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In silico study of the bioactive compounds in *Peronema canescens* Jack (*Sungkai*) leaf infusion targeting VEGFR-2: Insights into anticancer potential

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Original Article

ABSTRACT

Background: *Peronema canescens* Jack, commonly known as *sungkai*, is a medicinal plant traditionally consumed as a herbal infusion. It has demonstrated potential anticancer properties attributed to its bioactive constituents, particularly clerodane-type diterpenoids known as peronemins. The anticancer potential of these compounds can be preliminarily explored using *in silico* approaches. Of particular interest are the reported the cytotoxic effects of *sungkai* leaves against cancer cell lines and their interactions with angiogenesis-related targets, including vascular endothelial growth factor receptor-2 (VEGFR-2). VEGFR-2 is a critical tyrosine kinase receptor that regulates angiogenesisa and Its aberrant activation contributes to tumor growth and metastasis. Consequently, VEGFR-2 represents a well-established target for anticancer therapy development.
Objectives: This study aims to investigate the bioactive compounds present in *sungkai* leaf infusion using liquid chromatography-mass spectrometry (LC-MS), and to predict their interactions with VEGFR-2 through *in silico* molecular docking analysis.
Methods: Phytochemical profiling of the leaf infusion was conducted using LC-MS. Molecular docking was performed using PyRx 0.8 integrated with AutoDock Vina to assess ligand-protein interactions between selected compounds and VEGFR-2 (PDB ID: 2QU5). The resulting interactions were visualized using Discovery Studio.
Results: LC-MS analysis identified ten major compounds in the leaf infusion, consisting of one alkaloid (gentiatibetine) and nine flavonoids. Molecular docking analysis revealed that genkwanin and acacetin-7-galactoside exhibited strong binding affinities toward VEGFR-2 (−9.6 and −9.2 kcal/mol, respectively), approaching that of the reference sorafenib (−11.2 kcal/mol). Both compounds interacted with key catalytic residues, suggesting their potential to inhibit VEGFR-2 by stabilizing its inactive conformation.
Conclusion: This study provides the first to report that *sungkai* leaf infusion is rich in flavonoid compounds two of which exhibit strong anti-VEGFR-2 activity. These findings suggest the potential of *sungkai* leaf infusion as a natural anticancer agent and support the need for further *in vitro* and *in vivo* validation.

INTRODUCTION

Cancer is a condition characterized by impaired apoptotic activity and disrupted regulation of abnormal cell proliferation, which leads to the invasion and damage of surrounding tissues. Its uncontrolled spread can ultimately lead to death.[1] Cancer cells exhibit distinct biochemical

abnormalities.[2] Metastatic tumor growth and survival depend on a continuous supply of oxygen and nutrients, making the establishment of an independent vascular network within the tumor microenvironment (TME) essential.[3,4] Consequently, both primary tumors and metastatic lesions rely heavily on angiogenesis.[5] Angiogenesis is a multistep process involving the formation of new blood vessels from pre-existing capillaries, resulting in a structured, functional, and mature vascular network. This process degradation of the basement membrane, followed by endothelial cell (EC) activation, proliferation, and migration[6] Angiogenesis is tightly regulated by multipleprotein kinases and growth factors.[5] Among these, vascular endothelial growth factor (VEGF) is recognized as one of the most potent mediators of angiogenesis and plays a central role in tumor progression.[7]

VEGF was initially identified as a critical growth factor for vascular endothelial cells and is now recognized as a key regulator of angiogenesis. Its expression is significantly elevated in various tumors, and its role in promoting tumor angiogenesis is well established. In addition to endothelial cells, VEGF and its receptors are expressed in several non-endothelial cells types, including tumor cells.[8] The VEGF family encompasses a broad group of growth factors, including VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor (PlGF). Each member exhibits unique expression profiles, receptor affinities, and functional roles. VEGF-A, commonly referred to as VEGF, is the most extensively studied and exists in multiple isoforms.[9] VEGF family members exert their biological effects primarily as homo- or heterodimers that bind to structurally and functionally analogous tyrosine kinase (TK) receptors located on the cell surface, namely VEGFR1, VEGFR2, and VEGFR3.[10] Ligand-induced receptor activation triggers intracellular signaling cascades involving phosphatidylinositol-3 kinase (PI3K), mitogen-activated protein kinase (MAPK), Src family tyrosine kinases, and phospholipase C gamma (PLCγ), which collectively regulate endothelial cell survival, proliferation, and migration.[11]

Vascular endothelial growth factor receptor-1 and VEGFR-2 are high-affinity receptors for VEGF and, together with VEGFR-3, belong to the Flt subfamily of receptor tyrosine kinases (RTKs). Although VEGFR-1 binds VEGF with high affinity, it exhibits relatively weak signaling due to limited tyrosine phosphorylation. In contrast, VEGFR-2 exhibits both strong binding affinity towards VEGF and robust signaling activity, making it the primary receptor responsible for mediating VEGF-induced angiogenesis in endothelial cells. Vascular endothelial growth factor receptor 3 specifically binds to VEGF-C and VEGF-D, contributing to lymphangiogenesis.[12]

Vascular endothelial growth factor and its receptor-mediated signaling pathways have become one of the most important therapeutic targets for treating various cancers.[10] A promising approach in cancer therapy involves targeted anti-angiogenic treatment (AAT), which aims to inhibit the formation of new blood vessels that support tumor growth. Vascular endothelial growth factor and its receptors (VEGFRs) are key regulators of angiogenesis, making them critical targets for therapeutic intervention. Inhibiting this pathway has shown potential as part of combination therapies for cancer. Various agents have been employed in VEGF/VEGFR-targeted therapy, including native VEGF ligands and their derivatives, monoclonal antibodies against VEGF or VEGFR, VEGF-binding peptides, and small-molecule inhibitors of VEGFR tyrosine kinases. When labeled with diagnostic or therapeutic radionuclides, these agents can function as radiopharmaceuticals for either imaging or treatment. In this context, diagnostic radiotracers play an essential role by enabling tumor detection, monitoring therapeutic response, predicting outcomes, and, importantly, guiding patient selection for AAT.[13]

