GMR
The Original

COMPUTATIONAL TARGET-BASED SCREENING OF *VINCA MINOR* PHYTOCHEMICALS FOR URINARY BLADDER CARCINOMA USING NETWORK PHARMACOLOGY AND MOLECULAR DOCKING

Aswathi K Biju[1], Keerthana Perumal[2], K Hema Shree[3*], Parameswari R P[4]

[1]Department of Bioinformatics, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai-602105, India. Email: aswathikb04@gmail.com Orcid Id: 0009-0003-5426-3471
[2]Department of Microbiology, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai-602105, India. Email: Keersan24@gmail.com Orcid Id: 0009-0009-3768-0592
[3]Department of Pathology, Saveetha Institute of Basic Medical Sciences, Saveetha Institute of Medical and Technical Sciences, Chennai-602105, India. Email: hemashree9111990@gmail.com Orcid Id: 0000-0001-9616-6539
[4]Molecular Biochemistry Lab, Saveetha College of Physiotherapy, Saveetha Institute of Medical and Technical Sciences, Chennai-602105, India. Email: paramsarathy@gmail.com Orcid Id: 0000-0003-3167-2873

ABSTRACT

Background: Urinary bladder carcinoma (UBC) is a common and recurrence-prone malignancy for which current therapeutic options remain constrained by resistance and toxicity. Vinca minor (lesser periwinkle) is a rich source of monoterpenoid indole alkaloids structurally related to clinically established Vinca alkaloid chemotherapeutics, yet its multi-target anticancer potential against UBC has not previously been characterised.

Methods: Protein targets of five *V. minor* phytochemicals (Vincamine, Apovincamine, Vinburnine, Vincadifformine and Vincarubine) were predicted using SwissTargetPrediction and intersected with UBC-associated genes retrieved from GeneCards. The overlapping targets were mapped onto a STRING protein-protein interaction network, analysed in Cytoscape using the CytoHubba MCC algorithm to identify hub genes, and subjected to Gene Ontology and KEGG pathway enrichment via ShinyGO. The leading hub gene was validated by molecular docking with its corresponding phytochemical using AutoDock 4.2.6.

Results: Of 271 predicted phytochemical targets, 110 overlapped with 2,534 UBC-associated genes. The resulting network comprised 110 nodes and 961 edges (average degree 17.5; enrichment $p < 1.0 \times 10^{-16}$). MTOR, HIF1A, CASP3, MDM2, ERBB2, MMP9, IGF1R, AKT1, SRC and MMP2 emerged as the top-ranked hub genes. KEGG analysis showed significant enrichment of the Bladder cancer pathway among other cancer-related pathways. Docking of Vincarubine against MTOR (PDB: 8ERA) yielded a binding affinity of −9.4 kcal/mol and ligand efficiency of −0.32 kcal/mol, stabilised by two hydrogen bonds, three carbon-hydrogen bonds and one hydrophobic contact.

Conclusion: This integrated in silico analysis identifies a biologically coherent, MTOR-centred multi-target mechanism underlying the potential activity of *V. minor* against urinary bladder carcinoma and nominates Vincarubine as a mechanistically grounded candidate for further experimental validation.

KEYWORDS: *Vinca minor*, urinary bladder carcinoma, network pharmacology, molecular docking, vincarubine, mTOR, Healthy Lives, Well-being, Quality Care.

1. INTRODUCTION

Urinary bladder carcinoma (UBC) remains one of the most burdensome malignancies of the genitourinary tract worldwide. According to the most recent iteration of the Global Burden of Disease study, the global incidence of bladder cancer very nearly doubled between 1990 and 2021, and its overall disease burden continues to rise despite a gradual decline in age-standardised rates.1 Men are affected roughly three to four times more frequently than women, and the disease is characterised by a pronounced tendency towards recurrence and progression, which necessitates lifelong surveillance and imposes one of the highest per-patient lifetime treatment costs of any solid tumour.1 Established risk factors include tobacco smoking, occupational exposure to aromatic amines, and chronic bladder inflammation, although a considerable proportion of cases still arise without an identifiable exposure.2 The burden is also unevenly distributed geographically and socioeconomically, with a disproportionate share concentrated in countries of higher sociodemographic development, a pattern that reflects both differential exposure to risk factors and variation in diagnostic intensity.1

Clinically, the majority of newly diagnosed tumours are non-muscle-invasive at presentation and are managed by transurethral resection followed by intravesical instillation of Bacillus Calmette-Guerin (BCG), which remains the mainstay of adjuvant therapy. However, up to one-third of patients fail to respond, or subsequently relapse, and current second-line options, including systemic chemotherapy, immune checkpoint inhibition, and emerging gene- and antibody-based therapies, are constrained by moderate efficacy and considerable toxicity.3 In muscle-invasive disease, neoadjuvant platinum-based chemotherapy followed by radical cystectomy remains standard, yet a substantial proportion of patients

are cisplatin-ineligible or develop chemoresistant disease, further narrowing the available options.3 These shortcomings, together with the pronounced molecular heterogeneity of UBC, underline a continuing need for therapeutic strategies capable of engaging multiple oncogenic pathways simultaneously rather than a single, easily circumvented target.

Natural products have long occupied a central position in oncology drug discovery, underpinning a substantial share of currently prescribed anticancer agents, and modern cheminformatic and network-based approaches have considerably accelerated the pace at which their multi-target pharmacology can be characterised computationally, ahead of costly wet-laboratory validation.4 Among plant-derived agents, the Vinca alkaloids constitute one of the most clinically consequential families in cancer chemotherapy: vincristine, vinblastine, vindesine and vinorelbine remain indispensable components of contemporary regimens for haematological and solid malignancies, and structurally related monoterpenoid indole alkaloids, including vincamine, apovincamine, vinburnine, vincadifformine and vincarubine, are characteristic phytoconstituents of Vinca minor (lesser periwinkle), a species long employed in traditional European herbal medicine for its vasoactive and nootropic properties.5 Despite the pharmacological prominence of this alkaloid family, the great majority of mechanistic and computational investigation has concentrated on the clinically established compounds, leaving the anticancer target repertoire of the less-studied *V. minor* alkaloids, and their relevance to urothelial malignancy specifically, largely uncharacterised.

Network pharmacology offers a conceptually appropriate framework within which to address this gap, as it moves beyond the classical "one drug, one target" paradigm to map the intersection between a phytochemical's predicted protein targets and the gene set implicated in a given disease, thereby revealing candidate hub proteins and the biological pathways they govern.6 When complemented by molecular docking, which provides an atomic-resolution estimate of ligand-protein binding geometry and affinity, this integrated in silico pipeline yields testable, mechanistically grounded hypotheses capable of guiding targeted experimental validation, and has been used productively across a range of medicinal-plant-cancer pairings.4 In practice, this involves the successive intersection of predicted phytochemical targets with disease-associated gene sets, the construction and topological analysis of the resulting protein-protein interaction network to identify hub proteins of high connectivity, and the enrichment of these hubs against Gene Ontology and pathway databases, before the most biologically plausible ligand-target pairing is subjected to docking.6 Applying such an approach to *V. minor* is particularly well motivated given the structural kinship between its alkaloids and the clinically validated Vinca alkaloids already established in oncological practice. Healthy Lives, Well-being, and Quality Care are fundamental to achieving Good Health and Well-being.

To date, however, no study has systematically examined the multi-target anticancer potential of *V. minor* phytochemicals against UBC, despite the biological plausibility afforded by the pharmacology of related Vinca alkaloids. The present investigation therefore integrates phytochemical-target prediction, disease-gene mapping, protein-protein interaction network analysis, functional enrichment analysis and molecular docking in order to (i) identify the common therapeutic targets shared between *V. minor* constituents and UBC-associated genes, (ii) determine the hub proteins and signalling pathways through which these targets converge, and (iii) computationally validate the binding interaction between the leading hub protein and its corresponding phytochemical, so as to furnish a mechanistic rationale for the further preclinical evaluation of *V. minor* in bladder cancer.

