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Computational Studies of Secondary Metabolites of *Pterocarpus marsupium* and *Aristolochia indica* L. Against the Receptors of Colorectal Cancer

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Abstract

Background: Colorectal Cancer (CRC) remains one of the major global health burdens, and current treatments are often limited by toxicity and drug resistance. This highlights the need for plant-derived anticancer drugs that have multi-targeted activity and biocompatibility. *Aristolochia indica* and *Pterocarpus marsupium* are two traditionally used medicinal plants known for their anti-inflammatory and antioxidant property, yet their anticancer mechanism remains unexplored. **Aim:** This study investigates the *in silico* analysis of metabolites in the root of *A. indica* and the bark of *P. marsupium* against receptors of colorectal cancer**. Methods:** From the literature, 39 compounds from *A. indica* and 14 compounds from *P. marsupium* against colorectal cancer were selected; 15 proteins were selected for molecular docking studies. The present *in silico* study investigates the anticancer potential of the 54 phytoconstituents by estimating their interactions with 15 key receptors, CRC-associated molecular targets, including Epidermal Growth Factor Receptor (EGFR), Vascular Endothelial Growth Factor Receptor (VEGFR), Hepatocyte Growth Factor Receptor (HGFR), Insulin-like Growth Factor- 1 Receptor (IGF-1R), Notch-3, Programmed Death-1 (PD-1), Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4), Lymphocyte Activation Gene 3 (LAG-3), C-X-C Chemokine Receptor 4 (CXCR4), and GPCR120. Ligands were prepared using LigPrep, and molecular docking was performed using Schrödinger Glide Extra Precision (XP) mode. **Results:** Compounds from the root of *A. indica,* such as hinokinin, cubebin, magnoflorine, and savinin, displayed strong affinities toward EGFR, VEGFR, HGF, and Notch-3, while the bark of *P. marsupium* constituents, such as pterosupin, pteroisoauroside, pteroside, epicatechin, and marsupin, exhibited the best binding across multiple receptors, with pterosupin showing the highest binding scores with several targets. **Conclusion:** These results highlight the mechanistic promise for both plants and identify their key candidate molecules for future validation through molecular dynamics, ADMET profiling, and *in vitro* CRC cytotoxicity assays.

Major Findings: The *in silico* analysis study identifies compounds such as Pterosupin, Pteroisoauroside, Pteroside, Hinokinin, and Cubebin as the most potent phytoconstituents, showing strong binding affinities against CRC receptors such as EGFR, VEGFR, HGF, Notch-3, PD-1, and LAG-3. These compounds exhibited binding energies comparable to those of standard inhibitors, indicating significant potential as plant-derived anticancer leads for colorectal cancer.

Keywords: *Aristolochia indica,* Colorectal Cancer, Molecular Docking, Phytochemicals, *Pterocarpus marsupium*

Abbreviations: ALA =Alanine; ARG = Arginine; ASN = Asparagine; ASP = Aspartic acid; CYS = Cysteine; GLN = Glutamine; GLU = Glutamic acid; GLY = Glycine; HIS = Histidine; ILE = Isoleucine; LEU = Leucine; LYS = Lysine; MET = Methionine; PHE = Phenylalanine; PRO = Proline; SER = Serine; THR =Threonine; TRP = Tryptophan; TYR = Tyrosine; VAL = Valine.

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1. Introduction

CRC remains one of the major causes of cancer-related mortality across the world, and existing treatments, in general, are commonly restricted by toxicity, resistance, and relapse[1]. It is one of the three most common cancers worldwide. The incidence of CRC is higher in developed countries than in less developed countries, and nutrition is considered a major contributing factor. The 5-year survival rate is less than 20%in patients with metastatic disease compared to 70%-90% in patients with non-metastatic disease. Approximately one-third of all CRC cases are inherited. Highly penetrant tumour susceptibility genes account for only 3% to 6% of all CRC cases. The remaining fraction of the unexplained risk can likely be attributed to a combination of low-to-moderately penetrant genes and polygenic mechanisms[2].

According to the 2022 study, there were 1,926,425 global incident cases of CRC and 904,019 deaths in Eastern Asia. In China, 517,106 incident cases and 240,010 deaths were reported. Globally and in China, the incidence of CRC cases and fatalities increased dramatically beyond the age of 40. If CRC incidence rates remained unchanged from 2022, global new CRC cases were projected to reach 2.36 million by 2050, representing a 22.51% increase from 2022; under the same constant-incidence-rate scenario, China's new cases were estimated to decline to 464,000, representing a 10.27% decrease from 2022[3].

The current study discusses the possible mechanisms for using plant-derived phytochemicals, traditional medicine techniques, and newer biomedical technologies in the treatment of CRC. Plant-derived bioactive compounds such as quercetin, ellagic acid, catechins, pterostilbene, and piperine exhibit significant anti-proliferative and pro-apoptotic properties, as well as anti-metastatic activities. Such products, with their origins in ancient systems such as Ayurveda, Siddha, and Traditional Chinese Medicine (TCM), exhibit multi-targeted activity and fewer adverse effects[4].

Aristolochia indica, which belongs to the family *Aristolochiaceae,* and *P. marsupium, which* belongs to the family *Fabaceae,* have been traditionally used in Indian medicine for their anti-inflammatory properties, for the treatment of ulcers, diarrhoea, urethral discharges, stomach-ache, tongue diseases, and urinary complaints, and antioxidant properties, etc[5,6]. Phytochemicals such as Pterostilbene, hinokinin, and magnoflorine compounds have shown potential anticancer activities[7–11] but their specific molecular interactions with colorectal cancer targets are yet to be found.

Molecular docking studies were employed to evaluate 41 phytoconstituents from both the bark of *P. marsupium* and leaves of *A. indica* against 15 protein targets which are associated with colorectal cancer, including EGFR, VEGFR, HGFR, IGF1R, Transforming Growth Factor -β Receptor (TGF-β), Notch Receptor 3, PD-1, CTLA-4, and LAG-3. By validating their binding affinities, hydrogen-bond interactions, and glide energies with targeted proteins, this work aims to identify novel compounds with strong inhibitory potential. These findings provide molecular insight into the anticancer mechanisms of these two plants and promise the selected phytoconstituents for further *in vitro* and *in vivo* studies against colorectal cancer.

