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In Silico Analysis of Potential Nicotine Addiction Treatment by Cinnamomum verum Phytochemicals against nAChRα3 and nAChRα7

Zefo Kiyosi Wibowo[1], Athaya Syahira Ramadhani[1], Muhammad Auliya Rachman[1], Siti Khaerunnisa[2*]

[1]Faculty of Medicine (60132), Universitas Airlangga, Surabaya, Indonesia
[2]Department of Physiology and Medical Biochemistry,
Faculty of Medicine (60132), Universitas Airlangga, Surabaya, Indonesia
E-mail: zefo.kiyosi.wibowo-2018@fk.unair.ac.id;
athaya.syahira.ramadhani-2018@fk.unair.ac.id;
muhammad.auliya.rachman-2018@fk.unair.ac.id; st.khaerunnisa@fk.unair.ac.id

*Corresponding author details: Siti Khaerunnisa; st.khaerunnisa@fk.unair.ac.id

ABSTRACT

Nicotine addiction has a direct effect on the occurrence of smoking-related diseases. The receptor that can increase absorption is the neuronal acetylcholine receptor alpha-3 (nAChRα3) and alpha-7 (nAChRα7). This in silico study was conducted to determine the effect of *Cinnamomum verum* to overcome nicotine addiction by inhibiting the target protein nAChRα3 and nAChRα7. Two hundred and eighty-one phytochemicals *Cinnamomum verum* were screened into nine ligands by Swiss Adme and PyRx. Molecular visualization and docking analyzes were performed using Avogadro, AutoDock 4.2., and Biovia Discovery Studio 2016. The docking results showed that 2 of 9 ligands in 4zk4 and 6 of 9 ligands in 3sq9 had hydrogen bonds. Sesquiterpenes is the compound with the highest binding affinity in two proteins. However, in 4zk4, the highest affinity with H-bond is linalool. Phenols is the second ligand that effectively binds amino acids. Phytochemicals in *Cinnamomum verum* potentially reduce addiction to nicotine by inhibiting the receptor and improving the neuroinflammation due to nicotine. The sesquiterpenes is the primary ligand that binds to the 4zk4 and 3sq9.

Keywords: in silico; *Cinnamomum verum*; nicotine addiction; nAChRα; sesquiterpenes

INTRODUCTION

Tobacco use has caused as many as 7 million deaths worldwide.[1] Indonesia is a country in Southeast Asia with the highest prevalence of smokers based on data from the Southeast Asia Tobacco Control Alliance (SEATCA) in 2018.[2] Based on data from the WHO, as many as 225,700 people died from smoking or other diseases related to smoking.[3] Smoking can cause several diseases such as heart disease, stroke, diabetes, and COPD and increase the risk of developing tuberculosis, eye diseases, and some diseases that attack the immune system.[4] Diseases related to smoking result from exposure to toxins in cigarettes; nicotine addiction directly influences smoking-related diseases.[5]

The mechanism of action of nicotine is through three pathways, including ganglion transmission, nicotinic acetylcholine receptors (nAChRs) on chromaffin cells through catecholamines, the central nervous system nAChRs by stimulation.[6] The direct effects of nicotine use include irritation, burning sensation in the mouth and throat, increased saliva secretion, nausea and vomiting, stomach pain, and diarrhea.[7] Another effect of nicotine is driving cancer cell growth because it can trigger tumorigenesis by increasing cell proliferation, angiogenesis, and apoptotic pathways.[8] Nicotine is the primary substance that causes addiction in smokers. [6] Nicotine interacts with nicotinic acetylcholine receptors, which causes dopaminergic transmission, and causes an increase in mood and cognitive function. [6]

