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ORIGINAL ARTICLE

Evaluation of anti-leukemic activity of potentised *Catharanthus roseus*: An in-vitro study

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Alcoholic extract of potentised *Catharanthus roseus* (L.) G. Don. was evaluated for its anti-leukemic activity in in-vitro model. For this *in- vitro* study two cell lines namely, jurkat and K562 for promyelocytic leukemia and chronic myeloid leukemia were used. The cell lines were grown in Roswell Park Memorial Institute Medium (RPMI 1640 medium) containing 10% fetal bovine serum and 2mM L-glutamine. Doxorubicin (Adriamycin, ADR) was used as positive control drug. Growth inhibition of 50 % (GI50) was calculated from [(test growth in the presence of drug at the four concentration levels Ti – time zero Tz)/(control growth C-Tz)] x 100 = 50], which is the drug concentration resulting in a 50% reduction in the net protein increase in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) was calculated from Ti = Tz. The LC50 (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from [(Ti-Tz)/Tz] x 100 = -50 in sulforhodamine B (SRB) assay. The *Catharanthus roseus* in 2X potency showed maximum anti-leukemic activity in molar drug concentration of 20 µg/ml on human leukemic cell line K562 for chronic myeloid leukemia.

Keywords : anti-leukemic activity, Catharanthus roseus (L.) G. Don., chronic myeloid leukemia

INTRODUCTION

More than 100 alkaloids and related compounds have so far been isolated and characterised from the plant *Catharanthus roseus*.

The alkaloid contents in different parts of the plant show large variations as roots have 0.14-1.34%, stem 0.074-0.48%, leaves 0.32-1.16%, flowers 0.005-0.84%, fruits 0.40%, seeds 0.18% and pericarp 1.14%. These alkaloids include monomeric indole alkaloids, 2-acyl indoles, oxindole, α-methylene indolines, dihydroindoles, bisindole and others. Dry leaves contain vinblastine (vincaleucoblastine or VLB) 0.00013-0.00063%, and vincristine (leurocristine or LC) 0.0000003-0.0000153% which have anti-cancerous activity.[1]

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Both vinblastine and vincristine at relatively low doses (<1 mg/kg/day for 10 days) can prolong the life span of leukemic animals by 100% and more. Vincristine is particularly effective against leukemia.

Chronic myeloid leukemia (CML) is a clonal disorder of pluripotent stem cell (stem cell that has the potential to differentiate into any of the three germ layers) involving myeloid, erythroid, megakaryocytic and lymphoid cells. More than 90% of cases have a cytogenetic abnormality involving reciprocal translocation between the long arms of chromosomes 9 and 22 (t9; 22) (q34:q11). The oncogene *c-abl*, located on the long arm of chromosome 9, get translocated to chromosome 22, where a specific gene called breakpoint cluster region *(bcr)* is found. Both *abl* and *bcr* form a fusion gene, *abl/bcr*, which encodes an unregulated, cytoplasm-targeted tyrosine kinase that allows the cells to proliferate without being regulated by cytokines.[2]

In contrast to normal bone marrow, which is usually about 50% cellular and 50% fat, the marrow of a patient with CML is usually 100% cellular. This increase in cellularity of CML marrow is from the maturing granulocytic precursor. Increased number

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of megakaryocytes, often including small dysplastic forms, is frequently observed, whereas erythroid progenitors are usually present in normal or decreased number. Peripheral blood examination reveals a marked leukocytosis, often exceeding 100,000 cells/mm.[4] The circulating cells are predominantly neutrophils, metamyelocytes, and myelocytes, with less than 10% myeloblasts.[2]

OBJECTIVES

To evaluate the anti-leukemic activity of potentised whole plant extract of *Catharanthus roseus* in *in-vitro* model by SRB assay.

Determination of concentration or quantification of vincristine alkaloid by HPLC (High Performance Liquid Chromatography) in whole plant extract of *Catharanthus roseus.*

MATERIAL AND METHODS

Plant Material: Whole plant of *Catharanthus roseus* (Apocynaceae) was collected from the Beed district of Maharashtra state in the month of June 2009. It was authenticated as *Catharanthus roseus* (L.) G. Don. belonging to family Apocynaceae, by Prof. Dileep Pokle, Head of Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, Maharashtra and accession no. was 5844.

