EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 12
Issue: 4
Year: 2025

# PHARMACOLOGICAL POTENTIAL OF IPOMOEA OBSCURA EXTRACTS: IN VITRO EVALUATION OF ANTIOXIDANT, ANTI-INFLAMMATORY, ANTIBACTERIAL, AMYLASE INHIBITOR AND ANTICANCER ACTIVITIES

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**Article Received on 13/02/2025** **Article Revised on 03/03/2025** **Article Accepted on 23/03/2025**

## ABSTRACT
*Ipomoea obscura*, a plant with traditional medicinal significance, was investigated for its in vitro pharmacological potential through methanol extracts (IO ME) and derived compounds. This study evaluated antioxidant, anti-inflammatory, antibacterial, alpha-amylase inhibitory, and anticancer activities using standardized assays. Antioxidant activity was assessed via DPPH radical scavenging, yielding 64.82% inhibition for IO ME and 74.07% for Compound A, compared to ascorbic acid's 85.18%. Anti-inflammatory effects, measured by albumin denaturation inhibition, were modest, with IO ME at 3% and Compound A at 10%, far below diclofenac sodium's 85.56%. Antibacterial activity against *Streptococcus pneumoniae* and *Klebsiella pneumoniae* was robust, with IO ME producing zones of inhibition of 22.49–25.66 mm, Compound B at 28.63–25.68 mm, and a repeat of Compound A at 29.97–26.28 mm, rivaling streptomycin (27.07–24.17 mm). Alpha-amylase inhibition ranged from 45.71% (IO C) to 54.28% (IO B) at 500 µL, against acarbose's 81.42%, indicating moderate antidiabetic potential. Anticancer activity against SK-N-SH neuroblastoma cells showed IO ME with an IC50 of 58.73 µg/mL and a CC50 of 128.95 µg/mL against Vero cells, suggesting potent cytotoxicity with low normal cell toxicity. Variability in repeat DPPH assays (IO ME at 56.02%) highlights the need for standardization. These findings demonstrate *Ipomoea obscura*'s multifaceted bioactivity, with significant promise in combating oxidative stress, bacterial infections, hyperglycemia, and cancer, though its anti-inflammatory effects are limited. The strong antibacterial and anticancer profiles, coupled with moderate enzyme inhibition, validate traditional uses and position IO ME as a candidate for further phytochemical and in vivo studies to elucidate mechanisms and enhance therapeutic applications.

**KEYWORDS:** *Ipomoea obscura*, Antioxidant activity, Anti-inflammatory, Antibacterial, Alpha-amylase inhibition, Anticancer, DPPH assay, Methanol extract, Phytochemicals.

## INTRODUCTION
Natural products have long served as a cornerstone of medicinal practices across cultures, offering a rich reservoir of bioactive compounds to address an array of human ailments. Among these, *Ipomoea obscura*, a lesser-studied species within the Convolvulaceae family, has garnered attention for its traditional use in herbal medicine, particularly in regions where it is valued for its purported healing properties. Known for its distinctive heart-shaped leaves and small, obscure flowers, this plant thrives in tropical and subtropical environments, where it has been employed to treat conditions ranging from infections to inflammatory disorders. Despite its ethnobotanical significance, scientific validation of its pharmacological potential remains limited, prompting a deeper investigation into its therapeutic capabilities. This study aims to bridge this gap by systematically evaluating the in vitro antioxidant, anti-inflammatory, antibacterial, alpha-amylase inhibitory, and anticancer activities of methanol extracts (IO ME) and derived compounds from *Ipomoea obscura*, providing a comprehensive assessment of its multifaceted bioactivity.

The global burden of chronic and infectious diseases underscores the urgency of identifying novel therapeutic agents, particularly those derived from natural sources. Oxidative stress, driven by an imbalance between free radicals and antioxidants, is implicated in numerous pathological conditions, including cardiovascular diseases, neurodegenerative disorders, and cancer. Similarly, inflammation, a fundamental immune response, can become deleterious when dysregulated,

contributing to arthritis, diabetes, and other inflammatory states. Microbial infections, exacerbated by the rise of antibiotic resistance, pose a persistent threat to public health, while diabetes—a metabolic disorder affecting millions—requires innovative strategies to manage postprandial hyperglycemia effectively. Cancer, with its complex etiology and devastating impact, remains a leading cause of mortality, necessitating selective and potent anticancer agents. Natural products like *Ipomoea obscura* offer a promising avenue for addressing these challenges, as they often contain a diverse array of secondary metabolites—such as phenols, flavonoids, and alkaloids—that exhibit multi-target effects.

