Journal of Applied Biology & Biotechnology 2025. Article in Press
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2025.253184

# Phytochemical profiling and *in silico* target exploration of hexadecanoic acid from *Bergenia ciliata*: An integrative approach combining docking, absorption, distribution, metabolism, and excretion, and biological activity studies

Sagar Vishwakarma[1], Vijeta Chaudhry[1], Sumit Chand[1], Soban Prakash[2], Kajal Singh[1], Ajay Singh[3], Rajendra Prasad[4], Harish Chandra[1]*

[1]Department of Botany and Microbiology, Gurukula Kangri (Deemed to be University), Haridwar, Uttarakhand, India.
[2]High Altitude Plant Physiology Research Centre, H.N.B. Garhwal University (A Central University), Srinagar, Uttarakhand, India.
[3]Department of Chemistry, School of Applied and Life Sciences, Uttaranchal University, Dehradun, Uttarakhand, India.
[4]School of Agriculture, Uttaranchal University, Dehradun, Uttarakhand, India.

**ARTICLE INFO**

*Article history*:
Received on: 17/04/2025
Accepted on: 22/07/2025
Available online: ***

***Key words***:
Medicinal plant,
*Bergenia ciliata*,
Gas chromatography-mass spectrometry analysis,
Antioxidant,
Cytotoxicity

**ABSTRACT**

Natural products are essential to the development and discovery of new medications because of their abundant supply of bioactive chemicals, which fuel important advancements in medicine. *Bergenia ciliata* is one of the many ethnomedicinal plants found in the Western Himalayas, which are renowned for its varied medicinal flora. In this investigation, *B. ciliata* was extracted using methanol after being gathered from the high-altitude areas of Srinagar and Khirsu (Garhwal), Uttarakhand. Numerous phytochemical components were identified in the extract when analyzed using gas chromatography-mass spectrometry. When the agar well diffusion method was used to evaluate antibacterial efficacy, the methanolic extract showed significant inhibitory action, especially against *Staphylococcus aureus* (20.65 ± 0.49 mm). Notable antibacterial qualities of the extract were demonstrated by all eight tested bacterial strains. In addition, neutral red uptake cytotoxicity assays and 2,2-diphenyl-1-picrylhydrazyl radical scavenging were used to assess the extract's biological potential. The extract's $IC_{50}$ value of 317.9 ± 0.12 µg/mL showed encouraging antioxidant activity. Molecular docking of hexadecanoic acid with six protein targets exhibited varying degrees of affinity and interaction potential across different bacterial proteins. The highest binding affinity observed for Hexadec+4KRA, i.e., −6.4 kcal/mol, suggests its potential as a target for further studies.

## 1. INTRODUCTION

Plants that are medicinal (MPs) have been employed in both mainstream and alternative medicine since ancient times. They are a substantial source of biologically active substances, including antimicrobial, antifungal, antivirus, and cancer-preventing compounds. Medicinal plants may either produce or stimulate secondary metabolites. Humans employ secondary metabolites as both food constituents and pharmaceuticals [1]. A significant proportion of natural products that are sold as agrochemicals, personal care products, and pharmaceuticals are derived from higher medicinal plant sources [2]. The higher plants produce these kinds of secondary metabolites through a series of physical and chemical processes to promote therapeutic plant growth, enhance quality and production, and also contribute to biologically active efficacy, as well as interacting with outside microbial life and a variety of stresses, both abiotic and biotic, as part of the defense system. Typically, at least 25% of the active compounds in synthetic drugs that are routinely prescribed were initially discovered in natural sources. Approximately 4000 of the 20,000 plants used for medicinal purposes are used frequently, and 10% are commercially available [3].

*Bergenia* is a genus of 10 flowering plants in the *Saxifragaceae* family, native to Central Asia, spanning from Afghanistan to China and the Himalayas. They have been employed in traditional medicine for an extended period. The plant is known as Pashanbheda, which is derived from the Sanskrit words "pashan," meaning granite stone, and "bheda," meaning piercing. The extensive range of biological activity of *Bergenia ciliata* has been the subject of numerous traditional applications. An herb that has been utilized for centuries to alleviate stones in the kidneys, fever, vomiting, healing of wounds, antihelminthic, and tonic effects [4].

*Corresponding Author:
*Harish Chandra,*
*Department of Botany and Microbiology,*
*Gurukula Kangri (Deemed to be University), Haridwar,*
*Uttarakhand, India. E-mail: hreesh5@gmail.com*

The therapeutic value of bioactive substances is derived from their pharmacological actions, which include antibacterial, antioxidant, anti-inflammatory, analgesic, and anticancer properties. Quercetin, tocopherol, carotenoid, flavonoid, terpenes, and alkaloids are among the compounds found in medicinal plants such as caper (*Capparis* spp.) that have been demonstrated to possess antibacterial, anti-oxidative, anti-inflammatory, and antiviral properties through phytochemical analysis [5]. Phytochemicals present in medicinal plants are employed as food additives, as well as in pharmaceuticals and cosmetics. It is capable of inhibiting a diverse array of hazardous microorganisms, such as *Listeria monocytogenes* ATCC 19115 [6], *Escherichia coli* NCTC 12923, *Pseudomonas aeruginosa* NCTC 12924, *Pseudomonas fluorescens* ATCC13525, and *Staphylococcus aureus* NCTC 10788 [7]. Its broad-spectrum antibacterial properties make it a powerful antimicrobial agent with biological activity, including antibacterial activity. Its wide range of bioactive compounds is thought to be responsible for its efficacy, as they selectively block bacterial DNA gyrase, an enzyme necessary for bacterial reproduction. Flavonoids, which are strong antioxidants, are among the bioactive compounds found in the medicinal plant *Gymnema sylvestre*. Flavonoids mitigate oxidative stress and protect cells from damage by scavenging free radicals. This antioxidant activity is one of the plant's medicinal effects in traditional treatments, as it supports metabolic processes and general health [8]. To identify putative bioactive substances that are necessary for the antioxidant activity, the raw extract of *Costus spicatus* was profiled using high-resolution gas chromatography-mass spectrometry (GC-MS) and LC-MS/MS techniques. The aqueous extract exhibited the greatest scavenging activity for 2,2-diphenyl-1-picrylhydrazyl (DPPH) (2.24 ± 0.02 mm). Plants also possess antitumor properties that are beneficial. *Solanum* is the *Solanaceae* family that has garnered the most attention for its anticancer properties. Indigenous cultures in numerous regions of the world utilize all portions of the plants in this genus as remedies for cancer and comparable conditions. *Capsicum annuum*, *Camellia sinensis*, *Zingiber officinale*, *Punica granatum,* and *Nerium oleander* were identified as the most promising plants in 2024 [9], as they demonstrated anticancer efficacy in numerous clinical studies that focused on a variety of organs.

Medicinal plants have long been integral to traditional and modern healthcare systems due to their ability to produce diverse secondary metabolites with therapeutic potential, including antimicrobial, antioxidant, and anticancer effects. Among these, *B. ciliata* (family: *Saxifragaceae*), commonly known as "Pashanbheda," is a well-known ethnomedicinal herb native to the Himalayan region. The name "Pashanbheda," derived from Sanskrit, refers to its traditional use in dissolving kidney stones ("pashan" = stone, "bheda" = to break).

This plant has been extensively used in indigenous medicine for treating ailments such as urinary calculi, fever, respiratory infections, and inflammation. Recent pharmacological studies have confirmed several of its traditional claims, including antibacterial, antiurolithiatic, antioxidant, and wound-healing properties. These findings highlight the plant's bioactive potential and justify its selection for further phytochemical and molecular investigations.

