

Molecular Simulation-based Combinatorial Modeling and Antioxidant Activities of Zingiberaceae Family Rhizomes

Talambedu Usha^{1,2}, Sushen Pradhan³, Arvind Kumar Goyal⁴, Shanmugarajan Dhivya¹, H. P. Prashanth Kumar⁵, Manoj Kumar Singh⁶, Neelu Joshi⁶, Bharat Chandra Basistha³, K. R. Siddalinga Murthy⁷, Saravanakumar Selvaraj⁸, Sushil Kumar Middha^{1,9}

¹DBT-BIF Facility, Biotechnology Finishing School, Science Research Centre, Maharani Lakshmi Ammanni College for Women, Departments of ²Biochemistry and ³Biotechnology, Maharani Lakshmi Ammanni College for Women, Bengaluru, Karnataka, ⁴Sikkim State Council of Science and Technology, Gangtok, Sikkim, ⁵Centre for Bamboo Studies, Department of Biotechnology, Bodoland University, Kokrajhar, Assam, ⁶Department of Biotechnology, Saphthagiri College of Engineering, Bengaluru, Karnataka, ⁷Department of Biotechnology, Kumaun University, Nainital, Uttarakhand, India, ⁸Department of Biochemistry, Bangalore University, Bengaluru, Karnataka, ⁹Centre for Pharmacology and Toxicology, Hannover Medical School, 30625 Hannover, Germany

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ABSTRACT

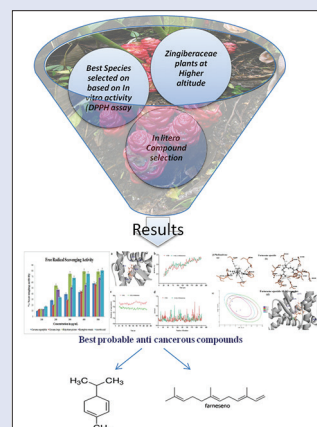
Objective: The main aim of this scientific report was to investigate a series of phytochemicals *in silico* and the pharmacology of four plants found at higher altitude in the ginger family, Zingiberaceae (incl. Costaceae) from North-East India, particularly Sikkim. First, the goal was to determine the biological activities of the four herbs (used under Zingiberaceae family) using antioxidant assays to identify the best species. Second, previously reported compounds *in vitro* were subsequently screened for their anticancerous activities using *in silico* methods. **Materials and Methods:** Using the methanolic extracts of herbs, quantitative detection of phytochemicals such as total phenols and total flavonoids was detected, and the free radical scavenging activity was also studied using 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assay. Docking process was studied, using Discovery Studio version 3.5, to identify suitable molecules at the protein-binding sites through annealing and genetic simulation algorithms. Grids centered on active sites were obtained with spacing of 54 × 55 × 56, and 0.503 grid spacing was calculated. The methods adopted and used in this study were comparisons of Global and Local Search Methods to determine the parameters such as maximum number of 250,000 energy evaluations as well as generations of 27,000, followed by mutation and crossover rates of 0.02 and 0.80. The number of docking runs was set to 10. Molecular dynamics study was done to check the stability of the complex. **Results:** Among all the genus of Zingiberaceae family investigated in this study, *Curcuma angustifolia* and *Hedychium sp.* exhibited the highest 537 ± 12.45; 292 ± 9.16 mg gallic acid equivalent/g total polyphenols and 38 ± 1.54; 75 ± 6.75 mg quercetin equivalent/g flavonoids, respectively. Depending on the concentration, the *Hedychium sp.* extract exerted the highest scavenging activity on DPPH radical (IC₅₀ 36.4 µg/mL). *In silico* result demonstrated that the synergetic effects of β-phellandrene with other compounds might be responsible for its anticancerous activity. β-phellandrene and farnesene epoxide showed bonding with Leu²⁹⁸, Ala³⁰², Met³³⁶, Leu³³⁹, Leu³⁴³, Phe³⁵⁶, Ala³⁰², Glu³⁰⁵, Met³⁴⁰, Leu³⁴³, Arg³⁴⁶, Phe³⁵⁶, Ile³⁷³, Ile³⁷⁶, Leu³⁸⁰, His⁴⁷⁵, Leu⁴⁷⁶, and Leu⁴⁹¹. **Conclusion:** Based on the current available literature, this is the first study to understand the interaction of compounds found in the rhizomes of Zingiberaceae family.

Key words: Zingiberaceae, *Curcuma*, cancer, CADD, molecular dynamics

SUMMARY

- The aqueous methanolic extract of Zingiberaceae family *Curcuma angustifolia* and *Hedychium sp.* has potent antioxidant activity as assessed by 2,2-diphenyl-1-picryl-hydrazyl assays

- Hedychium sp.* is understood to possess more active compounds than other varieties
- In silico* studies indicated synergetic effects of β-phellandrene and other compounds for its anticancerous activity.



