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In silico exploration and identification of key bioactive compounds of the molecular targets of *Piper guineense* in colon cancer through network pharmacology, and molecular docking

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Abstract

Colon cancer is a severe and rapidly progressing disease that represents a substantial global health challenge. *Piper guineense* is a plant with anticancer potential that requires experimental validation; however, its molecular mechanisms against colon cancer remain poorly understood. This research utilized a network pharmacology approach combined with molecular docking to explore possible therapeutic mechanisms. The bioactive compounds were identified using gas chromatography–mass spectrometry (GC–MS). Colon cancer–related genes were obtained from the GeneCards and DisGeNET databases, while predicted targets of the identified compounds were generated through SwissTarget Prediction. Overlapping targets between compounds and disease genes were identified and used to construct protein–protein interaction network via STRING. The network was visualized in Cytoscape, hub genes were screened using CytoHubba, and functional enrichment analysis was conducted using Gene Ontology and KEGG pathway databases. Molecular docking was performed using PyRx software. A total of 21 compounds from GC-MS corresponding to 452 non-redundant targets were identified, and 7,332 colon cancer targets were obtained from the databases. Five hub genes were identified as key targets: EGFR, MAP2K1, SRC, KDR, and PIK3CA. Molecular docking analysis demonstrated that α -selinene bound to EGFR, MAP2K1, SRC, and PIK3CA with docking scores of -7.6 , -7.7 , -5.1 , and -7.5 kcal/mol, respectively, while β -selinene had scores of -7.5 , -7.7 , -5.4 , and -7.6 kcal/mol with the same targets. β -humulene exhibited a strong docking score of -8.7 kcal/mol towards KDR. The α and β -selinene, and humulene showed better docking scores in comparison with 5-fluorouracil, which contributes to its anticancer potential.

Keywords: Colon cancer; *Piper guineense*; Molecular docking; Hub gene

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INTRODUCTION

Colon cancer (CC) is the most prevalent globally in the gastrointestinal tract and a significant public health concern worldwide, arising from the epithelial lining of the colonic mucosa (Chen et al., 2023). The development of this disease is progressive and multifactorial; however, despite significant advances in management, the five-year survival rate has not exceeded 40%. It was reported that a significant proportion of patients eventually experience disease recurrence, metastatic progression, and the development of resistance to therapeutic interventions (He et al., 2023). Furthermore, colon cancer has shown a steady rise in incidence, with a particularly rapid increase observed among younger populations (Bansod et al., 2021). Colon cancer constitutes a significant global health burden, accounting for about 10.2% of newly diagnosed cancer cases and approximately 9.2% of deaths attributable to cancer worldwide (Sung et al., 2021). Colon cancer arises from a wide range of contributing factors, and early-stage disease often remains clinically silent. However, as the condition progresses to advanced stages, patients may present with symptoms such as abdominal discomfort, mucopurulent stools, constipation, bowel obstruction, and lymphatic spread (Gupta et al., 2019). Current management strategies include modern therapeutic advances such as immunotherapy or precision oncology to improve relevance aside from surgical intervention. In addition, commonly used adjuvant chemotherapeutic agents such as fluorouracil, capecitabine, and oxaliplatin are frequently associated with significant adverse effects, including neutropenia, peripheral neuropathy, and diarrhoea (Gelibter et al., 2019). Therefore, there is an urgent need to develop more effective and safer therapeutic approaches for the management of colon cancer that reduce adverse effects while overcoming the shortcomings associated with current therapeutic options. Furthermore, investigating natural compounds using a reverse pharmacology framework, with an emphasis on favourable safety profiles, may offer a rational and promising approach for colon cancer management. It was reported that medicinal plants remain highly valuable, as they have historically contributed substantially to the discovery and development of contemporary therapeutic agents in modern healthcare systems (Qamar et al., 2023). *Piper guineense* (*P. guineense*) is an underexploited spice and flavouring agent, widely recognized in traditional medicine for the therapeutic use of its leaves, fruits, and roots across diverse cultural practices (Ojimelukwe, 2023). Locally, the plant is employed in the management of various ailments, including urogenital disorders as well as skin, hepatic, and gastrointestinal conditions. Pharmacological studies have reported that *P. guineense* exhibits antioxidant, antimicrobial, antifungal, and neuropharmacological activities (Salehi et al., 2019). The phytochemical analysis of *P. guineense* leaves showed the presence of alkaloids (wisamine, dihydrowisamine, piperine, and dihydropiperine), saponins, tannins, flavonoids, resins, and essential oil (dillapiol, elemicine, myristicine, and safrole), which have a lot of pharmacological properties in diseases such as diabetes mellitus (Sulaimon et al., 2020).

The network pharmacology approach enables the prediction of compound effectiveness across multiple disease targets, offering valuable tools for accelerating the drug development process while significantly lowering costs by limiting the reliance on extensive experimental studies (Gupta et al., 2024). Furthermore, it has gained increasing attention for its systems-level perspective, allowing the construction of integrated protein–compound and disease–gene interaction networks to uncover synergistic and multi-target therapeutic pathways (Zhou et al., 2020). Furthermore, molecular docking functions as an essential *in silico* approach for forecasting atomic-scale interactions between small-molecule ligands and specific protein targets (Dey et al., 2023). Collectively, *in silico* drug discovery tools also facilitate the evaluation of compound toxicity, pharmacological classification, and biological activity. Presently, this study provides computational insight and identification of key bioactive compounds of molecular targets of *Piper guineense* through network pharmacology and molecular docking.

