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Original Article

In-vitro Cytotoxic Activity of *Indianthus virgatus* (Roxb.) Suksathan and Borchs. On A549, A431, CaCo2, U87 and L929 Cell Lines

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ABSTRACT

Introduction: Medicinal plants play a key role to cure many diseases from time immemorial. The usage of medicinal plants in traditional medicinal system is the vital process of India. Cancer is one of the killing diseases and causes severe defects on human being. There are many types of cancer diseases in human beings affects the different organs. There is no proper medicine to cure such kind of cancer diseases. **Objective:** The purpose of the study is to evaluate the test substances for their cytotoxicity against selected cell lines. **Methods:** In the present study the *in-vitro* cytotoxicity potential of chloroform and methanolic leaf extract of *Indianthus virgatus* (Roxb.) Suksathan and Borchs. Was carried out against five cell lines, four of which were cancerous and one normal cell line i.e., A549, A431, CaCo2, U87 and L929. **Results:** The results revealed that the cytotoxicity potential of the leaf and rhizome increased with the increase in concentration of leaf and rhizome extracts. The chloroform leaf extract showed highest percentage of growth inhibition against A549 cell line. The methanol leaf extract showed highest percentage of growth inhibition against A431 cell line. The chloroform leaf extract showed highest percentage of growth inhibition against CaCo2 cell line. The chloroform rhizome extract showed highest percentage of growth inhibition against U87 cell line. The methanolic leaf extract showed highest percentage of growth inhibition against L929 cell line .This shows that for different cell lines the highest percentage growth of inhibition was shown by different extracts. **Conclusion**: The present study has suggested that the leaf and rhizome extracts of *Indianthus virgatus* (Roxb.) Suksathan and Borchs. , Possesses potent anticancer property which can be used to prepare anticancer drug with proper standardization methods.

Key words: *Indianthus virgatus* (Roxb.) Suksathan and Borchs, Anticancer activity, Cancer Cell Lines, Medicinal Plant.

INTRODUCTION

Cancer is the leading killing disease after the cardiovascular disorders. There is no proper medicine for controlling the growth of the cancer cells. There has long been standing interest in the identification of natural products for the treatment of various diseases for thousands of years. Ayurveda, a traditional Indian medicine of plant drugs has been successful from early times in using the natural drugs and preventing or suppressing various tumours using various lines of treatment.[1] Natural products possess immense pharmacological significance in the development of drugs including cancer.[2-4] The majority plant derived phytoconstituents, such as paclitaxel, camptothecin, etoposide, indole alkaloids, vinca alkaloids podophyllotoxin derivatives, teniposide and etoposide, currently used in clinical cancer chemotherapy. The efficacies of chemotherapy, radiotherapy, hormonal therapy, or surgery, which are mainly used for the treatment of cancer, are well-known for side effects. Hence, the identification of novel natural products that possess better effectiveness against cancer, but less harmful effects has become desirable and therefore, natural products are continuously being explored worldwide.

Indianthus virgatus (Roxb.) Suksathan and Borchs. belonging to the family Marantaceae is locally known as 'Malamkoova'. *Schumannianthus virgatus* (Roxb.) Rolfe is a synonym of this plant. It is an erect herb of 4 cm height with tuberous root stock. It has a compound leaf cluster on top of cane like stem. It is distributed in South India and Sri-Lanka. In Kerala, the plants are abundant in Western Ghats. It is used by the Kurichar tribes to treat skin diseases.[5] It is also used by tribal healers of Kerala to treat jaundice.[6] The present study framed to investigate the *in-vitro* cytotoxicity potential of leaf and rhizome extracts of *Indianthus virgatus* (Roxb.) Suksathan and Borchs.

MATERIALS AND METHODS

Collection and extraction of plant material

Fresh leaves and rhizome of *Indianthus virgatus* (Roxb.) Suksathan and Borchs. was collected from Palode, Kerala, India and authenticated by Foundation for Revitalisation of Local Health Traditions

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herbarium, Bangalore, India. Leaves and rhizomes were cleaned and dried at room temperature for a period of 25 days under shade. Finely ground dried leaf and rhizomes were weighed and extracted using Soxhlet apparatus by two different solvents chloroform and methanol.

Outline of the method

The *in-vitro* cytotoxicity was performed for Leaf Extract (Chloroform), Leaf Extrac t (Methanol), Rhizome extract (Chloroform), Rhizome extract (Methanol) on A549 (Human Lung Carcinoma), A431 (Human Skin Carcinoma), CaCo2 (Human Colon Cancer), U87 (Human Glioblastoma) and L929 (Mouse Fibroblast) cell lines to find toxic concentration of the extracts by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay.

