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Chemical Profiling, Antioxidant Potential, Molecular Docking And Molecular Dynamic Simulation Of Essential Oil Constituents Of Four Curcuma Species

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Abstract
The present study offers an extensive phytochemical characterization, antioxidant activity, and in silico analysis of the essential oils derived from the rhizomes of Curcuma amada, Curcuma angustifolia, Curcuma caesia, and Curcuma zedoaria. Gas Chromatography-Mass Spectrometry (GC-MS) analysis demonstrated species-specific variations, with C. amada characterized by major constituents such as myrcene, β-pinene, C. angustifolia by velleral, germacrone, and (2E,6Z)-Farnesol, C. caesia by trans-β-elemenone, curzerenone, whereas C. zedoaria by curzerenone, β-eudesmol acetate, 1,8-cineole. In vitro experiments revealed significant antioxidant capacity in all species, especially in C. angustifolia and C. caesia (DPPH IC_{50} = 31.2 ± 0.3 µg/mL and RPA EC_{50} = 23.1 ± 0.2 µg/mL) and (DPPH IC_{50} = 39.8 ± 0.5 µg/mL and RPA EC_{50} = 27.0 ± 0.4 µg/mL). In silico docking of primary ingredients against xanthine oxidase revealed sesquiterpenes, including germacrone (–6.5 kcal/mol) and velleral (–6.2 kcal/mol), as effective inhibitors, corroborated by molecular dynamics simulations demonstrating persistent protein ligand interactions. Curcuma rhizome essential oils show potential as natural antioxidants and xanthine oxidase inhibitors, suggesting their potential in managing oxidative stress-related disorders.

Keywords: *Curcuma species, essential oils, phytochemical profile, antioxidant activity, molecular docking*

INTRODUCTION

Curcuma, a genus in the Zingiberaceae family, is known for its medicinal, culinary, and aromatic properties due to its diverse phytoconstituents. *Curcuma amada* (mango ginger), *Curcuma angustifolia* (cone turmeric), *Curcuma caesia* (black turmeric), and *Curcuma zedoaria* (white turmeric) are significant in traditional Asian medicine and are gaining recognition in modern phytopharmacological research (Poudel et al., 2022; Gadnayak et al., 2022). *Curcuma* species rhizomes derive essential oils (EOs), which are complex mixtures of monoterpenes and sesquiterpenes, including terpenoid alcohols, ketones, and hydrocarbons. The volatile constituents are primarily responsible for the fragrance and therapeutic efficacy of oils (Poudel et al., 2022; Gadnayak et al., 2022). These substances are utilized in food flavoring, cosmetics, and traditional remedies for conditions like inflammation, microbial infections, neurological disorders, diabetes, and cancer (Poudel et al., 2022; Gadnayak et al., 2024).

Curcuma essential oils are extensively researched for their antioxidant activity, as oxidative stress significantly contributes to the development of chronic illnesses. *C. amada* rhizome oil is a potent blend of essential components, including myrcene, β-pinene, curzerenone, thymol, and linalool (Dosoky& Setzer, 2018), *C. caesia* is distinguished by various chemicals such as germacrone, curzerene, camphor, and ar-curcumene (Gangal et al., 2023). *C. angustifolia* exhibits various chemotypes, including neocurdione, germacrone, and velleral (Gangal et al., 2023), *C. zedoaria* is known for its high levels of β-pinene, 1,8-cineole, camphor, and linalool (Elhawary et al., 2024). All four *Curcuma* species, rich in volatile compounds, are known for their antioxidant, anti-inflammatory, antibacterial, and cytotoxic properties, indicating their therapeutic importance.

The antioxidant activity is a widely studied pharmacological characteristic of *Curcuma* essential oils, given that oxidative stress significantly contributes to the development of chronic illnesses. Extracts and essential oils of *C. amada*, *C. angustifolia*, *C. caesia*, and *C. zedoaria* have shown DPPH radical scavenging activity and ferric reduction antioxidant capacity (Nag et al., 2021; Nag et al., 2022; Chen et al., 2023). Nonetheless, published

papers exhibit considerable variability because of the differences in extraction methodologies, plant parts used, and geographical origin. Moreover, limited research has carefully examined these four species using standardized techniques (Chen et al., 2023). Moreover, while essential oils are acknowledged for their ability to scavenge free radicals, their mode of action concerning pivotal oxidative enzymes like xanthine oxidase, a primary source of reactive oxygen species, is inadequately defined (Gawlik-Dziki, 2012). The inhibition of xanthine oxidase is pharmacologically significant for treating ailments associated with oxidative stress, such as gout, cardiovascular diseases, and neurodegenerative disorders (Liu et al., 2021).

In silico studies, such as molecular docking and molecular dynamic simulation can be applied to study the interaction of phytoconstituents with desirable bioactivities of medicinal plants (Nayak et al., 2024). Available reports on bioactivity studies of *Curcuma* species do not include any work on comparative evaluation of *in vitro* and *in silico* bioactivity. However, such comparative *in vitro* and *in silico* bioactivity assessment has been reported in other medicinal plants (Rehman et al., 2020, Abbdullad et al., 2021, Nayak et al., 2024).

The present work was carried out to analyze the chemical composition and it's *in vitro* antioxidant activity of rhizome essential oil of *Curcuma amada*, *C. angustifolia*, *C. caesia*, and *C. zedoaria* in order to ascertain their therapeutic potential. *In silico* analysis, such as molecular docking and molecular dynamics simulation of essential oil constituents, was done to validate the experimental results by revealing the interaction of chemical constituents with the enzyme Xanthine oxidase.

