E-ISSN :2722-0877

ORIGINAL RESEARCH ARTICLES

Potential of Indole-3-Carbinol Derived from Kale Leaves (*Brassica oleracea L.*) Against Cervical Cancer Targeting IGF2BP2 and MYCBP: An In Silico Study

Farhan Abdullah Rizal[1], Humairah Medina Liza Lubis[2]

[1] *Department of Medicine, Faculty of Medicine, Universitas Muhammadiyah Sumatera Utara, Medan, Indonesia.*

[2] [b] *Dapartment of Anatomical Pathology, Faculty of Medicine, Universitas Muhammadiyah Sumatera Utara, Medan, Indonesia*

Corresponding Email: *Farhan.Rizal06@gmail.com*

Abstract:

Introduction: Cervical cancer remains one of the leading causes of morbidity and mortality worldwide, including in Indonesia, involving metabolic reprogramming such as the Warburg effect in tumorigenesis. The proteins IGF2BP2 and MYCBP are known to play important roles in promoting cancer cell proliferation through the regulation of cellular metabolism. **Objective:** This study aimed to evaluate the potential of Indole-3-Carbinol derived from kale leaves (*Brassica oleracea L*) as a candidate anticancer agent for cervical cancer using an in silico approach. **Method**: Included molecular docking targeting IGF2BP2 and MYCBP proteins, with 5-fluorouracil and doxorubicin as comparator drugs, along with ADMET and druglikeness analysis based on Lipinski's rule. **Result:** The results showed that Indole-3-Carbinol exhibited binding energy (ΔG) values of -3.20 kcal/mol for IGF2BP2 and -5.75 kcal/mol for MYCBP, with better binding affinity than 5-fluorouracil but lower than doxorubicin. Interaction analysis indicated stronger binding to IGF2BP2 compared to MYCBP, suggesting IGF2BP2 as the primary target protein. Furthermore, Indole-3-Carbinol fulfilled Lipinski's criteria, demonstrated high gastrointestinal absorption, the ability to penetrate the blood-brain barrier, and a favorable toxicity profile without carcinogenic effects. **Conclusion:** Indole-3-Carbinol shows potential as a candidate cervical anticancer agent targeting the IGF2BP2 pathway and modulating the Warburg effect, with the advantage of being a relatively safe natural compound.

***Keywords*:** *Cervical cancer, In-silico, Indole-3-Carbinol, Kale leaves, Warburg effect*

INTRODUCTION

Cervical cancer is a malignant tumor that occurs in the epithelial layer of the cervix and ranks fourth among all malignancies

E-ISSN :2722-0877

worldwide. The number of new cases is estimated to increase from 570,000 to 700,000 annually between 2018 and 2030, with annual deaths rising from 311,000 to 400,000 cases.[1] In Indonesia, based on data from the Global Burden of Cancer Study 2020, cervical cancer is the second most common cancer, with a total of 36,633 cases. It is also the third leading cause of cancer-related deaths, with 21,003 fatalities.[2]

High-risk human papillomavirus (HPV), particularly genotypes 16 and 18 that produce E6 and E7 oncoproteins, is the primary cause of cervical cancer. These viruses infect poorly differentiated squamous basal keratinocytes and trigger tumorigenesis. HPV infection alters tumor cell metabolism, leading to immunosuppression and immune evasion, thereby promoting oncogenic mutations. This process is characterized by increased expression of E6 and E7 proteins, which inactivate tumor suppressor proteins p53 and pRb, resulting in uncontrolled cell division. These oncoproteins also inhibit apoptosis, increase genomic instability, prevent telomere shortening, promote angiogenesis, and facilitate invasion and metastasis.[3] Changes in metabolic phenotype are among the microenvironmental alterations following infection. The post-HPV infection microenvironment actively regulates tumor cell metabolism, which is a key factor in HPV progression and cervical cancer development.[4]

