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ANTIPROLIFERATION ACTIVITY OF OCIMUM GRATISSIMUM AQUEOUS EXTRACT ON HUMAN BREAST CANCER MCF-7 CELL LINE

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ABSTRACT

Objective: *Ocimum gratissimum* (OG) has been used in traditional systems of medicine as it has marked remedial activities against many diseases including cancer. Our study is a part of an ongoing search for potential anticancer agents in the ethnomedicinal plants of Odisha. **Methods:** Different concentrations of OG aqueous extract were exposed to Human breast cancer MCF-7 cell line at different time intervals. *In-vitro* cytotoxicity study with evaluation of growth inhibition was done by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay, regardless of ER status. Observation of the changes in cell morphology & detection of apoptosis was done. The various concentrations of the aqueous extract of OG were used & effective dose was calculated from dose-response curve. **Results:** After treatment an increased rate of cell death was observed in the MCF-7 cells. The antiproliferation activity on MCF-7 cell line was evaluated & found to have significant growth inhibitory effect with IC(50) value 41.7 microg/ml. **Conclusion:** This proposed that the aqueous extract of OG be appropriate for potential anticancer activity through growth inhibition and apoptosis on human breast cancer cell. Further purification and characterization of the aqueous OG extract might bring more potential sources of bio active molecules.

KEYWORDS: Antiproliferation, *Ocimum gratissimum* (OG), Aqueous extract, Breast cancer, MCF-7 MTT assay.

INTRODUCTION

The significance of Tulsi is enormous both religiously and medicinally in India. The domesticated Tulsi is available in two forms: Rama Tulsi and Shyama Tulsi. Out of these two, Rama is lighter in colour, while Shyama is darker in colour. *Ocimum gratissimum* L., Rama Tulsi also known as African basil has been used in Ayurveda for its diverse healing properties for thousands of years. This basil (Ram Tulsi) is renowned for its important role in the traditional Ayurvedic and Unani system medicine.[1] Ram Tulsi is regarded as an adaptogen, take part in the body balancing mechanism, facilitate stress adaptation & it was mentioned in the Charaka Samhita. It is believed that it promotes marked longevity by its strong aroma and astringent taste.[2] For colds, headaches, stomach disorders, inflammation, heart disease, various forms of poisoning the leaf extracts are used as Ayurvedic remedies.[3] As a traditional source of medicine the plant has a number of medicinal applications like anti-diabetes, cardiac activity, wound healing activity, antioxidant, gastro protective, effect on CNS, anti-inflammatory, and anti cancer.[4-11]

(Ocimum sanctum)

(Ocimum gratissimum)

In this study we focused upon the effect of *Ocimum gratissimum* extracts from leaves to observe its efficacy as an anti-cancer agent on breast cancer. To elucidate the effectiveness of the extract we analyzed cell motility and survival assay to assess the phenotypic changes in MCF-7(breast) cancer cell line. To elaborate our study further, we also analyzed the cell survival, apoptosis, and cell cycle progression of MCF-7 cell line with the extract of OG. The rationale behind selecting these lines are; easy access to availability and more importantly, prevalence of breast cancer in a major population globally. Worldwide breast cancer is the deadly most cancer among women.[12] The breast cancer cells proliferate & invade throughout the extra cellular matrix, form secondary tumours in other body parts. From different plant species about 74% of the currently available anti-cancer medicines are derived.[13-14] There

are also several domestic dietetic goods which have marked anti-cancer potential with low margin of side effect, under clinical trials for cancer therapy.[15-17] Curcumin from turmeric & Lycopene from bael are very common having potential chemo preventive efficiency in cancer.[2,18] The remarkable effects of *Ocimum gratissimum* leaves on MCF-7 breast cancer cell line were publicized in this study & considerable level of apoptosis in breast cancer MCF-7 cell line was tested. Notable changes were also observed in the cells conforming anti-cancer potential of *Ocimum gratissimum*, against breast cancer.

MATERIALS AND METHODS

Plant material collection

Leaves of *Ocimum gratissimum* were collected from the Chandaka forest area, Bhubaneswar, Odisha, India around February 2017. Plant materials were air dried in the laboratory for a week at room temperature, then grinded to powder & kept in air tight container till use. It does not fall under the endangered plant species and hence safe to use for research purpose. The plant was identified by Dr.S.K.Nayak, PG Department of Botany, Utkal University, VC No.1349. The study was performed in the University Department of Pharmaceutical Sciences, Utkal University, Bhubaneswar, Odisha, India.

