Current Pharmacology Reports (2020) 6:429–440
https://doi.org/10.1007/s40495-020-00241-6

CANCER CHEMOPREVENTION (R AGARWAL, CV RAO AND S YU, SECTION EDITORS)

# Molecular Docking, Drug Likeness, and ADMET Analyses of Passiflora Compounds as P-Glycoprotein (P-gp) Inhibitor for the Treatment of Cancer

Serap Yalcin[1]

Accepted: 8 September 2020 / Published online: 18 September 2020


## Abstract

Cancer disease leads to deaths worldwide. Anti-cancer drugs have a high prevalence of side effects and cause multidrug resistance (MDR) that remains a significant barrier to major cancer therapy. To date, chemical and herbal substances have been analyzed for their MDR modulatory activity. However, research on new and safe molecules has been continued to overcome MDR in cancer. The plant compounds can be an effective inhibitor for successful cancer therapy. Recently, computational models have gained importance to discover new inhibitors. In the present study, we aimed to explore the various compounds of Passiflora species as P-gp inhibitor. P-gp protein was docked with the active substrate and inhibitor, respectively, including tamoxifen and verapamil. Besides, 3D structure of P-gp was docked with 11 compounds (luteolin, beta amyrin, beta-sitosterol, chimaphilin, chrysin, edulan I and II, apigenin, oleanolic acid, stigmasterol, hydroxyflavone) of plant origin using AutoDock4.2 program. Furthermore, the compounds were analyzed for ADMET and drug likeness properties of compounds determined as Lipinski, Veber, and Ghose's rules (http://www.swissadme.ch/). As obtained molecular docking analysis results, luteolin, chrysin, hydroxyflavone, and apigenin may be a candidate for being P-gp inhibitor. Hence, it may be of attention to consider these compounds for further in vitro and in vivo evaluation.

**Keywords** P-gp inhibitor · Passiflora · Molecular docking · Drug likeness · ADMET

## Introduction

Traditional cancer therapies are surgery, radiation therapy, and chemotherapy, or their combinations [1]. Chemotherapy generally is more difficult important in the treatment of metastatic malignancies and it also causes multiple drug resistance (MDR) and side effects on healthy cells [2]. MDR demonstrates a large field of resistance against functionally and structurally unrelated chemotherapeutic agents [3, 4], is the ability of cancer cells to escape and to survive from chemotherapeutics in cancer therapy, and this situation seriously disrupts the success of cancer chemotherapy [4–7].

ATP-binding cassette transporters (ABC transporters) are a complicated pump superfamily, in which substrate is transported across membranes against a concentration gradient in the efflux of small molecule drugs [8–11]. P-glycoprotein (P-gp) is one of the well-described ABC transporters which are currently considered to be one of the important barriers in cancer therapy [12]. P-gp has an important role in drug resistance and its overexpression has been associated with the MDR, so it has become a therapeutic target to overcome MDR [7, 9].

Since prehistoric times, flowers, berries, roots, and leaves of herbals have great importance and they have been used in traditional natural medicine, natural products have a key role in the discovery of new drugs, and they have been in constant use in therapy of different diseases [13]. Passiflora species are also one of the natural products. Studies have reported various pharmacological activity of Passiflora species including antioxidant [14] and anti-tumor [15] effects.

Recently, computational methods are a rapidly growing area and play an important role in drug discoveries in medicine and therapeutics [16]. Molecular dynamic, pharmacophore modeling, QSAR, and docking analyses can determine protein-ligand interaction, structural changes, binding sites, drug candidates, etc. [17–21]. Prompted by this, in the present study, we investigated new potential inhibitors of P-gp from compounds of Passiflora species with molecular docking analyses.

This article is part of the Topical Collection on *Cancer Chemoprevention*

✉ Serap Yalcin
syalcin@ahievran.edu.tr

[1] Faculty of Sciences and Arts, Department of Molecular Biology and Genetics, Kırsehir Ahi Evran University, Kırsehir, Turkey

## Material and Method

### Molecular Structure Preparing

To analyze the molecular docking between P-gp and potential inhibitors, we used the P-gp structures (PDB code: 6c0v) which were found by Kim and Chen et al. at the resolutions of 3.4 Å. The PDB file for P-gp proteins was obtained from the RCSB Protein Data Bank (available at http://www.rcsb.org). The water and other molecules were removed from P-gp protein, and then, only 3D structure of P-gp (Fig. 1) was hidden as pdb. file. 3D structures of 11 ligand molecules, including luteolin, beta amyrin, beta-sitosterol, chimaphilin, chrysin, edulan I and II, apigenin, oleanolic acid, stigmasterol, hydroxyflavone, and control drugs (tamoxifen and verapamil), were detected for molecular docking from Pubchem (https://pubchem.ncbi.nlm.nih.gov/) (Table 1).

### Molecular Docking Analyses

In this study, we performed AutoDock-Version 4.2 (http://autodock.scripps.edu) to analyze molecular docking. The AutoDock is designed as computational docking tools for the prediction of protein-ligand interaction [Morris et al. 1998]. Molecular docking calculations were analyzed via Lamarckian Generic Algorithm [22] in Autodock Vina [23, 24]. All bound water molecules and nonprotein molecules were removed from the proteins, non-polar hydrogen atoms were merged, and the polar hydrogen atoms were added. The Molegro Molecular Viewer 2.5 (Molegro Molecular viewer academic free software) and VMD (Visual Molecular Dynamic) [24] programs were used in the visualization of protein-ligand interaction [25].

### Drug Likeness and ADME Analysis

Recently, in silico ADMET analyses are gaining attention in computer-based drug discovery [26]. ADMET analyses are used to determine the pharmacological structure from the perspective of drug discovery (http://biosig.unimelb.edu.au/pkcsm/prediction). Pharmacokinetics and drug likeness prediction for compounds were also performed by online tool SwissADME (http://www.sib.swiss) (http://www.swissadme.ch/index.php) [27, 28]. In addition, pharmacokinetics and drug likeness predictions have been applied on Lipinski, Ghose, and Veber rules and bioavailability scores [29–31].

