



Anticancer potential of phytochemicals from *Oroxylum indicum* targeting Lactate Dehydrogenase A through bioinformatic approach

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ABSTRACT

In recent years, small molecule inhibition of LDHA (Lactate Dehydrogenase A) has evolved as an appealing option for anticancer therapy. LDHA catalyzes the interconversion of pyruvate and lactate in the glycolysis pathway to play a crucial role in aerobic glycolysis. Therefore, in the current investigation LDHA was targeted with bioactive phytochemicals of an ethnomedicinally important plant species *Oroxylum indicum* (L.) Kurz. A total of 52 phytochemicals were screened against LDHA protein through molecular docking, ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) assay and molecular dynamics simulation to reveal three potential lead compounds such as Chrysin-7-O-glucuronide (−8.2 kcal/mol), Oroxin A (−8.1 kcal/mol) and Oroxin A (−8.0 kcal/mol). ADMET assay unveiled favorable pharmacokinetic, pharmacodynamic and toxicity properties for all the lead compounds. Molecular dynamics simulation exhibited significant conformational stability and compactness. MM/GBSA free binding energy calculations further corroborated the selection of top candidates where Oroxin A (−46.47 kcal/mol) was found to be better than Chrysin-7-O-glucuronide (−45.72 kcal/mol) and Oroxin A (−37.25 kcal/mol). Aldolase reductase and Xanthine dehydrogenase enzymes were found as potential drug targets and Esculin, the FDA approved drug was identified as structurally analogous to Oroxin A. These results could drive in establishing novel medications targeting LDHA to fight cancer.

1. Introduction

Cancer is one of the major causes of mortality worldwide, accounting for approximately 10 million deaths in 2022, as reported by the WHO (<https://who.int/news-room>). Lactate Dehydrogenase A (LDHA) enzyme is considered as a key player in cancer progression and is targeted frequently to develop anticancer medications [1]. Cancer cells often switch their metabolic activities from oxidative phosphorylation to enhanced glycolysis [2]. Glycolysis is the breakdown of a whole glucose molecule into two pyruvate molecules. In cancer cells, glycolysis process breaks down glucose molecules partially and produces pyruvate molecules which are then transformed into lactate via a metabolic mechanism catalyzed by Lactate Dehydrogenase (LDH) enzyme [3]. This lactate synthesis is responsible for increased glycolysis, which contributes significantly to cancer growth and progression by lowering the pH

for invasion, replenishing NAD⁺ for glycolysis, and inducing immune escape [4].

LDH structure is well preserved across species, with very minor alterations in amino acid sequences [5], and the structural affinity of LDH provides a justification for developing small-molecule inhibitors to modify its catalytic action in cells. LDH's active pocket contains catalytically active residues such as His193, Asp168, Arg171, Thr246, and Arg106 [6]. LDH possesses five isozymes where the fifth form or LDH-5, also known as LDHA is upregulated in most of the tumor cells, thus inhibiting LDHA decreases tumor development and invasiveness [7]. LDHA expression has been dysregulated in endometrial cancer cells, squamous cell carcinoma and breast cancer cells [8–11]. Abnormal activation of these oncogenes may result in increased glucose absorption and lactate generation. Inhibition of LDHA, therefore, may limit the energy supply in tumors, reducing cancer cells' ability to spread and

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invade. As a result, LDHA is gaining popularity as a possible diagnostic or prognostic biomarker for cancer, as well as a therapeutic target for the development of future anticancer medicines [12–16].

Oroxylum indicum (L.) Kurz is a small to medium tree belonging to the family Bignoniaceae, and is native to Bangladesh and Indian subcontinent. The tree can attain a height of up to 12 m. The species is characterized by its pinnately compound leaves, ovate leaflets, pinkish red flowers in terminal raceme inflorescence, axile placentation and boat-shaped, sword-like fruits [17,18]. Traditional medicinal knowledge (TMK) has reported various components of this plant to cure different maladies including cancer [19]. Several *in vivo* and *in vitro* investigations have revealed its anticancer, antiulcer, hepatoprotective, anti-inflammatory, and immunostimulant properties [18]. *O. indicum* has been shown to possess a wide variety of bioactive phytochemicals, viz., alkaloids, flavonoids, cardiac glycosides, phenols, and other bioactive compounds that can help to accelerate the discovery of nature-based new LDHA inhibitors.

Computer-aided structure-based drug design approach has revolutionized the discovery of drugs by expediting the process through bioinformatic servers and tools involving molecular docking, ADME/T (Absorption, Distribution, Metabolism, Excretion and Toxicity) analyses and molecular dynamics simulation. This strategy is specific and effective in finding and refining new lead compounds and thus has contributed to enhance our current understanding of the discovery of novel therapeutics [20]. Molecular docking as a computational modeling approach elucidates the interactions between a small molecule (as ligand) and a protein (as receptor) at the atomic level, which permits to characterize the behavior of small molecules in the binding site of target protein as well as to understand fundamental biochemical processes [21]. Molecular docking relies on two basic steps – firstly, by sampling conformations of the ligand in the active site of the protein and secondly, ranking these conformations via a scoring function [22]. Scoring functions help to differentiate the correct poses from incorrect poses, or binders from inactive compounds in a reasonable computation time. Different methodologies are used for molecular docking such as, rigid ligand and rigid receptor docking, flexible ligand and rigid receptor docking, flexible ligand and flexible receptor docking and so on [23]. Molecular docking has been the most widely employed computational technique and the main application of the approach lies in the structure-based drug designing for identification of new active compounds towards a specific target protein [24]. ADME/T analyses is supportive to understand whether the pharmacokinetic (PK) and pharmacodynamic (PD) properties of the lead compounds are in the acceptable range or not. Pharmacokinetic properties examine how the drug is absorbed, distributed, metabolized, and excreted by the body, while pharmacodynamic properties interpret the relationship between drug concentration at the site of action and the resulting effect, including the time course and intensity of therapeutic and adverse effects [25]. A shocking 90% attrition rate of drug candidates is reported by the pharmaceutical industry during the transition from preclinical trials to phase 4 clinical trials [26]. The main causes of the high failure rate of drug discoveries include undesirable bioavailability of drugs due to inappropriate pharmacokinetic and pharmacodynamic properties. During the synthesis of drug molecules, a delicate balance between drug candidates and their ADME/T profiling can help prevent late-stage drug failure in the drug discovery process [27]. Therefore, earlier PK/PD property detection in conjunction with drug-likeness and ADME/T profiling can save time and money while simultaneously assuring the stability and safety of the designed drugs or candidate pharmaceuticals [28].

Though a few efforts on *in silico* have been made to explore medicinal properties of bioactive phytochemicals of *O. indicum*, no attempts have been taken so far to expose its anticancer efficacy targeting Lactate Dehydrogenase A. Therefore, the present study aimed at evaluating the bioactive compounds of *O. indicum* to unveil novel drug candidates targeting LDHA through bioinformatic approach.

2. Materials and methods

2.1. Target protein retrieval and preparation

Lactate Dehydrogenase A (LDHA) was retrieved from the RCSB (Research Collaboratory for Structural Bioinformatics) Protein Data Bank (PDB) database using the PDB ID '4OJN' (<https://www.rcsb.org>) [29]. The selected protein '4OJN' was solved experimentally using X-ray diffraction method with eight chains. The resolution and observed R-value was 2.40 Å and 0.215, respectively [30]. BIOVIA Discovery Studio Visualizer v21.1.0.20298 was employed for removing all the chains from the protein except the chain A. Subsequently, all the heteroatoms including water molecules and two small molecules such as Pentaethylene glycol and Glycerol were removed. Afterwards, the receptor was modified using MGL-AutoDockTools v1.5.6 and energy minimized using SWISS-PDB viewer v4.10 employing GROMOS96 43b1 force field. The PDB file from SWISS-PDB Viewer was converted to PDBQT format using Open Babel v.2.3.1 before performing docking [31–33], and the command has been provided in the Supplementary file 1.

2.2. Preparation of ligand library

Fifty-two phytochemicals of *O. indicum* were selected along with the control Sunitinib after thorough literature survey using SCOPUS, Google Scholar and PubMed databases to construct the ligand library for molecular docking and subsequent analyses [18,19,34]. All the phytochemicals and the control were retrieved from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov>) in 3D SDF (Structure Data File) format. Afterwards, energy minimization was conducted for all the ligands employing MMFF94 force field in 2000 steps employing steepest descent algorithm of the Open Babel v.2.3.1 software in Linux Ubuntu 18.04.6 LTS environment using specific command (Supplementary file 1) [33,35,36]. Subsequently, all the ligands were converted from SDF to PDBQT format in the same Linux environment using necessary commands (Supplementary file 1).

2.3. Virtual screening through molecular docking

Molecular docking was carried out employing AutoDock Vina v1.2.0 [37] in Linux command line along with the Perl programming script "Vina_windows.pl", the script has been provided in the supplementary file 2. The receptor macromolecule was considered as rigid, whereas the ligands as flexible during blind docking. Grid box was constructed using MGL-AutoDockTools v1.5.6 and the box covered the entire surface of the macromolecular receptor. Grid box was constructed before running the Perl script, and the size coordinates were $45.45 \times 84.07 \times 54.28$, and the center coordinates were $70.33 \times -15.09 \times -26.45$ for X, Y, and Z axes, respectively. Phytochemicals having similar or better scoring than Sunitinib were processed for further investigation. BIOVIA Discovery Studio Visualizer was used for visualization of the docked protein-ligand complexes.

