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Studies on NF-κB Docking with Common Bioactive Compounds in *Punica granatum* peel and *Vitis vinifera* Seeds

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KEYWORDS

Punica granatum

Vitis vinifera

NFkB

Guanosine

Pyrogallol

Palmitic acid

ABSTRACT

Plant-based products have long been utilized as traditional remedies throughout the world. Higher plants serve as a "reservoir" of phytochemicals known as bioactive compounds, which are used as valuable medicines to fight a variety of diseases across the world. The materials that are considered waste in plants possess bioactive components with potential medicinal properties due to the presence of important secondary metabolites known as phytochemicals. In this study, the interaction of phytochemicals that are present in both *Punica granatum* peel and *Viti vinifera* seeds was analyzed on protein NF-κB. Compounds 2,3- dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP), α-tocopherol-β-D-mannoside, gamma-sitosterol, glycerine, guanidine, pyrogallol, palmitic acid, and ethyl palmitate were the eight phytoconstituents which are present in both the selected plant materials and further investigated for *in-silico* analysis. The 3D protein structure of NF-κB was retrieved from the protein data bank. The structures of bioactive compounds were obtained from Chemspider and drawn using Chemsketch software. This study clearly shows that α-tocopherol-β.-D-mannoside interacts with target protein NF-κB with an energy level of -10.88 kcal/mol (2 hydrogen bonds). The interaction of α-tocopherol-β-D-mannoside with NF-κB may play a major role in anti-oxidant and anti-cancer potential and provide chemopreventive property for both *P. granatum* peel and *V. vinifera* seeds.

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1 Introduction

Plants have been used for therapeutic purposes long before the early civilization, and these are the basis of much contemporary medicine. Many traditional medications are derived from plants. The fruit of the pomegranate (*P. granatum*) is a rich source of bioactive chemicals with numerous medicinal values. Pomegranate extracts have also been shown to lower blood fat levels and have potent anticancerous, antiviral, and anti-inflammatory properties (Li et al. 2006; Hossin 2009; Lin et al. 2013; Bassiri-Jahromi 2018). Pomegranate polyphenols may enhance the effectiveness of cancer therapy by protecting normal cells from cancer-related toxicity (Mukherjee et al. 2021). It is also utilized in various herbal therapies to treat ailments, including flu and upper respiratory infections. Further, fruits of pomegranate are considered a dietary medicine due to its high nutritional content, health advantages, and antioxidant components. The peels of pomegranate fruits are one of the most common by-products of pomegranate food processing. All the waste components of the pomegranate fruit, such as the peel and seeds, may be processed into value-added products with industrial, medical, and cosmetic applications (Dhumal et al. 2014).

Grapes (*V. vinifera*) are also one of the world's most valuable traditional fruits (Zhu et al. 2015). Many kinds of research have shown that grapes may be associated with illness prevention and health promotion activities, which has piqued people's curiosity in recent years. Grape seeds are becoming more popular as a source of functional food components, including natural antioxidants and nutritional supplements (Girard and Mazza 1998; Ferrer-Gallego et al. 2010). The effects of *V. vinifera* stem extracts on cancer cells resulted in a decrease in cancer cell growth, death by apoptosis, and a decrease in the antioxidant enzyme TrxR1, which increases cellular levels of ROS capable of inhibiting NF-kB binding to the nucleus and causing proteasome upregulation (Quero et al. 2021).

Nuclear factor-B (NF-κB) is a family of inducible transcription factors that controls several genes involved in immunological and inflammatory response pathways (Oeckinghaus and Ghosh 2009). Akt/PKB stimulates the NF-κB survival pathway by phosphorylation of IκB kinase α (IKK α), and it suppresses p53 pro-apoptotic signaling by phosphorylation of the oncogene *mdm2* and activated the inhibition of p53 (Mayo and Donner 2002). In the transformation process, both NF-κB and mdm2 are activated inappropriately or over-expressed (Orlowski and Baldwin 2002; Chene 2003). The signaling pathways are less controlled during carcinogenesis (Zhang et al. 2017; Sun et al. 2017). In cancer, abnormal regulation of cell-signal-transduction pathways plays a crucial role and obstruction or anomalies in signaling pathways which can lead to excessive cell proliferation, angiogenesis, apoptotic resistance, invasion, and metastasis all of which can lead to cancer development and progression (Chen et al. 2006).

