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Original Research Article

Quality Analysis of Clove Essential Oil and *In Silico* Study of Metabolites on nAChR

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ABSTRACT

Nicotine addiction occurs through its interaction with nicotinic acetylcholine receptors (nAChR), particularly the α4β2 subtype, in the central nervous system. Clove (*Syzygium aromaticum*) essential oil has the potential to serve as a natural therapy to overcome nicotine dependence. This study evaluated the quality of clove flower and leaf essential oils from Jatijejer Village and examined the activity of bioactive compounds on the α4β2 nAChR receptor *in silico*. Clove essential oil quality was analysed using Thin Layer Chromatography (TLC) and Gas Chromatography-Mass Spectrometry (GC-MS). The *in silico* study included drug-likeness prediction, binding energy analysis, and molecular interaction assessment. Steam distillation produced a pale yellow oil with the characteristic clove aroma, yielding 2.4% (flowers) and 1.4% (leaves). TLC revealed two active compound spots, while GC-MS analysis identified eugenol as the major component (23.44% in flowers and 26.1% in leaves), along with caryophyllene and humulene as secondary components. The *in silico* analysis showed several compounds with binding energies equal to or lower than nicotine (-6 kcal/mol); eugenol (-5.6 kcal/mol), caryophyllene (-5.7 kcal/mol), and humulene (-5.8 kcal/mol) exhibited slightly higher energies but maintained good binding affinity. Amino acid interaction analysis indicated that 2-nonanol, 3-allyl-6-methoxyphenol, phenol, 2-methoxy-4-(2-propenyl)-acetate, and trans-isoeugenol shared the highest similarity with nicotine (83.33%), while 2-nonanol and linalool showed high similarity with varenicline (86.36%). All 23 compounds met Veber's Rule, and 20 compounds were predicted to cross the blood–brain barrier (BBB). Clove essential oil from Jatijejer demonstrates promising potential to be developed further as a natural therapy for overcoming nicotine addiction.

Keywords: Clove Essential Oil, Nicotine Addiction, Nicotinic Acetylcholine Receptors, *In Silico,* Thin Layer Chromatography, Gas Chromatography-Mass Spectrometry

Introduction

Cigarettes are tobacco-processed products that deliver nicotine into the body. Each cigarette contains approximately 1–1.5 milligrams of nicotine, the main addictive substance that causes dependence.[1-3] The type of cigarette most common in Indonesia is *kretek*, which is considered a cultural heritage with a distinctive aroma and flavor. It is made from a mixture of tobacco and cloves and produces a characteristic crackling sound when burned.[4] According to World Health Organization data in 2024, around 8 million people die each year due to tobacco use, including 7 million deaths from active smoking and 1 million from secondhand smoke exposure. Based on data from the Indonesian Central Bureau of Statistics in 2024, about 28.99% of Indonesians aged 15 years and older are smokers, with 7.04% of males and 0.20% of females aged ≤18 years being smokers. Factors that contribute to nicotine dependence include the age at which smoking begins, social environment, genetic predisposition, and psychological conditions such as stress or anxiety disorders.[5]

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Nicotine can penetrate the plasma membrane and enter the brain, where it stimulates the nicotinic acetylcholine receptors (nAChR), particularly the α4β2 subtype.[3] The activation of this receptor increases the release and metabolism of acetylcholine, which plays a role in cognitive processes and stress responses.[6] Nicotine dependence is associated with various medical conditions such as lung cancer, heart disease, chronic obstructive pulmonary disease, and mental disorders.[7] When nicotine intake ceases, dopamine deficiency occurs, leading to withdrawal symptoms such as anxiety and a strong urge to smoke. This cycle reinforces nicotine dependence, making it difficult to quit.[3]

Overcoming nicotine addiction can be achieved through various approaches, including behavioral and pharmacological therapy. The main therapies approved by the Food and Drug Administration (FDA) include Nicotine Replacement Therapy (NRT), bupropion, and varenicline, which are used to reduce nicotine dependence and smoking frequency.[8] Non-pharmacological approaches can also help reduce nicotine dependence, such as the use of natural products like essential oils. Essential oils can stimulate the sense of smell and help reduce the urge to smoke.[9]

Indonesia's agricultural sector plays an important role in the national economy.[10] One area with great agricultural potential is Jatijejer Village, located in Trawas, Mojokerto, East Java. The village lies at the foot of Mount Penanggungan and Mount Welirang. Its main agricultural commodity is lemongrass to produce essential oil. The people of Jatijejer Village also cultivate other plants, including clove (*Syzygium aromaticum*).

Clove is an aromatic plant belonging to the family *Myrtaceae*. It contains various volatile and antioxidant compounds such as eugenol, β-caryophyllene, and α-humulene.[11] Clove essential oil has potential as a smoking cessation therapy, as it contains eugenol as the main

compound with a distinctive aroma and a range of pharmacological activities, including antibacterial, antifungal, antioxidant, anti-inflammatory, analgesic, and anticancer effects. The extraction process generally utilizes clove flowers and leaves, which are rich in volatile compounds.[12] Clove essential oil can be extracted using steam distillation, an effective technique for isolating volatile compounds from natural materials. After extraction, essential oil quality analysis can be performed using Thin Layer Chromatography (TLC) to separate and identify existing components, while the essential oil content can be determined using the Stahl distillation method.[13] Gas Chromatography–Mass Spectrometry (GC-MS) can be used to identify and quantify active components, particularly eugenol. The computational methods, namely molecular docking was applied to predict the interaction between identified compounds and the α4β2 nAChR receptor, providing mechanistic insight into their potential anti-nicotine activity.

