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Phytochemical Profiling and In Vitro Cytotoxic Evaluation of Ethanolic Leaf Extract of *Ipomoea Pes- Tigridis* on Cancer Cell Lines

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Abstract: Medicinal plants remain an important source of bioactive compounds with therapeutic potential. The present study was carried out to evaluate the phytochemical composition and in-vitro biological activities of the ethanolic leaf extract of Ipomoea pes-tigridis, with emphasis on antioxidant, antibacterial, and cytotoxic properties. Leaves were extracted by the maceration method using ethanol, and the extract was subjected to preliminary phytochemical screening. Qualitative analysis revealed the presence of alkaloids, flavonoids, phenolics, tannins, saponins, glycosides, terpenoids, steroids, carbohydrates, and proteins. Antibacterial activity was assessed against Escherichia coli using the agar well diffusion method, where the extract exhibited concentration-dependent inhibition, with a maximum zone of inhibition of 18 mm at 200 µg/ml, while ethanol showed no activity. Antioxidant potential was evaluated using the DPPH radical scavenging assay, and the extract demonstrated moderate free-radical scavenging activity, showing 57.3% inhibition at the highest tested concentration. Cytotoxic activity was determined by the MTT assay on A549 human lung cancer cell lines. The extract produced a dose-dependent reduction in cell viability, with an IC_{50} value of 88.25 µg/ml, indicating moderate cytotoxic potential. Overall, the findings suggest that Ipomoea pes-tigridis leaves possess significant phytochemical diversity and exhibit notable antioxidant, antibacterial, and cytotoxic activities. These results support the traditional medicinal use of the plant and indicate its potential as a natural source of bioactive compounds for further pharmacological investigation.

Keywords: Ipomoea pes-tigridis, phytochemical analysis, antioxidant activity, antibacterial activity, cytotoxicity

1. Introduction

Medicinal plants have constituted an integral component of human health care systems since ancient times and continue to contribute significantly to disease prevention and therapy. Traditional medical systems such as Ayurveda, Siddha, Unani, and diverse indigenous practices rely extensively on plant-derived formulations for maintaining health and treating various ailments[1]. These systems are based

on long-standing empirical knowledge accumulated through careful observation and repeated therapeutic use of natural resources. Over generations, medicinal plants were systematically identified, processed, and administered according to their perceived healing properties. Despite major advances in modern medicine, plant-based therapeutics remain highly relevant due to their ability to provide bioactive compounds that are used either directly as herbal remedies or indirectly as lead molecules for pharmaceutical drug development. [3] In recent years, global interest in natural products and plant-derived medicines has increased considerably. This resurgence is driven by the growing incidence of chronic and lifestyle-related disorders, the rapid emergence of antimicrobial resistance, rising healthcare costs, and increasing concerns about the safety and long-term adverse effects of synthetic drugs. [4] Although synthetic medicines are often effective, their use may be limited by toxicity, side effects, and restricted accessibility in resource-poor regions. In contrast, medicinal plants are generally considered safer, cost-effective, and culturally acceptable. Moreover, the complex chemical composition of plant extracts enables them to exert multiple biological effects simultaneously by acting on diverse molecular targets, thereby offering broader therapeutic potential. [5] The therapeutic efficacy of medicinal plants is largely attributed to their rich diversity of secondary metabolites, including alkaloids, flavonoids, phenolic compounds, tannins, saponins, glycosides, terpenoids, and steroids. These phytochemicals play protective roles in plants and are responsible for a wide range of pharmacological activities in humans.[8] Alkaloids are known for their antimicrobial, analgesic, and anticancer properties, while flavonoids and phenolic compounds exhibit strong antioxidant and anti-inflammatory activities.Tannins possess astringent and antimicrobial effects, saponins demonstrate immunomodulatory and cytotoxic actions, and terpenoids and steroids are associated with anti-inflammatory and anticancer potential. [22]The presence of such diverse bioactive constituents provides a scientific rationale for the traditional medicinal use of plants and underscores their importance in contemporary drug discovery research. [12] Oxidative stress is a critical factor implicated in the pathogenesis of several chronic and degenerative diseases, including cancer, cardiovascular disorders, diabetes, and neurodegenerative conditions. It arises from an imbalance between the generation of reactive oxygen species and the body's antioxidant defense mechanisms, leading to cellular and molecular damage. [6] Plant-derived antioxidants play an essential role in scavenging free radicals, thereby protecting biomolecules from oxidative injury and preserving cellular function. In addition to oxidative stress–related disorders, infectious diseases remain a major global health concern, particularly due to the increasing prevalence of drug-resistant microbial strains.[9] Consequently, plant-based antimicrobial agents have gained considerable attention as potential alternatives or adjuncts to conventional antibiotics. [23] Cancer continues to be one of the leading causes of morbidity and mortality worldwide and is characterized by uncontrolled cell proliferation, evasion of apoptosis, and dysregulation of cellular signaling pathways. [2] Notably, many anticancer agents currently used in clinical practice are derived from natural sources or are structurally inspired by plant-based compounds. Numerous plant extracts and isolated phytochemicals have demonstrated the ability to inhibit tumor cell growth, induce programmed cell death, and suppress cancer progression, often with lower toxicity to normal cells.[22] Therefore, the exploration of medicinal plants for cytotoxic and anticancer activities remains a promising and active area of research. *Ipomoea pes-tigridis* is a medicinally important plant traditionally used in various regions for the management of infections, inflammation, wounds, and other health conditions. Despite its ethnomedicinal significance, systematic scientific investigations validating its phytochemical composition and biological activities are relatively limited. [14] Preliminary reports suggest that the plant may possess antioxidant, antimicrobial, and cytotoxic properties, potentially attributable to its diverse phytochemical constituents. [15] However, comprehensive experimental studies are required to substantiate these claims and to establish correlations between its chemical profile and therapeutic effects. Therefore, a scientific evaluation of *Ipomoea pes-tigridis* is necessary to validate its traditional applications and to assess its potential as a source of biologically active compounds. The use of standardized extraction methods, preliminary phytochemical screening, and in vitro biological assays provides a reliable framework for evaluating its medicinal properties. [19] Such studies not only contribute to the scientific validation of traditional medicinal knowledge but also serve as a foundation for future research, including the isolation of active principles, mechanistic investigations, in vivo studies, and the development of novel plant-based therapeutic agents. [26]

