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**Research Article** 

# A COMPARATIVE STUDY ON ANTICANCER POTENTIAL OF DIFFERENT EXTRACTS OF *MUCUNA PRURIENS* LINN. SEEDS AGAINST

### SERTOLI (GC) & ZR-75 CELL LINES

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#### ABSTRACTS

Mucunapruriens Linn is one of the popular and important medicinal plants of India. In about 200 indigenous drug formulations it has been used as a constituent. The plant is an important cover crop (or green manure crop) in many parts of the world. The plant is commonly known as Common Cowitch, Velvet bean and Cowhage, belonging to the family of Fabaceae and is indigenous to tropical countries like of India and West Indies. In the present study, comparison between the anticancer potential of different extracts of M. Pruriens Linn. Seeds in vitro against sertoli (GC) prostate cancer and ZR-75 breast cancer cell lines was investigated. In the turm of half maximum inhibitory concentration (IC<sub>50</sub>), or cytotoxicity, cell viability count, and growth inhibition of both cell lines at different concentration of each extracts. The MEMP (IC<sub>50</sub>-14.74 µg) and PEMP (IC<sub>50</sub>-15.50 µg) was found more cytotoxic than AEMP (IC<sub>50</sub>-16.64 µg) against GC cells, but MEMP (IC<sub>50</sub>-15.06 µg) and AEMP (IC<sub>50</sub>-15.35 µg) was more cytotoxic than PEMP (IC<sub>50</sub>-16.63 µg) against ZR-75 cells. The MTT assay method mainly used in study. The MEMP (15 µg concentration) exhibit significant cell inhibition against both of the cancer cell lines as compare to control after 48, 72 and 96 hrs intervals. The MEMP showed the significant decreased viable tumor cell count and improve tumor cells inhibition and percentage life span. From performed comparative study, exhibited that all extracts have anticancer potential but the MEMP showed significant anticancer potential against GC cells and ZR-75cells and that mean M.pruriens can be used in anticancer activity.

Keywords: Anticancer activity, IC<sub>50</sub>, MTT assay, mucunapruriens, sertoli, ZR-75.

#### INTRODUCTION

Carcinogenesis is a multi-step process it include initiation, promotion and progression stages and various require agents promote the development of cancer in each stages<sup>1,2</sup>.Cancer is a cellular disease and mainly caused by the misbalance of the normal cellular growth maturation and multiplication<sup>3</sup>. Main feature of the cancer is chromosomal change and disease primarily caused by alteration in the genome of the affected cells. Cancer is one of the leading causes of death around the world. According to the International Agency for Research on Cancer (IARC), in 2002,

cancer killed > 6.7 million people around the world, another 10.9 million new cases were diagnosed; and at the current rate, an estimated 15 million people will be diagnosed annually by 2020<sup>4</sup>. Now a day's chemotherapy is most commonly used technology for prevention of cancer it is also called as chemoprevention. In chemoprevention various synthetic and naturally agents are used to block the development of cancer. Various plants, vegetable and herbs used in folk and traditional medicine have been accepted as one of the main source of cancer chemoprevention drug discoverv and

development<sup>3</sup>. Medicinal herbs are significant source of synthetic and herbal drugs. Plants derived compounds such as navelbine, camptothecin, taxol and vinca alkaloids have a great significant to cancer therapy. Many herbs were the starting point of important chemotherapeutic drugs<sup>4</sup>.

