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Anticancer Activity of Ethanol Extract of *Kleinhovia hospita* L. Leaves Against HCT-116 Colorectal Cancer Cells

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ABSTRACT

Colorectal cancer is characterized by abnormal cell growth in the colon or rectum and is strongly associated with increased expression of cyclooxygenase-2 (COX-2). COX-2 activation enhances cell proliferation and suppresses apoptosis, thereby promoting cancer progression. Leaves of *Kleinhovia hospita* are known to contain alkaloids, flavonoids, and saponins, which may serve as anticancer agents by inhibiting COX-2 activity. This study aimed to evaluate the anticancer potential of bioactive compounds in the ethanol extracts of *Kleinhovia hospita* L. leaves using both in vitro and in silico approaches. The extract was obtained by maceration with 96% ethanol. Cytotoxicity against HCT-116 colorectal cancer cells was evaluated using the MTT assay at extract concentrations of 500, 250, 125, 62.5, and 31.25 µg/mL. LC-MS analysis identified the compounds, which were further screened using PASS Online, ProTox 3.0, and Lipinski's rule, followed by molecular docking against COX-2. The ethanol extract showed moderate cytotoxicity, with an IC_{50} value of 85.113 µg/mL. Fourteen compounds were predicted to possess anticancer-related bioactivities based on in silico screening. Among them, N-trans-Feruloyltyramine demonstrated strong binding to COX-2 with an affinity of –8.3798 kcal/mol, surpassing the native ligand. These findings indicate that the ethanol extract of *K. hospita* leaves and its bioactive constituents may contribute to anticancer activity, potentially through COX-2–related pathways, as suggested by in silico analyses.

Keywords: Anticancer, Cell line, Colorectal cancer, HCT-116, Molecular docking, *Kleinhovia hospita* leaves.

Introduction

Colorectal cancer is the abnormal growth of cells in the large intestine, colon, or rectum, or both. It is the third most common type of cancer worldwide. According to GLOBACON 2022 data, there were 1,926,118 new cases of colorectal cancer and 903,859 deaths due to the disease. In Indonesia, colorectal cancer ranks fourth in terms of incidence.[1] By 2040, the burden of colorectal cancer is projected to increase to 3.2 million new cases per year (a 63% increase) and 1.6 million deaths per year (a 73% increase).[2] Current cancer treatments have several limitations, including high costs, significant side effects, high recurrence rates, impaired immune function, and the development of therapy resistance.[3] Recent developments in colorectal cancer therapy increasingly emphasize molecularly targeted agents designed to overcome treatment resistance and improve long-term clinical outcomes.[4] Therapeutic agents for cancer treatment and protective effects can be obtained from bioactive compounds that act as antioxidants, anti-inflammatory agents, and anti-cancer agents.[5]

In colorectal cancer, COX-2 expression is significantly higher than in normal colorectal tissue. COX-2 overexpression promotes prostaglandin-mediated inflammation, enhancing cell proliferation, suppressing apoptosis, and facilitating metastatic progression.[6] Cancer inhibition of COX-2 in colorectal treatment is essential; therefore, recent research has shifted toward therapeutic agents that can reduce COX-2 expression and induce apoptosis in cancer cells, as apoptosis is a crucial mechanism in cancer treatment.[7]

Research on the colorectal anticancer potential of various plant extracts has been conducted in vitro, including studies using *Helianthus annus* seeds on the Caco-2 cell line,[8] and using *Artemisia sieversiana* ethanol extract on the HT-29, HCT-15, and COLO-205 cell lines, which showed potential as colorectal anticancer agents.[9] Additionally, clove constituent Eugenol has also been used in the treatment of colorectal cancer cells including the SW-620, CACO-2, HCT-116, HCT-15, and HT-29 cell lines.[10] Although Kleinhovia hospita has been examined for several pharmacological activities, its anticancer potential in colorectal cancer, specifically within the HCT-116 model, remains insufficiently characterized, indicating a clear gap that this study aims to address.

The *Kleinhovia hospita* plant, often referred to as timoho wood or paliasa, is commonly used as an anticancer, antidiabetic, and hepatoprotective medicine among indigenous communities. The leaves of *Kleinhovia hospita* are also believed by the community to treat hepatitis.[11] *Kleinhovia hospita* leaves contain alkaloids (2.83%), flavonoids (19.78%), and saponins (14.23%). A methanol extract of *Kleinhovia hospita* leaves exhibits strong antioxidant effects compared to vitamin C using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method.[12] This study was designed to evaluate the cytotoxic effects of *K. hospita* leaf extract on HCT-116 colorectal cancer cells and to explore the predicted interactions of its constituent compounds with COX-2 through in silico approaches. This study has significant implications for health sciences, particularly in the discovery of anticancer compounds for new drug candidates or the integrating of herbal medicines, thereby increasing the availability of effective anticancer drugs made from easily obtainable materials with minimal risks.

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Materials and Methods

Chemical and reagents
The ethanol extract of *Kleinhovia hospita* L. leaves was prepared and used as the test material. Ethanol (96%), trypan blue, RPMI-1640 medium, fetal bovine serum (FBS), penicillin–streptomycin, trypsin–EDTA (0.05%), phosphate-buffered saline (PBS), and the MTT assay kit were obtained from standard commercial suppliers. The HCT-116 colorectal cancer cell line was used for the in vitro assays. The COX-2 protein structure was retrieved from the Protein Data Bank (PDB ID: 6COX).

Preparation of Ethanol Extract of Kleinhovia hospita L. *Leaves*
Leaves of *Kleinhovia hospita* L. were collected, cleaned, cut into small pieces, and air-dried for three days. The dried leaves were then ground into a fine powder. The plant material was identified and authenticated at the Herbarium Universitas Andalas (ANDA) with voucher specimen number 61/K-ID/ANDA/I/2023. The resulting *K. hospita* simplicia was macerated in 96% ethanol at a 2:3 ratio in a dark bottle. Maceration was carried out for three days with twice-daily stirring (morning and evening). The macerated mixture was filtered, and the filtrate was evaporated using a rotary evaporator at 64.6 °C until a concentrated extract was obtained.

MTT Cytotoxicity Assay
HCT-116 cells were cultured in RPMI-1640 medium supplemented with 10% FBS and 1% penicillin–streptomycin, and incubated at 37 °C with 5% CO_2 until ~80% confluence. Cells were seeded into 96-well plates at a density of 2×10^4 cells per well in 200 µL of medium and incubated for 24 hours. Cells were then treated with *K. hospita* leaf extract at concentrations of 500, 250, 125, 62.5, and 31.25 µg/mL and incubated for 24 hours. For the MTT assay, 9 mL of complete medium was mixed with 1 mL of MTT reagent in a 15-mL centrifuge tube and homogenized. Then, 100 µL of this mixture was added to each well and incubated at 37 °C with 5% CO_2 for 24 hours until formazan crystals formed. After incubation, DMSO was added to dissolve the formazan crystals, and absorbance was measured at 550 nm using a Multiskan EX microplate reader.

