Published online in http://ijam.co.in ISSN No: 0976-5921

*International Journal of Ayurvedic Medicine, Vol 14 (1), 2023; 114-120*

# *In-Silico* Prediction of Phytoconstituents from *Phyllanthus niruri* for Anticancer Activity against Prostate Cancer Targeting PIM-1 Kinase

**Research Article**

**Apeksha P Motghare[1], Parimal P Katolkar[2*], Tina S Lichade[3], Jagdish Baheti[4]**

1. Research Student, 2. Associate Professor, 3. Research Student, 4. Principal,
Kamla Nehru College of Pharmacy, Butibori, Nagpur, Maharashtra. India.

## Abstract

Objective: Prostate cancer is a disease in which the malignant cells form in the tissues of the prostate that is characterised by unchecked cell proliferation in prostrate tissues. Oncemore, medicinal plants are being researched for the treatment of prostrate cancer. Prototypical compounds found in medicinal plants have been the source of many conventional medications. *In-silico* testing of *Phyllanthus niruri* (L.) Lour. phytoconstituents for anticancer efficacy was a part of our investigation. Design: Utilizing Discovery studio, molecular docking is done to assess the pattern of interaction between the phytoconstituents from the *Phyllanthus niruri* plant and the crystal structure of the anticancer proteins (PDB ID: 3A99). Later, SwissADME and pkCSM were used to screen for toxicity as well as the pharmacokinetic profile. Results: The docked results suggest that luteolin (-8.3 kcal/mol), and caffeic acid (-6.6 kcal/mol), for 3A99 macromolecule has best binding affinity towards PIM-1 kinase for anticancer activity on prostrate as compared to the standard drug lenvatinib mesylate (-3.5 kcal/mol). Furthermore, pharmacokinetics and toxicity parameters were within acceptable limits according to ADMET studies. Conclusion: Results from the binding potential of phytoconstituents aimed at anticancer activity were encouraging. It promotes the usage of *Phyllanthus niruri* and offers crucial details on pharmaceutical research and clinical care.

**Key Words:** In-silico*, Phyllanthus niruri,* Anticancer Activity, 3A99, Discovery studio.

## Introduction

The traditional medicine of South and Southeast Asia has employed the perennial tropical shrub *Phyllanthus niruri* to treat a variety of ailments, including but not limited to jaundice, diarrhea, dyspepsia, genitourinary infections, and kidney stones. *P. niruri* formulations are used as traditional treatments for renal and vesicular calculi in Brazil, where the plant is known as "*Chanca Piedra*" or "stone breaker." (1)

The fruit and leaves have been used as a treatment for gallstones and jaundice in traditional medical systems like Ayurvedic and Unani medicine. *P. niruri*, also referred to as "dukonganak" in colloquial Malay, is used to treat renal problems and coughs. (1) The herb, known as Bhumyamalaki in South India, is thought to be effective in treating syphilis, gonorrhea, and constipation. (3) This plant, colloquially referred to as "pitirishi," has developed a reputation in northern India as a go-to treatment for bronchitis, asthma, and even tuberculosis. (4) This herb's young shoots might occasionally be used as an infusion to treat chronic diarrhoea. (5) *P. niruri*, also known as "zhu zi cao," has long been used in traditional Chinese medicine to treat liver damage brought on by a variety of hepatotoxic substances. In fact, even since Venkateswaran and colleagues' seminal animal work, which showed for the first time in vivo that *P. niruri* may have anti-hepatitis B activity, (6) There have been a number of research looking at the varied therapeutic potential of this plant species as a result of the substantial scientific interest in this herb.