For preparation of alcoholic extract, the crude drug was dried for 15 days and then coarsely powdered. The coarse powder was loaded in thimble made up of Whatman filter paper no. 1 and extracted in the Soxhlet continuous extraction column with 3 litres of 95% alcohol for 10 cycles for each of two batches. The extract so obtained was viscous with characteristic odour. The excess of alcohol was evaporated on the water bath. The final extract was thick and was stored separately in amber colour bottle of 30 ml capacity.

The potencies up to 12X of *Catharanthus roseus* were made under Class IV of Hahnemannian classification by using 95% ethyl alcohol as solvent in ratio 1:5 (Dry drug: alcohol)[3] in Department of Pharmacy, Sonajirao Kshirsagar Homoeopathic Medical College, Beed, Maharashtra. The drug strength was 1:10.

a. ***Qualitative Analysis:*** Phytochemical evaluation of sample by Thin Layer Chromatography (TLC): 50mg of the sample was dissolved in 5ml of methanol. To 2ml of the solution was added a few drops of Dragendorfs reagent.

b. ***Quantitative Analysis:*** Analysis of sample by High Performance Liquid Chromatography (HPLC): 2.135 g of sample was dissolved in 10x2 ml methanol and sonicated for 30 minutes, filtered and made up volume to 25 ml with methanol.

For *In-vitro* Cytotoxicity study, total 5 compounds in coded form were submitted to Advanced Centre for Treatment Research & Education in Cancer (ACTREC), Tata Memorial Centre, Kharghar, Navi Mumbai, through Dr. A.S. Juvekar, Officer-in-charge, Anticancer Drug Screening Facility.

Control Group:

Dexorubicin (Adriamycin) procured from Pfizer Pharmacenticals with batch number BQL008-B 88J004 was used as control.

For analysis by Thin Layer Chromatography: 10 mg of standard vincristine sulphate was dissolved in 1 ml of methanol.

For analysis by High Performance Liquid Chromatography: 20 mg of standard vincristine sulphate was dissolved in 10 ml of methanol and subsequent dilutions were made to get the final concentration of 100 µg/ml.

Experimental Group:

In experimental group, the following codes were used for the drugs:

a. USA-1 = Crude Drug Substance
b. USA-2 = *Catharanthus roseus* Mother Tincture
c. USA-3 = *Catharanthus roseus* 2X
d. USA-4= *Catharanthus roseus* 6X
e. USA-5 = *Catharanthus roseus* 12X

Quality Control

The following procedures were followed to enhance quality and reliability of experiment:

1. Tests for alkaloids

a. Qualitative: Phytochemical evaluation of sample by Thin Layer Chromatography (TLC) by using Silica gel $60F_{254}$ pre-coated TLC plate (Merck) absorbent.

b. Quantitative: Analysis of sample by High Performance Liquid Chromatography (HPLC) by using HiQ sil C18 HS 4.6mmX250mm 5µm column.

2. *In-vitro* cytotoxic studies by using sulforhodamine (SRB) assay.

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Phytochemical Evaluation of Sample by TLC	
Sample preparation:	50mg of the sample dissolved in 5ml of methanol
Absorbent:	Silica gel $60F_{254}$ pre-coated TLC plate (Merck)
Chromatography solvent/mobile Phase:	Toulene:Ethylacetate:Diethyl amine: (70:20:10)
Technique of development:	Ascending
Length of run:	10cm
Spraying Reagent:	Dragendorfs reagent
Amount spotted:	10µl
Band width:	6mm
Phytochemical Evaluation of Sample by HPLC	
Column:	HiQ sil C18 HS 4.6mmX250mm 5µm
Mobile phase:	Acetonitrile: phosphate buffer (30:70) pH 3.6
Wavelength:	260nm
Run time:	30 min
Sample preparation:	2.13g of sample was dissolved in 10 X 2ml of methanol and sonicate for 30 min. filter and made up the volume to 250ml with methanol
In-vitro Cytotoxicity Studies	
Vehicle used for the test articles:	Ethyl Alcohol
Source of Cell Lines:	National Cancer Institute (NCI), USA and National Centre for Cell Science (NCCS), Pune.
Method of Testing:	Sulforhodamine B assay.