Preparation of test solution

For cytotoxicity studies, 10mg of all the four test substances were separately dissolved and volume was made up with MEM/DMEM-HG/Ham's F-12 supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by 0.22µ syringe filtration. Serial two- fold dilutions were prepared from this for carrying out cytotoxic studies.

Cell line and culture medium

A549 (Human Lung Carcinoma), A431 (Human Skin Carcinoma), CaCo2 (Human Colon Cancer), U87 (Human Glioblastoma), and L929 (Mouse Fibroblast) cell lines were procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in their respective media viz., MEM/DMEM-HG/Ham's F-12 supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5 µg/ml) in a humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 well microtiter plates (Tarsons India Pvt. Ltd., Kolkata, India).

Cytotoxicity studies

In all the cell lines, the monolayer cell culture was trypsinized and the cell count was adjusted to 100,000 cells/ml using respective media viz., MEM/DMEM-HG/Ham's F-12 containing 10% FBS. To each well of the 96 well micro titre plate, 0.1 ml of the diluted cell suspension was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, monolayer washed once with medium and 100µl of different test concentrations of test substances were added on to the partial monolayer in micro titre plates. The plates were then incubated at 37°C for 72 h in 5% CO_2 atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval.

MTT ASSAY

After 72 h incubation, the drug solutions in the wells were discarded and 50µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37°C in 5% CO_2 atmosphere. The supernatant was removed and 100µl of propanol was added and the plates were gently shaken to solubilise the formed formazan.[7-11] The absorbance was measured using a micro plate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the standard formula and concentration of test substances needed to inhibit cell growth by 50% (CTC_{50}) values was generated from the dose-response curves for each cell line.

RESULTS AND DISCUSSION

In the present study, *in-vitro* cytotoxicity effects of leaf and rhizome extracts of *Indianthus- virgatus* (Roxb.) Suksathan and Borchs. was carried out with various concentrations for the following cancer cell lines A549 (human lung carcinoma cell line), A431 (human skin carcinoma cell line) CaCo2 (human colon cancer cell line), U87 (human glioblastoma) and one normal cell L929 (Mouse fibroblast cell line). The leaf and rhizomes of the plant was collected from Kerala and shade dried powdered and extracted with chloroform and methanol solvent. Six different concentrations (31.25µg/ml, 62.5µg/ml, 125µg/ml, 250 µg/ml, 500 µg/ml and 1000µg/ml) of leaf extracts were used to study the cytotoxicity potential of the plant. The cytotoxicity potential of various concentrations of chloroform and methanolic extracts with CTC_{50} values of *Indianthus virgatus* (Roxb.) Suksathan and Borchs. is displayed in Tables 1 to 5 and Figure 1 to 5.

The results revealed that the cytotoxicity rate had increased when the concentrations of leaf and rhizome extracts increases. MTT assay measured the cell viability based on the reduction of yellow tetrazolium MTT to a purple formazan dye by mitochondrial succinate dehydrogenase enzyme. So, the amount of formazan produced reflected the number of metabolically active viable cells. The test substances Leaf Extract (Chloroform), Leaf Extract (Methanol), Rhizome extract (Chloroform), Rhizome extract (Methanol) exhibited a CTC50 value of 99.53±1.9, 176.61±1.4, 149.43±0.9, and 805.53±3.9 on A549, 292.56±1.8, 209.39±3.8, 492.31±4.7, and 404.30±1.1 on A431, 140.67±1.4, 181.91±1.1, 421.22±3.3 and 209.32±4.1 on CaCo2, 101.00±1.1, 51.68±4.4, 48.02±0.2, and 79.96±1.5 on U87 and 113.47±0.3, 53.83±0.3, 124.09±0.4 and 91.13±0.5 on L929 respectively. The chloroform leaf extract showed highest percentage of growth inhibition against A549 cell line. The

Table 1: Cytotoxic properties of test substances against A549 cell line.