The methanol extract of *Ipomoea obscura* (IO ME) was selected as the primary focus of this study due to methanol's efficacy in extracting polar and semi-polar compounds, which are likely responsible for the plant's bioactivity. Preliminary phytochemical screenings of *Ipomoea* species have identified antioxidants and anti-inflammatory agents, suggesting that IO ME may harbor similar constituents. To quantify its antioxidant potential, we employed the DPPH (1,1-Diphenyl-2-picrylhydrazyl) radical scavenging assay, a widely accepted method for measuring free radical neutralization, with ascorbic acid as the reference standard. This assay reflects the extract's ability to combat oxidative stress, a critical factor in disease prevention. Concurrently, the anti-inflammatory activity was assessed through the inhibition of albumin denaturation, a process linked to inflammatory cascades, using diclofenac sodium as the benchmark. These assays provide insight into IO ME's capacity to mitigate oxidative and inflammatory damage, two interconnected processes underlying many chronic conditions.

Beyond these foundational properties, the antimicrobial efficacy of IO ME and its derived compounds (e.g., IO A, IO B) was tested against clinically relevant pathogens, *Streptococcus pneumoniae* MTCC 39 and *Klebsiella pneumoniae* MTCC 39, using the agar well diffusion method, with streptomycin as the positive control. The emergence of multidrug-resistant bacteria has intensified the search for natural antimicrobials, and *Ipomoea obscura*'s traditional use in wound healing and infection control suggests it may contribute to this effort. In the context of diabetes management, the alpha-amylase inhibitory assay was conducted to determine IO ME's potential to reduce starch hydrolysis and glucose absorption, a key mechanism for controlling postprandial hyperglycemia. Acarbose, a standard enzyme inhibitor, served as the comparator, highlighting the extract's relevance to metabolic health.

Perhaps most compelling is the exploration of IO ME's anticancer activity, assessed against SK-N-SH neuroblastoma cells, with cytotoxicity to normal Vero cells measured to evaluate selectivity. Cancer remains a formidable challenge, and neuroblastoma—a pediatric malignancy—requires targeted therapies with minimal off-target effects. The methanol extract's performance in this assay, expressed as IC50 and CC50 values, offers a glimpse into its chemotherapeutic potential. By comparing IO ME's bioactivity across these diverse assays to established standards, this study establishes a robust framework for understanding its pharmacological spectrum.

The objectives of this research are twofold: first, to validate the traditional claims surrounding *Ipomoea obscura* through rigorous scientific methods, and second, to position it as a candidate for pharmaceutical development. The use of standardized in vitro assays ensures reproducibility and reliability, while the inclusion of multiple bioactivities reflects the plant's potential as a multi-target therapeutic agent. As natural product research continues to gain momentum, driven by the need for sustainable and accessible treatments, *Ipomoea obscura* emerges as a promising contender. This introduction sets the stage for a detailed analysis of its pharmacological properties, laying the groundwork for future in vivo studies and phytochemical characterizations that could unlock its full therapeutic potential.

## MATERIALS AND METHODS

### 2.1. Plant Material and Extract Preparation

The Plant Material of Ipomoea Obscura was Collected from Rural Kerala, authenticated and Processed at the Lab of Dr Joseph MarThoma Institute of Pharmaceutical Sciences and Research, Kattnam, Kerala Fresh aerial parts were washed, air-dried in the shade, and pulverized into a fine powder using a mechanical grinder. The methanol extract (IO ME) was prepared by macerating 500 g of the powdered material in 2 L of methanol (99.8% purity, Merck) for 72 hours at room temperature with occasional stirring. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator (Heidolph) at 40°C. The resulting crude extract (IO ME) was stored at 4°C in an airtight container. Derived compounds (e.g., IO A, IO B) were isolated from IO ME via column chromatography using silica gel (60–120 mesh) and gradient elution with hexane:ethyl acetate mixtures, followed by characterization (details not provided here). Stock solutions of IO ME and compounds were prepared at 1 mg/mL in methanol or distilled water, as appropriate, for subsequent assays.

### 2.2. In Vitro Antioxidant Activity (DPPH Assay)

The antioxidant activity of IO ME and its compounds was evaluated using the DPPH (1,1-Diphenyl-2-picrylhydrazyl) radical scavenging assay, adapted from Brand-Williams et al. (1995). A 0.1% DPPH solution was prepared by dissolving 1 mg of DPPH (Sigma-Aldrich) in 1 mL of methanol. For the assay, 100 µL of IO ME or compound solution (1 mg/mL) was pipetted into a 96-well microtiter plate, followed by the addition of 100 µL of 0.1% methanolic DPPH. The plate was incubated in the dark at 25°C for 30 minutes. Post-

incubation, the discoloration of DPPH from purple to yellow or pale pink was observed, indicating radical scavenging. Absorbance was measured at 490 nm using an ELISA plate reader (Bio-Rad). Ascorbic acid (1 mg/mL, Sigma-Aldrich) served as the positive control, while a mixture of 100 µL methanol and 100 µL DPPH acted as the negative control. All samples were tested in triplicate. The percentage of DPPH radical scavenging activity was calculated using the formula:

$$\%\text{Inhibition} = \left[\frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}}\right] \times 100$$

