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Short Communication

IN-VITRO CYTOTOXICITY OF FERMENTED INEDIBLE FRUIT AND VEGETABLE WASTES ON NORMAL FIBROBLAST CELLS AND MCF-7 BREAST CANCER CELLS

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ABSTRACT

In recent times, plant derived drugs are being utilized to compete the breast cancer, due to the variety of phytochemicals present in them and also because they have no or lesser adverse effects compared to the chemical drugs. In our study, the inedible wastes obtained from vegetable and fruits (Peel of *Allium cepa* and *Punica granatum*; Seed of *Syzygium cumini* and *Mangifera indica*) were fermented and their methanolic extracts were prepared. The cytotoxicity of extracts on human breast cancer cell line (MCF-7) and normal fibroblast cell line was studied by MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) assay. All the extracts turned out to be more cytotoxic for MCF-7 cell line and less cytotoxic for normal fibroblast cell line. The fermented methanolic extract of *Allium cepa* peel is found to be most toxic against MCF-7 cell line. Upon further investigations, these wastes may provide us potent natural anti-breast cancer compounds.

Keywords: Breast cancer, *Allium cepa*, *Punica granatum, Syzygium cumini, Mangifera indica*

1. INTRODUCTION

Breast cancer is the most frequently diagnosed cancer and the chief cause of death amongst women. In 2018, 2,088,849 new cases and 626,679 deaths were reported for breast cancer worldwide [1]. Thus breast cancer is the serious problem that needs to be solved.

Cancer is caused by genetic mutations over a period of time which leads to uncontrollable cell division that later gets converted to tumour. The treatment of cancer includes surgery, radiation therapy, chemotherapy, immunotherapy, target therapy, hormone therapy, stem cell transplant and precision medicine [2]. But these treatments are costly and also have certain side effects. Besides this, over a period of time drug resistance is also developed by the cancer cells [3]. Thus an alternative approach is made by researchers to fight cancers by using natural products.

The extracts of peel of *Allium cepa* (onion) and seed of *Mangifera indica* (mango) are reported to possess variety of phytochemicals and good antioxidant potential [4].The anti-cancer properties of natural products are by the virtue of their phytochemical composition.

2. MATERIAL AND METHODS

2.1. Collection of plant material

Allium cepa, *Punica granatum*, *Syzygium cumini* and *Mangifera indica* were bought from local fruit and vegetable market, Surat. The peels of *Allium cepa*, *Punica granatum*, and seed of *Syzygium cumini*, *Mangifera indica* were washed, shade dried, powered using electric blender and stored until further use.

2.2. Chemicals used

Analytical grade extra pure Methanol, Phosphate buffer saline (PBS), sodium dodecyl sulphate (SDS), Sodium Chloride (NaCl), Tryptone, Yeast Extract, Dulbecco's Modified Eagle's Medium (D-MEM), trypsin/EDTA solution (170000 U/L of trypsin and 0.2 g/L of EDTA), fetal bovine serum (FBS), Dimethyl sulfoxide (DMSO), and tetrazolium salt were used.

2.3. Fermentation

Slight modification of the method, described by Wen et al [5], was used for fermentation. Erlenmeyer flasks (250 mL) containing 10 g *Allium cepa* peel powder and 100 mL water, were sterilized at 121°C for 1 hour. After sterilization, the flasks were allowed to cool to room temperature and inoculated with *Saccharomyces cerevisiae*.

The inoculated flasks were statically incubated at 30°C for 5 days. Same protocol was followed for rest of wastes under study.

2.4. Preparation of extract by methanolic extraction

After fermentation, 100 mL of methanol was added to each flask and extraction was performed at room temperature in shaking condition for 24 hours. The extraction mixture was filtered through a Whatman No. 1 filter paper [5] and allowed to evaporate in preweighed glassware. After evaporation the extracted material was dissolved in dimethyl sufoxide to make the concentration of 10mg/mL.