Cell Lines details:

S. No.	Cell Line	Human Tissue Origin
1..	jurkat	Leukemia (Promyelocytic Leukemia)
2..	K562	Leukemia (Chronic Myeloid Leukemia)

Compound with G150 ≥ 50 is considered to be active at the respective concentration

Experimental Setup

Experimental Procedure for TLC[4]

Slurry of the stationary phase, generally in water, was applied to a glass, plastic or foil plate, generally 20 cm square, as a uniform thin layer by means of a plate spreader starting at one end of the plate and moving progressively to the other. The layer was 0.25 mm thick. Once the slurry layer had been prepared, the plates dried to leave the coating of stationary phase. Dried in an oven at 100 to 120°C served to activate Silica gel $60F_{254}$ pre-coated TLC plate (Merck) absorbent.

The 50 mg of the sample dissolved in 5 ml of methanol was applied to the plate 2.0 to 2.5 cm from the edge by means of a micropipette or microsyringe. The solvent was removed from the spot by gentle heating or by use of an air blower. It was then possible to apply more sample to the spot if necessary. In thin layer chromatography, the sample was applied as a band across the plate rather then as a single spot.

Plate development: Separation most commonly took place in a glass tank that contained the developing Toulene:Ethylacetate:Diethyl amine: (70:20:10) (mobile phase) to a depth of about 1.5 cm. This was allowed to stand for at least 1 hour with a lid over the top of the tank to ensure that the atmosphere within the tank became saturated with solvent vapour (equilibration). After equilibration, the lid was removed, and the thin layer plate was then placed vertically in the tank so that it stood in the solvent. The lid was replaced and separation of the compounds then occurred as the solvent travelled up the plate. It was possible to develop the plate in a horizontal plane by connecting the sample end of it to a reservoir of mobile phase by means of a suitable wick. It was preferable to keep the system at a constant temperature whilst the development was occurring, to avoid anomalous solvent-running effects.

Analyte detection: Examination of the plate under ultraviolet light at 254 nm showed the position of ultraviolet-absorbing or fluorescent compounds. Spraying of plates with Dragendorfs reagent would stain certain compounds. Although the movement of compounds on TLC characterised by specific Rf (distance moved by analyte from origin/ distance moved by solvent front from origin) values, these measurements

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were not always reproducible. Component identification was made based on comparison of the movement of the components with those of reference compounds chromatographed alongside the sample on the TLC plate.

On plate, quantification was achieved by using radiochromatograph scanning in the case of radiolabelled compounds or more generally by means of densitometry.

Experimental Procedure for HPLC[4]

The stainless steel column of HiQ sil C18 HS 4.6mmX250mm 5µm was made to withstand pressure of upto 5.5 X 107pa without cyclic variation.

Column packing: High pressure slurrying technique was used. A suspension of the packing was made in a solvent density equal to that of the packing material. The slurry was then pumped rapidly at high pressure into a column with a porous plug at its outlet.

Mobile phase and pump: Acetonitrite:phosphate buffer (30:70), pH 3.6 solvent purified as traces of impurities affect the column and interfere with the detection. Solvent was degassed before use because gassing is bad for aqueous methanol, alter column resolution and interfere with the continuous monitoring of the effluent.

Application of sample: Microsyringe used to inject the 2.135 g of sample was dissolved in 10 X 2ml of methanol and sonicated for 30 minutes filtered and made the volume to 250 ml with methanol.

Detection: The detection was carried out at 260 nm wavelength by use of diode array technique, which allowed the simultaneous measurement of absorbance at many or all wavelength within 0.01s.

Experimental Procedure for Sulforhodamine B Assay [5,6]

The cell lines were grown in Roswell Park Memorial Institute Medium 1640 (RPMI 1640 medium) containing 10% fetal bovine serum and 2 mM L-glutamine. For present screening experiment, cells were inoculated into 96 well microtiter plates in 100 µL at plating densities, depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37°C, 5 % CO_2, 95 % air and 100 % relative humidity for 24 h prior to addition of experimental drugs.

After 24 h, one 96 well plate containing 5*10* cells/ well was fixed in situ with TCA (Trichloroacetic Acid), to represent a measurement of the cell population at the time of drug addition (Tz). Experimental drugs were initially solubilised in dimethyl sulfoxide at 100mg/ml and diluted to 1mg/ml using water and stored frozen prior to use. At the time of drug addition, an aliquote of frozen concentrate (1mg/ml) was thawed and diluted to 100 µg/ml, 200 µg/ml, 400 µg/ml and 800 µg/ml with complete medium containing test article. Aliquots of 10 µl of these different drug dilutions were added to the appropriate microtiter wells already containing 90 µl of medium, resulting in the required final drug concentrations i.e.10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml.

