Prediction of Tyrosinase Inhibitory Activity of Compounds from *Zingiber zerumbet* L. using *In Silico* Docking and Drug-Likeness Methods

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Abstract

Tyrosinase is a polyphenol oxidase containing a metalloenzyme that catalyses the oxidation process in melanin synthesis, causing skin hyperpigmentation. Studies on tyrosinase inhibition are gaining significant attention for treating skin hyperpigmentation and may find further usage in agriculture, cosmetics and pharmacology. Zingiber zerumbet, also known as "bitter ginger" or "pinecone ginger", is a plant rich in anti-inflammatory and antioxidant bioactive compounds. In this researh, we applied molecular docking and drug-likeness to screen and predict the interaction between compounds from Zingiber zerumbet and tyrosinase. Molecular docking of these compounds onto tyrosinase was executed using Autodock Vina and the SwissADME online tool to evaluate the drug-likeness/ADME properties.

The screening results identified 5 compounds with the most effective docking energies: Germacrene B (-7,0 kcal/mol), 3-methyl kaempferol (-7,3 kcal/mol), kaempferol-3-O-methyl ether (-7,4 kcal/mol), α-Cedrene (-7,5 kcal/mol) and kaempferol (-7,6 kcal/mol). According to Lipinski's rule of five, the drug-likeness/ADME suggested that all 5 compounds from Zingiber zerumbet effectively suppress the activity of tyrosinase.

Keywords: Molecular docking, drug-likeness/ADME, *Zingiber zerumbet.* L, melanin, tyrosinase inhibition.

Introduction

Tyrosinase (TYR) is a dicopper oxidase containing metalloenzymes primarily found in all living organism, from bacteria to humans, where it produces melanin. TYR is a glycoprotein and is synthesed by melanocytes. TYR undergoes glycosylation, which assists in melanosome metabolism and the synthesis of melanin[25]. The substances L-tyrosine and L-DOPA are respectively needed in the initial and subsequent steps of melanin production[17]. Initially, TYR catalyses the conversion of L-tyrosine to L-DOPA through hydroxylation and subsequently, L-DOPA is changed to dopaquinone (the di-phenolase activity of TYR)[17]. Furthermore, TYR catalyses the oxidation of 5,6-dihydroxyindole (DHI) to form indole-5,6-quinone, a precursor of melanin[18]. As TYR participates in all three stages of melanin formation, its inhibition prevents melanin biosynthesis and provides a scientific basis for screening compounds to treat skin disorders[18].

Numerous methods have been reported for controlling melanin synthesis by targeting tyrosinase, primarily through inhibiting its activity. The most common tyrosinase inhibitors on the market include azelaic acid, magnesium L-ascorbyl-2-phosphate, phenol, hydroxyanisole, corticosteroids, N-acetyl-4-S-cysteaminylphenol, resinoids, arbutin, kojic acid, salicylic hydroxamic acid, hydroquinone (HQ), monobenzyl hydroquinone, tretinoin and mercury salts, which are frequently used in beauty products[11,24]. However, in clinical trials, arbutin and kojic acid have exhibited limited inhibition against melanin production in intact melanocytes. Hydroquinone has been deemed toxic to melanocytes and is potentially capable of causing mutations to animal cells[23]. Consequently, the search for new tyrosinase inhibitors from plant sources without adverse side effects has become a focus of research.

The utilization of traditional plants to combat skin diseases, particularly for cosmetic purposes, is a traditional remedy found in many cultures and new discoveries have provided more effective agents that help reduce pigmentation[4]. *Zingiber zerumbet* also known as "bitter ginger" or "pinecone ginger", is a medicinal herb found in India and the Malaysian peninsula[10,14]. Numerous studies have reported its biological activities like antibacterial, anti-allergic and immune-regulating properties[13,22], analgesic and anticancer properties[9] and especially the anti-oxidant properties[19]. The compounds found in the Z. *zerumbet*, with their anti-oxidant, are a natural source of medicine capable of inhibiting TYR activity.

This study aims to predict whether the active compounds in the essential oil of *Z. zerumbet* exhibit antioxidant properties and inhibit TYR thrrough *in silico* docking and drug-likeness/ADME assessments.