2. MATERIALS AND METHODS

2.1 Prediction of Target Molecules for Phytochemicals of *V. minor*

The phytochemical constituents of *V. minor* were retrieved from the Indian Medicinal Plants, Phytochemistry and Therapeutics (IMPPAT) database (https://cb.imsc.res.in/imppat/).7,8 The canonical SMILES notation for each molecule was extracted from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/)9 and submitted for target prediction via the SwissTargetPrediction server (http://www.swisstargetprediction.ch/),10 which infers probable human protein targets on the basis of chemical similarity to experimentally validated ligands. Predicted targets meeting the platform's confidence threshold (probability score > 0.1) were retained, and duplicate entries across the five phytochemicals were removed to yield a single, non-redundant set of candidate target proteins for downstream analysis.

2.2 Identification of Gene Targets Related to UBC

Genes associated with urinary bladder carcinoma were obtained from the GeneCards database (https://www.genecards.org/),11 a comprehensive, integrated repository of human gene annotation, using the search term “Urinary Bladder Cancer”. To ensure the biological relevance of the resulting gene set, only entries with a GeneCards relevance score of 10 or above were retained for subsequent analysis.

2.3 Identification of Common Therapeutic Targets

The overlap between the predicted phytochemical targets and the UBC-associated gene set was determined using the Venny 2.1.0 web tool (https://bioinfogp.cnb.csic.es/tools/venny/),12 which compares gene lists via Venn diagram analysis. The resulting intersection was taken to represent the candidate molecular targets through which *V. minor* may exert biological activity against UBC, and was carried forward for network and enrichment analysis.

2.4 Construction of the Protein-Protein Interaction Network

The common target genes were submitted to the STRING database (https://string-db.org/)13 to construct a protein-protein interaction (PPI) network, with Homo sapiens specified as the reference organism and a minimum required interaction confidence score of 0.400. Full network mode was selected so as to display both predicted and experimentally validated interactions.

2.5 Identification of Hub Genes and Network Construction

The PPI network exported from STRING was imported into Cytoscape (version 3.10.3)14 for topological analysis. Hub genes were identified using the CytoHubba plugin,15 applying the Maximal Clique Centrality (MCC) algorithm, whereby nodes of higher connectivity were assigned a correspondingly higher rank. A separate phytochemical-target interaction network was also constructed in Cytoscape,14 in which phytochemicals and their predicted protein targets were represented as distinctly shaped and coloured nodes connected by edges denoting a predicted interaction; this network was used to identify which of the five phytochemicals were predicted to engage each hub target.

2.6 Gene Ontology and KEGG Pathway Enrichment Analysis

To characterise the biological significance of the common targets, Gene Ontology (GO) enrichment analysis was performed using the ShinyGO 0.85 platform (https://bioinformatics.sdstate.edu/go/),16 with enriched terms classified into Biological Process (BP), Cellular Component (CC) and Molecular Function (MF) categories. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was subsequently performed to identify signalling pathways significantly associated with the target set, applying a false discovery rate (FDR) threshold of ≤ 0.05.

2.7 Molecular Docking Analysis

Among the hub genes identified by CytoHubba,15 MTOR occupied the highest-ranking position and was accordingly selected as the primary target for structure-based validation. Cross-referencing of the phytochemical-target interaction network identified Vincarubine as the corresponding *V. minor* phytochemical predicted to engage MTOR, and this ligand-target pairing was therefore taken forward for molecular docking. The three-dimensional crystal structure of MTOR (PDB ID: 8ERA) was retrieved from the RCSB Protein Data Bank (https://www.rcsb.org/),17 and the structure of Vincarubine was obtained from PubChem.9 Docking was performed using AutoDock 4.2.6,18 which estimates the most energetically favourable binding conformation(s) of the ligand within the target binding site and reports the corresponding binding free energy. The resulting protein-ligand complex was analysed for intermolecular interactions, including hydrogen bonding, carbon-hydrogen bonding and hydrophobic contacts, using BIOVIA Discovery Studio Visualizer.19

3. RESULTS

The overall analytical workflow, from phytochemical and disease-gene retrieval through to network construction, functional enrichment and molecular docking, is summarised in Figure 1.

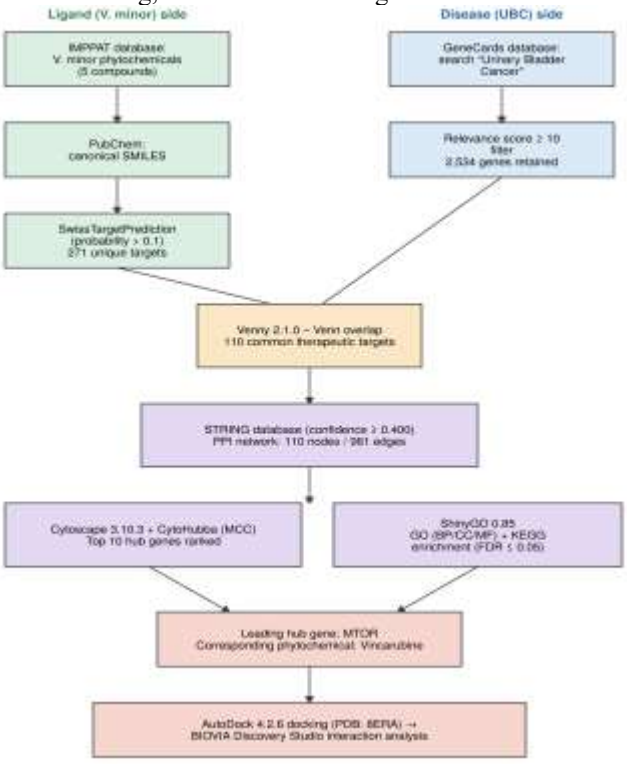

Figure 1. Overview of the study workflow, showing the successive stages of target prediction, disease-gene mapping, network construction, functional enrichment and molecular docking.

3.1 Prediction of Potential Therapeutic Targets

In total, five phytochemicals of *V. minor*, namely Vincamine, Apovincamine, Vinburnine, Vincadifformine and Vincarubine, were screened for potential therapeutic targets using SwissTargetPrediction with a probability score threshold of > 0.1. Following removal of duplicate entries, 271 unique human protein targets were retained. In parallel, 2,534 UBC-associated genes with a GeneCards relevance score of ≥ 10 were extracted. Comparison of the two sets by Venn diagram analysis identified 110 common targets (Figure 2), which were taken forward as the candidate molecular

targets underlying the potential activity of *V. minor* against UBC. Figure 3 summarises the successive reduction from the initial target and disease-gene sets to this final overlapping set.

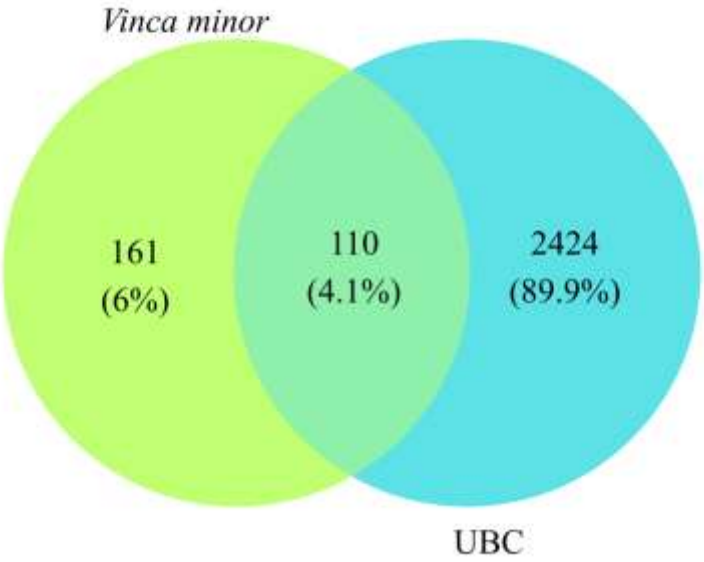

Figure 2. Venn diagram showing the common targets between predicted targets of *V. minor* phytochemicals and UBC-associated genes.

