RJC

RASĀYAN *J. Chem.*
Vol. 15 | No. 2 |1080-1084| April - June | 2022
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

IN SILICO DOCKING STUDIES OF PHYTOSTEROL COMPOUNDS SELECTED FROM *Ficus religiosa* AS POTENTIAL CHEMOPREVENTIVE AGENT

H.D. Syahputra[1,✉] M. Masfria[1,2], P.A.Z Hasibuan[3] and I. Iksen[4]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Sumatera Utara, Medan 20222, Indonesia

[2]Department of Nanomedicine, Faculty of Pharmacy, Universitas Sumatera Utara, Medan 20222, Indonesia

[3]Department of Pharmacology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan 20222, Indonesia

[4]Department of Pharmacy, Sekolah Tinggi Ilmu Kesehatan Senior Medan, Medan 20131, Indonesia

✉Corresponding Author: dharmawanhariyadi@gmail.com

ABSTRACT

Traditional medicine has employed *Ficus reliosa* (Moraceae) to treat a wide range of diseases such as central nervous system disorder, infectious disease, endocrine system, respiratory system, etc. This study aimed to evaluate phytosterol constituents that may serve as lead-drug such as 28-Isofucosterol, Stigmasterol, Sitosterol, and Campesterol towards their pharmacokinetic properties and chemopreventive activity to regulate nuclear receptor factor 2 (Nrf2) uptake bound by Kelch-like Activating Protein (Keap1). Structures were studied to evaluate their respective bioactivity using Milliprot followed by a docking study with Autodock Tools for macromolecule obtained from Protein Data Bank in 2FLU PDB file name. Chemopreventive activity from Sitosterol, Stigmasterol, Campesterol and 28-Isofucosterol in each respective bioactivity towards nuclear receptor target : 0.72; 0.74; 0.71; 0.91. A computational study performed by Autodock Tools against Keap1 targeted macromolecule covalent binding toward threonine 609 to obtain inhibition results. As respective Inhibition constant obtained (pM) : 522.97; 286.75; 620.16; 369.55. Free energy obtained (kcal/mol) : -8.57; -13.02; -12.56; -12.87. Hence the results revealed from the study showed that phytosterol compound from Ficus religiosa proven to be useful as a chemopreventive agent to promote antioxidant gene activity through regulation of Nrf2 factor by competing for receptor site with Keap1 during stressed conditions.

Keywords: Chemopreventive, Phytosterol, *Ficus religiosa*, *In silico*, Nrf2.

RASĀYAN *J. Chem.*, Vol. 15, No.2, 2022

INTRODUCTION

Tumorigenesis is a process that begins from the normal cellular transformation in the acquisition of invasive potential, angiogenic potency, avoiding apoptosis mechanism, and uncontrollable growth factors. This multistep of carcinogenic consists of three phases: tumor initiation, promotion, and progression.[1] Chemopreventive studies show significant progress in recent years. Sterol components derived from hydroxylated polycyclic isoterpenoids have a 1,2-cyclopentanophenanthrenen structure. With the consumption of phytosterol as a primary and secondary dietary chemopreventive which acts to prevent further free radicals or subject to any potential exposure to cancer, it proves beneficial to decreasing risk in some cancer forms such as breast, prostate, lung, and ovary.[2,3] In tropical and subtropical areas, the Ficus genus has about 800 species of trees, epiphytes, and shrubs, making it one of the largest angiosperms.[4] Among them is *Ficus religiosa* (FR) a sacred tree native to the south and south-east Asia reported to have numerous benefits in its leaf juice be used to treat asthma, Alzheimer's, cancer, diarrhea, haematuria, and gastric problems. Phytochemical research carried out mostly led to the isolation of phytosterol, phenolic compounds, aliphatic alcohols, amino acids, furocoumarins, hydrocarbons, essential oils, and a few other classes of secondary metabolites from different parts.[5-7] Campesterol, stigmasterol, sitosterol, and 28-isofucosterol (Fig.-1) are the common phytosterol found in FR. With the new opportunities for modern

http://dx.doi.org/10.31788/RJC.2022.1526801

computation modeling approaches, significant advances in computational chemistry and bioinformatics for pharmacological targets have increased significantly.[8,9] Each respective component undergoes a series of docking towards Keap1-Nrf2 (Kelch-like Activating Protein-Nuclear erythroid Factor 2) signaling pathway as chemopreventive activity properties, where the release of Nrf2 in stressed condition promote the expression of this onset during translocation in the cell will release antioxidant gene, for example, GST P1 (Glutathione S-Transferase Pi1) will protect the prostate cell from cytotoxicity and DNA damage by heterocyclic amine carcinogenic agent such as PHIP, as in some circumstances the release of Nrf-2 may be decreased in some genetic material deformities and prevent its translocation process that may act as primary or secondary chemopreventive agents.[10-14] This study will be focused on the docking analysis between selected phytosterol from FR against Keap1-Nrf2.

