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IN SILICO INSIGHTS INTO BIOACTIVE COMPOUNDS OF WILD SUMATRAN TURMERIC (*Curcuma sumatrana, ZINGIBERACEAE*) AS POTENT ANTIOXIDANT CANDIDATES

KAJIAN *IN SILICO* SENYAWA BIOAKTIF DARI KUNYIT LIAR SUMATRA (*Curcuma sumatrana*) SEBAGAI KANDIDAT ANTIOKSIDAN POTENSIAL

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Abstract

Oxidative stress, characterized by an imbalance between reactive oxygen species and antioxidant defenses, contributes to various diseases. Natural products, particularly plant-derived compounds, offer promising therapeutic avenues due to their antioxidant potential. This study investigates the antioxidant properties of wild Sumatran turmeric (*Curcuma sumatrana*), an endemic *Zingiberaceae* species from Sumatra, through *in silico* computational analyses of its rhizome's bioactive compounds. Twenty-two compounds were evaluated for drug-likeness using Lipinski's Rule of Five, revealing 21 compounds (95%) as orally bioavailable. Predictive bioactivity via PASS Online identified six compounds with moderate antioxidant activity (Pa >0.3). Molecular docking against antioxidant enzymes (GPx, SOD, CAT) and the Keap1-Nrf2 complex demonstrated significant binding affinities. Notably, 9,10-Epoxy-12-octadecenoate exhibited superior binding to SOD (-5.75 kcal/mol), GPx (-6.42 kcal/mol), and Keap1-Nrf2 (-8.39 kcal/mol), outperforming native ligands. Abietic acid and N-Octylgallate also showed strong interactions with Keap1-Nrf2, suggesting activation of antioxidant response pathways. The results highlight *C. sumatrana*'s potential to modulate enzymatic antioxidants and Nrf2 signalling. The findings underscore the species' role as a source of bioactive compounds with drug-like properties, advocating further exploration of its phytochemicals for mitigating oxidative stress-related pathologies while highlighting *C. sumatrana* as a viable candidate for developing natural therapeutics targeting redox imbalance.

Keywords: Antioxidant activity; *Curcuma sumatrana*; Keap1-Nrf2; Molecular docking; Oxidative stress

Abstrak

Stres oksidatif, yang ditandai oleh ketidakseimbangan antara spesies oksigen reaktif dan pertahanan antioksidan, berkontribusi terhadap berbagai penyakit. Bahan alami, khususnya senyawa dari tumbuhan, memiliki potensi terapeutik menjanjikan karena sifat antioksidannya. Penelitian ini menginvestigasi potensi antioksidan kunyit liar Sumatra (Curcuma sumatrana), spesies Zingiberaceae endemik Sumatra, melalui analisis in siliko komputasional senyawa bioaktif dari rimpangnya. Sebanyak 22 senyawa dievaluasi menggunakan Aturan Lipinski, dan terungkap 21 senyawa (95%) yang memiliki bioavailabilitas oral. Prediksi bioaktivitas melalui PASS Online mengidentifikasi enam senyawa dengan aktivitas antioksidan sedang (Pa >0,3). Penambatan molekuler terhadap enzim antioksidan (GPx, SOD, CAT) dan kompleks protein Keap1-Nrf2 menunjukkan afinitas pengikatan signifikan. Secara khusus, 9,10-Epoxy-12-octadecenoate menunjukkan pengikatan superior ke SOD (-5,75 kkal/mol), GPx (-6,42 kkal/mol), dan Keap1-Nrf2 (-8,39 kkal/mol), melampaui ligan alami. Asam abietat dan N-Oktilgallat juga menunjukkan interaksi kuat dengan Keap1-Nrf2, mengindikasikan aktivasi jalur respons antioksidan. Hasil ini mengindikasikan potensi C. sumatrana dalam memodulasi enzim antioksidan dan pensinyalan Nrf2. Studi ini menguak peran spesies tersebut sebagai sumber senyawa bioaktif dengan sifat mirip obat, mendorong eksplorasi lebih lanjut terhadap senyawa-senyawa potensialnya sebagai obat penyakit yang terkait dengan stres oksidatif sekaligus mengindikasikan bahwa C. sumatrana adalah kandidat potensial sebagai obat alami untuk mengatasi penyakit akibat ketidakseimbangan redoks.

***Kata Kunci**: Aktivitas antioksidan; Curcuma sumatrana; Keap1-Nrf2; Penambatan molekuler; Stres oksidatif*

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INTRODUCTION

Oxidative stress is a physiological state in which pro-oxidative processes overwhelm cellular antioxidant defenses due to disruptions in redox signaling and adaptive mechanisms. This imbalance between oxidants and antioxidants leads to the overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which modify macromolecules in living organisms and result in measurable cellular damage (Ji & Yeo, 2021). Oxidative stress is strongly implicated in the pathogenesis of chronic diseases, including neurodegenerative disorders, cardiovascular diseases, cancer, diabetes, and inflammatory conditions (Ermakov et al., 2021). Current strategies to mitigate oxidative stress involve lifestyle modifications, dietary interventions, supplementation, and targeted ROS-neutralizing therapies. Lifestyle approaches emphasize the consumption of antioxidant-rich foods (e.g., fruits, vegetables, nuts, and seeds), avoidance of calorie-dense refined sugars and saturated fats, and reduced exposure to environmental pollutants (Sharifi-Rad et al., 2020). However, synthetic antioxidants such as vitamins A, E, C, and β-carotene, though widely used in preventive and therapeutic applications, pose risks of hypervitaminosis and toxicity when administered at high doses over prolonged periods (Helwig & Smulders, 2020).