One of the receptors that can increase absorption is the neuronal acetylcholine receptor alpha-3 (nAChRα3). The binding of nicotine initiates nicotine addiction to nAChRs. This interaction increases dopamine (DA) release in the mesolimbic and mesocortical dopaminergic circuits.[9] The nicotinic acetylcholine receptor is a ligand ion channel activated by acetylcholine and nicotine, which mediates synaptic transmission in the brain and various functions in the periphery. [10] Subtype 3 is well expressed in the habenula-interpeduncular midbrain pathway (aversion pathway). In the brain basal ganglia, a different regulatory variant characterized by rs1948 increases the expression of nAChRα3 mRNA (nAChRα3 enhancer), suggesting additional regulation in brain regions associated with nicotine dependence.[11] In addition, nAChRα3 is also expressed in the brainstem, cerebellum, spinal cord, substantia nigra, medial habenula, pineal gland, hippocampus, cortex, thalamus, ventral tegmental area, and interpeduncular nucleus.[9] Association of variants of the nAChRαx3 gene cluster usually with nAChRα5 and nAChRB4 due to various nicotine-related behaviors. This receptor has a role in increasing susceptibility to tobacco dependence, namely nicotine and smoking-related diseases, especially lung cancer.[12]

Another receptor that plays a role in overcoming nicotine addiction is nAChRα7. The nAChRα7 receptor is associated with nicotine addiction therapy and is a receptor target for varenicline treatment. [13]

The nAChRα7 receptor causes complaints of dizziness when nicotine is inhaled for the first time. This receptor is strongly associated with smokers who have schizophrenia because smokers with schizophrenia will extract more nicotine.[14] Reducing the nAChRα7 receptor in people with schizophrenia will lead to an increase in nicotine use.

Cinnamomum verum has several roles such as antioxidant, antibacterial, antifungal, antiviral, antiulcer, antilipidemic, anticancer, antipyretic, antiplatelet, antiallergic, antihypertensive, insecticidal, nematicidal, antidiabetic, and anesthetic activity. Besides that, *Cinnamomum verum* also plays a role in several health problems such as flatulence, diarrhea, amenorrhea, toothache, fever, headache, leucorrhea, dizziness, and can control blood pressure.[15] Previous studies have found that cinnamon and ginger have a synergistic effect to enhance the development of nicotine in the prefrontal cortex through antioxidant effects, anti-inflammatory and neuroprotective.[16]

Thus, to reduce the rate of addiction in cigarette consumption, it is necessary to control the nicotine receptor. Our in silico study was conducted to determine the effect of *Cinnamomum verum* as a potential treatment for nicotine addiction by inhibiting the target protein nAChRα3 and nAChRα7, which acts as a nicotine receptor.

MATERIALS AND METHODS

• System Configuration

This study uses a Windows 10 laptop using an Intel Core i5-8265U CPU @ 1.60GHz 1.80 GHz and 4 GB of RAM. Applications in silico research include OpenBabel, PyRx, Avogadro, Autodock 4.2, Command Prompt, and Biovia discovery studio 2016.

• Protein Selection

We used two target proteins for this study, nAChRα3 (Cholinergic Receptor Nicotinic Alpha 3 Subunit) and nAChRα7 (Cholinergic Receptor Nicotinic Alpha 7 Subunit). They have different roles and locations for inhibiting the absorption of nicotine. The proteins were obtained from RCSB PDB using the ID of PDB, namely 4zk4 (nAChRα3) and 3sq9 (nAChRα7). Determination of protein structure was using the principle of X-ray crystallography which had a diffraction resolution of less than 2.00. The nAChRα7 had a resolution more than 2.00, so the selection by structure identification did not bond with other agonists. The proteins were searched for the native ligand by data from RCS PDB. The Biovia Discovery Studio could confirm visualization of the native ligand and the active site.

• Phytochemical Selection

Phytochemicals in *Cinnamomum verum* can be found at dr. Duke's Phytochemical and Ethno-botanical database (phytochem.nal.usda.gov/phytochem/ search). The 3D structure of the phytochemicals was obtained from PubChem (pubchem.ncbi.nlm.nih.gov) in .sdf format.

Canonical smiles from the compound were obtained from Pubchem to determine whether the compound met the Lipinski rule of five criteria. This selection was carried out using Swiss ADME. Phytochemicals in *Cinnamomum verum* were selected using PyRx, searched for a small binding affinity, and selected the 9 best compounds included with the criteria.

