Research Article

Anticancer And Antimicrobial Properties Of Catharanthus Roseus: An In Vitro Evaluation

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Abstract

Catharanthus roseus hydroalcoholic leaf isolate was tested for anticancer, antioxidant, cell-killing, and antibacterial effects in vitro and by GC–MS phytochemical profiling. To establish its therapeutic potential, the extract was tested for cytotoxic, anticancer, and antibacterial activity on HEK293 cell lines and selected bacterial pathogens.

Materials and Methods: Catharanthus roseus leaves were authenticated, shade-dried, and extracted in 80% methanol/1% HCl. Phytochemical analysis of extract. Ascorbic acid was used as a reference for DPPH radical scavenging antioxidant ability. The MTT test was used to investigate cytotoxic and anticancer effects on HEK293 cell lines using doxorubicin as the reference medication. The antibacterial activity of Staphylococcus aureus and E. coli was measured using Kirby-Bauer agar well diffusion methods.

Results: GC–MS analysis showed 44 phytoconstituents, including antioxidant, antibacterial, and anticancer components, including N-hexadecanoic acid, octadecanoic acid, phytol, lupeol, stigmasterol oleate, and indole derivatives. Compared to ascorbic acid (21.89 ± 0.02 µg/mL), the extract showed significant antioxidant ability in the DPPH radical scavenging experiment (IC_{50} value: 982.6 ± 0.02 µg/mL). The MTT assay showed that the extract was cytotoxic to HEK293 cells (IC50 = 75.54 ± 0.08 µM), whereas doxorubicin exhibited an IC50 of 11.26 ± 0.20 µg/mL. Microscopic examination demonstrates cytopathic effects such as cell shrinkage and density loss. Moderate antibacterial activity against Escherichia coli and Staphylococcus aureus was identified with 10.67 mm and 6.67 mm inhibition zones.

Conclusions: This study confirms the plant's long-term use in traditional medicine and suggests it might be used to generate phytopharmaceuticals and anticancer drugs.

Keywords: Catharanthus roseus, GC–MS, DPPH assay, antioxidant activity, cytotoxicity, antimicrobial activity, anticancer activity, HEK293 cell line.

Introduction

Cancer is the most frightening disease in the world, hereditary, caused by abnormal cell division. The International Agency for Research on Cancer reported that there were 18.1 million new cases and 9.6 million deaths in 2018, highlighting the ongoing global health crisis. The four most common cancers in humans are lung, breast, colon and rectum and female reproductive system cancers. Major factors affecting cancer survivability include early diagnosis, treatment resistance, tumour heterogeneity and metastatic potential [1]. Ayurveda, the ancient Indian medical system, extensively deals with the therapeutic properties of plants. Catharanthus roseus, a prized herb in Ayurveda, possesses antioxidative, antibacterial, anticancer & antidiabetic properties. This evergreen plant is found in Madagascar. Its opposite-pair leaves grow in opposing directions, and its blossoms may be any shade from pink to purple. The plant has 130 alkaloids, such as raubasine, vincine, reserpine, vincristine and ajmalicine. Vincristine and vinblastine are used in the treatment of a number of malignancies including Hodgkin's lymphoma, skin cancer, breast cancer and lymphoblastic leukaemia. with vincristine and vinblastine, the species is threatened with extinction and needs micropropagation and other conservation measures. The plant has tremendous medicinal efficacy and has huge anticancer potential that makes it worth for further research. Catharanthus roseus is known for its antibacterial and anticancer activities, due to the presence of many bioactive chemicals [2]. The plant contains several indole alkaloids, such as vincristine and vinblastine, which are widely used as anti-cancer drugs. These chemicals kill cancer cells by preventing the production of microtubules during cell division, preventing the growth of cancer cells. Vincristine is an effective agent in the fight against lymphoma and leukaemia and vinblastine is a therapy for breast cancer, testicular cancer and Hodgkin's disease [3]. Other phytochemicals including flavonoids, tannins, saponins, terpenoids, and phenolic compounds also contribute to antioxidant and cytotoxic activities that help reduce oxidative stress associated with cancer progression. In addition to anticancer potential, the plant exhibits strong antimicrobial activity against several pathogenic bacteria and fungi. Alkaloids, phenolics, and flavonoids damage microbial

membranes, inhibition of enzyme function, and disrupt nucleic acid synthesis, resulting in to microbial death. Extracts of C. roseus have demonstrated inhibitory effects against organisms such as Staphylococcal Infections-associated bacterial and fungal pathogens. The synergistic action of these phytoconstituents makes C. roseus an important medicinal plant with significant therapeutic potential in the development of novel anticancer and antimicrobial agents [4-5].

