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(RESEARCH ARTICLE)

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CADD studies on evaluation of cruciferous vegetable components on anti-oxidant activity via Nrf2 pathway

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Abstract

The current study directed to progress antioxidant activity of cruciferous vegetable components on adaptor proteins and peroxiredoxins. Nrf2 is a transcription factor (basic leucine zipper protein). Under basal conditions it is located in the cytosol and under unfavourable conditions it translocates into the nucleus and aids in transcriptional activation of cytoprotective genes. Nrf2 plays a crucial role in determining the sensitivity of cells to oxidative stress. Some of the receptor proteins such as 7k2f, 7k2j, 7k2m, 7kiz, 7kj0 has undergone In-silico evaluation with chemical constituents, Sulforphane (Standard) and Glucosinolates (ligand) which are abundantly present in Cruciferous vegetables. To evaluate the receptor-ligand interaction between Brassicaceae chemical constituents and receptor proteins in the activation of Nrf2 pathway; Chemical components of Cruciferous vegetables were constructed by Chemsketch (2022 1.2) and they were subjected to molecular docking using Autodock Vina tools with the respective proteins. The ligand have greater binding affinity with the proteins when compared with Sulforphane and that concluded, Glucosinolates has more interaction towards the proteins when compared to Sulforphane. The results clearly revealed that Cruciferous components have enhanced antioxidant activity in the activation of Nrf2 pathway under extreme oxidative stress conditions.

Keywords: Nrf2 pathway; Keap; Reactive oxygen species; Oxidative stress; In-silico; Sulforphane; Glucosinolates

1. Introduction

Oxidative stress is defined as an imbalance between increased levels of ROS and low activity of antioxidant mechanisms. An increased oxidative stress can induce damage to cellular structure and potentially destroy tissues.[1] Recent interest has focused on the intricate ways by which redox signals integrate these converse properties. Redox balance is maintained by prevention, interception, and repair and concomitantly the regulatory potential of molecular thiol-driven master switches such as Nrf2/Keap1 or NF-κB/IκB is used for system-wide oxidative stress response.[2] An antioxidant is a molecule which has the ability to prevent or slow the oxidation of macromolecules. The role of antioxidants is to lower or terminate these chain reactions by removing free radicals or inhibiting other oxidation reactions by being oxidized themselves. So, antioxidants are often reducing agents such as polyphenols or thiols.[3] Keap-1-Nrf2 pathway functions as an important regulator of both physiological and pathophysiological conditions. Oxidative stressors or electrophiles inhibit the ubiquitination-dependent degradation and increase nuclear accumulation of Nrf2. Nrf2 is a master regulator of the antioxidant response, and its functions are tightly regulated at transcriptional, translational, post-translation. [4]

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Natural antioxidants have a variety of biochemical actions such as inhibition of the production of ROS and scavenging of free radicals. Cruciferous vegetables belong to the mustard /Brassicaceae family of plants such as Brussel sprouts, kale, broccoli, cabbage and cauliflower.[5] Brassica plants, as a source of natural bioactive agents, have a great potential application to biological activities, mainly the antimicrobial and antioxidant capacity. Glucosinolates and sulforphane are a large group of plant secondary metabolites with nutritional effects and biologically active compounds.[6]

Sulforaphane (SFN) (1-isothiocyanato-4-methylsulfinylbutane) is a compound within the isothiocyanate group of organosulfur compounds and mainly found in cruciferous vegetables. It is produced when the enzyme myrosinase transforms glucoraphanin, a prodrug or storage form of SFN, into SFN upon damage to the plant (such as from chewing), which allows the two compounds to mix and react. Glucoraphanin is one of a few molecules known as isothiocyanates, existing alongside Sinigrin (metabolized into allylisothiocyanate). Crucially myrosinase is not present in mammalian cells but is found in the bacterial microflora of the gastrointestinal tract. Such conversion can therefore be influenced by external factors that modify gut bacteria. Sulforaphane is perhaps the best known and potent natural product inducer of Nrf2 and hence Phase II cell defense enzymes such as NQO1, HO-1 and GSH among others[7].

