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Quality analysis of citronella oil and in silico study of metabolites on nAChR

Stefany Halim, Marisca Evalina Gondokesumo* and Azminah

Faculty of Pharmacy, University of Surabaya, Universitas Surabaya, Surabaya, Indonesia

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ABSTRACT

Cigarettes are a product that contains thousands of dangerous chemicals that can disrupt the function of body organs and even threaten life. Nicotine is the most dangerous compound of cigarettes due to its addictive effect, and one way to reduce nicotine dependence is to use citronella oil. This study began with a quality test of citronella oil from Jatijejer Village using Thin Layer Chromatography (TLC), Stahl Distillation, and Gas Chromatography-Mass Spectrometry (GC-MS) instruments. Next, an *in silico* study was conducted to assess drug-likeness, bond energy, and bond interactions. The results of the quality test showed that citronella oil was proven to have good quality, and there were 5 spots indicating the presence of essential oils through TLC testing with a total essential oil content of 0.82% w/v, which was determined by the Stahl distillation method. The chemical content of citronella oil from Jatijejer Village has a content profile and chromatogram pattern that is quite similar to PT.N, as proven by GC-MS testing. The results of *in silico* testing show that one of the chemical contents, namely Geraniol (−6.3 kcal/mol), has a lower binding energy value (ΔG) than nicotine and can bind to important amino acids, so that it has the potential to replace nicotine. Based on the results of the drug-likeness analysis, the compound has also met Lipinski's rule of five and Veber's rule but does not meet the Ghose Filter Law. Meanwhile, based on the interaction, the chemical content of (1R)-cis-Verbenol and Geranial dimethyl acetal has the most appropriate %similarity to nicotine, and the chemical content of Chavibetol has the most appropriate %similarity to varenicline.

KEYWORDS

bond energy, bond interaction, citronella oil, Jatijejer Village, nAChR α4β2

INTRODUCTION

Cigarettes are tobacco-based processed products that may come in forms such as cigars or other variations, derived from the *Nicotiana tabacum* plant and other species or their synthetic forms. A single cigarette contains about 4,000 chemical compounds, 400 harmful substances, and 43 carcinogenic agents [1]. The most dangerous component of cigarettes, due to its addictive effects, is nicotine. According to the 2023 World Health Organization (WHO) report, more than 7 million deaths were caused by direct tobacco use among active smokers, while approximately 1.3 million deaths occurred due to exposure to secondhand smoke. Based on data from the Indonesian Central Statistics Agency (Badan Pusat Statistik, BPS) in 2023, 28.62% of Indonesians aged 15 years and older were cigarette users. Moreover, the prevalence of smoking in rural areas reached 31.09%, which was higher than in urban areas at 26.87%.

Cigarette smoking can cause various health problems, including cancer, coronary heart disease, cerebrovascular disease, heart attacks, impotence, pregnancy complications, fetal disorders, and even death [2]. These detrimental effects have led to the development of various pharmacotherapies to help individuals quit smoking, which can be achieved through pharmacological and non-pharmacological approaches. Pharmacologically, two main types of drugs have been approved by the U.S. Food and Drug Administration (FDA)

*Corresponding author.
Tel.: +62 878-5136-7988.
E-mail: marisca@staff.ubaya.ac.id

to reduce nicotine dependence—nicotine replacement therapy (NRT) and non-nicotine therapies (varenicline and bupropion) [3]. One of the commonly used non-pharmacological therapies involves essential oils. Indonesia, being an agrarian country, has abundant agricultural commodities [4]. One of the agricultural regions rich in such resources is Jatijejer Village, where one of the flagship crops is citronella (*Cymbopogon nardus*).

The main chemical constituents of citronella essential oil, as identified using Gas Chromatography-Mass Spectrometry (GC-MS), are citronellal (24.39%), geraniol (21.45%), and citronellol (15.11%) [5]. Geraniol acts as a GABA(_A) receptor agonist with neuroprotective effects by inhibiting glutamatergic receptors, blocking voltage-gated Na^+ channels, activating potassium channels, and attenuating neuronal inflammation and oxidative stress [6]. Meanwhile, citronellal can activate dopamine D2 receptors located in the striatum, nucleus accumbens, and olfactory tubercle, which play a role in regulating mood, emotional stability, learning, movement, and memory [7]. Therefore, citronella oil has potential as an alternative therapy for nicotine addiction.

Based on the above background, this study will examine the quality of citronella essential oil using Thin Layer Chromatography (TLC), content determination using the Stahl distillation method, and GC-MS. In addition, an *in silico* study will be conducted to evaluate drug-likeness, binding energy, and molecular interactions through molecular docking of the best secondary metabolites from citronella oil with the nAChR α4β2 receptor (PDB ID: 5KXI).

EXPERIMENTAL

Sample preparation

Citronella (*Cymbopogon nardus* (L.) Rendle) was collected from Jatijejer Village, Trawas, Mojokerto, East Java, and was identified by the Center for Information and Development of Traditional Medicines (PIPOT), University of Surabaya follow World Flora Online http://www.worldfloraonline.org/taxon/wfo-0000861056.

Citronella oil destillation

Essential oil extraction was carried out using steam distillation, with water distillation used as a comparison. A sample of citronella oil from PT. N was also used as a reference. The citronella leaves were cut into pieces of 1–2 cm in length, dried, and then subjected to steam distillation. The distillation flask was filled with 120 g of citronella simplicia, while the water flask was filled with distilled water according to its capacity. Steam distillation was then performed for 5–6 h until the essential oil was obtained, after which the oil phase was collected in a container. If water was present, it was separated using a separating funnel, and anhydrous Na_2SO_4 was added to bind the remaining moisture [8].