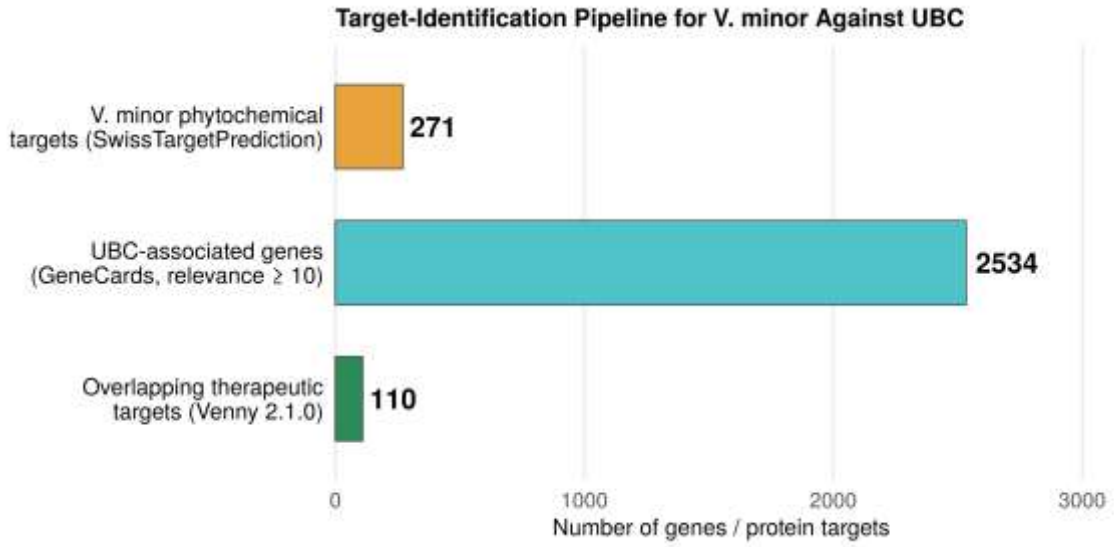

Figure 3. Target-identification pipeline showing the number of genes or protein targets retained at each successive stage of the analysis.

3.2 Analysis of Protein-Protein Interaction Networks

The 110 common targets were submitted to the STRING database to characterise their interactions, applying the minimum interaction confidence threshold of 0.400 specified in Section 2.4. The resulting protein-protein interaction (PPI) network comprised 110 nodes connected by 961 edges (Figure 4), with an average node degree of 17.5 and a highly significant PPI enrichment p-value of $< 1.0 \times 10^{-16}$ (Table 2), indicating substantially more interaction than would be expected for a random gene set of equivalent size. Hub-gene analysis using the CytoHubba plugin (MCC algorithm) within Cytoscape ranked MTOR, HIF1A, CASP3, MDM2, ERBB2, MMP9, IGF1R, AKT1, SRC and MMP2 as the ten most highly connected nodes, in descending order of connectivity (Figure 5, Figure 6). MTOR attained the highest MCC value of the set and was accordingly selected as the primary target for molecular docking. A plant-phytochemical-target-disease interaction network was additionally constructed in Cytoscape to visualise the relationships linking *V. minor*, its constituent phytochemicals, the common target genes and UBC as the associated disease (Figure 7).

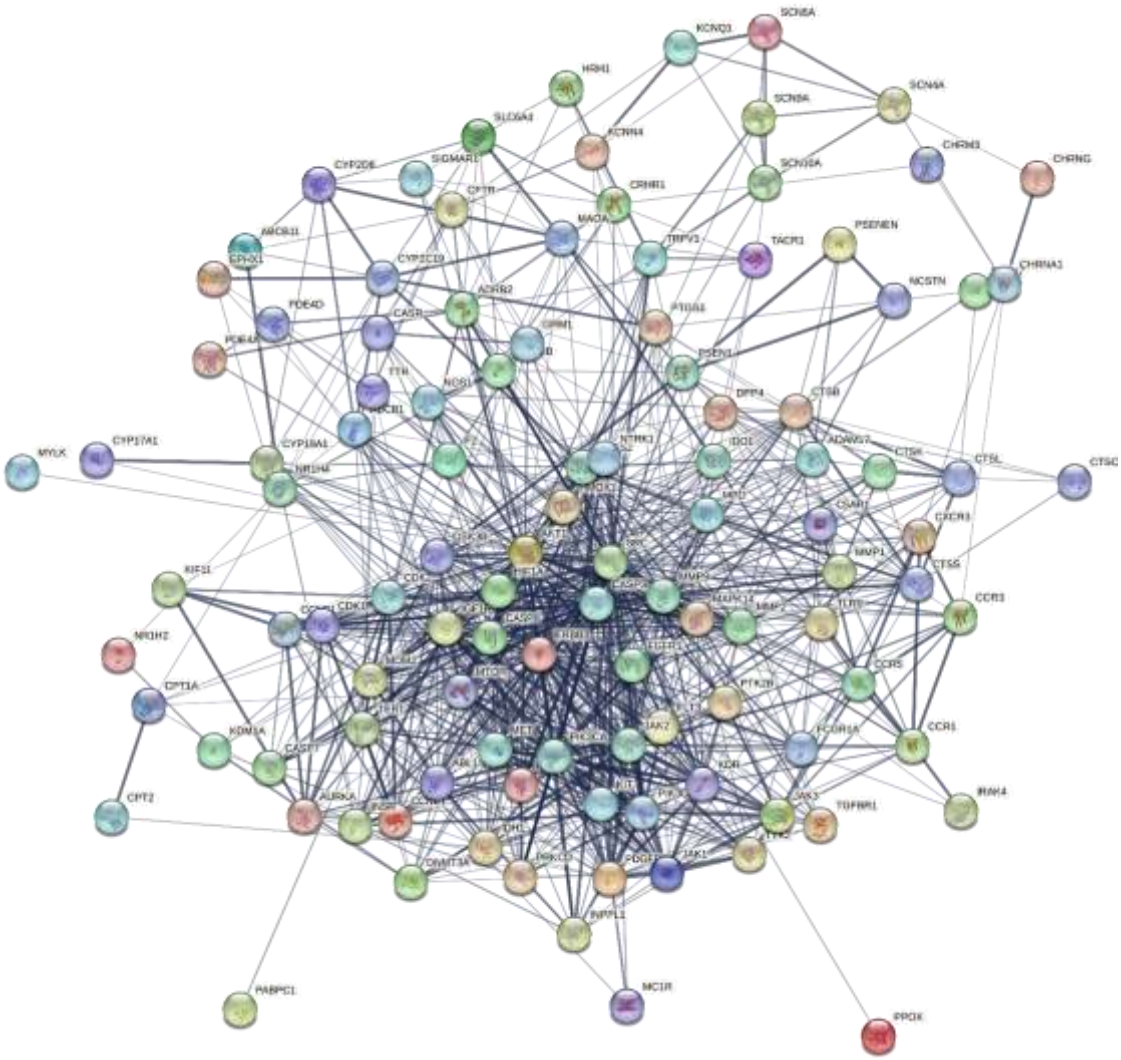

Figure 4. Protein-protein interaction network of the 110 overlapping targets, generated using STRING.