MATERIALS AND METHODOLOGY

Collection and Extraction of *Curcuma amada, Curcuma angustifolia, Curcuma caesia,* and *Curcuma zedoaria* Rhizome Essential Oils

Fresh rhizomes of *C. angustifolia* were collected from Similipal of Mayurbhanj district, whereas *C. amada*, *C. caesia*, and *C. zedoaria* were collected from Khurdha district of Odisha, meticulously cleaned to eliminate debris, and air-dried until surface dryness was achieved. Hundred grams of rhizomes were used for each species' essential oil extraction. Rhizomes were sectioned, coarsely pulverized, and subjected to hydrodistillation using a Clevenger apparatus for 6 to 7 hours. The essential oils (EOs) were isolated, dehydrated using anhydrous sodium sulfate, filtered, and preserved in glass vials, covered with aluminium foil, and stored at 4°C until analysis.

GC-MS analysis of rhizome essential oil

The chemical composition of the essential oil was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). The mass spectrometer was set up with a quadrupole and a source temperature of 150°C. The injector and interphase temperature were sustained at 250°C. The mass scan was conducted from 50 to 600 amu at a rate of 0.2 scans per second, with an inter-scan interval of 0.1 seconds. The electron ionization source was configured to a voltage of 70 eV. The volatile ingredients of *C. amada*, *C. angustifolia*, *C. caesia*, and *C. zedoaria* rhizome essential oils were identified by matching mass spectra to the NIST08 collection using Turbo mass™ software 6.1. Comparing experimental retention index values with literature values (Adams, 2017) and inserting authentic standards corroborated constituent identities. An n-alkane series (C8-C20, C21-C40) was performed in the same chromatographic conditions as the sample to compute experimental RI.

IN VITRO ANTIOXIDANT ACTIVITY

DPPH assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging study assessed the antioxidant property. Various quantities of the essential oil were combined with a methanolic DPPH solution (0.1 mM) and incubated in darkness for 30 minutes. The absorbance was recorded at 517 nm. Ascorbic acid (AA) and butylated hydroxytoluene (BHT) functioned as standards. The IC_{50} values, representing the concentration necessary to neutralize 50% of radicals, were determined.

FRAP assay

The lowering capacity of the essential oil was assessed using the Ferric Reducing Antioxidant Power assay (FRAP). The test sample was combined with phosphate buffer (0.2 M, pH 6.6) and potassium ferricyanide (1% w/v), incubated at 50°C for 20 minutes, and then treated with trichloroacetic acid (10% w/v). Following centrifugation, the supernatant was combined with $FeCl_3$ (0.1% w/v). Absorbance was measured at 700 nm. The EC_{50} values (effective concentration at which absorbance attained 0.5) were computed.

Pre-processing of ligand

Major bioactive chemicals found during GC–MS analysis were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in SDF format. The ligands underwent energy minimization and were transformed into PDBQT format via AutoDock Vina. Tautomers, stereoisomers, and protonation states were modified for docking preparation.

ADME and toxicity study

The SwissADME (http://www.swissadme.ch/) was used to predict medication resemblance in all phytocompounds. SwissADME analyzed molecular properties such as molecular weight, hydrogen acceptors and donors, rotatable bonds, and atoms for all phytocompounds using canonical SMILES from PubChem. To determine toxicity class, ProTox-II (https://tox-new.charite.de/protox_II/) was used. Then, substances with LD50 toxicity classes 3-6 were chosen.

Pre-processing of protein

The crystal structure of the target protein Xanthine oxidase (PDB ID: 3NRZ) was obtained from the RCSB Protein Data Bank (PDB) (https://www.rcsb.org/). Protein preparation was conducted using the AutoDock Vina program, including eliminating water molecules, incorporating hydrogen atoms, assignment of bond ordering, and energy minimization.

Protein binding site prediction

The PrankWeb server (https://prankweb.cz/) was used to identify the binding sites of target proteins, which were then confirmed by co-crystallized ligand locations. The active site residues were identified using molecular docking analyses within a grid box.

Molecular docking

AutoDock Vina was used for molecular docking to determine the binding affinity and orientation of ligands within the protein binding pocket. The docking settings were adjusted to ensure thoroughness and meet grid specifications. The study documented binding affinity scores (kcal/mol) and interaction profiles, including hydrogen bonds, hydrophobic and ionic interactions.

Desmond molecular dynamics simulation

The optimal docked complexes underwent 50 ns MD simulations with Desmond (D. E. Shaw Research: Resources) in an explicit solvent model (TIP3P water box, orthorhombic) for optimal analysis. Counter ions were introduced for system neutralization, and a physiological salt content of 0.15 M NaCl was sustained. The OPLS_2005 force field was used. System equilibration was achieved with the NPT ensemble at 300 K and 1 atm. Trajectories were examined for RMSD, RMSF, hydrogen bonding, hydrophobic contacts, ionic interactions, and water-bridge interactions to evaluate structural stability and binding durability.

STATISTICAL ANALYSIS

R Studio was used to do statistical visualization, and the data were shown via the use of a bar plot of antioxidant matrices with standard deviation error bars representing the results (Fig 1).