Materials and Methods

Extract preparation

The seeds of *P. guineense* were collected and taxonomically authenticated at the University of Ilorin Herbarium, Nigeria, where they were assigned the voucher number UILH/002/1203. The plant material was carefully cleaned and left to air-dry at room temperature. Once dried, the seeds were ground into a fine powder, and 100 g of this powder was soaked in 1 L of 80% methanol for 72 h by shaking and stirring at room temperature (20–30 °C). After maceration, the mixture was filtered using a muslin cloth, and the filtrate was concentrated with a rotary evaporator (temperature at 40 °C, 33.8 mmHg for pressure, and 90 rpm as rotation speed). The extraction yield was then determined following the method described by (Chike-Ekwughe et al., 2025)

Gas chromatography/mass spectrometry (GC–MS) evaluations

The chemical composition of *Piper guineense* was determined using GC-MS-5975C Agilent system composed of an auto sampler and a gas chromatograph interfaced with a mass spectrometer (GC-MS) instrument. It was worked with the following conditions using a fitted variant column Agilent JSW HP- 5 MS (30 m × 0.32 mm × 0.25 μM), sample was dissolved in hexane and 1 μL inject automatically into the column with the injector temperature set at 250 °C. Helium gas was used as the carrier gas at a flow rate of 1 ml/min. GC oven temperature was started at 50 °C held for 5 mins and then raised to 80 °C at the rate of 5 °C / min for 9 min. The temperature of injector and ion source were maintained at 250 °C and 280 °C respectively. For the GC-MS analysis an injector was done on a duplicate of the same operational conditions. The mass spectrum of compounds in sample was obtained by electron ionization at 7 eV, ion source temperature was 230 °C and interface temperature 250 °C and its detector was operated in scan mode from 45 to 450 amu (atomic mass units). A

scan interval of 0.5 s and fragment from 45 to 450 amu was maintained. The total running time was 60 mins. Identifications were made by comparing the acquired spectra with the National Institute of Standards and Technology (NIST) reference library by using the following matching criteria: peak intensity, retention time, molecular weight, and mass-to-charge ratios (Akubugwo et al., 2022).

Compound/ligand selection through pharmacokinetic properties and ADMET analysis

The oral bioavailability of the compounds was assessed using Lipinski's Rule of Five (Daina et al., 2017; Shahid et al., 2023). Key pharmacokinetic and safety properties, including absorption, distribution, metabolism, excretion, and toxicity (ADMET), were predicted through SwissADME and ADMETlab 2, providing insights into gastrointestinal absorption, blood–brain barrier permeability, P-glycoprotein interactions, and potential inhibition of cytochrome P450 enzymes (Mukhtar et al., 2023).

Compounds toxicity assessment

The compounds' toxicity was evaluated using the ProTox-II server, assessing their potential for carcinogenicity, immunotoxicity, cytotoxicity, hepatotoxicity, and mutagenicity. Furthermore, median lethal doses (LD₅₀) were predicted via ProTox-II server online rather than experimental calculation and each compound was assigned a toxicity class based on the Global Harmonization System (GHS) for chemical hazard classification (Nafisah et al., 2022).

Network pharmacology, potential target screening of active compounds, and colon cancer

The potential targets of the active compounds were predicted using Swiss Target Prediction, and colon cancer-related targets were retrieved from GeneCards and DisGeNET databases. The datasets were combined, duplicates removed, and target names verified using UniProtKB. Common targets between the compounds and disease were then identified and illustrated using a Venn diagram (Tabassum et al., 2022).

Prediction of protein–protein interaction network and hub genes

The overlapping target proteins were examined using the STRING database and visualized in Cytoscape v3.10.1. Key hub genes were identified with the CytoHubba plugin, and the strength of their interactions was evaluated using maximal clique centrality (MCC) scores (Shahzadi et al., 2024).

Gene ontology (GO) and KEGG pathway analysis

GO and KEGG pathway enrichment analyses were performed using the DAVID database, with *Homo sapiens* specified as the organism. GO terms were classified into three categories: biological processes (BP), cellular components (CC), and molecular functions (MF). At the same time, KEGG analysis was used to identify signaling

and metabolic pathways with significant enrichment. Only pathways with $p < 0.05$ were considered, and the top 20 GO terms from each category, along with the most relevant KEGG pathways, were selected. Visualization of the enrichment results was performed using bar and lollipop plots in ShinyGO (<http://bioinformatics.sdstate.edu/go/>).

Molecular docking

Ligand preparation

The structures of the compounds identified by GC-MS, along with the standard drug 5-Fluorouracil, were obtained from the PubChem database and converted to the specified format. Before performing molecular docking, all ligands were prepared by adding hydrogen atoms and assigning proper charges to enable accurate simulation of their interactions with the target proteins.

Target protein preparation

The 3D structures of the hub proteins, such as EGFR (1M17), MAP2K1 (3DY7), SRC (1O4N), KDR (6GQ0), and PIK3CA (6PYS) were obtained from the Protein Data Bank as suitable available proteins without mutation, high resolution, and co-crystallized with ligand. However, protein preparation in BIOVIA Discovery Studio involved removing water molecules and heteroatoms, adding polar hydrogens, and eliminating co-crystallized ligands. The spaces previously occupied by these ligands were then used to define the active site residues and construct the docking grid boxes for molecular docking (Tian et al., 2018).

