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Original Research Article

Assessment of the Antioxidant Capacity and Cytotoxic Activity of *Ipomoea pes-tigridis*

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ABSTRACT

Cancer, one of the deadliest diseases, is the second leading cause of mortality all over the world. The study aims to investigate the phytochemical profile and *in vitro* antioxidant activity of ethanol extracts from *Ipomoea pes-tigridis* and assess its cytotoxicity against three cell lines. Its qualitative and quantitative phytochemical analysis revealed that the plant possesses various secondary metabolites like tannins and flavonoids, which have potent antioxidant properties. The DPPH (2,2-Diphenyl-1-picrylhydrazyl) and nitric oxide (NO) free radical assays showed promising *in vitro* antioxidant potential. The ethanol extracts demonstrated significant cytotoxicity against A375, B-16-F10, and NHDF cell lines, with IC_{50} values ranging from 12.02 ± 3.14 µg/mL to 136.42 ± 2.92 µg/mL. These findings suggest that *Ipomoea pes-tigridis* may contain compounds with potential anticancer properties, which warrant further investigation for identification and characterisation. The observed activities of the plant can be credited to the polyphenolic compounds, and future research is needed to assess the detailed phytochemistry and biological activities of *Ipomoea pes-tigridis*.

Keywords: *Ipomoea pes-tigridis*, Phytochemical profile, Antioxidant, Cytotoxicity, MTT assay

Introduction

The intracellular release of free radicals plays a crucial role in preventing the invasion of parasites into the cytoplasm. However, overproduction of these radicals can induce cellular stress and trigger the intervention of free radical scavenging enzymes, namely superoxide dismutase and catalase. A balance between neutralising and generating free radicals is essential to maintain cellular homeostasis. Any disturbance in this equilibrium can predispose cells to stress, ultimately contributing to chronic or progressive conditions such as neurodegenerative and cardiovascular diseases, as well as the ageing process. Therefore, regular antioxidants are essential to sustain scavenging activity and preserve cellular integrity. Natural antioxidants like tocopherols and polyphenols, including tannins and flavonoids, are renowned for their robust adaptogenic properties as they offer health advantages with significantly fewer risks than synthetic alternatives.[1,2] Cancer is a serious disease characterised by the uncontrolled proliferation of cells that form tumours, which spread to other organs through metastasis and promote the growth of new tumours via angiogenesis, thereby aggravating hypoxia and malnutrition in healthy cells. The pathophysiology of cancer is multifactorial and complex, involving various factors such as chemical, environmental, genetic, and other unknown causes.[3] Developing potent anticancer drugs with few side effects is still challenging despite intensive research due to the chemical nature, drug compatibility, and other physicochemical characteristics of chemical moieties.

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Cancer is one of the considerable diseases that leads to the increasing deaths globally. Breast, lung, colon, and rectal cancers are among the most prevalent types cancers.[4] Men are at a higher risk than women, with lung cancer being the leading cause of death, followed by colon and rectum cancers. This increasing socio-economic burden affects both developed and developing countries alike.[5-7] Plants have traditionally served as a fundamental source of medicinal compounds and are often perceived as safer than synthetic medications, especially for prolonged treatments. They are also widely accepted across cultures around the globe. The numerous phytochemicals in these plants, acting independently or in synergy, play a vital role in rejuvenating health.[8-10] There is a growing inclination towards herbal medicine in contemporary drug discovery. Consequently, a multidisciplinary approach has been introduced to examine medicinal plants' phytochemical profile and therapeutic properties. These techniques have enabled the identification of biologically active compounds in plants and facilitated the development of new drugs. Natural medicines have gained popularity due to their minimal side effects, better patient tolerance, and low toxicity levels. Hence, natural medicines have the potential to serve as an alternative or adjunct to conventional therapies for the treatment of various ailments.[11] *Ipomoea pes-tigridis*, commonly known as morning glory or tiger foot, is a twinning or spreading herb belonging to the Convolvulaceae family. It is widely distributed in tropical and subtropical regions and contains various compounds, such as ergoline alkaloids, indolizidine alkaloids, nor-tropane alkaloids, flavonoids, glycolipids, lignin, and triterpenes.[12] Determination of the total phenolic content is an essential aspect of evaluating the health benefits of herbs. Phenolic compounds are well-known for their antineoplastic, antistress, and protective properties, which make them valuable for medicinal and nutritional purposes. Estimating the total phenolic content provides a method for assessing the presence of these beneficial compounds in a plant extract and allows for comparison and evaluation of different plant samples. It also helps maintain the quality and stability of plant-based products during storage and processing, thereby preserving their beneficial properties. Thus, determining the total phenolic content is crucial in analysing plant-based products and provides valuable information for product development, quality control, and consumer safety. This plant exhibits psychotropic, uterotonic, and hemostatic properties. It is widely recognised for its extensive medicinal benefits. It is used to treat peptic ulcers, joint problems, rheumatoid arthritis, inflammations, gout, venereal diseases, boils,