Glucosinolates are a large group of plant secondary metabolites with nutritional effects and biologically active compounds. Glucosinolates are mainly found in cruciferous plants such as Brassicaceae family, including common edible plants such as broccoli (Brassica oleracea var. italica), cabbage (B. oleracea var. capitata f. alba), cauliflower (B. oleracea var. botrytis), rapeseed (Brassica napus), mustard (Brassica nigra), and horseradish (Armoracia rusticana). Glucosinolates (GLNs) are made up of three compartments: β-thioglucose, thiohydroximate-O-sulfonate, and a variable aglycone side chain derived from an α-amino acid which then classifies the GLN as either aliphatic, indole, or aromatic. Glucosinolates are special nitrogen and sulfur-containing metabolites. The core structure of GLs is constituted of a β-D-thio-glucose group, a sulfonated aldoxime group, and a variable side chain derived from amino acids.[8]

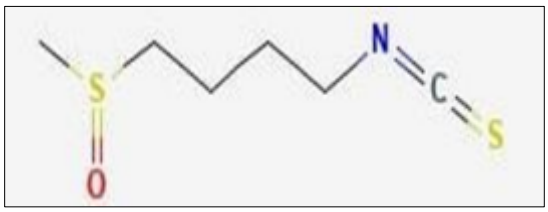


Figure 1 Structure of Sulforphane

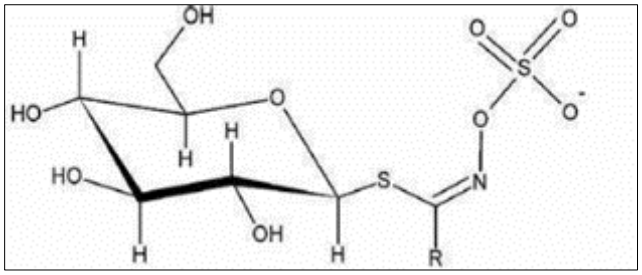


Figure 2 Structure of Glucosinolates

- Molecular Targets: Kelch-like ECH-associated protein 1 (KEAP) and nuclear factor erythroid 2 like 2 (Nrf2), an important drug targets.
- 7K2F, 7K2J, 7K2M: It is a Nrf2 cyclic peptide bound to Kelch domain of human KEAP1. Keap1 is a substrate adaptor protein for an ubiqitin ligase complex that targets the Nrf2 transcription factor for degradation. It binds Nrf2 through C-terminal Kelch domain.[9]
- 7KIZ: It is a peroxiredoxin 2 (Prdx2), thiol peroxidase with an active site Cys (C52). In addition to their antioxidant function, the eukaryotic peroxiredoxins (Prxs) facilitate peroxide-mediated signaling by undergoing controlled inactivation by peroxide-driven over-oxidation.
- 7KJ0: It is a hyperoxidized human peroxiredoxin 2 and has activity same as the above-mentioned target.[10]

In this study, our aim is to evaluate in-silico antioxidant potential of cruciferous vegetable components on Nrf2 pathway. By using Autodock Vina tools, molecular docking of adaptor proteins and peroxiredoxins with the constituent, predicted higher biological affinity than Sulforphane. These models can assist through virtual screening techniques.

2. Materials and Methods

The computational approaches were found to be the most reliable methods to start a research methodology. Hence, several computational tools were identified which may rely on current research work for efficient strategies to develop novel compounds. The designed derivatives were studied using web tools to understand their physicochenical properties, biological activities and also any toxicological effects.

Table 1 Different softwares required for In-silico study

S.no	Materials	Methodology
1	Chemsketch	Generation of ligand structures
2	Molinspiration	Molecular property prediction
3	Swiss ADME	Prediction of pharmacokinetic properties
4	GUSAR	Toxicological studies
5	Autodock vina 1.5.7	Molecular docking
6	Biovia Discovery studio 2021	Visualization of interactions

2.1. Methodology

Chemsketch: It is a software program specifically designed for drawing chemical structures. It is a valuable tool used in various scientific fields, particularly chemistry and allied fields.

2.1.1. Molinspiration

Molinspiration is a web tool that supports molecular management and processing, as well as SMILES and SDfile translation, standardization of molecules. Creation of tautomer, molecular fragmentation, calculation of numerous molecular properties desirable in QSAR studies, molecular modelling and novel drug design, higher quality molecule depiction, molecular database tools, auxiliary substructure and resemblance searches. Similarly, provisions fragment based virtual screening, bioactivity screening and visualization. Molinspiration web tools are used for calculation of significant molecular properties (polar surface area, log P, number of hydrogen bond acceptors and donors and others), as well as estimation of bioactivity score for the utmost significant drug targets (GPCR ligands, ion channel modulators, kinase inhibitors, nuclear receptors). The physicochemical properties predicted using this web tool.[11]

