International Journal of Pharmacological Research
ISSN: 2277-3312 (Online)
Journal DOI: https://doi.org/10.7439/ijpr **Original Research Article**

Isolation, Characterization and Antimicrobial Activity of *Convolvulus prostratus* Plant Extract

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Abstract

The aim of the present study is extraction, isolation and antimicrobial evaluation of *Convolvulus prostratus*. The main objective of the research was to determine the plant's potential medical uses. Various antimicrobial drugs are present in in the era still there is lacuna to halt microbial infection. The method of extraction was performed by Soxhlet extraction techniques. The compound was isolated by using column chromatography and identified by using various analytical methods like UV, FTIR, NMR and mass spectra. The antimicrobial study was perform by using well-diffusion method. The percentage yield of the chloroform extract obtained 60 %. One compound was isolated from chloroform extract whose structure were identified as (8-hydroxy-8-azabicyclo [3.2.1] octan-3-yl) 3, 4-dimethoxybenzoate by spectroscopic methods. The antimicrobial activity of isolated compound was show prominent inhibitory effect on *E. coli*. *Convolvulus prostratus* for its medicinal properties, focusing on extraction, isolation, and antimicrobial evaluation. This underscores *Convolvulus prostratus* potential as a source of antimicrobial agents, highlighting its significance in combating microbial infections.

Keywords: *Convolvulus prostratus; Extraction, Isolation; Antimicrobial; Spectroscopy*.

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***Article History:**
Received: 02/11/2025
Revised: 26/12/2025
Accepted: 28/12/2025
DOI: https://doi.org/10.7439/ijpr.v15i1.5903

How to cite: Danao K, Nagre N, Barole R, Walode V and Baheti J. Isolation, Characterization and Antimicrobial Activity of *Convolvulus prostratus* Plant Extract. *International Journal of Pharmacological Research* 2025; 15(1): e5875. Doi:10.7439/ijpr.v15i1.5903 Available from: https://ssjournals.co.in/index.php/ijpr/article/view/5903

1. Introduction

An infection occurs when pathogens invade tissues, multiply, and host tissues respond to the infectious agent and the toxins it produces. An infectious disease, usually referred to as a transmissible or communicable disease, is an ailment caused by an infection. A variety of pathogens may cause infections, the most common of which being bacteria and viruses. Hosts can fight infections with their immune systems [1]. Mammalian hosts respond to infections with an innate response, which frequently involves inflammation, followed by an adaptive response. Antibiotics, antivirals, antifungals, antiprotozoals, and antihelminthics are among the specific medications used to treat infections [2-4]. According to the WHO, antimicrobial resistance (AMR) is a significant global public health and development issue. It is estimated that bacterial AMR led to 4.95 million deaths worldwide in 2019 and caused 1.27 million deaths. AMR has significant financial costs in addition to death and harm. The World Bank estimates that by 2050, AMR might lead to an additional $1 trillion in healthcare costs and between $1 trillion and $3.4 trillion in GDP losses annually by 2030 [5,6]. Medicinal plants, often known as medicinal herbs, have been identified and employed in traditional healing practices since ancient times. Plants produce hundreds of chemical compounds for a variety of purposes, including defense and protection against insects, fungus, diseases, and herbivorous animals [7].

Convolvulus prostratus is a member of the Convolvulaceae family of plants. In northern India, bindweed is a prostrate, spreading, perennial, wild plant that is typically

found in xerophytic environments on rocky or sandy terrain [8]. The species is distinguished by a high degree of morphological variety, particularly in bloom size. Stems are either prostrate or ascending, 10–40 cm long, thickly velvety, and have hairs that are appressed to spreading. The leaves are practically stalkless, linear to oblong, inverted-lanceshaped, 0.8-3 cm long, 1.5-6 mm wide, pointy to blunt at the tip, and velvety to hairy. Flowers are produced in 1-3-flowered cymes held on stalks up to 2-3 cm long, but are frequently considerably shorter or missing. Bracts range in form from linear to lanceshaped and measure 3-7 mm in length. Flower stalks can be up to 3 mm long. Sepals are lance-shaped, long-pointed, and 4-8 mm long, with the outer two being longer and hairy. Flowers range from white to light pink and are 1-1.3 cm in length. Stigma lobes are 3–5 mm long [9, 10].