carbuncles, and dog bites. Additionally, it has diuretic, laxative, painkilling, and antidote properties for poisonous stings and snake bites. The extracts of this plant effectively treat acne scars and sores[13]. The numerous compounds in *Ipomoea pes-tigridis* act synergistically to exhibit their therapeutic effects, making it a valuable resource in traditional medicine. Understanding the phytochemical composition of the plant and its pharmacological effects is vital in developing new natural therapeutic agents.[14] The study could potentially lead to the discovery of new anticancer agents with fewer side effects than conventional chemotherapy drugs. Additionally, the antioxidant activity of the plant may have potential applications in preventing or treating oxidative stress-related diseases. This study aims to determine the potential of *Ipomoea pes-tigridis* as a source of natural compounds with antioxidant and cytotoxic properties.

Materials and Methods

Plant materials
The aerial parts of *Ipomoea pes-tigridis* were collected in September 2023 from Hyderabad, Telangana, and authenticated from Osmania University with Voucher specimen number OUAS-160.

Plant preparation and extraction
The aerial portions of *Ipomoea pes-tigridis* were harvested and dried in a shaded area. The dried leaves were powdered and defatted using n-hexane. The marc was then subjected to hydroalcoholic extraction through maceration with 70% ethanol. The resulting extract was filtered, and the solvent was removed through evaporation with a rotary evaporator at 40℃ to obtain a solid extract. The percentage yield was calculated and recorded.[15]

Phytochemical analysis
Preliminary screening of the ethanol extract of *Ipomoea pes-tigridis* for the presence of phytochemicals was performed according to the established protocols.[16]

Quantitative estimation of phytochemicals
Total phenolic content
The method for estimating the total phenolic content in *Ipomoea pes-tigridis* was adopted from the technique initially proposed by Singleton and colleagues in 1965, with slight modifications. We prepared a standard solution of gallic acid and diluted to different concentrations (25 to 100 µg/mL) using ethanol. 10% of Folin-Ciocalteu (FC) reagent solution was added and mixed with 7.5% w/v Na_2CO_3 for each sample. These mixtures/samples were left to incubate at an ambient temperature for two hours. A calibrated UV-visible spectrophotometer was used to measure the absorbance of the test solutions in triplicates at a wavelength of 765 nm. Based on these readings, a calibration graph was plotted. The quantification of total phenolics in all the samples was expressed in terms of gallic acid equivalents (GAE) in milligrams per gram of the dry weight of the sample (mg/g).[17]

Total flavonoid content
The Dowd method was utilised for total flavonoid content estimation in *Ipomoea pes-tigridis*. In this assay, 1 mL sample solution containing either plant extracts or quercetin was combined with one-fifth volume of $AlCl_3$ solution with 10% concentration (weight/volume), 0.2 mL of 1 M potassium acetate and enough distilled water to bring the total volume to 6 mL. This mixture was then left to incubate for 30 minutes, and the absorbance was calculated in triplicates using a UV-visible spectrophotometer at a wavelength of 415 nm. The findings were then calculated and reported as mg of quercetin equivalents per gram of the dry plant extract. The final values were expressed in milligrams per gram (mg QE/g).[18]

Total tannin content
The Broad Hurst method, with a slight modification, was employed for the quantification of tannins. In this procedure, 0.4 mL of the plant extract was added to 3 mL of vanillin mixture (4% vanillin in methanol), and concentrated hydrochloric acid was added to half the volume of this mixture (1.7 mL). This mixture was incubated for 15 minutes. The absorbance was measured at 500 nm. The tannin content in the test solution was denoted as mg of tannic acid equivalents per milligram of the sample's dry weight (mg TAE/mg dry weight). This procedure was replicated three times.[19]

In-vitro antioxidant assay
2, 2-diphenyl-1-picrylhydrazy (*DPPH) radical scavenging assay*
The ability of ethanolic extracts of *Ipomoea pes-tigridis* to neutralise free radicals was evaluated using the DPPH assay. In this procedure, 2 mL of a DPPH solution (concentration: 0.5 mM) was added to 0.2 mL of the extract solution and allowed to stand for 20 min at 25℃. Ascorbic acid was employed as a control standard for comparison. The absorbance of each sample was measured at a wavelength of 515 nm (in triplicates). The antioxidant activity was then calculated using a predetermined formula based on the absorbance readings.[20]

$$\% \text{ of Free radical scavenging activity} = [(A_{blank}-A_{test})/A_{blank}] \times 100$$

Where A_{blank} is the absorbance of the blank, and A_{test} is the absorbance of the samples or standard.

