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SCREENING OF PHYTOCHEMICALS OF *TRICHOPUS ZEYLANICUS* ssp. *Travancoricus* BY *INSILICO* APPROACH-NEW TRENDS

Manza M.M.[1] and Oommen P. Saj[2]*

[1]Research Scholar, University College, Thiruvananthapuram.

[2]Asso. Prof. (Rtd.) of Botany, University College, Thiruvananthapuram - 695034.

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***Corresponding Author**
Oommen P. Saj
Asso. Prof. (Rtd.) of Botany,
University College,
Thiruvananthapuram -
695034.

ABSTRACT

Traditional medicines were used by people all over the world as it is dependent on locally available plant species and plant based products and capitalizes on traditional wisdom-repository of knowledge. Computational (*in silico*) methods have been developed and widely applied to pharmacology hypothesis development and testing. It is clear that the preclinical studies in cancer biology are expensive. Such studies involving *in vitro* and *in vivo* animal experiments involve hypothesis generation and testing to determine whether further trials are warranted and are extremely costly both in terms of researchers‟ time and the associated financial investment. *In silico* experimental modeling of cancer involves combining findings from biological literature with computer-based models of biological systems in order to conduct investigations of hypotheses entirely in the computer laboratory.. All the four phytochemicals studied showed interaction with the target protein Bcl-2. Benzopyran showed hydrogen bonding with the 143rd His of Bcl2 target protein. Whereas both Vitexin and Vicinin showed hydrogen bonding with the 68th ARG. Beta Sitosterol and Vitexin showed a hydrogen bonding with the 22 Lys residue of the target protein Bcl2 receptor. Vicenin also showed bonding with Asn122andArg65oftargetprotein.Traditional laboratory-based cancer research may be followed as the results were promising.

KEYWORDS: T*richopus zeylanicus* ssp. *Travancoricus*, by *insilico* approach, molecular docking, molecular visualistion.

INTRODUCTION

Approximately 80% of world population relies on traditional herbal medicine for primary healthcare as plant and plant based medication in the base of many of the today's

pharmaceutical drugs used for various ailments. Likewise, ethnomedicinal discoveries are valuable sources of novel pharmacons for the development of modern medicine and.[29] Natural products possess enormous structural and chemical diversity that cannot be matched by any synthetic libraries and continue to inspire novel discoveries in chemistry, biology, and medicine. Taking into consideration the high ratio of pharmacons of natural origin to synthetic compounds, it is indeed the New Golden Age of natural product discovery. According to[24] natural products including plants, animals and minerals have been the basis of treatment of diseases from time immemorial. The art of healing by plants has its origin in the antiquity of human civilization.

Screening of phytochemicals by *Insilico* approach

The prime objective of the study is to provide molecular insights on the utilization of phytochemicals from plants as promising anticancer drugs. Drug discovery and development is an intense, lengthy and an interdisciplinary venture. In the traditional drug discovery process, identification of the suitable drug target and validation of target and to identify their pharmacological relevance is the first and foremost task, which are performed from the very basic levels like cellular, molecular to the whole animal level. Once the target validation is complete the need to identify the effective compounds like inhibitors, antagonists for the target arise, which leads to lead identification and further lead optimization, preclinical studies and clinical trials follows. The sophisticated process of drug discovery and development can be an extensive process lasting over 7-10 years which is time consuming and labor intensive.

In silico pharmacology alias computational therapeutics or computational pharmacology has recently gained significant momentum. The drug discovery process involves the identification of the lead structure followed by the synthesis of its analogs, their screening to get candidate molecules for drug development. The prime goal of the drug discovery is to identify novel drug molecules which can bind to a specific target known to be involved in causing a disease and change the target's function. *Insilico* drug design skills are used in nanotechnology, molecular biology, biochemistry etc. The main benefit of the *insilico* drug design is cost effective in research and development of drugs.