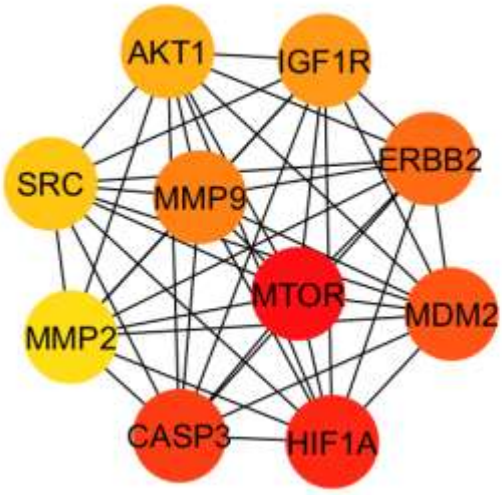

Figure 5. The ten top-ranked hub genes identified by the MCC algorithm within the CytoHubba plugin.

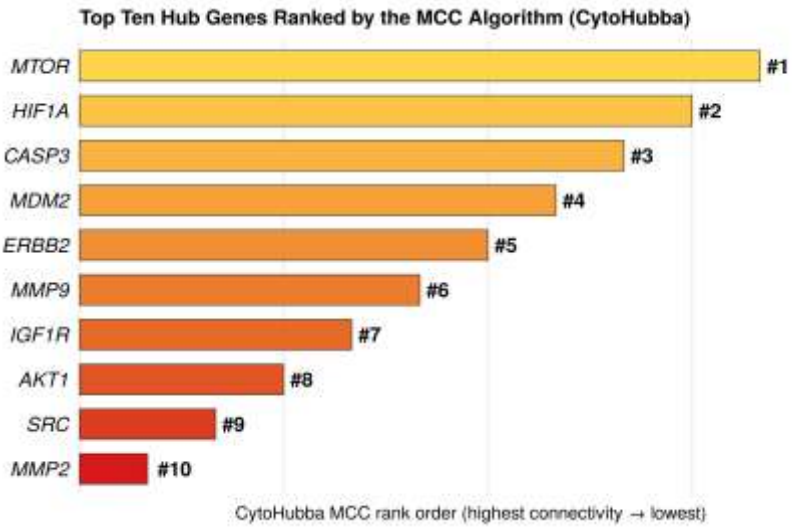

Figure 6. Rank order of the ten hub genes shown in Figure 5, illustrating their relative connectivity as determined by the MCC algorithm.

Table 1. Summary of protein-protein interaction network topology and molecular docking parameters.

Parameter	**Value**
Common therapeutic targets (network nodes)	110
PPI network edges	961
Average node degree	17.5
PPI enrichment p-value	$< 1.0 \times 10^{-16}$
Top-ranked hub gene (CytoHubba / MCC)	MTOR
GO / KEGG enrichment FDR threshold	≤ 0.05
Docking target (PDB ID)	MTOR (8ERA)
Docking ligand	Vincarubine
Binding affinity	−9.4 kcal/mol
Ligand efficiency	−0.32 kcal/mol
Hydrogen bonds	2
Carbon-hydrogen bonds	3
Hydrophobic contacts	1

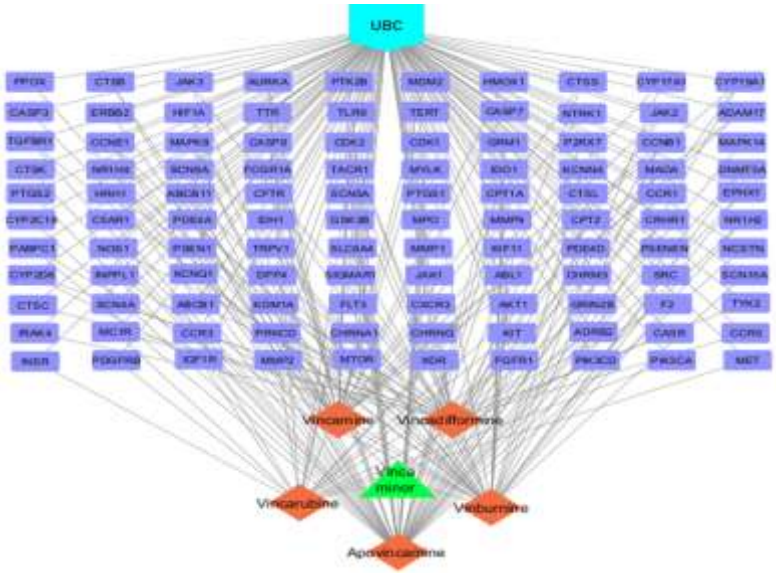

Figure 7. Plant-phytochemical-target-disease interaction network constructed in Cytoscape. Nodes are coloured green, orange, blue and sky-blue to denote *V. minor*, its phytochemicals, target proteins and the associated disease, respectively, with edges representing the corresponding interactions.

3.3 Functional Enrichment Analysis

Gene Ontology and KEGG pathway enrichment analyses were performed using ShinyGO to determine the biological relevance of the identified hub genes. GO enrichment grouped the target genes into Biological Process (BP), Cellular

Component (CC) and Molecular Function (MF) categories (Figure 8), in which bar length reflects fold enrichment and colour intensity reflects the statistical significance of enrichment, expressed as −log10(FDR). The most significantly enriched BP term was positive regulation of smooth muscle cell proliferation; caveola was the most significantly enriched CC term; and insulin receptor binding was the most significantly enriched MF term.

KEGG pathway analysis identified significant enrichment (FDR ≤ 0.05) of several cancer-relevant signalling pathways, including Bladder cancer, Endocrine resistance, EGFR tyrosine kinase inhibitor resistance, Prostate cancer, Platinum drug resistance, Proteoglycans in cancer, the HIF-1 signalling pathway, the Thyroid hormone signalling pathway, Kaposi sarcoma-associated herpesvirus infection, and the generic Pathways in cancer term. Grouping these ten terms thematically (Figure 9) shows that, alongside the Bladder cancer pathway itself, the largest categories relate to generic or cross-tumour cancer signalling and to resistance against established anticancer therapies, a pattern consistent with the multi-target, pathway-convergent mode of action generally proposed for network pharmacology-derived phytochemical candidates. Within the Bladder cancer KEGG pathway specifically, the constituent hub genes are highlighted in red (Figure 10). A pathway-target network was additionally constructed in Cytoscape to visualise the relationships between the enriched pathways and their associated hub genes (Figure 11).

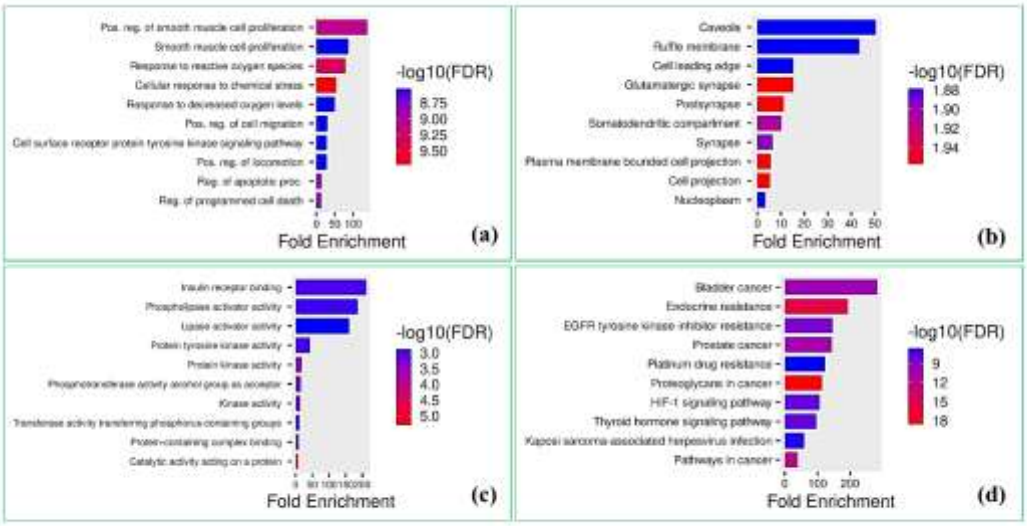

Figure 8. Gene Ontology (GO) and KEGG pathway enrichment analysis of the hub genes: (a) Biological Process, (b) Cellular Component, (c) Molecular Function, and (d) KEGG pathways.

