Harnessing the potential of *Catharanthus roseus*: From enhanced alkaloid extraction to targeted therapeutic applications

Keerthana S[1], Sarath Perumal[2], Subanu Priya Amirthalingam[3], Sasikumar Kandasamy[4], Pavithra Madhiyazhagan[3]*

1. Junior Executive, Department of Production Editing, SPi Technologies India Pvt Ltd, Chennai, Tamil Nadu, India
2. Department of Biotechnology, School of Bio Sciences and Technology, Vellore Institute of Technology, Vellore- 632014, Tamil Nadu, India.
3. PG Department of Biotechnology, Dwaraka Doss Goverdhan Doss Vaishnav College, Chennai-106, Tamil Nadu, India.
4. SS Herbals, Senguttai taluk, Dharmapuri, Tamil Nadu, India.

*Corresponding author:
Dr Pavithra Madhiyazhagan,
Assistant Professor,
PG Department of Biotechnology,
Dwaraka Doss Goverdhan Doss Vaishnav College,
Chennai-106, Tamil Nadu, India.

ABSTRACT

Our study focuses on the extraction, characterisation, and evaluation of pharmacological activities of alkaloids from *Catharanthus roseus*. Employing both Soxhlet and cold extraction techniques, the research optimised the extraction of bioactive compounds, including the clinically significant alkaloids vincristine and vinblastine. The extracts were systematically analysed using Gas Chromatography-Mass Spectroscopy (GC-MS) and subjected to a series of confirmatory tests to validate the presence of alkaloids. In addition to assessing their well-documented anticancer properties via MTT-based cell viability assays in colorectal cancer cells, the study also evaluated the extracts' antibacterial, antioxidant, and anti-inflammatory activities using standard in vitro assays. Novel aspects of this research include the exploration of the extracts' potential in modulating angiogenesis, a process crucial for tumour progression and wound healing, and the assessment of anti-diabetic activity through chick embryo assay. Furthermore, the study explored additional therapeutic applications such as sunscreen efficacy and wound healing, thereby highlighting the multifaceted pharmacological profile of *C. roseus*. Overall, the findings support the hypothesis that an optimised extraction process enhances the yield of key alkaloids but also improves their therapeutic efficacy, paving the way for novel, plant-based interventions in cancer, diabetes, and angiogenesis-related disorders.

KEYWORDS: *Catharanthus roseus,* alkaloids, cold extraction, chick embryo assay, sun screen activity, novel therapeutics

INTRODUCTION

Catharanthus roseus, commonly known as the Madagascar periwinkle, is a widely recognised medicinal plant with a rich botanical and cultural history. Native to Madagascar, this species has adapted to diverse habitats ranging from tropical forests to urban gardens in various parts of the world [1]. The plant's vibrant flowers and distinctive foliage not only make it a popular ornamental species but also an important resource in traditional medicine. Its ability to flourish in varied ecological niches underscores its evolutionary versatility and sets the stage for its extensive use in pharmacological research aimed at harnessing its therapeutic compounds [2]. Over the years, alkaloids have been the backbone of several life-saving drugs, providing treatments for infections, chronic diseases, and even complex conditions such as cancer. The molecular diversity and potent biological activities of these compounds enable them to interact with various cellular targets, leading to innovative therapeutic strategies [3]. In an era marked by increasing drug resistance and the emergence of new health challenges, the exploration of plant-derived alkaloids remains a vibrant area of research. Their multifaceted roles in human health have not only cemented their importance in traditional remedies but also spurred modern pharmacological investigations[4].

Within the vast family of plant alkaloids, vincristine and vinblastine have garnered significant attention due to their potent anticancer properties. Extracted from *C. roseus*, these compounds have revolutionised chemotherapy protocols for several types of cancer, including leukaemia, lymphoma, and various solid tumours. Despite their proven efficacy, the extraction of these alkaloids poses several challenges, with conventional methods often yielding suboptimal purity and quantity. Such an integrated approach is designed to enhance the yield and purity of these critical alkaloids, potentially leading to more effective and accessible cancer therapies [5]. Based on the promising pharmacological attributes of *C. roseus* and its bioactive alkaloids, the central hypothesis of this study is that an optimised extraction process will significantly improve the yield and efficacy of vincristine

and vinblastine. Moreover, the hypothesis extends to the possibility that the synergistic interaction of these alkaloids with other phytochemicals present in the plant may further potentiate their medicinal benefits. Ultimately, these findings are anticipated to contribute to the development of more effective cancer treatment strategies, while also highlighting the broader therapeutic potential of plant-based bioactive compounds in modern medicine.

MATERIALS AND METHODS

Collection of samples

The leaves and flowers of *C. roseus* were collected from Kolathur, Chennai, and Thiruvalangadu, Arakkonam. The collected samples were cleaned with water to get rid of impurities, shade-dried for 3 weeks, and then ground finely.

Extraction of alkaloids from leaves and flowers

Soxhlet Extraction

The powdered samples of leaves and flowers of *C. roseus* were taken and soaked in hexane overnight, and kept in an orbital shaker. The next day, the hexane was discarded, and the sample was dried to allow the remaining hexane to evaporate. Then, 50 grams of the sample was taken and packed into the Soxhlet chamber. 250 mL of Hexane was used as a solvent to run the Soxhlet. Then the process was repeated with chloroform and methanol, following the same procedure, along with 24 hours of evaporation time in between each solvent. The temperature was maintained between 50°C and 60°C. The marc after methanol extraction was then processed for cold extraction [6].

