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Chemical Compound Analysis of *Syzygium myrtifolium* Essential Oil and Molecular Docking Analysis on Phenylalanine Hydroxylase Enzyme

Analisis Kandungan Senyawa Kimia Minyak Asiri Syzygium myrtifolium *dan Analisis* Docking *Molekuler pada Enzim Phenylalanine Hydroxylase*

Zikri Hamidi[1*]

[1]Department of Forest Products, Faculty of Forestry and Environment, IPB University, Jalan Lingkar Akademik, Dramaga, 16680, Bogor, Indonesia
*Email: zikrihamidi62@gmail.com

RESEARCH ARTICLE

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Syzygium myrtifolium, Gas Chromatography-Mass Spectrometry, molecular docking, phenylalanine hydroxylase

ABSTRACT

Essential oil from the red shoot plant (*Syzygium myrtifolium*) had high potential as a non-timber forest product. Therefore, this research aimed to evaluate the effect of leaves storage duration on oil yield, essential oil quality, and compliance with the Indonesian National Standard (SNI) 06-237-2006 for clove leaf oil. This research performed steam distillation to produce essential oil from fresh leaves, leaves stored for 24 hours, and leaves stored for 48 hours. The results showed that the highest essential oil yield was obtained from the 24-hour stored leaves at 0.204%, in line with the SNI 06-237-2006. The Gas Chromatography-Mass Spectrometry (GC-MS) analysis showed that the main compounds in the product were beta-pinene (39.09%) and caryophyllene (23.74%). Based on the in silico results from molecular docking, there was a strong interaction between the essential oil compounds and the enzyme phenylalanine hydroxylase. In addition, humulene, ledene, and caryophyllene exhibited the highest binding affinities due to the molecular stability of the enzyme at residues Phe A:254 and Tyr A:325. The current research opened opportunities for developing essential oil-based products for health applications.

KATA KUNCI
Syzygium myrtifolium, *Gas Chromatography-Mass Spectrometry, penambatan molekul, phenylalanine hydroxylase*

INTISARI

*Minyak asiri dari daun pucuk merah (*Syzygium myrtifolium*) memiliki potensi tinggi untuk dikembangkan sebagai hasil hutan bukan kayu. Penelitian ini bertujuan untuk mengkaji pengaruh waktu penyimpanan daun terhadap rendemen, kualitas minyak asiri, serta kesesuaiannya dengan SNI 06-237-2006 tentang minyak daun cengkih. Distilasi uap dilakukan pada daun segar, daun yang disimpan selama 24 jam, dan 48 jam. Rendemen minyak asiri tertinggi diperoleh dari daun penyimpanan 24 jam sebesar 0,204% dan hasil mendekati Standar Nasional Indonesia 06-237-2006. Analisis Gas Chromatography-Mass Spectrometry (GC-MS) menunjukkan bahwa senyawa utama yang terkandung dalam minyak asiri daun pucuk merah adalah beta-pinene (39,09%) dan caryophyllene (23,74%). Penambatan molekul menunjukkan interaksi kuat antara senyawa minyak asiri dan enzim phenylalanine hydroxylase, dengan humulene, ledene, dan caryophyllene memiliki afinitas pengikatan tertinggi yang dipengaruhi oleh stabilitas molekuler enzim pada residu Phe A:254 dan Tyr A:325. Studi ini membuka peluang pengembangan produk berbasis minyak asiri untuk aplikasi kesehatan.*

Introduction

The paradigm of forest resource utilization has shifted towards prioritizing the benefits of non-timber forest products (NTFPs). This shift reflects global efforts to maintain the sustainability of forest ecosystems while providing more inclusive economic benefits to local communities (Bas et al. 2024). According to the Indonesian Ministry of Forestry Regulation No. 35 of 2007, NTFPs include forest products derived from plants and animals, including their derivatives and cultivated products, excluding timber. Previous research has shown that the leaves of the red shoot plant (*Syzygium myrtifolium*) have the potential to produce essential oils. While people often use this plan for ornamental purposes, its economic value remains relatively low. However, it has the advantage of being highly adaptable to various environmental conditions. One unique characteristic of the red shoot plant is that its leaves emit a distinctive aroma when crushed, which is associated with the essential oil content found in various *Syzygium* species (Loukili et al. 2023).