Material and Methods

In this study, we use the compounds found in the *Z. zerumbet* as a ligand for the tyrosine receptor through *in silico* docking and evaluating their binding energies. Based on the results, the compounds with the strongest TYR inhibition potential

were selected. Finally, the best compounds were analyzed for their biological applicability using the drug-likeness/ADME method.

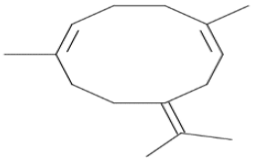
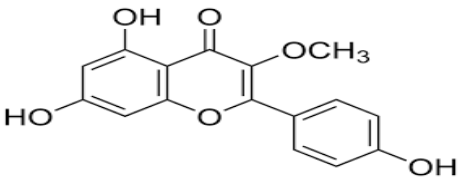
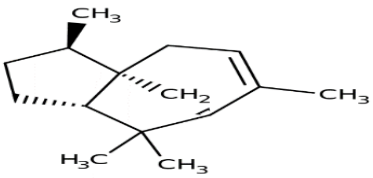
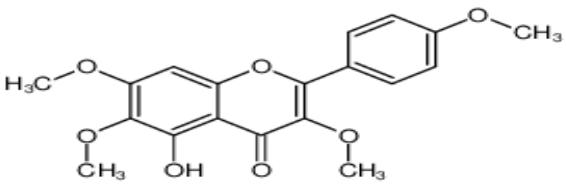
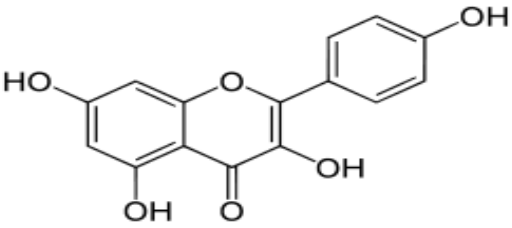
Data collection and ligand conformation of compounds from Zingiber zerumbet: The compound data for *Z. zerumbet* was collected from various sources including NCBI, Google Scholar and scientific articles, resulting in the identification of 114 compounds extracted from *Z. zerumbe*t. These compounds extracted from *Z. zerumbet* were selected as ligands. The structures of these ligands were collected from PubChem.

Target protein structure: The target protein structure was selected from the Protein Data Bank (PDB ID 2Y9X) presenting the 3D structure of TYR from *Agaricus bisporus*.

Molecular docking: This molecular docking study used Autodock Vina as the docking engine[6]. The docking grid was set at coordinates x = -10.04, y = -29.006 and z = -43.501 with a box size of x = 16, y = 16 and z = 16. The binding energies of the best ligands were organized into tables and the interaction details were illustrated using Biovia Discovery Studio 2017R2 Client. 2D and 3D interaction details of ligands-receptors were generated ligands with the best binding interactions with the target protein.

Drug-likeness /ADME analysis: Chemical evaluation of the drug-likeness is based on Lipinski's rule of five[15] including assessments of physicochemical properties, lipophilicity, water solubility, pharmacokinetics and overall drug-likeness. ADME was performed with the Swiss-ADME online tool[5]. Swiss-ADME was performed using the 3D structure of the ligands and the results were organized into tables.

Table 1
Five compounds predicted to be the most potent to inhibit tyrosinase enzyme

S.N.	Sign	Compound name	Structure
1	A	Germacrene B	
2	B	3-metyl kaempferol	
3	C	Kaempferol-3-O-methylether	
4	D	α-Cedrene	
5	E	Kaempferol	

Results

Studies on molecular docking: Research on molecular docking has identified the compatibility of the ligand-receptor interactions for the 144 compounds from the *Z. zerumbet* based on their binding affinities. The results show that all the compounds exhibited inhibitory activity against the TYR enzyme. Among them, five compounds were predicted to be the most potent: Germacrene B (A), 3-methyl kaempferol (B), kaempferol-3-O-methylether (C), α-cedrene (D) and kaempferol (E). The chemical structures of compounds are depicted in table 1 and their binding affinities are presented in table 2.

Based on the ligand-protein and target protein receptor binding energy results, the top 5 compounds with the highest binding energies are presented in both 3D and 2D models (Figures 1-5). Interaction between kaempferol ligand and TYR receptor (Figure 1) indicates that interactions are stabilized through hydrogen bonds interactions with amino acid residues Met 280 and Asn 260, as well as a carbon-hydrogen bond at His 85. Three stacked pi-pi interactions contribute to the interaction, acting as a scaffold between kaempferol and the TYR residues His 263, Phe 264 and Ser 282. Additionally, pi-sigma interactions at Val 283 and pi-alkyl interactions at Val 248 and Ala 286 provide binding energy to the complex.