2.1.2. SwissADME

swissADME allows us to calculate physicochemical properties and to forecast ADMET properties, pharmacokinetic parameters, drug likeliness and medicinal chemistry approachability of one or several small molecules to support novel drug discovery. The computer-based studies consist of not only lead generation, hit optimization, binding energy calculation, interaction design determination of the minor molecules to the enzyme pouch, and dynamic recreation studies but then again also the estimation of physicochemical properties and pharmacokinetic properties of the trivial new molecules. Numerous tools can be hired for the same purpose, amongst which swissADME is popular web tool due to its free access to all. Thus, obtained pharmacokinetic properties identification utilizing the swissADME.[12]

- Gusar: This software was developed to create QSAR/QSPR models based on the appropriate training sets represented as SD file contained data about chemical structures and endpoint in quantitative terms has been developed according to OECD principles and includes last achievements in the QSAR modelling.[13]

2.1.3. Molecular Docking Studies

Computational methods are identical obligatory and beneficial resources in the course of drug development. With the initiation of computational tools, scheming, searching, assessment, modelling, binding energy calculation, pharmacokinetic properties and pharmacokinetic predictions, and the procedure of lead optimization turn out to be significantly easier. Computational molecular docking is a significant web tool in structural biology and computer aided drug design. The main goal of ligand target protein docking is to forecast the principal binding model of a ligand molecule with a target protein of known three-dimensional molecular structure. Efficacious molecular docking methods

are used to search high-dimensional spaces excellently besides utilising a scoring function that will appropriately rank candidate dockings.[14]

Preparation of protein: In this step the structure of the protein is downloaded from the open source protein database. The water molecules are deleted, the bounded ligands are removed using Autodock vina software, the polar hydrogens were added and Kollman, Gasteiger charges were introduced, the structures were converted to the .pdbqt format and saved.

Preparation of ligand: The 2-D structures of the ligands were drawn, and it was converted into 3-D structures in Chemsketch which is suitable for understanding of ligand-protein interaction and saved in .pdb format. To perform the docking studies the .pdb is converted into .pdbqt format by Auto dock vina software.[15]

2.1.4. Performing docking

Molecular docking was performed with AutoDock 1.4.7. preparation of ligand and the target protein was done by using Auto Dock vina tools. Molecular dockings studies were performed to find the active binding site and their interaction with ligand molecules. Both ligand and macromolecule were selected and the rigid grid box was attained using Auto-grid, i.e., blind docking studies were performed as the active binding site for the newly synthesized compound was not known. The grid box dimensions were then documented in the config file as text document and saved in separate file for each target and each ligand. To predict the binding scores of these ligand – target complex, command prompt was utilized. The desired syntax for the prediction and path for the results were given. Thus, obtained scores were obtained as the output file in the given file. The docked output files were used to study interactions. The pose with best binding affinity was visualized using biovia discovery studio.[16]

2.1.5. Visualization

Ligand target Molecular complex visualization is a significant aspect of the investigation and communication of modeling studies. It permits a mechanistic understanding of a molecular structure to be visualized. BIOVIA Discovery Studio Visualizer is a free web tool, feature-rich modeling application for observing, allocation and analyzing protein and other small molecular data. The output files obtained for every ligand against each target were utilized separately in Biovia discovery studio where the best scoring output amongst 9 conformers were visualized for their interactions of ligand molecule with amino acids of the target, visualizing the active site and 2D interactions to know which atom is bonding with which amino acid of target molecule. Thus, visualized complexes were saved as image files.[17]

3. Results

3.1. ADMET properties of antioxidant compounds

Using Molinspiration we can get to know whether the compound can be used as a drug or not.

Table 2 ADMET properties of ligands

S.NO	FORMULA	Mol.wt	NHD	NHA	NRB	Log P	VIOLATIONS
1	C6H11NOS2	177.29	0	2	5	2.11	0
2	C10H17NO9S2	359.37	5	10	7	0.42	0

3.2. Pharmacokinetic properties using swiss ADME

Through this we get to know the route of administration of the drug 'Log Kp' -skin permeability measures the capacity of the compound to penetrate skin. If log kp value exceeds -2.5cm (about 0.98 in/s) it indicates low skin permeability. BBB permeability gives us an idea if the drug can cross the BBB or not. Low GI absorption indicates the drug cannot be given through oral route.