Previous research has shown that essential oil from the leaves of *Syzygium myrtifolium* contains active compounds, including alkaloids, triterpenoids, steroids, saponins, flavonoids, and phenolics (Loukili et al. 2023). The isolation of essential oil from *Syzygium aromaticum* using the hydrodistillation method produced a yield of 0.1089%, with 42 compounds identified through Gas Chromatography-Mass Spectrometry (GC-MS) (Benmakhlouf et al. 2022). However, there is no information on the quality of essential oils produced.

Phenylalanine hydroxylase plays an essential role in dopamine biosynthesis by converting phenylalanine into tyrosine (Daubner et al. 2011). Balanced dopamine supports bodily functions like mood regulation and movement (Speranza et al. 2021). Disruptions in the functions can lead to serious health issues that affect quality of life (Radwan et al. 2019). Research shows that clove essential oil (*Syzygium aromaticum*) exhibits antidepressant effects by enhancing dopamine production in the brain, which helps reduce symptoms of depression and anxiety, evidenced by behavioral tests, such as the Forced Swimming Test (FST) and Tail Suspension Test (TST), which show a decrease in immobility time in mice (Astuti et al. 2022).

However, little research has been conducted on *Syzygium myrtifolium* to date. Therefore, this research aimed to evaluate the effect of leaves storage duration on yield, essential oil quality, and compliance with the Indonesian National Standard (SNI) 06-237-2006 on clove leaf oil. This research examines how the bioactive compounds of *Syzygium myrtifolium* interact with enzymes that influence dopamine hormone secretion through molecular docking of phenylalanine hydroxylase enzyme using in silico testing.

Methods

Essential Oil Distillation

This research experiment employed a completely randomized design (CRD) with storage duration treatments replicated three times, namely fresh storage, 24-hour storage, and 48-hour storage, resulting in nine experimental units. It started with preparing raw materials, comprising young leaves of the *Syzygium myrtifolium* plant sourced from IPB University and steam distillation equipment. The raw materials were sliced to facilitate the evaporation of essential oils, increase the surface area for distillation, and maximize the exposure of oil glands in the raw materials. The distillation duration was six hours, using 1.5 kg of raw material and 6000 ml of water. Subsequently, the obtained essential oil was stored in 10 ml dark glass vials. Anhydrous sodium sulfate was used to separate the oil from water, resulting in water-free essential oil (Hamidi 2024).

Essential Oil Quality Testing

Essential oils have physical properties that could be determined through several testing methods to assess the quality of the distilled oil. The leaves of the *Syzygium myrtifolium* plant belonged to the same genus as clove plants, allowing the comparison of quality characteristics of *Syzygium myrtifolium* essential oil with those of clove leaf oil according to the SNI for clove leaf oil 06-237-2006 (Table 1).

Table 1. Indonesian National Standard (SNI) for clove leaf oil 06-237-2006

Test Type	Unit	Standard Requirement
Color	-	Yellow-brown
Odor	-	Characteristic of oil
Solubility in Ethanol	-	1 : 2 Clear
Refractive Index ($^{n}D_{20}$)	-	1,025-1,049

Source: National Standardization Agency (2006)

Yield

The destination process yielded essential oil, which was subjected to quality testing. Yield refers to the amount of oil obtained during the distillation process, expressed as a percentage (Hamidi et al.2024) obtained from the ratio of oil produced to the initial amount of raw material before processing. In addition to yield measurement, moisture content was measured to determine the water content in the essential oil, which affected its quality and stability. Moisture content was measured using the gravimetric method by heating the sample in an oven at 103 ± 2 °C for 24 hours until it reached a constant weight. The moisture percentage was calculated based on the weight difference before and after drying, ensuring an accurate assessment of the oil's purity and compliance with quality standards (Hamidi et al.2024).

Refractive Index

In this research, the refractive index of the essential oil was measured using a refractometer. Before the measurement, water was run through the refractometer to ensure the device reached the desired measurement temperature. In addition, before placing the oil into the instrument, the oil must be at the same temperature as the measurement temperature. Readings were observed once the temperature had stabilized to ensure the accuracy of the result.

Solubility in Ethanol

Solubility in ethanol was the ratio of essential oil dissolved completely in ethanol. In this research, 1 ml of oil was added to a 10 ml test tube, and then small amounts of 70% ethanol were added while stirring. Observation of any changes in the solution was carried out, and the volume and concentration of ethanol required for the solution to become clear were recorded. Ethanol was continuously added until the volume reached 10 ml. The solubility results of the oil were compared to the solubility standards for clove oil (Table1).

