**RESEARCH PAPER**

# Isolation, Characterization and Bronchodilator Potential of Plant Bougainvillea glabra Against Chemically -Induced Tussive in animals

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**ABSTRACT**

**Background:** The purpose of coughing is to clear the airways of mucus and foreign objects. It is the hallmark of inflammatory disorders affecting the airways. Suppressant medications are prescribed to alleviate coughing in clinical settings. The most common anti-tussive medications have limited use due to side effects such as sleepiness, constipation, hypotension, and respiratory depression. So, as an alternative to present drugs, there is an unfulfilled need for the development of safe and efficient cough suppressant therapeutic solutions for chronic cough. Potential sources of alternative therapy could be medicinal herbs, which contain anti-tussive activity according to traditional medicine.
**Objective:** The prime purpose of this research is to isolate and characterize the primary phyto constituents and verify the hypothesis that Bougainvillea glabra can protect animals against chemically induced cough.
**Materials and Methods:** After extraction, the active phytochemicals are isolated using column chromatography and initial phytochemical screening. The next step will be to use spectrophotometric analysis to identify and characterize the phytochemicals. Several animal strains and substances were subjected to in vivo studies after the probable compound was identified. Mice and guinea pigs were randomly assigned to both positive and negative control groups, each with six animals. After the test extracts are given orally to mice and guinea pigs, they are put in a Perspex box and subjected to ammonia and citric acid gases. By assessing the proportion of animals whose coughs were suppressed, the effectiveness against coughing was assessed.
**Results:** The methanolic extract was found to be suitable after phytochemical screening since it contained the majority of phytoconstituents, especially phenols. Column chromatography and the proper solvent system were used to isolate the active component. The spectral techniques that were employed to ascertain the structure of the active, separated phytoconstituents were FTIR, NMR, and GCMS analysis. Molecular docking studies were carried out using codeine phosphate and bovine serine protease (PDB ID: 1NC6) as benchmarks after structural elucidation. The structure of the isolated molecule may be 3,4,5-Trihydroxybenzoic acid and 4-hydroxy-3-methoxycinnamic acid based on the analysis of its spectrum data. To hypothesise on the molecule's possible mode of action, more molecular docking tests were conducted using normal codeine phosphate. By occupying most of the amino acid active sites, the compound was found to effectively block the target enzyme.Following the administration of tussigenic drugs such as citric acid and ammonia, all of the guinea pigs and mice exhibited a significantly elevated cough response, allowing for additional evaluation of the compounds' potency. On the other hand, the animals fully recovered and returned to nearly normal levels following the use of B. glabra methanolic and aqueous extracts. The methanolic extract-treated mice showed promising outcomes similar to those of a traditional treatment when compared to the aqueous group. Conclusions: The results of our experiment were statistically significant, indicating that the methanolic extract of B. glabra may shield animals from coughing caused by chemicals.

**Keywords:** Bougainvillea glabra, Citric acid, ammonia, anti-tussive, Cough suppressant, Guinea pig, Swiss albino rat.
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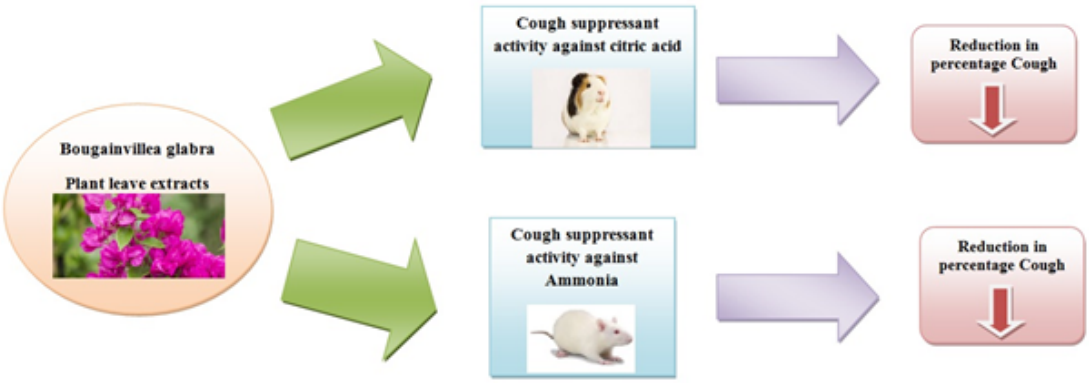
**GRAPHICAL ABSTRACT:**




## INTRODUCTION

Despite the Meth-01ct's assertion that coughing is a defensive response intended to rid the airways of mucus and bacteria, a number of respiratory inflammatory illnesses, such as asthma, chronic bronchitis, pneumonia, and postnasal drip syndrome, cause coughing. [1]. Codeine and dextromethorphan are two drugs that help control coughing at the moment; nevertheless, they come with side effects including Meth-01lling asleep or becoming dependent on the drug. Sputum persistence, which leads to sputum adhesiveness and cohesiveness, has a significant impact on sputum clearance [2]. It is believed that expectorants, which are drugs, can enhance the hydration of mucus or periciliary fluid [3]. Sputum hydration alone won't help with clearance when airway surMeth-01ce liquid is low, but increasing the hydration of the surMeth-01ce fluid may loosen secretions from the epithelium, which would reduce their tenacity and make them easier to transport and clear [4,5].This has led to a rise in the need for safer medications that have the same or similar anti-tussive and expectorant properties.

The Nyctaginaceae Meth-01mily includes the *Bougainvillea* genus, which contains about 110 species. Mexico is home to 18 of these genera, out of a total of 400 species. The three most studied and important species of bougainvillea are *B. glabra*, *B. peruviana*, and *B. spectabilis*, which are used in horticulture. The number of unstudied cultivars and hybrids is close to 100 [6,7].

Paper flower, or *Bougainvillea glabra*, is a climbing evergreen ornamental shrub that originally hails from Brazil but is now common in warmer climes across the globe, including the Americas, Asia, and Europe. Jacques Denys Choisy, a Swiss botanist, first recognized *B. glabra*in 1850. This is a perennial climbing shrub measuring 1–7 m in height, characterized by branches adorned with curved spines measuring 5–15 mm. The shrub features simple, dark green leaves that exhibit a glossy appearance on the upper surMeth-01ce, with a petiole length of 1 cm, glabrous adaxially and pubescent abaxially, approximately 10 cm in length. The bisexual flowers have a diameter of 0.4 cm and are grouped in a cymose inflorescence with three white to cream-coloured blooms. With a single carpel, an ovary, and six to eight stamens, the perianth is somewhat pubescent and ranges in length from 1 to 2.5 cm. The chartaceous, ovate bracts, which are 5 cm long and 1.54 cm wide, have a cardioid base and pointy points. They cling to the flowers at the middle rib's terminal region and exhibit a variety of hues. The fruit is a tiny, ribbed, one-seeded, dry achene. *B. glabra* Meth-01 prefers warm, temperate, semi-arid, and arid environments. [7,8].