RESULT & DISCUSSION

Essential oil composition

The hydrodistilled rhizome essential oils of *C. amada*, *C. angustifolia*, *C. caesia*, and *C. zedoaria* were light yellow with a pleasant aroma. The average essential oil yields were determined as follows: 1.00 ± 0.07% for *C. amada*, 1.10 ± 0.01% for *C. angustifolia*, 1.50 ± 0.06% for *C. caesia*, and 1.90 ± 0.05% for *C. zedoaria*, on a fresh-weight basis.

The chemical constituents of four *Curcuma* species were characterised using GC-MS, and identification was achieved by comparing the mass spectra and retention indices (RI) of each peak with literature-reported RI values and the NIST spectral library. Major components (≥5% area) were further confirmed by co-injection with authentic reference standards. GC-MS analysis identified 67 constituents, accounting for 82.43-95.6% of the total volatile content in the rhizome essential oils of four *Curcuma* species. Monoterpene hydrocarbons (2.68-87.56%) represented the predominant chemical class in the essential oils, followed by oxygenated sesquiterpenes (2.09-55.02%). *C. angustifolia*, *C. caesia*, and *C. zedoaria* rhizome essential oils were rich in oxygenated sesquiterpenes, whereas *C. amada* has the highest percentage of monoterpene hydrocarbons (Table 1). The major constituents of *C. amada* essential oil are myrcene (63.33%), β-pinene (14.24%), and (E)-β-

ocimene (6.28%). The predominant constituents of C. *angustifolia* are velleral (17.83%), germacrone (12.92%), and (2E,6Z)-Farnesol (11.53%), followed by others. Volatile profiling of C. *caesia* revealed trans-β-elemenone (27.75%), δ-elemene (7.83%), curzerene (7.01%), 1,8-cineole (6.11%), and germacrone (5.45%) as its major constituents. The essential oil of C*urcuma zedoaria* revealed a sesquiterpene-dominant composition, featuring curzerenone (24.73%), β-eudesmol acetate (14.93%), 1,8-cineole (5.63%), and curzerene (5.05%) as predominant constituents. The major constituents identified in this study exhibit notable biological activities and hold diverse applications in the pharmaceutical and perfumery industries. These phytochemicals have demonstrated antimicrobial, anti-inflammatory, anticancer, and antioxidant properties (Burt, 2004; Miguel, 2010).

Earlier studies on C. *amada* essential oil reported myrcene as the predominant constituent, comprising over 80% of the total composition (Choudhury et al., 1996; Singh et al., 2002; Padalia et al., 2013). George et al. (2015) reported myrcene and β-pinene as the significant components in C. *amada* essential oil, similar to our result. C. *angustifolia* rhizome essential oil is reported to have germacrone and camphor as major compounds (Srivastava et al., 2006; Jena et al., 2017). Some previous studies reported various compounds, viz. β-elemenone, curzerenone, α-bulnesene, eucalyptol, curzerene, and camphor, predominantly present in the C. *caesia* rhizome oil (Gangal et al., 2023; Mukunthan et al., 2014). Their results showed that the most predominant class of oil was sesquiterpenes, which is in agreement with our findings. Similarly, Poudel et al. (2022) revealed that the most dominant compounds in C. *zedoaria* were curzerenone, 1,8-cineole, curzerene, and camphor, mostly sesquiterpenoids. Also, another study by Dosoky et al. (2019) reported a similar kind of result for C. *zedoaria* essential oil, which predominantly consists of 1,8-cineole, curzerenone/epi-curzerenone, α-copaene, camphor, β-caryophyllene, elemol, germacrone, curzerene, and β-elemene as major compounds. The compounds identified in the current study were found to be in line with previous research.

Table 1: The GC-MS analysis of rhizome essential oils of *C. amada, C. angustifolia, C. caesia,* and *C. zedoaria*

Sl no.	Compound name	RI[a]	RI[b]	Concentration (%)			
				C. amada	***C. angustifolia***	***C. caesia***	***C. zedoaria***
1	α-Thujene	919	930	0.04	-	-	-
2	Tricyclene	927	926	2.11	0.48	0.38	0.51
3	Camphene	943	954	0.47	1.38	1	1.45
4	Sabinene	966	975	0.32	0.22	-	-
5	β-Pinene	974	979	14.24	-	0.65	0.46
6	Myrcene	997	990	63.33	0.24	0.22	0.26
7	α-Terpinene	1013	1017	0.04	-	-	-
8	ρ-Cymene	1020	1024	0.02	-	-	-
9	Limonene	1024	1029	0.63	0.61	0.58	-
10	1,8-Cineole	1029	1031	0.91	3.32	6.11	5.63
11	(E)-β-Ocimene	1042	1037	6.28	0.49	-	-
12	γ-Terpinene	1052	1059	0.04	-	-	-
13	Terpinolene	1079	1088	0.04	-	-	-
14	2-Nonanone	1085	1090	0.35	0.12	0.1	0.12
15	Linalool	1090	1096	0.08	2.55		0.1
16	n-Nonanal	1093	1100	0.25	-	-	-
17	trans-Thujone	1095	1110	0.17	-	-	-

