INVITRO ANTI BREAST CANCER ACTIVITY OF METHANOLIC EXTRACT OF Ocimum basilicum L leaves AGAINST MCF-7 Cell Line

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Abstract: In this study, different concentrations of the methanolic extract of leaves of the plant Ocimum basilicum L was subjected to in-vitro cytotoxic activity study against MCF-7cells using the MTT assay. Percentage cell viability of cell lines were carried out by using Trypan blue dye exclusion technique MTT assay was used to evaluate the reduction of viability of cell cultures in the presence and absence of the extract. Cell viability was inhibited to different extents by different concentrations of the extract. Phytochemical analysis showed the presence of alkaloids, tannins, flavonoids, terpenoids, phenols and saponins.

Keywords: **Cytotoxic activity, MCF-7 cell line, MTT assay, *Ocimum basilicum* L, phytochemicals**

I. INTRODUCTION

Plants have been used as medicines for thousands of years. They have always been used as a rich source of biologically active drugs. Medicinal plants and natural products have been used to treat a variety of human health issues and there has been renewed interest in their use for integrated cancer management. Breast cancer is a malignant tumor (a collection of cancer cells) arising from the cells of the breast. Breast cancer is the most common malignancy in women and accounts for one-tenth of all new cancers [1]. It is the second leading cause of cancer deaths in women after lung cancer. More than 1.2 million women are diagnosed with breast cancer every year worldwide. Younger people in the age group of 14 to 30 and older people (above40) are more prone to developing breast cancer due to late menopause or environmental factors. The different phytoconstituents present in medicinal plants are flavonoids, alkaloids, phenol and tannins, carboxylic acids, terpenes, amino acids and inorganic acids. These phytoconstituents give specific distinctiveness and properties to plants [2]. Many of the drugs that are derived from the secondary metabolites are simple synthetic modifications or copies of these naturally obtained substances [3].These phytochemicals can be used in treatment as anticancer, antimicrobial, antioxidant, anti-inflammatory agents etc [4]. Recent studies show that these phytochemicals are safe, broadly effective and have less adverse effects. However *in vivo* studies of these phytochemicals are necessary to demonstrate their efficacy, safety and to verify their bioavailability. It has been shown that *in vitro* screening methods could provide the needed preliminary observations to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations.

Therefore, there is an urgent need for novel therapeutic approaches to treat cancer. Accumulating evidence from *in vitro* and animal studies suggest that some plant flavonoids possess potent cancer chemoprevention activities, *invitro* anti-invasive activities and anti-metastasis activities in animal models [5]. Recently, more research has been focused on the role of flavonoids in cancer prevention because epidemiological investigations suggest that increased intake of fruits and vegetables are associated with the reduced risks of certain cancers. Hence, plant derived compounds can serve as a source for anticancertherapy. tific studies on these plants are likely to provide in valuable drugs to some of the diseases. So, in this view, the plant *Ocimum basilicum* L was selected for evaluating invitro anticancer (breast) activity.

Ocimum basillicum L (OB) or basil grows in the tropical region and it is a herb plant erect or bush, branched a lot, with height 1.3-5 meters, has a distinctive fragrant sourced from citric acid, especially the flowers and leaves. Studies have shown many pharmacological affect in several diseases, with potent antioxidant, anti-aging, anticancer, antiviral and antimicrobial properties [6]. The plants are used as diuretic, sedative, digestive, antiphrastic, carminative, appetizer, anti-convulsant, anti-inflammatory, anti-oxidant and stimulant. It is used to treat fever, cough, headaches, and stomachaches, wound healing, heart diseases and dysmenorrheal (Sarac and Aysel Ugur, 2007).

