Research Article

In Vitro Anti-Inflammatory and Antimicrobial Profiling of *Ipomoea Obscura* Linn. Extracts

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ABSTRACT

Background: *Ipomoea obscura* Linn. has long been used as a medicinal plant for various therapeutic applications, and its phytochemical components may underlie its antioxidant, anti-inflammatory, and antimicrobial effects.
Objective: The objective of the present study was to assess the phytochemical composition and the in vitro antioxidant, anti-inflammatory, and antimicrobial properties of the methanolic aerial stem extract of *Ipomoea obscura*.
Materials and Methods: The aerial stem parts of I. obscura were collected, verified, shade-dried, and extracted with methanol. A preliminary phytochemical screening was carried out to identify the major phytoconstituents, and the antioxidant activity was tested with the DPPH assay; the anti-inflammatory activity was determined by the protein denaturation inhibition method, and the antibacterial activity against Escherichia coli and Staphylococcus aureus was assessed using the disc diffusion method.
Results: The methanolic extract contained phenolics, flavonoids, alkaloids, tannins, and other phytoconstituents. The flavonoid content was 42.1 ± 2.0 mg QE/g. It showed good DPPH free radical-scavenging activity and dose-dependent anti-inflammatory activity, with maximum inhibition of 71.4% at 400 µg/mL. Against E. coli and S. aureus, the extract showed dose-dependent antibacterial activity, with maximum inhibition at 100 µg/mL, although it was less than that of amoxicillin.
Conclusion: The aerial stem extract of I. obscura in methanol showed promising antioxidant, anti-inflammatory, and antibacterial activities, indicating its potential as a source of bioactive phytoconstituents. However, further research is needed to isolate the active compounds and verify their pharmacological effects.

Keywords: *Ipomoea obscura*, anti-inflammatory activity, antioxidant, phytochemicals, anti-microbial activity.

1. Introduction

For centuries, medicinal plants have been an important part of traditional health care systems and remain valuable sources of bioactive compounds in modern drug discovery. Their therapeutic value stems from a wide variety of secondary metabolites, such as flavonoids, phenolic compounds, alkaloids, tannins, terpenoids, steroids, glycosides, and saponins[1,2]. These components are linked to a broad range of pharmacological actions, including antioxidant, anti-inflammatory, antimicrobial, analgesic, antidiabetic, hepatoprotective, and cytoprotective effects[3,4]. As a result, medicinal plants remain central to research on identifying and developing new pharmacologically active agents[5,6].

Inflammation is a complex defensive reaction to infection, tissue damage, exposure to toxic agents, or other unfavorable stimuli. Although acute inflammation is usually advantageous and helps repair tissue, ongoing or improperly regulated inflammation can contribute to the development and progression of many chronic diseases. The inflammatory response consists of a sequence of cellular and biochemical events which are controlled by various inflammatory mediators and signaling pathways7,8. As a result, a main aim of pharmacological research is to find drugs that are both safe and effective in modifying inflammatory processes.

Protein denaturation is linked to inflammatory conditions and is often used as the first indicator of anti-inflammatory activity in in vitro tests[9]. During inflammation, structural changes or protein denaturation may contribute to the development of autoantigens and subsequent inflammatory reactions[10]. Therefore, inhibiting protein denaturation is a simple and cost-effective way to test the possible anti-inflammatory activity of plant extracts[11].

Ipomoea obscura (L.) Ker Gawl., which is commonly called obscure morning glory and belongs to the Convolvulaceae family, is a plant that has medicinal importance and is found in tropical and subtropical areas. It has traditionally been used to treat several conditions, such as gastrointestinal disorders, ulcers, wounds, respiratory problems, and inflammatory diseases[12,13.] Since these traditional uses have been made, scientific

studies have been carried out on its phytochemical composition and pharmacological properties.
Analysis of the phytochemical composition of I. obscura has shown that it contains a variety of secondary metabolites, such as flavonoids, phenolic compounds, alkaloids, steroids, glycosides, and other phytoconstituents[12,14]. Alkaloids specific to the species, including those related to ipobscurine and calystegine-type alkaloids, have also been found. This chemical diversity likely accounts for the range of biological activities associated with the plant[15,16].