Figure 1 shows Catharanthus roseus. Also known as Vinca rosea, Catharanthus roseus is an evergreen shrub that can reach a height and width of 1 metre. This plant features a short, branching, erect stem. The leaves are 5cm long, glossy-green, oval, and of opposite orientation; their tips are acuminate. The 5 petals are open tubular, about 4 cm in diameter and lightly pink with reddish undertones. There are three colour varieties, white, pink and purple, flowering in spring and autumn.

METHODOLOGY

Collection and identification of the plant material: The present investigation was carried out on Catharanthus roseus. The plant materials were collected from Maharana Pratap College of Pharmacy and authenticated at Central Institute of Medicinal & Aromatic Plants, Lucknow. No. CIMAP-865 is the voucher specimen for this which is deposited in the plant systematics lab.

Extraction: Pulverised to a 20-mesh powder, Catharanthus roseus leaves were air-dried. We mixed 5 grams of the powdered material with 50 millilitres of a solvent combination consisting of distilled water, 1% hydrochloric acid, and 80% methanol. A rocker shaker was used to stir the ingredients for a whole day. After that, the mixture was dried in a 40°C oven after passing it through Whatman No. 1 filter paper. Placing the dried extracts in microcentrifuge tubes, they were then maintained at 4°C [6].

Figure-2 Hydroalcoholic extractions of Catharanthus roseus

IN VITRO ANTIOXIDANT ACTIVITY

DPPH radical scavenging Assay: A 96-well microplate was used to assess antioxidant activity by combining 10 µL of various test sample or standard quantities with 200 µL of 0.1 mM DPPH solution (SRL Chem, Cat. No. SR-29128). The experiment was carried out in triplicate using control wells that were not treated and with sample blanks that were sample + methanol without DPPH and reagent blanks that were also devoid of DPPH. After 30 minutes of darkness incubation, the plates were read at 517 nm for absorbance using a microplate reader (iMark, Bio-Rad). The test solution was mixed with 20 µL of deionized water to provide a control. In comparison to the control, the radical scavenging activity % was determined. The IC_{50} values were calculated by graphing the percentage of inhibition versus the concentration of the sample in GraphPad Prism 6[7].

Formula:

% RSA= (ABS control-Abs Sample)/Abs control) x100

GC-MS Analysis: A PerkinElmer Clarus 600 system, fitted with a Rtx-5MS capillary column, was used to conduct the GC-MS analysis of the hydroalcoholic extract. A flow rate of 1.0 mL/min was used using helium (99.99%) as the carrier gas. The volume of the injection was 1 µL, and the split ratio was 10:1. The scan rate was 0.2 s, the ionisation energy was 70 eV, and the mass range was 40-650 m/z. An injection temperature of 260°C was established. The temperature of the column oven was gradually increased from 50 to 300 degrees Celsius, starting at 50, and continued at a rate of 10 degrees per minute. We used mass spectra and retention durations to match compounds in the NIST and Wiley-8 databases. Cytotoxicity Assessment of the Substances in a Vitro Setting Using HEK293 Cells.

MTT Assay Method: The HEK293 cell line (NCCS, Pune, India; Passage 29; RRID: CVCL_0045) was used to assess the cytotoxicity of the test substances using the MTT technique. The cells were placed in 96-well plates with a density of 1×10^4 cells/well and left to incubate for 24 hours in Ham's F-12K medium, which included 10% FBS and 1% antibiotic-antimycotic solution, at 37°C with 5% CO_2. The cells were then subjected to different concentrations of the test substances. After being diluted in serum-free media, stock solutions were prepared in DMSO. Incubation with MTT reagent (5 mg/mL) was performed after 24 hours of exposure. There was no treatment in the control wells, and the blank wells did not contain any MTT. After incubation, the liquid above the solid was removed, and the formazan was dissolved in 100 µL of dimethyl sulfoxide. A Bio-Rad iMark microplate reader was used to record the optical density at 540 nm. The findings are shown as mean ± SEM, and IC_{50} was calculated using GraphPad Prism 6. An Olympus CK2 inverted microscope equipped with an AmScope digital camera was used to get the microscopic pictures [8-10].

Calculation

% Viability = (A sample / A control) × 100
A sample = Absorbance of sample
A control = Absorbance of control

Anti-microbial activity evaluation: The agar well diffusion test, also known as the Kirby-Bauer disc diffusion method, was used to assess the antibacterial effectiveness. A 100 µL solution of E. coli and S. aureus was added to Mueller-Hinton agar for inoculation. The concentration of inoculum was calibrated to 0.5 McFarland turbidity, which is 1.5×10^8 CFU/mL in Mueller-Hinton broth. Discs were placed on the plates after being saturated with 10 µL of plant extract at different doses (0-100 mg/mL). Negative controls consisted of ciprofloxacin discs (3 µg), and positive controls of solvent-only discs were used. The diameter of the inhibitory zones was measured and recorded after incubation at 37°C for 24 hours [6].

