

ISOLATION, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF *KALANCHOE PINNATA* LEAF PHYTOCONSTITUENTS: ANTIOXIDANT, ANTIMICROBIAL, AND *IN-SILICO* STUDIES

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Abstract

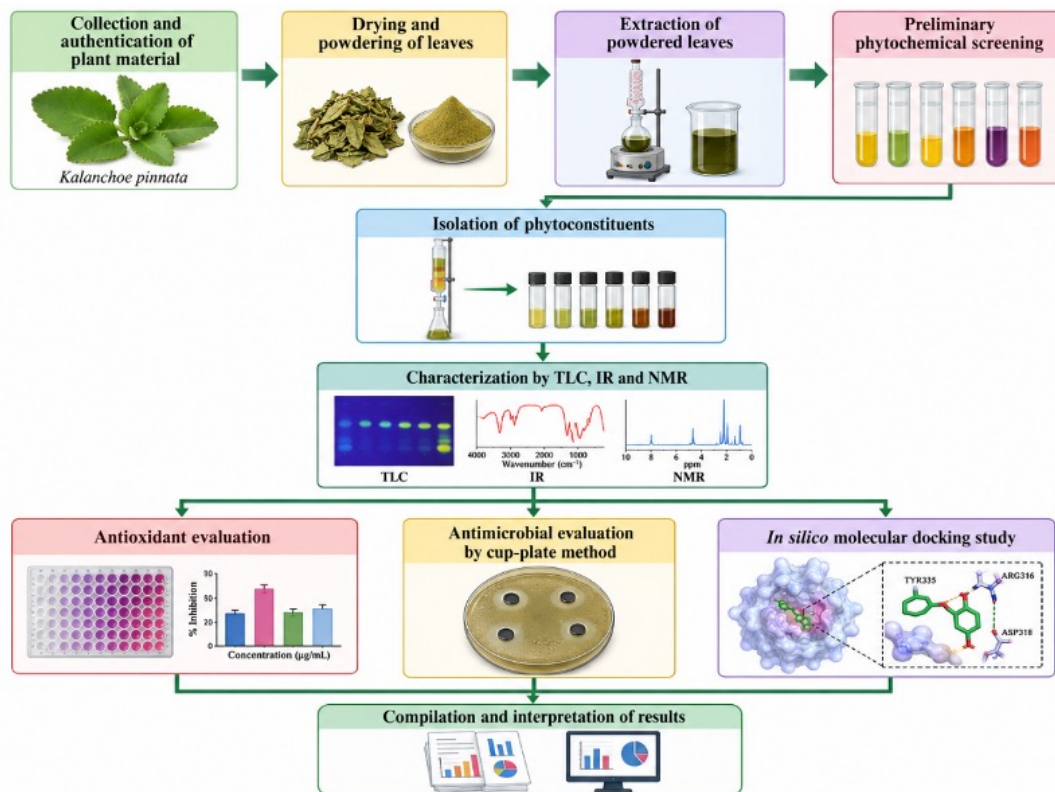
Kalanchoe pinnata (Lam.) Pers., commonly referred to as "Patharchatta" or "miracle leaf," has a variety of traditional medicinal applications. This study extracted and isolated quercetin from the leaves and characterized it, evaluated its in vitro antimicrobial and antioxidant activity and studied the molecular binding by docking. The leaf powder was standardized, shade dried and extracted with Methanol using Soxhlet apparatus. Cu-plate diffusion, DPPH scavenging and docking studies with bacterial DNA gyrase B (PDB ID: 6ZKK) were used to assess the antibacterial, antioxidant and target binding properties of quercetin, respectively, which was isolated by silica gel chromatography. The antibacterial, antioxidant and target binding properties of quercetin isolated by silica gel chromatography were assessed by cu-plate diffusion, DPPH scavenging and docking studies with bacterial DNA gyrase B (PDB ID: 6ZKK), respectively. ADMET/ProTox-3.0 predictions assessed safety. The leaves had 2.11 % loss on drying, 2.11 % total ash and 42.0 % water soluble extractives. Purified quercetin gave a single TLC band (R_f 0.54; toluene: ethyl acetate: formic acid, 5:4:0.5). Spectral data supported its flavonol structure: UV λ_{max} 370nm; FT-IR bands at 3431.36 cm⁻¹ (O–H) and 1672.28 cm⁻¹ (C=O); ¹H-NMR hydroxyl signals at δ 10.8–12.0 ppm and aromatic signals at δ 6.0–8.0 ppm; and an LC-MS/MS precursor ion at m/z 302.16 [M–H]. Quercetin bound to the DNA gyrase B with a binding affinity of -8.5 kcal/mol, and hydrogen bonds are formed with PRO79A, GLU50A and ASP73A while ciprofloxacin had an affinity of -6.9 kcal/mol. It yielded an inhibition zone of 16.0 mm at 10 µg/mL against *S. aureus* and 100.0% DPPH inhibition at 50 µg/mL which is in the same range as ascorbic acid (99.4%). The results obtained reveal that the antioxidant and antimicrobial properties of quercetin are derived from leaves, and this finding justifies further in-depth research on its possible application in drug development, but also provides scientific evidence for the traditional use of *K. pinnata*.

