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"A COMPARATIVE ANTICANCER ACTIVITY OF *PLUMBAGO ZEYLANICA* COLLECTED FROM NORTHERN AND SOUTHERN PARTS OF INDIA"

Dr. Rajesh Sharma[1*], Dr. Shraddha Naik[2], Dr. Vikas Saroch[3] and Dr. Gousia Chasoo[4]

[1]Lecturer, Dept. of Dravyaguna, Jammu Institute of Ayurveda and Research, Jammu.

[2]Head of Department, Dravyaguna, Shri KLE B.M.K Ayurved Mahavidhyalay, Belgaum 590003.

[3]Asst.Prof. PG Dept of Rasa and BK Desh Bhagat University, Punjab

[4]Scientist, Indian Institute of Integrative Medicine, Jammu.

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***Correspondence for Author**
Dr. Rajesh Sharma
Lecturer, Dept. of Dravyaguna, Jammu Institute of Ayurveda and Research, Jammu.

ABSTRACT

Cancer is one among the largest cause of deaths worldwide. Increasing interest and research on herbal medicine have revealed its importance in treating many diseases including cancer. The present study was carried out to evaluate the *invitro* anticancer activity of root extracts of *Plumbago zeylanica* Linn. Collected from two different geographical areas of India i.e. Himalayas (Jammu region) and Western Ghats (Belgaum region) against five different cancer cell lines. To determine the anticancer activity Alcoholic, Hydroalcholic and Aqueous extracts of *Plumbago Zeylanica* are prepared and anti-cancer activity is performed using Sulphorodamine B assay (SRB). Hydroalcholic extracts showed significant growth inhibition of HCT-15 cell line i.e. Colon cancer cells at all concentrations as compared to alcoholic and aqueous extracts and Aqueous extracts inhibits the growth of MCF-7 Breast cancer cells at 50,70 and 100 µg/ml concentrations. Comparison of IC_{50} values and linearity of the activity, it is observed that the sample of Northern region (Jammu) has promising results as compared to the sample collected from the Southern region.

KEYWORDS: Cancer, Geographical areas, *Plumbago zeylanica*, various extracts, anticancer activity, Sulphorodamine B assay.

INTRODUCTION

Ethno historical accounts shows that medicinal plants have been used as a remedy for various human ailments, the reason of using these plants is that they contain certain types of chemical constituent which is having greater therapeutic value that produces a definite pharmacological actions on human body with lesser side effects.[1] Cancer is one of the most life threatening diseases and possess many health hazard in both developed and developing countries.[2] As various types of cancers like breast cancer, colon cancer, leukemia etc. and management strategies for their curative aspect mainly are Chemotherapy, Radiation and Surgery so to overcome the side effects which are caused by these treatments we should go with the adjuvant therapy that can be achieved by using herbal drugs as so many herbal drugs are mentioned in Ayurveda for the treatment of cancer .A number of herbal drugs has been reported for their anticancer activity .The plant species *Plumbago zeylanica* popularly known as *Chitraka*, belongs to the family Plumbaginaceae, is distributed throughout the world. Plumbago species have been shown to contain compounds with significant activity and its extract has anti plasmodial, anti hyperglycemic and anti allergic properties.[3,4] Ethanolic extract of *Plumbago zeylanica* possessed significant anticancer activity and also reduce peroxidation levels due to higher content of terpenoids and flavonoids.[5] Plumbagin which is the active chemical constituent is also reported for its anticancer activity against lung cancer cells.[6] In Ayurveda it is said that the drugs which are collected from Northern region or from Himalayas are said to be potent than collected from other regions.[7]

So present study was undertaken to compare the anticancer activity of *Plumbago zeylanica* collected from two different geographical areas as Northern India and Southern India by taking different cancer cell lines.

MATERIAL AND METHODS

Materials

Drug procurement and Authentication

The roots of *Plumbago zeylanica* Linn.Were collected from Belgaum region (south) and Jammu (north) are Identified and authenticated at K.L.E U'S Central Research facility, Belgaum. (*Voucher no*: CRF/11/215-216).