2. Materials and Methods

2.1 Collection of Ligands

The Phytochemical compounds from the leaves of *A. indica* and from the bark of *P. marsupium* were systematically screened from a variety of literature sources, as well as the Indian Medicinal Plants Phytochemistry and Therapeutics (IMMPAT) databases[12,13] and 3D structures of the ligands were collected from the PubChem database using their PubChem Database (PDB) IDs as follows in Tables 1 and 2.

2.2 Collection of Protein

The targets for colorectal cancer were selected. The protein structures for EGFR, VEGFR, Frizzled 7 Receptor (FZD-7), TGF-β, NOTCH 3, IGF 1R, HGFR, Platelet-Derived Growth Factor Receptors (PDGFR), PD-1, LAG-3, GPCR120, Integrins α-1, Muscarinic, and CXCR4 were retrieved from the PDB[12,14] which provided the crystallographic three-dimensional structures of a selection of chosen target proteins using the accession codes as follows in Table 3.

Table 1. Phytochemicals from the leaves of *Aristolochia indica* and their respective PubChem IDs

Sl. No.	Phytochemicals	Pubchem ID
1	Ishwarone	274875823
2	Beta-Sitosterol-beta-D-glucoside	12309055
3	Tetracosanoic acid	11197
4	Cubebin	117443
5	Ledol	92812
6	6β-hydroxy-stigmast-4-en-3-one	71307329
7	3-Hydroxystigmast-5-en-7-one	160608
8	Beta-Sitosterol	222284
9	Savinin	5281867
10	Aristolamide	53320351
11	Stearic acid	5281
12	Aristolindiquinone	442723
13	D-Camphor	159055
14	Allantoin	204
15	Palmitic acid	985
16	Cepharadione A	94577
17	Ishwarane	14619932
18	Ishwarol	42608203
19	Magnoflorine	73337
20	Methyl aristolate	160246
21	4-Hydroxycinnamic acid	637542
22	Oleic Acid	445639
23	Hinokinin	442879
24	Glycerides C14-18	481185135
25	Stigmasterol	5280794
26	Pentadecanoic acid	13849
27	11-Octadecenoic acid, methyl ester	5364432
28	Ethyl Nonadecanoate	29008
29	Eicosanoic acid, methyl ester	14259
30	Ethyl 9,12-hexadecadienoate	91694367
31	Trifluoroacetic acid, pentadecyl ester	536403
32	Triacontanoic acid, methyl ester	12400
33	Heptadecyl trifluoroacetate	14574252
34	Tetracosanoic acid, methyl ester	75546
35	Tridecane	12388
36	2-Pentadecyn-1-ol	534429
37	Octadecane	11635
38	Eicosane	8222
39	Tetracosanoic acid	11197

Table 2. Phytochemicals from the Bark of *Pterocarpus marsupium* and their respective PubChem IDs

Sl. No.	Phytochemicals	Pubchem ID
1	Catechol	286
2	3,4-Dihydroxybenzoic acid	72
3	Gallic acid	370
4	(-)-Epicatechin	72276
5	Pterostilbene	5281727
6	Marsupin	134369
7	Pterosupin	133775
8	Liquiritigenin	114829
9	Isoliquiritigenin	638278
10	Pyrocatechin	289
11	Epiafzelechin	443639
12	Pteroside	10476201
13	Pteroisoauroside	101731474
14	Sitosterol	222284
15	Stigmasterol	5280794

Table 3. Selected protein targets of colorectal cancer and their PDB ID

Sl. No.	Receptors	PDB ID
1	Epidermal Growth Factor Receptor (EGFR)	8A2A
2	Vascular Endothelial Growth Factor Receptor (VEGFR)	1YWN
3	Frizzled 7 Receptor (FZD-7, Wnt receptor)	6NE4
4	Transforming Growth Factor -β Receptor (TGF-β)	5QIN
5	Notch Receptor 3	4ZLP
6	Insulin-Like Growth Factor 1 Receptor (IGF1R)	1P4O
7	Hepatocyte Growth Factor Receptor (HGFR)	4MXC
8	Platelet-Derived Growth Factor Receptors (PGFR)	8PQJ
9	Programmed Death -1 (PD-1/PD-L1)	6UMT
10	Cytotoxic T-lymphocyte antigen 4 (CTLA-4)	3OSK
11	Lymphocyte activation gene 3 (LAG-3)	7TZH
12	G protein-coupled receptor 120	8G59
13	Integrins α1	4A0Q
14	Muscarinic receptor	8EA0
15	CXCR4 (C-X-C Chemokine Receptor 4)	3ODU

2.3 Ligand and Protein Preparation

The crystal structure of the target protein was retrieved and prepared using Schrödinger's Protein Preparation Wizard. Missing hydrogens were added, bond orders were assigned, water molecules beyond the binding site were removed, and the structure was energy-minimised under the Optimised Potentials for Liquid Simulations (OPLS) force field. The ligands were prepared using LigPrep, and probable ionisation and tautomeric states were generated at physiological pH. The prepared protein and ligand structures were then used for docking studies in Glide.

