Research Article

# Evaluation of Anticancer Activity of *Mazus pumilus* Leaf Extracts on Selected Human Cancerous Cell Lines

**Pittu Vishnu Priya[1]*, Avanapu Srinivasa Rao[2]**

[1]Assistant Professor, Department of Biotechnology, Joginpally B.R Pharmacy College, Yenkapally (V), Moinabad (M), Telangana, India.
[2]Principal, Professor, Department of Pharmacology, Bhaskar Pharmacy College, Yenkapally (V), Moinabad (M), Telangana, India.
***Corresponding author's E-mail:** pittu.vishnupriya@gmail.com

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**ABSTRACT**

The present study was aimed to evaluate the anticancer activity of various leaf extracts of *Mazus pumilus* against PC 3 (prostate cancer *cell lines)*, A549 (lung adeno carcinoma cell line), Hep G2 (liver carcinoma cell line). MTT assay is based on the capacity of mitochondrial enzymes of viable cells to reduce the yellow soluble salt MTT i.e., [3-(4, 5-dimethyl –thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide] to purple blue insoluble formazan precipitate which is then quantified spectrophotometrically at 560 nm. Tryphan blue assay is based on staining of cells. The cells which exclude the stain are viable. The acetone and ethanol leaf extracts of *Mazus pumilus* has shown potent anticancer activity on A549 and Hep G2 human cancerous cell lines by MTT assay and Tryphan blue dye exclusion test. The aqueous, ethanol and acetone leaf extracts of *Mazus pumilus* has shown less anticancer activity on PC 3 cancer cell line by MTT assay and Tryphan blue exclusion assay. The results of the study shown that various leaf extracts of *Mazus pumilus* has high potential of anticancer activity on A549 and Hep G2 human cancerous cell line. Further detailed investigation of active components of the plant for exact mechanism of action will contribute greatly to the development of new pharmaceuticals.

**Keywords:** Anticancer activity, *Mazus pumilus*, PC 3, A549, Hep G2, ethanol, acetone, aqueous, MTT assay, Tryphan blue exclusion assay.

## INTRODUCTION

Cancer is a major public health burden in both developed and developing countries. Anticancer activity is the effect of natural and synthetic or biological and chemical agents to reverse, suppress or prevent carcinogenic progression. Several synthetic agents are used to cure the disease but they have their toxicity and hence the research is going on to investigate the plant derived chemotherapeutic agents.

Ayurveda, a traditional Indian medical practice using plant drugs has been successful from very early times in using these natural drugs and preventing or suppressing various tumours with different lines of treatment[1]. In India, people of different ethnic groups inhabiting various distinct culture, religious rites, food habit and a rich knowledge of traditional medicine[2].

Cancer is a group of diseases caused by loss of cell cycle control. Cancer is associated with abnormal uncontrolled cell growth[3]. Cancer is caused by both external factors (tobacco, chemicals, radiation and infectious organisms) and internal factors (inherited mutations, hormones, immune conditions, and mutations that occur from metabolism).

Cancer is a significant worldwide health problem generally due to the lack of widespread and comprehensive early detection methods, the associated poor prognosis of patients diagnosed in later stages of the disease and its increasing incidence on a global scale. Indeed, the struggle to combat cancer is one of the greatest challenges of mankind[4]. The National Cancer Institute collected about 35,000 plant samples from 20 countries and has screened around for anticancer activity[5]. Over 3000 species of plants with antitumour properties have been reported[6]. Cancer is one of the most prominent diseases in humans and currently there is considerable scientific and commercial interest in the continuing discovery of new anticancer agents from natural product sources[7]. Chemoprevention is recognized as an important approach to control malignancy and recent studies have focused on the search for desirable chemopreventive agents. Natural products, particularly dietary substances, have played an important role in creating new chemopreventive agents[8]. In any cancer drug discovery program, a paradigm based on ethnobotanical and ethnopharmacological data would be more economical and beneficial in identifying potential anticancer molecules than mass screening of plant species[9]. Natural products have been regarded as important sources of potential chemotherapeutic agents and many anticancer drugs have originated from natural sources[10]. Many medicinal plants that possess antioxidant activity also show anticancer activity. *Mazus pumilus* is a annual growing plant species. Habitats wet grass lands, along streams, and the edges of forests, grasslands on mountain slopes at elevations. It is native of China, India, Japan, South Korea and eastern Russia. *Mazus pumilus* has shown antimicrobial and antioxidant activity. So, an attempt was made to evaluate the anticancer activity of various leaf extacts on human cancerous cell lines *Mazus pumilus.*

