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Research Article

Formulation, Characterization, and In Vitro Evaluation of *Catharanthus roseus* Leaf Extract Tablets as Anticancer Agents

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ABSTRACT

Background- Catharanthus roseus is a well-known medicinal plant rich in vinca alkaloids with established anticancer properties. However, there is a need to develop stable and effective dosage forms to enhance its therapeutic application. Objective-The present study aimed to extract, standardize, formulate, and evaluate Catharanthus roseus leaf extract into a tablet dosage form and to assess its in vitro anticancer activity. Methods- Hydroalcoholic extraction of Catharanthus roseus leaves was performed using Soxhlet apparatus. The extract was subjected to phytochemical screening and standardization. Tablets were formulated by wet granulation using suitable excipients. Preformulation, granule, and tablet evaluations were carried out as per standard procedures. In vitro drug release studies were performed using USP dissolution apparatus. Anticancer activity was evaluated using the MTT assay on cancer cell lines, and IC_{50} values were determined. Stability studies were conducted under accelerated conditions. Results- The extract showed a percentage yield of 12.3% and contained alkaloids, flavonoids, tannins, glycosides, and saponins. Preformulation and granule studies indicated good flow and compressibility. All tablet formulations complied with pharmacopeial standards. Optimized formulations demonstrated rapid drug release, with nearly complete release within 45 minutes. The extract exhibited significant concentration-dependent cytotoxic activity with an IC_{50} value of 59.84 µg/mL, while the standard drug showed higher potency (IC_{50}: 33.10 µg/mL). Stability studies confirmed the robustness of the formulation. Conclusion- The study successfully developed a stable tablet formulation of Catharanthus roseus extract with significant anticancer activity. Although less potent than the standard drug, the extract demonstrates promising therapeutic potential and warrants further investigation.

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INTRODUCTION

Cancer remains one of the leading causes of mortality worldwide and poses a significant challenge to public health due to its complex pathophysiology and limitations associated with conventional therapies.[1] It is characterized by uncontrolled cell proliferation, invasion, and metastasis, which contribute to disease progression and poor prognosis. Although chemotherapy is widely used for cancer treatment, it is often associated with severe side effects, drug resistance, and non-specific toxicity to normal cells.[2] These limitations have driven the search for safer and more effective therapeutic alternatives, particularly from natural sources. Medicinal plants have long been recognized as valuable sources of bioactive compounds with diverse pharmacological activities.[3] A significant proportion of currently used anticancer drugs are derived from natural products, highlighting their importance in drug discovery. Among them, Catharanthus roseus (family: Apocynaceae) is a well-known medicinal plant that has gained considerable attention due to its potent anticancer properties.[4] It is a rich source of indole alkaloids, especially vinca alkaloids such as vincristine and vinblastine, which are widely used in chemotherapy. These compounds exert their anticancer effects primarily by inhibiting microtubule formation, thereby preventing cell division and inducing apoptosis in rapidly proliferating cancer cells.[5] In addition to alkaloids, Catharanthus roseus contains other phytoconstituents such as flavonoids, tannins, glycosides, and saponins, which contribute to its overall therapeutic potential. These compounds may act synergistically to enhance biological activity, including antioxidant and anticancer effects. However, the direct use of plant extracts in therapy is often limited by issues related to stability, dosage accuracy, and bioavailability, which may affect therapeutic outcomes.[6] To overcome these limitations, formulation of plant extracts into suitable dosage forms is essential. Tablet dosage forms offer several advantages, including ease of administration, accurate dosing, improved stability, and better patient compliance. Moreover, standardized formulations help in maintaining batch-to-batch consistency and reproducibility of therapeutic effects.[7]

Therefore, the present study was designed to extract and standardize Catharanthus roseus leaf extract, formulate it into tablets using the wet granulation method, and evaluate its physicochemical properties. Furthermore, the in vitro anticancer activity of the extract was assessed using the MTT assay to determine its cytotoxic potential against cancer cells. This study aims to provide a scientific basis for the development of a plant-based anticancer formulation with potential therapeutic applications.

MATERIALS AND METHODS

Materials

Fresh leaves of *Catharanthus roseus* were collected and used as the primary source of vinca alkaloids for the present study. Organic solvents such as ethanol (70%) and methanol, along with chemical reagents including hydrochloric acid, Mayer's reagent, Dragendorff's reagent, and Wagner's reagent, were of analytical grade and procured from certified suppliers for extraction and phytochemical analysis. Pharmaceutical excipients employed for tablet formulation comprised microcrystalline cellulose and lactose monohydrate as diluents, polyvinylpyrrolidone (PVP K-30) as a binder, croscarmellose sodium as a superdisintegrant, magnesium stearate as a lubricant, and talc as a glidant. Double-distilled water was used throughout the study for all experimental procedures.