Metabolic reprogramming due to oncogenic mutations plays a crucial role in tumorigenesis across various cancers, including cervical cancer. The activity of glucose transporters and metabolic enzymes contributes to the Warburg effect, which supports increased glucose uptake and lactate production in the tumor microenvironment, thereby promoting cancer cell proliferation. The Warburg effect enhances glycolysis, leading to increased lactic acid production. Cancer cells rely heavily on aerobic glycolysis, enabling lactic acid formation from glucose at rates 10–100 times faster than complete mitochondrial oxidation under normal conditions.[5] [6] Additionally, cancer cells regulate mitochondrial pathways within the tumor microenvironment to enhance resistance to apoptosis and promote survival.[7] Proteins involved in this signaling pathway include Insulin-like Growth Factor 2 mRNA-Binding Proteins (IGF2BPs) and MYCBP, which contribute to cancer progression by stabilizing methylated mRNA and promoting tumor cell proliferation. This study investigates the anticancer phytochemical effects of natural compounds in relation to cervical cancer pathogenesis through the IGF2BP2 and MYCBP pathways.[8] [9]

Indole-3-carbinol (I3C) is a natural compound found in vegetables of the Brassicaceae family. Numerous studies have demonstrated that I3C suppresses the proliferation of various cancer cells, including breast, colon, prostate, and endometrial cancers. For example, studies on

E-ISSN :2722-0877

non-tumorigenic and tumorigenic breast epithelial cells (MCF10A and MCF10CA1a) show that I3C induces apoptosis in cancer cells while sparing normal cells. I3C and its derivative, diindolylmethane (DIM), are involved in phase I detoxification enzyme induction, which helps degrade carcinogens. Both in situ and in vivo studies have demonstrated the chemoprotective effects of I3C in breast and prostate cancers.[10]

Due to limitations in current cervical cancer therapies, there is growing interest in targeting cellular signaling pathways. Therapies that focus on a single pathway are often insufficient; however, natural compounds such as Indole-3-Carbinol may act as multi-target agents by modulating various signaling pathways. Previous research has shown that Indole-3-Carbinol is effective in overcoming drug resistance and inhibiting metastatic tumor progression.[11]

This study employs an in silico approach to analyze the molecular interactions between compounds derived from kale leaves (Brassica oleracea L.) and target proteins. Molecular docking was conducted to evaluate the binding interactions between Indole-3-Carbinol and the IGF2BP2 and MYCBP proteins. Kale-derived phytochemicals, particularly Indole-3-Carbinol, are considered potential modulators of the Warburg effect with minimal side effects, as they specifically target cancer cells compared to conventional therapies such as chemotherapy and radiotherapy. Therefore, this study aims to analyze the anticancer potential of Indole-3-Carbinol in cervical cancer by targeting metabolic pathways, particularly IGF2BP2 and MYCBP signaling..

METHOD

After identifying compounds in kale leaves (Brassica oleracea L.), an in silico analysis was conducted using molecular docking to evaluate interactions between ligands and proteins. The natural compound Indole-3-Carbinol was used as a ligand and obtained from the PubChem database in a 3D structure format. The structure was downloaded in .sdf format and converted into .pdb format using OpenBabel software. Subsequently, the ligand was prepared for docking using AutoDockTools by converting the file into .pdbqt format.[12]

Target proteins were selected from the UniProt database using the criteria “Homo sapiens,” namely IGF2BP2 and MYCBP. Protein structures were obtained from the RCSB-PDB database based on the corresponding IDs, 6ROL and 2YY0. The selection of these proteins was based on the availability of crystal structures with good resolution, year of publication, and absence of mutations. Protein 6ROL has a resolution of 2.10 Å (published in 2019), while 2YY0 has a resolution of 2.40 Å (published in 2008), and both were determined using X-ray crystallography.[13]

Protein preparation was carried out using AutoDockTools by removing water

E-ISSN :2722-0877

molecules and native ligands, followed by the addition of polar hydrogen atoms and partial charges before conversion into .pdbqt format. For proteins requiring structural modeling, a homology modeling approach was performed using SWISS-MODEL. Structure validation was conducted using a Ramachandran plot to ensure protein model quality.[14]

Active site determination was performed based on the available protein structure and through a blind docking approach. The blind docking method allows the ligand to interact with the entire protein surface to identify potential binding sites. Through this approach, the amino acid residues involved in binding interactions can be predicted.[15] [16]

Molecular docking was performed using AutoDock Tools 4.0. Two-dimensional interaction visualization was conducted using LigPlot+, while three-dimensional visualization was performed using PyMOL and Discovery Studio 4.1.[17]

Docking validation was performed by comparing the position of the docked ligand with the reference structure using the Root Mean Square Deviation (RMSD) parameter. The RMSD value was used to assess the agreement of the ligand binding position with the protein structure.[18]