Abbreviations used: CADD: Computer-aided drug designing; ROS: Reactive oxygen species; ADMET: Absorption, distribution, metabolism, and excretion-toxicity; FeCl₃: Ferric chloride; DPPH: 2,2-diphenyl-1-picryl-hydrazyl; NaNO₂: Sodium nitrite; TCA: Trichloroacetic acid; K₂HPO₄: Di-potassium hydrogen phosphate; H₂O₂: Hydrogen peroxide; KH₂PO₄: Potassium di-hydrogen phosphate; K₂Fe (CN)₆: Potassium ferricyanide; KOH: Potassium hydroxide; NaOH: Sodium hydroxide; Na₂CO₃: Sodium carbonate; CH₃COONa: Sodium acetate; AlCl₃: Aluminum chloride.

Correspondence:

Dr. Sushil Kumar Middha,
Department of Biotechnology, Biotechnology
Finishing School, Science Research Centre,
DBT-BIF Facility, Maharani Lakshmi Ammanni
College for Women, Bengaluru - 560 012,
Karnataka, India.

E-mail: drsushilmiddha@gmail.com

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INTRODUCTION

Lack of scientific knowledge to find a cure for health ailments leads to the diversion of research toward new strategies such as exploration of traditional ethnobotanical knowledge. Plants constitute an important source of various secondary metabolites.^[1] High-altitude medicinal plants acquire a unique collection of secondary metabolites

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that can act as a potential therapeutic targets for diseases.^[2] Bioactive natural inventions with remedial potential have relied on external circumstances and some of these compounds are beyond exploration by traditional means. Therefore, the efficiency of computational tools to facilitate drug discovery are known and has been recognized even in case of natural products.^[1,3-5] Computer-aided drug designing (CADD) utilizes the existing information from the ethnobotanical data to provide valuable understanding to address the existing therapeutics and for the development of new nature-derived drugs. In addition, more than a few medicinal plants have been continuously utilized in various conventional medicinal systems since olden days throughout the globe. However, their mechanisms have not yet been explored properly. Therefore, the CADD approaches for plant-based drug discovery would yield immense gain in advancing the world's health population.

Antioxidants are the counterparts that can restrain single oxygen or free radicals prior to any noteworthy oxidant force taking place.^[2,6] Increasing knowledge and lookout for a healthier product having fewer preservatives and synthetic additives are motivating research endeavors toward the natural antioxidants. Plant-related dietary products are gaining consideration due to their increasing scientific evidence, having indicated their richness in several phenolic compounds; therefore, preventing pathological conditions such as diabetes, cancer, and neurological diseases.

Zingiberaceae family, an Angiosperm (flowering plants) group which contains 52 plant genera including genus such as *Curcuma angustifolia*, *Curcuma longa*, *Hedychium* sp., and *Kaempferia rotunda* Linn., is widely distributed in Asian and African regions, known to have diverse pharmacological activities (<http://www.theplantlist.org/1.1/browse/A/zingiberaceae/>).

Curcuma and Ginger are the most imperative known crops in the hill state of Sikkim, India, at North-East Himalayas (27° 00' 46" N–28° 07' 48" N latitudes and 88° 00' 58" E–88° 55' 25" E longitudes) next to *Citrus* sp. and *Amomum subulatum*. The Sikkimese tribes, namely, Lepcha and Bhutia use them for their religious activities besides culinary and medicinal use.^[7] Pradhan and Badola^[8] stated 118 medicinal plant species at the high altitude of Sikkim practiced by Lepcha tribe of Dzongu valley, distributed across 71 families and 108 genera. Sharma^[9] reported that *C. angustifolia* plant is used for curing worms and stomach ache. Curcumin isolated from *C. longa* is well known for its pharmacological properties. The antioxidant, antibacterial, antifungal, cytotoxic, and chemopreventive effects are the well-known pharmacological properties of *Hedychium* sp. (butterfly ginger) in addition to anti-urolithiasis, anti-angiogenic, neuro-pharmacological, fibrinogenolytic, coagulant, anti-allergic, larvicidal, anthelmintic, analgesic, anti-inflammatory, and hepatoprotective activities.^[10] The phytochemistry of *Hedychium* sp. and *K. rotunda* Linn. (Indian crocus) is still unknown, which makes these species studying worthwhile. Though the pharmacological properties of these rhizomes have been previously reported, the mechanisms of therapeutic principles are still unknown. Hence, the objective of this study was to investigate the pharmacological properties of four indigenous rhizomes in addition to interaction against an estrogen receptor and a series of previously reported compounds (phytochemicals) *in silico* found in Zingiberaceae (incl. Costaceae) family from Sikkim, India, reported at a higher altitude.

MATERIALS AND METHODS

Chemicals and reagents

Ascorbic acid, 2,2-diphenyl-1-picryl-hydrazyl (DPPH), ferric chloride, gallic acid, naphthyl ethylenediamine dihydrochloride, quercetin, sodium nitrite (NaNO₂), sulphanilamide, and trichloroacetic acid were obtained from HiMedia Laboratories Pvt. Ltd., Mumbai, India.