Organoleptic Test

Essential oils, in their fresh and pure state, were generally colorless. However, with prolonged storage, essential oils could undergo oxidation (Sharmeen et al. 2021). Visual observation using direct sensory perception was used to characterize the color of essential oils (Hamidi et al. 2024). The essential oil yield evaluation for its characteristic plant aroma used an organoleptic test, which required sensory assessment using the sense of smell. The organoleptic test employed a panel of 20 individuals comprising 10 males and 10 females. Before the evaluation, the panelists were asked to sniff the aroma of coffee to neutralize their sense of smell. Then, they were instructed to smell the aroma of crushed *Syzygium myrtifolium* leaves and the distilled essential oil yield.

Chemical Compound Analysis of Essential Oil from Red Shoot Plant

Based on the quality characteristics of *Syzygium myrtifolium* essential oil, one sample of the best extract was subjected to phytochemical profiling using the Gas Chromatography-Mass Spectrometry (GC-MS) analysis with an Agilent 19091S GC-MS system equipped with an HP-5 MS UI column (5% phenyl methyl siloxane) of 30 m length, 0.25 µm diameter, and 0.25 µm film thickness. The injector used was an auto-injector with an injection temperature of 280 °C, volume of 1 µl in split mode (50:1 v/v), and 3 µl/min speed. The oven temperature was programmed to start at 40 °C for 5 minutes, then increase by 5 °C/min until reaching 280 °C, where it was held for 20 minutes. Helium (He) functioned as the carrier gas at a 1 ml/min flow rate, a linear velocity of 36.26 cm/s, and a pressure of 7.07 psi. The ion source temperature was set at 230 °C, while the quadrupole temperature was maintained at 150 °C. Ionization

occurred at 70 eV using electron ionization (EI), classified as hard ionization. The tested compounds were identified and re-examined using the MassHunter software integrated with the Agilent GC-MS instrument, following database library analysis for compound validation. In addition, the quantification of identified compounds was performed using peak area normalization, where the relative percentage of each compound was calculated based on its peak area compared to the total chromatogram area. Internal standards using eugenol or external standards using standard solutions were recommended for quantification to enhance data reliability.

Prediction of Bioactive Metabolites In Silico (Molecular Docking)

Based on the metabolite profiling results, the 3D structures of the compounds were obtained from the PubChem and ChemSpider databases, resulting in a total of 14 structures in .sdf file format. The geometries of these compounds were then optimized using the Newton-Raphson algorithm within a molecular mechanics approach, implemented through the Open Babel (Obabel) software, generating .pdb files. Subsequently, partial charges were assigned to these structures using the Gasteiger method, producing pdbqt files. For the structure of the phenylalanine hydroxylase enzyme (PAH), partial charges were assigned using the same method as for the ligands, with the AutoDockTools 1.5.7 software, leading to pdbqt files. The receptor structure of phenylalanine hydroxylase with PDB code 6PAH was used in this research. Screening of 14 compounds was conducted using AutoDock Vina 1.2.5, with each compound tested in 10 repetitions. The screening results were ranked, and the top two compounds were further analyzed using PyMOL 3.1 and LigPlot 2.28 (Mustofa et al. 2023). The toxicity assessment of essential oil compounds from *Syzygium myrtifolium* was conducted using in silico predictive models. In addition, this was carried out where pharmacokinetic properties, including gastrointestinal absorption, blood-brain barrier permeability, and cytochrome P450 interactions, were analyzed using SwissADME (http://www.swissadme.ch/) during toxicity evaluations. This included hepatotoxicity, mutagenicity, carcinogenicity, and immunotoxicity, which were predicted using ProTox-III (https://tox.charite.de/protox3/), with cytotoxicity parameters such as IC_{50}, LC_{50}DM, and LC_{50}FM further analyzed to determine potential biological activity based on NR-Aromatase classification (Nursyarah et al.2023).

Result and Discussion

Essential Oil Quality

The essential oil yield obtained from *Syzygium myrtifolium* showed significant variation depending on the treatment time (Table 2). The fresh sample yielded 0.102%, which increased to 0.204% after 24 hours of treatment and then decreased to 0.140% after 48 hours. These results indicated that treatment time affected the efficiency of essential oil extraction, with the 24-hour treatment yielding the optimal result. Previous research had reported that essential oil yield was influenced by the moisture content of the material and the storage time, with materials left for a specific period before extraction tending to produce higher yields due to the release of volatile compounds from the tissue matrix (Hamidi et al. 2024), supported the findings of this research, which showed an increase in yield in the 24-hour sample compared to the fresh sample.

