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In-silico evaluation of novel BCL-XL inhibitors and PASS prediction of bioactive compounds from Catharanthus roseus

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ABSTRACT
Bcl-xL is the most prevalent Bcl-x protein, and it works to prevent apoptosis through a variety of mechanisms, including Bax inhibition. Bcl-xS, on the other hand, can bind to and block the anti-apoptotic Bcl-xL and Bcl-2 proteins, causing the pro-apoptotic Bak protein to be released.Traditional Chinese medicine has purportedly utilised Catharanthus roseus for generations to treat a variety of diseases ranging from diabetes to depression. Compounds with cancer-fighting capabilities have recently been discovered in this unique plant. Docking experiments demonstrated that chemicals discovered from the plant have considerable inhibitory action towards BCL-XL. Vincoline has the highest binding affinity for the BCL-X receptor of all the bioactive substances. PASS prediction was used to look at all of the bioactive compounds that were expected to have pharmacological activity.
Key-words:BCL X, Catharanthus roseus, Vincoline, PASS

I. INTRODUCTION

Catharanthus roseus, usually known as brilliant eyes, Cape periwinkle, burial ground plant, Madagascar periwinkle, old servant, pink periwinkle, rose periwinkle, is a type of blossoming plants in the family Apocynaceae [1]. It is local and endemic to Madagascar, however developed somewhere else as a decorative and restorative plant. It is a wellspring of the medications vincristine and vinblastine, used to treat disease. It was previously remembered for the class Vinca as Vinca rosea. Catharanthus roseus, which is an intense restorative plant a considerable lot of pharmacological activities like antimicrobial, cell reinforcement, antihelmintic, antifeedant, anti-sterility, antidiarrheal, antidiabetic impact, and so forth it is utilized to treat a large number of deadly illnesses[2]. The leaves of Catharanthus roseus comprise the main wellspring of the noticeable indolomonoterpenic alkaloids vincristine and vinblastine. In this work, we concentrated on the natural capability of the roots, which are utilized in a few nations as imperfections or high temperature water separates for the treatment of various conditions. Customarily C. roseus was utilized to fix different sorts of infections like hypertension, malignant growth, skin sickness, diabetes, feminine problems, acid reflux, ailment, dyspepsia. The plant is wealthy in bioactive mixtures and has a tremendous scope of pharmacological properties[3]. Announced that Catharanthus roseus contains more than 130 unique kinds of alkaloids comprehensively utilized in relieving different sorts of disease including bosom malignant growth, cellular breakdown in the lungs, melanomas because of the presence of vincristine and vinblastine[4]. Leaves of Catharanthus roseus contain 70 unique kinds of substance mixtures, for example, ajmalicine, reserpine, and serpentine[5]. Bcl-xL is the most prevalent Bcl-x protein, and it works to prevent apoptosis through a variety of mechanisms, including Bax inhibition. Bcl-xS, on the other hand, can bind to and block the anti-

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apoptotic Bcl-xL and Bcl-2 proteins, causing the pro-apoptotic Bak protein to be released [6]. Bcl-xL is a 233-amino-acid protein that contains four BH domains, a transmembrane region, and a loop between BH3 and BH4 [7].

II. MATERIALS AND METHODS

2.1. Plant material collection

Plant leaves were gathered from Vijayawada during June and washed multiple times in running regular water to eliminate residue and toxins. Then, at that point, the leaves are again squandered in refined water[8].

2.2. Extract preparation

Cleaned 1g of the leaf was taken and grind in mortar and pestle for homogenization utilizing 100ml refined water. This was then poured into a funnel-shaped cup and hatched at 37^0C for short-term in a shaker at 300rpm. The suspension was sifted and gathered in a funnel-shaped carafe utilizing Whatman's channel paper and store at 4^0C for some time later[9].

2.3. Preliminary phytochemical screening

2.3.1.Test for sugars and glycosides

Molisch's test: The filtrate was treated with 2-3 drops of 1% alcoholic alpha-naphthol and 2 mL of conc. H2SO4 was added to the walls of the test tube. The appearance of the earthy coloured ring at the intersection of two fluids shows the presence of sugars[10].

