International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Impact Factor 2024: 7.101

Cytotoxic Activity of *Sauromatum venousm (*Ait.) Kunth. Against Carcinoma Cell Line

M. U. Ghurde[1], S. N. Malode[2], R. R. Godse[3], P. A. Wadegaokar[3]

[1]Department of Botany, Vidya Bharati Mahavidyalaya, Amravati
Email: *monali.ghurde23[at]gmail.com*

[2]P. G. Department of Botany GVISH, Amravati

[3, 4]Department of Biotechnology, SGBAU, Amravati

Abstract: ***In the present study, human pancreatic cancer cell lines (MiaPaca - 2) were used to assess the cytotoxic effect of Sauromatum venosum corm extract. The MTT assay was used to measure the cytotoxic activity on human pancreatic cells. A calorimetric approach based on the ability of metabolically active cells to cleave the yellow tetrazolium salt MTT to an insoluble purple formazan crystal by the mitochondrial enzyme succinate dehydrogenase was used to quantify cell proliferation following a 48 - hour treatment with the extracts. The soluble formazan dye in DMSO was directly quantified by scanning multiwel spectrophotometer. The findings demonstrated that the corm`s acetone extract significantly inhibited the cell growth in dose dependent manner. It exhibited that the extract had a potent cytotoxic action against the MIAPaCa - 2cells with an IC50 value is 92.69µg/ml. The findings revealed that the high concentration of alkaloids, saponins, and polyphenolic chemicals in the corm acetone extract may be the cause of its cytotoxic action. Therefore, it can be concluded that Sauromatum venosum corm may kill MIAPaCa - 2 cancer cells making it a potentially effective therapeutic agent for the treatment of pancreatic cancer.***

Keywords: *Sauromatum venosum* corm, acetone extract, cytotoxicity, MTT assay, *5 - Flurouracil*

1. Introduction

The wild plants offer a good material for studying the phytochemical constituents responsible for therapeutic properties of the plants. In the developing nations, numerous wild plant species are exploited as food sources, providing an adequate level of nutrition to the inhabitants [5].

***Sauromatum venousm (*Ait.) Kunth.** var. *guttatum* Schott. Family Sauromatum venousm (Ait.) Kunth. var. guttatum Schott. Family Araceae known locally as "Sanp Ki Booti" and also frequently referred as "Voodoo lily or SnakePlant" [4]. This shade loving plant found in Melghat region of Amravati District. It`s corm is a condensed form of rhizome made up of solid, stout, fleshy underground stem. It contains heavy food material deposits. The globose - depressed petiole is long, the leaves are pedate, the berries are crimson, and the seeds are ovoid. The corm can be up to 13 cm broad. This plant's corm is utilized in traditional medicine as an anticancer and antidote for snake bites. Various chemical compounds found in plants have been linked to their antivenom activity, which is the ability of plants to counteract the effects of snake venom [15]. Lectins, dimethyl sulphides, p - caryophyllene, indole, ammonia, trimethylamine, and primary amines are among the components of the plant that have been documented to be present [16]. The preliminary qualitative screening of phytochemicals revealed the presence of alkaloids, flavonoids, saponins, terpenoids, phenolics and tannins in the acetone extract of *Sauromatum* corm [8].

Around the world, Pancreatic cancer (PC) is a highly aggressive human malignancy with an extremely poor prognosis [18]. The only treatment option that shows promise for most PC patients is cytostatic therapy with common chemotherapeutic medications like gemcitabine and 5 - FU (5 - fluorouracil) or their combination [13]. According to [9] cytotoxicity is the ability of a chemical molecule to kill cells independent from the mechanism of death. Cytotoxicity assay is suitable technique for quickly screening novel compounds to assess their cytotoxicity to cancer cells. The MTT assay is a rapid, simple, and reproducible technique that is frequently used in assessing the anticancer drugs and to measure cytotoxic properties [1]. Hence, the present study has been made to evaluate *In - vitro* cytotoxic properties of acetone extract of *Sauromatum venosum (*Ait.) Kunth. corm on human pancreatic carcinoma cell line (MIAPaCa - 2).

2. Materials & Methods

2.1 Selection and Collection of Plant

Sauromatum venosum corm were collected from Melghat region of Amravati District in the months of June to September.

Figure 1: Plant Habit

2.2 Identification and Authentication of plants

The plant was identified with the help of standard floras [6], [16] and authenticated by Taxonomist Dr. S. P. Rothe,

Professor and Head Department of Botany, Shri. Shivaji Science College, Akola.