Liquid Chromatography-Mass Spectrometry (LC-MS) Analysis
The *K. hospita* leaf extract was analyzed using Liquid Chromatography–Mass Spectrometry (LC-MS) to identify its bioactive compounds. A total of 10.00 mg of extract was dissolved in methanol in a 10 mL volumetric flask. Using a microsyringe, 5 µL of the methanol-dissolved extract was injected into the mobile phase stream. The components of the extract were detected by the LC-MS detector, and the results were recorded as a chromatogram.[13]

Prediction of Biological Activities of Bioactive Compounds from Ethanol Extract of Kleinhovia hospita L. *Leaves Using PASS Online*
The chemical formulas obtained were verified using the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) to retrieve the canonical SMILES. The SMILES codes were then entered and analyzed using the PASS Online server (http://way2drug.com/PassOnline/index.php). PASS Online generated Pa (probability of activity) and Pi (probability of inactivity) values, enabling preliminary prediction of pharmacological potentials.[14] These values were interpreted as follows: if Pa > 0.7, the probability of obtaining similar activity experimentally is considered high; if 0.5 < Pa < 0.7, the probability is moderate; and if Pa < 0.5, the probability is low.

Bioavailability Analysis of Bioactive Compounds from the Ethanol Extract of Kleinhovia hospita L. *Leaves*
Bioavailability prediction was performed to evaluate the permeability and solubility of compounds in the body. The prediction was based on Lipinski's Rule of Five for bioactive compounds identified in *Kleinhovia hospita* leaves extracts, which were further used as ligands in the molecular docking process. According to Lipinski's rules, compounds should meet the following criteria: partition coefficient (log P) < 5, molar refractivity between 40-130, molecular weight < 500 Da, no more than five hydrogen bond donors, and no more than ten hydrogen bond acceptors. The analysis was carried out using the SwissADME online tool (http://www.swissadme.ch/).

Molecular docking
The molecular docking procedure included ligand preparation followed by preparation of the COX-2 crystal structure. Redocking was performed to validate the docking protocol, with an RMSD threshold of < 2 Å. Bioactive compounds that met Lipinski's criteria were docked onto the target protein (COX-2) using MOE software. After obtaining the docking score, RMSD, and bond distances for each ligand, these values were recorded and visualized to determine the interactions between the protein and ligand. Docking was performed to predict the interaction between each ligand and the target protein.

Results and Discussion

Cytotoxicity of Ethanol Extract of Kleinhovia hospita L. *Leaves on HCT-116 Cells (MTT Assay)*
Ethanolic leaf extract of *Kleinhovia hospita* reduced HCT-116 cell viability in a concentration-dependent manner. Morphological changes observed included cell shrinkage, detachment, and membrane blebbing. No microbial contamination was detected, confirming aseptic culture conditions with antibiotics (Figure 1).

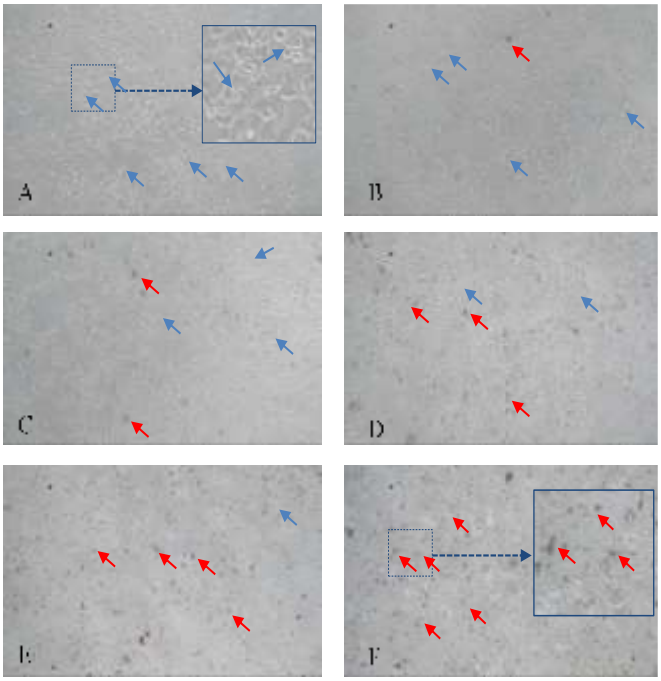

Figure 1: Phase-contrast images of HCT-116 cells after treatment with *Kleinhovia hospita* leaves extract and incubation for 24 hours: (A) control cells, (B) 31.25 µg/mL, (C) 62.5 µg/mL, (D) 125 µg/mL, (E) 250 µg/mL, and (F) 500 µg/mL. Note: blue arrows indicate viable cells, while red arrows indicate cell death.

The cytotoxic activity of the ethanol extract of *Kleinhovia hospita* leaves against the HCT-116 cell line yielded an IC_{50} value of 85.113 µg/mL (Table 1, Figure 2). According to the National Cancer Institute (NCI) criteria, this value is categorized as moderate cytotoxic activity, indicating that the ethanol extract of *Kleinhovia hospita* leaves possesses potential anticancer properties. Colorectal cancer, which is strongly associated with COX-2 overexpression, remains a significant global health challenge. In this study, ethanolic leaf extract of *Kleinhovia hospita* demonstrated moderate cytotoxicity against HCT-116 colorectal cancer cells, with an IC_{50} of 85.113 µg/mL, as classified by NCI standards. The concentration-dependent reduction in cell viability, characterized by morphological alterations such as shrinkage, detachment, and membrane blebbing, is consistent with previous

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reports on the antiproliferative effects of plant-derived metabolites.[15,16,17] Although the cytotoxicity observed was moderate, these results highlight the potential of *Kleinhovia hospita* leaf extract to interfere with colorectal cancer progression. Interestingly, a lower IC_{50} value was reported against MCF-7 cells,[3] suggesting that cytotoxic potency may be cell-type specific.

