

Phytochemical Profiling of *Tapinanthus globiferus* for the Identification of Marker Compounds guiding its Ethnomedicinal and Anticancer Potential

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Research Article

Keywords: *Tapinanthus globiferus*, Phytochemical profiling, Flavonoids, VEGF-A inhibition, BCL-2 inhibition, Anticancer potential

Posted Date: October 20th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-7608163/v1>

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Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at *In Silico Pharmacology* on February 26th, 2026. See the published version at <https://doi.org/10.1007/s40203-026-00562-2>.

Abstract

Medicinal plants remain a valuable source of structurally diverse natural products with therapeutic potential.

Tapinanthus globiferus (Loranthaceae) is widely used in African ethnomedicine, including for the treatment of cancer-related conditions, yet its chemical and pharmacological basis remains poorly defined. In this study, phytochemical profiling, cytotoxicity testing, and computational analyses were employed to characterize the bioactive potential of *T. globiferus* leaves. The butanol fraction demonstrated notable cytotoxic activity against HT-29 colon cancer cells, consistent with morphological evidence of apoptosis. High-performance liquid chromatography with diode array detection (HPLC–DAD) revealed the presence of abundant phenolic and flavonoid constituents, including kaempferol and quercetin derivatives, rutin, isoquercetin, catechin, and protocatechuic acid, which were identified as candidate marker compounds. To explore their therapeutic relevance, molecular docking was performed against angiogenic and apoptotic targets, particularly vascular endothelial growth factor A (VEGF-A) and the anti-apoptotic protein B-Cell Lymphoma 2 (BCL-2). To explore mechanisms, major identified compounds were docked in silico to VEGF-A and the anti-apoptotic protein BCL-2. Rutin, trifolin (a kaempferol glycoside), and epigallocatechin exhibited the strongest binding (e.g. rutin: -8.85 kcal/mol to VEGF-A), surpassing the reference inhibitor pazopanib (-3.56 kcal/mol) with multiple stabilizing interactions with these proteins, suggesting potential to interfere with tumor angiogenesis and cell survival pathways. Collectively, these findings provide a scientific basis for the traditional use of *T. globiferus* and support its fractions as promising sources of multi-targeted anticancer agents. The identification of marker compounds further establishes a foundation for bioassay-guided isolation, mechanistic validation, and future drug development.

1.1. INTRODUCTION

Medicinal plants remain a rich source of structurally diverse compounds that serve as leads in modern drug discovery. Among these, mistletoes of the genus *Tapinanthus* (family: Loranthaceae) occupy an important place in African traditional medicine, where they are used for the treatment of ailments ranging from epilepsy, diabetes, and hypertension to infectious and degenerative diseases (Adesina et al., 2013; Elekofehinti et al., 2019; Raceline et al., 2019). *Tapinanthus globiferus*, an African mistletoe distributed across West Africa, is especially valued in ethnomedicine for its restorative and anticancer properties. Despite its prominence in traditional healthcare systems, there is a limited availability of systematic phytochemical and pharmacological investigations on this species, and the compounds responsible for its reported bioactivities remain largely undefined. *Tapinanthus globiferus* (A. Rich.) Tiegh., in particular, has been widely reported in ethnomedicine for the management of rheumatism, fever, liver disorders, and cancer-related conditions (Jeremiah et al., 2019; Raceline et al., 2019). Its broad spectrum of folk uses has stimulated scientific investigations into its pharmacological potential.

Previous studies have provided evidence that *T. globiferus* possesses antioxidant, hepatoprotective, antihyperlipidaemic, immunomodulatory and analgesic activities (Musa et al., 2023; Oche et al., 2019; Oloruntola, 2023; Raceline et al., 2019). A methanolic extract of the leaves was shown to protect isolated rat liver mitochondria against Fe^{2+} -induced oxidative stress by improving mitochondrial membrane potential, thiol content, and reducing lipid peroxidation (Elekofehinti et al., 2019). Using HPLC–DAD, the authors reported the presence of a limited set of phenolic and flavonoid compounds, including gallic acid, caffeic acid, catechin, epigallocatechin, rutin, and quercetin, which were suggested to underlie the observed bioactivity. In vitro cytotoxicity assays against human cancer cell lines (prostate DU-145, breast JIMT-1, and pancreatic MiaPaCa-2/BxPC-3) confirmed that *T. globiferus* extracts possess anticancer activity by demonstrating significant cytotoxic effects, validating its traditional use in tumor management (Oriola et al., 2018). *T. globiferus*, despite its widespread ethnomedicinal use against tumors, was tested only at the crude level, where modest cytotoxicity led to its exclusion from deeper chemical or biological evaluation. While these

findings established a biochemical basis for the antioxidant property, hepatoprotective activity and anticancer activity, the chemical characterization was restricted to crude methanolic and ethanolic extracts respectively.

Consequently, its phytochemical composition remains poorly defined, and no marker compounds have been linked to its reported anticancer potential.

Phytochemical profiling of crude extracts and their solvent-partitioned fractions provides a rational approach for identifying bioactive secondary metabolites. Methanolic crude extracts often serve as rich reservoirs of both polar and moderately non-polar constituents, which can be further fractionated into solvents of varying polarity to enrich distinct classes of compounds (Lestari et al., 2023). For instance, butanol fractions typically concentrate phenolic compounds, flavonoids, and glycosides (Sharma et al., 2023), while ethyl acetate fractions often contain aglycones, terpenoids, and other moderately lipophilic metabolites (Abubakar et al., 2020). Such fractionation not only facilitates compound identification but also aids in associating specific phytochemicals with biological activities, ultimately enabling the recognition of marker compounds that can serve as chemotaxonomic indicators or quality-control standards.

In cancer research, plant-derived metabolites are known to exert cytotoxicity through diverse mechanisms including oxidative stress modulation, apoptosis induction, and interference with oncogenic signaling pathways (Vidjeyamannane et al., 2025). In this study, we profiled the butanol fraction obtained from methanolic crude extract of *T. globiferus*, which was used due to its efficiency in extracting a broad spectrum of secondary metabolites. Through HPLC-DAD analysis coupled with in vitro cytotoxic assay, we correlate fraction chemistry with anticancer activity using in silico studies, targeting a specific target protein, thereby identifying marker compounds that validate traditional use and highlight *T. globiferus* as a promising source of anticancer agents. The bioactive constituents of *T. globiferus* may extend beyond common phenolics and flavonoids to include alkaloids, terpenoids, saponins, and glycosides, which have been preliminarily reported in related studies (Oche et al., 2019).

1.2. MATERIALS AND METHODS

1.2.1 Plant Collection and Authentication

Fresh leaves of *Tapinanthus globiferus* (A. Rich.) Tiegh. parasitizing guava tree (*Psidium gwere*) were collected from Alheri, Zaria Road, Jos, Plateau State Nigeria Latitude: 9° 54' 59" N. Longitude: 8° 52' 46" E. Lat/Long (dec); 9.91646,8.87971. The plant was identified and authenticated by a taxonomist at the College of Forestry, Jos, Plateau State, Nigeria, where a voucher specimen FHJ284 was deposited for reference. The leaves were washed, air-dried at room temperature, and pulverized into fine powder using a mechanical grinder.

1.2.2 Preparation of Crude Extract and Fractions

The powdered leaf material (500 g) was macerated in 70% methanol for 72 h with intermittent shaking. The mixture was filtered using Whatman No. 1 filter paper and concentrated under reduced pressure in a rotary evaporator at 40 °C to obtain the crude extract.

The crude extract was suspended in distilled water and partitioned sequentially with n-butanol to yield fraction, leaving an aqueous residue. The fraction was concentrated to dryness, weighed to determine percentage yields, and stored at 4 °C until analysis.