## Results and Discussion

Cancer is a complex disease, and multiple drug resistance is a major drawback in cancer therapy. Therefore, the design and development of new drugs are becoming increasingly necessary. P-gp is a significant factor of MDR because its overexpression is associated with increased efflux of cancer drugs in cancer [10]. Here, we aimed at the discovery of new drug compounds with computer-based analyses and presented an opportunity for further experimental analysis.

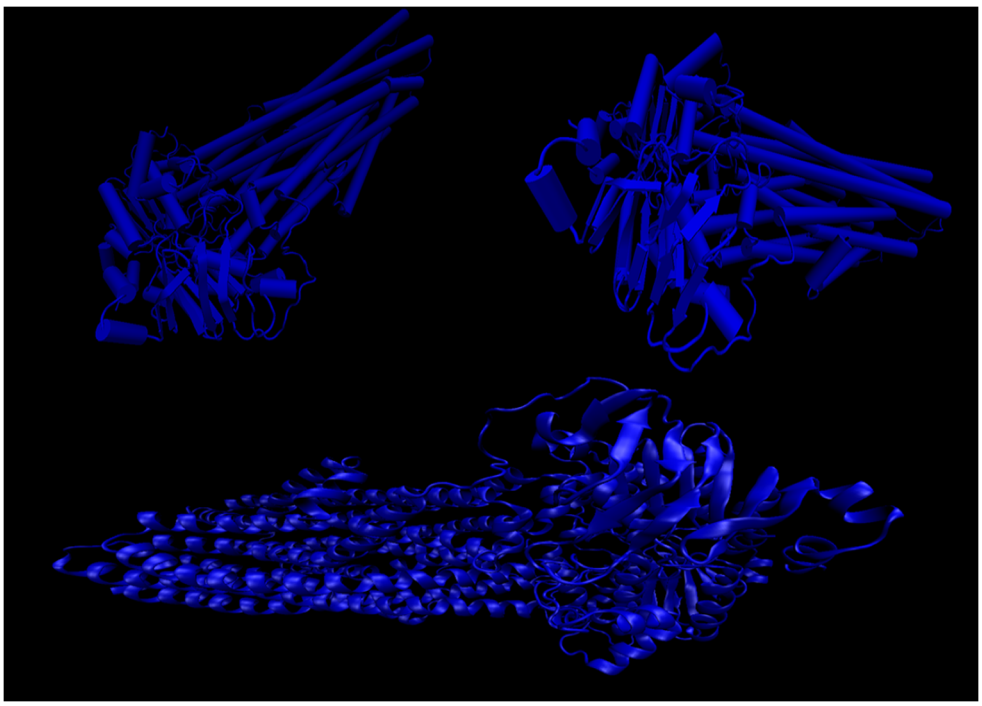


**Fig. 1** 3D structure of P-gp

**Table 1** Ligands used in the study and their properties

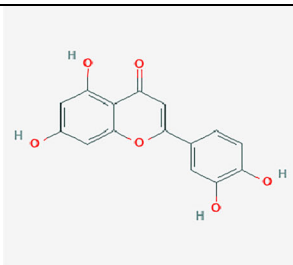
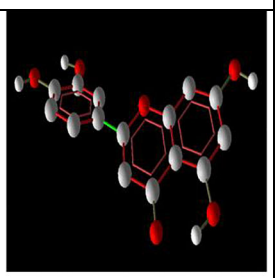
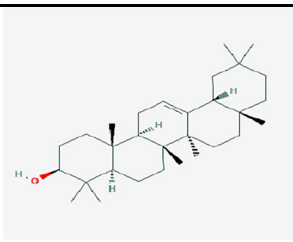
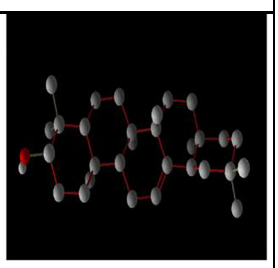
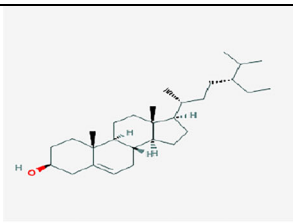
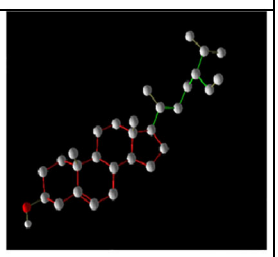
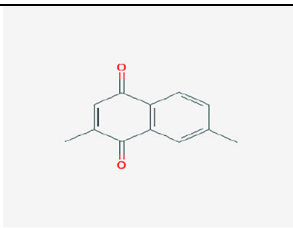
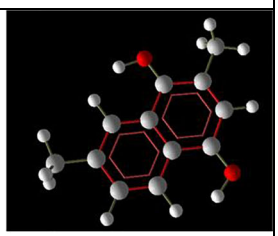
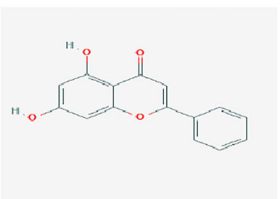
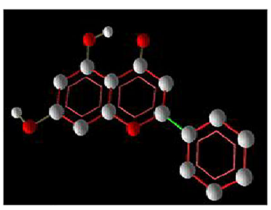
| No | Ligands | PubChem ID code | Molecular weight ($g.mol^{-1}$) | Structure(2D) | Structure(3D) |
|---|---|---|---|---|---|
| 1 | Luteolin | 5280445 | 286.24 g/mol |  |  |
| 2 | Beta -Amyrin | 73145 | 426.7 g/mol |  |  |
| 3 | Beta-Sitosterol | 222284 | 414.7 g/mol |  |  |
| 4 | Chimaphilin | 101211 | 186.21 g/mol |  |  |
| 5 | Chrysin | 5281607 | 254.24 g/mol |  |  |

