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Identification of potential lead compound from *Thuja occidentalis* as an inhibitor of FMS-like tyrosine kinase 3 (FLT3) in acute myeloid leukemia through virtual screening

Swetha S, Hema Priya Manivannan, Arul Prakash Francis*, Vishnu Priya Veeraraghavan, Gayathri R & Kavitha Sankaran

Centre of Molecular Medicine and Diagnostics (COMManD), Department of Biochemistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-600 077, Tamil Nadu, India

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The present study focus on the investigation of phytochemicals derived from *Thuja occidentalis* as potential inhibitors of FMS-like tyrosine kinase 3 (FLT3) in the context of acute myeloid leukemia (AML). The side effects associated with existing chemotherapy drugs have indeed spurred significant interest in the search for natural compounds from plants as alternative treatment options for cancer. Through *in silico* drug-likeness screening and pharmacokinetics analysis using the SwissADME web server, 25 phytochemicals were selected for further evaluation from the initial pool of 28 compounds. Among these, catechin, beta-eudesmol, and beta-thujaplicin exhibited remarkable binding affinities to FLT3, outperforming the control drug gilteritinib. Additionally, these compounds demonstrated favorable pharmacokinetic profiles and are predicted to have low toxicity, making them promising lead candidates for further studies. Radar plots representing oral bioavailability highlighted that the compounds fall within the optimal range, indicating their ability to be effectively absorbed. Our findings suggest that phytochemicals from *Thuja occidentalis* hold considerable promise as FLT3 inhibitors. But further experimental validation is required to explore their therapeutic potential

Keywords: Acute myeloid leukemia, Docking, Drug-likeness, Oral bioavailability, Phytochemicals, *Thuja occidentalis*

Acute myeloid leukemia (AML) is a hematological malignancy characterized by the rapid growth of abnormal myeloid cells in the bone marrow[1]. It is a highly aggressive and heterogeneous disease that poses significant challenges to effective treatment strategies[2]. FMS-like tyrosine kinase 3 (FLT3) is a receptor tyrosine kinase that plays a crucial role in the proliferation and survival of hematopoietic stem cells and progenitor cells[3]. Mutations in FLT3 are frequently found in AML patients and are associated with a poor prognosis[4].

Targeting FLT3 signalling has emerged as an attractive therapeutic approach for AML[5]. In recent years, virtual screening methods have gained prominence in drug discovery, allowing researchers to identify potential lead compounds efficiently from vast libraries of chemical compounds[6]. *Thuja occidentalis*, commonly known as white cedar, is an evergreen coniferous tree widely used in traditional medicine for various ailments[7]. It contains a diverse array of bioactive compounds, making it an intriguing source for drug discovery[8].

This study aimed to identify potential lead compounds from *Thuja occidentalis* through virtual screening, with a specific focus on their inhibitory activity against FLT3 in AML. Virtual screening involves the computational screening of chemical libraries to identify compounds that have the potential to interact with a target protein and modulate its activity[9]. The identification of a lead compound that effectively inhibits FLT3 holds great promise for the development of novel therapeutic strategies for AML treatment. Previously phytochemicals from various plants have been analyzed for their activity against disease conditions[10-12].

In addition, this study aimed to examine the drug-likeness, pharmacokinetics, and toxicity profiles of phytochemicals derived from *Thuja occidentalis*. Furthermore, it sought to explore the interaction between these bioactive compounds and FLT3 through docking analysis. Moreover, the oral bioavailability and molecular targets of the identified lead compounds were assessed.

*Correspondence:
E-mail: fdapharma@gmail.com; arulprakashf.sdc@saveetha.com

Materials and Methods

Ligand and protein

Phytochemicals from *Thuja occidentalis* were retrieved from Dr. Duke's Phytochemical and Ethnobotanical database (https://phytochem.nal.usda.gov/), which serves as a comprehensive source of information on the phytochemical composition of various plants, including *Thuja occidentalis*[13]. To obtain 2D, 3D, and simplified molecular-input line-entry system (SMILES) representations of the phytochemicals present in *Thuja occidentalis*, data were extracted from the PubChem database[14] (https://pubchem.ncbi.nlm.nih.gov/). PubChem is a publicly accessible repository that provides extensive chemical information including the structures and properties of diverse compounds. To ensure consistency and availability of structural information and associated properties, phytochemicals that were not present in the PubChem database were excluded from this study. A total of 28 phytochemicals from *Thuja occidentalis* were selected for further investigation based on their presence in the PubChem database and their reported relevance to potential anticancer properties documented in the literature. Gilteritinib, a well-known drug was used as a control in this study.