18	Camphor	1144	1146	-	3.64	3.96	4.31
19	Isoborneol	1156	1160	0.12	1.88	1.08	1.44
20	trans-β-Terpineol	1167	1163	-	0.43	0.43	0.44
21	α-Terpineol	1174	1188	0.1	0.4	0.56	0.53
22	γ-Terpineol	1190	1199	-	0.51	-	-
23	Isobornyl acetate	1281	1285	-	-	4.35	1.2
24	2-Undecanone	1286	1294	0.12	-	-	-
25	δ-Elemene	1327	1338	-	0.8	7.83	-
26	Eugenol	1344	1359	0.38	-	-	-
27	α-Copaene	1366	1376	-	0.11	-	-
28	β-Elemene	1380	1390	0.07	4.1		3.5
29	β-caryophyllene	1410	1419	1.75	2.92	0.9	0.5
30	γ-Elemene	1420	1436	-	0.31	0.24	0.2
31	Aromadendrene	1439	1441	-	0.32	0.32	0.19
32	α-Humulene	1444	1454	0.2	0.94	0.24	0.25
33	allo-romadendrene	1479	1460	-	0.19	0.19	0.12
34	Germacrene D	1487	1484	0.04	3.457	0.14	1.89
35	**γ**-Himachalene	1477	1482	-	2.7	0.13	0.95
36	β-Selinene	1479	1490	-	0.67	1.01	0.53
37	δ-Selinene	1483	1492	0.15	-	-	-
38	Curzerene	1488	1499	-	5.66	7.01	5.05
39	β-Bisabolene	1495	1505	0.03	-	2.97	-
40	Bicyclogermacrene	1499	1500	-	2.19	-	-
41	Germacrene-A	1508	1509	0.04	0.32	0.58	0.29
42	δ-Cadinene	1518	1523	-	0.67	0.32	0.22
43	10-epi-Cubebol	1531	1535	-	0.14	-	-
44	Germacrene B	1546	1561	0.14	2.78	3.47	1.28
45	(E)-Nerolidol	1560	1561	0.21	-	-	-
46	Himachalene epoxide	1576	1579	-	0.28	-	-
47	Caryophyllene oxide	1579	1583	-	0.4	0.43	0.39
48	1-Hexadecene	1589	1593	-	-	0.37	0.31
49	Viridiflorol	1590	1592	0.57	0.56	-	-
50	trans-β-Elemenone	1596	1602	-	0.18	27.75	-
51	5-epi-7-epi-α-Eudesmol	1603	1607	-	0.18	-	-
52	Curzerenone	1606	1606	-	-	-	24.73

53	β-Atlantol	1608	1608	-	1.39	1.32	-
54	Humulene epoxide II	1608	1605	0.14	-	0.34	0.22
55	2-epi-α-Cedren-3-one	1622	1627	-	0.75	-	-
56	Muurola-4,10(14)-dien-1-β-ol	1627	1631	0.07		0.37	1.77
57	γ-Eudesmol	1635	1635	-	-	0.18	-
58	α-Muurolol	1640	1648	-	1.2	1.18	-
59	β-Eudesmol	1654	1655	0.29	0.6	0.27	1.23
60	Germacrone	1680	1693	0.04	12.92	5.45	3.48
61	(2Z,6Z)-Farnesol	1702	1798	-	-	-	1.14
62	(2E,6Z)-Farnesol	1713	1715	-	11.53	-	1.38
63	Velleral	1730	1739	-	17.83	-	2.68
64	Xanthorrhizol	1754	1753	-	3.23	-	-
65	(2Z,6E)-Farnesol	1788	1781	-	-	-	1.55
66	γ-Eudesmol acetate	1785	1784	0.63	-	-	1.21
67	β-Eudesmol acetate	1801	1792	-		-	14.93
Monoterpene hydrocarbons				87.56	3.42	2.83	2.68
Oxygenated monoterpenes				2.48	12.85	16.59	13.77
Sesquiterpene hydrocarbons				2.28	28.28	25.35	14.97
Oxygenated sesquiterpenes				2.09	51.05	37.66	55.02
Total				94.41	95.60	82.43	86.44

RIa calculated from a homologous series of n-alkane (C8 – C20,) on the Elite-5 column.
RIb obtained from literature.

Antioxidant activity

Antioxidants are essential for the defence of human health by neutralizing free radicals, which are recognized as factors in aging and other degenerative disorders. Terpenoids in essential oils are known for their potent antioxidant properties (Sharifi-Rad et al., 2018; Nayak et al., 2024). The observed activity may be attributed to the predominant constituents and the influence of minor compounds, as their collective interactions can produce beneficial synergistic effects (Fadel et al., 2020). This present study assessed the antioxidant capacity of *C. amada*, *C. angustifolia*, *C. caesia*, and *C. zedoaria* by DPPH radical scavenging and reducing power assays, utilizing ascorbic acid (AA) and butylated hydroxytoluene (BHT) as reference standards.

The activity of DPPH radical scavenging showed significant interspecies variability. The ideal IC_{50} values were determined as follows: *C. angustifolia* EO (31.2 ± 0.3 µg/mL), *C. caesia* (39.8 ± 0.5 µg/mL), *C. amada* (52.5 ± 0.8 µg/mL), and *C. zedoaria* (58.7 ± 1.0 µg/mL). Four essential oils demonstrated significant scavenging value compared to ascorbic acid (14.7 ± 0.4 µg/mL) and BHT (21.6 ± 0.9 µg/mL). The reducing power activity (RPA) demonstrated a similar potency hierarchy: ascorbic acid (15.3 ± 0.6 µg/mL) > BHT (18.5 ± 0.8 µg/mL) > *C. angustifolia* (23.1 ± 0.2 µg/mL) > *C. caesia* (27.0 ± 0.4 µg/mL) > *C. amada* (32.4 ± 0.5 µg/mL) > *C. zedoaria* (38.2 ± 0.3 µg/mL) (Fig. 1).