NF-κB is a crucial signaling pathway involved in the genesis and development of cancer. The NF-κB pathway is involved in both inflammatory and programmed cell death processes, and, predictably, it is also involved in necroptosis (Verzella et al. 2020). NFκB regulates tumor cell proliferation, survival, and angiogenesis by modulating the expression of target genes such as TNFA, IL6, BCLXL, BCL2, BCLXS, XIAP, and VEGF (Baud and Karin 2009). NF-kB can be activated by cytokines (TNF-, IL-1), growth factors, bacterial and viral products (lipopolysaccharide (LPS), dsRNA, UV, and ionizing radiation, reactive oxygen species (ROS), DNA damage, and oncogenic stress from inside the cells. Exploring the naturopathic formulations may play an important role in identifying new NF-κB inhibitors considering the multi-target effects of phytochemicals, on various components of the NF-κB transduction pathway (Chauhan et al. 2021).

The advent of immune-informatics has made it feasible to design novel compounds *"in-silico"* and predict their functionality. This technique aids in the selection of better compounds for *in-vitro* or *in-vivo* testing. Using an immune-informatics approach, many research groups have created a variety of *in-silico* drugs (Kaliamurthi et al. 2018; Kar and Srivastava 2018).

Inflammation, cancer, and other human disorders are linked to the hyperactivation of NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) in the cellular system (Cheemanapalli et al. 2019) but, the *in-silico* docking studies of NF-κB and its interaction with bioactive compounds are yet to be studied in detail. Therefore, the purpose of this study was to analyze the interaction of NF-κB protein against bioactive compounds present in *P. granatum* peel and *V. vinifera* seeds using molecular docking. This study may pave the way to find whether any anti-cancer, anti-oxidant, and anti-inflammatory agents present in selected plant materials interacts with the NF-κB protein and play a role in its regulation. The inhibition of NF-κB may provide insights for future treatment strategies

2 Materials and Methods

2.1 Database

2.1.1 Protein Data Bank (Pdb)

The 3D structure of the target protein NF-κB of Homo sapiens was elucidated by X-ray diffraction at a resolution of 2.60Å (1SVC) (Space fill Model - Figure 1) (https://www.rcsb.org/structure/1SVC) a repository for the 3-D structural data of proteins and nucleic acids. The NF-κB molecule possesses two chains (Chain D and Chain P), with Chain D containing 76 polydeoxyribonucleotides and Chain P comprising 365 amino acids.