This study presents novelty by in silico analysis targeting the α4β2 nicotinic acetylcholine receptor, which has rarely been explored in previous studies focusing on smoking cessation. Based on this background, this study aims to evaluate the quality analysis of clove essential oil from Jatijejer Village using several instruments, including TLC, content determination by the Stahl Distillation method, and component characterization using GC-MS. Furthermore, an *in silico* study will be conducted to analyze binding energy and molecular interactions between the active compounds in clove essential oil and the nAChR α4β2 receptor through a molecular docking approach.

Material and Methods

Sample Preparation

Clove plants (*Syzygium aromaticum*) consisting of flowers and leaves were collected from Jatijejer Village, Trawas District, Mojokerto Regency, East Java, Indonesia (GPS coordinates: 7°36'43.2"S; 112°34'17.2"E). Samples were harvested in June 2025 from mature plants aged approximately 4–6 years. The plant materials were taxonomically identified by Dr. Marisca, as the Director of the Center for Information and Development of Traditional Medicine, Faculty of Pharmacy, University of Surabaya.

Steam Distillation of Clove Essential Oil

Steam distillation was carried out to extract essential oil from clove flowers and leaves. Each plant material was cleaned, ground, and weighed to 100 grams. The distillation was carried out using a Clevenger-type apparatus (Iwaki Glass, Japan). The distillation flask was filled with 500 mL of water (analytical grade) and a few boiling stones to prevent bumping during heating using a heating mantle (Thermo Scientific, USA). The distillation process was carried out for 4–6 hours until the resulting distillate appeared clear. The distillation product consisted of two phases, i.e., the oil phase and the aqueous phase. The oil phase was collected in a brown vial, while the aqueous phase was stored in a brown bottle. The essential oil was then treated with anhydrous sodium sulfate (Na_2SO_4, ≥99%, Merck, Germany) to remove residual moisture, stirred, and left to stand for 1–2 minutes. The oil phase was then transferred into a brown vial, labeled, and stored in a refrigerator at 4°C to maintain its stability.[14-16]

Determination of Essential Oil Content Using the Stahl Distillation

Essential oil content determination followed the Stahl distillation method according to the Indonesian Herbal Pharmacopoeia and WHO guidelines for medicinal plant materials. The distillation was conducted using a Stahl-type distillation apparatus (Pyrex®, Germany). A total of 50 grams of powdered simplicia were placed into a 1-liter round-bottom flask, along with a few boiling stones and 300–500 mL of distilled water. The round-bottom flask was connected to a condenser and a graduated burette. Heating was carried out gradually using an air bath to maintain a stable temperature for 3–4 hours. After distillation was complete, the distillate was allowed to stand for up to 15 minutes before recording the volume of essential oil collected in the burette. The oil phase was transferred to a brown vial, and the aqueous phase was stored in a brown bottle. Na_2SO_4 was added to the vial containing oil to remove remaining moisture, stirred, and left for 1–2 minutes. The oil was then transferred into a new brown vial. The essential oil content was calculated using the %v/w formula, i.e., equation 1.

$$\text{Essential oil content (\%)} = \frac{Volume\ of\ essential\ oil}{Weight\ of\ dried\ powder} x\ 100\% \qquad (1)$$

Thin Layer Chromatography Analysis

TLC analysis was performed to identify the chemical components in clove essential oil. Silica gel 60 F_{254} plates (Merck, Germany) were cut to a size of 6 × 20 cm, with spotting marks placed 2.5 cm from the lower edge. The mobile phase was prepared by mixing toluene and ethyl acetate (analytical grade, Merck, Germany) in a 93:7 (v/v) ratio and poured into a chamber lined with filter paper to allow vapor saturation. The test solution was prepared from clove essential oil distilled at a concentration of 5% in ethanol p.a., while the reference solution came from PT. N clove essential oil at the same concentration. Both solutions were spotted onto the silica plate using a 2 µL capillary tube and air-dried. The plate was observed under UV light at 254 nm to assess spot thickness. The plate was then placed in the chamber with the spotted end facing downward. The chamber was closed and left until the mobile phase reached the elution boundary. After elution, the plate was removed, dried, and sprayed with a vanillin–sulfuric acid reagent (Merck, Germany). The plate was then dried in an oven at 110°C for 5–10 minutes and re-observed under UV light at 254 nm and 366 nm. The distance of each spot from the origin was measured to calculate the Rf value using the following formula (equation 2).[17,18]

$$Rf = \frac{Distance\ traveled\ by\ analyte}{Distance\ traveled\ by\ eluent} \qquad (2)$$

Gas Chromatography–Mass Spectrometry analysis

GC–MS analysis was performed using a Shimadzu QP2010-SE instrument (Shimadzu Corporation, Japan) to determine the chemical constituents of the essential oil. During the analysis, the sample was introduced into the GC–MS system, with methanol used as a rinsing solvent between injections. The analytical conditions were optimized as follows: injector temperature maintained at 250°C, oven temperature programmed between 230°C and 250°C for a total runtime of 40 minutes, and helium employed as the carrier gas with a flow rate of 0.75 mL/min.[19] The resulting chromatogram was subsequently analyzed to identify compounds based on their retention times, relative peak areas, and similarity indices. Compound identification was performed by comparing mass spectra with the NIST library database.