• Docking and Visualization

Protein preparation using Autodock 4.2 application by removing water and separating it from the original ligand. Both 4zk4 protein and 3sq9 with native ligand were optimized by adding polar hydrogen only, then non-polar coupling and adding Kollman charges for protein and computing Gasteiger for native ligand. The position of the native ligand at the binding site is determined by setting the size of the XYZ box with the center coordinate (X, Y, Z). The grid is for 4zk4 set as follows: 30x24x20 (XYZ) with center coordinates, 26.537; -31.817; 48.378. Furthermore, 3sq9 has a grid set like this: 30x20x20 (XYZ) with center coordinates, 14.177; 29.075; -11.236. All of the protein grids are running with a spacing of 0.375

The optimization of the ligands was carried out using the Avogadro and Autodock 4.2 applications. The optimization stages of the ligands were the same as the optimization of the native ligands but without adding Kollman charges. Ligands that have been positioned at the binding site of the native ligand are stored in a .gpf file and processed on the grid. The docking process starts after everything is ready by inputting the ready ligands. Furthermore, proteins can be inputted for later integration of the two. So, the docking process begins with selecting proteins as rigid materials and compounds as ligands. Then the last step is to create Lamarckian GA output and save it in dpf format. Docking starts after the code is entered in the Command Prompt.

Visualization using Biovia discovery studio 2016. The stage begins with opening the docked file in .pdb format. Furthermore, it was observed that the bond between the ligand and protein was observed using show ligand binding interaction. *Show 2D diagrams* can be used to clearly see the bonds between ligands and amino acids and the types of bonds in two dimensions.

• Analyzing

Analysis of docking results can be done in Autodock 4.2 by inputting docking results in. dlg format. Furthermore, an analysis is carried out to determine the *binding energy* and other bond numbers. However, in Autodock 4.2, the interaction between ligands and amino acids is not visible, so visualization of other applications is needed. After getting the visual, each result, both pictures and interaction strengths, is summarized in one table.

RESULTS AND DISCUSSIONS

TABLE 1: Characteristics of ligands

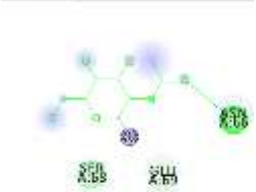
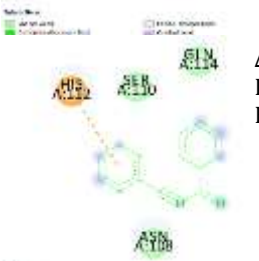
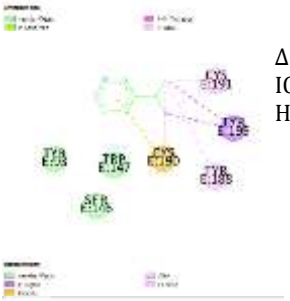
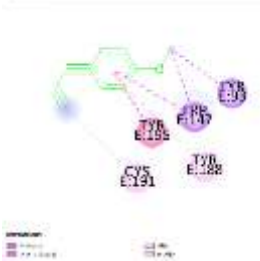
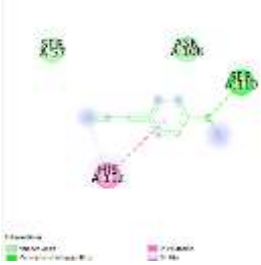
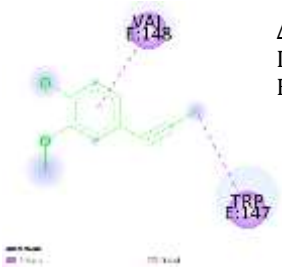
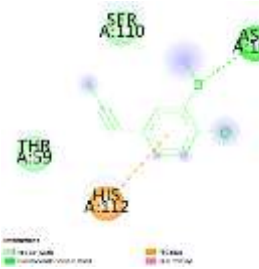
NO	LIGAND	CID	MOLECU-LAR FORMULA	MOLECULAR WEIGHT	H-BOND ACCEPTOR	H-BOND DONOR	LOG P	VIOLATION	TPSA	GI ABSORPTION	BBB PERMEANT
1	BENZYL BENZOATE	2345	$C_{14}H_{12}O_2$	212.24	2	0	3.25	0	26.3	High	Yes
2	CUMENE	7406	C_9H_{12} or $C_6H_5CH(CH_3)_2$	120,19	0	0	3,14	1	0,00	Low	Yes
3	ESTRAGOLE	8815	$C_{10}H_{12}O$	148.2	1	0	2,78	0	9.23	High	Yes
4	EUGENOL	3314	$C_{10}H_{12}O_2$	164.2	2	1	2,25	0	29.46	High	Yes

