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Elucidation of the inhibitory potential of flavonoids against PKP1 protein in non-small cell lung cancer

Pratibha Pandey[1], Fahad Khan[1*], Prashant Chauhan[1], Tarun Kumar Upadhyay[2], Fevzi Bardakci[3], Md Jahoor Alam[3], Nujud Almuzaini[3], Rania Abdeen Hussain Abdalla[4], Sujeet Kumar Singh[1], Mohd Saeed[3*]

[1] Department of Biotechnology, Noida Institute of Engineering & Technology, Greater Noida 201306, India
[2] Department of Biotechnology, Parul Institute of Applied Sciences and Centre of Research for Development, Parul University, Vadodara 391760, India
[3] Department of Biology, College of Sciences, University of Hail, Hail, Saudi Arabia
[4] Department of Obstetrics and Gynecology, College of Medicine, University of Hail, Hail, Saudi Arabia

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ABSTRACT

PKP1 has been crucially involved in enhancing the MYC translation leading to lung carcinogenesis via evading numerous tumour-suppressing checkpoint systems. Plakophilin 1(PKP1) is the part of armadillo and plakophilin gene families and it is a necessary component of desmosomes. Several researches reported PKP1 protein as one of the most overexpressed proteins in human lung cancer. Therefore, we have designed our research towards elucidating better plant-based compounds as drug candidates for the management of lung cancer with minimal adverse effects over other chemotherapeutic drugs such as afatinib. This study comprises forty-six flavonoids for targeting PKP1 using *in silico* approaches that were not used earlier as an anti-cancerous agent targeting PKP1 in lung cancer treatment. Flavonoids are plant-derived natural compounds that exhibited enormous anti-cancerous potential against several human cancers. NPACT database was used to screen potent flavonoids that have not been used to target the PKP1 protein in lung cancer. Patch Dock and CB Dock were employed to elucidate the PKP1 (1XM9) inhibitory potential of selected flavonoids. Analysis with both the docking tools has revealed that calyxins I showed maximum affinity in comparison to the standard drug, afatinib. Further PASS and BAS analyses were performed using SWISS ADME and molinspiration to investigate the pharmacokinetic profiling of potent flavonoids having significant binding energy. Visualization of complexes was done by using UCSF chimera. However, further detailed in vitro studies are needed to validate the candidature of calyxinsI for being developed as an anticancer drug for the management of lung cancer.

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Introduction

Cancer is one of the most deadly diseases and the second leading cause of death across the globe(1). Many chemotherapeutic drugs have been elucidated by scientists to cure and prevent cancer. However, these drugs have various side effects on the human body such as low platelet count, skin infection, diarrhoea, hair loss, etc(2,3). One of major cancer that is having a high mortality rate with no specific cure is lung cancer(4). Cigarette smoking is the predominant cause of lung cancer(5,6). Despite diagnostic upgrades and remedial advances, cellular breakdown in the lungs remains deadly and around 18% of patients in developed nations stay alive up to five years of post-detection(1). Non-small cell lung cancer (NSCLC) is a significant subtype of lung cancer which represents around 30% of all lung cancers(7). To treat NSCLC, various traditional therapies are being utilized but exhibiting numerous severe side effects. Interestingly, PKP1 has become a potential oncotarget in the administration of several chemotherapeutic treatments(8,9). Previously published reports have revealed the overexpression of PKP1 gene encoding in human prostate and breast cancer(10). This multifunctional oncoprotein plays an essential role in the development of various carcinomas, including non-small cell lung cancer (NSCLC) (11,12). Thus, it is essential to search for novel flavonoids that can suppress the production of these oncotargets involved in carcinogenesis.