The interactions between the ligands kaempferol-3-O-methylether and 3-methyl kaempferol with the TYR receptor (Figures 2-3) exhibit similar bonding patterns to those of kaempferol due to their similar chemical structures. However, these two receptors lack a hydrogen bond at position Asn 260, resulting in weaker binding energies to the TYR receptor compared to kaempferol. Additionally, the interaction of 3-methyl kaempferol involves an unfavorable donor-donor interaction at His 263 of TYR, which may introduce repulsive forces between the ligand and the target, reducing the binding energy.

The α-cedrene ligand forms stable bonds through alkyl and pi-alkyl interactions at residues Ala 286, His 263 and Val 283, as well as a pi-sigma bond with Phe 264, resulting in stronger binding energy for the complex (Figure 4). Similarly, the germacrene B ligand interacts with the TYR receptor via alkyl and pi-alkyl bonds at residues Val 28, His 85, His 61, His 244, along with a pi-sigma bond at His 263. However, due to the location of these bonds in the ligand's side chain, the binding energy is lower compared to the α-cedrene ligand (Figure 5).

Analysis of drug-likeness and ADME properties: The basic physical and chemical characteristics of the five prospective drug candidates from the docking results are illustrated in tables 3, 4 and 5. The molecular mass of the selected compounds ranges from 204.35 to 286.24 g/mol. The calculated consensus LogP values (indicating lipophilicity) for the selected compounds range from 1.58 to 4.60.

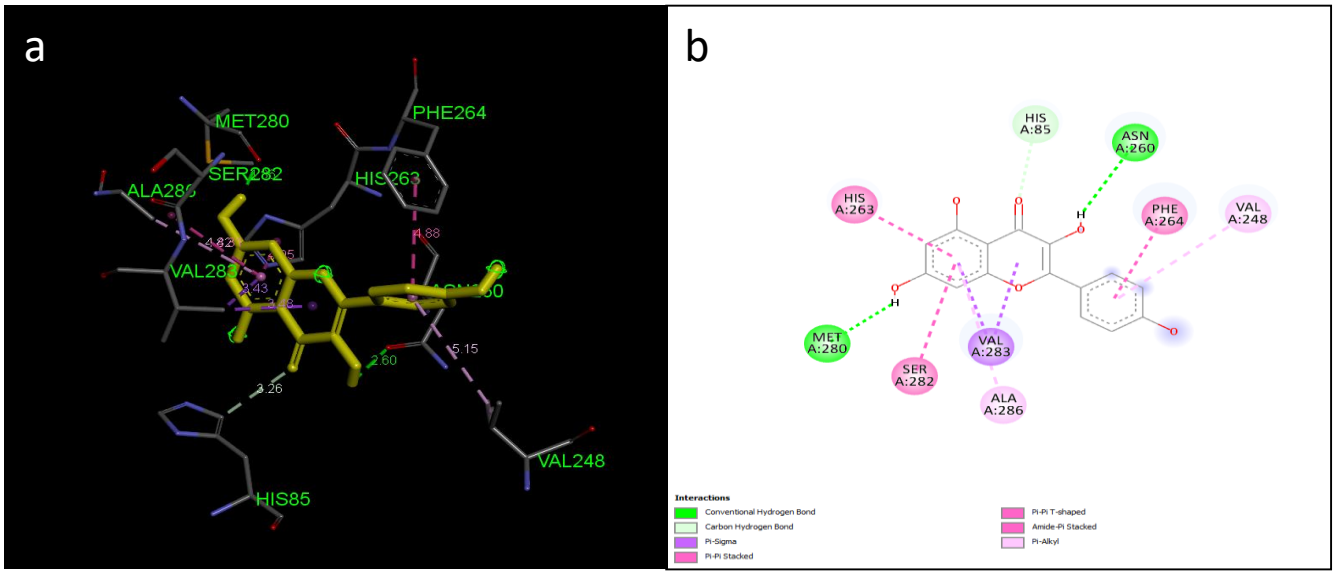


Figure 1: Interactions of TYR with kaempferol. a, 3 D interaction; b, 2 D interaction