Computational (*in silico*) methods have been developed and widely applied to pharmacology hypothesis development and testing. The *in silico* drug design is a vast field which incorporates multi-dimensional researches methodologies, which includes modern

techniques. These *in silico* methods include databases, quantitative structure-activity relationships, similarity searching, pharmacophores, homology models and other molecular modeling, machine learning, data mining, network analysis tools and data analysis tools that use a computer. Such methods have seen frequent use in the discovery and optimization of novel molecules with affinity to a target, the clarification of absorption, distribution, metabolism, excretion and toxicity properties as well as physicochemical characterization.

Bioinformatics software tools helps in generating the 3D structure of the target on the basis of the known 3D structures of templates. The Modeler is a popular tool in homology modeling, and SWISS-model repository is a database of protein structures created with homology modeling.[4] In the field of molecular modeling, docking is a technique which envisages the favored orientation of one molecule to a second, when bound to each other to form a stable complex.[28] Molecular docking denotes ligand binding to its receptor or target protein. Molecular docking is used to recognize and optimize drug candidates by examining and modeling molecular interactions between ligand and target macromolecules. Molecular docking is used to generate multiple ligand conformations and orientations and the most appropriate ones are selected. There are several molecular docking tools available that includes Argus Dock, DOCK, FRED, eHITS, Auto Dock and FTDock. Molecular modeling involves scoring methods that are used to rank the affinity of ligands to bind to the active site of a receptor. In virtual high throughput screening, compounds are docked into the active site and then scored to determine which one is more likely to bind tightly to the target macromolecule.[27]

Virtual screening is a computational technique where large libraries of compounds are evaluated for their potential to bind specific sites on target molecules such as proteins, and well-matched compounds tested. The research in the drug discovery process involves virtual screening (VS) which is a computational method used for the rapid exploration of large libraries of chemical structures in order to identify those structures that are most likely to bind to a drug target, usually a protein receptor or enzyme.[18]

During the process of selection of novel drug candidates many essential steps are taken to eliminate such compounds that have side effects and also show interaction with other drugs. *Insilico* drug designing soft wares play an important role to design innovative proteins or drugs in biotechnology or the pharmaceutical field. *Insilico* methods have been of great importance in target identification and in prediction of novel drugs.[28]

Application of structure-based design leads to the identification of new non-peptidic inhibitors of human renin, which is an important target of hypertension. These molecules include aliskiren, piperidines etc. These piperidines bind to and stabilize a different conformer of the protein termed 'open renin' whereas aliskiren binds to 'closed renin'.[5]

Estrogen Receptor

Estrogens attract great attention due to their role in promoting the proliferation of both the normal and the neoplastic breast epithelium. Recently estrogens are identified to be carcinogenic in the human breast. Estrogen receptors are nuclear transcription factors that are involved in the regulation of many complex physiological functions in humans especially menstrual and estrous reproductive cycles in women. They are involved in the regulation of various processes ranging from tissue growth maintenance to reproduction. Estrogen receptors include two subtypes namely ERα and ERβ which exhibit distinct cellular and tissue distribution patterns. These hormones bind to the receptors and generate a growth response in the form of a signal cascade leading to cell proliferation and growth.[17]

A number of chemical compounds derived from plants, known as phytoestrogens, demonstrates the ability to bind to the estrogen receptors, producing estrogenic or anti-estrogenic activity. Their potential anti-proliferative effects could be useful for the formulation of nutraceuticals or pharmaceutics. Recent literature has focused on selective estrogen ligands as highly promising agents for the treatment of some types of cancer, as well as for cardiovascular, inflammatory, and neurodegenerative diseases. Selective estrogen ligands are highly promising targets for treatment of some types of cancer, as well as for cardiovascular, inflammatory and neurodegenerative diseases. Tamoxifen (TX), a selective estrogen-receptor modulator (SERM), is nowadays the most effective and widely used anti-estrogen drug used for this purpose.[9]

Scientists reported that Tamoxifen has been the only drug of choice for more than 30years to treat patients with estrogen related (ER) positive breast tumors. However, several patients treated with TX develop a rapid onset of resistance. Other drugs include Raloxifene, Toremifene etc.[3] Eventhough all these drugs act as an estrogen receptor antagonist, they possess various side effects. Tamoxifen induced blood clots, strokes, uterine cancer, and cataracts. Raloxifene are known to cause serious blood clots to form in the legs, lungs, or eyes, leg swelling/pain, trouble breathing, chest pain, vision changes. Therefore, these side effects make these drugs unsuitable for use and require studies on a better alternate. Thus, the

identification of novel anti-estrogen compounds able to overcome resistance onset is still demanding.