Natural products from plants in the family of *Zingiberaceae* have emerged as promising candidates for oxidative stress management due to their rich repertoire of bioactive molecules (Zhang et al., 2022; Hafizah & Fitmawati, 2024). These compounds exhibit antioxidant properties that protect against ROS-mediated damage and oxidative stress-related pathologies (Chen et al., 2022). It has been shown that many bioactive compounds act as potent agents in treating oxidative stress and neurodegenerative disorders (Chandran & Abrahamse, 2020). Additionally, diverse phytochemical substances that could counteract oxidative stress are also capable of managing inflammation and cell death in metabolic and cardiovascular diseases, while offering protective benefits against conditions like diabetes and obesity (Kosuru et al., 2023; Park et al., 2022).

Curcuma sumatrana, a *Zingiberaceae* species endemic to Sumatra, Indonesia, has attracted attention for its prospective medicinal benefits (Ardiyani et al., 2011). Recent studies highlight its phenolic profile, antioxidant activity, and anticancer properties, positioning it as a viable source of natural antioxidants for oxidative stress treatment (Alamsjah et al., 2023; Rahman et al., 2022). Moreover, *C. sumatrana* extract has been reported to effectively manage non-alcoholic fatty liver disease in mice (Santoso et al., 2025). Despite these findings, research on the phytochemical mechanisms underlying the antioxidant effects of *C. sumatrana* rhizomes remains limited. *In silico* approach is one of the reliable methods to explore the mechanisms and potentials of bioactive compounds in medicinal plants (Park et al., 2023). Unfortunately, to our knowledge, *in silico* study to reveal the potentials and the underlying mechanisms of bioactive compounds from *C. sumatrana* as antioxidants remains limited. Hence, this investigation aims to fill the gap.

MATERIALS AND METHODS

Bioactive Compounds of *Curcuma sumatrana*

The data of bioactive compounds of *C. sumatrana* were referred from a previous study based on liquid chromatography-mass spectrophotometry (LC-MS) elucidation of ethanolic extract of *C. sumatrana* rhizomes (Rahman, 2024).

Lipinski's Rule of Five Test

This analysis was conducted by converting the compound formats downloaded from PubChem in SDF format into PDB format using PyMOL software. The saved compounds were then submitted to the scfbio-iitd software for evaluation. Specifically, these compounds were required to meet the criteria outlined by Lipinski's rules: a molecular weight (MW) below 500 Daltons, a lipophilicity (LogP) value under 5, fewer than 10 hydrogen bond donors (HBDs), and a molar refractivity (MR) within the range of 40–130 (Hartati et al., 2021).

PASS Online Test

The PASS test was performed using PASS Online software. First, SMILES notations for the compounds via PubChem were retrieved. Next, the SMILES were entered into the Way2Drug

software to predict bioactivity. Predictions generated by the Way2Drug server suggested that the metabolite compounds from *C. sumatrana* rhizomes, acting as ligands, complied with Lipinski's rules. Antioxidant bioactivity was selected as the parameter. The PASS prediction results indicate the number of potential active biological activities (*Pa*= probable activity) and potential inactivity (*Pi* probable inactivity). *Pa* and *Pi* values range from 0.000 to 1.000, and generally, $Pa + Pi \neq 1$. Compounds with $Pa > Pi$ were considered potentially active. If $Pa > 0.7$, the compound was deemed highly likely to exhibit experimental activity. If $0.5 < Pa < 0.7$, the likelihood of experimental activity was considered low and unfavorable compared to known drugs (Prasetyawan et al., 2024).

Molecular Docking

The following proteins were used for molecular docking: Keap1-Nrf2 (6QME), SOD (5YTO), Catalase (1DGB), and GPx (1GP1). The crystal structure of the target protein was obtained from the Protein Data Bank, and its native ligand was removed using MOE. MOE was used to add polar hydrogens, assign Gasteiger partial charges, and save protein structures in.moe format. The ligand binding site on the receptor was identified using the Find Receptor tool. Ligands selected for testing were those with potential antioxidant activity identified via the PASS assay. Ligand structures were retrieved from PubChem in SDF format. Each ligand underwent docking against all target proteins using MOE. MOE was utilized to manually analyze docking poses at the active site of each protein and calculate binding affinities. The docking method was considered valid if the RMSD value was ≤2 Å. A lower RMSD value indicates smaller deviations during docking. Free binding energy was used to evaluate ligand-receptor stability, with lower values corresponding to more stable interactions (Muthusamy et al., 2024).

RESULTS

Drug-Likeness of Compounds Based on Lipinski's Rule of Five

The majority of compounds (21 out of 22, 95.5%) from *C. sumatrana* adhered to Lipinski's Rule of Five, demonstrating favorable drug-like properties for oral bioavailability (Table 1). All compounds exhibited molecular weights (MW) below 500 Daltons, with only myricanone (356 D) and Ingenol (348 D) approaching the upper limit. LogP values remained under 5 for all compounds except benzyldioxidanyl (LogP= 5), which also exceeded the hydrogen bond donor (HBD) limit (HBD= 6) and was the sole compound to violate two rules. Molar refractivity (MR) values for all compounds fell within the acceptable range of 40–130, with myricanone (MR= 98.36) and 9,10-Epoxy-12-octadecenoate (MR= 86.51) representing the highest values. Notably, 7-Ethoxy-4-methylcoumarin, chamazulene, and dehydrocostus lactone exhibited zero violations, highlighting their strong compliance with drug-likeness criteria.

Drug-Like-Ness of Compounds Based on Lipinski's Rule of Five

Using scfbio-iitd software, from 22 compounds in the *C. sumatrana*, 4 compounds were found that violated one rule in the Lipinski rule, namely N-Octylgallate, artemisin, ingenol, and myricanone (hydrogen bond donors >5), but still passed the Lipinski's Rule of Five. Furthermore, one compound was found that violated 2 Lipinski's rules due to its higher H-bond donor and lipophilicity (>5), namely benzyldioxidanyl, which could not be declared to have passed the Lipinski's Rule of Five.

