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A comprehensive computational study of Millets derived phytochemicals as potential inhibitors of NACHT domain of NLRP3 inflammasome: Molecular docking, molecular dynamics simulation, MM-PBSA free energy calculation and DFT analysis

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The NLRP3 inflammasome is a vital constituent in the innate immune response, which regulates the caspase-1 activation for the production of proinflammatory cytokines IL-1 and IL-18. NLRP3 inflammasome activation is the main cause of many inflammatory diseases and can contribute to onset of metabolic disorders. There is an urgent need to develop drugs that can inhibit activation of inflammasome. Now a days, millets are known to be rich in polyphenols, which have potential to become drug molecules. In the current study, we investigated the efficacy of phytochemicals of millets against NACHT domain of NLRP3 inflammasomeby using various *In silico* techniques. The interaction affinities of polyphenolic compounds towards NLRP3 was evaluated *via* intramolecular by Quantum Mechanic, intermolecular by Molecular Docking, and spatial by Molecular dynamics simulations. Isovitexin, Kaempferol, Quercetin, Syringic acid Tricin showed highest (most negative) CDOCKER interaction energy among all phytochemicals and positive control MCC950. Two best compounds Isovitexin and Kaempferol along with MCC 950 were further studied through dynamic simulation, which showed their stability at active site. MM-PBSA binding free energy of phytochemicals Isovitexin, Kaempferol and MCC950 are -51.5813 kcal/mol, -26.6370 Kcal/mol and -12.7006 kcal/mol, respectively, which indicate high degree of binding compared to positive control further these compounds were studied through DFT to determine chemical stability.

Keywords: Isovitexin, MCC950, Millets, NLRP3 inflammasome, Phytochemicals

Immune system protects against dangers using adaptive immunity and innate immunity[1]. The innate immune reactions depend on pattern-recognition receptors (PRRs) to aim pathogenic microorganisms. PRRs are primarily expressed in immune cells such as dendritic cells and macrophages[2,3]. PRRs contribute to long-term protection by presenting antigens to the adaptive immune system[4]. PRR recognizes antigens which are common to a group known as Pathogen-associated molecular patterns (PAMPs)[5]. Inflammasome is the new PRR identified in the recent times, NLRP3 is one of the inflammasome. NLRP3 inflammasome comprises adapter protein apoptosis-associated speck-like protein (ASC) and procaspase-1[6]. Three proteins compactly regulate inflammasome activity. The principal protein of NLRP3 inflammasome, NLRP3 comprises a central nucleotide-binding and oligomerization (NACHT) domain which enables self-oligomerization and has ATPase activity. The C-terminal can modify NLRP3 activity and respond to endogenous signals and ligands from microbes. In contrast, the N-terminal pyrin domain (PYD) is essential for interactions with the adaptor protein ASC. ASC has downward transduction domain connected with caspase-1[7]. Caspase-1 is involved in the production of active forms-IL-1β, IL-18 and pyroptosis[8]. (TLR)/nuclear inflammasomes has critical role in the start and advancement of several diseases, including neurodegenerative diseases, metabolic disorders, cardiovascular diseases, and many additional diseases[9]. In addition, numerous auto inflammatory disorders associated with increased production of IL-1β are triggered by inflammasome activation. The wide-ranging association of the NLRP3 inflammasome in such a range of diseases makes it extremely desired drug target for treating inflammatory disorders[10]. Inhibitors of the NLRP3 inflammasome pathway from natural sources such as polyphenols have been

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validated in *in vitro* studies and *in vivo* studies of NLRP3-related diseases but their mechanism of action is not clear, therefore understanding the inflammasome complex and structural investigation is necessary for developing new therapeutic target[11].

The major types of Pearl millet, Proso millet or white millet (*Panicum miliaceum*), and Finger Millet (*Eleusine coracana*). Minor millets include Barnyard millet (*Echinochloa* spp.), Kodo millet (*Paspalum scrobiculatum*) and Little millet (*Panicum sumatrense*)[12.13]. Millets are gaining interest around the globe due to their beneficial effects like low glycemic index compared to other cereals like wheat, rice and maize[14]. Whole grains are rich sources of fibre, vitamins, minerals and phytochemicals such as phenolics, lignans, β-glucan, inulin, resistant starch, sterols and phytates[15]. Apart from the nutritional benefits, millets have the preventive effect on diseases. Flavonoids in millets have demonstrated a variety of therapeutic effects for use in medicine and clinical settings, including analgesic, anti-inflammatory, anti-hypertensive, diuretic, and anti-cancer activities[16-19].