2. MATERIALS AND METHODS

2.1 Preparation of Plant Extract by Maceration

The leaves of *Ipomoea pes-tigridis* were shade-dried, powdered, and subjected to extraction by the maceration technique. Five grams of the powdered plant material were soaked in 50 mL of ethanol, maintaining a 1:10 (w/v) ratio. The mixture was kept at room temperature for 72 hours with intermittent shaking to facilitate efficient extraction of phytoconstituents. After the maceration period, the extract was filtered using Whatman No. 1 filter paper. The filtrate was concentrated under reduced pressure to obtain a dried ethanolic extract, which was stored in an airtight container at 4 °C until further analysis[8]

2.2 Preliminary Phytochemical Screening

Preliminary phytochemical analysis of the plant extract was performed using standard qualitative chemical tests to identify the presence of major secondary metabolites. The screening focused on detecting alkaloids, glycosides, flavonoids, steroids, triterpenoids, phenolic compounds, tannins, saponins, carbohydrates, proteins, gums, mucilage, fixed oils, and fats. These tests provide a preliminary understanding of the bioactive constituents responsible for the biological activities of the plant. [8] [11]

2.2.1 Detection of Alkaloids

The extract was acidified with 2 M hydrochloric acid and treated separately with Dragendorff's, Mayer's, and Wagner's reagents. The formation of orange-red, white, or reddish-brown precipitates was considered indicative of the presence of alkaloids.

2.2.2 Detection of Glycosides

Glycosides were detected using the Borntrager's test. The extract was hydrolyzed with sulfuric acid, followed by extraction with chloroform. The chloroform layer was treated with dilute ammonia solution, and the development of a pink to crimson color indicated the presence of anthraquinone glycosides.

2.2.3 Detection of Flavonoids

The Shinoda test was used for flavonoid detection. Magnesium turnings and concentrated hydrochloric acid were added to the extract, and the appearance of a pink or crimson-red coloration confirmed the presence of flavonoids.

2.2.4 Detection of Steroids and Triterpenoids

Steroids and triterpenoids were identified using the Liebermann–Burchard test. The extract was treated with acetic anhydride followed by concentrated sulfuric acid. The formation of a brown ring at the interface or color changes to red indicated the presence of steroids and triterpenoids.

2.2.5 Detection of Tannins and Phenolic Compounds

Ferric chloride test was performed by adding 5% ferric chloride solution to the extract. The appearance of a blue-black coloration confirmed the presence of phenolic compounds and tannins. Formation of a white precipitate upon addition of lead acetate further supported the presence of tannins.