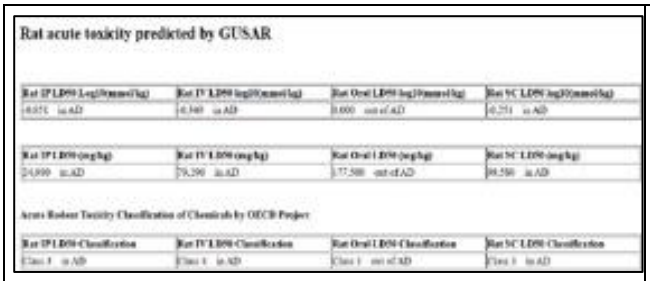
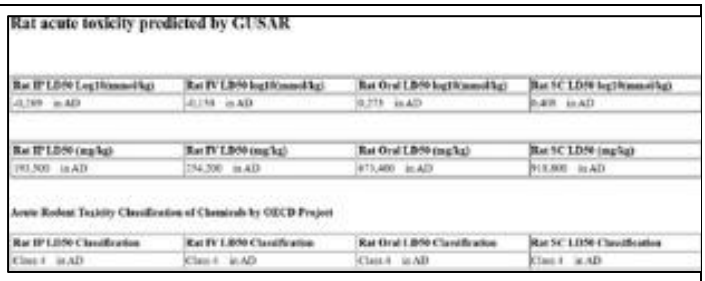
Table 3 Pharmacokinetic properties using swiss ADME

S.NO	FORMULA	logKp cm/ s	Gi abs	BBB Permeabili ty	INHIBITORY interactions					
					P-gp substra te	CY P 1A2	CYP2C19	CYP2C 9	CYP2D 6	CYP3A 4
1	C6H11NOS2	-6.38	High	No	No	No	No	No	No	No
2	C10H17NO9S2	-9.25	Low	No	Yes	No	No	No	No	No

3.3. Rat acute toxicity predicted by GUSAR

GUSAR measures the dose of drug. It determines the lethal dose of the drug through LD50 value. LD50 is the amount of drug given at once to cause the death of half of the subject population. It ensures the safety and prevent toxicity caused by the drug.

Table 4 Acute toxicity test of ligands predicted by GUSAR

	
Rat acute toxicity test of Sulforphane by GUSAR	Rat acute toxicity test of Glucosinolates by GUSAR

3.4. Docking studies

With ligand SULFORPHANE – BINDING AFFINITY (kcal/mol)

Table 5 Molecular docking results of all compounds with standard Sulforphane

MODE	7k2f	7k2j	7k2m	7kiz	7kj0
1	-4.3	-3.8	-3.4	-3.9	-3.7
2	-4.1	-3.7	-3.0	-3.6	-3.5
3	-4.0	-3.3	-2.9	-3.4	-3.3
4	-4.0	-3.2	-2.9	-3.0	-3.2
5	-3.5	-3.2	-2.8	-3.0	-3.2
6	-3.3	-3.1	-2.7	-2.9	-3.1
7	-3.3	-3.0	-2.7	-2.9	-2.9
8	-3.0	-3.0	-2.7	-2.9	-2.9
9	-2.9	-2.8	-2.7	-2.9	-2.8

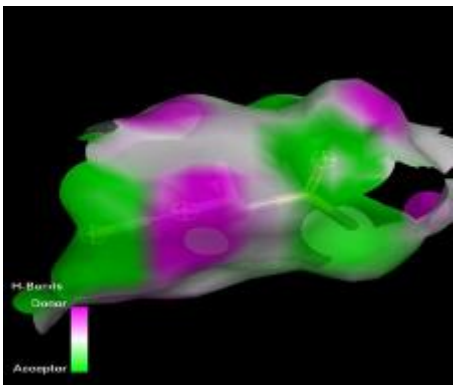
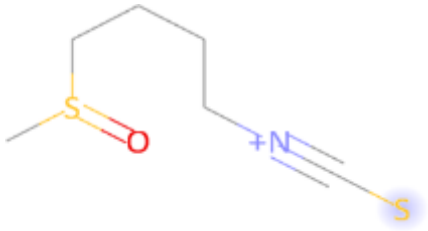
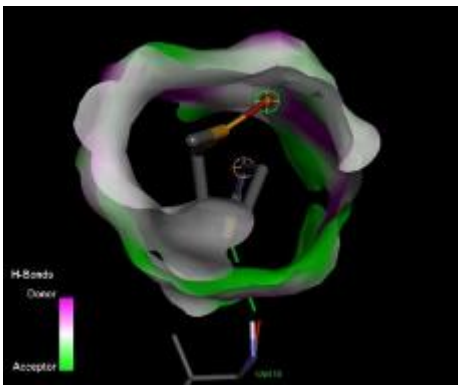
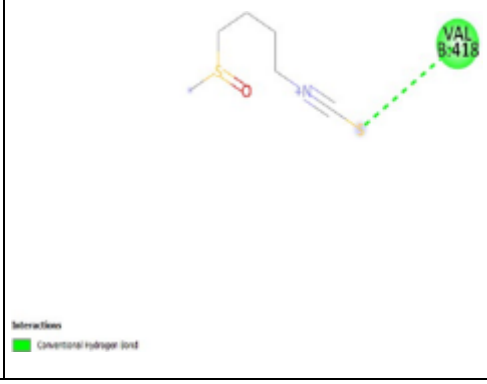
3.5. Biovia visualization