2. Materials and Methods

Fresh *Convolvulus prostratus* plants were obtained from a farm in May 2023 in the Nagpur region, Maharashtra. The plant was authenticate by botanist at department of botany, Nagpur University, Nagpur. The collected plant was rinsed with water and dried and further pulverized at coarse powder. Moreover, Crude extraction, phytochemical screening, isolation and purification of compounds, characterization of pure compounds using spectroscopic techniques such as Ultraviolet–visible spectroscopy (UV–Vis), Fourier Transform Infrared Spectroscopy (FT-IR), and Nuclear Magnetic Resonance (NMR) were conducted at STIC, Kochi, India. Antimicrobial activity assays were performed at the KNCP, Nagpur, India.

All the organic solvents and chemicals used for the phytochemistry tests were purchased from commercial sources at Sigma Aldrich, Bangalore, India. All the organic solvents hexane, chloroform, MeOH, distilled water, and ethyl acetate were purified by the solvent distillation method. NMR spectra were collected using a Bruker spectrometer with TMS as a reference. ^{1}H NMR and ^{13}C NMR data were collected at 600 MHz. Resonances are reported in parts per million (ppm), and coupling constants, J, are reported in hertz (Hz). Infrared spectra were recorded by the

KBr pellet method using Shimadzu FT-IR instruments. A Soxhlet apparatus, rotary evaporator, TLC plate, TLC chamber, column chromatography, Whatman No. 1, flasks of different sizes, UV lamb and UV–Vis spectra, bacterial strains for antimicrobial testing and appropriate media were used in this research work.

2.1 Sample preparation and crude extraction

The collected stem parts of Convolvulus *prostratus* were washed and dried in open air and shielded from direct sunlight to prevent degradation of the bioactive compounds. After drying, the stem parts were coarse powder using grinding mixer. This process ensure that the plant part is clean, dry and properly stored for further extraction[11].

Subsequently, 100 g of powdered *Convolvulus prostratus* was extracted by petroleum ether using a Soxhlet apparatus for 24 h at 45 °C. After filtration, the petroleum ether filtrate was concentrated using a rotary evaporator at 40 °C under reduced pressure. The resulting defatted residue was then air-dried at room temperature for further extraction. Next, 86.5 g of the defatted residue was collected after petroleum ether (P.E.) crude extraction for a second extraction using chloroform using the same procedure above. Finally, 80.4 g of the dried residue from the chloroform extraction step was collected for a third extraction using methanol following the same procedure. All the crude extract was then stored at 4°C until analysis.

2.2 Determination of the crude extract percentage yield

The crude extract percentage yield was determined using the following formula:

$$\text{Percentage yield (\%)} = \text{Weight of extract} / \text{Weight of sample} \times 100$$

The percentage of the crude extract obtained relative to the initial amount of plant material used in the extraction process is given. This calculation helps in assessing the efficiency of the extraction process and provides valuable information for further experimentation or processing of the crude extract.

2.3 Phytochemical screening tests

Convolvulus prostratus crude extract were subjected to phytochemical screening. Petroleum ether crude extract, Chloroform crude extract, and MeOH crude extract were among the plant extracts that are initially screened for the study using the prescribed standard procedures[12].

Test for Terpenoids When 5 ml of the extracts were combined with 2.5 ml of $CHCl_3$ and 3 ml of concentrated H_2SO_4, a reddish-brown color formed at the interface, indicating a positive terpenoid test result.

Test for Saponins: The presence of saponins was determined by diluting all extracts (0.5 g each) with distilled water to 20 ml and vigorously shaking them in a graduated cylinder for 15 min. Foam formation was observed as an indicator of the presence of saponins.

Test for Quinones: The P.E., $CHCl_3$, and MeOH crude extracts (0.2 g each) underwent individual treatment with alcoholic potassium hydroxide solution, resulting in the formation of a blue color from red, indicating the presence of quinones.

