Studies on quantification of phytochemicals in the Catharanthus roseus and Datura stramonium by HPLC and evaluation of their cytotoxic activity on HeLa Cell line

RAJAKUMAR

rajakumar.parabathina@gmail.com

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Research Article

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Abstract

The current study investigated on the phytochemical composition and cytotoxic potential of methanol extracts from *Catharanthus roseus* (Madagascar periwinkle) and *Datura stramonium* against HeLa cervical cancer cells. Alkaloids and flavonoids were isolated and characterized using Thin Layer Chromatography (TLC), the Shinoda test, and High-Performance Liquid Chromatography (HPLC). TLC analysis revealed distinct spots corresponding to flavonoids and phenolic compounds, while the Shinoda test confirmed flavonoid presence in all samples. HPLC profiling identified morin, naringin, quercetin, and rutin, with *C. roseus* leaves showing notably higher quercetin and rutin content, and higher naringin levels compared to *D. stramonium*. Cytotoxicity was evaluated using the MTT assay, revealing that *D. stramonium* leaf extract exhibited the highest cytotoxicity (78% cell death), followed by *C. roseus* leaf (71%) and stem (59%) extracts. These findings highlight *C. roseus* as a clinically established anticancer agent with a favorable safety profile, and *D. stramonium* as a potent but toxic candidate warranting dose-regulated investigation. The combined use of TLC, Shinoda, and HPLC techniques provides a robust approach for phytochemical and pharmacological studies of medicinal plants

Introduction

Cancer is one of the most prevalent diseases globally, with the World Health Organization (WHO) reporting approximately 20 million new cancer cases diagnosed in 2022. According to WHO projections, this burden is expected to rise dramatically to over 35 million new cases by 2050, representing a 77% increase from current levels. The rapidly growing cancer incidence reflects population aging, demographic changes, and evolving exposure to risk factors associated with socioeconomic development[1].

Periwinkle (*Vinca*), a genus in the Apocynaceae family, is valued for its ornamental flowers and medicinal uses. Traditionally employed to treat diabetes, hypertension, and infections, it is most notable for *Vinca rosea*, the source of the anticancer alkaloid's vincristine and vinblastine, used in chemotherapy for leukemia and Hodgkin's lymphoma. The plant also shows potential in managing hypertension and type 2 diabetes, reflecting its significance in both traditional and modern medicine [2,3].

Datura, a genus in the Solanaceae family, is known for its potent medicinal and psychoactive properties, historically used in Ayurveda and Unani medicine for asthma, pain, and muscle spasms [4]. It contains toxic tropane alkaloids such as atropine, scopolamine, and hyoscyamine, along with flavonoids, phenolic acids, steroids, triterpenoids, saponins, tannins, and essential oils that contribute antioxidant and anti-inflammatory effects [5]. Due to its toxicity, use requires strict professional supervision. High-Performance Liquid Chromatography (HPLC) enables precise identification and quantification of *Datura*'s alkaloids and flavonoids, supporting pharmacological, toxicological, and quality control studies [6].

In phytochemical analysis, HPLC is used to identify and quantify flavonoids, supporting the evaluation of their antioxidant, anti-inflammatory, and therapeutic potential [7]. The MTT assay assesses the cytotoxic and antiproliferative effects of these compounds by measuring cell viability through the enzymatic reduction of MTT to formazan, aiding in the determination of their pharmacological relevance [8].

Materials and methods

Plant Collection and Preparation

Fresh stems and leaves of the plants were collected and thoroughly washed with distilled water to remove debris. The cleaned plant material was air-dried in shade for 7–10 days, then ground into a fine powder using a grinder. The powdered samples were stored in airtight containers until further use.

Extraction of Alkaloids from Stem and Flower

Maceration Process:

A quantity of 20–50 g of powdered stem and flower material was mixed with 200–300 mL of 70% ethanol or methanol in a conical flask and macerated at room temperature for 48–72 hours with occasional shaking. The mixture was then filtered through Whatman filter paper or muslin cloth [9].

Concentration:

The filtrate was concentrated using a water bath or rotary evaporator at 40–50°C to obtain the crude ethanolic/methanolic extract.

Alkaloid Isolation:

The crude extract was dissolved in 100 mL of 1% HCl and filtered. The acidic solution was extracted with chloroform or ethyl acetate (three times) to remove non-alkaloid components. The aqueous layer was adjusted to pH 9–10 using ammonium hydroxide, then extracted with ethyl acetate or chloroform. The organic fractions were pooled and concentrated to yield the crude alkaloid fraction.

Qualitative and Quantitative Analysis

Thin-Layer Chromatography (TLC):

Alkaloid extracts were spotted on silica gel plates and developed using mobile phases such as chloroform: methanol (9:1) or ethyl acetate: hexane (7:3). Spots were visualized with Dragendorff's reagent.

HPLC Analysis

Prepared samples are filtered or diluted as necessary. The HPLC system is switched on, and solvent reservoirs are filled with the appropriate mobile phase. The column is equilibrated before sample injection. A small volume of the sample is injected into the port, and chromatographic separation is carried out with parameters (flow rate, temperature, etc.) adjusted as needed. Detection is performed using UV-Vis, fluorescence, or mass spectrometry, and chromatograms are recorded for peak identification and quantification using calibration curves or standards. After the analysis, the system is flushed with a clean mobile phase and switched to standby or turned off [9].

Cell Culture

HeLa cervical carcinoma cells are procured from the National Centre for Cell Science (NCCS), Pune, India. Cells are cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (HiMedia, Mumbai, India) and maintained at 37°C in a humidified 5% CO_2 incubator. Cells are subcultured upon reaching 80% confluence and used for subsequent cytotoxicity studies.

MTT Assay

Cell viability is assessed using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay. Cells are seeded at a density of 1×10^5 cells/well in 12-well plates and incubated for 24 h. The medium is then replaced, and

cells are treated with the test extracts, while untreated cells serve as controls. After 24 h of treatment, 200 µL of MTT solution (5 mg/mL in PBS) is added to each well (final concentration: 0.5 mg/mL), followed by incubation at 37 °C for 4 h. The medium is discarded, and 1 mL of DMSO is added to solubilize the formazan crystals. Absorbance is measured at 570 nm using a microplate reader [10].