The color of the essential oil from red shoot plants showed a significant change from yellow (fresh) and yellow-brown (24 hours) to bright yellow (48 hours) (Figure 1). Active compounds such as flavonoids and other essential oils susceptible to oxidation during storage influenced this change (de Sousa et al. 2023). According to the SNI, the ideal color for clove leaf

Table 2. Essential oil quality of *Syzygium myrtifolium* from three storage duration treatments.

Sampel	Fresh	24 Hours	48 Hours
Yield	0.102% ± 0.01	0.204% ± 0.015	0.140% ± 0.02
Moisture Content	70% ± 0.43%	70.73% ± 2.62%	71.51% ± 2.01%
Color	Yellow	Yellow-brown	Yellow-brown
Solubility in Ethanol	1:3	1:2	1:2
Organoleptic	5 People	11 People	People
Refractive Index	1.343	1.339	1.333

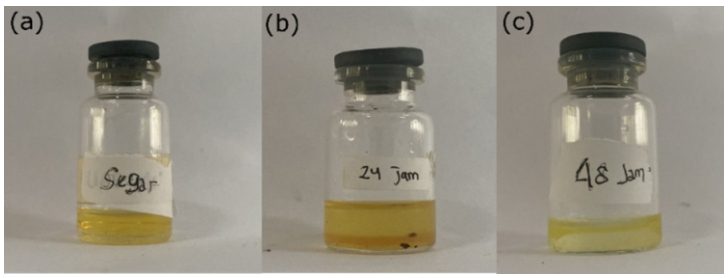

Figure 1. Color of essential oil from red shoot plants with storage duration treatments: (a) fresh, (b) 24 hours, (c) 48 hours.

essential oil was yellow-brown, which made the essential oil from the 24-hour treatment the closest to this standard. Previous research suggested that the color of essential oils was greatly affected by the oxidation process of phenolic compounds during storage, which could alter the visual characteristics of the oil (Chen & Sun 2023), aligned with these results, where the 24-hour storage treatment produced a color that more closely aligned with the standard.

The refractive index of the essential oil from red shoot plants ranged from 1.333 to 1.343, with the highest value observed in the fresh sample and a decrease in the 48-hour treatment. This decline was due to the degradation of volatile compounds, as indicated by GC-MS analysis, which showed a reduction in key monoterpenes and sesquiterpenes such as beta-pinene (39.09%) and caryophyllene (23.74%). The decrease in refractive index over storage time resulted from oxidation and polymerization of monoterpenes, leading to compositional changes in volatile compounds. According to the SNI, the refractive index of clove leaf essential oil ranged from 1.025 to 1.049, indicating that the essential oil from red shoot plants had a higher refractive index than this standard. This finding was consistent with previous literature stating that monoterpenes underwent oxidation during storage, forming oxygenated compounds with lower volatility, ultimately reducing the refractive index (Lima et al. 2021), reflecting the unique characteristics of this species. Previous research reported that the content of volatile compounds such as eugenol and methyl eugenol influenced the refractive index of essential oils (Siddiqui et al. 2024). The decrease in the refractive index in the 48-hour treatment was due to the degradation of volatile compounds during storage, particularly the reduction in monoterpenes such as beta-pinene (39.09%) and caryophyllene (23.74%) that were predominant in the 24-hour storage treatment. In addition, other compounds such as alpha-pinene (9.98%), D-limonene (2.36%), and aristol-(10)-ene (2.06%) also experienced slight reductions after prolonged storage. These findings suggested that monoterpenes and sesquiterpenes played a significant role in maintaining the refractive index of the essential oil. Therefore, the best treatment obtained in this research, which was closest to the SNI standard for clove leaf oil (SNI 06-237-2006), was the 24-hour storage treatment.

The Chemical Compound of Essential Oil

The chemical compounds in *Syzygium myrtifolium* essential oil were determined using the essential oil with the closest characteristics to the SNI standard for clove leaf oil (SNI 06-237-2006). The GC-MS chromatogram showed varying retention times and relative abundances (Figure 2). The main peak appeared at a retention time of approximately 25.119 minutes, indicating the compound with the highest relative abundance in this sample. There were extra peaks at retention times of 5.168 minutes and 48.873 minutes, indicating the presence of other volatile compounds with lower abundances. The presence of these peaks suggested a complex mixture of secondary metabolites (Ajilogba & Babalola 2019).