This investigation reports that the actions of *C. angustifolia* and *C. caesia* are distinct and consistent with previous research. Jena et al. (2017) documented an IC_{50} of 25.1 µg/mL for the essential oil of *C. angustifolia*

rhizome, while Albaqami et al. (2022) identified comparable IC_{50} values ranging from 26.4 to 28.7 µg/mL for leaf oils. *C. caesia* is widely recognized for its potent free radical scavenging ability. Paw et al. (2020) documented an IC_{50} of 18.65 µg/mL for rhizome essential oil, while Borah et al. (2019) established an IC_{50} of 22.7 µg/mL for leaf oils, and Kanglom et al. (2024) validated activity with an IC_{50} of 20.5 µg/mL in methanolic extracts. The investigation found that *Curcuma amada* exhibited modest activity, with an IC_{50} of 52.5 µg/mL. George et al. (2015) documented a higher IC_{50} of 41.2 µg/mL, which exceeded that of the synthetic antioxidant BHT (IC_{50} typically >50 µg/mL). The variation in activity can be attributed to variations in extraction techniques, the specific plant component used, or chemotypic diversity. Likewise, for *C. zedoaria* Mau et al. (2003) reported an IC_{50} of 32.9 µg/mL for DPPH scavenging, but Rahman et al. (2014) recorded a somewhat elevated value of 35.7 µg/mL. The modest scavenging activity and its robust electron-donating ability indicate that *C. zedoaria* mostly demonstrates antioxidant benefits via reducing pathways rather than hydrogen atom transfer.

All four *Curcuma* essential oils exhibited significant antioxidant activity compared to the synthetic standard BHT, and ascorbic acid. The variation in the antioxidant activity might be the variation of phytoconstituents (Nayak et al. 2024). The observed low IC_{50} and EC_{50} values, along with very low to no side effects of all four essential oils, underscore their potential as candidates for advancement in nutraceutical and pharmacological applications.

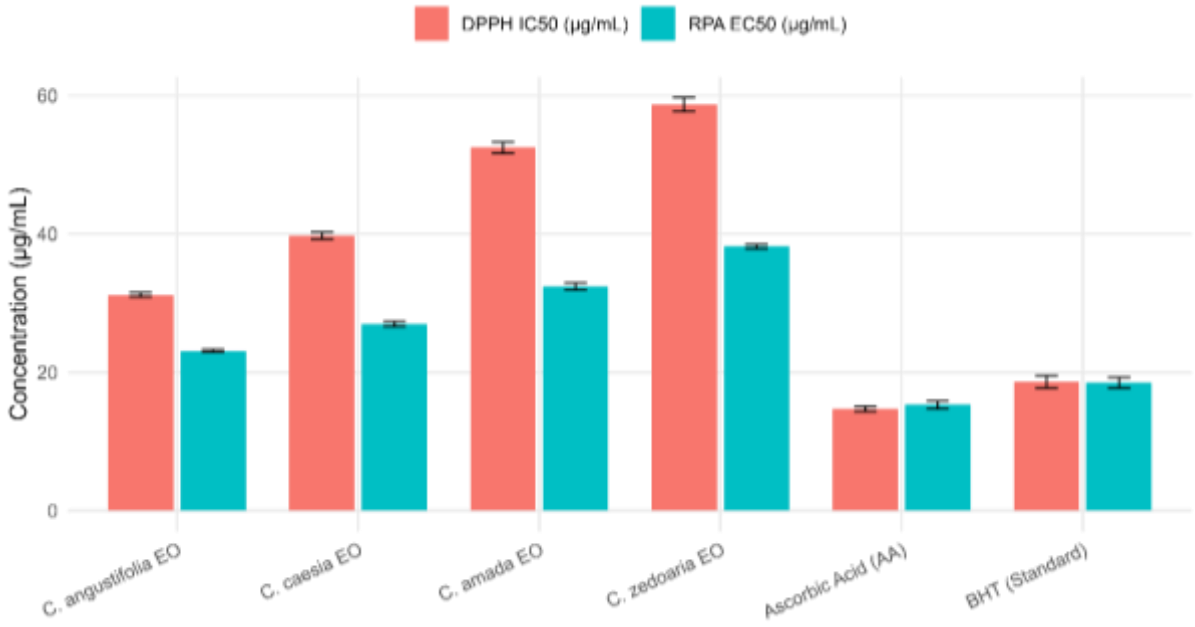

Fig 1: Anti-oxidant activity metrics of the four *Curcuma* species essential oils

IN SILICO ANALYSIS

Prediction of drug-likeness properties of selected ligands

The phytocompounds (<1% area) identified through GC-MS analysis of the essential oils from all four species were evaluated for drug-likeness properties with SwissADME (Sympli, 2021). The compounds lacking strong drug-like characteristics were discarded. The prediction was conducted by adhering to several parameters, including molecular weight (MW) < 300, number of hydrogen acceptors (nOHNH) ≤ 5, number of hydrogen donors (nON) < 10, compliance with the Lipinski Rule of 5, the Veber Rule, positive enzyme inhibitors score from bioactivity score prediction, and a lower toxicity class (Class 3-6) (Veber et al., 2002). Subsequently, the qualifying compounds underwent toxicity study via ProTox-II. All phytocompounds classified under LD50 classes 3, 4, 5, and 6 were selected. The selected compounds have met the criteria for drug-likeness and were determined to be non-toxic and non-mutagenic (Table S1). The top 11 compounds were selected for a molecular docking study (Mousavi et al., 2021) against Xanthine oxidase protein, which is responsible for causing oxidation reactions, to find the possible inhibitors among the compounds.