MucunapruriensLinn is one of the popular and important medicinal plants of India. In about 200 indigenous drug formulations it has been used as a constituent. The plant is an important cover crop (or green manure crop) in many parts of the world<sup>1</sup>. The plant is commonly known as Common Cowitch, Velvet bean and Cowhage, belonging to the family of Fabaceae and is indigenous to tropical countries like of India and West Indies. Whole plant of Mucuna pruriens heaving valuable medicinal properties<sup>5</sup> and demand of Mucuna in Indian and International drug markets is very high. This plant is widely used in Ayurveda, from ancient time<sup>6</sup>. The seed powder has recently been found to show the anti-Parkinsonism effects which are probably due to the presence of L-DOPA. It is well known that dopamine is the brain neurotransmitter. The dopamine content in the brain tissue gets reduced because of its blockade of crossing over the blood brain barrier to reach the site of action. As L-DOPA is the precursor of dopamine, it crosses the barrier and gets dopamine converted into resuming the neurotransmission<sup>6</sup>. The anti-epileptic and antineoplastic activity of methanol extract of M. prurienshas been reported<sup>7</sup>. The methanol extract of MP seeds showed significant in -vitro antioxidant activity while it has also been indicated that the methanol extract of *M. pruriens* can be a potential source of natural anti-oxidant anti-microbialagent<sup>8</sup>. and restores It antioxidantlevels and reduces lipid peroxide content<sup>9</sup>. And it has been studied forvarious activities like anti-diabetic<sup>10</sup>, aphrodisiac, antineoplastic, anti-epileptic, and antimicrobialactivities<sup>11</sup>. Infact, its learningand memory enhancement has been detailed by and

its antihelmintic activityhas been demonstrated by the alcoholic extract of *M. pruriens* seeds gave four alkaloids. viz. mucunine, mucunadine, prunieninine<sup>12-14</sup>.The major prurienine and portion of the alcoholicextract of seeds showed the presence of 5- indolic compounds, two of which were identified as tryptamine and 5hydroxy tryptamine. It is a natural source of Lphenyl 4-dihydroxy dopa(L-3, alanine). Interestingly, even after the wide clinical application of this herb, not much experimental work has been done to support the mechanism of action of the seeds of *M. pruriens* for different clinical applications<sup>15</sup>. its Four tetrahydroisoquinoline alkaloids have been isolated for the first time from M. pruriens seeds. Out of them, two are new whose structures have been elucidated by spectroscopic methods<sup>15</sup>. The effects of the Mucuna pruriens Linn seeds extract have been investigated on general mating behavior, libido and potency of normal male Wister albino rats and also compared with the standard reference drug, Sildenafil citrate. The antitumor effect and antioxidant role of Mucuna pruriens (Family: Fabaceae) have been evaluate against EAC bearing Swiss albino mice<sup>8</sup>. The quantitative determination of L-Dopa, L-3-7-dihydroxy-1,2,3,4carboxy-6, tetrahydroisoguinoline and 1-methyl-3-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline in Mucuna pruriens var. utilis seeds were assayed by Rapid reversed-phase high performance liquid chromatographic (HPLC) method<sup>16</sup>. In the resent study, we have done the comparative study regarding the in vitro cytotoxic effect, and capacity of growth inhibition of timorous cell lines, of the petroleum ether and methanolic and aqueous extracts of Mucuna pruriens Linn seeds on both of the tumors cell lines SP sertoli (prostate cancer cell lines) and ZR-75(breast cancer cell lines). We show that the methanolic extract possess strong cytotoxic effect against both tumors cells. We also report the comparative

data of cell viability of normal cell.

S.No.	Name of Drug and Chemical	Name of Manufacturer/Supplier								
1.	Mucuna pruriens Linn	Purchased from the local market of Sagar (M.P.) India.								
2.	Petroleum Benzene	Ashapur Group of Industries, Dn-Road, Gujrat.								
3.	Methanol	Mikado Enterprises, Mumbai, Maharashtra.								
4.	Dimethylsulfoxide	Darshan Chemicals, Thane, Maharashtra.								

MATERIAL AND METHODS

#### Tumor Cell lines

Daltons Ascites Lymphoma cell lines, GC cell lines or sertoli cells (SP) human prostate cancer cell lines, ZR-75 human breast cancer cell line were provided from the Bhopal memorial hospital and research center Bhopal M.P. These cell lines are routinely cultured in RPMI and DMEM medium.

#### **Preparation of Plant Material and Extraction**

The seeds of the plant *Mucuna pruriens Linn*were purchased from the local market of Sagar (M.P.) India. The seeds of the plant were identified and authenticated by the botany department of Dr. H. S. Gour University and stored as a voucher specimen (MPYMP07) in the same institute for future reference. The seeds were dried under shade for 7 days and pulverized under mechanical grinder and dehulled seeds were coarse powdered with a laboratory mill and passed through sieve No. 60 and stored in an airtight container for further use.