Since Ottow first isolated the lignan phyllanthin from this plant in 1861(7) until as recently as the isolation of the potential anti-HBV phytochemicals nirtetralin and niranthin(8,9), phytochemical studies on this plant have shown that it is rich in tannins, flavonoids, alkaloids, terpenes, coumarins, lignans, and phenylpropanoids, which are responsible for the numerous chemicals that have been identified from this herb employed in study are listed in Table 1 in brief. Although it has a wide variety of ethnomedicinal uses, most of these possible therapeutic uses have not been the subject of research that has advanced to the point of clinical trials. In fact, there is a lack of synthesis in the field of *P. niruri* studies about the current level of knowledge. The heterogeneity of the initial research on *P. niruri* has also prevented an objective evaluation of the plant's potential, and the mechanisms underlying the majority of this herb's medicinal effect are still unknown.As it should be emphasized, natural compounds from herbs are still essential sources of

*** Corresponding Author:**

**Parimal P Katolkar**
Associate Professor,
Kamla Nehru College of Pharmacy,
Butibori, Nagpur,
Maharashtra. India.
Email Id: parimal.katolkar@gmail.com

innovative therapeutic agents and new chemical entities, *P. niruri* may potentially be a significant medication lead. The relevance of researching natural products has also been reemphasized due to the past over-reliance on combinatorial chemistry and the fact that it does not always produce large and pharmacologically viable libraries. Exploring these natural products could result in the creation of novel natural product-like libraries, which, when combined with high-throughput screening assays, could produce new therapeutic candidates for further research. *P. niruri*, a common herb with several uses, can be used to create more affordable and accessible medications that target a variety of chronic conditions and have less adverse effects than synthetic pharmaceuticals. Consolidation of the scientific evidence and potential knowledge gaps must be addressed in order to facilitate more focused future study on this species. The current study aims to compile and synthesize the most recent body of research on the pharmacological properties of *P. niruri* that has been published in PubMed between 1980 and 2015. It will point out potential directions for additional research into the creation of new Phyllanthus-based medications as well as places where this herb could be improved as an affordable adjunct or perhaps a cutting-edge alternative therapeutic agent.

## Materials and Methods

### Platform for molecular docking

Using AutoDock Vina software, a computational docking analysis of all the phytoconstituents chosen as ligands with anticancer action as the target was carried out. (10)

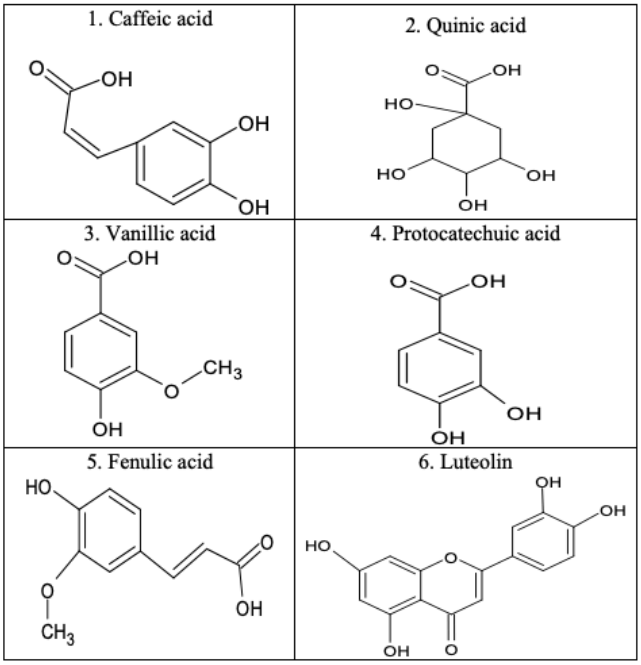
### Protein preparation

The 2.00 crystal structure of anticancer with inhibitor, (PDB ID:3A99, having resolution: 1.60Å, R-Value Free: 0.206, R-Value Work: 0.187, R-Value Observed: 0.187), which was retrieved from the protein data bank (https://www.rcsb.org), was subjected to *in-silico* analysis of a few phytoconstituents. 3A99 is used to treat prostrate cancer. Using Discovery Studio, all additional molecules were eliminated, including undesirable chains, nonstandard residues, and co-crystallized water molecules.(11)

### Ligand preparation

Using the Avogadro programme, all constituents' three-dimensional (3D) structures were extracted from the PubChem database on the NCBI website (https://pubchem.ncbi.nlm.nih.gov/). However, the ChemSketch application was used to sketch the geometrical 2D structure. The ligand structures were saved in the PDB format and the two-dimensional (2D) structures were converted into 3D models using the Avogadro software. **Figure 1** depicts each chemical structure.