1. **Compute percent cell viability** for each sample by dividing its absorbance by the control absorbance and multiplying by 100.
2. **Compute percent cytotoxicity** with the formula:

$$\text{Cytotoxicity (\%)} = \frac{\text{Sample Absorbance - Blank absorbance}}{\text{Control Absorbance- Blank absorbance}} \times 100$$

3. Apply this calculation to every treated sample to quantify how much each treatment reduced cell viability relative to the control.

Statistical Analysis

All experiments are performed in triplicate (n = 3), and the results are expressed as mean ± standard deviation (SD). Statistical significance, where applicable, is determined using appropriate methods, with a p-value of <0.05 considered statistically significant.

Results and Discussion

Thin Layer Chromatography was performed to analyze the phytochemical constituents of the methanolic extract of Periwinkle (Leaves and stems) and Datura leaves. The TLC plate was developed using a solvent system of methanol. Three distinct spots were observed at Rf values of 1.35, 1.2, and 0.74, indicating the presence of multiple compounds. The spot at Rf 1.35 was the most intense, suggesting it corresponds to a major component in the extract. The results suggest the presence of flavonoids and phenolic compounds shown in the Fig. 1- 3.

The HPLC analysis of the sample was performed using an RP-18 column, with a mobile phase consisting of Methanol at a flow rate of 1 mL/min. The detection was carried out at a wavelength of 350 nm, and the column temperature was maintained at 35°C. The injection volume was 20 µL. The retention times for standards Morin,
Naringin, Quercetin, and Rutin were observed at 4.047 min, 2.527 min, 2.940 min, and 2.727 min, with peak areas of 794.909, 62.786, 2312.724, and 1222.007 mAU*min shown in the Fig. 4-7.
The HPLC analysis of the sample followed the same protocol as that of the standard. The retention times observed for Naringin were 2.593 min, respectively, with peak areas of 1153.629 mAU*min, whereas in Datura leaves it was 2.673 min with a peak area of 361.80 shown in Fig. 8-9.

The MTT assay results shown in the table-1, that the control group had the highest cell viability (3.63 ± 0.23). Sample A (Datura leaf) showed the lowest absorbance (0.76 ± 0.03), indicating strong cytotoxicity. Sample B (Periwinkle leaf) had moderate cytotoxicity with an absorbance of 1.04 ± 0.03, while Sample C (Periwinkle stem) showed the least toxicity among the treated samples (1.47 ± 0.14). Cell viability followed: Control > Sample C > Sample B > Sample A.

The cytotoxic effects of the plant extracts were assessed using the MTT assay, where a reduction in absorbance at 570 nm indicates increased cytotoxicity. The Datura leaf extract showed the highest cytotoxicity of 78%, indicating a strong suppression of cell viability. The Periwinkle leaf and stem extracts showed notable cytotoxic activity with 71%

and 59% respectively. The control group served as a baseline for normal metabolic activity and exhibited no cytotoxic effects. The extract cytotoxicity is in the following order: Datura Leaf > Periwinkle Leaf > Periwinkle Stem > Control.

The MTT assay results indicate varying levels of cell viability upon treatment with different plant extracts. The control group exhibited the highest viability, noted as 100%. Confirming the presence of healthy, metabolically active HeLa cells. After the treatment with Datura leaf extract, cell viability decreased to 22%, suggesting a significant effect on cell viability. Whereas the Periwinkle leaf and stem extracts showed cell viability 29% and 41% respectively, which indicates a prominent effect on cytotoxicity of these two plants. Hence, the cell viability increased in the following order: Control > Periwinkle Stem > Periwinkle Leaf > Datura Leaf.

The results of the MTT assay performed in a 12-well plate visually demonstrate varying levels of cell death, as reflected by differences in purple coloration intensity. The top row (a), representing the Control group, shows deep purple wells, indicating minimal cell death and high cell viability. Row (b), treated with Datura leaf extract, displays a very faint purple coloration, suggesting the highest level of cell death due to strong cytotoxicity. Row (c), corresponding to Periwinkle leaf extract, shows a moderate purple hue, implying a significant degree of cell death but less than Datura. Row (d), with Periwinkle stem extract, presents a slightly darker purple than the leaf treatment, indicating comparatively lower cell death. Thus, the pattern of increasing cell death follows the order: Datura Leaf > Periwinkle Leaf > Periwinkle Stem > Control.

The microscopic images shown in Fig 13, labeled a) to d) illustrate the morphological changes in cells under different treatment conditions. Image a) Control represents the untreated group, where cells appear healthy, well-spread, and intact, with normal morphology and minimal debris. This indicates the typical growth pattern of healthy cells. In contrast, image b) Datura leaf shows signs of early cytotoxic effects, as evident from cell rounding, partial detachment, and a slight increase in cellular debris. These changes suggest the beginning of stress-induced cellular responses, likely due to exposure to Datura leaf extract. Image c) Periwinkle Leaf displays the most severe morphological alterations, including extensive cell shrinkage, fragmentation, and significant debris. This suggests a high degree of cytotoxicity, likely caused by the bioactive compounds in Periwinkle leaf extract, leading to cell death through apoptosis or necrosis. Image d) Periwinkle Stem presents moderate cellular damage compared to b) and c), with a mixture of intact and damaged cells, suggesting an intermediate level of cytotoxicity. Overall, the images indicate a progressive cytotoxic effect of the different plant extracts, with increasing severity from Datura leaf to Periwinkle leaf, while the Control group remains unaffected.