2.4. Evaluation of Drug profile through ADME and toxicity analyses

After initial screening, 18 phytochemicals were selected for drug profile evaluation via ADME/T (Absorption, Distribution, Metabolism, Excretion and Toxicity) analysis. SwissADME server was employed to analyze various pharmacokinetic and pharmacodynamic properties related to ADME analysis for their vital role in determining the pharmacological activity and performance of drugs [38]. For accurate prediction, Canonical SMILES format files were produced for all the top 18 phytochemicals using Open Babel v.2.3.1 in the Linux command line, which were subsequently imported to the SwissADME server for predicting ADME properties. For toxicity analysis, two different servers were utilized, e.g. ProToxII and StopTox [39,40]. ProTox-II server

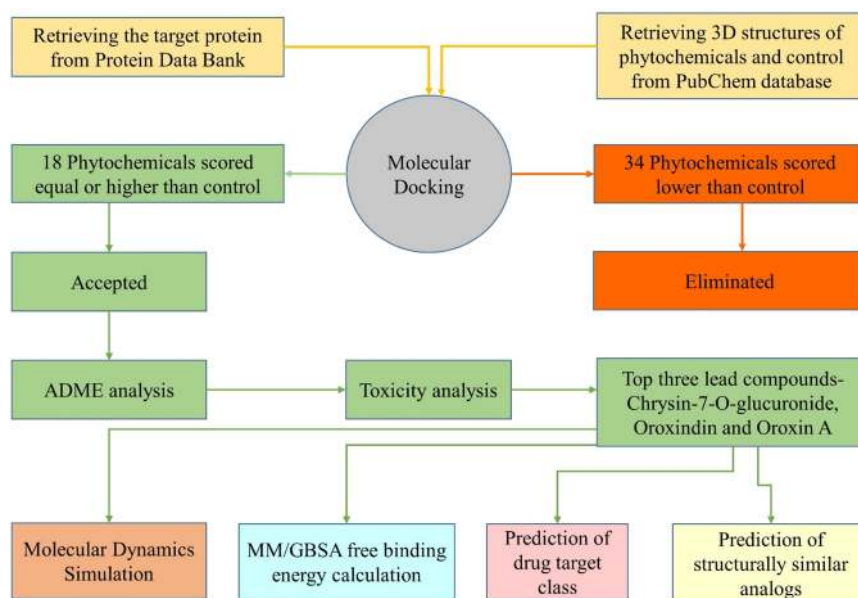


Fig. 1. Workflow of the current study showing stepwise *in silico* screening of bioactive phytochemicals of *O. indicum* targeting Lactate Dehydrogenase A (LDHA).

predicts various toxicity endpoints, such as acute toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity, immunotoxicity, adverse outcomes (Tox21) pathways, and toxicity targets using fragment propensities, molecular similarity, most frequent features, and machine learning methods. StopTox server utilizes an ensemble of quantitative structure-activity relationship (QSAR) models to evaluate the toxicity of compounds for various toxicity end points including acute inhalation toxicity, acute oral toxicity, eye irritation and corrosion, skin sensitization etc. after compiling, curating and integrating the largest publicly available datasets.

2.5. Molecular dynamics simulation

Molecular dynamics (MD) simulation was performed for the top selected protein-ligand complexes for 100 ns using the GROMACS v2019.2 software via WebGro server (<https://simlab.uams.edu>) [41]. Before performing simulation, initially ligand topology files were generated using PRODRG server [42]. SPC (Simple Point Charge) water model was used to solve the system with triclinic box. The system was neutralized by sufficient sodium and chloride ions (0.15 M salt). GROMOS96 43a1 force field in 5000 steps was used for energy minimization [43]. For equilibration and MD run, NVT/NPT was used fixing the temperature at 300 K and pressure at 1 bar. V-rescale, a modified Berendsen thermostat was utilized for temperature coupling. Leap-frog method, as MD integrator, was employed for updating positions and velocities [44]. Finally, a 100 ns MD production run was performed setting approximate number of frames 1000 per simulation. MD simulation results were retrieved from the server in CSV format and plotted subsequently using Microsoft Excel V.2013 to analyze RMSD (Root Mean Square Deviation), RMSF (Root Mean Square Fluctuation), Rg (Radius of Gyration), SASA (Solvent Accessible Surface Area) (total) and number of hydrogen bonds between the LDHA and lead compounds.

2.6. Molecular mechanics/generalized born surface area (MM/GBSA)

Prime module of Schrödinger v.2021–2 software package was used to calculate free binding energies of the top selected complexes [45]. The Prime module employing OPLS2005 force field [46] and VSGB continuum solvation model was applied to calculate free binding energies using the following formula:

$$\Delta G(\text{bind}) = \Delta G(\text{solv}) + \Delta E(\text{MM}) + \Delta G(\text{SA})$$

where $\Delta G(\text{solv})$ indicates the difference in GBSA solvation energy of the protein-inhibitor complex and the sum of the solvation energies for unliganded protein and inhibitor; $\Delta E(\text{MM})$ indicates difference in the minimized energies between protein-inhibitor complex and the sum of the energies of the unliganded protein and inhibitor; and $\Delta G(\text{SA})$ indicates the difference in surface area energies of the complex and the sum of the surface area energies for the unliganded protein and inhibitor.

2.7. Drug target class and structurally similar analogs prediction

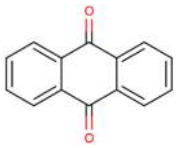
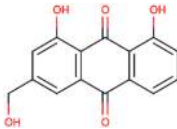
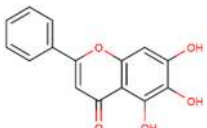
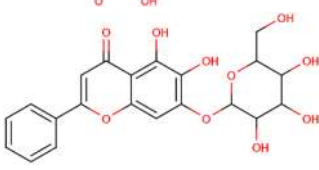
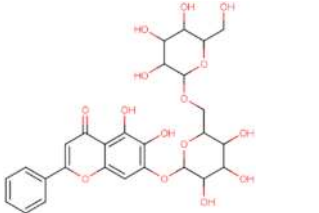
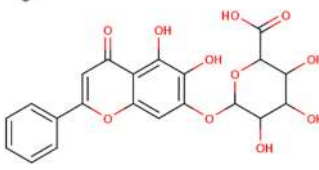
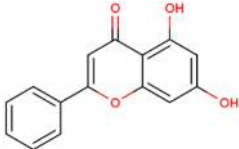
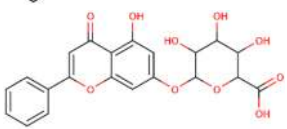
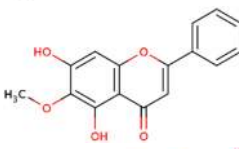
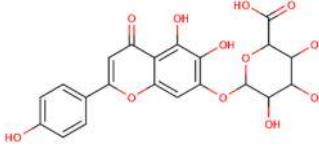
SwissTargetPrediction server was utilized to predict potential macromolecular targets for the top selected candidates [47]. The server utilizes a library of 3,76,342 identified bioactive chemicals on roughly 3068 proteins on the basis of 2D and 3D similarity. The SwissSimilarity server was used to identify potential structural analogs that may be repurposed against the chosen receptor [48]. We employed the path-based FP2 fingerprinting method on the DrugBank compound library (<https://go.drugbank.com>) to uncover bioactive compounds that might be used against the specified receptor. The SwissSimilarity service contains a screenable library of 2108 virtual compounds and applies a variety of complimentary methodologies such as chemical fingerprinting, superpositional 3D shape similarity, and rapid non-superpositional 3D form similarity. The workflow of present investigation has been elucidated in Fig. 1.

3. Results

3.1. Screening of phytochemicals via molecular docking

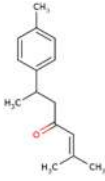
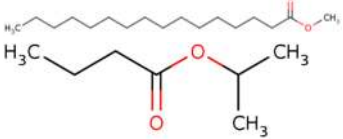
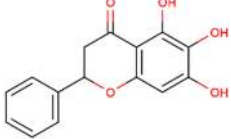
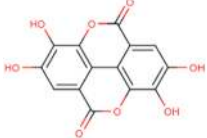
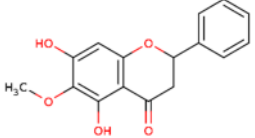
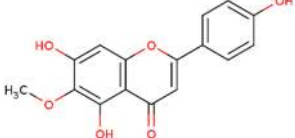
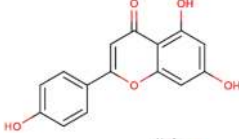
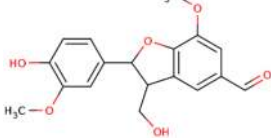
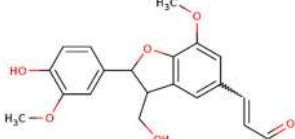
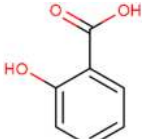
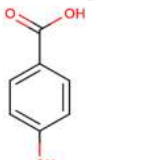
The present study revealed that all the 52 phytochemicals were docked successfully with binding affinities ranging from -4.0 to -9.3 kcal/mol. The control Sunitinib scored -7.6 kcal/mol when docked with LDHA (Table 1). A total 18 phytochemicals (35%) were accepted for scoring equal or higher than the control Sunitinib, while 34 phytochemicals (65%) were eliminated due to their low scores as compared to the control. Among the accepted phytochemicals, the highest binding affinity was found in Oroxin B (-9.3 kcal/mol). The remaining 16 accepted phytochemicals showed variation in binding affinities from -7.6 to -8.5 kcal/mol. In contrary, the binding

Table 1Phytochemicals of *O. indicum* used for first-step-virtual screening with their PubChem CID, 2D structures, molecular weight and binding affinities.

Phytochemicals	PubChem CID	Chemical formula	Molecular weight (g/mol)	Binding affinities (kcal/mol)	Two dimensional chemical structures	References
Anthraquinone	6780	C ₁₄ H ₈ O ₂	208.21	-6.3		[18]
Aloe-emodin	10207	C ₁₅ H ₁₀ O ₅	270.24	-6.8		[18]
Baicalein	5281605	C ₁₅ H ₁₀ O ₅	270.24	-7.6		[19]
Oroxin A	5320313	C ₂₁ H ₂₀ O ₁₀	432.4	-8.0		[19]
Oroxin B	10077207	C ₂₇ H ₃₀ O ₁₅	594.5	-9.3		[19]
Baicalin	64982	C ₂₇ H ₃₀ O ₁₅	446.4	-8.1		[19]
Chrysin	5281607	C ₁₅ H ₁₀ O ₄	254.24	-7.6		[19]
Chrysin-7-O-glucuronide	14135335	C ₂₁ H ₁₈ O ₁₀	430.4	-8.2		[19]
Oroxylin A	5320315	C ₁₆ H ₁₂ O ₅	284.26	-7.2		[19]
Scutellarin	185617	C ₂₁ H ₁₈ O ₁₂	462.4	-7.9		[19]
2-Methyl-6-(4-methylphenyl)hept-2-en-4-one	558221	C ₁₅ H ₂₀ O	216.32	-6.1		[19]

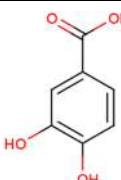
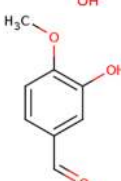
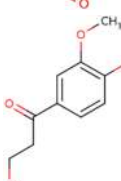
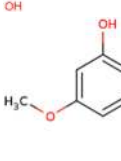
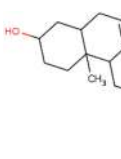
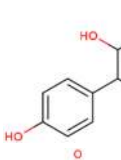
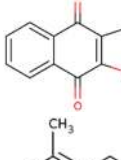
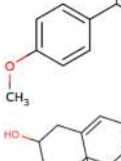
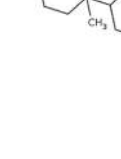
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Table 1 (continued)