Keywords: *Kalanchoe pinnata*, Quercetin, Column Chromatography, LC-MS/MS, Molecular Docking, DNA Gyrase, Antimicrobial, DPPH Antioxidant.

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GRAPHICAL ABSTRACT

ISOLATION, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF KALANCHOE PINNATA LEAF PHYTOCONSTITUENTS: ANTIOXIDANT, ANTIMICROBIAL, AND IN-SILICO STUDIES



INTRODUCTION

Kalanchoe pinnata, also known as *Bryophyllum pinnata*, is a perennial succulent native to Madagascar and a member of the Crassulaceae family, which includes 25 genera and 450 species. Locally called Patharchatta and often described as the "miracle plant," it is an erect plant that can grow to about 1.1–1.5 meters tall.¹ The name "miracle plant" refers to its diverse therapeutic properties, which are associated with chemical constituents in its different parts.² Key constituents of *Kalanchoe pinnata* include bersaldegenin, bryophylline, caffeic acid, campesterol, gallic acid, isorhamnetin, kaempferol, p-coumaric acid, quercitrin, quercetin, and stigmasterol.³ These compounds are linked to the plant's wound-healing, antioxidant, antitumor, anti-inflammatory, antiviral, immunomodulatory, nephroprotective, antimicrobial, antileishmanial, antidiabetic, antifungal, anthelmintic, hepatoprotective, hemoprotective, and antihypertensive properties.⁴ One notable chemical compound in *Kalanchoe pinnata* is quercetin. Its biological activity largely arises from interactions with various protein targets, mediated by hydrogen bonds and hydrophobic interactions involving its multiple hydroxyl groups and aromatic rings.⁵ By affecting enzyme and receptor activity, quercetin modulates a range of cellular processes. For instance, it has been shown to inhibit enzymes such as tyrosine kinase and phosphodiesterase, which have important roles in cell-

signaling pathways.⁶ This inhibition affects processes, such as cell proliferation, apoptosis and inflammation. The structural studies, such as molecular docking simulations and Swiss ADME / ProTox software analyses, have provided insights on the binding of quercetin to protein targets, which can help determine the most important binding sites and interactions responsible for the activity of quercetin. The knowledge gained can be used to develop quercetin derivatives with better biological activity, as well as to develop therapeutic strategies based on the mode of action of quercetin. *Bryophyllum pinnatum*, a plant of the Crassulaceae family, is a medicinal plant which is commonly called as Patharchatta in Hindi and Pashanabheda in Sanskrit as shown in figure 1. *B. pinnatum* is also referred to by the synonym *Bryophyllum calycinum*. Taxonomically, it belongs to the Kingdom Plantae, Division Magnoliophyta, Class Magnoliopsida, Order Saxifragales, Genus Kalanchoe, Section Bryophyllum, and Species *Kalanchoe pinnata*. Traditionally recognised for its medicinal significance, the plant has been widely studied for its pharmacological properties.⁹ *Kalanchoe pinnata* is rich in various bioactive compounds, including alkaloids, triterpenes, glycosides, flavonoids, cardenolides, steroids, bufadienolides, and lipids.¹⁰ The leaves specifically contain several phytochemicals such as bersaldegenin, bryophylline, caffeic acid, campesterol, gallic acid, isorhamnetin, kaempferol, p-coumaric acid, quercitrin,

quercetin, and stigmasterol. 11 These constituents exhibit diverse yet overlapping structural properties and biological activities. They contribute to a wide range of pharmacological effects, including wound healing, antioxidant, antitumor, anti-inflammatory, antiviral, immunomodulatory, nephroprotective, antimicrobial, antileishmanial, antidiabetic, antifungal, anthelmintic, hepatoprotective, hemoprotective, and antihypertensive properties.¹²

2. MATERIALS AND METHODS

2.1 Plant Material Collection and Authentication

Fresh, healthy leaves of *Kalanchoe pinnata* (Lam.) Pers. (Family: Crassulaceae) were collected from Nagpur, Maharashtra, India, and selected for the present study. Botanical authentication of the plant was carried out at Rashtrasant Tukadoji Maharaj Nagpur University (RTMNU), Nagpur, Maharashtra, India. A representative voucher specimen was prepared and deposited in the herbarium for future reference under Herbarium Sheet No. 3068. The specimen was authenticated as *Kalanchoe pinnata* (Lam.) Pers., belonging to the family Crassulaceae. The authenticated leaves were collected, cleaned of extraneous materials, and processed as required for the subsequent experimental procedures.