**Fig.1. Chemical structures of all selected phytoconstituents in the molecular docking studies**




### Molecular docking

In order to determine the scoring function based on geometry and forecast the binding affinity of the ligand molecule,(12,13) molecular docking analyses the interactions between the protein and the ligand. We used molecular docking experiments to examine the interactions between specific phytoconstituents (Fig.1), the conventional medication, and the crystal structure of a macromolecule with anticancer activity (PDB ID: 3A99). PyRx software was used to carry out the molecular docking investigation, and the Vina wizard tool was used to investigate binding affinity. With bound ligands as the benchmark, the final data were analysed and presented using Discovery Studio 2020 Client.(14) The number of contacts and active residues responsible for significant binding at the target enzyme's active site are reflected in the protein-ligand interaction visualisation.

### Absorption, distribution, metabolism, and excretion (ADME) and toxicity prediction

The chosen phytoconstituents and the reference medication were then examined for drug-like characteristics in accordance with Lipinski's rule. The tolerability of phytochemicals must be predicted during therapeutic development before they are consumed by people and animal models. SwissADME (http://www.swissadme.ch) and pkCSM (an online server database predicting small-molecule pharmacokinetic features using graph-based signatures, http://biosig.unimelb.edu.au/pkcsm/prediction) were used to determine the pharmacokinetic profile (ADME) and toxicity predictions of ligands. Simplified Molecular Input Line Entry System (SMILES) notations or PDB files were uploaded to examine the toxicological qualities of ligands, and then the necessary models were chosen to generate a wealth of information regarding effects associated to structure.(15,16)

**Standard Preparation**

Recent evidences suggest that the proto-oncogene product PIM-1 kinase induces the initiation and progression of prostate cancer.(17) The expression of PIM-1 has been reported to be up-regulated from the neoplastic state in the prostate and correlated with measures of clinical outcome in prostate cancer patients. PIM-1 could induce cell cycle progression by phosphorylating the cyclin-dependent kinase inhibitor p27Kip1 and inhibit apoptosis by phosphorylating the apoptosis- inducing factor Bad (18). Thus, PIM-1 seems to be an important drug target for cancer.

The standard is created in a series of phases, such as creating the 2D structure of the standard medicine using the chemsketch tool, then converting the 2D structure into a 3D model using the Avogadro Software, and finally saving it in PDB format. Lenvatinib mesylate's molecular docking with 3A99 was carried out utilizing PyRx.

## Results and Discussion

The objective of the current study was to investigate the phytoconstituents found in *P. niruri's* anticancer activity's inhibitory capacity. Using PyRx, we conducted molecular docking studies of all the phytoconstituents present in *P. niruri* for this investigation. We next looked at the interactions between the amino acid residues and how they affected the inhibitory potentials of the active components. Using SwissADME and pkCSM servers, selected phytoconstituents with the best fit were further assessed for their absorption, distribution, metabolism, excretion, and toxicity (ADMET) characteristics.

**Molecular docking**

The docking scores and binding energies of all chemical constituents of *P. niruri* targeting anticancer activity (PDB ID: 3A99) and binding interactions with amino acid residues are presented in **Table 1.**

**Table 1: Binding interaction of ligands from *Phyllanthus niruri* targeting prostrate cancer activity (PDB ID: 3A99)**

| Sr. No. | Chemical constituent | PubChem ID | Docking Score 3A99 |
|---|---|---|---|
| 1 | Caffeic acid | 689043 | -6.6 |
| 2 | Quinic acid | 6508 | -6.1 |
| 3 | Vanillic acid | 8468 | -5.9 |
| 4 | Protocatechuic acid | 528594 | -6.0 |
| 5 | Fenulic acid | 445858 | -6.2 |
| 6 | Luteolin | 5280445 | -8.3 |
| **Standard Drug** | | | |
| 7 | Lenvatinib mesylate | 11237762 | -3.5 |

The binding affinities of phytoconstituents ranged from -8.3 to -5.9 kcal/mol for 3A99 macromolecule. From the docked results, it is evident that the compounds, luteolin and caffeic acid for 3A99 exhibit the most favourable binding affinity (-8.3 and -6.6 kcal/ mol respectively) in complex with anticancer activity, as compared to other docked compounds i.e., fenulic acid (-6.2 kcal/mol), quinic acid (-6.1 kcal/mol), protocatechuic acid (-6.0 kcal/mol) and vanillic acid (-5.9 kcal/mol).