Peronema canescens Jack or *sungkai* is a medicinal plant that has been reported inhibit cancer properties. Previous studies have reported the cytotoxic effects of chloroform and ethanol extracts of *sungkai* leaves against HeLa cells,[14,15] while the methanol extract of *sungkai* has been shown to exhibit anticancer activity against HT-29 cells.[16] In addition, a derivative compound from peronenim has also been predicted to possess anticancer potential by targeting dihydrofolate reductase.[17] However, existing studies have predominantly focused on organic solvent extracts. In contrast, the present study investigates *sungkai* in the form of an aqueous infusion, which is more representative of traditional usage. Beyond reflecting customary preparation methods, aqueous infusion is expected to enrich polar phytochemicals that may be

underrepresented in organic extracts, resulting in a distinct, solvent-dependent phytochemical profile. Because the composition of such infusions cannot be assumed a priori, liquid chromatography-mass spectrometry (LC-MS) analysis was performed to characterize the constituent compounds prior to downstream biological evaluation. Building on previous evidence of *sungkai*'s anticancer potential, this study specifically aims to investigate its anti-angiogenic potential. Accordingly, this study evaluates the bioactive compounds in the leaf infusion and predicts their anticancer potential through *in silico* analysis. We focused on VEGFR-2 because it is the principal transducer of VEGF-A-driven endothelial proliferation, migration, and survival, thereby directly sustaining tumor perfusion.

METHODS

Study design

This study was conducted as a laboratory-based experimental investigation. Active compounds were identified using liquid chromatography–mass spectrometry (LC-MS). Antioxidant activity was evaluated based on the scavenging capacity against the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). The potential anti-VEGF activity of the compounds was predicted through molecular docking.

Plant material and extraction

Peronema canescens Jack, locally known as *sungkai*, was collected from Palangkaraya, Kalimantan. The specimen was authenticated by Prof. Dr. Kris Herawan Timotius, one of the authors, using the taxonomic determination keys of Sari and Aulya (2022), based on diagnostic macromorphological characters (leaf arrangement and morphology, bark, and branch features).[18] Ten grams of powdered *sungkai* leaves were boiled in 100 mL of distilled water at 95°C for 15 minutes. The filtrate was filtered and employed for phytochemical analysis and evaluation of antioxidant activity.

Figure 1. *Peronema canescens* Jack

Total phenolic content

Total phenolic content was quantified using the Folin-Ciocalteu colorimetric method with gallic acid as a reference standard. In brief, 0.5 mL of the sample was mixed with 2.5 mL of 10% Folin-Ciocalteu reagent and incubated for 10 minutes. Subsequently, 2.5 mL of sodium carbonate solution (75 g/L) was added. The reaction mixture was allowed to incubate at room temperature for two hours, and absorbance was measured at 765 nm. Results were expressed as milligrams of gallic acid equivalents per milliliter (mg GAE/mL) based on a calibration curve.[19]

Total flavonoid content

Total flavonoid content was determined using the aluminum chloride colorimetric assay. A standard curve was generated using quercetin dissolved in 95% ethanol (50 mg/mL) and serially

diluted. For each assay, 0.5 mL of the standard solution was mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% $AlCl_3 \cdot 6H_2O$, 0.1 mL of 1M sodium acetate, and 2.8 mL of distilled water. After 30 minutes of incubation at room temperature, absorbance was recorded at 415 nm using a UV-Vis spectrophotometer. Flavonoid concentration was calculated from the quercetin calibration curve and expressed as milligrams of quercetin equivalents per milliliter (mg QE/mL).[20]

Antioxidant activity via DPPH radical scavenging

The antioxidant activity of the hydroethanolic extract was assessed by mixing 500 µL of the extract at various concentrations with 150 mM DPPH prepared in absolute methanol. The reaction mixtures were then incubated in the dark at room temperature for 30 minutes. Butylated hydroxytoluene (BHT) was used as a positive control. The absorbance of each solution was subsequently measured at 517 nm to determine the free radical scavenging capacity.[20,21]

LC-MS analysis

The bioactive compounds present in the *sungkai* leaf infusion were profiled using LC-MS/MS-QTOF (Waters), operated in TOF MSE mode. Chromatographic separation was carried out on a C18 column, employing a mobile phase consisting of 0.1% formic acid in acetonitrile and 0.1% formic acid in distilled water. A 5% (w/v) methanolic extract was initially prepared and homogenized for 30 minutes, after which 10 µL of the filtrate was injected into the system. Compound identification was conducted using UNIFI software.[20]

***In silico* analysis**

Ligand preparation

Homology modeling was employed to construct predictive models of the anti-target, VEGF. These models were then utilized in molecular docking analyses, which were conducted using AutoDock Vina integrated in the PyRx 0.8 platform. Ligand structures, including 5,4'-dihydroxy-6,7,8-trimethoxyflavone, genistein-7-O-β-D-glucoside, genkwanin, luteolin-7-O-beta-D-glucopyranoside, yuankanin, gentiatibetine 3,5,6-trihydroxy-4',7-dimethoxyflavone, rhamnocitrin-3-glucoside, acacetin-7-galactoside, kaempferol-3,7-diglucoside, and sorafenib, were retrieved from the PubChem database in SDF format. Sorafenib was included as a reference compound due to its known inhibitory activity against VEGF.[22] Only compounds available in the PubChem database were selected for further in silico analysis. In such cases, representative compounds from the same chemical group or class were selected as substitutes for further in silico analysis. This approach was taken to maintain structural relevance and ensure a reasonable prediction of biological activity. All ligands underwent energy minimization to enhance structural stability, followed by conversion to the PDBQT format using Open Babel within the PyRx software.[23]