Phytochemicals are proved to be very successful to diminish the possibility of cancer. Isoflavones possess chemical structure similar to estrogen which blocks estrogen. They act rather like Tamoxifen. The protein-ligand interaction plays a significant role in structural based drug designing. Researchers studied the interaction between various phytochemicals and Human estrogen receptor by computational approaches and found that Genistein, Quercetin and Daidzein showed promising binding with estrogen receptor which denotes that Daidzein, Genistein and Quercetin could be the potential lead molecule for the inhibition of Human estrogen receptor.[6] Hence these natural compounds could be used as the template for designing therapeutic lead molecules which could results into massive reductions in therapeutics development time.

Molecular docking studies in human estrogen receptor against isoflavones from *Leopoldia comosa.* In the study two compounds namely have been selected as potential ligands of estrogen receptors (ERs) and the interaction with both isoforms of estrogen receptors have been investigated through molecular docking on their crystallographic structures.[8] The results provide evidence of the binding of these compounds to the target receptors and their interactions with key residues of the active sites of the two proteins, which give insights for the development of novel tools for the dissection of ER signaling and the development of new pharmacological treatments in hormone-sensitive cancers.

Bcl2- The Anti-Apoptotic Protein

Bcl-2 family proteins are key regulators of apoptosis. The inhibition of apoptosis is implicated in diseases such as cancer, autoimmune disorders and viral infections whereas excess apoptosis is linked to other pathological processes such as neurodegenerative disorders, AIDS and stroke. Bcl-2 family includes both anti- and pro-apoptotic proteins with opposing biological functions in either inhibiting or promoting cell death.[10] Deregulation of BCl2 family members is a frequent feature of human malignant diseases and causal for therapy resistance. The role of Bcl_2 in carcinogenesis was first identified more than 20 years ago in follicular lymphoma and ever since huge number of studies were carried out in mouse models to investigate their contribution to tumour formation, progression and therapy resistance.[7] Overexpression of Bcl_2 is common in many types of human cancer and has

frequently been correlated with decreased susceptibility to chemotherapeutics and to increased radio resistance.

The comparative efficacies of five neoplastic drugs which are targeted against Bcl-$_2$ to reduce its expression *in vivo*. The docking results suggested that Gemcitabine showed highest anti-Bcl activity than other drugs.[1] It also revealed that Cisplatin is one of the good inhibitory compounds of Bcl-$_2$. He further reported that the application of computational sciences to pharmaceutical research is a discipline, which is phenomenal. Structural studies of Bcl-$_2$ family members have provided many insights into the molecular mechanism of apoptosis and how Bcl-$_2$family members interact with one another. A lot of conventional therapies with chemical or synthetic drugs are available today for treating cancer, but the side effects and developing chemotherapy resistance has paved way for the search of novel pharmacons with less side effects.

Many phytochemicals are known to possess anti-oxidant as well as anti- apoptotic activity. For example quercetin, resveratrol and curcumin isolated from plants are known to have antioxidant activities and suppress the growth of cancer cells by inducing apoptosis and cell cycle arrest. Quercetin have shown to induce cell cycle arrest in cancer cells including breast, colon, and gastric cancers and exhibits cytotoxicity against lung cancer cells.in prostate cancer cells. Curcumin also has properties as an anti-inflammatory agent by inhibiting release of IL-8 and COX-2 expression as well as lowered expression level of Bcl-$_2$, CDK4, and cyclin D. These results suggest that phytochemicals, which can be found in a normal diet, inhibit lung cancer cell proliferation and regulate the expression of the proteins involved in apoptosis and cell cycle.[30]