Further pharmacogenetic and phytochemical studies have shown that I. obscura leaves contain flavonoids and phytosterols. Of the flavonoids identified, kaempferol and quercetin are especially important because of their known antioxidant and anti-inflammatory properties. The presence of these bioactive compounds provides a reasonable phytochemical explanation for the species' pharmacological potential[15,16].
Although evidence supports the pharmacological potential of I. obscura, little is known about the biological activity of its aerial stem extract. Therefore, it seems reasonable to conduct a systematic assessment of this plant part to clarify its phytochemical constituents and biological properties further. Investigating the aerial stem is especially relevant because differences in metabolite distribution among tissues may affect the extract's overall pharmacological activity.

The present study was intended to examine the phytochemical composition and some of the biological activities of the methanolic aerial stem extract of I. obscura. It involved evaluating antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay, assessing anti-inflammatory activity through the protein denaturation inhibition assay, and determining antibacterial activity against Escherichia coli and Staphylococcus aureus using the disc diffusion method. The aim was to provide initial scientific evidence of the pharmacological potential of the methanolic aerial stem extract and to provide a basis for further phytochemical and pharmacological research on I. obscura.

2. Materials and Methods

2.1 Collection of Plant Material

Fresh aerial stem parts of *Ipomoea obscura* Linn. were collected from Odisha, India, in May. The plant material was collected carefully in clean condition and transported to the laboratory for further processing.

2.2 Preparation of Plant Material

The collected aerial stem material was carefully inspected and cleaned of unwanted plant material and adhering soil particles. It was washed with clean water and subsequently shade-dried at room temperature. Direct exposure to intense sunlight was avoided to minimize possible degradation of thermolabile and photosensitive phytoconstituents. After complete drying, the material was converted into a coarse powder using a mechanical grinder. The powdered material was stored in an airtight container until extraction.

2.3 Preparation of Methanolic Extract[17]

The powdered aerial stem material was extracted with methanol. The 100 g of powdered plant material was placed in a Soxhlet apparatus and extracted with methanol. The extraction process continued for 8–12 hours until the solvent in the siphon tube turned colorless. After completion of extraction, the extract was filtered through suitable filter paper to remove insoluble plant debris.
The filtrate was concentrated using a rotary evaporator by reducing the pressure and controlling the temperature. The concentrated extract thus obtained was dried to yield a semisolid/crude extract, after which it was placed in a container that was tightly closed and kept until further analysis.

2.4 Preliminary Phytochemical Screening[17]

The methanolic extract was subjected to standard qualitative phytochemical tests to identify major groups of secondary metabolites. The extract was screened for: Alkaloids, Flavonoids, Phenolic compounds, Tannins, Saponins, Terpenoids, Steroids, Glycosides, and Proteins. The results were recorded based on characteristic colour changes, precipitates, frothing, or other positive reactions.

2.5 Quantitative Phytochemical Analysis of *Ipomoea obscura* Linn.

Evaluation of Total Phenolic Content[17,18]

The Folin–Ciocalteu colorimetric method was used to determine the total phenolic content (TPC) of the methanolic aerial stem extract of *Ipomoea obscura* Linn. In this procedure, 0.5 mL of the extract was combined with 3 mL of Folin–Ciocalteu reagent (which had been diluted 1:10, v/v), and the mixture was left to react for 5 minutes. Then, 4 mL of 20% (w/v) sodium carbonate (Na_2CO_3) solution was added, and the reaction mixture was incubated at 30°C for 15 minutes to promote colour development.
The absorbance of the resulting blue solution was measured at 765 nm with a UV–visible spectrophotometer. Gallic acid was used as the reference standard, and a calibration curve was constructed using different gallic

acid concentrations in methanol. The total phenolic content was calculated from this calibration curve and is given as milligrams of gallic acid equivalents per 100 mg of dried extract (mg GAE/100 mg dry extract).