Drug-likeness and pharmacokinetics

The drug-likeness and pharmacokinetics such as absorption, distribution, metabolism and excretion (ADME) of the 28 phytochemicals were assessed using the SwissADME webserver[15]. The SMILES representations of the 28 compounds obtained from PubChem were submitted to the SwissADME web server (http://www.swissadme.ch/) to predict their drug-likeness and pharmacokinetic properties. Linpinski's rule of 5 was used as a parameter to evaluate oral bioavailability and drug-likeness[16]. This rule considers several factors, including a molecular weight of 500 Da or less, 5 or fewer hydrogen bond donors, 10 or fewer hydrogen bond acceptors, and a computed logarithm of the partition coefficient (log P) of 5 or less. Additionally, the pharmacokinetic properties of the phytochemicals were also analyzed. This included assessment of their gastrointestinal (GI) absorption, blood-brain barrier (BBB) permeability, whether they act as substrates for the p-glycoprotein transporter, and their potential to inhibit cytochrome p-450 isoenzymes. These properties are crucial for determining the behavior and fate of a compound within the body, including its absorption, distribution, metabolism, and elimination.

Molecular docking

Molecular docking was conducted using AutoDock Tool 1.5.7, to explore the interaction between compounds exhibiting favorable drug-likeness and pharmacokinetic properties, and the FLT3. The docking procedure was performed according to the methodology described by Roy *et al*[17]. To prepare the target protein FLT3 for docking, water molecules, and the co-crystallized ligand were removed, and hydrogen bonds were added. Additionally, the Gasteiger charges were computed. The docking grid was established based on the grid of the co-crystallized ligand. During the docking process, 10 runs were performed to generate multiple conformations. Among these runs, the conformation that displayed the most promising binding affinity was selected for further analysis. The resulting conformation was visualized using BIOVIA Discovery Studio Visualizer v21.1.0.20298.

Toxicity analysis

Compounds demonstrating favorable binding affinities were subjected to toxicity analysis using the ProTox II (https://tox.charite.de/protox3/) server[18]. This server was used to assess the various toxicological properties of the compounds. Toxicity analysis involves evaluating the potential adverse effects of the compounds based on their predicted hepatotoxicity, carcinogenicity, immunogenicity, mutagenicity, and cytotoxicity profiles. Additionally, the LD_{50} (median lethal dose) in milligrams per kilogram (mg/kg) and the acute toxicity class of the compounds were analyzed. The compounds predicted to have no significant toxic effects were identified as potential hits.

Bioavailability radar and molecular target prediction

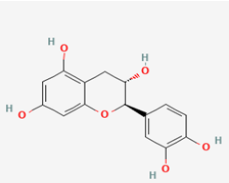
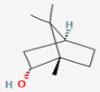
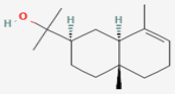
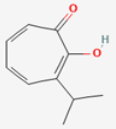
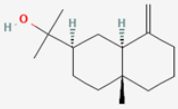
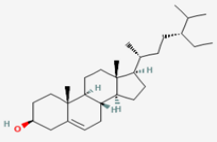
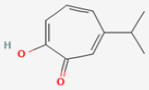
The identified leads were further analyzed for their oral bioavailability using a radar plot, which was generated using the SwissADME webserver. The radar plot provided a visual representation of multiple parameters related to oral bioavailability, Additionally, the molecular targets of the identified compounds were investigated using the Swiss Target Prediction Tool[19]. This analysis aimed to predict the specific proteins or biological targets with which leads may interact, providing insights into their potential mechanisms of action and therapeutic relevance.

Results and Discussion

Protein and targets

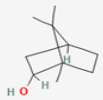
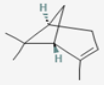
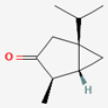
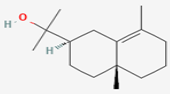
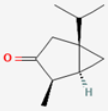
Table 1 shows the 2D structures of the 28 phytochemicals derived from *Thuja occidentalis*, along

Table 1 — 2D structure and PubChem ID of phytochemicals from *Thuja occidentalis*

S.No	Chemical	PubChem ID	2D structure
1	Catechin	9064	
2	(-) -Borneol	1201518	
3	Alpha-eudesmol	92762	
4	Alpha-thujaplicin	80297	
5	Beta-eudesmol	91457	
6	Beta-sitosterol	222284	
7	Beta-thujaplicin	3611	

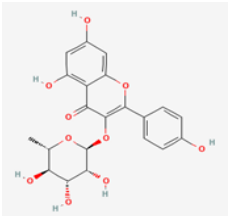
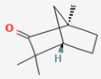
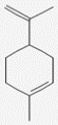
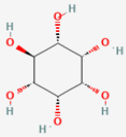
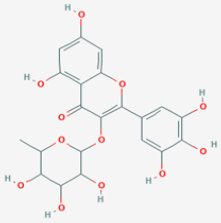
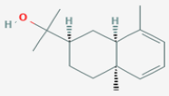
(*Contd.*)