Statistical analysis: Each experiment was conducted three times separately with a total of four replicates (n = 4). Mean ± standard error of the mean is how the results are presented. A logistic model with four parameters was used to determine the IC_{50}. The cytotoxicity data was compared statistically using one-way ANOVA followed by Dunnett's test. Statistical significance was defined as a p-value below 0.05. Finding the coefficient of variation (CV) between several runs allowed us to assess the reliability of the data.

RESULTS

Identification of components: Information on molecular mass, structural characteristics, and fragment ions allowed for chemical identification. GC-MS spectra were analysed using spectrum libraries maintained by Wiley and the National Institute of Standards and Technology (NIST), which together include over 62,000 reference patterns. Each component of the test samples was identified together with its molecular weight and chemical structure. Verifying the phytochemical profile of the plant extract was done by comparing the relative fraction of each discovered ingredient to reference spectra from the NIST database and Wiley 8 library. Traditional and modern therapeutic applications of the species may be based on the presence or combination of certain compounds. Finding the best methods for getting rid of harmful substances is another benefit of this. We were able to determine the test materials' chemical compositions (Table 1 and Figure 3).

Table 1- Compound of Catharanthus roseus

Peak Report TIC-Sample- Catharanthus roseus					
Peak	**R. Time**	**Area**	**Area %**	**Name**	**Common Names**
1	11.183	1223935	1.64	Tetradecane	Tetradecane
2	12.590	977363	1.31	2,6,6-trimethyltricyclo[...]Undecan-8-one	Trimethyl tricyclic ketone (camphor-like compound)
3	13.689	656967	0.88	Hexadecane	Hexadecane
4	14.750	474251	0.64	1-tetradecanol, acrylate	Tetradecanol acrylate
5	15.090	259023	0.35	Octadecanoic acid, methyl ester	Methyl octadecanoate (methyl stearate)
6	15.489	284331	0.38	Tetradecanoic acid	Tetradecanoic acid (Myristic acid)
7	15.850	358840	0.48	Ethyl nonadecanoate	Ethyl nonadecanoate
8	16.268	236687	0.32	Eicosyl acetate	Eicosyl acetate
9	16.883	578132	0.78	2-Oxazolidinone, 3-amino-5-(4-morpholinylmethyl)	Amino morpholine oxazolidinone derivative
10	17.090	473893	0.64	7,9-Di-tert-butyl-1-oxaspiro (4,5)deca-6,9-diene-2,8-dione	Tert-butyl spiro diketone (synthetic antioxidant type)
11	17.218	1290639	1.73	Hexadecanoic acid, methyl ester	Methyl palmitate (Hexadecanoic acid methyl ester)
12	17.590	16414046	22.01	N-Hexadecanoic acid	Palmitic acid (Hexadecanoic acid)
13	17.817	294501	0.39	3,5-di-tert-Butyl-4-hydroxyphenylpropionic acid	Di-tert-butyl hydroxypropionic acid derivative
14	17.891	757749	1.02	Hexadecanoic acid, ethyl ester	Ethyl palmitate
15	17.976	463018	0.62	Octadecane	Octadecane
16	18.354	256564	0.34	Hexadecane-1,2-diol	Hexadecanediol
17	18.920	1086185	1.46	8,11,14-Docosatrienoic acid, methyl ester	Docosatrienoic acid methyl ester
18	19.022	2377485	3.19	Phytol	Phytol
19	19.153	694246	0.93	Methyl stearate	Methyl stearate
20	19.279	10781071	14.46	(Z, z)-6,9-cis-3,4-epoxy-nonadecadiene	Epoxy nonadecadiene derivative
21	19.488	10240763	13.73	Octadecanoic acid	Stearic acid (Octadecanoic acid)
22	19.759	726997	0.97	Octadecanoic acid, ethyl ester	Ethyl stearate
23	19.831	286086	0.38	Heneicosane	Heneicosane
24	20.254	410088	0.55	1-docosanol	Docosanol (behenyl alcohol)
25	20.872	517545	0.69	Octacosyl acetate	Octacosyl acetate
26	21.476	760500	1.02	Eicosanoic acid, ethyl ester	Ethyl eicosanoate
27	21.995	428330	0.57	Octacosyl acetate	Octacosyl acetate (duplicate/isomer)
28	22.130	948243	1.27	Octadecanoic acid, 3-oxo-, ethyl ester	Oxo stearic acid ethyl ester
29	22.263	3466263	4.65	Palmitic acid	Palmitic acid
30	22.454	5389089	7.23	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl est	Glyceryl hexadecanoate derivative (monopalmitin-type)
31	23.064	653726	0.88	Docosanoic acid, ethyl ester	Ethyl docosanoate
32	23.590	133283	0.18	Acetic acid n-octadecyl ester	Stearyl acetate (acetic acid octadecyl ester)
33	23.683	390518	0.52	1h-indole-3-ethanamine	Tryptamine derivative (indole ethylamine)
34	24.032	2718868	3.65	Octadecanoic acid, 2,3-dihydroxypropyl ester	Diglyceride of stearic acid

