

Research Article



In Vitro Cytotoxicity Study on Polyherbal Formulation of *Moringa oleifera*, *Viola odorata*, *Allium sativum*

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ABSTRACT

This study is aimed at determining the anticancer ability of polyherbal formulations prepared through methanolic extracts from plants such as *Moringa oleifera*, *Viola odorata* and *Allium sativum* against the lung carcinoma cell line (Calu-6). The usage of ingredients from herbs and traditional medicines are being studied across various parts of world for their potential as therapeutic agents against cancer. In our current study we had investigated the effectiveness of polyherbal formulations on its clonogenic inhibition on lung cancer (Calu-6) cell lines. The cytotoxic effect of polyherbal formulations was evaluated by MTT assay on Calu-6. The polyherbal preparation PF-3 showed significant cytotoxicity against Calu-6, when equated to polyherbal formulations PF-2 and PF-1. Through MTT assay it was known that cytotoxicity of the PF-3 formulation was the utmost against Calu-6 cell lines followed by PF-2. It was found that polyherbal preparation PF-3 has shown significant cell line inhibition at 200 µg/ml of test dosage in comparison to other formulation which showed lesser anticancer activities against experimental cancer cell line. Thus, the above analysis can be concluded by saying that polyherbal formulation PF-3 has revealed maximum activity among three. This concludes that the study of polyherbal formulations by their synergistic effect showing the high potent cytotoxicity activity in dosages and time dependent manner. The outcome of this investigation recommends that the polyherbal preparations of studied plants could complement to basic medicine for cancer like diseases.

Keywords: Anticancer, MTT assay, Cell lines, polyherbal formulations.

INTRODUCTION

The plants contain many chemical agents known for their healing properties and hence are known to be source of many types of medicines. Since times immemorial it is known that herbal medicines are used to enhance color, taste, aroma and is also known to act as dietary supplements, preservative and medicinal properties.¹

The nature is known to provide unlimited source of novel chemotypes and pharmacophores and many number of modern day drugs have been known to explore their origin on herbal source. There has been unprecedented and diversified development in natural product chemistry currently focused on drug design and discovery. As many drug classes are being extracted from its natural form and are also acts as templates for synthetic modification and this has especially helped in therapeutic areas of infectious diseases and oncology. There has been a significant contribution in drug discovery currently from drugs discovered through herbal sources despite major scientific and technological progress in combinatorial chemistry. Many plants have been marked for its anti-cancerous attributes but their effectiveness has to be confirmed.²

The plants have complemented the western medicines by giving drug ingredients and have played a significant role in discovery of drugs. The herbal medicines are known to contain chemical entities which have helped in treating

few complex diseases. The natural ingredients found in herbal plants are biosynthetically derived compounds from primary metabolites namely amino acids, carbohydrates and fatty acids and categorized secondary metabolites. There are various parts of plants such as roots, seeds, leaves, barks, stems etc. that helps a specific function in human body. The drugs used for treating cancer doesn't only target cancers and may be prove to fatal even to normal surrounding tissue.^{3,4}

The usage of herbal medicines depends on diversity of plant species and their related knowledge of its use as an herbal medicine as both play a key part in their therapeutic use. The chemotherapy and radiation therapy being the method for treatment of cancer has lots of side effects. Hence people find solace in complementary modes of treatment through herbal medicine which are now playing significant part in treatment. The rural population have known to use herbal medicine as the primary healthcare mechanism as synthetic drugs prove to be expensive.^{5,6}

The herbal medicine is known to play an important role in prevention and treatment of cancer; they act on by confining the enzymes and hormones which trigger cancer. During the last decade there has been a change on the treatment cancer with new methods which combines surgery with chemotherapy, radiations and diverse phytochemicals derived from various plant species. The present day research is targeted towards plants with anti-cancer attributes with documented



details from various plants containing their name, parts used, active principle, families and numerous cell lines used in diverse studies. The plants can be used as they are or their active components are isolated through various solvent extractions for the treatment of cancer.^{7,8}

In this paper, we account a study based on anticancer properties of three polyherbal formulation (PF-1, PF-2 and PF-3) prepared from plants *Moringa oleifera*, *Viola odorata* and *Allium sativum* respectively.

MATERIALS AND METHODOLOGY

Plant materials

The plant samples were collected from Bangalore. The authentication was done by NISCAIR. The fresh samples were washed with water, shade dried and homogenized to powder and stored in the glass jars.

Extraction and formulation

Fifty grams of powdered plant samples were taken in 250 ml of 95% methanol for 8-9 hours in Soxhlet apparatus. The extract was filtered by Whatman No.1 filter paper and allowed to evaporate in rotary evaporator at 60°C. The extract was then dissolved in Dimethyl sulfoxide (DMSO) and stored in refrigerator for further experiments. All these three plants were extracted by methanol. These methanolic extracts were used for making polyherbal formulations PF-1, PF-2, PF-3.

Cell culture methods

The Calu-6 cell lines were procured. The cells were cultured in a humid environment at 37°C and 5% CO₂ in minimum essential medium supplemented with 15% fetal bovine serum and 1% penicillin/streptomycin (Invitrogen). At 85-90% confluence, cells were harvested using 0.25% trypsin/ EDTA solution and sub-cultured onto 96-well plates according to the experimental requirements.

