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Homo Sapien Bcr-Abl-Interacting Scaffolds from *Bryophyllum pinnatum* and *Cantharanthus roseus: Computational Studies*

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Abstract

Leukaemia is one of the ten life-threatening cancer of the world today. Chronic myelogenous leukaemia (CML), in particular constitutes about 15% of adult leukaemia and is characterised by over-production of immature myeloid cells in the bone marrow, spleen and peripheral blood resulting in the presence of the constitutively activated tyrosine kinase BCR-ABL, the cardinal therapeutic target. With the prevailing patients resistant to current drugs, it is imperative to come up with novel drugs particularly of plant origin considering the critical roles natural products (plants) play in the discovery and developments of new pharmaceuticals. In the present study, Virtual High Throughput Screening of phytochemicals from Bryophyllum pinnatum and Cantharanthus roseus (reported anticancer plants) against the Bcr-Abl catalytic site for possible drug scaffolds were carried out. A library of phytochemical compounds (ligands) was generated and multiple docking of the ligands against the Bcr-Abl catalytic site was executed with the Autodock Vina algorithm. Docking scores from Autodock vina were validated via the online available ChembL database and a correlation coefficient of 0.7 was obtained from a plot of the docking scores of 43 compounds from ChembL database with their corresponding pIC_{50} values. The lead compounds from Virtual High Throughput Screening, Cynaroside and Rutin have a binding energies of -9.6 kcal/mol and -9.4 kcal/mol respectively when compared with the co-crystallized nilotinib's -9.4 kcal/mol. Pharmacological kinetics analysis indicated that Cynaroside, with a bit of optimization could end up a potential anti-CML drug.

Keywords: Leukemia, BCR-ABL Oncoprotein, *Bryophyllum pinnatum*, *Cantharanthus roseus*, Molecular Docking

Introduction

Leukaemia has a 94% death rate in Nigeria, only a paltry one out of every 20 Nigerians with leukaemia survives (1). A person with leukaemia has bone marrow that produces abnormal white blood cells called leukaemia cells and leukemic blast cells. Chronic myelogenous leukaemia (CML) is characterised by over-production of immature myeloid cells and mature granulocytes in the bone marrow, spleen and peripheral blood (2). Chronic myelogenous leukaemia (CML), in particular constitutes about 15% of adult leukaemia (3). The median age at presentation is 53 years, but all age groups, including children, are affected. The hallmark of CML is chromosomal translocation on t(9;22)(q34;q11),which explains the chimeric gene BCR-ABL formed on chromosome 22 (2).The effect of this gene is the ability to induce leukemic phenotype.

The BCR-ABL gene constitute an active kinase activity capable of activating and interfering with several cellular processes such as cell proliferation and differentiation leading to malignant transformation (4,5,6). Historically, it has been treated with chemotherapy, interferon and bone marrow transplantation, although targeted therapies introduced at the beginning of the 21st century have radically changed the management of CML. Insight into the cellular pathophysiology of CML has given rise to the development of various drug therapies. CML is treated with inhibitors of tyrosine kinase, the first with crystallographic material of which was imatinib mesylate (marketed as Gleevec or Glivec; previously known as STI-571). Among the various tyrosine kinase inhibitors developed is nilotinib, a second-generation inhibitor developed to be effective in imatinib-resistant patients. Imatinib resistance results from the emergence of point mutations that result from the kinase domain of BCR-ABL reducing the effectiveness of the drug (7, 8). Nilotinib is highly selective for wild-type BCR-ABL with 20 times the binding affinity of imatinib, the first drug developed towards CML (9).

Natural products play critical roles in the discovery and developments of new pharmaceuticals (10). Natural products have brought forth the most important success in the chemotherapy of cancer, most of the foremost anticancer drugs are unmodified natural products derived from plants or microorganisms (11). *Cantharanthus roseus* was reported to possess anti-cancer properties against leukemia (12). On the other hand, the anti-cancer

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properties of *Bryophyllum pinnatum against leukaemia* have not been properly defined, however when investigated with the human cervical cancer it was seen to show inhibitory properties (13). In the present study, Virtual High Throughput screening of phytochemicals from *Bryophyllum pinnatum and Cantharanthus roseus* against the target BCR-ABL catalytic site were carried out. The drug likeness and bioactivity of our hit compounds were determined through the calculation of their molecular properties; calculated logP, polar surface area, number of hydrogen bond donor, number of hydrogen bond acceptors and molecular weight (Lipinski's rule) (14).