Evaluation of Total Flavonoid Content

The aluminium chloride ($AlCl_3$) colorimetric method was used to determine the total flavonoid content (TFC) the methanolic aerial stem extract of *Ipomoea obscura* Linn. To this end, 1.7 mL of methanol, 0.1 mL of 10% aluminium chloride solution, 0.1 mL of 1 M sodium acetate solution, and 3.0 mL of distilled water were added to a known amount of the extract. The mixture was then thoroughly mixed and left to stand at room temperature for 30 minutes to allow the flavonoid–aluminium complex to form. The absorbance was then measured at 415 nm with a UV–visible spectrophotometer. Quercetin was used as the reference standard, and a calibration curve was prepared using various quercetin concentrations in methanol. The total flavonoid content was calculated from the standard calibration curve and is given as milligrams of quercetin equivalents per 100 mg of dried extract (mg QE/100 mg dry extract)[19,20].

Determination of Total Tannin Content

The Folin–Ciocalteu colourimetric method was used, with a few modifications, to determine the total tannin content (TTC) of the methanolic aerial stem extract of *Ipomoea obscura* Linn., tannic acid serving as the reference standard and various concentrations of tannic acid being prepared in distilled water to produce a standard calibration curve.

In summary, 0.5 mL of the extract was combined with 2.5 mL of Folin–Ciocalteu reagent (which had been diluted 1:10, v/v) and left to react for 5 minutes. Then, 2.0 mL of 7.5% (w/v) sodium carbonate solution was added, after which the reaction mixture was mixed thoroughly and incubated at room temperature for 30 minutes to allow the colour to develop. The absorbance was determined at 725 nm with a UV–visible spectrophotometer, using an appropriate reagent blank as a reference. The total tannin content was calculated from the tannic acid calibration curve and is given as milligrams of tannic acid equivalents per 100 mg of dried extract (mg TAE/100 mg dry extract). All measurements were carried out in triplicate, and results are reported as the mean ± standard deviation (SD).

2.6 In Vitro Antioxidant Activity

DPPH Radical-Scavenging Assay

The antioxidant property of the methanolic extract obtained from the aerial parts of *Ipomoea obscura* Linn. Was investigated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay[21,22]. DPPH is a stable free radical that has a characteristic violet colour. When it reacts
with antioxidant substances that can donate hydrogen atoms or electrons, the radical is reduced, causing the colour intensity to drop. The extent of the decrease in absorbance is then used to indicate the extract's radical-scavenging activity[23,24].

Aerial stem extracts at concentrations of 5, 10, 25, 50, 100, and 200 µg/mL were combined with a DPPH solution, and the resulting mixtures were left in the dark under the given experimental conditions. Absorbance was determined spectrophotometrically at the relevant wavelength using an appropriate blank.

$$\textbf{DPPH radical-scavenging activity (\%)} = [(A_o - A_1) / A_o] \times 100$$

Where:

- **A_o** = Absorbance of the control
- **A_1** = Absorbance of the sample

The IC_{50} value—which is the concentration of the extract required to inhibit 50% of the DPPH radicals—was obtained from the concentration–response curve, the lower the IC_{50} value the greater the radical-scavenging activity.

2.7 In Vitro Anti-inflammatory Activity

Protein Denaturation Inhibition Assay

The anti-inflammatory activity of the methanolic aerial stem extract of *Ipomoea obscura* Linn. was tested using the protein denaturation inhibition assay. Since protein denaturation is linked to inflammatory conditions, inhibiting this denaturation may indicate possible anti-inflammatory activity[26,27,28].

To test their ability to inhibit protein denaturation, solutions of the methanolic aerial stem extract at concentrations of 50, 100, 200, and 400 µg/mL were prepared and examined. The respective extract concentrations were added to the mixture containing the protein substrate, and the system was incubated under the specified experimental conditions. After incubation and heating, the reaction mixtures were cooled to room temperature, and the absorbance was determined with a UV–visible spectrophotometer. Diclofenac sodium was used as the standard anti-inflammatory drug for comparison.

The percentage inhibition of protein denaturation was calculated using the following equation:

$$\textbf{Inhibition (\%)} = [(A_o - A_1) / A_o] \times 100$$

Where:

- **Ao** = Absorbance of the control
- **A_1** = Absorbance of the test sample

2.8 Antibacterial Activity

Test Microorganisms

The antibacterial activity of the methanolic extract was evaluated against:

1. Escherichia coli
2. Staphylococcus aureus

These organisms were selected to represent Gram-negative and Gram-positive bacteria, respectively.