Prediction of binding sites

The binding sites describe the location of a protein segment involved in the adhesion of small molecules. The crystal structures obtained from the complete models of Xanthine oxidase were predicted for the binding site utilizing PrankWeb server (Fig. 2). The structure contains many pockets at various site locations. The ligand binding site for Xanthine oxidase was identified as site-5 with coordinates (x = 45.98, y = -10.59, z = 18.002).

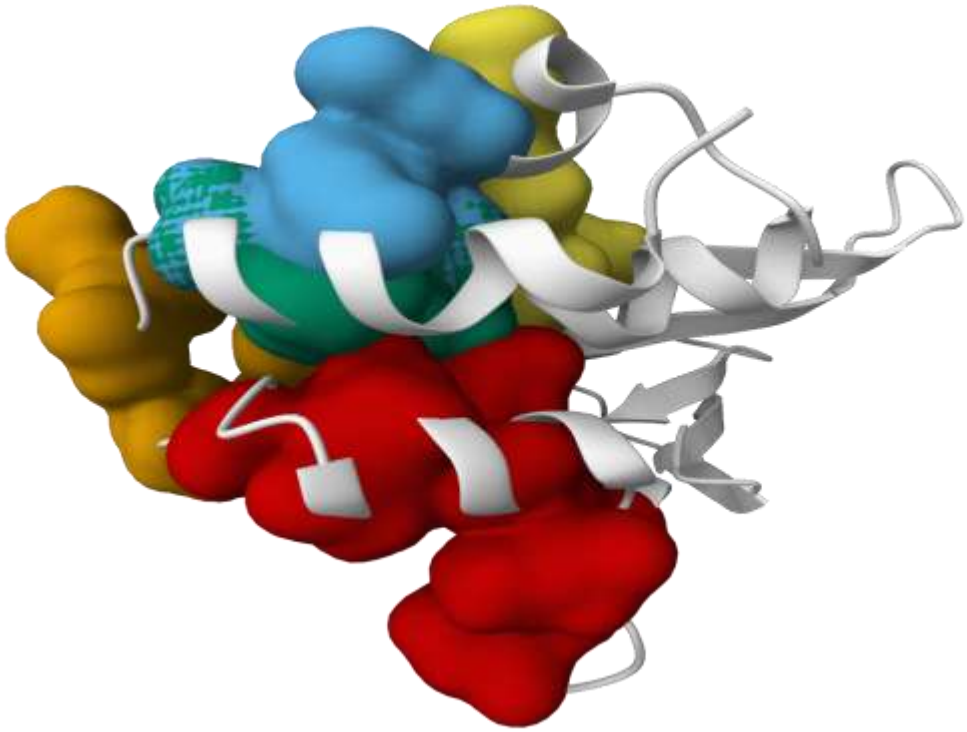

Fig 2: This figure shows the binding site of ligands to the active sites of the target protein, providing valuable insights into the interaction between the ligands and protein receptor (Red: Pocket 1, Blue: Pocket 2, Green: Pocket 3, Orange: Pocket 4, Mustard: Pocket 5).

Molecular docking

Molecular docking is a computational method used in drug discovery to predict the interaction and fit of a small molecule into a target protein's binding site, assessing its binding affinity and potential therapeutic efficacy (Gadnayak et al., 2025). Co-crystallized ligands are commonly used in molecular docking research to interact with their target proteins (Nayak et al., 2024). The study involved selecting ligands that passed ADMET screening and were then docked with xanthine oxidase crystal structures to evaluate their binding interactions. The docking was conducted at the selected protein binding site. AutoDock Vina was utilized for docking, revealing 11 compounds with high scores against the protein, with the calculated binding affinities presented in Table 4. The evaluated ligands, Germacrone (-6.5 kcal/mol), Velleral (-6.2 kcal/mol), β-Caryophyllene (-6.1 kcal/mol), Germacrene B (-6.0 kcal/mol), and Neocurdione (-6.0 kcal/mol) exhibited comparatively enhanced binding affinities, suggesting their capacity to involve with the XO active site. These results are advantageous in comparison to other evaluated chemicals, such as Linalool (-4.1 kcal/mol) and 1,8-Cineole (-4.8 kcal/mol), which demonstrated inferior binding interactions (Table 4). The docking scores indicate that sesquiterpenes, including Germacrone, Velleral, Germacrene B, and β-Caryophyllene, are pivotal in suppressing XO. Their hydrophobic frameworks may facilitate stable connections with the enzyme's active site via van der Waals forces and hydrophobic interactions. Monoterpenes like Linalool and 1,8-Cineole showed reduced binding affinities due to their smaller molecular size and limited ability to establish stable connections within the binding cavity. Xanthine oxidase is crucial for purine metabolism, uric acid and ROS production. Inhibiting XO can reduce oxidative stress, treat gout, hyperuricemia, and cardiovascular issues. The study suggests that *Curcuma* species sesquiterpenes like Germacrone and β-Caryophyllene may be effective natural XO inhibitors, along with their antioxidant properties. Previous studies have demonstrated that plant-

derived terpenoids and sesquiterpenes are antioxidants and XO-inhibitors (Mehmood et al., 2019; Koul et al., 2019). The investigation confirms Germacrone's binding affinity (-6.5 kcal/mol), indicating its potential as a bioactive molecule with anti-inflammatory and antioxidative properties. β-Caryophyllene, a studied sesquiterpene, may enhance the antioxidant properties of *Curcuma* essential oils by regulating oxidative enzymes. Germacrone and Velleral were the top-scoring compounds against the Xanthine oxidase protein, as depicted in Fig. 3A and B.