Predictive Antioxidant Bioactivity via PASS Online

PASS Online predictions identified six compounds with moderate antioxidant potential (*Pa* >0.3) (Table 2), namely osthol (*Pa* 0.535), protocatechuic aldehyde (*Pa* 0.443), cinnamic acid (*Pa* 0.489), 3,5-ditert-butyl-4-hydroxybenzaldehyde (*Pa* 0.576), N-Octylgallate (*Pa* 0.384), and 7-Ethoxy-4-methylcoumarin (*Pa* 0.333). The remaining 14 compounds, including Artemisinin (*Pa*= 0.190) and dehydrocostus lactone (*Pa* 0.138), showed low predicted activity (*Pa* <0.3), while a compound, namely loxoprofen, was inactive as an antioxidant (Pa <Pi). Surprisingly, 9,10-Epoxy-12-octadecenoate (*Pa* 0.210) displayed low antioxidant probability despite exhibiting robust molecular docking results.

Table 1. Physicochemical properties based on Lipinski's rule of five test of the compounds in *Curcuma sumatrana*

Compound	MW (D)	LogP	H-bond donor	H-bond acceptor	Molar refractivity	Rule violation	Justifi-cation
Standard	<500	<5	<5	<10	40–130	<2	
N-Octylgallate	282	3	5	3.32	75.09	1	Pass
Artemisinin	282	0	5	2.39	68.04	1	Pass
7-Ethoxy-4-methylcoumarin	204	0	3	2.40	56.89	0	Pass
Chamazulene	184	0	0	3.75	61.33	0	Pass
Dehydrocostus lactone	230	0	2	3.01	66.23	0	Pass
4-phenylbutyric acid	164	1	2	2.09	47.01	0	Pass
Helenalin	262	1	4	1.24	68.04	0	Pass
Octanal, 2-(phenylmethylene)	216	0	1	4.23	69.24	0	Pass
Nabumetone	228	0	2	3.36	69.50	0	Pass
Loxoprofen	246	1	3	2.78	68.54	0	Pass
Myricanone	356	2	5	4.47	98.36	1	Pass
3-(4-Hydroxyphenyl) propionic acid	166	4	4	0.34	47.42	0	Pass
Osthol	244	0	3	3.13	70.79	0	Pass
Protocatechuic aldehyde	138	2	3	0.91	35.15	0	Pass
9,10-Epoxy-12-octadecenoate	296	1	3	5.09	86.51	0	Pass
Ingenol	348	4	5	0.81	91.36	1	Pass
3,5-ditert-butyl-4-hydroxybenzaldehyde	234	1	2	3.79	70.89	0	Pass
Benzyldioxidanyl	123	5	6	-0.05	77.14	2	Not Pass
Benzoate hydrate	139	1	2	1.38	33.40	0	Pass
2-Isobutoxynaphthalene	200	0	1	3.87	64..28	0	Pass
Cinnamic acid	148	1	2	1.78	43.11	0	Pass
Abietic acid	302	1	2	5.20	89.53	0	Pass

Table 2. Predictive antioxidant bioactivity based on PASS Online test

Compound	Antioxidant (Pa)	Antioxidant (Pi)	Criteria
N-Octylgallate	0.384	0.014	Moderate
Artemisinin	0.190	0.061	Low
7-Ethoxy-4-methylcoumarin	0.333	0.018	Moderate
Chamazulene	0.244	0.049	Low
Dehydrocostus lactone	0.138	0.117	Low
4-phenylbutyric acid	0.200	0.055	Low
Helenalin	0.203	0.054	Low
Octanal, 2- (phenylmethylene)-	0.191	0.061	Low
Nabumetone	0.241	0.039	Low
Loxoprofen	0.216	0.784	Inactive
Myricanone	0.259	0.033	Low
3-(4-Hydroxyphenyl) propionic acid	0.285	0.026	Low
Osthol	0.535	0.005	Moderate
Protocatechuic aldehyde	0.443	0.009	Moderate
9,10-Epoxy-12-octadecenoate	0.210	0.050	Low
Ingenol	0.143	0.111	Low
3,5-ditert-butyl-4-hydroxybenzaldehyde	0.576	0.005	Moderate
Benzoate hydrate	0.199	0.055	Low
2-Isobutoxynaphthalene	0.162	0.088	Low
Cinnamic acid	0.489	0.007	Moderate
Abietic acid	0.202	0.054	Low

Molecular Docking with Antioxidant-Related Proteins

The molecular docking analysis (Table 3) revealed strong binding affinities between *C. sumatrana* compounds and key antioxidant proteins (GPx, SOD, CAT, Keap1-Nrf2) (Table 3). A 9,10-Epoxy-12-octadecenoate emerged as the most potent ligand, demonstrating superior binding energies across all targets: GPx (-6.42 kcal/mol), SOD (-5.75 kcal/mol), CAT (-7.80 kcal/mol), and Keap1-Nrf2 (-8.39 kcal/mol). Its affinity for Keap1-Nrf2 exceeded that of the native ligand glutathione (-6.71 kcal/mol). Other notable interactions included N-Octylgallate (-7.39 kcal/mol with Keap1-Nrf2), abietic acid (-7.50 kcal/mol with Keap1-Nrf2), and loxoprofen (-6.50 kcal/mol with Keap1-Nrf2). Most compounds exhibited binding energies ≤-5 kcal/mol, indicating stable ligand-receptor interactions.