Determination of essential oil content test

The determination of essential oil content was carried out using the Stahl distillation method based on the guidelines of the Indonesian Ministry of Health [1]. A total of 50 g of citronella powder was weighed and placed into a round-bottom flask, followed by the addition of 400 mL of distilled water. The flask was connected to a condenser and a graduated burette, then heated using a heating mantle. After distillation was completed, the volume of the essential oil was recorded from the burette, and the essential oil content was calculated using the following formula:

$$\text{Total essential oil content} = \text{Volume of essential oil} / \text{Weight of dried powder x } 100\%$$

Thin Layer Chromatography (TLC) test

The quality test of citronella essential oil was carried out using Thin Layer Chromatography (TLC) [9]. The chamber was prepared with a mobile phase of toluene and acetone (9:1), and a filter paper was placed inside to allow saturation. The TLC plate was then spotted with the test solution and the reference solution using a capillary tube, keeping a distance of 2.5 cm from the lower edge of the plate and an appropriate distance between spots, and allowed to dry. The plate was then placed vertically in the chamber and developed until the solvent front reached the upper limit mark. After development, the plate was dried and observed under visible light, short-wave ultraviolet (254 nm), and long-wave ultraviolet (366 nm). A positive result was indicated by a blue spot. The plate was then sprayed with anisaldehyde–concentrated H_2SO_4 reagent and heated in an oven at 100 °C for 5–10 min. The plate was then observed under visible light, where a positive result was indicated by a reddish-brown color. The distance of the spot from the application point was measured and recorded, and the Rf value was calculated using the formula:

$$\text{Rf} = \text{distance traveled by analyte} / \text{distance traveled by solvent front}$$

Gas Chromatography–Mass Spectrometry (GC-MS) test

The chemical composition of citronella essential oil was analyzed using Gas Chromatography–Mass Spectrometry (GC-MS). Before analysis, the column, injector, and detector were equilibrated with carrier gas flow until a constant signal was obtained. The sample was then injected through a septum or autosampler. Helium gas was used as the carrier gas. The column temperature was programmed from 80 to 200 °C (with a 4 °C/min increase), then from 200 to 240 °C (with a 10 °C/min increase), while the injector temperature was set at 250 °C. The mass spectrometer was operated using the electron impact ionization method (70 eV), with a scan time of 0.3 s and a mass range of 35–500 *m*/*z* [10]. After all parameters were set and the

system was ready, the analysis was performed, and the resulting chromatogram was analyzed. The identification of chemical constituents was conducted by comparing the obtained mass spectra with those in the NIST mass spectral library.

Molecular docking test

The *in silico* activity test of citronella oil was performed using molecular docking to analyze binding energy, interaction similarity (with nicotine and reference drugs), and anti-nicotine activity toward nAChR α4β2 (PDB ID: 5KXI). The molecular docking procedure began with the collection of secondary metabolite data from citronella oil through literature review and GC-MS results. The 1D structures (SMILES) of each metabolite were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/), and if unavailable, the structures were drawn using MarvinSketch version 23.16 and saved in *.mol* format. The structures were optimized by energy minimization using the Merck Molecular Force Field 94 (MMFF94) in Q-VS.exe, and the lowest-energy conformation was saved in *.pdbqt* format. The target receptor was downloaded from the Protein Data Bank (https://www.rcsb.org/structure/) with PDB ID 5KXI and processed using AutoDock Tools 1.5.7 to separate the receptor and ligand. Docking method validation was performed using Command Prompt by comparing the native ligand and redocked ligand based on the Root Mean Square Deviation (RMSD) value, which was visualized using BIOVIA Discovery Studio Visualizer 2024. The validation results obtained showed an RMSD value of 0.6211 Å with a binding energy (ΔG) of −6.0 kcal/mol. Molecular docking between secondary metabolites and the receptor was performed via Command Prompt using a grid box size of $16 \times 16 \times 16$ with three replications for each ligand, yielding binding energy (ΔG) values in kcal/mol, where the lowest value was considered the best result. The docking results were then visualized using BIOVIA Discovery Studio Visualizer 2024 to observe the interaction bonds formed between ligands and the receptor [11].

Drug-likeness analysis

The drug-likeness analysis of citronella oil secondary metabolites was carried out by converting the 3D structure of each metabolite into *.sdf* or *.mol* format, or by using the 1D structure in SMILES form. The SwissADME website was then accessed, and the metabolite file was imported via *Import* → *Choose file* → *Open*, or by pasting the SMILES code into the designated field. The analysis was initiated by clicking *Run* to evaluate the chemical characteristics of the metabolites. The observed parameters included Lipinski's Rule of Five, Veber's Rule, and the Ghose Filter to assess the compounds' suitability as drug candidates. The molecular weight (MW), log P, number of H-bond acceptors (HBA), H-bond donors (HBD), number of rotatable bonds (RB), topological polar surface area (TPSA), molar refractivity (A), and total number of atoms (TNA) were then recorded for further analysis [12].

RESULT AND DISCUSSION

Distillation of Citronella oil

Citronella (*Cymbopogon nardus* (L.) Rendle) was extracted into essential oil using steam distillation and water distillation as a comparison. Steam distillation is a method used to separate mixtures of chemical compounds with boiling points ≥200 °C and is typically applied to substances that are immiscible with water [13]. Therefore, this method was chosen to extract the essential oil from *C. nardus* leaves. The resulting citronella oil had a clear yellow color and an aromatic odor (Fig. 1), which met the quality standards specified in SNI 06-3953-1995. Quality testing of citronella oil was carried out using the Stahl distillation method, which operates similarly to water distillation but includes a burette to determine the oil content. The Stahl distillation in this study was conducted using method I, which excludes xylene. Although xylene can improve measurement accuracy and extraction efficiency [14], it was not used because the oil volume already met the burette's capacity, and the total oil yield satisfied the standards of the *Indonesian Herbal Pharmacopoeia* (2nd Edition). After approximately 3 h of distillation, 0.41 mL of essential oil was obtained, corresponding to a total oil content of 0.82% v/w (Table 1), fulfilling the pharmacopoeial requirements.