Considering the pivotal role played by the NLRP3 inflammasome in inflammation, it was thought worthwhile to study the role of inflammasome in preventive effect of millets in inflammatory disease conditions. As per our knowledge this is the first study where phytochemicals of millets are evaluated against NACHT domain of NLRP3 inflammasome. In the present study, we have studied the interaction of phytochemicals of millets against NLRP3 inflammasome using molecular docking, molecular dynamics simulation and DFT analysis.

Materials and Methods

Molecular docking studies

Preparation of Protein

The crystallographic structure of NACHT domain of NLRP3 inflammasome was retrieved from the Protein Data Bank (http://www.rcsb.org/pdb) of PDB ID: 7ALV. To optimize the protein structure for further analysis, we employed the BIOVIA Discovery Studio (DS) 2022 software "prepare protein" feature, ensuring the inclusion of loop construction and protonation. Furthermore, we eliminated water molecules, heteroatoms, and the native inhibitors from the protein structure to create a refined and suitable starting point for our research.

Preparation of Ligand

Phytochemicals sourced from the millets were identified and collected through a comprehensive review of existing literature and databases[20,21]. These compounds were then download from PubChem in the form of 3D structural data files (sdf). To ensure their suitability for subsequent docking analysis, the 3D structures of these ligands were processed by utilizing the "Small Molecule" tool within BIOVIA DS 2022.

Molecular docking

The prepared protein and ligand were docked using tool CDOCKER, a grid-based molecular dynamics simulation. For the molecular docking study, the binding site of native inhibitor to the NACHT domain of NLRP3 was selected as the active site of interest. The specific coordinates for this binding site on NLRP3 was selected as a grid in 3D Cartesian space X: 16.75; Y: 35.44; Z: 125.70, with a radius of 15 Å. Furthermore, CDOCKER interaction energies were determined to assess the interactions between the ligands and the receptor. As part of this analysis, MCC950 was employed as a positive control, based on previous research findings[22,23].

Molecular dynamics simulation and Molecular Mechanics-Poisson Boltzmann Surface Area (MM-PBSA)-based binding free energy calculation

Molecular dynamics (MD) simulation studies were performed using Biovia Discovery Studio software, 2022 based on the CHARMM molecular mechanics. Top ranked poses without any restraints were used for the study. The complexes were solvated in an orthorhombic box with a distance of 1 Å, 0.15 M NaCl by replacing randomly added TIP3 water molecules[24]. At the initial step, energy minimization was carried out using steepest descent algorithm with 500 max steps and RMS gradient of 1.0, followed by conjugated gradient as algorithm, with 500 maximum steps and RMS gradient of 0.1[25]. For the second step (heating) is carried by initial and target temperatures of 50 and 300 K, respectively. NAMD was used for the final production, a simulation time of 100,000 ps (100 ns) was set. 2 fs was used as time step for the integration. Multiple-time step algorithm was used to integrate the long- and short-range forces with Impulse/Verlet-I[26]. Results were saved at 40ps, root mean square deviation (RMSD), radius of gyration (rg) and root mean square fluctuations (RMSF) were calculated for all the generated conformers.

The binding free energies for each protein-ligand complex were calculated using MM-PBSA. The free energy of the protein-ligand binding (Δ G binding) was estimated using Equation.

Δ G binding = Δ G complex –[Δ G protein + Δ G ligand]

Frontier molecular orbital studies

The modeling of HOMO, LUMO orbitals of the most effective small molecules and mulliken atomic charges were calculated by density functional theory (DFT) on discovery studio 2022 by using function of B3LYP[27-30].