2.2.6 Detection of Saponins

The foam test was employed for saponin detection. The extract was shaken vigorously with sodium bicarbonate solution, and the formation of a stable froth indicated the presence of saponins.

2.2.7 Detection of Carbohydrates

Carbohydrates were identified using Molisch's test by adding alcoholic α-naphthol followed by concentrated sulfuric acid. The formation of a violet ring at the interface confirmed carbohydrates. Red precipitate formation in Fehling's test indicated the presence of reducing sugars.

2.2.8 Detection of Proteins and Amino Acids

Proteins were detected using the Biuret test. Addition of copper sulfate solution to the extract resulted in a violet coloration, confirming the presence of proteins and peptides.

2.2.9 Detection of Gums and Mucilage

The presence of gums and mucilage was confirmed by treating the extract with acetic anhydride followed by concentrated sulfuric acid. The formation of a purple-red coloration indicated positive results.

2.2.10 Detection of Fixed Oils and Fats

The filter paper test was used to detect fixed oils and fats. Formation of a permanent oily spot on the filter paper indicated their presence.

2.3 Antimicrobial Activity

The antimicrobial activity of the ethanolic extract was evaluated using the agar well diffusion method. Nutrient agar medium was prepared, poured into sterile Petri plates, and allowed to solidify. Each plate was inoculated with 0.2 ml of freshly prepared *Escherichia coli* culture using the spread plate technique. Wells of 6 mm diameter were aseptically bored into the agar plates. The extract was introduced into the wells at concentrations of 50, 100, 150, and 200 µg/ml. Ciprofloxacin was used as the standard drug, while ethanol served as the negative control. The plates were kept at room temperature for diffusion and incubated at 37 ± 2 °C for 24 hours. Zones of inhibition were measured in millimeters to assess antimicrobial activity. [27] [16]

2.4 Antioxidant Activity

(DPPH Radical Scavenging Assay)

The antioxidant potential of the extract was determined using the DPPH free radical scavenging assay. A 0.1 mM DPPH solution was freshly prepared in methanol and protected from light. Various concentrations of the extract (50, 100, 150, and 200 µg/ml) were prepared.

Each reaction mixture contained 1 mL of DPPH solution and 1 ml of extract. Methanol with DPPH served as the control, and ascorbic acid was used as the reference standard. The mixtures were incubated in the dark at room temperature for 30 minutes, and absorbance was measured at 517 nm using a UV–Visible spectrophotometer. [23] [24]

The percentage of radical scavenging activity was calculated using the formula:

$$\text{Antioxidant Activity (\%)} = \frac{(\text{Abs of control} - \text{Abs of sample}) \times 100\%}{(\text{Abs of control})}$$

2.5 Cytotoxicity Study

(MTT Assay)

The cytotoxic activity of the ethanolic extract was evaluated using the MTT assay on A549 human lung cancer cells. Cells were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum and 1% antibiotic–antimycotic solution.

Cells were seeded at a density of 1×10^4 cells per well in 96-well plates and incubated overnight at 37 °C in a humidified atmosphere containing 5% CO_2. After attachment, cells were treated with different concentrations of the extract (100, 50, 25, 12.5, 6.25, and 3.125 µg/ml). Untreated cells served as the negative control. Following 48 hours of incubation, cells were washed with phosphate-buffered saline and treated with MTT reagent (5 mg/ml). After incubation for 4 hours, formazan crystals were dissolved using DMSO, and absorbance was measured at 570 nm using a microplate reader. [18] [28] [25]

Cell viability was calculated using the formula:

$$\text{Cell viability (\%)} = \frac{\text{Mean OD of treated cells}}{\text{Mean OD of control cells}} \times 100$$

3. RESULTS AND DISCUSSIONS

3.1 Results

3.1.1 Extraction of *Ipomoea pes-tigridis* Leaves by Maceration

Ethanolic extraction of *Ipomoea pes-tigridis* leaves using the maceration technique resulted in a dark green to brownish semi-solid extract, indicating efficient extraction of phytoconstituents. The extract was clear after filtration, free from visible particulate matter, and remained stable when stored at 4 °C in an airtight container. These characteristics suggest that the extract was suitable for subsequent phytochemical and biological evaluations.