**2.3. In Vitro Anti-Inflammatory Activity (Albumin Denaturation Assay)**

The anti-inflammatory potential was assessed via inhibition of albumin denaturation, following the method of Padmanabhan and Jangle (2012). A reaction mixture was prepared by combining 0.05 mL of IO ME or compound solution (1 mg/mL in distilled water) with 0.45 mL of 5% bovine serum albumin (BSA, Sigma-Aldrich) in distilled water. The pH was adjusted to 6.3 using 0.1 N HCl (Merck). The mixture was incubated at 37°C for 20 minutes, then heated to 57°C for 30 minutes in a water bath to induce denaturation. After cooling to room temperature, the solution was transferred to a 96-well plate, and absorbance was measured at 660 nm using a microplate reader (Thermo Scientific). Diclofenac sodium (1000 µg/mL, Sigma-Aldrich) was used as the standard, while a mixture containing 0.05 mL distilled water instead of the sample served as the control. Triplicate analyses were performed. The percentage inhibition of albumin denaturation was calculated as:

$$\%\text{Inhibition} = \left[\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}}\right] \times 100$$

**2.4. Antibacterial Activity (Agar Well Diffusion Assay)**

Antibacterial activity was tested against *Streptococcus pneumoniae* MTCC 39 and *Klebsiella pneumoniae* MTCC 39. Bacterial cultures were maintained on nutrient agar (HiMedia) and adjusted to a 0.5 McFarland standard (~$1.5 \times 10^8$ CFU/mL). Petri dishes (10.5 cm diameter) containing Mueller-Hinton agar (HiMedia) were inoculated with 100 µL of bacterial suspension via spread plating. Wells (0.5 cm diameter) were punched using a sterile cork borer, and 50 µL of IO ME or compound solution (10 mg/well) was added. Streptomycin (1 mg/well, Sigma-Aldrich) served as the positive control, and sterile water was the negative control. Plates were incubated at 37°C for 24 hours, and zones of inhibition (mm) were measured using a calibrated ruler. Triplicates were conducted for each sample.

**2.5. Alpha-Amylase Inhibition Assay**

The alpha-amylase inhibitory activity was evaluated following Patil (2018). A 500 µL aliquot of IO ME or compound solution (1 mg/mL) was mixed with 500 µL of 0.1 M phosphate buffer (pH 6.9, Research Lab) containing 0.5% fungal α-amylase (Sigma-Aldrich). After incubation at 25°C for 10 minutes, 500 µL of 1% soluble starch (Loba Chemie) in 0.1 M phosphate buffer (pH 6.8) was added, followed by another 10-minute incubation at 25°C. The reaction was terminated by adding 1000 µL of 3,5-dinitrosalicylic acid (DNS, Loba Chemie), and the mixture was boiled in a water bath for 10 minutes. After cooling, absorbance was recorded at 540 nm using a spectrophotometer (Shimadzu UV-1800). Acarbose (1 mg/mL, Sigma-Aldrich) was the standard, and a control replaced the sample with buffer. Triplicates were analyzed, and inhibition was calculated as:

$$\%\text{Inhibition} = \left[\frac{\text{Absorbance of control} - \text{Absorbance of extract}}{\text{Absorbance of control}}\right] \times 100$$

**2.6. In Vitro Anticancer Activity (MTT Assay)**

Anticancer activity was assessed against SK-N-SH neuroblastoma cells and Vero cells (normal) using the MTT assay. Cells were cultured in DMEM (Gibco) with 10% FBS (Gibco) and seeded in 96-well plates at $5 \times 10^3$ cells/well. After 24 hours, cells were treated with IO ME at concentrations of 62.5, 125, 250, and 500 µg/mL and incubated for 48 hours at 37°C in 5% $CO_2$. Subsequently, 20 µL of MTT (5 mg/mL, Sigma-Aldrich) was added, and plates were incubated for 4 hours. Formazan crystals were dissolved in 100 µL DMSO, and absorbance was measured at 570 nm using a microplate reader. Cell viability (%) was calculated relative to untreated controls, and IC50 (concentration inhibiting 50% of cancer cells) and CC50 (50% cytotoxicity to normal cells) were determined using dose-response curves (GraphPad Prism).