Phytochemicals	PubChem CID	Chemical formula	Molecular weight (g/mol)	Binding affinities (kcal/mol)	Two dimensional chemical structures	References
Methyl hexadecanoate	8181	C ₁₇ H ₃₄ O ₂	270.5	-4.4		[19]
Isopropyl butyrate	61184	C ₇ H ₁₄ O ₂	130.18	-4.0		[19]
Dihydrobaicalein	9816931	C ₁₅ H ₁₂ O ₅	272.25	-7.6		[19]
Ellagic acid	5281855	C ₁₄ H ₆ O ₈	302.19	-7.2		[19]
Dihydrooroxylin A	5316733	C ₁₆ H ₁₄ O ₅	286.28	-7.0		[19]
Hispidulin	5281628	C ₁₆ H ₁₂ O ₆	300.26	-7.3		[19]
Apigenin	5280443	C ₁₅ H ₁₀ O ₅	270.24	-7.8		[19]
Ficusal	10496641	C ₁₈ H ₁₈ O ₆	330.3	-6.5		[19]
Balanophonin	23252258	C ₂₀ H ₂₀ O ₆	356.4	-6.8		[19]
Salicylic acid	338	C ₇ H ₆ O ₃	138.12	-4.7		[19]
4-Hydroxybenzoic acid	135	C ₇ H ₆ O ₃	138.12	-5.0		[19]

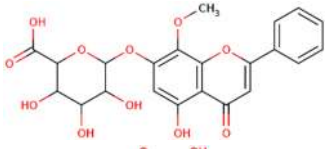
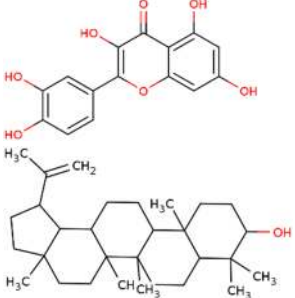
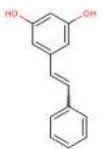
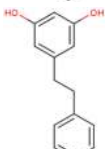
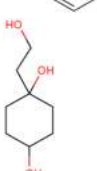
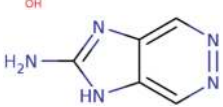
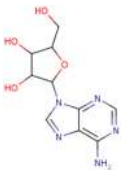
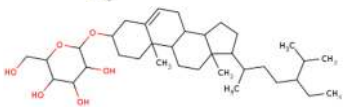
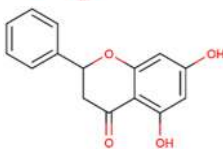
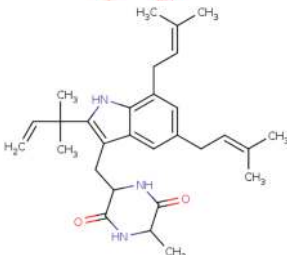
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Table 1 (continued)

Phytochemicals	PubChem CID	Chemical formula	Molecular weight (g/mol)	Binding affinities (kcal/mol)	Two dimensional chemical structures	References
3,4-Dihydroxybenzoic acid	72	C ₇ H ₆ O ₄	154.12	-5.4		[19]
Isovanillin	12127	C ₈ H ₈ O ₃	152.15	-4.7		[19]
Beta-Hydroxypropiovanillone	75142	C ₁₀ H ₁₂ O ₄	196.2	-5.2		[19]
5-Hydroxy-7-methoxy-2-phenylchroman-4-one	73201	C ₁₆ H ₁₄ O ₄	270.28	-6.8		[19]
Stigmast-7-en-3-ol	3080632	C ₂₉ H ₅₀ O	414.7	-8.2		[19]
Kaempferol	5280863	C ₁₅ H ₁₀ O ₆	286.24	-7.8		[19]
2-Isopropenyl-2,3-dihydronaphtho [2,3-b]furan-4,9-dione	364109	C ₁₅ H ₁₂ O ₃	240.25	-6.7		[19]
Lapachol	3884	C ₁₅ H ₁₄ O ₃	242.27	-6.4		[19]
Biochanin A	5280373	C ₁₆ H ₁₂ O ₅	284.26	-7.0		[19]
Beta-sitosterol	222284	C ₂₉ H ₅₀ O	414.7	-7.7		[19]
Oroxindin	3084961	C ₂₂ H ₂₀ O ₁₁	460.4	-8.1		[19]

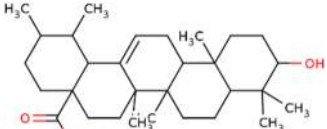
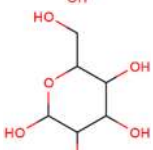
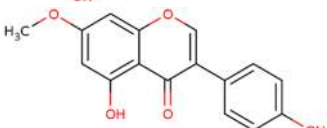
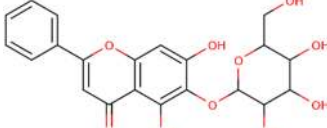
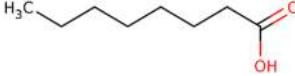
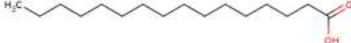
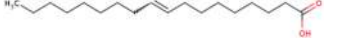
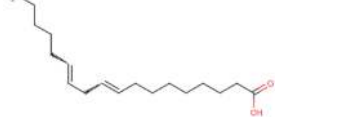
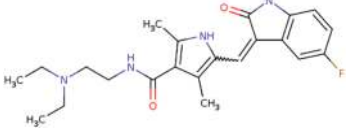
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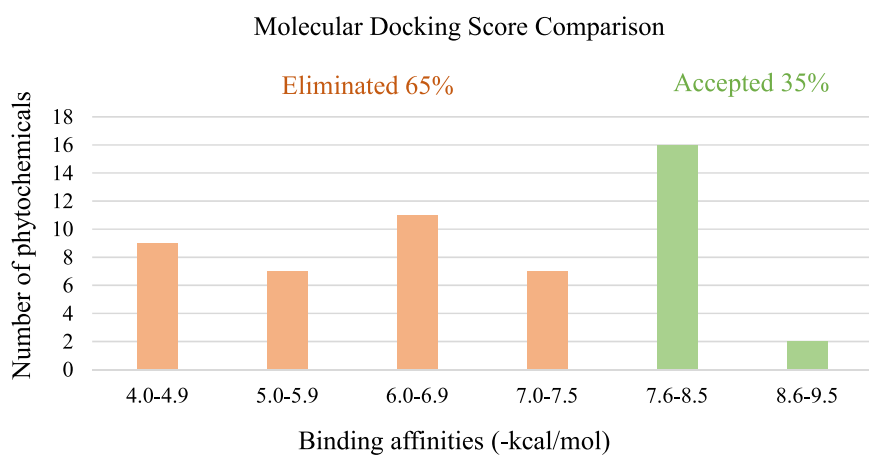
Table 1 (continued)

Phytochemicals	PubChem CID	Chemical formula	Molecular weight (g/mol)	Binding affinities (kcal/mol)	Two dimensional chemical structures	References
Quercetin	5280343	C ₁₅ H ₁₀ O ₇	302.23	-7.8		[19]
Lupeol	259846	C ₃₀ H ₅₀ O	426.7	-8.3		[19]
Pinosylvin	5280457	C ₁₄ H ₁₂ O ₂	212.24	-6.6		[19]
Dihydropinosylvin	442700	C ₁₄ H ₁₄ O ₂	214.26	-6.2		[19]
Rengyol	363707	C ₈ H ₁₆ O ₃	160.21	-5.4		[19]
Zarzissine	6400641	C ₅ H ₅ N ₅	135.13	-5.3		[19]
Adenosine	60961	C ₁₀ H ₁₃ N ₅ O ₄	267.24	-5.8		[19]
Sitogluside	5742590	C ₃₅ H ₆₀ O ₆	576.8	-8.5		[19]
Pinocembrin	68071	C ₁₅ H ₁₂ O ₄	256.25	-6.8		[19]
Echinulin	115252	C ₂₉ H ₃₉ N ₃ O ₂	461.6	-7.5		[19]

(continued on next page)

Table 1 (continued)

Phytochemicals	PubChem CID	Chemical formula	Molecular weight (g/mol)	Binding affinities (kcal/mol)	Two dimensional chemical structures	References
Ursolic acid	64945	C ₃₀ H ₄₈ O ₃	456.7	-9.1		[19]
D-Galactose	6036	C ₆ H ₁₂ O ₆	180.16	-4.9		[18]
Prunetin	5281804	C ₁₆ H ₁₂ O ₅	284.26	-7.0		[18]
Baicalein 6-O-glucoside	5321896	C ₂₁ H ₂₀ O ₁₀	432.4	-8.4		[18]
Octanoic acid	379	C ₈ H ₁₆ O ₂	144.21	-4.1		[18]
Myristic acid	11005	C ₁₄ H ₂₈ O ₂	228.37	-4.4		[18]
Palmitic acid	985	C ₁₆ H ₃₂ O ₂	256.42	-4.9		[18]
Oleic acid	445639	C ₁₈ H ₃₄ O ₂	282.5	-4.9		[18]
Linoleic acid	5280450	C ₁₈ H ₃₂ O ₂	280.4	-5.2		[18]
Sunitinib (Control)	5329102	C ₂₂ H ₂₇ FN ₄ O ₂	398.5	-7.6		[34]

**Fig. 2.** Binding affinities of the investigated phytochemicals via virtual screening.

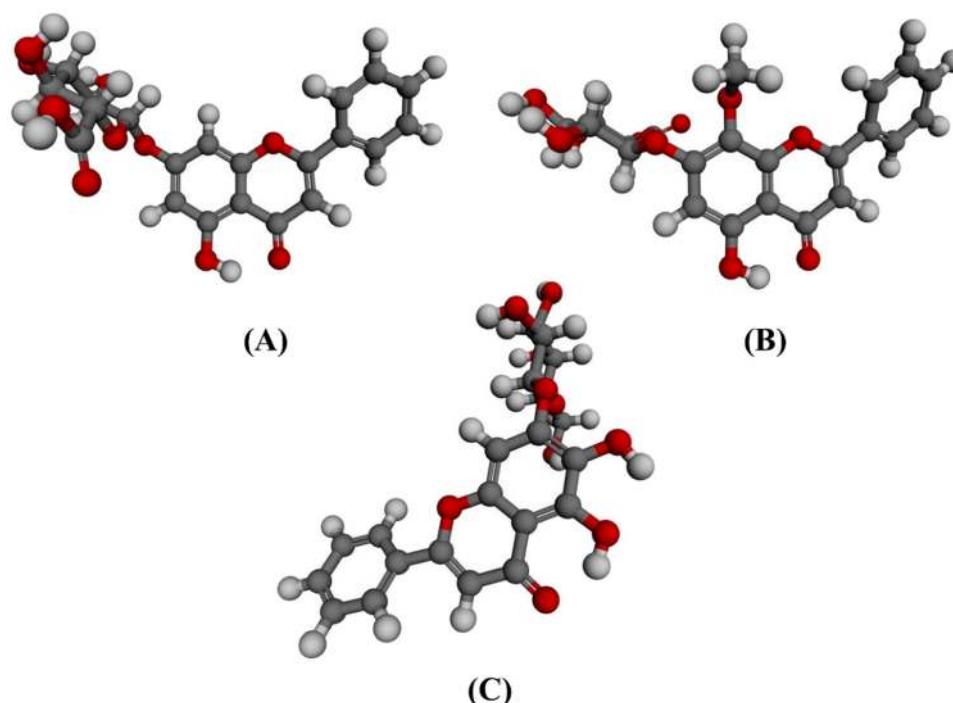


Fig. 3. Three dimensional structures of the top selected lead compounds: (A). Chrysin-7-O-glucuronide; (B). Oroxindin and (C). Oroxin A.

affinities of the eliminated 34 phytochemicals varied from -4.0 to -7.5 kcal/mol. The lowest binding affinity was detected in Isopropyl butyrate (-4.0 kcal/mol) (Table 1 & Fig. 2). ADME/T analyses of the accepted 18 phytochemicals revealed three final lead candidates, such as Chrysin-7-O-glucuronide (-8.2 kcal/mol), Oroxindin (-8.1 kcal/mol) and Oroxin A (-8.0 kcal/mol) as depicted in Fig. 3.