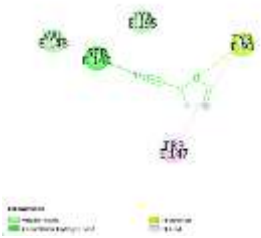
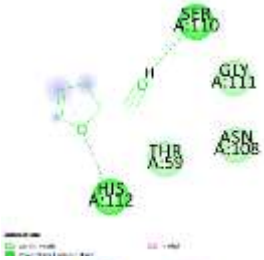
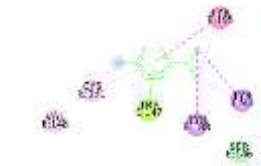
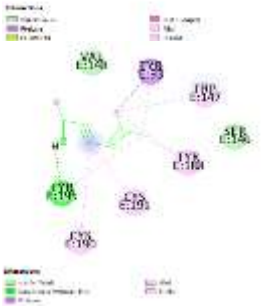
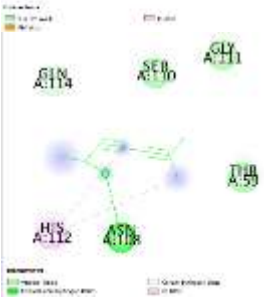
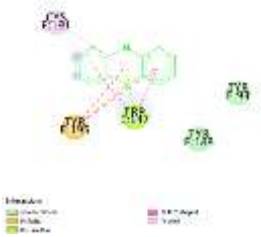
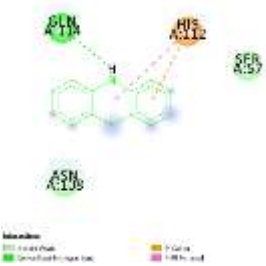
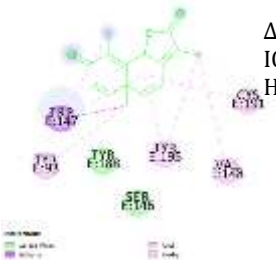
NO	LIGAND	CID	MOLECULAR FORMULA	MOLECULAR WEIGHT	H-BOND ACCEPTOR	H-BOND DONOR	LOG P	VIOLATION	TPSA	GI ABSORPTION	BBB PERMEANT
5	FURFUROL	7361	$C_5H_6O_2$	98.1	2	1	0,62	0	33.37	High	Yes
6	LIMONENE	22311	$C_{10}H_{16}$	136.23	0	0	3.37	0	0.00	Low	Yes
7	LINALOOL	6549	$C_{10}H_{18}O$ or $(CH_3)_2C=CH(CH_2)_2C(CH_3)(OH)CH=CH_2$	154.25	1	1	2.66	0	20.23	High	Yes
8	PHENOLS	7108	$C_{12}H_9NS$	199.27	0	1	3.32	0	37.33	High	Yes
9	SESQUITERPENES	667450	$C_{15}H_{18}O_3$	246.30	3	0	2.38	0	43.37	High	Yes

The nine best compounds were analyzed for Lipinski rules and other characteristics. All ligands are qualified Lipinski rules, and only cumene has one violation.

If the ligands are taken orally, the seven ligands have high GI absorption. All ligands are identified that have penetrated through BBB to be absorbed in the brain for addiction reduction.