Cold Extraction

The marc was acidified with 2M H_2SO_4 and incubated with trichloromethane for 30 minutes, followed by filtration with Whatman filter paper. This step was repeated three times. The aqueous layer of the sample was taken, and ammonium hydroxide was added until the pH of the sample reached 10. Following this, trichloromethane-methanol (3:1) extraction was carried out. The aqueous basic layer was separated and further evaporated with methanol, and alkaloids were extracted [7]. Further, the presence of alkaloids in the extract was determined using Dragendorff's, Mayer's, and Wagner's tests.

Gas Chromatography-Mass Spectroscopy analysis

This method is highly significant for the identification of various phytochemicals present in plants. The GC-MS analysis was performed using a QP-2010 Ultra instrument. The detection system employed an electron ionisation system with an ionising energy of 70 eV. Helium gas of 99.999% purity served as the carrier gas, maintained at a constant flow rate of 1 mL/min. A volume of 1 µl of the *C. roseus* extract was injected, with a split ratio of 10:1. The injector temperature was set at 250°C, while the ion-source temperature was maintained at 280°C. The total running time for each sample was approximately 76 minutes [8].

Anti-Bacterial Activity

A reference solution called the McFarland standard was used to standardise the density of bacterial suspensions used in this study. To prepare a 0.5 McFarland standard, 0.5 mL of 1.175% (w/vol) barium chloride dihydrate ($BaCl_2 \cdot 2H_2O$) solution was mixed with 99.5 mL of 1% (vol/vol) sulphuric acid. This will produce a turbidity that is equal to a given concentration of bacterial cells, which is usually measured spectrophotometrically at 625 nm. Petri dishes underwent sterilisation, and 20 ml of Muller-Hinton Agar was prepared for each dish. The medium was preserved for sterilisation and subsequently dispensed onto the dish post-sterilisation. A volume of 100 microliters of fresh cultures (5-6 hours) of Gram-positive bacteria (*Bacillus subtilus* and *Staphylococcus aureus*) and Gram-negative bacteria (*Pseudomonas aeruginosa*) culture was uniformly applied to the agar surface, and subsequently, a perforation of 6–8 mm in diameter was created using a cork-borer. Following this, the leaf and flower extracts of *C. roseus* were incorporated. Then the agar plate was incubated under optimal conditions, and the diffusion and antimicrobial effects of the phytochemical compound were discerned. The ensuing inhibition zone was quantified in terms of diameter after a 24-hour incubation period [9].

Anti-Oxidant Activity

The DPPH assay, as described by Chang *et al.* (2001) [10], was used to evaluate the radical scavenging activity of different extracts. At 517 nm, the decline in absorbance of the DPPH solution after the addition of an antioxidant was evaluated. As a standard, ascorbic acid (10 mg/ml DMSO) was used, and ethanol was used as a blank.0.1mM DPPH solution was prepared by dissolving 4mg of DPPH in 100 mL of ethanol. After diluting the extract with water, *C. roseus* extracts were taken in different volumes (20–100µl), and 2.96 ml of DPPH (0.1 mM) solution was added. The resulting mixture was allowed to incubate at room temperature for half an hour in the dark. The solution's absorbance was then determined at 517 nm. 3 ml of DPPH on its own was used as a control. The radical scavenging activity was calculated with the following formula:

$$\%RSA = \frac{Absorbance\ of\ Control - Absorbance\ of\ \_\_}{Absorbance\ of\ Control} \times 100$$

Anti-Inflammatory Activity

Tissue deterioration and inflammation are frequently linked to the denaturation process. Thus, substances or compounds that effectively decrease egg albumin's denaturation in this assay may show promising anti-inflammatory properties [11]. The fresh egg of a hen was cracked gently, and then 1 mL of the translucent part (egg albumin) into 100 mL of volumetric (w/v) distilled water [12]. A reaction mixture with a total volume of 5 mL is made using the following constituents: 0.2 mL of 1-2% egg albumin solution (from fresh hen's egg), 2 mL of the *C. roseus* extract dissolved in distilled water at varying concentrations (20–100µl), and 2.8 mL of phosphate-buffered saline (pH 7.4).

Similarly, 2 mL of triple-distilled water, 0.2 mL of 1-2% egg albumin solution, and 2.8 mL of phosphate-buffered saline are combined to create a control solution, which has a total volume of 5 mL. The reaction mixtures are prepared, incubated for 30 minutes at 37±2°C, and then heated for 15 minutes in a water bath at 70±2°C. After the solutions have cooled, their absorbance is measured at 280 nm using an appropriate UV/Vis spectrophotometer.

Triple-distilled water was used as the calibration blank, and Diclofenac sodium was used as the standard. The percentage inhibition of protein denaturation was evaluated by

$$\% \, Inhibition = \frac{Absorbance\ of\ Control - Absorbance\ of\ test\ sample}{Absorbance\ of\ Control} \times 100$$

Sunscreen Activity Test

The determination of SPF involves measuring the absorbance of extracts of *C. roseus* using a UV Vis spectrophotometer within the range of 290-320 nm. Solutions of *C. roseus* extracts are prepared at concentrations of 1%, 3%, 5%, 7%, and 10%. Subsequently, the obtained data is analysed using the Mansur equation to derive the SPF values.

$$SPF = CF \; x \sum 320 \; EE \; (\lambda) x \; I \; (\lambda) x \; Abs \; (\lambda)$$

The SPF value is determined by a formula involving the correction factor (CF=10), the erythema effect spectrum (EE), the intensity spectrum from sunlight (I), and the absorbance (Abs) of the lotion sample being tested. The SPF value is obtained specifically within the wavelength range of 290-320 nm, which is adjusted to match the wavelength of UV-B light [13].