Fig. 1. Results of Citronella steam distillation

Table 1. Results of Citronella oil content determination

Volume of essential oil (mL)	Weight of simplicia (g)	Essential oil content (% v/w)
0.41	50.0	0.82

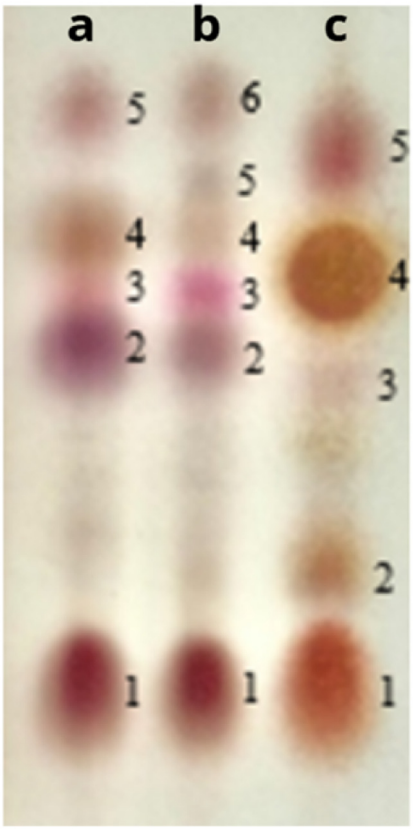


Fig. 2. Result of TLC Citronella Oil
*a: Steam destillation, b: Water destillation, c: Comparison from PT.N

Thin Layer Chromatography profile of Citronella oil

Thin-layer chromatography (TLC) was performed to qualitatively screen the chemical constituents of citronella oil. A TLC result is considered positive for essential oil if, after spraying with anisaldehyde–sulfuric acid reagent and heating at 110 °C for 5–10 min, it produces blue, purple, green, red, or brown spots under visible light [15]. The anisaldehyde–sulfuric acid reagent enhances the detection of terpenoids, steroids, and essential oil components such as alcohols and phenols [15, 16]. Heating the TLC plate is also necessary to remove residual solvent and increase activation energy, making the spots more visible [17].

The citronella oil samples obtained by steam distillation, water distillation, and from PT.N showed positive results for essential oil presence based on the spot colors that appeared after visualization and heating (Fig. 2).

Based on the identification results, the steam-distilled citronella oil sample showed five spots in sequence: red (Rf = 0.2857), purple (Rf = 0.5238), purple (Rf = 0.5595), orange (Rf = 0.6012), and purple (Rf = 0.6845). The water-distilled citronella oil sample showed six spots in sequence: red (Rf = 0.2857), purple (Rf = 0.5238), purple (Rf = 0.5595), orange (Rf = 0.5952), blue (Rf = 0.6309), and purple (Rf = 0.6845). Meanwhile, the citronella oil sample from PT. N also showed five spots in sequence: orange (Rf = 0.2857), orange (Rf = 0.3690), purple (Rf = 0.4940), orange (Rf = 0.5774), and purple (Rf = 0.6548).

The color patterns and Rf values observed indicate the presence of similar compounds among the three samples,

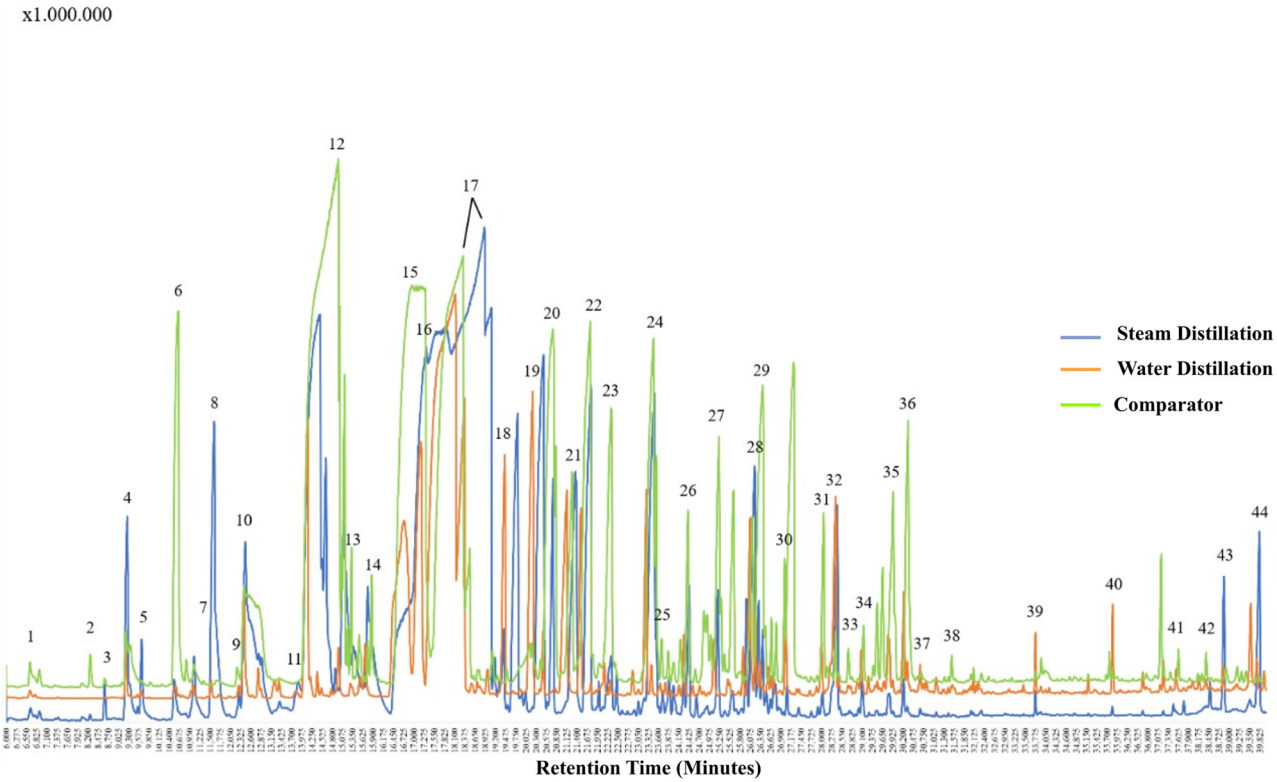


Fig. 3. Chromatogram of citronella oil compound

particularly in the spots with Rf values around 0.52–0.68, which are typically associated with the main components of citronella oil such as citronellal, citronellol, and geraniol. The orange spots with Rf values around 0.28–0.60 are presumed to correspond to terpenoid or aldehyde compounds, while the purple spots suggest the presence of oxidized alcohol compounds (such as geraniol and citronellol). This is in line with other studies that show that HPTLC shows that there is the same Rf as the standard compounds in citronella oil (*C. nardus*), namely citronellal, citral, and geraniol [18].