After compound addition, plates were incubated at standard conditions for 48 hours and assay was terminated by the addition of cold TCA. Cells were fixed in situ by the gentle addition of 50 µl of cold 30 % (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 minutes at 4°C. The supernatant was discarded; the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (50 µl) at 0.4 % (w/v) in 1 % acetic acid was added to each of the wells, and plates were incubated for 20 minutes at room temperature. After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1 % acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10 mM trizma base, and the absorbance was read on a plate reader at a wavelength of 540 nm with 690 nm reference wavelength.

Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells. Percent Growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells X 100.

Using the six absorbance measurements [time zero (Tz), control growth (C), and test growth in the presence of drug at the four concentration levels (Ti)], the percentage growth was calculated at each of the drug concentration levels. Percent growth inhibition was calculated as:

[(Ti-Tz)/(C-Tz)] x 100 for concentrations for which Ti>/=Tz (Ti-Tz) positive or zero

[(Ti-Tz)/Tz] x 100 for concentrations for which Ti<Tz. (Ti-Tz) negative

The dose response parameters were calculated for each test article.

Growth inhibition of 50 % (GI50) was calculated from [(Ti-Tz)/(C-Tz)] x 100 = 50, which is the drug concentration resulting in a 50% reduction in the net

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protein increase (as measured by SRB staining) in control cells during the drug incubation.

The drug concentration resulting in total growth inhibition (TGI) was calculated from Ti = Tz. The concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning (LC50) indicating a loss of cells following treatment is calculated from [(Ti-Tz)/Tz] x 100 = -50.

Values were calculated for each of these three parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the values for that parameter were expressed as greater or less than the maximum or minimum concentration tested.

Results

Qualitative: Phytochemical evaluation of methanol extract sample by Thin Layer Chromatography (TLC) shows three bands giving positive tests for alkaloids at Rf 0.37, 0.33 and 0.241 of which one band corresponds to vincristine sulphate. The details are given in Figure 1.

Quantitative: Analysis of alcoholic extract of potentised Catharanthus roseus (L.)G. Don. by high performance liquid chromatography (HPLC) showed the presence of 0.036% of alkaloid vincristine.

In processed sample by HPLC, total 26 constituents are identified in which vincristine have: (Table 1)