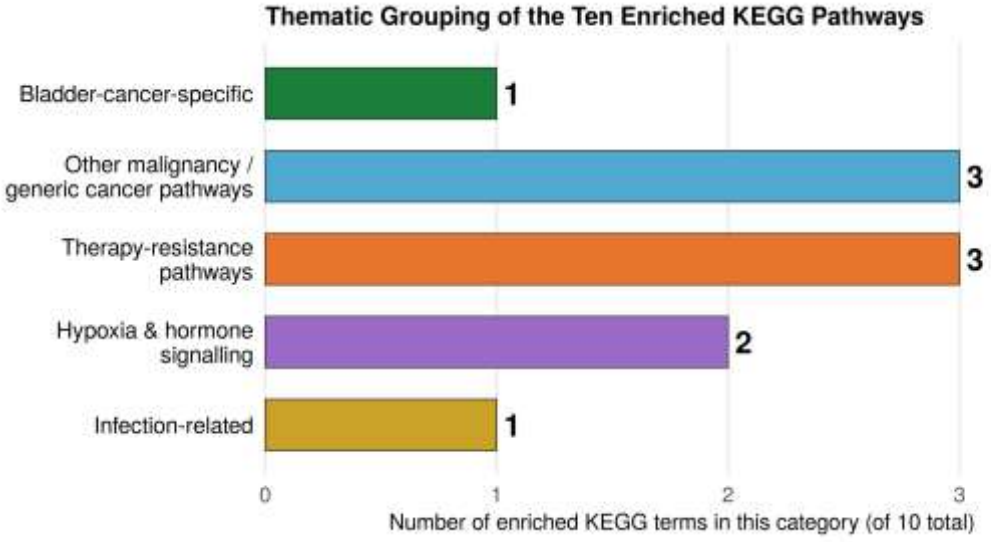

Figure 9. Thematic grouping of the ten KEGG pathways enriched among the hub-gene target set (categorisation of the pathways shown in Figure 8d).

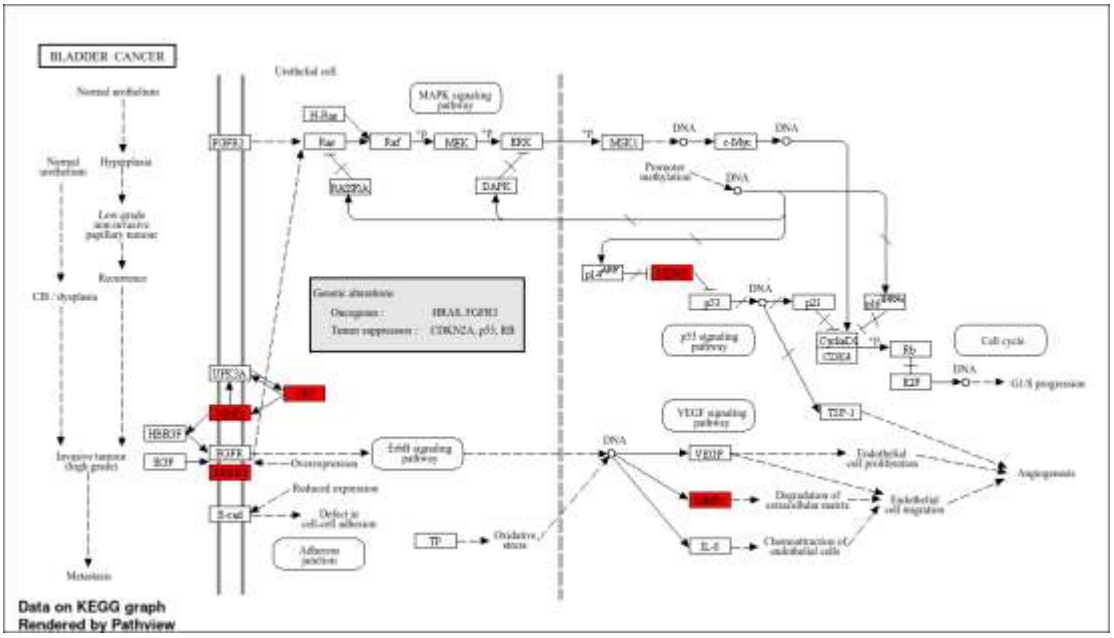

Figure 10. Bladder cancer KEGG pathway diagram, with hub genes involved in the pathway highlighted in red.

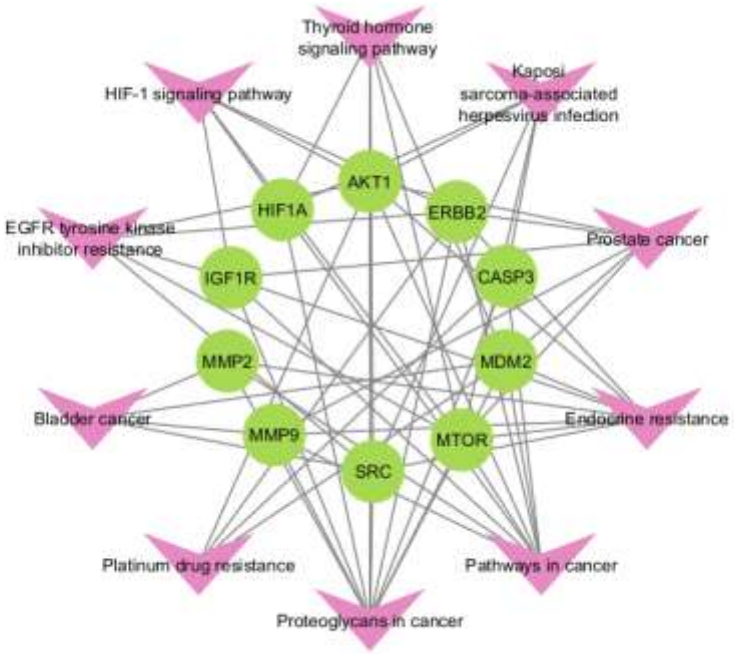

Figure 11. Pathway-target network generated in Cytoscape, in which green nodes represent target genes, pink nodes represent enriched pathways, and edges represent the interaction between a given pathway and its associated targets.

3.4 Molecular Docking Analysis

To evaluate the interaction between the leading hub gene and its corresponding phytochemical, molecular docking was performed between MTOR (PDB ID: 8ERA) and Vincarubine using AutoDock 4.2.6. Vincarubine exhibited a binding affinity of −9.4 kcal/mol and a ligand efficiency of −0.32 kcal/mol (Figure 12), a magnitude generally regarded in the docking literature as indicative of a favourable, energetically efficient protein-ligand interaction. Analysis of the docked complex using BIOVIA Discovery Studio Visualizer identified six intermolecular interactions underlying this binding (Table 1, Figure 13, Figure 14). Vincarubine formed two canonical hydrogen bonds, with Gln1937 (bond distance 2.46 Å) and Glu2196 (bond distance 1.96 Å), together with carbon-hydrogen bond interactions involving Leu1936 (3.54 Å), Gln1937 (3.78 Å) and Thr2207 (3.63 Å), and a hydrophobic contact with Ala1971 (4.13 Å). Taken together, this hydrogen-bonding and hydrophobic interaction profile is consistent with stable accommodation of Vincarubine within the MTOR binding pocket, supporting its candidacy as an MTOR-directed ligand warranting further investigation.