Residue-Specific Interactions and Visual Confirmation of Docking Poses

The molecular docking analysis identified specific amino acid residues critical for stabilizing interactions between *C. sumatrana* compounds and antioxidant-related proteins (Table 4). For GPx, 9,10-Epoxy-12-octadecenoate formed hydrophobic interactions with Ala138, Met140, and Trp158, while N-Octylgallate established hydrogen bonds with Asp135, Leu139, and Arg178 (Table 4; Figure 1). These interactions suggest complementary binding to the GPx active site, potentially enhancing antioxidant activity. In the case of SOD, 9,10-Epoxy-12-octadecenoate exhibited a hydrogen bond with Lys23 and alkyl interactions with Pro28 (Table 5; Figure 2), key residues involved in SOD's metal-binding and catalytic functions. For CAT, hydrogen bonds between 9,10-Epoxy-12-octadecenoate and Gln195/Tyr215 (Table 6; Figure 3) were observed, residues known to participate in substrate recognition and heme coordination. Notably, in Keap1-Nrf2, salt bridges formed between 9,10-Epoxy-12-octadecenoate and Arg415/Arg483, critical residues in the Keap1 binding pocket, while abietic acid engaged in alkyl/pi-alkyl interactions with Ala366 and Arg415 (Table 7; Figure 4). These interactions highlight the compound's ability to disrupt the Keap1-Nrf2 complex, potentially activating antioxidant response pathways.

Visualization of docking poses confirmed that 9,10-Epoxy-12-octadecenoate occupied the active sites of all four target proteins (GPx, SOD, CAT, Keap1-Nrf2) in orientations closely resembling those of native ligands (Figures 1–4). This structural mimicry suggests a competitive inhibition mechanism, where the compound competes with endogenous ligands for binding. The alignment of its binding mode with native antioxidants further supports its potential to emulate natural redox regulation mechanisms, underscoring its multi-target antioxidant capability.

Table 3. Binding affinity of standard drug, native ligand, and compounds from *Curcuma sumatrana* with GPx, SOD, CAT, and Keap1-Nrf2

Compound	CID	Binding affinity (kcal/mol)			
		GPx	SOD	CAT	Keap1-Nrf2
Glutathione (GPx native ligand)	124886	-6.1483	-	-	-
4-[(1~{R})-2-(naphthalen-2-ylmethylamino)-1-oxidanyl-ethyl]benzene-1,2-diol (synthetic analog)	134828057	-	-5.5172	-	-
NADPH (Native Ligand)	134821689	-	-	-9.4026	-
Glutathione (Native Ligand)	124886	-	-	-	-6.7140
Chamazulene	10719	-5.1972	-4.3006	-5.4070	-5.3109
Osthol	10228	-5.3904	-4.9980	-6.1978	-6.5901
Myricanone	161748	-5.9558	-4.9742	-6.1171	-6.2878
2-Isobutoxynaphthalene	16582	-5.7506	-4.7143	-5.9332	-5.5982
7-Ethoxy-4-methylcoumarin	66595	-5.4546	-4.5545	-5.5544	-5.5488
Nabumetone	4409	-6.0293	-4.8424	-6.5623	-5.8835
Cinnamic acid	444539	-4.7120	-4.2698	-4.9288	-5.0156
3-(4Hydroxyphenyl) propionic acid	77033	-5.2722	-4.3480	-5.0099	-5.2281
Loxoprofen	3965	-5.9452	-4.9501	-6.1995	-6.4958
Abietic Acid	10569	-4.7411	-4.7005	-5.9515	-7.4977

Compound	CID	Binding affinity (kcal/mol)			
		GPx	SOD	CAT	Keap1-Nrf2
9,10-Epoxy-12-octadecenoate	5283018	-6.4168	-5.7451	-7.7977	-8.3931
4-phenylbutyric acid	4775	-5.2347	-4.3575	-5.2803	-5.5249
N-Octylgallate	61253	-5.9653	-5.2330	-6.3975	-7.3937
Benzoate hydrate	22172796	-4.0795	-3.6707	-4.7267	-4.6511
Helenalin	23205	-3.8569	-4.3991	-5.2367	-6.7455
Octanal, 2(phenylmethylene)-	1550884	-5.8576	-5.0272	-5.7310	-6.2045
Dehydrocostus lactone	73174	-4.5930	-4.6127	-5.2257	-6.4748
Ingenol	442042	-4.9059	-4.3912	-5.4908	-5.7456
Artemisin	65030	-4.582	-4.5055	-5.3097	-6.0806
3,5-ditert-butyl-4hydroxybenzaldehyde	73219	-4.9414	-4.5337	-5.2320	-6.3857
Protocatechuic aldehyde	8768	-4.6100	-4.0040	-4.7610	-4.6820

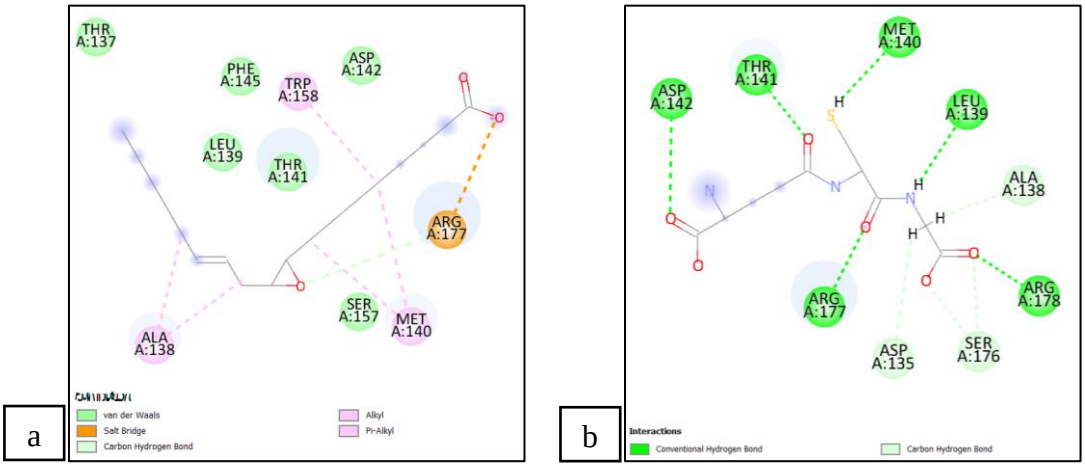

Figure 1. Visualization of molecular docking between GPx with the most potent ligand 9,10-Epoxy-12-octadecenoate (a) and the native ligand for GPx (b)