MTT assay^{9,10,11}

Cell Viability Analysis

Cell viability was estimated using 3-(4, 5- dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium (MTT) assay, which reflects the normal function of mitochondrial and cell viability. Briefly, cells were seeded onto flat bottomed 96-well culture plate at a density of 5×10⁴ cells/well in Dulbecco's modified eagle's medium culture medium. After 24 hrs, the cells were washed and placed in culture medium with different polyherbal formulations PF-1, PF-2, PF-3 for 48 hrs and 72 hrs. Next, non-FBS culture medium containing 10 % MTT was added to each well of a microtitre plate and the samples were then incubated for 4 hours at 37°C. After removing the culture medium, dimethyl sulfoxide (DMSO) was added to each well. The absorbance was then read on an enzyme-labelled detector at 570 nm. The absorbance of control cells (treated with DMSO) were considered as 100%. The formula used to calculate:

$$\% \text{ viability} = (\text{OD of test material} / \text{OD of control}) \times 100$$

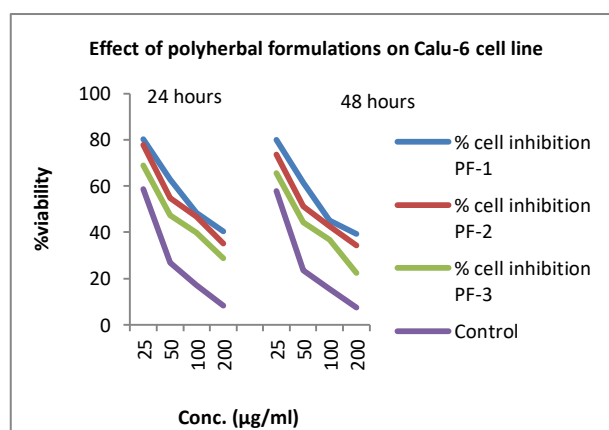
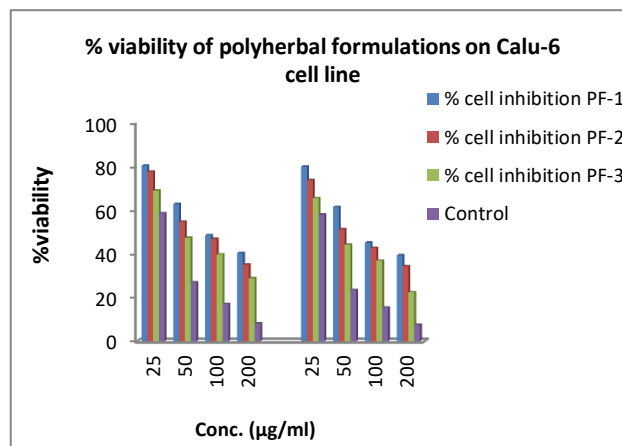
$$\% \text{ Inhibition} = 100 - (\% \text{ Viability})$$

RESULTS AND DISCUSSION

After experimental observation it's found that the methanolic extracts polyherbal formulation PF-1 and PF-2 are showing reasonable anticancer activities. However, PF-3 showed significantly high anticancer activity against Calu-6 cell lines. The % inhibitory and % viability effect of these formulations are evaluated with standard for Calu-6 cell line. The % inhibitory effect was found to be concentration dependent whereas % viability is inversely proportional to the dosage. The maximum concentration (µg/ml) used in the study was 200µg/ml (Graph-1). The data were statistically analyzed to find out the significance of test groups.

Effect of Polyherbal formulations PF-1, PF-2, PF-3 on calu-6 cells

To determine percent cell viability after treatment of different concentrations of PF-1, PF-2, PF-3 methanolic extract, MTT assay was performed on calu-6 cancer cell lines. The treatment of PF-3 methanolic extract on calu-6 cells resulted in a significant reduction in cell proliferation at 100 µg/ml (P<0.01), and 200µg/ml (P<0.001) after 24 hours treatment and at 50, 100 and 200µg/ml (P<0.001) after 48 hours treatment. Whereas, it found non-significant at 25µg/ml after 24 and 48 hours treatment and at 50µg/ml after 48 hours treatment.



Graph 1- Percent cell viability of polyherbal formulations on Calu-6 cell line (After 24 hours and 48 hours)



Values are Mean \pm SEM (of two independent experiments) one way ANOVA followed by Dunnett's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant.

CONCLUSION

The medicinal plants cure many diseases which also include cancers without any harmful side effects and also help in maintaining the health and vigor of an individual. It's well known that products discovered from medicinal plants have played a key role in treatment of cancer. This paper we have reported a study based on anticancer attributes found in three plants such *Moringa*, *Viola* and *Allium*. It's to be noted that anticancer activities are assayed with standard MTT colorimetric and flow cytometry procedure against Calu-6 cell lines. Based on the analysis it was noted that polyherbal formulation PF-3 of *Moringa*, *Viola* and *Allium* has shown considerable cell line inhibition at 200 μ g/ml tested dose while other formulation showed much lesser anticancer activities against experimental cancer cell line. The above analysis can be concluded by saying that polyherbal formulation PF-3 has shown highest activity among three.

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