Table 6 Molecular docking results of all compounds with ligand Glucosinolates

MODE	7k2f	7k2j	7k2m	7kiz	7kj0
1	-7.2	-6.9	-6.6	-6.4	-6.5
2	-6.6	-6.8	-6.4	-6.2	-6.4
3	-6.6	-6.6	-5.9	-6.1	-6.2
4	-6.5	-6.5	-5.7	-6.0	-5.8
5	-5.4	-6.4	-5.7	-5.9	-5.8
6	-5.4	-6.4	-5.7	-5.8	-5.7
7	-5.1	-6.2	-5.5	-5.7	-5.7
8	-4.9	-6.2	-5.5	-5.7	-5.7
9	-4.7	-6.0	-4.9	-5.5	-5.6

Highest scoring compounds are visualized for their interaction with the proteins in 2D and 3D images. The results are shown in the table below :

Table 7 Interaction of Sulforphane with respective receptors 7k2f, 7k2j, 7k2m, 7kiz, 7kj0

		
		VAL B:418 – CONVENTIONAL HYDROGEN BOND

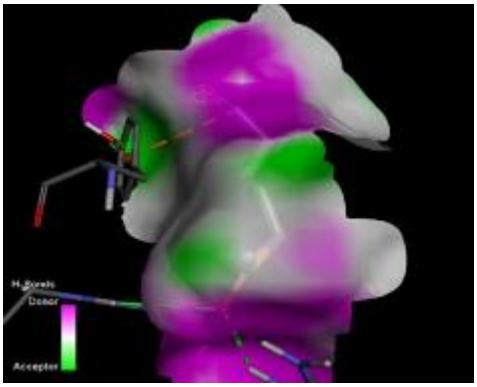
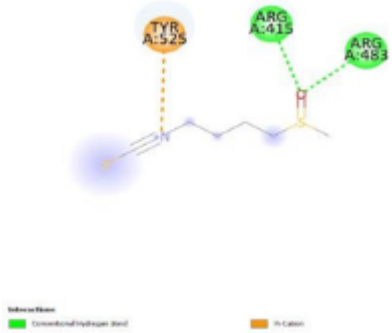
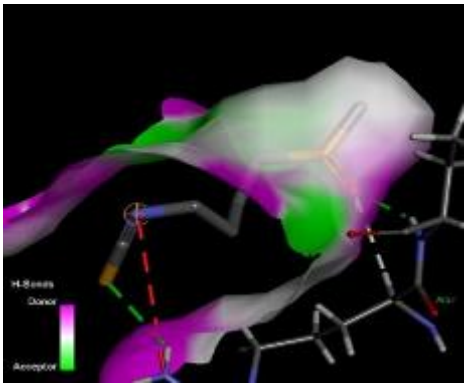
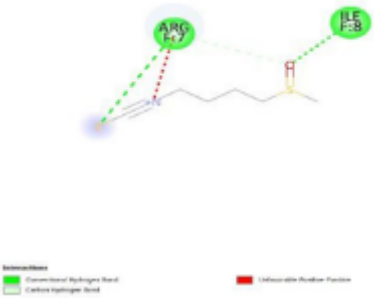
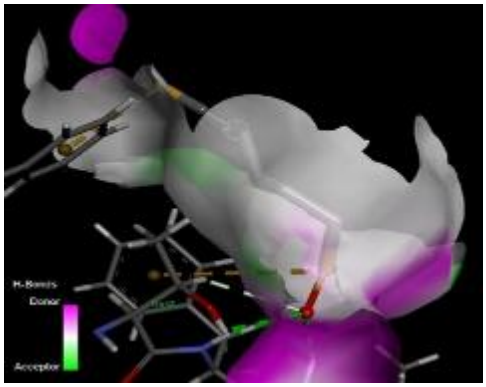