2.5. *In Vitro* toxicity testing

In vitro toxicity of extracts were studied by MTT(3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay. MTT assay was performed by modification of the method used by Labieniec et al [6] and Lapshina et al [7]. The steps of MTT assays are as followed:

2.5.1. Cell cultivation

The normal fibroblast cells were cultivated in RPMI (Roswell Park Memorial Institute) 1640 / D-MEM (Dulbecco's Modified Eagle Medium) growth medium which was supplemented with 10% foetal calf and incubated at 37 °C and 5% CO_2.

2.5.2. Seeding of Normal fibroblast cells

Confluent cells (70-80%) in Corning flasks were detached using a trypsin solution and later the trypsin was removed. The cells were again suspended in D-MEM growth medium and counted using a haemocytometer. Cell densities were adjusted to 1.0 x10^5 cells/mL. The suspension of cells (1000 µL) was seeded into micro centrifuge tubes. The tubes were incubated at 37 °C and 5% CO_2 for 24 hours.

2.5.3. Assay procedure

After seeding, the cells were inoculated D-MEM growth medium containing test compound and incubated for a further 24 hours at 37°C and 5% CO_2. Each compound was tested in ten different concentrations (1.25, 2.5, 5, 10, 20, 100, 250, 500, 750 and 1000 µg/mL). Thereafter 100 µL of MTT solution (5 mg/mL final concentration) was added to the wells and incubated for 1 hour at 37°C and 5% CO_2. At the end of the treatments, the medium was removed and DMSO (200 µL) was added into each well. The 24-wells plate was allowed to stand for 10 minutes followed by shaking for 15 seconds. The absorbance was measured at wavelength 562 nm.

3. RESULTS AND DISCUSSION

The result of cytotoxicity of methanolic extracts of fermented peels of *Allium cepa* & *Punica granatum* and seeds of *Syzygium cumini* & *Mangifera indica* on normal fibroblast cells and breast cancer cells MCF-7 are portrayed in graphs of inhibition (%) vs concentration of extracts (µg/mL) in Figure 1 and Figure 2 respectively.

