yes	20.23	Extracellular, membrane
Lavandulol	CC(=CCC(CO)C(=C)C)C	5464156	2.6	0.601	-2.27	No	No	Yes	20.23	Extracellular, membrane
Verbenone	CC1=CC(=O)C2CC1C2(C)C	29025	2.25	0.776	-2.32	No	No	Yes	17.07	Not available
Bornyl formate	CC1(C2CCC1(C(C2)OC=O)C)C	518472	2.81	0.584	-2.378	No	No	Yes	26.3	Extracellular, membrane
Neryl alcohol	CC(=CCCC(=CCO)C)C	643820	2.78	0.606	-2.159	No	No	Yes	20.23	Extracellular, membrane
p-Cumic aldehyde	CC(C)C1=CC=C(C=C1)C=O	326	2.48	0.438	-1.485	No	No	Yes	17.07	Extracellular, membrane
Linalyl acetate	CC(=CCCC(C)(C=C)OC(=O)C)C	8294	3.24	0.516	-2.379	No	No	Yes	26.3	Extracellular, membrane
Phellandral	CC(C)C1CCC(=CC1)C=O	89488	2.47	0.617	-2.17	No	No	Yes	17.07	Extracellular, membrane
Lavandulol acetate	CC(=CCC(COC(=O)C)C(=C)C)C	30247	3.11	0.565	-2.373	No	No	Yes	26.3	-

β-Caryophyllen	CC1=CCCC(=C)C2CC(C2CC1)(C)C	5281515	4.24	0.733	-2.172	No	No	Yes	0	Extracellular, membrane
α-Santalene	CC(=CCCC1(C2CC3C1(C3C2)C)C)C	94164	4.59	0.901	-1.745	No	No	Yes	0	Extracellular, membrane
d-Germacrene	CC1=CCCC(=C)C=CC(CC1)C(C)C	5373727	4.3	0.723	-2.138	No	No	Yes	0	Not available
γ-Cadinene	CC1=CC2C(CC1)C(=C)CCC2C(C)C	15094	4.18	0.809	-1.631	No	No	Yes	0	Extracellular, membrane
Cedrelanol	CC1=CC2C(CCC(C2CC1)(C)O)C(C)C	160799	3.43	0.596	-2.151	No	No	Yes	20.23	Extracellular, membrane

Table 2. Interaction of pregabalin, geranyl formate, neryl alcohol, and phellandral to α2δ subunit VGCC

Compounds	Interaction	Distance (Å)	Category	Type	Binding Affinity (kcal/mol)
Pregabalin	A:LIG1:H - D:ASP491:OD1	2.30	Hydrogen Bond;Electrostatic	Salt Bridge;Attractive Charge	-6.8
	A:LIG1:N - D:ASP452:OD1	4.83	Electrostatic	Attractive Charge	
	D:TYR236:OH - A:LIG1:O	3.03	Hydrogen Bond	Conventional Hydrogen Bond	
	D:ARG241:NE - A:LIG1:O	2.79	Hydrogen Bond	Conventional Hydrogen Bond	
	D:ARG241:NH2 - A:LIG1:O	2.96	Hydrogen Bond	Conventional Hydrogen Bond	
	D:TRP243:NE1 - A:LIG1:O	2.85	Hydrogen Bond	Conventional Hydrogen Bond	
	D:ALA453:N - A:LIG1:O	2.95	Hydrogen Bond	Conventional Hydrogen Bond	
	A:LIG1:O - D:ASP452:OD1	3.06	Hydrogen Bond	Conventional Hydrogen Bond	
	A:LIG1:H - D:TYR450:OH	2.48	Hydrogen Bond	Conventional Hydrogen Bond	
	A:LIG1:H - D:THR461:OG1	2.87	Hydrogen Bond	Conventional Hydrogen Bond	
	A:LIG1:H - D:TYR450:OH	2.60	Hydrogen Bond	Conventional Hydrogen Bond	
	A:LIG1:N - D:TRP243	3.63	Electrostatic	Pi-Cation	
	A:LIG1:N - D:TRP243	4.48	Electrostatic	Pi-Cation	
	D:ALA215 - A:LIG1:C	4.33	Hydrophobic	Alkyl	
	A:LIG1:C - D:VAL207	4.25	Hydrophobic	Alkyl	
	A:LIG1:C - D:LEU454	4.89	Hydrophobic	Alkyl	
	A:LIG1:C - D:VAL207	4.66	Hydrophobic	Alkyl	
	D:TRP205 - A:LIG1:C	4.78	Hydrophobic	Pi-Alkyl	
	D:TYR217 - A:LIG1:C	4.36	Hydrophobic	Pi-Alkyl	
	D:TYR217 - A:LIG1:C	4.24	Hydrophobic	Pi-Alkyl	
	D:TRP223 - A:LIG1:C	4.83	Hydrophobic	Pi-Alkyl	