**Figure 1:** *Mazus pumilus* plant

## MATERIALS AND METHODS

### Plant material

The plant was identified and authenticated by plant taxonomist Dr. K. Madhava Chetty, Asst. Professor, Botany Department, Sri Venkateshwara University, Tirupathi, A.P, India. The plants was collected at Kapalitheertham forest A.P, India. The fresh leaves were separated from the plants and shade dried. The separated leaves were powdered in a mechanical grinder and fine powder was collected by passing through sieve no: 40.

### Preparation of leaf extracts

For the preparation of various leaf extracts 100 g of plant material was separately extracted using ethanol, acetone and aqueous solvents using soxhlet extractor at a temperature 45°C. The extracts were concentrated and dried by using rotary evaporator and was stored in a refrigerator at 4°C.

### Chemicals

The chemicals used in the present study, (3-(4,5- dimethyl thiazolyl-2 - yl)-2, 5-diphenyl tetrazolium bromide (MTT), Fetal Bovine Serum (FBS), Phosphate Buffered Saline (PBS), RPMI 1640 medium, and antibiotics from Sigma Aldrich and Himedia, Mumbai. All the chemicals used were of analytical grade.

### Cell lines and culture conditions

PC3, A549 and Hep G2 were procured from National Centre for Cell Science at Pune was maintained in RPMI-1640 supplemented with 10% FBS, antibiotic 2% (Penicillin or Streptomycin) in a humidified atmosphere of 5% $CO_2$ at 37°C until confluent. The stock cultures were grown in culture flask and the experiments were carried out in 96 micro titer plate.

### Anticancer Studies

The techniques employed to determine the anticancer activity are MTT assay and Tryphan blue dye exclusion test.

### MTT Assay

The MTT assay is based on cleavage of the soluble yellow tetrazolium salt MTT [3-(4, 5-dimethyl –thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide] into a blue coloured formazan by the mitochondrial enzyme succinate dehydrogenase was used for assaying cell survival and proliferation. This assay is extensively used for measuring cell survival and proliferation.

There is a direct proportionality between the formazan produced and the number of viable cells. However, it depends on the cell type, cellular metabolism and incubation time with MTT. This method is based on the capacity of mitochondrial enzymes of viable cells to reduce the yellow soluble salt MTT to purple blue insoluble formazan precipitate which is quantified spectrophotometrically at 560 nm after dissolving in DMSO. Cells are plated on to 96 well plates at and allowed to grow in $CO_2$ incubator for 24 h (37 °C, 5 % $CO_2$). The medium is then removed and replaced by fresh medium containing different concentrations of sample for 48 h.

The cells are incubated for 24-48 h (37 ˚C, 5 % $CO_2$). Then, 20 µL MTT ([3- (4, 5-dimethylthiazol-yl)-2, 5-diphenyltetrazolium bromide]) stock solution (5 mg/mL in PBS) is added to each well and incubated for 5 h. The medium is removed and 200 µL DMSO is added to each well to dissolve the MTT metabolic product. Then the plate is shaken at 150 rpm for 5 min and the optical density is measured at 560 nm. Untreated cells (basal) are used as a control of viability (100 %) and the results are expressed as % viability (log) relative to the control.[11,12]

% Cell viability = [(O.D of control - O.D of test compound)/(O.D. of control)] X 100

(O.D=Optical density).