2.3 Preparation of ethanol extract¹³

The leaves of *Kalanchoe pinnata* were air dried coarse leaf powder and extracted with water using standardized pharmacognostical and pharmacopoeial extraction procedure. In hot extraction 50.0 g of shade-dried leaf powder was decocted with 500 mL distilled water (1:10 w/v) in a continuous reflux condition at 100°C. For cold maceration, 5.0 g of powdered material of leaf was macerated with 100 mL of the chloroform water (or sterile distilled water) for 24 hours at room temperature and shaken periodically during the first 6 hours. Crude mixture was filtered through clean muslin cloth and then Whatman No. 1 filter paper to remove insoluble particles. Filtrate was concentrated under reduced pressure on a vacuum rotary evaporator and evaporated to dryness in water bath at 105°C for further phytochemical quantification and bioassays, to get dried crude aqueous residue.

2.3 Preparation of Crude Methanolic Extract

Leaves were thoroughly washed with running tap water followed by distilled water to get rid of the dust and organic debris on the leaves. The leaves were shade dried, ground to moderately coarse powder in an electric industrial grinding machine. The dried leaf powder was packed in a muslin cloth thimble and extracted with 350 mL of pure methanol using Soxhlet extraction. The extraction was performed at a set temperature of 50°C for 7 continuous extractions during 72 hours. The methanolic extract obtained was concentrated under reduced pressure at 40°C in

vacuum rotary evaporator to obtain a dark greenish-viscous mass¹⁴.

2.4 Preliminary Phytochemical Screening¹⁵

Preliminary qualitative phytochemical screening of *Kalanchoe pinnata* leaf extracts revealed a diverse range of bioactive secondary metabolites in both methanolic and aqueous fractions. The tests used were Shinoda, alkali, and lead acetate for flavonoids; Dragendorff, Wagner, Hager, and tannic acid for alkaloids; ferric chloride for phenolics; Salkowski and Liebermann-Burchard for steroids and triterpenoids; Keller-Kiliani and Legal's for cardiac glycosides; and ninhydrin and precipitation tests for proteins and amino acids.

2.5 Physicochemical Evaluation

The physicochemical properties of shade-dried *Kalanchoe pinnata* leaf powder were characterised according to standard WHO and pharmacopoeial guidelines to assess the raw material's purity, authenticity, and quality. 13 Moisture content (loss on drying) was determined by heating a known quantity of leaf powder in a hot air oven at 105°C until a constant mass was reached. 16 To determine total ash, powdered leaf material was incinerated in a tared silica crucible in a muffle furnace at 500–600°C until carbon-free ash remained. 17 Acid-insoluble and water-soluble ash fractions were assessed by boiling the total ash with dilute hydrochloric acid or distilled water, respectively, filtering through ashless filter paper, igniting the residue in a muffle furnace, and calculating the insoluble matter. 18 Water- and alcohol-soluble extractive values were determined by macerating the powdered material in chloroform, water and methanol, respectively. The mixtures were then filtered, and the filtrates were evaporated to dryness in a water bath at 105°C. Soluble extractive yield was calculated relative to the initial air-dried sample. 19 The swelling factor was also assessed by measuring the volumetric expansion of the powdered leaf sample in distilled water using a graduated cylinder.²⁰

2.6 Column Chromatography Isolation of Quercetin²¹

In column chromatography, the selection of the stationary and mobile phases depends on the polarity of the sample. Silica gel is commonly used as the stationary phase, while the mobile phase is applied in a sequence ranging from less polar to more polar solvents (such as. Initially, a weaker band forms in the column, followed by a stronger band. Compounds with lower affinity for the stationary phase elute first, while those with higher affinity elute later. Both the weaker and stronger bands are collected in volumetric flasks and covered with silver foil to protect them from light. The isolated compound is then concentrated, analysed by FT-IR and utilised for pharmacological

studies. The compound was isolated in a fraction of solvents. Initially, the fraction (1-3) was collected using the solvent system hexane. Further, the mixture of solvent system hexane: ethyl acetate (5:5), in which the 4-7 fraction was collected, similarly, the 7-10 fraction was collected by the solvent system hexane: ethyl acetate (7:3). All the fractions collected and analysed by further identification tests.

2.7 Analytical and Spectroscopic Structural Characterisation

Characterisation of the isolated Compound was achieved through multi-analytical techniques:

2.7.1 Thin Layer Chromatography (TLC) Analysis²²

Analytical thin-layer chromatography (TLC) was conducted on the isolated fraction and commercial quercetin standard to confirm their chromatographic identity and assess purity. Pre-coated silica gel 60 F254 aluminum plates (0.25 mm layer thickness) were used as the stationary phase and activated in a hot-air oven at 105°C for 15 minutes before sample application. The isolated compound and reference standard were each dissolved in analytical-grade methanol and spotted along the baseline, 1 cm from the bottom edge, using fine glass capillary tubes. The plates were developed by ascending solvent migration in a twin-trough glass chamber pre-saturated for 15–20 minutes with an optimized mobile phase of toluene: ethyl acetate: formic acid (5:4:0.5 v/v/v). After the solvent had travelled 80–90% of the plate height, the chromatograms were air-dried and visualized under short-wave (254 nm) and long-wave (365 nm) ultraviolet (UV) light, as well as in an iodine vapor chamber. The retention factor (R_f) was calculated as the ratio of the distance travelled by the solute spot to the distance traveled by the solvent front.

