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Network pharmacology and Molecular docking-based activity of *Hemidesmus indicus* (L.) R.Br. in Acute myeloid leukemia : A Computational Study

Research Article

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Abstract

Acute myeloid leukemia (AML) is malignancy of the stem cell precursors of the myeloid lineage, occurs due to variations in genetics. In Ayurveda AML, can be considered into *Raktapitta* (~bleeding disorder) disease. *Hemidesmus indicus* (L.) R.Br. (~*H. indicus*) is described for treatment of *Raktapitta*. This study establish link for therapeutic activity of *H. indicus* in AML using Network pharmacology and molecular docking study. Active compound from root of *H. indicus* was retrieved from phytochemical based IMPPAT database. ADME (absorption, distribution, metabolism and excretion) done with SwissADME database, and target of active compound were obtained with SwissTargetPrediction database. Target of AML retrieved from GeneCard database. Cytoscape3.9.1 software was used to construct the "drug-active components-target" network diagram from common targets. The PPI (protein-protein interaction) network between proteins was constructed by STRING and result exported to Cytoscape3.9.1 for network analysis to get subnetwork with key target of subnetwork and core targets of overall PPI. GO and KEGG pathway analysis of key target from subnetwork done with g-profiler database. Core targets were docked with their corresponding active compound to get docking score. All core targets identified through network analysis of PPI network were linked to common active compound quercetin, and on molecular docking study all core targets showed good docking score to quercetin. Hence, based on this study conclusion can be drawn that the activity of *H. indicus* is AML might be due to presence of quercetin active compound in it. This study generated link for usefulness of *H. indicus* is AML.

Keywords: *Hemidesmus indicus (L.) R.Br., Acute myeloid leukemia,* Ayurveda, Network Pharmacology, Molecular docking.

Introduction

AML is a condition in which there is malignancy of the stem cell precursors of the myeloid lineage occurs, it results due to variations in genetics and lead to neoplastic changes (1). Incidence rate of childhood AML in Asian and Pacific Islanders, Hispanics, Caucasians and African Americans are 8.4, 8.1, 7.5 and 6.6 per million respectively (2). Children with AML shows pancytopenia, fatigue, bleeding, fever, pallor, bone pain and infections as a sign and symptoms (3). AML can be classified into *Raktapitta* disease in Ayurveda on the basis of its sign and symptoms, various studies classified leukemia into *Raktapitta* disease (4) (5)(6). Various Ayurvedic herbal medicines are being used widely since centuries for the management of *Raktapitta*. *Hemidesmus indicus* (L.) R.Br. is described one of best herb used for treatment of *Raktapitta* (7). It has traditionally been used for treating snakebites, scorpion stings, diabetes, urinary diseases, dyspnea, menorrhagia, sexually transmitted diseases and cancer (8).

Now a days network pharmacology, is being used for elucidating the multi-target effects of medicinal plants for curing various types of diseases and disorders (9). The compounds of *H. indicus* and the putative mechanism behind activity in AML were investigated utilizing network pharmacology integrating with molecular docking in the current study. According our understanding this is the leading study to classify the underlying mechanism of *H. indicus* in the treatment of AML using bioinformatics and network pharmacology.

Methodology

The overall design of this study is shown in Figure 1. The database used in this study is described in Table 1.

Active compounds in *H. indicus*

Information related to active compound from root of *H. indicus* was retrieved from literature and publicly available database. *H. indicus* related compounds were collected from phytochemical based IMPPAT database (10)(11). The keyword "*Hemidesmus indicus*" was used in the databases, while literature mining was conducted on PubMed, and Google Scholar. PubChem (12) were used to collect chemical information of predicted compounds.

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ADME screening of active compounds in *H. indicus*

A simplified molecular-input line-entry system (SMILES) was searched for active compounds identified from literature and publicly available databases of *H. indicus*. SMILES were screened for human gastrointestinal absorption (HIA). Drug-likeness calculated by SwissADME software (13). Parameters of HIA met "high," and two or more models among five drug-likeness models (Lipinski, Ghose, Veber, Egan, and Muegge) met "yes" and were selected as active compounds with good bioavailability.

Targets prediction of ADME qualified compounds

Targets of the identified active compounds were predicted by SwissTargetPrediction (14), and active compounds showing targets with a probability of more than 70% were only selected.

Retrieval of targets for acute myeloid leukemia

Targets of acute myeloid leukemia were obtained and screened with a relevance score ≥5.0 by using keyword "acute myeloid leukemia" from GeneCards, a database integrating all annotated and predicted targets associated with human diseases (15).

Screening common targets of drugs and diseases and construction of network diagram

Possible targets of *H. indicus* and AML related targets were screened for common targets using Venny 2.1.0 tool (16). Importing common targets into Cytoscape3.9.1 a network construction tool (17), to construct the network diagram of "drug- active components - target".

Protein protein interaction network construction and screening of core targets

The screened common targets were imported into STRING a protein-protein association networks analyser tool (18), the species was limited to "Homo sapiens", the medium confidence level was 0.4, other settings were default, and the protein-protein interaction (PPI) network was constructed. The obtained PPI result were imported into Cytoscape 3.9.1 for network analysing to get core targets.

GO and KEGG analysis

Top targets screened were subjected to GO enrichment analysis and KEGG pathway analysis using online functional annotation and enrichment tool g-Profiler (19), and species were restricted to "Homo sapiens" and P <0.05. Enrichment analyses include molecular function (MF), biological process (BP), and cellular component (CC).