Results and Discussion

Molecular docking

As an intracellular innate immune sensor, NLRP3 inflammasome is involved across several pathological states and diseases. Inflammation is usually always a factor in the disorders and diseases connected to inflammasomes. The emergence of various inflammasome activators is crucial to the development of pathological conditions[9]. Thus, it is claimed that IL-1 production, dysregulated inflammasome activation, and disease etiology are interrelated. Disease-related stresses that result in mutations in genes linked to the inflammasome, its underlying pathways, and inflammasome-dependent IL-1 production are frequently involved in the pathogenesis of the disease[31]. Understanding the pathways and mechanism of NLRP3 inflammasome activation can help treat a variety of illnesses that involve the inflammasome complex. It is beneficial to use natural compounds as they have a high binding affinity, high efficacy, and minimal adverse effects[32]. Although both natural and synthetic medicines have many advantages, the efficacy of treatments is mostly influenced by the extent of the ailment. Natural bioactive substances might be more beneficial with a lesser number of adverse effects. Among all the phytochemicals present in millets, phenolics are important sources of antioxidants. Phenolics have antioxidant properties, which importantly maintain oxidative balance in the body[33]. Apart from that they have potential to be a drug target for treating various disease conditions[34]. Considering the beneficial effects, the 75thsession of the UN General Assembly and members of the FAO Governing Bodies supported the Government of India's proposal to establish an International Year of Millets in 2023. However, the FAO of the United Nations formally commenced the International Year of Millets - 2023 on December 6, 2022 in Rome, Italy[35].

The NLRP3 NACHT domain consists of a nucleotide-binding domain, helical domain 1, winged-helix domain and helical domain 2. The NACHT domain is known as the “interaction domain” of the NLRP3.The key residues of the NACHT domain include ARG 578, GLU 629, ALA 227, ALA 228, and ARG 351. Binding into these residues could inhibit the conformational change essential for the activation of inflammasome[22]. The virtual screening of the phytochemicals from millets was completed by considering their highest CDOCKER interaction energy, number of non-bonding interactions, and hydrogen bonds. CDOCKER interaction energy is the interaction energy between the protein and ligand. It represents the strength of interaction between the proteins and ligands. Hydrogen bonds and Van der Waals interaction play an important role in the binding of ligand to the protein. Van der Waals interaction is the weakest intermolecular attraction between two molecules. However, increased number of Van der Waals forces has stronger interactions despite its weakest bond nature[36]. Molecular docking simulation revealed that five compounds Isovitexin, Kaempferol, Quercetin, Syringic acid and Tricin showed highest (most negative) CDOCKER interaction energy among all phytochemicals (Table 1) and positive control MCC 950. MCC950 (Fig. 1A) formed six hydrogen bonds ARGA:578, ALAA:228, ARGA:351, ILEA:574, ALAA:227, and PROA:352 at the active site and formed several Van der Waals interactions, Pi-Sulfur and alkyl-alkyl interactions GLYA:229, THR A:524,LEU A:413, TYR A:443, PHE A:579, VAL A:414, THR A:439, MET A:408, MET A:661, PHE A:410,TYR A:632, GLU A:629, LEU A:628, ASP A:662, THR A:659, VAL A:353, ILE A:411, PHE A:575 interacting with all the key residues of active site and forming four hydrogen bonds with the active site. CDOCKER interaction energy of MCC950 was -52.4598 (Kcal/ mol). Isovitexin (Fig. 1B) has showed highest CDOCKER interaction energy (-77.40 Kcal/mol), formed three hydrogen bonds, THR A:439, ALA A:228, ASP A:662 and hydrophobic interactions include VAL A:353, ILE A:574, GLN A:624, GLU A:629, SER A:626, ARG A:578, MET A:661, PHE A:575, MET A:408, VAL A:414, TYR A:443, ILE A:411, THR A:524, LEU A:628, GLY A:229, TYR

Table 1 — Results of the docking of compounds of millets on the crystal structure of NACHT domain of NLRP3