Di-potassium hydrogen phosphate, hydrogen peroxide, methanol, potassium di-hydrogen phosphate, potassium ferricyanide, potassium hydroxide, sodium hydroxide (NaOH), sodium carbonate (Na₂CO₃), sodium acetate, and sodium nitroprusside were procured from Merck, Mumbai, India, and Folin–Ciocalteu (FC) reagent was purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. Aluminum chloride (AlCl₃) and ortho-phosphoric acid were obtained from SD Fine Chemicals Limited, Mumbai, India. All chemicals and solvents were of analytical grade.

Plant materials and extraction

The fresh rhizomes of *C. angustifolia*, *C. longa*, *Hedychium* sp., and *K. rotunda* Linn. were collected in June 2015 from Sajong, Sikkim, India. The specimen vouchers have been identified by a plant taxonomist and deposited at Sikkim State Council of Science and Technology, Sikkim, India, and the given voucher numbers are GT07, GT08, GT09, and GT10. The washed rhizomes were air-dried and macerated using a grinder. The powdered rhizomes were defatted with 1:1 v/v petroleum ether (b.p. 40°C–60°C) in a Soxhlet apparatus. Then, 20 g of each processed sample was extracted using Soxhlet apparatus with 70% aqueous methanol in 1:15 w/v ratio at 70°C for 4 h in three cycles.^[11] The lyophilized extracts were stored at 4°C until used. The samples were dissolved in double-distilled water (DDW) in the required aliquots just before use.

Preliminary phytochemical screening

All four freshly prepared extracts were subjected to preliminary phytochemical analysis according to the standard protocols.^[12]

Determination of the yield of plant extract

The yields of evaporated rhizome extracts were calculated using the author's previous reports.^[3]

Determination of biochemical constituents

Determination of total phenolic content

FC reagent method^[13] with slight alteration was used to assess total phenolic content. A volume of 0.5 mL of the extracts was mixed with 0.5 mL of FC reagent (previously diluted 1:1 with DDW) and incubated at room temperature (RT) for 5 min. Followed by the addition of 1 mL of 20% Na₂CO₃ solution, the mixture was incubated at RT for 10 min. The absorbance was measured at 730 nm. Gallic acid monohydrate was used as a standard and the total phenolic content was defined as Gram gallic acid equivalents per 100 g of the extract.

Determination of total flavonoid content

The AlCl₃ method was used to determine the total flavonoid content of samples.^[14] The reactive mixture was prepared by adding the extract with 1.25 mL DDW, followed by 75 µl of NaNO₂ (5%). After 5 min of incubation at RT, 0.15 mL of AlCl₃ (10%) was added and kept at RT for 6 min. Then, the mixture was treated with 0.5 mL of 1 mM NaOH. Finally, the sample was diluted with 275 µl of DDW followed by incubation at RT for 20 min. The absorbance was measured at 510 nm. The absorbance was measured at 510 nm and the total flavonoid content was determined from quercetin standard curve.

2,2-diphenyl-1-picryl-hydrazyl radical scavenging activity

DPPH method was used to determine the free radical scavenging activity of the sample and the standard, ascorbic acid.^[15] A volume of 0.5 mL of 100–1000 µg of the extracts was mixed with freshly prepared 0.5 mL DPPH solution and the final volume was made up to 2 mL. A control sample was the same volume without any

extract. Methanol was used as a blank. The reaction mixture is allowed to stand for 30 min in dark. The discoloration was measured at 517 nm. The experiment was performed in triplicates. DPPH scavenging activity (%) was determined using the following equation: DPPH scavenging activity (%) = $A_0 - A_1/A_0 \times 100$

Where A_0 is the absorbance of the control and A_1 is the sample.

Docking studies

An *in silico* study was performed to understand the protein–ligand interaction similarly as per our previously designed protocol.^[16]

Generation of dataset

Eighty-one compounds of Zingiberaceae (incl. Costaceae) were selected from PubMed (<http://pubmed.ncbi.nlm.nih.gov/>) and downloaded from PubChem database (<http://pubchem.ncbi.nlm.nih.gov/>) as ligands considering their chemical and physical properties. The 2D structures of all these compounds were drawn using MarvinSketch, and biological activities were calculated using Molinspiration (www.molinspiration.org) server.

Preparation of the ligands

Drawn ligands were processed during the ligand preparation step for various stages having included addition and deletion of hydrogen atoms and heteroatom.^[16,17] In addition, charged group neutralization, low-energy ring conformers, geometry optimization, and saving of output files were performed^[5,18] using Discovery Studio (DS) version 3.5 (Biovia, USA). Lipinski's filter of 5 with various parameters such as hydrogen donors, acceptors, log *P* value, molecular weight, and the reactive filter were followed to discard the ligands with poor pharmacological properties and reactive functional groups.^[19]

Preparation of the receptors

The three-dimensional (3D) structure of the receptor (Protein Data Bank [PDB] ID-1U3Q) was downloaded from PDB (<http://www.rcsb.org>). The X-ray crystal structure of the estrogen receptor has 2.4 Å resolution, *R*-value free: 0.279, *R*-value work: 0.227, and is biologically active as well as in a stable state. PDB structure was processed as per the standard accessible protocol of DS version 3.5.^[5]