3.1.2 Preliminary Phytochemical Screening

Qualitative phytochemical screening of the ethanolic leaf extract of *Ipomoea pes-tigridis* revealed the presence of a wide range of bioactive secondary metabolites. These included carbohydrates, alkaloids, glycosides, tannins, phenolic compounds, terpenoids, steroids, flavonoids, saponins, and proteins, indicating the chemically diverse nature of the extract. Carbohydrates were confirmed through positive Molisch's and Fehling's tests, suggesting the presence of reducing sugars that contribute to metabolic and protective functions. Alkaloids were detected using Dragendorff's, Mayer's, and Wagner's reagents, indicating nitrogen-containing compounds with potential pharmacological relevance. Glycosides were identified by Borntrager's and modified Borntrager's tests, suggesting the presence of anthraquinone and cardiac glycosides. Tannins and phenolic compounds, confirmed by ferric chloride and lead acetate tests, are widely recognized for their antioxidant and antimicrobial properties. Terpenoids and steroids were detected using Salkowski and Liebermann–Burchard tests, indicating the presence of lipophilic bioactive compounds with anti-inflammatory and anticancer potential. Flavonoids identified by the Shinoda test are known for their strong antioxidant and free radical scavenging abilities. The presence of saponins, detected by the foam test, suggests possible immunomodulatory and cholesterol-lowering effects, while proteins and amino acids detected by the Biuret test indicate nutritional and physiological relevance. The identification of these phytochemical constituents provides a scientific basis for the observed biological activities and supports further pharmacological investigations.

3.1.3 Antimicrobial Activity

The antibacterial activity of the ethanolic extract of *Ipomoea pes-tigridis* was evaluated against *Escherichia coli* using the agar well diffusion method. The extract demonstrated a concentration-dependent increase in antibacterial activity. Zones of inhibition of 11 mm, 13 mm, 15 mm, and 18 mm were observed at concentrations of 50, 100, 150, and 200 µg/ml, respectively. The highest inhibitory effect was observed at 200 µg/ml. The negative control (ethanol) did not produce any inhibition zone, whereas the standard antibiotic ciprofloxacin produced a significantly larger zone of inhibition (28 mm). These findings indicate that the extract possesses moderate antibacterial activity against *E. coli*.

3.1.4 Antioxidant Activity

The antioxidant potential of the extract was assessed using the DPPH free radical scavenging assay. The method evaluates the ability of antioxidant compounds to neutralize DPPH radicals, resulting in a decrease in absorbance. Ascorbic acid served as the reference standard. The extract exhibited concentration-dependent radical scavenging activity. Maximum inhibition (57.3%) was observed at 100% concentration, indicating moderate antioxidant potential. A gradual decrease in activity was noted with decreasing concentrations, and no detectable activity was observed at 12.5% concentration. The observed antioxidant activity may be attributed to the presence of phenolic compounds and flavonoids in the extract.

3.1.5 Cytotoxicity Study

The cytotoxic effect of the ethanolic extract of Ipomoea pes-tigridis was evaluated against A549 human lung cancer cells using the MTT assay. The extract exhibited a dose-dependent reduction in cell viability, indicating cytotoxic activity.

At the highest concentration tested (100 µg/ml), cell viability was reduced to 50.60%, while 53.29% viability was observed at 50 µg/ml. Lower concentrations showed reduced cytotoxic effects, with maximum cell viability (76.05%) recorded at 6.25 µg/ml. Untreated control cells maintained high viability, confirming assay reliability.

The **IC_{50} value** of the extract was calculated to be **88.25 µg/ml**, suggesting that the ethanolic extract of *Ipomoea pes-tigridis* possesses **moderate cytotoxic activity** against A549 lung cancer cells.

Table 1: Extractive value and physical characteristics of ***Ipomoea pes-tigridis*** leaf extract

Solvent	Ethanol
Extraction value % w/w	28.64
Colour	Dark greenish brown
Odour	Characteristic
Consistency	Greesy

Table 2: Phytochemical screening of *Ipomoea pes-tigridis* leaf extract

Phytochemical Constituents	**Tests Performed**	**Result**
Carbohydrates	Molisch's test	+
	Fehling's test	+
Alkaloids	Dragendorff's test	+
	Mayer's test	+
	Wagner's test	+
Glycosides	Borntrager's test	+
	Modified Borntrager's test	+
Tannins	Ferric chloride test	+
	Lead acetate test	+
Phenols	Ferric chloride test	+
Terpenoids	Salkowski test	+
Steroids	Liebermann–Burchard's test	+
Flavonoids	Shinoda test	+
Saponins	Foam test	+
Proteins & Amino acids	Biuret test	+

Note: (+) Presence of phytochemical; (–) Absence of phytochemical.