Materials and Methods

Data Collection and Preparation

Phytochemicals characterised from *Cantharanthus roseus* and *Bryophyllum pinnatum* were obtained from literature. Fifty (50) phytochemicals each from *Cantharanthus roseus* and *Bryophyllum pinnatum* totalling one hundred phytochemicals from the two plants, were downloaded in sdf format from the PubChem database(https://pubchem.ncbi.nlm.nih.gov). The sdf format were converted to pdb format by Open Babel, and finally converted to pdbqt using ligprep command lines. The *BCR-ABL oncoprotein* structure with PDB ID: 3CS9 and crystallographic resolution of 2.50Aº was downloaded from the protein data bank (http://www.rcsb.org). The BCR-ABL oncoprotein in the crystal structure is in complex with the drug nilotilib (http://www.rcsb.org). PyMOL Autodock/Vina Plugin was employed to extract the receptor BCR-ABL and nilotinib. SWISS-MODEL software was used to rebuild missing residues and atoms of BCR-ABL (15).

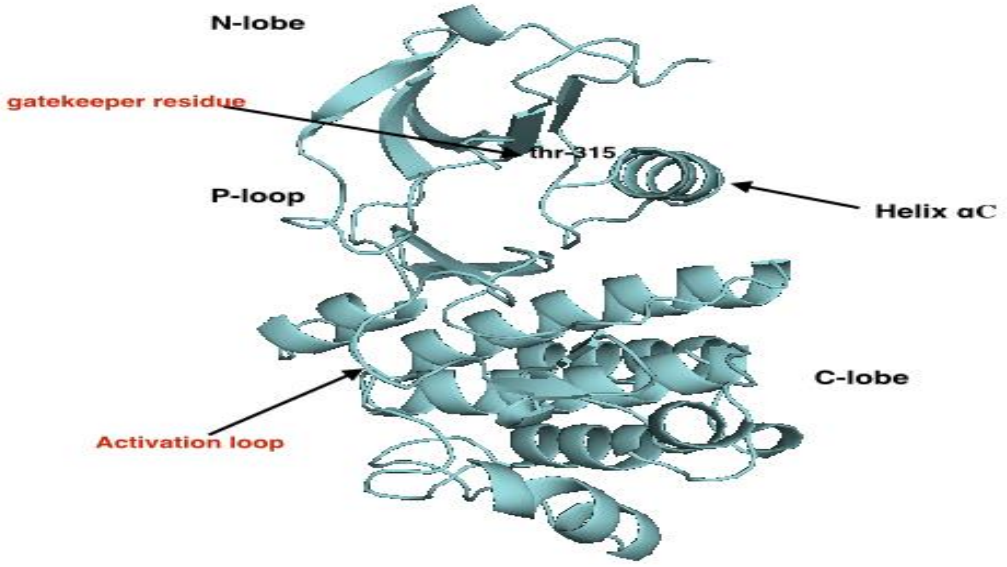


Figure 1*: Structure of the BCR-ABL Oncoprotein*

Virtual High Throughput Screening

A computational screening technique known as Virtual high throughput screening (vHTS), was used to screen a pool of compounds libraries to probe the binding affinity of the target receptor with the library compounds (16). The downloaded BCR-ABL from the protein data bank (http://www.rcsb.org), was uploaded in Pymol and a config file was generated, the grid coordinate was set as in the co-crystallized compound, x= -42.42, y= -50.7, z=-10.85. Our phytochemical compounds were converted to pdb, and to pdbqt, using command lines in auto dock vina and processed for docking.

Molecular Docking

The protein-ligand docking was carried out using Autodock Vina (17). The phytochemicals were docked into the exact BCR-ABL ATP catalytic site as occupied by the co-crystalized compound (nilotinib). Command lines in AutoDock Vina were used to carry out multiple docking of the phytochemicals.