35	24.195	504641	0.68	Tetrapentacontane, 1,54-dibromo-	Brominated long-chain hydrocarbon
36	24.472	1324046	1.78	13-docosenamide, (z)-	Erucamide (13-docosenamide)
37	24.538	643546	0.86	Docosanoic acid, ethyl ester	Ethyl docosanoate (isomer)
38	24.698	359387	0.48	,10,14,18,22-Tetracosapentaen-2-ol, 3-bromo-2,6,10,15,19,23-hexamethyl-, (all-E)	Brominated polyunsaturated fatty alcohol derivative
39	25.997	603543	0.81	Docosanoic acid, ethyl ester	Ethyl docosanoate (another isomer)
40	26.957	261242	0.35	1-propanone, 1-(4-methoxy-1-methyl-1h-indol-2-yl)-2-(methylsulfonyl)	Indole ketone/sulfonyl derivative (synthetic-like metabolite)
41	27.070	812326	1.09	Stigmast-5-en-3-ol, oleat	Stigmasterol oleate (plant sterol ester)
42	29.556	861249	1.15	25-homo-24-ketocholesterol	Oxygenated cholesterol derivative
43	30.398	1179382	1.58	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	BHT phosphite antioxidant (synthetic preservative)
44	30.923	1016859	1.36	Lupeol	Lupeol (plant triterpenoid)
		74575446	100.00		

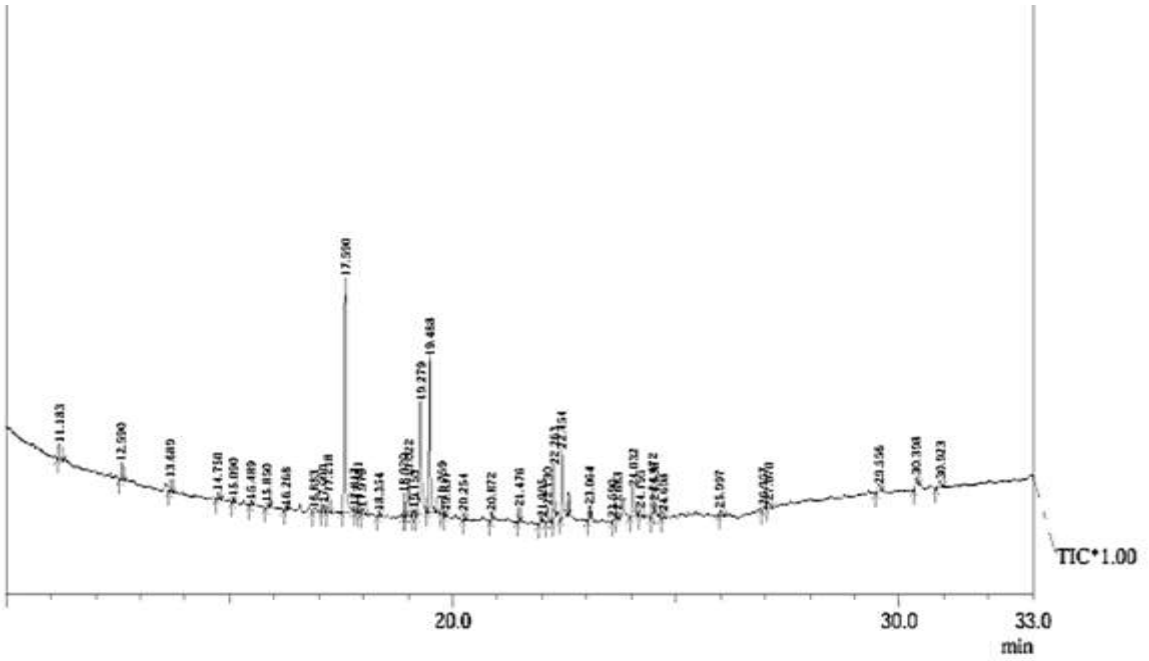


Figure-3 Catharanthus roseus Hydroalcoholic chromatogram

The Catharanthus roseus hydroalcoholic was discovered to have a wide variety of compounds when GCMS was run on it. The samples included forty-four chemicals, which are previously listed in the table above.

Antioxidants Activities: Oxidative stress has been linked to neurological diseases that occur with age, according to much research. Antioxidants' neuroprotective function in reducing or avoiding neuronal cell death under these circumstances has also been well studied. Total antioxidant capacity and free radical quenching ability are two important ways to measure how well an antioxidant works. In order to determine the plant extracts' antioxidant capacity, the DPPH test was used. To find out how well the different extracts scavenged free radicals, the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was used. Tabular form is used to summarize the data.