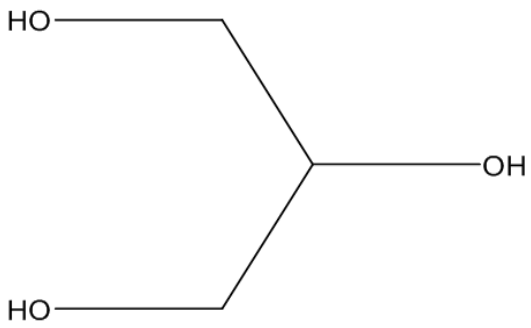

Figure 1 Structure of glycerine

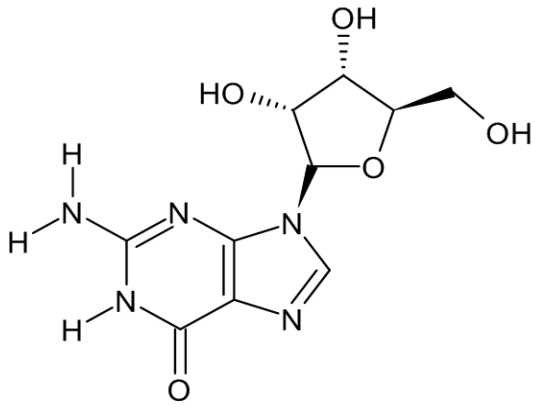

Figure 2 Structure of guanosine

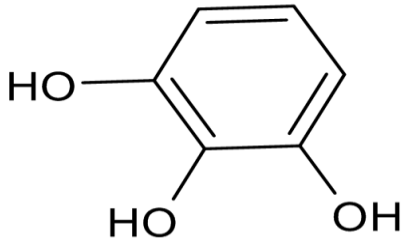

Figure 3 Structure of pyrogallol

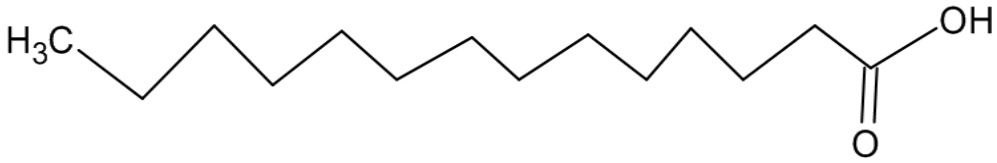

Figure 4 Structure of palmitic acid

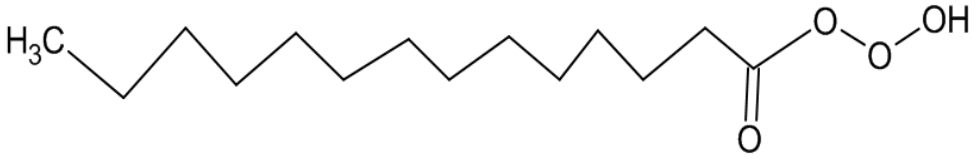

Figure 5 Structure of ethyl palmitate

2.1.2 Chemspider

The structures of bioactive compounds 2,3- dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one, α-tocopherol- β.-D-mannoside, and gamma-Sitosterol used for this study were obtained from ChemSpider. (http://www.chemspider.com/).

2.2 Tools Used for the Studies

2.2.1 Chemsketch

Chemsketch is a web-based drawing tool used in this study to design the structures for the five chemicals found in both the selected extracts: glycerine (Figure 1), guanosine (Figure 2), pyrogallol (Figure 3), palmitic acid (Figure 4), and ethyl palmitate (Figure 5). The structure of the selected ligands is provided in the following diagrams.

2.2.2 Argus Lab

Argus Lab is a molecular modeling, graphics, and drug design application that is available online and provides a rapid and reliable way of binding site optimization, which implies that the program can automatically discover binding sites and accelerate the docking process (Tangyuenyongwatana and Jongkon 2016). Many researchers utilize ArgusLab to execute their molecular

docking since it eliminates the requirement for blind docking, which takes a lot of time to calculate and often results in an erroneous binding site (Naz et al. 2009; Oda and Takahashi 2009). In this study, Argus Lab 4.0 is used for docking of the selected target protein NF-kB with eight selected ligands.

2.2.3 Pymol

The interaction between selected ligands and the target protein is visualized using PyMOL2.1 (Yuan et al. 2017), an open-source molecular visualization toolkit. PyMOL is used to visualize the docking in this study. PyMOL display Protein Residue Networks, results of numerous analyses, indicating the size and location of the binding site which may be modified interactively (Seeliger and de Groot 2010; Sladek et al. 2021).

2.3 Molecular Docking

The 3D structure of the target (p53), was obtained from PDB. Ligand structure was determined using Chemspider or Chemsketch. The interactions of target proteins with various ligands were analyzed using Argus Lab. Pymol 2.1 (2018) software was used to conduct the docking investigation and to predict the docking of a specific ligand with target proteins. The interactions with the lowest energy and the number of atoms involved in docking the ligand with a target protein are criteria to select the best docking. The docking technique involves the extrapolation of ligand/inhibitor conformation and orientation inside a specific binding site or active site. The promising posture with greater binding energy, ligand efficiency, and intermolecular Hydrogen-bonds was retained for extensive intermolecular interaction study based on docking simulations. Hydrogen bonds are required for molecular recognition as well as a protein's and its complex's overall stability. The protein-inhibitor complex system's intermolecular hydrogen bonds were investigated (Mukund et al. 2019).

3 Results

The eight compounds commonly present in both the fruit materials were selected for docking studies. The results of docking are displayed in Table 1, showing the details of the interaction between eight selected ligands from the two plant materials and the target protein NF-κB. (Figure 6)

The hydrogen bond formation between the amino acid residues of the target protein and the atoms of ligands selected for this study are tabulated in table 1. The table also provides details on the number of hydrogen bonds formed and the hydrogen bond distance between the target protein and the ligands. The energy value for the formation of the hydrogen bond is provided in table 1 which is a criterion to assess the better docking ligand with the target proteins. The interaction of the ligands with the target proteins visualized through Pymol was presented in Figures 7 – 11. In this *in-silico* study, the following ligands pyrogallol, guanosine, α-tocopherol-beta.-D-mannoside, 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one, and glycerin interaction with NF-κB via hydrogen bonds. Other ligands selected for the docking didn't show any interaction with NF-κB. Out of the above five ligands showing interaction, α -tocopherol-beta-D-mannoside forms interaction with an energy value of -10.88 kcal/mol with two hydrogen bonds, and pyrogallol forms two hydrogen bonds with an energy value of-7.07 kcal/mol.

