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Original Article

EVALUATING THE CYTOTOXIC POTENTIAL OF METHONOLIC LEAF EXTRACT OF *ALEO VERA* ON MCF-7 BREAST CANCER CELL LINES

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ABSTRACT

Objective: Breast cancer is the second leading cause of death among cancer pateints. Ayurvedic system of traditional medicine validated the use of plants as a useful source for occurrence of anticancer compounds. This study was attempted to investigate the *in vitro* anti-cancer potential of methanolic extract of *Aleo vera* on breast cancer MCF-7 cell lines.

Methods: The methanol extract of *Aleo vera* was screened for its anti-proliferative effect against MCF-7 (Breast cancer) cell lines seeded on 96 well plates.

Results: MCF-7 cells were treated with the extract for 24h and 72h at a range of increasing concentrations (0-500µg/ml) in order to obtain a dose-response graph and IC50 value. The *Aleo vera* extract showed cytotoxic effect in MCF-7cells with IC_{50} of 74.33 µg/ml.

Conclusion: The *Aleo vera* extract showed effective cytotoxic activity in a dose and time dependent manner. Future investigations will be interesting to find an active principle and also better understand its mechanism of action against cancer.

Keywords: *Aleo vera*, Anticancer, Breast cancer, Cytotoxic, Antraquninones, Drugs.

INTRODUCTION

The fighting against the highly wicked public adversary called "cancer" has been going on for decades, and we are often at the defeating side. Every year the prevalence of cancer just keep rising and lost its control. According to WHO, cancer becomes one of the leading causes of death worldwide, accounting for 8.2 million deaths in 2012, Among them, about 521,000 deaths were due to the breast cancer ("cancer", WHO). The incidence of breast cancer in Indian women is more at the age of 40 and the prevalence of breast cancer has been rising worldwide for many decades [1, 2]. The current treatment for cancer usually involves chemotherapy, surgery, radiation, biological therapy, hormone therapy, and targeted therapy. However, these therapies cause multiple side effects and also lead to toxic accumulation. Side effects and troublesome of these cocktail treatments have led to increased emphasis on the use of plant materials to combat against the serious enemy, cancer [3].

Now a day, growing tendency to "Go Natural" i.e. use of herbal medicines in some aspects of primary healthcare has been increased [4, 5]. Ayurvedic system of traditional medicine validated the use of plants as a useful source for an occurrence of anticancer compounds [6-8]. In fact, about 60% of anticancer drugs (vincristine, vinblastin) are derived from herbal source [9, 10]. Although most of the plants have been identified for its anti-cancerous properties, yet their efficacy is needed to be verified.

Aloe vera (Asphodeloideae) is a medicinal plant and is a perennial succulent xerophyte, which develops water-storage tissue in the leaves to survive in dry areas of low or erratic rainfall [11]. They are well known in folk medicine for their laxative properties [12]. *Aloe* gel is widely used for treatment of wounds and inflammatory skin disorders [11, 13]. *Aloe vera* has a very ancient history for healing wounds and also found in folk medicine for the treatment of burns and chronic wounds [11, 14]. Basic research over the past couple of decades has begun to reveal the extent of Aloe's pharmaceutical potential, particularly against neoplastic disease [12]. The leaf extracts of *A. vera* has been shown to possess cytotoxic effect [15]. Henceforth, in this study we have attempted to investigate the *in vitro* anti-cancer potential of methanolic extract of *A. vera* on breast cancer MCF-7 cell lines.

MATERIALS AND METHODS

Materials

All the chemical reagents and solvents of analytical grade were purchased from SRL Chemicals, India. 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT, No M5655) purchased from Sigma (St Louis, MO, USA).

Plant material and extracts preparation

The leaves of *A. vera* was collected from the Maharani Lakshmi Ammanni College For Women, Bangalore, India. The plant materials were authenticated by Dr. S. Sundara Rajan, a Taxonomist and the voucher specimen (MC-H-52) were stored at the college. The leaves were washed with distilled water, shade dried and crushed into fine powder by using electric grinder. The finely powdered leaf material was extracted with methanol in a Soxhlet apparatus for 24 h. The extract was dehydrated under reduced pressure using a flash evaporator (BuchiFlawil, Switzerland) and a portion of the residue was used for the experiments.

MTT assay

MCF-7 (breast cancer) cell lines were procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5 µg/ml) in an 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 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India). All these cell lines were cultured and Cytotoxicity test were carried out using MTT assay (Mossman, 1983; Edmondson

et al., 1988). The trypsinized 70-80% confluent cell lines (MCF-7) of 1×10^5 cells/well were seeded in a 96 well plate and incubate for 24 hr at 37 ºC, and varying concentrations (0-500 µg/ml) of *A. vera* are added and incubated at 48 and 72 hrs. After incubation, 20 µl of MTT reagent will be added to each well and incubated for 4 hr at 37 °C. The incubated cells were washed twice with PBS and DMSO (100µL/well) reagent which dissolved the insoluble crystalline formazan product. The efficacy of the sample was determined based on the reduced dye at 570 nm by UV spectrophotometer The effect of the samples on the proliferation of MCF-7 cell lines were expressed as the % cell viability, using the following formula: % cell viability = A570 of treated cells/A570 of control cells × 100%.