Disc Diffusion Method

The disc diffusion method was used to evaluate antibacterial activity. Fresh bacterial cultures were prepared and adjusted to an appropriate turbidity. Sterile agar plates were inoculated uniformly with the respective bacterial cultures. Sterile paper discs impregnated with different concentrations of the methanolic extract were placed on the inoculated agar surface[29,30].

The concentrations tested were:

- 60 µg/mL
- 80 µg/mL
- 100 µg/mL

Amoxicillin was used as the standard antibacterial drug.

The plates were incubated under suitable microbiological conditions. After incubation, the diameter of the zone of inhibition around each disc was measured in millimetres. A larger inhibition zone indicated greater antibacterial activity.

3. Result

3.1 Extraction yield

The percentage yield was calculated using:

Percentage yield = (Weight of dried extract / Weight of powdered plant material) × 100

Extraction yield: Crude methanolic extract of aerial stems: 7.43 gm

Table 1. Extraction yield of *Ipomoea obscura* Extract

Solvent/Extract	Weight of powdered material (g)	Weight of crude extract (g)	Yield (% w/w)
Methanol (IOME)	100	7.43	7.43

3.2 Preliminary Phytochemical Screening

Table 2. Preliminary phytochemical screening of *I. obscura* Extract

Phytochemical	*Extract*	*Observation*
Alkaloids *Mayer's test* *Wagner's test* *Dragendorff's Test*	 + + +	 *Orange- brown precipitate* *Characteristic precipitate* *Orange-brown precipitate*
Phenols & Tannins (Ferric chloride)	++	*Blue-black color*
Flavonoids (Shinoda test)	++	*Red coloration*
Saponins (Foam test)	++	*Persistent froth formation*

Terpenoids (Salkowski test)	+	*Reddish-brown interface*
Glycosides (Keller–Killiani)	+	*Brown ring at interface*
Steroids	–	*No color change*
Proteins & Amino acids	+	*Violet coloration (Biuret test)*

3.3 Quantitative Phytochemical Analysis

The quantitative phytochemical analysis of the methanolic aerial stem extract of *Ipomoea obscura* Linn. revealed appreciable amounts of phenolic compounds, flavonoids, and tannins. The total phenolic content was 18.6 ± 0.7 mg GAE/100 mg dry extract, and the total flavonoid content was 12.4 ± 0.5 mg QE/100 mg dry extract. The total tannin content was 6.8 ± 0.3 mg TAE/100 mg dry extract. Among the estimated phytochemical constituents, phenolic compounds showed the highest content, followed by flavonoids and tannins. These phytoconstituents may contribute to the biological activities observed in the antioxidant, anti-inflammatory, and antibacterial assays.

Table 3. Quantitative Phytochemical Content of the Methanolic Aerial Stem Extract of *Ipomoea obscura* Linn.

Phytochemical Parameter	Result (Mean ± SD)
Total Phenolic Content (TPC)	18.6 ± 0.7 mg GAE/100 mg dry extract
Total Flavonoid Content (TFC)	12.4 ± 0.5 mg QE/100 mg dry extract
Total Tannin Content (TTC)	6.8 ± 0.3 mg TAE/100 mg dry extract

Values are expressed as mean ± SD (n = 3).

Abbreviations: GAE, gallic acid equivalents; QE, quercetin equivalents; TAE, tannic acid equivalents; SD, standard deviation.