Fig.-1: Chemical Structures of Several Phytosterols. (A) Sitosterol, (B) Stigmasterol, (C) Campesterol, and (D) 28-Isofucosterol

EXPERIMENTAL

Material and Methods

In silico study performed with Asus ROG hardware with specification i7-77700HQ CPU@ 2.80 GHz. PDB code of Keap1-Nrf2: 2FLU obtained from Protein Data Bank (https://www.rcsb.org/) followed with target covalent binding for ligand onto THR609 cavity and smiles data of campesterol, stigmasterol, sitosterol, and 28-isofucosterol derived from Pubchem compound (https://PubChem.ncbi.nlm.nih.gov/). The computational analysis of each phytosterol component was conducted by using a variety of applications, including Chemdraw and Mollinspiration (https://molinspiration.com/cgi-bin/properties). Computational assay of pharmacokinetic properties (ADMET) for Sitosterol, Stigmasterol, Campesterol, and 28-isofucosterol determined with SMILES series followed an analysis by using pkCSM tools to predict its values (http://biosig.unimelb.edu.au/pkcsm/prediction).[15] Autodock Tools-15.6 and Discovery Studios 2016 (64-bit) to determine each ligand-based-receptor binding result.

RESULTS AND DISCUSSION

Target and Pharmacokinetic Prediction

Table-1 prediction of the ligand-target receptor for each phytosterol component respectively with Mollinspiration showed that most of the subject has a great potential to act with nuclear receptor binding whereas 28-isofucosterol gave the best possibility towards ligand-target, each component to give good compatibility result. Meanwhile, for G-protein, ion channel, and kinase inhibitor none had the potential except for sitosterol. This indicates NrF2 which belongs to the nuclear receptor family could prove to be beneficial in their interaction with the subjected phytosterol group (Bioactivity Probability ≤ 1). Table-2

showed most phytosterol may induce the activity of CYP3A4 if combined with multiple treatments it may compete with other pharmaceutical products that inhibit the enzyme which led to the toxicity of others. Whereas for the substances themselves even with their nature to act as CYP3A4 inducers, they won't cause any major harm to the body due to the less to no hepatoxicity effect is presented.[16]

Table-1: Bioactivity Prediction of each FR Phytosterol Component

Compound	G-Protein	Ion-channel	Kinase inhibitor	Nuclear receptor	Protease inhibitor	Enzyme inhibitor
Sitosterol	0.20	0.00	0.40	0.72	0.25	0.45
Stigmasterol	0.12	0.08	0.48	0.74	0.02	0.53
Campesterol	0.11	0.01	0.48	0.71	0.01	0.50
28-Isofucosterol	0.11	0.06	0.49	0.91	0.14	0.64

Table-2: Prediction of Pharmacokinetic Properties of each FR Phytosterol Component

Properties	Parameters	Sitosterol	Stigmasterol	Campesterol	28-Isofucosterol	Unit
Absorption	Water solubility	-7.402	-6.82	-7.056	-6.4	Numerical (Log mol/L)
Distribution	Volume of Distribution	0.269	0.102	0.282	0.039	Numerical (Log L/kg)
Metabolism	CYP3A4 Substrate	Yes	Yes	Yes	Yes	Categorical (Yes/No)
Excretion	Total clearance	0.498	0.618	0.562	0.61	Numerical (Log ml/min/kg)
Toxicity	Hepatotoxicity	No	No	No	No	Categorical (Yes/No)

Table-3 showed the rule of 5 for each phytosterol component predicted accordingly to Lipinski rule of five and met all the requirements whereas molecular weight should be below < 500; Log p indicated that four compounds acting more hydrophobic due to its value above 5. The number of donors and acceptor of protein are all molecule acceptable in the requirements whereas proton donor ≤ 5 as for acceptor proton ≤ 10. The number of pose strike more for sitosterol to reach its ligand respective target molecule and met the requirement < 10, while particle surface area is the same for each component. This proves that four components have almost the same high permeability and are easier to absorb based on the rules of five Lipinski.[17-19]

Table-3: Lipinski Rules of Five Prediction

Compound	Molecular weight	Log P	DP/AP	Rotb	TPSA
Sitosterol	456.80	8.95	1/1	6	20.23
Stigmasterol	412.70	7.87	1/1	5	20.23
Campesterol	400.69	8.30	1/1	5	20.23
28-Isofucosterol	412.70	7.69	1/1	5	20.23

*log P = Partition coefficient; DP/AP = Proton Donor/ Acceptor Donor; Rotb = number of Rotational Bond; TPSA = Total Particle Surface Area

Table-4 showed that each respective phytosterol compound identified from FR has a free affinity binding value of about -13.02 kcal/mol for stigmasterol indicating that it gave a strong and stable binding but low inhibition affinity of 286.75 pM. Meanwhile, sitosterol even though the lowest free energy binding values have better binding quality due to it had an attachment with two hydrogen bonds namely GLY462 and ARG415, indicated the abundance of hydrogen bond for sitosterol provide an inhibition activity to regulate Nrf2 intake in nuclear.