Extract preparation

The extractions were performed by maceration process. About 50gm of OG dried powdered leave specimen was taken in round bottom flask and 250ml of water was added. After keeping for 72hrs the crude extract was filtered using Whatman No. 1 filter paper (0.22µM). The filtrate was dried into powder using freeze dryer & the extract thus obtained, was collected and stored at 4°C until further use. The sterile extract was always used in a cell culture hood under aseptic conditions.

Cell lines

MCF-7 human breast cancer cell line used during the study was obtained from ICSCCB, Pune, Maharashtra, India. MCF-7 cells were maintained in RPMI-1640 medium supplemented with 10% FBS, glutamine, penicillin & streptomycin and cultured at 37^0C in a humidified 5% CO_2 incubator. The cells were & grown in 96-well tissue culture plates.

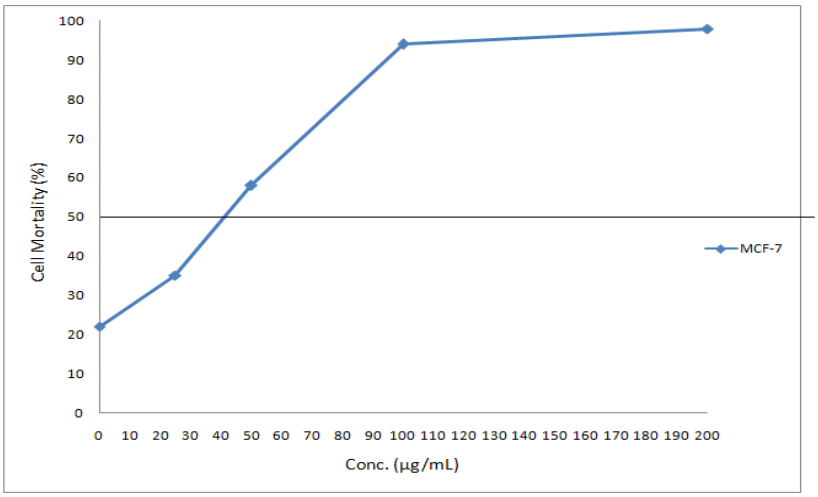
Cytotoxicity assay

The aqueous extract of *Ocimum gratissimum* leaf was tested for *in vitro* cytotoxicity, using MCF-7 cell line by MTT assay.[19] In brief, from row B to row G of the 96 -well plates 100

µL of RMPI 1640 (media) was added into each. Then, at different concentrations as 25µg/ml, 50µg/ml, 100µg/ml & 200µg/ml of *Ocimum gratissimum* aqueous leaf extract diluted with DMSO was added in row A and row B. 200 µL of solution containing 100 µL drug & 100 µL media were mixed starting from row B and 100 µl from row B were added into row C (next row)by micropipette and up to row G serial dilution was done. From row G excessive 100 µL were discarded and for each well the final volume was 100 µL.. By trypsinization the cultured MCF-7 cells were harvested & then plated 100 µL into 96-well micro-titre plates from row B to row G. For the control measure 200 µL of MCF-7 cells were added in row H. Test for each sample was replicated thrice and the cells were incubated in a 5% humidified CO_2 incubator at 37^0 C for 24 hrs. After the 24 hrs of incubation 20 µL of 5 mg/mL MTT in PBS was added into each well and the cells incubated for another 4 hrs.. Again 100 µL of DMSO was added followed by the shaking the plates for 5 min.. By using a micro plate reader at 540nm the absorbance for each well was measured. Anti-proliferation activity was established from the cell viability (CV) study which was calculated mathematically by using the following formula.

$$\text{Cell viability} = \frac{\text{Average absorbance of treated cells}}{\text{Average absorbance of untreated cells}} \times 100\%$$

