Original Article

Efficacy of Phytochemicals of Cassia Angustifolia in Chronic Myeloid Leukaemia – An In-silico Analysis

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Abstract

Objective: To measure the inhibitory potential of phytochemicals of Cassia angustifolia against the BCR-ABL fusion protein involved in the pathogenesis of Chronic Myeloid Leukaemia (CML) and to compare it with the previously developed inhibitor, nilotinib using in silico analysis techniques.

Methodology: The 3D structure of Human BCR-ABL fusion protein was obtained from PDB by RCSB (Protein Data Bank by Research Collaboratory for Structural Bioinformatics). The smiles and Chemical Structures of the ligands were obtained from PubChem. They were prepared in Mol SDF format by the Chem Bio Draw and then converted to PDBQT format using PyRx tool for generating the atomic coordinates for molecular docking. Molecular docking of Nilotinib, Quercimeritin, and Scutellarin with Human ABL Kinase was performed using Autodock4. The Absorption, Distribution, Metabolism, and Toxicity (ADMET) properties were described using Swiss ADME, a web-based tool.

Results: All the three compounds under study bond and made stable complexes with wild-type BCR ABL with the global energies of -12.46, -16.17kCal/mol and -15.41kCal/mol for Nilotinib Scutellarin and Quercimeritin respectively which means that these compounds can act as selective inhibitors of BCR-ABL fusion protein. Quercimeritin, also form Hydrogen bonds with GLU 286 and Asp 381,

Conclusion: The binding energies of the phytochemicals of Cassia are significantly higher in comparison with Nilotinib which has a binding energy of -12.46kCal/mol which suggests a better inhibitory potential of these compounds. Quercimeritin also forms Hydrogen bonds with Glutamine 286 and Aspartate 381, hence its potential to be a potent inhibitor of the BCR- ABL fusion protein is more promising than Nilotinib. Further in vitro and in vivo studies are suggested to elaborate the anti-neoplastic potential of Quercimeritin in CML.

Keywords: Chronic Myeloid Leukaemia, In-Silico Analysis, Cassia angustifolia, Senna Makki

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Introduction

WHO has categorized Chronic Myeloid Leukaemia (CML) under Chronic Myeloproliferative Disorders.[1,2] CML is a neoplasm of myeloproliferative type and is genetically associated with a balanced translocation involving the translocation of ABL gene from chro-mosome 9 to BCR chromosome 22, with their fusion resulting in BCR-ABL 1 gene,

which translates into the BCR-ABL protein, a protein with a size of 210kDa.[3,4] The BCR-ABL 1 fusion protein has a specific tyrosine kinase activity that triggers multiple transduction pathways leading to the proliferation of myeloid lineage of hematopoiesis.[5,6] The disease is divided into two phases. The initial phase, involves the overproduction of immature myeloid cells along with an increased production of mature granulocytes. This overproduction occurs in spleen, peripheral blood and bone marrow. The second phase is the 'Blast Crisis' phase which is characterized by a rapid overproduction of myeloid cells, and a diagnostic finding of more than 30% of blast cells (undifferentiated) in the bone marrow. The median survival rate in this phase is about 18 weeks.[7] Discovery of the BCR-ABL 1 gene and the BCR-ABL Fusion protein led to the design and development of a novel drug 'Imatinib' which acts as a Tyrosine Kinase Inhibitor targeting the ABL-Kinase, and other products of oncogenes such as PDGFR and c-Kit. Imatinib has been found to provide an effective treatment in the initial chronic phase of the disease in about 86% of the patients providing a complete remission of haematological overproduction (a normal TLC is achieved in about 98% of the newly diagnosed patients) whereas a complete cytogenetic response (characterized by an absence of detectable Ph+ cells in bone marrow aspirate) is also noted in about 86% of the patients with this drug.[8]

Although, Imatinib acts as an effective frontline drug for the initial phase of CML due to its tyrosine kinase inhibitory activity, but in the 'Blast Crisis' phase of CML and patients with a Ph+ AML often relapse despite the Imatinib therapy. This relapse is attributed to point mutations in BCR-ABL Tyrosine Kinase Domain resulting in the resistance to the Imatinib therapy. This prompted the development AMN107, Nilotinib, a new tyrosine-kinase inhibitor that selectively fit the ATP-binding site of BCR-ABL, has a higher affinity than Imatinib and thus overrides the resistance to Imatinib. Besides a better affinity than Imatinib, it also has a significant inhibitory effect against imatinib-resistant BCR-ABL mutant sub-types. It has produced haematological and cytogenetic responses in CML patients in clinical trials in patients with Imatinib-resistant Chronic Myeloid Leukemia.[7]