Table 1 Results of NF-κB docking with the selected ligands

S.No	Ligand name	Protein residue atom	Ligand atom	Hydrogen bond distance (^{0}A)	No. of Hydrogen bonds	Energy value (Kcal/ mol)
1	alpha.-tocopherol-beta.-D-mannoside	– H – N Gly 294 B – H – N Gly 294 B	O O	2.91 2.97	2	-10.88
2	Pyrogallol	Gly 296 N – H – Gly 296 O-H	O H	2.6 2.6	2	-7.07
3	Guanosine	Thr 313 OG1 – H Thr 313 OG1 – H Thr 313 OG1 – H Thr 316 N – H Thr 316 N – H	H N O O O	2.8 3.0 2.9 3.0 2.8	5	-6.40
4	2,3- dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one	Arg 164 N – H Tyr 166 O – H Lys 95 N – H Thr 125 OG1 – H	O O O O	2.76 2.22 2.80 2.52	4	-6.2
5	Glycerin	Thr 313 O – H	H	1.8	1	-5.65
6	gamma.-Sitosterol	No Interaction	No Interaction	Nil	Nil	-10.73
7	Palmitic acid	No Interaction	No Interaction	Nil	Nil	-8.56
8	Ethyl palmitate	No Interaction	No Interaction	Nil	Nil	-7.96

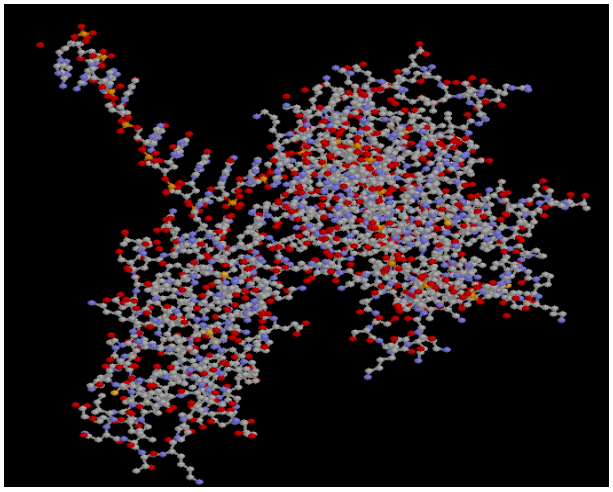

Figure 6 - 3D structure of the NF-κB protein (1SVC) Homo sapiens (2.60Å)

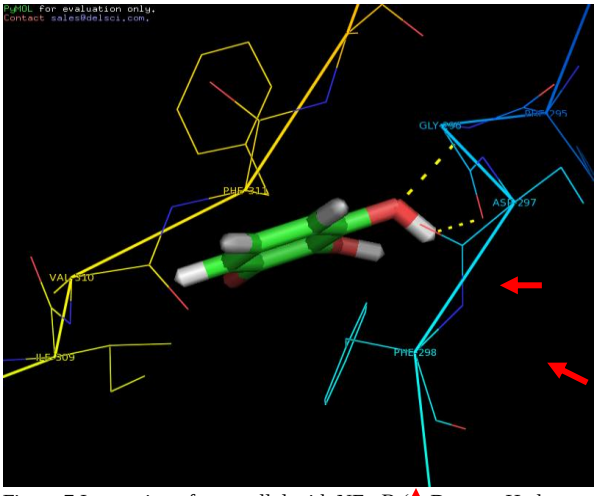

Figure 7 Interaction of pyrogallol with NF-κB (▲ Denotes Hydrogen bond)