Table-2DPPH Scavenging Assay of Catharanthus roseus

Compounds code	IC_{50} value (microgram/millilitre)
Ascorbic Acids	21.89±0.02
Catharanthus roseus-PI	982.6±0.02

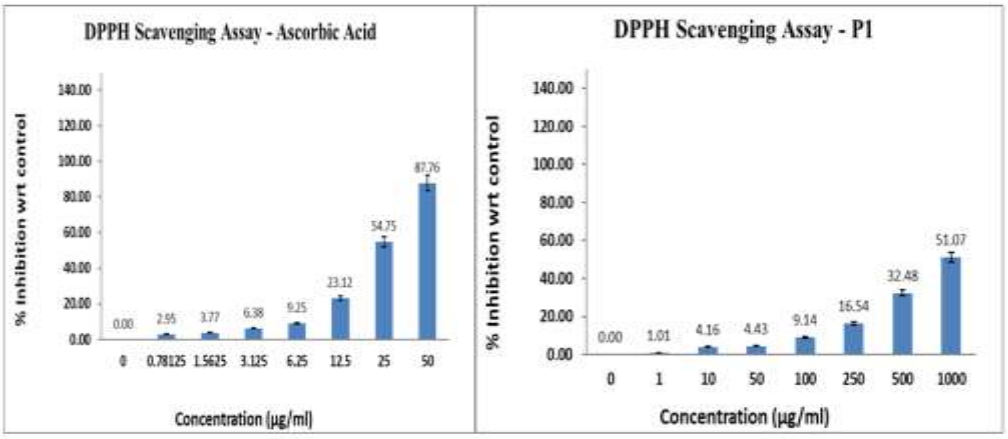


Figure-4 DPPH Scavenging Assay of Catharanthus roseus Hydroalcoholic Extract

Table 3 displays the results of the radical scavenging test, which was used to determine the sample's antioxidant activity, based on the experimental findings. The values of the 50% inhibitory concentration (IC_{50}) are also presented in the report. The anti-oxidant activity of Sample P1 was rather low. Similar to 21.89 µg of conventional ascorbic acid, 982 µg of sample P1 had antioxidant activity.

Table-3Testing Compound Toxicity in Cells Using the PC-12 MTT Assay

Sample code	IC_{50} value Mean ± SEM
Doxorubicin	11.26±0.20 (µg/ml)
P1	75.54±0.08 (µM)

The results of the MTT experiment showed that the sample's cytotoxic potential could be determined by exposing HEK293 cells to different doses of it; Table 4 displays the IC_{50} values. Extensive cytotoxicity was seen in Sample P1. A sample's or compound's IC_{50} value is the concentration at which 50% cell viability inhibition takes place.

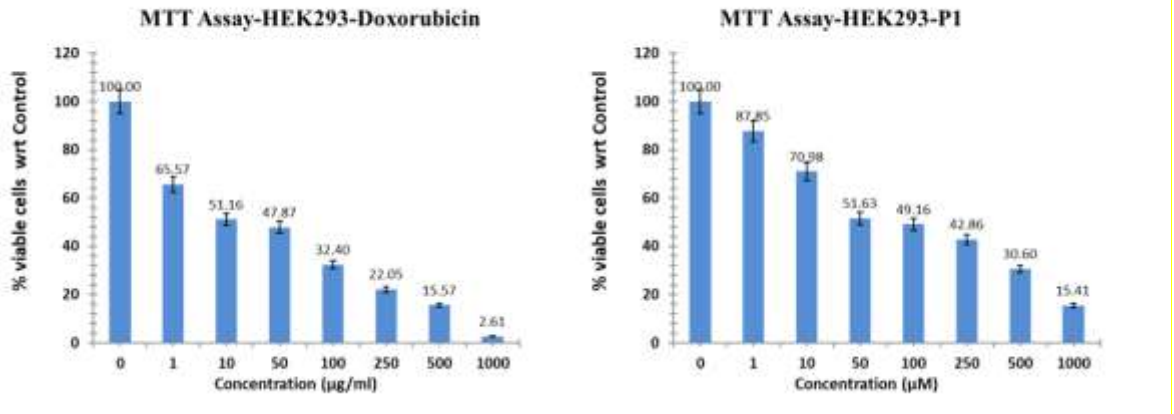


Figure-5 Cytopathic effects on the HEK293 cell line treated by Catharanthus roseus Hydroalcoholic Extract