Statistical analysis

The experiments were carried out in triplicate and results are given as the mean±standard deviation. The data in all the experiments were analyzed for statistical significance using Students *t*-test and differences were considered significant at $p<0.05$.

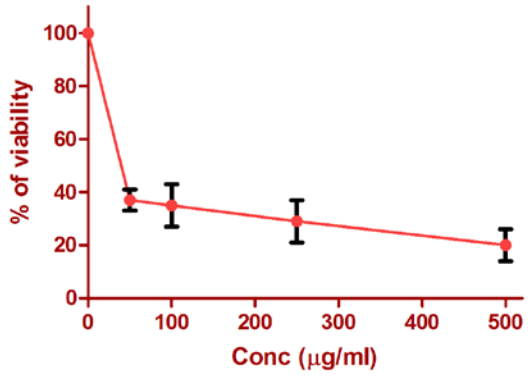

Fig. 1: Cytotoxic activity of *A. vera* extract in MCF-7 cell lines. Extract were incubated with 10^5 viable cells at concentrations ranging from 0 to 500 µg/ml for 48 h. Cell viability was determined by the MTT method

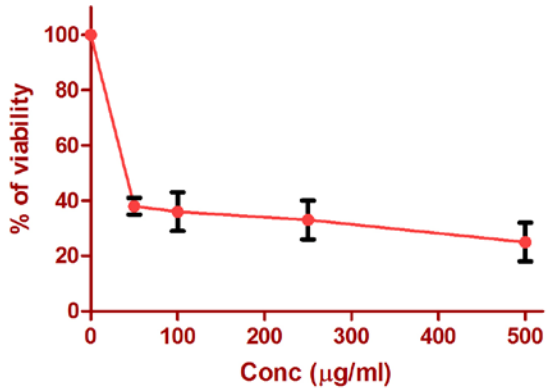

Fig. 2: Cytotoxic activity of *A. vera* extract in MCF-7 cell lines. Extract were incubated with 10^5 viable cells at concentrations ranging from 0 to 500 µg/ml for 72 h. Cell viability was determined by the MTT method

RESULTS AND DISCUSSION

Plants have always been a potential source of new drug molecule and research work in this domain has resulted in discovery of more efficient drugs for cancer treatment [9, 10]. MTT is a simple, reliable technique, which measures cell viability and can be used for screening anti-proliferative agents [16, 17]. MTT assay is a a spectrophotometric analysis, which uses (3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyl tetrazolium bromide), known as MTT, a yellow collor and water soluble compound. The MTT enters the cells through the plasma membrane and, in contact with superoxide produced by the mitochondrial activity, is oxidized to MTT-formazan, a slat purplish color, which is insoluble in water. Then the oxidation of MTT is proportional to the mitochondrial activity and therefore to cell viability [16, 17]. When the methanol extracts of *A. vera* was screened for their anti-proliferative effect against MCF-7 (Breast cancer) cell lines, it was found that, the extract was effective in a dose and time dependent manner in inducing cytotoxic effect (fig. 1 and fig. 2). The assessment of *A. vera* extract cytotoxicity is generally performed on MCF-7 cell seeded 96 well plate. MCF-7 cell seeded plate was incubated with the extract for 24h and 72h at a range of increasing concentrations (0-500µg/ml) in order to obtain a dose-response curve and IC50 value. T. h. e *A. vera* extract showed cytotoxic effect in MCF-7cells with IC_{50} of 74.33µg/ml.

The Aleo species are known to be rich sources of biologically active molecules that includes vitamins, minerals, enzymes, sugars, anthraquinones or phenolic compounds, lignin, saponins, sterols, amino acids and salicylic acid [11, 12]. The aleo`s antineoplastic property is due to at least three different mechanisms based on antiproliferative, immunostimulatory, and antioxidant effects [12]. Anthraquinones (tricyclic aromatic quinones), which represent the richest class of *A. v. e. ra* secondary metabolites are known to be rich in leafs [12], and are known to induce in antiproliferative activity [15, 18]. Similarly in this extract the anthraquinones that are present in the methanolic extract of *A. vera* might be bringing out the anti-proliferative or cytotoxic effect. Therefore, it will be interesting to understand the chemical composition and better understand the mechanism of action of bioactive constituents of the extract for development it as drug for therapeutic application.

CONCLUSION

The results of this study authenticate the anticancer activities of *A. vera* leaf extracts. The potential anticancer activities of *A. vera* extracts is may be due to the presence of anthraquinones. The experimental evidence could be useful for validate the traditional use of plant as the source of easily available effective anticancer agents to the people, particularly in developing countries. Future investigations will be interesting to find an active principle and also better understand its mechanism of action against cancer.

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Ethical Issues

There is none to be applied.

CONFLICTS OF INTERESTS

Declared None

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