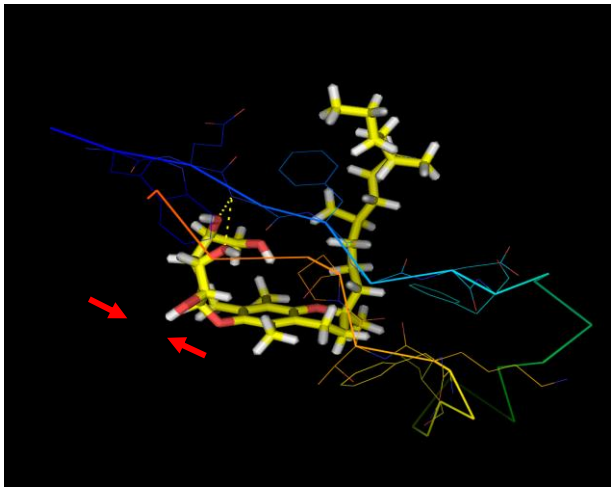

Figure 8 Interaction of α-tocopherol-beta.-D-mannoside with NF-κB (▲ Denotes Hydrogen bond)

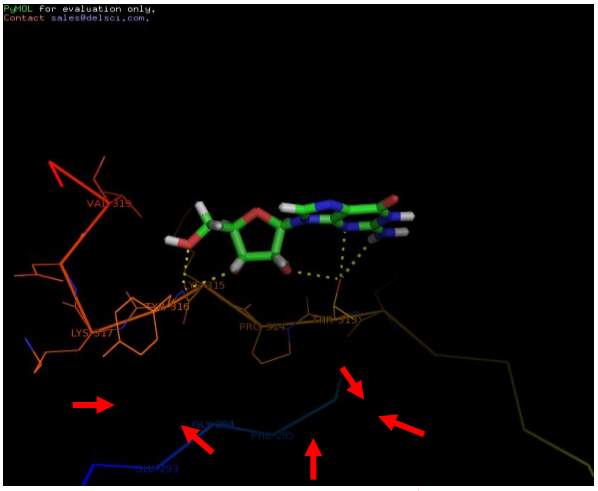

Figure 9 Interaction of guanosine with NF-κB (▲ Denotes Hydrogen bond)

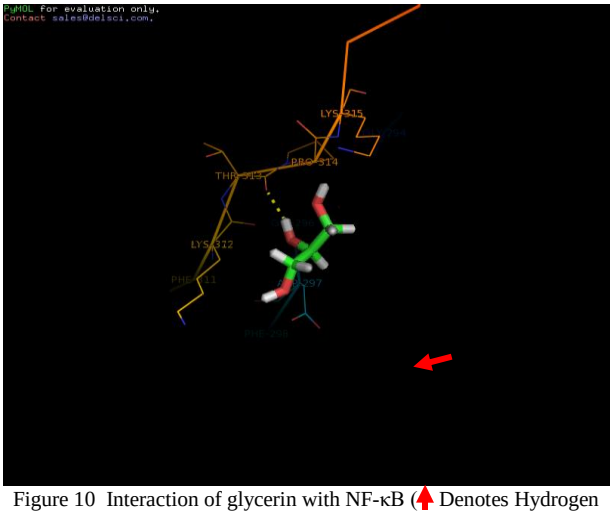

Figure 10 Interaction of glycerin with NF-κB (▲ Denotes Hydrogen bond)

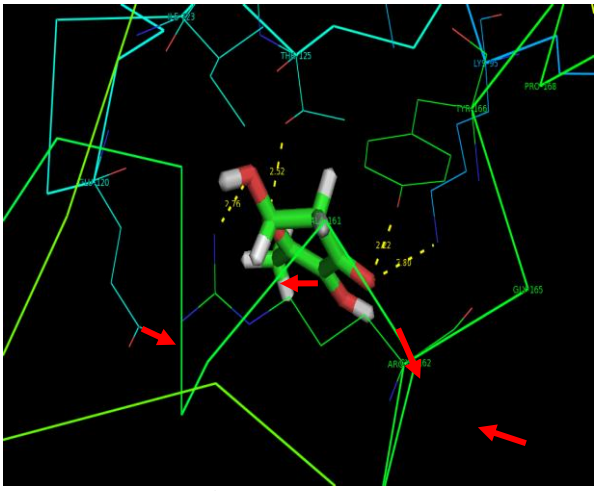

Figure 11 Interaction of 2, 3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one with NF-κB (▲ Denotes Hydrogen bond)

The guanosine forms five hydrogen bonds with an energy value of -6.40 kcal/mol and 2,3- dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one interacts with an energy value of -6.2 kcal/mol with four hydrogen bonds. The Glycerin interacts with an energy value of -5.65 kcal/mol with one hydrogen bond. The docking study clearly shows that α-Tocopherol-beta.-D-mannoside and pyrogallol forms interaction with the NF-κB target proteins at the lower energy level.