**Table 1** (continued)

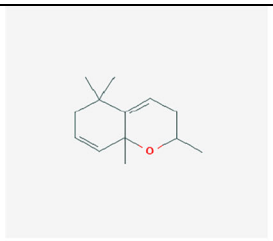
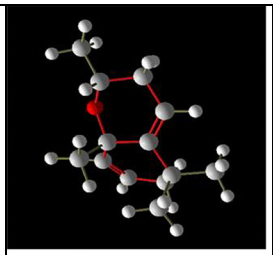
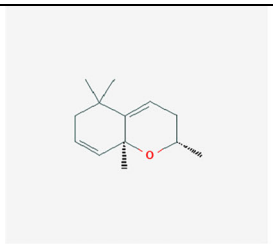
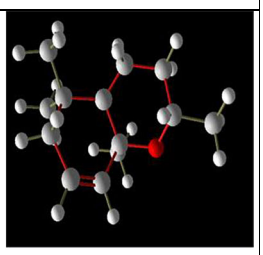
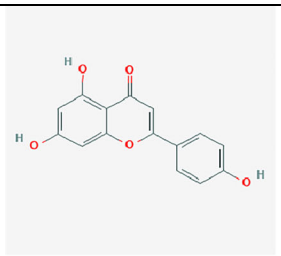
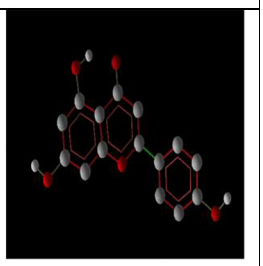
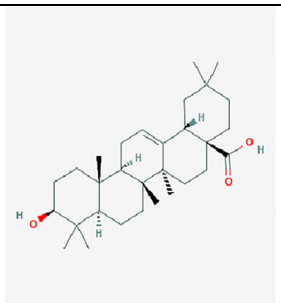
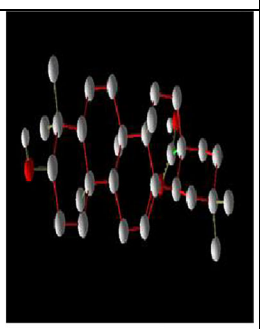
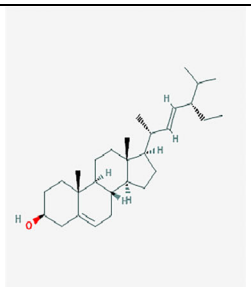
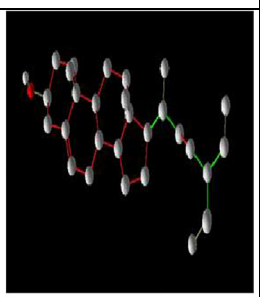
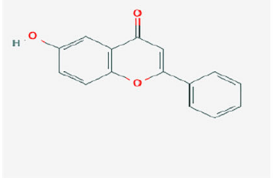
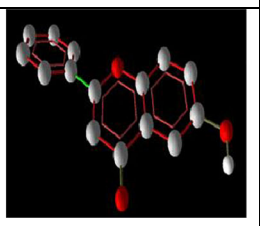
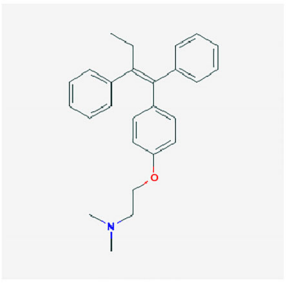
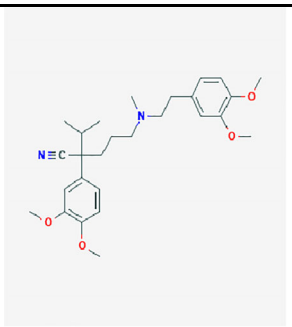
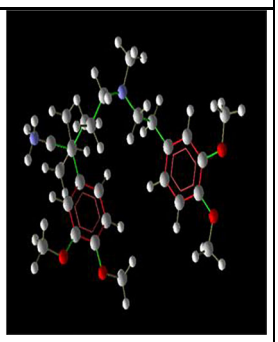
| | | | | | |
|---|---|---|---|---|---|
| 6 | Edulan I | 521066 | 192.3 g/mol |  |  |
| 7 | Edulan II | 6432428 | 192.3 g/mol |  |  |
| 8 | Apigenin | 5280443 | 270.24 g/mol |  |  |
| 9 | Oleanolic Acid | 10494 | 456.7 g/mol |  |  |
| 10 | Stigmasterol | 5280794 | 412.7 g/mol |  |  |
| 11 | Hydroxyflavone | 72279 | 238.24 g/mol |  |  |

**Table 1** (continued)

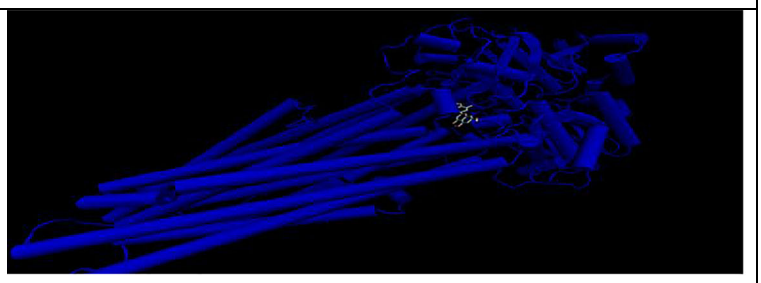
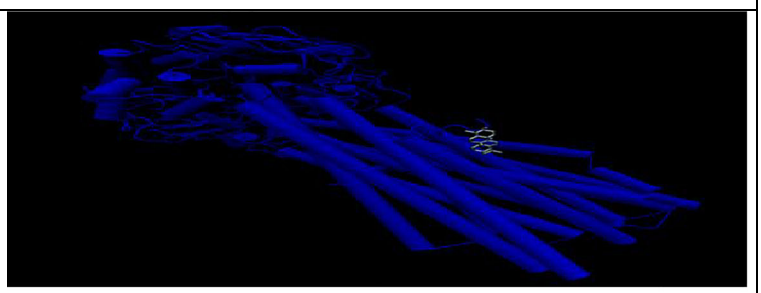
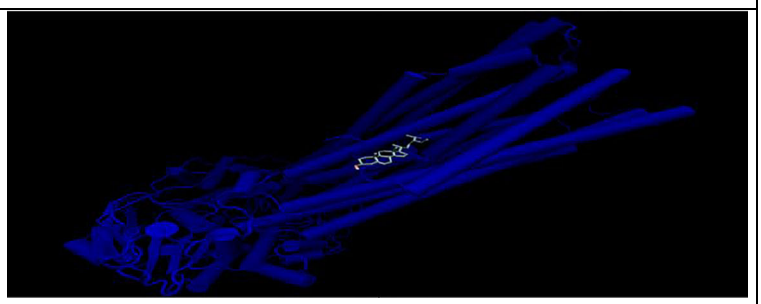
| | | | | | |
|---|---|---|---|---|---|
| 12 | Tamoxifen | 2733526 | 371.5 g/mol |  |  |
| 13 | Verapamil | 2520 | 454.6 g/mol |  |  |