The binding affinity of the standard (lenvatinib mesylate) for 3A99 is -3.5 kcal/mol.(19)

In addition, an analysis of the interactions of the 3A99 protein complex and ligand lenvatinib mesylate was performed, which showed that the ligand molecule is oriented due to one unfavorable donor-donor with GLY 50(A) amino acid, one Pi-alkyl with LEU 174(A), six alkyl interaction with LEU 44(A), ALA 65(A), ARG 122(A), ILE 185(A), LYS 67(A) and LEU 43(A), two conventional hydrogen bonds with VAL 52(A) and GLY 47(A) and nine Van der Waals interactions with amino acid residues SER 51(A), GLY 45(A), ASP 128(A), VAL 126(A), LEU 120(A), ASP 186(A), SER 46(A), GLY 48(A), and PHE 49(A) were also found.(Fig.2)

An analysis of the interactions between the 3A99 protein complex and the ligand luteolin was also carried out, and it was discovered that the ligand molecule is oriented as a result of three Pi-Alkyl interactions with VAL 69(A), ILE 74(A) and ARG 73(A), one Pi-Anion interactions with GLU 70(A), two conventional hydrogen bond with HIS 68(A), and MET 88(A), one unfavourable donor-donor interactions with ASP 76(A), one carbon hydrogen bond with GLY 50(A) and six Van der Waals interaction with PHE 49(A), SER 51(A), GLY 188(A), SER 75 (A), PRO 87(A), GLU 89(A) were also found. (Fig.3.a).

Additionally, an analysis of the interactions between the 3A99 protein complex and the ligand caffeic acid was carried out, and it was discovered that the ligand molecule is oriented as a result of one Pi-sigma interaction with ILE 74(A), one unfavourable donor-donor interactions with GLU 89(A), one conventional hydrogen bonds with SER 75(A), and seven Van der Waals interaction with ARG 73(A), GLU 70(A), GLY 188(A), PRO 87(A), MET 88(A), ASP 76(A), VAL 69(A) were also found. (Fig.3.b).

**ADMET study**

Pharmacokinetic profile (ADME) and toxicity predictions of the ligands are important attentive parameters during the transformation of a molecule into a potent drug. In the present study, these parameters were assessed using SwissADME and pkCSM. The absorption potential and lipophilicity are characterized by the partition coefficient (Log *P*) and topological polar surface area (TPSA), respectively. For better penetration of a drug molecule into a cell membrane, the TPSA should be less than 140 Å. However, the value of Log *P* differs based on the drug target. The ideal Log *P* value for various drugs are as follows: oral and intestinal absorption, 1.35 − 1.80; sublingual absorption, > 5; and central nervous system (CNS). The aqueous solubility of ligands ideally ranges from –6.5 to 0.5 , while the blood brain barrier (BBB) value ranges between –3.0 and 1.2 (20). In addition, non-substrate P-glycoprotein causes drug resistance (21).

In our study, all the selected ligands followed the TPSA parameter, P-glycoprotein non-inhibition, thereby showing good intestinal absorption and an acceptable