Cytotoxicity Screening Method

The following method is used for this study and List of cancer cell lines used, their source, growth medium and positive control are shown in Table No.1.

- **Reagents:** Phosphate Buffer Saline (PBS), Penicillin Solution, Trypsin EDTA, TCA, Acetic acid, Tris buffer.
- **Media:** Incomplete growth media (RPMI 1640), Complete growth medium, FBS (Fetal Bovine Serum) (Bioclot, Lot No: 07310).
- **Apparatus:** Tissue culture flasks, Incomplete growth medium (NUNC), ,Micropipettes (Eppendorf), 1.5 ml and 0.5 ml eppendorf centrifuge tubes (Tarsons), 15ml centrifuge tubes (Tarsons), 50 ml centrifuge tubes, 96-Well cell culture plates (NUNC), Sterile centrifuge tubes, Cryovials, Glass bottles to store media etc. (Schott Duran), *Glass pipettes (*Tarsons*), Syringe (*Dispo Van*).*
- **Instruments needed:** Haemocytometer,Cryocontainers (Thermo Electron),Water bath (Genei), Filtration assembly (Millipore), Deep freezer (ScienTemp), Mechanical shaker, Centrifuge (Beckman), Autoclave, ELISA Reader (Tecan), Liquid Nitrogen Cylinder,CO2 Gas Incubator (Hera Cell).

Methods

Preparation of Extraction

The roots collected were shade dried; Aqueous, Alcoholic and Hydro alcoholic extracts were prepared by reflux extraction method.

The dried coarse powder (25g) was taken in the iodine flask, solvents were added in the ratio of 4:1 and were kept for 5 hours with intermittent shaking then condenser was attached to it and was placed in the heating mantle with proper temperature and water supply attached to the condenser. Mixture was refluxed for 2 -3 hours and this process was repeated for 3 times and filtrate is collected.

ANTICANCER ACTIVITY

Sulphorodamine B[7]

The anticancer activities of extracts were studied at Indian institute of Integrative medicine(IIIM)Canal Road Jammu where 5 cell lines were maintained in ideal laboratory conditions. The cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2mM L-glutamine. For present screening experiment, cells were inoculated into 96 well microtiter plates 90 µL/well at appropriate plating densities, depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37°C, in 5% CO2, 95% air and 100 % relative humidity for 24 h prior to addition of experimental drugs. After 24 h, cells from one plate of each cell line were fixed in situ with

TCA, to represent a measurement of the cell population for each cell line at the time of drug addition(Tz). Experimental extracts were solubilized in appropriate solvent at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to 10 times the desired final maximum test concentration with complete medium. Aliquots of 10 µl of these different dilutions were added to the appropriate micro-titer wells already containing 90 µl of cell suspension, resulting in the required final drug concentrations of 10,30,50,70 and 100 µg/ml. For each of the experiments a known anticancer drug was used as a positive control.

End point measurement

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. Sulphorodamine 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 an Elisa Plate Reader at a wavelength of 540 nm with 690 nm reference wavelength.[8]

% Growth inhibition =

$$100 - \frac{\text{Mean OD of individual Test Group}}{\text{Mean OD of control Group}} \times 100$$

RESULTS AND DISCUSSION

Six samples coded as M-4529 to M-4534(Table no. 2) were evaluated for their *Invitro* cytotoxicity against five human cancer cell lines at 10, 30 ,50 ,70 , 100 µg/ml concentrations. All the samples exhibited concentration dependent cytotoxity as described in the Table No 3. All six samples showed cell growth inhibition at all concentrations in HCT-15 colon cell lines, sample M-4534 i.e. (hydroalcoholic extract Jammu) is found to be the active sample at all concentrations against HCT-15 colon cell lines having IC_{50} value 1µg/ml as compared to the sample M-4533 (Hydroalcholic extract Belgaum) having IC_{50} value 16µg/ml. Sample M-4530 i.e. (Aqueous extract Jammu) has also shown cell growth inhibition against HCT-15 colon cell lines at all concentrations i.e. 10,30,50,70,100 that is more as compared to sample