## Preparation of petroleum ether and methanolic extract

The powder was loaded in to soxhlet extractor in 3 batches of 500g each and extracted with petroleum benzene (60-80) followed by methanol for near about 72 hrs at temperature not exceed then 30'C. After extraction the solvent was distilled off and the extract was concentrated under reduced pressure at 50oc and dried in vacuum (Yield: 9.65%, methanol extract). The phytochemical studies were performed as described. The dried extract thus obtained was dissolved in isotonic normal saline solution and used directly for the assessment of In-vitro anti tumor activity.

#### Preparation of aqueous extract

2 Kg of Powdered drug was taken in the closed container and boiled water was added to the container to completely soaked the drug in the water. Than very small amount of methanol was added in the flask as a preservative and shake well. The container was kept at room temperature for 72 hr. but continuously shaking had done every 2-3 hr. than filter the content through the vacuum, filtrate was discard and precipitate was dried under vacuum to give a completely dried product than collected the product and calculate the yield. The % yield of aqueous extract was calculated as 7.88%.

#### MTT cell viability test Principal of MTT assay

This is the colorimetric assay. In this assay water soluble Yellow MTT [3-(4,5 Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole] is reduced to insoluble purple formazan in the mitochondria of living cells. This reduction based on the mitochondria succinate dehydrogenase enzymes activity. The absorbance of this colored solution can be quantified by measuring at a certain wavelength by a spectrophotometer<sup>16</sup>.

The resulting purple solution is spectrophotometrically measured. An increase in cell number results in an increase in the amount of MTT formazan formed and an increase in absorbance<sup>17</sup>.

#### Procedure

The methyl thiazoletetrazolium (MTT) test was used to determine the effect of the Petroleum ether, methanolic and aqueous extracts on the growth of non-adherent cells petroleum ether extract dissolved in the DMSO and methanolic and aqueous extracts dissolved isotonic normal saline solution (0.9% Nacl w/v) for different concentration prepared. The test was performed as described.

Daltons Ascitic Lymphoma cell lines (DAL) was subcultured from stock and incubated with RPMI media. Then after DAL cells were incubated with media (control) and with different concentration of extracts (treated) in tohumidifiedCO<sub>2</sub> (5%) incubator at 37°C for overnight. After that Treated cells along with control were treated with MTT dye (10µI/100µI per well of 96 well plate) and incubate at 37°C for 3.0 hours. A Stop mix solution added in 100µI concentration and shake plate for an hour at room temperature for formazan formation. Then Incubate for an hour to precipitate formazan. Dissolve the precipitate and read the plate in plate reader at 570nm wavelength<sup>18</sup>.

#### RESULTS

Different extracts used for *In-vitro* determination of their toxicity on sertoli cells and ZR-75 cell lines. Percentage of viable cells and Cytotoxicity study were carried out by using MTT assay

with varying concentration using Will Tassay method										
S. No.	Concentration (mcg)	Categorie s	Pet. ether extract (PEMP)		Methanolic (MEM	extract P)	Aqueous extract (AEMP)			
			Mean absorbance	%cell viability	Mean absorbance	%cell viability	Mean absorbance	%cell viability		
1		Control RPMI media	0.273	100	0.273	100	0.273	100		
2	5	Treated	0.556	49.10	0.605	48.90	0.517	52.80		
3	10		0.695	39.28	0.687	43.08	0.566	48.23		
4	15		0.801	34.08	0.791	37.42	0.697	39.16		
5	20		0.904	30.19	0.895	33.07	0.777	33.13		
6	25		1.232	22.15	1.128	26.24	0.899	30.36		
7	30		1.919	14.22	1.920	15.41	0.990	27.57		
8	35		2.170	12.58	2.152	13.75	1.342	20.34		
9	40		2.307	11.83	2.312	12.80	1.975	13.82		

Cell Viability study Table 1: % Cell viability of different extract of *M.pruriens* seeds with varying concentration using MTT assay method



#### Cytotoxicity activity

The Cytotoxicity study was carried out for different extracts of plant *Mucuna pruriens Linn* seeds. These extracts was screened for its

Cytotoxicity against sertoli (SP) prostate cancer cell lines and ZR-75 breast cancer cell lines at different concentrations to determine the IC50 (50% growth inhibition) by MTT assay<sup>19</sup>.