1.2.3. HPLC–DAD Analysis

High-performance liquid chromatography coupled with diode array detection (HPLC–DAD) was employed for phytochemical profiling. The dried fractions were dissolved in methanol, diluted in the corresponding initial mobile phases, and filtered through a 0.22 µm PTFE syringe filter (Millipore®, São Paulo, Brazil). Phenolic compound quantification followed the procedure described by Quatrin et al. (2019). Analyses were performed on a Shimadzu CBM-20A Prominence HPLC system (Kyoto, Japan) equipped with a degasser (DGU-20A5 Prominence), a column oven (CTO-20A Prominence), and a diode array detector (SPD-M20A Prominence). A 20 µL sample volume was injected into a C18 Hypersil Gold column (150 × 4.6 mm, 5 µm particle size; Thermo Fisher Scientific, Massachusetts, USA) maintained at 38 °C.

The mobile phases consisted of 5% (v/v) methanol in water acidified with 0.1% formic acid (eluent A) and 0.1% (v/v) formic acid in acetonitrile (eluent B), at a flow rate of 1.0 mL/min. Chromatographic separation was carried out under reverse-phase conditions using the following gradient: 4% B from 0–10 min, held until 21 min; 16% B from 21.1–55 min; 50% B from 55.1–70 min; 100% B from 70.1–72 min, held until 80 min; then returned to 0% B from 80.1–83 min, maintained until 92.1 min.

Absorbance spectra were recorded between 200–800 nm. Phenolic compounds were identified by comparing retention times and UV–Vis spectral data with those of authentic standards. Quantification was performed according to compound class: hydroxybenzoate derivatives at 280 nm, hydroxycinnamate derivatives at 320 nm, flavonoid derivatives at 360 nm, and total phenolic content as described by Quatrin et al. (2019). Results were expressed as mg per 100 g of dry extract weight.

1.2.4 Cell Culture and Cytotoxicity Assays

Cell Culture

The human colorectal adenocarcinoma cell line (HT29) was obtained from [insert source if available] and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin. Cells were maintained in T25 culture flasks under standard conditions of 5% CO₂ and 95% relative humidity in a CO₂ incubator (CHP-16, Precision Biomedical). All procedures followed the standard operating protocols of the Precision Biomedical tissue culture facility.

Cytotoxicity Assay (MTT Method)

The cytotoxic effects of the butanol fraction of *Tapinanthus globiferus* and Lorlatinib (a standard anticancer drug) were evaluated on HT29 cells using the MTT assay as previously described by Waziri et al. (2016), with slight modifications. Briefly, monolayer cells were harvested with trypsin–EDTA to obtain single-cell suspensions. Viable cells were counted using a hemocytometer following Trypan blue exclusion.

Cells were seeded in 96-well plates at a density of 5×10^3 cells per well in 100 µL of culture medium and incubated overnight at 37 °C in 5% CO₂. The following day, cells were treated with 100 µL of either the fraction or Lorlatinib at concentrations of 12.5, 25, 50, 100, and 200 µg/mL. Wells containing only culture medium served as negative controls.

After 48 h of treatment, 20 µL of MTT solution (5 mg/mL in PBS, Sigma-Aldrich, Germany) was added to each well, yielding a final concentration of 0.5 mg/mL. Plates were wrapped in foil to prevent light exposure and incubated for an additional 4 h. The medium was then carefully removed, and 150 µL of dimethyl sulfoxide (DMSO) was added to solubilize the purple formazan crystals. Absorbance was read at 490 nm using an ELISA microplate reader. Each treatment was performed in quintuplicate.

The percentage cytotoxicity was calculated according to the formula:

$$\text{Cytotoxicity (\%)} = 1 - \frac{\text{ODT}}{\text{ODC}} \times 100$$

Where:

ODT = optical density of treatment well

ODC = optical density of negative control well.

Phase-Contrast Microscopy

For morphological assessment, HT29 cells were seeded into 6-well plates at a density of 5×10^4 cells per well in 1 mL of culture medium and incubated overnight under standard culture conditions. Cells were then treated with 1 mL of the test fraction at concentrations of 12.5, 25, 50, 100, and 200 $\mu\text{g/mL}$. Medium-only wells served as negative controls. After 48 h of treatment, changes in cell morphology were observed and documented using an AmScope MU303 camera mounted on an inverted phase-contrast microscope at $10\times/0.25$ pH magnification.

1.2.5 Molecular Docking

Ligand Preparation

The bioactive compounds of *Tapinanthus globiferus* identified through HPLC–DAD analysis were used for ligand preparation. Using the LigPrep module of Maestro 13.4 (Schrödinger Suite 2022-4), both the identified compounds and the standard ligands for each protein target were converted into low-energy 3D structures with correct stereochemistry. Ionization states were generated at physiological pH (7.2 ± 0.2). For each ligand, stereoisomers were computed by maintaining specified chiral centers while varying the others, following the approach described by Johnson, et al. (2021). The target proteins used were Vascular Epithelial Growth Factor A (VEGF-A PDB ID: 5O4E) and B-Cell Lymphoma 2 (BCL2 PDB ID: 6GL8).

Protein Preparation

The target proteins, along with their PubChem CIDs, standard inhibitors, and co-crystallized ligands (Table 2), were retrieved and prepared using the Protein Preparation Wizard in Maestro (Schrödinger Suite 2022-4). During preparation, hydrogen atoms were added, bond orders assigned, and disulfide bonds generated. Missing side chains and loops were modeled using Prime. Water molecules beyond $3.0 \text{ \AA}$ from heteroatoms were removed. The protein structures were energy-minimized with the OPLS4e force field and protonation states optimized with PROPKA, as reported by Johnson et al. (2021).

Receptor Grid Generation

Receptor grids were generated using the Grid Generation tool in Maestro 13.4, following the protocol of Johnson et al. (2021). The grids defined the active site boundaries of each protein, with the binding position of the co-crystallized ligand serving as the reference for grid placement.

Protein–Ligand Docking

Molecular docking was performed using the Glide module in Maestro 13.4 (Schrödinger Suite 2022-4). The prepared ligands were docked into the receptor grids employing the Extra Precision (XP) docking mode. For all proteins, the van

der Waals scaling factor was set to 1.0, with a partial charge cut-off of 0.25 applied to ligand atoms. Ligand sampling was carried out flexibly to allow conformational variation, in line with Johnson, et al. (2021).

Pharmacokinetics and Toxicity Profiling

Determination of the pharmacokinetics (ADME) property of the compounds was performed using the SWISS-ADME as described by Daina et al. (2017). Toxicity profile was carried out using Protox 3.0 (<https://tox.charite.de/protox3/>)

Statistical Analysis

Statistical analyses were performed using Graphpad prism (ver 10.4.2). Results express as mean ± standard error

1.3. RESULTS

1.3.1 Cytotoxicity of Butanol Fraction against HT29 Colon Cancer Cells

The butanol fraction of *Tapinanthus globiferus* leaves demonstrated a strong, dose-dependent cytotoxic effect against HT29 colon cancer cells compared with the standard drug, lorlatinib. (Figure 1). Following 48 h of treatment (0–200 µg/mL), the butanol fraction yielded an IC₅₀ of 8.15 µg/mL, while lorlatinib exhibited a higher potency with an IC₅₀ of 3.23 µg/mL (Figure 3).

Phase-contrast microscopy revealed distinct morphological alterations characteristic of apoptosis, including cell shrinkage, rounding, and membrane rupture. These effects were observed in a concentration-dependent manner in cells treated with both the butanol fraction and lorlatinib (Figure 2), suggesting similar mechanisms of cytotoxic action.