Figure 1. Fresh leaves of *Catharanthus roseus*

Extraction of the plant material was carried out using a Soxhlet apparatus, and the obtained extract was concentrated using a digital rotary evaporator. Drying of the extract was performed in a temperature-controlled hot air oven, and the dried material was powdered using a mechanical grinder followed by sieving through mesh No. 40 to obtain uniform particle size. Physicochemical parameters were evaluated using a high-precision digital analytical balance and a digital pH meter. Quantitative analysis, including drug content estimation, was performed using a double-beam UV–visible spectrophotometer, while Fourier-transform infrared (FTIR) spectroscopy was employed to assess drug–excipient compatibility.

Tablet formulation was carried out using a single-punch or rotary tablet compression machine. The prepared tablets were evaluated for hardness using a Monsanto hardness tester, thickness using Vernier calipers, and friability using a Roche friabilator. Disintegration and dissolution studies were conducted using USP disintegration and dissolution test apparatus (Type II, paddle method), respectively. Stability studies were performed under controlled temperature and humidity conditions using a stability chamber. For in vitro anticancer evaluation, SH-SY5Y human neuroblastoma cells were maintained in a controlled CO_2 incubator. Cell viability and cytotoxic effects were assessed using the MTT assay, and absorbance was measured using an ELISA-based microplate reader.

Collection and Authentication of Plant Material

Leaves of *Catharanthus roseus* were collected from a local medicinal plant source during the flowering season. The plant material was authenticated by a qualified taxonomist, and a voucher specimen was deposited in the departmental herbarium for future reference. The collected material was washed thoroughly to remove adhering impurities and air-dried under shade at ambient temperature.[8]

Preparation of Vinca Extract

Drying and Powdering

The cleaned plant material was shade-dried for 7–10 days until constant weight was achieved. The dried leaves were pulverized using a mechanical grinder and sieved through mesh No. 40 to obtain uniform powder.[9]



Figure 2. Drying and Powdering

Extraction Procedure

The extraction of bioactive constituents was carried out using the Soxhlet extraction method, which is a continuous hot extraction technique widely used for plant materials. Approximately 200 g of the powdered plant material was placed in a thimble and extracted using 500 mL of 70% hydroalcoholic solvent (ethanol:water). The choice of hydroalcoholic solvent was based on its ability to dissolve a wide range of phytoconstituents, including alkaloids. The extraction process was conducted for 6–8 hours, corresponding to approximately 10–12 extraction cycles, until the solvent in the siphon tube became colorless, indicating exhaustive extraction. The extract obtained was filtered to remove insoluble plant residues and then concentrated using a rotary evaporator under reduced pressure at a temperature not exceeding 45°C to prevent thermal degradation of active constituents.[10]

Concentration and Drying

The concentrated extract was further dried in a vacuum desiccator to remove residual solvent and obtain a semisolid or dry extract. The final extract was weighed, transferred into airtight containers, and stored at refrigerated conditions (4°C) to maintain stability and prevent microbial contamination.[11]

Percentage Yield

The percentage yield of the extract was calculated to evaluate the efficiency of the extraction process. It was determined using the following formula:

Yield (%) = (Weight of dried extract / Initial weight of plant material) × 100

This parameter is important for standardization and reproducibility of the extraction method.[12]

Phytochemical Screening

Preliminary phytochemical screening was carried out to qualitatively identify the presence of major classes of secondary metabolites in the extract. These compounds are responsible for the biological and pharmacological activities of the plant. Standard chemical tests were performed using specific reagents to detect alkaloids, flavonoids, tannins, glycosides, and saponins. The appearance of characteristic color changes or precipitates indicated the presence of respective phytoconstituents.[13]

Standardization of Extract

Total Alkaloid Estimation

Quantitative estimation of total alkaloids was performed using UV–visible spectrophotometry. The extract was treated with appropriate reagents

to form a colored complex, and absorbance was measured at a specific wavelength. A calibration curve was prepared using a suitable standard, and the alkaloid content was expressed in terms of equivalent concentration.[14]

Thin Layer Chromatography (TLC)

TLC analysis was performed to separate and identify different components present in the extract. Silica gel-coated plates were used as the stationary phase, and an appropriate solvent system was selected as the mobile phase. The extract was spotted on the plate and developed in a TLC chamber. After development, the plate was visualized under UV light (254 nm and 366 nm), and spots were recorded.[15]

The retention factor (Rf) value was calculated using the formula:

Rf = Distance travelled by compound / Distance travelled by solvent front

pH Determination

The pH of a 1% w/v solution of the extract was measured using a calibrated digital pH meter to assess its acidity or alkalinity, which is important for formulation stability.[16]

Moisture Content

Moisture content was determined by the loss on drying (LOD) method. A known quantity of extract was heated at 105°C until constant weight was obtained. This parameter helps in assessing the stability and shelf life of the extract.[17]

