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ANTI METASTATIC EFFECT OF *PERGULARIA DAEMIA* ON BREAST (MCF-7) CANCER CELL LINE

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ABSTRACT

Background: Breast cancer develops as a result of genetic mutations or damage to DNA. On average only 35% women with advanced breast cancer are alive five year after diagnosis. However, search for new anticancer drugs for effective treatment is on as the plant- based system of medicine does not pose serious problems. So, the aim of this study is to analyze the antimetastatic effect of *Pergularia daemia* on Breast (MCF-7) cancer cell lines

Materials and Methods: *P.daemia* was collected, powdered and Soxhlet extracted with 70% ethanol. It was then filtered and solvent evaporated to get a viscous mass, which was then stored at 4° C until used.

Results: By optimizing the concentration of treated *P.daemia* it significantly decreases cell viability. Bcl-2 and Bcl-xL show significant decrease in expression with higher concentration of *P.daemia* treated cells. A significant increase in the Caspase-3 mRNA activity was observed after incubation with the leaf extract of *P.daemia***.**
Conclusion: *Pergularia daemia* exhibits antimetastatic effect on breast (MCF-7) cancer cell lines. The active component of *P.daemia* may promote the antimetastatic effect on breast cancer cell lines.

Keywords: *Pergularia daemia*, breast cancer, cancer cell lines, MCF-7, antimetastatic, anticancer property

INTRODUCTION:

Cancer is a disease in which some of the body's cells grow uncontrollably and spread to the other parts of the body. Although breast cancer is the most commonly spread to nearby lymph nodes [1]**.** It can spread further through the body through blood vessels and / or lymph nodes to areas such as the bones, lungs, liver and brain. This is called metastatic or stage IV breast cancer and is the most advanced stage of the disease [2].

On average only 35% women with advanced breast cancer are alive five year after diagnosis. In 2020, there were 2.3 million women diagnosed with breast cancer and 685,000 deaths reported globally. The first symptom of breast cancer is usually an area of thickened tissue in the breast or a lump in the breast or in the armpit. Other symptoms include armpit or breast pain, redness in the skin of the breast, rash around or one on nipple, discharge from nipple, which may contain blood, change in the size or shape of the breast, scaling of the skin of the breast or nipple [3]. Breast cancer develops as a result of genetic mutations or damage to DNA. These can be associated with exposure to estrogen, inherited genetic defects or inherited genes that can cause cancer, such as the BRCA1 and BRCA2 genes [4].

Although various treatment regimes are available for breast cancer, including surgery, radiotherapy and chemotherapy, severe side effects are still observed [5]. Therefore, prognosis and treatment must be performed before the cancer develops further. Thus, different medicinal plants have been studied using modern scientific methodologies to identify various biological components of these plants [6]**.**

Many indigenous Indian medicinal plants have been found to be successfully used to manage cancer and some of them have been tested and active principles isolated. However, search for new anticancer drugs for effective treatment is on as the plant- based system of medicine does not pose serious problems.

Pergularia daemia is a perennial twinning herb found in the plains throughout the hot parts of India and is said to have multiple applications in different folk medicine. [7] Phytochemical investigation of this plant shows the presence of alkaloids, flavonoids, terpenoids and steroids [8]**.** Traditionally, *P.daemia* is used as an antipyretic, anthelmintic and expectorant, as well as to treat infantile diarrhea and malarial intermittent fevers [9]. The excellent anti-fertility, antidiabetic [10], anti-inflammatory [11]and cardiovascular effects of this plant have been

reported in folk and Ayurvedic medicine [12]. However, there is little scientific investigation carried out on anti-cancer activity of this plant and there is a wide scope for investigation. Our team has extensive knowledge and research experience that has translate into high quality of publications [13–26]

The aim of the study is to analyze the antimetastatic effect of *Pergularia daemia* on Breast (MCF-7) cancer cell lines and the main objective is to prepare the powdered extract of *P.daemia* and to check the anti-cancer activity of *P.daemia* on MCF-7 cancer cell lines.

MATERIALS AND METHODS::

Chemicals:

Trypsin-EDTA, fetal bovine serum (FBS), antibiotics-antimycotics, Dulbecco's modified Eagle's medium (DMEM) and phosphate buffered saline (PBS) were purchased from Gibco, Canada. JC-1(5,5,6,6-tetrachloro-1,1,3,3-tetraethyl benzimidazole carbocyanine iodide) and real time PCR kit (MESA Green) were purchased from Invitrogen, USA. All the chemicals used were extra pure of analytical grade.