**2.7. Statistical Analysis**

All experiments were performed in triplicate, and results are expressed as mean ± standard deviation where applicable. Percentage inhibition values were calculated

using the aforementioned formulas, and data consistency was verified across repeats.

## RESULTS

### 3.1. In Vitro Antioxidant Activity (DPPH Assay)

The antioxidant potential of *Ipomoea obscura* methanol extract (IO ME) and its compounds was assessed using the DPPH radical scavenging assay. Table 1 summarizes the results from the initial and repeat experiments. In the initial assay, IO ME (1 mg/mL) exhibited an average radical scavenging activity of 64.82%, with individual measurements of 66.67%, 61.11%, and 66.67% (control optical density [COD] = 0.36; sample optical density [SOD] = 0.12–0.14). Compound A showed a higher average of 74.07% (COD = 0.36; SOD = 0.09–0.10), while Compound B averaged 64.82% (COD = 0.36; SOD = 0.10–0.16). The standard, ascorbic acid (1 mg/mL), achieved 85.18% inhibition (COD = 0.36; SOD = 0.04–0.06).

In the repeat assay, IO ME's average scavenging activity decreased to 56.02% across six replicates (COD = 0.30–0.36; SOD = 0.15–0.17), with values ranging from 52.78% to 58.33%. Compound A's activity dropped to 42.59% (COD = 0.30; SOD = 0.20–0.21), while ascorbic acid remained consistent at 85.18%. Additionally, a variant extract, IO (S) ET, showed 62.96% inhibition (COD = 0.36; SOD = 0.13–0.14). These results indicate that IO ME and Compound A possess notable antioxidant activity, though less potent than ascorbic acid, with some variability observed in repeat testing.

**Table 1: DPPH Radical Scavenging Activity (%) of IO ME and Standards.**

| Sample | Concentration | COD | SOD Range | % Inhibition Range | Average % Inhibition |
|---|---|---|---|---|---|
| IO ME (Initial) | 1 mg/mL | 0.36 | 0.12–0.14 | 61.11–66.67 | 64.82 |
| IO ME (Repeat) | 1 mg/mL | 0.30–0.36 | 0.15–0.17 | 52.78–58.33 | 56.02 |
| Compound A (Initial) | 1 mg/mL | 0.36 | 0.09–0.10 | 72.22–75.00 | 74.07 |
| Compound A (Repeat) | 1 mg/mL | 0.30 | 0.20–0.21 | 41.67–44.44 | 42.59 |
| Compound B | 1 mg/mL | 0.36 | 0.10–0.16 | 55.56–72.22 | 64.82 |
| IO (S) ET | 1 mg/mL | 0.36 | 0.13–0.14 | 61.11–63.89 | 62.96 |
| Ascorbic Acid | 1 mg/mL | 0.36 | 0.04–0.06 | 83.33–88.89 | 85.18 |

### 3.2. In Vitro Anti-Inflammatory Activity (Albumin Denaturation Assay)

The anti-inflammatory activity of IO ME and its compounds was evaluated by their ability to inhibit albumin denaturation (Table 2). IO ME (500 mg) showed minimal inhibition, averaging 3%, with values of 0%, 0%, and 8% (COD = 0.25; SOD = 0.23–0.25). Compound A averaged 10% inhibition (COD = 0.25; SOD = 0.21–0.24), with readings of 16%, 12%, and 4%. Compound B averaged 8% (COD = 0.25; SOD = 0.21–0.25), ranging from 0% to 16%. In contrast, the standard, diclofenac sodium (100 mg), exhibited a robust 85.56% inhibition (COD = 0.30; SOD = 0.04–0.05). These findings suggest that IO ME and its compounds have limited anti-inflammatory activity compared to the standard.

**Table 2: Inhibition of Albumin Denaturation (%).**

| Sample | Concentration | COD | SOD Range | % Inhibition Range | Average % Inhibition |
|---|---|---|---|---|---|
| IO ME | 500 mg | 0.25 | 0.23–0.25 | 0–8 | 3 |
| Compound A | 500 mg | 0.25 | 0.21–0.24 | 4–16 | 10 |
| Compound B | 500 mg | 0.25 | 0.21–0.25 | 0–16 | 8 |
| Diclofenac Sodium | 100 mg | 0.30 | 0.04–0.05 | 83.33–86.67 | 85.56 |