Preformulation Studies

Preformulation studies were conducted to evaluate the physicochemical properties of the extract and to ensure its suitability for tablet formulation. Organoleptic properties such as color, odor, and appearance were recorded. Solubility studies were performed in different solvents to determine the solubility profile. Flow properties were assessed by determining parameters such as angle of repose, bulk density, tapped density, Carr's index, and Hausner ratio. These parameters are essential for predicting the flow behavior and compressibility of the powder. Drug–excipient compatibility studies were carried out using FTIR spectroscopy to ensure that there were no interactions between the extract and excipients.[18]

Formulation of Vinca Extract Tablets

Vinca extract tablets were formulated using the wet granulation method to obtain tablets with acceptable mechanical strength and drug release characteristics. Different formulations were prepared by varying the concentration of diluents and superdisintegrant while maintaining constant drug content. Microcrystalline cellulose and lactose were used as diluents, whereas croscarmellose sodium served as a superdisintegrant. Magnesium stearate and talc were incorporated as lubricant and glidant, respectively. The composition of different tablet formulations is presented in Table 6.4.[19]

Table 1: Composition of Vinca Extract Tablet Formulations (F1–F5)

Ingredients	Function	F1 (mg)	F2 (mg)	F3 (mg)	F4 (mg)	F5 (mg)
Vinca Extract	Active ingredient	100	100	100	100	100
Microcrystalline Cellulose (MCC PH-102)	Diluent	95	85	75	65	55
Lactose Monohydrate	Diluent	55	65	75	85	95
Polyvinylpyrrolidone (PVP K-30)*	Binder	15	15	15	15	15
Croscarmellose Sodium	Superdisintegrant	8	12	16	20	24

Magnesium Stearate	Lubricant	3	3	3	3	3
Talc	Glidant	2	2	2	2	2
Total Tablet Weight	—	**278 mg**	**282 mg**	**286 mg**	**290 mg**	**294 mg**

Evaluation of Granules

The granules prepared by the wet granulation method were evaluated for their micromeritic and flow properties to ensure their suitability for compression into tablets. Proper evaluation of granules is essential to achieve uniform die filling, consistent tablet weight, and optimal mechanical strength. Various parameters such as flow behavior, density characteristics, and moisture content were assessed using standard procedures.[20]

The following parameters were evaluated:

Angle of Repose: Determined by the fixed funnel method, where granules were allowed to flow freely to form a conical heap. The angle between the surface of the pile and the horizontal plane was measured. It indicates the flowability of granules, where lower values suggest better flow properties.

$$\theta = \tan^{-1}\left(\frac{h}{r}\right)$$

Where:

- h = height of the pile
- r = radius of the base

Bulk Density: Measured by introducing a known mass of granules into a graduated cylinder and recording the volume occupied without tapping. It reflects the packing ability of the granules under normal conditions.

$$\rho_b = \frac{M}{V_b}$$

Where:

- M= mass of granules
- V_b= bulk volume (untapped)

Tapped Density: Determined by mechanically tapping the cylinder containing granules until a constant volume was achieved. This parameter indicates the maximum packing capacity of the granules.

$$\rho_t = \frac{M}{V_t}$$

Where:

- M= mass of granules
- V_t= tapped volume

Compressibility Index (Carr's Index): Calculated using bulk and tapped density values. It provides an indication of flowability and compressibility, where lower values indicate better flow properties.

$$\text{Carr's Index} = \frac{\rho_t - \rho_b}{\rho_t} \times 100$$

Hausner Ratio: Derived from the ratio of tapped density to bulk density. A value close to 1 indicates good flow characteristics of granules.

$$\text{Hausner Ratio} = \frac{\rho_t}{\rho_b}$$

Moisture Content: Determined using the loss on drying method at a specified temperature. It is important to control moisture levels, as excess

moisture can adversely affect flow properties, stability, and compressibility of granules.[21]

$$\text{Moisture Content (\%)} = \frac{W_i - W_f}{W_i} \times 100$$

Where:

- W_i= initial weight
- W_f= final weight after drying

Evaluation of Tablet

The prepared tablets were evaluated according to standard pharmacopeial specifications to ensure their quality, uniformity, mechanical strength, and performance. These evaluation parameters are essential to confirm that the tablets meet the required standards for safety, efficacy, and patient compliance. Various physical and chemical tests were carried out using appropriate instruments and standard procedures.[22]

The following parameters were evaluated:

- **Weight Variation:** Twenty tablets were individually weighed using a digital analytical balance, and the average weight was calculated. The individual weights were compared with the average weight to ensure compliance with pharmacopeial limits, indicating uniformity in tablet weight.
- **Thickness:** Tablet thickness was measured using Vernier calipers. Uniform thickness ensures consistent tablet size and is important for packaging and patient acceptability.
- **Hardness:** The hardness of tablets was determined using a Monsanto hardness tester and expressed in kg/cm². This test evaluates the mechanical strength of tablets and their ability to withstand handling and transportation.
- **Friability:** Friability was assessed using a Roche friabilator. Pre-weighed tablets were subjected to 100 revolutions at 25 rpm for 4 minutes. The percentage weight loss was calculated, and values below 1% indicate acceptable resistance to abrasion.
- **Disintegration Time:** The disintegration time was determined using a USP disintegration test apparatus in a suitable medium maintained at 37 ± 0.5°C. This test measures the time required for tablets to break down into smaller particles, which is essential for drug release.
- **Drug Content Uniformity:** Tablets were crushed, and a known quantity was dissolved in a suitable solvent. After appropriate dilution, the solution was analyzed spectrophotometrically. This test ensures uniform distribution of the active ingredient within the tablets.
- **In Vitro Dissolution Study:** Dissolution studies were performed using a USP Type II (paddle) dissolution apparatus. The tablets were placed in an appropriate dissolution medium maintained at 37 ± 0.5°C with constant paddle rotation. Samples were withdrawn at predetermined time intervals, filtered, and analyzed spectrophotometrically to determine the drug release profile.[23]

Drug Release Kinetic Studies

Dissolution data obtained were fitted into various kinetic models including zero-order, first-order, Higuchi, and Korsmeyer–Peppas equations to elucidate the mechanism of drug release from formulated tablets.[24]

In Vitro Anticancer Activity

The anticancer activity of the vinca extract formulation was evaluated using the MTT assay, which is a widely used colorimetric assay for assessing cell viability and cytotoxicity. Selected human cancer cell lines were cultured in appropriate growth media under controlled conditions of temperature, humidity, and carbon dioxide. The cells were seeded into microtiter plates and allowed to adhere. Various concentrations of the extract were added to the cells and incubated for 24–48 hours. After incubation, MTT reagent was added to each well and further incubated. Viable cells with active metabolism reduced the yellow-colored MTT to insoluble purple-colored formazan crystals. The crystals formed were dissolved using a suitable solvent such as dimethyl sulfoxide (DMSO). The absorbance of the resulting solution was measured at 570 nm using a spectrophotometer or microplate reader. The percentage of cell viability was calculated by comparing treated cells with control cells. The IC_{50} value, representing the concentration required to inhibit 50% of cell growth, was determined from the dose–response curve, indicating the cytotoxic potential of the formulation.[25,26]

Stability Studies

Stability studies were conducted to evaluate the effect of environmental factors such as temperature and humidity on the physical and chemical stability of the formulated tablets. The studies were performed according to ICH guidelines under accelerated conditions of 40°C ± 2°C and 75% ± 5% relative humidity for a period of three months. The tablets were packed in suitable containers and stored in a stability chamber. Samples were withdrawn at specific time intervals (0, 1, 2, and 3 months) and evaluated for various parameters including physical appearance, hardness, friability, drug content, and dissolution profile. Any significant changes observed during the study were recorded and analyzed. These studies help in predicting the shelf life of the formulation and ensuring that the product maintains its quality, safety, and efficacy throughout its intended storage period.[27]

RESULTS AND DISCUSSION

Extraction and Percentage Yield

The hydroalcoholic extraction of *Catharanthus roseus* leaves was carried out using Soxhlet apparatus to ensure exhaustive extraction of phytoconstituents. The selection of 70% ethanol as the solvent system played a crucial role in extracting a wide spectrum of bioactive compounds, particularly alkaloids, due to its intermediate polarity. The extraction process yielded a dark green semisolid mass with a characteristic odor, indicating the presence of plant-derived secondary metabolites. The efficiency of extraction was evaluated in terms of percentage yield, which is an important parameter for standardization and reproducibility of herbal formulations. A satisfactory yield suggests optimal extraction conditions and effective solvent penetration into plant tissues.

Table 2: Percentage Yield of Extract

Parameter	Observation
Weight of dried plant material (g)	200
Weight of dried extract (g)	24.6
Percentage yield (%)	12.3%

The percentage yield obtained indicates efficient extraction of phytoconstituents. The yield is within the acceptable range for hydroalcoholic extraction, supporting its suitability for further formulation development.

Phytochemical Screening

Preliminary phytochemical screening was conducted to qualitatively identify the major classes of secondary metabolites present in the extract. These phytoconstituents are responsible for the biological activities of the plant, including anticancer effects. Standard chemical tests were performed using specific reagents, and the formation of characteristic color changes or precipitates confirmed the presence of various compounds. The detection of alkaloids is particularly important, as *Catharanthus roseus* is known for its vinca alkaloids, which possess potent cytotoxic properties.