Table 1 — 2D structure and PubChem ID of phytochemicals from *Thuja occidentalis* (*Contd.*)

S.No	Chemical	PubChem ID	2D structure
8	Borneol	64685	
9	Camphene	6616	
10	Camphor	2537	
11	D-alpha-pinene	82227	
12	Alpha-thujone	261491	
13	Gamma-eudesmol	6432005	
14	Isothujone	12304613	

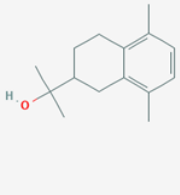
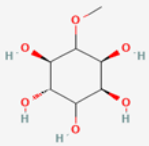
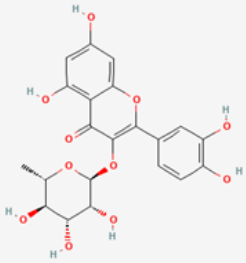
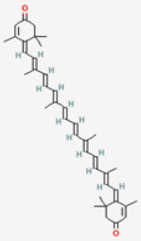
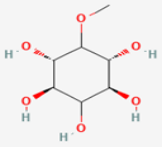
(*Contd.*)

Table 1 — 2D structure and PubChem ID of phytochemicals from *Thuja occidentalis* (*Contd.*)

S.No	Chemical	PubChem ID	2D structure
15	Kaempferol-3-rhamnoside	5316673	
16	L-Alpha-fenchene	28930	
17	L-fenchone	2794921	
18	Limonene	22311	
19	Myo-inositol	892	
20	Myricetin-3-rhamnoside	5352000	
21	Occidentalol	11138655	

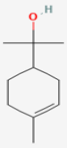
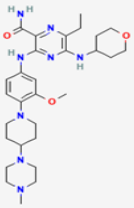
(*Contd.*)

Table 1 — 2D structure and PubChem ID of phytochemicals from *Thuja occidentalis* (*Contd.*)

S. No	Chemical	PubChem ID	2D structure
22	Occidol	11020369	
23	Pinitol	164619	
24	Quercitrin	5280459	
25	Rhodoxanthin	5281251	
26	Sabinene	18818	
27	Sequoyitol	439990	

(*Contd.*)

Table 1 — 2D structure and PubChem ID of phytochemicals from *Thuja occidentalis* (*Contd.*)

S. No	Chemical	PubChem ID	2D structure
28	Terpineol	17100	
Control	Gilteritinib	49803313	

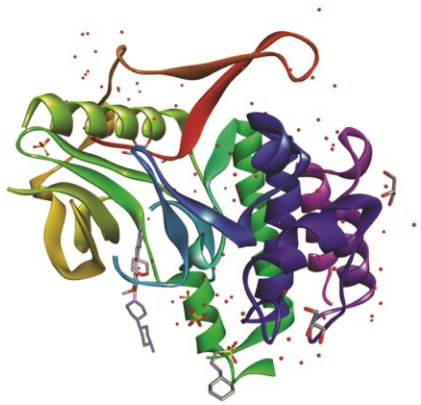


Fig. 1 — 3D structure of Target protein FLT3 (PDB ID: 6JQR)

with a control drug. Figure 1 depicts the 3D structure of the target protein FLT3, (PDB ID:6JQR).

Drug-likeness and pharmacokinetics

Among the 28 screened compounds, myricetin-3-rhamnoside, quercitrin, and rhodoxanthin displayed two Lipinski's rule violations, leading to a poor bioavailability score of 0.17. Conversely, the remaining 25 compounds adhered to Lipinski's rule and exhibited a higher bioavailability score of 0.55. Catechin, myo-inositol, pinitol, rhodoxanthin, and sequoyitol were identified as substrates of P-glycoprotein (P-gp), suggesting their potential involvement in P-gp mediated efflux. Catechin, beta-sitosterol, kaempferol-3-rhamnoside, myo-inositol, myricetin-3-rhamnoside, pinitol, quercitrin, rhodoxanthin, and sequoyitol were not found to be permeant across the blood-brain barrier (BBB). On the other hand, catechin, borneol, alpha-eudesmol, alpha-thujaplicin, beta-eudesmol, beta-thujaplicin, camphor, gamma-eudesmol, isothujone, fenchone, occidentalol, occidol, and terpineol demonstrated high gastrointestinal (GI) tract absorption. These findings provide valuable insights into the drug-likeness and pharmacokinetic properties of the screened compounds, which are essential for understanding their potential as drug candidates. Table 2 provides an overview of the drug-likeness properties of the screened phytochemicals. Table 3 presents a comprehensive summary of the pharmacokinetic properties of the screened phytochemicals. After identifying three compounds with Lipinski's rule violations, they were excluded from further consideration. Consequently, the remaining 25 compounds were chosen for subsequent investigations.