Absorption, distribution, metabolism, and excretion/TOxicity Prediction by Komputer-assisted Technology

The energy-minimized natural compounds were subjected to absorption, distribution, metabolism, and excretion (ADME)/Tox (ADMET) calculations using ADME-TOxicity Prediction by Komputer-assisted Technology (TOPKAT) (DS 3.5). The TOPKAT predicts structurally significant candidates and pharmaceutically relevant properties such as Ames mutagenicity, carcinogenicity, rat oral LD₅₀, rat maximum tolerated dose, rat inhalation toxicity LC₅₀, and Log *P* of the probable candidates. ADMET descriptor includes various parameters such as human intestinal absorption, CYP2D6 binding, plasma protein binding, blood–brain barrier (BBB) penetration, and aqueous solubility.

Simulation studies

The study was followed to explore the protein–ligand interactions. Docking allows virtually screening compounds and predicting the strongest binders based on their scoring functions. The software used here to do docking simulation was iGEMDOCK against all prepared compounds. This system's total energy alias Gibbs energy (kcal/mol) is calculated and recorded by the system.

LigandFit/Discovery Studio 3.5

Ligand fit is a shape-directed docking method of ligands to bind with the specific active sites of the protein.^[20] To initiate docking study in DS

3.5, receptor or target protein was prepared by removing heteroatoms, water molecules. The Chemistry at HARvard Macromolecular Mechanics (CHARMM) force field was applied using the simulation tool DS 3.5. The active site was identified using cavity-based method from receptor cavities. A virtually screened compound after ADME and TOPKAT was subjected to docking process. Top 20 poses/scores were generated for each interaction. Each pose was saved, evaluated, and ranked based on DS consensus scoring function that includes JAIN, Ligscore 1, Ligscore 2, PLP1, PLP2, and potential of mean force. Hydrogen bond interactions between the ligands and active site residues were also assessed.

Molecular dynamic simulation

The final screened compound having the best pose of docked complex from the docking studies (1U3Q-apo protein complex) was subjected to 200 picosecond (ps) of MD simulations using leap-frog verlet integration algorithm in DS 3.5. In addition, SHAKE constraint was introduced to fix all bonds involving hydrogen in the simulation. The executed simulation cascades were minimization with CHARMM force field followed by minimization with steepest descent of 500 steps and conjugate gradient of 500 steps. Consequently, the docked complex is heated upto 300K with 4 ps of simulation time. Finally, production in the canonical ensemble was subjected with equal T_{mass} and P_{mass} at 300 k for 200 ps. In addition, spherical cutoff method of electrostatics was implemented to study all nonbonded energy without using periodic boundary condition.

Statistical analysis

All experiments reported were done in triplicates. The Tukey range test was used to check their statistical significance at $P < 0.05$.

RESULTS AND DISCUSSION

In the modern era, Zingiberaceae family has attained an interest as a potential treatment for many health ailments,^[21] including anti-inflammatory disorders^[22] and cancer.^[23] Till March 2016, only 56 scientific articles were found for the keyword “Zingiberaceae” and “Phytochemistry in the pubmed repository. Most of the species reported were *Zingiber* sp. (23), *Curcuma* sp. (9), *Kaempferia* sp. (3), *Hedychium* sp. (3), *Aframomum* sp. (1), *Alpinia* sp. (1), *A. subulatum* (1), *Boesenbergia* sp. (1), *Renealmia* sp. (1), *Siphonochilus* sp. (1), and *Xiphidium* sp. (1) with 73% of all the available publications. However, the authors were not able to find any report with regard to *A. purpurata* and *H. coronarium* from North-East India, specifically at high latitude (27.20 N) and longitude (88.40 E). Moreover, there were lots of scientific lacunae in case of any works addressing the phytochemistry of *Hedychium* sp., *K. rotunda* Linn., and *Curcuma* sp. To the authors, it was important to notice that well-documented species of this Zingiberaceae family only represent 5%–10% of the species, and the vast majority belonging to this family remain poorly studied or biochemically unknown. Hence, the present study focused on the phytochemistry and *in silico* findings of selected species of Zingiberaceae family.

Biochemical investigation

Antioxidants are the molecules to quench the free radicals synthesized in the plants as the inevitable byproducts of turning food into energy and formidable threats faced by them in disease condition.^[3] Polyphenols, naturally occurring in our food, are scientifically proven for the major antioxidant activities.^[24] The preliminary tests indicated the presence of various constituents such as phenols, saponins, carbohydrates, tannins, flavonoids, and alkaloids in different edible rhizome extracts. The phenols and flavonoids are the most investigated phytochemicals

with regard to curing health ailments. Several reports suggested that these phytochemicals are active against diabetes,^[1,25] cancer,^[16] oxidative diseases,^[6,15] and inflammatory diseases.^[3,26] The present study aimed to analyze the comparative antioxidative properties of the herbs classified under Zingiberaceae family and their possible anticancer potentials against an estrogen receptor. The extracting solvent used was methanol, and the conditions were based on previous reports.^[27,28] Total extract yield was ranging between 1.65% and 3.20% (w/w) for all the four rhizomes [Table 1].