Wavelength (λ) nm	EE x I
290	0.015
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.018
Total	1

EE x I values for respective wavelengths.

Anti-Cancer Activity

MTT-based colourimetric assay is a cell-based assay to demonstrate cell proliferation and viability. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5- 2,5-diphenyl tetrazolium bromide), which is a yellow-colored tetrazole, is reduced to purple formazan in the mitochondria of living cells, and the absorbance of the formed colour in every well was quantified by measuring at a wavelength of 570 nm by a Spectrophotometer. Vero and HeLa cells were seeded in 96-well plates (10,000 cells/ well), and the cells were treated with different concentrations (0.2 µL/mL-2 µL/mL) of the *C. roseus* extracts. On the very next day. The surfactant and water mixture was used as a vehicle control. A treatment period of 24 h, 48 h, and 72 h was employed in this assay. The supernatant was discarded after the exposure period, and MTT solution (0.5 mg/mL) was added to each well, and incubated for 3 h. When solubilised with DMSO, purple-color formazan crystals were formed in live cells, which were then measured at

570 nm using a microplate reader. The percentage of cell viability was calculated by comparing the absorbance values of control and treated cells [14].

Wound Healing Activity

Vero cells were seeded onto 6-well plates at a density of 1×10^5 cells per well and cultured until reaching 90-95% confluence under optimal culture conditions. To simulate wound formation, a scratch was generated in the middle of the cell monolayer using a P10 pipette tip, and any cellular debris was eliminated by washing with a fresh medium. The scratch was then treated with the test sample and incubated for 24-48 hours at 37°C in a humidified environment with 5% CO_2. Negative control cells were maintained without any treatment. Evaluation of scratch wound closure was performed in two ways: first, by capturing digital images at time 0 hours (T0), 24 hours (T1), and 48 hours (T2) using a digital inverted microscope (static imaging). The closure of the scratch was quantified by measuring the difference in wound widths between T0 and T1/T2 using ImageJ software. The scratch closure rate (SCR) was calculated according to the formula established by Felice *et al.*[15] which is expressed as:

$$SCR = \left(T0 - \frac{T1}{T2} \right) \div TO \times 100$$

where T0 represents the scratch area at time 0 hours, and T1/T2 represents the scratch area at 24 and 48 hours, respectively.

Anti-diabetic activity

Fertilised chicken eggs were incubated at 37 °C with controlled humidity for nine days. Windows were created over the air sacs to access the chorioallantoic membrane (CAM). The extract of *C.roseus* was diluted in PBS and applied to the CAM; sodium nitroprusside was used as a positive control. Incubation continued for 23 more days, after which the CAMs were microscopically examined. Angiogenic changes were recorded through image capture and observation. Vessel density and branching were analysed quantitatively using image analysis software [16].

RESULTS AND DISCUSSION

Confirmatory tests for the presence of alkaloids:

The leaves and flowers of C. roseus were collected from Kolathur, Chennai, and Thiruvalangadu, Arakkonam. The collected samples were cleaned with water to get rid of impurities, shade-dried for 3 weeks, and then powdered finely for Soxhlet extraction. The Soxhlet extraction was performed, followed by cold extraction and the extract was stored for further studies. Confirmation of alkaloids by Dragandorf's test, Wagner's test, and Mayer's test was done, and all results concluded with the presence of alkaloids (Figure 1)

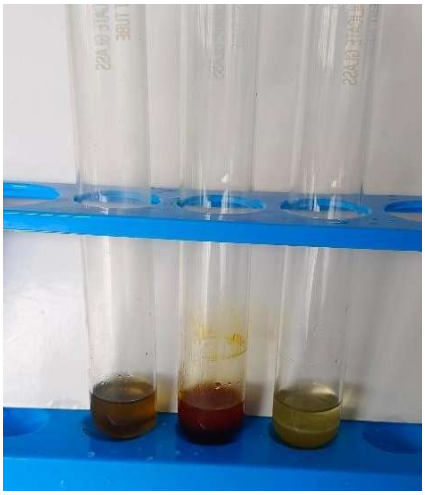


Fig 1: Confirmatory tests for the presence of alkaloids: Dragendorff's test, Wagner's test, and Mayer's test, respectively

Medicinal plants have long been valued for their therapeutic properties, contributing significantly to both traditional and modern medicine. Among these, *Catharanthus roseus*, belonging to the *Apocynaceae* family, is recognised for its diverse pharmacological applications. The study by multiple authors [17,18] revealed that the extraction of alkaloids from any plant material had the same procedure, which was employed in our study. Although the method of extraction was the same, a slight variation was made in the protocol, which resulted in a shorter duration of the extraction process with a greater amount of yield and an enhanced alkaloid was extracted. The study made by Agarwal and Paridhavi (2017) [19] also gave similar results to quantify the sample. The quantitative and qualitative studies of the extracted alkaloid from the plant *C. roseus* were assigned to further clarify the phytochemicals. As per the report [20,] the formation of a white or yellow-colored cream precipitate, reddish-brown precipitate, and a yellowish-brown precipitate indicates the presence of alkaloids using Mayer's test, Dragendoff's test, and Wagner's test and the same was achieved in our study.