In this study, we focused on characterizing the phytochemicals present in *B. ciliata* using GC-MS, assessing its biological activities (antioxidant, cytotoxic, and antibacterial), and exploring the molecular interactions of its major compound, hexadecanoic acid, with bacterial target proteins through docking and absorption, distribution, metabolism, and excretion (ADME) analyses. This integrative approach aims to validate the traditional uses of *B. ciliata* and uncover its potential in modern drug discovery. This study represents the first integrative report on the biological validation and molecular interaction profiling of hexadecanoic acid derived from *B. ciliata*, combining GC-MS, bioassays, and *in silico* approaches to highlight its therapeutic

## 2. MATERIALS AND METHODS

### 2.1. Collection of plant materials and Preparation of Methanolic Extract

During the 2021 monsoon season, plants of the species *B. ciliata* were gathered from the high-altitude areas of Srinagar, Khirsu (Garhwal), Uttarakhand, India. The medicinal plants that were collected were cleansed and allowed to dry in the shade. Discover powder was produced from the desiccated plant material. The Soxhlet apparatus was loaded with approximately 30 g of each plant powder and 100 mL of 96% methanol solvent. The extract was filtered through a Whatman Filter paper No. 42 filter, concentrated using a rotary vacuum distillation apparatus, and desiccated in an oven set at 40°C to remove the solvent after 8 h. Finally, the purified extract that was obtained was stored in the refrigerator to facilitate additional microbiological testing, ensuring that it was not exposed to direct sunlight [10].

### 2.2. GC-MS Analysis of Methanolic extract of *B. ciliata* leaves

GC-MS was employed to identify the components of the extract in this investigation [11]. The methanolic extract of the plant was injected into the GC-MS using a micro syringe, with an injection volume of approximately 1 µL. Each peak in the chromatogram represented the signal generated by a compound that eluted from the gas chromatography analysis using the AOC-20i Auto injector (Model: GCMS-TQ8040) in the SH-Rxi-5Sil MS (5% biphenyl 95% dimethyl polysiloxane) (30 M, 0.25 mmID, 0.25 µm df) column during a temperature range of 320–350°C. The carrier gas is helium, and its minimum rate is 1.0 mL/min. The injector was operated at 250°C, and the furnace temperature was initially set at 60°C for 15 min. Subsequently, the temperature gradually increased to 280°C over a period of 3 min. The component identification was analyzed using the National Institute of Standards and Technology library.

### 2.3. Molecular Docking Analysis of Hexadecanoic Acid with Selected Proteins

Hexadecanoic acid, identified as the major bioactive component of the methanolic extract of *B. ciliata* through GC-MS analysis, was selected for molecular docking studies to evaluate its interaction with key pathogenic targets from *S. aureus* and *Salmonella typhi* – two clinically relevant human pathogens. The 3D structure of hexadecanoic acid was retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/), whereas the crystal structures of bacterial target proteins were downloaded from the Protein Data Bank (PDB) (https://www.rcsb.org/). The selected protein targets included 5TW8: Crystal structure of wild-type *S. aureus* penicillin binding protein 4 (PBP4) in complex with ceftaroline, 7AHL: Alpha-hemolysin from *S. aureus*, 4KRA: *Salmonella typhi* OmpF complex with Ciprofloxacin, 5ZTJ: Crystal Structure of GyraseA C-Terminal Domain from *S. typhi* at 2.4A Resolution, 4OZ5: *Bacillus subtilis* HmoB, 1EX2: Crystal structure of *B. subtilis* Maf protein. These proteins are crucial for bacterial cell wall synthesis, DNA replication, and membrane permeability, making them attractive targets for antibacterial drug development. Protein-ligand binding was studied using the CB-dock-2 online tool, and the docking was performed within a grid box large enough to

encompass the entire active site: The grid box size was set to 23 × 23 × 23 Å (unless otherwise noted), centered at the binding pocket coordinates [Table 1]. Default docking parameters were used, and the exhaustiveness was set to 8 to ensure sufficient sampling. The top-ranked conformations based on binding affinity (kcal/mol) were selected for interaction analysis. To validate the docking protocol, redocking of the native ligand into its co-crystallized binding site was performed where available, and root mean square deviation (RMSD) values <2.0 Å were considered acceptable for protocol accuracy. Visualization and interaction analysis were conducted using PyMOL and Discovery Studio Visualizer.

**Table 1:** Phytocomponents identified in the methanolic extract of *Bergenia ciliata* by GC-MS.

| Rt | Metabolites | Formula | Relative concentration (%) |
|---|---|---|---|
| 16.85 | 1-Azabicyclo[2.2.2] octane-3-carboxylic acid | $C_8H_{13}NO_2$ | 0.49 |
| 17.12 | 3,7-Diazabicyclo[3.3.1] nonane, 9,9-dimethyl- | $C_9H_{18}N_2$ | 0.24 |
| 19.73 | 1,2,3-Benzenetriol | $C_6H_6O_3$ | 4.34 |
| 25.75 | n-Butyric acid 2-ethylhexyl ester | $C_{12}H_{24}O_2$ | 1.09 |
| 26.2 | Lauryl acrylate | $C_{15}H_{28}O_2$ | 1.34 |
| 27.89 | Tetradecanoic acid | $C_{14}H_{28}O_2$ | 0.31 |
| 28.27 | 8,11,14-Eicosatrienoic acid | $C_{20}H_{34}O_2$ | 0.03 |
| 29.19 | Phytol acetate | $C_{22}H_{42}O_2$ | 4.16 |
| 29.7 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | $C_{20}H_{40}O$ | 1.12 |
| 30.06 | Phytol | $C_{20}H_{40}O$ | 1.69 |
| 30.15 | Phthalic acid, isobutyl octadecyl ester | $C_{30}H_{50}O_4$ | 0.24 |
| 31.02 | Palmitic acid, methyl ester | $C_{17}H_{34}O_2$ | 1.58 |
| 32.05 | Palmitic acid | $C_{16}H_{32}O_2$ | 12.65 |
| 34.28 | Linoleic acid, methyl ester | $C_{19}H_{34}O_2$ | 0.4 |
| 34.37 | 9-Octadecenoic acid (Z)-, methyl ester | $C_{19}H_{36}O_2$ | 0.15 |
| 34.43 | Linolenic acid | $C_{18}H_{30}O_2$ | 0.6 |
| 34.6 | Ethanol, 2-(9-octadecenyloxy)-, (Z)- | $C_{20}H_{40}O_2$ | 1.39 |
| 34.81 | Methyl isostearate | $C_{19}H_{38}O_2$ | 0.43 |
| 35.33 | Trans-13-Octadecenoic acid | $C_{18}H_{34}O_2$ | 7.28 |
| 35.48 | 6-Octadecenoic acid | $C_{18}H_{34}O_2$ | 5.14 |
| 35.75 | Stearic acid | $C_{18}H_{36}O_2$ | 6 |
| 38.87 | Z-(13,14-Epoxy) tetradec-11-en-1-ol acetate | $C_{16}H_{28}O_3$ | 0.04 |
| 49.45 | Stigmasta-5,22-dien-3-ol, acetate, (3β)- | $C_{31}H_{50}O_2$ | 1.13 |
| 49.73 | Cholesta-8,24-dien-3-ol, 4-methyl-, (3β,4α)- | $C_{28}H_{46}O$ | 1.07 |
| 50.18 | Vitamin E | $C_{29}H_{50}O_2$ | 0.76 |
| 51.96 | Stigmasterol | $C_{29}H_{48}O$ | 0.86 |

GC-MS: Gas chromatography-mass spectrometry.

### 2.4. Redocking-Based Docking Validation

AutoDock Vina was used to redock native ligands to validate the molecular docking procedure. Protein-ligand complex crystal structures were obtained from the PDB. After being removed, the native ligand was redocked into the active site of the relevant protein. PyMOL was used to compute the RMSD between the native ligand pose and the redocked pose. The docking approach is deemed capable of accurately reproducing the experimental binding configuration if the RMSD value is <2.0 Å.