The present study provides a comprehensive evaluation of the phytochemical profile and cytotoxic activity of methanolic extracts from *Catharanthus roseus* and *Datura stramonium* against HeLa cervical cancer cells. The combined use of TLC, Shinoda test, and HPLC proved effective in confirming and quantifying key bioactive constituents, particularly flavonoids and alkaloids. TLC analysis revealed distinct bands corresponding to multiple phytochemicals, with the major spot at Rf 1.35 indicating a high concentration of flavonoids and phenolic compounds, consistent with previous reports on plant-derived flavonoids [2]. The positive Shinoda test further confirmed flavonoid presence in both species, aligning with earlier documentation of quercetin and related flavonoids in *C. roseus* and *D. stramonium* [1].

HPLC profiling identified four major flavonoids—morin, naringin, quercetin, and rutin—across the samples. Quantitatively, *C. roseus* demonstrated higher concentrations of quercetin and rutin, both of which are widely recognized for their antioxidant, anti-inflammatory, and anticancer activities [2-5]. Naringin levels exhibited a pronounced difference between species, being significantly higher in *C. roseus* (1153.629 mAU·min) than in *D. stramonium* (361.803 mAU·min), suggesting species-specific variations in flavonoid biosynthesis. These findings

substantiate existing literature describing *C. roseus* as a rich source of bioactive flavonoids with therapeutic potential [12].

Cytotoxic evaluation through the MTT assay revealed significant anticancer activity in all plant extracts. *D. stramonium* leaf extract exhibited the strongest cytotoxic effect, inducing 78% cell death, followed by *C. roseus* leaf (71%) and stem extracts (59%). These results are in line with previous studies reporting the potent but dose-sensitive cytotoxicity of *Datura* species due to tropane alkaloids such as atropine, scopolamine, and hyoscyamine [5,9]. Despite its potency, *D. stramonium*'s therapeutic use remains limited by its narrow safety margin and high toxicity, necessitating controlled dosing and further toxicological assessments [9-12].

In contrast, *C. roseus* displayed substantial cytotoxicity coupled with a more favorable clinical safety record. Its anticancer activity is well established and largely attributed to vinca alkaloids vincristine and vinblastine—which disrupt microtubule assembly and inhibit mitosis [4,9]. The present findings reinforce *C. roseus* as a validated phytopharmaceutical agent, supporting its historical and ongoing clinical relevance in cancer treatment.

The integration of qualitative (TLC, Shinoda test) and quantitative (HPLC) analyses allowed precise characterization of bioactive markers, while the MTT assay provided functional evidence of cytotoxicity. Together, these results underscore the pharmacological potential of both plants, particularly their roles in anticancer, antioxidant, and antimicrobial applications [2,4,12]. Furthermore, the study highlights the utility of combining chromatographic and spectrophotometric techniques for robust phytochemical investigations and provides a foundation for future mechanistic and isolation-based research.

The present study demonstrates that both *Catharanthus roseus* and *Datura stramonium* possess significant phytochemical diversity and notable anticancer potential against HeLa cells. The combined use of TLC, Shinoda testing, and HPLC enabled the precise detection and quantification of key bioactive compounds, particularly morin, naringin, quercetin, and rutin. *D. stramonium* leaf extract exhibited the highest cytotoxic activity, indicating the potency of its alkaloids and flavonoids, though its clinical application is limited by toxicity concerns. *C. roseus*, in contrast, showed substantial cytotoxicity alongside a well-documented clinical safety record, largely attributable to its vinca alkaloids. These findings underscore the value of integrating phytochemical profiling with in vitro cytotoxicity assays for the identification of promising plant-derived anticancer agents. Future studies should focus on mechanistic evaluations, bioactive compound isolation, and toxicity assessments to support the potential therapeutic applications of these medicinal plants.

Declarations

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Conflicts of interest

NIL.

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Table

Table No. 1- Absorbance Values Indicating Cytotoxicity of Datura and Periwinkle (Leaf & Stem) Extracts in HeLa Cells (570 nm, 24 h)

Average	Absorbance ± SD
Control	3.63 ± 0.23
Sample A	0.76±0.03
Sample B	1.04±0.03
Sample C	1.47±0.14

Figures

Figure 1

(TLC of Periwinkle Leaf, Periwinkle stem and Datura leaf)

Figure 2

(TLC of Periwinkle Leaf, Periwinkle stem and Datura leaf)

Figure 3

(TLC of Periwinkle Leaf, Periwinkle stem and Datura leaf)