Gas Chromatography-Mass Spectroscopy Analysis

To identify the bioactive compound present in extracts of *Catharanthus roseus*, GC-MS analysis was done, and the compounds have been found in the study (Figure 3 and Table 1). GC-MS analysis was employed to identify the various therapeutic phytoconstituents of *C.roseus*. The presence of Dasycarpidan-type alkaloids in plant extracts is frequently reported as a biochemical marker of alkaloid biosynthesis in medicinal plants, particularly those belonging to the Apocynaceae family, including Catharanthus roseus (Madagascar periwinkle). Structurally, the dasycarpidan skeleton represents a β-carboline alkaloid core, a key structural feature in many indole alkaloids. Therefore, the identification of Dasycarpidan-1-methanol, acetate (ester) strongly confirms the presence of alkaloids in the analysed sample. This finding aligns with previous phytochemical investigations on Catharanthus roseus, which consistently report a wide range of indole alkaloid [21]. Referring to the same, the results obtained for GC-MS analysis in this study and the study made by Rani, J *et al*., [22] were found similar and consisted of other parameters that help to identify it as an alkaloid with the help of literature. The GC-MS results ensure the presence of alkaloids, which have various therapeutic properties.

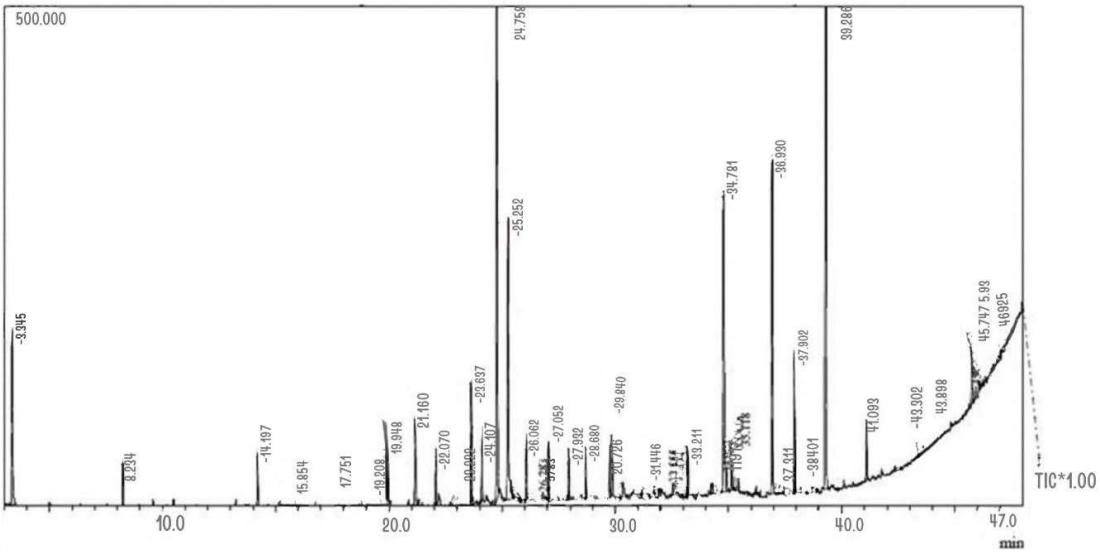


Figure 2: GC-MS Analysis of the obtained extracts of *C. roseus*

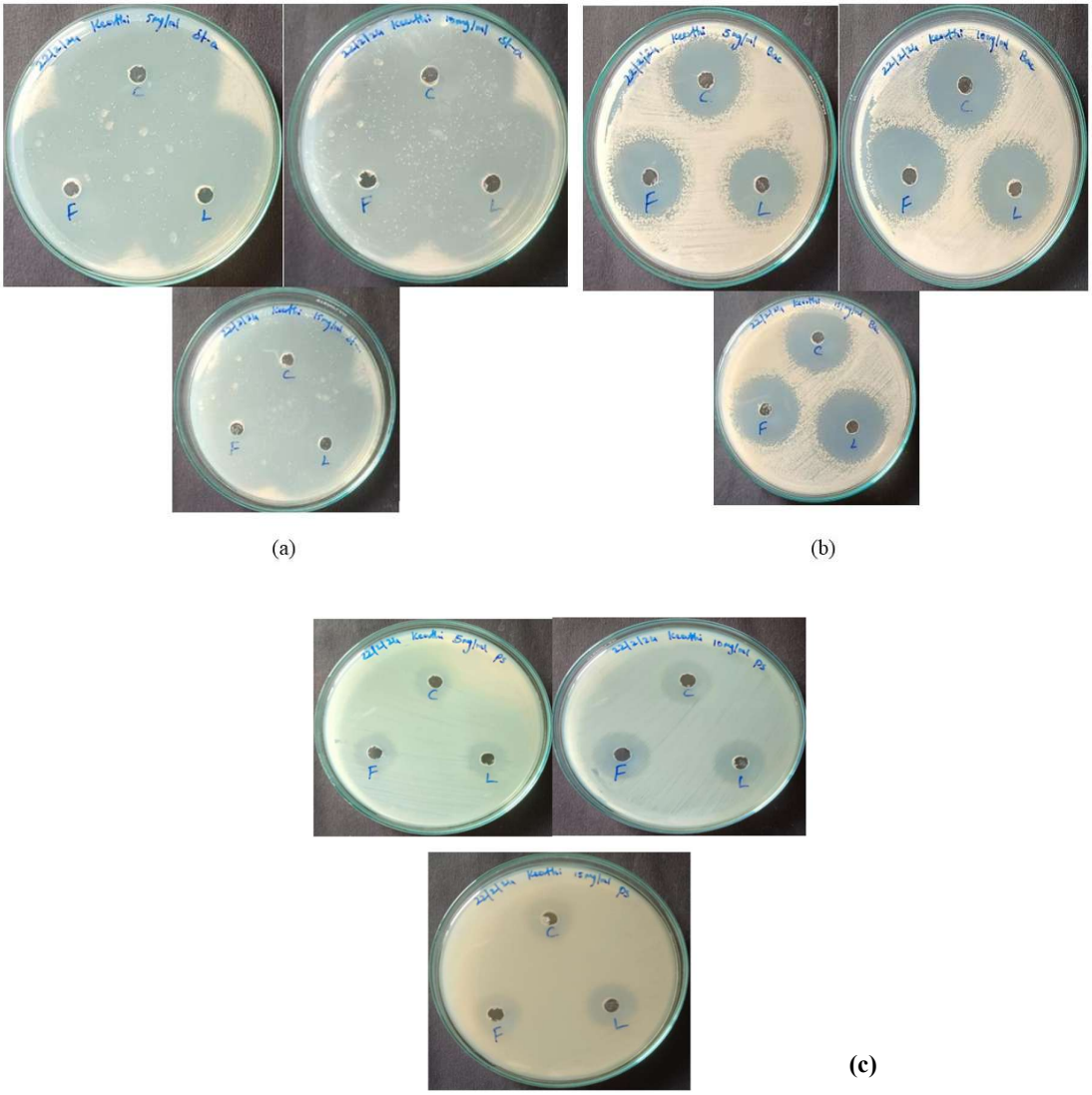
Table 1: Compounds present in the extract of *C. roseus*