The protein docking studies in Bcl_2 would interpret the role of key amino acids in the protein's active site which enables to study the functional properties of the protein. Since it is a target based approach for drug discovery it may help in the identification and synthesis of novel drug candidates.[25]

Previous studies have revealed that ginseng has preventive activity against the development of cancer. Administration of ginseng extract has been demonstrated to increase resistance and decrease effects of colon, lung, liver, pancreas, pharynx, skin, stomach, ovarian, uterine cervix, mammary gland, and kidney cancer. In the light of this docking studies on twelve ginsenoside compounds and anti-apoptotic proteins were performed and reported that

ginsenosides were found to have a good binding affinity with BCL_2 proteins, and concluded that ginsenosides are effective compounds to control the overexpression of anti-apoptotic proteins such as BCL-2, BCL-XL, and MCL-1.[11]

Annona muricata Linn is a potential anticancer plant that has been widely reported to contain valuable chemopreventive agents known as annonaceousacetogenins. Many invitro studies were carried out on this plant and were reported to be anti-proliferative.[20] *Annona muricata* L. inhibits different types of cancer cells by apoptosis.He further elucidated the binding interaction of ten bioactive phytochemicals of *Annona muricata* to three Bcl-2 family of anti-apoptotic proteins viz. BCL-2, BCL-w and MCL-1 using an *in silico* molecular docking analysis and found that anonaine, a benzylisoquinoline alkaloid showed high affinity towards the Bcl-2, thus indicating that this compound is a potent inhibitor of the Bcl-2 antiapoptotic family of proteins.

MATERIALS AND METHODS

Insilico Studies

The term '*in silico*' is a modern word usually used to mean experimentation performed by computer and is related to the biological terms *in vivo* and *in vitro*. *In silico* pharmacology or computational therapeutics or computational pharmacology is a rapidly growing area that globally covers the development of techniques for using software to capture, analyse and integrate biological and medical data from many diverse sources.[5]

Pharmacology over the past hundred years had a rich tradition of scientists with the ability to form qualitative or semi-quantitative relations between molecular structure and activity *incerebro*. To test these hypotheses the conventional or traditional pharmacology tools like *in vivo* and *in vitro* models were used. *In silico* pharmacology was established in the early 1960s when quantitative relationships between chemical structure and pharmacodynamics(PD) and Pharmacokinetic (PK) effects in biological systems began to be unveiled by computational means. The initial focus was in computing the bioactivity of molecules.[5]

Over the last decade computational (*in silico*) methods have been developed and applied to pharmacology hypothesis development and testing. These *in silico* methods include databases, quantitative structure-activity relationships, pharmacophores, homology models and other molecular modelling approaches, machine learning, network analysis tools and data analysis tools. *In silico* methods are primarily used alongside of *in vitro* data both to create

the model and to test it. Such models have seen frequent use in the discovery and optimisation of novel molecules with affinity to a target, the clarification of absorption, distribution, metabolism, excretion and toxicity properties as well as physicochemical characterization.[5]

The process of scoring and ranking molecules in large chemical libraries according to their affinity for a certain target is generally referred to as virtual screening. Virtual screening is a knowledge-driven approach that requires structural information either on bioactive ligands for the target of interest (ligand-based virtual screening) or on the target itself (target-based virtual screening).Comparative studies have suggested that information about a target obtained from known bioactive ligands is as valuable as knowledge of the target structures for identifying novel bioactive scaffolds through virtual screening.[5]

Docking methods have also resulted in the discovery of novel inhibitors for several kinase targets, including cyclin-dependent kinases, epidermal growth factor receptor kinase and vascular endothelial growth factor receptor 2 kinase among others.[5]

In silico experimental modeling of cancer involves combining findings from biological literature with computer-based models of biological systems in order to conduct investigations of hypotheses entirely in the computer laboratory. Traditional laboratory-based cancer research involves expensive trial and error experimental strategies applied to humans, animals, and their harvested tissues. *In silico* experimentation involves the coupling of current computing technologies with mathematical or theoretical characterizations of cancer cell biology, provides a novel approach to guiding the early stages of hypothesis development and experimental design that has the potential to create subsequent efficiencies and cost savings in the laboratory. This computational approach is advantageous because it allows vast numbers of experiments to be carried out that are easily observed at any desired level of detail and can be repeated and controlled at will.