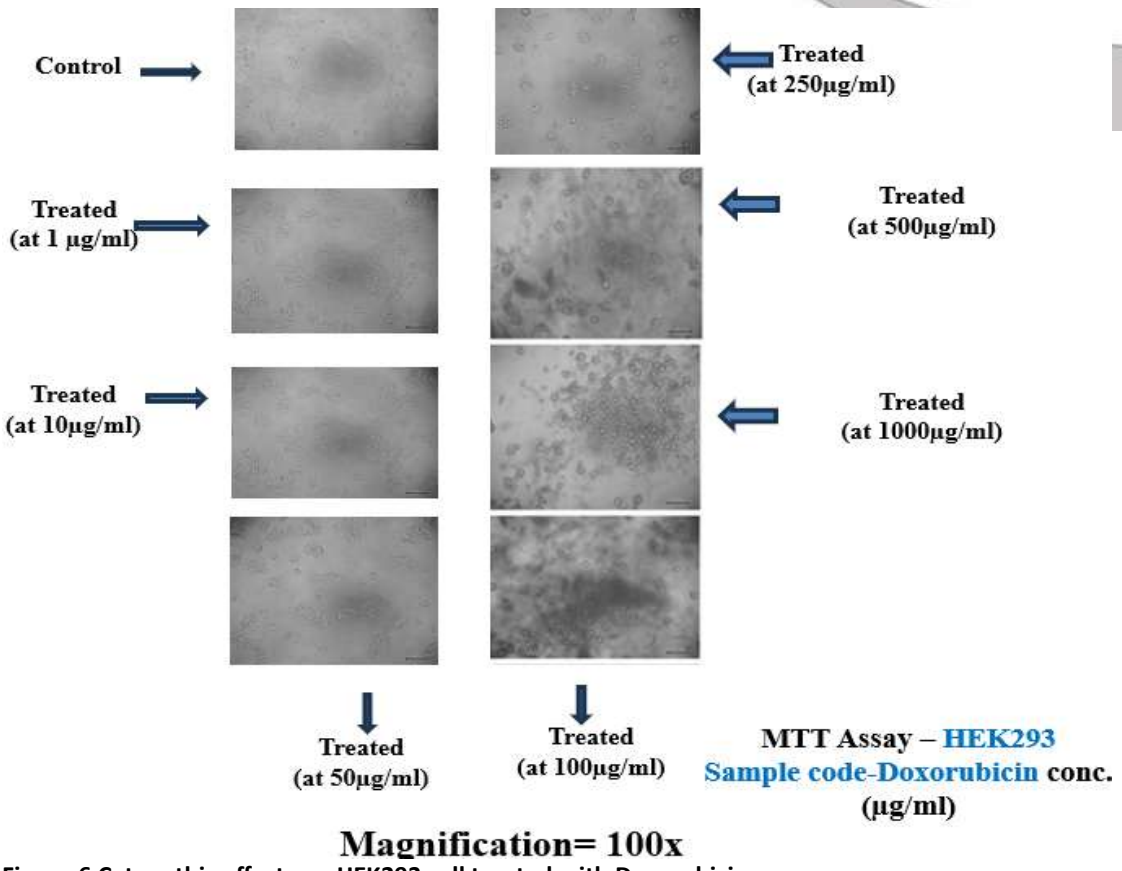
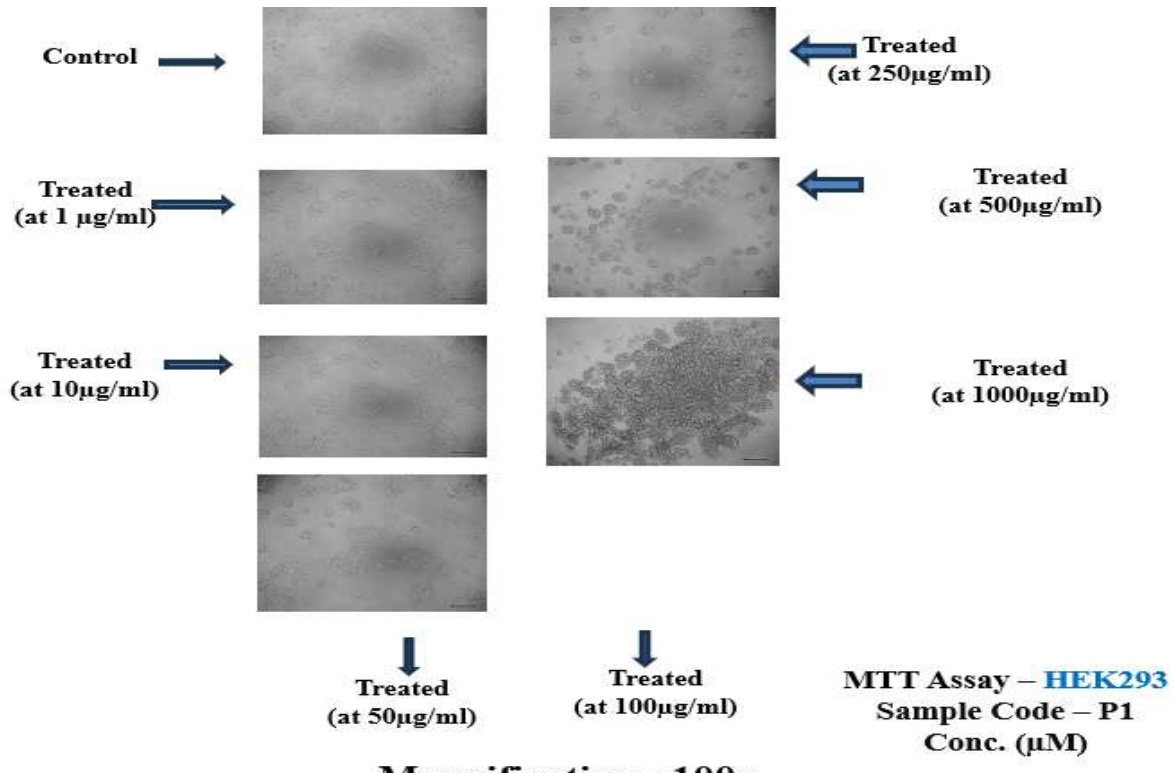