The structures of bioactive compounds were obtained from PubChem in .sdf format. For ADME and toxicity analysis, ADMETlab 2.0 was used by inputting the SMILES data of the compounds. Compound evaluation was carried out based on ADME parameters, toxicity, and Lipinski's Rule of Five to determine their suitability as drug candidates.[19]

RESULTS

The docking results consist of three types: 3D visualization, 2D visualization, and Admetox. In silico testing is conducted between a protein and ligands. The protein used is IGF2BP2 and MYCBP found in cervical cancer cells, which is docked with ligands from compounds found in kale leaves, specifically Indole-3-Carbinol. The comparison ligands used are drugs commonly used in chemotherapy for cervical cancer, namely 5-Fluorouracil and Doxorubicin.

Table 1. Docking Results of IGF2BP2 protein

Bioactive Compound	Binding Energy	Inhibition Constant
Indole-3- Carbinol	-3.30	4.50
5-Fluorouracil	-3.08	5.55
Doxorubicin	-6.14	31.45

The ΔG (Gibbs Energy) values for Indole-3-Carbinol, 5-fluorouracil and Doxorubicin are -3.20, -3.08 and -6.14 kcal/mol, respectively. Meanwhile, the pKi values are 4.50, 5.55, 31.45 respectively. From the above results, using molecular simulations, we found that Indole-3-Carbinol has a higher binding affinity compared to 5- fluorouracil.

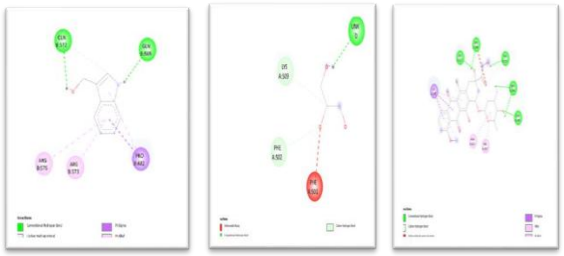
Table 2. Docking Results of MYCBP protein

Bioactive Compound	Binding Energy	Inhibition Constant
Indole-3- Carbinol	-5.75	61.23
5-Fluorouracil	-4.45	549.10
Doxorubicin	-6.90	8.72

The ΔG (Gibbs Energy) values for Indole-3-Carbinol, 5-fluorouracil and Doxorubicin are -5.75, -4.45 and -6.90 kcal/mol, respectively.

E-ISSN :2722-0877

Meanwhile, the pKi values are 61.23, 549.10, and 8.72 respectively. From the above results, using molecular simulations, we found that Indole-3- Carbinol has a higher binding affinity compared to 5-fluorouracil.



(A) (B) (C)

Figure 1. 2D Visualization of IGF2BP2 protein, (A) Indole- 3-Carbinol, (B) 5-Fluorouracil, (C) Doxorubicin

2D visualization of Indole-3-Carbinol with the IGF2BP2 protein shows that there are 5 bonds. From the 5-Fluorouracil ligand with the IGF2BP2 protein, it was found that there were 4 bonds. From the Doxorubicin ligand with the IGF2BP2 protein, it was found that there were 8 bonds. showed that indole-3-carbinol had stronger binding compared to 5-fluorouracil.

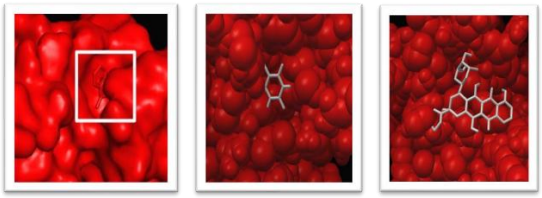
Table 3. Amino Acid Residue Q9Y6M1

Protein	Bioactive Compound	GLN 52	GLN 569	PRO 482	ARG 573	ARG 576
Q9Y6 M1	*Indole-3-Carbinol*	✓	✓			
	Total	1	1			

Table 4. Amino Acid Residue Q99417

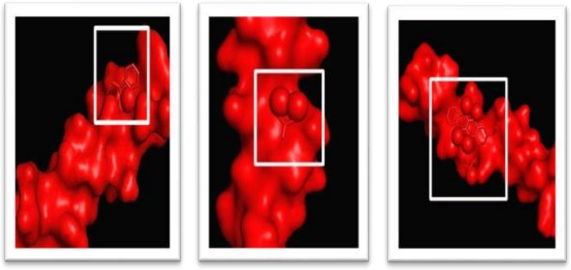
Protein	Compound	LEU 86	LYS 89	LEU 90	TYR 93
Q99417	*Indole-3-Carbinol*	✓			
	Total	1			