Test for Steroids: The crude extract was subjected to the Liebermann–Burchard test by heating to a boil, followed by cooling and mixing with a few drops of acetic anhydride. Upon injecting concentrated sulfuric acid from the sides of the test tube, a brown ring formed at the intersection of the two layers, with a positive test result for steroids indicated by an upper layer turning green.

Test for Phenols: After treating the hexane, $CHCl_3$, and MeOH crude extracts (0.2 g) with 3–4 drops of ferric chloride solution, the presence of phenols was determined by determining the formation of a bluish black color.

Cardiac Glycoside: *Test* In the Keller-Killiani test, the test solution was treated with a few drops of glacial acetic acid and ferric chloride solution. Following the addition of concentrated sulfuric acid, two layers developed: a reddish-brown bottom layer and an upper layer of acetic acid that turned bluish green, indicating the presence of glycoside.

Test for Flavonoids: Five millilitres of diluted ammonia solution was added to an aliquot of the plant extract obtained by hexane, $CHCl_3$, and MeOH as an aqueous filtrate. Concentrated H_2SO_4 was then added. The appearance of a yellow color confirmed the presence of flavonoids, which disappeared upon standing.

Test for Tannins: Approximately 0.25 g of the extract was boiled in 10 ml of water in a test tube and subsequently filtered. A blue–black or brownish-green color was observed when a few drops of 0.1% Fe_2O_3 solution were added, indicating the presence of tannins.

Test for alkaloids: For the alkaloid test, approximately 0.25 g of the crude extracts of hexane, $CHCl_3$, and MeOH were mixed in 5 ml of diluted hydrochloric acid. The mixture was then filtered. Wagner's reagent (iodine in potassium iodide) was applied to the filtrates in two millilitres. The formation of a brown or reddish precipitate indicates the presence of alkaloids.

2.4 Isolation and characterization of the bioactive molecule

Extraction of plant material was perform by using Soxhelt extraction method. Followed by isolation and purification via column chromatography. The purified extract was subsequently Characterized using spectroscopic techniques such as FT-IR, NMR, and Mass spectra to analyze its bioactive structure. Among the three crude extracts (P.E., chloroform, and MeOH), chloroform was selected due to its superior separation compared to the P.E. and methanol extracts [13]. The crude extracts were chromatographed using a glass column with an internal diameter of 1.0 inches and a height of 18 inches. In the column, approximately 10 g of the crude extract was subjected to chromatography with 50 g of medium-sized silica gel (200 mesh) serving as the stationary phase 25 fractions were obtained. The elution process involved sequentially increasing the polarity of the n-hexane and ethyl acetate mixtures as eluents (ratio 3:1), aiming to identify the most suitable solvent for checking complete spot resolution.

The purity of each of the collected fractions was monitored using TLC. Among the 25 fractions obtained from the chloroform crude extract, chloroform twelfth fractions (CTF) appeared pure based on TLC observations, while the others exhibited mixed spots. Subsequent NMR analysis revealed that the twelfth fraction was confirmed to be pure after NMR analysis and was chosen for further spectral data collection. While our objective was to identify and characterize as many compounds as possible, it is important to recognize that certain compounds may have been ignored due to impurities or the complexity of plant chemistry[14].

2.5 Screening for antimicrobial activity

Screening for antimicrobial activity involves various methods to identify potential compounds for combating infectious diseases [15]. Traditional well-diffusion technique is commonly used but may have limitations in reproducibility and speed [16].

The chloroform crude extract, isolated fractions from *Convolvulus prostratus* tests against bacterial strains one gram-negative bacteria *Escherichia coli.* As per the European Committee for Antimicrobial Susceptibility Testing, the well-diffusion method was used to determine the minimum inhibitory concentration (MIC) of compounds against rapidly growing bacterial pathogens[17,18].