Table 1: Cytotoxicity results of the ethanolic extract of *Kleinhovia hospita* leaves determined by the MTT assay

Concentration (ug/mL)	Cell viability (%)	IC_{50}
31.25	72.863	
62.5	61.656	
125	44.812	85.113 µg/ml
250	14.216	
500	4.539	

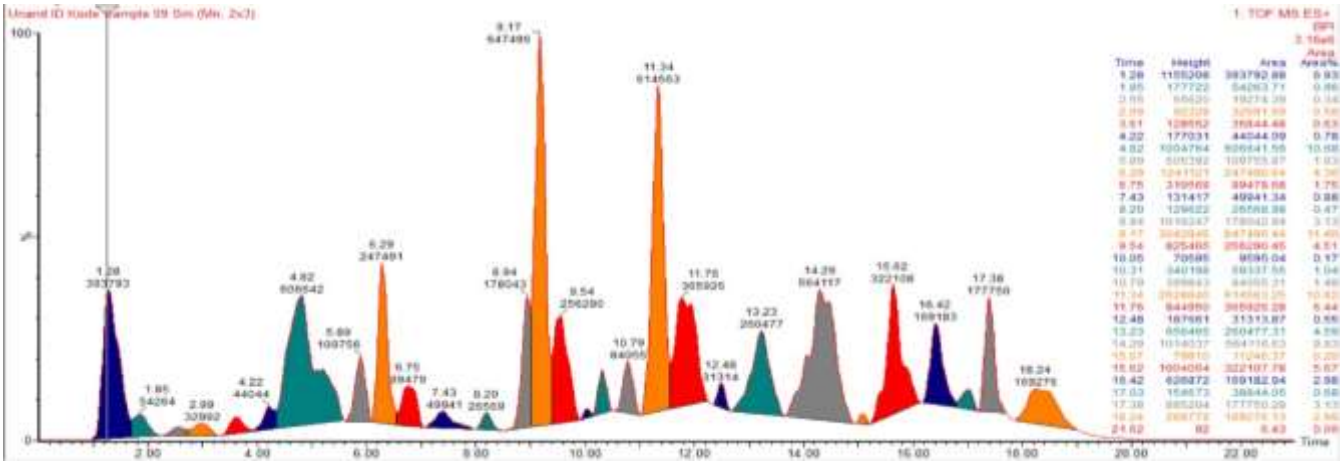

Figure 2: Chromatographic profile of the ethanol extract of *Kleinhovia hospita* leaves obtained from LC-MS analysis.

LC-MS Identification of Bioactive Compounds from Ethanol Extract of Kleinhovia hospita L. *Leaves*

The identification of bioactive compounds from the ethanol extract of *Kleinhovia hospita* leaves was performed using LC-MS analysis, which revealed 29 peaks (Figure 2). Based on MassLynx 4.1 interpretation, 28 compounds were identified due to two peaks sharing the same molecular formula, as summarized in Table 2. Among them, Dihydrodaidzein 7-O-glucuronide showed the highest peak area (10.68%) (Table 2).

The moderate cytotoxic activity of *Kleinhovia hospita* ethanol extract against HCT-116 cells, as previously discussed, is likely attributed to the presence of diverse secondary metabolites identified through LC-MS analysis. A total of 28 bioactive compounds were detected, with Dihydrodaidzein 7-O-glucuronide showing the highest percentage area (10.68%). This isoflavonoid derivative has been reported to exhibit strong antioxidant and anticancer properties by modulating cellular metabolism and scavenging free radicals.[18] In addition, phenolic compounds such as N-trans-feruloyltyramine and 2-hydroxybenzaldehyde oxime were also identified, which are known to suppress cancer progression through inhibition of NF-κB and AP-1 signaling pathways.[19]

The presence of both flavonoids and phenolics supports the cytotoxic potential observed in HCT-116 cells, as these compounds may act synergistically to inhibit cancer cell proliferation. Compared with the previous study, which identified only 10 compounds using LC-MS and 9 compounds using GC-MS, this study detected a broader profile of metabolites.[3] Such variations can be explained by differences in extraction methods, analytical techniques, and environmental growth conditions.[20]. The diverse profile of flavonoids and phenolic compounds identified through LC-MS provides a plausible biochemical basis for the observed cytotoxicity, as these metabolite classes are frequently associated with antiproliferative activity in colorectal cancer models.

Prediction of Anticancer Properties of Bioactive Compounds in Ethanol Extract of Kleinhovia hospita L. *Leaves through PASS Online Analysis*

PASS Online analysis revealed that 14 compounds from *Kleinhovia hospita* leaves exhibited antineoplastic, anticarcinogenic, and anti-inflammatory properties. Among these, 12 compounds showed high antineoplastic activity, while 2 compounds demonstrated low antineoplastic activity, although the latter were specifically predicted to target colorectal cancer. The PASS predictions highlight several metabolites with potential anticancer relevance; however, these activities remain hypothetical and require experimental verification before their functional significance can be established (Table 3).

In silico toxicity profiling using ProTox 3.0 classified compounds across classes II–VI, with loliolide displaying higher toxicity (LD50 34 mg/kg) and N-trans-feruloyltyramine lower toxicity (LD50 500 mg/kg), supporting the relevance of safety evaluation in early drug discovery (Supplementary 1).[21-24] Furthermore, PASS analysis predicted antineoplastic, anticarcinogenic, and anti-inflammatory activities for 14 compounds, with Pa values above 0.5-0.7, indicating high probability of biological activity.[25] Notably, loliolide has been reported to inhibit EMT and metastasis in colorectal and breast cancers,[26,27] while brassicasterol demonstrated strong anticancer and anti-infective potential.[28,29] These findings underscore the multifaceted pharmacological potential of *Kleinhovia hospita* leaf constituents, particularly in targeting inflammation-driven carcinogenesis.[30,31]

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Table 2: Bioactive Compounds in the Ethanol Extract of *Kleinhovia hospita* Leaves