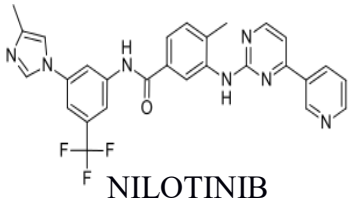
Validation of Docking Results

The Validation of result was performed with the multiple alignments of the BCR-ABL kinase domain receptor sequences obtained from pubmed. Using the online available ChemBL Database, the BCR-ABL sequences were blasted on www.ebiac.uk/chembl/. The result produced with the identity of 100%, IC50 value of 1862 and KI value of 721, was downloaded in text format and converted to pdb format by Data Warrior version 2(www.openmolecules.org), and finally converted to pdbqt . The 43 compounds obtained were docked into the Bcr-Abl catalytic site, as it was with the phytochemicals, being dictated by the config file (centre x=-42.42 ,

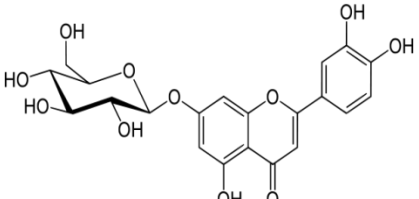
centre y=-50.7, centre z=-10.5, size x=22.50, size y=22.50, size z=22.50,). The config file indicates the grid map of the catalytic site of BCR-ABL kinase domain generated by AutoDock Vina. A correlation coefficient graph was plotted between the docking scores of the 43 compounds generated and their corresponding pIC_{50} values.

Molecular Properties, Lipinski Rule "Rule Of Five"

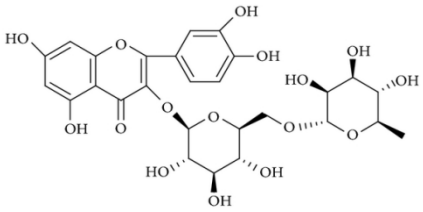
According to Christopher Lipinski *et al*. (14), the rule of 5' predicts an orally active drug. Poor absorption or permeation is more likely when there are more than 5 H-bond donors, 10 H-bond acceptors, molecular weight (MWT) greater than 500 and the calculated Log P (CLogP) greater than 5 (or MlogP>4.15). The rule of five also describes the molecular properties, which are vital to ascertain the pharmacokinetics, which include the absorption, distribution, metabolism and excretion of compounds. We applied the Lipinski rule of five to determine the drug likeness and the probable biological activities of our lead ligands. The Mavin Viewer software (www.chemaxon.com) was employed to establish the conformity of our hit compounds to the rule of five. The number of rotable bonds and polar surface area which are known to discriminate between compounds that are orally active and those that are not, for a large data set of compounds (18) were also derived. Compounds with 10 or fewer rotable bonds as well as polar surface area equal or less than 140Å² have good oral bioavailability (18).



NILOTINIB



CYNAROSIDE



RUTIN

Figure 2: Chemical structure of Nilotinib and the lead compounds

Results and Discussion

Table 1: Docking scores of the phytochemicals from the anti-cancer plants (*Cantharanthus roseus* and *Bryophyllum pinnatum*). The two leads (cynaroside with the docking of -9.6 kcal/mol and rutin with the docking of -9.4kcal/mol) are from *Bryophyllum pinnatum*

Cantharanthus roseus

Phytochemicals	Docking Scores
1-dodecanol	-4.8
3-phenyldodecane	-6.1
ajmalicine	-8.8
alpha-pinene	-5.1
alpha-terpineol	-5.6
beta-bisabolene	-6.2
beta-cadinene	-7.6
beta-phellandrene	-5.5
beta-pinene	-5
camphor	-4.9
catharathine	-8
cedrol	-7.8
davanone	-6.6
dehydoabietic acid	-7.7
delta-cadinene	-6.2
dodecane	-4.8
dodecyl acetate	-5.2
farnesyl acetone	-6.4
gamma-teripene	-4.6
geranial	-5.4
geraniol	-5.4
hexadecane	-5.1
iso-dihydrocarveol	-5.4
isopulegol	-5.5
lanosterol	-7
limonene	-5.4
linalool	-5.4
methyleugenol	-5.5
neral	-5.4
pentadecane	-5.2
perillaldehyde	-5.6
phytol	-6.1
reserpine	-7.4
roridin D	-7.9
tabersonine	-7.7
terpinen-4-ol	-5.3
tetracontane	-6.1
tetradecanal	-5
trans-alpha-bergamot	6.7
trans-sabienene hydr	5.1
tridecane	-4.7
trimethyldodecane	-5.9
vanillic acid	-5.4
vinblastine	-5.7
vincamine	-8.5
vincristine	-7.1
vindesin	-6.3
vitamin E	7.7
widdrol	-5.3
(Z)-beta-ocimene	-5.4