### 2.5. ADME and Drug-likeness Prediction of Active Molecule, i.e., Hexadecanoic Acid

Swiss ADME, a free online tool for early-phase pharmacokinetic screening, was used to assess the drug-likeness and ADME properties of hexadecanoic acid. Lipinski's Rule of Five (Ro5), which forecasts a compound's oral bioavailability, served as the foundation for the evaluation. The rule states that a molecule is likely to show good oral absorption if it satisfies the following requirements: a calculated LogP (lipophilicity) of no more than 5, a molecular weight (MW) of <500 Da, and no more than 5 hydrogen bond donors (HBD) and 10 hydrogen bond acceptors (HBA) [12]. Furthermore, to provide sufficient permeability, the topological polar surface area (TPSA) should ideally be ≤140 $Å^2$.

### 2.6. Antibacterial Activity Assay

Antimicrobial susceptibility testing was performed on Mueller–Hinton agar using the agar well diffusion technique, as per Ikeagwuonu and Adjeroh [13]. The antimicrobial capacity of *B.ciliata* methanolic extract (100 mg/mL) was evaluated against a diverse array of Gram-positive (*B. cereus*, *B. subtilis*, *S. aureus*, and *S. pneumoniae*) and Gram-negative (*E. coli*, *S. typhi*, *P. aeruginosa*, and *K. pneumoniae*) microorganisms. The bacterial culture was uniformly disseminated across the agar surface to ensure consistent growth. After that, the Petri dishes were incubated at 37°C for 24 h. Utilize a Vernier Caliper to determine the diameter of the clear inhibition zones surrounding the wells following incubation. Furthermore, the zone of inhibition (in mm) was compared to the standard antibiotics gentamicin (30 µg/disc) and chloramphenicol (25 µg/disc), with DMSO (dimethylsulphoxide) serving as the negative control.

### 2.7. Antioxidant Activity by DPPH Assay

With minor adjustments, the DPPH radical scavenging test was carried out in compliance with the guidelines provided by Virginia *et al.* [14]. A 0.002% DPPH solution was combined with a variety of concentrations of extracts (10, 50, 100, 150, 200, 250, 500, and 1000 µg/mL). The absorbance at 517 nm was measured following a 30-min process. Using an ultraviolet-visible dual-beam spectrophotometer. The color transition from pink to yellow was associated with the scavenging of the DPPH radical. The DPPH radical scavenging was ascertained using this equation:

$$RSC\ (\%) = ([A_C - A_S]/A_C) \times 100$$

$A_c$ represents the absorbance of the DPPH methanolic solution, while $A_S$ denotes the absorbance of the sample. The samples' mean RSC (in percentage) was determined for each extract concentration. The concentration of extract that provided 50% inhibition $EC_{50}$ – that is, the concentration at which 50% of DPPH was efficiently scavenged – was determined by interpolating from linear regression analysis. The standard antioxidant was ascorbic acid.

### 2.8. Neutral Red Uptake (NRU) Assay

The cytotoxic potential of the methanolic extract was evaluated using the NRU assay on the human breast cancer cell line MCF-7. Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin under standard conditions (37°C, 5% $CO_2$). MCF-7 cells (RRID: CVCL_0031) were obtained from the National Centre for Cell Science (NCCS), Pune, India. Cells were authenticated by the supplier and used within six passages of revival.

The cells were cultivated in 96-well plates for 24 h in DMEM media (Dulbecco's Modified Eagle media-AT149-1L) supplemented with 1% antibiotic solution and 10% FBS (fetal bovine serum – HiMedia-RM 10432) at 37°C with 5% $CO_2$. The cells were cultivated at a density of 5–8000 cells per well. 0.1% DMSO (final concentration), used to assess solvent-related cytotoxicity. Doxorubicin (10 µM) was used as a positive control, a well-established anticancer drug, used to validate assay sensitivity. Cells receiving only culture medium serve as a baseline for comparison.

The medium was removed the following day, and new culture media were introduced into each plate as well. To acquire varying concentrations of the samples in inadequate cell culture medium (without FBS), a stock solution of the samples was prepared in DMSO and subsequently diluted. The treated plates were incubated for 24 h after 5 µL of treatment dilutions (of variable concentrations) were added to the designated wells. The Heal Force-Smart cell $CO_2$ incubator-Hf-90 was used to incubate 100 µL of NRU (SRL Chem-36248) (40 µg/mL in PBS-phosphate buffered saline) in the designated wells for 1 h. After the medium was removed, cells that were devoid of NRU dye were referred to as blank, while cells that were not treated were referred to as control. NRU was dissolved using 100 µL of NRU destain solution. Finally, the plates were read at 550 nm using the ELISA Plate Reader (iMarkBioRad-USA). The $IC_{50}$ was determined using GraphPad Prism-6 software. Images were captured under an inverted microscope (Olympus ek2) using an AmScope digital camera (10 MP Aptima CMOS). The 50% inhibitory concentration ($IC_{50}$) was represented as the mean ± standard error of mean (SEM) [15].

## 3. RESULTS AND DISCUSSION

### 3.1. GC-MS Analysis

The components in the methanolic extract of the plant *B. ciliata* were analyzed using gas chromatography and mass spectroscopy. Figure 1 displays the 26 peaks of the chemicals found in the plant extract. In the methanolic extract of plant *B. ciliata*, the compounds are palmitic acid (12.65%), trans-13-octadecenoic acid (7.28%), Stearic acid (6%), 6-octadecenoic acid (5.14%), 1,2,3-benzenetriol (4.34%), and phytol acetate (4.16%). Palmitic acid shows antimicrobial and antioxidant activity [16], trans-13-octadecenoic acid also shows antimicrobial activity [17], stearic acid shows anticancer activity [18], 6-octadecenoic acid shows antiviral, antibacterial, and antitumor activity [19] [Table 1].

### 3.2. Molecular Docking Analysis of Hexadecanoic Acid with Selected Proteins

Molecular docking was performed to evaluate the binding affinity and pocket characteristics of hexadecanoic acid with six different target proteins. The docking results, summarized in Table 2, provide insight into the potential interactions between the ligand and the target proteins based on binding affinity, pocket volume, and spatial coordinates [20,21].

Binding affinity values (in kcal/mol) indicate the strength of interaction between hexadecanoic acid and each protein. The highest binding affinity was observed for the Hexadec-4KRA complex (−6.4 kcal/mol), suggesting a relatively strong interaction. This was followed by Hexadec-7AHL (−6.0 kcal/mol), Hexadec-4OZ5 (−5.7 kcal/mol), Hexadec-5TW8 (−5.5 kcal/mol), Hexadec-1EX2 (−5.3 kcal/mol), and Hexadec-5ZTJ (−4.5 kcal/mol). The lower binding affinity observed for Hexadec-5ZTJ suggests weaker interactions, making it less favorable compared to other complexes [22].