Construction of network for "drug-active compound-target-pathway"

Screened top 10 KEGG pathways related to AML, and the targets corresponding to the pathways were identified and imported into Cytoscape3.9.1 to build a "drug-active component -target-pathway" network diagram.

Molecular docking

The top five core targets were selected for molecular docking with their corresponding active compound, CB-Dock (20) is used for docking purpose. The crystal structure of targets was downloaded from the Protein Data Bank (PDB) (21), and structure of active compound were downloaded from PubChem database. The best vina score after docking was selected for evaluation.

Results

Active compounds in *H. indicus*, ADME screening, target prediction and retrieval of targets for AML

Active compound from root of *H. indicus* was retrieved, total of 66 active compound were obtained, On ADME screening 49 active compounds qualified. ADME qualified active compound screened for target on SwissTargetPrediction and only 13 active compounds shown >70% target prediction with 149 target, and after removal of duplicates 81 target were remained (table 2). Targets of AML were obtained and screened with a relevance score ≥5.0 by using keyword "acute myeloid leukemia" from GeneCards database, it reported 806 target.

Identification of common target and network construction

The targets of active compound after removal of duplicate and disease targets retrieved form GeneCards database were imported into Venny2.1.0 database for intersection, and 19 common targets were obtained (Figure 2, table 3). A network diagram is constructed between drug - active component and common target by using Cytoscape3.9.1 (Figure 3), this network diagram includes 23 nodes and 22 edges.

PPI network construction and core target screening

Importing screened 19 targets in STRING database to construct protein-protein interaction (PPI) network (Figure 4), it consists of 19 nodes and 56 edges having average node degree 5.89. The obtained result imported into Cytoscape 3.9.1 and the target network was analyzed by sub-clustering using "MCODE" plug-in 1 sub networks with 11 key targets were obtained (Table 4). MCC (Maximal Clique Centrality) was selected as the computational method to identify top five *H. indicus* core targets for AML using "CytoHubba" plug-in (Table 5, figure 5).

GO and KEGG analysis

GO and KEGG enrichment analyses were performed on the above-mentioned 11 key targets (table 4) for activity of *H. indicus* in AML using the g-profiler. Values of P < 0.05 and species were set to "Homo sapiens". From the GO bioaccumulation analysis, 44 MF (Molecular function), 166 BP (Biological process), 12 CC (Cellular component) and 55 pathways were obtained from the KEGG pathway analyses (Supplementary table 1). Top five enriched GO scores include ATP binding (GO:0005524), adenyl ribonucleotide binding (GO:0032559), adenyl nucleotide binding (GO:0030554), protein tyrosine

kinase activity (GO:0004713), purine ribonucleoside triphosphate binding (GO:0035639) as MF, phosphatidylinositol 3-kinase signaling (GO:0014065), transmembrane receptor protein tyrosine kinase signaling pathway (GO:0007169), phosphatidylinositol-mediated signaling (GO:0048015), inositol lipid-mediated signaling (GO:0048017), cellular response to chemical stimulus (GO:0070887) as BP, membrane raft (GO:0045121), membrane microdomain (GO:0098857), cell periphery (GO:0071944), external side of apical plasma membrane (GO:0098591), side of membrane (GO:0098552) as CC details given in table 6.

Top 10 key signaling pathways for activity of *H. indicus* in AML are EGFR tyrosine kinase inhibitor resistance (KEGG:01521), Endocrine resistance (KEGG:01522), Proteoglycans in cancer (KEGG:05205), Focal adhesion (KEGG:04510), Prostate cancer (KEGG:05215), Rap1 signaling pathway (KEGG:04015), Relaxin signaling pathway (KEGG:04926), Estrogen signaling pathway (KEGG:04915), Fluid shear stress and atherosclerosis (KEGG:05418) and VEGF signaling pathway (KEGG:04370) detail given in table 7.

Drug-active component -target -pathway Network

Target of active compound present in *H. indicus* related to AML were screened and the pathway of corresponding target were identified and imported into Cytoscape3.9.1 to build a "drug - active compound - target pathway" network diagram (Figure 6). The network diagram includes 33 nodes and 78 edges, The network shows activity of *H. indicus* in AML is achieved through multi-compound, multi-target and multi-pathway.

Molecular Docking

The top five core targets EGFR, SRC, AKT1, KDR and IGF1R obtained from PPI network after applying MCC computational method (table 5, figure 5) were selected for molecular docking with their corresponding active compound. In docking study, lower docking score between ligands and receptors implies stronger binding, and more stable docking.

Crystal's structure of the EGFR (PDB code 1IVO) at resolution 3.30 Å, SRC (PDB code 1A07) at resolution 2.20 Å, AKT1 (PDB code 1H10) at resolution 1.40 Å, KDR (PDB code 1VR2) at resolution 2.40 Å and IGF1R (PDB code 1IGR) at resolution 2.60 Å imported into CB-Dock along with 3D SDF file of their corresponding target Quercetin (table 2) downloaded from PubChem (PubChem ID 5280343) for cavity detection based blind docking. Best docking score and best docking position are detailed in table 8 and figure 7a, 7b, 7c, 7d, 7e respectively. It was found that the docking score between of Quercetin to EGFR was the lowest with 8.4 kcal. mol^{-1}, and between Quercetin - AKT1 was highest -6.0 kcal. mol^{-1}. This molecular docking study shows that active constituents of *H. indicus* may play a role in AML management.