Flavonoids are a class of plant-derived(13) compounds having a large number of medicinal properties (14). Plant-based compounds are the major focus of research in terms of their bioactivity as an anti-cancerous property (15,16). Their distinct molecular structures are linked to the metabolic processes of the body (17) and their metabolites get used in multiple trials to prevent and cure a wide range of illnesses including; obesity, diabetes, hypertension, hyperlipidaemia, cardiovascular diseases, neurological disorders, and osteoporosis (18). Flavonoids are frequently found in natural resources (19), making it simple for humans to get metabolized easily without causing any issues in their physiology or biochemistry. The inhibitory potential of flavonoids against PKP1 in lung cancer cells remains unelucidated. Furthermore, the development of a novel anticancerous drug is a very time-consuming, costly

* Corresponding author. Email: fahadintegralian@gmail.com, mo.saeed@uoh.edu.sa

and tedious task (16). Therefore, a systematic and planned approach is required for rational drug discovery to conquer the limitations associated with chemotherapeutic approaches. *In silico* approaches, employing the medicinal benefits of phytocompounds have been a fundamental element of drug design and development in this era of personalized medicine. Thus, our current study is focused on elucidating the inhibitory potential role of selected flavonoids against PKP1 in lung cancer via employing *in silico* approaches. In this research report, forty-six flavonoids were selected to determine their inhibitory potential in lung cancer against PKP1, using a variety of *in silico* methods. However, *in vitro* method is necessary to gain detailed insight into the mechanism involved.

Materials and Methods

A total of forty-six phytocompounds and one standard drug have been selected from the NPACT (20) and PubChem databases. Only those flavonoids were selected for this study which have not been studied earlier for the treatment of lung cancer were. The compounds which are being considered for this research are (-)-Epicatechin, (+)-Gallocatechin, (3S)-3',7-dihydroxy-2',4',5',8-tetramethoxyisoflavan, (3S)-7-hydroxy-2',3',4',5',8-pentamethoxyisoflavan, candenatenin A, 2',3',5,7-Tetrahydroxy flavone, 2',5,6',7-Tetrahydroxy Flavanone, 2',5,6'7-Tetrahydroxy flavone, 2',5-Dihydroxy-6,7,8-trimethoxyflavone, 4'-bromoflavone, 6-hydroxycalyxin F, 7-OH-flavanone, candenatenin B,apigenin-7-O-beta-D-glucopyranoside, Artelasticin, Artobiloxanthone, Artoindonesianin B, Artoindonesianin P, Artoindonesianins A, Artoindonesianins U, Artoindonesianins V, Baicalein, Cajanol, Biochanin A, Blepharocalyxins A7, Blepharocalyxins B, Blepharocalyxins C, Blepharocalyxins D, Blepharocalyxins E, Burttinone, Butlin, Calyxins A, Calyxins B, Calyxins C, Calyxins D, Calyxins E, Calyxins F, Calyxins G, Calyxins H, Calyxins I, Calyxins J, Calyxins K, Calyxins L, Catechin, Chrysin, and candenatenin C. Among all the standard drugs afatinib is used to treat metastatic non-small cell lung cancer (NSCLC). Along with its advantages, afatinib(21) shows some severe side effects like diarrhoea, skin problem, hair loss, etc, hence it is required to identify a new drug that will be more effective than afatinib with no harmful side effects on the human body. And the 3D structure of the targeted PKP1(1MX9)(22) protein was downloaded from the protein data bank PDB(RSMD) database in .pdb format.

Preparation of ligands

All Forty-six flavonoids were selected for ligand preparation. Which have not been elucidated as potent drug candidates for non-small cell lung cancer. (Table S1) consists of all forty-six flavonoids with their respective PubChem Id and 2D or 3D structures.

Preparation of target proteins

The 3D structure of the target PKP1 (1MX9) protein, was obtained from the protein data bank (RCSB PDB) database and the target optimization was done by using UCSF Chimera for molecular docking. Throughout the docking period, the ligand was considered to be flexible, and the protein was considered inflexible.

Molecular Docking analysis using Patchdock and CB Dock

Patchdock

PatchDock(23) is an online available docking server used to perform the molecular docking of selected forty-six phytocompounds and one standard drug against one target PkP1(1XM9) protein. PatchDock server works on a geometry-based docking algorithm(24). Docking was done by giving 4.0 as a default value of cluster RMSD and protein-protein ligand interaction as a complex type in the given slot.