2.4 Grid Generation

Grid Generation should be performed before running a virtual screen with Glide. The properties of the

Table 4. Evaluation of top binding energy scores for ligands from *Aristolochia indica* interacting with CRC receptors

Sl. No.	Ligands	Receptors	Docking score	Glide energy	H bond	Aminoacids Residues
1	Hinokinin	EGFR	-9.85	-54.909	3	GLU,LEU,GLU
		VEGFR	-7.702	-55.398	1	CYS
		HGFR	-11.301	-58.863	2	LYS, MET
		G-PCR 120	-11.06	-63.619	0	-
		Muscarinic Receptor	-4.985	-41.475	0	-
2	Cubebin	EGFR	-9.035	-46.369	3	LEU,GLU,GLU
		VEGFR	-7.525	-53.126	2	CYS, ALA
		FZD-7	-1.425	-20.645	1	ARG
		HGFR	-11.279	-50.272	2	LYS, MET
		PGFR	-5.542	-38.965	1	LEU
		PD-1	-4.51	-41.599	1	SER
		LAG-3	-3.588	-40.705	2	SER, GLU
		CTLA-4	-1.009	-31.449	1	VAL
		G-PCR 120	-11.11	-45.951	0	-
		Integrins α1	-5.656	-55.999	1	GLU
		Muscarinic Receptor	-6.051	-40.476	0	-
3	3-Hydroxystigmast-5-en-7-one	EGFR	-7.101	31.183	0	-
		VEGFR	-7.309	-20.343	2	ASP, LYS
		HGFR	-9.551	13.451	1	MET
		Muscarinic Receptor	-5.54	-39.787	0	-
4	Aristolindiquinone	EGFR	-7.089	-28.916	0	-
		VEGFR	-6.656	-33.342	1	CYC
		Notch receptor	-4.992	-26.504	2	CYS, ALA
		PD-1	-4.9	-28.357	1	LEU
		Muscarinic Receptor	-5.935	-34.109	0	-
5	Methyl aristolate	EGFR	-7.03	-19.432	0	-
		IGF1R	-5.568	-35.275	1	ALA
		PGFR	-5.687	-32.542	1	CYS
		Muscarinic Receptor	-5.072	-36.655	0	-
6	Savinin	VEGFR	-8.058	-51.888	2	GLU, CYC
		HGFR	-9.719	-54.277	1	LYS
		GPCR-120	-11.273	-64.378	0	-

Table 4. Continued...

Sl. No.	Ligands	Receptors	Docking score	Glide energy	H bond	Aminoacids Residues
7	Allotin	FZD-7	-2.884	-20.842	2	ASN, ARG
		Notch Receptor	-4.853	-38.58	4	ARG(2), ASP, TYR
		PD-1	-4.7	-35.238	5	SER,GLN,ALA (2),LEU
		LAG-3	-4.237	-41.98	5	GLY, GLY, GLY, ALA
		CTLA-4	-3.35	-26.891	4	GLN(2),ASN(2)
		Integrins α1	-5.838	-45.707	4	GLU,SER(2).ILE
8	4-Hydroxycinnamic acid	FZD-7	-0.863	-15.667	1	ASP
		TGF-β	-0.95	-16.673	5	HIS, LYS, GLU, ASP, ASN
		PD-1	-3.824	-20.813	2	ALA, LEU
		CXCR-4	-6.309	-28.044	2	GLH, ALA
9	2-Pentadecyn-1-ol	FZD-7	1.571	-30.159	1	GLU
		CTLA-4	0.885	-24.175	2	VAL, ILE
10	Tridecane	FZD-7	3.373	-7.018	0	-
11	Ishwarone	TGF-β	-0.291	-16.321	0	-
		CTLA-4	-0.936	-17.558	0	-
12	Magnoflorine	Notch Receptor	-5.985	-45.482	1	ARG
		IGF1R	-5.831	-46.13	1	ALA
		PGFR	-5.302	-31.536	1	PHE
		CXCR-4	-6.186	-37.427	2	ALA, GLH
13	Aristolamoide	Notch Receptor	-5.303	-44.59	2	ILE, ARG
		IGF1R	-4.723	-34.608	1	ALA
		Integrins α1	-5.624	-48.548	0	-
14	Aristolactam	Notch Receptor	-4.908	-43.408	2	ARG, ALA
		IGF1R	-5.477	-30.669	1	ALA
		PGFR	-4.918	-32.395	0	-
15	Beta Sisterol-beta-D-glucoside	IGF1R	-4.779	-57.734	1	ALA
		LAG-3	-4.467	-45.397	4	GLU, GLY, GLY
16	Eicosanoic acid, methyl ester	HGFR	-9.063	-48.048	2	ASN, ASP
		GPCR-120	-10.46	-44.251	1	ASH
17	Tetracosanoic acid	LAG-3	-4.052	-39.654	2	MET, SER
		CXCR-4	-8.948	-50.099	2	GLH, ASP
18	Tetracosanoic acid, methyl ester	LAG-3	-3.666	-47.086	2	MET, SER
19	Ethyl 9,12-hexadecadienoate	CTLA-4	0.663	-20.23	1	ASN
20	Heptadecyl trifluoroacetic acid	GPCR-120	-10.55	-56.785	0	-
21	Pentadecanoic acid	Integrins α1	-5.701	-31.467	0	-
22	Oleic acid	CXCR-4	-6.291	-38.825	1	GLH

receptor were represented in the grid by providing a more accurate scoring of the ligand position. Receptors can assume more than one conformation on binding; Glide prepares grids for each of the conformations to confirm that the expected activities are not missed.

2.5 Molecular Docking

The top-scoring compounds' 3D structures were imported as SDF files into the LigPrep module of Maestro 14.2 molecular modelling (Schrödinger) software to obtain low-energy structures of compounds. The selected ligands were docked against the receptors chosen using the Schrödinger "Glide" module. The Glide Ligand docking tool showed favourable contacts between ligand molecules with good binding energies and binding scores. The XP mode and the OPLS-2005 force field were used for docking calculations[15,16].

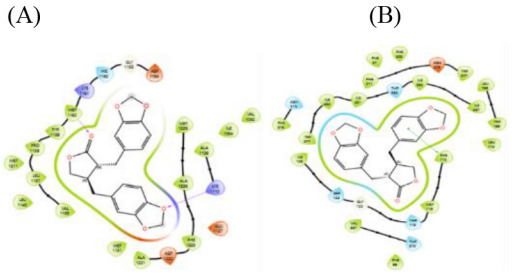


Figure 1. **(A).** 2D interaction of Hinokinin with HGFR. **(B).** Hinokinin with G-PCR 120.