Docking

The compounds identified by GC-MS, together with the standard drug (5-fluorouracil), were docked against the active sites of their respective target proteins using PyRx version 8.0. The active amino acid residues were obtained from the co-crystallized ligands of respective proteins in order to generate a grid box area via re-docking. However, the criteria for selecting binding score out of nine (9) conformation is due lowest energy score, and highest binding score. The Compounds exhibiting the highest binding affinities and demonstrating significant interactions across multiple targets were selected (Mir et al., 2023).

Results and Discussions

Percentage yield and GC-MS analysis of *Piper guineense*

The methanolic extract of *P. guineense* seeds produced a yield of 18.5% (Table 1). In contrast, a previous study (Ogbonna et al., 2013) reported that ethanolic extraction of the same seeds resulted in a lower yield of 13.5%, suggesting that methanol was a more effective solvent under the conditions used in this study. In this study, GC-MS analysis revealed a variety of bioactive compounds, which may contribute to its pharmacological effects. Table 2 lists the identified compounds along with their respective peak numbers, retention times (RT), and relative percentage areas, including caryophyllene

(5.55%), humulene (1.71%), β -farnesene (9.44%), α -copaene (0.82%), n-hexadecanoic acid (2.23%), piperine (14.99%), and benzamide N-(2,5-dimethoxyphenyl)-3-

methoxy, which exhibited the highest area (17.28%), while D-limonene showed the lowest (0.56%). The chromatogram of the extract is presented in Figure 1.

Table 1: Percentage yield of the methanolic extract of *Piper guineense* seed

Sample	Extraction solvent	Seed powder (g)	Extracted seed powder (g)	Yield (%)
<i>Piper guineense</i>	Methanol	100	18.51	18.51

Table 2: Gas chromatograph coupled with mass spectrometer analysis of methanolic extract of *Piper guineense* seed

Peak number	RT	Compound name	Area (%)
1	10.241	D-limonene	0.56
2	13.356	1,6-octadien-3-ol, 3,7-dimethyl	1.64
3	22.745	alpha copaene	0.82
4	23.333	1,Z-5, E-7-dodecatriene	1.65
5	24.084	Caryophyllene	5.55
6	25.110	Humulene	1.71
7	25.304	β -humulene	1.85
8	25.585	Cis-beta-farnesene	9.44
9	25.998	β -copaene	2.83
10	26.13	β -selinene	2.27
11	26.43	α -selinene	1.41
12	26.993	β -bisabolene	3.1
13	27.393	cyclohexene,3-(1,5-dimethyl-4-hexenyl)-6-methylene	0.77
14	28.644	1,6, 10-dodecatrien-3-ol, 3,7, 11-trimethyl	1.01
15	37.452	n-hexadecanoic acid	2.23
16	38.603	9,12-Octadecadienoic acid (Z,Z)-	3.23
17	39.973	1-piperonylpyrazole-3-carboxylic acid hydrazide	5.8
18	41.205	4-methoxy-N'-(1-(2-thienyl)ethylidene)benzohydrazide	9.75
19	41.386	benzamide-N-(2,5-dimethoxyphenyl)-3-methoxy-	17.28
20	41.624	Piperine	2.15
21	42.35	Piperine	0.92
22	43.882	carbamic acid, N-(2-naphthyl)-methyl ester	9.23
23	44.358	Piperine	14.99