Bryophyllum pinnatum

Phytochemicals	Docking Scores
24-epiclerosterol	-8
alpa-amyrin	-5.9
alpha-amyrin acetate	-6.2
astragalin	-8.2
bersaldegenin	-7.5
beta-amyrin	-5.4
beta-amyrinacetate	-5.6
beta-sorbitol	-8.6
bufa-20,22-dienolide	-6.1
campesterol	-8.8
chamomile	-8.3
citric acid	-4.4
clerosterol	-7.1
cynaroside	-9.6
cysteine	-3.2
dimethylcaffeic acid	-6.2
d-sorbitol	-4
ferulic acid	-6.4
friedelin	-7.6
glucose	-4.6
glutamic acid	-4.3
glycine	-3
isocitric acid	-4.6
isoquercitrin	-8.2
malic acid	-4.1
methione	-3.8
nicotinic acid	-4.6
oxalic acid	-3.5
palmitic acid	-5.4
patuletin	-8.2
p-coumaric acid	-6.2
peposterol	-7.7
phenylalnine	-5.7
psi-taraxerol	-8
pyridoxine	-5
quercitrin	-8.4
raffinose	-6.6
riboflavin	-7.8
rutin	-9.4
salicylic acid	-5.1
soyasaponin	-7.3
stearic acid	-5.6
stigmasterol	-7.7
succinic acid	-4
syringic acid	-5.6
taraxerol	-5.6
thiamine	-6.3
tyrosine	-6.1
vanillic acid	-5.4
vit.E acetate	-7.1

Table 2. Molecular properties of the lead compounds (cynaroside and rutin) and the standard drug (nilotinib)

S/N	Compounds	M w	HBD	HBA	ClogP	RTY	RB	PSA
1	Nilotinib (standard Drug)	529.53	2	9	4.9	152.85	6	97.62
2	Cynaroside	448.38	7	11	0.5	107.04	4	186.37
3	Rutin	610.52	10	16	-1.3	140.15	6	265.52

M w= Molecular Weight
HBD= Hydrogen Bond Donor
HBA= Hydrogen Bond Acceptor
ClogP= Calculated Log P(lipophilicity)
RTY= Refractivity
RB= Rotatable Bond
PSA= Polar Surface Area.