Protein preparation

Three-dimensional crystal structures of the VEGF receptor-2 (VEGFR-2) (PDB ID: 2QU5) was downloaded from the Protein Data Bank (http://www.rcsb.org). Prior to docking, non-essential molecular components, such as water and solvents, were removed. Hydrogen atoms were added to the protein structures to prepare them for docking simulations, which were subsequently carried out using PyRx 0.8. Based on Discovery Studio Visualizer (DSV, v25.1.0.24284) analysis, the binding grid dimensions for VEGFR-2 was set to 65.67 Å (X-axis), 94.15 Å (Y-axis), and 44.83 Å (Z-axis).[23,24]

Protein–ligand docking

Molecular docking was performed using the predefined grid dimensions to ensure comprehensive coverage of the active sites. Grid boxes were accurately positioned in PyRx 0.8, and docking simulations were executed to assess the potential interactions between ligands and target receptors. The resulting docked complexes were visualized and analyzed using DSV.[23] Pose plausibility was evaluated by binding-site alignment to sorafenib-bound VEGFR-2, conservation

of hinge or DFG contacts in the predicted poses, and clustering of top docking modes. Although a re-docking RMSD could not be reported in the current workflow, we applied orthogonal, widely used checks: (i) binding-site alignment to sorafenib-bound VEGFR-2 structures (conservation of hinge/DFG geometry), (ii) conservation of key contacts in the predicted poses (interactions at Cys919/Glu885 and the DFG motif at Asp1046–Phe1047), (iii) pose clustering (convergence among top-ranked modes under identical settings). These criteria support the suitability of 2QU5 for docking in this study.

Data analysis

The LC-MS analysis identified a range of bioactive compounds, which were subsequently classified based on their respective chemical groups. Descriptive analysis was used to summarize the findings comprehensively, without conducting advanced statistical inference. Linear regression analysis was performed to determine the relationships among total phenolic content, total flavonoid content, and antioxidant activity.

Ethical statement

This study involved only *in vitro* and *in silico* analyses and did not include human participants or experimental animals; therefore, ethical approval was not required

RESULTS

The *sungkai* leaf infusion showed a total phenolic content of 34.11±0.33 µg GAE/mL and a total flavonoid content of 18.86±0.44 µg QE/mL, indicating a substantial polyphenolic composition in which flavonoids constitute an essential fraction. Consistent with this profile, the infusion exhibited concentration-dependent DPPH scavenging with an IC_{50} of 16.93 ± 0.06 µg/mL, while BHT, tested under identical conditions, showed an IC_{50} of 6.21 ± 0.21 µg/mL. Based on commonly used thresholds, an IC_{50} < 50 µg/mL denotes robust antioxidant activity; thus, despite being less potent than BHT, the infusion still demonstrates robust radical-scavenging capacity. The observed activity is in line with the presence of phenolic and flavonoid constituents, which are known to quench DPPH radicals via hydrogen-atom or electron donation.[25]

Liquid chromatography–mass spectrometry (LC-MS) was employed to identify active compounds in the infusion due to its high sensitivity, specificity, and effectiveness in detecting and characterizing complex mixtures of bioactive substances. This method is particularly suitable for herbal infusions, which often contain diverse and structurally variable phytochemicals.[26] One alkaloid compound was identified, namely gentiatibetine. A total of nine flavonoid compounds were also identified, namely genkwanin, yuankanin, and the derivatives of trimethoxyflavone, dimethoxyflavone, genistein, luteolin, kaempferol, dan acacetin (Table 1).

Table 1. Major bioactive compounds identified in sungkai leaf infusion by LC–MS

No	Identified compounds	Ionization mode	RT	MZ	Molecular formula	Response
		Alkaloid				
1	Gentiatibetine	+	2.29	120 166 311	$C_9H_{11}NO_2$	12.564
		Flavonoid				
2	5,4'-dihydroxy-6,7,8-trimethoxyflavone	+	16.64	367 760 786	$C_{18}H_{16}O_7$	134.532
3	Genistein-7,4'-diO-β-D-glucoside	+	12.49	301 617 633	$C_{27}H_{30}O_{15}$	84.286
4	Genkwanin	+	11.13	242 285 469	$C_{16}H_{12}O_5$	425.050

No	Identified compounds	Ionization mode	RT	MZ	Molecular formula	Response
5	Luteolin-7,4'-di-O-β-D-glucopyranoside		11.42	317 611 633	$C_{27}H_{30}O_{16}$	91.627
6	Yuankanin	+	10.57	285 579 601	$C_{27}H_{30}O_{14}$	104.502
7	3,5,6-Trihydroxy-4',7-dimethoxyflavone	+	14.98	136 270 331	$C_{17}H_{14}O_7$	42.181
8	Rhamnocitrin-3-O-β-D-glucoside	+	9.96	258 301 463	$C_{22}H_{22}O_{11}$	42.069
9	Acacetin-7-galactoside	-	11.01	268 283 445	$C_{22}H_{22}O_{10}$	124.799
10	Kaempferol-3,7-diglucoside	-	11.29	299 314 609	$C_{27}H_{30}O_{16}$	190.030

Note: LC–MS data are reported as annotations (ionization mode, retention time, m/z, molecular formula) with instrumental response values serving as semi-quantitative indicators rather than absolute concentrations

The *in silico* analysis revealed that the compounds with the strongest potential to inhibit VEGFR-2 activity belonged to the flavonoid class. Genkwanin and acacetin-7-galactoside exhibited the highest binding affinities, with docking scores of -9.6 and -9.2 kcal/mol, respectively (Table 2). These values are relatively close to that of sorafenib (-11.2 kcal/mol), a small-molecule inhibitor known to suppress tumor proliferation and angiogenesis by targeting the receptor tyrosine kinase activity of VEGFRs.[27] Table 2 shows the interactions between active compounds and the residues of VEGFR-2 active sites.