CB Dock

For further validation, the results of the PatchDock, target protein and selected flavonoids were again docked with CB-Dock(25) which is also an online docking server. The number of cavities of the compound were given five as the default value of the software.

Analysis and visualization of docked ligand-protein complexes

Depending upon the highest (negative) binding scores of compounds, the best compounds were selected for adding amino acid residues. And the visualization of the best orientation pose of ligand and receptor complex was done by using UCSF chimera (26).

Prediction of activity spectra for substances (PASS) analysis

The PASS analysis predicts the biological activity range of a compound underworked on its structure-activity relationship with a known compound (27). In this research, the PASS examination was performed by using several online software mentioned below.

Lipinski's rule of five

The drug-likeness of selected flavonoids and the standard drugs was evaluated using Lipinski's rule of five (28). The parameters of drug-likeness such as MW =<500, logP=< 5, number of hydrogen bond donors (NOHNH) =<5 and hydrogen bond acceptor sites (NON) =<10, topological polar surface area (TPSA) (=< 140 Å2), and the number of rotatable bonds (=<10) were determined. In this study drug-likeness of phytocompounds was calculated by using the online tool swissADME (29) and compared with that standard drug.

Bioactivity score (BAS) prediction

BAS analysis suggests the compound's overall ability to be a potent drug candidate. Molinspiration (30) software was used to predict the drug score of selected phytocompounds concerning many human receptors such as GPCRs, kinases, nuclear receptors, ion channels, proteases and enzymes. In general, the higher the activity score, the greater the chance of the compound being active (31).

Pharmacokinetic (PK) parameters prediction

The ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) properties of selected flavonoids and the standard drug were examined by using an online tool SwissADME. These tools predict the significant pharmacokinetic properties of a compound like dissemination viz. blood-mind boundary (BBB) and skin permeability (LogKp), and its digestion as far as it is a P-glycopro-

tein (P-GP) substrate, Cytochrome P450 viz. CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4 inhibitors as well as their lipophilicity for plasma membrane absorption (28, 32).

Toxicity Potential assessment

Toxicity assessment is very essential to avoid unsuitable compounds. Toxicity analysis is a very crucial step in the process of finding new drugs is the early prediction of various features of molecules. The ProTox-II(33) programme was used to examine the drug toxicity risk criteria such as Hepatotoxicity, mutagenicity, tumorigenicity, carcinogenicity, and cytotoxicity (34).

Results

Docking analysis of phytocompounds of flavonoids against the targeted protein of lung cancer

Patchdock and CBdock (online docking servers) were utilised for the docking investigation of forty-six flavonoids against the targeted PKP1(1XM9) protein of lung cancer. (Table S2 and S3) have the docking results of all forty-six compounds. Consequently, a total number of forty-six protein-ligand complex structures were produced. Based upon their highest binding energy only three common flavonoids named as calyxinsI, Blepharocalyxins E and calyxinsJ were screened out from all forty-six compounds. Furthermore, clarifications of complex structures and important amino acid residues interaction within the complex has been displayed by using UCSF chimera. (Table S4) has shown the comparative analysis of these three best-scoring compounds with the standard drug afatinib. When the results of molecular docking were compared with the referenced drug afatinib to the same targeted PKP1(1XM9) protein, calyxinsI calyxinsJ and Blepharocalyxins E showed more binding affinity with targeted protein in comparison to the standard drug afatinib.

PASS analysis of selected phytocomponents using Lipinski's rule of five

Lipinski's rule of five shows the molecular properties of a compound which are essential for lead selectivity and optimization of a potential orally active drug in clinical applications. (Table 1) shows the PASS analysis of calyxinsI, calyxinsJ and, BlepharocalyxinsE versus the standard drug afatinib. Generally, an orally active compound should not have more than one Lipinski's violation in any case its bioavailability is compromised. Interestingly, calyxinsI and calyxinsJ have shown one Lipinski violation, on the other hand, BlepharocalyxinsE showed three Lipinski violations and, afatinib did not show any Lipinski violation.