Table 2
Binding energy of the five ligands

Phối tử	Ligand	Binding energy [a] (kcal/mol)
A	Germacrene B	-7,0
B	3-metyl kaempferol	-7,3
C	kaempferol-3-O-methylether	-7,4
D	α-Cedrene	-7,5
E	Kaempferol	-7,6

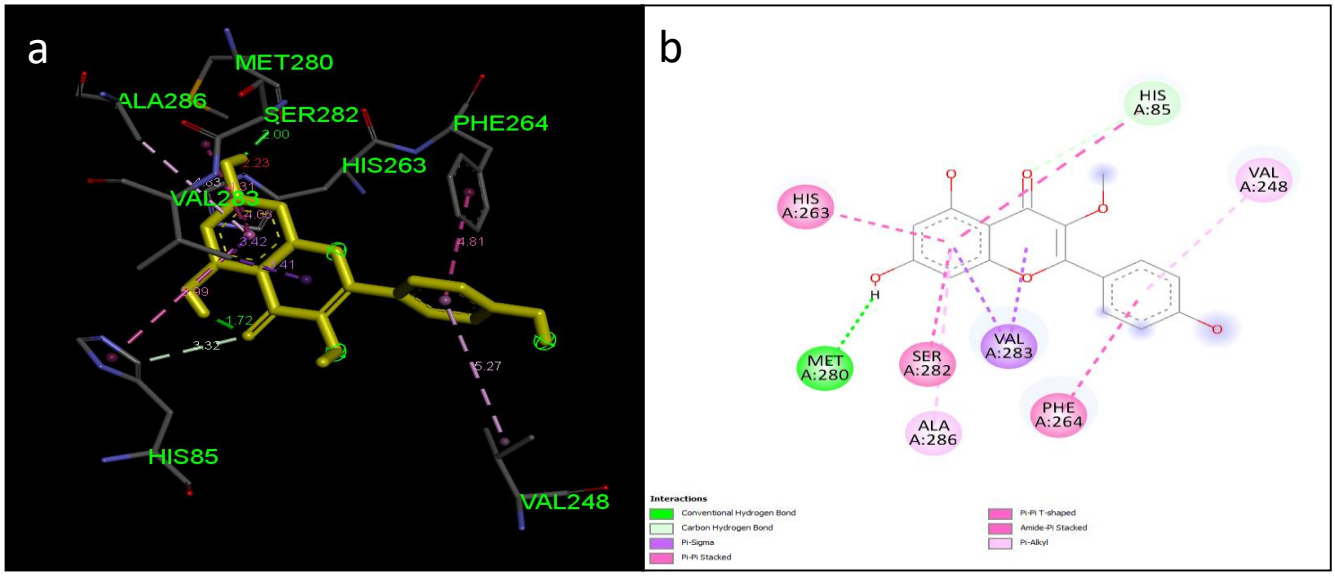


Figure 2: Interactions of TYR with kaempferol-3-O-methylether. a, 3 D interaction; b, 2 D interaction