1.3.2 Phytochemical Profiling of Tapinanthus globiferus

HPLC–DAD analysis identified several phenolic compounds in the fractions of *T. globiferus* leaves, in the butanol fraction. Hydroxybenzoate derivatives such as protocatechuic acid (23.85 ± 1.23 mg/100 g) and syringic acid (24.95 ± 4.26 mg/100 g) were detected, while gallic acid was below the limit of quantification. Hydroxycinnamate derivatives including caffeic acid, chlorogenic acid, p-coumaric acid, and t-ferulic acid were below detection or quantification thresholds. In contrast, flavonoids were abundantly represented, particularly rutin (1633.77 ± 7.71 mg/100 g), isoquercetin (1318.37 ± 1.70 mg/100 g), catechin (192.04 ± 23.96 mg/100 g), epicatechin (32.49 ± 0.16 mg/100 g), and kaempferol derivatives (8848.81 ± 12.70 mg/100 g). Quercetin derivatives were also prominent (7517.89 ± 74.25 mg/100 g), resulting in a total phenolic content of 24,099.60 ± 100.25 mg/100 g (Table 1; Figure 3-4).

Table 1

Bioactive Compounds identified in the Butanol Fraction of *Tapinanthus globiferus* Leaf

	Butanol mg/100 g of lyophilised extract	
	λ (nm)	
<u>Hydroxybenzoate derivates (HBD)</u>		
Gallic acid	280 nm	$\leq$ LoQ
Protocatechuic acid	280 nm	23.85 ± 1.23
Syringic acid	280 nm	24.95 ± 4.26
<u>Hydroxycinnamate derivatives (HCD)</u>		
Caffeic acid	320 nm	$\leq$ LoD
Chlorogenic acid	320 nm	$\leq$ LoQ
<i>p</i> -Coumaric acid	320 nm	$\leq$ LoQ
<i>t</i> - Ferulic acid	320 nm	$\leq$ LoQ
<u>Flavonoids</u>		
Rutin	360 nm	1633.77 ± 7.71
Quercetin-3-glucoside (Isoquercetin)	360 nm	1318.37 ± 1.70
Catechin	280 nm	192.04 ± 23.96
Epigallocatechin	280 nm	$\leq$ LoD
Epicatechin	280 nm	32.49 ± 0.16
Kaempferol derivatives*	360 nm	8848.81 ± 12.70
(Kaempferol-o- β -D-galactopyranoside)		
Quercetin derivaties**	360 nm	7517.89 ± 74.25
Total phenolic compounds		24099.60 ± 100.25

Results express as mean $\pm$ standard error; LoQ: limit of quantification; LoD: Limit of Detection

** Quercetin derivatives were quantified as equivalent to quercetin-3-glycoside

***Total phenolic compounds were determined through the sum of the hydroxybenzoate derivatives, hydroxycinnamate derivatives, and flavonoids; LoQ: limit of quantification.

1.3.3 *In silico* Therapeutic Potential of Identified Bioactive Compounds

The predominance of phenolic compounds—particularly flavonoids—in the active fractions highlights their potential role in the anticancer activity of *T. globiferus*. Given their established pharmacological significance, these compounds were subjected to further *in silico* analyses, including molecular docking to evaluate their interaction with VEGF-A.

Molecular Docking with VEGF-A

Docking studies against vascular endothelial growth factor A (VEGF-A; PDB ID: 5O4E) in Table 2, revealed favorable binding affinities for several bioactive compounds compared to the co-crystallized ligand, pazopanib (docking score – 3.560 kcal/mol). Rutin displayed the highest binding affinity (–8.850 kcal/mol), followed by trifolin (–7.041 kcal/mol) and epigallocatechin (–6.554 kcal/mol).

The compounds exhibited diverse interaction patterns with VEGF-A. Rutin engaged with the backbone atoms of PRO247, LYS248, SER375, ASP376, ILE377, ALA378, and VAL379, while additional contacts were formed with the side chains of LYS246, PRO247, PRO248, SER375, ASP376, ILE377, ALA378, VAL379, GLU380, THR393, and LEU406. Polar interactions were established with LYS246, LYS248, ARG255, SER375, ASP376, GLU380, and THR393, whereas hydrophobic contacts occurred with PRO247, ILE377, ALA378, VAL379, and LEU406. Hydrogen bonding involved ASP376, ILE377, and GLU380 acting as hydrogen acceptors, while SER375 and VAL379 donated hydrogen atoms to rutin. In addition, electrostatic interactions were observed with LYS246, LYS248, ARG255, ASP376, and GLU380, and a pi–cation interaction was identified with LYS246 (Figure 5a).

Similarly, trifolin established interactions with the backbone of ILE377, VAL379, TYR391, LEU392, THR393, TRP394, and PRO395, alongside side chain contacts with PRO247, LYS248, GLU380, TYR391, LEU392, THR393, PRO395, and PRO396. Polar interactions were noted with LYS248, GLU380, and THR393, while hydrophobic contacts occurred with PRO247, TYR391, LEU392, PRO395, and PRO396. Hydrogen bonding involved donation of hydrogen atoms to THR398 and acceptance by GLU380, TYR391, and THR393 (Figure 5b).

Epigallocatechin formed interactions with the backbone of SER 375, ASP 376, ILE 377, ALA 378 and VAL 379; sidechains of SER 375, ASP 376, ILE 377, ALA 378, THR 393, PRO 395, PRO 396 and LEU 406. Polar interactions were also formed with SER 375, ASP 376 and THR 393. Hydrophobic interactions were formed with ILE 377, ALA 378, PRO395, PRO 396 and LEU 406. Hydrogen bonds were formed with ILE 377, VAL379 and SER 375, while an electrostatic interaction was formed with ASP 376 (Figure 5c).

In comparison, pazopanib—the co-crystallized standard VEGF-A inhibitor—interacted with the backbone of LYS248, SER375, ASP376, ILE377, ALA378, and ILE399. Side chain contacts were observed with PRO244, LYS246, PRO247, LYS248, ARG255, SER375, ASP376, VAL379, GLU380, TYR391, THR393, and PRO396. Polar interactions involved LYS246, LYS248, ARG255, SER375, ASP376, GLU380, and THR393, while hydrophobic contacts were formed with PRO244, PRO247, VAL379, TYR391, and PRO396. Hydrogen bonds were formed reciprocally between VAL379 and the ligand, with the residue both donating and accepting hydrogen atoms (Figure 5d).

These results suggest that key flavonoids of *T. globiferus*, particularly rutin and trifolin, possess strong affinity for VEGF-A, supporting their potential as anti-angiogenic agents in cancer therapy.

Table 2

Docking scores of the bioactive compounds of *Tapinanthus globiferus* Leaf against VEGFA

Compounds	CID	Docking score (kcal/mol)
Rutin	5280805	-8.850
Trifolin	5282149	-7.041
Epigallocatechin	72277	-6.554
Epicatechin	72276	-6.375
Gallic acid	370	-6.337
Quercetin	5280343	-6.330
Isoquercetin	5280804	-6.271
Protocatechuic acid	72	-5.772
Kaempferol	5280863	-5.544
Chlorogenic acid	1794427	-5.397
Syringic acid	10742	-5.269
Catechin]	9064	-5.211
Caffeic acid	689043	-4.411
Pazopanib	10113978	-3.560
T-ferulic acid	445858	-3.120
P-coumaric acid	637542	-2.783

Molecular Docking with BCL2

The docking score of the compounds against BCL2 in Table 3 showed favour binding affinities to the protein compared to the standard drug venetoclax (-4.445 kcal/mol). Compounds with the highest scores were rutin (-7.402 kcal/mol), kaempferol (-5.873 kcal/mol), and trifolin (-5.575 kcal/mol). Compared to the standard drug, these and several other compounds also showed higher preferential binding the protein. This could be attributed to the different interactions that were established between the compounds and the protein.

Venetoclax demonstrated extensive interactions within the binding pocket, engaging both backbone and side-chain residues (Figure 6d). Backbone contacts were observed with ALA100, ASP103, PHE104, GLU136, GLY145, ALA149, and PHE153, while side-chain interactions included ASP103, PHE104, ARG107, PHE112, MET115, GLN118, ASP140, ASN143, ARG146, VAL148, PHE153, and TYR202. Polar contacts were established with ASP103, ARG107, GLN118, ASP140, ASN143, and ARG146. Hydrophobic interactions were mediated by PHE104, PHE112, MET115, VAL148, PHE153, and TYR202, with π - π stacking observed between venetoclax and PHE104, PHE112, PHE153, and TYR202. Hydrogen bonds were formed with ASP140 and ARG146, while electrostatic contacts involved ASP103, ARG107, ASP140, and ARG146. This combination of salt bridges, hydrogen bonds, and π - π stacking likely underpins the high binding affinity and specificity of venetoclax.

