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EVALUATION OF ANTI CANCER ACTIVITY OF NOVEL POLYHERBAL FORMULATION

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ABSTRACT

Cancer is multi pathological disease that is being considered immortal in today's global medicine world. Therefore, the present study was aimed to develop and evaluate polyherbal formulation (PHF) for its anticancer activity. PHF was prepared by mixing of equal proportion of extracts of Azadirachta indica (leaves), Trigonella foenum-graecum (seeds), Canthium coromandelicum (leaves) and Barringtonia acutangula (bark). The polyherbal formulation was evaluated for their cytotoxic effect using MTT (3-(4,5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) assay on cell line of human breast adenocarcinoma (MCF-7). Results revealed that the developed polyherbal formulation, showed significant effect on cancer cells (in vitro) by increasing cytotoxicity without causing toxicities and could render prospective candidate for the therapy of cancer. Further clinical trial will be of use in establishing it as a potential anticancer formulation.

KEYWORDS: Invitro cancer activity, polyherbal formulation, cytotoxicity, MTT assay, MCF-7.

INTRODUCTION

Natural products have played an imperative role in the lead-finding of candidates for the development of present-day cancer chemotherapy due to their low toxicity and side effects. They offer a valuable source of a wide variety of chemical structures with biological activities (lead molecules) for the development of novel drugs.[1] Approximately 60% of all drugs currently undergoing clinical trials for cancer treatment are natural products or compounds derived from natural products.[2] These substances embrace some of the most exciting new chemotherapeutic agents currently available for use in a clinical setting.[3] Many traditional healers and folklore medical practitioners in our country have been treating cancer patients for many years using various medicinal plant species. There is evidence of case reports on cancer cure using traditional knowledge, but they are not scientifically investigated. In addition to use of a single plant, poly herbal formulations of drugs are intensively used. The present study aimed to assess anticancer, efficacy of novel poly herbal formulation.

MATERIALS AND METHODS

Preparation of Polyherbal formulation: Polyherbal formulation (PHF) which consists dried plant powders of *Azadirachta indica* (leaves), T*rigonella foenum-graecum* (seeds), *Canthium coromandelicum*(leaves) and *Barringtonia acutangula* (bark) (Fig.1). After collection, the plants materials are washed and shade dried. After 15 days, the polyherbals were pulverized individually into fine powder. Each plant powder was weighed accurately and mixed together in equal proportions. The poly herbal drug (5 g) soaked in distilled water (100 mL) was kept in the rotary shaker for 48 hours in an air tight dark bottle. The extract was then filtered through a layer of muslin cloth and filtrate was centrifuged at 3,000 rpm for 15 minutes at 4oC to remove any debris. The supernatant was freeze dried, and stored at -20°C in an air tight vial until used. The freeze dried extract was reconstituted with distilled water for experimental purposes.

Fig.1: Plants used in PHF.

Human Breast Cancer Cells (MCF-7) collection: MCF-7 (Human Breast Cancer cells) and normal lymphocytes were obtained from National Centre for Cell Sciences, Pune, India.

Preparation of culture media:-DMEM (Dulbecos Modified Eagle's medium) was prepared by mixing DMEM powder of about 1.03gm in autoclaved triple distilled water. To this 1.95gm of Herpes buffer, 3.75gm sodium bicarbonate and antibiotics like Penicillin (100µg/ml), Streptomycin(100µg/ml), Amphotericin-B(100µg/ml) were added. This is the amount of drugs should be added in 1000ml of triple distilled water. The pH was confirmed to be 7.2-7.4 using pH meter and adjustments made if needed. It was filtered under negative pressure using 0.22µm cellulose filter. 10% FBS (Foetalbovein serum) was mixed with the medium before used for culture.

In vitro assay for Cytotoxicity activity (MTT assay): The cytotoxicity of sample on MCF-7 cells were determined by the MTT assay.[4-7] Cells (1×10^5/well) were plated in 1ml of medium/well in 24-well plates (Costar Corning, Rochester, NY). After 48 hours incubation the cell reaches the confluence. Then, cells were incubated in the presence of various concentrations of the samples in 0.1% DMSO for 48h at 37°C. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 200µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2 -thiazolyl)-2,5-diphenyl--tetrazolium bromide cells (MTT) phosphate- buffered saline solution was added. After 4h incubation, 0.04M HCl/ isopropanol were added. Viable cells were determined by the absorbance at 570 nm. Measurements were performed and the concentration required for a 50% inhibition of viability (IC_{50}) was determined graphically. The absorbance at 570 nm was measured with a UV-Spectrophotometer using wells without sample containing cells as blanks. The effect of the sample on the proliferation of MCF-7 was expressed as the % cell viability, using the following formula:

% cell viability = (A_{570} of treated cells / A_{570} of control cells) × 100%.