In traditional medicine, the plant has a number of applications, including the treatment of diarrhea, reduction of acidity, coughing, and sore throats. A decoction of dried flowers is used to treat blood vessel problems and leucorrhea, and a decoction of the stem is used to treat hepatitis. Leaf material is the most common. The therapeutic qualities of *Bougainvillea glabra* are extensive and varied, including anti-diabetic [9], anti-lipidemic[10], anti-viral[11], anti-fertility[8], anti-inflammatory[11], anti-microbial [7,14], anti-ulcer[14], and larvicidal effects[11]. In traditional medicine, *B. glabra* is used to cure a variety of illnesses, including those affecting the respiratory system (the common cold, flu, cough, bronchitis, asthma, etc.) and the digestive system (dysentery, diarrhea, etc.)[15].

Not only that, but *B. glabra*, or "glory of the garden," has been found to have antioxidant, insecticide, larvicide, hepatoprotective, anthelmintic, antipyretic, and anticancer properties, as well as to promote collagen formation and inhibit tyrosinase and TNF activity [16,17,18].

Due to the insufficiency of data on the cough suppressing properties of *B. glabra*, we intended to conduct a study on the leaves of this plant in order to assess its phytochemical composition. We also intended to test various *B. glabra* leaf extracts for their ability to alleviate chemically-induced coughing in both mice and guinea pigs.

## MATERIALS AND METHOD

### The process of Sampling and verification plant material

Botanist used morphological features to help identify *B. glabra* leaves collected during the flowering stage in and near the institute in Khargone district. The plant specimen

was sent to BN University, Udaipur, Rajasthan, after being authorized by Professor Dr. Mahajan, a Govt. professor from Botany stream of Post graduate Government College Khargone, MP. Professor Mahajan confirmed the specimen's identification as *B. glabra* Choisy, belonging to the Nyctaginaceae Meth-01mily. The flowers and stems were carefully detached after authentication, and the leaves were gathered for additional screening.

**Preparation of Extracts**

After gathering the leaves, they were left to air-dry in a shaded area. After that, they were split and pulverized using a mechanical grinder to a fine to coarse powder. The next step was to strain the powder using sieves 40 and 10. Initially, 250 g of dried powdered *B. glabra* leaves were deMeth-01tted with pet ether at 60-80°C. Afterwards, they underwent polarity index-based extraction using chloroform, ethyl acetate, and ethanol in a Soxhlet device utilizing the continuous hot extraction method. Lastly, the maceration with heat and agitation method was used for water extraction. The potential of the resulting extracts as cough suppressants in living organisms was evaluated after they were standardized.

**Initial Assessment of Phytochemical Properties**

Using conventional phytochemical techniques, the methanolic and water-based extracts were qualitatively examined for the presence of different major phytoconstituents, including flavonoids, Carbohydrate, alkaloids, tannins, proteins, Phenol, Phlobatanins and saponins. Research by Shah, Quadry, and Kokate et al. was followed by phytochemical analyses [19,20].

**TLC study of plant *B. glabra*** [21]

The methanolic plant extract was subjected to TLC analysis. TLC plates were created by applying iodine vapour, examined under a UV lamp, or coated with a reagent to facilitate visualization. In order to determine which solvent solution offered the optimum separation for TLC, it was customary to try a number of different ones.

**Column chromatography** [22]

The wet packing technique was applied for column packing. A funnel was used to move the neutral activated silica gel slurry into the column after it had been prepared in a solvent system. An suitable solvent was used to combine approximately 20 grams of plant extract in methanol and 80 grams of silica gel for CC (60-120 mesh).Using a pestle, the mixtures were triturated until they were consistent, dry, and free-flowing. As previously mentioned, a glass funnel was used to help create and gently feed the mixes into the column without disturbing the silica substrate. After that, the column was filled with an appropriate solvent system to reveal the components. The elutes from column chromatography were subjected to preparative TLC as needed to obtain pure chemicals.

**Spectrum identification, Structure Identification of the isolated phytoconstituents from the plant species**

The structural characterization of natural compounds is most commonly accomplished using spectroscopic techniques such as Fourier Transform Infrared Spectroscopy (FTIR), Nuclear Magnetic Resonance (NMR), and Mass Spectrometry (MS).

The FTIR spectra of the isolated compounds were recorded using the KBr pellet method on a Shimadzu 8400S FTIR spectrophotometer at the Central Instrumentation Laboratory, Panjab University, Chandigarh. Spectral data were collected over the range of 650–4000 $cm^{-1}$. The obtained spectra were analyzed to identify characteristic absorption bands corresponding to key functional groups, including hydroxyl (–OH), carbonyl (C=O), aromatic C=C, and C–O stretching vibrations, thereby facilitating structural confirmation of the isolated compounds.[23]

The Indian Institute of Science Education and Research Bhopal (IISER Bhopal) used a Bruker Advance Neo (500 MHz) NMR spectrophotometer to record the C-NMR spectra of isolated chemicals. Tetramethylsilane (TMS) was utilised as an internal standard after the samples were dissolved in deuterated dimethyl sulfoxide (DMSO-$d_3$). Parts per million (δ, ppm) were used to express chemical changes. The spectra were examined to confirm the compounds' proton environments by identifying aromatic, olefinic, methoxy, hydroxyl, and carboxylic acid protons. [24].

All GC–MS analyses were carried out at the Sophisticated High-Tech Laboratory, Indian Institute of Science Education and Research (IISER), Bhopal. Mass spectral analysis was performed using an electrospray ionization mass spectrometer (ESI–MS) operated in both positive and negative ionization modes. The molecular ion peaks and characteristic fragmentation patterns were recorded and systematically analyzed to determine the molecular weight and to confirm the structural integrity of the isolated compounds.

The compounds isolated from the ethanolic extract of *Bougainvillea glabra* were identified by comparing their mass spectral data, including molecular ion peaks, with reference spectra available in the NIST library database at IISER Bhopal. The GC–MS chromatogram further indicated the presence of a bioactive phenolic phytoconstituent in the isolated compound obtained from the ethanolic extract of *B. glabra*. [25]

**Computational study of Isolated compounds**

**Molecular Properties and drug likeness properties (https://molsoft.com/mprop/)**

Drug-likeness properties describe the structural and physicochemical characteristics of a compound that influence its suitability as an orally active drug. These properties include molecular weight, lipophilicity (logP), hydrogen bond donors and acceptors, solubility, polarity, and molecular flexibility, all of which affect absorption, distribution, and overall bioavailability. Evaluating drug-likeness helps in early screening of compounds to identify promising candidates with Meth-01vorable pharmacokinetic behavior and reduced risk of Meth-01ilure in later stages of drug development[26,27].