In Silico Analysis

The *in silico* analysis was performed using molecular docking to evaluate the interaction between secondary metabolites of clove essential oil and target receptors computationally. The chemical compounds were identified based on GC-MS results and retrieved from the PubMed database (https://pubmed.ncbi.nlm.nih.gov/, National Center for Biotechnology Information, USA). The 2D structures of the compounds were visualized using BIOVIA Discovery Studio Visualizer 2025 (Dassault Systèmes, France), and optimized using Avogadro software version 1.2.0 (Open Chemistry, USA) via energy minimization. The 3D structure of the target protein was downloaded from the RCSB Protein Data Bank (https://www.rcsb.org/) with PDB ID: 5KXI, and the native ligand was removed using AutoDock Tools 1.5.7 (The Scripps Research Institute, USA). Docking validation was performed via Command Prompt by evaluating the Root Mean Square Deviation (RMSD) value between the native ligand and docking results, and the RMSD value obtained was 0.8888 Å. Docking was carried out with a grid size of 22×22×22 and splitless mode. Each compound was tested three times, and the binding energy (ΔG, in kcal/mol) was recorded, with the lowest value indicating the best binding affinity. The docking results were visualized to evaluate molecular interaction types and binding sites using BIOVIA Discovery Studio Visualizer 2025.

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Drug-likeness evaluation of the compounds was conducted using SwissADME (https://www.swissadme.ch/, Swiss Institute of Bioinformatics, Switzerland, accessed 2025) by uploading structures in *.sdf or *.mol format or by using SMILES obtained from PubChem. The analysis included molecular weight, log P, number of hydrogen bond donors and acceptors (HBD and HBA), number of rotatable bonds (RB), Topological Polar Surface Area (TPSA), molar refractivity, and total atom count. The results were compared against Lipinski's Rule of Five, Veber's Rule, the Ghose Filter, and Blood–Brain Barrier (BBB) permeability predictions.

Results and Discussion

Steam Distillation of Clove Plants

This study aimed to evaluate the quality of clove flower and leaf essential oils obtained from Jatijejer Village by comparing them with reference samples from PT N. The results showed that the essential oil obtained from the steam distillation of clove flowers and leaves possessed a pale yellow color and a characteristic clove aroma. The yield of essential oil from flower distillation using the Stahl method was 2.4% v/w, while the essential oil from clove leaves was 1.42% v/w.

Thin Layer Chromatography Analysis

Essential flower and leaves oil samples from steam distillation, water distillation, and the commercial reference oil from PT. N was analyzed using TLC. The chromatographic profiles are shown in Figure 1. TLC analysis revealed two distinct spots for each distilled oil sample, while the reference sample showed only one spot, presumed to be pure eugenol. After spraying with a vanillin-sulfuric acid reagent, the spots developed yellowish-brown and purple colors, indicating the presence of eugenol compounds.[20,21]

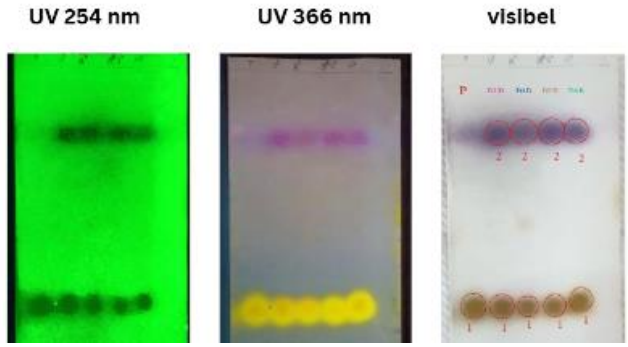

Figure 1: TLC Results of Clove Oil

Note: P = Reference; DUD = Steam Distillation of Leaves; DAD = Water Distillation of Leaves; DUB = Steam Distillation of Flowers; DAB = Water Distillation of Flowers.