2.3.2.Detection of fixed oils

A little amount of concentrate was squeezed independently between the channel paper. The appearance of an oil stain on the paper demonstrated the presence of fixed oils[11].

2.3.3.Detection of proteins and free amino acids

To the above pre-arranged concentrates equivalent volumes of 5% NaOH and 1% CuSO4 arrangement was added. Violet tone shows the presence of proteins and free amino acids[12].

2.3.4.Detection of tannins and phenolic compounds

In a test tube, a small measure of the ethanolic separate was blended in with 1 mL water and 1 to 2 drops of Iron III chloride (FeCl3). A positive test brings about a blue, green, red, or purple tone[13].

2.3.5.Detection of phytosterol

To 1 ml of the above extricate, not many drops of conc. H2SO4 was added. Earthy coloured tone delivered showed the presence of phytosterols [14].

2.3.6.Detection of alkaloids Arrangement

(a): 0.425 g essential bismuth nitrate in 5 mL chilly acidic corrosive and 20 mL water under warming. Arrangement (b): 4 g potassium iodide in 15 mL water. Stock arrangement: (a)+(b) were blended 1:1. To around 3 mL of concentrate, a couple of drops of Dragendorff's reagent were added. Orange earthy coloured encourage demonstrated the presence of alkaloid[15].

2.3.7.Detection of flavonoids

Remove was broken up independently in watery NaOH. The appearance of a yellow tone showed the presence of flavonoids. To little part of each concentrate, conc. H2SO4 was added. The yellow-orange tone showed the presence of flavonoids [16].

2.4. GC-MS

The investigation was directed utilizing an Agilent 7890 N, outfitted with an Eclipse Plus section (60 m × 0.25 mm × 0.25 µm). Helium gas is utilized at a steady stream pace of 1.0 mL/min. The injector port was warmed to 250°C, utilizing the spitless infusion mode. The underlying broiler temperature was kept up with at 40°C for 3 min, then, at that point, raised to 150°C at a pace of 5°C/min and held for 1 min, lastly raised to 220°C at a pace of 10°C/min and kept up with for 2 min [17].

2.5. Molecular docking

Sub-atomic docking was performed utilizing Autodock. This product is utilized for the assessment of energy during cooperation and distinguishes the best adaptable ligand present with the least energy. The scoring capacity depends on the intramolecular communication of ligand and protein during docking[18].

2.5.1.Preparation of receptor

For the sub-atomic docking study, a delegate construction of the protein was gotten from the Protein Data Bank (PDB); the BCL X structure.All of the necessary hydrogen particles were added, the water atoms were eliminated from the cavity, the lower inhabitance build-up structures were erased, any missing deposits were filled in, side chains were produced, and any incomplete side chains were re-established utilizing the AutoDock Tools (ADT) form 4.0. The suitable ligand structures for docking were recorded in the AutoDock PDBQT document design[19].

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2.5.2.**Preparation of Ligands**

Pubchem gave the 2D construction of ligands. Sub-atomic Mechanics (MM)2 was utilized to diminish the energy level. For docking assessment, these energy-streamlined ligands were utilized. At long last, all ligand structures were saved as PDB records, which can be utilized as contributions to Auto Docking examinations (AutoDock 4.0). Subsequent to utilizing Auto Dock apparatuses to break down the ligand structures, they were saved in PDBQT design[20].

2.5.3.**Analysis of docked protein-ligand complex construction**

The best direction for the ligand-protein complex was chosen dependent on the H-bond energy score, restricting fondness, rmsd/ub, and rmsd/lb esteem[21].

2.6. Prediction of activity spectrum of substances (PASS)

The expectation of methanolic concentrate of A. reticulata pull for antibacterial and antifungal action was finished with the assistance of the PC program, PASS (Prediction of action spectra for substances)[22]. PASS is a PC-based program utilized for the forecast of various sorts of pharmacological exercises for various substances including phytoconstituents. The expectation of this range by PASS depends on primary movement relationship (SAR) examination of the preparation set containing more than 205,000 mixtures displaying more than 3750 sorts of natural exercises. The anticipated movement range of a compound is assessed as likely action (Pa) and plausible idleness (Pi). The mixtures showing more Pa esteem than Pi are the main constituents considered as workable for a specific pharmacological action[23].