2.6 Molecular docking study

Pyrx in autodock vina software was used to perform a molecular docking analysis on the isolated compound CTF of Convolvulus prostratus, which was chosen as a ligand against the target *E.coli*. The crystal structure of *E.coli* DNA gyrase B (PDB ID: 6KZV) was downloaded in PDB format and chosen. The three-dimensional structure of all phytoconstituents was obtained from the PubChem database on the NCBI website (https://pubchem.ncbi.nlm.nih.gov/). The PyRx-virtual screening technique supplemented energy reduction, geometrical confirmation, and hydrogen bonding. Ligand CTF were entered into the PyRx virtual screening program via the Open Babel control. SDF ligand files are translated to PDB format using the Open Babel tool. Furthermore, the Autodock Vina tool (http://vina.scripps.edu/) may be used to determine atomic coordinates for molecules by detecting the torsion root, adjusting torsion angles, changing charges, and optimizing the universal force field [19].

2.7 Molecular properties analysis

The pharmacokinetic study and toxicological parameters of the molecule are critical for identifying prospective therapeutic candidates. We used the pkCSM and SwissADME web server tools to evaluate molecular characteristics and conduct toxicity studies. The selected ligands were subsequently submitted to Lipinski rule of five screening, utilizing the parameters of molecular weight 500, logP 5, hydrogen bond acceptor 10, and topological polar surface area 140 (Å) [20].

3. Results and discussion

The air-dried powdered stem of *Convolvulus prostratus* (100 g) were pulverized and extracted with petroleum ether at 45 °C for 24 h by a Soxhlet apparatus to yield a green extract (25 g, 17.2%). After petroleum ether extraction, 86.5 g of defatted powder was weighed and extracted with chloroform at 45 °C for 24 h using the same apparatus with petroleum ether to yield a dark green extract (30.2 g, 28.9%). Finally, after chloroform extraction, 80.4 g of defatted powder was extracted with methanol at 45 °C with a Soxhlet apparatus for 24 h to yield a brown extract (30.8 g, 39.2%). Based on their polarity, three organic solvents (petroleum ether, chloroform, and methanol) were chosen; the average values of crude extracts percentage yield are calculated for each organic solvent.

3.1 Phytochemical screening results

Phytochemical screening tests conducted against all crude extracts indicated that petroleum ether extracts constitute saponins, terpenoids, steroids, and cardiac glycosides. Chloroform extracts include saponins, terpenoids, phenols, and cardiac glycosides. Methanol extracts contain alkaloids, saponins, tannins, terpenoids, phenols, and cardiac glucosides. The phytochemical screening test results for the petroleum ether, $CHCl_3$ and MeOH crude extracts of *Convolvulus prostratus* showed the following results (Table 1).

3.2 Characterization of CTF

Compound **CTF** was isolated from the $CHCl_3$ crude extract of *Convolvulus prostratus and characterized*. The **CTF** compound was isolated as a yellowish solid with an R*f* value of 0.25 in n-hexane: ethyl acetate (3:1). Structure elucidation of the compound was based on the spectroscopic data obtained from FT-IR, NMR (^{1}H-NMR and ^{13}C-NMR), Mass spectra and UV– Vis spectral data.

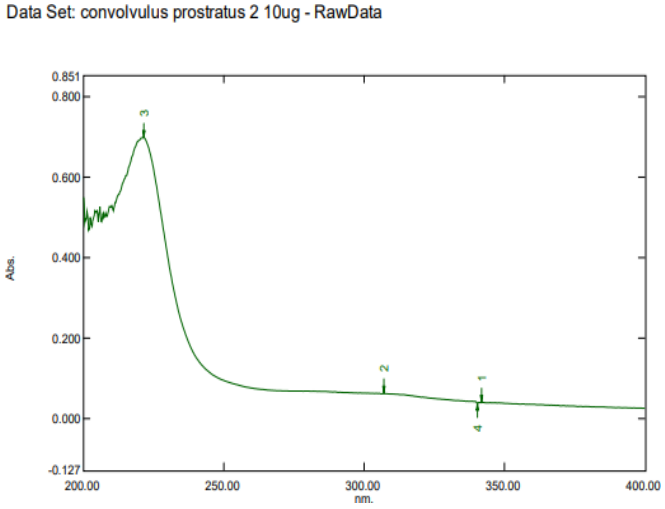
MP 158-160ºC; R*f* 0.25 (n-hexane: ethyl acetate, 3:1 v/v); FT-IR: 3018.60 (C-H, stretching), 1473.62 (C-H, bending), 1132.21 (C-C); ^{1}H NMR: 1.02-1.30 (m, 4H, C-H), 2.29-2.71 (m, 12H, CH_2), 1.02-1.30 (m, 8H, CH_3), ^{13}C NMR: 73.71 (C-H), 38.91-39.91 (CH_2, CH_3), EI-MS: 439.1068,