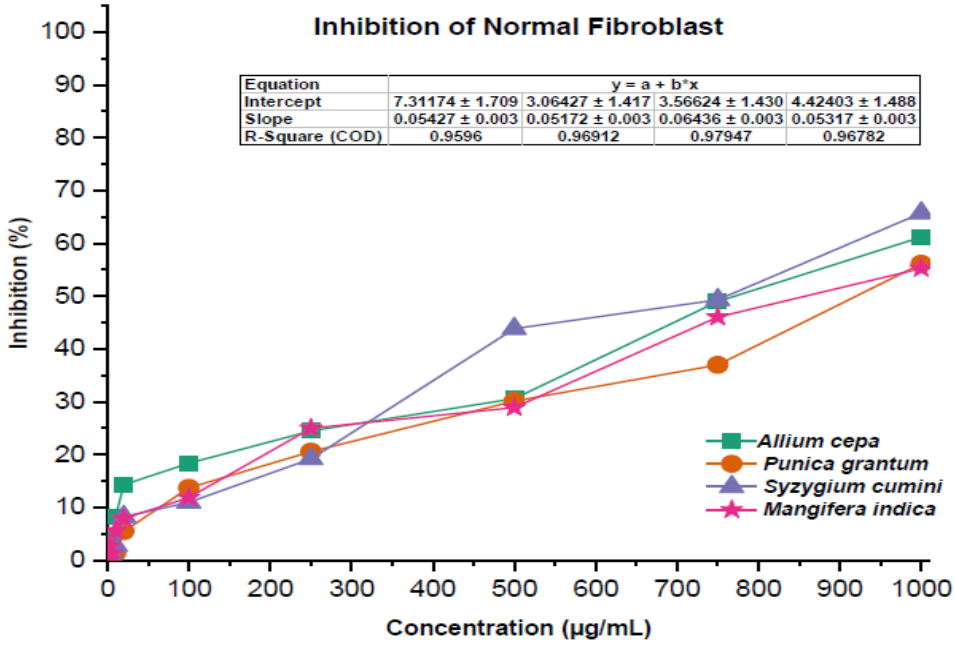

Fig. 1: Inhibitory effect of fermented waste extracts on Normal Fibroblast cells

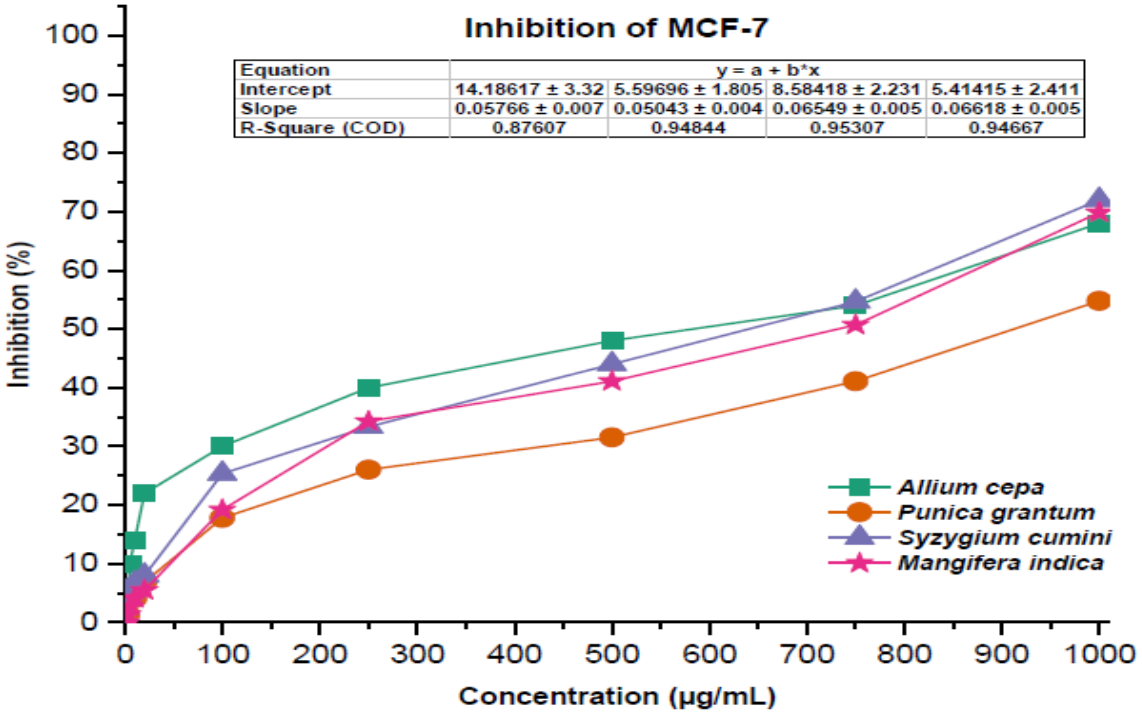

Fig. 2: Inhibitory effect of fermented wastes on Breast Cancer cells MCF-7

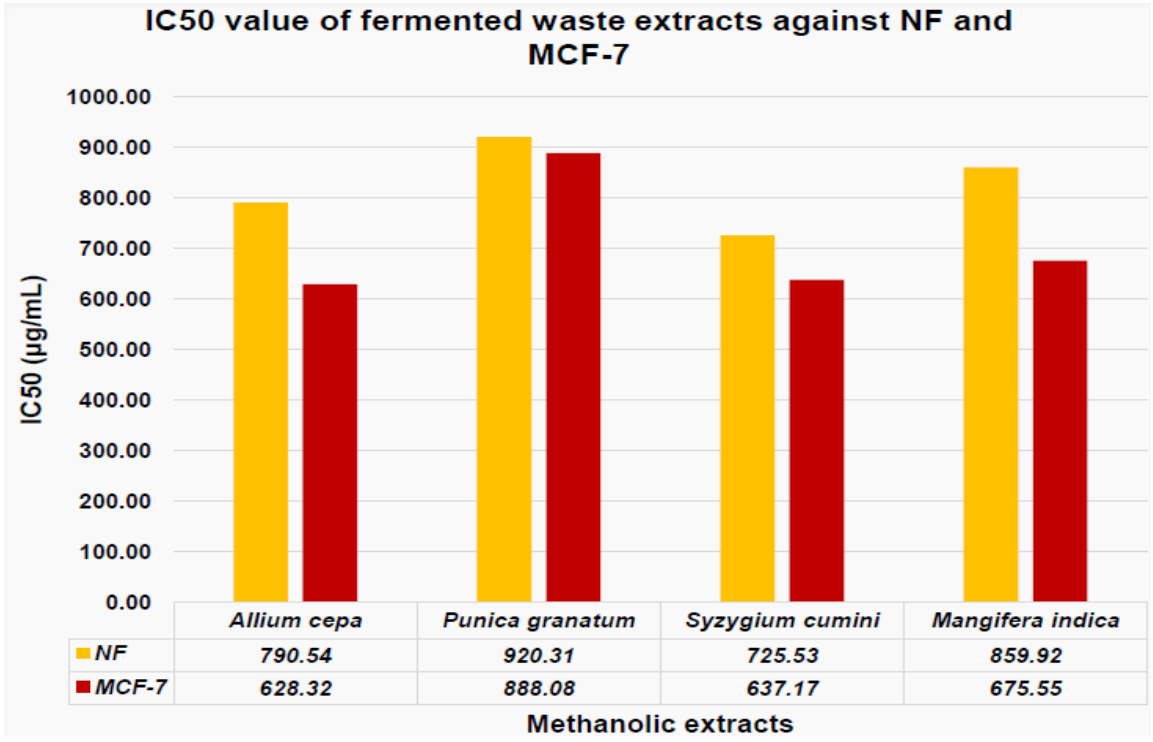

Fig. 3: IC50 values of fermented waste extracts for Normal Fibroblast cells (NF) and Breast Cancer cells (MCF-7)

By using linear regression analysis provided by Origin: A Data Analysis and Graphing Software, the values of IC50 were calculated and noted in Figure 3.

Cytotoxicity assay of methanolic extract of fermented *Allium cepa* peel, *Punica granatum* peel, *Syzygium cumini* seed and *Mangifera indica* seed showed that all of the extracts under study are able to inhibit the breast cancer cells to a greater extent compared to the normal fibroblast cells**.** The results of inhibition by the extracts, at concentration 1000 µg/mL on breast cancer cells

MCF-7 were as; *Allium cepa* peel, *Punica granatum* peel, *Syzygium cumini* seed and *Mangifera indica* seed- 68%, 54.79%, 72% and 69.86% respectively.

The lowest concentration for 50% inhibition of breast cancer cells MCF-7 was 628.32 µg/mL of *Allium cepa* peel.

4. CONCLUSION

In this study, we investigated the cytotoxic effects of methanolic extracts of *Allium cepa* peel, *Punica granatum* peel, *Syzygium cumini* seed and *Mangifera indica* seed on normal fibroblast cells and breast cancer cells MCF-7 by MTT assay. Different concentrations of extracts were used and their IC50 values were determined. The exposure to lower doses did not impose noteworthy cytotoxic effect on either of the cell lines used. Later, as the concentration increased the viability of the cell decreased. The IC50 values indicate that the concentration of extracts required to inhibit the normal fibroblast cells is higher compared to that required to inhibit breast cancer cells. Amongst all four extracts, the methanolic extracts of fermented *Allium cepa* peel showed lowest IC50 value for MCF-7 cells.

The consequence of this study suggests that extracts of *Allium cepa* peel can be studied further for identification of active compound and molecular mechanism of cell death can be deduced.

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