S. No	RT	Name of the compound	Molecular formula	Molecular Weight (Da)	Peak area (%)
1	5.75	4H-Pyran-4-one	$C_6H_8O_4$	144	0.63
2	6.39	Catechol/Resorcinol	$C_6H_6O_2$	110.1	0.06
3	6.53	Benzofuran, 2,3-dihydro	C_8H_8O	120.1	0.61
4	6.77	1,2,3-Propanetriol, 1-acetate/acetin	$C_5H_{10}O_4$	134.1	0.84
5	7.09	Chlorozotocin	$C_9H_{16}C_1N_3O_7$	313.6	0.01
6	7.24	1,3-Diazacyclooctane-2-th	$C_6H_{12}N_2S$	144.2	0.82
7	7.44	Ascaridole epoxide	$C_{10}H_{16}O_3$	184	0.14
8	7.71	Chlorozotocin	$C_9H_{16}C_1N_3O_7$	313.6	0.17
9	8.21	l-Gala-l-ido-octonic lactone	$C_8H_{14}O_8$	238.19	0.05
10	8.48	Limonen-6-ol, pivalate	$C_{15}H_{24}O_2$	236.3	0.06
11	8.73	Sucrose	$C_{12}H_{22}O_{11}$	342.2	3.14
12	9.07	Desulfosinigrin	$C_{10}H_{17}NO_6S$	279.3	0.06
13	9.16	2-Pyrrolidinone, 1-butyl-	$C_8H_{15}NO$	141.2	0.13
14	9.38	3',5'-Dimethoxyacetophenone	$C_{10}H_{12}O_3$	180.2	0.17
15	9.61	2H-1-Benzopyran-3,4-diol	$C_{18}H_{20}O_5$	316.3	0.35
16	9.83	α-D-Glucopyranoside,	$C_{18}H_{32}O_{16}$	504	0.06
		O-α-D-glucopyranosyl			
17	9.89	Ethyl N-(o-anisyl) formimidate	$C_{10}H_{13}NO_2$	179.2	0.36
18	10.05	1,2,3,5-Cyclohexanetetrol	$C_6H_{12}O_4$	148.1	5.12
		2-Methyl-9-á-d-			

19	10.28	ribofuranosylhypoxanthine	$C_{11}H_{14}N_4O_5$	282	0.03
20	10.42	Tetraacetyl-d-xylonic nitrile	$C_{14}H_{17}NO_9$	343.3	0.19
21	10.94	Myo-Inositol, 4-C-methyl-	$C_7H_{14}O_6$	194.1	30.45
22	11.18	2-Pentyne-1,4-diol, 4- methyl-1-(2-thienyl)-	$C_{10}H_{12}O_2S$	196.2	0.33
23	11.54	Psicofuranine	$C_{11}H_{15}N_5O_5$	297.2	0.11
24	11.61	Cis-Inositol	$C_6H_{12}O_6$	180.6	0.09
25	11.90	Muco-Inositol	$C_6H_{12}O_6$	180.1	2.38
26	12.29	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	370	0.97
27	12.64	Pentadecanoic acid	$C_{15}H_{30}O_2$	242.39	10.41
28	13.05	3-Phenylbicyclo (3.2.2) nona-3,6-dien- 2-one	$C_{15}H_{14}O$	210.2	0.06
29	13.56	Dasycarpidan-1-methanol, acetate	$C_{20}H_{26}N_2O_2$	326.4	0.11
30	14.22	9,12-Octadecadienoic acid (Z, Z)-, methyl ester	$C_{19}H_{34}O_2$	294.4	0.67
31	14.32	9,12,15-Octadecatrienoic acid, methyl Ester	$C_{19}H_{32}O_2$	292.2	2.70

Anti-bacterial activity

The well diffusion assay was conducted to evaluate the antibacterial properties of *C. roseus* leaf and flower extracts. It was observed that the extracts produced a distinct and visible zone of inhibition against both gram-positive (*Bacillus subtilis, Staphylococcus aureus*) and gram-negative bacteria (*Pseudomonas aeruginosa*), as shown in Figure 3. Patil, P. J., and Ghosh, J. S. [23] elucidated that alkaloids show greater antimicrobial activity. The antimicrobial qualities of the flower and leaf extracts were the main focus of the investigation. The results of this study showed the antibacterial potential of the alkaloid sample obtained from *C. roseus.* Concerning the literature, the sample obtained possesses antibacterial activity against Gram-negative (*Pseudomonas aeruginosa*) and Gram-positive pathogens (*Bacillus subtilis, Staphylococcus aureus*).