III. RESULT AND DISCUSSIONS

3.1.Preliminary phytochemical screening

Phytochemical analysis of Catharanthus roseus leaves employing aqueous extract revealed the presence of phytoconstituents. The end product is Protein, Amino acids, Flavonoids, Tannins, Phenolics, and other compounds are detected. It can be used as a source of useful pharmaceuticals for industrial manufacturing in the treatment of a variety of disorders.

S No	Phytochemical	Result
1	sugars and glycosides	**+ve**
2	fixed oils	**-ve**
3	proteins and free amino acids	**+ve**
4	tannins and phenolic	**+ve**
5	phytosterol	**-ve**
6	alkaloids	**-ve**
7	flavonoids	**+ve**

3.2. Gas Chromatography-Mass spectroscopy

Gas chromatography mass spectrometer analysis reveals a total of 9 compounds. Out of 9 bioactive molecule Vindoline was present in the greatest ratio of about 8.6%. Analysis of the mass spectrum of the GC-MS was carried out with the use of the National Institute of Standards and Technology (NIST) database, which has over 62,000 patterns. The unknown component's spectra were compared to the known component's spectrum contained in the NIST collection. The components of the test materials were identified by their name, molecular weight, and structure. The name and composition of the compounds are given below.

S.NO	Name of the compound	Composition
1	Methyltetradecanoate	0.4%
2	Phythol	0.8%
3	Vincoline	1.5%

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4	Pericyclivine	4.8%
5	Vindoline	8.2%
6	B-Sitosterol	7.6%
7	Hexadecanoic acid	8.6%
8	Oleic acid	1.1%
9	Tetracosane	0.1%

3.3. Molecular docking results

All the compounds obtained from GC-MS analysis are submitted for molecular docking analysis with BCL X receptor. Out of the nine compounds Pericyclivine, Vincoline and B-Sitosterol shows a greater binding potential. Because of its high resolution and generally intact structure, the crystal structure of BCL X from the PDB data library was chosen. Based on the protonation statuses of the ionizable side chains, hydrogens were added to the protein. The average binding affinity (kcal/mole) for BCL X family proteins was used to arrive at these conclusions. These findings show that B-Sitosterol has the highest binding affinity for BCL X (kcal/mole).

S.NO	Compound	Binding affinity	Rmsd l.b	Rmsd u.b
1	Methyltetradecanoate	-4.5	27.576	29.399
2	Phythol	-5.1	31.307	34.470
3	Vincoline	-9.5	1.623	3.610
4	Pericyclivine	-8.4	1.977	4.517
5	Vindoline	-9.8	35.372	38.142
6	B-Sitosterol	-9.5	2.379	7.061
7	Hexadecanoic acid	-4.4	4.640	10.251
8	Oleic acid	-4.2	15.361	17.497
9	Tetracosane	-6.6	0.954	3.136

3.4. PASS (prediction of activity spectra for substances)

Each of the key chemicals in EEOR has potential targets and likely pharmacological activity, according on PASS analysis. Based on the values of Pa > Pi and Pa > 7, we evaluated nine biological features for each molecule. This method of prediction suggested that the chemicals tested have antibacterial, anthelmintic, anti-inflammatory, spasmolytic, and antiprotozoal effects, all of which are relevant to our current

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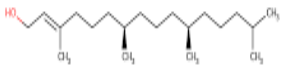
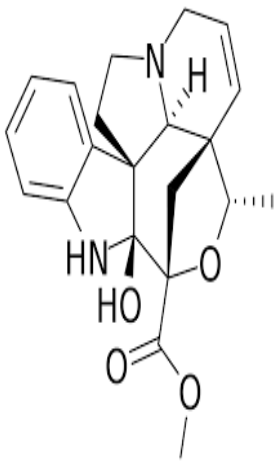
investigation. All-important chemicals anticipated pharmacological activity profiles are shown below.