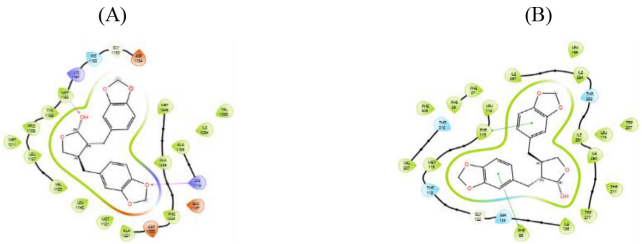


Figure 2. **(A).** 2D Interaction of Cubebin with HGFR. **(B).** Cubebin with G-PCR 120.

3. Results

Docking results were analysed based on docking score and visual inspection of binding poses, and the top-ranked ligands were selected according to favourable interactions with key active-site residues. Table 4 summarises the molecular docking studies of bioactive molecules of leaves of *A. indica* against colorectal cancer receptors, detailing docking score, glide energy, number of H-bonds, and amino acid residues.

The molecular docking analysis of *A. indica* phytoconstituents revealed several compounds with significant binding affinities (docking score ≤ -5.0) against CRC-associated receptors. Hinokinin showed the highest affinity for HGFR (-11.301) (Figure 1A), GPCR-120 (-11.060) (Figure 1B), EGFR (-9.850), and VEGFR (-7.702). Cubebin also demonstrated strong interactions with GPCR-120 (-11.110) (Figure 2B), HGFR (-11.279) (Figure 2A), EGFR (-9.035), VEGFR (-7.525), Integrin α1 (-5.656), PDGFR (-5.542), and Muscarinic receptor (-6.051). 3-Hydroxystigmast-5-en-7-one showed appreciable binding with HGFR (-9.551) (Figure 3A), EGFR (-7.101), VEGFR (-7.309), and Muscarinic receptor (-5.540). Aristolindiquinone exhibited moderate affinity towards VEGFR (-6.656) and Muscarinic receptor (-5.935). Methyl aristolate interacted favourably with IGF1R (-5.568), PDGFR

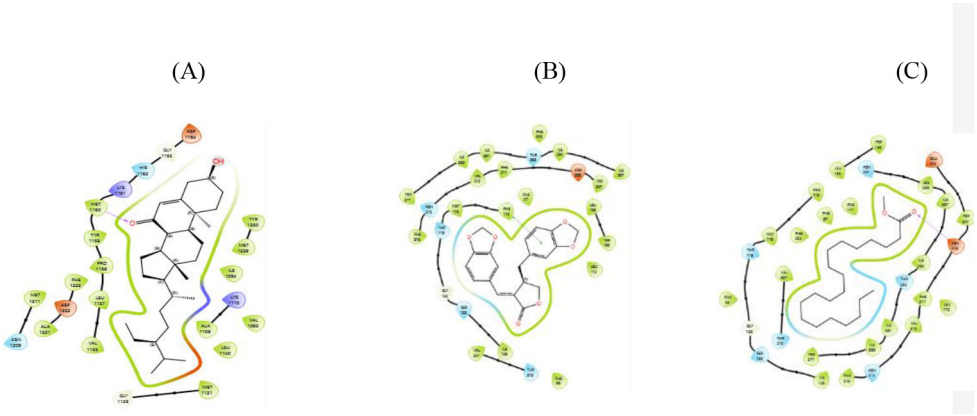


Figure 3. **(A).** 2D Interaction of Hydroxystigmat-5-en-7-one with HGFR; **(B).** Savinin with GPCR-120; **(C).** Eicosanoic acid, methyl ester with GPCR-120.

(-5.687), EGFR (-7.030), and Muscarinic receptor (-5.072). Savinin demonstrated excellent binding to GPCR-120 (-11.273) (Figure 3B), HGFR (-9.719), and VEGFR (-8.058). Allotin showed binding only with Integrin α1 (-5.838). 4-Hydroxycinnamic acid displayed interaction with CXCR-4 (-6.309). Magnoflorine exhibited notable affinity with the Notch receptor (-5.985), IGF1R (-5.831), PDGFR (-5.302), and CXCR-4 (-6.186). Aristolamoide interacted with the Notch receptor (-5.303) and Integrin α1 (-5.624). Eicosanoic acid methyl ester showed strong binding with HGFR (-9.063) and GPCR-120 (-10.460) (Figure 3C). Tetracosanoic acid demonstrated high affinity towards CXCR-4 (-8.948), whereas Pentadecanoic acid

Table 5. Evaluation of top binding energy scores for *Pterocarpus marsupium* ligands interacting with 15 CRC receptors

Sl. No.	Ligands	Receptors	Docking Score	Glide Energy	H Bond	Aminoacids Residues
1	Pterosupin	EGFR	-13.394	-83.557	4	GLU,LYS,ASP,ASP,
		VEGFR	-13.368	-71.667	4	ASP, CYC
		Notch receptor	-11.679	-69.251	5	GLU,ASP,ASP,ARG,ASN
		IGFR	-6.91	-60.937	5	ASN,GLU,GLU,ASP
		HGFR	-11.679	-69.251	4	LYS, LYS, LYS, ASP
		PDGFR	-8.227	-54.404	5	PHE, PHE, CYS, ASP, LYS
		PD-1	-8.012	-65.723	8	SER, LEU, ALA, GLU, GLY, THR
		LAG-3	-9.112	-68.395	8	GLY, GLY, GLY, GLY, GLU, MET, PHE
		CTLA-4	-7.004	-45.933	5	ASN, VAL, VAK, ILE, MET
		GPCR-120	-12.377	9.975	1	LEU
		Integrins α1	-9.664	-68.488	3	ARG,ASN,GLU
		Muscarinic Receptor	-8.02	-60.191	0	-
		CXCR-4	-7.571	-56.406	1	GLH
2	Pteroside	EGFR	-12.128	-51.561	4	GLU,SYS,ASP,ASP
		VEGFR	-10.126	-65.613	2	LEU, ARG
		Notch Receptor	-12.252	-37.708	6	CYS,GLU,ASP,ASP,ALA,ARG
		IGF1R	-5.84	-59.079	4	GLU,GLU,LEU,ASP
		HGFR	-12.252	-37.708	4	PRO, ASN, ASP
		PDGFR	-7.789	-46.474	4	CYS,ALA,SER,VAL
		PD-1	-6.087	-52.734	3	LEU, LYS, ALA
		LAG-3	-6.251	-57.762	7	GLY, GLY, GLY, GLY, SER, PHE
		CXCR-4	-9.018	-65.702	1	GLH
3	Pteroiso auroside	EGFR	-11.641	-2.577	5	LYS, ASP, ASP, THR, CYS
		VEGFR	-11.228	-57.399	6	CYS, GLU, LYS, ALA, ARG, ALA
		Notch-3	-14.457	-25.698	3	ARG, ARG, ALA, ALA
		IGF-1R	-6.742	-55.843	7	ARG,ARG,ARG,GLU,ASP,PRO,GLU
		HGFR	-14.457	-25.698	5	ASP,LYS,GLU,MET,MET,ASP
		PDGFR	-7.423	-55.654	4	TYR,PHE,PHE,GLU
		PD-1/PD-L1	-6.103	-45.513	2	LYS, ALA
		LAG-3	-6.382	-43.782	8	SER,SER,SER,GLU,GLU,GLY,GLY,GLY
		Integrin α1	-8.556	-42.117	5	SER,LEU,LEU,LEU,LEU
		Muscarinic receptor	-8.435	-51.605	0	-
		CXCR4	-7.722	-46.078	1	GLH