Table 4. Interaction of amino acid residues of GPx with selective compounds from *Curcuma sumatrana*

Ligands	Interactions with GPx						
	Salt bridge	Carbon hydrogen	Alkyl	Pi-alkyl	Conventional hydrogen bond	Pi-donor hydrogen bond	Pi-cation
Glutathione (native ligand)	-	Asp:135 Ser:176	-	-	Leu:139 Met:140 Thr:141 Asp:142 Arg:177 Arg:178	-	-
Myricanone	-	-	Ala:138	-	Leu:139 Arg:177	Thr:141	Arg:177
Nabumetone	-	Ala:138 Ser:157	Ala:138	Ala:138	Thr:141 Asp:142	Thr:141	-
Loxoprofen	-	-	-	Met:140 Trp:158	Thr141 Asp:142	Thr:141	Arg:177
9,10-Epoxy-12-octadecenoate	Arg:177	Arg:177	Ala:138 Met:140	Trp:158	-	-	-
N-Octylgallate	Arg:177	-	Ala:138 Met:140 Trp:158 Arg:177	Ala:138 Phe:145 Tro:158	Asp:135 Leu:139 Thr:141 Arg:178	-	Arg:177

Ligands	Interactions with GPx						
	Salt bridge	Carbon hydrogen	Alkyl	Pi-alkyl	Conventional hydrogen bond	Pi-donor hydrogen bond	Pi-cation
Octanal, 2(phenylmethylene)-	-	Thr:141	Met:140	Ala:138 Met:140 Phe:145 Trp:158 Arg:177	-	-	-

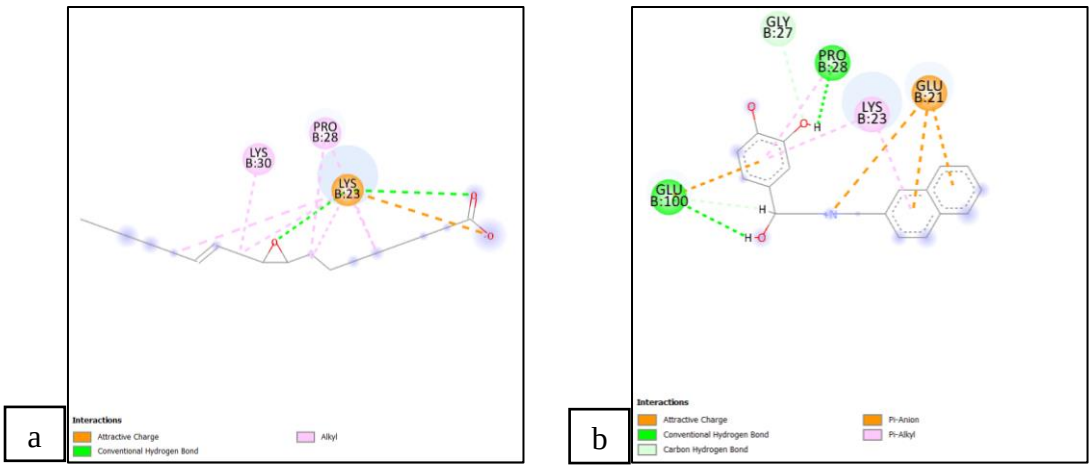

Figure 2. Visualization of molecular docking between SOD with the most potent ligand 9,10-Epoxy-12-octadecenoate (a) and the native ligand for SOD (b)

Table 5. Interaction of amino acid residues of SOD protein with selective compounds from *Curcuma sumatrana*

Ligands	Interactions with SOD					
	Attractive charge	Carbon hydrogen	Alkyl	Pi-alkyl	Conventional hydrogen bond	Pi-anion
4-[(1~{R})-2-(naphthalen-2-ylmethylamino)-1-oxidanyl-ethyl]benzene-1,2-diol (SOD control drug)	Glu:21	Gly:27 Glu:100	-	Lys:23 Pro:28	Pro:28 Glu:100	Glu:21 Glu:100
9,10-Epoxy-12-octadecenoate	Lys:23	-	Lys:23 Pro:28 Lys:30	-	Lys:23	-

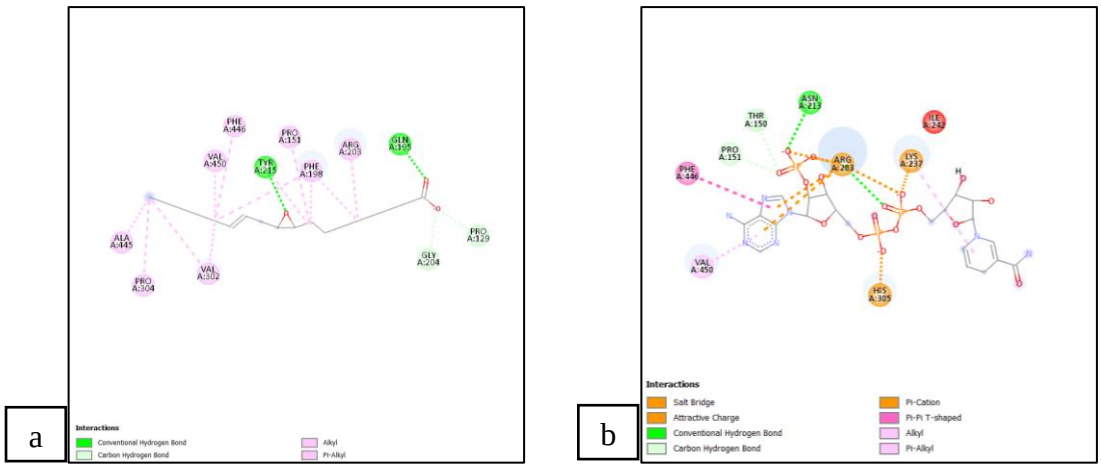

Figure 3. Visualization of molecular docking between CAT with the most potent ligand 9,10-Epoxy-12-octadecenoate (a) and the native ligand for CAT (b)