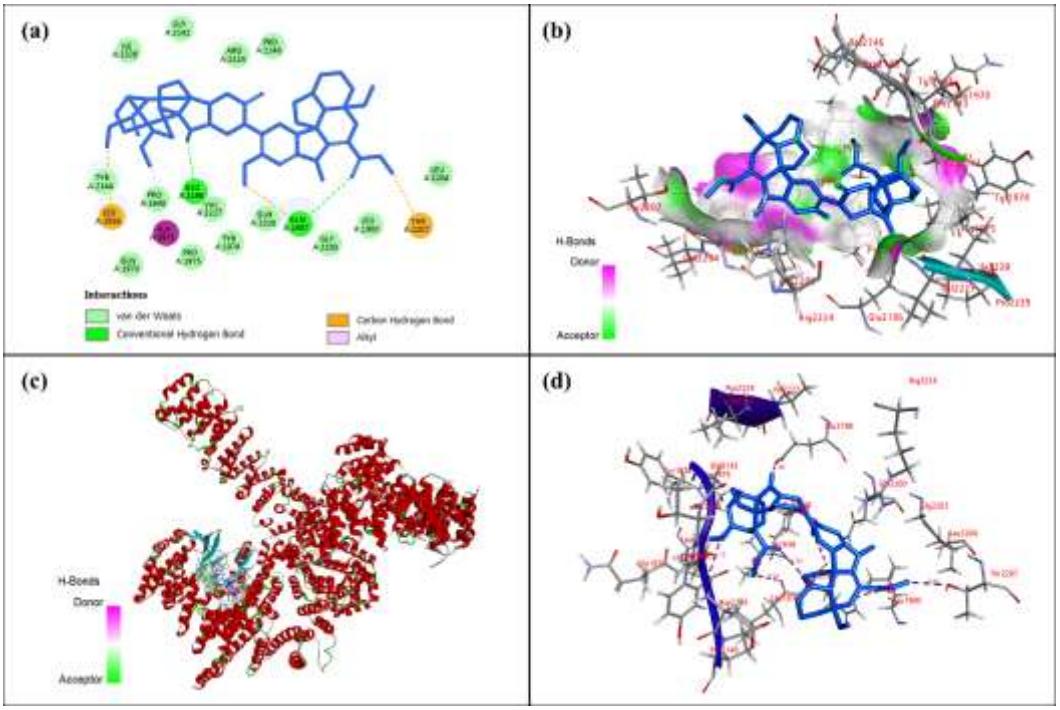

Figure 12. Molecular docking analysis of MTOR with Vincarubine, showing (a) the two-dimensional interaction map, (b) hydrogen bond donor and acceptor sites surrounding the bound ligand, (c) the three-dimensional ribbon representation of the protein-ligand complex, and (d) the amino acid interaction profile with corresponding bond distances.

Table 2. Intermolecular interactions between MTOR and Vincarubine in the docked complex.

Complex	Amino acid residue…ligand atom	Type	Distance (Å)
MTOR–Vincarubine	GLN1937/HE21…LIG/O	Hydrogen bond	2.46
	GLU2196/OE2…LIG/HN	Hydrogen bond	1.96
	LEU1936/O…LIG/C	Carbon-hydrogen bond	3.54
	GLN1937/O…LIG/C	Carbon-hydrogen bond	3.78
	THR2207/OG1…LIG/C	Carbon-hydrogen bond	3.63
	ALA1971…LIG	Hydrophobic	4.13

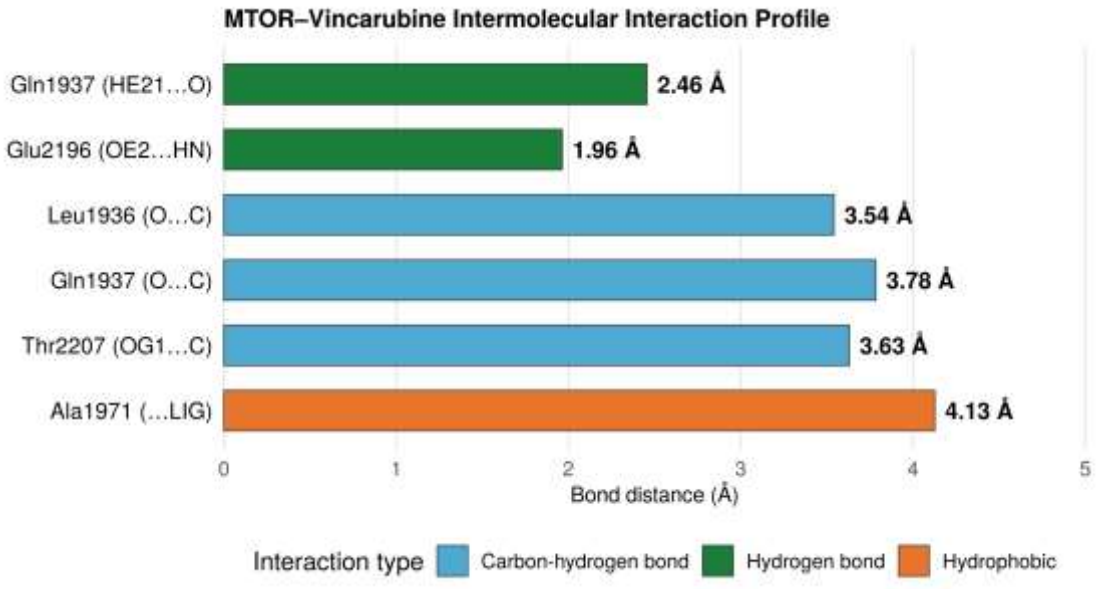

Figure 13. Bond-distance profile of the intermolecular interactions between MTOR and Vincarubine, categorised by interaction type.

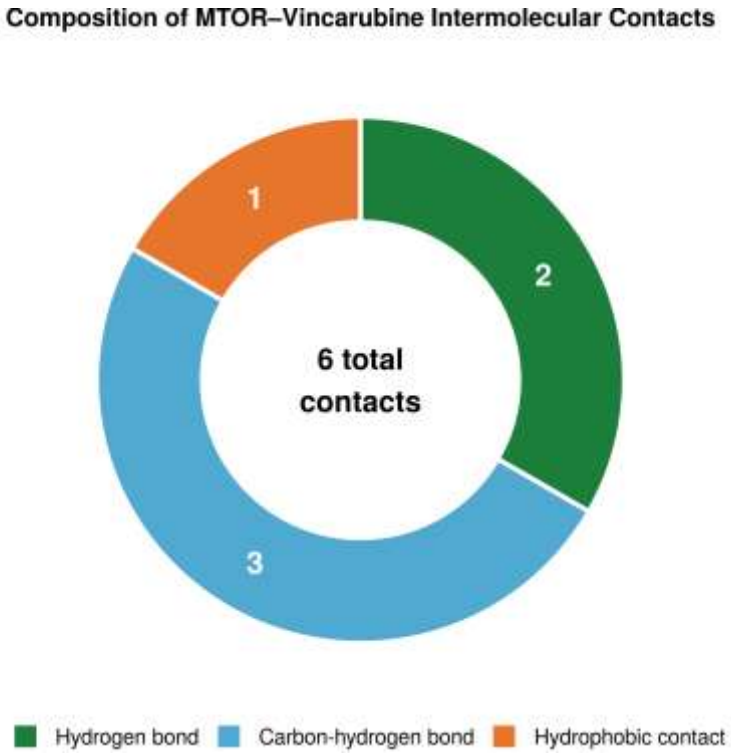

Figure 14. Proportional composition of the six MTOR–Vincarubine intermolecular contacts listed in Table 1, by interaction type.