Sl. No	Name of Test Substance	Test Conc. (µg/ml)	% Cytotoxicity	CTC_{50} (µg/ml)
1	Leaf extract(Chloroform)	1000	92.10±0.3	
		500	81.43±0.6	
		250	70.20±0.3	99.53±1.9
		125	56.65±0.2	
		62.5	40.26±1.3	
		31.25	28.93±0.2	
2	Leaf extract(Methanol)	1000	73.67±0.3	
		500	64.14±0.4	
		250	59.36±0.3	176.61±1.4
		125	43.42±0.4	
		62.5	40.33±0.6	
		31.25	34.30±0.5	
3	Rhizome extract(Chloroform)	1000	85.38±0.8	
		500	72.11±0.3	
		250	65.25±0.4	
		125	46.29±0.3	149.43±0.9
		62.5	39.43±1.4	
		31.25	32.92±1.6	
4	Rhizome extract(Methanol)	1000	53.47±0.2	
		500	44.56±0.2	
		250	30.70±0.3	805.53±3.9
		125	23.60±1.1	
		62.5	15.63±0.8	
		31.25	13.34±0.2	

Table 2: Cytotoxic properties of test substances against A431 cell line.

Sl. No	Name of Test Substance	Test Conc. (µg/ml)	% Cytotoxicity	CTC_{50}(µg/ml)
1	Leaf extract(Chloroform)	1000	90.62±0.4	
		500	89.10±0.4	
		250	41.97±0.4	292.56±1.8
		125	32.84±1.2	
		62.5	25.58±0.6	
		31.25	23.42±0.5	
2	Leaf extract(Methanol)	1000	89.89±0.3	
		500	72.75±0.5	
		250	56.50±0.6	209.39±3.8
		125	36.48±0.7	
		62.5	31.17±0.3	
		31.25	29.55±0.4	
3	Rhizome extract(Chloroform)	1000	86.55±0.4	
		500	50.47±0.3	
		250	35.64±0.9	492.31±4.7
		125	30.54±0.3	
		62.5	27.15±0.5	
		31.25	25.28±0.8	
4	Rhizome extract(Methanol)	1000	57.83±2.1	
		500	54.74±0.3	
		250	42.37±0.4	404.30±1.1
		125	35.10±0.7	
		62.5	28.62±0.5	
		31.25	22.88±0.4	

Table 3: Cytotoxic properties of test substances against CaCo2 cell line.

Sl. No	Name of Test Substance	Test Conc. (µg/ml)	% Cytotoxicity	CTC_{50}(µg/ml)
1	Leaf extract(Chloroform)	1000	82.67±0.3	
		500	78.93±1.6	
		250	62.67±2.1	140.67±1.4
		125	48.18±0.4	
		62.5	15.02±0.4	
		31.25	8.18±0.9	
2	Leaf extract(Methanol)	1000	82.31±0.6	
		500	81.16±0.4	
		250	56.00±0.5	181.91±1.1
		125	44.98±0.6	
		62.5	21.60±1.0	
		31.25	13.33±1.0	
3	Rhizome extract(Chloroform)	1000	68.00±0.3	
		500	55.81±0.2	
		250	37.33±1.1	421.22±3.3
		125	31.20±1.5	
		62.5	21.42±1.4	
		31.25	12.18±0.4	
4	Rhizome extract(Methanol)	1000	68.18±0.4	
		500	63.91±1.1	
		250	56.09±0.9	209.32±4.1
		125	37.42±0.6	
		62.5	21.69±0.8	
		31.25	6.31±0.67	

methanol leaf extract showed highest percentage of growth inhibition against A431 cell line. The chloroform leaf extract showed highest percentage of growth inhibition against CaCo2 cell line. The chloroform rhizome extract showed highest percentage of growth inhibition against U87 cell line. The methanolic leaf extract showed highest percentage of growth inhibition against L929 cell line. This shows that extracts showing the highest percentage growth of inhibition varies with respect to different cell lines. The leaf methanolic extracts also showed cytotoxicity against the normal mouse fibroblast cell line at 50% cytotoxicity concentration at 53.83±0.3µg'ml.

The plant exhibits some medicinal properties and has a potential to cure certain diseases. Though this plant does not show any antibacterial and antifungal activity,[12] reports have revealed that the rhizomes of this plant have hepatoprotective property.[6] The present investigation reveals that both rhizomes and leaves of this plant have cytotoxic potential. The leaf extracts show better cytotoxic activity compared to rhizome extracts.

CONCLUSION

Indianthus virgatus (Roxb.) Suksathan and Borchs. is one of the potent medicinal plants used by the Kani tribes of Kerala. The rhizomes of the plant have the potent medicinal property and generally used to jaundice. The current research also added one more potent activity of the leaf and rhizome of the plant. The further research is necessary to design the drugs for cancer diseases in pharmaceutical industries.

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Table 4: Cytotoxic properties of test substances against U87 cell line.