Geranyl formate	D:TYR236:OH - A:LIG1:O	2.97	Hydrogen Bond	Conventional Hydrogen Bond	-6.6
	D:ARG241:NE - A:LIG1:O	2.86	Hydrogen Bond	Conventional Hydrogen Bond	
	D:TRP243:NE1 - A:LIG1:O	3.07	Hydrogen Bond	Conventional Hydrogen Bond	
	D:TYR450:OH - A:LIG1:O	2.85	Hydrogen Bond	Conventional Hydrogen Bond	
	A:LIG1:C - D:ASP491:OD1	3.43	Hydrogen Bond	Carbon Hydrogen Bond	
	D:ALA215 - A:LIG1:C	3.09	Hydrophobic	Alkyl	
	D:ALA215 - A:LIG1:C	4.08	Hydrophobic	Alkyl	
	A:LIG1:C - D:VAL207	3.58	Hydrophobic	Alkyl	
	A:LIG1:C - D:VAL207	5.39	Hydrophobic	Alkyl	
	A:LIG1:C - D:VAL207	4.09	Hydrophobic	Alkyl	
	D:HIS167 - A:LIG1:C	5.05	Hydrophobic	Pi-Alkyl	
	D:TRP205 - A:LIG1:C	4.94	Hydrophobic	Pi-Alkyl	
	D:TYR217 - A:LIG1:C	4.37	Hydrophobic	Pi-Alkyl	
	D:TRP223 - A:LIG1:C	4.56	Hydrophobic	Pi-Alkyl	
	D:TYR236 - A:LIG1:C	5.38	Hydrophobic	Pi-Alkyl	
	D:TYR236 - A:LIG1:C	5.11	Hydrophobic	Pi-Alkyl	
	D:TRP243 - A:LIG1:C	5.27	Hydrophobic	Pi-Alkyl	
Neryl alcohol	D:ARG241:NH2 - A:LIG1:O	2.99	Hydrogen Bond	Conventional Hydrogen Bond	-6.4
	A:LIG1:H - D:ASP452:OD1	2.12	Hydrogen Bond	Conventional Hydrogen Bond	
	A:LIG1:C - D:TYR236:OH	3.52	Hydrogen Bond	Carbon Hydrogen Bond	
	A:LIG1:C - D:TYR217	3.67	Hydrophobic	Pi-Sigma	
	A:LIG1:C - D:TRP243	3.64	Hydrophobic	Pi-Sigma	
	D:ALA215 - A:LIG1:C	3.79	Hydrophobic	Alkyl	
	A:LIG1:C - D:VAL207	4.84	Hydrophobic	Alkyl	
	A:LIG1:C - D:LEU454	4.16	Hydrophobic	Alkyl	
	A:LIG1:C - D:VAL207	4.49	Hydrophobic	Alkyl	
	D:TYR217 - A:LIG1:C	5.06	Hydrophobic	Pi-Alkyl	
	D:TRP223 - A:LIG1:C	4.85	Hydrophobic	Pi-Alkyl	
	D:TRP223 - A:LIG1:C	4.67	Hydrophobic	Pi-Alkyl	