The search for herbal compounds cannot be easy to use them for experiments in vitro and in vivo. Recently, predicted data of these compounds were obtained by applying computer-based studies. The absorption, distribution, metabolism, elimination, and toxicity (ADMET) analysis have a big importance in drug discovery studies. In silico ADMET predictions have been designed to evaluate the pharmacokinetic and toxicity properties. In present work, human intestinal absorption, aqueous solubility levels, BBB penetration levels, CYP inhibition, hepatotoxicity, etc. of luteolin, beta amyrin, beta-sitosterol, chimaphilin, chrysin, edulan I and II, apigenin, oleanolic acid, stigmasterol, hydroxyflavone, and control drugs (tamoxifen and verapamil) were determined. ADMET and pharmacokinetics results are presented in the supplementary file (supplementary data). ADMET analysis shows that most of the compounds are predicted good human intestinal absorption, no toxicity, and water solubility.

**Table 2** A drug likeness results of potential inhibitors

| Ligand | Drug likeness | | | Bioavailability Score |
|---|---|---|---|---|
| | Lipinski | Ghose | Veber | |
| Luteolin | Yes | Yes | Yes | 0.55 |
| Beta-amyrin | Yes 1 violation: MLOGP > 4.15 | No 3 violations: WLOGP > 5.6, MR > 130, #atoms > 70 | Yes | 0.55 |
| Beta-sitosterol | Yes 1 violation: MLOGP > 4.15 | No 3 violations: WLOGP > 5.6, MR > 130, #atoms > 70 | Yes | 0.55 |
| Chimaphilin | Yes | Yes | Yes | 0.55 |
| Chrysin | Yes | Yes | Yes | 0.55 |
| Edulan I | Yes | Yes | Yes | 0.55 |
| Edulan II | Yes | Yes | Yes | 0.55 |
| Apigenin | Yes | Yes | Yes | 0.55 |
| Oleanolic acid | Yes 1 violation: MLOGP > 4.15 | No 3 violations: WLOGP > 5.6, MR > 130, #atoms > 70 | Yes | 0.56 |
| Stigmasterol | Yes 1 violation: MLOGP > 4.15 | No 3 violations: WLOGP > 5.6, MR > 130, #atoms > 70 | Yes | 0.55 |
| Hydroxyflavone | Yes | Yes | Yes | 0.55 |

**Table 3** Protein-ligand molecular docking results

| Protein | Ligand | Binding Energy (kcal/mol) | Interaction |
|---|---|---|---|
| **P-gp** | Luteolin | -10.7 kcal/mol |  |
| **P-gp** | Beta -Amyrin | -10.0 kcal/mol |  |
| **P-gp** | Beta-Sitosterol | -8.7 kcal/mol |  |

In addition, drug likeness results of potential inhibitors are shown in Table 2. According to Lipinski's rule (Pfizer's rule, Lipinski's rule of five, RO5), the active drug has no more than one violation of the following properties including molecular weight (MW) ≤ 500, LogP ≤ 5, hydrogen bond acceptors ≤ 10, and hydrogen bond donors ≤ 5 [29]. According to Veber rules, the active drug has total hydrogen bonds ≤ 12, rotatable bonds ≤ 10, and polar surface area (PSA). Polar surface area ≤ 140 tend to have oral bioavailability ≥ 20% [30]. According to Ghose rules, active drug has Log P(− 0.4~5.6), MR (molar refractivity (40~150), MW (160~480), number of atoms (20~70), and polar surface area (PSA) < 140 [31]. Based on the drug likeness analysis, all the compounds were found by the Lipinski's and Veber rule. Furthermore, luteolin, chimaphilin, chrysin, edulan I, edulan II, apigenin, and hydroxyflavone complied with Ghose's rules.

To better understand interaction with P-gp of luteolin, beta amyrin, beta-sitosterol, chimaphilin, chrysin, edulan I and II, apigenin, oleanolic acid, stigmasterol, and hydroxyflavone compounds, a molecular docking analysis was performed by Autodock-Vina program. For this purpose, tamoxifen and verapamil were selected as reference drugs. The general properties of molecules are described in Table 1. The results of molecular docking analyses of 11 compounds and the number of hydrogen bonds are summarized in Tables 3 and 4. In the procedure, luteolin, beta amyrin, beta-sitosterol, chimaphilin, chrysin, edulan I and II, apigenin, oleanolic acid, stigmasterol, and hydroxyflavone were docked to the proteins with a binding free energy of − 10.7, − 10.0, − 8.7, − 6.8, − 8.6, − 6.2, − 7.5, − 8.1, − 8.9, − 8.6, and − 8.7 kcal $mol^{-1}$, respectively. For P-gp [32] protein and luteolin interaction, five hydrogen bonds were identified with amino acid residue Thr 1174, Phe 904, Arg 905, Asp 167, and Val 168. In human, the maximum number of hydrogen bond interactions was detected between luteolin and P-gp protein. In the P-gp protein and apigenin interaction, hydrogen bonds can be observed with residue Tyr 1044, Ser 1077, and Lys 1076. Hydrogen bonds of other ligands and P-gp interaction are shown in Table 4.