II. MATERIALS AND METHODS

2.1. Collection of plant material and extraction

The plant material of *Ocimum basillicum* L leaf used for the investigation was collected from in and around the village nearby Thanjavur. The plant was identified and authenticated by Dr. P. Jayaraman, Director, Plant Anatomy Research Centre (PARC), Chennai and a voucher specimen is kept in the herbarium. 300gm of coarsely ground powder was packed into soxhlet column and extracted with 250ml of 70% methanol for 48 hours (64.5-65.5°C). The extract was filtered and concentrated on water bath at reduced pressure (bath tem 50°C) to syrup consistency (yield: 15%). Then the dried extract was stored in air tight container for further use.

2.2. Preliminary phytochemical screening

Chemical tests for the screening and identification of phytochemicals in methanolic plant extract were carried out using standard procedures as described by [7] [8] [9].

2.3. Cell Culture

Human breast cancer MCF-7 cell line was obtained from Sigma-Aldrich, Bangalore. MCF-7 is a cell line used in many studies due to its characteristics and being easy to culture. The MCF-7 cell line is adherent and grows in clumps. The cells were maintained in RPMI-1640 supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO2 at 37 °C.

2.4.1. *In Vitro* Anti-Breast Cancer Activity

2.4.1.1. IC_{50} determination

MTT assay was used for cytotoxicity testing in MCF-7 cells, for *O. basillicum* leaf extract (10, 20, 40, 60, 80, 100, 150, 200 and 250 µg/ml) IC_{50} values were calculated in a dose dependent manner.

2.4.2. Viability Staining by Trypan blue dye exclusion method

Trypan Blue is a vital blue acid dye that has two azo chromophores group. The reactivity of Trypan blue was based on the fact that the chromophore was negatively charged and does not interact with the cell unless the membrane was damaged. Therefore, all the cells which exclude the dye are viable. Trypan blue will not enter into the cell wall of plant cells grown in culture. It is used in estimating the number of viable cells present in a population. Human Liver cancer MCF-7 cells suspension was mixed gently and an aliquot was added to the trypan blue solution (100 µl cell suspension: 100 µl dye) and was then counted in a haemocytometer by using the calculation.

Total no of viable cells $= A \times B \times C \times 10^4$

Total dead count $= A \times B \times D \times 10^4$

Total cell count = Viable cell count × Dead cell count

% of cell viability = Viable cell count × 100 /Total cell count

Where,

A = Volume of cells

B = Dilution factor in trypan blue

C = Mean number of unstained cells

D = Mean number of dead cells or stained cells 10^4 is the conversion factor for 0.1 mm^3 to 1ml

2.4.3. Micro culture tetrazolium (MTT) assay

The cytotoxicity of *O. basillicum* leaf extract in MCF-7cells was determined by the method of [10]. MTT is cleaved by mitochondrial dehydrogenase of viable cells yielding a measurable purple formation product. This formation production is proportionate to the viable number of cells and inversely proportional to the degree of cytotoxicity.

2.4.3.1. Principle

MTT assay, the present yellow tetrazolium salt was metabolized by NAD- dependent dehydrogenase (in active mitochondria) to form a dark blue formazan product)

2.4.3.2. MTT stock solution

MTT (50 mg) dye was dissolved in 10 ml of PBS. After vortexing for 1 min, it was filtered through 0.45 micro filters. The bottle was wrapped with aluminum foil to block the light, as MTT is light sensitivity. The preparation was stored at 4ºc.

2.4.3.3. Procedure

The cells were plated separately in 96 well plates at a concentration of 1×10^5 cells/well. After 24 hours, cells were washed twice with 100 µl of serum-free medium and starved for an hour at 37°C. After starvation, cells were incubated in a CO_2 incubator at 37 ºC at 24 h. At the end of the treatment period the medium was aspirated and serum free medium containing MTT (0.5 mg/mL) was added and incubated for 4 hours at 37°C in a CO_2 incubator. The MTT containing medium was then discarded and the cells were washed with PBS (200 µl). The crystals were then dissolved by adding 100 µl of DMSO and this was mixed properly by pipetting up and down. Spectrophotometrical absorbence of the purple, blue formazan dye was measured in a microplate reader at 570 nm (BioRad 680). The results are expressed in the percentage of stable cells with respect to the control. The half maximal inhibitory concentration (IC_{50}) values were calculated and the optimum doses were analyzed at different time period.

$$\text{Inhibition of cell proliferation (\%)} = \frac{\text{Mean absorbence of the control} - \text{Mean absorbence of the sample}}{\text{Mean absorbence of the control}}$$

The medium effective dose (IC_{50}) is the amount of samples able to inhibit cell proliferation by 50%, which was calculated graphically for each well proliferation curve.