M-4529 i.e. (Aqueous extract Belgaum) having their IC_{50} values 11µg/ml and 85 µg/ml.and Sample M-4529 and M-4530 also showed cell growth inhibition at concentrations 50, 70, 100 µg/ml in MCF- 7 Breast cancer cell lines, having IC_{50} values 33µg/ml and 52 µg/ml.SampleM-4532 had shown cell growth inhibition at 50, 70, 100 µg/ml concentrations against P C 3 – Prostate cancer lines. The samples with 50% or more than 50% growth inhibition in different concentrations on different cell lines possess anticancer activity.

Table No 1: List of Cancer Cell Lines, Their Source, Growth Medium and Positive Control Used

S .no	Name of cell line	Source	Medium for growth	Positive control
1	Colo-205	Colon	RPMI	5-FA
2	MCF-7	Breast	RPMI	5-FA
3	HCT-15	Colon	RPMI	5-FA
4	THP-1	Leukemia	RPMI	5-FA
5	PC-3	Prostate	RPMI	5-FA

Table No.2: Samples used for the Cytotoxic toxic Evaluation

S. No	Institute Code	Samples(Root of *Plumbago Zeylanica)*
1	M-4529	Aqueous extract (Bgm)
2	M-4530	Aqueous extract (Jmu)
3	M-4531	Alcoholic exract(Bgm)
4	M-4532	Alcoholic exract(Jmu)
5	M-4533	Hydro Alcoholic extract(Bgm)
6	M-4534	Hydro Alcoholic extract(Jmu)

Table No. 3: In vitro Cytotoxicity against Human Cancer Cell Lines

Cell line type			HCT-15	Colo-205	MCF-7	PC-3	THP-1
	Tissue		Colon	Colon	Breast	Prostrate	leukemia
Institution code	Code	Conc.	% Growth inhibition				
M-4529	Aq.Bgm	10	0	0	28	8	0
		30	3	0	43	15	0
		50	7	0	69	28	2
		70	71	0	78	46	2
		100	92	74	79	49	5
IC50 (µg/ml)			85	>100	33	>100	>100
M-4530	**Aq.Jmu**	10	52	0	10	0	0
		30	56	0	30	0	0
		50	76	0	51	30	0
		70	70	90	0	74	65
		100	100	96	0	80	68
IC50 (µg/ml)			11	>100	52	>100	>100
M-4531	Alc.Bgm	10	60	0	0	1	28
		30	89	15	0	4	35

		50	95	35	0	9	50
		70	99	50	5	15	67
		100	100	70	30	31	73
IC50 (µg/ml)			3	69	>100	>100	45
M 4532	Hyd.Alc Bgm	10	35	0	0	0	0
		30	74	0	0	0	0
		50	100	0	0	3	0
		70	100	5	17	36	9
		100	100	13	20	36	42
IC50 (µg/ml)			13	>100	>100	>100	>100
M-4533	Hyd.Alc Jmu	10	70	0	0	0	0
		30	80	0	0	0	0
		50	100	0	0	0	4
		70	100	54	20	0	10
		100	100	90	30	15	30
IC50 (µg/ml)			1	>100	>100	>100	>100
	5-FU		54	57	-	-	50
	Doxorubicin				60		

CONCLUSION

As per the aim of the study a comparative anticancer activity of Plumbago zeylanica was performed and from the results obtained from SRB Assay method, by the comparison of IC_{50} values and linearity of the activity, it is observed that the sample which was collected from Northern region (Jammu) has promising results as compared to the sample collected from the Southern region. This justifies the statement claimed by Ayurveda texts that medicinal plants collected from Himalayas are more potent as compared with else others.

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