Cavity volume plays a crucial role in determining ligand accommodation and interaction. The Hexadec-5ZTJ complex exhibited the largest cavity volume (4754 Å$^3$), followed by Hexadec-7AHL (2638 Å$^3$), Hexadec-4KRA (1494 Å$^3$), and Hexadec-1EX2 (1015 Å$^3$). The Hexadec-4OZ5 and Hexadec-5TW8 complexes displayed relatively smaller cavity volumes of 882 Å$^3$ and 594 Å$^3$, respectively. Notably, despite its high binding affinity, Hexadec-4KRA did not have the largest cavity, suggesting efficient ligand accommodation within a compact binding site. Significant binding energy and interaction of hexadecanoic acid were reported against *P. aeruginosa* [23]. The spatial coordinates of the docking centers, defined by x, y, and z values, reflect the positioning of the ligand within each protein. The docking box sizes varied slightly, with Hexadec-5ZTJ having the largest dimensions (35 × 33 × 23), whereas

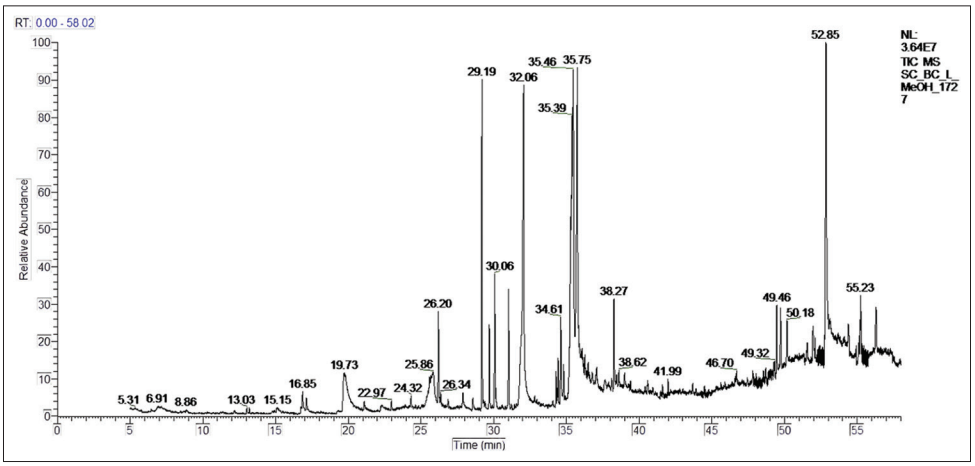


**Figure 1:** Gas chromatography-mass spectrometry chromatogram of the plant *Bergenia ciliate.*

the others were mostly confined to a uniform size (23 × 23 × 23), indicating structural constraints within the binding pockets.

Molecular docking analysis revealed that hexadecanoic acid exhibited binding affinities ranging from −4.5 to −6.4 kcal/mol with selected bacterial protein targets. The highest binding affinity was observed with the *Salmonella typhi* OmpF protein (4KRA) at −6.4 kcal/mol, followed by interactions with *S. aureus* α-hemolysin (7AHL, −6.0 kcal/mol) and others. While these values suggest favorable interactions, it is important to note that binding affinities in this range are considered moderate and not indicative of strong ligand-receptor binding on their own (Fig. 2).

However, it is also recognized that hexadecanoic acid is a long-chain saturated fatty acid whose bioactivity may not solely depend on high binding affinity to specific protein pockets. Instead, its antibacterial effects may result from membrane perturbation, disruption of lipid bilayers, or modulation of membrane-associated proteins – mechanisms that are not fully captured by docking scores alone [19]. Therefore, the docking data are used here as complementary mechanistic support rather than a standalone prediction of bioactivity.

Notably, the docking findings are corroborated by *in vitro* biological assays, where the methanolic extract of *B. ciliata*, rich in hexadecanoic acid, demonstrated significant antibacterial and cytotoxic activities. This integrative approach strengthens the rationale for further investigation into hexadecanoic acid's potential as a lead molecule, especially when supported by experimental validation.

### 3.3. Validation of Molecular Docking via Redocking

The results of the redocking validation are summarized in Table 3. For three target proteins with known co-crystallized ligands – penicillin-binding protein 4 (PDB ID: 5TW8), alpha-hemolysin (PDB ID: 7AHL), and OmpF porin (PDB ID: 4KRA) – the RMSD values were 1.42 Å, 1.65 Å, and 1.38 Å, respectively. These values are all within the acceptable range, confirming the reliability of the docking protocol. For targets lacking co-crystallized ligands (PDB IDs: 5ZTJ, 4OZ5, and 1EX2), redocking was not applicable. Overall, the validation confirms that the docking settings used are robust and suitable for further analysis of ligand interactions with the selected protein targets.

In conclusion, hexadecanoic acid exhibited varying degrees of affinity and interaction potential across different bacterial proteins. The highest binding affinity observed for Hexadec-4KRA suggests its potential as a target for further studies. At the same time, pocket volume and spatial properties provide additional insights into ligand accommodation within the protein structures.

### 3.4. ADME and Drug-likeness Prediction of Active Molecule, i.e., Hexadecanoic Acid

The molecule has the molecular formula $C_{16}H_{32}O_2$ with a MW of 256.42 g/mol. It consists of 18 heavy atoms with no aromatic heavy atoms and exhibits a high degree of aliphatic nature (Fraction $Csp^3$ = 0.94). The compound has 14 rotatable bonds, indicating high flexibility, and possesses two HBAs and one HBD. The TPSA is 37.30 Å², suggesting moderate permeability [24]. The lipophilicity profile presents various log $P_o/w$ values across different models, with a consensus log $P_o/w$ of 5.20, indicating high hydrophobicity. Individual models show a range from 3.85 (iLOGP) to 7.17 (XLOGP3), highlighting varying predictions of partitioning behaviour between octanol and water. The molecule is categorized as moderately soluble based on ESOL (log S = −5.02, solubility = 2.43e-03 mg/mL). High logP values are typically associated with good membrane permeability but poor aqueous solubility [12]. However, Ali's model predicts it to be poorly soluble (log S = −7.77), indicating variability in solubility predictions depending on the applied model. The gastrointestinal (GI) absorption is high, and the molecule is capable of blood–brain barrier (BBB) penetration. It is not a substrate for P-glycoprotein (P-gp), which suggests a lower likelihood of being actively pumped out of cells [24]. Among cytochrome P450 enzyme interactions, the molecule inhibits CYP2C9, whereas it does not inhibit CYP1A2, CYP2C19, CYP2D6, or CYP3A4. The skin permeability (log Kp) is -2.77 cm/s, suggesting moderate dermal absorption. The molecule adheres to Lipinski's rule

**Table 2:** Molecular docking interactions between the ligand and the target proteins based on binding affinity.

| Ligand+Protein | Cur Pocket Id | Binding affinity | Cavity Vol (A°) | Center (x, y, z) | Docking size (x, y, z) |
|---|---|---|---|---|---|
| Hexadec+5TW8 | C2 | −5.5 | 594 | 24, −63, 39 | 23, 23, 23 |
| Hexadec+7AHL | C1 | −6.0 | 2638 | 33, 51, 44 | 23, 23, 29 |
| Hexadec+4KRA | C3 | −6.4 | 1494 | −29, −2, −27 | 23, 23, 23 |
| Hexadec+5ZTJ | C1 | −4.5 | 4754 | 25, 22, 26 | 35, 33, 23 |
| Hexadec+4OZ5 | C1 | −5.7 | 882 | −2, 10, 34 | 23, 23, 23 |
| Hexadec+1EX2 | C2 | −5.3 | 1015 | 1, 3, 24 | 23, 29, 30 |

Hexadec: Hexadecanoic acid; 5TW8: Crystal structure of wild-type *S. aureus* penicillin binding protein 4 (PBP4) in complex with ceftaroline, 7AHL: Alpha-hemolysin from *S. aureus*, 4KRA: *Salmonella typhi* OmpF complex with Ciprofloxacin, 5ZTJ: Crystal Structure of GyraseA C-Terminal Domain from *S. typhi* at 2.4A Resolution, 4OZ5: *Bacillus subtilis* HmoB, 1EX2: Crystal structure of *B. subtilis* maf protein.