2.7.2 Fourier-Transform Infrared (FT-IR) Spectroscopy²³

Fourier-transform infrared (FT-IR) spectroscopy was used to characterize the functional groups in the isolated quercetin. Briefly, 1 to 2 mg of the isolated crystalline sample was thoroughly mixed and ground with spectroscopic-grade, dry potassium bromide (KBr) powder at a 1:100 (w/w) ratio using an agate mortar and pestle. The homogeneous mixture was compressed into a thin, transparent KBr disk with a hydraulic pellet press at a pressure of 8 to 10 tons for 2 minutes. The pellet was placed in the sample holder of an FT-IR spectrophotometer (e.g., Shimadzu/Bruker) and scanned over the mid-infrared wavenumber range of 4000 cm⁻¹ to 400 cm⁻¹ at a spectral resolution of 4 cm⁻¹. Atmospheric water vapor and carbon dioxide signals were automatically subtracted before spectrum acquisition, and characteristic transmission absorption bands were

recorded to identify the key functional groups in the isolated compound.

2.7.3 Tandem Mass Spectrometry (LC-MS/MS)²⁴

Isolated quercetin was characterised by tandem mass spectrometry (LC-MS/MS) using high-performance liquid chromatography coupled to a tandem mass spectrometer with an Electrospray Ionization (ESI) interface. Briefly, 1.0 mg of the isolated crystalline sample was dissolved in LC-MS-grade methanol and filtered through a 0.22 µm PTFE membrane before analysis. Mass spectra were acquired in negative ESI mode ([M-H]⁻). High-purity nitrogen was used for nebulization and desolvation, while argon served as the collision gas. Collision-induced dissociation (CID) was performed at collision energies of 20–30 eV to induce targeted fragmentation of the C-ring and A-ring regions. Full-scan mass spectra were recorded over an m/z range of 50–500, and characteristic precursor-to-product daughter-ion transitions were monitored to establish the molecular skeleton.

2.7.4 Proton Nuclear Magnetic Resonance (1H-NMR) Spectroscopy²⁵

The structure and proton environments of the isolated quercetin were characterised using proton nuclear magnetic resonance (1H-NMR) spectroscopy. Briefly, 5.0–10.0 mg of the purified crystalline isolate was fully dissolved in 0.6 mL of deuterated dimethyl sulfoxide (DMSO-d₆, 99.9 atom % D) and transferred to a standard 5 mm precision NMR tube. Spectra were recorded at ambient temperature (298 K) using a 400 MHz NMR spectrometer operating at 400.13 MHz for 1H nuclei¹². Chemical shifts (δ) were reported in parts per million (ppm) relative to tetramethyl silane (TMS, δ = 0.00 ppm) as an internal reference, or calibrated against the residual proton signal of DMSO-d₆ (δ = 2.50 ppm). Spin-spin coupling constants (J) were calculated in hertz (Hz), and proton signal multiplicities were classified as singlet (s), doublet (d), or doublet of doublets (dd) to assign the aromatic and phenolic hydroxyl proton environments.

2.7.5 UV-Vis Spectrophotometric Analysis²⁶

Spectral characterization and quantitative calibration of isolated quercetin were performed using a double-beam UV-Vis spectrophotometer equipped with 1.0 cm matched quartz cuvettes. A primary stock solution (100 µg/mL) was prepared by dissolving 10.0 mg of isolated quercetin in 100 mL of analytical-grade methanol, from which working calibration standards (10 to 50 µg/mL) were prepared via serial dilution. Baseline correction was conducted using pure methanol as a solvent blank. Absorption spectra were acquired across the 200 to 400 nm wavelength range to identify characteristic absorption maxima, recording Band at = 370nm. Absorbances of the serial dilutions were measured at 370 nm to construct a linear

calibration plot confirming Beer-Lambert law compliance.

2.8 *In Silico* Molecular Docking, ADMET, and Toxicity Studies

Molecular docking studies²⁷⁻²⁹ were performed to investigate the binding interactions of selected phytoconstituents of *Kalanchoe pinnata* against bacterial DNA gyrase B/topoisomerase IV subunit complexes (PDB ID: 6KKZ) Crystal structure of *E. coli* DNA gyrase B in complex with 2-oxo-1,2-dihydroquinoline derivative.³⁰ The three-dimensional structures of 11 major phytoconstituents, namely bersaldegenin, bryophyllin, caffeic acid, campesterol, gallic acid, isorhamnetin, kaempferol, p-coumaric acid, quercitrin, quercetin, and stigmasterol, along with ciprofloxacin as the reference drug, were retrieved from the PubChem database.³¹ The selected protein structures were prepared using Discovery Studio Visualizer (DSV) by removing unwanted water molecules and other non-essential components and preparing the protein structures for docking. Molecular docking was performed using PyRx, with the docking grid defined around the respective active binding sites. The resulting protein–ligand complexes were subsequently analyzed using Discovery Studio Visualizer to identify key interactions, including hydrogen bonding, hydrophobic interactions, π – π interactions, and other relevant non-covalent interactions. The docking scores and interaction profiles of the phytoconstituents were compared with those of ciprofloxacin as the reference ligand.³² Ramachandran plot analysis was used to assess the stereochemical quality of the selected protein structures.³³ Furthermore, drug-likeness and pharmacokinetic properties were predicted using Swiss ADME. Potential toxicity and LD₅₀ values were predicted using ProTox-3.0.^{34,35}