**Table 2: Binding interactions of ligands with the binding site of PIM-1 kinase**

| No. | Inhibitor | Binding energy (kcal/mol) | Amino acids interaction with hydrogen bond | Amino acids with hydrophobic interaction |
|---|---|---|---|---|
| 1 | Caffeic acid | -6.6 | SER 75(A) | ARG 73(A), GLU 70(A), GLY 188(A), PRO 87(A), MET 88(A), ASP 76(A), VAL 69(A) |
| 2 | Quinic acid | -6.1 | SER 75(A), ASP 76(A), MET 88(A) | SER 189(A), ARG 73(A), GLY 188(A), ILE 74(A), VAL 69(A), PRO 87(A), VAL 90(A) |
| 3 | Vanillic acid | -5.9 | SER 75(A), ASP 76(A), ARG 73(A), GLY 188(A) | MET 88(A), GLU 89(A), PRO 87(A) |
| 4 | Protocatechuic acid | -6.0 | SER 75(A), ASP 76(A) | MET 88(A), VAL 69(A), PRO 87(A), GLY 188(A), ARG 73(A) |
| 5 | Fenulic acid | -6.2 | SER 75(A), ASP 76(A), MET 88(A), GLU89(A), GLY 188 (A) | GLU 70 (A), ARG 73 (A), VAL 69 (A), PRO 87 (A), SER 189(A) |
| 6 | Luteolin | -8.3 | HIS 68(A), MET 88(A), GLY 50(A) | PHE 49 (A), SER 51(A), GLY 188(A), SER 75 (A), PRO 87(A), GLU 89(A) |
| 7 | Lenvatinib mesylate | -3.5 | VAL 52(A), GLY 47(A) | SER 51(A), GLY 45(A), ASP 128(A), VAL 126(A), LEU 120(A), ASP 186(A), SER 46(A), GLY 48(A), PHE 49(A) |

range of BBB values. All the compounds showed aqueous solubility values within the range. Further, it was predicted that the selected ligands do not show AMES toxicity, hepatotoxicity, and skin sensitivity. In addition, it did not inhibit hERG-I (low risk of cardiac toxicity). Lipinski's rule violations, *T. pyriformis* toxicity, minnow toxicity, maximum tolerated dose, rat acute oral toxicity, and chronic toxicity are depicted **in table 3.(22)**

**Table 3: ADME and toxicity predicted profile of ligands with superior docking scores**

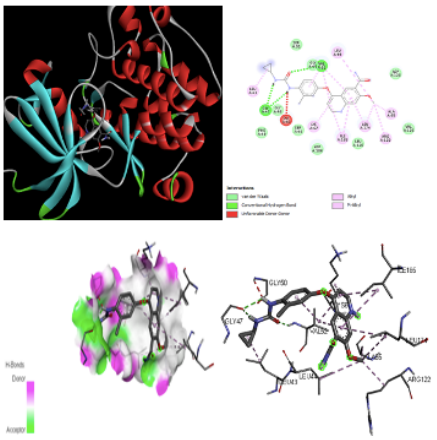
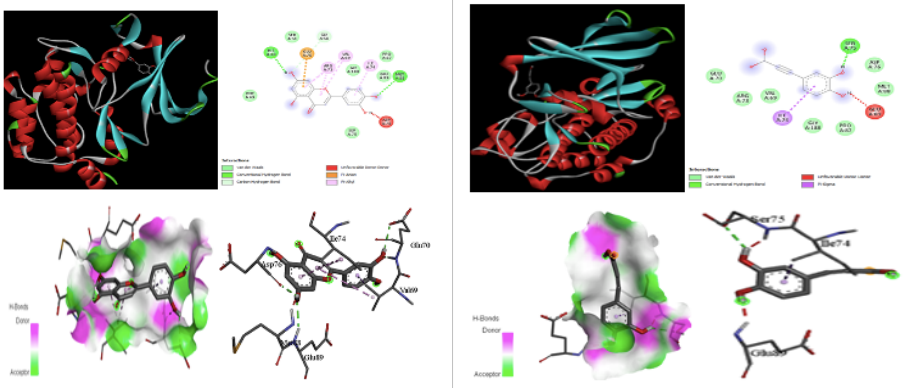
| ADMET Properties | Formula | MW (g/mol) | Log P | TPSA (Å²) | HB donor | Hb acceptor | Aqueous solubility (Log mol/ L) | Human intestinal absorption (%) | Blood-brain barrier |
|---|---|---|---|---|---|---|---|---|---|
| Caffeic acid | $C_9H_8O_4$ | 180.16 | 1.19 | 77.76 | 3 | 3 | -2.16 | 56.503 | -0.652 |
| Quinic acid | $C_7H_{12}O_6$ | 192.17 | -2.32 | 118.22 | 5 | 5 | -1.67 | 14.745 | -0.999 |
| Vanillic acid | $C_8H_8O_4$ | 168.15 | 1.09 | 66.76 | 2 | 3 | -1.85 | 77.248 | -0.326 |
| Protocatechuic acid | $C_7H_6O_4$ | 154.12 | 0.79 | 77.76 | 3 | 4 | -1.99 | 75.77 | -0.692 |
| Fenulic acid | $C_{10}H_{10}O_4$ | 194.18 | 1.49 | 66.76 | 2 | 4 | -2.90 | 93.22 | -0.28 |
| Luteolin | $C_{15}H_{10}O_6$ | 286.24 | 2.28 | 111.13 | 4 | 6 | -3.26 | 79.08 | -1.101 |
| Lenvatinib mesylate | $C_{22}H_{23}ClN_4O_7S$ | 522.96 | 4.07 | 178.32 | 4 | 8 | -3.37 | 88.88 | -1.342 |