TABLE 2: The binding interaction ligands and proteins

	4zk4 (nAChRα3)	**3sq9 (nAChRα7)**
Native Ligand (TII & NAG)	 ΔG: -6.23 kcal/mol IC: 27.07 mM H-bond: 2	 ΔG: -0.93 kcal/mol IC: 206.75 mM H-bond :1
CID 2345	 ΔG: -5.57 kcal/mol IC: 82.74 mM H-bond: -	 ΔG: -1.59 kcal/mol IC: 68.36 mM H-bond: -
CID 2406	 ΔG: -4.51 kcal/mol IC: 491.74 mM H-bond: -	 ΔG: -2.02 kcal/mol IC: 32.81 mM H-bond: -
CID 8815	 ΔG: -4.14 kcal/mol IC: 925.17 mM H-bond: -	 ΔG: -1.75 kcal/mol IC: 52.04 mM H-bond :1
CID 3314	 ΔG: -1.29 kcal/mol IC: 114.05 mM H-bond: -	 ΔG: -1.71 kcal/mol IC: 55.49 mM H-bond :1

	4zk4 (nAChRα3)	3sq9 (nAChRα7)
CID 7361	 ΔG: -2.7 kcal/mol IC: 10.57 mM H-bond :1	 ΔG: -1.76 kcal/mol IC: 51.04 mM H-bond :2
CID 22311	 ΔG: -4.86 kcal/mol IC: 272.08 mM H-bond: -	 ΔG: -2.06 kcal/mol IC: 30.92 mM H-bond: -
CID 6549	 ΔG: -3.62 kcal/mol IC: 2.24 mM H-bond: 1	 ΔG: -1.44 kcal/mol IC: 87.29 mM H-bond :1
CID 7108	 ΔG: -5.78 kcal/mol IC: 58.12 mM H-bond: -	 ΔG: -2.48 kcal/mol IC: 15.11 mM H-bond :1
CID 667450	 ΔG: -6.98 kcal/mol IC: 7.6 mM H-bond: -	 ΔG: -3.42 kcal/mol IC: 3.11 mM H-bond :2

The ligands were tried to bind with two protein targets, namely 4zk4 and 3sq9. The docking process determines the strength of the affinity between the ligand and protein. The native ligand 4zk4, namely TII has an association with 4zk4 (ΔG: -6.23 kcal/mol), and there were two hydrogen bonds (H-bond) to the serine and tyrosine. Only one ligand exceeds the binding affinity of the native ligand, namely sesquiterpenes, and this ligand has the highest affinity (ΔG: -6.98 kcal/mol). However, sesquiterpenes did not have H-bond, only hydrophobic bonds. The other ligand with low binding affinity is eugenol (ΔG: -1.29 kcal/mol). Only two of nine ligands have H-bond; furfurol (ΔG: -2.7 kcal/mol) and linalool (ΔG: -3.62 kcal/mol).

Linalool with tyrosine has an affinity higher than furfurol with serotonin.

NAG in 3sq9 is a native ligand with an H-bond to asparagine (ΔG: -0.93 kcal/mol). All ligands of *Cinnamomum verum* exceed this binding affinity. The highest strength is in the sesquiterpenes (ΔG: -3.42 kcal/mol), with two H-bonds with asparagine and serotonin. The other with a low affinity between ligand and 3sq9 is linalool (ΔG: -1.44 kcal/mol). Six ligands have hydrogen bonds; two of them have two hydrogen bonds. Fufurol binds with serotonin and histidine (ΔG: -1.76 kcal/mol); other ligands are sesquiterpenes, including this.