Rutin interacted primarily with backbone residues VAL133 and ALA149, and with side-chains of PHE104, TYR108, ASP111, PHE112, MET115, GLN118, LEU119, VAL133, GLU136, LEU137, ALA149, and PHE153. Polar contacts were observed with ASP111, GLN118, and GLU136, while hydrophobic contacts included PHE104, TYR108, PHE112,

MET115, LEU119, VAL133, LEU137, ALA149, and PHE153. Hydrogen bonds were established with ASP111, GLN118, and GLU136. Aromatic stabilization was mediated by PHE104, TYR108, PHE112, and PHE153, and electrostatic interactions were formed with ASP111 and GLU136. The interaction profile suggests rutin relies more heavily on hydrogen bonding and polar contacts compared to venetoclax, but still exploits hydrophobic and aromatic residues for stabilization (Figure 6a).

Kaempferol engaged backbone atoms of ALA149 and PHE153, and side-chains of PHE104, PHE112, MET115, GLN118, VAL133, GLU136, LEU137, ALA149, and PHE153. Polar interactions were contributed by GLN118 and GLU136, while hydrophobic contacts included PHE104, PHE112, MET115, VAL133, LEU137, ALA149, and PHE153. Hydrogen bonding occurred with GLN118, GLU136, and ALA149, and π - π stacking was observed with PHE104, PHE112, and PHE153. Electrostatic interactions were limited to GLU136 (Figure 6b). These results indicate that kaempferol primarily exploits aromatic stacking and a smaller set of hydrogen bonds, which may account for moderate binding affinity relative to venetoclax.

Trifolin formed backbone contacts with TYR108, ASP111, PHE112, and GLY145, and interacted with side-chains of PHE104, TYR108, ASP111, PHE112, MET115, GLN118, VAL133, LEU137, ARG146, ALA149, and PHE153. Polar contacts were mediated by ASP111, GLN118, and ARG146, while hydrophobic interactions involved PHE104, TYR108, PHE112, MET115, VAL133, LEU137, ALA149, and PHE153. Hydrogen bonding was observed with ASP111, while aromatic stabilization involved PHE104, TYR108, PHE112, and PHE153. Electrostatic interactions were contributed by ASP111 and ARG146. The binding profile indicates a balanced contribution of hydrogen bonding, polar, and aromatic interactions, with additional electrostatic stabilization via ARG146 (Figure 6c).

Table 3

Docking scores of the bioactive compounds of *Tapinanthus globiferus* Leaf against BCL2

Compounds	CID	Docking score (kcal/mol)
Rutin	5280805	-7.402
Kaempferol	5280863	-5.873
Trifolin	5282149	-5.575
Quercetin	5280343	-5.291
Caffeic Acid	689043	-5.039
Protocatechuic Acid	72	-4.989
Isoquercetin	5280804	-4.968
Epicatechin	72276	-4.903
Venetoclax	49846579	-4.445
Ferulic Acid	445858	-3.947
P-Coumaric Acid	637542	-3.934
Epigallocatechin	72277	-3.822
Catechin	9064	-3.487
Gallic Acid	370	-3.43
Chlorogenic Acid	1794427	-3.329
Syringic Acid	10742	-2.332

In silico ADME-Tox (Table 4-5) profiling indicated that major glycosides (rutin, isoquercetin, trifolin), despite strong VEGF-A/BCL-2 binding, have low GI absorption, poor bioavailability scores, and multiple Lipinski violations, suggesting limited oral drug-likeness. Conversely, aglycones such as kaempferol and quercetin show high GI absorption, no violations, and better oral potential. Toxicity predictions were favorable: rutin and isoquercetin (LD50 $\approx$ 5000 mg/kg, class 5) and catechins/epigallocatechin (LD50 $\approx$ 10000 mg/kg, class 6) all indicated low acute toxicity, with no major hepatotoxicity or mutagenicity signals, though some renal alerts were noted. These findings support kaempferol and quercetin as promising oral leads, while formulation strategies (e.g., nanoparticles, prodrugs) may enhance glycoside bioavailability for further development.

Table 4

Pharmacokinetics

Compound	Molecular Weight	Mean LOGP	Lipinski violations	Bioavailability Score	PAINS alerts	GI absorption
Protocatechuic acid	154.12	0.40	0	0.56	1	High
Gallic acid	170.12	-0.16	0	0.56	1	High
Syringic acid	198.17	0.49	0	0.56	0	High
Chlorogenic acid	354.31	-1.05	1	0.11	1	Low
Isoquercetin	464.38	-2.59	2	0.17	1	Low
Rutin	610.52	-3.89	3	0.17	1	Low
T-ferulic acid	194.18	1.00	0	0.85	0	High
Kaempferol	286.24	-0.03	0	0.55	0	High
Quercetin	302.24	-0.56	0	0.55	1	High
P-coumaric acid	164.16	1.28	0	0.85	0	High
Caffeic acid	180.16	0.70	0	0.56	1	High
Epigallocatechin	306.27	-0.29	1	0.55	1	High
Epicatechin	290.27	0.24	0	0.55	1	High
Catechin	290.27	0.24	0	0.55	1	High
Trifolin	448.38	-2.10	2	0.17	0	Low

Table 5

Toxicity Profile

Compound	LD50	Toxicity Class	DILI	Neuro	Nephro	Cardio	Carcino	Immuno	Muta	Cyto
Protocatechuic acid	2000 mg/kg	4	-	-	+	-	+	-	-	-
Gallic acid	2000 mg/kg	4	-	-	+	-	+	-	-	-
Syringic acid	1700 mg/kg	4	-	-	+	-	-	-	-	-
Chlorogenic acid	5000 mg/kg	5	-	-	+	-	-	+	-	-
Isoquercetin	5000 mg/kg	5	-	-	+	-	-	+	-	-
Rutin	5000 mg/kg	5	-	-	+	-	-	+	-	-
T-Ferulic acid	1772 mg/kg	4	-	-	+	-	-	+	-	-
Kaempferol	3919 mg/kg	5	-	-	+	-	-	-	-	-
P-Coumaric acid	2850 mg/kg	5	-	-	+	-	+	-	-	-
Caffeic acid	2980 mg/kg	5	-	-	+	-	+	-	-	-
Epigallocatechin	10000 mg/kg	6	-	-	+	-	-	-	-	-
Epicatechin	10000 mg/kg	6	-	-	+	-	-	-	-	-
Catechin	10000 mg/kg	6	-	-	+	-	-	-	-	-
Trifolin	5000 mg/kg	5	-	-	+	-	-	-	-	-

1.4. DISCUSSION

1.4.1 Phytochemical Composition and Correlation with Cytotoxicity

In this study, the butanol fraction was used. This was because our previous study had shown a more consistent antioxidative activity by the butanol fraction compared to the ethyl acetate and methanol fraction, in an in vivo study on the redox modulatory activity of *T. globiferus* (Oche et al., 2024; Oche et al., 2025). The butanol fraction of *T. globiferus* leaves exhibited potent cytotoxicity against HT-29 colon cancer cells, only modestly less active than the reference chemotherapeutic lorlatinib. This pronounced activity correlates with the phenolic profile of the fraction. HPLC-DAD analysis revealed that it is dominated by flavonoids: kaempferol derivatives, quercetin derivatives, rutin, and isoquercetin were among the most abundant constituents. Such flavonoids are well-known for diverse anticancer effects (Amjad et al., 2022; Kopustinskiene et al., 2020). In general, dietary polyphenols modulate cell signaling, induce cell-cycle arrest and apoptosis, and suppress tumor proliferation and metastasis (Kopustinskiene et al., 2020).