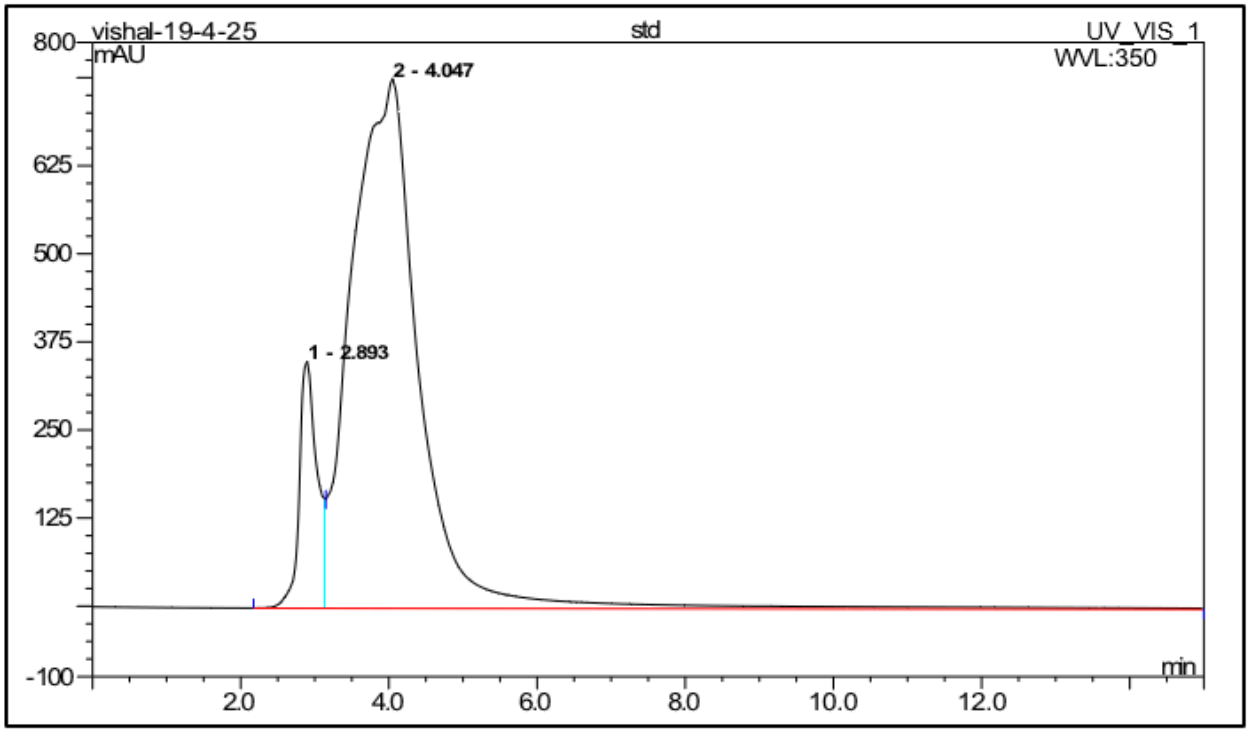

Figure 4

Chromatogram of standard Morin

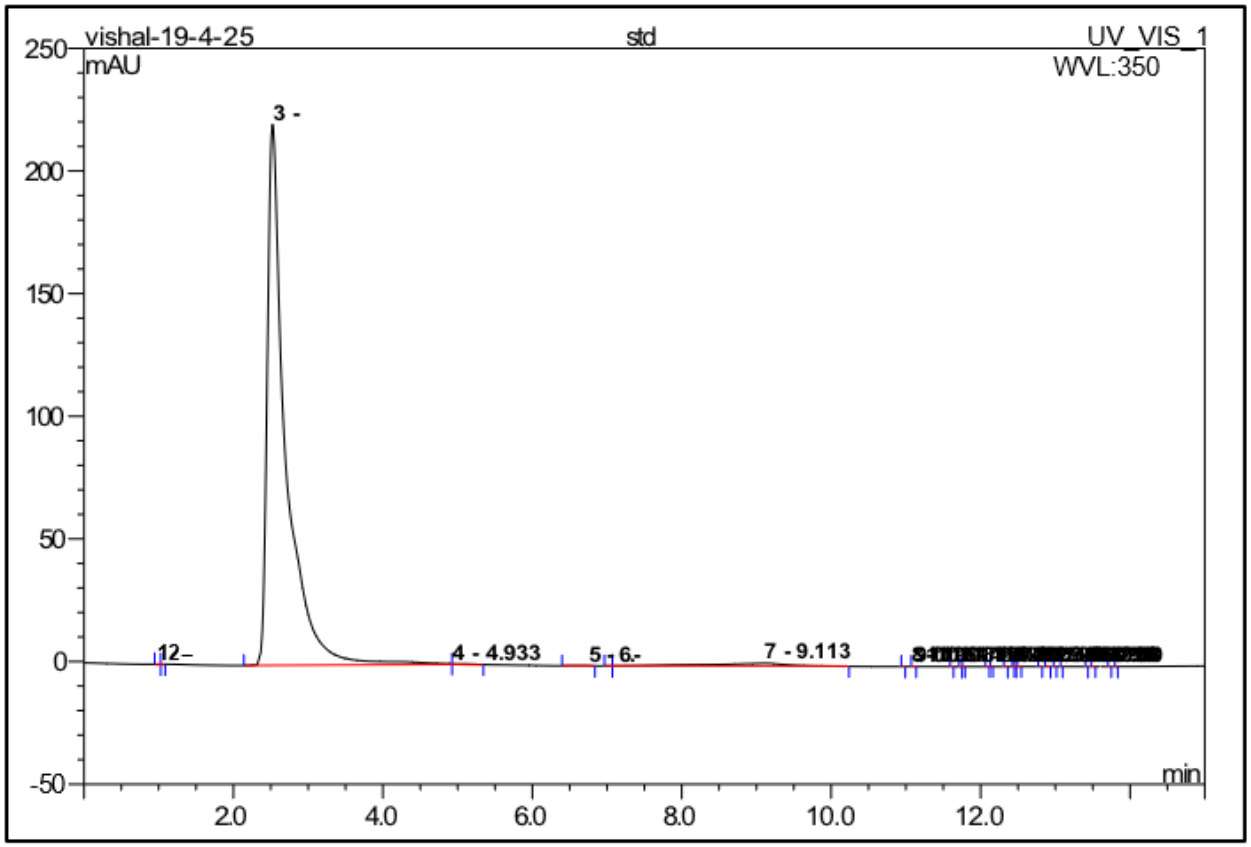

Figure 5

Chromatogram of Standard Naringin.