Table 4 The study examines the docking results of compounds from all four *Curcuma* species with Xanthine oxidase

Sl no.	Ligand	PubChem CID	Binding Affinity
1	1,8-Cineole	CID_2758	-4.8
2	Beta-Caryophyllene	CID_5281515	-6.1
3	Beta-Elemene	CID_6918391	-5.5
4	Camphor	CID_2537	-5.1
5	Curzerene	CID_572766	-5.8
6	Gamma-Cadinene	CID_15094	-5.8
7	Germacrene B	CID_5281519	-6
8	Germacrone	CID_6436348	-6.5
9	Linalool	CID_6549	-4.1
10	Neocurdione	CID_5316216	-6
11	Velleral	CID_14412869	-6.2

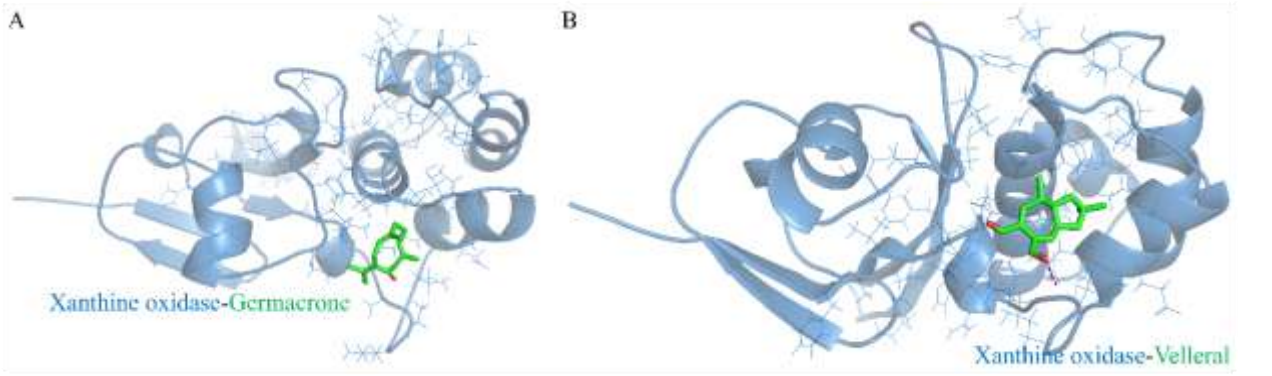

Fig 3: The molecular docking results of active constituents with target protein (A) Xanthine oxidase against Germacrone and (B) Xanthine oxidase against Velleral were examined.

Molecular dynamics simulation

Molecular dynamics simulation was performed using Desmond for Germacrone (-6.5 kcal/mol) and Velleral (-6.2 kcal/mol) complexed with xanthine oxidase to assess dynamic stability and interaction patterns beyond static docking predictions. MD provides a temporal perspective on ligand-protein interactions, aiding in confirming docking outcomes and predicting plausible binding configurations (Hollingsworth &Dror, 2018; Hospital et al., 2015). The RMSD trajectory indicated that Germacrone generated structural stability after 25 ns and maintained this stability until the conclusion of the 50 ns simulation, exhibiting fluctuations within 5.6 Å (Fig. 4A). This signifies a comparatively stable binding shape, appropriate for efficient inhibition of XO. The RMSF study exhibited satisfactory flexibility among critical residues, revealing no significant destabilizing variations (Fig. 4A). The examination of interaction fraction histograms revealed persistent hydrogen bonding with LYS40 and water-bridge interactions with ARG37, GLY38, LYS40, SER93, THR94, GLN102, GLN112, and THR117. Additionally, hydrophobic connections were preserved with VAL88, LEU98, ILE105, ALA106, PRO118, and VAL121, in conjunction with ionic interactions involving LYS40, GLN112, and THR117 (Fig. 5 A). Several stable hydrogen and hydrophobic interactions indicate a robust affinity, enhancing Germacrone's exceptional docking score and dynamic stability. These results align with previous

terpenoid–protein interactions studies that demonstrate comparable stability characteristics (Mehmood et al., 2019; Koul et al., 2019).

RMSD study for Velleral demonstrated stability from approximately 20 ns to 45 ns, with slight variations observed from 45 ns to 50 ns, peaking at around 9 Å. Notwithstanding the elevated RMSD relative to Germacrone, the complex exhibited considerable stability, indicating that Velleral had modest conformational flexibility while retaining its binding orientation (Fig. 4 B). RMSF readings remained within acceptable limits, indicating permissible variations of protein side chains (Fig. 4 B).The interaction fraction histogram indicated robust hydrogen bonds with GLY38, LYS40, THR94, GLN102, and GLU103, whereas water bridges involving ARG37, GLY38, LYS40, SER93, THR94, LYS95, GLN102, GLU103, ALA106, SER111, GLY114, and THR117 further enhanced the stability of the complex. Hydrophobic interactions were established with VAL88, LEU98, ILE105, PRO118, and VAL121 (Fig. 5 B). Compared to Germacrone, Velleral exhibited more ephemeral hydrogen bonds, which may explain its marginally diminished stability in the later phase of the simulation.