Table 6. Interaction of amino acid residues of the CAT protein with selective compounds from *Curcuma sumatrana*

Ligands	Interactions with CAT							
	Attractive charge	Carbon hydrogen	Alkyl	Pi-alkyl	Conventional hydrogen bond	Pi-cation	Pi-Pi T-shaped	Salt bridge
NADPH (native ligand)	Arg:203 Lys:237 His:305	Thr:150 Pro:151	Lys:237	Val:450	Arg:203 Asn:213	Arg:203	Phe:446	Arg:203 Lys:237
9,10-Epoxy-12-octadecenoate	-	Gly:204 Pro:129	Pro:151 Arg:203 Arg:302 Arg:304 Arg:445 Arg:446	Phe:198 Tyr:215 Phe:446	Gln:195 Tyr:215	-	-	-

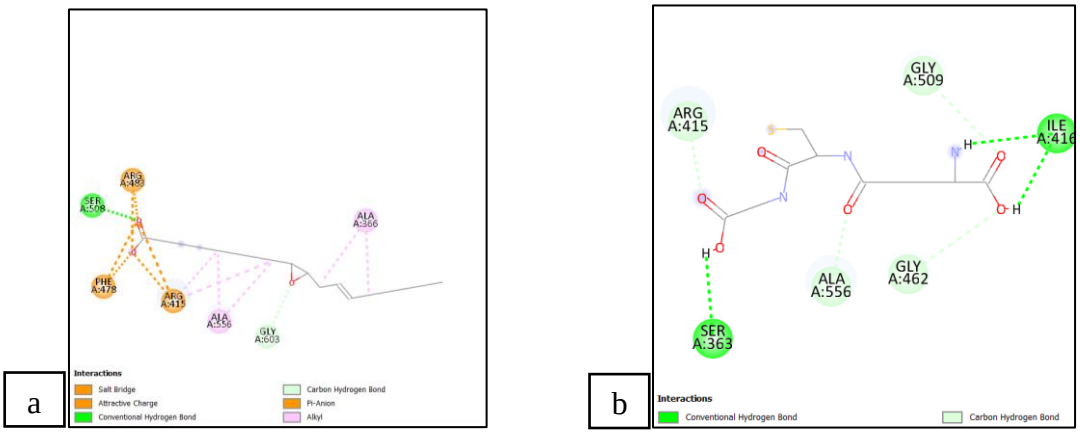

Figure 4. Visualization of molecular docking between Keap1-Nrf2 with most potent ligand 9,10-Epoxy-12-octadecenoate (a) and native ligand for Keap1-Nrf2 (b)

Table 7. Interaction of amino acid residues of Keap1-Nrf2 protein with the most potent compound from *Curcuma sumatrana*

Ligands	Interactions with Keap1-Nrf2			
	Attractive charge	Carbon hydrogen	Alkyl	Pi-alkyl
Glutathione (native ligand)	-	Arg:415 Gly:462 Gly:509 Ala:556	-	-
Osthol		Gly:462 Ala:510 Gly:605	-	Ala:366
Abietic acid	-	Ala:366	Ala:366 Arg:415 Val:512	-
9,10-Epoxy-12-octadecenoate	Arg:415 Arg:483	Gly:603	Ala:366 Arg:415 Ala:556	-
N-Octylgallate		Ala:366 Gly:558 Gly:605	Arg:415 Ala:556	-
Helenalin	-	Ala:366 Gly:509 Ala:556 Gly:605	Val:465 Val:512	-

DISCUSSION

The *in silico* exploration of *Curcuma sumatrana* ethanolic extract has unveiled a compelling pharmacochemical profile, positioning this plant as a rich source of multi-target antioxidant agents with promising drug-like properties. This study bridges traditional ethnopharmacological knowledge with modern computational methodologies, offering a blueprint for harnessing natural products in combating oxidative stress, a root cause of chronic diseases such as cancer, neurodegeneration, and cardiovascular disorders. Below, we contextualize these findings within broader scientific paradigms, address mechanistic nuances, and discuss their implications for drug discovery and functional food development.

The prioritization of specific metabolites from *C. sumatrana* rhizomes identified via LC-MS by Rahman (2024) for *in silico* antioxidant profiling is mechanistically justified by compelling *in vivo* evidence. Our prior experimental study established that ethanolic extracts of *C. sumatrana* significantly reduced malondialdehyde levels - a key biomarker of lipid peroxidation - in both hepatic tissue and plasma of mice with non-alcoholic fatty liver disease (Santoso et al., 2025). Furthermore, Rahman (2024) demonstrated that the same extract containing these 22 metabolites substantially alleviated oxidative stress in colonic tissues while elevating catalase (CAT) activity, suggesting antioxidant potential. This dual evidence of systemic and tissue-specific antioxidant efficacy confirms the presence of bioactive compounds capable of modulating redox homeostasis *in vivo*. Consequently, adopting the full suite of LC-MS-detected metabolites for computational analysis represents a pharmacologically sound strategy to identify the constituents responsible for the extract's observed antioxidant effects.