A commonly used method for determining oral drug-likeness is the Lipinski Rule of Five. This rule states that a chemical with a molecular weight of less than 500 Da, a logP of less than 5, five hydrogen bond donors, and ten hydrogen bond acceptors is more likely to have acceptable oral bioavailability. Compounds may show poor absorption or permeation if they breach more than one of these requirements. Lipinski's rule is a useful filter in medicinal chemistry for creating and choosing orally active drug candidates, despite its limitations [28].

**ADMET study**

PreADMET is a widely utilized web-based computational tool in drug discovery for the early prediction of ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties of chemical compounds prior to experimental evaluation. It employs validated quantitative structure–activity relationship (QSAR) models to estimate key pharmacokinetic parameters, including human intestinal absorption, Caco-2 cell permeability, blood–brain barrier penetration, plasma protein binding, and cytochrome P450 enzyme inhibition. In addition, it predicts important toxicity endpoints such as mutagenicity and carcinogenicity.

This in silico approach enables rapid screening and prioritization of potential lead compounds, thereby minimizing late-stage drug development failures and facilitating the optimization of drug-likeness in a cost-effective and time-efficient manner [29,30].

**Sequence Retrieval and Phylogenetic Analysis**

Phylogenetic analysis of PDB INC6 was performed using protein sequences retrieved from the NCBI database. Homologous sequences were identified by BLASTp, aligned using Clustal Omega, and the phylogenetic tree was constructed using the Neighbor-Joining method in NCBI Tree Viewer [31,32].

**Molecular Docking study**

The docking software Molegro virtual docker 6.0 (MVD) was employed to conduct ligand docking investigations within the binding pocket of the bovine serine protease (trypsin) receptor. The crystal structures of bovine serine protease (trypsin) were identified from the Protein Data Bank (PDB ID: 1NC6**)** [16]. 1NC6 is the crystal structure of a bovine serine protease (trypsin) in complex with a potent small-molecule inhibitor designed as a transition-state analogue with a benzothiazole ketone moiety. The structure was solved by X-ray diffraction at 1.90 Å resolution and deposited in 2002. It is used to understand how these inhibitors bind in the active site of serine proteases and has relevance in designing anti-asthmatic compounds targeting human mast cell tryptase**,** as such inhibitors may block allergic or inflammatory responses. The model includes the protease and bound ligand(s), with key interactions helping explain specificity and potency [33].

MVD is based on an accurate and efficient molecular docking approach to predict ligand–protein interactions. It uses a guided differential evolution algorithm to investigate the best ligand binding positions inside the target protein's designated active region. Protein preparation (removal of water molecules, addition of hydrogens, and cavity detection) precedes ligand optimisation in the docking process. Binding affinity is evaluated using the MolDock scoring function**,** which combines piecewise linear potentials, electrostatic interactions, and hydrogen bonding terms, with optional re-ranking using more detailed energy components. Multiple poses are generated and ranked based on their docking scores, allowing identification of the most stable and biologically relevant ligand–receptor interactions **Fig. 1** [34,35].

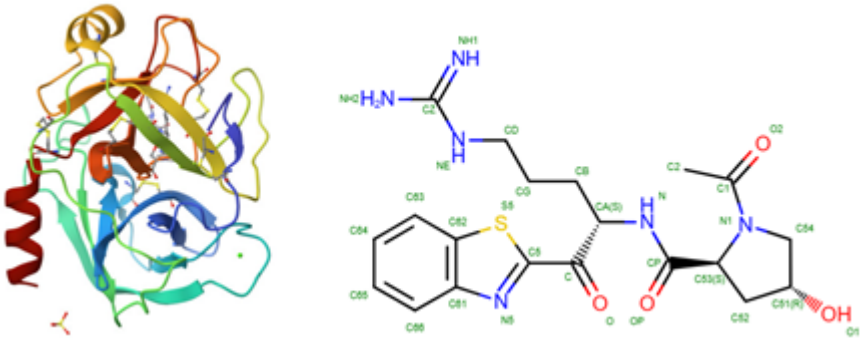



**Figure 1:** Secondary structure of PDB: 1NC6 and its co-crystallized ligand

**Animal Husbandry**

The study adhered to the standards set by the CCSEA on the treatment and utilization of animals. The CCSEA members gave their approval to the animal housing conditions at BNCP in Udaipur, Rajasthan. The institute's IAEC has given its approval to the study, and the accepted protocol's proposal number is 26/BNCP/IAEC/2025.Animals ranging in weight from 250 to 300 grams of guinea pigs and 20 to 25 gms of mice, were used in the study. In addition to being acclimated with the laboratory setting for a minimum of 14 days, the testing animals will be evaluated daily for clinical indicators. Light and dark cycles of 12 hours each, temperatures between 22 and 28 degrees Celsius, and humidity between 35 and 55% were all features of the animal housing environment that mimicked a typical laboratory. Each animal in its cage has access to water and food pellets stored in a stainless steel mesh top grill. The

mice and guinea pigs were given regular laboratory rodent food and had access to plenty of drinkable water.

**Acute Oral Toxicity Study**

Following the protocols laid out in OECD No. 423, we performed this experiment using adult Swiss albino mice weighing between 20-25 gm. The acute toxicity test involved dosing ten mice per group with varying concentrations of the methanolic and aqueous extract, ranging from 500 to 3500 mg/kg. Using instruments such as the actophotometer and Rota rod, researchers meticulously examined the mice's behavior throughout the inquiry. We looked for things like decreased spontaneous activity, altered skin or fur, dilated pupils, drowsiness, hypothermia, convulsions, hyperactivity, and any other abnormalities or toxic signs. The following day, for the next seven days, the animals were examined at predetermined intervals [36,37,38].

**Experimental Procedure:**

Research on anti-tussive effects was conducted using guinea pigs and mice, respectively, using the citric acid-induced cough model and the ammonia-induced cough model, all in accordance with established protocols and guidelines [39,40,41].