**Table 3:** Validation of molecular docking via redocking – RMSD values between native and redocked ligand poses.

| S. No. | Protein PDB ID | Target protein | Native ligand | RMSD (Å) | Validation status |
|---|---|---|---|---|---|
| 1. | 5TW8 | Penicillin-binding protein 4 (*Staphylococcus aureus*) | Ceftaroline | 1.42 | Validated (<2.0 Å) |
| 2. | 7AHL | Alpha-hemolysin (*Staphylococcus aureus*) | Native peptide toxin | 1.65 | Validated (<2.0 Å) |
| 3. | 4KRA | OmpF porin (*Salmonella typhi*) | Ciprofloxacin | 1.38 | Validated (<2.0 Å) |
| 4. | 5ZTJ | GyraseA C-terminal domain (*Salmonella typhi*) | None (no co-ligand) | NA | Not applicable |
| 5. | 4OZ5 | HmoB (*Bacillus subtilis*) | None (no co-ligand) | NA | Not applicable |
| 6. | 1EX2 | Maf protein (*Bacillus subtilis*) | None (no co-ligand) | NA | Not applicable |

RMSD: Root mean square deviation.

of +5, with one violation (MLOGP > 4.15) [12]. It also violates Veber's rule (more than 10 rotatable bonds) and Muegge's criteria (XLOGP3 >5), potentially impacting bioavailability [25]. However, it satisfies Ghose, Egan, and bioavailability score criteria (0.85). The molecule shows no PAINS or Brenk alerts, indicating no red flags for reactive or toxic functional groups [26]. However, lead-likeness was violated due to excess rotatable bonds (>7) and XLOGP3 (>3.5), affecting its potential as a lead compound. The synthetic accessibility score of 2.31 suggests moderate ease of synthesis. These findings are consistent with previous computational studies evaluating fatty acid derivatives for drug-likeness and pharmacokinetic properties [27].

In the ADME analysis [Figure 3], hexadecanoic acid displayed favorable pharmacokinetic properties, including high gastrointestinal absorption and BBB permeability. It was not identified as a substrate for P-glycoprotein and exhibited moderate synthetic accessibility (score = 2.31). Although the compound showed one violation of Lipinski's rule (logP >5) and exceeded the rotatable bond limits under Veber's and Muegge's criteria, it satisfied other drug-likeness filters such as Ghose and Egan rules. These attributes suggest that while hexadecanoic acid shows potential as a lead molecule, further structural optimization may enhance its bioavailability and drug-likeness.

### 3.5. Antibacterial Activity Assay

The results obtained show that *B. ciliata's* methanolic extract has significant antibacterial action against a variety of bacterial strains [Table 4], but it is often less effective than the common antibiotics gentamicin and chloramphenicol. The extract showed the greatest inhibition against *E. coli* (19.5 ± 0.84 mm) and *S. aureus* (20.65 ± 0.49 mm), indicating that it may be worth looking into further as a natural antibacterial agent, and the lowest inhibition (13.45 ± 0.91 mm) against *S. typhi,* but as compared to conventional antibiotics, its inhibition zones are comparatively smaller.

Medicinal plants are indispensable to the pharmaceutical industry due to their capacity to prevent and treat illnesses. The ethanol extracts of *Solanum somalense*, *Withania somnifera* (L.), and *Calpurnia aurea* exhibited antibacterial efficacy against specific bacterial strains (MICs of 1.5 mg/mL for *S. agalactiae*, 2 mg/mL for *S. aureus* and *E. coli*, and 3 and 3.5 mg/mL for *E. coli* and *K. pneumoniae*, respectively). The results indicated that the medicinal botanicals selected possessed promising antibacterial efficacy against specific bacterial isolates [28]. The branches were extracted using acetone, whereas the foliage and roots of *Delphinium denudatum* were extracted using methanol. Every plant part – leaf, stem, and root – displayed antibacterial activity

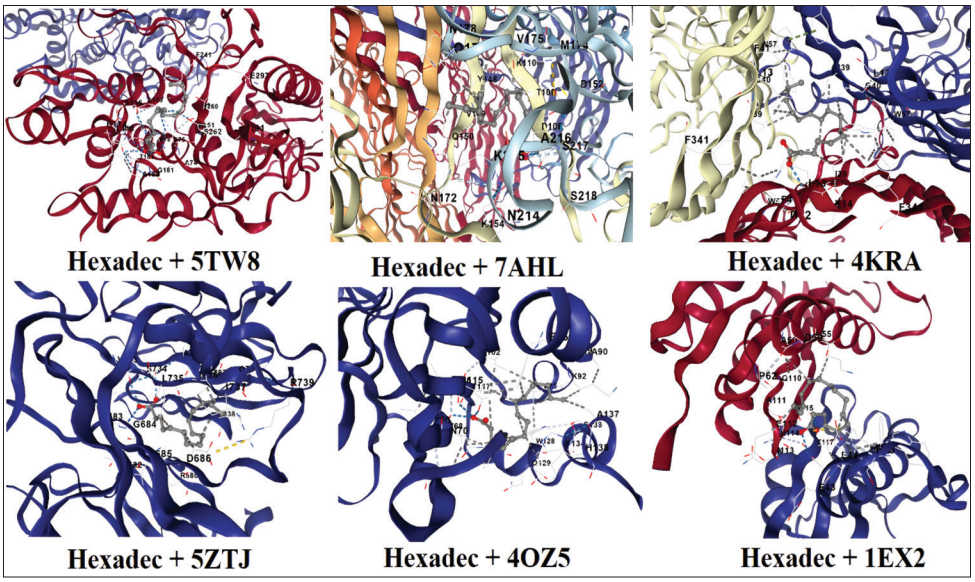


**Figure 2:** Molecular docking of hexadecanoic acid with different target proteins.

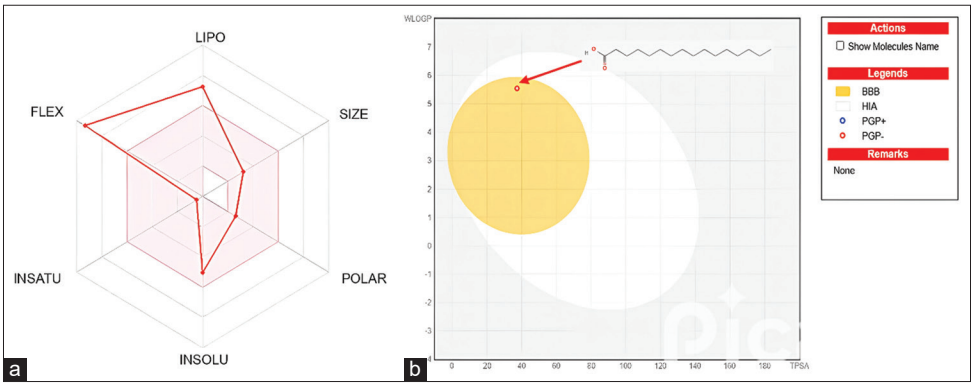


**Figure 3:** (a) Physicochemical, pharmacokinetic, lipophilicity, drug-likeness, and medicinal chemistry properties of hexadecanoic acid. (b) Boiled egg plot between lipophilicity and polarity showing hexadecanoic acid physicochemical properties favorable for both blood-brain barrier penetration and high intestinal absorption.