Gas Chromatography–Mass Spectrometry Analysis

GC–MS analysis was performed on five chromatogram (flower clove oil steam distillation, flower clove oil water distillation, leaves clove oil steam distillation, leaves clove oil water distillation, and comparation from PT. N) results using retention time (Rt) as the X-axis and absolute intensity—scaled down by a factor of 1,000,000—as the Y-axis, followed by an overlay comparison (Figure 2). GC–MS results identified 39 compounds present in clove flower and leaves essential oil (Table 1). The dominant identified compounds were eugenol, caryophyllene, and humulene. The eugenol content of steam-distilled clove flower oil (23.44%) and leaf oil (26.1%) was considerably lower than that of the reference sample from PT N (49.19%), the literature (55.60%)[16], and the SNI standards, which specify 80–95% for flower oil and a minimum of 78% for leaf oil (SNI 06-4267-1996; SNI 06-2387-2006). However, the concentrations of caryophyllene and humulene were higher than reported values in the literature (15% and 3%, respectively).[16] The lower eugenol content in this study may be attributed to the use of a simple steam distillation method with relatively low temperature and pressure, which could result in incomplete hydrolysis and extraction of eugenol, as well as possible compound loss through the aqueous phase or thermal degradation, consistent with other findings.[15] Although clove flowers are theoretically richer in eugenol due to their role as the main biosynthesis site,[22] the leaf samples exhibited slightly higher eugenol levels (26.1%) than the flowers (23.4%), similar to other results.[23] This could be due to a more even distribution of oil glands in the leaves, the conversion of eugenyl acetate into free eugenol, and less dependence on seasonal variation. In contrast, some eugenol in the flowers may remain bound as eugenyl acetate, which does not completely hydrolyze during distillation. Consequently, only free eugenol is detected by GC–MS, leading to a higher apparent eugenol concentration in the leaf oil. A total of 39 compounds were detected in both the samples and reference chromatograms. 23 major compounds were selected for further analysis based on their Similarity Index, consistency across samples, and NIST library matching.

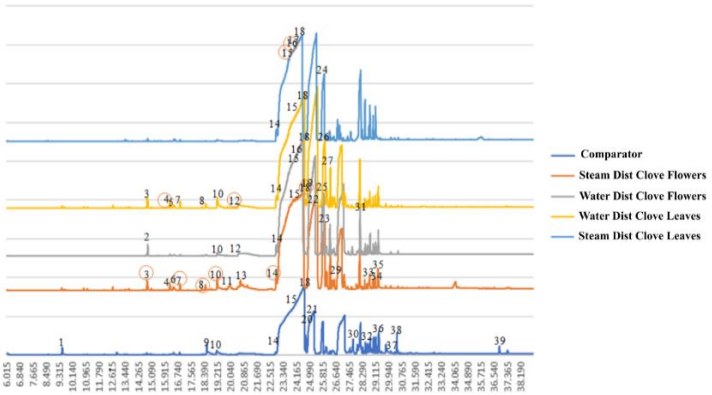

Figure 2: GC–MS Chromatograms of Clove Oil

In Silico Analysis of Clove Essential Oil

According to SwissADME analysis, among 23 compounds tested, three did not meet Lipinski's Rule of Five, all satisfied Veber's Rule, eight did not comply with the Ghose Filter, and twenty compounds were predicted to be able to cross the BBB (Table 2). Three compounds that do not meet Lipinski's rules because their M log P values are ≤ 4.15. These compounds include α-Cubebene, Caryophyllene, and Humulene. A high Log P value indicates that a compound has increasingly hydrophobic properties and is potentially toxic because it can survive longer in the lipid bilayer membrane or spread more widely in the body, thereby reducing its level of selectivity towards the target enzyme.[24] Analysis on the Ghose Filter, there are 8 compounds that do not meet the requirements, these 8 compounds have molecular weights that are outside the range of 160–480, including 2-Nonanol acetate, 2-Nonanone, 2-Nonanol, Linalool, (E)-4,8-Dimethylnona-1,3,7-triene, Benzoic acid, ethyl ester, Methyl salicylate, Geraniol, (Z)-3-Phenylacrylaldehyde. There are 2 other compounds whose total number of atoms does not fit the range of 20–70, namely Methyl salicylate, (Z)-3-Phenylacrylaldehyde. If a compound does not meet the requirements, then the compound is considered not to meet the drug-likeness criteria, this indicates that the compound may have less than optimal pharmacokinetic characteristics, such as low absorption, distribution, or ability to bind biological targets.

The binding energy (ΔG) values of the secondary metabolites were compared with those of the native ligand (nicotine) and the reference compound (varenicline). 23 Compounds showed ΔG values equivalent to or lower than that of the native ligand (≤6.0 kcal/mol). A visualization comparing ΔG values among the secondary metabolites, native ligand, and reference compound is shown in Figure 3. Fourteen compounds exhibited ΔG values lower than or slightly higher than both nicotine and varenicline. Benzyl benzoate exhibited the strongest binding affinity (–7.7 kcal/mol), surpassing that of varenicline (–6.6 kcal/mol).