Secondary metabolite analysis by GC-MS

Chemical profiling of citronella oil was carried out using GC–MS, an analytical technique suitable for separating volatile compounds, making it ideal for essential oil analysis [19]. The analysis produced chromatograms and chemical composition data for each sample (Fig. 3). Differences in chromatogram patterns and chemical profiles were observed among samples, which can be attributed to various factors such as distillation method, plant origin, climate, soil characteristics, nutrients, and land preparation [20].

Several secondary metabolites exhibited prominent peaks at specific retention times, indicating that these metabolites were present in high abundance. These compounds include 6-Methyl-5-hepten-2-one, 4-Nonanone, Linalool, Citronellal, Isocitral, Decanal, (1R)-cis-Verbenol, Geraniol, Geranial dimethyl acetal, 2,6-Dimethyl-2,6-octadiene, Chavibetol, *β*-Elemene, Caryophyllene, Gamma-cadinene, and Caryophyllene oxide. These compounds were subsequently selected for further *in silico* analysis.

However, the steam-distilled citronella oil and the PT.N sample exhibited similar profiles, sharing 23 identical compounds, whereas the water-distilled oil differed significantly in both chemical composition and chromatogram pattern. This difference likely results from the lower pressure and temperature of water distillation, which yields less oil and can alter its chemical profile [21]. Overlaying the chromatograms revealed 44 major chemical constituents across all samples (Table 2).

The analysis identified terpenoid compounds—including monoterpenes, diterpenes, and sesquiterpenes—with monoterpenes and sesquiterpenes being the most abundant. Eight key compounds were consistently detected in all samples: 6-methyl-5-hepten-2-one, linalool, *cis*-rose oxide, citronellal, geraniol, *β*-elemene, caryophyllene oxide, and *α*-cadinol, all belonging to the terpenoid group. Terpenoids are volatile, nonpolar compounds that are easily detected by GC–MS due to their high vapor pressure.

The results of this study are in line with previous studies that analyzed the GC-MS of *C. nardus* essential oil. The study revealed the presence of several constituents, including monoterpenes, diterpenes, sesquiterpenes, and phenolic compounds. The main monoterpenes identified were citronellal (11.35%), z-Citral (11.34%), *β*-Myrcene (6.70%), and *β*-Trans-ocimene (6.03%) [22].

Table 2. Secondary metabolites of Citronella oil

No	Compound	Group
1	1-Hexanol	Alcohol
2	alpha-Pinene	Monoterpenes
3	Camphene	Monoterpenes
4	6-Methyl-5-hepten-2-	Ketones
5	One Octanal	Aldehydes
6	D-Limonene	Monoterpenes
7	beta-cis-Ocimene	Monoterpenes
8	4-Nonanone	Ketones
9	Terpinolene	Monoterpenes
10	Linalool	Monoterpenes
11	cis-Rose oxide	Monoterpenes
12	Citronellal	Monoterpenes
13	Isocitral	Monoterpenes
14	Decanal	Aldehydes
15	Citronellol	Monoterpenes
16	(1R)-cis-Verbenol	Monoterpenes
17	Geraniol	Monoterpenes
18	Geranial dimethyl acetal	Monoterpenes
19	Citronellyl acetate	Monoterpenes
20	2,6-Octadiene, 2,6-dimethyl	Monoterpenes
21	Chavibetol	Sesquiterpenes
22	Geranyl acetate	Monoterpenes
23	β-Elemene	Sesquiterpenes
24	Caryophyllene	Sesquiterpenes
25	(E)-beta-Farnesene	Sesquiterpenes
26	Humulene	Sesquiterpenes
27	β-Copaene-4α-ol	Sesquiterpenes
28	Gamma-cadinene	Sesquiterpenes
29	Delta-cadinene	Sesquiterpenes
30	Elemol	Sesquiterpenes
31	Germacrene D-4-ol	Sesquiterpenes
32	Caryophyllene oxide	Sesquiterpenes
33	Trans-farnesol	Sesquiterpenes
34	Epicubenol	Sesquiterpenes
35	Tau-muurolol	Sesquiterpenes
36	Alpha-cadinol	Sesquiterpenes
37	14-Hydroxycaryophyllene	Sesquiterpenes
38	Farnesol	Sesquiterpenes
39	Neophytadiene	Diterpenes
40	Geranly caprylate	Monoterpenes
41	Citronellyl citronellate	Monoterpenes
42	Trans-geranylgeraniol	Diterpenes
43	5,9,13,17-Tetramethyl 4,8,12, 16-octadecatetraenoic acid	Fatty acids
44	6-Methyl-4,6-bis(4-methylpent-3-en-1-yl) cyclohexa-1,3-dienecarbaldehyde	Aldehydes

Results of molecular docking between Citronella oil compounds and 5KXI receptor

Molecular docking analysis was performed to evaluate the interaction between citronella oil constituents and nicotine at the nicotinic acetylcholine receptor (nAChR) α4β2 (PDB ID: 5KXI). In *in silico* studies, Gibbs free energy (ΔG) reflects the strength and stability of ligand–receptor interactions; a more negative ΔG indicates a stronger and more stable interaction [23]. Fifteen major constituents (with %area >1%) from the steam-distilled citronella oil were

selected for docking analysis, as they represent the dominant compounds [24].

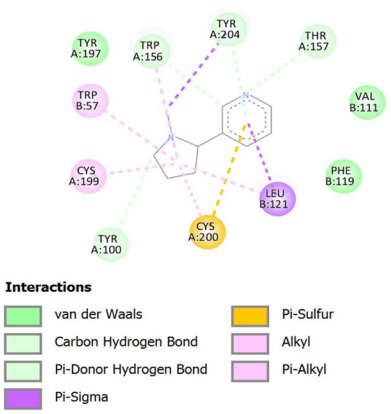
The results showed that the compounds from citronella oil exhibited relatively low binding affinity values against the 5KXI receptor, ranging from −2.4 to −6.3 kcal/mol, when compared with nicotine (−6 kcal/mol) as the native ligand and varenicline (−6.6 kcal/mol) as the reference drug (Table 3). There were six secondary metabolites with binding energy (ΔG) values comparable to or lower than that of nicotine, namely Linalool, Citronellal, Isocitral, Geraniol, Geranial dimethyl acetal, and 2,6-Dimethyl-2,6-octadiene.