**Table 3** (continued)

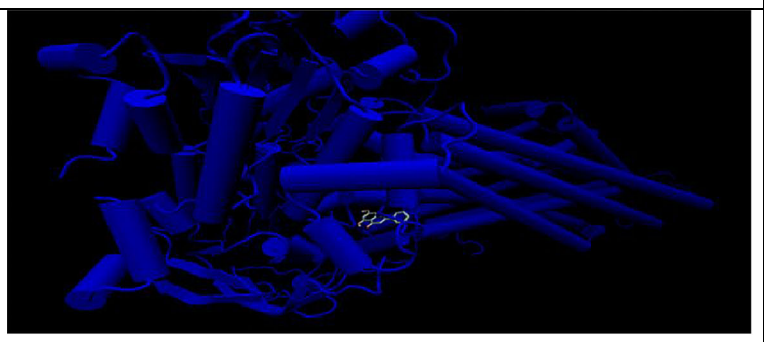
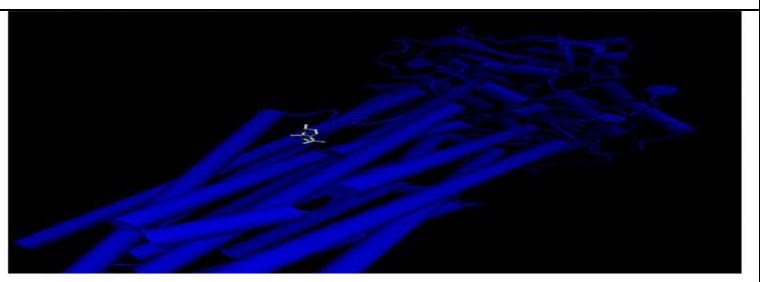
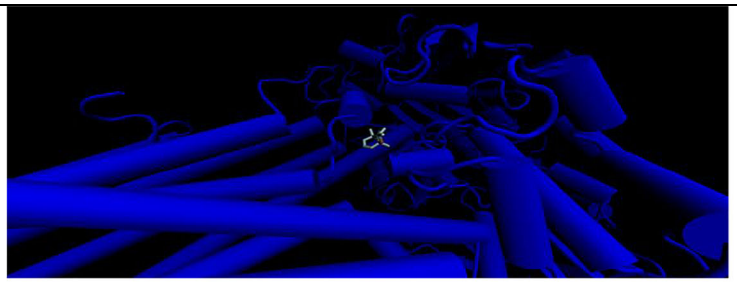
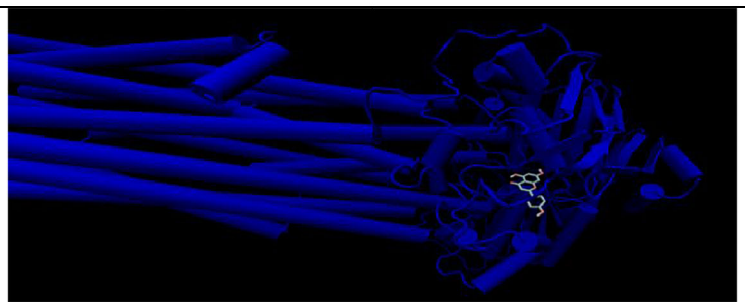
| | | | |
|---|---|---|---|
| **P-gp** | Chimaphilin | -6.8 kcal/mol |  |
| **P-gp** | Chrysin | -8.6 kcal/mol |  |
| **P-gp** | Edulan I | -6.2 kcal/mol |  |
| **P-gp** | Edulan II | -7.5 kcal/mol |  |
| **P-gp** | Apigenin | -8.1 kcal/mol |  |

**Table 3** (continued)

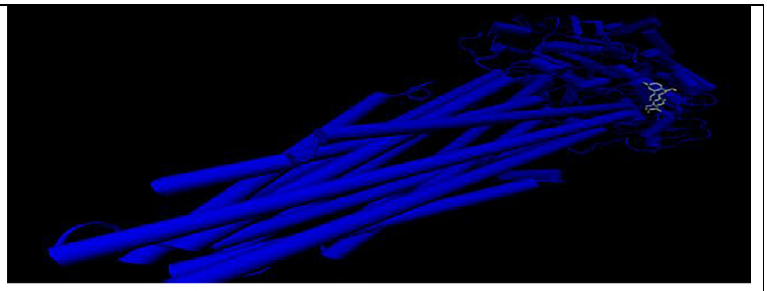
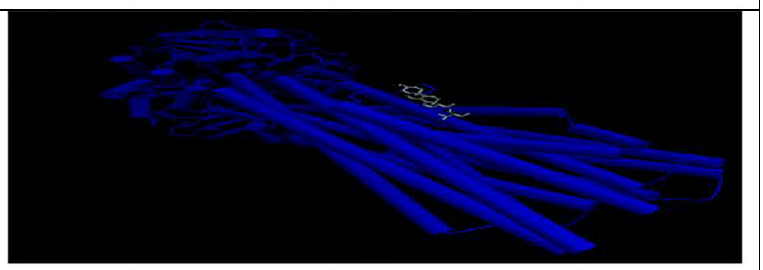
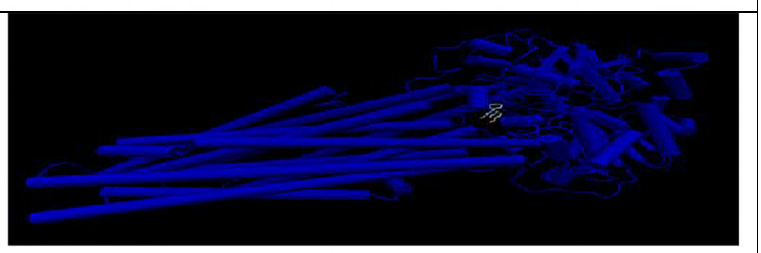
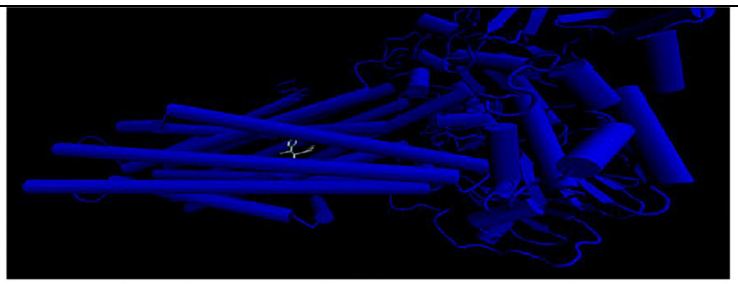
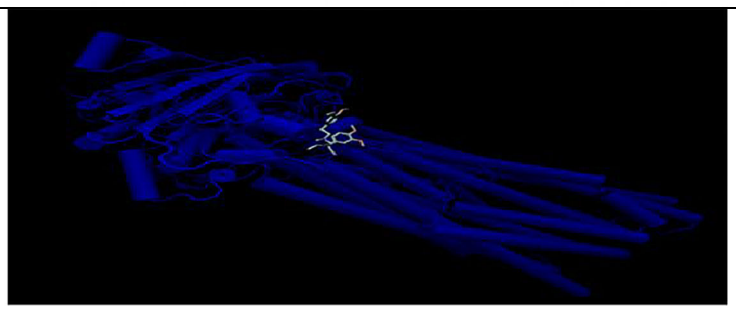
| | | | |
|---|---|---|---|
| **P-gp** | Oleanolic Acid | -8.9 kcal/mol |  |
| **P-gp** | Stigmasterol | -8.6 kcal/mol |  |
| **P-gp** | Hydroxyflavone | -8.7 kcal/mol |  |
| **P-gp** | Tamoxifen | -8.8 kcal/mol |  |
| **P-gp** | Verapamil | -6.8 kcal/mol |  |