**Table 3: Continued**

| ADMET Properties | P-glycoprotein substrate | Total clearance (Log ml/ (min.kg)) | Bioavailability score | AMES toxicity | Max tolerated dose (Log mg/ (kg.d)) | hERG I inhibitor | hERG II inhibitor |
|---|---|---|---|---|---|---|---|
| Caffeic acid | YES | 0.52 | 0.56 | NO | 0.89 | NO | NO |
| Quinic acid | NO | 0.63 | 0.56 | NO | 2.08 | NO | NO |
| Vanillic acid | NO | 0.61 | 0.85 | NO | 1.40 | NO | NO |
| Protocatechuic acid | NO | 0.55 | 0.56 | NO | 1.37 | NO | NO |
| Fenulic acid | YES | 0.61 | 0.85 | NO | 1.44 | NO | NO |
| Luteolin | YES | 0.60 | 0.55 | NO | 0.55 | NO | NO |
| Lenvatinib mesylate | YES | 0.21 | 0.17 | NO | 0.42 | NO | YES |

**Table 3: Continued**

| ADMET Properties | Acute oral rat toxicity, LD50(mol/kg) | Oral rat chronic toxicity (Log mg/kg bw/day) | Hepatotoxicity | Skin sensitisation | *T. Pyriformis* toxicity (Log µg/L) | Minnow toxicity (Log mmol/L) | Lipinski's rule violations |
|---|---|---|---|---|---|---|---|
| Caffeic acid | 2.22 | 1.847 | NO | NO | 0.135 | 2.33 | YES (0) |
| Quinic acid | 1.28 | 3.481 | NO | NO | 0.285 | 4.37 | YES (0) |
| Vanillic acid | 2.20 | 1.982 | NO | NO | 0.158 | 2.14 | YES (0) |
| Protocatechuic acid | 2.18 | 1.95 | NO | NO | 0.267 | 2.53 | YES (0) |
| Fenulic acid | 2.32 | 1.79 | NO | NO | 0.255 | 2.07 | YES (0) |
| Luteolin | 2.37 | 1.67 | NO | NO | 0.459 | 1.51 | YES (0) |
| Lenvatinib mesylate | 2.22 | 1.7 | YES | NO | 0.309 | -0.005 | NO (2) |

| Standard Drug | Drugs to be considered |
|---|---|
| **Fig. 2: Docking scores and binding interaction of lenvatinib mesylate (PDB ID: 3A99)** | **Fig. 3: Docking scores and binding interaction for anticancer activity (PDB ID: 3A99)** |
|  |  |
| The ligand is shown in line and stick representation along with its 2D diagram and hydrogen bond interaction. | The ligand is shown in line and stick representation along with its 2D diagram and hydrogen bond interaction. |

**Combine Boiled Egg Diagram**

**Fig. 4: Combined boiled egg diagram of all phytoconstituents with standard.**

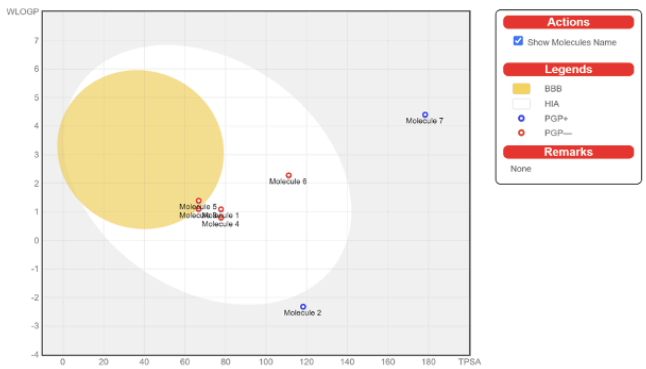



**Table 4: Molecule names in boiled egg diagram**

| Molecule No. | Drug Name |
|---|---|
| 1 | Caffeic acid |
| 2 | Quinic acid |
| 3 | Vanillic acid |
| 4 | Protocatechuic acid |
| 5 | Fenulic acid |
| 6 | Luteolin |
| 7 | Lenvatinib mesylate |