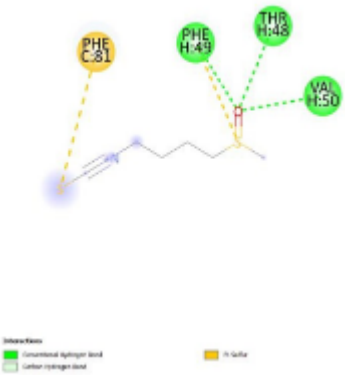
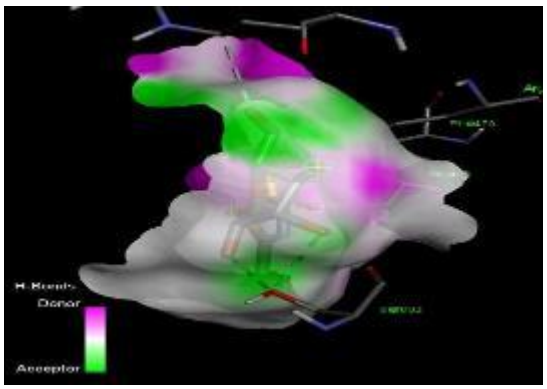
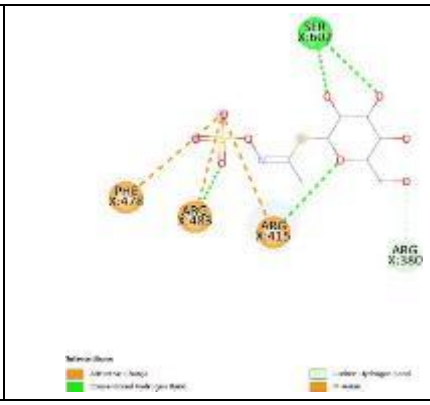
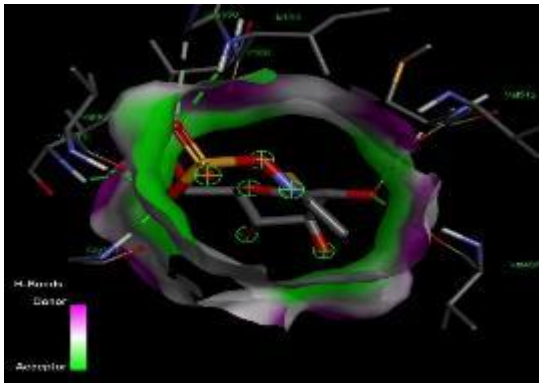
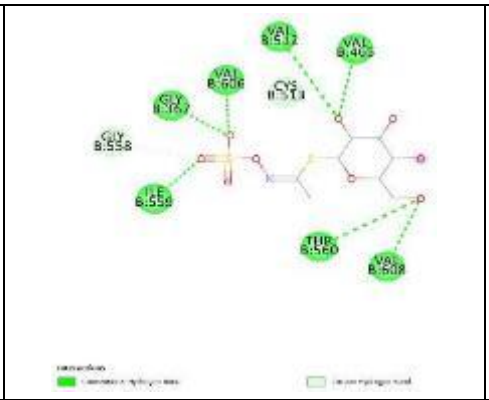
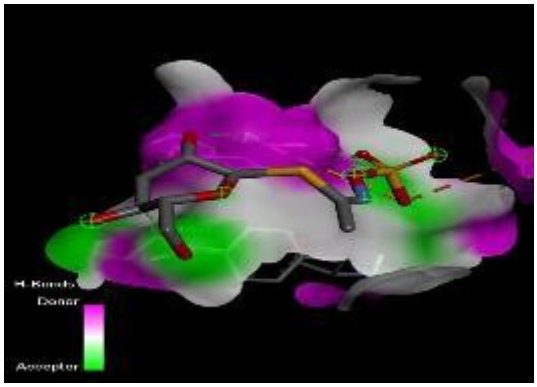
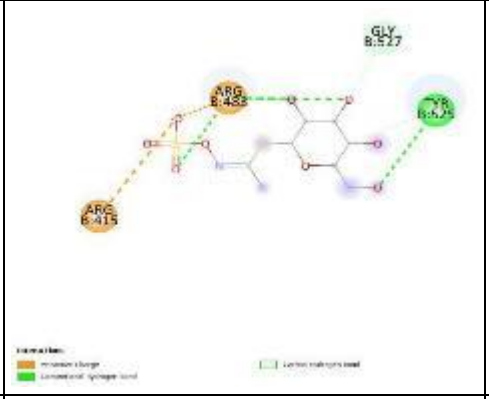
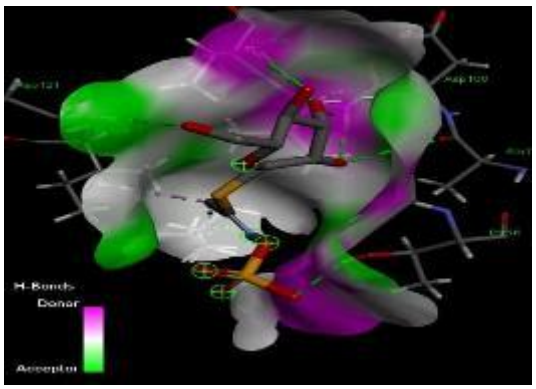
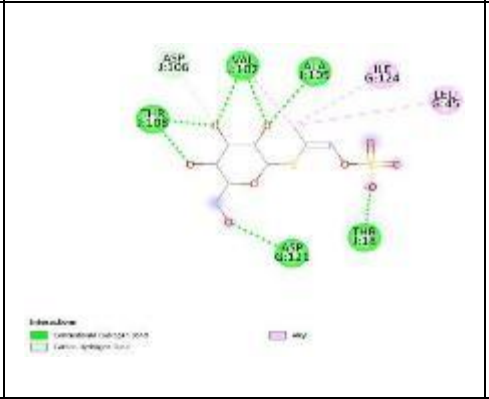
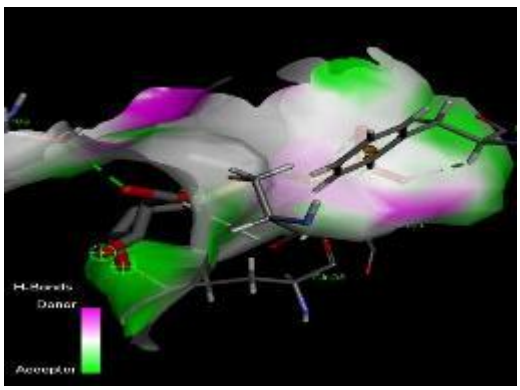
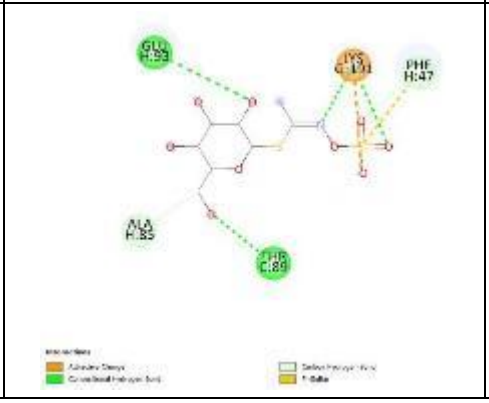
		ARG A:415, ARG A:483 – CONVENTIONAL HYDROGEN BOND TYR A:525 – Pi-Cation
		ARG F:7 – CONVENTIONAL HYDROGEN BOND ARG F:7 – CARBON HYDROGEN BOND ILE F:8 – CONVENTIONAL HYDROGEN BOND +N – UNFAVOURABLE POSITIVE-POSITIVE
		PHE H:49 – CONVENTIONAL HYDROGEN BOND, Pi-Sulfur THR H:48 – CONVENTIONAL HYDROGEN BOND VAL H:50 – CONVENTIONAL HYDROGEN BOND PHE C:81 – Pi-Sulfur