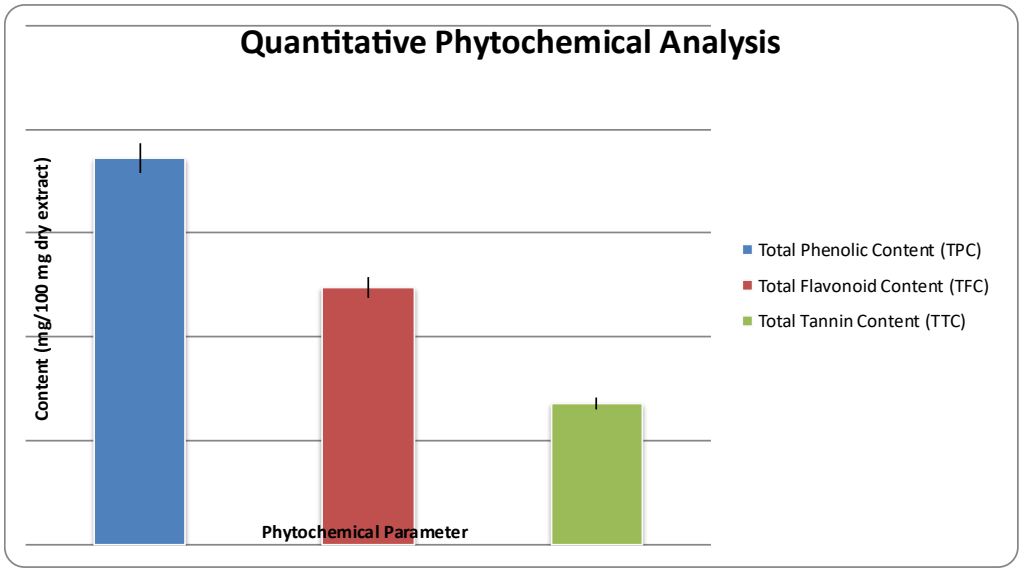


Figure 1: Concentration of Quantitative Phytochemical

3.4 Results of DPPH Radical-Scavenging Activity

The aerial stem extract of *Ipomoea obscura* Linn. showed concentration-dependent DPPH radical-scavenging activity. As the extract concentration increased, the percentage inhibition rose steadily, from 15.4% at 5 µg/mL to 89.0% at 200 µg/mL. At 25 µg/mL, the extract gave 38.8% inhibition, and at 50 µg/mL, inhibition was greater than 50% (55.2%). This shows that the extract has considerable free-radical-scavenging activity.
The increase in DPPH radical-scavenging activity with increasing concentration may be due to the phenolic and flavonoid components detected in the phytochemical screening. These compounds may exert their antioxidant effect by donating hydrogen atoms or electrons and stabilising free radicals.

Table 4. DPPH Radical-Scavenging Activity of the Methanolic Aerial Stem Extract of *Ipomoea obscura* Linn.

Concentration (µg/mL)	Methanolic Extract (% Inhibition)
5	15.4
10	23.7
25	38.8
50	55.2
100	71.3
200	89.0

Table 5. IC5o Value of the Methanolic Aerial Stem Extract in the DPPH Assay

Assay	Sample	IC5o (µg/mL)
DPPH radical-scavenging assay	Methanolic aerial stem extract	Approximately 42.1 µg/mL*

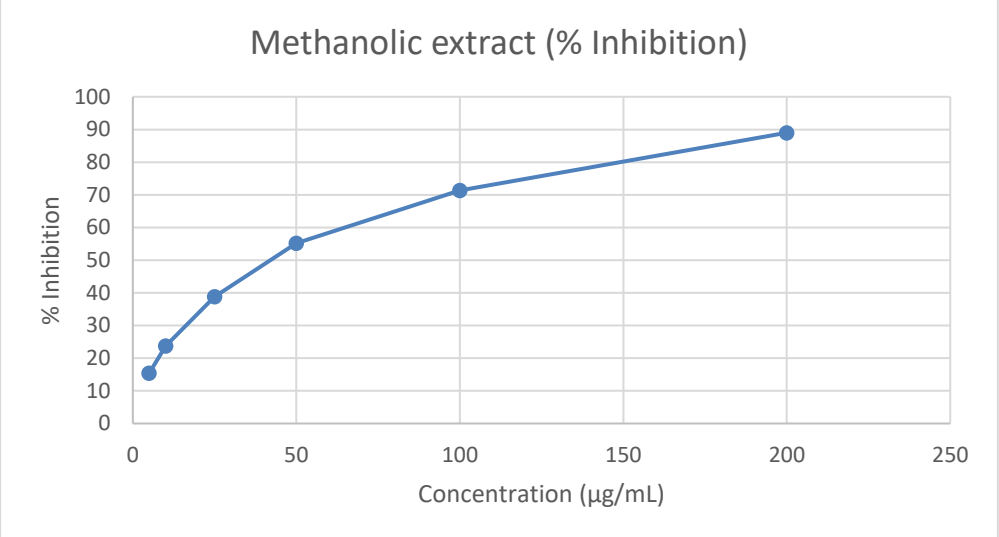


Figure 2: DPPH Radical scavenging activity of *ipomoea obscura* Methanolic extracts