(a) (b)

(c)

Fig 3: Anti-microbial activity of extracts of *C. roseus* against (a) *Staphylococcus aureus,* (b) *Bacillus subtilus,* (c) *Pseudomonas aeruginosa* at different concentrations.

Table 2: The resultant antimicrobial activity of extracts of *C. roseus* at different concentrations.

Concentration of extracts	*Bacillus subtilis*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
5 mg/ml	8mm	14mm	6mm
10 mg/ml	10mm	16mm	8mm
15 mg/ml	10mm	20mm	8mm

Anti-oxidant activity

Plant-based products are widely employed in pharmaceuticals and cosmetics due to their antioxidant properties. Therefore, it's essential for the formulation used in encapsulation to preserve the antioxidant activity. This formula is utilised to determine the percentage of radical scavenging. Table 3 provides an overview of the antioxidant potential of *Catharanthus roseus.* The antioxidant assay performed by [24] by the DPPH assay method is more accurate and widely practised. The values obtained in this study for alkaloids of C. roseus were similar to those in the literature. The alkaloid sample obtained from the flower part of C. roseus has better antioxidant activity to scavenge free radicals than the sample obtained from the leaf part of C. roseus, and thus, confirms that the flower sample possesses potential antioxidant activity.

Table 3: The resultant antioxidant activity of the extract of *C. roseus* at different concentrations

Concentration of the Sample	Percentage of Radical Scavenging
20 µg/ml	26.13%
40 µg/ml	40.23%
60 µg/ml	59.56%
80 µg/ml	69.64%
100 µg/ml	79.82%

Anti-inflammatory activity

The primary objective of the egg albumin denaturation assay is to assess whether the sample can inhibit or impede the denaturation of egg albumin under specific conditions. This assay serves as a means to gauge the anti-inflammatory effects of a compound by measuring its ability to prevent or reduce egg albumin denaturation. The anti-inflammatory activity is assessed for the *C. roseus* extracts and presented in a tabulated form (Table 4). The anti-inflammatory activity was performed using the egg albumin denaturation method; by following the same procedure, the results were obtained for the study of the C. roseus alkaloid sample. The protection percentage was higher in the alkaloids extracted from the flower part than that of the alkaloids extracted from the leaves part [25]. So, we elucidate that the sample obtained from the flower part of the plant possesses better anti-inflammatory activity as per the literature.

Table 4: The resultant Anti-inflammatory activity of *C. roseus* extracts at different concentrations

Concentration of the Sample	Percentage Inhibition
20 µg/ml	20.32%
40 µg/ml	31.66%

60 µg/ml	42.67%
80 µg/ml	54.32%
100 µg/ml	65.45%

Sunscreen test

The sunscreen activity test was carried out using the ultraviolet spectrometry method. The absorbance value at each concentration is entered into the Mansur equation to determine the SPF value. Measurements were taken in the UV-B region because these rays are the main cause of skin cancer. The results of determining the sunscreen activity of cinnamon oil are shown in Table 5. The sunscreen activity results obtained proved the ability of the extracts to absorb UV radiation, and hence, the extracts have UV protection ability. This supported the prophylactic utility of *C. roseus* and its role in anti-solar formulation. This will be a better, cheaper and safer alternative to harmful chemical sunscreens that are used nowadays in the industry. Besides its antisolar activity and effects, making it a useful sun care as well as skin care product [26].

Table 5: The resultant Sunscreen activity of *C. roseus* extracts at different percentages

Percentage of extracts	SPF Values
1%	5
3%	13
5%	25
7%	39
10%	47

Anti-Cancer activity

The Vero cell lines and HeLa cell lines were treated with extracts of C. roseus, and the IC_{50} value was found to be 72.33 and 66.44, respectively, for Vero cell lines and HeLa cell lines respectively (Figure 4). The anti-cancer activity of *C.rosues* was studied by [27,] which showed that the flower extract didn't show better results, but the leaf extract showed better anti-cancerous activity, which coincided with our results. With reference to the literature on anti-cancerous activity, alkaloids obtained from the leaf sample could be identified as a good anti-cancerous agent.