UV–Vis: ƛ max 221 nm. Experimental data obtained of FT-IR, 1H-NMR, 13C-NMR, Mass spectra and UV–Vis were obtained from Sophisticated Test and Instrumentation Centre (STIC) Kochi, India.

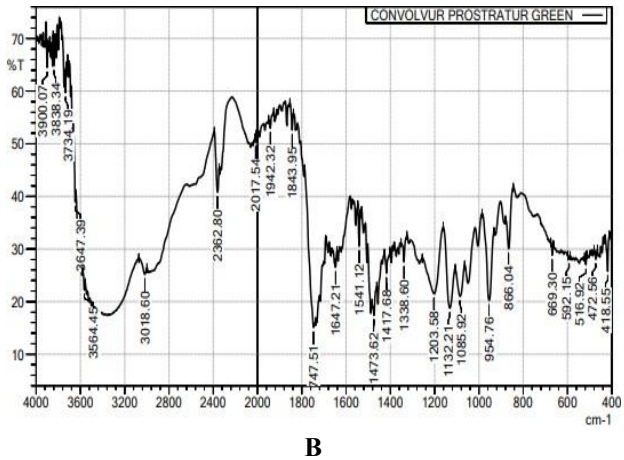
Table 1. Phytochemical screening results for *Convolvulus prostratus.*

Test	Crude extract		
	Petroleum ether	Chloroform	Methanol
Alkaloids	-	+	-
Terpenoids	+	+	+
Saponins	-	+	-
Steroids	+	-	+
Phenols	+	-	+
Cardiac Glycosides	-	-	-
Flavonoids	+	+	+
Tannins	-	-	-

+ve presence of phytochemical constituents
–ve absence of phytochemical constituents



A



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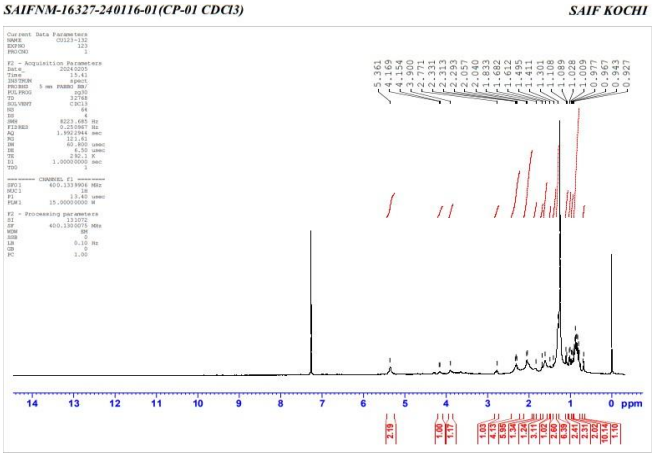

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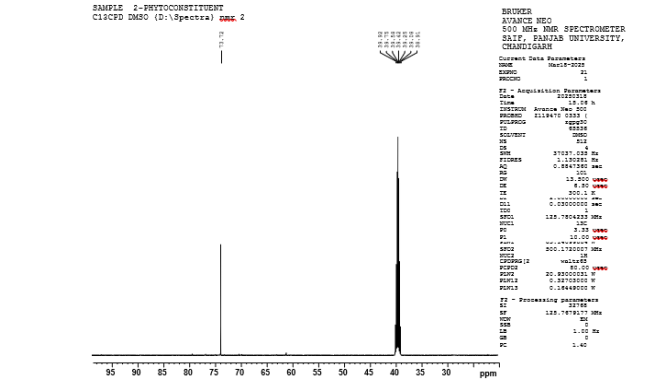