A brief summary of these hub genes and their established relevance to UBC is presented in Table 3.

Table 3. Established relevance of the ten hub genes to urinary bladder carcinoma.

Hub gene	Rank	Established function	Relevance to UBC
MTOR	1	Serine/threonine kinase; downstream effector of PI3K/AKT signalling	Pathway abnormalities in ~70% of urothelial carcinomas 20
HIF1A	2	Master transcriptional regulator of the hypoxic adaptive response	Stabilisation promotes bladder cancer survival under hypoxia 21
CASP3	3	Principal executioner caspase of the apoptotic cascade	Incorporated into apoptosis-related prognostic gene models 22
MDM2	4	E3 ubiquitin ligase; principal negative regulator of p53	Component of the TP53/RB1 driver-gene axis 23
ERBB2 (HER2)	5	Receptor tyrosine kinase; growth-factor signalling	Positive in up to 68% of urothelial carcinomas; ADC target 24
MMP9	6	Gelatinase; degrades type IV basement-membrane collagen	Elevated in higher-grade, higher-stage tumours 25
IGF1R	7	Receptor tyrosine kinase upstream of PI3K/AKT/mTOR	Implicated in proliferation and chemoresistance 23
AKT1	8	Serine/threonine kinase; core PI3K/AKT/mTOR node	Core driver pathway in precision oncology 23
SRC	9	Non-receptor tyrosine kinase	Drives migration, invasion and metastasis via PD-L2/c-Src/FAK 26
MMP2	10	Gelatinase; degrades type IV basement-membrane collagen	Elevated in higher-grade, higher-stage tumours 25

4. DISCUSSION

This study applied an integrated network pharmacology and molecular docking workflow to characterise, for the first time, the multi-target anticancer potential of *V. minor* phytochemicals against urinary bladder carcinoma. Of the 271 protein targets predicted for the five constituent phytochemicals, 110 (approximately 41%) overlapped with UBC-associated genes, a proportion that compares favourably with equivalent network pharmacology analyses of other

medicinal plants against solid tumours and supports the biological plausibility of a genuine, rather than coincidental, mechanistic relationship between *V. minor* and bladder carcinogenesis.4 The resulting PPI network displayed a markedly non-random topology (961 edges among 110 nodes, average degree 17.5, enrichment $p < 1.0 \times 10^{-16}$), consistent with the expectation that a therapeutically coherent target set should cluster around a small number of densely connected hub proteins rather than being dispersed across functionally unrelated genes.

The identity of the ten hub genes recovered by the MCC algorithm lends considerable biological coherence to this network. MTOR, which occupied the highest-ranking position and was consequently selected for docking, is a serine/threonine kinase situated downstream of PI3K/AKT signalling and is aberrantly activated in as many as 70% of urothelial carcinomas, where it drives proliferation, invasion and resistance to conventional cytotoxic therapy; despite this prominence, mTOR-directed agents have thus far yielded only modest clinical benefit in bladder cancer, underscoring the continued need for novel chemotypes capable of engaging this node.20 HIF1A, ranked second, mediates the hypoxic adaptive response that is increasingly recognised as a driver of bladder cancer proliferation, migration and stemness, with recent work demonstrating that its stabilisation actively promotes tumour survival under hypoxic stress.21 CASP3, the principal executioner caspase of the apoptotic cascade, has been incorporated into apoptosis-related prognostic gene models for bladder cancer, reflecting the clinical relevance of apoptotic dysregulation in this malignancy.22 MDM2 and AKT1 map onto the TP53/RB1 and PI3K/AKT/mTOR axes that are now recognised as core driver pathways in bladder cancer precision oncology.23 ERBB2 (HER2) is an increasingly important biomarker and therapeutic target in urothelial carcinoma, with HER2 positivity reported in up to 68% of tumours when HER2-low disease is included, and with antibody-drug conjugates against this receptor now showing meaningful clinical activity.24 MMP9 and MMP2, the two matrix metalloproteinases in the hub set, degrade basement-membrane collagen and are consistently elevated in higher-grade, higher-stage bladder tumours, where they facilitate local invasion.25 SRC, finally, sits downstream of sphingosine-1-phosphate and PD-L2 signalling in a pathway recently shown to drive bladder cancer migration, invasion and metastasis.26 This convergence of independently curated hub genes onto pathways with well-documented roles in bladder oncogenesis, most directly reflected in the KEGG-level enrichment of the Bladder cancer pathway itself, strengthens confidence that the network derived computationally in this study captures pharmacologically meaningful biology rather than a statistical artefact of the underlying databases. The enrichment of insulin receptor binding as the leading Molecular Function term, together with the involvement of IGF1R among the hub genes, is also noteworthy, as insulin-like growth factor signalling acts immediately upstream of the PI3K/AKT/mTOR axis and has been implicated in bladder cancer proliferation and chemoresistance, providing a further point of convergence with the MTOR-centred mechanism highlighted by the docking analysis.

The docking result itself merits comment. A binding affinity of −9.4 kcal/mol for Vincarubine at the MTOR ATP-binding region is comparable to, or more favourable than, affinities typically reported for small-molecule kinase-directed leads at an early discovery stage, and the observed interaction profile, two canonical hydrogen bonds below 2.5 Å together with supporting carbon-hydrogen and hydrophobic contacts, is consistent with a stable and specific binding mode rather than a weak or non-specific association. This is mechanistically plausible given that Vincarubine shares the pentacyclic indole alkaloid scaffold common to clinically validated Vinca alkaloids, several of which are already established components of cytotoxic chemotherapy, and that a related family member, vincamine, has long been used clinically for its vasoactive and neuroprotective properties, illustrating the pharmacological versatility of this alkaloid class.5 Nonetheless, docking scores are estimates rather than direct measurements of binding, and recent methodological appraisals have highlighted that molecular docking predictions, particularly where the docking search space or scoring function is not rigorously specified, can diverge from experimentally determined poses and affinities; such predictions should therefore be regarded as hypothesis-generating rather than confirmatory.27,28

This study has several limitations that should be acknowledged. First, target prediction via chemical similarity, disease-gene curation via literature-derived relevance scores, and PPI inference via computational confidence thresholds are all subject to the coverage and quality constraints of their respective source databases, and may consequently omit genuine targets or include false positives. Second, the docking analysis was restricted to a single ligand-target pairing identified from the network; it does not exclude the possibility that other *V. minor* phytochemicals engage MTOR, or the remaining hub proteins, with comparable or superior affinity. Third, and most importantly, the entire analysis is computational in nature: no cell-based, biochemical or in vivo evidence is presented here, and the binding interaction, network topology and pathway enrichment reported should accordingly be interpreted as testable mechanistic hypotheses rather than demonstrated pharmacological effects.27,28. Computational approaches such as network pharmacology and molecular docking have emerged as valuable strategies in cancer research for exploring complex molecular interactions, identifying potential therapeutic targets, and elucidating the mechanisms underlying disease progression. The integration of these complementary approaches enables systematic evaluation of compound–target relationships and prediction of ligand–protein interactions, thereby supporting the identification and prioritization of promising candidates for further experimental investigation. 29, 30, 31 Future work should prioritise in vitro cytotoxicity and mechanistic assays in UBC cell lines, orthogonal validation of the MTOR-Vincarubine interaction by biophysical or cell-free binding assays, molecular dynamics simulation to assess the temporal stability of the docked complex, and, contingent on these results, in vivo evaluation of efficacy and pharmacokinetics.