3.5 Anti-inflammatory activity (Protein Denaturation)

The methanolic aerial stem extract of *Ipomoea obscura* Linn. showed a concentration-dependent ability to inhibit protein denaturation, with inhibition rising from 28.6 ± 1.4% at 50 µg/mL to 71.4 ± 2.3% at 400 µg/mL. Diclofenac sodium showed a higher degree of inhibition at each concentration tested, increasing from 45.1 ± 1.1% at 50 µg/mL to 92.3 ± 1.9% at 400 µg/mL. Using linear interpolation of the concentration–response data, the estimated IC_{50} values for the methanolic extract and diclofenac sodium were approximately 156.6 µg/mL and 78.3 µg/mL, respectively. The lower IC_{50} value for diclofenac sodium indicates that it is more potent than the methanolic extract under the experimental conditions.

The methanolic aerial stem extract showed a considerable inhibition of protein denaturation which was dependent on concentration, the maximum inhibition being found at a concentration of 400 µg/mL (71.4 ± 2.3%). This activity may be linked to phenolic compounds, flavonoids, tannins, and other secondary metabolites in the extract. Nevertheless, further research using additional in vitro anti-inflammatory assays, bioactivity-guided fractionation, and mechanistic studies is needed to verify the anti-inflammatory potential and identify the components responsible for the observed effect.

Table 6: Effect of Methanolic Aerial Stem Extract of *Ipomoea obscura* Linn. on Protein Denaturation

Concentration (µg/mL)	Methanolic Extract (% Inhibition)	Diclofenac Sodium (% Inhibition)
50	28.6 ± 1.4	45.1 ± 1.1
100	42.3 ± 1.7	62.4 ± 1.4
200	55.9 ± 1.9	78.6 ± 1.7
400	71.4 ± 2.3	92.3 ± 1.9
Estimated IC_{50} (µg/mL)	156.6	78.3

Values are expressed as mean ± SD (n = 3). IC_{50} values were estimated from the concentration–response data by linear interpolation.

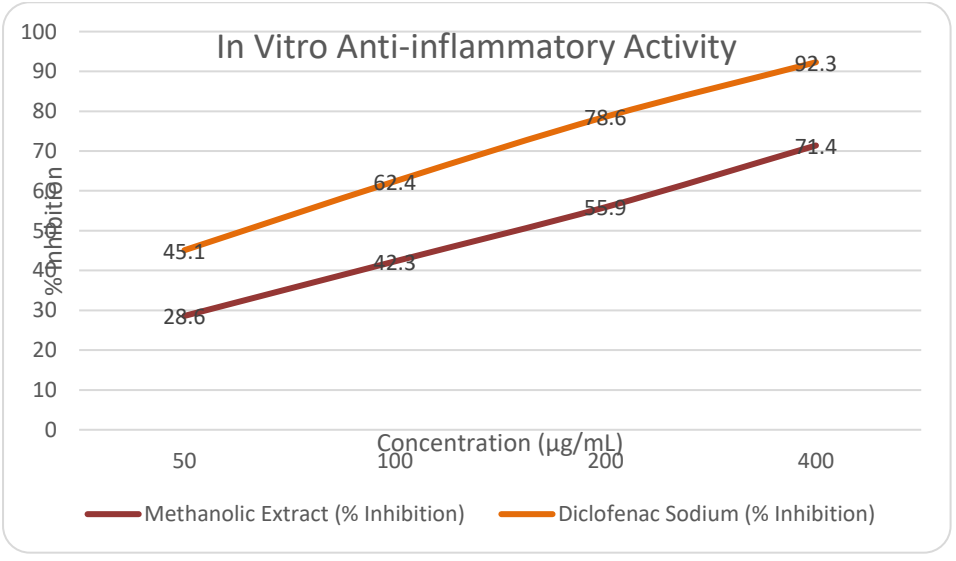


Figure 3: Anti-inflammatory activity comparison graph

3.6. Antibacterial Activity

The methanolic extract demonstrated antibacterial activity against both E. coli and S. aureus. The antibacterial activity increased with increasing concentration from 60 to 100 µg/mL. The maximum inhibition was observed at 100 µg/mL.