**BOILED** means **B**rain **O**r**I**ntestina**LE**stimate**D** permeation predictive model.

The boiled egg diagram shows two regions white and yellow.

The white region is the physicochemical space of molecules with highest probability of being absorbed by the gastrointestinal tract, and the yellow region (yolk) is the physicochemical space of molecules with highest probability to permeate to the brain.

In addition, the points are coloured in blue if predicted as actively effluxed by P-gp(PGP+) and in red if predicted as non-substrate of P-gp(PGP-).

Previously, flow cytometry and caspase-3 immunostaining were used to demonstrate that *Phyllanthus niruri* extracts significantly inhibited human hepatocellular carcinoma cells (HepG2, Huh-7), colorectal carcinoma cells (Ht29), and keratinocytes (HaCaT). These findings suggest that the spray-dried extract of *Phyllanthus niruri* is protective of normal cells while selectively harmful to cancer cell lines. (23)

*Phyllanthus niruri* extract was also tested for its impact on hospitalised colorectal cancer patients as well as its impact on cell growth by assessing granzyme expression. On patients with colorectal cancer, *Phyllanthus niruri* extract boosted granzyme expression, pointing to its potential as an anticancer drug.(24)

This research backs up our in-silico research that suggests luteolin and fenuric acid, which have the lowest binding energies (-8.3 kcal/mol and -6.2 kcal/mol, respectively) in complex with PIM 1 kinase, may be useful in the therapy of cancer. However, regardless of the type of phytoconstituents and cancer mediators involved, the previously reported action is consistent with the extract's overall activity. Thus, it is clear from our work that the screened phytoconstituents had better interactions with the conserved catalytic residues, higher docking scores, and stronger binding energies, which resulted in the inhibition or blocking of the PIM 1 kinase in cancer. Our research thus offers convincing evidence that, among phytoconstituents, luteolin and fenuric acid have the ability to treat cancer by specifically targeting PIM 1 kinases.

## Conclusion

The serine/threonine kinase PIM-1 plays an important role in cell cycle progression and apoptosis inhibition, resulting in prostate tumorigenesis. Therefore, PIM-1 inhibition has been expected to be an attractive target for developing new anti-cancer drugs.In this study, we have carried out an *in-silico* screening of the phytoconstituents of *Phyllanthus niruri*. This study demonstrates that six compounds from selected phytoconstituents showed docking results from -8.3 to -5.9 kcal/mol. Among all, luteolin gave the lowest binding energy (-8.3 kcal/mol) with 3A99 macromolecule, whereas the reference compound, lenvatinib mesylate showing a docking score with a binding energy -4.8 kcal/mol.