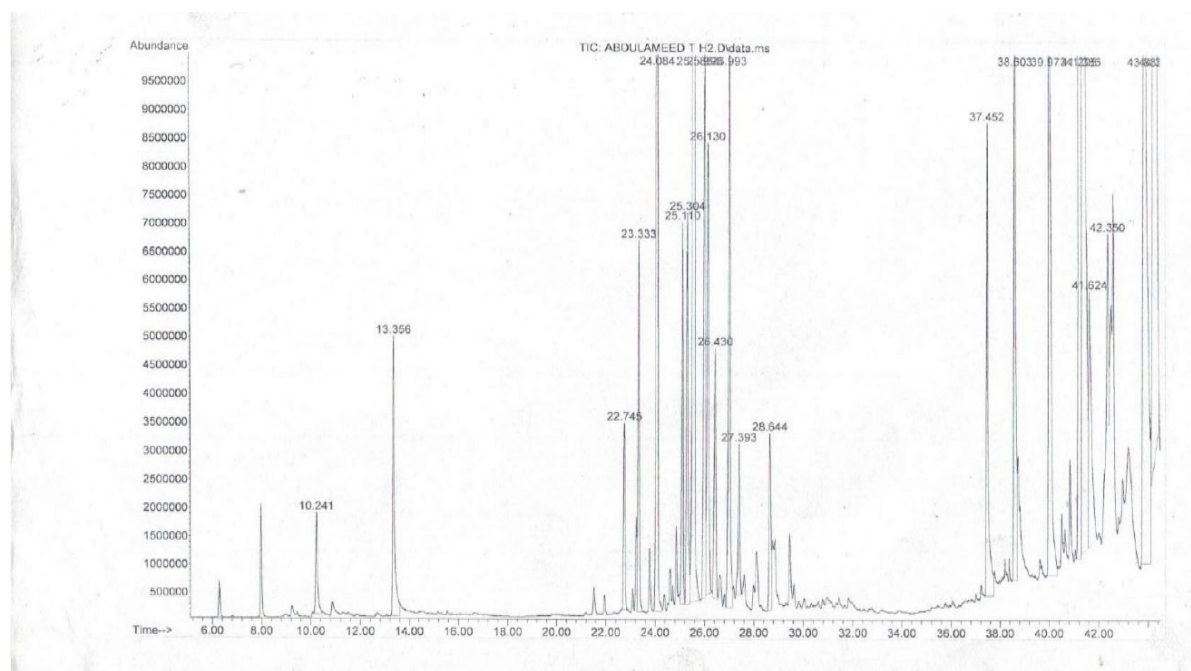


Figure 1: Chromatogram of *Piper guineense* seed

Screening of active compounds

Computational screening of bioactive compounds with favourable pharmacokinetic and pharmacodynamic profiles offers an efficient and cost-effective approach to drug discovery (Siddiqui et al., 2022). In Table 2, GC-MS results revealed twenty-one (21) compounds since piperine had different retention time (41.624, 42.35, and 44.358) and was counted as one compound. Ten (10) compounds showed no violations of Lipinski's criteria, while the remaining eleven had only a single violation, which still falls within acceptable limits for oral bioavailability. Pharmacokinetic evaluation indicated that nine compounds were unable to penetrate the blood-brain barrier, such as caryophyllene, β -copaene, humulene, β -humulene, cis- β -farnesene, β -bisabolene, β -selinene, cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene, α -selinene, and 1-piperonylpyrazole-3-carboxylic acid hydrazide. This barrier, composed of endothelial cells lining the cerebral vasculature, acts as a protective filter that restricts the entry of many potentially harmful substances into the brain (Alajangi et al., 2022). P-glycoprotein (P-gp) is an important drug transporter and represents a crucial consideration in pharmacology and drug development because of its significant influence on drug bioavailability and therapeutic efficacy (Attia et al., 2023). The findings indicated that compounds not recognized as P-gp substrates tend to persist longer within cells. Among the tested compounds, all showed negative responses except for 1-piperonylpyrazole-3-carboxylic acid hydrazide, which exhibited a positive response. Moreover, to improve absorption and maintain consistent plasma concentrations, most of the compounds were predicted to inhibit the five major cytochrome P450 isoforms. In contrast, 1-piperonylpyrazole-3-carboxylic acid hydrazide and 1,6-octadien-3-ol-3,7-dimethyl showed no inhibitory activity toward these enzymes. However, cytochrome P450 enzymes are crucial for metabolizing drugs and xenobiotics; their inhibition can enhance bioavailability, prolong drug half-life, and minimize drug-drug interactions (Xing et al., 2023). In this study, some compounds also exhibited high gastrointestinal absorption, suggesting they are efficiently taken up in the human intestine (Table 4).

Toxicity prediction of compounds

In silico tools provide an efficient and affordable way to estimate drug toxicity, enabling the evaluation of the safety profiles of compounds (Roney & Mohd Aluwi, 2024). In this study, the 21 compounds obtained via GC-MS were screened out based on the toxicological parameters (mutagenicity, hepatotoxicity, carcinogenicity, immunotoxicity, and cytotoxicity) and the result revealed that fifteen (15) compounds such as alpha-copaene, caryophyllene, 1,6,10-dodecatrien-3-ol (3,7,11-trimethyl), D-limonene, humulene, 1,Z-5,E-7-dodecatriene, β -humulene, cis- β -farnesene, 1,6-octadien-3-ol (3,7-dimethyl), β -copaene, β -selinene, cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene, α -selinene, 9,12-octadecadienoic acid (Z,Z) and n-hexadecanoic acid were predicted to be non-mutagenic and non-carcinogenic, with

no evidence of hepatotoxic, immunotoxic, or cytotoxic effects (Table 5). The degree of toxicity was found to be dose-dependent, and acute toxicity was evaluated using the median lethal dose (LD₅₀), which serves as an indicator of short-term toxic potential (Saganuwan, 2017). As shown in Table 6, the estimated LD₅₀ values and toxicity categories of the evaluated compounds revealed that three compounds; 1,Z-5, E-7-dodecatriene, benzamide-N-(2,5-dimethoxyphenyl)-3-methoxy and 9,12-octadecadienoic acid (Z,Z) were classified under drug toxicity class VI, with LD₅₀ values of 5700 mg/kg, 10,000 mg/kg, and 9750 mg/kg, respectively, indicating they may or may not be harmful if ingested. In contrast, eleven compounds were assigned to toxicity class V, suggesting they exhibit low toxicity. However, Seven (7) compounds such as limonene, caryophyllene, n-hexadecanoic acid, 1-piperonylpyrazole-3-carboxylic acid hydrazide, piperine, 4-methoxy-N'-(1-(2-thienyl)ethylidene)benzohydrazide and carbamic acid N-(2-naphthyl)-methyl ester were classified as drug toxicity class IV, with LD₅₀ values of 330, 2000, 900, 1000, 1120, 330, and 1500 mg/kg, respectively, indicating that they can be harmful if ingested.