***In vivo* chemical induced cough suppressant activity:**

***Citric Acid-Induced Cough in the animal species Guinea Pigs*:** Twenty-four guinea pigs were divided into four groups at random, each consisting of six animals, in order to conduct pre-clinical testing. The guinea pigs were given a 5-minute exposure to a 7.5% aerosol of citric acid by an ultrasonic nebulizer after being pre-screened in a 24 × 12 × 24 cm Perspex box. The cough was identified by its distinctive sound and by the patient's limbs being extended in response to inspiration and subsequent expiration. Cough reflexes and the total number of coughs were recorded from the guinea pigs. The test was conducted on animals that coughed ten times or more. The animals that were chosen for the experiment were then given water to drink while they Meth-01sted overnight. The chosen guinea pigs were fasted overnight, then split into four groups of six animals each, and subjected to the following treatments: The first group served as a toxic controls, which consisted of 7.5% citric acid aerosol alone. Second group received with standard drug codeine phosphate with a dose of 25 mg/kg. Third and Fourth group received methanolic and aqueous extracts of the equal dose of 250 mg/kg of *B. glabra*. All the guinea pigs were exposed to the citric acid aerosols except normal control and their cough bout counts were taken one hour after the usual medication, distilled water, and extract had been administered. For every animal, the percentage of cough suppression was determined as:

% Cough Suppression (C1-C2)/C1) × 100

(Where, C1 = frequency of cough episodes prior to medication introduction and C2 = the frequency of coughs following medication administration)

***Ammonia induce cough in mice:*** The following procedures were used to treat twenty four mice of four treatment groups, ranging in weight from 20 to 30 grams, with six mice per group. In Group 1 served as a hazardous control group, ammonia was the only substance given. Second Group received with 25 mg/kg of codeine phosphate. Group third and fourth received, 250 mg/kg of *B. glabra* methanolic extract and aqueous extract. Oral administration was used for all purposes. Following one hour of dosing, the mice were subjected to 25% $NH_3OH$ for 45 seconds in a specially designed chamber with a diameter of 1000 ml and cotton wool embedded within. The mice were placed in a chamber with a top opening, and their cough frequency was recorded for 5 minutes. The percentage of anti-tussive activity was determined by calculating the proportion of coughs that were inhibited by using the formula mentioned earlier in previous protocol [42,43,44].

**Statistical analysis**

Results of anti-tussive tests were tabulated using mean values ± SEM. We found the statistical dissimilarity and significance by analyzing the variance and doing group evaluations using the "Tukey-Kramer" multiple comparison test. A significance level of $p<0.05$ was used. The toxic control group was evaluated in respect to the other treatment groups using *in vivo* techniques.

## RESULTS AND DISCUSSION

An important aspect in the accuracy of the evaluation of botanical phytoconstituents is the choice of solvents for their extraction from plants. Phytochemical screenings revealed that *B. glabra* leaves contained a number of phytoconstituents that represents promising pharmacological agents, including glycosides, alkaloids, tannins, carbohydrates, saponins, phenol, phlobatanins, and flavonoids. The phytochemical study's findings showed that, in comparison to other extracts, methanolic extract contained the majority of phytochemicals which is indicated in Table No 1.

**Table 1:** Qualitative Phytochemicals Screening *B.glabra* Extracts

| S.No. | Phytochemical constituents | Methanolic extract | Aqueous extract |
|---|---|---|---|
| 1 | Carbohydrate | + | - |
| 2 | Glycoside | + | + |
| 3 | Proteins | - | - |
| 4 | Flavonoids | + | - |
| 5 | Tannins | + | + |
| 6 | Alkaloids | + | - |
| 7 | Saponin | + | + |
| 8 | Phenol | + | - |
| 9 | Phlobatanins | + | - |

In order to better support the plant *B. glabra*'s potential as a bronchodilator, we chose to separate the phytoconstituents phenols from the methanolic extract. We discovered that the solvent systems Hexan:ethyl Acetate: Methanol:Formic Acid with a varied ratio were the most suitable for isolating phenol compounds present in the methanolic extract of *B. glabra* after analysing the plant's methanolic extract using the TLC method by applying various solvent systems and visualizing the TLC plates under a UV lamp of 254 nm after spraying the 10% aqueous H2SO4. Thus, the solvent system mentioned above was chosen for additional column chromatographic research. To create the material adsorbed in the silica gel, 15 grams of dried ethanolic extract of *B. glabra* were combined with 80 grams of silica gel (60–120 mesh). The chemical was isolated in the column using several solvent systems based on the polarity. To determine the precise ratio in which chemicals were eluted and fractions were collected, the column was first eluted with increasing amounts of solvent from hexane alone, then ethyl acetate with methanol, and finally formic acid with varying ratios in increasing polarity. From the solvent system, one chemical was extracted. Meth-01 and Meth-02 are the two chemicals that were separated from the column.

**Spectral Characterization of Isolated compounds through various Spectrophotometric Analysis**

**Spectral Characterization of** Meth-01:

IR (KBr, $cm^{-1}$): 3425 (O–H stretching), 3078 (aromatic C–H), 1686 (C=O), 1602 (C=C), 1265 and 1032 (C–O stretching).
$^{1}H$ NMR (400 MHz, DMSO-$d_6$, δ ppm): 3.83 (3H, s, –$OCH_3$), 6.41 (1H, d, CH=CH), 7.52 (1H, d, CH=CH), 6.88 (1H, d, Ar–H), 7.01 (1H, d, Ar–H), 7.10 (1H, s, Ar–H), 9.68 (1H, s, –OH), 12.36 (1H, s, –COOH).
$^{13}C$ NMR (100 MHz, DMSO-$d_6$, δ ppm): 168.5 (C=O), 150.6 (C-4), 148.3 (C-3), 145.2 (Cα), 126.4 (C-1), 123.7 (C-6), 116.8 (Cβ), 111.9 (C-2), 56.1 (–$OCH_3$).
MS (ESI-MS, m/z): 194 ($[M]^+$), 193 ($[M–H]^-$), 178 ($[M–OH]^+$), 149 ($[M–COOH]^+$).

Spectral Characterization of Meth-02

IR (KBr, $cm^{-1}$): 3360 (O–H stretching), 3062 (aromatic C–H), 1708 (C=O), 1606 (aromatic C=C), 1235 (C–O stretching), 758 (aromatic C–H bending).
$^{1}H$ NMR (400 MHz, DMSO-$d_6$, δ ppm): 7.02 (2H, s, Ar–H), 9.12 (3H, br s, –OH), 12.24 (1H, s, –COOH).
$^{13}C$ NMR (100 MHz, DMSO-$d_6$, δ ppm): 167.8 (C=O), 145.6 (C-3, C-5), 138.9 (C-4), 121.3 (C-1), 110.2 (C-2, C-6).
MS (ESI-MS, m/z): 170 ($[M]^+$), 169 ($[M–H]^-$), 125 ($[M–COOH]^+$).

According to the study of the results of the various spectrophotometric readings the first isolated compound, Meth-01, may be 4-hydroxy-3-methoxycinnamic acid, and Meth-02 may be 3,4,5-trihydroxybenzoic acid,. Figure No. 2 depicts their structure.