**Table 4** Hydrogen bonds between ligands and P-gp protein

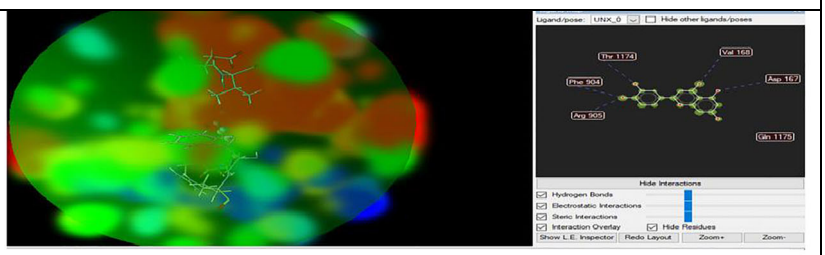
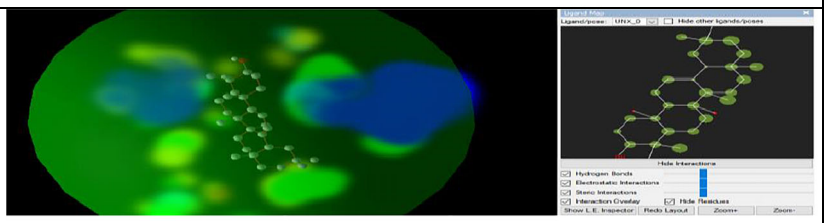
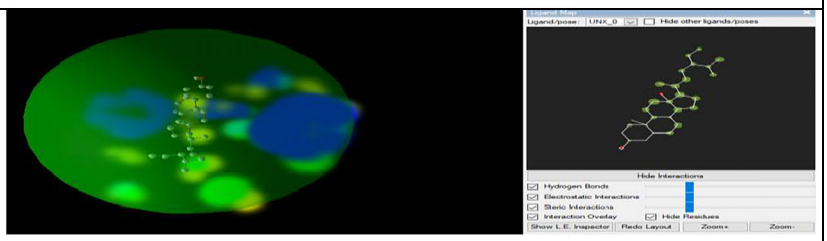
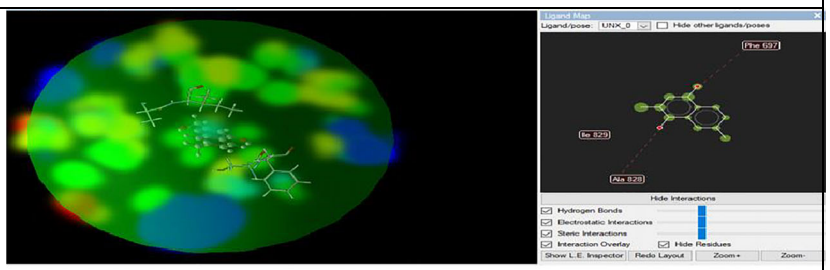
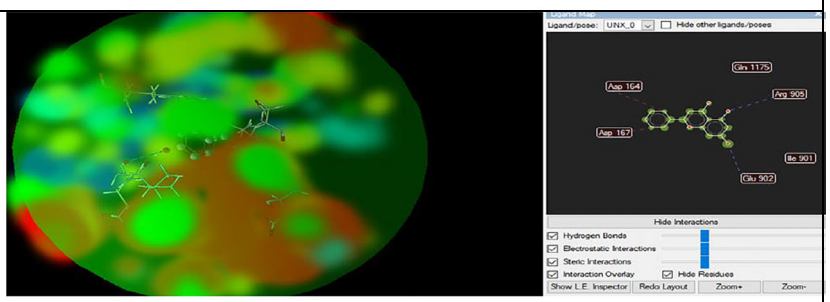
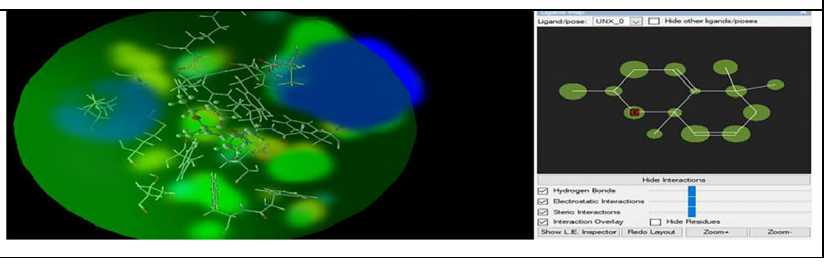
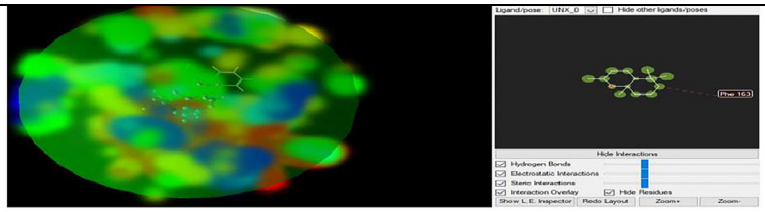
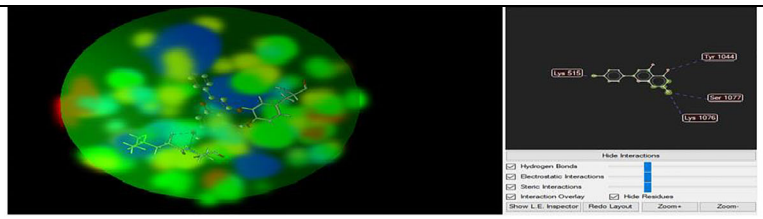
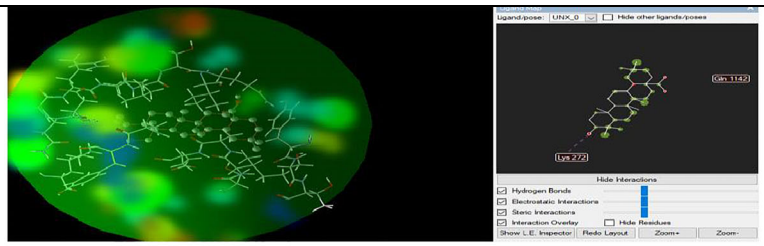