2.3 Preparation of test solution

1 gm powder of the leaf part was crushed in a mortar and pestle by adding 10 ml DW, then centrifuged at 4000 rpm for 10 min. Concentrations (10 - 50 µg/ml) were prepared by using the supernatant accordingly.

2.4 In - vitro Cytotoxic Activity Assay

Cell line and Cell Treatment Procedure

The Human Pancreatic Carcinoma cell (MiaPaCa - 2) was procured from the National Centre for Cell Sciences (NCCS), Pune, and Maharashtra. MiaPaca - 2cells were cultured in a Dulbecco's Minimum Essential Medium (DMEM) and incubated with different concentrations (10, 20, 30, 40 and 50µg/ml) of acetone extract of *Sauromatum venosum* corm for 48 hrs. with standard positive control chemotherapeutic agent viz.5 - Flurouracil. At the end of incubation period, the medium was replaced by 150µl fresh medium and 50 µl MTT (1mg/mL) was added to each well, followed by an incubation period for a further 4 hours at 37^0C. Later, 150 µl of DMSO was added to each well for solubilization of the formazan products. Absorbance was taken at 630 nm using a Bio - Tek microplate reader. The percent cell cytotoxicity was calculated by using the following formula.

$$\% \text{ Cytotoxity} = \frac{(\text{Absorbance of control sample} - \text{Absorbance of treated sample})}{Absorbance\ of\ control\ sample} X100$$

Statistical Analysis

The results are presented as means ± SD of three independent experiments. Statistical differences among means were determined by one - way ANOVA. Differences were considered significant at $P<0.05$. The IC_{50} values were calculated using GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA). Every experiment included a set of negative controls (untreated cultures) and a positive control treated with 5 - fluorouracil (a Standard anticancer drug).

3. Results and Discussions

The results for cell growth inhibition by the acetone extract of *Sauromatum venosum* corm against MIAPaCa - 2 cell lines at 10 - 50µg/ml concentrations is shown in Table 1 and graphically represented in Fig.2. The acetone extract of corm was tested *in vitro* for its potential human cancer cell growth inhibitory effect on MIAPaCa - 2 cancer cell line using MTT assay, a non - radioactive, fast and economical assay widely used to quantify cell viability and proliferation. In the present study MIA paca - 2 cells showed growth inhibition in a dose - dependent manner when treated with acetone extract at concentrations ranging from 10 - 50µg/ml (Table 1 & Fig.2). The percentage of dead cells for each concentration was found to be 6.53, 9.45, 16.66, 22.52 and 26.80. The 50% cytotoxic effect (IC_{50}) of acetone extract of *Sauromatum venosum* corm was found to be 93.68 ± 3.69 µg/ml. The IC_{50} for the 5 - FU standard control was found to be 25.9 ±0.68µg/ml.

Table 1: Effect of acetone extract of *Sauromatum venosum corm* on growth of MIAPaCa - 2 cell line after the incubation for 48 hrs

Conc. (µg/ml)	% Inhibition	
	AC	5 - FU
Control	0	0
10	6.53 ± 0.39	43.02 ±1.00
20	9.45 ± 1.17	45.78 ± 0.10
30	16.66 ± 2.73	51.02 ± 0.36
40	22.52 ± 2.73	59.37 ± 0.72
50	26.80 ± 0.39	61.07 ± 0.52
IC_{50}	93.68 ± 3.69	25.9 ± 0.68

*Results are represented as an average of three± replicates [**AC** - Acetone extract; 5 - FU - 5 - Flurouracil]

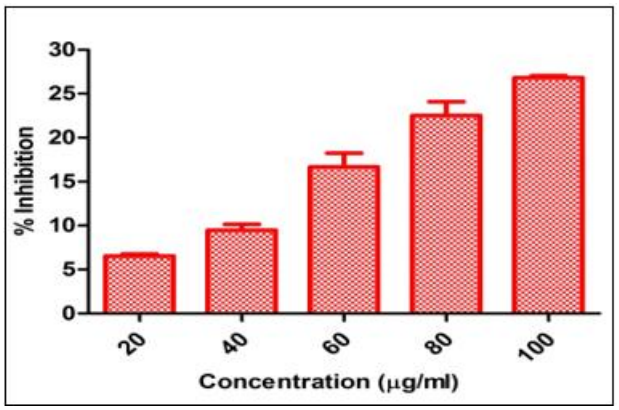

Figure 2: Inhibition of *Sauromatum venosum* acetone extract of corm against Miapaca - 2 cells