Table 1: Cytotoxicity activity of PHF extract against MCF-7 Cell Line at Different Concentrations by MTT Assay.

S.No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	1000	Neat	0.03	6.8
2	500	1:1	0.08	18.8
3	250	1:2	0.14	31.8
4	125	1:4	0.18	40.9
5	62.5	1:8	0.23	50.0
6	31.2	1:16	0.30	68.1
7	15.6	1:32	0.35	79.5
8	7.8	1:64	0.39	88.6
8	Cell control	-	0.44	100

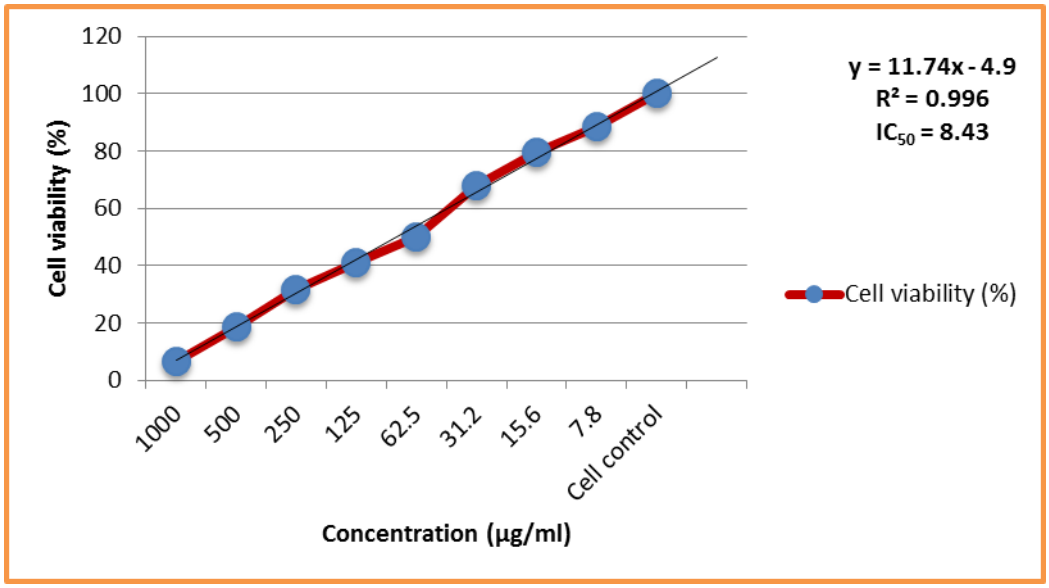

Fig.2: Cytotoxicity activity of PHF extract against MCF-7 Cell Line (IC_{50}=8.43 µg/ml)

Cell viability: The effect of PHF extract on cell viability of MCF-7 Cell Line is shown in Table 1. Cells were incubated with different concentrations (7.8 to 1000µg mL–1) of the extract. The results showed that the PHF extract exhibits a moderate to strong growth inhibition of MCF-7 cell line. The relationship between surviving fraction and extract concentration was plotted to obtain the survival curve of the cell line. The response parameter calculated was the IC50 value, which corresponds to the concentration required for 50% inhibition of cell viability. The PHF extract showed cytotoxicity against the breast carcinoma cell line (MCF-7), with IC50 values of 8.43µg mL–1 (Fig. 2).

DISCUSSION

Cancer has become the most leading cause of death worldwide. It has become an emerging health problem affecting both developing and developed countries. The most effective treatment approaches in cancer are chemotherapy and radiotherapy. Nevertheless, the higher incidence in side effects, have made investigators engage in finding novel anticancer compounds with less adverse effects. As a result of this, plants and other natural sources have provided nearly 60% of anti cancer agents which are currently in use.[8] Traditional folklore medicine which involves the use of natural elements uses either single or multiple herbs as a mixture (polyherbs) for treatment of diseases. The use of polyherbal formulation is found to be more effective than the use of a single herb as the active phytochemical constituents of the individual plants are insufficient to achieve the desirable therapeutic effect. But when combining the herbs in a particular ratio the synergistic effect produced by the active phytochemicals of several plants give a better therapeutic effect.[9] Polyherbal preparations are used worldwide for treatment of various disease conditions including cancer. As the polyherbal formulations have gained more attention, many studies have been conducted to identify the mechanism of action and the efficacy of these polyherbal preparations.[10]

CONCLUSION

The present study provides strong evidence for potent anti-cancer activity of the poly herbal drug on MCF-7 cells. To the best of our knowledge, this is the first investigation about cytotoxic, effects associated with novel poly herbal formulation. Results of this study indicated that this PHF has potential as a source of anticancer agents for breast cancer treatments. Further studies are required to assess the active ingredients, involved in the antiproliferative or cytotoxic effects of this poly herbal formulation. The type(s) of effects

applied by the plant component(s) involved in its activity must be further analyzed by molecular or other relevant techniques.

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