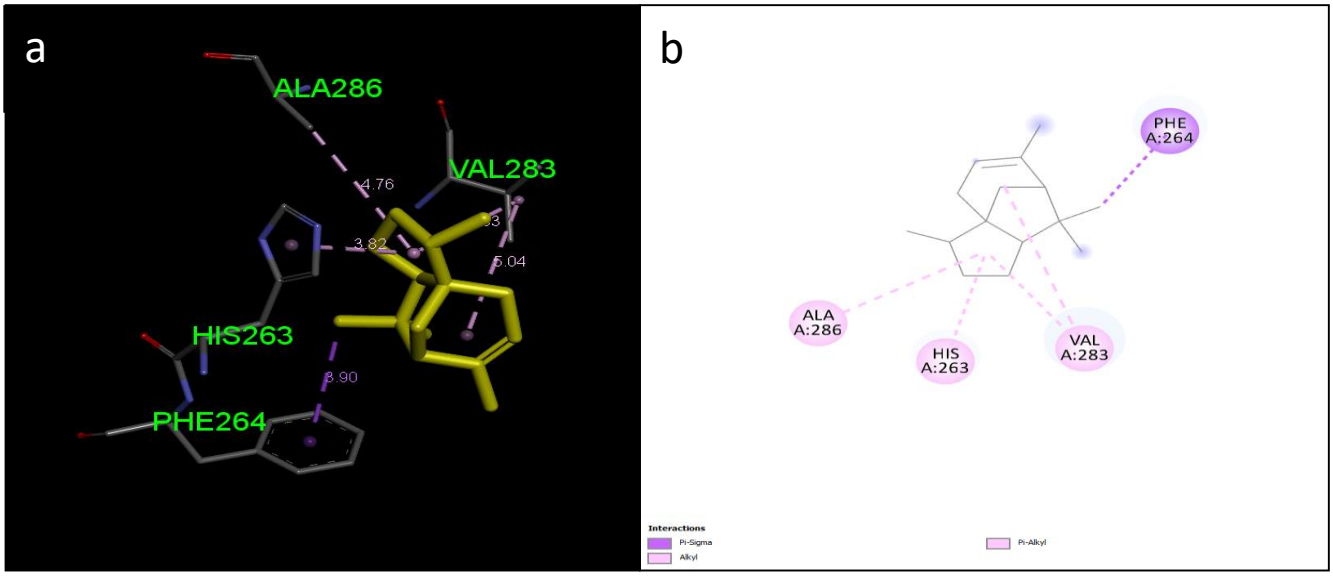


Figure 3: Interactions of TYR with 3-metyl kaempferol a, 3 D interaction; b, 2 D interaction.

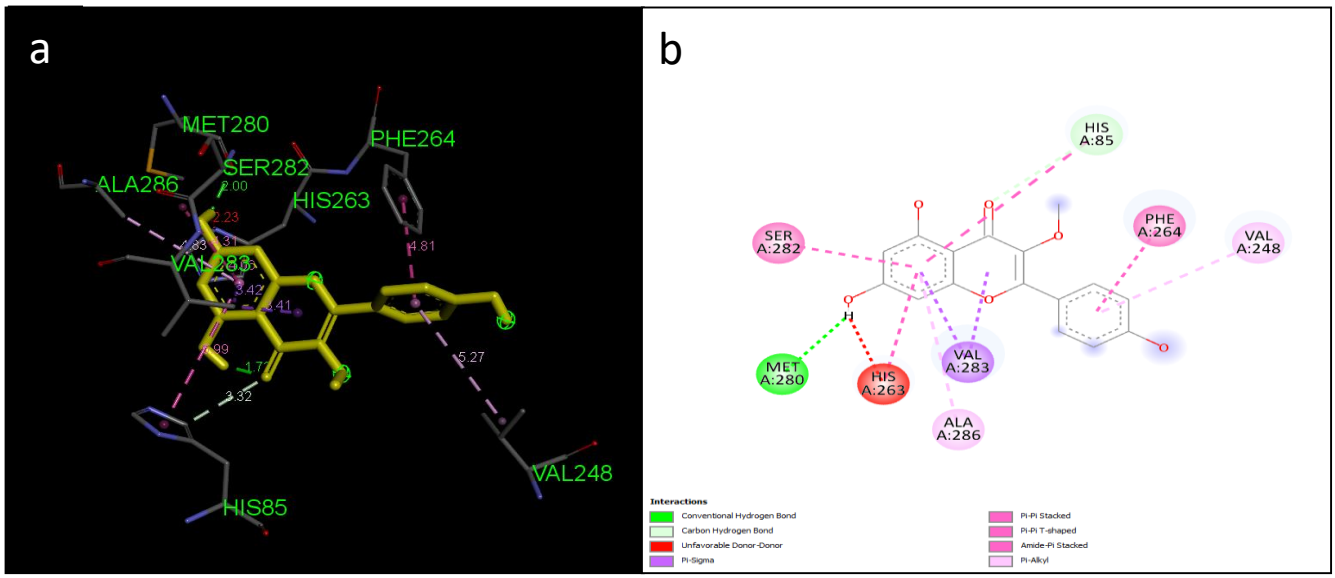


Figure 4: Interactions of TYR with α-Cedrene. a, 3 D interaction; b, 2 D interaction

ADME Parameters: The calculated ADME properties (gastrointestinal absorption, blood-brain barrier penetration, cytochrome P450 inhibition and P-glycoprotein substrances) of these compounds are presented in table 6. The results indicated that compounds E, C and B exhibited high gastrointestinal absorption, while compounds A and D showed low absorption.