***In Silico* pharmacological evaluation of isolated compounds**

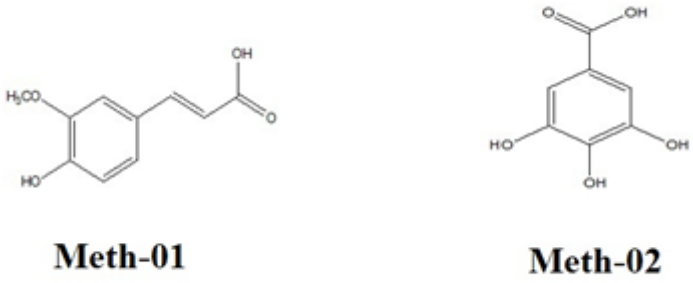



**Figure 2:** Design Structure of isolated compounds

According to Lipinski's rule of five, lead compounds with molecular weights (MW) of no more than 500 Da, hydrogen-bond donors (HBD) of no more than 5, hydrogen-bond acceptors (HBA) of no more than 10, and logP of less than 5 are considered the best absorption medications. Additionally, molecules having rotatable bonds (nrotb) fewer than 10 and total polar surface area (TPSA) less than 140 Å exhibit high bioavailability.

The in silico evaluation results, including the drug likeness score of the new candidate molecules METH-01 and METH-02, have good oral bioavailability and satisfy Lipinski's criterion, as Table 3 and Figure 3, 4 make clear. Furthermore, every new chemical has a molecular weight of less than 500 Da, suggesting that our molecules are readily absorbed and disseminated. These results show that the recommended bovine serine protease medications have good bioavailability.

**Table 3:** Molecular properties Lead compounds

| S. No | Molecular properties | Meth-01 | Meth-02 |
|---|---|---|---|
| 1 | Molecular formula | $C_{10}$ $H_{10}$ $O_4$ | $C_7$ $H_6$ $O_5$ |

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| | | | |
|---|---|---|---|
| 2 | Molecular weight | 194.06 | 170.02 |
| 3 | Number of HBA | 4 | 5 |
| 4 | Number of HBD | 2 | 4 |
| 5 | MolLogP | 1.61 | 0.78 |
| 6 | MolLogS | -1.90 | -1.10 |
| 7 | MolPSA | 52.80 $A^2$ | 77.22 $A^2$ |
| 8 | MolVol | 194.88 $A^3$ | 142.94 $A^3$ |
| 9 | pKa of most Basic/Acidic group | <0. / 4.54 | <0. / 5.43 |
| 10 | Drug Likeness Score | -0.61 | -0.22 |
| 11 | Bioavailability Score | 0.85 | 0.56 |
| 12 | Molar Refractivity | 51.63 | 39.47 |
| 13 | TPSA | 93.76 Å² | 97.99 Å² |

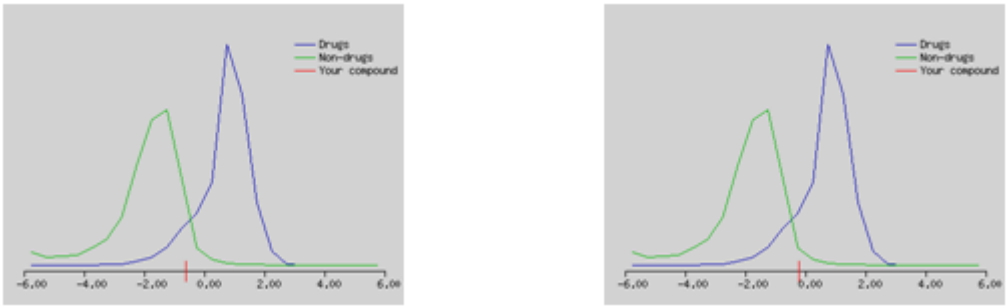


**Figure 3:** Drug likeness score of Meth-01 and Meth-02

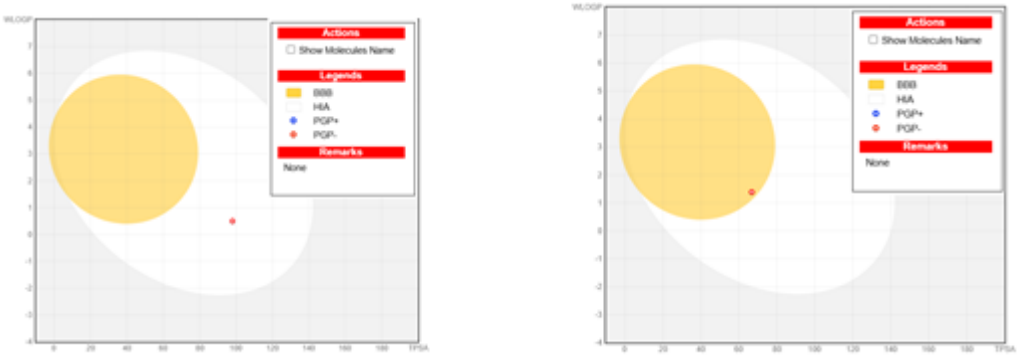


**Figure 4:** ADMET Properties of design compounds by boiled egg model

The ADMET properties of Meth-02 and Meth-01 were predicted using the online web tool PreADMET to evaluate their pharmacokinetic behavior and safety profile. Meth-02 showed moderate human intestinal absorption, which may be attributed to its high polarity and multiple hydroxyl groups that can limit passive membrane permeability; its predicted Caco-2 permeability was moderate, indicating reasonable but not optimal oral absorption. In contrast, Meth-01 exhibited comparatively higher intestinal absorption and better Caco-2 permeability, likely due to its balanced hydrophilic–lipophilic character and fewer hydrogen bond donors, suggesting improved oral bioavailability.

Distribution analysis indicated that Meth-02 has limited blood–brain barrier penetration, implying restricted central nervous system distribution, whereas Meth-01 demonstrated slightly higher BBB permeability, allowing broader tissue distribution. Both compounds showed moderate plasma protein binding, which supports adequate free drug availability in systemic circulation. Metabolic predictions revealed that neither Meth-02 nor Meth-01 inhibits major cytochrome P450 enzymes such as CYP3A4 and CYP2D6, indicating a low potential for metabolic drug–drug interactions; however, the presence of phenolic and carboxylic acid functional groups suggests that both compounds may undergo rapid phase II metabolism, potentially reducing their systemic half-life.