Among the 28 screened compounds, none exhibited inhibitory effects on CYP1A2, CYP2C19, or CYP3A4, which are essential enzymes involved in the metabolism of various drugs in the liver. Beta-eudesmol, limonene, and occidentalol demonstrated inhibitory activity against CYP2C9, another significant liver enzyme responsible for drug metabolism. This finding suggests that these compounds may influence the metabolism of drugs that are substrates of CYP2C9, possibly affecting their efficacy and safety profiles. Occidol was identified as a CYP2D6 inhibitor.

In their study, Sravika *et al*. conducted a screening of the phytochemicals found in *Bauhinia acuminata* to assess their drug-likeness and pharmacokinetic properties using the SwissADME web server[20]. The authors obtained promising compounds with drug-likeness properties. The result of this study was found to adhere to our present study.

Table 2 — Drug likeness of phytochemicals in *Thuja occidentalis*

S.No	Phytochemicals	Molecular weight	NHBD	NHBA	RB	LogP	MR	TPSA	Lipinski's violation	Bioavailability Score
1	Catechin	290.27	5	6	1	1.22	74.33	110.38	0	0.55
2	Borneol	154.25	1	1	0	2.19	46.6	20.23	0	0.55
3	Alpha-eudesmol	222.37	1	1	1	3.92	70.46	20.23	0	0.55
4	Alpha-thujaplicin	164.2	1	2	1	1.88	49.32	37.3	0	0.55
5	Beta-eudesmol	222.37	1	1	1	3.92	70.46	20.23	0	0.55
6	Beta-sitosterol	414.71	1	1	6	8.02	133.23	20.23	1	0.55
7	Beta-thujaplicin	164.2	1	2	1	1.88	49.32	37.3	0	0.55
8	Borneol	154.25	1	1	0	2.19	46.6	20.23	0	0.55
9	Camphene	136.23	0	0	0	3	45.22	0	1	0.55
10	Camphor	152.23	0	1	0	2.4	45.64	17.07	0	0.55
11	Alpha-pinene	136.23	0	0	0	3	45.22	0	1	0.55
12	Alpha-thujone	152.23	0	1	1	2.26	45.9	17.07	0	0.55
13	Gamma-eudesmol	222.37	1	1	1	4.06	70.46	20.23	0	0.55
14	Isothujone	152.23	0	1	1	2.26	45.9	17.07	0	0.55
15	Kaempferol-3-rhamnoside	432.38	6	10	3	0.78	106.97	170.05	1	0.55
16	Alpha-fenchene	136.23	0	0	0	3	45.22	0	1	0.55
17	Fenchone	152.23	0	1	0	2.4	45.64	17.07	0	0.55
18	Limonene	136.23	0	0	1	3.31	47.12	0	0	0.55
19	Myo-inositol	180.16	6	6	0	-3.83	35.81	121.38	1	0.55
20	Myricetin-3-rhamnoside	464.38	8	12	3	0.19	111.02	210.51	2	0.17
21	Occidentalol	220.35	1	1	1	3.7	69.98	20.23	0	0.55
22	Occidol	218.33	1	1	1	3.18	69.43	20.23	0	0.55
23	Pinitol	194.18	5	6	1	-3.18	40.54	110.38	0	0.55
24	Quercitrin	448.38	7	11	3	0.49	109	190.28	2	0.17
25	Rhodoxanthin	562.82	0	2	9	10.74	184.36	34.14	2	0.17
26	Sabinene	136.23	0	0	1	3	45.22	0	1	0.55
27	Sequoyitol	194.18	5	6	1	-3.18	40.54	110.38	0	0.55
28	Terpineol	154.25	1	1	1	2.5	48.8	20.23	0	0.55
C	Gilteritinib	552.71	3	7	9	2.21	168.43	121.11	2	0.17

NHBD- Number of hydrogen bond donors; NHBA- Number of hydrogen bond acceptors, RB- Rotatable bonds, MR- Molar refractivity, TPSA- Topological polar surface area

Table 3 — Pharmacokinetics of *Thuja occidentalis* phytochemicals

S.No	Phytochemicals	GIA	BBB Permeant	p-gp substrate	CYP1A2 inhibitor	CYP2C19 Inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
1	Catechin	High	No	Yes	No	No	No	No	No
2	Borneol	High	Yes	No	No	No	No	No	No
3	Alpha-eudesmol	High	Yes	No	No	No	No	No	No
4	Alpha-thujaplicin	High	Yes	No	No	No	No	No	No
5	Beta-eudesmol	High	Yes	No	No	No	Yes	No	No
6	Beta-sitosterol	Low	No	No	No	No	No	No	No
7	Beta-thujaplicin	High	Yes	No	No	No	No	No	No
8	Borneol	High	Yes	No	No	No	No	No	No
9	Camphene	Low	Yes	No	No	No	Yes	No	No
10	Camphor	High	Yes	No	No	No	No	No	No
11	Alpha-pinene	Low	Yes	No	No	No	Yes	No	No
12	Alpha-thujone	High	Yes	No	No	No	No	No	No
13	Gamma-eudesmol	High	Yes	No	No	No	No	No	No
14	Isothujone	High	Yes	No	No	No	No	No	No
15	Kaempferol-3-rhamnoside	Low	No	No	No	No	No	No	No
16	Alpha-fenchene	Low	Yes	No	No	No	No	No	No
17	Fenchone	High	Yes	No	No	No	No	No	No

(*Contd*.)