In addition, 12 compounds were identified (Table 3), with beta-pinene as the main compound (39.09%), followed by caryophyllene at 23.74%. Previous research revealed that these two compounds positively impacted dopamine hormones and general neurological health (Juárez Olguín et al. 2016). Beta-pinene could reduce inflammation and improve cognitive function in animal models subjected to oxidative stress (Salehi et al. 2019). Others reported that caryophyllene could reduce neuroinflammation and protect dopaminergic neurons from damage

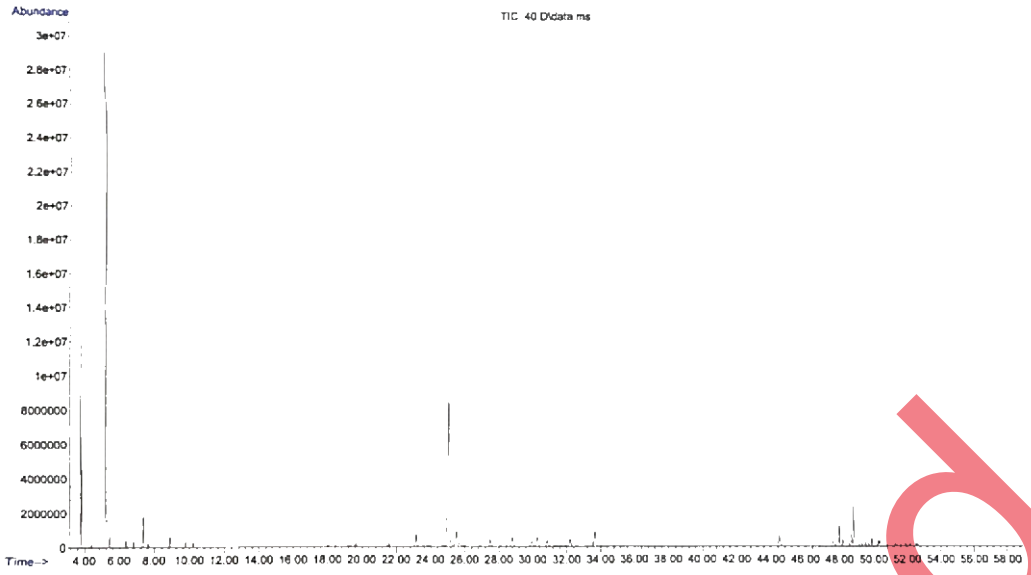

Figure 2. Composition of secondary metabolites from essential oil distillation.

Table 3. Composition of secondary metabolites of *Syzygium myrtifolium* essential oil of 24-hour storage duration treatment.

RT (Minute)	Similarity Index	Compound	Component Percentage (%)
5.167	94	Beta-pinene	39.09
25.121	99	Caryophyllene	23.74
3.739	95	Alpha-pinene	9.98
33.663	99	Cadina-1(10), 4-diene	2.68
7.338	98	D-Limonene	2.36
48.872	96	Aristol-1(10)-ene	2.06
44.485	83	Khusilal	1.74
25.529	99	Aromandendrene	1.71
23.137	92	Cyclofenchene	1.51
48.003	98	γ-Gurjunene	1.4
28.8	96	Humulene	1.28
32.201	98	Ledene	1.09

Table 4. Cytotoxic properties of *Syzygium myrtifolium* essential oil of 24-hour storage duration treatment.

Compound	IGC50	LCD50DM	LC50FM	NR-A
Beta-pinene	4.597	5.333	5.465	O
Caryophyllene	4.381	5.169	5.194	O
Alpha-pinene	4.397	5.115	5.233	O
Cadina-1(10), 4-diene	4.598	5.115	5.568	O
D-Limonene	4.457	5.234	5.190	O
Aristol-1(10)-ene	4.853	5.198	5.776	X
Khusilal	3.901	5.426	4.967	O
Aromandendrene	4.830	5.375	5.887	O
Cyclofenchene	4.634	5.475	5.708	X
γ-Gurjunene	4.610	4.973	5.401	X
Humulene	4.479	5.444	5.712	O
Ledene	4,761	5.118	5.830	X

Note: O = active; X = inactive; IGC50 = Inhibitory Growth Concentration 50; LCD50DM = Lethal Concentration 50 in Daphnia Magna; LC50FM = Lethal Concentration 50 in Fathead Minnow; NR-A = Nuclear Receptor – Aromatase.