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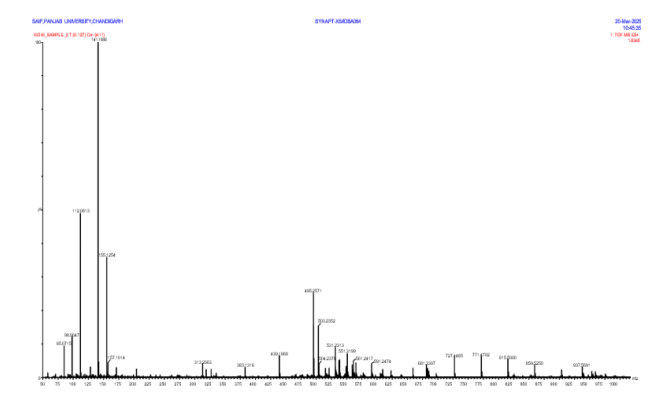


E

Figure 1: Spectrum analysis of *Convolus prostatus* A: Ultraviolet spectra B: FTIR spectra C: 1HNMR spectra D: 13 CNMR spectra E: Mass spectra

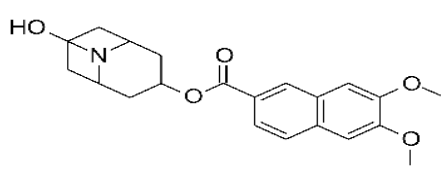


Figure 2. The proposed structure of CTF ($C_{16}H_{21}NO_5$)

3.3 Antimicrobial assay

Isolated fractions **CTF** from *Convolvulus prostratus* showed the following results of inhibition against five bacterial strains gram-negative bacteria *Escherichia coli* using the well-diffusion method (Figure 3).

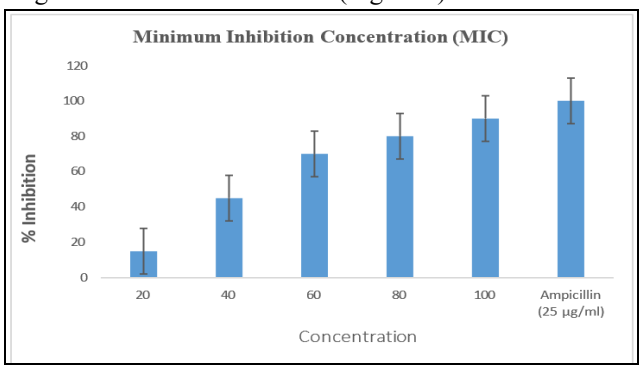


Figure 3: Graphical representation of MIC of isolated compound CTF

The isolated compound **CTF** from chloroform extract *Convolvulus prostrates* stem showed significant antibacterial activity against pathogenic bacteria. The MICs of isolated compound **CTF** having concentration 20-100 µg/mL, for *E.colli* strains were near to standard ampicillin concentration 25 µg/mL. Salehi, B., has demonstrating that *Convolvulus prostrates* exhibits potent antimicrobial activity against *Escherichia coli*. However, our results were not in accordance with those of other reports. This discrepancy may be due to differences in the samples used, the extraction process, the composition of the samples used and the solubility process. Researchers suggest that the antimicrobial activity of bioactive components from *Convolvulus prostrates* relies on both the quantity of bioactive components and the specific types of microorganisms involved.