### Tryphan blue dye exclusion assay

This the most commonly utilized test for measuring cell viability. The assay is based on fact that the chromophore is negatively charged and does not interact with the cell unless the membrane is damaged. Therefore, all the cells which exclude the dye are viable. In this assay, the cells are washed with HBSS (Hank's Buffered Salt Solution) and centrifuged for 10-15 mn at 10,000 rpm. The procedure is repeated thrice. The cells are suspended in known quantity of HBSS and the cell count is adjusted to 2 x $10^6$ cells/ml. The cell suspension is distributed into Eppendorf tubes. The cells are exposed to various leaf extracts separately and incubated at 37 °C for 3 h. After 3 h, dye exclusion test is performed. The cell suspension was diluted with 0.4% tryphan blue solution (1:1). Mixed thoroughly and was allowed to stand for 5 min at room temperature. Then hemocytometer was for cell counting. When observed under the microscope, non-viable cells were stained blue, viable cells remain unstained.[13,14]

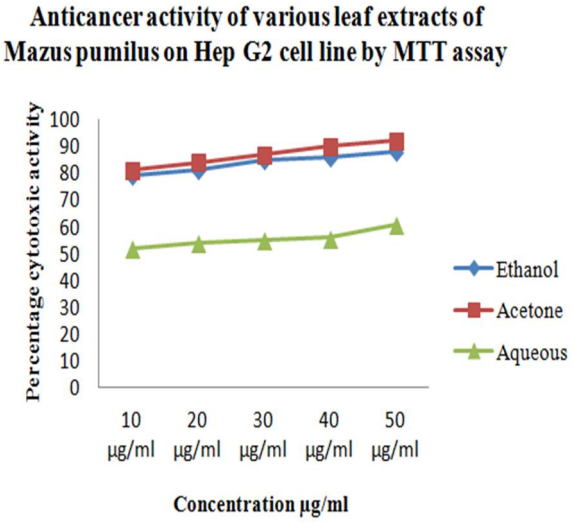
% Dead cell = No. of dead cells / (Sum of the live cells and dead cells) X 100.

## RESULTS

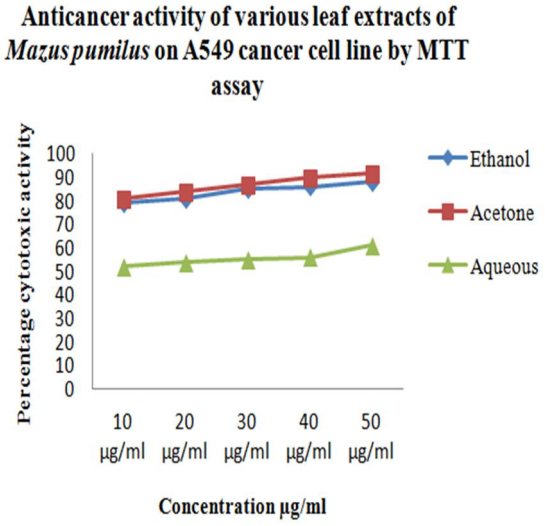
Effect of anticancer activity of various leaf extracts of *Mazus pumilus* was estimated by MTT assay and Tryphan blue dye exclusion test on selected cancerous cell lines i.e., PC 3, A549 & Hep G2. The acetone and ethanol leaf extracts of *Mazus pumilus* has shown potent anticancer activity on A549 and Hep G2 human cancerous cell lines by MTT assay and Tryphan blue dye exclusion test. The aqueous, ethanol and acetone leaf extracts of *Mazus pumilus* has shown less anticancer activity on PC 3 cancer cell line by MTT assay and Tryphan blue exclusion assay.

The results of MTT assay of ethanol, acetone and aqueous leaf extracts of *Mazus pumilus* on Hep G2, A549 and PC3 cancerous cell lines were represented in graph 1, 2 & 3 respectively.