The extract showed activity against both Gram-positive and Gram-negative bacteria, suggesting that its phytochemical constituents may possess broad antibacterial potential. However, the antibacterial activity of the crude extract was lower than that of the standard antibiotic amoxicillin.

Table 7: Antibacterial Activity Zone of Inhibition (mm)

Sl No	Organism	Methanolic Extract (Zone of Inhibition in mm)			Amoxicillin (5 µg/disc) (mm)
		60 µg/mL	80 µg/mL	100 µg/mL	
1	Escherichia coli	10.2 ± 0.5	13.6 ± 0.7	17.1 ± 0.6	24.5 ± 0.8
2	Staphylococcus aureus	11.4 ± 0.6	14.8 ± 0.5	18.3 ± 0.7	26.2 ± 0.9

Values should be expressed as mean ± SD of zone of inhibition in mm.

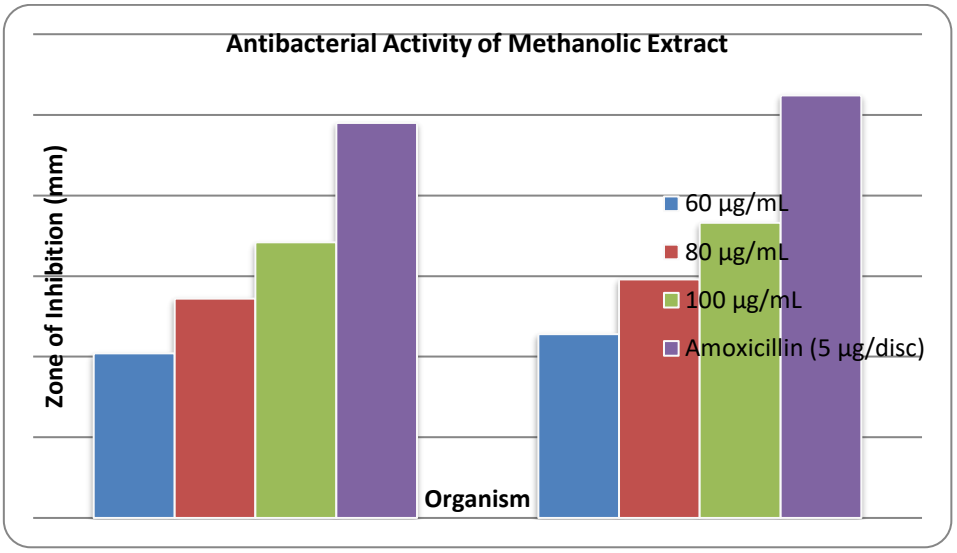


Figure 4: Antibacterial Activity of methanolic extract

4. Discussion

The present study investigated the in vitro antioxidant, anti-inflammatory, and antibacterial potential of the methanolic aerial stem extract of *Ipomoea obscura* Linn., along with its preliminary and quantitative phytochemical characteristics. The methanolic extract yielded 7.43% w/w, indicating that methanol extracted a measurable proportion of constituents from the powdered aerial stem material. Preliminary phytochemical screening revealed alkaloids, phenolic compounds, flavonoids, tannins, saponins, terpenoids, glycosides, and proteins/amino acids, whereas steroids were not detected. These diverse secondary metabolites may provide a phytochemical basis for the biological activities observed in the present study. Quantitative phytochemical analysis further demonstrated the presence of phenolic, flavonoid, and tannin constituents in the methanolic extract. The total phenolic content was 18.6 ± 0.7 mg GAE/100 mg dry extract, while the total flavonoid and tannin contents were 12.4 ± 0.5 mg QE/100 mg and 6.8 ± 0.3 mg TAE/100 mg dry extract, respectively. Phenolic and flavonoid compounds are widely recognized for their ability to donate hydrogen atoms or electrons and may therefore contribute to the antioxidant properties of plant extracts. The quantitative findings are consistent with the antioxidant activity observed in the DPPH assay; however, a direct causal relationship cannot be established from the present data alone.