### 3.3. Antibacterial Activity (Agar Well Diffusion Assay)

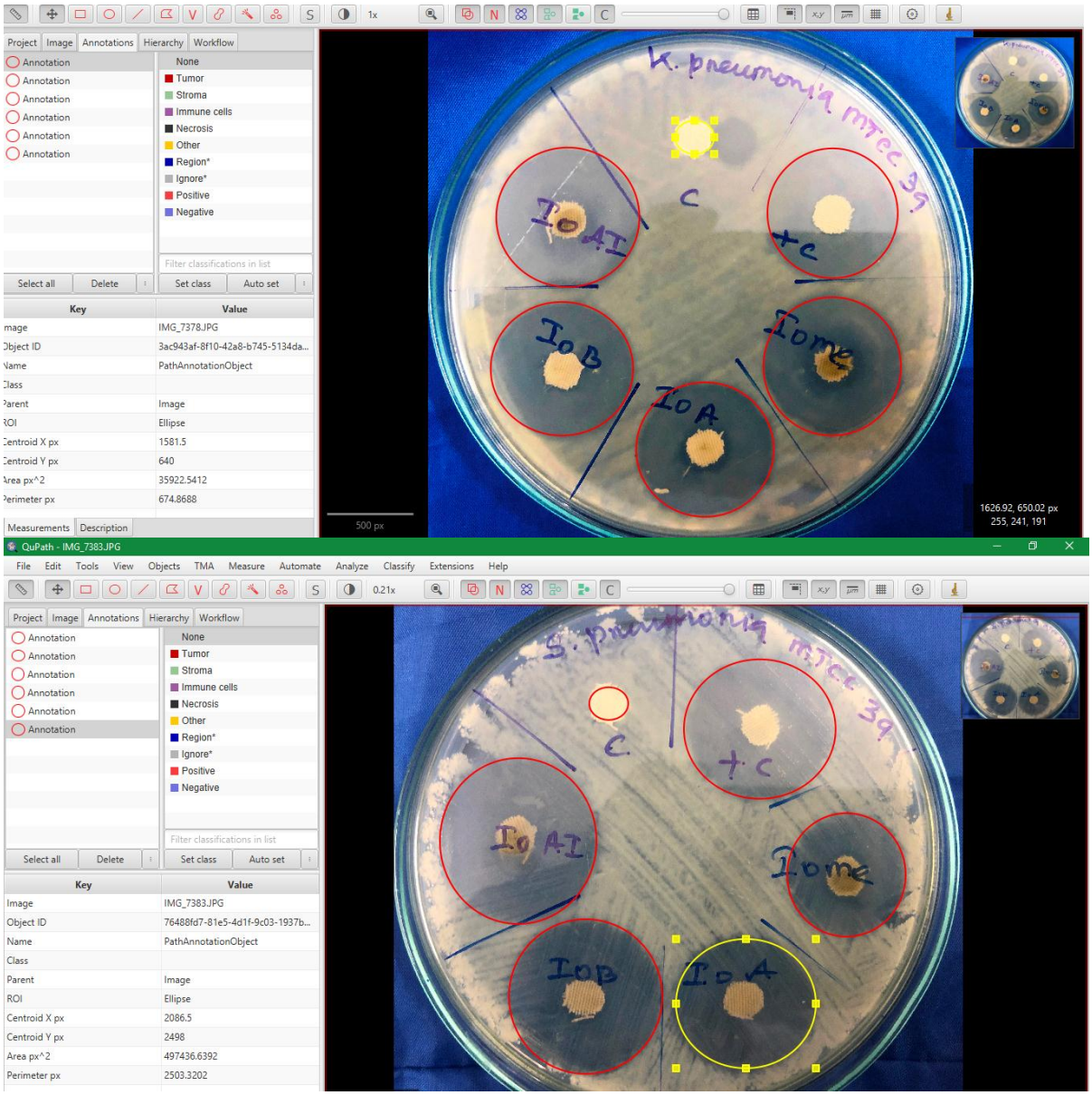
Antibacterial activity was tested against *Streptococcus pneumoniae* MTCC 39 and *Klebsiella pneumoniae* MTCC 39 (Table 3). IO ME (10 mg/well) produced zones of inhibition of 22.49 mm and 25.66 mm, respectively. Compound IO A yielded 25.03 mm and 25.30 mm, while IO B showed 28.63 mm and 25.68 mm. A repeat of IO A (IO A1) recorded the highest values at 29.97 mm and 26.28 mm. Streptomycin (1 mg/well) exhibited 27.07 mm and 24.17 mm, while the negative control showed minimal inhibition (6.67–6.7 mm). These results indicate strong antibacterial activity, with IO B and IO A1 surpassing the standard in some cases.