4 Discussion

Several natural chemopreventive compounds, such as curcumin, resveratrol, and lycopene are effective inhibitors of NF-κB (Aggarwal and Shishir 2006). The transcription factor NF-κB is a newly discovered target molecule that helps develop anti-tumor, anti-inflammatory, and pro-apoptotic medicines (Piccagli et al. 2008). Carcinogens, tumor promoters, and inflammatory drugs all activate NF-κB. NF-κB regulates apoptosis suppression, and chemopreventive drugs might inhibit NF-κB activity (Aggarwal and Shishodia 2004). *Withania somnifera's* with anolides beneficial chemicals interact with the NF-κB protein to modulate its activity and may be employed in medication development (Nithya et al. 2009). In comparison to pulp extract, peel extract contains more total phenolics, flavonoids, and proanthocyanidins. Peel extract's high phenolic content may account for its potent antioxidant activities (Li et al. 2006).

Pyrogallol and α-tocopherol-beta-D-mannoside found in the EPGP and EVVS showed interaction with NF-kB protein in this study. The α-tocopherol-beta-D-mannoside has anti-oxidant, anti-mutagenic, and anti-proliferative properties (Duke's Phytochemical and Ethnobotanical Databases database 2016). Pyrogallol shows anti-bacterial, anti-oxidant, and anti-tumor activities. The pomegranate peel and grape seeds possess chemopreventive potential (Ashok Kumar and Vijayalakshmi 2015) and, the interaction of these two ligands with the chosen target protein may provide the specific property to these plant materials. In the current *in-silico* study, five phytochemicals of the selected plant extracts interact with the target protein via hydrogen bonds. The α -tocopherol-beta -D-mannoside and Pyrogllol show the highest docking with NF-κB protein with the energy value of -10.88 kcal/mol and -7.07 kcal/mol. The docking studies of resveratrol, a naturally occurring antioxidant with NF-κB, showed strong interaction with the NF-κB and might prevent its binding ability with DNA (Banagalapalli et al. 2013). This result is similar to our study as the antioxidants pyrogallol and α-tocopherol-beta-D-mannoside present in selected plant materials show interaction with NF-κB and may inhibit its activity.

Similarly, the epigallocatechin-gallate (EGCG), which possesses protective action against inflammatory colitis showed a high binding profile against NF-κB in docking studies which might play a role in its anti-inflammatory activity (Varthya et al. 2020). The genistein which, possesses anti-cancer potential against NF-κB activated breast cancer, showed a strong binding affinity with NF-κB with four hydrogen bonds similar to our results. This interaction might play an important role in its anti-cancer potential against breast cancer (Mukund et al. 2019). The α.-tocopherol-beta-D-mannoside interacts with amino acid glycine at 294 positions of NF-κB via two hydrogen bonds with hydrogen bond lengths of 2.91^{0}A and 2.97^{0}A**,** respectively. The pyrogallol interacts with NF-κB at amino acid glycine in position 296 via two hydrogen bonds with the same bond length of 2.6 ^{0}A.

Conclusion

This *in-silico* docking study was carried out to show the interaction of bioactive constituents present in *P. granatum* peel and *V. vinifera* seeds with the selected target protein NF-κB. The five phytochemicals in selected plant materials showed a binding affinity with the NF-κB via Hydrogen bonds. The α-tocopherol-beta-D-mannoside and pyrogallol show higher interaction with NF-κB than other selected ligands. These two bioactive constituents with antioxidant and anti-cancer properties might play a role in providing anti-cancer potential for the selected plant materials by inhibiting NF-κB activity. This study provides insights for *in-vitro*, *in-vivo*, and simulation studies, which might pave the way for future personalized treatment strategies against cancer and inflammation activated by NF-κB and the signaling pathways involved in the inhibition of NF-κB.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Aggarwal, B. B., & Shishir, S. (2006). Molecular target of dietary agents for prevention and therapy of cancers. *Biochemical Pharmacology, 71*, 1397-1421.