All five compounds were predicted not to infiltrate the blood-brain barrier (BBB) and were not substrates for P-glycoprotein. Inhibition of cytochrome P450 (CYP) isoforms revealed that compounds B, C and E inhibited three isoforms. Compound D inhibited two isoforms and compound A inhibited only one isoform.

Drug-likeness and pharmacokinetic parameters of the compounds: Drug-likeness and pharmacokinetic and lead-likeness of the compounds are calculated and illustrated in table 7.

Based on research findings, compounds B, C and E did not violate any of the five filters (Lipinski, Ghose, Veber, Egan and Muegge), while compounds A and D presented violations of two criteria (Lipinski and Muegge). The bioavailability scores of all compounds were calculated and found to be 55%. Compounds A and D had two violations of the BRENK criteria.

Table 3
Physicochemical of the compound

Criteria	Formula	Molecular weight (g/mol)	No. of heavy atoms	No. of aromatic heavy atoms	Fraction Csp3	No. of rotatable bonds	No. of H-bond acceptors	No. of H-bond donors	Molar Refractivity
A	$C_{15}H_{24}$	204.35	15	0	0.6	0	0	0	70.68
B	$C_{16}H_{12}O_6$	300.26	22	16	0.06	2	6	3	80.48
C	$C_{16}H_{12}O_6$	300.26	22	16	0.06	2	6	3	80.48
D	$C_{15}H_{24}$	204.35	15	0	0.87	0	0	0	66.88
E	$C_{15}H_{10}O_6$	286.24	21	16	0	1	6	4	76.01

Table 4
Lipophilicity of the compound

Compound	TPSA	iLOGP	XLOGP3	WLOGP	MLOGP	Silicos-IT Log P	Consensus Log P
A	0.00	3.27	5.77	5.18	4.53	4.25	4.6
B	100,13	2,11	2,22	2,59	0,22	2,55	1,94
C	100.13	2.11	2.22	2.59	0.22	2.55	1.94
D	0.00	3.2	4.62	4.42	5.65	3.91	4.36
E	111.13	1.7	1.9	2.28	-0.03	2.03	1.58

Table 5
Water solubility of the compounds

Class	Compound	log S	Solubility [mg/ml]	Solubility [mol/l]	Level of solubility
ESOL	A	-4.74	3.70E-03	1.81E-05	Moderately soluble
	B	-3.51	9.36E-02	3.12E-04	Soluble
	C	-3.51	9.36E-02	3.12E-04	Soluble
	D	-4.02	1.96E-02	9.60E-05	Moderately soluble
	E	-3.31	1.40E-01	4.90E-04	Soluble
ALI	A	-5.54	5.91E-04	2.89E-06	Moderately soluble
	B	-3.96	3.31E-02	1.10E-04	Soluble
	C	-3.96	3.31E-02	1.10E-04	Soluble
	D	-4.35	9.23E-03	4.51E-05	Moderately soluble
	E	-3.86	3.98E-02	1.39E-04	Soluble
Silicos-IT	A	-3.75	3.63E-02	1.78E-04	Soluble
	B	-4.52	9.07E-03	3.02E-05	Moderately soluble
	C	-4.52	9.07E-03	3.02E-05	Moderately soluble
	D	-3.52	6.19E-02	3.03E-04	Soluble
	E	-3.82	4.29E-02	1.50E-04	Soluble