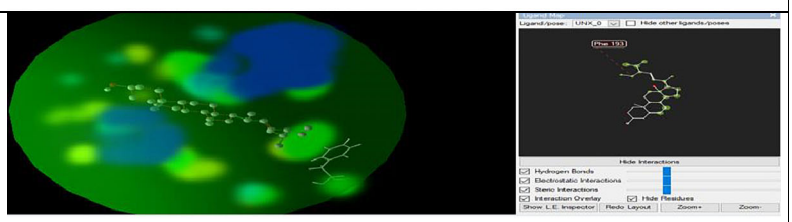
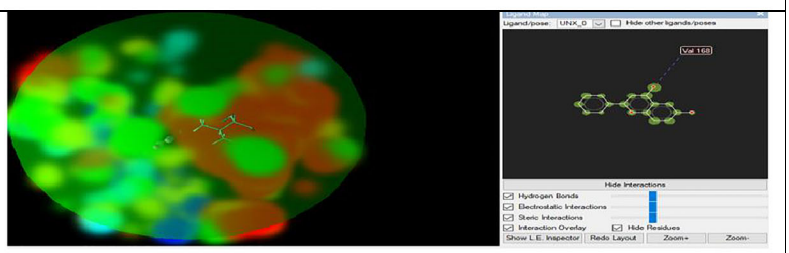
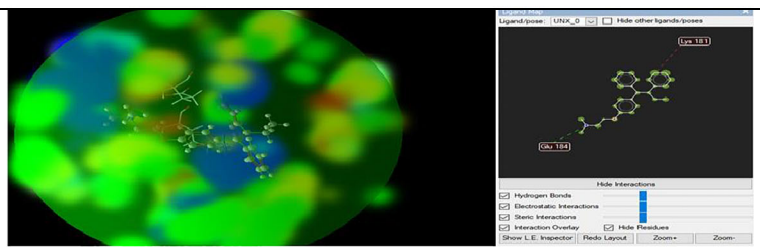
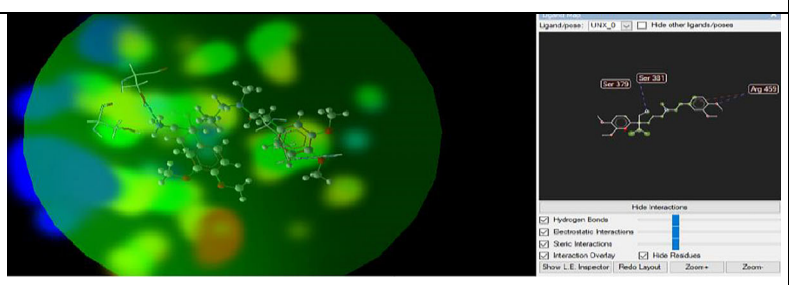
| Protein | Ligand | H bound | Ligand-protein interaction |
|---|---|---|---|
| **P-gp** | Luteolin | 5 |  |
| **P-gp** | Beta -Amyrin | 0 |  |
| **P-gp** | Beta-Sitosterol | 0 |  |
| **P-gp** | Chimaphilin | 0 |  |
| **P-gp** | Chrysin | 2 |  |
| **P-gp** | Edulan I | 0 |  |