It is well known that the preclinical studies in cancer biology are expensive. Such studies involving *in vitro* and *in vivo* animal experiments involve hypothesis generation and testing to determine whether further trials are warranted and are extremely costly both in terms of researchers' time and the associated financial investment. Costs, such as laboratory setup, equipment and space, time spent by academics training others and the time, equipment, and

materials costs involved in repetitive, hands-on experimental work, all contribute to the expense of laboratory-based experimental research.[26]

The docking affinity of commercially available breast cancer drugs against Human estrogen receptor using HEX docking software. Estrogen and progesterone bind to the receptors and side by side may work with growth factors which cause further proliferation of cancer. Tamoxifen, Raloxifene, Toremifene etc are commercially available breast cancer drugs, which inhibits the estrogen activity on these cells. Depending on the energy values obtained by docking the drugs and Estrogen receptor, Raloxifene and Toremifene were found to be best and was chosen for further study.[13]

The effect of diosgenin in inhibiting alpha amylase and determined inhibition mechanism for yeast α-glucosidase by diosgenin through computational methodology. Computational docking studies provided immense information about the mechanism behind active site binding interactions. They reported that α-glucosidase-diosgenin inhibitor complex showed lowest energy with highest binding affinity as compared to α-amylase-diosgenin inhibitor complex. Hence Diosgenin exhibited potent inhibition against both porcine pancreatic α-amylase and α-glucosidase as well as against crude murine amylase and glucosidase, which give insights about antidiabetic effect of diosgenin as well as give immense evidences of its chance to become a novel antidiabetic drug.[8]

RESULTS AND DISCUSSION

Protein Preparation

Three-dimensional crystal structure of Human Estrogen Receptor protein (PDB ID:1X7R) and Human Apoptotic Regulator protein Bcl_2 (PDB ID: 2W3L)was downloaded in pdb format from the protein data bank (http://www.rcsb.org/pdb/home/home.do). Further details of the protein for modeling purposes were retrieved from UNIPORT data base (PO3372) & (P10415) which was downloaded in FASTA canonical format, and similarity search was carried out using SWISS PROT software tool. For the validation of the protein RAMPAGE software tool was used. The protein was purified using the PyMOL software tool and the purified protein was exported to PyRx for docking studies.

Ligand Preparation

Trichopus zeylanicus was reported to possess the active principles such as 1, 2-Benzopyran, β-sitosterol, Vitexin and Vicinin-2. Initially the chemical structures of all the mentioned

compounds were retrieved from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) for creation of ligand library. Commercially available drug Femara (Letrozole) was selected as control for this study. 3D structures of the compounds were downloaded in SDF format and further converted to Mol (MDL mol file) and PDB file (Protein Data Bank) format using Open Babel (http://openbabel.org/wiki/Main_Page) chemical tool box.

Evaluation of Selected Ligands

Determination of Drug Likeness of Ligand Using Molsoft

MOLSOFT tool was used to predict the drug likeness of molecules (http://www.molsoft.com/mprop/). The whole library compound was analyzed for the Lipinski's rule of five online cheminformatics software tool Molsoft. Mol (.mol) formate was used to analyze the results. The rules describe molecular properties important for a drug's pharmacokinetics in the human body, including their absorption, distribution, metabolism and excretion ("ADME"). Molecular properties such as log P, number of hydrogen bond acceptors, no of hydrogen bond donors and molecular weight, as well as prediction of drug likeness source is an important parameter in screening of drug molecules.