Table 3 : Zone of Inhibition of *Ipomoea pes-tigridis* Extract against E. coli

Sample	**Concentration (µg/mL)**	**Zone of Inhibition (mm)**
Plant extract A	50	11
Plant extract B	100	13
Plant extract C	150	15
Plant extract D	200	18
Control (Ethanol)	-	0
Standard (Ciprofloxacin)	50	28

Table 4: DPPH Free Radical Scavenging Activity of the Extract

Concentration	**Absorbance (OD)**	**% Inhibition**
100%	0.296	57.3
50%	0.361	48.0
25%	0.452	34.8

12.5%	ND	ND

ND – Not Detected; Absorbance calculated using control OD = 0.694

Table 5: Cytotoxic Effect of *Ipomoea pes-tigridis* Extract on A549 Cells

Concentration (µg/mL)	**Cell Viability (%)**
100	50.60
50	53.29
25	61.08
12.5	65.57
6.25	76.05
3.125	68.86



Fig 1: Dried leaves, powderd leaves, extract of Ipomoea pes-tigridis



Fig 2: Dried extract of Ipomoea pes-tigridis

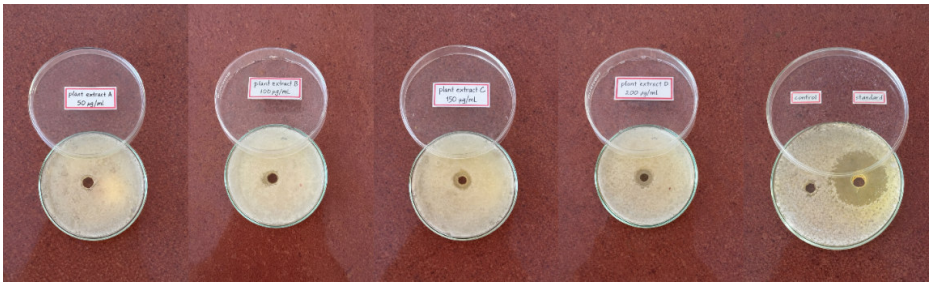

Fig 3: Zone of inhibition of antimicrobial activity

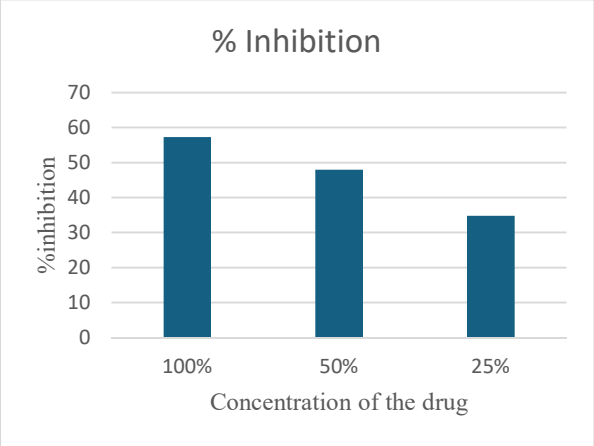

Fig 4: DPPH Radical Scavenging Activity of the Extract

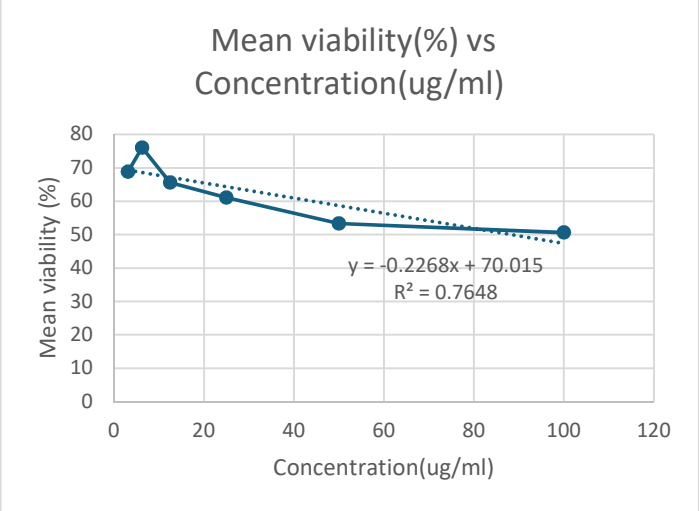

Fig 5: Mean viability vs concentration graph

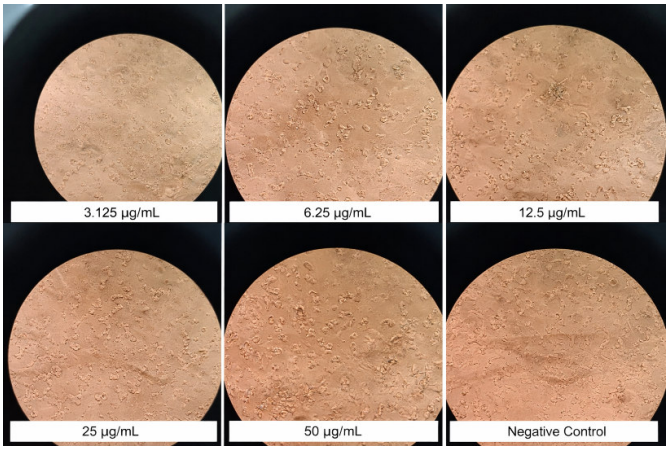

Fig 6: A549 cells under different concentrations of Ipomoea pes-tigridis extract

The present study demonstrated that ethanolic extraction of *Ipomoea pes-tigridis* leaves using maceration yielded a stable extract with a relatively high extractive value, suggesting efficient recovery of bioactive constituents. Phytochemical screening confirmed the presence of diverse secondary metabolites, including alkaloids, flavonoids, phenolic compounds, tannins, glycosides, terpenoids, steroids, saponins, carbohydrates, and proteins. Many of these compounds are known to possess antioxidant, antimicrobial, and cytotoxic properties, and their combined presence may result in synergistic biological effects. Biological evaluations further supported the therapeutic potential of the extract. The antibacterial activity against *Escherichia coli* showed a clear concentration-dependent pattern, although the activity was lower compared to the standard antibiotic ciprofloxacin. The antioxidant study demonstrated moderate DPPH radical scavenging activity, likely due to the presence of phenolic and flavonoid compounds capable of neutralizing free radicals. The cytotoxicity assay revealed a dose-dependent reduction in viability of A549 lung cancer cells, with a moderate IC_{50} value, indicating potential anticancer activity. Overall, these findings support the traditional medicinal use of *Ipomoea pes-tigridis* and suggest that the plant is a promising source of bioactive compounds. Further studies focusing on isolation of active constituents, mechanistic investigations, and in vivo evaluations are warranted to establish its therapeutic potential.