Figure 2: Cytotoxic effect of acetone extracts of *Sauromatum venosum corm* on growth of MIAPaCa - 2 cells after the incubation for 48 hrs. using MTT assay. (A) 5 - FU as standard (positive control) and (B) % inhibition of CU - L extract. Data are represented by mean ± SD (n=3). Statistical significance between untreated and treated cells was determined using one - way ANOVA where $p < 0.05$ verses untreated control cells

The ability of mitochondrial succinate dehydrogenase enzymes in living cells to convert the yellow water - soluble substrate 3 - (4, 5 dimethyl thiazol - 2 - yl) - 2 - 5 - diphenyl tetrazolium bromide (MTT) into blue formazan crystals, which can be measured spectrophotometrically, is the basis for the cytotoxicity assay [12], [14]. Since only metabolically active cells can reduce MTT, the activity level is a measure of the cells viability. The amount of formazan produced by the cells utilized was found to be proportional to the number of cells [7]. Human cancer cell lines have aggregated a readily available and useable collection of biological models to study cancer biology in recent decades [10].

MTT proliferation was used to measure the rate of cells growth. *Sauromatum venosum* corm acetone extract has shown notable growth inhibition on the Miapaca - 2 cell line in this investigation. When acetone extract was applied to Miapaca - 2 cell lines, the growth rate significantly decreased in comparison to the control. This activity may be caused by

the presence of carbohydrate and glycosides, protein and amino acids, alkaloids, phenolic compounds & flavonoids, phytosterols, saponins, and terpenoids [8]. The utility of cell lines acquired from tumor allows the investigation of tumor cells in a simplified and controlled environment [2].

Additionally, it has protected against cancer via influencing signal transduction in cell proliferation and angiogenesis. *Allium cepa* L. root tip cells and the Hep - 2 cell line are used as two model in - vitro systems to examine the cytotoxic properties of the various solvent extracts of *Amorphophallus paeoniifolius* tuber. Among the seven solvent extracts of *Amorphophallus* tuber, petroleum ether and ethanolic extract had the highest levels of cytotoxicity and showed dose - dependent antiproliferative activity against HE - 2 cells [19]. From the tubers of a wild monocotyledonous plant, *Sauromatum venosum*, a novel lectin with strong mitogenic and in vitro anti - proliferative activity was isolated. It significantly inhibited nine human and four murine cancer cell lines and demonstrated a strong mitogenic response against BALB/c and human lymphocytes, outperforming standard Con A, a well - known plant mitogen [3]. The anticancer potential of *Sauromatum venosum* (SV) tuber by gas chromatography with high - resolution mass spectrometry (GC - HRMS) analysis of ethanolic (eSV), hydroalcoholic (hSV), and aqueous extracts (wSV), and in silico study were performed to investigate the main targets of 12 - O - acetylingol 8 - tiglate by computational docking. Computation docking analysis was performed for the prediction of the main target of the cancer proliferation of active compound of the *Sauromatum venosum* tuber extract in cancer therapy. A total of 45 phytocompounds were detected including diterpenoids, esters of fatty acid, hydrocarbons, and alkanes in the tuber of SV. When treated with SaOS2 cells, eSV displayed the lowest IC50 value of all the crude samples examined. One of the phytocompounds contained in eSV extract, 12 - O - acetylingol 8 - tiglate, has been shown in studies to have cytotoxic effects on a variety of cancer cells [11].

4. Conclusion

The current investigation showed that the acetone extract of *Sauromatum venosum* corm was cytotoxic to human MIApaca - 2 cells in MTT assay with a concentration of 93.68 ± 3.69 µg/ ml needed to cause 50% cell death. The findings demonstrated the efficacy of *Sauromatum* corm for the cytotoxicity towards MIApaca - 2 cells and suggested that it might be a novel lead structure for pancreatic cancer drugs.

References

[1] M. C. Alley, D. A. Scudiro, A. Monks, M. L. Hursey. M. J. Czerwinski and D. L. Fine, “Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay”, Cancer Res.48, pp.589 - 601, 1998.

[2] V. Arya, C. P. Kshyap, T. Bandana, S. Shiksha, K. Sweta, P. Verma, and Sharma, Swati, “ Human cancer cell lines - A brief communication”, J. Chem. Pharm. Res, 3 (6), 514 - 520, 2011.