Extract Preparation:

The *Pergularia daemia* powder was extracted with 70% ethanol. The extract was then filtered with Whatman no. 1 filter paper and the solvent evaporated at reduced pressure by using a Rotary evaporator apparatus to get a viscous mass, which was then stored at 4°C until used.

Procurement and culture of MCF-7 cell line

The breast cancer cells, MCF-7 cell line was obtained from The National Center for Cell Science (NCCS), Pune, India and cultured according to the cell culture instructions provided. Briefly, MCF-7 cells were grown in DMEM containing 10% FBS at 37˚C in an atmosphere containing 5% CO_2.

Cell viability assay.

MCF-7 breast cancer cells were seeded at a density of 5x105 cells/well in 96-well plates and allowed to attach to the well overnight. After incubation, cultured cells were stimulated with various concentrations of *Pergularia daemia* in triplicate and incubated at 37˚C in a 5% humidified CO2 incubator for 24 h. Subsequently, 3-(4,5-dimethylthiazol2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to each well, and incubation was continued for a further 4 h at 37˚C. To dissolve the formazan formed from MTT, the cells were resuspended in 200 µl dimethyl sulfoxide (DMSO), and the optical density (OD) of the solution was determined using a spectrometer at a wavelength of 570 nm. The experiments were repeated 3 times, independently. The mean optical density (OD) ± SD for each group of replicates was calculated. The entire procedure was repeated 3 times. The inhibitory rate of cell growth was calculated using the equation:

% Growth inhibition = (1 – OD extract treated)/OD negative control x 100.

Gene expression analysis by Real Time PCR:

mRNA expression levels were examined using real-time PCR. The total RNA was isolated by using Tri Reagent (Sigma). Total RNA (2 µg) from each sample was reverse transcribed using a commercial Superscript III first strand cDNA synthesis kit (Invitrogen, USA) according to the manufacturer's protocol. Real time-PCR was carried out in a MX3000p PCR system (Stratagene, Europe). Reaction was performed using MESA Green PCR master mix (It contains all the PCR components along with SYBR green dye.) Eurogentec, USA. The specificity of the amplification product was determined by melting curve analysis for each primer pair. The data were analyzed by comparative CT method and the fold change is calculated by 2−CT method described by Schmittgen and Livak (2008) using CFX Manager Version 2.1 (Bio Rad, USA).

Statistical analysis:

Data were expressed as the means ± SD of 3 individual experiments performed in triplicate. Statistical analysis was performed using the one-way ANOVA and $p<0.05$ was considered to indicate a statistically significant result.

RESULT:

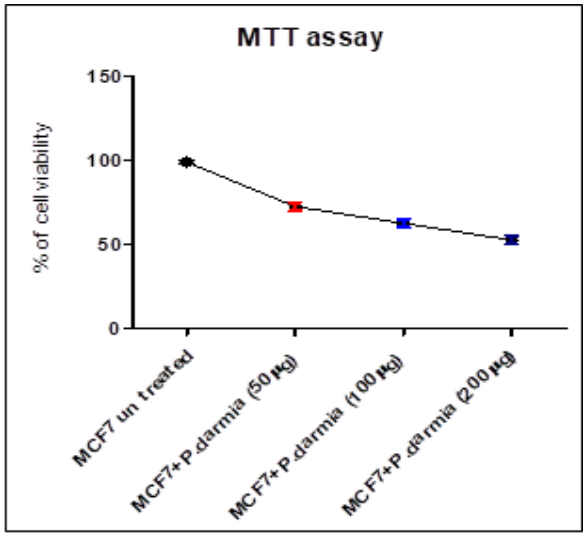

Figure 1: MTT assay

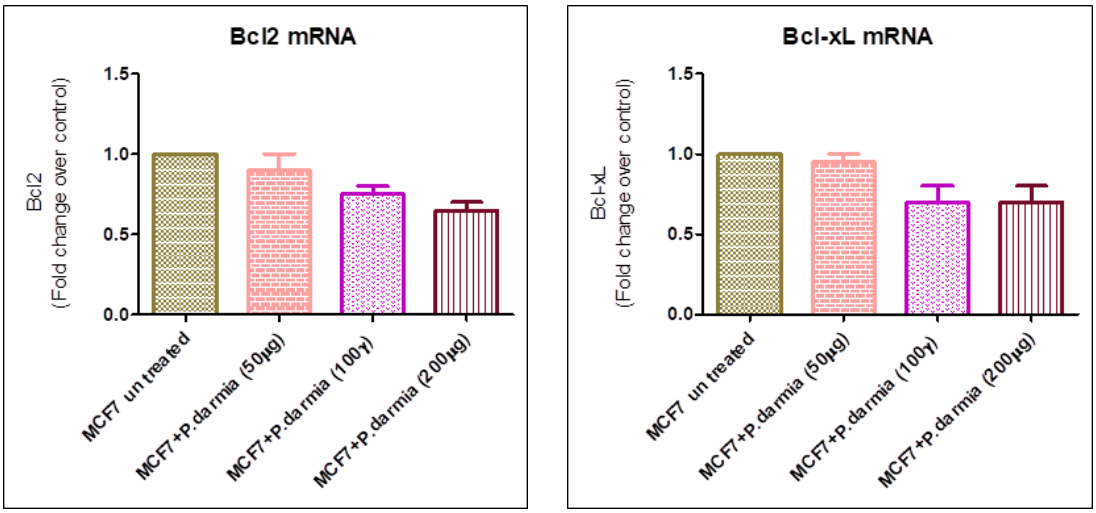

Figure 2:

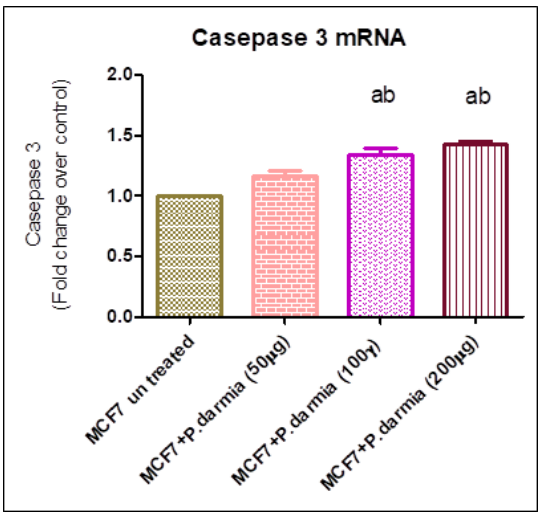

Figure 3:

By optimizing the concentration of treated *P.daemia* it significantly decreases cell viability. Bcl-2 and Bcl-xL show significant decrease in expression with higher concentration of *P.daemia* treated cells. A significant increase in the Caspase-3 mRNA activity was observed after incubation with the leaf extract of *P.daemia***.**

DISCUSSION:

The various parts of the plant are reported to possess numerous pharmacological properties such as wound healing, anti-diabetic, and antibacterial activity. It is believed that more new detailed information would help the researchers to get aware of this plant and extensive research should be undertaken on *P.daemia* for establishing new therapeutic drugs for mankind.

Bcl-xL belongs to the Bcl-2 family and has anti-apoptotic activity. It has been proposed that Bcl-xL modulates apoptosis by controlling mitochondrial membrane permeability and regulating the release of cytochrome c. [27]**.** In a liver tumor study, Bcl-xL was localized mainly in the cytoplasm and mitochondria of both normal and cancer cells. It was interesting that Bcl-xL expression was also found in the nuclei of liver cancer cells in the tumor margins. This suggests that Bcl-xL participates in the progression of the liver cancer cells [28]**.**

Similar study was conducted by Hamad Ghaleb Dailah which aimed to assess the anticancer and antioxidant activity of *P.daemia* inspired zinc oxide nanoparticles against lung cancer (A549) cell line. *P.daemia* is a therapeutic plant that has been explored and identified with excellent pharmacological activity, but less reported. In this study, ZnO NPs were manufactured from *P.daemia* aqueous extract and their property was evaluated by MTT assay and BAX and PARP gene expression by PCR. Results exhibited decrease in cell viability and increase in the cytotoxicity in green synthesized ZnO nanoparticles with increasing doses. Based on the above findings, green synthesized ZnO nanoparticles with *P.daemia* are effective against A549 cancer cell lines proving their anticancer and antioxidant activity [29].

A study conducted by Sunitha Marin et al on the cytotoxic activity of *P.daemia* against ovarian cancer cell lines OAW-42 and PA-1[30]**.** The antioxidant activity of the plant extract was tested spectrophotometrically by DPPH assay, reducing power assay, ABTS assay, FRAP assay and nitric oxide radical scavenging assay. The findings provide evidence that the crude methanolic extract of *P.daemia* is a potential source of natural antioxidants and this justifies its use in folk medicines and in cancer therapy.

CONCLUSION:

The results suggested that the Bcl-2 and Bcl-xL may induce the cellular metastasis on breast cancer. The active component of *P.daemia* may promote the anti-metastatic effect on breast cancer cell lines.

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Author Contributions:
Aditya Mantri: Literature search, Data collection analysis, Manuscript drafting
Dr.R. Gayatri Devi : Data Verification, Manuscript draft
Dr.J.Selvaraj: Data collection analysis, Data Verification, Manuscript draft

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