Plants have served as a foundation in the development of various modern drugs in the treatment of diseases in an efficient way. About 60% of the currently utilized anti-neoplastic drugs and anti-biotics are derived from plants.9 Cassia angustifolia (Senna Makki) is an herb native to Saudi Arabia. Studies to determine the chemical composition of Cassia have been conducted. Various phytochemicals isolated include "Sitosterol, Sennosides A, B, C, Anthraquinone, Quercimeritin, Scutellarin, Cathartic acid, Rhamnetin, Gluco-sennin, Chrysophenic acid, nigrin, Kaemphrin, Rhein, Flavonoids, Emodin and Salicylic acid."10-12 Medicinal benefits such as anti-inflammatory, laxative, purgative, anti-neoplastic, anti-biotic, anti-malarial, anti-pyretic, and antioxidant have been studied and described based on the chemical composition of this herb.[12]

In-silico molecular docking and software-based drug like properties analyses tools have been an efficient method in the screening of bioactive compounds from a pool of phytochemicals.[13] The in-silico molecular uses a computer-based program to energize a reaction between a ligand and a protein to predict the possibility of binding of the two. It also calculates the binding energies for the compounds with a negative binding energy predicting a feasible reaction. The programs for Drug-like Properties Analysis use specific algorithms on the structure of a compound to predict the Pharmacokinetic properties such as Absorption, Distribution, Metabolism, and Toxicity (ADMET) of the compound.[14]

Using the molecular docking, ADMET and other in-silico studies, this study was aimed to discover

the compounds of Cassia having activity against the BCR-ABL 1 fusion protein involved in the pathogenesis of Chronic Myeloid Leukaemia and to compare it with previously developed inhibitor, nilotinib.

Methodology

This was an In-silico study based on computer software programs Autodock[4]. The 3D structure of Human BCR-ABL fusion protein was obtained from PDB (RSCB). The SMILES and Chemical Structures of the ligands were obtained from PubChem. They were prepared in Mol SDF format by the Chem Bio Draw and then converted to PDBQT format using PyRx tool for generating the atomic coordinates for molecular docking. Molecular docking of Nilotinib, Quercimeritin, and Scutellarin with Human ABL Kinase was performed using Autodock.[4] The ADMET properties were described using Swiss ADME, a web-based tool. Molecular docking is used to compute the binding energies by modelling interactions between receptors and ligands with a purpose of discovering the optimal drug candidates. It creates multiple spatial conformations for the receptor and ligand calculating the energies for each and then most appropriate interactions with the best binding energies are selected as potential drug candidates.[16]

Results

Table 1 shows the results of docking and Drug-Like Properties Analysis. Docking results show high binding affinity of both Quercimeritin and Scutellarin to the wild type ABL Kinase Receptor with binding energies of -15.41 and -16.17 even higher compared to the novel drug Nilotinib which shows a binding energy of -12.46 kCal/mol. Drug-like Properties Analysis using SwissADME divulges comparable pharmacokinetic properties (Table 1). The interactions of the compounds with the amino acid residues are of quite interest. Table 2 provides the list of the amino acid residues to which the compounds bind. Fig 1, 2, and 3 shows the interactions along with the type of bond present and the bond length (in Angstroms). The results of molecular inte-

Table 1: *Results of Molecular Docking Analysis (Binding Energies) and Drug Like Properties Analysis*

	Nilotinib	Quercimeritrin	Scutellarein
Binding Energy (kCal/mol)	**-12.46**	**-15.41**	**-16.17**
Molecular Formula	$C_{28}H_{22}F_3N_7O$	$C_{21}H_{20}O_{12}$	$C_{15}H_{10}O_6$
Molecular Weight (g/mol)	529.52	464.38	286.24
Log P	4.64	-0.37	1.81
Solubility (Ali)	-6.69	0.02	-4.65
GI Absorption	Low	Low	High
BBB Permeability	No	No	No
Skin Permeation (cm/s)	-6.05	-8.88	-6.16
Drug Likeness (Ghose,Veber)	No; 3 violations: MW>480, WLOGP>5.6, MR>130 Yes	No; 1 violation: WLOGP<-0.4 No; 1 violation: TPSA>140	Yes Yes
BRENK	0	1 (catechol)	1 (catechol)
PAINS	0	1 (catechol)	1 (catechol)
Leadlikeness	No	No	Yes
Bioavailability Score	0.56	0.17	0.55
Synthetic Accessibility	3.81	5.31	3.04
CYP1A2	NI	NI	I
CYP2C9	I	NI	NI
CYP2C19	I	NI	NI
CYP2D6	I	NI	I