A low-to-high pattern such as *K. rotunda* Linn. < *Hedychium* sp. < *C. longa* < *C. angustifolia* was seen in case of water contents [Table 1]. *Hedychium* sp. was found to be the richest in flavonoids, followed by both *Curcuma* species, though in case of the total phenol, *C. angustifolia* showed highest phenols followed by *Hedychium* sp., *C. longa*, and *K. rotunda* Linn [Table 1].

Free radical scavenging activity (2,2-diphenyl-1-picryl-hydrazyl method)

All the rhizomes showed free radical scavenging activity as measured by DPPH with the concentrations ranging from 100 to 500 µg/mL [Figure 1]. A dose-dependent approach in DPPH scavenging activity was noticed in the methanolic extract of different rhizomes of Zingiberaceae family. The maximum percentage inhibition was observed at a concentration of 500 µg/mL with 79.5% inhibition by *Hedychium* sp. (IC₅₀ 36.4 µg/mL), whereas that of the standard (ascorbic acid) was found to be 81.76%. The highest radical scavenging activity suggests that all the indigenous rhizomes of Zingiberaceae family might be full of potent-free radical inhibitors, which can act as potential antioxidants.^[29] Therefore, as a result, these extracts can scavenge the DPPH*radicals to form constantly reduced DPPH molecules.^[27] The methanolic extract of all the four species found to have high DPPH scavenging activities and have already been reported in comparison to the aqueous and acetone extract by other researchers in different plant materials.^[15,16,24,27] The DPPH activity in a dose-dependent manner

suggested that these rhizomes can serve as a potential targets for several health problems.

In silico studies

Molecular docking, a vital tool, has gained a lot of popularity in structural molecular biology and CADD. The aim of the ligand–protein docking is to determine the interaction of a chemical entity (ligand) with a receptor (protein) of already existing 3D structure. First, the purpose of this stochastic interaction study is hypothesized to know a better candidate and second how the drug like molecules (ligands) inhibit the target.

The prediction methods use scoring functions to approximately calculate the binding energies of envisaged ligand–receptor complex. The estimated binding energy (combination of binding constant [Kd] and the Gibbs-free energy [ΔGL]) is carried out by evaluating the most vital physical–chemical phenomena involved in inter-molecular interactions and entropic effects.^[29] Based on scoring function, a probable molecule would be ranked.

In this study, we have collected common molecules found in Zingiberaceae family *in vitro*. Lead optimization was done using Lipinski rule of 5 for the molecules reported earlier.^[5] Only 71 entities have passed out of 81 compounds.

Lowest energy stabilization is directly proportional to maximum hydrogen bonding interactions with regard to protein–ligand complex and selected as the best pose possible. β-phellandrene (−96.8) has shown the best docking energy score as followed by t-linalool oxide (−87.6) and zingerone (−83.4) [Table 2]. There are previous studies showing β-phellandrene as a potent candidate for cancer studies.^[30,31] Song

Table 1: Quantitative phytochemical assay in different rhizomes

	<i>Curcuma angustifolia</i>	<i>Curcuma longa</i>	<i>Hedychium spp.</i>	<i>Kaempferia rotunda</i> Linn.
Sample yield (%)	2.14±0.12	2.60±0.43	1.65±0.03	3.20±0.01*
Water contents (/100 g)	509.31±16.75*	188.20±10.9	162.43±5.63	132.22±3.99
Flavonoid ^a	38±11.54	30.42±2.14	75.45±6.75*	26.13±5.41
Total phenol ^b	537.26±12.45*	195.12±24.86	292.54±9.16	102.24±4.67

^aTotal flavonoid content analyzed as µg QE/g of extract, values are the average of triplicates. ^bTotal phenol content analyzed as mg GAE/g of extract, *Significant difference between different rhizome extract at P<0.05. All values are in average of triplicates. QE: Quercetin equivalent; GAE: Gallic acid equivalent

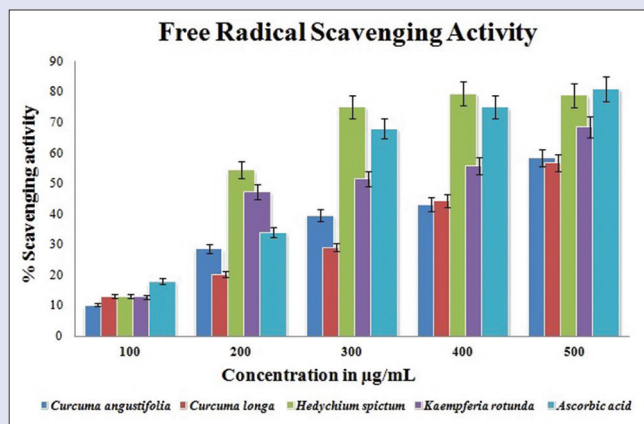


Figure 1: Comparative 2,2-diphenyl-1-picryl-hydrazyl scavenging activity of methanolic extract of different rhizomes of Zingiberaceae family