(Viveros-Paredes et al. 2017).

The *Syzygium myrtifolium* essential oil quality was compared to the SNI for clove leaf oil 06-237-2006 (Table 1). Previous research reported that the ethanol extract from *Syzygium jambos* leaves exhibited strong antioxidant activity (Sobeh et al. 2018). This research tested the toxicity of selected compounds from the GC-MS analysis against Inhibitory Growth Concentration 50 (IGC50), Lethal Concentration 50 in Daphnia Magna (LC50DM), Lethal Concentration 50 in Fathead Minnow (LC50FM), and Nuclear Receptor – Aromatase (NR-A) (Table 4), not on the therapeutic potential through toxicity parameters.

The research results indicated that the compounds beta-pinene, caryophyllene, and alpha-pinene in *Syzygium myrtifolium* essential oil exhibited toxicity potential, with IGC50 values ranging from 4 to 5 µg/ml, reflecting their ability to influence cellular biological activity. However, the positive activity against NR-Aromatase suggested the intervention of these compounds in the metabolic pathway of the aromatase enzyme, which played a significant role in dopamine biosynthesis. Previous research had reported that beta-pinene and caryophyllene possessed neuromodulatory potential, including the possibility of affecting the dopamine pathway. This was crucial in neurotransmitter function, particularly in mood regulation, stress response, and cognitive function, making these compounds potential candidates for therapeutic applications in neurological disorders (Ricardi et al. 2024).

Molecular docking with the phenylalanine hydroxylase receptor is a key enzyme involved in the metabolism of aromatic amino acids. This enzyme was particularly crucial in converting phenylalanine to tyrosine, as tyrosine was the primary precursor for dopamine synthesis (Meiser et al. 2013). The dopamine biosynthesis pathway included the conversion of phenylalanine to tyrosine by the phenylalanine hydroxylase (PAH) enzyme, after which tyrosine was converted to L-3,4-dihydroxyphenylalanine (L-DOPA) by the tyrosine hydroxylase (TH) enzyme. L-DOPA was then converted to dopamine by DOPA decarboxylase (Harding et al. 2014). Based on Table 5 and Figure 3, the molecular docking results of the phenylalanine hydroxylase receptor with the DAH ligand showed binding affinity and efficiency parameters.

The Binding Affinity results showed the free binding energy of the ligand on the enzyme, with negative values indicating stable interactions. For the DAH ligand, the flexible binding affinity was -6.477 kcal/mol, while under rigid conditions, it was stronger at -7.925 kcal/mol. This difference suggested that the ligand or enzyme's flexibility affected the interaction's stability. Negative free energy values below -5 indicated stronger interactions (Mohanty & Mohanty 2023). The binding energy efficiency per unit molecular weight of the ligand was also better under

Table 5. Affinity of phenylalanine hydroxylase (PAH) enzyme structure binding and co-crystalline ligands.

PDB ID	Code Ligan	Binding Affinity-Fleks (kkal/mol)	Binding Affinity-Rigid (kkal/mol)	Ligand Efficiency-Fleks (kkal/mol)	Ligand Efficiency-Rigid (kkal/mol)	RMSD-Fleks (Å)	RMSD-Rigid (Å)
6PAH	DAH	-6,477	-7,925	-0.4626	-0.5661	0.6101	0.6251

Note: PAH = phenylalanine hydroxylase; DAH= co-crystallized ligand (Dihydroxyphenylalanine Analog); RMSD = Root Mean Square Deviation.