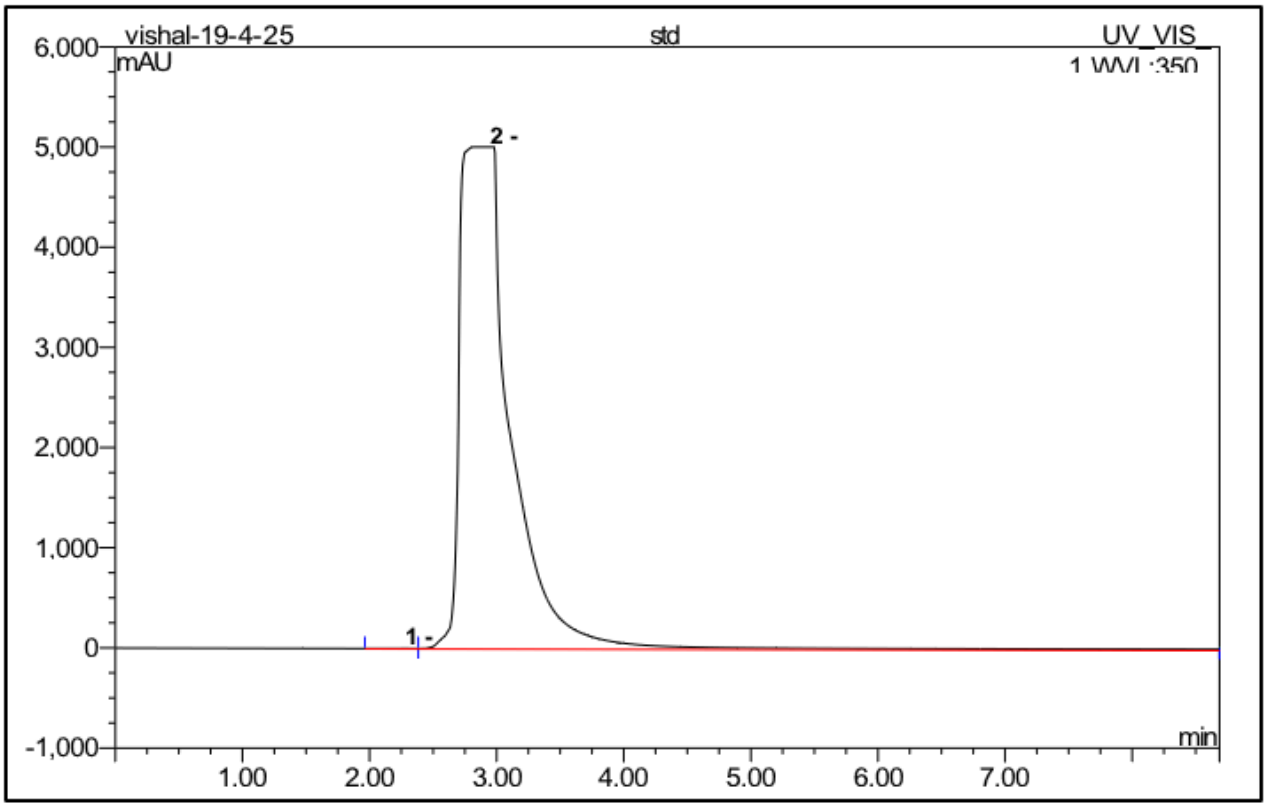

Figure 6

Chromatogram of standard Quercetin

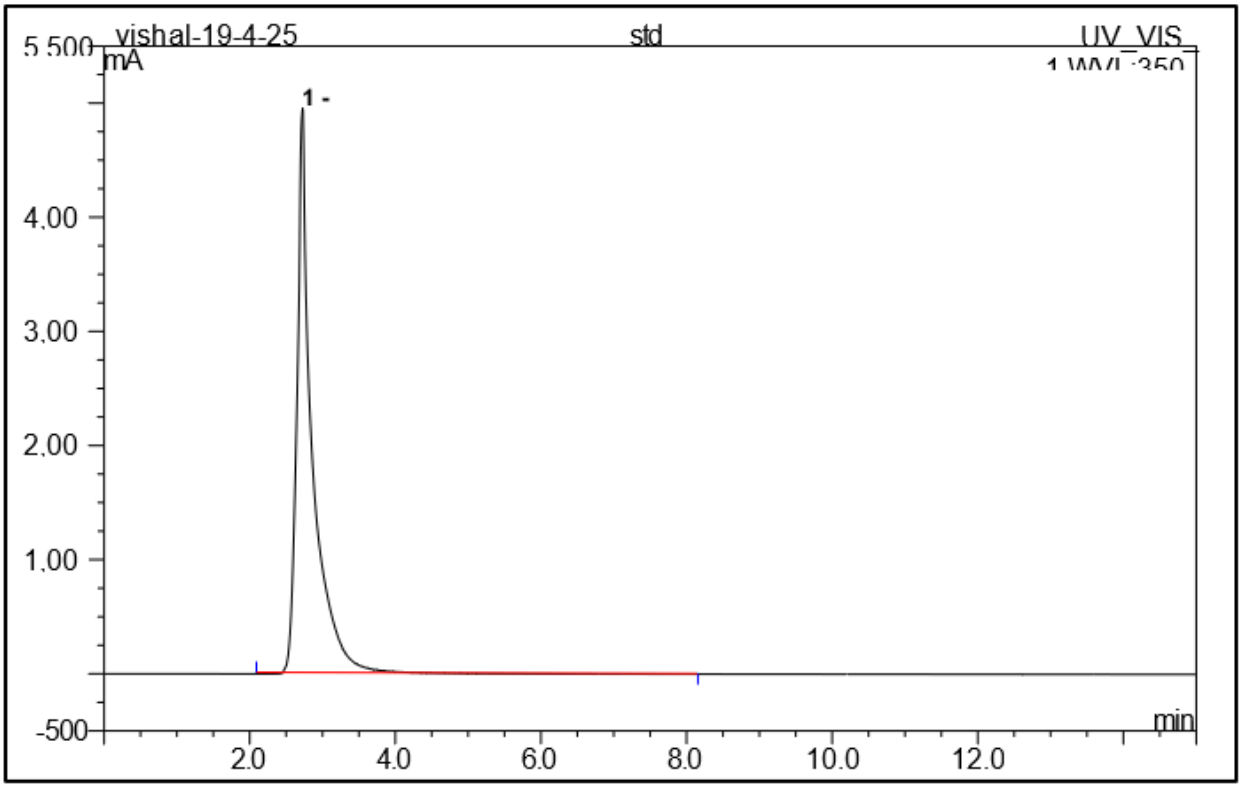

Figure 7