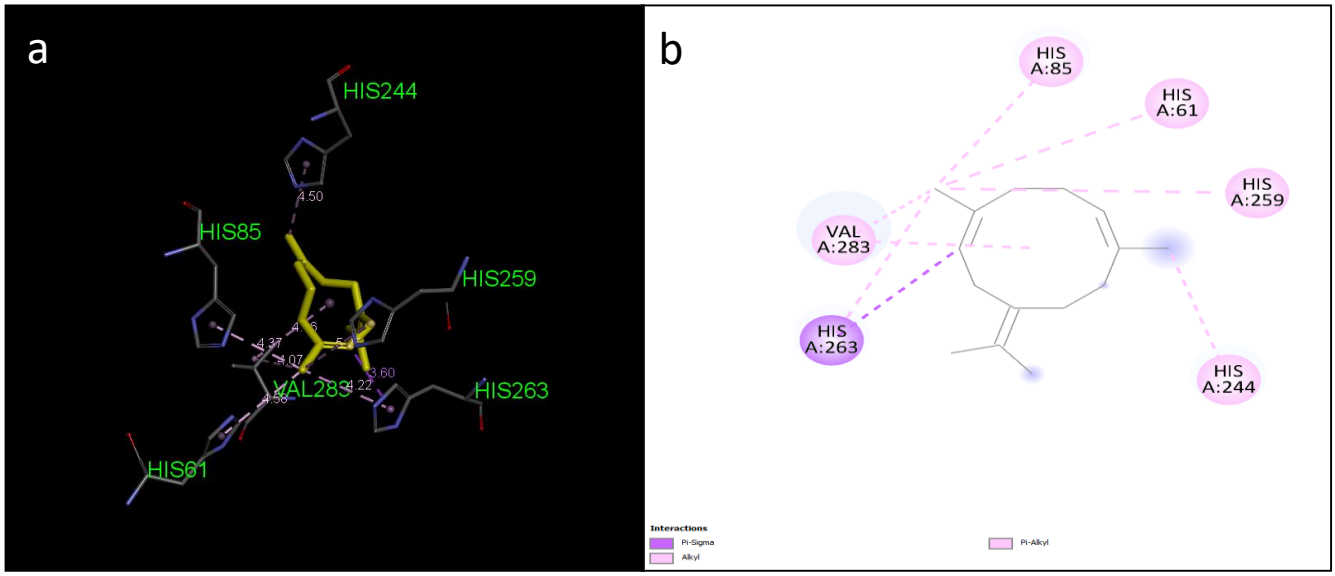


Figure 5: Interactions of TYR with Germacrene. a, 3 D interaction; b, 2 D interaction

Table 6
ADME properties of the compounds

	GI absorption	BBB permeant	Pgb Substrate	CYP Inhibitor					Log Kp (cm/s)
				CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4	
A	Low	No	No	No	No	Yes	No	No	-3.45
B	High	No	No	Yes	No	No	Yes	Yes	-6.56
C	High	No	No	Yes	No	No	Yes	Yes	-6.56
D	Low	No	No	No	Yes	Yes	No	No	-4.27
E	High	No	No	Yes	No	No	Yes	Yes	-6.70

Table 7
Drug-likeness and pharmacokinetic parameters of the compounds

	ABS	Violations						Alerts		Synthetic accessibility
		Lipinski	Ghose	Veber	Muegge	Egan	Lead-likness	PAINS	Brenk	
A	0.55	1	0	0	2	0	2	0	1	3.14
B	0.55	0	0	0	0	0	0	0	0	3.20
C	0.55	0	0	0	0	0	0	0	0	5.53
D	0.55	1	0	0	1	0	2	0	2	3.20
E	0.55	0	0	0	0	0	0	0	0	3.65

Discussion

Molecular docking has become an important phase of drug development based on *in silico* these past few years. This technique is related to simulating the molecular interactions with a protein on an atomic level. The binding energy obtained from docking can be considered a function of the binding afinity of the ligand to the target protein and can be evaluated, screened and predicted[20]. In this research, molecular docking was employed to predict the potential of compounds from *Z. zerumbet* to inhibit the TYR (ID: 2Y9X) protein from *Agaricus bisporus*. The 5 compounds germacrene B (A), 3-metyl kaempferol (B), kaempferol-3-O-methylether (C), α-cedrene (D), kaempferol (E) exhibit potential connection and high compatability to the target protein. Overall, the result of docking shows that most ligands with high binding affinity (at the binding site) also exhibit binding to the active site the target protein.

In this study, a common ligand-protein interaction was observed between all ligands at positions Val 283 and His 263. Compounds B, C and E exhibited interactions of the hydrogen bond with catalytic residues at the active site Met 280 of the TYR receptor. This is expected to impact the function of the target protein leading to its inhibition. To be considered a drug candidate, a compound must both therapeutic potential and safety pharmacokinetic profiles[8]. This study aims to evaluate 5 compounds from the *Z. zerumbet* chosen by the docking simulation to predict the pharmacokinetic, drug-likeness and optimal physicochemical properties using the Swiss-ADME *in silico* tool.