2.9 *In Vitro* Antimicrobial Cup Plate Assay³⁶

The *in vitro* antibacterial activity of Quercetin isolated from *Kalanchoe pinnata* was determined against the pathogenic strain of Gram-positive bacteria *Staphylococcus aureus* in nutrient agar by agar well diffusion method. The medium was made by dissolving the necessary quantity of agar powder in distilled water and autoclaving the medium at 121°C and 15 lbs pressure (103.4 kPa) for 15 minutes. The molten agar was cooled to 45-50°C and poured into sterile Petri dishes, under the aseptic conditions of a laminar-flow cabinet. A suspension of *Staphylococcus aureus* was prepared in sterile normal saline and adjusted to a turbidity standard of 0.5 McFarland (about 1.5×10⁸ CFU/mL). The standardized inoculum was evenly distributed on the surface of the agar by using a sterile cotton swab for the purpose of forming a confluent lawn. The wells, 6 mm in diameter, were punched into the agar with a sterile stainless-steel cork

borer, and the plugs were carefully removed. Purified quercetin solutions were prepared (10 µg/mL and 50 µg/mL) in dimethyl sulfoxide (DMSO) and 100 µL of these solutions were placed in the corresponding wells. The positive antibiotic control used was ciprofloxacin (30µg/mL) while the negative antibiotic control was DMSO. The bioactive components were allowed to pre-diffuse into the medium for 30-60 minutes at room temperature prior to bacterial growth. These plates were then turned over and incubated at 37°C for 24 hours. Bacterial inhibition against *Staphylococcus aureus* was determined by measuring the clear zones of inhibition (ZOI, mm) around each well in triplicate (n = 3), using the digital Vernier Callipers.

2.10 *In Vitro* DPPH Free Radical Scavenging Antioxidant Assay^{37, 38}

The isolated quercetin in *Kalanchoe pinnata* was quantitatively assessed for *in vitro* free radical scavenging activity with respect to ascorbic acid, through the spectrophotometric method using 2,2-diphenyl-1-picrylhydrazyl (DPPH). A new solution of DPPH radical was prepared in a new solution of 0.1 mM by dissolving DPPH powder in analytical grade methanol and kept in an amber volumetric flask away from light. Isolated quercetin and reference standard ascorbic acid were dissolved in methanol to get isolated concentration of 10, 20, 30, 40, and 50µg/mL and the reference standard ascorbic acid concentration of 10, 20, 30, 40, and 50µg/mL respectively. In each glass test tube, 1 mL of the 0.1 mM DPPH methanolic solution was thoroughly mixed with 3 mL of the various sample or standard solution. At the same time, a control reaction solution was prepared by adding 1.0 mL of the DPPH solution to 3.0 mL of pure methanol. The reaction mixtures were vortexed and placed in the dark at ambient room temperature for 30 minutes for the complete radical-antioxidant electron transfer. After dark incubation, the absorbance of each mixture was measured at 517 nm using a UV-Vis spectrophotometer and a blank of methanol. Each experimental measurement was done in triplets (n = 3) and the percentage inhibition in DPPH radical scavenging was calculated using the formula.

3. RESULTS AND DISCUSSION

3.1 Physicochemical Evaluation

Shade dried *Kalanchoe pinnata* leaf powder was evaluated for physicochemical parameters to establish quality standardization parameters. Crude material had low moisture content (loss on drying) of 2.11% w/w which means that it is very stable and will be very safe for storage from microbial contamination and enzymatic degradation. Inorganic mineral content analysis also showed the total ash value of 10.0% w/w, which indicated that there was little non-physiological silica or soil contamination. Extractive value determinations showed a predominant water-soluble

extractive value (42.0% w/w) over alcohol-soluble extractive value (18.0% w/w), which correlated directly with the final percentage yield of crude extract (25.0% w/w for aqueous extract versus 2.32% w/w for methanolic extract) and a swelling factor of 8%.

3.2 Analysis of phytochemical screening

The qualitative phytochemical screening of extracts of the *Kalanchoe pinnata* leaf indicated that there is a broad array of secondary metabolites with unique extraction patterns. From the methanolic extract, the positive results obtained included flavonoids, alkaloids, phenolics, steroids, triterpenoids, cardiac glycosides, and amino acids. In contrast, the aqueous extract of *Kalanchoe pinnata* had the saponins, tannins, anthraquinones, carbohydrates, flavonoids, and phenolics. The presence of flavonoids and phenolic substances, especially from the methanolic extract, was one reason why this extract was selected for further column chromatography and isolation of quercetin. Furthermore, the aqueous extract indicated positive results for saponins, tannins, anthraquinones, amino acid and Protein.