2.4.4. Trypan blue and colony survival assay

Cell feasibility was measured by the capacity of living cells to eliminate tryphan blue vital dye [11]. Cells were seeded in 96-well micro plates at a concentration of 10^4 cells / well were treated within the presence and absence of O. basillicum leaf extract for 24 h. After this, the MCF-7 cells were trypsinzed from the micro plates, combined with any moving cells prevailing in the media and pelleted by centrifugation at 1000 g for 10 min at 4 ºC. Cells were washed twice with PBS and tryphan blue was added at a final concentration of 0.2%. Living cells will be counted in a haemocytometer and articulated as the % of the whole count in vehicle control. A dose dependent study was accepted for *O.basillicum* leaf extract to find out maximum inhibition.

2.4.5. Statistical Analysis

Values were presented as mean ± SD of three independent experiments (one way analysis of variance [ANOVA] followed by Duncan's multiple range test (DMRT).

III. RESULTS AND DISCUSSION

3.1. Preliminary Phytochemical Analysis

Different phytochemical components and anti-oxidants are present in the herbal extract that can be of significant therapeutic uses, much of the protective effect of leaves and fruits has been attributed by phytochemicals which are the non-nutrient plant compound [12].

In the present study, methanolic extract of *Ocimum basilicum* L leaves used for screening phytochemical compounds and the results are presented in Table 1 .This plant was choosing on the basis of their medicinal value. Table 1 represents the qualitative phytochemical analysis of methanolic extract of *O.basilicum* L leaves.

Methanolic extract of *O. basillicum* L contained the secondary metabolites such as alkaloids, flavonoids, carbohydrades, glycosides, phenol, saponin, phytosterol, tannins, terpenoids, protein & , steroids, fixed oil& fat and coumarin, but the extract did not contains resin, gum & mucilage and chlorogenic acids based on the presence (or) absence of colour changes.

S.No	Phytochemical constituents	Methanolic extract
1	Alkaloids	+
2	Flavonoids	+
3	Carbohydrates	+
4	Phenol	+
5	Glycosides	+
6	Saponin	+
7	Phytosterol	+
8	Tannins	+
9	Terpenoids	+
10	Protein &aminoacids	+
11	Steroids	-
12	Resin	-
13	Gum & mucilage	-
14	Coumarin	-
15	Fixed oil & fat	+

Table 1: Preliminary phytochemical screening of methanolic extracts of *O.basilicum* L.

3.2. Effect of *O. basillicum* leaf extract on cell viability, growth of MCF-7 cells in a dose dependent manner

The cytotoxicity effect of *O. basillicum* leaf extract in MCF-7 cells has been assessed by MTT assay. Figure.1 shows the % of viable cells was significantly decreased with respect to treatment of *O. basillicum* leaf extract with increasing concentrations after 24h. The MTT assay indicated that cell viability decreased to 90%, 78%, 68% and 38% when cells were exposed to *O. basillicum* leaf extract at concentrations of 10, 20, 40, and 80 µM/ml respectively. 50% of viable cells were observed at 60 µg on MCF-7 cells at 24 hrs. From this observation the IC_{50} values of *O. basillicum* leaf extract were considered as 200 µM. The survival of MCF-7 cells decreased significantly in a concentration dependent manner with an IC_{50} (the concentration causing 50% growth inhibition) value at 60 µg/ml 0.48 ± 0.052. There is complete destruction of cells above 250 µg/ml. It is likely that *O. basillicum* leaf extract 40µM/ml concentration affected the HOC MCF-7 cells, whereas the induced anti- proliferative responses were observed in the HOC KB cells.