Chromatogram of standard Rutin

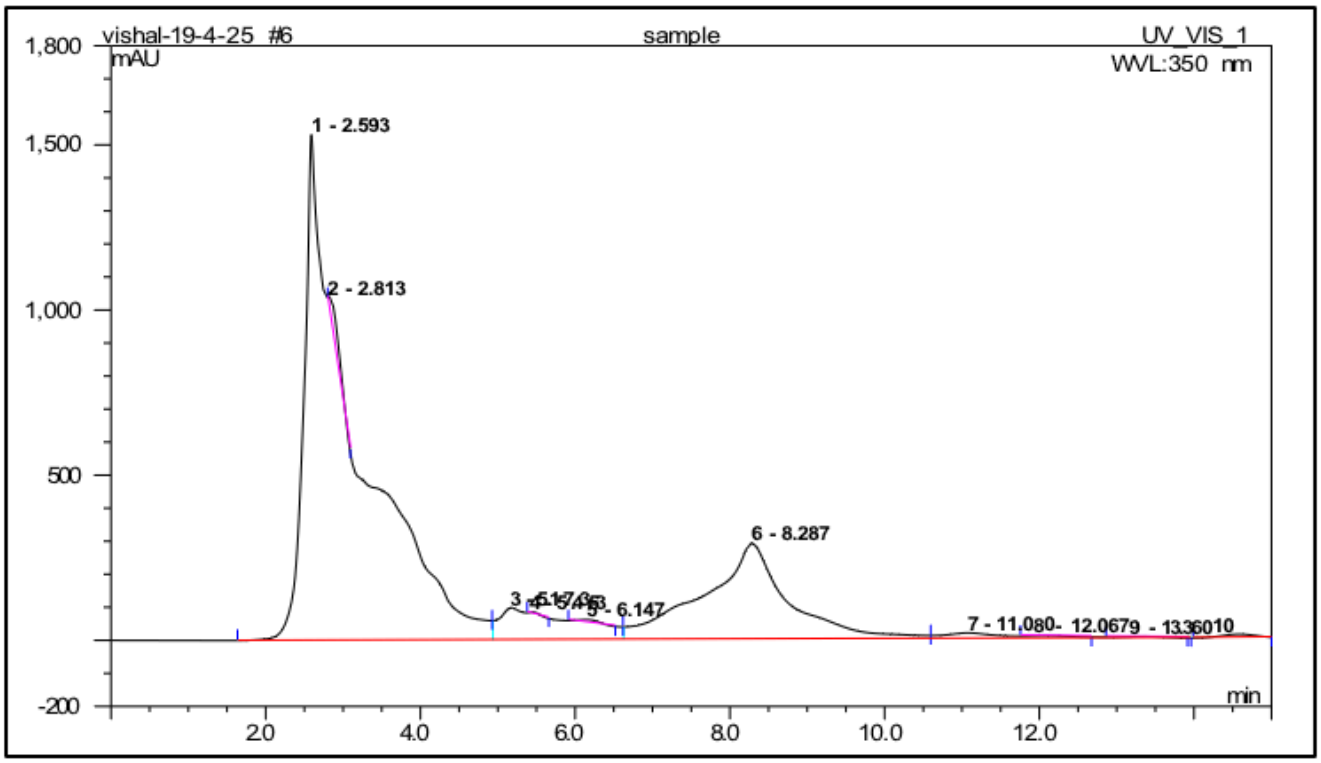

Figure 8

Chromatogram of Periwinkle Leaves

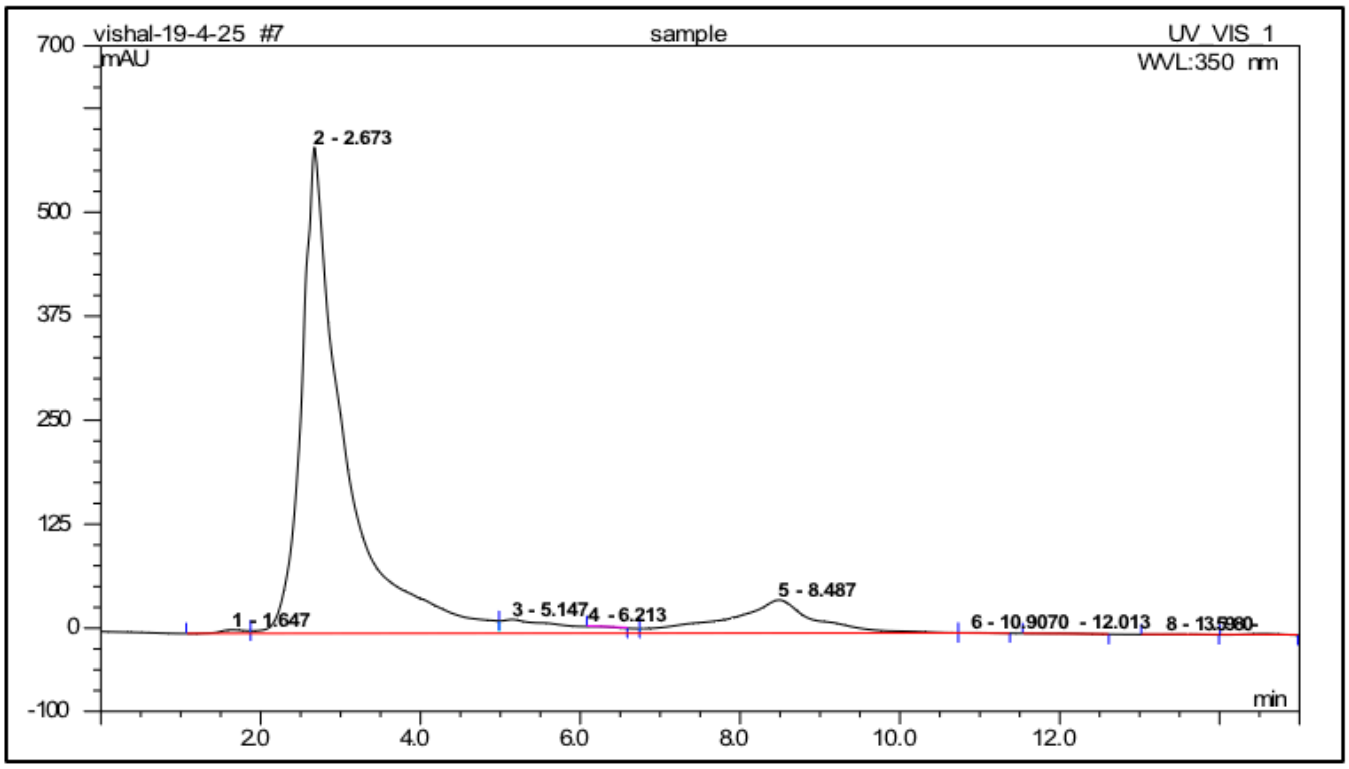