No	Compounds	Molecular Formula	Retention Time (minutes)	Area (%)	Compound Class	Molecule Mass (g/mol)
1.	2-Hydroxybenzaldehyde oxime	$C_7H_7NO_2$	1.28	6.93	Phenols	137.138
2.	O-Glutarylcarnitine	$C_{12}H_{21}NO_6$	1.85	0.96	Fatty Acyls	275.301
3.	Isoindoline	C_8H_9N	2.55	0.34	Isoindoles	199.167
4.	3-[[(2S)-2,4-Dihydroxy-3,3-dimethylbutanoyl]amino]propanoic acid	$C_9H_{17}NO_5$	2.99	0.58	Carboxylic Acids	219.235
5.	Indoleacrylic acid	$C_{11}H_9NO_2$	3.61	0.63	Indoles	187.198
6.	Apiosylglucosyl 4-hydroxybenzoate	$C_{18}H_{24}0_{12}$	4.22	0.78	Organooxygen compounds	432.376
7.	Dihydrodaidzein 7-O-glucuronide	$C_{21}H_{20}O_{10}$	4.82	10.68	Isoflavonoids	432.3775
8.	Loliolide	$C_{11}H_{16}O_3$	5.89	1.93	Benzofurans	196.2429
9.	azane;1-[4-[(2S,3R,4R,5R,6S)-4,5-dihydroxy-6-methyl-2-[(2R,3R,4S,5S,6R)-2,4,5-trihydroxy-6-(hydroxymethyl)oxan-3-yl]oxyoxan-3-yl]oxy-2,6-dihydroxyphenyl]-3-(3-hydroxy-4-methoxyphenyl)propan-1-one	$C_{28}H_{39}NO_{15}$	6.29	4.36	Unknown	629.6
10.	N-trans-feruloyltyramine	$C_{18}H_{19}NO_4$	6.75	1.75	Phenols	313.3478
11.	(9S)-9-Acetyl-7-[(2R,4S,6S)-4-amino-5-hydroxy-6-methyloxan-2-yl]oxy-6,9,11-trihydroxy-8,10-dihydro-7H-tetracene-5,12-dione	$C_{26}H_{27}NO_9$	7.43	0.88	Anthracyclines	497.4939
12.	Nandrolone	$C_{18}H_{26}O_2$	8.20	0.47	Steroids	274.3978
13.	methyl (2R,3S,4S,5R,6R)-5-acetyloxy-6-[(2R,3S,4R,5R,6S)-4-acetyloxy-2-(acetyloxymethyl)-6-methoxy-5-(phenylmethoxycarbonylamino)oxan-3-yl]oxy-3-hydroxy-4-phenylmethoxyoxane-2-carboxylate	$C_{35}H_{43}NO_{16}$	8.94 and 9.17	3.13 and 11.40	Unknown	733.7
14.	[(1S,3R,14S,15S,18S,19R,20R,21S,22S,24R,25R,26R)-19,20,22,25-tetraacetyloxy-3,14,15,26-tetramethyl-6,16,23-trioxo-2,5,17-trioxa-8-azapentacyclo[16.7.1.01,21.03,24.07,12]hexacosa-7(12),8,10-trien-21-yl]methyl acetate	$C_{37}H_{45}NO_{16}$	9.54	4.51	Unknown	759.7
15.	Neoacrimarine G	$C_{29}H_{25}NO_8$	10.05	0.17	Benzena	515.5107
16.	(19,20,22,25-tetraacetyloxy-13-ethyl-26-hydroxy-3,26-dimethyl-6,16,23-trioxo-2,5,17-trioxa-11-azapentacyclo[16.7.1.$0^{1,21}$.$0^{3,24}$.$0^{7,12}$]hexacosa-7(12),8,10-trien-21-yl)methyl acetate	$C_{37}H_{45}NO_{17}$	10.31	1.04	Unknown	775.7
17.	[(3*R*,6*S*,7*R*,8*S*,12*R*,13*S*,14*S*,15*R*)-6-[(2*S*,3*R*,4*S*,6*R*)-4-(dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy-8-[(2*R*,4*S*,5*S*,6*S*)-4-methoxy-6-methyl-5-propanoyloxyoxan-2-yl]oxy-5,7,9,12,13,15-hexamethyl-10,16-dioxo-1,11-dioxaspiro[2.13]hexadecan-14-yl] propanoate	$C_{41}H_{69}NO_{14}$	10.79	1.48	Unknown	800.0

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18.	4*S*)-5-[[(2*S*)-1-[2-carboxy-2-[3-[furan-2-ylmethyl(pyridin-4-ylmethyl)carbamoyl]oxirane-2-carbonyl]hydrazinyl]-3-methyl-1-oxobutan-2-yl]amino]-4-[[(2*S*)-3-carboxy-2-(phenylmethoxycarbonylamino)propanoyl]amino]-5-oxopentanoic acid	$C_{38}H_{43}N_7O_{15}$	11.34	10.82	Unknown	837.8
19.	(2*S*,6*S*,8*S*,9*R*,12*Z*,14*E*,16*R*,25*S*,27*S*)-16-ethyl-6,8,9-trihydroxy-12-(methoxymethyl)-25,27-dimethyl-2-propyl-1-oxa-4-azacyclooctacosa-12,14-diene-3,20,24,28-tetrone	$C_{35}H_{59}NO_9$	11.76	6.44	Unknown	637.8
20.	(3alpha,20R,24Z)-3-Hydroxy-21-oxoeupha-8,24-dien-26-oic acid	$C_{30}H_{46}O_4$	12.48	0.55	Prenol lipids	470
21.	methyl (2*R*,4*aS*,6*aR*,6*aS*,14*aS*)-10-hydroxy-2,4*a*,6*a*,6*a*,9,14*a*-hexamethyl-11-oxo-1,3,4,5,6,13,14,14*b*-octahydropicene-2-carboxylate	$C_{30}H_{40}O_4$	13.23 & 14.29	4.59 & 9.93	Unknown	464.6
22.	3-[[(E)-4-(dimethylamino)but-2-enoyl]amino]-N-[4-[[4-(2-phenylpyrazolo[1,5-a]pyridin-3-yl)pyrimidin-2-yl]amino]phenyl]benzamide	$C_{36}H_{32}N_8O_2$	15.07	0.20	Unknown	608.7
23.	Scortechinone B	$C_{34}H_{40}O_9$	15.62	5.67	Unknown	529.7
24.	Ganoderal A	$C_{30}H_{44}O_2$	16.42	2.98	Prenol lipids	436.6692
25.	[(2*R*,3*S*,4*R*,5*R*,7*S*,9*S*,10*S*,11*R*,12*S*,13*R*)-2-[(2*S*)-1-[(2*R*,3*R*,4*R*,5*R*,6*R*)-5-hydroxy-3,4-dimethoxy-6-methyloxan-2-yl]oxypropan-2-yl]-10-[(2*S*,3*R*,4*E*,6*R*)-3-hydroxy-4-methoxyimino-6-methyloxan-2-yl]oxy-3,5,7,9,11,13-hexamethyl-6,14-dioxo-7-(oxolan-2-ylmethylcarbamoyloxy)-12-[[(2*S*,7*R*)-2,4,5-trimethyl-1,4-oxazepan-7-yl]oxy]-oxacyclotetradec-4-yl] 3-methylbutanoate	$C_{56}H_{97}N_3O_{19}$	17.03	0.68	Unknown	1116.4
26.	DG(14:1n5/0:0/22:5n6)	$C_{39}H_{64}O_5$	17.38	3.13	Glycerolipids	612.936
27.	Brassicasterol	$C_{28}H_{46}O$	18.24	2.98	Steroids dan Steroids derivatives	398.3549
28.	6-*N*-[2-[[4,6-bis(dimethylamino)-1,3,5-triazin-2-yl]amino]ethyl]-2-*N*,2-*N*,4-*N*,4-*N*-tetramethyl-1,3,5-triazine-2,4,6-triamine	$C_{16}H_{30}N_{12}$	21.62	0.00	Unknown	390.49

Prediction of Bioavailability of Bioactive Compounds from Kleinhovia hospita Leaves Ethanol Extract Using Lipinski's Rule of Five

In the bioavailability study of 14 compounds predicted by PASS Online, seven compounds were found to comply with Lipinski's Rule of Five (Table 4). The bioavailability of bioactive compounds is an essential consideration in drug discovery, as it determines whether a compound can be absorbed and utilized effectively in the human body. In this study, PASS online analysis was applied to evaluate the drug-likeness of 14 compounds from *Kleinhovia hospita* leaf extract using Lipinski's Rule of Five. The analysis showed that seven compounds fulfilled Lipinski's criteria, indicating their potential as orally active candidates. According to Lipinski's rule, ideal drug-like compounds should have a molecular weight < 500 Da, logP < 5, no more than five hydrogen bond donors, and no more than ten hydrogen bond acceptors.[32] These parameters collectively serve as indicators of oral bioavailability and absorption.