Bioactivity scores (BAS) of phytocompound

The BAS analysis of calyxinsI, calyxinsJ and BlepharocalyxinsE with the standard drug afatinib is mentioned in (Table 2). According to the rules, compounds having BAS >0.00 is most likely to possess considerable biological activities, while compounds having values between -0.50 and 0.00 are supposed to be moderately active and compounds having BAS < -0.50, are expected to be inactive (34). The results of the bioactivity scores demonstrated that all three flavonoids are biologically inactive molecules because none of them had bioactivity scores >0.00. After connecting with GPCR ligands, afatinib showed BAS 0.27, nuclear receptor ligands or by interacting with inhibitors of proteases and different enzymes. All three phytoconstituents showed as inactive but afatinib showed an active compound BAS 0.21. The same process was carried out for the ion channel modulator then all phytocompounds including the standard drug afatinib showed as inactive compounds and for kinase inhibitors, all three phytocompounds showed as inactive compounds whereas afatinib showed as active compound BAS 0.73.

ADMET properties of phytocomponents

To check the pharmacokinetics of selected phytocom-

Table 1. PASS analysis of phytocompounds and standard drug afatinib.

S.No.	Phytoconstituents	calyxinsI	CalyxinsJ	Afatinib	Blepharocalyxins E
	topological Polar Surface Area (Å)^2 (TPSA)^b (<160 Å)	145.91	134.92	88.61	197.37
	MW (<500)	686.76	685.76	485.54	879.00
	C logP^c (<5)	5.56	5.54	3.73	7.42
	heavy atom count (n atoms)	51	51	34	65
	Hydrogen Bond Donors (nOHNH) (5)	5	4	2	8
	Hydrogen Bond Acceptors (nON) (10)	9	9	8	11
	Number of Rotatable bonds (10)	9	7	8	17
	Lipinski's violation	1	1	0	3

Table 2. BAS analysis of phytocompounds and standard drug afatinib.

S no.	Phytocomponents	GPCR ligand	Ion Channel Modulator	Kinase Inhibitor	Nuclear receptor ligand	Protease Inhibitor	Enzyme Inhibitor
1.	calyxinsJ	-0.53	-1.73	-1.35	-0.95	-0.41	-0.91
2.	calyxinsI	-0.56	-1.68	-1.33	-1.00	-0.45	-0.93
3.	Afatinib	0.21	-0.11	0.73	-0.38	-0.07	0.21
4.	Blepharocalyxins E	-3.02	-3.61	-3.60	-3.51	-2.72	-3.28

pounds, ADMET (Absorption, distribution, metabolism, excretion and toxicity) properties (36) of compounds were determined by utilizing an online available software SwissADME. (Table 3) has mentioned the determined LogP value, all parts were viewed as lipid dissolvable (lipophilic) which shows great retention of all parts across the skin. Interestingly, all three phytocompounds did not show blood-brain barrier (BBB) permeability and none were supposed to act as permeability-glycoprotein (P-GP) substrates except afatinib and blepharocalyxinsE. P-GP is an ATP-dependent bioavailability protein pump that eliminates drugs from biological systems. The normal discharge of drugs back into the stomach lumen by PGPp diminishes the pharmacokinetics and viability of pharmaceutical drugs (which are supposed to be PGPp substrates). Cytochromes P450 (CYPs) are a superfamily of major metabolic compounds engaged with the biotransformation of xenobiotics. Drugs and other xenobiotics can go about as the two substrates and inhibitors of cytochromes P450 and they are engaged with the digestion of most medications drugs that inhibit the five classes of CYPs viz. CYP3A4, CYP1A2, CYP2C9, CYP2C19 and CYP2D6 would cause an expansion in their plasma concentrations thus contributing to improved bioavailability. In the current study, calyxinsI and calyxinsJ were not found to behave as inhibitors of CYPS but blepharocalyxinsE was found to behave as inhibitors of CYP3A4.and standard drug afatinib was found to act as an inhibitor of CYP2C19, CYP2C9, CYP2D6 and CYP3A4 (Table 3). Skin permeability (Kp) is generally used to quantitatively elaborate the rate of substance penetration through the outermost layer (epidermis) of the skin. Interestingly, all three phytocompounds including afatinib showed negative Kp value which demonstrates less chance of effective ingestion of these phytocompounds.