3.3 Chromatographic Purity and TLC Profile

Thin-Layer Chromatography (TLC) was carried out to assess the purity and retardation factor (R_f) of the flavonoid isolated via silica gel column chromatography. Using a solvent system of toluene: ethyl acetate: formic acid (5: 4: 0.5 v/v/v) on pre-coated silica gel 60F254 plates, the isolated compound resolved into a single, sharp yellow spot with a calculated R_f value of 0.54 as given in Figure 1 and 2 Visualization under short- and long-wavelength UV radiation (254 nm and 365 nm) as well as iodine vapor revealed no secondary spots or trailing, confirming high chemical purity. Co-chromatography alongside authentic standard quercetin demonstrated identical mobility ($R_f = 0.54$) and fluorescence properties, verifying the successful isolation of pure quercetin from *Kalanchoe pinnata* leaves.

3.4 FT-IR Spectral Analysis:

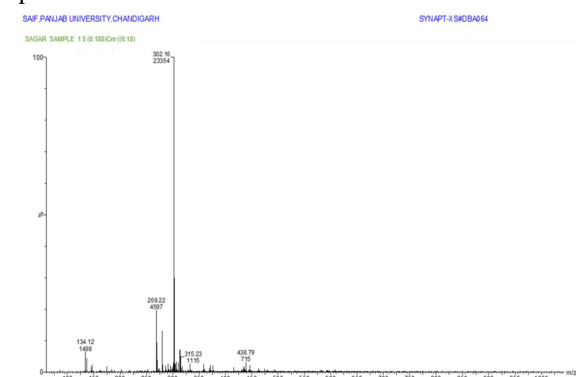
The IR spectrum of Quercetin exhibits distinct absorption peaks that correspond to its functional groups. These peaks confirm the molecular structure and key functional groups present in the compound. The significant peaks observed in the spectrum given in Figure 3.

FTIR spectrum of quercetin showed characteristic peaks at 3431.36 cm^{-1} (O–H stretching), 2968.45 and 2829.57 cm^{-1} (C–H stretching), 1672.28 cm^{-1} (conjugated C=O stretching), 1618.28 and 1517.98 cm^{-1} (aromatic C=C stretching), and 1317.38 and 1247.94 cm^{-1} (C–O stretching). The absorption bands in the fingerprint region below 1500 cm^{-1} were associated with various bending and skeletal

vibrations, consistent with the characteristic functional groups of quercetin.

3.5 Tandem mass spectroscopy (LC/MS/MS)

The isolated compound from the column chromatography was identified by spectroscopic methods and spectral analysis. From the fragmentation pattern observed in the spectrum, the mass/charge ratio (m/z) of 302.16 was detected, which indicates that the compound is quercetin as shown in figure 4. This identification was further verified by comparing the obtained data with a reference standard and a spectral library which identified the isolated compound as quercetin.



conjugation, structural integrity, and chemical purity of isolated quercetin. Table 1 presents the UV absorbance of quercetin at different concentrations. The calibration curve for quercetin is shown in Figure 6.

Table 1: Observation of UV absorbance of quercetin

Name of drug	Concentration (µg)	Absorbance		Absorptivity (Abs/Conc)	
		$\lambda_1=255$ Nm	$\lambda_2=320$ nm	$\lambda_1=255$ Nm	$\lambda_2=320$ nm
Quercetin	0.0005	0.498	0.269	996	538
	0.0010	0.973	0.489	973	489
	0.0015	1.557	0.73	1038	486.00
	0.0020	1.958	0.895	979	447.50
	0.0025	2.467	1.101	986.8	440.40
	Mean	$A_1=1.49$	$A_2=0.69$	$a_1=973$	$a_2=441$

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A1=absorbance of Quercetin at λ1
A2=absorbance of Quercetin at λ2

3.8 *In Silico* Molecular Docking and Target Binding Mechanics

Molecular docking simulations against bacterial DNA gyrase B/ topoisomerase IV (PDB ID: 6KKZ) demonstrated exceptional binding affinities for *K. pinnata* constituents. Isolated quercetin achieved the top binding score of -8.5 kcal/mol, significantly outperforming frontline antibiotic ciprofloxacin (-6.9 kcal/mol). Figures 8 to 18 show the docking complex with ligand and proteins.

Molecular docking analysis revealed that the investigated phytoconstituents exhibited docking scores ranging from -5.9 to -8.5 kcal/mol. Among the tested ligands, quercetin showed the highest binding affinity (-8.5 kcal/mol), forming hydrogen-bond

interactions with PRO79A, GLU50A, ASP73A, ARG76A, and GLY77A, along with hydrophobic interactions involving ILE78A, ILE94A, and ASN46A. Isorhamnetin (−8.4 kcal/mol) interacted through hydrogen bonds with ASP73A, GLY77A, and GLU50A, while stigmasterol (−8.1 kcal/mol), bryophylline (−8.0 kcal/mol), campesterol (−8.0 kcal/mol), and quercitrin (−8.0 kcal/mol) also demonstrated favorable binding interactions. Kaempferol exhibited a docking score of −7.9 kcal/mol, whereas bersaldegenin showed −7.5 kcal/mol. The reference drug ciprofloxacin displayed a docking score of −6.9 kcal/mol. Other compounds, including caffeic acid, *p*-coumaric acid, and gallic acid, showed docking scores of −6.6, −6.1, and −5.9 kcal/mol, respectively. Overall, the docking results indicated favorable interactions of several *Bryophyllum pinnatum* phytoconstituents with the target protein, with quercetin exhibiting the most favorable docking score among the investigated compounds as given in table 2.