Nitric oxide free radical scavenging assay
The study aims to test the ability of *Ipomoea pes-tigridis* extracts to scavenge nitric oxide radicals. Briefly, 0.5 mL of the extract was added to 10 mM sodium nitroprusside solution (2 mL) and 0.5 mL of a phosphate buffer at pH 7.4, followed by incubation at 25°C for 150 minutes. Ascorbic acid was used as the standard, and DMSO was used as a control. For analysis, equal amounts of the experimental samples and Griess reagent were incubated at 25°C for 30 minutes.[21] Absorbance measurements were then taken at a wavelength of 540 nm to determine the nitric oxide scavenging activity using the above equation.

Cytotoxicity screening of the plant extract
The cytotoxicity of *Ipomoea pes-tigridis* extract was studied using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay against mouse melanoma cells (B-16-F10) and human melanoma cells (A375). Normal human dermal fibroblast (NHDF) was also selected to compare their toxicity. These cell lines, sourced from the American Type Culture Collection (ATCC), were cultured in 96-well plates under controlled conditions (37°C and 5% CO_2 for 72 hours). The cells were treated with MTT reagent (20 µL of a 2 mg/mL solution in phosphate-buffered saline) and incubated for three hours. The purple formazan product, resulting from the reduction of MTT by mitochondrial enzymes, was dissolved in DMSO (100 µL). The colour intensity of the formazan solution, indicative of cell viability, was quantified using a spectrophotometer at 540 nm wavelength, with measurements taken in triplicates. Cell viability was inferred from the formazan intensity, which correlated with the number of live cells and was expressed in terms of IC_{50} values. These values were then contrasted with those obtained from standard treatments (Doxorubicin) and blank control.[22]

Statistical analysis
The outcomes of the experiments and observations were presented as Mean + Standard Deviation (SD). GraphPad Prism 9.0 software was utilised for statistical analysis, employing suitable statistical methods to ascertain the significance of the findings.

Results and Discussion

The ethanolic extract of *Ipomoea pes-tigridis* was subjected to a preliminary phytochemical analysis, revealing several plant metabolites (Table 1). Flavonoids and tannins are secondary metabolites that plants synthesise as part of their detoxification process. These polyphenolic compounds possess strong structural features that neutralise excessive

free radicals, reinforcing the protective mechanism. Extensive scientific evidence supports the adaptogenic effect of polyphenolic compounds in managing metabolic and lifestyle disorders linked with stress. Therefore, plant-derived antioxidants, such as polyphenolic compounds, are preferred over synthetic agents in nutraceuticals to promote health due to their minimal side effects.[23] Examining plant extracts with qualitative and quantitative methods helps to understand their chemical components. Testing plant extracts for antioxidant properties using methods like the DPPH test and the nitric oxide assay is essential to know how they combat oxidative stress and to measure their possible protective effects.[24] We followed established procedures to measure the total phenolic, flavonoid, and tannin levels in ethanol extracts of *Ipomoea pes-tigridis*. The *in vitro* antioxidant screening was conducted using the DPPH and NO free radical assays, and the results were compared with ascorbic acid as a standard.

Table 1: Qualitative phytochemical constituents of *Ipomoea pes-tigridis*

Phytochemical	Inference
Carbohydrates	+
Proteins	-
Amino acids	+
Flavonoids	+
Tannins	+
Steroids	-
Saponins	+
Glycosides	-
Alkaloids	+

Present (+)/absent (-)

Phenolic compounds are recognised as antistress agents that prevent cellular damage within the body. The total phenols in the *Ipomoea pes-tigridis* were estimated by constructing a calibration graph using the detected absorbance levels at varying gallic acid concentrations. From this graph's regression formula ($y = 6.7662x + 0.2114$; $R^2 = 0.9906$), the phenolic content in the mixture quantified, in terms of milligrams of gallic acid equivalent (GAE) per gram of dried substance (mg/g) (Table 2). The phenolic content in the *Ipomoea pes-tigridis* was determined to be 93±0.54 mgGE/g. Flavonoids are an essential class of polyphenols that serve as adaptogens. They help the body cope with various environmental stresses and promote health by combating chronic diseases. In the ethanolic extract of *Ipomoea pes-tigridis*, the flavonoid content was quantified using the Dowd colourimetric method (Table 2).