Table 3: Lipinski rule compliance data

S/N	Compound name	Molecular weight (g/mol) (MW < 500))	Oral bioavailability (OB ≥ 30)	Clog P (P < 5)	H-bond acceptors (<10)	H-bond donor (<5)	No. of rotatable bonds (<10)	Lipinski's rule of five violations	Polar surface area (A ²) (≤140)
1	D-limonene	136.23	0.55	3.37	0	0	1	0	0
2	1,6-octadien-3-ol, 3,7-dimethyl	154.25	0.55	2.66	1	1	4	0	20.23
3	alpha copaene	204.35	0.55	4.3	0	0	1	1	0
4	1,Z-5, E-7-dodecatriene	164.29	0.55	4.16	0	0	7	0	0
5	Caryophyllene	204.35	0.55	4.24	0	0	0	0	0
6	Humulene	204.35	0.55	4.26	0	0	0	1	0
7	β-humulene	204.35	0.55	4.37	0	0	0	1	0
8	Cis-beta-farnesene	204.35	0.55	4.97	0	0	7	1	0
9	β-copaene	204.35	0.55	3.73	0	0	1	1	0
10	β-selinene	204.35	0.55	4.51	0	0	1	1	0
11	α-selinene	204.35	0.55	4.4	0	0	1	1	0
12	β-bisabolene	204.35	0.55	4.83	0	0	4	1	0
13	cyclohexene,3-(1,5-dimethyl-4-hexenyl)-6-methylene	204.35	0.55	4.57	0	0	4	1	0
14	1,6, 10-dodecatrien-3-ol, 3,7, 11-trimethyl	222.37	0.55	4.18	1	1	7	0	20.23
15	n-hexadecanoic acid	256.42	0.55	5.2	2	1	14	1	37.3
16	9,12-Octadecadienoic acid (Z,Z)-	392.66	0.55	8.23	2	0	22	1	26.30
17	1-piperonylpyrazole-3-carboxylic acid hydrazide	260.25	0.55	0.62	5	2	4	0	91.4
18	4-methoxy-N'-(1-(2-thienyl)ethylidene)benzohydrazide	346.5	0.55	3.73	3	0	6	0	70.14
19	benzamide-N-(2,5-dimethoxyphenyl)-3-methoxy-	287.31	0.55	2.83	4	1	6	0	56.79
20	Piperine	285.34	0.55	3.03	3	0	4	0	38.77
21	carbamic acid, N-(2-naphthyl)-methyl ester	201.22	0.55	2.46	2	1	3	0	38.33

Table 4: Pharmacokinetic properties of phytoconstituents

S/N	Compound name	CYP1A2	CYP3A4	CYP2C19	CYP2D6	CYP2C9	BBB	P-gp substrate	LogKp	GI absorption
1	D-limonene	No	No	No	No	Yes	Yes	No	-3.89	Low
2	1,6-octadien-3-ol, 3,7-dimethyl	No	No	No	No	No	Yes	No	-5.13	High
3	alpha copaene	Yes	No	Yes	No	Yes	Yes	No	-4.37	Low
4	1,Z-5, E-7-dodecatriene	Yes	No	No	No	No	Yes	No	-3.87	Low
5	Caryophyllene	No	No	Yes	No	Yes	No	No	-4.44	Low
6	Humulene	No	No	No	No	Yes	No	No	-4.32	Low
7	β-humulene	No	No	No	No	Yes	No	No	-4.15	Low
8	Cis-beta-farnesene	Yes	No	No	No	Yes	No	No	-3.27	High
9	β-copaene	No	No	Yes	No	No	Yes	No	-4.2	Low
10	β-selinene	No	No	Yes	No	Yes	No	No	-3.68	Low
11	α-selinene	No	No	Yes	No	Yes	No	No	-3.85	Low
12	β-bisabolene	No	No	No	No	Yes	No	No	-2.98	Low
13	cyclohexene,3-(1,5-dimethyl-4-hexenyl)-6-methylene	No	No	Yes	No	Yes	No	No	-3.71	Low
14	1,6, 10-dodecatrien-3-ol, 3,7, 11-trimethyl	No	No	No	No	Yes	Yes	No	-4.23	High
15	n-hexadecanoic acid	Yes	No	No	No	Yes	Yes	No	-2.77	High
16	9,12-Octadecadienoic acid (Z,Z)-	Yes	No	No	No	No	No	No	-1.38	Low
17	1-piperonylpyrazole-3-carboxylic acid hydrazide	No	No	No	No	No	No	Yes	-7.53	High
18	4-methoxy-N'-(1-(2-thienyl)ethylidene)benzohydrazide	Yes	No	Yes	No	Yes	Yes	No	-5.12	High
19	benzamide-N-(2,5-dimethoxyphenyl)-3-methoxy-	Yes	Yes	Yes	Yes	Yes	Yes	No	-5.66	High
20	Piperine	Yes	No	Yes	No	Yes	Yes	No	-5.58	High
21	carbamic acid, N-(2-naphthyl)-methyl ester	Yes	No	Yes	No	No	Yes	No	-5.57	High

Table 5: Toxicity prediction of effective compounds

S/N	Compound name	Mutagenicity	Hepatotoxicity	Carcinogenicity	Immunogenicity	Cytotoxicity
1	D-limonene	Inactive	Inactive	Inactive	Inactive	Inactive
2	1,6-octadien-3-ol, 3,7-dimethyl	Inactive	Inactive	Inactive	Inactive	Inactive
3	alpha copaene	Inactive	Inactive	Inactive	Inactive	Inactive
4	1,Z-5, E-7-dodecatriene	Inactive	Inactive	Inactive	Inactive	Inactive
5	Caryophyllene	Inactive	Inactive	Inactive	Inactive	Inactive
6	Humulene	Inactive	Inactive	Inactive	Inactive	Inactive
7	β -humulene	Inactive	Inactive	Inactive	Inactive	Inactive
8	Cis-beta-farnesene	Inactive	Inactive	Inactive	Inactive	Inactive
9	β -copaene	Inactive	Inactive	Inactive	Inactive	Inactive
10	β -selinene	Inactive	Inactive	Inactive	Inactive	Inactive
11	α -selinene	Inactive	Inactive	Inactive	Inactive	Inactive
12	β -bisabolene	Inactive	Inactive	Inactive	Active	Inactive
13	cyclohexene,3-(1,5-dimethyl-4-hexenyl)-6-methylene	Inactive	Inactive	Inactive	Inactive	Inactive
14	1,6, 10-dodecatrien-3-ol, 3,7, 11-trimethyl	Inactive	Inactive	Inactive	Inactive	Inactive
15	n-hexadecanoic acid	Inactive	Inactive	Inactive	Inactive	Inactive
16	9,12-Octadecadienoic acid (Z,Z)-	Inactive	Inactive	Inactive	Inactive	Inactive
17	1-piperonylpyrazole-3-carboxylic acid hydrazide	Active	Active	Active	Active	Inactive
18	4-methoxy-N'-(1-(2-thienyl)ethylidene)benzohydrazide	Active	Inactive	Inactive	Inactive	Inactive
19	benzamide-N-(2,5-dimethoxyphenyl)-3-methoxy-	Active	Active	Active	Active	Inactive
20	Piperine	Inactive	Inactive	Active	Active	Inactive
21	carbamic acid, N-(2-naphthyl)-methyl ester	Active	Active	Active	Inactive	Inactive