Table 3. Binding energy (ΔG) results of the native ligand, reference compound, and Citronella oil secondary metabolites against 5KXI receptor

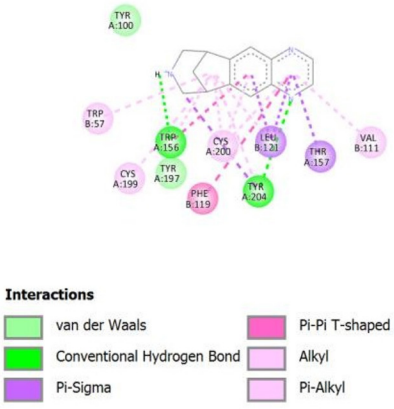
No	Secondary metabolites	Compound of Citronella oil (%)	ΔG (kkal/mol)	Interaction types
	Native Ligand (NCT)	–	−6	van der Waals forces, carbon-hydrogen bonds, π-donor hydrogen bonds, π–sigma, alkyl, π–alkyl, and π–sulfur interactions
	Varenicline (Comparator)	–	−6.6	van der Waals forces, conventional-hydrogen bonds, π–sigma, π-π T-shaped, alkyl, and π–alkyl
1.	6-Methyl-5-hepten-2-one	1.15	−5.6	van der Waals forces, π–sigma, and π–alkyl
2.	4-Nonanone	2.36	−5.7	van der Waals forces, π–sigma, alkyl and π–alkyl
3.	**Linalool***	2.38	−6.1	van der Waals forces, conventional-hydrogen bonds, π–sigma,alkyl, and π–alkyl
4.	**Citronellal***	8.99	−5.9	van der Waals forces, alkyl and π–alkyl
5.	**Isocitral***	2.85	−5.9	van der Waals forces, π–sigma, alkyl and π–alkyl
6.	Decanal	1.76	−5.6	van der Waals forces, π–sigma, alkyl and π–alkyl
7.	(1R)-cis-Verbenol	24.19	−5.3	van der Waals forces, π-donor hydrogen bonds, π–sigma, alkyl, and π–alkyl
8.	**Geraniol***	26.33	−6.3	van der Waals forces, conventional-hydrogen bonds, π–sigma,alkyl, and π–alkyl
9.	**Geranial dimethyl acetal***	2.60	−6.0	van der Waals forces, conventional-hydrogen bonds, carbon-hydrogen bonds, π–sigma, alkyl, and π–alkyl
10.	**2,6-Dimethyl 2,6-octadiene***	4.88	−6.1	van der Waals forces, π–sigma, alkyl and π–alkyl
11.	Chavibetol	2.28	−5.3	van der Waals forces, π–sigma, π-π T-shaped, alkyl and π–alkyl
12.	β-Elemene	3.45	−4.3	van der Waals forces, π–sigma, alkyl and π–alkyl
13.	Caryophyllene	3.46	−2.4	van der Waals forces, π–sigma, π-π T-shaped, alkyl and π–alkyl
14.	Gamma-cadinene	2.91	−3.6	van der Waals forces, π–sigma, alkyl and π–alkyl
15.	Caryophyllene oxide	1.27	−3.3	van der Waals forces, π–sigma, alkyl and π–alkyl

*secondary metabolites with the best ΔG.

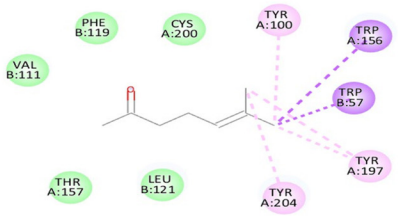
Binding energy is influenced by specific interactions between ligand and receptor amino acid residues. The nAChR receptor belongs to the Cys-loop receptor family, a ligand-gated ion channel activated by neurotransmitters such as acetylcholine, serotonin, glycine, and GABA [25]. Key residues related to nicotine dependence at the 5KXI receptor are CYS200, LEU121, and TRP156 [26]. Nicotine forms hydrogen bonds between its pyrrolidine nitrogen and the carbonyl group of TRP156, establishing a characteristic cation interaction [26, 27]. Additionally, CYS200 and LEU121 influence binding through steric effects [26, 28]. The six citronella oil compounds that interacted with these residues demonstrated favorable affinity and stability, supported by hydrophobic and hydrogen bonding. These interactions help stabilize ligand–receptor complexes and modulate binding affinity [29]. Furthermore, van der Waals forces contribute to stabilizing ligand–receptor interactions



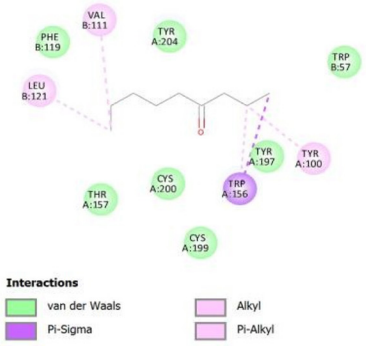
Nicotin (Native Ligand)



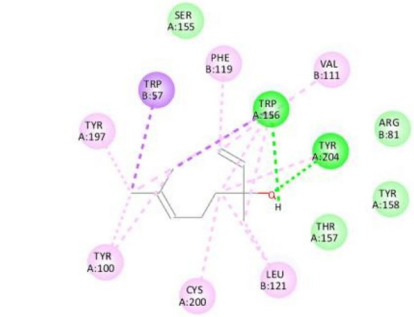
Varenicline (Comparator)



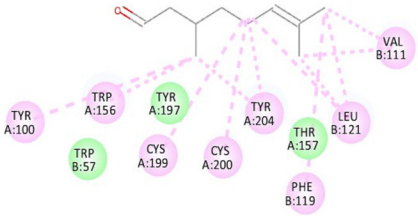
6-Methyl-5-hepten-2-one



4-Nonanone



Linalool



Citronellal

Fig. 4. Visualization results of 5KXI receptor interaction with compounds from Citronella oil