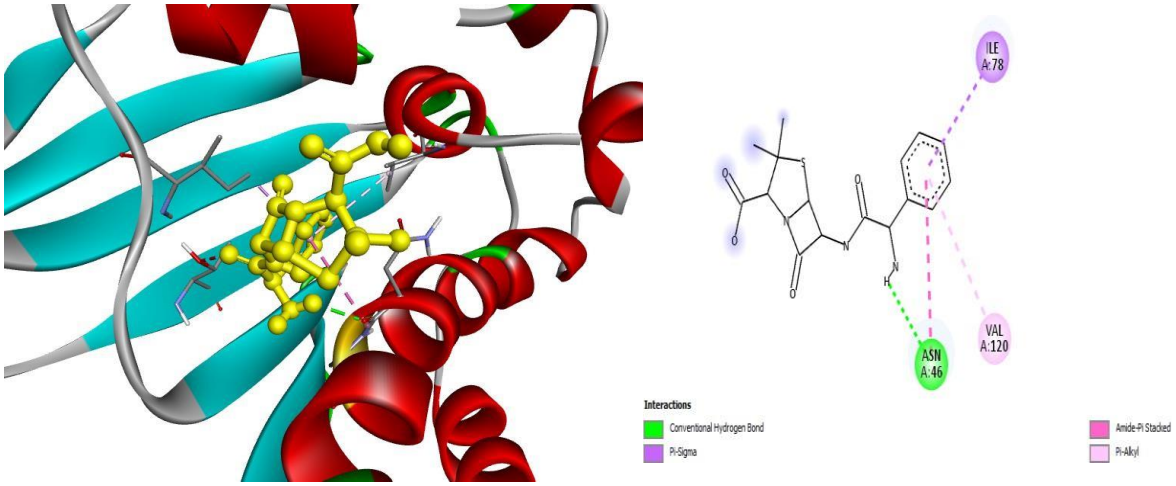
3.4 In-Slico study

In-silico study of isolated compound CTF was perform against the crystal structure of *E.coli* DNA gyrase B (PDB ID: 6KZV) by using computational methods. The purpose of the molecular docking study is to identify the interaction of protein and ligands.

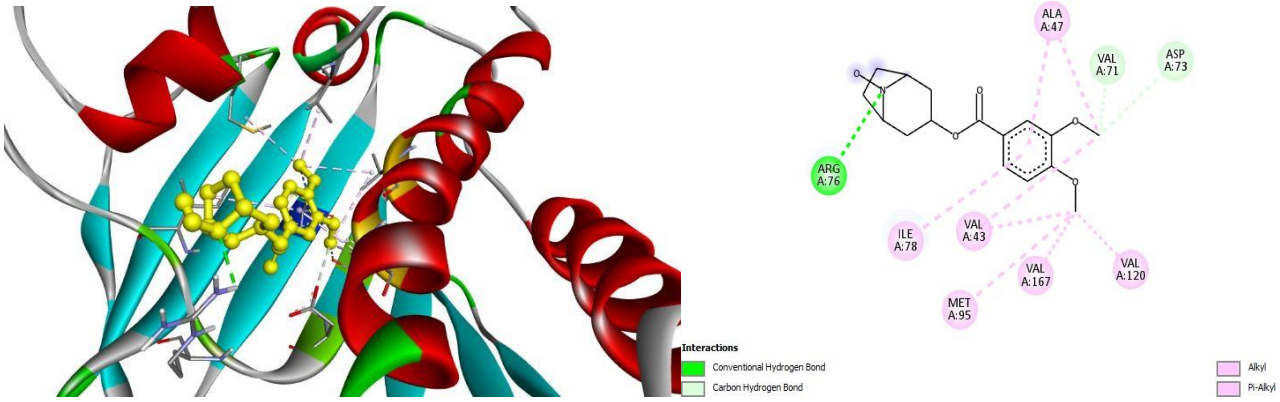
Table 2. Interaction of isolated compound CTF with protein pdb id: 6KZV

Ligands	Binding Energy (kcal/mol)	Amino acid interactions	
		Hydrogen bonding	Hydrophobic bonding
Ampicillin (Standard)	-7.2	ASN A:46	ASN A:46, ILE A:78, VAL A:120
(8-hydroxy-8-azabicyclo[3.2.1]octan-3-yl) 3,4-dimethoxybenzoate	-7.1	ARG A: 76, VAL A:71, ASP A:73	ALA A:47, VAL A:43, ILE A:78, MET A:95, VAL A:120, VAL A:167

Based on visual inspection, molecular docking of ligands on protein 6KZV substantially involves many types of interactions, including hydrogen bonds and hydrophobic bonds. Moreover, isolated compound and ampicillin showed a similar binding pattern to *E.coli* target protein (Table 4). From the Figure 8, the isolated compound CTF and standard ampicillin fitted with amino acid recidue pocket. The isolated compound showed prominent result of inhibition of target protein pdb id: 6KZV as compared to standard ampicillin.