S.No.	Concentration of	N	Number of cells (in lacks)						
	extracts (in µg)	Pet. ether extract	Methanolic extract	Aqueous extract					
1.	0	5.82	5.82	5.82					
2.	5	4.11	3.78	4.29					
3.	10	3.66	3.41	3.83					
4.	15	3.01	2.96	3.11					
5.	20	1.48	2.55	1.05					
6.	25	0.97	1.32	0.64					
7.	30	0.38	0.76	0.19					
8.	35	0.11	0.001	0.08					
9.	40	0.03	Nil	nil					
10.	45	0.01	Nil	-					
11	50	Nil	_	_					

### Table 2: IC-50 value of different extracts of *Mucuna pruriens Linn* seeds onsertoli(SP) cell lines after 24 hours

Table 3: IC-50 value of different extracts of Mucuna pruriens Linn
seeds on ZR-75 cell lines after 24 hours

S.No.	Concentration of	Number of cells (in lacks)						
	extracts (in µg)	Pet. ether extract	Methanolic extract	Aqueous extract				
1.	0	6.11	6.11	6.11				
2.	5	5.51	5.72	5.55				
3.	10	4.27	4.88	4.32				
4.	15	3.75	3.11	3.34				
5.	20	2.31	2.79	2.39				
6.	25	1.26	1.21	1.67				
7.	30	0.79	0.98	1.02				
8.	35	0.18	0.13	0.58				
9.	40	0.03	0.06	0.11				
10.	45	0.004	0.01	0.02				
11.	50	Nil		0.001				

The cytotoxic effect of Petroleum ether, Methanolic and Aqueous extracts on different tumor cell lines (SP and ZR-75) is summarized in Table 2 & Table 3. The table's shows that the *M.pruriens Linn* seed extracts have a differential cytotoxic activity on both of the cell lines tested. The Methanolic extract was found more cytotoxic as compared to the other extracts on both of the cancer cell lines tested, and the aqueous and petroleum ether extract have variable Cytotoxicity on both of the cancer cell lines, as indicated by the IC50 values.

The Cytotoxicity status of different extracts are that the methanolic and petroleum ether extracts

were more cytotoxic than aqueous extract against the sertoli (SP) cell line (IC50 ( $\mu$ g) = 14.74, 15.50 and 16.64 respectively). Cytotoxicity was observed against ZR-75 cell line (IC50 ( $\mu$ g): 15.06, 15.35 and 16.63 for the methanol, aqueous and petroleum ether extracts respectively).

These results show that the Cytotoxicity of each extract depends on the tumor cell type tested. The cell growth arrest or 100% mortality of the methanol, aqueous and petroleum ether extracts against sertoli (SP) cell lines were found 30µg, 35µg and 40µg and against ZR-75 cell lines 45 µg, 50 µg and 40 µg respectively<sup>20.</sup>

#### Evaluation of growth of GC (sertoli) cells Table 4: Growth comparison of GC (sertoli) cells with different concentration of all three extracts and RPMI media

S. No.	Time interval (in hrs)	Control(M) NCL	Methanolic extract(L) NCL		Aqueous extract (B) NCL			Pet. Ether extract (P) NCL			
			M+L1	M+L2	M+L3	M+B1	M+B2	M+B3	M+P1	M+P2	M+P3
1	24	5.82	3.78	3.41	2.96	4.29	3.83	3.11	4.11	3.66	3.01
2	48	8.92	7.47	6.17	5.68	8.1	7.39	6.74	8.18	7.91	6.86
3	72	14.4	8.63	7.2	7.13	11.71	9.2	8.33	13.57	13.15	10.87
4	96	48.3	33.7	29.1	21.3	40.01	35.06	29.91	47.55	45.11	39.22