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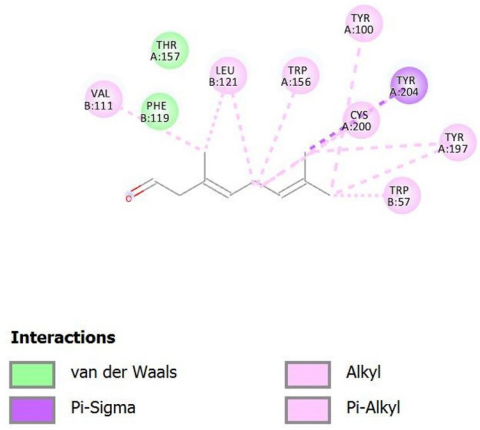
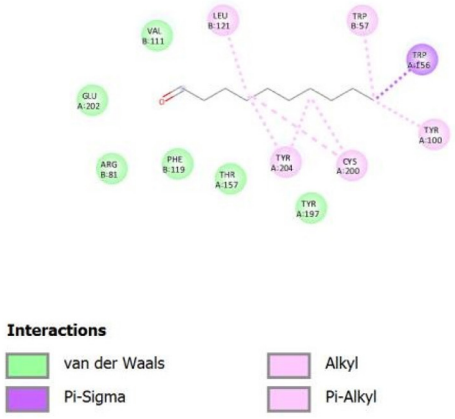
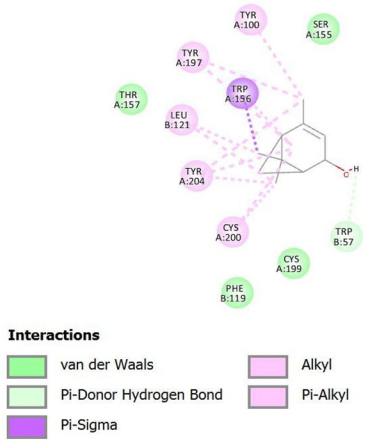