	D:TYR236 - A:LIG1:C	4.86	Hydrophobic	Pi-Alkyl	
	D:TRP243 - A:LIG1:C	4.17	Hydrophobic	Pi-Alkyl	
Phellandral	D:ARG241:NE - A:LIG1:O	2.95	Hydrogen Bond	Conventional Hydrogen Bond	-6.2
	D:ARG241:NH2 - A:LIG1:O	2.89	Hydrogen Bond	Conventional Hydrogen Bond	
	A:LIG1:C - D:TYR217	3.29	Hydrophobic	Pi-Sigma	
	D:VAL207 - A:LIG1	4.94	Hydrophobic	Alkyl	
	D:ALA215 - A:LIG1:C	3.79	Hydrophobic	Alkyl	
	D:ALA453 - A:LIG1	4.97	Hydrophobic	Alkyl	
	A:LIG1:C - D:LEU454	3.87	Hydrophobic	Alkyl	
	A:LIG1:C - D:VAL207	4.52	Hydrophobic	Alkyl	
	D:TYR217 - A:LIG1:C	4.42	Hydrophobic	Pi-Alkyl	
	D:TRP223 - A:LIG1:C	4.44	Hydrophobic	Pi-Alkyl	
	D:TRP223 - A:LIG1:C	5.00	Hydrophobic	Pi-Alkyl	
	D:TRP243 - A:LIG1	5.46	Hydrophobic	Pi-Alkyl	

Stability interaction of geranyl formate, neryl alcohol, and phellandral with α2δ subunit of VGCC

In order to assess the stability of protein and ligand interaction, we performed molecular dynamic simulations of geranyl formate, neryl alcohol, and phellandral with α2δ subunit of VGCC. RMSD result showed stability differences of protein and ligan for each trajectory after docked. Although the fluctuations remain high, compared with pregabalin, these three ligands geranyl formate, neryl alcohol, and phellandral required longer time to reach their stability. It can be observed on Figure 3, that all RMSD for ligand-protein interaction showed that the three ligands were stable at 5 ns. Similar to ligand, protein rigidity observed from RMSD backbone of all trajectories also increased around time and still had moderate fluctuation. RMSD backbone measured the average distance between backbone atoms during simulation compared with starting structure, while RMSD ligand-protein measure the distance of ligand motion compared with supposed binding pocket after superimposed. Lower RMSD value indicating stable pose of the backbone atoms or ligand compared with initial structure and positions (Kim, et al., 2016). Considering all the ligands affect the structure of protein backbone, its presumably causing conformational change leads to functional impairment of the protein, thus inhibit the function of pore forming α1 voltage gated calcium channel.