Ligand	CDOCKER interaction energy (-Kcal/ mol)	Hydrogen bond interaction	Hydrophobic interaction
MCC950	52.4598	ARGA:578, ALAA:228, ARGA:351, ILEA:574, ALAA:227, PROA:352	GLYA:229, THR A:524, LEU A:413, TYR A:443, PHE A:579, VAL A:414, THR A:439, MET A:408, MET A:661, PHE A:410, TYR A:632, GLU A:629, LEU A:628, ASP A:662, THR A:659, VAL A:353 ILE A:411, PHE A:575
Caffeic acid	38.5439	ALA A:228, ALA A:227, ARG A:351, THR A:439	VAL A:414, TYR A:443, THR A:545, PHE A:575, PRO A:352, ILE A:574, TYR A:632, MET A:661, MET A:408,
Caprolactam	17.397	-	MET A:408, TYR A:632, MET A:661, PHE A:410, ARG A:578, ALA A:227, PHE A:575, ALA A:228, VAL A:414, THR A:439,
Apigenin	52.3301	ALA A:228, GLN A:180, SER A:658, ASP A:662,	ALA A:227, PRO A:352, LEU A:628, SER A:626, ASN A:656, THR A:659, ARG A:578, ILE A:574, PHE A:575, ARG A:351
Campesterol	50.223	GLN A:180	SER A:626, ASN A:656, GLY A:229, PRO A:352, LEU A:628, ALA A:228, ASP A:662, LEU A:413, PHE A:575, THR A:524, PHE A:579, TYR A:443, VAL A:414, THR A:439, THR A:659, ILE A:411, PHE A:410, MET A:661, ARG A:578, TYR A:632, ILE A:574, ALA A:227, GLU A:629, GLN A:624, VAL A:353,
Cinnamic acid	35.3146	ALA A:228, PRO A:352	ARG A:351, PHE A:575, ALA A:227, TYR A:632, LEU A:628, ASP A:632, THR A:659, PHE A:410, MET A:661, ARG A:578, ILE A:411, VAL A:353, ILE A:574
Coumaric acid	37.6033	ASP A:662, ALA A:228, PRO A:352	THR A:659, MET A:661, PHE A:410, ARG A:578, ILE A:411, ALA A:227, VAL A:353, ARG A:351, ILE A:574, PHE A:575, LEU A:628, TYR A:632
Gallic acid	51.145	THR A:439, TYR A:443	ARG A:578, PHE A:575, ALA A:227, ALA A:228, THR A:524, GLY A:229, LEU A:413, VAL A:414ILE A:417, ILE A:411, MET A:408, MET A:661, TYR A:632
Gamma sitosterol	50.8923	GLN A:180	ILE A:623 SER A:626, VAL A:353, GLN A:624, PRO A:352, GLU A:629, ALA A:227, ARG A:578, ILE A:574, ARG A:351, ALA A:228, PHE A:575, GLY A:229, LEU A:413, THR A:524, TYR A:443, VAL A:414, ILE A:411, THR A:439, MET A:408, MET A:661, THR A:659, PHE A:410, TYR A:632, LEU A:628, ASP A:662
Gentisic acid	37.3324	ARG A:578, ALA A:228	TYR A:632, LEU A:628, ALA A:227, PRO A:352, VAL A:353, ARG A:551, ILE A:574, PHE A:575
Isovitexin	77.40	THR A:439, ALA A:228, ASP A:662	VAL A:353, ILE A:574, GLN A:624, GLU A:629, SER A:626, ARG A:578, MET A:661, PHE A:575, MET A:408, VAL A:414, TYR A:443, ILE A:411, THR A:524, LEU A:628, GLY A:229, TYR A:632, PHE A:410, THR A:659, SER A:658, ALA A:227, PERO A:352, ARG A:351
Kaempferol	61.44	ARG A:578, THR A:659	ILE A:411, THR A:524, ALA A:228, PHE A:575, TYR A:632, LEU A:628, ASP A:662, SER A:658, GLU A:369, PHE A:410, ILE A:230, MET A:661, VAL A:414, THR A:439, TYR A:443
Luteolin	39.82	THR A:439, TYR A:443, PHE A:575, ALA A:227, ALA A:228, ARG A:351	MET A:408, MET A:661, TYR A:632, VALV353, PRO A:352, ARG A:578, THR A:524, GLY A:229, LEU A:413, VAL A:414
Quercetin	58.6012	THR A:439, ASP A:662	ILE A:411, ARG A:351, ARG A:578, ALA A:228, PHE A:575, PRO A:352, LEU A:628, SER A:658, THR A:659, PHE A:410, TYR A:632, ALA A:227, MET A:661, MET A:408, VAL A:414
Sinapic acid	43.316	ALA A:228, TYR A:632, ASP A:662, PRO A:352	ILE A:574, ARG A:351, PHE A:575, ILE A:628, SER A:658, THR A:659, PHEV410, MET A:661, MET A:408, THR A:439, ILE A:411, ARG A:578, ALA A:227, VAL A:353
Syringic acid	54.94	TYR A:443, THR A:439	ILE A:417, MET A:661, VAL A:414, METV408, TYR A:632, ARG A:578, ARG A:351, ILE A:411, ILE A:574, ALA A:227, ALA A:228, PHE A:575, THR A:524, GLY A:229, LEU A:413

(*contd*.)