4. CONCLUSION

The purpose of this study was to provide scientific evidence for the traditional medicinal use of *Ipomoea pes-tigridis* by examining its phytochemical composition and selected in vitro biological activities. Through a systematic experimental approach, the work aimed to highlight the medicinal relevance of this plant and to establish its potential value in contemporary natural product research. The results of the investigation demonstrate that *Ipomoea pes-tigridis* leaves contain a wide spectrum of biologically important secondary metabolites, including alkaloids, flavonoids, phenolic compounds, tannins, saponins, glycosides, terpenoids, and steroids. The presence of these constituents is significant, as they are commonly associated with antimicrobial, antioxidant, and anticancer properties. The observed antibacterial activity against *Escherichia coli*, along with measurable free radical scavenging capacity and a concentration-dependent cytotoxic effect on A549 lung cancer cells, confirms the biological relevance of the plant extract and supports its pharmacological potential.Beyond validating traditional knowledge, the findings of this study have broader implications. The demonstrated biological activities suggest that *Ipomoea pes-tigridis* may represent a valuable natural source of lead compounds for the development of novel therapeutic agents. Additionally, the experimental methods employed in this study, including ethanolic extraction, phytochemical screening, and in vitro bioassays, provide a practical and reproducible framework that can be extended to the investigation of other medicinal plants.

Importantly, this research establishes a foundation for future scientific exploration. The outcomes encourage further studies focusing on the isolation and structural characterization of active compounds, investigation of underlying molecular mechanisms, evaluation of in vivo efficacy, and assessment of safety and toxicity profiles. Such studies are essential for translating laboratory findings into clinically relevant applications.In summary, this work emphasizes the medicinal significance of *Ipomoea pes-tigridis* and reinforces the importance of plant-based research in drug discovery and development. By integrating traditional knowledge with scientific validation, the study offers a meaningful contribution to natural product research and opens new avenues for the development of plant-derived therapeutic agents.

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