Table 8 Interaction of Glucosinolates with respective receptors 7k2f, 7k2j, 7k2m, 7kiz, 7kj0

		SER X : 602 – CONVENTIONAL HYDROGEN BOND ARG X : 380 – CARBON HYDROGEN BOND PHE X : 478, ARG X : 483, ARG X : 415 – ATTRACTIVE CHARGE

		VAL B : 606, VAL B : 512, VAL B : 465, VAL B : 608, THR B : 560, ILE B : 559, GLY B : 367 – CONVENTIONAL HYDROGEN BOND GLY B : 558, CYS B : 513 – CARBON HYDROGEN BOND
		TYR B : 525 – CONVENTIONAL HYDROGEN BOND, GLY B : 527 – CARBON HYDROGEN BOND, ARG B : 483, ARG B : 415 – ATTRACTIVE CHARGE
		THR J : 108, VAL J : 107, ALA J : 105, THR J : 18, ASP G : 121 – CONVETIONAL HYDROGEN BOND, ASP J : 106 – CARBON HYDROGEN BOND, ILE G : 124, LEU G : 45 – ALKYL BOND FORMATION
		GLU H : 93, THR C : 89 – CONVENTIONAL HYDROGEN BOND, ALA H : 85, PHE H : 47 – CARBON HYDROGEN BOND, LYS G : 191 – ATTRACTIVE CHARGE