Toxicity potential assessment

The toxicity potential assessment of all three flavonoids in comparison to the standard drug afatinib was performed by using ProTox-II (35). (Table 4) has the results shown by the ProToxII, revealing that calyxinsI, calyxinsJ and blepharocalyxinsE are highly inactivity compounds toward cytotoxicity and hepatotoxicity but less inactivity toward carcinogenicity and mutagenicity on the other hand the standard drug afatinib displayed less activity toward hepatotoxicity and highly active toward immunotoxicity but less inactive towards carcinogenicity, mutagenicity and cytotoxicity.

Discussion

Various reports have demonstrated that PKP1 has been crucially involved in several oncogenic signalling pathways such as enhancing the MYC translation(37) which is leading to lung carcinogenesis and the most overexpressed protein in NSCLC(38). Therefore PKP1 suggesting as a potential therapeutic target for non-small cell lung cancer(39). Hence we focused our study on identifying the inhibitory potential of those flavonoids which remain unelucidated for non-small cell lung cancer. Flavonoids are plant-derived compounds well known for their fascinating properties(16). These phytocompounds are broadly involved in health-promoting effects and crucial components in a variety of pharmaceutical, nutraceutical, medicinal and cosmetic applications. (14) These are also associated with their anti-oxidative(40), anti-mutagenic(41), anti-inflammatory(42), and anti-carcinogenic(43) properties. Some researchers reported the tremendous scope of flavonoids as potential drugs in preventing chronic diseases and future research directions. Thus we have planned our study in a very effective manner to find the best compound out of all forty-six selected flavonoids for the treatment of non-small cell lung cancer by using *in silico* techniques(34). All forty-six compounds have been docked using an online server named Patchdock and CB-Dock. Then based on their docking results, three compounds named as calyxinsI, Blepharocalyxins E and calyxinsJ have shown the highest binding affinity with PKP1(1XM9) protein rather than standard drug afatinib which is being used for the treatment of non-small cell lung cancer with its severe side-effects. Results of molecular docking analysis of these three compounds including one standard drug afatinib by using patchdock have shown that calyxinsJ(BE= 8322) has the best binding affinity with PKP1 protein followed by Blepharocalyxins E(BE= 7150),calyxinsI(BE=5908) and afatinib (BE= 5610) and the docking results of CB-Dock has demonstrated that calyxinsI(BE = -9.4) have shown best binding affinity with PKP1 protein followed by calyxinsJ(BE = -8.3), Blepha-

Table 3. ADME properties of phytocompounds versus standard drug afatinib.

S.No.	Phytocomponents	Lipophilicity (Consensus Log Po/w)	BBB permeant	P-GP substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Log Kp (skin permeation)
1.	calyxinsJ	5.54	No	No	No	No	No	No	No	-5.57 cm/s
2.	calyxinsI	5.56	No	No	No	No	No	No	No	-5.56 cm/s
3.	Afatinib	3.73	No	Yes	No	Yes	Yes	Yes	Yes	-6.68 cm/s
4.	Blepharocalyxins E	7.42	No	Yes	No	No	No	No	Yes	-4.34 cm/s

Table 4. Toxicity of phytocompounds and standard drug afatinib.