Table 6: Prediction of LD₅₀ and toxicity class of compounds

S/N	Compound name	Predicted LD ₅₀ (mg/kg)	Predicted toxicity class
1	D-limonene	330	class 4
2	1,6-octadien-3-ol, 3,7-dimethyl	5000	Class5
3	alpha copaene	3700	Class5
4	1,Z-5, E-7-dodecatriene	5700	Class 6
5	Caryophyllene	2000	Class 4
6	Humulene	3650	Class 5
7	β-humulene	5300	Class 5
8	Cis-beta-farnesene	5000	Class 5
9	β-copaene	4700	Class 5
10	β-selinene	5000	Class 5
11	α-selinene	5000	Class 5
12	β-bisabolene	4400	Class 5
13	cyclohexene,3-(1,5-dimethyl-4-hexenyl)-6-methylene	5000	Class 5
14	1,6, 10-dodecatrien-3-ol, 3,7, 11-trimethyl	5000	Class 5
15	n-hexadecanoic acid	900	Class 4
16	9,12-Octadecadienoic acid (Z,Z)-	10000	Class 6
17	1-piperonylpyrazole-3-carboxylic acid hydrazide	1000	Class 4
18	4-methoxy-N'-(1-(2-thienyl)ethylidene)benzohydrazide	1120	Class 4
19	benzamide-N-(2,5-dimethoxyphenyl)-3-methoxy-	9750	Class 6
20	Piperine	330	Class 4
21	carbamic acid, N-(2-naphthyl)-methyl ester	1500	Class 4

Construction of protein–protein-interaction (PPI) and hub genes

Protein–protein interaction (PPI) networks reveal the complex functional relationships between cellular proteins, offering deeper insight into biological processes and disease mechanisms (Rodina et al., 2023). In this study, a PPI network was built for 14 overlapping target genes, and the network consisted of 14 nodes connected by 25 edges, highlighting the strong interconnections and functional associations among these proteins (Figure 2).

To determine the most influential genes within the network, the cytoHubba plugin was applied, utilizing the maximum clique centrality (MCC) algorithm for ranking. Based on their notably high MCC scores, the five principal hub genes identified were EGFR, MAP2K1, SRC, KDR, and PIK3CA (Table 7). The EGFR is a critical regulator of cellular proliferation, differentiation, and survival pathways. Its overexpression has been documented in approximately 25–77% of colorectal cancer cases and is frequently correlated with unfavourable clinical outcomes (Lu et al., 2023). The MAPKs constitute a group of serine/threonine kinases that transmit extracellular stimuli into coordinated intracellular responses, thereby governing essential cellular functions such as growth, differentiation, and stress adaptation (Chen et al., 2025). Additionally, Src family kinases (SFKs), contribute significantly to the pathogenesis of colorectal cancer. The expression and activity of Src family kinases (SFKs) are elevated in patients with colorectal cancer, where their activation drives tumor cell proliferation, metastasis, chemoresistance, and the formation of cancer stem cells (CSCs) (Jin, 2020). The kinase insert domain receptor (KDR), is a key receptor for vascular endothelial growth factor (VEGF), which mediates signaling by VEGF ligands binding. The VEGF/KDR signaling is the primary pathway promoting angiogenesis through endothelial cell proliferation and increased vascular permeability (Taheri et al., 2019). The Phosphatidylinositol 3-kinase pathway (PI3K) signaling cascade is a key regulator of cellular survival, proliferation, and growth, and it is commonly altered in various solid malignancies (Fakih et al., 2023). Activation of function mutations in the PIK3CA oncogene occurs in roughly 20% of patients with colorectal cancer, where they drive tumor development and are often associated with adverse clinical outcomes (Voutsadakis, 2021). Current evidence indicates that there is no direct experimental study (*in vitro* or *in vivo*) specifically demonstrating that α - or β -selinene and copaene modulate EGFR, KDR, PI3K, MAP2K1, or SRC pathways. However, both *in vitro* and *in vivo* studies stated that α -humulene has been shown to suppress tumor cell proliferation and induce apoptosis primarily through inhibition of the PI3K/AKT pathway and MAPK/ERK pathway (Chen et al., 2019)