Excretion profiles predicted efficient clearance for both molecules through renal and hepatic pathways, consistent with their small molecular size and polar nature. Toxicity assessment using PreADMET showed that both Meth-02 and Meth-01 are non-mutagenic in the AMET test and non-carcinogenic, indicating a Meth-01vorable safety profile. Overall, the ADMET analysis suggests that both compounds possess acceptable pharmacokinetic and toxicity characteristics, with Meth-01 demonstrating slightly superior absorption and distribution properties, supporting their potential as safe and drug-like bioactive candidates. The results were shown in **Table 4,5,6.**

**Table 4:** Result of drug likeness properties

| Properties | Score/ Value | |
|---|---|---|
| | **METH-01** | **GA** |
| CMC_like_Rule | Quaalified | Not qualified |
| CMC_like_Rule_Violations | 0 | 2 |
| Lead_like_Rule | Suitable | Violated |
| Lead_like_Rule_Violations | 0 | 1 |
| MDDR_like_Rule | Mid-structure | Mid-structure |
| MDDR_like_Rule_Violation_Fields | No_Rings, No_Rotatable_bonds | No_Rings, No_Rotatable_bonds |
| MDDR_like_Rule_Violations | 2 | 2 |
| Rule_of_Five | Suitable | Suitable |
| **ADME properties** | | |
| BBB | 0.758419 | 0.348084 |
| Caco2 | 21.1177 | 13.8492 |
| CYP_2D6_inhibition | Non | Non |
| HIA | 90.603297 | 53.696852 |
| MDCK | 228.559 | 9.53976 |
| Pgp_inhibition | Non | Non |
| Plasma_Protein_Binding | 50.414225 | 65.384676 |
| Skin_Permeability | -1.87204 | -3.62686 |
| **Toxicity study** | | |
| Ames_test | mutagen | mutagen |
| Carcino_Mouse | negative | negative |
| Carcino_Rat | positive | positive |
| hERG_inhibition | medium_risk | low_risk |
| algae_at | 0.0848386 | 0.0780307 |

**Correlation of RCSB 1NC6 protein information with phylogenetic analysis**

The RCSB Protein Data Bank entry 1NC6 corresponds to a hydrolase enzyme derived from *Bostaurus*, a placental mammal belonging to the order Artiodactyla (even-toed ungulates). The protein was heterologously expressed in *Escherichia coli* without any reported mutations, ensuring that the resolved structure closely represents the native bovine enzyme. The use of *E. coli* as an expression host is purely experimental and does not influence evolutionary or phylogenetic interpretations.

Phylogenetic analysis based on homologous sequences reveals that *Bostaurus* clusters within the even-toed ungulates clade, which shows a close evolutionary association with cetaceans (whales and dolphins). This clustering is consistent with well-established molecular and fossil evidence supporting the evolutionary origin of cetaceans from terrestrial artiodactyl ancestors. The position of *Bostaurus* in the phylogenetic tree thus validates the evolutionary relevance of 1NC6 as a representative placental mammalian hydrolase **Fig. 4**.

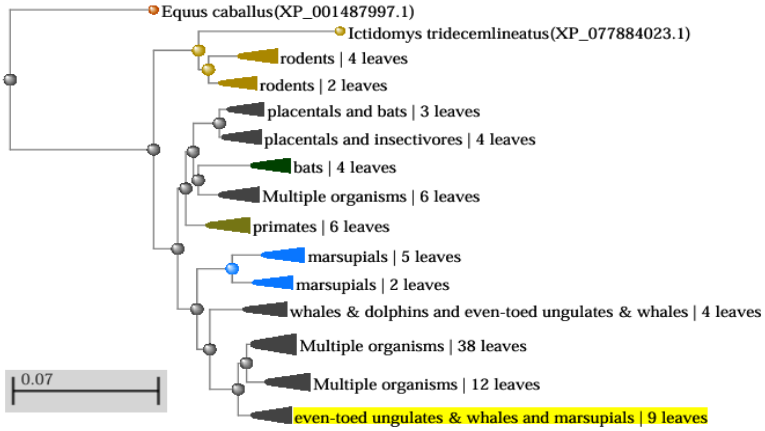


**Figure 4:** Phylogenetic analysis of RCSB protein 1NC6

Hydrolase enzymes are known to be highly conserved across mammalian species, particularly among placental mammals, due to their essential roles in metabolic and physiological processes. The conservation of sequence and structural features observed in 1NC6 supports the close phylogenetic relationship among ungulates and related mammalian taxa depicted in the tree. Therefore, the phylogenetic placement of *Bostaurus* correlates well with the functional conservation and evolutionary stability of the hydrolase Family.

Overall, the combined structural, functional, and phylogenetic evidence confirms that 1NC6 is evolutionarily conserved within placental mammals, and its phylogenetic clustering reflects both shared ancestry and conserved enzymatic function, reinforcing the reliability of the constructed phylogenetic tree.

Molecular docking studies were performed using MVD to investigate the binding interactions of Meth-02 and Meth-01 with the target protein 1NC6. The docking protocol was validated by selecting the active site cavity with the highest volume and biological relevance. Both ligands were docked flexibly, and binding affinity was evaluated based on MolDock score**,** re-rank score**,** and interaction profiles. Meth-02 exhibited a Meth-01vorable binding orientation within the active site of 1NC6, forming 7 hydrogen bond interactions with key amino acid residues i.e. Ser190, Ser195, Asp189, Gly219, Trp215, Val227, Ser214 with MolDock score -91.83. The presence of three hydroxyl groups and one carboxylic acid group contributed to strong hydrogen bonding and electrostatic interactions, enhancing binding stability. The MolDock score indicated a strong binding affinity, supported by additional vander Waals interactions within the binding pocket. Meth-01 also showed effective binding within the active site cavity of 1NC6. 4 Hydrogen bond interactions Ser190, Ser195, Asp189, Gly193 were observed between the hydroxyl and carboxyl groups of Meth-01 and active site residues with moldock score 101.17, along with hydrophobic interactions involving the aromatic ring and methoxy group. Although the binding affinity of Meth-01 was slightly lower compared to Meth-02, its interaction pattern suggested stable ligand–protein complex formation. Comparative analysis revealed that Meth-02 demonstrated stronger binding affinity toward 1NC6 than Meth-01, likely due to its higher hydrogen bond–forming capability. Overall, the docking results suggest that both Meth-02 and Meth-01 can interact effectively with the active site of 1NC6, supporting their potential biological relevance and warranting further experimental validation. Interaction results were shown in Table 5 and Figure 5.