3.2. Molecular interaction analysis

Molecular interactions between the phytoligands and the target protein were analyzed after visualization through BIOVIA Discovery Studio Visualizer that revealed noteworthy results. Both conventional hydrogen bonding and hydrophobic interactions were found in all the lead candidates (Figs. 4 and 5).

Among the three top scoring candidates, Chrysin-7-O-glucuronide showed interactions with Val28, Gly29, Val31, Val53, Thr95, Gly97, Arg99 and Ile116 amino acid residues. This lead compound formed 6 conventional hydrogen bonds with Val28, Gly29, Val31, Thr95, Gly97 and Arg99 residues using bond distance of 3.04, 1.96, 3.06, 2.67, 2.07 and 2.61 Å, respectively. Only two residues, such as Val53 and Ile116 were associated in hydrophobic interactions with Chrysin-7-O-glucuronide. The second lead candidate Oroxindin interacted with Ala168, Arg169, Arg171, Tyr172, Pro182, Ala251, Leu254 and Asp258 amino acid residues where Arg171 formed two conventional hydrogen bonds with bond distances of 2.38 and 2.44 Å, respectively, and Asp258 formed one conventional hydrogen bond with a bond distance of 2.26 Å (Figs. 4 and 5). Five residues, viz., Ala168, Arg169, Tyr172, Pro182 and Leu254 were involved in hydrophobic interactions. The third lead candidate, Oroxin A interacted with Asn164, Ala168, Arg169, Arg171, Tyr172, Leu254, Ser255, Asp258, Arg269 and His271 where four residues formed conventional hydrogen bonds. Asn164 formed two conventional hydrogen bonds with bond distances of 2.12 and 2.44 Å and the other three residues Arg171, Asp258 and Arg269 formed one conventional hydrogen bond each with a bond distance of 1.97, 2.61 and 2.69 Å, respectively. Hydrogen bond distance varied from 1.96 to 3.06 Å where the minimum (Gly29) and maximum (Val31) distances were observed with Chrysin-7-O-glucuronide (Table 2).

3.3. Drug likeness analysis

Different pharmacodynamic and pharmacokinetic properties were evaluated via ADME/T analyses where all the top candidates revealed noteworthy results (Table 3). Oroxindin showed the highest molecular weight (460.39 g/mol) among the three candidates. Gastro-intestinal absorption property was found lower for all the lead compounds. None of the compounds revealed blood brain barrier permeability. Water solubility results were satisfactory as all the three candidates were found to be soluble in water. Drug likeness was estimated based on Lipinski's Rules of Five and Ghose filter, where Chrysin-7-O-glucuronide showed zero violations in the Lipinski and Ghose criteria. Both Oroxindin and Oroxin A followed Ghose parameter with zero violations but showed one violation each in Lipinski's Rules of Five which was acceptable. Cytochrome P450 enzyme inhibitory properties were also evaluated based on five isoforms, such as CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4 where all the three lead candidates showed no inhibition of these five isoforms.

Pan-Assay Interference Compounds (PAINS) criterion revealed zero alerts for both Chrysin-7-O-glucuronide and Oroxindin and one alert for Oroxin A. Considering synthetic accessibility parameter, Chrysin-7-O-glucuronide scored better (5.03) than Oroxin A (5.16) and Oroxindin (5.23) (Table 3).

Toxicity properties were analyzed using two different servers for reliability of prediction where both the servers provided coherent satisfactory results (Table 4). ProToxII server revealed noteworthy results in the various parameters. Both Chrysin-7-O-glucuronide and Oroxindin revealed inactive status for all the criteria and subcriteria in ProToxII server. All the three lead compounds were predicted to have toxicity class 5 with an oral LD₅₀ dose of 5000 mg/kg with 69.26% accuracy. Oroxin A also showed inactive status for all the parameters except one subcriterion, i.e., Phosphoprotein p53 parameter of the Tox21-Stress response pathway where it indicated active status with 50% probability. StopTox server evaluated toxicity based on five toxicity endpoints, such as acute inhalation toxicity, acute oral toxicity, eye irritation and corrosion, skin sensitization and skin irritation and corrosion criteria where all the three lead candidates were found

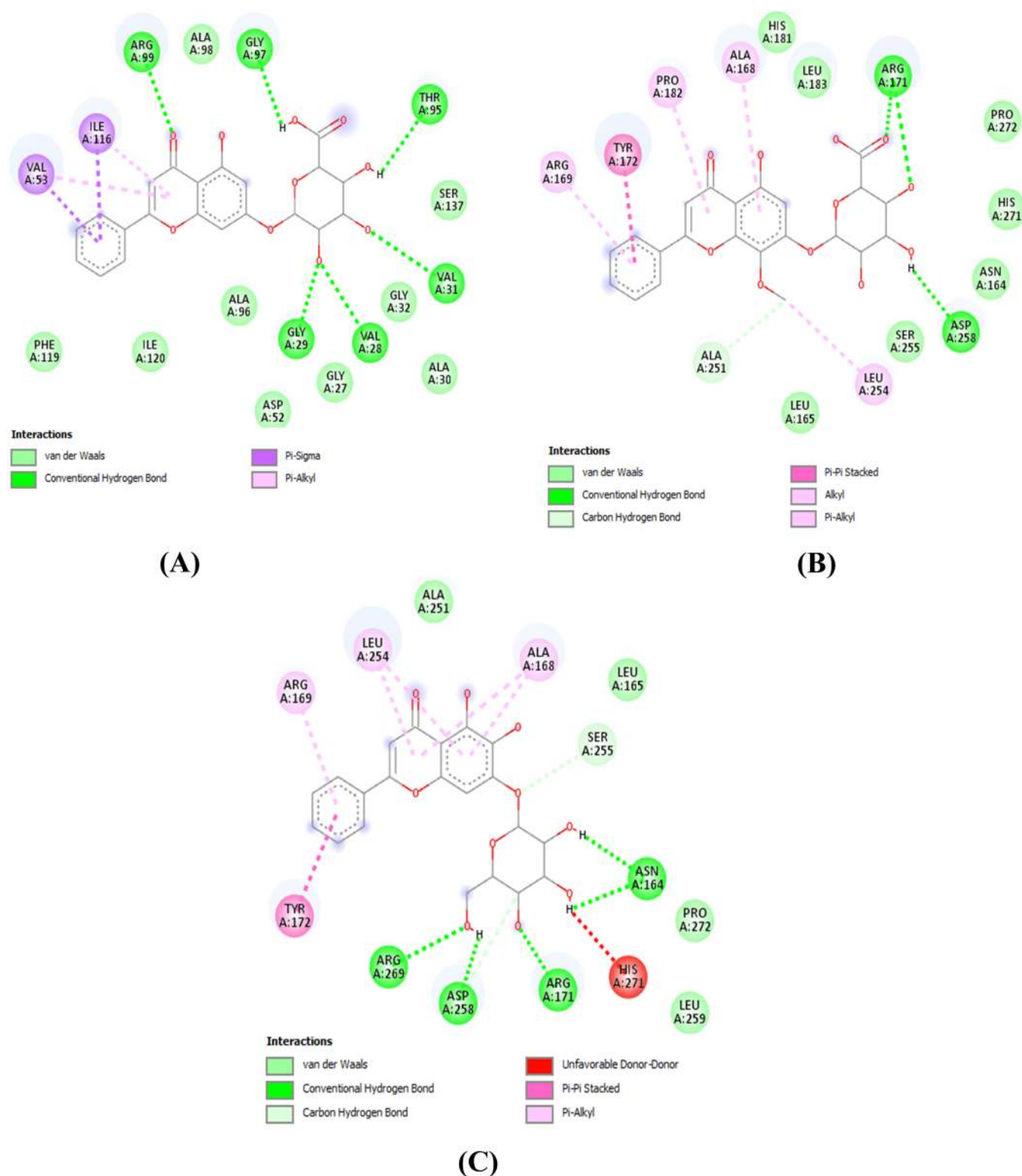


Fig. 4. Two-dimensional molecular interactions of three lead compounds with amino acid residues of the macromolecular receptor: (A). Chrysin-7-O-glucuronide; (B). Oroxindin and (C). Oroxin A.

harmless and satisfactory (Table 4).

3.4. Molecular dynamics simulation

Molecular dynamics (MD) simulation exhibited small variation in RMSD, RMSF, Rg, SASA and intermolecular hydrogen bonds in the three

lead compounds, such as, Chrysin-7-O-glucuronide, Oroxindin and Oroxin A. The MD trajectory was investigated for evaluating different systems employed in MD simulations and the findings are presented in Table 5.

RMSD, RMSF and Rg properties were evaluated for the whole 100 ns trajectory as shown in Fig. 6. RMSD graph revealed some initial