4. Discussion

The objective of this investigational study is to evaluate the antioxidant potential of Cruciferous vegetable components i.e.Glucosinolates on Nrf2 pathway. Antioxidant activity denotes the ability of a bioactive compound to maintain cell structure and function by effectively clearing free radicals, inhibiting lipid peroxidation reactions, and preventing other oxidative damage. Oxidation reaction depending upon site of occurrences presents specific repercussions. When oxidation occurs in biological cell system, it causes damage or death to the cell. These antioxidants in cruciferous

vegetables have the highest singlet oxygen quenching properties which are thought to protect against oxidative stress and prevent osteoporosis. The receptor-ligand interaction evaluated by computer simulations gave evidence for greater potential activity of cruciferous chemical constituents against extreme oxidative stress. The receptor proteins exhibited higher binding affinities with Glucosinolates when compared with standard ligand Sulforphane. Chemical constituents have shown more binding residues with the receptors than the standard ligand. The in-silico evaluation of cruciferous vegetable components exhibited antioxidant potential against extreme oxidative stress via Nrf2 defense system.

5. Conclusion

In the present investigation molecular docking findings gave the impression of antioxidant potential of Sulforphane and Glucosinolates as they activate the process of Nrf2pathway under extreme oxidative stress conditions.

References

[1] Hang Li, Yu Xia, Hong-Yan Liu, Huan Guo, Xiao-Qin He, Yi Liu, Ding-Tao Wu, Ying-Hui Mai, Hua- Bin Li, Liang Zou, Ren-You Gan, Nutritional values, beneficial effects, and food applications of broccoli (Brassica oleracea var. italica Plenck), Volume 119, Elsevier, 2022.

[2] Ying Huang, Wenji Li, Zheng-yuan Su, Ah-Ng Tony Kong, The complexity of the Nrf2 pathway: beyond the antioxidant response, Volume 26, Elsevier, 2015.

[3] V. Lobo, A. Patil, A. Phatak, N. Chandra – Free radicals, antioxidants and functional foods: Impact on human health Volume 4, Pharmacognosy reviews, 2010.

[4] Qiang Ma – Role of Nrf2 in oxidative Stress and Toxicity, Annual Review Pharmacology Toxicology 2013.

[5] Kenia Mirozlava Favela, Ayerim Yedid Hernandez, Norma Margarita – The value of bioactive compounds of cruciferous vegetables (Brassica) as antimicrobials and antioxidants: A review, Journal of Food Biochemistry, 2020.

[6] Duygu Agagunduz, Teslime Ozge Sahin, Birsen Yilmaz, Kubra Damla, Sehriban Duyar, Raffaele Capasso – Cruciferous Vegetables and Their Bioactive Metabolites: from Prevention to Novel Therapies of Colorectal Cancer, Evidence-based Complementary and Alternative medicine, Volume 2022.

[7] Jae Kwang Kim, Sang Un Park – Current potential health benefits of Sulforphane, EXCIL Journal 2016.

[8] M A Prieto, Cecilia Jimenez, Jesus Simal – Glucosinolates: Molecular structure, breakdown, genetic, bioavailability, properties and healthy and adverse effects, Advances in Food and Nutrition Research, Volume 90, 2019.

[9] https://doi.org/10.2210/pdb7K2F/pdb

[10] https://doi.org/10.2210/pdb7KIZ/pdb

[11] Bilal Shaker, Sajjad Ahmad, Jinyu Lee, Chanjin Jung, Dokyun Na - In silico methods and tools for drug discovery, Computers in biology and Medicine, Volume 137, 2021.

[12] ADME Toxicity - Qik Prop, Vol Surf, Gastro Plus, ALOGPS, Swiss ADME

[13] www.way2drug.com/gusar

[14] Estari Mamidala, "Molecular Docking studies in drug discovery" First edition 2019

[15] Osman Ghani – Chemsketch; Jul 2019

[16] Vikram Aditya – Molecular Docking using Autodock Tools; 2020

[17] www.3ds.com/products/biovia/discovery-studio/visualization