Table 2. *In silico* analysis results of anticancer activity and interactions between compounds

Active compound	VEGFR-2 (kcal/mol)	Interaction	Residues
Gentiatibetine	-6.1	Van der Waals	Val867, Val914, Ala866, Thr916, **Glu885**, Leu889, **Asp1046**, Leu1035, Val848
		Conventional H bond	-
		C-H bond	-
		Pi-sulfur	**Cys1045**
		Pi-Pi stacked	**-**
		Pi-Cation	Lys868
		Pi-alkyl	Val899
		Pi-sigma	-
		Unfavorable acceptor-acceptor	-
5,4'-dihydroxy-6,7,8-trimethoxyflavone	-7.4	Van der Waals	Val899, Cys1045, Thr916, Glu917, Val848, **Phe1047**, Gly922, Thr926, Lys920
		Conventional H bond	Asn923
		C-H bond	Leu840
		Pi-sulfur	**Cys919**
		Pi-Pi Stacked	Phe918
		Pi-Cation	-
		Alkyl/Pi-alkyl	Leu1035, Ala866

Active compound	VEGFR-2 (kcal/mol)	Interaction	Residues
Genkwanin	-9.6	Pi-sigma	-
		Unfavorable acceptor-acceptor	-
		Van der Waals	Gly922, **Phe1047**, Thr916, Val867, Val914, **Asp1046**, Glu917
		Conventional H bond	**Glu885**
		C-H bond	**Cys1045**
		Pi-Sulfur	**Cys919**
		Pi-Pi stacked	Phe918
		Pi-Cation	Lys868
		Alkyl/Pi-alkyl	Leu889, Val848, Leu840, Val899, Ala866, Leu1035
Yuankanin	-8,1	Pi-sigma	-
		Unfavorable acceptor-acceptor	-
		Van der Waals	Asn923, Gly922, **Phe1047**, Glu917, Thr916, Val899, **Cys1045**, Val848, Lys920, Glu850
		Conventional H bond	Ser930
		C-H bond	Thr926, Tyr927
		Pi-Sulfur	**Cys919**
		Pi-Pi stacked	Phe918
		Pi-Cation	-
		Alkyl/Pi-alkyl	Ala866, Leu1035
3,5,6-Trihydroxy-4',7-dimethoxyflavone	-7.2	Pi-sigma	Leu840
		Unfavorable acceptor-acceptor	Phe921
		Van der Waals	Ala1050, **Glu885**, **Asp1046**, Thr916, Val914, Val898, Leu1019, Ile892, His1026, Cys1024, Ile1025
		Conventional H bond	Ile1044
		C-H bond	**Cys1045**
		Pi-sulfur	**-**
		Pi-Pi Stacked	**-**
		Pi-Cation	Lys868
		Alkyl/Pi-alkyl	Ile888, Leu889, Ala866, Val848, Val899
Rhamnocitrin-3-glucoside	-6.5	Pi-sigma	-
		Unfavorable acceptor-acceptor	-
		Van der Waals	Asp857, Thr859, Asn900, Glu917, Lys920, Thr864, **Cys919**, Ser1037, Lys1043, Lys1039
		Conventional H bond	Phe918, Glu1038
		C-H bond	Arg868
		Pi-sulfur	-
		Pi-Pi Stacked	-
		Pi-Cation	-
		Alkyl/Pi-alkyl	Leu896, Val1041
		Pi-sigma	-
		Unfavorable acceptor-acceptor	-

Active compound	VEGFR-2 (kcal/mol)	Interaction	Residues
Acacetin-7-galactoside	-9.2	Van der Waals	Ile888, **Asp1046**, **Glu885**, Val867, Thr916, Ile1044, Val898, Leu1019, Ile892
		Conventional H bond	Arg1027, Ile1025, His1026, Ala1050
		C-H bond	-
		Pi-Sulfur	**Cys1045**
		Pi-Pi stacked	**-**
		Pi-Cation	**Lys868**
		Alkyl/Pi-alkyl	Val848, Ala866, Val899
		Pi-sigma	Leu889
		Unfavorable acceptor-acceptor	-
Kaempferol-3,7-diglucoside	-8.1	Van der Waals	Pro839, Arg1066, Arg842, Phe845, Gly841, Gly1048, **Val848**, Gly1102, Pro1105, Ser1104, Ser925, Ala1031, Trp1071, Thr926, **Leu1035**, Gly922
		Conventional H bond	Asn923, **Phe1047**, Ala1103, Arg1032, Glu1097
		C-H bond	Leu840
		Pi-Sulfur	-
		Pi-Pi stacked	-
		Pi-Cation	-
		Alkyl/Pi-alkyl	-
		Pi-sigma	-
		Unfavorable acceptor-acceptor	Arg929
Sorafenib	-11.2	Conventional H bond	**Glu885**, **Cys919**, **Asp1046**
		C-H bond/Pi-donor H	Lys920, Phe918, **Cys1045**
		Pi-sulfur	Phe918
		Pi-Pi stacked/Pi-Pi T-shaped	**Phe1047, Phe918**
		Pi-Cation	**-**
		Alkyl/Pi-alkyl	Val848, Ile888, Leu889, Ala866, Leu840, Leu1035, Cys1045
		Pi-sigma	**-**
		Unfavorable acceptor-acceptor	-