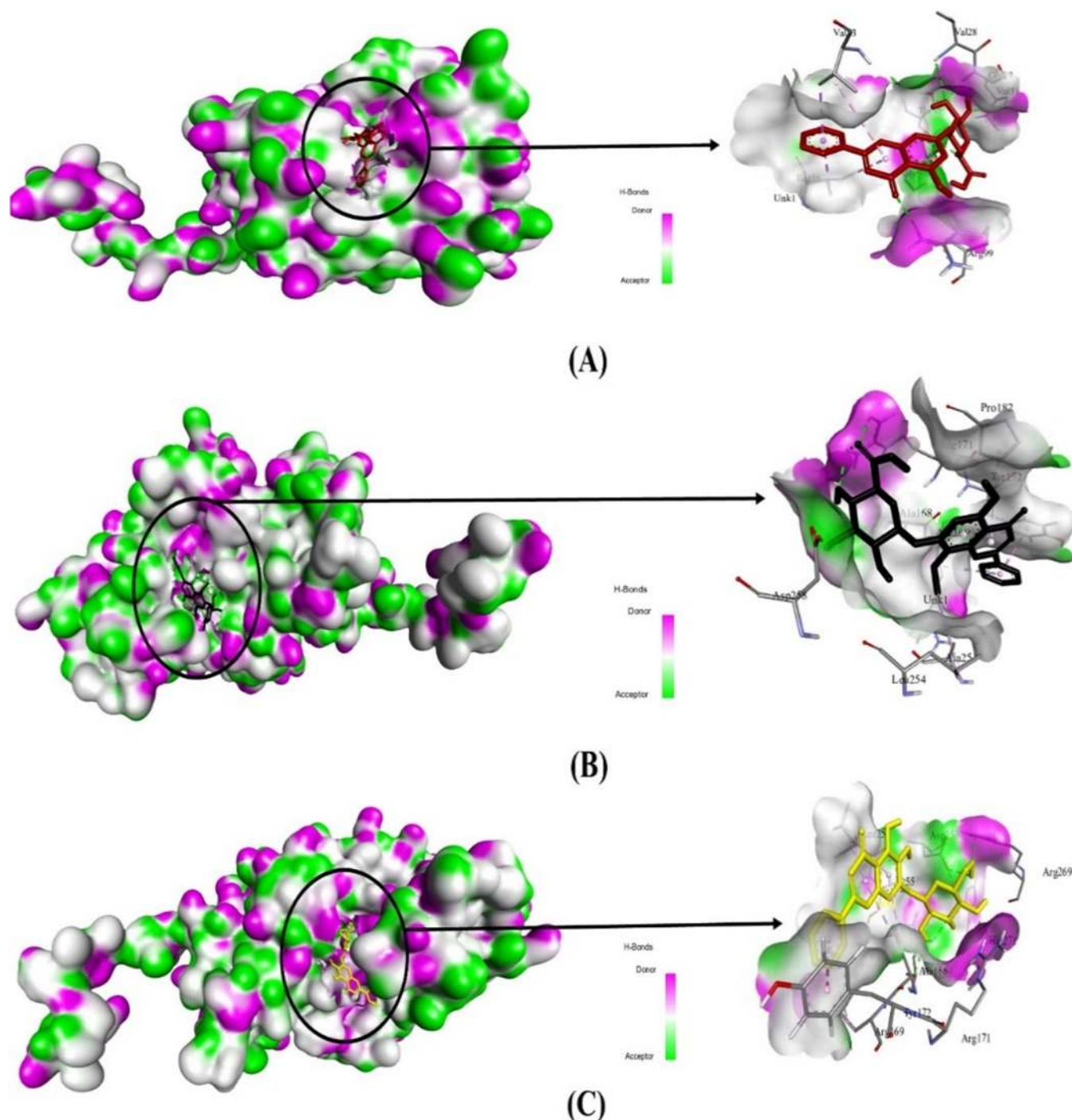


Fig. 5. Docked complexes with three dimensional molecular interactions between the macromolecular receptor and top selected candidates to show the surface of hydrogen bond donating and accepting regions in the target protein LDHA: (A). Chrysin-7-O-glucuronide; (B). Oroxindin and (C). Oroxin A.

fluctuations from 0 ns to 18 ns. After 18 ns, all the compounds showed good amount of stability up to 100 ns without any major fluctuations. Mean RMSD values were found the same (0.50 nm) in apoprotein and Sunitinib-protein complex. The other three systems involving the three lead compounds showed mean RMSD values of 0.64, 0.70 and 0.61 nm, for Chrysin-7-O-glucuronide, Oroxindin and Oroxin A complexes, respectively. Therefore, considering mean RMSD criterion, Oroxin A showed better stability among the three lead candidates (Table 5). After 68 ns, Oroxin A aligned closely with the Sunitinib-protein complex and the apoprotein.

Mean RMSF values were evaluated where both apoprotein and

Sunitinib-protein complex showed same value (0.18 nm). The mean RMSF in Chrysin-7-O-glucuronide was found to be 0.19 nm which was slightly higher than those of apoprotein and the control-complex. The Oroxindin-protein complex and Oroxin A-protein complex showed the same mean RMSF value (0.17 nm). Therefore, both Oroxindin and Oroxin A complexes were found to have better regional flexibility profile than Chrysin-7-O-glucuronide, apoprotein and the Sunitinib-protein complex. The average distance of the lead compounds at binding pocket was analyzed that revealed a mean RMSF distance of 1.6 Å for Chrysin-7-O-glucuronide, 1.3 Å for Oroxindin and 1.0 Å for Oroxin A.

Rg graph demonstrated satisfactory results as compared to the

Table 2

Molecular interactions of Chrysin-7-O-glucuronide, Oroxindin, Oroxin A and Sunitinib with different amino acid residues of LDHA.

Ligands	Binding sites	Residues in conventional hydrogen bond formation (Distance in Å)	Hydrogen bond formed	Residues in hydrophobic interactions	Binding affinity (kcal/mol)
Chrysin-7-O-glucuronide	Val28, Gly29, Val31, Val53, Thr95, Gly97, Arg99, Ile116	Val28 ^(3.04) , Gly29 ^(1.96) , Val31 ^(3.06) , Thr95 ^(2.67) , Gly97 ^(2.07) , Arg99 ^(2.61)	6	Val53, Ile116	-8.2
Oroxindin	Ala168, Arg169, Arg171, Tyr172, Pro182, Ala251, Leu254, Asp258	Arg171 ^(2.38, 2.44) , Asp258 ^(2.26)	3	Ala168, Arg169, Tyr172, Pro182, Leu254	-8.1
Oroxin A	Asn164, Ala168, Arg169, Arg171, Tyr172, Leu254, Ser255, Asp258, Arg269, His271	Asn164 ^(2.12, 2.44) , Arg171 ^(1.97) , Asp258 ^(2.61) , Arg269 ^(2.69)	5	Ala168, Arg169, Tyr172, Leu254	-8.0
Sunitinib (Control)	Asn164, Leu165, Ala168, Arg169, Arg171, Tyr172, Pro182, Leu183, Ser237, Ala251, Leu254, Ser255, Asp258, His271	Ala168 ^(2.63)	1	Leu165, Arg169, Tyr172, Pro182, Ala251	-7.6

Table 3

Evaluation of drug candidacy of Chrysin-7-O-glucuronide, Oroxindin and Oroxin A via ADME analysis.

Parameters		Chrysin-7-O-glucuronide	Oroxindin	Oroxin A
Physicochemical properties	Formula	C ₂₁ H ₁₈ O ₁₀	C ₂₂ H ₂₀ O ₁₁	C ₂₁ H ₂₀ O ₁₀
	Molecular weight	430.36 g/mol	460.39 g/mol	432.38 g/mol
	H-bond acceptor	10	11	10
	H-bond donors	5	5	6
Lipophilicity	Molar refractivity	104.70	111.19	106.11
	TPSA	166.89 Å ²	176.12 Å ²	170.05 Å ²
	Log <i>P</i> _{o/w} (iLOGP)	2.05	1.30	2.54
	Log <i>P</i> _{o/w} (SILICOS-IT)	0.37	0.44	0.35
Pharmacokinetics	Consensus Log <i>P</i> _{o/w}	0.64	0.44	0.44
	GI absorption	Low	Low	Low
	BBB permeant	No	No	No
	CYP1A2 inhibitor	No	No	No
Water Solubility	CYP2C19 inhibitor	No	No	No
	CYP2C9 inhibitor	No	No	No
	CYP2D6 inhibitor	No	No	No
	CYP3A4 inhibitor	No	No	No
Drug likeness	Log <i>K</i> _p (skin permeation)	-7.89 cm/s	-8.09 cm/s	-8.33 cm/s
	Log <i>S</i> (SILICOS-IT)	-2.81	-2.91	-2.69
	Solubility	6.62e-01 mg/ml; 1.54e-03 mol/l	5.65e-01 mg/ml; 1.23e-03 mol/l	8.77e-01 mg/ml; 2.03e-03 mol/l
	Class Lipinski	Soluble Yes; 0 Violation	Soluble Yes; 1 Violation	Soluble Yes; 1 Violation
Medicinal Chemistry	Ghose Bioavailability	Yes	Yes	Yes
	Score	0.11	0.11	0.55
	PAINS	0 alert	0 alert	1 alert
	Synthetic accessibility	5.03	5.23	5.16

apoprotein and control-complex. Mean Rg values were 2.05, 2.01, 2.02, 1.99 and 2.01 nm for apoprotein, control-complex, Chrysin-7-O-glucuronide-complex, Oroxindin-complex and Oroxin A-complex, respectively which indicated that Oroxindin-complex system showed the best structural compactness among all the five systems tested. Although fluctuations were observed for the top three lead complexes at various points of the trajectory, all of them interestingly converged to a single

level thus representing close proximity with Sunitinib-complex near to 100 ns. SASA graph revealed compactness of the systems where the mean values of all the systems were almost similar with slight variations (Fig. 7). Among the lead candidates, Oroxin A-complex revealed the mean SASA value of 141.97 nm² that showed a close proximity with the mean SASA value of Sunitinib-complex (140.62 nm²). Mean values of Chrysin-7-O-glucuronide-complex (147.37 nm²) and Oroxindin-complex (144.16 nm²) were found to be very close to the apoprotein (146.59 nm²).

All these findings indicated good structural compactness of the lead complexes. Number of hydrogen bonds between the LDHA and lead compounds revealed the highest average for Chrysin-7-O-glucuronide followed by Oroxin A and Oroxindin. All the lead candidates showed higher number of hydrogen bonds when compared to the control-complex during the 100 ns trajectory (Fig. 8).

3.5. Molecular mechanics/generalized born surface area (MM/GBSA) calculation

MM/GBSA free binding energy was calculated to eliminate false positive results and to corroborate the docking protocol. Prime module of Schrödinger revealed free binding energy values of −45.72 kcal/mol, −46.47 kcal/mol and −37.25 kcal/mol for Chrysin-7-O-glucuronide-complex, Oroxindin-complex and Oroxin-A complex, respectively. All of the top selected complexes scored higher than the control Sunitinib-complex (−35.36) kcal/mol indicating that all the lead candidate-complexes were energetically more favorable than the control-complex (Table 6).

3.6. Exploration of drug target class and similar structural analogs

Aldolase reductase and Interleukin-2 were predicted as potential targets for Chrysin-7-O-glucuronide. Xanthine dehydrogenase was predicted as potential target for both Oroxindin and Oroxin A. In addition, Adenosine A1 receptor was found as potential target for Oroxindin and Aldolase reductase was revealed as promising target for Oroxin A. Most of the targets predicted for all the three leads belonged to enzymes (Table 7, Fig. 9). SwissSimilarity server predicted structurally similar analogs with good percentage of probability where Daidzin (68%) and Troxerutin (67%) were predicted to have similar structures of the lead compound Chrysin-7-O-glucuronide (Table 8, Fig. 10). Daidzin was found to be in the experimental stage and Troxerutin to be in the investigational stage. An FDA approved drug Esculin and another investigational drug Icariin were predicted to be analogous to Oroxindin with 55% and 62% probability, respectively. Elsamitrucin (59%) and Isoquercitrin (69%) were predicted as structurally similar analogs for Oroxin A, where both of the predicted drugs were found to be in the investigational stage.

4. Discussion

Computer aided structure-based drug design approach provides a

Table 4
Toxicity analysis for Chrysin-7-O-glucuronide, Oroxindin and Oroxin A.