Table 1 — Results of the docking of compounds of millets on the crystal structure of NACHT domain of NLRP3 (*contd.*)

Ligand	CDOCKER interaction energy (-Kcal/ mol)	Hydrogen bond interaction	Hydrophobic interaction
Tricin	55.91	ILE A:370, GLY A:226, SER A:658	THR A:439, MET A:408, METV661, ILE A:411, PHE A:410, TYR A:632, ILE A:230, ALA A:227, THR A:569, PRO A:352, LEU A:371, GLU A:369, GLN A:225, HIS A:177, ASP A:662, LEU A:628, ALA A:28, ILE A:574, ARG A:351, PHE A:575, GLU A:629, ARG A:578
Vanillic acid	50.6085	VAL A:353, ARG A:578	GLU A:629, PRO A:352, ARG A:351, ILE A:574, ALA A:228, ALA A:227, PHE A:575, ARG A:578, LEU A:628, TYR A:632
Xanthone	24.56	ARG A:578	VAL A:353, ARG A:351, ALA A:228, PRO A:352, PHE A:575, ILE A:411, MET A:661, PHE A:410, TYR A:632, THR A:659, ASP A:662, ALA A:227, ILE A:574
Protocatechuic acid	47.85	VAL A:353	ARG A:578, TYR A:632, ALA A:227, ILE A:574, PRO A:352, ARG A:351, ALA A:228, PHE A:575
Ferulic acid	41.12	ASP A:662, TYR A:632, ALA A:228	MET661, THR A:659, PHE A:410, ILE A:411, ARG A:578, VAL A:353, ILE A:574, PHE A:575, ARG A:351, ALA A:227, PRO A:352, SER A:658, LEU A:628