**Table 4:** Antimicrobial activity of the methanolic extract of the plant *B. ciliata* against pathogenic bacteria.

| Tested microorganism | Zone of inhibition (in mm) | | | P-value | 95% confidence interval | Significance |
|---|---|---|---|---|---|---|
| | Methanolic extract (100 mg/ml) | Chloramphenicol (25 µg/disc) | DMSO | | | |
| *Escherichia coli* | 19.5±0.84 | 23.5±0.21 | NA | 0.0000 | 3.00–4.96 | Significant |
| *Salmonella typhi* | 13.4±0.91 | 25.2±0.56 | NA | 0.0000 | 1.10–13.52 | Significant |
| *Pseudomonas aeruginosa* | 17.2±0.77 | 28.9±0.42 | NA | 0.0000 | 10.20–12.18 | Significant |
| *Klebsiella pneumoniae* | 12.6±0.35 | 28.7±0.21 | NA | 0.0000 | 15.84–16.76 | Significant |
| *Bacillus cereus* | 15.1±0.56 | 30.3±0.70 | NA | 0.0000 | 14.16–16.18 | Significant |
| *Staphylococcus aureus* | 20.6±0.49 | 26.4±1.83 | NA | 0.0001 | 4.49–8.78 | Significant |
| *Bacillus subtilis* | 16.5±1.55 | 28.9±0.28 | NA | 0.0004 | 10.60–14.16 | Significant |
| *Streptococcus pneumoniae* | 16.3±1.48 | 25.3±0.21 | NA | 0.0000 | 8.60–11.98 | Significant |

NA*: No activity.

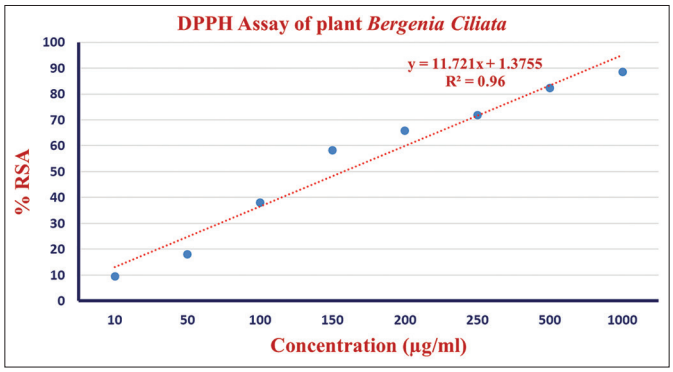


**Figure 4:** Antioxidant activity of the methanolic extract of the plant *Bergenia ciliata* by the 2,2-diphenyl-1-picrylhydrazyl assay.

against *Bacillus subtilis*, *Escherichia coli,* and *Serratia marcescens*, with MICs ranging from 400 to 900 µg/mL. Among all the plant components that have been investigated, leaf extracts in polar solvents are a significant source of antibacterial activity [29].

A paired t-test was performed to compare the antibacterial activity of the *B. ciliata* methanolic extract with standard antibiotics (chloramphenicol) across eight bacterial strains. Moreover, the 95% confidence intervals for the mean differences confirm that the activity of the extract, although lower than that of chloramphenicol, was within a biologically meaningful range. This suggests that the extract possesses broad-spectrum antibacterial potential, albeit with lower potency than a standard antibiotic. The highest statistical difference was observed in *K. pneumoniae*, where the extract showed considerably less inhibition ($p < 0.0001$), highlighting the strain-specific sensitivity.

These findings underscore the potential of the extract as a natural antibacterial agent, supporting its further purification and characterization for bioactive constituents.

### 3.6. Antioxidant Activity by DPPH Assay

The DPPH test graph for the plant *B. ciliata* illustrates the correlation between the concentration of the plant extract (in µg/ml) and its percentage radical scavenging activity (% RSA). The data points indicate a progressive increase in antioxidant activity as the extract concentrations increase. The plant extracts exhibited the highest degree of decolorization at a concentration of 1000µg/ml (88.67%) [Figure 4], as evidenced by a coefficient of determination (R2) of 0.96. This suggests a strong correlation between concentration and

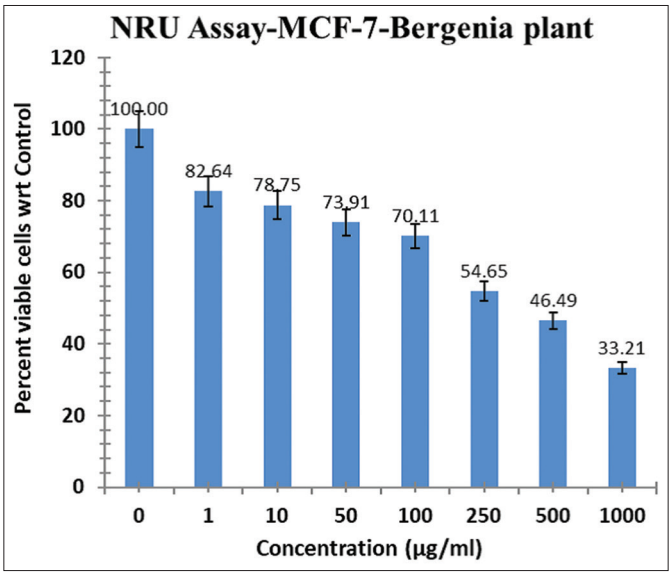


**Figure 5:** Cytotoxicity activity of the methanolic extract of the plant *Bergenia ciliata* by the nitrogen rejection unit assay.

RSA percentage. The dotted red line, which is the most appropriate trend line, emphasizes the potential antioxidant properties of the plant extract.

A diverse array of bioactive compounds is present in medicinal plants, which have a variety of beneficial effects on human metabolism. The *Zygophyllum gaetulum,* an indigenous plant, is found in the Moroccan Sahara. The methanolic extracts exhibited the strongest antioxidant activity and the maximum levels of flavonoid and phenolic compounds in the DPPH and FRAP assays [30]. The Soxhlet extraction technique was employed to produce *Sesamum prostratum* plant extracts that exhibited antioxidant activity. The solvents used were acetone, ethyl acetate, and ethanol. The ethanol extract exhibited the lowest IC50 value of 80.84 µg/mL in the DPPH assay, the acetone extract had the lowest IC50 value of 35.13 µg/mL in the hydrogen peroxide test, and the ethyl acetate extract had an IC50 value of 65.49 µg/mL in the ABTS assay, as per [31]. According to the findings, the plant contains bioactive substances with antioxidant potential that may be further studied for possible anticancer and other therapeutic applications. The FRAP, DPPH, CAT, and ABTS techniques are used to evaluate the antioxidant activity of the methanolic and aqueous extracts of the *Melitotus albus* plant. According to Ed-Dahmani [32].

The aqueous extract is the most effective solvent for antioxidants, DPPH (0.087 ± 0.015), ABTS (0.014 ± 0.001), and the plant's overall antioxidant capacity.

### 3.7. NRU Assay

The NRU Assay findings for MCF-7 breast cancer cells treated with several doses of the *Bergenia* plant extract are shown in the graph. 100% of the cells in the control group (0 µg/mL) are viable. Cell viability declines with increasing extract concentration, suggesting a dose-dependent cytotoxic impact. At lower doses (1–100 µg/mL), cell viability gradually declines: 82.64% at 1 µg/mL and 70.11% at 100 µg/mL. At doses between 250 and 1000 µg/mL, cell viability dramatically declines: 54.65% at 250 µg/mL and 33.21% at 1000 µg/mL. The trend indicates that the cytotoxic effects of the *Bergenia* plant extract on MCF-7 cells are concentration-dependent [Figure 5]. Stronger cytotoxic effects at higher doses point to possible anticancer qualities. For example, the plant extract's $IC_{50}$ is 317.9 ± 0.12 µg/mL.

The global public health community has been significantly concerned by the increase in the morbidity and mortality rates of cancer in recent years. In addition, breast cancer is the second most prevalent cause of mortality among women. The anticancer effects of the traditional medicinal herb *Aloe perryi*, which is a member of the genus Aloe (family: *Asphodelaceae*), were investigated on human breast cancer (MDA-MB-231) and normal (HEK-293) cell lines using the NRU and MTT assays. MDA-MB-231 cells demonstrate concentration-dependent significant cytotoxic effects, with an $IC_{50}$ value of 24.5 µg/mL, in contrast to HEK-293 ($IC_{50}$ value >100 µg/mL). The results of the NRU assay were comparable to the previous study reported [33]. *Dennettia tripetala* fruit extracts in ethanol and water exhibited anticancer properties against the MCF-7 cell line at concentrations of 25.0 and 50.0 µg/mL. The half inhibitory concentrations of the aqueous and ethanol extracts were 37.42 and 61.74 µg/mL, respectively. The percentage of inhibition increased as a result of cytotoxicity on the MCF-7 cells [34]. The proliferation and survival of MCF-7 breast cancer cells were also investigated about the effects of pomegranate seed extract. The promotion of apoptosis by pomegranate seeds, in turn, impeded cell proliferation. In addition, the administration of pomegranate seeds resulted in a dose-dependent decrease in mitochondrial membrane potential, which was indicative of mitochondrial dysfunction and initiated apoptosis [35,36].