Classification	Target	Prediction and probability		
		Chrysin-7-O-glucuronide	Oroxindin	Oroxin A
Organ toxicity	Hepatotoxicity	Inactive (0.73)	Inactive (0.79)	Inactive (0.82)
Toxicity end points	Carcinogenicity	Inactive (0.51)	Inactive (0.53)	Inactive (0.85)
	Immunotoxicity	Inactive (0.96)	Inactive (0.56)	Inactive (0.92)
	Mutagenicity	Inactive (0.74)	Inactive (0.61)	Inactive (0.76)
	Cytotoxicity	Inactive (0.81)	Inactive (0.91)	Inactive (0.69)
Tox21-Nuclear receptor signaling pathways	Aryl hydrocarbon Receptor (AhR)	Inactive (0.56)	Inactive (0.50)	Inactive (0.92)
	Androgen Receptor (AR)	Inactive (0.99)	Inactive (0.99)	Inactive (0.90)
	Androgen Receptor Ligand Binding Domain (AR-LBD)	Inactive (0.96)	Inactive (0.98)	Inactive (0.98)
	Aromatase	Inactive (0.94)	Inactive (0.93)	Inactive (1.0)
	Estrogen Receptor Alpha (ER)	Inactive (0.73)	Inactive (0.75)	Inactive (0.91)
	Estrogen Receptor Ligand Binding Domain (ER-LBD)	Inactive (0.83)	Inactive (0.79)	Inactive (0.99)
	Peroxisome Proliferator	Inactive (0.88)	Inactive (0.95)	Inactive (0.99)
	Activated Receptor Gamma (PPAR-γ)			
	Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element	Inactive (0.95)	Inactive (0.92)	Inactive (0.98)
	Heat shock factor response element (HSE)	Inactive (0.95)	Inactive (0.92)	Inactive (0.98)
Tox21-Stress response pathways	Mitochondrial Membrane Potential (MMP)	Inactive (0.61)	Inactive (0.52)	Inactive (0.98)
	Phosphoprotein (Tumor Suppressor) p53	Inactive (0.81)	Inactive (0.83)	Active (0.50)
	ATPase family AAA domain-containing protein 5 (ATAD5)	Inactive (0.83)	Inactive (0.71)	Inactive (1.0)
Acute inhalation toxicity		No	No	No
Acute oral toxicity		No	No	No
Eye irritation and corrosion		No	No	No
Skin sensitization		No	No	No
Skin irritation and corrosion		No	No	No

rapid and cost-effective strategy to explore anticancer potential of various natural compounds to develop lead candidates targeting cancer [49–51]. In the current study, 52 phytochemicals from *O. indicum* were investigated utilizing a structure-based drug design approach to determine their anticancer potential targeting Lactate Dehydrogenase A enzyme *in silico*. Molecular docking analysis aided to screen out 65% of the total compounds immediately and further ADME/T analyses

Table 5
MD trajectory analysis using mean values for different systems.

No.	Systems	RMSD (nm)	RMSF (nm)	Rg (nm)	SASA (nm ²)
1	Apoptin	0.50	0.18	2.05	146.59
2	Sunitinib-protein complex	0.50	0.18	2.01	140.62
3	Chrysin-7-O-glucuronide-protein complex	0.64	0.19	2.02	147.37
4	Oroxindin-protein complex	0.70	0.17	1.99	144.16
5	Oroxin A-protein complex	0.61	0.17	2.01	141.97

unveiled the top three lead candidates, such as Chrysin-7-O-glucuronide (−8.2 kcal/mol), Oroxindin (−8.1 kcal/mol) and Oroxin A (−8.0 kcal/mol). Molecular interaction displayed that both Oroxindin and Oroxin A interacted with Ala168, Arg171, Tyr172 and Asp258 residues which can be potential drug surface hotspots. Both hydrogen bonding and hydrophobic interactions were found in the lead candidates. Considering the number of conventional hydrogen bonds, all the three lead compounds showed much higher number than the control drug Sunitinib. Hydrogen bonds are crucial in regulating the specificity of ligand-macromolecule interactions [52]. Hydrophobic interactions between the lead candidates and receptor enhance the stability of docked complexes [53].

Chrysin-7-O-glucuronide is commonly present in *Scutellaria baicalensis* as a flavonoid [54]. This compound has been reported to exert inhibitory effects *in vitro* against alpha-amylase and alpha-glucosidase enzymes and thereby plays a vital role in treating diabetes [55]. Certain types of cancer cells express Breast Cancer Resistance Protein (BCRP), and its overexpression has been shown to be a significant contributor to the resistance of chemotherapeutic treatment [56]. Chrysin-7-O-glucuronide production considerably decreased when BCRP was silenced, showing that glucuronide is a substrate of BCRP, according to RNA-mediated silencing studies in HeLa cells [57]. While in an animal study, it was revealed that the impact of BCRP on Chrysin and its conjugates was limited as the PK parameters were not altered significantly in BCRP knockout mice compared to those in wild-type mice [58]. Kseibati et al. [59] reported that Chrysin administered orally at a dose of 50 mg/kg improved bleomycin-induced pulmonary fibrosis. Chrysin reduced hydroxyproline content, decreased TGF1 protein expression, lowered the activity of lactate dehydrogenase (LDH), and decreased lipid peroxidation. Chrysin-7-O-glucuronide, as a conjugate of Chrysin, may inhibit certain biotransformation enzymes (CYP2C9). Therefore, high intake of Chrysin-7-O-glucuronide might interrupt the transport and/or biotransformation of drugs [60]. In an *in vivo* rodent experiment peak plasma concentration of Chrysin-7-O-glucuronide was found considerably higher than that of Chrysin [61]. In mice, 20 mg/kg oral dose of Chrysin caused 160 nM peak plasma concentrations of Chrysin-7-O-glucuronide [58].

Oroxindin is a flavonoid which is commonly found in *O. indicum* and several other plants. This phytochemical has been reported in various *in vitro* studies to possess antimicrobial and anti-inflammatory properties [62,63]. Oroxin A is another flavonoid usually isolated from *O. indicum* and it has been reported to possess significant inhibitory properties against breast cancer proliferation by generating significant endoplasmic reticulum stress and senescence [64].

Servers involving computational ADME and toxicity analyses have improved greatly in recent years with the incorporation of machine learning methods which have facilitated rapid analyses to evaluate various pharmacokinetic, pharmacodynamic and toxicity properties of drug-like compounds [65]. The present investigation revealed favorable ADME/T properties for Chrysin-7-O-glucuronide, Oroxindin and Oroxin A. In current drug discovery, interaction of lead compounds with cytochrome P450 (CYP) is considered as a crucial indicator. CYP isoforms exert regulatory effects in transformation, cellular metabolism, and excretion of drugs, and found to be critical in detoxification of foreign substances [66]. All lead candidates were found not to inhibit any of the

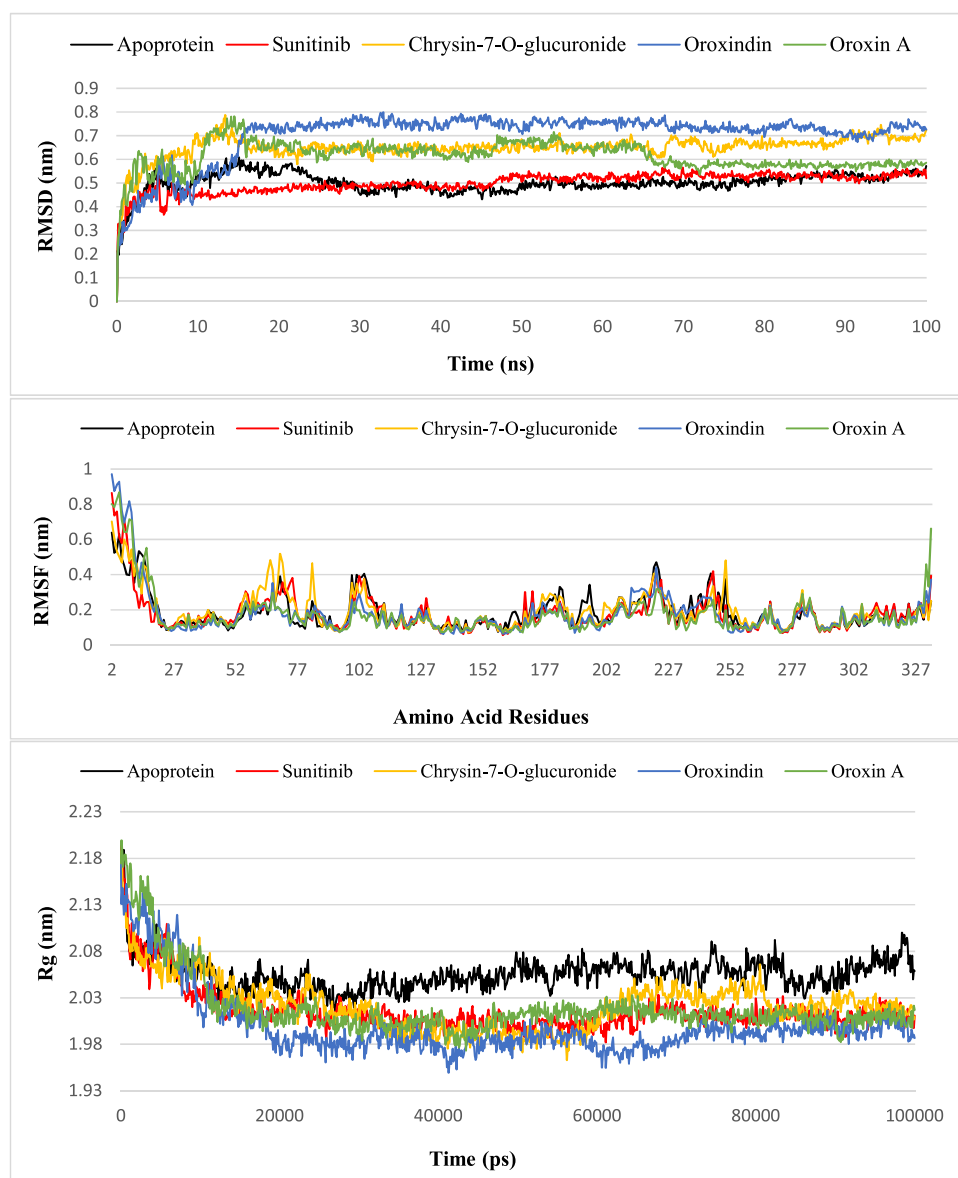


Fig. 6. Molecular dynamics simulation for 100 ns depicting RMSD, RMSF and Rg properties of the lead candidates Chrysin-7-O-glucuronide, Oroxindin and Oroxin A along with Sunitinib and apoprotein.