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Table 1: Chemical Composition of Clove Flower and Clove Leaf Essential Oils from Jatijejer Village Identified by GC–MS

No	Compound Name	R. Time (min)	Flower Oil	Leaf Oil	Chemical Class
1	Furfural	9.500		V	Aromatic aldehyde
2	Eucalyptol	14.860	V		Monoterpene
3	2-Nonanol, acetate	14.819	V	V	Aliphatic ester
4	2-Nonanone	16.250	V	V	Aliphatic ketone
5	2-Nonanol	16.450		V	Aliphatic secondary alcohol
6	Linalool	16.495	V		Monoterpene alcohol
7	(E)-4,8-Dimethylnona-1,3,7-triene	16.850	V	V	Monoterpene
8	Benzoic acid, ethyl ester	18.485	V	V	Aromatic ester
9	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-	18.730		V	Monoterpene alcohol
10	Methyl salicylate	19.210	V	V	Phenolic ester
11	Geraniol	20.015	V		Monoterpene alcohol
12	Phenol, 4-(2-propenyl)-, acetate	20.545	V		Phenolic ester
13	(Z)-3-Phenylacrylaldehyde	20.670	V		Aromatic aldehyde
14	α-Cubebene	22.895	V	V	Sesquiterpene
15	3-Allyl-6-methoxyphenol	23.600	V	V	Phenylpropanoid
16	trans-Isoeugenol	23.905	V		Phenylpropanoid
17	Phenol, 2-methoxy-6-(2-propenyl)- (o-Isoeugenol)	23.780	V	V	Phenylpropanoid
18	Eugenol	24.595	V	V	Phenylpropanoid
19	Phenol, 2-methoxy-3-(2-propenyl)-	24.595	V		Phenylpropanoid
20	Phenol, 2-methoxy-4-(1-propenyl)-, (Z)-	24.660		V	Phenylpropanoid
21	Methyleugenol	24.765		V	Phenylpropanoid
22	Caryophyllene	25.485	V		Sesquiterpene
23	Humulene	25.945	V		Sesquiterpene
24	1-Isopropyl-4,7-dimethyl-1,2,3,4,5,6-hexahydronaphthalene	26.015		V	Sesquiterpene
25	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl	26.040	V		Sesquiterpene
26	γ-Muurolene	26.085		V	Sesquiterpene
27	α-Farnesene	26.300		V	Sesquiterpene
28	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl	26.465	V	V	Sesquiterpene
29	Phenol, 2-methoxy-4-(2-propenyl)-, acetate	27.005	V		Phenolic ester
30	Caryophyllene alcohol	27.955		V	Sesquiterpene alcohol
31	Caryophyllene oxide	28.165	V		Sesquiterpene epoxide
32	4a(2H)-Naphthalenol, 1,3,4,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-	28.620		V	Sesquiterpene alcohol
33	Caryophylla-4(12),8(13)-dien-5.alpha.-ol	28.755	V		Sesquiterpene alcohol
34	14-Hydroxycaryophyllene	29.105	V		Sesquiterpene alcohol
35	2',3',4'-Trimethoxyacetophenone	29.275	V		Aromatic ketone
36	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol	29.790		V	Phenylpropanoid
37	Benzyl Benzoate	30.070		V	Aromatic ester
38	Neophytadiene	30.470		V	Diterpene hydrocarbon
39	Estragole	36.885		V	Phenylpropanoid

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Table 2: SwissADME analysis of Clove Essential Oil

No	Compound	Lipinski's Rule of Five	Veber's Rule	Ghose Filter	BBB
1	2-Nonanol, acetate	Yes	Yes	Yes	Yes
2	2-Nonanone	Yes	Yes	No[2]	Yes
3	2-Nonanol	Yes	Yes	No[2]	Yes
4	Linalool	Yes	Yes	No[2]	Yes
5	(E)-4,8-Dimethylnona-1,3,7-triene	Yes	Yes	No[2]	Yes
6	Benzoic acid, ethyl ester	Yes	Yes	No[2]	Yes
7	Methyl salicylate	Yes	Yes	No[2]	Yes
8	Geraniol	Yes	Yes	No[2]	Yes
9	(Z)-3-henylacrylaldehyde	Yes	Yes	No[2]	Yes
10	α-Cubebene	No[1]	Yes	Yes	Yes
11	3-Allyl-6-methoxyphenol	Yes	Yes	Yes	Yes
12	Phenol, 2-methoxy-3-(2- propenyl)-	Yes	Yes	Yes	Yes
13	Eugenol	Yes	Yes	Yes	Yes
14	Caryophyllene	No[1]	Yes	Yes	No
15	Humulene	No[1]	Yes	Yes	No
16	Phenol, 2-methoxy-4-(2- propenyl)-, acetate	Yes	Yes	Yes	Yes
17	Caryophyllene oxide	Yes	Yes	Yes	Yes
18	Caryophylla-4(12),8(13)-dien-5.alpha.-ol	Yes	Yes	Yes	Yes
19	14-hydroxycaryophyllene	Yes	Yes	Yes	Yes
20	2',3',4'-Trimethoxyacetophenone	Yes	Yes	Yes	Yes
21	Benzyl Benzoate	Yes	Yes	Yes	Yes
22	Trans-isoeugenol	Yes	Yes	Yes	No
23	Methyeugenol	Yes	Yes	Yes	Yes