Ampicillin



(8-hydroxy-8-azabicyclo[3.2.1]octan-3-yl) 3,4-dimethoxybenzoate

Figure 4: Ligands fitted with protein pocket

3.5 ADME and toxicity studies

A ligand has pharmacokinetic and toxicological characteristics are essential for turning a chemical into an effective medication. In this study, we do ADMET investigations using the SwissADME and pkCSM servers. The partition coefficient (log P) and total polar surface area (TPSA) of the molecule determine its absorption and lipophilicity potential, respectively. When the TPSA is larger than 140, the drug molecule may readily cross the cell membrane. However, the ideal Log P value of medicines is crucial for achieving the desired pharmacological objective. The Log P- value for oral and sublingual absorption ranges from 1.35 to 1.80; sublingual absorption exceeds 5, whereas the central nervous system is less than 2. Furthermore, medication penetration across the blood-brain barrier (BBB) ranges from -3.0 to 1.2. Furthermore, human intestinal absorption (HIA,%) of ligands is divided into low, medium, and high value ranges: 0 - 29%, 30 - 79%, and 80 - 100%, respectively.

Table 3: ADMET profiles of ligands

ADMET Properties	Molecular formula	Molecular weight [g/mol]	Log P	TPS A [A^0]	HB Donor	HB Acceptor	Human intestinal absorption (%)
Ampicillin	$C_{16}H_{19}N_3O_4$	349.4	0.318	143.1	3	5	43.034
(Standard)	S		1	21			
(8-hydroxy-8-azabicyclo[3.2.1]oc tan-3-yl) 3,4-dimethoxybenzoate	$C_{16}H_{21}NO_5$	307.34	2.245 2	129.0 79	1	6	94.265

ADMET Properties	Hepatoxicity	Neurotoxicity	Cardio toxicity	Carcinogenicity	Mutagenicity	Lipinski's rule violation
Ampicillin (Standard)	Inactive	Inactive	Inactive	Inactive	Inactive	No
(8-hydroxy-8- azabicyclo[3.2.1]octan-3-yl) 3,4- dimethoxybenzoate	Inactive	Inactive	Inactive	Inactive	Inactive	No

The ADMET data (Table 5) revealed that isolated compound (8-hydroxy-8-azabicyclo [3.2.1] octan-3-yl) 3,4-dimethoxybenzoate has obey Lipinski's RO5 rule and does not showed toxicity.

4. Conclusion and Future perspectives

Over the past ten years, Soxhlet equipment-based natural product extraction has become more and more popular since it is considered a traditional method, with a greater focus on safety, economic, and environmental considerations. The stem of Convolvulus prostratus was extracted using a Soxhlet apparatus with three organic solvents: petroleum ether, chloroform, and methanol, in order of polarity differences. The chloroform crude extract was subjected to column chromatography with increasing polarities of n-hexane and ethyl acetate mixtures, yielding twenty-five fractions, including the characterized compound **(8-hydroxy-8-azabicyclo[3.2.1]octan-3-yl) 3,4-dimethoxybenzoate**, from fraction twelfth using various spectroscopic techniques. These findings confirm the existence of the azabicyclo moiety as a natural antibacterial agent with broad-spectrum activity against pathogenic microbes, similar to penicillin antibiotics. We hope that the gaps in our research paper will inspire other researchers to gather all of the necessary information in this field in order to investigate additional applications by synthesizing nanoparticles for biosensors and evaluating the cytotoxicity and antioxidant activities of *Convolvulus prostratus*.

Acknowledgments

Authors are thankful to Management and Dr. Jagdish Baheti, Principal, Kamla Nehru College of Pharmacy, Nagpur for giving moral supports throughout the work.

Author contributions

In the present paper, all authors have equally contributed.

Abbreviation

CTF: Chloroform Twelfth Fraction, **PDB:** Protein Data Bank, **HB:** Hydrogen Bond, **MP:** Melting Point

Funding

The authors have no relevant financial or non-financial interests to disclose.

Data availability

The authors have no financial or proprietary interests in any material discussed in this article.

Conflict of interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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