The amino acid residue in the IGF2BP2 (Q9Y6M1) protein has 2 amino acids, while the MYCBP (Q99417) protein has 1 amino acid. This shows that the Indole-3-Carbinol ligand has better adhesion to the IGF2BP2(QY6M1) protein



(A) (B) (C)

Figure 2. 3D Visualization of IGF2BP2 protein, (A) Indole- 3-Carbinol, (B) 5-Fluorouracil, (C) Doxorubicin



(A) (B) (C)

Bonds between proteins and ligands are found in protein gaps. The bond that occurs is a strong bond because based on the nature of proteins, there are many active binding sites between protein gaps. Active binding sites that bind one protein to another form a strong bond.

Table 5. ADMETOX bioactive compounds by druglikeness

Compound	Molecular Weight	(H) Bond Acceptor	(H) Bond Donor	LogP
Indole-3-	147.07	10	2	1.39

E-ISSN :2722-0877

Carbinol				
5-Fluorouracil	130.020	4	2	-0.773
Doxorubicin	543.52	12	6	2.012

Based on Lipinski's 5 rules, it is stated that the molecular weight should not exceed 500 g/mol, Hydrogen Bond Donors should not be more than 5, Hydrogen Bond Acceptors should not be more than 10, and the Log P value should not exceed 5. Therefore, according to Lipinski's 5 rules, the compound Indole-3-Carbinol has satisfied all these criteria.

Table 6. ADMETOX bioactive compounds at Q9Y6M1 by Pharmacokinetics

Compound	GI	BBB	CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4
Indole 3-Carbinol	High	✓	✓	X	X	X	X
5-Fluorouracil	High	X	X	X	X	X	X
Doxorubicin	Low	X	X	X	X	X	X

Based on the inhibitor study of the drug-metabolizing enzyme CYP, based on Lipinski's rule that no more than 2 CYP inhibitors should be inhibited. This shows that the indole-3-carbinol compound does not violate these regulations and is still within safe limits.This means that the compound can be metabolized very well by the body. Additionally, it exhibits high absorption in the gastrointestinal tract and has the ability to penetrate the blood-brain barrier.

Table 7. ADMETOX bioactive compounds by Toxicities

Compound	AMES	Oral	Carci	Respi	LC50FM	LC50DM	Lipinski	
Indole 3-Carbinol	--	+ + +	--	+	3.815	4.78	✓	
5-Fluorouracil	---	--	---	++	2.630	4.69	✓	
Doxorubicin	+++	--	+++	++	4.976	6.859	X	

Based on toxicity tests, it was found that Indole-3-Carbinol is not harmful if ingested, indicating its potential as a for oral drug formulations. Indole-3-Carbinol also does not induce carcinogenicity. Therefore, according to Lipinski's 5 rules, the compound Indole-3-Carbinol is considered acceptable.

The molecular docking results indicate that Indole-3-Carbinol exhibits favorable binding affinity toward both IGF2BP2 and MYCBP proteins, with stronger interactions observed in IGF2BP2.[21] This suggests that IGF2BP2 may represent a more relevant molecular target than MYCBP in the context of cervical cancer. Mechanistically, IGF2BP2 functions as an RNA-binding protein involved in the stabilization of oncogenic transcripts, particularly through the regulation of N6-methyladenosine (m6A) modification. This regulation contributes to increased cancer cell proliferation and metabolic reprogramming. In cervical cancer, this mechanism is associated with the Warburg effect, in which cancer cells preferentially rely on glycolysis even under aerobic conditions. Therefore, targeting IGF2BP2 may potentially disrupt cancer cell

E-ISSN :2722-0877

metabolism and growth.[22]

The binding energy values further support these findings, where Indole-3-Carbinol demonstrates better affinity than 5-Fluorouracil, although still lower than Doxorubicin. This indicates that while the compound shows promising molecular interactions, its activity has not yet surpassed that of clinically established chemotherapeutic agents.[23] [24]