Table-5 and Figure-2 showed the number of hydrogen bonds of each phytosterol compound with 2FLU in some mostly attached to ARG415, differ from stigmasterol whose chemical structure could provide van der walls bond with GLY462. The alkyl and Pi-alkyl chains through binding with receptors still provide a weak

bond and do not contribute much to binding stability, as for sitosterol due to its covalent bond presence within TYR525 and TYR527 may provide a good affinity and more stable attachment toward receptors making it a good competitive inhibitor amongst the other three compounds

Table-4: *In silico* Result of Each Phytosterol Against 2FLU

No	Ligand	Inhibition Affinity (pM)	Free Energy (kcal/mol)
1	Sitosterol	522.97	-8.57
2	Stigmasterol	286.75	-13.02
3	Campesterol	620.16	-12.56
4	28-Isofucosterol	369.55	-12.87

Table-5: Amino Acids Interaction Between Phytosterol Compounds With 2FLU

Compound	Chemical bonds				
	Hydrogen Bond	Van Der Walls	Alkyl	Pi-Alkyl	Covalent
Stigmasterol	ARG415	GLY462`	CYS513 VAL514 ALA466 VAL467 VAL418 CYS368	ILE559 ALA366 VAL606	-
Sitosterol	ARG380	-	TYR334 ALA556	PHE577	TYR572 TYR525
Campesterol	ARG415	-	ALA486 CYS368 VAL418	ILE559 VAL606	-
Isofucosterol	ARG415	-	ALA366 ALA607 CYS368	CYS513 VAL465 ILE559	-

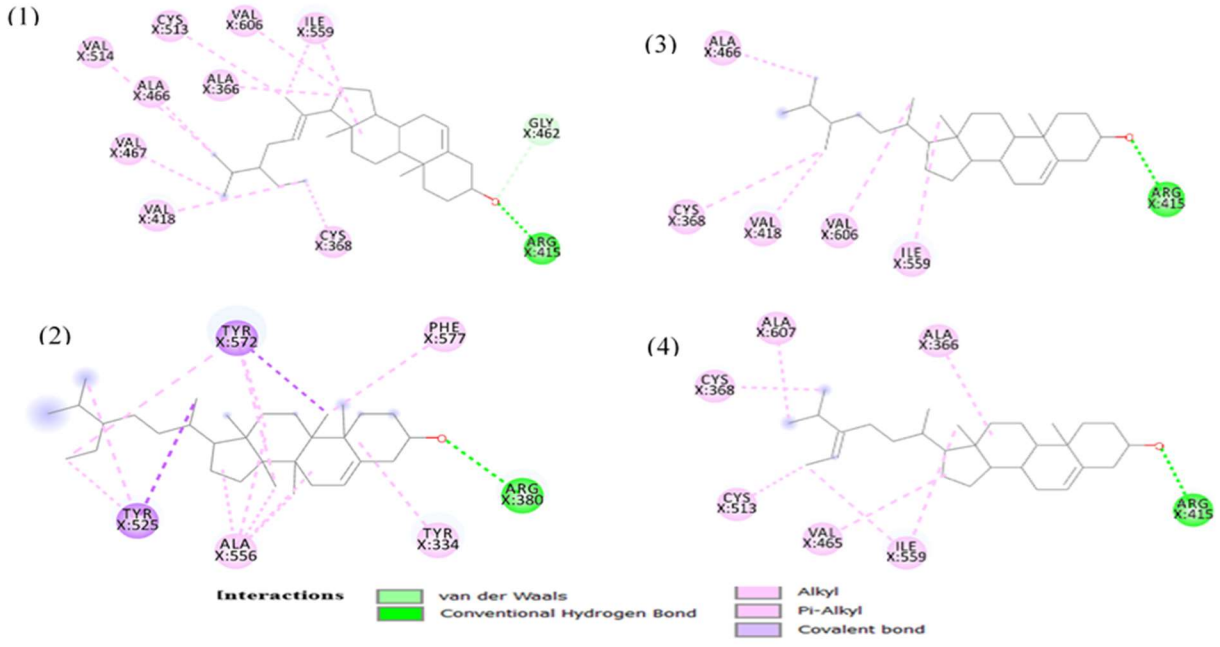

Fig.-2: 2D Interaction between Amino Acids and Phytosterol Compounds with 2FLU

CONCLUSION

According to the findings of this research, it can be concluded that consuming phytosterol from FR as a supplement during tumor/cancer incidence could provide primary or secondary chemopreventive to prevent

any possibility of malignant occurrence for the subject. These phytosterols act as competitive agents to counteract against keap1 for Nrf2 to promote more antioxidant genes during stressed conditions, thus furthering the possibilities as primary.