SNo	Compound	Structure	Bioactivities
1	Methyltetradecanoate		Saccharopepsin inhibitor Acrocylindropepsin inhibitor Chymosin inhibitor Phobic disorders treatment Polyporopepsin inhibitor Pro-opiomelanocortin converting enzyme inhibitor Acylcarnitine hydrolase inhibitor Ubiquinol-cytochrome-c reductase inhibitor Testosterone 17beta-dehydrogenase (NADP+) inhibitor
2	Phythol		PrenPrenyl-diphosphatase inhibitor Retinol dehydrogenase inhibitor Ubiquinol-cytochrome-c reductase inhibitor Phobic disorders treatment Undecaprenyl-phosphate mannosyltransferase inhibitor Testosterone 17beta-dehydrogenase (NADP+) inhibitor Mucomembranous protectoryl-diphosphatase inhibitor
3	Vincoline		Antineoplastic (non-small cell lung cancer)Antineoplastic (non-small cell lung cancer) Antineoplastic CYP2H substrate Polarisation stimulant Antineoplastic alkaloid Leukopoiesis inhibitor P-glycoprotein substrate Antineoplastic (non-Hodgkin's lymphoma) Myc inhibitor

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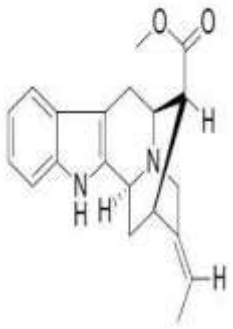
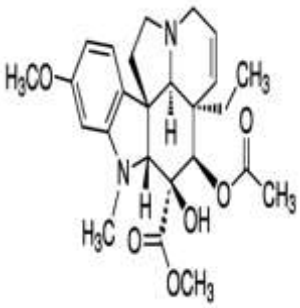
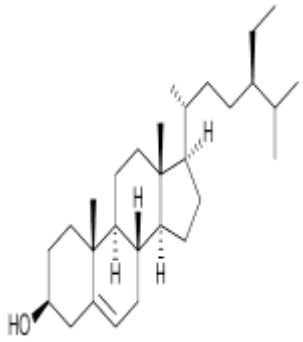
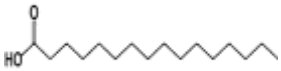
4	Pericyclivine		Hydroxytryptamineuptake inhibitor CYP2H substrate Adrenaline uptake inhibitor Analeptic Angiogenesis stimulant
5	Vindoline		P-glycopP-glycoprotein substrate Tubulin antagonist Leukopoiesis inhibitor CYP3A4 substrate TP53 expression enhancer Xenobiotic-transporting ATPase inhibitor CYP3A substrate P-glycoprotein inhibitorrotein substrate
6	β-sitosterol		DELTA14-sterol reductase inhibitor Antihypercholesterolemic Prostaglandin-E2 9-reductase inhibitor Cholesterol antagonist Alkenylglycerophosphocholine hydrolase inhibitor Alkylacetylglycerophosphatase inhibitor Acylcarnitine hydrolase inhibitor HypolipemicDELTA14-sterol reductase inhibitor
7	Hexadecanoic acid		Alkylacetylglycerophosphatase inhibitor Acylcarnitine hydrolase inhibitor CYP2J2 substrate

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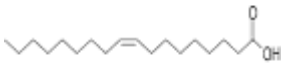
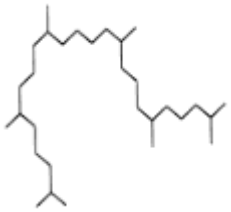
8	Oleic acid		Antieczematic CYP2J2 substrate Saccharopepsin inhibitor
9	Tetracosane		Acylcarnitine hydrolase inhibitor Acylcarnitine hydrolase inhibitor

IV. CONCLUSION

A virtual screening technique found a novel BCL X inhibitor from the plant extract in this work, and Vindoline inhibits the BCL X receptor with increased potency. The preliminary phytochemical screening reveals that plant extract has a large number of bioactive chemicals, which accounts for its increased use in pharmacological activity. The probable bioactivities expressed by the chemical discovered from the plant extract using GC-MS analysis are predicted using the PASS programme.

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