Aggarwal, B. B., & Shishodia, S. (2004). Suppression of the nuclear factor kappa B activation pathway by spice derived phytochemicals: reasoning for seasoning. *Annals of the New York Academy of Sciences, 1030*, 434 - 441.

Ashok Kumar, K., & Vijayalakshmi, K. (2015). Protective Effect of *Punica granatum* Peel and *Vitis vinifera* Seeds on DEN-Induced Oxidative Stress and Hepatocellular Damage in Rats. *Applied Biochemistry and Biotechnology, 175*, 410-420.

Banaganapalli, B., Mulakayala, C. D. G., Gowsia, D., Mulakayala, N., et al. (2013). Synthesis and biological activity of new resveratrol derivative and molecular docking: dynamics studies on NFkB. *Applied Biochemistry and Biotechnology, 171*, 1639-1657.

Bassiri-Jahromi, S. (2018) *Punica granatum* (Pomegranate) activity in health promotion and cancer prevention. *Oncology Review*, *12*, 345-349.

Baud, V., & Karin, M. (2009). Is NF-κB a good target for cancer therapy? Hopes and pit falls. *Nature reviews drug discovery*, *8*,33–40.

Chauhan, A., Islam, A., Prakash, H., & Singh, S. (2021). Phytochemicals targeting NF-κB signaling: Potential anti-cancer interventions, *Journal of Pharmaceutical Analysis*, 2021, https://doi.org/10.1016/j.jpha.2021.07.002.

Cheemanapalli, S., Chinthakunta, N., & Shaikh, N. M. (2019) Comparative binding studies of curcumin and tangeretin on up-stream elements of NF-kB cascade: a combined molecular docking approach. *Network Modeling Analysis in Health Informatics and Bioinformatics*, *8***,** 15-19.

Chen, H., Huang, Q., Dong, J., & Lan, Q. (2006). Cancer initiating cell theory: popularity and controversies. *AiZheng*, *25*, 779–784.

Chene, P. (2003). Inhibiting the p53-MDM2 interaction: an important target for cancer therapy. *Nature review cancer*, *3*, 102-109.

Dhumal, S. S., Karale, A. R., Jadhav, S. B., & Kad, V. P. (2014). Recent advances and the developments in the pomegranate processing and utilization: A review. *Journal of Agriculture Crop Science*, *1*, 1-17.

Duke's Phytochemical and Ethnobotanical Databases database, 2016.

Ferrer-Gallego, R., García-Marino, M., Hernández-Hierro, J. M., Rivas-Gonzalo, J. C., et al. (2010). Statistical correlation between flavanolic composition, colour and sensorial parameters in grape seed during ripening. *Analytica Chimica Acta*, *660*, 22-28.

Girard, B., & Mazza, G. (1998) Functional grape and citrus products. In Mazza, *Functional Foods* (pp.139-154).Technomic Publishing, PA, USA.

Hossin, F. L. A. (2009). Effect of pomegranate (*Punica granatum*) peels and it's extract on obese hypercholesterolemic rats. *Pakisthan Journal of Nutrition*, *8*, 1251–1257.

Kaliamurthi, S., Selvaraj, G., & Junaid, M. (2018). Cancer Immunoinformatics: a promising era in the development of peptide vaccines for human papillomavirus-induced cervical cancer. *Current Pharmaceutical Design*, *24*, 3791–3817.

Kar, P. P., & Srivastava, A. (2018). Immuno-informatics analysis to identify novel vaccine candidates and design of a multi-epitope based vaccine candidate against theileria parasites. *Frontiers in Immunology*, *9*, 2213-2218.

Li, Y., Guo, C., Yang, J., Wei, J., et al. (2006) Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. *Food Chemistry*, *96*, 254-260.

Lin, L. T., Chen, T. Y., Lin, S. C., Chung, C. Y., et al. (2013). Broad-spectrum antiviral activity of chebulagic acid and punicalagin against viruses that use glycosaminoglycans for entry. *BMC Microbiology*, *13*, 187.

Mayo L. D. & Donner D. B., (2002). The PTEN, Mdm2, p53 tumor suppressor oncoprotein network. *Trends in Biochemical Sciences*, *27*, 462-467.

Mukherjee, S., Ghosh. S., Choudhury. S., Gupta, P., et al. (2021). Pomegranate Polyphenols Attenuate Inflammation and Hepatic Damage in Tumor-Bearing Mice: Crucial Role of NF-κB and the Nrf2/GSH Axis. *Journal of Nutritional Biochemistry*, *97*, 108812. doi: 10.1016/j.jnutbio.2021.108812.