Sl. No	Name of Test Substance	Test Conc. (µg/ml)	% Cytotoxicity	CTC_{50}(µg/ml)
1	Leaf extract(Chloroform)	1000	93.33±0.5	
		500	89.44±0.3	
		250	68.82±0.2	101.00±1.1
		125	56.47±0.6	
		62.5	39.64±0.2	
		31.25	29.05±0.09	
2	Leaf extract(Methanol)	1000	93.77±0.4	
		500	93.14±0.3	
		250	77.37±1.5	51.68±4.4
		125	65.19±2.2	
		62.5	56.63±2.4	
		31.25	37.13±3.6	
3	Rhizome extract(Chloroform)	1000	93.73±0.2	
		500	91.96±0.4	
		250	89.41±0.4	48.02±0.5
		125	76.60±0.3	
		62.5	61.67±0.6	
		31.25	36.48±0.3	
4	Rhizome extract(Methanol)	1000	93.84±0.0	
		500	92.93±0.2	
		250	78.59±0.7	79.96±1.5
		125	68.06±0.3	
		62.5	42.99±0.7	
		31.25	12.49±0.6	

Table 5: Cytotoxic properties of test substances against L929 cell line.

Sl. No	Name of Test Substance	Test Conc. (µg/ml)	% Cytotoxicity	CTC_{50} (µg/ml)
1	Leaf extract(Chloroform)	1000	92.19±0.2	
		500	90.59±0.8	
		250	63.20±0.2	113.47±0.3
		125	58.57±0.3	
		62.5	12.10±0.2	
		31.25	5.77±0.25	
2	Leaf extract(Methanol)	1000	. 93.64±0.2	
		500	91.75±0.2	
		250	91.03±0.2	53.83±0.3
		125	63.98±0.3	
		62.5	54.75±0.2	
		31.25	37.65±0.1	
3	Rhizome extract(Chloroform)	1000	91.98±0.2	
		500	90.10±0.4	
		250	88.34±0.8	124.09±0.4
		125	50.25±0.1	
		62.5	33.44±0.4	
		31.25	18.23±0.2	
4	Rhizome extract(Methanol)	1000	91.83±0.3	
		500	87.90±0.6	
		250	75.07±0.2	91.13±0.5
		125	65.53±0.3	
		62.5	36.88±0.2	
		31.25	34.08±1.1	

CTC_{50}- Cytotoxicity concentration.

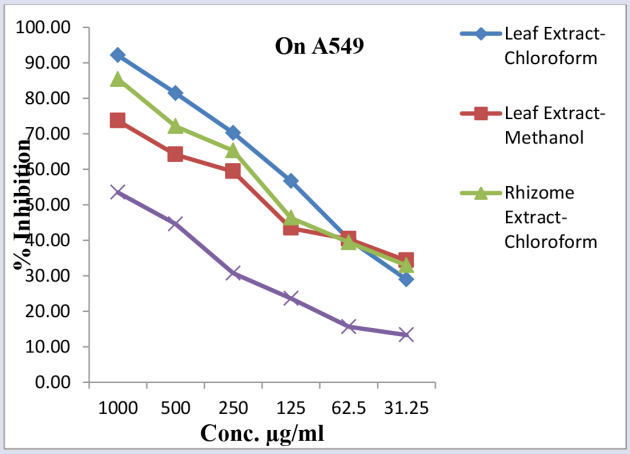

Figure 1: Graph of cytotoxic effect of Leaf Extract (Chloroform), Leaf Extract (Methanol), Rhizome extract (Chloroform), Rhizome extract (Methanol) on A549.

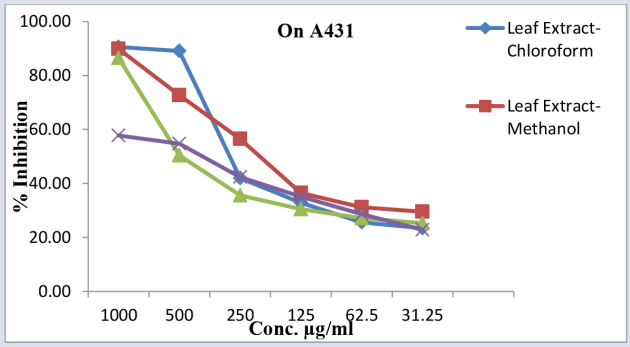

Figure 2: Graph of cytotoxic effect of Leaf Extract (Chloroform), Leaf Extract (Methanol), Rhizome extract (Chloroform), Rhizome extract (Methanol) on A431.