ACKNOWLEDGMENT

The authors are grateful to the Faculty of Pharmacy, Universitas Sumatera Utara to provide the means and facilities for the support in this study.

REFERENCES

1. T. Dorai and B.B. Aggarwal, *Cancer Letters,* **215(2)**, 129(2004), https://doi.org/10.1016/j.canlet.2004.07.013
2. V.R. Ramprasath and A.B. Awad, *Journal of AOAC International,* **98(3)**, 735(2015), https://doi.org/10.5740/jaoacint.SGERamprasath
3. K.R. Landis-Piwowar and N.R. Iyer, *Cancer Growth Metastasis*, **7**, 19(2014), https://doi.org/10.4137/CGM.S11288
4. D. Singh, B. Singh and R.K. Goel, *Journal of Ethnopharmacology,* **134(3)**, 565(2011), https://doi.org/10.1016/j.jep.2011.01.046
5. V. Gulecha and T. Sivakuma, *Asian Pacific Journal of Tropical Medicine,* **4(7)**, 526(2011), https://doi.org/10.1016/S1995-7645(11)60139-9
6. M.K. Kwak, N. Wakabayashi, and T.W. Kensler, *Journal of Biological Chemistry,* **278(10)**, 8135(2003), https://doi.org/10.1074/jbc.M211898200
7. I. Iksen, S. Pothongsrisit, and V. Pongrakhananon, *Molecules*, **26(13)**, 1(2021), https://doi.org/10.3390/molecules26134100
8. C. Isaac, R. Narayanaswamy, and K. Vallivitan, *Rasayan Journal of Chem*istry, **14(1)**, 659(2021), http://dx.doi.org/10.31788/RJC.2021.1416107
9. F.M. Ibrahim, H.A. Holik, and A. Achmad, *Rasayan Journal of Chem*istry, **14(3)**, 1469(2021), http://doi.org/10.31788/RJC.2021.1436172
10. B.K. Tiwari, N.P. Brunton, and C.S. Brennan, Handbook of Plant Food Phytochemicals: Source, Stability and Extraction, John Wiley & Sons Ltd, West Sussex, p.1,4 (2013).
11. S.B. Chandrasekar, M. Bhanumathy, A.T. Pawar, and T. Somasundaram, *Pharmacognocy Reviews,* **4(8)**, 195(2010), http://doi.org/10.4103/0973-7847.70918
12. A.S. Choudhari, S.A. Suryavanshi, and R. Kaul-Ghanekar, *Plos One*, **8(7)**, 70127(2013), https://doi.org/10.1371/journal.pone.0070127
13. W.V. Berghe, *Pharmacological Research,* **65(6)**, 565 (2012), https://doi.org/10.1016/j.phrs.2012.03.007
14. S. Shankar, D. Kumar, and R.K. Srivastava, *Pharmacology & Therapeutics,* **138(1)**, 1(2013), https://doi.org/10.1016/j.pharmthera.2012.11.002
15. D.E.V. Pires, T.L. Blundell, and D.B. Ascher, *Journal of Medicinal Chemistry,* **58(9)**, 40166(2015), https://doi.org/10.1021/acs.jmedchem.5b00104
16. R.A. Syahputra, U. Harahap, A. Dalimunthe, P. Nasution, G. Haro, W. Widodo, et al., *Rasayan Journal of Chemistry*, **13(2)**, 1220(2020), http://dx.doi.org/10.31788/RJC.2020.1325638
17. C.A. Lipinski, *Drug Discovery Today: Technol*ogies, **1(4)**, 337(2004), https://doi.org/10.1016/j.ddtec.2004.11.007
18. C.M. Chagas, S. Moss, and L. Alisaraie, *International Journal of Pharmaceutics,* **5(1-2)**, 133(2018), https://doi.org/10.1016/j.ijpharm.2018.07.046
19. S. Johansson, A. Thakkar, T. Kogej, E. Bjerrum., S. Genheden, T. Bastys, et al., *Drug Discovery Today: Technol*ogies, **32-33**, 65(2019), https://doi.org/10.1016/j.ddtec.2020.06.002

[RJC-6801/2021]