## References

1. Calixto JB et al. A review of the plants of the genus Phyllanthus: their chemistry, pharmacology, and therapeuticpotential. Med Res Rev 1998;18: 225–258.
2. Burkill IH et al. A dictionary of the economic products of the Malay peninsula. Kuala Lumpur, Malaysia: Published on behalf of the governments of Malaysia and Singapore by the Ministry of Agriculture andcooperatives; 1966.
3. Chopra RN et al. Glosssary of Indian medicinal plants. Ranchi: CatholicPress, 1986.
4. Dhar ML et al. Screening of Indian plants for biological activity: I. Indian J Exp Biol 1968; 6: 232–247.
5. Nadkarmi NK. India Materia Medica. Bombay: Popular Prakashan Private Ltd., 1993.
6. Venkateswaran PS et al. Effects of an extract from Phyllanthus niruri on hepatitis B and woodchuck hepatitis viruses: in vitro and in vivo studies. Proc Natl Acad Sci USA 1987; 84: 274–278.
7. Row LR et al. New lignands from Phyllanthus niruri Linn. Tetrahedron Lett 1964; 5: 1557–1567.
8. Wei W et al. Lignans with antihepatitis B virus activities from Phyllanthus niruri L. Phytother Res: PTR 2012; 26: 964–968.
9. Murugaiyah V, Chan KL. Analysisof lignans from PhyllanthusniruriL. in plasma using a simple HPLCmethod with fluorescence detectionand its application in a pharmacokineticstudy. J ChromatogrBAnalytTechnol Biomed Life Sci2007; 852: 138–144.
10. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. Journal of computational chemistry. 2009 Dec; 30(16): 2785-91.
11. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE. UCSF Chimera—a visualization system for exploratory research and analysis. Journal of computational chemistry. 2004 Oct;25(13):1605-12.
12. Verdonk ML, Cole JC, Hartshorn MJ, Murray CW, Taylor RD. Improved protein–ligand docking using GOLD. Proteins: Structure, Function, and Bioinformatics. 2003 Sep;52(4):609-23.
13. Leach AR, Shoichet BK, Peishoff CE. Prediction of protein− ligand interactions. Docking and scoring: successes and gaps. Journal of medicinal chemistry. 2006 Oct 5;49(20):5851-5.
14. BIOVIA DS. Discovery studio modeling environment, release 2017, San Diego: DassaultSystèmes, 2016. Available from:(Accessed 1 September 2016). 2016 Sep.
15. Arora S, Lohiya G, Moharir K, Shah S, Yende S. Identification of potential flavonoid inhibitors of the SARS-CoV-2 main protease 6YNQ: a molecular docking study. Digital Chinese Medicine. 2020 Dec 1;3(4):239-48.
16. Shah S, Chaple D, Arora S, Yende S, Moharir K, Lohiya G. Exploring the active constituents of Oroxylum indicum in intervention of novel coronavirus (COVID-19) based on molecular docking method. Network Modeling Analysis in Health Informatics and Bioinformatics. 2021 Dec;10(1):1-2.

17. Dhanasekaran SM, Barrette TR, Ghosh D, Shah R, Varambally S, Kurachi K, Pienta KJ, Rubin MA, and Chinnaiyan AM. (2001) Nature 412, 822–826.
18. Macdonald A, Campbell DG, Toth R, McLauchlan H, Hastie CJ, and Arthur JS. (2006) BMC Cell. Biol. 7, 1
19. Yende SR, Shah SK, Arora SK, Moharir KS, Lohiya GK. In silico prediction of phytoconstituents from Ehretialaevis targeting TNF-α in arthritis. Digital Chinese Medicine. 2021 Oct 1;4(3):180-90.
20. Kaloni D, Chakraborty D, Tiwari A, Biswas S. In silico studies on the phytochemical components of Murrayakoenigii targeting TNF-α in rheumatoid arthritis. Journal of Herbal Medicine. 2020 Dec 1;24:100396.
21. Joshi T, Sharma P, Joshi T, Chandra S. In silico screening of anti-inflammatory compounds from Lichen by targeting cyclooxygenase-2. Journal of Biomolecular Structure and Dynamics. 2020 Aug 12;38(12):3544-62.
22. Nisha CM, Kumar A, Vimal A, Bai BM, Pal D, Kumar A. Docking and ADMET prediction of few GSK-3 inhibitors divulges 6-bromoindirubin-3-oxime as a potential inhibitor. Journal of Molecular Graphics and modelling. 2016 Apr 1;65:100-7.
23. De Araújo Júnior RF, de Souza TP, Pires JG, Soares LA, de Araujo AA, Petrovick PR, Mâcedo HD, de Sá Leitão Oliveira AL, Guerra GC. A dry extract of Phyllanthus niruri protects normal cells and induces apoptosis in human liver carcinoma cells. Experimental biology and medicine. 2012 Nov;237(11):1281-8.DOI: 10.1258/ebm.2012.012130.
24. Sayuti M, Riwanto I, Boediono BP, Akbar TI. Anticancer Activity of Phyllanthus Niruri Linn Extract in Colorectal Cancer Patients: A phase II Clinical Trial. Studies. 2020 Oct 1;10:17.

*****