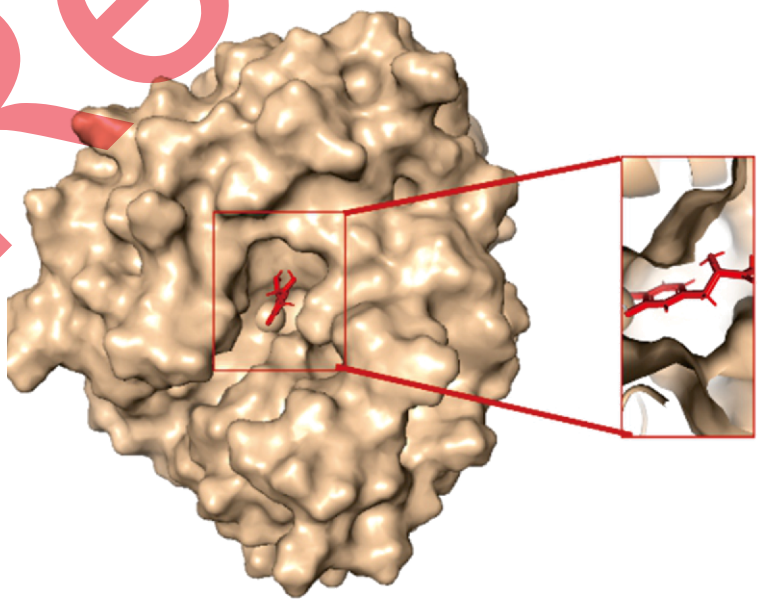

Figure 3. Results of molecular docking on the 6PAH enzyme.

rigid conditions (-0.5661 kcal/mol) compared to flexible conditions (-0.4626 kcal/mol), indicating that rigidity enhanced optimal interactions. In addition, the Root Mean Square Deviation (RMSD) was a validation parameter showing the deviation in docking ligand atom positions. An RMSD < 2 Å was ideal, indicating that the docking results successfully mimicked the ligand's position as in the crystal structure (Torres et al. 2019). The flexible RMSD (0.6101 Å) and rigid RMSD (0.6251 Å) showed that the docking results successfully mimicked the ligand's position compared to the crystal structure.

Virtual screening used AutoDock Vina through docking 12 compounds from the metabolite profiling onto the 6PAH enzyme structure, resulting in affinity energy data. The screening results in Table 6 ranked 12 compounds according to the highest concentration abundance in the essential oil of *Syzygium myrtifolium*. The more negative the value of binding affinity, the higher the interaction. The ligand efficiency values, provided information about how efficiently the compounds bind to the receptor compared to their molecular size (Torres et al. 2019).

The virtual screening indicated that the *Syzygium myrtifolium* essential oil bioactive compounds had a strong binding affinity toward the phenylalanine hydroxylase receptor. Humulene had the highest binding affinity (-8.598 kcal/mol), followed by ledene (-8.411 kcal/mol) and caryophyllene (-8.444 kcal/mol). Beta-pinene had more negative ligand efficiency values (-0.6380 kcal/mol) than other compounds, indicating optimal binding energy efficiency. In contrast, D-Limonene (-6.240 kcal/mol) showed a weaker interaction due to low affinity. Compounds with lower torsion values (torsion = 0 in humulene) tended to be more stable and precise in binding to the enzyme's active site than more flexible ligands. Previous research reported that beta-sitosterol from the *Syzygium* genus contributed to brain health by enhancing cell membrane function and supporting synaptic transmission of dopamine (Mehla et al. 2020). This interaction suggested terpenoid compounds, such as those found in *Syzygium myrtifolium*, had great potential as inhibitors of phenylalanine hydroxylase through stable and selective binding mechanisms.

Figure 4 indicated the binding positions to the specific amino acid receptor and suggesting a selective interaction pattern within the active site of phenylalanine hydroxylase. Beta-pinene and caryophyllene demonstrated molecular binding mechanisms that required dominant interactions such as Pi-Alkyl and Van der Waals, stabilizing their association with key active residues Phe A:254, Val A:245, and Leu A:248. The smaller and more flexible structure of beta-pinene allowed it to adapt efficiently within the hydrophobic core of the enzyme, facilitating transient but significant binding interactions. However, with its larger cyclic and rigid structure, caryophyllene formed multiple contact points, integrating hydrophobic and aromatic interactions, including stabilization through Tyr A:325 and His A:264, contributing to the binding network. The Glu A:286 enhanced the interaction through hydrophilic contributions, further reinforcing ligand stability within the active pocket. The greater binding stability of caryophyllene, as indicated by its increased interaction points, suggested a higher affinity for phenylalanine hydroxylase. This result aligned with previous findings, showing that hydrophobic and aromatic interactions significantly enhanced ligand affinity toward this enzyme (Conde-giménez et al. 2022). These interactions reduced the likelihood of ligand

Table 6. Binding affinity and ligand efficiency of *Syzygium myrtifolium* essential oil bioactive compounds.