Table 3: Phytochemical Screening of Extract

Phytoconstituent	Test Performed	Observation	Result
Alkaloids	Mayer's test	Cream precipitate	Present
Alkaloids	Dragendorff's test	Reddish-brown ppt	Present
Alkaloids	Wagner's test	Brown ppt	Present
Flavonoids	Shinoda test	Pink color	Present
Tannins	Ferric chloride test	Blue-black color	Present
Glycosides	Keller–Killiani test	Brown ring	Present
Saponins	Foam test	Persistent foam	Present

The presence of multiple phytoconstituents confirms the therapeutic potential of the extract. Alkaloids contribute to anticancer activity, while flavonoids and tannins provide antioxidant support.

Standardization of Extract

The standardization of herbal extract is essential to ensure quality, consistency, and reproducibility. Various physicochemical and analytical parameters such as alkaloid content, TLC profile, pH, and moisture content were evaluated. These parameters provide insight into the chemical composition and stability of the extract, which are critical for formulation development.

Table 4: Standardization Parameters

Parameter	Result
Total alkaloid content	8.5 mg/g
pH (1% solution)	6.2
Moisture content (%)	4.1%
TLC Rf values	0.32, 0.58, 0.74

The alkaloid content confirms the richness of active compounds. The pH and moisture content are within acceptable limits, indicating stability.

The TLC profile suggests the presence of multiple constituents.

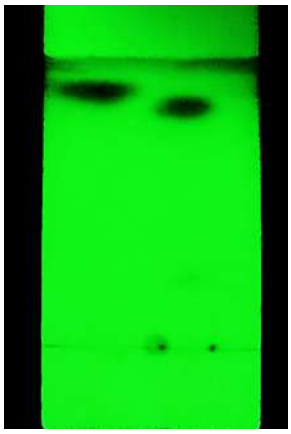

Figure 3. Thin Layer Chromatography

Preformulation Studies

Preformulation studies were performed to evaluate the physicochemical and flow properties of the extract powder. These studies are crucial in determining the suitability of the extract for tablet formulation. Parameters such as angle of repose, bulk density, tapped density, Carr's index, and Hausner ratio were assessed. The results indicate the flow behavior and compressibility of the powder, which directly affect tablet uniformity and mechanical strength.

Table 5: Preformulation Parameters

Parameter	Result	Interpretation
Angle of repose (°)	27.5°	Good flow
Bulk density (g/cm³)	0.42	—
Tapped density (g/cm³)	0.49	—
Carr's index (%)	14.2%	Good compressibility
Hausner ratio	1.16	Good flow

The results indicate good flow and compressibility, making the extract suitable for wet granulation and tablet compression.

Evaluation of Granules

Granules prepared by the wet granulation method were evaluated to determine their suitability for tablet compression. The assessment of micromeritic properties is essential to ensure proper flow behavior, uniform die filling, and consistent tablet weight. Parameters such as angle of repose, bulk density, tapped density, Carr's index, and Hausner ratio were analyzed using standard methods. The angle of repose was found to be 25.8°, indicating excellent flow properties. Bulk density and tapped density were recorded as 0.45 g/cm³ and 0.52 g/cm³, respectively, suggesting good packing ability. The Carr's index (13.5%) and Hausner ratio (1.15) further confirmed good compressibility and flowability of the granules.

Table 6: Granule Evaluation

Parameter	Result
Angle of repose (°)	25.8°
Bulk density (g/cm³)	0.45
Tapped density (g/cm³)	0.52
Carr's index (%)	13.5%
Hausner ratio	1.15

Overall, the granules showed improved flow properties compared to the raw extract, which can be attributed to the formation of uniform and dense particles during granulation. These characteristics ensure smooth compression and minimize processing issues.

Evaluation of Tablets

The prepared tablets (F1–F5) were evaluated for quality control parameters to ensure compliance with pharmacopeial standards. Parameters such as weight variation, hardness, friability, thickness, disintegration time, and drug content were studied to assess the mechanical strength and performance of tablets. All formulations showed acceptable weight uniformity, indicating proper die filling. Hardness values ranged from 4.2 to 5.2 kg/cm², which is sufficient to withstand handling. Friability values were below 1%, confirming good mechanical resistance, while thickness remained consistent across all batches.

Table 7: Physical Evaluation of Tablets

Formulation	Weight (mg)	Hardness (kg/cm²)	Friability (%)	Thickness (mm)
F1	278	4.2	0.85	3.1
F2	282	4.5	0.72	3.2
F3	286	4.8	0.65	3.3
F4	290	5.0	0.58	3.4
F5	294	5.2	0.52	3.5

Disintegration time decreased progressively with increasing concentration of croscarmellose sodium, demonstrating its effectiveness as a superdisintegrant. Drug content across all formulations was within acceptable limits, indicating uniform distribution of the active ingredient.