DISCUSSION

The *sungkai* leaf infusion demonstrated antioxidant capacity, indicated by measurable total phenolic and total flavonoid contents and by a concentration-dependent DPPH radical-scavenging activity. The relatively high phenolic and flavonoid values suggest that polyphenols, particularly flavonoids, contribute substantially to the infusion's antioxidant profile. While the infusion was less potent than the synthetic antioxidant BHT under identical assay conditions, an IC_{50} of <50 µg/mL is within the range commonly considered indicative of vigorous radical-scavenging activity. This finding is consistent with the ability of phenolic and flavonoid compounds to neutralize DPPH radicals via hydrogen atom transfer and/or single-electron transfer, and with the commonly reported correlation between total polyphenol content and DPPH activity across plant matrices.[25]

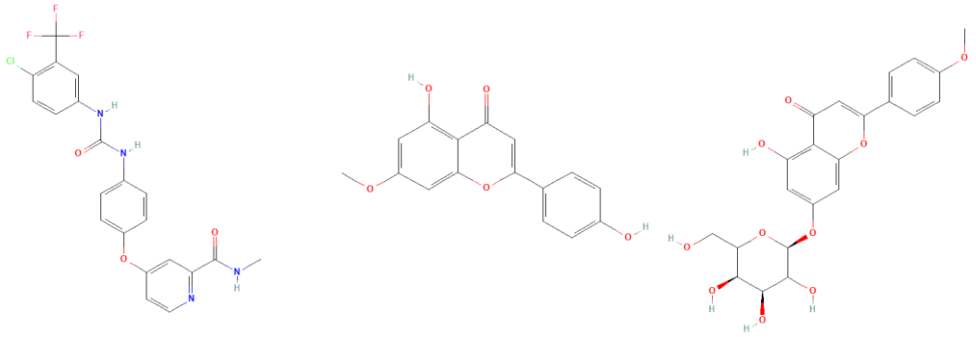
The findings of this study indicate that the *sungkai* leaf infusion predominantly contains flavonoid compounds, with nine active compounds classified within this group (Table 1).

Flavonoids possess significant therapeutic potential across a broad spectrum of diseases, including cardiovascular diseases, immune-related disorders, and various types of cancer.[28] Flavonoids exert their anticancer activity by suppressing cellular proliferation and promoting both apoptotic and autophagic cell death. Additionally, they have been reported to induce necrosis, promote cell cycle arrest, and inhibit cell migration, invasion, and tumor angiogenesis. These mechanisms contribute to their potential role in overcoming or preventing chemoresistance, partly through the modulation of reactive oxygen species (ROS)-scavenging enzymes.[29] Given the ability of flavonoids to interfere with multiple cancer-related pathways, including angiogenesis, it is plausible to investigate their interaction with specific molecular targets such as VEGF and its associated receptors. One of the most critical pathways in tumor angiogenesis involves VEGF signaling through VEGFR-2.[30]

Vascular endothelial growth factor is a key regulator of angiogenesis, and its overexpression is indicative of tumor progression and metastasis.[31] Vascular endothelial growth factor signaling occurs primarily through VEGFR-2, also known as kinase insert domain receptor (KDR), which is a member of the receptor tyrosine kinase (RTK) family that facilitates the transmission of signals to polypeptides, protein hormones, cytokines, and growth factors. Vascular endothelial growth factor signaling is essential for neovascularization processes, including endothelial cell survival, proliferation, migration, and vascular permeability.[24] Therapeutic agents targeting dysregulated activity of receptor tyrosine kinases (RTKs) are generally classified into two primary groups: biologics and small-molecule inhibitors, commonly known as tyrosine kinase inhibitors (TKIs). Biologics exert their effects by either directly preventing RTK activation or by sequestering their specific ligands at the ligand-binding domain. In contrast, TKIs inhibit tyrosine kinase function and downstream signaling pathways by targeting the ATP-binding pocket or allosteric regulatory sites.[32] Targeting VEGFR-2 is a promising approach for cancer treatment, as it interferes with tumor vascularization, slowing its growth and metastasis. Research highlights the importance of targeting VEGFR-2 in cancer therapies.

Upon VEGF binding to the extracellular domain of VEGFR-2, receptor dimerization and autophosphorylation occur, subsequently activating angiogenic signaling pathways that support the development of cancer cells. VEGF inhibitors are employed to block this process. The catalytic domain of VEGFR-2, which serves as the ATP-binding pocket, comprises a small N-lobe and a large C-lobe, connected by a hinge region and a convex motif. Between these two lobes lies a cleft known as the ATP-binding pocket, where the phosphorylation process takes place. Protein kinases such as VEGFR-2 possess a highly conserved Asp-Phe-Gly (DFG) motif located at the N-terminal end of the activation segment. This motif plays a crucial role in phosphorylation by participating in ATP coordination and magnesium ion (Mg^{2+}) binding, both of which are essential for enzymatic activity. The aspartate residue facilitates the coordination of ATP phosphates and magnesium, while the phenylalanine stabilizes both the aspartate and the αC-helix through hydrophobic interactions with the catalytic HRD motif. Although the function of the glycine residue is less well defined, it is believed to support structural flexibility within the activation segment.[33,34]