From a drug-likeness perspective, Indole-3-Carbinol satisfies all Lipinski's Rule of Five criteria, suggesting favorable pharmacokinetic properties. This may indicate improved oral bioavailability and absorption potential compared to Doxorubicin, which does not meet several of these parameters. In line with this, ADMET analysis shows that Indole-3-Carbinol has high gastrointestinal absorption, an acceptable CYP inhibition profile, and lower predicted toxicity compared to standard drugs. However, these findings remain predictive and require further biological validation.[25]

Overall, the in silico approach provides valuable insights into ligand–protein interactions, structural visualization, and pharmacokinetic as well as toxicity parameters, which can serve as a foundation for further studies. However, molecular docking has inherent limitations, as it provides only a static representation of ligand–protein interactions and does not account for protein flexibility, solvent effects, or the complexity of biological systems. In addition, ADMET predictions are computational estimates that may not fully reflect in vivo conditions, limiting the direct clinical applicability of these findings.[26]

Furthermore, although Indole-3-Carbinol demonstrates better interaction than 5-Fluorouracil, its binding affinity remains lower than that of Doxorubicin; therefore, it cannot yet be considered superior to existing therapies. Further in vitro and in vivo studies are required to validate its biological activity and therapeutic relevance.[27] [28] [29]

CONCLUSION

The compound Indole-3-Carbinol from kale leaves (Brassica oleracea L.) shows potential interactions with IGF2BP2 and MYCBP proteins associated with cervical cancer. In the in silico analysis, Indole-3-Carbinol fulfilled all five of Lipinski's rules, indicating favorable drug-likeness properties. Based on ADME-Tox predictions compared to standard positive controls (5-Fluorouracil and Doxorubicin), Indole-3-Carbinol demonstrated better potential than 5-Fluorouracil, although it was not superior to Doxorubicin.

Overall, Indole-3-Carbinol shows preliminary in silico potential targeting IGF2BP2 signaling and the Warburg effect; however, these findings require further validation through in vitro and in vivo studies.

ACKNOWLEDGMENTS

This article work was supported by

E-ISSN :2722-0877

Dapartment of Anatomical Pathology and Universitas Muhammadiyah Sumatera Utara.

REFERENCES

1. World Health Organization. Global strategy to accelerate the elimination of cervical cancer as a public health problem and its associated goals and targets for the period 2020 – 2030. Vol. 2, United Nations General Assembly. 2020. 1–56 p.
2. Sutnick AI, Gunawan S. Cancer in Indonesia. JAMA J Am Med Assoc. 2020;247(22):3087–8.
3. Shamseddine A, Burman B, Lee N, Zamarin D, Riaz N. Tumor Immunity and Immunotherapy for HPV-related cancers. Cancer Discov. 2021;11(8):1896–912.
4. Hu C, Liu T, Han C, Xuan Y, Jiang D, Sun Y, et al. HPV E6/E7 promotes aerobic glycolysis in cervical cancer by regulating IGF2BP2 to stabilize m6A-MYC expression. Int J Biol Sci. 2022;18(2):507– 21.
5. Fan T, Sun G, Sun X, Zhao L, Zhong R. Tumor Energy Metabolism and Potential of 3-Bromopyruvate as an Inhibitor of Aerobic Glycolysis : Implications in Tumor Treatment. 2019;
6. Pascale RM, Calvisi DF, Simile MM, Feo CF, Feo F. The warburg effect 97 years after its discovery. Cancers (Basel). 2020;12(10):1–33.
7. Boese AC, Kang S. Mitochondrial metabolism-mediated redox regulation in cancer progression. Redox Biol [Internet]. 2021;42:101870. Available from: https://doi.org/10.1016/j.redox.2021.10187 0
8. Qi Y, Zhang C, Wu D, Zhang Y, Zhao Y, Li W. Indole-3-Carbinol Stabilizes p53 to Induce miR-34a, Which Targets LDHA to Block Aerobic Glycolysis in Liver Cancer Cells. Pharmaceuticals. 2022;15(10).
9. Popolo A, Pinto A, Daglia M, Nabavi SF, Farooqi AA, Rastrelli L. Two likely targets for the anti-cancer effect of indole derivatives from cruciferous vegetables: PI3K/Akt/mTOR signalling pathway and the aryl hydrocarbon receptor. Semin Cancer Biol [Internet]. 2017;46(May):132–7. Available from: http://dx.doi.org/10.1016/j.semcancer.2017.06.002
10. Chamovitz DA, Katz E, Nisani S. Indole-3-carbinol: A plant hormone combatting cancer [version 1; referees: 2 approved]. F1000Research. 2018;7(0):1–9.
11. Biersack B. 3,3’-Diindolylmethane and its derivatives: Nature-inspired strategies tackling drug resistant tumors by regulation of signal transduction, transcription factors and microRNAs. Cancer Drug Resist. 2020;3(4):867–78.
12. Lin X, Li X, Lin X. A review on applications of computational methods in drug screening and design. Molecules.