Lipophilicity is a scale to test how well is the medicine's ability to dissolve in fats or oils. This property greatly impacts the overall ADMET profile of the drug and contributes a key role in the ability of a drug to pass through cell membranes[1]. According to Lípinki's rule about drug-likeness, lipophilicity value between 0 and 5 is commonly regarded as the ideal range for drug development[16,26]. In this study, all 5 compounds germacrene B (A), 3-methyl kaempferol (B), kaempferol-3-O-methylether (C), α-cedrene (D), kaempferol (E) demonstrated lipophilicity, the evaluation results suggest that these compounds will be easily taken in across membranes into the systemic circulation, leading to easily distribution throughout the body.

On the other hand, solubility is a physical and chemical property of drugs that significantly influences their pharmacokinetics[5]. To facilitate absorption, the drug is anticipated to be in solution form to ensure optimal absorption[21]. All five compounds evaluated in this study exhibited a moderate level of solubility, indicating a potential for high oral bioavailability.

The ADME scale is used to evaluate whether the drug is suitable for human consumption[3]. Orally administered drugs must be well absorbed in the gastrointestinal tract to achieve optimal pharmacokinetics. Therefore, the BBB criteria are crucial to restrict drug passage through the central nervous system. In this study, the 3 compounds B, C and E were well absorbed into the bloodstream via the gastrointestinal tract, while compounds A and D were poorly absorbed. Furthermore, none of the compounds were capable of penetrating the BBB. This is considered a positive sign as concerning side effects related to the nervous system.

P-gp commonly functions as an efflux, pumping foreign substances or drugs out of the gastrointestinal tract (GIT) and into the bloodstream. As a result, it decreases drug plasma concentrations and tissue levels, thereby impacting treatment efficacy[7,12]. According to the study results, all compounds were estimated to be non-substrates of P-gp.

The interaction between the compound and the cytochrome P450 system (CYP) is needed to illustrate the absorption, distribution, metabolism and excretion of the potential drugs as these interactions are crucial for the metabolism and removal of drug from the body[5]. Inhibiting different isoforms of this enzyme system by drugs can lead to poor drug clearance, causing drug toxicity. As a result, these compounds must exhibit limited inhibition against isoforms of this enzyme system. Evaluation results indicate that compound A only inhibits 1 enzyme in the CYP system, while compound D inhibits 2 and compounds B, C and E inhibit 3.

This suggests that compound A will be metabolized efficiently and will be easily eliminated from the body while compounds B, C, D and E will be metabolized less efficiently. Drug-likeness assessment is a quantitative method to measure how well the physicochemical and structural properties of a compound align with those of most common medications. This is often anticipated using Lipinski's Rule of Five (a consensus of predictions from Lipinski at Pfizer, Ghose at Amgen, Verber at GSK, Egan at Pharmacia and Muegge at Bayer). The research results indicate that compounds B, C and E adhere to Lipinski's Rule of Five, not violating any of the five rules. Compounds A and D violate two rules: Lipinski's and Muegge's.

All of the compounds have been screened to identify BRENK and PAIN alerts. Results indicated that none of the compounds in the study flagged a PAIN alert. This suggests the lack of any promiscuous substructures or fragments. These structural features may generate incorrect result *in silico*, regardless of the protein target[2]. If present, such alerts require careful consideration. On the other hand, BRENK alerts describe potentially toxic, reactive and metabolically unstable fragments. Compounds B, C and E did not flag any BRENK alerts, while compounds A and D had one BRENK alert each. The concern may be minor, depending on the nature of the fragment triggering the alert.

However, this aspect should be considered when prioritizing drug-like compounds. Overall, compounds B, C, aiberd E appear to show the greatest potential in terms of target protein binding interactions, ADMET and drug-like properties. These compounds represent novel TYR inhibitors, previously unreported.

Conclusion

Through *in silico* docking simulations, this study aimed to identify potential TYR inhibitory compounds from ginger extracts for the treatment of hyperpigmentation. The screening results revealed that 5 out of 114 compounds exhibited promising inhibitory activity against tyrosinase, demonstrating strong binding affinity to the target TYR protein.

Subsequent drug-likeness assessment identified three compounds, namely 3-methyl kaempferol (B), kaempferol-3-O-methylether (C) and kaempferol (E), possessing desirable drug-like properties. These compounds warrant further investigation including *in vitro* and *in vivo* studies, to evaluate their TYR inhibitory activity, to elucidate their mechanism of action and to explore potential optimization strategies.

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