Discussion

Ayurveda is a rapidly developing in scientific research, and the demand of Ayurvedic medicine are growing globally, in COVID pandemic time Ayurveda played very vital role in healthcare system of India as a result of that it is getting recognition globally for other disorder also. Ayurvedic herbal medicines consists of multiple active compounds and they act on multiple targets for a single disease, this can be seen with the help of network pharmacology. *H. indicus* is known as *Sariva* in Ayurveda, is very useful in various disorders, it is described for treatment of *Raktapitta* (~bleeding disorders) and AML can be considered as *Raktapitta*. In current study *H. indicus* is explored for its use in AML based on computational work, total 19 common targets from *H. indicus* were obtained for having activity in AML, out of these EGFR, SRC, AKT1, KDR and IGF1R were identified as core target play role in AML.

Expression of EGFR, an important proto-oncogene, regulates cell differentiation, proliferation, cell migration and survival in most of the cancer types (22). The EGFR inhibitor has been shown to induce complete remission of AML (23). SRC belongs to SRC-family kinases (SFKs) play crucial roles in normal hematopoiesis, essential for membrane receptor downstream signaling in AML and number of studies generated evidence that SFKs are rational therapeutic targets in AML (24). The AKT1 gene belongs to a class of genes known as oncogenes. AKT inhibitor, induces growth inhibition and apoptosis in leukemia cell lines (25). PI3K-Akt signaling pathway (KEGG:04151) is obtained through KEGG analysis of 11 key targets of *H. indicus* (Supplementary table 1), and the PI3K/AKT Pathway inhibitor induces apoptosis and inhibits growth of leukemia in preclinical models of AML (26). KDR is markedly upregulated in many types of cancer cells (27), studies reported over expression of vascular endothelial growth factor and its cellular receptor KDR in patients with acute myeloid leukemia (28). IGF1R signaling has been profusely implicated as a critical contributor to cancer cell proliferation, survival, migration, and resistance to anticancer therapies, thus targeting IGF signaling is an attractive therapeutic strategy (29). IGF-1R is always expressed in acute myeloid leukemia blast cells and is constitutively activated in samples that also show constitutive activation of the PI3K/Akt pathway (30).

Among top 10 signaling pathway all core target work through EGFR tyrosine kinase inhibitor resistance (KEGG:01521), Proteoglycans in cancer (KEGG:05205), Focal adhesion (KEGG:04510) and Rap1 signaling pathway (KEGG:04015). SRC, AKT1 and KDR use Fluid shear stress and atherosclerosis (KEGG:05418) and VEGF signaling pathway (KEGG:04370). EGFR, SRC and AKT1 involved in Relaxin signaling pathway (KEGG:04926) and Estrogen signaling pathway (KEGG:04915). EGFR, AKT1and IGF1R takes Prostate cancer (KEGG:05215) signaling pathway, and all core target except KDR shows their activity in AML by Endocrine resistance (KEGG:01522) signaling pathway.

Conclusion

All core targets identified through network analysis of PPI network were linked to common active compound quercetin, and on molecular docking study all core targets showed good docking score to quercetin, with quercetin – EGFR having best docking score among all. Studies support that the quercetin is useful in management of AML and induces apoptosis via Downregulation of Vascular Endothelial Growth Factor/ Akt Signaling Pathway in AML cells (31). Hence, based on this study conclusion can be drawn that the activity of *H. indicus* is AML might be due to presence of quercetin active compound in it.

In Ayurveda *H. indicus* is used for *Raktapitta* and other various blood related disorders, there is no exact disease described in Ayurveda which could be termed as AML, but based on signs and symptoms it can be grouped into *Raktapitta*.

This study generated link for usefulness of *H. indicus* is AML.

Limitation

ADME study of active compound Tannic acid was not performed as its SMILES crossed the maximum limit of 200 characters in SwissADME database. In Ayurveda root of *H. indicus* is used, so only active compound of root from IMPPAT database were assed in this study, active compound from other part of plant can also be used for further study to know activity of *H. indicus* in AML.

Source of Funding

None.

Declaration of competing Interest

The authors declare no conflicting interest.