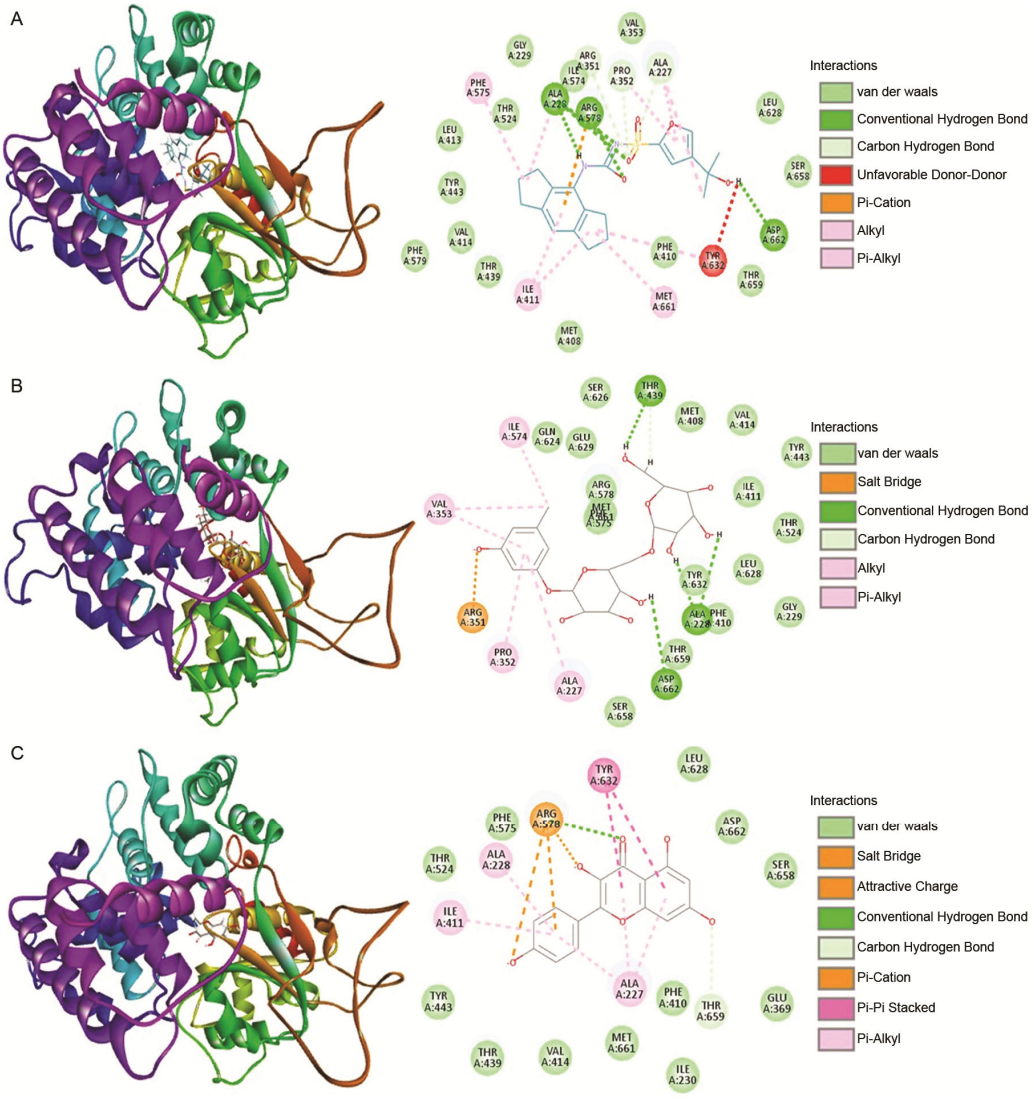

Fig. 1 — 3D and 2D representations of the predicted binding mode of (A) MCC950; (B) Isovitexin; and (C) Kaempferol inside the active site of NACHT domain of NLRP3

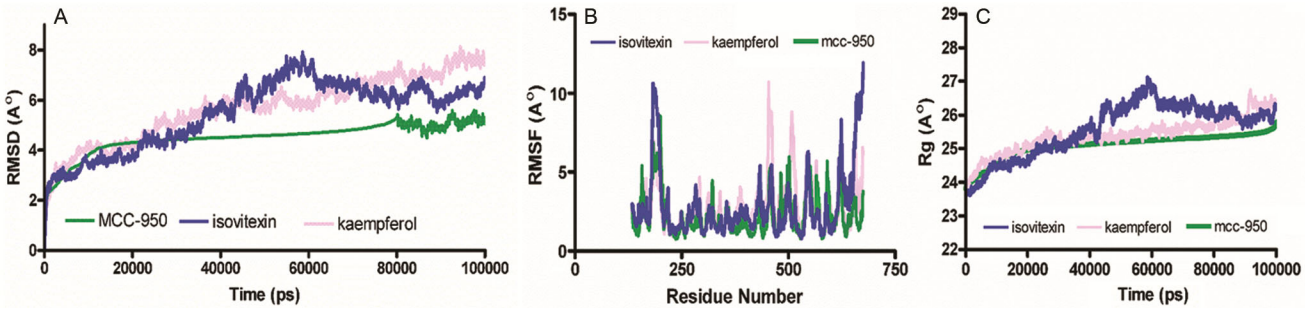

Fig. 2 — Molecular dynamics simulation graph (A) RMSD; (B) RMSF; and (C) Rg of phytochemicals and MCC950

Table 2 ─ Results of MM-PBSA analysis of compounds of millets along with MCC950

Energy Parameters (Kcal/mol)	Kaempferol	Isovitexin	MCC950
Final binding energy ($G_{binding}$)	-26.6370 Kcal/mol	-51.5813 kcal/mol	-12.7006 kcal/mol
Complex Energy ($G_{complex}$)	-25,664.12	-25,654.43423	-25,228
Ligand Energy (G_{Ligand})	-205.890377	-91.20283507	70.6857
Protein Energy ($G_{protein}$)	-25,431.59716	-25,511.64854	-25,275.10

A:632, PHE A:410, THR A:659, SER A:658, ALA A:227, PRO A:352, ARG A: 351. Isovitexin has interacted with all the residues of active site of NACHT domain. Kaempferol (Fig. 1C) showed CDOCKER interaction energy of -61.44 Kcal/mol and formed 2 hydrogen bonds at residues ARG A:578, THR A:659and hydrophobic interactions of ILE A:411, THR A:524, ALA A:228, PHE A:575, TYR A:632, LEU A:628, ASP A:662, SER A:658, GLU A:369, PHE A:410, ILE A:230, MET A:661, VAL A:414, THR A:439, TYR A:443.