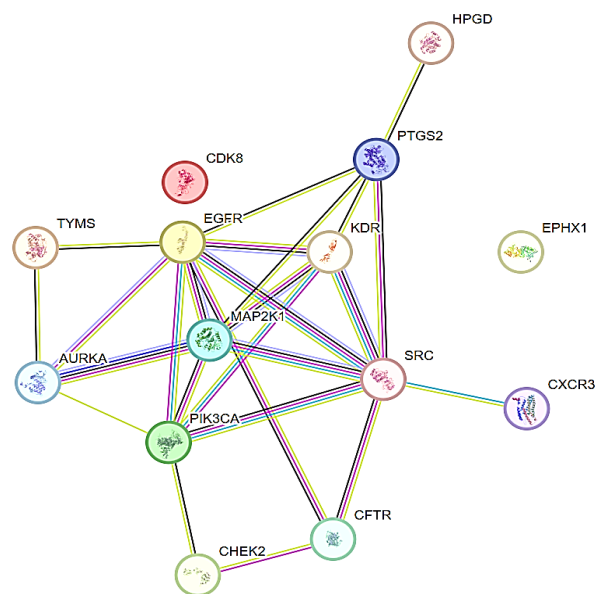


Figure 2: Protein–protein interaction Network: The nodes (14) represent proteins while the edges (25) indicate interactions between proteins. The edge thickness indicates the strength of interaction and the node colour indicate expression level.

Table 7: List of ten (10) hub genes

Rank	Name	Score
1	EGFR	58
2	MAP2K1	54
3	SRC	51
4	KDR	48
5	PIK3CA	31
6	PTGS2	25
7	AURKA	8
8	CFTR	3
9	CHEK2	2
9	TYMS	2

GO and KEGG enrichment analysis of intersecting targets

The GO offers a comprehensive and standardized system for classifying and describing gene functions and their corresponding products across different biological contexts (Wang et al., 2022). GO functional annotation and KEGG pathway enrichment analyses were conducted on 14 colon cancer–related target genes to provide insights into the possible molecular mechanisms of the bioactive compounds against colon cancer. The top 20 enriched GO terms for biological processes, cellular components, and molecular functions are illustrated as bubble plots in Figures 3A–D. The BP analysis highlighted processes such as regulation of anoikis, peptidyl-tyrosine autophosphorylation, protein autophosphorylation, and peptidyl-serine phosphorylation. In terms of CC, the most enriched components included the phosphatidylinositol 3-kinase complex, germinal vesicle, multivesicular body, and female germ cell nucleus. The MF analysis primarily

highlighted protein serine kinase activity, scaffold protein binding, and transmembrane receptor protein kinase activity while KEGG pathway enrichment revealed 151 signaling pathways, including VEGF signaling, bladder cancer, and Rap1 signaling, which are involved in the regulation of vascular tone and the function of endothelial

cells. Additionally, pathways such as EGFR tyrosine kinase inhibitor resistance, endometrial cancer, and ErbB signaling were enriched. These pathways may play critical roles in mediating the therapeutic effects of the studied compounds against colon cancer, with significance determined at a cutoff of $p < 0.05$.

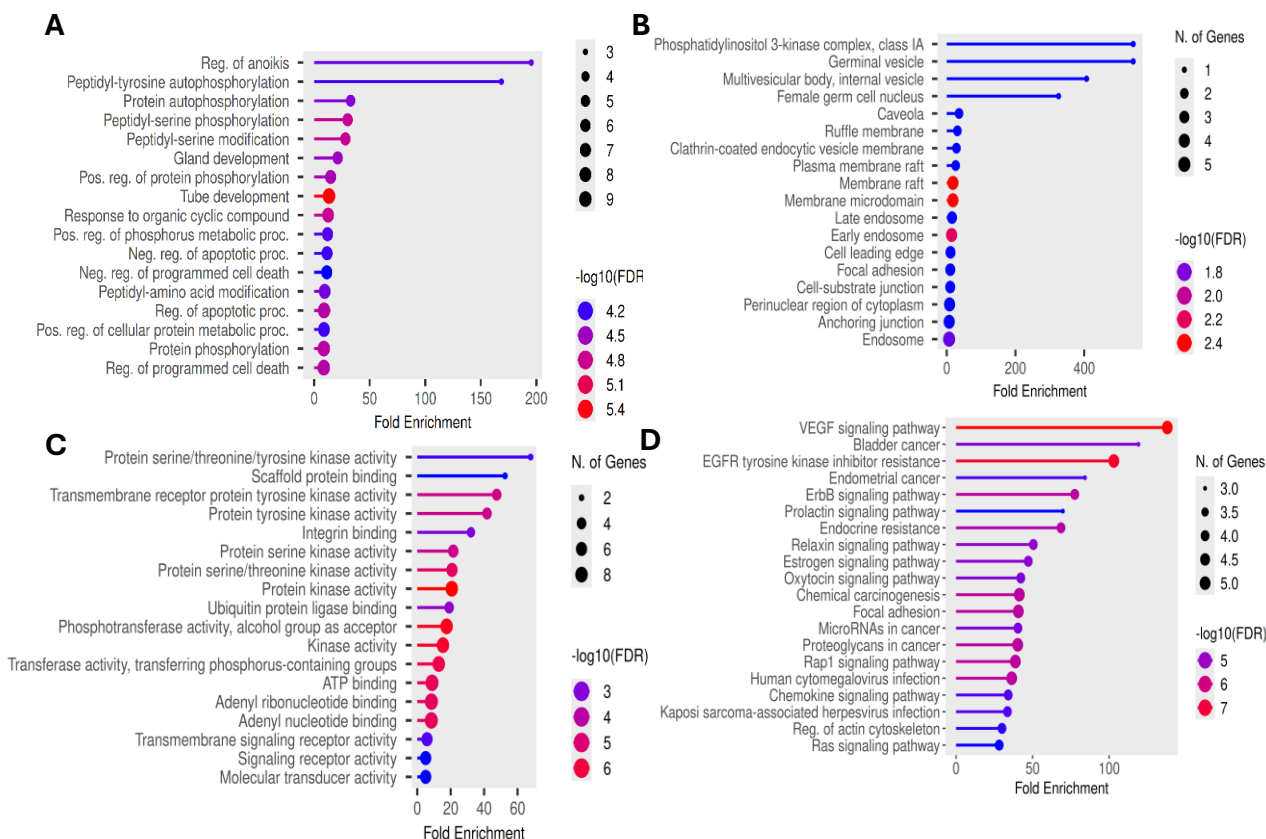


Figure 3: Gene ontology enrichment categories and functional insights such as A. Biological process B. Cellular Components C. Molecular function D. KEGG pathway.