Figure 1: Overall design of study

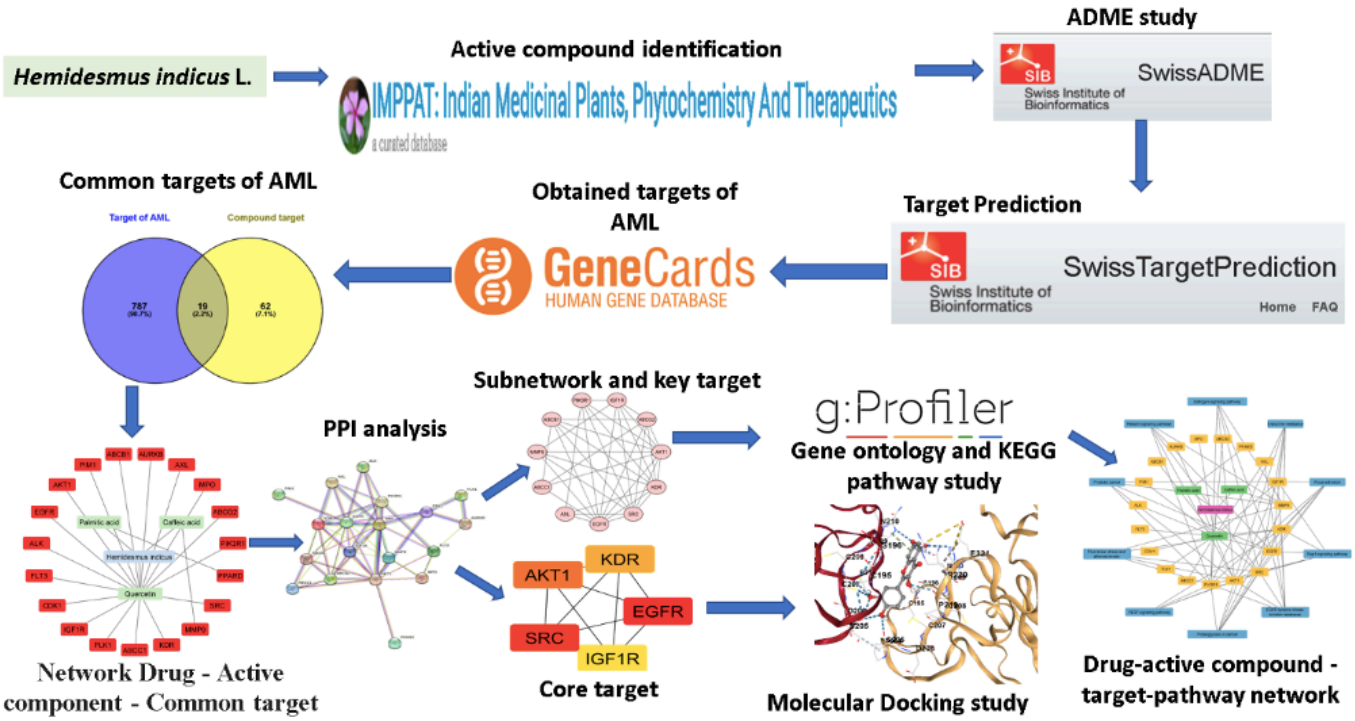


Table 1: Database used in this study

S. No.	Database and Software	Website
1	IMPPAT	https://cb.imsc.res.in/imppat/
2	PubChem	https://pubchem.ncbi.nlm.nih.gov/
3	SwissADME	http://www.swissadme.ch/index.php
4	SwissTargetPrediction	http://www.swisstargetprediction.ch/
5	GeneCard	https://www.genecards.org/
6	Venny 2.1	https://bioinfogp.cnb.csic.es/tools/venny/
7	Cytocsape (3.9.1)	https://cytoscape.org/
8	STRING	https://string-db.org/
9	g:Profiler	https://biit.cs.ut.ee/gprofiler/gost
10	CB-Dock	https://cadd.labshare.cn/cb-dock2/
11	Protein Data Bank	https://www.rcsb.org/

Table 2: Bioactive with their SMILES, ADME status and their corresponding targets

S. No.	Bioactive	ADME qualified	Targets	SMILES
1	Myrtenol	Yes	Not qualified	CC1(C2CC=C(C1C2)CO)C
2	Syringic acid	Yes	CA2	COC1=CC(=CC(=C1O)OC)C(=O)O
			CA7	
			CA1	
			CA3	
			CA6	
			CA12	
			CA14	
			CA9	
			CA5A	
3	Vanillin	Yes	Not qualified	COC1=C(C=CC(=C1)C=O)O
4	Pinocarvone	Yes	Not qualified	CC1(C2CC1C(=C)C(=O)C2)C
5	Isovanillin	Yes	Not qualified	COC1=C(C=C(C=C1)C=O)O
6	Hemidescine	No	Not qualified	C[C@@H]1[C@H]([C@H](C[C@@H](O1)O[C@@H]2[C@H](O[C@H](C[C@H]2OC)O[C@H]3CC[C@@]4([C@H]5CC[C@@]6([C@H](CC[C@@]6([C@@H]5CC=C4C3)O)[C@H](C)OC(=O)C)C)C)C)O)O
7	Lauric acid	Yes	FFAR1	CCCCCCCCCCCC(=O)O
8	Methyl salicylate	Yes	Not qualified	COC(=O)C1=CC=CC=C1O
9	Decanoic acid	Yes	Not qualified	CCCCCCCCCC(=O)O
10	2,5-Dihydroxybenzoic acid	Yes	CA2	C1=CC(=C(C=C1O)C(=O)O)O
			CA1	
			CA12	
			CA9	
11	Octanoic acid	Yes	Not qualified	CCCCCCCC(=O)O
12	4-Methoxybenzaldehyde	Yes	Not qualified	COC1=CC=C(C=C1)C=O
13	Benzophenone	Yes	Not qualified	C1=CC=C(C=C1)C(=O)C2=CC=CC=C2
14	beta-Amyrin acetate	No	Not qualified	CC(=O)O[C@H]1CC[C@]2([C@H](C1(C)C)CC[C@@]3([C@@H]2CC=C4[C@]3(CC[C@@]5([C@H]4CC(CC5)(C)C)C)C)C)C
15	Dihydrocarvyl acetate	Yes	Not qualified	CC1CCC(CC1OC(=O)C)C(=C)C
16	Verbenone	Yes	Not qualified	CC1=CC(=O)C2CC1C2(C)C
17	Quercetin	Yes	NOX4	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O)O
			AVPR2	
			AKR1B1	
			XDH	
			MAOA	
			IGF1R	
			FLT3	
			CYP19A1	
			EGFR	
			F2	
			CA2	
			PIM1	
			ALOX5	
			AURKB	
			DRD4	
			ADORA1	
			CA7	
			GLO1	