Germacrone exhibited superior stability during MD compared to Velleral, as indicated by its reduced RMSD deviations and enhanced hydrogen bond persistence. Both ligands exhibited substantial water-mediated and hydrophobic interactions, suggesting that these terpenoids are crucial in maintaining the ligand-XO complex. These findings underscore the potential of *Curcuma*-derived compounds as natural xanthine oxidase inhibitors, consistent with other research that highlighted the significance of terpenoids in the control of oxidative stress (Kumar et al., 2016; Kanyal et al., 2023).

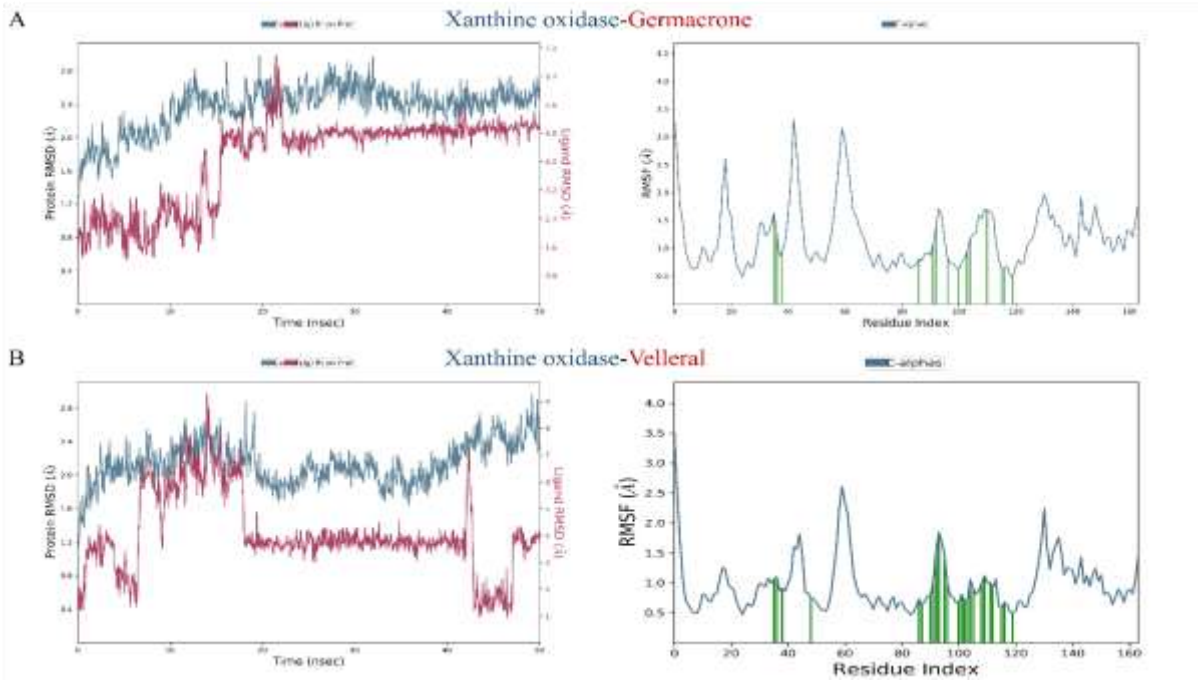

Fig 4: Molecular dynamic simulation was utilized to assess conformational changes, stability, and compatibility of protein-ligand complexes, as shown in RMSD and RMSF plots for (A) Xanthine oxidase-Germacrone and (B) Xanthine oxidase-Velleral.

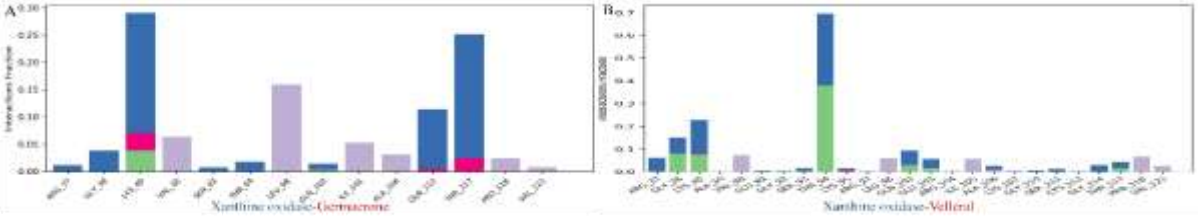

Fig 5: The simulation analysis reveals minor fluctuations in the (A) Xanthine oxidase-Germacrone interaction and the (B) Xanthine oxidase-Velleral interaction.

CONCLUSION

The comparative phytochemical and biological assessment of essential oils from four *Curcuma* species highlights their chemical variety and therapeutic potential. The prevalence of bioactive terpenoids, including germacrone, curzerenone, and β-caryophyllene, accounts for the significant antioxidant properties noted. *In silico* predictions and molecular docking established that sesquiterpenes, including germacrone and velleral, have favorable interactions with xanthine oxidase, while molecular dynamics simulations corroborated their binding stability. The results indicate that *Curcuma* rhizome essential oils exhibit significant free-radical scavenging capabilities and demonstrate potential as natural inhibitors of oxidative enzymes, making them suitable candidates for further exploration in nutraceuticals, pharmaceuticals, and functional food applications. Subsequent research should prioritize in vivo validation and clinical assessment to facilitate the translation of these results into therapeutic applications.

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