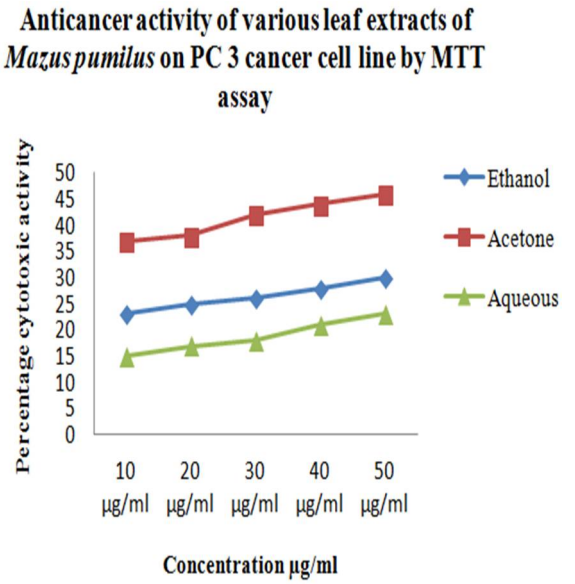
The results of Tryphan blue exclusion assay of ethanol, acetone and aqueous extracts of *Mazus pumilus* on Hep G2, A549 and PC3 cancerous cell lines were represented in graph 4, 5 & 6 respectively.