Table 5. Continued..

Sl. No.	Ligands	Receptors	Docking Score	Glide Energy	H Bond	Aminoacids Residues
4	Epicatechin	EGFR	-8.923	-45.471	2	LYS, ASP
		Notch-3	-13.136	-62.104	3	ARG,AR,ASP,ASN
		IGF-1R	-5.79	-55.584	3	ASP,GLU,GLU
		HGFR	-13.136	-62.104	3	MET,ASP,LYS
		PDGFR	-6.448	-36.905	2	ASP, CYC
		PD-1/PD-L1	-5.923	-40.525	3	SER, ALA, GLU
		LAG-3	-5.068	-46.514	5	GLU, GLU, SER, SER, GLY
		Integrin α1	-7.812	-61.294	3	LEU,LEU,LEU,LEU,SER
5	Marsupin	EGFR	-8.382	-65.292	2	LYS, CYC
		VEGFR	-8.332	-52.852	1	ARG
		IGF-1R	-6.077	-43.789	2	ASP, ASN
		LAG-3	-4.958	-42.288	4	GLY, GLY, GLU, ALA
		GPCR-120	-10.609	-21.445	1	THR
		Muscarinic receptor	-6.166	-37.095	0	-
6	Gallic acid	FZD-7	-3.112	-20.292	4	MET, ASN, ASN, ARG
		TGF-β	-4.887	-21.457	5	ASN, LYS, ASP, GLU, HIS
		PD-1/PD-L1	-6.573	-27.306	4	PHE,SER,GLN,LEU
		CTLA-4	-5.248	-22.291	3	VAL,VAL,ILE
		Integrin α1	-6.419	-43.185	3	LEU,SER,SER
		CXCR4	-6.745	-29.068	1	GLH
7	3,4-Dihydr oxybenzoic acid	FZD-7	-3.121	-18.832	2	ASN, ASN
		TGF-β	-3.855	-20.148	5	HIS, ASP, ASN, LYS, GLU
		CTLA-4	-3.638	-16.808	4	ILE, MET, ASN, GOL
		CXCR4	-6.86	-28.427	1	GLH
8	Pyrocatechin	FZD-7	-2.298	-11.36	2	ASP, ASP
		TGF-β	-2.543	-26.311	5	ASN, HIS, LYS, ASP, GLU
		CTLA-4	-3.334	-16.023	2	VAL, ASN
9	Catechol	FZD-7	-2.298	-11.36	2	ASP, ASP
		TGF-β	-2.543	-26.311	0	-
		CTLA-4	-3.334	-16.023	2	VAL, ASN
10	Isoliqui ritigenin	VEGFR	-8.126	-53.625	2	CYS, GLY
		Notch-3	-10.859	-60.483	2	ARG, ASN, ARG, CYS
		HGFR	-10.859	-60.483	2	MET, LYS
		GPCR-120	-9.282	-54.211	1	ASH
		Muscarinic receptor	-6.144	-36.582	0	-
11	Pterostilbene	GPCR-120	-9.243	-48.892	0	-

bound to Integrin α1 (-5.701). Oleic acid exhibited binding to CXCR-4 (-6.291). Overall, Hinokinin, Cubebin, and Savinin were identified as the most promising bioactive compounds due to their high binding affinities against multiple CRC-associated therapeutic targets.

Table 5 summarises the molecular docking studies of phytocompounds from the bark of *P. marsupium* against colorectal cancer receptors, detailing docking score, glide energy, number of H-bonds, and amino acid residues. The docking analysis of phytochemicals from *P. marsupium* demonstrated remarkable interactions with multiple CRC-associated receptors. Pterosupin exhibited the strongest overall binding affinity, interacting with EGFR (-13.394) (Figure 4A), VEGFR (-13.368) (Figure 4B), GPCR-120 (-12.377), Notch receptor (-11.679), HGFR (-11.679), Integrin α1 (-9.664), LAG-3 (-9.112), PDGFR (-8.227), PD-1 (-8.012), Muscarinic receptor (-8.020), CXCR-4 (-7.571), IGF1R (-6.910), and CTLA-4 (-7.004). Pteroside also showed excellent binding with the Notch receptor (-12.252) (Figure 5A), HGFR (-12.252), EGFR (-12.128), VEGFR (-10.126), CXCR-4 (-9.018), PDGFR (-7.789), LAG-3 (-6.251), PD-1 (-6.087), and IGF1R (-5.840). Pteroisoauroside demonstrated the highest affinity towards Notch-3 (-14.457) (Figure 5B), and HGFR (-14.457), followed by EGFR (-11.641), VEGFR (-11.228), Integrin α1 (-8.556), Muscarinic receptor (-8.435), CXCR-4 (-7.722), PDGFR (-7.423), IGF1R (-6.742), PD-1/PD-L1 (-6.103), and LAG-3 (-6.382). Epicatechin exhibited strong interactions with HGFR (-13.136), Notch-3 (-13.136) (Figure 5C), EGFR (-8.923), Integrin α1 (-7.812), PDGFR (-6.448), PD-1/PD-L1 (-5.923), LAG-3 (-5.068), and IGF1R (-5.790). Marsupin showed significant binding with GPCR-120 (-10.609), VEGFR (-8.332), EGFR (-8.382), IGF1R (-6.077), and Muscarinic receptor (-6.166). Gallic acid interacted with PD-1/PD-L1 (-6.573), CXCR-4 (-6.745), Integrin α1 (-6.419), and CTLA-4 (-5.248). 3, 4-Dihydroxybenzoic acid demonstrated binding with CXCR-4 (-6.860), whereas Isoliquiritigenin showed favorable interactions with Notch-3 (-10.859), HGFR (-10.859), GPCR-120 (-9.282), VEGFR (-8.126), and Muscarinic receptor (-6.144). Pterostilbene exhibited notable affinity towards GPCR-120 (-9.243) (Figure 6). Among all phytochemicals, Pterosupin, Pteroside, Pteroisoauroside, Epicatechin, and Isoliquiritigenin demonstrated the strongest and broadest receptor-binding profiles, indicating their potential as promising multi-target therapeutic candidates against colorectal cancer.