The methanolic extract demonstrated a concentration-dependent DPPH radical-scavenging activity, with inhibition increasing from 15.4% at 5 µg/mL to 89.0% at 200 µg/mL. The progressive increase in radical-scavenging activity with increasing concentration indicates that the extract contains constituents that interact with and neutralize DPPH radicals. The higher activity at higher concentrations may be associated, at least in part, with the phenolic and flavonoid constituents detected in the extract. These findings suggest that the aerial stem of I. obscura may serve as a potential source of naturally occurring antioxidant constituents. Nevertheless, further studies involving individual compound isolation and antioxidant mechanism-based assays are needed to establish the contribution of specific phytochemicals.

The extract also exhibited concentration-dependent inhibition of protein denaturation, indicating potential in vitro anti-inflammatory activity. Protein denaturation increased progressively from 28.6 ± 1.4% inhibition at 50 µg/mL to 71.4 ± 2.3% at 400 µg/mL. Diclofenac sodium produced higher inhibition at all tested concentrations, reaching 92.3 ± 1.9% at 400 µg/mL. The estimated IC_{50} values were approximately 156.6 µg/mL for the methanolic extract and 78.3 µg/mL for diclofenac sodium, indicating greater potency of the standard drug under the experimental conditions. The extract's activity may be associated with its phenolic, flavonoid, tannin, and other secondary metabolites. However, protein denaturation inhibition represents an initial screening model, and additional anti-inflammatory assays are necessary to confirm the activity and clarify its mechanism.

The methanolic extract further showed concentration-dependent antibacterial activity against both Escherichia coli and Staphylococcus aureus. As the extract concentration increased from 60 to 100 µg/mL, inhibition zones increased from 10.2 ± 0.5 to 17.1 ± 0.6 mm against E. coli and from 11.4 ± 0.6 to 18.3 ± 0.7 mm against S. aureus. Although the extract showed activity against both Gram-negative and Gram-positive organisms, it was less active than amoxicillin. The antibacterial effect may be related to the combined presence of phenolics, flavonoids, tannins, alkaloids, and other phytoconstituents that may interfere with microbial growth and cellular functions. However, the present findings demonstrate activity only under the tested conditions and do not establish a specific antimicrobial mechanism.

Taken together, the findings indicate that the methanolic aerial stem extract of *Ipomoea obscura* possesses promising in vitro antioxidant, protein-denaturation inhibitory, and antibacterial activities, which are broadly consistent with its phytochemical composition. The concentration-dependent responses across the assays strengthen evidence for the crude extract's biological activity. However, the activities should not be interpreted as evidence of therapeutic efficacy because the study was limited to preliminary in vitro models. Further research involving bioactivity-guided fractionation, chromatographic and spectroscopic characterization of active constituents, additional mechanistic assays, toxicity evaluation, and in vivo studies is necessary to validate the pharmacological potential of I. obscura aerial stem extract.

5. Conclusion

The present study demonstrated that the methanolic aerial stem extract of *Ipomoea obscura* Linn. contains several important secondary metabolites, including alkaloids, phenolic compounds, flavonoids, tannins, saponins, terpenoids, glycosides, and proteins/amino acids. Quantitative phytochemical analysis confirmed the presence of phenolic, flavonoid, and tannin constituents, which may contribute to the extract's biological activities. The methanolic extract exhibited concentration-dependent DPPH radical-scavenging activity, with maximum inhibition of 89.0% at 200 µg/mL. It also demonstrated concentration-dependent inhibition of protein denaturation, reaching 71.4 ± 2.3% at 400 µg/mL, although the activity was lower than that of diclofenac sodium. Furthermore, the extract showed antibacterial activity against both Escherichia coli and Staphylococcus aureus, with the highest inhibition observed at 100 µg/mL.

Overall, the findings suggest that the aerial stem of I. obscura possesses promising in vitro antioxidant, anti-inflammatory, and antibacterial potential, which may be associated with its diverse phytochemical constituents. However, these findings are preliminary and cannot be directly extrapolated to therapeutic efficacy. Further studies involving isolation and characterization of bioactive compounds, mechanistic investigations, toxicity evaluation, and in vivo validation are required to establish the pharmacological potential of I. obscura and identify the constituents responsible for the observed activities.

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7. Conflict of Interest
The authors declare that there is no conflict of interest associated with the present study.

8. Funding
The authors declare that no specific external funding was received for the present study.

9. Ethical Statement
The present investigation was conducted using in vitro experimental methods and did not involve experimental animals or human participants. Therefore, animal or human ethical approval was not required.

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