Table 3 — Pharmacokinetics of *Thuja occidentalis* phytochemicals (*Contd*.)

S.No	Phytochemicals	GIA	BBB Permeant	p-gp substrate	CYP1A2 inhibitor	CYP2C19 Inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
18	Limonene	Low	Yes	No	No	No	Yes	No	No
19	Myo-inositol	Low	No	Yes	No	No	No	No	No
20	Myricetin-3-rhamnoside	Low	No	No	No	No	No	No	No
21	Occidentalol	High	Yes	No	No	No	Yes	No	No
22	Occidol	High	Yes	No	No	No	No	Yes	No
23	Pinitol	Low	No	Yes	No	No	No	No	No
24	Quercitrin	Low	No	No	No	No	No	No	No
25	Rhodoxanthin	Low	No	Yes	No	No	No	No	No
26	Sabinene	Low	Yes	No	No	No	No	No	No
27	Sequoyitol	Low	No	Yes	No	No	No	No	No
28	Terpineol	High	Yes	No	No	No	No	No	No
C	Gilteritinib	High	No	No	No	Yes	No	No	No

GIA- gastrointestinal absorption, p-gp – p-glycoprotein

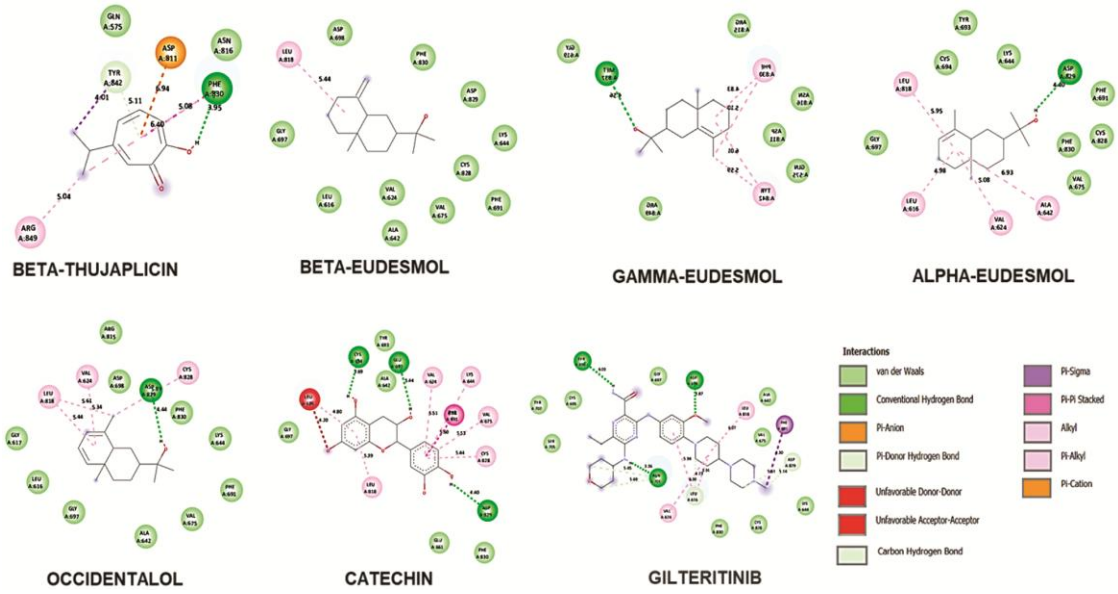


Fig. 2 — 2D docked structure of the phytochemicals with best binding affinity with FLT3