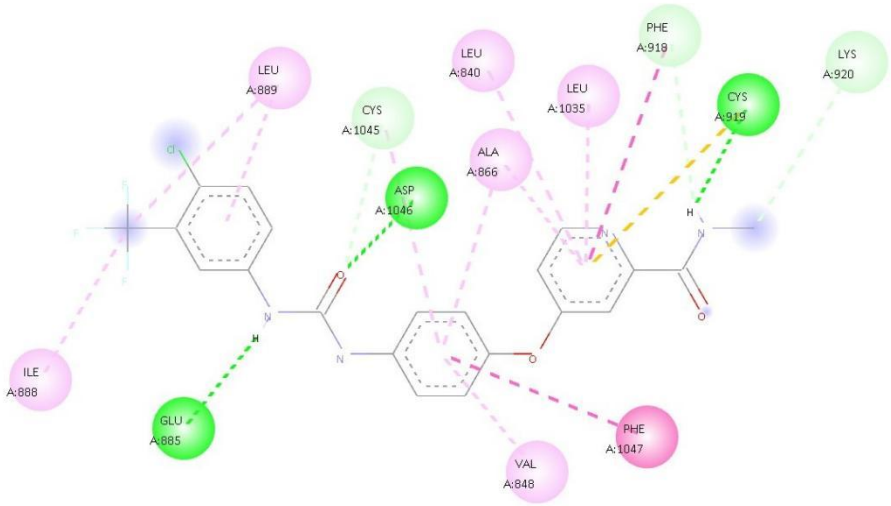
This study examines the interactions and effects of genkwanin, acacetin-7-galactoside, and sorafenib on VEGFR-2, a key receptor involved in angiogenesis and cancer development. Sorafenib is a multi-kinase inhibitor that targets VEGFR-2, along with several other kinases, and is used to treat advanced cancers, such as hepatocellular carcinoma (HCC) and renal cell carcinoma. It has demonstrated effectiveness in inhibiting tumor growth by blocking angiogenesis, a process that relies significantly on VEGFR signaling. As a proven multi-kinase inhibitor, sorafenib is used in the management of multiple cancers, including HCC and renal cell carcinoma.[35] Investigating the potential interactions with or combined effects of these compounds on VEGFR-2 may reveal new insights into alternative therapeutic approaches.



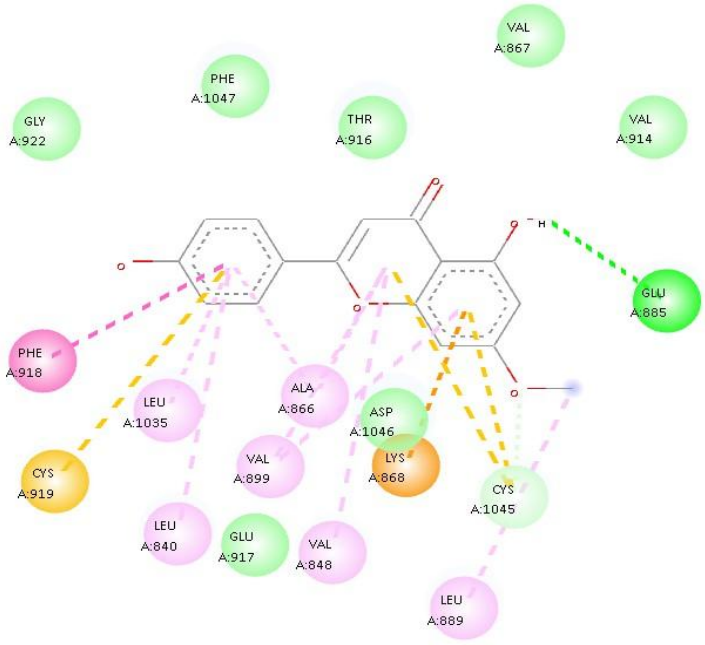
(a) (b) (c)

Figure 2. Chemical structure of Sorafenib (a), Acacetin-7-galactoside (b), and Genkwanin (c)

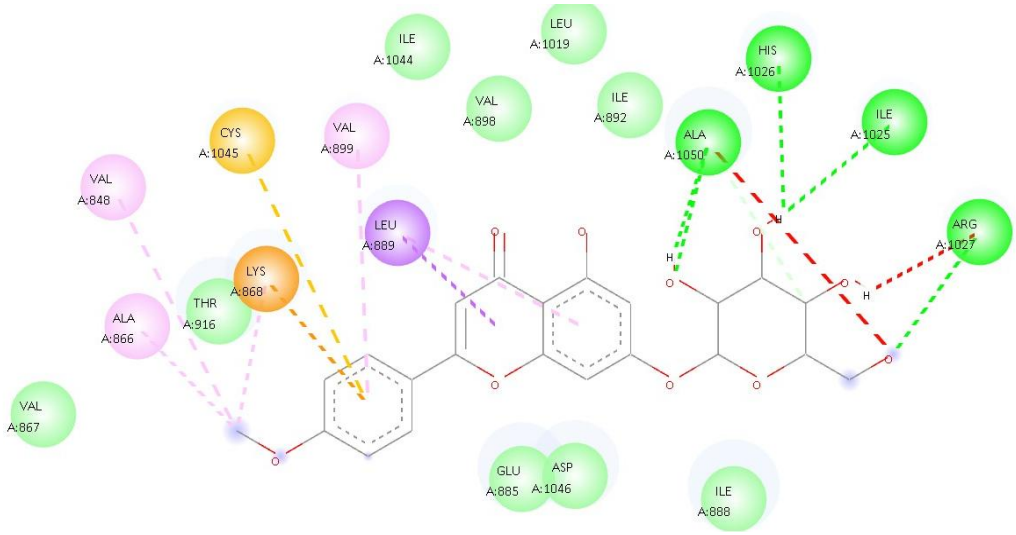
The catalytic site of VEGFR-2 (PDB ID: 2QU5) comprises key residues such as Cys919 (hinge), Glu885 (αC-helix), and the DFG motif (Asp1046–Phe1047–Gly1048) that govern ATP-site recognition and catalytic regulation. Effective inhibition typically combines a hinge hydrogen bond to Cys919, polar contacts to Glu885 and/or Asp1046, engagement of the DFG region to stabilize the DFG-out inactive conformation, and occupation of the hydrophobic back pocket adjacent to the gatekeeper Val916. These features are associated with enhanced binding stabilization, target selectivity, and overall inhibitory efficacy.[24] *In silico* analysis was conducted to predict the anticancer potential of bioactive compounds present in the *sungkai* leaf infusion. A molecular docking study was undertaken to assess the potential of these compounds as ligands that inhibit VEGFR-2 activity. As shown in Table 3, genkwanin and acacetin-7-galactoside emerged as the most promising candidates, demonstrating stronger predicted binding affinities compared to sorafenib, which was used as the reference control.