Figure-6 Cytopathic effects on HEK293 cell treated with Doxorubicin

Figure-7 Cytopathic effects on the HEK293 cell treated with Catharanthus roseus Hydroalcoholic Extract



Antimicrobial activity – Table 4 presents the microbial growth inhibitory effect of Catharanthus roseus. The extract exhibited potent antibacterial activity against E. coli and Staphylococcus aureus.

S. No.	Microbial Species	Sample Id	Effective Amount	Average Zone (in mm) at Effective Amount
1	Escherichia coli	Ciprofloxacin (PC)	3 µg	26.00
2	Escherichia coli	P1	1000µg	10.67
3	Staphylococcus aureus	Ciprofloxacin (PC)	3 µg	26
4	Staphylococcus aureus	P1	62.5 µg	6.67

DISCUSSIONS

The hydroalcoholic test leaf sample of Catharanthus roseus included many phytoconstituents that showed promise as antioxidants, anticancer agents, cytotoxic agents, and antimicrobials, according to the GC-MS (Gas Chromatography-Mass Spectrometry) study. By using retention duration, molecular weight, fragmentation profile, and matching with NIST and Wiley8 spectrum databases, a total of 44 substances have been found. The most abundant chemicals found were N-hexadecanoic acid (22.01%), octadecanoic acid (13.73%), glyceryl hex decanoate (7.23%), palmitic acid (4.65%), phytol (3.19%), and octadecanoic acid glyceride derivatives (3.65%). Fatty acid esters, tryptamine derivatives, doconazole, stigmasterol oleate, and lupeol were among the significant phytochemicals discovered [11]. The presence of these compounds indicates the therapeutic significance of C. roseus. Fatty acids such as palmitic acid, stearic acid, along with their and esters are recognized for anti-inflammatory and antimicrobial potential. Phytol possesses antioxidant, antimicrobial, and anticancer properties by inducing apoptosis and reducing oxidative stress. Lupeol, an important pentacyclic triterpenoid, has been widely reported for its anticancer and anti-inflammatory activities through modulation of signaling pathways involved in tumour progression. The occurrence of indole derivatives further supports the medicinal value of C. roseus, as indole alkaloids are known precursors of anticancer agents such as vincristine and vinblastine. The hydroalcoholic preparation's capacity to neutralise free radicals was assessed using the DPPH test. A value of 21.89 ± 0.02 µg/mL was reported by the standard, ascorbic acid, compared to the test material's IC_{50} of 982.6 ± 0.02 µg/mL. As a result, the extract revealed comparatively [12-13]. The plant extract has poorer radical scavenging action compared to the conventional antioxidant, as shown by its somewhat larger IC_{50} value. The existence of phytoconstituents that donate hydrogen and may neutralise free radicals The extract's demonstrable antioxidant potential suggests the presence of phytoconstituents that donate hydrogen and may neutralise free radicals. by the extract's demonstrable antioxidant potential, nevertheless. Possible GC-MS-detected antioxidants include phytol, lupeol, phenolic compounds, and fatty acid esters. These bioactive components may reduce oxidative damage and neutralise free radicals. The antioxidant activity of C. roseus might greatly enhance its therapeutic potential, since oxidative stress is associated with the development of cancer and neurological disorders [14]. The MTT test was used to assess the extract's cytotoxicity in relation to the HEK293 cell line. With an IC_{50} value of 75.54 ± 0.08 µM, Extract P1 demonstrated notable cytotoxic capability, in contrast to the reference medication doxorubicin's 11.26 ± 0.20 µg/mL. Bioactive components that may inhibit cell proliferation and trigger cell death are responsible for the post-treatment decrease in cell viability. Microscopic examination of treated cells showed marked cytopathic effects, including cell shrinkage, membrane damage, and reduction in cell density, confirming the cytotoxic action of the extract. The observed anticancer activity may be associated with bioactive compounds such as lupeol, phytol, indole derivatives, and fatty acid components identified in the GC–MS profile. These compounds are known to induce apoptosis, inhibit DNA replication, alter mitochondrial function, and suppress tumor cell growth. these results corroborate the traditional therapeutic application of C. roseus and suggest its promise as a source of natural anticancer compounds. The hydroalcoholic solution's antimicrobial activity was tested by exposing it to E. coli and S. aureus via agar well diffusion. Both test organisms were moderately inhibited by the material. At 1000 µg/mL, an inhibitory zone of 10.67 mm was seen for E. coli, but at 62.5 µg/mL, S. aureus displayed a zone of 6.67 mm. Ciprofloxacin, on the other hand, showed an inhibition zone of 26 mm against both strains when used as a reference.The antibacterial properties of C. roseus could be due to the combined action of phytochemicals including fatty acids, phytol, lupeol, docosanol, and phenolic compounds detected in the GC-MS analysis. These constituents may damage microbial cell membranes, inhibit enzymatic activity, and disrupt microbial metabolic pathways, thus limiting bacterial proliferation. Even though the efficacy was less than that of the standard antibiotic, the results suggest that the plant has broad-spectrum antimicrobial capacity [15-16].