Molecular docking

The binding affinities of the 25 compounds to the FLT3 target protein ranged from -9.2 kcal/mol to -4.6 kcal/mol. From this range, compounds exhibiting binding affinities between -6.5 kcal/mol and -8 kcal/mol were selected for further studies. Based on the specified criterion, six compounds were selected for further studies based on their respective binding affinities to the FLT3 target protein. Catechin, occidentalol, alpha-eudesmol, gamma-eudesmol, beta-eudesmol, and beta-thujaplicin demonstrated binding affinities of -7.7 kcal/mol, -7.7 kcal/mol, -7.6 kcal/mol, -7.3 kcal/mol, -7.1 kcal/mol, and -6.5 kcal/mol, respectively. These selected compounds exhibited strong interactions and favorable binding energies with the FLT3 protein. Comparing the binding affinity of these compounds with the control drug, Gilteritinib (with a binding affinity of -7 kcal/mol), it is evident that the compounds possess comparable binding affinities to the FLT3 target protein. Figure 2 illustrates the 2D docked structures of the ligands interacting with FLT3, highlighting the complex formed with the best binding affinity. Table 4 presents comprehensive details concerning the binding affinity and specific amino acid interactions established between FLT3 and the bioactive compounds. Supplementary Figure S1 illustrates the 2D docked structure of ligands with affinity <-6.5 and >-8 Kcal/mol towards FLT3.

In their study, Egbuna *et al*. examined the interactions between selective phytochemicals and the FLT3 target protein[21]. During the screening process, several phytochemicals demonstrated improved binding affinities towards FLT3 compared to others.

Table 4 —Binding affinity and amino acid interactions between FLT3 and the bioactive compounds

No	Phytochemical	Binding affinity	NHB	Hydrogen bond	Carbon Hydrogen / Pi-anion / Pi-Sulfur / Pi-Pi stacked / Pi-Alkyl bond	van der Waal
1	Catechin	-7.7	3	Cys694 (3.69), Glu692 (5.44), Asp829 (4.40)	Leu616, Val624, Lys644, Phe691, Val675, Cys828, Leu818	Gly697, Thr693, Ala642, Glu661, Phe830
2	(-) -Borneol	-5.5	0	Not available	Asp829, Ala642, Val624, Phe691, Cys828, Val675	Phe830, Leu818, Lys644, Glu692
3	Alpha-eudesmol	-7.6	1	Asp829 (4.40)	Leu818, Leu616, Val624, Ala642	Gly697, Cys694, Tyr693, Lys644, Phe691, Cys828, Phe830, Val675
4	Alpha-thujaplicin	-6.3	2	Cys694 (3.43), Glu692 (5.38)	Ala642, Leu616, Leu818, Val624, Phe691	Cys828, Val675, Lys644, Tyr693
5	Beta-eudesmol	-7.1	0	Not available	Leu818	Gly697, Leu616, Val624, Ala642, Val675, Cys828, Phe691, Lys644, Asp829, Phe830, Asp698
6	Beta-sitosterol	-9.2	0	Not available	Phe691, Val675, Val624, Cys828, Ala642, Leu616, Leu818	Asp829, Phe830, Lys644, Gly697, Asp698, Cys694, Tyr693, Cys695
7	Beta-thujaplicin	-6.5	1	Phe830 (3.95)	Arg849, Tyr842, Asp811	Gln575, Asn816
8	Borneol	-5.3	1	Asp829 (3.88)	Val675, Ala642, Cys828, Val624	Lys644, Phe691, Glu692, Phe830, Leu818\
9	Camphene	-5.7	0	Not available	Phe691, Ala642, Cys828, Val675, Leu818, Val624	Lys644, Asp829, Phe830
10	Camphor	-4.8	0	Not available	Not available	Arg849, Tyr842, Met837, Gly619, Phe830, Ala620
11	d-Alpha-pinene	-5.9	0	Not available	Cys828, Val675, Ala642, Phe691, Lys644, Val624	Phe830, Leu818, Asp829
12	Alpha-thujone	-5.7	0	Not available	Phe691, Val624 ,Cys828, Val675	Lys644, Ala642, Leu616, Asp829, Leu818, Phe830
13	Gamma-eudesmol	-7.3	1	Met837 (4.56)	Tyr842, Phe830	Arg849, Gln575, Asp811, Asn816, Arg815, Gly619
14	Isothujone	-5.3	1	Lys644 (3.86)	Not available	Ala642, Asp829, Val624, Phe830, Leu616, Leu818, Val675, Cys828, Phe691
15	Kaempferol-3-rhamnoside	-8.4	2	Glu692 (5.95), Asp829 (4.01)	Gly697, Cys828, Val675, Ala642, Leu818, Val624, Phe830	Phe691, Cys694, Tyr693, leu616, Asp698, Ser618, Gl617, Lys644
16	l-Alpha-fenchene	-6	0	Not available	Ala642, Val624, Phe691, Val675, Cys828	Leu818, Phe830, Lys644, Asp829
17	l-Fenchone	-5.2	0	Not available	Leu818, Val675, Cys828, Phe691, Val624	Glu692, Ala642, Lys644, Asp829, Phe830
18	Limonene	-6.1	0	Not available	PHe691, Val675, Cys828, Val624, Ala642, Leu818, Leu616	Asp829, Lys644, Phe830
19	Myo-inositol	-4.7	3	Gln575 (5.16), Glu573 (4.63), Asn847 (4.17)	Not available	Phe830, Tyr572, Arg849, Ala848

(*Contd*.)