			MPO	
			PIK3R1	
			ADORA2A	
			DAPK1	
			PYGL	
			CA1	
			GSK3B	
			SRC	
			PTK2	
			HSD17B2	
			KDR	
			MMP13	
			MMP3	
			CA3	
			ALOX15	
			ABCC1	
			PLK1	
			CA6	
			CDK1	
			MMP9	
			CA12	
			MMP2	
			PKN1	
			CA14	
			CA9	
			CSNK2A1	
			ALOX12	
			MET	
			CA4	
			NEK2	
			CXCR1	
			CAMK2B	
			ALK	
			AKT1	
			ABCB1	
			NEK6	
			PLA2G1B	
			CA5A	
			BACE1	
			CYP1B1	
			AXL	
			ABCG2	
			NUAK1	
			AKR1C2	
			AKR1C1	
			AKR1C3	
			AKR1C4	
			CA13	
			AKR1A1	
			GPR35	
18	4-Methoxysalicylic acid	Yes	Not qualified	COC1=CC(=C(C=C1)C(=O)O)O
19	Myrtenal	Yes	Not qualified	COC1=CC(=C(C=C1)C(=O)O)O
20	Thymol	Yes	Not qualified	CC1=CC(=C(C=C1)C(C)C)O
21	2-Hydroxy-4-methoxybenzaldehyde	Yes	Not qualified	COC1=CC(=C(C=C1)C=O)O
22	Salicylaldehyde	Yes	Not qualified	C1=CC=C(C(=C1)C=O)O
23	Hydroquinone	Yes	CA2	C1=CC(=CC=C1O)O

			CA3	
			CA12	
24	Linalyl acetate	Yes	Not qualified	CC(=CCCC(C)(C=C)OC(=O)C)C
25	Vanillic acid	Yes	Not qualified	COC1=C(C=CC(=C1)C(=O)O)
26	Palmitic acid	Yes	FABP4	CCCCCCCCCCCCCCCC(=O)O
			PPARA	
			FABP3	
			FABP5	
			PPARD	
			FABP2	
27	Oleanane	No	Not qualified	C[C@@]12CC[C@@]3([C@@H]([C@@H]1CC(CC2)(C)C)CC[C@H]4[C@]3(CC[C@@H]5[C@@]4(CCCC5(C)C)C)C)C
28	(1S,3aR,5aR,5bR,7aS,11aS,11bR,13aR,13bR)-3a,5a,5b,8,8,11a-hexamethyl-1-propan-2-yl-1,2,3,4,5,6,7,7a,9,10,11,11b,12,13,13a,13b-hexadecahydrocyclopenta[a]chrysene	No	Not qualified	CC(C)[C@@H]1CC[C@]2([C@H]1[C@H]3CC[C@@H]4[C@]5(CCCC([C@@H]5CC[C@]4([C@@]3(CC2)C)C)(C)C)C)C
29	Ursane	No	Not qualified	CC1CCC2(C(C1C)C3CCC4C5(CCC(=O)C(C5CCC4(C3(CC2=O)C)C)(C)C)C)C
30	Hexatriacontane	No	Not qualified	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
31	2,10-Epoxypinane	Yes	Not qualified	CC1(C2CCC3(C1C2)CO3)C
32	Eucalyptol	Yes	Not qualified	CC1(C2CCC(O1)(CC2)C)C
33	beta-Elemene	No	Not qualified	CC(=C)[C@@H]1CC[C@@]([C@@H](C1)C(=C)C)(C)C=C
34	Benzyl benzoate	Yes	Not qualified	C1=CC=C(C=C1)COC(=O)C2=CC=CC=C2
35	trans-2,cis-6-Nonadienal	Yes	Not qualified	CC/C=C\CC/C=C/C=O
36	3-Methoxyphenol	Yes	Not qualified	COC1=CC=CC(=C1)O
37	Phenethyl cinnamate	Yes	Not qualified	C1=CC=C(C=C1)CCOC(=O)/C=C/C2=CC=CC=C2
38	Pentyl cinnamate	Yes	Not qualified	CCCCCOC(=O)/C=C/C1=CC=CC=C1
39	4-Carvomenthenol	Yes	Not qualified	CC1=CCC(CC1)(C(C)C)O
40	Guaiacol	Yes	CA2	COC1=CC=CC=C1O
41	alpha-Terpinyl acetate	Yes	Not qualified	CC1=CCC(CC1)C(C)(C)OC(=O)C
42	d-Borneol	Yes	Not qualified	C[C@@]12CC[C@@H](C1(C)C)C[C@@H]2O
43	alpha-Amyrin	No	Not qualified	C[C@@H]1CC[C@@]2(CC[C@@]3(C(=CC[C@H]4[C@]3(CC[C@@H]5[C@@]4(CC[C@@H](C5(C)C)O)C)C)[C@@H]2[C@H]1C)C)C
44	Tannic acid	NA*	Not qualified	C1=C(C=C(C(=C1O)O)O)C(=O)OC2=CC(=CC(=C2O)O)C(=O)OC[C@@H]3[C@H]([C@@H]([C@H]([C@@H](O3)OC(=O)C4=CC(=C(C(=C4)OC(=O)C5=CC(=C(C(=C5)O)O)O)O)O)OC(=O)C6=CC(=C(C(=C6)OC(=O)C7=CC(=C(C(=C7)O)O)O)O)O)OC(=O)C8=CC(=C(C(=C8)OC(=O)C9=CC(=C(C(=C9)O)O)O)O)O)OC(=O)C1=CC(=C(C(=C1)OC(=O)C1=CC(=C(C(=C1)O)O)O)O)O
45	Ferulic acid	Yes	CA2	COC1=C(C=CC(=C1)/C=C/C(=O)O)O