Molecular dynamics simulation

RMSD (root-mean square deviation)

RMSD reveals the degree of the positional change of the molecular structure over time. After simulation studies, the graph obtained by calculating RMSD indicates the structural changes in the structure specifically the deviation between several structures can be best interpreted. The calculation of RMSD shows spatial differences between the backbone atoms present in the protein all through the simulation time[37,38]. The average RMSD value (Fig. 2A), observed for the MCC950 was calculated to be 4.5 Å. The trajectory remains almost same throughout the entire simulation period after 10,000 ps without any deviation indicating stable complex between ligand and protein. Isovitexin has showed highest CDOCKER energy interaction and exhibited an average RMSD value of 5.4 Å. RMSD value reached peak around 60,000 ps and started to decrease after that point of time. In the case of Kaempferol the average RMSD value is about 5.6 Å, started to stabilize after 20,000 ps.

RMSF (root mean square fluctuation)

The root mean square fluctuation (RMSF) value represents the mobility and flexibility of a structure[39]. To examine the binding efficiency of phytochemicals and MCC950, the root mean square fluctuation (RMSF) values for C-α atoms of all the residues were measured based on 100 ns trajectory data. Values are analysed for phytochemicals and MCC950. Results of RMSF are represented in the (Fig. 2B). The average RMSF value for MCC950, Isovitexin and Kaempferol are 2.28 Å, 2.83 Å and 2.81Å, respectively. All the compounds have not shown much fluctuations around the residues of active site ARG 578, GLU 629, ALA 227, ALA 228, and ARG 351.

Radius of Gyration (Rg)

The compactness and unbending nature of a molecule can be determined using the Rg value[40-42]. The results observed during the simulation period of 100,000 ps, the average Rg value (Fig. 2C), for MCC950, Isovitexin and Kaempferol are 25.10 Å, 28.73 Å and 25.58 Å, respectively. All the compounds except Isovitexin showed constant value till the entire time period. Rg of Isovitexin fluctuated till 55,000 ps and started to stabilize after that.

MM-PBSA of the complex

The MM-PBSA based binding free energies were calculated for all the generated conformations after the MD simulations (Table 2). The binding free energy of phytochemicals- Isovitexin, Kaempferol and MCC950 are -51.5813 kcal/mol, -26.6370 Kcal/mol

Table 3 —Results of the DFT of compounds of millets along with MCC950

	Isovitexin	Kaempferol	MCC950
Total dft	-1737.46733765	-1089.17542890	-1748.7962705
Dfbinding energy(eV)	-108.97132644	-69.70433259	-101.6525855
E_{homo}(eV)	0.00904479	-0.00509297	-0.2351627
E_{lumo}(eV)	0.1050519	0.09676735	-0.04575703
Band gap (EHOMO -E LUMO) (eV)	0.09600715	0.10186033	0.18940572

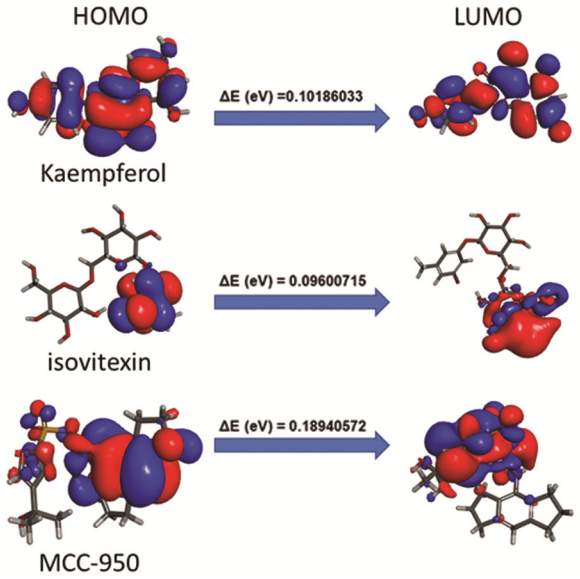

Fig. 3 — 3D plots frontier orbital energies using DFT method for Kaempferol, Isovitexin, and MCC950

and -12.7006 kcal/mol, respectively. Binding energies of Isovitexin and Kaempferol are greater as compared to MCC950, which indicate stable favourable thermodynamic complex formation than MCC950.