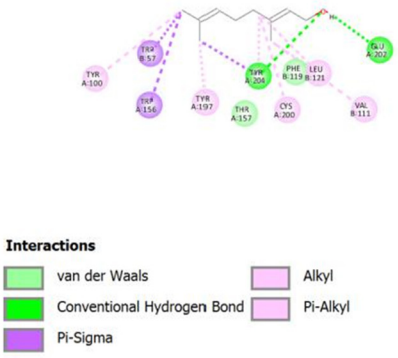
Isocitral



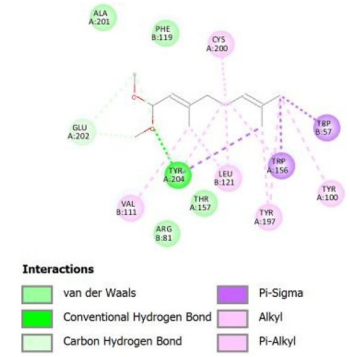
Decanal



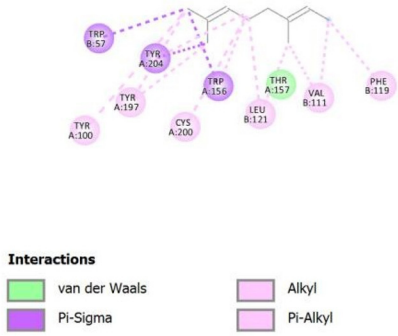
(1R)-cis-Verbenol



Geraniol



Geranial dimethyl acetal



2,6-Dimethyl 2,6-octadiene

Fig. 4. Continued

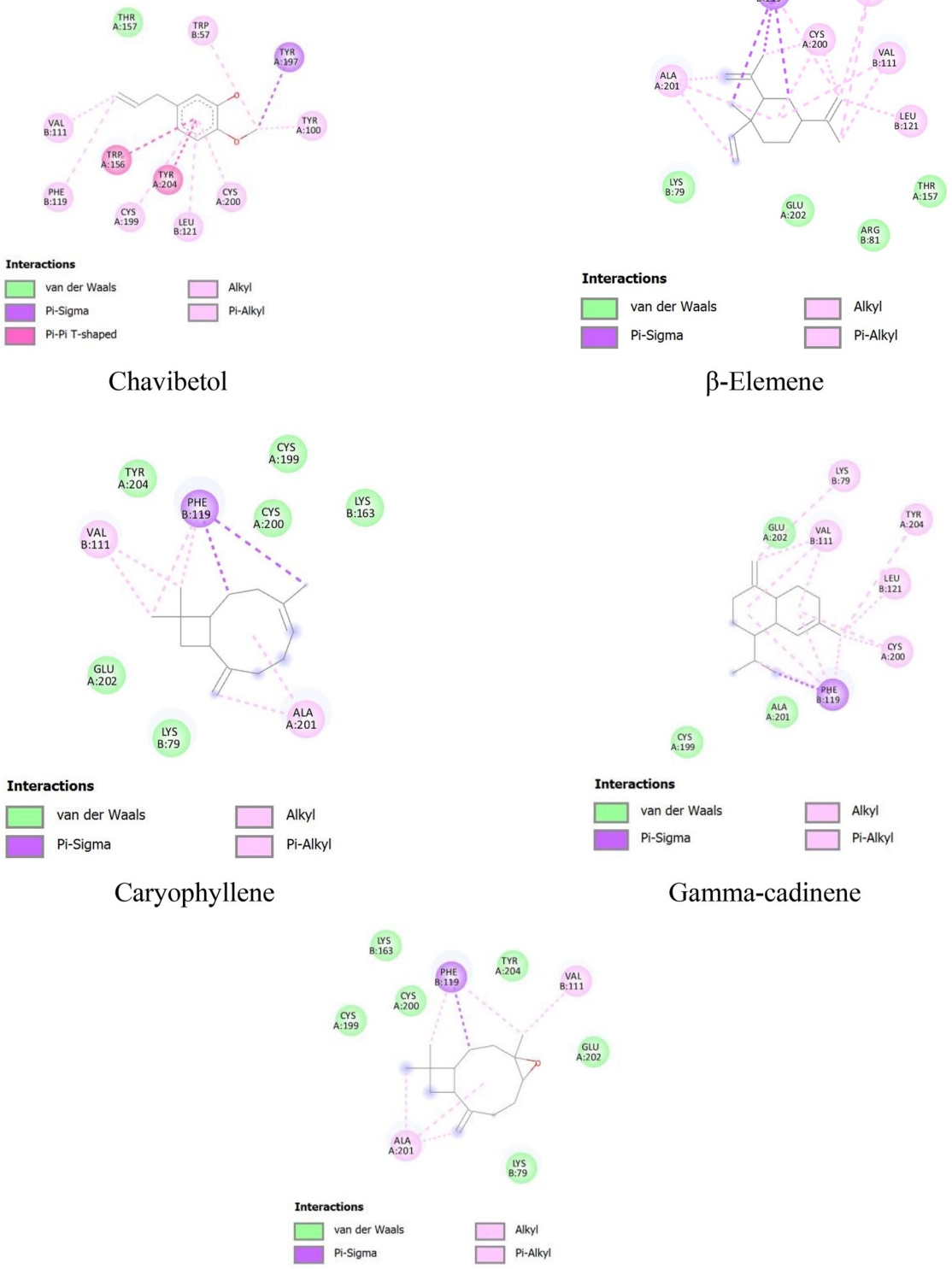


Fig. 4. Continued

at α4β2 [26]. Conversely, chavibetol, despite forming interactions similar to nicotine and varenicline, exhibited a higher (less favorable) ΔG, likely due to weaker overall binding strength [30].

The visualization results of molecular interactions between the 5KXI receptor and the reference ligands (nicotine and varenicline), as well as various secondary metabolites from citronella oil, showed that most compounds exhibited interaction patterns similar to the native ligand. These interactions involved key amino acid residues such as TYR100, TRP156, TYR204, LEU121, VAL111, CYS199, and CYS200 (Fig. 4). The nicotine ligand interacted through hydrogen bonds with TYR100, THR157, TRP156, and TYR204, while varenicline formed hydrogen bonds with TRP156 and TYR204, accompanied by additional hydrophobic interactions with VAL111, LEU121, and CYS200. Several compounds, including linalool, geraniol, and geranial dimethyl acetal, also showed hydrogen bonding in addition to hydrophobic interactions, indicating a potentially strong binding affinity toward the 5KXI active site. Meanwhile, other compounds such as citronellal, isocitral, decanal, 2,6-dimethyl-2,6-octadiene, and *β*-elemene only exhibited hydrophobic interactions, suggesting weaker affinity compared to the reference ligands.

Amino acid residue similarity analysis of the 5KXI receptor

Interaction similarity analysis was conducted to evaluate the resemblance between the test ligands and the amino acid residues involved, as well as the bonding interactions compared to the native ligand and the reference drug. The results of the analysis are presented in Fig. 5. Similarity analysis of amino acid interactions revealed that (1R)-*cis*-verbenol and geranial dimethyl acetal showed the highest

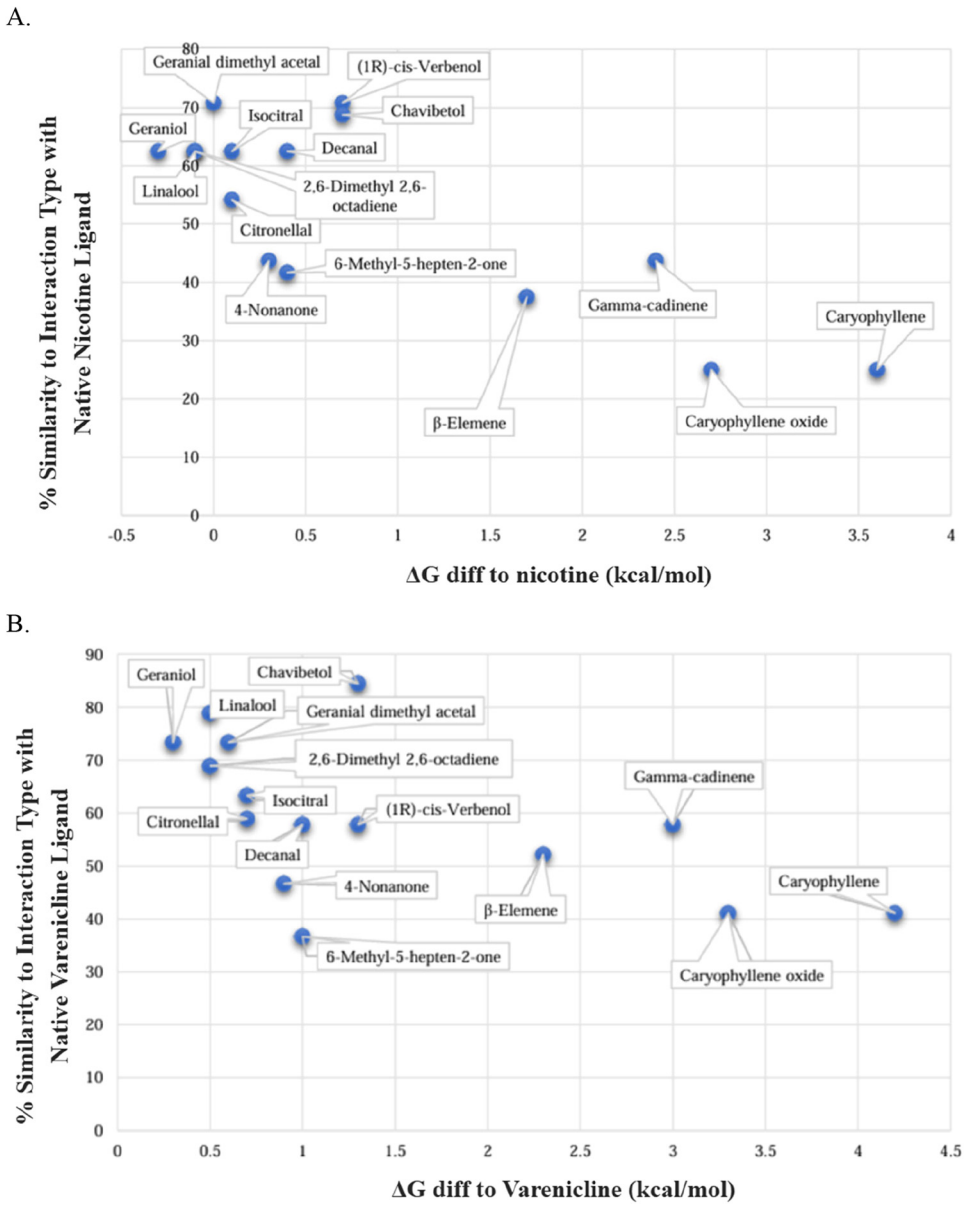


Fig. 5. Similarity Graph of Interactions with Differences in Energy Binding on the 5KXI Receptor for (A) nicotine and (B) varenicline

similarity (70.83%) to nicotine, while chavibetol showed the greatest similarity (84.44%) to varenicline (Fig. 5). This indicates that certain citronella oil constituents share interaction patterns with known nicotinic ligands.