Comparative examination with non-cancerous human cell lines, such as HEK-293, was not carried out in the current work, although the cytotoxic potential of the test chemical was assessed using MCF-7 breast cancer cells utilizing the NRU assay. This is a barrier to properly evaluating the compound's specific toxicity. At this point, the main goal was to look at early anticancer activities, for which MCF-7 cells are a reliable model. Non-tumorigenic cell lines will be used in future research to ascertain the compound's selectivity index and have a better understanding of its safety profile. Such comparative data must be included to minimize off-target effects on normal cells and validate the therapeutic potential.

## 4. CONCLUSION

The methanolic extract of *B. ciliata* and the bioactive substance hexadecanoic acid are the main subjects of this integrative study, which emphasizes the plant's diverse medicinal potential. We showed that the plant has promising antibacterial, antioxidant, and cytotoxic qualities using a combination of phytochemical studies, biological tests, and computational methods. Numerous pharmacologically active substances were detected by GC-MS analysis, including trans-13-octadecenoic acid, stearic acid, phytol acetate, and hexadecanoic acid (palmitic acid), each of which contributes to unique bioactivities. The extract's broad-spectrum effectiveness against both Gram-positive and Gram-negative bacterial strains was validated by the antibacterial assay. The significant inhibition zones observed against *S. aureus* and *Escherichia coli* underscore the plant's potential as a source of natural antibacterial agents. However, its activity was modest compared to conventional antibiotics. Still, such findings are notable, especially given the global rise in antimicrobial resistance and the urgent demand for alternative therapeutic strategies derived from natural sources. The antioxidant capacity of the extract, evidenced by a robust DPPH radical scavenging effect ($IC_{50}$ = 317.9 ± 0.12 µg/mL), underscores its potential in neutralizing free radicals and combating oxidative stress. These findings are aligned with traditional uses of *B. ciliata* in folk medicine and validate its relevance in modern phytotherapy. Molecular docking studies further provided mechanistic insights into the interaction of hexadecanoic acid with critical bacterial protein targets. The strongest binding affinity was observed with the 4KRA protein (−6.4 kcal/mol), suggesting selective interaction and potential for inhibiting bacterial membrane transport or function. The observed docking parameters, such as pocket volume and spatial orientation, reinforce the suitability of hexadecanoic acid as a candidate for rational drug design targeting microbial pathogens.

Furthermore, hexadecanoic acid has favorable pharmacokinetic properties, such as high gastrointestinal absorption and BBB permeability, according to the ADME and drug-likeness predictions made by SwissADME. However, some rule violations, such as high lipophilicity and rotatable bonds, may affect its lead-likeness.

Dose-dependent cytotoxicity against MCF-7 breast cancer cells was validated by the NRU assay, suggesting that *B. ciliata* extract has the potential to develop anticancer properties, especially in hormone-responsive breast malignancies. Despite indicating modest potency, the $IC_{50}$ value provides a solid basis for more tuning and mechanistic investigation.

In conclusion, *B. ciliata* exhibits a useful pharmacological profile that demands additional preclinical verification, particularly for its ingredient, hexadecanoic acid. In order to improve bioavailability and therapeutic efficacy, future research should investigate formulation options and clarify molecular pathways *in vivo*. These discoveries enhance our knowledge of traditional medicinal plants and facilitate their incorporation into current drug development processes.

## 5. AUTHORS' CONTRIBUTION

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

## 6. FUNDING

There is no funding to report.

## 7. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

## 8. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

## 9. DATA AVAILABILITY

All the data are available with the authors and shall be provided upon request.

## 10. PUBLISHER'S NOTE

All claims expressed in this article are solely those of the authors and do not necessarily represent those of the publisher, the editors and the reviewers. This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