CONCLUSION

Overall, the study confirms that Catharanthus roseus leaves contain several biologically active phytoconstituents responsible for antioxidant, cytotoxic, anticancer, and antimicrobial activities. The results scientifically support the traditional therapeutic use of the plant and suggest that it could serve as a promising origin for the development of novel phytopharmaceutical activity.

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AUTHOR CONTRIBUTIONS

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Ms. Pragati Katiyar, Mrs. Swati Trivedi, Dr. Vikram Kumar Sahu: Methodology, Laboratory Analysis.
Dr. Sribatsa Lanchhana Dash, Dr. Amit Mishra: Data Curation, Formal Analysis.
Ms. Pragati Katiyar, Mrs. Swati Trivedi: Supervision, Writing—Review & Editing.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS STATEMENT

Ethical approval was not required for this study.

DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES:

1. Noble RL. The discovery of the vinca alkaloids—chemotherapeutic agents against cancer. Biochem Cell Biol. 1990;68(12):1344–1351.
2. van Der Heijden R, Jacobs DI, Snoeijer W, Hallard D, Verpoorte R. The Catharanthus alkaloids: pharmacognosy and biotechnology. Curr Med Chem. 2004;11(5):607–628.
3. Gupta M, Sharma A, Gautam A. Phytochemical analysis and antimicrobial activity of Catharanthus roseus extracts against pathogenic microorganisms. J Pharm Res. 2013;7(2):97–101.
4. Ferreres F, Pereira DM, Valentão P, Andrade PB, Seabra RM, Sottomayor M. New phenolic compounds and antioxidant potential of Catharanthus roseus. Food Chem. 2008;109(4):974–979.
5. Gajalakshmi S, Vijayalakshmi S, Devi RV. Phytochemical and pharmacological properties of Catharanthus roseus: A review. Int J Pharm Sci Rev Res. 2013;20(2):123–129.
6. Kumari M, Shukla D, Shukla D, Kumar U, Dubey A, Tripathi BP. Unlocking the bioactivity of lemongrass (Cymbopogon flexuosus): A dual assessment of antioxidant and antimicrobial efficacy. IP Int J Med Microbiol Trop Dis. 2025;11(4):510-516.
7. Surveswaran S, Cai YZ, Corke H, Sun M. Systematic evaluation of natural phenolic antioxidants from medicinal plants. Food Chem. 2007;102(3):938–953.
8. Dubey, A., Ghosh, N., & Singh, R. (2023). An in-depth and in vitro evaluation of the antioxidant and neuroprotective activity of aqueous and ethanolic extract of Asparagus racemosus Linn seed. Research Journal of Chemistry and Environment, 27(10), 46–66.
9. Dubey, A.; Ghosh, N. S. and Singh, R. (2023) A Toxicological Study on Seed Extracts of Asparagus Racemosus Linn (Ethanolic and Water) in Experimental Animals. Journal of Advanced Zoology, 44, 71–78.
10. Dubey Et Al., A. (2023). Role of aqueous and ethanolic seed extract of asparagus racemosus on acr-induced neurotoxicity in adult zebrafish: Emergence of neuroprotective results. Egyptian Journal of Aquatic Biology and Fisheries, 27(6), 285–296.
11. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT - Food Science and Technology. 1995;28(1):25–30.
12. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev. 1999;12(4):564–582.
13. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd ed. London: Chapman and Hall; 1998.

14. Parekh J, Chanda S. In vitro antimicrobial activity and phytochemical analysis of some Indian medicinal plants. Turk J Biol. 2007; 31:53–58.
15. Silva GL, Lee IS, Kinghorn AD. Special problems with the extraction of plants. In: Cannell RJP, editor. Natural Products Isolation. New Jersey: Humana Press; 1998. p. 343–364.
16. Prakash OM, Kumar A, Kumar P, Ajeet. Anticancer potential of plants and natural products: A review. Am J Pharmacol Sci. 2013;1(6):104–115.