E-ISSN :2722-0877

2020;25(6):1–17.
13. O'Boyle NM, Banck M, James CA, Morley C, Vandermeersch T, Hutchison GR. Open Babel: An open chemical toolbox - 1758-2946-3-33.pdf. J Cheminform. 2011;3(33):1–14.
14. Allouche AR. Gabedita - A graphical user interface for computational chemistry softwares. Vol. 32, Journal of Computational Chemistry. 2011. p. 174–82.
15. Garrett M. Morris. AutoDock Version 4.2 - User Guide. Guide. 2010;1–49.
16. Pinzi L, Rastelli G. Molecular docking: Shifting paradigms in drug discovery. Int J Mol Sci. 2019;20(18).
17. Maia EHB, Assis LC, de Oliveira TA, da Silva AM, Taranto AG. Structure- Based Virtual Screening: From Classical to Artificial Intelligence. Front Chem. 2020;8(April).
18. 18. Opo FADM, Rahman MM, Ahammad F, Ahmed I, Bhuiyan MA, Asiri AM. Structure based pharmacophore modeling, virtual screening, molecular docking and ADMET approaches for identification of natural anti-cancer agents targeting XIAP protein. Sci Rep [Internet]. 2021;11(1):1–18. Available from: https://doi.org/10.1038/s41598-021-83626- x
19. Stitou M, Toufik H, Bouachrine M, Lamchouri F. Quantitative structure–activity relationships analysis, homology modeling, docking and molecular dynamics studies of triterpenoid saponins as Kirsten rat sarcoma inhibitors. J Biomol Struct Dyn. 2021;39(1):152–70.
20. Zeng Q, Zhao RX, Chen J, Li Y, Li XD, Liu XL, et al. O-linked GlcNAcylation elevated by HPV E6 mediates viral oncogenesis. Proc Natl Acad Sci U S A. 2016;113(33):9333–8.
21. Ji F, Lu Y, Chen S, Yu Y, Lin X, Zhu Y, et al. IGF2BP2-modified circular RNA circARHGAP12 promotes cervical cancer progression by interacting m6A/FOXM1 manner. Cell Death Discov [Internet]. 2021;7(1):1–9. Available from: http://dx.doi.org/10.1038/s41420-021-00595-w
22. Ekins S, Mestres J, Testa B. In silico pharmacology for drug discovery: Applications to targets and beyond. Br J Pharmacol. 2007;152(1):21–37.
23. Pope DJ, Fryer R, Masouros SD. In Silico Models. Blast Inj Sci Eng A Guid Clin Res Second Ed. 2023;(January):279–84.
24. Casini A, Scozzafava A, Supuran CT. Cysteine-modifying agents: A possible approach for effective anticancer and antiviral drugs. Environ Health Perspect. 2002;110(SUPPL. 5):801–6.
25. Liao J, Wang Q, Wu F, Huang Z. In Silico Methods for Identification of Potential Active Sites of Therapeutic

E-ISSN :2722-0877

Targets. Vol. 27, Molecules. 2022.
26. Drwal MN, Banerjee P, Dunkel M, Wettig MR, Preissner R. ProTox: A web server for the in silico prediction of rodent oral toxicity. Nucleic Acids Res. 2014;42(W1):53–8.
27. El-Din HMA, Loutfy SA, Fathy N, Elberry MH, Mayla AM, Kassem S, et al. Molecular docking based screening of compounds against VP40 from Ebola virus. Bioinformation. 2016;12(3):192–6.
28. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev. 2012;64(SUPPL.):4–17.
29. Guttman Y, Kerem Z. Computer- Aided (In Silico) Modeling of Cytochrome P450-Mediated Food–Drug Interactions (FDI). Int J Mol Sci. 2022;23(15).