Table 2: Docking score of phytoconstituents of kalanchoe pinnata leaves

S. No	ligands	Docking score	Hydrogen bond interaction	Hydrophobic bond interaction
1.	Ciprofloxacin	-6.9	ASN46A	GLY77A, ARG76A, ILE78A, GLU50A
2.	Bersaldegenin	-7.5	ASN46A, ASP73A	ALA47A, THR165A, ILE78A
3.	Bryophylline	-8	ASN46A, ASP73A	ALA47A, THR165A, ILE78A
4.	Caffeic Acid	-6.6	ASP73A, THR165A, ALA47A	ASN46A, ILE78A, GLU50A
5.	Campesterol	-8	THR165A	ILE78A, ILE94A

6.	Gallic Acid	-5.9	GLY77A, GLU50A	ILE78A
7.	Isorhamnetin	-8.4	ASP73A, GLY77A, GLU50A	VAL71A, ALA47A, ILE87A, PRO79A
8.	Kaempferol	-7.9	GLU50A, VAL43A, ASN46A	PRO79A, ILE78A, THR165A, ALA47A
9.	P-Coumaric Acid	-6.1	VAL167A	ILE78A
10.	Quercitrin	-8	ASN46A, GLY77A	ALA47A, ARG76A, ILE78A, PRO79A, GLU50A
11.	Quercetin	-8.5	PRO79A, GLU50A, ASP73A, ARG76A, GLY77A	ILE78A, ILE94A, ASN46A
12.	Stigmasterol	-8.1	-	GLY77A, ILE78A, ILE94A

3.9 Ramachandran Plot Analysis

Stereochemical backbone validation of the target protein (PDB ID: 6ZKK) was verified via Ramachandran plot as given in figure 22. Over 92% of non-glycine/non-proline residues resided in the most favored core regions, confirming high structural quality suitable for docking.

Observational analysis of the Ramachandran plot shown in Table 2. Most of the observed dihedral angles (ϕ and ψ) in the quercetin plot fell within allowed or favorable regions, indicating a predominantly stable backbone conformation. One data point fell in the disallowed region, suggesting a minor unfavorable torsional deviation. Overall, the plot showed a predominantly stable conformation with only one minor unfavorable torsional angle.

Table 3: observational analysis of Ramachandran plot

Parameter	Description
X-Axis (Φ , Phi)	Dihedral angle ranging from -180° to $+180^\circ$
Y-Axis (Ψ , Psi)	Dihedral angle ranging from -180° to $+180^\circ$
Contour Colors	- Blue: Allowed/Favorable regions - Magenta: Less favorable regions
Data Points	- Green circles: Observed dihedral angles - Red triangle: Outlier point
Observation	Most data points lie in allowed regions; one outlier exists in a disallowed region
Interpretation	Quercetin's backbone conformation is mostly stable, with a minor unfavorable torsion

3.10 Toxicity studies

ProTox-3 software is used to predict the various toxicity endpoint for chemical compound. To study the toxicity profile of quercetin the proTox-3 software was used. According to this study the quercetin poses risks for liver, nervous system, respiratory system and immune system toxicity. However, it does not show significant carcinogenic, mutagenic, nephrotoxic, cardiotoxic or cytotoxic potential as given in table 4. The high probability values for neurotoxicity, respiratory toxicity, and immunotoxicity suggest that caution should be exercised in its use, especially in biological systems or human exposure scenarios.

Table 4: ProTox-3.0- Prediction of toxicity of quercetin

Classification	Target	Short hand	Prediction	Probability
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Organ toxicity	Hepatotoxicity	dili	Active	0.69
Organ toxicity	Neurotoxicity	neuro	Active	0.87
Organ toxicity	Nephrotoxicity	nephro	Inactive	0.90
Organ toxicity	Respiratory toxicity	respi	Active	0.98
Organ toxicity	Cardiotoxicity	cardio	Inactive	0.77
Toxicity end points	Carcinogenicity	carcin	Inactive	0.62
Toxicity end points	Immunotoxicity	immun	Active	0.96
Toxicity end points	Mutagenicity	mutagen	Inactive	0.97
Toxicity end points	Cytotoxicity	cyto	Inactive	0.93

3.11 Antimicrobial activity

The study demonstrates that Quercetin exhibits antimicrobial properties, with its efficacy increasing at higher concentrations. Greater doses result in more pronounced inhibition of microbial growth. However, compared to the standard antibiotic ciprofloxacin, Quercetin is less effective as given in **figure 23**. These findings indicate its potential as an antimicrobial agent.