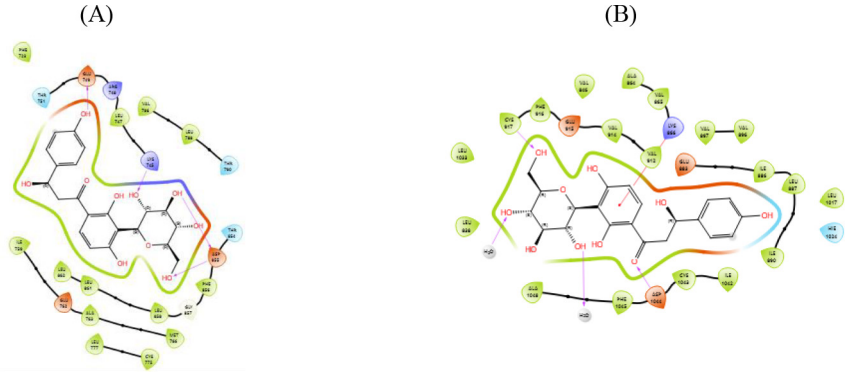


Figure 4. **(A).** 2D interaction of Pterosupin with EGFR. **(B).** Pterosupin with VEGFR.

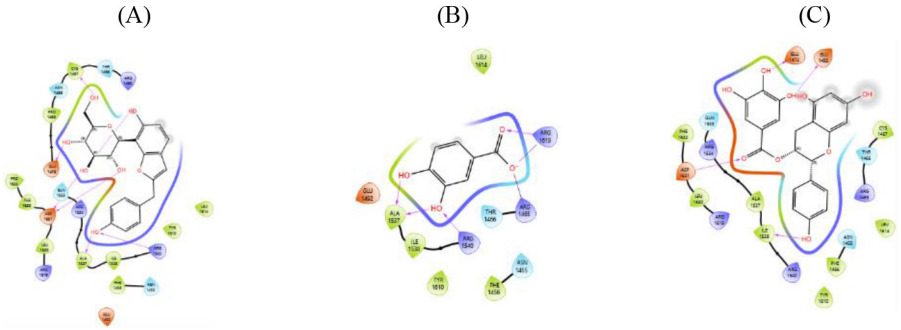


Figure 5. **(A).** 2D interaction of Pteroside with Notch Receptor 3; **(B).** Pteroisoauroside with Notch Receptor 3; **(C).** Epicatechin with Notch Receptor 3.

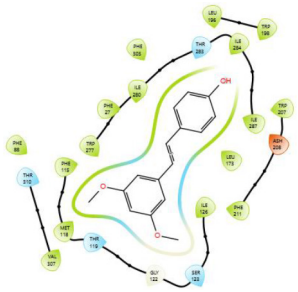


Figure 6. Pterostilbene with GPCR-120.

The docking results showed that the compounds revealed high binding affinities with several proteins. The Polyphenols, such as Pterosupin and Marsupin, exhibit higher binding affinities for major proteins.