Time:	18.865 minutes
Height:	927.8 µV
Area%:	0.419%
Area:	459.6 µV. minutes

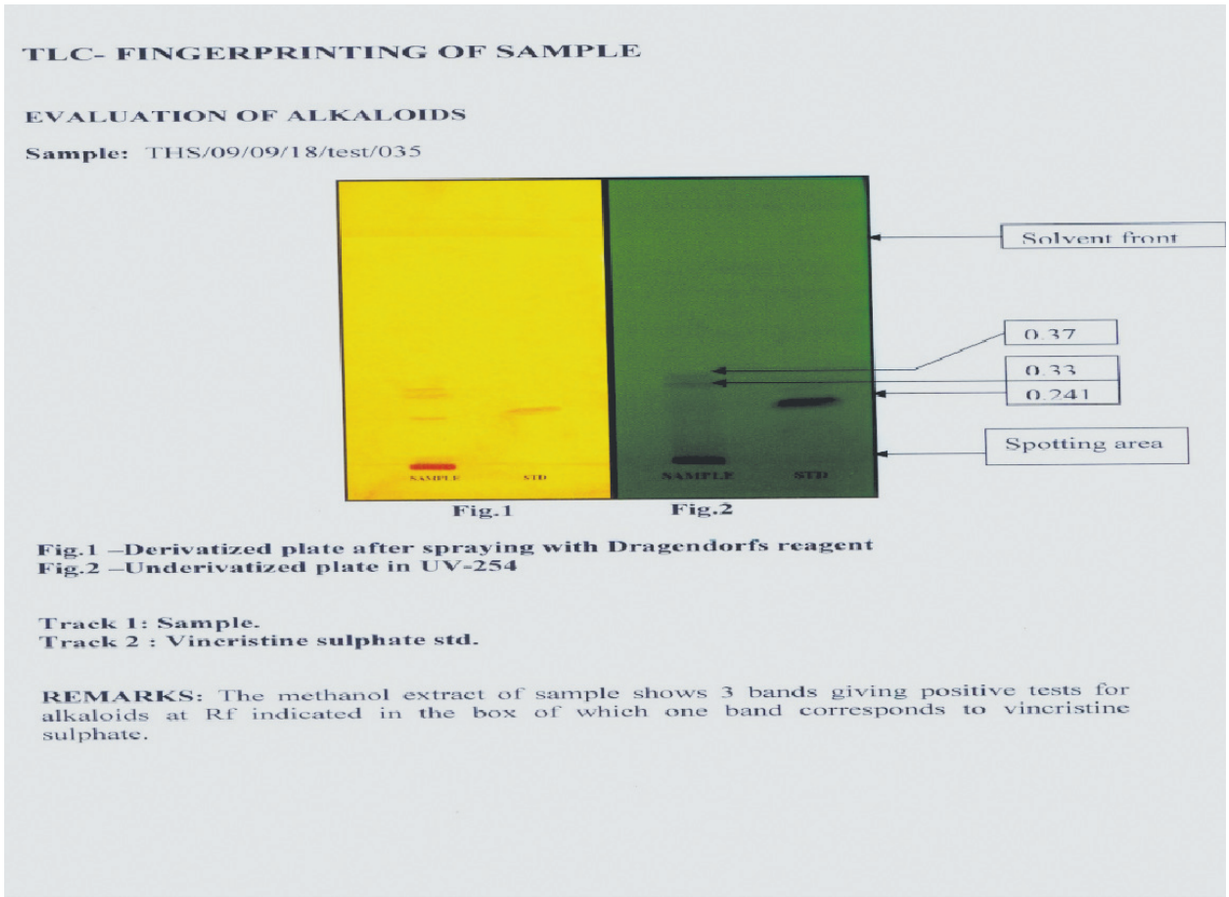

Figure 1: TLC-Fingerprinting of sample

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Table 1: 26 processed constituents of extract sample at Vincristin_Sample Run 3 9 10 94 Data- 260.00 nm

Index	Name	Time(Minutes)	Height(μV)	Area(%)	Area(μV. minutes)
1	UNKNOWN	2.346	121657.5	49.894	54755.7
2	UNKNOWN	3.160	529.8	0.040	44.4
3	UNKNOWN	3.280	463.6	0.048	52.7
4	UNKNOWN	4.000	154631.4	20.924	22963.0
5	UNKNOWN	4.360	9511.8	1.388	1523.7
6	UNKNOWN	4.560	4683.7	0.632	693.6
7	UNKNOWN	4.720	3500.8	0.429	470.7
8	UNKNOWN	4.880	3159.7	0.392	430.4
9	UNKNOWN	5.066	7216.2	1.373	1506.4
10	UNKNOWN	5.373	2154.8	0.288	316.3
11	UNKNOWN	5.746	5377.1	0.764	838.0
12	UNKNOWN	6.466	2812.6	0.618	678.0
13	UNKNOWN	7.013	1405.8	0.309	338.7
14	UNKNOWN	7.346	872.7	0.187	205.4
15	UNKNOWN	7.719	1505.9	0.346	379.9
16	UNKNOWN	8.546	789.6	0.186	204.0
17	UNKNOWN	9.373	1222.2	0.392	430.4
18	UNKNOWN	10.426	5129.4	2.059	2260.1
19	UNKNOWN	11.919	2500.3	0.850	932.6
20	UNKNOWN	13.186	1489.4	0.518	568.6
21	UNKNOWN	13.546	1623.0	0.488	535.3
22	UNKNOWN	14.626	3119.0	1.274	1398.2
23	UNKNOWN	15.892	29979.9	13.724	15061.8
24	VINCRISTINE	18.865	927.8	0.419	459.6
25	UNKNOWN	21.012	597.8	0.262	287.4
26	UNKNOWN	23.278	4214.5	2.195	2408.7
Total			**371076.2**	**100.000**	**109743.8**