Kaempferol in particular has been repeatedly shown to inhibit tumor cell growth and induce cancer cell death across multiple malignancies (Amjad et al., 2022). Thus, the high concentrations of kaempferol and quercetin glycosides in the extract likely underlie much of the observed cytotoxicity. Moreover, the combination of multiple flavonoids may produce synergistic effects. For example, quercetin and kaempferol derivatives can generate pro-oxidant stress and inhibit pro-survival pathways in cancer cells (Amjad et al., 2022; Kopustinskiene et al., 2020). In this context, antiproliferative potency of the butanol fraction is possibly attributable to the combined action of its phenolic constituents, each of which is documented to exert anticancer activity in vitro.

1.4.2 Induction of Apoptosis in HT-29 Cells

Phase-contrast microscopy of treated HT-29 cultures revealed hallmark features of apoptosis that intensified with dose. Cells became markedly shrunken and rounded, and membrane blebbing and nuclear condensation were evident – changes virtually identical to those induced by lorlatinib. These morphological alterations strongly indicate programmed cell death. Notably, they mirror effects reported for individual flavonoids: quercetin has been shown to inhibit HT-29 viability in a dose-dependent manner and to elicit classic apoptotic morphology (cell shrinkage, chromatin condensation, DNA fragmentation) (Yang et al., 2016). Similarly, rutin produces apoptosis in HT-29 cells by activating both extrinsic (death-receptor) and intrinsic (mitochondrial) pathways (Guon & Chung, 2016). In this study, rutin treatment increased levels of cleaved caspase-3, -8 and -9, upregulated pro-apoptotic Bax and downregulated anti-apoptotic Bcl-2, a feature that aligns with our observations of membrane rupture and cellular collapse. It has also been reported that rutin induces reactive oxygen species (ROS) generation and loss of mitochondrial membrane potential in colon cancer cells, further driving apoptosis (ben Sghaier et al., 2016). Thus, the dose-dependent apoptotic phenotype seen with the *T. globiferus* fraction is consistent with the known pro-apoptotic actions of its flavonoids. In aggregate, these data suggest that the fraction triggers apoptotic cell death, likely via activation of caspase cascades and mitochondrial pathways, paralleling the mechanisms of isolated kaempferol/quercetin glycosides in colon cancer cells (Nouri et al., 2020; Yang et al., 2016).

1.4.3 VEGF Binding and Anti-Angiogenic Potential

Our molecular docking results indicate that several of the phenolic compounds identified in the butanol fraction bind strongly to human VEGF-A (PDB ID: 5O4E). Rutin showed especially high affinity, with trifolin (a kaempferol glycoside) and epigallocatechin also binding more tightly than the reference VEGF inhibitor pazopanib. These flavonoids formed multiple hydrogen bonds, hydrophobic and polar contacts, and electrostatic interactions with residues in the VEGF-A active site. Although VEGF-A is a signaling ligand rather than an enzyme, such tight binding is likely to disrupt its interaction with VEGF receptors. Indeed, a prior study demonstrated that EGCG from green tea can directly bind VEGF and prevent VEGFR2 activation, potentially inhibiting VEGF-induced angiogenesis at nanomolar concentrations (Moyle et al., 2015). By analogy, our in silico data suggest that rutin, kaempferol derivatives, and catechins in the fraction could similarly sequester VEGF-A or alter its conformation, thereby blocking downstream angiogenic signaling. Supporting this, isoquercetin (quercetin-3- β -glucoside), one of the major compounds here, was recently shown in vivo to reduce tumor vascularization and growth in a colon cancer model (da Silva et al., 2022). Mice treated with isoquercetin had ~65% fewer blood vessels in the tumor than controls, outperforming even bevacizumab (anti-VEGF) (da Silva et al., 2022). Thus, the docking findings align with an anti-angiogenic mechanism for the fraction. In sum, the strong computational binding of *T. globiferus* phenolics to VEGF-A, together with known polyphenol interference with VEGF signaling (da Silva et al., 2022), implies that inhibition of tumor angiogenesis may contribute to the observed anticancer activity.

Targeting the amino acids in the VEGF-A binding site is therapeutically significant. Aspartate, glutamate, arginine, and proline residues facilitate the interaction of VEGF-A with its receptor VEGFR2, enabling signal transduction during

angiogenesis. These residues also mediate VEGF-A binding to Neuropilin-1 (NRP1) and extracellular matrix (ECM) components through Ig-like domains 2 and 3 of VEGFR2, processes essential for angiogenesis (Peach et al., 2018). Tyrosine residues support hydrogen bonding and hydrophobic contacts, while lysine enhances the stability of the VEGF-A–VEGFR2 complex (Mukherjee et al., 2024). Disrupting these interactions could therefore block vascularization, depriving cells of blood supply, since VEGF-A requires VEGFR2-induced conformational changes to promote angiogenesis (Peach et al., 2018). Moreover, aspartate residues in VEGF-A serve as key regulatory elements. Their ability to bind metal ions or undergo post-translational modifications such as phosphorylation and glycosylation influences protein function, including receptor binding, downstream signaling, angiogenesis, and cellular localization (Woolard et al., 2009). The interactions of these compound with the amino acids can influence receptor binding to the protein thus inhibiting enzyme activities.

1.4.4 Docking with BCL-2

BCL-2 is a key anti-apoptotic member of the BCL-2 family that preserves mitochondrial outer membrane integrity by sequestering pro-apoptotic BH3-only proteins; its overexpression confers resistance to apoptosis in many cancers, including colorectal carcinoma. Direct inhibition or displacement of BH3-binding groove of BCL-2 is therefore a validated anticancer strategy (venetoclax is the clinical archetype). Our in silico docking of *T. globiferus* phenolics against BCL-2 indicates that several abundant flavonoids, especially rutin, kaempferol, and trifolin, are capable of occupying the BCL-2 binding pocket with energetically favorable poses that are consistent with competitive disruption of BH3 interactions.

The hydrophobic groove formed by residues such as Phe, Tyr, Met, Val, Leu, Ala, in BCL-2 family proteins is crucial for binding BH3-only proteins. Molecular dynamics simulations show that this groove is solvent-exposed yet stabilized by internal hydrophobic interactions (Lama et al., 2013). The groove exhibits plasticity, allowing it to accommodate diverse BH3 ligands through conformational changes, particularly in the loop connecting helices H2 and H3 (Sankararamakrishnan, 2011). Conserved “knob” residues, including Gly, Leu, Ala, and Glu, are thought to play a pivotal role in determining binding specificity within the BH3/BCL-2 interface. In contrast, residues such as Asp, Asn, and Val contribute to the formation of complementary “socket” surfaces that accommodate these knobs (Yi et al., 2023). This structural complementarity provides valuable insight for the rational design of BH3 mimetics with selective affinity for pro-survival BCL-2 proteins, thereby advancing targeted cancer therapeutics (Yi et al., 2023). The docking analyses demonstrated that rutin, kaempferol, and trifolin engage the BH3-binding groove of BCL-2 with interaction patterns closely resembling those of the standard inhibitor venetoclax. Notably, hydrophobic and aromatic residues such as PHE104, PHE112, MET115, VAL133, PHE153, and TYR202 provided van der Waals and π – π stacking interactions, while polar and charged residues including GLN118, GLU136, ASP111, and ARG146 contributed stabilizing hydrogen bonds and electrostatic contacts. These residues are essential for the role of BCL-2 in sequestering pro-apoptotic BH3-only proteins and thereby preventing mitochondrial outer membrane permeabilization (Kvansakul & Hinds, 2015). Occupation of these key positions by *T. globiferus* flavonoids suggests direct disruption of BCL-2 function, effectively mimicking BH3 peptides. This mechanistic inference aligns with the observed apoptotic features in HT-29 cells and supports the notion that the butanol fraction acts, at least in part, through BCL-2 modulation. Collectively, these findings highlight the potential of *T. globiferus* flavonoids as natural BH3 mimetics and justify further validation through biophysical binding assays and functional apoptosis studies.