a. 2QU5-sorafenib



b. 2QU5-Genkwanin



c. 2QU5-Acacetin-7-galactoside

Figure 3. Interactions between compounds and VEGFR2

Sorafenib works by inhibiting VEGFR-2, a key receptor involved in the process of anti-angiogenesis. This mechanism has been validated through clinical studies, which have demonstrated improved survival rates in patients with advanced HCC.[36] However, its effectiveness is tempered by side effects and resistance, leading to the need for exploring combination therapies or alternative treatments.[37] Sorafenib binds to key residues within the catalytic site of VEGFR-2, including Glu885, Cys919, Asp1046, Cys1045, and Phe1047. Sorafenib establishes hydrogen bonds with Glu885, Cys919, and Asp1046 that lock the ligand in the ATP site and favor the DFG-out inactive state, thereby blocking ATP coordination and catalysis and underpinning its inhibitory efficacy. The interaction with the DFG motif residues, Asp1046 and Phe1047, highlights its potential as a potent inhibitor by stabilizing its position within the catalytic pocket. Additionally, sorafenib exhibits a crucial interaction with the hinge region at Cys919, which is a primary target in kinase inhibitor design. The hinge region plays an essential role in ATP and inhibitor binding by facilitating the formation of stable hydrogen bonds.[38] The

affinity of the ligand for the VEGFR-2 residues was reinforced by a range of hidrophobic interactions such as Pi-sulfur, Pi-Pi stacked/Pi-Pi T-shaped, and alkyl/Pi-alkyl.

Given the aromatic nature of many ligands, including flavonoid derivatives, additional non-covalent interactions such as Pi-sulfur, Pi-Pi stacked, and alkyl/Pi-alkyl contacts play a pivotal role in stabilizing ligand and receptor complexes. Pi-sulfur interactions support ligand intercalation within the binding site, particularly in aromatic compounds.[39,40] Pi-Pi stacked interactions between the ligand and residues contribute to stronger receptor affinity.[41] Moreover, alkyl and Pi-alkyl interactions help stabilize the ligand's orientation within the hydrophobic pocket of the protein, reinforcing binding through nonpolar contacts.[40]

Genkwanin binds to VEGFR-2 at the catalytic site, interacting specifically with Glu885, Asp1046, Phe1047, and Cys919. A key hydrogen bond is formed with Glu885, one of the primary residues within the catalytic domain. Additionally, genkwanin exhibits van der Waals interactions with the DFG motif residues Asp1046 and Phe1047, suggesting its potential to stabilize the kinase in an inactive DFG-out conformation, thereby inhibiting tyrosine kinase activity.[22] Several other interactions, including Pi-sulfur, Pi-Pi stacked, and alkyl/Pi-alkyl, enhance genkwanin's binding affinity to VEGFR-2. Genkwanin may suppress the expression of VEGFR-2. By inhibiting VEGFR-2, genkwanin disrupts the VEGF signaling pathway, leading to reduced angiogenesis and tumor growth. This inhibition occurs through the downregulation of VEGFR-2 mRNA and protein levels, which is vital for achieving antiangiogenic outcomes. Genkwanin's ability to inhibit VEGFR-2 presents itself as a potential candidate for treating tumors that rely on angiogenesis for growth and spread.[42] Clinical studies are essential to explore genkwanin's therapeutic potential and improve its application in cancer treatment.

Acacetin-7-galactoside binds to the catalytic residues of VEGFR-2, including Asp1046, Cys1045, and Glu885. Its interactions with Asp1046 and Glu885 occur through van der Waals forces. Although these are relatively weak interactions, they indicate that acacetin-7-galactoside can reach the active site. Conventional hydrogen bonding with residues such as Arg1027, Ile1025, His1026, and Ala1050 helps maintain the polar orientation of the ligand's hydroxyl and carbonyl groups, thereby contributing to the overall stability of the ligand within the binding pocket. These interactions may influence drug specificity, metabolism, and absorption.[43] Additional interactions that contribute to the binding affinity include Pi-sulfur and Pi-cation interactions involving Cys1045 and Lys868, respectively. Moreover, alkyl and Pi-alkyl interactions with Val848, Ala866, and Val899 further strengthen the binding through nonpolar contacts. The Pi-Pi stacked interaction between the ligand and Phe918 contributes to stronger receptor affinity. Overall, acacetin-7-galactoside occupies an optimal position within the catalytic pocket, and its interaction with the DFG motif at Asp1046 suggests its potential to inhibit tyrosine kinase activity.[22]

For both genkwanin and acacetin-7-galactoside, binding at the catalytic site is dominated by van der Waals contacts (including interactions with Glu885 and the DFG motif at Asp1046–Phe1047), with only limited classical hydrogen bonding. Van der Waals contacts are less directional and generally provide smaller per-contact enthalpic anchoring than DFG hydrogen bonds, the overall binding stabilization is expected to be weaker unless compensated by extensive pocket occupancy.[44] Consistent with this interaction pattern, the predicted binding scores for both ligands were lower than those of sorafenib under identical docking settings, suggesting a reduced inhibitory potency that warrants experimental confirmation. A heavy-atom re-docking RMSD of the co-crystallized ligand could not be computed in the current workflow due to atom-mapping mismatches after format conversion. This is acknowledged as a limitation and will be addressed with a dedicated re-docking tool in future work.