Molecular docking

Molecular docking was employed to predict the interactions between compounds and target proteins, providing insights for the development of more effective and selective therapies (Nag et al., 2023). In this study, the selected phytochemicals exhibited strong binding affinities. The detailed docking outcomes for fifteen compounds such as D-limonene, 1,6-octadien-3-ol (3,7-dimethyl), alpha-copaene, 1,Z-5,E-7-dodecatriene, caryophyllene, humulene, beta-humulene, cis-beta-farnesene, beta-copaene, beta-selinene, alpha-selinene, cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene, 1,6,10-dodecatrien-3-ol (3,7,11-trimethyl), n-hexadecanoic acid, and 9,12-octadecadienoic acid (Z,Z) are summarized in Table 8. These compounds were docked against the five principal hub genes implicated in colon cancer, with 5-Fluorouracil serving as the reference standard for comparative evaluation. The 5-fluorouracil is a chemotherapeutic drug, which serves as a clinical benchmark to validate whether ligand shows better binding affinity than the current compounds. Although the direct mechanism of 5-Fluorouracil might differ from targeted inhibitors. Aside

from that, 5-fluorouracil acts as a pyrimidine analog that interrupts DNA and RNA synthesis. This allows the ligand to be compared against a broad-acting agent that affects multiple cellular pathways in rapidly dividing cancer cells. In molecular docking, lower (more negative) binding energies indicate stronger predicted interactions between a ligand and its target protein (Kumar et al., 2023). The results of this study showed that alpha- and beta-selinene exhibited favorable docking interactions with EGFR, MAP2K1, SRC, and PIK3CA, with binding energies of -7.6, -7.7, -5.1, -7.5 kcal/mol and -7.5, -7.7, -5.4, -7.6 kcal/mol, respectively. beta-Humulene displayed the most favourable binding energy of -8.7 kcal/mol with KDR among the hub genes. Overall, the majority of the compounds demonstrated strong predicted binding affinities with all five hub genes, often comparable to or better than the standard anticancer drug 5-fluorouracil.

Table 8: The binding affinity (kcal/mol) of compounds and core targets

Compound name	EGFR (PDB ID: 1M17)	MAP2K1 (PDB ID: 3DY7)	SRC (PDB ID: 104N)	KDR (PDB ID: 6GQ0)	PIK3CA (PDB ID: 6PYS)
D-limonene	-5.6	-6.4	-4.0	-6.6	-5.8
1,6-octadien-3-ol, 3,7-dimethyl	-5.3	-5.8	-4.2	-6.2	-5.1
alpha copaene	-7.2	-6.6	-4.9	-7.6	-7.1
1,Z-5, E-7-dodecatriene	-5.0	-5.9	-3.5	-6.2	-5.8
Caryophyllene	-7.1	-6.7	-5.0	-7.6	-7.5
Humulene	-6.8	-6.8	-4.7	-7.8	-7.6
β-humulene	-6.8	-7.3	-4.9	-8.7	-7.5
Cis-beta-farnesene	-5.9	-6.8	-3.8	-6.9	-5.6
β-copaene	-6.8	-7.5	-4.8	-8.0	-7.4
β-selinene	-7.5	-7.7	-5.4	-8.0	-7.6
α-selinene	-7.6	-7.7	-5.1	-8.4	-7.5
cyclohexene,3-(1,5-dimethyl-4-hexenyl)- 6-methylene	-6.6	-7.6	-4.1	-8.4	-6.3
1,6, 10-dodecatrien-3-ol, 3,7, 11-trimethyl	-6.0	-6.9	-4.7	-7.6	-6.0
n-hexadecanoic acid	-5.2	-6.0	-3.6	-6.5	-5.2
9,12-Octadecadienoic acid (Z,Z)-	-5.9	-6.6	-4.0	-7.0	-6.2
5-fluorouracil (standard drug)	-5.2	-5.0	-4.0	-5.5	-5.4

Conclusion

In summary, fifteen (15) active compounds were identified from *P. guineense* seeds as potential candidates require experimental validation. Furthermore, this study employed GC-MS analysis, network pharmacology, and molecular docking to systematically identify key bioactive components, core targets, and potential mechanisms of action. Network pharmacology and molecular docking predict the molecular mechanism of action and binding affinity, which require further investigation into gene expression modulation via *in vitro* and *in vivo* studies. α-selinene, β-selinene, β-copaene, and humulene were highlighted as the most promising compounds that demonstrated favourable binding affinities toward key colorectal cancer-associated targets out of the 15 compounds that passed the toxicological evaluation and Lipinski rule for oral drug. This study offers preliminary computational insights into potential therapeutic strategies for colon cancer and provides a foundation for the development of new anticancer agents. This study recommends *in vitro* and *in vivo* studies as an experimental validation of those bioactive compounds against colorectal cancer.

Conflict of Interest

The authors declare no conflict of interest.

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