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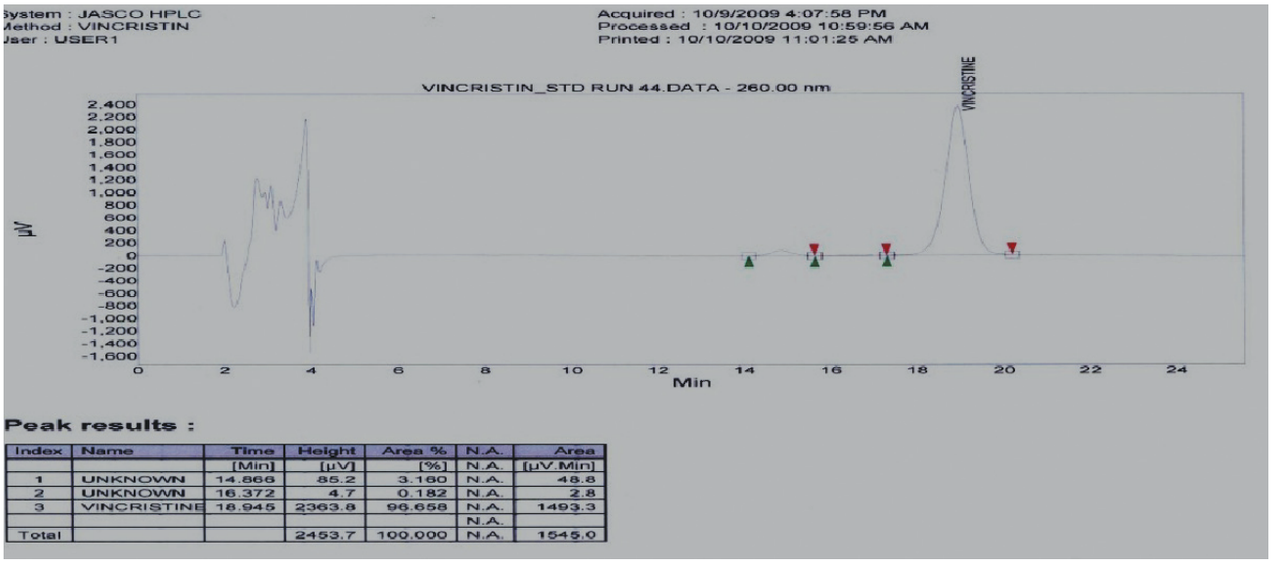

Index	Name	Time [Min]	Height [μV]	Area % [%]	N.A. N.A.	Area [μV.Min]
1	UNKNOWN	14.866	85.2	3.160	N.A.	48.8
2	UNKNOWN	16.372	4.7	0.182	N.A.	2.8
3	VINCRISTINE	18.945	2363.8	96.658	N.A.	1493.3
					N.A.	
Total			2453.7	100.000	N.A.	1545.0

Figure 2 : Peak results of *Catharanthus roseus* 1X potency during analysis by HPLC

In-vitro cytotoxic studies by using sulforhodamine assay

The extract of *Catharanthus roseus* in individual potencies used during study showed maximum cytotoxic activity at following molar drug concentration in μg/ml:

1. Cell line K562

Drug used	*Average value**	*Molar drug concentration*
Crude drug substance	49.4	80
Catharanthus roseus 1X	33.1	80
Catharanthus roseus 2X	12.5	20
Catharanthus roseus 6X	22.5	80
Catharanthus roseus 12X	33.1	40
ADR	9.0	80

Catharanthus roseus 2X showed maximum cytotoxicity of 12.5 in K562 cell line at molar drug concentration of 20 μg/ml. The details are given in Figure 3.

2. Cell line jurkat

Drug used	*Average value**	*Molar drug concentration*
Crude drug substance	38.6	80
Catharanthus roseus 1X	38.0	80
Catharanthus roseus 2X	6.6	80
Catharanthus roseus 6X	42.9	80
Catharanthus roseus 12X	37.9	80
ADR	0.8	80

*Average value indicates the mean of all the three experiments done

The Catharanthus roseus extract in human leukemic cell line jurkat showed maximum cytotoxicity of 6.6 at molar drug concentration of 80 μg/ml in 2X potency as shown in Table 2.

DISCUSSION

It is known that vincristine sulphate is an antineoplastic agent which may act by arresting mitosis at the metaphase. It is given intravenously in the treatment of acute leukemia of children.[7] This experimental work evaluated the anti-leukemic activity of potentised whole plant extract of *Catharanthus roseus* (L.)G. Don. The potencies up to 12X of *Catharanthus roseus* were made under Class IV of Hahnemannian classification by using 95% ethyl alcohol as solvent.