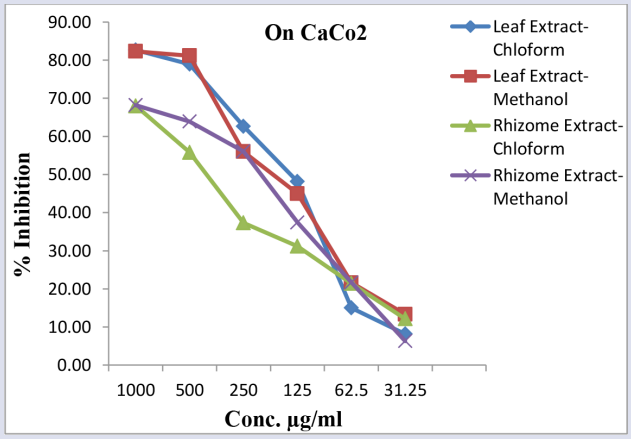

Figure 3: Graph of cytotoxic effect of Leaf Extract (Chloroform), Leaf Extract (Methanol), Rhizome extract (Chloroform), Rhizome extract (Methanol) on CaCo2.

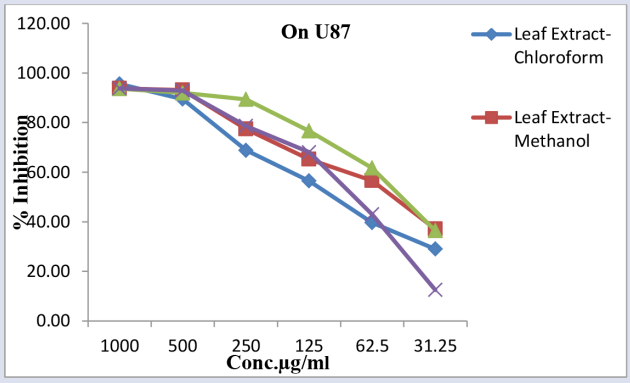

Figure 4: Graph of cytotoxic effect of Leaf Extract (Chloroform), Leaf Extract (Methanol), Rhizome extract (Chloroform), Rhizome extract (Methanol) on U87.

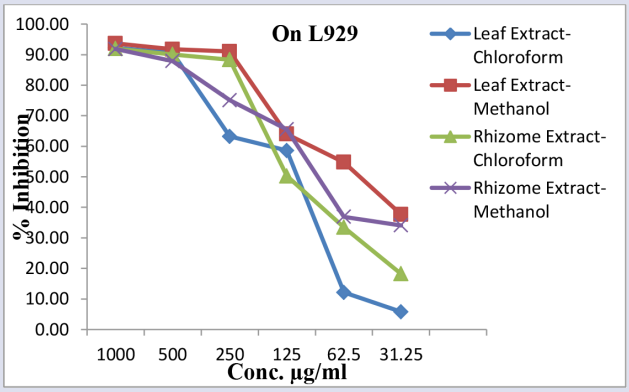

Figure 5: Graph of cytotoxic effect of Leaf Extract (Chloroform), Leaf Extract (Methanol), Rhizome extract (Chloroform), Rhizome extract (Methanol) on L929.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

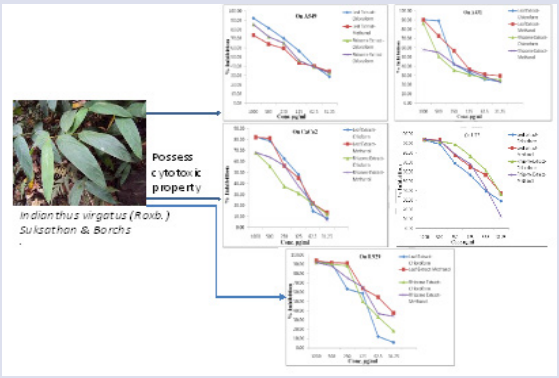
ABBREVIATIONS

NCCS: National Centre For Cell Science; **FBS:** Fetal bovine serum; ºC: Degree Centigrade; **% :** Percentage; **gm:** Gram; **hr:** Hour; **mg:** Milli gram; **mL:** Milli litre; **nm:** Nano meter; **µl:** Micro litre; **µg:** Micro gram; **MTT**: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; **TPVG:** Trypsin Phosphate Versene Glucose Solution; **DMEM :** Dulbecco's Minimum Essential Media; **MEM :** Minimum Essential Media; Ham's F-12: Nutrient Mixture F12; **DMSO :** Dimethyl sulfoxide; **CTC_{50} :** Cytotoxicity concentration; **EDTA :** Ethylenediaminetetraacetic acid.