The antibacterial activity of quercetin was evaluated by measuring the zone of inhibition. Ciprofloxacin (30 $\mu\text{g/mL}$) produced a zone of inhibition of 19 mm, indicating strong antibacterial activity. Quercetin at 10 $\mu\text{g/mL}$ exhibited a zone of inhibition of 16 mm, indicating moderate inhibitory activity, whereas at 50 $\mu\text{g/mL}$, a zone of inhibition of 12 mm was observed. The sterile water control showed no zone of inhibition (0 mm), confirming the absence of antimicrobial activity from the solvent/control as given in table 8. Overall, quercetin demonstrated measurable antibacterial activity under the tested conditions.

Table 5: Zone of inhibition observation in antimicrobial assay of quercetin

Sr.no	Concentration of quercetin/standard	Zone of inhibition	observation
1	Ciprofloxacin (30µg/ml)	19mm	Strong inhibition
2	Quercetin (10µg/ml)	16mm	Moderately inhibition
3	Quercetin (50µg/ml)	12mm	Active inhibition
4	Sterile water	0mm	No inhibition

3.12 Antioxidant assay of quercetin

In vitro free radical scavenging activity of isolated quercetin was evaluated using the DPPH spectrophotometric assay across higher concentrations ranging from 50 to 250 µg/mL and compared with standard ascorbic acid. Across this concentration range, isolated quercetin displayed near-saturation radical inhibition, recording 100.0% at 50 µg/mL, 99.4% at 100 µg/mL, 99.2% at 150 µg/mL, 98.9% at 200 µg/mL, and 98.2% at 250 µg/mL. Standard ascorbic acid maintained steady, near-maximal radical scavenging across the same range (99.4% from 50 to 200 µg/mL and 99.1% at 250 µg/mL). These results confirm that isolated quercetin possesses outstanding antioxidant capacity comparable to ascorbic acid, achieving peak scavenging saturation at concentrations as low as 50 µg/mL as shown in **table 6** and graphically given in **figure 21**.

Table 6: % inhibition of quercetin and ascorbic acid in DPPH activity

Sr.no	Conc.(µg/ml)	Quercetin (%inhibition)	Ascorbic acid (% inhibition)
1.	50	100	99.4
2.	100	99.4	99.4
3.	150	99.2	99.4

4.	200	98.9	99.4
5.	250	98.2	99.1

DISCUSSION

The leaves of *Kalanchoe pinnata* (Lam.) were used for quercetin isolation, extraction and identification in this study. In addition to the evaluation of its antioxidant potential and in-vitro antibacterial activities, also in-silico pharmacology was conducted. Some physical properties such as low moisture percentage (2.11%), Total Ash and high percentage of Water soluble extractive (42.0%) were among the properties that showed good quality of the powdered plant material. The phytochemical screening revealed the presence of flavonoids, phenols, alkaloids, steroids, triterpenoids and cardiac glycosides, which is why this extract was subjected to further isolation of plant compounds. The individual quercetin exhibited a strong antibacterial activity against the *S aureus* with 16 mm inhibition at 10 µg/mL and 12 mm at 50 µg/mL, and the control group did not show any inhibition, confirming the effect of the tested solution. The DPPH assay yielded significant radical scavenging activity with almost 100% inhibition at all the concentrations of the test material. The molecular docking study revealed that the docking score of quercetin with the selected DNA gyrase protein was -8.5 kcal/mol, indicating the presence of favorable interaction between the two molecules. Hydrogen bonds with PRO79A, GLU50A, ASP73A, ARG76A, and GLY77A may play a role in the predicted interaction. Please note that the docking score does not ensure either the antibacterial mechanism or efficiency in practice of the product being tested. Furthermore, ProTox-3.0 predictions are recommended to be interpreted only as a computational warning of toxicity, and not as a statement of toxicity. In summary, the results presented indicate that the biological activity of quercetin in *K. pinnata* can be identified. A single compound was obtained by silica gel column chromatography and TLC with an R_f value of 0.54. The isolated compound was further authenticated by UV-Vis, FT-IR, ¹H-NMR and LC-MS/MS. The presence of flavonol was confirmed by the presence of UV maxima at 370 nm, FT-IR bands of hydroxyl group, carbonyl group, aromatic C=C group and C-O group. The isolated compound was identified as quercetin by LC-MS/MS at the m/z of 302.16 [M-H]⁻.

CONCLUSION

The isolation and the characterization of quercetin from *Kalanchoe pinnata* leaves were successfully carried out by chromatographic and spectroscopic methods. Quercetin showed strong antioxidant and significant antibacterial activity against

Staphylococcus aureus. Computational safety data was provided and molecular docking showed good results for binding with bacterial DNA gyrase B. The results presented herein pave the way for additional pharmacological and mechanistic studies of quercetin, a natural bioactive compound.

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CONFLICT OF INTEREST

Authors declare no conflict of interest associated with this research publication.

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