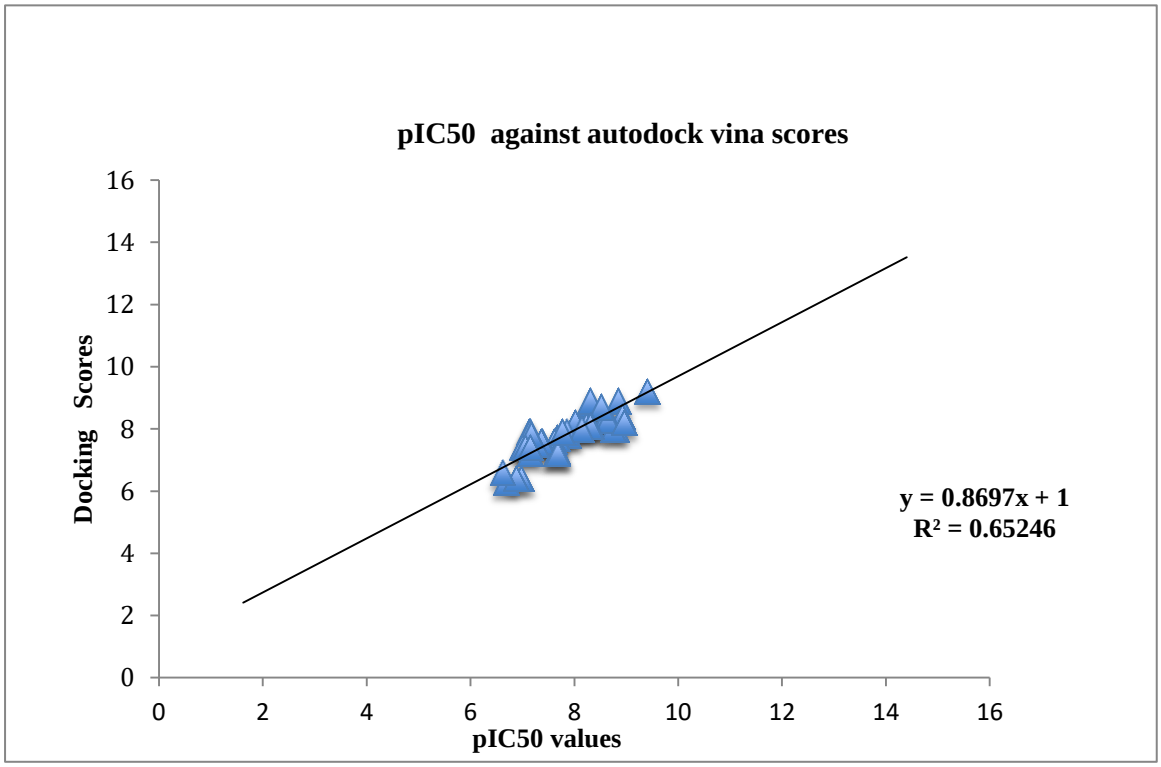
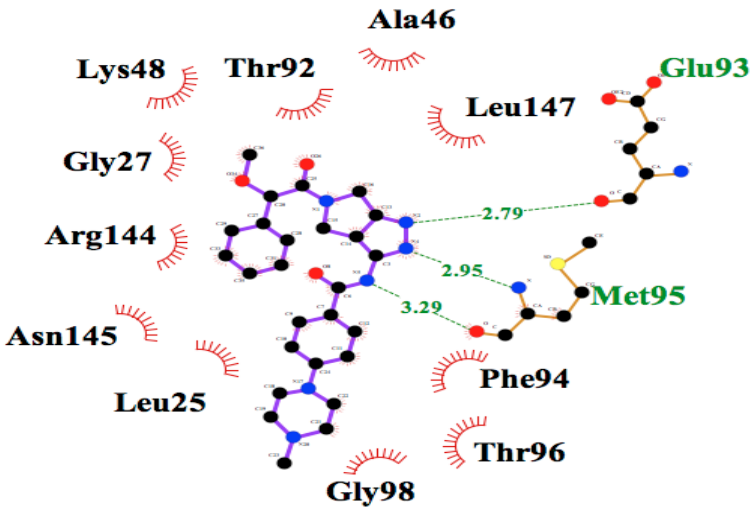


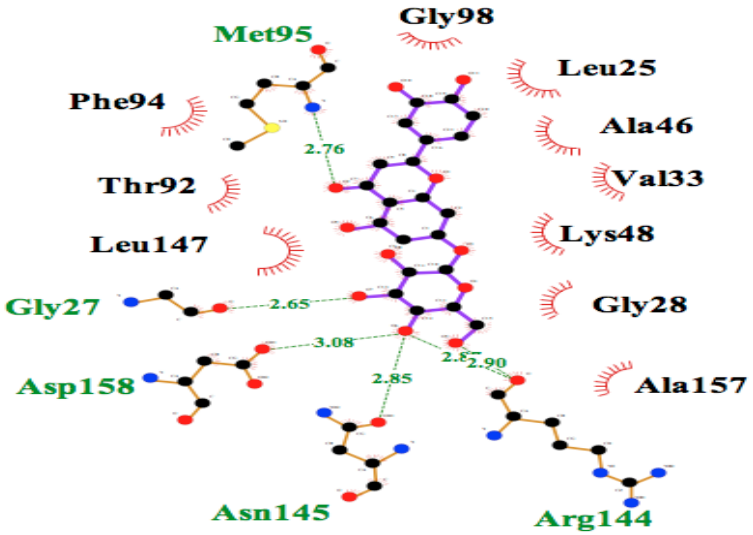
Figure 4: Autodock vina Scores vs Experimental pIC $_{50}$ values for 43 compounds docked into the Bcr-Abl catalytic site.

a).