Table 8: Disintegration Time and Drug Content

Formulation	Disintegration Time (min)	Drug Content (%)
F1	12.5	96.2
F2	10.2	97.5
F3	8.6	98.3
F4	6.8	99.1
F5	5.5	99.5

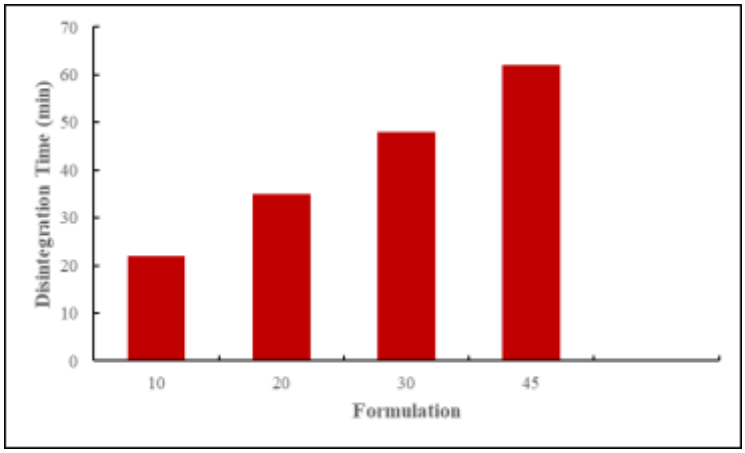

Figure 4. Effect of Superdisintegrant on Disintegration Time

Formulations F4 and F5 showed faster disintegration and better drug uniformity, indicating optimized performance due to higher superdisintegrant concentration.

In Vitro Dissolution Study

The dissolution study was performed to evaluate the drug release behavior of the tablets. This test is essential for predicting in vivo performance and ensuring therapeutic efficacy. The results showed a clear increase in drug release with higher concentrations of croscarmellose sodium. Formulations F1 and F2 exhibited slower release, while F4 and F5 showed rapid and nearly complete drug release within 45 minutes. This can be attributed to faster disintegration and improved wettability of the tablet matrix.

Table 9: Drug Release Profile (% Release)

Time (min)	F1	F2	F3	F4	F5
10	22	28	35	42	48
20	35	45	55	65	72
30	48	60	70	82	90
45	62	75	85	95	98

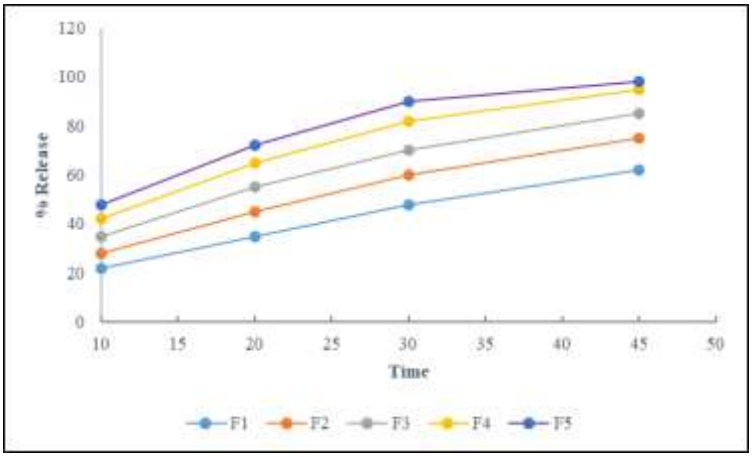

Figure 5. In Vitro Drug Release Profile of Vinca Extract Tablets (F1–F5)

Among all formulations, F5 showed the highest drug release, followed by F4, indicating superior dissolution performance.

In Vitro Anticancer Activity

The cytotoxic potential of vinca extract tablets was evaluated using the MTT assay and compared with the standard drug Vincristine. The assay is based on the reduction of MTT to formazan crystals by viable cells, where a decrease in absorbance indicates reduced cell viability. The results demonstrated a clear concentration-dependent inhibition of cancer cell growth for both the standard and the extract. The control group exhibited maximum cell viability, whereas increasing concentrations resulted in a progressive reduction in cell viability and increased percentage inhibition. The standard drug showed higher cytotoxic activity at all concentrations, with maximum inhibition of 84.87% at 100 µg/mL. In comparison, the vinca extract exhibited 70.57% inhibition at the same concentration, indicating significant but comparatively lower potency. The IC_{50} value for the extract was found to be 59.84 µg/mL, whereas the standard drug showed a lower IC_{50} value of 33.10 µg/mL, confirming higher potency of the standard. The observed anticancer activity of the extract can be attributed to the presence of vinca alkaloids, which interfere with microtubule formation and inhibit cell division.

These findings demonstrate that the extract possesses significant cytotoxic potential and supports its possible use as a natural anticancer agent.