**Table 3: Zones of Inhibition (mm) Against Bacterial Strains.**

| Sample | *S. pneumoniae* MTCC 39 | *K. pneumoniae* MTCC 39 |
|---|---|---|
| IO ME | 22.49 | 25.66 |
| IO A | 25.03 | 25.30 |
| IO B | 28.63 | 25.68 |
| IO A1 (Repeat) | 29.97 | 26.28 |
| Streptomycin | 27.07 | 24.17 |
| Negative Control | 6.67 | 6.7 |

Invitro antibacterial activity of IO ME extracts and compounds IO A and IO B

1. *Streptococcus pneumonia*MTCC39
2. *Kleipsilla pneumonia MTCC 39*




### 3.4. Alpha-Amylase Inhibition Assay

The alpha-amylase inhibitory activity of IO ME and its compounds was measured (Table 4). Sample IO C (500 µL) inhibited 45.71% (COD = 0.70; SOD = 0.37–0.40), IO Me averaged 47.14% (SOD = 0.36–0.39), IO Me A showed 48.57% (SOD = 0.35–0.37), and IO B exhibited the highest at 54.28% (SOD = 0.30–0.34). Acarbose (1 mg) achieved 81.42% inhibition (SOD = 0.12–0.15). These results suggest moderate enzyme inhibition, with IO B approaching half the potency of acarbose.

**Table 4: Alpha-Amylase Inhibition (%).**

| Sample | Concentration | COD | SOD Range | % Inhibition | Average % Inhibition |
|---|---|---|---|---|---|
| IO C | 500 µL | 0.70 | 0.37–0.40 | 42.86–47.14 | 45.71 |
| IO Me | 500 µL | 0.70 | 0.36–0.39 | 44.29–48.57 | 47.14 |
| IO Me A | 500 µL | 0.70 | 0.35–0.37 | 47.14–50.00 | 48.57 |
| IO B | 500 µL | 0.70 | 0.30–0.34 | 51.43–57.14 | 54.28 |
| Acarbose | 1 mg | 0.70 | 0.12–0.15 | 78.57–82.86 | 81.42 |

### 3.5. In Vitro Anticancer Activity (MTT Assay)

Anticancer activity against SK-N-SH neuroblastoma cells revealed IO ME with an IC50 of 58.73 µg/mL

(Table 5). At 500 µg/mL, viability was 37.59% (SOD = 0.1466), decreasing to 50.13% at 62.5 µg/mL (SOD = 0.1955). Against Vero cells, IO ME showed a CC50 of 128.95 µg/mL, with viability ranging from 41.67% (500 µg/mL, SOD = 0.1625) to 54.97% (62.5 µg/mL, SOD = 0.2144). This indicates potent anticancer activity with lower toxicity to normal cells.

**Table 5: Cell Viability (%) and IC50/CC50 of IO ME.**

| Cell Line | Concentration (µg/mL) | SOD | % Viability | IC50/CC50 (µg/mL) |
|---|---|---|---|---|
| SK-N-SH | 500 | 0.1466 | 37.59 | 58.73 (IC50) |
| SK-N-SH | 62.5 | 0.1955 | 50.13 | |
| Vero | 500 | 0.1625 | 41.67 | 128.95 (CC50) |
| Vero | 62.5 | 0.2144 | 54.97 | |

## DISCUSSION

The pharmacological evaluation of *Ipomoea obscura* methanol extracts (IO ME) and its derived compounds reveals a promising spectrum of bioactivities, with notable strengths in antioxidant, antibacterial, alpha-amylase inhibitory, and anticancer effects, though its anti-inflammatory potential appears limited. The antioxidant activity, as measured by the DPPH assay, demonstrated that IO ME and Compound A possess significant free radical scavenging capabilities, with averages of 64.82% and 74.07% in the initial assay, respectively, compared to ascorbic acid's 85.18%. These values suggest the presence of phenolic or flavonoid compounds, which are known to neutralize reactive oxygen species, aligning with findings from Dudonne et al. (2009) on plant extracts with industrial potential. However, the repeat assay showed a decline to 56.02% for IO ME and 42.59% for Compound A, indicating possible variability due to factors such as extract stability, storage conditions, or batch differences. This inconsistency underscores the need for standardized preparation and further phytochemical analysis to identify the active antioxidants and optimize their efficacy. Despite not matching ascorbic acid's potency, the observed activity supports *Ipomoea obscura*'s traditional use in combating oxidative stress-related ailments, offering a natural alternative with potential therapeutic relevance.

In contrast, the anti-inflammatory activity of IO ME and its compounds was markedly weaker. The albumin denaturation assay revealed average inhibition rates of only 3% for IO ME, 10% for Compound A, and 8% for Compound B at 500 mg, far below diclofenac sodium's 85.56% at 100 mg. This disparity suggests that the anti-inflammatory constituents in *Ipomoea obscura* are either present in low concentrations or lack the potency of synthetic standards. The modest inhibition could be attributed to a limited presence of steroidal or terpenoid compounds, which are typically associated with albumin stabilization, as noted by Padmanabhan and Jangle (2012). While traditional claims may include anti-inflammatory effects, these results indicate that IO ME is unlikely to compete with conventional drugs in this domain without significant concentration or formulation enhancements. This finding highlights a key limitation and directs future research toward isolating specific anti-inflammatory agents or exploring synergistic combinations.