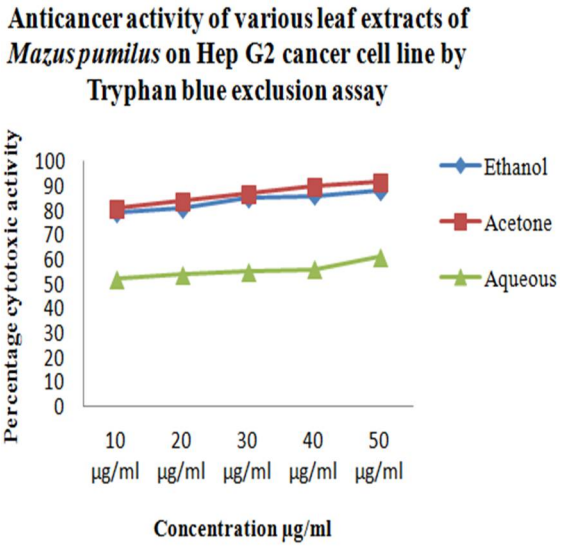

**Graph 1:** Anticancer activity of *Mazus pumilus* on HepG2 cancer cell line by MTT assay



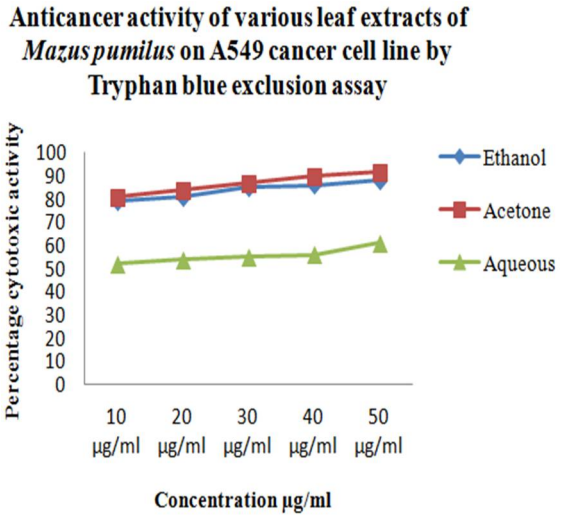

**Graph 2:** Anticancer activity of *Mazus pumilus* on A549 cancer cell line by MTT assay



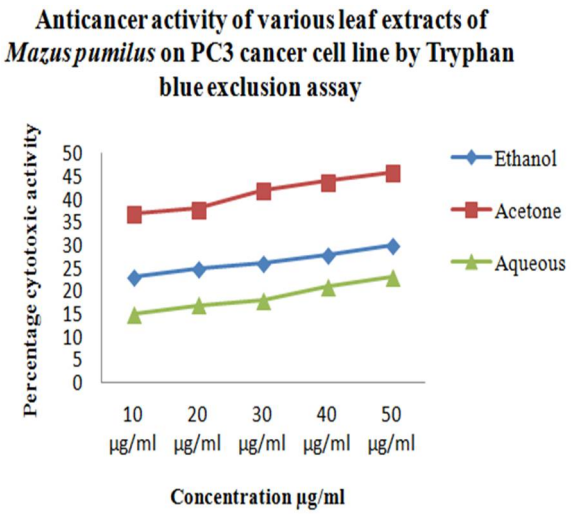

**Graph 3:** Anticancer activity of *Mazus pumilus* on PC 3 cancer cell line by MTT assay




**Graph 4:** Anticancer activity of *Mazus pumilus* on HepG2 cancer cell line by Tryphan blue exclusion assay




**Graph 5:** Anticancer activity of *Mazus pumilus* on A549 cancer cell line by Tryphan blue exclusion assay




**Graph 6:** Anticancer activity of *Mazus pumilus* on PC3 cancer cell line by Tryphan blue exclusion assay

## DISCUSSION

Cancer is a major public health burden in both developed and developing countries. Anticancer activity is the effect of natural and synthetic or biological and chemical agents to reverse, suppress or prevent carcinogenic progression. Several synthetic agents are used to cure the disease but they have their toxicity and hence the research is going on to investigate the plant derived chemotherapeutic agents. Anticancer properties of many natural compounds isolated from different Indian plant extracts have been reported. Research is being carried out throughout the world to find a lead compound which can block the development of cancer in humans. Nature has always been a great contributor towards this goal. Plant-derived natural products such as flavonoids, terpenoids and steroids have received considerable attention due to their diverse pharmacological properties, which include cytotoxic and chemopreventive effects[15]. The isolation of the vinca alkaloids, vinblastine and vincristine from the Madagascar periwinkle, Catharanthus roseus introduced a new era in the use of plant material as anticancer agents. They were the first agents to advance into clinical use for the treatment of cancer[16]. The medicinal plants contain many antioxidants such as vitamins (A, C, E, K), carotenoids, flavonoids (flavones, isoflavones, flavonones, anthocyanins, catenchins, isocatechins), polyphenols (ellagic acid, gallic acid, tannins), saponins, enzymes and minerals (selenium, copper, manganese, zinc, chromium, iodine, etc)[17]. In this review, anticancer medicinal plants of Indian origin belonging to 35 families are reported along with detailed information plants continue to be used against various types of tumours such as sarcoma, lymphoma, carcinoma and leukemia. Many of these medicinal plants have been found to be very effective in experimental as well as clinical cases of tumours/cancers. Some medicinal plants have been studied in various *in vivo* and *in vitro* experimental models of cancer and have shown significant inhibition of cancer cell proliferation. For eg. Abrus precatorius in Yoshida's sarcoma, carcinoma and Dalton's lymphoma ascites cancer[18,19].

So, an attempt has made to study the cytotoxic activity of various extracts *Mazus pumilus* against various human cancerous cell lines.

## CONCLUSION

The aim of this study was to evaluate the anticancer activity of various leaf extracts of *Mazus pumilus.* We have tried to explore the *in vitro* anticancer activity by MTT assay and Tryphan blue exclusion assay. The acetone and ethanol leaf extracts of *Mazus pumilus* has shown potent anticancer activity on A549 and Hep G2 cancerous cell lines, but the leaf extracts of *Mazus pumilus* has shown less anticancer activity on PC3 cell line. However, the mechanism of the anticancer activity has not yet been fully elucidated and further research is needed to explore the molecular mechanism of this herbal plant.

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