Sno.	Compounds name	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
1.	CalyxinsI	High inactive	Less inactive	High active	Less inactive	High Inactive
2.	CalyxinsJ	High inactive	Less inactive	High active	Less inactive	High Inactive
3.	BlepharocalyxinsE	High inactive	Less inactive	High active	Less inactive	High inactive
4.	Afatinib	Less active	Less inactive	High active	Less inactive	Less inactive

rocalyxins E(BE = -8) and afatinib (BE=-7.8). So these results have shown that calyxinsI and calyxinsJ are the best compounds that can lead to better drug options than afatinib. To further validate the efficiency of these two compounds few more tests have been taken to get the accuracy in the final results.

To check the efficiency of the above-screened compounds as a better drug candidate, all four compounds were tested for their drug likeness using Lipinski's rule of five(44). Lipinski's rule of five predicts that strong absorption/ permeation is more likely when the MW <500, the calculated LogP (cLog P) 5.0, there are 5 H-bond donors and 10 H- bond acceptors. Generally, an orally active compound should have no more than one Lipinski's violation otherwise its bio-availability is compromised(45). Interestingly, calyxinsI and calyxinsJ have exhibited one Lipinski violation whereas blepharocalyxins E has shown three Lipinski violations and standard drug afatinib has shown zero as a Lipinski violation hence it can be postulated that calyxinsI and calyxinsJ phytocomponents have the potential to be evaluated further from a drug development perspective. Further toxicity potential assessment is required for avoiding unsuitable substances for further drug development(46-55). All four compounds were further screened to check their hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity and cytotoxicity. All three selected phytocompounds were found to be safe with less or highly inactive toxicity parameters whereas the standard drug afatinib has shown less activity towards hepatotoxicity. Furthermore, all phytocomponents displayed a lipophilic nature, indicating good absorption and transport kinetics through the gut. Hence calyxinsI and calyxinsJ both phytocompunds found to be better potent drug candidates in comparison to the standard drug afatinib. But based on molecular docking and other preliminary analysis, this study concludes that calyxinsI can be the better competitor in comparison to the standard drug afatinib.

Conclusion

PKP1 has been crucially involved in various oncogenic signalling pathways including non-small cell lung cancer so, there is a crucial need to identify a potent drug candidate to suppress the mechanism of action of this protein in lung cancer progression. Hence this research concludes that CalyxinsI is a better inhibitory potential drug competitor in comparison to the standard drug afatinib which is being used for the treatment of NSCLC with its adverse side effects. CalyxinsI has not been elucidated to date but this research has demonstrated that calyxinsI has shown better interaction with PKP1(1MX9) protein rather than the standard drug afatinib and also has passed all the eligibility criteria as per our research findings including Lipinski's rule of five, ADMET, BAS and PASS analysis which is required to being as an effective and potential drug candidate for further drug development. As this research is preliminary research, more *in silico* and in vitro experimentation are needed to validate the candidature of calyxinsI for the effective management of non-small cell lung cancer.

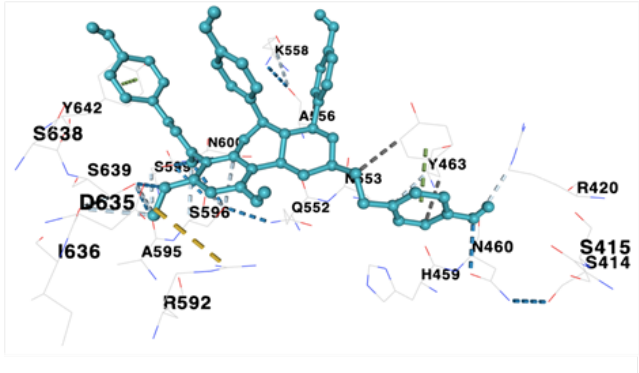

Figure 1. Docked result of calyxinsI against PKP1 protein in non-small cell lung cancer.

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Interest conflict

The authors declare no conflict of interest.

Consent for publications

The author read and proved the final manuscript for publication.

Availability of data and material

All data generated during this study are included in this published article

Authors' Contribution

All authors had equal role in study design, work, statistical analysis and manuscript writing.

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Ethics approval and consent to participate

No human or animals were used in the present research.

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