[1] One violation: molecular weight < 160 Da
[2] One violation: MLogP > 4.15

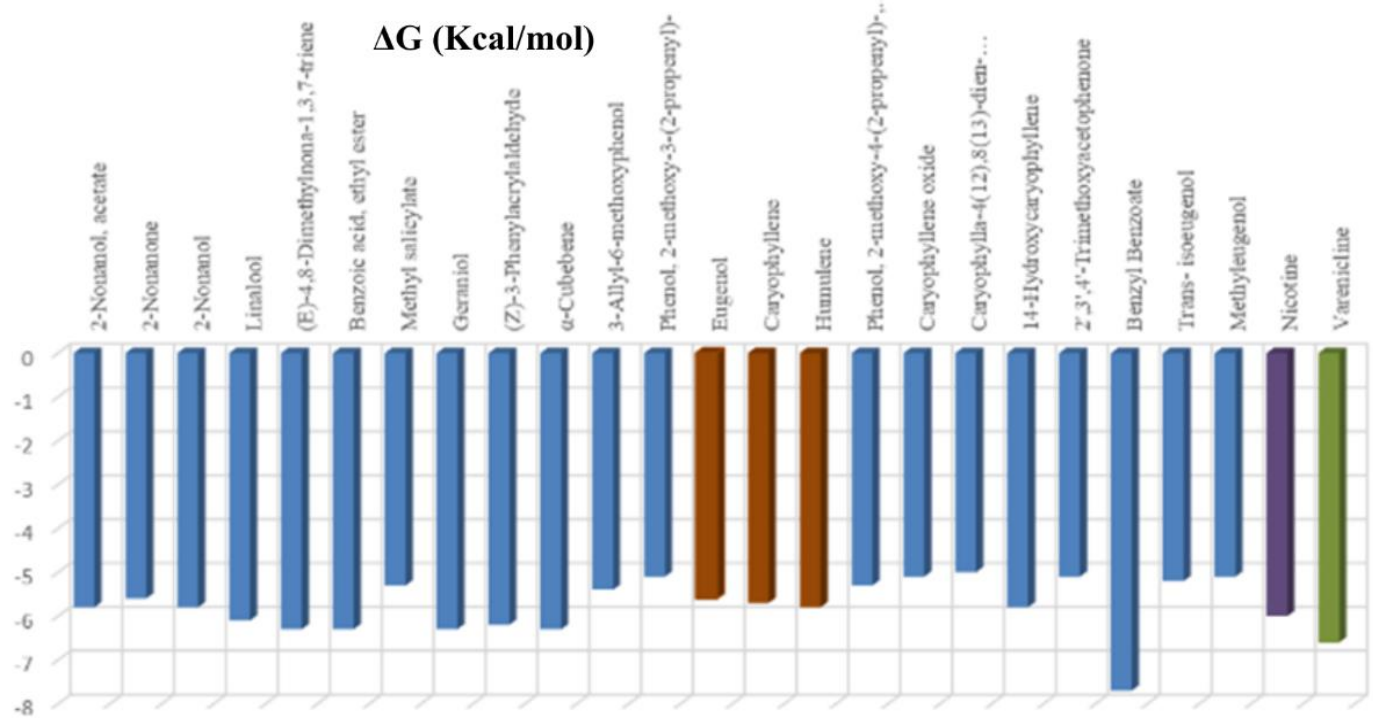

Figure 3: Visualization of Binding Energy (ΔG) Comparison among Secondary Metabolites, Native Ligand, and Reference Compound *Amino Acid Interaction Similarity on Receptor 5KXI*

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Molecular docking analysis further indicated that the 23 metabolites formed various types of interactions with amino acid residues in the α4β2-nAChR receptor, including hydrogen bonding, π–σ, π–alkyl, and alkyl interactions. Interaction similarity analysis showed that 2-Nonanol, 3-Allyl-6-methoxyphenol, Phenol 2-methoxy-4-(2-propenyl)-acetate, and trans-isoeugenol had the highest similarity (83.33%) with nicotine, while 2-Nonanol and Linalool displayed the highest similarity (86.36%) with varenicline (Figures 4 and 5). These findings suggest that several secondary metabolites in clove essential oil may share similar ligand–receptor interaction patterns with nicotine and varenicline, indicating their potential as active candidates for developing clove oil–based anti-smoking therapies.