Compound	Average Binding Affinity	Average Ligand Efficiency	Number of Torsion
Beta-pinene	-6.375	-0.6380	0
Caryophyllene	-8.444	-0.5629	0
Cadina-1(10), 4-diene	-8.173	-0.5428	1
D-Limonene	-6.240	-0.6242	1
Aristol-1(10)-ene	-7.516	-0.5015	0
Aromandendrene	-8.415	-0.5610	0
Cyclofenchene	-6.034	-0.6040	0
γ-Gurjunene	-8.332	-0.5550	1
Humulene	-8.598	-0.5736	0
Ledene	-8.411	-0.5619	0

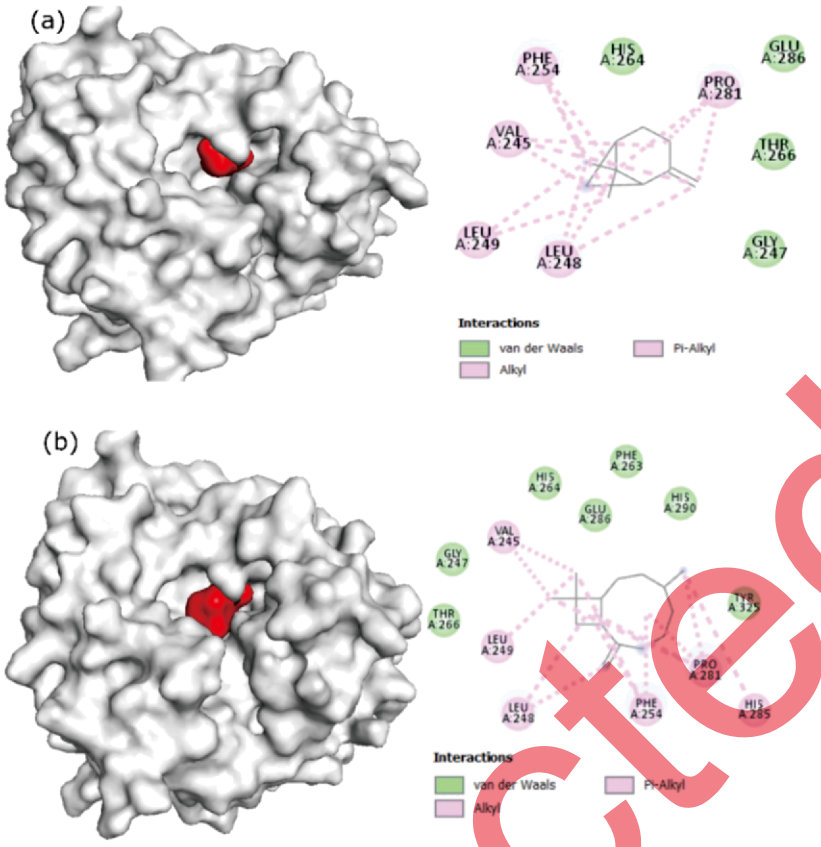

Figure 4. Ligand-receptor interactions for ligands with the best molecular binding activity on compounds (a) beta-pinene and (b) caryophyllene.
Note: The amino acid residues GLU (glutamate), LEU (leucine), VAL (valine), PHE (phenylalanine), THR (threonine), PRO (proline), GLY (glycine), and HIS (histidine) interact through van der Waals, Alkyl, and Pi-Alkyl bonds, supporting the stability of molecular binding.

dissociation, which improved inhibitory potential. These results emphasized the potential role of beta-pinene and caryophyllene as phenylalanine hydroxylase inhibitors, supporting their applicability in developing natural compound-based therapies targeting dopamine-related metabolic pathways.

Conclusion

In conclusion, this research demonstrated that raw material storage duration significantly affected essential oil yield, quality, and bioactivity from *Syzygium myrtifolium* leaves. A 24-hour storage duration resulted in an optimal yield of 0.204%, with a yellow-brown color, approaching the SNI for clove leaf oil (06-237-2006). The refractive index of the essential oil ranged from 1.333 to 1.343, which was higher than the standard, indicating the unique chemical composition of this species. The GC-MS analysis identified 12 major compounds, with beta-pinene (39.09%) and caryophyllene (23.74%) as dominant compounds. Toxicity testing revealed that these compounds exhibited significant biological activity with IGC50 values ranging from 4-5 µg/ml. The virtual screening revealed strong interactions between the essential oil compounds and phenylalanine hydroxylase enzyme, with humulene, ledene, and caryophyllene showing the highest binding affinity. The active enzyme residues, such as Phe A:254 and Tyr A:325, reinforced these interactions by ensuring molecular stability, playing a crucial role in the compound binding process.

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