			CA7	
			CA1	
			CA6	
			CA12	
			CA14	
			CA9	
			CA5A	
46	3,4-Dihydroxybenzoic acid	Yes	CA2	C1=CC(=C(C=C1C(=O)O)O)O
			CA7	
			CA1	
			CA6	
			CA12	
			CA14	
			CA9	
			CA4	
47	Caffeic acid	Yes	CA2	C1=CC(=C(C=C1/C=C/C(=O)O)O)O
			ALOX5	
			CA7	
			CA1	
			CA6	
			MMP9	
			CA12	
			MMP1	
			MMP2	
			PTPN1	
			CA14	
			CA9	
			CA5B	
			CA5A	
48	Cinnamic acid	Yes	HCAR2	C1=CC=C(C=C1)/C=C/C(=O)O
49	4-Hydroxycinnamic acid	Yes	AKR1B1	C1=CC(=CC=C1/C=C/C(=O)O)O
			CA2	
			CA7	
			ESR2	
			CA1	
			CA3	
			CA6	
			CA12	
			CA14	
			CA9	
			CA4	
			CA5B	
			CA5A	
50	Gallic acid	Yes	CA2	C1=C(C=C(C(=C1O)O)O)C(=O)O
			CA7	
			CA1	
			CA3	
			CA6	
			CA12	
			CA14	
			CA9	
			FUT7	
			CA4	
			CA5B	
			CA5A	
			CA13	
51	Camphor	Yes	Not qualified	CC1(C2CCC1(C(=O)C2)C)C
52	alpha-Terpineol	Yes	Not qualified	CC1=CCC(CC1)C(C)(C)O

53	beta-Amyrin	No	Not qualified	C[C@@]12CC[C@@]3(C(=CC[C@H]4[C@]3(CC[C@@H]5[C@@]4(CC[C@@H](C5(C)C)O)C)C)[C@@H]1CC(CC2)(C)C)C
54	Lupeol	No	Not qualified	CC(=C)[C@@H]1CC[C@]2([C@H]1[C@H]3CC[C@@H]4[C@]5(CC[C@@H](C([C@@H]5CC[C@]4([C@@]3(CC2)C)C)(C)C)O)C)C
55	Levomenol	Yes	Not qualified	CC1=CC[C@H](CC1)[C@](C)(CCC=C(C)C)O
56	Isocaryophyllene	No	Not qualified	C/C/1=C/CCC(=C)[C@H]2CC([C@@H]2CC1)(C)C
57	beta-Selinene	No	Not qualified	CC(=C)[C@@H]1CC[C@]2(CCCC(=C)[C@@H]2C1)C
58	Aromadendrene	No	Not qualified	CC1CCC2C1C3C(C3(C)C)CCC2=C
59	beta-Sitosterol	No	Not qualified	CC[C@H](CC[C@@H](C)[C@H]1CC[C@@H]2[C@@]1(CC[C@H]3[C@H]2CC=C4[C@@]3(CC[C@@H](C4)O)C)C)C(C)C
60	Bornyl acetate	Yes	Not qualified	CC(=O)OC1CC2CCC1(C2(C)C)C
61	Ledol	Yes	Not qualified	C[C@@H]1CC[C@H]2[C@@H]1[C@H]3[C@H](C3(C)C)CC[C@@]2(C)O
62	Limonene	No	Not qualified	CC1=CCC(CC1)C(=C)C
63	Lupeol acetate	No	Not qualified	CC(=C)[C@@H]1CC[C@]2([C@H]1[C@H]3CC[C@@H]4[C@]5(CC[C@@H](C([C@@H]5CC[C@]4([C@@]3(CC2)C)C)(C)C)OC(=O)C)C)C
64	alpha-Muurolol	Yes	Not qualified	C[C@H]1CC[C@@H]2[C@H](C1)[C@H](CC[C@@]2(C)O)C(C)C
65	Nerolidol	Yes	Not qualified	CC(=CCC/C(=C/CCC(C)(C=C)O)/C)C
66	Isobornyl acetate	Yes	Not qualified	CC(=O)O[C@H]1C[C@@H]2CC[C@]1(C2(C)C)C
*: ADME not identified in SwissADME database				

Table 3: Common targets between active compound target and target of AML retrieved from database

S. No.	Target	Uniprot ID
1	FLT3	P36888
2	MPO	P05164
3	ABCB1	P08183
4	AKT1	P31749
5	ABCC1	P33527
6	ABCG2	Q9UNQ0
7	KDR	P35968
8	PIM1	P11309
9	AXL	P30530
10	MMP9	P14780
11	AURKB	Q96GD4
12	EGFR	P00533
13	CDK1	P06493
14	PLK1	P53350
15	SRC	P12931
16	PIK3R1	P27986
17	IGF1R	P08069
18	ALK	Q9UM73
19	PPARD	Q03181

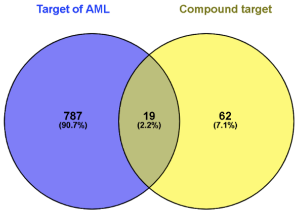
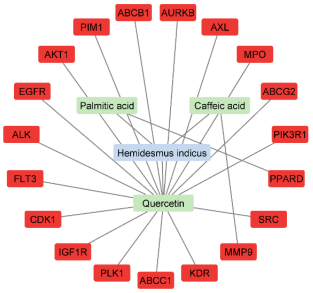
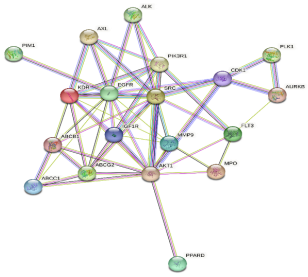
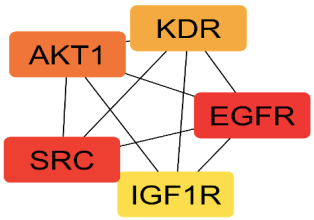
Figure 2: Venn diagram of common targets between active compound target and target of AML retrieved from database	Figure 3: Network diagram showing Drug - Active component - Common target	Figure 4: Protein-protein interaction analysis diagram	Figure 5: Core targets of *H. indicus* in AML
			