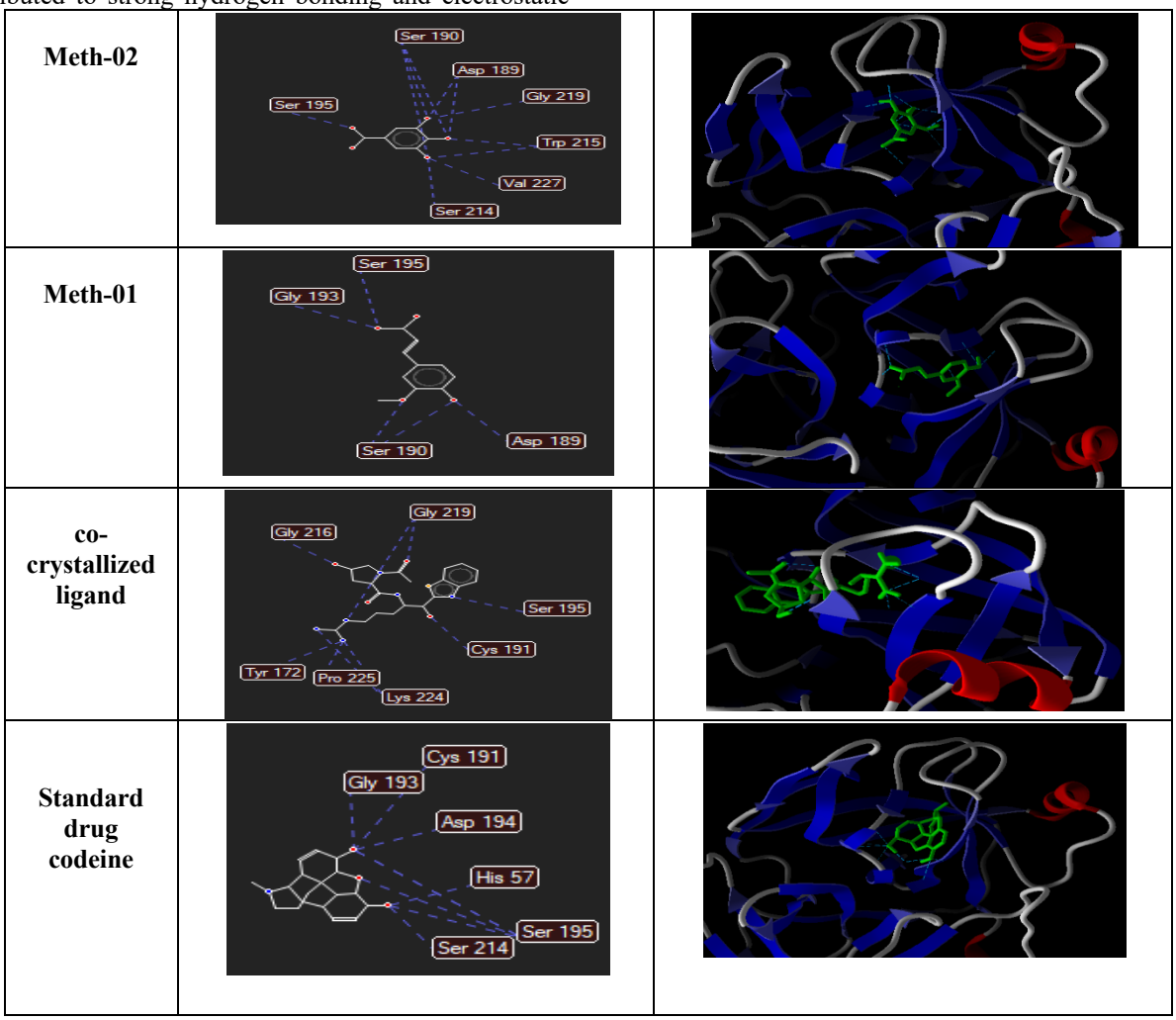


**Figure 5:** Docking pose of Meth-01, Meth-02, co-crystallized ligand and Standard drug codeine

**Table 5:** Result of docking interaction of compounds

| Comp | Mol Dock score | Hydrogen bond Interaction |
|---|---|---|
| Meth-02 | -91.83 | Ser190, Ser195, Asp189, Gly219, Trp215, Val227, Ser214 |
| Meth-01 | 101.17 | Ser190, Ser195, Asp189, Gly193 |
| Co-crystallized ligand | -132.38 | Ser195, Gly216, Gly219, Cys191, Lys224,, pro225, Tyr172 |

| | | |
|---|---|---|
| Codeine | -71.26 | Ser195, Ser214, His57, Asp194, Gly193, Cys191 |

**Evaluation of Antitussive Effect Using Citric Acid–Induced Cough Model**

Table 6 presents the outcomes of citric acid-induced cough in guinea pigs. Compared to the control group, *B. glabra* at doses of 250 mg/kg of methanolic and aqueous extracts demonstrated cough suppressant percentages of 26.83 and 14.5, respectively, in contrast to the conventional medication codeine phosphate at 25 mg/kg, which exhibited a significant percentage of 58.33 (Figure 6). In the ammonia-induced coughing in rats, compared to the control group, animals administered an methanolic and aqueous extract of *B. glabra* at a dosage of 250 mg/kg exhibited cough suppression of 37.5% and 17.5%, respectively, relative to the standard group, while animals receiving the standard treatment demonstrated a highest degree of cough suppression about 58.5%. (Figure 7)

**Table 6:** The effect the plant *B. glabra* on cough induced by citric acidand ammonia

| **TestGroups** | **Frequency of Cough bouts Before drug administration** | **Frequency of Cough bouts after drug administration** | **Cough Suppression percentage** |
|---|---|---|---|
| **The effect the plant *B. glabra* on cough induced by citric acid in guinea pigs** | | | |
| Gr-I ( Toxiccontrol ) | 14.5± 1.33 | 25.63±1.49 | -76.75±7.29 |
| Gr-II(Standard) | 13.66±1.33** | 6.0±1.29*** | 58.33±5.73 |
| Gr-III (Eth250mg/kg) | 14.16±2.19* | 10.5±2.09* | 26.83±3.97 |
| Gr-IV (Aq 250mg/kg) | 14.5±1.66*** | 11.56±1.52** | 14.5±1.75 |
| **The effect the plant *B. glabra*on cough induced by Ammonia in mice** | | | |
| Gr-I ( Toxiccontrol ) | 72.66± 5.45 | 16.16±1.49 | -10.94±1.39 |
| Gr-III(Standard) | 19.5±3.52** | 8.33±1.97*** | 58.5±6.03** |
| Gr-IV (Eth250mg/kg) | 27.16±5.0* | 17.16±3.70* | 37.5±4.10 |
| Gr-IV (Aq 250mg/kg) | 33.33±5.11*** | 28.16±5.29** | 17.5±3.73** |