Molecular Docking

Molecular docking works in a two-step process which starts with compiling different ligand conformations in the identified active site of the receptor and then ranking them according to their binding conformation energies for each individual binding conformation. Various softwares like Auto Dock, Argus Lab, FLEX, and GOLD are extensively used for estimating docking binding energies of various ligand-receptor conformations. Fig-2 depicts the schematic representation of docking study of phytochemicals from the plant against the protein ER. In the present study docking studies were carried out using an open source software for computational drug discovery called PyRx. PyRx aids in virtual screening. It is a combination of several softwares such as AutoDockVina, AutoDock 4.2, Mayavi, Open Babel, etc. PyRx uses AutoDockVina and AutoDock 4.2 as docking softwares. After successfully loading the target purified protein and ligand molecules in PDB format, it was autodocked and converted into autodock input files or (.pdbqt) files and the Vina software was used to the evaluate the docking energies. The identification of the most appropriate binding conformations is carried out by exploring the large conformational space depicting various binding sites and then accurately predicting the interaction energy associated with the respective binding conformations. This process is carried out recursively until converging to

a solution of minimum energy. More negative the binding affinity better the orientation of the ligand in the binding site. Results can be exported to other software programs like UCSF Chimera or Pymol for analysis.[22]

Visualisation of Protein –Ligand Interactions

PyMOL was used for molecular docking, virtual screening and binding site analysis. It is the most frequently used program for generating high definition pictures of molecular structures and offers multiple advanced rendering options. The Autodock / Vina-plugin for PyMOL makes it easier to export the results from PyRx. Additionally it provides exceptional 3D-viewing functionalities which can be very useful in structure-based drug design. Since visualization is crucial for structure-based drug design, several tools have been developed to add visual support for the auto dock suite. The visualizer Auto Dock Tools offers a complete molecular viewer and a graphical support for all steps required for set up and analysis of docking runs.[23]

***IN SILICO* DRUG ANALYSIS**

Insilico analysis of genes and proteins has been receiving greater attention with particular emphasis to find suitable biomarkers for rapid identification of designing of drugs to combat the life threatening diseases like cancer, cardiovascular diseases, diabetics, pathogenic microbes and superbugs, in diagnosis of infectious diseases and discovery of potent pharmacons to diseases[19]. The current study is carried out to study the properties of phytochemicals present in *Trichopus zeylanicus* leaves.

Protein Selection & Preparation

Three-dimensional crystal Structure of Human Estrogen Receptor (PDB ID:1X7R) and Apoptotic regulator protein (PDB ID: 2W3L) was downloaded in. pdb format from the protein data bank. Further details of the protein for modeling purposes were retrieved from UNIPORT data base (P30556), which was downloaded in FASTA canonical format and homology modeling was carried out using SWISS PROT software tool. A high percentage of sequence similarity should be more accurate alignment between the target sequence and template structure. Through homology modelling using Swiss Prot of Estrogen Receptor Protein 11protein models were acquired, of that the one with highest similarity was chosen for the study. The model 1 (4znh.1.A) with a sequence similarity or template alignment of 99.61% was taken for docking studies whereas homology modeling of Bcl2 yielded two

model and the model with a sequence similarity of 90.96% (1gjh.1.A) was taken for further studies.

The validation of the protein model was carried by using Ramachandran plot and was analyzed by the RAMPAGE software tool. The plot statistics showed that 97.9% of the residues were in most favored regions, 2.1% in additional allowed regions, and no residues were observed in disallowed region. This explains the reliable validity of the models with acceptable stereo chemical quality.

Purification of Protein

The native protein human estrogen receptor and BCl2 was purified before docking with the PYMOL tool software. The purification was done based on common procedures and the small molecules and ligands present in the protein were removed. The data regarding the ligands and small molecules were retrieved from the RCSB Protein Data Bank. The purified protein was saved in. pdb format for further studies.

Preparation of Ligands

Rational design of new chemical entities typically involves learning from past experiences and developing a knowledge base that can be used to predict future successes. For example QSAR methodology involves development of predictive models based on a list of compounds with known biological activities and their structural attributes. pharmacophore methodology involves development of a model that represents the common 3D functional attributes of known active compounds deemed responsible for biological effect.[12]

The ligand molecules that are selected for *Insilico* analysis was 1, 2 Benzopyran, Beta-Sitosterol, Vicenin-2 and Vitexin. Considering the diverse pharmacological properties of 1, 2 Benzopyran, Beta-Sitosterol, Vicenin-2 and Vitexin, the present study is undertaken for it's *in silico* analysis. Molecular property of these ligands like hydrophobicity, molecular size, flexibility were studied to evaluate drug likeness and to determine if the candidate drugs with a certain pharmacological or biological activity has specific physiochemical properties that would make it a likely orally active drug in humans.