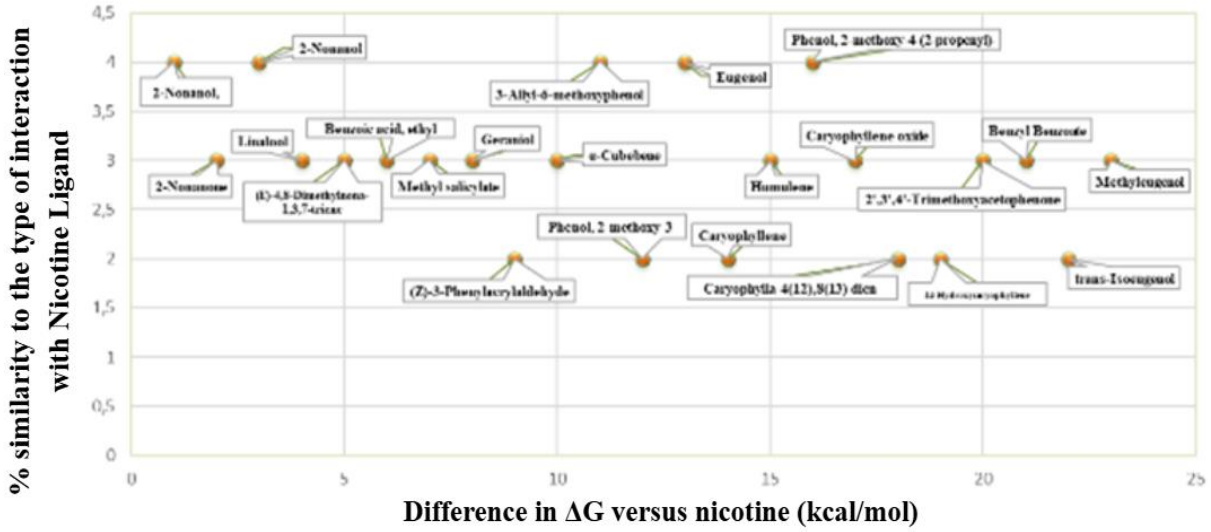
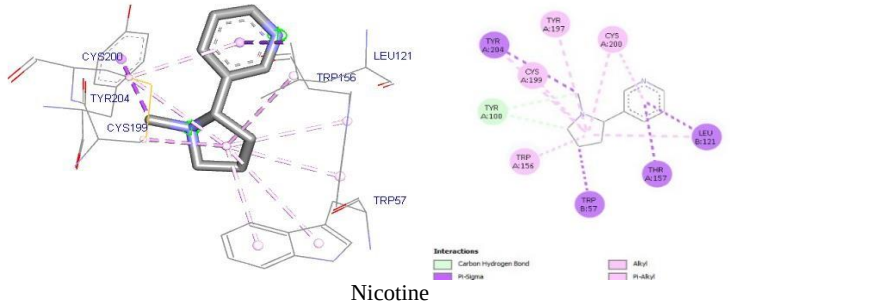
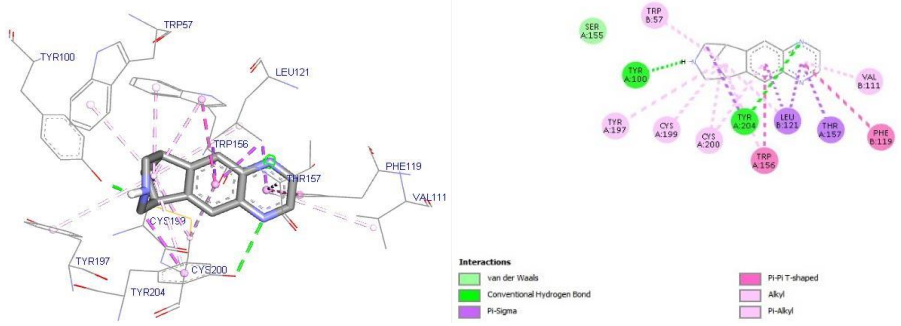


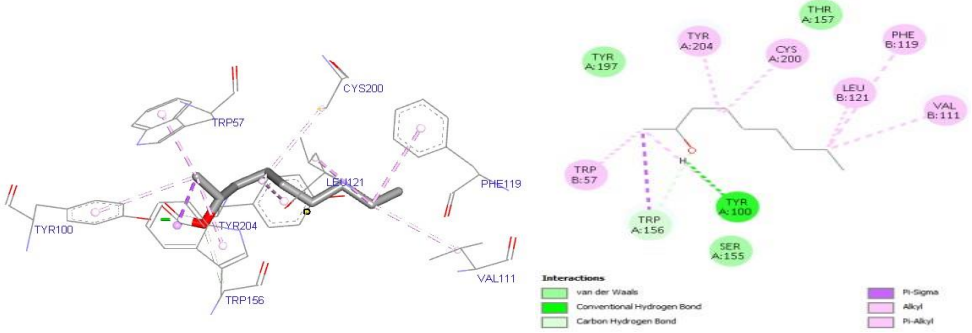
Figure 4: Interaction Similarity Graph of Amino Acid Binding on 5KXI Receptor Compared to Varenicline and Nicotine