**Table 4** (continued)

| | | | |
|---|---|---|---|
| **P-gp** | Edulan II | 0 |  |
| **P-gp** | Apigenin | 3 |  |
| **P-gp** | Oleanolic Acid | 0 |  |
| **P-gp** | Stigmasterol | 0 |  |
| **P-gp** | Hydroxyflavone | 1 |  |
| **P-gp** | Tamoxifen | 0 |  |
| **P-gp** | Verapamil | 2 |  |

## Conclusion

The objective of this work was to obtain and evaluate molecular docking, predicted drug likeness, and ADMET analyses in potential compounds of Passiflora species. The binding energies, ADMET, and drug likeness for ligands were compared with the control drug, tamoxifen, and verapamil. As a result, luteolin, chrysin, apigenin, and hydroxyflavone may be potential inhibitors for P-gp and be helpful in cancer therapy.

## References


1. Yura Y. Presage of oncolytic virotherapy for oral cancer with herpes simplex virus. Jpn Dent Sci Rev. 2017;53:53–60. https://doi.org/10.1016/j.jdsr.2016.10.001.
2. Pluchino KM, Hall MD, Goldsborough AS, Callaghan R, Gottesman MM. Collateral sensitivity as a strategy against cancer multidrug resistance. Drug Resist Updat. 2012;15:98–105. https://doi.org/10.1016/j.drup.2012.03.002.
3. Saunders NA, Simpson F, Thompson EW, Hill MM, Endo-Munoz L, Leggatt G, et al. Role of intratumoural heterogeneity in cancer drug resistance: molecular and clinical perspectives. EMBO Mol Med. 2012;4(8):675–84. https://doi.org/10.1002/emmm.201101131.
4. Gottesman MM, Fojo T, Bates SE. Multidrug resistance in cancer: role of ATP-dependent transporters. Nat Rev Cancer. 2002;2(1):48–58. https://doi.org/10.1038/nrc706.
5. Chai S, To KK, Lin G. Circumvention of multi-drug resistance of cancer cells by Chinese herbal medicines. Chin Med. 2010;5:26. https://doi.org/10.1186/1749-8546-5-26.
6. Wang J, Seebacher N, Shi H, Kan Q, Duan Z. Novel strategies to prevent the development of multidrug resistance (MDR) in cancer. Oncotarget. 2017;8(48):84559–71. https://doi.org/10.18632/oncotarget.19187.
7. Iyer AK, Singh A, Ganta S, Amiji MM. Role of integrated cancer nanomedicine in overcoming drug resistance. Adv Drug Deliv Rev. 2013;65(13–14):1784–802. https://doi.org/10.1016/j.addr.2013.07.012.
8. Moitra K. Overcoming multidrug resistance in cancer stem cells. Biomed Res Int. 2015;2015:635745. https://doi.org/10.1155/2015/635745.
9. Jabr-Milane LS, van Vlerken LE, Yadav S, Amiji MM. Multi-functional nanocarriers to overcome tumor drug resistance. Cancer Treat Rev. 2008;34(7):592–602. https://doi.org/10.1016/j.ctrv.2008.04.003.
10. Gillet JP, Efferth T, Remacle J. Chemotherapy-induced resistance by ATP-binding cassette transporter genes. Biochim Biophys Acta. 2007;1775(2):237–62. https://doi.org/10.1016/j.bbcan.2007.05.002.
11. Allikmets R, Gerrard B, Hutchinson A, Dean M. Characterization of the human ABC superfamily: isolation and mapping of 21 new genes using the expressed sequence tags database. Hum Mol Genet. 1996;5(10):1649–55. https://doi.org/10.1093/hmg/5.10.1649.
12. Nobili S, Landini I, Giglioni B, Mini E. Pharmacological strategies for overcoming multidrug resistance. Curr Drug Targets. 2006;7(7):861–79. https://doi.org/10.2174/138945006777709593.
13. Yuan H, Ma Q, Ye L, Piao G. The traditional medicine and modern medicine from natural products. Molecules. 2016;21(5):559. https://doi.org/10.3390/molecules21050559.
14. Lourith N, Kanlayavattanakul M. Antioxidant activities and phenolics of Passiflora edulis seed recovered from juice production residue. J Oleo Sci. 2013;62(4):235–40. https://doi.org/10.5650/jos.62.235.
15. Silva DC, Freitas ALP, Barros FCN, Lins KO, Alves APN, Alencar NM, et al. Polysaccharide isolated from *Passiflora edulis*: characterization and antitumor properties. Carbohydr Polym. 2012;87(1):139–45. https://doi.org/10.1016/j.carbpol.2011.07.029.
16. Brewer FK, Follit CA, Vogel PD, Wise JG. In silico screening for inhibitors of p-glycoprotein that target the nucleotide binding domains. Mol Pharmacol. 2014;86(6):716–26. https://doi.org/10.1124/mol.114.095414.
17. McCormick JW, Vogel PD, Wise JG. Multiple drug transport pathways through human P-glycoprotein. Biochemistry. 2015;54(28):4374–90. https://doi.org/10.1021/acs.biochem.5b00018.
18. Prajapati R, Singh U, Patil A, Khomane KS, Bagul P, Bansal AK, et al. In silico model for P-glycoprotein substrate prediction: insights from molecular dynamics and in vitro studies. J Comput Aided Mol Des. 2013;27(4):347–63. https://doi.org/10.1007/s10822-013-9650-x.
19. Shahraki O, Zargari F, Edraki N, Khoshneviszadeh M, Firuzi O, Miri R. Molecular dynamics simulation and molecular docking studies of 1,4-dihydropyridines as P-glycoprotein's allosteric inhibitors. J Biomol Struct Dyn. 2018;36(1):112–25. https://doi.org/10.1080/07391102.2016.1268976.
20. Pajeva IK, Globisch C, Wiese M. Combined pharmacophore modeling, docking, and 3D QSAR studies of ABCB1 and ABCC1 transporter inhibitors. Chem Med Chem. 2009;4(11):1883–96. https://doi.org/10.1002/cmdc.200900282.
21. Zhou S, Lim LY, Chowbay B. Herbal modulation of P-glycoprotein. Drug Metab Rev. 2004;36(1):57–104. https://doi.org/10.1081/dmr-120028427.
22. Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, et al. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. J Comput Chem. 1998;19(14):1639–62. https://doi.org/10.1002/(SICI)1096-987X(19981115)19:14<1639::AID-JCC10>3.0.CO;2-B.
23. Trott O, Olson AJ. Auto Dock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. 2010;31(2):455–61. https://doi.org/10.1002/jcc.21334.
24. Humphrey W, Dalke A, Schulten K. VMD: visual molecular dynamics. J Mol Graph. 1996;14(1):33–8, 27-8. https://doi.org/10.1016/0263-7855(96)00018-5.
25. Thomsen R, Christensen MH. MolDock: a new technique for high-accuracy molecular docking. J Med Chem. 2006;49(11):3315–21. https://doi.org/10.1021/jm051197e.
26. Ntie-Kang F, Lifongo LL, Mbah JA, Owono Owono LC, Megnassan E, Mbaze LM, et al. In silico drug metabolism and pharmacokinetic profiles of natural products from medicinal plants in the Congo basin. In Silico Pharmacol. 2013;1:12. https://doi.org/10.1186/2193-9616-1-12.
27. Zoete V, Daina A, Bovigny C, Michielin O. SwissSimilarity: a web tool for low to ultra high throughput ligand-based virtual screening. J Chem Inf Model. 2016;56:1399–404. https://doi.org/10.1021/acs.jcim.6b00174.
28. Daina A, Michielin O, Zoete V. Swissadme: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep. 2017;7:427–517. https://doi.org/10.1038/srep42717.

29. 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. 2001;46(1–3):3–26. https://doi.org/10.1016/s0169-409x(00)00129-0.
30. Veber DF, Johnson SR, Cheng HY, Smith BR, Ward KW, Kopple KD. Molecular properties that influence the oral bioavailability of drug candidates. J Med Chem. 2002;45(12):2615–23. https://doi.org/10.1021/jm020017n.
31. Ghose AK, Viswanadhan VN, Wendoloski JJ. A knowledge-based approach in designing combinatorial or medicinal chemistry libraries for drug discovery. 1. A qualitative and quantitative characterization of known drug databases. J Comb Chem. 1999;1(1):55–68. https://doi.org/10.1021/cc9800071.
32. Kim Y, Chen J. Molecular structure of human P-glycoprotein in the ATP-bound, outward-facing conformation. Science. 2018;359(6378):915–919. https://doi.org/10.1126/science.aar7389.