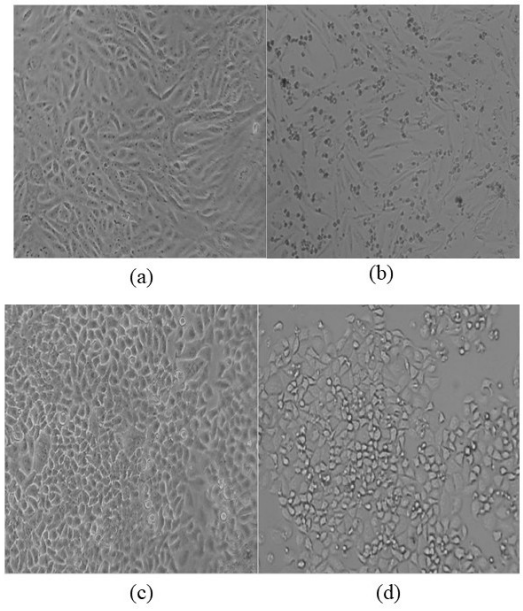


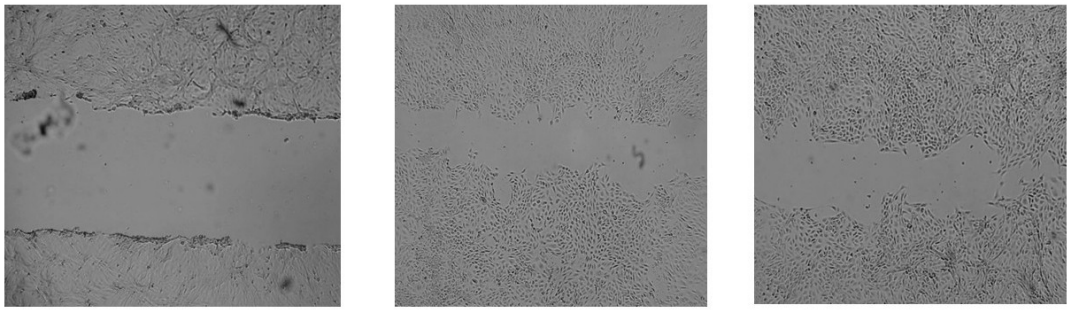
Figure 4: Vero cell lines (a) before, (b) after and HeLa cell lines (c) before, (d) after treatment with *C. roseus* extracts.

Table 6: The resultant anti-cancer activity of *C. roseus* extract at different concentrations

Concentration (µg/ml)	Vero cell lines (%)	HeLa cell lines (%)
10	88.98	87.59
20	84.38	82.63
30	78.57	75.89
40	72.68	70.89
50	66.29	64.75
60	59.69	58.68
70	53.57	50.36
80	47.19	42.57
90	40.18	35.51
100	32.28	28.68

Wound Healing assay

The extracts of *C. roseus* exhibited wound healing activity greater than control, which was and has given a maximum scratch closure rate as shown in Figure 7. The leaf extract of *C. roseus* reveals wound-healing activity on mouse fibroblast L929 cell lines. Here, the alkaloid obtained from the leaf extract of *C. roseus* showed wound-healing activity. With reference to the literature on wound-healing activity, alkaloids obtained from the leaf extracts could be identified as a preferable wound-healing agent [28].



(a) (b) (c)

Figure 5: Wound Healing activity on (a) Vero Cell lines when treated with (b) control and (c) extracts of *C. roseus* after 24 hours

	Scratch assay (0 hrs)	Scratch assay (24 hrs)	Percentage (%)
Control	0.52	0.39	25.37
C. roseus extract	0.52	0.28	46.15

Table 7: The resultant wound healing activity of the extract of *C. roseus*

Anti-diabetic activity

The chick embryos treated with *C. roseus* extracts exhibited improved vascular development compared to controls, indicating potent anti-diabetic activity as elucidated in Figure 6. Our study explored the antidiabetic potential of *C. roseus* using the CAM assay to assess its effect on angiogenesis. The results indicate that the plant extract influences blood vessel formation, with higher concentrations showing anti-angiogenic effects, suggesting a possible role in regulating vascular complications in diabetes. The presence of bioactive alkaloids like vincristine and vinblastine would have contributed to its hypoglycemic activity [29]. While the findings support its pharmacological relevance, further in vivo studies and compound isolation are needed to confirm its mechanisms.

(a) **(b)**

Figure 6: Chick embryo assay to assess anti-diabetic property (a) Incubated egg (b) The well-developed blood vessels

CONCLUSION

Catharanthus roseus, commonly known as Madagascar periwinkle, is a commonly found plant in many of our localities. The traditional folklore medicine, particularly in the Indian subcontinent, has vivid properties in many therapeutic applications. The plant of our extract has been showing many therapeutic properties, including antibacterial, antioxidant, anti-inflammatory, sun screen activity, wound healing, and anti-cancer activity has been reported with both flower and leaf extracts. The conclusion drawn from the study reinforces the efficacy of *C. roseus* in folk medicine practices. It provides scientific evidence for the traditional uses of this plant and highlights its potential as a source of novel pharmaceutical agents. Integrating traditional knowledge with scientific research can lead to the development of effective and culturally relevant healthcare solutions.

CONFLICT OF INTEREST

The authors declare no conflict of interest, and all authors have read and agreed to the published version of the manuscript.

DECLARATION

The manuscript is original, has not been published previously, and is not under consideration for publication elsewhere.

ACKNOWLEDGEMENT

The authors are grateful to the management of Dwaraka Doss Goverdhan Doss Vaishnav College, Chennai, for their support in pursuing research.

FUNDING

The authors have no relevant financial or non-financial interests to disclose. The authors also declare that no funds, grants, or other support were received during the preparation of this manuscript.