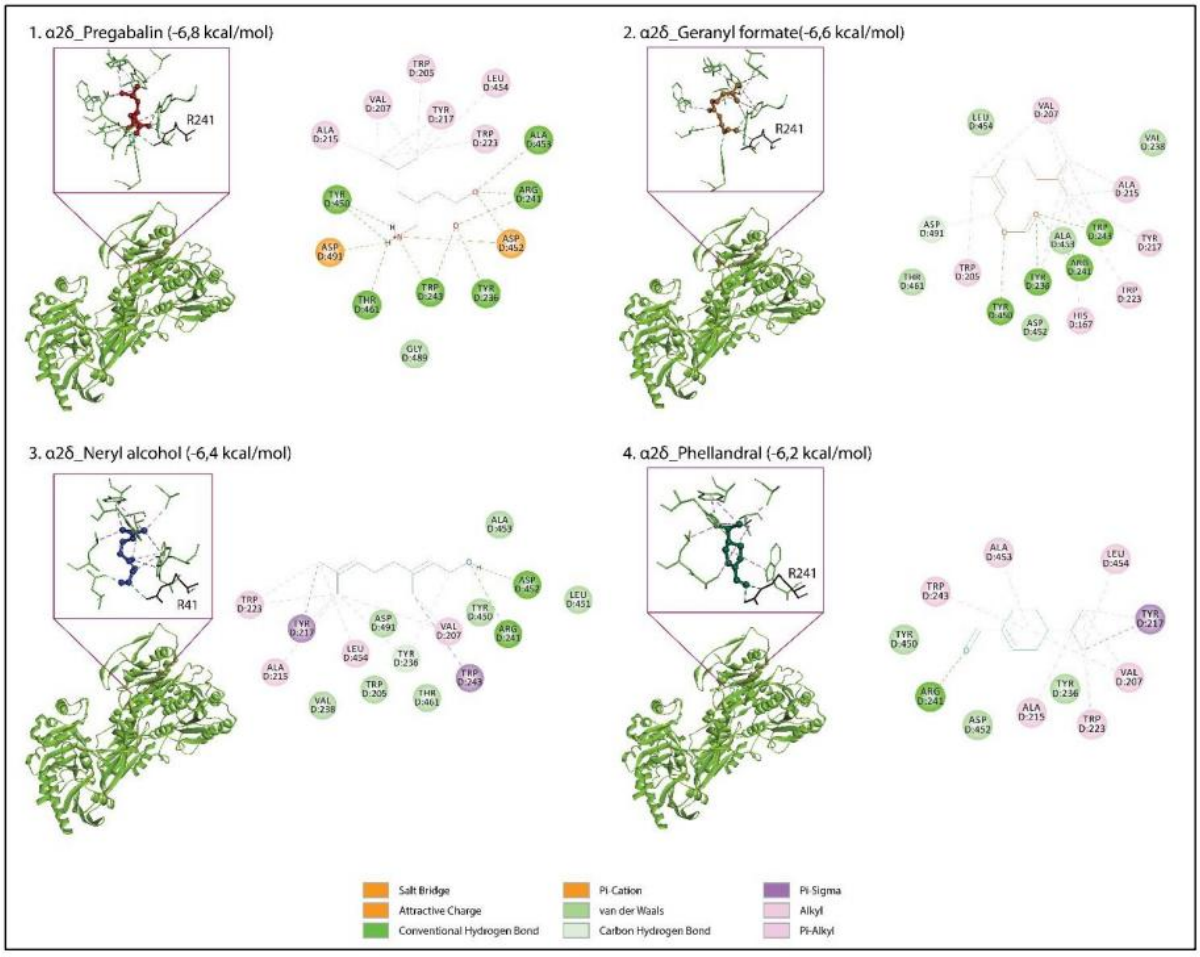

Figure 2. Molecular Interaction between α2δ subunit and pregabalin, geranyl formate, neryl alcohol, and phellandral

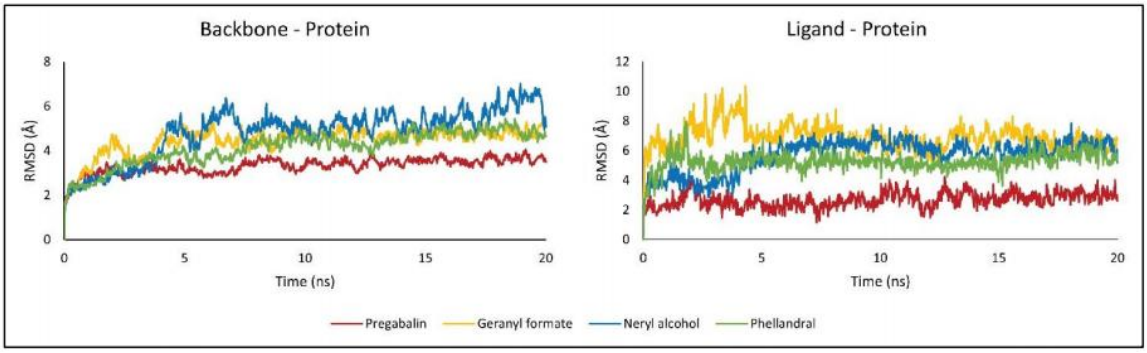

Figure 3. Molecular dynamic results showing RMSD for ligand and backbone after superimposed to protein

Discussion

Based on studies by Nasca et. al. (2012), increased expression of α2δ especially α2δ-1 subunit VGCC around the amygdala complex always associated with anxiety. The α2δ subunit overexpression of VGCC increases intracellular calcium influx through electrochemistry gradient and involves SNARE complex proteins to release neurotransmitter on presynaptic membrane. Induction of action potential stimulus caused by hyperexcitation of glutamate neurotransmitter in certain areas such as hippocampus, BLA, and PFC induces anxiety symptoms (Antonucci, et al., 2016; Dolphin, 2016). VGCC consist of several subunit including α1, α2δ, and β. Calcium channel α1 subunit bound to auxiliary α2δ subunit to enhance calcium channel membrane stability and modulating channel kinetics therefore increases exocytosis. Interactions between those two proteins are formed on the extracellular loops of domain I-III α1 subunit with area von-Willebrand homology A (VWA) domain as well as metal ion-dependent adhesion site (MIDAS) motif of α2δ subunit (Dolphin, 2016; Taylor & Harris, 2020). VWA is a highly conserved domain within α2δ which critical to help α1 trafficking as well as VGCC abundance to active zone on presynaptic terminal. Therefore, MIDAS motif within VWA causes robust intracellular change in response to gabapentinoid family binding targeting α2δ-1 subunit (Taylor, et al., 2007; Hoppa, et al., 2012). The presence of two calcium channel and chemotaxis (Cache I and Cache II) domain on the carboxyl terminal side of VWA domain also speculated to be binding basis of gabapentinoid drugs (Dolphin, 2016; Cui, et al., 2021; Briot, et al., 2016). Pregabalin itself is a compound originated from gabapentinoid family which regularly used as analgesic drugs mediated by inhibition of N-type or P/Q-type VGCC. Clinically, pregabalin is well established and approved to treat nerve pain and epilepsy. Earlier investigation of pregabalin in reducing neurotransmitter release have been demonstrated in vivo and in vitro model of neuronal hyperexcitability. The effect of anxiolytic pregabalin mediated reduction in calcium currents decreased several neurotransmitters implicated in pathological anxiety such as glutamate and monoamine in animal models of anxiety, Vogel conflict test (Mico´ & Prieto, 2012).