Cytoxicity in vehicle control and *O. basillicum* leaf extract treated MCF-7 cells for 24 h were showed at different concentration (10, 20, 40, 60, 80, 100, 150 and 250 µg/ml) respectively. Interest in the pharmacological effects of bioactive compounds on cancer treatments and prevention has increased dramatically over the past twenty years. It has been shown to possess numerous anti-cancer activities in various cancer cells through different forms of cytotoxic effects without exhibiting considerable damage to normal cells [13] .Our observations on toxicity on MCF-7 cells showed, in agreement with our previous studies [14]. Accruing evidences suggest that *O. basiicum* L may be a potential chemotherapeutic or a chemo preventive agent based on its ability to induce apoptosis in cancer cells with relatively low toxicity to normal cells. Further studies with *in vivo* and clinical trials needs to be conducted to establish *O. basilicum* as a safe agent for cancer therapy.

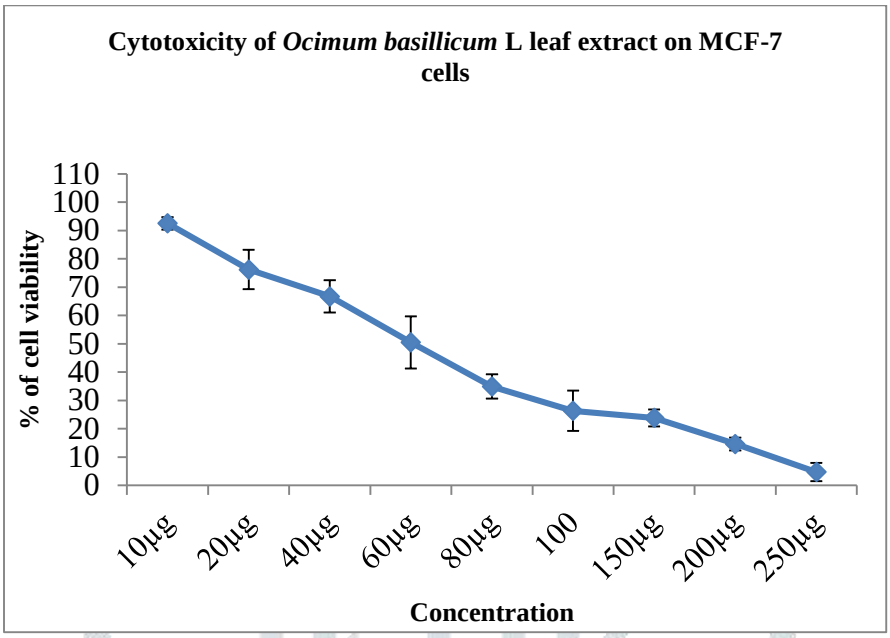

Figure 1: Cytotoxicity of *Ocimum basillicum* L leaf extract on MCF-7 cells

IV. CONCLUSION

Natural therapeutic approaches for breast cancer was emerging one in respect to plant, microorganism secreted proteins and marine sources. Studies have shown differential sensitivities to several natural compounds between tumor and normal cells in vitro or in vivo, and the results obtained from the present study show that the methanol extract from fruit pulp of *Ocimum basilicum* L had anti breast cancer activity against MCF-7 cell lines. Our phytochemical screening revealed the presence of flavonoids, alkaloids, steroids in the methanolic extract of *Ocimum basilicum* L, which could be responsible for this activity. This in sake represents a natural therapy for breast cancer therapy. In future, this will be a milestone in breast cancer research with less side effectives.

V. ACKNOWLEDGEMENT

The authors would like to thank the Management, Principal, Head of the Department, and Staff members of Deparment of Biochemistry, Rabiammal Ahamed Maideen College for Women, Thiruvarur, for support in carrying out this work.

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