Control – growth of the GC cell with RPMI media M = RPMI media

L1, L2 and L3 = 5  $\mu$ g, 10  $\mu$ g and 15  $\mu$ g concentration of methanolic extract B1, B2 and B3 = 5  $\mu$ g, 10  $\mu$ g and 15  $\mu$ g concentration of aqueous extract P1, P2 and P3 = 5  $\mu$ g, 10  $\mu$ g and 15  $\mu$ g concentration of petroleum ether extract NCL= number of cells in lacks



Evaluations of growth of ZR-75 cells
Table 5: Growth comparison of ZR-75 cells with different
concentration of all three extracts and RPMI media

S. No	Time interval	Control(M) NCL	Methanolic extract(L) NCL		Aqueous extract (B) NCL			Pet. Ether extract (P) NCL			
	(in hrs)		M+L1	M+L2	M+L3	M+B1	M+B2	M+B3	M+P1	M+P2	M+P3
1.	24	6.11	5.72	4.88	3.11	5.55	4.32	3.34	5.51	4.27	3.75
2.	48	11.89	10.10	8.40	6.13	9.12	7.97	4.13	11.08	10.93	8.48
3.	72	21.34	19.68	16.85	8.49	18.59	15.76	7.21	20.75	18.75	15.16
4.	96	45.8	42.34	35.10	14.43	39.92	30.11	12.53	44.0	41.62	35.62

Control - growth of the GC cell with RPMI media M = RPMI media

L1, L2 and L3 = 5  $\mu$ g, 10  $\mu$ g and 15  $\mu$ g concentration of methanolic extract

E1, E2 and B3 = 5  $\mu$ , 10  $\mu$ g and 15  $\mu$ g concentration of aqueous extract P1, P2 and P3 = 5  $\mu$ g, 10  $\mu$ g and 15  $\mu$ g concentration of petroleum ether extract NCL= number of cells in lacks



Fig. 3

Growth evaluation of GC sertoli cells





#### Fig. 4:For Growth Comparison of GC Sertoli cells with different concentration of all three extracts

Growth evaluation of ZR-75 cells

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Fig. 5:For evaluation of ZR-75 cells with different concentration of all three extracts

#### DISCUSSION

The present study was performed to providecomparative data on the in vitro cytotoxicity study of different extracts of M. pruriens Linn seeds against (SP) sertoli cells, prostate cancer cell lines and ZR-75 cells, Breast cancer cell lines. The methanol extract exhibited a significant cytotoxic effect against both of the cell lines taken for study. While aqueous and petroleum ether extracts showed little cytotoxic activity. But petroleum ether extracts found more cytotoxic compared to aqueous extract against the (SP) sertoli cells and aqueous extract found more cytotoxic compared to petroleum ether extracts against ZR-75 cells.

Our results agree with previous research in which investigated that, to evaluate the antitumor activity and antioxidant status of methanol extract of Mucuna pruriens (MEMP) in EAC tumor bearing mice. The MEMP treated animals at the doses of 125 and 250 mg/kg significantly inhibited the tumor volume, packed cell volume, tumor (viable) cell count.(Rajeshwar et.al.) the percentage growth inhibition increase with the increasing the concentration of the different extract of M. pruriens Linn on both of cell lines used in study. The present study demonstrates that the cytotoxic activity of *M. pruriens* extracts is a complex phenomenon depending not only on the nature of the extract and its components, but also on the tumor cell type. Now overall study evaluate

that *M. pruriens Linn* has potential activity on (SP) sertoli cells, prostate cancer cell lines and ZR-75 cells, Breast cancer cell lines these drug has considerable anticancer activity on prostate and breast cancer.

Additional research is now necessary in order to determine the agents responsible for these *invitro* anti-cancer activities as well as the molecular mechanisms involved in their effects.

#### CONCLUSION

In the resent study, we have done the comparative study regarding the *in vitro* cytotoxic effect, and capacity of growth inhibition of tumourous cell lines, of the petroleum ether and methanolic and aqueous extracts of *Mucuna pruriens* Linn seeds on both of the tumors cell lines SP sertoli (prostate cancer cell lines) and ZR-75(breast cancer cell lines). We show that the methanolic extract possess strong cytotoxic effect against both tumors cells. We also report the comparative data of cell viability of normal cell.

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