Prediction of Molecular Properties of the Ligand Molecules

Computational sensitivity analysis and structural analysis have been used to study the drug-likeness of the candidate drug. The study was carried out by the MOLSOFT tool. Molecular

volume determines transport characteristics of molecules, such as intestinal absorption or blood- brain barrier penetration. Volume is therefore often used in QSAR studies to model molecular properties and biological activity. Molecular polar surface area (PSA) is very useful parameter for the prediction of drug transport properties. PSA is a sum of surfaces of Polar atoms (usually oxygen, nitrogen and attached hydrogen) in a molecule. The molecular properties of the selected candidate are recorded in Table 1.

Table: 1 Result of molecular properties of ligands.

Phytochemical	Molecular properties		
	MF	MPSA	MV
1, 2 Benzopyran	C_9H_8O	8.11 A^2	138.22 A^3
β-sitosterol	C_{29} H_{50} O	16.28 A^2	519.36 A^3
Vicenin 2	$C_{27}H_{30}O_{15}$	214.06 A^2	521.52 A^3
Vitexin	$C_{21}H_{20}O_{10}$	177 A^2	391.88 A^3
Femara (Control)	$C_{17}H_{11}N_5$	58.48 A^2	290.67 A^3

Lipinsk's Rule of Five

Lipinski's rule of 5 helps in distinguishing between drug like and non-drug like molecules. It predicts high probability of success or failure due to drug likeness for a molecule. The prediction results are recorded in Table 2. After successful prediction of molecular properties, ligands were considered for the next level of molecular docking studies. Virtual screening of acetylcholinesterase inhibitors using the Lipinski's Rule of Five and ZINC Databank was used and they screened around 382 compounds which obey the Lipinski's rule of five which was later docked with the enzymes.[15]

Table: 2 Result of Lipinski's Rule analysis & Drug Likeness.

Phyto Chemicals	Lipinski's rule				Drug Likeness Score
	MW <500	LOGP<5	HBA<10	HBD <5	
1, 2 Benzopyran	132.16	2.56	1	0	-1.65
Beta- Sitosterol	414	9.48	1	1	0.88
Vitexin	432.381	0.17	10	7	0.79
Vicenin-2	432	-2.71	15	11	0.37
Femara	285	2.51	4	0	-0.85

Docking Using PyRx

The screened ligands were docked with the target protein Human Estrogen Receptor and Bcl2 receptor with PDB ID: 1X7R AND 2W3L using PyRx. The minimum binding affinities needed by the ligand to occupy the catalytic pocket is displayed as a result of docking using PyRx is depicted in table 3. Muhammad and Fathima (2015) studied the *In silico* analysis and

molecular docking studies of potential angiotensin-converting enzyme inhibitor using quercetin glycosides using PyRx, Auto Dock Vina option where quercetin showed optimum binding affinity with a angiotensin-converting-enzyme which indicated that quercetin glycosides could be one of the potential ligands to treat hypertension, myocardial infarction, and congestive heart failure.

Based on the result, both the docking scores are evaluated and Vitexin showed the highest docking score of -8.9 against the apoptotic regulator protein Bcl_2 and Beta sitosterol showed the highest docking scores satisfying Estrogen receptor protein with a docking score of -7.9 followed by Vicenin -2 and is showing more affinity that the standard drug fumara, which clearly indicates the potentiality of drug candidate. The docking results show that ligands have higher binding affinities with the protein, indicating they must be capable of acting as a potent drug against breast cancer diseases.

Table: 3Ligand Structure and Molecular Docking scores.