By analysis of sample by HPLC, total 26 constituents were identified in which one alkaloid was vincristine. Therefore, it is considered that medicinal properties of the whole plant extract are better than those of the alkaloid vincristine/vinblastine alone because homoeopathic medicines are prepared using the whole plants, leaves, fruits *etc*. as the case may be without

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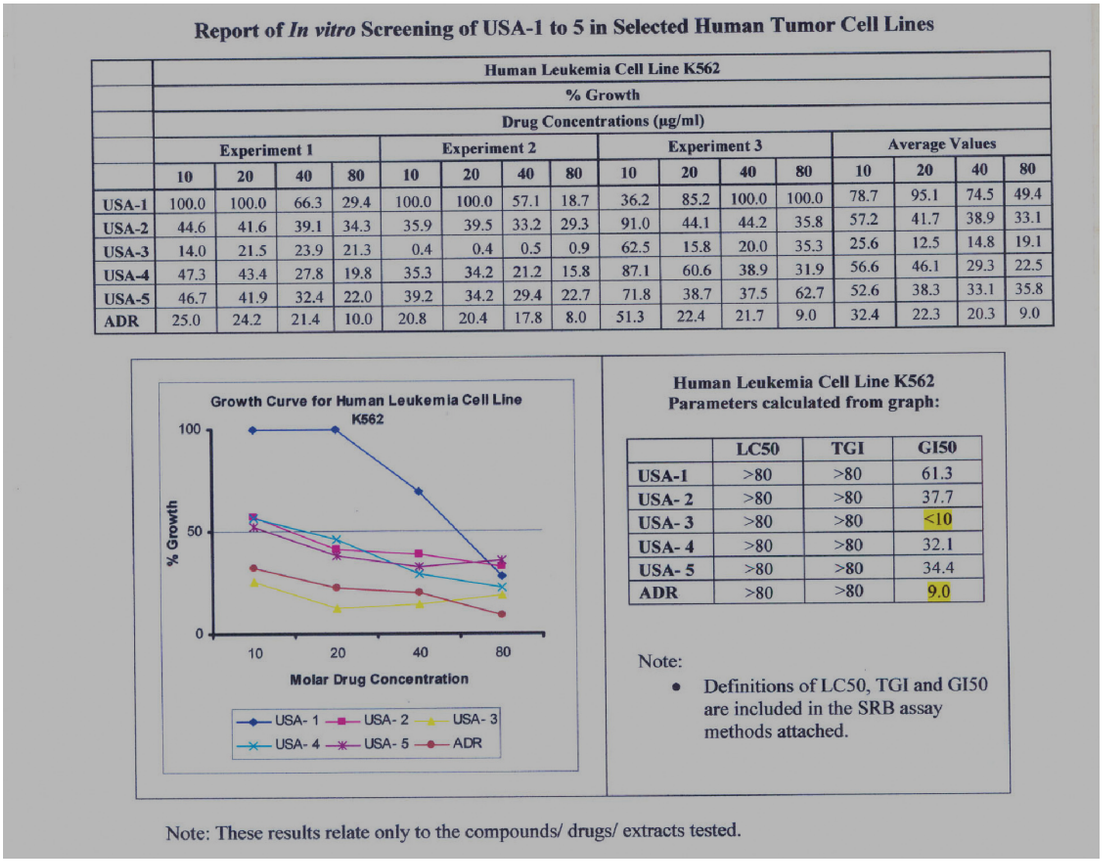

Report of *In vitro* Screening of USA-1 to 5 in Selected Human Tumor Cell Lines

	Human Leukemia Cell Line K562															
	% Growth															
	Drug Concentrations (µg/ml)															
	Experiment 1				Experiment 2				Experiment 3				Average Values			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
USA-1	100.0	100.0	66.3	29.4	100.0	100.0	57.1	18.7	36.2	85.2	100.0	100.0	78.7	95.1	74.5	49.4
USA-2	44.6	41.6	39.1	34.3	35.9	39.5	33.2	29.3	91.0	44.1	44.2	35.8	57.2	41.7	38.9	33.1
USA-3	14.0	21.5	23.9	21.3	0.4	0.4	0.5	0.9	62.5	15.8	20.0	35.3	25.6	12.5	14.8	19.1
USA-4	47.3	43.4	27.8	19.8	35.3	34.2	21.2	15.8	87.1	60.6	38.9	31.9	56.6	46.1	29.3	22.5
USA-5	46.7	41.9	32.4	22.0	39.2	34.2	29.4	22.7	71.8	38.7	37.5	62.7	52.6	38.3	33.1	35.8
ADR	25.0	24.2	21.4	10.0	20.8	20.4	17.8	8.0	51.3	22.4	21.7	9.0	32.4	22.3	20.3	9.0

Human Leukemia Cell Line K562
Parameters calculated from graph:

	LC50	TGI	GI50
USA-1	>80	>80	61.3
USA- 2	>80	>80	37.7
USA- 3	>80	>80	<10
USA- 4	>80	>80	32.1
USA- 5	>80	>80	34.4
ADR	>80	>80	9.0

Note:
- Definitions of LC50, TGI and GI50 are included in the SRB assay methods attached.