Table 2: Quantitative phytochemical content of *Ipomoea pes-tigridis*

Quantitative Phytochemical	Values
Total phenolic content (TPC)	93 ± 0.54 mgGE/g
Total flavonoid content (TFC)	67 ± 0.32 mgQE/g
Total tannin content (TTC)	106.1 ± 1.45 mgTAE/g

Values are expressed as Mean ± SD of triplicate measurement

From the regression equation ($y = 7.6393x + 0.0817$; $R^2 = 0.9956$), the total flavonoid content was estimated as 67 ± 0.32 mg of Quercetin equivalents per gram of dry sample weight (mg QE/g). Various concentrations of tannic acid were used to create the calibration curve of $y = 7.1449x + 0.0911$ and a coefficient of determination (R^2) of 0.9964. The resulting data was used to calculate the total condensed tannin content in the extract of *Ipomoea pes*-tigridis. The tannin content was estimated as 106.1±1.45 mg of tannic acid equivalence per gram of sample at 500 nm wavelength (Table 2). According to the results, *Ipomoea pes-tigridis* showed a significant antioxidant capacity and increased effectiveness with the dose. A reference graph was constructed with various ascorbic acid concentrations to determine antioxidant capacity. The plant exhibited an inhibition of 47.53% (IC_{50} = 71.16 µg/ml) at a dose of 75 µg/mL, whereas ascorbic acid showed a higher inhibition of 82.44% (IC_{50} = 38 µg/ml) (Table 3). The ability of *Ipomoea pes-tigridis* to counteract DPPH suggests that it may prevent oxidative damage and provide protective advantages for the body, as shown in Figure 1. *Ipomoea pes-tigridis* proved to mitigate the effects of nitric oxide (NO) radicals. The observed inhibition exhibited an upward trend concerning the administered dosage (Table 3). The extract showed an inhibitory effect of 48.89% on NO radicals at 75 µg/mL concentration, resulting in an IC_{50} value of 74.76 µg/mL. It was observed that ascorbic acid had a significant inhibitory effect of 83.12% on these radicals, with an IC_{50} value of 41.9 µg/mL (Figure 2).

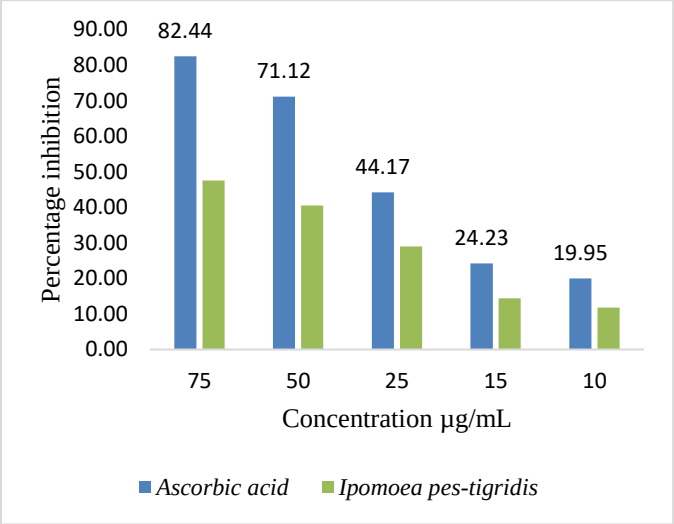

Figure 1: Antioxidant capacity of *I. pes-tigris* extract using the DPPH Assay

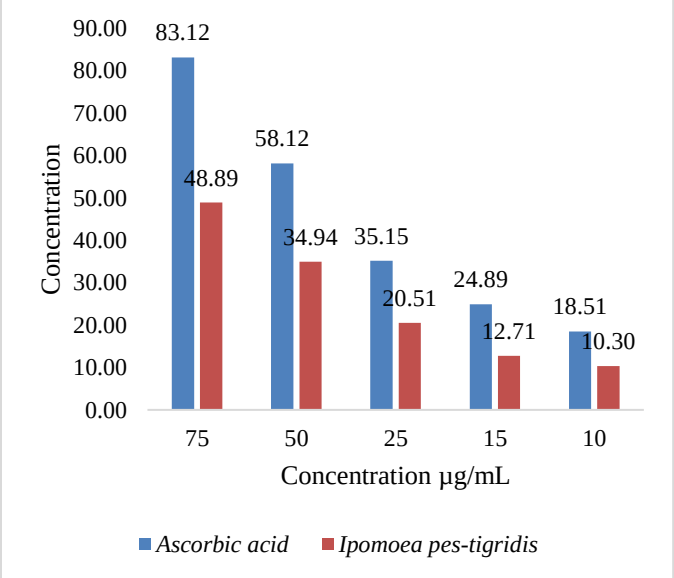

Figure 2: Nitric oxide free radical scavenging activity of *I. pes-tigris extract*