Table 2: Interaction score of the best molecule with bonding pattern using iGemDock

Compound	Energy	E (pharma)	H-S-Glu ³⁰⁵	H-S-Arg ³⁴⁶	V-M-Leu ²⁹⁸	V-S-Leu ²⁹⁸	V-S-Leu ³³⁹	V-S-Leu ³⁴³	V-S-Phe ³⁵⁶	V-S-Leu ⁴⁷⁶
β-phellandrene	−96.8	−114.3	0	−3.5	−3.60798	−7.08378	−6.3714	−4.63273	−6.8414	−5.41568
Trans linalool oxide	−87.6	−94.5	0	0	−4.65881	−4.23399	1.64548	−5.30051	−6.74036	−6.32999
Zingerone	−83.4	−93.3	0	0	−5.02241	−5.57698	−6.06812	−2.74392	−6.3676	−6.03061
E, E-alpha-farnesene	−80.8	−80.8	0	0	0	0	0	0	0	0
Farnesene epoxide	−80.4	−96.3	0	−3.5	−5.20999	−4.23829	−5.97088	−4.03972	−4.43555	−6.89351
e-nerolidol	−79.7	−97.7	−1.95968	−3.5	−4.76047	−6.33018	−6.49341	−4.53189	−2.69365	−5.19354
Neryl acetate	−78.9	−87.5	0	0	−5.64722	−5.69329	−4.12797	−2.45744	−4.93197	−6.10002
Bisabool	−76.7	−89.8	−2.5	0	−5.75345	−9.2785	−2.8395	−1.00903	−7.25221	−4.62203
Sabinol	−76	−85.4	0	0	−5.27801	−7.38587	−4.95733	−2.95024	−5.22693	−5.23058
Farnesene	−74.5	−83.4	0	0	−4.98203	−4.59783	−6.04783	−3.71285	−4.42416	−6.74707
Undacanone	−74.5	−86.2	0	0	−7.05172	−8.71618	−4.18385	−3.69435	−8.23452	−5.45295

et al.^[32] indicated zingerone to ameliorate lipopolysaccharide-induced acute kidney injury by inhibiting toll-like receptor-4 signaling pathway. Chang *et al.*^[33] showed the potency of linalool oxide against cancer cell lines. They reported that linalool can promote gene expression for p53, p21, p27, p16, and p18 genes that can arrest the G0/G1 phase as well as G2/M phase.

A hydrogen bonding interaction could be pragmatic between the β -phellandrene and the hydroxyl group of Arg³⁴⁶ [Table 2]. Therefore, this could only functional as a weak hydrogen bond acceptor, which is not very physiologically relevant. Few van der Waals interactions are incidental and formed. The major three ligands such as β -phellandrene, trans linalool oxide, and zingerone mutually interacted with amino acids such as Arg³⁴⁶, Leu²⁹⁸, Leu^{349/343/476}, Phe³⁵⁶, and Leu⁴⁷⁶ [Table 2].

Molecular dynamics (simulations) studies

The best pose of 1U3Q – β -phellandrene – complex from virtual screening and LigandFit was selected, and the apo-form of protein (1U3Q) was subjected to 200 ps molecular dynamics (MD) simulations using DS version 3.5. The stability of the protein was analyzed and confirmed by plotting root mean square deviation (RMSD) and radius of gyration (Rg) of protein [Figure 2a]. The RMSD is a measure of the deviation of the conformational stability of the proteins from backbone structure to the early starting structure and fundamental property investigation in MD studies.^[34] The RMSD plot of apo-form of protein and 1U3Q – β -phellandrene complex structure has shown deviation between 2.15 and 2.25 Å [Figure 2b]. Root mean square fluctuation (RMSF) of main chain C α atoms is also calculated with reference to a primary structure for average fluctuation of all residues during the simulation and to locate the flexibility of each amino acid residue along the polypeptide chain. An insight of computed results of RMSF showed that the major alpha-helices are more stable and stabilized [Figure 2c]. Perhaps, slight changes in deviation were observed in C-terminal alpha helices of apo-form of protein and the loop regions are flexible in both structures. The Rg is also calculated to analyze the folding pattern of the protein during simulation^[35] and is defined as the root mean square distance of the collection of atoms from their common center of gravity. The results of gyration showed not much of deviation in folding pattern of protein [Figure 2d]. The overall simulation of 1U3Q – β -phellandrene complex showed more stability (–12585.238 kcal/mol) than an apo-form of protein (–12438.166 kcal/mol) variation in energy parameters with respect to time and temperature [Table 3]. Figure 3a-d shows the two best molecules, namely, β -phellandrene and farnesene epoxide interacting with 1U3Q.