TABLE 3: The binding affinity and amino acids site

No	Ligands	4zk4 Binding Affinity (kcal/mol)	3sq9 Binding Affinity (kcal/mol)	Amino acids in binding site (4zk4) (3sq9)
1	CID 2345	-5.57	-1.59	CYS E:190, TYR E:188, TYR E:93, SER R:146, TYR E:195, TRP E:147, VAL E:148, CYS E:191 ASN A:108, GLN A:114, SER A:110, HIS A:112
2	CID 2406	-4.51	-2.02	TYR E:93, SER E:146, TYR E:188, CYS E:190, TRP:147, VAL E:148, CYS E:191, TYR E:195, VAL E:148 GLN A:114, ASN A:108, HIS A:112
3	CID 8815	-4.14	-1.75	TYR E:93, TRP E:147, TYR E:195, CYS E:191, TYR E:188 SER A:110, SER A:57, ASN A:108, HIS A:112
4	CID 3314	-1.29	-1.71	VAL E:148, TRP E:147 ASN A:108, SER A:110, THRR A:59, HIS A:112
5	CID 7361	-2.7	-1.76	SER E:146, VAL E:148, TYR 3E:195, TYR E: 93, TRP E:147 SER A:110, HIS A:112, GLY A:111, THR A:59, ASN A:108
6	CID 22311	-4.86	-2.06	SER E:146, TRP E:147, VAL E:148, CYS E:191, TYR E:188, TYR E:93, TYR E:195 GLN A:114, ASN A:108, HIS A:112
7	CID 6549	-3.62	-1.44	TYR E:195, SER E:146, VAL E: 148, CYS E:190, CYS E:191, TYR E:188, TRP E:147, TYR E:93 ASN A:108, THR A:59, GLY A:111, SER A:110, GLN A;114, HUS A:112
8	CID 7108	-5.78	-2.48	TYR E:93, TYR E: 188, TRP E:147, TYR E:195, CYS E:191 GLN A:114, HUS A:112, SERA:57, ASN A:108
9	CID 667450	-6.98	-3.42	TRP E:147, TYR E:93, TYR E:188, TYR E: 195, VAL E:148, CYS E:191, SER E:146 ASN A:108, SER A:110, HUS A:112, GLN A:114

We analyzed the binding affinity between *Cinnamomum verum* and two protein targets; the sesquiterpenes was the highest strength (Table 3). However, only sesquiterpenes in 3sq9 have H-bond. The second one is phenols with high affinity in both proteins, but only in 3sq9 has H-bond. The high affinity in 4zk4 that has 4zk4 is linalool. The dominant amino acids in their binding are tyrosine, serotonin, and asparagine. The other amino acids have a hydrophobic bond with ligands.

Nicotine plays a role in providing comfort due to an increase in dopamine (DA) release in the mesolimbic and mesocortical dopaminergic circuits resulting in addiction. [9] Relevant to previous studies that tyrosine in nAChRα3 is a precursor for the production of dopamine which plays a role in the work of addictive substances in the body.[17] The sensitivity of serotonin was affected by nicotine use, and this amino acid can be targeted to reduce the absorption of nicotine.[18] Asparagine had potentially related to tobacco addiction.[19]

Sesquiterpenes had a potential inhibition activity for Acetylcholinesterase (AChE). [20] The binding activity with active site amino acids was able to form non-polar solid contacts. [21] The effect could improve Aβ plaque, neuron excitability, and oxidative stress, which had a function to alleviate neuroinflammation.[22] This role effectively improves inflammation due to nicotine, and the other side could slowly reduce the addiction.[23] Coherent with this study, sesquiterpenes had a potential reduced nicotine reward by different variants.[24]

The second high binding affinity for 3sq9 was phenols, which reduced the oxidative stress burden and played a neuroprotective agent.[25] Coherent with our finding, the history as a psychotic drug, phenols inhibit α7-nicotinic acetylcholine receptors as therapeutic targets (nAChRα7).[26] Optimizing phenols potencies for new derivatives can lead to a new therapy for nicotine addiction.[27]

Other results also show that linalool is a potent inhibitor of neuronal nAChR. The homology of this finding is still considering the co-use of many allosteric modulators between muscle type and neuronal nAChR. [28] Mainly, the herbs contained in linalool can inhibit Ach release (a presynaptic mechanism) and nAchRs (a postsynaptic mechanism). [29] Linalool can be the therapy of choice to reduce the effects of nicotine as an addictive substance.[30] However, further research on the mechanism of action of linalool is still needed.

CONCLUSIONS

Sesquiterpenes are the primary substance in *Cinnamomum verum* that potentially inhibits nAChRα3 (4zk4) and nAChRα7 (3sq9) as a role receptor of nicotine. Phenols and linalool are the other ligands that have high affinity also. *Cinnamomum verum* is a candidate herbal for reducing nicotine addiction based on this in silico study.

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