In Silico Molecular Docking of Bioactive Compounds from ethanol extract of Kleinhovia hospita L. *leaves against COX-2*

Docking validation was performed by re-docking the native ligand (S58) into the COX-2 protein (PDB ID: 6COX). The re-docking yielded an RMSD value of 1.1676 Å (Table 5), which is below the 2 Å threshold, indicating a valid docking protocol. A lower RMSD confirms that the predicted ligand pose closely aligns with the crystallographic conformation. Molecular docking of seven compounds from the ethanolic leaves extract of *Kleinhovia hospita* leaves, together with the native ligand (S58) and the reference drug celecoxib, showed RMSD values below 2 Å.

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Table 3: Predicted Anticancer Activity of Bioactive Compounds from the Ethanol Extract of Leaves

No	Compounds	Pa	Pi	Bioactivity
1	Apiosylglucosyl 4-hydroxybenzoate	0.828	0.009	Antineoplastic
		0.818	0.005	Anticarcinogenic
		0.744	0.011	Antiinflammatory
2	Dihydrodaidzein 7-O-glucuronide	0.713	0.024	Antineoplastic
		0.839	0.004	Anticarcinogenic
		0.613	0.029	Antiinflammatory
3	Loliolide	0.746	0.019	Antineoplastic
		0.437	0.026	Anticarcinogenic
		0.515	0.053	Antiinflammatory
4	azane;1-[4-[(2S,3R,4R,5R,6S)-4,5-dihydroxy-6-methyl-2-[(2R,3R,4S,5S,6R)-2,4,5-trihydroxy-6-(hydroxymethyl)oxan-3-yl]oxyoxan-3-yl]oxy-2,6-dihydroxyphenyl]-3-(3-hydroxy-4-methoxyphenyl)propan-1-one	0.730	0.021	Antineoplastic
		0.865	0.003	Anticarcinogenic
		0.628	0.026	Antiinflammatory
5	N-trans-feruloyltyramine	0.149	0.090	Antineoplastic (colorectal cancer)
		0.373	0.036	Anticarcinogenic
		0.333	0.049	Antiinflammatory
6	Nandrolone	0.720	0.023	Antineoplastic
		0.393	0.032	Anticarcinogenic
		0.456	0.071	Antiinflammatory
7	methyl (2R,3S,4S,5R,6R)-5-acetyloxy-6-[(2R,3S,4R,5R,6S)-4-acetyloxy-2-(acetyloxymethyl)-6-methoxy-5-(phenylmethoxycarbonylamino)oxan-3-yl]oxy-3-hydroxy-4-phenylmethoxyoxane-2-carboxylate	0.781	0.014	Antineoplastic
		0.495	0.020	Anticarcinogenic
		0.519	0.051	Antiinflammatory
8	Neoacrimarine G	0.735	0.020	Antineoplastic
		0.357	0.039	Anticarcinogenic
		0.281	0.180	Antiinflammatory
9	[(3*R*,6*S*,7*R*,8*S*,12*R*,13*S*,14*S*,15*R*)-6-[(2*S*,3*R*,4*S*,6*R*)-4-(dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy-8-[(2*R*,4*S*,5*S*,6*S*)-4-methoxy-6-methyl-5-propanoyloxyoxan-2-yl]oxy-5,7,9,12,13,15-hexamethyl-10,16-dioxo-1,11-dioxaspiro[2.13]hexadecan-14-yl] propanoate	0.807	0.110	Antineoplastic
		0.260	0.077	Anticarcinogenic
		0.388	0.102	Antiinflammatory
10	(3alpha,20R,24Z)-3-Hydroxy-21-oxoeupha-8,24-dien-26-oic acid	0.860	0.006	Antineoplastic
		0.506	0.019	Anticarcinogenic
		0.605	0.031	Antiinflammatory
11	methyl (2*R*,4*aS*,6*aR*,6*aS*,14*aS*)-10-hydroxy-2,4*a*,6*a*,6*a*,9,14*a*-hexamethyl-11-oxo-1,3,4,5,6,13,14,14*b*-octahydropicene-2-carboxylate	0.829	0.009	Antineoplastic
		0.207	0.113	Anticarcinogenic
		0.821	0.005	Antiinflammatory
12	Scortechinone B	0.806	0.004	Antineoplastic (colorectal cancer)
		0.740	0.007	Anticarcinogenic
		0.320	0.143	Antiinflammatory
13	Ganoderal A	0.733	0.021	Antineoplastic
		0.290	0.062	Anticarcinogenic
		0.668	0.020	Antiinflammatory
14	Brassicasterol	0.735	0.020	Antineoplastic
		0.385	0.033	Anticarcinogenic
		0.561	0.040	Antiinflammatory

N-trans-feruloyltyramine had the lowest binding affinity (-8.3798 kcal/mol), stronger than the native ligand (-7.3046 kcal/mol), while loliolide showed a docking score of -5.2308 kcal/mol (Table 6), the docking visualizations are shown in Figure 3. Most compounds from *Kleinhovia hospita* leaves ethanol extract were found to have molecular weights below 500 Da, which suggests good membrane permeability. However, five compounds exceeded this threshold, indicating possible challenges in absorption.[33] Lipophilicity, represented by logP, also plays a critical role in absorption. Compounds with logP values below 5 are generally well-absorbed because they can diffuse through lipid membranes without losing solubility. Several *Kleinhovia hospita* leaves compounds exhibited favorable logP values, supporting their potential for good permeability, although excessively negative values may reduce membrane transport efficiency.[34] Hydrogen bond donors and acceptors were also within acceptable limits for most compounds, suggesting efficient passive diffusion through membranes.[35] In addition, molar refractivity values, which reflect molecular polarizability, indicated that most compounds fall within the safe range of 40-130. However, a few compounds outside this range may pose potential toxicity risks.[36] Taken together, these results suggest that *Kleinhovia hospita* leaves contains several bioactive compounds with physicochemical properties suitable for drug development. Potential molecular interactions were further explored through docking simulations, which revealed favorable binding orientations for several metabolites within the COX-2 active site. Colorectal cancer is strongly associated with COX-2 overexpression, which promotes tumor growth, angiogenesis, and resistance to apoptosis. Docking simulations revealed favorable binding orientations for several *K. hospita* metabolites within the COX-2 active site, suggesting a potential interaction that warrants further biochemical validation. One compound yielded a positive docking score, indicating an energetically unfavorable or non-binding interaction; therefore, this ligand was excluded from mechanistic interpretation to maintain analytical rigor.