Table 10. Cytotoxic Activity of Vinca Extract

SR NO	SAMPLE CODE	Conc. (µg/ml)	OD			Mean	% Of Inhibition	% Of Viability	IC50 (µg/ml)
1	Control		1.573			-	-	-	-
2	Standard	20	0.813	0.819	0.815	0.815	48.19%	51.81%	
	(Vincristine)	40	0.679	0.673	0.682	0.678	56.90%	43.10%	**33.10**
		60	0.536	0.539	0.532	0.535	65.99%	34.01%	
		80	0.401	0.403	0.409	0.404	74.32%	25.68%	
		100	0.239	0.235	0.241	0.238	84.87%	15.13%	
3	Vinca Extract	20	1.125	1.129	1.122	1.125	28.48%	71.52%	
		40	0.922	0.921	0.925	0.922	41.39%	58.61%	**59.84**
		60	0.783	0.782	0.788	0.784	50.16%	49.84%	
		80	0.613	0.617	0.623	0.617	60.78%	39.22%	
		100	0.462	0.460	0.467	0.463	70.57%	29.43%	

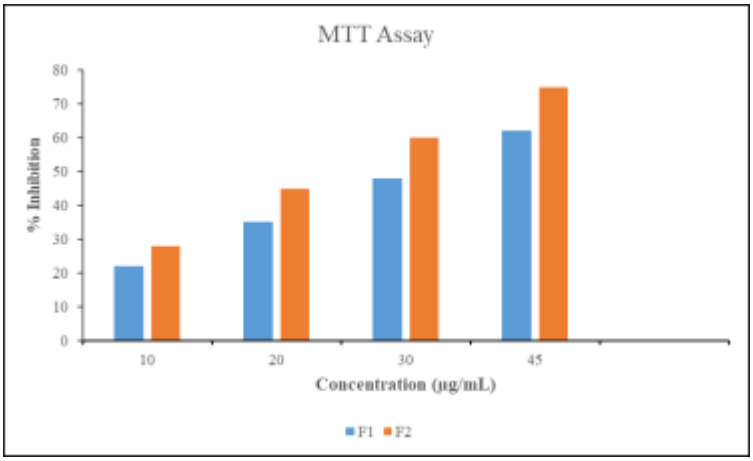

Figure 6. Effect of Vinca Extract and Vincristine on Cell Viability using MTT Assay