Absorption, distribution, metabolism, and excretion and toxicity study

ADME-T plot [Figure 3c] shows 95% and 99% confidence ellipsoids for absorption and BBB, respectively. Farnesene epoxide (55.86) solubility is lower than β -phellandrene (36.61) with good absorption, nonhepatotoxic activity, and high penetration across BBB [Table 1]. Unlike other compounds, β -phellandrene had showed a nontoxic effect in developmental toxicity potential. Hence, authors considered β -phellandrene a better target for estrogen receptor. The two best molecules, i.e., β -phellandrene and farnesene epoxide are forming 11 and 14 favorable nonbond interactions, respectively [Figure 3a and b]. The bond-forming residues in case of compound β -phellandrene are Leu²⁹⁸, Ala³⁰², Met³³⁶, Leu³³⁹, Leu³⁴³, and Phe³⁵⁶ and farnesene epoxide are Ala³⁰², Glu³⁰⁵, Met³⁴⁰, Leu³⁴³, Arg³⁴⁶, Phe³⁵⁶, Ile³⁷³, Ile³⁷⁶, Leu³⁸⁰, His⁴⁷⁵, Leu⁴⁷⁶, and Leu⁴⁹¹ [Figure 3a and b]. Figure 3d depicts 1U3Q-farnesene epoxide complex. TOPKAT employs robust and cross-validated Quantitative Structure Toxicity Relationship representation for

Table 3: Energy parameter variations in different stages of molecular dynamics

Name	Stages	Initial potential energy (kcal/mol)	Total energy (kcal/mol)	Potential energy (kcal/mol)	Kinetic energy (kcal/mol)	Temperature (K)	Van der waals energy (kcal/mol)	Electrostatic energy (kcal/mol)
1U3Q- β -phellandrene	Minimization	1,557,565.2		-10,838.333			-1657.045	-10,511.107
	Minimization 2	-10,838.333		-13,783.657			-1587.706	-13,834.755
	Heating	-13,783.657	-8691.246	-11,448.136	2756.890	302.745	-1356.232	-13,941.223
	Equilibration	-11,448.136	-9658.247	-12,402.171	2743.924	301.321	-1381.955	-14,885.163
	Production	-12,402.171	-9837.765	-12,585.238	2747.474	301.711	-1345.029	-15,231.853
1U3Q	Minimization	1,557,566		-10,828.93			-1634.132	-10,504.052
	Minimization 2	-10,828.93		-13,842.796			-1572.575	-13,895.137
	Heating	-13,842.795	-8721.153	-11,487.419	2766.266	305.844	-1327.895	-14,037.373
	Equilibration	-11,487.419	-9333.019	-12,102.764	2769.745	306.229	-1308.346	-14,804.978
	Production	-12,102.764	-9757.219	-12,438.166	2680.947	296.411	-1350.825	-14,991.909

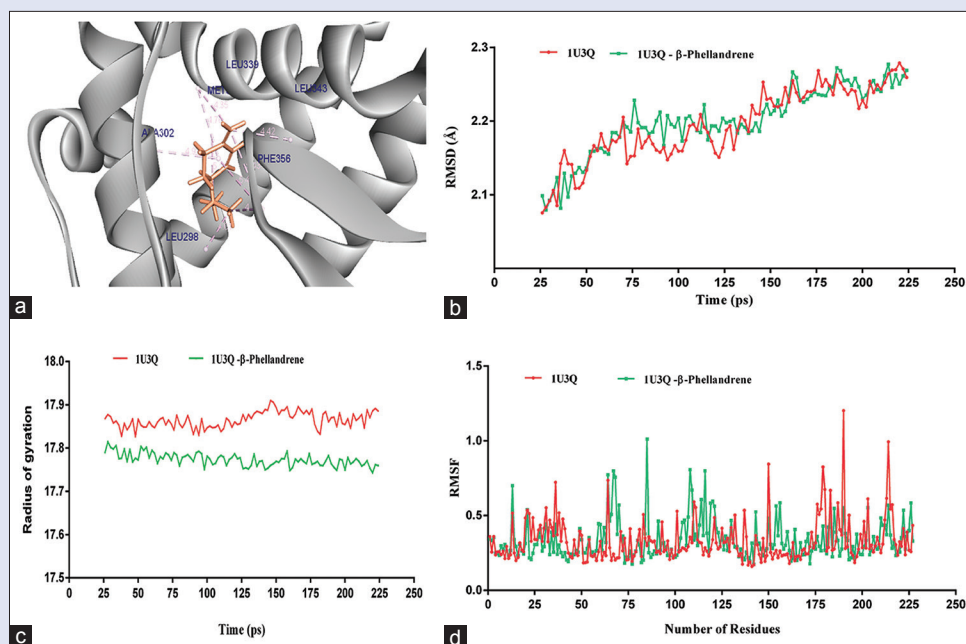


Figure 2: (a) Molecular interaction of β -phellandrene, (b) molecular interaction of farnesene epoxide, (c) absorption, distribution, metabolism, and excretion-Tox plot, (d) 1U3Q-farnesene epoxide complex