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Complementing these in silico observations, the extract produced measurable anticancer activity in vitro, inducing a concentration-dependent decline in HCT-116 cell viability accompanied by characteristic morphological features of cell death, including shrinkage, membrane blebbing, and detachment.

Table 4: Lipinski's rule screening of bioactive compounds from *Kleinhovia hospita* leaves extract with anticancer potential

No	Compounds	Molecule weight < 500 Da	LogP ≤ 5 (Å)	H-acceptor < 10	H-donor < 5	Mol. Refractivity (40-130)
1	Dihydrodaidzein 7-O-glucuronide	432	0.022	10	5	102.06
2	Loliolide	196	1.409	3	1	51.60
3	N-trans-feruloyltyramine	313	2.478	5	3	88.51
4	(9S)-9-Acetyl-7-[(2R,4S,6S)-4-amino-5-hydroxy-6-methyloxan-2-yl]oxy-6,9,11-trihydroxy-8,10-dihydro-7H-tetracene-5,12-dione	497	3.356	9	5	122.23
5	Nandrolone	274	3.489	2	1	122.23
6	[(3*R*,6*S*,7*R*,8*S*,12*R*,13*S*,14*S*,15*R*)-6-[(2*S*,3*R*,4*S*,6*R*)-4-(dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy-8-[(2*R*,4*S*,5*S*,6*S*)-4-methoxy-6-methyl-5-propanoyloxyoxan-2-yl]oxy-5,7,9,12,13,15-hexamethyl-10,16-dioxo-1,11-dioxaspiro[2.13]hexadecan-14-yl] propanoate	312	-0.053	6	5	77.145
7	Brassicasterol	389	7.410	1	1	123.50

Table 5: COX-2 Protein Crystal Structure Validation

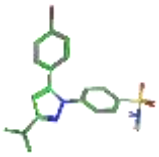
PDB ID	Resolution (Å)	Description	ID Native ligand	RMSD (Å)	Visuallization Re-docking	Validation
6COX	2.80	Cyclooxygenase-2 (prostaglandin synthase-2) complexed with a selective inhibitor, sc-558 in i222 space group	S58	1.1676		Valid

Table 6: Binding affinity and interaction types of the tested compounds with the cox-2 receptor

No	Compound/ ligand	Binding affinity (kcal.mol-1)	RMSD	Bond Distance (Å)	Bond Type	Amino Acid
1	Dihydrodaidzein 7-O-glucuronide	-5.2281	1.1843	2.48	H- acceptor	Arg 120
2	Loliolide	-5.2308	1.2332	4.61	Arane-H	Try 355
				2.89	H-donor	Leu 352
3	N-trans-feruloyltyramine	-8.3798	1.8710	4.64	Arene-H	Try 355
4	(9S)-9-Acetyl-7-[(2R,4S,6S)-4-amino-5-hydroxy-6-methyloxan-2-yl]oxy-6,9,11-trihydroxy-8,10-dihydro-7H-tetracene-5,12-dione	-3.6589	1.4325	2.45	H-acceptor	Arg 120
5	Nandrolone	-4.876	0.9522	2.89	H- acceptor	Arg 120
6	[(3*R*,6*S*,7*R*,8*S*,12*R*,13*S*,14*S*,15*R*)-6-[(2*S*,3*R*,4*S*,6*R*)-4-(dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy-8-[(2*R*,4*S*,5*S*,6*S*)-4-methoxy-6-methyl-5-propanoyloxyoxan-2-yl]oxy-5,7,9,12,13,15-hexamethyl-10,16-dioxo-1,11-dioxaspiro[2.13]hexadecan-14-yl] propanoate	45.1841	1.4513	3.27	H- donor	Ser 530
				2.71	H- acceptor	Try 385
				3.24	H- acceptor	Ile 345
7	Brassicasterol	-0.9722	0.9736	-	-	-
8	S58 (Native ligand)	-7.3046	1.1676	2.95	H- donor	Leu 352
				2.90	H- donor	Ser 353
				2.86	H- acceptor	Arg 120
9	Celecoxib (Obat Paten antiinflamasi)	-7.1402	1.0702	2.89	H- acceptor	Try 385
				3.51	Arene-H	Ala 527