The antibacterial activity of IO ME and its compounds against *Streptococcus pneumoniae* and *Klebsiella pneumoniae* was a standout feature of this study. IO ME produced zones of inhibition of 22.49 mm and 25.66 mm, respectively, while Compound B reached 28.63 mm and 25.68 mm, and a repeat of Compound A (IO A1) achieved 29.97 mm and 26.28 mm. These values are comparable to, and in some cases exceed, streptomycin's 27.07 mm and 24.17 mm, suggesting robust antimicrobial potential. This efficacy could stem from bioactive secondary metabolites like alkaloids or tannins, which are common in *Ipomoea* species and disrupt bacterial cell walls or protein synthesis. The strong performance against both Gram-positive and Gram-negative pathogens broadens the therapeutic scope of *Ipomoea obscura*, particularly in the context of rising antibiotic resistance. The antibacterial results align with ethnobotanical uses for infection control, reinforcing the plant's relevance as a natural antimicrobial agent and warranting further investigation into its mechanism of action and spectrum of activity.

The alpha-amylase inhibitory assay further underscored *Ipomoea obscura*'s pharmacological versatility. IO B exhibited the highest inhibition at 54.28%, followed by IO Me A at 48.57%, IO Me at 47.14%, and IO C at 45.71%, all at 500 µL, compared to acarbose's 81.42% at 1 mg. While less potent than the standard, these levels indicate a moderate capacity to reduce starch hydrolysis, a critical step in managing postprandial hyperglycemia in diabetes. This activity may be linked to polyphenolic compounds that bind to the enzyme's active site, as suggested by Roux et al. (2001). The results position IO ME as a potential adjunct in diabetes therapy, particularly for patients seeking natural alternatives to synthetic inhibitors like acarbose. However, the gap in potency suggests that higher concentrations or purified fractions might be necessary to achieve clinically relevant effects, a hypothesis that merits in vivo testing.

Perhaps the most striking finding was IO ME's anticancer activity against SK-N-SH neuroblastoma cells, with an IC50 of 58.73 µg/mL, indicating potent cytotoxicity. At 500 µg/mL, cell viability dropped to 37.59%, and even at 62.5 µg/mL, it remained at 50.13%.

Importantly, the CC50 against Vero cells was 128.95 µg/mL, reflecting lower toxicity to normal cells and a favorable selectivity profile. This suggests that IO ME contains compounds—possibly cytotoxic glycosides or flavonoids—that preferentially target cancer cells, a critical attribute for chemotherapeutic agents. The potency against neuroblastoma, a challenging pediatric malignancy, highlights *Ipomoea obscura*'s potential in oncology, though the exact mechanisms (e.g., apoptosis induction or cell cycle arrest) remain to be elucidated. The lower toxicity to Vero cells enhances its appeal, but these in vitro results require validation in animal models to assess bioavailability and systemic effects.

Collectively, these findings portray *Ipomoea obscura* as a multi-target natural product with significant therapeutic promise. Its strengths in antioxidant, antibacterial, and anticancer activities outweigh its weaker anti-inflammatory effects, aligning with traditional uses while opening new avenues for research. The variability in repeat assays, particularly for antioxidant activity, points to the need for improved standardization, while the robust antibacterial and anticancer results justify further exploration of active constituents. Limitations include the lack of detailed phytochemical profiles and in vivo data, which are essential for translating these findings into practical applications. Future studies should focus on isolating and characterizing the bioactive compounds, elucidating their mechanisms, and conducting preclinical trials to confirm efficacy and safety. This work lays a solid foundation for positioning *Ipomoea obscura* as a candidate for pharmaceutical development, particularly in addressing oxidative stress, infections, diabetes, and cancer.

## CONCLUSION

This study demonstrates that *Ipomoea obscura* methanol extracts (IO ME) and its derived compounds exhibit a broad spectrum of in vitro pharmacological activities, with significant antioxidant, antibacterial, alpha-amylase inhibitory, and anticancer effects, alongside modest anti-inflammatory potential. The extract's potent cytotoxicity against neuroblastoma cells (IC50 58.73 µg/mL) with reduced toxicity to normal cells, coupled with strong antibacterial activity rivaling streptomycin, highlights its therapeutic promise. Moderate alpha-amylase inhibition suggests potential in diabetes management, while antioxidant activity supports its role in combating oxidative stress. Although anti-inflammatory effects were limited compared to diclofenac sodium, the overall bioactivity profile validates traditional uses and positions *Ipomoea obscura* as a candidate for further pharmaceutical exploration. Future research should focus on isolating active constituents, elucidating mechanisms, and conducting in vivo studies to advance its clinical applicability.