1.4.5 Pharmacokinetics and Safety Implications

The ADME–Tox predictions add translational context to these findings. Glycosides such as rutin, isoquercetin, and trifolin, though potent in vitro and in docking, exhibited poor oral bioavailability due to high molecular weights, multiple Lipinski violations, and low gastrointestinal absorption. In contrast, aglycones like kaempferol and quercetin displayed

favorable pharmacokinetic properties, including high GI absorption, compliance with Lipinski's rule, and acceptable bioavailability, making them more suitable for oral therapy.

Toxicity predictions were reassuring, with most compounds falling within low-toxicity classes ($LD_{50} \geq 5000$ mg/kg) and minimal alerts for hepatotoxicity, mutagenicity, or carcinogenicity. Although some renal toxicity signals were noted, the overall safety profile supports selective anticancer activity rather than nonspecific cytotoxicity. These results indicate that kaempferol and quercetin are promising oral leads, while glycosides may require formulation strategies—such as nanoparticle delivery, prodrug approaches, or structural simplification—to improve systemic exposure.

1.4.6 Comparison with Established Effects of Flavonoids

These results are consistent with a large body of literature demonstrating anticancer effects of flavonoids. Rutin and quercetin glycosides have been reported to inhibit proliferation of colon cancer cells by inducing apoptosis, arresting the cell cycle, and modulating inflammatory pathways (Nouri et al., 2020; Yang et al., 2016). For example, dietary rutin was found to significantly reduce HT-29 cell viability by activating caspases and upregulating Bax (Guon & Chung, 2016), while quercetin triggered S-phase cell-cycle arrest and Akt-pathway inhibition in HT-29 cells (Yang et al., 2016). Kaempferol itself has a broad anticancer profile, affecting diverse oncogenic pathways (PI3K/Akt, MAPK, etc.) and inducing apoptosis in multiple tumor types (Amjad et al., 2022). More generally, flavonoids often act as dual antioxidants/pro-oxidants: they scavenge free radicals under normal conditions but generate ROS in cancer cells to trigger cell death (Kopustinskiene et al., 2020). They also tend to suppress NF- κ B and other pro-survival inflammatory signals (Kopustinskiene et al., 2020). Together, our findings corroborate these established patterns: the high-flavonoid content of *T. globiferus* fraction provides the means to both initiate apoptosis and impede angiogenesis, matching prior reports of their multi-targeted anticancer activity.

1.4.6 Traditional Use and Mechanistic Implications

Tapinanthus globiferus is an African mistletoe valued in traditional medicine for treating pain, fever, rheumatism, and even tumors (Musa et al., 2023). Our data offer a pharmacological rationale for these uses. The identified flavonoids have known anti-inflammatory and analgesic properties in addition to anticancer activity. For instance, quercetin and kaempferol derivatives are documented to inhibit pro-inflammatory mediators (TNF- α , IL-6, NF- κ B) and to modulate opioid and adrenergic receptors (Kopustinskiene et al., 2020; Musa et al., 2023). Such effects could underlie the reported analgesic actions of the plant and its use in inflammatory conditions. Importantly, chronic inflammation is a driver of carcinogenesis, so the anti-inflammatory phytochemicals in *T. globiferus* may also help prevent tumor development or progression. In light of the docking results, constituents of the fraction could simultaneously target multiple cancer hallmarks – suppressing angiogenesis via VEGF interference and promoting apoptosis via mitochondrial, death-receptor pathways and BCL2 inhibition. Thus, the traditional role of *T. globiferus* in folk cancer therapies is supported by our mechanistic findings: its rich phenolic composition likely acts on both cancer cells and the tumor microenvironment to exert antineoplastic effects.

1.4.7 Implications for Drug Development and Future Directions

The present findings position *T. globiferus* as a promising source of anticancer agents. The activity of the butanol fraction (IC_{50} in the low μ g/mL range) meets or exceeds thresholds often used to flag crude extracts for drug discovery. Moving forward, bioassay-guided fractionation should be employed to isolate the most active constituents (e.g. rutin, kaempferol glycosides). These pure compounds can then be tested individually and in combination to assess their specific cytotoxic and anti-angiogenic properties. Integrating the phytochemical, cytotoxicity, docking,

and ADMETox findings highlights a dual path for drug discovery: aglycones as directly translatable scaffolds for lead optimization, and glycosides as potent compounds that may be developed through advanced formulations. Together, these findings validate the ethnomedicinal use of *T. globiferus* and underscore its potential as a source of multi-targeted anticancer agents. Future studies should prioritize bioassay-guided fractionation, mechanistic assays, and in vivo models to establish efficacy and safety.

In parallel, detailed mechanistic studies are warranted: for example, measuring caspase activation, Bcl-2/Bax ratios, and reactive oxygen species generation in treated cells, as well as VEGF secretion and endothelial cell tube formation. In vivo investigations in colon cancer xenograft models will be critical to determine antitumor efficacy and angiogenesis inhibition in a whole-organism context. Finally, the observed interactions with VEGF-A and BCL2 suggest that *T. globiferus* compounds might serve as leads or adjuncts for anti-angiogenic therapy.

In summary, our results justify further research on *T. globiferus* and its flavonoids as multi-targeted anticancer agents. The integration of ethnobotanical knowledge with modern bioassays could expedite the development of novel therapeutics derived from this traditional medicinal plant.

1.5 CONCLUSION

Tapinanthus globiferus was shown to be rich in flavonoids and phenolic compounds, particularly kaempferol and quercetin derivatives, rutin, and isoquercetin, which correlated with strong cytotoxicity against HT-29 colon cancer cells. Molecular docking further revealed high binding affinities to VEGF-A and BCL-2, suggesting dual anti-angiogenic and pro-apoptotic mechanisms. These findings provide a pharmacological basis for its ethnomedicinal use and identify marker compounds with promise for anticancer drug discovery, warranting further in vivo validation and formulation studies.

Declarations

ACKNOWLEDGEMENT

The authors gratefully acknowledge Precision Biomedicals, Kaduna State, Nigeria, and the Department of Food Technology and Science, Center of Rural Sciences, Federal University of Santa Maria, Camobi, Santa Maria, RS, Brazil, for providing the facilities and technical support required to carry out the analyses in this study.

Funding: This work was supported by the 2024 TETFUND Institution Based Research (IBR) Intervention

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Conceptualization: Jane-Rose Oche, Titilayo Johnson; Methodology: Jane-Rose Oche, Titilayo Johnson, Jonathan Dabak; Investigation: Jane-Rose, Oluwabukola Farodoye, Camila Sant'Anna Monteiro; Data curation: Jane-Rose Oche, Camila Sant'Anna Monteiro, Oluwabukola Farodoye; Formal analysis: Jane-Rose Oche, Titilayo Johnson; Writing – original draft: Jane-Rose Oche, Titilayo Johnson; Writing – review & editing: Jane-Rose Oche, Titilayo Johnson, Jonathan Dabak; Visualization: Jane-rose Oche, Jonathan Dabak; Supervision: Titilayo Johnson, Jonathan Dabak; Project administration: Titilayo Johnson

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data availability is based on reasonable request

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Figures

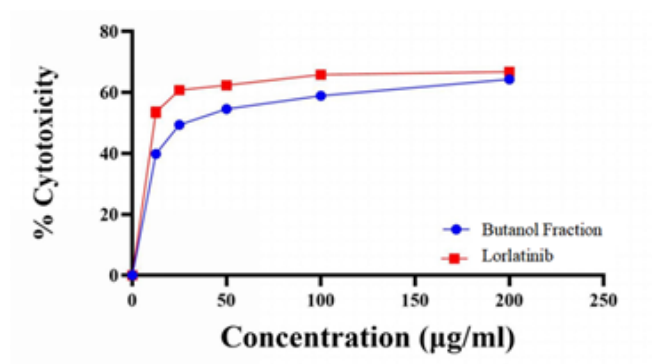


Figure 1

Dose-dependent cytotoxicity of *T. globiferus* butanol fraction (blue) compared with reference drug lorlatinib (red) against HT-29 colon cancer cells. Data are expressed as mean $\pm$ SD of triplicate experiments.