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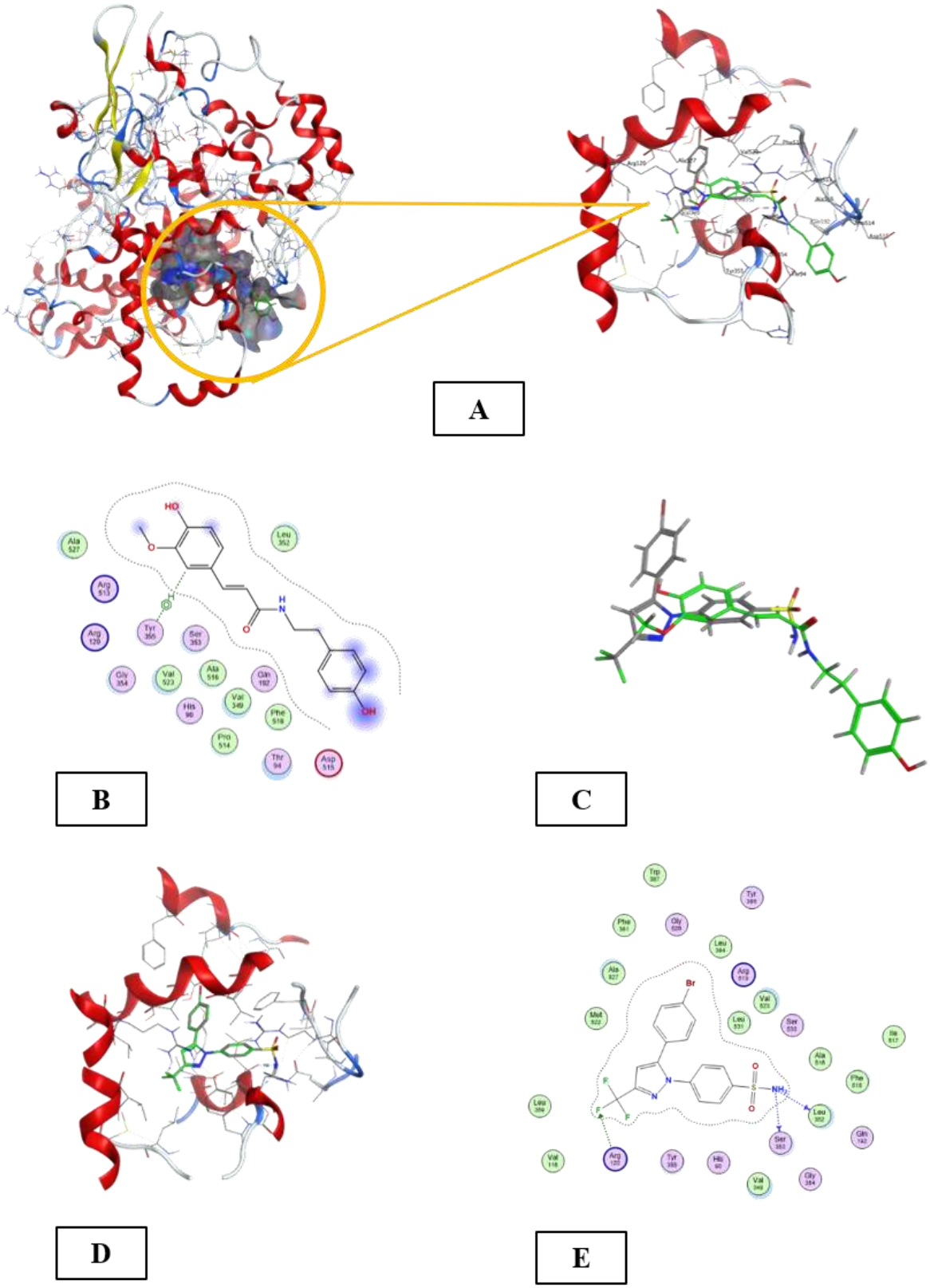

Figure 3: Visualization of molecular docking results: (A) 3D docking pose of N-trans-feruloyltyramine with the COX-2 target protein; (B) 2D binding site interactions of N-trans-feruloyltyramine with COX-2 amino acid residues; (C) overlay of native ligand (grey) and N-trans-feruloyltyramine (green); (D) 3D re-docking pose of the native ligand with COX-2; and (E) 2D binding site interactions of the native ligand with COX-2 amino acid residues.

The inhibitory effect on HCT-116 cell proliferation is consistent with previous reports showing that K. hospita–derived triterpenoids exert antiproliferative effects on colon cancer cells.[37] Such antiproliferative effects may involve modulation of signaling pathways linked to COX-2/PGE_2-associated PI3K/Akt and MAPK/ERK activity,[38] although these mechanistic connections remain provisional without direct molecular analyses.

Conclusion

The present study demonstrated that the ethanol extract of *Kleinhovia hospita* leaves exhibited cytotoxic activity against the HCT-116 colorectal cancer cell line with an IC_{50} value of 85.113 µg/mL, indicating a moderate potency. LC-MS analysis identified 28 bioactive compounds, of which 14 were predicted to possess antineoplastic, anti-inflammatory, and anticarcinogenic activities, suggesting their potential as colorectal anticancer agents with toxicity classes ranging from 2 to 6. Among the identified metabolites, N-trans-feruloyltyramine exhibited the strongest predicted interaction with the COX-2 binding site. Together, these combined in vitro and in silico findings position N-trans-feruloyltyramine as a promising lead candidate for further anticancer investigation