Phytochemical	Molecular Docking – Binding Score-1X7R	Molecular Docking-Binding Score- Bcl2 2W3L
Vitexin	-6.8	-8.9
Vicenin 2	-7.5	-8.6
1, 2 Benzopyran	-5.9	-5.6
Beta Sitosterol	-7.9	-7.6
Femara	-6.4	-

Molecular Visualisation Using pyMOL

The binding pockets of the protein – ligand interactions were visualized using pyMOL. The table: shows the interacting amino acid residues and the no of hydrogen bond interactions.

All the ligands showed good interaction towards the human estrogen receptor 1X7R. The highest number of bonding was observed in Vicenin-2. Vicenin interacts twice with ARG 503 and thrice with ASN 439th residue, which is followed by Vitexin. Vitexin showed four hydrogen bonding with Leu 511, ASN 455 and interacting twice with ARG 515th residue. Beta Sitosterol showed a hydrogen bonding with GLN 441.All the four drug candidates showed much higher docking score than the standard drug Femara, which indicates that all the compounds can be potent anti-cancer drugs.

Table: 4 Molecular Docking Studies.

Target	Compound name	No. of H bonds	H bond interactions
Human Estrogen Receptor Protein	1,2-Benzopyran		
	Beta-Sitosterol	1	GLN 441
	Vicenin 2	5	ARG 503*, ASN 439**
	Vitexin	4	ARG 515*, LEU 511, ASN 455

*Interacting twice **- INTERACTING THRICE

Table: 5: Result of the amino acid interacting residue and their positions.

Target	Compound name	No. of H bonds	H bond interactions
Bcl-2	1,2-Benzopyran	1	His143
	Beta-Sitosterol	1	Lys22
	Vicenin 2	3	Asn122, Arg65, Arg68
	Vitexin	4	22Lys, 75Ser, 68Arg

All the four phytochemicals studied showed interaction with the target protein Bcl-2 which is enlisted in table 4B. Benzopyran showed hydrogen bonding with the 143rd His of Bcl2 target protein. Whereas both Vitexin and Vicinin showed hydrogen bonding with the 68th ARG. Beta Sitosterol and Vitexin showed a hydrogen bonding with the 22 Lys residue of the target protein Bcl2 receptor. Vicenin also showed bonding with Asn122 and Arg65 of target protein.

Any anticancer drug exerts its activity through several biological mechanisms namely anti-inflammatory, anti-oxidant, simulation of anticancer immunity, modulation of oncogene expression. Hayashi *et al.*, 2003 have reported that over expression of estrogen receptor alpha is the main reason for the occurrence of breast cancer. Therefore the drug has to be a potent ER inhibitor. Commercially available drug Femara was used a positive control in this study. In case of Femara, it binds to 520 LYS & 548 ARG residue of ER and inhibits the role of ER. Tamoxifen, a commercially available drug for breast cancer binds to Arg394 and blocks the function of estrogen receptor and inhibits the function of ER-α.

Molecular docking results provided insights on the interaction of phytochemicals as a drug to the ER and Bcl_2 via hydrogen bonding. Thus *Insilico* approach using structure-based drug design can be used as a priorto *in vitro* methods for screening of phytochemicals as potent anticancer compounds from *Trichopuszeylanicus.* Among all the phytochemicals, Vitexin exhibited higher binding affinity to apoptotic regulator protein Bcl_2 and yielded positive value for drug likeliness. 548 ARG was found to be the most interacting site of the protein. Vitexin (apigenin-8-C-glucoside) has recently received increased attention due to its wide

range of pharmacological effects, including but not limited to anti-oxidant, anti-cancer, anti-inflammatory, anti-hyperalgesic, and neuroprotective effects. Latest research has suggested that Vitexin could be potential substitute medicines for diversity diseases, and may be adjuvants for stubborn diseases or health products.[14] Hence the present study gives insights into the anti-cancerous activity of phytochemicals present in *Trichopus zeylanicus*, which can be potent anti-cancer drugs in future. But extensive *in vitro* and *invivo* followed by clinical studies are studies are required to prove the same.

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