Table 2: *Comparison of Nilotinib, Scutellarin, and Quercimeritin, various interactions with amino acids*

Nilotinib	MET 318, LEU 370, LEU 248, TYR 253, ALA 269, ILE 313, PHE 382, VAL 256, LYS 271, THR 315, VAL 299, **ASP 381,** MET 290, VAL 379, LEU 298, ALA 380
Quercimeritrin	VAL 299, ALA 380, **ASP 381, GLU 286,** PHE 382, VAL 256, LEU 370, GLU 316, MET 318, LEU 248, ALA 269
Scutellarein	PHE 317, MET 318, LEU 248, ALA 269, TYR 253, LEU 370, VAL 256, PHE 382, LYS 271

ractions in our study also reveal that Quercimeritin, a phytochemical present in Cassia, also form Hydrogen bonds with GLU 286 and Asp 381.

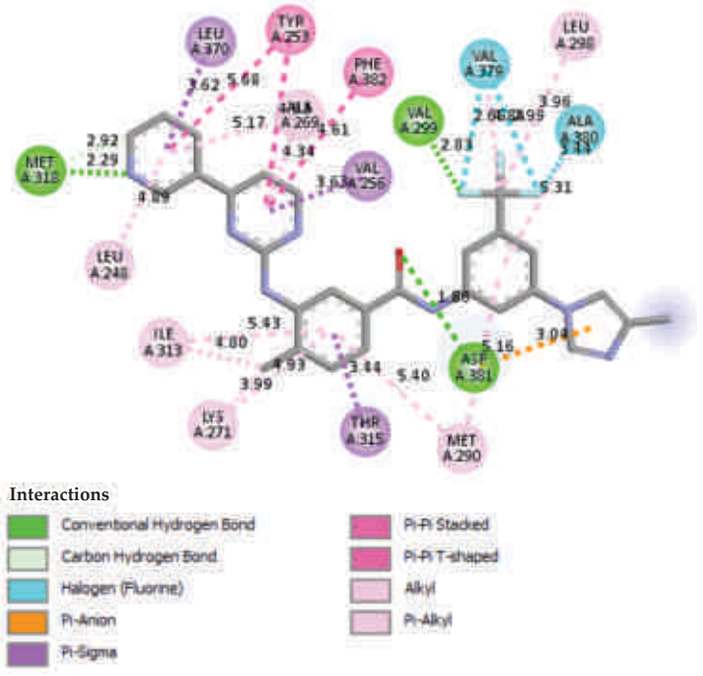

Fig 1. *Interactions of Nilotinib*

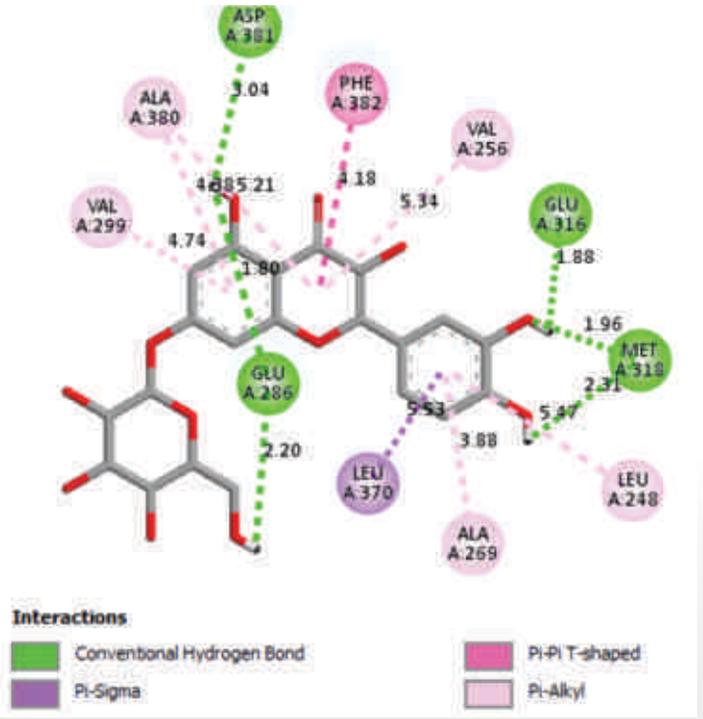

Fig 2. *Interactions of Quercimeritin*

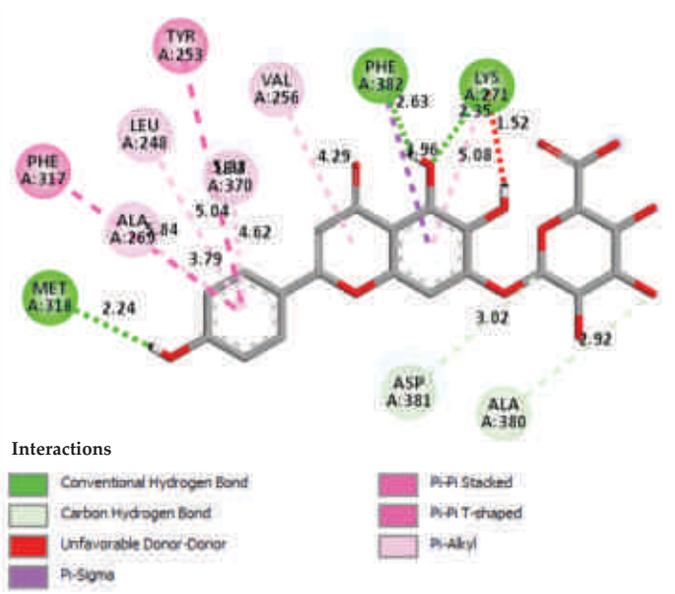

Fig 3. *Interactions of Scutellarin*

Discussion

Out of the phytochemicals of Cassia, Scutellarin and Quercimeritin showed interaction with the wild-type BCR-ABL gene with binding energies of -16.17kCal/mol and -15.41kCal/mol respectively.

Binding energy is the measure of the binding affinity of the ligand. Highest contact energies are shown by Quercimeritin and Scutellarin. Global Energy refers to the energy of Receptor Ligand Complex.[17] A negative global energy shows a stable ligand-receptor complex. All the three compounds under study bind and make stable complexes with wild-type BCR ABL with the binding energies of -12.46, -16.17kCal/mol and -15.41kCal/mol for Nilotinib, Scutellarin, and Quercimeritin respectively which means that these compounds can act as selective inhibitors of BCR-ABL fusion protein. The binding energies of the phytochemicals of Cassia are higher in comparison with Nilotinib which has a binding energy of -12.46 kCal/mol which suggests a better inhibitory potential of these compounds.

It was hypothesized that targeting the Glutamine 286 and Aspartate 381 with an amide pharmacophore while maintain the binding to inactive conformation