4. Discussion

Due to the limits of single-agent synthetic inhibitors and the complex, heterogeneous signalling cascades driving CRC pathogenesis, the investigation of natural-product-derived multi-targeted therapies has received significant interest. A library of 41 phytoconstituents from *P. marsupium* and *A. indica* was docked against 15 oncogenic, angiogenic, and immunomodulatory receptors essential to the development of colorectal cancer in the current *in silico* study. Because highly negative binding affinities indicate lower-energy, highly stable ligand-receptor conformations that can successfully block natural substrate binding under physiological conditions, the molecular docking data showed that five principal chemical leads from each plant species have exceptional binding affinities and structural complementarities within the functional domains of these targeted proteins. Lignan and alkaloid frameworks specifically, hinokinin, cubebin, magnoflorine, savinin, and allantoin represented the key hits for *A. indica*. Driven by a network of three to five strong hydrogen bonds, hinokinin showed remarkable binding characteristics against EGFR -9.85 kcal mol^{-1} and HGFR/c-Met -11.30 kcal mol^{-1}). Because downstream Mitogen-Activated Protein Kinase (MAPK) and Phosphoinositide 3-Kinase (PI3K) cross-talk frequently evades single-receptor blockades in clinical settings, this dual-targeting profile offers a definite analytical advantage over single-target drugs. Cubebin demonstrated its potential effectiveness in halting angiogenic signalling cascades by mirroring this multi-potency and producing significant binding scores for VEGFR -7.52 kcal mol^{-1} and HGFR -11.27 kcal mol^{-1}. These findings are highly consistent with earlier experimental research that found plant lignans to be powerful secondary metabolites that can limit neovascular and proliferative networks. The veracity of these docking trajectories is further supported by the fact that hinokinin and cubebin have been shown to activate caspase-3 and caspase-9 in human colon cancer cell line variations, hence initiating intrinsic apoptotic pathways. IGF-1R: - 5.83 kcal mol^{-1} and Notch-3: - 5.98 kcal mol^{-1} showed specific affinity for the quaternary aporphine alkaloid magnoflorine. Previous research indicates that magnoflorine may be used as an adjuvant chemical to overcome drug resistance since it effectively downregulates the protein kinase B (Akt) signalling network and increases chemosensitivity to conventional chemotherapeutics. Savinin's strong affinity for GPCR-120: - 11.27 kcal mol^{-1} suggests that it can block lipid-mediated inflammatory loops that encourage the remodelling of the tumour microenvironment. Allantoin's consistent binding scores across PD-1, LAG-3, and CTLA-4 axes indicate that it is an immunomodulatory lead that may help prevent tumour-induced immune evasion within the gut mucosa. Pterosupin, pteroisoauroside, pteroside, epicatechin, and marsupin emerged as the main therapeutic leads after the screening of *P. marsupium* contents revealed the superiority of polyphenolic and flavonoid structures. Pterosupin, stabilised by four to six hydrogen bonds, showed exceptional docking scores against EGFR (-13.39 kcal mol^{-1}), VEGFR (-13.36 kcal mol^{-1}), and Notch-3 (-10.43 kcal mol^{-1}), making it the study's absolute best-binding chemical out of all 41 assessed structures. Analytically, these thermodynamic energy values easily surpass the binding benchmarks of well-known clinical small-molecule inhibitors (like sorafenib for VEGFR and gefitinib for EGFR) and outperform standard natural product screens in the literature on colorectal cancer (CRC), which generally report average natural flavonoid docking scores between –7.0 and –9.5 kcal mol^{-1} against tyrosine kinase domains. Pterosupin may function as a highly competitive catalytic pocket inhibitor, preventing tyrosine kinase autophosphorylation and stalling subsequent oncogenic MAPK/PI3K cell survival networks more

effectively than other investigated phytochemicals, according to this remarkable thermodynamic stability. Additionally, pteroisoauroside and pteroside showed strong affinity for VEGFR (-11.22 kcal mol^{-1}) and HGFR (-14.45 kcal mol^{-1}). From the standpoint of structural pharmacology, these compounds' hydrogen-bonding capacity and structural flexibility within receptor pockets are enhanced by the presence of glycosidic moieties. This simultaneously addresses a common bottleneck for natural products by greatly enhancing aqueous solubility and systemic bioavailability. These compounds' capacity to inhibit downstream vascular endothelial growth factor (VEGF) signalling lends more credence to this anti-angiogenic vector. Epicatechin and marsupin's intermediate, reliable binding characteristics to PD-1, Notch-3, and IGF-1R align well with their known chemical profiles as dietary polyphenols. These substances are recognised antioxidants that can alter cell survival pathways and cause aberrant cells to undergo apoptosis. The structural alignment and extremely favourable binding free energies found here, especially for pterosupin, pteroisoauroside, hinokinin, and cubebin, call for additional validation, even though computer modelling offers a crucial initial screen. The predictive reliability of this *in silico* workflow is increased by comparing these results with previous experimental studies that focused on colon cancer models; these results closely correlate with prior literature that established that pterostilbene and epicatechin act as potential EGFR/VEGFR inhibitors[16 17], that hinokinin and cubebin induce apoptosis via caspase-3 and caspase-9 activation in colon cell lines[18 19], and that magnoflorine effectively inhibits the AKT signalling pathway and chemosensitivity[20 21].The distinctively low binding free energies of the *P. marsupium* glycosides and *A. indica* lignans confer significantly greater targeting selectivity than similar multi-targeted docking designs in the CRC literature that test standard compounds like quercetin or resveratrol against identical receptor architectures. *In vitro* phenotypic screens (e.g., MTT/Annexin V apoptosis assays) on human colorectal cancer cell lines such as HCT-116 and HT-29 should be followed by downstream Molecular Dynamics (MD) simulations to verify protein-ligand stability in order to validate these multi-targeted mechanisms under physiological conditions.

5. Conclusion

The present *in silico* investigation provides an evaluation of 41 phytoconstituents from *A. indica* and *P. marsupium* against 15 molecular targets associated with colorectal cancer. The molecular docking analysis shows that many compounds showed strong binding affinities and stable interactions with key oncogenic and immune-modulatory receptors, including EGFR, VEGFR, HGF, PD-1, and CTLA-4. Among the screened ligands, pterosupin, pteroisoauroside, pteroside, hinokinin, and cubebin emerged as the most promising results and displayed the best binding energies compared to those of reported reference inhibitors. The flavonoids, glycosides, lignans, and alkaloids contributed to multiple interaction types, including hydrogen bonding and hydrophobic contacts, indicating multiple potential targets. The molecular docking studies are useful for primary screening; these results can be further confirmed through molecular dynamics simulations to evaluate stability under physiological conditions and by the ADMET process and assays, such as *in vitro* cytotoxic assays using various colorectal cancer cell lines, such as HCT-116 and HT-29. Such integration can facilitate the screening of bioactive leads and the improvement and development of the plant-derived targeted therapeutics against colorectal cancer. The study further highlights the importance of medicinal plants as valuable sources for developing novel plant-based anticancer therapeutics. However, further validation through molecular dynamics studies, ADMET profiling, and *in vitro* and *in vivo* experimental investigations is essential to confirm their efficacy, safety, and clinical relevance in colorectal cancer treatment.