Note: These results relate only to the compounds/ drugs/ extracts tested.

Figure 3 : Report of in-vitro screening of USA 1-5 in human leukemic cell line K562

Table 2: Molar drug concentration and cytotoxic activity during study in Human Leukemic Cell Line jurkat

Molar drug concentration (µg/ml)	USA-1	USA-2	USA-3	USA-4	USA-5	ADR
10	100.0	100.0	95.1	88.8	92.5	7.5
20	100.0	100.0	77.2	83.7	90.1	4.2
40	85.3	59.2	32.4	58.9	56.3	1.6
80	38.6	38.0	**6.6**	42.9	37.9	0.8

The details of LC50, TGI and G150 calculation are given in Table 3.

Table 3: Results of LC50, TGI and G150 calculation of human cell lines K562 and jurkat

	K562			Jurkat		
	LC50	TGI	G150	LC50	TGI	G150
Crude drug substance	> 80	> 80	61.3	> 80	> 80	74.3
Catharanthus roseus 1X	> 80	> 80	37.7	> 80	> 80	64.6
Catharanthus roseus 2X	> 80	> 80	< 10	> 80	> 80	40.8
Catharanthus roseus 6X	> 80	> 80	32.1	> 80	> 80	65.5
Catharanthus roseus 12X	> 80	> 80	34.4	> 80	> 80	61.8
ADR	> 80	> 80	9.0	> 80	60.1	< 10

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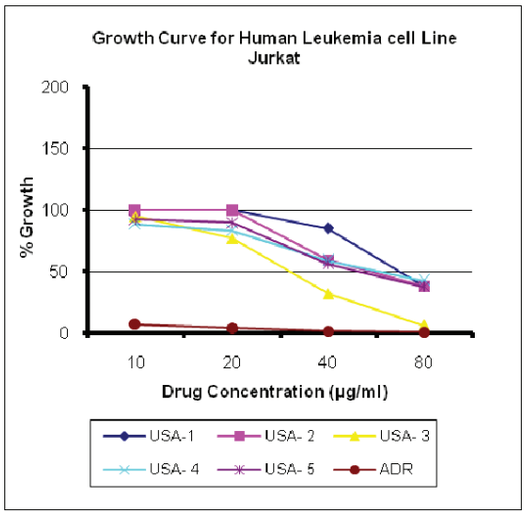

Figure 4: showing the growth curve for human leukemic cell line jurkat.

considering the alkaloid contents in the medicinal substances.

Alcoholic extract of potentised *Catharanthus roseus* (L.)G. Don. showed the presence of 0.036% of alkaloid vincristine with area of 1493.3µV.min by analysis of sample by HPLC.

The alcoholic extract of potentised *Catharanthus roseus* in 2X potency shows maximum cytotoxicity of 12.5 in human leukemic cell line K562 for chronic myeloid leukemia as compared to the positive control drug used in experimental procedure which has cytotoxicity of 22.3 at molar drug concentration of 20 µg/ml.

The extract of potentised *Catharanthus roseus* 2X potency appeared to have maximum cytotoxicity of 6.6 in human leukemic cell line jurkat for promyelocytic leukemia as compared to the positive control drug which has cytotoxicity of 0.8 at molar drug concentration of 80 µg/ml.

The study shows the maximum cytotoxic activity against the chronic myeloid leukemia.

LIMITATIONS OF THE STUDY

a. For preparation of homoeopathic potencies under Decimal scale, 1 ml of tincture or solution is added to 9 ml of alcohol. Ethanol in small concentration causes inhibition of cell proliferation and increases apoptosis during *in-vitro* study. This may give false-positive result.

b. No guidance has been given until now for standardisation of finished preparations by any authority in Homoeopathy. However, Homoeopathic Pharmacopoeia Laboratory (HPL), Ghaziabad, Govt. of India, has undertaken measures in this direction.

c. The vincristine is present in extremely low content in the plant viz. 0.0002%. It requires about 500 kg crude drug to extract 1 gm of vincristine.

This experimental work needs to be replicated with other potencies of *Catharanthus roseus*.

CONCLUSION

The alcoholic extracts of potentised *Catharanthus roseus* 2X showed antileukemic activity in chronic myeloid leukemia on human leukemic cell line 562, with a G150< 10.

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