Figure 9

Chromatogram of Datura Leaf

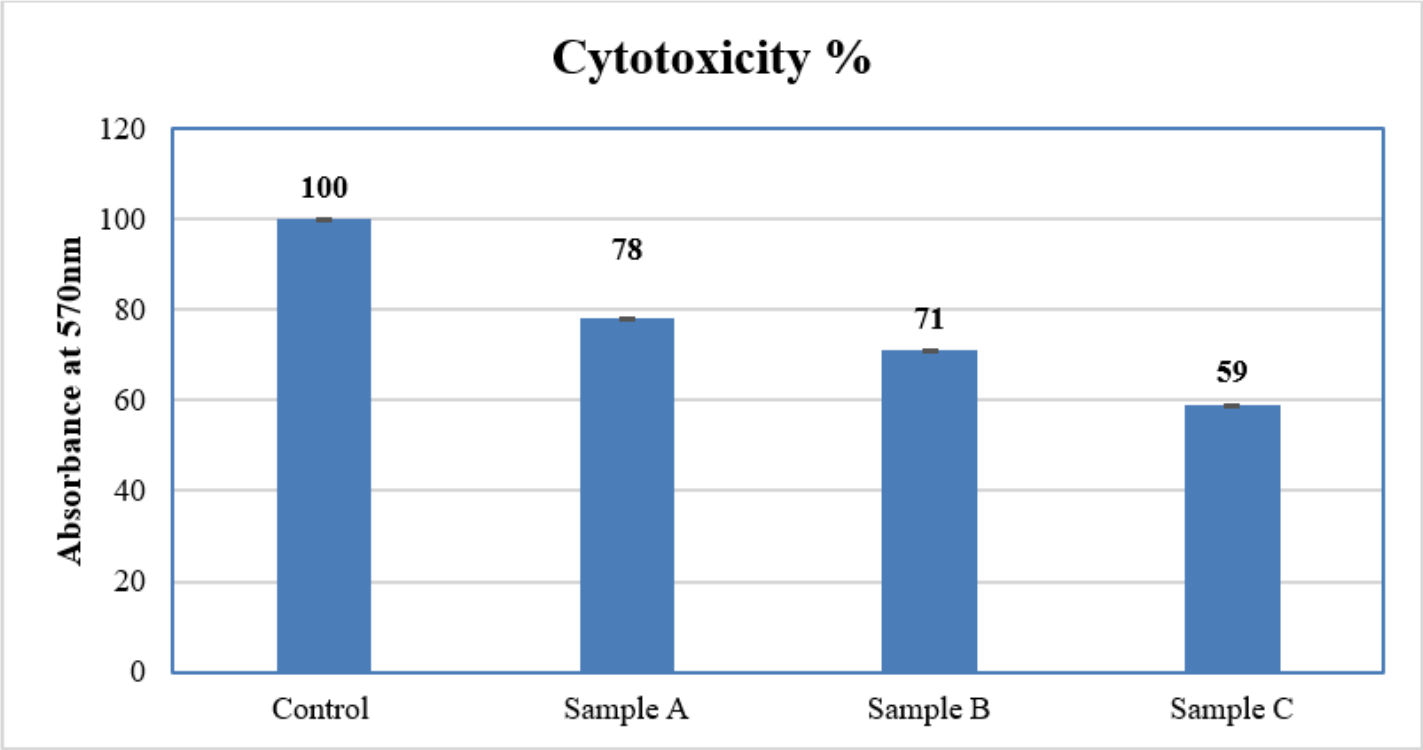

Figure 10

Cytotoxicity effect by MTT assay.