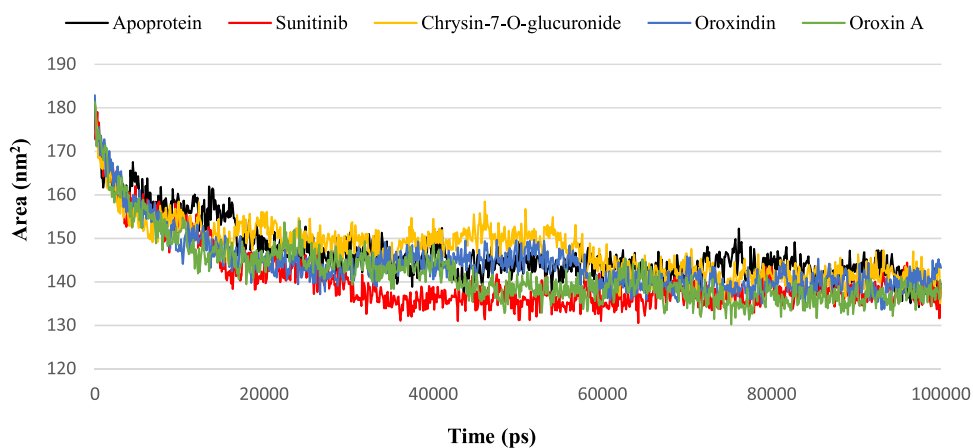


Fig. 7. Molecular dynamics simulation for 100 ns depicting SASA values for the lead candidates Chrysin-7-O-glucuronide, Oroxindin and Oroxin A along with the control Sunitinib and apoprotein.

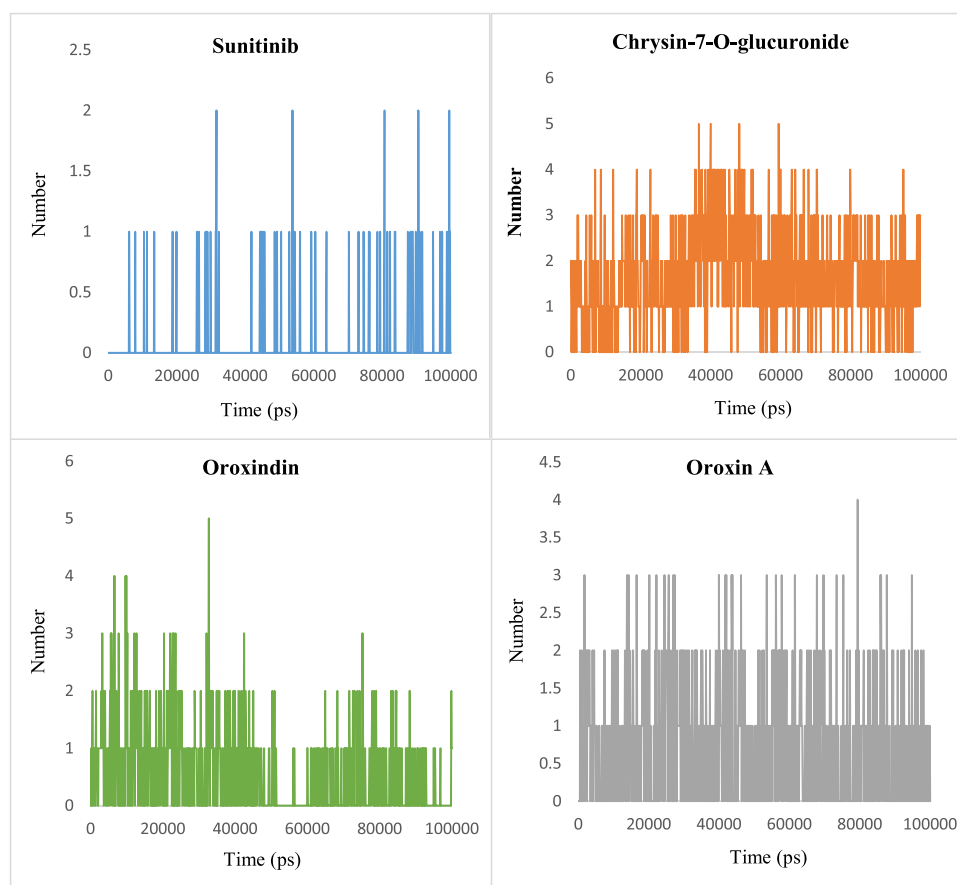


Fig. 8. Number of hydrogen bonds formed between Lactate Dehydrogenase A and top three lead compounds along with the control Sunitinib.

Table 6

Binding free energy estimation employing Prime MM/GBSA.

Complexes	MM/GBSA ΔG Bind (kcal/mol)	MM/GBSA ΔG Coulomb (kcal/mol)	MM/GBSA ΔG Covalent (kcal/mol)	MM/GBSA ΔG Hbond (kcal/mol)	MM/GBSA ΔG Lipo (kcal/mol)	MM/GBSA ΔG Solve GB (kcal/mol)	MM/GBSA ΔG Bind vdW (kcal/mol)
Sunitinib-protein complex (control)	-35.56	-12.56	5.82	-0.27	-16.42	22.67	-33.63
Chrysin-7-O-glucuronide-protein complex	-45.72	-9.56	0.80	-0.71	-22.77	23.17	-36.65
Oroxindin-protein complex	-46.47	-23.76	3.74	-1.51	-16.35	20.371	-27.4
Oroxin A-protein complex	-37.25	-27.26	0.83	-2.74	-12.92	28.36	-23.53

Table 7

Target class prediction for Chrysin-7-O-glucuronide, Oroxindin and Oroxin A.

Ligands	Drug Targets	Common name	UniProt ID	Target Class
Chrysin-7-O-glucuronide	i. Aldolase reductase ii. Interleukin-2	AKR1B1 IL2	P15121 P60568	Enzyme Secreted protein
Oroxindin	i. Adenosine A1 receptor ii. Xanthine dehydrogenase	ADORA1 XDH	P30542 P47989	Family A G protein-coupled receptor Oxidoreductase
Oroxin A	i. Aldolase reductase ii. Xanthine dehydrogenase	AKR1B1 XDH	P15121 P47989	Enzyme Oxidoreductase

CYP isoforms that was satisfactory. Lipinski's rules of five was proposed when the drug discovery paradigm transitioned from phenotypic screening to combinatorial chemistry and high-throughput screening during mid- to late 1990's [67]. The five principles constituted an investigation of compounds that passed Phase I and entered into the Phase II (clinical trials) by linking physicochemical characteristics, permeability, solubility, and oral bioavailability, thus acting as a crucial component in drug-likeness evaluation [68]. In the present study, Chrysin-7-O-glucuronide followed all the five rules of Lipinski with zero violations and the other two lead compounds followed the parameter with 1 violation each, which were acceptable. Pan Assay Interference Compounds (PAINS) criterion revealed zero alerts for both Chrysin-7-O-glucuronide and Oroxindin indicating that these leads would not cause false positive results [69]. Oroxin A only showed one violation in PAINS alert test, which was acceptable. All the investigated compounds revealed moderate synthetic accessibility where Chrysin-7-O-glucuronide scored better among the three compounds [70]. Toxicity properties revealed satisfactory results with no major side

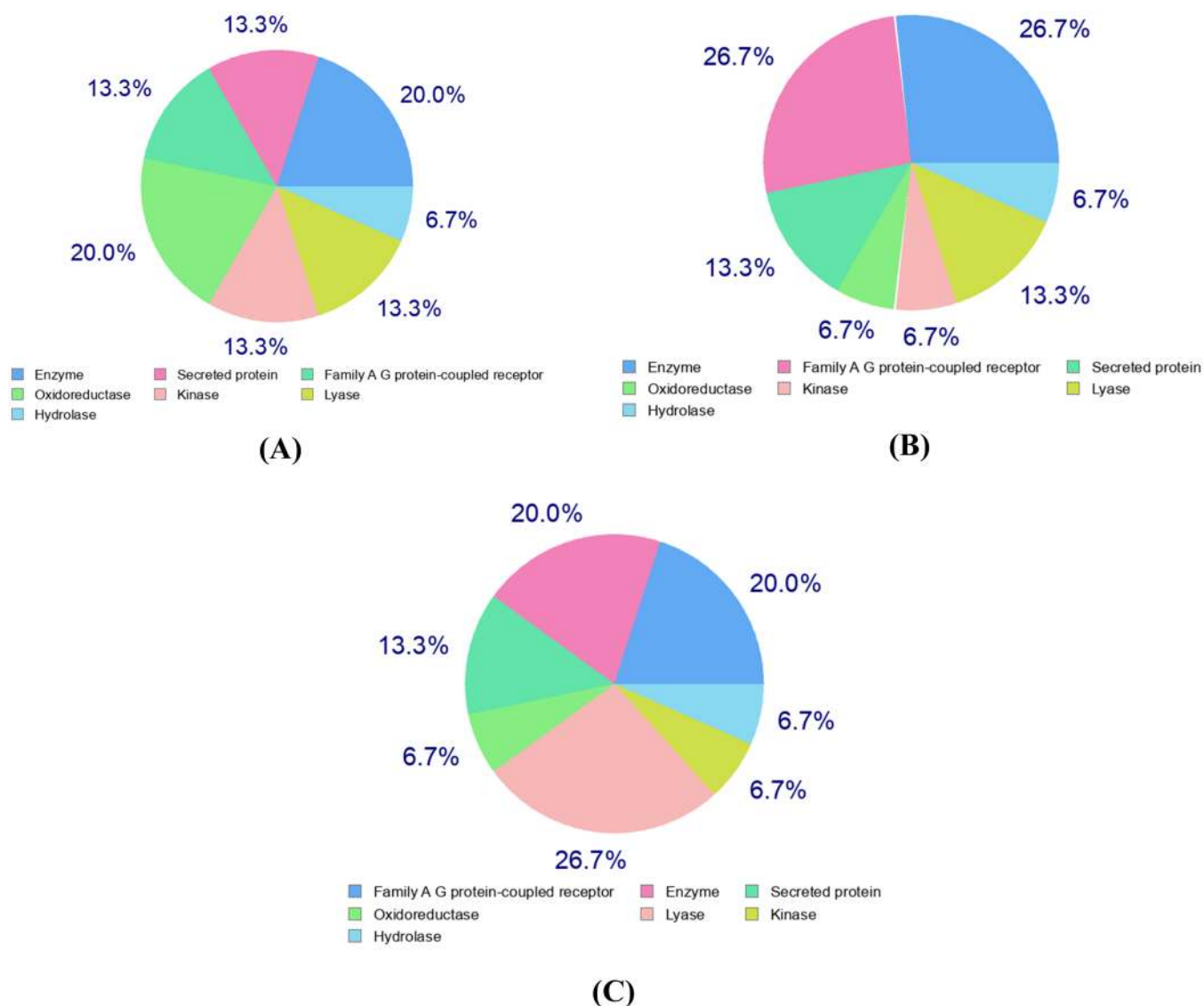


Fig. 9. Drug target class predicted for Chrysin-7-O-glucuronide (A), Oroxindin (B) and Oroxin A (C).

Table 8

Structurally similar analogs for Chrysin-7-O-glucuronide, Oroxindin and Oroxin A.