Table 4 —Binding affinity and amino acid interactions between FLT3 and the bioactive compounds (*Contd*.)

No	Phytochemical	Binding affinity	NHB	Hydrogen bond	Carbon Hydrogen / Pi-anion / Pi-Sulfur / Pi-Pi stacked / Pi-Alkyl bond	van der Waal
20	Occidentalol	-7.7	1	Asp829 (4.44)	Leu818, Val624, Cys828	Arg815, Asp698, Phe830, lys644, Phe691, Val675, Ala642, Gly697, Leu616, Gly617
21	Occidol	-8.2	1	Asp829 (4.92)	Leu616, Leu818, Ala642, Val624, Cys828	Phe830, Lys644, Phe691,
22	Pinitol	-4.6	2	Tyr693 (6.00), His821 (5.36)	Gln640	Cys694, Cys695, Thr820, Lys826, Glu692, Val641, Leu678, ile639
23	Sabinene	-6.3	0	Not available	Lys644, Cys828, Val624, Val675, Phe691	Leu818, Ala642, Leu616, Phe830, Asp829
24	Sequoyitol	-4.6	1	Cys694 (3.69)	Not available	Leu616, Gly697, Asp698, Tyr693, Glu692, Leu818, Ala642, Val675, Val624, Cys828, Phe830
25	Terpineol	-6	0	Not available	Asp829, Phe691, Val624, Ala642, Leu818, Cys828, leu616	Phe830, Lys644, Val675
C	Gilteritinib	-7	3	Tyr696 (6.03), Asp698 (3.87), Asn701 (3.36)	Val624, Leu616, Asp829, Phe691, Leu818	Ser705, Tyr702, Cys695, Gly697, Ala642, Val675, Lys644, Cys828, Phe830

NHB- Number of hydrogen bonds.

Table 5 — Toxicity profiles of the Phytochemicals

No	Phytochemicals	LD_{50} (mg/Kg)	Acute toxicity class	Hepatoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
1	Catechin	10000	6	Inactive	Inactive	Inactive	Inactive	Inactive
2	Alpha-eudesmol	5000	5	Inactive	Inactive	Inactive	Inactive	Active
3	Beta-eudesmol	2000	4	Inactive	Inactive	Inactive	Inactive	Inactive
4	Beta-thujaplicin	1030	4	Inactive	Inactive	Inactive	Inactive	Inactive
5	Gamma-eudesmol	4300	5	Inactive	Inactive	Inactive	Inactive	Active
6	Occidentalol	288	3	Inactive	Inactive	Inactive	Inactive	Active
C	Gilteritinib	1500	4	Inactive	Inactive	Active	Inactive	Inactive

LD- lethal dose

Notably, the identified compounds exhibited superior binding affinities in comparison to the control drug gilteritinib. These results pave the way for further exploration of these phytochemicals as potential inhibitors of FLT3 in our study.

Toxicity analysis

Table 5 presents the toxicity profiles of the compounds investigated in this study. Among the compounds analyzed using ProTox II, catechin, beta-eudesmol, and beta-thujaplicin exhibited no predicted toxic endpoints. These compounds were selected for further studies as they demonstrated promising safety profiles in terms of their toxicity. Catechin was classified in acute toxicity class 6, indicating a very low potential for acute toxicity. The LD_{50} value for Catechin was 10,000 mg/kg, indicating a relatively high dose required to cause lethality in 50% of test subjects. Beta-eudesmol and beta-thujaplicin were classified in acute toxicity class 4, indicating a moderate potential for acute toxicity. The LD_{50} values for beta-eudesmol and beta-thujaplicin were 2,000 mg/kg and 1,030 mg/kg. The results from ProTox II provide important insights into the potentially toxic effects of the investigated compounds.

Pokharkar *et al*. analyzed the toxicity profiles of bioactive compounds derived from marine sponges[22]. The study revealed that the compounds from marine sponges exhibited acute toxicity classes 4 and 5, indicating moderate to high potential for acute toxicity. Furthermore, the LD_{50} values for the