A proposition has suggested that the presence of substances such as tannins and flavonoids in the solution of *Ipomoea pes-tigridis* could potentially neutralise the radicals generated by nitroprusside throughout the experiment. This counteraction may provide protective advantages to cells under stress, as illustrated in Figure 2. The findings indicate

notable cytotoxicity towards the chosen cancer cell lines after a 72-hour incubation with the ethanolic extract of *Ipomoea pes-tigridis* at 37°C and 5% CO_2 (Table 4). The MTT test, a colourimetric method, measures the conversion of the MTT tetrazolium dye into formazan by mitochondrial enzymes in living cells. IC_{50} values of the *Ipomoea pes-tigridis* extract were determined for three cell lines: A375, B-16-F10, and NHDF. The findings suggest that *Ipomoea pes-tigridis* displays varying degrees of cytotoxicity towards the selected three cell lines. The extract exhibited an IC_{50} value of 12.02±3.14µM against A375 cells, and the relatively low IC_{50} value suggests that the extract has potent cytotoxic effects against the rapidly dividing malignant A375 cells. Against the B-16-F10 cells, the extract showed an IC_{50} value of 18.25±1.82µM, which was slightly higher than that of A375 cells, indicating that the extract is less effective in inhibiting the growth or viability of these cells. In contrast, the IC_{50} value exhibited against NHDF cells was 136.42±2.92µM, much higher than that for the other two cancer cell lines. It indicates that the extract is less toxic to normal human cells. IC_{50} values for Doxorubicin were much lower than those of the extract. The results suggest that the *Ipomoea pes-tigridis* extract has varying levels of cytotoxicity against different cell lines. It has higher potency against the two cancer cell lines (A375 and B16F10) than normal cells (NHDF) (Table 4). These results indicate that the extract may contain compounds with potential anticancer properties and warrant further investigation to identify and characterise these compounds.

Table 3: Antioxidant activity of ethanol extract of *Ipomoea pes-tigridis*

Concentration µg/mL	DPPH assay		NO free radical assay	
	Ascorbic acid	*Ipomoea pes-tigridis*	Ascorbic acid	*Ipomoea pes-tigridis*
75	82.44±0.35	47.53±1.36	83.12±1.33	48.89±1.05
50	71.12±1.06	40.48±1.08	58.12±1.28	34.94±1.54
25	44.17±0.96	28.95±1.34	35.15±1.06	20.51±1.39
15	24.23±1.07	14.42±1.26	24.89±1.54	12.71±1.57
10	19.95±1.24	11.82±1.11	18.51±1.27	10.30±1.21
IC_{50}	**38 µg/mL**	**71.16 µg/mL**	**41.9 µg/mL**	**74.76 µg/mL**

Table 4: IC_{50} values from MTT assay

Cell lines	***Ipomoea pes-tigridis* (µg/mL)**	**Doxorubicin (µM)**
A375	12.02±3.14	0.14±1.18
B16F10	18.25±1.82	0.73±1.93
NHDF	136.42±2.92	112.3±2.54

Conclusion

The ethanolic extract of *Ipomoea pes-tigridis* was analysed to determine the total quantity of flavonoids, tannins, and phenolics, as they possess potent antioxidant properties crucial for plant detoxification. These compounds are significant in managing stress-related chronic disorders. The significant antioxidant capacity of the plant extract indicated by the DPPH and NO free radical scavenging assays shows that the extract has the potential for preventing/controlling oxidative damage of cells. The cytotoxicity tests on various cell lines suggested potent anticancer properties, especially against the A375 cell line, highlighting the extract's potential therapeutic applications. The observed activities of *Ipomoea pes-tigridis* may be ascribed to phenolic compounds, such as flavonoids and tannins. Future research is underway to thoroughly assess the plant's phytochemical and pharmacological characteristics to validate its traditional uses comprehensively and report other biological activities.

Conflict of Interest

The authors declare that there is no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by authors.

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