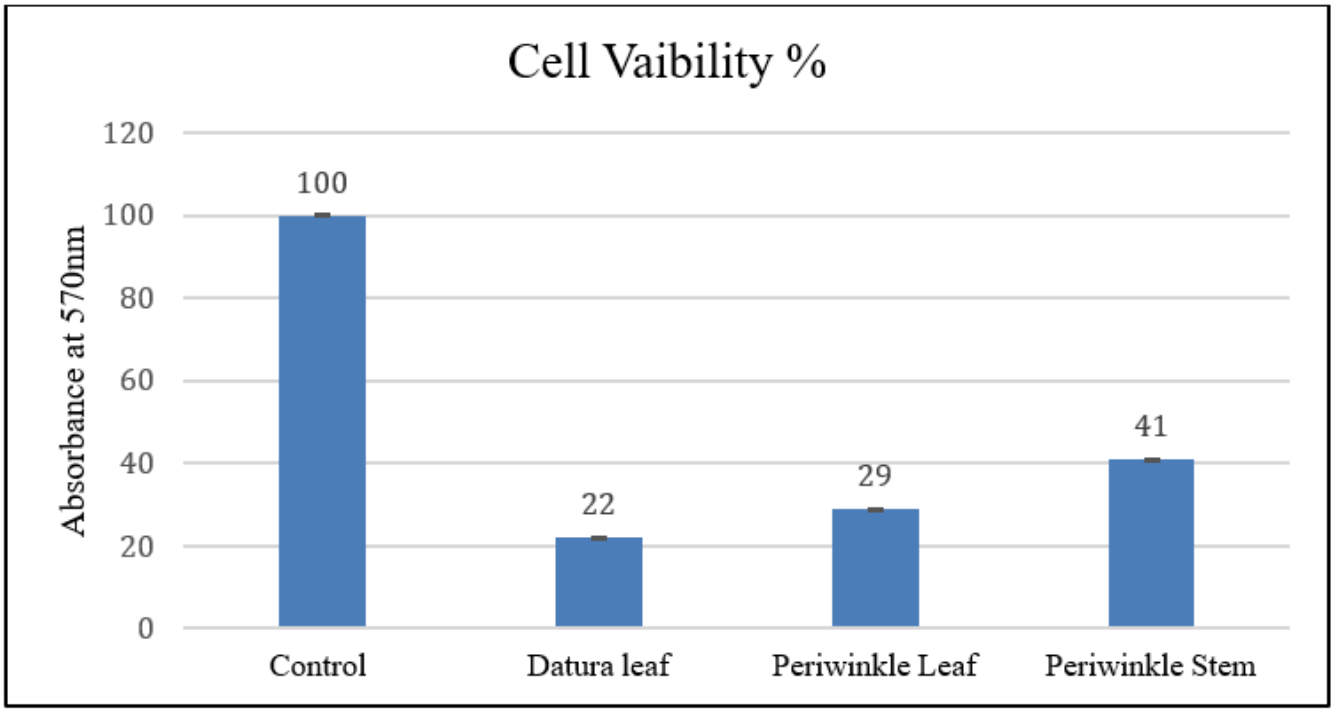

Figure 11

Cell viability by MTT Assay.

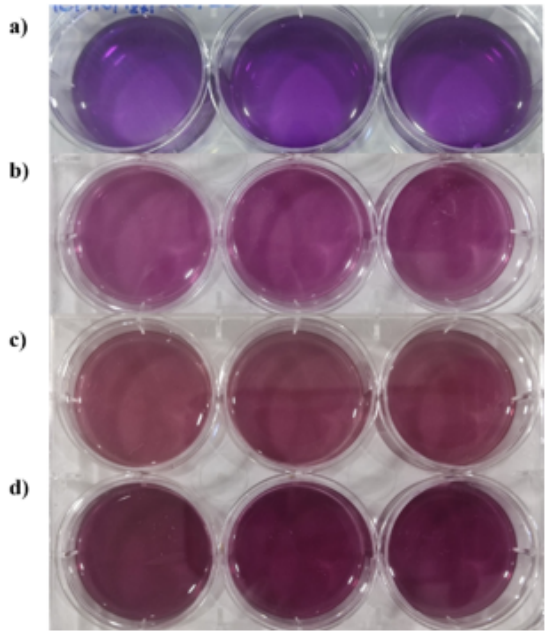

Figure 12

a) Control, b) Datura leaf, c) Periwinkle Leaf, d) Periwinkle Stem.

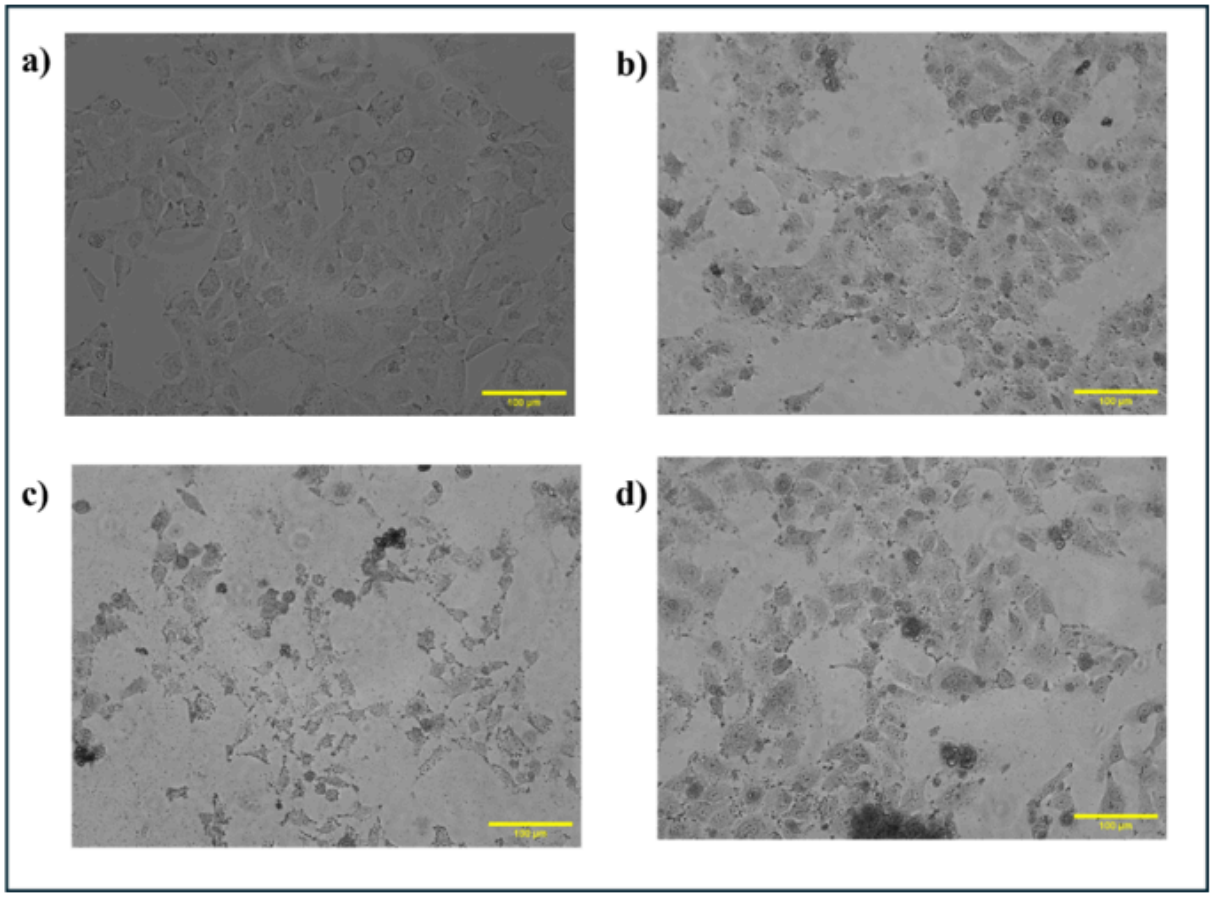

Figure 13

a) Control, b) Datura leaf, c) Periwinkle Leaf, d) Periwinkle Stem.