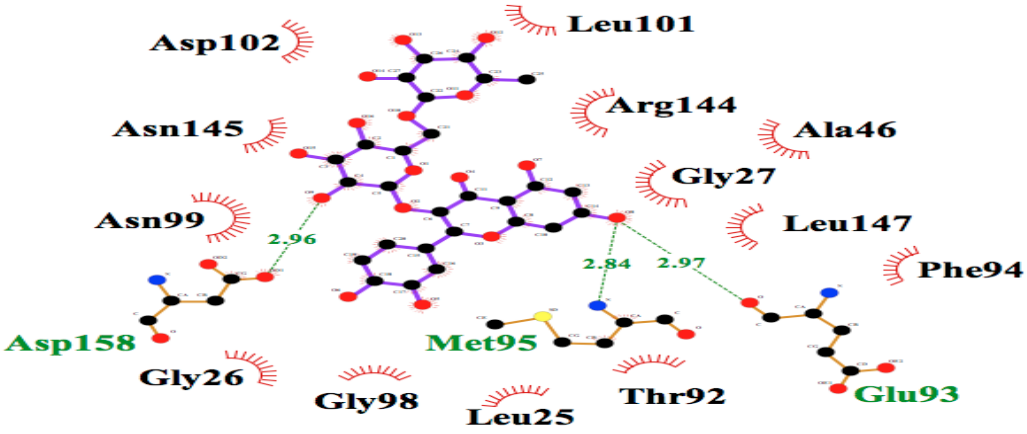
Interaction of co-crystalized nilotinib with Bcr-Abl

b).



Interaction of Cynaroside with key residues of Bcr-Abl

c).



interaction of rutin with key residues of Bcr-Abl

Figure 3: Interactions of nilotinib and the hit compounds with key residues of the therapeutic target, Bcr-Abl

Chronic myeloid leukaemia (CML) is a haematological cancer of the white cell, that contributes about 15% of adult leukaemia (19). With the increased cases of chronic myeloid leukaemia associated death (19), there is need for more potent drugs especially of plants origin; plants have brought forth the most important success in the chemotherapy of cancer (11). In this present study phytochemicals from *Cantharanthus roseus* and *Bryophyllum pinnatum* were docked into the catalytic site of Bcr-Abl using nilotinib , an FDA approved drug as the standard. From our virtual highthrough-put screening (vHTS) it is worthy of note that only bryophyllum pinnatum has phytochemicals with lower binding energy (Cynaroside with -9.6kcal/mol and rutin with -9.4kcal/mol) when screened against the Bcr-Abl catalytic site (Table 1), though *Cantharanthus roseus* has been reported to have anti-leukemic properties (12).

Cynaroside, with binding energy -9.6kcal/mol and rutin with -9.4kcal/mol were found to have a better inhibitory effect on Bcr-Abl catalytic site when compare with the co-crystalized nilotinib (-9.2kcal/mol). The closely related binding energies between cynaroside, rutin and the standard nilotinib, is believed to be as a result of the hydrogen bond interactions with key residues. Cynaroside forms six hydrogen bond interactions with ASP 158, ASN 145, ARG 144, GLY 27 (Figure 3 b), rutin forms three hydrogen bond interactions with GLU 93, MET 95, ASP 158, while nilotinib forms three hydrogen bonds with GLU 93 and MET 95(Figure 3 c). Cynaroside, rutin and the standard drug nilotinib all share a common hydrogen bond interaction with MET 95, which explains the closely related binding affinities.

Cynaroside with a better binding affinity is probably due to the greater number of hydrogen bond interations (6H-bond) when compared with rutin and nilotinib with three hydrogen bond each (3H-bond) (Figure 3). Our lead compounds were subjected to lipinski's rule of five (14) to evaluate their possible oral bioavailability and pharmacokinetics. It was shown that cynaroside obeyed the rule with a molecular weight of 448.38 and ClogP value of 0.5 and a refractivity of 107.04 and rotable bond of 4 while rutin violated the lipinski's rule. It is worthy of note that both cynaroside and rutin have been reported elsewhere to possess anti-cancer effects (20, 21). Both cynaroside and rutin are potential anti-leukaemia phyto-compounds. A positive correlation of 0.67 (Figure 4) shows that our docking scores using AutoDock Vina algorithm is dependable and depict possible experimental pIC_{50} values obtainable.

Conclusion

Virtual High Throughput screening of Phytochemicals from *Cantharanthus roseus* and *Bryophyllum pinnatum* against the Bcr-Abl catalytic site, revealed rutin and cynaroside from *Bryophyllum pinnatum* as the hits compounds Both cynaroside and rutin are potential anti-leukaemia phyto-compounds.

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