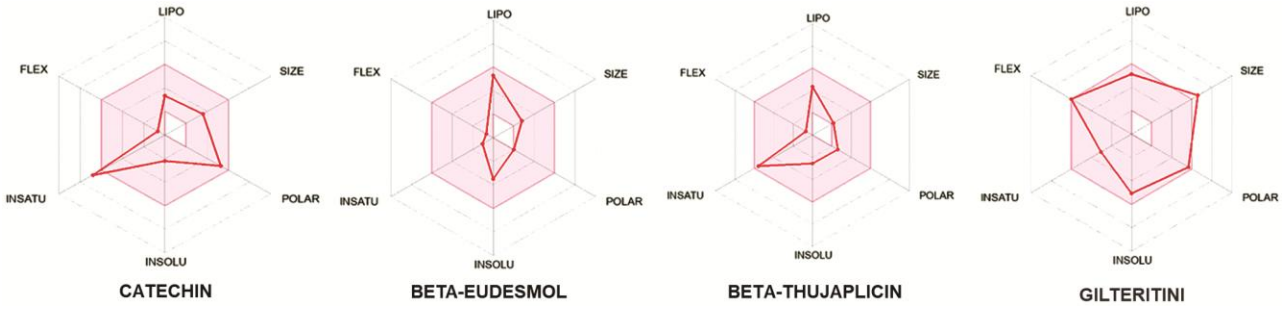


Fig. 3 — Radar plots for identified leads

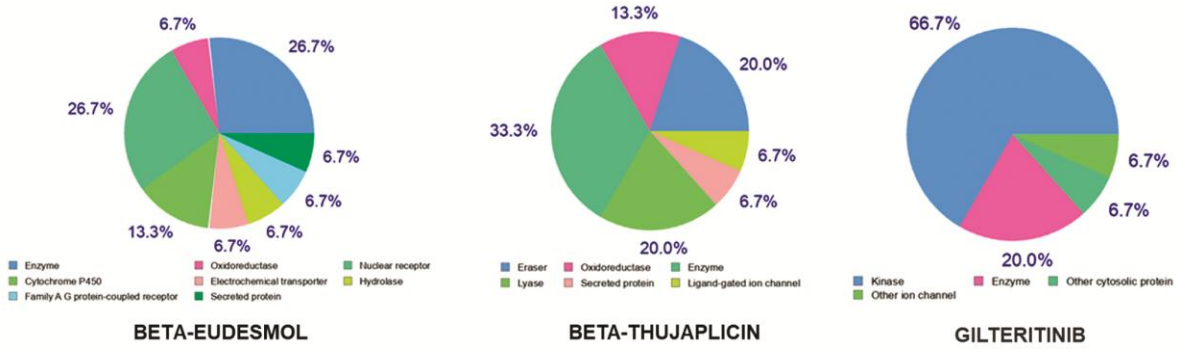


Fig. 4 — Molecular targets of the identified hits elucidating the potential therapeutic interaction

compounds ranged from 5000 to 350 mg/kg, The research conducted by Pokharkar *et al*. laid the groundwork for our current study, providing valuable insights and information that formed the basis for our investigations.

Bioavailability radar and molecular targets

Figure 3 showcases radar plots for catechin, beta-eudesmol, and beta-thujaplicin obtained from the SwissADME web server. The plots utilize the pink color to emphasize the optimal range for each compound, indicating favorable oral bioavailability. Within the pink region of the radar plot, these compounds demonstrate characteristics that make them more likely to be effectively absorbed and reach their intended targets within the body. By falling within the pink region, catechin, beta-eudesmol, and beta-thujaplicin demonstrate favorable drug-likeness and pharmacokinetic properties, indicating their potential as promising lead candidates for further exploration in drug development.

The molecular target analysis of the beta-eudesmol, beta-thujaplicin, and Gilteritinib obtained from Swiss target prediction was presented in (Fig. 4). This analysis involved predicting the specific molecular targets with which the selected compounds interact. In the realm of drug discovery, molecular target analysis assumes a vital role by revealing the proteins or biological processes that may be influenced or modulated by the compounds under investigation. While the molecular targets for beta-eudesmol and beta-thujaplicin provide valuable information for further investigation.

Choudhury *et al* screened the natural and synthetic compounds against the human secretory phospholipase A2 (PLA2) through *in silico* approach[23]. The authors reported that natural compounds possess ADME properties and binding affinity toward the target of inflammatory diseases. This study is in line with our research, where we explore the potential of natural compounds to target the disease. Similarly, Agarwal *et al* investigated the plant alkaloids as a potent inhibitor of Poly (ADP-ribose) polymerase (PARP) using *in silico* study[24]. The authors obtained leads with significant drug-likeness, pharmacokinetics, and binding affinity toward the target in their study. This study adds as an additional reference to support our research. Our analysis results were consistent with the findings of previous research demonstrating the reliability of our findings.

Conclusion

Our study highlights the potential of phytochemicals from *Thuja occidentalis* as promising FLT3 inhibitors. The identified leads catechin, beta-

eudesmol, and beta-thujaplicin exhibit desirable drug-like properties, safety profiles, and binding affinities, offering a strong foundation for their development as potential therapeutic agents in the treatment of acute myeloid leukemia. To validate the identified lead compounds effectively, further studies are essential, involving the isolation of these identified hits from *Thuja occidentalis* and conducting *in vitro* and *in vivo* investigations in the future.

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Conflict of interest

All authors declare no conflict of interest.

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