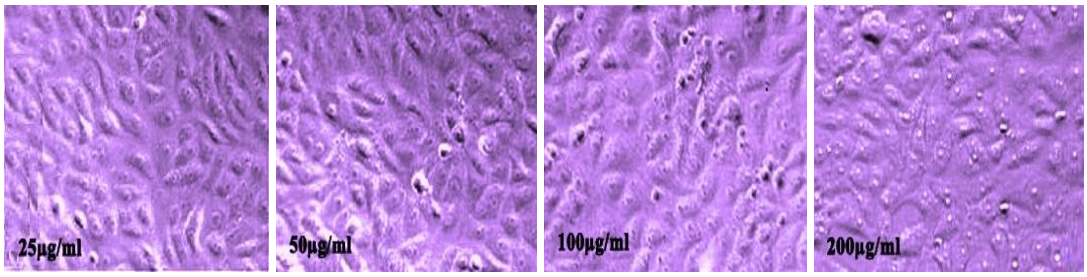
To enable the calculation of the concentration that killed 50% of the MCF-7 cells (IC_{50}) a dose-response curve was plotted.



Morphological analysis

Cells treated with *Ocimum gratissimum* extract brought the morphological changes & observation was done to establish cytotoxicity study. Cell shrinkage, membrane blebbing,

condensed chromatin, and apoptotic body formation like changes were observed which predict apoptosis, the mechanism for cell death.



A1 **A2** **A3** **A4**

(Morphological changes of MCF-7 cells (A1-A4) after treatment at various concentrations for 24 hrs.).

RESULTS

After exposure to the *Ocimum gratissimum* leaf extracts the morphological alteration of MCF -7 cells were indicated & observed under phase contrast microscope. The anticancer effect of Ocimum gratissimum aqueous extract (OGAE) on MCF-7 cell line was evaluated through micro culture MTT assay. The multiple concentrations of OGAE were used & effective dose was calculated from the dose response curve. OGAE exhibited significant antiproliferation activity against MCF-7 cell line with an IC50 value 41.7 µg/ml. Morphological changes or alteration of MCF-7 cells upon exposure using OGAE was observed under phase contrast microscope. The number of dead cells increased with the concentration increment of the extract treatment. Apoptotic bodies were seen in the extract treated MCF-7 cells with 25µg/ml. The cells with condensed nuclei and shrunken cytoplasm were considered apoptotic. Autophagosome like structures were seen in 50µg/ml & 100µg/ml treated MCF-7 cells. At highest i.e 200µg/ml concentration of OGAE treatment, the cells become sunken & detached from the surface of wells denoting cell death.

DISCUSSION

Plants contain new and novel chemotherapeutics, which account for control the chemotherapy of some cancers. This fortifies the researchers to search for new anticancer compounds in plant as medicines based on ethnomedicinal knowledge. Consequently, by using MTT assay aqueous leaf extract of *Ocimum gratissimum* was assessed as new anticancer agent in this study. Ethnomedicinal plants used by traditional people have been scientifically established as leads for therapeutic drug development in recent medicine.

Ocimum gratissimum was chosen for this study due to its use as an immunostimulant as well as an agent for wound healing among the tribes of Odisha and as medicine worldwide. A large number of bioactive compounds in the aqueous leaf extract of *Ocimum gratissimum* plant were observed including alkaloids, steroids, tannins, terpenoids and flavonoids. These compounds are immense potential as drug due to safety, minimal toxicity and their extensive appreciation by people. The cancerous MCF-7 breast cancer cell line was selected & investigated the cytotoxicity effect of *Ocimum gratissimum* extract on breast cancer cell, throughout this study. At the end of 24 hours incubation with extract, measure of percentage cell mortality calculated by MTT assay in MCF-7 breast cancer cells in a dose dependent manner indices the cytotoxicity. The MCF -7 breast cancer cell line was used as the test system in our study. The anti proliferation activity of *Ocimum gratissimum* aqueous leaf extract on the MCF-7 breast cancer cell was at 41.75 µg/mL. Result from the current study illustrates the capable cytotoxic effect on MCF-7 cells with *Ocimum gratissimum* aqueous leaf extract. Reduction in viable cell number after 24 hours of treatment with the extract manifests the cytotoxicity. More over the morphological observations like extensive blebbing and vacuolation suggest apoptosis mechanism of cell death. Our results show that *Ocimum gratissimum* aqueous leaf extract inhibits proliferation on treated MCF-7 cells having a higher anti proliferation activity. This reveals the potential cancer-fighting ability of *Ocimum gratissimum*. Therefore, *Ocimum gratissimum* leaf could potentially be a source of novel anti cancer compounds in future if successfully isolated, purified and identified. More clinical trials should be conducted to sustain its therapeutic use in formulating new treatments for breast cancer.

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