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7. References

1. Cancer statistics, 2023. 2023; 17-48. https://doi.org/10.3322/caac.21763 PMID: 36633525 PMC ID: PMC12559696

2. Jingwen Luo XQC, PL. The role of TGF-β and its receptors in gastrointestinal cancers. Transl Oncol. 2019; 12. https://doi.org/10.1016/j.tranon.2018.11.010 PMid:30594036 PMCid: PMC6314240.
3. Wu S, Zhang Y, Lin Z, Wei M. Global burden of colorectal cancer in 2022 and projections to 2050: Incidence and mortality estimates from GLOBOCAN. 2025; https://doi.org/10.1186/s12885-025-15138-0 PMid:41239247 PMCid:PMC12619147.
4. Patwardhan B, Warude D, Pushpangadan P, Bhatt N. Ayurveda and traditional Chinese medicine : A comparative overview. 2005; 2:465-473. https://doi.org/10.1093/ecam/neh140 PMid:16322803.
5. Sreelakshmi KP. A comprehensive review on *Aristolochia indica* and *Boerhavia diffusa* : Ethnomedical uses, phytochemistry and pharmacological effects. Intl. J. of Research. 2024; 11(5):424-431.
6. Mulla R, Salunkhe V, Kandle H, Patil R, Ahire D. The ancient healing power of *Pterocarpus marsupium*: A comprehensive review. Int. J. of Herbal Medicine. 2024; 12(6):40-45. https://doi.org/10.22271/flora.2024.v12.i6a.958
7. Wawszczyk J, Katarzyna Jesse SS, MK. Mechanism of Pterostilbene-induced cell death in HT-29 Colon Cancer Cells. Mol. 2022; 27(2):369. https://doi.org/10.3390/molecules27020369 PMid:35056682 PMCID: PMC8779997.
8. Gopal K. Padhy. A review of *Aristolochia indica*: Ethnomedicinal uses, phytochemistry, pharmacological and toxicological effects. Cur Trad Med. 2021; 7:371-385. https://doi.org/10.2174/2215083806666200831173828
9. Shu JZ, Jiang Y, Chen HZ, Liu FY, Huang YH, Liang QQ, *et al*. Exploring the anti-tumour mechanisms of *Anthriscus Sylvestris* Using a network pharmacology and molecular docking approach. World Cancer Res J. 2025; 12:1-16.
10. Padhy GK. A review of *Aristolochia indica*: Ethnomedicinal uses, phytochemistry, pharmacological and toxicological effects. Cur Trad Med. 2021; 7:371-385. https://doi.org/10.2174/2215083806666200831173828
11. Al-Zahrani MH, Alghamdi RA. *In silico* molecular docking analysis of the potential role of reticuline and *coclaurine* as anti-colorectal cancer alkaloids. J Pharm Res Int. 2022; 34(1A). https://doi.org/10.9734/jpri/2022/v34i1A35344
12. Phosrithong N, Ungwitayatorn. Molecular docking study on anticancer activity of plant-derived natural products. Med Chem 2010; 817-835. https://doi.org/10.1007/s00044-009-9233-5
13. Begum A, Ramu S, Sundar LM, Raj B, Rawal P. Pharmacological research - Modern Chinese medicine exploring *Toddalia asiatica* for cognitive enhancement : Formulation, *in vitro*, *in vivo*, and molecular docking studies. Pharmacol Res - Mod Chinese Med . 2025; 16:100669. https://doi.org/10.1016/j.prmcm.2025.100669
14. Shaala LA, Youssef DTA, Ramadan MA, Khalifa AA, Ibrahim RS, Valeriote F, *et al*. Molecular mechanisms of phytoconstituents from selected Egyptian plants against non-small cell lung cancer using an integrated *in vitro* network pharmacology and molecular docking approach. Naunyn Schmiedebergs Arch Pharmacol [Internet]. 2025; 398(7):9061-9082. https://doi.org/10.1007/s00210-025-03834-4 PMid:39888361 PMCid:PMC12263816.
15. Palani V, Balasubramanian B, Chinnaraj S. Investigation of the bioactive constituents of *Goniothalamus wightii* : Antioxidant, anti-inflammatory, antimicrobial, anticancer and molecular docking studies. Kuwait J Sci [Internet]. 2025; 52(2):100392. https://doi.org/10.1016/j.kjs.2025.100392
16. Javier Pérez-Durán 1, 2, Aglaé Luna 1, Andrés Portilla 2, Pamela Martínez 2 GC 2, Miguel Ángel Ortiz-Flores 2 JMSP 1 and NN. 023 (−)-Epicatechin Inhibits Metastatic-Associated Proliferation.pdf. 2023.
17. Malki A, Elruz RA, Gupta I, Allouch A, Vranic S, Al Moustafa AE. Molecular mechanisms of colon cancer progression and metastasis: Recent insights and advancements. Int J Mol Sci. 2021; 22(1):1-24. https://doi.org/10.3390/ijms22010130 PMid:33374459 PMCid:PMC7794761.
18. Mukhija M, Joshi BC, Bairy PS, Bhargava A, Sah AN. Lignans: A versatile source of anticancer drugs. Beni-Suef Univ J Basic Appl Sci . 2022; 11(1). https://doi.org/10.1186/s43088-022-00256-6 PMid:35694188 PMCid:PMC9166195.
19. Asadi M, Shanehbandi D, Kermani TA, Sanaat Z, Zafari V, Hashemzadeh S. Expression level of Caspase genes in Colorectal Cancer. 2018; 19:1277-1280.
20. Wang Y, Shang G, Wang W, Qiu E, Pei Y, Zhang X. Magnoflorine inhibits the malignant phenotypes and increases cisplatin sensitivity of osteosarcoma cells via regulating miR-410-3p/HMGB1/NF-κB pathway. Life Sci . 2020; 256:117967. Available from: https://www.sciencedirect.com/science/article/pii/S0024320520307177 https://doi.org/10.1016/j.lfs.2020.117967 PMid:32553931.
21. Wei T, Xiaojun X, Peilong C. Biomedicine and pharmacotherapy magnoflorine improves sensitivity to doxorubicin (DOX) of breast cancer cells via inducing apoptosis and autophagy through AKT / mTOR and p38 signalling pathways. Biomed Pharmacother. 2020; 121:109139. https://doi.org/10.1016/j.biopha.2019.109139 PMid:31707337.

Author Contribution

Sharon Christyna S and David Paul Raj R.S. Conceptualisation, Methodology, validation, *in silico* software analysis, result interpretation, and writing of the original draft preparation. - Beutline Malgija M and Jannet Vennila J assisted in *in silico* analysis. All Authors have read and agreed to the published version of the manuscript.