Here we demonstrate that binding of geranyl formate, neryl alcohol, and phellandral to Arg217 of α2δ subunit VGCC is the most favorable pose of three ligands, represented from negative charge of oxygen atoms in ligands pointing to positive charge on Arginine residue forming hydrogen bonds. Other hydrogen bonds including Tyr236, Trp243, and Asp452 are also common interaction between the three ligands and α2δ subunit VGCC. Both Tyr236 and Trp243 are located between VWA and Cache 1 domains, while Trp243 on Cache 1 domain. Previous experimental studies have demonstrated that Arg217 (Arg 241 on PDB: 7MIY) located between VWA and Cache 1 domains is a crucial residue for pharmacological action of pregabalin to inhibit α2δ subunit. When this residue mutated to alanine (R217A), it reduces anxiolytic and analgesic effect of pregabalin. This mutation also produces smaller enhancement of calcium currents on N-type VGCC (Field, et al., 2006). Binding disruption of certain compounds to this residue presumably causes a large conformational change that interfere its interaction with α1 subunit. Moreover, it is necessary to remind that arginine residue has an essential role as carrier of voltage-dependent gating charge (Taylor & Harris, 2020; Meneses, et. al., 2021).

This study discovers detailed molecular mechanism of geranyl formate, neryl alcohol, and phellandral as Lavender small molecules to reduce anxiety. Among the three compounds, neryl alcohol (nerol) and phellandral (phellandrene) were known to have a physiological function. Both compounds are terpenoid family that can be extracted as secondary metabolites from several fragrant plants such as lavender (*Lavandula angustifolia*), bitter orange (*Citrus aurentium*), cinnamon (*Cinnamomum verum*), and *Eucalyptus*. In α-phellandrene form, this compound exhibits antinociceptive and anti-tumor effects in cancer animal model (Pires, et al., 2022). This effect also contributes to suppress synaptic excitatory neurotransmitter release including glutamate, serotonin, dopamine, and acetylcholine (Pinheiro-Neto, et al., 2021). Like phellandrene, nerol in neroli oil form can be used to relieve pain and anxiety. Randomized clinical trial to anxiety presented by pregnant women compared with childbirth moment after neroli oil inhalation showed a reduction in anxiety levels. Neroli oil is frequently used for medicinal purposes to treat tachycardia, rheumatism, and reducing central nervous system disorders (Borba, et al., 2021; Scandurra, et al., 2022). Toxtree evaluation showed that neryl alcohol belong to Class I classification of cramer decision tree, indicating that the compound was simple shemical structures and efficient metabolism modes, suggesting low toxicity properties identified. This class also include normal constituent of common terpenes (EFSA Scientific Committee et al., 2019). Exploring the voltage gated calcium channel subunits as potential target and developing these compounds as anxiolytic agents will be important research to find natural sources as anti-anxiety and pain-reducing drugs.

This study predicted that Lavender essential oil small molecules including geranyl formate, neryl alcohol, and phellandral had anxiolytic properties similarly to pregabalin by inhibit the α2δ subunit VGCC. Our findings discover the molecular mechanism of these compounds to exerts its beneficial function to reduce anxiety and pain. However, in vitro studies still required for further investigation of these compounds as potent anxiolytic therapeutic.

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