Table 4: Sub network and key target

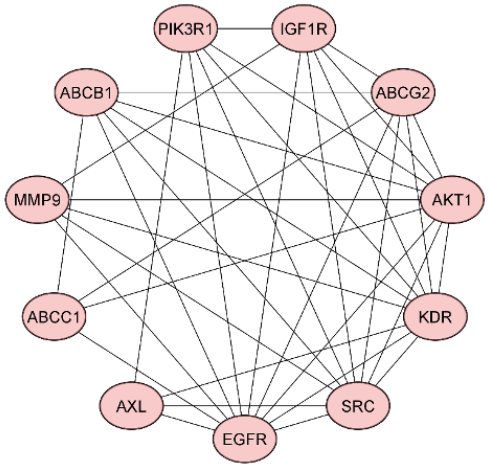
S. No.	Nodes	Edges	Gene	Network
1	11	38	PIK3R1, IGF1R, ABCG2, AKT1, KDR, SRC, EGFR, AXL, ABCC1, MMP9, ABCB1	

Table 5: Ranking of core targets of *H. indicus* in AML

Rank	Name	Score
1	EGFR	541
2	SRC	522
3	AKT1	521
4	KDR	504
5	IGF1R	360

Table 6: GO enrichment analysis

Class	GO ID	Enrichment analysis	Adjusted P value	Number of enriched
MF	GO:0005524	ATP binding	5.93E-07	9
MF	GO:0032559	Adenyl ribonucleotide binding	8.56E-07	9
MF	GO:0030554	Adenyl nucleotide binding	1.49328E-06	9
MF	GO:0004713	Protein tyrosine kinase activity	1.61223E-06	5
MF	GO:0035639	Purine ribonucleoside triphosphate binding	3.88042E-06	9
BP	GO:0014065	Phosphatidylinositol 3-kinase signaling	5.59E-08	6
BP	GO:0007169	Transmembrane receptor protein tyrosine kinase signaling pathway	1.24E-07	8
BP	GO:0048015	Phosphatidylinositol-mediated signaling	1.81E-07	6
BP	GO:0048017	Inositol lipid-mediated signaling	2.07E-07	6
BP	GO:0070887	Cellular response to chemical stimulus	2.12E-07	11
CC	GO:0045121	Membrane raft	2.95642E-05	5
CC	GO:0098857	Membrane microdomain	3.00193E-05	5
CC	GO:0071944	Cell periphery	0.000105079	11
CC	GO:0098591	External side of apical plasma membrane	0.000354536	2
CC	GO:0098552	Side of membrane	0.001168372	5

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Table 7: KEGG pathway analysis

KEGG ID	Enrichment analysis	Adjusted P value	Number of targets involved in pathway
KEGG:01521	EGFR tyrosine kinase inhibitor resistance	1.09E-10	7
KEGG:01522	Endocrine resistance	5.24E-08	6
KEGG:05205	Proteoglycans in cancer	9.68E-08	7
KEGG:04510	Focal adhesion	4.98857E-06	6
KEGG:05215	Prostate cancer	5.23408E-06	5
KEGG:04015	Rap1 signaling pathway	6.28887E-06	6
KEGG:04926	Relaxin signaling pathway	2.19151E-05	5
KEGG:04915	Estrogen signaling pathway	2.95975E-05	5
KEGG:05418	Fluid shear stress and atherosclerosis	3.06911E-05	5
KEGG:04370	VEGF signaling pathway	4.38906E-05	4