Acacetin-7-galactoside shows promise as a VEGFR-targeting therapeutic agent due to its antiangiogenic properties. It inhibits angiogenesis by downregulating VEGF and STAT signaling, which are vital for the proliferation and migration of endothelial cells. This suggests that acacetin-7-galactoside could be a promising candidate for anti-cancer treatments that target tumor vasculature. Acacetin reduces the growth and survival of human umbilical vein endothelial cells (HUVECs) by 92% and disrupts capillary-like tube formation by 98% in vitro, indicating

significant antiangiogenic activity. It causes the retraction and breakdown of capillary networks and inhibits HUVEC migration and invasion, which are critical stages in angiogenesis. Acacetin also downregulates pro-angiogenic factors such as VEGF, eNOS, and MMP-2, as well as restraining the phosphorylation of STAT-1 and STAT-3, which are also key in angiogenesis and tumor proliferation. Furthermore, acacetin reduces the nuclear localization of phosphorylated STAT3, thereby lowering the transcription of genes related to angiogenesis. In animal studies, it has been shown to inhibit angiogenesis in rat aortic rings and chicken egg chorioallantoic membranes, as well as in Matrigel plug assays in mice.[45] Acacetin markedly enhanced apoptosis in gastric cancer (GC) cells by modulating the STAT3 and ERK signaling pathways, suggesting its promising potential as a therapeutic candidate for GC treatment.[46] By downregulating VEGF and STAT signaling, it may enhance therapies targeting the angiopoietin-TIE pathway, which is crucial for vascular stabilization and tumor angiogenesis.[47] While acacetin-7-galactoside holds promise as a VEGFR-targeting agent, it is essential to consider the broader context of angiogenesis inhibition within cancer treatment. Targeting tumor vasculature provides a genetically stable target, thereby lowering the risk of resistance.[45,48] Nevertheless, the complexity of angiogenesis suggests that a multi-targeted approach may be needed for effective intervention. [49]

Genkwanin and acacetin-7-galactoside are predicted to bind VEGFR-2 at the catalytic site, displaying interaction patterns that partially overlap with those of sorafenib, thereby supporting their potential as anti-angiogenic leads targeting VEGFR-2. Nevertheless, several limitations should be considered. First, docking scores provide only an in silico estimate and do not directly translate into experimental binding affinity or inhibitory activity; moreover, our models suggest that the predicted binding is largely driven by van der Waals interactions. Accordingly, biochemical kinase assays and cell-based studies are required to quantify their inhibitory potency and confirm functional VEGFR-2 blockade in relevant cancer models. Second, further mechanistic investigations, followed by appropriate in vivo and clinical evaluations, are necessary to establish therapeutic efficacy, safety, pharmacokinetics, and potential off-target effects before these compounds can be advanced toward cancer therapy. Finally, botanical identification was conducted in-house without independent herbarium authentication. Although morphological photographs (Figure 1) are provided for transparency, formal taxonomic verification would strengthen the reproducibility and generalizability of the findings..

CONCLUSION

Sungkai leaf infusion contains a diverse range of bioactive compounds, predominantly flavonoids, along with a single alkaloid. *In silico* molecular docking analysis indicated that two flavonoids, genkwanin and acacetin-7-galactoside, exhibit favorable binding affinities toward VEGFR-2 and interaction profiles similar to those of sorafenib, suggesting their potential as VEGFR-2 inhibitors. These findings provide a preliminary mechanistic rationale for exploring sungkai-derived flavonoids as anti-angiogenic leads targeting the VEGFR-2 axis in cancer. Future studies should validate these *in silico* predictions through *in vitro* and *in vivo* experiments, including VEGFR-2 kinase inhibition assays and cell-based assessments of anti-proliferative and anti-angiogenic effects, followed by appropriate animal studies to confirm efficacy and safety.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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DATA AVAILABILITY

The authors confirm that all data supporting the findings of this study are included within the article.

SUPPLEMENTAL DATA

No supplementary material is associated with this study. All relevant data that support the conclusions of this study are fully presented within the main body of the article.

AUTHOR CONTRIBUTIONS

IR and SES conceptualized the study. IR and SES conducted data curation, while KHT performed validation. IR carried out formal analysis. IR and SES prepared the original draft, and all authors (IR, SES, and KHT) contributed to the review and editing of the manuscript. KHT provided supervision of the study.

DECLARATION OF USING AI IN THE WRITING PROCESS

This study utilized artificial intelligence (AI) tools, specifically Grammarly, to assist in manuscript writing, primarily for language refinement. All AI-assisted processes were thoroughly reviewed by the authors to ensure the accuracy and reliability of the findings. The authors affirm that all final decisions and interpretations presented in this article were made independently by the authors.

LIST OF ABBREVIATIONS

VEGF: Vascular Endothelial Growth Factor; VEGFR: Vascular Endothelial Growth Factor Receptor; PlGF: Placental Growth factor; TK: Tyrosine Kinase; PI3K: Phosphatidylinositol-3 Kinase; MAPK: Mitogen-Activated Protein Kinase; PLCγ: Phospholipase C Gamma; RTKs: Receptor Tyrosine Kinases; AAT: Anti-angiogenic Treatment; DPPH: 2,2-diphenyl-1-picrylhydrazyl; LC-MS: Liquid Chromatography–Mass Spectrometry; ROS: Reactive Oxygen Species; KDR: Kinase Insert Domain Receptor; TKIs: Tyrosine Kinase Inhibitors; HCC: Hepatocellular Carcinoma; HUVECs: Human Umbilical Vein Endothelial Cells; eNOS: endothelial Nitric Oxide Synthase; MMP: Matrix Metalloproteinase; STAT: Signal Transducers and Activators of Transcription; GC: Gastric Cancer; TIE: Tyrosine kinase with Immunoglobulin-like and EGF-like domains.

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