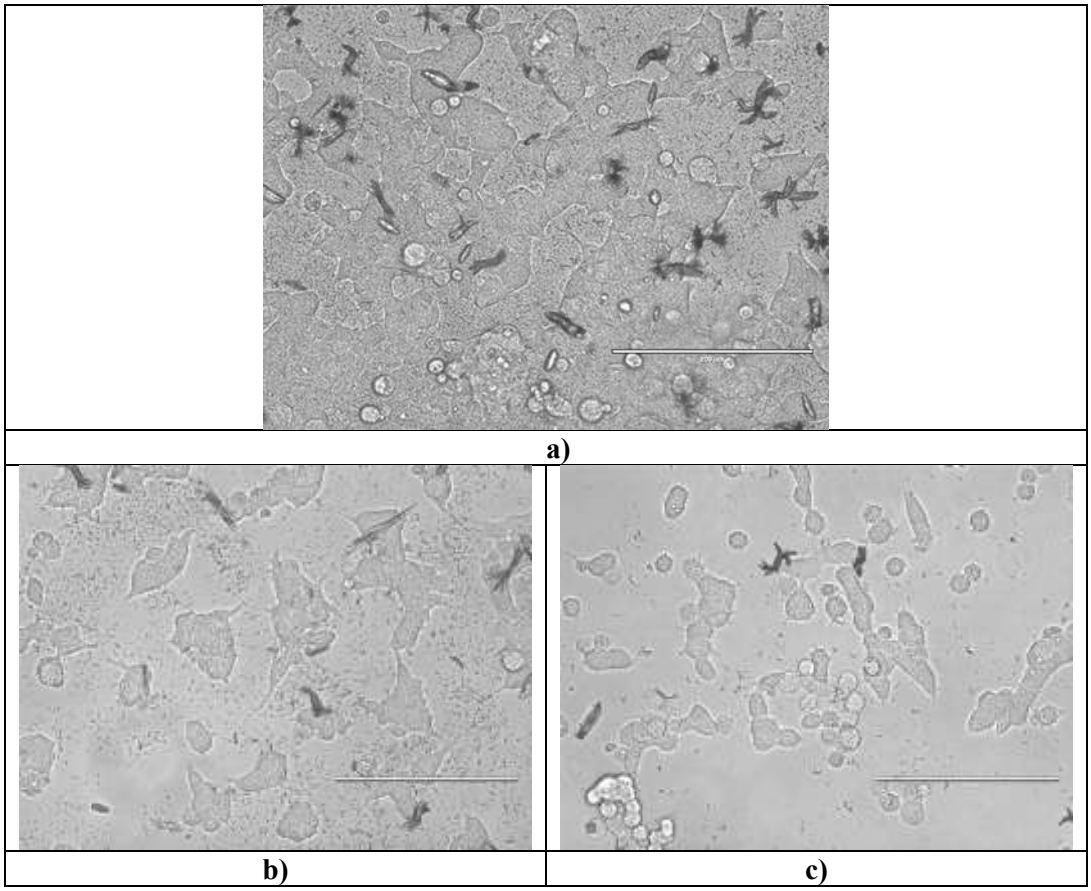

Figure 7. Microscopic images showing cytotoxic effects. (a) Control cells with normal morphology. (b) Cells treated with standard (vincristine) showing high cytotoxicity. (c) Cells treated with vinca extract showing moderate cytotoxic effects.

Stability Studies

Stability studies were conducted under accelerated conditions to evaluate the effect of environmental factors on tablet quality over time. Parameters such as hardness, friability, drug content, and dissolution were monitored for three months. The results showed minimal variation in all parameters, indicating good stability of the formulation. Although slight decreases in hardness and drug content were observed, they remained within acceptable limits.

Table 11. Stability Study Results

Time	Hardness	Friability	Drug Content (%)	Release (%)
0 month	5.0	0.58	99.1	95
1 month	4.9	0.60	98.7	94
2 months	4.8	0.63	98.2	93
3 months	4.7	0.65	97.9	92

These findings confirm that the formulation maintains its quality under stress conditions and is suitable for storage.

The present study aimed to develop and evaluate a tablet formulation of *Catharanthus roseus* leaf extract and assess its in vitro anticancer activity. The hydroalcoholic extraction yielded 12.3% extract, indicating efficient recovery of

phytoconstituents using 70% ethanol. Phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, glycosides, and saponins, while standardization showed a total alkaloid content of 8.5 mg/g, pH 6.2, and moisture content of 4.1%, ensuring extract quality and stability. Preformulation studies demonstrated good flow properties (angle of repose: 27.5°, Carr's index: 14.2%, Hausner ratio: 1.16), which improved after granulation (angle of repose: 25.8°, Carr's index: 13.5%, Hausner ratio: 1.15). All tablet formulations complied with pharmacopeial limits, with hardness ranging from 4.2–5.2 kg/cm² and friability below 1%. Disintegration time decreased from 12.5 to 5.5 minutes with increasing croscarmellose sodium concentration, indicating effective superdisintegrant action. Dissolution studies showed enhanced drug release with optimized formulations, achieving up to 98% release within 45 minutes (F5), compared to 62% in F1. The MTT assay revealed concentration-dependent cytotoxicity, with an IC_{50} value of 59.84 µg/mL for the extract, compared to 33.10 µg/mL for the standard drug. Maximum inhibition observed was 70.57% at 100 µg/mL, confirming significant anticancer activity. Stability studies indicated minimal variation, with drug content decreasing slightly from 99.1% to 97.9% and drug release from 95% to 92% over three months under accelerated conditions. Overall, the study demonstrates that *Catharanthus roseus* extract can be formulated into a stable tablet with appreciable anticancer activity, although further in vivo and mechanistic studies are required to establish clinical relevance.

CONCLUSION

The present study successfully established the formulation and evaluation of *Catharanthus roseus* leaf extract into a stable and effective tablet dosage form with promising anticancer potential. The hydroalcoholic extraction method yielded a phytochemically rich extract containing key bioactive constituents such as alkaloids, flavonoids, tannins, glycosides, and saponins, which are known to contribute to therapeutic activity. The formulated tablets exhibited satisfactory physicochemical and mechanical properties, complying with pharmacopeial standards for weight variation, hardness, friability, disintegration, and drug content uniformity. Optimized formulations demonstrated rapid and efficient drug release, indicating suitability for oral delivery and effective bioavailability. In vitro anticancer evaluation using the MTT assay confirmed significant, concentration-dependent cytotoxic activity of the extract against cancer cell lines, with a measurable IC_{50} value. Although the extract showed comparatively lower potency than the standard drug, its activity is notable considering its crude, multi-component nature. The observed effects are primarily attributed to vinca alkaloids, supported by possible synergistic contributions from other phytoconstituents. Stability studies further confirmed the robustness and reliability of the developed formulation under accelerated conditions, indicating good shelf-life potential. Overall, this study highlights the feasibility of developing a plant-based anticancer formulation from *Catharanthus roseus*. The findings support its potential as a safer, natural therapeutic alternative; however, further investigations involving isolation of active compounds, detailed mechanistic studies, and in vivo evaluations are essential to validate its clinical applicability.

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