Nicotine



Varenicline



2-Nonanol

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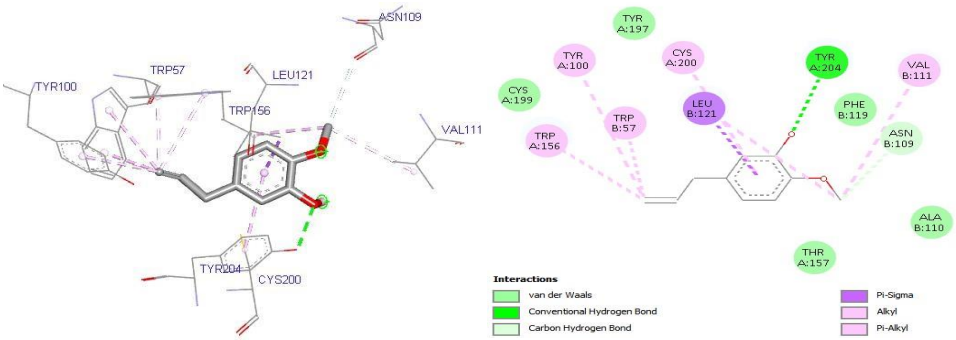
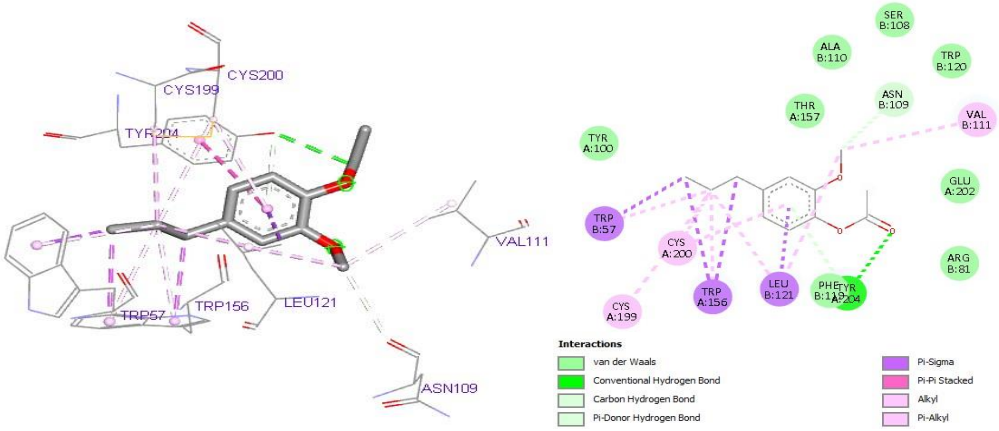
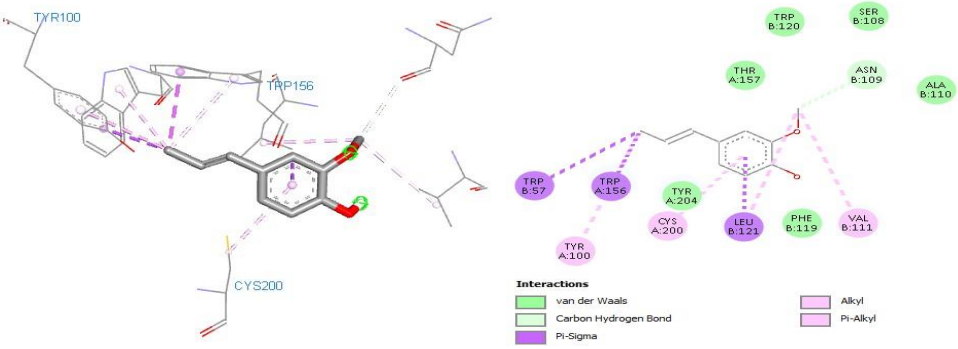


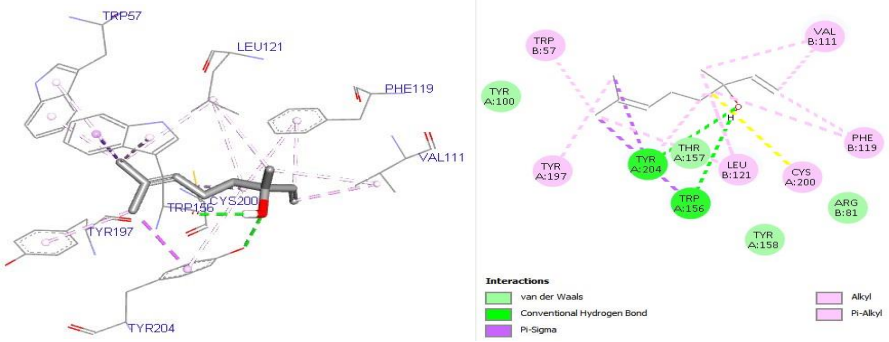
3-Allyl-6-methoxyphenol



Phenol, 2-methoxy-3-(2-propenyl)-acetate



Trans-isoeugenol



Linalool

Figure 5: Two-dimensional (2D) and three-dimensional (3D) molecular interactions of nicotine, varenicline, 2-Nonanol, 3-Allyl-6-methoxyphenol, Phenol 2-methoxy-4-(2-propenyl)-acetate, trans-isoeugenol, Linalool with the α4β2-nAChR receptor (PDB ID: 5KXI).

Conclusion

The clove flower and leaf essential oils from Jatijejer Village obtained through steam distillation exhibited organoleptic characteristics consistent with SNI standards. However, the eugenol content produced from both steam and water distillation methods remained below the SNI requirements. Based on the drug-likeness evaluation, all compounds met Veber's Rule, although some did not comply with Lipinski's Rule of Five and the Ghose Filter. Additionally, twenty compounds were predicted to cross the BBB, suggesting potential activity in the central nervous system. Molecular docking analysis of the nAChR α4β2 receptor revealed that major compounds such as eugenol, caryophyllene, and humulene exhibited good binding affinity with ΔG values close to that of nicotine. The compounds 2-Nonanol, 3-Allyl-6-methoxyphenol, Phenol, 2-methoxy-4-(2-propenyl)-acetate, and trans-isoeugenol showed the highest similarity percentage with nicotine, while 2-Nonanol and Linalool exhibited the highest similarity with varenicline. Further *in vitro* studies are required to confirm that these secondary metabolites in clove essential oil possess anti-nicotine activity.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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