Figure 6: Drug-active compound -target-pathway network

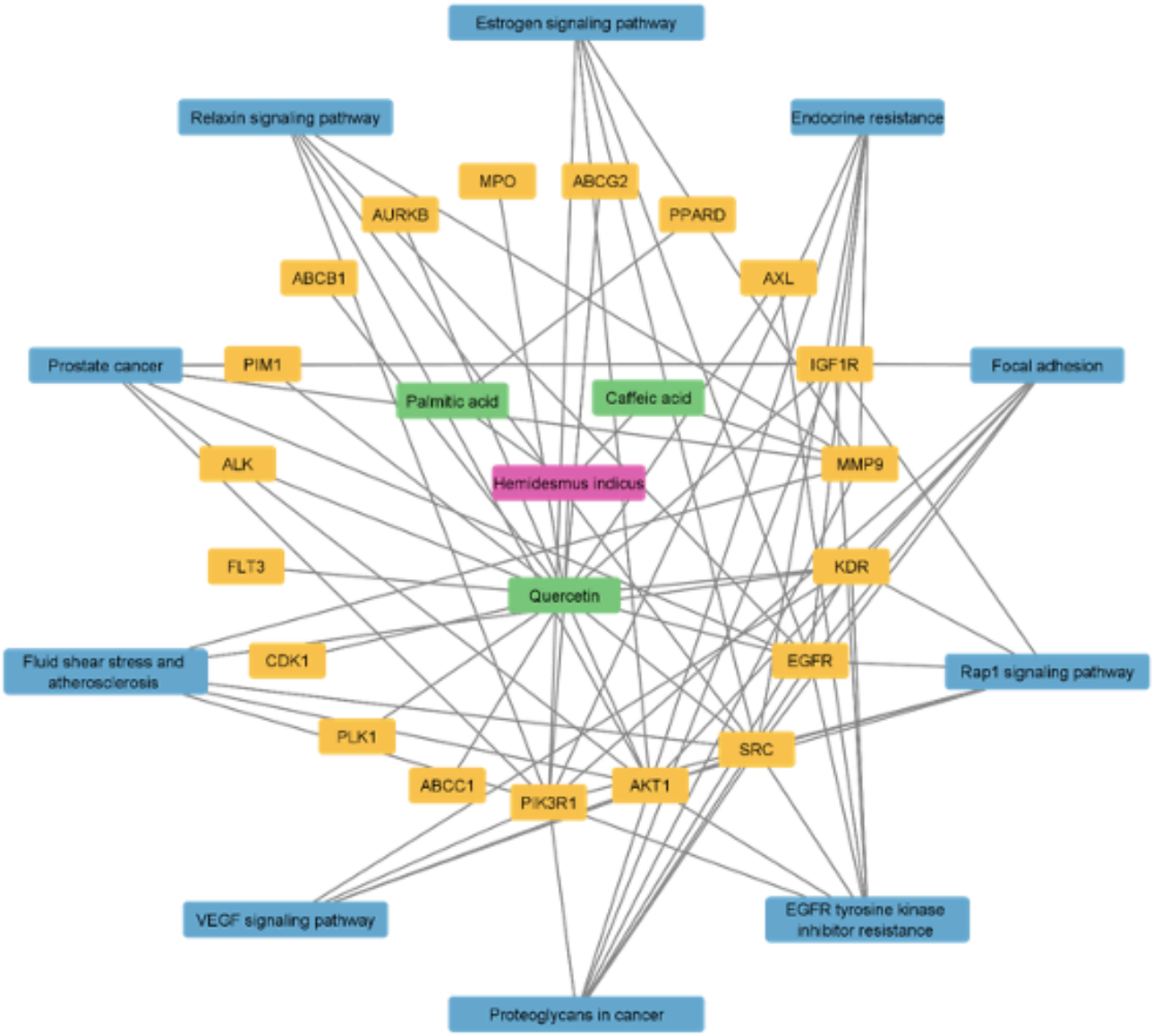
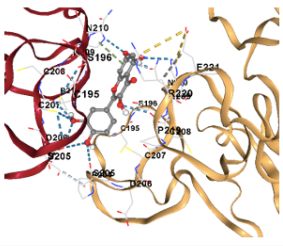
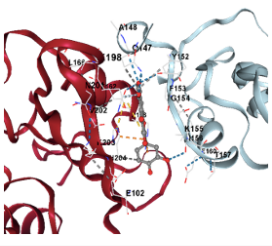
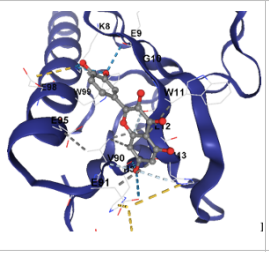
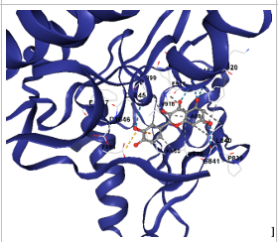
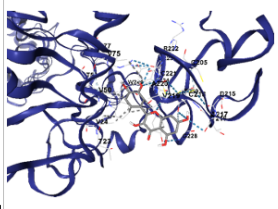


Table 8: Molecular docking result

Ligand-Protein	Vina score	Cavity Volume (Å^3)	Centre			Size			Contact residues
			x	y	z	x	y	z	
Quercetin - EGFR	-8.4	1458	73	78	42	21	21	21	**Chain A:** CYS195 SER196 SER205 ASP206 CYS207 CYS208 HIS209 ASN210 PRO219 **Chain B:** CYS195 SER196 PRO204 SER205 ASP206 CYS207 CYS208 HIS209 ASN210 PRO219 ARG220 GLU221
Quercetin - SRC	-8.1	1209	42	12	28	21	21	21	**Chain A**: ARG158 GLU162 LEU166 LYS198 ASN201 VAL202 LYS203 HIS204 **Chain C**: GLU102 **Chain B**: GLN147 ALA148 TYR152 PHE153 GLY154 LYS155 ILE156 THR157
Quercetin - AKT1	-6.0	110	17	5	14	21	21	21	**Chain A**: LYS8 GLU9 GLY10 TRP11 LEU12 HIS13 ARG69 HIS89 VAL90 GLU91 GLU95 GLU98 TRP99
Quercetin - KDR	-6.8	539	35	32	17	21	21	21	**Chain A:** PRO839 LEU840 GLY841 VAL848 ALA866 LYS868 GLU885 VAL899 VAL916 GLU917 PHE918 CYS919 LYS920 LEU1035 CYS1045 ASP1046 PHE1047
Quercetin – IGF1R	-8.1	202	48	14	75	21	21	21	**Chain A**: THR23 VAL24 VAL50 THR52 VAL75 ARG77 CYS205 ASP215 THR216 ALA217 CYS218 VAL219 ALA220 CYS221 ARG222 HIS223 TYR224 TYR225 GLY228 CYS230 TRP244

Figure 7a, 7b, 7c, 7d, 7e: Best docking position

Figure 7a: Best Molecular docking position of Quercetin – EGFR	Figure 7b: Best Molecular docking position of Quercetin – SRC	Figure 7c: Best Molecular docking position of Quercetin to AKT1	Figure 7d: Best Molecular docking position of Quercetin to KDR	Figure 7e: Best Molecular docking position of Quercetin to IGF1R
				

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