Mukund, V., Behera, S. K., Alam, A., & Nagaraju, G. P. (2019). Molecular docking analysis of nuclear factor-κB and genistein interaction in the context of breast cancer. *Bioinformation*, *15*(1), 11-17.

Naz, A., Bano, K., Bano, F., Ghafoor, N. A., et al. (2009). Conformational analysis (geometry optimization) of nucleosidic antitumor antibiotic show domycin by Argus Lab 4 software. *Pakistan Journal of Pharmacy*, *22*,78-82.

Nithya, K., Shanthi, N., & Kalaiselvi, K. (2009). Molecular docking of Withanolides against the transcription factor Nuclear Factor kappa B (NFkB) Using Glide. *Advanced Biotechnology*, *9*, 23-27.

Oda, A., & Takahashi, O. (2009). Validation of ArgusLab efficiencies for binding free energy calculations. *Chem-Bio informatics Journal*, *9*, 52-61

Oeckinghaus, A., & Ghosh, S. (2009). The NF-kappaB family of transcription factors and its regulation. *Cold Spring Harbor Perspectives in Biology*, *1*, 1-14

Orlowski, R. Z., & Baldwin, A. S. (2002). NF-kappaB as a therapeutic target in cancer. *Trends Molecular Medicine*, *8*, 385-389.

Piccagli, L., Fabbri, E., Birgatti, M., Bezzerri, V., et al. (2008). Docking of molecule identified bioactive medicinal plant extract into the p50 NF-kappa B transcription factor: Correlation with inhibition of NFkappa B/ DNA interactions and Inhibitory effects on IL- 8 gene expression. *BMC Structural Biology*, *8*, 1-13.

Quero. J., Moreno. N., Esparza. I., Osada. J., et al. (2021). Grape Stem Extracts with Potential Anticancer and Antioxidant Properties. *Antioxidants, 10,* 243. https:// doi.org/10.3390/antiox10020243

Seeliger, D., & de Groot, B. L. (2010). Ligand docking and binding site analysis with PyMOL and Autodock/Vina. *Journal of Computer-aided Molecular Design, 24*, 417-422.

Sladek, V., Yamamoto, Y., Harada, R., Shoji, M., et al. (2021). pyProGA—A PyMOL plugin for protein residue network analysis. *PLoS ONE, 16*, 1-17.

Sun, Y., Fan, X., Zhang, Q., Xu, G., et al. (2017). Cancer-associated fibroblasts secrete FGF-1 to promote ovarian proliferation, migration, and invasion through the activation of FGF-1/FGFR4 signaling. *Tumour Biology, 39*, 1-9.

Tangyuenyongwatana, P., & Jongkon, N. (2016). Molecular docking study of tyrosinase inhibitors using ArgusLab 4.0.1: A comparative study. *Thai Journal of Pharmaceutical Sciences, 401*, 1-53.

Varthya, S. B., Sarma, P., Bhatia, A., Shekhar, N., et al. (2020). Efficacy of green tea, its polyphenols and nanoformulation in experimental colitis and the role of non-canonical and canonical nuclear factor kappa beta (NF-kB) pathway: a preclinical in-vivo and in-silico exploratory study. *Journal of Biomolecular Structure Dynamics*, *39*, 5314-5326.

Verzella, D., Pescatore, A., Capece, D., Vecchiotti, D., *et al.* (2020). Life, death, and autophagy in cancer: NF-κB turns up everywhere. *Cell Death and Disease, 11***,** 210 https://doi.org/ 10.1038/s41419-020-2399-y.

Yuan, S., Chan, H. C. S., & Hu, Z. (2017). Using PyMOL as a platform for computational drug design. *WIREs Computational Molecular Science, 7*, e1298.

Zhang, W., Yin, G., & Dai, J. (2017). Chemoprevention by quercetin of oral squamous cell carcinoma by suppression of the NF-κB signaling pathway in DMBA-treated hamsters. *Anticancer Research, 37*, 4041–4049.

Zhu, F., Du, B., Zheng, L., & Li, J. (2015). Advance on the bioactivity and possible uses of dietary fiber from grape pomace. *Food Chemistry, 186*, 207-212.