REFERENCES

1. Srivastav Y, Chauhan AS, Hameed A. *Catharanthus roseus* (Vinca rosea): taxonomy, phytochemicals and pharmacological (anti-cancer) properties – a comprehensive overview.
2. Pandey P, Tripathi A, Dwivedi S, Lal K, Jhang T. Deciphering the mechanisms, hormonal signalling, and potential applications of endophytic microbes to mediate stress tolerance in medicinal plants. *Front Plant Sci*. 2023;14:1250020.
3. Pinjari RR, Siddiqui A, Ahmed NA. *Concepts of alkaloids from natural sources*. Newcastle upon Tyne: Cambridge Scholars Publishing; 2024.
4. Seal S, Kar S, Pal S, Datta S, Debnath A, Bannerji S, Sinha D. Alkaloids of natural origin with promising anti-diabetic properties. In: *Advances in pharmacognosy and phytochemistry of diabetes*. 2024. p. 61.
5. Varela C, Silva F, Costa G, Cabral C. Alkaloids: their relevance in cancer treatment. In: *New insights into glioblastoma*. Cambridge: Academic Press; 2023. p. 361–401.
6. De Castro ML, García-Ayuso LE. Soxhlet extraction of solid materials: an outdated technique with a promising innovative future. *Anal Chim Acta*. 1998;369(1–2):1–10.
7. Benbott A, Bahri L, Boubendir A, Yahia A. Study of the chemical components of *Peganum harmala* and evaluation of acute toxicity of alkaloids extracted in the Wistar albino mice. *J Mater Environ Sci*. 2013;4:558–65.
8. Naz S, Moreira dos Santos DC, García A, Barbas C. Analytical protocols based on LC–MS, GC–MS and CE–MS for nontargeted metabolomics of biological tissues. *Bioanalysis*. 2014;6(12):1657–77.
9. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: a review. *J Pharm Anal*. 2016;6(2):71–9.
10. Chang LW, Yen WJ, Huang SC, Duh PD. Antioxidant activity of sesame coat. *Food Chem*. 2002;78(3):347–54.
11. Dharmadeva S, Galgamuwa LS, Prasadinie C, Kumarasinghe N. In vitro anti-inflammatory activity of *Ficus racemosa* L. bark using albumin denaturation method. *AYU*. 2018;39(4):239–42.
12. Chandra S, Chatterjee P, Dey P, Bhattacharya S. Evaluation of in vitro anti-inflammatory activity of coffee against the denaturation of protein. *Asian Pac J Trop Biomed*. 2012;2(1):S178–80.
13. Malsawmtluangi C, Nath DK, Jamatia I, Zarzoliana E, Pachuau L. Determination of sun protection factor (SPF) number of some aqueous herbal extracts. *J Appl Pharm Sci*. 2013;3(9):150–1.
14. Morgan DM. Tetrazolium (MTT) assay for cellular viability and activity. In: Morgan DM, editor. *Polyamine protocols*. Totowa: Humana Press; 1998. p. 179–84.
15. Felice F, Zambito Y, Belardinelli E, Fabiano A, Santoni T, Di Stefano R. Effect of different chitosan derivatives on in vitro scratch wound assay: a comparative study. *Int J Biol Macromol*. 2015;76:236–41.
16. Haselgrübler R, Stübl F, Essl K, Iken M, Schröder K, Weghuber J. Gluc-HET, a complementary chick embryo model for the characterization of antidiabetic compounds. *PLoS One*. 2017;12(8):e0182788.
17. Senizza B, Rocchetti G, Okur MA, Zengin G, Yıldıztugay E, Ak G, Montesano D, Lucini L. Phytochemical profile and biological properties of *Colchicum triphyllum* (Meadow Saffron). *Foods*. 2020;9(4):457.
18. Al-Subaie SF, Alowaifeer AM, Mohamed ME. Pyrrolizidine alkaloid extraction and analysis: recent updates. *Foods*. 2022;11(23):3873.

19. Agarwal SS, Paridhavi M. *Herbal drug technology*. 2nd ed. Hyderabad: Universities Press; 2007.
20. Yusuf AZ, Zakir A, Shemau Z, Abdullahi M, Halima SA. Phytochemical analysis of the methanol leaves extract of *Paullinia pinnata* Linn. *J Pharmacogn Phytother*. 2014;6(2):10–6.
21. Maharajasri V, Devamalar D. Characterization of antibacterial, anticancer properties and bioactive compounds of methanolic leaf extract of catharanthus roseus. Int. J. Humanit. Arts Med. Sci. 2015;1:35-42.
22. Rani J, Kapoor M, Dhull SB, Goksen G, Jurić S. Identification and assessment of therapeutic phytoconstituents of *Catharanthus roseus* through GC-MS analysis. *Separations*. 2023;10(6):340.
23. Patil PJ, Ghosh JS. Antimicrobial activity of *Catharanthus roseus* – a detailed study. *Br J Pharmacol Toxicol*. 2010;1(1):40–4.
24. Goboza M, Meyer M, Aboua YG, Oguntibeju OO. In vitro antidiabetic and antioxidant effects of different extracts of *Catharanthus roseus* and its indole alkaloid, vindoline. *Molecules*. 2020;25(23):5546.
25. Samaraweera T, Samaraweera T, Senadeera NN, Ranaweera CB. In vitro anti-inflammatory activity of leaves of *Jeffreycia zeylanica* using the egg albumin denaturation method and human red blood cell stabilization method. *Asian Plant Res J*. 2023;11(6):56–64.
26. Tolambiya P, Jagetiya BL, Mathur S. Extraction and identification of various phytopharmaceutical constituents of *Catharanthus roseus* L. (Alba) leaves. *Plant Arch*. 2023;23(2).
27. Harshini M, Sheeba L, Selvanayaki M. Anticancer activity of *Catharanthus roseus* and *Murraya koenigii*. *J Crit Rev*. 2020;7:10.31838.
28. Akkara PJ, Martin SA, Tomy AM, Menon AS, Takri G. Methanolic leaf extract of *Catharanthus roseus* reveals wound healing activity on mouse fibroblast L929 cell lines. *Med Plants Int J Phytomed*. 2023;15(3):587–94.
29. Arshad I, Ali Q, Rashid MS, Malik A. Evaluation of *Catharanthus roseus* plant extract grown under multiple stress environmental conditions for its biochemical activities. *Plant Cell Biotechnol Mol Biol*. 2020;21(53–54):71–8.