REFERENCES

1. Balachandran P, Govindarajan R. Cancer- An ayurvedic prespective. Pharmacol Res. 2005;51:19-30.
2. Newman DJ, Cragg GM. Natural products as sources of new drugs over the 30 years from 1981 to 2010. J Nat Prod. 2012;75(3):311-35.
3. Kuno T, Testuya T, Akira H, Takuji T. Cancer chemoprevention through the induction of apotosis by natural product. J Biophysical chem. 2012;3(2):156-73.
4. Wen T, Jinjian L, Mingqing H, Yingboli, Meiwanchen, Guosheng W, *et al.* Anticancer natural products isolated from Chinese medicinal herbs. Chin Med. 2011;6(1):27.
5. Binu T, Rajendran A. Less known ethnomedicinal plants used by Kurichar tribe of Wayanad District, Southern Western Ghats Kerala, India. BRI. 2013;6(2):32-5.
6. Neethu V, Latha PG, Suja SR, Vilash V, Ragesh R, Shoumya S, Aneesh Kumar AL. Hepatoprotective property of *Schumannianthus virgatus* (Roxb.)Rolfe against D- galactosamine induced hepatotoxicity in wistar rats. Int J Adv Res. 2017;5(7):2301-7.
7. Francis D, Rita L. Rapid colorometric assay for cell growth and survival modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. J Immunol Methods. 1986;89:271-77.
8. David GC, Ruili H, Anders W. Anticancer medicines in development: assessment of bioactivity profiles within the National Cancer Institute anticancer screening data. Mol Cancer Ther. 2007;6(8):2261-70.
9. Chris H. Takimoto. Anticancer drug development at the US National Cancer Institute. Cancer Chemother Pharmacol. 2003;52:29-33.
10. Rubinstein LV, Shoemaker RH, Paull KD, Simon RM, Tosino S, Skehan P, *et al.* Comparision of *in-vitro* Anticancer –Drug-Screening Data Generated with a Tetrazolium Assay Versus a Protein Assay Against a Diversal Panel of Human Tumour cell lines. J Natl Cancer Inst. 1990;82:1113-7.
11. Samuel BT, Molly M, Dhanabal SP. *In vitro* cytotoxic activity of menispermaceae plants against HeLa cell line. Anc Sci Life. 2013; 33(2):81-4.
12. Goel AK, Kulshreshtha DK, Dubey MP, Rajendran SM. Screening of Indian plants for biological activity. Indian J Exp Biol. 2002;40:812-17.

GRAPHICAL ABSTRACT



SUMMARY

- *In-vitro* cytotoxicity of the test substances leaf extract (chloroform), leaf extract (methanol), rhizome extract (chloroform), rhizome extract (methanol) were tested by MTT against Cancerous and Normal cell line *i.e.* A549 (Human Lung Carcinoma), A431 (Human Skin Carcinoma), CaCo2 (Human Colon Cancer), U87 (Human Glioblastoma) and L929 (Mouse Fibroblast) cell lines. The test substances were taken at concentrations ranging from 1000 µg/ml to 31.25µg/ml to determine the percentage growth inhibition on the cell lines A549, A431, CaCo2, U87 and L929.
- The test substances Leaf Extract (Chloroform), Leaf Extract (Methanol), Rhizome extract (Chloroform), Rhizome extract (Methanol) exhibited a CTC50 value of 99.53±1.9, 176.61±1.4, 149.43±0.9, and 805.53±3.9 on A549, 292.56±1.8, 209.39±3.8, 492.31±4.7, and 404.30±1.1 on A431, 140.67±1.4, 181.91±1.1, 421.22±3.3 and 209.32±4.1 on CaCo2, 101.00±1.1, 51.68±4.4, 48.02±0.2, and 79.96±1.5 on U87 and 113.47±0.3, 53.83±0.3, 124.09±0.4 and 91.13±0.5 on L929 respectively.
- The chloroform leaf extract showed highest percentage of growth inhibition against A549 cell line. The methanol leaf extract showed highest percentage of growth inhibition against A431 cell line. The chloroform leaf extract showed highest percentage of growth inhibition against CaCo2 cell line. The chloroform rhizome extract showed highest percentage of growth inhibition against U87 cell line. The methanolic leaf extract showed highest percentage of growth inhibition against L929 cell line.

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