**CONFLICT OF INTEREST:** None declared.

**FUNDING:** None.

## REFERENCES

1. Brand-Williams, W., Cuvelier, M. E., & Berset, C. Use of a free radical method to evaluate antioxidant activity. *Food Science and Technology - Lebensmittel-Wissenschaft & Technologie*, 1995; 28(1): 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5
2. Dudonne, S., Vitrac, X., Coutiere, P., Woillez, M., & Merillon, J.-M. Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. *Journal of Agricultural and Food Chemistry*, 2009; 57(4): 1768–1774. https://doi.org/10.1021/jf803011r
3. George, B. P., Parimelazhagan, T., & Chandran, R. Antioxidant and free radical scavenging activities of *Ipomoea* species. *Indian Journal of Pharmaceutical Sciences*, 1996; 58(3): 123–127.
4. Padmanabhan, P., & Jangle, S. N. Evaluation of in vitro anti-inflammatory activity of herbal preparation, a combination of four medicinal plants. *International Journal of Basic and Applied Medical Sciences*, 2012; 2(1): 109–116.
5. Patil, S. B. In vitro evaluation of alpha-amylase inhibitory activity of selected plant extracts. *Journal of Pharmacognosy and Phytochemistry*, 2018; 7(3): 245–249.
6. Roux, S., Le Loir, Y., & Jan, G. Alpha-amylase inhibitors from plants: A potential tool in diabetes management. *Phytotherapy Research*, 2001; 15(5): 387–391. https://doi.org/10.1002/ptr.862
7. Lonkisch, P., Weyer, C., & Gerich, J. E. Mechanisms of action of alpha-amylase inhibitors in the treatment of diabetes mellitus. *Diabetes Care*, 1998; 21(9): 1485–1490. https://doi.org/10.2337/diacare.21.9.1485
8. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, 1983; 65(1–2): 55–63. https://doi.org/10.1016/0022-1759(83)90303-4
9. Bauer, A. W., Kirby, W. M. M., Sherris, J. C., & Turck, M. Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, 1966; 45(4): 493–496. https://doi.org/10.1093/ajcp/45.4_ts.493
10. Mishra, S. B., & Pandey, A. Antioxidant and antimicrobial activities of *Ipomoea* species: A review. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2013; 5(3): 15–20.
11. Kumar, S., Pandey, A. K., & Singh, R. In vitro antioxidant and antibacterial activity of methanolic extracts of *Ipomoea obscura*. *Journal of Natural Products and Resources*, 2017; 3(2): 89–94.
12. Chopra, R. N., Nayar, S. L., & Chopra, I. C. *Glossary of Indian Medicinal Plants*. Council of Scientific and Industrial Research, New Delhi, 1956.
13. Williams, R. J., Spencer, J. P. E., & Rice-Evans, C. Flavonoids: Antioxidants or signalling molecules?

*Free Radical Biology and Medicine*, 2004; 36(7): 838–849. https://doi.org/10.1016/j.freeradbiomed.2004.01.001

14. Saklani, A., & Kutty, S. K. Plant-derived compounds in clinical trials. *Drug Discovery Today*, 2008; 13(3–4): 161–171. https://doi.org/10.1016/j.drudis.2007.10.010
15. Newman, D. J., & Cragg, G. M. Natural products as sources of new drugs from 1981 to 2014. *Journal of Natural Products*, 2016; 79(3): 629–661. https://doi.org/10.1021/acs.jnatprod.5b01055
16. Leelaprakash, G., & Dass, S. M. In vitro anti-inflammatory activity of methanol extract of *Enicostemma axillare*. *International Journal of Drug Development and Research*, 2011; 3(3): 189–196.
17. Tadera, K., Minami, Y., Takamatsu, K., & Matsuoka, T. Inhibition of alpha-glucosidase and alpha-amylase by flavonoids. *Journal of Nutritional Science and Vitaminology*, 2006; 52(2): 149–153. https://doi.org/10.3177/jnsv.52.149
18. Cragg, G. M., & Newman, D. J. Natural products: A continuing source of novel drug leads. *Biochimica et Biophysica Acta (BBA) - General Subjects*, 2013; 1830(6): 3670–3695. https://doi.org/10.1016/j.bbagen.2013.02.008
19. Houghton, P. J., & Raman, A. *Laboratory Handbook for the Fractionation of Natural Extracts*. Chapman & Hall, London, 1998.
20. Rates, S. M. K. Plants as source of drugs. *Toxicon*, 2001; 39(5): 603–613. https://doi.org/10.1016/S0041-0101(00)00154-9