of the BCR-ABL fusion protein could result in a better activity against the Imatinib-resistant protein. This led to the development of the drug nilotinib.[8] The results of molecular interactions in our study reveal that Quercimeritin, a phytochemical present in Cassia, also form Hydrogen bonds with GLU 286 and Asp 381, hence its potential to be a potent inhibitor of wild-type BCR-ABL is more promising Nilotinib.

Our study is consistent with the previous studies. Three flavonoids Quercimeritin, Scutellarin, and Rutin from Cassia have been known to show significant antineoplastic activity against oncogenes such as MCF-7, HeLa, Hep2, and low cytotoxicity against HCEC[18]. Cassia has been used in the treatment of Leukaemia traditionally[19], however to our knowledge, this is the first study that provides a look into the molecular basis of anti-cancer properties of Cassia angustifolia in Chronic Myeloid Leukaemia.

Though in-silico studies provide a good foundation for drug discovery and drug interactions, yet they are not a substitute to experimental analysis and clinical studies. This is the major limitation of our study. Further research should look into in vitro analysis and experimental trials for the efficacy of Cassia angustifolia in the treatment of CML.

Conclusion

The binding energies of the two phytochemicals of Cassia, i.e., Quercimeritin and Scutellarin are higher in comparison with Nilotinib which suggests a better inhibitory potential of these compounds. Quercimeritin also forms Hydrogen bonds with Glutamine 286 and Aspartate 381, hence its potential to be a potent inhibitor of the BCR- ABL fusion protein is more promising Nilotinib. Further in vitro and in vivo studies are suggested to elaborate the anti-neoplastic potential of Quercimeritin in CML.

Conflict of Interest *None*

Funding Disclosure *None*

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Authors Contribution

FM: Conceptualization of study
HM: Drafting
UG, UA : Critical Revision, Final Approval
UA, FN, MQA: Data Collection and Analysis

All authors are equally accountable for accuracy, integrity of all aspects of the research work.