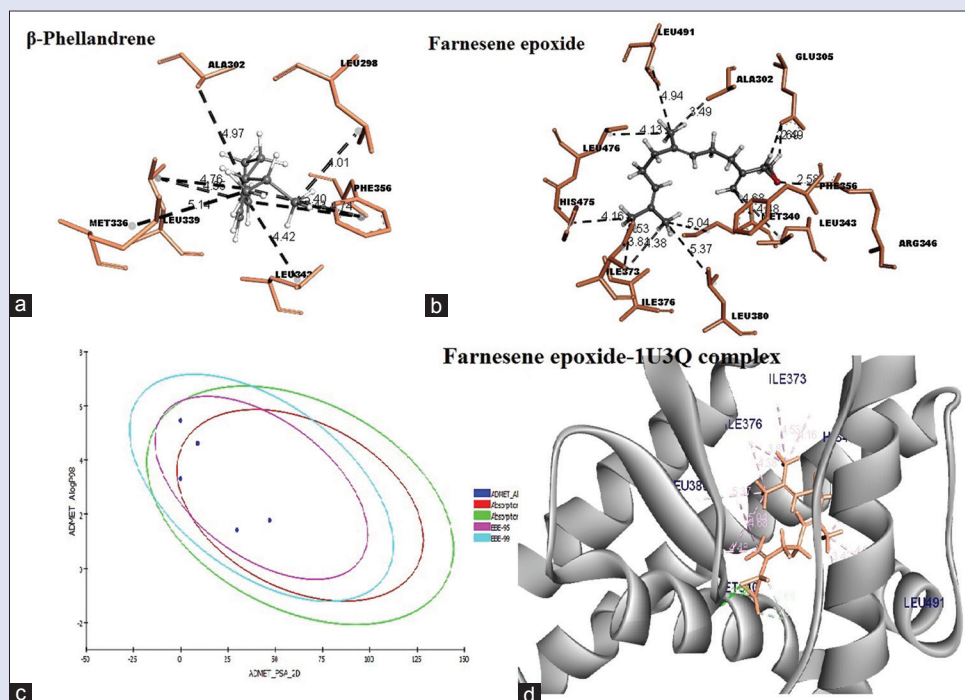


Figure 3: (a) 1U3Q- β -phellandrene complexes, (b) root mean square deviation plot of 1U3Q- β -phellandrene complexes and 1U3Q-apo protein, during 200 ps simulations. (c) Root mean square fluctuation plot of 1U3Q- β -phellandrene complexes and 1U3Q-apo protein, during 200 ps simulations. (d) Radius of gyration plot of 1U3Q- β -phellandrene complexes and 1U3Q-apo protein

assessing various measures of toxicity and utilizing the patented optimal predictive space validation method to assist in interpreting the results.^[16] Screened compounds showed a nonmutagenic effect when subjected to Ames test [Table 4]. Trans linalool oxide and zingerone showed optimal solubility and were less favorable to cross BBB while E, E alpha-farnesene had a moderate absorption.

CONCLUSION AND FUTURE PROSPECTS

To conclude, among all the genus of Zingiberaceae family, *Hedychium* sp. possess more active compounds than other varieties, followed by *K. rotunda* Linn., *C. angustifolia*, and *C. longa*. This study is an effort to investigate the interaction of Zingiberaceae family compounds and

Table 4: Absorption, distribution, metabolism, and excretion and toxicity descriptors for top five drug-like compounds

Principal descriptors	β -phellandrene	E, E, α -farnesene	Farnesene epoxide	Trans linalool oxide	Zingerone
ADMET descriptors					
Solubility level	3	2	2	4	4
BBB level	0	0	0	2	2
Absorption level	0	1	0	0	0
Hepatotoxic prediction	False	False	False	False	False
CYP2D6 prediction	False	True	False	False	False
Toxicity descriptors					
Carcinogenicity potency Rat _{TD50} (mg/kg body weight/day)	83.1015	213.789	63.8847	25.7805	39.6583
Rat maximum tolerated dose (g/kg body weight)	0.0491628	0.082759	0.0855131	0.0586508	0.25343
Rat oral LD50	1.47155	4.88145	4.86229	3.26057	5.95974
Rat inhalation toxicity LC50 (mg/m ³ /h)	52,022.90	16,774.20	18,535	20,318.80	6544.37
Daphnia EC50 (mg/L)	2.68827	0.413978	2.2326	65.4259	45.8544
Fathead minnow LC50 (g/L)	0.0003895	2.506e-05	0.0001414	0.0405962	0.060732
AM	NM	NM	NM	NM	NM
Developmental toxicity potential	NT	T	T	T	T

Absorption level 0 as good absorption and 1 as moderate absorption, BBB level 0 as very high penetrant and 2 as medium, solubility level 2 as low, 3 as good, and 4 as optimal. AM: Ames mutagenicity; NM: Nonmutagen; NT: Nontoxic; T: Toxic; BBB: Blood–brain barrier; ADMET: Absorption, distribution, metabolism, and excretion-Tox

predict the best active compound for anticancerous activity. The bioassay-guided isolation and identification of the bioactive components are still need to be explored. In addition, a detailed research is also required to reveal the structure–activity relationship of these active compounds. The outcome of this research in the aforementioned areas will provide a convincing support for the future clinical uses of these rhizomes in modern medicine.

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Conflicts of interest

There are no conflicts of interest.

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