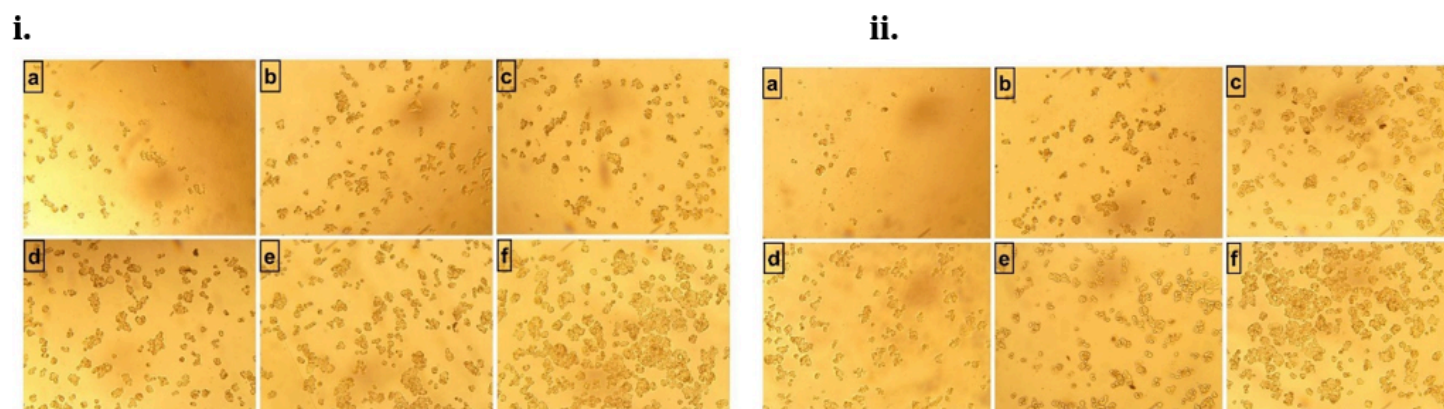


Figure 2

Phase contrast microscopy of HT29 cells treated with (i) Butanol fraction and (ii) Lorlatinib at varying concentrations (a) 200 µg/ml, (b) 100 µg/ml, (c) 50 µg/ml (d) 25 µg/ml (e) 12.5 µg/ml (F) negative control (DMEM media only).

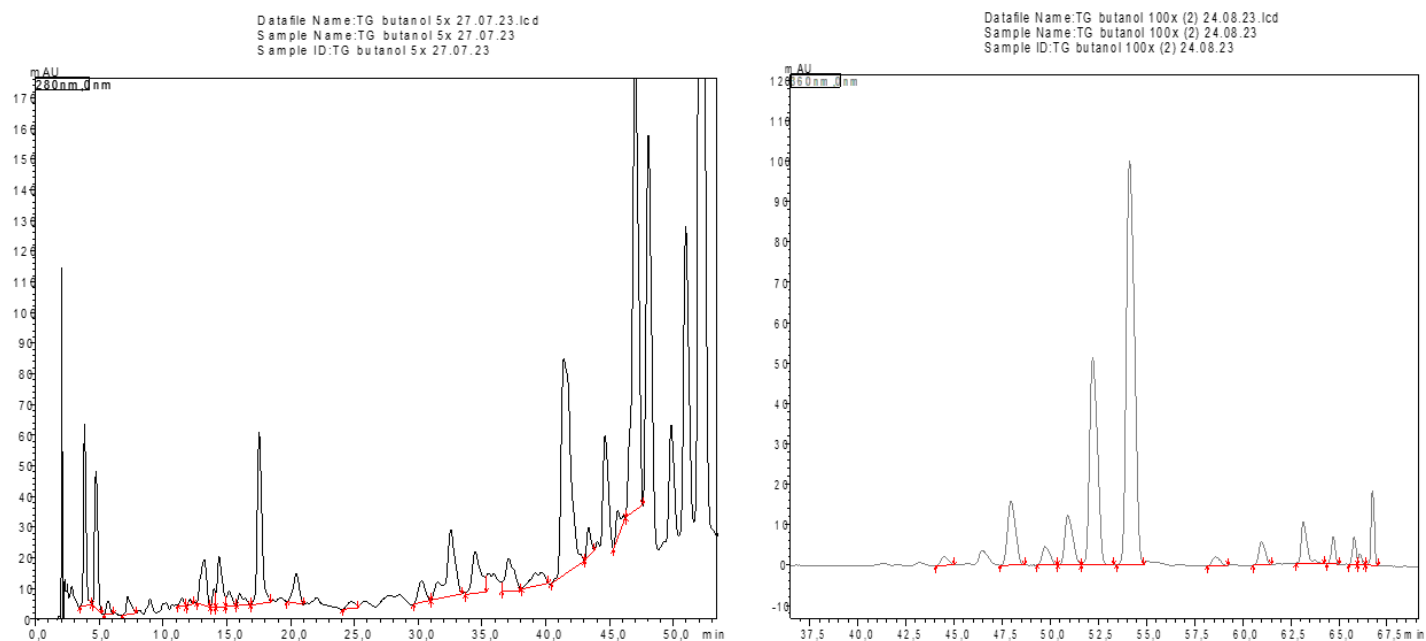
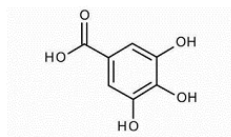


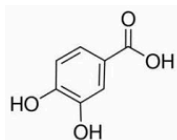
Figure 3

Representative HPLC–DAD chromatogram showing retention peaks of phenolic and flavonoid compounds. Chemical structures include rutin, isoquercetin, kaempferol derivatives, quercetin derivatives, catechin, epicatechin, and syringic acid.

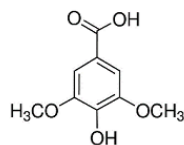
Hydroxybenzoate Derivatives



Gallic acid (CID: 370)

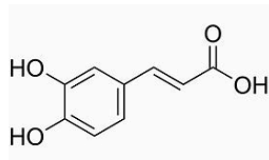


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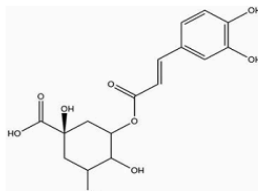


Syringic acid
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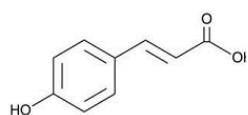
Hydroxycinnamate Derivatives



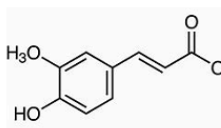
Caffeic acid (CID: 689043)



Chlorogenic acid (CID: 1794427)

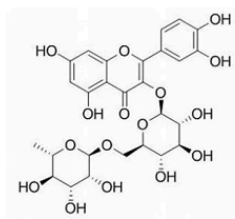


P-coumaric acid (CID: 637542)

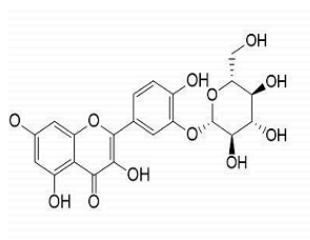


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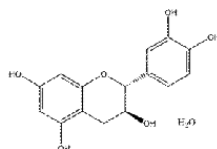
Flavonoids



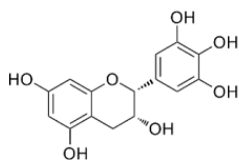
Rutin (CID: 5280805)