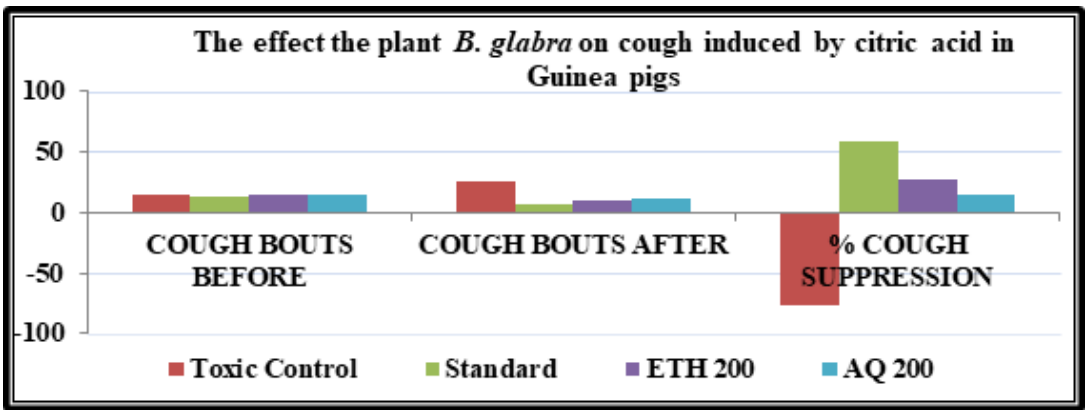


**Figure 6:** Histogram of cough suppressant potential of the plant against citric acid induced cough

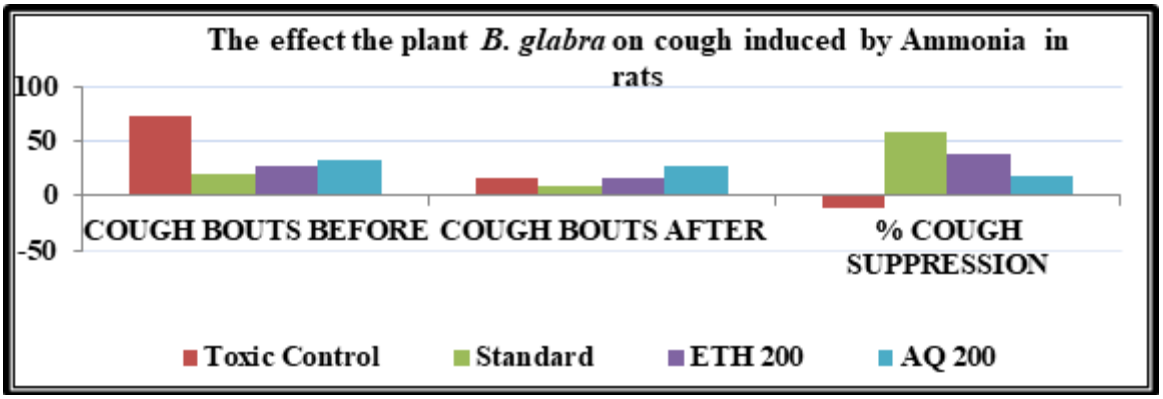


**Figure 7:** Histogram of cough suppressant potential of the plant against Ammonia induced cough in mice

## DISCUSSION

Traditional medicine has long made use of *B. glabra* for the relief of a variety of respiratory issues, including coughs, asthma, inflammation, discomfort, and other similar conditions. Methanolic extract from *B. glabra* plants decreased the frequency of cough episodes in

guinea pigs when subjected to citric acid-induced coughing. Citric acid is a well-studied tussigenic drug because it raises transient receptor potential on C fibers when inhaled, leading to the release of tachykinnins that mediate broncho-constriction and mucus secretion. There is an increase in the percentage of cough suppression with all of the plant extracts, especially the methanolic extract, and with codeine phosphate, the standard medicine comparable to the standard medicine codeine phosphate, the extracts had an anti-tussive effect by increasing the percentage of cough suppression and reducing the number of cough bouts.

Both the conventional medication Codeine Phosphate and extracts of *B. glabra* plants decreased the frequency of cough bouts in a mouse model of ammonia-induced coughing. All doses, both plant extracts, but especially the methanolic extract, reduced the frequency of cough episodes and increased the percentage of suppression. One member of the morphine family of opioids is codeine phosphate. The way it inhibits the cough reflex is by binding to the μ-receptors in the central nervous system, which in turn affects the cough center in the medulla directly. This is how it demonstrates its anti-tussive properties. Historically, *B. glabra* preparations have been used to treat coughs because to their expectorant and anti-tussive properties. The pharmacological and therapeutic actions of plants are due to their secondary metabolites, which include alkaloids, flavonoids, tannins, triterpenoids, sterols, quinins, and phenols. These metabolites are present in both plant extracts. Antioxidant capabilities are also exhibited by these secondary metabolites. Free radicals, produced by oxidative stress, are a known carcinogen and a cause of age-related, cardiovascular, cancerous, and neurological illnesses. Antioxidants help scavenge and mop up these harmful particles. When it comes to coughing, alkaloids are said to have strong anti-tussive and expectorant properties. One of the major components of a cough is inflammation. The anti-inflammatory action of flavonoids and triterpenoids contributes to the anti-tussive and expectorant properties of plant extracts.The presence of alkaloids, flavonoids, and triterpenoids in the plants may explain how the extracts work. We infer that the substantial anti-tussive properties of the plant's methanolic extract are due to its active phytochemicals, in contrast to the negligible impact of the water-based extract, which is devoid of these substances. This study lends credence to the ethno-medical practice of using the *B.glabra* herb to alleviate coughing and pain. Our results suggest that the cough suppressant properties of *B. glabra* in our study are caused by the active phytochemicals found in the plant's methanolic extract. These phytochemicals could be a single molecule or a complex combination of molecules. To move forward in this field of research, we must identify the specific *B. glabra* phytochemicals that may have cough suppressant properties.

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**CONFLICT OF INTEREST**
No conflicts of interest entire research has been reported by any of the Writers.

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**ETHICAL APPROVAL:**
Animal testing at BN University, Udaipur, followed protocols authorized by the CCSEA. The experimental techniques have been accepted by the appropriate university's IAEC. The acceptable protocol proposal number is 26/BNCP/IAEC/2025.

**ABBREVIATION USED:**
*B. glabra*: *Bougainvillea glabra,* TNF: tumor necrosis Meth-01ctor,IAEC: Institutional Animal Ethical Committee, CCSEA: committee for the control and supervision on experimental animals.

**Author Contribution**
Entire plan of work was developed by the corresponding author Dr. Prabhat Kumar Das. Dr. Nitin Deshmukh and Mrs. Mohini Patidar performed all Spectrophotometric analysis and docking study, while Dr. Prabhat Das performed the development of pharmacological tests for anti-tussive activity. The final version of the manuscript was drafted by Dr. Prabhat Kumar Das with the help of all the authors. The final version of the manuscript was authorized after all writers had seen it.

**CONCLUSION**
This pharmacological investigation compared the cough suppression effects of citric acid and ammonia-derived coughing agents with those of methanolic and water-based *B. glabra* plant leaf extracts. This investigation found that both *B. glabra* plant extracts had expectorant and anti-tussive properties. In comparison to the water-based extract, the methanolic *B. glabra* extract significantly reduced coughing, according to our results. Effectiveness was moderate in the water-based extract and competent, strong, and comparable in the methanolic extract of the plant at 250 mg/kg. More research is required to confirm alternative likely mechanisms of action by which these plants exert their benefits; however it is important to investigate the plant's bioactive components first.

Research into *B. glabra* and its protective mechanism has to be thorough and multi-Meth-01ceted at the molecular level to fill in the gaps in our current knowledge. These findings support the concept that *B. glabra* could be a helpful anti-tussive plant species. Antioxidant and free radical scavenging capacities, toxicology, and anti-tussive studies in various chronic models are potential areas for further research.

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