[3] B. J. Singh, Singh J., Singh S. K, Nijjar K. K., Agrewala J. N., V. Kumar, Ashok Kumar, Saxena A. K, Mitogenic and anti - proliferative activity of a lectin from the tubers of Voodoo lily (*Sauromatum venosum*). Biochimica et Biophysica Acta (BBA), 1723 (1 - 3), pp.163 - 174, 2005.

[4] S. R. Baquer, “Poisonous Plants of Pakistan”, Printas Karachi, 278: 368, 103Medica, 60: 90 - 100.

[5] Borkataky, Munmi., Kakoty, B. B. and Saikia, L. R. “Proximate analysis and antimicrobial activity of *Ecliptaalba* (L.) Hassk. - a traditionally used herb. Int J Pharm Pharm Sci. Vol 5, Suppl 1: 149 - 154, 2013.

[6] M. A. Dhore, Flora of Amravati District with special reference to the distribution of Tree species, 2002.

[7] D. Francis and L. Rita, “Rapid calorimetric assay for cell growth and survival modification to the tetrazolium dye procedure giving improved sensitivity and reliability”, Journal of Immunological Methods, 89, pp.271 - 277, 1986.

[8] M. U. Ghurde, “Phytochemical evaluation and Cytotoxicity studies of *Scilla hyacinthine (*Roth.) Macbr., *Sauromatum venosum (*Ait.) Schott. and *curcuma inodora* Blatt. ” Ph. D. Thesis.2018.

[9] B. Graham - Evans, P. B. Tchounwou and Cohly, H. H, Cytotoxicity and proliferation studies with arsenic in established human cell lines: keratinocytes, melanocytes, dendritic cells, dermal fibroblasts, microvascular endothelial cells, monocytes and Tcells, Int. J. Mol. Sci.4, pp.13 - 21.2003.

[10] J. E. Green, “Mouse model of human breast cancer: evolution or convolution”, Brest Cancer Res, 5 (1), 1, 2003.

[11] N. Kahar, P. Mishra, R. Bhatt and R. Seth, “Chemical characterization of the crude extract of *Sauromatum venosum* (voodoo lily) and docking study with 12 - O - acetylingol 8 - tiglate for cytotoxicity testing in $SaOS_2$ (osteoblastic osteosarcoma cells) ”, Anal Sci, 40 (1), pp.151 - 162, 2024.

[12] R. W. Masters, “Animal cell cultures, cytotoxicity and viability assays”, 3rd ed, pp.202 - 203, 2000.

[13] P. Michl and T. M. Gress, “Current concepts and novel targets in advanced pancreatic cancer”, Gut.62, pp.317 - 326, 2013.

[14] T. Mosmann, “Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays”, J Immunol Methods, 65, pp.55 - 63, 1983.

[15] N. A. Pereira, B. M. Ruppelt, M. C. Nascimento, J. P. Parente, and M. B. Mors, “Pharmacological screening of plants recommended by folk medicine as snake venom antidotes. IV: Protection against jararaca venom by isolated constituents”, Plant, 1994.

[16] B. D. Sharma, S. Kartikeyan, and Singh, N. P. “Flora of Maharashtra State Monocotyledones, Botanical Survey of India”, pp.220 - 221, 1996.

[17] Shi - Yong Gao, Jun Li, Long Wang, Qiu - Jia Sun, Yun - Fei Gong, Jian Gang, Yi - Jun Su, Yu - Bin Ji, “Ethanol but not Aqueous Extracts of Tubers of *Sauromatum giganteum* (Engl.) Cusimano and Hett Inhibit Cancer Cell Proliferation,” Asian Pacific Journal of Cancer Prevention, Vol 15 (14), pp.10613 - 10619, 2014.

[18] R. Siegel, K. D. Miller, and A. Jemal, “Cancer Statistics, 2016”, CA. Cancer J. Clin,.66 (1), pp.7 - 30, 2016.

[19] J. Angayarkanni, K. M. Ramkumar, T. Poornima and U. Priyadarshini "Cytotoxic Activity of *Amorphophallus paeoniifolius* Tuber Extracts In vitro," American - Eurasian J. Agric. & Environ. Sci., 2 (4), pp.395 - 398, 2007.

Author Profile

Dr. M. U. Ghurde received the B. S. degree in CBZ from Brijlal Biyani Science College, Amravati (Maharashtra) in 2000 and M. S. degree in Botany at GVISH, Amravati (M. S.) in 2002. Research areas of interest are Cytogenetics, Phytochemistry and Molecular biology.