## 11. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

## REFERENCES


1. Tekman E, Asgarlı T, Yuca H, Atila A, Çeçen Ö, Karakaya S. Exploring quantitative biological major, trace, and ultratrace elements composition and qualitative primary-secondary metabolites in *Lamiaceae* medicinal plants from Turkey. Biol Trace Elem Res. 2024;203(2):1188-201. https://doi.org/10.1007/s12011-024-04219-z
2. Zaman W, Ayaz A, Park S. Climate change and medicinal plant biodiversity: Conservation strategies for sustainable use and genetic resource preservation. Genet Res Crop Evol. 2025;398:4673-87. https://doi.org/10.1007/s10722-025-02410-2
3. Singh K, Gupta JK, Chanchal DK, Shinde MG, Kumar S, Jain D, *et al*. Natural products as drug leads: Exploring their potential in drug discovery and development. Naunyn Schmiedebergs Arch Pharmacol. 2024;398:4673-87. https://doi.org/10.1007/s00210-024-03622-6
4. Moktan N, Gajbhiye RL, Sahithi TV, Roy DN, Kundu R, Banerjee A. Antibacterial and antibiofilm activities of extract and bioactive compounds from *Bergenia ciliata* (Haw.) Sternb. Flowers against *Streptococcus mutans* through cell membrane damage. J Ethnopharmacol. 2025;339:119144. https://doi.org/10.1016/j.jep.2024.119144
5. Gull T, Sultana B, Bhatti IA, Jamil A. Antibacterial potential of *Capparis spinosa* and *Capparis decidua* extracts. Int J Agric Biol. 2015;17(4):727-33. https://doi.org/10.17957/IJAB/14.0007
6. Kuete V. Potential of Cameroonian plants and derived products against microbial infections: A review. Planta Med. 2010;76(14):1479-91. https://doi.org/10.1055/s-0030-1250027
7. Guo F, Chen Q, Liang Q, Zhang M, Chen W, Chen H, *et al*. Antimicrobial activity and proposed action mechanism of linalool against *Pseudomonas fluorescens*. Front Microbiol. 2021;12:562094. https://doi.org/10.3389/fmicb.2021.562094
8. Laha S, Paul S. *Gymnema sylvestre* (Gurmar): A potent herb with anti-diabetic and antioxidant potential. Pharmacogn J. 2019;11(2):201-6. https://doi.org/10.5530/pj.2019.11.33
9. Elshnoudy IA, Elkhouly AM, Masoud M, Rabea HA, Mansour FR. Medicinal plants cultivated in Egypt with anticancer potential; a systematic review. Phytochem Rev. 2024;24(1):527-83. https://doi.org/10.1007/s11101-024-09957-5
10. Nawaz I, Nawaz S, Naqvi ST, Nasir A, Shahzadi I, Ahmed R, *et al*. Antimicrobial, phytochemical, and antioxidant characterization of the leaf extracts of *Clematis montana* and *Clematis grata*. Kuwait J Sci. 2025;52(1):100305. https://doi.org/10.1016/j.kjs.2024.100305
11. Chandra H, Chaudhry V, Sagar K, Pradhan S, Gupta SC, Thakur GS, *et al*. Effect of hawan samagri used for the agnihotra against human pathogenic bacteria responsible for foodborne and airborne infections. Vegetos. 2024. https://doi.org/10.1007/s42535-024-00928-x
12. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev. 2001;46(1-3):3-26. https://doi.org/10.1016/S0169-409X(00)00129-0
13. Ikeagwuonu CM, Adjeroh LA. Preliminary phytochemical screening, anti bacterial and anti fungal effects of leave extracts of *Indigofera suffruticosa* Mill. World Sci News. 2024;188:146-59.
14. Virginia F, Cathrine L, Fernandez S. Isolation, purification, and characterization of betulin, a pentacyclic triterpenoid from the ethanolic extracts of *Coleus forskohlii* tuberous roots; its antimicrobial, antioxidant, anti-inflammatory and cytotoxicity appraisals. Moroc J Chem. 2024;12(4):1531-53. https://doi.org/10.48317/IMIST.PRSM/morjchem-v12i4.47463
15. Dubey A, Ghosh NS, Singh R. A toxicological study on seed extracts of *Asparagus racemosus* Linn (ethanolic and water) in experimental animals. J Adv Zool. 2023;44(2):1770-80. https://doi.org/10.17762/jaz.v44i2.194
16. Shawer EE, Sabae SZ, El-Gamal AD, Elsaied HE. Characterization of bioactive compounds with antioxidant activity and antimicrobial activity from freshwater cyanobacteria. Egypt J Chem. 2022;65(9):723-35. https://doi.org/10.21608/EJCHEM.2022.127880.5681
17. Abubakar MN, Majinda RR. GC-MS analysis and preliminary antimicrobial activity of *Albizia adianthifolia* (Schumach) and *Pterocarpus angolensis* (DC). Medicines (Basel). 2016;3(1):3. https://doi.org/10.3390/medicines3010003
18. Lerata MS, D'Souza S, Sibuyi NR, Dube A, Meyer M, Samaai T, *et al*. Encapsulation of variabilin in stearic acid solid lipid nanoparticles enhances its anticancer activity *in vitro*. Molecules. 2020;25(4):830. https://doi.org/10.3390/molecules25040830
19. Karthikeyan SC, Velmurugan S, Donio MB, Michaelbabu M, Citarasu T. Studies on the antimicrobial potential and structural characterization of fatty acids extracted from Sydney rock oyster *Saccostrea glomerata*. Ann Clin Microbiol Antimicrob. 2014;13(1):332. https://doi.org/10.1186/s12941-014-0057-x
20. Morris GM, Lim-Wilby M. Molecular docking. Methods Mol Biol. 2008;443:365-82. https://doi.org/10.1007/978-1-59745-177-2_19
21. Kitchen D, Decornez H, Furr JR, Bajorath J. Docking and scoring in virtual screening for drug discovery: Methods and applications. Nat Rev Drug Discov. 2004;3:935-49. https://doi.org/10.1038/nrd1549
22. Trott O, Olson AJ. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. 2010;31(2):455-61. https://doi.org/10.1002/jcc.21334
23. El-Sapagh S, El-Shenody R, Pereira L, Elshobary M. Unveiling the potential of algal extracts as promising antibacterial and antibiofilm agents against multidrug-resistant *Pseudomonas aeruginosa*: *In vitro* and *in silico* studies including molecular docking. Plants. 2023;12:3324. https://doi.org/10.3390/plants12183324
24. Veber DF, Johnson SR, Cheng HY, Smith BR, Ward KW, Kopple KD. Molecular properties that influence the oral bioavailability of drug candidates. J Med Chem. 2002;45(12):2615-23. https://doi.org/10.1021/jm020017n
25. Doan KM, Humphreys JE, Webster LO, Wring SA, Shampine LJ,

Serabjit-Singh CJ, *et al*. Passive permeability and P-glycoprotein-mediated efflux differentiate central nervous system (CNS) and non-CNS marketed drugs. J Pharmacol Exp Ther. 2002;303(3):1029-37. https://doi.org/10.1124/jpet.102.041921
26. Muegge I, Heald SL, Brittelli D. Simple selection criteria for drug-like chemical matter. J Med Chem. 2001;44(12):1841-6. https://doi.org/10.1021/jm015507e
27. Baell JB, Holloway GA. New substructure filters for removal of pan assay interference compounds (PAINS) from screening libraries and for their exclusion in bioassays. J Med Chem. 2010;53(7):2719-40. https://doi.org/10.1021/jm901137j
28. Daina A, Michielin O, Zoete V. SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep. 2017;7(1):42717. https://doi.org/10.1038/srep42717
29. Alemu M, Lulekal E, Asfaw Z, Warkineh B, Debella A, Abebe A, *et al*. Antibacterial activity and phytochemical screening of traditional medicinal plants most preferred for treating infectious diseases in Habru district, North Wollo Zone, Amhara region, Ethiopia. PLoS One. 2024;19(3):e0300060. https://doi.org/10.1371/journal.pone.0300060
30. Kumari K, Adhikari P, Pandey A, Samant SS, Lal M, Pande V. Influence of solvent polarity on phytochemicals, antioxidants, and antimicrobial properties of *Delphinium denudatum*: A medicinal herb from Sainj Valley, Himachal Pradesh, India. Bioactivities. 2024;2(1):30-40. https://doi.org/10.47352/bioactivities.2963-654x.214
31. Mammass B, Rachid B, Lahbib F, Mohammed H, Abdelhafed E, Mohamed A. Phytochemical screening and *in vitro* evaluation of antioxidant and antibacterial activity of aqueous and organic extracts of the Moroccan endemic medicinal plant *Zygophyllum gaetulum*. Int J Environ Stud. 2025;82(1):330-47. https://doi.org/10.1080/00207233.2025.2456447
32. Dhanaraj FI, Kalimuthu JK, Balamurugan PS, Subramani P, Katerere DR, Gurusamy M. Investigating the phytochemical profile and antioxidant activity of different solvent extracts of *Sesamum prostratum* Retz. Plants. 2025;14(4):519. https://doi.org/10.3390/plants14040519
33. Ed-Dahmani I, El Fadili M, Kandsi F, Conte R, El Atki Y, Kara M, *et al*. Phytochemical, antioxidant activity, and toxicity of wild medicinal plant of *Melitotus albus* extracts, *in vitro* and *in silico* approaches. ACS Omega. 2024;9(8):9236-46. https://doi.org/10.1021/acsomega.3c08314
34. Farshori NN, Al-Oqail MM, Al-Sheddi ES, Al-Massarani SM, Alam P, Siddiqui MA, *et al*. HPTLC estimation and anticancer potential of *Aloe perryi* petroleum ether extract (APPeE): A mechanistic study on human breast cancer cells (MDA-MB-231). J King Saud Univ Sci. 2022;34(4):101968. https://doi.org/10.1016/j.jksus.2022.101968
35. David SI, Danborno B, Bauchi ZM. Anticancer effect of aqueous and ethanol extract of pepperfruit (*Dennettia tripetala*) fruit on MCF-7 breast cancer cell line using neutral red uptake assay. Bio Sci. 2024;4:763774. https://doi.org/10.55006/biolsciences.2024.4402
36. Alqahtani RA, Almutairi B, Al-Zharani M, Alkahtane AA, Al-Johani NS, Alkeraishan N, *et al*. The potential therapeutic efficacy of pomegranate (*Punica granatum*) seeds on MCF-7 breast cancer cell line. Nat Prod Commun. 2024;19(12):1-11. https://doi.org/10.1177/1934578X241302555

**How to cite this article:**
Vishwakarma S, Chaudhry V, Chand S, Prakash S, Singh K, Singh A, Prasad R, Chandra H. Phytochemical profiling and *in silico* target exploration of hexadecanoic acid from *Bergenia ciliata*: An integrative approach combining docking, absorption, distribution, metabolism, and excretion, and biological activity studies. J Appl Biol Biotech 2025. Article in Press. http://doi.org/10.7324/JABB.2025.253184