Lipinski's Rule of Five analysis, which is one of the rules in determining a compound that can be considered to have the ability as an active drug in humans (Chen et al., 2020). A compound or drug is considered to pass the Lipinski's Rule of Five only when it does not violate more than one Lipinski's rule (Hartati et al., 2021). In this study, the adherence of 21 out of 22 compounds to Lipinski's Rule of Five (RO5) underscores *C. sumatrana*'s potential as a reservoir of orally bioavailable therapeutics. RO5 compliance is a critical gatekeeper in drug discovery, as molecules violating these rules often face bioavailability challenges due to poor absorption or excessive metabolism (Karami et al., 2022).

The zero violations observed for 7-Ethoxy-4-methylcoumarin, chamazulene, and dehydrocostus lactone align with their structurally compact frameworks; low molecular weight (<300 Da), minimal hydrogen bond donors (HBD ≤3); and moderate lipophilicity (LogP <3). These features are hallmarks of successful oral drugs. The exceptions, myricanone (MW= 356 Da) and ingenol (MW= 348 Da), approach but do not exceed the 500 Da threshold, retaining their drug-like status. Their moderate LogP values (2–4) and acceptable HBD counts (≤5) suggest they may still achieve oral bioavailability through active transport mechanisms, as seen with similarly sized natural products like curcumin (MW= 368 Da) (El-Hack et al., 2021). In contrast, benzyldioxidanyl's dual violations (LogP= 5, HBD= 6) render it a poor candidate, likely due to excessive hydrophobicity and polar interactions that hinder membrane permeability. This dichotomy highlights the importance of RO5 as a filtering tool, though exceptions exist for compounds leveraging alternative absorption pathways (e.g., peptide transporters) (Liu, 2019).

The PASS Online predictions revealed six compounds with moderate antioxidant potential (*Pa* >0.3), including osthol (*Pa* 0.535) and 3,5-ditert-butyl-4-hydroxybenzaldehyde (*Pa* 0.576). These compounds share phenolic and hydroxyl groups; structural motifs that donate protons to neutralize free radicals, a mechanism well-documented in gallic acid and quercetin (Patil & Killedar, 2021). Protocatechuic aldehyde (*Pa* 0.443), with its catechol moiety, mirrors the redox activity of epigallocatechin gallate (EGCG), a green tea antioxidant known for chelating metal ions and scavenging ROS (Zhang et al., 2023).

The molecular docking results position 9,10-Epoxy-12-octadecenoate as a standout multi-target antioxidant ligand. Its superior binding to Keap1-Nrf2 (-8.39 kcal/mol vs. glutathione: -6.71 kcal/mol) suggests a potent ability to disrupt the Keap1-Nrf2 complex, liberating Nrf2 to translocate to the nucleus and activate antioxidant response elements (ARE). This mechanism is central to

cellular defense against oxidative stress and mirrors the action of sulforaphane, a broccoli-derived Nrf2 activator (Matsagar & Singh, 2024). The salt bridges formed with Arg415 and Arg483 in Keap1-Nrf2 are critical, as these residues anchor the hinge-and-latch interaction that sequesters Nrf2. By competing with Keap1's DLG motif, 9,10-Epoxy-12-octadecenoate could mimic endogenous disruptors like nitric oxide, offering a novel phytochemical strategy for Nrf2 pathway activation. For GPx, the hydrophobic interactions of 9,10-Epoxy-12-octadecenoate with Ala138 and Trp158 likely stabilize the enzyme's selenocysteine active site, enhancing glutathione oxidation, a key step in neutralizing hydroperoxides (Xu et al., 2021). Similarly, its hydrogen bond with Lys23 in SOD may facilitate superoxide dismutation by stabilizing the enzyme's catalytic copper-zinc cluster, akin to the role of histidine residues in native SOD. Abietic acid's strong affinity for Keap1-Nrf2 (-7.50 kcal/mol) and N-Octylgallate's interactions with GPx (-7.39 kcal/mol) further highlight the extract's polypharmacological potential. Such multi-target activity is advantageous in oxidative stress, where ROS generation involves interconnected pathways (e.g., NADPH oxidase, mitochondrial electron transport chain) (Juan et al., 2021). By simultaneously modulating GPx, SOD, CAT, and Nrf2, *C. sumatrana* compounds may synergistically amplify antioxidant defenses, a strategy superior to single-target inhibitors in complex diseases.

Beyond their shared roles in mitigating oxidative stress, the proteins targeted in this docking study represent two fundamentally distinct arms of the redox homeostasis machinery: enzymatic detoxification and transcriptional regulation. GPx, SOD, and CAT function as frontline enzymatic antioxidants, each neutralizing specific ROS (Ji & Yeo, 2021). GPx reduces hydrogen peroxide and organic hydroperoxides to water and corresponding alcohols using glutathione, thereby preventing lipid peroxidation and maintaining membrane integrity (Juan et al., 2021). SOD catalyzes the dismutation of the superoxide radical into oxygen and hydrogen peroxide, the latter of which is subsequently degraded by CAT into water and oxygen, ensuring sequential ROS detoxification (Park et al., 2023). The observed interactions between 9,10-Epoxy-12-octadecenoate and catalytic or structurally relevant residues of these enzymes suggest potential enhancement of their detoxifying efficacy. In contrast, the Keap1-Nrf2 complex governs a slower but broader transcriptional response. Under oxidative stress, Nrf2 dissociates from Keap1 and translocates to the nucleus to induce expression of a spectrum of cytoprotective genes, including those encoding GPx, SOD, and CAT themselves (Matsagar & Singh, 2024). Thus, ligands like 9,10-Epoxy-12-octadecenoate that can disrupt the Keap1-Nrf2 interaction hold dual promise: initiating immediate enzymatic ROS neutralization and sustaining long-term cellular resilience through upregulation of endogenous antioxidant systems. This bifunctional mode of action underscores the therapeutic advantage of compounds that span both enzymatic and transcriptional regulatory pathways in oxidative stress-related pathologies.