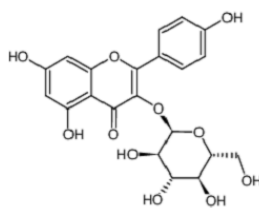
Quercetin-3- glycoside
(Isoquercetin)
(CID: 5280804)



Catechin (CID: 9064)



Epigallocatechin (CID: 72277)

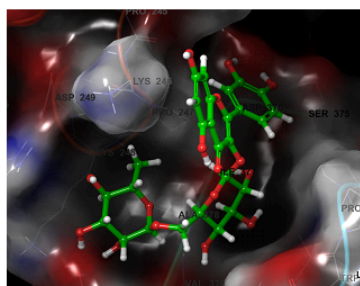


Kaempferol Derivative
kaempferol 3-o- β -D-glucopyranoside
(CID: 5282102)

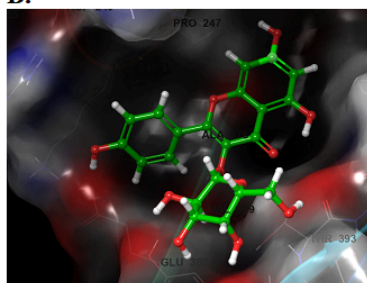
Figure 4

Bioactive compounds identified in *Tapinanthus globiferus* using HPLC-DAD

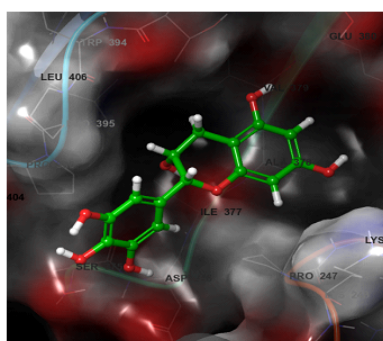
A.



B.



C.



D.

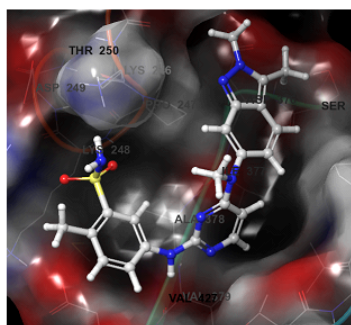
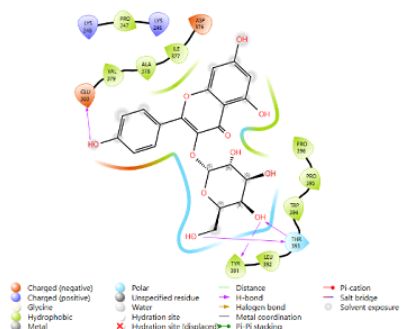
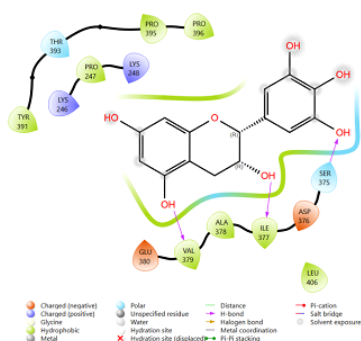
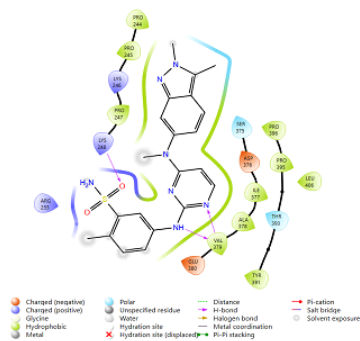


Figure 5

2-D and 3-D molecular interaction profiles of (a) rutin (b) trifolin (c) epigallocatechin, and (d) the reference VEGF inhibitor pazopanib with VEGF-A. Rutin demonstrated the highest number and strength of interactions within the binding site compared to other compounds.

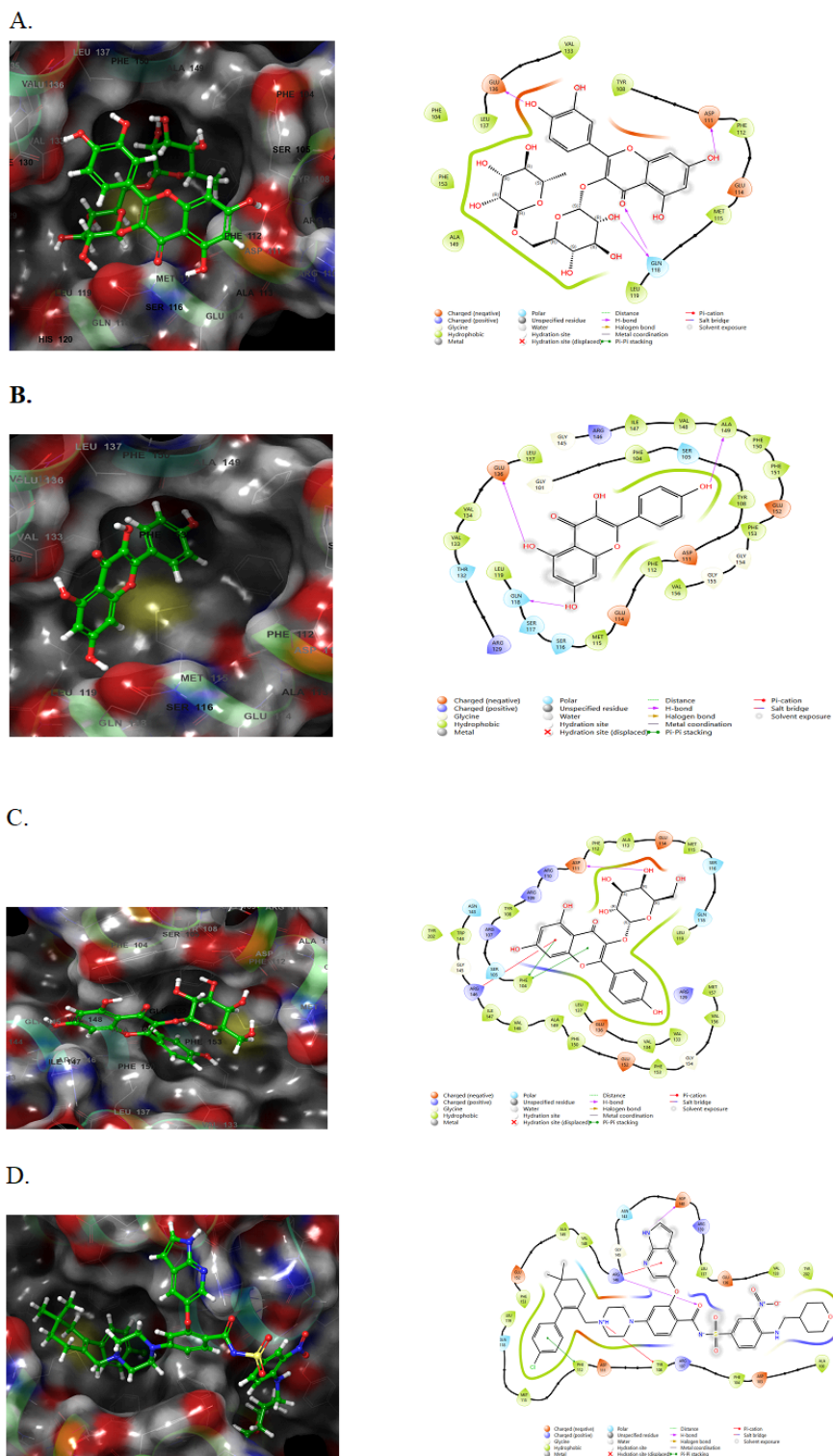


Figure 6

3D and 2D Molecular Interactions of the Amino Acids in BCL2 with (a) Rutin (b) Kaempferol (c) trifolin and (d) Venetoclax. Docking pose of the standard BCL-2 inhibitor, venetoclax, demonstrating stabilizing interactions compared with the top phytochemicals.