Drug-likeness analysis of Citronella oil secondary metabolites

Drug-likeness analysis was conducted to predict the absorption potential and drug-like behavior of citronella oil constituents using Lipinski's Rule of Five, Veber's Rule, and the Ghose Filter [31]. According to Lipinski's Rule of Five, a compound is considered drug-like if it has a molecular weight (MW) $\leq$ 500 Da, log $P \leq$ 4.15, hydrogen bond acceptors (HBA) $\leq$ 10, and hydrogen bond donors (HBD) $\leq$ 5. This rule emphasizes that small molecules with moderate polarity are more likely to permeate lipid cell membranes. Veber's Rule adds that the number of rotatable bonds (RB) should be $\leq$10 and the topological polar surface area (TPSA) $\leq$ 140 Å^2, as these parameters influence molecular flexibility and membrane permeability. Meanwhile, the Ghose Filter evaluates the balance of physicochemical properties, with criteria including molecular weight between 160 and 480 Da, log P between −0.4 and 5.6, molar refractivity (A) of 40–130 cm^3, and total number of atoms (TNA) between 20 and 70.

Table 4. Drug-likeness analysis of Citronella Oil Secondary metabolites

No	Secondary Metabolites	Lipinski's rule of five	Veber's rule	Ghose filter
1.	6-Methyl-5-hepten-2-one	Yes	Yes	No, 1 Violation (BM < 160)
2.	4-Nonanone	Yes	Yes	No, 1 Violation (BM < 160)
3.	Linalool	Yes	Yes	No, 1 Violation (BM < 160)
4.	Citronellal	Yes	Yes	No, 1 Violation (BM < 160)
5.	Isocitral	Yes	Yes	No, 1 Violation (BM < 160)
6.	Decanal	Yes	Yes	No, 1 Violation (BM < 160)
7.	(1R)-cis-Verbenol	Yes	Yes	No, 1 Violation (BM < 160)
8.	Geraniol	Yes	Yes	No, 1 Violation (BM < 160)
9.	Geranial dimethyl acetal	Yes	Yes	Yes
10.	2,6-Dimethyl 2,6-octadiene	Yes	Yes	No, 1 Violation (BM < 160)
11.	Chavibetol	Yes	Yes	Yes
12.	β-Elemene	No, 1 violation (M Log $P > 4.15$)	Yes	Yes
13.	Caryophyllene	No, 1 violation (M Log $P > 4.15$)	Yes	Yes
14.	Gamma-cadinene	No, 1 violation (M Log $P > 4.15$)	Yes	Yes
15.	Caryophyllene oxide	Yes	Yes	Yes

Twelve compounds met all drug-likeness criteria (Table 4). However, β-elemene, caryophyllene, and gamma-cadinene exceeded the acceptable MLogP (>4.15), indicating high lipophilicity. These nonpolar compounds are more soluble in lipids, enhancing membrane permeability but potentially reducing distribution efficiency due to higher volume of distribution [32]. The LogP value correlates with TPSA, which reflects polarity and permeability; low TPSA values indicate few polar groups and greater brain penetration [33]. All compounds met Veber's Rule, suggesting good oral absorption potential, while nine compounds failed the Ghose Filter molecular weight criterion (<160 Da), including 6-methyl-5-hepten-2-one, 4-nonanone, linalool, citronellal, isocitral, decanal, (1R)-*cis*-verbenol, geraniol, and 2,6-dimethyl-2,6-octadiene. Compounds with low molecular weight exhibit high permeability but may lack specificity and stability in receptor binding [34, 35].

Because citronella oil is administered via inhalation, the blood–brain barrier (BBB) parameter was also evaluated. Essential oils are known to penetrate the BBB through the nasal–brain route [36]. Twelve compounds—6-methyl-5-hepten-2-one, 4-nonanone, linalool, citronellal, isocitral, decanal, (1R)-*cis*-verbenol, geraniol, geranial dimethyl acetal, 2,6-dimethyl-2,6-octadiene, chavibetol, and caryophyllene oxide—were predicted to cross the BBB passively according to the Boiled-Egg (Egan Egg) model, consistent with their TPSA and WLogP values [37].

CONCLUSION

The essential oil of citronella (*Cymbopogon nardus*) from Jatijejer Village exhibited good quality. This was indicated by its clear yellow color, aromatic fragrance, and the presence of five spots confirming essential oil components through Thin Layer Chromatography (TLC) analysis. The total essential oil yield was 0.82% v/w obtained via Stahl distillation. GC-MS analysis revealed that citronella oil contained major components such as citronellal and geraniol, with a compositional profile and chromatogram pattern closely resembling those of PT.N's product. Several chemical constituents of citronella oil—namely Linalool, Citronellal, Isocitral, Geraniol, Geranial dimethyl acetal, and 2,6-dimethyl-2,6-octadiene—showed lower binding energy values than nicotine and were capable of interacting with key amino acid residues. Drug-likeness analysis further indicated

that these compounds complied with Lipinski's Rule of Five and Veber's Rule, although several did not fully meet the Ghose Filter criteria. Based on interaction similarity analysis, the compounds (1R)-cis-Verbenol and Geranial dimethyl acetal exhibited the highest similarity percentages to nicotine, while Chavibetol showed the highest similarity to varenicline. These findings suggest that lemongrass oil constituents possess computationally predicted binding potential to nicotine-related targets and may serve as potential adjuvant agents, such as in aromatherapy-based approaches. However, their pharmacological efficacy and potential role in modulating nicotine addiction remain speculative and require further validation through in vitro and in vivo studies.

Conflict of interest: The authors declare no conflict of interest, financial or otherwise.

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