The paradox of 9,10-Epoxy-12-octadecenoate—low Pa (0.210) yet exceptional docking performance, highlights a critical discrepancy between ligand-based and structure-based predictive models. While PASS Online evaluates biological activity probabilities based on structural similarity to known pharmacologically active compounds (Jamkhande et al., 2024), it may fail to capture nuanced physicochemical interactions that emerge during target-ligand binding. In the case of 9,10-Epoxy-12-octadecenoate, the compound demonstrated high binding affinity across multiple antioxidant targets, particularly Keap1-Nrf2, suggesting meaningful bioactivity despite its low Pa value. This discrepancy underscores that docking studies, which model spatial and energetic complementarity with specific protein sites (Rigby, 2024), can uncover potential interactions that PASS may overlook, especially when evaluating structurally unique or underrepresented scaffolds in the training database. The observed binding patterns (such as salt-bridge formation and stabilization of catalytic residues) indicate mechanistic plausibility that extends beyond conventional ligand-based prediction.

This inconsistency also reveals broader limitations inherent to PASS-based models. As a machine learning tool trained on curated databases of known bioactivities (Jamkhande et al., 2024), PASS is inherently biased toward well-characterized chemotypes and may underestimate compounds with unconventional or multi-target mechanisms. For example, 9,10-Epoxy-12-octadecenoate's

epoxy and conjugated diene groups could confer activity through allosteric effects, membrane interactions, or redox cycling capabilities, features not readily encoded in PASS's probability estimations. This aligns with previous findings on compounds like resveratrol, where *in silico* PASS predictions undervalued its wide-ranging antioxidant and signaling effects (Huo et al., 2022). Moreover, the failure of PASS to predict loxoprofen's inactivity as an antioxidant, despite its potent non-stereoidal anti-inflammatory drug classification, further illustrates the need for caution in interpreting Pa scores, particularly for phytochemicals or novel derivatives. These limitations affirm that computational docking and ligand-based predictions should be viewed as complementary tools rather than definitive stand-alone assessments, with experimental validation remaining essential in drug discovery workflows.

While this study evaluates individual compounds in isolation to characterize their specific antioxidant mechanisms, it is important to acknowledge that plant extracts function as complex mixtures whose bioactivity often arises from synergistic or additive interactions among multiple constituents (Rigby, 2024). The PASS Online predictions identified several compounds with moderate to high antioxidant potential, such as osthol, 3,5-ditert-butyl-4-hydroxybenzaldehyde, and protocatechuic aldehyde, all of which contain phenolic or catechol groups known to donate hydrogen atoms and stabilize free radicals. These shared structural features suggest potential cooperative effects in radical scavenging and redox cycling. Moreover, the polypharmacological docking profiles (particularly for 9,10-Epoxy-12-octadecenoate, which showed strong binding to Keap1-Nrf2, GPx, and SOD) highlight the relevance of multi-target modulation in oxidative stress contexts. Framing these findings within a network pharmacology perspective reveals how these phytochemicals might engage in both parallel and convergent pathways, reinforcing the antioxidant defense system at multiple levels: direct enzymatic detoxification, membrane protection, and transcriptional regulation via Nrf2. Therefore, future work integrating interaction networks and combination modeling could provide a more holistic understanding of *C. sumatrana*'s antioxidant efficacy, better reflecting the complex interplay of compounds in whole plant extracts.

In summary, this *in silico* study illuminates *Curcuma sumatrana* as a treasure trove of multi-target antioxidant agents, with 9,10-Epoxy-12-octadecenoate emerging as a mechanistically novel candidate. Its compliance with Lipinski's rules, coupled with robust binding to GPx, SOD, CAT, and Keap1-Nrf2, positions it as a promising lead for combating oxidative stress, a ubiquitous driver of chronic disease. By integrating computational predictions with traditional knowledge, this work advances the paradigm of reverse pharmacology, where natural products guide targeted drug discovery. Future studies must bridge these *in silico* insights with experimental and clinical validation to unlock the full therapeutic potential of *C. sumatrana*.

While this study provides robust *in silico* evidence for the antioxidant potential of *C. sumatrana* compounds, several limitations must be acknowledged. First, PASS Online's reliance on existing bioactivity data may overlook novel mechanisms of action, necessitating experimental validation through direct assays. Hence, *in vitro* antioxidant activity tests by means of 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and ferric reducing antioxidant power (FRAP), and oxygen radical absorbance capacity (ORAC) assays are required to validate the radical-scavenging capacity of the *C. sumatrana* extract and particular compounds isolated from the extract. Second, the absence of absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiling (including CYP450 inhibition and plasma protein binding) limits translational insights, though tools like SwissADME or ProTox-II could address these gaps. Accordingly, a pharmacokinetics study using animal models and cell culture could unveil the ADMET profile under real biological circumstances. Third, molecular docking captures static binding poses, neglecting dynamic conformational changes and solvation effects; molecular dynamics simulations would refine stability assessments over time. Finally, the study evaluates individual compounds, ignoring potential synergies within the extract, which could be explored through network pharmacology approaches to unravel multi-compound interactions and holistic efficacy. Addressing these limitations in future work will strengthen the translational relevance of *C. sumatrana* as a therapeutic agent.

CONCLUSION

This *in silico* study highlights *Curcuma sumatrana* as a promising source of multi-target antioxidants, with most compounds adhering to Lipinski's Rule of Five, ensuring oral bioavailability. Despite moderate PASS predictions, 9,10-Epoxy-12-octadecenoate emerged as a potent candidate, exhibiting strong binding to GPx, SOD, CAT, and Keap1-Nrf2 based on molecular docking simulation, suggesting competitive inhibition and Nrf2 pathway activation. While computational insights reveal mechanistic promise, further *in vitro* and *in vivo* validation is essential to confirm efficacy and translate findings into therapeutic applications.

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