Lead candidates	Structurally Similar Analogs	Drug Bank ID	Score	Status
Chrysin-7-O-glucuronide	i. Daidzin	DB02115	68%	Experimental
	ii. Troxerutin	DB13124	67%	Investigational
Oroxindin	i. Esculin	DB13155	55%	Approved
	ii. Icaritin	DB12052	62%	Investigational
Oroxin A	i. Elsamitrucin	DB05129	59%	Investigational
	ii. Isoquercitrin	DB16403	69%	Investigational

effects considering various toxicity endpoints and other parameters, and further corroborated our choice of the top three lead candidates. However, Computational ADME/T analyses encounter some limitations and are not completely error-free. Three essential elements make up a predictive ADME/T model: experimental data used to train the model; molecular structure descriptors that can be associated with the experimental data; and the right modeling technology. Lack of high-quality experimental data on which to develop models is one of the obstacles to improved prediction of organism-based features [71].

Molecular dynamics simulation unveiled significant structural stability and compactness for the top ligand-protein complexes compared to the control-complex and apoprotein LDHA. Higher values of RMSD, RMSF, Rg and SASA are indicative of higher degrees of flexibility and instability [66]. Rg and SASA provide insights regarding the global stability of the protein tertiary structures and ligands [72]. As mass-weighted RMSD for a group of atoms relative to their common mass center, Rg is often used to estimate whether a complex is stably folded or not [73]. Thus, the structure stability, within a valid MD simulation, is correlated to Rg values reaching a plateau around the average values [74]. In the current study, Oroxindin-complex showed better Rg values from 20 to 65 ns as compared to the control-complex and other two lead candidates, which is suggestive of significant stability, compactness and good accommodation within the protein pocket. After that with a very slight fluctuation, Oroxindin-complex became stable again at close to 80 ns up to 100 ns with lower Rg values than the control-complex. Oroxin A-complex followed the control-complex closely from 20 up to 100 ns indicating that Oroxin A holds the folding behavior of the protein. Chrysin-7-O-glucuronide-complex slightly fluctuated from 60 to 80 ns due to floppy packing but became stable again near 100 ns. With no major abrupt fluctuations after 20 ns, all the three lead candidate-complexes maintained the folding behavior

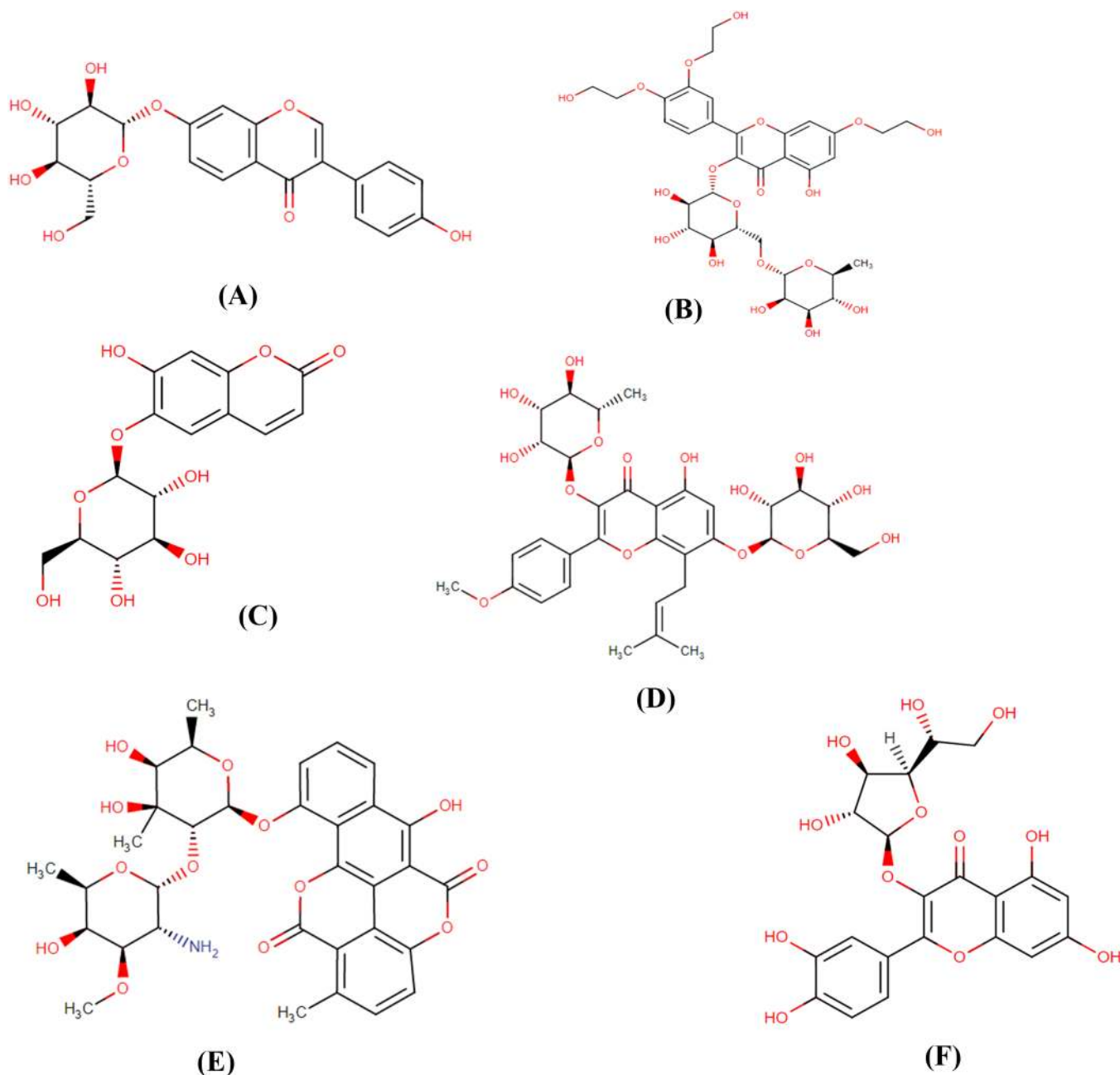


Fig. 10. Two dimensional chemical structures of structurally similar analogs predicted for the top three phytochemicals: (A). Daidzin and (B). Troxerutin for Chrysin-7-O-glucuronide; (C). Esculin and (D). Icaritin for Oxindin; (E). Elsamitrucin and (F). Isoquercitrin for Oxindin A.

of the protein Lactate Dehydrogenase A.

SASA measures the biomolecular surface area of the protein that is accessible to the solvent molecules. Reduced SASA values indicate relative structural shrinkage of protein-ligand complexes due to the influence of solvent surface charges, resulting in more compact and stable conformations [75]. The present investigation showed similar kind of SASA profiles for all the three lead candidate-complexes up to 40 ns that indicates similar kind of protein-ligand interactions. After 40 ns, SASA values decreased for Oxindin A-complex up to 50 ns and then became almost stable up to 100 ns showing close proximity with the control complex. This suggests the truncated nature of the complex across the simulation time that attributes to better stability. Oxindin-complex showed reduced SASA value after 60 ns and closely aligned with the control-complex up to 100 ns for better compactness. After 40 ns, Chrysin-7-O-glucuronide-complex showed elevated SASA values close to

60 ns that might confer the migration of Chrysin-7-O-glucuronide towards the solvent side during this simulation time frame where the protein cavity became highly solvated and minimally compacted. After 60 ns, Chrysin-7-O-glucuronide-complex showed better stability up to 100 ns with no major fluctuations.

The present study showed close similarity among the lead complexes with lower values of the aforesaid criteria as compared to the control complex which strengthened Chrysin-7-O-glucuronide, Oxindin and Oxindin A as lead candidates. MM/GBSA free binding energy was evaluated for all the three top ligand-protein complexes where Oxindin-protein complex showed better results than other systems tested. This is considered as a revolutionary strategy involving quantum-mechanics/molecular-mechanics properties for estimating relative binding energies [76].

Drug target prediction aids in the discovery of new targets for the top

selected candidates, whereas prediction of structurally similar analogs facilitates drug design process targeting the same receptor [77]. Various enzymes have been found as the target class in most of the cases for all the top three candidates which would be useful for conducting further studies. Daidzin and Troxerutin were predicted as similar analogs for Chrysin-7-O-glucuronide. Daidzin has been reported to show suppressive effects on rat prostate carcinogenesis as well as to inhibit growth and induce apoptosis in HeLa cell lines [78,79]. Several *in vitro* investigations using various cancer cell lines demonstrated anticancer and cytotoxic properties of Troxerutin [80–82]. Esculin, an FDA approved drug was predicted to be analogous to Oroxindin which has been shown to have anticancer activities in glioblastoma, lung cancer, and breast cancer [83–85]. Similarly, Icaritin, another analog of Oroxindin has been found to inhibit glioblastoma and gallbladder cancer [86,87]. Elsamitruicin and Isoquercitrin were predicted as structurally similar analogs to Oroxin A, where both have been reported to have anticancer properties [88,89].

Various computational studies have been attempted to explore novel inhibitors targeting LDHA [90–92]. Medicinal plants can unleash new avenues for discovering new effective inhibitors with insignificant side effects that can exert anti-cancer potential targeting LDHA. Although few bioinformatic approaches have been taken to explore the inhibitory potentials of *O. indicum* phytochemicals [93–95], no attempts have been undertaken to evaluate its anti-cancer potential *in silico* targeting Lactate Dehydrogenase A. Therefore, the present study could unveil a new window on the discovery of novel LDHA inhibitors employing *Oroxylum indicum* phytochemicals against cancer.

5. Conclusion

The present investigation explored the anticancer efficacy of *Oroxylum indicum* using a variety of bioinformatic approaches to reveal novel drug candidates targeting Lactate Dehydrogenase A. Molecular docking unleashed 18 compounds initially from 52 phytoligands which were further processed through ADME and toxicity analyses. Finally, three potential candidates were identified, such as Chrysin-7-O-glucuronide, Oroxindin and Oroxin A wherein Chrysin-7-O-glucuronide showed the best binding affinity in molecular docking analysis. All the three lead compounds revealed favorable pharmacokinetic and pharmacodynamic properties with no major side effects. Furthermore, the top selected drug candidates exhibited noteworthy conformational stability and compactness in 100 ns molecular dynamics simulation. MM/GBSA study revealed Chrysin-7-O-glucuronide as the best lead candidate. Finally, we recommend further *in vivo* investigation for experimental validation of our findings.

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CRediT authorship contribution statement

Sheikh Sunzid Ahmed: Conceptualization, Methodology, Software, Data curation, Writing – original draft preparation. **M. Oliur Rahman:** Conceptualization, Methodology, Data curation, Visualization, Validation, Supervision, Writing – review & editing. **Ali S. Alqahtani:** Visualization, Validation, Writing – review & editing, Funding acquisition. **Nahid Sultana:** Investigation, Visualization, Writing – original draft preparation. **Omer M. Almarfadi:** Methodology, Software, Visualization. **M. Ajmal Ali:** Visualization, Writing – review & editing. **Joongku Lee:** Visualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

Data availability

No data was used for the research described in the article.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.toxrep.2022.12.007.

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