Frontier molecular orbital studies

Frontier molecular orbitals (FMOs) are the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO). In general HOMO is electron donor and LUMO is electron acceptor as it has space for electrons[43,44]. There is an inverse relationship between energy gap and reactivity of the molecule, if the energy gap is less the molecule is more reactive. Best-docked polyphenol compound's eV values are represented in the (Table 3). Isovitexin showed a lesser energy gap of 0.09600715eV (Fig. 3) followed by Kaempferol (0.16241782). MCC950 showed energy gap of 0.18940572eV slightly higher than both phytochemicals. Isovitexin, Kaempferol, and MCC950 exhibited total energy values of -1737.46733765, -1089.17542890 and -1748.7962705 kcal/mol, respectively. Isovitexin and MCC950 have higher total energy indicating a higher reactivity

Table 4 — Calculated Mulliken atomic charges of Kaempferol, Isovitexin, and MCC950

Kaempferol	Isovitexin	MCC950
O (1) -0.602007	O (1) -0.472487	S (1) **1.652405**
O (2) **-0.651312**	O (2) -0.498012	O (2) -0.382237
O (3) -0.596517	O (3) -0.494907	O (3) -0.437466
O (4) -0.495645	O (4) -0.589390	O (4) -0.537057
O (5) **-0.641742**	O (5) -0.585808	O (5) -0.654892
O (6) -0.637762	O (6) **-0.594909**	O (6) -0.670765
C (7) -0.020972	O (7) -0.568625	N (7) -0.689843
C (8) **0.334580**	O (8) -0.582495	N (8) **-0.872727**
C (9) 0.171911	O (9) -0.583245	C (9) 0.064705
C (10) 0.289456	O (10) -0.590266	C (10) 0.073979
C (11) 0.235537	O (11) -0.585447	C (11) 0.046725
C (12) 0.068018	O (12) **-0.774080**	C (12) 0.055403
C (13) 0.323965	C (13) 0.129095	C (13) -0.307563
C (14) -0.217704	C (14) 0.068415	C (14) -0.384757
C (15) **0.344183**	C (15) 0.108648	C (15) -0.301159
C (16) -0.222506	C (16) 0.122157	C (16) -0.305349
C (17) -0.120571	C (17) 0.119112	C (17) 0.152701
C (18) -0.059343	C (18) 0.322452	C (18) -0.273674
C (19) -0.130357	C (19) 0.060675	C (19) -0.284138
C (20) -0.173307	C (20) 0.111907	C (20) -0.188626
C (21) 0.314795	C (21) 0.136176	C (21) 0.694452
	C (22) 0.060644	C (22) 0.015720
	C (23) 0.318908	C (23) 0.089169
	C (24) 0.045083	C (24) -0.062906
	C (25) 0.340541	C (25) -0.067302
	C (26) -0.223754	C (26) -0.375768
	C (27) -0.203727	C (27) -0.424409
	C (28) 0.088489	C (28) 0.090669
	C (29) 0.325685	
	C (30) -0.192998	
	C (31) -0.303864	

against the biological target. Mulliken charges were analyzed by DFT. Values are represented in the Table 4; the most negative values and the most positive values for all the best docked molecules are presented in the bold. Positive values indicate electron deficient positions and are susceptible to nucleophilic attack and most negative values are susceptible to electrophilic attack[45].

Conclusion

In this study, molecular docking was performed for phytochemicals of millets against NACHT domain of NLRP3 inflammasome. In comparison to MCC950,

Isovitexin and Kaempferol exhibited better binding affinity in docking studies. Molecular simulations exhibited that these compounds show better stability after binding to the target. MM-PBSA energy calculation revealed phytochemicals have strong binding energy than MCC950. The findings implicate that these phytochemicals can act as drug candidates or adjuvant therapy for treating inflammatory disorders involving NLRP3 inflammasome. Further *in vitro* and *in vivo* studies are warranted to confirm their experimental value.

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Conflict of interest

All authors declare no conflict of interest.

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