Conflict of Interest

The author declares no conflicts of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024; 74(3):229-263. doi:10.3322/caac.21834.
2. Lewandowska A, Rudzki G, Lewandowski T, Stryjkowska-Góra A, Rudzki S. Risk factors for the diagnosis of colorectal cancer. Cancer Control. 2022; 29:1-15.
3. Elisya Y, Junaedi, Saleha M, Safrina U. Identification and anticancer evaluation of Paliasa leaves (*Kleinhovia hospita* Linn) extracts obtained by subcritical extraction. Int J Sci Res Sci Technol. 2024; 11(4):398-409. doi:10.32628/IJSRST52411142.
4. Nalli M, Puxeddu M, La Regina G, Gianni S, Silvestri R. Emerging therapeutic agents for colorectal cancer. Molecules. 2021; 26(24):7463. doi:10.3390/molecules26247463.
5. Subramaniam S, Selvaduray KR, Radhakrishnan AK. Bioactive compounds: natural defense against cancer? Biomolecules. 2019; 9(12):758. doi:10.3390/biom9120758.
6. Wu QB, Sun GP. Expression of COX-2 and HER-2 in colorectal cancer and their correlation. World J Gastroenterol. 2015; 21(20):6206-14. doi:10.3748/wjg.v21.i20.6206.
7. Venkatachala S, Rajendran M. Correlation of COX-2 expression in colorectal carcinoma with clinicopathological features. Turk J Pathol. 2017; 33(3):228-234. doi:10.5146/tjpath.2016.01389.
8. Smith LF, Patterson J, Walker LT, Verghese M. Chemopreventive potential of sunflower seeds in a human colon cancer cell line. Int J Cancer Res. 2016;12(1):40-50. doi:10.3923/ijcr.2016.40.50
9. Tang J, Zhao J, Li Z. Ethanol extract of Artemisia sieversiana exhibits anticancer effects and induces apoptosis through a mitochondrial pathway involving DNA damage in COLO-205 colon carcinoma cells. Bangladesh J Pharmacol. 2015; 10(3):518-523. doi:10.3329/bjp.v10i3.23856.
10. Padhy I, Paul P, Sharma T, Banerjee S, Mondal A. Molecular mechanisms of action of eugenol in cancer: recent trends and advancement. Life (Basel). 2022; 12(11):1795. doi:10.3390/life12111795.
11. Paramita S. Tahongai (*Kleinhovia hospita* L.): a review of herbal medicine from East Kalimantan. J Tumbuh Obat Indones. 2016; 9(1):29-36.
12. Solihah I, Herlina, Anggraini F, Fitria N, Kirana M. The potential healing effect of *Kleinhovia hospita* L. leaves extract on second-degree burns in rats. J Adv Pharm Educ Res. 2023; 13:109-118. doi:10.51847/VusD92SyAU
13. Pratima NA, Gadikar R. Liquid chromatography–mass spectrometry and its applications: a brief review. Arch Org Inorg Chem Sci. 2018; 1(1):26-34. doi:10.32474/AOICS.2018.01.000103.
14. Maliza R, Syaidah R, Agusta I. Incision-wound healing activity of sunflower seed oil (*Helianthus annuus* L.): in vivo and in silico study. *Farmacia*. 2023;71(5):1001-1012. doi:10.31925/farmacia.2023.5.15
15. Bharadwaj N, Manimuthu MS, Vimal S, Radhakrishnan N. Evaluation of in vitro anti-cancer activity of methanolic leaf extract of *Phoenix pusilla* on colon cancer cell line. J Pharm Bioallied Sci. 2024; 16(Suppl 2):S1181-S1185. doi: 10.4103/jpbs.jpbs_522_23.
16. Dewa WJ, Handharyani E, Purwaningsih S, Mariya S. Cytotoxic activity of red eye snail (*Cerithidea obtusa*) extract on colon cancer cell WiDr. J Sain Vet. 2024; 42(2):129-135.
17. Hyun S, Kim B, Lin D, Hyun S, Yoon SH, Seo J. The effects of gentamicin and penicillin/streptomycin on the electrophysiology of human induced pluripotent stem cell-derived cardiomyocytes in manual patch clamp and multi-electrode array system. J Pharmacol Toxicol Methods. 2018; 91:1-6. doi: 10.1016/j.vascn.2017.12.002
18. Kopustinskiene DM, Jakstas V, Savickas A, Bernatoniene J. Flavonoids as anticancer agents. Nutrients. 2020; 12(2):457. doi:10.3390/nu12020457
19. Wahle KW, Brown I, Rotondo D, Heys SD. Plant phenolics in the prevention and treatment of cancer. Adv Exp Med Biol. 2010; 698:36-51. doi:10.1007/978-1-4419-7347-4_4
20. Saxena S, Rao PB. Comparative GS-MS and FT-IR analysis of Portulaca oleraceae L. and Portulaca quadrifida L. leaf extracts. Pharma Innov J. 2021; 10:40-48.
21. Banerjee P, Kemmler E, Dunkel M, Preissner R. ProTox 3.0: a webserver for the prediction of toxicity of chemicals. Nucleic Acids Res. 2024;52(W1):W513-W520. doi:10.1093/nar/gkae303
22. Maheshwari DG, Shaikh NK. An overview on toxicity testing method. Int J Pharm Technol. 2016; 8:3834-3849.
23. Supandi, Yeni, Merdekawati F. In silico study of pyrazolylaminoquinazoline toxicity by Lazar, Protox, and Admet predictor. J Appl Pharm Sci. 2018; 8(2):119-129.
24. Yuliana Y, Herawati S. Phytochemical content and protective effect of *Kleinhovia hospita* L. leaves extract on pancreatic cytotoxicity in hyperglycemic rats. J Vet. 2016; 17(3):411-417.
25. Riyadi PH, Anggo AD, Suharto S, Tanod WA, Aryani A. Anti-inflammatory potential from tilapia (*Oreochromis niloticus*) viscera hydrolysate with bioinformatics analysis (Prediction of Activity Spectra for Substances–PASS). IOP Conf Ser Earth Environ Sci. 2021; 750:012056. doi:10.1088/1755-1315/750/1/012056.
26. Lee YR, Bae S, Kim JY, Lee JH, Cho DH, Kim HS, An IS, An S. Monoterpenoid loliolide regulates hair follicle inductivity of human dermal papilla cells by activating the Akt/β-catenin signaling pathway. J Microbiol Biotechnol. 2019; 29:1830-40. doi:10.4014/jmb.1908.08018.
27. Yang MH, Ha IJ, Ahn J, Kim C, Lee M, Ahn KS. Potential function of loliolide as a novel blocker of epithelial–mesenchymal transition in colorectal and breast cancer cells. Cell Signal. 2023; 105:110610. doi: 10.1016/j.cellsig.2023.110610.

28. Hassan STS. Brassicasterol with dual anti-infective properties against HSV-1 and Mycobacterium tuberculosis, and cardiovascular protective effect: Nonclinical in vitro and in silico assessments. Biomedicines. 2020; 8:132. doi:10.3390/biomedicines8050132.
29. Xu Y, Ryu S, Lee YK, Lee HJ. Brassicasterol from edible aquacultural Hippocampus abdominalis exerts an anti-cancer effect by dual-targeting AKT and AR signaling in prostate cancer. Biomedicines. 2020; 8:370. doi:10.3390/biomedicines8090370.
30. Kariyil BJ. Antineoplastic drugs: Treatment principles and toxicity. Vet World. 2010; 4:380-382.
31. Kummar S, Murgo AJ, Tomaszewski JE, Doroshow JH. Therapeutic targeting of cancer cells: Era of molecularly targeted agents. In: Niederhuber JE, Armitage JO, Doroshow JH, Kastan MB, Tepper JE, editors. Abeloff's clinical oncology. 5th ed. Philadelphia: Churchill Livingstone; 2014. p. 407-22. doi:10.1016/B978-1-4557-2865-7.00028-X.
32. Lipinski CA. Rule of five in 2015 and beyond: Target and ligand structural limitation, ligand chemistry structure and drug discovery project decisions. Adv Drug Deliv Rev. 2016; 101:34-41.
33. Butnarasu C, Garbero OV, Petrini P, Visai L, Visentin S. Permeability assessment of a high-throughput mucosal platform. Pharmaceutics. 2023; 15:380. doi:10.3390/pharmaceutics15020380.
34. Wulandari RP, Gabriel K, Nurdin HA, Pakhrul DHF, Harits SS, Prameswari N, Pribadi APA, Aulifa DL. In silico study of secondary metabolite compounds in parsley (*Petroselinum crispum*) as a drug therapy for blood cancer (myeloproliferative neoplasm) targeting JAK-2. Indones J Chem Sci. 2023; 12:216-228.
35. Weni M, Safithri M, Seno DSH. Molecular docking of active compounds *Piper crocatum* on the α-glucosidase enzyme as antidiabetic. Indones J Pharm Sci Technol. 2020; 7:64-70.
36. Ojo OA, Ojo AB, Okolie C, Nwakama MC, Iyobhebhe M, Evbuomwan IO. Deciphering the interactions of bioactive compounds in selected traditional medicinal plants against Alzheimer's disease via pharmacophore modeling, Auto-QSAR, and molecular docking approaches. Molecules. 2021; 26:1996. doi:10.3390/molecules26071996.
37. Mo J, Bai Y, Liu B, Zhou C, Zou L, Gan L. Two New Cycloartane Triterpenoids from Kleinhovia hospita. Helv Chim Acta. 2014;97(6):887-894. doi:10.1002/hlca.201300331.
38. Sobolewski C, Cerella C, Dicato M, Ghibelli L, Diederich M. The role of cyclooxygenase-2 in cell proliferation and cell death in human malignancies. Int J Cell Biol. 2010:1-21. doi:10.1155/2010/215158.