In summary, this integrated network pharmacology and molecular docking analysis identifies a biologically coherent, MTOR-centred multi-target mechanism through which *V. minor* phytochemicals, and Vincarubine in particular, may exert activity against urinary bladder carcinoma, and provides a mechanistic rationale to prioritise this understudied Vinca alkaloid for experimental validation.

5. CONCLUSION

This study presents the first integrated network pharmacology and molecular docking investigation of *V. minor* against urinary bladder carcinoma. Screening of five *V. minor* phytochemicals identified 110 targets shared with UBC-associated genes, from which a densely interconnected protein-protein interaction network and ten hub genes, headed by MTOR, HIF1A and CASP3, were resolved. Functional enrichment linked this target set directly to the Bladder cancer KEGG pathway and to insulin receptor signalling, while molecular docking demonstrated a favourable, multi-point binding interaction between Vincarubine and MTOR. Collectively, these findings position *V. minor*, and Vincarubine specifically, as a mechanistically plausible, MTOR-directed candidate meriting dedicated in vitro and in vivo evaluation in urinary bladder carcinoma.

REFERENCES

1. Su X, Tao Y, Chen F, Han X, Xue L. Trends in the global, regional, and national burden of bladder cancer from 1990 to 2021: an observational study from the global burden of disease study 2021. Sci Rep. 2025;15:7655.
2. Jubber I, Ong S, Bukavina L, Black PC, Compérat E, Kamat AM, et al. Epidemiology of bladder cancer in 2023: a systematic review of risk factors. Eur Urol. 2023;84(2):176-90.
3. Qu F, Darji S, Thompson DH. Recent advances in drug delivery strategies for high-risk BCG-unresponsive non-muscle invasive bladder cancer: a brief review from 2018 to 2024. Pharmaceutics. 2024;16(9):1154.
4. Chunarkar-Patil P, Kaleem M, Mishra R, Ray S, Ahmad A, Verma D, et al. Anticancer drug discovery based on natural products: from computational approaches to clinical studies. Biomedicines. 2024;12(1):201.
5. Dhyani P, Quispe C, Sharma E, Bahukhandi A, Sati P, Attri DC, et al. Anticancer potential of alkaloids: a key emphasis to colchicine, vinblastine, vincristine, vindesine, vinorelbine and vincamine. Cancer Cell Int. 2022;22:206.
6. Zhai Y, Liu L, Zhang F, Chen X, Wang H, Zhou J, et al. Network pharmacology: a crucial approach in traditional Chinese medicine research. Chin Med. 2025;20:8.
7. Mohanraj K, Karthikeyan BS, Vivek-Ananth RP, Chand RPB, Aparna SR, Mangalapandi P, et al. IMPPAT: a curated database of Indian Medicinal Plants, Phytochemistry And Therapeutics. Sci Rep. 2018;8(1):4329.
8. Vivek-Ananth RP, Mohanraj K, Sahoo AK, Samal A. IMPPAT 2.0: an enhanced and expanded phytochemical atlas of Indian medicinal plants. ACS Omega. 2023;8(9):8827-45.
9. Kim S, Chen J, Cheng T, Gindulyte A, He J, He S, et al. PubChem 2023 update. Nucleic Acids Res. 2023;51(D1):D1373-80.
10. Daina A, Michielin O, Zoete V. SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules. Nucleic Acids Res. 2019;47(W1):W357-64.
11. Stelzer G, Rosen N, Plaschkes I, Zimmerman S, Twik M, Fishilevich S, et al. The GeneCards suite: from gene data mining to disease genome sequence analyses. Curr Protoc Bioinformatics. 2016;54(1):1.30.1-1.30.33.
12. Oliveros JC. Venny. An interactive tool for comparing lists with Venn's diagrams [Internet]. 2007. Available from: https://bioinfogp.cnb.csic.es/tools/venny/
13. Szklarczyk D, Kirsch R, Koutrouli M, Nastou K, Mehryary F, Hachilif R, et al. The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. Nucleic Acids Res. 2023;51(D1):D638-46.
14. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003;13(11):2498-504.
15. Chin CH, Chen SH, Wu HH, Ho CW, Ko MT, Lin CY. cytoHubba: identifying hub objects and sub-networks from complex interactome. BMC Syst Biol. 2014;8(Suppl 4):S11.
16. Ge SX, Jung D, Yao R. ShinyGO: a graphical gene-set enrichment tool for animals and plants [Internet]. 2020.
17. Burley SK, Bhikadiya C, Bi C, Bittrich S, Chen L, Crichlow GV, et al. RCSB Protein Data Bank: powerful new tools for exploring 3D structures of biological macromolecules for basic and applied research and education in fundamental biology, biomedicine, biotechnology, bioengineering and energy sciences. Nucleic Acids Res. 2021;49(D1):D437-51.
18. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, et al. AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. J Comput Chem. 2009;30(16):2785-91.
19. BIOVIA. Discovery Studio modeling environment. San Diego: Dassault Systèmes; 2017.
20. Huan J, Grivas P, Birch J, Hansel DE. Emerging roles for mammalian target of rapamycin (mTOR) complexes in bladder cancer progression and therapy. Cancers (Basel). 2022;14(6):1555.
21. Zhou Q, Huang W, Xiong J, Guo B, Wang X, Guo J. CDCA8 promotes bladder cancer survival by stabilizing HIF1α expression under hypoxia. Cell Death Dis. 2023;14(10):658.
22. Song DM, Feng K, Luo WF, Lv DS, Zhou LP, He YB, et al. Predicting survival in bladder cancer with a novel apoptotic gene-related prognostic model. Discov Oncol. 2024;15:702.
23. Xiang J, Luo Y, Zhang B, Ke K, Li H. Molecular mechanisms and translational advances in bladder cancer: from driver genes to precision therapy. Front Oncol. 2025;15:1670574.
24. Raggi D, Crupi E, Pederzoli F, Martini A, Briganti A, Alhalabi O, et al. HER2 and urothelial carcinoma: current understanding and future directions. Nat Rev Urol. 2026;23:110-32.
25. Kudelski J, Tokarzewicz A, Gudowska-Sawczuk M, Mroczko B, Chłosta P, Bruczko-Goralewska M, et al. The significance of matrix metalloproteinase 9 (MMP-9) and metalloproteinase 2 (MMP-2) in urinary bladder cancer. Biomedicines. 2023;11(3):956.
26. Kao WH, Liao LZ, Chen YA, Lo UG, Pong RC, Hernandez E, et al. SPHK1 promotes bladder cancer metastasis via PD-L2/c-Src/FAK signaling cascade. Cell Death Dis. 2024;15(9):678.

27. Agu PC, Afiukwa CA, Orji OU, Ezeh EM, Ofoke IH, Ogbu CO, et al. Molecular docking as a tool for the discovery of molecular targets of nutraceuticals in diseases management. Sci Rep. 2023;13(1):13398.
28. Che X, Zhang L. Blind docking methods have been inappropriately used in most network pharmacology analysis. Front Pharmacol. 2025;16:1566772.
29. Venkatesan LS, Ali S, Sathishkumar P. Prevalence of Drug-Resistant Acinetobacter baumannii Infections and their Threats in Public Health on Global Scale. Current Clinical Microbiology Reports. 2026 Dec;13(1):5.
30. Aruchamy M, Ramanathan S, Shanmugam N, Muthupandian S. Deciphering autophagy-metabolism interactions in cancer drug resistance: a comprehensive review. Cancer Chemotherapy and Pharmacology. 2026 Apr 29;96(1):38.
31. Sidhambaram J, Iruthayaraj A, Viswaresh Babu S, Thayumanavan P. Integrative network pharmacology and molecular dynamics analysis of a curcumin–indole-3-propionic acid conjugate for diabetes-associated cognitive decline: J. Sidhambaram et al. Journal of Biological Physics. 2026 Dec;52(1):27.